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DEVELOPMENT AND *EX-VIVO* EVALUATION OF ATORVASTATIN MICROEMULSIONS FOR TRANSDERMAL DELIVERY USING BOX-BEHNKEN DESIGN

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ABSTRACT

Atorvastatin is a lipid lowering drug with low oral bioavailability and associated side effects. Hence atorvastatin microemulsions and microemulsion based gels for transdermal delivery were developed using Box-Behenken design and evaluated physico chemical parameters and ex vivo permeation on rat abdominal skin. Based on solubility of atorvastatin, Isopropyl Myristate, Tween 80 and Propylene Glycol were selected as oil, Surfactant and co surfactant respectively. Microemulsions were prepared by water titration method. Pseudo ternary phase diagrams were constructed to choose the levels of surfactants and oil. The influence of independent variables such as oil, Smix and water on responses Size, Zeta potential and flux were studied with the help of polynomial equations and response surface plots generated by design expert software. optimized microemulsion formulation (ME18D) composed of oil, Smix, water, drug and DMSO in the ratio of 5: 50: 45: 1: 5. The size, Zeta potential and flux of the optimized microemulsion was 62.5nm, -28.9mV, and107.2 µg/cm^2/h respectively. The optimized microemulsion was converted to gel by adding to either Carbopol 934 or HPMC K4M gels. The flux of ME 18D was 5.81 times, ME 18(without DMSO) was 4.91 times to that of drug solution. The flux of HPMC gel and Carbopol gel was significantly lower than optimized formulation ME18D. Indicating potential advantage of microemulsion formulation.

KEY WORDS

Atorvastatin; Microemulsion; Box-Behnken design; Transdermal delivery; ex vivo permeation.

INTRODUCTION

Hyperlipidemia is a disorder which increases the risk of cardiovascular disease. It was reported that a 10% reduction in serum cholesterol level results in a 50% reduction in occurrence of cardiac disease [1]. Statins are first line drugs to lower the elevated lipid levels. There is extensive evidence that statin therapy can provide protection against the cardiovascular disease [2]. Statins therapy is associated with many side effects as they are continued for life time. The adverse effects include gastrointestinal disorders (0.5%), myalgia (0.1%), arthralgia (0.1%), induction of type II diabetes and liver damage. Atorvastatin is one of the most frequently used statins for hyperlipidemia. It is a potent competitive inhibitor of 3-hydroxy-3-metylglutaryl coenzyme A reductase, an enzyme which is responsible for the conversion of HMG CoA to mevalonate, a precursor of cholesterol synthesis [3]. Atorvastatin bioavailability is 12% due to its extensive first pass metabolism by the liver. Therefore, an alternative route of drug administration is required to reduce side effects and to increase bioavailability.

The present investigation of transdermal delivery of atorvastatin microemulsions was designed to overcome potential adverse effects associated with oral administration. Transdermal drug delivery is an

effective route for systemic delivery of drugs. It gained importance due to its potential advantages such as avoidance of gastrointestinal tract environment, first pass metabolism by the liver, convenient in instances like vomiting/diarrhea and withdrawal of medication is possible at any point of time in case of adverse effects [4, 5]. Use of carrier systems like liposomes, microemulsions, nanoemulsions etc. has become one among the promising methods employed for enhancing transdermal permeation of drugs [6–9]. Recently many drugs such as theophylline, hydrocortisone, peniciclovir, meloxicam and estradiol were studied transdermally as microemulsions and proven effective in overcoming the barrier of stratum corneum [10–13].

Microemulsions are optically clear and thermodynamically stable solutions consisting of oil, surfactant, co surfactant and water. Microemulsions (o/w) have potential advantage of enhanced solubilization of lipophilic drugs and enhanced permeation across the skin due to their nano sized globules.

The formulation ingredients of microemulsions might increase the skin permeation of the drug by acting as permeation enhancers [14, 15]. The main objective of the present study was to develop atorvastatin microemulsion and microemulsion based gels for transdermal delivery. The optimized formulations were further evaluated for physico chemical characterization and *ex vivo* permeation.

MATERIALS AND METHODS

Materials

Atorvastatin was obtained as gift sample from Aurobindo Pharma Ltd., Hyderabad, T.S, India. Tween 80, Isopropyl myristate, Polyethylene glycol 400, Isopropyl alcohol, Ethanol, Dimethyl Sulfoxide, Sodium lauryl sulfate and Triton X 100 were purchased from SD Fine chemicals, Mumbai, India. Transcutol P, Capmul MCM, Captex R, Lauroglycol were from Gattefosse India pvt. Ltd, Mumbai, India. Oleic acid was from Sigma Aldrich, Bangalore, India. Double distilled water was used for the preparation of microemulsions. All the solvents were of HPLC grade from Merck.

Methods

Estimation of atorvastatin by UV method

100 mg of Atorvastatin was accurately weighed in to 100 ml volumetric flask. The drug was dissolved in 5 ml of methanol and the volume was made up with pH 7.4 Phosphate buffer saline. From the stock solution (1mg/mL) dilutions were made to get the concentrations between 2µg/mL to 24µg/mL. The absorbance of the samples was measured by U.V visible spectrophotometer at 245 nm and standard graph was constructed.

Solubility studies

The solubility of atorvastatin in various oils, surfactants and co-surfactants was determined by using equilibrium solubility method. Two ml of solvent was taken in a glass vial to which excess amount of atorvastatin was added. The mixture was agitated a shaker at room temperature for 48 hours. The supernatant was filtered through 0.22 µ membrane filter and the filtrate was diluted suitably with methanol and analyzed by UV-method.

Construction of Pseudo ternary phase diagrams

The microemulsion region and the concentration range of ingredients were determined by constructing pseudo-ternary phase diagrams, using water titration method. Microemulsions were prepared at different ratios (1:1, 2:1, 3:1, 1:2 and 1:3) of surfactant and co-surfactant (Smix). The surfactant mixture (S/Co-S) and oil phase were mixed at different weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. The oil and surfactant mixtures were added with aliquots of distilled water drop by drop until the mixture turns to turbid. The amount of water added to the mixture was noted and Pseudoternary phase diagrams were constructed using CHEMIX software [16].

Experimental design

A three level, three factor Box-Behnken experimental design (Design expert software, Version 10.0.2. Stat-Ease Inc., MN) was used for the formulation optimization[17]. Box-Behnken design is categorized under the response surface designs, with 3 levels, coded as -1, 0 and +1. The three major factors affecting the formulation, oil (X_1), Smix (X_2) and water(X_3) were selected as independent variables and particle size (Y1), zeta potential (Y2), Flux(Y3) were selected as dependent variables. The design is suitable for determining the influence of factors on responses. The characteristic feature of design is replicated (n=5) center points lying at the midpoint of each edge and center point of the multi-dimensional cube. Design matrix was comprised of 17 experimental runs. The polynomial equation generated for nonlinear quadratic model was as follows:

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

Where Y_i is the measured response of each factor level combination; b_0 is intercept; $b_1, b_2, b_3, b_{12}, b_{13}, b_{23}, b_{11}, b_{22}$ and b_{33} are regression coefficients calculated from the measured experimental response Y. X_1, X_2 and X_3 are the coded levels of independent variables. The terms X^2 and X_1X_2 represent the quadratic and interaction terms respectively. All 17 experiments were conducted and the responses were measured.

Preparation method of microemulsions

Microemulsions were prepared by dissolving accurately weighed amount of atorvastatin in a mixture of oil and surfactant by vortexing followed by the addition of weighed quantity of water with stirring to form a clear and transparent microemulsion.

Characterization of microemulsions

Physical appearance

Physical appearance of the microemulsion was observed visually for the transparency.

Droplet Size, Zeta potential, Polydispersity Index (PDI)

Droplet size, Polydispersity Index (PDI) and Zeta potential of diluted (100 times with millipore water) microemulsion samples were determined by photon correlation spectroscopy using Zeta Sizer (Nano – ZS 90, Malvern Instruments, Ltd., UK).

Measurement of pH and Viscosity

The pH of microemulsion formulations was determined by using digital pH meter. The viscosity of the microemulsion was measured using Brookfield's viscometer, Spindle C-50 (Brookfield, USA).

Drug Content

The microemulsion was suitably diluted using methanol to obtain a drug solution of 10 µg/ml and the content was analyzed by UV method.

% Transmittance

Percentage transmittance of the microemulsion formulations was determined at 633 nm, spectrophotometrically using UV–Visible spectrophotometer (Shimadzu, Japan).

Preparation of rat abdominal skin

The animal study was conducted in accordance and approval of CPCSEA and Institutional Animal Ethical Committee (IAEC), vide No- IAEC/29/UCPSc/KU/2016, University College of Pharmaceutical Sciences, Kakatiya University, India. Male Wistar rats weighing between 150–200 g were sacrificed by cervical dislocation method. The full thickness of skin from the abdominal region was removed from the rats after the trimming of hair carefully using electrical clippers. The epidermis was separated by heat separation technique. The entire skin was immersed in water at 60 °C for a period of 45 seconds, immediately after taking out from hot water epidermis was separated using blunt forceps. The isolated skin was washed with water and preserved at -20° C until use [18].

Ex vivo permeation studies

Ex vivo permeation studies were conducted by using vertical Franz diffusion cells. The phosphate buffer saline pH 7.4 was filled in receptor compartment, the isolated epidermis of rat was mounted on franz diffusion cell by facing epidermis towards donor compartment. Formulation was added to the donor cell and the receptor cell contents were stirred at 400 rpm on magnetic stirrer. Samples of 2 mL were collected at time points of 0, 1, 2, 3, 4, 8, 12, 18 and 24 hours and replenished with PBS pH 7.4. The samples were analyzed by UV method. Similarly, permeation studies were carried out for drug solution (atorvastatin dissolved in 30% Propylene Glycol), Drug dissolved in mixture of isopropyl myristate and Smix and microemulsion based gels. The cumulative amount of atorvastatin permeated through the rat skin was calculated by the equation

$$Q_n = [C_nV + \sum_{i=1}^{n-1} C_iS]$$

Where,

Q_n= Cumulative amount of drug permeated at n^{th} time

C_n= Concentration of drug (µg/mL) determined at n^{th} sampling interval

V= Volume of individual Franz diffusion cell,

ΣC_iS =Sum of concentrations of samples (n-1) determined at sampling points 1 through n-1

i =1 multiplied with sampling volume (S).

Permeation data analysis

The graph was plotted between Cumulative amount of drug permeated through the skin (µg) and time (h) for each formulation. Drug flux (µg/cm^2/h) at steady state (Jss) was calculated by dividing slope of the linear portion of the regression line with the effective diffusion cell area. Permeability coefficient (Kp,) was calculated by dividing the Jss with the initial concentration (µg/cm^3) of the drug in the donor cell. Enhancement ratio (ER) is ratio between steady state flux (Jss) of formulation and drug solution. The lag time was

obtained from the X-axis intercept by extrapolating the plot to the time axis.

Check point analysis and model validation

Six formulations were selected for the check point analysis from design matrix by grid search. The formulations were prepared and evaluated for the response properties. The results of experimental values were compared with predicted values and the percentage prediction error was calculated. The optimized formulation was selected by exhaustive feasibility and grid search, based on desirability (near to 1).

Microemulsion with permeation enhancer and gelling agents

Dimethyl sulfoxide (DMSO) was added to optimized formulation (ME18) at 5% level as permeation enhancer (ME18D). The optimized microemulsion with 5% DMSO (ME18D) was converted into gel form by adding microemulsion to the gel under continuous stirring. Gelling agents HPMC K4M at 4% concentration and Carbopol 934 at 2% concentration were used. Carbopol gel was prepared by neutralizing with triethanolamine.

Stability studies

Stability of optimized formulations ME18 and ME18D were studied at room temperature and at refrigerated temperature for three months. The samples were withdrawn at monthly intervals and analyzed for particle Size, PDI, Zeta potential and drug content. Physical stability of microemulsion was evaluated by centrifugation at 10,000 rpm for 30 minutes.

Statistical analysis

Results were expressed as mean ± S.E (n=6). Statistical significance among the groups was carried out by one-way analysis of variance (ANOVA) followed by Tukey-Karmer multiple comparison tests using Graph pad prism software.

RESULTS AND DISCUSSION

Calibration curves of Atorvastatin

The calibration curves obtained in methanol and pH 7.4 PBS showed good linearity with correlation coefficient values of 0.999.

Solubility studies

In order to screen appropriate solvent system for the preparation of microemulsions, the solubility of atorvastatin was determined in various oils, surfactants and co surfactants and the results were shown in Table 1. The solubility of atorvastatin was highest in isopropyl myristate (IPM), surfactant tween 80 and cosurfactant propylene glycol (PG). IPM is an ester and increases the permeation by disruption of lipids in the skin [19]. Tween 80 is non ionic surfactant and is widely used in topical and transdermal formulations as solubilizing agent. PG is safe component as co surfactant. Based on results of preliminary studies, IPM, tween 80 and propylene glycol were selected as the oil phase, surfactant and cosurfactant for the formulation of microemulsions in this study.

Construction of pseudoternary diagrams

IPM (Oil phase), Tween 80, PG (Smix) at different ratios and water were selected for the construction of pseudo ternary phase diagrams (Fig.1).

The transparent microemulsion region was represented as shaded area. Maximum isotropic region was obtained at 2:1 ratio of Tween 80 and PG. Hence used at 2:1 ratio in the microemulsion formulations.

Formulation optimization by Box- Behnken design

All 17 experimental runs with their independent variables (factors) and the measured responses were given in Table 2. The high-medium-low levels of oil were 5–6.25-7.5, Smix were 50-55-60, water was 32.5-38.75-45.

Table: 1 Solubility studies

S.No	Oils	Solubility (mg/mL)
1	Oleic Acid	49 ± 1.5
2	Isopropyl Myristate	62±1.6
3	Capmul MCM	22 ±1.2
4	Capmul GMO	20 ±1.7
5	Captex 355	19 ±1.3
6	Labrafil	14±1.3
7	Olive oil	9 ±1.3
8	Soya oil	8 ±0.8
9	Lauroglycol	13 ± 1.5
Surfactants and Co Surfactants		
10	Tween 80	116± 1.3
11	Isopropyl Alcohol	45 ±0.9
12	Ethanol	50 ±1.1
13	Transcutol P	86 ± 1.2
14	Poly ethylene Glycol 400	36 ±1.3
15	Propylene Glycol	168.4 ±1.4

Data shown as mean ± SD (n=3)

Table 2: Composition of formulations and optimized formulations generated by Box Behnken design and measured responses

Formulation	Oil (A)	Smix (B)	Water (C)	Size (nm)	ZP (mV)	Flux (µg/cm²/h)
ME1	7.5	60	38.75	117.9	-23.4	30.35
ME2	5	55	32.5	55.2	-31.7	70.83
ME3	6.25	55	38.75	88.4	-26.6	50.34
ME4	7.5	55	32.5	129.2	-23.2	35.14
ME5	5	55	45	59.8	-29.8	82.1
ME6	6.25	50	32.5	98.7	-25.3	54.06
ME7	5	50	38.75	64.9	-28.4	76.6
ME8	6.25	55	38.75	90.6	-25.3	45.7
ME9	7.5	55	45	138.6	-22.8	37.54
ME10	6.25	50	45	103.4	-24.5	61.09
ME11	6.25	55	38.75	92.1	-24.8	49.26
ME12	6.25	55	38.75	89.3	-23.6	42.79
ME13	7.5	50	38.75	145.4	-22.1	40.98
ME14	5	60	38.75	46.3	-32.5	68.14
ME15	6.25	60	32.5	77.3	-28.4	41
ME16	6.25	60	45	82.7	-27.5	48.34
ME17	6.25	55	38.75	91.1	-25.1	44.58

Data shown as mean ± SD (n=3)

Note: Atorvastatin 1part is common in all formulations

Table 3: Characterization of design formulations and optimized formulation

S.No	pH	Viscosity (mPa-s)	PDI	Lag Time (h)	Kp×10−3 (cm/h)	% T	DC	ER
ME1	6.56	184	0.13	2.59	3.04	98.8	98.4	1.64
ME2	6.51	173	0.15	4.51	7.08	98.6	98.6	3.84
ME3	6.62	182	0.15	3.57	5.03	99.2	99.2	2.73
ME4	6.74	194	0.12	2.81	3.51	98.5	99.3	1.90
ME5	6.58	187	0.20	4.78	8.21	98.7	98.5	4.45
ME6	6.78	185	0.18	3.23	5.41	98.4	99.8	2.93
ME7	6.85	193	0.18	4.1	7.66	99.3	99.6	4.15
ME8	6.73	215	0.13	3.6	4.57	99.1	98.3	2.48
ME9	6.81	263	0.12	2.62	3.75	98.6	98.2	2.03
ME10	6.75	257	0.13	4.23	6.11	99.5	98.5	3.31
ME11	6.57	283	0.14	4.05	4.93	99.1	99.1	2.67
ME12	6.79	218	0.15	2.88	4.28	98.2	99.3	2.32
ME13	6.53	316	0.15	2.73	4.10	98.3	98.5	2.22
ME14	6.48	305	0.19	4.13	6.81	98.4	99.3	3.69
ME15	6.5	223	0.16	2.95	4.10	98.8	98.4	2.22
ME16	6.42	269	0.20	3	4.83	99.2	98.6	2.62
ME17	6.85	319	0.17	3.07	4.46	98.5	99.1	2.41
ME18	6.47	199	0.10	3.1	9.06	99.6	99.2	4.91
ME18D	6.76	285	0.14	2.4	10.72	99.1	99.4	5.81

Data shown as mean ± SD (n=3)

Note: PDI: Poly dispersity index; Kp: Permeation coefficient; ER: Enhancement ratio; % T: Percentage of transmittance; DC: Drug content.

Table 4: ANOVA and Regression values for the quadratic model

Parameter	Source	D.F	S.S	M.S	F-value	P-value	Adeq. Pre	%C.V	PRESS
Size	Model	9	12776.9	1419.65	792.31	< 0.0001	95.725	1.45	76.81
	Residual	7	12.54	1.79					
	Lack of fit	3	3.96	1.32	0.62	0.6402			
	Pure error	4	8.58	2.14					
ZP	Model	9	149.34	16.59	24.53	0.0002	16.803	3.14	8.95
	Residual	7	4.74	0.68					
	Lack of fit	3	0.11	0.036	0.031	0.9916			
	Pure error	4	4.63	1.16					
Flux	Model	9	3572.73	396.97	58.44	< 0.0001	25.523	5.04	176.84
	Residual	7	47.55	6.79					
	Lack of fit	3	7.1	2.37	0.23	0.8686			
	Pure error	4	40.45	10.11					
R^2Analysis									
	R^2		R^2 Adjusted		R^2 Predicted		R^2 Predicted-R^2Adjusted		
Size	0.999		0.9978		0.994		-0.0038		
ZP	0.9693		0.9297		0.9419		0.0122		
Flux	0.9869		0.97		0.9512		-0.0188		

Note: DF: Degrees of freedom; SS: Sum of squares; MS: Mean squares; CV: Coefficient of Variation; Adeq.Pre: Adequate Precision; PRESS: Predicted Residual Error Sum of Squares; ZP: Zeta potential.

Table 5: Check point analysis of design formulations

Formulation number	Formulation composition (A:B:C)	Response variable	Experimental value	Predicted value	Percentage prediction error
1	5: 50: 45	Y1	64.4	67.1	-4.1
		Y2	-29.3	-28.28	3.48
		Y3	90.63	87.43	3.53
2	5: 51: 45	Y1	62.8	65.6	-4.4
		Y2	-29.7	-28.5	4.04
		Y3	88.8	85.02	4.25
3	5 : 56.5 : 45	Y1	58.5	56.7	3.07
		Y2	-29.5	-30.54	-3.5
		Y3	78.3	76.04	2.88
4	5 : 54.36 : 39.4	Y1	56.5	57.7	-2.12
		Y2	-29.1	-29.5	-1.37
		Y3	71.4	73.5	-3.9
5	5 : 53.7 : 45	Y1	62.5	61.4	1.76
		Y2	-28.7	-29.3	-2.09
		Y3	77.4	79.79	-3.08
6	5 : 52.8 : 45	Y1	63.6	62.9	1.1
		Y2	-28.1	-29	-3.2
		Y3	79.7	81.3	-2

Table 6: Composition of optimized formulation and measured responses

Formulation	Oil (A)	Smix (B)	Water (C)	Size (nm)	ZP (mV)	Flux (µg/cm ^{2}/h)	ER
ME18	5	50	45	64.4	-29.3	90.63	4.91***
ME18D	5	50	45	62.5	-28.9	107.2	5.81****
DS	atorvastatin in 30% Propylene Glycol					18.46	1
Drug in oil and Smix	atorvastatin in oil and Smix (5:50)					25.82	1.3
ME18DH	HPMC gel					58.7	3.17**
ME18DG	Carbopol gel					65.5	3.5**

Note: ** indicates significance at p<0.0001, *** at p<0.001, ** p<0.01 at when compared with DS (Drug solution).**

Table 7: Stability studies

Time	Size(nm)		PDI		ZP (mV)		Drug content (%)	
Months	4°C	25°C	4°C	25°C	4°C	25°C	4°C	25°C
0	65.4±0.13	64.6±0.24	0.169±0.23	0.167±0.17	-28.3±0.14	-28.1±0.16	99.12±0.14	99.24±0.18
1	66.6±0.14	66.5±0.17	0.171±0.12	0.168±0.18	-27.6±0.12	-27.8±0.15	99.15±0.13	99.22±0.17
2	68.1±0.17	67.3±0.19	0.172±0.15	0.168±0.14	-27.4±0.17	-27.1±0.14	99.13±0.19	99.20±0.16
3	68.9±0.19	68.7±0.16	0.174±0.16	0.170±0.12	-26.9±0.15	-26.9±0.17	99.10±0.16	99.13±0.11

Data shown as mean ± SD (n=3)

Characterization of microemulsions

The experimental values of physico chemical properties of microemulsions were shown in Tables 2 & 3. The mean globule size of microemulsions varied between 55.2-145.4 nm. The poly dispersity index was found in between 0.102 to 0.195, the zeta potential varied between -22.1mV to -32.5 mV. The drug content of formulations was 98.2-99.8. The pH of the formulations varied between 6.42 - 6.86. The percentage of transmittance varied from 98.2 to 99.8 %. The viscosities of formulations were found to be in the range of 173 – 319 mPa-s.

Ex-vivo permeation studies

The permeation profiles of atorvastatin formulations through rat skin were shown in (Fig.2 and 3). Flux of optimized formulations was shown in Fig. 4. The steady state flux, lag time, enhancement ratio and permeation coefficient (Kp) of all experimental formulations were shown in Tables 2 &3. Steady state flux ranged from 30.35 to 82.1 µg/cm^2 /h and lag time ranged from 2.6 h to 3.9 h. Permeation coefficient values ranged from 3× 10^{-3} to 8.21× 10^{-3} cm/h. Drug solution (control) showed a flux of 18.46 µg/cm^2/h and a lag time of 4.5h. Drug in oil and Smix permeated with a flux of 25.82 µg/cm^2/h and a lag time of 4.3h. The enhancement ratio of microemulsions was 1.6–4.4 folds higher than the drug solution.

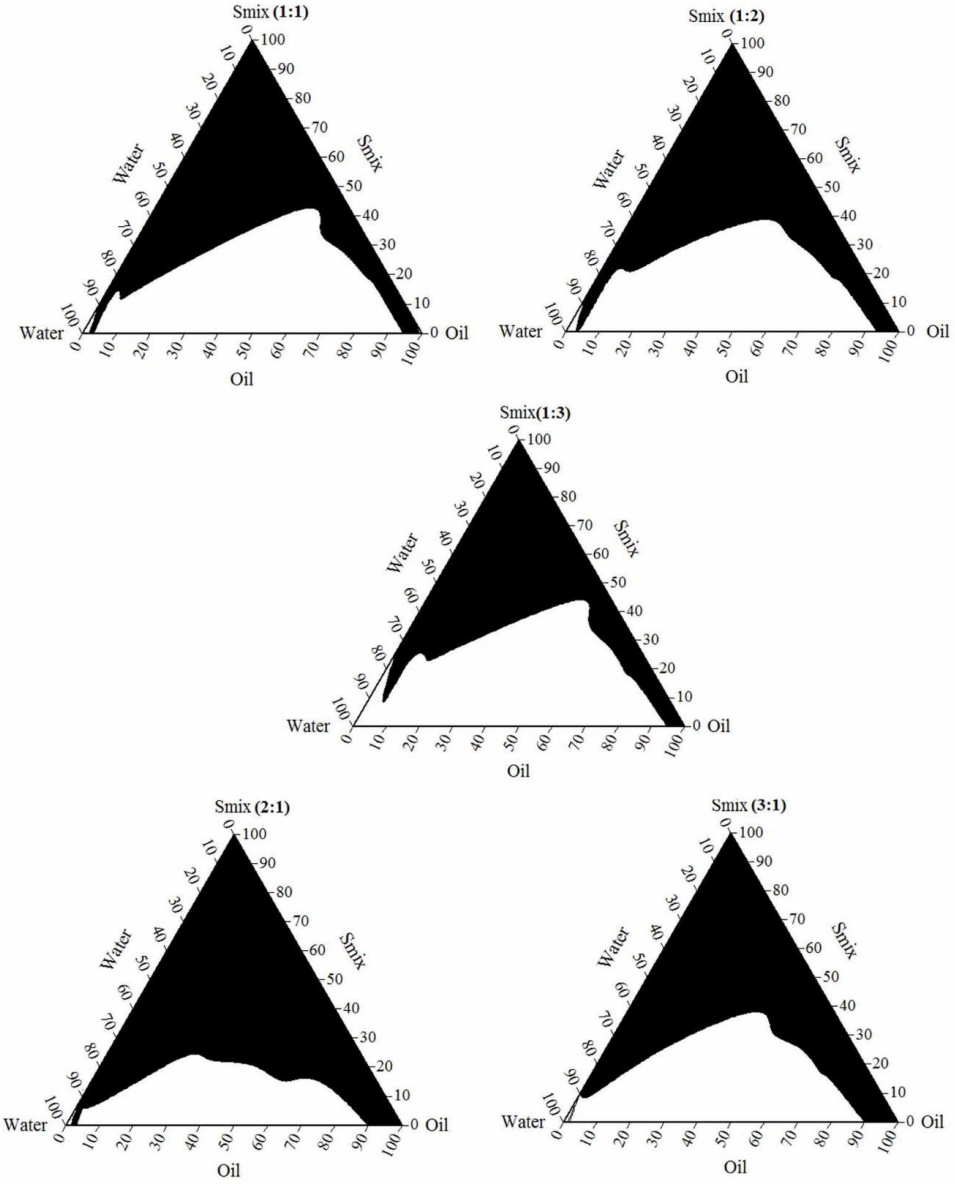

Figure 1: Pseudo-ternary phase diagrams of microemulsions composed of oil (IPM), Smix (Tween 80: PG) and water. Shaded area represents microemulsion region

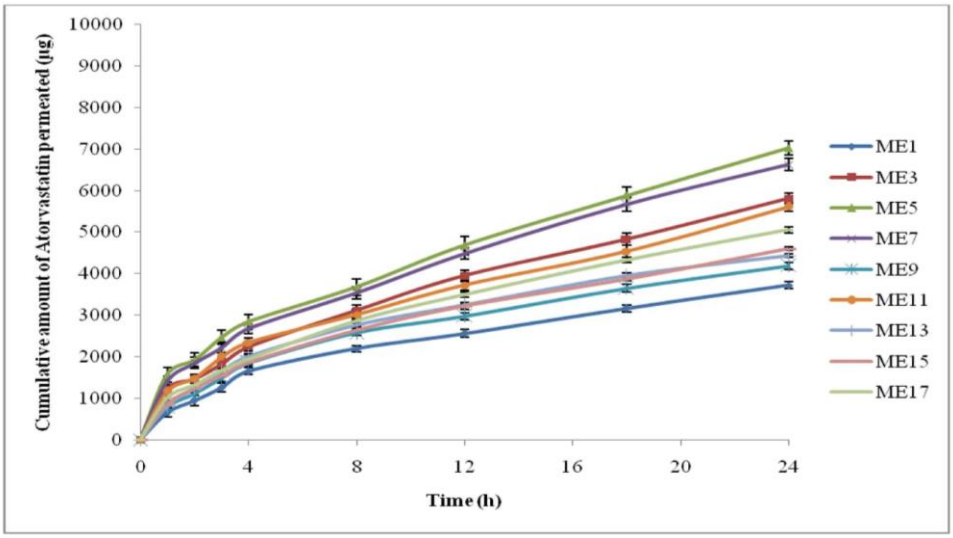

Figure 2: *Ex- vivo* permeation profiles of microemulsion formulations

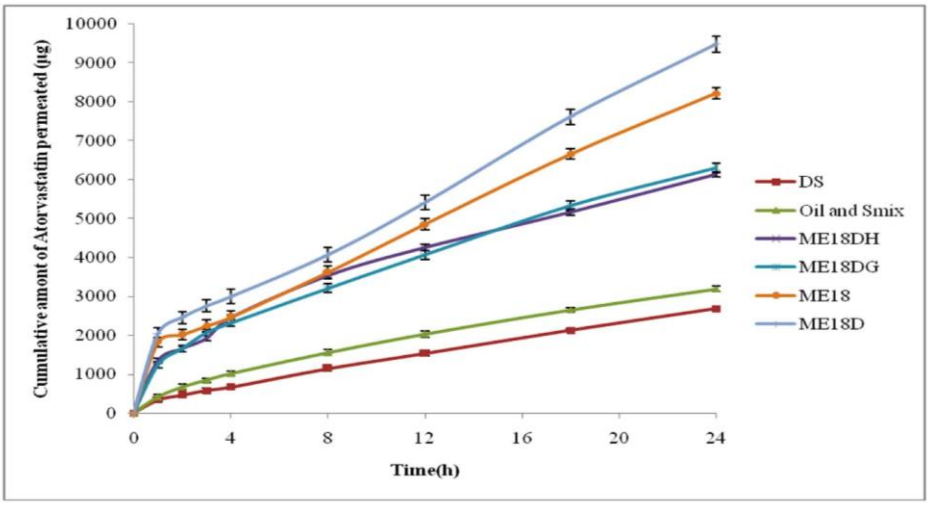

Figure 3: *Ex- vivo* permeation profiles of microemulsion formulations including DS (Drug solution), Oil and Smix , ME18, ME18D, ME18DH (HPMC Gel) and ME18DG (Carbopol Gel).

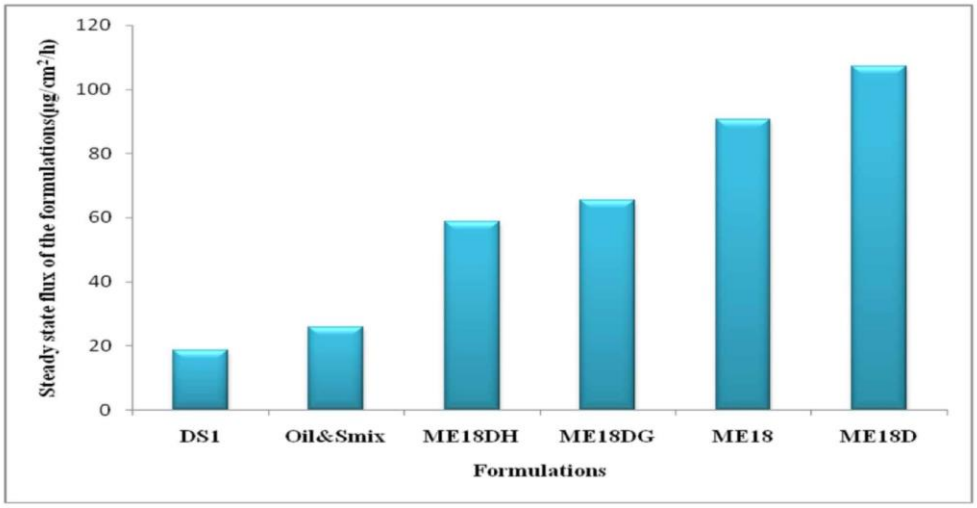

Figure 4: Steady state flux of DS (Drug solution), Drug in oil and Smix, ME18, ME18D, ME18DH (HPMC Gel) and ME18DG (Carbopol Gel).

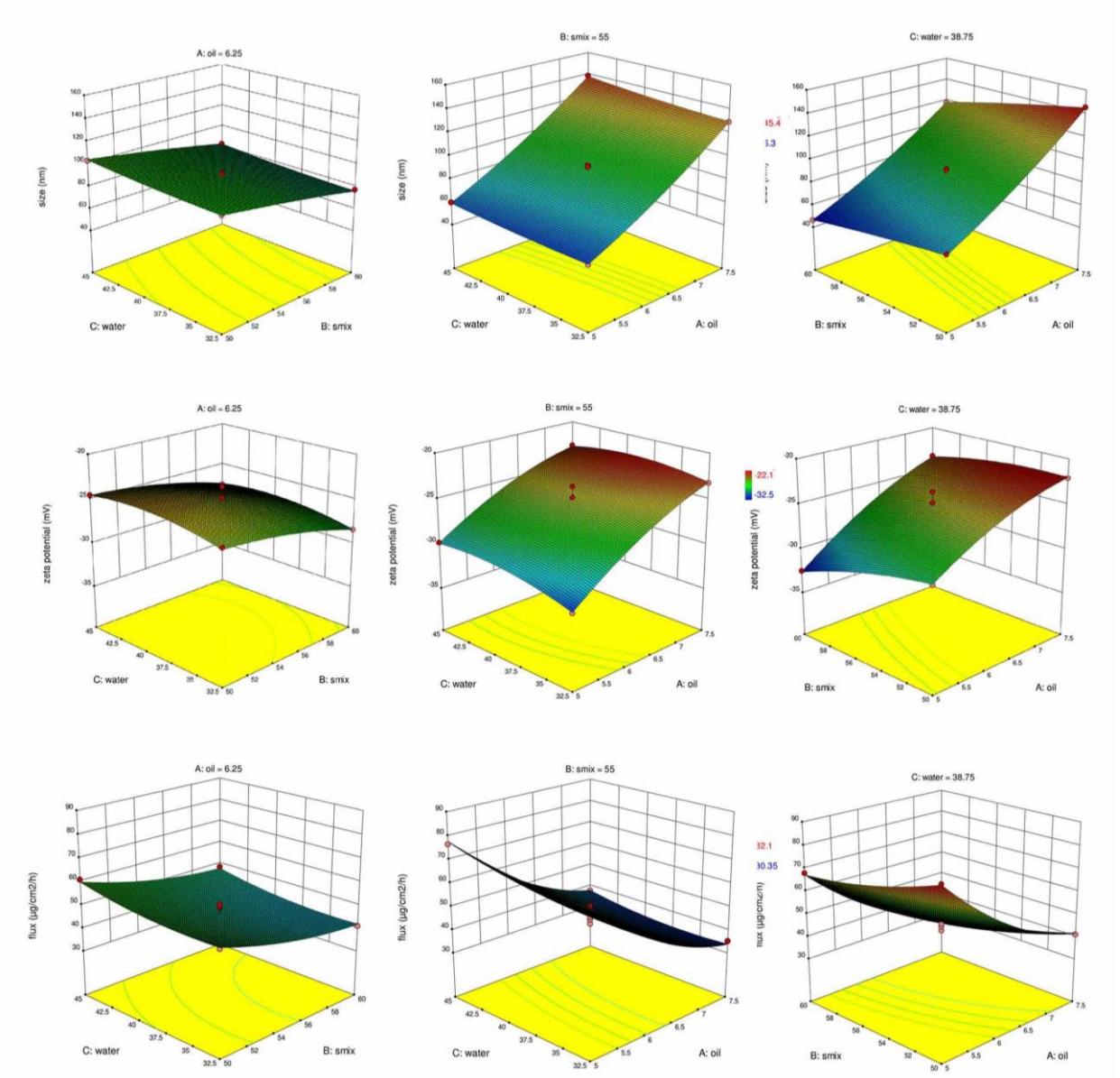

Figure 5: 3D Response surface plots of size, zeta potential and flux showing effects of oil, Smix and water

Experimental design- data analysis

The experimental data were analyzed using ANOVA and the results were presented in Table 4.

The model's fit was confirmed by the difference between predicted R^2 value and adjusted R^2 value and the probability (P-value) greater than F-Value [20]. The Polynomial equations generated by design expert software for the responses size; flux and zeta potential were given in equations 1, 2 and 3 respectively.

The model p- values for the responses size, flux and zeta potential were significantly high. Quadratic model PRESS value was low. These two parameters indicate the significance of the Quadratic model. The lack of fit, F-values of all the three responses was not significant. Non-significant lack of fit indicates model fitness. For all the three responses the difference between Predicted R^2 value and the Adjusted R^2 value was below 0.2 indicates validity of the model. Adequate Precision measures the signal to noise ratio. For all 3 responses the ratio was greater than 4, indicates a sufficient signal to move in the design space. The Variance Inflation Factor (VIF) value of all terms in the polynomial equation for all three responses was nearly 1 indicating that one factor is orthogonal to all other factors in the model.

The effect of independent factors on the dependent responses was further studied and quantified their relationship by polynomial equations and 3D response surface plots (Fig.5)

Effect of formulation variables on response Globule size (Y1)

The polynomial equation for the quadratic model in coded factors for the response size was given below.

Size (Y1) = + 90.30 + 38.11A – 11.03B + 3.01C – 2.22AB + 1.20AC + 0.17BC + 4.25A^2 –0.92B^2 + 1.15C^2 …….(1)

The model terms A^2, A, B, C, AB, were significant model terms and influencing globule size in the decreasing order. The oil (A) has greater positive effect on globule size, Smix (B) has negative effect on globule size and water has less positive influence on globule size. These responses were observed within studied range of factors. The influence of oil could be due to high concentration of oil at constant level of Smix leads to larger size globules to compensate increase in interfacial tension. Increment in the Smix leads to reduction in interfacial tension as a result globule size was decreased.

Effect of formulation variables on response Zeta potential (Y2)

The polynomial equation obtained for the response zeta potential was given below

Zeta potential (Y2) = +25.08 - 3.86A + 1.44B - 0.50C - 0.70AB + 0.37AC - 0.025BC + 0.98A^2 + 0.53B^2 + 0.81 C^2 ……(2)

In this case A, B, A^2 are significant model terms. All coefficients were very low in value. Oil (A) showed negative influence as oil content increased zeta potential was decreased and Smix (B) showed positive influence as Smix content increased zeta potential also increased within studied range. Water (C) showed very low effect on zeta potential[21].

Effect of formulation variables on response Flux (Y3)

The polynomial equation obtained for the response flux given below

Flux (Y3) = + 46.53 – 19.21A – 6.30B + 2.82C + 0.83AB – 0.85AC + 0.078BC + 6.38A^2 + 2.47B^2 + 2.11C^2 ……. (3)

A, A^2 B, C, were significant model terms. Oil (A), Smix (B) had negative impact and water (C) had positive impact on flux of the formulation. As Oil and Smix content increased within the studied range the permeation flux was decreased. This may be due to globule size of the formulation increased as result skin permeability was decreased and thermodynamic activity of the drug in the formulation was became low which results in reduction of flux respectively[22] [10]. As water content increased within the studied range flux also increased. This could be due to hydration effect of water on the skin leads to increase in the flux [23, 24].

Check point analysis

Check point analysis was done to validate the response surface model. Compositions and measured, predicted responses of check point formulations were shown in Table 5.

The predicted values of three responses of check point formulations were compared with experimental values and percentage prediction error was calculated. The percentage prediction error of all the check point formulations was below± 5% which indicates validity of the model.

Optimization of the formulation

The desirability of optimized formulation was 0.954. The composition of the optimized formulation (ME18) was 1% drug, 5% oil, 50% Smix, 45% water and responses size, zeta potential, flux were 64.4nm, - 29.3 mV, and 90.63µg/cm^2/h respectively. Flux and enhancement ratios of formulations were shown in Table 6. The formulation with DMSO (ME18D) as permeation enhancer showed significant increase (7.5%) in flux. There was no significant difference between size and zeta potential of ME18 and ME18D. Formulation flux of HPMC gel and Carbopol gel was significantly lower than micro emulsion. This could be due to the entrapment of oil globules in gel matrix which lowered their mobility and hence lower flux. Optimized formulation with DMSO (ME18D) showed significantly high flux compared with Drug solution ($p < 0.0001$), Drug in Oil and Smix ($p < 0.001$), ME18DH ($p<0.01$), ME18DG ($p < 0.01$) and ME18 ($p < 0.05$). Enhancement ratios of ME18D, ME18, ME18DH and ME18DG were 5.8, 4.9, 3.17 and 3.54 compared to drug solution. In order to determine the formulation influence on flux of drug dissolved in oil, surfactant mixture without adding water was also studied. Addition of surfactants to oil enhanced the flux to an extent of 7.5%. However, conversion into microemulsion form enhanced the flux significantly that is 3.51 times.

Enhancement ratio of ME18D was 1.1 folds higher than ME18. In the present study 5% DMSO was used as permeation enhancer at this low concentration DMSO was nonirritant to the skin. DMSO is one of the earliest and most widely used permeation enhancers [25, 26].

The flux of formulation with DMSO (ME18D) was significantly high at $p<0.05$ compare to ME18. All microemulsion formulations showed significantly high compared to that of Drug solution. The main reason for significant increase in flux of microemulsion formulation could be hydrodynamic permeation of nano sized oil globules through the epidermal layer. The presence of oil globules in the receptor fluid substantiated the hypothesis.[27]. There was no significant difference between fluxes of HPMC & Carbopol gel formulations.

Stability studies

The Size, PDI, Zeta potential and drug content were determined for the formulation ME18D after storage of 3 months at room temperature and at refrigerator temperature (4-8 ^{o}C). The results were shown in Table 7. There was no significant change in size, PDI and Zeta potential and drug content after three months storage indicating the stability of formulation. No phase separation was observed in microemulsion after centrifugation indicating physical stability of formulation.

CONCLUSION

The atorvastatin microemulsions for the transdermal delivery were developed. The optimization of formulation was done by Box- Behnken experimental design. The permeation of all microemulsion formulations (flux) was significantly high compared to drug solution. The optimized formulation (ME18D) enhancement ratio of 5.81 to that of drug solution. The present study established the potential advantage of microemulsion formulation for the trans dermal delivery.

REFERENCES

1. Venkitachalam L., Wang K., Porath A., Corbalan R., Hirsch AT., Cohen DJ. Global variation in the prevalence of elevated cholesterol in outpatients with established vascular disease or 3 cardiovascular risk factors according to national indices of economic development and health system performance. *Circulation,125*:1858–69,(2012).
2. Ramkumar S., Raghunath A., Raghunath S. Statin therapy: Review of safety and potential side effects. *Acta Cardiol , 32*:631–9, (2016).
3. Tokudome Y., Katayanagi M., Hashimoto F. Esterase activity and intracellular localization in reconstructed human epidermal cultured skin models. *Ann. Dermatol, 27*:269–74, (2015).
4. Lopez RF V., Vitoria M., Bentley LB., Begona M., Guy RH. O ptimization of aminolevulinic acid delivery by iontophoresis. *J. Control. Release, 88*: 65–70, (2003).
5. Megrab NA., Williams AC., Barry BW. Oestradiol permeation through human skin and silastic membrane: effects of propylene glycol and supersaturation. *J. Control. Release, 36* : 277–94, (1995).
6. Cevc G., Blume G. Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. *BBA - Biomembr, 1104*:226–32, (1992).
7. Mezei M., Gulasekharam V. Liposomes - a selective drug delivery system for the topical route of administration. Lotion dosage form. *Life Sci, 26*:1473–7, (1980).
8. Schreier H., Bouwstra J. Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery. *J. Control. Release , 30*:1–15, (1994).
9. El Maghraby GM., Barry BW., Williams AC. Liposomes and skin: From drug delivery to model membranes. *Eur. J. Pharm. Sci, 34*:203–22, (2008).
10. El Maghraby GM. Transdermal delivery of hydrocortisone from eucalyptus oil microemulsion: Effects of cosurfactants. *Int. J. Pharm, 355*:285–92, (2008).
11. Zhu W., Yu A., Wang W., Dong R., Wu J., Zhai G. Formulation design of microemulsion for dermal delivery of penciclovir. *Int. J. Pharm, 360*:184–90, (2008).
12. Yuan Y., Li S ming., Mo F kui., Zhong D fang. Investigation of microemulsion system for transdermal delivery of meloxicam. *Int. J. Pharm, 321*:117–23, (2006).
13. Peltola S., Saarinen-Savolainen P., Kiesvaara J., Suhonen TM., Urtti A. Microemulsions for topical delivery of estradiol. *Int. J. Pharm*, 254:99–107, (2003).
14. Kreilgaard M. Influence of microemulsions on cutaneous drug delivery. *Adv. Drug Deliv. Rev, 54* SUPPL, (2002).
15. Kogan A., Garti N. Microemulsions as transdermal drug delivery vehicles. *Adv. Colloid Interface Sci*, 123–126 SPEC. ISS.:369–85, (2006).
16. Syed HK., Peh KK. Identification of phases of various oil, surfactant/co-surfactants and water system by ternary phase diagram. *Acta Pol. Pharm. - Drug Res, 71*:301–9, (2014).
17. Box GEP., Behnken DW. Some New Three Level Designs for the Study of Variables Quantitative. *Technometrics, 2*:455–75, (1960).
18. Levang AK., Zhao K., Singh J. Effect of ethanol/propylene glycol on the in vitro percutaneous absorption of aspirin, biophysical changes and macroscopic barrier properties of the skin. *Int. J. Pharm*, *181*:255–63, (1999).
19. Lee PJ., Ahmad N., Langer R., Mitragotri S., Prasad Shastri V. Evaluation of chemical enhancers in the transdermal delivery of lidocaine. *Int. J. Pharm, 308*:33–9, (2006).

20. Aslan N., Cebeci Y. Application of Box-Behnken design and response surface methodology for modeling of some Turkish coals. *Fuel, 86*:90–7, (2007).
21. Sathyamoorthy N., Magharla D., Chintamaneni P., Vankayalu S. Optimization of paclitaxel loaded poly (e-caprolactone) nanoparticles using Box Behnken design. *Beni-seuf univ. J. Appl. Sci*, 6:362–73, (2017).
22. Chen H., Chang X., Weng T., Zhao X., Gao Z., Yang Y. A study of microemulsion systems for transdermal delivery of triptolide. *J. Control. Release, 98*:427–36, (2004).
23. Alvarez-Figueroa MJ., Blanco-Méndez J. Transdermal delivery of methotrexate: Iontophoretic delivery from hydrogels and passive delivery from microemulsions. *Int. J. Pharm, 215*:57–65, (2001).
24. Thacharodi D., Panduranga Rao K. Transdermal absorption of nifedipine from microemulsions of lipophilic skin penetration enhancers. *Int. J. Pharm, 111*:235–40, (1994).
25. Pathan IB., Setty M. Chemical Penetration Enhancers for Transdermal Drug Delivery Systems. *Trop. J. Pharm, 8*:173–9, (2009).
26. Embery G., Dugard PH. The isolation of dimethyl sulfoxide soluble components from human epidermal preparations: a possible mechanism of action of dimethyl sulfoxide in effecting percutaneous migration phenomena. *J. Investig. Dermatol, 57*:308-311, (1971).
27. Paulsson M, Edsman K. Controlled Drug Release from Gels using Surfactant Aggregates. *J. Pharm. Sci, 90*:1216, (2001).

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