ORIGINAL ARTICLE

Formulation, Characterization, and Optimization of a Better and Novel Transfersome Formulation Containing Olmesartan Using Design-Expert 11 Software

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Abstract

Aim: The present study was aimed to optimize, formulate, and characterize a better and novel transferosome formulation containing olmesartan (OS). **Materials and Methods:** The preformulation studies were performed by Fourier transform infrared spectrophotometer. OS transferosome gel was fabricated and optimized asper Design Expert 11 Software. A three-factor, three-level, Box–Behnken design was applied to optimize transfersome formulation, using response surface methodology. The amount of phospotidyl choline 90 G (PC 90G) (A), surfactant (B), and sonication time (C) were selected as independent variables. The OS optimized formulation was evaluated for Scanning Electron Microscopy (SEM), Entrapment efficiency (EE) (PDE), and *in vitro* studies. The ANOVA was done for EE and *in vitro* studies. **Results and Discussion:** Optimized formulation prepared with phospholipid (A) 451.84 mg, Surfactant (B) 144.93 mg, sonication time (C) 6 min. It was evaluated for EE and *in vitro* drug release and values were found to be in close agreement with the predict values. *In vitro* studies confirmed that release of OS for >24 h. It was found to be spherical from SEM analysis. **Conclusion:** Hence, in the prepared transferosome formulation, phospholipid and surfactant were effectively working for prolonged delivery of OS.

Key words: ANOVA, entrapment efficiency, *In vitro* studies, probe sonicator, reverse phase evaporation method, transdermal, transferosome

INTRODUCTION

One of the oldest and most successful drug delivery systems in targeting the drugs to target site or systemic effect or local therapy is transdermal drug delivery system (TDDS), achieved through skin at pre-determined rate with minimal inter and intra-patient variation. At present, also transdermal delivery stands as one of the most promising and helpful methods for drug application. It mitigates the load that the oral route commonly places on the digestive tract and liver, enhances patient compliances, minimizes harmful side effects of a drug caused from temporary overdose, and is convenience in transdermal delivered drugs that require only once weakly application.[1]

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Hypertension, also known as high or raised blood pressure, is a condition in which the blood vessels have persistently raised pressure. The higher the pressure the harder the heart has to pump.[2] The natural treatment is best regulated through diet and lifestyle adjustments are the standard first-line treatment for hypertension and regular physical exercise, stress reduction, avoiding using alcohol, drugs, smoking, and unhealthy eating to cope. A range of drug types is available to help lower blood pressure, including diuretics, including thiazides, chlorthalidone, and indapamide, beta-blockers and alpha-blockers, calcium-channel blockers, central agonists, peripheral adrenergic inhibitor, vasodilators, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers. The choice of drug depends on the individual and any other conditions they may have.[3,4]

Hypertension is one of the common disorders for the mankind. It is not a disease in itself but is an important risk factor for cardiovascular mortality and morbidity. The present article delivers a brief view on the work being done to increase the bioavailability of various antihypertensive drugs by formulated and delivered as transdermal patches.

Advantages of TDDS

1. They can avoid gastrointestinal drug absorption difficulties caused by gastrointestinal pH, enzymatic activity, and drug interactions with food, drink, and other orally administered drugs
2. They avoid the first-pass metabolism and avoid drug deactivation by liver enzymes
3. They can substitute for oral administration of medication when that route is unsuitable, as in case of vomiting and diarrhea
4. Provides suitability of self-medication, reduction of dosing frequency, and enhancement of patient compliance
5. Enhance therapeutic efficacy, reduced fluctuations (rapid blood level spikes – low and high) due to optimization of blood concentration – time profile.

Disadvantages of TDDS

The limitations of transdermal drug delivery are mainly associated with barrier function of skin, so it is limited to potent drug molecules. Skin irritation or contact dermatitis due to drug, excipients, and enhancers is another limitation.[5]

Physical approaches for transdermal drug delivery

Numerous approaches for transdermal drug delivery can be done through physical methods such as iontophoresis, sonophoresis, electroporation, magnetophoresis, microneedles, and radiofrequency.[6-12]

Chemical approaches of TDDS

The constitutes of approaches are synthesis of lipophilic analogs, delipidization of stratum corneum, and co-administration of skin permeation enhancers. The necessary approach according to chemical ingredients in the class of sulfoxides, alcohols, polyols, alkenes, esters, amines and amides, pyrrolidones, trepenes, surface active agents, and cyclodextrins and the examples were dimethylsulfoxide, decylmethalsulfoxide, ethanol, propylene glycol, long-chain alkanes (C7-C16), oleic acid, isopropyl myristate, urea, dimethyl acetamide, dimethyl formamide, N-methylpyrrilidone, azones, eugenol and cationic surfactants and cyclodextrines.[13]

Transfersomes

The transfersome means "carrying body" and is derived from the Latin word "transferre" means "to carry across," and the Greek word "soma" means for a "body." It is a novel, elastic, or ultra-deformable vesicular drug carrier system composed of phospholipids, surfactant, and water for enhanced transdermal delivery and overcomes the filtration and skin penetration difficulty by squeezing themselves along the intracellular sealing lipid of the stratum corneum. Figure 1 shows the structure of transferosome.

Composition and structure

Transfersomes are composed of phospholipids like phosphatidylcholine which self assembles into lipid bilayer in aqueous environment and closes to form a vesicle. A lipid bilayer softening component (biocompatible surfactant or an amphiphilic drug) is added to increase lipid bilayer flexibility and permeability and known as edge activator. An edge activator consists usually of single chain surfactant of non- ionic nature that causes destabilization of the lipid bilayer thereby increasing its fluidity and elasticity. Flexibility of transfersomes membrane can be altered by mixing suitable surface active agents in the proper ratios.[14]

Mechanism of penetration

Transfersome ensure penetration through the skin by getting squeezed through the lipid present in the cells of the stratum corneum. This is possible due to the ability of the vesicle to deform to a great extent which provides the mechanical stress needed to enter the skin. To maintain optimum flexibility of transfersome is to have a suitable mix of surface-active agents in proper ratios with phospholipids. This flexibility minimizes the possibility of complete rupture of the vesicle in the skin and helps the vesicles to follow a natural aqueous gradient across the outer layer of non-occluded skin. There are two routes through which the transfersomes can penetrate the stratum corneum through the intracellular lipid and

differ in the properties of the bilayer.[15,16] Figure 2 shows the mechanism of penetration of Transferosomes across skin barrier.

MATERIALS AND METHODS

The novel transfersome formulation containing olmesartan (OS) was carried out using Phosphatidylcholine, Cholesterol, Tween 80, Diethyl ether, Ethanol, Methylcellulose gel, and Span 20. All the chemicals used in the formulation were analytical grade. The instruments Digital Balance, ultraviolet (UV)-Vis spectrophotometer, Magnetic Stirrer, Rotary evaporator, Franz Diffusion cell, and probesonicator were used for research.

Preformulation studies

Fourier transform infrared (FTIR) spectrophotometer[16,17]

IR spectra of physical mixture of drug and excipients were recorded by KBr method using FTIR Spectrophotometer. A baseline correction was made using dried potassium bromide pellet. The potassium bromide-drug pellet of approximately 1 mm diameter was prepared by grinding 3–5 mg of physical mixture of drug-excipients with 100–150 mg of potassium bromide in pressure compression machine. The sample pellet was mounted in IR compartment and scanned at wavelengths 4000 cm^{-1}–400 cm^{-1}.

Construction of calibration curve

100 mg of OS was weighed accurately and transferred to a 100 mL volumetric flask containing 10 mL of methanol, which was further sonicated for 15 min with frequent shaking. The volume was brought up to 100 mL by methanol. The 100 mL of primary stock solution in 10 mL is taken in 100 mL VF and make up the water (secondary solution) than 0.2 mL is taken in 2° stock solution to get the 10 mL. This was subsequently analyzed for OS medoxomil using a double-beam UV-VIS spectrophotometer and water as the blank.[18]

Preparation of transfersomes

Transferosomes were formulated by reverse phase evaporation method with some modifications as described in the literature. At first, phospotidyl choline 90 G (PC 90G) and cholesterol were taken in a cleaned beaker as lipids. Then, Tween 80 as surfactant was poured in the same beaker and dissolved in a solvent mixture of diethyl ether and methanol (3:1). The beaker was kept at room temperature for 24 h until the thin film formed. OS was added onto the thin film and sonicated by a probe sonicator at a frequency of 20 KHz for 2 min. After that, the film was hydrated using edge deactivator, spans 20 in phosphate buffer saline (PBS) (pH 7.4), and then further sonicated for 2 min to obtain transfersomal suspensions. Then, these transferosomal suspensions were transferred to 5% w/v methylcellulose gel and stored in cool and dark place.[19]

Optimization of transferosome

A three-factor, three-level, Box-Behnken design (BBD) was applied, to optimize transferosome formulation, using response surface methodology (RSM). The amount of phospotidyl choline 90 G (PC 90G) (A), surfactant (B), and sonication time (*C*) were selected as independent variables, considering their overall effects on the formulation's stability, as reported by many researchers accordingly, entrapment efficiency (EE) (Y_1) and *in vitro* drug release (Y_2) were selected as dependent variables for the optimization process. After a significant number of preliminary studies, the amount of PC90G (A), SDC (B), and sonication time (C) was selected in the range of 250–750 mg, 50–150 mg, and 2–6 min, respectively, as independent variables for OS-loaded transferosomes formulation optimization by RSM. Fifteen trial formulations of various factor combinations, including five center points, were prepared according to the BBD. All other formulation and processing variables were kept constant throughout the study.[20] The selected dependent and independent variables for optimization are listed in Table 1.

Characterization of transferosomes

EE Percentage Drug Entrapment (PDE)

Entrapped transfersomal vesicles were separated from untrapped OS by centrifugation at 10000 rpm for 45 min. The concentration of OS in supernatant was measured by UV spectrophotometer. Percent drug entrapment was calculated using following equation.[21,22]

$$\text{Entrapment efficiency}\ (\%) = \frac{(\text{Total amount of drug}) - (\text{Amount of free drug})}{(\text{Total amount of drug})} \times 100$$

Scanning electron microscopy (SEM)

The surface morphology of optimized transfersome was examined by SEM. 1–2 drops of vesicular dispersion were mounted on a glass and paste over grid using double-sided carbon adhesive tape and sputter-coated with conductive gold palladium. A round coverslip was gently placed over the stub to enable uniform conductivity and a silver paint lining was applied to the edges of the coverslip to fill the narrow spacing between the stub and coverslip. They were viewed with an EVO LS 10 (Carl Zeiss, Brighton, Germany) SEM operating at an accelerating voltage of 200 kV under high vaccum. The particles were examined for surface characteristics such as shape, size, pores, pits, and presence of aggregation.[23]

Table 1: Observed responses in Box–Behnken design for optimization of Transferosomes formulations

F. No.	Factor A (Phospholipid)		Factor B (surfactant)		Factor C (sonication time)	
	Coded	Actual (mg)	Coded	Actual (mg)	Coded	Actual (min)
F1	−1	250	−1	50	0	4
F2	0	500	0	100	0	4
F3	−1	250	+1	150	0	4
F4	0	500	−1	50	+1	6
F5	0	500	+1	150	−1	2
F6	0	500	0	100	0	4
F7	0	500	+1	150	+1	6
F8	0	500	0	100	0	4
F9	0	500	−1	50	−1	2
F10	+1	750	−1	50	0	4
F11	−1	250	0	100	+1	6
F12	+1	750	+1	150	0	4
F13	+1	750	0	100	−1	2
F14	+1	750	0	100	+1	6
F15	−1	250	0	100	−1	2

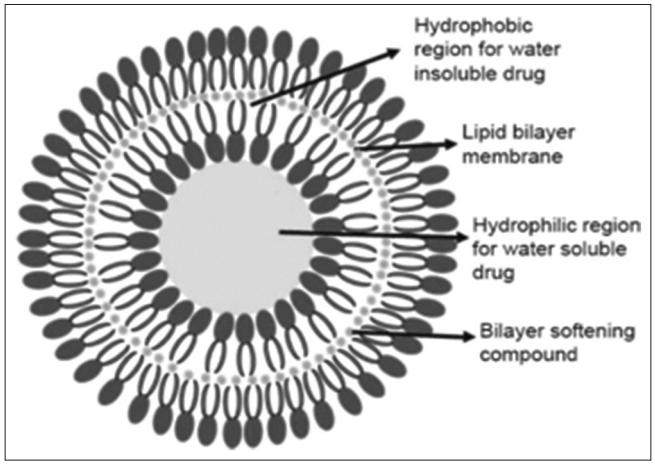

Figure 1: Structure of transferosomes

Drug content

Drug content 1 mL of OS-loaded transfersomal suspension was taken and diluted with 10 mL 7.4 ph phosphate buffer. It was ultra-centrifuged at 15,000 rpm for 40 min. The pellet formed after centrifugation was disrupted with 10 mL methanol to come out the drug from vesicles. 1 mL of this solution was taken and suitable dilutions were made and analyzed by UV spectrophotometer at 285 nm which gives the concentration of entrapped drug. The concentration of drug in supernatant and pellet collectively gives the amount of drug present in 1 mL of suspension. % drug content was calculated by dividing with theoretical drug content present in 1 mL of suspension.[24]

Drug Content (%) = Actual drug content/Theoretical yield × 100.

In vitro studies

In vitro skin permeation studies were carried out using Franz diffusion cell. The cell consists of two chambers, the donor and the receptor compartments with a diffusion area of 1.43 cm^2. The donor compartment was open at the top and was exposed to atmosphere. The excised porcine ear skin was mounted between the compartments of the diffusion cell with stratum corneum facing the donor compartment and clamped into position. Magnetic stirrer bars were added to the receptor chambers and filled with the receptor phase. PBS, pH 7.4 was used as receptor medium. The entire setup was placed over magnetic stirrer and the temperature was maintained at 37 ± 0.5 °C. 1 mL of medium was collected from the receptor compartment at predetermined intervals over study period and replaced with the same amount of fresh buffer. The amount of permeated drug was measured using UV–Vis spectrophotometer by measuring absorbance at 214 nm.[25]

RESULTS AND DISCUSSION

FTIR spectrophotometer

The FTIR graphs are given below group's identification in Figures 3 and 4 and data were presented in Table 2.

Construction of calibration curve

The values are presented in Table 3 and Figure 5 shows the calibration curve of Olmesartan.

Table 2: Functional groups present in the phospholipid and olmesartan

S. No.	Functional groups present in the phospholipid		Functional groups present in the drug	
	Wavelength	Functional groups	Wavelength	Functional groups
1	3443.93	3^0 amine	3412.23	3^0 amine
2	2975.53	C-H Alkane	2905,52	C-H Alkane
3	1616.51	C=O	1751.55	C=O
4	1142.23	CH_2	1123.99	CH_2

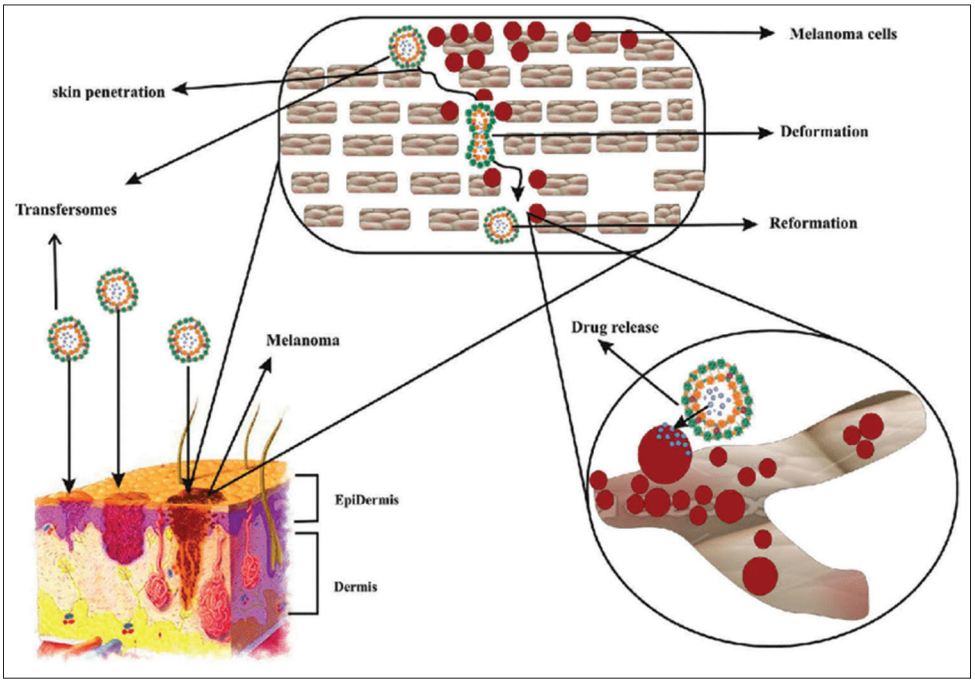

Figure 2: Mechanism of penetration

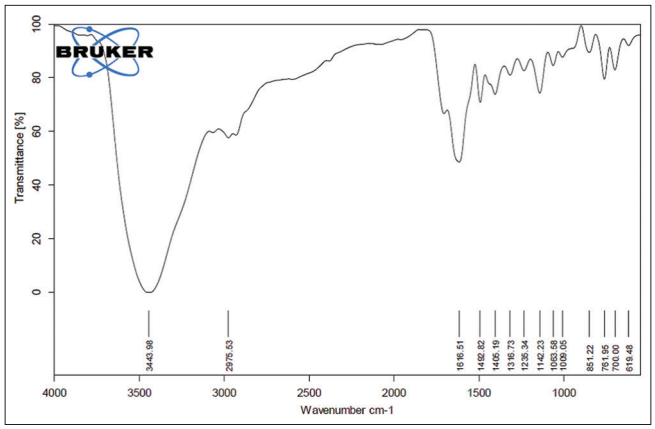

Figure 3: Fourier transform infrared graph for phospholipid

Optimization of transferosome

Using design expert software 11 optimized transfersomal gel was prepared with optimized concentration of phospholipid (A), surfactant (B), and sonication time (C) as per desirability approach and evaluated for 2 responses EE (Y_1), *in vitro* drug release (Y_2), in addition also studied for drug content, SEM, vesicle shape, and other.

Equations:

Entrapment efficiency % = $90+0.7500A+3.62B+4.63C-0.5000AB-5.00AC+0.7500BC-11.37A^2-0.625B^2$ (1)

In vitro drug release = $27.67+5.00A+2.12B+0.625C+0.5000AB+0.5000AC+1.75BC-2.21A^2-2.46B^2+1.04C^2$ (2)

$P < 0.0500$ indicates model terms are significant. In this case B, C, AC, A^2 are significant model terms. Values >0.1000 indicate that the model terms are not significant.

$P < 0.0500$ indicates model terms are significant. In this case, A, B, BC, A^2, B^2 are significant model terms. Values >0.1000 indicate that the model terms are not significant.

Equation (1) describes that

Factor B, C, AC, and A^2 have significant effect on EE. This

was evidenced by above Table 5. It describes that surfactant (B) and sonication time (C) have positive effect on EE. If B and C increase, EE also increases. Combination of AC and A^2 has negative effect on EE. If AC and A^2 increase, EE also decreases. Among this, all factors AC have highest co-efficient value that is 5.00. Hence, it has more profound effect which was evidenced by 2D and 3D Figure 6.

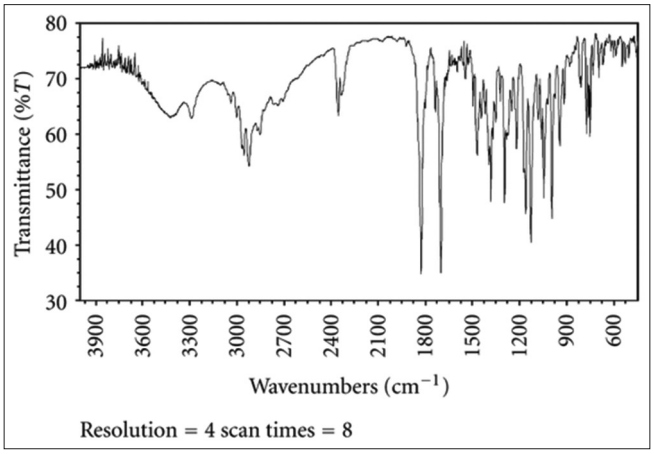

Figure 4: Fourier transform infrared graph of olmesartan

Table 3: Absorbance values obtained for the given standard concentration of drug

S. No.	Concentration (µg/mL)	Absorbance (nm)
1	0	0
2	20	0.216
3	40	0.355
4	60	0.525
5	80	0.686
6	100	0.862

Equation 2 describes that

Factors A, B, BC, A^2, and B^2 have significant effect on *in vitro* drug release which was evidenced by Table 6. It describes that phospholipid (A), surfactant (B), and combination of surfactant and sonication time (BC) have positive effect on *in vitro* drug release. Hence, *in vitro* drug release increases with increase in A, B, and BC. A^2 and B^2 have negative effect on *in vitro* drug release, i.e., *in vitro* drug release decreases with increase in A^2 and B^2. Among this, all factors A have highest co-efficient value that is 5.00. Hence, it has more profound effect, which was evidenced by 2D and 3D Figure 7.

OS Optimized Formulation (OSOF) as per desirability approach and Numerical optimization Optimized formulation (OSOF) prepared with phospholipid (A) 451.84 mg, Surfactant (B) 144.93 mg, sonication time (C) 6 min.

EE (PDE)

It was evaluated for EE and value was found to be 97.91%. This value is in close agreement with the predict value 97.89%.

SEM

The SEM evaluation is shown in Figure 8.

Table 4: Results of two responses of all formulations by Box–Behnken design

F. No.	Factor A (phospholipid)		Factor B (surfactant)		Factor C (sonication time)		EE (Y_1) (%)	*In vitro* drug release (Y_2) (%) 24 h
	Coded	Actual (mg)	Coded	Actual (mg)	Coded	Actual (min)		
F1	−1	250	−1	50	0	4	73	51
F2	0	500	0	100	0	4	89	84
F3	−1	250	+1	150	0	4	79	57
F4	0	500	−1	50	+1	6	87	69
F5	0	500	+1	150	−1	2	89	78
F6	0	500	0	100	0	4	91	84
F7	0	500	+1	150	+1	6	98	96
F8	0	500	0	100	0	4	90	81
F9	0	500	−1	50	−1	2	81	72
F10	+1	750	−1	50	0	4	78	78
F11	−1	250	0	100	+1	6	89	63
F12	+1	750	+1	150	0	4	82	90
F13	+1	750	0	100	−1	2	77	93
F14	+1	750	0	100	+1	6	78	96
F15	−1	250	0	100	−1	2	68	66

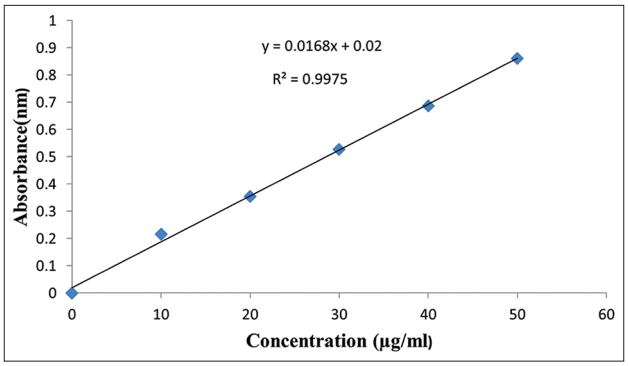

Figure 5: Calibration curve of olmesartan

Drug content

The drug content was found to be 99%.

In vitro studies

It was evaluated for *in vitro* drug release and value was found to be 89.60%. This value is in close agreement with the predict values 90.05%.

15 runs of formulations were prepared as per Design Expert 11 Software with different compositions of phospholipid,

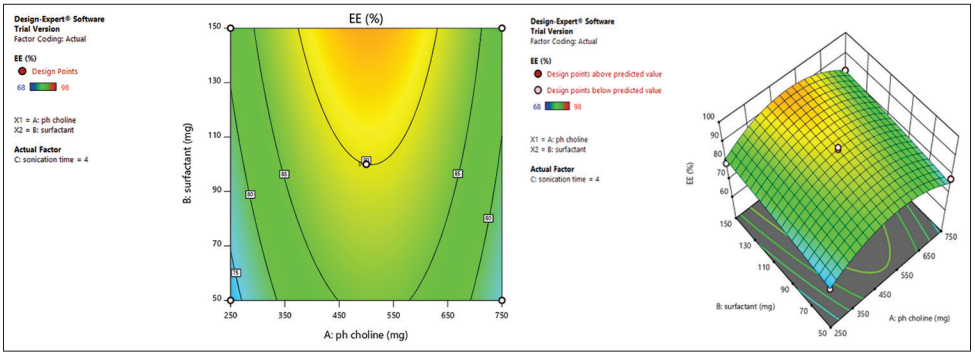

Figure 6: 3D an 2D-Response surface plots presenting the effect of three selected factors on % Entrapment Efficiency.

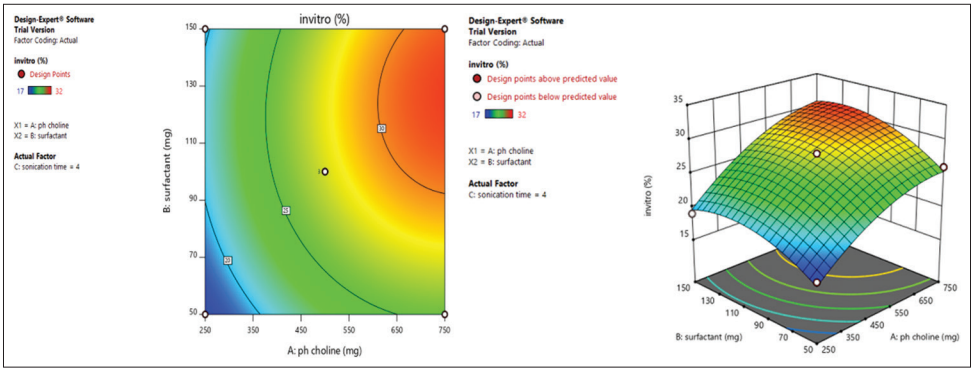

Figure 7: 3D an 2D-Response surface plots presenting the effect of three selected factors on *Invitro* release.

Table 5: ANOVA table of entrapment efficiency (Y_1)

Source	Sum of squares	df	Mean square	F-value	*P*-value	
Model	298.02	9	33.11	23.94	0.0014	Significant
A-Phosphatidyl Choline	200.00	1	200.00	144.58	< 0.0001	Significant
B-surfactant	36.12	1	36.12	26.11	0.0037	Significant
C-sonication time	3.13	1	3.13	2.26	0.1932	Non-significant
AB	1.0000	1	1.0000	0.1626	0.7034	Non-significant
AC	100.00	1	100.00	16.26	0.0100	Significant
BC	2.25	1	2.25	0.3659	0.5717	Non-significant
A^2	477.75	1	477.75	77.68	0.0003	Significant
B^2	1.44	1	1.44	0.2345	0.6487	Non-significant
C^2	1.44	1	1.44	0.2345	0.6487	Non-significant

Table 6: ANOVA table of ***in vitro*** drug release (Y_2)

Source	Sum of squares	df	Mean square	F-value	*P*-value	
Model	298.02	9	33.11	23.94	0.0014	Significant
A-phosphotidyl Choline	200.00	1	200.00	144.58	<0.0001	Significant
B-surfactant	36.12	1	36.12	26.11	0.0037	Significant
C-sonication time	3.13	1	3.13	2.26	0.1932	Non-significant
AB	1.0000	1	1.0000	0.7229	0.4340	Non-significant
AC	1.0000	1	1.0000	0.7229	0.4340	Non-significant
BC	12.25	1	12.25	8.86	0.0309	Significant
A^2	18.01	1	18.01	13.02	0.0154	Significant
B^2	22.31	1	22.31	16.13	0.0102	Significant
C^2	4.01	1	4.01	2.90	0.1495	Non-significant

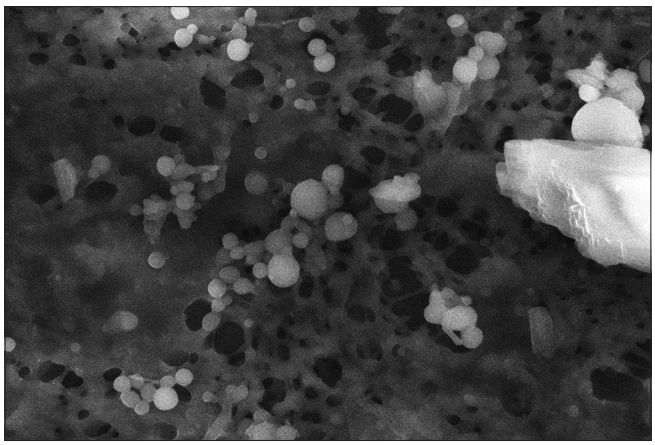

Figure 8: Scanning electron microscopy (SEM) image of optimized formulation (olmesartan transfersomes) was found to be spherical from SEM analysis

surfactant, and sonication time. These are selected as factors A, B, and C, respectively, and effect of these on responses Y_1 (EE %) and Y_2 (*in vitro* drug release at 24 h) were evaluated practically. The drug content was found to be 99%.

It was evaluated for EE and *in vitro* drug release and values were found to be 97.91%, 89.60%. This values are in close agreement with the predict values 97.89% and 90.05%.

The results of EE, *in vitro* drug release by BBD, ANOVA Table of EE (Y_1), and ANOVA Table of *in vitro* drug release (Y_2) were shown in Tables 4-6, Figures 6 and 7.

Application of contour plots and response surface plots revealed that EE increases linearly with factors A, B, and C. In contrast to this, *in vitro* drug release showed non-linearity with factors A, B, and C. Hence, increase in A and B increases Y_2. This is evidenced by so many research articles.

Finally, an optimized formulation (OSOF) was suggested by overlay plot and graphical optimization with A, B, and C concentrations 451.845 mg, 144.930 mg, and 6 min, respectively. Based on these, concentrations prepared OS transfersomes were evaluated for Y_1 and Y_2 and compared with predicted values and relative error was calculated and it was within the limit. Further, OS transfersomes were evaluated for EE, *in vitro* drug release, drug content, and SEM and all were found to be within the limits.

This study revealed the importance of combined effects of phospholipid and surfactant to improve drug release with desirable EE.

CONCLUSION

OS transfersomal gel was prepared as per Design Expert 11 Software to optimize concentrations of phospholipid (A), surfactant (B), and sonication time (C). All 15 formulations containing high concentrations of B and C showed increase in Y_1 and high concentrations of A, B, and BC showed increase in Y_2. Based on numerical optimization and graphical optimizations, an optimized formulation (OSOF) with desirable values of EE and *in vitro* drug release at 24 h was 97.910 and 90.15%, respectively. These values indicated the reliability and validity of the model. *In vitro* studies confirmed that the release of OS for >24 h. Hence, the phospholipid and surfactant were effectively working for prolonged delivery of OS.

REFERENCES

1. Jalwal P, Jangra A, Dhaiya L, Sangwan Y, Saroha R. A review on transdermal patches. Pharm Res J 2010;3:139-49.
2. Kumar JA, Pullankandam N, Prabhu SL, Gopal V. Transdermal drug delivery system: An overview. Int J Pharm Sci Rev Res 2010;3:49-54.
3. Bavaskar, K, Jain A, Patil M, Kalamkar R. The impact of penetration enhancers on transdermal drug delivery

system: Physical and chemical approach. Int J Pharma Res Rev 2015;4:14-24.
4. Shingade GM. Review on: Recent trend on transdermal drug delivery system. J Drug Deliv Ther 2012;2:66-75.
5. Saini S, Chauhan SB, Agrawal SS. Recent development in penetration enhancers and techniques in transdermal drug delivery system. J Adv Pharm Educ Res 2014;4:31-40.
6. Murthy SN. Magnetophoresis: An approach to enhance transdermal drug diffusion. Pharmazie 1999;54:377-9.
7. Tripathy S, Patel DK, Barob L, Naira SK. A review on phytosomes, their characterization, advancement and potential for transdermal application. J Drug Deliv Ther 2013;3:147-52.
8. Hussain A, Khan GM, Wahab A, Akhlaq M, Ur Rahman S, Altaf H, *et al*. Potential enhancers for transdermal drug delivery: A review. Int J Basic Med Sci Pharm 2014;4:19-22.
9. Raza R, Mittal A, Kumar P, Alam S, Prakash S, Chauhan N. Approaches and evaluation of transdermal drug delivery system. Int J Drug Dev Res 2015;7: 222-33.
10. Jawale NR, Bhangale CD, Chaudhari MA, Deshmukh TA. Physical approach to transdermal drug delivery: A review. J Drug Deliv Ther 2017;7:28-35.
11. Tamar H, Sachdeva R. Transdermal drug delivery system: A review. Int J Pharm Sci Res 2017;59:10-5.
12. Nagasamy Venkatesh D, Kalyani K, Tulasi K, Swetha Priyanka V, Abid Ali SK, Kiran HC. Transfersomes: A Novel technique for transdermal drug delivery. Int J Res Pharm Nano Sci 2015;20:266-76.
13. Jain S, Umamaheshwari RB, Bhadra D, Jain NK. Ethosomes: A novel vesicular carrier for enhanced transdermal delivery of an anti-HIV agent. Indian J Pharm Sci 2004;66:72-81.
14. Kumar KP, Radhika PR, Sivakumar T. Ethosomes-A priority in transdermal drug delivery. Int J Adv Pharm Sci 2010;1:111-21.
15. Haag SF, Fleige E, Chen M, Fahr A, Teutloff C, Bittl R, *et al*. Skin penetration enhancement of core-multishell nanotransporters and invasomes measured by electron paramagnetic resonance spectroscopy. Int J Pharm 2011;416:223-8.
16. Sudhamani T, Priyadarisini N, Radhakrishnan M. Proniosomes - A promising drug carrier. Int J Pharm Tech Res 2010;2:1446-54.
17. Martin AN, Swarbrick J, Cammarata A. Physical Pharmacy. 3rd ed. Philadelphia, PA: Lea & Febiger; 1991. p. 303.
18. Vijaya R, Ruckmani K. In vitro and in vivo characterization of the transdermal delivery of sertraline hydrochloride Films. Daru 2011;19:424-32.
19. Barani S, Ali M, Amin S. Formulation and characterization of flexible phosphatydilcholine vesicles for systemic delivery of piroxicam. J Pharm Sci Res 2017;9:972-85.
20. Duangjit S, Opanasopit P, Rojanarata T, Ngawhirunpat T. Evaluation of meloxicam-loaded cationic transfersomes as transdermal drug delivery carriers. AAPS PharmSciTech 2013;14:133-40.
21. Ahad A, Al-Mohizea AM, Al-Jenoobi FI, Aqil M. Transdermal delivery of angiotensin II receptor blockers (ARBs), angiotensin-converting enzyme inhibitors (ACEIs) and others for management of hypertension. Drug Deliv 2016;13:579-90.
22. Guo F, Wang J, Ma M, Tan F, Li N. Skin targeted lipid vesicles as novel nano-carrier of ketoconazole: Characterization, *in vitro* and *in vivo* evaluation. J Mater Sci Mater Med 2015;16:175-80.
23. Jain S, Sapre R, Tiwary AK, Jain NK. Proultraflexible lipid vesicles for effective transdermal delivery of levonorgestrel: Development, characterization, and performance evaluation. AAPS PharmSciTech 2005;6:22-30.
24. Basak SC, Vellaiyan K. Transdermal drug delivery system. East Pharm 1997;40:63-70.
25. Irfan M, Verma S, Ram A. Preparation and characterization of ibuprofen loaded transferosome as a novel carrier for transdermal drug delivery system. Asian J Pharm Clin Res 2012;5:162-5.

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