

FORMULATION DEVELOPMENT AND EVALUATION OF MIGRAINE ALMOTRIPTAN LOADED ETHOSOMES USING BOX BEHNKEN DESIGN

Abstract

One of the most common nervous system illnesses is headache disorders, which are characterized by chronic headaches. In Present investigation Almotriptan loaded Ethosomes were prepared by water phase addition method. The three independent factors including Phosphotidylcholine: Cholestrol: DSPE (6:3:1molar ratio), Surfactant concentration and sonication time. A factorial design 3*3(3 factor 3 level) was applied to prepare 17 formulation. Optimization of ethosomal preparation was carried out by applying Box Behnken response surface randomized factorial design following quadratic model using Design of Experiment (DOE) software version 11.04.0. The factor Soya PC: Cholesterol: DSPE in molar ration (6:3:1), Concentration of Tween-80 and sonication time were selected as dependable process and formulation factors that can be effect formulation characteristics like entrapment efficiency, average vesicle size, Polydispersity Index (PDI). All other factors like sonication speed and rotation speed was kept constant All the formulation were prepared by simple solvent evaporation thin film formation method and characterized for the drug entrapment, average vesicle size and PDI, shape morphology. Formulations were optimized on the basis of responses such as average vesicle size, PDI, and entrapment efficiency. All the characterized values of the responses were putted in the formulation design table and analyse to best fitted model for the design. It was observed that, quadaratic model is best fitted model for the design. The prepared ethosomes formulation can further incorporated in situ gel for effective treatment of migraine.

Key words: Almotriptan, Ethosomes, Formulation, Characterization, Migraine

INTRODUCTION

One of the most common nervous system illnesses is headache disorders, which are characterized by chronic headaches. A painful and incapacitating characteristic of a few main headache diseases, such as migraine, tension-type headache, and cluster headache [1], is headache. Although the migraine headache is the most frequent, prevalent, debilitating, and mostly curable of them, it is underappreciated and undertreated [2]. Migraine is a common chronic headache condition marked by pulsing headaches of mild to severe severity, nausea, vomiting, photophobia, or phonophobia, and frequent attacks lasting 4–72 hours.

Our understanding of the biology of migraine has greatly advanced over the last two decades, thanks to a series of fundamental science and imaging studies that show how vascular alterations, which were once considered to be the cause of migrainous pain, are neither essential nor sufficient in migraine [3, 4]. The research has progressed from vascular ideas to neuronal hypotheses involving the central or peripheral nervous system, or both. Many studies have centered on specific brain regions considered to be at the root of pain, which is perhaps the most common migraine symptom. With these advancements, it is obvious that the notion of a single migraine generator may be obsolete, given the diversity of overlapping phases that make up a migraine episode. Migraine is now largely acknowledged as a complex brain network illness with a strong hereditary foundation involving various cortical, subcortical, and brainstem areas to account for the pain and a wide range of symptoms that characterize the attack [4–6]. The encapsulated drug in the ethosomes will be almotriptan. The sulfonamide triptan almotriptan has extracerebral and intracranial vasoconstrictor action. Almotriptan causes extracerebral, intracranial blood vessel constriction via binding to serotonin 5-HT 1B and 1D receptors in the central nervous system (CNS). Vascular headaches may be relieved as a result of this. Almotriptan can also help with vascular headaches by preventing the release of vasoactive neuropeptides from perivascular trigeminal axons in the dura mater during a migraine, lowering extravasation of plasma proteins, and lowering the release of other inflammatory mediators from the trigeminal nerve. Thus the prepared ethosomes will help to manage migraine with minimum side effect.

MATERIALS AND METHODS

Material

Soya PC, DSPE and cholesterol were purchased from the Aldrich pvt ltd, USA. Almotriptan was obtained as a gift sample from Yarrow Chem Products, Mumbai. Other other chemicals and reagents were of pharmaceutical quality and were bought from Loba Chemise laboratory reagents and Fine Chemicals Ltd, Mumbai, as were all materials and solvents.

Methods

Preparation of drug (Almotriptan) loaded ethosomes

Ethosomes were prepared by water phase addition method reported by Hallan *et al.*, [7]. In brief the soya lecithin (3-5% (w/v) and cholesterol (2-4% w/v) was accurately weighed and taken in 250 mL capacity of flask containing ethanol (10.0 mL). It was kept on the magnetic stirrer for stirring, covered with aluminum foil to stop the evaporation of ethanol. The lipid solution was stirred using magnetic stirrer at 500 rpm. Then distilled water (5.0 mL) containing with or without drug was added in the lipid solution dropwise with continuous

stirring. After complete addition mixture was stirred for 30 min. The ethosomal suspension was sonicated for 30-90 sec using probe sonicator to minimize the vesicle size of ethosomes.

Optimization

Ethosomes were optimized by factorial design using Design of Experiment (DOE) software version 11.0 [8]. The three independent factors including Phosphotidylcholine: Cholesterol: DSPE (6:3:1 molar ratio), Surfactant concentration and sonication time. A factorial design 3*3(3 factor 3 level) was applied to prepare 17 formulation. Optimization of ethosomal preparation was carried out by applying Box Behnken response surface randomized factorial design following quadratic model using DOE software version 11.04.0. The applied design was suggested 17 run with varying the dependable and in dependable process and formulation variables. The factor SOYA PC: Cholesterol: DSPE in molar ration (6:3:1), Concentration of Tween-80 and sonication time were selected as dependable process and formulation factors that can be effect formulation characteristics like entrapment efficiency, average vesicle size, Polydispersity Index (PDI). All other factors like sonication speed and rotation speed was kept constant. All the formulation were prepared by simple solvent evaporation thin film formation method and characterized for the drug entrapment, average vesicle size and PDI, shape morphology. Formulations were optimized on the basis of responses such as average vesicle size, PDI, and entrapment efficiency. All the characterized values of the responses were putted in the formulation design table and analyse to best fitted model for the design. It was observed that, quadaratic model is best fitted model for the design.

Table 1: Formulation factors and their dependable and in dependable variables

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	SOYA PC: Cholesterol: DSPE (6.0:3.0:1.0)	% (W/V)	Numeric	0.5000	1.50	-1 ↔ 0.50	+1 ↔ 1.50	1.0000	0.3536
B	Tween 80	% (W/V)	Numeric	1.0000	2.00	-1 ↔ 1.00	+1 ↔ 2.00	1.50	0.3536
C	Sonication Time	(Sec)	Numeric	30.00	90.00	-1 ↔ 30.00	+1 ↔ 90.00	60.00	21.21

The Ethosomes formulation was optimized by applying response surface factorial design. The 17 formulation were prepared with varying the formulation variables such as concentration of lipids, concentration of tween-80 and the process variables such as sonication time. The

optimization was done on the basis of average vesicle, PDI and entrapment efficiency. The optimized formulation was further validated by preparing the formulation using the formula suggested by the DOE response surface optimization design.

Final Equation in Terms of Coded Factors

$$\text{Vesicle Size} = +222.22+65.30A-16.70B-6.67C+4.20AB+25.00AC+12.55BC+148.69A^2+14.24B^2+11.09C^2$$

Final equation in terms of actual factors

$$\text{Vesicle Size} = +1122.85000-1184.12000\text{SOYA PC: Cholesterol: DSPE (6.0:3.0:1.0)}-271.28000\text{Tween 80}-4.62283\text{Sonication Time}+16.80000\text{SOYA PC: Cholesterol: DSPE (6.0:3.0:1.0)} * \text{Tween 80}+1.66667\text{SOYA PC: Cholesterol: DSPE (6.0:3.0:1.0)} * \text{Sonication Time}+0.836667\text{Tween 80} * \text{Sonication Time}+ 594.76000\text{SOYA PC: Cholesterol: DSPE (6.0:3.0:1.0)}^2+56.96000\text{Tween 80}^2+0.012322 \text{Sonication Time}^2$$

Final Equation in Terms of Coded Factors

$$\text{PDI} = +0.3104-0.0144A+0.0589B-0.0390C-0.0553AB+0.0500AC-0.0205BC-0.0183A^2+0.0072B^2+0.3769C^2$$

Final Equation in Terms of Actual Factors

$$\text{PDI} = +1.48500 +0.249350 \text{SOYA PC: Cholesterol: DSPE (6.0:3.0:1.0)} +0.334650\text{Tween 80}-0.052840 \text{Sonication Time}-0.221000 \text{SOYA PC: Cholesterol: DSPE (6.0:3.0:1.0)} * \text{Tween 80}+0.003333 \text{SOYA PC: Cholesterol: DSPE (6.0:3.0:1.0)} * \text{Sonication Time}-0.001367\text{Tween 80} * \text{Sonication Time}-0.073300 \text{SOYA PC: Cholesterol: DSPE (6.0:3.0:1.0)}^2+ 0.028700\text{Tween 80}^2+0.000419.$$

Final Equation in Terms of Coded Factors

$$\text{Entrapment Efficiency:} = +72.56+8.45A-1.14B-1.61C+0.6500AB-2.25AC+7.88BC-3.04A^2-2.97B^2-11.72C^2$$

Final Equation in Terms of Actual Factors

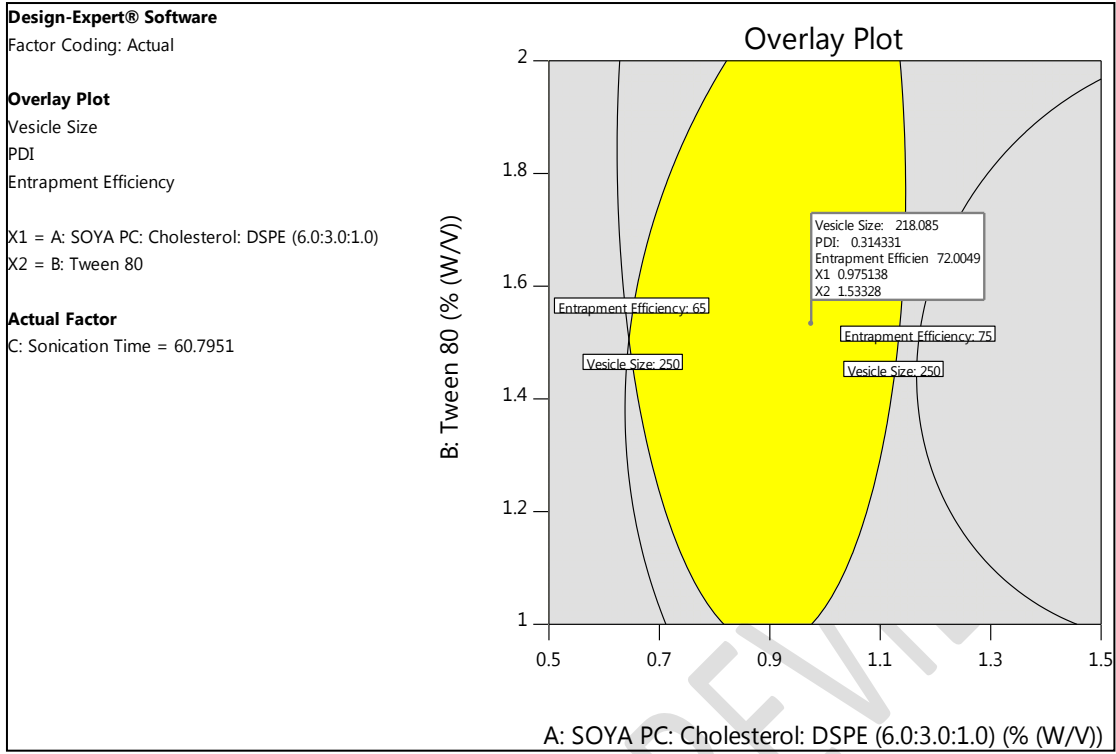
$$\text{Entrapment Efficiency} = +18.70000+46.34000 \text{SOYA PC: Cholesterol: DSPE (6.0:3.0:1.0)} -0.765000 \text{Tween 80}+0.871083 \text{Sonication Time}+2.60000\text{SOYA PC: Cholesterol: DSPE (6.0:3.0:1.0)} * \text{Tween 80} -0.150000 \text{SOYA PC: Cholesterol: DSPE (6.0:3.0:1.0)} * \text{Sonication Time} +0.525000 \text{Tween 80} * \text{Sonication Time} -12.17000 \text{SOYA PC: Cholesterol: DSPE (6.0:3.0:1.0)}^2-11.87000 \text{Tween 80}^2 -0.013019 \text{Sonication Time}^2$$

Table 2: Formulation design for Almotriptan loaded ethosomes

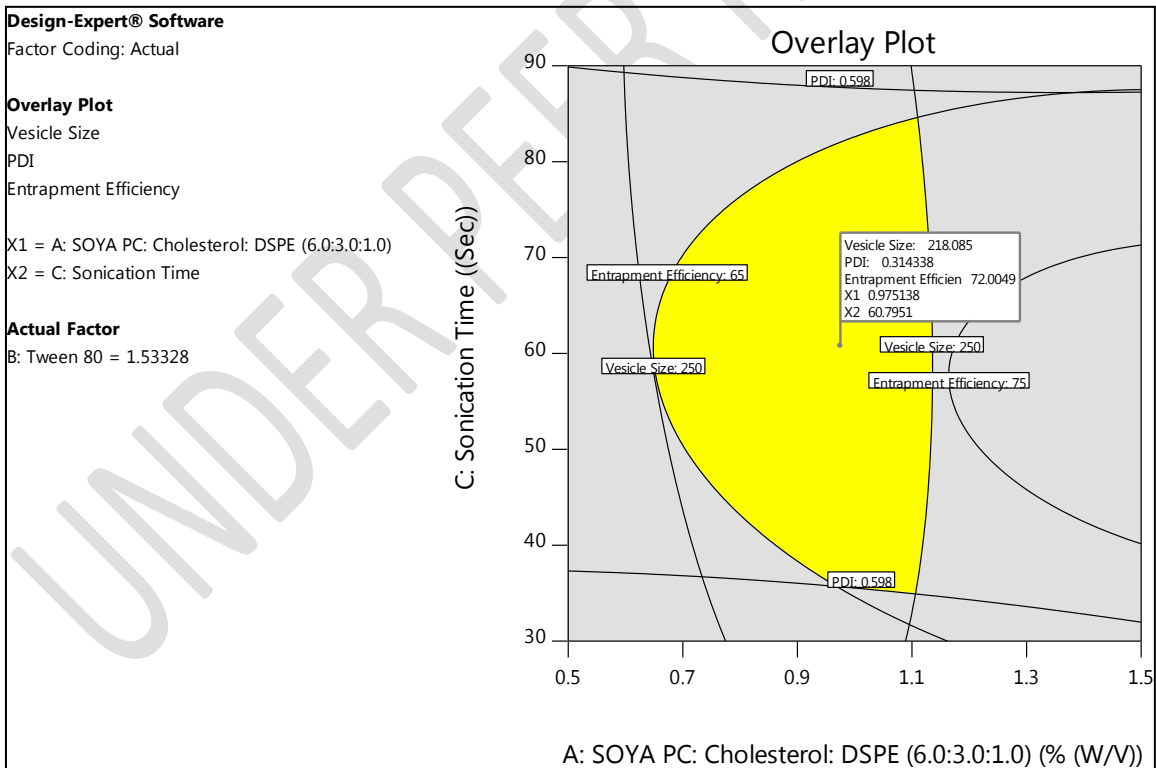
Std	Run	Factor 1	Factor 2	Factor 3
-----	-----	----------	----------	----------

		A:Soya PC:	B:Tween 80	C:Sonication Time
		Cholesterol: DSPE (6.0:3.0:1.0)		
		% (W/V)	% (W/V)	(Sec)
15	TSOM-1	1	1.5	60
8	TSOM- 2	1.5	1.5	90
13	TSOM-3	1	1.5	60
6	TSOM-4	1.5	1.5	30
12	TSOM-5	1	2	90
7	TSOM-6	0.5	1.5	90
16	TSOM-7	1	1.5	60
9	TSOM-8	1	1	30
2	TSOM-9	1.5	1	60
17	TSOM-10	1	1.5	60
11	TSOM-11	1	1	90
5	TSOM-12	0.5	1.5	30
3	TSOM-13	0.5	2	60
4	TSOM-14	1.5	2	60
10	TSOM-15	1	2	30
1	TSOM-16	0.5	1	60
14	TSOM-17	1	1.5	60

Overlay plot for actual factor showing optimized area



(A)



(B)

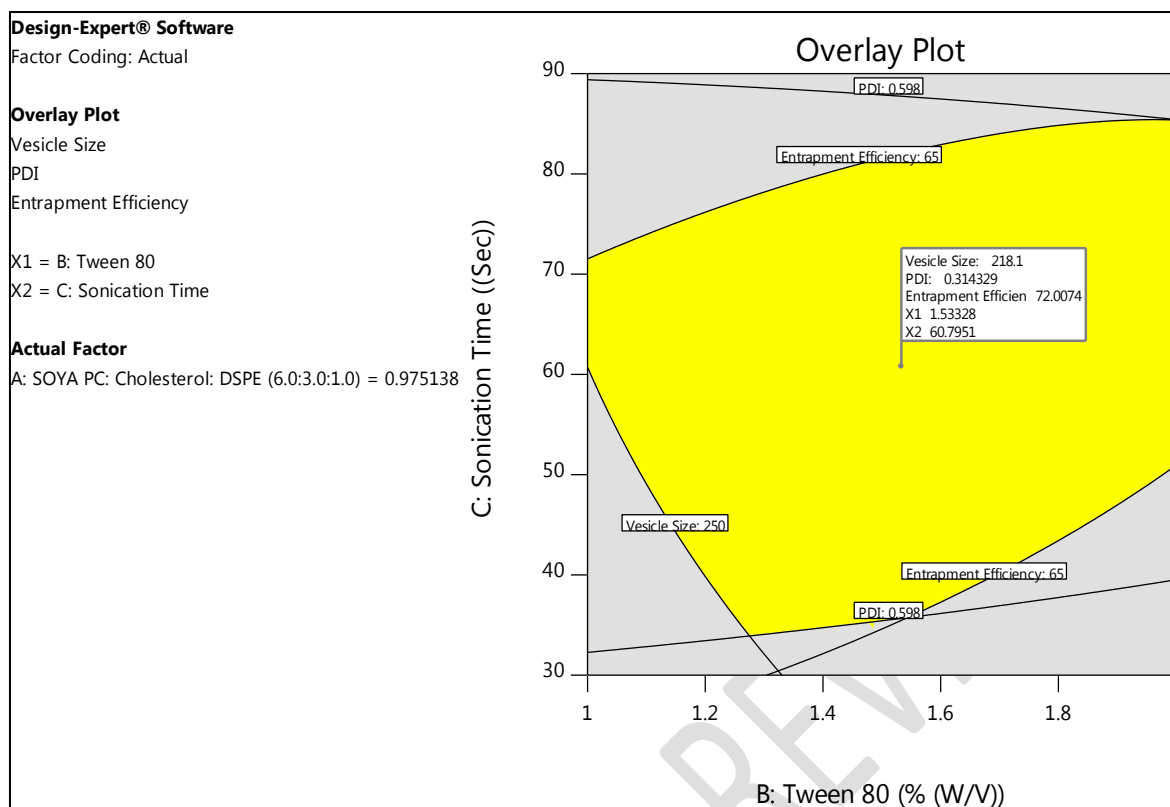


Fig. 1: (A, B, C) Overlay plot for actual factor showing optimized area.

Characterization of ethosomal formulation

Formulation Design: Each numeric factor is set to 3 levels. If categorical factors are added, the Box-Behnken design will be duplicated for every combination of the categorical factor levels. These designs have fewer runs than 3-Level Factorials. Formulations were characterized for the Vesicle size, Polydispersity index (PDI) and Zeta potential, Entrapment Efficiency, Surface morphology and in vitro drug release.

Vesicle size, Polydispersity index (PDI) and Zeta potential

The average vesicle size of ethosomes was measured using dynamic light scattering, Malvern zetasizer (Malvern zetasizer, Worcestershire, UK) from SAIF RGPV, Bhopal. The sample was kept in polystyrene cuvettes and observations were found at 90 fixed angle. The dispersion sample was diluted to 1:9 v/v with de-ionized water and distilled water to ensure that the light scattering intensity was within the instrument sensitivity range [9]. The polydispersity index is a range of particle size measurements within a sample. The polydispersity index is determined by dividing the average molecular weight by the number of average molecular weights. It's used to show the range of vesicle diameter dispersion.

The electro kinetic potential in a colloidal system is known as the zeta potential. Malvern Zetasizer calculated the zeta potential (DTS version 4.10, UK). The zeta potential is

significant because its value may be linked to colloidal dispersion stability and reflects the degree of repulsion between neighboring, similarly charged particles in dispersion. A high zeta potential confers stability to particles and molecules, i.e., the solution or dispersion will resist aggregation. Tween 80 used in the formulation also provides steric stability to achieve stable formulation.

Entrapment efficiency

The entrapment efficiency of ethosomes was determined by separation of ethosomes from the aqueous medium. Sephadex G-50 mini-columns was prepared by using weighed amount of Sephadex G-50 and mixed with sufficient amount of double distilled water and stand for 24 h for swelling. Then it placed in a 1 ml PVC syringe (Dispovan) previously packed with a plug of glass wool and a small piece of Whatman filter paper at the bottom end. These columns were used to determine entrapment efficiency of ethosomes [10].

The amount of un-entrapment drug was removed by passing 2.0 ml of Ethosomes formulation from Sephadex column and then centrifuged at 3000 rpm to complete removal of Ethosomes. Un-entrapped drug was remain in Sephadex column and Ethosomes eluted from it. Eluted Ethosomes treated with 1% of triton X-100 to analyze the Ethosomes. Solution was centrifuged at 10000 rpm and supernatant was analyzed under UV Spectrophotometer (Simadzu 1700) for Almotriptan. The entrapment efficiency was calculated by following formula

$$\% \text{ Drug Entrapment} = \frac{\text{Amount of drug entrapped in Ethosomes formulation.}}{\text{Initial amount of drug taken for loading}} \times 100$$

In vitro drug release

The drug release was performed in PBS (pH 6.6) for Ethosomes with drug separately using dialysis bag (Cellophane membrane) technique. In this study of Ethosomes formulation equivalent to 10 mg of drug was taken in dialysis tubing and placed in a beaker containing 100 ml of PBS pH 6.6. The release profile of drug loaded ethosomes was determine using pretreated membrane with 100ml of phosphate buffer solution. The dialysis bag retains Ethosomes and allows passing of free drug into the dissolution media. Temperature was maintained at $37 \pm 2^{\circ}\text{C}$ throughout the study. The samples were withdrawn after specified time intervals i.e. 0, 0.5, 1, 2, 4, 8, 24, 48, and 72 h and replaced with the same volume of fresh PBS pH 6.6 and analyzed for drug concentration by using UV spectrophotometer [11].

Results and Discussion

The percent entrapment efficiency was found 71.5, 59.7, 70.2, 70.8, 64.7, 49.3, 73.4, 66.8, 76.5, 73.4, 51.2, 51.4, 55.3, 75.5, 48.8, 58.9, and 74.3 respectively for TSOM-1, TSOM-2,

TSOM-3, TSOM-4, TSOM-5, TSOM-6, TSOM-7, TSOM-8, TSOM-9, TSOM-10, TSOM-11, TSOM-12, TSOM-13, TSOM-14, TSOM-15, TSOM-16 and TSOM-17 coded formulation. The drug entrapment efficiency was decrease with increasing the mechanical force by sonicating the formulation using probe sonicator. It helps to reduce the vesicle size and impart the uniformity in the prepared ethosomes. It may chances of drug leaking from the ethosomes during deform and reform the transferosomal vesicles upon size reduction of the ethosomes. It is also revealed that drug entrapment efficiency was decrease with decrease in vesicle size of the ethosomes. High mechanical force is responsible for permanent breaking of the vesicles of ethosomes due to lipid bilayer disruption. High mechanical force is generate heat in the formulation that may sufficient to disrupt the lipid bilayers of the vesicles. So it was very necessary to optimize the time of sonication to formulate the traferosomes with uniform in size and high drug loading efficiency. In the optimization process minimum and maximum sonication time was kept 30 and 90 sec respectively.

Drug loading and size vesicles are directly affected by the concentration of lipids and their ratios. It was reveled that the viscosity of the lipid solution was increase and form multi layers with high thickness lipid film in the round bottom flask and upon hydration of the film, It may chances to form multi lamalar vesicle or big size of ethosomes. So it also very important to optimize the lipid concentration and it was optimized response surface factorial design by taking 0.5 to 1.5% concentration of lipids in 6:3:1 molar ratio of Soya PC: Chlestrol: DSPE.

The validation of the formulation design was done by preparing the formulation that was suggested by factorial design and some formulation were selected by using the overlay plot. Overlay plot provideda optimized region that provide the optimized value of formulation and process variable. The formulation TSOMV-1, TSOMV-2, TSOMV-3, TSOMV-4 and TSOMV-5 were prepared and found that the formulation were shown similar response value like the value which were showing in suggested formulation. The formulation TSOMV-1 was prepared by using 1.5 % W/V of Soya PC: Cholesterol: DSPE in the molar ratio of (6:3:1) and 1.5 %W/V of Tween-80. The sonicaion time was kept 30 second. The formulation TSOMV-1 was showing 464.5 nm of vesicle size, 0.623 of PDI value and 69.8 % of entrapment efficiency. In case of TSOMV-2 formulation which was prepared by using 1.0 %w/v lipid 1,5 % w/v of Tween-80 and 60 second of sonication time that shown 275.4 of average particle size, 0.298 of mean PDI, 70.2% of entrapment efficiency and -17.9 mV of zeta potential. The formulation prepared by using 0.9% of lipid, 1.4 % of Tween 80 and 65 second of sonication time shown the 215.3 nm of average particle size, 0.314 of PDI, 2.3% of

drug entrapment efficiency and -18.4 mV of zeta potential. In case of TSOV-5 that was prepared by taking 0.95 % of lipid, 1.0% of Tween-80 and 60 second of sonication time that shown 434.1 of average vesicle size, 0.237 of mean PDI, 70.2% of entrapment efficiency. A selected formulation provided by the factorial design coded as TSOMV-4 was prepared by taking 0.95% molar mixture of lipid, 1.53% W/V of Tween-80 and 62 second of sonication time. It was shown 218.6 nm of average vesicle size, 0.305 of mean PDI, -18.3 mV of zeta potential and 73.5% of entrapment efficiency.

To reduce the size of Ethosomes vesicles with uniform in size, sonication is the best means to reduce the size. Sonication process creates high mechanical force with high intensity that is capable to break the particle or vesicles in very small size within short period of time. It is also possible that size of vesicle bring in uniform size by optimizing the time of sonication.

Drug concentration was kept constant during the optimization process. But it reported that, the concentration of drug was increases then drug entrapment was increase. But drug entrapment was not significantly increase with further increasing the drug concentration in the formulation.

It was found that the size of vesicle was increase with increasing the lipid concentration and ratio and also increase when the increasing the concentration of lipids in the formulation. It is due to the cholesterol which enhance the strengthens the vesicle layer make more intact that not break easily on applying constant mechanical force. The zeta potential value represents good stability of formulation. Tween 80 used in the formulation also provides stearic stability to achieve stable formulation.

The drug release was performed in PBS (pH 6.6) for ethosomes separately using dialysis bag (Cellophane membrane) technique. In this study 6ml of Ethosomes formulation equivalent to 10 mg of drug was taken in dialysis tubing (MWCO, 15 KDa, Himedia) and placed in a beaker containing 100ml of PBS pH 6.6. The dialysis bag retains ethosomes and allows passing of free drug into the dissolution media. Temperature was maintained at $37 \pm 20^{\circ}\text{C}$ throughout the study. The samples were withdrawn after specified time intervals i.e. 0, 0.5, 1, 2, 4, 8, 24, 48, 72 h and replaced with the same volume of fresh PBS pH 6.6 and analysed for drug concentration by using UV spectrophotometer.

In vitro drug release estimation done by a comparative study between ethosomes and ethosomes loaded with drug. The cumulative percentage release of drug (almotriptan) for optimized ethosomes formulation were carried out over a period of 72 hours. The drug release was found above 90% with plain drug solution within 5hr while the drug release was found 47.35% after 24 hr. The drug release after 72 hr was found 63.32%.

The release of the drug from ethosomes is complicated. It involves drug diffusion, interface movement and various interactions. The rate of release process is described by correlation coefficient values comparing of the lines collected from the graphical presentation of different mathematical models.

Table 3: Results of characterization of prepared ethosomal formulation

Formulation Code	Response 1	Response 2	Response 3
	Vesicle Size (nm)	PDI	Entrapment Efficiency (%)
TSOM-1	270.6	0.268	71.5
TSOM- 2	473.7	0.695	59.7
TSOM-3	275.4	0.298	70.2
TSOM-4	464.5	0.623	70.8
TSOM-5	243.4	0.629	64.7
TSOM-6	249.5	0.615	49.3
TSOM-7	198.5	0.562	73.4
TSOM-8	276.8	0.719	66.8
TSOM-9	434.1	0.237	76.5
TSOM-10	192.9	0.189	73.4
TSOM-11	265.8	0.632	51.2
TSOM-12	340.3	0.743	51.4
TSOM-13	327.8	0.472	55.3
TSOM-14	423.2	0.324	75.5
TSOM-15	204.2	0.798	48.8
TSOM-16	355.5	0.164	58.9
TSOM-17	174.4	0.235	74.3

Table 4: Validation of formulation and formulation design

Formulation code	Soya PC: Cholesterol: DSPE (m.mol)	Tween-80 (% W/V)	Sonication Time (Sec)	Mean Vesicle size (nm)	Mean PDI	% EE	Zeta Potential (mV)
TSOMV-1	1.5	1.5	30	464.5	0.623	69.8	-17.5
TSOMV-2	1	1.5	60	275.4	0.298	70.2	-17.9
TSOMV-3	0.9	1.4	65	215.3	0.314	72.3	-18.4
TSOMV-4	0.95	1.53	62	218.6	0.305	73.5	-18.3

TSOMV-5	1.5	1.0	60	434.1	0.237	70.2	-18.2
---------	-----	-----	----	-------	-------	------	-------

Table 5: In vitro drug release study of optimized formulation of Ethosomes (TSOMV-4)

S. No.	Time (hr.)	Plain Drug	Ethosomes
1	0	0	0
2	0.5	18.5	8.9
3	1	38.2	12.3
4	2	56.3	18.9
5	3	73.5	23.6
6	4	91.4	29.93
7	6	-	33.77
8	8	-	36.65
9	10	-	39.63
10	12	-	41.91
11	16	-	44.73
12	20	-	47.35
13	24	-	49.31
14	48	-	57.61
15	72	-	63.32

Table 6: r² value of optimized formulations (TSOMV-4)

Correlation coefficient	
Kinetic Models	Ethosomes
Zero order plot	R ² = 0.865
First order plot	R ² = 0.963
Higuchi plot	R ² = 0.943
Korsmeyer's Peppas plot	R ² = 0.976

CONCLUSION

The results obtained in this research work clearly indicated a promising potential of ethosomal carrier system of almotriptan for migraine treatment with a topical approach for

sustained action. It is concluded that ethosomes loaded with drug follow the Korsmeyer's Peppas equation because its r^2 value is 0.976 and, respectively which is very near to unity. It means drug release follows the Korsmeyer's Peppas model, in which the drug release occurs by diffusion process from the polymeric matrix of ethosomes, so it was selected as best fit model.

REFERENCES

1. Global Burden of Disease Study 2013 Collaborators (2015) Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study. *Lancet (Lond, Engl)* 2013; 386:743–800
2. Goadsby PJ. Bench to bedside advances in the 21st century for primary headache disorders: migraine treatments for migraine patients. *Brain* 2016; 139:2571–2577
3. Amin FM, Asghar MS, Hougaard A, Hansen AE, Larsen VA, de Koning PJ, Larsson HB, Olesen J, Ashina M. Magnetic resonance angiography of intracranial and extracranial arteries in patients with spontaneous migraine without aura: a cross-sectional study. *Lancet Neurol* 2013; 12:454–461
4. Goadsby PJ, Holland PR, Martins-Oliveira M, Hoffmann J, Schankin C, Akerman S. Pathophysiology of migraine—a disorder of sensory processing. *Physiol Rev.* 2017; 97(2):553–622
5. Charles A. Migraine: a brain state. *Curr Opin Neurol* 2013; 26:235–239
6. Ferrari MD, Klever RR, Terwindt GM, Ayata C, van den Maagdenberg AM. Migraine pathophysiology: lessons from mouse models and human genetics. *Lancet Neurol* 2015; 14:65–80
7. Hallan SS, Sguizzato M, Mariani P, Cortesi R, Huang N, Simelière F, Marchetti N, Drechsler M, Ruzgas T, Esposito E. Design and characterization of ethosomes for transdermal delivery of caffeic acid. *Pharmaceutics.* 2020; 12(8):740.
8. Anju K, Priya S, Sandeep DS, Nayak P, Kumar P, Kumar A, Lobo CL, Krithi SP. Formulation and Optimization of Zaltoprofen Loaded Ethosomal Gel by using 23 Full Factorial Designs. *Journal of Pharmaceutical Research International.* 2021; 17:30-44.
9. Jain S, Umamaheshwari RB, Bhadra D and Jain NK. Ethosomes: Novel vesicular carrier for enhanced Transdermal delivery of an antiHIV agent. *Ind J Pharm Sci,* 2004; 66(1):72-81.
10. Jain S, Tiwary AK, Sapra B, Jain NK. Formulation and evaluation of ethosomes for transdermal delivery of lamivudine. *AAPS PharmaSciTech.* 2007; 12(21):1-9.

11. Touito E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes-novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. *J Control Release*.2000; 65:403-418.

UNDER PEER REVIEW