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DEVELOPMENT AND EVALUATION OF INTRANASAL LAMOTRIGINE TRANSFEROSOMES FOR TREATMENT OF EPILEPSY

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ABSTRACT:

Objective: The aim of the present work was to prepare and optimize the lamotrigine loaded transferosomes for treatment of epilepsy by design expert software. Method: Using the thin film rehydration method, lamotrigine transferosomes were created with phospholipon, cholesterol, and tween 80 as the primary constituents. Preparation was optimized by Box-Behnken design for establishing the functional relationships between variables on responses i.e. particle size, entrapment efficiency, % cumulative release. Particle size, % entrapment efficiency, invitro drug release, surface morphology, and stability analysis were assessed for prepared transferosomes. Result: The optimized transferosomes had a vesicle size of 100.02±0.16 nm, entrapment efficiency of 69.75±1.23% and invitro release of 80.42±1.82 %. Conclusion: Transferosomes optimized by 3-level Box-Behnken design response surface methology generated a quadratic response surface. The optimized transferosome showed vesicle size in nano range with high release and high entrapment efficiency. The prepared transferosomes will have direct effect on the brain when delivered through nose in the management of epilepsy.

Keywords: epilepsy, lamotrigine, transferosomes, Box-Behnken, vesicle size, quadratic response surface

1. INTRODUCTION

Epilepsy, the most common neurological disorder of brain in which one out of 50 individuals are having epileptic convulsions[1] The cause of epilepsy is unidentified and is characterized by multiple seizures.[2] According to epidemiology commission epilepsy is defined as more than 2 uncontrolled seizure occurring in 24 hrs.[3]The wrong diagnosis of epilepsy is very much common[4]Most widely used antiepileptic drug Lamotrigine is very much effective in treatment of uncontrolled seizures. As the solubility of Lamotrigine is very poor in water, hence it has very less probability to cross BBB.[5]Lamotrigine belongs to BCS class II, hence it is rapidly and completely absorbed orally. [6] Intranasal administration has been effectively accomplished with transferosomes. [7] The hydrophilic nature of liposome gave a good contribution to drug entrapment and sustained or controlled release.[8]The most effective pathway to deliver the drug directly into the brain is the intranasal route.[9]Intranasal route helps in avoiding the hepatic metabolism and also bypasses the blood brain barrier.[10] Introduced in 1990, transferosomes are among the most pliable forms of liposomes.[7]Transferosomes can pass through very small tiny pores and therefore it is very much useful in skin permeation process.[11]

2. MATERIALS AND METHODS

2.1 Material

A gift sample of lamotrigine was received from CTX Lifesciences Pvt Limited in Surat, Gujarat, India. Spectrochem Pvt Ltd, India provided a gift sample of phospholipid; SD Fine Chemicals, India sold NaCl; Spectrochem Pvt Ltd, India supplied cholesterol; SD Fine Chemicals, India supplied Tween 80; and SD Fine Chemicals, India supplied chloroform.

2.2 Method of Preparation of lamotrigine loaded transferosomes

The thin film rehydration approach was used to prepare Lamotrigine transferosomes.[12] Required quantity of phospholipon, surfactant and drug were taken in RBF and dissolving was done in the ratio of 2:1 of chloroform and ethanol by shaking. A thin layer was produced by evaporating solvent for 20 minutes at 100 rpm and 25°C in a rotary evaporator. Overnight, the thin film was preserved to eliminate organic solvents. Tween 80 was added followed by hydration with NSB (pH 6.5). The obtained vesicles were sonicated and stored at 2-8°C for further characterization.

2.3 Optimization of lamotrigine loaded transferosomes by design of experiment

The main effect, interaction effect, and quadratic effect of particular independent variables with dependent responses were assessed using Box-Behnken Design (BBD). [13] It utilizes three most significant factors by Design Expert software version (10.0.5.0).(Aslan and Cebeci 2007)The independent variables selected were phospholipid(X_1), cholesterol(X_2) and sonication time(X_3) in respect to particle size(Y_1), entrapment efficiency(Y_2) and invitro release(Y_3) as dependent responses (Table 1). This design showed 17 experimental runs (Table 2).

2.4 Vesicle size

Developed lamotrigine transferosomes size was determined by photon correlation spectroscopy by use of Malvern particle size analyzer. The prepared sample was examined at 25°C after being diluted ten times with PBS.

2.5 Zeta potential measurement

Malvern Zeta Seizer was used to assess the zeta potential after diluting the transferosome suspension with purified water..(Erdogan et al. 2019)

2.6 Entrapment efficiency

Drug loaded transferosomes was taken out from its aqueous phase at 15000 rpm by centrifugation process for 45 min or till a clear supernatant was obtained. (Singh, Ahamad, et al. 2024)The sample was washed with PBS to remove free drug and diluted with methanol and analyzed by UV spectrophotometer at 306.5 nm

$$\%EE = \frac{initial\ amount\ of\ drug - amount\ of\ drug\ in\ supernatant}{initial\ amount\ of\ drug} \times 100$$

2.7 In-vitro release

In- vitro dialysis bag was used for determination of amount of lamotrigine in prepared transferosomes. 2 ml of prepared transferosomes was filled in dialysis tube and completely submerged in 100ml of NSB of pH 6.5 at 37◦C ±0.5◦C at 100 rpm for 12 hrs. 2ml of sample was withdrawn and immediately filled with same volume of fresh NSB.(Singh, Maheshwari, et al. 2024) The sample was analyzed by UV Spectrophotometer and cumulative release was calculated.

2.8 Surface morphology

SEM was used to determine the morphology of lamotrigine transferosome formulation. Grid was prepared by placing one drop of carbon coated copper grid and it was dried at room temperature and then analyzed by scanning electron microscope.[18]

2.9 Stability study

Stability study was determined at two temperatures, one at 25±2◦C/ 60±5%RH and other at 4±0.5◦C for a period of 6 months.(Singh, Ahamad, et al. 2024) The procedure followed was filling the sample in the glass vial, tightly packing and then storing at above mentioned conditions After time interval of 0 days, 1 week, 2 weeks, 1 month, 3 months and 6 months, sample was analyzed for phase separation, pH, particle size, PDI and entrapment efficiency.(Praveen et al. 2018)

3. RESULTS

3.1 Optimization by Design of experiment (DOE)

3.1.1 Impact of independent variables on vesicle size (Response Y_1)

Because smaller size facilitates easier diffusion through the nasal mucosa, which offers a larger area of absorption, vesicle size is an important property of transferosomes. [20] Model is quadratic with F value 9651.41. Model term is significant as p value was less than 0.0500. The correlation coefficient value was found to be 0.9999 (Table 3) which showed a good model fit. The foreseen R^2 value was 0.9987 which was in suitable harmony with adjusted R^2 0.9998 (Table 3).

$Y_1=118.87+12.72X_1+6X_2-20.19X_3+1.32\ X_1\ X_2-7.07\ X_1\ X_3-2.37\ X_2\ X_3-12.02X_1^2-1.15X_2^2-8.59X_3^2$ … eq. (1)

Vesicle size was positively impacted by cholesterol and phospholipid, as demonstrated by the polynomial equation (1). The minimum average vesicle size observed of lamotrigine transferosomes was 72.44 nm and maximum average vesicle size observed of lamotrigine transferosomes was 138.22 nm (Table 2). It was observed that the size of lamotrigine transferosomes increases with increase in phospholipid and cholesterol concentration and decreases with increase in sonication time.

3.1.2 Impact of independent variables on entrapment efficiency (Response Y_2)

The entire amount of drug integrated into the lipid bilayer model of transferosomes can be determined using entrapment efficiency, and the percentage is assessed using UV spectroscopy.

Model is quadratic with F value 1439.19. Model terms are noteworthy when p value is less than 0.0500. The correlation coefficient value R^2 was found to be 0.9995 which showed a good model fit. The predicted R^2 value was 0.9914 which was in adequate harmony with adjusted R^2 0.9988 (Table 3).

$Y_2=68.79+12.12X_1+3.70X_2-4.30X_3-1.55\ X_1\ X_2-0.8850\ X_1\ X_3+2.56\ X_2\ X_3-9.26X_1^2-0.6363X_2^2-0.2962X_3^2$ .. eq. (2)

The maximum and minimum entrapment efficiency was found to be 41.28% and 76.04%. entrapment efficiency is also greatly affected by nature of drug. As lamotrigine is a lipophilic drug which shows more entrapment in the lipid bilayer of phospholipid. [21]

The process of ultrasonication is what reduces vesicles' size.

By breaking the vesicles, the sonication process increases drug leakage from the vesicles and decreases drug entrapment within them, which has an impact on drug entrapment.[22]

In comparison to formulation B17, which had a lower sonication time of 120 seconds and a higher entrapment efficiency of 76.04%, formulation B12 had a lower entrapment efficiency with a longer sonication time of 600 seconds. Cholesterol has a encouraging outcome on entrapment efficiency. It increases drug entrapment via hardening the lipid bilayer's structure by integrating in between the phospholipid layer, it strengthens the lipid bilayer model and contributes to the drug's increased entrapment efficiency. Additionally, it aids in boosting vesicle stability and preventing phospholipid bilayer leaking.[23]

3.1.3 Impact of independent variables on invitro drug release (Response Y_3)

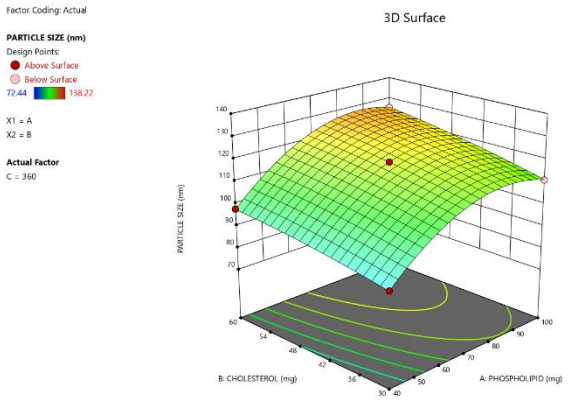
The in vitro drug release of transferosomes was carried out using the dialysis bag method, which produced a polynomial equation (3) after optimization by response surface approach.

$Y_3=74.10+3.77X_1-1.48X_2+7.62X_3-0.2075X_1\ X_2+1.98\ X_1\ X_3+1.07\ X_2\ X_3-5.75X_1^2-1.12X_2^2-0.2912X_3^2$ .. eq. (3)

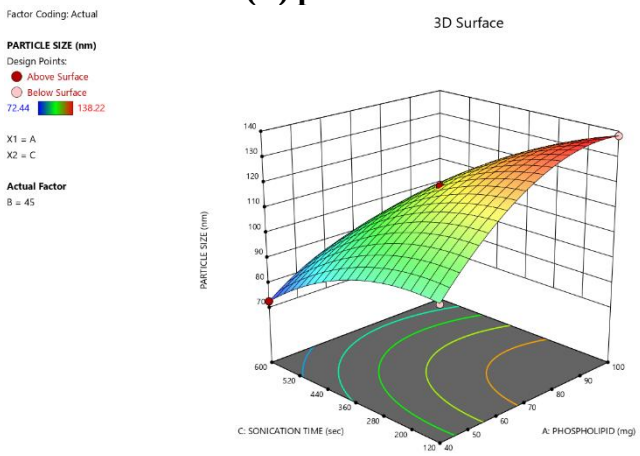
Equation (3) demonstrated the quadratic nature of the model with a F value of 151.85. Given that the P value was less than 0.0500, the model terms are significant. A good fit was shown by the correlation coefficient value R2, which for drug release in vitro was determined to be 0.9949. The predicted R^2 value was 0.9185 which was in acceptable harmony with adjusted R^2 0.9884 (Table 3).

Maximum release for B1 formulation, with 81.43% across prepared cellophane membrane, was observed in the in vitro release profile of transferosomes. The aim for better release is the flexibility which comes by adding of tween 80. The vesicle integrity is attained by addition of cholesterol which is also responsible for passing of transferosomes through pores of cellophane membrane.[24]

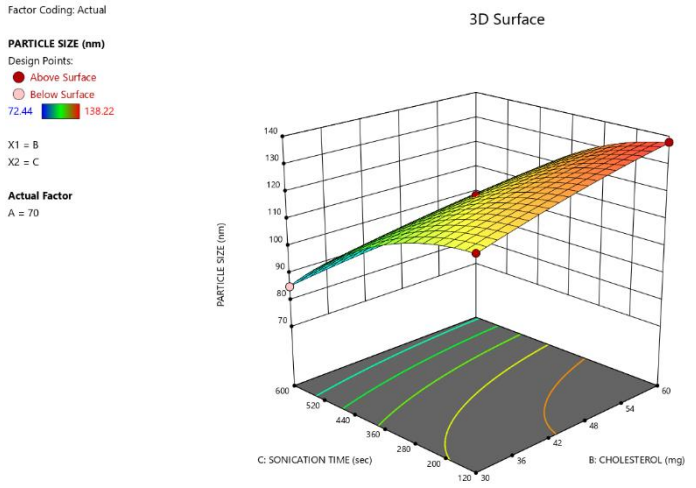
Figure 1: 3D-Contour plot for (A) particle size; (B) Entrapment efficiency (%EE); (C) In-vitro release.



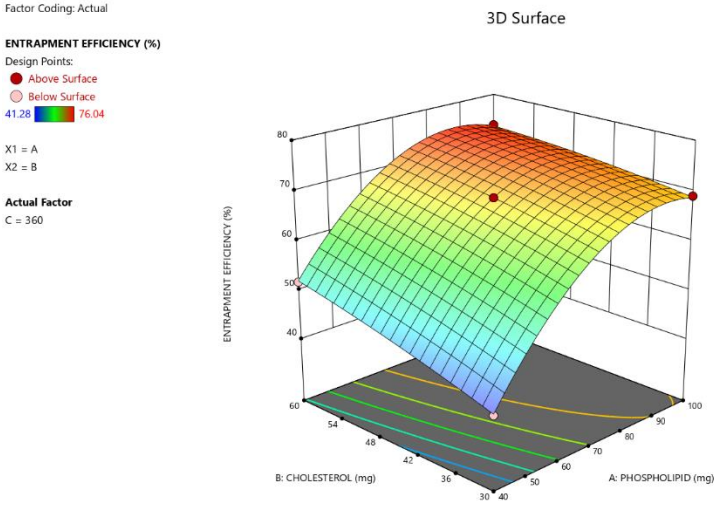
(A) particle size



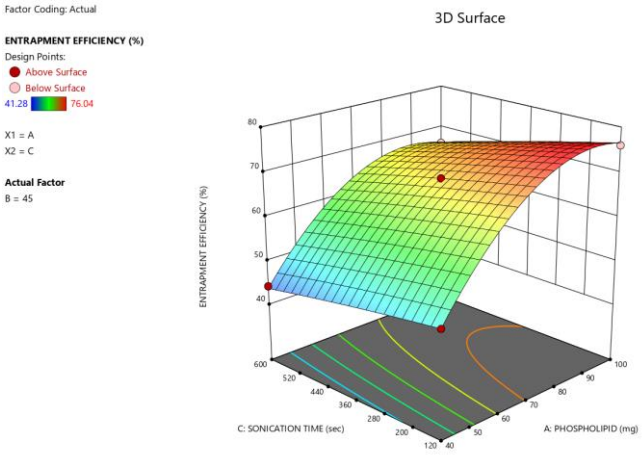
(A) particle size



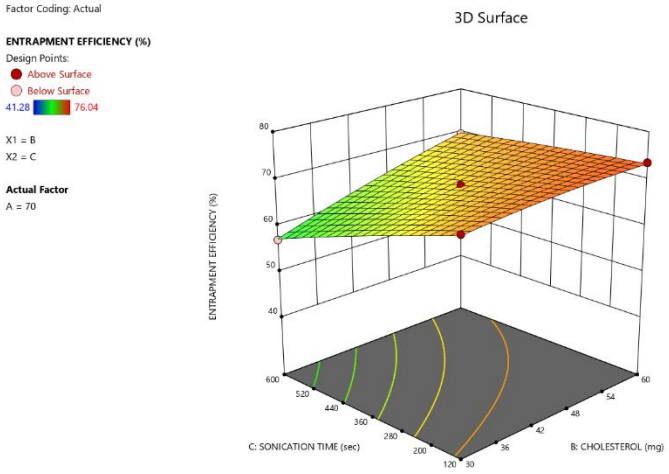
(A) particle size



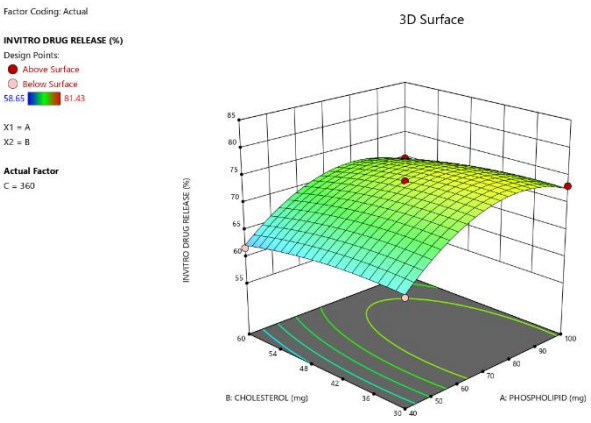
(B) Entrapment efficiency (%EE)



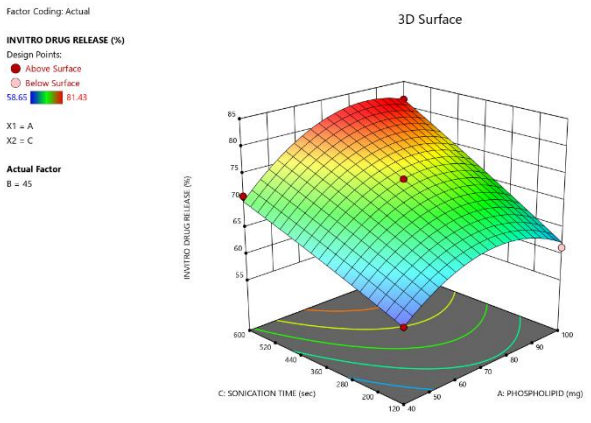
(B) Entrapment efficiency (%EE)



(B) Entrapment efficiency (%EE)



(C) In-vitro release



(C) In-vitro release

(C) In-vitro release

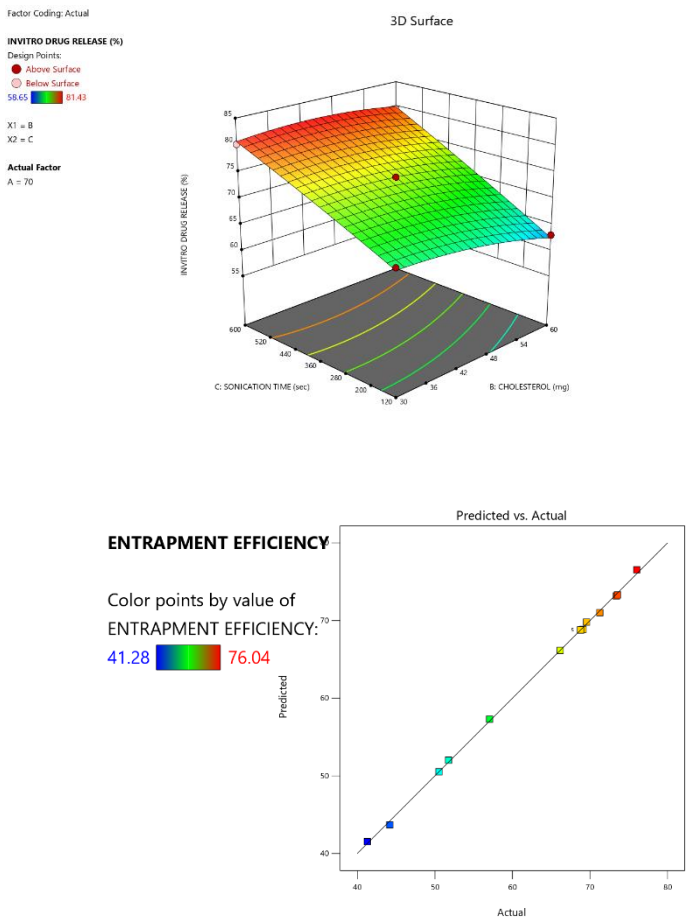
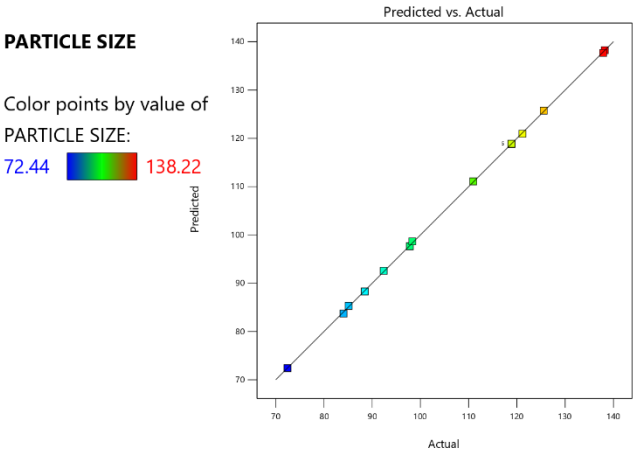
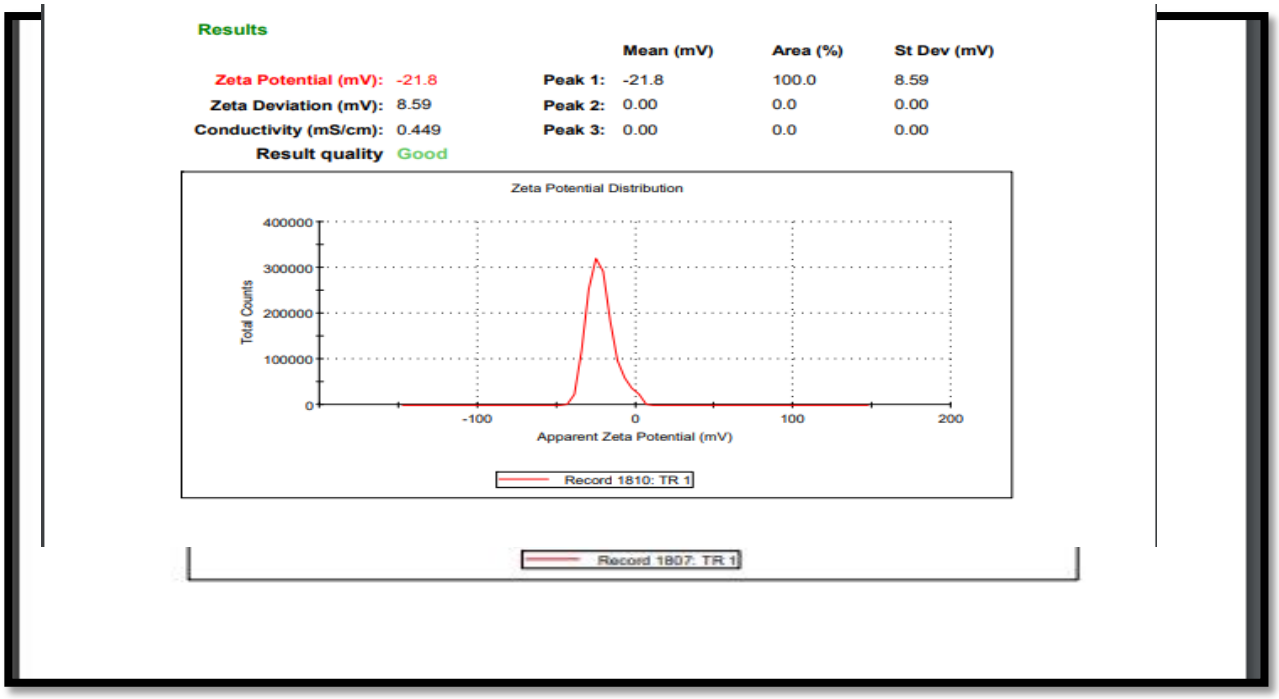
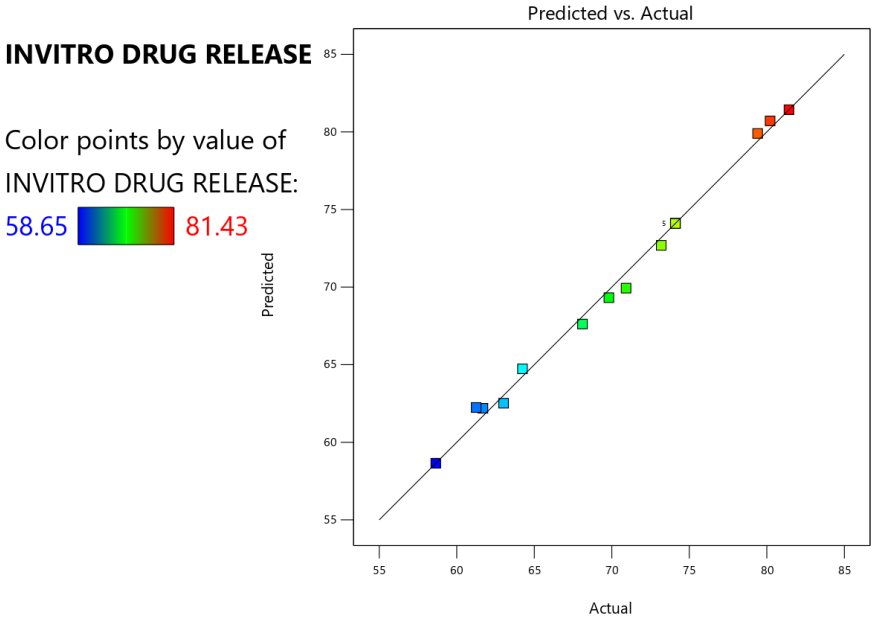


Figure 2: Linear correlation plots (A) particle size, (B) Entrapment efficiency, (C) *In-vitro* release.







3.2 Vesicle size and size distribution

It directly affects the material's property. The polydispersity index (PDI) of transferosomes was determined to be 0.189 and their mean average particle size to be 131.3 nm. (Figure 3)

Figure 3: Vesicle size and size distribution

3.3 Zeta potential measurement

Zeta potential is the attraction between the particles and the ecectroststic charge repulsion magnitude. The zeta potential was found to be -21.8mV which indicated a good stability. (Figure 4)

Figure 4: Zeta potential measurement

3.4 Surface morphology

SEM image of lamotrigine transferosomes indicated that the vesicles are spherical in shape and uniform in size. the process of sonication helped in getting vesicles of uniform size. (Figure 5)

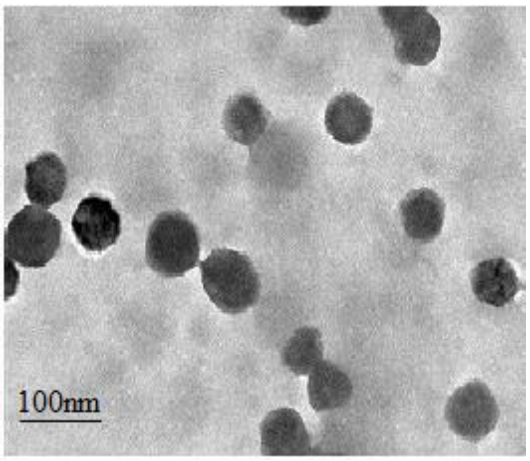


Figure 5: SEM of optimized formulation (B12)

3.5 Stability study

Stability of transferosomes stored at 4◦C shows that there is no significant change in phase separation, no evidence of sedimentation, pH of formation was under the range of 6.41 to 6.52. Vesicle size of formation was found to be between the range of 88.80 to 132.61 nm with PDI from 0.23 to 0.28 for the period of 6 months.(Table 4) Optimized lamotrigine transfersomes kept at 25◦C shows phase separation after 6 months, with slight acidic pH. Vesicle size increases to 683.74 at the end of 6 months. (Table 5) Reason for increasing particle size are aggregation of vesicles. Leakage of drug from bilayer of liposome decreases their entrapment efficiency to 38.60 %. These all data determine the suitable storage temperature for optimized transfersomes is 4º C for period of six months according to experimental results.

Table 1: Response surface methodology (RSM) used for Independent variables and responses in optimizing lamotrigine loaded transfersomes

Independent variables	**Level**		
	Low (-1)	**Medium (0)**	**High (+1)**
Phospholipid(X_1) mg	40	70	100
Cholesterol (X_2) mg	30	45	60
Sonication time (X_3) sec	120	360	600
Responses	**Preferences**		
Particle size (nm) (Y_1)	Minimizes		
Entrapment efficiency (%) (Y_2)	Maximizes		
In-vitro release (%) (Y_3)	Maximizes		

Table 2: Box-Behnken design (BBD) for optimization of lamotrigine loaded transfersomes formulations created by Design Expert Software

	Independent variables			**Dependent responses**			
FC	**RUN**	**X_1 mg**	**X_2 mg**	**X_3 sec**	**Y_1 nm**	**Y_2 %**	**Y_3 %**
B1	1	100	45	600	84.06±1.53	66.16±0.14	81.43±0.96
B2	2	70	45	360	118.87±0.32	68.79±2.33	74.1±1.65

B3	3	70	45	360	118.87±2.66	68.79±2.31	74.1±1.71
B4	4	40	30	360	88.44±2.91	41.29±0.05	64.21±2.55
B5	5	40	45	120	98.31±1.26	50.53±0.82	58.65±0.33
B6	6	70	30	600	85.12±1.22	57.05±1.12	80.21±0.93
B7	7	100	30	360	110.94±0.84	69.11±0.03	73.19±0.82
B8	8	70	45	360	118.87±1.33	68.79±2.44	74.1±0.55
B9	9	70	60	600	92.38±0.55	69.56±0.04	79.41±1.52
B10	10	70	30	120	121.13±0.49	71.27±1.66	68.11±2.55
B11	11	70	45	360	118.87±1.12	68.79±0.14	74.1±1.66
B12	12	40	45	600	100.02±0.16	69.75±1.23	80.42±1.82
B13	13	70	60	120	137.88±0.08	73.55±0.12	63.02±0.43
B14	14	70	45	360	118.87±1.12	68.79±2.54	74.1±2.45
B15	15	100	60	360	125.56±0.33	73.41±1.12	69.81±2.56
B16	16	40	60	360	97.81±0.42	51.76±1.23	61.69±0.66
B17	17	100	45	120	138.56±1.13	78.04±2.10	65.25±0.65

*All the results mentioned are reported as mean ±SD where n=3
FC: Formulation code, X_1: Phospholipid (mg) X_2: Cholesterol(mg) X_3: Sonication time(sec)
Y_1: Particle size (nm) Y_2: Entrapment efficiency (%) Y_3: *In-vitro* release (%)

Table 3: Analysis of variance of calculated model for responses

Result of the analysis of variance	Particle size	Entrapment efficiency	Invitro drug release
Sum of square	6063.32	1841.18	765.45
Df	9	9	9
Mean square	673.70	204.58	85.05
F- value	9651.41	1439.19	151.85
p-value	<0.0001	<0.0001	<0.0001
Residual			
Sum of square	0.4886	0.9950	3.92
Df	7	7	7
Mean square	0.0698	0.1421	0.5601
Lack of fit			
Sum of square	0.4886	0.9950	3.92
Df	3	3	3
Mean square	0.1629	0.3317	1.31
F- value	Non-significant	Non-significant	Non-significant
Standard deviation	0.2642	0.3770	0.7484
R^2	0.9999	0.9995	0.9949
Adjusted R^2	0.9998	0.9988	0.9884
Predicted R^2	0.9987	0.9914	0.9185
CV%	0.2432	121.0734	39.6872

Table 4: Stability parameters at 25◦C and 4◦C for 6 months Storage condition (4◦C±2◦C)

STORAGE TIME	APPEARANCE	SEDIMENTATION	PH	VESICLE SIZE(nm)	PDI	ENTRAPMENT EFFICIENCY(%)

0 day	opalescence	no	6.51	88.80	0.23	68.72
1 week	opalescence	no	6.52	92.36	0.30	66.80
2 weeks	opalescence	no	6.45	106.42	0.25	66.07
1 month	opalescence	no	6.43	118.62	0.26	65.42
3 months	opalescence	no	6.43	134.28	0.27	66.91
6 months	opalescence	no	6.41	132.61	0.28	61.36

Table 5: Storage condition (25◦C±2◦C/60 ± 5 % RH)

STORAGE TIME	APPEARANCE	SEDIMENTATION	PH	VESICLE SIZE(nm)	PDI	ENTRAPMENT EFFICIENCY(%)
0 day	opalescence	no	6.67	88.89	0.23	68.74
1 week	opalescence	no	6.39	124.83	0.36	63.46
2 weeks	opalescence	no	6.18	173.19	0.23	54.32
1 month	Turbid	yes	5.70	236.42	0.36	48.36
3 months	Milky	yes	5.40	442.33	0.43	43.86
6 months	Milky	yes	5.07	683.74	0.66	38.60

4. CONCLUSION

Using the thin film rehydration method, lamotrigine transferosomes were created with phospholipon, cholesterol, and tween 80 as the primary constituents. The formulation was optimized by 3- level Box-Behnken design response surface methology which generated a quadratic response surface. The lamotrigine-loaded transferosome optimization process was carried out using a three-level Box-Behnkem design response surface methodology. Sonication time, quantity of cholesterol and amount of phospholipid were selected as independent variables while vesicle size, entrapment efficiency and invitro release were the responses to produce a quadratic response surface. The 3D contour plots showed that as the content of phospholipid and cholesterol is increased, the size of vesicle also increased while with rise in sonication time the particle size decreased. The entrapment efficiency also increased with rise in concentration of phospholipid and the concentration of phospholipid was responsible for maximum loading of drug. As the sonication time increases the entrapment efficiency decreases. Cholesterol showed a positive effect on entrapment efficiency. The lipid bilayer's rigid structure was created by cholesterol. The invitro drug release increased with increase in phospholipid content. Phospholipid is flexible in nature and by adding of tween 80, it makes the vesicles ultra-deformable to diffuse easily. As the sonication time increased, the invitro drug release also increased. The optimized

transferosome showed vesicle size in nano range with high release and high entrapment efficiency. The prepared transferosomes will have direct effect on the brain when delivered through nose in the management of epilepsy.

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Conflict of Interest

The author states that there were no economic interests or individual relationships that could have seemed to impact the work described in this paper. There is no conflict of interest among the authors.

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