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DEVELOPMENT OF CARBAMAZEPINE MUCOADHESIVE MICROEMULSIONS FOR BRAIN TARGETING: PHARMACODYNAMIC EVALUATION

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ABSTRACT

Objectives: *Carbamazepine (CBZ), an anticonvulsant drug has low oral bioavailability and gastrointestinal side effects, in order to overcome these problems, the present study was to development and pharmacodynamic evaluations of carbamazepine mucoadhesive microemulsions (MME) for brain targeting via nasal route.* ***Methods:*** *Based on solubility, oleic acid, Labrasol and Transcutol P were selected as oil, surfactant and co-surfactant. Pseudo ternary phase diagrams were constructed to identify microemulsion region. A three factor three level Box Behnken design was used to optimize formulation. The micro emulsions (ME) were also evaluated for size, PDI, zeta potential, flux, pH, viscosity, content, surface morphology. Chitosan was added to the optimized ME at 0.5% concentration as permeation enhancer. Ex-vivo permeation studies were performed on excised porcine nasal mucosa using Franz diffusion cells. Histopathological changes in mucosa were studied. Pharmacodynamic activity of ME, MME, drug solution (D.S) and i.v CBZ solution were evaluated by Maximal Electroshock seizures (MES) method in male Wistar rats.* ***Results:*** *Optimized MME, composed of oleic acid (5%), Surfactant mixture (53.78%) water (45%) and chitosan (0.5%) showed mean globule size 97.43nm, PDI 0.213 and zeta potential +16.32. MME showed significantly ($p<0.001$) high flux of 651.53 µg/cm^2/h compared to D.S (101.8 µg/cm^2/h) and ME18 (551.23µg/cm^2/h). The reduction seizure recovery time of MME was significantly ($p<0.001$) high compared to MEs and D.S by i.v route.* ***Conclusion:*** *The efficacy of ME and MME formulations via nasal route for brain targeting in comparison to i.v route was improved.*

KEY WORDS

Box Behnken design, Carbamazepine, Chitosan, Epilepsy, Intranasal microemulsion, Maximal electroshock seizures.

INTRODUCTION

Carbamazepine (CBZ), an anticonvulsant drug is the drug of choice in the treatment of partial and secondarily generalized seizures. CBZ is also effective in trigeminal neuralgia and diabetic neuropathy [1,2]. It is a poorly water-soluble drug (0.17mg/ml) with low bio availability of less than 50%. Absorption of tablets by oral route is slow and irregular [3]. Oral administration of CBZ therapy is associated with adverse effects such as drowsiness, dizziness, headaches and migraines, motor coordination impairment, nausea, vomiting**.** In order to overcome gastro intestinal side effects, liver toxicity other side effects and also to increase bioavailability of carbamazepine using less dose of CBZ, present work of CBZ microemulsions for brain targeting via nasal route was taken up.

Nasal route has several advantages like rapid onset of therapeutic action, lower doses, avoidance of liver and

gastrointestinal metabolism, noninvasive and brain targeting via olfactory pathway, self-medication, and improved patient compliance [4,5]. Drug delivery to the central nervous system through olfactory path way bypassing Blood Brain Barrier (BBB) is studied by number of researchers and reported improvement in bioavailability of drugs [6,7,8]. Zhang et al developed nimodipine-loaded microemulsion system for brain targeting [9]. Intranasal delivery of sumatriptan mucoadhesive microemulsion was studied by Vyas et al and proved most efficient in the treatment of migraine [10]. Most important limiting factors for nasal drug delivery were nasal mucociliary clearance [11]. Mucoadhesive preparations have been reported to increase the contact time between the nasal mucosa and dosage form and reduce rapid nasal clearance [12]. Micro Emulsions (MEs) were thermodynamically stable having small globule size of 10-100 nm composed of water, oil and surfactant. MEs of o/w are suitable for poorly soluble drugs due to their solubilization capacity. Many researchers reported increased bioavailability of MEs and Mucoadhesive Micro Emulsions (MME) with Chitosan [13,14,15]. The present work was development and evaluations of ME and MME of CBZ by Box-Behnken design [16]. The antiepileptic efficacy of formulations was measured by Maximal Electroshock seizures (MES) in comparison to intravenous route [17].

MATERIALS & METHODS

Carbamazepine was procured as a gift sample from Novartis, Hyderabad, India. Oleic acid, sesame oil, sunflower oil was purchased from Hi Media, (Mumbai, India). Capmul MCM was from Abitec Corporation Ltd. (Mumbai, India). Labrafil M 1944 CS, Lauroglycol 90, Labrasol from Gattefosse Pvt. Ltd. (Mumbai, India). Chitosan (CH;low molecular weight), Transcutol-P were from Sigma-Aldrich (Bangalore, India). Propylene glycol, Polyethylene glycol 400 Tween 80 were purchased from S.D Fine Chemicals (Mumbai, India). All other chemicals were of analytical reagent grade.

Animals

Male Wistar rats, weighing between 220-250 g were obtained from Sainadh agencies, Hyderabad. The animal study protocol was approved by "Committee for the Purpose of Control and Supervision of Experiments on Animals" (CPCSEA) and Institutional Animal Ethics Committee, wide number IAEC/46/ UCPSc /KU/2016. Animals were maintained under standard laboratory diet, water *ad libitum* and acclimatized to laboratory conditions (22 ± 2°C, 12-hour light-dark cycle and 55% - 65% humidity) one week prior to initiation of experiments.

Spectrophotometric determination, UV Method

CBZ was dissolved in sufficient quantity of methanol, made up with PBS pH 6.4 and the solutions of concentrations between of 2 to 20 µg/ml solutions were prepared. The absorbance of the samples was measured at 284 nm and calibration curve of CBZ was plotted. The standard graph in methanol was also plotted similarly [18].

Solubility studies

Solubility studies were performed by equilibrium solubility method at room temperature, by adding excess amount of drug into screw caped vials containing solvent. The samples withdrawn at 24h, 48h intervals were centrifuged at 4000rpm for 20min [19]. The supernatant was filtered through a 0.45µm filter, diluted suitably with methanol and the content was determined by UV spectroscopy.

Pseudo-ternary Phase diagrams

Pseudo-ternary phase diagrams were constructed to determine the microemulsion region using CHEMIX software. To the homogenous mixture of oil, surfactant and cosurfactant, water was added drop by drop under gentle stirring until it turned to turbid and the weight of water added was determined. Phase diagrams were constructed by varying oil and Smix (surfactant and co surfactant mixture) ratio from 1:9 to 9:1. The composition of Smix also varied in the ratios of 1:1, 2:1, 3:1, 1:2 and 1:3 [14].

Preparation of micro emulsions (ME) and mucoadhesive microemulsions (MME)

The CBZ-loaded micro emulsions were prepared by phase titration method. Predetermined amount of CBZ was added to the mixture of oil phase (oleic acid) and Smix, vortexed continuously for 15minutes. Required amount of distilled water was added dropwise to the above mixture and stirred continuously for 5 minutes until transparent and homogeneous micro emulsion was produced [8,14]. Chitosan was dissolved in minimum volume of 1 % acetic acid solution and added to the optimized microemulsion formulation at 0.5% concentration and stirred continuously to obtain clear formulation [20,21]. CBZ-SDC was prepared by addition of 0.5% of Sodium deoxy cholate to the microemulsion.

Preparation of drug solutions (D.S)

Carbamazepine solution for nasal administration was prepared by dissolving the 20 mg of drug in 1mL mixture of Poly ethylene glycol 400, water and ethanol in 60:30:10 ratio. Carbamazepine solution for iv injection was prepared at 2mg/ml concentration by dissolving in mixture of PEG 400 and normal saline (20: 80). It was sterilized through 0.22 µm membrane filters.

Characterization of Microemulsions

Globule size, Polydispersity Index (PDI) and Zeta potential

The globule size, PDI and zeta potential were determined using Zeta Sizer (Nano-ZS 90, Malvern Instruments Ltd.UK) on 100 times diluted sample.

The pH of micro emulsion (ME) and muco adhesive micro emulsions (MME) were determined using calibrated digital pH meter at room temperature. Viscosity of the ME and MME were determined using Brookfield viscometer (Brookfield, model No. LVDV-E *8542328*, USA) [13].

Drug Content

Accurately weighed micro emulsion was suitably diluted with methanol and analyzed for the drug content using UV method at 284nm. Microemulsions without drug were similarly diluted were used as blank.

Ex-vivo permeation studies [22]

An Ex-vivo permeation studies were conducted on porcine nasal mucosa using vertical type of Franz diffusion cells. The nose of porcine was collected from the slaughter house and kept in Krebs bicarbonate ringer's solution, used within 1 hour of isolation. The nasal mucosa was isolated carefully using scalpel blade and blunt forceps. It was rinsed with PBS pH 6.4 and allowed to equilibrate in PBS pH 6.4 for 30 min at room temperature. Nasal mucosa was sandwiched between the receptor and donor compartment. The donor chamber was replaced with formulation and the receptor was filled with fresh buffer. Samples were withdrawn at regular intervals up to 8 h and replaced with fresh medium. The samples were analyzed by UV method. The cumulative amount of drug permeated at different time points was calculated using the following formula.

$$Q = [\, C_n.V + \sum_{i=1}^{n-1} C_i S \,]$$

Where

Q = Cumulative amount of drug permeated

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

V = Volume of Franz diffusion cell

n-1

$\sum_{i=1}^{n-1} C_i$ =Sum of concentration of (µg/mL) determined at sampling intervals 1 through n-1

S = sampling volume.

The cumulative amount of drug permeated across the nasal mucosa was plotted against time. The flux at steady state (J_{ss}, µg/cm^2/h) was calculated by dividing the slope of the linear portion of the curve by the effective surface area of nasal mucosa (3.8cm^2). Permeability coefficient (Kp, cm/h) was calculated by dividing Steady state flux by the initial concentration of drug in the formulation (J_{ss} /C_0). Enhancement ratio (ER) was calculated from the ratio between flux at steady state (J_{ss}) of the respective formulation and J_{ss} of the drug solution.

Experimental design [16]

A three factor, three level Box-Behnken statistical design generated by Design- Expert software, Version 11.0.2. Stat-Ease Inc., MN was used for optimization of formulation and to determine relationship between factors and responses. Independent variables were oil-X_1, Smix-X_2 and water-X_3 and the dependent variables were size -Y_1, flux-Y_2 and zeta potential-Y_3.

The nonlinear quadratic model is given as

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

Where Y_i is the measured response at each factor level combination, b_0 is an intercept; b_1 to b_{33} are the regression coefficients; and X_1, X_2, and X_3 are the coded levels of independent variables. The terms X_1X_2 and X^2_i (i = 1, 2 or 3) represent the interaction and quadratic terms respectively. Analysis of variance (ANOVA) was performed to determine the significant of the main and interactive effects of factors on responses. The independent variables were used at low, medium and high levels. The constraints chosen were minimum size, maximum flux and within the range of zeta potential.

Check point analysis and optimization

The experimental design was validated by preparing six experimental formulations given by feasibility search and the responses were measured. The percent prediction error was calculated from the difference

between measured and predicted values. The optimum levels of the factors were determined from the polynomial equations generated by software.

Scanning electron microscopy (SEM)

The morphology of optimized microemulsion and muco adhesive micro emulsion were studied by scanning electron microscope (JSM-6510LA, JEOL, Indonesia). The formulation adhered on to the carbon-coated metallic stub was sputter coated with Platinum coating machine (JFC-1600 Auto fine coater, JEOL) and the imaging was carried out under high vacuum.

Nasal cilio-toxicity studies

Freshly excised porcine nasal mucosa were treated with PBS pH 6.4 (negative control), isopropyl alcohol (positive control), microemulsion and mucoadhesive microemulsion for 1 h separately. After 1 h, the mucosa were rinsed with PBS pH 6.4 and preserved in 10% formalin for the preparation of slides using microtome technique. The histopathology of mucosa was stained with hematoxylin, eosin and studied under an optical microscope and the images were taken [23].

Stability study

The optimized micro emulsion formulation and CBZ-MME were subjected to stability study for a period of three months at room temperature. At monthly intervals samples were subjected to centrifugation cycle, characterized for size, zeta potential and drug content [24].

Pharmacodynamic study

Maximal Electroshock seizure (MES) model was used to evaluate antiepileptic activity [17]. Male Wister rats weighing between 200 - 250 gms were selected for the study. Rats were divided into 6 groups each containing 6 animals. Group 1 received the placebo, Group 2 -Drug solution, Group 3- ME18, Group 4- CBZ-MME, Group-5 CBZSDC (Sodium Deoxy Cholate) and Group-6 CBZ *i.v* preparation. All the preparations were administered intranasally using rat nasal catheter of Impel Neuropharma (Figure 1) to the anaesthetized rat.

Figure 1: Administration of drug by using IMPEL intranasal catheter

The rat was anaesthetized by exposing the rat to ether vapors in a chamber, until the rat becomes just unconscious such that within 3-4 minutes the rat will become normal. The carbamazepine dose was 2 mg/ kg body weight (20-25 µL). Group-6 received sterile CBZ solution through tail vein at the same dose of 2mg/kg. Five minutes after treatment the rats were subjected to shock using 150 mA currents for 0.2 sec delivered via ear electrodes using electro convulsiometer and different phases of seizures were recorded.

RESULTS AND DISCUSSIONS

Calibration curves of Carbamazepine

The calibration curves obtained in methanol and PBS pH 6.4 showed good linearity with correlation coefficient values of above 0.999.

Solubility studies

Table 1: Solubility studies of carbamazepine

S.No	Exciepients	Solubility (mg/mL)
1	Oleic acid	43.23 ± 1.71
2	Capmul MCM	28.47 ± 2.16
3	Lauroglycol™ 90	19.21 ± 2.13
4	Iso propyl myristate	13.11 ± 1.56
5	Labrafil M1225	11.32 ± 2.35
6	Soyabean oil	7.11 ± 1.57
7	Sesame oil	4.21 ± 1.32
8	Sunflower oil	3.27 ± 1.11
9	Labrasol	33.22 ± 1.23
10	Tween 80	13.56 ± 1.35
11	Tween 20	11.21 ± 1.12
12	Transcutol P	53.21 ± 0.45
13	PEG 400	24.31 ± 1.63
14	Propylene glycol	15.11± 1.63

Data shown as Mean ± SD

Solubility of CBZ in various oils, surfactants and co-surfactants was shown in Table 1. CBZ has maximum solubility (43.23 ± 1.71mg/mL) in oleic acid among the oils. High solubilization potential of oil is very important for ME for attaining the larger microemulsion region in ternary plots [25]. Labrasol was selected as surfactant and Transcutol P as co-surfactant based on their solubilizing power. Combination of nonionic surfactants mixture in a definite proportion is crucial for the stability and globule size of micro emulsion. Co-surfactant imparts sufficient flexibility to the interfacial film [25,26,27].

Pseudo-ternary phase diagrams

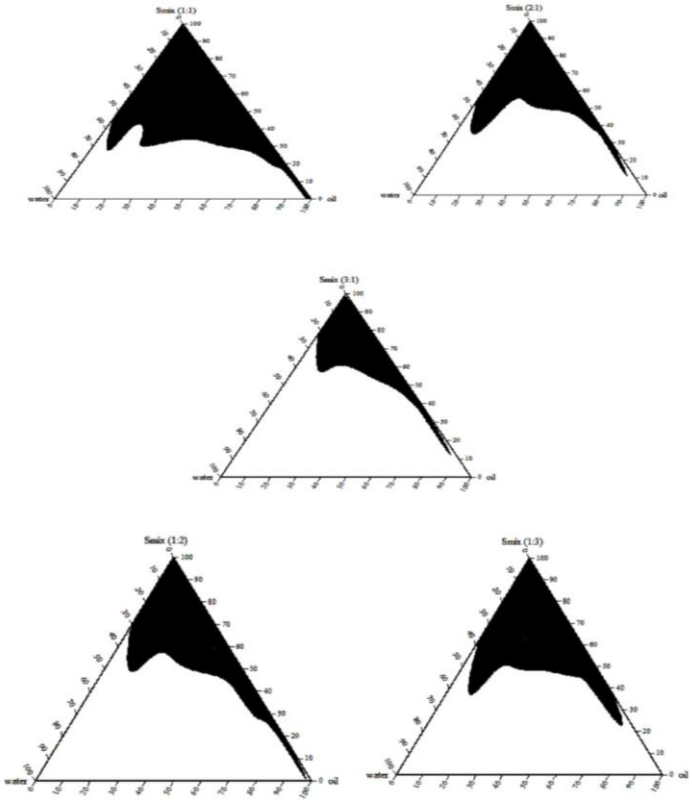

Figure 2: Pseudo-ternary phase diagrams of micro emulsions composed of oil (Oleic acid), surfactant mixture (Smix; Labrasol : Trascutol P) and water. Shaded area represents the microemulsion region.

The Pseudo-ternary phase diagrams constructed with different weight ratios of Labrasol: and Transcutol P (Smix; 1:1,1;2,1:3,2:1) were shown in Figure 2. Shaded area represents the microemulsion region. It was observed that maximum microemulsion region was obtained at 1:1 ratio of Labrasol and Transcutol P (Smix). Hence 1:1 ratio of Smix was selected for formulation of microemulsions.

Formulation optimization by experimental design

Table 2. Compositions of formulations generated by Box Behnken design, optimized formulations and the measured responses

Formulation	X_1 Oil (mg)	X_2 Smix (mg)	X_3 Water (mg)	Y_1 Size (nm)	Y_2 Flux (µg/cm²/h)	Y_3 Zeta potential (mV)	PDI	ER	Kp *10⁻³/cm
ME1	10	60	40	184	132.32	-27.83	0.23	1.22	6.62
ME2	7.5	45	50	165	323.32	-27.31	0.20	2.98	16.17
ME3	7.5	52.5	40	141	346.76	-29.74	0.22	3.20	17.34
ME4	10	52.5	50	213	183.22	-26.84	0.15	1.69	9.16
ME5	5	52.5	30	83	503.45	-36.42	0.18	4.64	25.17
ME6	7.5	60	50	123	252.21	-31.21	0.18	2.33	12.61
ME7	7.5	60	30	103	210.52	-31.23	0.20	1.94	10.53
ME8	10	45	40	234	153.42	-25.3	0.27	1.42	7.67
ME9	7.5	52.5	40	135	350.54	-29.11	0.27	3.23	17.53
ME10	5	52.5	50	95	541.24	-34.52	0.10	4.99	27.06
ME11	5	60	40	65	423.43	-36.83	0.15	3.91	21.17
ME12	7.5	45	30	152	294.32	-28.63	0.18	2.71	14.72
ME13	7.5	52.5	40	134	362.34	-29.8	0.16	3.34	18.12
ME14	7.5	52.5	40	139	357.02	-29.5	0.24	3.29	17.85
ME15	5	45	40	112	478.82	-33.43	0.12	4.42	23.94
ME16	10	52.5	30	194	163.42	-27.85	0.14	1.51	8.17
ME17	7.5	52.5	40	137	354.63	-29.01	0.28	3.27	17.73
ME18	**5**	**53.78**	**50**	**90.16**	**551.25**	**-35.18**	**0.11**	**5.08**	**27.56**
MME	**5**	**53.78**	**50**	**97.43**	**651.63**	**+16.32**	**0.21**	**6.01**	**32.58**

Note: Carbamazepine20mg, Smix:Labrasol : Transcutol P1:1,ER: Enhancement Ratio. ME: Microemulsion. MME: Mucoadhesive microemulsion (0.5% Chitosan), PDI:Polydispersity index, Kp : Permeation coefficient

A three-factor, three-level Box–Behnken statistical experimental design was used to optimize the formulation variables. Based on the results of pseudo-ternary phase diagrams, oleic acid at 5-7.5-10, Smix (1:1) at 45-50-60 and water at 30- 35-40 were selected as three levels of each factor. The measured responses for 17 experimental runs were given in Table 2. The contour plots drawn using Design -Expert software were shown in Figure 3.

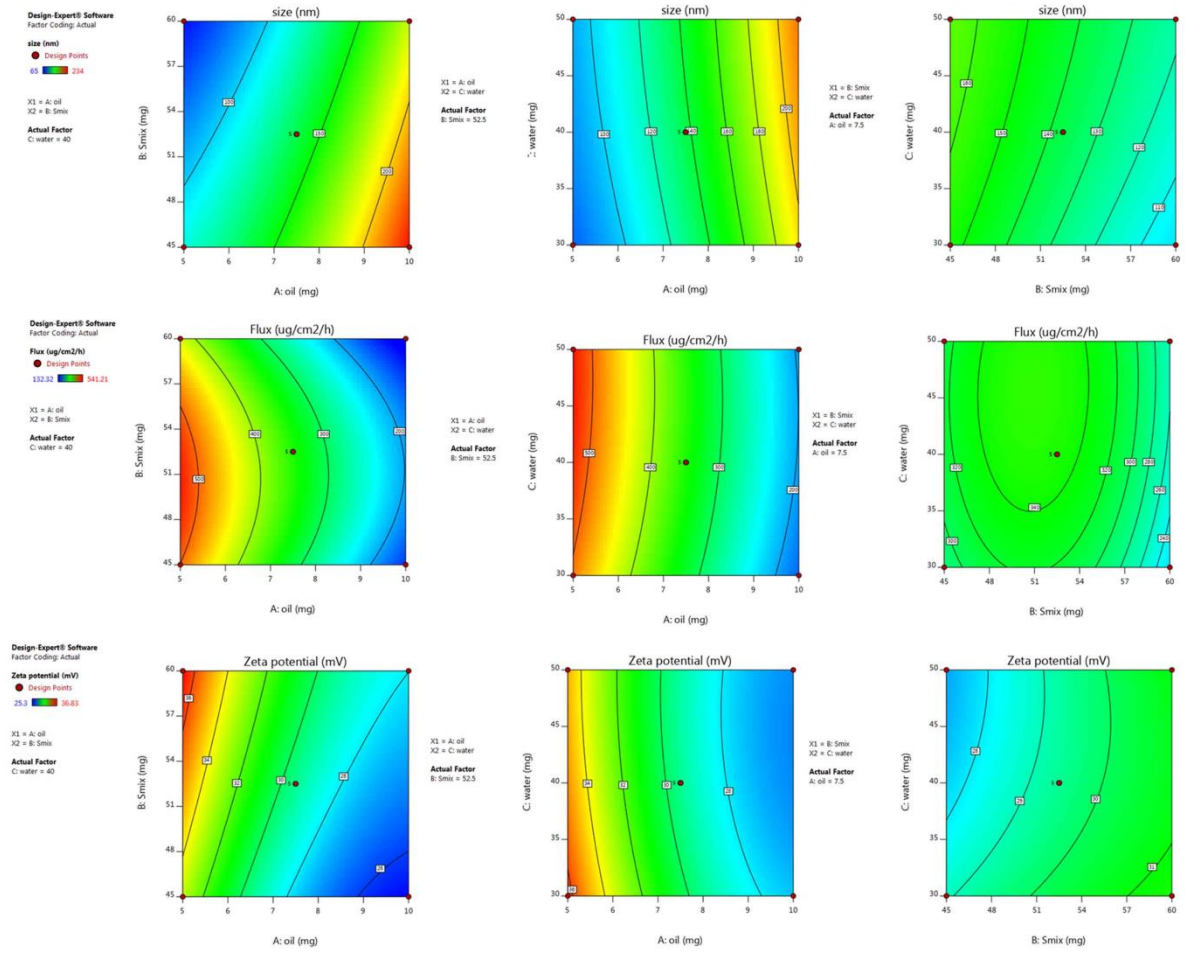

Figure 3: Contour plots represent the effects of oil, Smix and water on size, flux and zeta potential.

Characterization of microemulsions

The measured values of Size, PDI and Zeta potential of the formulations were shown in Table 2. The mean globule size of micro emulsions varied between 65 nm to 213 nm, PDI between 0.107 to 0.271 and Zeta potential between -25.3 to -36.42. mV. The pH of the microemulsion formulations was between 5.8 to 6.1. The viscosity of the optimal ME formulations was 78 to 84 cP and CBZ-MME viscosity was found to be 134 cP. To overcome the muco ciliary clearance, chitosan, a muco adhesive, cationic, biocompatible polymer was added at 0.5% level to the optimized microemulsion. The drug content of formulations was within limits. Polydispersity index value below 0.2 indicates uniform globule size distribution of all formulations.

Ex-vivo permeation studies

The ex-vivo permeation profiles of microemulsion formulations, optimized microemulsion (ME18), CBZ-MME, CBZ-SDC and D.S were shown in Figure 4&5 and flux values in Table 2.

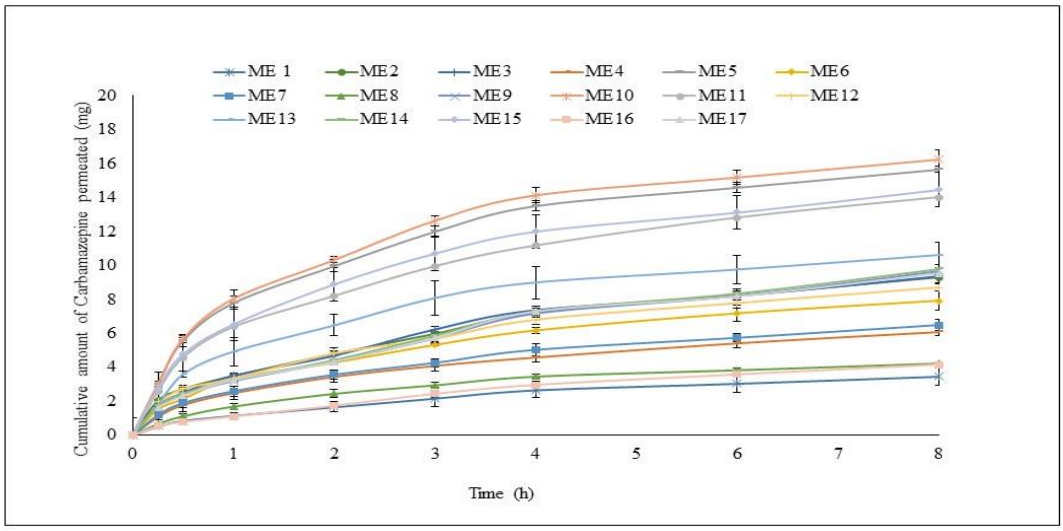

Figure 4: *Ex-vivo* permeation profiles through porcine nasal mucosa of CBZ microemulsion formulations, generated by Box-Behnken design

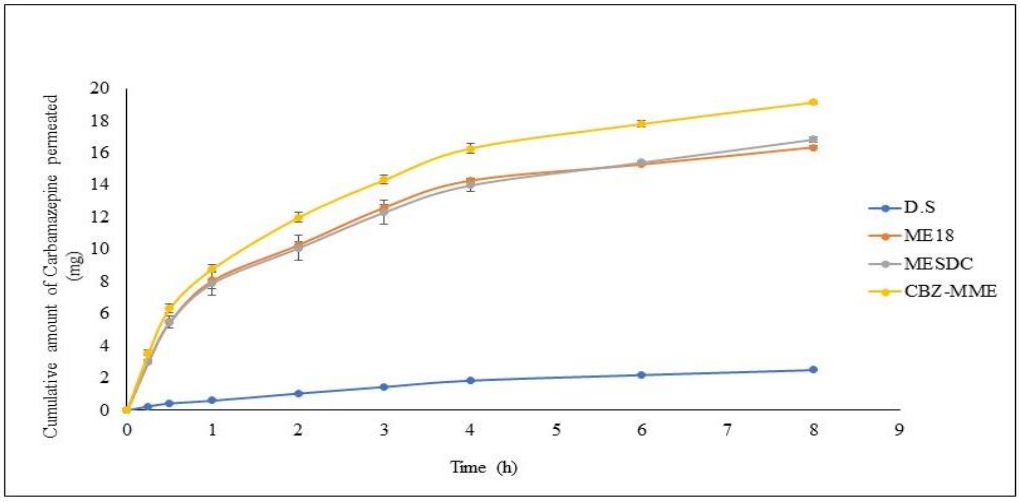

Figure 5: *Ex-vivo* permeation profiles through porcine nasal mucosa of D.S, ME18, CBZ-MME and CBZ-SDC

Flux values were observed between 131 to 541 µg /cm^2 /h for ME1 to ME 17 formulations. CBZ-MME showed maximum flux of 651.63 ± 16.94 µg/cm^2/h which was significantly high compared to D.S (**P<0.001) and ME18 (*P < 0.01). The enhancement ratio of CBZ- MME was 6 folds when compared to drug solution and 1.18 folds compared to micro emulsion (ME18). The permeation of carbamazepine from micro emulsions through porcine nasal mucosa was influenced by the microemulsion compositions and the results were in agreement with previous reports. From the results of ex-vivo permeation studies, the role of chitosan in improving the permeation of drug was proved [28].

Statistical analysis of data

The polynomial equations generated by design expert software taking coded values of factors were shown in equations1, 2 and 3 respectively, which described the individual, interaction and the quadratic effects of the selected independent variables. The significance of effects was analyzed using ANOVA for the responses – size, flux and zeta potential (Table 3). The model validity in predicting the responses was verified.

Table 3: ANOVA and Regression values for quadratic model

Parameter	Source	D.F	S. S	M.S	F- Value	p >F value	S. D	%C. V	Adequate precision	R^2	Adj R^2	Pred R^2	PRESS
Size	Model	9	33093.73	3677.08	461.28	< 0.0001	2.82	1.99	75.9665	0.9983	0.9962	0.9874	419.25
	Residual	7	55.8	7.97									
	Lack of fit	3	23	7.67	0.935	0.502							
	Pure error	4	32.8	8.2									
Flux	Model	9	2.44E+05	27126	79.25	< 0.0001	18.5	5.83	29.624	0.9903	0.9778	0.8899	27143
	Residual	7	2395.84	342.26									
	Lack of fit	3	1620.75	540.25	2.79	0.1736							
	Pure error	4	775.1	193.77									
Zeta potential	Model	9	173.5	19.28	140.25	< 0.0001	0.3708	1.22	40.2754	0.9945	0.9875	0.9545	7.94
	Residual	7	0.9622	0.1375									
	Lack of fit	3	0.4455	0.1485	1.15	0.4312							
	Pure error	4	0.5167	0.1292									

Note: ANOVA: analysis of variance, D F: Degrees of Freedom, SS: Sum of squares, M.S: Mean Squares. F: Fischer's ratio, P: Probability factor, C.V: Coefficient of variation. Adeq precis: Adequate precision, Adj: Adjusted, Pred: Predicted, PRESS: Predicted Residual Error Sum of Squares. ***ANOVA for the responses indicated that the quadratic model was found to be significant and valid for each of the responses Size (Y_1), Flux(Y_2) and Zeta potential (Y_3) (P<0.0001).

The adjusted, predicted, model R squared values were near to one and the difference between adjusted and predicted values was lowest for quadratic model. The Predicted Residual Error Sum of Squares (PRESS) value of quadratic model is low, suggesting that the model is validated in predicting responses. F value and P>F value indicate the model fitness and significance of each factor on response. The Model F-value was found to be high for all the three responses. P-value less than 0.05 was considered as significant. The Lack of Fit F-value is not significant relative to the pure error indicating fitness of the model. High values of adequate precision proved the fitness of model in predicting the optimal values of factors. Adequate precision is a used to measure of signal to noise ratio. The value above 4 indicates adequate precision.

Effect of formulation variables on responses

The polynomial equations in coded factors for the responses size, flux and zeta potential were shown in Eq 1, 2 and 3 respectively. The positive sign before the factor represents synergistic effect and the negative sign denotes antagonistic effect between the factor and the response. The interaction between the factors and responses were further represented graphically by contour plots.

Size (Y_1) = + 137.20 + 58.75 X_1 – 23.50X_2 + 8.00X_3 - 0.75X_1X_2 + 1.75 $X_1X_3$0 – 1.75 X_2X_3
+ 11.3 X_1^2 + 0.5250X_2^2 - 1.98X_3^2 (1)

Flux (Y_2) = + 347.90 -164.32 X_1 – 28.92X_2 +16.03X_3 +8.57 X_1X_2 - 4.49X_1X_3 + 3.17X_2X_3
+13.41X_1^2 – 64.32X_2^2 +2.49X_3^2 (2)

Zeta potential (Y_3) = +29.43 - 4.17 X_1 + 1.55X_2 - 0.5313X_3 – 0.2175 X_1X_2 +0.2225X_1X_3 +0.3250 X_2X_3 + 1.61 X_1^2 - 0.1985 X_2^2 + 0.3615X_3^2 (3)

Size (Y_1): The model terms X_1, X_2, X_3, X_1^2 were significant, influencing the size of the globule. The oil (X_1) has greater positive influence on the globule size. Smix (X_2) has a negative effect on particle size. Increase in oil content, increased the size of globules within the range studied (5-10mg). The observation was in compliance with the previous reports [29]. Influence of water on size was not significant.

Flux (Y_2): X_1, X_2, X_3, X_2^2 are significant model terms. The equation 2 and contour plots indicate negative influence of oil and Smix on flux. Increase in oil quantity significant reduction in flux is observed which could be due to increase in size of oil globules [29]. At high concentrations of Smix, thermodynamic activity was decreased which lowers the flux. Water had positive effect on flux.

Zeta potential (Y_3): In this case X_1, X_3 are negative terms and X_2, X_1^2 are positive terms
significant (eq -3). As Oil and water concentration increases, the size of the globule also increases there by the zeta potential is decreased. Smix concentration increases the zeta potential value within in the range studied. This could be due to decrease in size at high Smix concentrations [29].

Check point analysis

The model was validated for accurate prediction of responses by check point analysis. Predicted values of size, flux, zeta potential were compared with measured values and the prediction error was calculated. The prediction error was below 5% which confirms the validity of Response Surface Quadratic model.

Optimization

The optimized CBZ microemulsion formulation was selected based on the desirability factor near to 1 by exhaustive feasibility and grid search. The desirability of optimized microemulsion was 0.961. The composition of the optimized formulation ME 18 was 5%oleic acid, 53.78% Smix (Labrasol and Transcutol P, 1:1) 50% water. The measured values for size, flux and zeta potential of ME 18 were found to be 90.16 nm, 551.25 µg /cm^2 /h and -35.18mV respectively. CBZ - MME showed globule size of 97.43 nm, flux 651.63 µg /cm^2 /h and zeta potential +16.32mV and PDI ranged in between 0.23 to 0.28. The results were proved that role of chitosan in the formulation. Chitosan, a linear polysaccharide extracted from chitin and biocompatible natural polymer, safe, nontoxic, easily binds to mammalian and microbial cells [30]. It is a mucoadhesive agent and permeation enhancer. It has positively charged and act by electrostatic interaction with negatively charged mucosal surface [31]. The results were in agreement with the earlier findings. Aspden et al. 1996 reported nasal absorption of insulin with chitosan as a bio adhesive polymer and also Illum et al 1994 also proved that, Chitosan as a 0.5% solution, has increased seven times higher AUC of insulin in sheep's [32].

Nasal ciliary toxicity: Nasal mucosa treated with formulations ME 18 and CBZ-MME did not show any sign of damage of mucosal epithelial layer indicating the safety of formulation on mucosa (Figure 6). The mucosa treated with isopropyl alcohol showed complete disruption of epithelial layer and cilia.

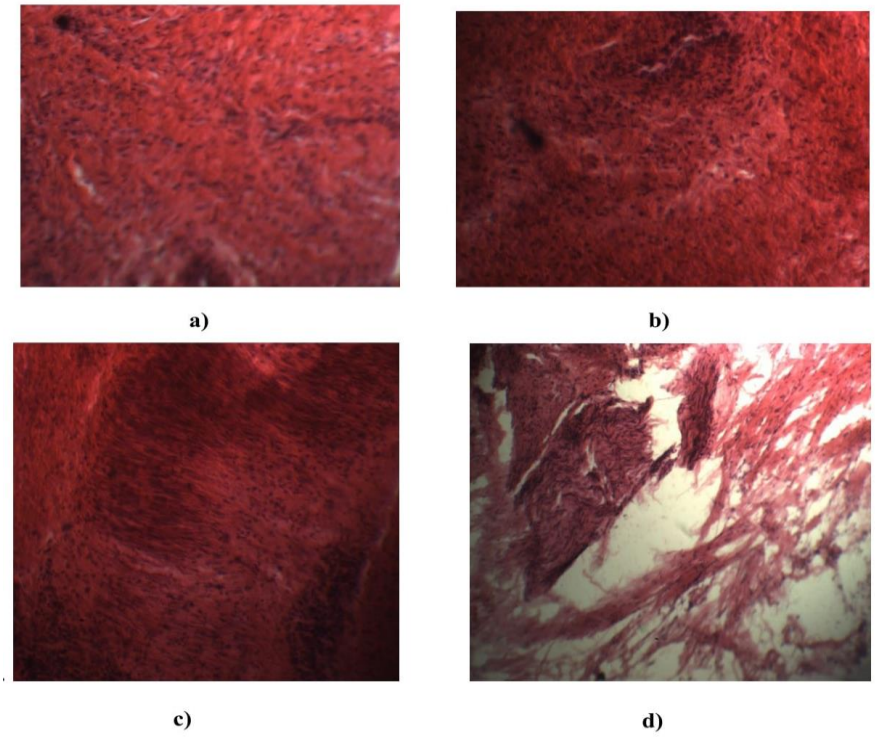

Figure 6: Histopathology changes of porcine nasal mucosa after treatment with a. PBS pH 6.4 b.ME18 c. MME d. Isopropyl alcohol

SEM Study: The SEM image of micro emulsion (ME18) and optimized formulation (CBZ-MME) contained spherical shaped globules (Figure 7).

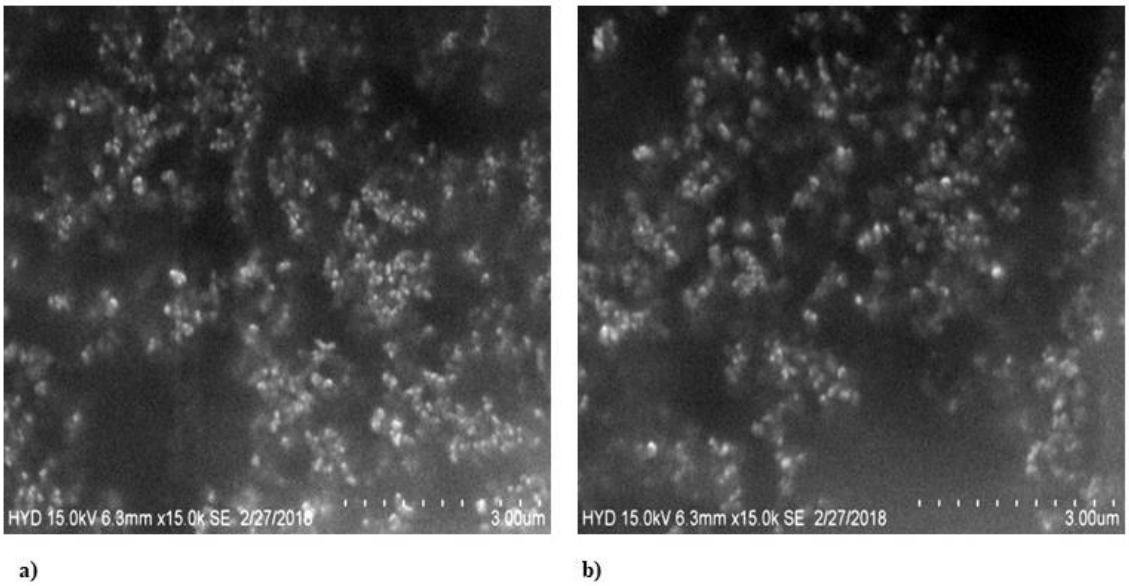

Figure 7: Scanning Electron Microscopy(SEM) images of optimized formulations ME18 and MME

Stability studies

The Size, PDI and Zeta potential of CBZ-MME samples after 3 months storage at room temperature were found to be 115.16 nm, 0.213 and +14.18mV. Initial day size, Zeta potential, PDI and drug content were found to be 91.44nm, 0.13, +15.32mV 99.12. The results concluded that the formulation was stable during the

study. There is no significant change in size, zeta potential and PDI and drug content. The size of the emulsion increased 25% of initial value.

Pharmacodynamic studies

MES induced convulsions were divided into five phases such as, Phase of tonic limb flexion, tonic limb extension, clonic convulsions, Stupor and Recovery or death. Immediate severe After flexion, tonic phase (Extension phase) was observed which was characterized by maximal extension of the anterior and posterior legs. At the end of tonic phase, clonic phase starts which was characterized by paddling movement of the hind limb and shaking of body. During stupor phase which was observed after tonic and clonic phase rat remained silent without any movement. Abolition or decrease in the duration of extensor phase should be taken as an index of anti-epileptic activity. The duration of these phases were recorded and shown in Figure 8**.**

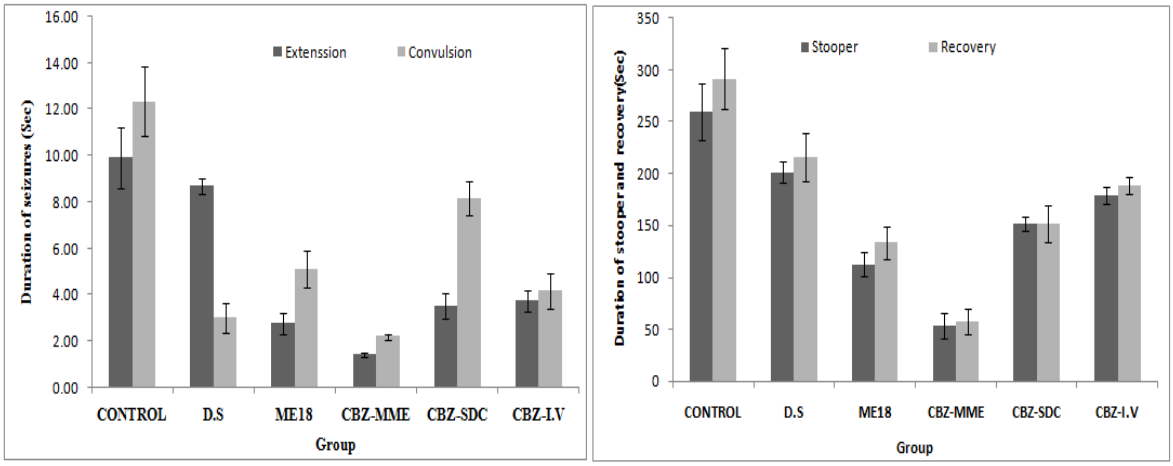

Figure 8: Pharmacodynamic evaluations of CBZ formulations in MES induced seizures in rats (CBZ MME significantly($p<0.001$) high vs D.S & i.v CBZ.)

Figure 8 represents the Extension and clonus phases and Figure 9 represents the stooper and recovery phases of different groups. The results clearly indicated that CBZ-MME was having lesser intensity of seizures and rapid recovery from seizures and significantly difference ($p<0.001$) was observed in rats treated with D.S, ME18 and CBZ solution (*i.v*).

CONCLUSION

The present study demonstrated the use of a Box–Behnken statistical design in optimization of microemulsion formulations. *Ex-vivo* permeation studies and the antiepileptic activity of microemulsion formulations and mucoadhesive microemulsion formulations of carbamazepine via nasal route were significantly high compared to *i.v* injection. The antiepileptic activities of mucoadhesive microemulsions were significantly high when compared to ME, CBZ drug solution and CBZ *i.v.* solution. Further clinical studies are required to prove this hypothesis

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