

## DESIGN AND DEVELOPMENT OF CARBAMAZEPINE MUCOADHESIVE MICROEMULSION FOR INTRANASAL DELIVERY: AN EX-VIVO STUDY.

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### ABSTRACT

Carbamazepine has got poor brain uptake due to its poor aqueous solubility (0.0061 mg/ml) and very high plasma protein binding (>78%) so the aim of this study was to develop its microemulsion formulation to enhance the aqueous solubility and to accomplish the intranasal delivery of the drug to brain. Carbamazepine microemulsion (CME) was prepared using Labrafil M 1944 CS as oil, Accenon CC as surfactant and Transcutol P as co-surfactant by titration method. Pseudo ternary phase diagram was constructed to determine the microemulsion existing zone. Optimized microemulsion was characterized for their % Transmittance, globule size, zeta potential, and % drug content. Mucoadhesive microemulsion containing Carbamazepine (CMME) was prepared using Carbopol 934. Nasal ciliotoxicity studies were carried out for CME and CMME using in situ toad palate model. Developed formulations were subjected for stability study as per the ICH guidelines. Drug release from the formulations was carried out through excised sheep nasal mucosa using modified Franz diffusion cell. CME was transparent with mean globule size  $29.6\text{nm} \pm 1.56$  and zeta potential  $-15.8\text{ mV} \pm 0.89$ . % drug content in CME and CMME were found out to be  $99.3 \pm 0.6$  and  $99.6 \pm 0.4$ . Stability study revealed that CME and CMME were stable for 6 months. Scanning electron microscope investigation revealed that no marked damage on the sheep nasal mucosa after nasal application of CME and CMME for 1hr. Results from 8 hrs ex-vivo release study revealed that CME and CMME showed 29% and 17.6% higher drug release than that of plain drug solution of Carbamazepine (PDSC). Based on the results, CMME and CME were stable and can target the drug to brain through intranasal route and thus can play as an alternative for conventional dosage form.

**Keywords:** Intranasal delivery, Microemulsion, Carbamazepine, Ciliotoxicity study.

### INTRODUCTION

Epilepsy is a common chronic neurological disorder characterized by recurrent unprovoked seizures. These seizures are transient signs and/or symptoms of abnormal, excessive or synchronous neuronal activity in the brain. About 50 million people worldwide have epilepsy, with almost 90% of these people being in developing countries. Epilepsy is more likely to occur in young children or people over the age of 65 years; however it can occur at any time<sup>1</sup>. Carbamazepine (CBZ) is an anticonvulsant and mood stabilizing drug used primarily in the treatment of epilepsy and bipolar disorder, as well as trigeminal neuralgia. It is also used off-label for a variety of indications, including attention-deficit hyperactivity disorder (ADHD), schizophrenia, phantom limb syndrome, paroxysmal extreme pain disorder, and post-traumatic stress disorder. Majority of the epileptic patients not only suffer from gastric stasis but have also been associated with severe nausea and vomiting which may lead to erratic absorption of the drug from the gastrointestinal tract thus may result into ineffective treatment<sup>2</sup>. Because of having poor water solubility, its absorption is dissolution rate limited, which often results in irregular and delayed absorption. Reports in the literature reveal that Carbamazepine has got variable oral bioavailability ranging from 69%-74% due to hepatic first-pass metabolism principally through CYP3A4. Carbamazepine has got very high plasma protein binding (78%) with the half-life is 25 hrs<sup>3</sup>. Moreover, the transport of Carbamazepine across the blood-brain barrier (BBB) is very poor. Carbamazepine and carbamazepine-10, 11-epoxide were present in CSF in concentrations ranging from 19 to 34% and 26 to 71% of the serum concentrations, respectively. Presently, Carbamazepine is

available on the market in conventional tablet forms which can't increase the oral bioavailability and brain uptake. Therefore, an alternative route of drug delivery and dosage form that can selectively target the drug directly into various regions of the brain, including vasculature is needed for the treatment of epilepsy<sup>4</sup>. In recent years systemic drug delivery through nasal route has received a lot of attention, because it offers some advantages including avoidance of hepatic first-pass metabolism and the preferential drug delivery to brain via the olfactory region. Intranasal drug delivery system must be meticulously designed to provide rapid transport of drug across nasal mucosa and longer residence time in nasal cavity. Microemulsions, by virtue of its lipophilicity and low globule size, are explored widely as a delivery system to enhance uptake across nasal mucosa. Addition of a mucoadhesive agent such as a polyelectrolyte polymer helps in retention of formulation in nasal cavity<sup>5</sup>. Hence the objective of this investigation was to prepare and to characterize microemulsion/mucoadhesive microemulsion of Carbamazepine, to study the release of drug through ex-vivo study. It was hypothesized that Microemulsion / mucoadhesive microemulsion based drug delivery system will result in effective nose-to brain transport, enhanced rate and extent of drug transport, and greater distribution of Carbamazepine into and within the brain. This can help to maximize the therapeutic index of the drug, reduce its side effects, reduce the dose and frequency of dosing, and perhaps result into cost effectiveness<sup>6</sup>.

### MATERIALS

Carbamazepine was received as gift from Relax Pharmaceuticals Ltd, Vadodara, India. Labrafil M 1944 CS, Labrasol (caprylocaproyl macrogol-8-glycerides), Plurol Oleique (polyglyceryl-6-dioleate), and Labrafac

(medium-chain triglyceride, C8-C10 fatty acids) Capmul MCM, Captex, Cremophor EL, Accenon CC and Transcutol P were received as gifts from ABTEC, Mumbai, India. Isopropyl Myristate (IPM), Oleic Acid (OA), Tween-20, Tween-40, Tween-60, Tween-80, Polyethylene Glycol 400 (PEG 400), PEG 600, Glycerol, Isopropyl alcohol, Isobutyl alcohol, Carbomer-934, Citric acid (monohydrate) and disodium phosphate (dihydrate) were purchased from Baroda Chemicals, Vadodara, India. All other chemicals and solvents were of analytical reagent grade and were used without further purification.

## METHODS

**Solubility Studies:** The solubility of Carbamazepine in various oils (Capmul MCM, Labrafil M 1944 CS, Isopropyl Myristate (IPM), sunflower oil, soya bean oil, labrafac CC), surfactant (Cremophor EL, Accenon CC, Labrasol<sup>R</sup>), and co-surfactants (Transcutol P, Isopropyl alcohol (IPA), PEG-600, and Glycerol) was determined. Excess drug (100 mg) was added to each cap vial containing 5 ml each of the selected vehicle. After sealing the cap vials, the mixtures were shaken on a shaker at 25°C for 48 hrs. Each vial was then centrifuged at 10,000 rpm for 10 min. The undissolved drug in the pellet as well as solubilized drug in the supernatant was quantified by UV spectroscopy and mass balance was obtained<sup>7</sup>.

**Pseudo Ternary Phase Diagram Study:** Pseudo-ternary phase diagrams of ME were developed by titration method using Labrafil M 1944 CS (Oil Phase), Accenon CC (Surfactant), Transcutol P (Cosurfactant), and distilled water (Aqueous Phase). The mixture of oil and surfactant / co-surfactant at certain weight ratio was titrated with water in a drop wise manner. Four phase diagrams were constructed taking ratio of surfactant / co-surfactant as 4:1, 3:1, 2:1, and 1:1 respectively. After the identification of ME region in the phase diagrams, the ME formulations were selected at desired component ratios. The effect of drug on the phase diagram was also investigated<sup>8</sup>.

**Preparation of Formulations:** Carbamazepine solution (PDSC, 10 mg/ml) was prepared by addition of Carbamazepine (100 mg) to 8 ml of distilled water and ethanol (9:1) with continuous stirring. The pH was adjusted to  $6.8 \pm 0.25$  using glacial acetic acid (approximately 0.06 ml/ml). The mixture was stirred for 10 min and a clear solution was obtained. Final volume was made up to 10 ml using distilled water<sup>9</sup>.

Microemulsion containing Carbamazepine (CME, 10 mg/ml) was prepared by titration method using Labrafil M 1944 CS as oil phase, Accenon CC as surfactant and Transcutol P as co-surfactant. A mixture of surfactant and cosurfactant was added to the solution of drug in oil. This mixture was titrated by the aqueous phase which resulted in transparent and homogenous CME<sup>10</sup>. Carbamazepine mucoadhesive microemulsion (CMME) was prepared by addition of Polyvinyl carboxy polymer (0.3% wt/wt of Carbomer-934) to CME and the dispersion was stirred for 15minutes<sup>11</sup>. Finally the pH of the gel was maintained to  $6.8 \pm 0.25$  by adding diethanolamine.

**Optimization of Microemulsion:** Various batches of microemulsion were prepared by titration method and

optimization was done in terms of % transmittance, particle size, and % drug content. Carbamazepine mucoadhesive microemulsion was also optimized for particle size, pH and % drug content<sup>12</sup>.

**Characterization of Microemulsion:** The optimized microemulsion was characterized for % Transmittance, globule size, zeta potential, pH and drug content. % Transmittance was measured by UV Spectrophotometer at 520nm. Distilled water was employed as the reference solution with a transmittance of 100%<sup>13</sup>. Globule size and zeta potential were determined by Zetasizer (Nano ZS, Malvern Instruments, and UK)<sup>14</sup>. Carbamazepine content in the formulations was determined by UV Spectroscopy method at 220nm wavelength ( $\lambda_{max}$ ).

**Nasal Ciliotoxicity studies:** Nasal ciliotoxicity studies were carried out using in situ toad palate model<sup>15</sup>. Upper palate of toad ( $40 \pm 5$  g) was exposed and treated with 0.5 ml test sample i.e., CME and CMME at 10 mg/ml for 1 h, then rinsed with saline. The palate was dissected out and the mucocilia was examined with an optical microscope (Nikon Fx-35A, Japan). Saline and propranolol (a serious nasal mucociliary toxicity agent, 1% w/v solution) were used as a negative and positive control, respectively.

**Stability study:** Stability of the developed microemulsion was carried according to ICH guideline<sup>16</sup>. Both CME and CMME were stored at three different temperature ranges for 6 months i.e., refrigerator condition ( $2^{\circ}\text{C} - 8^{\circ}\text{C}$ ), room temperature and elevated temperature ( $50 \pm 2^{\circ}\text{C}$ ). The shelf life of the stored of the developed systems were evaluated by visual inspection (phase separation), % Transmittance, Particle size and % Assay. In order to estimate the metastable systems, the optimized microemulsion formulation was also centrifuged (Remi Laboratories, Mumbai, India) at different speeds like 5,000, 10,000 and 15,000 rpm for 30 minutes and observed for any change in homogeneity<sup>17</sup>.

**Ex-Vivo Release Studies:** In vitro release studies were performed using a modified Franz diffusion cell at  $37 \pm 2^{\circ}\text{C}$ . Sheep nasal mucosa was removed carefully and washed with distilled water to remove tissues. Then size of 2.4 cm of excised nasal mucosa was cut and mounted on the receptor compartment of Franz diffusion cell. Donor compartment was fixed and temperature of the diffusion medium was maintained to  $37 \pm 1^{\circ}\text{C}$  by circulation distilled water of temperature of  $37 \pm 1^{\circ}\text{C}$ . CME, CMME and PDSC (each equivalent to 200  $\mu\text{g}$ ) were placed in the donor compartment and the receptor compartment was filled with dialysis medium (23 ml of phosphate buffer pH 6.8). At fixed time interval of 1 hr, 2 ml of the sample was withdrawn from the receiver compartment through a side tube and analyzed spectrophotometrically at 220 nm<sup>18</sup>.

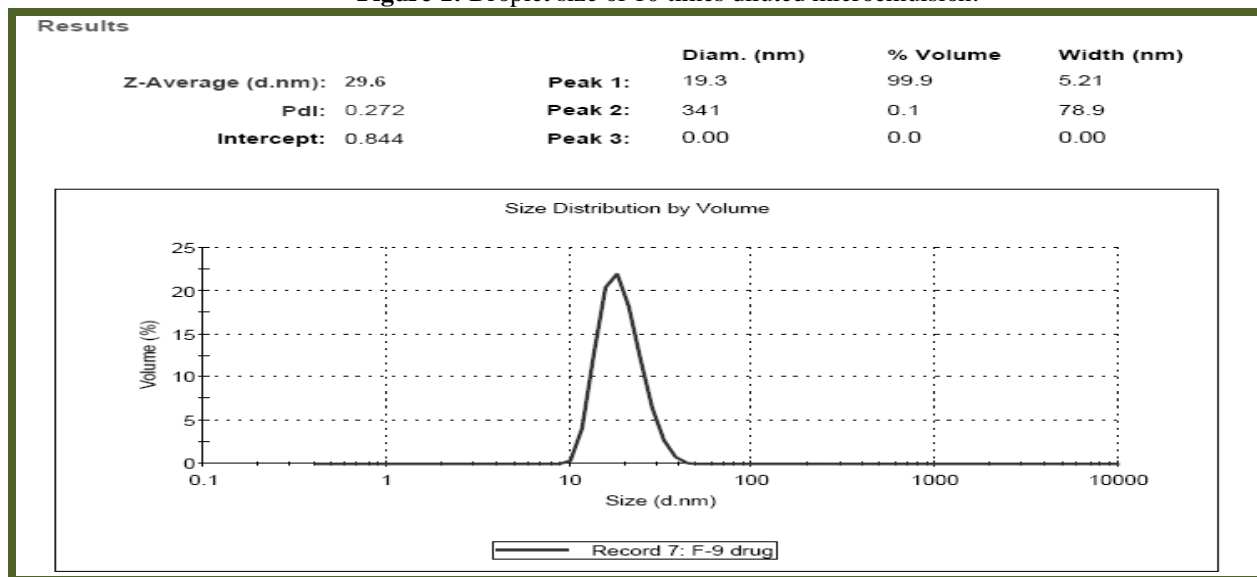
**Statistical analysis:** All data are reported as mean  $\pm$  SEM and the difference between the groups were tested using Student's t test at the level of  $P < 0.05$ . More than two groups were compared using ANOVA and differences greater at  $P < 0.05$  were considered significant.

**RESULTS AND DISCUSSION:**

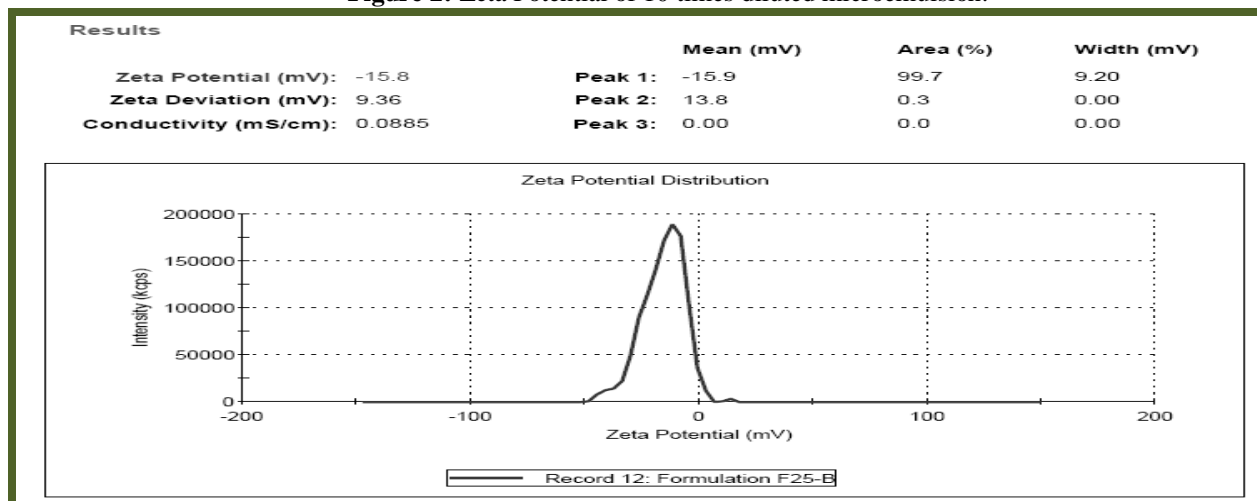
Labrafil M 1944 CS showed higher solubilizing capacity compared to other vehicles. Hence, Labrafil M 1944 CS was selected as oil phase. Accenon CC and Transcutol P were selected as surfactant and co-surfactant respectively for the preparation of optimized microemulsion. Pseudo-ternary phase diagrams were constructed to identify the ME existing zone from which appropriate concentration ranges of different components for CME can be obtained. 4:1 and 3:1 ratios of Accenon CC & Transcutol P gave clear ME. However the ME existing region with 3:1 ratio

was comparatively larger than with 4:1 and it also required less quantity of surfactant; hence 3:1 ratio of surfactant and co-surfactant was selected. Percentage transmittance of microemulsion after 10 times and 100 times dilution was  $98.62 \pm 0.43\%$  and  $98.85 \pm 0.29\%$ , respectively indicating transparency and stability of optimized microemulsion<sup>19</sup>. The average droplet size of 10 and 100 times diluted form of optimized microemulsion was found to be  $27.9 \pm 0.3$  nm and  $26.7 \pm 0.2$  nm respectively as shown in the Figure-1 (For 10 times diluted microemulsion).

**Figure 1:** Droplet size of 10 times diluted microemulsion.



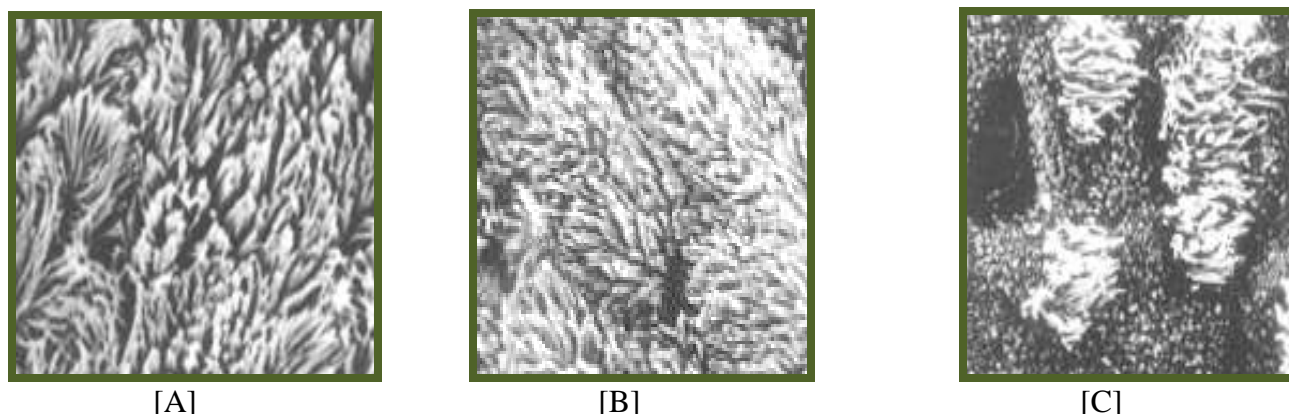
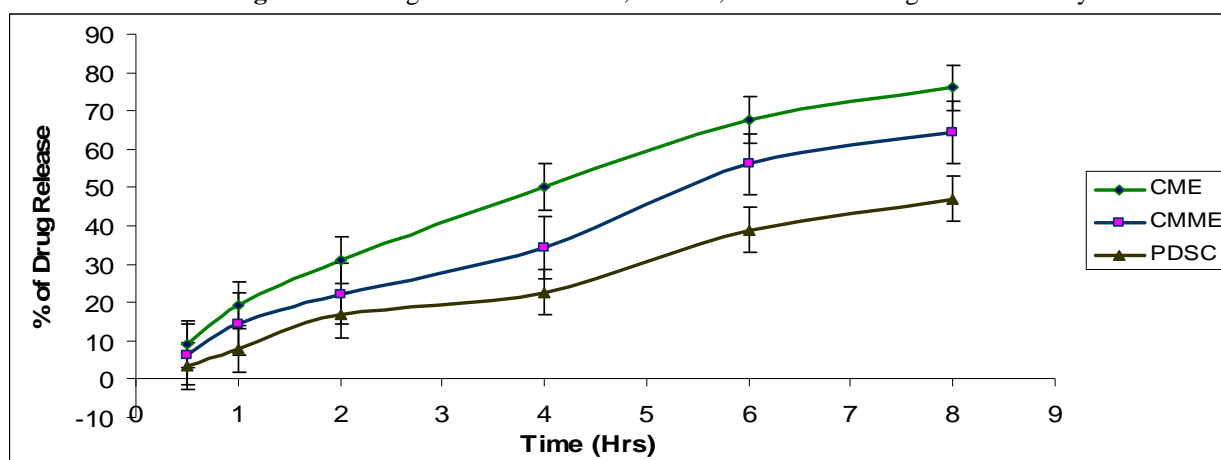
**Figure 2:** Zeta Potential of 10 times diluted microemulsion.



**Table 1:** Results of stability studies of microemulsion.

Temperature (°C)	Phase separation		% Transmittance		Particle size (nm)		% of Assay	
	After 4 months	After 6 months	After 4 months	After 6 months	After 4 months	After 6 months	After 4 months	After 6 months
2°C-8°C	Yes	Yes	98.2±0.8	Hazy	27.9±2.4	Hazy	98.3±1.5	Hazy
Room Temp	No	No	99.1±1.2	98.8 ±1.3	27.2±2.1	29.1±3.1	99.4±1.9	99.1±2.1
Elev. Temp (50 ± 2°C)	No	Hazy	99.1±0.8	Hazy	28.2±2.6	29.7±3.2	99.2±0.9	Hazy

\* Parameters are derived using mean ± SEM values of 4 different estimations

**Figure 3:** Results of Nasal ciliotoxicity study by Scanning electron microscope.**Figure 4:** % drug release from CME, CMME, and PDSC through Ex-vivo study

\*Parameters are derived using mean  $\pm$  SEM values of 3 different estimations

The Polydispersity Index was found to be well below 1.0 which confirms that the optimized ME remains stable upon dilution<sup>20</sup>. Zeta potential of 10 and 100 times diluted CME was found to be  $-15.8 \text{ mV} \pm 0.89$  and  $-16.62 \text{ mV} \pm 1.26$  respectively as shown in the Figure 2 (For 10 times diluted microemulsion), indicating that dilution does not significantly affect the zeta potential of the ME.

% drug content in CME and CMME were found out to be  $99.3 \pm 0.6$  and  $99.6 \pm 0.4$ . Results of stability studies of optimized microemulsion (Table 1) indicated that the optimized microemulsion became phase separated at cold and hazy at elevated temperature in 6<sup>th</sup> months. So it was considered to be stable at room temperature up to 6 months as it showed no change in particle size, assay, and transmittance value.

Scanning electron microscope results showed that there were a great number of cilia with fast rate beating on the edge of mucosa that was treated with CME and CMME for 1 hr [Figure 3 (A) and 3 (B)], and the same phenomenon was observed when the contact time was extended up to 4 hr, indicating that developed CME had no obvious effect on the cilia movement. While 1% propranolol had serious irritation. It was observed that some cilia fell off from the edge of the mucosa, and the remaining was still beating, but the duration of ciliary movement decreased as shown in Figure 3(C).

*In Vitro* Release of Drug: Results of Ex vivo drug release from the optimized CME, CMME and PDSC are shown in the Figure 4. Drug release in 8 hours from CME, CMME and PDSC was found to be 76.04%, 64.6% and 47% respectively. CME showed higher drug release as compared to CMME and PDSC which may be due to the solubility enhancing of the surfactant and co-surfactant.

## CONCLUSION

The studies demonstrated that microemulsion system comprising Carbamazepine Labrafil M 1944 CS (3% v/v), Accenon CC (18% v/v), Transcutol P (7% v/v) and distilled water (72% v/v) and mucoadhesive microemulsion system (0.4% carbopol based microemulsion) were optimal for intranasal delivery of Carbamazepine. The microemulsion systems are transparent and stable at ambient conditions for 6 months. Results of nasal ciliotoxicity study confirmed that developed microemulsion and Mucoadhesive microemulsion containing Carbamazepine had no cilio toxicity. Enhanced rate and extent Carbamazepine release following application on excised nasal mucosa from CME and CMME may help in decreasing the dose and frequency of dosing and possibly maximize the therapeutic index. However, clinical benefits to the risk ratio of the

formulation developed in this investigation will decide its appropriateness in the clinical practice.

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