Formulation Development and **Characterization of Oxcarbazepine Microemulsion for Intranasal Delivery**

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ABSTRACT

The objective of this study was to develop novel intranasal microemulsion containing oxcarbazepine (OXC) for treatment of epilepsy. Optimized ratio of Tween 80: Polyethylene glycol and isopropyl myristate was selected after developing pseudoternary phase diagrams and microemulsions were prepared. The prepared microemulsions were characterized for drug content, pH, particle size, polydispersity index, zeta potential, conductivity, viscosity and in vitro release. Ex vivo permeation study for selected microemulsion was performed through sheep nasal mucosa. Further pharmacodynamic performance was evaluated in mice by electrically induced seizures. It was found that selected microemulsion was transparent with average globule size of 20.5 nm and cumulative percentage drug permeated was 95.60 %. Pharmacodynamic evaluation of selected formulation also indicated lesser intensity of seizures with low dose in mice in comparison to oral suspension of OXC. OXC intranasal delivery system is an effective alternate therapy for treatment of epilepsy.

Keywords: Oxcarbazepine, intranasal, microemulsion

INTRODUCTION

Several methods have been reported in the literature to enhance the drug penetration across biological membranes¹. Nasal drug delivery is an alternate route to oral and parenteral route for the drug to reach systemic circulation. As nasal drug delivery shows various benefits in comparison to other forms of drug deliveries.

Nasal cavity is lined by vascularized epithelium which provides larger surface area useful for drug absorption. It has low enzymatic activity in contrast to the di-

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gestive system. It bypasses hepatic first pass metabolism. So there is negligible irritation of gastrointestinal membrane²⁻³. Nasal drug delivery can be preferred over other routes of drug delivery as it is non-invasive, convenient method with better patient compliance, easy and cost effective⁴⁻⁵. Nasal drug delivery also offers advantage of transporting the drugs to brain by detouring the blood brain barriers.

Microemulsions (ME) are clear, thermodynamically stable and isotropic mixtures⁷ (Oil, water and surfactant, mostly along with cosurfactants). ME should be kept under proper storage conditions8. There are three types of ME microstructures. They are: Micellar (oil in water), inverted micellar (water in oil) and bicontinuous structure. ME is a new approach to sparingly water soluble drugs, in order to enhance their dissolution and improve bioavailability.

Epilepsy is one of the most common and devastating neurological disorders which is estimated to have a worldwide prevalence of about 0.5-1% 9. There are several antiepileptic drugs currently available to control and suppress seizures. However, despite the ongoing development of new pharmacological therapies, more than 30% of the patients do not become seizure free mainly due to the pharmacoresistance phenomena¹⁰.

In order to ameliorate the antiepileptic drug regimen, various strategies of administration have been explored. An intranasal microemulsion is one of the advanced drug delivery option, which can be given orally, topically and through nasal cavity as aerosol11.

Intranasal ME will transport the drug from nose to brain at a very faster rate. By improved distribution and dissolution of drug within the brain, one can assume reaching higher levels of the rapeutic index along with the benefit of reduced side effects, low dosages and also reduction in the cost of therapy¹².

Intranasal microemulsion formulations have been developed for a number of drugs such as Quetiapine fumarate¹³, Fexofenadine¹⁴, Diazepam, Lorazepam, Alprazolam¹⁵, Eucalyptus oil¹⁶, Ibuprofen¹⁷ and Zolmitriptan¹⁸.

Oxcarbazepine (OXC) is an anticonvulsant and mood stabilizing drug, used primarily in the treatment of epilepsy and is also used to treat anxiety / mood disorders. It is a derivative of carbamazepine. Chemically it is 10, 11-dihydro-10-oxo- 5H-dibenz (b,f)azepine-5-carboxamide (As shown in Figure 1). It is poorly soluble in water (308 mg/L) and has a partition coefficient of 1.31. It belongs to iminostilbene category of antiepileptic's and act on convulsions by post tetanic potentiation of synaptic transmission, also act on neuropathy by sodium channel blockade and calcium channel blockade mechanism and act on bipolar disorder by decreasing abnormal electrical activity in brain¹⁹.

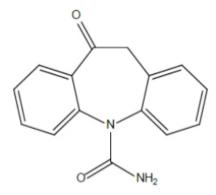


Figure 1: Structure of oxcarbazepine.

The objective of this investigation is to develop intranasal OXC and to compare with oral formulation available in market.

METHODOLOGY

Materials

OXC was obtained from Aurobindo Pharma Ltd., Hyderabad, India. Sunflower oil from local market, Isopropyl myristate, Tween 20, Tween 80, PEG 400, PEG 600, glycerol, propylene glycol, oleic acid, methanol, sodium hydroxide & potassium dihydrogen phosphate were obtained from S.D. Fine-Chem Ltd., Mumbai. Dialysis membrane was procured from Himedia, Mumbai. All the chemicals were of analytical grade and purchased commercially. Double distilled water was used throughout the study.

Software used: Ternary phase diagram (CHEMIX School 3_60).

Methods

Solubility Studies

The solubility of OXC in various components (oils, surfactants and cosurfactants) was determined by adding an excess of drug to 25 ml conical flask, containing 10 ml of selected vehicle and vortexed for half an hour and placed on a rotary shaker for 48 hours at room temperature. Then contents were centrifuged (REMI R-8C) at 3000 rpm for 5 minutes. The supernatant was filtered through a membrane filter (0.45µm) and the drug concentration in filtrate was determined by UV-Visible (UltraViolet) spectroscopy (Thermo Fisher Scientific Model Evolution 201 series). The oil, surfactant and cosurfactant that showed high soloubility of OXC was used in preparation of microemulsions.

Preparation of Pseudoternary Phase Diagram²⁰

Pseudoternary phase diagrams were constructed to obtain the appropriate ratio of surfactant: cosurfactant which can result in to large existence of microemulsion area. They were constructed using water titration method. Surfactant (Tween 80) and cosurfactant (PEG 600) were mixed (Smix) in different weight ratios (1:1, 2:1, 4:1) and represented as A-series, B-series and C-series respectively. Oil (isopropyl myristate) and Smix (Tween 80 and PEG 600) were mixed thoroughly in different weight ratios from 1:8 to 8:1 in different test tubes and diluted with distilled water in a drop wise manner till it changed from opaque to transparent (composition shown in Table 1). The concentrations of components were recorded in order to complete pseudoternary phase diagrams, and then the contents of oil, surfactant, cosurfactant and water at appropriate weight ratios were selected based on these results.

Table 1: Formulation composition of microemulsions

Formulation code	Smix (Tween 80 and PEG 600)	Oil and Smix
A-Series, A1- A8	1:1	1:8 to 8:1
B-Series, B1 - B8	2:1	1:8 to 8:1
C-Series, C1 - C8	4:1	1:8 to 8:1

By joining the change points the boundaries of phases formed were obtained in the phase diagrams. All samples exhibiting a transparent and homogenous state were assigned to a microemulsion area, a monophasic area, in the phase diagram. The pseudoternary phase diagrams were constructed by using CHEMIX School 3_60 software (As shown in Figure 2).

Preparation of Microemulsion

The OXC microemulsion was prepared by phase titration method employing Isopropyl myristate (IPM) as oil, Tween 80 as surfactant and PEG 600 as cosurfactant.

Preparation of Microemulsion with 1:1, 2:1 And 4:1 Ratio of Smix

Accurately weighed 50 mg of the drug was added to test tube containing Smix (Tween 80 and PEG 600) in 1:1 ratio. The mixture was shaken on a cyclo mixer until the drug gets properly mixed. Oil (Isopropyl myristate) was then added to the Smix and again shaken for about 10 min. The mixture was diluted with distilled water in a drop wise manner under constant stirring till a transparent microemulsion was achieved.

IPM and Smix were mixed thoroughly in different weight ratios from 1:8 to 8:1

(A1 to A8). The pseudoternary phase diagrams were constructed to know the area of the microemulsion formed. Similarly microemulsions with 2:1 and 4:1 ratios of Smix and varying ratios of Oil: Smix (B1 to B8 and C1 to C8 respectively) were prepared and pseudoternary phase diagrams were constructed.

Characterization of Microemulsion

For the selected three formulations viz., A2, A5 and B3 different characterization tests were done. These three formulations were selected based on transparency, viscosity and amount of water that can be incorporated. C-series of formulations resulted in microemulsions having high viscosity, turbidity and few resulted in gel like consistency after addition of water. These are the reasons for not selecting C-series (Smix 4:1) for further studies.

Drug Content

The drug content of microemulsion formulation was determined by dissolving 1 ml of the formulation in 10 ml of methanol. After suitable dilutions with methanol, absorbance was determined using the UV-Visible spectrophotometer keeping blank microemulsion as control at wavelength 256 nm.

pH Determination

The pH values of the microemulsions were measured by a pH meter (Digisun Electronics, India) at ambient temperature with glass electrode.

Particle Size Distribution, Polydispersity Index (PDI), Zeta Potential **And Conductivity**

Physical characteristics of microemulsion (particle size distribution, polydispersity index, zeta potential and conductivity) were determined by using Dynamic light scattering (DLS) method using a zetasizer (Horiba SZ-100Z, Japan).

Viscosity Measurement

Microemulsions are generally low viscosity systems. The viscosity of prepared microemulsion was measured using Brookfield viscometer (Brookefield viscometer LVDV-E, US).

In vitro Release Studies

Preparation of Calibration curve of OXC in 40% v/v PEG 400 plus phosphate buffer pH 6.4

Accurately weighed 10 mg of drug was dissolved in 100 ml of 40% v/v PEG 400 + phosphate buffer pH 6.4 which gives stock solution of 100 μg/ml.

From this stock solution aliquots of 0.5 ml, 1 ml, 1.5 ml, 2 ml, 2.5 ml, 3 ml, 3.5 ml, 4 ml, 4.5 ml were pipetted out into a series of 10 ml volumetric flasks and make up to mark with phosphate buffer which gives 5, 10, 15, 20, 25, 30, 35, 40, 45 µg/ml respectively.

The absorbance of the resulting solution was then measured at 256 nm using UV spectrophotometer. The calibration curve was obtained by plotting Absorbance vs. Concentration in μg/ml (Figure 3).

In vitro Release Studies

Based on characterization results three formulations were selected i.e., A2, A5 and B3 for in vitro release studies. The composition of selected formulations is shown in Table 2. The *in vitro* release study was carried out using Franz diffusion cell (Fabricated locally). The donor compartment was open at the top and was exposed to atmosphere. The dialysis membrane with molecular weight in the range 12,000 to 14,000 (Himedia, Mumbai) was previously soaked for 24 h in phosphate buffer pH 6.4. The donor and receptor compartments were held together using a clamp. The receptor compartment contained 13 ml of 40% v/v PEG 400+ phosphate buffer pH 6.4 and stirred with a magnetic capsule operated by a magnetic stirrer (REMI 2MLH, India). The temperature was maintained at 37 ± 0.5 °C and the receptor compartment was provided with a sampling port. Samples were collected at preset time points. At each sampling time, 3 ml of sample was removed using a syringe with syringe filter and replaced with fresh 40% v/v PEG 400 in phosphate buffer pH 6.4²¹.

The concentration of drug was determined using a UV-Visible spectrophotometer at a wavelength of 256 nm. The percentage drug released was calculated and plotted against time (Figure 4).

Ex vivo Permeation Studies

Two formulations were selected for permeation study based on in vitro release studies. The freshly excised sheep nasal mucosa, except the septum part, was collected from the slaughter house. The membrane was kept in 40% v/v PEG 400 + phosphate buffer pH 6.4 for 15 min to equilibrate. The superior nasal concha was identified and separated from the nasal membrane²². The excised superior nasal membrane was mounted on a Franz diffusion cell²³. Franz diffusion cell used for ex vivo permeation studies had a diameter of 2 cm and mucosa of thickness 0.2 ± 0.01 mm. The receptor compartment was filled with 14 ml of diffusion media. Diffusion media was continuously stirred with a Teflon-coated magnetic bar at a constant rate, in a way that the nasal membrane surface just flushes the diffusion fluid24.

Two ml of OXC microemulsion was placed in the donor compartment of Franz diffusion cell. Samples were collected at preset time points and analyzed using U.V spectrophotometer. Each sample removed was replaced by an equal volume of diffusion media (3 ml).

Each study was carried out for a period of 3 h, during which the drug in the receptor compartment (µg/ml) across the sheep nasal membrane was calculated at each sampling point.

The cumulative amount of OXC permeated through mucosa was determined by the following equation:

$$Q_n = C_n x V_{\circ} + \sum_{i=1}^{n-i} C_i x V_i$$

Where C_n is OXC concentration of receptor medium after each sampling time, C_n is oxcarbazepine concentration for i sample, Vo and V are the volumes of receiver solution and sample respectively, and S is the effective diffusion area²⁵.

Pharmacodynamic Studies

Maximal Electroshock: Mice weighing about 25 gm and exhibiting clear hind limb extension phase during electrically induced convulsions were included in the present study. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC/SUCP/03/2014). Mice were divided into 3 groups (n=6). The first and second groups were treated orally with OXC suspension (0.5% Na CMC) and intranasally [23] with OXC microemulsion respectively containing OXC equivalent to 13.5 mg/kg body weight (using a micropipette attached with low density polyethylene (LDPE) tubing, having 0.1 mm internal diameter at the delivery site). For intranasal administration, 60 µl (0.2542 mg drug) of microemulsion was instilled equally divided in both the nares of mice. The third group was not subjected to any treatment, served as control. Electroconvulsions were produced by applying current (50 mA, 0.2 s) through ear clip electrodes using electroconvulsiometer after 30 min of administration of formulations and different phases of seizures were measured.

Briefly after application of current an immediate severe tonic phase (E 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 mice remained silent without any movement. Recovery time was recorded as total time from starting of tonic phase till animal regains its normal movement (Figure 8).

RESULTS AND DISCUSSION

It is estimated that more than 98% of all small molecules and nearly 100% of large molecular weight drugs systemically delivered to the central nervous system (CNS), either by oral or intravenous routes, do not readily cross the blood brain barrier and reach the brain parenchyma at pharmacologically active concentrations²⁶.

In the light of the current knowledge, drug transport across the nasal mucosa into the CNS depends on a variety of factors that can range from the physicochemical properties of the drug to the formulation design and physiological conditions at the absorption site27-28.

Solubility Studies

The solubility of the drug was determined in each component of microemulsion (oils, surfactants and cosurfactants) and was reported. Based on absorbance values (qualitative) oil, surfactant and co surfactant was selected.

Oils - IPM > Oleic acid > Sunflower oil

Surfactants - Tween 80 > Tween 20 > Cremophor EL

Co surfactant – PEG 600 > PEG 400 > Isopropyl alcohol > Glycerol > Propylene glycol

Depending on solubility results isopropyl myristate was selected as oil for preparation of microemulsion, tween 80 showed good solubility for OXC and previous studies have reported improved nasal absorption²⁹. Thus tween 80 was selected as surfactant and based on solubility results PEG 600 was selected as cosurfactant. The water solubility of the drug was found to be 308 mg/L.

Microemulsions were prepared by the spontaneous emulsification method (phase titration method) and can be depicted with the help of phase diagrams.

Preparation of Pseudoternary Phase Diagram

The components that showed maximum solubility were further optimized using pseudoternary phase diagrams as shown in Figure 2. The zone of microemulsion was obtained.

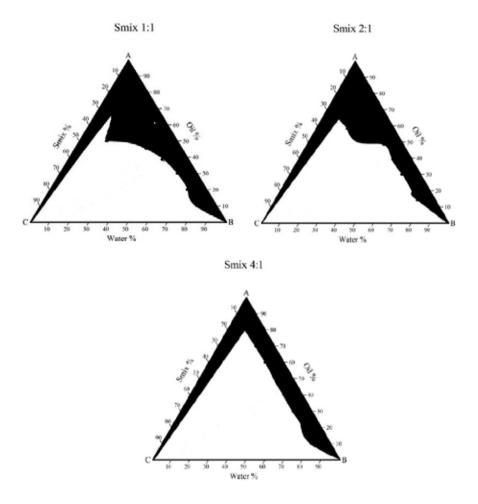


Figure 2: Pseudoternary phase diagram using isopropyl myristate as oil, tween 80 as surfactant, polyethylene glycol 600 as cosurfactant and water (Tween 80: PEG 600 = 1:1, 2:1, 4:1).

Preparation of Microemulsion

Eight formulations (1:8 to 8:1 ratio) from each ratio of Smix (1:1, 2:1 and 4:1) were prepared and the selected formulations were characterized thoroughly. Highest microemulsion area was obtained with ratio of 1:1, 2:1 and thus selected for further studies.

The OXC loaded microemulsion formulations were prepared as per the compositions shown in Table 2. Microemulsion systems were obtained by mixing oil, surfactant and cosurfactant together and adding appropriate quantity of OXC and adding precisely distilled water drop by drop to these oily phases with continuous stirring at ambient temperature. The final concentration of OXC in microemulsion systems was 5 mg/ml. Microemulsions have several specific physicochemical properties such as transparency, optical isotropy, low viscosity³⁰. The formulations having these specific physicochemical properties were selected for characterization, in vitro release and ex vivo permeation studies.

Table 2: Formulation composition of selected microemulsions.

Formulation code	OXC (mg)	Smix (%)	IPM (%)	Water (%)
A2	50	50	14.28	35.72
A5	50	38.46	48.07	13.47
B3	50	50.84	25.42	23.74

Characterization of Microemulsion

Drug content percentage of all the three selected formulations was found to be 99.25 \pm 1.30. The pH was found to be 5.47 \pm 0.42. Viscosity of all formulation was found to be 60.53 ± 5.93 cps. Physical characteristics of microemulsion (particle size distribution, polydispersity index, zeta potential and conductivity) were shown in Figures 3 & 4.

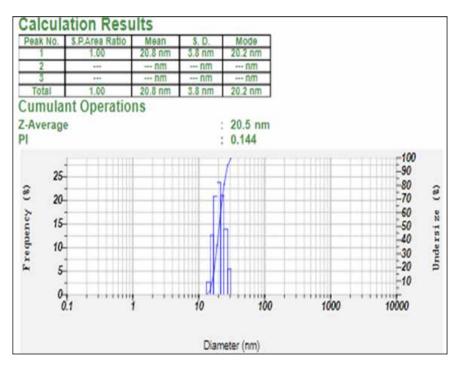


Figure 3: Particle size measurement of Formulation A2.

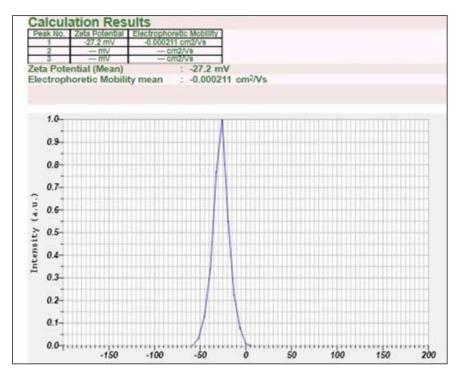


Figure 4: Zeta potential measurement of Formulation A2.

Result of globule size indicated that smallest globule size was obtained with formulation A2 with poly dispersity index 0.144 which is close to zero, indicating that the prepared microemulsion had uniform globule size and thus it was selected for further studies as faster permeation is expected when the globule size is small. Zeta potential was negative which indicated the stability of formulations as there were less chances of globules aggregation. The conductivity of the results confirmed the formation of solution type of microemulsion with water in continuous phase.

Calibration curve of Oxcarbazepine in 40% v/v PEG 400 + phosphate buffer pH 6.4:

Calibration curve of OXC in 40% v/v PEG 400 + phosphate buffer pH 6.4 was shown in Figure 5 and it was found to be linear.

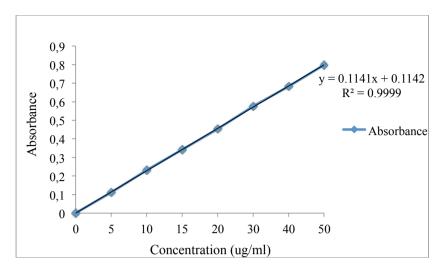


Figure 5: Calibration curve of OXC in 40% v/v PEG 400 + phosphate buffer pH 6.4

In vitro release studies: The cumulative percentage of drug release after 210 min was found to be maximum with formulation A2 (98.65 %). In vitro release profile of OXC was shown in Figure 6. The formulation with more amount of water has shown maximum percentage drug release compared to other formulations.

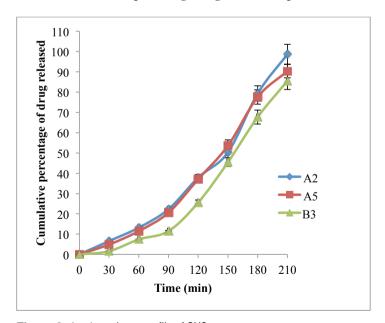


Figure 6: In vitro release profile of OXC.

Ex vivo permeation studies: Although human nasal mucosa would be the ideal substrate for nasal permeation studies, its limited availability has made to use suitable alternative. It was reported³¹ that the sinus anatomy (including the placement of nasal cavity, turbinates, frontal and maxillary sinuses) in sheep is comparable to humans. Histology of the sheep's nasal mucosa is also identical to that of humans³². Hence ex vivo permeation study was performed by using sheep nasal mucosa for optimized formulations A2 and A5. The cumulative percentage of drug permeated after 210 min was found to be maximum with formulation A2 (95.60 %) and formulation A5 shown 81.25 %. Ex vivo permeation profile of OXC from sheep nasal mucosa was shown in figure 7.

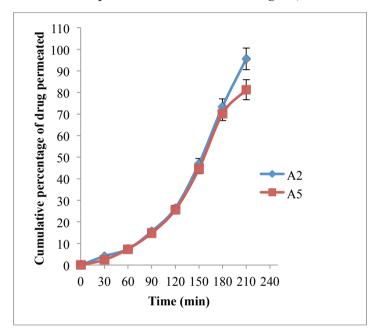


Figure 7: Ex vivo permeation profile of OXC through sheep nasal mucosa.

Pharmacodynamic studies

The antiepileptic activity was assessed by observing the extent of different phases of seizures including duration of seizures, extension phase, clonus phase and stupor phase and results were represented in Figure 8 and different phases in Figure 9. The results clearly indicated lesser intensity of seizures and rapid recovery from seizures in mice treated with intranasal OXC microemulsion.

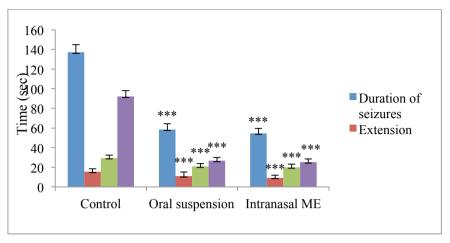


Figure 8: Duration of seizures, extension, clonus and stupor phase for two treatments of oxcarbazepine - OXC oral suspension and OXC microemulsion (IN) where, *** indicates significant difference in comparison to control (p < 0.05).

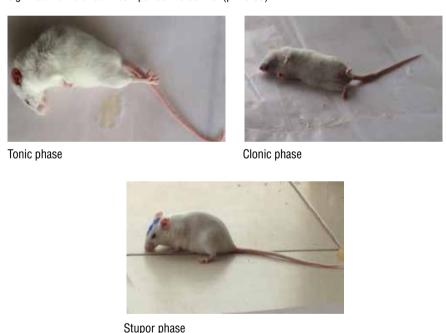


Figure 9: Different phases of seizures in mice.

The intranasal OXC microemulsion demonstrated lesser intensity of seizures which may be due to larger extent of selective nose to brain delivery of drug in comparison to oral suspension of OXC. This may help in decreasing the dose and frequency of administration of drug and may possibly maximize therapeutic benefits and may also reduce the cost of therapy.

CONCLUSION

In comparison to oral formulation, intranasal microemulsion of OXC was shown significant difference in antiepileptic activity. However detailed animal study followed by thorough clinical trials is required to establish clinical safety and efficacy of this formulation.

CONFLICT OF INTEREST

There is no conflict of interest between the authors of the article.

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