

Formulation and evaluation of an anti-epileptic drug-loaded microemulsion for nose to brain delivery

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The aim of the present study was to formulate an anti-epileptic drug-loaded microemulsion for nose-to-brain delivery. The oil system evaluated for the preparation of a stable microemulsion was iso-propyl myristate. A non-ionic surfactant like Tween 80 was used with polyethylene glycol 400 as a co-surfactant. A pseudoternary phase diagram for various proportions of S_{mix} :oil was constructed by the water titration method. The effect of changing concentration of alcohol as a co-surfactant was also studied. The t-phase diagram shows that the water consumption capacity of the system was increased as the surfactant concentration was increased. It was also found that as the concentration of the alcohol was increased, the viscosity of the microemulsion decreased. After the identification of the microemulsion region, the composition of the microemulsion was fixed at oil 9-10%, S_{mix} 35-40% and water 45-50%. The formulated microemulsion was evaluated for various parameters like pH, conductivity, zeta potential, viscosity and *in vitro* drug diffusion studies. All the evaluation parameters showed satisfactory results. Using this microemulsion, the solubility of valproic acid was increased from 1.29 to 36 mg/ml. Diffusion studies have shown a lag period of 45 min. Thirteen percent drug diffusion was achieved in 3 h. The prepared microemulsion was stable at 40°C and 75% relative humidity.

Key words: *Isopropyl myristate, microemulsion, nose to brain delivery, valproic acid*

INTRODUCTION

It is now well established that the blood–brain barrier (BBB) is a unique membranous barrier that tightly segregates the brain from the circulating blood.^[1,2] The central nervous system (CNS) consists of blood capillaries that are structurally different from the blood capillaries in the other tissues. These structural differences result in a permeability barrier between the blood within the brain capillaries and the extracellular fluid in the brain tissue.^[3] The BBB also has an additional enzymatic aspect along with a high concentration of P-glycoprotein and an active-drug-efflux-transporter protein in the luminal membranes of the cerebral capillary endothelium.^[4] These strategies to circumvent the BBB are manipulating drugs, disrupting the brain and finding alternative routes of drug delivery.^[5] Alternative routes to CNS drug delivery are intraventricular, intrathecal route and olfactory pathway. Drugs delivered intranasally are

transported along the olfactory sensory neurons to yield significant concentrations in the cerebro spinal fluid (CSF) and the olfactory bulb.^[6] The nasal route offers some advantages such as rapid absorption, evidence of hepatic first-pass metabolism and the preferential drug delivery to the brain via the olfactory region.^[7] During the epileptic attack, rapid access of drug to the brain is very important. In such a case, intranasal drug delivery presents a promising alternative pathway. Valproic acid is a poorly water-soluble drug. Using the microemulsion system, an attempt was made to increase its solubility, thereby increasing its bioavailability.

Microemulsion is a thermodynamically stable, isotropically clear product that has a droplet size of $<0.15 \mu\text{m}$. It consists of an oil phase, surfactant, co-surfactant and an aqueous phase. O/W microemulsions represent a promising prospect for the development of formulations suitable for the incorporation of a poorly water-soluble drug due to its high solubilization capacity as well as the potential for enhanced absorption.

The aim of the present study was to formulate an anti-epileptic drug-loaded microemulsion for nose-to-brain delivery using generally regarded as safe materials for the solubilization and rapid onset intranasal

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delivery of valproic acid. The solution-like features of the o/w microemulsion could provide advantages over regular emulsion in terms of sprayability, dose uniformity and formulation physical stability. The diffusion efficiency was also studied using the *in vitro* model for nose-to-brain delivery of the drug.

MATERIALS AND METHODS

Materials

Valproic acid was provided by Briocia Pharma Pvt. Ltd. (Jejuri) (Pune, India). Isopropyl myristate (IPM) and polyethylene glycol-400 monostearate were procured from Loba Chemicals Pvt. Ltd. (Mumbai, India). Tween 80 was procured from Merck Chemicals, Mumbai, India. All the ingredients used were of analytical grade.

Determination of the partition coefficient

The partition coefficient of the drug in the water and oil was determined. The drug in the liquid state was partitioned in the water and oil phase. After shaking for 1.5 h, the mixture was kept aside and after appropriate dilution, the concentration in the oil was determined on a UV Spectrophotometer (UV-1700, Shimadzu, USA) at 210 nm.

Phase diagram preparation and microemulsion formulation

Pseudoternary phase diagrams were constructed using the water titration method to obtain the components and their concentration ranges that resulted in a large existence area of the microemulsion. IPM with Tween 80 (non-ionic surfactant) as the surfactant and polyethylene glycol-400 as the co-surfactant was used as a component in phase diagram construction. For each phase diagram construction, the desired concentration of the surfactant and the co-surfactant (S/CoS) at certain weight ratios were prepared. Nine transparent and homogeneous mixtures of oil (IPM):S_{mix} (Tween 80/polyethylene glycol-400 monostearate) at 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 w/w were prepared by mixing with a mechanical stirrer. Each mixture was then titrated with water and visually observed for the phase clarity and the flow ability. Three such phase diagrams for S/CoS at 3:1, 2:1 and 1:1 w/w ratios and four phase diagrams using ethanol as a co-emulsifier for S/CoS/Co-emulsifier at 1:3:0.25, 3:1:0.25, 1:1:0.25 and 1:1:1 w/w ratios were prepared. After the identification of the microemulsion region in the phase diagram, the microemulsion compositions were selected at a desired component ratio. The preparation of the selected microemulsion was simply performed by adding the weighed components together and stirring to form the clear microemulsion. The drug-loaded microemulsion can be prepared by adding the drug in the oily phase and then mixing all the other components together.

Characterization of the microemulsion

The physical and chemical stability of the plain and the

valproic acid drug-loaded microemulsions were studied via clarity and phase separation observation, refractive index determination, viscosity and drug content determination.^[7]

The stability of the microemulsion was also measured with the help of electrical conductivity and zeta potential by means of a zeta meter equipped with a platinum electrode used for the measurement of electrophoresis mobility of the diluted microemulsion solutions. The potential drop in a cell was measured by a digital voltmeter. All measurements were made at zero electro-osmotic flow, determined previously by the method suggested by Sehoft and Young, at a field strength of 10.0 v/cm.

In vitro drug diffusion studies

The Wruster horizontal diffusion chamber was used for the present study using bovine nasal mucosa. Phosphate buffer solution of pH 6.4 was used in the receptor chamber. Before starting the study, the mucosa was pre-incubated with phosphate buffer solution of pH 6.4. It was performed to saturate the mucosa so that further change in permeability should not occur. The microemulsion solution was taken into the donor compartment. The quantity of the microemulsion was so taken that the solution contained about 35-55 mg of the drug (i.e., about 1-1.5 ml of microemulsion). The speed of the magnet was kept at an optimum. Sampling was carried out at regular intervals, i.e. 15, 30, 45, 60, 75, 90, 120 and 180 min. The sink condition was maintained with phosphate buffer solution. The samples were diluted with methanol and further measurements were carried out on the UV spectrophotometer at 210 nm. To study the mucosal toxicity of the present formulation, the bovine nasal mucosa was placed in contact with the drug-containing microemulsion for about 48 h.^[8]

RESULTS AND DISCUSSION

Partition coefficient of the drug

One milliliter of the drug was partitioned in 10 ml of oil and 10 ml of water. The drug-retaining capacity of the oil was found to be 901 µg/ml.

Study of the pseudoternary phase diagram

From these pseudoternary phase diagrams, the microemulsion region was identified and it was found that within each microemulsion region, the solution of the microemulsion was transparent and was with a low viscosity. No distinct conversion from oil in water to water in oil microemulsion was seen. Therefore, this single isotropic region was considered to be a bicontinuous microemulsion. The rest of the region in the t-phase diagram shows either a turbid solution of microemulsion or the gel form of the mixture.

IPM/Tween 80/polyethylene glycol-400 system

In case of IPM, the microemulsion region was decreased with an increase in the gel area. During the water titration method of IPM, it was found that oil and the S_{mix} itself forms a very

thick mixture and addition of water turns it to the gel. The phase changes were increased as the concentration of the oil was increased. In this case also, three phase diagrams were studied with a change in the concentration of the emulsifier (Tween 80) and the constant concentration of the co-emulsifier (polyethylene glycol-400 monostearate) (3:1, 2:1 and 1:1 w/w).

From these, the phase diagram having the largest area of the microemulsion was selected. It was found that the phase diagram with a composition of emulsifier (Tween 80) and co-emulsifier (polyethylene glycol-400 monostearate) 3:1 w/w had the maximum area of microemulsion and hence was selected as the best composition for the microemulsion. It was possible to incorporate a maximum of 10 ml of oil into the microemulsion when the S_{mix} in the ratio of 3:1 w/w was used. The results of the phase study are shown in Figure 1a-c.

Effect of ethanol as a co-emulsifier

The effect of the changing concentration of ethanol as a co-emulsifier was also studied. Ethanol was used in the concentration of 0.25, 0.5 and 1%. Pseudoternary

phase diagrams of Tween 80:polyethylene glycol-400 monostearate:ethanol in the ratios of 1:3:0.25, 3:1:0.25, 1:1:0.5 and 1:1:1 w/w were studied using IPM as an oil phase.

IPM/Tween 80/polyethylene glycol-400/ethanol system

For this also, four-phase diagrams were studied for ratios 1:3:0.25, 3:1:0.25, 1:1:0.5 and 1:1:1 w/w. But, it was found that except 3:1:0.25, all the other three diagrams had a greater gel area. Hence, the microemulsion region under the phase diagram of 3:1:0.25 was considered for further study. The resulting phase diagram is shown in Figure 2.

Selection of the microemulsion composition

As the present formulation is meant for intranasal delivery, a less-viscous microemulsion formulation was desired for the sprayability of the nasal formulation by a pump device. The selection of the components of the microemulsion^[9,10] was decided on the basis of three criteria:

1. The solubility of the valproic acid should be increased to such a level that the therapeutic concentration of the

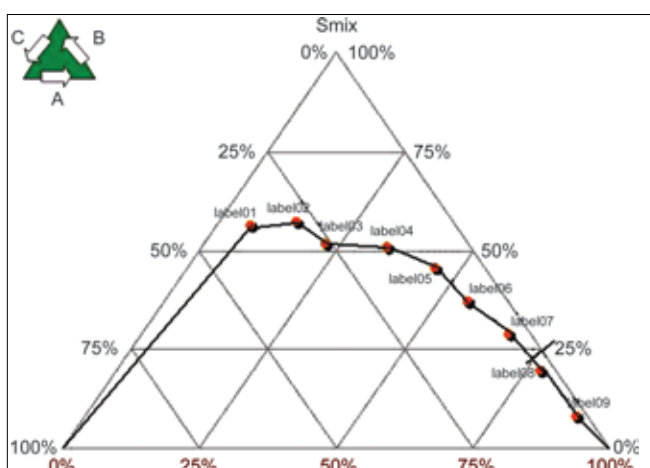


Figure 1a: Pseudoternary phase diagram for the composition isopropyl myristate (IPM)/Tween 80/polyethylene glycol-400 system (1:1)

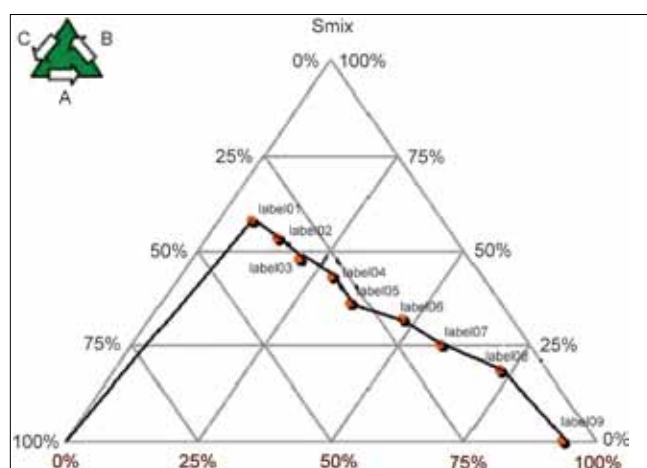


Figure 1b: Pseudoternary phase diagram for the composition isopropyl myristate (IPM)/Tween 80/polyethylene glycol-400 system (2:1)

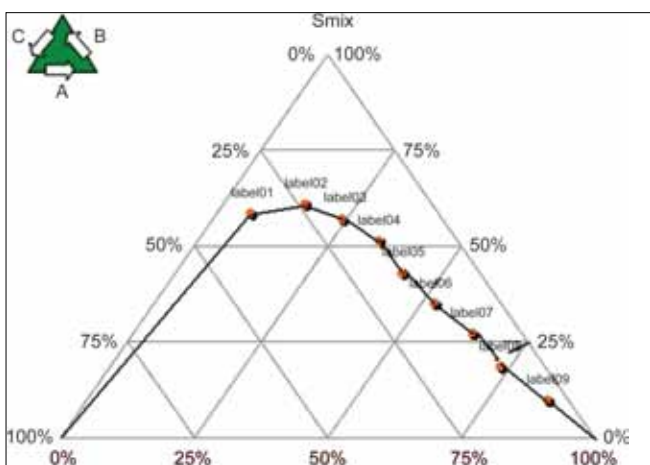


Figure 1c: Pseudoternary phase diagram for the composition isopropyl myristate (IPM)/Tween 80/polyethylene glycol-400 system (3:1)

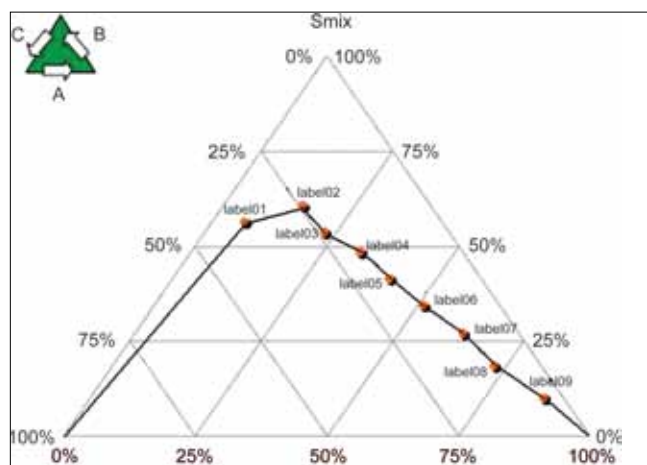


Figure 2: Pseudoternary phase diagram for the composition isopropyl myristate (IPM)/Tween 80/polyethylene glycol-400/ethanol (3:1:0.25)

- drug should be achieved in the CSF after administration.
- The viscosity of the microemulsion should be low enough to deliver the solution to the brain through the olfactory region.
 - The amount of water should be greater than 10% for minimum nasal irritation.

The concentration of the valproic acid that should be present in the CSF for its therapeutic activity is $78 \mu\text{g/ml}$.^[11] If $400 \mu\text{l}$ of the preparation can be instilled at a time, the target concentration of the drug in the microemulsion is fixed at $56 \mu\text{g/ml}$. Considering the oil-retaining capacity, the oil concentration of the microemulsion system was fixed as 10%.

Three microemulsion regions were chosen for the final formulation from which two were with a 3:1 w/w composition and were defined as IPM: Tween 80:polyethylene glycol-400 (formulation 1 and formulation 3) and one with 3:1:0.25 w/w composition and was defined as IPM:Tween 80:polyethylene glycol-400:ethanol (formulation 2), respectively. The detailed compositions of these formulations are shown in Table 1.

Evaluation of the selected microemulsion composition

The microemulsion prepared by the selected composition was evaluated for parameters like viscosity, zeta potential, pH, conductivity and refractive index. The results are shown in Table 2.

In vitro drug diffusion studies

The drug content in the formulations was found to be in the range of 1.5-1.9%.

Table 1: Composition of the selected microemulsion formulations

Formulation no.	Composition of S_{mix} (surfactant: co-surfactant)	Name of the ingredients	% used
1	(3:1)	IPM (oil)	9
		Tween 80	26
		PEG-400	9
		Water	46
2	(3:1:0.25)	IPM (oil)	8
		Tween 80	24
		PEG-400	9
		Alcohol	2
3	(3:1)	Water	47
		IPM (oil)	10
		Tween 80	28
		PEG-400	9
		Water	43

Table 2: Evaluation parameters with observations of the selected microemulsion formulation

Characteristic formulation	pH	Conductivity	Zeta potential (mv)		Refractive index	Viscosity (cps)
			At 30°C	At 45°C		
1	6.24	-0.3	0.19×10^{-4}	0.21×10^{-4}	1.45	46.52
2	6.04	-0.2	0.20×10^{-4}	0.26×10^{-4}	1.52	27.88
3	6.13	-0.3	0.26×10^{-4}	0.37×10^{-4}	1.40	39.29

The drug release through the bovine nasal mucosa was studied and it was found that the drug release pattern shows a lag time of about 45 min, after which it increased linearly. Table 3 shows the %drug diffused of the formulations.

From Table 3, it is clear that the release of the drug from all the formulations showed a lag time of 45 min and then an increase in drug release was observed, but up to 90 min only 1.40-1.64% of the drug was released. From the drug diffusion study, it was found that formulation 3 shows a maximum drug diffusivity with a lag period of 45 min. Figure 3 shows the %drug diffused with time.

Stability studies

Stability studies were carried out for 3 months at 40°C and 75%. After 3 months, the formulation were taken out and again evaluated for parameters like viscosity, conductivity, pH and clarity. All the formulations were found to be stable after stability studies.

CONCLUSION

The microemulsions prepared by the selected compositions from the pseudoternary phase diagram using different proportions of oil, surfactant and co-surfactant were found to be stable and clear for a substantial period of time. Hence, the microemulsion solutions prepared by IPM:Tween 80:polyethylene glycol-400:ethanol (3:1:0.25 w/w) and IPM:Tween 80:polyethylene glycol-400 (3:1 w/w) were evaluated for further studies. The prepared microemulsions were found to be stable at ambient temperature for 3 months. In the *in vitro* drug diffusion study, it

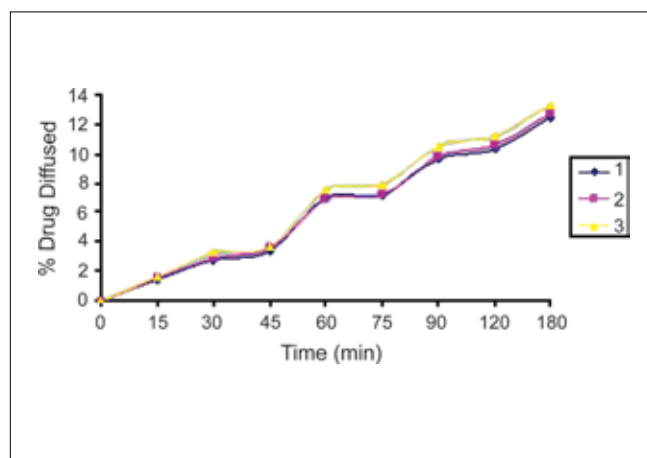


Figure 3: Comparison of %drug diffused from the three selected microemulsion formulations

Table 3: Percent drug diffused of the formulations

Time (min)	% drug diffused		
	Formulation 1	Formulation 2	Formulation 3
15	1.46	1.56	1.69
30	2.83	2.91	3.33
45	3.45	3.56	3.69
60	6.98	6.94	7.49
75	7.19	7.15	7.91
90	9.63	9.79	10.53
120	10.34	10.56	11.19
180	12.45	12.65	13.29

was found that the microemulsion system containing valproic acid showed a fractional diffusion efficiency and hence a better brain bioavailability efficiency.

In conclusion, the microemulsion system is a promising approach for bioavailability improvements. Further studies are necessary to investigate and increase the drug-loading capacity of the microemulsion system containing valproic acid as a therapeutic agent.

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