

PREPARATION AND EVALUATION OF TRANSNASAL MICROEMULSION OF VIGABATRIN

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ABSTRACT

Introduction: The objective of this study was to develop novel transnasal microemulsion containing Vigabatrin for treatment of epilepsy. **Method:** Oleic acid was selected as oil while Tween 80 and ethanol were selected as surfactant and co-surfactant respectively based on solubility results. Optimized ratio of Tween 80 and Ethanol was selected after developing pseudoternary phase diagrams for different ratio and microemulsions were prepared. The prepared microemulsions were evaluated for globule size, viscosity, pH, and % transmittance. *Ex-vivo* diffusion study for optimized microemulsion was performed through goat nasal mucosa where in diffusion flux and permeability coefficients were determined. Pharmacological performance was screened in rats by electrically induced seizures. **Result:** It was found that optimized microemulsion was stable and transparent. Pharmacological evaluation indicated significant reduction ($p < 0.001$) of seizures in rats treated with optimized formulation in comparison to rats treated with oral Vigabatrin microemulsion and nasal Vigabatrin solution which suggested Vigabatrin transnasal delivery system as an effective alternate therapy for treatment of epilepsy. **Conclusion:** Transnasal microemulsion of Vigabatrin was successfully formulated using Tween 80 as surfactant and ethanol as co-surfactant in the formulation to treat epilepsy.

Key words: Transnasal Microemulsion, Vigabatrin, Epilepsy.

INTRODUCTION

Intranasal Drug Delivery for Brain Targeting: Nasal drug delivery system used conventionally for local delivery of drugs for treatment of nasal allergies and infections. It has been found that the nasal route is safe and acceptable alternate to oral and parenteral administration of drugs. Nasal route is found to have potential benefit for targeting drugs to CNS via different mechanisms. Many researchers have reported observations presented in support of assertions of nose-to-brain transport. Many previously relinquished potent CNS drug candidates promise to become successful CNS therapeutic drugs via intranasal delivery. Many nasal formulations, such as ergotamine (Novartis), sumatriptan (GlaxoSmithKline), and zolmitriptan (AstraZeneca) have been marketed to treat migraine recently. Now-a-days studies are focused toward intranasal administration for drug delivery to the brain especially for the treatment of diseases, such as epilepsy, migraine, eme-

sis, depression angina pectoris and erectile dysfunction [1-3].

It has always been challenging to treat CNS disorders because of a variety of formidable obstacles for effective and persistent delivery of drugs. Although the drugs used for the treatment of CNS disorders are potent, their clinical failure is not only due to lack of drug efficacy but also due to shortcomings in the drug delivery approach. Hence, scientists have been exploring the novel approaches so that delivery of the drugs can be enhanced and /or restricted to the brain and CNS.

Recently many advanced and effective approaches to the CNS drug delivery systems have been emerged. Intranasal drug delivery is one of the focused delivery option for brain targeting as brain and nose compartments are connected to each other via olfactory/ trigeminal route via peripheral circulation [4-6].

Realization of nose to brain transport and the therapeutic viability of the route can be traced from the ancient times and has been successfully investigated for rapid and effective transport in last two decades. Transnasal drug delivery delivers drug directly to the brain besetting BBB and reduces drug delivery to the non-targeted sites, which results in reduction in dose, systemic dilution and first pass metabolism of the drug.



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Direct nose to brain transport results into rapid and higher uptake in the brain, which provides an alternative option of self-medication in the management of emergencies. Thus, nasal drug products development for brain targeting is extremely challenging task. For overcoming the obstacles, better understanding in terms of factors which are involved in the direct nose to brain transport (physicochemical factors and formulation factors) and transport mechanisms is of utmost importance. Many sophisticated and effective approaches to the CNS drug delivery have emerged in recent years [7,8].

Certain pharmaceutical challenges like low bioavailability, local irritation and toxicity upon long term usage; can be eliminated by synthesis of more lipophilic analogues, enzyme inhibitors, permeation enhancers, colloidal, bio adhesive and novel drug delivery systems like microemulsion, liposomes and nanoparticles[9].

With all its ingrained advantages, intranasal route has been signified as the most potential approach for delivery of drugs to the brain/CNS.

MATERIALS & METHODS

Materials

Drug sample: Vigabatrin was donated by Lupin Pharma, Pune, India. Oleic acid, Tween 80 and Ethanol were purchased. All other chemicals were of analytical grade and purchased commercially. Water used was double distilled throughout the study.

Study design: An experimental animal based study

Ethics approval: The experimental protocol was approved by the Institutional ethics Committee. Committee (COPH/IAEC/2017/11).

Study place: DVVP Foundation's College of Pharmacy, Ahmednagar.

Study duration: 1 week

Sample size: Thirty rats were used in the present

Inclusion criteria: Maximal Electroshock: Albino rats of Wistar strain weighing between 150-200gm and exhibiting clear hind limb extension phase during electrically induced convulsions were included in the present study.

Methods

Solubility: The solubility of Vigabatrin in various components (oils, surfactants and co-surfactants) was determined by adding an excess of Vigabatrin to each cap vial containing 5 ml of selected vehicles. The mixture was heated at 40°C in water bath to facilitate the solubilisation. Formed suspensions were then stirred for 48hrs on magnetic stirrer. Then each suspension was centrifuged at 3000 rpm for 5 min, and supernatant

was taken and diluted with methanol and Vigabatrin was determined by UV Spectrophotometer at wavelength 222.2 nm[10].

Preparation of pseudoternary phase diagram:

Pseudoternary phase diagrams were constructed to obtain the appropriate ratio of surfactant: co-surfactant which can result into large existence of ME area. Water titration method was used to construct the phases. Surfactant (Tween 80) and co-surfactant (ethanol) were mixed (Smix) in different weight ratios (1:1, 1:2 and 2:1). Oil (oleic acid) and Smix (tween 80 & ethanol) were mixed thoroughly in different weight ratios from 1:9 to 9:1 in different glass vials and diluted with distilled water in a drop wise manner till it changed from transparent to opaque. By joining the change points, the boundaries of phases formed were obtained in the phase diagrams. All samples exhibiting a transparent and homogeneous state were assigned to an ME region, a monophasic area, in the phase diagram. The pseudoternary phase diagrams were constructed by using software [11].

Physicochemical characterization of Vigabatrin: Percentage transmittance determination of each sample was done at 222 nm using distilled water as reference. One drop of Microemulsion (ME) was placed on slide and refractive indices of MEs were measured by using Abbe refractometer. Isotropic nature of MEs was verified by placing a drop of ME on slide with cover slip on it and observing it under polarized light using polarizing microscope. Viscosity of the MEs was measured by a Brookfield viscometer at room temperature by using LV III spindle. Electrical conductivity of ME was measured using a conductivity meter at ambient temperature and the pH of ME was measured by using pH meter. Droplet size and Zeta potential distribution were also determined. Each sample was suitably diluted five times with filtered distilled water and placed in a disposable zeta cell. Samples were centrifuged at 3000 rpm for 15 min to determine centrifugation stability. Experiments were performed in triplicate for each sample[12].

Ex-vivo diffusion study: The use of natural membranes is vital for predicting the potential drug release characteristic. Freshly excised goat nasal mucosa was obtained from slaughter house and dipped immediately in phosphate buffer (pH 6.4). The isolated mucosal membrane was washed with phosphate buffer (pH 6.4). Ex-vivo drug diffusion study was performed using a Franz-type diffusion cell with a diameter of 10 mm and mucosa thickness of 0.20 mm. Phosphate buffer (pH6.4) was used to stabilize the tissue. The receptor compartment was filled with 10 ml diffusion media (phosphate buffer pH 6.4 & 30% PEG 400) to maintain perfect sink condition while 2 ml ME (30 mg/ml) was placed in donor compartment. Continuous slow stirring was maintained in receptor compartment. Similarly ex-vivo diffusion of pure drug was conducted by placing 2 ml of drug solu-

tion in PEG 400 (30 mg/ml). At periodic intervals, the withdrawn samples from the receptor compartment were filtered through 0.45 mm nylon filter paper and analysed using a UV Visible spectrophotometer at 222 nm. Equal volume of diffusion medium was added to replace each removed sample. The cumulative amount of Vigabatrin permeated through the skin was determined [13].

Pharmacological screening:

Grouping: Rats were divided into 5 groups (n=6). Group 1 was not subjected to any treatment served as control. Groups 2, 3 were treated intranasally with Vigabatrin solution and Vigabatrin microemulsion respectively containing Vigabatrin equivalent to 8.18mg/kg body weight (using a micropipette attached with low density polyethylene (LDPE) tubing, having 0.1 mm internal diameter at the delivery site). Groups 4, 5 animals were treated orally with Vigabatrin solution and Vigabatrin microemulsion respectively containing Vigabatrin equivalent to 8.18mg/kg body weight.

Methodology [13]

Electroconvulsions were produced by applying current (150 mA. 0.2s) through ear clip electrodes using electro convulsimeter after 60 min of administration of formulations and different phases of seizures were measured. Tonic phase (E phase) was observed after application of current which was characterized by maximal extension of the anterior and posterior legs. When the tonic phase ceases, clonic phase begins; which was characterized by paddling movement of hind limb and shaking of body. Stupor phase was observed after tonic and clonic phase, during which rat remained silent without any movement. Total time from starting of tonic phase till animal regains its normal movement was recorded as recovery time.

RESULT

Solubility:

The saturated solubility of Vigabatrin in various oils was reported. Nasal formulations should have the least volume. It can be done if the components of the ME that are chosen have highest solubility for the drug. The solubility of the drug was determined in each component of ME sequentially oil, surfactant and then cosurfactant. Highest solubility was observed in oleic acid. Thus oleic acid was selected as oil for preparation of ME. Among the surfactants studied, Tween 80 showed the highest solubility for Vigabatrin and previous studies have reported improved nasal absorption when Tween 80 was used as one of the ingredient.

Pseudoternary phase diagrams: The components that showed maximum solubility were further optimized using pseudoternary phase diagram as shown in figure 1. The zone of ME was obtained. Six formulations were

then taken from each corner at random and the best formulation was characterized thoroughly. According to the ME area in the phase diagram, the Vigabatrin loaded ME formulations were prepared as per the composition shown. ME systems were obtained by mixing oil, surfactant and co-surfactant together and adding appropriate quantity of Vigabatrin and adding precisely distilled water drop by drop to these oily phases with magnetic stirring at ambient temperature. The final concentration of Vigabatrin in ME systems was 30 mg/ml.

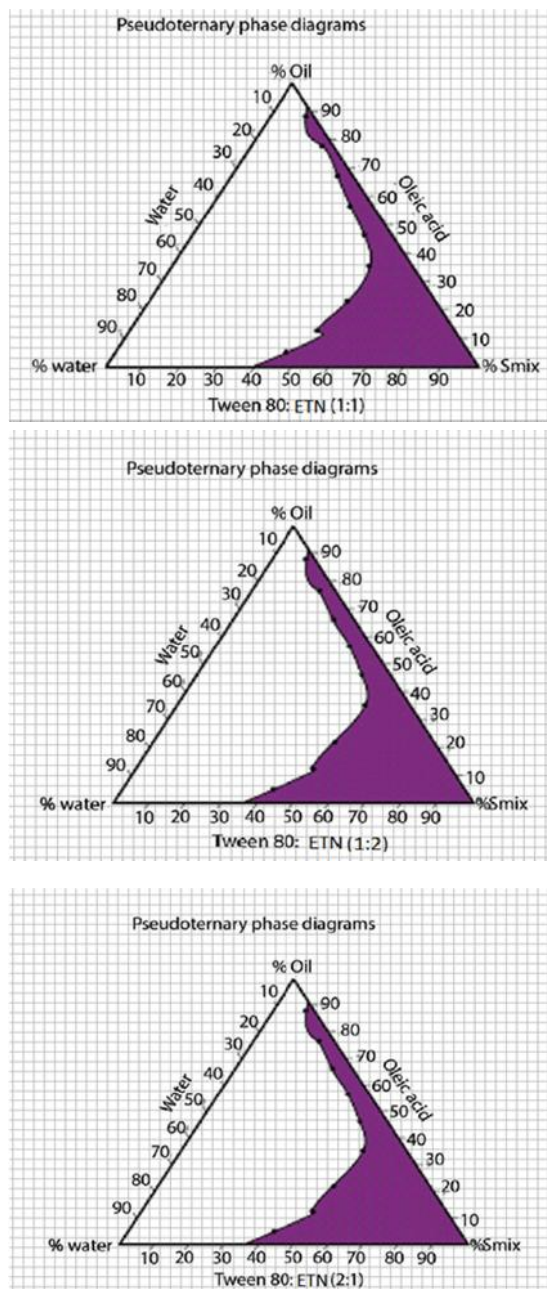


Figure 1. Pseudo ternary phase diagram using oleic acid as oil, Tween 80 as surfactant, Ethanol as co-surfactant and Water (Tween 80: Ethanol = 2:1,1:1 and 1:2)

Table 1. Composition of selected ME

	F1	F2	F3	F4	F5	F6
Vigabatrin	3	3	3	3	3	3
Oleic Acid	10	10	10	15	15	15
Tween 80	32.5	34.5	36.5	32.5	34.5	36.5
Ethanol	32.5	34.5	36.5	32.5	34.5	36.5
Water	25	21	17	24	16	12

Physicochemical characterization of Vigabatrin microemulsion

The microemulsions for nasal administration are expected to show higher permeation rate with minimum globule size. Prepared microemulsions are expected to have good physical stability with respect to phase separation and/or flocculation. This can be achieved when zeta potential values are negative. The pH values need to be close to nasal secretions (4.5-6.5) and viscosity should be moderate. Viscosity and pH are important factors affecting mucociliary action which may cause another set of complications. Additionally the pH deviations may cause irritation to the patient. Higher viscosity is preferred as it increases residence time but permeation rate also decreases with increase in viscosity and hence formulation should have moderate viscosity. The refractive index was 1.33 and % transmittance was found to be greater than 99% which confirmed that prepared Vigabatrin ME was transparent. Viscosity of the optimized formulation is suitable for nasal administration. Zeta potential was negative -52.68 which indicated the stability of formulation as there were less chances of globules aggregation. After centrifugation cycle it was found that ME F3 was stable and no separation was observed which indicated centrifugation stability. The optimized ME F3 remained clear and transparent even after 3 months of storage. ME F3 was optimized from the prepared formulations as per the attributes decided earlier in this section. Pharmacological screening was performed by using optimized ME F3.

Ex-vivo Diffusion Study:

From the data we have found that the prepared transnasal microemulsion releases drug faster due to presence of oleic acid, tween 80 and ethanol which act as permeation enhancer. A biphasic release profile was obtained due to Vigabatrin release in which initial faster release was due to solubilized drug in continuous phase while slower rate was due to Vigabatrin.

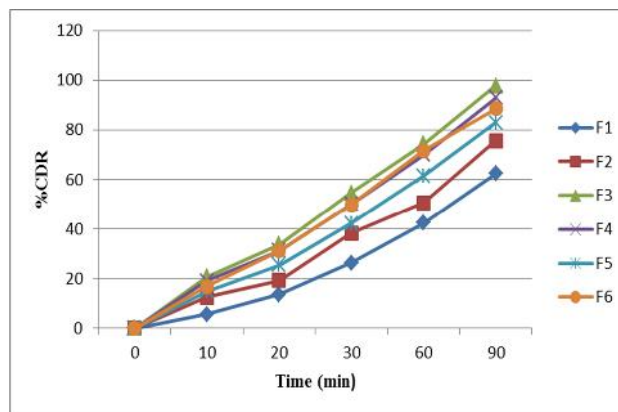


Figure 2. CDR Vs Time

Pharmacological Screening

The antiepileptic activity was assessed by observing the extent of different stages of seizures including duration of seizure, extension phase (E), clonus phase (F) and stupor phase (S) and results were represented in Figs. 3 and 4. One way ANOVA followed by Tukey test were employed to determine significant differences between the control and treated groups for exact comparison in pharmacodynamic study. Significant reduction in E phase, clonus, stupor and duration of seizure was observed in the rats treated with Vigabatrin ME by intranasal route and in comparison to group of rats treated VIGABATRIN solution administered IN and Vigabatrin ME administered intranasally (P< 0.05, n=5). The results clearly indicated lesser intensity of seizure and rapid recovery from seizures in the rats treated with intranasal Vigabatrin ME.

Table 2. Physicochemical parameters

	F1	F2	F3	F4	F5	F6
Globule size (nm)	368 ± 21	325 ± 15	196± 8	392± 23	384±12	335± 10
pH	5.32	5.34	5.48	5.61	5.32	5.74
Viscosity (cps)	0.8872 ±0.57	0.8875 ±0.57	0.8874 ±0.23	0.8877 ±0.57	0.9574 ±0.5	1.121 ±0.14
Absolute drug content (%)	85±0.013	88±0.024	94±0.02	90±0.08	78±0.052	74±0.06
% Transmittance	98.88	99.21	99.85	98.73	98.23	96.23
All values are expressed as mean of three readings						

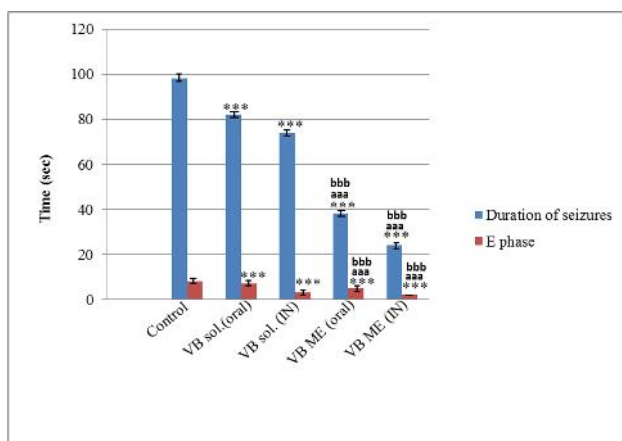


Figure 3. Duration of seizure and E phase for different treatments of Vigabatrin.

where, *** indicate significant difference in comparison to control, ^{aaa} indicate significant difference in comparison to Vigabatrin solution (IN), ^{bbb} indicate significant difference in comparison to Vigabatrin ME (oral).

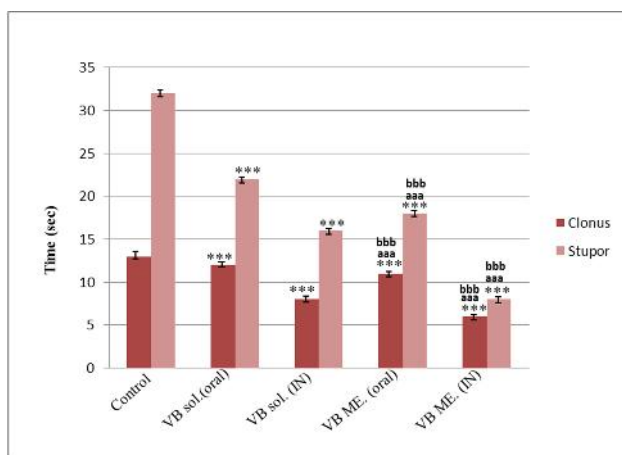


Figure 4: Duration of clonus and stupor for different treatments of Vigabatrin.

where, *** indicate significant difference in comparison to control, ^{aaa} indicate significant difference in comparison to Vigabatrin solution (IN), ^{bbb} indicate significant difference in comparison to Vigabatrin ME (oral).

DISCUSSION

In present study Tween 80, surfactant with HLB 14, was selected as a surfactant and Ethanol was selected as cosurfactant, which also acts as permeation enhancer [10-12].

Drug from ME permeates rapidly through nasal mucosa in comparison to solution. The graphical representation of in-vitro drug diffusion study of transnasal microemulsion. 3rd formulation had more diffusion as compared to others [13].

Vigabatrin are coupled to diglycerides or modified diglycerides. While increased lipophilicity may improve movement across the BBB, it also tends to increase uptake into other tissues, causing an increased tissue

burden. This selectivity in delivery is especially detrimental [14]. The Vigabatrin transnasal 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 solution of Vigabatrin. This may help in decreasing dose and frequency of administration of drug and may possibly maximize therapeutic benefits and may also reduce cost of therapy. To establish clinical safety and efficacy of this formulation, detailed animal study and thorough clinical trials are required.

CONCLUSION

Transnasal microemulsion of Vigabatrin could be successfully formulated using Tween 80 as surfactant and ethanol as co-surfactant in the formulation to treat epilepsy.

Conflict of interest: Nil

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