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Original Article

Microemulsion for Simulataneous Intransal Delivery of Carbamazepine and Vitamin B_6 for Treatment of Epilepsy

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ABSTRACT

Background: Status epilepsy is a neurological emergency characterized by severe bouts of seizure. It has been reported that combination of antiepileptic drug with Vitamin supplements reduce the drug resistance and rapid improvement was also observed in epilepsy patients. In this study, we tried to develop novel intranasal microemulsion for simultaneous delivery of carbamazepine and Vitamin B_6 for treatment of epilepsy. **Materials and Methods:** For simultaneous estimation of Carbamazepine and Vitamin B_6, second derivative spectroscopy method was developed and validated. The ME containing CBZ, in lipophilic phase and Vitamin B_6 in aqueous phase was studied for physicochemical characters and *ex vivo* diffusion through sheep nasal mucosa. The maximal electroshock (MES)-induced seizure models was used to establish the pharmacodynamic credentials of the microemulsion. **Results:** The wavelength selected for Carbamazepine and Vitamin B_6 were 231 nm and 250 nm respectively. It was found that prepared microemulsion is stable with globule size of 395+12 nm. Diffusion across sheep nasal mucosa followed zero order kinetics for carbamazepine and Vitamin B_6 in the prepared microemulsion. It was found that the hind limb extension time and the recovery time were significantly different from control group (p<0.05). The microemulsion remained stable after dilution, centrifugation and freeze thaw cycle as tested for particle size, zeta potential and percentage transmission. **Conclusion:** The proposed microemulsion should form the basis for treatment of epilepsy which warrants co-administration of micronutrients.

Key words: Carbamazepine, Intranasal Microemulsion, Pharmacodynamic, Simultaneous Delivery, Vitamin B_6.

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INTRODUCTION

Status epilepsy is a neurological emergency characterized by severe bouts of seizure. Single drug therapy is generally preferred therapy for treatment of epilepsy.[1] Carbamazepine (CBZ) is a major antiepileptic drug used for the treatment of epilepsy. Currently CBZ is available only in the form of oral dosage forms. Oral administration of CBZ has certain limitations like poor absorption, prolonged tmax along with GIT side effects. It has been observed prolonged CBZ therapy reduces plasma B6 level significantly.[2,3]

Vitamin B_6 plays an important role in treatment of epilepsy. Pyridoxal phosphate, active from of Vitamin B_6 is essential for formation of inhibitory neurotransmitter GABA. In absence of GABA the neurons continues to fire and this leads to epileptic seizure. However it has been observed that drug resistance occurs often in epilepsy patients. Carbamazepine is a first line therapy used for epilepsy treatment since long and hence chances of development of drug resistance are high. Previous studies reported that combination of antiepileptic drug with Vitamin supplements reduce the drug resistance and rapid improvement was also observed in epilepsy patients.[3,4] Thus the novel formulation containing CBZ and Vitamin B_6, is required which can provide rapid onset of action and reduce occurrence of drug resistance is required.

Aqueous solubility of CBZ is very poor while Vitamin B_6 is hydrophilic in nature and hence microemulsion seems to be convincing carrier as it can incorporate both lipophilic (CBZ) and hydrophilic (Vitamin B_6) in the same system. A microemulsion is thermodynamically stable transparent system containing oil, surfacatnat, cosurfactant and aqueous phase. Along with the other advantages, a peculiar advantage of ME is it can enhance drug permeation through mucosal membrane due to its lipophilic nature and smaller globule size.[5]

In recent year's drug delivery to brain through the nasal route has received a lot of attention, because it offers several advantages including rapid onset of action due to bypass of BBB, ease of administration, avoidance of first pass metabolism.[6,7] This has been proven by our previous researcher for Diazepam and Phenytoin, other anticonvulsant drugs.[8,9] Thus, it is expected that intranasal and simultaneous delivery of CBZ and Vitamin B_6 may show faster recovery from epilepsy. The aim of this investigation was to incorporate Vitamin B_6, in hydrophilic phase of the optimised CBZ intranasal ME. The ME containing CBZ, in lipophilic phase and Vitamin B_6 in aqueous phase was further evaluated for physicochemical parameters. *Ex vivo* diffusion study was performed through sheep nasal mucosa. The % release of CBZ and Vitamin B_6 was calculated by developed second derivative UV spectroscopy method. The CBZ ME and the ME containing CBZ and Vitamin B_6 are further evaluated by pharmacodynamics parameters, in rats after inducing seizures electrically. It was hypothesised that simultaneous release of CBZ and Vitamin B_6 would result into rapid recovery of patients and would help to maximize therapeutic advantages of drugs.

MATERIALS AND METHODS

Materials

CBZ was obtained as gratis sample from Lincoln Pharma (India) and diethylene glycol monoethyl ether (Transcutol®), polyglyceryloleate (PlurolOleique CC497®) were received as gratis samples from Gattefossé (Toronto, Canada). Tween 80, Oleic acid, propylene glycol and polyethylene glycol 400 were purchased from Sigma Aldrich (India). The study was performed by using double distilled water throughout the study. All analytical chemicals were used for study and were used as received.

Animal

Sprague Dawley rats of either sex aged between six and eight weeks, weighing between 200 and 250 g, were procured from the central animal facility of L. J. Institute of Pharmacy and Research Centre, Ahmedabad. The rats were maintained in a room at a temperature of 25±5°C and in relative humidity of 55±5 % under a cycle of 12-hr light/dark. Free access to food (standard pellet diet, Pranav Agro, India) and water is provided to animals.

METHODS

Preparation of microemulsion containing CBZ and Vitamin B_6 (MB6)

The microemulsion (MB6) was prepared by water titration method. Optimized formulation (MCBZ), from our previous study[10] was selected and Vitamin B_6 was incorporated in aqueous phase. The oleic acid was taken as oil, while Tween 80 and Transcutol were taken as surfactant and cosurfactant respectively and water was added drop by drop slowly. The resultant formulation was stirred on magnetic stirrer at 35°C for 30 min.

Development of simultaneous estimation method for CBZ and Vitamin B_6

All reagents were tested for stability in solution and stability of the same during the actual analysis. The behaviour of the analytes remained unchanged up to about 24 hr from their preparation at the room temperature and the same for 48 hr from their preparation in refrigerator. Both CBZ and Vitamin B_6 were found to be stable during each kind of experimental measurements. Each measurement was done at room temperature throughout the method development and validation.

The diluted samples of CBZ (10µg/ml) and Vitamin B_6 (10µg/ml) were scanned individually in UV spectrophotometer in 200-300 nm range. The second order derivative spectra of CBZ and Vitamin B_6 were obtained by UV prob software of version 2.0. The scaling factor was set as 100 and delta lambda was set as 4 for all type of derivative measurement. After examining the overlain second order derivative spectra, wavelengths were selected for further measurement and evaluation of both the drug simultaneously. Serial dilutions of 6,12,15,18 and 24 µg/ml were prepared for measuring zero-order and subsequently for the measurement of second derivative spectra. Second derivative spectra of CBZ, Vitamin B_6 and overlay spectra of CBZ and Vitamin B_6 were taken. The method was validated as per the ICH guidelines for analytical method.[11,12]

Accuracy

Accuracy of proposed method and interference from excipients was determined by recovery study. The recovery study was performed by using spiking method. This study was performed by addition of known amounts of CBZ and Vitamin B_6 to a known concentration of the newly developed formulation. The amounts of standard recovered were calculated in the terms of mean recovery with the upper and lower limits of percent relative standard deviation.

Precision

Inter and intra day precision for the developed methods were calculated in form of % RSD. The experiments were repeated for five different days for interday precision and five times a day for intra day precision. The concentration values for both inter and intraday precision were calculated five times separately and percent relative standard deviation were calculated. Finally the mean of % RSD was calculated.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated according to the 3 s/m and 10 s/m criterions, respectively, where *s*, is the standard deviation of the absorbance (n = 10) of the sample and *m* is the slope of the respective calibration curve.

Reproducibility

Shimadzu UV 1700 and Shimadzu UV 1601 were used to check reproducibility of the developed method. The average value of % RSD was determined.

Physicochemical characterization of MB6

The prepared MB6 formulation was tested for various physicochemical parameters. The pH of the formulation was measured by using pH meter (Systronic 335, India). The conductivity was measured by using conductivity meter (CM 180ELICO, India) at room temperature. The refractive index of formulation was measured by using Abbe refrectometer. A drop of MB6 was placed on slide covered with coverslip and observed under polarized light using polarizing microscope (Carl Zeiss, Germany). The formulation was 5 times diluted with filtered distilled water and placed in a disposable zeta cell of Malvern zetasizer (NanoZS, Malvern Instrument UK) for measurement of globule size and zeta potential. %Transmittance was checked against distilled water using UV visible spectrophotometer at 650 nm. Viscosity of formulation was measured by using Brookfield viscometer at room temperature using LV IIII spindle.[13,14] Drug content measured by using UV-visible spectrophotometer (UV 1800,Shimadzu, Japan). Stability was also another important attribute to be considered for ME. Thus, it was evaluated by subjecting developed MEs to centrifugation test (3000 rpm for 15 min), and freeze thaw cycle.[15]

Ex vivo diffusion study

Comparative *ex vivo* nasal diffusion study was performed between MB6, CBZ solution (SCBZ) and Vitamin B_6 solution (SB6) using Franz diffusion cell method. Piece of freshly excised sheep nasal mucosa was rinsed thoroughly using phosphate buffer saline (PBS) pH 6.4 to remove any adhered tissues from the mucosa which was used as a dialyzing membrane. The nasal mucosa having uniform thickness of 0.20 nm was mounted in the cell such that mucosal surface faced donor compartment and serosal surface faced receptor chamber. Receptor chamber was filled with 10 ml diffusion media (PBS pH 6.4 and 30% PEG 400) and the contents were kept on slow stirring speed. The receptor compartment temperature was maintained at 37 ± 2°C. The arrangement allowed the nasal mucosa to be sandwiched between the donor compartment and the receiver compartment using clamps. 2 ml of the formulation was placed in the donor compartment and diffusion was allowed to run for 2 hr. Aliquots of 1 ml were withdrawn at different time points from the receptor chamber, and replaced with an equal volume of fresh PBS. The percentage drug diffusion through the membrane was calculated after analysis of the sample using UV spectroscopic method.[10]

Pharmacodynamic study

Maximal electroshock method: Sprague Dawley rats having weight in the range of 200 and 250 g and exhibiting clear hind limb extension

phase during electrically induced convulsions were incoporated in the present study. Total four groups of animals were prepared and each group contains 6 animals. The different groups received the following treatments. SCBZ (60% PEG 400)/ ME6 containing 3.52 mg CBZ and 0.88 mg Vitamin B_6 was administered to each nostril (using a micropipette attached with LDPE tubing, having 0.1 mm internal diameter at the delivery site) to the first and second group. The third group of rats received intraperitoneal (IP) administration of SCBZ containing 3.52 mg CBZ while the fourth group of rats was treated with kept as control, not receiving any treatment.[16]

Group 1: SCBZ Intransally (IN)

Group 2: MB6 administered IN

Group 3: SCBZ Intraperitoneally

Group 4: No treatment

Electroconvulsions were produced by applying current (150 mA, 0.2 s) through ear clip electrodes using an electroconvulsiometer (INCO, Ambala, India) after 60 min of administration of formulations. The experimental protocol was approved by the Institutional Animal Ethics Committee (No. LJIP/IAEC/09/2011–2012, Dated-09/07/2011).

RESULTS

Preparation of microemulsion containing CBZ and Vitamin B_6

The optimized formulation was selected from our previous study and the optimized formulation contains oil (oleic acid) 8% w/w, Surfactant-cosurfactant mixture (Tween 80-Transcutol (1:1)) 78% and water 14% w/w. CBZ (40 mg/ml) was dissolved in oil phase while Vitamin B_6 (10 mg/ml) was dissolved in aqueous phase. The recommended dose of Vitamin B_6 is 10 mg.[17] Thus the prepared optimized microemulsion contained required dose in 1 ml preparation.

Development and validation of method for simultaneous estimation for CBZ and Vitamin B_6

For determination of CBZ, in the presence of Vitamin B_6, 231 nm wavelength was selected where there is no interference of Vitamin B_6. For determination Vitamin B_6, in the presence of CBZ, 250 nm wavelength was selected where there is no interference of CBZ. Second derivative overlay spectra of CBZ and Vitamin B_6. B_6 were shown in Figures 1,2. Thus both the drugs were determined accurately at the zero crossing points of each other using second derivative spectroscopy. The Regression equation for CBZ and Vitamin B_6 were found to be 0.9996 and 0.9998 respectively. The percentage recoveries for CBZ and Vitamin B_6 were found to be 99.87± 0.247 and 101.22 ± 0.547 respectively. The relative standard deviation for precision studies were found to be less than 2 for both the drugs. The value of Limit of detection was found to be 0.247 mcg/ml and 0.297 mcg/ml for CBZ and Vitamin B_6 respectively. Similarly the value of Limit of quantitation was found to be 2.47 mcg/ml and 2.97 mcg/ml for CBZ and Vitamin B_6 respectively.

Physicochemical characterization of prepared microemulsion

The MB6 was thoroughly characterized (Table 1). The pH of MB6 was found to be 5.28± 0.02 which is closer to that of nasal secretion; thus, it was expected that MB6 will not show any irritancy. The conductivity of the MB6 was found to be 0.24 ± 0.04 mS^{cm-1}. The MB6 was observed as completely dark under cross polarizer that confirms that the prepared ME was colloidal dispersion and optically isotropic in nature. The globule size obtained was 395 ±12 nm with 0.24 PDI indicated that the globule size was suitable and uniform for nasal administration. Zeta potential of MB6 was −10.85 mv which indicates that the MB6 is stable. Greater than

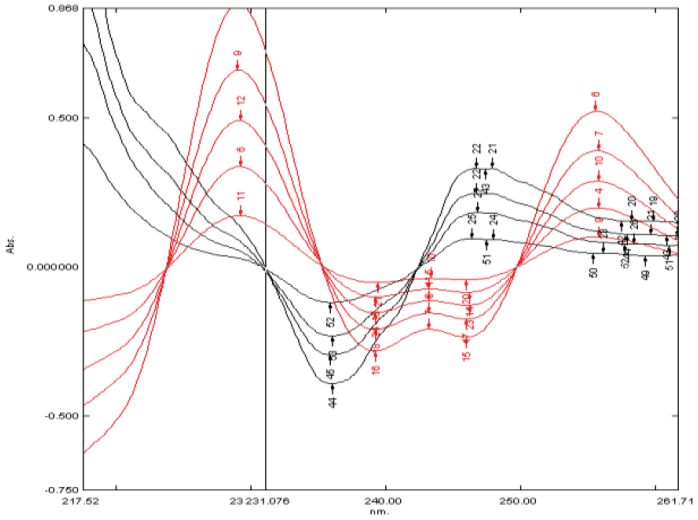

Figure 1: Second derivative overlay spectra of CBZ and Vitamin B_6 reflecting zero crossing point of Vitamin B_6 at 231 nm.

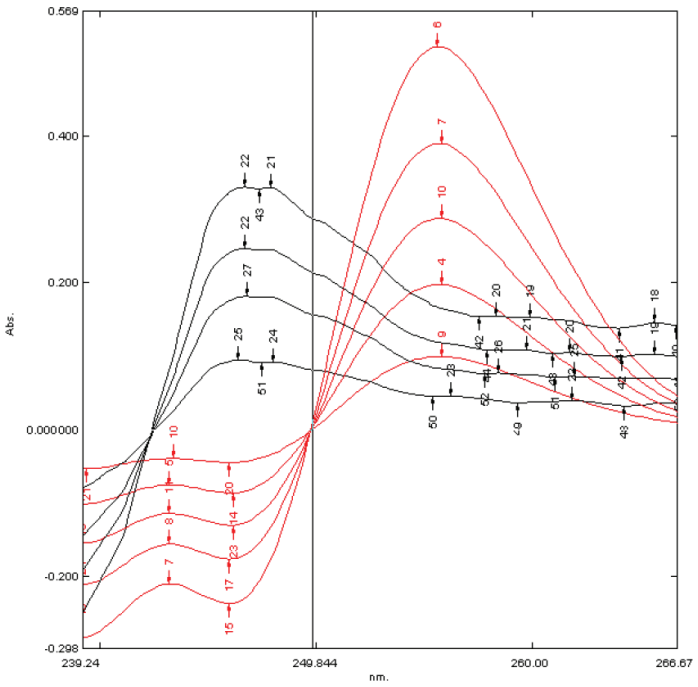

Figure 2: Second derivative overlay spectra of CBZ and Vitamin B_6 reflecting zero crossing point of CBZ 250 nm.

99% transmittance indicated the prepared microemulsion is transparent. The viscosity of MB6 was found to be 194 ± 3 cps, which is suitable for nasal administration.[10] No phase separation was observed I MB6 after centrifugation cycle. The stability study results were recorded in Table 2 and indicates formation of stable microemulsion.

Ex vivo diffusion study

In case of seizure faster delivery of drug to brain is required to achieve rapid recovery from seizure. The % CBZ and Vitamin B_6 diffused from MB6 were graphically represented in Figure 3. The drug permeation kinetics was assessed through statistical model fitting, using Microsoft Excel™ with DDSolver. The CBZ and B6 permeation profile for MB6 through the mucosa was fitted to Zero Order, First order and Higuchi

Table 1: Composition and characterization of MB6.

Carbamazepine	40 mg/ml
Vitamin B_6	10 mg/ml
Characterization	
% Assay (CBZ)	99.54+ 0.78
% Assay (Vitamin B_6)	99.87+ 0.56
Zeta potential (mV)b	−10.85 mv
Globule size (nm)b	395 +12 nm
Polydispersity index	0.24
% Transmittance at 630 nm	99
pH	5.28 ± 0.02
Viscosity at 33°C (cP)	194 + 3 cps
Refractive index at 22°C	
Conductivity	0.24 + 0.04 mS^{cm-1}

Table 2: Stability Study of Final Microemulsion.

	MB6		
	Zeta Potential (mV)	Size (nm)	% Transmittance
Initial	-10.85	395.0	99.56
After centrifugation	-10.27	396	99.45
After Freeze-thaw cycle	-10.15	399.5	99.40

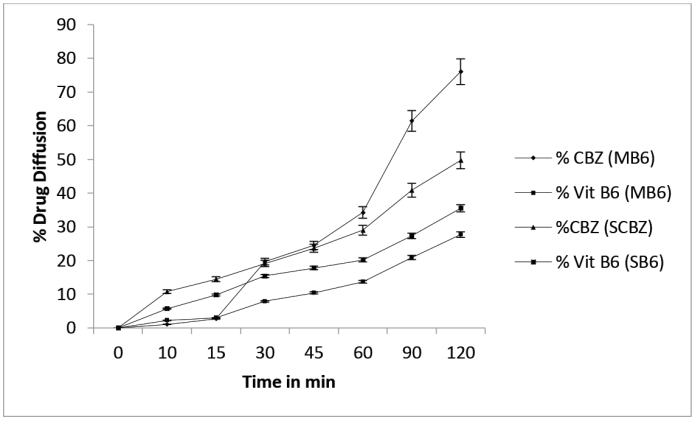

Figure 3: *Ex vivo* diffusion of CBZ and Vitamin B_6 through sheep nasal mucosa from various formulations. All the values are mean ± SEM (*n*=3).

model. Drug permeation followed zero order kinetics for CBZ as well as B6. Similar results were recorded by previous scientists.[18,19]

Pharmacodynamic study

One-way ANOVA revealed a significant difference in the THE of the 4 groups (P<0.001)(Figure 5). The Tukey HSD test compared the THE of the four group and showed that the THE for intranasal solution of CBZ was different from that of microemulsion containing CBZ and B6 and Intraperitoneal solution of CBZ (*P*<0.001). While the THE for MB6 and

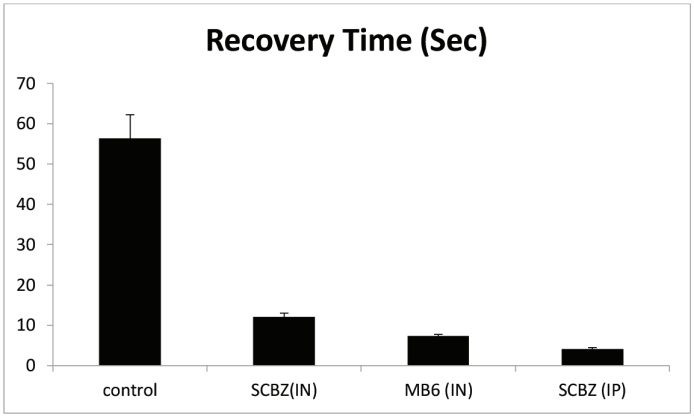

Figure 4: Duration of Recovery time for rats after Intransasal administration of different formulations. All the values are mean ± SEM (*n*=3).

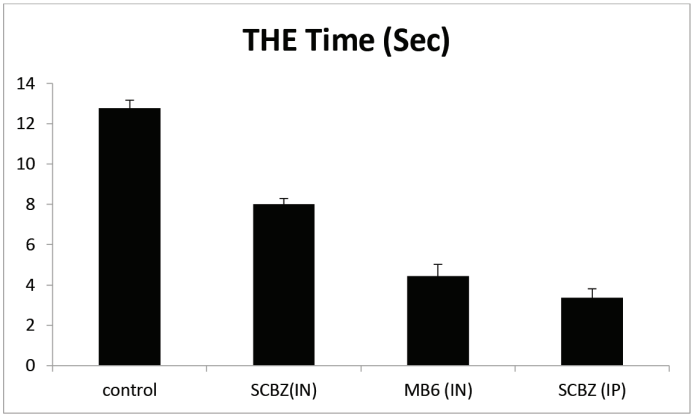

Figure 5: Duration of seizure for different treatment of CBZ formulations. All the values are mean ± SEM (*n*=3).

IP solution did not differ significantly. The Dunnett's test compared the THE for the three groups with that of the control group and showed that THE value for the all the three groups was significantly lower than that of control group (*P*<0.001). The analysis therefore indicates that the reduction in THE value for MB6 and IP solution was similar and significantly lower than that of control group.

One-way ANOVA revealed a significant difference in the recovery time of the 4 groups (P<0.001) (Figure 4). The Tukey HSD test compared the recovery time of the four group and showed that the recovery time for intranasal solution of CBZ was similar to the microemulsion containing CBZ and B6 and Intraperitoneal solution of CBZ. The Dunnett's test compared the recovery time for the three groups with that of the control group and showed that recovery time value for the all the three groups was significantly lower than that of control group (*P*<0.001). The analysis therefore indicates that the reduction in recovery time was similar for the all the three treatment groups.

DISCUSSION

The simultaneous administration of Vitamin B_6 and CBZ plays important role in epilepsy management, even it can reduce occurrence of drug resistance in epilepsy patients. CBZ is lipophilic while Vitamin B_6 is hydrophilic thus for simultaneous administration of drugs, CBZ was

added in oil phase and Vitamin B_6 was loaded in aqueous phase of MB6. The recommended dose of Vitamin B_6 is 10 mg.[17] Thus the prepared MB6 contained required dose in 1 ml preparation.

Simultaneous UV estimation method was developed and validated in the present study. The absorption spectra of CBZ and Vitamin B_6 are completely overlapped which resist the direct measurement of both CBZ and Vitamin B_6 by zero order spectroscopy. To resolve this, second order derivative zero crossing spectroscopic technique is used in which CBZ is measured at zero crossing point of Vitamin B_6 and Vitamin B_6 is measured at Zero crossing point of CBZ. By this method, one can accurately determine both CBZ and Vitamin B_6 without any prior separation of individual drugs. The specificity ad resolution of the method was improved with the use of second order derivative spectroscopy. Thus the method was found to be accurate, precise, simple and easy to perform for simultaneous determination of CBZ and Vitamin B_6 using second derivative spectroscopy.[12]

The physicochemical results indicated the prepared MB6 is suitable for nasal administration as its pH is near to nasal secretion p and hence less chances of irritation. The conductivity results indicated the presence of water in the continuous phase observed in bicontinuous or solution type of MB6 which confirms the formation of solution-type ME.[15] As viscosity of nasal formulation increases residence time of formulation also increases but higher viscosity of system decreases diffusion rate thus moderate viscosity in the range of 100-200 cps is preferred for nasal formulation.[10] The prepared MB6 showed viscosity in the desired range. The globule size below 400 nm indicated that the globule size was suitable and provides faster permeation through nasal mucosa. The uniformity in globule size was confirmed with narrow PDI. The negative results of zeta potential indicates less chances of globule aggregation and the stability of MB6 is further confirmed with the results of stability and centrifugation study. The diffusion from MB6 was very slow initially as compared to SCBZ but this was followed by much higher release. This could be attributed to the partitioning of drug, the drug was trapped in the oil globules so there might be initial slow release but after the lag time the drug could diffuse quickly and completely giving diffusion rate that was higher than the SCBZ. In spite of the fact that Vitamin B_6 is soluble in water higher permeation of Vitamin B_6 from MB6 as compared to SB6 can be correlated to the presence of surfactants in MB6. Oil, surfactant and cosurfactant used in the MB6 were already reported by previous researcher as safe ingredients for nasal delivery by histopathological evaluation.[8,14,16]

The pharmacodynamics data indicates the THE value was significantly lower in the groups of animals treated with MB6 and IP solution in comparison to control animals. The recovery time observed for MB6 group was also significantly rapid in comparison to control group. These outcomes are indicative of rapid and direct nose-to-brain delivery of CBZ and Vitamin B_6 after MB6 intra nasal administration. The results may be accredited to the fact that ME increases permeation of drug through nasal mucosa. These outcomes are in consonance with the results reported by previous researchers that microemusion enhances drug diffusion through nasal mucosa and hence delivers drugs rapidly to brain by nose to brain pathway.[14,16] The microemulsion can also be used for potential carrier for simultaneous delivery of lipophilic and hydrophilic drug.

CONCLUSION

The novel intranasal ME was developed in this study containing CBZ in oil phase and Vitamin B_6 in aqueous phase. An accurate, precise, rapid and simple method was developed and validated successfully for simultaneous determination of Vitamin B_6 and CBZ which indicate the simple use of the developed method for simultaneous determination of Vitamin B_6 and CBZ with variety of formulations. Studies revealed that simultaneous delivery of Vitamin B_6 and CBZ showed faster recovery and shorten THE time in comparison to control group in rats. The study signifies that the developed formulation can be a good platform for brain targeting and can be used for coadministration of micronutrient for treatment of epilepsy. However, thorough animal studies including different animal studies and substantial clinical trials require to be conducted to initiate practice of this formulation in clinical practice.

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CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

ABBREVIATIONS

CBZ: Carbamazepine; **ME:** Microemulsion; **MB6:** Microemulsion containing CBZ and Vitamin B_6; **LOD:** Limit of Detection; **LOQ:** Limit of Quantitation; **IP:** Intraperitoneal.

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