Bulletin of Faculty of Pharmacy, Cairo University (2013) 51, 243–253

Cairo University
Faculty of Pharmacy

Cairo University
Bulletin of Faculty of Pharmacy, Cairo University

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www.sciencedirect.com

ORIGINAL ARTICLE

Formulation consideration and characterization of microemulsion drug delivery system for transnasal administration of carbamazepine

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Received 19 March 2013; accepted 15 July 2013
Available online 9 August 2013

KEYWORDS

Carbamazepine;
Diffusion study;
In vitro mucoadhesion study;
Microemulsion;
Nasal ciliotoxicity study;
Transmission electron microscopy

Abstract The purpose of the present study was to formulate and characterize carbamazepine loaded microemulsion and mucoadhesive microemulsion drug delivery system for its intranasal administration. Carbamazepine microemulsion and mucoadhesive microemulsion were prepared by titration method. The drug-loaded microemulsions were successfully prepared which contain 6% Labrafil M 1944 CS as an oily phase, 32% surfactant mixture of Cremophor RH 40: Transcutol P (4:1) and 62% (wt/wt) aqueous phase. Microemulsion formulation which displayed an optical transparency of 99.95%, globule size of 34.32 ± 1.09 nm, and polydispersity index of 0.127 ± 0.012 was selected for the incorporation of mucoadhesive component. The drug-loaded mucoadhesive microemulsion that contains 0.5% wt/wt of polycarbophil displayed higher *in vitro* mucoadhesive potential (21.0 ± 3.0 min) and diffusion coefficient (0.3172 ± 0.03) than microemulsion. All formulations were found free from nasal ciliotoxicity and stable for 6 months.

1. Introduction

The treatment of brain disorders is the greatest challenge because of a variety of formidable obstacles in effective drug delivery and maintaining therapeutic concentration in the brain. General methods that can enhance drug delivery to the brain are, therefore, of great interest. An alternative central nervous system (CNS) drug delivery strategy that has received relatively little attention is the intranasal route. Drugs delivered intranasally are transported along olfactory sensory neurons to yield significant concentrations in the cerebrospinal fluid (CSF) and olfactory bulb. Recent evidence of direct nose-to-brain transport and direct access to CSF of certain drugs bypassing the bloodstream has been shown in human trials.[1–4]

Carbamazepine (CBZ) is used for the management of epilepsy and several psychiatric diseases. It is traditionally given

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Peer review under responsibility of Faculty of Pharmacy, Cairo University.

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by oral administration but due to its poor water solubility (about 170 mg/L at 24 °C) it is characterized by slow and irregular gastrointestinal absorption. Reportedly, it has an oral bioavailability of less than 50%.[5]

Advantages of microemulsion (ME) include its ease of preparation due to spontaneous formation, thermodynamic stability, transparent and elegant appearance, increased drug loading, and enhanced penetration through the biological membranes, increased bioavailability and less inter- and intra-individual variability in drug pharmacokinetics. These advantages make ME attractive drug delivery systems.[6,7] The advantages of nasal route are rapid absorption, higher bioavailability allowing lower doses, fast onset of therapeutic action, avoidance of liver or gastrointestinal metabolism, avoidance of irritation of the gastrointestinal membrane, reduced risk of overdose, non-invasive administration, ease of convenience and self-medication, and improved patient compliance.[7,8] Targeting the brain via nasal administration of ME has been reported in the literature.[9–14]

Barakat et al. designed hypromellose and Carbopol 974P based gel formulation for intranasal administration of CBZ. Hypromellose and Carbopol were used as mucoadhesive polymers in the formulation to increase the residence time of the gel on the mucosa. Results of this study revealed that concentration of CBZ in the brain was found to be much higher than that in the plasma following intranasal administration; showing that there existed a direct transport pathway from the nose to the brain and that transport was comparatively rapid.[5] Gavin et al. prepared chitosan glutamate based microspheres for the nasal administration of CBZ. The results obtained indicate that the loading of CBZ in chitosan glutamate microspheres increases the amount of the drug absorbed through the nose.[15] However, few formulation factors need to be addressed while designing the drug delivery system for the intranasal administration. A lipophilic component in the formulation seems to be advantageous, but in the case of nasal administration, incompatibilities can be indicated by impairment of ciliary movement. Use of ME may minimize these side effects by administering a lipophilic component in a transparent, water-continuous, less viscous system. Nasal mucociliary clearance is one of the most important limiting factors for nasal drug delivery. However, mucoadhesive preparations have been developed to increase the contact time between the dosage form and mucosal layers of nasal cavities, thus enhancing drug absorption and preventing rapid nasal clearance. This study aims at using polycarbophil as a mucoadhesive component in designing mucoadhesive microemulsion (MME).

Although IV administration is probably the most rapid way of seizure suppression, an alternative and more convenient approach like MME is highly needed when IV administration is not immediately available, for instance, because of the delay in transferring the patient to hospital or the arrival of emergency medical personnel. This study aimed to develop and characterize new ME and MME formula for intranasal delivery of CBZ and it was hypothesized that it will be able to reduce the side effects, decrease the dose and frequency of administration, and perhaps even the cost of the therapy.

2. Experimental

2.1. Drugs and reagents

Pure powdered CBZ was obtained as gratis sample from Max Pharma (India) with 99.9% purity. Labrafil M 1944 (Oleoyl polyoxylglycerides), Transcutol P (Diethylene glycol monoethyl ether), Labrafac CC (Gattefosse Saint-Priest, France) was procured as gratis sample from Gattefosse Asia Ltd. (Mumbai, India). Cremophor RH 40 (Polyoxyl 40 Hydrogenated Castor Oil) was procured as gratis sample form BASF (Mumbai, India). Polycarbophil (AA-1, pharmagrade, molecular weight approximately 3.5 million) was procured as gratis sample from Lubrizol Advance Material India Pvt. Ltd. (Mumbai, India). Potassium dihydrogen phosphate, methanol, propylene glycol were purchased from SDfine Chemicals (Ahmedabad, India). Ethanol was purchased from Baroda Chemical Ind. Ltd. (Dabhoi, India). Double distilled water was used throughout the study. All other chemicals and solvents were of analytical reagent grade and used as received without further purification.

2.2. High performance thin layer chromatography method and chromatographic conditions

The standard and formulation samples of CBZ were spotted on pre-coated high performance thin layer chromatography(HPTLC) plates in the form of narrow bands of lengths 6 mm, with 10 mm from the bottom and left margin and with 9 mm distance between two bands. Samples were applied using Linomat V autosprayer under continuous drying stream of nitrogen gas at a constant application rate of 150 nL/s. Plates were developed using a mobile phase consisting of ethyl acetate–toluene–methanol (5.0 + 4.0 + 1.0 v/v/v). Linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber equilibrated with the mobile phase. The optimized chamber saturation time for mobile phase was 20 min at 25 ± 2 °C. Ten milliliters of the mobile phase (5 mL in trough containing the plate and 5 mL in other trough) was used for each development and allowed to migrate a distance of 70 mm, which required 10 min. After development, the HPTLC plates were dried completely. Densitometric scanning was performed on Camag TLC scanner III (Camag, Muttenz, Switzerland) in absorbance mode and operated by winCATS planar chromatography version 1.3.4. The source of radiation utilized was a deuterium lamp. The spots were analyzed at a wavelength of 285 nm. The slit dimensions used in the analysis were length and width of 5 mm and 0.45 mm, respectively, with a scanning rate of 20 mm/s. These are selected as recommended by the CAMAG TLC Scanner III manual. It covers 70–90% of the application band length, which in the present case is 6 mm. The monochromator bandwidth was set at 20 nm. Concentrations of compound chromatographed were determined from the intensity of diffusely reflected light and evaluated as peak areas against concentrations using linear regression equation. The method was validated for precision, recovery, repeatability, and robustness as per the International Conference on Harmonization guidelines.[16] Fig. 1 shows a typical chromatogram obtained by the proposed HPTLC

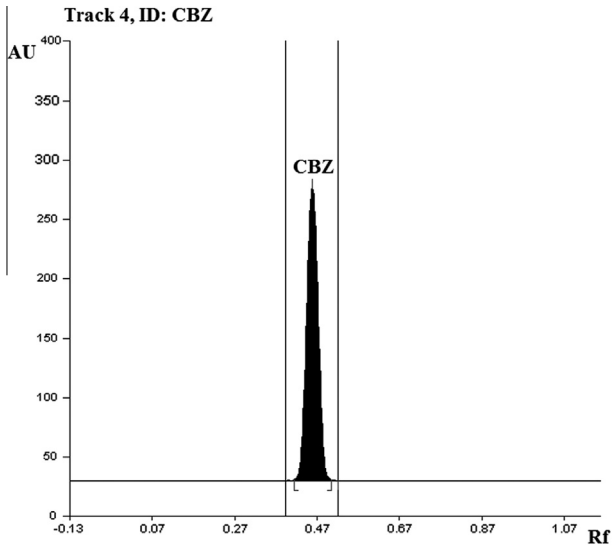

Figure 1 Chromatogram of standard solution containing 400 ng/spot Carbamazepine.

method, demonstrating the symmetrical peak corresponding to CBZ.

2.3. Solubility determination

Drug powder of CBZ was added in excess to each of the oils, surfactant (S) and cosurfactant (CoS) and vortexed for mixing. After vortexing the samples were kept for 72 h at ambient temperature for attaining equilibrium. The equilibrated samples were then centrifuged at 5000 rpm for 30 min to remove the undissolved drug.[6] The aliquots of supernatant were filtered through 0.45 m membrane filters and the solubility of CBZ was determined by HPTLC[16] method.

2.4. Pseudo-ternary phase diagram

In order to find out the concentration range of components for the existing range of ME, pseudo-ternary phase diagrams were constructed using water titration method[6] at ambient temperature. Four phase diagrams were prepared with the 1:1, 2:1, 3:1 and 4:1 weight ratios of Cremophor RH 40 to Transcutol P, respectively. For each phase diagram at a specific S/CoS mixing ratio (km), the ratios of oil to the mixture of S/CoS (Smix) were varied as 0.5:9.5, 1:9, 1.5:8.5, 2:8, 2.5:7.5, 3:7, 3.5:6.5, 4:6, 4.5:5.5, 5:5, 5.5:4.5, 6:4, 6.5:3.5, 7:3, 7.5:2.5, 8:2, 8.5:1.5, 9:1, 9.5:0.5. The mixtures of oil and S/CoS at certain weight ratios were diluted with water dropwise, under moderate magnetic stirring. After being equilibrated at ambient temperature for 24 h, the mixtures were assessed visually and determined as being ME, crude emulsions or ME gels. The stable ME formulations were also observed under polarizing light to confirm their isotropic nature. No attempt was made to distinguish between oil-in-water, water-in-oil or bicontinuous type ME. Gels were claimed for those clear and highly viscous mixtures that did not show a change in the meniscus after being tilted to an angle of 90°.

Table 1 Composition of carbamazepine microemulsions (CME) and carbamazepine mucoadhesive microemulsions (CMME).

Ingredients	CME[a] (% wt/wt)	CMME[a] (% wt/wt)
Labrafil M 1944	6.00	6.00
Cremophor RH 40	8.00	8.00
Transcutol P (4:1)	24.00	24.00
Water	62.00	62.00

[*] The formulations contain carbamazepine 5 mg/mL; CMME additionally contain 0.5% wt/wt of polycarbophil as mucoadhesive agent.

2.5. Optimization of microemulsions

Considering the amount and solubility of drug to be incorporated in the ME, certain Oil–Smix–water mixture within the ME region was prepared and the final composition of ME was optimized based on transparency, dilution characteristics, and globule size. For optimization process, the ME formulations were prepared by varying the stirring speed and stirring time and the globule size was taken as response.

2.6. Preparation of microemulsions and mucoadhesive microemulsions

The ME for CBZ was prepared by the titration method. The calculated amount of drug (5 mg/mL of CBZ) was added to the oily phase of ME and magnetically stirred until dissolved followed by the addition of Smix in a fixed proportion to produce a clear mixture. Then a defined proportion of water was added and stirred to produce clear ME of CBZ (CME). The MME of CBZ (CMME) was prepared by initially preparing ME of the drug using a a minimum volume of external phase and then adding the required volume of polymer solution (1%, wt/vol) so that the final concentration of polymer in the MME was 0.5% (wt/wt). After the addition of polymer solution the MME was allowed to homogenize for 10 min. Composition of CME and CMME is shown in Table 1.

2.7. Preparation of drug solution

The CBZ solution (CS) meant for comparative evaluation of MME-based systems was prepared by dissolving CBZ (50 mg) in a mixture of 5 mL propylene glycol, 3 mL water and 2 mL ethanol (95%, vol/vol) resulting in a solution of 5 mg/mL.

2.8. Physicochemical characterization of MEs

2.8.1. Particle size and zeta potential measurements

The average droplet size and polydispersity index (PDI) of ME were measured by photon correlation spectroscopy (PCS) with an in-built Zetasizer (Nano ZS, Malvern Instruments, UK) at 633 nm. Helium–neon gas laser having intensity of 4 mW was the light source. The droplet size was calculated using the Stokes–Einstein relationship by Zetasizer Software. Electrophoretic mobility (μm/s) was measured using small volume disposable zeta cell and converted to zeta potential[17] by in-built

software using the Helmholtz–Smoluchowski equation. All determinations were made in triplicate.

2.8.2. Transmission electron microscopy (TEM)

TEM was used to characterize the microstructure of CBZ loaded ME. CME was placed on a carbon-coated copper grid and then a drop of 1% phosphotungstic acid was covered on ME. The superfluous phosphotungstic acid on ME was wiped off by filter paper. The TEM images were obtained using a Tecnai G2 20 TEM (Philips, Holland).

2.8.3. Refractive index and percent transmittance measurement

The refractive index of the system was measured by a digital Abbe refractometer (Atago Co. Ltd., Tokyo, Japan) by placing 1 drop of ME on the slide. The percent transmittance of the system was measured using a colorimeter (Digital Colorimeter, D-801, Photocon) at 570–590 nm.

2.8.4. Polarizing microscopy

In order to verify the isotropic nature of ME, samples were examined using cross-polarized light microscopy (Polarizing Microscope RPL-55 Series, Radical Instruments, India). A drop of ME was placed between a cover slip and a glass slide and then observed under cross-polarized light.[6]

2.8.5. pH measurement

The pH value of ME was determined using digital pH meter (Orion pH meter 420A, Allometric Ltd., Baton Rouge, LA), standardized using pH 4 and 7 buffers before use.

2.8.6. Osmolarity determination

The osmolarity of the formulations was determined by the following expression, [18]

$$\text{mOsm/liter} = (\text{concentration in gram per liter} / \text{molecular weight in grams}) \times 10,000. \quad (1)$$

2.8.7. Viscosity measurement

The viscosity of ME was measured using a Brookfield Viscometer LVDV – IIIU (Brookfield Engineering LABS, Stoughton, MA) with spindle SC 18 at 100 rpm using interval of 30 s. All aspects of testing were controlled using Rheocalc Software.[6]

2.8.8. Conductivity measurement

The electrical conductivity of ME was measured with a conductivity meter (Equip-Tronics, EQ – 664, Mumbai, India) equipped with an inbuilt magnetic stirrer. This was done by using conductivity cell (with a cell constant of 1.0) consisting of two platinum plates separated by desired distance and having liquid between the platinum plate acting as a conductor.[6]

All determinations were made in triplicate.

2.8.9. Infrared study

The infrared (IR) spectra of CBZ, plain ME, optimized CME and CMME were taken using an IR spectrophotometer (Spectrum GX FT-IR, Perkin Elmer, Norwalk, CT). The plain ME, CME and CMME were spread as a thin layer onto a potassium bromide cell and then scanned between 4000 and 400 cm^{-1}. The resulting IR spectra of CBZ and plain ME were then compared with CME and CMME to detect any possible interaction between the drug and different components used.

2.9. Ex vivo evaluation of carbamazepine formulations

2.9.1. Ex vivo diffusion study of carbamazepine formulations

The freshly excised sheep nasal mucosa,[19] except the septum part, was collected from the slaughter house in phosphate buffer saline (PBS), pH 6.4. The membrane was kept in PBS at pH 6.4 for 15 min to equilibrate. The superior nasal concha was identified and separated from the nasal membrane. The excised superior nasal membrane was then mounted on a Franz diffusion cell. The tissue was stabilized using phosphate buffer pH 6.0 in both the compartments and allowed to stir for 15 min on a magnetic stirrer. After 15 min, solution from both the compartments was removed and fresh phosphate buffer pH. 6.0[5] was filled in the receptor compartment. The mounting of the nasal membrane was done using glue at the brim of the donor compartment to avoid leakage of the test sample and supported with thread crossing over the cell.

Franz diffusion cell used for ex vivo diffusion studies had a diameter of 10 mm and mucosa of thickness 0.2 ± 0.1 mm. The temperature of the receiver chamber containing 25 mL of diffusion media (phosphate buffer, pH 6.0) was controlled at 37 °C ± 1 using circulating equibath, (Model 8506, Medica Instrument Mfg. CO, Mumbai, India). 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 fluid.

A volume of 1 mL of each CS, CME, and CMME was placed in the donor compartment of Franz diffusion cell. Samples from the receptor compartment were withdrawn at predetermined time intervals and analyzed using the HPTLC method. Each sample removed was replaced by an equal volume of diffusion media (1 mL). Each study was carried out for a period of 4.0 h, during which the drug in the receiver chamber (μg/mL) across the sheep nasal membrane was calculated at each sampling point. The formulations were studied in triplicate for diffusion studies and the mean cumulative values for % of drug diffused were plotted against time. The slopes of the graphs were used to calculate the diffusion coefficients and the results were subjected to one-way ANOVA.

2.9.2. Test for nasal cilio toxicity of microemulsion

Freshly excised sheep nasal mucosa, except for the septum, was collected from the slaughter house in saline phosphate buffer pH 6.4. Three sheep nasal mucosa pieces (S1, S2, and S3) with uniform thickness were selected and mounted on Franz diffusion cells. S1 was treated with 0.5 mL of PBS pH 6.4 (negative control), S2 with 0.5 mL of isopropyl alcohol (positive control), and S3 was treated with ME for 1 h. After 1 h, the mucosae were rinsed with PBS at pH 6.4 and subjected to histological studies to evaluate the toxicities of ME photographed by microscope.

2.9.3. Ex vivo mucoadhesion study

The mucoadhesive potential of the CME and CMME was evaluated by an *in vitro* method reported by Bachhav and Patravale.[20] Briefly, an agar plate (1%, w/w) was prepared in pH 6.0 phosphate buffer, CME and CMME formulations, each

50 mg was placed at the center of plate. After 5 min, the agar plate was attached to a USP disintegration test apparatus and moved up and down in pH 6.0 phosphate buffer at 37 ± 1 °C. The CMME formulation on the plate was immersed into the solution at the lowest point and was out of the solution at the highest point. The residence time of the CMME on the plate was noted visually.

2.10. Stability studies

The formulations, CME and CMME, were subjected to stability testing for a period of 6 months at room temperature to simulate patient usage conditions. After 6 months of storage, the formulations were studied for physical stability by means of creaming, phase separation, or flocculation, accelerated centrifugation cycle (3000×*g* for 15 min) and chemical stability by means of drug content, particle size, and zeta potential determinations.[17]

3. Results and discussion

3.1. Preparation and optimization of microemulsion formulations

The solubility of practically insoluble CBZ was determined in the selected oily phases and was highest in Labrafil M 1944 CS (Table 2). Hence, Labrafil M 1944 CS was selected as the oily phase for the preparation of ME. The type of ME formed depends on the properties of the oil, S, and CoS. Most single-chain S does not lower the oil–water interfacial tension sufficiently to form ME and short-to-medium-chain length alcohols are necessary as CoS. The CoS also ensures that the interfacial film is flexible enough to deform readily around each droplet as their intercalation between the primary S molecules decreases both the polar head group interactions. In this study, cremophor RH 40 and transcutol P were selected as the S–CoS system. An important criterion for selection of the surfactants is that the required hydrophilic lipophilic balance (HLB) value to form the oil–water ME be greater than 10. The right blend of low and high HLB S leads to the formation of a stable ME formulation.[21] In this study, we selected cremophor RH 40 as S with a HLB value of 15. Transient negative interfacial tension and fluid interfacial film are rarely achieved by the use of single surfactant; usually, addition of a CoS is necessary. The presence of CoS decreases the bending stress of interface and allows the interfacial film with sufficient flexibility to take up different curvatures required to form ME over a wide range of compositions.[22] Thus, the CoS selected for the study was transcutol P, which has an HLB value of 4.2.

A ternary phase diagram explains the selection of the formulations from the phase diagrams to avoid metastable formulations having minimum surfactant concentration, in the least possible time. Ternary phase diagrams were constructed by varying cremophor RH 40: transcutol P ratios as 1:1, 2:1, 3:1, and 4:1 (Fig. 2). The shaded areas of phase diagrams show the ME regions, whereas the nonshaded area displays the

Table 2 Solubility of carbamazepine in various excipients.

Excipients	Solubility[a]
Oily phases	
Labrafil M 1944 (oleoyl polyoxylglycerides)	37.7 ± 4.21
Labrafac CC (caprylic/capric triglycerides)	10.47 ± 3.6
Isopropyl myristate	21.9 ± 3.9
Labrafac lipophile (medium chain triglycerides)	0.9 ± 0.7
Labrafac PG (propylene glycol dicaprylocaprate)	1.1 ± 0.7
Miglyol 810 (Caprylic/Capric Triglyceride)	1.31 ± 1.0
Miglyol 812 (Caprylic/Capric triglyceride)	1.6 ± 0.9
Miglyol 840 (propylene glycol dicaprylate/dicaprate)	0.7 ± 0.36
Lauryl alcohol	1.8 ± 0.6
Isostearylic isostearate	1.44 ± 0.6
Isopropyl palmitate	0.35 ± 0.21
Captex 200 (propylene glycol dicaprylate/dicaprate)	3.8 ± 0.9
Captex 355 (glycerol caprylate caprate)	1.8 ± 0.7
Surfactants	
Labrasol (caprylocaproyl polyoxylglycerides)	165.8 ± 6.16
Tween (polysorbate) 80	75.0 ± 6.18
Plurol stearique WL (polyglyceryl-6-distearate)	0.8 ± 0.15
Plurol diisostearique (Polyglyceryl diisostearate)	1.05 ± 0.7
Cremophor RH 40 (polyoxyl 40 hydrogenated castor oil)	25.34 ± 6.11
Cosurfactants	
Plurol Oleique CC (polyglyceryl oleate)	4.13 ± 1.1
Plurol oleique 5203 (Polyglyceryl 6 – dioleate)	1.7 ± 0.8
Lauroglycol 90 (propylene glycol monolaurate)	49.8 ± 3.1
Capryol 90 (Propylene glycol monocaprylate)	87.44 ± 5.8
Transcutol P (diethylene glycol monoethyl ether)	157.23 ± 7.34
Capmul MCM (glyceryl mono- and dicaprate)	11.14 ± 4.1
Propylene glycol	150.9 ± 4.6

[a] Data expressed as mg/g, mean ± *SD*, $n = 3$.

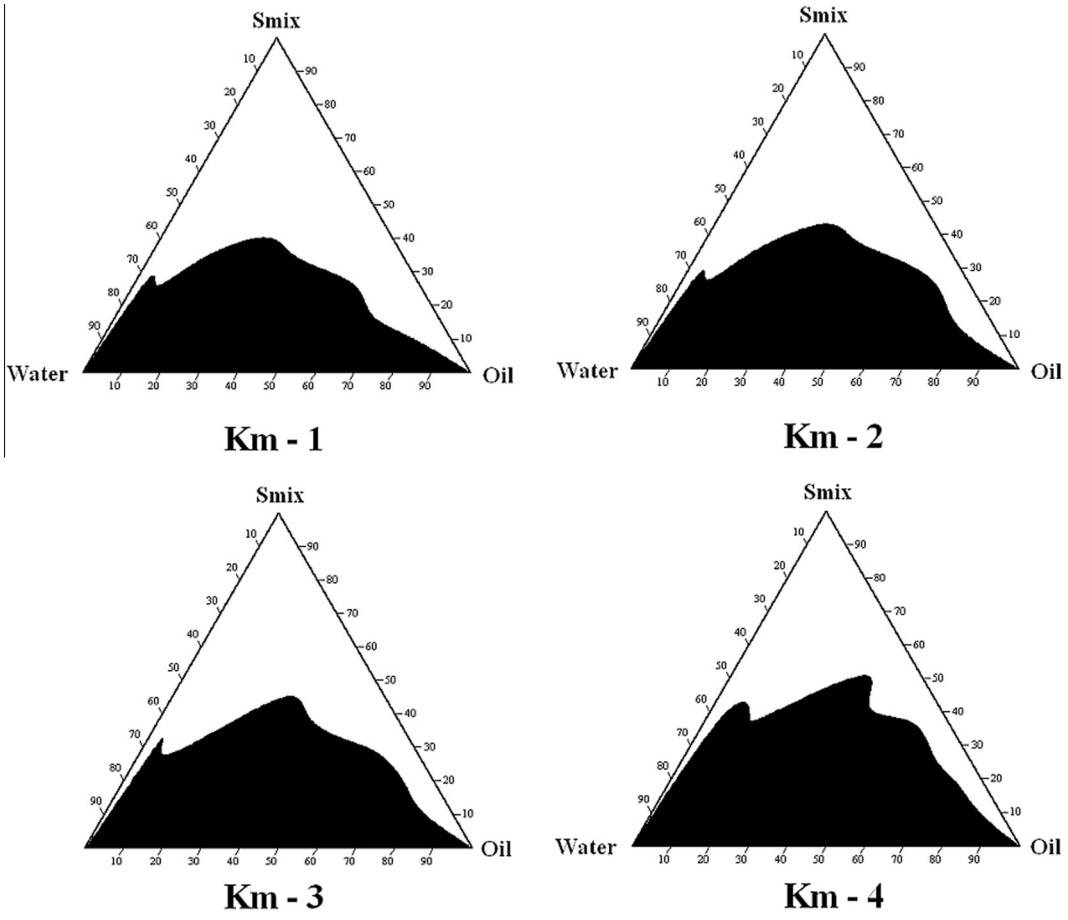

Figure 2 The pseudoternary phase diagrams of the oil-surfactant mixture-water system at the 1:1, 2:1, 3:1 and 4:1 weight ratio of Cremophor RH 40 to Transcutol P at ambient temperature, dark area represent microemulsion region.

Table 3 Various ternary phase compositions and characterization parameters of plain microemulsions.

Batch	Km	Oil (% wt/wt)	Smix (%wt/wt)	Water (%wt/wt)	Globule Size (nm) ± *SD*	PDI ± *SD*	Zeta potential (mV) ± *SD*	% *T*
L1	4	6	22	72	139.0 ± 4.17	0.226 ± 0.021	19.12 ± 2.79	96.35
L2	4	6	24	70	113.0 ± 2.88	0.442 ± 0.016	11.34 ± 2.74	97.12
L3	4	6	26	68	107.0 ± 2.63	0.332 ± 0.027	16.45 ± 2.46	99.78
L4	4	6	28	66	67.0 ± 1.28	0.122 ± 0.010	25.09 ± 1.88	99.23
L5	4	6	30	64	45.0 ± 1.44	0.136 ± 0.014	24.72 ± 2.25	99.61
L6	4	6	32	62	34.32 ± 1.09	0.116 ± 0.012	36.29 ± 2.03	99.95
L7	4	6	34	60	34.16 ± 1.36	0.127 ± 0.023	36.79 ± 2.66	99.87
L8	4	6	36	58	32.53 ± 1.78	0.129 ± 0.038	36.52 ± 2.18	99.69

turbid region. Thus, the ternary phase system of Smix (4:1, Km 4) that exhibited maximum area for ME formation was selected for the optimization of ME batches. Apart from the ternary phase diagrams, globule size determinations were also performed as it could provide supportive evidence for the selection of phase diagram of ratio 4:1. It was clearly evident that an increase in the concentration of Cremophor RH 40 resulted in a decrease in globule size. Thus, at the lowest concentration of S, the globule size was 68.31 ± 2.27 nm, whereas at the highest concentration of Cremophor RH 40 it reduced to 30.32 ± 1.09 nm, and hence the ratio of Smix 4:1 (Km 4) was selected for optimization studies. The optimization of ME was carried out on the basis of percentage transmittance (%*T*), globule size, and zeta potential, and the results are

tabulated in Table 3. According to the solubility study of CBZ in Labrafil M 1944 CS, a minimum of 6% by the weight of oily phase was required to fulfill the dose requirement, and with Smix maintained at 4:1 and water at 62% by weight, eight batches of CBZ-loaded ME formulations were prepared and characterized.

The globule size decreased with the increase in the concentration of Smix in the formulations (Table 3). The globule size of batch L1, containing 22% of Smix, was highest (139.0 ± 4.17 nm) and was least (32.53 ± 1.78 nm) for highest concentration (36%) of the Smix. All the formulations had droplets in the nano range, which is very well evident from the low PDI values. PDI is the ratio of standard deviation to mean droplet size; hence, it indicates the uniformity of droplet size within the formulation. The higher the PDI, the lower the uniformity of the droplet size in the formulation.[6] Although the PDI values of all formulations were very low, indicating uniformity of droplet size within each formulation, it was least for L6 (0.116 ± 0.012).

The batch L6 (oil: S–CoS: water, 6:32:62) was selected as the optimized batch as it displayed optimum response variables of 99.95% optical transparency, low globule size (34.32 ± 1.09 nm), polydispersity of 0.116 ± 0.012, and zeta potential to the tune of −36.29 ± 3.03. Although batches L7 and L8 showed lower values for globule size and PDI that may be attributed to higher Smix concentrations, the difference was insignificant ($p < .05$) when compared with L6. Moreover, higher concentrations of Smix may cause damage to nasal mucosa; hence, L6 was selected for further processing.

The process for the preparation of ME was optimized by varying the stirring speed (200, 500, and 800 rpm) and time (5, 10, and 20 min for each stirring speed) followed by the globule size determination. Based on the minimum globule size of 32.53 ± 1.78 nm, a stirring speed of 800 rpm and stirring time of 10 min were selected as the optimized process parameters to obtain drug-loaded ME. ME form spontaneously without the aid of high shear equipment or significant heat input (heat and gentle mixing are required only if it is necessary to melt any of the ingredients), and their microstructures are independent of the order of addition of the excipients.

3.2. Characterization of microemulsion

Characterization data of CME are tabulated in Table 4. The narrow globule size range of 34–41 nm and PDI of 0.127 and 0.134 for CME and CMME, respectively, indicated that the ME approached a monodispersed stable system and could deliver the drug effectively owing to larger surface area. The globule size of the batch L6 containing carbamazepine was not significantly affected by incorporation of the drug when compared to the globule size of ME prepared without drug. The presence of zeta potential to the tune of –36.29 and –48.58 mV on the globules of CME, and CMME, respectively, conferred physical stability to the system. CME showed net negative charge and addition of mucoadhesive agent further contributed negatively to the system. This may be attributed to the fact that the increase in surfactant level resulted in a decrease in surface tension and surface free energy of the formed micelles. Therefore, net negative charge (anionic) of the ME increased.[10] The microemulsions were expected to have good physical stability (phase separation) as zeta potential is less than −30 mV.[11] Moreover, addition of a

Table 4 Characterization parameters of optimized CBZ microemulsions (CME) and CBZ mucoadhesive microemulsions (CMME).

Formulation	% assay	pH	% *T*	Globule size (nm) ± *SD*	PDI ± *SD*	Zeta potential(mV) ± *SD*	Conductivity (mS)	Viscosity (cp)	Refractive index
CS	99.46 ± 1.38	5.17 ± 0.11	–	–	–	–	-	-	1.3834
CME	99.88 ± 1.25	5.53 ± 0.15	99.95	34.11 ± 1.41	0.127 ± 0.012	36.29 ± 3.03	0.169 ± 0.05	186 ± 4.63	1.3902
CMME	100.14 ± 1.03	5.54 ± 0.19	–	40.57 ± 1.26	0.134 ± 0.023	48.58 ± 4.46	0.103 ± 0.07	201 ± 6.82	1.4021

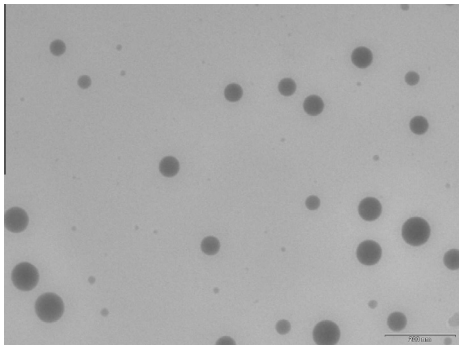

Figure 3 Transmission electron microscopy image of carbamazepine loaded microemulsion.

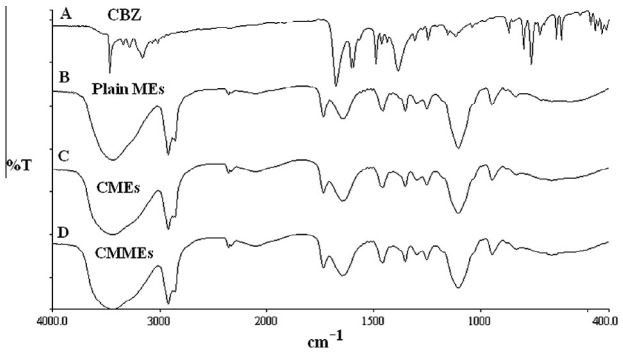

Figure 4 Infra Red spectra of (A) Carbamazepine (CBZ), (B) plain microemulsion (ME) (C) Drug loaded microemulsion (CME) and (D) drug loaded mucoadhesive microemulsion (CMME).

mucoadhesive polymer (Polycarbophil) may further stabilize the system since it increased the negative charge of the system.[12]

The TEM imaging of CME is shown in Fig. 3. The globule size of CME from TEM images accords with that from PCS. The imaging showed that CME exhibited a spherical shape and had a narrow size distribution.

The refractive index values of the developed ME formulation, CME and CMME were found to be 1.3902 and 1.4021, respectively. A percentage transmittance of 99.95 for CME indicated clear dispersion, whereas CMME, no transmittance was hazy due to the presence of mucoadhesive component in the formulations.

The samples were examined by ocular inspection in a cross polarizer for sample homogeneity and birefringence. The ME appeared completely dark when observed under a cross polarizer. The observations indicated that all the ME formulations were optically isotropic colloidal dispersions.

The pH of all the ME ranged between 4.5 and 6.5, approximating the normal pH range of nasal fluids,[4] which is one of the formulation considerations that may help reducing the irritation produced upon instillation.

Although the nasal passages can tolerate a wide range of tonicity without pain, isotonicity is significantly important. Nasal solutions with osmolarity comparable to aqueous 0.5–2.0% (equivalent to 85.47–341.88 mOsmol/L) sodium chloride solution are relatively comfortable and do not harm nasal cilia. Calculated osmolarity for both CME and CMME formulations was 211.62 mOsmol/L, which is within the suggested limits and the preparations are unlikely to cause potential discomfort upon instillation. All the formulations were of an osmolarity of almost similar magnitude as the presence of high molecular weight (~300,000) mucoadhesive agents (0.5% by weight of polycarbophil) contributed negligibly to the osmolarity.

It was observed that the viscosity of the ME formulations generally was very low. This was expected, because one of the characteristics of ME formulations is of a lower viscosity.[23] Low viscosity values of CME and CMME were 186 ± 4.63 and 201 ± 6.82 cp respectively, ensure easy handling, packing, and hassle-free nasal administration of formulations.

Conductivity measurements rely on the poor conductivity of oil compared with water and give low values for water in oil ME where oil is the continuous phase. The reverse happens for oil in water ME.[7] The conductivity measurements (0.169–0.103 mS) indicate the ME to be of oil-in-water type.

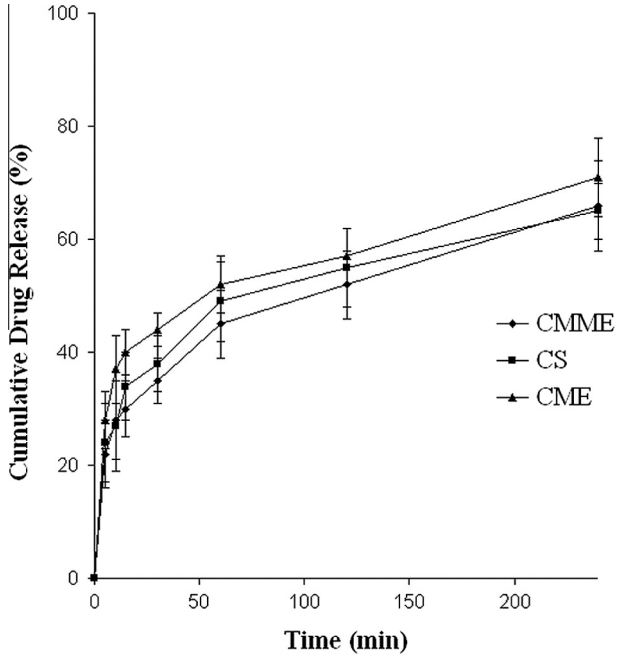

Figure 5 Percentage cumulative drug diffused versus time profiles of Carbamazepine solution (CS), microoemulsion (CME), and mucoadhesive microemulsions (CMME).

Table 5 Diffusion coefficients and modeling parameters of CBZ solution (CS), CBZ microemulsions (CME) and CBZ mucoadhesive microemulsions (CMME).

Formulation	Diffusion coefficient (cm^2/min)	Zero order r^2	First order r^2	Higuchi r^2
CS	0.3421 ± 0.08	0.8735	0.8988	0.9355
CME	0.2913 ± 0.11	0.9067	0.9013	0.9745
CMME	0.3172 ± 0.03	0.9128	0.9527	0.9873

The infrared spectra of CBZ pure powder, plain ME, CME and CMME are as shown in the Fig. 4. The spectrum of CBZ shows two principle absorption bands, one absorption band at 3460 cm^{-1} due to –NH valence vibration and other at

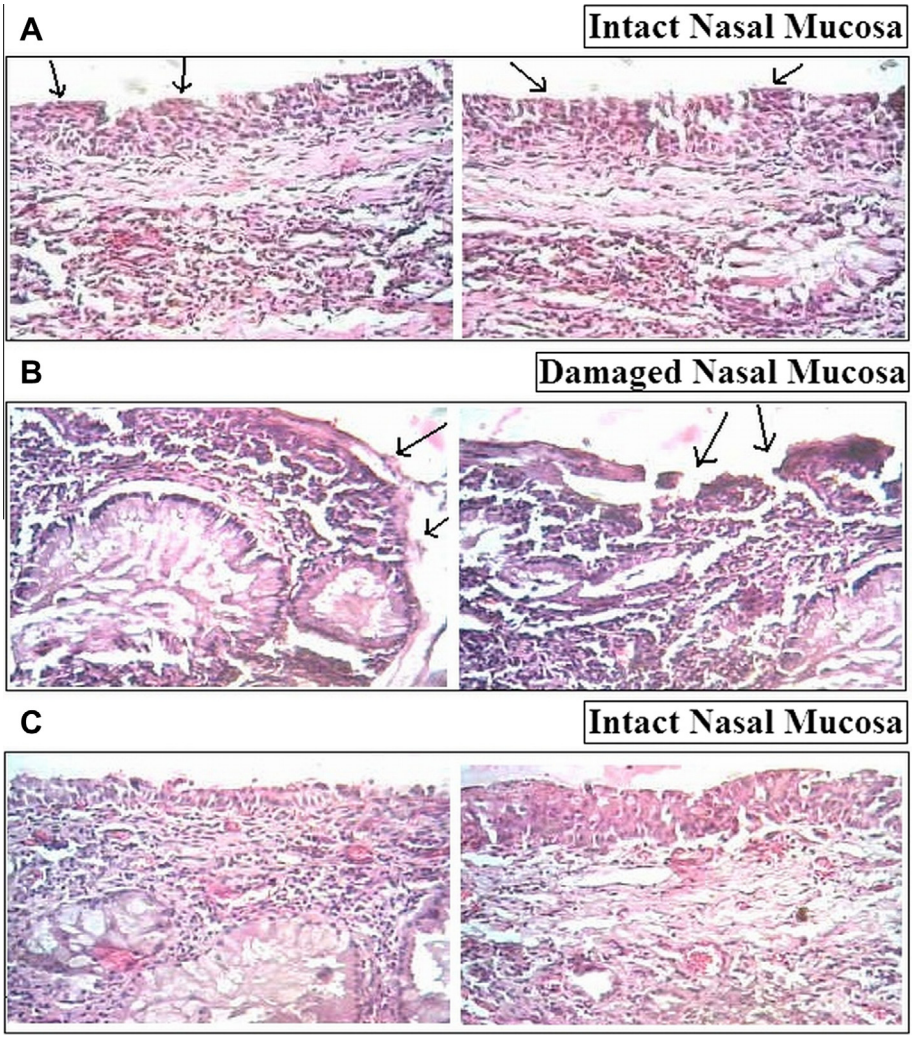

Figure 6 Photographs of sheep nasal mucosa demonstrating histological characteristics when treated with (A) phosphate buffer saline pH 6.4 (B) isopropyl alcohol and (C) mucoadhesive microemulsion of carbamazepine.

1674 cm^{-1} due to –CO–R– vibration. This band was not affected by components of formulation present in ME and MME, which emphasized the absence of any possible interaction between the drug and formulation components used.

3.3. Ex vivo diffusion study

In ex vivo diffusion studies of CBZ formulations, the recorded successful diffusion through sheep nasal mucosa and the results obtained are presented in Fig. 5, and the calculated diffusion coefficients are tabulated in Table 5 along with the regression coefficients (r^2) for first-order, Higuchi, and zero-order modeling of the diffusion profiles for each formulation. The decreasing order of diffusion coefficient for the tested formulations was CME < CMME < CS (although not significantly different at $p < .05$). For CS, the drug exhibited the highest diffusion coefficient, whereas it was least for CNE. The CMME exhibited a higher diffusion due to the presence of mucoadhesive agent that probably due to its intrinsic character tends to adhere to the mucosa thereby causing increased contact and hence increased diffusion.[24–26]

On modeling, the diffusion of drug from CBZ formulations exhibited higher r^2 values for the Higuchi model compared with zero- and first-order model(s). This may be due to the fact that the diffusion system used has a reservoir compartment (donor compartment) and sheep mucosa acts as a barrier or controlling membrane; hence, the diffusion process will mimic and shall be closer to the reservoir system than zero-order (concentration independent) or first-order (concentration gradient) diffusion.[25–27]

3.4. Nasal ciliotoxicity study

Nasal cilio-toxicity studies were carried out in an attempt to evaluate any potential toxic effects of excipients used in the formulation on the nasal mucosa. The nasal mucosa treated with PBS (pH 6.4, negative control) showed no nasociliary damage (Fig. 6A) and the nasal membrane remained intact, whereas an extensive damage to nasal mucosa coupled with loss of nasal cilia (Fig. 6B) could be observed with positive control. However, with ME, no damage to nasal mucosa could be observed (Fig. 6C), thus substantiating the safety of the excipients used in the formulation.

Table 6 Results of stability testing of the cbz microemulsions (CME) and CBZ mucoadhesive microemulsions containing 0.5% (wt/wt) polycarbophil (CMME) at room temperature.

Test	CME	CMME
% Assay	98.43	99.72
% Transmittance	99.80	97.56
Globule size (nm)	36.48	44.32
Polydispersibility index	0.143	0.159
Zeta potential (mV)	−37.24	−49.37

3.5. In vitro mucoadhesion study

The mucoadhesive potential of CMME was evaluated by *in vitro* method. The retention times showed by CME and CMME were 5.0 ± 1.0 and 21.0 ± 3.0 min, respectively ($n = 3$). The retention time on agar plate showed by CMME was significantly higher than CME. Thus it was hypothesized that the developed mucoadhesive preparation, CMME able to increase the contact time between the dosage form and mucosal layers of nasal cavities which can be attributed due to the presence of polycarbophil (0.5% wt/wt).

3.6. Stability study

In stability studies, the ME exhibited no precipitation of drug, creaming, phase separation, and flocculation on visual observation and was found to be stable after centrifugation (3000×*g* for 15 min) at room temperature. The results of stability studies (Table 6) showed that there are negligible changes in the parameters of CME and CMME after 3 months of storage, thus substantiating the stability of ME for 3 months.

4. Conclusion

On the basis of low droplet size and PDI, optimum S and CoS concentrations, the mucoadhesive formulation CMME of CBZ that contained 0.5% by weight of polycarbophil as the mucoadhesive component displayed highest diffusion coefficient. The formulation was free from nasal ciliotoxicity and was found to be stable for 3 months. The Ex vivo studies demonstrated the potential of developed CMME for intranasal delivery of CBZ.

Further *in vivo* studies are necessary to demonstrate the potential of MME based drug delivery system and to confirm the existence of a transport pathway for a drug (CBZ) to the brain directly from the nasal cavity. Thus authors are currently working on the brain targeting study of technetium-labeled CMME formulation in rabbit.

5. Conflict of interest

None.

Acknowledgments

Authors are thankful to Max Pharma (India) for the gift sample of CBZ pure powder, Sophisticated Instrumentation Center for Applied Research and Testing (SICART) (Vallabh Vidyanagar, India) for providing facilities to carry out analytical work, Gattefosse (Saint-Priest, France), Colorcon (Asia) Pvt. Ltd. (Mumbai, India), Abitec Corporation (Janesville, USA), BASF (Mumbai, India), Sasol (Witten, Germany), Lubrizol Advance Material India Pvt. Ltd. (Mumbai, India), and Noveon (Cleveland, USA) for providing gratis samples of excipients.

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