ANNEXURE 1

DRUG AND EXCIPIENTS PROFILE
DRUG PROFILE

CARBAMAZEPINE [173-176]

An anticonvulsant used to control grand mal and psychomotor or focal seizures. This compound belongs to the dibenzazepines. These are compounds with two benzene rings connected by an azepine ring.

IUPAC name: 5H-dibenzo[b,f]azepine-5-carboxamide

Chemical Formula $\text{C}_{15}\text{H}_{12}\text{N}_{2}\text{O}$

\[
\begin{array}{c}
\text{CONH}_2 \\
\text{N}
\end{array}
\]

Molecular weight: 236gm/mole

Melting point: 190° C

CAS number: 298-46-4

Pharmacodynamics: Carbamazepine, an anticonvulsant structurally similar to tricyclic antidepressants, is used to treat partial seizures, tonic-clonic seizures, pain of neurologic origin such as trigeminal neuralgia, and psychiatric disorders including manic-depressive illness and aggression due to dementia. The response to carbamazepine is variable and may be due to its variable transport, especially across the blood-brain-barrier. The transporter that may confer drug resistance is RALBP1.

Absorption: In clinical studies, carbamazepine suspension, conventional tablets, and extended-release tablets delivered equivalent amounts of drug to the systemic circulation. However, it has been observed that the suspension is somewhat faster absorbed. Furthermore, the extended-release tablet is slightly slower than the conventional tablet. Plasma levels of
carbamazepine are variable. The time to peak concentration for the different formulations are as follows: Suspension = 1.5 hours; Conventional tablets = 4-5 hours; Extended-release tablets = 3-12 hours. Oral bioavailability 80%

**Metabolism:** Hepatic metabolism by CYP3A4 enzymes. CYP3A4 is the primary isoform responsible for the formation of carbamazepine-10,11-epoxide. This metabolite is active and shown to be equipotent to carbamazepine as an anticonvulsant. Carbamazepine is more rapidly metabolized to the aforementioned metabolite in younger patients than in adults. It also undergoes glucuronidation via UGT2B7, however this finding has been disputed

Excretion: Urine 72 %, Faces 28%

Protein binding: 70-80 %

Half life: 36 hrs (single dose), 16-24 hrs (Repeated dosing)

Mechanism of action: The mechanism of action of carbamazepine and its derivatives is relatively well-understood. Carbamazepine stabilizes the inactivated state of Voltage-gated sodium channels, making fewer of these channels available to subsequently open. This leaves the affected cells less excitable until the drug dissociates. Carbamazepine has also been shown to potentiate GABA receptors made up of alpha1, beta2, gamma2 subunits. This may be relevant to its efficacy in neuropathic pain and manic-depressive illness.

Adverse effects: Mild ingestions cause vomiting, drowsiness, ataxia, slurred speech, nystagmus, dystonic reactions, and hallucinations.

Available Dosage Form: Tablet, Capsule and Suspension
PHENYTOIN [177-182]

An anticonvulsant that is used in a wide variety of seizures. It is also an anti-arrhythmic and a muscle relaxant.

IUPAC Name: 5,5-diphenylimidazolidine-2,4-dione

Chemical formula: C₁₅H₁₂N₂O₂

Molecular weight: 252.268 gm/mole

Melting point: 295° C

CAS Number: 57-41-0

Pharmacodynamics: Phenytoin is an antiepileptic drug which can be useful in the treatment of epilepsy. The primary site of action appears to be the motor cortex where spread of seizure activity is inhibited. Phenytoin reduces the maximal activity of brain stem centers responsible for the tonic phase of tonic-clonic (grand mal) seizures. Phenytoin acts to dampen the unwanted, runaway brain activity seen in seizure by reducing electrical conductance among brain cells. It lacks the sedation effects associated with phenobarbital. There are some indications that phenytoin has other effects, including anxiety control and mood stabilization, although it has never been approved for those purposes by the FDA.

Absorption: oral bioavailability 70%, Rectal: 25% bioavailability. T_{max} obtained with oral tablets is around 3 hrs.

Metabolism: Hepatic. The majority of the dose (up to 90%) is metabolized to 5-(4'′-hydroxyphenyl)-5-phenylhydantoin (p-HPHP). This metabolite undergoes further
glucuronidation and is excreted into the urine. CYP2C19 and CYP2C9 catalyze the aforementioned reaction.

Excretion: Primary through bile and urine

Plasma protein binding: Highly protein bound, 90%

Elimination half-life: 22 hours

Mechanism of action: Phenytoin produces its anticonvulsant activity through blocking sustained high frequency repetitive firing of action potentials. This is accomplished by reducing the amplitude of sodium-dependent action potentials through enhancing steady state inactivation. Sodium channels exist in three main conformations 1.Resting state 2.Open state 3.Inactive state. Phenytoin binds preferentially to the inactive form of the sodium channel. Because it takes time for the bound drug to dissociate from the inactive channel, there is a time dependent block of the channel. Since the fraction of inactive channels is increased by membrane depolarisation as well as by repetitive firing, the binding to the inactive state by phenytoin sodium can produce voltage-dependent, use-dependent and time-dependent block of sodium-dependent action potentials

Side effects: Symptoms of overdose include coma, difficulty in pronouncing words correctly, involuntary eye movement, lack of muscle coordination, low blood pressure, nausea, sluggishness, slurred speech, tremors, and vomiting.

Available Dosage Form: tablet, Capsule, Suspension and Injection
EXCIPIENTS PROFILE [183-184]

Oleic acid:

Oleic acid is a fatty acid that occurs naturally in various animal and vegetable fats and oils. It is an odorless, colourless oil, although commercial samples may be yellowish. In chemical terms, oleic acid is classified as a monounsaturated omega-9 fatty acid, abbreviated with a lipid number of 18. Oleic acid is a common monounsaturated fat in human diet. Monounsaturated fat consumption has been associated with decreased low-density lipoprotein (LDL) cholesterol, and possibly increased high-density lipoprotein (HDL) cholesterol. However, its ability to raise HDL is still debated. Oleic acid may hinder the progression of adrenoleukodystrophy (ALD), a fatal disease that affects the brain and adrenal glands. Oleic acid may be responsible for the hypotensive (blood pressure reducing) effects of olive oil. Adverse effects also have been documented, however, since both oleic and monounsaturated fatty acid levels in the membranes of red blood cells have been associated with increased risk of breast cancer although the consumption of oleate in olive oil has been associated with a decreased risk of breast cancer. Oleic acid should be stored in a well-filled, well-closed container, protected from light, in a cool, dry place.

- Formula: C_{18}H_{34}O_{2}
- Acidity/alkalinity: pH = 4.4 (saturated aqueous solution)
- Physical state: Pale yellow colour liquid
- Density: 0.89 gm/ml
- Molar mass: 282.4614 g/mol
- Boiling point: 360 °C
- Melting point: 13-14°C
- CAS Number 112-80-1
- Solubility: miscible with benzene, chloroform, ethanol (95%), ether, hexane, and fixed and volatile oils; practically insoluble in water.
- Vapor pressure: 133 Pa (1 mmHg) at 176.58°C
- Viscosity (dynamic): 26 mPa s (26 cP) at 258C
Capmul MCM C8

Capmul products are mono-, di- and triglyceride (58% monoglyceride, 36% diglyceride, and 5% triglyceride consisting of 80% w/w caprylic acid (C8), 20% capric acid (C10), and 2% caproic acid (C6). It is prepared through the glycerolysis of select fats and oils. They can be prepared by esterification of glycerin with specific fatty acids. They are lipophilic, insoluble in water and soluble in oils at elevated temperatures. They are used to produce stable emulsions and to modify viscosity. Caprylic and capric mono-diglyceride esters function as very effective carriers and solubilizers of active compounds. Mono-diglyceride medium chain esters are particularly recommended for the dissolution of difficult compounds such as sterols and have also exhibited bacteriostatic activity.

- IUPAC Name: 2,3-dihydroxypropyl octanoate
- Chemical Formula: C$_{11}$H$_{22}$O$_4$
- Average Molecular weight: 219 gm/mole
- Physical state: slightly yellow colour liquid
- Chemical Name: Glyceril Monocaprylate
- CAS Number: 26402-26-6
- Specific gravity : 0.99 gm/ml
- Boiling point : 340 °C
- Viscosity 23 Cps. at 25 °C
**Tween 80**

Tween 80 is a non ionic surfactant and emulsifier derived from polyoxylated sorbitan and oleic acid, and is often used in foods. It is a viscous, water-soluble yellow liquid. It is having a characteristic odor and a warm, somewhat bitter taste. The hydrophilic groups which are polymers of ethylene oxide. Hydrophilic nonionic surfactants that is used widely as emulsifying agents in the preparation of stable oil-in-water pharmaceutical emulsions. Polysorbates 20, 40, 60, and 80 are included in the FDA Inactive Ingredients Guide (IM, IV, oral, rectal, topical, and vaginal preparations). Polysorbates are included in parenteral and nonparenteral medicines licensed in the UK.

IUPAC name: Polyoxymethylene(80) sorbitan monooleate

- Physical state: yellow coloured viscous liquid
- Formula: $C_6H_{12}O_{26}$
- Acidity/alkalinity: pH = 6.0–8.0 for a 5% w/v aqueous solution.
- Flash point: 149⁰C
- Acid value : 2
- Hydroxyl value: 65-80
- Moisture content: 3.0
- Saponification value: 45-55
- Solubility: Soluble in ethanol and water. Insoluble in vegetable and mineral oil.
- Density: 1.06-1.09 g/mL
- Boiling point: >100 ℃
- HLB : 15
- Viscosity 425 mPas
- CMC 0.012mM at room temperature
- CAS Number: 9005-65-6
Transcutol P

It is also known under trade names Carbitol, Carbitol cellosolve, Transcutol, Dioxitol, Poly-solv DE, and Dowanol DE, is an industrial solvent. It is a clear, colorless liquid. Structurally it is an alcohol and an ether, a triethylene glycol with missing one hydroxyl group

- **IUPAC name**: 2-(2-Ethoxyethoxy)ethanol
- **Empirical formula**: \( C_6H_{14}O_3 \)
- **Physical state**: colourless liquid
- **Molecular weight**: 134.2
- **Boiling point**: 197 – 205 °C
- **Density**: 0.988 gm/ml
- **CAS Number**: 111-90-0
- **HLB value**: 4
Labrasol:
A non-ionic water dispersible surfactant composed of well-characterised polyethylene glycol (PEG) esters, a small glyceride fraction and free PEG. It is able to self-emulsify on contact with aqueous media forming a fine dispersion i.e. microemulsion (SMEDDS). It increases bioavailability due to strong inhibition of the enterocytic efflux transporter (known as P-gP inhibition).

- IUPAC name: CAPRYLCAPROYL MACROGOL GLYCERIDES
- Physical state: colourless liquid
- HLB value: 14
- CAS Number : 52622-27-2
- Saponification value: 85-105
- Acid value: Less than 0.2
- Density : 1.063 gm/ml
**Certificate of Analysis**

**Product Name**: Carbamazepine  
**A.R. Number**: RD115471  
**Batch No.**: CBD130099  
**Batch quantity**: N.A.  
**Mfg. Date**: --  
**Date of Analysis**: 23/09/2013  
**Specification**: EP  
**Page No.**: 1 of 1

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<th>S.No.</th>
<th>Tests</th>
<th>Observations</th>
<th>Specifications</th>
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<tr>
<td>1</td>
<td>Description</td>
<td>Almost white crystalline powder.</td>
<td>White or almost white, crystalline powder.</td>
</tr>
<tr>
<td>2</td>
<td>Solubility</td>
<td>Very slightly soluble in water, freely soluble in methylene chloride, sparingly soluble in acetone and in ethanol (96%).</td>
<td>Very slightly soluble in water, freely soluble in methylene chloride, sparingly soluble in acetone and in ethanol (96%).</td>
</tr>
<tr>
<td>3</td>
<td>Identification</td>
<td>A) By Melting Point (°C) 192.8</td>
<td>Between 189 and 193</td>
</tr>
<tr>
<td></td>
<td>D) By IR</td>
<td>IR spectrum of sample recorded in KBr exhibits absorption maxima and minima at the same wavelengths as that of the IR spectra of similar preparation of Carbamazepine standard.</td>
<td>IR spectrum of sample recorded in KBr should exhibit absorption maxima and minima at the same wavelengths as that of the IR spectra of similar preparation of Carbamazepine standard.</td>
</tr>
<tr>
<td>4</td>
<td>Acidity or Alkalinity</td>
<td>Meets the requirement.</td>
<td>As per Ph. Eur monogram.</td>
</tr>
<tr>
<td>5</td>
<td>Related substances (% w/w, by HPLC)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Impurity A</td>
<td>0.019</td>
<td>Not more than 0.1</td>
</tr>
<tr>
<td></td>
<td>Impurity B</td>
<td>0.001</td>
<td>Not more than 0.10</td>
</tr>
<tr>
<td></td>
<td>Impurity D</td>
<td>0.002</td>
<td>Not more than 0.10</td>
</tr>
<tr>
<td></td>
<td>Impurity E</td>
<td>Not Detected</td>
<td>Not more than 0.1</td>
</tr>
<tr>
<td></td>
<td>Impurity F</td>
<td>Not Detected</td>
<td>Not more than 0.10</td>
</tr>
<tr>
<td></td>
<td>Any unknown impurity</td>
<td>Not Detected.</td>
<td>Not more than 0.5</td>
</tr>
<tr>
<td></td>
<td>Total impurities</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Chlorides (ppm)</td>
<td>Less than 140</td>
<td>Not more than 140</td>
</tr>
<tr>
<td>7</td>
<td>Heavy metals (ppm)</td>
<td>Less than 20</td>
<td>Not more than 20</td>
</tr>
<tr>
<td>8</td>
<td>Loss on drying (% w/w, at 105°C for 2 hrs.)</td>
<td>0.13</td>
<td>Not more than 0.5</td>
</tr>
<tr>
<td>9</td>
<td>Sulphated ash (% w/w)</td>
<td>0.07</td>
<td>Not more than 0.1</td>
</tr>
<tr>
<td>10</td>
<td>Residual solvents (μg/g, By GC-HS)</td>
<td>Not performed</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Assay (% w/w, on dried basis, by HPLC)</td>
<td>99.09</td>
<td>Not less than 98.0 and not more than 102.0 of C14H12N2O.</td>
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*Sample is prepared in R&D Laboratory for research evaluation only.  
**Status**: The Product complies with above specifications.

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<th>Prepared by</th>
<th>Checked by</th>
<th>Approved by</th>
</tr>
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<tr>
<td>Nitin V. Patel</td>
<td>B.B. Rajaguru</td>
<td>Dr. B.K. Srivastava</td>
<td></td>
</tr>
<tr>
<td>Signature</td>
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<td>Date</td>
<td>09/10/2013</td>
<td>09/10/2013</td>
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# CERTIFICATE OF ANALYSIS

## RAW MATERIAL

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<tr>
<th>Name of Raw Material</th>
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<th>Receipt Date</th>
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<td>Phenytoin</td>
<td>27/08/2009</td>
<td>IG900537</td>
<td>21/08/2009</td>
<td>APH010</td>
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<tr>
<th>A.R. No.</th>
<th>B.No</th>
<th>Quantity Received</th>
<th>Manufacturer</th>
<th>Supplier</th>
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<td>12901052</td>
<td>09100289</td>
<td>03 x 25.00 kgs</td>
<td>Recordati Industrial Chem</td>
<td>Recordati Industrial Chem</td>
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## Test

<table>
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<th>Test</th>
<th>Specification</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance #</td>
<td>White or almost white, slightly hygroscopic, crystalline powder.</td>
<td>White slightly hygroscopic, crystalline powder.</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in water and in ethanol (96 per cent), practically insoluble in methylene chloride.</td>
<td>Conforms</td>
</tr>
<tr>
<td>Identification</td>
<td>A. Conform By IR. #</td>
<td>Conforms</td>
</tr>
<tr>
<td></td>
<td>B. A pink, crystalline precipitate is formed</td>
<td>Conforms</td>
</tr>
<tr>
<td></td>
<td>C. The solution gives reaction (b) of sodium.</td>
<td>Conforms</td>
</tr>
<tr>
<td>Related Substances (HPLC) #</td>
<td>EP Impurity C (USP related compound A) : NMT 0.20%</td>
<td>0.049%</td>
</tr>
<tr>
<td></td>
<td>EP Impurity E (USP related compound B) : NMT 0.30%</td>
<td>0.26%</td>
</tr>
<tr>
<td></td>
<td>EP Impurity D : NMT 0.10%</td>
<td>0.080%</td>
</tr>
<tr>
<td></td>
<td>EP Impurity A (Benzophenone) : NMT 0.10%</td>
<td>Not detected</td>
</tr>
<tr>
<td></td>
<td>EP Impurity B : NMT 0.10%</td>
<td>Not detected</td>
</tr>
<tr>
<td></td>
<td>Benzoin : NMT 0.10%</td>
<td>Not detected</td>
</tr>
<tr>
<td></td>
<td>Benzhydrol : NMT 0.10%</td>
<td>Not detected</td>
</tr>
<tr>
<td></td>
<td>Each Unspecified impurity : NMT 0.10%</td>
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</tr>
<tr>
<td></td>
<td>Total Impurity : NMT 0.50%</td>
<td>0.39%</td>
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Prepared By: [Signature]
Quality Control Date: 10/09/12

Checked By: [Signature]
Quality Control Date: 10/09/12

Approved by: [Signature]
Quality Assurance Date: 10/09/12

Contd...
ANNEXURE 2

PUBLICATIONS
Original Research Paper

Preparation and evaluation of transnasal microemulsion of carbamazepine

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ABSTRACT

The objective of this study was to develop novel transnasal microemulsion containing carbamazepine for treatment of epilepsy. Oleic acid was selected as oil while Tween 80 and propylene glycol were selected as surfactant and cosurfactant respectively based on solubility results. Optimized ratio of Tween 80: propylene glycol was selected after developing pseudoternary phase diagrams for different ratio and microemulsions were prepared. The prepared microemulsions were evaluated for globule size, viscosity, pH, conductivity and % transmittance. Ex-vivo diffusion study for optimized microemulsion was performed through sheep nasal mucosa wherein diffusion flux and permeability coefficients were determined. Further pharmacodynamic performance was evaluated in rats by electrically induced seizures. It was found that optimized microemulsion was stable and transparent with average globule size of 190 nm and diffusion flux of 75.77 μg cm⁻² min⁻¹ and showed no toxicity during histopathological evaluation on sheep nasal mucosa. Pharmacodynamic evaluation also indicated lesser intensity of seizures in rats treated with optimized formulation in comparison to rats treated with oral carbamazepine microemulsion and nasal carbamazepine solution which suggested carbamazepine transnasal delivery system as an effective alternate therapy for treatment of epilepsy.

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1. Introduction

Status epilepsy is a serious neurological emergency characterized by severe bouts of seizures. It requires rapid termination of seizure activity because if the episode of epilepsy remains untreated, it may lead to a permanent damage to the brain [1]. About 50 million people worldwide suffer from epilepsy and nearly two out of every three new cases are reported in developing countries. Epilepsy is more likely to occur in young children or people over the age of 65 years however it may occur at any age.

Treatments of epilepsy include treatment with antiepileptic drugs or surgery. Carbamazepine (CBZ) is a major antiepileptic drug for the treatment of different form of seizures [2]. Currently CBZ is available only in the form of oral dosage forms including tablets, capsules, suspensions etc. The major
limitation with CBZ oral formulation is its slower and erratic absorption and thus a novel formulation of carbamazepine to overcome the stated limitation is mandatory [3].

In recent years, systemic drug delivery through nasal route has received a lot of attention, because of its advantages including rapid absorption, avoidance of hepatic first-pass metabolism and the ability for preferential drug delivery to brain via the olfactory region [4, 5]. In comparison with oral administration, intranasal drug delivery may provide an improvement in bioavailability and rapid onset of action [6]. Thus, it is expected that intranasal delivery of CBZ may also achieve rapid onset of action and improved bioavailability by avoiding the first-pass effect in the liver and intestine. CBZ transnasal formulations are not available in market. Some researchers have worked on CBZ nasal formulations but thorough characterization with pharmacodynamic studies has not been reported by the researchers as per the authors’ knowledge [7].

CBZ aqueous solubility is very poor. Hence solubility enhancement is necessary for transnasal delivery of CBZ as nasal delivery cannot permit administration of large volumes of liquids. Microemulsion (ME) seems to be convincing approach for administration of CBZ. ME is defined as a thermodynamically stable and transient dispersion consisting of oil, surfactant, cosurfactant and aqueous phases [8]. The advantages of ME as a drug delivery system are the enhancement of drug solubilization and absorption across mucosal membranes due to its lipophilic nature and smaller globule size [9].

The objective of this investigation was to prepare and optimize transnasal CBZ microemulsion by using various physicochemical parameters including globule size, % transmittance, pH, viscosity, etc. Optimized ME was further evaluated for ex-vivo diffusion study through sheep nasal mucosa. Pharmacodynamic activity of CBZ ME was evaluated in rats after inducing seizures electrically.

2. Materials and methods

2.1. Materials

Carbamazepine was donated by Lincoln Pharma, Ahmedabad, India. Capmul MCM (glyceryl monocaprylate) and Labrafac (propylene glycol dicapryloctrate) were obtained as gift samples from Gattefosse. Oleic acid, Tween 80, Tween 20, Span 80, polyethylene glycol (PEG 400) and propylene glycol (PG) were purchased from Sigma Aldrich. All other chemicals were of analytical grade and purchased commercially. Double distilled water was used throughout the study.

2.2. Determination of solubility of drug

The solubility of CBZ in various components (oils, surfactants, and cosurfactants) was determined by adding an excess of (1 g) CBZ to each cap vial containing 5 ml of the selected vehicles. The mixture was heated at 40 °C in a water bath to facilitate the solubilization. Formed suspensions were then stirred for 48 h on magnetic stirrer. Then each suspension was centrifuged at 3000 rpm for 5 min, and supernatant was taken and diluted with methanol and CBZ was quantified by UV spectrophotometer (UV 1800, Shimadzu) at wavelength of 284 nm.

2.3. Preparation of pseudoternary phase diagram

Pseudoternary phase diagrams were constructed to obtain the appropriate ratio of surfactant: cosurfactant which can result in to large existence of ME area. They were constructed using water titration method. Surfactant (Tween 80) and cosurfactant (propylene glycol) were mixed (Smix) in different weight ratios (1:1, 2:1 and 1:2). Oil (oleic acid) and Smix (Tween 80 and propylene glycol) 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 [10]. The pseudoternary phase diagrams were constructed by using CHEMIX software.

2.4. Physicochemical characterization of carbamazepine loaded MEs

Percentage transmittance of each sample was determined at 630 nm using distilled water as reference. One drop of MEs was placed on slide and refractive indices of MEs were measured by using Abbe refractometer (ELICO, India). 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 (Carl Zeiss, Germany). 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 (CM 180 ELICO, India) at ambient temperature and the pH of ME was measured by using pH meter (Systronic 335, India). Droplet size and Zeta potential distribution was measured using Malvern zetasizer (Nano ZS, Malvern Instruments, UK). Each sample was suitably diluted five times with filtered distilled water and placed in a disposable zeta cell [11]. Samples were centrifuged at 3000 rpm for 15 min to determine centrifugation stability. Experiments were performed in triplicate for each sample.

2.5. Ex-vivo diffusion studies

The use of natural membranes is very important for predicting the potential drug release characteristic. Freshly excised sheep nasal mucosa was obtained from slaughter house and dipped immediately in phosphate buffer (pH 6.4). Cartilages were removed properly and the mucosal membrane was isolated and 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. The tissue was stabilized in phosphate buffer (pH 6.4). 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 solution in PEG 400 (30 mg/ml). Samples from the receptor compartment were withdrawn at periodic time intervals, filtered through 0.45 µm nylon filter paper and analyzed using a UV–Visible spectrophotometer at 284 nm. Each removed sample was replaced by an equal volume of diffusion medium. The cumulative amount of CBZ permeated through the skin was determined by the following equation [12]:

\[ Q_n = \frac{C_n \times V_o + \sum_{i=1}^{n-1} C_i \times V_i}{S} \]

Where, \( C_n \) is the CBZ concentration of receptor medium after each sampling time, \( C_i \) is the CBZ concentration for \( i \) sample, \( V_o \) and \( V \) are the volumes of the receiver solution and sample, respectively, and \( S \) is the effective diffusion area. The steady state flux (\( J_{ss} \)) was calculated from the slope of the steady state portion of the line in the plot of drug amount permeated \( V_o \) time (min) [13]. Permeability coefficient (\( K_p \)) was calculated by dividing the flux with concentration of the drug in ME.

2.6. Nasal ciliotoxicity studies

The method described by Sheetal et al. was used for this study; pieces of freshly excised sheep nasal mucosa with a thickness of 0.2 mm were exposed to CBZ ME for 2 h followed by thorough rinsing with PBS pH 6.4. In two other different sets of experiments isopropyl alcohol (a strong mucociliary toxin) and PBS pH 6.4 were used instead of CBZ ME for arriving at a comparative analysis of the extent of damage caused by the preparation. These pieces of mucosa were fixed in paraffin blocks and fine sections were taken that were stained by eosin and hematoxylin. The prepared slides were examined with an optical microscope (Olympus, Model BX10, Japan) and photomicrographs (magnification 400×) were taken. Similar procedure was used in our previous study [14].

2.7. Pharmacodynamic studies

Maximal Electroshock: Sprague Dawley rats weighting between 200 and 250 g and exhibiting clear hind limb extension phase during electrically induced convulsions were included in the present study. The experimental protocol was approved by the Institutional Animal Ethics. Committee (No. LJIP/IAEC/09/2011–2012). Rats were divided into 5 groups (n = 6). The first and second groups were treated intranasally with CBZ solution (60% PEG 400) and CBZ ME respectively containing CBZ equivalent to 8.18 mg/kg body weight (using a micropipette attached with low-density polyethylene (LDPE) tubing, having 0.1 mm internal diameter at the delivery site). In the third group CBZ solution containing CBZ equivalent to 8.18 mg/kg body weight was administered intraperitoneally (IP) while fourth group was treated with oral CBZ ME containing CBZ equivalent to 8.18 mg/kg body weight. The fifth group, not subjected to any treatment, served as control. Electroconvulsions were produced by applying current (150 mA, 0.2 s) through ear clip electrodes using electroconvulsometer (INCO, Ambala, India) after 60 min of administration of formulations and different phases of seizures were measured. Briefly after application of current an immediate severe tonic phase (E phase) was observed which was characterized by maximal extension of the anterior and posterior legs. At the end of tonic phase clonic phase starts which was characterized by paddling movement of the hind limb and shaking of body. During stupor phase which was observed after tonic and clonic phase rat remained silent without any movement. Recovery time was recorded as total time from starting of tonic phase till animal regains its normal movement [15].

3. Results and discussion

3.1. Solubility of drug

The saturated solubility of CBZ in various oils was reported in Table 1. It is important that the nasal formulation have the least volume. It can be done if the components of the ME that are chosen have highest solubility for the drug. This was analyzed using one variable at a time (OVAT) type of optimization. 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 carbamazepine and previous studies have reported improved nasal absorption when Tween 80 was used as one of the ingredient [16]. Thus, Tween 80, surfactant with HLB 14, was selected as a surfactant and depending on the solubility results PG was selected as cosurfactant, which also acts as permeation enhancer [16].

3.2. The pseudoternary phase diagrams

The components that showed maximum solubility were further optimized using pseudoternary phase diagram as shown in Fig. 1. The zone of ME was obtained. Six formulations were then taken from each corner at random and the best formulation was characterized thoroughly. It was found that increasing concentration of Tween 80 incorporation of water can be increased but solubility of drug decreases while by increasing concentration of PG drug solubility increase but incorporation of water decreases and highest ME area was obtained with ratio of 1:1 and thus selected for further studies. According to the ME area in the phase diagram, the carbamazepine loaded ME formulations were prepared as per the composition shown in Table 2. ME systems were obtained by mixing oil, surfactant and cosurfactant together and adding

| Table 1 – Solubility of CBZ in various excipients. |
|----------------|------------------|
| Material       | Solubility (mg/ml) |
| Sunflower oil  | 9.55 ± 0.25       |
| Soybean oil    | 16.22 ± 0.97      |
| Labrafac       | 5.46 ± 0.20       |
| Capmul MCM     | 10.11 ± 0.47      |
| Oleic acid     | 32.08 ± 2.54      |
| Tween 80       | 29.05 ± 1.13      |
| Propylene glycol | 54.18 ± 1.76    |
| Alcohol        | 51.55 ± 1.79      |

All values are expressed as mean of three readings.
appropriate quantity of CBZ and adding precisely distilled water drop by drop to these oily phases with magnetic stirring at ambient temperature. The final concentration of CBZ in ME systems was 30 mg/ml.

3.3. Physicochemical characterization of carbamazepine loaded MEs

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 have to be close to nasal secretions (4.5–6.5) and viscosity has to be moderate. pH and viscosity are important factors affecting mucociliary action which if affected may cause another set of complications. Additionally the pH deviations may cause irritation to the patient [7]. 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. Reported values indicates viscosity in between 100 and 200 cps is suitable for nasal administration [16]. It also has been observed the formulation containing water in external phase shows less irritancy on nasal mucosa. Based on the above rationale critical quality attributes and their desirable ranges were defined as follows. i) External medium – water ii) globule size – minimum iii) pH – 4.5–6.5 iv) viscosity – 100–200 cps. The pH, viscosity, conductivity, globule size and zeta potential of the prepared formulations are shown in Table 3. Result of globule size indicated that smallest globule size was obtained with formulation S3 with PDI 0.20, which is close to zero, indicating that the prepared ME had uniform globule size and thus it was selected for further studies as faster permeation is expected when the globule size is small. The pH of the ME S3

<table>
<thead>
<tr>
<th>Table 2 – Composition of selected ME.</th>
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<tr>
<td>S1</td>
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<tr>
<td>Carbamazepine (g)</td>
</tr>
<tr>
<td>Oleic acid (%)</td>
</tr>
<tr>
<td>Tween 80 (%)</td>
</tr>
<tr>
<td>PG (%)</td>
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<tr>
<td>Water (%)</td>
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</tbody>
</table>
was also near to above stated limit which indicated less chances of irritancy on nasal mucosa. The refractive index was 1.44 and % transmittance was found to be greater than 99% which confirmed that prepared CBZ ME was transparent. Isotropic nature of ME was also proved as ME appeared completely dark under polarized microscope [17]. The conductivity of the results confirmed the formation of solution type of ME with water in continuous phase. Viscosity of the optimized formulation was 193 cps which is suitable for nasal administration. Zeta potential was negative which indicated the stability of formulation as there were less chances of globules aggregation. After centrifugation cycle it was found that ME S3 was stable and no separation was observed which indicated centrifugation stability. The optimized ME S3 remained clear and transparent even after 3 months of storage. ME S3 was optimized from the prepared formulations as per the attributes decided earlier in this section. Ex-vivo permeation study and pharmacodynamic study was performed by using optimized ME S3.

### 3.4. Ex-vivo diffusion through sheep nasal mucosa

Ex-vivo diffusion study was performed by using sheep nasal mucosa for optimized formulation S3 and CBZ solution prepared in 60% PEG 400 and results were shown in Fig. 2. It was found that cumulative amount of CBZ permeated through sheep nasal mucosa was 32744.5 µg with a flux of 57.26 µg cm⁻² min⁻¹ and permeability coefficient was found to be 0.00253 cm⁻² min⁻¹ from CBZ ME while in case of CBZ solution cumulative amount of CBZ permeated through sheep nasal mucosa was 24537.06 µg with a flux of 57.26 µg cm⁻² min⁻¹ and permeability coefficient was found to be 0.00191 cm⁻² min⁻¹. The results clearly indicated faster diffusion of CBZ from CBZ ME in comparison to CBZ solution. The diffusion is faster due to presence of oleic acid, Tween 80 and PG which act as permeation enhancers. A biphasic release profile was obtained in which initial faster release was due to solubilized drug in continuous phase while slower rate was due to CBZ release from the oil droplets. CBZ from ME permeates rapidly (more than 50% of drug in 90 min) through nasal mucosa in comparison CBZ solution (less than 40% in 90 min).

### 3.5. Nasal ciliotoxicity studies

Results of nasal ciliotoxicity studies were shown in Fig. 3. Nasal ciliotoxicity studies revealed that nasal mucosa treated with PBS pH 6.4 (negative control) showed intact epithelium layer without any necrosis while nasal mucosa treated with isopropyl alcohol (positive control mucociliary toxic agent) showed complete destruction of epithelium layer, necrosis and even the deeper tissue parts were also destroyed. CBZ ME prepared in our studies did not exhibit any toxicity as no change could be noticed in the gross morphology and histology of the nasal mucosa. Our results are on similar lines of the observations reported by other workers on toxicity of oleic acid and Tween 80 which are the major components of our preparation [18].

### 3.6. Pharmacodynamic studies

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. 4 and 5. Significant differences between the control and treated groups were calculated using one way ANOVA followed by Tukey test for exact comparison in pharmacodynamic study. Significant reduction in E phase, clonus, stupor and duration of seizure was observed in the rats treated with CBZ ME by intranasal route and in comparison to group of rats treated CBZ solution administered IN and CBZ ME administered intranasally (p < 0.05, n = 6). The results clearly indicated lesser intensity

![Fig. 2 — Ex-vivo CBZ diffusion through sheep nasal mucosa from CBZ ME and CBZ solution.](image-url)

<table>
<thead>
<tr>
<th>Table 3 – Physicochemical parameters of ME.</th>
</tr>
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<tbody>
<tr>
<td>% Assay</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>Globule size (nm)</td>
</tr>
<tr>
<td>Conductivity (ms/cm)</td>
</tr>
<tr>
<td>Viscosity (cps)</td>
</tr>
<tr>
<td>% Transmittance</td>
</tr>
<tr>
<td>Zeta potential</td>
</tr>
</tbody>
</table>

All values are expressed as mean of three readings.
of seizure and rapid recovery from seizures in the rats treated with intranasal CBZ ME.

4. Conclusion

The carbamazepine loaded transnasal ME demonstrated lesser intensity of seizures which may be due to larger extent of selective nose to brain delivery of drug in comparison to oral ME, oral solution and nasal solution of carbamazepine. 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. However detailed animal study followed by thorough clinical trials is required to establish clinical safety and efficacy of this formulation.

Acknowledgments

This study was supported by a grant from Gujarat Council of Scientific Technology (GUJCOST), Ahmedabad.

REFERENCES


Development of carbamazepine transnasal microemulsion for treatment of epilepsy

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Development of carbamazepine transnasal microemulsion for treatment of epilepsy

Sheetal Porecha Acharya · K. Pundarikakshudu · Ashish Panchal · Anita Lalwani

Abstract Carbamazepine is widely preferred therapy for the treatment of epilepsy. However, oral therapy results in slower brain uptake and systemic side effects. Intranasal route can achieve faster brain uptake, but poor aqueous solubility of carbamazepine is the main obstacle for administration by nasal route. The purpose of this study was to prepare and evaluate intranasal oil in water microemulsion of carbamazepine to improve its solubility and enhance the brain uptake. Intranasal microemulsion of carbamazepine was prepared by water titration method using oleic acid as oil, Tween 80 as surfactant and Transcutol® as cosurfactant. Microemulsions were evaluated for various physical parameters including globule size, viscosity, pH and conductivity. Toxicity study of microemulsion was carried out by employing sheep nasal mucosa. The microemulsion was also evaluated by maximal electric shock, and the brain uptake study was done using HPLC method. The microemulsion was stable and transparent with average globule size of 21.03 nm and did not show any toxic symptoms. It showed reduction in the hind limb extension phase and faster recovery from seizures in comparison to oral microemulsion and nasal solution. Higher brain/plasma ratio was obtained with nasal microemulsion in comparison to ratio obtained after intraperitoneal injection of carbamazepine solution.

Keywords Brain targeting · Carbamazepine · Epilepsy · Intranasal · Microemulsion

Introduction

Carbamazepine (CBZ) has been the mainstream therapy for the treatment of different forms of seizures for a long time. However, its administration by oral route has certain side effects like GIT disturbances, severe skin reactions, liver failure, pulmonary disturbances, etc. A further limiting factor in oral dosage form is its poor absorption and prolonged $t_{\text{max}}$ [1]. This warrants the exploration of possibilities for alternative route of administration that may surmount the above problems.

Intranasal dosage forms have proved to be a promising tool for the pharmaceutical technologist in that they ensure a direct delivery of drug into the central nervous system via the olfactory region, prevention of loss of drug into the peripheral system, faster onset of action, noninvasiveness, ease of application and circumvention of blood–brain barrier. Lianli Li et al. (2002) and Vyas et al. (2006) have demonstrated the advantages of intranasal drug delivery system with diazepam and sumatriptan, respectively [2, 3].

Aqueous solubility of CBZ is very poor, and nasal delivery system cannot permit larger volumes for administration [4]. Thus, a microemulsion seems to be a convincing alternative for administering drugs like CBZ. A microemulsion is a thermodynamically stable transparent dispersion system containing oil, surfactant, cosurfactant and aqueous phases. Microemulsion also improves absorption across mucosal membranes due to its lipophilic nature and smaller globule size, which allow the reduction of dose and systemic side effects and may also be effective to achieve faster onset of action [5].

The objective of this investigation was to develop CBZ-loaded microemulsion containing the oil, surfactant and cosurfactant and evaluate the prepared formulation for physicochemical and pharmacodynamic parameters and also to perform brain uptake study in rats for CBZ. It was hypothesized that microemulsion-based intranasal delivery would
result in rapid nose to brain transport of carbamazepine in comparison to available oral formulation and intraperitoneal administration of CBZ solution. Hence, greater drug transport and distribution of CBZ is expected into and within the brain which in turn would help to maximize the therapeutic benefit of drug, reduce side effects, decrease the dose and frequency and perhaps even cost of therapy.

Materials and methods

Materials

CBZ was obtained as a gift from Lincoln Pharma, Ahmedabad, India and diethylene glycol monoethyl ether (Transcutol®), polyglyceryl oleate (Plurol Oleique CC497®) were gifted by Gattefossé (Toronto, Canada). Oleic acid, Tween 80, polyethylene glycol 400 and propylene glycol were purchased from Sigma Aldrich, India. All other chemicals were of analytical grade.

Selection and housing of animal

Sprague Dawley rats of either sex weighing between 200 and 250 g were procured from the central animal facility of L. J. Institute of Pharmacy and Research Centre, Ahmedabad. They were given standard pellet diet (Pranav Agro, Baroda, India) and water. All rats were maintained in a light-controlled room kept at a temperature of 25±5 °C and in relative humidity of 55±5 %. The experimental protocol was approved by the Institutional Animal Ethics Committee (No. LJIP/IAEC/09/2011–2012).

Methods

Determination of solubility of drug

The solubility of CBZ was determined by adding an excess of (1 g) CBZ to each cap vial containing 5 ml of the selected vehicles (Table 1). The mixture was heated at 40 °C, and the resulting suspension was shaken at room temperature for 24 h on a magnetic stirrer followed by centrifugation at 3,000 rpm for 10 min. The supernatant was diluted appropriately with methanol, and the quantity of solubilized drug was determined spectrophotometrically on a double beam spectrophotometer at 284 nm (UV 1800, Shimadzu, Japan) [6].

Preparation of pseudoternary phase diagram

Since oleic acid, surfactant and cosurfactant were to be used in the microemulsion, it was necessary to optimize the ratio of surfactant (Twee 80)/cosurfactant (Transcutol®) (Smix).

<table>
<thead>
<tr>
<th>Material</th>
<th>Solubility(mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunflower oil</td>
<td>9.55±0.25</td>
</tr>
<tr>
<td>Soyabean oil</td>
<td>16.25±0.97</td>
</tr>
<tr>
<td>Labrafac</td>
<td>5.46±0.20</td>
</tr>
<tr>
<td>Capmul MCM</td>
<td>10.11±0.47</td>
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<tr>
<td>Oleic acid</td>
<td>32.08±2.54</td>
</tr>
<tr>
<td>Tween 80</td>
<td>29.05±1.13</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>54.18±1.76</td>
</tr>
<tr>
<td>Transcutol</td>
<td>115.4±2.87</td>
</tr>
<tr>
<td>Alcohol</td>
<td>51.55±1.79</td>
</tr>
</tbody>
</table>

* Values are mean±S.D. for n=3

The ratio of surfactant/cosurfactant (Smix) was optimized by preparing pseudoternary phase diagrams for three different ratios, i.e. 1:1, 2:1 and 1:2. Oil (oleic acid) and Smix were mixed in different ratios, from 1:9 to 9:1, and distilled water was added dropwise till the solution converts from transparent to opaque. End points for different oil/Smix combinations were determined. By joining the end points, the boundaries of phases formed were obtained in the phase diagrams. Microemulsion region was assigned to samples demonstrating transparent and homogeneous state [7, 8]. CBZ was loaded in microemulsion with optimized surfactant/cosurfactant ratio (Table 2).

Characterization of CBZ-loaded microemulsions

Physicochemical characterization of CBZ-loaded microemulsions The viscosity of the prepared MEs was measured by a Brookfield Viscometer (Brookfield HADV III+, Brookfield Engineering Laboratory, USA) using 61 number spindle rotating at 100 rpm at room temperature. Electrical conductivity of microemulsions was measured using a conductivity meter (CM 180 ELICO, India) at room temperature, and the pH of microemulsion was measured using a pH meter (Systronic 335, India). Experiments were performed in

<table>
<thead>
<tr>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBZ</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>8</td>
<td>8</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Tween 80</td>
<td>32.5</td>
<td>39</td>
<td>35</td>
<td>37.5</td>
<td>35</td>
</tr>
<tr>
<td>Transcutol</td>
<td>32.5</td>
<td>39</td>
<td>35</td>
<td>37.5</td>
<td>35</td>
</tr>
<tr>
<td>Water</td>
<td>27</td>
<td>14</td>
<td>15</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>

* Amount in grams
b Amount in percent w/w

Table 1 The solubility of CBZ in various vehicles at 37 °C

Table 2 Composition of selected microemulsion formulations
triplicate for each sample. Droplet size distribution and zeta potential of the formulations were measured by dynamic light scattering using Malvern Zetasizer (Nano ZS, Malvern Instruments Ltd, UK). Percentage transmittance of prepared formulations was measured at 630 nm using distilled water as reference by UV spectrophotometer (UV 1800, Shimadzu, Japan).

**Ex vivo permeation studies**

Freshly excised sheep nasal mucosa was taken from sheep nose, obtained from a slaughter house. The mucosal membrane was freed from cartilage and dipped in phosphate buffer (pH 6.4). In order to mimic the physiological condition of the nasal cavity (the pH of nasal secretions is normally in the region 5.5–6.5), pH 6.4 phosphate buffer was selected in the present study. Ex vivo drug permeation study was performed using a Franz diffusion cell (diameter of 10 mm). Sheep nasal mucosa having uniform thickness of 0.20 mm was mounted on Franz diffusion cell. The receptor compartment was filled with 10 ml diffusion media (phosphate buffer pH 6.4+30 % PEG 400) to maintain perfect sink condition and maintained at 37 °C. Two-milliliter ME was placed in donor compartment with continuous slow stirring in receptor compartment. Samples from the receptor compartment were withdrawn at periodic time intervals, filtered through a 0.45-μm nylon filter paper and analysed using a UV–visible spectrophotometer. Equal volume of fresh diffusion medium was replaced after each withdrawal. Ex vivo permeation of pure drug solution was done similarly by placing 2 ml of drug solution in PEG 400 (40 mg/ml) in the donor compartment. Percentage drug perfused from CBZ ME and CBZ solution was calculated from the calibration prepared for CBZ in perfusion media.

The release data obtained were fitted in to the power law to determine release mechanism [9].

\[ \frac{M_t}{M_\infty} = k t^n \]

where \( M_t/M_\infty \) is the fraction of drug release time \( t \) and \( k \) is the rate constant and \( n \) is the perfusion coefficient related to the mechanism of the drug release. The value of \( n \) between 0.5 and 1.0 indicates non-Fickian of anomalous perfusion, while \( n=0.5 \) indicates Fickian release and \( n=1 \) indicates zero-order release.

**In Vivo nasal ciliotoxicity studies**

Microemulsion containing CBZ 40 mg/ml was administered intranasally to Spargue Dawley rats weighing 250±50 g at dose of 8.18 mg/kg [using a micropipette attached with a low-density polyethylene (LDPE) tubing, having 0.1 mm internal diameter at the delivery site]. After 2 h of dosing, the nasal mucosa from the bottom of the inferior meatus was dissected after sacrificing the rats. The mucosa was immersed in 10 % neutral formalin overnight and dehydrated with alcohol. These pieces of mucosa were fixed in paraffin blocks, and fine sections (10 μ) were taken and stained by eosin and hematoxylin. The prepared slides were examined with an optical microscope (Olympus, Model OIG, Japan), and photomicrographs (magnification ×400) were taken [6].

**Pharmacodynamic studies**

**Maximal electroshock method** Sprague Dawley rats weighing between 200 and 250 g and exhibiting clear hind limb extension phase during electrically induced convulsions were included in the present study. Rats were divided into
five groups ($n=6$). The different groups received the following treatments. CBZ solution (60 % PEG 400)/CBZ ME containing 3.52 mg CBZ (equivalent to 8.18 mg/kg body weight) was administered in 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. CBZ solution containing 3.52 mg CBZ was administered intraperitoneally (IP) to the third group of rats, while the fourth group was treated with oral CBZ ME containing 3.52 mg CBZ. The fifth group, not subjected to any treatment, served as control. Electroconvulsions were produced by applying current (150 mA, 0.2 s) through ear clip electrodes using an electroconvulsometer (INCO, Ambala, India) after 60 min of administration of formulations.

**Determination of CBZ uptake in the brain**

**High-performance liquid chromatography method** The method developed by Owen et. al. was used for estimation of CBZ in brain homogenate and plasma [10]. Briefly, the method involves high-performance liquid chromatography (HPLC) analysis (Model LC Module I) using C$_{18}$ column (250 mm×4 mm×5μm) with UV detector (Waters, USA) and Millennium® software. Mobile phase consists of acetonitrile/water (40:60), and flow rate was 1 ml/min.

**Extraction of CBZ from brain homogenate**

Sprague Dawley rats weighing 200 to 250 g were used after overnight fasting. Three groups of rats were employed in the
study (n=6). CBZ solution containing 3.52 mg CBZ in 60 % PEG 400 (equivalent to 8.18 mg/kg body weight) was administered intraperitoneally to the first group, while in the second group, CBZ ME containing 3.52 mg CBZ was administered intranasally by the method described in the “Pharmacodynamic studies” section.

Fifteen minutes after drug administration, animals were killed after stunning, and blood was collected from the carotid artery after decapitation, and intact brains were excised from the skull and homogenized immediately at 5,000 rpm for 10 min under chilled condition. Brain homogenate was made alkaline by adding 1 ml of 0.1 N NaOH, and drug was extracted by adding 2 ml ethyl acetate. Organic layer was separated after centrifugation at 7,000 rpm for 15 min at 4 °C and evaporated under vacuum. Residue was resuspended in 40 % acetonitrile and 60 % HPLC grade water. Plasma was also treated similarly, and CBZ in brain homogenate and plasma was estimated by HPLC method. Measurements were made using three rats at each time point for each formulation.

**Data analysis**

All the experiments in the study were performed at least three times, and the data were expressed as the mean ± standard deviation (S.D.). Significant differences between the control and treated groups were calculated using one-way ANOVA followed by Tukey test for exact comparison in pharmacodynamic study.

### Results and discussion

**Solubility of drug**

CBZ exhibited highest solubility in oleic acid, and hence, oleic acid was employed in the preparation of ME. Additionally, fatty acids present in oleic acid reduce zeta potential and suppress coalescence which provides supplementary stabilizing effect for microemulsion. Oleic acid has been reported to have minimal toxic effect on the nasal mucosa as shown by previous studies including nasal preparation of Sildenafil [11]. Tween 80 exhibited very good solubility for CBZ. It was preferred keeping in mind its role in improving nasal absorption [11]. Transcutol was also shown to act as a permeation enhancer [12].

**The pseudoternary phase diagrams**

The pseudoternary phase diagrams were constructed for identification of microemulsion region and optimization of ratio of surfactant/cosurfactant. The isotropic region was presented in the phase diagram as shaded part (Figs. 1, 2 and 3) as the one-phase microemulsion region. The remainder of the phase diagram represents the turbid region, represented as multi-phase, conventional emulsions. In our experiment, we noted that maximum monophasic region was obtained with 1:1 ratio of Smix. The ratio was ideal as increase in surfactant definitely enhanced the incorporation of water but limited solubility of CBZ. These results are in agreement with those reported by Rania et al. (2010) where in they used testosterone, also a poor water-soluble drug [13].

**Characterization of CBZ-loaded microemulsions**

**Physicochemical characterization of CBZ-loaded microemulsions**

All the prepared systems were transparent and clear even after storage for 3 months at room temperature. The pH of ME was found to be closer to that of nasal secretion; thus, it was expected that prepared ME will not show any irritancy. Conductivities ≥0.001 mScm⁻¹ indicate the presence of

| Table 3 Physicochemical characteristics of CBZ-loaded ME |
|-----------------|-------|-------|-------|-------|-------|-------|
|                 | S1    | S2    | S3    | S4    | S5    | S6    |
| % Assay          | 98.88±0.57 | 98.79±0.42 | 99.75±0.25 | 98.56±0.32 | 98.54±0.21 | 98.02±0.32 |
| pH               | 5.78±0.02 | 5.85±0.02 | 5.95±0.01 | 5.94±0.03 | 5.89±0.02 | 5.93±0.02 |
| Globule size (nm)| 51.3±2.5 | 21.0±0.9 | 145±3.5 | 100±3.2 | 91.2±2.7 | 80.0±2.2 |
| Conductivity (mS/m)| 0.24±0.05 | 0.23±0.05 | 0.23±0.04 | 0.23±0.04 | 0.24±0.03 | 0.22±0.02 |
| Viscosity (cps)  | 123±6  | 129±8  | 196±8  | 193±7  | 172±7  | 180±7  |

Values are mean±S.D. for n=3

![Fig. 4 Permeation study of CBZ ME and CBZ solution from sheep nasal mucosa](image)
water in the continuous phase observed in bicontinuous or solution type of ME [14]. Thus, conductivity results confirmed the formation of solution-type ME. Among the six ME (Table 2) prepared, ME S5 and S6 exhibited larger globule size (Table 3) and hence were not taken up for further study. Viscosity studies revealed ME S3 and S4 to be more viscous and hence showed less diffusion rate. Slow permeation rate is due to decrease in spreading area caused by viscous ME. Thus, ME S1 and S2 were selected for further studies. It has been reported by Lianli et al. (2002) that the nasal liquid formulations with more than 10 % (w/w) of water and less surfactant are expected to show better permeation and less irritancy on the nasal mucosa [2]. Thus, for ME S1 that had smaller globule size (PDI 1), maximum water incorporation and lesser viscosity were expected to show better permeation and less irritancy on the nasal mucosa. ME is considered to be stable if zeta potential is in the range of −30 to +30 mv. Zeta potential of ME S1 was −0.111 mv which indicates that the prepared ME is stable. ME S1 was studied further for pharmacodynamic, ciliotoxicity and brain uptake studies.

Ex vivo permeation study

The drug from ME permeates rapidly compared to that from CBZ solution (Fig. 4). This is expected as Tween 80, oleic acid and Transcutol® are known to enhance permeation of many drugs across the nasal mucosa [10, 12]. In our study, biphasic release profile of CBZ was noted. There was faster initial release of drug within the first 10 min followed by slower rate later. A convincing explanation for this phenomenon can be that the initial higher permeation rate may be obtained due to micellar solubilization of drug, while the slower rate later is may be due to drug release from the oil droplets to the receiver chamber through the continuous phase in the donor chamber [12]. The release mechanism was found to be non-Fickian or anomalous diffusion as n value obtained after data fitting in to power law was 0.5479. The results conformed to the release kinetics of CBZ nanoemulsion in water shown by Kelmann et al. [15].

In vivo nasal ciliotoxicity studies

In vivo nasal ciliotoxicity studies were conducted to check the changes in the gross morphology and histology of rat nasal mucosa induced by CBZ ME. The studies revealed that the CBZ ME prepared in our studies did not exhibit any toxicity as no change could be noticed in the gross morphology and histology of the rat nasal mucosa obtained from rats treated with CBZ ME in comparison to mucosa obtained normal rats (Fig. 5). Our results are on similar lines of the observations reported by other workers on toxicity of oleic acid, Transcutol® and Tween 80 which are the major components of our preparation [16, 17]. Hong-Mei et al. have reported that the formulation containing greater than 10 %
water and free from alcohol minimizes the irritation on the nasal mucosa. As optimized formulation contains 27% water and is free from alcohol, it is expected to be a non-nasal irritant.

**Pharmacodynamic studies**

Antiepileptic activity of prepared CBZ ME was assessed by observing the duration of the hind limb extension phase (THE) and by measuring time required for complete recovery after electrically induced seizures [18]. Significant differences between the control and treated groups were calculated using one-way analysis followed by Tukey test for exact comparison in pharmacodynamic study. A longer THE duration or higher time required for recovery indicated a more severe seizure. In control animals, mean THE recorded was 12.2317±1.6914, and the mean recovery time recorded was 62.316±1.969. The rats treated with CBZ ME orally did not show any significant difference with respect to THE duration (10.5267±1.9589) and recovery time (54.667±2.765) in comparison to untreated rats. The third group of animals which was treated with CBZ ME intranasally significantly reduced (p<0.05, n=6) THE phase (0.95167±0.4254) and recovery time (3.48±0.8347). Rats treated with CBZ solution intraperitoneally showed decrease in THE duration (0.9667±0.2439) and recovery time (3.7242±0.5272; p<0.05, n=6). The results clearly indicate that the CBZ ME administered intranasally protected rats from electrically induced seizure which is comparable to the intraperitoneal route (Figs. 6 and 7).

**Determination of CBZ uptake in the brain**

The brain/plasma ratio for different formulations administered by different routes indicated that the brain/plasma ratio for CBZ ME administered by intranasal route was significantly higher (p<0.05, n=6) in comparison to CBZ administered by IP route. At 10 min, the ratio obtained by nasal route was about 14 times higher than that obtained by IP route. Similarly, after 30 min of administration, 12.61 times higher ratio was obtained (Fig. 8). In short, these results support the existence of an alternative brain entry pathway for CBZ by formulating in o/w microemulsion by nasal route as indicated by rapid drug uptake obtained after intranasal administration of CBZ ME in comparison to CBZ solution administered through IP route.

**Conclusion**

Higher brain uptake of CBZ from ME for intranasal administration compared to oral and intraperitoneal route indicates that the developed formulation can be more useful for drugs like CBZ. This is specifically true if oral administration is not possible during severe bouts of seizure.

**Acknowledgments** The authors are thankful to Gujarat Council on Science and Technology for providing financial assistance for the project GUJCOST/MRP/201594/10-11/3765.

**References**


ANNEXURE 3

CPCSEA APPROVAL LETTER
TO WHOMSOEVER IT MAY CONCERN

This is to certify that Ms. Sheetal Acharya faculty of L. J. Institute of Pharmacy, Ahmedabad in the department of Pharmaceutics has been permitted to conduct animal experiment at our college. The experimental Protocol no. LJIP/IAEC/11-12/9 was subjected to scrutiny of Institutional Animal Ethics Committee and it was cleared by the same before beginning the experiment.

Title: Evaluation of Anti-epileptic activity by electric induced seizures & estimation of anti-epileptic drug in brain.

Ph.D. Thesis Title: "Microemulsion based Transnasal Delivery System for Brain Targeting"

Sign of Chairman

IAEC COMMITTEE
ANNEXURE 4

GUJCOST APPROVAL LETTER
Sanction Order:
Gujarat Council on Science and Technology, Gandhinagar is running different schemes for nurturing & promoting Research and Development activities in the state. With reference to the above letter, we have received an application titled “Microemulsion based transnasal delivery system for antiepileptic drugs” from Mrs. Sheetal T Acharya, Lecturer, L. J. Institute of Pharmacy, Sarkhej-Gandhinagar Highway, Ahmedabad, under the Minor Research Project Scheme. On the basis of recommendation of technical experts and approval by GUJCOSTs 27th EC meeting, financial assistance of Rs. 1,25,000/- (In words: Rupees One Lakh Twenty Five Thousand only) is sanctioned for period of Two Year as per the following details and conditions.

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Permission is hereby granted for release of First year Grant of Rs. 62,500/- under the following conditions.

(1) To get the above mentioned financial assistance an M.O.U has to be signed between the Principal/Head of the Institute, Principal Investigator of the project and GUJCOST. On submission of the MOU to GUJCOST financial assistance will be released.

(2) Six monthly and annual spiral bound progress reports of the project in triplicate with photographs, Copies of Vouchers and Bills of the spent amount will have to be submitted to GUJCOST.

(3) Unspent amount will have to be submitted to GUJCOST at the end of Project.
The Cheque/DD of Rs. 62,500/- (In words: Rupees Sixty Two Thousand Five Hundred only) shall be issued in favor of The Director, L. J. Institute of Pharmacy payable at Ahmedabad.

The expenditure involved is debitable to:
Demand No. 90:
Major Head-3425-other-Scientific Research,
60-other-200-Assistance to other Scientific Institutes
(02) STP (19) Gujarat Council on Science & Technology,
5000 - Other charges (Plan).

The sanction has been issued under the powers delegated to the Advisor by Governing Board meeting dated 15/02/2000 Agenda No: 10-13.

Dr. A. M. Prabhakar
Member Secretary & Advisor

Copy to:
(1) Dy. Accountant, GUJCOST, Gandhinagar (Three Copies).
(2) Director L. J. Institute of Pharmacy, Sarkhej- Gandhinagar Highway, Ahmedabad-382210.
(3) Mrs. Gheetal T Acharya, Lecturer, L. J. Institute of Pharmacy, Sarkhej-Gandhinagar Highway, Ahmedabad-382210.
(4) Concerned Project Manager.
(5) Office file.