RESEARCH ARTICLE

Rizatriptan Benzoate Nanoemulsion for Intranasal Drug Delivery: Preparation and Characterization

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ABSTRACT

Rizatriptan benzoate is a selective 5-HT (1B/1D) receptor agonist used to treat migraine with an oral bioavailability equal to 45%. This research aimed to develop and optimize an intranasal nanoemulsion formulation to bypass first pass effect, enhance residence time, and permeability through nasal preparation. The aqueous titration method was used to prepare NE formula and assessed for droplet size, polydispersity index (PDI), thermodynamic stability, drug content, viscosity, pH measurement also in-vitro drug dissolution studies. The selected formula was subjected to Fourier transform infrared (FTIR) compatibility studies and ex-vivo permeation studies. Ten formulas were prepared by using oleic acid as an oil phase, tween80, ethanol as a surfactant and co-surfactant, respectively. The results showed that the formula NE1 with oil: Smix(1:1):water (5:55:40) ratio was the optimized formula which has droplet size equal to 59.6 nm, polydispersity index (0.308), pH (5.6), drug content (98.78%), percent transmittance (99.4%), viscosity equal to (91.9 mPas.sec), and high release profile. Ex-vivo permeation study revealed that 88.27% of drug permeated within 5 hours; there was no interaction between the drug and the excipient, according to FTIR studies. In conclusion, intranasal NE was an efficient method to bypass first pass effect, enhance permeation and residence time, and increase patient compliance.

Keywords: Nanoemulsion, Pseudoternary phase diagram, Rizatriptan benzoate.


Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Rizatriptan benzoate is a 5-hydroxytryptamine (5-HT) 1B/1D receptor agonist that has been used to treat migraine headaches. Migraine symptoms are thought to be caused by local cranial vasodilation and/or the release of vasoactive and pro-inflammatory peptides from sensory nerve endings in an activated trigeminal system. In 70–80% of individuals, rizatriptan benzoate relieves migraine pain within two hours.1 In treating migraine symptoms, rizatriptan is often more effective than sumatriptan, specifically for headache and nausea.2 Rizatriptan benzoate has a 40–45% oral bioavailability. Monoamine oxidase isoenzyme (MAO-A) catalyzes the considerable hepatic metabolism of rizatriptan benzoate to an inactive indole acetic acid metabolite. Various approaches have been tried to address this issue, such as orodispersible tablets, mucoadhesive buccal films, and elastic liposome-based transdermal drug delivery systems. The intranasal route has been considered a potential alternative for drugs with considerable first-pass metabolism.1 Nasal drug delivery has been developed as a noninvasive alternative route. The nasal mucosa has been proposed as a possible route of drug delivery to produce a faster and higher amount of drug absorption. This is due to the large surface area, porous endothelial membrane, high total blood flow, avoidance of first-pass metabolism, and ease of access. Several researchers have investigated the morphological and physiological characteristics of the nasal membrane, especially its vascular nature, as they pertain to drug administration.3 According to mounting evidence, intranasal medication delivery allows both tiny and big molecules to cross the BBB by passing through the olfactory and the trigeminal neurons present in the nasal cavity. For instance, the olfactory “neuroepithelium” is the sole component of the central nervous system (CNS) that is not protected by the blood-brain barrier and thus in communication with the external environment. As a result, it is an unparalleled access port to the brain.4 Nanoemulsion (NE) formulations enhance nose-to-brain drug delivery by protecting the encapsulated drug from biological and/or chemical degradation.5 NE were produced from the dispersion of two immiscible phases with nanometric diameters ranging from 20 to 200 nm. Using a surfactant, or a mixture of surfactant and a co-surfactant, allows immiscible liquids to become miscible in a single phase.
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by lowering the interfacial tension between them, resulting in kinetic stability of nanoemulsions.  

The purpose of this research was to develop and optimize a rizatriptan benzoate nanoemulsion for nasal delivery, which will enhance permeation and avoid first pass metabolism, coupled with an ease of administration, is a promising dosage form to enhance patient compliance.

MATERIALS

Rizatriptan benzoate was purchased from Baoji Guokang Bio-Technology (China), Oleic acid and Tween20 have been bought from Thomas Baker (chemicals) Pvt Ltd, India. Olive oil provided by Pomace olive oil oilex, S.A. Spain. Sesame oil and sweet almond oil obtained from Shaanxi Guanjie Technology Co. Ltd. China. Tween80 was obtained from alpha chemika, India. Transcutol-p was obtained from Hyper-Chem. Ltd. Co., China. All of the substances and reagents utilized were of analytical grade.

METHODS

Differential Scanning Calorimeter (DSC)
The thermal characteristics of drug powder samples were investigated using a DSC/TA-60thermal analysis controller in conjunction with an intercooler-2 cooling system (DSC-60, Shimadzu, Japan). Each sample set was heated to 50–250°C at a rate of 10°C/min, with nitrogen used as a blank gas melting range, and variation of enthalpy in the process of characterizing medicines is one of the principal requirements evaluated in quality control of the pharmaceutical industry. In this study, the method of purity determination using DSC was outlined, as well as the application of this technique for the evaluation of commercial samples of zidovudine (AZT).

Solubility Studies
At 25°C, rizatriptan benzoate solubility tests were conducted using various oils, surfactants, and co-surfactants. The saturated solutions were constructed by shaking 5 mL of vehicles for 72 hours with an excess of rizatriptan benzoate under steady vibration on a water bath shaker. Following this time frame, samples were centrifuged at 3000 rpm for 15 minutes. The supernatant layer for each sample was filtered by using filter membrane (0.45 μm), sufficiently diluted by using methanol and detected by ultraviolet/visible (UV-vis) spectrophotometer at λ_{max} (227 nm) of rizatriptan benzoate. Three determinations executed for each vehicle (Table 1).

Pseudo-ternary Phase Diagrams Construction
NEs components used were oleic acid employed as an oil phase, tween80 used as a surfactant. And ethanol is used as a co-surfactant after thoroughly examining several oils, surfactants, and co-surfactants. Smix was made up of surfactant and co-surfactant in various weight ratios, including 1:1, 1:2, 1:3, 2:1, and 3:1, to clearly outline the boundaries of phases, at a temperature of 25°C using the aqueous titration technique. For each diagram, oil and required Smix ratios were entirely mixed in different weight ratios ranging from 1:9 to 9:1 in separate glass vials. Under mild magnetic agitation, water was added for each combination of oil and Smix drop by drop until a stable, transparent system was achieved. The samples were visually tested after reaching equilibrium and determined to be clear/transparent. A pseudo ternary phase diagram component was used to depict the physical state of nanoemulsion, and the aqueous phase is represented on one axis, oil on the other, and a combination of surfactant and co-surfactant at a defined weight ratio (Smix ratio) on the third axis.

Nanoemulsion Formulation Preparation
Nanoemulsions were made by dissolving a certain amount of drug in oleic acid and mixing it with a Tween80 and ethanol. The entire liquid was then mixed using a vortex mixer. After that, at room temperature, the system was gradually mixed with double distilled water until it was transparent. Table 2 shows the composition of the preparation.

Studies of Thermodynamic Stability
Physical thermodynamic stability studies are usually constructed to overcome the issue of metastable formulations. All formulations went through centrifugation, a heating-cooling cycle, and a freeze-thaw cycle.

Droplet Size Measurement and Poly-dispersity Index (PDI)
The formulation (0.1 mL) was properly mixed with vigorous shaking in 50 mL of water in a volumetric flask, and light scattering was measured at 25°C at a 90° angle using a particle size analyzer instrument (Brookhaven Corp 90 Plus, NY, USA), which investigates light scattering fluctuation caused by Brownian particle motion. The homogeneity of droplet distribution inside the formed nanoemulsion was determined by measuring PDI.

Measurement of pH
The pH of the nasal preparation should be measured in order to minimize irritation of the nasal mucosa, the growth of pathogenic microorganisms, and the maintenance of normal physiological ciliary activity as well as 5-HT1B/1D subtypes. It is effective for acute migraine attacks, but has a short half life (about 2 hours. pH measurement was done by using a digital pH meter.

Percent Transmittance Measurement
Using deionized water as a blank and a UV-vis spectrophotometer set at 650 nm, the clarity of the nanoemulsion formulations was measured spectrophotometrically.
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Measurement of Viscosity
The nanoemulsion preparation’s viscosity was detected by using a digital viscometer (NDJ-5S, U.K.) with spindle-1 at 25 ± 1°C. In a graduated cylinder, 40 mL of sample of prepared NE was placed and the spindle was revolved at 6, 12, 30, and 60 rpm.

Drug Content Measurement
The nanoemulsion formulation was diluted with methanol in a volumetric flask, then filtered using 0.45 μm filter syringe. Determining the contents of rizatriptan benzoate NEs via UV-vis spectrophotometer at the selected λ max.

In-vitro Drug Dissolution Study
The quantitative in vitro release test was performed in 500ml phosphate buffer (6.4) utilizing a dialysis bag technique (Molecular cut off 12000 Dalton “Da”) and a USP dissolution apparatus type II (paddle method) at 50 rpm and 34 ± 0.5°C. About 0.1-mL (0.1 g) of the prepared formulas, equipollent to 2.906 mg of rizatriptan benzoate, was integrated in the dialysis bag. 5 mL samples were collected at a regular interval (5, 10, 15, 30, 45, 60, 90, and 120 minutes), then filtered through a 0.45 μm filter syringe and were analyzed by UV-vis spectrophotometer at the λ max. of the drug (225 nm). An equivalent volume of fresh medium was added to maintain the volume of dissolution medium at 500 mL.

Selection of the Optimum Formula
The best formula could be chosen now, and this accomplished according to the following criteria: particle size analysis, polydispersity index (PDI), pH, viscosity, drug content and in vitro release studies.

Evaluation of the Selected Rizatriptan Benzoate Optimum Formula

Ex-vivo Permeation Study
Fresh nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. Nasal mucosa was integrated into phosphate buffer pH 6.4 until used for the ex-vivo study. First the sheep nasal mucosal membrane was positioned between the diffusion cell’s donor and receptor compartments. Then Phosphate buffer solution pH 6.4 at 34 ± 1°C was added to the receptor compartment. After a pre-incubation time of 20 min, a formulation equivalent to 2.906 mg of drug was deposited in the donor compartment, which was connected to the membrane’s mucosal surface. The magnetic stirrer was used to stir the contents of the donor compartment continuously. For 5 hours, 1-mL samples were withdrawn from the acceptor compartment at specified time points, with the sampled volume being replaced with phosphate buffer pH 6.4 after each sampling. The samples were filtered before being used for analysis. A UV-visible spectrophotometer was used to assess the amount of permeated drug at 225 nm. The permeability coefficient (P) is computed using the equation below.

\[ P = \frac{dQ/dt}{CoXA} \]

Where (dQ/dt) is the permeability rate or flux (mg/h), (Co) is the donor compartment initial concentration, and (A) is the effective surface area of nasal mucosa.

Drug and Excipient Compatibility Study by FTIR
FTIR could be used to show the interaction between the drug and the excipients in the chosen formula. The KBr disc method was used to produce the sample, and spectra were obtained in the range of 400–4000 cm⁻¹. Cuvette, specialized in the liquid sample, was employed for the specified nanoemulsion formula. The spectra that were obtained were analyzed.

RESULT AND DISCUSSION

Differential Scanning Calorimeter (DSC)
Pure rizatriptan benzoate powder showed a characteristic endothermic peak at 184.43, which displayed a prominent endothermic melting peak. Such peak signifies that the drug was in a pure crystalline state and near the reported one.

Solubility Study
The selection of the main component (oil, surfactant, and co-surfactant) was considered an important factor for preparing a stable nanoemulsion. Furthermore, Nonionic surfactants were selected since they’re typically considered safe and biocompatible. Because of toxicological concerns, ionic surfactants were precluded from the study. All of the co-surfactants studied are pharmaceutically acceptable ingredients. Table 1 represents the results of the solubility studies with oleic acid as an oil phase, tween80 as a surfactant, and ethanol as a co-surfactant used for preparing the nanoemulsion.

Construction of Pseudo-ternary Phase Diagrams
The pseudo-ternary phase diagram for the nanoemulsion employing oleic acid as an oil phase, tween80 as a surfactant, and ethanol as a co-surfactant is shown in Figures 1 and 2.


Table 1: Saturated solubility study of rizatriptan benzoate

<table>
<thead>
<tr>
<th>Oil</th>
<th>Solubility (mg/mL) mean ± SD</th>
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<tbody>
<tr>
<td>sweet almond oil</td>
<td>0.93 ± 0.12</td>
</tr>
<tr>
<td>oleic acid</td>
<td>31.36 ± 0.23</td>
</tr>
<tr>
<td>olive oil</td>
<td>1.92 ± 0.37</td>
</tr>
<tr>
<td>sesame oil</td>
<td>0.79 ± 0.15</td>
</tr>
<tr>
<td>Span80</td>
<td>4.32 ± 0.34</td>
</tr>
<tr>
<td>Tween80</td>
<td>35.43 ± 0.25</td>
</tr>
<tr>
<td>Tween20</td>
<td>9.55 ± 0.23</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Co-surfactant</th>
<th></th>
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<tbody>
<tr>
<td>Ethanol</td>
<td>25.00 ± 0.52</td>
</tr>
<tr>
<td>transcutol-p</td>
<td>4.58 ± 0.43</td>
</tr>
<tr>
<td>polyethylene glycol</td>
<td>20.27 ± 0.49</td>
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</table>
Preparation of Rizatriptan Benzoate-loaded Nanoemulsion

Many formulae can be created using the pseudo-ternary phase diagram. As shown in Table 2, different amounts of oil were used to cover the greatest number of formulations from the nanoemulsion area.

Thermodynamic Stability Studies

The thermodynamic stability studies were accomplished on all of the developed nanoemulsion formulations. There was no evidence of phase separation, cracking, or a change in odor or color as shown in Table 3.

Droplet Size Measurement and Polydispersity Index (PDI)

All of the nanoemulsions developed were in nanosize. The result illustrated that as the oil concentration increased, the globule size would also be increased. When the surfactant concentration was increased, the particle size was diminutive because a high surfactant concentration during homogenization lowers surface tension and stabilizes newly formed surfaces. All of the formulations had a polydispersity index of less than 0.5, indicating a homogeneous and narrow globule size distribution.
Ahmad et al. found that nanoemulsion with droplets of 80 or 200 nm show some retention in the nasal cavity up to 16 and 12 hours, respectively, as the formula may penetrate the mucus layer and reach deep into the nasal mucosa. Depending on what is mentioned, only formulas with less than 200 nm will be further characterized (Table 4).

**pH Measurement**
The pH of all nanoemulsion formulation has been determined as illustrated in Table 5, and it was established to be acceptable for nasal administration.

**Drug content**
The NEs formulas concurrent with the British Pharmacopeia requirement range (95–105%) as shown in Table 5, indicating high content uniformity and revealed the adequacy of the preparation method.

**Percent Transmittance Measurement**
The result of % transmittance illustrated that the formulated nanoemulsions were transparent, clear, and easily transmitted light as shown in Table 5, their transparency is imputed to their small size.

**Viscosity Measurement**
The result of the viscosity measurement revealed that as the surfactant concentration increased, the viscosity increased correspondingly. This could be due to the entrapment of water molecules in cross-linking surfactant chains, as well as the dispersion medium becoming more rigid with the highest surfactant content, the viscosity result illustrated in Figure 3.

**In-vitro Drug Dissolution Study**
The release of the drug from several NE formulations was found to be near 100% at the end of 90 minutes; The drug's quantitative release from a nanoemulsion formulation is impacted by droplet size. As the concentration of tween80 increase, the viscosity increases and the drug release decreases from the formulas. Increasing the co-surfactant (ethanol) concentration decreases the viscosity and increases the dissolution rate. The co-surfactant role in nanoemulsion systems decrease interfacial tension and increases the interface’s fluidity. Also, it increases the hydrocarbon tail’s mobility, enabling greater oil penetration in nanoemulsion region. Figure 4 demonstrates the release profile of nanoemulsion formulations.

**Selection of Optimum Formula of Rizatriptan Benzoate Nanoemulsions.**
Based on the result obtained from the characterization study of the prepared nanoemulsions, NE1 was picked as the optimum formula, since it is characterized by good droplet size (59.6), good PDI (0.308), pH (5.6), which is within the range of the nasal cavity pH, good percent transmittance (99.40), a high percentage of drug content (98.78), proper viscosity (91.9 mPa.sec) and high release of the drug from the formula. The optimized formula would be further characterized.

**Ex-vivo Permeation Study**
Figure 5 shows the NE1 ex-vivo permeation profile where (88.27%) is the amount of the drug permeated within 5 hours.
while only (34.67%) permeated from drug solution (control), and this revealed there is an increase in the permeability of rizatriptan benzoate in the nanoemulsion formulation. The permeability coefficient of NE1 is \((3.17 \times 10^{-3} \text{ cm}^2/\text{min})\) and the flux \((9.2003 \mu\text{g/cm}^2/\text{min})\), while the permeability coefficient of drug solution is \((1.1 \times 10^{-3} \text{ cm}^2/\text{min})\) and the flux \((3.1902 \mu\text{g/cm}^2/\text{min})\).

**Drug and Excipient Compatibility Study by FTIR**

The characteristic peak of pure rizatriptan benzoate was shown in Figure 6, and agrees with previous reported study.\(^{33,34}\) The peaks are \((C=C)\) stretching of aromatic ring at 1604.77 cm\(^{-1}\), \((C=N)\) stretching at 1504.48 cm\(^{-1}\), \((C=O)\) stretching of carboxylic acid at 1292.31 cm\(^{-1}\), \((CH_2;CH_3)\) bending at 1458.18 cm\(^{-1}\) and 1373.32 cm\(^{-1}\), respectively. The FTIR spectrum of the selected nanoemulsion formulation is shown in Figure 7.

**CONCLUSION**

Based on the results obtained from this study, it can be concluded that rizatriptan benzoate intranasal NE formulation was successfully prepared using oleic acid, Tween80, ethanol for the preparation. *Ex-vivo* permeation study showed that 88.27% amount permeated within 5 hours. NE formula was considered as a promising approach to enhance permeation and residence time for intranasal preparation.
REFERENCES


