Formulation and Evaluation of Fish Oil-based Rizatriptan Microemulsion for Intranasal Migraine Treatment

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ABSTRACT

In the present study microemulsion based intranasal gel of rizatriptan using fish oil was prepared for treatment and management of migraine to sustain the drug release and improve the drug residence time in nasal cavity. Fish oil is reported to have antimigraine activity hence it has been used in the present formulation along with cremophore EL as surfactant and Transcutol P as co-surfactant. The pseudoternary phase diagram plotted with these components shown the microemulsion existence region in ratio of (1:9-9:1) surfactant and co-surfactant. The optimized micro-emulsion contained fish oil (45.29%), cremophore E/transcutol P (2:1) and was characterized for pH (6.3±0.02), viscosity (114 ± 3.00cp), % transmittance (99.5 ± 1.01), refractive index (1.335±0.01)). The prepared microemulsion gels were optimized and characterized for in-vitro studies, pH, drug content, rheological studies and stability study. The release of rizatriptan from micro-emulsion gel prepared from carbopol 934 (98.01%) was found to be higher and prolonged than plain gel. Thus, microemulsion based gel was able to prolong drug release and improve drug residence time in the nasal cavity.

KEYWORDS: Microemulsion; Fish oil; Migraine; Rizatriptan; Mucoadhesion; Sustain release.

Introduction

Microemulsions are defined as a system of water, oil, and amphiphile which is a single, optically isotropic and thermodynamically stable liquid solution. Microemulsions forms spontaneously with an average droplet diameter of 10 to 140 nm (Senthil et al., 2011). Microemulsions can be applied as liquid membrane carriers to transport lipophilic substance through an aqueous medium or to carry hydrophilic substances across lipid medium. Microemulsions behave as a Newtonian liquid (Vandamme et al., 2010). The surfactant chosen for the microemulsion must be able to lower the interfacial tension and stabilize microemulsion system. (Khairnar et al., 2011) The addition of a co-surfactant along with surfactants reduces the surfactant concentration required for microemulsion formation (Fanun et al., 2010).

Drug delivery by nasal microemulsion increases the systemic delivery of drugs by various mechanisms that make them suitable a vehicle for delivery of drugs. Nose-to-brain delivery also avoids blood brain barrier which is an important factor to be considered in formulation of CNS targeting drugs. (Kulkarni et al., and Kamble et al., 2013). This route of administration is painless and useful in emergency conditions. The nasal cavity offers a number of unique advantages such as easy accessibility, good permeability especially for lipophilic, low molecular weight drugs, avoidance of harsh environmental conditions and hepatic first pass metabolism, potential direct delivery to the brain (Tyagi et al., 2012).

The anti-migraine drug of rizatriptan benzoate acts through activation of postsynaptic 5-HT1B receptors within cerebral and dural vessel walls, causing vasoconstriction of perivascular nerve terminals. Rizatriptan undergoes hepatic first pass, hence it shows poor bioavailability. Administration of the drug as a microemulsion via nasal route thus would be helpful to overcome its poor bioavailability. Also it could increase the drugs resident time and prolong drug release. Use of fish oil may further increase antimigraine activity by having a synergistic effect. This premise is tested in this study using a suitable formulation technology.

Materials and Methods

Materials

Rizatriptan benzoate (Cipla, mumbai), Reconstituted fish oil (Arbeebiomarine extract, Kottayam Kerala), Carbopol 940, Hydroxy propyl methyl cellulose, Methyl cellulose (Lobachemie, Mumbai), Poloxamer 407, Cremophore EL (BASF, Mumbai), TranscutolP (Gattefosse India Pvt. Ltd., Mumbai).

Solubility studies and Construction of pseudoternary phase diagram

Solubility studies of rizatriptan benzoate were carried out in various solvents and results are given in Table 1
From the results of solubility studies, reconstituted fish oil was chosen as the oil phase. Similarly cremophore EL, as surfactants and transcutol P, as co-surfactants were used as they showed maximum solubility of rizatriptan benzoate. For construction of pseudo-ternary phase diagram two methods can be employed, such as water titration or oil titration. In the present investigation, water titration method was employed to construct a pseudo-ternary phase diagram.

Fig. 1 Method of preparation.

Characterisation of Microemulsion

1. Transmittance and visual clarity
The droplets of the microemulsions being smaller than \( \frac{1}{4} \)th the wavelength of visible light, permit white light to pass through the dispersed system making it transparent or translucent (Patil et al., 2010, Dhakare et al., 2003 and Magdum et al., 2009). The microemulsion systems were inspected for optical transparency and homogeneity by usual observation against strong light. The system was also checked for the presence of undissolved drug or other solid ingredient. The results are shown in Table 3.

2. Centrifugation
Physical stability of the microemulsions was studied by centrifugation at 3,000 rpm for 2 hours. After centrifugation the samples were observed for clarity and any phase separation or precipitation. The results are shown in Table 3.

3. pH measurement
The pH measurement of the microemulsions was determined by using a pH meter which was calibrated before use with standard buffer solutions at pH 4 and 7. The results are shown in Table 3.

4. Refractive index
The refractive index of medicated formulation was determined using an Abbe type refractometer. The results are shown in Table 3.

5. Viscosity
The viscosity of the prepared microemulsions was measured using Brookfield viscometer (LVDVE) using spindle number S 64, at 100 rpm. Experiments were carried out in triplicate for each sample and the results are presented as an average ± standard deviation in Table 3.

6. Color
The color of the microemulsion may vary according to the percentage of mixture of oil, surfactant, co-surfactant and water. The results are shown in Table 3.

7. Particle size Analysis of formulated microemulsion
Mean globule size of the optimized microemulsion was determined by photon cross-correlation spectroscopy. Microemulsion was placed in transparent polystyrene cuvette (path length = 1 cm) which was placed in thermostatic sample chamber maintained at 25 °C. Sample temperature was set at 25 °C and 3 runs of 60s were performed. Detection was carried out at a scattering angle of 90°. From the resulting correlation curves, a 2nd order analysis was performed to calculate the mean globule size and standard deviation. The results are represented in Figure 3.

Preparation of Microemulsion Gel
Various gelling agents namely, methyl cellulose, hydroxyl propyl methyl cellulose, poloxamer 407 and carbopol 934 were evaluated for their ability to gel drug loaded liquid microemulsion (Shinde U, et al., 2012). Gelling agent was dispersed slowly in the medicated microemulsion with the help of overhead stirrer. In case of carbopol 940 and carbopol 934, the dispersion was neutralized by adding triethanolamine or sodium hydroxide to obtain the gel. The results are represented in Table 4.

Characterisation of Microemulsion-Gel
The prepared rizatriptan microemulsion-gel was inspected for homogeneity, grittiness, viscosity, spreadability, pH, drug content, consistency, in-vitro drug release and stability studies (Kumar S, et al., 2010). The results are depicted in Table 5.

1. Homogeneity
All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates. The results are shown in Table 5.

2. Grittiness
All the formulations were evaluated microscopically for the presence of particles. The results are shown in Table 5.

3. Viscosity
The measurement of viscosity of the microemulsion gel was done with a Helipath Brookfield viscometer. The gels were rotated at 20 rpm,
50 rpm and 100 rpm using spindle #F 96. At each speed, the corresponding dial reading was noted. The results are shown in Table 5.

4. **pH measurement**
   The pH measurement of the microemulsions was determined by using a pH meter which was calibrated before use with standard buffer solutions at pH 4 and 7. The results are shown in Table 5.

5. **Spreadability**
   The spreadability of the gel formulations was determined by measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm x 20 cm) after one min. The standard weight applied on the upper plate was 125 g. The results are shown in Table 5.

6. **Drug content**
   To ensure uniform distribution of drug in gel formulation, it was sampled from the different locations in the mixer and assayed for the drug content. Drug content of the gel was determined by dissolving an accurately weighed quantity of gel (about 1 gm) in about 100 mL of phosphate buffer pH 7.4. These solutions were quantitatively transferred to volumetric flasks and appropriate dilutions were made with the same buffer solution. The resulting solutions were then filtered from membrane filters (0.45 mm size) before subjecting the solution to spectrophotometric analysis at 225 nm and the drug content was determined. The results are shown in Table 5.

7. **Consistency**
   The measurement of consistency of the prepared gels was done by dropping a glass rod by means of a thread attached to a holding rod from a fix distance of 10cm in such way that it should fall on the center of the glass cup filled with the gel. The penetration by the glass rod was measured from the surface of the gel to the tip of the inside the gel. The distance traveled by glass rod was noted down after 10sec.
   \[ \% \text{ consistency} = \frac{\text{CT}}{\text{CD}} \times 100 \]
   Where, CT is length of glass rod which penetrate inside the gel, CD is the length of glass cup filled with gel.

8. **In-vitro drug release study**
   The Franz diffusion cell assembly consisted of donor and receptor compartments. The receptor compartment was filled with 20 mL phosphate buffer (pH 5.5). A standard dialysis membrane (soaked in pH 5.5 for 24 hours before use) was clamped between the two half of the cell. The position of the donor compartment was adjusted so that the membrane just touches the buffer medium. The cells were thermostated at 37 ± 1 °C and the receptor solution was stirred with a magnetic stirrer at 100 rpm.

   The prepared rizatriptan MEG was placed in donor compartment. The samples were withdrawn from the receiver solution at predetermined time intervals. After each withdrawal, cells were replenished to their marked volumes with fresh buffer solution. The withdrawn samples were taken in volumetric flask and analyzed by UV spectrophotometer at 225nm. The results are shown in Figure 4.

   To analyze the in-vitro release data, all the formulation were studies with various kinetic models to describe the release kinetics.

9. **Comparison between optimized Microemulsion gel, Gel and Pure drug**
   Optimized microemulsion based gel and plain gel containing carbapol were formulated. Pure drug solution was prepared. The drug release from the optimized preparation was compare with that from the gel and pure drug solution. The results are depicted in fig 4 and 5.

10. **Stability study**
    The accelerated stability studies of the rizatriptan microemulsion-gel were carried out. All the formulated rizatriptan microemulsion-gels were kept in glass containers and undisturbed, in the chamber. The analytical condition was 40 ± 2 °C temperature and 75 ± 5% RH. Then at specific intervals the samples were withdrawn for pH, viscosity and drug content determination. The results are depicted in Table 6.

**Results and Discussion**

1. **Solubility studies**
   Solubility studies of rizatriptan were carried out in various solvents.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Solvents</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chloroform</td>
<td>Insoluble</td>
</tr>
<tr>
<td>2</td>
<td>Glacial acetic acid</td>
<td>Slightly soluble</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol</td>
<td>Insoluble</td>
</tr>
<tr>
<td>4</td>
<td>Water</td>
<td>Soluble</td>
</tr>
</tbody>
</table>

   From the result of solubility of rizatriptan, it was observed that, rizatriptan was insoluble in chloroform, slightly soluble in glacial acetic acid, Insoluble in ethanol and soluble in water as reported in USP.

2. **Pseudoternary Diagram**
   From these pseudoternary phase diagram, the microemulsion region was identified and it was found that within each microemulsion region, the solution of microemulsion was transparent. The rest of the region in the diagram showed either a turbid solution of microemulsion or the gel form of the mixture. The results are shown in Figure 2. The microemulsion region was indicating the formation of w/o microemulsion.
For each phase diagram, the ratio of oil to surfactant or mixture of surfactant and co-surfactant was varied from 1:9 to 9:1. Water was added drop by drop, under gentle agitation, to each oily mixture until mixture become turbid. Transparent to translucent fluid systems were characterized as microemulsion. The pseudoternary phase diagram showing the largest area of the microemulsion was selected. It was found that the phase diagram with a composition of surfactant cremophore EL and cosurfactant Transcutol P in the ratio of 2:1 had the maximum area of microemulsion and hence was selected as the best composition for the microemulsion for further study.

Preparation of microemulsion by water titration method

The microemulsion formulations were selected at different component ratios as described in Table 2. Rizatriptan benzoate was dissolved into the different mixture of fish oil (oil) to surfactant Cremophore EL/Transcutol P cosurfactant ratio i.e., (2:1) and a transparent homogeneous ME was obtained by water titration method and results in a stable and clear microemulsion.

On the basis of solubility study and pseudoternary phase diagram four batches of ME were prepared with composition shown in Table 2.

TABLE 2
Composition of microemulsion.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>ME1</th>
<th>ME2</th>
<th>ME3</th>
<th>ME4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rizatriptan %</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Fish oil %</td>
<td>45.29</td>
<td>45.5</td>
<td>46.0</td>
<td>46.5</td>
</tr>
<tr>
<td>Cremophore EL and Transcutol P (2:1%)</td>
<td>50.31</td>
<td>45.50</td>
<td>46.00</td>
<td>46.50</td>
</tr>
<tr>
<td>Water %</td>
<td>4.40</td>
<td>4.50</td>
<td>4.00</td>
<td>3.50</td>
</tr>
<tr>
<td>Metabisulphite %</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Characterisation of microemulsion

The formulated microemulsions were characterized for percent transmittance, pH, refractive index, centrifugation, transparency/translucency, viscosity, globule size analysis, in-vitro drug release study and stability studies.

Particle size analysis of formulated microemulsion

The particle size of plain microemulsion and rizatriptan benzoate microemulsion was found to be 160.44 nm and 150.03 nm, respectively. The particle size was within the range indicating the microemulsion of very low globule size. The results are represented in Figure 2a and b.
TABLE 3
Physicochemical characteristics of microemulsions.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Formulations</th>
<th>ME 1</th>
<th>ME 2</th>
<th>ME 3</th>
<th>ME 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>%Transmittance</td>
<td>99.5 ± 1.01</td>
<td>98.6 ± 1.03</td>
<td>98.0 ± 1.02</td>
<td>96.7 ± 1.03</td>
</tr>
<tr>
<td>2</td>
<td>Centrifugation</td>
<td>No phase separation</td>
<td>No phase separation</td>
<td>No phase separation</td>
<td>No phase separation</td>
</tr>
<tr>
<td>3</td>
<td>pH</td>
<td>6.3 ± 0.02</td>
<td>5.5 ± 0.02</td>
<td>6.02 ± 0.01</td>
<td>6.7 ± 0.02</td>
</tr>
<tr>
<td>4</td>
<td>Refractive index</td>
<td>1.335 ± 0.01</td>
<td>1.331 ± 0.002</td>
<td>1.379 ± 0.01</td>
<td>1.378 ± 0.002</td>
</tr>
<tr>
<td>5</td>
<td>Viscosity at 100 rpm(cp)</td>
<td>114 ± 3.00</td>
<td>120 ± 1.00</td>
<td>96 ± 1.00</td>
<td>114 ± 2.00</td>
</tr>
<tr>
<td>6</td>
<td>Colour</td>
<td>Light yellow</td>
<td>Dark yellow</td>
<td>Dark yellow</td>
<td>Dark yellow</td>
</tr>
</tbody>
</table>

Fig. 3. Particle size analysis of formulated microemulsion.

Preparation of Microemulsion Gel

The optimised concentration of gelling agents for microemulsion gel is given in Table 4. During development of microemulsion gel; carbopol 934 and poloxamer 407 were found to be the best as it swelled into a reasonable viscous form with very little concentration and in a shorter time period than the other gelling agents.
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TABLE 4
Composition of microemulsion gel.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Formulation code of gel</th>
<th>Gelling Agent</th>
<th>Conc. in % w/v</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MEG 1</td>
<td>Carbopol 934</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>MEG 2</td>
<td>Hydroxy propyl methylcellulose</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>MEG 3</td>
<td>Poloxamer 407</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>MEG 4</td>
<td>Methyl cellulose</td>
<td>5</td>
</tr>
</tbody>
</table>

Characterization of Microemulsion Gel

The prepared gels were subjected to characterization as homogeneity, grittiness, spreadability, pH, viscosity, drug content, in-vitro drug release study and stability studies.

TABLE 5
Characterization of microemulsion gel.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>MEG 1</th>
<th>MEG 2</th>
<th>MEG 3</th>
<th>MEG 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Homogeneity</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>Grittiness</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Viscosity at 100 rpm (%)</td>
<td>3984 ± 1.542.5</td>
<td>3975 ± 2.642.4</td>
<td>3919 ± 2.541.8</td>
<td>3909 ± 141.7</td>
</tr>
<tr>
<td>4</td>
<td>pH</td>
<td>6.3 ± 0.20</td>
<td>5.5 ± 0.14</td>
<td>6.02 ± 0.07</td>
<td>6.0 ± 0.19</td>
</tr>
<tr>
<td>5</td>
<td>Spreadability Diameter(cm)</td>
<td>3.46 ± 0.05</td>
<td>3.01 ± 0.01</td>
<td>2.40 ± 0.03</td>
<td>2.11 ± 0.05</td>
</tr>
<tr>
<td>6</td>
<td>% Consistency</td>
<td>95.65 ± 0.044</td>
<td>92.56 ± 0.044</td>
<td>88.24 ± 0.043</td>
<td>53.33 ± 0.29</td>
</tr>
<tr>
<td>7</td>
<td>Drug content (% w/w)</td>
<td>98.02 ± 0.13</td>
<td>97.31 ± 0.17</td>
<td>95.40 ± 0.16</td>
<td>93.57 ± 0.21</td>
</tr>
</tbody>
</table>

Cumulative percentage drug release of the drug from the gels

The order of drug release was as follows: MEG1 > MEG4 > MEG2 > MEG3

Since MEG 1 was stable and showed good release, it was considered as optimized microemulsion.

Comparison between optimized microemulsion gel, gel and pure drug solution

Stability studies: (Data were expressed as mean ± S.D (n=3)).

The result of stability studies shown that there were no significant changes in the pH, drug content and viscosity of the gel, after storing at a temperature of 40 °C ± 2 °C / 75% ± 5% relative humidity for two months.

Fig. 4. Cumulative percentage drug release from the microemulsion.

Fig. 5. Cumulative percent drug release from MEG gel and pure drug solution.
Table 6
Stability studies of optimized microemulsion

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Stability study</th>
<th>PH</th>
<th>Drug content (%w/w)</th>
<th>Viscosity at 100 rpm (cp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 day</td>
<td>6.30 ± 0.20</td>
<td>98.02 ± 0.13</td>
<td>3909 ± 2.00</td>
</tr>
<tr>
<td>2</td>
<td>After 15 days</td>
<td>6.05 ± 0.65</td>
<td>98.02 ± 1.65</td>
<td>3909 ± 3.00</td>
</tr>
<tr>
<td>3</td>
<td>After 30 days</td>
<td>6.03 ± 0.04</td>
<td>97.96 ± 1.63</td>
<td>3903 ± 1.00</td>
</tr>
<tr>
<td>4</td>
<td>After 45 days</td>
<td>5.80 ± 0.04</td>
<td>97.47 ± 1.60</td>
<td>3883 ± 2.00</td>
</tr>
<tr>
<td>5</td>
<td>After 60 days</td>
<td>5.81 ± 0.03</td>
<td>97.03 ± 1.57</td>
<td>3883 ± 5.00</td>
</tr>
</tbody>
</table>

Conclusions

In the present work, we formulated a microemulsion based intranasal gel of rizatriptan for the treatment of migraine. As it was earlier known that fish oils have been helpful with recurrent migraines, the use of reconstituted fish oil for making intranasal delivery for the treatment of migraine provides a better and more rapid improvement in anti-migraine effect by its omega-3 phospholipid which might give good anti-inflammatory effect in the brain.

References


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