Development of Microemulsion Formulation for the Solubility Enhancement of Flunarizine

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Abstract

Flunarizine, a piperazine derivative, is a selective Ca++ channel blocker coupled with its antihistaminic property claimed to be effective in prophylaxis of migraine. Oral bioavailability of flunarizine is very low (less than 18%) due to poor water solubility and extensive first pass metabolism. The aim of the investigation was to design and develop microemulsion of Flunarizine for enhancing its solubility hence the oral bioavailability. Solubility of flunarizine was determined in various vehicles. Pseudo-ternary phase diagrams were constructed to identify the microemulsion existing zone. Optimization of formulation was done by process and formulation optimization. Optimized microemulsion was characterized for its transparency, droplet size, zeta potential, viscosity, % assay, and stability study etc. Particle size and zeta potential of optimized microemulsion were found to be 12.3 nm, -6.34 mV respectively. Drug content of the microemulsion formulation was 98.29±0.91. The viscosity data indicated the microemulsion to be O/W type. 78.49% and 71.53% of the drug was found to be released in 8hrs in the in-vitro and ex-vivo studies respectively. Solubility of flunarizine was successfully enhanced by 123 times by Capmul MCM microemulsion compared with distilled water (pH=7.4). Hence, by formulating into microemulsion, the solubility of Flunarizine was significantly enhanced which may increase its bioavailability.

Keywords: Flunarizine; Microemulsion; Solubility; Zeta potential; Pseudo-ternary phase diagrams.

INTRODUCTION

Oral route still remains the favorite route of drug administration in many diseases and till today it is the first way investigated in the development of new dosage forms. The major problem in oral drug formulations is low and erratic bioavailability, which mainly results from poor aqueous solubility. This may lead to high inter- and intra subject variability, lack of dose proportionality...
and therapeutic failure. Successful oral delivery of drugs has always remained a challenge to the drug delivery field because almost 40-50% of new drugs candidates have poor water solubility thus oral delivery is frequently associated with implications of low bioavailability. To overcome these bioavailability problems, various formulations strategies have been reported including use of surfactants, cyclodextrine inclusion complexes, solid dispersions, nanoparticles and absorption enhancers, but the possible formulations and their metabolic products are still to worth to be further investigated [1].

Microemulsions have attracted considerable amount of interest as potential drug delivery vehicles largely due to their simple method of preparation, stability and their abilities to incorporate a wide range of drugs of varying solubility [2]. O/W microemulsion is expected to increase the solubility by dissolving low water solubility compounds into its dispersed phase and to enhance the oral bioavailability by protecting the drug increasing the rate of absorption and wettability due to surfactants induced permeability changes and smaller droplet size (< 100 nm) and most importantly able to target lymphatic system [3].

Flunarizine, a piperazine derivative, is a selective Ca\(^{2+}\) channel blocker coupled with its antihistaminic property claimed to be effective in prophylaxis of migraine. It is effective in migraine by reducing intracellular Ca\(^{2+}\) overload due to brain hypoxia and thus prevents the deleterious effects of cellular calcium overload. Flunarizine has got variable oral bioavailability ranging from 18%-27% due to hepatic first-pass metabolism principally through N-oxidation and aromatic hydroxylation and it is sparingly water soluble [4]. Hence, the objective of this study was to improve the solubility of flunarizine by formulating into o/w microemulsion.

**MATERIALS AND METHODS**

**Materials:**
Flunarizine was received as a gift sample from Zyodus Cadila Pharmaceutical Ltd., Ahmedabad. Capmul MCM, Transcutol P, Captex, Labrafac CC, Labrafil M 1944 CS, Cremophor EL, Accenon CC were obtained from ABTEC, Mumbai, India. Polyethylene Glycol 400 (PEG 400), PEG 600, Glycerol, Isopropyl alcohol, soyabeen oil, Isobutyl alcohol, Tween-20, Tween-40, Tween-60, Tween-80, Isopropyl Myristate (IPM), Oleic Acid (OA), Ethanol were purchased from Gujarat Chemicals, Baroda, India. All other reagents are of AR grade.

**Methods:**
**Selection of oil phase:** Selection of the oil was based on the solubility of the drug. Different oils like Isopropyl Myristate (IPM), Capmul MCM, Labrafac CC, Oleic acid, Labrafil M 1944CS and soyabeen oil were taken for solubility studies. A total of 5 mL of each of the selected vehicle were added to each cap vial containing an excess of lovastatin. After sealing, mixtures were shaken with shaker at 25°C for 48 hr. After reaching equilibrium, each vial was centrifuged at 10000 rpm for 10 min and excess insoluble lovastatin was separated by filtration using Whatman filter. Both free drug as well as solubilized drug concentration was quantified by UV spectroscopy and mass balance was done [5].
Selection of surfactant and co-surfactant: The criteria for the selection of surfactant were its HLB value, drug solubility and non-toxic nature. Several surfactants like Tween-20, Tween-40, Tween-60, Tween-80, Captex-355, Accenon CC and Cremophor EL were screened. Above method was carried out for the selection of surfactants. Cosurfactants were selected based on their ability to form stable and clear microemulsion at a minimum concentration. Based on this, several cosurfactants like Polyethylene Glycol 400 (PEG 400), Glycerol, Polyethylene Glycol 600 (PEG 600), Isopropyl alcohol, Isobutyl alcohol and Transcutol P were screened.

Pseudo-ternary phase diagram: Pseudo-ternary phase diagrams were constructed to obtain the appropriate components and their concentration ranges that result in large existence area of microemulsion. To optimize the concentration of oil phase, surfactant and cosurfactant, different batches of varied concentration were prepared and titrated with distilled water till transparency persists. Ternary phase diagram was prepared by using a constant ratio of surfactant to cosurfactant. Four ratios of surfactant (Tween 80) and cosurfactant (Transcutol P) were selected (1:1, 2:1, 3:1 and 4:1) [6]. Pseudo-ternary phase diagrams were shown in Figure 1.

Preparation of microemulsion: Microemulsion was prepared by water titration method. Predetermined amount of the drug was dissolved in the required quantity of oil. Surfactant and cosurfactant in a fixed ratio were added to it. Finally the above mixture was titrated by distilled water with continuous stirring until a transparent and homogenous microemulsion is produced. Then microemulsion formulation was optimized through process and formulation optimization [7].

Characterization of microemulsion:
Transmittance Test: Transparency of optimized microemulsion formulation was checked by measuring % transmittance (UV Spectrophotometer (UV-1601-220x. Shimadzu) at 650nm) and by measuring refractive Index (by Abbe’s Refractometer) [8].

Globule size and zeta Potential Measurements: The globule size and zeta potential of the microemulsion was determined by dynamic light scattering with Zetasizer HSA 3000 (Malvern Instruments Ltd., Malvern, UK) [9]. Results were given in Figure 2 and Figure 3 respectively.

Viscosity measurements: Rheological behavior of the microemulsion and its diluted forms (10 and 100 times with 0.1N HCl) was evaluated using a Brookfield viscometer (DVIII+Rheometer) using rheocal software at a temperature of 25°C at shear rate 19.2sec⁻¹ [10]. Results were given in Table 1.

Determination of Drug Content in the microemulsion: Flunarizine from microemulsion formulation was analyzed spectrophotometrically at 254 nm.

Drug Stability: Optimized microemulsion was kept at cold temp (4-8 °C), room temperature and at elevated temperature (50 ± 2 °C). After every 2 month the microemulsion was analyzed for phase separation, % transmittance, Globule size and % assay [11]. Results were tabulated in Table 2.

Drug solubility study: Drug was added in excess to the optimized microemulsion formulation as well as each individual ingredient of the formulation. After continuous stirring for 24 hours at room temperature, samples were withdrawn and centrifuged at 6000

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rpm for 10 minutes. The amount of drug soluble in optimized formulation as well as each individual ingredient of the formulation was calculated by subtracting the drug in the sediment from the total amount of drug added. The solubility of drug in microemulsion was compared with respect to its individual ingredients [12]. The results were shown in Figure 4.

Drug release studies

In-vitro drug release: The diffusion study was carried out on a modified Franz diffusion cell of volume 20ml. The receptor compartment was filled with 20 ml of Phosphate buffer (pH 7.4). The donor compartment was fixed with cellophane membrane (Cut Off weight = 1000 Da) contains microemulsion (equivalent to 5 mg of Flunarizine) and plain drug solution separately. At predetermined time intervals samples were withdrawn from receptor compartment and analyzed for drug content by UV Spectrophotometer at 254 nm. Results were shown in Figure 5.

In Vitro Intestinal Permeability Studies: The methods employed were modified from experimental procedures well described in the literature [13]. Male albino rats (250-300 g) were killed by overdose with pentobarbitone administered by intravenous injection. To check the intraduodenal permeability, the duodenal part of the small intestine was isolated and taken for the in vitro diffusion study. Then this tissue was thoroughly washed with cold Ringer’s solution to remove the mucous and lumen contents. The equivalent dose of microemulsion, Flunarizine* and plain drug solution were prepared. One side of the intestine was tightly closed, resultant samples (2 mg/mL) were injected into the lumen of the duodenum separately using a syringe and then other side of the intestine was tightly closed. Then the tissue was placed in a chamber of organ bath with continuous aeration and a constant temperature of 37°C. The receiver compartment was filled with 30 mL of phosphate-buffered saline (pH 5.5). The absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 254nm, keeping the respective blank. The percent of cumulative drug diffusion was calculated against time and plotted on a graph. Results were shown in Figure 6.

RESULTS AND DISCUSSIONS

Preparation and optimization of microemulsion: Maximum amount of drug was found to dissolve in Capmul MCM (54.21 ± 1.27 mg/ml). Therefore this oil was selected for microemulsion formulation. Tween-80 was selected as surfactant to prepare O/W microemulsion as its HLB value is 15.4 and is non toxic. Transcutol P was selected as cosurfactant due to its ability to form transparent and stable microemulsion. Drug loaded microemulsion was prepared by water titration method.

Pseudo-ternary phase diagrams were constructed to obtain the appropriate components and their concentration ranges that can result in large existence area of microemulsion. From the ternary phase diagrams shown in Figure 1, it was concluded that highest microemulsion zone was achieved for the microemulsions having Tween-80/ Transcutol P ratio of 4:1.
Surfactant: Co-Surfactant = 1:1                           Surfactant: Co-Surfactant = 2:1

Surfactant: Co-Surfactant = 3:1                      Surfactant: Co-Surfactant = 4:1

Figure 1: ternary phase diagrams of microemulsion.

Optimization of the microemulsion was done through process and formulation optimization. Different batches of microemulsion were prepared by varying the conc. of the formulation parameter and varying the process parameters (speed and time).

Characterization of microemulsion:
Transmittance Test: % Transmittance of optimized microemulsion formulation as well as its 100 times dilution with 0.1N HCl was checked at 650 nm and were found to 99.76 ± 0.18 and 99.82 ± 0.17 respectively. Refractive Index of the formulation was found to be 1.34.

Globule size Measurement: Globule size of microemulsion and its 100 times diluted form was given in Figure 2. Optimized Capmul MCM microemulsion showed very small particle size i.e., 12.3nm and upon 100 folds dilution with 0.1N HCl and placed for 3 Hrs diluted microemulsion formulation show very little change in particle size i.e., 15.8nm. The value of polydispersity index (PI) of both cases were found to be below 1.0 suggesting that upon dilution with gastric fluid in body, optimized microemulsion formulation will remain stable.
Figure 2: Average globule size of optimized microemulsion

Zeta Potential Measurements: Zeta potential result of optimized microemulsion and its diluted form (100 times diluted with 0.1N HCl) was found to be -6.34mV and -3.02mV respectively as shown in the Figure 3. Aggregations will not take place due to slightly negative charge of the droplets.
Figure 3: Zeta potential of optimized microemulsion

<table>
<thead>
<tr>
<th>Results</th>
<th>Mean (mV)</th>
<th>Area (%)</th>
<th>Width (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zeta Potential (mV):</td>
<td>-3.02</td>
<td>100.00</td>
<td>4.06</td>
</tr>
<tr>
<td>Zeta Deviation (mV):</td>
<td>4.06</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Conductivity (mS/cm):</td>
<td>0.200</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Viscosity measurements: Viscosity of microemulsion was found very high (35.64cP) but diluted 10 times and 100 times with 0.1N HCl, viscosity of the system was decreased, which indicates that when microemulsion formulation will be diluted with the stomach fluid its viscosity will be decreased and therefore absorption from stomach will be rapid.
Table 1: Viscosity of microemulsion and its diluted forms

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Viscosity (cP)</th>
<th>Temperature(°C)</th>
<th>Shear Rate (sec⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microemulsion</td>
<td>15.64</td>
<td>25</td>
<td>19.2</td>
</tr>
<tr>
<td>10 times dilutions</td>
<td>1.76</td>
<td>25</td>
<td>19.2</td>
</tr>
<tr>
<td>100 times dilutions</td>
<td>0.72</td>
<td>25</td>
<td>19.2</td>
</tr>
</tbody>
</table>

**Determination of Drug Content:** Drug content of the microemulsion formulation was 98.29±0.91, which is in the limit.

**Drug solubility:** Solubility of drug in microemulsion formulation and the individual ingredients of the microemulsion are recorded in Figure 4. The data indicates enhanced solubility of Flunarizine in optimized microemulsion as compared to its respective individual ingredients.

Figure 4: Solubility of Flunarizine in different components of microemulsion and optimized microemulsion.

**Stability studies:** Results of temperature stability and centrifugal stability studies of optimized microemulsion were recorded in Table 2. From the data it was indicated that the optimized Capmul MCM based microemulsion was stable up to 6 months.

Table 2: Results of stability studies

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Phase separation</th>
<th>% transmittance</th>
<th>Particle size (nm)</th>
<th>% of Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After 4 month</td>
<td>After 6 months</td>
<td>After 4 month</td>
<td>After 6 month</td>
</tr>
<tr>
<td>2°C-8°C</td>
<td>No</td>
<td>No</td>
<td>98.2±0.8</td>
<td>17.9±1.4</td>
</tr>
<tr>
<td>Room Temp</td>
<td>No</td>
<td>No</td>
<td>99.1±0.2</td>
<td>17.2±1.1</td>
</tr>
<tr>
<td>Elevated Temp (50 ± 2°C)</td>
<td>No</td>
<td>No</td>
<td>99.1±0.8</td>
<td>18.2±1.6</td>
</tr>
</tbody>
</table>

**In-Vitro Drug release studies:** Release profile of Flunarizine was carried out from optimized Microemulsion, Flunarizine* (Marketed Formulation) and plain drug solution and the results are
shown in Figure 5. After 8 Hrs, drug released from plain solution, Flunarizine* and Capmul microemulsion was 56.76%, 51.19% and 78.53% respectively. From this study it can be concluded that the extent of diffusion of Flunarizine from microemulsion is greater than plain drug solution and Flunarizine*. Capmul microemulsion shows faster release of Flunarizine as compared to plain solution due to its smaller droplet size of microemulsion (12.3 nm).

![Graph showing drug release profile](image)

**Figure 5: In-Vitro Drug release profile of Flunarizine from microemulsion and Plain drug solution**

**Ex Vivo release study:** As shown in Figure 6, after 8 Hrs, drug released from microemulsion, Flunarizine* and plain solution was 71.20%, 47.52% and 51.72% respectively. From this results, extent of diffusion of Flunarizine from Capmul microemulsion is greater than Flunarizine* and plain drug solution. This may be due to the penetration enhancing effect surfactant and cosurfactant of microemulsion as well the smaller globule size of the microemulsion. Again microemulsion show relatively higher sustained action which may be due to the fact that drug has high partition coefficient and reservoir action of the formulation.

![Graph showing drug release profile](image)

**Figure 6: Ex-Vivo release profile of Flunarizine microemulsion, Flunarizine*and plain drug solution**

**CONCLUSION**

The study demonstrates that the developed microemulsion formulation containing Capmul MCM (3%), Tween-80 (28%), PEG-400 (7%) and distilled water is a transparent, less viscous system and a stable system with a particle size of 12.3 nm. Results from the in-vitro and ex-vivo studies revealed that developed microemulsion possessed higher rate and extent of absorption compared to plain drug solution and marketed formulation. The solubility profile of drug indicates that
microemulsion enhances the solubility by 123 folds compared to that of distilled water which may increase the oral bioavailability of Flunarizine [14]. However, further studies in higher animals and human being need to be performed before this formulation can be commercially exploited.

Acknowledgement
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REFERENCES