ENHANCING THE BIOAVAILABILITY OF SIMVASTATIN USING MICROEMULSION DRUG DELIVERY SYSTEM

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ABSTRACT

The objective of the present study was to develop a novel Microemulsion drug delivery system (ME) to enhance the solubility, dissolution rate and ultimately the oral bioavailability of a poorly water soluble drug, Simvastatin. Phase solubility studies were conducted using various oils and non-ionic surfactants for the maximum solubility of Simvastatin. Terinary phase diagrams were constructed to evaluate the microemulsion regions and were also for the optimum concentrations of oil (Oleic acid) and surfactant (Cremophore RH 40), Co-Surfactant (Transcutol p) and water in the formulation. The globule size analysis, and zeta potential of all the developed formulations were studied using Zeta Sizer (Horiba Instruments, Japan). In vitro release studies are conducted using USP Type II dissolution test apparatus and in-vitro intestinal permeation studies are conducted using Rat duodenum. The formulation of microemulsion was compared with marketed tablet. The results of the studies indicated that, the dissolution rate and intestinal permeation of the developed ME formulation containing simvastatin was 2.5 to 3 folds increased compared with that of marketed tablet. The mean globule size (n=3) was observed to be (< 100nm) for the optimized formulation and the zeta potential was negative which may interfere in the absorption of the simvastatin. Therefore the developed microemulsion formulation improved the Solubility and in-vitro drug release of simvastatin when compared with commercial tablet formulation.

Keywords: Microemulsion, Simvastatin, Non-ionic surfactant, Conductivity, Zeta potential, Intestinal permeation.

INTRODUCTION

Drug solubility enhancement is one of the most important challenges in the field of pharmaceutics. Nearly 40% of all new pharmacologically potent molecules show poor aqueous solubility, leading to their low effective concentration in biofluids and therefore poor bioavailability.[1] Simvastatin is chemically (1S,3R,7S,8S,8aR)-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-(2-[(2R,4R)-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl]ethyl)-1-naphthyl-2,2-dimethyl butyrate[1] is obtained from the fermentation of Aspergillus terreus. This compound, acts as a highly potent and effective cholesterol-lowering agent, is being used in the control of hypercholesterolemia. It exhibits a very important hepatic first-pass metabolism, acting by blocking the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA), and thereby reducing the low-density lipoproteins. Simvastatin is a potent inhibitor of HMG-CoA reductase, which is a rate limiting enzyme in cholesterol bio-synthesis.[2]

Simvastatin absorption in the gastrointestinal tract is slow, variable, and incomplete. The bioavailability of simvastatin after oral is 5%. Approximately 95% of an oral dose is never absorbed and is excreted through the feces. The main excretory organ for simvastatin is the kidney. The plasma half-life of oral simvastatin on average is 3 hours in adults with normal renal function.

In the present study, a microemulsion was prepared using the non-ionic cremophore (as surfactant, hydrophile-lipophile balance [HLB] 16), Transcutol p (as co-surfactant, HLB 15), Oleic acid, and water. Pseudoternary phase diagrams were constructed to find out the zone of microemulsion at different ratios of surfactant to cosurfactant (1:1:2:1:3:1). The effect of formulation variables on different physicochemical characteristics such as globule size, electroconductivity, and viscosity was studied. In vitro release studies are conducted using USP Type II dissolution test apparatus and in-vitro intestinal permeation studies are conducted using Rat duodenum. The formulation of microemulsion was compared with marketed tablet.[14-18]

MATERIALS AND METHODS

Simvastatin and Cremophore RH 40 were obtained as a gift samples from (Bright Labs, Hyderabad, India), Transcutol p was purchased from Sd fine chemicals, ltd, Mumbai, India, Oleic acid was purchased from Merck specialties pvt limited, Mumbai, India. HPLC Grade Acetonitrile and all other buffering agents of analytical grade were purchased from Sd fine chemicals, ltd, Mumbai, India, HPLC grade water prepared by using SG-LABOSTAR™ 3 TWF-ultra pure water system.

Solubility studies

The solubility of simvastatin in various oils, surfactants, and cosurfactants was determined, respectively. 2 ml of each of the selected vehicle were added to each cap vial containing an excess of simvastatin (ca. 500 mg). After sealing, the mixture was heated at 40°C in a water-bath to facilitate the solubilization using a vortex mixer. Mixtures were shaken with shaker at 25°C for 48 h. After reaching equilibrium, each vial was centrifuged at 3000 rpm for 5 min, and excess insoluble simvastatin was discarded by filtration using a membrane filter (0.45 μm, 13 mm, Whatman, USA). The concentration of simvastatin was quantified by HPLC.[15] The solubility of simvastatin in various oils and surfactants were represented in graph.

Construction of Phase Diagrams

The pseudo-ternary phase diagrams of oil, surfactant: cosurfactant, and water were developed using surfactant titration method: the mixtures of oil and water at certain weight ratios were titrated with

Figure 1. chemical structure of Simvastatin.
surfactant/co-surfactant mix in a dropwise manner. Three types of surfactant phases were prepared Cremophore RH40 + Transcutol p (1:2:1:3:1)] For each phase diagrams at a specific ratio of surfactant/co-surfactant transparent and homogenous mixture of oil and drug was formed under the mixing by magnetic stirring. Then, visually observed for phase clarity and flow ability. After the identification of microemulsion region in the phase diagrams, the microemulsion formulations were selected at desired component ratios. In order to form the microemulsion.20,21

Preparation of Microemulsion Formulation

A series of microemulsions were prepared in each of formulation with varying ratio of oil, surfactant, co-surfactant, and simvastatin. In all the formulations, the amount of simvastatin was constant (10 mg/mL). Briefly, simvastatin was dissolved by cosurfactant, such as Transcutol-p in glass vials. Oil and surfactant were accurately weighed and incorporated into glass vials. Then, the components were mixed by gentle stirring and vortex mixing, and heated at 37°C in incubator, until simvastatin perfectly has dissolved. Then the water is added and stirred on a magnetic stirrer to get a clear and transparent mixture. The mixture was stored at room temperature until used.

Characterization

Drug-excipients compatibility studies

FTIR spectrums of simvastatin and drug microemulsion formulation were obtained by means of a FTIR spectrophotometer (Bruker Alpha T). The samples were prepared by the potassium bromide disk method and measurements were attempted with the accumulation of 20 scans and a resolution of 4 cm⁻¹ over the range of 400–4000 cm⁻¹. After running the spectra, significant peaks relating to major functional groups were identified; spectra of the subsequent sample of the same compound were compared with the original.20

Droplet size and surface charge determination

The droplet size and surface charge of the emulsions was determined by photon correlation spectroscopy (which analyses the fluctuations in light scattering due to Brownian motion of the particles) using Nano Zetasizer (Horiba Instruments, Japan) able to measure sizes between 10⁻⁹–300 nm. Light scattering was monitored at 25°C at a 90° angle. The dispersed formulations were measured after dilution (1:100) to produce the required count rate (50–200) to enable the accurate measurement.21

Viscosity

The rheological property of the microemulsion was evaluated by Brookfield DV-11+ or viscometer using spindle 00 UL adaptor at 25±0.5°C at 5 rpm. Experiments were performed in triplicate for each sample, and results were presented as average ± standard deviation.22

Electroconductivity Study

The electroconductivity of the resultant system was measured by using (LABINDIA pico+ conductor) in a non-linear temperature compensation mode, according to EN 27888. Conductivity was determined between 45 & 90°C under magnetic stirring at an agitation of 250 rpm. This temperature ranges permit the steady state to be achieved, either as an emulsion o/w (high steady state) or as an emulsion w/o (low steady state) in different condition tested. The recording of conductivity relative to temperature permits the determination of phase inversion temperature. Conductivity values lower than 10 micromho cm⁻¹ means that the continuous phase is oil, where as a highly steady state shows that water is the continuous phase. For the conductivity measurements, the tested microemulsions were prepared with a 0.01N aqueous solution of sodium chloride instead of distilled water.23

Refractive Index and Percent Transmittance

The refractive index of the drug was measured by an Abbe refractometer (Bausch and Lomb Optical Company, Rochester, NY) by placing 1 drop of solution on the slide. The percent transmittance of the system was measured at 238 nm using a UV spectrophotometer (Shimadzu model 1800 Japan), keeping distilled water as a blank.

Thermodynamic Stability Studies

The microemulsion formulations were put into empty hard gelatin capsules (size 0) and subjected to stability studies at 25°C/60% relative humidity (RH), 30°C/65% RH, and 40°C/75% RH. Samples were charged in stability chambers with humidity and temperature control. They were withdrawn at specified intervals for analysis over a period of 3 months for intermediate and accelerated conditions and 6 months for long-term conditions. Drug content of the capsules was analyzed using a previously developed and validated stability indicating HPLC method.25

In vitro drug release studies

The release of Simvastatin from the optimized microemulsion and marketed tablet was determined according to USP dissolution apparatus type II. To permit the quantitative drug release from microemulsion and marketed tablet, 900 mL of phosphate buffer pH 5.5 was placed in the dissolution vessel and then the microemulsion formulation filled in hard gelatin capsule and tablet was placed in the dissolution medium and was agitated at 50 rpm at 37°C. At predetermined time intervals of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 min (up to 1 hour), 5 mL of the samples were withdrawn and the drug concentration was determined by HPLC at maximum wavelength 238 nm. The volume withdrawn was replaced each time with fresh dissolution medium. Cumulated released amounts were plotted as a function of time.26

In Vitro Intestinal Permeation Studies

The methods employed were modified from experimental procedures well described in the literature. Male Sprague-Dawley rats (250-300 g) were killed by overdose with pentobarbitone administered by intravenous injection. To check the intra duodenal 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 microemulsion sample was diluted with 1 mL of distilled water (outside mixing for 1 minute by vortex mixer), and for the tablet sample a suspension of tablet was made in distilled water. The resultant sample (1 mg/mL) was injected into the lumen of the duodenum using a syringe, and the 2 sides of the intestine were 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). At predetermined time intervals of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 min (up to 1 hour), 2 mL of the samples were withdrawn and the drug concentration was determined by HPLC at maximum wavelength 238 nm. The percent diffusion of drug was calculated against time and plotted on a graph.27

RESULTS AND DISCUSSION

Solubility studies

The microemulsion formulations consisted of oil, surfactants, cosurfactants, water, and drug should be a clear and monophasic liquid and should have good solvent properties to allow presentation of the drug in solution. The solubility of simvastatin in various vehicles is presented in Figure 2. Cremophore RH 40 and Transcutol P provided higher solubility than other vehicles and Oleic acid as oil, was selected respectively, for the optimal microemulsion formulation resulting in improved drug loading capabilities.
Figure 2: Graph showing solubility of Simvastatin in various Oils and Surfactants. The solubility of Simvastatin was determined in various vehicles by HPLC. The solubility of Simvastatin in surfactant was found to be high in cremophore RH40 & Transcutol P, among oils oleic acid exhibited the highest solubility.

Pseudoternary phase diagram

A pseudoternary phase diagram of the investigated quaternary system water/oleic acid/cremophore RH 40/Transcutol P, is presented in Figure 3. Formation of microemulsion systems (the shaded area) was observed at room temperature. Phase behavior investigations of this system demonstrated the suitable approach to determining the water phase, oil phase, surfactant concentration, and cosurfactant concentration with which the transparent, one-phase low-viscous microemulsion system was formed. The phase study revealed that the maximum proportion of oil was incorporated in microemulsion systems when the surfactant-to-cosurfactant ratio was 1:1. From a formulation viewpoint, the increased oil content in microemulsions may provide a greater opportunity for the solubilization of simvastatin. Moreover, when the composition (% w/w) of surfactant mixture (Smix) in a microemulsion preparation was <50%, the formulation was less viscous. The optimum formulation of microemulsion contained Oleic acid (18.70%), Smix (48.30%), and water (32.90%).

*Smix = surfactant/co-surfactant mixture.
Figure 3: Pseudo-ternary phase diagrams indicating the efficient microemulsion region containing (Cremophore RH 40/Transcutol p) = (a) 1:1 (w/w), (b) 2:1 (w/w), (c) 3:1 (w/w). The shaded area represents o/w microemulsion existence range and (a) 1:1 (w/w) cremophore/Transcutol p exhibited the highest microemulsion region.

Drug and Excipients compatibility studies

The compatibility of drug and excipients used in the microemulsions were characterized by their FTIR spectra. The FTIR spectrum of pure simvastatin has three characteristic peaks at 3548, 2969, and 1698 cm⁻¹ for O–H stretching vibration, C–H vibration and ester stretching vibration and lactone carbonyl functional group respectively. The FTIR spectrum of pure Formulation has three characteristic peaks at 3417 cm⁻¹, 2925 cm⁻¹ and at 1732 cm⁻¹. The FTIR spectrum of pure Simvastatin and microemulsion formulation were almost similar because of the same functional groups. It indicates that there was no interaction between Simvastatin and excipients used in the formulation. Depicted on figure 4.

Figure 4: Comparative FTIR Spectra of Simvastatin microemulsion with individual excipients

Droplet Size and surface charge Determination

The mean droplet size of the formulation, containing Oleic acid as oil, Transcutol p as co-surfactant and Cremophore RH 40 as surfactant (i.e in 1:1 ratio) found to be 60.1 nm. Furthermore, the decrease in the droplet size reflects the formation of a better close-packed film of surfactant at the oil-water interface, thereby stabilizing the oil droplets.

The zeta potential of microemulsion was determined using Nano Zeta sizer (Horiba Instruments, Japan). Charge on emulsion droplets and their mean Zeta potential values (±SD) was obtained from the instruments. Zeta Potential of the ME formulation is found to be negative charge (-75 mV).
Physicochemical Characterization of Microemulsion

The physicochemical characteristics of the developed microemulsion appear in Table 1. It was clear from the physicochemical data that the developed system had low viscosity (20.7253 cP). The investigated microemulsion system containing the non-ionic surfactant mixture, oil, and water showed electroconductive behavior in spite of its non-ionic nature. From the viscosity and electroconductive study it can be concluded that the system is of the o/w type. The refractive index of the developed system was similar to the refractive index of water (1.843). In addition, the developed system showed percent transmittance >99%. The refractive index and percent transmittance data prove the transparency of the system. The nanometric size range of the particle was retained even after 100 times dilution with water, which proves the system’s compatibility with excess water.

Table 1: Physicochemical parameters of developed formulation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Droplet size (nm)</td>
<td>60.1</td>
</tr>
<tr>
<td>Zetapotential (mV)</td>
<td>-75</td>
</tr>
<tr>
<td>Viscosity (cP)</td>
<td>20.7253</td>
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<tr>
<td>Electro conductivity (mΩ)</td>
<td>320.47 ± 5</td>
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<tr>
<td>Refractive index</td>
<td>1.843</td>
</tr>
<tr>
<td>Percent transmittance at 238nm</td>
<td>99.3 ± 0.6</td>
</tr>
</tbody>
</table>

Thermodynamic stability

The developed formulation was subjected to stability studies to evaluate its stability and the integrity of the dosage form. Table 4 gives the results of the evaluation test conducted on stability sample. The formulation was found to be stable for 3 months at intermediate and accelerated conditions and 6 months at long-term conditions. There was no significant change in the drug content, or particle size of the resultant emulsion. It was also seen that the formulation was compatible with the hard gelatin capsule shells, as there was no sign of capsule shell deformation. Furthermore, the formulation was found to show no phase separation, drug precipitation, or capsule leaks. Thus, these studies confirmed the stability of the developed formulation and its compatibility with hard gelatin capsules.

Table 4: Evaluation data of formulation subjected to stability studies.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Sampling point</th>
<th>Droplet size (nm)</th>
<th>% Drug release</th>
</tr>
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<tbody>
<tr>
<td>A=(25°C/60%RH)</td>
<td>0 days</td>
<td>60.1</td>
<td>99.67</td>
</tr>
<tr>
<td></td>
<td>3 months</td>
<td>59.9</td>
<td>96.37</td>
</tr>
<tr>
<td>B=(30°C/65%RH)</td>
<td>0 days</td>
<td>60.1</td>
<td>96.67</td>
</tr>
<tr>
<td></td>
<td>3 months</td>
<td>58.9</td>
<td>97.87</td>
</tr>
<tr>
<td>C=(40°C/75%RH)</td>
<td>0 days</td>
<td>60.1</td>
<td>96.67</td>
</tr>
<tr>
<td></td>
<td>3 months</td>
<td>59.4</td>
<td>97.12</td>
</tr>
</tbody>
</table>

In-vitro drug release studies

The in-vitro drug release studies for marketed tablet (simvas 10mg) and microemulsion was determined in USP dissolution medium pH 5.5. The results are shown in Figure 6. At the end of 1 h, the release of simvastatin from the microemulsion was significantly greater (98.62%) than that for marketed tablet (45.19%). This may be the result of surfactant molecules which leads to the enhancement of solubility of the drug in dissolution medium. The in-vitro intestinal permeability results exhibits the drug diffusion at a faster rate from the microemulsion system than from the tablet dosage form. After 1 hour of diffusion, 74.32% of drug was diffused from the microemulsion system, as compared with 31.54% diffused from the tablets.

Table 5: In Vitro Intestinal Permeability studies performed for the developed formulation and the commercial tablet

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>F1 (% CDR)</th>
<th>Tablet (% CDR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>12.35</td>
<td>2.34</td>
</tr>
<tr>
<td>10</td>
<td>24.65</td>
<td>7.54</td>
</tr>
<tr>
<td>15</td>
<td>35.73</td>
<td>9.87</td>
</tr>
<tr>
<td>20</td>
<td>43.23</td>
<td>11</td>
</tr>
<tr>
<td>25</td>
<td>51.11</td>
<td>13.83</td>
</tr>
<tr>
<td>30</td>
<td>60.34</td>
<td>18.04</td>
</tr>
<tr>
<td>35</td>
<td>64.96</td>
<td>22.32</td>
</tr>
<tr>
<td>40</td>
<td>67.32</td>
<td>24.32</td>
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<tr>
<td>45</td>
<td>69.41</td>
<td>25.42</td>
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<tr>
<td>50</td>
<td>70.87</td>
<td>28.52</td>
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<tr>
<td>55</td>
<td>72.52</td>
<td>30.23</td>
</tr>
<tr>
<td>60</td>
<td>74.32</td>
<td>31.54</td>
</tr>
</tbody>
</table>

In Vitro Intestinal Permeability Study

The drug concentration was determined by High performance liquid chromatography at maximum wavelength 238nm and the percent diffusion of drug was calculated against time and plotted on a graph. The in-vitro intestinal permeability results exhibits the drug diffusion at a faster rate from the microemulsion system than from the tablet dosage form. After 1 hour of diffusion, 74.32% of drug was diffused from the microemulsion system, as compared with 31.54% diffused from the tablets.

CONCLUSION

An optimized simvastatin microemulsion formulation consisting of Oleic acid (18.70% wt/wt), Cremophore RH40 and transcutol mixture (48.38% wt/wt) and water (32.90% wt/wt) was successfully developed with an increased dissolution rate, increased solubility, and, ultimately, increased bioavailability of a poorly...
water-soluble drug, simvastatin. The developed formulation showed higher intestinal permeability rate as compared with marketted tablet of simvastatin. The stability of the developed formulation was confirmed by the stability studies. Thus, from the results it can be predicted that the microemulsion formulation can be used as a possible alternative to traditional oral formulations of simvastatin to improve its bioavailability.

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