Development of Self-Microemulsifying Drug Delivery System for Simvastatin

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Abstract

The objective of the present investigation was to prepare the self microemulsifying drug delivery system (SMEDDS) of simvastatin in order to improve its oral bioavailability. Among the tested oils, simvastatin exhibited significantly higher solubility in oleic acid compared to all other oils.Emulsification studies showed that Span 60 was able to produce clear microemulsion with oleic acid upon dilution, and hence, it was employed as the surfactant in further studies. Ethanol was used as the co-solvent for the formulation of SMEDDS.The results revealed that span 60 and ethanol used in ratios of 1:1 (F7-8) and 2:1 (F15-16) exhibited largest microemulsion area and shortest emulsification time (less than 1 min).A fixed simvastatinconcentration of 5% w/w was selected to be loaded in all self-emulsifying formulations.All the formulations passed the thermodynamic stability studies without any signs of phase separation and precipitation during alternative temperature cycles (4°C and 40°C), freeze thaw cycles (-21°C and +25°C) and centrifugation at 10,000 g indicating good stability of formulations and their emulsions.The *in vitro* dissolution studies revealed the drug release profiles for the L-SMEDDS. All the formulations exhibited quick drug release characteristics and almost complete drug release in 15-45 minutes.

Keywords

Simvastatin, antihyperlimidemic, oleic acid, ternary phase diagram, self microemulsifying

Introduction

Oral delivery route is the most convenient route for drug administration to achieve desired therapeutic effects and the greatest degree of patient compliance, especially for chronic condition diseases [1].More than 40% of new drug candidates of recent years possess poor aqueous solubility, and approximately 40% of the marketed immediate-release (IR) oral drugs are categorized as practically insoluble [2]. Self-emulsifying drug delivery systems (SEDDS) are isotropic mixtures of oil, surfactant, co-surfactant and drug that rapidly form fine oil-in-water (o/w) nanoemulsions when introduced into aqueous medium under mild agitation.The agitation required for formation of nanoemulsions is provided by digestive motility of the gastrointestinal tract [3].For the lipophilic drugs that exhibit poor water solubility and rate−limited dissolution, SEDDS may offer an improvement in the rate and extent of absorption and result in more reproducible blood−time profiles.

In India, 28% of the entire population is attributable to cardiovascular diseases of which Ischemic heart disease is the most leading cause for the deaths among people accounting for about 12.4% (1215.4 thousand people) in 2012 [4,5]. According to a study carried out by ICMR, about 7.7% of the adult population had three lipid abnormalities (hypercholesterolemia + hypertriglyceridemia + low HDL-C) and 4.8% of the population had all four lipid abnormalities (hypercholesterolemia + hypertriglyceridemia + low HDL-C + high LDL-C) [6].

Initial therapy for any lipoprotein disorder is dietary restriction of total saturated fat and cholesterol and an increase in polyunsaturated fat intake along with regular exercise [7]. Several different classes of drugs are used to treat hyperlipidemia which differ not only in their mechanism of action but also in the type and magnitude of lipid reduction [8]. Majority of the

traditionally used anti-hyperlipidemic drugs like Atorvastatin, Fluvastatin, Pravastatin, Simvastatin, Lovastatin and Rosuvastatin are well absorbed but undergo extensive hepatic first pass metabolism, which leads to very low absolute bioavailability [9]. The aim of this study was to design and formulate suitable liquid SMEDDS of simvastatin using different combinations of essential oils, surfactant and co-surfactants, with a view to enhancing the solubility of the drug.

Material and Methods

Simvastatin was purchased from Yarrow Pharmaceuticals, other reagents and chemicals were purchased from various sources and were used as obtained.

Drug solubility

The solubility of simvastatinin different oils, surfactants and co-surfactants was determined according to the method of Date and Nagarsenker [10]. Briefly, an excess amount of simvastatin was mixed with definite amount of the oils (castor oil, sesame oil, coconut oil, peanut oil, sunflower oil, eucalyptus oil, oleic acid, Soyabean oil), surfactants (Tween 80, Tween 20, Span 20, Span 60) and cosurfactants (PEG 400, Propylene glycol, ethanol, butanol) and the mixtures were shaken for 48 hours at 25°C to attain equilibrium. The samples were then centrifuged to remove the undissolved drug, filtered through a 0.45 µm membrane filter, and the supernatant was suitably diluted before spectrophotometric analysis at 238 nm using UV-visible spectrophotometer to determine the amount of the drug dissolved in each excipient.

Surfactant and oil miscibility

The oil and surfactant in the ratio of 1:1 were shaken at 40°C in 3 ml transparent glass vials. The miscibility was monitored optically and considered to be good when the mixture was transparent.

Screening of surfactants/cosurfactants for emulsifying ability

The emulsification ability of different surfactants was evaluated by mixing the surfactant with the selected oily phase in a 1:1 weight ratio. The mixtures were vortex mixed and diluted up to 200 fold dilution. The ease of formation of an emulsion was assessed by observing the number of inversion of the volumetric flask required to obtain a uniform emulsion. The resulting emulsion was also examined visually for relative turbidity according to different grading systems (Grades A – E) described by Khoo et al [11] that depict the spontaneity and appearance of the nanoemulsion formed upon dilution.

Construction of ternary phase diagrams

Based on the solubility of simvastatin, eucalyptus oil was chosen as the oil phase. Span 60 was used as the surfactant and PEG 400 was employed as the cosurfactant. Distilled water was used as the aqueous phase for development of these phase diagrams. The surfactant and co-surfactant (Smix) in were mixed in different weight ratios (1:1, 2:1, 3:1) so that the concentration of surfactant increases with respect to co-surfactant.

The oil phase and each Smix were blended thoroughly in 9 different weight ratios (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9). From these each ratio, 0.1 ml of mixtures was transferred to separate glass beakers. To these contents, 100 ml distilled water was added gently stirrer using a magnetic stirrer at 37°C.The resulted emulsions were examined for clarity, phase separation, and coalescence of oil droplets on standing for 2 h. When the oil droplets easily spread out in water and formed a clear, transparent emulsion, the emulsion was judged as ‘‘good’’ emulsion, and when there was poor or no emulsion formation with immediate coalescence of oil droplets, especially when stirring was stopped, the emulsion was judged as ‘‘bad’’ emulsion.

Table 1Composition for construction of ternary phase diagram (%w/w)

Formulation	Oil	Smix ratio 1:1	Smix ratio 2:1	Smix ratio 3:1
F1	9	1	-	-
F2	8	2	-	-
F3	7	3	-	-
F4	6	4	-	-
F5	5	5	-	-
F6	4	6	-	-
F7	3	7	-	-
F8	2	8	-	-
F9	9	-	1	-
F10	8	-	2	-
F11	7	-	3	-
F12	6	-	4	-
F13	5	-	5	-
F14	4	-	6	-
F15	3	-	7	-
F16	2	-	8	-
F17	9	-	-	1
F18	8	-	-	2
F19	7	-	-	3

F20	6	-	-	4
F21	5	-	-	5
F22	4	-	-	6
F23	3	-	-	7
F24	2	-	-	8

Preparation of simvastatin-loaded self-microemulsifying formulations (L-SMEDDs)

Simvastatinwas added to the optimized blank ternary systems at a drug loading concentration of 5% w/w. Final mixtures were mixed and shaken for 24 hours at 25°C in a shaking water bath to ensure complete solubilization.

Table 2 Composition of optimized ternary systems for L-SMEDDs

Formulation	Oil %w/w	Surfactant %w/w	Cosurfactant %w/w	Smix ratio
F7	70	20	10	2:1
F8	60	26.6	13.3	2:1
F15	40	45	15	3:1
F16	30	52.5	17.5	3:1

Evaluation of optimized L-SMEDDS formulation

Thermodynamic stability studies and cloud point

Stability of the optimized L-SMEDDS formulation was evaluated at different stress conditions such as heating cooling cycles (4°C and 40°C) and freeze thaw cycles (-21°C and +25°C) along with storage at specified temperature for 48 h. In order to carry out centrifugation stress study, 1 mL of the formulation was diluted to 100 mL with distilled water and centrifuged at 10000 g for 20 min and visually observed for any phase separation [12]. In order to determine cloud point temperature, 10 mL of diluted L-SMEDDS formulation were gradually heated on a water bath and observed for cloudiness using thermometer. The temperature at which cloudiness appeared was denoted as cloud point.

Measurement of particle size and zeta potential

The particle size, polydispersity index and zeta potential of the L-SMEDDS was obtained using calibrated ocular micrometer using a microscope. The particle size and polydispersity index of the best formulation was also determined using a dynamic light scattering particle size analyzer.

Determination of drug content of simvastatin-loaded solid SMEDDS

An accurately weighed amount of the resulting drug-loaded SMEDDS formulation was dispersed in a suitable quantity of methanol and shaken thoroughly to ensure release and dissolution of the drug in methanol. The samples were centrifuged at 3000 rpm for 15 minutes and the supernatant was filtered through a 0.45 µm membrane filter and the filtrate was assayed spectrophotometrically for the drug at a wavelength of 238 nm. The drug content in each sample was calculated as milligrams of the drug per gram of the product using the following equation:

$$drug\ content = \frac{drug\ content\ in\ the\ weight\ taken\ from\ solid\ \ SNEDDS}{weight\ of\ the\ solid\ SNEDDS\ taken}$$

***In vitro* dissolution study**

The *in vitro* dissolution studies of different simvastatin SMEDDS formulations were carried out in dissolution apparatus II (Paddle method) according to the requirements specified for simvastatin capsules. The dissolution medium composed of 900 ml phosphate buffer pH 7.2 maintained at 37 ± 0.5°C and the rotational speed was adjusted at 50 rpm. Phosphate buffer pH 7.2 was prepared by mixing 50 ml of 0.2M potassium dihydrogen orthophosphate with 35 ml of 0.2M sodium hydroxide and diluting to 200 ml with water. Volumes of these solutions were corrected accordingly to prepare the total volumes required for dissolution studies. An amount of SMEDDS formulation equivalent to 25 mg of simvastatin was filled in dialysis membrane and used for dissolution studies. Samples were withdrawn at predetermined time intervals. An equal volume of fresh dissolution medium maintained at the same temperature was added to keep constant volume during dissolution study. The collected samples were filtered through 0.45 µm syringe filter, suitably diluted using methanol and then assayed for the content of simvastatin by UV spectrophotometry at 238 nm.

Results and Discussion

Solubility Studies

The solubility of simvastatin was determined in oils, surfactants, co-surfactants, mixture of oils and mixture of surfactants (Figure 1).

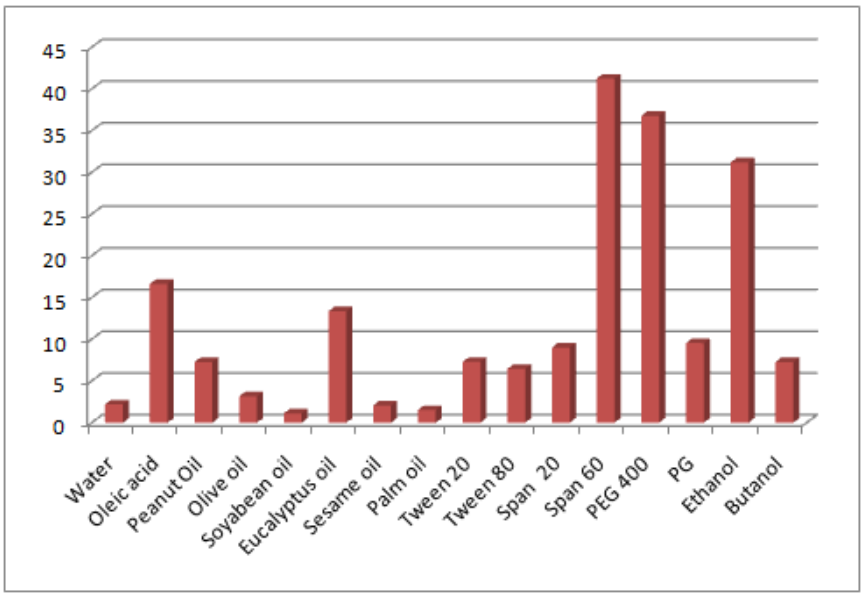

Figure 1 Solubility of simvastatin in various components

Among the tested oils, simvastatin exhibited the highest solubility in oleic acid compared to all other oils. To obtain a clear micro-emulsion proper selection of oil, surfactant, co-surfactant/cosolvent and oil to surfactant/co-surfactant ratio is significant. Oleic acid was selected as the oil phase form preparing the micro-emulsion. The highest solubility was exhibited by Span 60 and it has an HLB value of 4.7.

Selection of surfactant and cosurfactant

Emulsification studies showed that Span 60 was able to produce clear microemulsion with oleic acid upon dilution, and hence, it was employed as the surfactant in further studies.Blends of span 60 and Ethanol were used for the formulation of the microemulsions.

Construction of ternary phase diagram

In order to identify the self-emulsifying regions and to optimize the percentages of different liquid SMEDDS components, a ternary phase diagram was constructed in the absence of simvastatin.

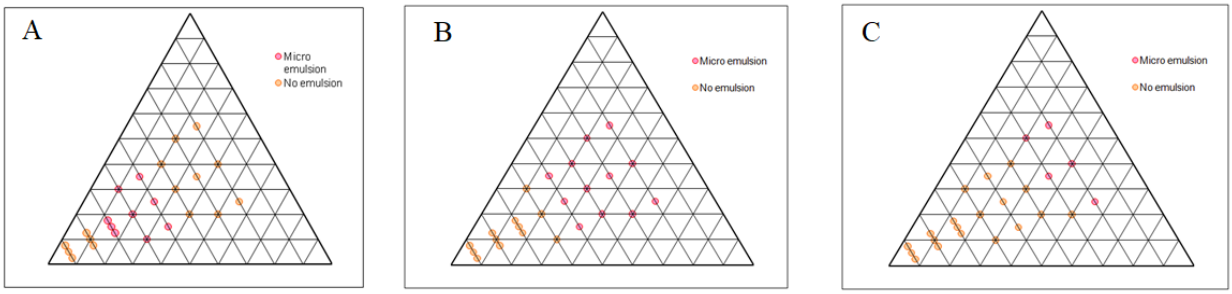

Figure 2 Ternary phase diagram (A)Smix (1:1)-water-oleic acid (B) Smix (2:1)-water-oleic acid (C) Smix (3:1)-water-oleic acid

Different batches of SEDDS were formulated and visually observed for their self-emulsifying properties. The ternary phases were judged as microemulsionand no emulsion formation on the basis of their turbidity measurements and visual observations for transparency. The concentration of components was expressed as percent volume/volume (%v/v) in ternary phase diagram. The results revealed that span 60 and ethanol used in ratios of 1:1 (F7-F8) and 2:1 (F15-F16) exhibited largest microemulsion area and shortest emulsification time (less than 1 min). It was observed that with increase in the ratio of the ethanol, spontaneity of the self-emulsification process got increased. It was observed that higher concentration of surfactant mixture (Smix) or lower concentration of oil resulted in formation of clear transparent emulsions with micro-sized droplets. The transparent emulsions (F7, F8, F15, F16) were visually evaluated for clarity and

stability after 48h at room conditions. All tested emulsions remained clear transparent even at the end of 48h. Hence, these ternary phases were selected for simvastatin loaded SMEDDs.

Simvastatin-loaded self-microemulsifying formulations (L-SMEDDs)

The ternary phase diagrams revealed the optimum concentration of the oil and the surfactant mix that could be used for the formulation of simvastatin loaded SMEDDs.

Thermodynamic stability and cloud point determination

All the formulations passed the thermodynamic stability studies without any signs of phase separation and precipitation during alternative temperature cycles (4°C and 40°C), freeze thaw cycles (-21°C and +25°C) and centrifugation at 10,000 g indicating good stability of formulations and their emulsions. Determination of cloud point is an essential parameter for the selection of a stable L-SMEDDS particularly when composed with non-ionic surfactants. "The cloud point temperature (lower consolute temperature) indicates the temperature at which the transparent monophasic system was transformed into cloudy biphasic system as dehydrated surfactant molecules associated together as precipitate, which can affect the formulation adversely. It is recommended that the cloud point for SMEDDS should be higher than body temperature (37°C), which will avoid phase separation occurring in the gastrointestinal tract. The cloud point temperature of the tested L-SMEDDS was found to be in the range of 90.47-93.22°C (Table 3). Thus, it can be inferred that the developed formulation was stable and do not require a precise storage temperature and it develops a stable emulsion upon administration at physiological temperature in vivo.

Droplet Size, Polydispersity and zeta potential of L-SMEDDs

The mean droplet size and polydispersity index (PDI) determined for different simvastatin-loaded SMEDDS (F7-8, F15-16) are shown in Table 3.

Incorporation of different amount of Smixinto simvastatin-loaded SMEDD formulations resulted in significantly different droplet size. Among the tested formulations, SMEDDS formulations prepared with 2:1 Smix ratio exhibited lower droplet size compared to formulations in which the amount of surfactant was low.

Table 3 Stability and characterization of L-SMEDDS

Formulation	Thermodynamic Stability				Surface characterization		
	Cloud point (°C)	Centrifugation	Cooling/ Heating	Freeze/Thawing	Mean droplet size (µm)	PDI	Zeta potential
F7	90.47	No phase separation	No Phase inversion	No Phase inversion	571.92 ± 7.07	0.961± 0.008	-27.4
F8	91.26	No phase separation	No Phase inversion	No Phase inversion	483.11 ± 6.03	0.729 ± 0.004	-26.8
F15	91.71	No phase separation	No Phase inversion	No Phase inversion	462.03 ± 9.08	0.963 ± 0.003	-28.9
F16	93.22	No phase separation	No Phase inversion	No Phase inversion	446.35 ± 8.05	0.918 ± 0.008	-27.5

It was observed from the results that decreasing the oil content of the formulations resulted in a decrease in the size of formulation droplets.

Self-emulsifying formulations possess a negative charge on the oil droplets due to the presence of anionic groups of free fatty acids contained in their composition; the oil, surfactant and co-surfactant. The obtained high negative values of zeta potential indicate that the tested formulations are less likely to flocculate or aggregate during storage or in biological environment.

In vitro dissolution study

The *in vitro* dissolution studies revealed the drug release profiles for the L-SMEDDS. All the formulations exhibited quick drug release characteristics and almost complete drug release in 15-45 minutes (Figure 3). In contrast, the pure drug exhibited only a maximum of 43.22% release in 60 min duration.

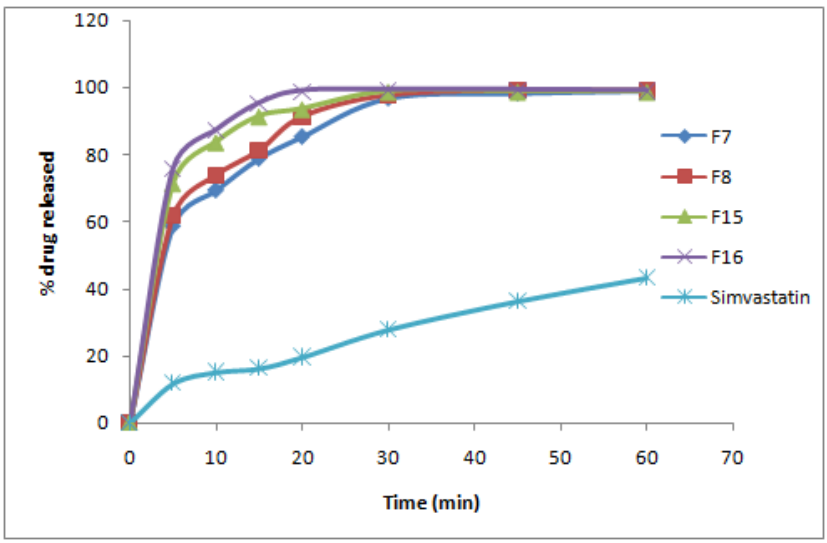

Figure 3 *In vitro* dissolution profile of L-SMEDDS and simvastatin

Simvastatin-loaded liquid SMEDDS formulations (F7, F8, F15& F16) exhibited optimal dissolution performance.High dissolution profiles of liquid SMEDDS are due to quick formation of o/w microemulsions with small droplet size upon exposure to dissolution medium with gentle agitation. In addition, the presence of the drug in a dissolved state in liquid SMEDDS formulations avoids the dissolution rate-limiting step required for crystalline drugs.

Conclusion

The bioavailability of the lipophilic drugs can be enhanced by formulating them as SMEDDS. From the release behavior witnessed through the present investigation it could be proven that the bioavailability of the lipophilic drug (simvastatin) could be almost doubled by formulating it as SMEDDS.

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