IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Development of self-microemulsifying drug delivery system for simvastatin using essential oil as carrier

Arvind*, Atul Kaushik

IPS College of Pharmacy, Gwalior, Madhya Pradesh, India

Abstract

The present investigation was undertaken with an objective to prepare the SMEDDS of simvastatin in order to improve the bioavailability of lipophilic drugs. Emulsification studies showed that Span 60 was able to produce clear microemulsion with eucalyptus oil upon dilution, and hence, it was employed as the surfactant in further studies. PEG 400 was used as the cosurfactant for the formulation of SMEDDS. 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. The results revealed that span 60 and PEG 400 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). It was observed that with increase in the ratio of the PEG 400, spontaneity of the self-emulsification process got increased. A fixed simvastatin concentration of 5% w/w was selected to be loaded in all self-emulsifying formulations. The prepared formulations were kept in closed containers and tested for thermodynamic stability. 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 15-20 minutes. In contrast, the pure drug exhibited only a maximum of 47.15% release in 60 min duration.

Keywords

Self-emuslifying, microemulsino, simvastatin, anti-hyperlipidemic, stability

Introduction

Simvastatin is an anti-hyperlipidemic drug that lowers the level of lipoproteins in blood. It has attracted considerable attention due to its potential to prevent cardiovascular diseases by retarding the accelerated atherosclerosis in hyperlipoproteinemic individuals [1].

According to World Health organization (WHO), one-third of ischemic heart disease is attributable to high cholesterol which has caused 2.6 million deaths and 29.7 million disability adjusted life years (DALYS), globally. In 2008, the global prevalence of raised total cholesterol among adults was 39% [2]. In a survey conducted in United States in the year 2009-2012, 13.4% adults aged 20 years and above had high serum total cholesterol. The mean serum total cholesterol levels for the same group of people were 196 mg/dL [3]. 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]. As most of the antihyperlipidemic drugs approved for clinical use are known to be possessing poor aqueous solubility and poor bioavailability, newer delivery systems are the need of the hour for these drugs. SEDDS provide an alternate to overcome the problems related to the solubility and bioavailability of these antihyperlipidemic drugs.

Self-emulsifying drug conveyance frameworks (SEDDS) represent a vital tool in improving oral bioavailability of lipophilic medications. Lipophilic medications can be solubilized in SEDDS formulations, empowering them to be administered as a unit dosage form for oral administration. The overall goal of the present postulation was to improve the dissolvability, dissolution pace, conceivably the intestinal penetratability and bioabavailability of lipophilic medications by using self-microemulsifying drug delivery systems (SMEDDS) for oral administration.

Material and Methods

Preformulation Studies

Calibration curve of simvastatin in methanol

A stock solution of simvastatin (100 mg/100 ml) was prepared in methanol. Diluted simvastatin solution (10 mg / 100 ml) in methanol was prepared from the stock solution. Then, serial dilutions were prepared from that diluted into simvastatin solution in ethanol to obtain different concentrations ranging from 2.5 to 45 µg/ml. The absorbance of these serial dilutions was determined spectrophotometrically at λmax 238 nm, using methanol as a reference. Each sample was analyzed in triplicate and the results are presented as mean ± SD. The measured absorbance was plotted against the corresponding concentrations to obtain the standard calibration curve.

Drug solubility

The solubility of simvastatin in different oils, surfactants and co-surfactants was determined according to the method of Date and Nagarsenker [10]. In this method, an excess amount of the drug was mixed with fixed amounts of the oil (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 and co-surfactant for emulsifying ability

The emulsification ability of different surfactants and co-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. Mixtures that showed grades A and B upon dilution were assigned for further evaluation.

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.

In plotting ternary phase diagram, one axis represents the oil phase, the second represents the Smix and the third represents the aqueous phase. The phase diagram was constructed to identify the microemulsifying region, using oil and Smix ratios which form ‘good’ emulsions upon dilution with purified water.

Table 1 Composition 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)

Simvastatin was 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
F15	70	20	10	2:1
F16	60	26.6	13.3	2:1
F23	40	45	15	3:1
F24	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

The particle size and polydispersity index 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:

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

The experiments were repeated in triplicate for each produced batch and then the results were averaged ± standard deviation.

***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

The standard calibration curve of simvastatin was constructed in methanol to obtain different concentrations ranging from 2.5 to 45 µg/ml, for which the absorbance readings were determined spectrophotometrically at λmax 238 nm (Figure 1).

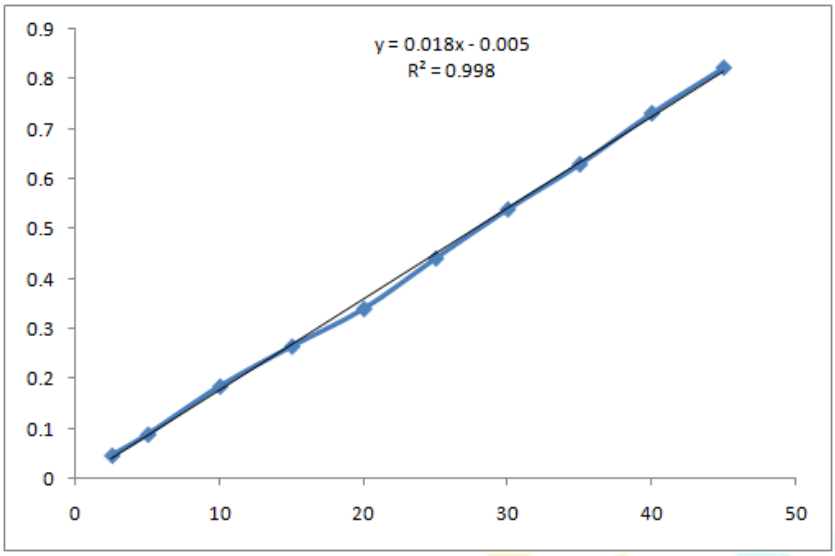

Figure 1 Standard calibration curve of simvastatin in methanol

Solubility Studies

The solubility of simvastatin was determined in oils, surfactants, co-surfactants, mixture of oils and mixture of surfactants (Figure 2).

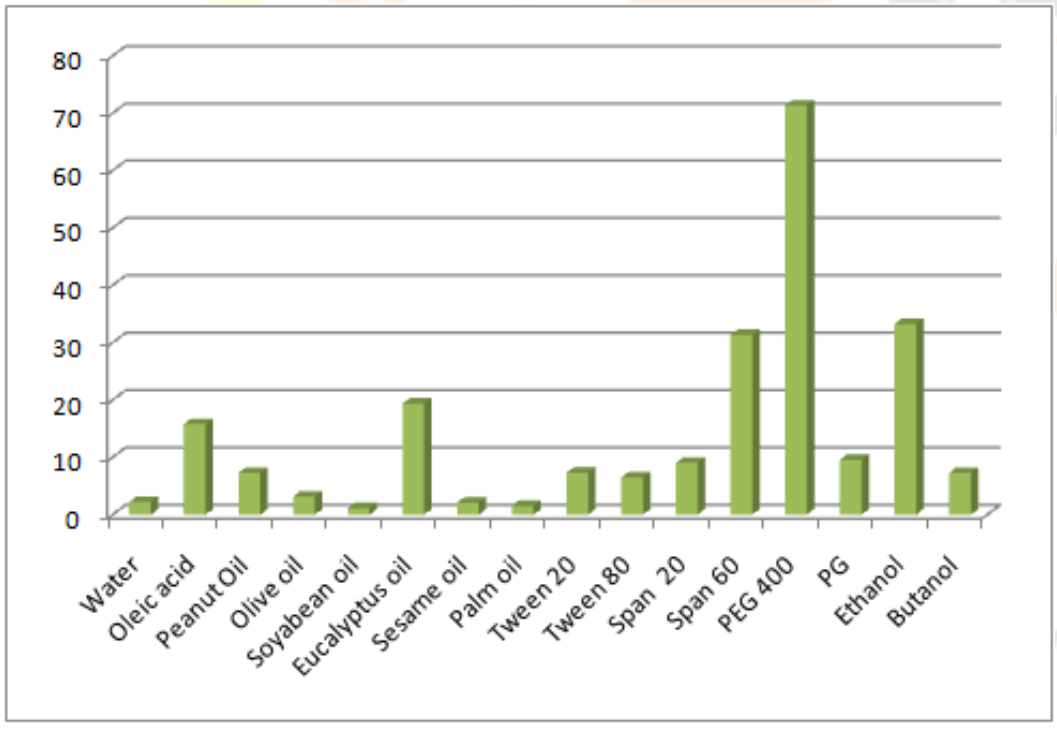

Figure 2 Comparative chart of solubility of simvastatin

Among the tested oils, simvastatin exhibited the highest solubility in eucalyptus oil compared to all other oils, though the solubility in oleic acid close enough to eucalyptus oil. To obtain a clear micro-emulsion clever selection of oil, surfactant, co-surfactant and oil to surfactant/co-surfactant ratio is significant. For achieving this, it is suggested that a surfactant should have hydrophilic-lipophilic balance (HLB) value more than 10 to form an o/w

emulsion. Eucalyptus oil 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 while PEG 400 has HLB value of 13.1.

Selection of surfactant and cosurfactant

Selection of surfactants should be based on its emulsification efficiency for the selected oil more than its solubilizing potential for the drug [13]. Therefore, the miscibility of the above surfactants with the selected oil (eucalyptus oil) at a 1:1 weight ratio was investigated according to the method reported by Balakrishnan [14] and Date and Nagarsenker [10]. Emulsification studies showed that Span 60 was able to produce clear microemulsion with eucalyptus oil upon dilution, and hence, it was employed as the surfactant in further studies.

The use of a single surfactant may not be enough to achieve a transient negative interfacial energy or a fluid interfacial film. Hence, addition of a co-surfactant may provide sufficient flexibility to the interfacial film so that various curvatures can be available to form microemulsions over a wide range of composition. The co-surfactant and co-solvents used were equivalent in improving emulsification ability of surfactants as demonstrated by grades A and B produced upon dilution with distilled water. Blends of span 60 and PEG 400 were used for the formulation of the microemulsions. The appropriate amounts of the selected oil, surfactants and co-surfactant were determined by constructing phase diagrams.

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.

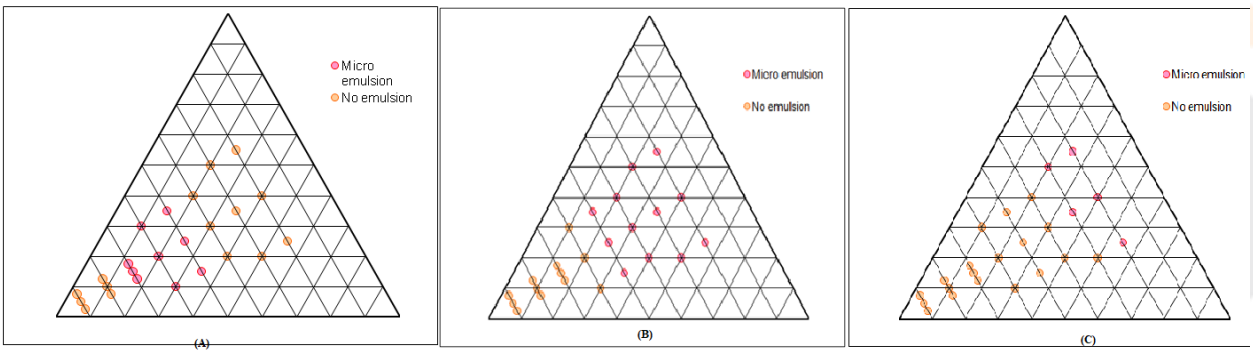

Figure 3 Ternary phase diagram for Smix -water-eucalyptus oil (A) Smix 1:1 (B) Smix 2:1 (C) Smix 3:1

The ternary phases were judged as microemulsion and 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 (Figure 3a-3c). The results revealed that span 60 and PEG 400 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 PEG 400, 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. This could be due to higher HLB value of Smix 80 and better solubilization in PEG. 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. A fixed simvastatin concentration of 5% w/w was selected to be loaded in all self-emulsifying formulations. It was expected to provide spontaneous emulsification of SMEDDS with a low tendency of drug precipitation upon aqueous dilution. Also, using fixed concentration of simvastatin in all formulations was proposed to exclude the effect of varying the drug concentration on the self-emulsifying efficiency of the systems.

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 90.45-94.18°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 Smix into 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	91.33	No phase separation	No Phase inversion	No Phase inversion	563.52 ± 9.07	0.396± 0.002	-29.6
F8	90.45	No phase separation	No Phase inversion	No Phase inversion	471.11 ± 3.06	0.297 ± 0.004	-25.4
F15	91.86	No phase separation	No Phase inversion	No Phase inversion	454.30 ± 8.01	0.405 ± 0.003	-29.8
F16	94.18	No phase separation	No Phase inversion	No Phase inversion	431.53 ± 7.05	0.401 ± 0.008	-26.9

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 15-20 minutes (Figure 4). In contrast, the pure drug exhibited only a maximum of 47.15% release in 60 min duration.

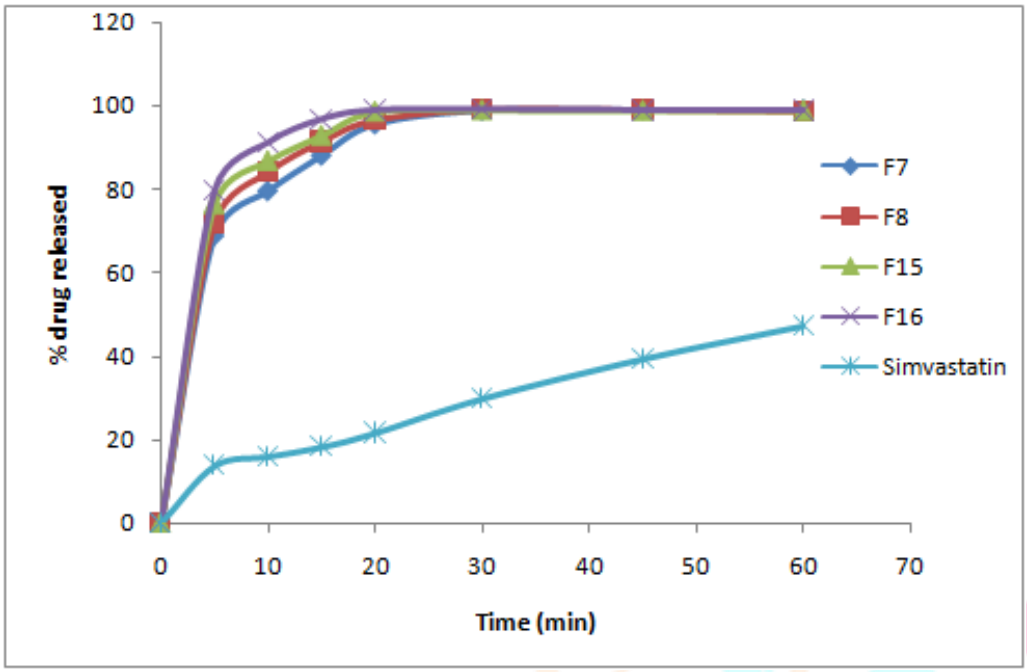

Figure 4 *In vitro* dissolution profile of L-SMEDDS and simvastatin

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 SNEDDS. 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 SNEDDS.

References

1. https://go.drugbank.com/drugs/DB00641
2. Global Health Observatory data. World health Organization Updates. WHO updates, 2015. Available at http://www.who.int/gho/ncd/risk_factors/cholesterol_text/en/. [Last accessed on 22nd June 2021]
3. Center of Disease Control and Prevention. National Center for Health Statistics. United States, 2014. Available at http://www.cdc.gov/nchs/fastats/cholesterol.htm [Last accessed on 22nd June 2021]
4. World Health Organization- Non communicable Diseases (NCD) Country Profiles, 2014
5. Global Health Observatory data. World health Organization Updates. WHO updates, 2015. Available at http://www.who.int/chp/chronic_disease_report/en/ [Last accessed on 22nd June 2021]
6. Joshi SR, Anjana RM, Deepa M, Pradeepa R, Bhansali A. Prevalence of Dyslipidemia in Urban and Rural India: The ICMR–INDIAB Study. PLoS ONE 9(5): e96808, 2014 doi:10.1371/journal.pone.0096808

7. Grundy SM. Pathogenesis of hyperlipoproteinemia. Journal of Lipid Research 1984; 25: 1611-1618.
8. Mckenney JM. Pharmacologic Characteristics of Statins. Clinical Cardiology, 2003; 26(3): III-32–III-38.
9. Srinivasa Rao K, Prasad T, Mohanta GP, Manna PK. An Overview of Statins as Hypolipidemic Drugs. International Journal of Pharmaceutical Sciences and Drug Research 2011; 3(3): 178-183
10. Date AA, Nagarsenker M.. Design and evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) for cefpodoxime proxetil. International Journal of Pharmaceutics 2007; 329:166-72.
11. Khoo S-M, Humberstone AJ, Porter CJH, Edwards GA, Charman WN. Formulation design and bioavailability assessment of lipidic self-emulsifying formulations of halofantrine. International Journal of Pharmaceutics 1998; 167:155-64.
12. Inugala S, Eedara BB, Sunkavalli S. Solid self-nanoemulsifying drug delivery system (S-SNEDDS) of darunavir for improved dissolution and oral bioavailability: in vitro and in vivo evaluation. European Journal of Pharmaceutical Sciences 2015; 74: 1-10
13. Duc Hanh N, Mitrevej A, Sathirakul K, Peungvicha P, Sinchaipanid N. Development of phyllanthin-loaded self-microemulsifying drug delivery system for oral bioavailability enhancement. Drug development and industrial pharmacy 2015; 41:207-17.
14. Balakrishnan P, Lee B-J, Oh DH, Kim JO, Hong MJ, Jee J-P, Kim JA, Yoo BK, Woo JS, Yong CS, Choi H-G. Enhanced oral bioavailability of dexibuprofen by a novel solid Self-emulsifying drug delivery system (SEDDS). European journal of pharmaceutics and biopharmaceutics 2009; 72: 539-45.