ORIGINAL ARTICLE

Development and Evaluation of Liquid and Solid Self-microemulsifying Drug Delivery System of Lovastatin

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Abstract

Aim: The present investigations was aimed to improve the solubility, dissolution rate and ultimately the bioavailability of a poorly water soluble BCS class II drug Lovastatin, by formulating it as self-microemulsifying drug delivery system (SMEDDS). **Materials and Methods:** Liquid SMEDDS of the drug were formulated using Labrafil M 1944, Acrysol EL 135 and Lauroglycol as oil, surfactant and co-surfactant respectively. The prepared systems were characterized for self-emulsification time, robustness to dilution, % transmittance, globule size and thermodynamic stability. Ternary phase diagrams were plotted to identify the area of microemulsification. The optimized liquid SMEDDS was transformed into free flowing powder using Neusilin US2 as the adsorbent. **Results and Discussion:** Self microemulsifying powder retained the self microemulsifying property of the liquid SMEDDS. Differential scanning calorimetric and X-ray powder diffraction studies confirmed solubilization of the drug in the lipid excipients and or transformation of crystalline form of the drug to amorphous one in solid-SMEDDS. This was supported by scanning electron microscopy studies which did not show evidence of precipitation of drug on the surface of the carrier. *In-vitro* dissolution studies revealed enhanced release of the drug from solid-SMEDDS as compared to pure drug and the marketed formulation. Similarly the in-vitro absorption studies revealed significant enhancement in drug release from the SMEDDS as compared to plain drug suspension. **Conclusion:** It can be concluded that the SMEDDS formulation of Lovastatin has the potential of improved delivery of the lipophilic drug and is amenable to development of solid dosage form using Neusilin US2 as the porous carrier.

Key words: Crystallinity, droplet size, drug release, lovastatin, Neusilin US2, self-microemulsifying drug delivery system

INTRODUCTION

Oral ingestion is the most convenient and commonly used the route of drug administration because it provides ease of administration, patient compliance, cost effectiveness, and flexibility in dosage design. A poorly bioavailable drug is one of the major challenges for formulation scientists because it can lead to compromised product performance and the drug is unlikely to reach its molecular target.[1] Lipid-based drug delivery system has attained increasing interest in the oral route of administration of poorly bioavailable drug as a means to bypass the drug passage in the hepatic portal vein and consequently its hepatic degradation. This hypothesis was believed to be attained chiefly, by lymphatic transport via Peyer's patches along the gastrointestinal (GI) tract.[2] Self-microemulsifying drug delivery system (SMEDDS) is one among the lipid-based drug delivery system that has been currently investigated for its advantages, providing a large interfacial area for partitioning the drug between oil and GI fluid.[3] This technique improves the oral bioavailability of poorly soluble drugs by enhancing the solubility and maintaining the drug in a dissolved state,

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in small droplets of oil, all over its transit through the GI tract.[4,5] However, the traditional SMEDDS as liquid dosage forms has limitations such as low drug loading capacity, drug leakage, low stability, few choices of dosage forms, excipient-capsule incompatibility, and possibility of irreversible drugs/excipients precipitation.[6] To overcome these complications, the liquid SMEDDS are adsorbed onto inert carriers to produce solid-SMEDDS. This approach of solid-SMEDDS has advantages such as stability, facility of manufacturing process, accuracy, and patient compliance. Thus, incorporation of liquid SEDDS into solid dosage forms combines the advantages of lipid-based drug delivery systems with those of solid dosage forms.[7]

Lovastatin (LVS), categorized as a Class II compound according to the Biopharmaceutics Classification System exhibits poor oral bioavailability (<5%) because of its low solubility (1.3 µg/mL in water) extensive metabolism in the gut and liver and transmembrane efflux via P-glycoprotein.[8] Its bioavailability can be improved by increasing the dissolution rate and/or decreasing pre-systemic clearance.[9-11] In the present investigation, we formulated liquid SMEDDS to enhance the solubility and absorption of LVS using Labrafil M1944 as oil, Acrysol EL 135 as surfactant, and Lauroglycol 90 as co-surfactant. As solid dosage forms are more stable and beneficial than liquids, the optimized liquid SMEDDS of the drug was transformed into free-flowing powder thus combining the solubilization effects of lipids and stabilizing effects of the carrier system. Solid-SMEDDS was formulating using Neusilin US2 as the porous carrier and evaluated for flow properties, solid state characteristics, and *in vitro* dissolution and absorption profiles.

MATERIALS AND METHODS

Materials

LVS was obtained as a generous gift from Themis Medicare Ltd., Mumbai, Maharashtra, India. Captex 300, Captex 355, Capmul MCM, and Acconon were kindly supplied by Abitec Corporation, Janesville, USA while Labrafil M 1944, Lauroglycol 90, Labrafac Lipophile WL 1349 and Labrasol were gifted by Gattefosse Ltd., Mumbai, Maharashtra, India. Neusilin US2 was supplied by Gangwal Chemicals, Mumbai, Maharashtra, India. Acrysol EL 135 and Acrysol K 140 were obtained as gift samples from Corel Pharma Chem, Ahmedabad, Gujarat, India. Tween 20, Tween 80, PEG 400, and Propylene glycol were purchased from Merck (Mumbai, Maharashtra, India). The tablets of LVS containing 10 mg of the drug (Brand name, Aztatin) were used for comparative dissolution studies. All the excipients and reagents were of analytical grade, and double-distilled water was freshly prepared whenever required throughout the study.

METHODS

Solubility studies

Solubility studies were carried by placing an excess amount of LVS in a screw capped vials containing 2 mL of vehicles (oils, surfactants, and co-surfactants). The suspensions of vehicles were heated on a water bath at 40°C to facilitate the solubilization using vortex mixer. The suspensions were then continuously agitated on a rotary shaker for 48 h at ambient temperature. After reaching equilibrium, the samples were centrifuged at 5000 rpm for 15 min and the supernatant was taken, filtered through 0.45 µm membrane filter. The filtrates were suitably diluted with methanol and analyzed spectrophotometrically for the dissolved drug at 238 nm. Blank was prepared by dissolving respective vehicles in methanol with the same dilution as for the samples. The experiment was performed in triplicate and results were represented as mean value (in milligram/mL) ± standard deviation (SD).

Preliminary screening of surfactants and co-surfactants

The surfactants and co-surfactants were screened for emulsification ability as per method reported in the literature.[12] Briefly, 300 mg of surfactant was added to 300 mg of the selected oily phase. The mixture was gently heated at 45-60°C for homogenizing the components. The isotropic mixture, 50 mg, was accurately weighed and diluted with double-distilled water to 50 mL to yield fine emulsion. The ease of formation of emulsions was monitored by noting the number of volumetric flask inversions required to give a uniform emulsion. The resulting emulsions were observed visually for the relative turbidity. The emulsions were allowed to stand for 2 h, and their transmittance was assessed at 650 nm by a UV-visible double beam spectrophotometer (Jasco V-630, Japan). The experiment was performed in triplicate. Similarly, various co-surfactants were screened for improving the emulsification ability in the SMEDDS formulation. Mixtures of 100 mg of co-surfactant, 200 mg of selected surfactant, and 300 mg of selected oil phase were prepared and evaluated in the same manner as for the screening of the surfactant. The experiment was performed in triplicate.

Construction of pseudoternary phase diagrams

The selected oil, surfactant, co-surfactant on the basis of solubility and preliminary screening studies were used to develop pseudoternary phase diagrams using the water titration method.[13] The various surfactant/co-surfactant (S_{mix} w/w) ratios were prepared using different proportions of surfactant and co-surfactant (1:1, 2:1, and 1:2) for plotting the phase diagrams. A series of oil/S_{mix} mixtures were prepared at all nine combinations (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1) and titrated with water to identify the microemulsion

region. The total water consumed was noted in terms of w/w and during titration of oil-S_{mix} ratio observations were made for phase clarity. The concentration of water at which turbidity-to-transparency and transparency-to-turbidity transitions occur was derived from the weight measurements. These values were used to determine the boundaries of the microemulsion region corresponding to the selected value of oil and S_{mix} ratio. Phase diagrams were constructed using CHEMIX school software, version 3.6.

Preparation of liquid SMEDDS

A series of SMEDDS formulations were prepared with varying ratios of oil (20-40%), surfactant (30-70%), and co-surfactant (10-50%). A single dose of LVS (10 mg) was incorporated in all formulations. The total weight of the formulations were kept at 160 mg. The formulations were prepared by dissolving the drug in oil followed by addition of surfactant and co-surfactant in glass vials. The resulting mixtures were stirred continuously by vortex mixing followed by sonication for few minutes to obtain a homogenous isotropic mixture. The SMEDDS formulations were stored at ambient temperatures until further use.

Characterization of SMEDDS

Visual assessment of self-emulsification

A visual test to assess the self-emulsification properties reported by Craig *et al*.[14] was modified and adopted in the present study. In this method, a unit dose of the formulation was introduced into 250 mL of water in a glass beaker that was maintained at 37°C ± 0.5°C and the contents mixed gently using a magnetic stirrer. The tendency to emulsify spontaneously and the time taken for the emulsion formation were assessed visually. Formulations forming transparent, clear bluish emulsion within less than 1 min were categorized as Grade A emulsions, whereas formulations forming slightly less clear emulsions within 1 min were categorized as Grade B emulsions. Grade C emulsions were those which were milky in appearance that formed within 2 min. All the trials were carried out in triplicate with similar observations being made between repeats.

Effect of pH and robustness to dilution

Formulations were subjected to 50-, 100-, 250-, and 1000-fold dilution with distilled water, pH 1.2 and pH 6.8 buffer. The resultant diluted emulsions were monitored for any physical changes such as (coalescence of droplets, precipitation, or phase separation) after 24 h storage.[15]

% Transmittance

The SMEDDS were reconstituted with distilled water, and the resulting nanoemulsions were observed visually for any turbidity. Thereafter, its % transmittance was measured at 650 nm using the UV-visible spectrophotometer (Jasco V-630, Japan) against distilled water as the blank. The studies were conducted after 100 times dilution.

Droplet size analysis

The mean droplet size (SMD) and polydispersity index (PDI) of the formulations were determined by photon correlation spectroscopy using nanosizer (Nanophox NX0088, Sympatec Germany). Each formulation was diluted with filtered (0.45 µm, Millipore) double-distilled water before analysis. Size analysis was carried at 25°C with an angle of detection of 90°.

Cloud point, refractive index, zeta potential, and thermodynamic stability

The cloud point measurement was carried out for the formulations as reported earlier.[16] The formulation was diluted up to 100-folds with distilled water and kept in a water bath which was maintained at a temperature of 25°C with a gradual increase of temperature at a rate of 5°C/min and the corresponding cloud point temperatures were read at the first sign of turbidity by visual observation. Refractive indices of the liquid SMEDDS formulations were determined using Abbes refractometer at 25°C ± 1°C while zeta potential of the liquid SMEDDS formulations was measured on Zetasizer (ZS 90, Malvern Zetasizer, Malvern, UK) after diluting the SMEDDS formulation with 100 mL double-distilled water. For evaluating the thermodynamic stability, the formulations were subjected to heating-cooling cycle (4°C and 45°C) and freeze-thaw cycle (−21°C and +25°C) with storage at each temperature of not less than 48 h. For centrifugation stress, the formulations were centrifuged at 3500 rpm for 15 min, and the extent of phase separation was monitored.[17]

Drug release studies

In vitro drug release studies were performed using a modified dialysis technique.[18] Initially, the dialysis tubing was soaked in the dialysis medium for 12 h at room temperature which was treated at 40°C before the start of the experiment. The diluted SMEDDS formulation (equivalent to 10 mg) was placed in dialysis tubing (Hi media membrane, Mumbai, cut off 12000-14000 Da) and clamped on both sides. The secured dialysis tube was allowed to rotate freely in the dissolution vessel of USP XXIV type-II dissolution apparatus (Electrolab TDT-06 T, Mumbai, India) containing 500 mL of pH 1.2 buffer as dialysis medium at 37°C ± 0.5°C and stirred at 50 rpm. An aliquot of 5 mL was withdrawn at predetermined time intervals and filtered through 0.45 µm filter. The withdrawn volume was replenished immediately with the same volume of fresh medium to keep total volume constant and maintain sink conditions. The concentration of LVS in

the filtrate was analyzed using the UV spectrophotometer at 238 nm. The blank SMEDDS without drug was processed similarly and used as a reference to avoid interference from the formulation components if any. Each release study was performed in triplicate. The data were analyzed using the PCP Disso v 3.0 software, India.

Transmission electron microscopy (TEM)

The morphology of the optimized SMEDDS formulation was observed using TEM (JEM-2100 F, M/s Jeol, Tokyo, Japan) with AMT image capture engine software. SMEDDS formulation was diluted with distilled water in 1:200 and mixed by gentle shaking. One drop of the diluted sample was deposited on a film coated copper grid, stained with one drop of phosphotungstic acid and allowed to dry before observation under the transmission microscope. The image was magnified and focused on a layer of photographic film.

Preparation of Solid-SMEDDS

Solid-SMEDDS was prepared by mixing liquid SMEDDS containing LVS with Neusilin US2 in 1:2, 1:1, and 2:1 proportions. In brief, liquid SMEDDS was added gradually over the carriers contained in a mortar. After each addition, the mixture was mixed vigorously and homogenized to ensure uniform distribution of formulation. Resultant damp mass was passed through sieve no. 120 and dried at ambient temperature and stored until further use.

Characterization of solid-SMEDDS

Micromeritic properties of solid-SMEDDS

The bulk density, tapped density, Carr's Compressibility Index, and Hausner's ratio were determined for the optimized solid-SMEDDS. The angle of repose of self-emulsifying powder was determined by funnel method.[19] Briefly, the sample was poured through a funnel with its tip positioned at a fixed height (h) on a horizontal surface until the apex of pile touches the tip of the funnel. The angle of repose was calculated using the formula $\tan \theta = h/r$ where r is the radius of the pile of powder. In addition, the flow rate was determined by measuring the time required for 1.0 g of the formulation to flow through the funnel with an orifice of 1.5 cm diameter. The powder flow property was noted on the basis of the time required to pass through the orifice as less than 1 s (excellent), less than 5 s (good), less than 10 s (average), and more than 10 s (poor).[20]

Morphological analysis

The outer macroscopic structure of the drug and that of solid self-microemulsifying powder was investigated by scanning electron microscopy (JEOL, JSM-6390 LV, Japan) at 15 keV accelerating voltage.

Differential scanning calorimetry (DSC)

The physical state of the LVS in solid-SMEDDS was characterized by DSC studies. The DSC thermograms of the LVS, a physical mixture of drug and carrier, carrier as well as that of solid-SMEDDS were recorded using DSC (Perkin Elmer, USA). The samples were heated in an open aluminum pan from 30°C to 450°C at a scanning rate of 10°C/min under the stream of nitrogen.

X-ray powder diffraction studies

X-ray powder scattering measurements of the LVS, physical mixture of LVS, and Neusilin US2, Neusilin US2 and that of solid self-microemulsifying powder were carried out with X-ray diffractometer (D8 Advance, Bruker AXS, Germany). The Powder X-ray diffraction patterns were recorded at room temperature using monochromatic CuKα-radiation ($k = 1.5406$ Å) at 40 mA and at 45 kV over a range of 2 θ angles from 3° to 50° with an angular increment of 0.02° per second.

Emulsion droplet size

The SMD and PDI of solid-SMEDDS were determined by photon correlation spectroscopy using nanosizer (Nanophox NX0088, Sympatec Germany). The formulation was diluted with filtered (0.45 µm, Millipore) double-distilled water before analysis. Size analysis was carried at 25°C with an angle of detection of 90°.

Drug content estimation

Liquid SMEDDS and solid-SMEDDS containing LVS, each equivalent to 10 mg was dispersed in suitable quantity of methanol. The samples were mixed thoroughly to dissolve the drug in methanol, centrifuged at 3000 rpm for 15 min using 12C micro-centrifuge (Remi motors, Mumbai, India) to separate the undissolved excipients. The supernatant was suitably diluted and analyzed spectrophotometrically at 238 nm using the Jasco UV-visible spectrophotometer. The content of LVS was calculated from the standard curve of the drug in methanol using the Beer-Lambert's equation ($y = 0.0674 \times \text{concentration} + 0.0098$).

In vitro dissolution studies

Drug release from solid-SMEDDS was performed using USP type II dissolution apparatus (Electrolab, TDT-06 T Mumbai, Maharashtra, India). Solid-SMEDDS formulation equivalent to 10 mg of LVS was used for the dissolution studies which were performed in dissolution medium containing 900 mL of pH 1.2 buffer with paddle rotation speed of 100 rpm. An aliquot of 5 mL was withdrawn at predetermined time intervals and filtered through 0.45 µm filter. The concentration of LVS in the filtrate was analyzed using the UV spectrophotometer

at 238 nm after suitable dilution. Similarly, the dissolution profiles of pure drug and the marketed formulation of LVS (Aztatintablet, 10 mg) was also carried out.

Calculation of dissolution parameters

Various dissolution parameters such as dissolution efficiency (DE) and mean dissolution time (MDT) were calculated.[21] The DE is defined as the area under the dissolution curve up to a certain time (t), expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time. DE at 15 min was calculated using the following equation:

$$D.E.=\frac{\int_0^t y\times dt}{y_{100}\times t}\times 100\%$$

The MDT can be calculated by the following expression:

$$MDT=\frac{\sum_{j=1}^{n} t_j \hat{} \Delta M_j}{\sum_{j=1}^{n} \ddot{A}M_j}$$

Where, j is the sample number, n is the number of dissolution sample times, t_j^ is the time at the midpoint between t_j and t_{j-1}, and ΔM_j is the additional amount of drug dissolved between t_i and $t_{i-1.}$

Statistical analysis

All the results were expressed as mean ± SD. The dissolution data obtained was subjected to one-way analysis of variance followed by Dunnett's multiple comparison test. The difference at $P < 0.05$ was considered to be statistically significant. The statistical analysis was performed with Instant Graph Pad Prism software (version 4.00; Graph Pad Software, San Diego California).

In vitro absorption studies

In vitro absorption profile of plain drug suspension, liquid SMEDDS, and solid-SMEDDS were carried out through everted rat intestinal segment using an in-house fabricated perfusion apparatus.[22] The apparatus (Figure 1) consists of two cylindrical glass tubes; one joined to other via J-shaped tapering end. Both the tubes are held together by a glass joint on the upper end. On the lower ends of both tubes, a bulge is given for proper mounting of tissue. After mounting the everted intestinal segment on the apparatus and setting it in the beaker, the inside of the glass tubes serve as the serosal compartment, and the beaker serves as the mucosal compartment. For the experimental purpose, the rat was sacrificed humanely by cervical dislocation and the abdomen was opened by a midline incision. A 9 cm intestinal segment corresponding to duodenal region was carefully removed and transferred to a petri dish containing Krebs medium (118.0 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4 7H_2O$, 25.0 mM $NaHCO_3$, 1.2 mM KH_2PO_4, and 5.5 mM glucose). The intestinal segment was cleaned with the Krebs solution and gently everted using a glass rod. A 6.0 cm everted segment was then mounted in the apparatus which was placed in a 600.0 mL beaker containing the drug suspended in 500 mL of pH 5.8 buffer solution. The total volume of the absorption compartment (tubes of perfusion apparatus) was 30 mL of Krebs solution. This assembly (beaker and apparatus with tissue) was placed on a magnetic stirrer, and a magnetic bead was allowed to rotate at 25 rpm in the beaker, and the temperature was maintained at 37°C ± 0.5°C with adequate aeration. The drug diffused from phosphate buffer pH 5.8 (mucosal side) to the Krebs solution contained in the tubes (serosal side). The samples were collected at different time points (at every 15 min for 2 h) and analyzed for the drug content by the UV spectrophotometer. Similarly, the absorption studies were carried out for the optimized liquid SMEDDS and solid-SMEDDS. All the experiments were performed in triplicate. The study protocol for *in vitro* absorption was approved by the Institutional Animal Ethics Committee of Department of Pharmaceutical Sciences, Nagpur and is in accordance with the guidance of committee for the purpose of control and supervision of experiments on animals, Ministry of Social Justice and Empowerment, Government of India.

RESULTS AND DISCUSSION

Solubility studies

Solubility studies were performed to identify suitable excipients with maximum potential to solubilize the drug and having good miscibility with each other which helps in minimizing the final volume of SMEDDS and potentiates optimal drug loading.[23] Thus, the solubility of LVS was assessed in a variety of oils, surfactants, and co-surfactants. The data is presented in the Figures 2 and 3. It was revealed from the data that the drug exhibited poor solubility in natural edible oils. Synthetic oil like Captex 355 also could solubilize the drug to a marginal extent. Highest solubility was displayed by the drug in Labrafil M 1944(52.13 ± 1.36 mg/mL)

Figure 1: In-house fabricated perfusion apparatus

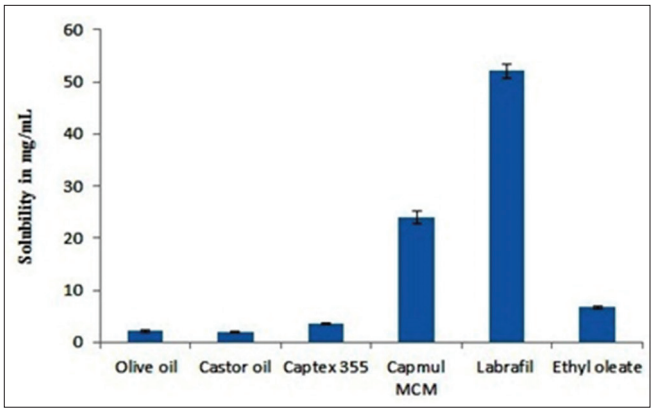

Figure 2: Solubility studies of Lovastatin in various oils. Data expressed as mean ± standard deviation, *n* = 3

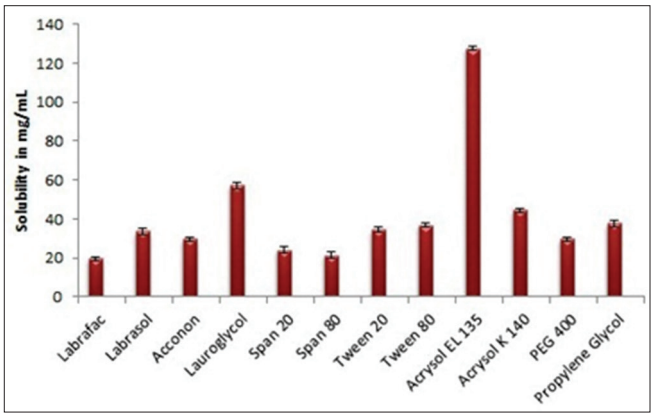

Figure 3: Solubility studies of Lovastatin in various surfactants and co-surfactants. Data expressed as mean ± standard deviation, *n* = 3

followed by Capmul MCM (23.9 ± 1.25 mg/mL). From the data obtained from the solubility studies, Labrafil 1944 was selected as oil phase for further studies. The surfactant and the co-surfactant were selected based on two parameters, ability to solubilize LVS and their emulsification ability.

Preliminary screening of surfactants and co-surfactants

Nonionic surfactants are generally considered safer than the ionic surfactants and are usually accepted for oral ingestion. They are also reported to provide better stability to emulsion over a wide range of pH and ionic strength.[24] Thus, various nonionic surfactants were screened to evaluate their ability to emulsify the selected oil phase, Labrafil M 1944. For oil-surfactant mixture to be used in SMEDDS formulations, it was essential to determine whether it could disperse efficiently to form spontaneous nanoemulsion. Tween 80, Tween 20, Labrasol, Acrysol EL 135, and Acrysol K 140 were selected for the emulsification study as they showed good solubility potential for LVS. The % transmittance values of the various dispersions are quoted in Table 1. It was observed that the emulsifying ability of Acrysol EL 135 was highest among the surfactants screened as judged by the % transmittance values

Table 1: Emulsification ability of various surfactants

Surfactant	% Transmittance*
Labrasol	52.9±0.67
Acrysol EL 135	95.3±0.29
Acrysol K 140	71.7±0.13
Tween 20	33.8±4.13
Tween 80	31.34±0.22

*Data expressed as mean±SD (*n*=3). SD: Standard deviation

Table 2: Emulsification ability of various co-surfactants

Co-surfactant	% Transmittance*
PEG 400	87.9±0.42
Propylene glycol	93.1±0.04
Lauroglycol	98.7±0.47
Labrafac	45.1±0.54

*Data expressed as mean±SD (*n*=3). SD: Standard deviation

of the various dispersions obtained. Acrysol EL 135 was therefore selected as the surfactant for further investigations.

The addition of a co-surfactant to a surfactant-containing formulation is reported to improve the emulsification ability of the surfactant and drug absorption from the formulation.[25] Here in the present investigations, the co-surfactants screened for the emulsification ability included Lauroglycol 90, Labrafac, PEG 400, and Propylene Glycol. The % transmittance values of various dispersions are depicted in Table 2. It was evident from the observations that the spontaneity of self-emulsification process was excellent with the combination Labrafil M 1944-Acrysol EL 135-Lauroglycol 90 as adjudged by the % transmittance of the resulting microemulsion obtained. Lauroglycol 90, which is propylene glycol mono and diester of lauric acid with an HLB value of 4, was considered as the desired co-surfactant in the present case to be used along with a hydrophilic surfactant, i.e., Acrysol EL 135.

Construction of pseudoternary phase diagrams

Pseudoternary phase diagrams were constructed as depicted in Figure 4a-c to identify the self-microemulsifying regions and to optimize the concentration of oil, surfactant, and co-surfactant in the liquid SMEDDS formulations. For the development of SMEDDS formulations, optimum ratios of excipients concentrations established by means of phase diagram studies provided the area of the monophasic region. It is important to determine this area to ensure successful aqueous dilution without breaking the microemulsions.[26] In the present study, the phase diagrams were plotted taking three ratios of surfactant/co-surfactant as 1:1, 2:1, and 1:2. It was observed that the area of microemulsion existence was higher at surfactant/co-surfactant ratio of 2:1 and 1:1

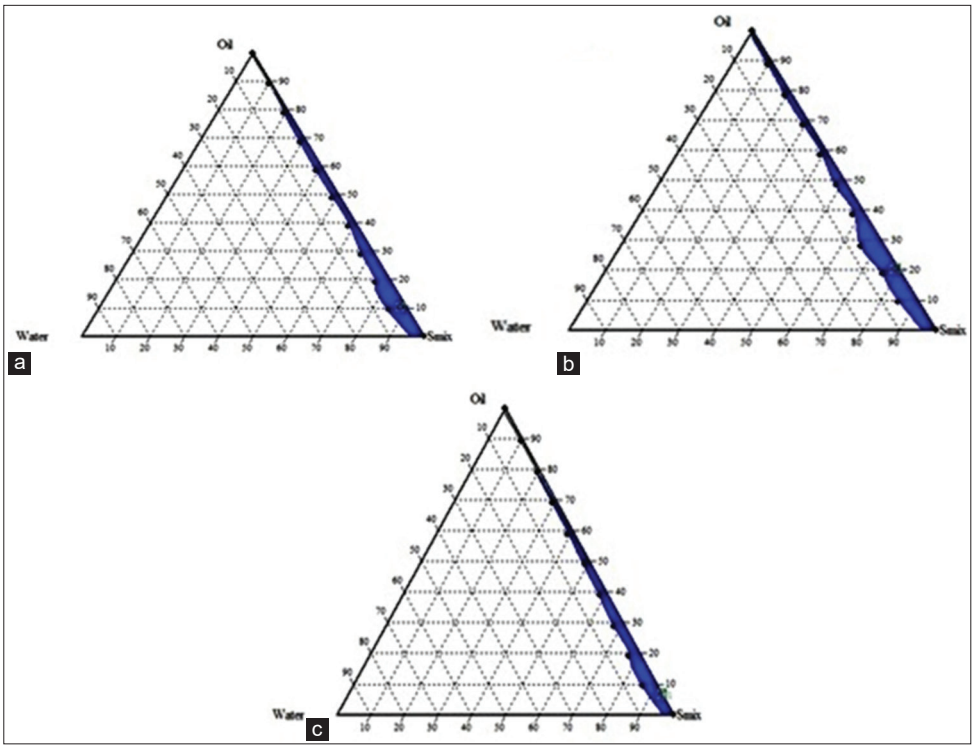

Figure 4: Pseudoternary phase diagrams involving Labrafil M 1944 (oil), Acrysol EL 135 + Lauroglycol (S_{mix}) and water. Ratio of surfactant to co-surfactant in (a) is 1:1, in (b) is 2:1 and in (c) is 1:2.

as compared to that of 1:2. Hence, for the formulation of SMEDDS, the ratio of surfactant/co-surfactant was kept at 1:1 and 2:1 and even higher to arrive at optimized composition.

Preparation of Liquid SMEDDS

14 batches of liquid SMEDDS were prepared as per the composition depicted in Table 3 which was then characterized for various parameters as shown in the same Table 3.

Characterization of SMEDDS

Visual assessment of self-emulsification

The SMEDDS formulation should have the ability to disperse rapidly on being administered orally to form a fine emulsion (micro/nano) with the aid of GI fluid. This self-emulsification process should occur within seconds paving the way for rapid absorption of the drug. The liquid SMEDDS formulations (F1-F14) in the present case were subjected to assessment of self-emulsification ability visually in terms of time required for self-emulsification as well as in terms of quality of the resulting emulsion being formed. It was observed from the data that all the formulations containing 20% and 30% of the oil phase had the ability to emulsify rapidly within less than 1 min forming clear, transparent bluish emulsions which can be categorized as Grade A emulsions. Increase in the proportion of the oil content to 40% resulted in slower self-emulsification time (>1 min) as well as reduced clarity of the dispersions being formed (Grade c). This may be attributed to increased interfacial tension between oil and aqueous phase due to insufficient concentration of surfactant system.

Effect of pH and robustness to dilution

It is desirable with regards to SMEDDS formulations that they should have not only the ability to emulsify rapidly but also should be able to form stable emulsions at different dilutions. The formulations are expected to undergo gradual dilution in contact with the GI fluid, and the process should not lead to precipitation of the drug. Hence, robustness to dilution was monitored by diluting the SMEDDS 50, 100, 250, and 1000 times with distilled water and with pH 1.2 and pH 6.8 buffer. It was found that the liquid SMEDDS formulations remained stable at different dilutions indicating the possibility of uniform release of the drug after its oral administration. Even after 24 h, neither precipitation of the drug nor any phase separation was observed when the SMEDDS were diluted up to 1000 times, showing the stability of the reconstituted emulsion.

% Transmittance and droplet size analysis

The liquid SMEDDS were characterized for % transmittance, average droplet size, and PDI. The droplet size of the emulsion is a crucial factor in self-emulsification performance because

Table 3: Formulation and characterization of SMEDDS

Batch	X	Y	Z	SET	%TM	Avg. size	PDI
F1	20	80	0	40±5	80.4±0.81	202.2±1.3	0.25±0.018
F2	20	70	10	39±1	96.7±0.83	137±2.0	0.287±0.014
F3	20	60	20	46.3±3.2	89.9±0.1	174±4.5	0.293±0.015
F4	20	50	30	47.3±2.5	85.6±0.46	191±3.6	0.39±0.066
F5	20	40	40	40.6±4.9	74.2±2.07	226±2.0	0.239±0.021
F6	30	70	0	38.3±3.5	73.2±1.3	224.2±2.6	0.238±0.100
F7	30	60	10	47±2.6	98±0.1	65.37±1.6	0.36±0.018
F8	30	50	20	31.6±2.8	95±0.52	58.5±1.3	0.296±0.012
F9	30	40	30	45±5	90.5±0.26	115.5±1.6	0.38±0.014
F10	30	30	40	41.6±3.5	85.6±0.67	188.2±1.6	0.296±0.200
F11	40	60	0	128.3±7.6	72.3±2.45	355.1±3.2	0.33±0.016
F12	40	50	10	140±10	78.1±1.53	283.4±2.2	0.227±0.012
F13	40	40	20	171.6±7.6	74.4±1.13	243±1.8	0.23±0.014
F14	40	30	30	181.6±10.4	62.9±1.47	372.6±2.1	0.296±0.016

X: % oil, Y: % surfactant, Z: % co-surfactant, SET: Self-emulsification time in seconds, %TM: % Transmittance. *Data expressed as mean±SD (*n*=3). SD: Standard deviation, SMEDDS: Self-microemulsifying drug delivery system

it determines the rate and extent of drug release as well as absorption.[27] Smaller droplet size presents a large surface area for drug absorption. Here in the present investigations, it was revealed that the formulations containing 20% of the oil phase (F1-F5) had % transmittance greater than 80% with the corresponding droplet size of formulations F2, F3, and F4 less than 200 nm and for formulation F1 and F5 it was determined to be less than 250 nm. The % transmittance of formulations containing 30% of the oil phase were greater than 85%, and the corresponding droplet size of the formulation F7, F8, and F9 were found to be less than 150 nm while for formulations F6 and F10 the average droplet size were found to be less than 250 nm. With the increase in the proportion of co-surfactant, the droplet size of the formulations were found to increase with both 20% and 30% of the oil phase, however, the formulations containing 30% of the oil phase had far less droplet sizes as compared to the ones containing 20% of the oil phase suggesting that the proportion of 30% of oil and 70% surfactant mixture were optimum to generate a microemulsion. The % transmittance of the formulations containing 40% of the oil phase was assessed to be below 80% with the corresponding droplet sizes of the formulations were determined to be greater than 250 nm. The PDI of most of the formulations were found to below 0.3 indicating a homogenous distribution of the oil globules.

Cloud point, refractive index, zeta potential, and thermodynamic stability

The physicochemical parameters such as cloud point, refractive index, zeta potential, and thermodynamic stability were determined of select formulations having a droplet size in the range of less than 150 nm. The cloud point is the temperature above which the clarity of the formulation turns to cloudiness. This may happen due to phase separation in microemulsion or due to precipitation of the drug. Thus, the stability of the formulation will get affected as well as the absorption of the drug depending on the cloud point. To avoid this phenomenon, the cloud point for SMEDDS should be above body temperature (37°C). In the present case, the cloud point temperatures of select formulations determined were in the range of 76-82°C (Table 4). This indicates that the formulated SMEDDS will be able to form stable microemulsions in a biological environment without any risk of precipitation of the drug. Higher cloud point also infers stability of the formulation during its shelf life.

The refractive indices of the formulations were found to in the range 1.382-1.421 which is an indication that the selected formulations were clear and transparent.

The zeta potential of the formulations (Table 4) was measured after dilution with double-distilled water. It was found that the zeta potential of the formulations was in the range −7.67 - −10.9 mV. Predictably, these values were found to be negative due to the presence of anionic groups of fatty acids and glycols present in the oil and the surfactant. High absolute zeta potential values (above 30 mV) should preferably be achieved to make sure about the repulsion of globules due to charge which will stabilize the system against coalescence. These recommended zeta potential values are predicted based on experiments. However, a wide range of zeta potential values have been reported for stable SMEDDS in previous studies.[28]

Thermodynamic stability study was designed to identify and avoid the metastable SMEDDS formulations. In

Table 4: Physicochemical characterization of the developed liquid SMEDDS formulations

Formulations	Cloud point (°C)	Refractive index	Zeta potential (mV)
F2	76±2.52	1.421±0.012	−7.91
F7	77±3.15	1.413±0.012	−9.14
F8	80±2.78	1.398±0.013	−7.91
F9	82±3.82	1.382±0.020	−10.92

*Data expressed as mean±SD (*n*=3). SD: Standard deviation, SMEDDS: Self-microemulsifying drug delivery system

thermodynamic stability studies, formulations were subjected to different stress tests such as centrifugation and freeze-thaw test. The observations revealed that microemulsions could withstand a wide range of temperature changes and centrifugal stress without any phase separation and drug precipitation.

Drug release studies

Conventional dissolution testing of SEDDS has a limitation in mimicking its real time *in vivo* dissolution, and such a technique can only provide a measure of dispersibility of SEDDS in the dissolution medium.[29] SMEDDS after oral administration spontaneously forms O/W emulsion on contact with GI fluids under mild agitation. During this process, a part of drug (free drug) will be dissolved in GI fluids, and remaining amount of drug is entrapped in the fine emulsion droplets. The main obstacle is the difficulty to find out the free drug concentration, i.e., to differentiate drug present in the surfactant supramolecular assemblies and in aqueous solution. To assess the real drug release pattern of formulations drug dissolved in aqueous medium should be separated from emulsion associated portion of the drug.[30] For this purpose, dialysis bag method was employed to facilitate the actual drug release pattern. In this study, dialysis bag with a molecular weight cutoff of 12,000 was used, through which only the dissolved drug molecules were permeated out providing actual release pattern from SMEDDS.

During this study, dilution of liquid SMEDDS with water avoids sticking of the formulations to the dialysis membrane as reported earlier.[31] Figure 5 shows the *in vitro* drug release profiles from the SMEDDS formulations over a period of 6 h. Formulations F2, F7, F8, and F9 were subjected to *in vitro* release studies. It was revealed from the data that the highest release of the drug was obtained from formulation F8 at the end of 6 h (98.9%) followed by formulation F7 (92.3%). The drug was least released from the formulation F2 (74.6%) during the same period signifying the influence of droplet size on drug release. Similar results have been reported earlier.[32]

Based on the aforementioned physicochemical parameters, release profile and stability the optimized composition

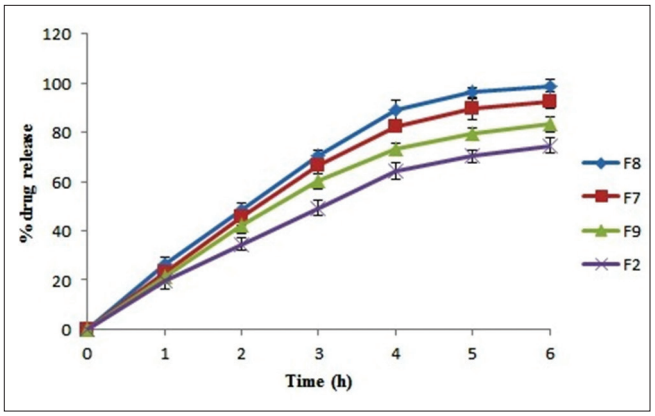

Figure 5: *In vitro* release profiles of lovastatin from self-microemulsifying drug delivery system formulations (mean ± standard deviation; *n* = 3)

of the liquid SMEDDS was selected for adsorption onto porous carriers for formulating solid-SMEDDS. Herein, the formulation F8 with the composition of 30% oil, 50% surfactant, and 20% co-surfactant was considered as the optimized one with the average droplet size of 58.5 nm and zeta potential of −7.97 mv (Figure 6).

TEM

Figure 7 portrays the electron microscopic images, depicting the morphology of the reconstituted optimized formulation, F8. As is evident from the figures, all the globules were of uniform shape, with globule size of most of them as less than 100 nm. The figure clearly illustrates that there are no signs of coalescence, indicating thereby the enhanced physical stability of the formulation.

Preparation of solid-SMEDDS

Transforming liquid SMEDDS of a poorly water-soluble drug into solid enables the development of capsules or tablets. In addition to providing, the obvious benefit of the SMEDDS, a high content of liquid SMEDDS can be loaded onto a variety of carriers which maintains good micromeritic properties and helps in the production of solid dosage forms. Various options are available for the transformation of liquid SMEDDS into solid like adsorption onto solid carriers, spray drying, freeze drying, and other techniques. The adsorption process is simple and just involves the addition of the liquid formulation onto carriers by mixing in a blender. The resulting powder may then be filled directly into capsules or alternatively, mixed with suitable excipients before compression into tablets. The adsorption process was adopted in the present study for preparing solid-SMEDDS for which the carrier chosen was Neusilin US2. Thus, the liquid SMEDDS containing LVS were adsorbed onto Neusilin US2 in 1:1, 1:2, and 2:1 proportions. Neusilin US2 is an ultra-light granular synthetic, amorphous form of magnesium alumino- metasilicate, prepared by spray

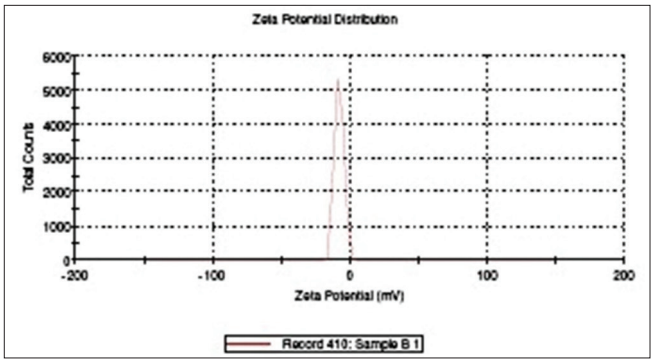

Figure 6: Zeta potential of optimized liquid self-microemulsifying drug delivery system

drying process. It has a highly porous structure with large surface area and good oil adsorbing capacity. A 1:1 proportion of Neusilin US2:liquid SMEDDS was sufficient to obtain a free-flowing powder. The appearance of the powder was such that one would have difficulty to recognize that the powder contained an equal weight of oily liquid.

Micromeritic properties of solid-SMEDDS

The micromeritic properties of solid-SMEDDS prepared with Neusilin US2 were determined to evaluate the flow properties of the powders (Table 5). It was revealed that the bulk and tap densities of powders prepared with Neusilin US2 was found to be 0.3677 ± 0.030 and 0.4186 ± 0.032, respectively. The solid-SMEDDS exhibited good flow characteristics with Carr's index between 11 and 15, Hausner's ratio less than 1.18, and angle of repose (θ) <30. Thus, it can be inferred that the prepared solid-SMEDDS with the porous carrier have the ability to be processed into the solid dosage form.

Morphological analysis

The scanning electron micrographs in Figure 8 revealed LVS as crystalline powder with rhombohedral crystals. The carrier Neusilin US2 appeared as granular, porous powders with good-flowing ability. The solid-SMEDDS prepared with Neusilin appeared as rough surfaced particles with no evidence of precipitation of the drug on the surfaces of the carriers indicating that the liquid SMEDDS was absorbed or coated inside the pores of Neusilin.

DSC

DSC allows determination of thermotropic phase transition behavior in a quantitative manner. The thermograms recorded during analysis display pronounced melting peaks. The narrow peak at 169.99°C for pure LVS (Figure 9) infers the presence of the crystalline form of the drug.

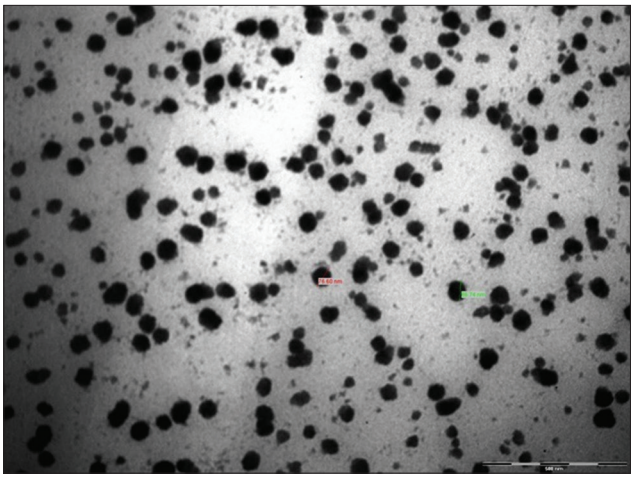

Figure 7: Transmission electron microscopy of optimized liquid self-microemulsifying drug delivery system

Table 5: Micromeritic properties of solid-SMEDDS

Properties of solid-SMEDDS	Results
Bulk density (g/cc)*	0.3677±0.030
Tap density (g/cc)*	0.4186±0.032
Carr's compressibility index*	12.4±1.2
Hausner ratio*	1.13±0.01
Angle of repose (degree)*	24.2±2
Flow rate*	<1 s

*Values expressed as mean±SD (*n*=3). SD: Standard deviation, SMEDDS: Self-microemulsifying drug delivery system

The physical mixture of LVS and Neusilin US2 displayed melting peak of the drug but with reduced intensity. The carrier did not show any prominent peak over the entire range of temperatures tested. No representative peaks for the drug were observed for solid-SMEDDS indicating the solubilization of the drug in the lipid excipients and/or possibility of transformation of the crystalline form of the drug to the amorphous one.

X-ray powder diffraction studies

The X-ray powder diffraction pattern of LVS, the physical mixture of drug and the carrier, carrier as well as that of solid-SMEDDS is shown in Figure 10. The diffraction pattern of LVS showed high-intensity peaks at 2 theta values of 9.559, 15.526, 17.6, 22.664, 25.743, and 28.417, respectively. Sharp, intense peaks may be due to the presence of the crystalline form of the drug. The diffractograms of the carrier were characterized by diffuse spectra which are typical of amorphous material. The diffractograms of solid-SMEDDS prepared with Neusilin US2 was characterized by diffuse peak suggesting that the drug was present in solubilized state in the lipid excipients and/or transformed from crystalline to amorphous form.

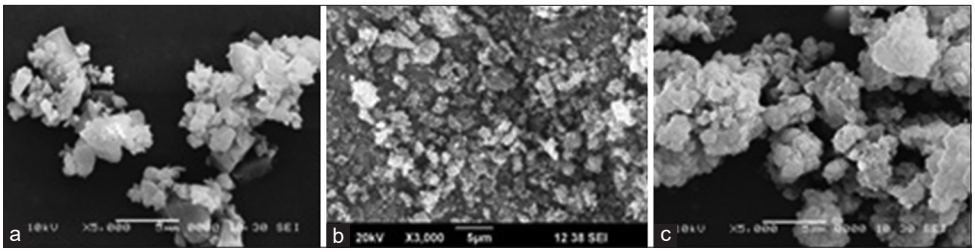

Figure 8: Scanning electron micrographs of (a) Pure drug (b) Neusilin US2 and (c) Solid self-microemulsifying drug delivery system

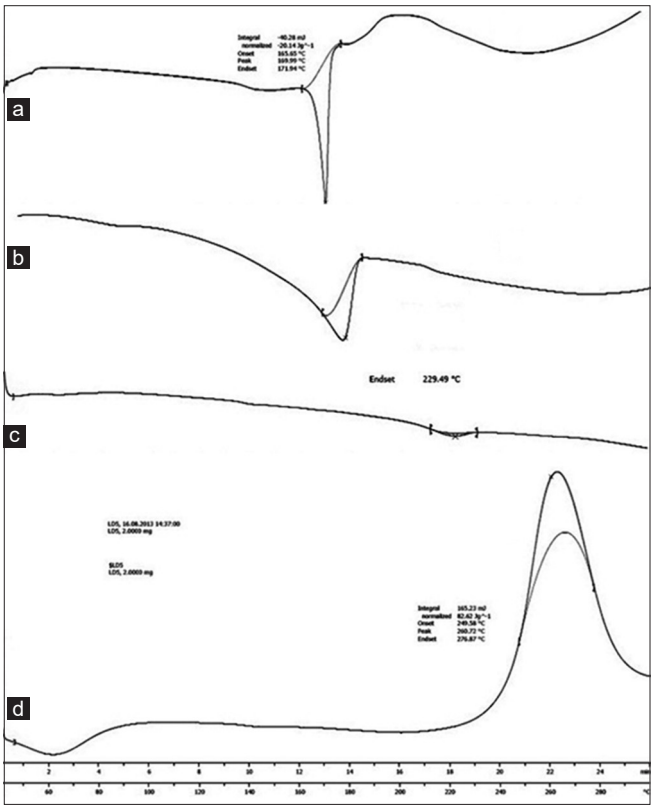

Figure 9: Differential scanning calorimetric thermograms of (a) lovastatin (b) physical mixture of lovastatin and Neusilin (c) Neusilin and (d) solid self-microemulsifying drug delivery system

Emulsion droplet size

The average droplet size of the optimized liquid SMEDDS formulation (F8) and those of solid-SMEDDS are depicted in Figure 11. The results of droplet size analysis revealed that as compared to the liquid SEDDS though the droplet sizes of solid-SMEDDS were slightly greater nevertheless they were found to below 100 nm. Thus, the solid-SMEDDS prepared with Neusilin US2 as the carrier was able to retain the droplet size of the microemulsion produced.

Drug content estimation

The drug content of liquid and solid-SMEDDS were found to 99.7 ± 0.152% and 99.2 ± 0.152%, respectively, inferring that the carrier Neusilin is an effective adsorbent in retaining the drug content in the formulation.

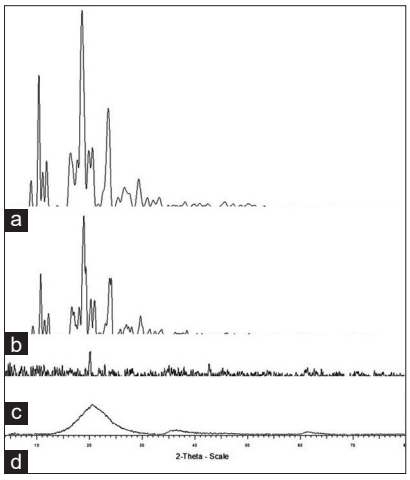

Figure 10: X-ray powder diffraction spectra of (a) Lovastatin (b) Physical mixture of drug and Neusilin (c) Neusilin and (d) Solid self-microemulsifying drug delivery system

In vitro dissolution studies

The *in vitro* dissolution studies for the optimized solid-SMEDDS were carried out in comparison to pure drug and the marketed formulation of LVS in pH 1.2 buffer. It was revealed from the data (Figure 12) that solid-SMEDDS formulations gave significant higher dissolution rates as compared to the pure drug and the marketed formulation. This goes on to prove the superiority of SMEDDS in enhancing the solubility and dissolution rate of the drug. Comparison of dissolution parameters such as DE_{15} and MDT substantiated the superiority of SMEDDS in enhancing the solubility and dissolution rate of the drug (Table 6). The faster drug release from SMEDDS is attributed due to spontaneous formation of microemulsion due to low surface free energy at the oil-water interface, which causes immediate solubilization of drug in the dissolution medium. During emulsification with water, oil, surfactant, and co-surfactant effectively swells and decreases; the globule size leads to decrease in surface area and surface free energy, thus eventually increases the drug release rate.[33]

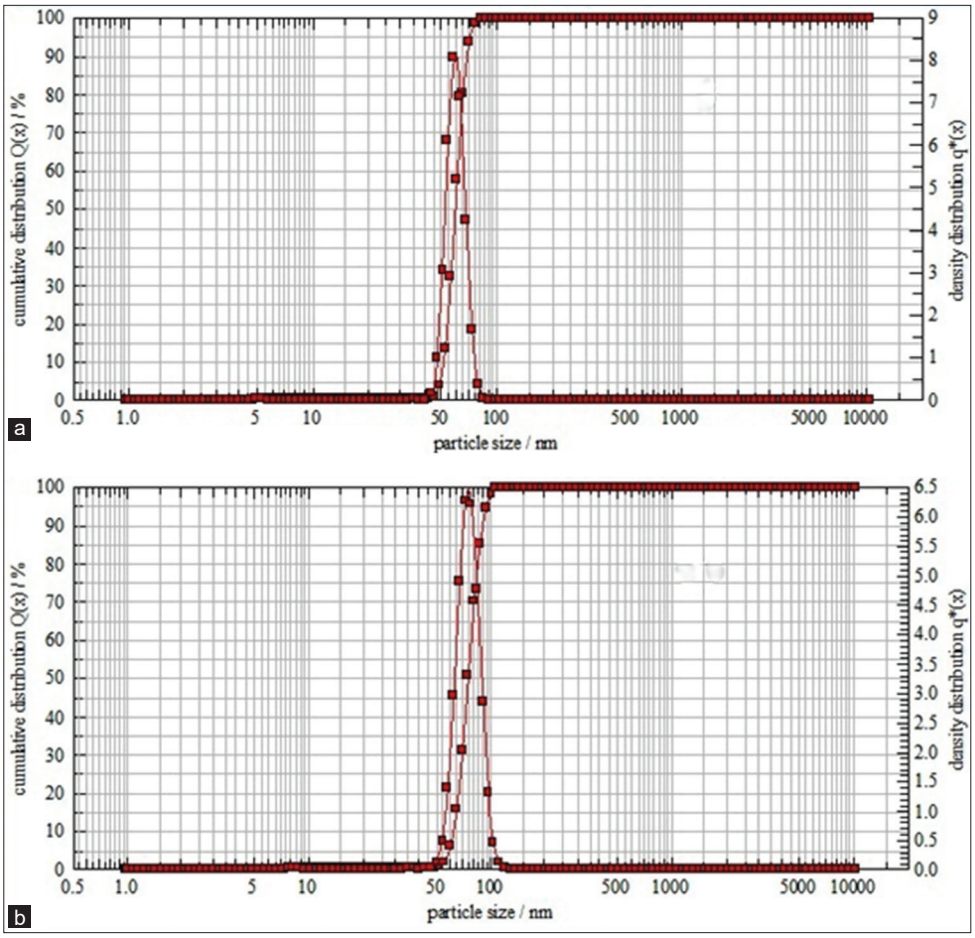

Figure 11: Droplet size distribution of (a) Optimized liquid self-microemulsifying drug delivery system (SMEDDS), F8 and (b) Solid-SMEDDS

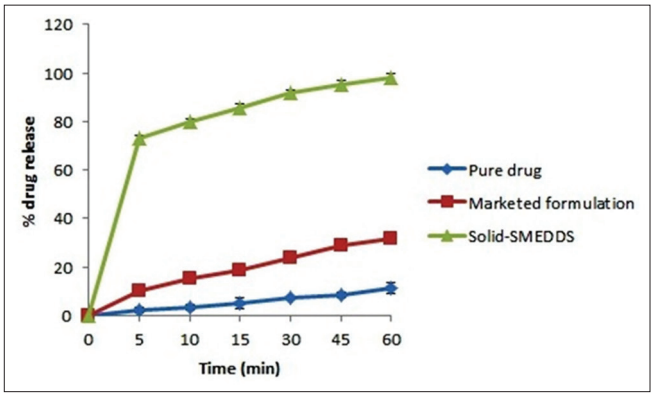

Figure 12: *In vitro* dissolution profiles of pure drug, marketed formulation and solid self-microemulsifying drug delivery system (mean ± standard deviation; *n* = 3)

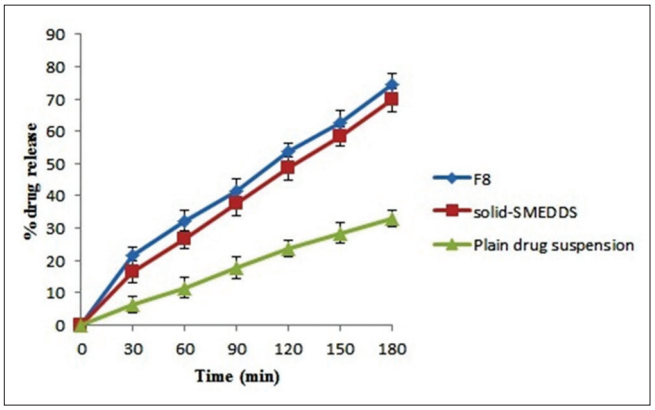

Figure 13: *In vitro* absorption profiles of plain drug suspension, optimized liquid self-microemulsifying drug delivery system (SMEDDS) and solid-SMEDDS (mean ± standard deviation; *n* = 3)

In vitro absorption profile

The release profile of the drug from the everted intestinal segment of the rat is shown in Figure 13. It was observed that the release of the drug was enhanced from SMEDDS as 74.6% ± 3.4% and 69.6% ± 3.6% of the drug was released within 180 min from optimized liquid SMEDDS formulation F8 and solid-SMEDDS, respectively. Comparatively, the drug was released only to the extent of 32.8% ± 2.6% from the plain drug suspension. Though the release of the drug from the solid-SMEDDS was slightly slower as compared to the liquid SMEDDS but the release profiles among the two were not statistically significant. Thus, it can be inferred that absorption of the drug from the solid-SMEDDS has been

Table 6: Dissolution parameters of SMEDDS formulations along with pure drug and the marketed formulation

Formulation	DE_{15}	MDT
Pure drug	2.65±0.16	7.2±0.23
Marketed formulation	11.53±1.19	5.59±0.49
Solid-SMEDDS	65.31±0.95***	3.55±0.12***

Mean±SD (n=3). ***P<0.0001 when compared with pure drug and marketed formulation using one-way analysis of variance followed by Dunnett's multiple comparison test. SMEDDS: Self-microemulsifying drug delivery system, SD: Standard deviation, MDT: Mean dissolution time, DE: Dissolution efficiency

enhanced, and inevitably solid-SMEDDS has the potential of improved oral delivery of the drug.

CONCLUSION

In the current investigations, SMEDDS of LVS was prepared and evaluated for various parameters. The optimized liquid SMEDDS, F8 was successfully transformed into free-flowing powder using Neusilin US2 without affecting the self-microemulsifying ability of the liquid SMEDDS. DSC and PXRD data of the solid self-microemulsifying powder confirmed the solubilization of the drug in the lipid excipients and or transformation of the crystalline form of the drug to amorphous one. The enhanced *in vitro* dissolution and absorption profile from the solid-SMEDDS is an indication of improvement in solubility, dissolution rate, and bioavailability of the drug.

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