INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

ENHANCEMENT OF BIOAVAILABILITY OF POORLY WATER-SOLUBLE DRUG BY SOLID SMEDDS

Monica RP. Rao*, Amruta S. Badhe, Jidnyasa Pawar, Sushant Deshpande, Omkar Kudumble
AISSMS College of Pharmacy, Pune-411001, India.

ARTICLE INFO

Article history
Received 04/05/2022
Available online
01/06/2022

Keywords
Solid SMEDDS,
Adsorbent,
Lecardipine Hcl,
AUC.

ABSTRACT

Lecardipine HCl (LCD) is a highly lipophilic dihydropyridine calcium antagonist indicated for the treatment of mild to moderate hypertension. It has poor aqueous solubility (less than 5 µg/ml) and its oral bioavailability is around 10%. The primary objectives of the present work was to develop, optimize and characterize the composition of a stable liquid and solid self-micro emulsifying drug delivery system (SMEDDS) of LCD HCl and to evaluate its oral bioavailability in rats. The LCD SMEDDS was prepared using Capmul MCM L8 (oil), Cremophor ELP (surfactant), and propylene glycol (cosurfactant). Liquid SMEDDS were converted to solid SMEDDS by adsorbing it on inert solid carrier. Flow properties were determined and solid-state characterization was done by SEM, DSC and XRPD. Optimized formulation showed complete release within 60 min. Stability study was done to check any drug precipitation or instability. HPTLC method was used to interpret *in vivo* studies of solid SMEDDS in rats. Area under curve and time were compared with those of pure drug suspended in 1% CMC solution. The area under curve and time showed significant improvement as the values obtained were 7680.19 ng h/ml and 1h for SMEDDS in comparison to 3574. 191 ng h/ml and 1.5 h for pure drug suggesting significant increased in oral bioavailability of SMEDDS of LCD HCl.

Corresponding author
Dr. Monica RP Rao
Department of Pharmaceutics,
AISSMS College of pharmacy,
Kennedy Road, Near R.T.O,
Pune, Maharashtra 411001, India.
9822916490
monicarp_6@hotmail.com

*Please cite this article in press as **Monica RP Rao** et al. Enhancement of Bioavailability of Poorly Water-Soluble Drug by Solid Smedds. Indo American Journal of Pharmaceutical Research.2022:12(05).*

INTRODUCTION

Lecardipine HCl is a new generation dihydropyridine calcium antagonist indicated for the treatment of mild to moderate hypertension. It is highly lipophilic and has a low solubility in gastrointestinal fluids and also it undergoes extensive first pass metabolism in the liver, which leads to the low absolute oral bioavailability (approximately 10%) [1]. SMEDDS is a novel approach to improve water solubility and ultimate bioavailability of drugs for which dissolution is a rate-limiting step. The self-emulsifying formulations have the ability to present the drug to GIT in 1 - 100 nm globule size and subsequent increase in specific area enables more efficient drug transport through the intestinal aqueous boundary layer leading to improvement in bioavailability. Self-micro emulsifying drug delivery systems (SMEDDS) are physically stable, isotropic mixtures of oil, surfactant, cosurfactant and solubilized drug substance that are suitable for oral delivery in capsules which on dilution with aqueous phases forms fine oil-in-water (o/w) microemulsions under gentle agitation (the digestive motility of the stomach and intestine provide the agitation required for self-emulsification in the lumen of the gut).This spontaneous formation of an emulsion in the gastrointestinal tract presents the drug in a solubilized form with smaller globule size. A droplet size less than 50 nm (SMEDDS) provides a large interfacial surface area for drug absorption. Additionally, the specific components of SMEDDS promote the intestinal lymphatic transport of drugs which are very useful in reducing the first pass effect of the drugs thus improving its bioavailability [2,3].

SMEDDS formulations are either filled in soft gelatin or hard gelatin capsules. With soft gelatin capsules they generally show incompatibility. Low molecular weight polar molecules present in capsule formulations are able to penetrate and plasticize gelatin capsule shells, which restricts the concentration of propylene glycol and related co-solvents that can be used in capsule fills. Surfactants can also destabilize capsule shells but there are differences between soft and hard gelatin capsules. A better alternative is to adsorb the SMEDDS on various solid carriers or formulate in the solid state using different methods like spray drying, extrusion spheronization technique, adsorption technique etc. In the present study adsorbent carriers were used for preparation of solid SMEDDS [4].

The objectives of the present study were (1) to develop and evaluate a SMEDDS of LCD HCl and convert it to solid by physical adsorption using an inert solid carrier and characterization of solid state (2) to compare the pharmacokinetic profile of solid SMEDDS with pure LCD HCl.

MATERIALS AND METHODS

Materials

Lecardipine HCl was a kind gift from Sun Pharmaceuticals (Baroda, India). Capmul MCM L8, Capmul MCM, Capmul MCM C8, Capmul MCM L, Capmul 908 P, Acconon CC6, Captex-200, Miglyol 812 were gifted by Abitec Corporation, USA. Labrafil M 1944 CS, Labrasol, Lauroglycol 90, Capryol 90, were gifted by Colorcon Mumbai Private Ltd, Mumbai, India. Tween-20, Tween-60, Tween-80, Polyethylene glycol (PEG)-400, Propylene glycol were purchased from S.D.Fine Chemicals, Mumbai, India. Cremophor ELP was gifted by BASF Corporation and Neusilin US2 was a gift from Gangwal Chemicals Pvt. Ltd. (Mumbai, India).

Methods

Solubility studies

Saturation solubility was determined using shake flask method in various oils, surfactants and cosurfactants as follow: 1 g of each of the selected vehicles was added to each vial containing an excess of drug (500mg). The mixture was heated in a water bath at 40ºC to facilitate the solubilization. Mixing of the systems was performed using a cyclo-mixer. Formed suspensions were then shaken in mechanical shaker at 25ºC for 48 h to attain equilibrium. Samples were then centrifuged at 3000 rpm for 20 min and the aliquots of supernatant were analyzed at 236 nm after proper dilution with methanol using UV-visible double beam spectrophotometer (Jasco V-730)

Pseudo Ternary Phase Diagram

The pseudo-ternary phase diagrams were constructed using water titration method. The coordinates of the ternary phase diagram represent three components of microemulsion system i.e. Oil phase, Smix (Surfactant-Cosurfactant) and water. At desired ratio of surfactant to co-surfactant value (1:1, 2:1, 3:1, 4:1 and 1:2) i.e Smix, Smix and oil were mixed at ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 in pre-weighed vial. To the resultant mixtures, deionized water was added dropwise till turbidity appears and the weight of vial was noted. The resultant mixtures were checked visually for phase clarity and flow ability. After identifying highest microemulsion region at desired Smix value, randomly different formulations were selected to prepare liquid SMEDDS. Phase diagram was constructed by using Chemix software, CHEMIX School Ver. 3.50 software (MN, USA, Trial version).

Preparation of liquid SMEDDS formulations

From the solubility study and comparison of ternary phase diagrams, SMEDDS component were selected for drug incorporation and further series of SMEDDS formulations were prepared using Cremophor ELP and propylene glycol as the surfactant and cosurfactant (Smix) combination and Capmul MCM L8 as the oil. LCD HCl SMEDDS were prepared by incorporating 10 mg drug into mixture of accurately weighed quantity of Smix and oil in glass vial. Components were mixed and heated (40–50 °C) to form a homogenous mixture and stored at room temperature.

Macroscopic evaluation

Microemulsions were observed for homogeneity or any change in color and transparency or phase separation during normal storage condition (37±2 °C).

Transmission test for liquid SMEDDS

Stability of optimized SMEDDS formulation on dilution was checked by measuring transmittance through UV-visible double beam spectrophotometer (Jasco V-730). Transmittance of samples was measured at 533 nm and for each sample three replicate measurements were performed. In this study 100 mg of formulations were diluted to 100 ml with 0.1 N HCl, phosphate buffer pH 6.8 and distilled water.

Globule size and zeta potential determination of liquid SMEDDS

SMEDDS formulations (500 mg) were diluted with 250 ml deionized water in a beaker with constant stirring on a magnetic stirrer. The average droplet size, polydispersity index and zeta potential of micro emulsion were assessed by dynamic light scattering (DLS) technique using Malvern zeta sizer at 25 ºC (Nano-ZS, Malvern Instruments, UK).

Freeze-thaw cycle

SMEDDS of LCD HCl was kept for physical stability under the following conditions: refrigeration and ambient room temperature. The stability studies were followed for 12 weeks. The stored samples were evaluated every 4 weeks for appearance, color, drug content and Percent transmission. For % transmission study, 100 mg of SMEDDS formulation was diluted to 100 ml distilled water and Percent transmission was taken at 533 nm in UV-visible double beam spectrophotometer.

Centrifugation test

In order to estimate stability of systems, 100mg of SMEDDS formulation was diluted with 100 ml distilled water. The formed microemulsion was centrifuged (Remi Laboratories, Mumbai, India) at 10,000 rpm for 30 min and observed for any change in homogeneity of microemulsions.

***In vitro* release of liquid SMEDDS**

For *in vitro* release studies, SMEDDS containing 10 mg LCD HCl was filled in HPMC capsule. The dissolution studies were performed using USP dissolution apparatus-II at a rate of 50 rpm. Dissolution studies were performed separately using 500 ml of 0.1 N HCl and phosphate buffer (pH 6.8). No enzymes were added to the media. The temperature was set at 37 °C ±0.5 °C. Samples of 5 ml of the media were collected and replaced with equal volume of fresh media. The samples were analyzed using UV method (Jasco V-730).

Preparation Of solid SMEDDS

The liquid SMEDDS was added dropwise over the solid adsorbent contained in a mortar. Various solid carriers namely dibasic calcium phosphate, anhydrous lactose, microcrystalline cellulose, calcium carbonate, magnesium carbonate, Aerosil 200 and Neusilin US2 were used in various ratios (1:1, 1:2 and 1:3). After each addition, the mixture was homogenized using glass rod to ensure uniform distribution of the droplet. The adsorbent that was required in a small amount to give a free flowing solid SMEDDS was selected for the further studies [5].

Dilution study by visual observation

Dilution study was done to study the effect of dilution on phase stability of SMEDDS, because dilution may better mimic the condition of stomach after oral administration. In this study, 100 mg of solid SMEDDS and liquid SMEDDS were introduced into 100 ml of demineralized water in a glass beaker that was maintained at 37 ºC and the contents mixed gently using a magnetic stirrer (Harco & Co). The tendency to emulsify spontaneously and progress of emulsion droplets were observed with respect to time. The emulsification ability of SMEDDS was judged qualitatively “good” when clear microemulsion formed and “bad” when there was turbid or milky white emulsion formed after stopping of stirring [6]. Percent transmittance of the formed microemulsion was measured at 533 nm by UV double beam spectrometer (Jasco V-730 using demineralized as blank.

Globule size and zeta potential determination for solid SMEDDS

SMEDDS formulations (500 mg) were diluted with 250 ml deionized water in a beaker with constant stirring on a magnetic stirrer. The average droplet size, polydispersity index and zeta potential of microemulsion droplets from SMEDDS were assessed by dynamic light scattering technique (DLS) using Malvern zeta-sizer at 25 ºC (Nano-ZS, Malvern Instruments, UK).

Determination of Flow Properties of solid SMEDDS

Flow properties of solid SMEDDS were done by finding Angle of repose, Carr’s Compressibility Index (CI) and Hausner’s ratio.

Characterization of solid SMEDDS

Differential Scanning Calorimetry (DSC)

The DSC thermogram of LCD HCl was recorded using Differential scanning calorimeter (DSC 823 Mettler Toledo, Japan). Approximately 2 to 5 mg of sample was heated in a closed pierced aluminum pan from 30 °C to 180 °C at a heating rate of 5°C/min under a stream of nitrogen at a flow rate of 50 ml/min.

Powder X- ray Diffraction (XRPD)

Powder X- ray diffraction pattern of LCD HCl and solid SMEDDS was investigated using powder X-ray diffractometer (PW 1729 X-ray Generator, Philips, Netherlands). The X-rays were Ni filtered CuKα1 radiation with 40 kV and 30mA over 0-100˚/2θ.

Scanning Electron Microscopy (SEM)

The surface morphology of samples was determined using analytical scanning electron microscope (JSM-6360A, JEOL, Tokyo, Japan). The samples were lightly sprinkled on a double adhesive tape stuck to an aluminum stub. The stubs were then coated with platinum to a thickness of about 10 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. Afterwards, the stub containing the coated samples was placed in the scanning electron microscope chamber.

Drug content determination for solid SMEDDS

Samples (100 mg) were dissolved in 10ml methanol and stirred by vortex mixing. The solutions were filtered using 0.45 µm membrane filters. The content was estimated by UV method**.**

***In vitro* dissolution studies of solid SMEDDS**

Solid SMEDDS equivalent to 10 mg of LCD HCl was filled in HPMC capsule. The dissolution studies were performed using USP dissolution apparatus-II at a rate of 50 rpm. Medium used were 500 ml of 0.1 N HCl and phosphate buffer (pH 6.8). No enzymes were added to the media. The temperature was set at 37 °C±0.5 °C. Samples of 5 ml of the media were collected and replaced with equal volume of fresh media. The samples were analyzed using UV method at λ_{max} of 236nm.

Stability studies for solid SMEDDS

Chemical and physical stability of solid SMEDDS was assessed at 40±2 °C/75±5% RH and room temperature as per ICH Guidelines. SMEDDS equivalent to 10 mg LCD HCl was filled in HPMC capsule 0 size packed in aluminum strips and stored for 3 months. Samples were analyzed drug content, mean globule size and % transmittance.

***Invivo* studies of solid SMEDDS**

Pharmacokinetic studies

Approval to carry out in-vivo study was obtained from Institutional Animal Ethics Committee, AISSMS College of Pharmacy and their guidelines were followed for the studies. The approval no. was CPCSEA/IAEC/PT-01/05-K11. The animals used for in vivo experiments were male rats (250-300 gm). Rats were divided into two groups for the study containing six rats in each group. The rats were fasted for 12 h before drug administration but were allowed free access to water. Each group animal received one of the following dosage forms: the solid SMEDDS and pure Lercanidipine HCl, corresponding to human dose (10mg)**.** Bioavailability of LCD HCl in SMEDDS was compared with LCD HCl suspension. LCD HCl suspension was prepared by mixing LCD HCl powder with 1% (w/v) carboxy methylcellulose (CMC) and diluted to a definite volume to yield LCD HCL of 2 mg/ml concentration [7]. Solid SMEDDS (2 mg/kg of LCD) and pure LCD HCL were administered by oral route using oral feeding needle no 18. The rats were anesthetized using ether and the blood samples were withdrawn from retro-orbital vein. The time interval was 0.5, 1, 1.5, 2, 4, and 6 h. Plasma was separated by centrifuging blood samples at 3000 rpm for 15 min. Plasma samples were stored at -5 °C until further analysis. Analysis was done by HPTLC. (Camag Linomat V, Camag, Switzerland.)

Instrumentation of HPTLC

The samples were spotted on Merck TLC aluminium plates, precoated with silica gel 60F 254 (10 cm x 10 cm with 250 µm layer thickness) using a Camag Linomat V applicator (Camag, Switzerland). The samples were applied onto the plates in the form of bands of 6 mm width with a Camag 100 µl sample syringe (Camag, Switzerland) under a nitrogen. Linear ascending development was carried out in a twin trough glass chamber (10cm x 10 cm). Densitometric scanning of the developed plates was performed using Camag TLC scanner 3, operated with win CATS software (Version 1.4.3 Camag) in the absorbance mode at 236 nm. Scanning speed was kept at 100 mm/s.

Pharmacokinetic sampling

Plasma samples (0.1 ml) were then transferred to labeled glass tubes. To each tube 1 ml of acetonitrile was added as a protein precipitating agent and the contents were vortexed for 3 minutes. The contents were further centrifuged for 5 minutes at 2500 rpm and 50 µl supernatant of each was then applied on TLC plate. The densitometric scanning at wavelength 236 nm was done and the concentration of LCD HCl in each sample was calculated using the areas obtained by densitometric scanning. Area under curve was calculated using the trapezoidal rule –

$$AUC_t = \frac{(T_2 - T_1)(C_1 + C}{2} \qquad \text{Equation 1}$$

The area under the drug concentration–time curve from zero to 6 h (AUC0–6h) was calculated using the trapezoidal rule. The maximal plasma concentration of drug (Cmax) and the time to reach maximum plasma concentration (Tmax) were directly obtained from plasma data. The relative bioavailability (BA) of LCD HCl solid SMEDDS formulations to the LCD HCl suspension was calculated using the following equation:

$$\text{Relative BA}\,(\%) = \left[\frac{(AUC_{test}/AUC_{reference})}{(Dose_{reference}/Dose_{test})}\right] \times 100 \qquad \text{……Equation 2}$$

Pharmacodynamic Study:

After recording the initial Blood Pressure (BP) of rats, the animals were divided into 3 groups with 6 animals each in which one group was taken as control and the other two groups were for test. Hypertension was induced in the test groups by subcutaneous injection of methyl prednisolone acetate (20 mg/kg/wk) for 2 weeks. Mean arterial blood pressure was measured in conscious rats using Non-Invasive Blood Pressure Recorder by Tail–Cuff method. One of the test groups received pure drug (2 mg/kg) after suspending in 1% carboxymethyl cellulose (CMC) solution and other test group received solid SMEDDS (2mg/kg). The mean arterial blood pressure was recorded after 0.5, 1, 1.5 and 2 h administration of the formulation and compared with control.

***Ex vivo* intestinal permeability studies:**

A small part of the male albino rat's small intestine was isolated and used in an in vitro diffusion study to investigate intraduodenal permeability. The microemulsion sample was diluted with 1 mL distilled water and a tablet suspension was made in distilled water for the tablet sample. The tissue was thoroughly washed with cold Ringer's solution, and the resulting sample (1 mg/mL) was injected into the tissue lumen using a syringe, with the two sides of the intestine tightly closed. The tissue was placed in an organ bath with continuous aeration at 37°C, with 30 mL of phosphate buffer in the receiver compartment (pH 6.8). The absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 236 nm, keeping the respective blank. The percent diffusion of drug was calculated against time and plotted on a graph.

RESULTS AND DISCUSSIONS

Solubility studies

The results of solubility of LCD HCl in oils were shown in Figure 1 and solubility of LCD HCl in surfactants and cosurfactants were shown in Figure 2. Solubility studies showed that LCD HCl was having maximum solubility in following components: Oils- Capmul MCM L8 (51.54 mg/ml), Surfactants- Cremophor ELP (169.12 mg/ml), co-surfactants- Propylene glycol (77.24 mg/ml). Capmul MCM L8 is chemically monoglycerides of caprylic acid (unsaturated fatty acids are used for improving bioavailability) which contains caprylic acid about 97% and capric acid 3%. Capmul MCM L8 was selected as oily phases for further study due to its maximum solubilization of the drug LCD HCl and relative ease of self-micro emulsification. Cremophor ELP showed maximum solubilization of drug. Cremophor ELP is PEG-35 castor oil formed from ethoxylation (etherification) of ricinoleic acid (present in glyceride) of castor oil. This reaction made castor oil hydrophilic. The amphiphilic nature, its ability to dissolve large quantity of drugs and good self –emulsification property, Cremophor ELP was selected as a surfactant. Addition of co-solvent with surfactant was reported to improve dispersibility. Cosurfactants also reduce the quantity of surfactant in SMEDDS formulation and thus make the formulation more acceptable. Polyalcohol esters of fatty acids are newer oil derivatives that possess co-surfactant properties because of its amphiphilic nature. Maximum solubility of drug was found in Propylene glycol. Hence Propylene glycol was selected as cosurfactant/cosolvent.

Pseudo Ternary Phase Diagrams

The Figures 3-7 show ternary phase diagrams of Cremophor ELP-Propylene Glycol (Smix) and as Capmul MCM L8 oil phase. Selection of oil and surfactant, and the mixing ratio of oil to Surfactant: Co-surfactant mixture (Smix), play an important role in the formation of the microemulsion. **V**ery minute difference was seen in the region of microemulsion for different Smix ratios. Amongst five ternary phase diagram with five Smix ratios, microemulsion region for Smix (1:1) was found to be marginally more than Smix ratio of 1:2 and relatively larger than rest all other ratios of Smix. For better stability and emulsification, Smix (1:1) was chosen. As the concentration of surfactant with respect to co-surfactant increases microemulsion region decreases. Thus, in the phase diagrams, it can be seen that the free energy of microemulsion formation can be considered to depend on the extent to which the surfactant lowers the surface tension of the oil-water interface and the change in dispersion entropy. Thus, a negative free energy of formation is achieved when a large reduction in surface tension is accompanied by significant favorable entropic changes. In such cases, microemulsion formation is spontaneous and the resulting dispersions are thermodynamically stable.[8-10] The surfactant or Smix, which are able to increase the dispersion entropy, reduce the interfacial tension, increase the interfacial area, and thus lower the free energy of the system to a very low value with the minimum concentration (weight ratio), which is thermodynamically stable.

Selection of SMEDDS component

Based on the solubility studies and pseudo ternary phase diagrams, Cremophor ELP as the surfactant, Propylene glycol as the cosurfactant and Capmul MCM L8 as the oil phase were selected. Surfactant-cosurfactant ratio i.e Smix (1:1) was fixed. Six formulations were prepared using this composition and is given in Table 1.

Macroscopic evaluation

All Formulations appeared homogenous and clear. There was no phase separation observed during normal storage (25°C±2°C) condition.

Transmission test for liquid SMEDDS

Transmittance of samples was measured at 533 nm and for each sample three replicate studies were performed as shown in Table 2. There was decrease in percent transmittance with increase in oil concentration. Among all the formulations the Percent transmittance was above 95% that indicate the droplet size in nm range and formation of transparent microemulsion. The data shows that Percent transmission increases with increase in Smix concentration and decreases in oil concentration.

Globule size and zeta potential determination of liquid SMEDDS

Globule size distribution, polydispersity index and zeta-potential are shown in Table 3. The average globule size of microemulsion dispersed from the LCD HCl SMEDDS of formulation F1-F6 was between 12.51 to 14.99 nm. The zeta-potential ranged from 9.67 to 10.56 mV for all the formulations. The polydispersity index was found to be between 0.023 to 0.133 for formulations F1-F6. The average globule size of microemulsion dispersed from the LCD HCl SMEDDS of formulation F1-F6 was between 12 to 16nm. The increase of globule size is due to decrease of surfactant content and increase in oil. Capmul MCM L8 is used as oil in just 10-20% range. Generally, an increase of electrostatic repulsive forces between microemulsion droplets prevents the coalescence of microemulsion droplets. On the contrary, a decrease of electrostatic repulsive forces will cause phase separation. As all the excipients used were nonionic in nature, low zeta potential values could be attributed to the drug molecules having ionizable group $–OCH_3$, $-NO_2$ and –NH. [11,12] In study we get the zeta-potential of formulation around 10 mV for all the formulations. SMEDDS is isotropic mixture of oil, surfactant and co-solvent. The water is absent in SMEDDS that leads to no aggregation of globules. Zeta-potential come in picture when the microemulsion formation in-vivo.

Selection of Optimum Composition:

Optimized composition was selected based on highest % transmittance, higher amount of oil than surfactant, lower mean droplet size and polydispersity index. Accordingly, formulation F6 was selected for further experiments and its components are shown in Table 4.

Freeze-thaw cycle

The formulation F6 did not showed any phase separation, flocculation or precipitation. There was no change in color, transparency and phase separation of the formulation at both the temperatures under study.

Centrifugation stability of Liquid SMEDDS

The formulation F6 showed no phase separation when subjected to centrifugation at 5000 rpm for 30 min.

***In vitro* release of Liquid SMEDDS**

Figure 8 displays the dissolution profile of formulations F1-F6 in 0.1 N HCl. Within 20 minutes 87.27 % release was obtained for F6 and complete release within 1 h. All other formulations were also showing more than 95 % release in 1 h. Figure 9 shows dissolution profile of formulation F1-F6 in buffer (pH 6.8). Within 20 minutes 84.53 % release was obtained for F6 while 98.26% within 1 h. It could be inferred that the SMEDDS formulation resulted in spontaneous formation of a microemulsion with a small droplet size, which permitted a faster rate of drug release into the aqueous phase. Thus, greater availability of dissolved LCD HCl from the SMEDDS could lead to higher absorption and higher oral bioavailability.

Preparation of solid SMEDDS

Adsorption of Liquid SMEDDS (F6) on Neusilin US2 in ratio of 1: 3.3 (NeusilinUS2: liquid SMEDDS) gave free flowing powder.

Reconstitution properties of solid SMEDDS

Dilution study by visual observation

A visual test was carried out to assess self emulsification of solid SMEDDS in 100 ml deionized water at 37 ºC under gentle agitation. The solid SMEDDS formulation showed spontaneous micro emulsification (< 1 min) as same as liquid SMEDDS and there was no sign of phase separation or phase inversion of microemulsion.

Globule size and zeta potential determination

The mean droplet size and polydispersity index of the reconstituted solid SMEDDS and liquid SMEDDS microemulsions are presented in Table 5. The droplet size of the microemulsion from the solid SMEDDS was slightly increased as compared to the liquid SMEDDS.

Flow Properties of solid SMEDDS

The prepared solid SMEDDS were evaluated for the powder property like bulk density, tapped density, Carr's index, Hausner's ratio and angle of repose and are given in Table 6. All the values were within the range and hence solid SMEDDS had good flow properties and can be filled in capsules.

***In vitro* drug release for solid SMEDDS**

In vitro drug release studies were performed for solid SMEDDS and pure LCD HCl, shown in Figure 10. The drug release from the solid SMEDDS in 30 min was more than 90% and the drug release from the pure LCD HCl showed 14 % which was significantly less as compared to solid SMEDDS. However, there was no significant difference in drug release between solid SMEDDS and liquid SMEDDS because conversion to solid state was done simply by adsorption process and an inert solid carrier have been used which rarely affected release process.

Characterization of solid SMEDDS

Differential Scanning Calorimetry (DSC)

DSC curves of pure LCD HCl, Neusilin US2 and solid SMEDDS are shown in Figure 11. Pure LCD HCl showed a sharp endothermic peak at about 181.13°C due to crystalline state (curve C). Neusilin US2 did not show any peak over the entire range of the tested temperatures because of the amorphous state (curve B). There was no drug peak found in the solid SMEDDS of LCD HCl (curve A), indicating that the drug must be present in molecularly dissolved state in solid SMEDDS. [13,14]

Powder X- ray Diffraction (XRPD)

X-ray powder diffractogram for Pure drug is shown in Figure 12 and for solid SMEDDS is shown in Figure 13. The further confirmation was carried out by X-ray powder diffractograms. The pure LCD HCl shows sharp peak that indicates the drug is in crystalline state. The LCD HCl in the solid SMEDDS shows no peaks representing LCD HCl in molecularly dissolved state. [15,16]

Scanning Electron Microscopy (SEM)

The scanning electron micrographs of NeusilinUS2 and solid SMEDDS are shown in Figure 14. NeusilinUS2 appeared with a rough surface with pores. However, the solid SMEDDS appeared as smooth-surfaced. Neusilin US2 appeared with a rough surface with pores. However, the solid SMEDDS appeared as smooth-surfaced, indicating that the liquid SMEDDS is absorbed or coated inside the pores of NeusilinUS2**.**

Drug content determination

The drug content of optimized formulation F6 (solid SMEDDS) was found to be 0.989 mg/ 100mg.

Stability studies

Stability studies of solid SMEDDS are given in Table 7. Solid SMEDDS on dilution shows no precipitation of drug and % transmission was near 99% that indicates formation of the microemulsion having globule size less than 100 nm. Formation of the microemulsion with globule size less than 50 nm for solid SMEDDS indicated that solid SMEDDS of LCD HCl was physically and chemically stable.

***In vivo* studies**

Table 8 shows the data for the calibration curve of Lercanidipine HCl in plasma and the calibration curve is represented in Figure 15. The value of regression coefficient was found to be 0.997 and Rf value in plasma found to be 0.68. Table 9 shows the data of the plasma concentrations of pure LCD HCl and solid SMEDDS analyzed by HPTLC. The plasma concentration time profile of pure LCD HCl and solid SMEDDS is represented in Figure 16. The pharmacokinetic data in Table 10 showed improved bioavailability of solid SMEDDS as compared to pure LCD HCl. The pharmacokinetic data shows improved bioavailability of solid SMEDDS as compared to pure LCD HCl. The improvement of bioavailability may be due to P-gp (P-glycoprotein) inhibition, cytochrome P_{450} inhibition and surfactants which increase the permeability of the epithelial cell membrane [17-20]. Furthermore, SMEDDS, a lipid-based formulation, is considered to be partially absorbed via the lymphatic route as well. That may reduce the opportunity for hepatic first pass metabolism and therefore enhance the bioavailability of drugs. The data shows relative bioavailability of solid SMEDDS is improved 2.14fold as compared to pure LCD HCl.

Pharmacodynamic studies:

Solid SMEDDS was found to be effective in lowering blood pressure from mean initial values of 164.67 mm Hg to 129 mm Hg for up to 1.5 h. The mean initial value of blood pressure was found to be decreased from 164.33 mm Hg to 134.33 mm Hg after 1.5 h when plain drug suspension was given. There was no further decrease in blood pressure after 2 h. The results of Blood pressure measurement are given in (Table 11).

***Ex vivo* intestinal permeability studies**

Ex vivo intestinal tissue permeation studies were conducted across the small intestine of male Wistar Albino rats to better understand the properties of drug penetration. The profile of drug permeation is shown in Figure 17. The permeability of drug through intestinal membrane from the pure drug Lercanidipine HCl was found to be 14.966 % after 3.5 h, while the release of solid SMEDDS was found to be 53.54 %.

FIGURES

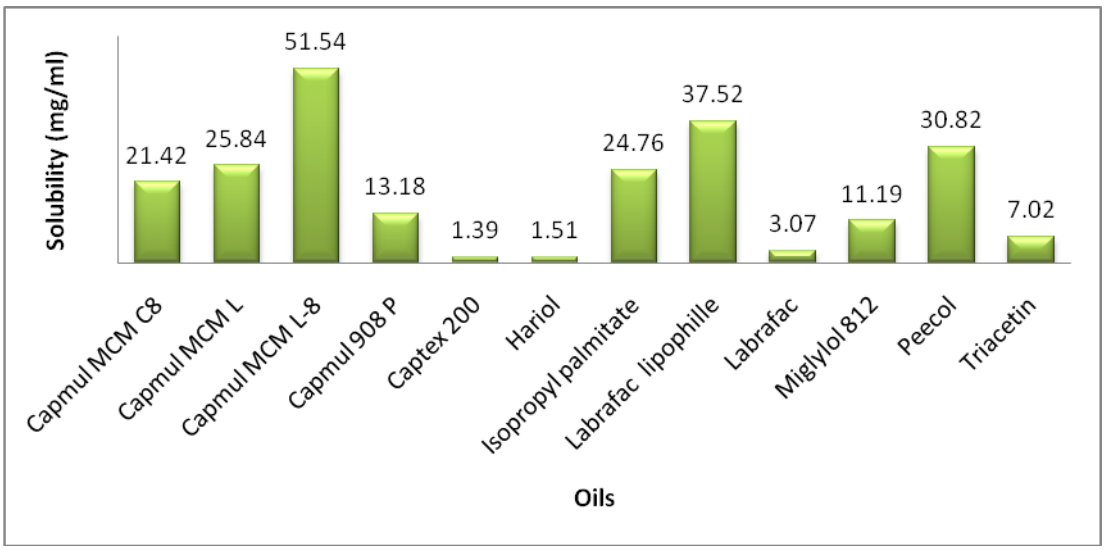

Fig 1: Solubility of LCDHCl in oils.

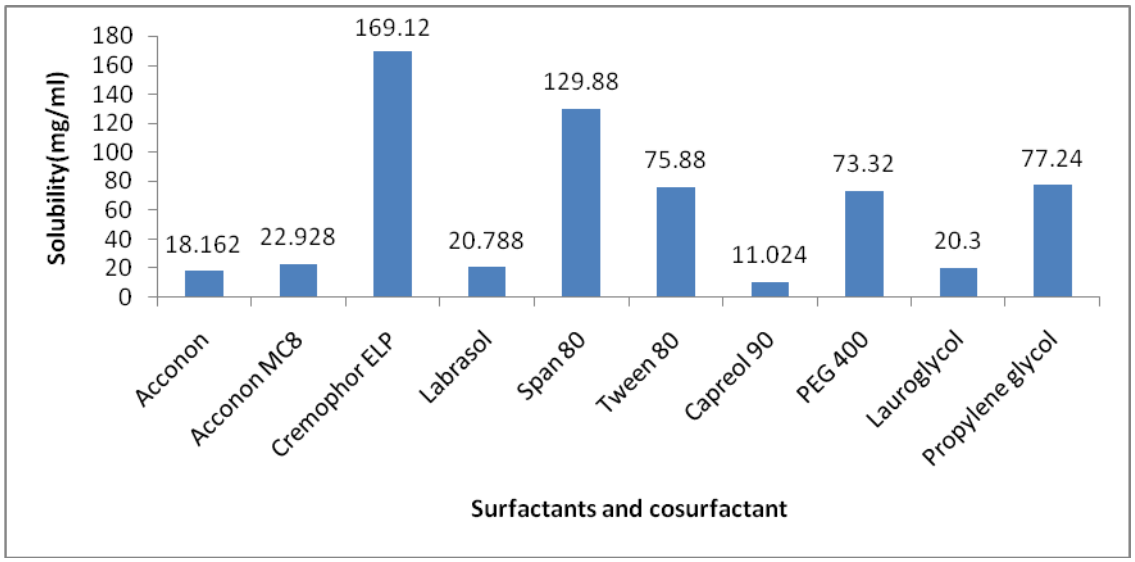

Fig 2: Solubility of LCD HCl in surfactants and co-surfactants.

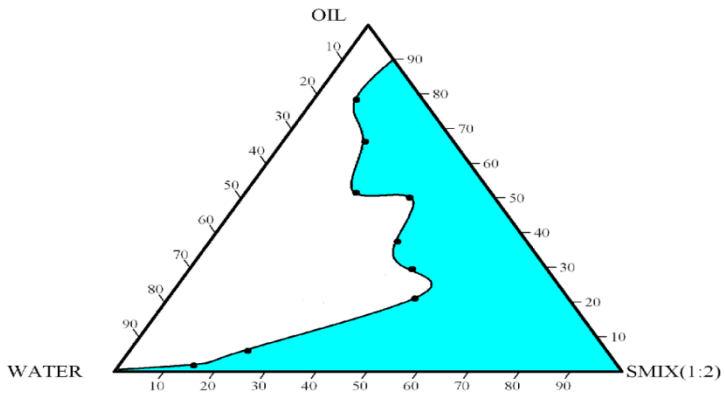

Figure 3: Pseudo-ternary phase diagram of Smix 1:2(Cremophor ELP: Propylene glycol) and oil Capmul MCM L8.

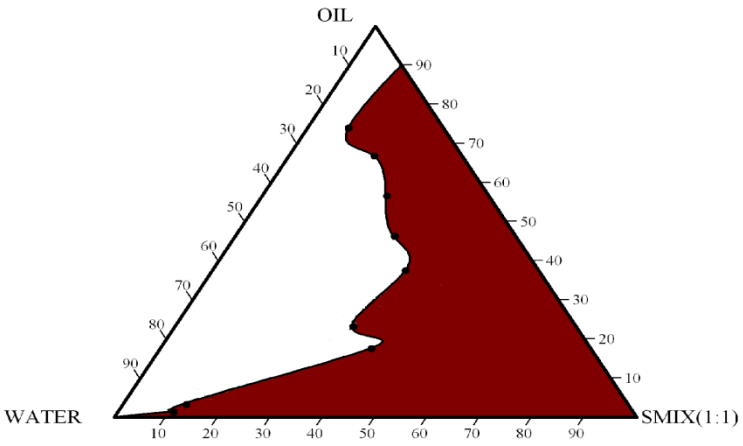

Figure 4: Pseudo-ternary phase diagram of Smix 1:1(Cremophor ELP: Propylene glycol) and oil Capmul MCM L8.

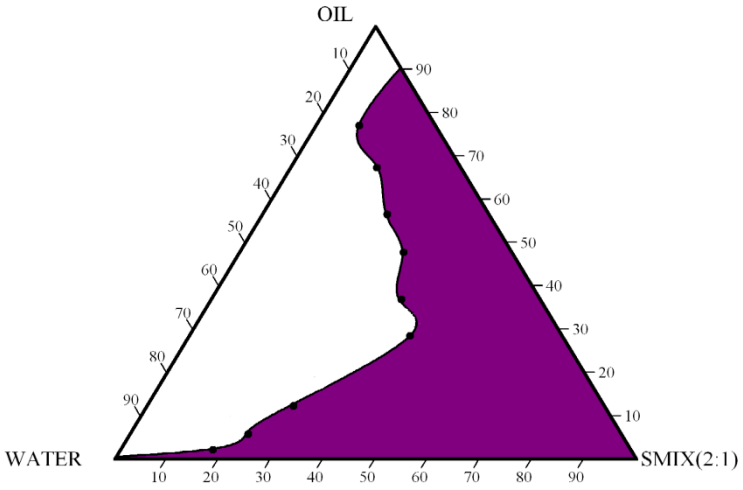

Figure 5: Pseudo-ternary phase diagram of Smix 2:1(Cremophor ELP: Propylene glycol) and oil Capmul MCM L8.

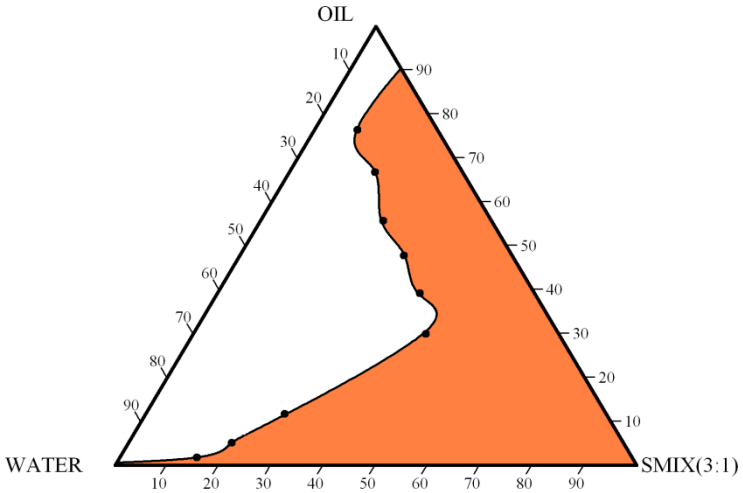

Figure 6: Pseudo-ternary phase diagram of Smix 3:1(Cremophor ELP: Propylene glycol) and oil Capmul MCM L8.

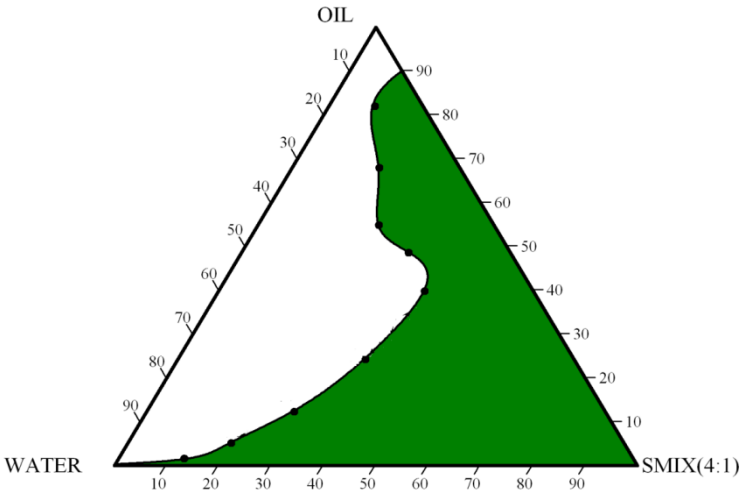

Figure 7: Pseudo-ternary phase diagram of Smix 4:1(Cremophor ELP: Propylene glycol) and oil Capmul MCM L8.

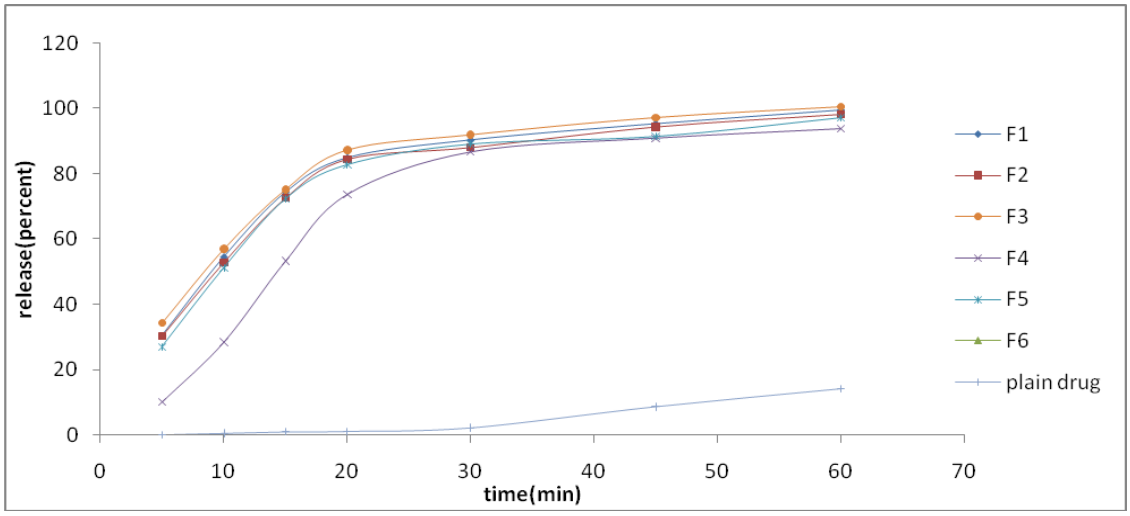

Figure 8: Dissolution profile of formulation F1-F6 in 0.1 N HCl.

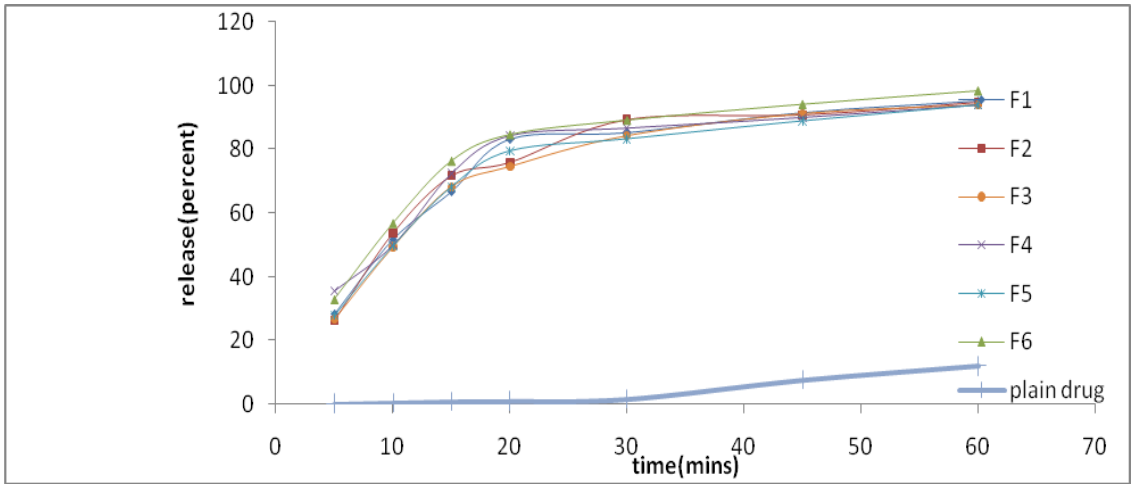

Figure 9: Dissolution profile of formulation F1-F6 in buffer (pH 6.8).

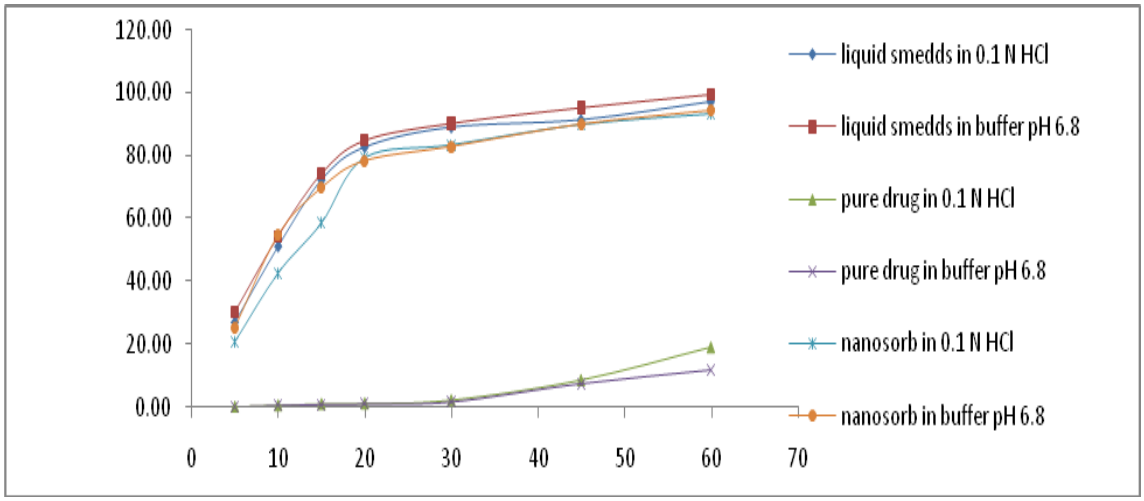

Figure 10: Dissolution profile of solid SMEDDS, liquid SMEDDS and pure LCD HCl.

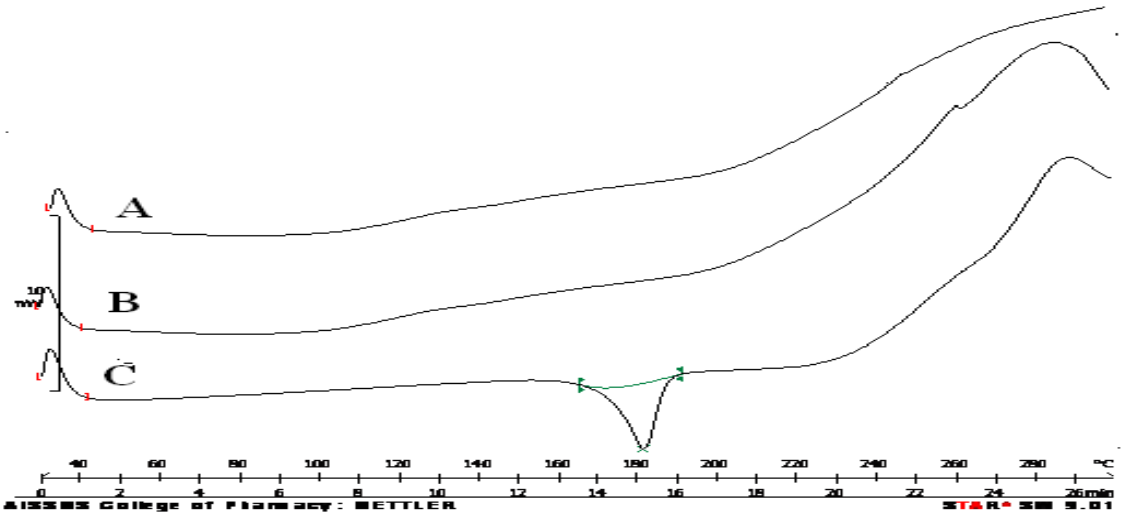

Figure 11: DSC curves of pure LCD HCl, Neusilin US2 and solid SMEDDS.

A= Solid SMEDDS, B= Neusilin US2 and C= LCD HCl

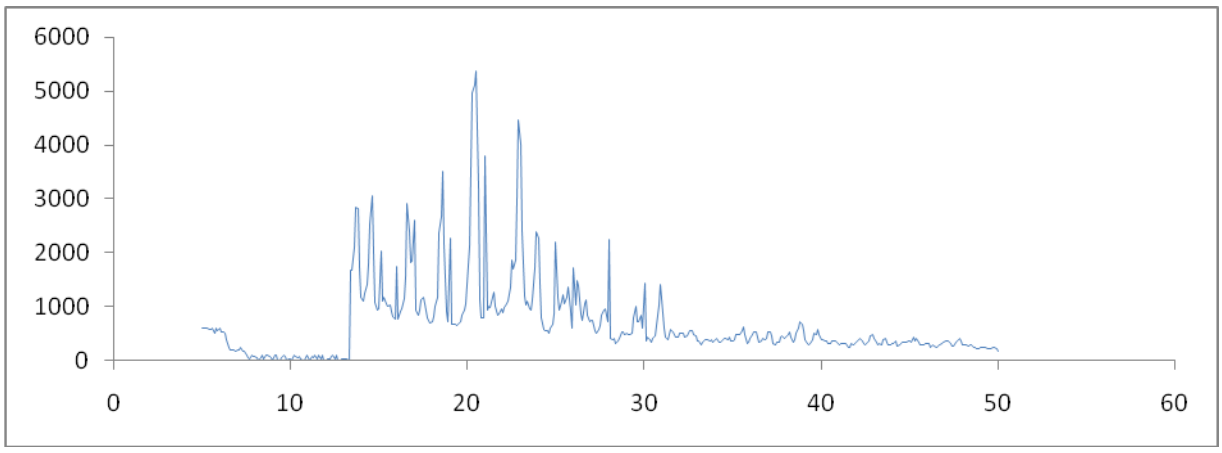

Figure 12: X-ray powder diffractogram for Pure drug.

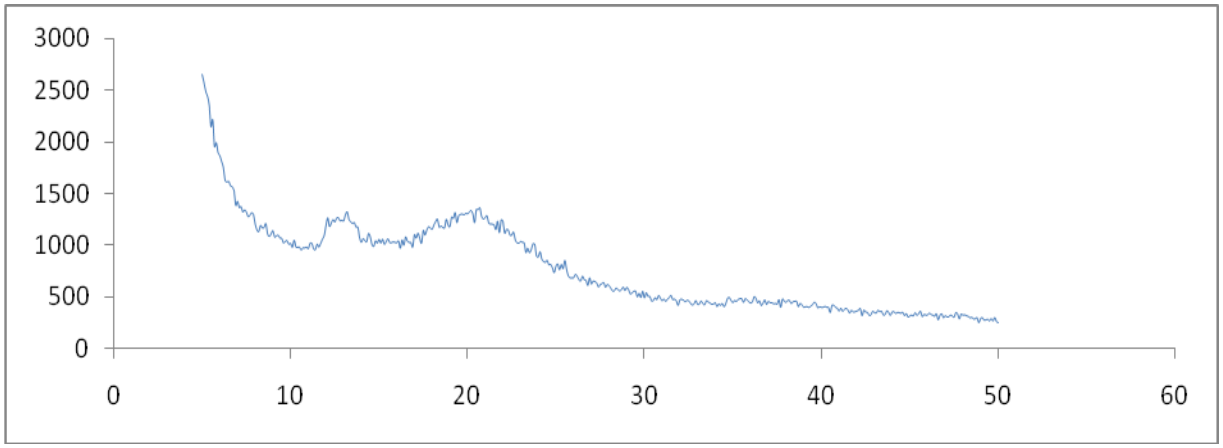

Figure 13: X-ray powder diffractogram for Solid SMEDDS.

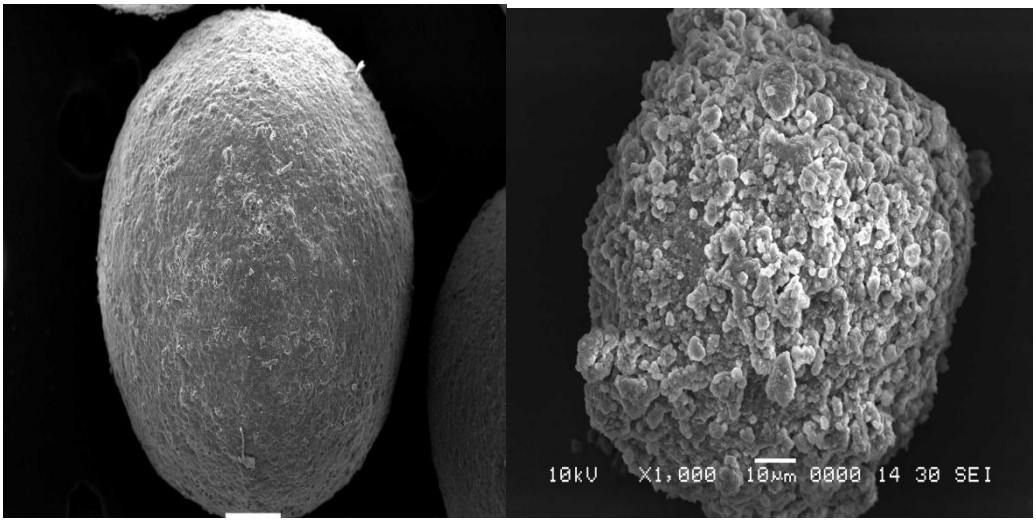

Figure 14: SEM of NeusilinUS2 and solid SMEDDS.

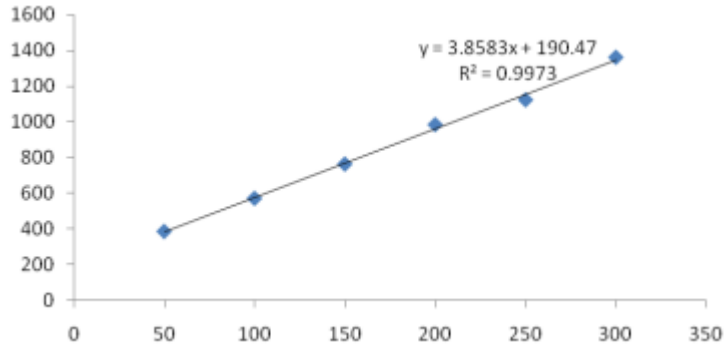

Figure 15: Calibration curve for Lercanidipine HCl in plasma.

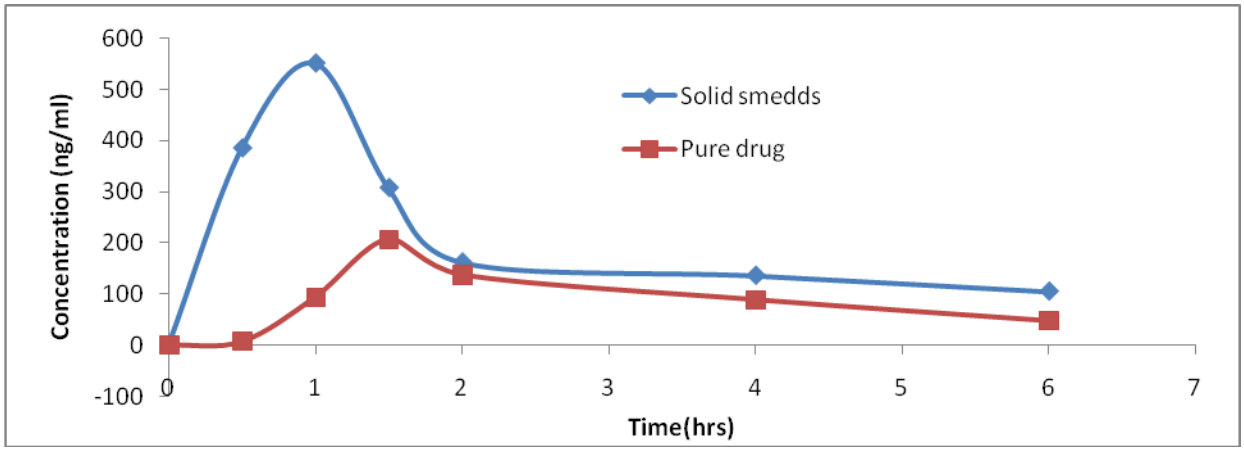

Figure 16: Plasma concentration profile of LCD HCl in rats from solid SMEDDS.

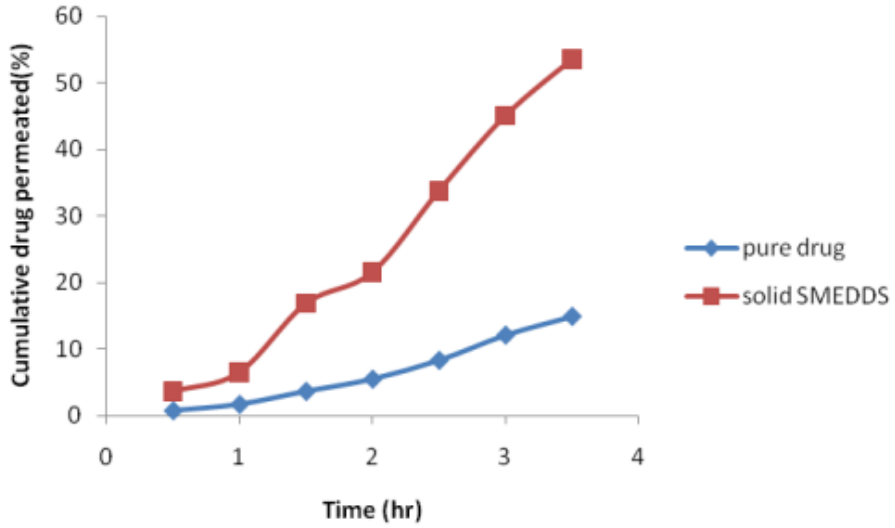

Figure 17: Ex vivo intestinal permeation profile of pure drug Lercanidipine HCl and solid SMEDDS in buffer pH 6.8.

Table 1: Composition comprising of Smix (1:1):Cremophor ELP-Propylene glycol, Oil: Capmul MCM L8.

Code	Smix (mg)	Oil (mg)	Drug (mg)
F1	900	100	10
F2	880	120	10
F3	860	140	10
F4	840	160	10
F5	820	180	10
F6	800	200	10

Table2: Percent Transmittance studies in different medium.

Code	Percent transmittance		
	In DM water	In 0.1 N HCl	In buffer pH 6.8
F1	99.91	99.98	96.9
F2	99.77	99.56	97
F3	99.15	99.29	99.15
F4	99.14	98.99	99.77
F5	98.34	97.55	99.14
F6	98.00	97.23	99.91

Table 3: Droplet size, Zeta-potential and PDI of the formulation F1-F6.

Code	Globule size (nm)	Zeta-potential (mv)	PDI
F1	12.51	9.67	0.069
F2	13.41	10.5	0.023
F3	13.42	10.37	0.128
F4	13.64	10.5	0.069
F5	13.72	9.90	0.133
F6	14.99	10.56	0.056

Table 4: Optimized composition of LCD HCl SMEDDS.

Ingredient	Quantity (mg)
Capmul MCM C8	200
Smix	800
Drug	10
Total	1010

Table5. Droplet size and Zeta-potential of the formulation F6.

Code	Globule size (nm)	Zeta-potential (mV)	PDI
F6(Liquid SMEDDS)	15.18	10.83	0.056
F6(Solid SMEDDS)	15.28	10.56	0.103

Table 6: Flow properties of solid SMEDDS.

Formula code	Angle of repose (°)	Bulk density	Tapped density	Carr's index (%)	Hausner's ratio
F6 solid SMEDDS	29.00	0.42g/ml	0.51 g/ml	14 %	1.17

Table 7: Stability study of SMEDDS.

Tests	40±2 °C/75±5% RH	Room temperature
Drug content	0.986 mg/ 100mg of solid SMEDDS	0.989 mg/ 100mg of solid SMEDDS
% Transmittance (533nm) in D.M. water	98.73%	99.01%
Droplet size (nm)	15.28	14.99
Zeta potential (mv)	10.5	10.56

Table 8: Calibration curve data for Lercanidipine HCl in plasma (n=6).

Concentration (ng/band)	Area
50	387
100	572
150	764
200	985
250	1124

Table 9: Plasma concentration profile data for LCD HCl rats from SMEDDS (n=6).

Time (h)	Concentration (ng/ml) SMEDDS	Concentration (ng/ml) pure LCDHCl
0	0	0
0.5	384.8	7
1	550.6	93.6
1.5	307.2	206.6
2	161.8	137.4
4	135.8	88.6
6	104.8	47.6

Table 10. Pharmacokinetic parameters and relative bioavailability of LCD HCl.

Parameters	Pure LCD HCl	SMEDDS
Cmax (ng/ml)	550.6	206.6
t max (h)	1.5	1
AUC (0-6 h) (ng/ml h)	3574.191	7680.194
Relative bioavailability (%)	100	214.87

Table 11: Blood pressure measurement after administration of plain drug, Solid SMEDDS and vehicle control. (n=6).

Time (h)	Mean Arterial Blood Pressure in mm Hg		
	Plain drug	Solid SMEDDS	Control
0	164.33	164.67	159
0.5	151.67	145.67	160
1	145.67	131.33	159
1.5	134.33	129	158
2	132.33	127	160

CONCLUSION

Solid SMEDDS for a poorly water-soluble drug, LCD HCl was prepared by adsorbing liquid SMEDDS on solid inert carrier Neusilin US2. The final composition of SMEDDS was obtained based on solubility evaluation, pseudo ternary phase diagram, and droplet size analysis. The solid SMEDDS showed good flow properties and rapid self-micro emulsification. XRPD studies suggested that LCD HCl in the solid SMEDDS is in molecular dispersion state. In-vitro dissolution testing showed faster rate of drug release than pure drug product in a discriminating dissolution media. From the pharmacokinetics data, there was an improvement in the bioavailability as compared to the pure drug. Thus, solid SMEDDS may provide a useful oral solid dosage form for poorly aqueous drug LCD HCl.

REFERENCES

1. Beg S, Rahman M, Panda SK, Alharbi KS, Alruwaili NK, Singh PK, Thappa M, et al. Nasal mucoadhesive microspheres of lercanidipine with improved systemic bioavailability and antihypertensive activity. J Pharma Innov. 2021;16(2):237-246.
2. Yan B, Ma Y, Guo J, Wang Y. Self-micro emulsifying delivery system for improving bioavailability of water insoluble drugs. J Nanopart Res. 2020;22(1):1-4.
3. Suram D, Narala A, Veerabrahma K. Development, characterization, comparative pharmacokinetic and pharmacodynamic studies of iloperidone solid SMEDDS and liquid solid compact. Drug Dev Ind Pharm. 2020;46(4):587-96.
4. Kyatanwar A, Gajbhiye N, Jadhav K, Kadam V. Solid self-emulsifying drug delivery systems: A review. J Pharm Res, 2010; 3(4):877-882.
5. Madan J, Patil K, Awasthi R, Dua K. Formulation and evaluation of solid self-micro emulsifying drug delivery system for azilsartan medoxomil. Int J Polym Mater and Polym Biomater. 2021;70(2):100-16.
6. Nekkanti V, Karatgi P, Prabhu R, Pillai R, Solid self-micro emulsifying formulation for candesartan cilexetil, AAPS Pharm sci tech., 2010; 11: 9-17.
7. Reagon-Shaw S,Nihal M, Nihal A .Dose translation from animal to human studies revisited, The FASEB Journal,2007;22:659-661.
8. Patil S, Tagalpallewar A, Kokare C. Natural anti-proliferative agent loaded self-micro emulsifying nanoparticles for potential therapy in oral squamous carcinoma. J Pharm Investig. 2019;49(5):527-41.
9. Rani S, Rana R, Saraogi GK, Kumar V, Gupta U. Self-emulsifying oral lipid drug delivery systems: advances and challenges. AAPS Pharm Sci Tech. 2019;20(3):1-2.
10. Zakkula A, Gabani BB, Jairam RK, Kiran V, Todmal U, Mullangi R. Preparation and optimization of nilotinib self-micro-emulsifying drug delivery systems to enhance oral bioavailability. Drug Dev Ind Pharm. 2020;46(3):498-504.
11. Yi T, Wan J, Xu H, Yang X. A new solid self-micro emulsifying formulation prepared by spray-drying to improve the oral bioavailability of poorly water-soluble drugs. Eur J Pharm Biopharm. 2008; 70:439–444.
12. Singh D, Singh A, Singh D, Kesavan A, Arora S, Tiwary A, et al. Enhanced oral bioavailability and anti-diabetic activity of canagliflozin through a spray dried lipid based oral delivery: a novel paradigm. DARU J Pharm Sci. 2020;28(1):191-208.
13. Kim D, Cho J, Park J, Kim J, Song E, Kwon J, et al. Self-micro emulsifying drug delivery system (SMEDDS) for improved oral delivery and photostability of methotrexate. Int J Nanomedicine. 2019; 14:4949.
14. Siddaramaiah N, Gowda D. Formulation And Evaluation Of Self-Micro emulsifying Dispersible Tablets Of Ibuprofen. Int J Pharm Res. 2021;13(1).
15. Dholakiya A, Dudhat K, Patel J, Mori D. An integrated QbD based approach of SMEDDS and liquid solid compacts to simultaneously improve the solubility and processability of hydrochlorthiazide. Journal of Drug Del Sci Tech. 2021; 61(1):102162.
16. Mandić J, Kosmač I, Kovačević M, Hodnik B, Hodnik Ž, Vrečer F, et al. Evaluation of solid carvedilol-loaded SMEDDS produced by the spray drying method and a study of related substances. Int J Pharm. 2021; 605:120783.

17. Wang Y, Chen L, Adu-Frimpong M, Wei C, Weng W, Wang Q, et al. Preparation, In Vivo and In Vitro Evaluation, and Pharmacodynamic Study of DMY-Loaded Self-Micro emulsifying Drug Delivery System. Eur J Lipid Sci Technol. 2021;123(6):2000369.
18. Liu J, Wang Q, Omari-Siaw E, Adu-Frimpong M, Liu J, Xu X, et al. Enhanced oral bioavailability of Bisdemethoxycurcumin-loaded self-micro emulsifying drug delivery system: Formulation design, in vitro and in vivo evaluation. Int J Pharm. 2020; 590:119887.
19. Salem H, El-Menshawe S, Khallaf R, Rabea Y. A novel transdermal nano ethosomal gel of lercanidipine HCl for treatment of hypertension: optimization using Box-Benkhen design, in vitro and in vivo characterization. Drug Deliv 2020;10(1):227-240.
20. Sun C, Gui Y, Hu R, Chen J, Wang B, Guo Y, et al. Preparation and pharmacokinetics evaluation of solid self-micro emulsifying drug delivery system (S-SMEDDS) of Osthole. AAPS Pharm sci tech. 2018;19(5):2301-10.

5487847845**1210508**