Chapter 6

Oral tablet formulation of LSF-LA conjugate polymeric micelles

1. Introduction

In **chapter 5**, LSF-LA PLM were prepared and evaluated for oral delivery of LSF. LSF-LA PLM enhanced the oral bioavailability of LSF and reduced its *in vivo* interconversion into PTX by protecting the LSF-LA ester linkage from degradation in the GIT environment,[1] thus enabling oral delivery of LSF-LA PLM and providing a sustained release of LSF in comparison to LSF-LA conjugate. Although polymeric micelles provided considerable control over the pharmacokinetics and pharmacodynamics of LSF owing to their nano-size, enhanced surface area and shielding the ester linkage in LSF-LA conjugate in the acidic GI fluid, nonetheless, administration of these nano sized particles to the patients still remains a challenge.[2,3] To meet this objective, the PLMs could have been administered in the form of an aqueous dispersion as final dosage form (lyophilized for reconstitution).[4] However, this creates an additional requirement of sterile water to be provided along with PLMs for reconstitution. Moreover, poor taste of the drug may require the incorporation of colloidal particles into solid dosage forms (e.g. granules, pellets or tablets).[5] Here, granules and pellets which require capsule as final dosage form have more complex preparation than directly compressible tablet dosage form.[6,7] Although, capsules are generally easier to swallow than tablets but are more costly and highly prone to degradation. For a chronic disorder like diabetes, multiple dosing is required to maintain the plasma concentration of the drug to maintain blood glucose levels.[8] Tablets have many advantages over other dosage forms, such as ease of transportation, application and production, high patient compliance, better formulation stability, accurate dosing and control of drug release.[9-11] Therefore, the drug delivery system preferred to carry and orally deliver the LSF-LA PLM were tablets.

In this work, we report the preparation and pharmaceutical profiling of a hybrid dosage form consisting of self-assembling, amphiphilic polymeric micelles of LSF-LA (LSF-LA PLM), compressed into tablets and optimized for oral delivery. To develop tablet dosage

form, initially, LSF-LA PLM formulation was prepared in scale-up batches while retaining its particle size and encapsulation efficiency followed by batch lyophilization and direct compression of these into tablets using suitable excipients. LSF-LA PLM tablets were characterized for all the tablet evaluation parameters, hardness, weight variation, friability and disintegration as recommended by USP. Further, to ensure the integrity of LSF-LA PLMs after compression into tablets, the tablets were crushed and subjected to fractional centrifugation to obtain LSF-LA PLM which were then characterized for particle size and drug content. In-vitro release of LSF-LA from tablet dosage form was carried out in simulated gastric and intestinal fluids (in presence of enzymes) as per USP. This tablet formulation was further evaluated for intestinal permeability in rat model using single pass intestinal perfusion (SPIP) study and in the oral PK studies. PK of LSF-LA PLM tablet showed greater Cmax than LSF, LSF-LA and LSF-LA PLM, which would enable immediate decrease in the postprandial glucose levels in diabetic patients.

2. Materials and Methods

2.1. Materials, reagents and experimental animals

LSF and LSF-LA conjugate were synthesized and characterized *in house* before use (***details mentioned in Chapter 2 and 4 respectively***). Poly(ethylene glycol) (PEG, Mn 2000) was purchased from Sigma Aldrich (St. Louis, MO). Avicel® PH102, a cellulose microcrystalline (MCC) grade was purchased from FMC BioPolymer, USA. Fujicalin® SG was procured as a gift sample from Gangwal Chemicals Pvt. Ltd, Mumbai, INDIA. Aerosil 200, pepsin, pancreatin and magnesium stearate were purchased from Sisco Research Laboratories Pvt. Ltd., INDIA. All other chemicals and reagents were of analytical or extra pure grade and used as obtained. Wistar rats (male; 8–10 weeks, 200–220 g) were procured from Central Animal Facility, BITS-PILANI (Pilani, India). All the animal experiments were performed as per CPCSEA guidelines and according to protocols approved by Institutional

Animal Ethics committee (IAEC) (Animal testing protocol no. IAEC/RES/23/26 and IAEC/RES/27/10; Central animal facility (CAF), BITS-Pilani).

2.2. Preparation of tablets of LSF-LA PLM

For preparation of the tablets of LSF-LA PLM, polymeric [mPEG-b-P(CB-co-LA)] micelles of LSF-LA were initially prepared in scaled-up batches followed by its lyophilization using PEG 2000 as a lyoprotectant. Thereafter, the resultant lyophilized powder was compressed in the form of tablet.

2.2.1. Preparation of scale up batches LSF-LA PLM

Scale-up batches of LSF-LA PLM were prepared in batch size of up to 6 gm. LSF-LA PLM were prepared by a thin-film hydration method as reported previously *(Chapter 5 section 2.3)* with slight modification to enable scale up. In brief, LSF-LA conjugate and polymer, mPEG-b-P(CB-co-LA) were dissolved in dichloromethane and dried under vacuum to form a thin film in a 1000 mL round bottom flask and dried overnight. The resultant film was reconstituted with ultra-pure water (100 mL) aided by bath sonication for 2 min followed by stirring for 1 h. Resulting formulation was probe sonicated for 2 min at 25% amplitude using a 13 mm probe. Theoretical drug loading for scale-up batch was kept at 12.5% w/w. LSF-LA PLM so formulated were characterized for particle size, zeta potential and entrapment efficiency.

2.2.2. Lyophilization of LSF-LA PLM formulations

PEG 2000 was selected as the lyoprotectant and dissolved in freshly prepared LSF-LA PLM formulations to obtain a concentration of 5% *w/v* of lyoprotectant and loaded into a bench top lyophilizer (FreeZone® Triad® Freeze Dry System (Labconco, USA)). The lyophilization cycle was carried out in three sequential steps, namely freezing, primary drying and secondary drying for a total duration of 56 h (**Table 6.1**). During the lyophilization

process, the temperature of the product was monitored using active vials probed with thermocouples.

2.2.3. LSF-LA PLM directly compression into tablets

LSF-LA PLM tablets were prepared by a direct compression method. Commercially available Avicel® PH 102 and Fujicalin® SG are directly compressible excipients which were used as diluent and adsorbent respectively. For preparation of tablets (batch size: 60 tablets), Aerosil® 200 was sifted through sieve #30. Avicel® PH 102 and Fujicalin® SG were used as obtained as directly compressible grade were procured. For preparation of powder blend, lyophilized LSF-LA PLM, Avicel® PH 102 and Fujicalin® SG were initially mixed thoroughly followed by addition and mixing of Aerosil® 200, magnesium stearate and talc. Detailed tablet composition has been provided in **Table 6.2.** The prepared powder blend was compressed into LSF-LA PLM tablets (9 mm diameter and 150 mg weight) using Rimek minipress (10 station tablet compression machine). Blank tablets were also prepared using the same method except for omitting the LSF-LA PLM. The blank and LSF-LA PLM tablets shall be designated as T1 and T2 throughout the text.

2.3. Pre-compression characteristics of powder blend of LSF-LA PLM tablet

Powder blend used for direct compression of LSF-LA PLM tablet was evaluated for various rheological properties like bulk density, tapped density, compressibility index, flow properties (angle of repose) using standard procedures.[12,13] All studies were carried out in triplicate (n = 3) and average values were reported.

Bulk and tapped density: Bulk density was determined by measuring the volume occupied by a known weight of the powder using a measuring cylinder. Tapped density was measured by tapping the powder bed 100 times to obtain a constant volume.

Hausner's ratio and compressibility index (Carr's Index): Hausner's ratio and Carr's index were determined by placing the powder in a measuring cylinder and the initial volume (V_0) (before tapping) and final volume (after 100 tapings) (V) were noted. Hausner's ratio and Carr's index were calculated using **Equation 1 and 2**.

$$\text{Hausner's ratio} = \text{Tapped density} / \text{bulk density} \qquad \textbf{Equation 1}$$

$$\text{Carr's index} = (1 - V/V_0) \times 100 \qquad \textbf{Equation 2}$$

Where, V_0 = volume of powder before tapping and,

V = volume of powder after 100 tappings

Angle of Repose (Ø): Angle of repose was determined by measuring the height and radius of the heap of the powder formed when the powder was allowed to flow freely through a funnel fixed at a height of 5 cm above the plane. Value of Ø was calculated using following formula (**Equation 3**).

$$\tan ø = h / r \qquad \textbf{Equation 3}$$

where, h = height of heap of powder and, r = radius of heap of the powder

2.4. Evaluation of LSF-LA PLM tablets

2.4.1. Drug content

For the determination of drug content from LSF-LA PLM tablets, LSF-LA PLMs were first separated/recovered from the compressed tablet by the method shown in **Figure 6.1**. Briefly, tablets of T2 formulation were allowed to undergo complete dissolution in 1 mL of distilled water at 100 rpm, 37 ºC for 6 h. The dissolution media after 6 h was subjected to fractional centrifugation at 1500 and 5000 rpm for 10 min each sequentially. The supernatant so obtained after centrifugation at 5000 rpm was analyzed by HPLC to determine the content of LSF-LA. Here, T1 tablets were processed only as control to prove recovery of PLM.

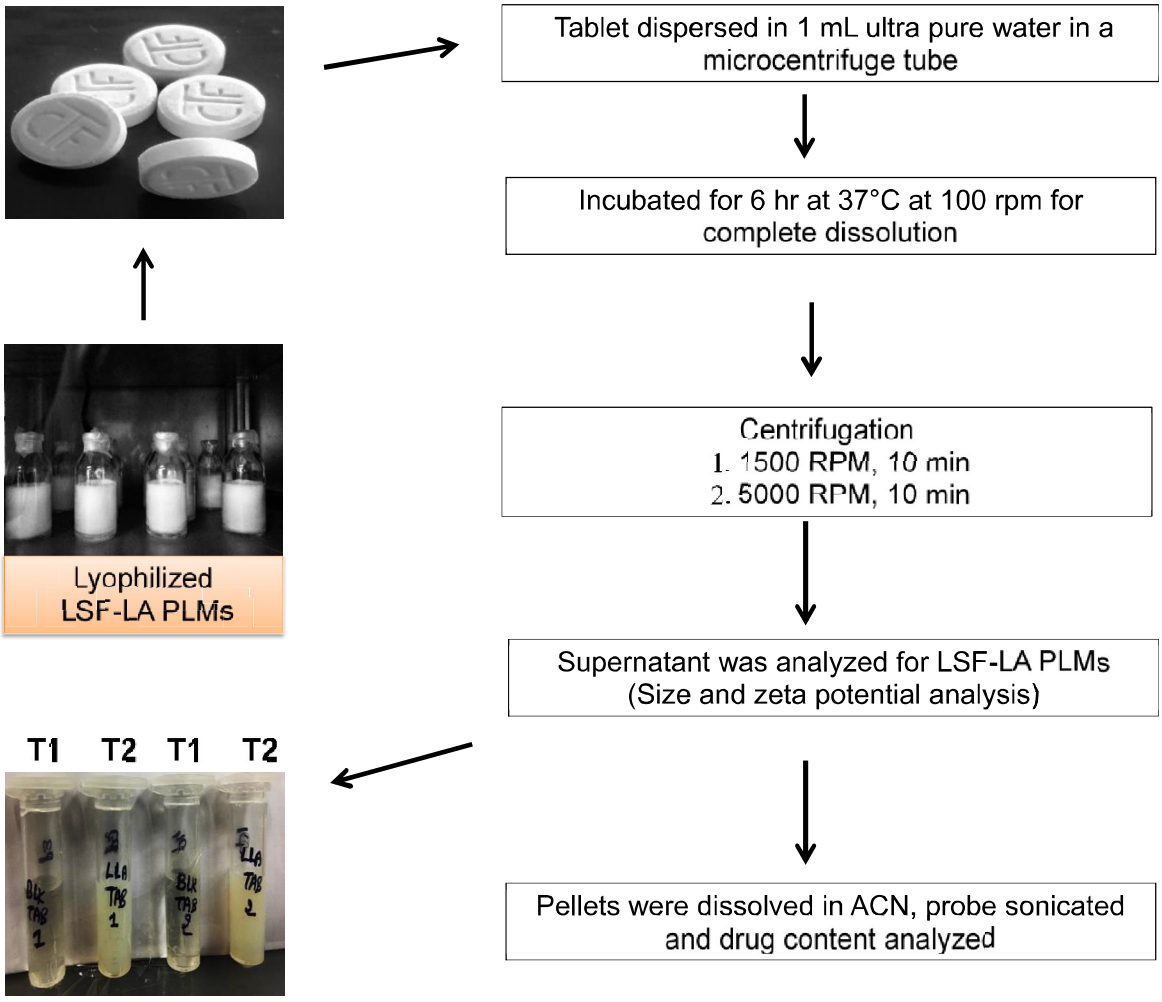

Figure 6.1 Recovery of LSF-LA PLM from tablets.

2.4.2. Weight variation, hardness, friability and disintegration test

Weight variation: *It* was carried out to ensure that each of tablets contains an equivalent amount of drug. The test was carried out by weighing 20 tablets individually using analytical balance, then calculating the average weight, and comparing the individual tablet weights to the average. For each formulation (T1, T2), twenty tablets were randomly picked, weighed individually; the average weight and variation were calculated.

***Tablet hardness*:** The resistance of tablets to capping, abrasion or breakage under conditions of storage, transportation and handling before usage depends upon its hardness. Tablet hardness is defined as the load required for crushing or fracturing a tablet placed on its edge. Sometimes it is also termed as tablet crushing strength. For hardness testing, six tablets of each formulation were randomly picked and the hardness of the tablets was determined by Monsanto hardness tester (Labpro, India).

Friability: The friability of the tablets was determined by tablet Roche friability tester. Twenty pre-weighed tablets were placed in the tablet friability tester and rotated at 25 rpm for 4 min. Next, the tablets were reweighed after dusting them and crushed tablets were removed and percentage loss was calculated.

Disintegration: Standard disintegration apparatus (DBK instruments, Mumbai, India) as described in USP was used for disintegration testing of the tablets. One tablet was placed in each of the tubes of the basket rack and positioned in 1-L beaker containing distilled water at 37±2°C. Time for complete disintegration of the tablets was recorded.

2.5. In vitro evaluation of LSF-LA PLM tablets

2.5.1. In vitro release study of LSF-LA from tablet in simulated biological fluids

In vitro release of drugs/dosage forms in simulated biological fluids indicates the likelihood of oral bioavailability. *In vitro* release of LSF-LA from tablet was studied in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 6.8) in the presence of enzymes (prepared as per USP).

Composition of SGF, pH 1.2

SGF was prepared by dissolving NaCl (200 mg) and HCl (36% v/v, 0.7 mL) in triple distilled water and volume was made up to 100 mL. The pH of the solution was adjusted to

1.2 using HCl. To prepare SGF with enzyme, 3.2 mg pepsin (porcine pepsin 3000 units/mg of protein) per mL of media was added after adjustment of pH to1.2.

Composition of SIF, pH 6.8

SIF was prepared by dissolving the KH_2PO_4 (680 mg) and NaOH (616 mg) in triple distilled water and volume was made up to 100 mL, then the pH of the solution was adjusted to 6.8 with 0.2N HCl/ 0.2N NaOH. To prepare SIF with enzyme, 10 mg pancreatin per mL of media was added after pH adjustment to 6.8.

***In vitro* release study**

06 T2 tablets (containing LSF-LA PLM) were added to 10 mL of SGF and SIF (n=3) individually and incubated at 37 °C and 100 rpm in shaking water bath. An aliquot of sample (200 µL; without replacement of media) was collected at each time point (0, 10, 20, 30, 45, 60, 90, 120, 180 and 240 min) for SGF and (0, 10, 20, 30, 45, 60, 90, 120, 180, 240 min and 360 min) for SIF followed by quenching of the sample with ice cold acetonitrile (800 µL), vortexing and centrifugation, then the supernatant was collected and LSF-LA conjugate in samples was analyzed by HPLC. The graph between percent LSF-LA remaining intact in the medium *vs.* time was plotted.

2.5.2. Cell viability assay

To evaluate the cell viability of LSF-LA PLM in diabetic conditions, mouse insulinoma cells, MIN-6 were used. Cells were grown in RPMI media supplemented with 10% FBS and 1% antibiotic solution and incubated at 5% CO_2 and 37°C. The aim of this experiment was to determine if LSF-LA PLM tablet, induces any toxicity in the insulinoma cells. MIN6 cells (5×10^3/well) were seeded in a 96 well cell culture plate and allowed to adhere for 24 h. For cell viability, T1 and T2 tablets as a whole as well as LSF-LA PLM recovered from T2 were tested. For testing the whole tablet, T1 and T2 were crushed and suspended in ultra-pure water and added to the cells (designated as T1 and T2 suspension).

For testing of LSF-LA PLM recovered from T2, LSF-LA PLM were recovered from T2 tablet (T1 tablet solution as control) as detailed in *section 2.4.1* (designated as T1 and T2 solutions) followed by adding to the cells and incubating at 37°C/5% CO_2 for 48 h. Untreated cells and cells treated with free LSF, LA, LSF-LA SM and LSF-LA PLM at equivalent concentrations (all at ~20 µM) and T1 tablet solution and suspension (Equal volume to T2 tablet) were kept as controls. After 48h, MTT assay was performed and optical density (OD) was recorded at 560 nm and at reference wavelength 630 nm. The percentage cell inhibition was determined by comparison with untreated cells.

2.6. In situ absorption studies of LSF-LA PLM: SPIP

2.6.1. Preparation of perfusion solution

Perfusion solution for SPIP assays was prepared with the following composition: NaCl 48 mM, KCl 5.4 mM, Na_2HPO_4 28 mM, NaH_2PO_4 43 mM, mannitol 35 mM, PEG-4000 1 g/L and anhydrous D-glucose 10 mM in ultra-pure water.[14,15] As needed, pH was adjusted to 6.5 with HCl or NaOH solution. Phenol red as a non-absorbable marker (50 µg/mL) was added to the perfusion solution to correct for any water absorption and secretion that may be encountered during the experiment.[16,17] The SPIP assays were performed at concentration of drug/formulation as ~30 µg/mL of free LSF.

2.6.2. SPIP procedure

Preliminary studies were carried out in the SPIP study to ensure that the loss of drug observed during the perfusion results only from its absorption and not due to other reasons such as non-specific binding of the drug to the tubing and/or its degradation.[15,18] In order to evaluate the effect of binding of the drug to the tubing, the perfusion solutions of LSF, LSF-LA SM and LSF-LA PLM were incubated at 37 °C with tubing for 2 h. Aliquots were collected after 2 h and drug content determined by HPLC. In order to evaluate the stability of the drug at 37 ºC, the perfusion solutions containing LSF/LSF-LA SM/LSF-LA PLM were

incubated at 37 ºC for 2 h. Aliquots were withdrawn and analyzed by HPLC. Here, LSF-LA SM was prepared as per our previous report.[19]

The *in situ* SPIP studies were conducted as per protocol approved by IAEC, BITS-Pilani, Pilani. Briefly, Wistar rats (220-250 gm) were kept in a 12 h light–dark cycle and fasted for 12–18 h, water was provided *ad libitum* before experiment. For experiment, rats were anaesthetized using an intraperitoneal injection of ketamine–xylazine mixture (0.1 g/kg and 0.02 g/kg, respectively), and a heating pad and lamp were used to maintain the body temperature at 37 ± 1 ºC. Laparotomy was performed wherein, midline incision of 3-4 cm was made in the abdomen to isolate the small intestine and approximately 15-20 cm of the proximal jejunum portion was carefully cannulated with plastic tubing (2 mm o.d.) at both the ends. The intestinal segment was rinsed with blank perfusion solution (free of drug) maintained at 37 ºC for approximately 25-30 min at 0.5 mL/min flow rate until the solution coming from the outlet was visually clear. Afterwards, the perfusion fluids (containing LSF/LSF-LA SM/LSF-LA PLM) were infused at a rate of 0.2 mL/min into the intestinal lumen of the rat. Initially, the perfusion solution (containing drug/formulation) was perfused for 1 h to achieve steady state (drug equilibrium with intestinal membrane). Once the steady state was achieved, the perfusate was collected at every 15 min interval (15, 30, 45, 60, 75, 90, 105, 120 min). Samples were immediately frozen at -80 ºC until analysed. At the end of the study, animals were euthanized with saturated potassium chloride (KCl) solution by intracardiac injection, according to the protocol for euthanasia in experimental animals.[16] After the death of the animal, the intestinal segment was carefully removed for measurement of its length and radius (L and r, respectively).

2.6.3. Instrumentation, chromatographic conditions and sample analysis

A Shimadzu HPLC system (Kyoto, Japan) equipped with a binary pump (LC-20AD), PDA detector (SPD-M20A) and auto sampler (SIL-HTC, Shimadzu, Japan) was used to

analyze the drug content in perfusate samples. The HPLC system was equilibrated for approximately 40 min before beginning the sample analysis. Eluents (LSF and LSF-LA) were monitored at a wavelength of 273 nm. Control of hardware and data handling was performed using LC solution software version 1.22 SP1.

The concentrations of LSF and phenol red were determined simultaneously using sodium acetate buffer (10 mM, pH 3.5) and 1:1 mixture of methanol and acetonitrile at 50:50 %v/v as a mobile phase at a flow rate of 1.0 mL/min. For analysis of LSF-LA and phenol red, sodium acetate buffer (10 mM, pH 3.5) and acetonitrile (05:95, %v/v) were used as a mobile phase at a flow rate of 1.0 mL/min. For sample analysis, perfusate samples were centrifuged at 15,000 rpm for 10 min. The upper layer was filtered through a membrane filter and diluted with acetonitrile and subjected to HPLC analysis.

2.6.4. Determination of drug permeability

Drug permeability was calculated after attainment of steady state of drug absorption in the intestine.[20] Steady state was confirmed by plotting the ratio of outlet to inlet drug concentration versus time. The corrected ratio of outlet to inlet drug concentration (C_{out}/C_{in}) was obtained with respect to phenol red inlet and outlet concentrations using **Equation 4** .[17,21]

$$C'_{out}/C_0'_{in} = [C_{out}/C_{in}] \times [C_{in\ (phenol\ red)} / C_{out\ (phenol\ red)}] \qquad \textbf{Equation 4}$$

Where, C_{out} is the concentration of LSF /LSF-LA SM/LSF-LA PLM in the outlet perfusate, C_{in} is the LSF/LSF-LA SM/LSF-LA PLM concentration in the initial perfusate at entry, and $C_{in\ (phenol\ red)}$ and $C_{out\ (phenol\ red)}$ are the inlet and the outlet concentrations of phenol red, respectively. The effective permeability coefficient (P_{eff} cm/s) was calculated according to a parallel tube model using **Equation 5.**[17,21]

$$P_{eff} = [-Q \ln (C'_{out}/C'_{in})]/A \qquad \textbf{Equation 5}$$

Where Q is the flow rate (mL/min) of entering perfusate, C'_{out}/C'_{in} is corrected ratio of outer concentration to entering perfusate, and A is the surface area (cm^2) of the intestinal segment assumed to be equivalent to the area of a cylinder ($2\pi rL$) with the length (L) of 15-20 cm and radius (r) of 0.21 cm for the jejunum. The apparent first-order absorption rate constant (Ka min^{-1}) was calculated as given in **Equation 6.**[17]

$$Ka = [1-(C'_{out}/C'_{in})]Q/\ \pi r^2 L] \qquad \textbf{Equation 6}$$

2.7. Pharmacokinetics of LSF-LA PLM tablet

PK studies of LSF-LA PLM tablet were performed on Wistar rats (200-220 g). LSF-LA PLM tablet was crushed and dispersed in distilled water and administered orally at the dose of 10 mg/kg (~LSF) with maximum dosing volume of 1 mL to each rat after overnight fasting (n=4). After dosing, blood samples were collected at each preset time point of 10, 20, 30 min, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h. Plasma concentration-time profile of LSF released from LSF-LA PLM tablet was plotted and analyzed by non-compartmental model approach using Phoenix 2.1 WinNonlin (Pharsight corporation, USA) to determine various PK parameters. I*n vivo,* PTX plasma concentration-time profiles were also generated in all the PK studies of LSF and its formulations to understand the rate and extent of interconversion of LSF to PTX.

3. Results

3.1. Preparation of scale-up batches of LSF-LA PLM

Scale up batches of LSF-LA PLM prepared by thin-film hydration method exhibited self-assembly demonstrating average particle size and zeta potential of 145.3 nm (PDI: 0.121) and -2.92 ± 3.74 mV respectively (**Figure 6.2**). Entrapment efficiency of LSF-LA in PLM was found to be 78.45 ± 6.65 % with a practical drug loading of 11.49 ± 0.76% (**Table 6.3A**).

3.2. Lyophilization of LSF-LA PLM

In the present study, use of PEG 2000 as lyoprotectant resulted in the formation of an intact cake of LSF-LA PLM with a good appearance (**Figure 6.1**)**.** After the cake formation,

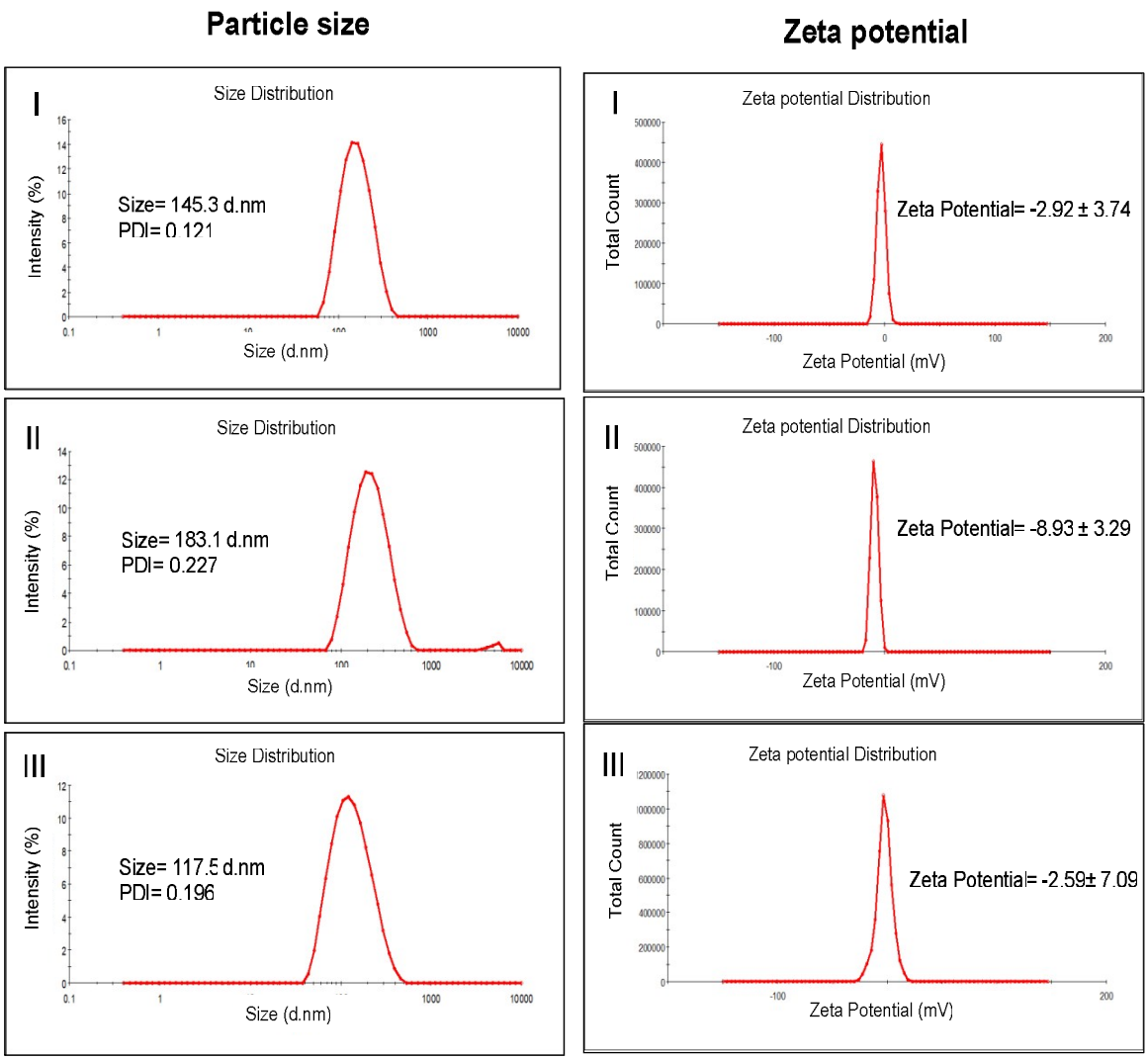

Figure 6.2. Particle size and zeta potential of LSF-LA PLM. (I) From scaled-up batches (before lyophilization), (II) after lyophilization and, (III) after recovery from the tablet

when lyophilized micelles were reconstituted in water, cake got redispersed immediately (within 30 s upon addition of water), and the redispersity index was found to be 1.26. Average particle size and zeta potential were found to be 183.1 nm (PDI: 0.227) and -8.93 ± 3.29 mV respectively (**Table 6.2**).

3.3. Pre-compression characteristics of powder blend of LSF-LA PLM tablet

Powder blend of tablets mainly included excipients such as Avicel® PH 102, Aerosil® 200, Fujicalin® SG, magnesium stearate and talc. Physical properties of powder blend are shown in **Table 3B.** Hausner's ratio and Carr's index were found to be 1.11 ± 0.01, 1.16 ±

0.02 and 10.17 ± 0.63, 13.69 ± 1.19 for T1 and T2 tablets respectively. Angle of repose was found to be 29.0 ± 1.59 and 29.16 ± 0.33 for T1 and T2 tablet respectively.

3.4. Evaluation of LSF-LA PLM tablets

3.4.1. Drug content, weight variation, hardness, friability and disintegration tests

LSF-LA PLMs loaded tablets (T2) as well as control tablets (T1) were uniform in size and color, clean and showed absence of any mottling on the surface (**Figure 6.1).** As shown in **Table 6.3C**, drug content in the tablets was uniform in the range of 85-115% and tablet weight ranged from 145-156 mg lying within the stipulated limit of weight variation as per USP. (±7.5%). Upon dissolution of the LSF-LA PLMs tablets, LSF-LA PLM were still found to be intact with an average particle size and zeta potential of 117.5 nm (PDI: 0.196) and -2.59 ± 7.09 mV respectively (**Figure 6.2**).

Table 6.1

Lyophilization cycles used for lyophilization of LSF-LA PLM.

Thermal treatment: Freezing (Vacuum: OFF)			
Segment/Step	Temperature (°C)	Hold time (h)	RAMP (°C/min)
1	10	0.5	5
2	0	2.0	2
3	-10	1.0	1
4	-30	3.0	1
5	-55	10.0	0.25
Primary drying (Vacuum: 200 mtorr)			
1	-55	6.0	0.25
2	-20	6.0	0.25
3	-10	5.0	0.25
4	4	5.0	0.25
5	20	5.0	0.25
Secondary drying (Vacuum: 100 mtorr)			
1	25	12	0.25

Table 6.2.
Composition of tablet formulation.

Tablet composition (Direct compression)		
Ingredients	**Composition per tablet (150 mg)**	
	T1 (mg)	**T2 (mg)**
LSF-LA PLMs	-	50 (~3 mg LSF-LA)
PEG 2000	50	-
Avicel® PH 102	60	60
Fujicalin® SG	30	30
Aerosil 200	5	5
Mg Stearate	2.5	2.5
Talc	2.5	2.5

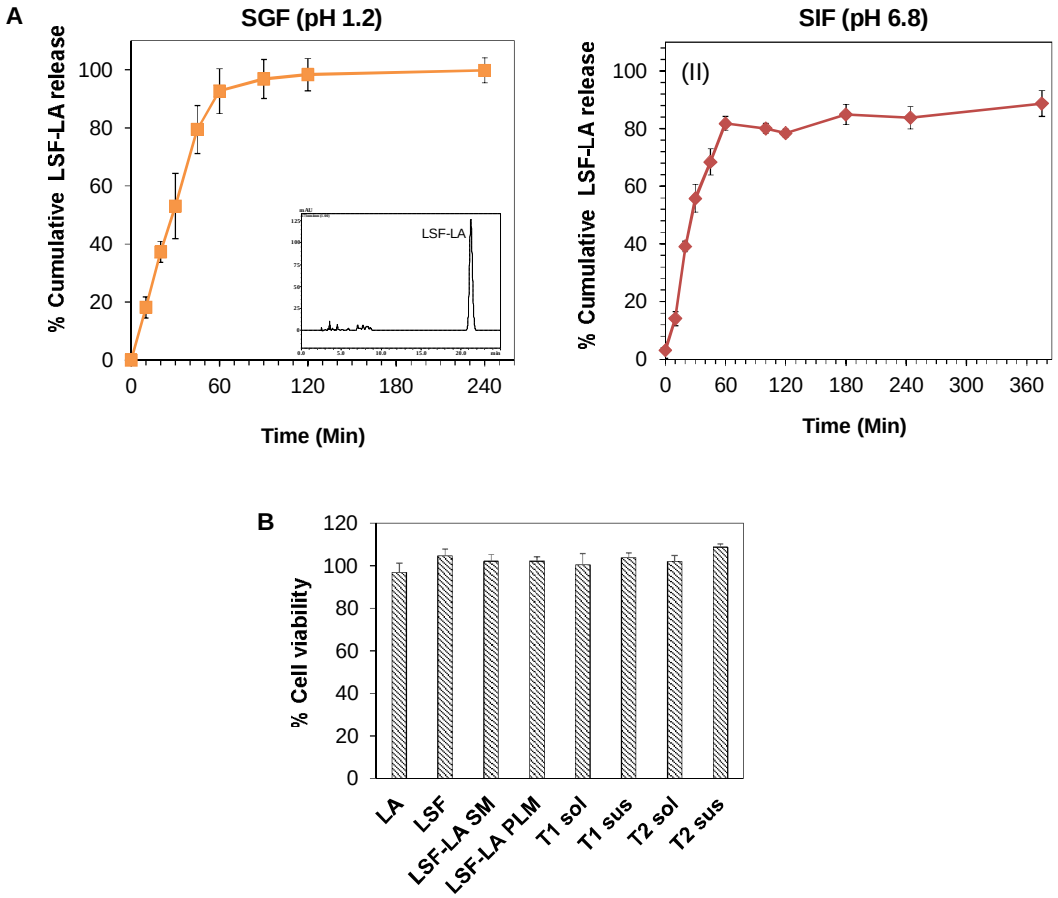

Figure 6.3. *In-vitro* evaluation of the LSF-LA PLM tablet. (A) *In vitro* release of LSF-LA from LSF-LA PLM tablet (T2) in SGF (also shown representative chromatogram for 2 h sample) and SIF, both containing enzymes and, (B) Cytotoxicity assay in MIN6 cells

Table 6.3.
(A) Evaluation of LSF-LA PLM before and after lyophilization

LSF-LA PLMs	Before lyophilization	After lyophilization
Particle size (nm)	145.3	183.1
PDI	0.121	0.227
Zeta Potential (mV)	-2.92 ± 3.74	-8.93 ± 3.29
% EE	78.45 ± 6.65	--
% DL	12.5	11.49 ± 0.76
% Yield	--	87.26 ±2.20

(B) Evaluation of physical properties of powder blend prepared for LSF-LA PLM tablets

Parameters	T1	T2
Bulk density	0.35 ± 0.01	0.21 ± 0.01
Tapped density	0.39 ± 0.01	0.24 ± 0.01
Hausner's ratio	1.11 ± 0.01	1.16 ± 0.02
Carr's Index	10.17 ± 0.63	13.69 ± 1.19
Angle of Repose	29.0 ± 1.59	29.16 ± 0.33

(C) Evaluation of LSF-LA PLM tablets

Parameters	T1	T2
LSF content (%)	--	93.75 ± 2.55
Mean weight	149.71 ± 2.30	150.47 ± 2.60
Hardness (kg)	3.75 ± 0.29	4.0 ± 0.41
Friability (%)	0.69 ± 0.08	0.63 ± 0.10

The hardness (~4 kg) and friability (<1%) results indicated that tablets possessed good mechanical strength and resistance to breakage. Disintegration time of tablets was found to be ~6 min. In conclusion, the LSF-LA PLM tablets conformed to all the tablet evaluation parameters in accordance to USP.

3.4.2. In vitro release study of LSF-LA from tablets in simulated biological fluids

As shown in **Figure 6.3A**, LSF-LA released from LSF-LA PLM tablets (T2) was found to be stable in SGF and SIF (even in the presence of enzyme) wherein, 99.80 ± 4.35% of

LSF-LA conjugate was released in SGF after 2 h and 88.73 ± 4.49% after 6 h of incubation in SIF. This data clearly indicated the feasibility of delivering LSF as LSF-LA PLM tablet by oral route of administration.

3.4.3. Cell viability assay

Cell viability of MIN-6 was assessed to determine the cytotoxicity of the LSF-LA PLM tablet formulation. As shown in **Figure 6.3B**, LSF-LA PLM and other tablet excipients were non-toxic to MIN6 cells.

3.5. In situ absorption studies of LSF-LA PLM: SPIP

The intestinal permeability of LSF/LSF SM/LSF-LA PLM was studied using the jejunum segment of rat's intestine by SPIP technique, wherein, diffusion of the drug molecule across the intestine at steady state was examined. The steady state is confirmed by plotting the ratio of the outlet to inlet concentrations of drug/conjugate after correction for water uptake or loss against time. As shown in **Figure 6.4A-C**, the C_{out}/C_{in} ratios attained a plateau with time in all the test solutions. Meanwhile, the preliminary study on nonspecific binding of the drug to the tubing revealed no adsorption of LSF/LSF-LA on the tubing. The drug was also found to be stable in the perfusion solution during the entire period of the experiment. The P_{eff} and Ka values of the different perfusion solutions were calculated using average of C_{out}/C_{in} data gathered at all the subsequent 15 min intervals over 2 h period. As shown in **Figure 6.4D,** in comparison to the control solution that contained solubilized LSF, both the test solutions including LSF-LA SM and LSF-LA PLM showed significant increase in permeability. Compared to the control solution LSF, LSF-LA SM exhibited 3.1 folds (1.58 ± 0.39 vs. 4.95 ± 0.21 ($\times 10^{-4}$) cm/s) increase in intestinal permeability. Encapsulation of LSF-LA into polymeric micelles (LSF-LA PLM) resulted in further increase in the permeability of LSF-LA by 1.8 folds (9.36 ± 0.94 ($\times 10^{-4}$) cm/s) and ~5.9 folds with respect to free LSF. The

apparent first order absorption rate constant (Ka) was found to be 14.90 ± 3.60, 45.70 ± 1.70 and 84.10 ± 7.70 min^{-1} respectively.

3.6. Pharmacokinetics of LSF-LA PLM tablet

As shown in **Table 6.5,** LSF-LA PLM tablet exhibited some improvement in oral pharmacokinetic parameters of LSF as compared to LSF, LSF-LA conjugate and LSF-LA PLM (**Table 5.2B and C**). Upon oral administration, LSF-LA PLM showed a C_{max} of 2710.34 ± 434.66 which is ~2 folds higher than LSF, LAF-LA and LSF-LA PLM (1138.64 ± 134.72, 1168.40 ± 89.73, 1449.39 ± 119.09 respectively). Although, LSF-LA PLM tablet exhibited reduced half-life (0.80 from 2.09 h) and MRT (0.98 from 2.41 h^{-1}) of LSF than LSF-LA PLM. Further, AUC_{0-t} for LSF-LA PLM tablet was found to be 2340.40 ± 155.06 ng.h/mL which is ~1.68 times higher than AUC_{0-t} observed in LSF-LA SM (1389.33 ng.h/mL) after oral administration. PTX was also detected in the PK studies due to the well proved LSF-PTX in vivo interconversion. As shown in **Figure 6.5**, plasma concentration of PTX was much lower upon administration of LSF-LA PLM tablet in comparison to LSF, LSF-LA SM and LSF-LA PLM PK (**Table 6.4**) which indicated that there was a significant decrease in the rate and extent of LSF-PTX in vivo interconversion upon administering LSF-LA as polymeric micelles, LSF-LA PLM which was further reduced upon incorporating of LSF-LA PLM in tablet dosage form.

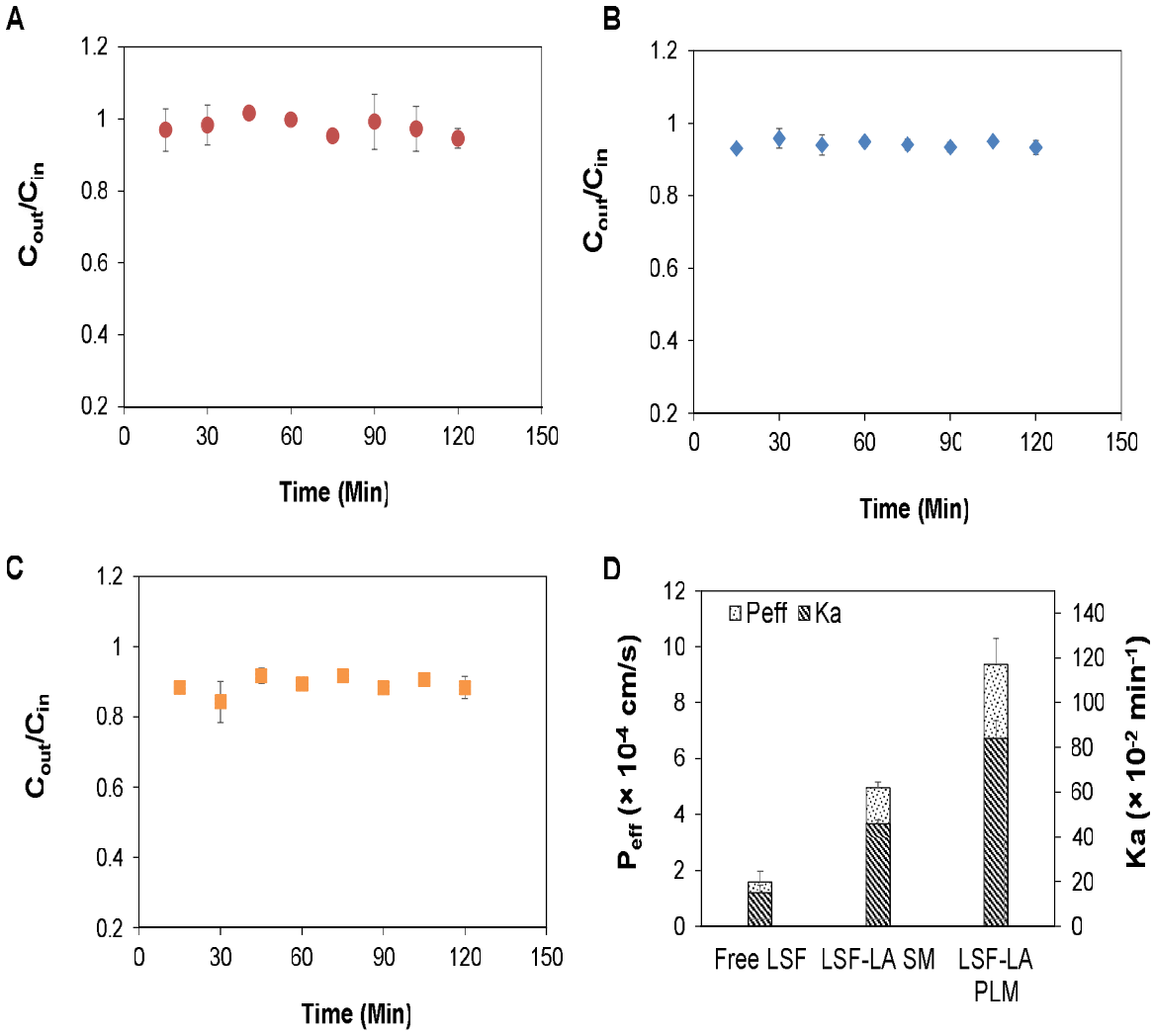

Figure 6.4. *In-situ* permeability studies of LSF, LSF-LA SM and LSF-LA PLM using SPIP rat model. Representative plots for the ratio of outlet to inlet drug steady state concentration (C_{out}/C_{in}) versus time for (A) LSF, (B) LSF-LA SM and, (C) LSF-LA PLM and, (D) effective permeability coefficient (P_{eff}) and apparent first order absorption rate constant (Ka) of LSF, LSF-LA SM and LSF-LA PLM. Values represent mean ± SEM (n = 3)

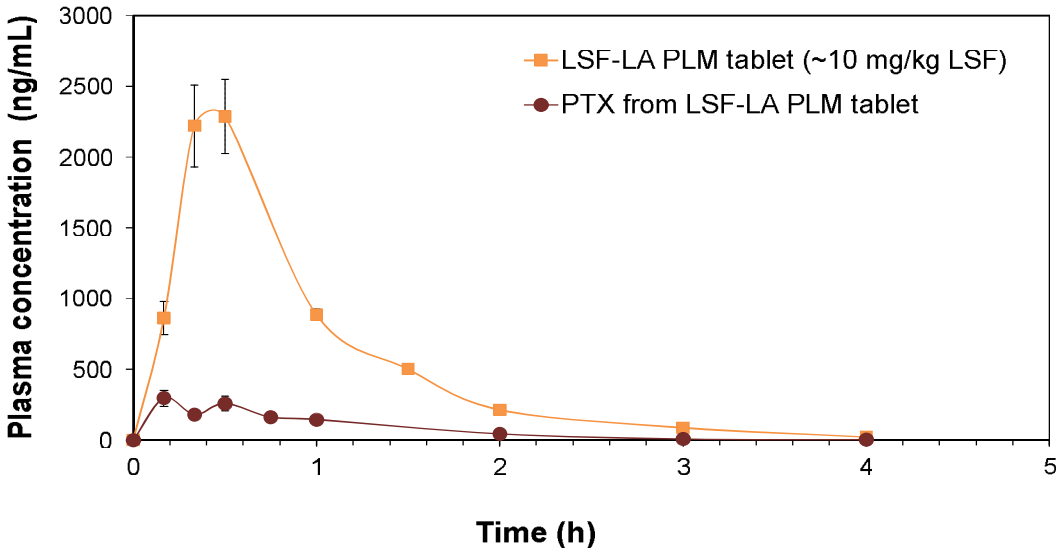

Figure 6.5. Pharmacokinetics of LSF-LA PLM tablet [mean (n=4) ± SEM]

Table 6.4.
The non-compartmental pharmacokinetic parameters for LSF-LA PLM tablet.

Parameters	**LSF-LA PLM tablet (**mean (n=4) ± SEM)	
	LSF	**PTX**
C_{max} (ng/mL)	2710.34 ± 434.66	222.79 ± 41.89
$t_{1/2}$ (h)	0.80 ± 0.02	0.68 ± 0.05
Ke (1/h)	0.86 ± 0.03	1.06 ± 0.08
$AUC_{0\text{-last}}$ (ng.h/mL)	2340.40 ± 155.06	279.30 ± 31.91
$AUC_{0\text{-}\infty}$ (ng.h/mL)	2364.88 ± 147.30	286.10 ± 32.56
$AUMC_{0\text{-last}}$ (ng.h/mL)	2047.21 ± 60.62	294.71 ± 27.11
$AUMC_{0\text{-}\infty}$ (ng.h/mL)	2170.68 ± 65.78	343.08 ± 29.96
MRT (h)	0.98 ± 0.04	1.22 ± 0.02

4. Discussion

Oral route remains the preferred mode of delivery for most of the drugs particularly intended for chronic ailments largely due to its convenience. This route is correlated with the highest degree of patient compliance (especially for chronic conditions such as diabetes) as it ensures ease, allows self-administration and provides great versatility in

dosage regimen.[2,22] Oral products do not require sterile conditions for their manufacture, which reduces production costs. For delivery of peroral NPs (here polymeric micelles), hybrid dosage forms of NPs, wherein, NPs are compressed directly into a tablet dosage form serve as superior alternative to direct administration of lyophilized NPs as well as conventional drug tablets.[5] Careful selection of the polymeric carriers for the preparation of PLMs and direct compression into tablets can provide a desirable sustained release profile along with improved PK and PD profile of short half-life drugs, such as LSF to ensure better management of the disease, improved patient compliance and improved dosing regimen.[23,24] Similar attempts have also been made previously wherein, naringin (NG), an anti-inflammatory compound was loaded into mPEG-PCL polymeric micelles (PLM) by thin film hydration method followed by freeze drying and directly compressed into buccal tablets. NGPLM tablet demonstrated increased solubility of NG and gave improved release profile which may enable better treatment as anti-ulcer agent in oral diseases.[25] Polymeric micelles have been used widely to prevent *in vivo* drug degradation due to enzymatic and environmental factors until it reaches the target site.[1] Likewise, we previously reported LSF-LA PLM to protect the ester linkage of synthesized conjugate LSF-LA in GIT and render it suitable for oral administration. Further, to ensure efficient drug delivery, patient compliance and considering a multiple dosing regimen in diabetes, LSF-LA PLMs were compressed into a tablet dosage form.

A typical pharmaceutical dosage form consists of active pharmaceutical ingredient (API) and excipients. Scaling up of the formulation is essential for clinical use but is often considered a major challenge for nanoformulations.[26] The methodology designed for formulation of LSF-LA PLM was amenable to scale-up as observed by preparation of larger batch sizes of LSF-LA PLM.

Lyophilization is a process of sublimation of water from frozen samples comprising of freezing and drying (primary and secondary) cycles. Lyophilization process has been extensively investigated to stabilize a broad variety of lipidic and polymeric drug nanocarriers.[27] Lyophilization of the fresh micellar formulation was optimized using PEG 2000 (5% *w/v*) as a lyoprotectant (**Table 6.1**) to improve its stability, handling during transportation and to improve its commercial viability. Since use of sugars as lyoprotectant is generally not recommended in anti-diabetic formulations, so, we avoided the use of lyoprotectants which can increase blood glucose levels such as glucose, sucrose, dextrose, mannitol, sorbitol, trehalose, fructose and lactose etc. PEG 2000 was selected as the lyoprotectant of choice that showed good appearance of cake and high redispersibility. Since the availability of such lyoprotectants for use in diabetic formulations was limited, screening study for selection of lyoprotectant could not be performed.

Finally, lyophilized LSF-LA PLM powder was used as API in the tablet formulation. Freeze drying process involving freezing, dehydration and mechanical stresses can destabilize the micelles and lead to secondary aggregation and fusion.[27] However, several reports in literature confirm that the micelles maintain their structure after lyophilization process such as rifampicin loaded polymeric micelles made of poly(e-caprolactone)–b-poly(ethylene glycol)–poly(e-caprolactone) (PCL–PEG–PCL) block copolymers and Amphotericin B loaded poly(ethylene glycol) poly(lactide) (PEG-PLA) micelles.[28,29]

Excipients are added to the tablet formulations to facilitate bulkiness, disintegration, stability, patient compliance and for efficient drug delivery.[30] Well known excipients were selected for the preparation of tablets of LSF-LA PLMs according to their established uses in literature and their concentrations were chosen as per the recommendations of the Handbook of Excipients.[31] As LSF-LA *per se* was semisolid in nature, lyophilized powder of LSF-LA PLMs was quite hygroscopic, hence Fujicalin® SG was used as an adsorbent. Avicel® PH 102 and Aerosil® 200 were added to the formulation as directly compressible diluents. Additionally, Avicel® PH 102 also served as a binder and improved the flow property of the powder blend while Aerosil® 200 acted as a tablet disintegrant and improved the powder

flow. Avicel PH 102, Fujicalin SG® and Aerosil 200 used in tablet composition also possess adsorbent property. Specifically, Fujicalin SG® a spherically granulated anhydrous dicalcium phosphate, is considered as an innovative tableting excipient with interesting properties.[32] Spherical particles of anhydrous dicalcium phosphate are characterized to be highly porous with large specific surface area bearing a high liquid adsorption capacity.[33] These properties impart faster disintegration to Fujicalin based tablets, when in contact with the disintegration medium, wherein, Fujicalin adsorbs water quickly leading to the observed rapid fragmentation of the tablet.[32] Apart from Fujicalin, Aerosil 200 also exhibits disintegration properties. Hence, disintegrating agent was not used in tablet as it showed acceptable disintegration time (~6 min) with Fujicalin SG® and Aerosil 200. Magnesium stearate was added as a lubricant, to reduce the frictional forces between particles, and between particles and metal contact surface of tablet punches and dies. Talc was used as a glidant.

Powder blend was characterized before compression for its physical and flow properties such as bulk density, tapped density, Hausner's ratio, Carr's index and angle of repose. Flow properties of T1 and T2 powder blends indicated good to excellent powder flow. Prepared tablets exhibited adequate physical properties typical of an immediate release dosage form. Prepared tablets exhibited adequate physical properties typical of an immediate release dosage form. Particle size and drug release results of LSF-LA PLMs based tablet formulations showed that the manufacturing process did not influence LSF-LA PLMs release.

In our previous experiments, LSF-LA PLM exhibited stability in SGF and SIF (without enzymes) indicating their stability in the hostile environment of the GIT and being nano-sized would also be easily absorbed into the systemic circulation. Further, LSF-LA PLM tablet when assessed for the *in vitro* release of LSF in the simulated biological fluids (in the presence of enzymes) exhibited ~98 and 88% drug release into SGF and SIF respectively (**Figure 6.3A**). In SIF, complete release of LSF-LA was not observed which might be

attributed to partial cleavage of LSF-LA into free LSF by proteolytic enzymes (12-15%). LSF-LA released in SGF and SIF in the presence of enzymes from LSF-LA PLMs directly and from tablets (composed of LSF-LA PLMs) demonstrated a similar release profile with no significant difference which proved the stability of LSF-LA PLM even in presence of enzymes. LSF-LA PLM recovered from T2 tablet was assessed in MIN6 cells for cytotoxicity and it was found to be non-toxic in the presence of T2 formulation excipients also.

Stabilization of the micellar structure by the employment of amphiphilic polymers is well recognized to enhance not only the solubilization capacity but also the intestinal absorption of hydrophobic drug molecules.[21,34-36] So, after successful release of LSF-LA from LSF-LA PLM present in LSF-LA PLM tablet, the absorption of LSF-LA PLM from intestine was determined in comparison to LSF-LA SM and free LSF by intestinal permeability rat model using SPIP. In these studies, impact of LSF-LA PLM formulation on absorption of LSF-LA was assessed. LSF-LA permeability from LSF-LA PLM was increased by 1.8 folds than LSF-LA SM formulation and ~5.9 folds in comparison to free LSF.

Further, LSF-LA PLM tablet was assessed in PK studies by oral administration of crushed tablets. PK studies suggested that C_{max} increased 2 folds higher than LSF, LSF-LA conjugate as well as LSF-LA PLMs; indicating the possibility of use of LSF-LA PLM tablets in control of glucose levels after meal in diabetic conditions. Increased C_{max} resulted in lower relative bioavailability of LSF from LSF-LA PLM tablet (55.03 %) in comparison to LSF-LA PLM as AUC_{0-t} was decreased in case of tablets. Nevertheless, bioavailability of LSF-LA PLM tablet was more than that of free LSF and LSF-LA conjugate (~22 and 24 %) after oral administration. Further, LSF-PTX conversion was further reduced in comparison to LSF-LA PLM (AUC_{0-t} 279.30 ± 31.91 in tablets *vs.* 440.16 ± 30.75 in LSF-LA PLM).

5. Conclusion

Polymeric micelles have drawn considerable attention for encapsulation of conjugates bearing an ester linkage due to their ability to shield the conjugate from the enzymatic degradation by esterase in GIT. In this study, we successfully prepared scale-up batches of LSF-LA PLMs in nano-size range (145.3 nm) and narrow size distribution (PDI-0.121). Lyophilized LSF-LA PLMs were compressed into tablets using directly compressible excipients. These tablets released LSF-LA conjugate into SGF and SIF under *in-vitro* enzymatic conditions while maintaining the integrity of ester linkage. LSF-LA PLM from tablet did not show any toxicity in MIN6 cells and significantly improved the intestinal permeability of LSF-LA as observed in intestinal permeability rat model using SPIP. In PK studies, LSF-LA PLM tablet showed increased C_{max} (~2 folds) than LSF-LA PLM alone indicating the clinical application of the oral tablet dosage form for effective control of post prandial glucose levels in T1DM.

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