AAPS PharmSciTech (2021) 22: 114
DOI: 10.1208/s12249-021-01980-5

Research Article

Nanoparticulate tablet dosage form of lisofylline-linoleic acid conjugate for type 1 diabetes: *in situ* single-pass intestinal perfusion (SPIP) studies and pharmacokinetics in rat

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Received 1 January 2021; accepted 1 March 2021; published online 24 March 2021

Abstract. Lisofylline (LSF) is an anti-inflammatory molecule with high aqueous solubility and rapid metabolic interconversion to its parent drug, pentoxifylline (PTX) resulting in very poor pharmacokinetic (PK) parameters, necessitating high dose and dosing frequency. In the present study, we resolved the physicochemical and pharmacokinetic limitations associated with LSF and designed its oral dosage form as a tablet for effective treatment in type 1 diabetes (T1D). Self-assembling polymeric micelles of LSF (lisofylline-linoleic acid polymeric micelles (LSF-LA PLM)) were optimized for scale-up (6 g batch size) and lyophilized followed by compression into tablets. Powder blend and tablets were evaluated as per USP. LSF-LA PLM tablet so formed was evaluated for *in vitro* release in simulated biological fluids (with enzymes) and for cell viability in MIN-6 cells. LSF-LA PLM in tablet formulation was further evaluated for intestinal permeability (*in situ*) along with LSF and LSF-LA self-assembled micelles (SM) as controls in a rat model using single-pass intestinal perfusion (SPIP) study. SPIP studies revealed 1.8-fold higher oral absorption of LSF-LA from LSF-LA PLM as compared to LSF-LA SM and ~5.9-fold higher than LSF (alone) solution. Pharmacokinetic studies of LSF-LA PLM tablet showed greater C_{max} than LSF, LSF-LA, and LSF-LA PLM. Designed facile LSF-LA PLM tablet dosage form has potential for an immediate decrease in the postprandial glucose levels in patients of T1D.

KEY WORDS: LSF-LA PLM; nanoparticulate tablet; SPIP; phenol red; simulated biological fluids; type 1 diabetes; pharmacokinetics.

INTRODUCTION

Lisofylline (LSF) is an immunomodulatory, anti-inflammatory molecule and one of the major active metabolites of pentoxifylline (PTX) which has been used for intermittent claudication along with inflammation and autoimmune disorders over the last three decades (1, 2). LSF is one of the small molecules with proven efficacy in type 1 diabetes (T1D) (2). In T1D, LSF acts by protecting pancreatic beta cells, preventing autoimmunity, and suppressing the response of the host to proinflammatory cytokines. In our studies, we have also observed that LSF restores the glucose-stimulated insulin secretion under inflammatory conditions to a certain extent and mitochondrial metabolism to normal levels in T1D (3, 4). Therapeutically, it is a nonselective phosphodiesterase (PDE) inhibitor that undergoes metabolic interconversion to its parent drug PTX (5, 6). Thus, irrespective of the fact that which of these (LSF-PTX) is administered, both the molecules are detected in blood. It also has a challenging pharmacokinetic (PK) profile with a rapid metabolic rate, short half-life (~0.75 h), and a rapid clearance resulting in a high dose (in clinical trials, 12 mg/kg, s.c. and 9–12 mg/kg i.v. infusion over 10 h) and patient non-compliance

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Abbreviations: *Ø*, Angle of repose; *AUC*, Area under curve; *AUMC*, Area under first moment curve; *CAF*, Central animal facility; C_{max}, Maximum (or peak) concentration; *GIT*, Gastrointestinal tract; *HPLC*, High-performance liquid chromatography; *IAEC*, Institutional Animal Ethics Committee; *Ka*, Apparent first-order absorption rate constant; *LA*, Linoleic acid; *LSF*, Lisofylline; *LSF-LA*, Lisofylline-linoleic acid; *LSF-LA PLM*, Lisofylline-linoleic acid polymeric micelles; *LSF-LA SM*, Lisofylline-linoleic acid self-assembled micelles; *MCC*, Microcrystalline cellulose; *MIN-6*, Mouse insulinoma 6; *mPEG-b-P(CB-co-LA)*, Methoxy-polyethylene-glycol-b-poly(carbonate-colactide); *MRT*, Mean residence time; *MTT*, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; *PD*, Pharmacodynamics; *PDA*, Photodiode array; *PDE*, Phosphodiesterase; *PDI*, Polydispersity index; *PEG-2000*, Polyethylene glycol 2000; P_{eff}, Permeability coefficient; *PK*, Pharmacokinetic; *PLM*, Polymeric micelles; *PTX*, Pentoxifylline; *RPMI*, Roswell Park Memorial Institute Medium; *SGF*, Simulated gastric fluid; *SIF*, Simulated intestinal fluid; *SPIP*, Single-pass intestinal perfusion; *T1*, Tablets without LSF-LA PLM (blank tablets); *T2*, Tablets with LSF-LA PLM; $t_{1/2}$, Half-life; *T1D*, Type 1 diabetes; *USP*, United States Pharmacopeia.

(2, 7–9). Furthermore, clinical translation of this molecule has been severely impeded because of its physicochemical characteristics such as aqueous solubility (approx. 60 mg/mL) exhibiting low bioavailability and poor encapsulation in any delivery system (10, 11). Although it is a potent molecule, it is quite less explored and very few studies have been undertaken to improve the physicochemical and pharmacokinetic issues associated with LSF. In one such study, Cui *et al.* synthesized 32 analogs based on the structural motif of LSF wherein only two of these analogs were found to be effective in protecting β-cells under inflammatory conditions and in maintaining cell culture–based assessment of insulin secretory capability. Nonetheless, *in vivo* PK and pharmacodynamic data on these analogs has not been reported to evaluate if the synthesized analogs have improved the metabolic stability and oral bioavailability of LSF (12). Previously, our group has reported self-assembling micelles of LSF-fatty acid conjugate (LSF-LA SM) and its polymeric micelles (PLM)–based formulation (LSF-LA PLM) for oral delivery of LSF (3, 13). LSF-LA PLM enhanced the bioavailability of LSF and reduced *in vivo* LSF-PTX interconversion by protecting the ester bond of LSF-LA conjugate in harsh environment of GIT thus enabling oral delivery of LSF-LA PLM and providing a sustained release of LSF with respect to free LSF/LSF-LA conjugate (14). Although polymeric micelles provided considerable control over the PK and pharmacodynamics (PD) of LSF owing to their nano-size, enhanced surface area and shielding the ester linkage in LSF-LA in the acidic GI fluid; nonetheless, administration of these nano-sized particles to the patients still remains a challenge (15, 16). To meet this objective, the PLMs could have been administered as an aqueous dispersion in form of lyophilized powder for reconstitution (17). However, this creates an additional requirement of dispersion medium to be provided along with PLMs and reconstitution. Considering the drug's poor taste, the incorporation of nanoparticles (LSF-LA PLM) into solid dosage forms such as pellets, granules, or tablet appears to be a better alternative (18). Here, granules and pellets require capsules as final dosage form and a complex preparation method than directly compressible tablet dosage form (19, 20). Gelatin capsules are generally easier to swallow than tablets but are more expensive than tablets and are prone to degradation. For a chronic disorder like diabetes, multiple dosing is necessary to control blood glucose levels (21). Tablets offer multiple benefits including self-administration, being patient-friendly, longer storage stability, unit dosing, and controlled release of drug (22–24). Tablet dosage form of a lyophilized product is more advantageous than lyophilized powder itself as the lyophilized powder exhibits greater hygroscopicity than its tablets during storage. Upon reconstitution, lyophilized formulation may/may not be uniformly dispersed and after reconstitution must not be stored beyond a predetermined time period to avoid changes in particle size, size distribution, and zeta potential etc.; thus, tablet dosage form is more convenient for use (25–27). Therefore, tablet dosage form was preferred to carry and orally deliver LSF-LA PLM.

In this work, we present the preparation and pharmaceutical profiling of nanoparticulate tablet dosage form consisting of lyophilized LSF-LA PLM compressed into tablets and optimized for delivery by oral route. Prepared tablet formulation was further evaluated in *in vitro* release studies, for intestinal permeability using single-pass intestinal perfusion (SPIP) and in the oral PK studies in Wistar rat.

MATERIALS AND METHODS

Materials, Chemicals, and Experimental Animals

LSF (purity ≥99%, HPLC) was obtained from Cayman Chemicals Inc. (MI, USA). Linoleic acid (purity ≥99%) was procured from Hi-Media Laboratories (Mumbai, India). LSF-LA conjugate and mPEG-b-P(CB_{26}-co-LA_{50}) were synthesized and characterized in house before use (3, 13). Poly(ethylene glycol) (PEG, Mn 2000) was procured 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. Male Wistar rats (200–220 g) were purchased from Central Animal Facility (CAF), BITS PILANI (Pilani Campus). Animal testing was carried out as per the Institutional Animal Ethics Committee (IAEC, BITS Pilani)–approved protocols (IAEC/RES/27/10 and IAEC/RES/23/26) and CPCSEA guidelines.

Preparation of Tablets of LSF-LA Polymeric Micelles

For preparation of the LSF-LA polymeric micelle–compressed tablets, LSF-LA PLM were initially prepared using *in house* synthesized amphiphilic polymer, mPEG-b-P(CB_{26}-co-LA_{50}), in scaled-up batches (6 g) keeping theoretical loading as 12.5% w/w followed by its lyophilization using PEG 2000 as a lyoprotectant as reported by our group previously (13). In brief, the LSF-LA conjugate and the 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. The particle size and zeta potential of the polymeric micelles (at 10-fold dilution with ultra-pure water) were measured using a Zetasizer Nano-ZS (Malvern Instrument Ltd., UK) with a helium laser at 633 nm and the scattering angle was fixed at 173°. Thereafter, the resultant lyophilized powder along with other excipients was compressed in the form of tablet.

LSF-LA PLM Directly Compression into Tablets

LSF-LA PLM tablets were formulated using commercially available directly compressible excipients, Avicel® PH 102 (as diluent) and Fujicalin® SG (as adsorbent) by a direct compression method. For preparation of tablets (batch size: 60 tablets), Aerosil® 200 (as adsorbent and disintegrating agent) was sifted through sieve #30. Avicel® PH 102 and Fujicalin® SG were used as obtained since directly compressible grades 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 (1.66%), and talc (1.66%). Detailed tablet composition has been provided in Table I. 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 API. The blank and LSF-LA PLM tablets shall be designated as T1 and T2 throughout the text.

Pre-Compression Characteristics of Powder Blend of LSF-LA PLM Tablet

Powder blend used for direct compression of LSF-LA PLM tablet was assessed for the different characteristics of powder blend such as tapped and bulk density, compressibility index (Carr's index), and angle of repose following standard procedures as recommended by the pharmacopeia (28–30). All parameters were determined in triplicate ($n = 3$).

Bulk and Tapped Density

The bulk density was measured by calculating the volume occupied by a known powder weight using a measuring cylinder. For tapped density measurement, powder bed was tapped approx. 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 using bulk and tapped density parameters using Eqs. 1 and 2.

$$\text{Hausner's ratio} = \text{Tapped density/bulk density} \quad (1)$$

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

where V_0 = the initial volume of powder blend before tapping and V = the final constant volume of powder blend after approx.100 tappings.

Angle of Repose (Ø)

The powder blend was allowed to pass freely through a glass funnel (fixed at a height of 5 cm above the surface). Angle formed between a heap of powder blend and plane surface was determined by measuring the radius (r) and height (h) of the heap of the powder blend formed. The value of Ø was obtained using Eq. 3.

$$\text{Tan}\varnothing = h/r \quad (3)$$

Table I. Composition of Tablet Formulations

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

Evaluation of LSF-LA PLM Tablets

Drug Content

For the assessment of drug content from LSF-LA PLM tablets, LSF-LA polymeric micelles are first separated/recovered from the compressed tablet by the method depicted in Fig. 1. Briefly, T2 tablets were allowed to undergo complete dissolution in 1 mL of distilled water at 100 rpm, 37°C for 6 h. The dissolution media after 6 h was processed by fractional centrifugation at 1500 rpm (to settle down larger fragments/particles of excipients) and 5000 rpm (to remove residual particles of excipient) for 10 min each sequentially. The supernatant so obtained after centrifugation at 5000 rpm (containing polymeric micelles) was analyzed for content of LSF-LA using HPLC. Here, T1 tablets were processed only as control to prove recovery of PLM.

Weight Variation, Hardness, Friability, and Disintegration Test

Weight Variation. For carrying out this test, twenty randomly selected tablets (each of T1 and T2) were individually weighed on the analytical balance and the average mean weight and variation were calculated.

Tablet Hardness. The tolerance of tablets to capping or breakage during packaging, shipping, and handling environments prior to use depends on their hardness or crushing strength. So, tablet hardness for randomly picked six tablets of each formulation was determined during tablet compression using Monsanto hardness tester (Labpro, India).

Friability. Roche friability tester was used for tablet friability testing wherein 20 tablets were weighed, placed into the tablet compartment of the machine, and made to undergo 25 rotations at fixed rpm (25) for 4 min. Any crushed tablets were removed. After dusting, all tablets were reweighed and percentage loss in tablet weight was determined.

Disintegration. A standard disintegration apparatus (DBK Instruments, Mumbai, India) as described in USP was used for disintegration testing of the tablets. One T1 or T2 tablet was placed in each of the tubes in the basket rack assembly and suspended in a 1-L beaker containing disintegration medium (distilled water) at controlled temperature 37 ±2°C. Disintegration time of the tablets was recorded.

In vitro evaluation of tablets

LSF-LA Release Study from the Tablet

The oral bioavailability of any drug can possibly be demonstrated by determining the release of the drug in

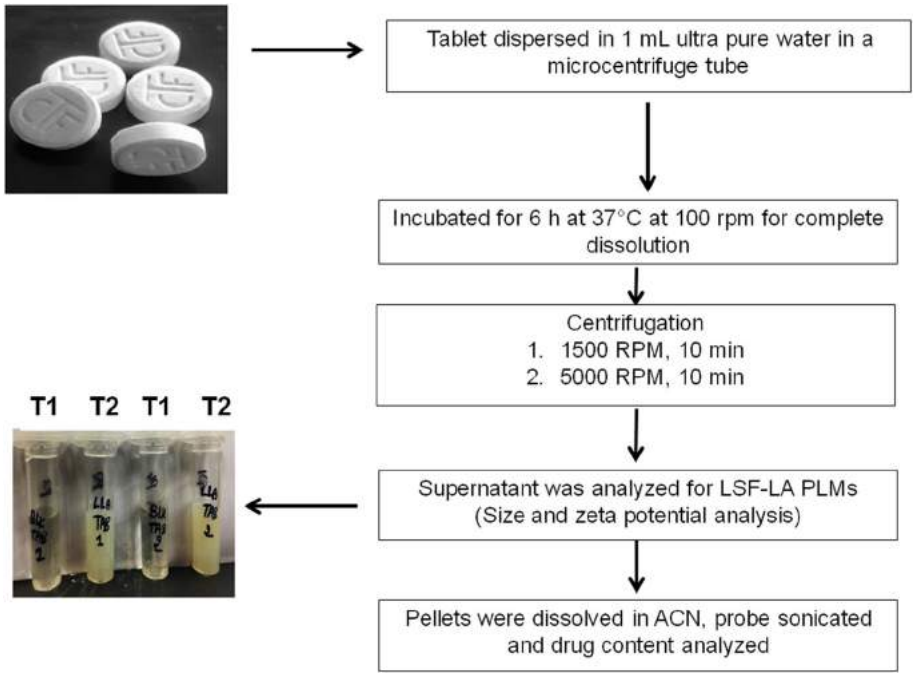

Fig. 1. Recovery of LSF-LA PLM from tablets

physiological fluids/media such as simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). So, *in vitro* release of LSF-LA from the tablet was studied in SGF and SIF in the presence of enzymes as per USP. *Composition of SGF (with Enzyme).* It was prepared by dissolving NaCl (200 mg) and HCl (36% v/v, 0.7 mL) in triple-distilled water (100 mL) and the pH of the solution was adjusted to 1.2 (using HCl). After adjustment of pH, 3.2 mg pepsin (porcine pepsin 3000 units/mg of protein) per milliliter of media was added (31).

Composition of SIF (with Enzyme). It was prepared by dissolving the KH_2PO_4 (680 mg) and NaOH (616 mg) in 100-mL triple-distilled water, then the pH of the solution was set to 6.8 using 0.2 N HCl/0.2 N NaOH. After pH adjustment, 10 mg pancreatin per milliliter of media was added (31).

In Vitro Release Study. T2 tablets (06; containing LSF-LA PLM) were added to 10 mL of SGF and SIF (in triplicate) individually and incubated at 37 ± 0.5°C at 100 rpm. 200 μL of sample was withdrawn at each predetermined time point from SGF (0, 10, 20, 30, 45, 60, 90, 120, and 240 min) and from SIF (0, 10, 20, 30, 45, 60, 90, 120, 180, 240, and 360 min) without replacement of release media. Each sample aliquot was quenched with acetonitrile (ice-chilled; 800 μL) and vortexed for 30 s followed by centrifuged to remove the enzymes and tablet excipients, then the clear supernatant was analyzed for content of LSF-LA using HPLC.

Cell Viability Study

MIN-6 (mouse insulinoma-6) cells, which mimic the characteristics of pancreatic beta cells, were used to assess toxicity of LSF-LA PLM tablet. In RPMI media, cells were grown and incubated at 5% CO_2 and 37°C. In 96 well cell culture plate, 5×10^3 MIN6 cells (per well) were seeded 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). LSF-LA PLM was recovered from T2 tablet as detailed in the "Drug Content" section and the supernatant was designated as T2 solution. T2 suspension and solution were added to the cells and incubated at 37°C/5% CO_2 for 48 h. Cells without any treatment and cells treated with free LA, LSF, LSF-LA SM, and LSF-LA PLM formulations at equivalent concentrations (all at ~20 μM) and T1 tablet solution and suspension (in equal volume to T2 tablet solution and suspension) were kept as controls. After 48 h of treatment, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was performed for cell viability as per standard protocol.

In situ absorption studies of LSF-LA PLM: SPIP

Preparation of Perfusion Solution

For SPIP, perfusion solution/media was prepared with the following composition: 48 mM NaCl, 28 mM Na_2HPO_4, 43 mM NaH_2PO_4, 5.4 mM KCl, 35 mM mannitol, 10 mM D-glucose, and 1 g/L PEG-4000 in ultra-pure water (32, 33). pH of the perfusion solution was adjusted to 6.5 using HCl or NaOH. To compensate for any water absorption or discharge that could occur during the experiment, a non-absorbable marker, phenol red (50 μg/mL), was added to the perfusion solution (34, 35). The SPIP assays were performed at concentration of drug/formulation as ~30 μg/mL of free LSF.

SPIP Procedure

In the SPIP studies, preliminary experiment was carried out to make sure that any loss of the drug observed is only due to its absorption/permeation and not due to any other reason, such as the drug's nonspecific binding and/or degradation in the tube (33, 36). To determine the impact of drug binding to the tube, LSF, LSF-LA SM, and LSF-LA PLM perfusion solutions were incubated at 37°C with perfusion tubing for 2 h. Likewise, the perfusion solutions containing LSF/LSF-LA SM/LSF-LA PLM were incubated at 37°C for 2 h to test the drug's stability in perfusion solution. After 2 h, aliquots were collected and drug content was determined by HPLC at λ=273 nm. Here, LSF-LA SM was prepared as per our previous report (3).

For *in situ* SPIP studies, Wistar rats (220–250 g) were exposed to 12-h light–dark cycle, and giving a fasting period of 12–18 h, water was provided *ad libitum* before conducting an experiment. For experiment, rats were anaesthetized by i.p. administration of ketamine (100 mg/kg) and xylazine (20 mg/kg) mixture, and body temperature was maintained at 37 ± 1°C using a heating pad and lamp. Laparotomy was carried out wherein midline incision of approx. 3–4 cm was made in the abdomen to isolate the small intestine and approx. 15–20 cm of the proximal jejunum area of intestine was cannulated using plastic tubing (2 mm o.d.) at both ends. The intestinal segment was rinsed with blank perfusion solution (free of drug) maintained at 37 ± 1°C for approximately 25–30 min at a flow rate of 0.5 mL/min until the solution coming from the outlet was visually clear. Thereafter, the perfusion fluids (containing drug/formulation) were infused at a 0.2-mL/min flow rate into the intestinal lumen of the rat. Initially, the perfusion solution (containing drug/formulation) was perfused for a period of 1 h to achieve a steady state (drug equilibrium with intestinal membrane). After reaching the a steady state, perfusate was collected at every 15-min time interval (15, 30, 45, 60, 75, 90, 105, 120 min). Samples withdrawn were immediately frozen at −80°C until analyzed. After completion of the study, rats were euthanized by intracardiac injection of saturated KCl solution (34). Afterwards, the cannulated intestinal segment was carefully isolated for measurement of its length (L) and radius (r).

Instrumentation, Chromatographic Conditions, and Sample Analysis

HPLC method for simultaneous detection of eluents (LSF and LSF-LA) and phenol red was developed by modifying the reported method (37, 38). HPLC (Shimadzu, Japan) with photodiode array detector (PDA) was used to analyze the LSF/LSF-LA content in perfusate samples. Before starting the sample analysis, the HPLC column (Inertsil® C18, 25 cm × 4.6 μm, 5 μm) was equilibrated for 40 min. The wavelengths of the eluents (LSF and LSF-LA) and phenol red were selected as 273 and 398 nm respectively.

LSF and phenol red contents were simultaneously determined using sodium acetate buffer (10 mM; pH 3.5) and 1:1 mixture of methanol and acetonitrile at 50:50% v/v mobile phase ratio at flow rate of 1.0 mL/min. Furthermore, for the analysis of LSF, LSF-LA, and phenol red, 10 mM sodium acetate buffer (pH 3.5) and acetonitrile (05:95, % v/v) were used as mobile phase at 1.0-mL/min flow rate. Sample analysis was carried out by centrifuging the perfusate samples (15,000 rpm for 10 min); supernatant was filtered through a 0.22 μm membrane filter and diluted with acetonitrile and subjected to HPLC analysis.

Determination of LSF/LSF-LA Permeability

Once the drug absorption attained a steady state in the intestine, LSF/LSF-LA permeability was determined (39). Here, achievement of steady state was verified by plotting the ratio of outlet to inlet drug concentration *vs* 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 Eq. 4 (7, 35).

$$C'_{out}/C_0'_{in} = [C_{out}/C_{in}] \times \left[C_{in\ (phenol\ red)}/C_{out\ (phenol\ red)}\right] \quad (4)$$

where C_{out} is the concentration of free 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. Equation 5 was used to calculate the value of effective permeability coefficient (P_{eff} cm/s) (7, 35).

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

where Q denotes the flow rate (mL/min) of entering perfusate, C'_{out}/C'_{in} is corrected ratio of outer concentration to inlet perfusate, and A is the surface area (cm^2). Considering the intestinal portion used here to be cylindrical ($2\pi rL$), surface area was calculated taking length (L) at 15–20 cm and radius r equivalent to 0.21 cm for the jejunum segment of intestine. The apparent first-order absorption rate constant (Ka min^{-1}) was determined as per Eq. 6 (35).

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

Pharmacokinetics of LSF-LA PLM Tablet

LSF-LA PLM tablet PK experiment was conducted on healthy Wistar rats (200–220 g; n=4). LSF-LA PLM tablet was crushed and dispersed in distilled water. After overnight fasting, LSF-LA PLM tablet dispersion (10 mg/kg ~LSF) was given to fasted normal rats by oral route using oral gavage. Upon dosing, blood sampling was performed from retro-orbital plexus at predetermined time points up to 24 h. Blood samples were subjected to plasma separation and immediately stored at −80°C until the analysis. Plasma samples were analyzed for both LSF and PTX (since LSF and PTX are interconvertible *in vivo*) using our previously reported and validated bioanalytical method (40). Briefly, 200 μL of plasma sample was taken in a glass tube (5 mL), followed by addition of 50 μL of I.S. (IBMX: 3-isobutyl methylxanthine; 2 μg/mL). Then, the sample was extracted by using 2 mL of dichloromethane and centrifuged at 3500 rpm for 15 min at 4°C.

Resulting organic layer was collected, dried, and reconstituted with 100-μL mobile phase (methanol: water; 1:1) and LSF and PTX were analyzed by HPLC. Plasma concentration *vs* time profiles of LSF and PTX were plotted and various PK parameters were determined using non-compartmental approach by WinNonlin software.

Statistical Analysis

Statistical analysis was performed using one-way ANOVA followed by Tukey–Kramer multiple comparison post-test using graph pad prism (Version 6.01) software.

RESULTS

Preparation and Lyophilization of Scale-Up Batches of LSF-LA PLM

LSF-LA PLM prepared by thin-film hydration method exhibited similar particle size and zeta potential values as reported earlier in our paper (13). It exhibited self-assembly with an average particle size and zeta potential of 147.2 nm (PDI: 0.103) and −2.92 ± 3.74 mV respectively. EE was calculated as 77.66 ± 1.86% with a theoretical drug loading of 12.5%. After lyophilization, LSF-LA PLM exhibited a particle size of 183.1 nm (PDI: 0.227) and zeta potential as −8.93 ± 3.29 mV (Fig. 2) with a practical drug loading of 11.49 ± 0.76%.

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. Pre-compression properties of powder blend are shown in Table IIA. Hausner's ratio and compressibility index were estimated 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 tablets respectively.

Evaluation of LSF-LA PLM Tablets

Drug Content, Weight Variation, Hardness, Friability, and Disintegration Tests

T1 (control) and T2 (LSF-LA PLM tablets) were clean and exhibited uniformity in size as well as color and were free of any mottling on the surface (Fig. 1). As shown in Table II B, LSF-LA content in the tablets was uniform in the range of 85–115% and tablet weight ranged from 145 to 156 mg falling within the stipulated limit of weight variation as per USP (±7.5%). Upon dissolution of the T2 tablets, LSF-LA PLM was still found to be intact with an average particle size of 117.5 nm (PDI: 0.196) and zeta potential of −2.59 ± 7.09 mV (Fig. 2).

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

LSF-LA Release Study from the Tablet in Simulated Biological Fluids

LSF-LA PLM tablets (T2) release studies demonstrated that the released LSF-LA was stable even in the presence of enzymes, 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 (Fig. 3a). This study clearly hinted at the feasibility of delivering LSF as LSF-LA PLM tablet by oral route of administration.

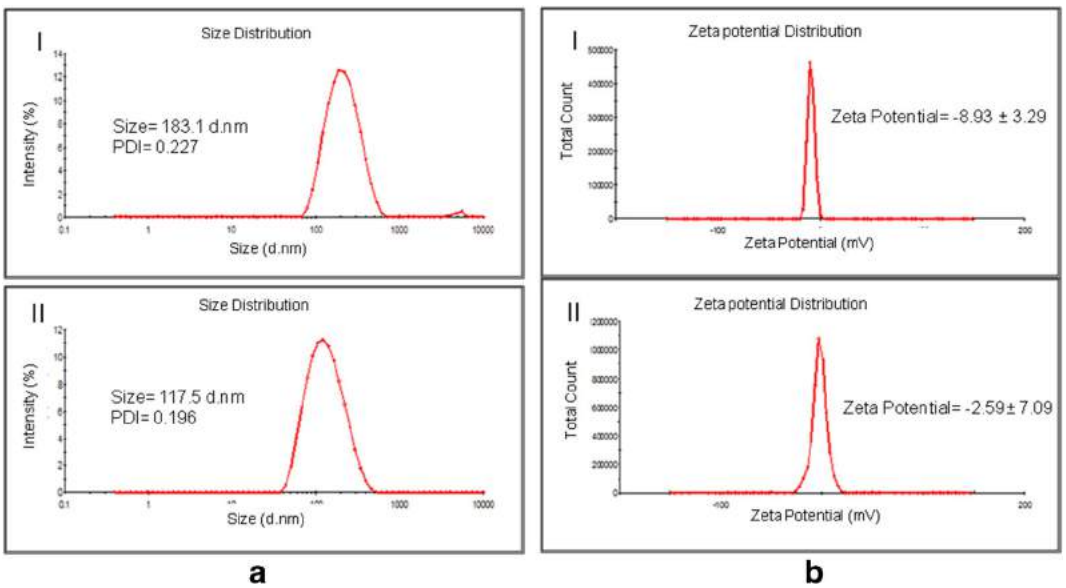

Fig. 2. a, **b** Lyophilized LSF-LA PLM formulation: particle size and zeta potential. (I) In powder blend (before compression) and (II) after recovery from the tablet

Table II. (A) Evaluation of Physical Properties of Powder Blend Prepared for LSF-LA PLM Tablets. (B) Evaluation of LSF-LA PLM Tablets

Parameters	T1	T2
(A)		
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
(B)		
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

Cell Viability Assay

After co-incubating the cells with LSF-LA PLM tablet solution and suspension, cell viability of MIN-6 cells was assessed to confirm cytotoxicity of any of the tablet excipients or LSF-LA PLM used in T1 or T2 tablet. LSF-LA PLM and other tablet excipients are found to be non-toxic to MIN6 cells as depicted in Fig. 3b.

In Situ Absorption Studies of LSF-LA PLM: SPIP

In situ intestinal permeability of free LSF/LSF-LA formulations was studied using the jejunum segment of rat's intestine by SPIP technique, wherein permeation of the LSF or LSF-LA through the intestine at its steady state was examined. Representative chromatogram for simultaneous detection of LSF, LSF-LA, and phenol red is shown in Fig. 3c. As shown in Fig. 4a–c, the C_{out}/C_{in} ratios attained a plateau with time in all the test solutions which confirmed the steady state of drug with intestinal tissue. Meanwhile, no nonspecific binding of LSF/LSF-LA was seen to the tubing and drugs were stable in the perfusion solution during the entire period of the experiment. Using average of C_{out}/C_{in} data gathered for the different perfusion solutions at all the subsequent 15-min intervals over a 2-h period, P_{eff} and *Ka* values were calculated. As shown in Fig. 4d, in comparison to the free LSF solution, both the LSF-LA formulations showed significantly improved permeability of LSF. Compared to free LSF solution, LSF-LA SM exhibited 3.1-fold (1.58 ± 0.39 *vs* 4.95 ± 0.21 ($\times 10^{-4}$) cm/s) increase in intestinal permeability of LSF. Encapsulation of LSF-LA conjugate into the amphiphilic polymer resulted in further significant (p <0.05) increase in the permeability of LSF-LA by 1.8-fold (9.36 ± 0.94 ($\times 10^{-4}$) cm/s) and ~5.9-fold with respect to free LSF. The *Ka* values for free LSF, LSF-LA SM formulation, and LSF-LA PLM

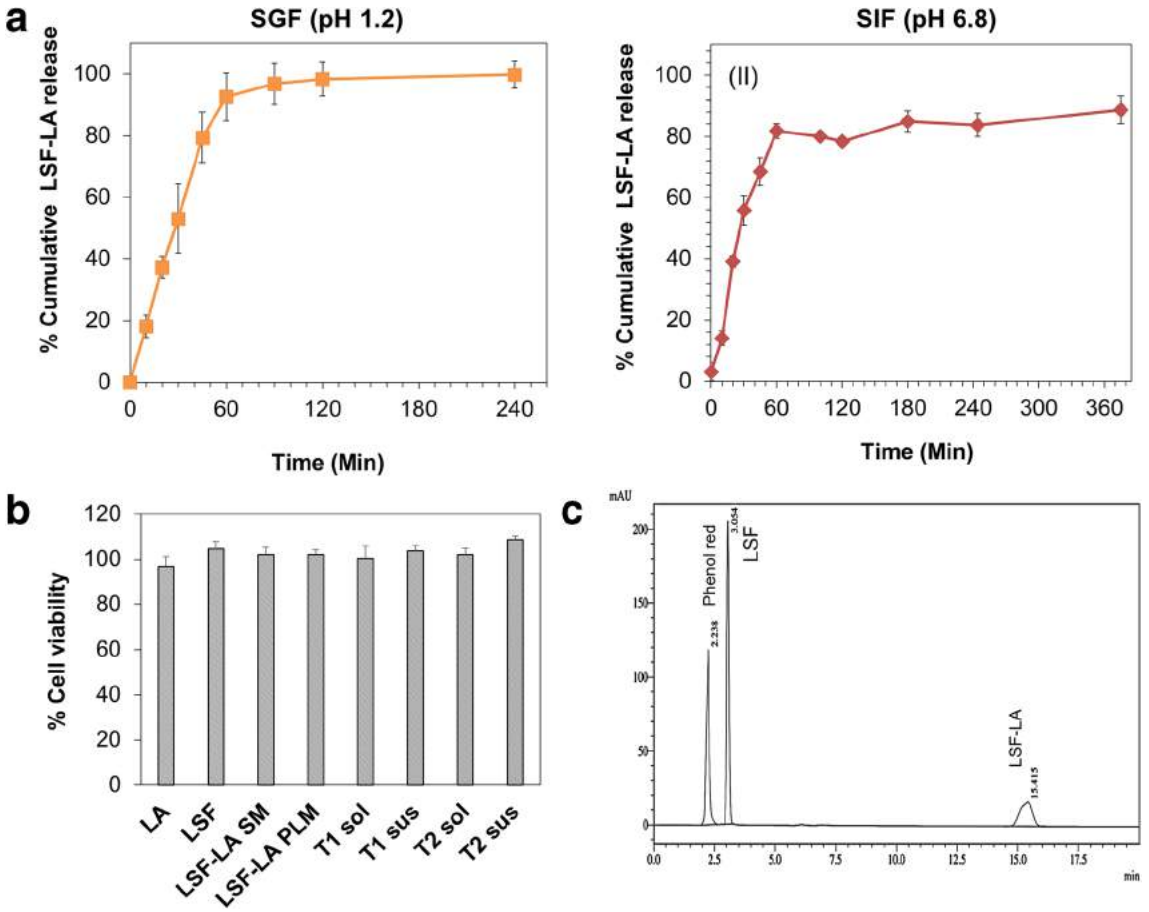

Fig. 3. Characterization of the LSF-LA PLM tablet. **a** Drug release from LSF-LA PLM tablet (T2) in SGF and SIF, both containing enzymes and **b** cytotoxicity assay in MIN6 cells. **c** Representative HPLC chromatogram for simultaneous determination of LSF, LSF-LA, and phenol red

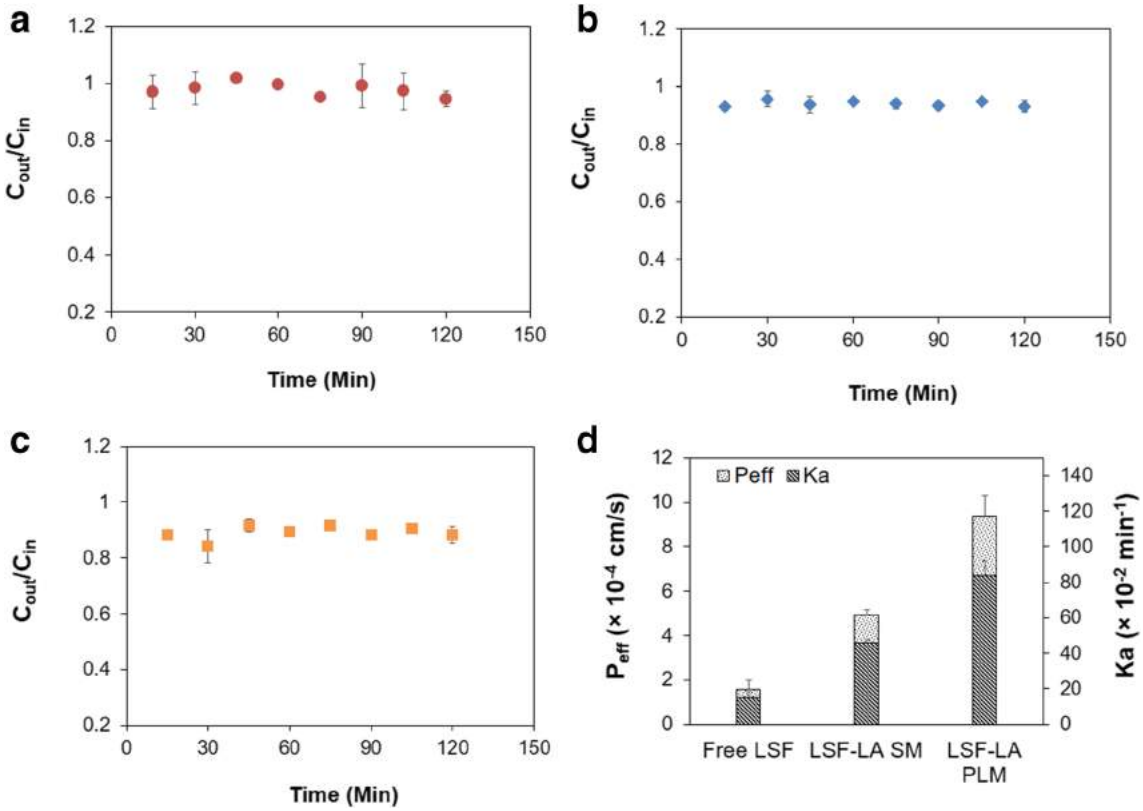

Fig. 4. *In situ* permeability studies using SPIP rat model. Representative plots for the ratio of outlet to inlet drug steady-state concentration (C_{out}/C_{in}) *vs* time for **a** LSF, **b** LSF-LA SM, and **c** LSF-LA PLM, and **d** effective permeability coefficient (P_{eff}) and apparent first-order absorption rate constant (*Ka*) of LSF, LSF-LA SM, and LSF-LA PLM. Values represent mean ± SEM (n = 3)

were found to be 14.90 ± 3.60, 45.70 ± 1.70, and 84.10 ± 7.70 min^{-1} respectively.

Pharmacokinetics of LSF-LA PLM Tablet

LSF-LA PLM tablet PK study revealed that there was some improvement in oral PK parameters of LSF in comparison to LSF and its nanoformulations (Table IIIA and B). LSF-LA PLM tablet showed a C_{max} of 2710.34 ± 434.66 ng/mL which is ~2-fold higher than LSF, LSF-LA SM formulation, and LSF-LA PLM (1138.64 ± 134.72, 1168.40 ± 89.73, 1449.39 ± 119.09 respectively) (13). However, LSF-LA PLM tablet exhibited a reduced half-life (0.80 h from 2.09 h) and MRT (0.98 h^{-1} from 2.41 h^{-1}) of LSF than LSF-LA PLM. By oral route, AUC for LSF-LA PLM tablet was ~1.68 times higher than LSF-LA SM formulation (2340.40 ± 155.06 *vs* 1389.33 ng/h/mL). Independent *t*-test was applied between LSF-LA PLM and LSF-LA PLM tablet group to verify a significant difference which indeed was found to be significant. For significant difference, p values for AUC, AUMC, and half-life were found to be 0.0313 ($p < 0.05$), 0.001($p < 0.001$), and 0.0001 ($p < 0.001$) respectively between these groups. The plasma concentration (Fig. 5) profile of PTX revealed a substantial decrease in the metabolism of LSF to PTX when LSF-LA was administered as a tablet in comparison to free LSF or its nanoformulations (13) which also suggested that the rate of LSF-PTX *in vivo* metabolism decreased substantially when LSF-LA was administered as polymeric micelles, and it decreased further when LSF-LA PLM was compressed into a tablet dosage form.

DISCUSSION

Insulin is considered the gold standard for the treatment of T1D patients. Several conventional and polymeric and lipid-based nanoformulations of insulin have been reported in literature; however, several drawbacks are associated with long-term administration of insulin such as a too late plasma peak of insulin due to changes in gastric emptying rate of T1D patients (resulting in improper balance of postprandial glucose absorption), increased cardiovascular risks, obesity, and hyperglycemia. Thus, novel non-insulin adjunct therapies need to be explored in patients with T1D (41, 42). Apart from insulin, several plant-derived therapeutics are reported for treatment of diabetes (43). Ganugula *et al.* reported a PLGA based-curcumin nanosuspension (nanocurcumin) which was found to be safe and efficacious in improving beta cell function and may prevent T1D (44). For most of the drugs intended for chronic ailments, oral formulations are the first choice for patients owing to the ease of self-administration and being non-invasive in nature (15, 45). For oral products, sterile manufacturing conditions are not required which minimizes their production costs. For peroral delivery of NPs (here polymeric micelles), hybrid dosage forms of NPs, for example, NPs compressed into a tablet dosage form serve as a superior alternative in comparison to the direct

Table III. (A) The Non-Compartmental Pharmacokinetic Parameters for Free LSF and LSF-LA SM (Oral) PK*. (B) The Non-Compartmental Pharmacokinetic Parameters for LSF-LA PLM and LSF-LA PLM Tablets (Oral) PK*

Parameters	LSF	PTX	LSF	PTX
	Free LSF, oral (mean ± SEM)		**LSF-LA SM, oral (mean ± SEM)**	
(A)				
C_{max} (ng/mL)	1138.64 ± 134.72	504.36 ± 38.44	1168.40 ± 89.73	409.55 ± 50.92
$t_{1/2}$ (h)	0.61 ± 0.06	0.69 ± 0.07	0.72 ± 0.03	0.67 ± 0.09
K_e (1/h)	0.94 ± 0.06	1.04 ± 0.11	0.96 ± 0.04	1.12 ± 0.18
AUC0-last (ng/h/mL)	1186.75 ± 70.40	491.12 ± 22.86	1389.73 ± 111.51	406.33 ± 71.28
AUC0-∞ (ng/h/mL)	1321.69 ± 63.34	547.20 ± 18.29	1473.64 ± 124.12	471.32 ± 100.42
AUMC0-last (ng/h/mL)	962.17 ± 35.82	379.06 ± 8.68	1273.34 ± 122.10	303.23 ± 64.17
AUMC0-∞ (ng/h/mL)	1379.63 ± 27.71	549.76 ± 37.80	1614.18 ± 176.66	508.83 ± 167.07
MRT (h)	0.81 ± 0.02	0.77 ± 0.02	0.91 ± 0.02	0.72 ± 0.05
	LSF-LA PLM, oral (mean ± SEM)		**LSF-LA PLM tablet, oral (mean ± SEM)**	
(B)				
C_{max} (ng/mL)	1449.39 ± 119.09	121.12 ± 12.30	2710.34 ± 434.66[#]	222.79 ± 41.89
$t_{1/2}$ (h)	2.09 ± 0.05[@]	2.96 ± 0.35	0.80 ± 0.02[$]	0.68 ± 0.05
K_e (1/h)	0.33 ± 0.01	0.24 ± 0.03	0.86 ± 0.03	1.06 ± 0.08
AUC0-last (ng/h/mL)	4252.69 ± 125.07	440.16 ± 30.75	2340.40 ± 155.06[§]	279.30 ± 31.91
AUC0-∞ (ng/h/mL)	4409.34 ± 132.71	492.49 ± 28.78	2364.88 ± 147.30	286.10 ± 32.56
AUMC0-last (ng/h/mL)	10,264.00 ± 531.25	1195.61 ± 82.78	2047.21 ± 60.62	294.71 ± 27.11
AUMC0-∞ (ng/h/mL)	11,993.57 ± 568.57	1851.07 ± 172.48	2170.68 ± 65.78	343.08 ± 29.96
MRT (h)	2.41 ± 0.07	2.72 ± 0.09	0.98 ± 0.04	1.22 ± 0.02

*LSF (lisofylline), LSF-LA SM (lisofylline-linoleic acid self-assembled micelle), and LSF-LA PLM (lisofylline-linoleic acid polymeric micelle) formulation PK (pharmacokinetic) data has been adapted with permission from Italiya *et al.* (2019). Copyright (2019) American Chemical Society. (13)

[#$] LSF-LA PLM tablet *vs* LSF-LA PLM, LSF-LA SM, and LSF ([#] $p<0.05$; [$] $p<0.001$)

[$] LSF-LA PLM tablet *vs* LSF-LA PLM ([$] $p<0.001$)

[@] LSF-LA PLM *vs* all groups ([@] $p<0.001$)

administration of lyophilized NPs as well as conventional tablets (18).

Nanomedicines formulated for oral route of administration offer several benefits including enhancement of the drug solubility and its half-life, controlled release, targeted delivery, improved bioavailability, and minimized side effects. This can address the major drawbacks associated with existing anti-diabetics such as poor solubility, permeability, and hence poor bioavailability (46, 47). Freeze-drying of nanomedicines further adds to these advantages as reported in literature wherein freeze-dried febuxostat-loaded self-nanoemulsifying self-nanosuspension systems (SNESNS) demonstrated improved solubility and enhanced oral bioavailability (2-fold increase) of febuxostat in comparison to the marketed formulation (48). Judicious screening of the polymeric carriers for designing the nanoformulation such as NPs which are further lyophilized and compressed into tablets holds immense potential to provide a targeted release profile along with improving the PK and PD profile of drugs particularly, those having a short half-life, such as LSF. This ensures better management of disease and can also improve patient compliance as well as dosing regimen (49, 50). Similar

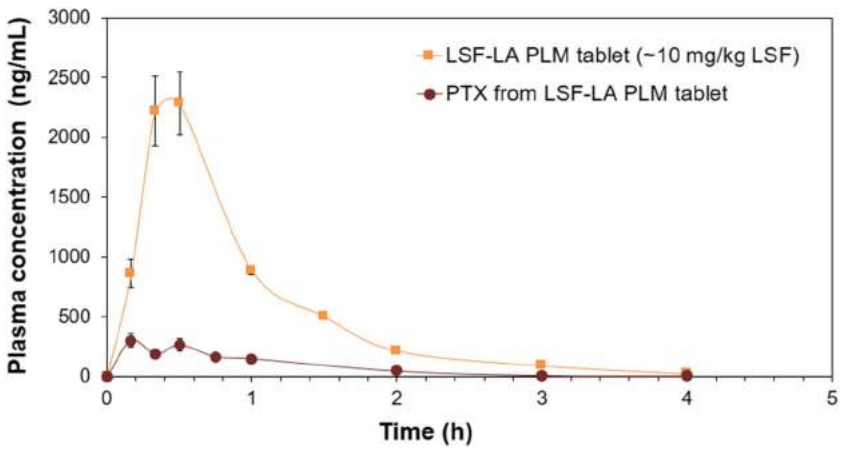

Fig. 5. Plasma concentration-time profile for LSF-LA PLM tablet in rats (~10 mg/kg LSF, oral; mean ± SEM, *N*=4)

attempts have also been made previously wherein naringin (NG), an anti-inflammatory compound, was loaded into mPEG-PCL polymeric micelles (PLM) followed by freeze-drying and direct compression into buccal tablets. NGPLM tablet demonstrated increased solubility of NG and improved release profile which might enable its better efficacy as an anti-ulcer agent in oral diseases (51). Polymeric micelles have been reported to prevent *in vivo* degradation of the drug which might result due to enzymes or other environmental factors (14). Likewise, we previously reported LSF-LA polymeric formulation, LSF-LA PLM which protected the ester linkage of synthesized LSF-LA conjugate in GIT and render it suitable for oral administration. Furthermore, to ensure efficient drug delivery and patient compliance and considering a multiple dosing regimen in diabetes, in this study, LSF-LA PLMs were compressed into a tablet dosage form.

A typical pharmaceutical dosage form consists of API and excipients. Using our previously optimized scale-up and lyophilization conditions, LSF-LA PLM was prepared and lyophilized using PEG 2000 as lyoprotectant. Finally, lyophilized LSF-LA PLM powder (containing PEG 2000) was used as API in the tablet formulation. Excipients are used in the tablet formulations to improve bulkiness, disintegration, and stability for efficient drug delivery with better patient compliance (52). Commercially available excipients were selected for the preparation of tablets of LSF-LA PLMs based upon their established uses in literature and their concentrations were chosen as per the recommendations of the "Handbook of Pharmaceutical Excipients" (53). 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 (microcrystalline cellulose) and Aerosil® 200 were added to the tablet formulation as directly compressible diluents. Additionally, Avicel® PH 102 also served as a binder while Aerosil® 200 acted as glidant to improve the powder flow. Avicel PH 102, Fujicalin SG®, and Aerosil 200 used in tablet composition are also possess adsorbent property. Specifically, Fujicalin SG® is a highly porous granulated form of anhydrous dicalcium phosphate having a large specific surface area that facilitates liquid adsorption and faster disintegration (54). Apart from Fujicalin, Aerosil 200 also exhibits disintegration properties. Hence, no other disintegrating agent was used in the tablet as it showed acceptable disintegration time (~6 min) with Fujicalin SG® and Aerosil 200. Mg stearate was added as a lubricant to reduce the friction between tablet and the surface of dies and Talc was used as a glidant.

Powder blend was characterized before compression for its physical and flow properties. Flow properties of T1 and T2 powder blends indicated good to excellent powder flow. Prepared tablets exhibited adequate post compression characteristics typical of an immediate release dosage form.

In our previous experiments, LSF-LA PLM exhibited stability in SGF and SIF (without enzymes) indicating their stability in GIT and being nano-sized, these would also get absorbed quickly into the systemic circulation (13). Furthermore, LSF-LA PLM tablet when assessed for the *in vitro* release of LSF in the simulated biological fluids (containing enzymes) exhibited ~98 and 88% drug release into SGF and SIF respectively (Fig. 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 simulated biological fluids from LSF-LA PLM 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 polymeric micelles 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.

It is well recognized that the micelles formulated using amphiphilic polymers improve not only the absorption of the hydrophobic drugs through intestinal membrane but also their solubilization (7, 55–57). So, after successful release of LSF-LA from its polymeric micelles present in LSF-LA PLM tablet, the absorption of LSF-LA polymeric micelles from intestine was determined in comparison to LSF-LA self-assembling micelles and free LSF by intestinal permeability rat model using SPIP. In these studies, impact of LSF-LA loaded PLM on absorption of LSF-LA when compared to its SM formulation and free LSF revealed ~1.8- and 5.9-fold increase (p <0.05) in permeability respectively.

Furthermore, LSF-LA PLM tablet was assessed in PK studies by oral administration of crushed tablets. PK studies suggested that C_{max} increased by 2-fold higher than free LSF, LSF-LA nanoformulations, indicating the possibility of use of LSF-LA nanoparticulate tablets for control of glucose levels after meal in diabetic conditions. Increased C_{max} resulted in lower relative bioavailability of LSF from LSF-LA PLM tablets (55.03%) in comparison to LSF-LA PLM as AUC_{0-t} was decreased in the case of tablets. Nevertheless, bioavailability of LSF-LA PLM tablet was more than that of free LSF and LSF-LA prodrug (~22 and 24%) after oral administration. Moreover, LSF-PTX conversion was much 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).

CONCLUSION

Polymeric micelles have drawn considerable attention for encapsulation of prodrugs bearing an ester linkage due to their ability to shield the prodrug from the enzymatic degradation by esterase in GIT. In this study, we successfully prepared LSF-LA PLM in 6 g batch size while still retaining their nano-size (145.3 nm with 0.121 PDI). Lyophilized LSF-LA PLMs were compressed into tablets using directly compressible excipients. These tablets released LSF-LA SM 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-fold) 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.

ACKNOWLEDGMENTS

The authors acknowledge Gangwal Chemicals Pvt. Ltd. (Mumbai, INDIA) for providing Fujicalin SG® as a gift sample for this work.

FUNDING

The work elaborated in the study was funded by SERB-DST, Govt. of India research grant #YSS/2014/000551 and DST-INSPIRE, fellowship to K.S.I. [#IF160659].

DECLARATIONS

Conflict of interest The authors (DC and AM) are the founding directors of Nanobrid Innovations Private Limited that is involved in the development of nanotechnology-based products. They have business and/or financial interest in the operations of the company. The same could be disclosed on request. The authors declare that they have no conflict of interest pertaining to the work outlined in this study.

REFERENCES

1. McCarty MF, O'Keefe JH, DiNicolantonio JJ. Pentoxifylline for vascular health: a brief review of the literature. Open Heart. 2016;3(1):e000365.
2. Yang Z, Chen M, Nadler JL. Lisofylline: a potential lead for the treatment of diabetes. Biochem Pharmacol. 2005;69(1):1–5.
3. Italiya KS, Mazumdar S, Sharma S, Chitkara D, Mahato RI, Mittal A. Self-assembling lisofylline-fatty acid conjugate for effective treatment of diabetes mellitus. Nanomedicine: NBM. 2019;15(1):175–87. https://doi.org/10.1016/j.nano.2018.09.014.
4. Chen M, Yang Z, Wu R, Nadler JL. Lisofylline, a novel antiinflammatory agent, protects pancreatic β-cells from proinflammatory cytokine damage by promoting mitochondrial metabolism. Endocrinology. 2002;143(6):2341–8. https://doi.org/10.1210/endo.143.6.8841.
5. Świerczek A, Wyska E, Pociecha K, Baś S, Mlynarski J. Influence of inflammatory disorders on pharmacokinetics of lisofylline in rats: implications for studies in humans. Xenobiotica. 2019;49(10):1209–20.
6. Wyska E, Pekala E, Szymura-Oleksiak J. Interconversion and tissue distribution of pentoxifylline and lisofylline in mice. Chirality. 2006;18(8):644–51.
7. Song WH, Yeom DW, Lee DH, Lee KM, Yoo HJ, Chae BR, et al. In situ intestinal permeability and in vivo oral bioavailability of celecoxib in supersaturating self-emulsifying drug delivery system. Arch Pharm Res. 2014;37(5):626–35.
8. Wyska E, Świerczek A, Pociecha K, Pomierny KP. Physiologically based modeling of lisofylline pharmacokinetics following intravenous administration in mice. Eur J Drug Metab Pharmacokinet. 2016;41(4):403–12.
9. Wyska E, Szymura-Oleksiak J, P kala E, Obruśnik A. Pharmacokinetic modelling of pentoxifylline and lisofylline after oral and intravenous administration in mice. J Pharm Pharmacol. 2007;59(4):495–501.
10. Striffler JS, Nadler JL. Lisofylline, a novel anti-inflammatory agent, enhances glucose-stimulated insulin secretion in vivo and in vitro: studies in prediabetic and normal rats. Metabolism. 2004;53(3):290–6. https://doi.org/10.1016/j.metabol.2003.10.008.
11. Lillibridge JA, Kalhorn TF, Slattery JT. Metabolism of lisofylline and pentoxifylline in human liver microsomes and cytosol. Drug Metab Dispos. 1996;24(11):1174–9.
12. Cui P, Macdonald TL, Chen M, Nadler JL. Synthesis and biological evaluation of lisofylline (LSF) analogs as a potential treatment for type 1 diabetes. Bioorg Med Chem Lett. 2006;16(13):3401–5.
13. Italiya KS, Basak M, Mazumdar S, Sahel DK, Shrivastava R, Chitkara D, et al. Scalable self-assembling micellar system for enhanced oral bioavailability and efficacy of lisofylline for treatment of type-I diabetes. Mol Pharm. 2019;16(12):4954–67. https://doi.org/10.1021/acs.molpharmaceut.9b00833.
14. Irby D, Du C, Li F. Lipid–drug conjugate for enhancing drug delivery. Mol Pharm. 2017;14(5):1325–38.
15. Date AA, Hanes J, Ensign LM. Nanoparticles for oral delivery: design, evaluation and state-of-the-art. J Control Release. 2016;240:504–26.
16. Xu W, Ling P, Zhang T. Polymeric micelles, a promising drug delivery system to enhance bioavailability of poorly water-soluble drugs. J Drug Deliv. 2013;2013:340315.
17. Wu L, Zhang J, Watanabe W. Physical and chemical stability of drug nanoparticles. Adv Drug Deliv Rev. 2011;63(6):456–69.
18. Schmidt C, Bodmeier R. Incorporation of polymeric nanoparticles into solid dosage forms. J Control Release. 1999;57(2):115–25.
19. Nikolakakis I, Partheniadis I. Self-emulsifying granules and pellets: composition and formation mechanisms for instant or controlled release. Pharmaceutics. 2017;9(4):50.
20. Patel HP, Patel J, Patel RR, Patel MP. Pellets: A general overview. Int J Pharm World Res. 2010;1(2):1–15.
21. Usman F, Javed I, Hussain SZ, Ranjha NM, Hussain I. Hydrophilic nanoparticles packed in oral tablets can improve the plasma profile of short half-life hydrophobic drugs. RSC Adv. 2016;6(97):94896–904.
22. Ilhan E, Ugurlu T, Kerimoglu O. Mini tablets: a short review-revision. Peertechz J Med Chem Res. 2017;3(1):012–22.
23. Ansari M. Oral delivery of insulin for treatment of diabetes: classical challenges and current opportunities. J Med Sci. 2015;15(5):209–20.
24. Balducci AG, Magosso E, Colombo G, Sonvico F. From tablets to pharmaceutical nanotechnologies: innovation in drug delivery strategies for the administration of antimalarial drugs. J Drug Deliv Sci Technol. 2016;32:167–73.
25. Ilhan E, Ugurlu T, Kerimoglu O. Mini tablets: a short review-revision. Open J Chem. 2017;3(1):012–22.
26. El-Nabarawi MA, Elshafeey AH, Mahmoud DM, El Sisi AM. Fabrication, optimization, and in vitro/in vivo evaluation of diclofenac epolamine flash tablet. Drug Deliv Transl Res. 2020;10:1–13.
27. Ahmed IS, Shamma RN, Shoukri RA. Development and optimization of lyophilized orally disintegrating tablets using factorial design. Pharm Dev Technol. 2013;18(4):935–43.
28. Chavan P, Ughade S. Preparation, characterization and evalution of tablet for colonic delivery. Int J Pharm Sci Res. 2018;9(5):2027–33.
29. Hadi MA, Rao NR, Rao AS. Formulation and evaluation of ileo-colonic targeted matrix-mini-tablets of naproxen for chronotherapeutic treatment of rheumatoid arthritis. Saudi Pharm J. 2016;24(1):64–73.
30. United States Pharmacopeia and National Formulary (USP 41-NF 36). United States Pharmacopeial Convention; General tests and assays. 2016. Accessed Jan 26, 2021 https://online.uspnf.com/uspnf/document/GUID-AC788D41-90A2-4F36-A6E7-769954A9ED09_1_en-US.2016.
31. United States Pharmacopeia and National Formulary (USP 41-NF 36). United States Pharmacopeial Convention; Reagents: solutions: test solutions. 2016. Accessed Jan 26, 2021 https://online.uspnf.com/uspnf/document/GUID-AC788D41-90A2-4F36-A6E7-769954A9ED09_1_en-US.2016.
32. Jain R, Duvvuri S, Kansara V, Mandava NK, Mitra AK. Intestinal absorption of novel-dipeptide prodrugs of saquinavir in rats. Int J Pharm. 2007;336(2):233–40.
33. Dezani TM, Dezani AB, da Silva Junior JB, dos Reis Serra CH. Single-Pass Intestinal Perfusion (SPIP) and prediction of fraction absorbed and permeability in humans: a study with antiretroviral drugs. Eur J Pharm Biopharm. 2016;104:131–9.
34. Sutton SC, Rinaldi M, Vukovinsky K. Comparison of the gravimetric, phenol red, and 14C-PEG-3350 methods to determine water absorption in the rat single-pass intestinal perfusion model. AAPS PharmSci. 2001;3(3):E25.
35. Kang MJ, Kim HS, Jeon HS, Park JH, Lee BS, Ahn BK, et al. In situ intestinal permeability and in vivo absorption characteristics of olmesartan medoxomil in self-microemulsifying drug delivery system. Drug Dev Ind Pharm. 2012;38(5):587–96.
36. Rathore R, Jain JP, Srivastava A, Jachak S, Kumar N. Simultaneous determination of hydrazinocurcumin and phenol red in samples from rat intestinal permeability studies: HPLC method development and validation. J Pharm Biomed Anal. 2008;46(2):374–80.

37. Zakeri-Milani P, Barzegar-Jalali M, Tajerzadeh H, Azarmi Y, Valizadeh H. Simultaneous determination of naproxen, ketoprofen and phenol red in samples from rat intestinal permeability studies: HPLC method development and validation. J Pharm Biomed Anal. 2005;39(3-4):624–30.
38. Tabatabayi H, Valizade PZ-MH, Azarmi Y, Jalali MB, Tajerzadeh H. An HPLC method development for simultaneous determination of metoprolol, propranolol and phenol red: application in perfusion studies. Res Pharm Sci. 2012;7(5):637.
39. Escribano E, Sala XG, Salamanca J, Navarro CR, Regué JQ. Single-pass intestinal perfusion to establish the intestinal permeability of model drugs in mouse. Int J Pharm. 2012;436(1-2):472–7.
40. Italiya KS, Sharma S, Kothari I, Chitkara D, Mittal A. Simultaneous estimation of lisofylline and pentoxifylline in rat plasma by high performance liquid chromatography-photodiode array detector and its application to pharmacokinetics in rat. J Chromatogr B. 2017;1061:49–56.
41. Frandsen CS, Dejgaard TF, Madsbad S. Non-insulin drugs to treat hyperglycaemia in type 1 diabetes mellitus. Lancet Diabetes Endocrinol. 2016;4(9):766–80.
42. Bahman F, Greish K, Taurin S. Insulin nanoformulations for nonparenteral administration in diabetic patients. Theory Appl Nonparenter Nanomed. 2021;2021:409–43.
43. Dewanjee S, Chakraborty P, Mukherjee B, De Feo V. Plant-based antidiabetic nanoformulations: the emerging paradigm for effective therapy. Int J Mol Sci. 2020;21(6):2217.
44. Ganugula R, Arora M, Jaisamut P, Wiwattanapatapee R, Jørgensen HG, Venkatpurwar VP, et al. Nano-curcumin safely prevents streptozotocin-induced inflammation and apoptosis in pancreatic beta cells for effective management of Type 1 diabetes mellitus. Br J Pharmacol. 2017;174(13):2074–84.
45. Souto EB, Souto SB, Campos JR, Severino P, Pashirova TN, Zakharova LY, et al. Nanoparticle delivery systems in the treatment of diabetes complications. Molecules. 2019;24(23):4209.
46. Pathak K, Raghuvanshi S. Oral bioavailability: issues and solutions via nanoformulations. Clin Pharmacokinet. 2015;54(4):325–57.
47. Mudshinge SR, Deore AB, Patil S, Bhalgat CM. Nanoparticles: emerging carriers for drug delivery. Saudi Pharm J. 2011;19(3):129–41.
48. ElShagea HN, ElKasabgy NA, Fahmy RH, Basalious EB. Freeze-dried self-nanoemulsifying self-nanosuspension (SNESNS): a new approach for the preparation of a highly drug-loaded dosage form. AAPS PharmSciTech. 2019;20(7):1–14.
49. Friedrich R, Bastos M, Fontana M, Ourique A, Beck R. Tablets containing drug-loaded polymeric nanocapsules: an innovative platform. J Nanosci Nanotechnol. 2010;10(9):5885–8.
50. Wang K, Liu T, Lin R, Liu B, Yang G, Bu X, et al. Preparation and in vitro release of buccal tablets of naringenin-loaded MPEG-PCL nanoparticles. RSC Adv. 2014;4(64):33672–9.
51. Fan H, Zhang P, Zhou L, Mo F, Jin Z, Ma J, et al. Naringin-loaded polymeric micelles as buccal tablets: formulation, characterization, in vitro release, cytotoxicity and histopathology studies. Pharm Dev Technol. 2020;25(5):1–31.
52. Kalasz H, Antal I. Drug excipients. Curr Med Chem. 2006;13(21):2535–63.
53. Rowe RC, Sheskey P, Quinn M. Handbook of pharmaceutical excipients: Libros Digitales-Pharmaceutical Press. 2009.
54. Schlack H, Bauer-Brandl A, Schubert R, Becker D. Properties of Fujicalin®, A new modified anhydrous dibasic calcium phosphate for direct compression: comparison with dicalcium phosphate dihydrate. Drug Dev Ind Pharm. 2001;27(8):789–801.
55. Brüsewitz C, Schendler A, Funke A, Wagner T, Lipp R. Novel poloxamer-based nanoemulsions to enhance the intestinal absorption of active compounds. Int J Pharm. 2007;329(1-2):173–81.
56. Chen L, Sha X, Jiang X, Chen Y, Ren Q, Fang X. Pluronic P105/F127 mixed micelles for the delivery of docetaxel against Taxol-resistant non-small cell lung cancer: optimization and in vitro, in vivo evaluation. Int J Nanomedicine. 2013;8:73.
57. Varma MV, Panchagnula R. Enhanced oral paclitaxel absorption with vitamin E-TPGS: effect on solubility and permeability in vitro, in situ and in vivo. Eur J Pharm Sci. 2005;25(4-5):445–53.