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List of Abbreviations

%	Percentage
% CV	Percentage coefficient of variance
% DL	Percentage drug loading
% EE	Percentage entrapment efficiency
% F	Percentage fraction of drug released
% RSD	Percentage relative standard deviation
% v/v	Percentage volume by volume
% w/v	Percentage weight by volume
% w/w	Percentage weight by weight
λ_{max}	Wavelength of maximum absorbance
<	Less than
>	More than
≤	Less than or equal to
≥	More than or equal to
=	Equal to
~	Approximately equal to
±	Plus or minus
º C	Degree centigrade
Mg	Milligram
G	Gram
Cm	Centimeter
µm	Micrometer
Nm	Nanometer
mL	Milliliter
µL	Microliter
mg/mL	Milligram per milliliter
µg/mL	Microgram per milliliter
ng/mL	Nanogram per milliliter
B	Beta
K	Capacity factor
ABC	ATP-binding cassette transporters

CAN	Acetonitrile
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
API	Active pharmaceutical ingredient
AST	Aspartate aminotransferase
AUC	Area under curve
AUMC	Area under first moment curve
BCS	Biopharmaceutical classification system
BGL	Blood glucose level
BSA	Bovine serum albumin
CAF	Central animal Facility
CC	Calibration curve
CD4	Cluster of differentiation 4
CD8	Cluster of differentiation 8
$CDCl_3$	Deuterated chloroform
CFSC	Carboxyfluorescein succinimidyl ester
Cl	Clearance
C_{max}	Maximum concentration
CMC	Critical micellar concentration
CPC	Cetyltrimethylammonium chloride
CPCSEA	Committee for the purpose of control and supervision of experiments on animals
CYP	Cytochromes P450
DCM	Dichloromethane
DHA	Docosahexaenoic acid
DLS	Dynamic light scattering
DM	Diabetes mellitus
DMAP	4-Dimethylaminopyridine
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DPP-4	Dipeptidyl peptidase 4
DSC	Differential scanning calorimetry
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme linked immunosorbent assay
EPA	Eicosapentaenoic acid
ESI	Electrospray ionization
FBS	Fetal bovine serum
GDM	Gestational diabetes mellitus
GIT	Gastrointestinal tract
GLP-1	Glucagon-like peptide 1
GPC	Gel permeation chromatography
h or hr	Hour
H&E	Hematoxylin and eosin
Hb1Ac	Glycated hemoglobin
HCl	Hydrochloric acid
HDL	High-density lipoproteins
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HETP	Height equivalent to theoretical plates
HPLC	High performance liquid chromatography
HQC	Higher quality control
HR-MS	High-resolution mass spectrometry
HR-TEM	High-resolution transmission electron microscopy
i.p.	Intraperitonial
i.v.	Intravenous
IAEC	Institutional animal ethical committee
IBMX	3-isobutyl-1-methylxanthine
ICH	International council for harmonization
IFN-γ	Interferon gamma
IgG	Immunoglobulin G
IHC	Immunohistochemistry
IL-12	Interleukin 12
IL-1β	Interleukin 1 beta
IP	Indian Pharmacopoeia
IPA	Isopropyl alcohol
IS	Internal standard

ISR	Injection site reaction
IU	International Unit
K_b	Apparent binding site
LA	Linoleic acid
LDL	Low-density lipoproteins
LLE	Liquid-liquid extraction
LLOQ	Lower limit of quantification
LOD	Limit of detection
Log P	Partition co-efficient
LOQ	Limit of quantification
LQC	Lower quality control
LSF	Lisofylline
LSF-LA	Lisofylline-linoleic acid
LSF-LA PLM	Lisofylline-linoleic acid polymeric micelles
LSF-LA SM	Lisofylline-linoleic acid self-assembled micelles
MBC	5-methyl-5-benzyloxycarbonyl-1, 3-dioxane-2-one
MeOH	Methanol
Min	Minute
MIN-6	Mouse insulinoma 6
mPEG	Methoxy-(polyethylene glycol)
mPEG-b-P(CB-co-LA)	Methoxy-polyethylene-glycol-b-poly(carbonate-co-lactide)
MQC	Medium quality control
MRT	Mean residence time
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
mV	Millivolt
MWCO	Molecular weight cut off
N	Number of theoretical plates
Nagg	Aggregation number
NCCS	National Centre for Cell Science
NDDS	Novel drug delivery system
NMR	Nuclear magnetic resonance spectroscopy

NOD	Non obese diabetic
NPD	Normal pellet diet
NPs	Nanoparticles
OD	Optical density
ODS	Octadecylsilyl
p value	Significance level in statistical tests
PBMC	Peripheral blood mononuclear cell or cells
PBS	Phosphate buffer or buffered saline
PD	Pharmacodynamic
PDI	Polydispersity index
P-gp	Permeability glycoprotein
pH	Negative log to the base 10 of hydrogen ion concentration
PHA	Phytohemagglutinin
pKa	Acid dissociation constant
PLA	Poly (D, L-lactide)
PLGA	Poly (D, L-lactide-co-glycolide)
PLM	Polymeric micelles
PTX	Pentoxifylline
PVA	Polyvinyl alcohol
Q	Quencher
QC	Quality control
R_2	Regression coefficient
R_f	Retention factor
RP-HPLC	Reversed phase high performance liquid chromatography
Rpm	Rotations per minute
RPMI	Roswell Park Memorial Institute Medium
Rs	Resolution
Rt	Retention time
RT	Room temperature
S/N	Signal to noise
SD	Standard deviation
Sec	Second
SGF	Simulated gastric fluid

SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SGTL-2	Sodium-glucose transport protein 2
SIF	Simulated intestinal fluid
SLN	Solid-lipid nanoparticles
SPIP	Single pass intestinal perfusion
Sn(Oct)2	Stannous octoate
SNEDDS	Self-nanoemulsifying drug delivery systems
SPE	Solid phase extraction
STAT-4	Signal transducer and activator of transcription 4
STZ	Streptozotocin
$t_{1/2}$	Half-life
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TDP	Transdermal patch
TDW	Triple distilled water
TEM	Transmission electron microscopy
Th1	T helper cells
TLC	Thin-layer chromatography
T_{max}	Time to reach maximum concentration
TNF-α	Tumour necrosis factor-α
TPGS	D-α-tocopheryl polyethylene glycol succinate
USFDA	United States Food and Drug Administration
USP	United States Pharmacopeia
Vz	Apparent volume of distribution
WS	Working standard
ZP	Zeta potential

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Abstract

Lisofylline (LSF) is an anti-inflammatory and immunomodulatory agent with proven therapeutic benefit in Type 1 diabetes. Its high solubility and rapid rate of metabolism along with a high interconversion rate to its metabolite Pentoxifylline (PTX), results in poor oral bioavailability and short half-life, thus limiting its widespread clinical utility.

Our goal is to improve its physicochemical and pharmacokinetic (PK) properties by conjugating the hydrophilic LSF with a hydrophobic fatty acid; linoleic acid (LA). In this work, LSF-LA conjugate containing a hydrolysable ester linkage was synthesized and found to self-assemble into micelles (LSF-LA SM) without any surfactant. LSF-LA SM exhibited potent activity and efficacy in *in vitro* and *in vivo* experiments at a reduced dose and dosage frequency mainly attributed to reduced (~50 %) interconversion of LSF to its inactive metabolite PTX by blocking free hydroxyl group in the side chain of LSF.

LSF-LA SM was found to be non-toxic, boosted the insulin production and also protected insulin secreting MIN6 cells in the presence of pro-inflammatory cytokines. It also suppressed the proliferation of activated peripheral blood mononuclear cells and reduced the production of inflammatory cytokines from them. PK studies revealed that the synthesized conjugate markedly improved PK parameters (~5 folds) in comparison to free LSF. The significant improvement in PK parameters was reflected in improved efficacy of LSF-LA conjugate in streptozotocin (STZ) induced T1DM rat model at a reduced dose (~15 mg/kg of LSF, once daily) as compared to ~25 mg/kg, twice daily dose of free LSF.

LSF-LA SM is the first reported injectable nanoformulation of LSF which made its sustained delivery possible for a variety of autoimmune disorders. LSF-LA SM, when tested by oral route of administration showed a very low bioavailability due to the ease of cleavage of ester linkage between LSF and LA in the GIT before reaching the systemic circulation.

As LSF-LA SM was unable to show appreciable oral bioavailability, a polymeric delivery system of the synthesized LSF-LA conjugate was designed which could exhibit oral bioavailability and thus enhance the potential for its clinical translation. LSF was encapsulated in the form of its ester conjugate (LSF-LA) into biodegradable self-assembling polymeric micelles (LSF-LA PLM) of methoxypoly(ethylene glycol)-b-poly(carbonate-co-L-lactide) (mPEG-b-P(CB-co-LA) block copolymer. LSF-LA PLM was found to be equally effective as LSF-LA conjugate in cell culture studies in MIN6 cells and showed excellent stability in simulating biological fluids and plasma.

PK of LSF-LA PLM (10 mg/kg dose) revealed significant improvement in oral bioavailability of LSF (74.86%; 3.3 fold increase in comparison to free LSF). Shielding the ester bond between LSF and LA against cleavage in GIT by encapsulating it in a polymeric carrier not only demonstrated equivalent therapeutic activity by oral and parenteral route but also decreased the interconversion of LSF to PTX substantially in STZ induced T1DM rat model.

Further, few additional experiments were performed to understand commercial feasibility of LSF-LA PLM. So, LSF-LA PLM formulation was prepared in scale-up batches and its lyophilization was also optimized at large scale. To further facilitate its delivery and ensure patient compliance, LSF-LA PLM in lyophilized form was directly compressed into tablets and evaluated for intestinal permeability (SPIP) and efficacy in PK studies.

It can be concluded that LSF-fatty acid conjugate and its oral nanoformulation were successfully designed and evaluated. The proposed method of preparation was simple and reproducible which has scope for commercialization. *In-vitro* and *in-vivo* performance of designed formulation proved their potential to regulate glucose levels and to minimize dose and the frequency of dosing.