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'Gratitude is a currency that we can mint for ourselves and spend without fear of bankruptcy'

-Fred De Witt Van Amburgh

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List of Abbreviations

AOT	1,4-bis-(2-ethylhexoxy)-1,4-dioxybutane-2-sulfonate
ACN	Acetonitrile
API	Active pharmaceutical ingredient
ARV	Antiretroviral
ATLAS	Antiretroviral therapy as long acting suppression
AUC	Area under curve
BCS	Biopharmaceutical Classification System
BDDS	Biopharmaceutical Drug Disposition Classification System
CAB LAP	Cabotegravir Long acting parenteral
cART	Combination antiretroviral therapy
CD	Circular Dichroism
C _{max}	Maximum concentration
DAPI	4',6-diamidino-2-phenylindole
DIR	1.1'-Diocetadecyl-3,3',3'-Tetramethylindotricarbocyanine iodide
DMEM	Dulbecco's modified eagle medium
DcNP	Combination drug nanoparticle
DS	Docusate sodium
DSC	Differential scanning calorimetry
% DL	% drug loading
%EE	% entrapment efficiency
Efa	Efavirenz
Enf	Enfuvirtide
FESEM	Field emission scanning electron microscopy
FTIR	Fourier transform infrared
GIT	Gastrointestinal tract
HIV	Human immunodeficiency virus
HIC	Hydrophobic ionic complex
HETP	Height equivalent to theoretical plate
HQC	High quality control
RP-HPLC	Reverse Phase High Performance Liquid Chromatography

ISMM	Isometamidium chloride
ISMM-DS LNP	Isometamidium chloride-docusate sodium complexed lipid nanoparticles
FACS	Fluorescence activated cell sorting
HAART	Highly active antiretroviral therapy
IM	Intramuscular
IV	Intravenous
LA	Long acting
LASER	Long acting slow effective release
LDN	Lipid drug nanoaprticle
LOD	Limit of detection
LLOQ	Lower limit of Quantification
LQC	Low quality control
MDM	Monocyte derived macrophage
MEC	Minimum effective concentration
MQC	Medium quality control
MTT	Thiazolyl blue tetrazolium bromide
PDI	Polydispersity index
PLN	Polymer-lipid hybrid nanoparticles
PSA	Polar surface area
MRT	Mean residence time
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
PLGA	Poly(lactic-co-glycolic acid)
OLS	Ordinary least square
RPMI	Rosewell Park Memorial Institute
RSD	Relative standard deviation
SC	Subcutaneous
MTT	Thiazolyl blue tetrazolium bromide
PMA	Phorbol-12-myristate-13-acetate
WLS	Weighted least square

Abstract

Perennial relapse of infectious diseases in humans and animals emanating from failure in disease treatment is a major cause of solicitude for grave socio-economic loss. Long-acting (LA) nanoformulations would overcome the pitfalls associated with existing anti-infective agents including inadequate plasma and tissue concentration, incompetence in treatment adherence due to high pill burden, frequent dosing interval, injection site reaction and ascendancy in treatment cost; further worsen the disease conditions. LA nanotechnology involves secondary depot genesis in immune cells infiltrated at the injection site (primary depot) after subcutaneous/intramuscular (SC/IM) administration. Furthermore, the slow effective release of drugs from the immune cell depot transpires due to their traverse through the lymphatic vesicles, sequestration into lymph node, and slow drainage into systemic circulation preceding thoracic duct which upon reaching various organs differentiate into tissue macrophages forming secondary tissue depot. Therefore, current research work involved the development and evaluation of LA nanoformulations for targeted delivery of antiretroviral and anti-trypanosomal drugs. Although hydrophobic drugs with longer half-lives were found to be suitable for LA potential; yet LA nanoformulations are desirable for drugs with a short half-life (<10 h) with frequent dosing. Therefore, the aim of the present work involves the development and characterization of LA nanoformulations incorporating hydrophilic high molecular weight peptide (Enfuvirtide; Enf) with short half-life (3.8 h) co-loaded with hydrophobic Efavirenz (Efa) in polymer-lipid hybrid nanoparticles (PLN). It further involved the development and characterization of hydrophilic charged anti-trypanosomal drugs (Isometamidium chloride; ISMM) loaded into solid lipid nanoparticles (ISMM-DS LNP) eliciting LA effect.

The development of suitable analytical and bioanalytical methods is indispensable for the estimation of each drug from its nanoformulation, release media, and biological matrices.

Therefore, the present work involved RP-HPLC and spectrophotofluorimetric method development for the estimation of Efa and Enf from Efa-Enf PLN. While, RP-HPLC analytical and bioanalytical method development was done for estimation of ISMM from ISMM-DS LNP, release media, and different biological matrices. The bioanalytical method development involved a novel ion-pairing approach for estimation of ISMM using 0.1% v/v formic acid in sodium lauryl sulfate buffer (5 mM, pH 3, 55% v/v) and 45% v/v acetonitrile. The method could suitably elute ISMM from biological matrices due to decreased interaction of free ISMM with free silanol groups and ion-pairing with ISMM. The developed analytical and bioanalytical method were validated as per ICH Q2(R1) and USFDA guidance for industry, respectively.

LA Efa-Enf PLN were prepared by a double emulsion solvent evaporation method. Box-Behnken design was utilized to optimize three high-risk factors namely, Efa amount, sonication time for primary emulsion, and sonication time for aqueous nano-dispersion obtained from preliminary studies. The optimized Efa-Enf PLN was found to have with particle size, PDI % EE of Efa and Enf of $346.4 \text{ nm} \pm 30.41 \text{ nm}$, 0.440 ± 0.06 , $27.1\% \pm 0.78\%$ and $69.7\% \pm 3.79\%$ respectively. Lyophilized Efa-Enf PLN using trehalose elicited spherical morphology, drug amorphization on incorporation depicted by DSC studies, and absence of drug-excipient interaction evaluated by FTIR spectroscopy. *In vitro* release studies revealed extended-release of both drugs from PLN with differential release profile of Efa and Enf upto 33 h and 120 h; respectively. Blood compatibility studies of Efa-Enf PLN revealed low hemolytic, platelet and leukocyte aggregation. While, Efa-Enf PLN exhibited low cytotoxicity in Jurkat E6.1 T cells and U937 macrophage cells. Circular dichroism spectra confirmed the presence of an α -helix form of Enfuvirtide after encapsulation in PLN. Coumarin-6 loaded PLN exhibited enhanced cellular uptake in Jurkat E6.1 T cells and U937 macrophage cells in comparison to free coumarin-6 as evidenced by fluorescent microscopy

and flow cytometry. *In-vivo* biodistribution studies after intravenous administration of near-infrared dye (DIR) loaded PLN (surrogate for Efavirenz-Enfuvirtide PLN) using bioimaging technique revealed non-uniform distribution within 2h in order of spleen \geq liver > lymph node > thymus > lungs > female reproductive tract (FRT) > heart > kidneys > brain. While, subcutaneous administration caused non-uniform biodistribution after 3 days eliciting long-acting slow release from injection site depot until day 5 in infection spread site (lymph nodes and female reproductive tract), reservoir sites (liver and spleen), and difficult-to-access site (brain). Furthermore, it presents a vital illustration of available tissue-specific drug concentration prediction from simulated surrogate PLN.

The second aim of current research involved the development of LA nanoformulation for a charged hydrophilic drug with a short half-life (<10 h) to attain protracted plasma drug release as well as secondary tissue depot. ISMM was adopted as a representative molecule and incorporated suitably into solid lipid nanoparticles (LNP) to achieve the established goal. The ISMM LNP were developed by a novel *in situ* complexation and solvent evaporation method. Docusate sodium (DS) was selected as an anionic complexing agent upon pretesting the available complexing agents by n-octanol and water solubility studies. ISMM-DS elicited maximum complexation (358.3 ± 108.41 $\mu\text{g/ml}$ ISMM-DS LNP solubility in n-octanol) at 1:4 charge molar ratio of ISMM and DS respectively. ISMM-DS LNP were developed using Precirol® ATO 5 and tween-80 as solid lipid and surfactant respectively to yield particle size, PDI, zeta potential, and ISMM % EE of $173.17 \text{ nm} \pm 10.70 \text{ nm}$, 0.22 ± 0.02 , $-48.3 \text{ mV} \pm 2.02 \text{ mV}$, and $69.14\% \pm 1.27\%$, respectively. *In vitro* drug release studies of ISMM-DS LNP revealed $46.3\% \pm 8.9\%$ drug release within 1 h. Thereafter, sustained drug release was observed leading to plateau until 24 h, mimicking the behaviour of ISMM-DS LNP upon exposure to systemic circulation. Blood compatibility studies revealed non-haemolytic potential (0-2.52% haemolysis) of ISMM-DS LNP. While, cytotoxicity studies of ISMM-DS LNP revealed

74.6±3.87% cell viability in Vero cells at the minimum effective concentration (MEC; 1-4 µg/ml) of ISMM. Furthermore, cellular uptake studies in THP-1 macrophage-like cells using fluorescence microscopy with apotome attachment revealed 2.3-folds higher uptake of ISMM-DS LNP compared with free ISMM. While, the presence of a significantly higher amount of ISMM in PBMC (68.4±19.0 ng/ml) at day 7 upon subcutaneous (SC) administration of ISMM-DS LNP confirmed the potential of ISMM-DS LNP for secondary depot genesis *in vivo*. Moreover, the ISMM-DS LNP engender protracted plasma drug release for 7 days with 3.0-, 4.5-, and 4.2- folds enhanced $AUC_{0-\infty}$, $MRT_{0-\infty}$, and $t_{1/2}$, compared with free ISMM. Further, the Cl/F was decreased by 4.2-folds upon SC administration of ISMM-DS LNP compared with free ISMM. Therefore, ISMM-DS LNP overcome the drawbacks associated with free ISMM including miniscule plasma retention and quick disposition leading to parasite relapse. Biodistribution studies revealed accumulation of ISMM in various reticuloendothelial (RES) organs and non-RES organs in the order of liver>spleen>kidneys>lymph node>lungs on day 7. Interestingly, the amount of ISMM was 2.9-, 4.2- and 2.0-folds higher in RES organs like liver (Kupffer cells), spleen (spleenotropic macrophages and 15% T-lymphocytes), and lymph nodes (75% T-lymphocytes), respectively in LA ISMM-DS LNP group compared with free ISMM which confirmed the mechanism of primary and secondary depot genesis of LA ISMM-DS LNP. To conclude, current research work was an invaluable contribution for long-term treatment and prophylaxis of drugs with varied physicochemical properties, short half-life (<10 h), desirable of frequent administration, and high adherence.

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