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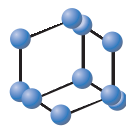
Formulation and *in-vivo* Evaluation of Novel Topical Gel of Lopinavir for Targeting HIV

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Abstract: ***Background***: Lopinavir is a specific reversible inhibitor of the enzyme HIV protease with mean oral bioavailability of less than 20 % due to extensive hepatic metabolism by cytochrome P450 3A4. The reported half-life of Lopinavir is 5-6 hours and the maximum recommended daily dose is 400 mg/day. All the marketed tablet and capsule formulations of lopinavir are generally combined with Ritonavir, a potent inhibitor of cytochrome P450 3A4, to minimize presystemic metabolism of lopinavir. Hence, to overcome limitations associated with oral administration of lopinavir and to promote single drug administration, utilization of vesicular nanocarriers through topical route could prove to be effective, as the approach combines the inherent advantages of topical route and the drug-carrying potential of vesicular nanocarriers across the tough and otherwise impervious skin barrier layer, *i.e.*, stratum corneum.

Objective: The objective was to develop solid lipid nanoparticles (SLN) of lopinavir and formulate a topical gel for improved systemic bioavailability of lopinavir.

Method: SLNs were prepared using high-pressure homogenization technique and optimized. The nanoparticles were characterized by SEM to confirm their spherical shape. Differential Scanning Calorimetry (DSC) analysis was carried out to ensure the entrapment of drug inside the SLNs. A comparative evaluation was done between SLN based gel and plain gel of drug by performing *ex-vivo* skin permeation studies using Franz diffusion cell. To explore the potential of topical route, *in-vivo* bioavailability study was conducted in male Wistar rats.

Results: The optimized formulation composed of Compritol 888ATO (0.5 %) as a lipid, Poloxamer 407 (0.25 %) as a surfactant and Labrasol (0.25 %) as a co-surfactant gave the maximum entrapment of 69.78 % with mean particle size of 48.86nm. The plain gel of the drug gave a release of 98.406 ± 0.007 % at the end of 4hours whereas SLN based gel gave a more sustained release of 71.197 ±0.006 % at the end of 12hours *ex-vivo*. As observed from the results of *in-vivo* studies, highest Cmax was found with SLN based gel (20.3127 ± 0.6056) µg/ml as compared to plain gel (8.0655 ± 1.6369) µg/ml and oral suspension (4.2550 ± 16.380) µg/ml of the drug. Also, the AUC was higher in the case of SLN based gel indicating good bioavailability as compared to oral suspension and plain gel of drug.

Conclusion: Lopinavir SLN based gel was found to have modified drug release pattern providing sustained release as compared to plain drug gel. This indicates that Lopinavir when given topically has a good potential to target the HIV as compared to when given orally.

1. INTRODUCTION

One of the newly arising and lethal diseases in the prior 1980s was the acquired immunodeficiency syndrome (AIDS) caused by a retrovirus known as human immunodeficiency virus (HIV) [1]. It is estimated that, currently 35 million subjects are living with HIV worldwide, out of which 19 million are unaware that they harbour the disease [2, 3]. Despite a considerable reduction in the number of HIV infections worldwide, yet 2.5 million new infections and 1.7 million AIDS-related deaths occurred [4]. Antiretroviral therapy (ART), educational tools and AIDS counseling have helped in transforming HIV infection from a fatal to manageable chronic infectious disease [5]. Currently, there are two main types of HIV: HIV-1 and HIV-2, out of which HIV-1 is more prevalent and transmittable responsible for majority of HIV infections worldwide [6, 7].

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The 'highly active antiretroviral therapy' also known as HAART uses multiple drugs having the ability to attack different viral targets. To reduce the rate of advancement of disease from HIV to AIDS, a chronic combined ART with an adherence rate of 95 % is needed [8-11].

Protease inhibitors (PIs) prevent the maturation of HIV polyproteins, thereby blocking the maturation of newly formed virions. This results in improper viral assembly preventing the spread of new infections. The PIs approved by USFDA are: saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, atazanavir and lopinavir-ritonavir co-formulation [12].

Lopinavir, (2S)-N-[(2S,4S,5S)-5-[2-(2,6-dimethylphenoxy)-acetamido]-4-hydroxy-1,6 diphenylhexan-2-yl]- 3-methyl-2-(2-oxo1,3-diazinan-1-yl)-butanamide, is an antiretroviral of the protease inhibitor class. It exerts its effect against HIV infection by blocking the ability of the protease to cleave the Gag-Pol polyprotein, resulting in the production of immature, noninfectious viral particles. However, the systemic availability of lopinavir via oral route is severely limited by its sensitivity towards cytochrome P450 3A4, susceptibility for P glycoprotein efflux transporters, poor aqueous solubility (~0.01mg/ml), moderately high molecular weight (~628 Da), and high log P value (~4.56) [13]. Though the marketed tablet and capsule formulations of lopinavir are generally combined with Ritonavir, a potent inhibitor of cytochrome P450 3A4, to minimize presystemic metabolism of lopinavir, other challenges contributing to poor oral absorption remain unanswered. Hence, to overcome limitations associated with oral administration of lopinavir and to promote single drug administration, utilization of vesicular nanocarriers through topical route could prove to be effective, as the approach combines the inherent advantages of topical route and the drug-carrying potential of vesicular nanocarriers across the tough and otherwise impervious skin barrier layer, *i.e.*, stratum corneum. Also, the topical route would eliminate the need for frequent dosing administration and there would be avoidance of pharmacokinetic 'peaks and valleys in plasma drug concentrations [14]. Since lopinavir is extensively metabolized by cytochrome P450 3A4, giving the drug by topical route would bypass the hepatic first pass metabolism and there would be high localized concentrations of the drug directly at the target site.

Patel *et al.* compared niosomal gel with ethosomal gel of lopinavir for enhanced transdermal delivery. The results of *in-vivo* bioavailability study showed that niosomal gel had the higher extent of absorption AUC (AUC(0→∞), 72.87 h × μg/ml) as compared to ethosomal gel and oral suspension of lopinavir. The study demonstrated that nanosized gel has substantial potential for improved transdermal delivery of lopinavir than by oral route [15].

A more efficient and less deleterious solution to overcome the limitations of HAART therapy is to integrate targeted drug delivery with controlled release technology [16]. Nanotechnology-based systems are well known for their adaptability to encapsulate all drugs essentially, feasibility to modify the drug release and high drug payload. Also, they can incorporate non-orally administered anti-HIV drugs and

improve their bioavailability. This leads to the prolonged residence of the drug at the target site thus eliminating the need for frequent dosing [17]. The pharmacokinetic barriers such as P-gp efflux, first pass metabolism and rapid clearance can be successfully surpassed by nanotechnology [18].

Solid lipid nanoparticles (SLN) are colloidal particles in the size range of 10 and 1000nm, composed of physiological lipid (*e.g.*: high carbon chain fatty acids, fatty acid esters and waxes), dispersed in water or in an aqueous surfactant solution [19-21]. SLN are aqueous in nature and do not require the use of organic solvents. They enhance the tissue distribution by improving the rate of dissolution, thereby protecting the drug from *in-vivo* processes like metabolism and providing targeted drug delivery [22, 23]. Factors such as solubility of the drug in lipid matrix, nature of the lipid and surfactant used as well as the manufacturing method regulate the amount of drug that could be incorporated into SLNs [24]. Apart from having higher entrapment efficiency as compared to other nanocarriers, they are easy to scale up and sterilize [25]. Fatma *et al.* developed solid lipid nanoparticles (SLN) of Diflucortolone valerate (DFV) as a semisolid topical delivery system. SLN formulations possessed average particle size ranging from 203.71± 5.61 to 1421.00±16.32 nm. Incorporation of lipid-based surfactants (Labrasol or Labrafil) was found to significantly increase DFV encapsulation efficiency (up to 45.79± 4.40 %). Thus, the proper selection of the type of lipids and surfactants, and their incorporated concentrations, is critical in the formation of SLN-based semisolid formulations that have the desirable properties for potential topical applications [26]. Ho Seong Jeon *et al.* prepared retinyl palmitate (Rpal) loaded solid lipid nanoparticles. In order to enhance the skin distribution properties of Rpal, Dicetyl phosphate (DCP) was added to negatively charge the surfaces of the SLNs. DCP modified negative SLNs enhanced the skin distribution of Rpal 4.8-fold and delivered Rpal to a greater depth that is to the viable layers of the epidermis and dermis than did neutral SLNs [27].

So, the aim of the present work was to develop lopinavir loaded SLN gel using Compritol 888ATO as the solid lipid matrix for the entrapment of lopinavir. SLN formulations containing lopinavir were prepared using the hot homogenization technique. SLN were further characterized for their mean particle size, loading parameters, and morphology. The drug-carrying potential of SLN was evaluated through porcine ear skin *ex vivo* and comparision of the bioavailability assessment was done for oral suspension, plain gel of drug and SLN based gel *in-vivo*.

2. MATERIALS

Lopinavir was received as a gift sample from Hetero Labs limited. Compritol 888 ATO (glyceryl behenate) and Labrasol were obtained as a gift sample from Gattefosse Mumbai, India. Poloxamer 407 was supplied by BASF Pvt Ltd, India. Carbopol 980, methanol, acetonitrile, sodium lauryl sulphate and ammonium acetate were purchased from S. D. Fine Chemicals, India. Dialysis membrane (150 Daltons) was purchased from Himedia Laboratories. Water (distilled) prepared in laboratory by distillation. All the chemicals and reagents used were of analytical grade.

Table 1.   Levels of independent factors used in the factorial design.

Variables / Levels	Concentration of Lipid (%)	Concentration of Surfactant (%)	Concentration of Co-surfactant (%)
-1	0.5	0.25	0.25
+1	1	1	1

Table 2.   Composition and Characterization of Lopinavir-Loaded solid lipid nanoparticles.

Formulation Code	Conc. of Lipid (%)	Conc. of Surfactant (%)	Conc. of Co-surfactant (%)	Entrapment Efficiency (%)
F1	0.5	0.5	0.5	69.78
F2	1	0.5	0.5	40.6
F3	0.5	1	0.5	11.27
F4	1	1	0.5	16.07
F5	0.5	0.5	1	26.77
F6	1	0.5	1	48.93
F7	0.5	1	1	18.47
F8	1	1	1	25.52

3. METHODS

3.1. Preparation of Lopinavir-loaded SLNs

Various SLN formulations were prepared using the process of hot homogenization by changing the proportions of lipid, surfactant and co-surfactant (Table **1**). Briefly, 100mg lopinavir was weighted accurately and added to 0.5 g Compritol 888 ATO previously melted at 80 °C. Poloxamer 407 was dissolved in double distilled water to obtain 0.25 % solution and heated up to 80 °C in a beaker. When a clear homogenous lipid phase was obtained, the hot aqueous surfactant solution was added to hot lipid phase and stirred using a magnetic stirrer for 15mins. The temperature was maintained at 80°C during stirring [28, 29]. After stirring, hot homogenization was carried out using Emulsiflex high-pressure homogenizer at 10,000psi, for 10mins. The obtained nanoemulsion (o/w) was cooled down at room temperature to form SLNs and finally diluted up to 100ml with distilled water. SLN dispersions were stored at 4 °C for further analyses.

3.2 Optimization of SLNs Using Factorial Design

3.2.1. Pre-optimization Studies

The principal aim was to formulate particles in the size range between 50-100nm. To achieve this, pre-optimization studies were done before deciding the level of each independent factor. Various placebo SLNs were prepared by changing the concentration of lipid, surfactant and co-surfactant. The prepared nanoparticles were evaluated based on particle size and zeta potential.

3.2.2. Factorial Design

A 2^3 factorial design was applied, having 3 factors at 2 levels giving a total of 8 batches (Table **2**). The independent variables were concentration of lipid, concentration of surfactant and concentration of co-surfactant. The dependent variables were particle size and entrapment efficiency. The amount of drug was randomly fixed to be 100 mg.

3.2.3 Statistical Evaluation of the Factorial Design

The responses obtained from the factorial design related to particle size and entrapment efficiency were statistically evaluated using Design-Expert® 10.0 software (Statease, MN, USA).

3.3. Preparation of Gel

The gel of optimized SLN dispersion was prepared by dispersing 0.8 % w/v of carbopol 980 NF in it and allowing it to hydrate for 2 h. Finally, neutralization of gel was done by adding triethanolamine which made it transparent. Plain drug gel was also prepared by using the same procedure by dispersing the Lopinavir in gel base.

3.4. Particle Size Analysis and Zeta Potential Measurements

Mean diameter of the main population, polydispersity index (PDI) as a measure of the width of particle size distribution and zeta potential were assessed using Malvern Nano ZS90, Zetasizer (Malvern Instruments, Malvern, UK zeta potential). SLN formulations were diluted (1:100) to weaken opalescence before particle size analysis. All measurements were done in triplicate.

3.5. Determination of Entrapment Efficiency

Amount of lopinavir entrapped SLNs was estimated using first derivative UV–visible spectroscopic technique (UV-

1700, Shimadzu). 10ml of the dispersion was centrifuged at 8000rpm at 4°C for 20mins. The supernatant obtained was then collected, diluted with ethanol and analyzed for free drug by taking the absorbance at 255nm [30-32]. Entrapment Efficiency was determined by using the following formula:

$$\% \text{ Entrapped drug} = \frac{\text{Total drug-Free drug}}{\text{Total drug}} * 100$$

3.6. Drug Content of SLN Based Gel

0.5 g of gel was dissolved in a mixture of chloroform and methanol (1:1) and volume was made up with the same and then Lopinavir was assayed by means of UV spectrophotometer.

3.7. HPLC Analysis of Lopinavir in Plasma

For quantitative estimation of lopinavir in samples obtained from *in vivo* studies, a more sensitive Shimadzu HPLC system equipped with an LC 20AT pump, a SPD-20A UV visible detector, a Thermosil® C-18 column (250×4.6×5 μ) was used. 10 mM of ammonium acetate buffer (pH 6.5) mixed with acetonitrile in a ratio of 35:65 was used as mobile phase at a flow rate of 1.0 ml/min [15]. Column eluent was monitored at 215 nm as λ_{max} and concentrations of lopinavir were compared against a standard calibration curve of lopinavir in the mobile phase. The method was found to obey Beer's law between concentration range of 2-12 μg/ml with LOD and LOQ values as 0.456 and 1.387μg/ml, respectively.

3.8. Differential Scanning Calorimetry (DSC)

DSC analysis was carried out using a DSC Q20 (TA instruments, US) to determine the entrapment of drug into SLN. The samples were sealed in aluminium pans and subjected to a heating run over the temperature range of 25 to 200°C at a heating rate of 5°C /min under nitrogen atmosphere.

3.9. SEM Analysis

The SEM analysis was performed to investigate the morphological characteristics of the particles. Prior to analysis, the sample was diluted with ultrapurified water, placed on a double side carbon tape mounted onto an aluminium stud, and dried in a desiccator. The sample was then sputter coated with gold in order to make it conducting. SEM images were recorded on Hitachi S-3000 N SEM at an acceleration voltage of 10 Kv and a magnification of 5000X.

3.10. *In-vitro* Release Studies of SLN Dispersion

The water bath shaker method was used to determine the release of drugs from the nanoparticulate formulations. Cellophane membrane was soaked in pH 7.4 phosphate buffer overnight. 5 mL of vesicular dispersion was added by tying one end of the membrane and hanging into a beaker containing 50 mL of pH 6.8 phosphate buffer (3 % SLS). All the beakers were kept in a shaker water bath maintained at 37 ∘C and 50 rpm [33]. 2 mL aliquots were withdrawn at predetermined time intervals from the beaker and were replaced with an equal volume of fresh buffer maintained at the same tem-

perature. The samples were analyzed spectrophotometrically at 256 nm for lopinavir.

3.11. *Ex-vivo* Diffusion Study of Gel

Franz diffusion cell with an effective diffusion area of 2 cm2 was used for the experiment. Porcine ear skin was placed between the donor and receptor compartment of Franz diffusion cell with the stratum corneum facing the donor compartment. SLN based gel containing Lopinavir was placed on the membrane and release profiles were taken. The receptor chamber was filled with 22ml of diffusion medium (pH 6.8 phosphate buffer with 3 % SLS). The receptor medium was maintained at 37±2°c and stirred magnetically at 50rpm. Samples were withdrawn at predetermined time intervals and were analyzed by UV spectrophotometer. The fresh buffer was immediately replenished into the receptor compartment after each sampling [34]. Percent cumulative drug release was plotted as a function of time.

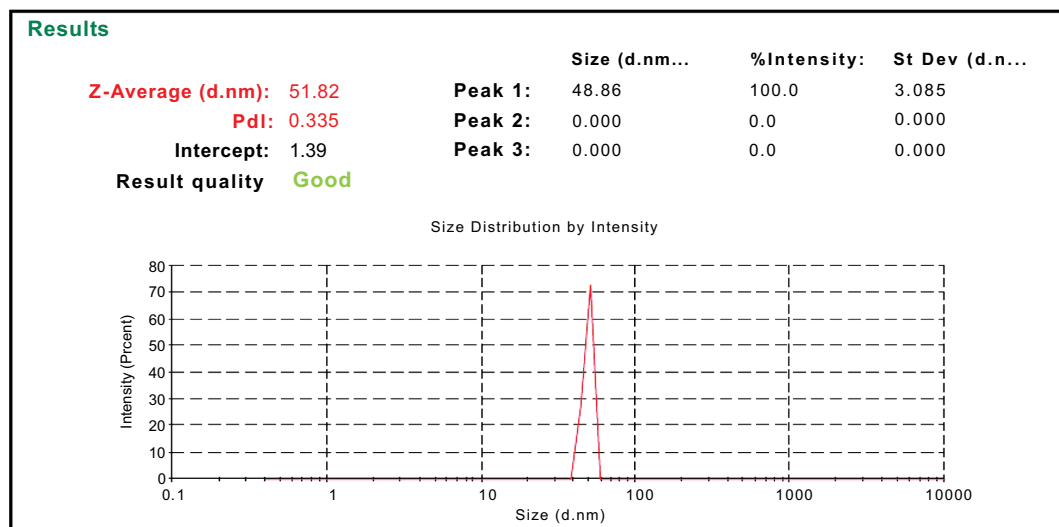### 3.12. *In-vivo* Bioavailability Study

Protocol for *in vivo* study was approved by Institutional Animal Ethics Committee of SVKM's Animal Facility, Vile Parle (W), Mumbai and is in accordance with the guidance of Committee for control and supervision of experiments on animals, Ministry of social justice and empowerment, Government of India.

Male Wistar rats weighing 240-270gms were taken and randomly divided into three test groups of nine animals each. One group was used as control which received oral lopinavir suspension and the other two groups were used as test groups. One test group received lopinavir SLN gel applied topically while to the other test group plain gel of drug was applied topically. The animals of control group were fasted overnight and administered with 7.2mg/kg of lopinavir suspension in distilled water. The rats of test group were anaesthetized and hair from the abdominal area were removed using an electrical clipper and lopinavir SLN gel (an equivalent amount containing 7.2mg/kg of lopinavir) was applied. Serial blood sampling (0.5ml) was done from the retro-orbital vein at time intervals of 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hours after administration.
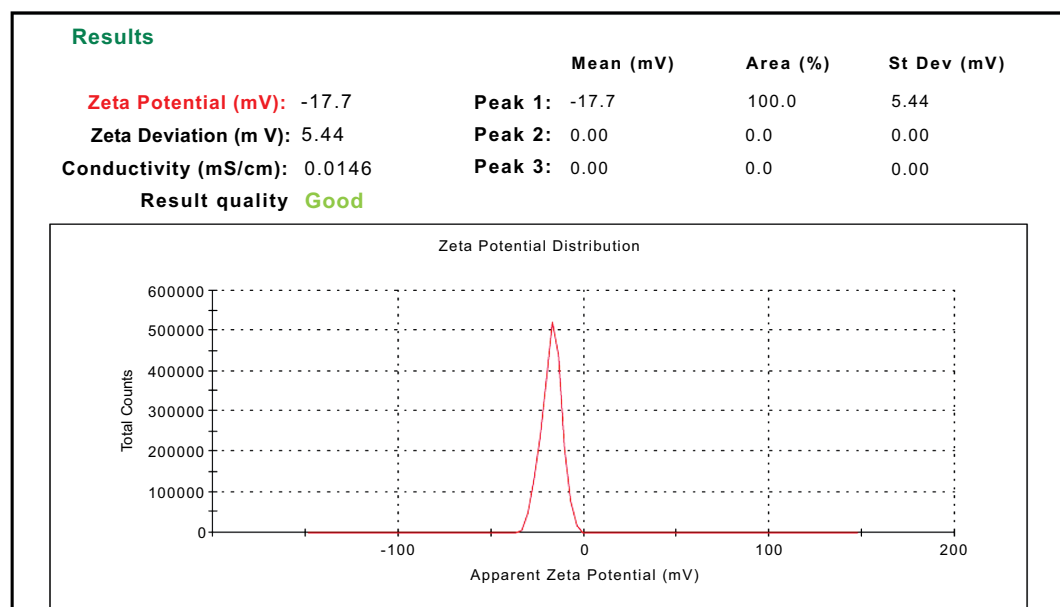
Plasma was separated by centrifugation at 3000 rpm, 4°C, for 15 min and 4 ml methanol was added to 200 μl plasma samples for deproteination and for extraction of the drug. The mixture was then vortexed for 2 min, followed by centrifugation for 5 min at 3,200 rpm. The organic layer was separated and filtered using 0.2-μm membrane syringe filter. About 20 μl of the filtrate was injected into the HPLC for estimation of lopinavir concentrations [35].

3.13. Accelerated Stability Studies

The accelerated stability studies were carried out according to on ICH Q1A (R2) guidelines with the optimized lopinavir-loaded SLN based gel. SLN based gel of the optimized formulation was tested for stability kept under two conditions for a period of three months. Gel was placed in aluminum tubes and stored in stability chambers maintained at 40°C±2°C /75 % RH ± 5 % RH and room temperature (25°C ±2°C/60RH ± 5 % RH). Formulation was evaluated for

Results

		Size (d.nm...	%Intensity:	St Dev (d.n...
Z-Average (d.nm): 51.82	**Peak 1:**	48.86	100.0	3.085
PdI: 0.335	**Peak 2:**	0.000	0.0	0.000
Intercept: 1.39	**Peak 3:**	0.000	0.0	0.000
Result quality Good				

Size Distribution by Intensity

Fig. (1a). Particle size of F1 SLN.

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -17.7	**Peak 1:**	-17.7	100.0	5.44
Zeta Deviation (m V): 5.44	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm): 0.0146	**Peak 3:**	0.00	0.0	0.00
Result quality Good				

Zeta Potential Distribution

Fig. (1b). Zeta potential of F1 SLN.

in vitro drug release and drug content at the end of 30 days, 60 days and 90 days of storage period.

3.14. Statistical Analysis

Data analysis was carried out using Microsoft Excel (version 2007), and results are expressed as mean ± standard deviation (n=3 independent samples). Statistical analysis was performed using one-way ANOVA at the probability level of 0.05.

4. RESULTS AND DISCUSSIONS

The formulation having the highest encapsulation efficiency was selected. The mean particle size of optimized SLN formulation was found to be 48.86±4.6nm with a PDI of 0.335±0.008 (Fig. **1a**). Mean zeta potential value was about -17.7±0.54mV (Fig. **1b**). The presence of behenic acid

residues on to the surface of lipid matrix attributed to the negative zeta potential of the nanoparticles [36]. The highly lipophilic nature of the drug imparted high solubility in the lipid giving an entrapment of 69.78 % and the drug content of gel was found to be 98.78 %.

### 4.1. Statistical Evaluation of the Factorial Design

The final equation for response 1 as per DOE was found to be:

Entrapment Efficiency= 32.17625 + 0.60375 (Conc. of lipid) - 14.3438 (Conc. of Surfactant)

The above model equation carries factors along with co-efficients both positive and negative, which quantify response values. Synergistic effects are indicated by positive sign while antagonistic effects are indicated by negative sign.
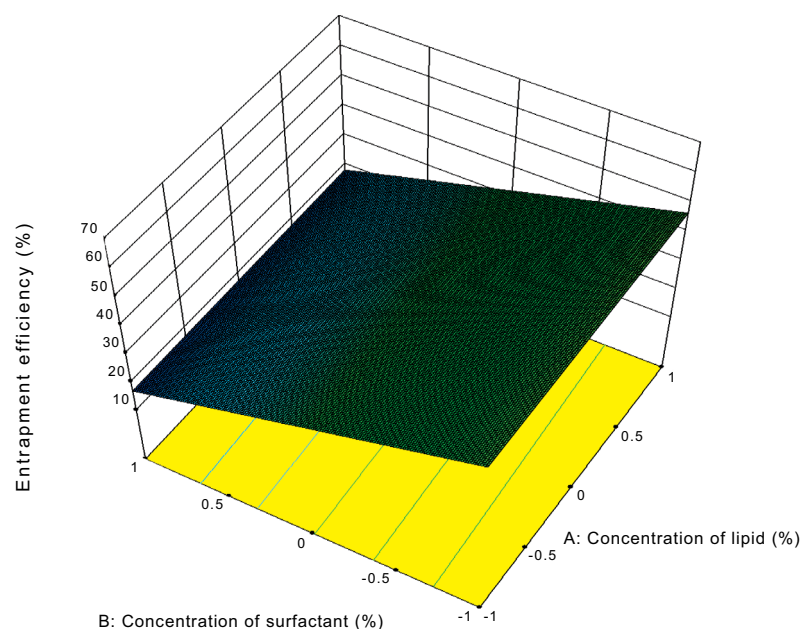
Fig. (2a). 3D surface plot of response 1 (% Entrapment Efficiency).
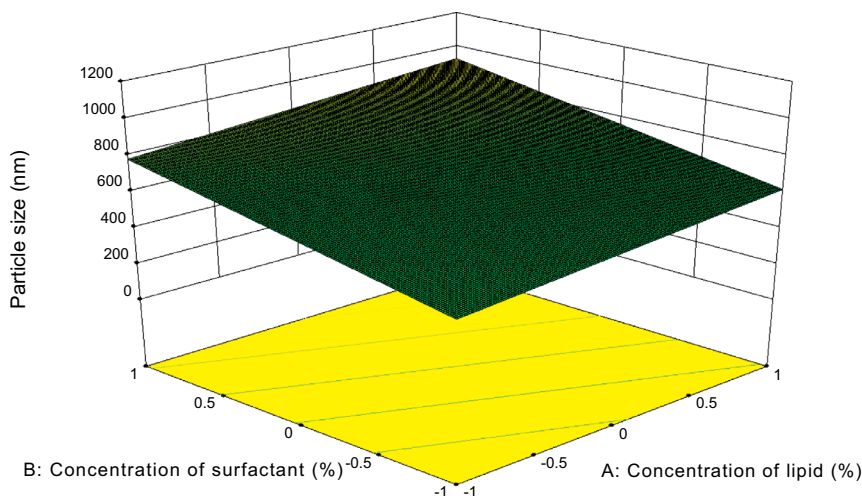
Fig. (2b). 3D surface plot of response 2 (Particle size in nm).

Interpretation: Application of Design of Experiment (DOE), a linear relation was observed between the variables and the factor as shown in Fig. (**2a**). As seen from the graph obtained through design expert software, increase in the concentration of surfactant is inversely proportional to entrapment efficiency. Whereas, the concentration of lipid is directly proportional to entrapment. As the concentration of lipid is increased the solubility of the drug in lipid increases which leads to an increase in the loading efficiency of the nanoparticles. The p-value obtained from DOE was found to be non-significant *i.e.* 0.0978 and the r^2 value was found to be 0.6054.

The final equation for response 1 as per DOE was found to be:

Particle Size = 704.345 + 72.855 (Conc. of lipid) + 155.08 (Conc. of Surfactant) + 123.155 (Conc. of Co-surfactant)

Interpretation: Application of DOE, a linear relation was observed between the variables and the factor as shown in Fig. (**2b**). The model indicates that particle size depends on conc. of lipid, surfactant and co-surfactant. As seen from the graph obtained through design expert software, as the concentration of lipid, surfactant and co-surfactant is decreased, the particle size also decreases. The p-value obtained from DOE was found to be non-significant *i.e.* 0.4614 and the r^2 value was found to be 0.4411 [37-39].

DSC thermogram of bulk compritol showed a sharp melting peak at 71.49°C, whereas lopinavir exhibited a melting peak at 129°C. A peak was observed in the DSC thermogram of placebo SLN which indicated melting point of the lipid, Compritol 888 ATO (71.49 °C). The DSC thermogram of drug loaded SLN also showed a peak at 72.14°C, again indicating the melting point of lipid and no other peak of drug was seen. This indicates that the drug is completely entrapped inside the nanoparticles. Fig. (**3a** and **b**) shows the DSC thermogram of unloaded and loaded SLNs.
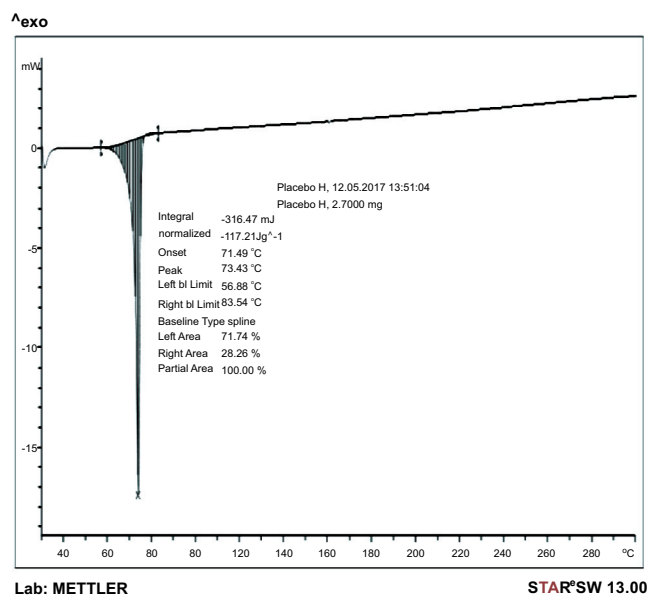
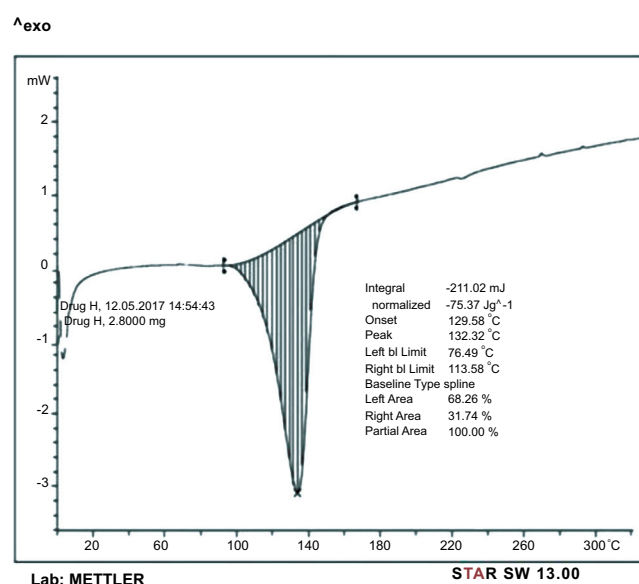**Fig. (3a).** DSC thermogram of unloaded SLN.

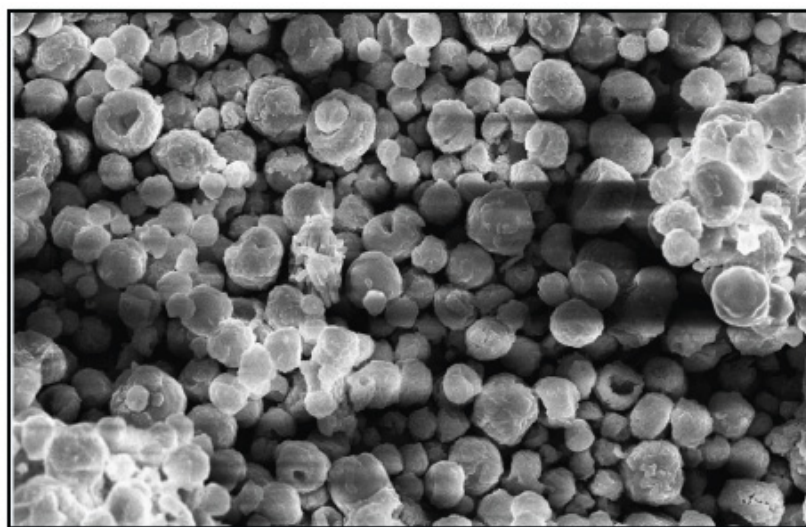**Fig. (3b).** DSC thermogram of loaded SLN.

Fig. (4). SEM image of batch 1 SLN.

Scanning electron photomicrograph used signals to produce an image, resulting from the interactions of the electron beam with atoms at various depths within the sample. SEM produced very high-resolution image of the sample surface, revealing the formation of smooth surfaced nanoparticles possessing vesicular characteristics and spherical shape (Fig. **4**).

In-vitro drug release of lopinavir SLNs in phosphate buffer pH 6.8 (3 % SLS) was found to be 99.82 ± 0.123 % at the end of 10 hours. The lipid matrix prolonged the release of lopinavir from SLN, indicating good solubility of the drug in compritol as well as homogeneous distribution of the drug within the lipid phase.

In order to evaluate the penetrating power of F1 gel, *ex-vivo* studies were conducted using porcine ear skin and compared with the plain gel of the drug (Fig. **5**).

Lopinavir in F1 gel efficiently delivered deeper into the skin and released 71.19 ±0.006 % at the end of 12h, whereas plain gel of the drug gave almost a complete release of 98.406 ± 0.007 % at the end of 10h. The hydrophobicity of the lipid matrix of SLNs attributed to less skin deposition and better penetration through the skin. Flux of SLN based gel was found to be 11,617.57μg/cm^{-2} hr^{-1} in 12 hours whereas flux of plain gel of drug was found to be 12,606.36μg/cm^{-2} hr^{-1}. This indicates that, the SLN gel penetrates through the skin efficiently which is essential for the activity of the drug [40].

To determine the advantages of topical route as well as F1 gel on lopinavir bioavailability *in-vivo* bioavailability study was performed and compared with plain gel of drug applied topically and orally administered lopinavir suspension. The chromatogram exhibited a single peak of lopinavir at a retention time of 7.37 mins. The plasma concentration of
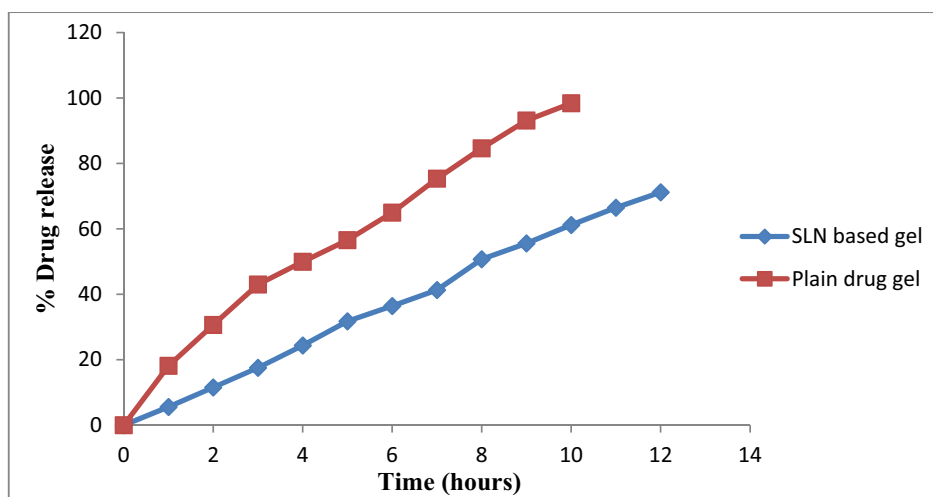
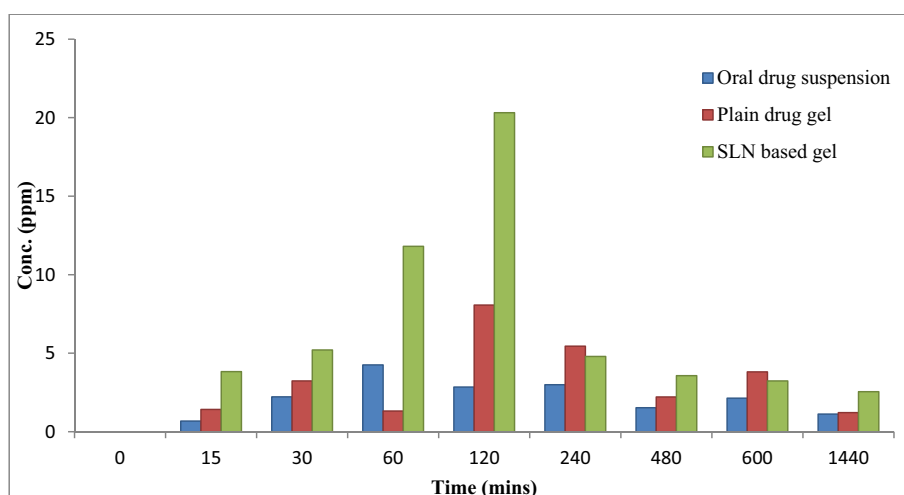**Fig. (5).** *Ex-vivo* drug release profile of SLN based gel and plain gel of drug.

Fig. (6). *In-vivo* drug release profile of oral drug suspension, plain drug gel and SLN based gel.

Table 3.   Results of *in-vivo* bioavailability study.

Parameters	Oral Drug Suspension	SLN Based Gel	Plain Drug Gel
AUC (µg/ml.hr)	2934.258 ± 51.095	7827.57 ± 26.55	4595.324 ± 18
T_{max} (hrs)	4 ± 0.115	6 ± 0.1	6 ± 0.152
C_{max} (µg/ml)	4.2550 ± 16.380	20.3127 ± 0.6056	8.0655 ± 1.6369

lopinavir at different time intervals evaluated by HPLC is shown in Fig. (**6**)

The blood withdrawn from all the three groups were evaluated for different parameters like AUC, Cmax and Tmax. The Cmax was 4.2550 ± 16.380, 8.0655 ± 1.6369 and 20.3127 ± 0.6056 with a Tmax of 4 ± 0.115, 6 ± 0.152 and 6 ± 0.1min, for the lopinavir suspension, plain drug gel and SLN based gel, respectively. The AUC was 2934.258 ± 51.095, 4595.324 ± 18 and 7827.57 ± 26.55 µg/ml.hr respectively for the lopinavir suspension, plain drug gel and SLN based gel, respectively. The results showed that (Table **3**), Tmax, Cmax and AUC was highest in the case of SLN based gel. The Cmax of SLN based gel was 5 times and 2 times higher as compared to oral suspension and plain gel of drug

respectively. Also, the AUC was higher in the case of SLN based gel indicating good bioavailability as compared to oral suspension and plain gel of drug. The difference was also found to be significant ($p < 0.001$). The results fulfilled the two characteristic features that (a) by opting for topical route, the extensive presystemic metabolism observed with oral administration of lopinavir can be overcomed and (b) solid lipid nanoparticles have the potential to carry the drug efficiently through the layers of skin.

The stability studies of the optimized F1 gel was conducted by subjecting the formulation to an accelerated temperature of 40°C ± 2°C /75 % RH ± 5 % RH, the data is presented in Table **4**.

Table 4.   **Stability study of optimized SLN based gel.**

Formulation	Time (Months)	In-vitro Drug Release (%)		Drug Content (%)	
		RT	40°C/75% RH	RT	40°C/75% RH
F1 gel	1	91.92	95.45	97.12	97
	2	92.99	92.37	97.50	97.92
	3	92	91.80	96.76	97.23

Stability studies of the optimized formulation of SLN based gel revealed, that there were no significant changes in the physical parameters when stored in accelerated temperature and humidity conditions. No significant reduction in the content of the active drug was observed over a period of three months; hence no special storage conditions are required. The optimized formulation did not show any significant change in the drug release profile. Hence, it can be concluded that optimized batch is stable at various accelerated storage and humidity conditions.

CONCLUSION

Solid lipid nanoparticles have been recognized widely for the topical delivery of several drugs. Lopinavir is a specific reversible inhibitor of the enzyme HIV protease with mean oral bioavailability of less than 20 % due to extensive hepatic metabolism by cytochrome P450 3A4. Hence, a formulation that is semi-solid in nature, having particle size in the nanometer range and enough entrapment efficiency was prepared and characterized. The solubility of the lipophilic drug lopinavir was found to be enhanced using a lipid-based surfactant (Labrasol®). This enhanced solubility led to an increase in the entrapment of drug inside the SLNs. Thus, a proper selection of the type of lipids and surfactants along with their concentration is crucial for the formulation of SLN based gel that has desirable properties to inhibit the virus. The high biocompatibility, biodegradability and nontoxicity again make SLNs excellent carriers for topical delivery of lopinavir. The plain gel of the drug gave a release of 98.406 ± 0.007 % at the end of 4hours whereas SLN based gel gave a more sustained release of 71.197 ±0.006 % at the end of 12hours *ex-vivo*. As observed from the results of *in-vivo* studies, highest C_{max} was found with SLN based gel (20.3127 ± 0.6056) as compared to plain gel (8.0655 ± 1.6369) and oral suspension (4.2550 ± 16.380) of the drug. Hence SLN based gel of lopinavir overcomes the limitations associated with oral administration of lopinavir and promotes single drug administration.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Protocol for *in vivo* study was approved by Institutional Animal Ethics Committee of SVKM's Animal Facility, Vile Parle (W), Mumbai.

HUMAN AND ANIMAL RIGHTS

No humans were used in this research. All animal research procedures followed were in accordance with the guidance of Committee for control and supervision of experiments on animals, Ministry of social justice and empowerment, Government of India.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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