

Research Article

Design and characterisation of lopinavir nanocrystals for solubility and dissolution enhancement

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ABSTRACT

The objective of present work was to prepare nanocrystals of Lopinavir (LPN) to enhance its solubility and dissolution rate with aim of dose reduction and minimising the side effects associated with its oral administration. Nanocrystals of LPN were prepared by anti-solvent precipitation method using a 3² full factorial design, employing stirring speed (X1) and concentration of surfactant (X2) as independent variables. The nanocrystals obtained were characterised mainly for particle size (PS), zeta potential (ZP), crystallinity, saturation solubility, in vitro dissolution and permeability. Results demonstrated profound effect of concentration of surfactant (pluronic F-68) on both the PS and polydispersity index (PDI) values. The optimised nanocrystals formulation had particle size 265nm, PDI 0.260 and ZP in the range of -18.0 to -22.5mv. X-Ray diffraction studies (XRPD) and Differential scanning calorimetry (DSC) studies suggested nanocrystal formation and absence of crystalline peaks, indicating loss of crystallinity, additionally confirmed by scanning electron microscopy (SEM). Nanocrystals showed 30.45 fold enhancements in aqueous solubility, and 38.5 fold in phosphate buffer pH 6.8, as compared to pure LPN. In vitro release studies have demonstrated 92.20% cumulative drug release within 3 hrs from nanocrystals compared to 42.65% from pure LPN. Even, increase in permeation flux from 423.1 µg/cm²/hr to 632.93 µg/cm²/hr in case of nanocrystals was also indication of enhanced dissolution. Stable LPN nanocrystals formulated by anti-solvent precipitation method shows improved solubility and dissolution. It has been concluded that LPN nanocrystals were obtained with significant improvement in saturation solubility and drug losing its crystalline nature when compared with plain drug.

1. INTRODUCTION

It is well explained that solubility, dissolution and gastrointestinal permeability are fundamental parameters that control rate and extent of drug absorption and its bioavailability. Hence, poor aqueous solubility is a major challenge for development of formulations, thus scientists are concerned with improving oral bioavailability of poorly soluble drugs¹⁻³. LPN is a potent protease inhibitor indicated for the treatment of HIV-1 infection. LPN is classified as a Class IV (poorly soluble and poorly

permeable) as per the biopharmaceutical classification system (BCS). Oral administration of LPN shows poor bioavailability due to its low aqueous solubility (0.01mg/ml), poor dissolution, high efflux by P-glycoprotein and extensive first pass metabolism in the liver by hepatic microsomal enzymes CYP3A4 and CYP3A5⁴⁻⁵. Numerous attempts have been made to overcome the solubility of LPN and improve its dissolution. Some of these attempts include alginate based nanoparticles, glyceryl behenate solid lipid nanoparticles, peptide based prodrug of LPN, Poly(lactic-co-glycolic acid) (PLGA) based nanoparticles⁶. However these nanoparticles formulations suffer from poor drug loading and the involvement of the multiple complicated steps in their formulations. Even their commercial use is limited due to stability issues of the amorphous nature of the produced drug.

Nowadays, nanocrystals are considered as a formulation choice for drug showing poor solubility and dissolution rate⁷. Nanocrystals consists of stabilized submicron sized crystalline drug particles in liquid medium, usually water^{8,9}. They can be produced either by precipitation technique (bottom-up approach) or by size reduction (top-down approach)^{10,11}.

Drug nanocrystals are nanoparticles being composed of 100% drug without any matrix material and mean particles size is below 1 μ m. The term drug nanocrystal implies a crystalline state of the discrete particles but depending on the production method, they can be partial or completely amorphous¹². Nanonization of hydrophobic drug in the presence of surfactant is one of the important approach for increasing the dissolution velocity of poorly soluble component. In the nanonization process, the coarse hydrophobic drug particles are converted small sized particle. Size reduction of drug particle is usually carried out in presence of different surfactant that imparts the physical stability to the nanocrystals vice-versa increasing the wetting property of nanosized drug particles¹³.

The aim of the present investigation was to prepare pluronic F68 stabilised LPN nanocrystals using anti-solvent precipitation method¹⁴⁻²⁰. The 3² full factorial design was employed to investigate the influence of formulation variables on nanocrystals characteristics. Solid state characterisation, particle size (PS), zeta potential (ZP), polydispersity index, saturation solubility, in

vitro release, and drug crystallinity parameters of freeze dried nanocrystals were studied.

2. MATERIALS AND METHODS

2.1. Materials

Lopinavir was obtained as a gift sample from Abbott Laboratories, Mumbai, India. Pluronic F 68 was purchased from Sigma chemicals, Mumbai. All other solvents and reagents in this work were of analytical/HPLC grade and used as provided.

2.1.1. Formulation of Lopinavir Nanocrystals

Lopinavir nanocrystals were prepared by anti-solvent precipitation method²¹⁻²⁴. Briefly, drug LPN of concentration 10 mg was dissolved in methanol & 0.5%, 1.0%, 2.0% concentration of Pluronic F-68 was dissolved in water (anti-solvent) for different batches. The anti-solvent was cooled to below 5°C in an ice-water bath. Then, drop wise addition of organic solution into 50 ml of the pre-cooled anti-solvent at a stirring speed of 800, 1000, 1200 rpm was carried out for different batches. Nanosuspension was prepared by adding the μ L quantity of drug solution to milliliter quantity of water quickly with continuous stirring on magnetic stirrer.

Optimization of Parameters for preparation of LPN nanocrystals were, rate of addition of organic phase 0.5 ml/min, needle size 26 ½ gauges, stirring time 2 hrs., ratio of organic: aqueous phase 1:1. LPN nanosuspension was lyophilized, 5ml of nanocrystal suspension was filled in 10 ml glass vials, covered with stoppers and placed in a freeze dryer (LABCONCO)²⁵⁻²⁹.

2.1.2. Saturation solubility study

Weighed amount of LPN, 10mg and the nanocrystal equivalent to 10 mg of the drug was separately introduced into 25ml stoppered conical flasks containing 10 ml distilled water, and phosphate buffer pH 6.8. The sealed flasks were agitated by using thermostatically controlled rotary shaker for 24 hrs at 37°C and equilibrated for 2 days. An aliquot was passed through 0.45 μ m membrane filter and the filtrate was suitably diluted and analyzed spectrophotometrically at the predetermined λ_{max} (260 nm).

Table 1: Translation of coded and actual levels of independent variables in factorial design.

Factors	Levels used		
	Low (-1)	Medium (0)	High (+1)
X1- Stirring speed (rpm)	800	1000	1200
X2- Surfactant concentration (Pluronic-F68)	0.5%	1%	2%

2.2. Optimization of formulation using factorial design

A statistical model incorporating interactive and polynomial term was used to evaluate the responses.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1^2X_2^2 + b_{11}X_1X_1 + b_{22}X_2X_2$$

Where, Y is dependent variable, b_0 is the arithmetic mean response of the 9 runs, and b_i (b_1 , b_2 , b_{12} , b_{11} and b_{22}) is the estimated coefficient for the factor X1). The main effect (X1 and X2) represents the average results of changing one factor at a time from its low to high values. The interaction term (X1X2) show how the response changes, when 2 factors are changed simultaneously. The polynomial term (X_1^2 and X_2^2) are included to investigate nonlinearity. The formulations were fabricated according to a 3^2 full factorial design, allowing the simultaneous evaluation of two formulation independent variables and their interaction^(30,31). The effect of the variables were investigated on the responses of the PS, ZP were selected as dependent variables and stirring speed (X1) and surfactant concentration (X2) were selected as independent variables. The replicate experimental runs were carried out in complete randomized manner. A multilinear stepwise regression analysis was performed using microsoft excel software. The full models were used to plot two dimension contour plots for both PS and ZP. All the statistical operations were carried out by PCP Disso 2000 V3 software. Factorial design parameters and experimental conditions are the factors used stirring speed (rpm) levels 800, 1000, 1200 and surfactant concentration (pluronic F68) levels 0.5%, 1.0%, 2.0%. 3^2 factorial designs formulation and experimental conditions factors levels used given in Table 1.

2.3. Characterization of lopinavir nanocrystals

2.3.1. Particle size analysis

Particle size of the nanocrystals formulation was determined by using particle size analyzer (Malvern Zetasizer Ver. 7.11 UK). Size and size distribution of the nanocrystals particles were determined through particle size analyser, after dilution with water and the diameters reported were calculated using mean particle size distribution. Measurements were performed in triplicate using 90° scattering angle at 25°C .

2.3.2. Zeta Potential Analysis

Measurement of zeta potential is also a prerequisite to know the stability of nanosuspension. Zeta potential is a measure of surface charge of particles and thus it imparts the colloidal stability due to particle-particle repulsion, as particle aggregation is less to occur for charged particles (a high zeta potential). So the prediction of zeta potential also allows the prediction of stability of nanocrystals. Zeta potential of the nanosuspended particles surface was determined by electrophoretic mobility in an apparatus such as a Malvern zetasizer (Malvern Instruments, UK) equipped with suitable software and calibrated with the supplied standard.

2.3.3. Percent drug content

The lyophilized nanocrystal powder (10mg) was dissolve in 1 ml methanol and volume was made up to mark in 10 ml volumetric flask with phosphate buffer pH 6.8. 0.1 ml of above solution was further diluted to 10 ml and analyzed by spectrophotometrically at 260 nm. The LPN content in nanocrystals (% w/w) was calculated using calibration curve.

2.3.4. *In vitro* release study

In vitro release of LPN from nanocrystals was evaluated by the dialysis bag diffusion technique. The diffusion medium was used phosphate buffer pH 6.8. The nanocrystals equivalent to 1 mg of LPN was dissolved in buffer and placed in the dialysis bag and sealed at both the ends. The dialysis bag was immersed in 70 ml of the receptor compartment (beaker), which was stirred at 50 rpm and maintained temperature at $37 \pm 2^\circ\text{C}$. The receptor compartment was covered to prevent the evaporation of medium. A sample (5ml) of the solution was withdrawn at every 5 minutes interval for first 1hr and 15 mins interval for next 2 hrs. The same volume was replaced by dissolution medium in the flask to maintain a constant volume. The samples were filtered and suitably diluted. The amount of drug dissolved determined by UV spectroscopy at λ_{max} of 260 nm. The percentage drug release was calculated.

2.3.5 *Permeability study:*

The optimised formulation and pure drug were subjected to *in-vitro* diffusion through cellophane membrane by using franz diffusion type cell with diffusional surface area of 3cm^2 and a receptor compartment of volume 22 ml. Cellophane membrane (pore size 0.45μ) was used as barrier between donor and receptor compartment. Cellulose acetate membrane was soaked in distilled water over night. The membrane was mounted on a franz diffusion cell. The receptor compartment was filled with phosphate buffer pH 6.8. The medium was magnetically stirred for uniform drug distribution and maintained temperature of $37 \pm 1.0^\circ\text{C}$. The nanocrystals equivalent to 5 mg drug was placed in the donor compartment along with cellulose acetate membrane used as the barrier between donor and receptor compartment.

Aliquots of 2 ml was withdrawn at definite time interval of 0, 15, 30 mins and 1hr up to 8 hrs. The amount of drug diffused was estimated spectrophotometrically at 260 nm. Permeability coefficient is the velocity of drug passage through the membrane in cm/hr. Permeability coefficient (P) was calculated from the slope graph of % of drug transported v/s time as using following formula.

$$P = \text{slope} \times V_d / S$$

Where, V_d = Volume of donor solution,
S = Surface area of tissue.

Flux is defined as the amount of material flowing through a unit cross sectional barrier in unit time. It is calculated by following formula,

$$\text{Flux (J)} = P \times C_D$$

Where, C_D = concentration of donor solution

2.3.6. *Fourier transforms infrared spectrophotometry*

FTIR spectrum shows the fundamental peaks corresponding to the chemical nature of the drug and excipients. FTIR studies were carried out in order to determine any possible interaction among drug and excipients used. IR absorption spectrum of LPN was determined by Fourier transform infrared spectrophotometer (Jasco- V-530 model). Spectra were recorded over the wave number $400\text{-}4000\text{ cm}^{-1}$. Infrared spectrums of pure drug and optimized batches were recorded. From the spectrum analysis the compatibility of ingredients in the formulations were determined.

2.3.7. *X-Ray diffraction studies*

The XRD patterns were recorded on X-ray diffractometer (PW 1729, Philips, Netherlands). Samples were irradiated with monochromatized Cu-K α radiation (1.542 \AA) and analyzed from 50 to 500 2θ . The voltage and current used were 30 kV and 30 mA, respectively. The XRD procedure to estimate the degree of crystallinity was based upon the measurement of the total scattering and the scattering from the crystalline region of formulations & pure drug.

2.3.8. *Differential scanning calorimetry*

DSC studies were carried out using (Mettler-Toledo DSC 821 instrument). Indium and zinc standards were used to calibrate the DSC temperature and enthalpy scale. Freeze dried nanocrystals of optimized batch and pure drug were hermetically sealed in aluminium crucibles and heated at a constant rate of $10^\circ\text{C}/\text{mins}$ over a temperature range of $25\text{-}300^\circ\text{C}$. Inert atmosphere was maintained by purging nitrogen gas at flow rate of 50 ml/min. An empty aluminium pan was used as standard reference and results were obtained in triplicates for each sample.

2.3.9. Scanning electron microscopy:

The surface characteristics of the selected formulation were observed using a scanning electron microscope (JSM-6360; JEOL, Tokyo, Japan). The samples were gold-coated under vacuum and then examined. Acceleration during the observation was 25 kV.

2.3.10. Stability studies:

Stability studies were carried out according to ICH guidelines Q1A (R²). The stability studies of LPN loaded nanocrystals, optimized formulation F6 batch were carried out temperature at 5°C ±3°C and 25°C ±2°C and 60% ± 5% RH for 3 months. The samples were withdrawn at specified intervals for analysis over a period of 30, 60 and 90 days. Particle size and percent drug contents were determined.

3. RESULTS & DISCUSSION

Lopinavir nanocrystals were successfully prepared by the anti-solvent precipitation method.

The obtained nanocrystals have been assessed for particle size analysis, zeta potential, saturation solubility and solid state characterisation by XPRD, DSC, FTIR and SEM analysis.

3.1. Selection of surfactant & its concentration

Selection of best surfactant from Pluronic F-68, Pluronic F-127 and Poly vinyl alcohol (PVA) on the basis of its nanocrystal size and polydispersibility index. For each surfactants of 1.0% concentrations was prepared and fixed stirring speed 1000 rpm. The concentration of surfactants was optimized depending on the resultant particle size and poly dispersability index of each batch. When surfactant pluronic F-68 was used, particle size and PDI observed 296.4±0.95nm & 0.147±0.002 as compared to pluronic F-127, 355.95±5.05nm and 0.101±0.04 and PVA 484.85±6.35nm and 0.13±0.03 respectively.

Pluronic F-68 was used as surfactants for stabilization of nanocrystals surface more effectively with smallest particle size and narrowest particle size distribution, observed as compared to pluronic F-127 and PVA. Hence Pluronic F-68 was used as a surfactant for further development of nanocrystals formulation with smallest particle size.

3.2. Saturation solubility study:

The optimized batch F6 showed highest solubility in water (0.067 mg/ml), as compared to pure LPN (0.0022 mg/ml), which was 30.45 fold greater than pure LPN drug respectively. The solubility of formulation batch F6 in phosphate buffer pH 6.8 was found to be 0.136 mg/ml. Thus solubility of formulation batch F6 was improved 38.85 fold as compared to pure LPN. It has been observed that saturation solubility of nanocrystals formulations were significantly higher than LPN pure drug in water and phosphate buffer pH 6.8 could be attributed to pH dependent solubility of LPN. The smaller PS and the higher surface area of the nanocrystals were associated with high potential energy, which resulted in an increase in solubility. Saturation solubility of nanocrystals in distilled water and phosphate buffer pH 6.8 shown in Table 2.

3.3. Characterization of Lopinavir nanocrystals

3.3.1. Particle size analysis:

PS and PDI of all the formulations were measured by dynamic light scattering (DLS; Malvern Zeta Sizer, Nano-ZS90, UK). It was found that the smallest particle size 265 nm and PDI 0.260 of optimized batch F6 as compared to other formulation batches. Factorial analysis of variance showed a significant effect of speed of rotation (X1) & concentration of surfactant (X2) on the PS. The 2.0 % surfactant concentration showed the lowest PS as compared with the 0.5 & 1.0% concentrations. This could be explained by the decrease in surface tension by increasing the surfactant concentration, which facilitates the

Table 2: Saturation solubility (mg) of nanocrystals in distilled water and phosphate buffer pH 6.8

Medium	Pure drug	Batches								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
Distilled Water	0.0022	0.065	0.058	0.059	0.062	0.059	0.067	0.056	0.060	0.058
Phosphate buffer pH 6.8	0.0035	0.129	0.119	0.116	0.121	0.127	0.136	0.118	0.120	0.119

Table 3: 3² factorial design, Data for particle size, PDI, zeta potential of nanocrystals

Batches	Coded Values		Particle size (nm)	PDI	Zeta potential (mV)	% Drug content*
	*X ₁	*X ₂				
F1	-1	-1	323	0.778	-18	90.26 ± 0.54
F2	-1	0	280	0.573	-19.3	86.05 ± 0.33
F3	-1	+1	307	0.281	-20.7	89.07 ± 0.93
F4	0	-1	277	0.288	-18.5	95.26 ± 0.37
F5	0	0	270	0.339	-17.9	94.65 ± 0.44
F6	0	+1	265	0.260	-22.5	96.01 ± 0.49
F7	+1	-1	426	0.126	-15.7	90.67 ± 0.35
F8	+1	0	450	0.144	-18.4	93.67 ± 0.28
F9	+1	+1	478	1.000	-19.1	92.67 ± 0.85

*Indicates average triplicates ±SD (n=3)

*X₁-Stirring speed (rpm), *X₂-Surfactant concentration*-1, 0, +1 – Low, Medium and High Levels

size reduction and stabilizes the formed nanocrystals with inhibition of aggregation. Moreover, PDI was significantly decreased by increasing the concentration of surfactant. The lowest PDI value was observed in the presence of 0.5 % surfactant concentration. These results were consistent with increasing the surfactant concentration leads to a significant decrease in the nanocrystals size and PDI. Particle size distribution of batches F1-F9 shown in Table 3 & Particle size distribution of batch F6 shown in Figure 1.

3.3.2. Zeta Potential Analysis:

The ZP value of all the batches were found to be in the range of -18 to -22.5mV, optimized batch F6 shows -22.5mV, which means optimized batch F6 have more stable than other batches. High ZP values indicate the physical stability of the prepared nanocrystals with low probability of aggregation and crystal growth. The highest surfactant concentration (2.0%) had showed significantly higher values, when compared to 0.5 & 1.0% surfactant concentration. The zeta potential of batches F1-F9 shown in Table 3 and Figure 2.

3.3.3. Drug content:

The drug content of freeze dried nanocrystals was determined by UV- visible spectroscopic method wavelength at 260nm. Low loss of drug content of optimized batch during freeze drying resulted in good recovery of nanocrystals. The drug content of freeze dried nanocrystals batches were shown in Table 3.

3.3.4. In vitro release study:

Nanocrystals formulation batch F6 was showed significantly higher % release of drug as compared with other formulation batches. The cumulative percentage drug release of optimized batch F6 was observed 92.20% within 3 hrs. Moreover, the increase in the dissolution rate caused due to PS reduction can be explained by the decrease in diffusion layer thickness. The increased surface area described by the Noyes-Whitney equation and the higher surface-to-volume ratio enabled hydration over a larger surface area and, consequently, resulted in increased drug dissolution. Hence decrease in the particle size achieved will have significant effect

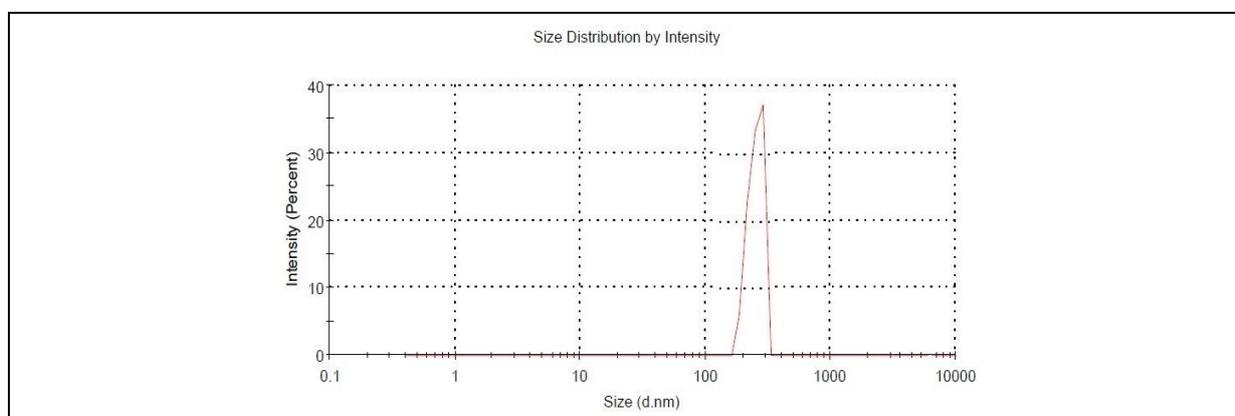


Figure 1. Particle size distribution of batch F6

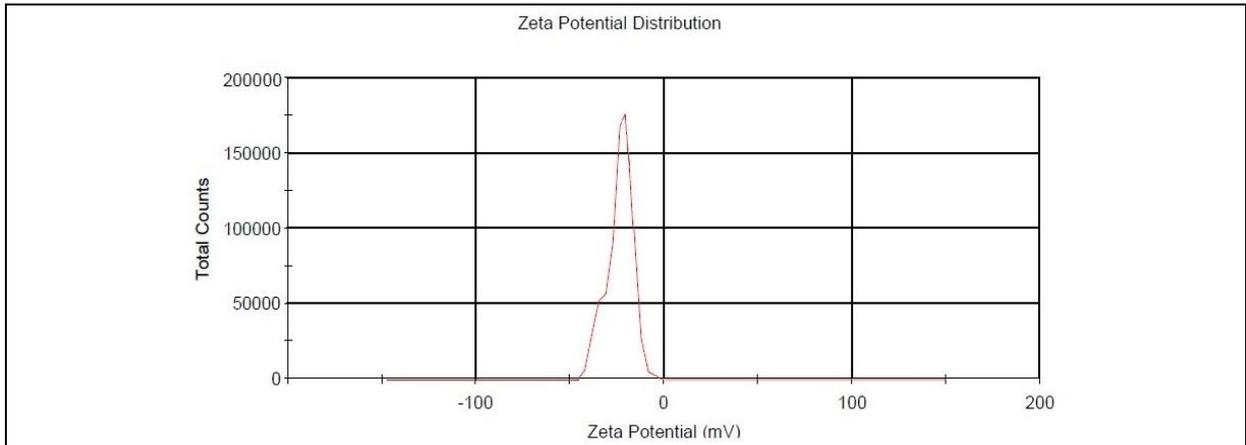


Figure 2: Zeta potential of batch F₆

in the drug solubility and dissolution. In vitro drug release for formulation batches F1-F9 were shown in Figure 3.

3.3.5. Permeability study:

The permeability coefficient of pure drug and optimized batch F₆ was found to be 62.1cm/hr and 95.37 cm/hr. The flux of pure drug and optimized batch F₆ was found to be 423.1 and 632.93(μg/cm²/hr) respectively.

3.3.7. Development of polynomial equations:

The experimental design for factorial formulations F1 to F9, polynomial equations for two dependent variables particle size and zeta potential have been derived using PCP Disso 2000 V.3 software.

Response Surface Plots:

The equation derived for particle size is:

$$Y1 = 268.3667 + 105.1083 X1 + 21.6667 X2 + 137.7750 X1X2 + 3.4500X2^2 + 48.3500 X1X2$$

The equation derived for Zeta potential is:

$$Y2 = -19.030 + 1.2275X1 - 1.8500X2 + 0.5275X^2 - 0.9050X2^2 - 0.5650X1X2$$

In Y1 equation positive sign for coefficient of X1 indicates that the particle size of nanocrystals increases, when speed of stirring is increased and positive sign for coefficient of X2 indicate positive effect of concentration of surfactant on particle size.

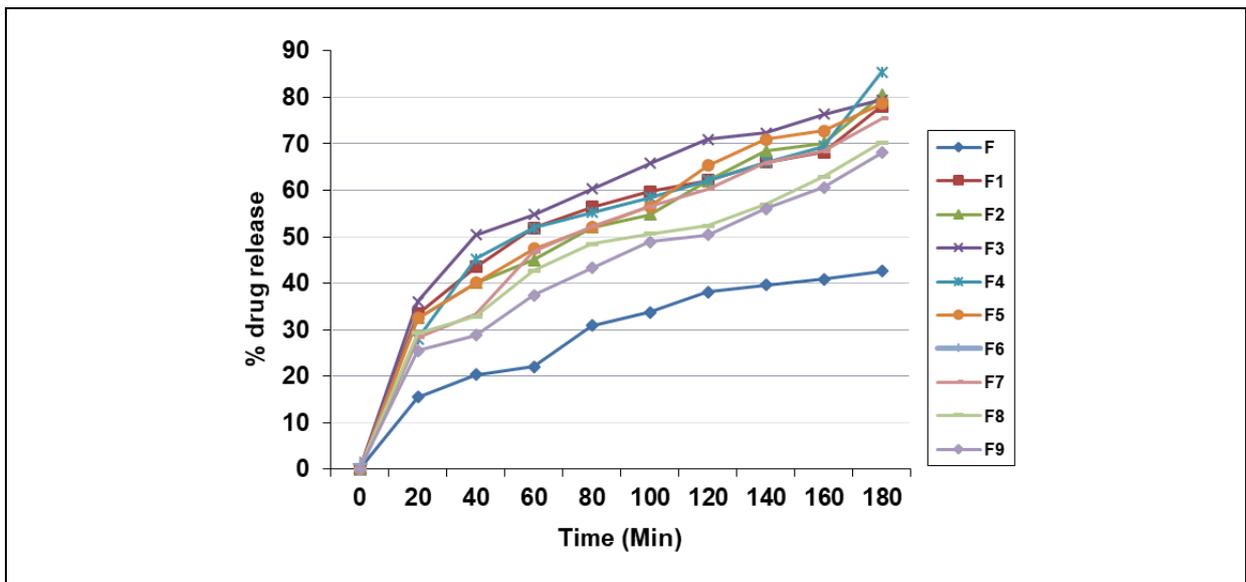


Figure 3: In vitro release profile of formulation F1-F9 and F pure drug

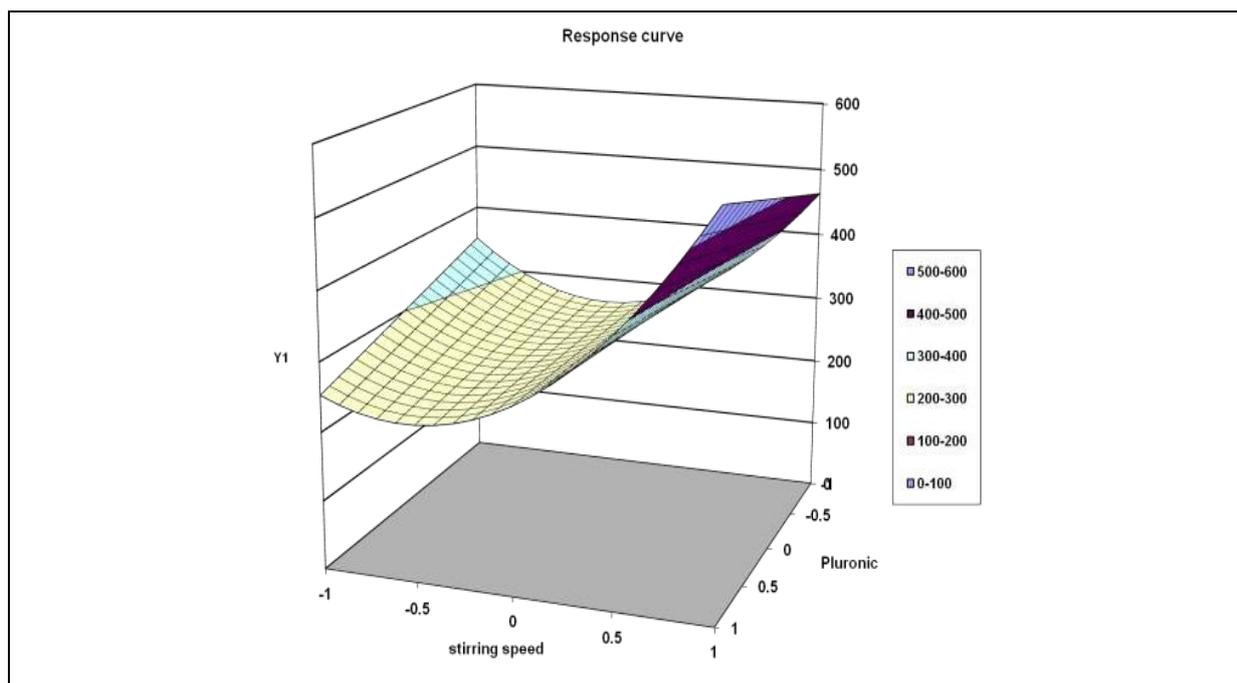


Figure 4: Response surface plots of particle size

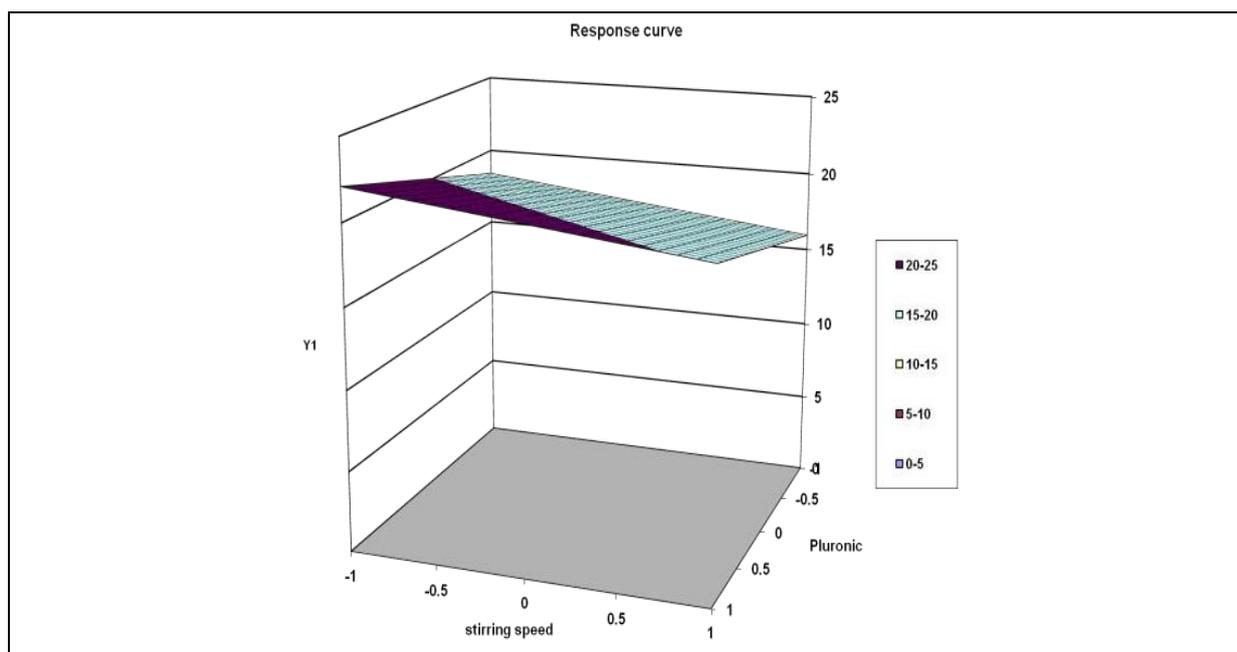


Figure 5: Response surface plots of zeta potential

In Y2 equation, positive sign for coefficient of X1 indicates that the zeta potential increases, when speed of stirring increases and negative sign for coefficient of X2 indicates that zeta potential of nanocrystals increases, when concentration of surfactant decreases. The closeness of predicted and observed values for particle size and zeta potential indicates validity

of derived equations for dependent variables. The data clearly indicates that the particle size and zeta potential are strongly dependent on the selected independent variables i.e speed of stirring and surfactant concentration. The values of the correlation coefficient indicate good fit. Response surface plots of particle size and zeta potential are shown in Figure 4 and 5.

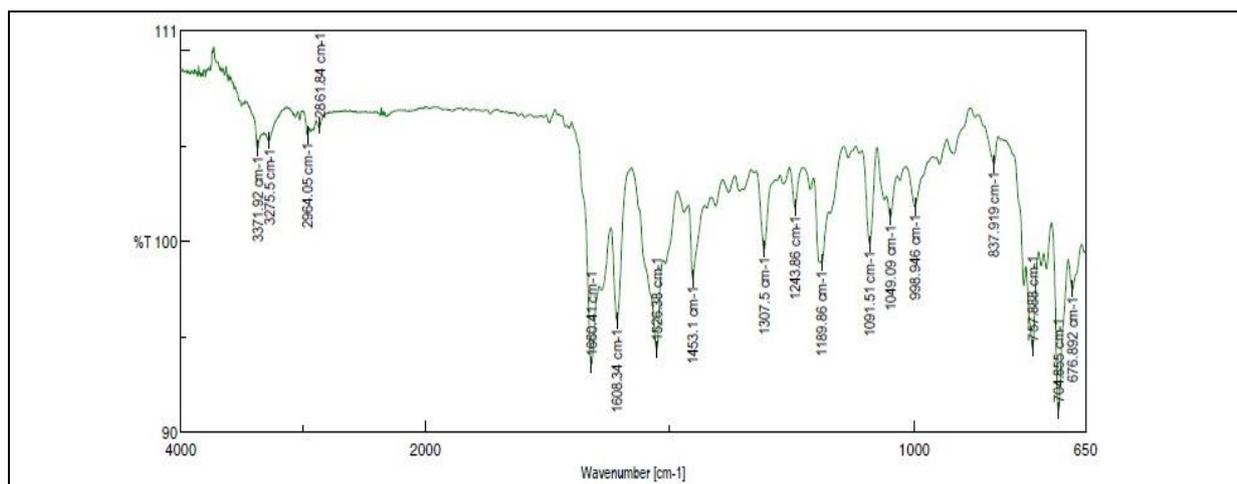


Figure 6. FTIR Lopinavir pure drug

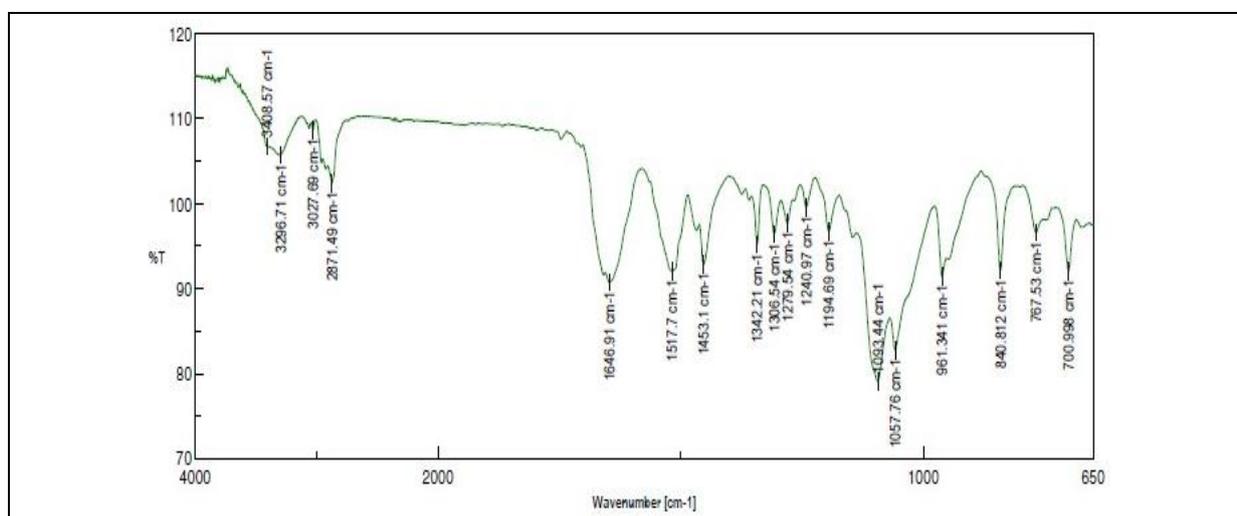


Figure 7. FTIR optimized batch F6

3.3.6. Fourier transform infrared spectrophotometry

FTIR studies revealed that the fundamental peaks of lopinavir were retained in the physical mixture indicating absence of any chemical interaction between LPN and excipients used. The characteristics peak of the hydroxyl group (OH stretching) at 3436.22cm^{-1} , a band peak at 3399.33cm^{-1} owing to imino group (N-H stretching), peak of the carbonyl group (C=O stretching) present in the amide group at 1653.35cm^{-1} , confirm the presence of lopinavir. FTIR studies revealed that the fundamental peaks of LPN was retained in the optimized formulation and indicating absence of any chemical interaction between LPN and excipients used. The characteristics peak of the hydroxyl group (OH stretching) at 3296.71cm^{-1} , a band peak at 3408.57cm^{-1} owing to imino group (N-H

stretching), the characteristics peak of the carbonyl group (C=O stretching) present in the amide group at 1646.91cm^{-1} . It has been observed that there was no appreciable change in the position and nature of the characteristics band of drug in the formulations. It can be concluded that the drug maintain it's identity without going any chemical interaction. FTIR of lopinavir pure drug and batch F6 shown in Figure 6 and 7.

3.3.7. X-Ray diffraction studies:

X-ray diffraction has been used to analyze potential changes in the inner structure of LPN crystals. The diffraction spectrum of pure LPN showed the drug was of crystalline nature as indicated by numerous, relative sharp and distinct peaks at a diffraction angle 2θ of 10.2, 15.1, 18.3, 21.4 and confirm the crystalline structure of drug

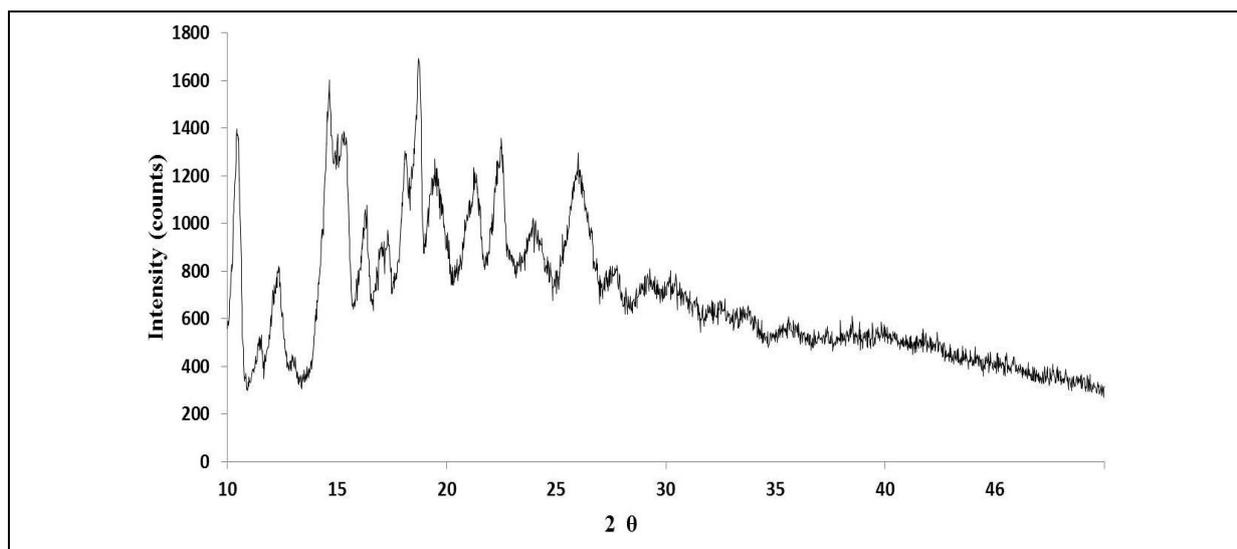


Figure 8. XRD Pattern of Lopinavir pure drug

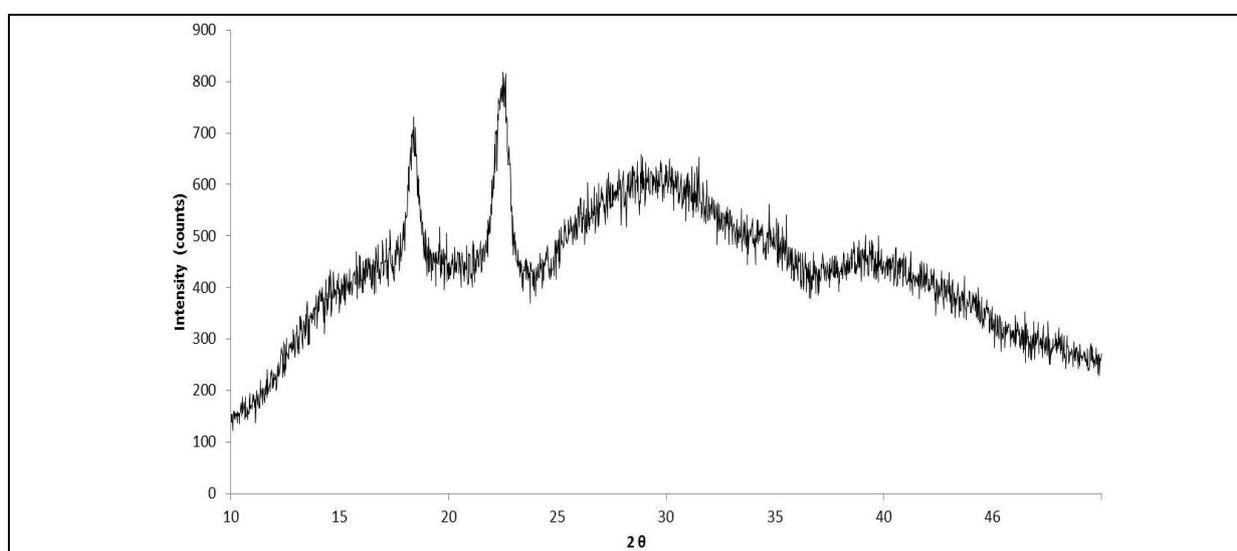


Figure 9. XRD Pattern of batch F6

shown in Figure 8. The decrease in peak intensity for nanocrystals can be attributed to the particle size reduction in formulation batches. The nanocrystals of optimized batch F6 was characterized by less intensity of the diffraction peak, when compared with LPN pure drug, which demonstrates that the chemical structure of the drug was not changed before and after precipitation process. This clearly indicates that significant reduction in the crystallinity of the LPN nanocrystals and the less ordered crystals were majority and the amorphous state would contribute to the higher drug loading capacity shown in Figure 9. It was confirmed that LPN existed in amorphous state in the LPN nanocrystals because of the disappeared sharp

peak of LPN in the diffraction pattern. Furthermore, maintenance of the initial crystalline state is advantageous for long-term stability

3.3.8. Differential Scanning Calorimetry:

DSC was performed to explore the physical changes that occurred in the drug after processing into nanocrystals. The pure drug exhibited a large and sharp endothermic peak at 93.95°C indicated its melting point shown in figure 10. DSC thermogram of formulation F6 showed an endothermic peak at 53.27°C ascribed to the melting of LPN, indicated the slight change in the crystalline nature shown in Figure 10. It

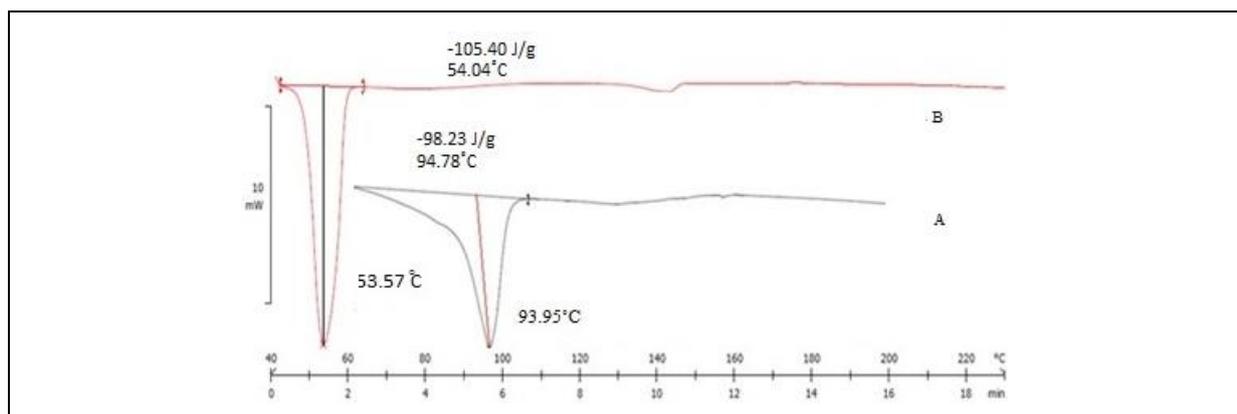


Figure 10: Overlain peak of DSC of lopinavir pure drug (A), batch F6 (B).

should be noted that reduction in melting temperature could increase the dissolution rate. No additional peaks were found to demonstrate the significant changes in the melting characteristics of LPN in the formulation, indicating no polymorphic changes during nano sizing process. The peaks were found to be nearly identical, with a calculated enthalpy (ΔH) of pure drug and F6 batch were found to be -98.23 J/g, -105.40 J/g, respectively. The DSC results were in support of the XRD analysis, which showed decrease in drug crystallinity.

3.3.9. Scanning Electron Microscopy:

Surface morphology of the formed nanocrystals was determined by using SEM and found that crystalline nature of all the formulations remains with slight change in crystallinity. It was clear that the investigated

lyophilized matrix possessed a highly porous nature, which led to the rapid penetration of water resulting in rapid drug dissolution. The SEM images and particle size distributions and its morphology of the LPN nanocrystals were presented in Figure 11. The nanocrystals were found to be flaky in shape with a narrow particle size distribution. Upon further magnification, lyophilized particles occurred in the form of rods with smooth and uniform surfaces.

3.3.10. Stability Studies

The stability studies of LPN loaded nanocrystals in terms of drug content and particle size distribution was monitored for 3 months at 2-8°C and RT 25-30°C. The nanocrystals showed physical stability for a period of 3 months at refrigerated conditions. At room temperature, results found to be particle size was increased at

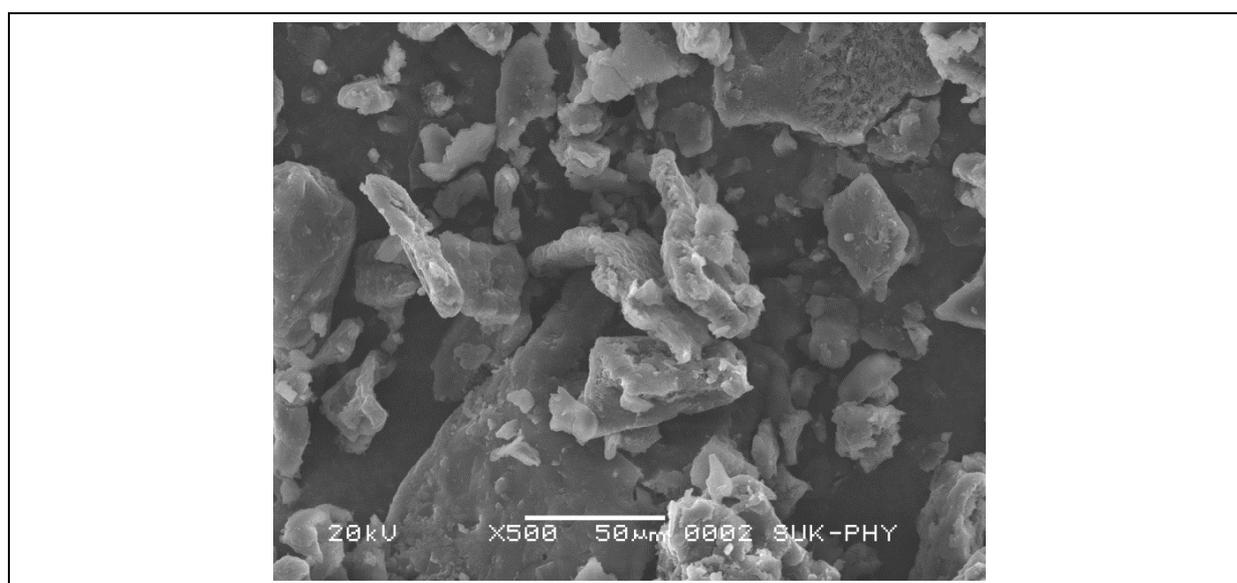


Figure 11: SEM images of batch F6 at 20 kv x500

Table 4: Stability data for lopinavir loaded nanocrystals at RT and (2-8 °C) *

Sr. No.	Time (Month)	Drug content (%) (2-8°C)	Drug content (%) (RT)	Particle size (nm) (2-8 °C)	Particle size (nm) (RT)
1	Initial	96.01±0.49	94.65 ± 0.44	265.14±12.6	265.14±8.3
2	1	97.6±1.3	93.5±2.4	268.67±10.2	280.3±13.5
3	2	96.6±1.0	90.4±2.5	270.16±15.3	288.6±14.2
4	3	96.9±1.6	89.8±3.4	271.6±13.9	304.1±8.9

*Indicates average triplicates ±SD (n=3)

265.14 to 271.6 and drug content 99.65 to 89.8 %. It was found that no significant difference was observed in the particle size and drug content of nanocrystals after 3 months at refrigerated conditions indicating its suitability for storage at 2-8°C. Stability of LPN loaded nanocrystals given in Table 4.

4. CONCLUSIONS

In this study it revealed that nanocrystallisation is promising approach for enhancing solubility and dissolution property of poorly soluble BCS IV drugs. The present work was a satisfactory preliminary study of improving solubility and dissolution of LPN by forming LPN nanocrystals using anti-solvent precipitation technique. Optimized formulation batch F6 having stirring speed 1000 rpm & 2.0 % pluronic F6 concentration, showed smallest particle size (265 nm), high saturation solubility in phosphate buffer pH 6.8 (0.136mg/ml) and rapid drug release 92.20% within 3 hrs. The aqueous solubility of optimized batch F6 was 30.45 fold greater than pure LPN. DSC and PXRD data revealed that there was partial decrease in crystallinity revealing improved solubility and dissolution. Even, increase in permeation flux from 423.1 µg/cm²/hr to 632.93 µg/cm²/hr in case of nanocrystals was also indication of enhanced dissolution. Further detailed investigations and *in vivo-in vitro* correlation need to be established to assure the efficiency and bioavailability of the formulation and it can be a promising tool in the treatment of HIV-1 infection. Preparation of nanocrystal formulations is simple and reproducible, and thus, could be used to improve the dissolution profiles of other poorly water-soluble active drug substances.

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Conflict of interest

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