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FORMULATION, DEVELOPMENT AND *IN-VITRO* EVALUATION OF LOPINAVIR LOADED SOLID LIPID NANOPARTICLES

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ABSTRACT: Lopinavir (LPV) is potent and poorly bioavailable molecule which is frequently administered protease inhibitors in the treatment of the HIV. Solid Lipid Nanoparticles (SLNs) of LPV were prepared using High Speed Homogenization. LPV were successfully encapsulated in the mixture of Glyceryl Behenate and Glyceryl monostearate. Poloxamer 188 was used as a surfactant for the preparation of the SLNs. Formulation composition and process parameter were optimized to get the desired quality of the SLNs. Formulation PF5 have mean particle size 185 ± 5 nm with Polydispersity index 0.11 ± 0.01 which indicates very narrow particle size distribution. % Entrapment efficiency was 86.2 ± 1.5. Slow drug release profile indicates the homogeneous dispersion of LPV in lipid matrix. SLNs have spherical shape and smooth surface which has been confirmed using SEM analysis. XRPD spectra show the reduction in crystalline behaviour of the drug and the lipid after formation of the SLNs. There was no significant change in the mean particle size and Polydispersity index after 6 month storage at 25°C/60% RH.

INTRODUCTION: Lopinavir (LPV) is potent and one of the frequently administered protease inhibitors in the treatment of the HIV [1, 2]. LPV is used in combined chemotherapy which is commonly referred as Highly Active Anti-Retroviral Therapy (HAART) [3]. LPV has very poor bioavailability when it is administered orally [4, 5]. Low bioavailability of LPV is due to P-glycoprotein efflux transport and high first pass metabolism which primarily mediated by cytochrome P450 [6, 7].

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Due to its high first pass metabolism of LPV, it is given in combination with Ritonavir (RTV) in most of the marketed formulation as RTV inhibits/suppress cytochrome P450 enzyme [8]. But, combination therapy with the RTV leads to major adverse effects such as Perioral Parasthesias, Elevations of lipid level, Glucose as well as Gastrointestinal intolerance [9, 10]. Therefore there is a requirement of a RTV free formulation strategy to improve the oral bioavailability of LPV.

Solid lipid nanoparticles (SLNs) have been recognized as an effective formulation and drug delivery because of its advantages like higher bioavailability, better stability, high compatibility, low toxicity, ease of process scale up and large scale production [11-15]. SLNs may improve the oral bioavailability by reaching the systemic circulation

through bypassing efflux transport as well avoiding first pass metabolism [16]. Gastro-intestinal tract (GI Tract) is richly supplied with blood as well as lymphatic vessels. Additionally, rate of fluid flow in portal blood is approximately 500 fold higher than that in intestinal lymph, maximum dietary compounds are transported to portal blood [17]. SLNs induce the stimulation of chylomicron which promote the absorption of lipid matrix through intestinal lymphatics [18].

Several methods had been utilized for the formulation of SLNs like High pressure homogenization, Micro-emulsion, Sonication, Solvent diffusion, Solvent evaporation. High pressure homogenization and Sonication are used widely as efficient and promising technique for SLNs preparation [19, 20]. Being highly lipophilic, LPV seems to be a suitable candidate for entrapment in the lipid matrix [21-23].

Poloxamer 188 has been used to coat the SLNs prepared with the lipid matrix of Glyceryl Monostearate and Glyceryl Behenate (Compritol 888 ATO) to entrap LPV. Paliwal et al. (2009) investigated the role of Glyceryl Behenate for improving the oral bioavailability of Methotrexate in comparison to other lipid matrices such as Stearic acid, Monostearin, Tristearin and Glyceryl Behenate.

In the present research, High pressure homogenization was utilized followed by ultrasonication for the preparation of SLNs. Prepared SLNs were characterized mean particle size, zeta potential, entrapment efficiency, assay as well as *in-vitro* release of the formulation[24,25]. Also, SEM and XRPD evaluation were performed on the Initial and Stability samples of the optimized formulation batch. **1.1.1**

MATERIALS:

LPV (melting point 124–127°C) was kindly supplied by Hetero Labs Limited, Andhra Pradesh, India as a gift sample. Glyceryl Monostearate and Glyceryl Behenate (Compritol 888 ATO) were obtained as a gift sample from Gattefosse, Mumbai, India. Poloxamer 188 were supplied by Sigma–Aldrich, Bangalore, India and Pearlitol-PF was supplied by Signet Chemicals, Mumbai, India as a gift sample. All remaining reagents and chemicals were of analytical grade. Purified water used for all experiments was MilliQ Plus, Millipore.

METHODS:

Analytical Method Development using HPLC-UV Technique:

The HPLC system used consists of LC-10AD/20AD pumps coupled with a Ultra-violet (UV) detector. The conditions on which these instruments run are as below.

Manufacturing Model: Perkin Elmer 200 series

Pump: Series 200 Binary pump

Detector: Variable single wavelength UV/VIS

Operating Software: Totalchrom

Sampling Method: Auto sampler

Mobile Phase: Methanol, Acetonitrile, Deionise water.

HPLC Column: Luna 5µ Phenyl-Hexyl column

Wavelength: 210 nm

Injection Volume: 10 µl

The LPV stock solutions were prepared by dissolving appropriate quantities of LPV in methanol, sonicated for 30 minutes for complete dissolution and make up to the mark in the volumetric flask to yield a final concentration of 1mg/ml (25mg or 0.025gms of LPV was accurately weighed and dissolved in 25ml of ethanol).

Pre-formulation Studies:

Solubility Studies in Different Solvent:

The solubility of LPV was determined by mixing an excess quantity of drug with approximately 2 ml of the solvent which was taken in a screw-capped bottle. The bottles were rotated on a Glass-Col (Terre Haute, IN) laboratory rotator at room temperature. Preliminary studies indicated that this time period was adequate to obtain equilibrium solubility. After the particles had settled, the supernatant was carefully withdrawn and filtered through a 0.22-µm filter and analysed by UV.

Physical Compatibility Study:

Compatibility studies were carried out for appropriate selection of excipients. Studies were carried out by mixing the drug with various excipients in required proportion in glass vials. Vials were closed with rubber stopper and kept at three conditions, namely 40ºC/75 % RH; 25ºC/60 % RH; and Photo stability for 1 month. Physical observations of the blend were done during the study at regular intervals. Compatibility of LPV with selected excipients was confirmed by Differential scanning calorimetry.

DSC scans of powdered sample of LPV, and Physical mixtures were carried out. DSC analyses were recorded using DSC - Shimadzu 60 with TDA trend line software. The pans were positioned on sample pan holder of a DSC 60. The thermal traces were obtained by heating from 50°C to 300°C. Thermograms were obtained by the DSC 60 thermal analyser program. Differential scanning calorimetry enables the quantitative detection of all processes in which energy is required or produced (i.e., endothermic or exothermic phase transformations).

Formulation Development of LPV Loaded SLNs

Preparation of SLNs:

SLNs were prepared using hot homogenization process followed by ultrasonication (Castelli et al., 2005). LPV 100 mg was weighed accurately and added to a suitable amount of Glyceryl Monostearate and Glyceryl Behenate (Compritol 888 ATO) which were previously melted at 80°C. Poloxamer 188 was dissolved in double distilled water 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 homogenization was carried out using a high-speed homogenizer (Ultra Turrax T25, IKA, GmbH). The temperature was maintained at 80°C or above during homogenization.

The obtained pre-emulsion was ultrasonicated using a probe sonicator t 100 W for 5 min. The obtained nanoemulsion (o/w) was cooled down in an ice bath to form SLN and finally diluted up to 200 ml with deionised water. The SLN dispersions were stored at 2°C-8°C until further analysis.

Optimization of the Composition and Process:

In present study concentration of surfactant (Poloxamer 188) was increased from 30% to 70% and proportionally concentration of lipid (Glyceryl Behenate) was decreased from 60% to 20%. Process parameter for the High Speed Homogenization will keep constant which is 15000 RPM for 15 min. The effects of lipid and surfactant concentration on the LPV SLN were evaluated as mentioned in **Table 1**.

TABLE 1: COMPOSITION FOR THE PREPARATION OF THE SLN

Batch No.		F1	F2	F3	F4	F5
Sr. No.	Ingredients	% w/w (Solid Content)				
1	LPV	1.2	1.2	1.2	1.2	1.2
2	Glyceryl Monostearate	8.8	8.8	8.8	8.8	8.8
3	Glyceryl Behenate	60.0	50.0	40.0	30.0	20.0
4	Poloxamer	30.0	40.0	50.0	60.0	70.0
5	Purified Water	Quantity Sufficient				
Total		100%	100%	100%	100%	100%

High pressure homogenizations are used widely as efficient and promising technique for SLNs preparation. Optimized and robust process of High Speed Homogenization may help to achieve the desired LPV loaded SLNs. Process parameter for High Speed Homogenization are optimized as mentioned in **Table 2**.

TABLE 2: PROCESS PARAMETER FOR HIGH SPEED HOMOGENIZATION

Batch No.	PF1	PF2	PF3	PF4	PF5
Parameter	5000 RPM for 2 min	20000 RPM for 2 min	10000 RPM for 2 min	10000 RPM for 5 min	10000 RPM for 10 min

Evaluation of the SLN:

Particle Size Analysis:

The average particle size and Polydispersity index as a measure of the particle size distribution were assessed by photon correlation spectroscopy using a Zetasizer Nano ZS (Malvern Instruments, UK). SLN formulations were diluted with double distilled water to weaken opalescence before particle size analysis.

Zeta Potential Measurement:
The surface charge was determined by measuring the zeta potential of SLN using Zetasizer Nano ZS (Malvern Instruments, UK). Zeta potential measurements were run at 25°C with electric field strength of 23 V/cm.

Entrapment Efficiency:
The entrapment efficiency was calculated by determining the amounts of non-encapsulated LOP in the aqueous surfactant solution, against the total amount of drug added to the formulation. LPV loaded SLNs were centrifuged in a cooling centrifuge and the filtrate was diluted appropriately and analysed by HPLC. Entrapment Efficiency was determined by using following formula:

Entrapment Efficiency = [(Amount of LOP in SLNs)/ (Total weight of LOP)] X 100

Assay:
Assay was determined by estimating concentration of LOP in 100 mg freeze dried SLNs against the lipid content of SLNs using following formula.

Assay = [(Amount of LOP in SLNs) / (Weight of Lipid in SLNs)] X 100

Freeze Drying:
Diluted SLN dispersions were filtered using a stirred cell ultra filtration unit 8050 (Millipore, Milan, Italy) equipped with a polyethersulfone membrane. Filtration was carried out by applying suitable positive pressure with Nitrogen. The lipid suspension was freeze-dried in table top freeze drier using Pearlitol-PF as a cryoprotectant. The pre-freezing and freeze drying times, temperature and vacuum conditions were appropriately adjusted to recover completely dried nanoparticles. The obtained freeze-dried SLN were stored at 2°-8°C.

Characterization of the SLNs using SEM and XRPD:
The surface morphology of the LPV loaded SLNs was visualized by a high-resolution field emission scanning electron microscope (FE-SEM; JSM-5610). The samples on conductive carbon paint were placed in a specimen holder, vacuum-dried and analysed for the surface characteristics using suitable magnification. Powder X-ray diffractometry of LPV and lyophilized sample were obtained at room temperature using Xpert MPD - XRD instrument by Philips, Holland. The sample was spread on a graticule and pressed in such a way that sample did not fall on keeping the graticule in vertical position. The graticule was placed in sample holder and exposed for radiation.

***In-vitro* Release Study:**
In-vitro release of LPV-SLNs was evaluated in USP-II apparatus at 50 RPM. Dissolution was performed in 0.1 N HCl for 2 h followed by Phosphate buffer pH 6.8 for 12 h. SLNs dispersion containing 10.0 mg LPV was transferred to USP-II apparatus. Samples were withdrawn at predefined time interval and analysed using HPLC.

Stability Study:
Stability studies were carried out with the optimized LPV loaded SLN. Sealed vials of freshly prepared freeze-dried SLN were placed in stability chamber maintained at 25° C/60% RH for 6 month and analysed.

RESULTS AND DISCUSSIONS: A suitable stability-indicating analytical method development is very critical. The standard curve was generated for the entire range from 0 to 100µg/ml. The results of standard curve preparation are shown in **Figure1.**

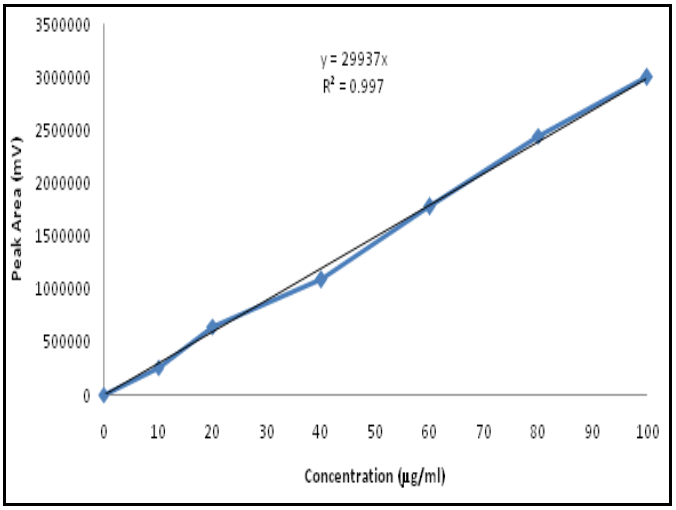


FIGURE 1: LINEARITY CURVE OF LPV

The solubility of LPV in various solvents was estimated. LPV is insoluble in water and aqueous buffer of pH 1 to 8. It is Soluble in organic solvents like methanol, ethanol, dichloromethane and DMF. From the extensive literature search and considering regulatory points, excipients were

selected for the compatibility study with LPV. vials. Vials were closed with seal and kept at 40ºC/75 % RH; 25ºC/60 % RH; and Photo stability for 1 Month. Drug Excipients compatibility study was performed with the ratio mentioned in above Studies were carried out using USP type I glass **Table 3**. There is no significant change in the appearance of the binary mixture as well as in physical mixture 1& 2 after 1 month accelerated condition.

TABLE 3: OBSERVATION OF DRUG-EXCIPIENTS COMPATIBILITY STUDIES

Sr. No.	Raw Materials	Ratio	Observation	Initial and after 1 Month		
				Light	25°C / 60% RH	40°C / 75% RH
1.	LPV	—	White powder	NC	NC	NC
2.	LPV + Glyceryl Monostearate	10:90	White powder	NC	NC	NC
3.	LPV + Glyceryl Behenate	10:90	White powder	NC	NC	NC
4.	LPV + Poloxamer 188	10:90	White powder	NC	NC	NC
5.	LPV + Pearlitol-PF	10:90	White powder	NC	NC	NC
6.	LPV + Glyceryl Monostearate + Glyceryl Behenate + Poloxamer 188 (Physical Mixture 1)	10:50:20:20	White powder	NC	NC	NC
7.	LPV + Glyceryl Monostearate + Glyceryl Behenate + Poloxamer 188 + Pearlitol-PF (Physical Mixture 2)	05:50:20:20:05	White powder	NC	NC	NC

Compatibility of LPV and physical mixture are also confirmed by Differential scanning calorimetry. DSC scans of LPV, Physical Mixture 1 and Physical Mixture 2 were carried out. The thermograms of LPV, Physical Mixture 1 and Physical Mixture 2 were shown in below **Figure 2**.

The melting point of LPV is about 124 – 127 °C. In DSC Spectra, LPV melting peak was shown at 124.1 ºC; in Physical Mixture 1 and Physical Mixture 2, it was present at 125.2 ºC and 124.9 ºC respectively.

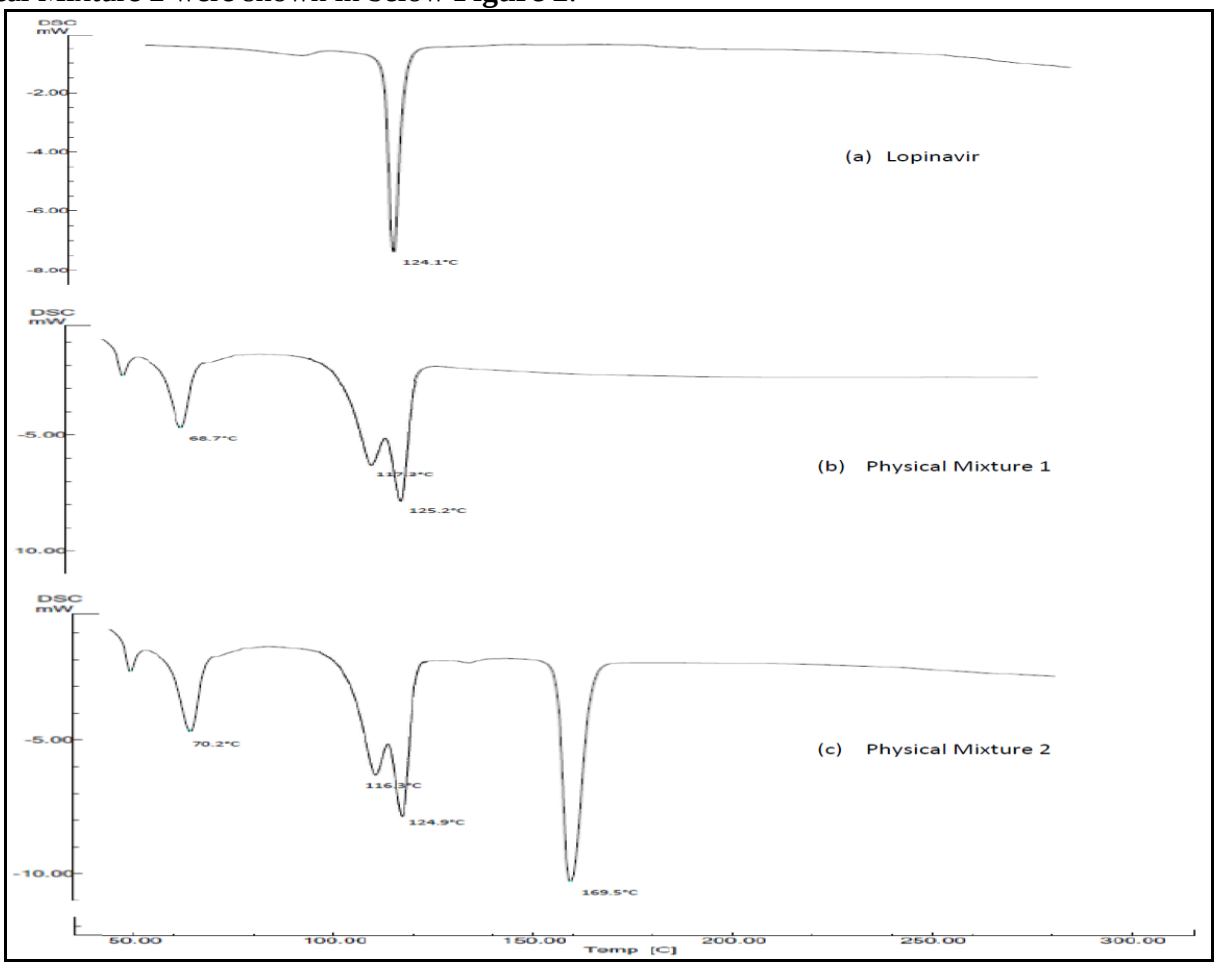


FIGURE 2: DSC THERMOGRAMS OF (A) LPV, (B) PHYSICAL MIXTURE 1 & (C) PHYSICAL MIXTURE 2

Ideal SLN have small particle size with narrow particle size distribution. PDI (Polydispersity Index) <1 % and higher % Entrapment efficiency depends on the formulation composition and process parameter. In present study concentration of surfactant (Poloxamer 188) was increased from 30% to 70% and proportionally concentration of lipid (Glyceryl Behenate) was decreased from 60% to 20%. Process parameter for the High Speed Homogenization will keep constant which is 15000 RPM for 15 min. The effect of lipid concentration on the LPV SLN was evaluated as mentioned in **Table 4**.

TABLE 4: EVALUATION OF THE LPV SLN

Sr. No.	Evaluation	F1	F2	F3	F4	F5
1	Mean Particle Size (nm)	364 ± 8	227 ± 10	189 ± 10	227 ± 42	219 ± 47
2	Polydispersity Index	0.15 ± 0.03	0.13 ± 0.01	0.17 ± 0.03	0.65 ± 0.18	0.91 ± 0.17
3	Zeta Potential (mv)	-25.2 ± 0.42	-31.3 ± 0.19	-27.4 ± 0.27	-12.2 ± 0.11	-11.8 ± 0.09
4	% Entrapment Efficiency	87.3 ± 0.8	85.4 ± 1.1	84.9 ± 1.0	62.1 ± 4.9	48.9 ± 6.1
5	Assay	92.4 ± 1.3	93.7 ± 1.3	94.2 ± 0.8	89.9 ± 1.8	90.5 ± 1.0

The effect of lipid concentration on the % entrapment efficiency was shown in **Figure 3.**

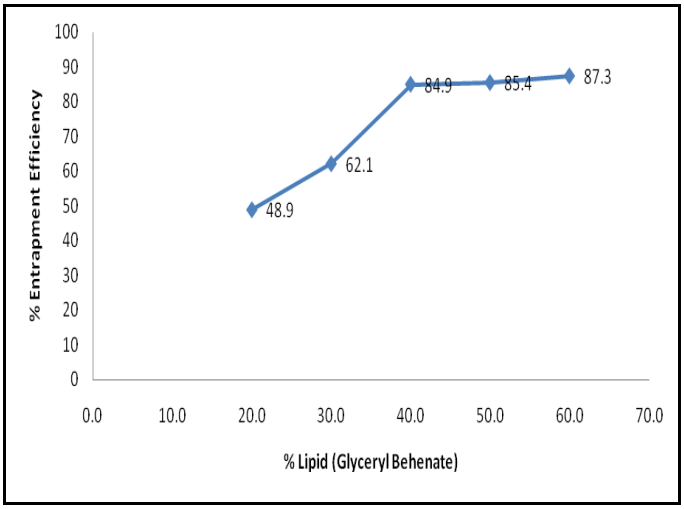


FIGURE 3: EFFECT OF LIPID CONCENTRATION ON % ENTRAPMENT EFFICIENCY

As the lipid concentration is increased with the simultaneous surfactant reduction, the entrapment efficiency was also increased. % Entrapment efficiency was increased from 48.9 ± 6.1% to 87.3 ± 0.8% by increasing lipid concentration from 20% to 60%. Formulation F1, F2 and F3 has around 85 – 90 % Entrapment Efficiency. While entrapment efficiency of formulation F4 and F5 are 62.1 ± 4.9 and 48.9 ± 6.1 % respectively.

Also % lipid content is responsible for the size distribution of the SLN. Wide variation in particle size of SLN leads to content uniformity issues. Also it impacts the release of the drug from the formulation. Increasing % lipid content resulted in more uniform distribution of particle size (Decrease in the PDI) as mentioned in the **Figure 4**.

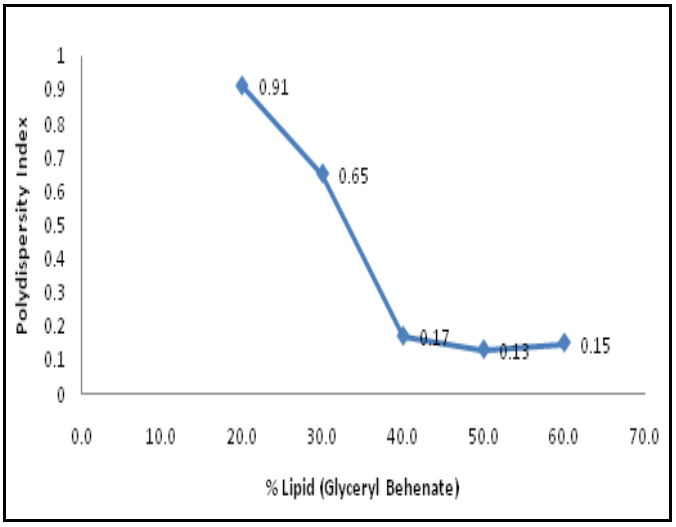


FIGURE 4: EFFECT OF LIPID CONCENTRATION ON POLYDISPERSITY INDEX

Formulation F1, F2 and F3 has the PDI of around 0.13 to 0.17 which is indicate very narrow particle size distribution. The viscosity of the system was also increased with the increased in lipid Concentration which resulted in the larger particle Size of the SLN. Formulation F1 has the particle size of 364 ± 8 nm and F2 has 273 ± 10 nm which might be due to more viscous system compare to other formulation. Hence composition of Formulation F3 is taken forward for time and RPM optimization of the high speed homogenization process.

Optimized and robust process of High Speed Homogenization may help to achieve the desired LPV loaded SLNs. Composition kept as similar to formulation F3 and process parameter was kept as mentioned in the **Table 5**.

TABLE 5: PROCESS PARAMETER FOR HIGH SPEED HOMOGENIZATION

Batch No.	PF1	PF2	PF3	PF4	PF5
Parameter	2500 RPM for 2 min	5000 RPM for 2 min	10000 RPM for 2 min	10000 RPM for 5 min	10000 RPM for 10 min

Above prepared batches are evaluated as mentioned in **Table 6**.

TABLE 6: EVALUATION OF THE SLN

Batch Code	PF1	PF2	PF3	PF4	PF5
Physical Appearance	Phase separation observed	Phase separation observed	Homogenous Emulsion	Homogenous Emulsion	Homogenous Emulsion
Mean Particle Size (nm)	--	--	169 ± 18	182 ± 7	185 ± 5
PDI	--	--	1.18 ± 0.09	0.52 ± 0.11	0.11 ± 0.01
Zeta Potential (mV)	--	--	-25.6 ± 0.15	-26.7 ± 0.27	-32.7 ± 0.13
% Entrapment Efficiency	--	--	42.4 ± 1.2	65.3 ± 1.8	86.2 ± 1.5
Assay	--	--	92.1 ± 0.9	89.2 ± 1.7	92.8 ± 0.5
Process Feasibility	--	--	Acceptable	Acceptable	Acceptable

By optimizing the process parameter such as Homogenization time, speed we can able to produce the SLN up to 200 nm particle size with PDI < 1, Which indicates narrow particle size distribution. Phase separation was observed in formulation PF1 and PF2 which may be due to low RPM and time of the High Speed Homogenization resulted in improper mixing of the aqueous and lipid phase. In batch no. PF3 RPM was increasing by keeping time constant as 2 min. Particle size of the SLN in formulation PF3 was < 200 nm as increasing homogenization speed resulted in decrease in particle size due to increase of applied shear forces which helps to breaking of the droplets with significant reduction of the particle size.

But, the % Entrapment Efficiency was < 50 % in formulation PF3 which may be due to improper mixing time can be responsible for the leakage. In formulation PF4 and PF5 time was increase by keeping RPM 10000. The mean particle size of optimized SLN formulation PF5 was found to be 185 ± 5 nm with a PDI of 0.11 ± 0.01. Mean zeta potential value was about −32.7 ± 0.13mV. The negative zeta potential was attributed to the presence of behenic acid residues onto the lipid matrix surface. The isoelectric point of Compritol was found to be 5.27, indicating that the produced SLN exhibit neutral charge at the pH of dispersions (pH between 6.8 and 7.3). The EE was around 85 % which may be due to the highly lipophilic nature of the drug and high solubility in Glyceryl Behenate and Glyceryl Monostearate.

In-vitro drug release study was performed for formulation PF5 in 750 ml of 0.1 N HCl at 50 RPM for 2 Hour followed by 1000 ml of pH 6.8 Phosphate Buffer at 50 RPM for 12 Hour as mentioned in **Figure 5**. Drug release in 0.1 N HCl after 2 Hour was around 4% and around 90% at the end in phosphate buffer. Slow drug release is due to homogeneous dispersion of LPV in lipid matrix.

For stability study freeze dried SLNs were kept at 25°C/60% RH up to 6 months. Results are as mentioned in the **Table 7**. There is no significant change in mean particle size as well as drug release profile after 6 months stability data. This indicates that freeze dried SLNs were remain stable up to 6 months at 25°C/60% RH.

TABLE 7: STABILITY DATA OF THE LPV SLN

Evaluation	25°/60%RH Condition				
	Initial	1 Month	2 Month	3 Month	6 Month
Mean Particle Size (nm)	185 ± 5.2	188 ± 4.1	188 ± 2.3	189 ± 3.9	191 ± 4.7
Polydispersity Index	0.11 ± 0.01	0.11 ± 0.03	0.13 ± 0.04	0.12 ± 0.03	0.14 ± 0.07
Zeta Potential (mV)	-32.7 ± 0.13	-29.2 ± 0.09	-30.5 ± 0.12	-32.2 ± 0.15	-33.1 ± 0.12
% Entrapment Efficiency	86.2 ± 1.5	85.3 ± 1.3	86.0 ± 1.0	84.9 ± 1.7	87.4 ± 1.6
Assay	92.8 ± 0.5	91.3 ± 0.8	92.1 ± 0.5	90.9 ± 0.9	91.8 ± 1.2

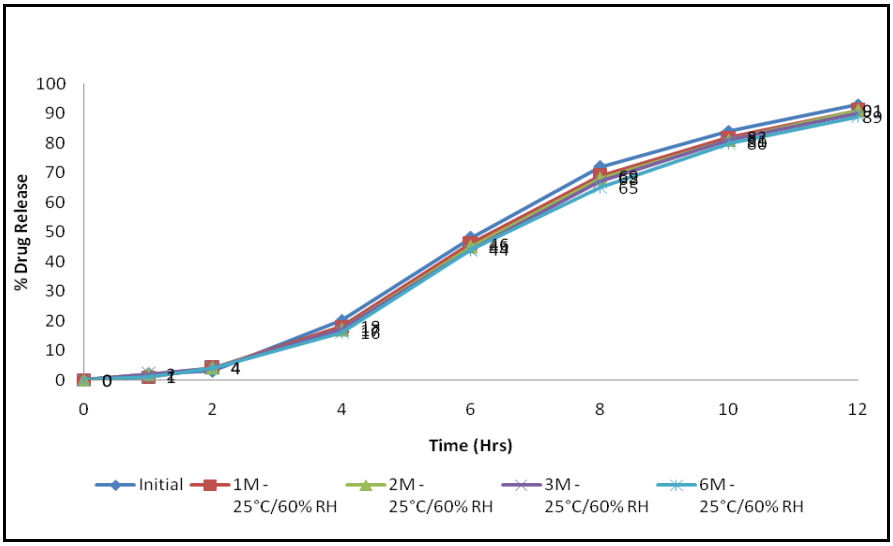
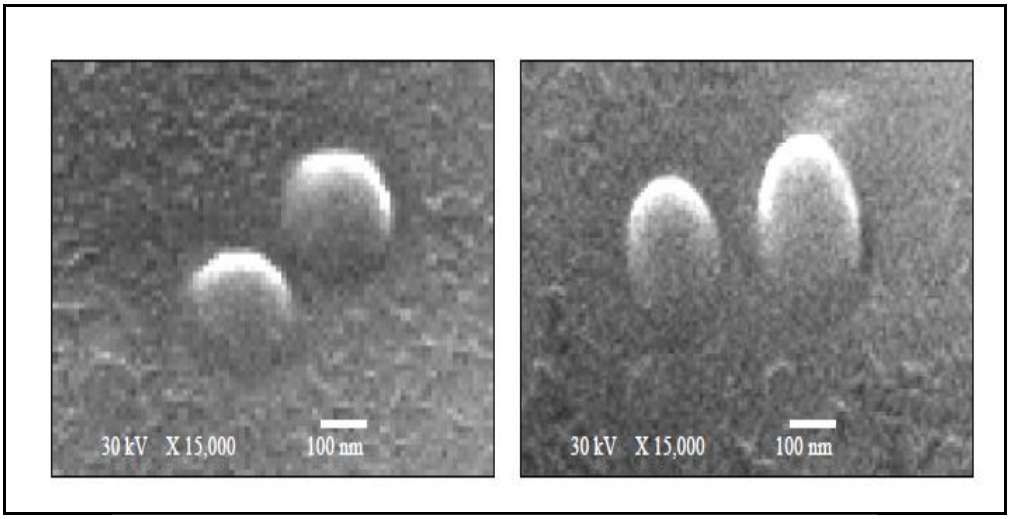


FIGURE 5: *IN-VITRO* DISSOLUTION OF THE LPV SLN

Figure 6 shows the typical SEM image of the LPV loaded SLNs which indicates that SLNs have spherical feature. SLNs showed bright periphery with the smooth surfaces. In addition to this, there is no significant difference in the SLNs sphericity, surface properties and size upon the stability study.



(a) (b)

FIGURE 6: SEM IMAGES OF THE LPV SLN (a) INITIAL AND (b) AFTER 6 MONTH 25°C/60% RH

In the XRPD evaluation as mentioned in **Figure 7**, the drug sample showed intense peaks as LPV is crystalline in nature. XRPD pattern of loaded and unloaded SLNs looks similar in terms of peak positioning and intensity as mentioned in below spectra. Absence of intense peaks of LPV in SLNs indicates presence of LPV in more amorphous form and efficient miscibility of LPV in lipids. Unloaded SLN and LPV loaded SLN shows less intense peaks at same or nearer 2θ values which shows that the crystalline nature of drug is reduced which might be due to increase in the amorphous \ tendency of the drug as well as lipid after SLNs formation. This further proves presence of molecular level dispersion of drug in lipid matrix after SLN formation which results in the crystalline reduction of drug and lipid.

XRPD spectra of the LPV and the LPV loaded SLNs (Initial and after 6M 25°C/60% RH sample) were carried out as mentioned in **Figure 8**. There is no significant change in the intensity of the peaks at same or nearer 2θ values on the stability samples which indicates that formulation is stable as there is no form conversion upon the stability.

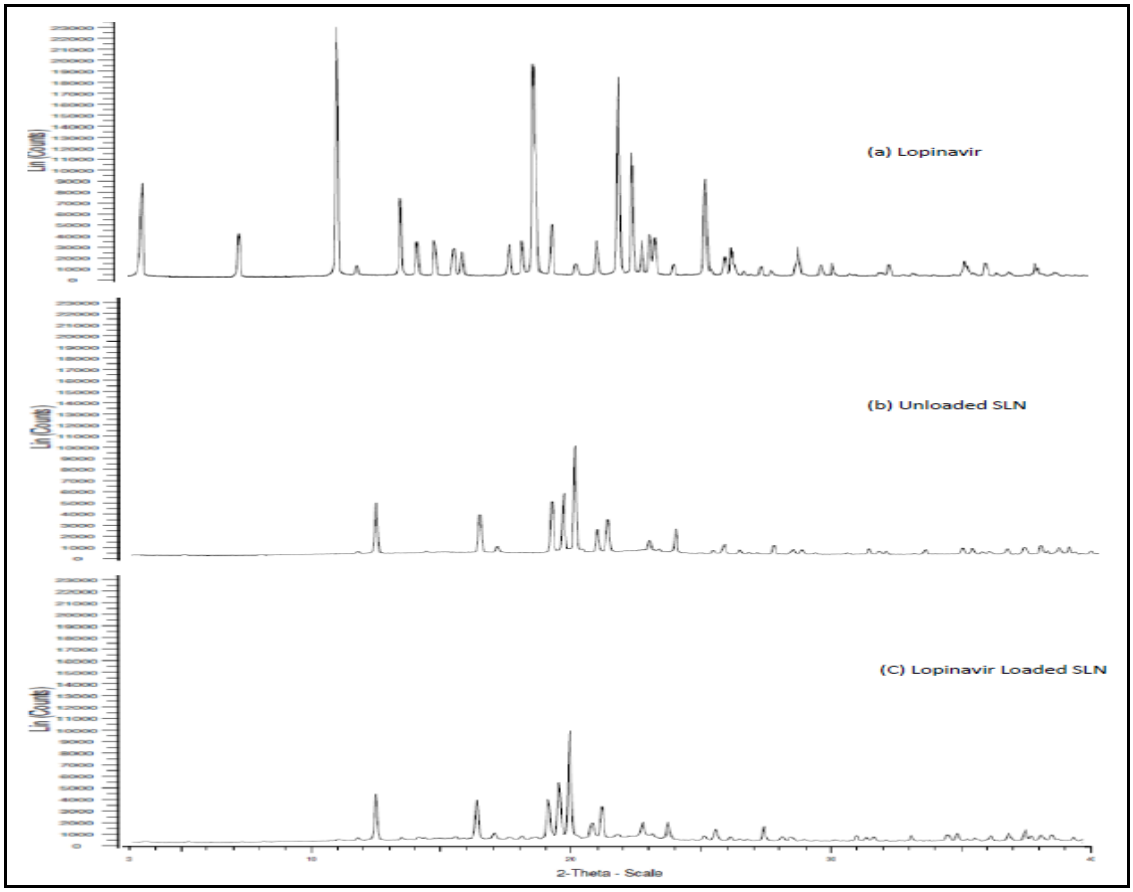


FIGURE 7: XRPD SPECTRA OF THE (a) LPV (b) UNLOADED SLN (c) LPV LOADED SLN

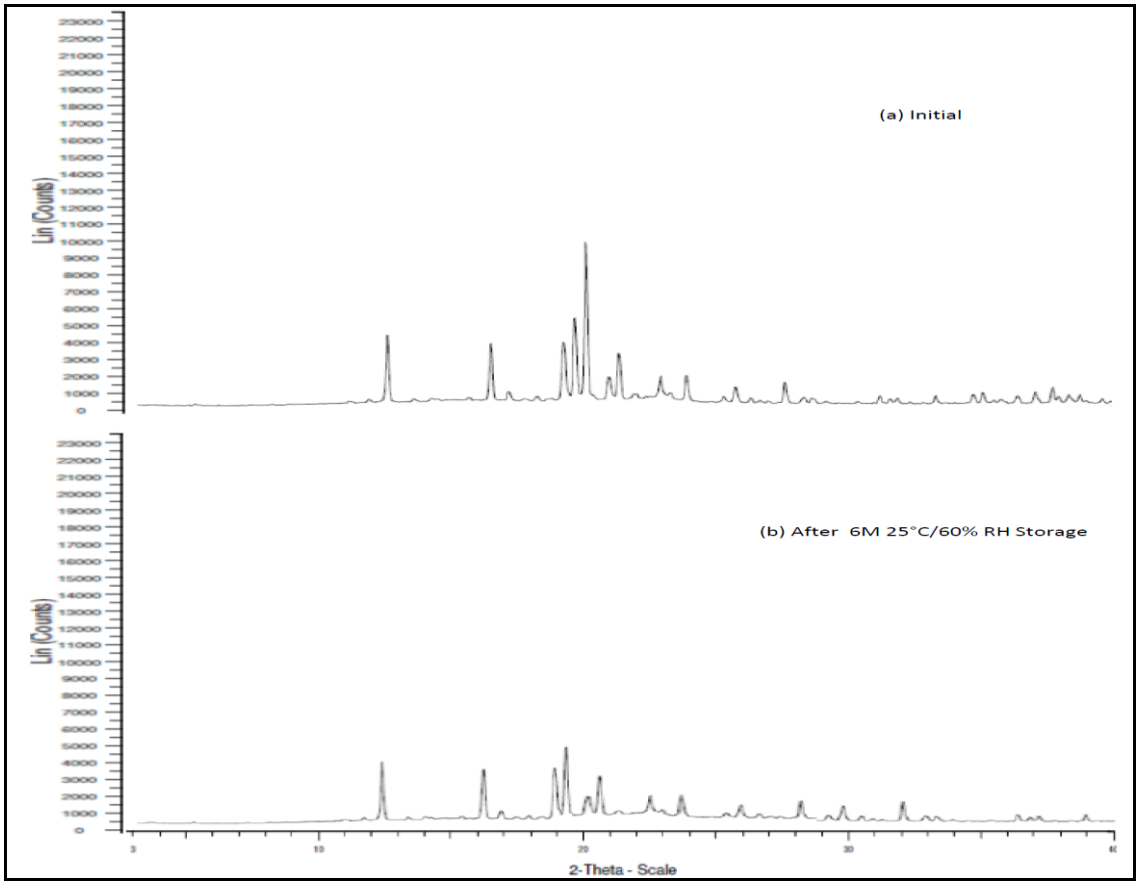


FIGURE 8: XRPD SPECTRA OF THE LPV LOADED SLN (a) INITIAL (b) AFTER 6M 25°C/60% RH

CONCLUSIONS: In the present investigation, LPV loaded SLNs were successfully manufactured using High shear hot homogenization technique. The effect of the formulation composition and process parameter on the LPV loaded SLNs were evaluated and optimized. Optimized formulation shows desired particle size with narrow particle size distribution, high % entrapment efficiency and longer *in-vitro* drug release profile. The good entrapment efficiency and drug loading were observed for LPV which may be due to good miscibility of LPV with lipids and surfactant. By suitable selection of the lipids and surfactant, we can achieve desired characteristics of SLNs. Stability data shows that there is no significant difference in the optimized formulation of LPV loaded SLNs after 6 Month 25°C/60% RH conditions. These storage conditions of SLNs were found appropriate for drug delivery. The study opens the chances of manufacturing of the LPV loaded SLNs by competitive cost at commercial level.

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