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In Vivo Evaluation of Palbociclib Loaded Solid Lipid Nanoparticles by Design of Experiment

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Abstract:

The purpose of the present study was to optimize palbociclib loaded solid lipid nanoparticles (SLNs) by evaluating the relationship between design factors and experimental data. A three factor, three-level Box-Behnken design (BBD) was used for the optimization procedure, choosing the amount of tricapric, cremophor RH40 and soy lecithin, as independent variables. The chosen dependent variables were particlesize, entrapment efficiency and % cumulative drug released. The generated polynomial equations and response surface plots were used to relate the dependent and independent variables. The optimal nanoparticles were formulated with 08%tricapric, 09%cremophor RH40, and 6% soy lecithin. Three formulations were prepared according to these levels and found that the observed responses were close to the predicted values of the optimized formulation. The formulation PF13 was chosen for characterization as it displayed minimum particle size (103 nm), PI of 0.47, zeta potential of -16 mV, maximum drug release of 98% in 12h. The formulation was stable when stored according to ICH guidelines for 6 months. In vivo study results in rat plasma show that at any time, point, the drug plasma concentrations in animals administrated with optimised solid lipid nanoparticles was higher than that of pure drug. C_{max} of the palbociclib optimised solid lipid nanoparticles 583.525±1.05 ng/ml was significant ($p<0.05$) as compared to the pure drug suspension formulation 110.45±1.64 ng/ml. T_{max} of both optimised solid lipid nanoparticles formulation and pure drug was 2.0±0.03and 4.0±0.04 h, respectively. $AUC_{0-\infty}$ infinity for palbociclib optimised solid lipid nanoparticles formulation was higher (10159.1±1.85 ng.h/ml) than the pure drug suspension 2587.3±0.37 ng.h/ml.

Keywords —Palbociclib,breast cancer, solid lipid nanoparticles, Box-Behnken design, in vivo and in vitro evaluation, bioavailability

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I. INTRODUCTION

Palbociclib is a medication developed by Pfizer for the treatment of HR-positive and HER2-negative breast cancer. It is a selective inhibitor of the cyclin-dependent kinases CDK4 and CDK6. Palbociclib was the first CDK4/6 inhibitor to be approved as a cancer therapy. It exhibits poor oral bioavailability of 46 % owing to its poor aqueous solubility and first pass metabolism. Hence solid lipid nanoparticles can be considered as a suitable drug delivery system to improve the poor oral bioavailability of palbociclib [1].

The drugs with poor oral bioavailability are unable to reach the minimum effective concentration to exhibit therapeutic action. Some of the reasons for poor bioavailability include poor solubility, inappropriate partition coefficient as it influences the permeation of drug through lipid membrane, first-pass metabolism; P-glycoprotein mediated

efflux, and degradation of drug in the gastrointestinal (GI) tract due to pH of the stomach or enzymatic degradation or by chemical. Solid lipid nanoparticles (SLNs) are sub-micron colloidal carriers having a size range of 50–1000nm. These are prepared with physiological lipid and dispersed in water or aqueous surfactant solution. SLNs were developed in the past decade as an alternative system to the existing traditional carriers, i.e., emulsions, liposomes, and polymeric nanoparticles [2,3]. These are related to emulsions, where the liquid lipid oil is substituted by a solid lipid. SLNs offer unique properties such as small size, large surface area, and high drug loading and are attractive for their potential to improve the performance of active pharmaceutical ingredients. The advantages of SLNs include drug targeting, biocompatibility, nontoxicity, drug release modulation, and small-scale production [4]. SLNs are also useful for the improvement of bioavailability of poorly water-soluble drugs, such as cyclosporine A [5], and to prolong the release of lipophilic drugs, such as camptothecin[6]. The mechanism proposed for enhancement of bioavailability of poorly water-soluble drugs by use of oral lipids includes promotion of lymphatic transport, which delivers drug directly to the systemic circulation while avoiding hepatic first-pass metabolism and by increasing GI membrane permeability [7,8]. It is known that drug delivery through SLNs improved the pharmacokinetic (PK) behaviour. Hence, there is a need to improve the solubility and bioavailability through this delivery system.

Response surface methodology (RSM) includes optimization procedures for the settings of factorial variables, such that the response reaches a desired maximum or minimum value. The response is in effect modelled by factorial techniques and ANOVA, but these are extended for more detailed modelling of the effects. RSM is based on the factorial study results (screening, then three-level factorial), and is a type of augmentation where extra treatments are added to focus the effects and improve the predictive power of the model. [9-14]

In the present research, palbociclib SLN was formulated and optimized using Box-Behnken design (BBD) followed by in vitro and in vivo evaluation.

II. MATERIAL AND METHODS

A. Materials used

The drug palbociclib was obtained from Hetero drugs Ltd, Hyderabad.. The excipients tricapric, cremophor RH 40, soy lecithin and solvents chloroform, methanol were purchased from Merck Specialties Pvt Ltd, Mumbai, India.

B.Preparation Of Palbociclib SLN

Palbociclib SLNs were prepared by a hot emulsification / ultrasonicationmethod using.Palbociclib (125 mg),tricapric (%) dissolved in chloroform and methanol [15].

C.Experimental Design

A 3^3 BBD employed for optimizing the main, interaction, and quadratic effects of formulation components on characteristics of SNEDDS. Seventeen experiments run randomly for chosen independent variables that include 5 repetitions at center (asterisk-marked) obtained from 3 factor, 3-level BBD and their subsequent responses noted. The variables that were chosen as dependent and independent are specified in **Table 1 and 2**.

The BBD matrix obtained using Design Expert® software (Version7.0, Stat-Ease Inc., Silicon Valley, CA, USA), the second-order quadratic equations are as:

$$Y = \beta_1 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1 X_2 + \beta_5 X_2 X_3 + \beta_6 X_1 X_3 + \beta_7 X_1^2 + \beta_8 X_2^2 + \beta_9 X_3^2$$

Y - Level of the measured response

β_0 – intercept

β_1 to β_9 - regression coefficient

X_1, X_2, and X_3 - main effects

X_1X_2, X_2X_3, and X_1X_3 - interaction between the main effects

X_1^2, X_2^2 and X_3^2 - quadratic terms of independent variables.

TABLE 1
COMPOSITION OF PALBOCICLIB SLNS FORMULATION BYBBD

F. No	Palbociclib (mg)	Tricapric (%)	Cremophor RH40 (%)	Soy Lecithin (%)	Tween 80 (ml)	Chloroform :Methanol (1:1)	Distilled Water (mL)
PF 1	125	4	3	4	0.5	20	Q.S
PF 2	125	8	3	4	0.5	20	Q.S
PF 3	125	4	9	4	0.5	20	Q.S
PF 4	125	6	6	4	0.5	20	Q.S
PF 5	125	4	6	2	0.5	20	Q.S
PF 6	125	8	6	2	0.5	20	Q.S
PF 7	125	4	6	6	0.5	20	Q.S
PF 8	125	8	6	6	0.5	20	Q. S
PF 9	125	6	3	2	0.5	20	Q.S
PF 10	125	6	9	2	0.5	20	Q.S
PF 11	125	6	3	6	0.5	20	Q.S
PF 12	125	6	9	6	0.5	20	Q.S
PF 13	125	8	9	6	0.5	20	Q.S
PF 14	125	6	9	4	0.5	20	Q.S
PF 15	125	6	6	4	0.5	20	Q.S
PF 16	125	4	3	6	0.5	20	Q.S
PF 17	125	4	9	4	0.5	20	Q.S

TABLE 2
LIST OF DEPENDENT AND INDEPENDENT VARIABLES IN IN BOX-BEHNKEN DESIGN

Independent variables			*Levels*		
Variable	**Name**	**Units**	**Low (-1)**	**Middle (0)**	**High (+1)**
A	Amount of Tricapric	%	4	6	8
B	Amount of Cremophor RH40	%	3	6	9
C	Amount of Soy Lecithin	%	2	4	6
Dependent variable			*Goal*		
Y1	Particle size	Nm	Minimize		
Y2	Entrapment Efficiency	%	Minimize		
Y3	Drug release after 12 Hrs	%	Maximize		

D.CharacterizationOfPalbociclib Loaded SLN

Measurement of particle size, zeta potential, drug Content and entrapment efficiency (EE%)[15], [16, 17] were calculated as per reported procedures.

E.In vitro release

Invitro release studies were performed in 0.1N HCl (pH 1.2) using modified franz diffusion cell and dialysis membrane having pore size 2.4 nm, molecular weight cut-off between 12,000-14,000 was used. Membrane was soaked in double distilled water for 12 h. SLN dispersion (2mL) was placed in the donor compartment and the receptor compartment was filled with 50 mL of release media. During the experiments, the solution in receptor side was maintained at 37°C ± 0.5°C and stirred at 50 rpm with magnetic stirring bars for 2 hours. Then, the pH was increased to pH 6.8 for the remaining 10 hours. An aliquot of the sample (5 mL) was taken from the dissolution medium at different time 0.5, 1,2,3,4,6,8 and12 h time points, samples were withdrawn and analysed by UV-visible spectrophotometer at 263 nm. Data obtained from *invitro* release studies were fitted to various kinetic equations to find out the mechanism of palbociclib release from SLN[18].

F.Kinetic Model Fitting

To elucidate the mode and mechanism of drug release, the data from the invitro release study were fitted into various kinetic models such as zero-order, first-order, Higuchi's, and Korsmeyer–Peppas model [19,20].

G. Characterization Of Optimized SLN Formulation

Characterization of optimized formulation by Fourier transform infrared spectroscopy (FT-IR), particle size, zeta potential, SEM studies and Stability studies were performed as per the procedures mentioned in reference.[21-24]

H. Pharmacokinetic Studies Of Palbociclib

Animal preparation

Healthy Wistar rats were (Weighing 180-200 g) selected for this study, all the animals were healthy during the period of the experiment. All efforts were made to maintain the animals under controlled environmental conditions (Temperature 25^0C, Relative Humidity 45% and 12 h alternate light and dark cycle) with 100 % fresh air exchange in animal rooms, uninterrupted power and water supply. Rats were fed with standard diet and *waterad libitum.* The protocol of animal study was approved by the institutional animal ethics committee

(IAEC NO:1477/PO/RE/S/11/CPCSEA-53/A).

Study Design[25,26]

Rats were divided in to two groups at random. The rats were fasted for 24 hours prior to the experiments. After 4 hours of dosing, foods were reoffered. First group was administered with pure Palbociclib (as such) made suspension with 0.5% methocel and second group was administered Prepared Palbociclibоptimised solid lipid nanoparticles diluted in 0.5% methocelby oral route at a dose of 0.15625mg. Then approximately 250 µL of blood samples were collected from retro-orbital plexus into heparinized 1.5 mL centrifuge tube at 0.5, 1, 2, 3, 4, 8, 12, 24 and 48 h post-dosing. Plasma was separated by centrifugation of the blood at 5000 rpm in cooling centrifuge for 5min to 10 minutes and stored frozen at –20°C until analysis.

Pharmacokinetic analysis

The pharmacokinetic parameters employed to evaluate were maximum plasma concentration (C_{max}), time to attain C_{max}i.e., T_{max} and t ½ values, area under plasma concentration–time curve from zero to the last sampling time (AUC_{0-t}), area under plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$).[27-29]

III. RESULTS AND DISCUSSION

A. Physico-Chemical Evaluation Of Palbociclib SLN

Developed palbociclib SLNs (PF1-PF17) were physicochemical evaluated in terms of particle size, entrapment efficiency, drug content and zeta potential. The drug content of all formulation ranged between 96-99% with formulation PF13 displaying maximum drug content. The EE of all formulation ranged between 78-93%with maximum value of 93.32% displayed by formulation PF13.The particle size of the drug loaded nanoparticles was found to be in the range of 103 to 239 nm. The nanoformulations exhibited negative surface charge with the inclusion of palbociclib which clearly suggested the orientation of palbociclib in the lipid matrix with long-term stability. The zeta potential of all 17 formulations ranged between 15-29mV.

B. Invitro Dissolution Testing Of Palbociclib SLN

The dissolution profiles of plain palbociclib and palbociclib SLNs formulation in simulated intestinal[23]. The drug release from the formulation PF13was shown to be 98.91%, whereas marketed product was shown to be 86.28% after 12 h (figures 1-3).

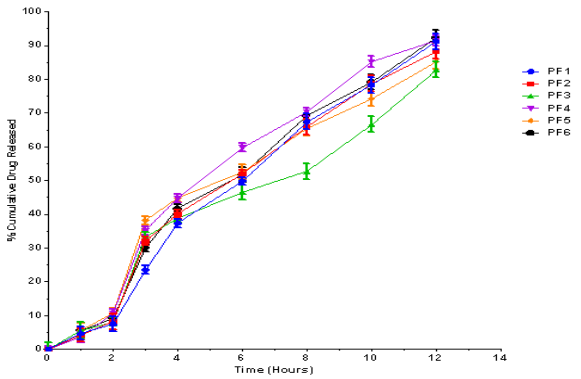

Figure 1: *In vitro*drug released profile of preparedpalbociclib loaded solid lipid nanoparticles PF1-PF6

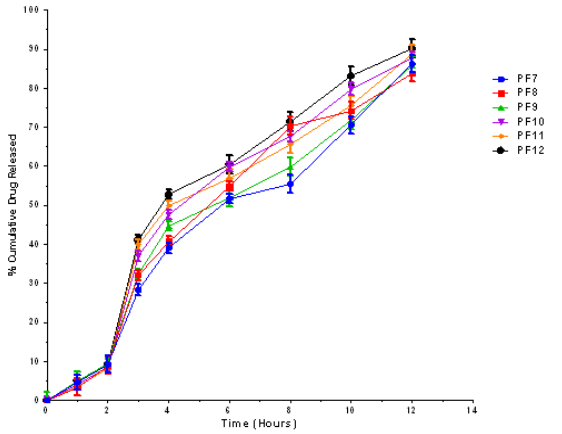

Figure 2: *In vitro*drug released profile of preparedpalbociclib loaded solid lipid nanoparticles PF7-PF12

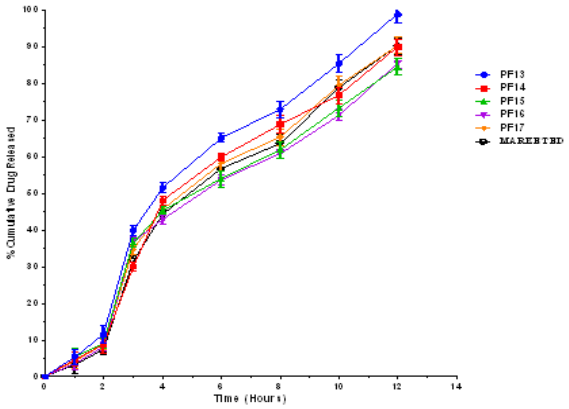

Figure 3: *In vitro*drug released profile of preparedpalbociclib loaded solid lipid nanoparticles PF13-PF17

C. Kinetic Analysis Of Palbociclib Release Data of PF13

It is apparent from the results that the regression coefficient value closer to unity in case of zero order plot i.e.,0.961 indicates that the drug release follows a zero-order mechanism Further the n value obtained from the Korsmeyer-Peppas plots i.e., 0.944 indicating nonFickian (anomalous) transports thus it projected that delivered its active ingredient by coupled diffusion and erosion for both optimized and marketed formulation.

C. Design of Experiments

Statistical analysis of the designed experiment

The range of particle size (Y1) for all batches was 103 – 239 nm. Similarly, the range for % entrapment efficiency(Y2) was 78.63% – 93.32% and the range for cumulative percentage of drug released in 12 h (Y3) was 83.88 –98.91 %. All responses were fitted to a second quadratic model and the adequacy of this model was verified by ANOVA; tests provided by Design- Expert software. For all the responses, the second-order quadratic model generated the highest F value so it was identified as the fitting model as shown in **Table 3.**

TABLE 3
STATISTICAL ANALYSIS RESULTS OF PARTICLE SIZE,EE AND % CUMULATIVE DRUG RELEASE

Source	Particle size		Entrapment efficiency		% Cumulative drug release	
	Sum of squares	p>F	Sum of squares	p>F	Sum of squares	p>F
Residual	4833.53	-	4232.45		2431.66	
Lack of fit	2838.17	< 0.05	3250.17	< 0.05	3485.19	< 0.05
	R-squared analysis		R-squared analysis		R-squared analysis	
R^2	0.9995		0.9991		0.9998	

Effect on particle size(Y1)

$$\mathbf{Y1}=95+73X_1-33X_2-17X_3-15X^2_1+42X_1X_3+71X^2_2-19X_2X_3+66X^2_3$$

The particle size of the nanoparticles was found to be in the range of 103-239 nm as shown in **Table 3.** The quadratic model generated revealed that the amount of tricapric, amount of cremophor RH40 and amount of soy lecithin have a significant influence on the Y1. The "Lack of Fit F-value" of 0.0259 implies the Lack of Fit is significant relative to the pure error. The theoretical (predicted) values and the observed values were in reasonably good agreement. The figures 4A and 4B clearly show that Amount of Cremophor RH40 has the main and the major effect on Y1 followed by Amount of Tricapric & Amount of Soy Lecithin which has moderate effect on Y1.

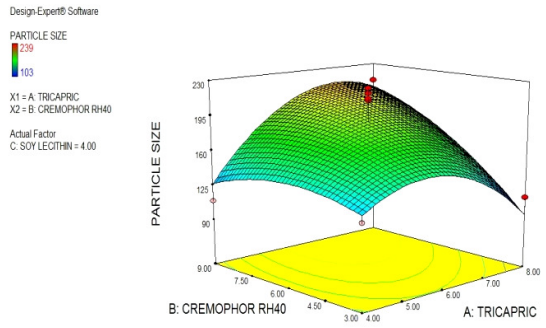

Figure 4A: Response 3D surface plot showing the influence of amount of Tricapric and amount of Cremophor RH40 on particle size fixed level of C

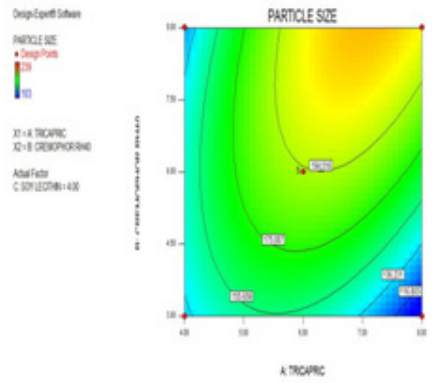

Figure 4B: Contour plot showing the influence of amount tricapric and amount of cremophor RH40 on particle size fixed level of C

Entrapment efficiency (%)

The entrapment efficiency (%) of the SLNs was found to be in the range of 78.63% to 93.32 %.The quadratic model generated revealed that the amount of tricapric and amount of cremophor RH40 have a significant influence on the entrapment efficiency (%). The theoretical (predicted) values and the observed values were in reasonably good agreement as seen. The "Lack of Fit F-value" of 0.0291 implies the Lack of Fit is significant relative to the pure error. The factorial equation for Entrapment Efficiency (%) showed a good correlation coefficient (0.9991).The figures 5A,5Bshow that shows that Amount of Cremophor RH40 has the main and the major effect on Y2 followed by Amount of Soy Lecithin which has moderate effect on Y2.

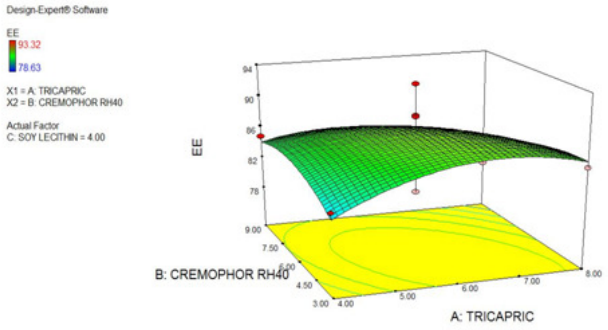

Figure 5A. Response 3D surface plot showing the influence of amount of tricapric and amount of cremophor RH40 on Entrapment Efficiency (%) fixed level of C

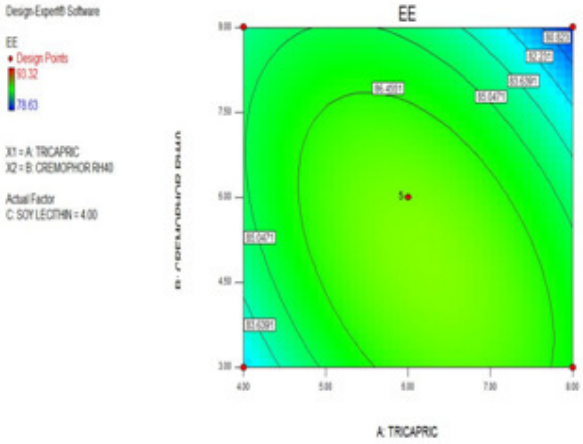

Figure 5B. Contour plot showing the influence of amount of tricapric and amount of Cremophor RH40 on entrapment efficiency (%) fixed level of C

Cumulative percent drug released

The cumulative percent drug release in 12 h from the SLNs was found to be in the range of 78.37 – 98.87%**.** The quadratic model generated revealed that the amount of tricapric, amount of cremophor RH40 and amount of Soy lecithin have a significant influence on the particle size. The "Lack of Fit F-value" of 0.0214implies the Lack of Fit is significant relative to the pure error. The results show that the effect of Amount of Cremophor RH40 is more significant than Amount of Trcapric and Amount of Soy Lecithin.

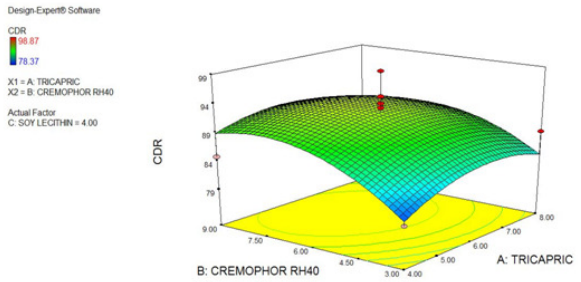

Figure 6A: Response 3D surface plot showing the influence of amount of Tricapric and amount of cremophor RH40 on cumulative % Drug Released fixed level of C

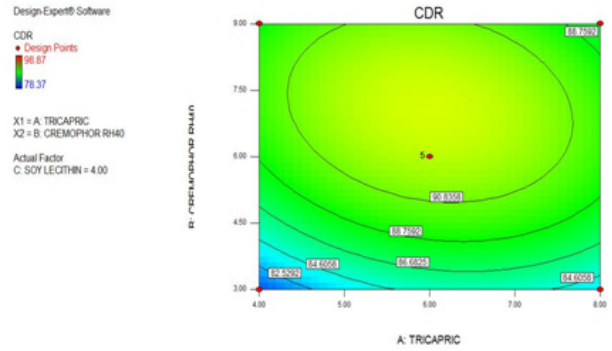

Figure 6B: Contour plot showing the influence of amount of Tricapric and amount of Cremophor RH40 on Cumulative % Drug Released fixed level of C

D. Optimization By Desirability Function

An optimization process was undertaken with desirability function to optimize the three responses simultaneously. The results are shown in **Table 4**.The model was proven to be validated since a fine agreement existed between the predicted and observed results. The maximum function value was obtained at X1:08, X2:09 and X3:06.It can be seen that the experimental values were in very close agreement with the predicted values, indicating the success of the Box–Behnken design combined with a desirability function for the evaluation and optimization of SLNs formulations.

TABLE 4

OPTIMIZED VALUES OBTAINED BY THE CONSTRAINTS APPLIES ON Y1, Y2 AND Y3

Independent variable	Nominal values %	Predicted values			Batch	Particle size (Y1) (nm)	Entrapment Efficiency (%) (Y2)	Percent drug release in 12 hrs (Y3)
		Particle size (Y1) (nm)	Entrapment Efficiency (%) (Y2)	%CDR (Y3)				
Amount ofTricapric(A)	8	103	93.32	98.91	1	103	93.25	98.75
Amount of Cremophor RH40(B)	9				2	106	93.21	98.84
Amount of Soy Lecithin (C)	6				3	105	93.18	98.82

E. Characterization Of Optimized SLN

FTIR study

The FTIR of pure drug displays the carbonyl vibration in the 1900–1650 cm^{-1} regions of the IR spectra. The N–H stretching exhibited a bathochromic shift from 3421.24 cm^{-1} to 3413.79 cm^{-1}, whereas the N–H bending vibration appear at 1549.67 cm^{-1}.No variation is observed in principal peaks of drug in optimized formulation indicating the compatibility between drug and excipients (**Figures 7 and 8**).

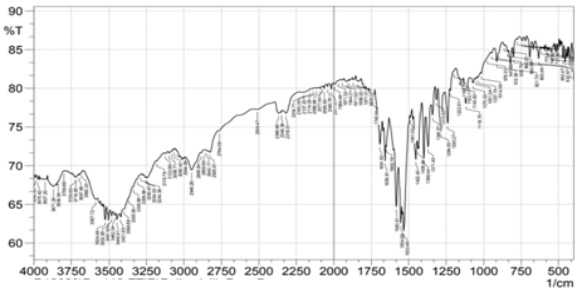

Figure 7:FTIR of Palbociclib pure drug

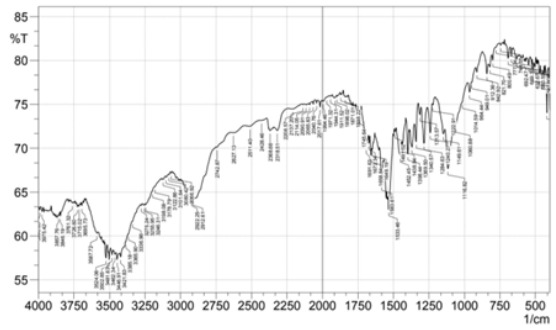

Figure 8: FTIR of optimized formulation PF13

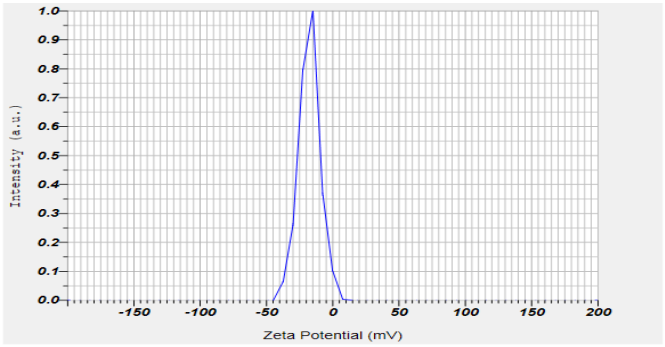

Figure 10: Zeta potential of optimized SLN PF13

Droplet size and zeta potential

Droplet size distribution following nanoemulsification is a critical factor to evaluate a nanoemulsion system. The mean globule size of selected SLN formulation PF 13 was 103 nm with low polydispersity index, which is indicated the ability of the present technology to produce nanoemulsion that offers larger interfacial surface area required for drug absorption.A narrow polydispersity index 0.47 means that the colloidal particles are homogenous in nature.The optimized SLN showed high absolute zeta potential value of -16.0 mV. The emulsion stability is directly related to the magnitude of the surface charge. (**Figure 9 and 10**)

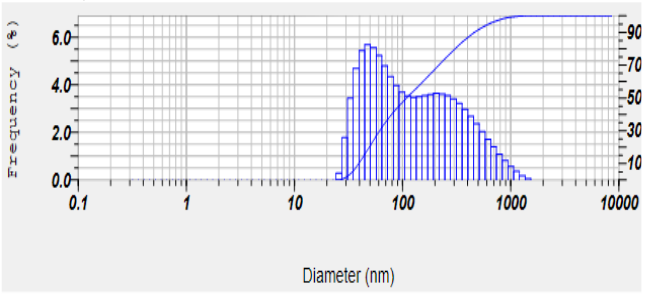

Figure 9: Particle size of optimized formulation PF13

SEM analysis

The SEM data indicates spherical and uniform particles of Palbociclib optimized formulation PF 13, that are slightly porous with rougher surfaces. The roughness of surface is due to quick moisture loss from wet mass possessing higher liquid content due to porous surface (**Figure 11**).

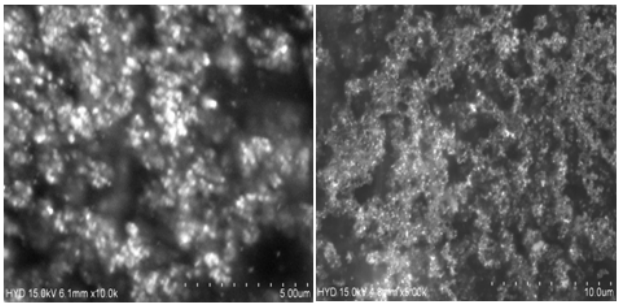

Figure 11:SEM of optimized formulation

F. Stability Studies

The formulation PF13 is found stable for 6 months with no significant variations in the values of particle size, entrapment efficiency, drug release profile and drug content values (**Table 5**).

TABLE 5
STABILITY STUDIES OF OPTIMIZED FORMULATION

Retest Time for Optimized formulation (PF13)	Particle Size (nm)	Entrapment Efficiency (%)	*In-vitro* drug release profile (%)	Drug content (%)
0 days	103	93.32±1.18	98.91±1.06	99.83±1.65
30 days	103	93.25±1.09	98.84±1.23	99.83±2.12
60 days	103	93.24±1.15	98.72±1.15	99.79±2.37
120 days	103	93.24±1.11	98.68±1.20	99.75±2.55
180 days	103	93.23±1.05	98.65±1.13	99.73±2.29

Values are expressed in mean± SD :(n=3)

G. Pharmacokinetic Data Of Palbociclib

Palbociclib concentrations in plasma following oral administration of pure drug and optimized palbociclib solid lipid nanoparticle formulation administered oral route and respective plasma concentration-time curves are shown in **Figures 12A,12B.**

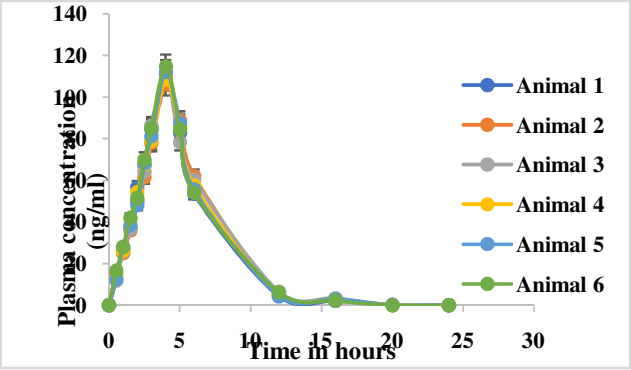

Figure 12A: Plasma concentration-time profile of palbociclib pure drug in rat plasma

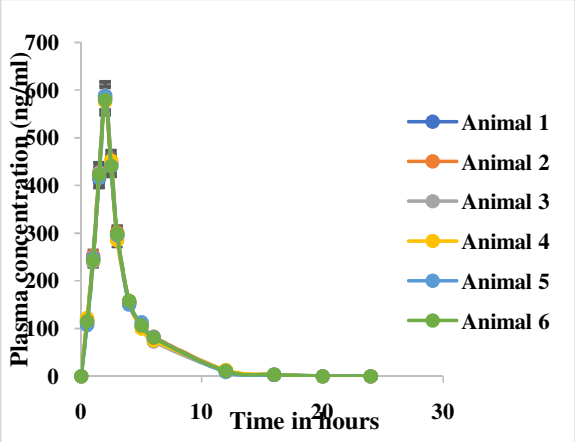

Figure 12B: Plasma concentration-time profile of palbociclib optimized solid lipid nanoparticles in rat plasma

Figure 13 indicates plasma concentration–time curve recorded post single oral dose of palbociclibоptimised solid lipid nanoparticles formulation in comparison to palbociclib pure drug suspension. At any time, point, the drug plasma concentrations in animals administrated with optimised solid lipid nanoparticles was higher than that of pure drug.

C_{max} of the palbociclib optimised solid lipid nanoparticles 583.525±1.05 ng/ml was significant ($p<0.05$) as compared to the pure drug suspension formulation 110.45±1.64 ng/ml. T_{max} of both optimised solid lipid nanoparticles formulation and pure drug was 2.0±0.03and 4.0±0.04 h, respectively. AUC is an important parameter in evaluating bioavailability of drug from dosage form, as it represents the total integrated area under the blood concentration time profile and represents the total amount of drug reaching the systemic circulation after oral administration. $AUC_{0-\infty}$ infinity for palbociclib optimised solid lipid nanoparticles formulation was higher (10159.1±1.85 ng.h/ml) than the pure drug suspension 2587.3±0.37 ng.h/ml. Statistically, AUC_{0-t} of the optimised solid lipid nanoparticles formulation was significantly higher ($p<0.05$) as compared to pure drug suspension formulation. Higher amount of drug concentration in blood indicated better systemic absorption of palbociclibfrom optimised solid lipid nanoparticles formulation as compared to the drug suspension formulation.(Table 6)

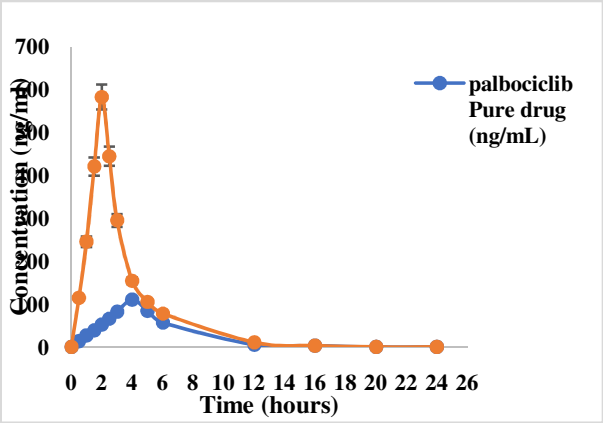

Figure 13: Plasma concentration profiles of palbociclib optimised solid lipid nanoparticles and pure drug

TABLE 6
PHARMACOKINETIC PARAMETERS OF PALBOCICLIB OPTIMISED SOLID LIPID NANOPARTICLES FORMULATION AND PURE DRUG

Pharmacokinetic parameters	Palbociclib Pure drug	Palbociclib –SLN Optimized formulation
C_{max} (ng/ml)	110.45±1.64	583.525±1.05
AUC_{0-t} (ng. h/ml)	1572.8±2.13	7236.1±1.92
AUC_{0-inf} (ng. h/ml)	2587.3±0.37	10159.1±1.85
T_{max} (h)	4±0.04	2.0±0.03
$t_{1/2}$ (h)	23.12±0.07	8.0±0.05

The pharmacokinetic data was subjected to statistical analysis to test the significant differences between the pharmacokinetic parameters of two formulations. The results are shown in **Table 7.** The data indicated that there was significant difference in C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, between palbociclib pure drug and palbociclib optimised solid lipid nanoparticle formulation.

TABLE 7

STATISTICAL ANALYSIS OF PK PARAMETERS OF PALBOCICLIB

PK parameters	palbociclib pure drug vs. palbociclib optimized solid lipid nanoparticle formulation	
	Significant difference between means (P < 0.05)	P
C_{max}	Yes	<0.0001
T_{max}	Yes	<0.0001
$AUC_{(0-t)}$	Yes	<0.0001
$AUC_{0-\infty}$	Yes	<0.0001
$t_{½}$	Yes	<0.0001

IV. CONCLUSIONS

In the present study, BBD was used for the optimization of SLN formulation. Controlled release profiles for more than 12 h were obtained by incorporating palbociclib into the solid matrix of tricapricbased lipid nanoparticles. The use of surfactants cremophor RH40 and soy lecithin resulted in the formation SLNs with decreased particle size. For optimization, the desirable goal was fixed for various responses particle size, entrapment efficiency and % cumulative drug release. The optimized single dose of SLN obtained using BBD the formulated SLNs were considered promising approach to improve drug solubility and long-term stability. In vivo studies highlighted the effectiveness of such formulations, enabling a concomitant increased absorption and a sustained drug release and, consequently, enhanced oral bioavailability.

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