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IMPROVEMENT OF POMALIDOMIDE SOLUBILITY BY SOLID LIPID NANOPARTICLES BY DESIGN OF EXPERIMENT

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Keywords:

Pomalidomide, Solid-lipid nanoparticles(SLN), Box-Behnken design (BBD) independent variables, Release kinetics

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ABSTRACT: The aim of the present work is to develop and optimize solid lipid nanoparticles (SLNs) of pomalidomide with the aid of Box-Behnken design (BBD). A design space with three formulation variables at three levels was evaluated in BBD. Amount of lipid (A), surfactant (B), and co-surfactant (C) were selected as independent variables, whereas particle size (Y1), entrapment efficiency (Y2), and percentage drug release after 12 h (Y3) as responses. Pomalidomide-SLNs were prepared by hot emulsification/ ultra-sonication method and evaluated for responses to obtain optimized formulation. The effect of different levels of factors was evaluated for the particle size, PDI, zetapotential, entrapment efficiency, and % cumulative drug released. Kinetic model fitting for all pomalidomide SLN formulations was done to interpret the release rate. Characterization for the optimized formulation was done by FTIR, SEM, and stability studies. The optimized formulation PLF15 with 6% of tricapric as lipid, 6% of poloxamer 188 as surfactant and 2% eupikuron 200 of co-surfactant used in the nanoparticles with 126.59 ± 1.17 nm of size, 0.172 ± 0.02 of polydispersity index, -25.13 ± 4.69 mV zeta potential 89.16±2.72% of entrapment efficiency, 99.53±2.18% of content uniformity and 98.74±2.46% of drug release. The release kinetics suggest that drug release followed zero-order and release was anomalous non- fickian diffusion super case II transport. FTIR studies revealed that there is no incompatibility between drug and excipients, SEM studies confirmed the spherical shape of SLN formulations. Stability studies indicated formulation was stable for 6months. The enhancement in the drug release of pomalidomide from solid lipid nano-particles, explicated the potential of lipid-based nanoparticles as a potential carrier in improving the oral delivery of this poorly soluble drug.

INTRODUCTION: Colloidal systems are the most promising alternative drug delivery systems for improving the bioavailability and therapeutic availability of the drugs. The colloidal systems include micelles, vesicles, liposomes, liquid crystals, nanocrystals, nanoparticles, *etc*. The particle size of the mentioned nano-carriers varies from 10 to 800 nm.

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Solid lipid nanoparticles (SLN) comprise solid lipid core stabilized by a surfactant at interfacial region. The solid lipids are used for the preparation of SLN instead of liquid lipids (used in case of liposomes) to overcome the disadvantages associated with the liquid state and to improve physical stability.

SLN possesses advantages over other colloidal delivery systems of increased physical stability, high drug payload, and absence of carrier biotoxicity. The preparation of SLN can also be extrapolated to large-scale production. The methods *viz.*, high-pressure homogenization, solvent evaporation, solvent emulsification, ultrasonication etc., are reported to be used for the preparation of solid lipid nanoparticles [1].

Pomalidomide is classified as a thalidomide analogue, immunomodulatory agent and an anti-angiogenic agent [2]. It is used for the treatment of relapsed and refractory multiple myeloma. The drug is practically insoluble in water (Pomalyst approved by FDA). Various literature suggested solid lipid nanoparticle as an efficient way to improve solubility and enhance drug release along with improved physical stability [3]. Thus, the present study was carried out with the aim to improve the solubility and dissolution profile of pomalidomide by preparing its solid lipid nanoparticles using the hot emulsification method. The prepared nanoformulation was characterized and evaluated for *in-vitro* release, FTIR, SEM, and stability studies.

MATERIALS AND METHODS: Pomalidomide was purchased from Hetero drugs Ltd, Hyderabad. Tricapric, poloxamer 188, cremophor RH 40, Eupikuron 200, tween80, chloroform and methanol were purchased from Gattefosse, Mumbai. All the reagents used were of analytical grade.

Marketed Product-pomalidomide 4 mg Capsules:

Preparation of Pomalidomide SLN: Pomalidomide SLNs were prepared by a hot emulsification / ultrasonication method. According to the partitioning results, Tricapric was selected as a solid lipid for the formulation of Pomalidomide SLNs. Pomalidomide (4 mg), Tricapric (%) and was dissolved in a mixture of chloroform and methanol (1:1) (20 mL).

The solvent was then completely removed using arotary evaporator. The drug-embedded lipid layer was melted by heating at 75 °C. An aqueous phase was formulated by dissolving the surfactant and co-surfactant(%), such as Poloxamer 188&Eupikuron 200 in double-distilled water and adding it to the molten lipid phase. This was followed by homo genization for 3 min. Coarsehotoilina water emulsion was obtained, which was then ultrasonicated using a probe sonicator. Finally, the obtained hot nanoemulsion was allowed to cool to room temperature to prepare Pomalidomide SLNs [4].

Box–Behnken Design (BBD): A three-factor, three-level Box-Behnken Design was used to explore and optimize the main effects, interaction effects, and quadratic effects of the formulation ingredients on the performance SLN. This design is suitable for exploring quadratic response surfaces and constructing second-order polynomial models. **Table 2** shows 17 randomized experimental runs for the selected independent variables, including five replicates at the center point (asterisk-marked) generated from a three-factor, three-level BBD and their corresponding responses. Five replicates at the center point were taken in this study for a more uniform estimate of the prediction variance over the entire design space. The amount of drug added to the formulation was kept constant. Based on the boundary of the solid lipid nanoparticles domain, three levels of independent or formulation variables (amount of lipid, surfactant, and co-surfactant) were identified: low (coded as−1), middle (coded as 0), and high (coded as +1). The range for each independent variable was selected for solid lipid nanoparticles: that is, the amount of Tricapric (Lipid, X1) was 4–8 %, the amount of Poloxamer 188(surfactant, X2) was 2–6 % and the amount of Eupikuron 200(co-surfactant, X3) was 1-3 %5 **Table 1**.

The BBD matrix was generated using Design Expert® software (Version7.0, Stat-Ease Inc., Silicon Valley, CA, USA) and the data obtained were analyzed by the same software [6]. The composition of Pomalidomide SLNs formulation by Box Behnken Design is given in **Table 2**.

TABLE 1: LIST OF DEPENDENT AND INDEPENDENT VARIABLES 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 Poloxamer 188	%	2	4	6
C	Amount of Eupikuron 200	%	1	2	3
Dependent variable				***Goal***	
Y1	Particle size	Nm		Minimize	
Y2	Entrapment Efficiency	%		Minimize	
Y3	Drug release after 12Hrs	%		Maximize	

TABLE 2: COMPOSITION OF POMALIDOMIDE SLNS FORMULATION BY BBD

F. no.	Pomalidomide (mg)	Tricapric (%)	Poloxamer 188 (%)	Eupikuron 200(%)	Tween 80 (ml)	Chloroform: Methanol (1:1)	Distilled Water (mL)
PLF1	4	4	2	2	0.5	20	Q.S
PLF2	4	8	2	2	0.5	20	Q.S
PLF3	4	4	6	2	0.5	20	Q.S
PLF4	4	6	4	2	0.5	20	Q.S
PLF5	4	4	4	1	0.5	20	Q.S
PLF6	4	8	4	1	0.5	20	Q.S
PLF7	4	4	4	3	0.5	20	Q.S
PLF8	4	8	4	3	0.5	20	Q.S
PLF9	4	6	2	1	0.5	20	Q.S
PLF10	4	6	6	1	0.5	20	Q.S
PLF11	4	6	2	3	0.5	20	Q.S
PLF12	4	6	6	3	0.5	20	Q.S
PLF13	4	**8**	**6**	**3**	**0.5**	**20**	**Q.S**
PLF14	4	6	6	2	0.5	20	Q.S
PLF15	4	6	4	1	0.5	20	Q.S
PLF16	4	4	2	3	0.5	20	Q.S
PLF17	4	4	6	2	0.5	20	Q.S

Optimization Using the Desirability Function: In the present study, all three responses were simultaneously optimized by a desirability function that uses the numerical optimization method introduced by Derringer and Suich in the Design-Expert software (Version8.0, Stat-Ease Inc., Silicon Valley, CA, USA). Recently, the desirability function approach was reported in several articles for the optimization of multiple responses7.

Characterization of Pomalidomide SLN:

Measurement of Particle Size, Polydispersity Index and Zeta Potential: The particle size and polydispersity index (PDI) of the SLN were determined by photon correlation spectroscopy (PCS) with a Zetasizer Nano ZS-90 (Malvern Instruments Ltd., Worcestershire, UK).

Prior to analysis, samples of all SLN formulations were diluted with double distilled water. The zeta potential measurements were done by laser-doppler-anemometer coupled with Zetasizer Nano ZS-90 (Malvern Instruments Ltd., Worcestershire, UK) to validate the electrophoretic mobility of particles. All the analyses were repeated in triplicate [8].

Drug Content: 1 ml SLNs dispersion was taken into 100 ml volumetric flask, and volume was made up with methanol. It was sonicated for 5 min in a bath sonicator. The solution was filtered through cellulose Whatman filter paper (0.45µ), and the filtrate was analyzed at UV-visible spectrophotometer at 254 nm [9].

Determination of Entrapment Efficiency (%): Entrapment efficiency (EE%) was determined by measuringthe concentration of free drug (unentrapped) in an aqueous medium as reported. The aqueous medium was separated by ultra-filtration using centrisart tubes (Sartorius, USA), which consists of a filter membrane (M.wt. cut off 20,000 Da) at the base of the sample recovery chamber. About 1ml of the formulation was placed in the outer chamber and the sample recovery chamber was placed on top of the sample and centrifuged at 4000 rpm for 15 min. The SLN along with encapsulated drug, remained in the outer chamber, and aqueous phase moved into the sample recovery chamber through filter membrane [10]. The amount of Pomalidomide in the aqueous phase was estimated by the HPLC method, and the entrapment efficiency was calculated by the equation:

% EE = analysed weight of drug in SLN / theoretical weight of drug loaded × 100

***In-vitro* Release Study:** *In-vitro* 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. The membrane was soaked in double-distilled water for 12 h. SLN dispersion (2 mL) was placed in the donor compartment, and the receptor compartment was filled with 50 mL of release media. During the experiments, the solution in the 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 h. An aliquot of the sample (5 mL) was taken from the dissolution medium at different times 0.5, 1, 2, 3, 4, 6, 8, and 12 h time points, samples were withdrawn and analyzed by UV-visible spectrophotometer at 254 nm. Data obtained from *in-vitro* release studies were fitted to various kinetic equations to find out the mechanism of Pomalidomide release from SLN [11].

Kinetic Model Fitting: The model-dependent methods all rely upon a curve-fitting procedure. Different mathematical functions have been used to model the observed data. Both the linear and non-linear models are being used in practice for dissolution modeling. Linear models include Zero-order, Higuchi, Hixon – Crowell, Quadratic and Polynomials, whereas the nonlinear models include First order, Weibull, Korsmeyer – Peppas, Logistic *etc*. [12, 13]

Characterization of the Optimized Pomalidomide SLN:

Fourier Transform Infrared Spectroscopy (FT-IR): The FTIR of pure pomalidomide and optimized formulations recorded using FTIR spectrometer Prestige-21 (Shimadzu- Japan). The scanning range adjusted between 400-4000 cm^{-1} and resolution to 1 cm^{-1} [14].

SEM Studies: The surface and shape morphology of optimized SLN analyzed using scanning electron microscopy by placing the formulations on metal stub and photographs were taken at proper magnification [15].

Stability Studies: Among all batches of pomalidomide SLN were subjected to immutability studies in accordance with guidelines of ICH stability protocol. The test specifications include Temperature of 40 ºC ± 2 ºC and relative humidity of 75 ± 5% RH for a time period of 6 months in Humidity chamber (REMI, Mumbai).

The specifications to be evaluated in the stability study period include particle size, entrapment efficiency, *in-vitro* drug released [16, 17].

RESULTS AND DISCUSSIONS:

Characterization of SLNs: Developed Pomalidomide SLNs were physicochemically evaluated in terms of particle size, entrapment efficiency, zeta potential, PDI, and content uniformity **Table 3**.

% Drug Content: The drug content for all the formulations was within satisfactory limits and found to be between 96.31 and 99.53 % and found to increase accordingly with an increase in the concentration of surfactant in all the formulations **Table 3**. The highest drug content was observed for PLF15 formulation.

Entrapment Efficiency: Entrapment efficiency is an important parameter for characterizing solid lipid nanoparticles. In order to attain optimal encapsulation efficiency, several factors were varied, including the type and concentration of the lipid and surfactant material used. The entrapment efficiency of the SLN dispersions was found to be in the range of 74.23to 89.16% **Table 3**.

Particle Size: The particle size of the drug-loaded nanoparticles were found to be in the range of 126.59 ± 1.17 to 217.76± 3.51 **Table 3**. The nanoformulations exhibited a negative surface charge with the inclusion of Pomalidomide which clearly suggested the orientation of pomalidomide in the lipid matrix. It is believed that the negative surface charge of nanoparticles facilitates intestinal permeation *via* Peyer's patches leading to enhanced lymphatic absorption. On the other hand, the gastrointestinal mucus layer is negatively charged, and negative surface particles are not entangled in that layer because of repulsion of like charges. This could also result in enhanced intestinal permeability of prepared nanoparticles.

Polydispersity Index: The polydispersity index of all SLNs was significantly varying from 0.172 to 0.279 as depicted in Table 3, indicating narrow size distribution, which reveals the higher stability of Pomalidomide SLN. Similar findings were reported in earlier studies on cyclosporine A incorporated cationic Pomalidomide solid lipid nanoparticles for drug delivery [18, 19].

Zeta Potential: It was currently admitted that zeta potentials between -15mV to -25mV were required for full electro-static stabilization **Table 3**.

In these studies, it seemed that the value of zeta potential of drug-loaded SLNs was not sufficient to keep the particles dispersing stably. However, the

particle size did not change significantly within 30 days, contributing to the following point. A high surfactant mixture can easily compensate for missing electrostatic repulsion to stabilize the dispersion for a long time. Tween 80 provides steric stability for maintaining the stability of SLN.

TABLE 3: PHYSICO-CHEMICAL PARAMETERS OF POMALIDOMIDE SLNS

F. No	#Content uniformity (%)	% Entrapment Efficiency	Mean particle size (nm)	Zeta potential (mV)	Polydispersity Index
PLF1	96.31±1.15	75.16±1.62	197.53 ± 4.28	20.35 ± 4.28	0.176± 0.03
PLF2	97.53±2.58	81.47±1.75	144.38 ± 4.91	18.89 ± 5.64	0.282 ± 0.08
PLF3	97.12±2.19	83.58±1.22	139.87 ± 8.93	19.13 ± 2.31	0.164 ± 0.02
PLF4	96.41±1.25	74.23±2.45	156.43 ± 4.22	21.38 ± 4.19	0.188 ± 0.09
PLF5	98.63±2.65	81.79±1.91	177.76 ± 3.58	28.67 ± 2.24	0.214 ± 0.03
PLF6	97.26±2.52	84.57±1.43	214.63± 4.21	22.35 ± 2.28	0.195 ± 0.06
PLF7	98.42±2.38	80.24±2.46	155.17± 5.15	24.83 ± 6.69	0.204 ± 0.01
PLF8	98.74±2.17	79.88±1.23	189.69 ± 7.61	19.87 ± 5.62	0.182 ± 0.087
PLF9	97.53±2.64	83.71±1.68	148.52 ± 4.20	27.883 ± 2.69	0.277 ± 0.04
PLF10	98.16±2.52	85.43±2.17	217.76± 3.51	16.75 ± 2.27	0.212 ± 0.09
PLF11	97.22±2.95	81.48±1.22	165.42 ± 2.55	27.92 ± 1.68	0.193 ± 0.02
PLF12	96.48±1.11	76.21±2.18	139.18 ± 3.64	20.27 ± 2.13	0.245 ± 0.07
PLF13	97.85±1.69	80.35±.2.47	169.32 ± 6.27	17.83 ± 6.68	0.233 ± 0.06
PLF14	98.44±2.21	77.24±1.89	201.25 ± 8.73	27.25 ± 6.17	0.259 ± 0.04
PLF15	99.53±2.18	89.16±2.72	126.59 ± 1.17	25.13 ± 4.69	0.172 ± 0.02
PLF16	97.23±2.47	81.65±1.13	198.76 ± 3.54	26.48 ± 4.41	0.266 ± 0.06
PLF17	98.29±2.43	84.83±2.88	173.89 ± 4.31	22.91 ± 3.16	0.297± 0.02

Above parameters are communicated as Average ± Standard Deviation; (n=3)

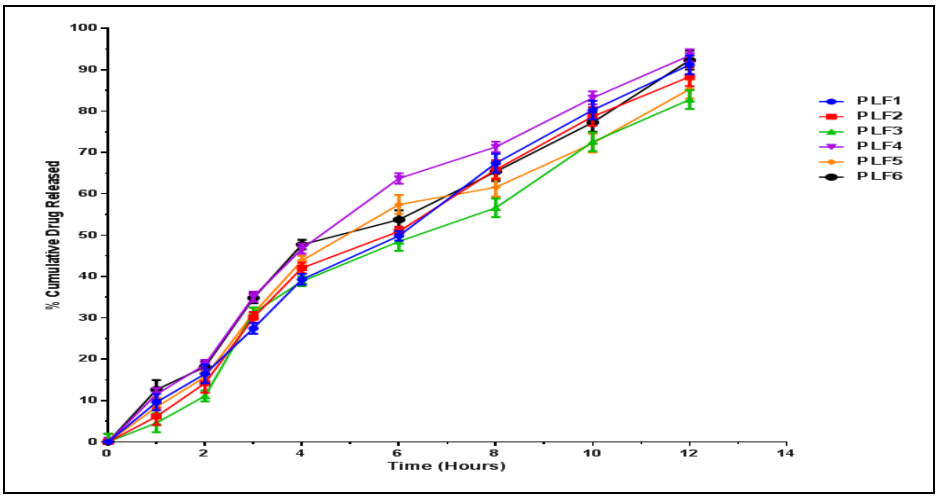

FIG. 1: *IN-VITRO* DRUG RELEASE OF POMALIDOMIDE SLN (PLF1-PLF6)

***In-vitro* Dissolution Testing of Pomalidomide SLNs:** The dissolution profiles of plain Pomalidomide and Pomalidomide SLNs formulation in simulated intestinal is shown in fig 1, 2 and 3. As shown in **Fig. 3**, more than 85% of drug was dissolved from PLF15 after 12 h. However, the original Pomalidomide powder showed only approximately 86% dissolved after the same time period. The enhanced dissolution may be due to the decrease in crystallinity and the increase in solubility of the drug. The increase in cumulative drug released is mainly attributed to rapid self-emulsification of the formulations due to instantaneous dispersion in the medium after dissolution of the capsule shell. As the amount of free energy required in the formation of an emulsion is very low, this results in the spontaneous formation of an oil-water interface. This increases the water penetration of lipid droplets, resulting in disruption of the interface and thereby decreasing the particle size and eventually increasing the release rate. From the relationship of formulation composition factors and the particle size, a higher drug release rate correlates with particle size that gives a larger surface area and subsequent water penetration.

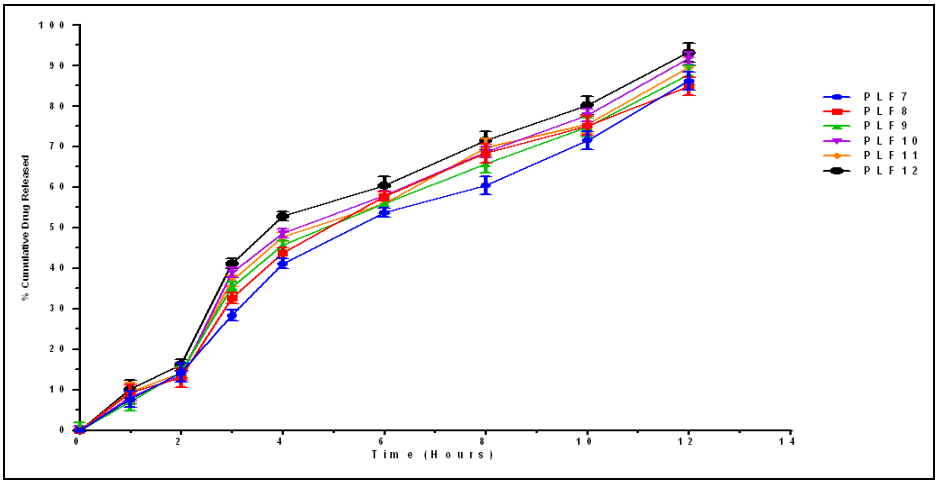

FIG. 2: *IN-VITRO* DRUG RELEASE OF POMALIDOMIDE SLN (PLF7-PLF12)

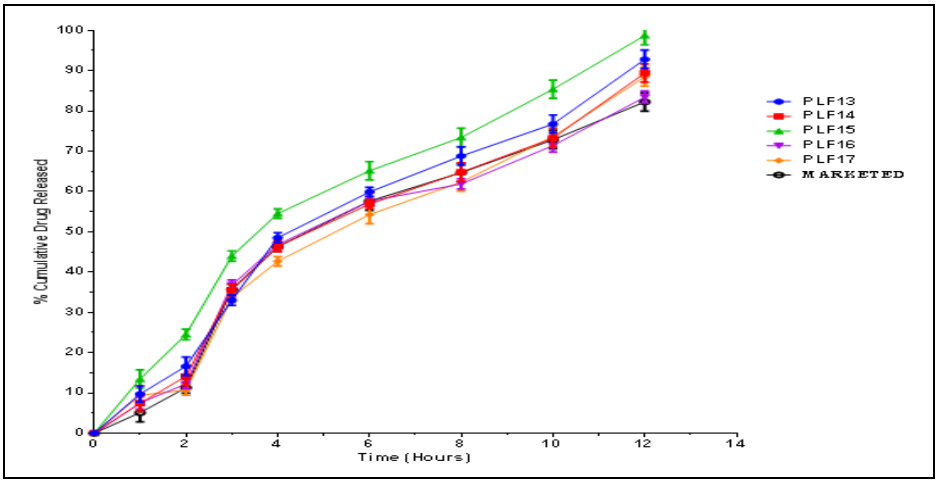

FIG. 3: *IN-VITRO* DRUG RELEASE OF POMALIDOMIDE SLN (PLF13-PLF17)

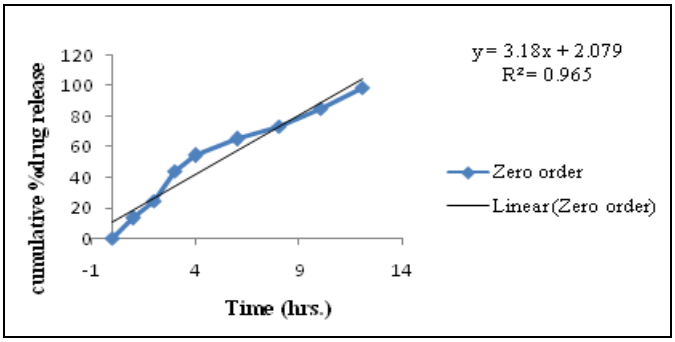

FIG. 4: ZERO-ORDER PLOT FOR THE OPTIMIZED FORMULATION (PLF15) OF POMALIDOMIDE SLNS

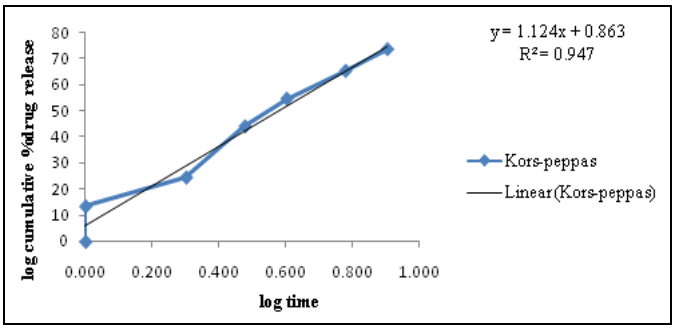

FIG. 5: KORSMEYER-PEPPAS PLOT FOR THE OPTIMIZED FORMULATION (PLF15) OF POMALIDOMIDE SLNS

Kinetic Analysis of Pomalidomide Release Data: Drug release data for optimized formulation and the marketed formulation was fitted into various kinetic equations to find out the order and mechanism of drug release **Fig. 4** and **5**.

From the above results, it is apparent that the regression coefficient value closer to unity in case of zero-order plot *i.e.*, 0.965 indicates that the drug release follows a zero-order mechanism. This data indicates a lesser amount of linearity when plotted by the first-order equation. Hence it can be concluded that the major mechanism of drug release follows zero-order kinetics.

Further, the translation of the data from the dissolution studies suggested the possibility of understanding the mechanism of drug release by configuring the data into various mathematical modeling such as Higuchi and Korsmeyer-Peppas plots. Further, the n value obtained from the Korsmeyer-Peppas plots *i.e.*, 0.947 indicating non-

Fickian (anomalous) transports; thus, it projected that it delivered its active ingredient by coupled diffusion and erosion.

Statistical Analysis of the Designed Experiment: The range of particle size (Y1) for all batches was 126.59± 1.17 – 217.76±3.51 nm. Similarly, the range for % entrapment efficiency (Y2) was 74.23 ± 2.45 % – 89.16±2.72 % and the range for the cumulative percentage of drug released in 12 h (Y3) was 82.27±2.35–98.74±2.46 %. 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. All three responses were individually fitted to a second-order quadratic model, and each obtained model was validated by ANOVA.

Particle Size: A smaller particle size provides a larger interfacial surface area for drug absorption. In addition, smaller particle size may permit a faster release rate. The particle size of the nanoparticles was found to be in the range of 126.59± 1.17 – 217.76±3.51 nmas shown in **Fig 6A, 6B.** The mathematical model generated for particle size (Y1) was found to be significant with F-value of 0.0284 implies the model is significant.

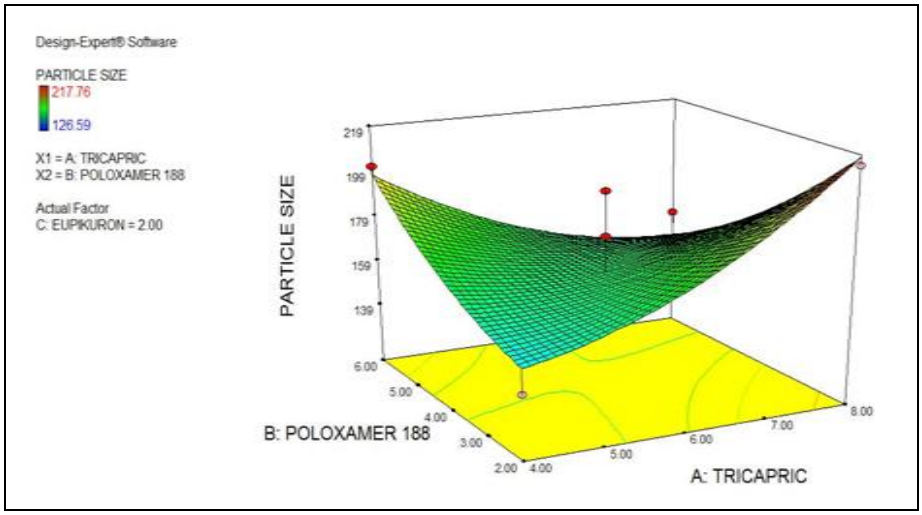

FIG. 6A: RESPONSE 3D SURFACE PLOT SHOWING THE INFLUENCE OF AMOUNT OF TRICAPRIC AND AMOUNT OF POLOXAMER 188ON PARTICLE SIZE FIXED LEVEL OF C

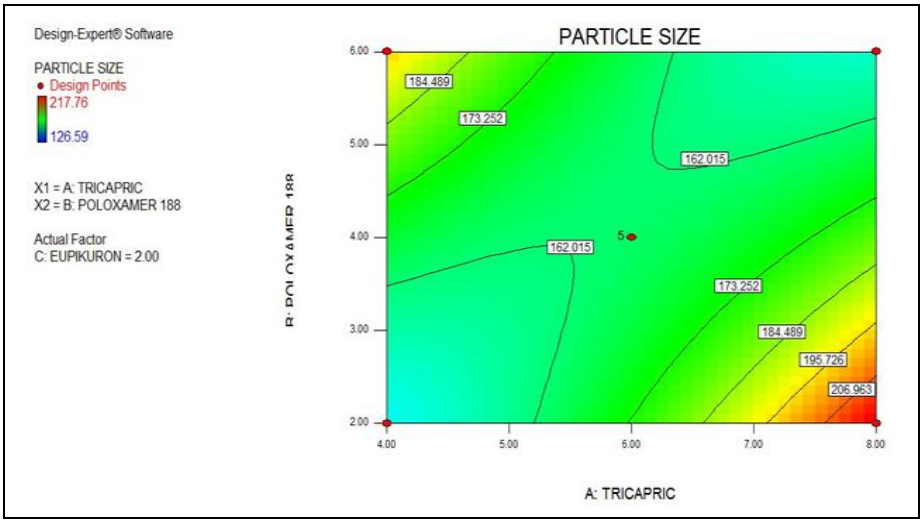

FIG. 6B: CONTOUR PLOT SHOWING THE INFLUENCE OF AMOUNT TRICAPRIC AND AMOUNT OF POLOXAMER 188ON PARTICLE SIZE FIXED LEVEL OF C

Entrapment Efficiency (%): The entrapment efficiency (%) of the SLNs was found to be in the range of 74.23 ± 2.45 % to 89.16 ± 2.72 % as shown in **Fig 7A** and **B**. The mathematical model generated for entrapment efficiency (%) (Y2) was found to be significant, with an F-value of 0.0264 implies the model is significant.

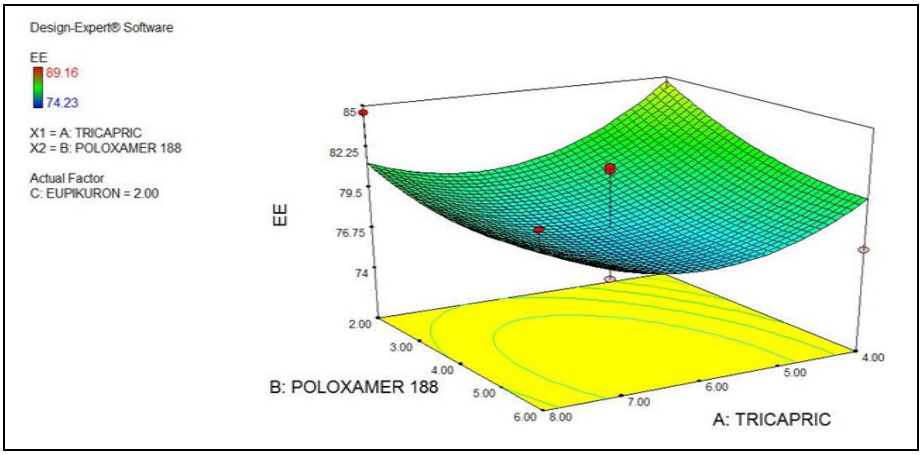

FIG. 7A: RESPONSE 3D SURFACE PLOT SHOWING THE INFLUENCE OF AMOUNT OF TRICAPRIC AND AMOUNT OF POLOXAMER 188ON ENTRAPMENT EFFICIENCY (%) FIXED LEVEL OF C

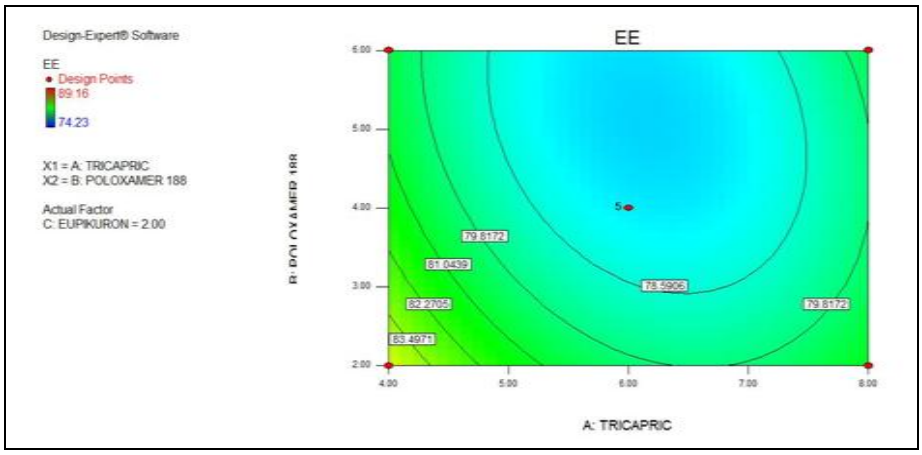

FIG. 7B: CONTOUR PLOT SHOWING THE INFLUENCE OF AMOUNT OF TRICAPRIC AND AMOUNT OF POLOXAMER 188ON ENTRAPMENT EFFICIENCY (%) FIXED LEVEL OF C

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%, as shown in **Fig. 8A** and **B**.

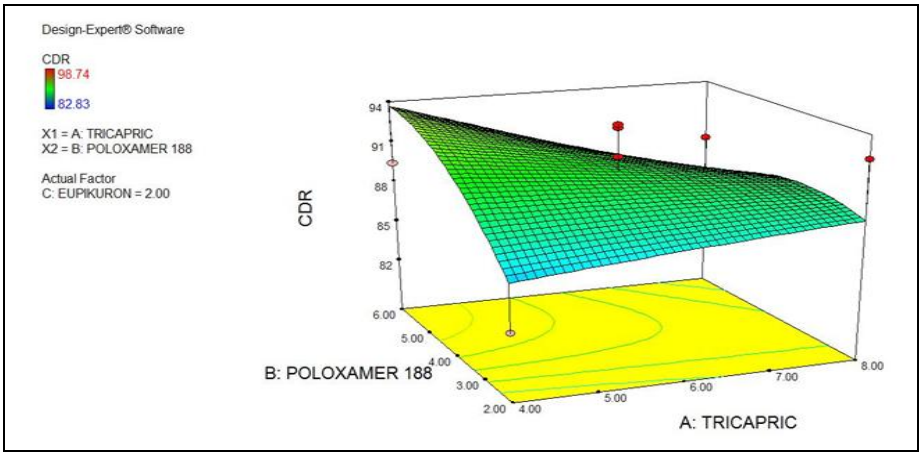

FIG. 8A: RESPONSE 3D SURFACE PLOT SHOWING THE INFLUENCE OF AMOUNT OF TRICAPRIC AND AMOUNT OF POLOXAMER 188ON CUMULATIVE % DRUG RELEASED FIXED LEVEL OF C

The mathematical model generated for percent drug released in 12 h (Y3) was found to be significant, with an F-value of 0.0265 implies the model is significant. The amount of surfactant was mainly responsible for the increase in the cumulative percentage of drugs released from the formulation. The increase in cumulative drug release was mainly attributed to rapid self-emulsification of the

formulations due to instantaneous dispersion in the medium after dissolution of the capsule shell.

As the amount of free energy required in the formation of an emulsion is very low, this results in the spontaneous formation of a lipid–water interface. This increases the water penetration of lipid droplets, resulting in disruption of the interface and thereby decreasing the particle size and eventually increasing the release rate.

It was also seen that the cumulative percentage of drug released was further improved by the addition of the co-surfactant.

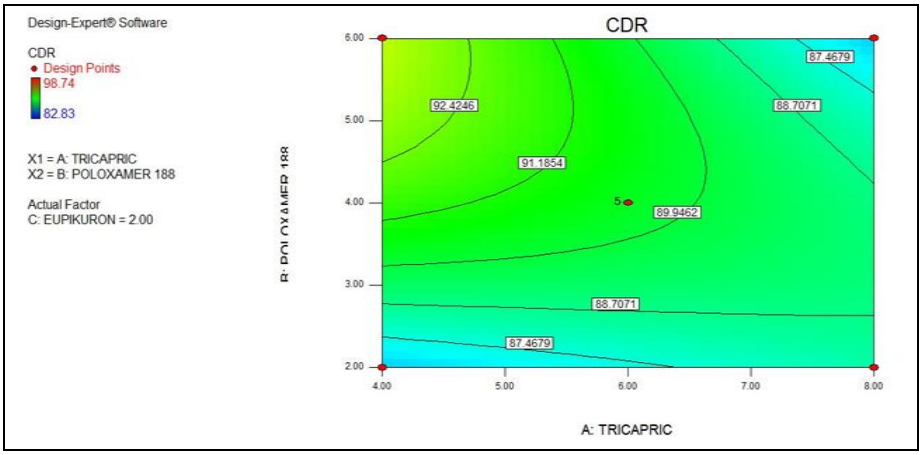

FIG. 8B: CONTOUR PLOT SHOWING THE INFLUENCE OF AMOUNT OF TRICAPRIC AND AMOUNT OF POLOXAMER 188ON CUMULATIVE % DRUG RELEASED FIXED LEVEL OF C

Optimization by Desirability Function: An optimization process was undertaken with the desirability function to optimize the three responses simultaneously.

The responses: particle size (Y1), entrapment efficiency (%) (Y2), and cumulative percentage of drug released in 12 h (Y3) were transformed into the desirability scale, respectively. Among them, Y1 and Y2 had to be minimized, while Y3 had to be maximized. For the individual desirability function, Y_{max} and Y_{min} were taken as the highest objective function (D) was calculated by Equations for each response. The maximum function value was obtained at X1:08, X2:06 and X3:03. 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. 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

		Predicted values						
Independent variable	**Nominal values %**	**Particle size (Y1) (nm)**	**Entrapment Efficiency (%) (Y2)**	**%CDR (Y3)**	**Batch**	**Particle size (Y1) (nm)**	**Entrapment Efficiency (%) (Y2)**	**Percent drug release in 12h (Y3)**
Amount of Tricapric(A)	8	126.59	89.16	98.74	1	125.33	88.19	98.45
Amount of Poloxamer 188(B)	9				2	126.48	89.58	98.36
Amount of Eupikuron 200 (C)	6				3	125.65	89.81	98.47

Stability Studies: Optimized formulation PLF15 was loaded for stability studies for 6months as per ICH guidelines, and formulation was found to be stable. There was no significant change in particle size, entrapment efficiency, in-vitro release and drug content observed at 40 °C±2 °C/75% RH±5%, and at 25 °C±2 °C/60% RH±5% the values are shown in **Table 5**.

TABLE 5: STABILITY STUDIES OF OPTIMIZED FORMULATION

Retest Time For Optimized formulation (PLF15)	Particle Size (nm)	Entrapment Efficiency (%)	*In-vitro* drug release profile (%)	Drug content (%)
0 days	126.59±1.17	89.16±2.72	98.74±2.46	99.53±2.18
30 days	126.59±1.17	89.16±2.72	98.72±1.55	99.44±2.67
60 days	127.23±1.35	88.73±2.44	98.63±1.78	99.31±2.55
120 days	127.61±1.78	88.62±2.23	98.59±1.12	99.23±2.47
180 days	127.74±1.89	88.55±2.14	98.45±1.84	98.89±2.38

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

Characterization of Optimized Formulation of Pomalidomide SLN:

FTIR Studies: The FTIR spectrum of pure pomalidomide **Fig. 9** exhibited N-H stretching vibrations at 3728.53, 3697.66 cm^{-1}, the C-N stretching modes are assigned at 1381.08 cm^{-1}, phenyl CH stretching vibrations occur above 3000 cm^{-1} and are typically exhibited as a multiplicity of weak to moderate bands compared with the aliphatic CH stretching. For the pomalidomide pure drug compound, this calculation gives CH stretching vibrations of the phenyl rings at 3217 and 3189 cm^{-1}. The aliphatic CH stretching vibrations are at 3198.08, 3117.07, 3080.42, 3064.99 and 3032.2 cm^{-1}. **Fig. 10** presents similar prominent peaks in optimized formulation same as that of pure drug and these results indicate the absence of any chemical interactions between the drug pomalidomide and used excipients in the formulation.

SEM Studies: SEM photographs for pure drug and optimized formulation of pomalidomide PLF16 are shown in **Fig. 11A,** and **B**. Spherical particles were observed with drug particles incorporated in lipid matrix. The surface of the drug appeared to be porous in nature in pomalidomide optimized formulation solid lipid nanoparticles (PLF16). The results could be attributed to the dispersion of the drug in the molten mass of the polymer, which leads to the sustained release of the drug upto 12 h.

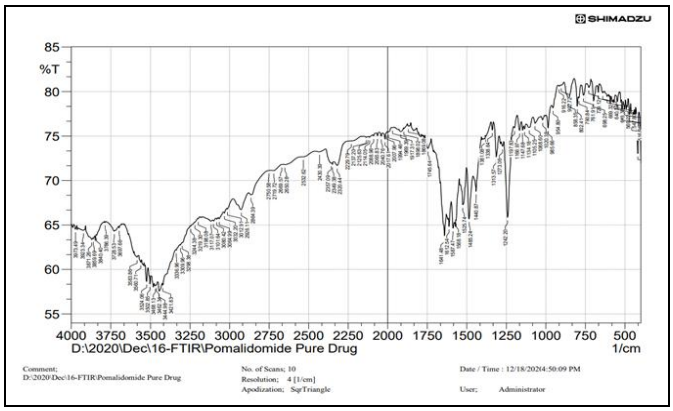

FIG. 9: FTIR SPECTRUM OF PURE DRUG POMALIDOMIDE

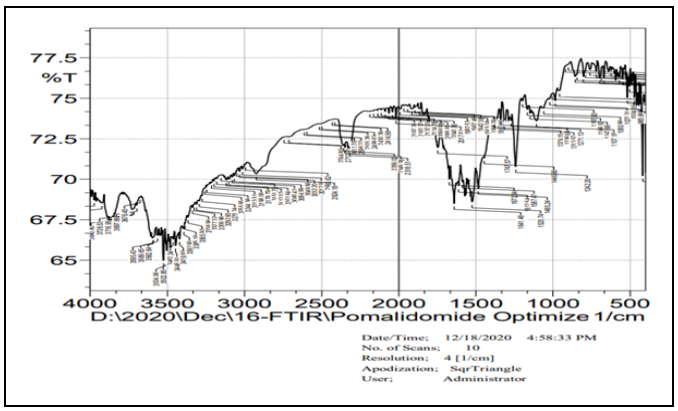

FIG. 10: FTIR SPECTRUM OF POMALIDOMIDE OPTIMIZED FORMULATION (PLF15)

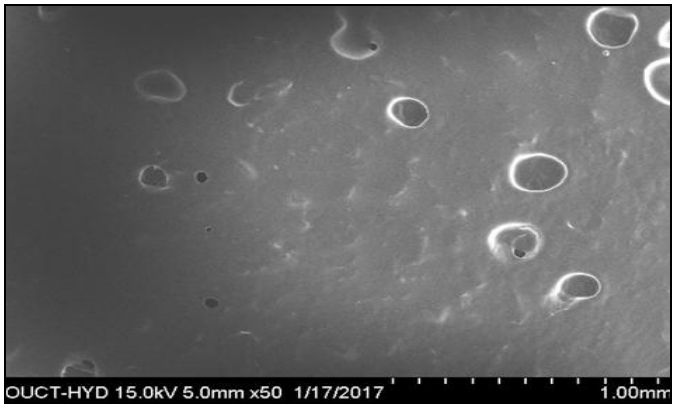

FIG. 11A: SEM IMAGES OF SOLID LIPID NANOPARTICLES OF POMALIDOMIDE (PLF15)

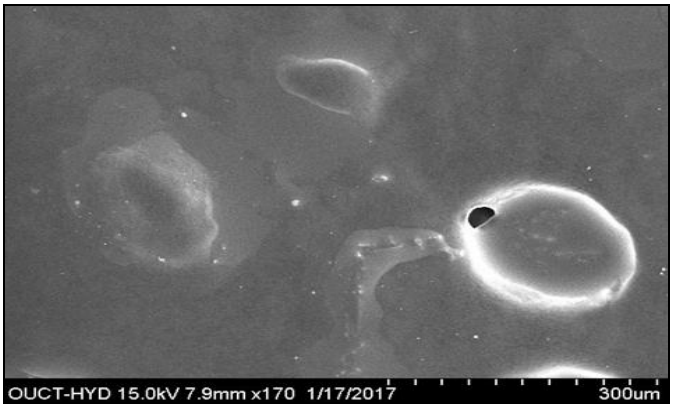

FIG. 11B: SEM IMAGE OF SOLID LIPID NANOPARTICLES OF POMALIDOMIDE (PLF15)

In this study, the pomalidomide loaded SLNs were designed and prepared by the hot emulsification / ultrasonication technique. The SLNs were optimized using the 3-level 3-factor Box Behnken statistical design. The optimized formulation (PLF15) exhibited particle size of 126.59 ± 1.17 nm, zeta potential 89.16±2.72%, 0.172 ± 0.02 of polydispersity index, entrapment efficiency 89.16 ± 2.72% and 98.74±2.46 % of drug release. The release kinetics suggest that drug release followed zero-order and release was anomalous non- fickian diffusion super case II transport. FTIR studies revealed that there is no incompatibility between drugs and excipients. The morphology of optimized SLNs was roughly spherical in shape. Stability studies indicated formulation was stable for 6months. Thus it is concluded that the development and optimization of SLNs for pomalidomide using a 3-factor, 3-level Box Behnken design was successful in enhancing the release drug release sustainably.

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