Plant Archives Vol. 21, Supplement 1, 2021 pp. 621-627 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
doi link : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.095

OPTIMISATION OF APREMILAST LOADED NANOSTRUCTURED LIPID CARRIERS: A PROSPECTIVE TOOL FOR PSORIASIS THERAPY

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ABSTRACT

This study aimed at screen and optimise the nanostructured lipid carriers (NLC), prepared by melt emulsification-Ultrasonication technique, as a carrier for Apremilast (MTX), highlighting the application of Design of Experiments(DoE). Preliminary screening was done to select the GMS, Sefsol 218, Tween 80 and Transcutol P as the solid lipid, liquid lipid, Surfactant and co-surfactant respectively. Then, a 3-level, 3-factor Box-Behnken design was used to study the interaction effects and optimise the formulation. The correspondence between the predicted values (Optimised by software) and those measured experimentally confirmed the robustness of the design. The results clearly showed that quality by design concept could be effectively applied to optimize Apremilast-loaded Nanostructured lipid carriers.

Keywords **:** Apremilast, Emulsification, Ultrasonication, Screening, Optimisation

Introduction

Psoriasis is a chronic inflammatory skin disease that affects 1 to 3% of the world population, with equal gender distribution. Incidence rates vary from 50 to 140 new 11 cases per 100,000 people per year (Pinto *et al*., 2014). Treatment modalities include common systemic agents such as methotrexate, acitretin, and cyclosporine that are associated with end-organ toxicities and treatment-related side effects, the biological agents have the limitations of added costs to the care and inconvenient mode of administration apart from the possibility of iatrogenic immuno suppression (Afra *et al*., 2019). Apremilast (APM) is a systemically administered PDE-4 antagonist approved by the FDA, which has shown great promise in treating patients with psoriasis (Bubna, 2016). Apremilast inhibits the enzyme phosphodiesterase 4, which leads to spontaneous inhibition of tumour necrosis factor-alpha production from the human rheumatoid synovial cell (Priyanka *et al*., 2019). However, APR is a BCS class IV drug with low solubility and low permeability. It has very low oral bioavailability (20–33%) (Muvva *et al*., 2020). Generally, long-term treatment with APM is recommended because of the chronic nature of the disease. Conventional immediate-release formulation of APM has issues in terms of tolerability and dose regimen, which could impair patient compliance and thus jeopardize the effectiveness of the treatment. An alternative drug delivery system is therefore urgently needed to overcome the frequent daily dosing and improve bioavailability of APM (Anwer *et al*., 2019). Nanotechnology has been the fastest-growing strategy in the last decade to increase the solubility and permeability of BCS Class IV drugs. Recently, NLC has gained popularity among various nano carriers for the delivery of lipophilic drugs. Their exclusivity lies in their unique matrix composition which contains an appropriate and permissible mixture of incompatible liquid lipids and solid lipids. Unlike solid lipid nanoparticles and nanoemulsions, the presence of solid cum liquid in the NLC results in greater drug encapsulation and loading and long-term colloidal stability (Khan *et al*., 2015). Many statistical experimental designs are often used to optimize formulations using fewer runs, and to estimate the relative significance between variables. Several Response Surface Methodologies (RSMs), such as Central Composite Design (CCD), D-optimal Design, and Box-Behnken Design (BBD) are useful to optimise formulations and to assess the relationship between independent and dependent factors. BBD has a three-factor experimental design in which the three-factor levels are located at the midpoints and edges of the process Since this cubic design has no vertices, it is easy to avoid experiments with extreme factors in BBDs. Additionally, BBD requires fewer runs than any other three-level response surface design model. Hence in the present study apremilast loaded NLCs were optimised by BBD using desirability function (Kim *et al*., 2019).

Materials and Methods

Apremilast was received as a gift sample from Glenmark life Sciences Ltd, Ankleshwar, Gujarat. Sefsol 218, Sefsol 228, Transcutol P were collected from Gattefosse, France. Glyceryl Monostearate, Tween 80, propylene glycol, tween 20, oleic acid, olive oil, PEG 400, Isopropyl alcohol, n butanol were procured from Himedia Laboratories Pvt. Ltd. Stearic acid, palmitic acid, and myristic acid were obtained from Daejung Chemical

(Cheongwon, Korea). Ultra-pure deionized water was used throughout the experiments. All other solvents and chemicals used were of analytical grades or higher.

Methodology

Solubility in Solid lipids

Solid lipid was selected by checking the drug's solubility in melted solid lipid by means of visible observation under normal light with naked eyes. Palmitic acid, Myristic acid, stearic acid and GMS were the lipids used for this study. In a temperature-regulated water bath in 10 mL glass vials, weighted amount of drug (100 mg) separately with specific lipids (1 g each) was heated past the lipid melting point. Then apremilast solubility in the melted lipid was visibly observed under normal light (Shah *et al*., 2016; Ghate *et al*., 2016).

Partition coefficient studies

The apremilast partition behavior in the solid lipid was assessed using isothermal shaker method. Weighed drug quantity (100 mg) was dispersed in a mixture of melting lipid (1 g) and hot phosphate buffer (PB) (1mL) pH 7.4, following which it was shaken at 100°C in a hot water bath shaker for 30min. After cooling, the aqueous phase was then segregated from lipid by centrifugation for 20min at speeds of 10,000rpm. The transparent supernatant collected was diluted sufficiently and was measured using UV Spectrophotometer (Moghddam *et al*., 2017; Nagaich *et al*., 2016).

Partition coefficient was calculated as:

$$PC = (Ci - C)/C$$

where, Ci = initial amount of drug added (10mg),

C = conc. of drug in pH 7.4 PBS

Solubility in liquid lipids

Excess of apremilast was added to the capped vial containing 2 ml of the vehicle. These vials were stirred on a magnetic stirrer for 72 hours at 25±0.5°C to equilibrium. The equilibrated sample was then removed from stirrer and centrifuged at 3000 rpm for 15 min and dissolved apremilast was -quantified by UV-spectrophotometer at 230 nm (Motawea *et al*., 2018; Kaur *et al*., 2018).

Physical compatibility of solid and liquid lipid

In different glass vials, solid lipidhaving the maximum affinity to the drug were mixed in the ratio 1:1 with the liquid lipid, and the admixture was then melted and cooled to congeal at room temperature. Visually the glass vials were examined for lack of separating layers in the congealed lipid mass (Thakkar *et al*., 2014).

Selection of a binary lipid phase

To determine the miscibility of the two lipids, the solid and liquid lipid with the best-solubilizing ability for apremilast were mixed in different ratios viz., 95:5, 90:10, 85:15,80:20, 70:30, and 60:40. A magnetic stirrer (Remi Instruments Ltd., Mumbai, India) was used to agitate the lipid mixtures at 200 rpm for 1 h at 85°C. The cooled sample of the solid mixture was then smeared onto a filter paper to examine the miscibility between the two elements, accompanied by visual inspection to assess the presence of any liquid oil droplets on the filter paper (Gaba *et al*., 2015).

Screening of Surfactants and co-surfactants

Method

Surfactants and co-surfactants were selected based on the capacity of surfactants and co-surfactants to emulsify optimized binary mixture of liquid lipid and solid lipid.

Emulsification ability of surfactants and co-surfactants

In brief, 100 mg of the binary mixture was dissolved in acetone (2mL), followed by 5% w/v of the surfactant solution. A magnetic stirrer was used to stir the resulting mixture at 40°C. After this 1mL of the mixture was diluted with 10 mL of purified water and the percentage transmittance at 630 nm was measured using a triplicate UV spectrophotometer at 25 ° C. The same technique used for co-surfactant screening (Negi *et al*., 2014).

Preparation of NLC

Method of preparation of Apremilast loaded NLCs

In the present study, melt emulsification-Ultrasonication technique was used to prepare NLCs. In short, solid lipid and liquid lipid(Lipid phase) were taken at a ratio of 6:4. The aqueous phase was prepared by dissolving the surfactant and the co-surfactant in Milli-Q water. Both these phases were maintained at 75 °C. Apremilast was and added to the melted lipid mixture and with continuous stirring, followed by addition of the aqueous phase to the lipid phase with continuous stirring for 30 minutes at 2000 rpmtill the emulsion formation. The formed primary emulsion was ultrasonicated for 15min with 60% amplitude. The resulting solution was then cooled to room temperature to obtain the NLC dispersion (Waghule, 2019).

Optimization of the APM_NLC by Box-Behnken design

Method

Based on the results of preliminary experiments, the APM-NLC was optimized by Box–Behnken design with three factors and three responses. The design of experiments and statistical analysis was conducted by Design Expert® 11 software (Stat-Ease Inc, Minneapolis, MN, USA).The independent variables selected were the Drug concentration (A), Total lipid concentration (solid lipid: liquid lipid) B) and Co surfactant concentration (C) with their low, medium and high levels used to prepare the 17 formulations as these are reported to play an important role in the formulating stable NLCs. The dependent variables were particle size (Y1) and zeta potentialY2) and polydispersity index (Y3) with constraints applied to the formulation of NLC (Table 1). The amount of drug (0.3%), sonication amplitude (60%), sonication time (15 min), Stirring time (30 min) and Stirring speed (2000 rpm) were set at fixed levels. To optimize the fitting model for each response, various statistical parameters, such as sequential p-values, lack of fit, squared correlation coefficient (R^2), adjusted R^2, and adequate precision were considered by comparing various statistical parameters provided by analyses of variance (ANOVA). After fitting the statistical model, the desirability value according to the goal of responses was obtained by numerical optimization and the APM-NLC with the highest desirability value was prepared as the selected composition. A recovery test was performed to compare the error between the predicted and actual values.

Results and Discussion

Solubility Studies

The selection of an appropriate solid lipid, liquid lipid, and surfactant is crucial in formulating an NLC dispersion method for a poorly water-soluble drug like apremilast. The NLC's drug-loading capacity largely depends on the drug's solubility in the lipid ingredients. Solid lipids and oils that were used topically in the generally recognized as safe (GRAS) group were screened for their solubilizing ability for apremilast in order to choose an suitable lipid for the apremilast loaded NLC.

Solubility in Solid Lipids

It was found from the study that drug solubility in palmitic acid, Myristic acid and stearic acid was indistinct but found fairly visible in GMS (Fig. 1). This is because of the fact that Glycerol monostearate tends to form amorphous forms rather than crystalline forms by incorporation with drugs. In contrast, the saturated fatty acids (stearic acid, palmitic acid, myristic acid)are inherently high crystalline and can incorporate only a small amount of drugs.

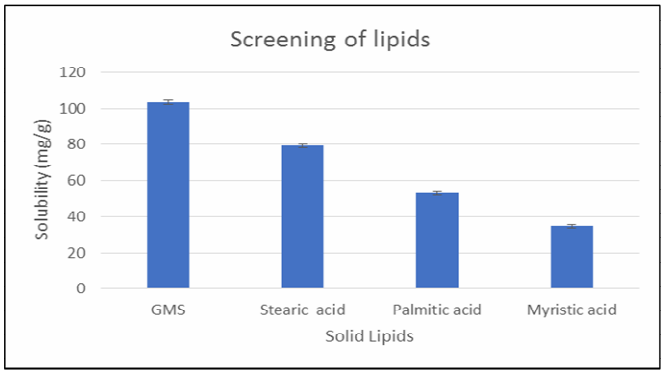


Fig. 1: Solubility studies of solid lipid

Partition coefficient studies

Determination of the partitioning behaviour of the drug is an important criterion, as it affects the entrapment efficiency as well as the release of the drug from the formulation. From the result shown in Fig. 2 it was found that APM had higher partitioning in GMS compared to other lipids. This finding also supported the high solubility of drug in GMS as discussed earlier. Therefore, GMS was chosen as solid lipid for development of NLCs owing to its high potential for solubilization and partition coefficient thereby entrap more amount of drug in NLCs formulation.

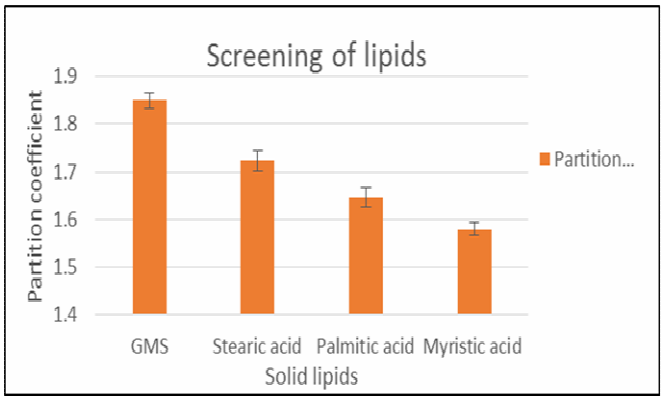


Fig. 2 : Partition coefficient Studies of Solid Lipids

Solubility in Liquid lipids

The highest solubility of apremilast among the screened oils was found in Sefsol 218 (220 ± 1.6 mg/mL) (Fig 3). Sefsol® 218 is a propylene glycol ester of monocaprylic acid. It is a medium chain saturated fatty acid in which eight carbons are bonded together into aliphatic carbon chain. It also serves as a penetration enhancer, skin conditioner, emollient and solubilizer. It is not prone to oxidation and rancidity. It has been used as oil phase carrier for topical drug delivery of poorly water-soluble drugs.

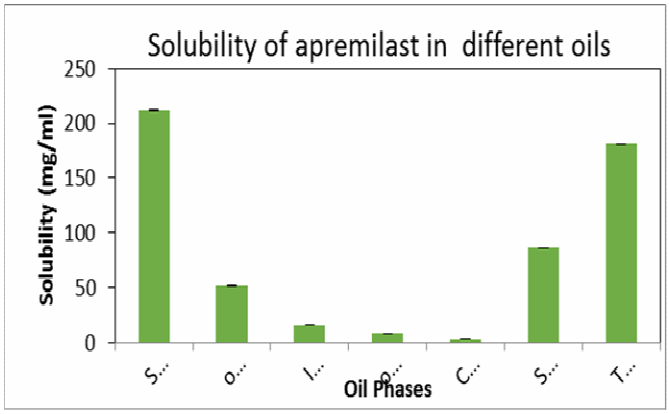


Fig. 3: Solubility of apremilast in liquid lipids

Physical compatibility of solid and liquid lipid

The solid lipid GMS and oil sefsol 218 was selected for physical compatibility with apremilast and it was found that both formed a homogenous mixture when checked visually and there was also no phase separation observed even after 48h in the lipid mixture.

Selection of a binary lipid phase

Optimal Liquid-solid lipid ratio was selected with the intention to have good entrapment efficiency with proper melting point to maintain the solid/semisolid consistency at room temperature. A higher ratio of liquid lipid could be useful for higher drug entrapment efficiency as the drug solubility is more in the oil compared to the solid lipid. However, at the same time, the consistency of the lipid mix cannot be compromised. It was observed that the liquid-solid lipid mixture in the ratio up to 60:40 was having a sufficient melting point. On the further increase of oil content, the melting point of the mixture decreased below the desired level. Based on the smear test, binary lipid phase was selected in the 60:40 w/w ratio of solid and liquid lipid for developing NLC.

Screening of Surfactants and co-surfactants

The transmittance of dispersion of Sefsol 218 and GMS (in 4:6 ratio) with Tween 80, Tween® 20, span 20 and span 80 were found to be 98.01 ± 0.10%, 95.40 ± 0.7%, 89.25 ± 1.30% and 73.67 ± 0.90% respectively. The transmittance of dispersion of binary mixture with co-surfactants Transcutol® P, PEG 400, n butanol and soya lecithin were found to be 97.06 ± 0.60%, 90.80 ± 0.12%,85.80 ± 0.27% and 77.80 ± 0.10% respectively. On account of their excellent solubility and emulsification capacity, tween 80 and transcutol were selected as surfactants and co-surfactants respectively. Also additionally, Tween 80 acted as a colloidal stabilizer since it increased the colloidal stability of the NLC *via* steric hindrance effects with minimal use and Transcutol served as the penetration enhancer.

Optimization of the APM_NLC by Box-Behnken design

Design of experiment (DoE) is a technique used to study the effect of various variables on the outcome of an experiment. With the help of DoE, one can obtain an optimized formulation in a minimum number of experiments and lower the chances of product failure. It consists of various designs such as factorial design, Box Behnken Design, Fractional Factorial Design, Central Composite Design, etc. Compared with central composite design, mixture design, and three-level factor design, the advantages of Box–Behnken design is lower cost and more time savings due to fewer experiments. Additionally, since there is no axial point in the design, all design points can belong to a safe operating area.

BBD design used for the optimization of apremilast NLC formulation has generated 17 experimental runs for the prepared formulations with 5 centre points as shown in Table 1. The optimized quantities of formulation variables including drug (A), total lipid (solid: liquid lipid) (B) and surfactant (C) was found in the range of 100-300 mg, 2000-6000 mg and 4000-8000 mg respectively. The particle size (Y1), PDI (Y2) and EE (Y3) were found in the range of 161.21-250.84 nm, 0.21-0.56, and 66.02% to 94.97% respectively. ANOVA was applied to determine the model significance. The result showed that model terms were highly significant corresponding to all three responses and showed a good fit to the quadratic model.

Response 1 (Y1): Effect of Independent Variables on Particle Size

Particle size is an important quality attribute for the preparation of NLC because particle size governs the rate of drug penetration and extent of drug deposition in skin layers. After fitting the response data into various models it was found that the quadratic model was significant ($p<0.0001$) with model F-value of 162.83. The difference between predicted R-Squared (0.95) and adjusted R-Squared (0.98) was less than 0.20 which indicated a reasonable agreement between them. The influence of independent variables on particle size was predicted from the following equation.

$$PS = 211.6 + 14.5 * A + 17.375 * B - 16.875 * C + 8 * AB + 9 * AC + 22.75 * BC - 6.925 * A^2 + 3.325 * B^2 + 3.325 * C^2$$

The positive sign in the polynomial equation represents a synergistic effect on the response, while the negative sign represents an antagonistic effect on the response. The independent factor drug concentration (A) and Lipid concentration (B)was found to have a positive effect on particle size whereas surfactants concentration (C) had an inverse effect on the particle size. The high value of the coefficient of variable B indicated that it had more significant effect on particle size than variable A. It was observed in response surface plot (Fig. 4) when the amount of total lipid was increasing in the formulation, the PS of the NLCs also increased. This effect may be attributed to the lipid concentration in the NLCs because increase in the lipid increased resulted in higher surface tension between the particles and which lead to an increase in particle agglomeration and hence bigger PS. It was also noticed that the surfactant concentration also had an antagonistic effect on the PS because when the surfactant concentration was increased, there was a decrease in particle size This could be attributed to the major influence of surfactant in preventing the coalescence of formed emulsion droplets due to the reduced surface tension of melted lipid droplets and thereby resulting in smaller PS. Least sizes were obtained at low concentration of lipid with higher surfactant concentration. Hence selection of lipid &surfactant concentration is crucial in NLC preparation.

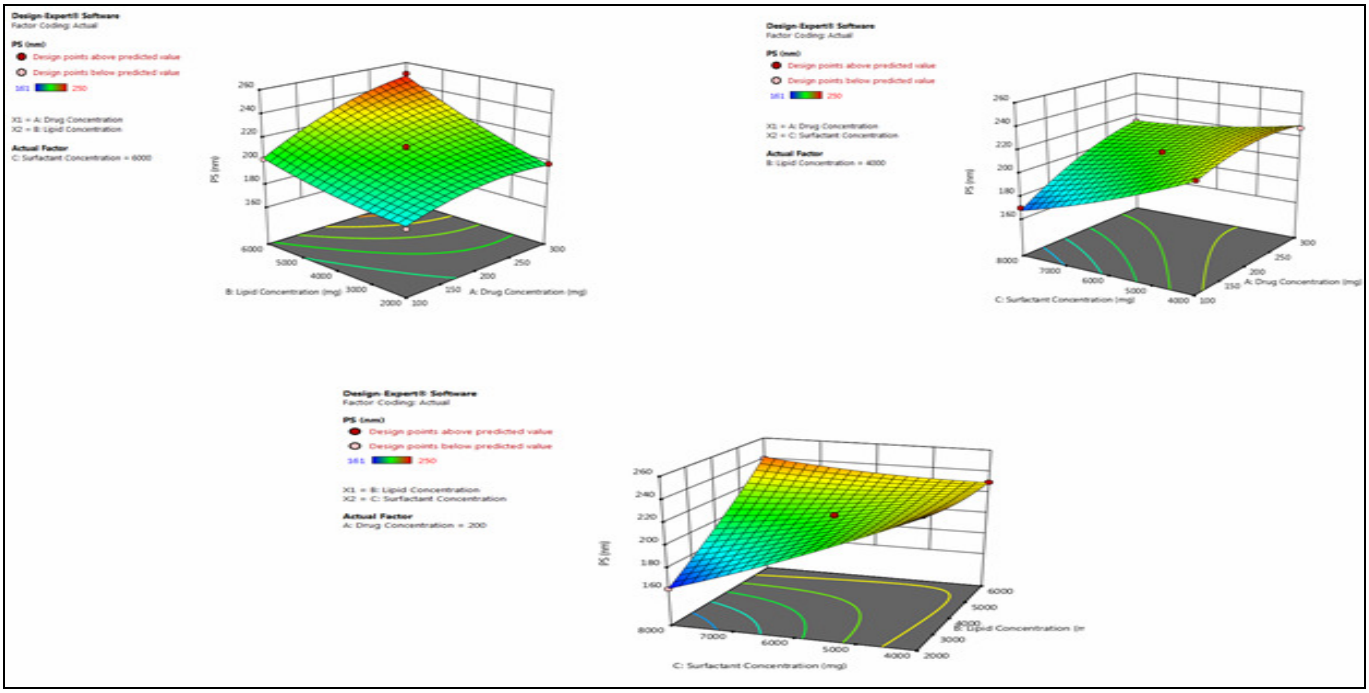
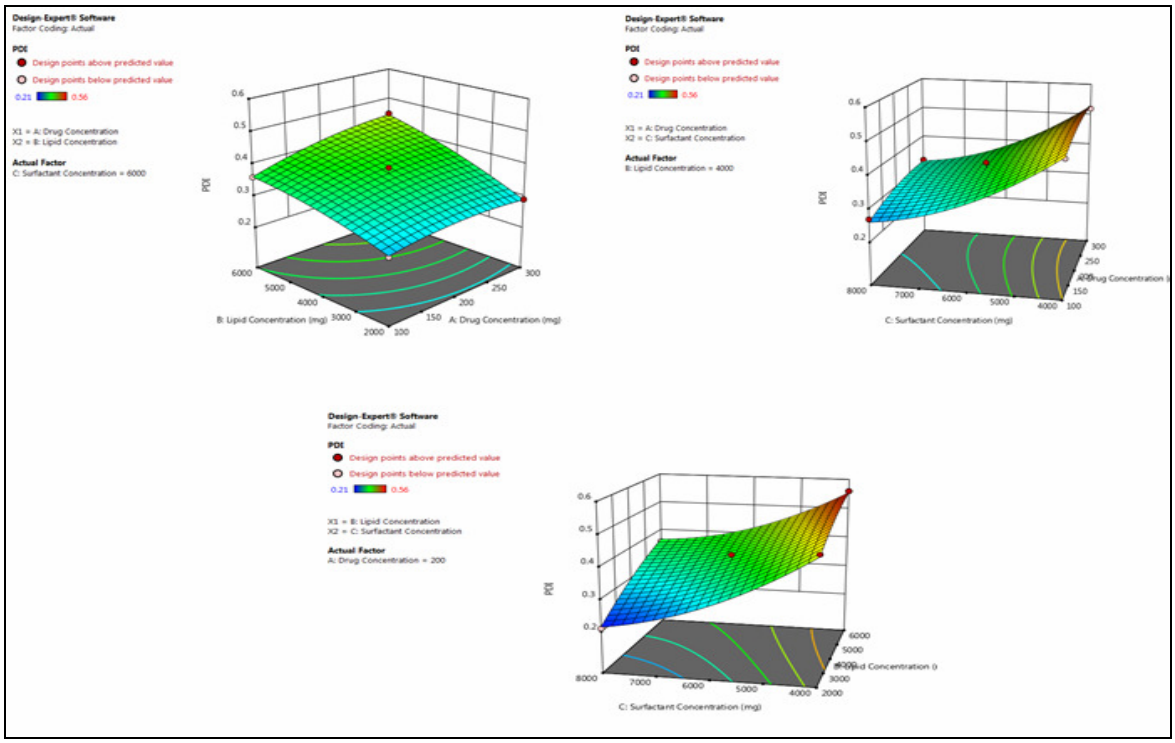


Fig. 4 : 3D Surface plots showing the effects of significant factors on Particle size

Response 2 (Y2): Effect of Independent Variables on PDI

PDI is a measure of the heterogeneity of particle sizes in the NLC. The smaller value of PDI is highly desirable in order to have uniform size distribution in the formulations. After fitting the response data into various models it was found that quadratic model was significant ($p<0.0001$) with

model F-value of 127.77 The difference between predicted R-Squared (0.95) and adjusted R-Squared (0.98) was less than 0.20 which indicated a reasonable agreement between them. The influence of independent variables on particle size was predicted from the following equation.



$$PDI = 0.372 + 0.025 * A + 0.06125 * B - 0.10875 * C + 0.0175 * AB + 0.0025 * AC + 0.015 * BC - 0.016 * A^2 - 0.0135 * B^2 + 0.0415 * C^2$$

Fig. 5 : 3D Surface plots showing the effects of significant factors on PDI

Both Lipid Concentration and Surfactant Concentration had a significant effect on the polydispersity index as compared to Drug Concentration. Contour plot analysis for the response of PDI showed similar results as in particle size this is because the Polydispersity index is directly related to the particle size. As seen from the counter plot (Fig. 5) The concentration of surfactant had an inverse effect on the narrow size distribution of the NLC because, as the concentration of surfactant increased, the PDI decreased. This narrow size distribution of the NLCs might be due to the higher concentration of surfactant results used in the formulation which resulted in an excellent steric hindrance which prevented the nanoparticles from aggregation. It was observed that drug concentration and lipid concentration had a positive effect on the particle size. This is due to the fact that the increase in lipid concentration causes uneven distribution of surfactant over the lipid droplet which results into coagulation of the particles and non-uniform size distribution.

Response 3(Y3): Effect of Independent Variables on Entrapment efficiency

Entrapment efficiency is an important parameter and plays a significant role in the formulation as the delivery of the desired dose of the drug for the clinical efficacy, which is incorporated in NLC is dependent on it. Entrapment efficiency of the obtained 17 formulations was found in the range of 66.02% to 94.97%.The repose data was fitted by quadratic model. The Predicted R^2 was in reasonable agreement with the Adjusted R^2, i.e., the difference was less than 0.20.The following quadratic equation were obtained, which described the effect of terms on entrapment efficiency.

$$\% EE = 90.636 + 1.1375 * A + 11.2912 * B - 1.13125 * C - 0.895 * AB - 1.63 * AC + 1.4075 * BC - 1.33425 * A^2 - 5.66675 * B^2 - 4.04175 * C^2$$

The higher value before lipid concentration in the equation indicates indicate the fact that Lipid concentration had the greatest impact on the % entrapment efficiency. The positive effect of lipid on entrapment can be understood by analyzing effect of lipid level on particle size whereby the larger particles could entrap mored drugs. From the counterplot (Fig. 6), it can be seen that as lipid concentration also increased the entrapment efficiency of the drug into the lipid matrix also increased. This is due to the increased amount of GMS leads into an increased concentration of mono, di and triglyceride that act as solubilizing agents for lipophilic drugs and also increased amount of sefsol 218 decreased imperfection in the crystal lattice which offers greater space to accommodate more drug and increase the entrapment efficiency. The positive correlation between the size of a particle and its ability to entrap materials may justify the negative effect of the surfactant level on entrapment efficiency. Additionally, increased surfactant concentration in the external phase leads to increased partitioning of the drug from the internal to external phase resulting in reduced drug entrapment efficiency.

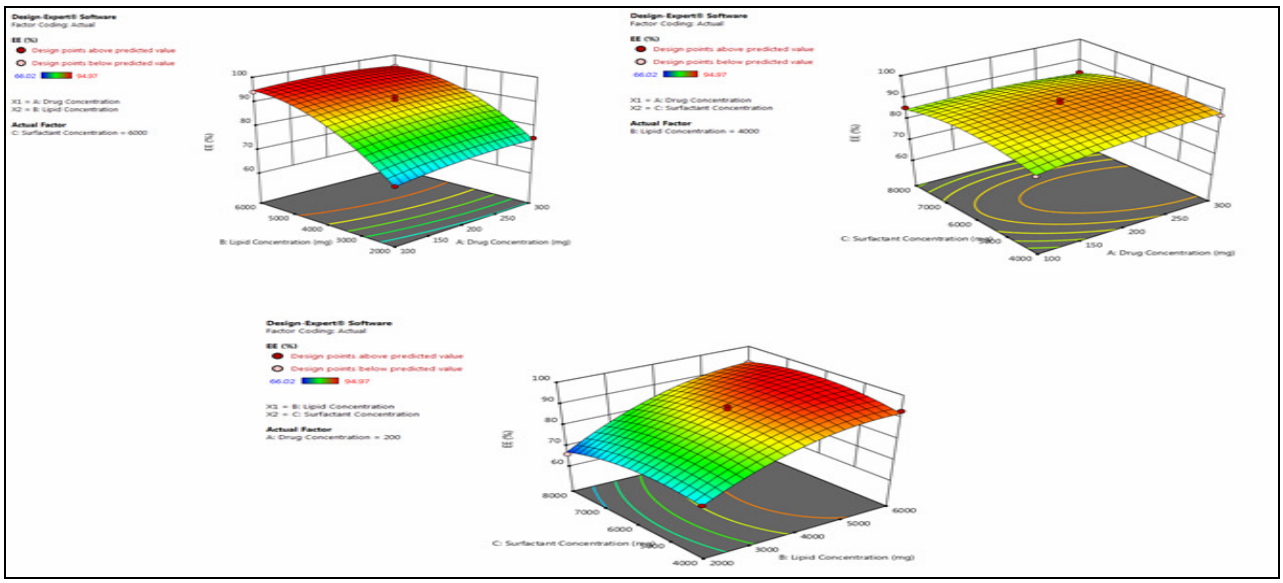


Fig. 6: 3D surface plots showing the effect of Significant factors on %EE

Optimization of the Apremilast Loaded NLC

The desirability function was explored using Design-Expert software to attain the optimized formulation. The formulation was optimized on the basis of the set criterion of maximum EE and minimum particle size and PDI After a comprehensive analysis, the Predicted formulation comprising drug (0.1%), lipid (4.2 % w/w) and Smix (7.8 %w/w) was selected as optimized formulation because it had the desirability factor 0.8 which was close to 1 and it also fulfilled the maximal criteria for optimal performance. The optimized formulation obtained exhibited particle size of 170.32±2.14 nm, PDI of 0.267 and entrapment efficiency 89.26±1.22%. The predicted value of particle size and % EE for the optimized formulation was found to be 172.5, PDI 0.270 and 86.61 respectively. The % error for the optimized formulation was observed to be less than 5% for Particle size, PDI and Entrapment efficiency.

Conclusion

In this study, experimental statistical designs were involved to investigate the effects of formulation and process variables on the particle size, PDI and %EE. The application of Box Behnken design proved to be a useful tool for optimizing Apremilast loaded NLC prepared by melt emulsification-ultrasonication technique. An analysis of these results was processed by polynomial equations and multiple regression. According to our studied factors, the selected optimum formulation with 89.26% of EE, 170.32 nm of particle size with PDI of 0.267, and was processed by 4200 mg(4.2%), 100 mg(0.1%) of drug and 7.8% of Smix. Observed response is in close agreement with the predicted values of the optimized formulation. Therefore, the statistical experimental design methodology has clearly shown the feasibility of the optimization procedure in developing APM loaded NLC for psoriasis therapy.

Table 1: Composition of NLC batches obtained by Box Behnken Design

	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Run	A: Drug Concentration (mg)	B: Lipid Concentration (mg)	C: Surfactant Concentration (mg)	PS (nm)	PDI	EE (%)
1	100	6000	6000	202	0.36	94.05
2	200	4000	6000	212	0.36	90.12
3	200	4000	6000	208	0.37	90.38
4	200	6000	8000	240	0.36	92.25
5	100	2000	6000	182	0.27	70.51
6	200	6000	4000	230	0.56	93.02
7	200	2000	4000	242	0.47	72.42
8	200	4000	6000	213	0.39	89.54
9	300	4000	8000	214	0.32	84.08
10	300	6000	6000	250	0.45	94.97
11	200	4000	6000	213	0.37	90.92
12	300	4000	4000	228	0.52	88.28
13	200	2000	8000	161	0.21	66.02
14	200	4000	6000	212	0.37	92.22
15	100	4000	4000	220	0.48	83.18
16	300	2000	6000	198	0.29	75.01
17	100	4000	8000	170	0.27	85.5

Acknowledgments

The authors are thankful to the authorities of N.G.S.M. Institute of Pharmaceutical Sciences and NITTE (Deemed to be University), Mangalore, Karnataka, India, for providing all the necessary facilities to support this research project.

Conflict of Interest

Authors declare no conflict of interest

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