IJPSR (2019), Volume 10, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 18 July 2019; received in revised form, 16 August 2019; accepted, 19 August 2019; published 01 September 2019

FACTORIAL DESIGN BASED OPTIMIZATION AND *IN-VITRO – EX-VIVO* EVALUATION OF CLOBETASOL LOADED NANO STRUCTURED LIPID CARRIERS

K. Ramesh Reddy [1, 3], S. V. Satyanarayana [2] and V. Jayasankar Reddy [* 3]

Department of Pharmaceutical Sciences [1], Department of Chemical Engineering [2], Jawaharlal Nehru Technological University, Anantapur, Ananthapuramu - 515002, Andhra Pradesh, India.
Department of Pharmacology [3], Krishna Teja Pharmacy College, Chittoor - 517506, Andhra Pradesh, India.

Keywords:

Nanostructured lipid carriers, Clobetasol-17- propionate, 3^3 full factorial design, Franz diffusion cell

Correspondence to Author:
Dr. V. Jayasankar Reddy

M. Pharm., Ph.D., Professor, Department of Pharmacology, Krishna Teja Pharmacy College, Chittoor - 517506, Andhra Pradesh, India.

E-mail:k.rameshreddy88@gmail.com

ABSTRACT: The aim of the present work was to develop and evaluate the potential of nanostructured lipid carriers (NLCs) loaded with Clobetasol-17-propionate (CP) as a new approach for the topical treatment of psoriasis. CP-loaded NLCs were prepared by melt emulsification and ultra-sonication method and optimized using 3^3 full factorial designs (Design-Expert software 11.0), by using solid lipid (compritol ATO 888) and liquid lipid (oleic acid) and Tween 80 as a surfactant. Drug loaded NLCs were evaluated for various parameters like particle size, surface charge, polydispersity index, entrapment efficiency, surface morphology, thermal analysis, *in-vitro* drug release through the skin (Franz diffusion cell), drug deposition study and stability. The optimized formulation has a particle size of 91.2 ± 2.37 nm, the zeta potential of -34.7 ± 1.49 mV, polydispersity index of 0.173 ± 0.035 and entrapment efficiency of 85.4 ± 2.89%. Release study demonstrated prolonged CP release from NLCs following Higuchi release kinetics with r^2 = 0.9838, while pure CP suspension showed quicker drug release obeying zero-order kinetics with r^2 value of 0.9904. Skin permeation study of CP loaded SLNs suspension showed prolonged drug release up to 24 h. The maximum *ex-vivo* drug deposition was obtained after developing the drug into NLCs (51.23 µg/ml) when compared to the pure drug (18.34 µg/ml). The prepared NLCs based formulation has proved to be a promising carrier system for the treatment of psoriasis.

INTRODUCTION: Psoriasis is a polygenic, chronic autoimmune disease resulting from the derangement in the inflammatory pathways in the dendritic cells, T cells and the keratinocytes that make up most of the skin structures [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(9).4374-83

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(9).4374-83

The Psoriasis disease involves a series of linked cellular changes in the skin involving hyperplasia of epidermal keratinocytes, vascular hyperplasia, and infiltration of T-lymphocytes, neutrophils and other types of leucocytes in affected skin [2]. For the management of psoriasis, topical treatment was most commonly used in the majority of patients.

However, challenges associated with psoriatic skin such as skin being rough, absence of Normal Moisturising Factors (NMFs) like water and imbalance of skin lipids poses a stiff challenge in designing an effective topical delivery system [3].

Psoriasis is a life-long disease, and the management and treatment of psoriasis are different depending on the severity of the disease the first-line treatment was topical therapy. Clobetasol propionate (CP), a highly potent drug of all the available corticosteroids is widely used in the treatment of various skin disorders including psoriasis, atopic dermatitis [4]. NLC comprises of a mixture of liquid and solid lipids which creates a less perfect crystalline structure with many imperfections, thus offering more space for drug accommodation[5]. Examples of solid lipids are triglycerides (tristearine, tripalmitine, trimiristine), Compritol 888 ATO, Precirol, fatty acids (stearic acid, palmitic acid), and waxes (carnauba, cetyl palmitate), whereas liquid lipids include, *e.g.*, medium-chain triglycerides, oleic acid, and isopropyl myristate. NLCs are promising drug carriers for topical application because of their improved skin retention properties [6].

Therefore, transdermal delivery is a superior way to achieve efficacy and avoid toxicity, especially for treating inflammatory diseases like psoriasis or rheumatoid arthritis [7]. NLCs are potential carriers for improving the drug retention at the skin layers and to reduce the risks of both local and systemic side effects associated with topical corticosteroids. The aim of the present work was to develop and optimize solid lipid nanoparticles using design expert software and to explore the in vitro characterization of the optimized formulation for particle size (PS), zeta potential (ZP), percentage entrapment efficiency (% EE), transmission electron microscopy, differential scanning calorimetry (DSC) and drug release studies.

MATERIALS AND METHODS:

Materials: Clobetasol-17-propionate was purchased from Yarrow Chemicals (Mumbai, India). Compritol 888ATO, Precirol ATO5, Gelucire 44/14 and Capryol 90 were procured as gift samples from Gattefosse Co. (Lyon, France). Stearic acid, Glyceryl monostearate (GMS), Isopropyl myristate, Soybean oil, Coconut oil, Oleic acid, Poloxamer® 188 (Pluronic® F68), Tween 80, dimethyl sulfoxide (DMSO), sodium lauryl sulfate (SLS), Acetone and ethyl alcohol were purchased from Sigma–Aldrich Co. (Mumbai). All other chemicals and reagents were of analytical grade.

Screenings of Components: The solubility of Clobetasol in various solid lipids (Compritol, cetyl palmitate, Sorbitan monostearate, glyceryl monostearate, cetyl alcohol, and stearic acid) were determined by adding Clobetasol in increments of 1 mg until it failed to dissolve in the molten solid [7]. The amount of solid lipids required to solubilize Clobetasol was determined. The solubility of Clobetasol in various liquid lipids and surfactants were determined by adding an excess amount of drug in 5 ml of each of the lipids in glass vials. The vials were vortexed and kept at 37 ± 1.0 °C in an isothermal shaker (Royal Scientific, Mumbai, India) for 48 h to reach equilibrium. The samples were filtered through 0.45µm membrane through vacuum filtration and analyzed spectrophotometrically (Agilent 8453 UV-visible Spectroscopy System) at 239 nm after appropriate dilution with methanol [7].

Selection of a Binary Phase: The solid and liquid lipids were mixed in ratios of 95:5, 90:10, 85:15, 80:20, 70:30 and 60:40 w/w to establish the miscibility of two lipids using magnetic stirrer 200 rpm. The miscibility between the two components were investigated by smearing cooled sample of a solid lipid mixture onto a Whatman filter paper, followed by visual observation to determine the presence of any liquid oil droplets on the filter paper.

Preparation of Nano-Structured Lipid Carriers: Clobetasol loaded nanostructured lipid carriers were prepared by using melt emulsification and ultra-sonication method. Initially, the organic phase was prepared by mixing solid and liquid lipid on a magnetic stirrer at a temperature of 5 °C above the melting point of solid lipid. Drug loaded NLC preparations were prepared by adding an ethanolic solution of CP (50 mg) to the above organic phase and heated till organic solvent was completely removed from the lipid melt. 30 ml of preheated aqueous phase comprising of 1.5% w/v surfactant was added dropwise to the lipid blend under stirring, followed by its mixing at 800 rpm for 15 min. The suspension was later subjected to probe sonication (3 mm diameter, Sonic Vibra Cell, VCX 130, USA) cycle for 10 min (30-sec run and 5-sec break) at 40% amplitude maintained at a temperature of 60-80 °C employing oil bath. The solution so obtained was cooled in an ice bath for

15 min and later maintained under refrigerated conditions for 24 h. After that samples were lyophilized employing 2% w/v mannose (cryoprotectant) and were stored in an airtight container till further use [8].

Optimization of NLCs: The interaction effect of dependent and independent variables were investigated through optimization using Design Expert Software (Stat-Ease; MN Trial Version 11.04). 3^3 Full Factorial Design was generated a quadratic polynomial model which will describe the non-linear equation. In this study, 3 independent variables were selected the ratio of lipid: drug (A), surfactant concentration (B) and sonication time (C) as independent variables while PS, PDI and percentage EE as dependent variables. All variables with their levels, codes, and constraint were illustrated in **Table 1**. Perturbation plots were prepared using reduced polynomial equation and 2D contour and 3D response surface plots were generated to determine the effect of the independent variable on particulate characteristics. Desirability was evaluated to determine the closeness of response to ideal value and goodness of fit of a model was predicted from R^2 values.

TABLE 1: SELECTION OF INDEPENDENT VARIABLES AND THEIR LEVELS FOR EXECUTION OF 3^3 FACTORIAL DESIGNS

Types of variable Independent variable	Levels		
	Low (-1)	Medium (0)	High (+1)
X1 = Drug: lipid Ratio (D:L) (%w/w)	1:5	1:7.5	1:10
X2 = Surfactant concentration (S) (%w/v)	1	1.5	2
X3 = Sonication time (min)	5	10	15
Dependent variable			
Y1 = Particle Size (nm) Y2 = Poly Dispersibility Index Y3 = % Entrapment Efficiency			

Characterization of NLCs:

Mean Particle Size, Poly Dispersity Index and Zeta Potential: The average particle size, polydispersity index, and zeta potential of NLCs were measured using particle size analyzer (Malvern Zetasizer ZS 90, UK). For this, the NLCs loaded with Clobetasol were dispersed in adequate quantity of distilled water to overcome the opalescence. Measurement was done at a scattering angle of 90° [9].

Percentage Entrapment Efficiency (%EE): The % EE of NLCs dispersion was estimated indirectly, by determining the amount of free drug Clobetasol (un-entrapped) present in the aqueous phase of dispersions, the calculation was done using the following equations:

% EE = (Total amount of CP - Amount of free CP) / (Total amount of CP) × 100

The un-entrapped amount of Clobetasol was separated by centrifugation and filtration using a cooling centrifuge and Millipore filtration assembly, respectively. The aqueous dispersion of NLCs was centrifuged at 11,000 rpm for 10 min. maintained at 4 ºC. After centrifugation, the supernatant was analyzed using UV-visible spectrophotometer at 239 nm [10].

Differential Scanning Calorimetry Study (DSC): DSC analysis was done to predict the compatibility between drug and excipients. The thermograms of blank and drug laded NLCs were recorded by heating the samples at a temperature range from 25 ºC to 350 ºC maintained at 10 ºC per minute. An accurately weighed sample of CP-NLCs was placed in aluminum pans, sealed, and DSC analysis was carried out (Perkin-Elmer Instruments, USA). Then the pans were placed under the isothermal condition at 25 ± 1 ºC for 10 min. An empty aluminum sealed pan was used as a reference.

Transmission Electron Microscopy (TEM): Transmission Electron Microscope (TEM; Philips, Tecnai 20, Holland) was used to examine the surface morphology of CP-NLCs. A drop of the diluted sample was placed on the surface of the carbon-coated copper grid and stained with a drop of 1% (w/w) aqueous solution of phosphotungstic acid (negative stain) for the 30s. Excess staining was drained, and the grid was air-dried. TEM images were captured in the microscope [11].

***In-vitro* Release Study:** *In-vitro* release studies of NLCs suspension containing Clobetasol and pure Clobetasol suspension were characterized for its release behavior and mechanism using locally designed Franz diffusion cell [12]. For the estimation, pretreated dialysis membrane (MWCO- 12 000–14 000 Da, pore size- 2.4 nm, HIMEDIA, Mumbai, India) was positioned over the FD Cell (Franz diffusion cell having diffusion area 1.95 cm^2).

Before mounting over the diffusion cell, the membrane was drenched in distilled water for 24 h. NLCs suspension (equivalent to 2 mg/ml of Clobetasol) was placed in the donor compartment. Release media (phosphate buffer pH 7.4) was filled in the receptor compartment. The study was conducted at 37 ± 0.5 ºC by continuous stirring the suspension at 200 rpm by a magnetic stirrer. A definite time intervals (0, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h), the sample was withdrawn from the receptor compartment and replaced with the exact volume of release medium. The samples were analyzed using UV spectrophotometer at 239 nm after appropriate dilutions. Percent drug release was calculated and the graph was plotted between percent drug release against time. The cumulative percentage drug release obtained at the various time period (Qt) was calculated, and the data obtained were fitted to Zero-order release, Higuchi release kinetics, and first-order kinetic model in order to reveal the mechanism of drug release from NLCs formulation [13]. The drug release data were reported as the Mean ± SD (standard deviation) of three replicates.

***Ex-vivo* Diffusion Study:** An *ex-vivo* diffusion studies were carried out to evaluate the distribution of CP delivered through CP loaded NLCs suspension and plain CP suspension using sheepskin. Sample of sheepskin was obtained from the local slaughterhouse (Tirupati, Andhra Pradesh). The skin diffusion studies were performed using the Franz diffusion cells according to this method. Skin samples were separately treated with the formulations (plain CP suspension and CP loaded NLCs) containing drug equivalent to 400 mg (6h with each test formulation). At the end of the experiment, the skin samples were washed and removed from the Franz cells. Epidermal and dermal layers were manually separated using tweezers. CP was extracted in 10 ml methanol by sonication of each skin layer, and extracted samples were assayed for CP concentration using UV spectroscopy at 239 nm [14].

Stability Studies: To ensure the stability of prepared nano-lipid carriers to withstand environmental stress, the stability study was conducted as per ICH guidelines (Q1AR2) [12]. NLCs were stored at normal condition, *i.e.*, room temperature (25 ± 2C, º60 ± 5% RH) and accelerated temperature (40 ± 2 ºC, 75 ± 5% RH) for 180 days. At regular intervals, the NLCs were evaluated PS, ZP, PDI, and EE.

RESULTS AND DISCUSSION:

Screening of Components: The solubility of Clobetasol in various solid and liquid lipids were given in **Table 2**. Among the various lipids selected for screening, the solubility of the drug was found to highest in oleic acid. Further, the solubility of a drug in various solid lipids used topically and was found to be highest in Compritol 888 ATO. Liquid-solid lipid ratio was selected with the intention to have the more drug-carrying capacity with a 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 [15].

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 70:30 was having a sufficient melting point (70.0°-80.0 °C). 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 70:30% w/w ratio of solid and liquid lipid for developing nanocarriers. Surfactant blend of Tween 80 (1.5% w/v) and Poloxamer (Stabilizer 1% w/v) showed maximum stable formulations due to a reduction in interfacial tension and increased in the fluidity of the interface, thereby increasing the entropy of the system. So, this ratio was selected for development of NLC dispersion.

TABLE 2: SOLUBILITY OF DRUG IN DIFFERENT SOLID LIPIDS AND OILS

Components	Solubility (mg/ml)
Glyceryl monostearate	4.25 ± 0.26
Stearic acid	3.82 ± 0.07
Compritol 888 ATO	6.23 ± 0.25
Cetyl palmitate	2.23 ± 0.49
Gelucire	2.99 ± 0.42
Capryol 90	4.25 ± 0.25
Soybean	2.35 ± 0.21
Coconut	2.05 ± 0.37
Isopropyl myristate	3.31 ± 0.29
Oleic acid	5.32 ± 0.44

* All values were expressed as mean ± SD, n=3

Optimization of NLCs Preparation Method: Based upon the findings of preliminary evaluations, variables *viz*. total lipid: drug ratio, surfactant

concentration and sonication time, were found to significantly affect the particle characteristics. Therefore, DOE (Stat-Ease; MN Trial Version 11.04) was applied to prepare various combinations varying the above independent variables within a specified limit *i.e.* total lipid: drug ratio (5:1 to 10:1), surfactant concentration (1-2% w/v) and sonication time (5-15 min), keeping fixed values of solid: liquid lipid ratio (70:30) and amplitude (40%). 3^3 full factorial design was employed to study the influence of the independent variable on particle characteristics at 4 concentration levels. **Table 3** depicts a set of 30 runs generated and their respective responses. The optimized NLCs batch was selected based on lowest PS and highest percent EE. Perturbation, 2D contour, and 3D response surface plots were utilized to determine the influence of the independent variable on the responses.

A 2^{nd} order polynomial equation determining the relationship between independent and dependent variables were depicted in the following equation (1):

$$Y = A_0+A_1X_1+ A_2X_2+ A_3X_3+ A_4X_1 X_2+ A_5X_2 X_{3+} A_6X_1 X_2+ A_7X_1^2{}_+ A_8X_2^2{}_+ A_9X_3^2$$

Where Y is the response, X_0 is the intercept and X_1 to X_3 are the regression coefficients computed from the observed experimental values of Y. A, B and C represents total lipid: drug ratio, surfactant concentration, and sonication time, respectively. The positive sign before the coefficient values indicate the synergistic effect while a negative sign represents the antagonistic effect.

TABLE 3: LAYOUT OF 3^3 FULL FACTORIAL DESIGN SHOWING THE VALUES OF DEPENDENT VARIABLES OF 30 POSSIBLE CP SLNS FORMULATIONS

Run	X1	X2	X3	Y1	Y2	Y3
1	5	1.5	5	199.8 ±3.25	0.326 ± 0.028	77.8 ± 3.21
2	7.5	1.5	10	95.3 ± 4.21	0.187 ± 0.033	82.4 ± 2.69
3	10	1	10	185.2 ± 3.69	0.262 ± 0.052	79.3 ± 1.89
4	5	2	5	235.1 ± 4.23	0.355 ± 0.085	54.4 ± 3.25
5	5	1	15	145.2 ± 3.52	0.323 ± 0.057	50.3 ± 2.49
6	7.5	2	5	241.3 ± 4.21	0.322 ± 0.043	58.5 ± 3.26
7	7.5	1.5	10	91.2 ± 2.37	0.173 ± 0.035	85.4 ± 2.89
8	10	2	5	261.4 ± 2.59	0.352 ± 0.042	68.1 ± 2.75
9	10	1	15	201.2 ± 3.56	0.345 ± 0.052	65.3 ± 3.47
10	10	2	15	207.3 ± 3.43	0.332 ± 0.028	61.8 ± 3.23
11	10	2	10	195.2 ± 3.89	0.298 ± 0.041	73.3 ± 3.55
12	7.5	1	10	123.5 ± 3.95	0.222 ± 0.021	75.4 ± 3.41
13	7.5	1.5	10	95.3 ± 2.56	0.192 ± 0.011	81.3 ± 2.59
14	5	1.5	15	145.1 ± 3.53	0.322 ± 0.028	52.6 ± 1.99
15	5	2	15	205.7 ± 2.97	0.333 ± 0.054	47.5 ± 2.67
16	5	1.5	10	130.2 ± 3.46	0.251 ± 0.027	71.3 ± 2.26
17	10	1.5	10	156.5 ± 4.23	0.284 ± 0.074	85.3 ± 4.21
18	10	1.5	5	201.2 ± 4.57	0.328 ± 0.029	77.5 ± 3.58
19	7.5	2	15	220.3 ± 5.26	0.354 ± 0.088	53.4 ± 3.87
20	10	1	5	243.3 ± 4.89	0.326 ± 0.075	70.2 ± 4.29
21	5	1	5	216.4 ± 5.74	0.309 ± 0.088	60.2 ± 3.54
22	10	1.5	15	179.1 ± 4.88	0.351 ± 0.072	67.2 ± 3.85
23	7.5	1	5	157.3 ± 4.52	0.257 ± 0.027	72.3 ± 3.52
24	7.5	1	15	138.2 ± 3.48	0.321 ± 0.059	55.3 ± 2.55
25	7.5	1.5	15	179.2 ± 3.59	0.342 ± 0.035	67.3 ± 4.26
26	7.5	2	10	195.4 ± 4.21	0.295 ± 0.058	73.2 ± 3.56
27	7.5	1.5	10	112.3 ± 1.99	0.284 ± 0.098	85.2 ± 3.56
28	5	1	10	144.2 ± 3.69	0.247 ± 0.072	66.3 ± 4.25
29	7.5	1.5	5	157.6 ± 4.21	0.257 ± 0.036	72.3 ± 3.22
30	5	2	10	168.3 ± 3.56	0.295 ± 0.089	60.2 ± 4.83

* All values were expressed as mean ± SD, n=3

Effect of Formulation Variables on Particle Size: The minimum average particle size of executed batch was 91.2 ± 2.37 nm whereas; maximum particle size was 261.4 ± 3.45 nm. The Quadratic model was suggested for particle size giving the Equation (2):

PS = 109.84+13.36A+20.86B-16.23C-5.76AB+3.08AC+ 2.33BC+25.87A^2+36.55B^2+44.92C^2

The individual factor A, lipid to drug ratio had a positive effect on particle size as showed by the positive coefficient estimate value. The particle size increased with the ratio of lipid to the drug, whereas surfactant concentration (B) showed a positive effect and sonication time showed a negative effect. The combined effect of lipid to drug ratio and surfactant concentration were negative, whereas the sonication time with lipid to drug ratio and surfactant concentration showed a positive effect. An increase in the particle size was observed with a concomitant increase in the proportion of lipid and reduction in the surfactant concentration. Larger particle size with 10:1 lipid to drug ratio could be attributed to the increased viscosity of the system due to higher lipid concentration. Another possible explanation to this may be a film of loosely arranged surfactant molecules at the interface of the two layers in nanodispersion due to the lower concentration of surfactant in comparison to the lipid. On the other hand, a lower lipid with higher surfactant resulted in fine nanodispersion with smaller sized particles.

The size of NLC decreased with increase in sonication time. This could be due to the sheer energy induced by sonication. The effect of lipid: drug ratio, sonication time and surfactant concentration on the particle size are presented in the form of response surface plots in **Fig. 1**.

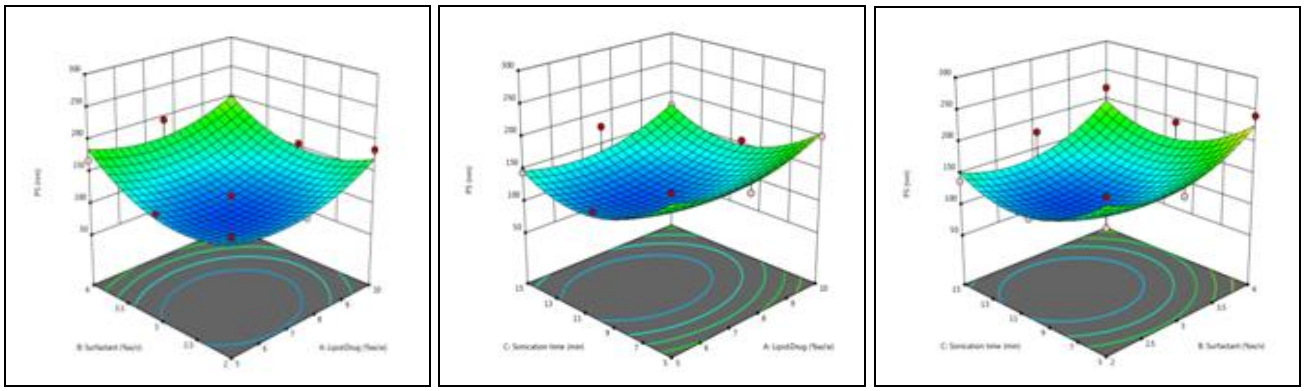

FIG. 1: 3D RESPONSE SURFACE PLOT FOR EFFECT OF LIPID: DRUG RATIO, SONICATION TIME AND SURFACTANT CONCENTRATION ON PARTICLE SIZE

Effect of Formulation Variables on PDI: The minimum PDI of the executed trials were 0.173 ± 0.035 whereas; maximum PDI was 0.355 ± 0.085.

A Quadratic model was suggested and Equation (3) was obtained for PDI.

PDI = 0.2248+0.0065A+0.0180B+0.0106C-0.0046AB+ 0.0028AC-0.0089BC+0.0320A^2+0.0168B^2+0.0680C^2

As per the polynomial equation, the effect of lipid to drug ratio, surfactant concentration, and sonication time was shown a positive effect. The interaction effect of lipid to drug ratio and surfactant concentration was negative effect, lipid to drug ratio and sonication time showed positive effect and surfactant concentration and sonication time showed a negative effect with the PDI.

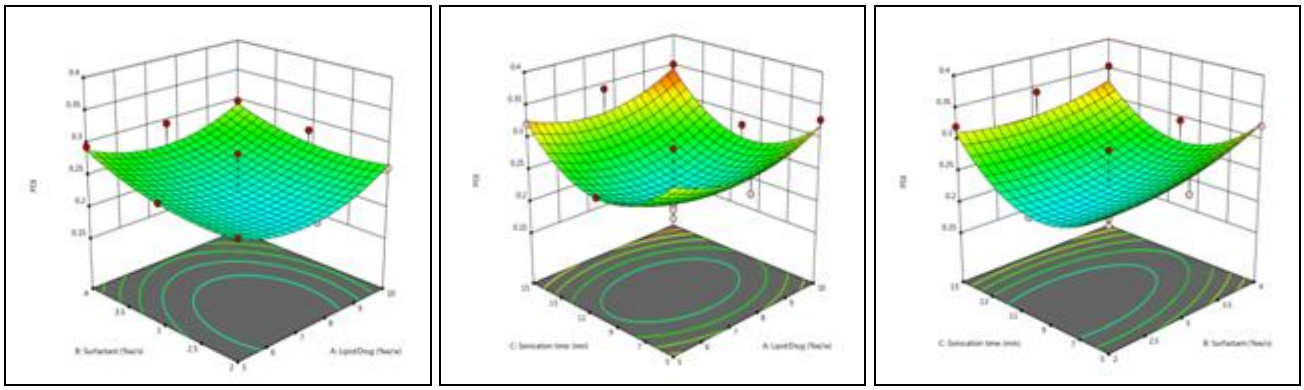

FIG. 2: 3D RESPONSE SURFACE PLOT FOR EFFECT OF LIPID: DRUG RATIO, SONICATION TIME AND SURFACTANT CONCENTRATION ON POLYDISPERSITY INDEX

It was observed that when 2% w/w tween 80 was used, PDI was increased **Table 3**. This was because; during the sonication process alkyl chain of the surfactant molecule covers the surface of the lipid particle *via* hydrophobic interaction to form a stable lipid matrix. Once this stable matrix is formed, an excess surfactant may lead to accumulation of surfactant particles on the surface of stable lipid matrix causing an increase in PDI as was observed in the form of response surface plots in **Fig. 2**.

Effect of Formulation Variables Entrapment Efficiency: Entrapment efficiency obtained was in the range of 47.5 ± 2.89% to 85.4 ± 2.89%. The Quadratic model was suggested for entrapment efficiency, and Equation (4) was obtained.

$$EE\ (\%) = 82.45+5.97A-2.46B-5.03C+0.2583AB+1.71AC+1.13BC-2.26A^2-9.53B^2-11.70C^2$$

The term A, lipid to drug ratio showed a positive coefficient estimate value indicating the direct relationship with the entrapment efficiency, whereas surfactant concentration and sonication time showed a negative effect. The interaction effect of lipid to drug ratio with surfactant concentration and sonication time showed a positive effect. Formulation with lipid: drug ratio of 7.5:1 showed greater drug entrapment, i.e., 85.4%. A higher % EE could be due to the presence of the higher amount of lipid which provides additional space for a drug molecule to embed in, thereby decreasing total surface area.

Table 3 this can lead to a reduction in the diffusion rate of the solute molecule as the viscosity of the lipidic phase is higher and thus showed a higher % EE compared to others. % EE was found to increase with the increasing amount of lipid to drug molar ratio. Thus, % EE was found to be mainly dependent on the drug: lipid ratio of the formulation. **Fig. 3** represents the response surface plots depicting the effect of various factors on % EE.

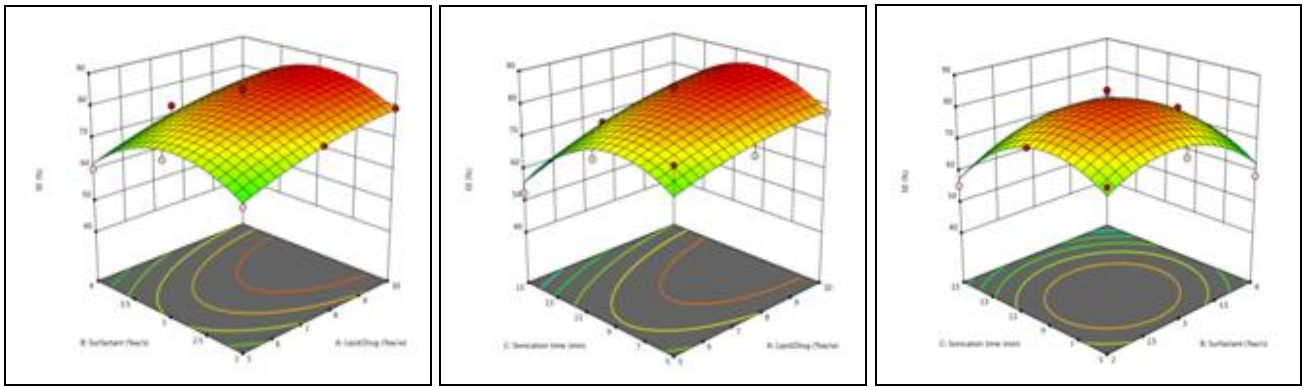

FIG. 3: 3D RESPONSE SURFACE PLOT FOR EFFECT OF LIPID: DRUG RATIO, SOICATION TIME AND SURFACTANT CONCENTRATION ON ENTRAPMENT EFFICIENCY

Optimization and Validation: Method validation was investigated by Design-Expert software to acquire the optimized formulation. The set criteria for the optimization were a minimum particle size of NLCs, minimum polydispersibility index, and maximum entrapment efficiency of the drug. The solutions generated by the software were sorted in the descending order of the desirability and the formulation with the highest desirability factor was considered for further formulation. The predicted values were compared with actual values by calculating the relative error. The relative error was found to be less than 5% indicating a minimum variation in the batch **Table 4.**

TABLE 4: PREDICTED AND OBSERVED RESPONSES FOR THE OPTIMIZED CP-NLCS FORMULATION

Factor 1 Lipid: Drug Ratio	Factor 2 Surfactant Concentration	Factor 3 Sonication time	Responses	Observed	Predicted	Relative error (%)
5.93	2.42	13.24	PS (nm)	115.90 ± 5.23	117.32 ± 6.42	1.42
			PDI	0.264 ± 0.23	0.266 ± 0.18	0.002
			%EE	66.87 ± 2.87	67.33 ± 2.45	1.54

* All values were expressed as mean ± SD, n=3

Transmission Electron Microscopy: Shape and size of the optimized batch of nanoparticles were evaluated by TEM. TEM images of the NLCs demonstrated the nearly spherical or oval shape of the nanoparticle with non aggregated particles having narrow size distribution **Fig. 4**. The diameters of the nanoparticles observed in the micrographs were in good agreement with the data obtained from Malvern particle size analyzer.

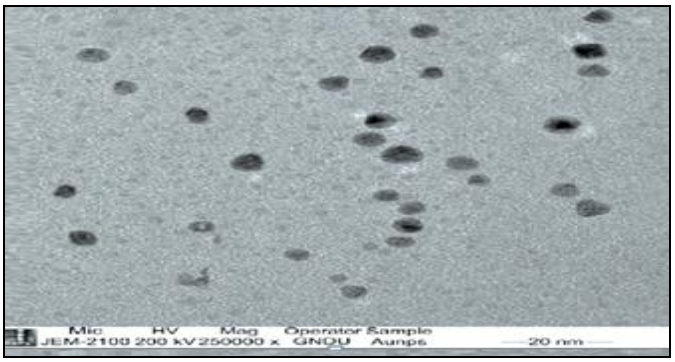

FIG. 4: TEM MICROGRAPH OF OPTIMISED NLC DISPERSION

Differential Scanning Calorimetry: Fig. 5 shown the DSC thermograms of pure CP, Compritol 888 ATO, physical mixture of CP and Compritol 888 ATO, and CP loaded NLCs. Compritol 888 ATO exhibited a sharp endothermic event ascribing to the melting point around 75.41 ºC **Fig. 5B**. Pure CP showed a sharp endothermic peak at 196.24 ºC corresponding to its melting point, indicating its characteristic crystalline nature **Fig. 5C**. These sharp melting endothermic peaks of bulk lipid and drug indicated that the starting materials were crystalline. Characteristic peak for CP was completely absent in lyophilized CP loaded NLCs **Fig. 5A**, while it was clearly evident in the physical mixture of CP (196.24 ºC) and Compritol 888 ATO 75.41 ºC as showed in **Fig. 5D**.

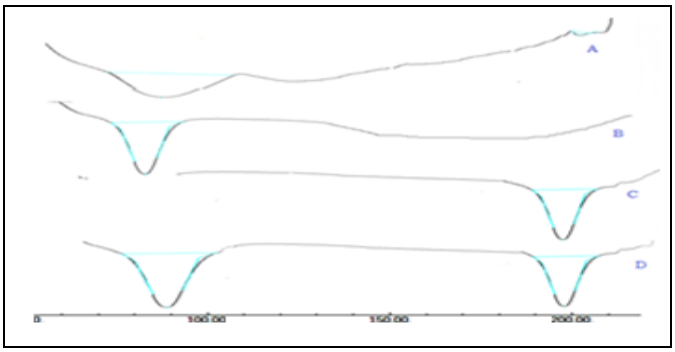

FIG. 5: DSC STUDY. (A) CP LOADED NLCS (B) PURE COMPRITOL 888 (C) PURE CP (D) PHYSICAL MIXTURE OF CP AND COMPRITOL 888 ATO

It has been reported that when the drug does not show its endothermic peak in the CP SLNs formulations, it is said to be in the amorphous state [16]. Consequently, it could be concluded that the drug was homogeneously dispersed inside the nanocarrier in its amorphous state.

***In-vitro* Release Study:** Drug release study was carried out for CP suspension, and CP loaded NLCs suspension *in-vitro* for a period of 24 h. Each sample was examined in triplicate, and the release curve has been presented in **Fig. 6.** It was indicated that plain CP suspension released almost 94.56 ± 1.23% of the drug at the end of 7 h, while CP loaded NLCs suspension released about 75.23 ± 1.55% of the drug after 24 h. CP loaded NLCs suspension exhibited a biphasic drug release pattern with an initial burst release phase followed by a sustained release of the drug. Release kinetics from the optimized formulation of CP loaded NLCs were compared to different kinetic models. Regarding the drug release profiles, CP loaded NLCs and plain CP suspension followed Higuchi release kinetics (r^2 = 0.9838) and zero-order release kinetics (r^2 = 0.9904) respectively. Similar results for novel and conventional formulation were obtained [17].

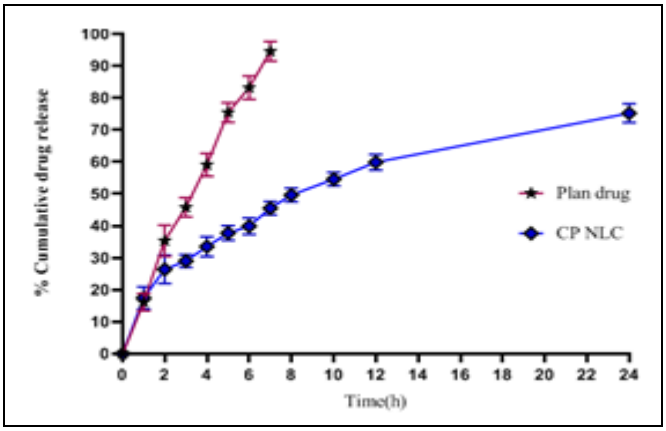

FIG. 6: *IN-VITRO* DRUG RELEASE PROFILE FROM PURE CP SUSPENSION AND CP LOADED SLNS SUSPENSION IN PHOSPHATE BUFFER (pH 7.4)

***Ex-vivo* Skin Distribution Study:** The drug delivery potential of developed novel drug formulation and pure drug suspension across sheepskin was examined. Quantification of CP in the epidermis, dermis, and receptor was examined using UV spectroscopy. Results showed that plain CP suspension delivered the maximum quantity of drug in the receptor compartment (142.21 µg/ml)

with the minimum quantity of CP in the epidermal layer (18.34 µg/ml) and dermal layer (34.05 µg/ml), when examined through 1cm^2 diffusion areas **Fig. 7**. This designates that plain CP suspension distributed maximum of its drug in systemic circulation following 6 h of study. But under similar condition CP loaded NLCs suspension delivered its maximum amounts of CP into the epidermis (51.23 µg/ml), which is considered to be the prime site for keratinocytes hyperproliferation thereby contributing to psoriasis development. Furthermore, the least quantity of CP was observed to be present in the dermis (16.19 µg/ml) as well as the receptor compartment (3.99 µg/ml) of the Franz cell. This proves the ability of drug-loaded NLCs to restrict the systemic flee of a steroidal drug.

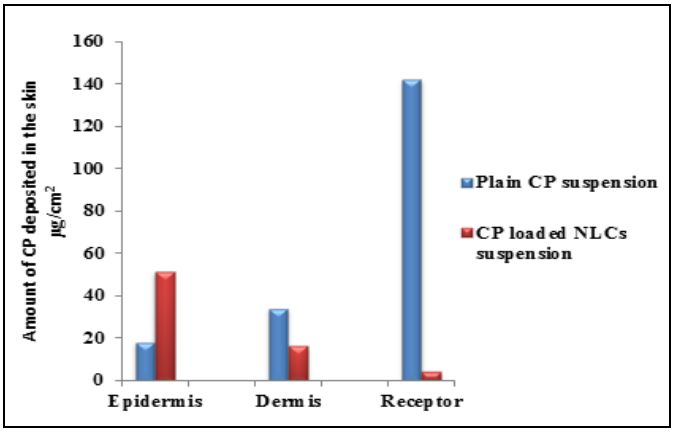

FIG. 7: *IN-VITRO* SKIN DISTRIBUTION STUDY OF PURE CP LOADED SUSPENSION AND CP LOADED NLCS

Stability study: Stability studies were done to evaluate the stability and integrity of CP loaded NLCs. Particle size, PDI, ZP and % EE obtained for fresh NLCs formulation were 91.2 ± 2.37 nm, 0.173 ± 0.035, -34.7 ± 1.49 mV and 85.4 ± 2.24% respectively. While Particle size, PDI, ZP and % EE obtained for NLCs formulation after 180days were 97.52 ± 3.17nm, 0.186 ± 0.019, -32.26 ± 0.57mV and 81.78 ± 1.59% respectively. During the storage of developed novel formulation at 4 ºC for 180 days, the changes observed were insignificant which indicates good physical stability of the NLCs.

CONCLUSION: In the present study, the CP loaded NLCs drug delivery system was successfully prepared by using compritol 888 ATO and oleic acid as solid and liquid lipid respectively by using melt emulsification and ultra-sonication method. The important parameters like a drug: lipid ratio, surfactant concentration, and homogenization time were optimized by 3^3 full factorial design to obtain a minimal PS, Narrow PDI, and highest % EE. Thus, desirable goals could be achieved by systematic formulation approach the shortest possible time with the reduced number of experiments. The maximum zeta potential of -32.26 ± 0.57 mV was attributed to the anionic nature of the lipid matrix and indicated, physical stability of the CP NLCs.

DSC studies confirmed the absence of any interaction between CP and lipids. TEM imaging of CP loaded NLCs exhibited the spherical shape of NLCs. Drug release from NLCs dispersion and CP suspension showed a sustained release of the drug over a prolonged period as compared to free drug. Drug release from NLCs based suspension followed the Higuchi model. Stability studies indicate a significant change in PS when stored at room temperature.

ACKNOWLEDGEMENT: The authors are thankful to the principal and management of Krishna Teja Pharmacy College, for providing necessary facilities to carry out this work.

AUTHORS CONTRIBUTIONS: All the authors have contributed equally.

CONFLICT OF INTEREST: Declared none

REFERENCES:

1. Ghate VM, Kodoth AK, Shah A, Vishalakshi B and Lewis SA: Colloidal nanostructured lipid carriers of pentoxifylline produced by microwave irradiation ameliorates imiquimod-induced psoriasis in mice. Colloids Surf B Biointerfaces 2019; 181: 389-99.
2. Krueger J and Bowcock A: Psoriasis pathophysiology: Current concepts of pathogenesis. Ann Dermatol 2005; 64(2): 30-36.
3. Katare OP, Raza K, Singh B and Dogra S: Novel drug delivery systems in topical treatment of psoriasis: Rigors and vigors. Indian J Dermatol VenereolLeprol 2010; 76(6): 612-21.
4. Kumari J: Vitiligo treated with topical clobetasol propionate. Arch Dermatol 1984; 120(5): 631-35.
5. Müller RH, Petersen RD, Hommoss A and Pardeike J: Nanostructured lipid carriers (NLC) in cosmetic dermal products. Adv Drug Del Rev 2007; 59: 522-30.
6. Xin K, Zhao Y, Quan P and Liang F: Development of a topical ointment of betamethasone dipropionate loaded nanostructured lipid carrier. Asian J Pharm 2016; 11(2): 248-54.

7. Qian K, Jia L and Xin YL: Application of quality by design approach to formulate and optimizetripterine loaded in nanostructured lipid carriers for transdermal delivery. Joul of Drug Delivery Sci and Technol 2019; 52: 1032-42.
8. Gaba B, Fazil M, Khan S, Ali A, Baboota S and Ali J: Nanostructured lipid carrier system for topical delivery of terbinafine hydrochloride. Bulletin of Faculty of Pharmacy, Cairo University 2015; 53: 147-59.
9. Ghate VM, Lewis SA, Prabhu P, Dubey A and Patel N: Nanostructured lipid carriers for the topical delivery of tretinoin. Eur J Pharm Biopharm 2016; 108: 253-61.
10. Wang L, Liu Z, Liu D, Liu C, Juan Z and Zhang N: Docetaxel-loaded-lipid-based-nanosuspensions (DTX-LNS): preparation, pharmacokinetics, tissue distribution and antitumor activity. Int J Phar 2011; 413(1-2): 194-01.
11. Das S, Ng WK, Kanaujia P, Kim S and Tan RB: Formulation design, preparation and physicochemical characterizations of solid lipid nanoparticles containing a hydrophobic drug: effects of process variables. Colloids Surf B Biointerfaces 2011; 88(1): 483-9.
12. Patil GB, Patil ND, Deshmukh PK, Patil PO and Bari SB: Nanostructured lipid carriers as a potential vehicle for Carvedilol delivery: Application of factorial design approach. Art Cells Nanomed Biotech 2016; 44(1): 12-19.
13. Gidwani B and Vyas A: Preparation, characterization, and optimization of altretamine-loaded solid lipid nano-particles using Box-Behnken design and response surface methodology. Artif Cells Nanomed Biotechnol 2016; 44(2): 571-80.
14. Lasoń E, Sikora E, Miastkowska M, Escribano E, Garcia-Celma MJ, Solans C, Llinas M and Ogonowski J: NLCs as a potential carrier system for transdermal delivery of forskolin. ActaBiochim Pol 2018; 65(3): 437-42.
15. Khurana S, Bedi PM and Jain NK: Preparation and evaluation of solid lipid nanoparticles based nanogel for dermal delivery of meloxicam. Chem Phys Lipids 2013; 175: 65-72.
16. Fits LV, Mourits S and Voerman JSA: Imiquimod induced psoriasis like skin inflammation in mice is mediated *via* the IL-23/ IL-17 axis. J Immunol 2012; 182(9): 5836-45.
17. Agrawal Y, Petkar KC and Sawant KK: Development, evaluation and clinical studies of Acitretin loaded nanostructured lipid carriers for topical treatment of psoriasis. Int J Pharm 2010; 401: 93-02.
18. Harshad V, Abhinesh K and Sawant K: Development of solid lipid nanoparticles based controlled release system for topical delivery of terbinafine hydrochloride. Eur J Pharm Sci 2013; 49: 311-22.

How to cite this article:
Reddy KR, Satyanarayana SV and Reddy VJ: Factorial design based optimization and *in-vitro – ex-vivo* evaluation of clobetasol loaded nano structured lipid carriers. Int J Pharm Sci & Res 2019; 10(9): 4374-83. doi: 10.13040/IJPSR.0975-8232.10(9).4374-83.