Research Article

FORMULATION AND EVALUATION OF NANO PARTICULATE DRUG DELIVERY SYSTEM FOR AN EFFECTIVE TREATMENT OF ACNE.

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ABSTRACT

The aim of the present study was to formulate and develop Nanoparticulate drug delivery system (Nanostructured Lipid Carrier) for the treatment of Acne. The nanostructured lipid carrier was prepared by high speed and high pressure homogenizer by using different types of solid lipid, liquid lipid and concentration of surfactants. The NLC was characterized by particle size, zeta potential, polydispersity index. Stearic acid and oleic acid were used as solid lipid and liquid lipid and Pluronic F-68 was used as surfactant. The drug excipient compatibility study was performed by Fourier transform infrared spectrophotometer and the result revealed that all the excipients and drugs were compatible with each other. For the drug estimation HPLC method was developed for the combined drug Clindamycin phosphate and Nicotinamide. Formulation of nanoemulsion was prepared by high speed and high pressure homogenization technique. This emulsion was incorporated into the gel which was prepared by using Carbopol 934P as gel base and evaluated for the *In-vitro* release. Factorial design was used for the evaluation and optimization of the nanoparticles. Short term stability study was performed for 30 days and there was no significant change in consistency, pH and viscosity.

Keywords: NLC (Nanostructured Lipid Carriers), High pressure homogenizer, Clindamycin Phosphate, Nicotinamide.

INTRODUCTION

Acne is one type of skin diseases which in turn leads to abnormalities in sebum production and sebaceous follicles inflammation followed by the formation of microcomedo. It evolves blackheads, whiteheads and pimples on body parts. The etiology of Acne as dermal disease is still unknown. It has multifactorial pathogenesis condition; polysebaceous canal may be infected by the *Propioni-bacterium acne* and other chemical factors and may be due to the hormonal changes. On an average 85% of the populations are suffering from Acne in which the sufferers are mostly teenagers and adults aged 25-34. Topical and systemic drug therapies are available for the treatment ofacne [4,5]. Acne can destroy the structure of parent tissue and get inflammation which may genetically encoded [6]. For the treatment of acne many antibiotics and antifungal drugs can be used like vitamins, benzoyl peroxide, retinoids, tetracyclines, Niacin, Nicotinamide, benzoyl peroxide, Clindamycin and other antibiotics as topical therapy in form of gel, lotion and cream. Oral antibiotics, tretinoin, hormonal therapy can be included in systemic therapy. Symptoms of the acne can be identified by the comedones, papules and pimples.Nanoparticles are the colloidal drug carriers which are made up from phospholipid with particle size ranging from 10 nm to1000 nm [11]. Basically, it contains encapsulated drug in the lipid being stabilized by surfactant.Nanoparticles provide control release, Lipidicnanoparticles increase the elasticity of skin, Improve the permeation to the skin, Prolong activity of drug can be achieve, Excellence skin tolerability, Increase the residence time of actives on skin, Single drug therapy cause the problem of resistant strain of Propionibacterium Acne. Thus, combine drug therapy is preferred. Nano structured lipid carrier is one type of Nanosystem. NLC can be formed by using solid lipids and liquid lipids.[16,17]Matrix is formed by the application of lipids.Solid lipid and liquid lipids are mixed in different ratio. In formulation of SLN solid lipid and surfactant was used where in case of the nanostructured lipid carrier solid lipid, liquid lipid and surfactant was used. Due to solid lipid and liquid lipid matrix structure chances of drug leaching and degradation of drug decreases. Taking NLC problems into consideration NLC was developed. They are the 2nd generation nanoparticle drug delivery system [18] and having numerous advantages such as Higher drug loading capacity as compare to SLN, Organic solvents can be avoid by using water base, Lessexpensive than other polymeric and surfactant base NLC, Higher Entrapment efficiency, Leaching of drug is less so prolong action can be achieve, Increase the patient compliance by reducing the dosing frequency, Enhanced bioavailability etc.Clindamycin phosphate is an antibacterial as well as antiseptic drug. It inhibits the protein synthesis by binding with bacterial species. Nicotinamide is the derivative acid of niacin and nicotinic acid, act as a CYP450 inhibitorsand functioning as a component of coenzyme NAD[30]and skin tonic. Combine therapy of drug gives better efficacy and higher drug effectiveness.

Materials and methods

Clindamycin phosphate and Nicotinamide was obtained from Vinayak Pharmaceuticals, Gujarat; Stearic acid from SD fine chem. Pvt. Ltd, Mumbai; Oleic acid from Krishna chemical industry, Baroda; Pluronic F-68 from Loba cheimi. Pvt. Ltd., Mumbai; Carbopol 934P from Chemdyes, Mumbai.

PREFORMULATION STUDY

Characterization of Drug (Clindamycin phosphate and Nicotinamide):

Characterization of drug: colour, odour and appearance were observed visually [55].

Melting Point Determination

Melting point was carried out by open capillary method. Powdered drug of Clindamycin and Nicotinamide was filled into sealed capillary and dipped into paraffin bath. Thermometer was attached with capillary and temperature was noted down. The melting point was observed nearby 113-135°C and 130-132°C respectively.

Solubility of Drug

10 mg drug was taken in a test tube and solvent added drop by drop. So, the quantity required for the soluble all the drug material of the amount of solvent, according to that volume solubility was checked in pharmacopoeia and observed that both drugs were very soluble in water, ethanol, acetone but slightly soluble in Chloroform [56].

Drug-Excipient compatibity study

Drug-drug compatibility and drug-excipients compatibilities of the drug and excipients is an important parameter of the formulation. Spectra of Clindamycin phosphate and Nicotinamide, were taken with all ingredients individually and in combination. Then physical mixture was taken for both drug combination with other formulation combinations and then their pellets were prepared by using potassium bromide in disc method. The pallet was prepared by compressing the mixture using potassium bromide press from 8 to 10 Kpa. Prepared sample charged into the sample disc of FTIR and was analyzed at transmission mode in the region of 4000-5000 cm^{-1}[57].

Table: 1.1IR bands of Clindamycin phosphate and Nicotinamide

Functional group	Observed peak value
NH (Amino)	3366.06
OH (hydroxyl)	3165.33
-CONH	1680.15
-CH-	2785.51
P=O	1255.75
NH(Nicotinamide)	3366.06
$CONH_2$	1680.15
C=C	1592.79
Aromatic ring	3165.33

METHOD DEVELOPMENT ON HPLC

Selection of wavelength

On the basis of literature survey wavelength was selected i.e. 211nm.

Preparation of Mobile Phase

70 ml of acetonitrile and 30 ml of water taken and mixed. Mobile phase was filtered through 0.45µ filter and sonication was done by bath sonication for 20 minutes.

Preparation of Stock Solution

10 mg of clindamycin phosphate and 40 mg of Nicotinamide was taken in 10 ml volumetric flask (1000 µg/ml). 1 ml of stock solution was withdrawn and diluted upto 10 ml (100 µg/ml). This solution was considered as final stock solution. Mobile phase was used as diluent.

DEVELOPMENT OF DOSAGE FORMS

As shown in Figure: 1, Solid lipid and liquid lipid was mixed in a beaker and in another beaker, water and surfactant was mixed. Heat both the beaker at temperature above the melting point of solid lipid. Both the phases were mixed at the same temperature and homogenized at optimal pressure and cycles.Prepared NLCs were incorporated into the Carbopol-934P aqueous dispersion (1%w/v).The carbopol 934P was overnight soaked with water and the triethanolamine was added dropwise to adjust the pH of the Carbopol 934P. Gelled NLC was evaluated for viscosity, spreadability and transparency (visually).

OPTIMIZATION

Formulation optimization by 3^2 Factorial Design

By using trial and error technique, different NLC batches were prepared and from the data it was observed that both ratio of SL to LL and surfactant concentration significantly altering the parameters like particle size, Size distribution and Entrapment efficiency. Design expert software (trail version 7.0.0) was used for the determination of influence of the factors. To study the possible combinations of factors, three factors and two level full factorial design was constructed in a fully randomized order. For the optimization, two independent variables, ratio of solid lipid to liquid lipid (X1= 1.5, 2.33, 4.0) and concentration of surfactant (X2= 0.5, 1,1.5) at three different levels. High and low levels of variables

were coded as 1 and -1 respectively. Zero was taken as mean value. There were three dependent variables as particle size, polydispersity index and entrapment efficiency. The result was subjected to ANOVA and regression analysis that lead to effect of dependent variables on independent variables on the selected responses [60]. Optimization of the Nanostructured lipid carriers of Clindamycin Phosphate and Nicotinamide where three independent variables: particle size (X1), PDI (X2) and entrapment efficiency(X3) were selected on the basis of its preliminary experiments and suitable model of polynomial were selected according its significant value. multiple correlation coefficient like R^2 and adjusted multiple correlation coefficient like adjusted R^2 that provided by software i.e. Design-Expert® This model was design by the selection of the upper and lower value. A design shown total 10 models of Clindamycin phosphate and Nicotinamide were randomly arranged then selected ratio of solid lipid to liquid lipid and concentration of surfactant then Particle size, PDI and entrapment efficiency (%) were studied.

Table: 1.2System Suitability Data

No. of runs	Retention time (min)		Theoretical plates		Tailing factor		Resolution
	Clin.P.	Nico.	Clin.P.	Nico.	Clin.P.	Nico.	
1	2.733	5.472	38858	21510	1.251	1.564	10.576
2	2.770	5.480	38051	21011	1.239	1.563	10.634
3	2.757	5.474	38525	21061	1.236	1.581	10.652
4	2.755	5.442	38213	21844	1.238	1.573	10.623
5	2.767	5.459	38384	21234	1.239	1.568	10.414
6	2.754	5.476	38448	21112	1.240	1.582	10.490
% RSD	0.012	0.014	-	-	-	-	-
Limit	< 2%		>2000		<2		>2

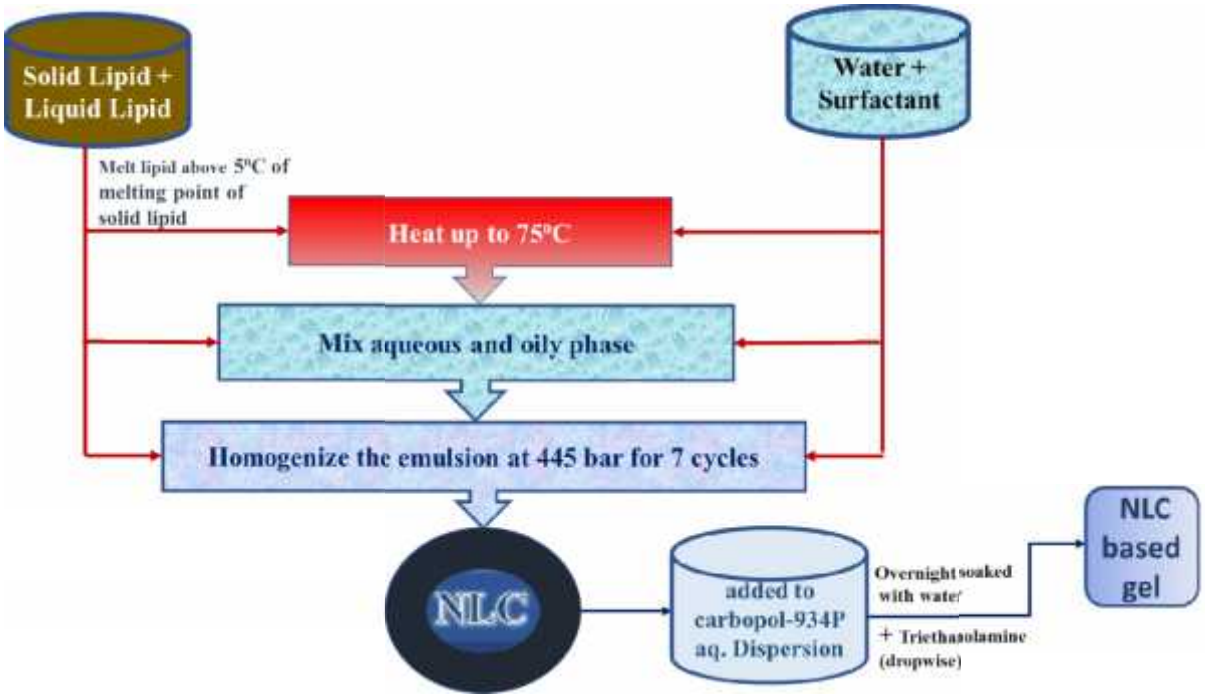

Figure: 1 Method of preparation of NLC based gel

Check Point Batch

As the optimization was done, 3 check point batches were prepared and evaluated. The better combination in terms of closeness between the predicted and obtained value was treated as optimized batch and was carried forward to process optimization.

Process optimization by 3^2 Factorial Design

Design expert software (trail version 7.0.0) was again also used for the determination of influence of the factors. To study the possible combinations of factors, three factors and two level full factorial design was constructed in a fully randomized order. For the optimization, two independent variables, HPH pressure (X1= 300,500 & 700) and no of cycles (X2= 3, 6 & 9) at three different levels. High and low levels of variables were coded as 1 and -1 respectively. Zero was taken as mean value. There were three dependent variables as particle size, polydispersity index and entrapment efficiency. The

result was subjected to ANOVA and regression analysis that lead to effect of dependent variables on independent variables on the selected responses [60].

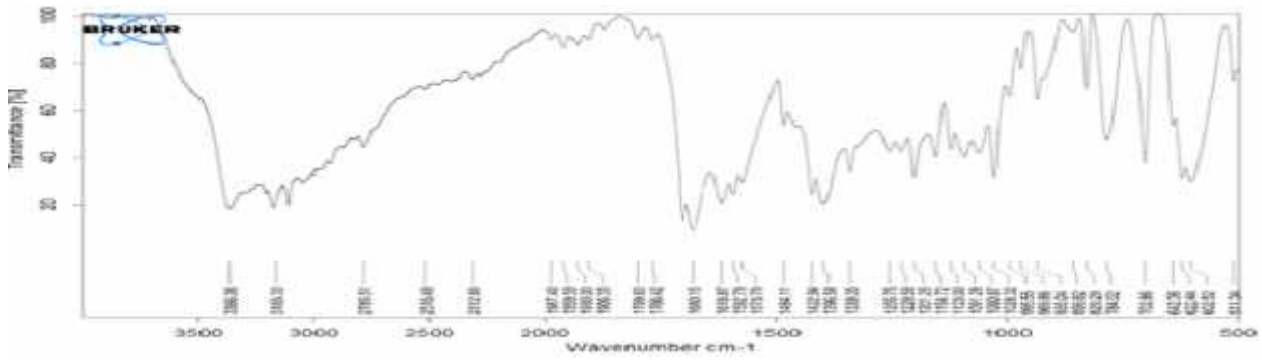

Figure:2.1 Drug-Excipients compatibility

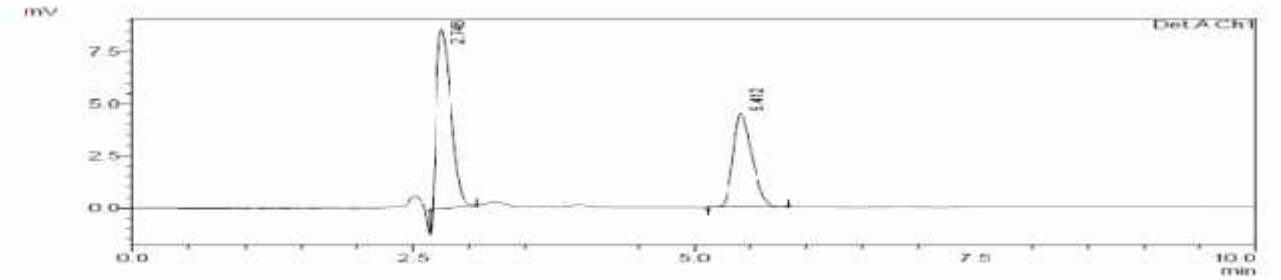

Figure:2.2 Buffer (pH-3.5): Methanol

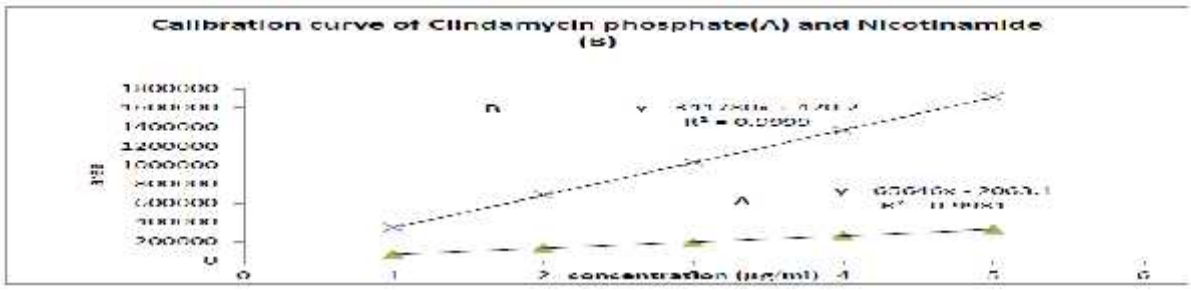

Figure: 2.3 Calibration curve of drugs

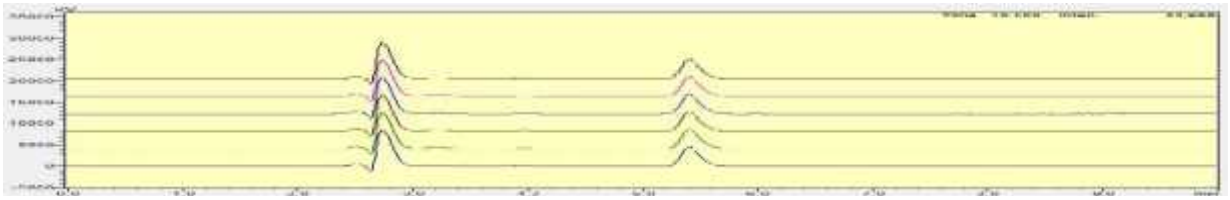

Figure: 2.4 System suitability

EVALUATION OF NANOSTRUCTURED LIPID CARRIERS

Particle Size, PdI and Zeta potential

Particle size and PDI are the most effective parameters in the Nanoparticulate drug delivery systems which directly affected on stability, entrapment efficiency and release rate. Particle size and PDI was determined by Malvern Zetasizer nano ZS. Zeta potential was measured to ascertain the stability of the formulation.

Entrapment Efficiency

Entrapment drug in NLC has prime influence on release profile. The drug containing NLC (in dispersion form) was dried using suitable cryo-protectant in lyophilizer. The dry NLC was dispersed in water and filtered to remove the un-entrapped drug. The NLC was then further dispersed in water and centrifuged for 30 min at 10000 rpm to break the lipidic barrier so that the entrapped drug released from the NLC. Then supernatant was discarded (lipidic material) and then the supernatant

was quantified for entrapped drug content by HPLC at 211 nm. The entrapment efficiency was determined by following equations:

$\% EE = (W_{initial\ drug} - W_{free\ drug}) / W_{initial\ drug} * 100$

pH of formulation

Digital pH meter was used for the determination of pH of optimized formulation.

Transparency of gel

Transparency of nanostructured lipid carriers based gel was observed visually.

Spreadability

Spreadability was determined by the method describe in literature. It contains two glass slides in which 1 gm of formulation was put between the slides. 50gm of weight was put on the slide and till constant and uniform thickness achieved, the diameter was measured along with the time. The spreadability was calculated by the following formula:

$S = (m \times l)/t$

where, S = Spreadability

m = Weight tied to the upper slides

l = length of glass slide and

t = Time in seconds.

Table:2.1.1 Formulation optimization by factorial design

Sr no.	Solid lipid: Liquid Lipid (Factor 1)	Surfactant concentration (Factor 2)	Particle size (nm)	PDI	Entrapment Efficiency (%)
1	1.50	0.5	295.5	0.38	38
2	2.75	0.5	462.5	0.51	42
3	4	0.5	516.4	0.59	58
4	1.50	1	236.8	0.36	47
5	2.75	1	376.8	0.46	54
6	4	1	412.4	0.48	62
7	1.50	1.5	311.8	0.51	50.13
8	2.75	1.5	426.2	0.55	57.17
9	4	1.5	407.5.	0.56	67

Table: 2.1.2Analysis of Variance of particle size of NLC

Source	Sum of Squares	D.f.	Mean Squares	F- Value	P-Value	
Model	61451.27	1	12290.25	272.02	< 0.0001	Significant
SL:LL	40376.81	1	40376.81	893.65	< 0.0001	
Sur. conc.	2756.33	1	2756.33	61.01	0.0015	
Residual	180.73	1	45.18			
Core total	61632.00	1				

Table: 2.1.3Check Point Batch

SL:LL	Surf conc (%)	Particle size (nm)		PdI		Entrapment efficiency (%)	
		Predicted value	Observed value	Predicted value	Observed value	Predicted value	Observed value
1.64	0.95	258.83	249.8	0.374	0.318	44.5 %	43.2%
1.60	**1.10**	**257.74**	**252.3**	**0.40**	**0.387**	**46.57%**	**46.14%**
1.64	0.89	260.43	258.7	0.36	0.325	43.64%	42.8%

Drug content

1gm of NLC was diluted suitably using solvent (Phosphate buffer, 3.5: Methanol, 70:30) in 100 ml of volumetric flask, test sample was prepared by filtering the diluted sample through 0.44 µm filter followed by sonication, and drug content was determined by using HPLC at 211 nm.

In- vitro drug diffusion study

In-vitro drug diffusion study was performed by the modified dissolution apparatus. The dialysis membrane soaked up overnight in Phosphate buffer pH-6.4 that has 10,000 D molecular weight. 1gm of NLC was put into dialysis membrane and tied with thread then put into the modified dissolution apparatus that contained the 100 ml of phosphate buffer pH-6.4 at 37±1°C and 50 rpm then at 1hr time interval, samples were withdrawn and drug content was quantified at 211 nm in HPLC.

Release kinetics

To analyse the mechanism of the drug release rate kinetics of the formulation, the dissolution data obtained were fitted into zero order, first order, Higuchi model, Hixson-Crowell and Korsmeyer's equation release models.

Skin Irritation Study

Skin irritation study was done by usinggoat ear skin and analysed it microscopically.

Stability Study of Nanostructured Lipid Carrier:

Stability was carried out as per ICH guideline. At three different temperatures and humidity that in

refrigerator, room temperature and evaluated temperature, 2-8 °C (45% ± 5), 37°C ± 2 (55 % ± 5) and 50°C±2 (75 % ± 5) respectively. Finally, the formulation was analyzed for particle size, PdI, zeta potential and pH.

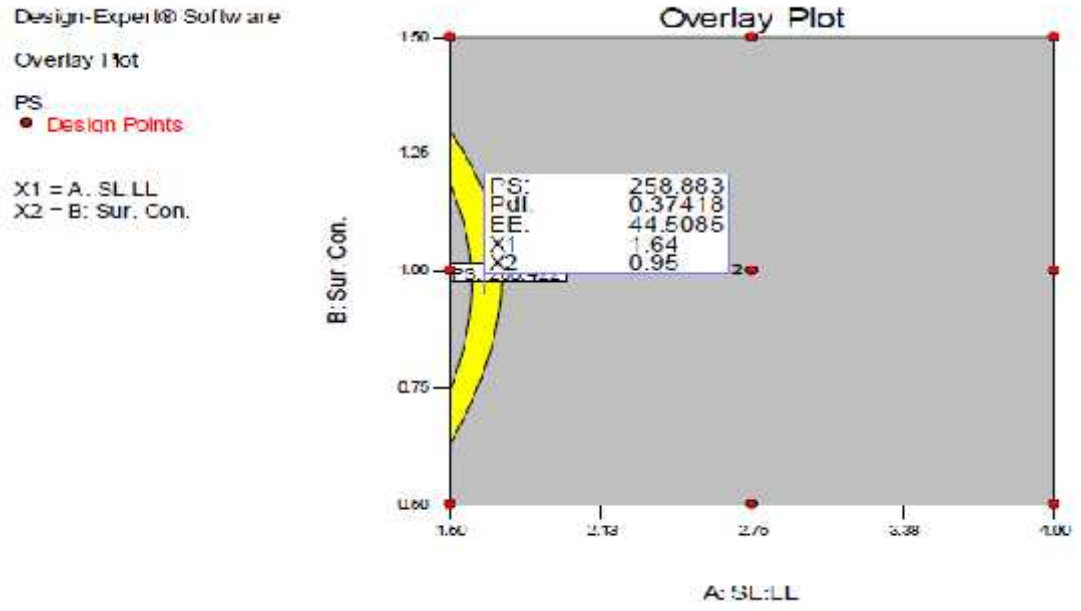

Figure: 3.1(a)Overlay plot of Formulation parameters

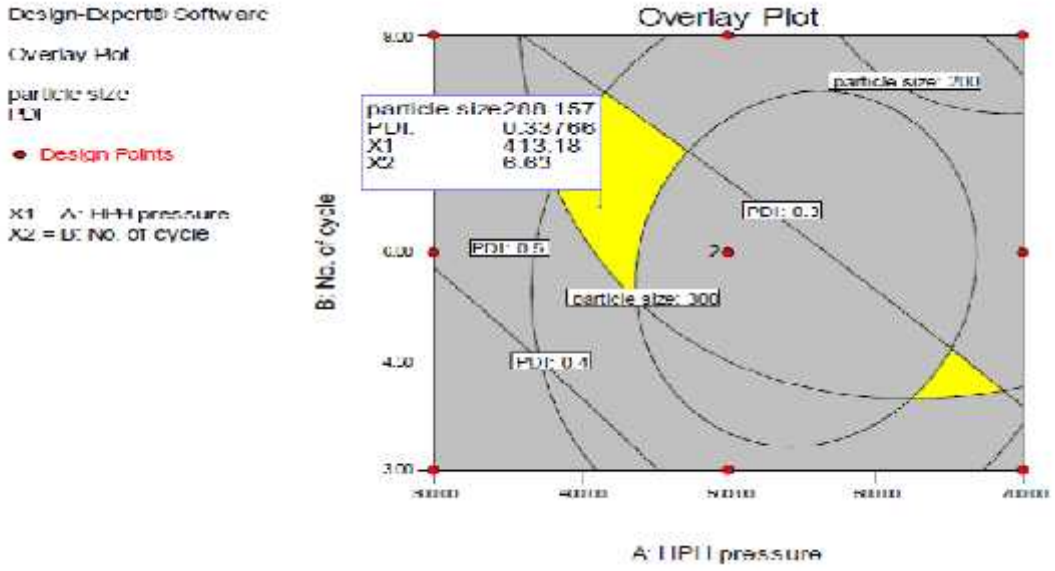

Figure: 3.1(b) Overlay plot of process parameters

Results And Discussion

PREFORMULATION STUDIES

Identification of Drugs

Characterization of Drugs: Clindamycin phosphate and Nicotinamide were observed visually for the characterization of drug. They were found white powder which having no odour.

Melting Point Determination: Melting point of Clindamycin Phosphate and Nicotinamide was found to be 113-115°C and 129-132 °C respectively, which is comply with reported data.

Determination of Partition co-efficient (log P) value: Log P value was determined as a part of preformulation which indicated the water solubility nature of the drugs. log P values were found to be 0.53±0.12 and 0.34±0.2 for Clindamycin phosphate and Nicotinamide respectively.

Solubility Study: Solubility data of Clindamycin Phosphate and Nicotinamide showed that both the drugs were very soluble in water that complying the reported value [56].

Discussion: From the results of melting point, log P value, IR interpretations and solubility of drugs, it was confirmed that the procured drugs were Clindamycin phosphate and Nicotinamide.

Table: 2.2.1 Process optimization by full factorial design

Sr no.	Pressure (bar) (Factor 1)	No of cycles (Factor 2)	Particle size (nm)	PDI
1	300	3	421.6	0.465
2	500	3	352.5	0.37
3	700	3	342.2	0.32
4	300	6	361.7	0.41
5	500	6	263.7	0.32
6	700	6	243.51	0.24
7	300	9	342.57	0.31
8	500	9	223.69	0.29
9	700	9	183.3	0.17

Table:2.2.2Analysis of Variance of particle size of NLC

Source	Sum of Squares	Dilution factor	Mean Squares	F- Value	P-Value	
Model	50153.4	5	10030.68	904.93	< 0.0001	Significant
Pressure	21224.84	1	21224.84	1914.83	< 0.0001	
No. of cycles	22417.59	1	22417.59	2022.43	<0.0001	
Residual	44.34	4	11.08			
Core total	50197.75	9				

Table: 2.2.3 Check batch point

HPH pressure (bar)	No. of cycle	Particle size (nm)		PdI	
		Predicted value	Observed value	Predicted value	Observed value
445	6	263.29	286.9	0.334	0.372
442	7	278.32	301.1	0.303	0.366
445	**7**	**276.84**	**258.8**	**0.321**	**0.374**

SCREENING

Screening of Solid Lipid (from drug solubility data)

From the results obtained, stearic acid was showing comparatively more drug solubilizing capacity (7.5±0.4mg and 5.3±0.4mg for Clindamycin phosphate and Nicotinamiderespectively)over other screened solid lipid and hence was used for the development of proposed formulation (Nanostructured lipid carriers).

Screening of Liquid Lipid

From the results obtained, solubility of drug was highest in oleic acid i.e.,9.2±0.5mg and 10.1±0.26mg for Clindamycin phosphate and Nicotinamide respectively. Hence,Oleic acid was selected as the liquid lipid for the formulation.

Screening of Binary mixture

Binary mixture of solid lipid and liquid lipid is depending on drug solubility in both the lipids. Drug-lipid compatibility also plays an important role in formulation of NLC[24]. As the concentration of the lipid increases, the entrapment efficiency increases too. At the time of formulation, the consistency of lipid should not be changed. Clindamycin phosphate and Nicotinamide having maximum solubility in stearic acid (solid lipid) and oleic acid (liquid lipid). So, these substances were selected for the formulation of NLC. Screening of lipid was done by oil spot method. Ratio so selected was 50:50 to 90:10.

Screening of Surfactants

For the screening of surfactants, HLB value and solubilizing capacity of surfactant are the factors influencing on particle size and PdI of the formulated nanostructured lipid carriers. Here Pluronic F-68 was used as the best surfaceactive agent for these drugs and this formulation based on the particle size and size distribution (PdI). Homogenization parameters set were 500 bar and 7 cycles. With different concentration of surfactants, according to the particle size (178.0) and PdI (0.241), Pluronic F-68 found ascomparatively better surfactant for this formulation.

Compatibility study by FTIR

The compatibility study was performed by FTIR. The obtained results of IR bands of Clindamycin phosphate and Nicotinamide are shown in table 1.1 and Compatibility between drugs and other excipients are shown in figure 2.1.

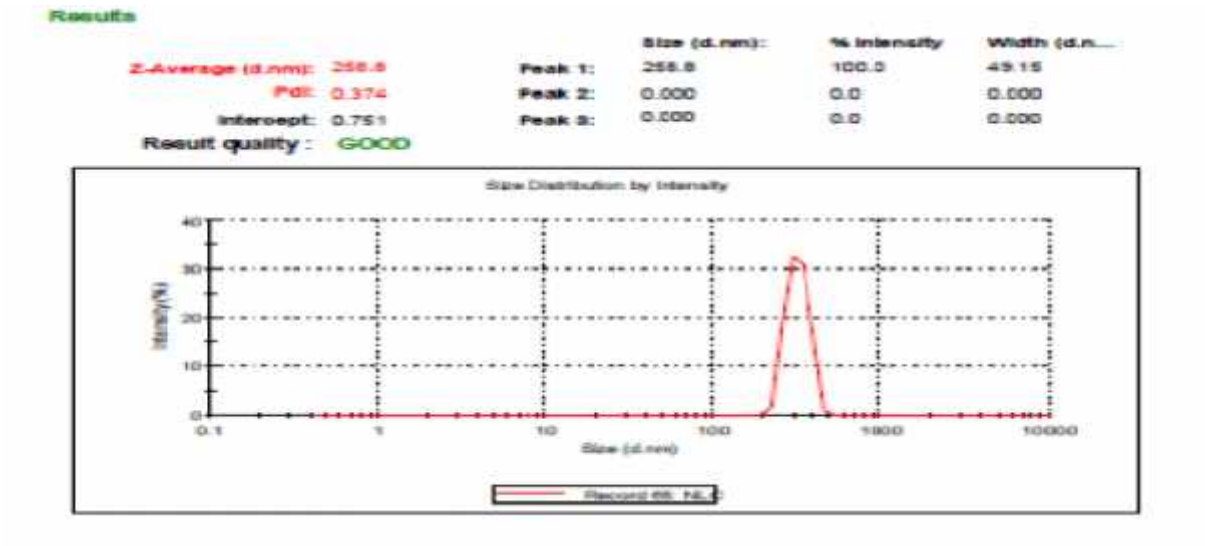

Figure: 3.2 Particle size

Method development on HPLC[61]:

Mobile phase: Buffer (pH-3.5): Methanol (70:30)
Flow rate: 1ml/min
Wave length: 211 nm
As shown in figure 2.2
When the mobile phase ratio was 70:30 both the peaks were resolved and hence were taken as better or optimized ratio for the quantification of the drugs.

Calibration curve of drugs:

The calibration curve was prepared by made a suitable dilutions. The obtained calibration curve of Clindamycin phosphate and Nicotinamide is shown in figure 2.3.
System suitability data:
As per table 1.2 the obtained data of Clindamycin phosphate and Nicotinamide are shown in figure 2.4.
Discussion: Since all the observed data for retention time (min), theoretical plates, tailing factor and resolution were found to be well within the standard limit and hence the developed HPLC method may be said to have system suitable for the quantification of drugs (Clindamycin Phosphate and Nicotinamide).

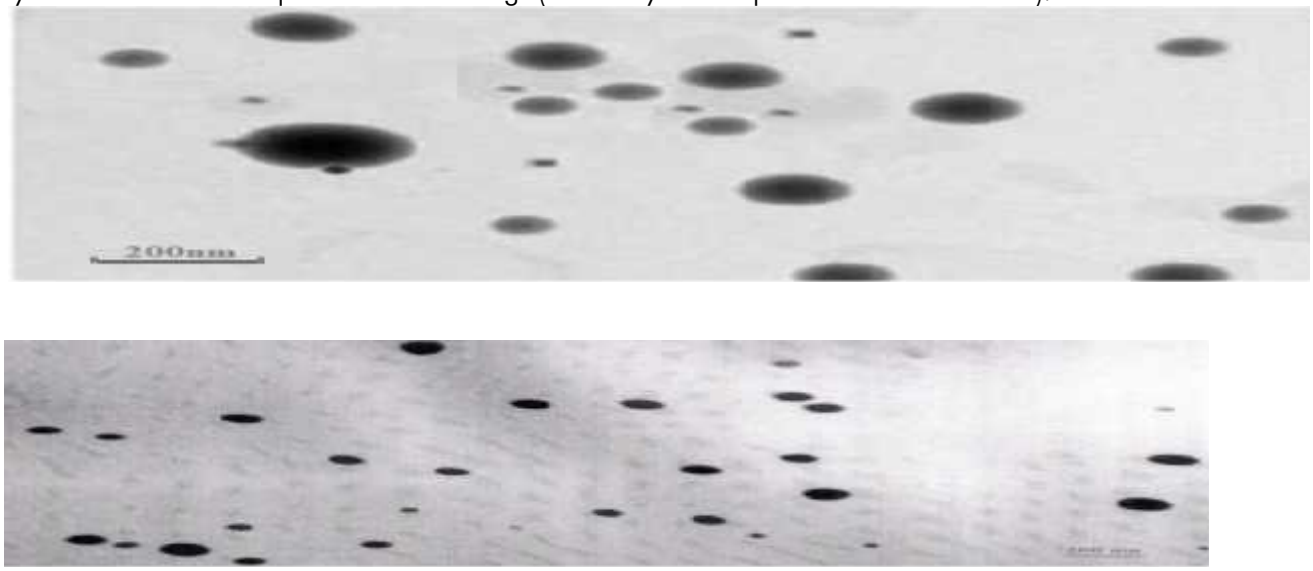

Figure: 3.3 TEM of optimized NLC and Gel

Formulation optimization by 3^2 Full Factorial Design

3^2 Full Factorial Design of design expert (software version 7.0.0) was used for the development of the formulation. As shown in Table 2.1.1 two independent factors like ratio of solid lipid to lipid and conc. of surfactant were taken. The levels for SL: LL were 4.0 (80:20), 2.33 (70:30) and 1.5 (60:40) while the levels for concentration of surfactant were

varied from t was observed that the particle size and % entrapment efficiency was found in ranging of 183.3 to 421.6 nm and 37 to 68% respectively.

Particle size = +72.35276 + 19.53981 × SL:LL - 27.08952 × Surf.Conc. -50.080 × SL:LL × Sur. Con. -17.06057 × $SL:LL^2$ + 19.97143 × $Surf.conc.^2$ ---------- (1).

From the polynomial equation (1), it was found that particle size of NLC was positively influenced by ratio of SL to LL while sur. conc. was indirectly influencing the particle size. This may be due to the fact that when SL:LL increased interfacial tension was increased due to their lipophilic nature and as because surfactant reduced the surface tension and hence particle size was reduced. However, their combined affect was negatively influencing the size.

Check Point Batch:

As shown in table 2.1.3 the check point batch was prepared by using solid lipid: liquid lipid ratio of 2.75 and surfactant concentration was 1 %. According to those parameters, particle size, entrapment efficiency and PDI was found as significant.

Process optimization by 3^2 Full Factorial Design

3^2 Full Factorial Design of design expert (software version 7.0.0) was again used for optimizing the process parameters of NLC formulation. As shown in table 2.2.1 two independent factors homogenization pressure (Bar) and no of cycles per batch were taken and their effect on the particle size and PdI were evaluated. The levels for pressure was set from 300 to 700 while no of cycle was from 3 to 9.

Particle size = +749.63 - 32.117 × HPH pressure - 42.71637 × No. of cycle - 23.0332 × HPH pressure × No. of cycle + 9.06018E-004 × HPH pressure2 + 2.41508 × $No. of cycle^2$-----(2)

From the polynomial equation (2) it was found that both HPH Pressure and numbers of cycle was negatively influencing the particle size due to the fact that increased pressure reduced the size by crushing the particles. However, it was also observed that number of cycle was having more size reduction over homogenization pressure.

EVALUATION OF NANOSTRUCTURED LIPID CARRIERS

Particle Size, PdI

The formulation of NLC was developed by High Pressure Homogenizer using stearic acid and oleic acid as a solid lipid, liquid lipid respectively. NLC showed optimal particle size in nano range and Nano dispersed system. As shown in figure 3.2 the PdI value of NLC was found below 0.374 which is near to zero polydispersity value.

Table: 2.3Results of the characterization of optimized batch

Composition	Particle size (nm)	PdI	Zeta potential (mV)	pH	Spreada-bility	Viscosity (cP)	Transparency
SL:LL (1.6)	258.83	0.374	-19.0	6.4	Good	452.6	Visually Transparent
Surf. (1%)							
HPH pressure (445 Bar)							
No of cycles (7)							

TEM: Transform Electron Microscopy:

From the results of TEM, it was confirmed that the particle size of the formulation was in nano size and having narrow size distribution which was again supported by particle size and PdI data.

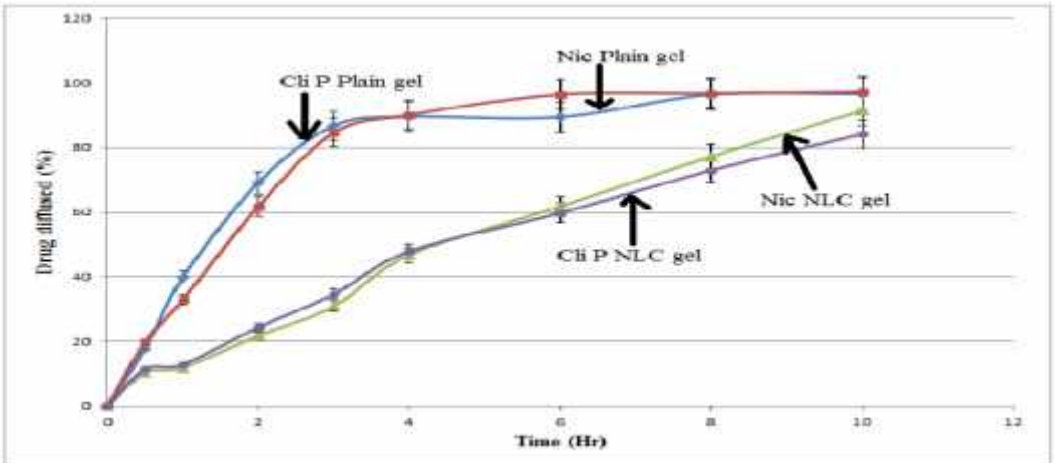

Figure: 3.4Drug release from NLC

Table: 3.1 Dissolution of drugs

Time (hrs)	Drug loaded plain gel		NLC loaded developed gel	
	Clindamycin Phosphate	Nicotinamide	Clindamycin Phosphate	Nicotinamide
0	0	0	0	0
0.5	11.8	19.7 ± 1.12	10.2	11.4 ± 1.56
1	21.7	32.8 ± 1.56	11.8	12.8 ± 1.89
2	46.1	61.7 ± 1.38	21.7	24.3 ± 1.74
3	66.7	84.5 ± 1.46	30.8	34.5 ± 1.67
4	85.5	90.1 ± 2.11	46.7	47.8 ± 2.12
6	89.4	96.4 ± 2.64	61.7	59.7 ± 2.89
8	96.4	96.7 ± 1.89	77.2	72.9 ± 2.56
10	96.6	97.2 ± 3.1	93.4	84.1 ± 2.78

Dissolution study:

The results obtained from Table 3.1, it was found that developed NLC was showing perfectly control of Clindamycin Phosphate and Nicotinamide. However, the plain gel was showing burst release and almost 100% of drugs were released from the gel.

Release kinetic modelling study:

Table: 3.2 Release Kinetic Model

Time (hrs)	Log t	SQRT t	Drug release (%)		Log % Drug release		Cube root of drug release (%)	
			Clin.P.	Nico.	Clin.P.	Nico.	Clin.P.	Nico.
0.5	-0.301	0.70	10.2	11.4	1.00	1.05	3.4	3.8
1	0	1	11.8	12.8	1.07	1.10	3.93	4.26
2	0.301	1.41	21.7	24.3	1.33	1.38	7.23	8.1
3	0.477	1.73	30.8	34.5	1.48	1.53	10.26	11.5
4	0.602	2	46.7	47.8	1.66	1.67	15.56	15.93
6	0.778	2.44	61.7	59.7	1.79	1.77	20.56	19.9
8	0.903	2.8	77.2	72.9	1.88	1.86	25.73	24.3
10	1	3.16	93.4	84.1	1.97	1.92	31.13	28.03

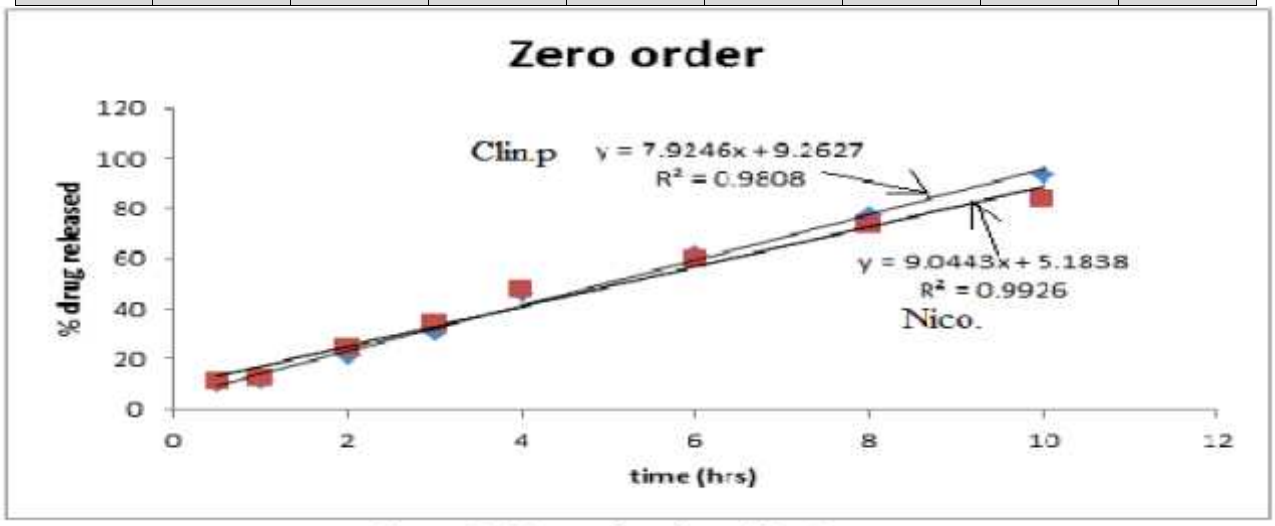

Figure: 3.5 Zero order release kinetic

Discussion: The release kinetics of the developed formulation i.e., Shown in figure 3.4 NLC for both the drugs was showing zero order kinetics and hence it will show perfectly control over the release of the drug.

Skin Irritation Study

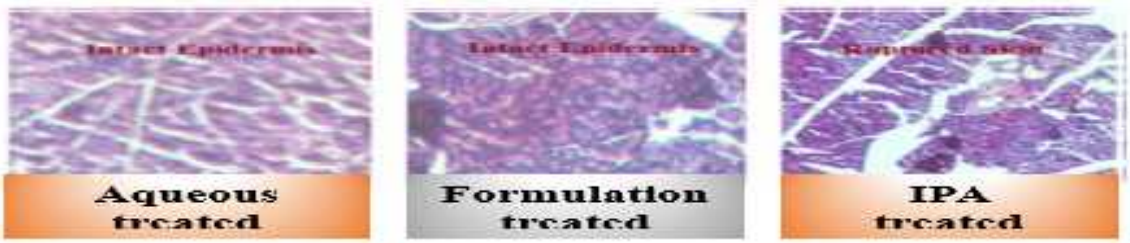

Figure: 4 Skin toxicity study

F

As shown in the figure 4, the developed NLC based gel was not showing any epidermal deformation, so said to be non-toxic to skin and can be used for topical application.

Stability study

Table: 4 Stability of Formulation

Storage Conditions	Particle Size (nm)	PdI	Zeta potential (mV)	pH
2-8 °C (45% ± 5)	266.4	0.359	-19.56	6.7
37°C ± 2 (55 % ± 5)	284.1	0.338	-18.77	6.5
50°C ± 2 (75 % ± 5)	356.8	0.451	-14.56	6.7

From the above result it was confirmed that the developed NLC based gel formulation of Clindamycin phosphate and Nicotinamide was found to be stable at cool temperature and room temperature.

Conclusion

The nanostructured lipid carriers are the colloidal drug carriers rather than other traditional drug carrier systems. They were developed based on industrial requirements like, scale up, low costing and convenience to the patients. Screening of solid lipid, liquid lipid and surfactant helpful to identify most suitable excipients. For the preparation of NLC containing Clindamycin phosphate and Nicotinamide, High pressure homogenization was used. The optimized batch was containing stearic acid and oleic acid as solid lipid and liquid lipid respectively. Investigation of drug was done by using FTIR study. The optimized batch was formulated by Stearic acid and Oleic acid and Pluronic F-68 as solid lipid, liquid lipid and surfactant respectively. The stability study reveals optimized NLC formulation was stable. Thus, the developed nanostructured lipid carrier may provide better prospects for other drug delivery of Clindamycin phosphate and Nicotinamide. However, the developed formulation will be subjected for preclinical as well as clinical study before it to be commercialized.

Acknowledgement

We are very thankful to Parul University for providing infrastructure for conducting research work.

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