EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 5
596-606
Year: 2019

FORMULATION DEVELOPMENT OF NANOSTRUCTURED LIPID CARRIER LOADED EMULGEL OF BUTENAFINE HYDROCHLORIDE

S.B. Gondkar[1], Sonali D. Yelmame[2]* and R. B. Saudagar[3]

[1]Department of Pharmaceutics, R. G. Sapkal College of Pharmacy, Anjaneri, Nashik-422213, Maharashtra, India.
[2]Department of Pharmaceutics, R.G. Sapkal College of Pharmacy, Anjaneri, Nashik-422213, Maharashtra, India.
[3]Department of Pharmaceutical Chemistry, R.G. Sapkal College of Pharmacy, Anjaneri, Nashik-422213, Maharashtra, India.

***Corresponding Author: Dr. Sonali D. Yelmame**
Department of Pharmaceutics, R.G. Sapkal College of Pharmacy, Anjaneri, Nashik-422213, Maharashtra, India.

Article Received on 20/03/2019 **Article Revised on 09/04/2019** **Article Accepted on 30/04/2019**

ABSTRACT
The aim of the study was to prepare and evaluate emulgel incorporating the Nanostructured Lipid Carrier (NLC) of Butenafine HCl for the localized delivery of the active ingredient after topical application. Butenafine HCl has been used as model drugs for the treatment of the fungal infection. Butenafine HCl loaded NLC was designed for topical administration, were prepared by the hot high pressure homogenization technique. This Butenafine loaded NLC was characterized for particle size, zeta potential, entrapment efficiency and SEM. The lipid nanoparticles were then incorporated in gel for convenient topical application and were evaluated for particle size, rheology analysis, texture analysis, *in vitro* drug release studies and *ex vitro* skin permeation studies. The preparation of NLC dispersion with a mean particle size 272.9 nm has been obtained with uniform size distribution (PI <0.518). It was observed that prepared NLC formulation showed that sustained activity for 24hr as compared to the conventional cream which was 12hr. Research work could be concluded as successful development of Butenafine HCl loaded NLC in emulgel formulation.

KEYWORDS: Nanostructured lipid carrier, emulgel, homogenization, topical administration.

INTRODUCTION

- Butenafine HCl is a broad-spectrum antifungal agent of the antimycotic of the allylamines class with activity against a wide range of pathogenic fungi, especially dermatophytes. It act by interfering with the ergosterol biosynthesis by inhibiting the enzymes squalene monooxygenase (squalene 2,3-epoxidase), which ultimately decreased amount of sterols, especially ergosterol, and there is accumulation of squalene which is toxic component for the fungal cell. The drug primarily used as a topical treatment for dermal infections: *teniapedis*, *teniacruris*, *candida albicans* etc.
  The drug has the greater skin-penetration capability can be effectively used for the topical application. For the effective treatment, the drug must be delivered in sufficient concentrationto the site of infection.
- Biodegradable nanoparticles like Nanostructured lipid carrier (NLC), Solid lipid nanoparticle (SLN) are colloidal systems with notable advantage as drug delivery systems, i.e. physicochemical stability, versatility, biocompatibility and controlled release profiles for many substances. Aqueous dispersions of lipid nanoparticles are being used as drug delivery systems for different therapeutic purposes.

Compared with traditional carriers, NLC have small size and relatively narrow size distribution permits, site-specific drug delivery and have high bioavailability hence, a nice targeting effect and are amenable to large scale production. However, due to high crystallization of the solid lipid particles, drugs often released from nanoparticles, thus leading to drug expulsion and low loading capacity. To overcome the limitation of SLN formulation the Nanostructured lipid carriers have been developed. The NLC is generally prepared by mixing solid lipid and liquid lipid together. Stearic acid and oleic acid is used by varying the concentration of the ingredients. The incorporation of oil results in thecrystalline state of the solid lipid. There can be formation of the Nano compartment within the solid matrix. It showed that high oil loads may lead to phase separation.

The objective of the study is to develop topical emulgel containing Butenafine HCl incorporated into NLC. The NLC were prepared by high pressure homogenization technique. Nanoparticles were characterized in terms of

particle size, morphology, encapsulation efficiency. The influence of the NLC on ex-vivo drug skin permeation was compared with conventional formulation[1,2,3,4,5,6,7,8,9,10]

MATERIALS AND METHODS

Materials Butenafine HCl was gifted by Glenmark Pharmaceuticals, Ltd., Nashik, India. Excipients such as Stearic Acid, Oleic Acid and SLS were gifted by Glenmark Pharmaceuticals, Ltd., Nashik, India. All the other chemicals were of the analytical grade. Water was used in double-distilled quality.

Screening Of Components

Loading capacity of the drug is determined by the lipid solubility of drug in melted lipid. 10 mg of Butenafine HCl was dispersed in a mixture of melted lipid (1g) and 1 ml of hot distilled water and shaken for 30 min in a hot water bath.

There was separation of aqueous phase after cooling by ultracentrifugation and then analysed for drug content by spectrophotometric method at 282 nm. Solubility of drug in the lipid phase is the most important factors that determine the loading capacity of the drug in the lipid carrier. The solubility of Butenafine HCl was determined in different liquid lipids and surfactants. An excess of drug was added individually to liquid lipids and surfactants (5 ml each) in screwcapped tubes. After 24 h, each sample was centrifuged and 0.5 ml of the clear supernatant layer was diluted suitably with methanol, and analysed by spectrophotometric method at 282 nm.

Preparation of Nlc

The NLC dispersions were prepared using hot High Pressure Homogenization method (HPH). Table 1, 2 reports the composition of the prepared NLC dispersion. In order to prepare NLC, the lipid phase has been melted at 5-10ºC above the melting point of the solid lipid. At the same time, an aqueous surfactant solution has been prepared and heated at the same temperature. The hot lipid phase was then dispersed in the hot surfactant solution using an homogenizer at 25000, 20000, 15000 rpm for 10 min. The obtained pre-emulsion was homogenized at a temperature 5ºC to 10ºC higher than the melting point of the bulk lipid, using anhomogenizer. The obtained dispersion was cooled in an ice bath in order to solidify the lipid matrix and to form NLC.[1,2,3,4,5,6,7,8,9,10]

LIST OF VARIABLES USED IN THE OPTIMISATION

Table 1: Variables in optimization study.

Variables	Code	Factor
Independent	X1	Stearic acid
	X2	Speed of homogenizer
Dependent	Y1	Entrapment efficiency
	Y2	Antifungal activity

COMPOSITION OF NLC

Table 2: Composition of formulations as per 3^2 full factorial designs.

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Ingredient	%								
Butenafine HCl (w/v)	1	1	1	1	1	1	1	1	1
Stearic acid (w/v)	60	60	60	50	50	50	35	35	35
Oleic acid (v/v)	1.85	1.85	1.85	1.75	1.75	1.75	1.6	1.6	1.6
SLS (w/v)	1.3	1.3	1.3	1.2	1.2	1.2	1.1	1.1	1.1
Water q.s (ml)	100	100	100	100	100	100	100	100	100

Table 3: Composition of Gel.

Sr. no	Ingredients %w/w	Quantity (%)
1	Carbopol 934	1%
2	Triethanolamine	0.1%
3	Water (q. s)	100

EVALUATION OF NANOSTRUCTURED LIPID CARRIERS

Entrapment efficiency

Entrapment efficiency is defined as the percentage amount of drug which is entrapped by the Nanostructured Lipid Carrier. For the determination of entrapment efficiency, the unentrapped drug was first separated by centrifugation at 15000 rpm for 30 minutes. The resulting solution was then separated and supernatant liquid was collected. The collected supernatant was then diluted appropriately and estimated using UV visible spectrophotometer at 282 nm.[10,11,12,13]

$$\% \text{ Entrapment efficiency} = \frac{\text{Amount of drug entrapped}}{\text{Total drug added}} \times 100$$

Particle size, Polydispersity index

Particle size analysis of optimized batch was determine by the (Nano ZS, Malvern, Worcestershire, UK) instrument at 25ºC, Which is based on the Brownian motion. Samples were diluted in particle free purified water to scattering intensity approximately 150300keps. The mean z-average diameter and polydispersity indices were obtain by cumulative analysis using MALVERN software.[11,12,13,14,15,16]

Zeta potential

Zeta potential is key indicator of the stability of formulation. The magnitude of zeta potential indicates the degree of electronic repulsion between adjusts, similarly charge particle in dispersion. Zeta potential of

optimised batch was measured by folded capillary cells using the zetasizer. 1ml sample was taken from formulated nanosuspension and dispersed with 10 ml double distilled water. The samples were ultrasonicated for 5 min prior size determination to measure the primary particle size. Then the sample was taken in disposable cuvette and placed in the instrument for size and zeta potential measurement.[19,20,21,22,23]

Scanning Electron Microscopy

The morphology i.e, shape and surface characteristics of optimized batch of NLC was studied by scanning electron microscopy (SEM) (model JSM 840A, JEOL, Japan). The sputtering was done for nearly 5 minutes to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage of about 25KV with load current of about 80MA.[20,21,22,23,24]

EVALUATION OF EMULGEL FORMULATIONS

pH

The pH of each formulation was determined using digital pH meter previously calibrated by pH 5 and pH 7. The pH values were recorded immediately after preparation.[20,21,22,23,24]

Viscosity

The viscosity of different emulgel formulation was determined at room temperature using a Brookfield viscometer type DV-II + PRO at 10, 20, 30, 40, 50 rpm using spindle (LPV) no. 64.[25,26,27,28,29,30]

Spreadability

One of the criteria for an emulgel to meet the ideal quantities is that it should possess good spreadability. It is term expressed to denote the extent of area to which gel readily spread on application to skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreading value. It is performed by 'slip and drag method' by modified spreading apparatus. It consists of glass slide having dimensions 10×5 cm fixed on tripod stand. An excess of 2gm of emulgel is placed on the fixed slide over which another slide is placed to which a weight is attached by thread. A weight of 500gm is placed over both the slides to expel air for 5 minutes. Then weight is removed over the two slides. Weight of 80gm is attached and time required for slide to travel per marked distance i.e. 7.5 cm was noted. Lesser the time taken for separation of two slides, better the spreadability. It is calculated by using the formula.[25,28,29,30,31,32,35]

$$S = M \times L / T$$

Where, M = weight tied to upper slide

L = Length of glass slides

T =time taken to separate the slides

Drug Content

Emulgel was taken containing 100mg of drug in a volumetric flask & sufficient quantity of methanol was added to dissolve the formulation completely and volume was made up to 100mL with methanol to get a concentration of 1000µg/mL. The absorbance of prepared solution was measured at 282nm by using UV visible spectrophotometer and % drug content was calculated. According to literature, the drug content should be in the range of 94.0% to 98 %.[25,26,27,28,29,30]

***In-vitro* drug release study (Diffusion study)**

Laboratory-assembled apparatus resembling a Franz diffusion cell was used to determine the release profile of drug emulgel. The cell consisted of two chambers, the donor and the receptor compartment between which a diffusion membrane (egg membrane) was mounted. The donor compartment, with inner diameter 24 mm, was open i.e. exposed to the atmosphere at one end and the receptor compartment was such that it permitted sampling. The diffusion medium used was phosphate buffer pH 6.8. 1 mL of the drug containing emulgel was placed in the donor compartment separated from the receptor compartment by the egg membrane. The egg membrane was previously soaked for 24 hr. in phosphate buffer pH 6.8. The donor and receptor compartments were held together using a clamp. The position of the donor compartment was adjusted so that egg membrane just touches the diffusion medium. The whole assembly was fixed on a magnetic stirrer. The receptor compartment with 100 mL phosphate buffer pH 6.8 was placed on a thermostatically controlled magnetic stirrer. It was maintained at 37 ± 0.5°C and stirred constantly at 50 rpm. Samples of 1 mL were collected at predetermined time intervals diluted sufficientlywith methanol to get 10ml and analysed for drug content by UV Spectrophotometer at 282nm against blank. The receptor phase was replenished with an equal volume ofphosphate buffer at each time of sample withdrawal. The release kinetics was also studied for the formulations.[34,35,36,37,38,39,40]

Antifungal activity

An agar diffusion method used for determination the antifungal activity of Formulation. Standard Petri dish 9 cm containing medium to depth of 0.5cm were used. The sterility of the lots was controlled before used. Inoculums were prepared by Suspending 1-2 colonies of Butenafine HCl (NCIM NO.3102) from 24 hr. Cultures in Potato dextrose agar medium in to tube contain 10 ml of sterile saline. The tubes were diluted with saline. The inoculums spread over the surface of agar medium. The plate was dried at 35º C for 15 min prior to placing the formulation. The boars of 0.5 cm diameter were prepaid and 20µl sample of formulation (1% w/v) were added in the bores. After incubation at 35ºC for 24 hr. the zone of inhibition around the boars are measure.[36,37,38]

Optimization study

Optimization of the formulations was studied by 3^2 full factorial designs. The amounts of Stearic acid (X_1) and Speed of homogenizer (X_2) were selected as independent variables and the dependent variables were Entrapment

efficiency (Y1) and Antifungal activity (Y2). The data obtained were treated using Design expert software (version 7.1.6) and analyzed statistically using analysis of variance (ANOVA). The data were also subjected to 3-D response surface methodology to study the effect of Stearic acid and Speed of homogenizer on the dependent variables. I.e. Entrapment efficiency and Antifungal activity.[35,36,37]

Accelerated Stability studies

Test conditions for Accelerated stability studies as per ICH guidelines are shown in the following table. The optimized formulation was used for the Accelerated Stability Studies.

Table 4: Test conditions for stability studies.

Test Conditions	
Duration of study	3 months
Temperature conditions	40°C± 2°C
Relative humidity conditions	75%RH ± 5%RH
Frequency of testing	3 months

The formulations were evaluated mainly for their physical characteristics at 0 and 3 months. Physical appearance such as: clarity, pH, viscosity, drug content were evaluated for optimized batch.[33,34,35]

EVALUATION OF NANOSTRUCTURED LIPID CARRIER

Entrapment Efficiency:

The maximum Entrapment efficiency was found to be 97.5% and the minimum Entrapment efficiency was found to be 70% in figure 1. The effect of Stearic acid on drug entrapment efficiency in NLC was investigated. It has been observed that the drug entrapment efficiency was highest for optimised batch (F1) containing 60:40 (solid lipid: liquid lipid) and 1.3% surfactant at 25000 RPM. It might be due to the incorporation of liquid lipids into solid lipids which have led to massive crystals order disturbance. Greater imperfection in the crystal lattice leaves enough space to accommodate drug molecules, which ultimately improved drug entrapment efficiency.[33,34,35,36,37]

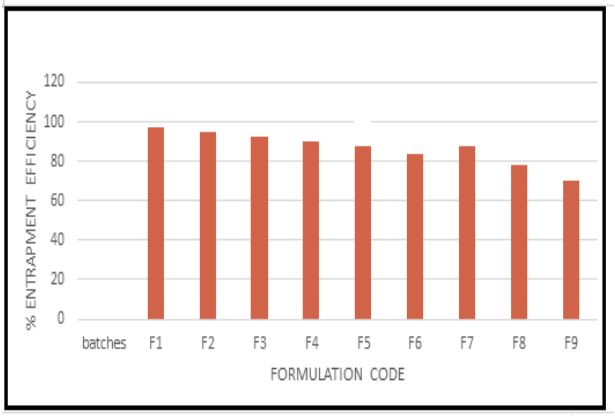

Figure 1: Entrapment efficiency of F1 to F9.

Particle Size and Polydispersibility Index:

The particle size of the optimised batch (F1) is given in Table 5.

Table 5: Size distribution and PDI.

Formulation code	Particle size Z average (nm)	Particle size (nm)	PDI
Optimized Batch (F1)	272.9	Peak 1: 201.3	0.518

The particle size of the Nanostructured Lipid Carrier formulation of optimised batch was found to be 272.9 nm. It is seen with increase in concentration of stearic acid with high speed of homogenizer lead to decrease in particle size.[33,35,37,38]

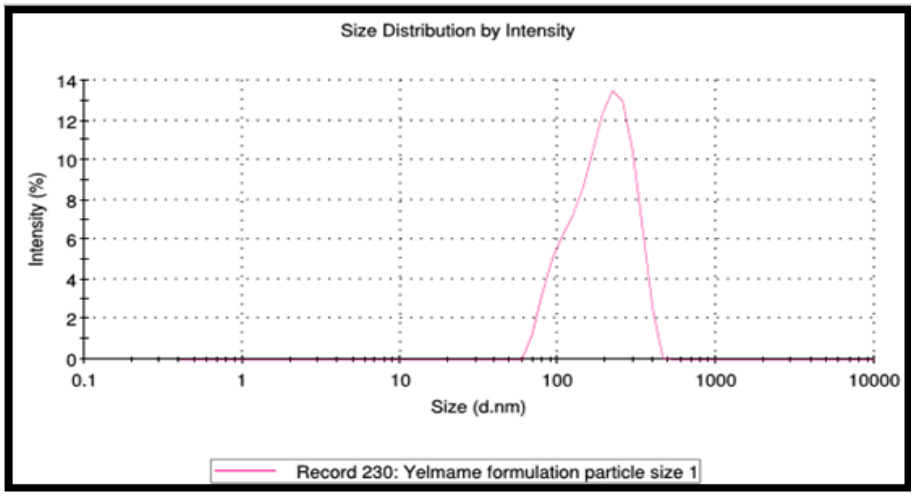

Figure 2: Graph of Particle size.

Zeta Potential

Zeta potential of optimized batch (F1) is given in Table 6:

Table 6: Zeta potential.

Formulation	Zeta potential (mV)	Zeta deviation(mV)
Optimized Batch (F1)	-16.1	3.90

Zeta potential shows the stability of the (colloidal dispersion) nanostructured lipid carrier under the stress testing condition according to ICH guidelines of stability studies of various pharmaceutical formulations. Zeta potential is affected by particle size, lowest particle size in nanosize i.e. 272.9, shows -16.1 mV zeta potential which indicates the thermodynamic instability of the dispersion.[36,37,38]

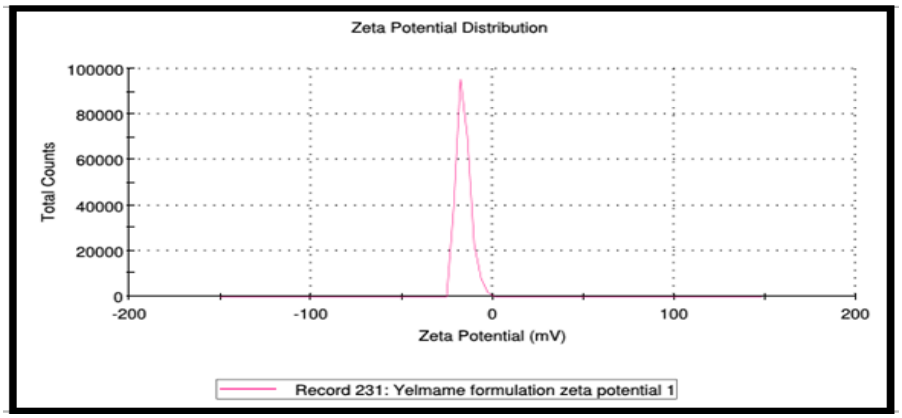

Figure 3: Graph of Zeta potential.

Scanning Electron Microscopy

Scanning electron micrograph of NLC is shown in Figure 4. The shape of the NLC was spherical and the size of the NLC was below micrometer range. Moreover, the micrograph also revealed the some agglomeration of nanoparticle which might be due to the evaporation of water present in formulation during sample preparation prior to SEM analysis.[40,41,42]

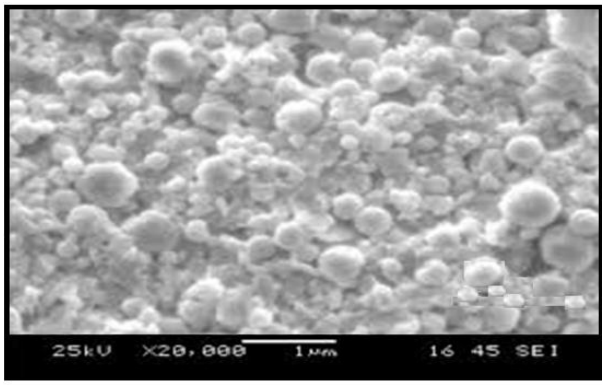

Figure 4: Scanning Electron Microscopy.

EVALUATION OF EMULGEL

The physical appearance of Emulgel was found to be **Translucent Gel**.

Viscosity

The viscosity is resistance to flow which is important physical property for topical preparations because it influences spredability and drug release as well as jellification the rheological behaviour of the emulgel indicate that the systems were shear thinning in nature showing decrease in viscosity at increasing shear rate. This viscosity result reflects that the decrease in proportion of stearic acid and increase in speed of homogeniser results in decrease in viscosity.[39,40,41,42]

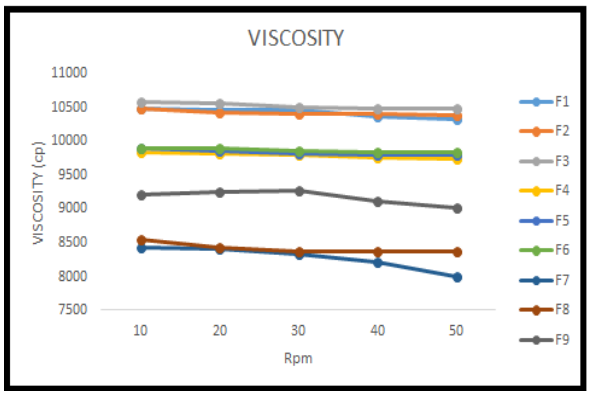

Figure 5: Viscosity results.

Viscosity v/s rpm plots for all formulations shows decrease in viscosity as shear rate (rpm) was increased. Concentration of Stearic acid and Speed of homogenizer was a major factor affecting viscosity of formulations.

Spreadability

Spreadability of emulgel is very important in the topical emulgel formulations. Spreadability shows inverse relationship with the viscosity of the emulgel. Formulation with higher viscosity are very thick in

nature, difficult to spread; on the contrary emulgels having very low viscosity have fluid like appearance, both the extremes are not suitable for any of the topical preparation. Hence gel having optimum viscosity provides proper Spreadability to the formulations.Formulation F1, F4 and F7 having good Spreadability due to maximum concentration of oil present. It was also observed that there was a marginal difference in spredability results amongst the formulations; all the formulations were found to have good spredability.[36,37,40,41]

Table no 7: Spreadability results.

Sr. no.	Formulation code	Spreadability (gm.cm/sec) ± S.D.
1.	F1	**15.80 ± 0.19**
2.	F2	**15.20 ± 0.26**
3.	F3	**15.00 ± 044**
4.	F4	**15.76 ± 0.26**
5.	F5	**15.66 ± 0.43**
6.	F6	**15.50 ± 0.40**
7.	F7	**15.88 ± 0.26**
8.	F8	**15.60 ± 0.43**
9.	F9	**14.10 ± 0.39**

Drug Content

The Drug content of formulations is shown in the following Table 8.

Table 8: Drug content of formulations.

Sr no.	Formulation code	Drug content (%) (±S.D.)
1.	F1	98.60 ± 0.05
2.	F2	97.00 ± 0.25
3.	F3	95.05 ± 0.05
4.	F4	96.00 ± 0.25
5.	F5	97.15 ± 0.50
6.	F6	96.26 ± 0.50
7.	F7	95.00 ± 0.50
8.	F8	97.63 ± 0.37
9.	F9	97.00 ± 0.25

The drug content was carried out to ascertain the concentration of drug in each Proportion was uniform. The percentage drug content of all prepared emulgel Formulations were found to be in the range of 95-99%. Therefore uniformity of content Was maintaining in all formulations. F1 formulations drug content was found to be 98 %.[40,41,42,43]

In-vitro drug release study

The in-vitro release study of formulation is shown in following Figure 6.

***In-vitro* drug release study**

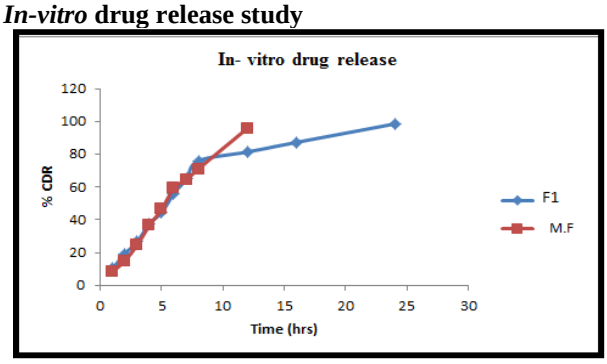

Figure 6: Comparative In- vitro drug release.

It was observed that the release of the drug from optimized (F1) emulgel formulation was higher than the commercial cream. (Fintop 1% Cream). The drug release of optimised formulation shows the controlled release up to 24 hours (98.60%) and marketed formulation shows 96% drug release upto 12 hrs. Formulation F1 showed steady state release up to 24 hours which also indicates that this formulation would show better contact with biological membrane. The drug is entrapped in the lipid phase, hence when formulation was applied on the egg membrane the penetration takes place upto 24 hrs[39,40,41,42]

Antifungal activity

Observed value of (1% Drug suspension) for Butenafine HCl against *Butenafine HCl* (NCIM no. 10231) for zone of inhibition is 14mm. The study indicates that Butenafine HCl retained its antifungal activity when formulated in Nanostructured lipid carrier loaded emulgel and Butenafine HCl was active against selected strain of micro-organism. F1 shows a zone of inhibition of 22mm.[28,29,30,31,32]

STATISTICAL ANALYSIS

Optimization

The experimental design in correlation with optimization study has been outlined in Table 9. X1 and X2 are the amounts of Stearic acid and Speed of homogenizer Respectively, and Y1 Entrapment efficiency and Y2 Antifungal activity respectively. The values of X 1 and X 2 were found to be significant at p ≥0.05, hence it can be Confirmed that both the variables have a significant effect on the selected responses. From this data optimum concentration of Stearic acid was found to be 60% w/v and That for Speed of homogenizer was found to be 25000 rpm.[34,35,36]

Final Equation in Terms of Actual Factors:

Y1 (Entrapment efficiency) = 87.72 – 4.66 *(A) – 7.68 *(B)

Table 9: Experimental design and Optimization study.

Formulation code	Factor X1 (Stearic acid %)	Factor X1 (Speed of Homogenizer rpm)	Response Y1 (Antifungal activity mm)	Response Y2 (% Entrapment efficiency)
F1	60	25000	22.00	97.5
F2	60	20000	21.00	95
F3	60	15000	20.14	92.5
F4	50	25000	19.00	90
F5	50	20000	19.50	87.5
F6	50	15000	18.00	84
F7	35	25000	17.20	87.5
F8	35	20000	15.70	78.5
F9	35	15000	15.50	70

In order to compare the results ANOVA (Design expert version) was used. Stability Data were compared using ANOVA test.

Antifungal activity

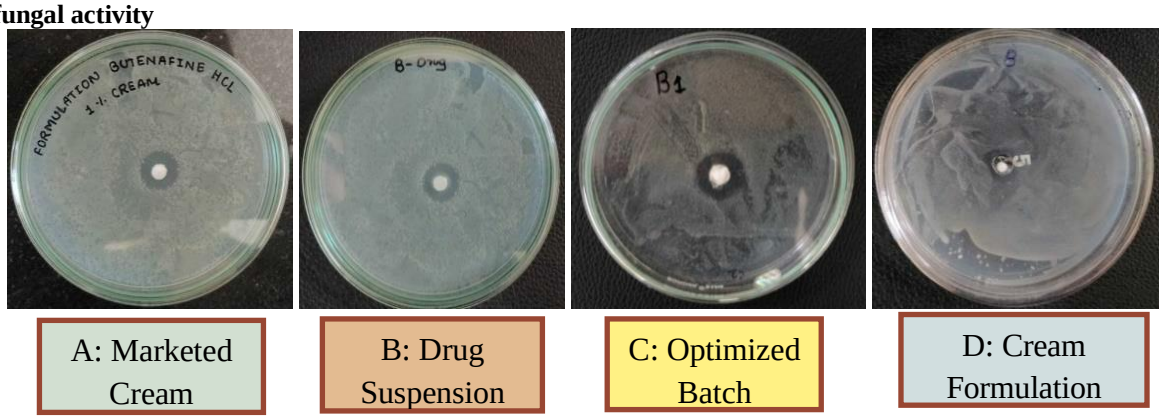

Figure 7: Zone of inhibition of formulation F1toF9, Comparative study with A:Marketed cream, B:Drug suspension, C:Optimized batch (F1), D:Formulated cream.

Table 10: Analysis of variance for % Entrapment efficiency.

Source	F Value	p-value Prob >F	Model significant/non-significant	Standard Deviation	R-squared
Model	53.85	0.0001	Significant	2.12	0.9472
A-Stearic acid	28.99	0.0017			
B-Speed of homogenizer	78.71	0.0001			

The Variance Inflation Factor (VIF) measured the extent to which the variance of the Model coefficient was inflated by the lack of orthogonally in the design and was Calculated for % Entrapment efficiency. It was found to be near to one, this indicated A good estimation of the coefficient. Similarly R-squared was near to zero which led To a good model. The values of Prob ≥ F were less than 0.05, which indicated model Terms were significant. The linear model obtained from the regression analysis was Used to build a 3-D graph in which the responses were represented by curvature Surface as a function of independent variables. The relationship between the responses And independent variables can be directly visualized from the response surface plots.

The response surface plot presented in figure 8 was generated using Design Expert 8.0.4.1 Software. It can be used to observe the effects of independent variables on the Responses studied. From response surface methodology a 3 level factorial design was Chosen using linear design mode. The range was set from a minimum value of 75.32% To a maximum of 97.40% for Entrapment efficiency. The 9 runs performed for the Response % Entrapment efficiency were found to be linear[39,40].

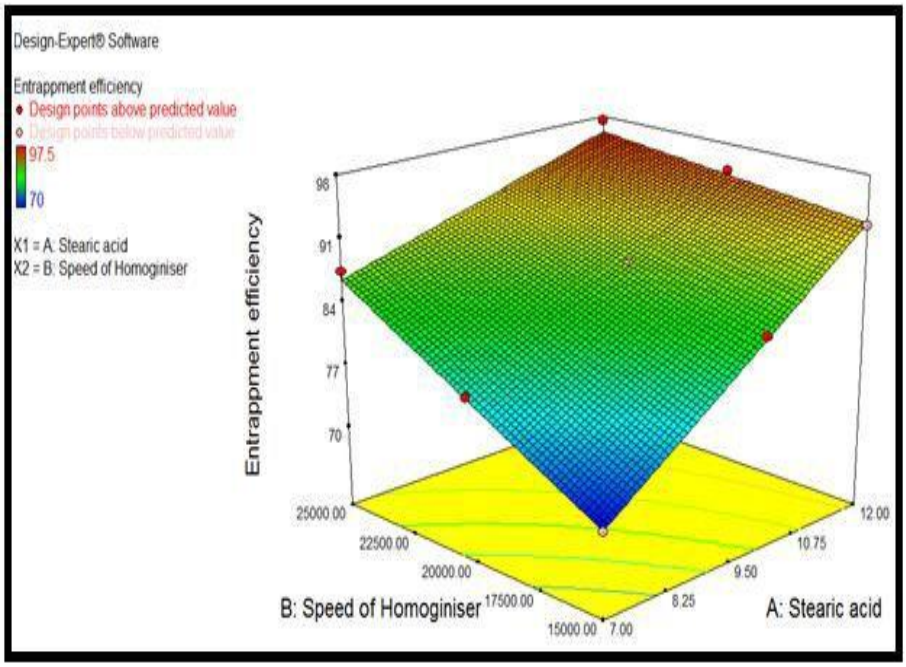

Figure 8: Surface Response Plot showing effect of Stearic acid and Speed of homogenizer on Entrapment efficiency.

The above figure shows the effect of concentration of Stearic acid and Speed of homogenizer on Entrapment efficiency. It is shown that both the independent variables have a significant effect on the dependent variable.

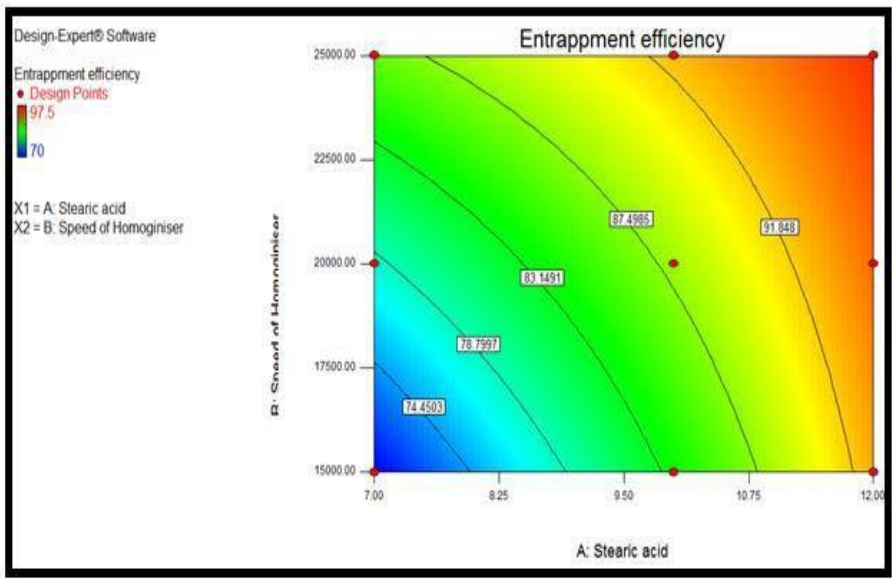

Figure 9: Contour Plot showing Effect of Stearic acid and Speed of homogenizer on Entrapment efficiency.

In the above figure, it can be seen that as the concentration of Stearic acid and Speed of homogenizer increases Entrapment efficiency goon increasing. Hence It can be concluded that the two factors: X1 and X2 have a combined effect on drug release.

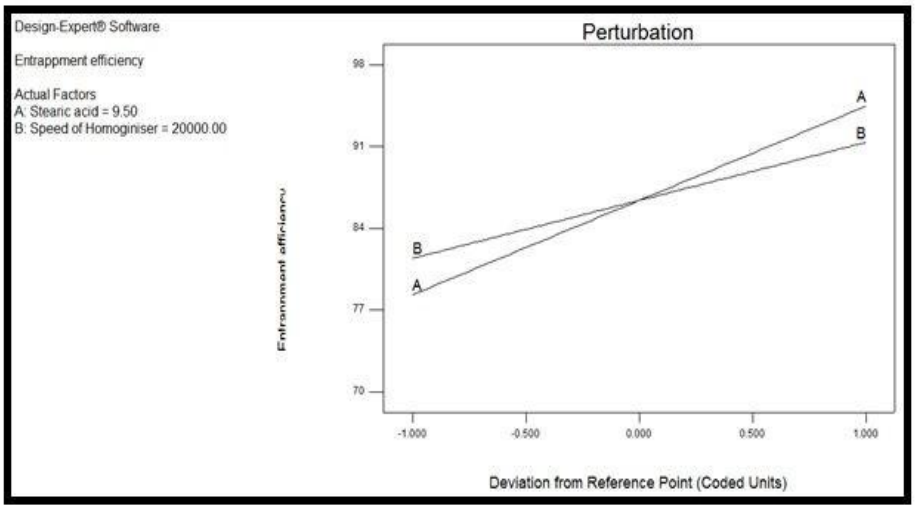

Figure 10: Perturbation Plot showing Effect of Stearic acid and Speed of homogenizer on Entrapment efficiency.

It can be seen from the above figure, that the effect of concentration of Speed of homogenizer on Entrapment efficiency is more pronounced than that of Stearicacid.Table11 shows design summary for Entrapment efficiency and table 12 shows response summary for Entrapment efficiency.

Table 11: Design summary.

Factor	Name	Units	Type	Min	Max	-1 actual	+1 actual	Mean	SD
A	**Stearicacid**	% w/v	Numeric	35	60	35	60	86.94	1.33
B	**Speed of homogenizer**	Rpm	Numeric	15000	25000	15000	25000	86.94	1.33

Table 12: Response summary.

Response	Name	Units	Obs.	Analysis	Minimum
Y1	Entrapment efficiency	%	9	Polynomial	70
Maximum	Mean	SD	Ratio	Trans	Model
97.5	86.94	1.33	29.49	None	Linear

Accelerated Stability studies

The optimized formulation was evaluated after storage at Refrigerated condition and Room temperature. The results of stability studies show t hat the formulation was stable at Accelerated temperature conditions ($40^0C\pm2^0C$, 75%±5%RH) and also at Room temperature conditions (25^0 $C\pm2^0C$, 60% RH±5%RH). Results have been Given in Table 13 and Table 14. A slight increase in pH and viscosity and a slight decreased in drug content were observed however, these were not significant so as to affect the quality and safety of the formulation after storage.[44,45,46]

Table 13: Stability studies data for F1 formulation at Accelerated temperature conditions ($40^0C\pm2^0C$, 75%±5%RH).

Sr. no.	Observation	Before study	During study	
			3rd month	
1.	**Clarity**	Opaque	Opaque	
2.	**Ph**	7.61±0.005	7.32±0.005	
3.	**Viscosity(rpm)**	**10**	10480	10460
		20	10411	10409
		30	10403	10400
		40	10400	10390
		50	10398	10371
4.	**Drug content**	98.33±0.05	98.08±0.15	

Table 14: Stability studies data for F1 formulation at Room temperature (25^0 C±2^0 C, 60% ±5%RH).

Sr. no.	Observation	Before study	During study 3rd month	
1.	**Clarity**	Opaque	Opaque	
2.	**pH**	7.61±0.05	7.50±0.005	
3.	**Viscosity(rpm)**	**10**	10485	10480
		20	10410	10409
		30	10405	10400
		40	10402	10399
		50	10395	10380
4.	**Drug Content**	98.30±0.05	98.10±0.10	

CONCLUSION

NLC's were prepared by high speed homogenization and studied for different parameters. The problems associated with emulsion stability and also lipid stability of nano carriers was also over come by formulating drug loaded NLC emulgel by 3^2 full factorial design in which stearic acid (%) and speed of homogenization (rpm) were taken as independent factors in 3 different levels. before formulating this formulations Preformulation testing were performed for drug characterisation and to analyse its purity and compatibility. organoleptic properties melting point, solubility testing, UV spectroscopy studies and FTIR were performed for the Butenafine hydrochloride and the drug sample procured was found to be pure and compatible with the excipients used in formulation. for optimization design expert software (version 7.1.6) was used. The drug loaded NLC's were evaluated for entrapment efficiency, particle size, Polydispersibility index, zeta potential and scanning electron microscopy analysis. Drug loaded NLC emulgel were evaluated for physical appearance, pH, viscosity, spredability, drug content, *in vitro* drug release study (diffusion study), antifungal activity and Accelerated stability studies.

REFERENCES

1. Anajwala CC, Jani JK, *et. al.* Current Trends of Nanotechnology for Cancer Therapy, International Journal of Pharmaceutical Sciences and Nanotechnology, October- December, 2010; 3(3): 1043-1056.
2. Nikalje AP, Nanotechnology and its Applications in Medicine, Medicinal Chemistry, 2015; 5(2): 81-87.
3. Shlyakhtenko LS, Rychahou PG, *et. al*. Ultrastable Synergistic Tetravalent RNA Nanoparticles for Targeting to Cancers. Nano Today, 2012; 7: 245-257.
4. Lippacher A, Muller RH, *et.al.* Semisolid SLN Dispersions for Topical Application: Influence of Formulation and Production, Parameters on Microstructure, European Journal of Pharmacy, 53(2): 155- 1.
5. Muiller RH, Mehnert W, *et. al*. Solid Lipid Nanoparticles (SLN): An Alternative Colloidal Carrier System for Controlled Drug Delivery, European Journal of Pharmacy and Biopharmaceutics, 1995;l 41: 62-69.
6. Muller RH, Wissing SA, *et. al*. Nanostructured Lipid Matrices for Improved Microencapsulation of Drugs, International Journal of Pharmacy, Volume 242, (2002): 121-128.
7. Mehnert W, Mader K, *et. al*. Solid Lipid Nanoparticles: Production, Characterization and Applications, Advance Drug Delivery, Volume 47, Issue 2-3, (2001):165-96.
8. Patidar A, Thakur DS, *et. al.* A Review on Novel Lipid Based Carrier, International Journal of Pharmacy & Pharmaceutical sciences, Volume 2, (2010): 30-35.
9. Radtke M, Muller RH, *et. al.* Nanostructured Lipid Carriers: The New Generation of Lipid Drug Carriers, New Drugs, Volume 2, (2001): 48–52.
10. Muller RH, Radtke M, *et. al*. Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC) in Cosmetic and Dermatological Preparations, Advance Drug Delivery, Volume 54, Issue 1, (2002):131-55.
11. Muller RH, Radtke M, *et. al.* Nanostructured Lipid Matrices for Improved Microencapsulation of Drugs, International Journal of Pharmacy, Volume 242, Issue 1-2, (2002):121-8.
12. Jenning V, Gohla S, *et. al*. Vitamin A-Loaded Solid Lipid Nanoparticles for Topical use: Drug Release Properties, Journal of Control Release, Volume 66, Issue 2-3, (2000):115-26.
13. ShekhawatPrachi B. Preparation and Evaluation of Clotrimazole Nanostructured Lipid Carrier for Topical Delivery, International Journal of Pharmacy and Biosciences, Volume 4, Issue 1, (2013): 407-416.
14. SanapGajanan, Guruprasad M, Design and Evaluation of Miconazole Nitrate Loaded Nanostructured Lipid Carriers for improving the antifungal therapy, Journal of Applied Pharmaceutical Sciences, Volume 3, Issue 1, (2013): 46-54.
15. PaluvaiVenkatesh, Britoraj S, Design, Optimisation, Formulation and In-vitro, Pharmacokinetic Evaluation of Carvedilol Nanostructured Lipid Carriers in suspension form, International Journal of Pharmacy and Technology, Volume 7, Issue 3, (2015): 9638-9654.
16. LeelapornpisidPimporn, ChansakaowSunee, *et. al.* Development of Cream Containing Nanostructured Lipid Carriers Loaded Marigold Flowers Extract for Anti-wrinkles Application, International Journal of Pharmacy and Pharmaceutical Sciences, Volume 6, Issue 5, (2014): 313-314.
17. Tri Suciati, *et. al.* Development of Jacalin Conjugated Nanostructured Lipid Carriers Formula for Transcutaneous Vaccine Delivery, International Journal of Pharmacy and Pharmaceutical Sciences, Volume 6, Issue 6, (2014): 542-556.

18. Vega E, Egea MA, *et. al.*Flurbiprofen Loaded Biodegradable Nanoparticles for Ophtalmic Administration, Journal of Pharmaceutical Sciences, Volume 95, Issue 11, (2016): 2393-405.
19. Lachman L, Lieberman HA, Kanig JL, Theory and Practice of Industrial Pharmacy, Varghese Publishing House Mumbai, Edition 3, (1991): 502-508.
20. Martin A, Swarbrick J, *et. al*. Physical Pharmacy: Physical, chemical Principles in the Pharmaceutical Science, Varghese Publishing House Mumbai, Edition 3, (1991): 553-572.
21. Banker GJ, Rhodes CT, Modern Pharmaceutics, Marcel Dekker Inc. New York, Edition 4, (2002): 265-266, 121:611.
22. Pawar A, Gaud RS, Modern Dispensing Pharmacy, Career Publication, Nashik, Edition 2, (2008): 187-188.
23. Bhardwaj S, Gupta GD, Sharma VK. Topical Gel: A Novel approach for drug delivery, Journal of Chemical, Biological and Physical Sciences, Volume 2, Issue 2, (2012):856-67.
24. Roychowdhury S, Singh DH, Gupta R, Masih D, A Review on Pharmaceutical Gel, International Journal of Pharmaceutical Research and Bio-Science, Volume 1, Issue 5, (2012): 21-36.
25. Joshi, B, Singh, G, *et.al*. Emulgel: A Comprehensive Review on the Recent Advances in Topical Drug Delivery, International Research Journal of Pharmacy, Volume 2, Issue 11, (2011): 66-70.
26. Joshi M, Formulation and Evaluation of Nanostructured Lipid Carrier Based Gel of Valdecoxib, Drug Development and Industrial Pharmacy, Volume 32, Issue 8, (2006): 911-918.
27. Dai W, Preparation and Characteristics of Oridonin-loaded Nanostructured Lipid Carriers as a Controlled Release Delivery System, Journal of Microencapsulation- Micro and Nano Carriers, Volume 27, Issue: 3, (2010): 234-241.
28. Souto EB, Investigation of the Factors Influencing the Incorporation of Clotrimazole in SLN and NLC Prepared by Hot High Pressure Homogenization, Journal of Microencapsulation- Micro and Nano Carriers, Volume 23, Issue: 4, (2006): 377-388.
29. Mendes AI, Miconazole-loaded Nanostructured Lipid Carriers (NLC) for local Delivery to the oral Mucosa Improving Antifungal Activity Colloids and Surfaces B: Biointerfaces, 2013; 111: 755-763.
30. Rachit K, Saini S, *et. al*. Emulgels: A Surrogate Approach for Topical use Hydrophobic Drugs, International Journal of Pharmacy and Biological Sciences, 2011; 1(3): 117-128.
31. Kalia S, Singh P, *et. al.* Emulgel: A Novel Formulation Approach to Topical Drug Delivery, International Journal of Universal Pharmacy and Bio Sciences, July-August 2014; 3(4): 51-62.
32. Deng L, Michel Poncet, Topical Steroids with Anti-infective activity; Pharmacopeial Forum, 2009; 76-78.
33. Erbagci Z, Topical Therapy for Dermatophytoses: should Corticosteroids be Included, 2007; 37-39.
34. Flynn GL, Cutaneous and Transdermal Delivery: Processes and Systems of Delivery, Drugs and the Pharmaceutical Sciences, Edition, 2002; 4(121): 296-324.
35. Gungor S, Erdal MS, *et. al*. New formulation Strategies in Topical Antifungal Therapy, Journal of Cosmetics, Dermatological Sciences and Applications, 2013; 3: 56-65.
36. International Conference on Harmonization Steering Committee, ICH Harmonized Tripartite Guideline-Stability Testing of New Drug Substances and Products, February 6, (2003); ICH Q1A (R2).
37. The Japanese Pharmacopoeia, Published by PMRJ (Former Society of Japanese Pharmacopoeia), Edition 16, 483-485.
38. Dash S, *et. al.* Kinetic Modelling on Drug Release from Controlled Drug Delivery Systems, ActaPoloniae Pharmaceutics- Drug Research, 2010; 67(3): 21-33.
39. SyamalaUrmilaSri, Development and Optimization of Allyl amine Antifungal Nanoemulgel using 23 factorial designs: for the treatment of tineapedis, European Scientific Journal, December, 2013; 4: 597-605.
40. Salome CA, *et. al.* Kinetics and Mechanisms of Drug Release from Swellable and Non-swellable Matrices: A Review, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2013; 4(2): 97-103.
41. Sharma YR, Elementary Organic Spectroscopy, Principles and Chemical Applications, S. Chand, Multicolour edition: 69-148.
42. Bolten S, Bon C, Pharmaceutical Static Practical and Clinical Applications, Marcel Dekker, Edition 4, 2004: 135; 265-288.
43. https://www.drugs.com/butenafine/pharmacologicaluses/.%20Cited%20on%2020/7/2013 (assessed on 28 July 2016).
44. Rashmi, MS. Topical Gel: A Review, 2008. Available from: http://www.pharmainfo.net/reviews/topic al-gel-review (assessed on 2 August 2016).
45. Singh S, Gajra B, *et. al.* Enhanced Transdermal Delivery of Ketoprofen from Bioadhesive Gels, Available from: http://www.google.com (assessed on 3 December 2016).
46. Sharma S, Topical drug delivery system. [Cited in 2011 Aug 9]. Available from: http://www.pharmainfo.net/Section/science-news/ (assessed on 23 February 2017).