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# Development, evaluation and clinical studies of Acitretin loaded nanostructured lipid carriers for topical treatment of psoriasis

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ABSTRACT

The objective of the present study was to formulate and characterize Acitretin loaded Nanostructured Lipid Carriers (ActNLCs), to understand *in vitro* drug release and clinically evaluate the role of the developed gel in the topical treatment of psoriasis. ActNLCs were prepared by solvent diffusion technique using $3^2$ full factorial design. The mean diameter and surface morphology of ActNLC was evaluated. ActNLCs were lyophilized and crystallinity of NLC was characterized by Differential Scanning Calorimtery (DSC) and powder X-Ray Diffraction (XRD). The NLCs were incorporated in 1% w/w Carbopol 934 P gel base and *in vitro* skin deposition studies in Human Cadaver Skin and double-blind clinical studies in psoriatic patients were conducted. The optimized ActNLCs were spherical in shape, with average particle size of 223(±8.92) nm, zeta potential of −26.4 (±0.86) mV and EE of 63.0(±1.54)%. DSC and XRD data confirmed the formation of NLCs. Significantly higher deposition of Acitretin was found in human cadaver skin from ActNLC gel (81.38 ±1.23%) as compared to Act plain gel (47.28 ± 1.02%). Clinical studies demonstrated significant improvement in therapeutic response and reduction in local side effects with ActNLCs loaded gel indicated its effectiveness in the topical treatment of Psoriasis.



## 1. Introduction

Psoriasis is a skin disease affecting millions of persons worldwide. It is clinically characterized by erythematous, sharply demarcated papules and rounded plaques, and covered by silvery micaceous scale, epidermal hyper proliferation overlying immune-mediated dermal inflammation, leading to profound adverse effects on patient's physical, social and mental well-being (Christophers, 2001). Management of this disease can be done based on the knowledge of its symptoms and major factors affecting the disease. This includes the occurrence, causative factors, behaviour of disease in different individuals, aggravating factors, efficacy of the available drug, availability and cost of different therapies on a long-term basis etc. (Kaur et al., 1997). Various topical therapies are available for the treatment of psoriasis such as calcipotriene, corticosteroids, tar, anthralin phototherapy with ultraviolet B radiation (UVB) and methoxsalen (psoralen) with ultraviolet A radiation (PUVA). The systemic treatment includes drugs like methotrexate, cyclosporine and Acitretin (Krueger et al., 2001). Treatment with topical steroids suppress the body's immune system when used for prolonged period of time, whereas systemic drugs like methotrexate, cyclosporine impair liver and kidney functioning and decrease RBCs, WBCs and Platelets counts.

Nanostructured Lipid Carriers (NLCs) are the second generation of Solid Lipid Nanoparticles (SLN). In contrast to the more or less highly ordered SLN being yielded from solid lipids or blends of solid lipids, the incorporation of liquid lipids to solid lipids leads to massive crystal order disturbance. The resulting matrix shows great imperfections in the crystal lattice and leaves enough space to accommodate drug molecules, leading to improved drug loading capacity, preventing its leakage and giving more flexibility for modulation of drug release (Jenning et al., 2000b,c,d; Jenning and Gohla, 2001; Souto et al., 2004). In recent years, NLCs have been used in topical drug formulations and cosmetic products. Their small particle size ensures close contact to the stratum corneum and the lipid provides selective drug delivery to skin layers (Sarah Küchler et al., 2010; Lombardi et al., 2005; Jenning et al., 2000a; Feste, 1999). NLCs possess a solid matrix, which has the potential to modulate the drug release over a prolonged period with a reduced rate of systemic absorption (Müller and Dingler, 1998). Another benefit of NLCs for topical delivery of active compounds is the short time required to market these products (Müller et al., 2002; Feste, 1999; Müller and Dingler, 1998).

Acitretin (Act) or 13 *cis–trans* retinoic acid, a metabolite of vitamin A, has gained great interest due to its multitude of physiological effects such as regulation of epithelial cell growth and differentiation, sebum production and collagen synthesis (Allen and Bloxham,

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**Table 1**
Formulation of Acitretin loaded NLCs by $3^2$ factorial design: factors, their levels and transformed values, response: MPS and EE.

| Batch No. | Real Value | | Transformed values | | | | | Response | |
|---|---|---|---|---|---|---|---|---|---|
| | Solid lipid (mg) | Liquid lipid (mg) | $X_1$ | $X_2$ | $X_{12}$ | $X_{22}$ | $X_1X_2$ | MPS ± SD* (nm) | EE ± SD* (%) |
| A1 | 50 | 20 | −1 | −1 | 1 | 1 | 1 | 189 ± 11.2 | 36 ± 0.86 |
| A2 | 50 | 30 | −1 | 0 | 1 | 0 | 0 | 169 ± 8.64 | 41 ± 1.23 |
| A3 | 50 | 40 | −1 | 1 | 1 | 1 | −1 | 112 ± 18.7 | 31 ± 1.25 |
| A4 | 70 | 20 | 0 | −1 | 0 | 1 | 0 | 249 ± 10.2 | 57 ± 0.74 |
| A5 | 70 | 30 | 0 | 0 | 0 | 0 | 0 | 223 ± 8.92 | 63 ± 1.54 |
| A6 | 70 | 40 | 0 | 1 | 0 | 1 | 0 | 216 ± 22.31 | 54 ± 1.82 |
| A7 | 90 | 20 | 1 | −1 | 1 | 1 | −1 | 630 ± 12.04 | 51 ± 2.1 |
| A8 | 90 | 30 | 1 | 0 | 1 | 0 | 0 | 561 ± 10.12 | 54 ± 2.13 |
| A9 | 90 | 40 | 1 | 1 | 1 | 1 | 1 | 489 ± 8.24 | 47 ± 1.24 |

* Data represented as mean ± SD ($n = 3$).

1989; Lee and Koo, 2005). Oral Acitretin is currently indicated for the treatment of severe psoriasis in adults, but its use is limited by systemic side effects and teratogenicity. Topical administration of Acitretin may lessen the risk of systemic toxicity while increasing local bioavailability in the skin (Hsia et al., 2008). However, despite these interesting features, its utility is strongly limited by several disadvantages such as skin irritation, very low water solubility and high instability in the presence of air, light and heat. The low solubility of Acitretin (0.0729 mg/L) may limit its incorporation in a suitable vehicle, while its poor photostability may render the topically applied drug ineffective. Furthermore, the topical application of Acitretin often leads to local irritation such as erythema, peeling and burning at the application site and increased susceptibility to sunlight, which often limits its acceptability by patients (Shah and Date, 2007). Thus, Acitretin poses a great challenge to the formulation scientists due to these unique problems. Therefore, the aim of the present work was to explore the potential benefits of NLCs in improving the topical delivery of Acitretin.

## 2. Materials and methods

### 2.1. Materials

Acitretin was obtained as a gift from Glenmark Pharmaceuticals, Nashik, India and Precirol ATO 5 was gifted by Colorcon Asia Pvt. Ltd., Mumbai, India. Oleic Acid and Tween 80 were purchased from S.D. Finechem Ltd., India. Dialysis Bag (Molecular weight cut off 10 kDa) was purchased from Hi-media Pvt. Ltd., Trehalose dehydrate was purchased from Sigma Chemicals Co. Human cadaver skin (forearm region of the healthy bodies) was obtained from All India Institute of Medical Sciences (AIIMS) Hospital, Delhi, India. All other chemicals used were of analytical grade or spectroscopic grade.

*Reagents*: Phosphate buffer (pH 7.4) was prepared as per Indian Pharmacopoeia (1996). Dialysis Medium containing 3% w/v SLS in PBS (7.4) was prepared as per British Pharmacopoeia (2001).

**Table 2**
Response of full model for Acitretin loaded NLCs.

| Response | Mean particle size (MPS) | | % Entrapment efficiency (% EE) | |
|---|---|---|---|---|
| | $X$ coeff | $p$ value | $X$ coeff | $p$ value |
| $X_1$ | 201.6667 | 0.000357* | 7.3333 | 0.000479* |
| $X_2$ | −41.8333 | 0.032179* | −2 | 0.020275* |
| $X_{12}$ | 129 | 0.006638* | −14.6667 | 0.000312* |
| $X_{22}$ | −3.5 | 0.866344 | −6.6667 | 0.003202* |
| $X_1X_2$ | −16 | 0.321613 | 0.25 | 0.676111 |
| Intercept | 231.6667 | 0.001411# | 62.4444 | 4.78E−06# |

* Statistically significant ($p < 0.05$).
# Significance of $F$ value.

### 2.2. Preparation of Acitretin loaded NLC

The NLCs (with or without drug) were prepared by solvent diffusion method in an aqueous system (Hu et al., 2002). In brief, Oleic acid was added to the melted Precirol ATO 5 containing 5% of drug and Tween 80 and was dissolved into a mixture of acetone:tetrahydrofuran (1:1) in water bath at 70 °C. The resultant solution was quickly dispersed into 25 ml distilled water under mechanical agitation using high speed homogenizer, Eurostar (IKA Labortechnik, Germany) at 1000 rpm for 10 min at 70 °C. The obtained pre-emulsion was then cooled under stirring at room temperature, yielding drug loaded NLC dispersion. Blank NLC dispersion was prepared exactly in the same manner as above process without using drug. Nanoparticles were aggregated by adjusting the pH of the NLC dispersion to 1.20 using 0.1 M HCl and centrifuged at 25,000 rpm for 30 min (3K30, Sigma, Germany). The NLC pellet was redispersed in distilled water (1.0% of lipid content w/v) and the resultant dispersion was kept in a deep-freezer (Sanyo Ultra Low Temperature Freezer MDF-192, Japan) at −75 °C for 5 h. The sample was then subjected to freeze-drying (Freezone 2.5 L, LABCONCO, USA) using trehalose as a cryo protectant in the weight ratio of 1:1 with the total solid content (samples).

#### 2.2.1. Experimental design

A $3^2$ factorial design was applied for formulation of Acitretin loaded NLCs to determine the effect of two independent variables, amount of Precirol ATO 5 as a solid lipid and amount of Oleic acid as a liquid lipid on entrapment efficiency (EE) and mean particle size (MPS) (response variables). Each factor was tested at three levels designated as −1, 0 and +1. The values of the factors were transformed to allow easy calculation of coefficient in polynomial equation. Interactive multiple regression analysis and $F$ statistics was utilized to evaluate the response. The regression equation for the two responses were calculated using following equations:

$$\text{Response}: \quad Y_1 \ (\text{MPS}) = b_0 + b_1X_1 + b_2X_2 + b_1{}^2X_1{}^2 + b_2{}^2X_2{}^2 + b_{12}X_1X_2 \tag{1}$$

$$\text{Response}: \quad Y_2 \ (\text{EE}) = b_0 + b_1X_1 + b_2X_2 + b_1{}^2X_1{}^2 + b_2{}^2X_2{}^2 + b_{12}X_1X_2 \tag{2}$$

where, $Y_1$ and $Y_2$ are dependent variables viz. mean particle size (MPS) and entrapment efficiency (EE), respectively, indicating the quantitative effect of the formulation components; while $b_0$ is the intercept and $b_1$, $b_2$ represent the regression coefficients for the second order polynomial equations. Amount of solid lipid ($X_1$) and liquid lipid ($X_2$) represent the level of independent variables. $X_1{}^2$ or $X_{11}$, $X_2{}^2$ or $X_{22}$ and $X_1X_2$ stand for

**Table 3**
Clinical evaluation of plain Acitretin gel and ActNLC gel after 4 weeks.

| Treatment period follow up ($n=6$) | ACI G1 score[a] | ACI G2 score[a] |
|---|---|---|
| Mean psoriasis area and severity index (PASI) score[b] ($n=6$) | | |
| Week 1 | 5.2 ± 0.226 | 5.4 ± 0.353 |
| Week 2 | 4.4 ± 0.108 | 4.0 ± 0.126 |
| Week 3 | 3.9 ± 0.101 | 3.2 ± 0.333 |
| Week 4 | 3.1 ± 0.122 | 2.4 ± 0.319 |

| Patient | Initial score | ACI G1 score | ACI G2 score |
|---|---|---|---|
| Overall lesion severity (OLS) scale[c] | | | |
| 1 | 5 | 5 | 4 |
| 2 | 6 | 5 | 4 |
| 3 | 4 | - | - |
| 4 | 4 | 3 | 2 |
| 5 | 5 | - | 2 |
| 6 | 6 | 5 | 4 |
| Physician assessment (PA) score[d] | | | |
| 1 | 6 | 5 | 4 |
| 2 | 7 | 7 | 5 |
| 3 | 5 | - | - |
| 4 | 5 | 4 | 3 |
| 5 | 6 | - | 4 |
| 6 | 7 | 6 | 4 |
| Psoriasis symptoms assessment (PSA) score[e] | | | |
| 1 | 14 | 12 | 10 |
| 2 | 16 | 14 | 10 |
| 3 | 12 | - | - |
| 4 | 10 | 10 | 8 |
| 5 | 14 | - | 12 |
| 6 | 16 | 12 | 08 |

| Adverse event | ACI G1 score | ACI G2 score |
|---|---|---|
| Safety and tolerability (STA)[f] | | |
| Nausea | 0 | 0 |
| Headache | 1 | 0 |
| Chills | 0 | 0 |
| Hepatotoxicity | 0 | 0 |
| Diarrhea | 0 | 0 |
| Irritation | 3 | 1 |

($n=6$ patients in each group).
3rd patient from both the groups and 5th patient from ACI G1 dropped out.
[a] ACI G1, plain Act gel; ACI G2, NLC loaded Acitretin gel.
[b] PASI: Score was calculated as the sum of the severity of the main symptoms multiplied by the numerical value of the area involved with various % of 3 main body areas ($n=6$).
[c] OLS: Static assessment was done with 6 categories (clear, minimal, mild, moderate, severe, and very severe) based on the characteristics of plaque elevation, scaling and erythema, giving scoring from 1 (clear) to 6 (very severe).
[d] PAS: The categories included were worse, unchanged, slight, fair, good, excellent and cleared, giving score from 1 (cleared) to 7 (worse).
[e] PSA: The PSA is a measure of 8 psoriasis-related cutaneous symptoms (hurt, burning or stinging, itched, bothered by water, irritated, sensitive, skin condition bled, scaling). The PSA contains 2 subscales, one measuring the frequency of the 8 symptoms and the other assessing how troublesome or bothersome psoriasis symptoms are.
[f] STA: Safety and Tolerability Assessment was a measure of 6 adverse events. The study was based on the score from 0 (none) to 6 (very severe).

the quadratic effects of the variables. $X_1X_2$ indicates the interactions between two factors. Multiple regression was applied using Microsoft excel in order to deduce the factors having significant effect on the responses. The variables having $p$ value < 0.05 in the model were considered to have significant effect on the formulations.

### 2.2.2. Contour plots and surface response curves

Contour plots and surface response curves are diagrammatic representation of the values of the response. They are helpful in explaining the relationship between independent and dependent variables. The full models were used to plot both the contour plots and surface response curve using Microsoft excel and STATISTICA® Students' version 1998 at the values of $X_1$ and $X_2$ between −1 and +1 at predetermined values of particle size and EE (Mehta et al., 2007). Full model equation for MPS and EE was developed as per Eqs. (1) and (2), respectively.

## 2.3. Characterization of Acitretin loaded NLCs

### 2.3.1. Mean particle size, polydispersity index and zeta potential

The mean particle size (MPS), polydispersity index (PI) and zeta potential of blank (empty) and ActNLCs were determined by laser light diffractometry using Zetasizer nanoseries, SM 2000K (Malvern Instruments Inc., UK). Samples were prepared by dispersing NLC in sufficient amount of water (pH 7) to achieve obscuration between 10% and 20%.

### 2.3.2. Percent drug entrapment (EE)

The free drug (unentrapped) in the NLCs dispersion was sedimented by controlled centrifugation at 2000 rpm, 4 °C for 30 min using Sigma centrifuge and the NLC dispersion was decanted without disturbing the drug pellet. NLCs dispersion and Act pellet were used for estimation of entrapped and unentrapped drug content, respectively. Fixed volume of NLCs dispersion and Acitretin pellet were dissolved separately in tetrahydrofuran and analyzed for entrapped and unentrapped drug content using UV spectropho-

tometry at 353 nm (Shimadzu 1601, Japan). EE was calculated using Eq. (3).

$$EE = \frac{\text{Entrapped drug}}{\text{Entrapped drug+Free drug}} \times 100 \quad (3)$$

*2.3.3. Surface morphology by TEM*

The morphological observation of Acitretin loaded NLCs was performed to analyze the size, size distribution and shape of the NLC particles by Transmission Electron Microscopy (TEM) (Morgagni 268 D, Fei Co., The Netherlands) after sufficient dilution.

*2.3.4. Differential scanning calorimetry*

DSC studies of pure Acitretin, Precirol ATO 5, physical mixture of Acitretin and Precirol ATO 5 (at a ratio equivalent to that in the formulation) and the lyophilized NLC powder were carried out. Accurately weighed samples were carefully placed in DSC boats and heating curves were recorded in temperature range of 25–250 °C at a heating rate of 10 °C/min under inert atmosphere ($N_2$ 8 ml/min). The study was carried out using Differential Scanning Calorimeter (DSC, Mettler Toledo e822, Japan).

*2.3.5. X-ray diffraction study*

XRD study was performed to analyze crystalline or amorphous nature of the Acitretin loaded NLCs. X-ray powder diffraction studies of pure Acitretin, Precirol ATO 5, physical mixture of Acitretin and Precirol ATO 5 (at a ratio equivalent to that in the formulation) and lyophilized NLC were carried out using Bruker AXS D8 Advance X-ray Diffractometer from 2° to 80° on $2\theta$ scales.

*2.4. In vitro release study*

The *in vitro* drug release profile of the ActNLCs was determined by dispersing 50 mg of the NLCs in 25 ml of phosphate buffer saline (PBS – pH 7.4) containing 3% w/v SLS and stirring on magnetic stirrer at 100 rpm at 37 ± 1 °C. One milliliter of the dispersion was withdrawn from the medium and replaced with the same amount of medium at definite time intervals. It was filtered using 0.22 μm filter (Millipore, USA) and drug release was analyzed by UV–visible spectroscopy at 353 nm. Similarly, *in vitro* drug release profile of the Act suspension was determined by suspending 2.5 mg of the Act in 25 ml of PBS (pH 7.4) containing 3% w/v SLS.

*2.5. Preparation of Acitretin loaded NLC gel*

The Acitretin–NLC gel was prepared by dispersing required quantity of Carbopol 934 P (1% w/w) in small quantity of distilled water and allowing to hydrate for 4–5 h. Propylene glycol (10% w/w) and glycerol (30% w/w) were subsequently added to the aqueous dispersion. Inclusion of air was avoided by addition of 0.5 ml of triethanolamine (Maia et al., 2000) and lyophilized ActNLC powder equivalent to 150 mg of the drug was incorporated into the gel under gentle stirring. Finally, rest of the water (58.85% w/w) was added to make up the volume of aqueous dispersion up to 100% w/w.

*2.6. In vitro skin deposition study*

Human cadaver skin (HCS) was washed thoroughly with hot distilled water and subcutaneous fat was removed. Full thickness HCS membrane was prepared by shaving the skin, punching out a disc of approximately 2.5 $cm^2$ in area and slicing to a 500 mm thickness using a Davis Dermatome 7 (Anthony Products, Indianapolis, Indiana, US). These slices were hydrated with PBS for 24 h at room temperature prior to use. (Kalariya and Misra, 2004). Thereafter, 0.1 g of gel was weighed, spread over the skin and allowed to stand for 36 h. The amount of drug remaining on the surface of the skin was determined by washing the surface three times with PBS and measuring the absorbance at 353 nm. The residual washing solvent was carefully wiped off from the skin with a cotton swab, the skin was digested overnight in PBS containing 3% SLS at 40 °C and the drug deposited in the skin was quantified by UV–visible spectrophotometery at 353 nm.

*2.7. Clinical study*

Acitretin gels were evaluated clinically by a parallel group design. The clinical study was conducted on 12 psoriasis patients for a period of 4 weeks in Skin-VD Department of All India Institute of Medical Sciences, New Delhi, India, with prior permission of the Hospital Ethics Committee. Written consent was obtained from the patients after explaining the objective and possible consequences of the studies. Twelve outpatients consisting of 8 male and 4 female candidates, ranging between 18 and 50 years of age with chronic psoriasis joined the study and were randomized into two parallel groups of 6 patients. One group received Act plain gel whereas another received ActNLC.

The mean duration of the disease at entry was ranging from 1 to 10 years. Patients were provided plain Acitretin gel for twice daily application and ActNLC gel was given as once a day application. Treated lesions were a maximum of 25% body surface area (Gordon et al., 2003). Before beginning the studies, each patient underwent a general physical examination and standard laboratory tests including hematologic analysis, analysis of serum chemistry and urine analysis profile. All previous treatments were discontinued at least 30 days prior to studies. The lesions selected were located on the forearm, elbow, knee, chest region, back and were thoroughly cleaned with lukewarm water before applying the gels.

Clinical evaluations were done on the basis of improvement in clinical manifestations and reduction of adverse effects. Progress was evaluated on a weekly basis by a group of physicians in terms of improvement in Psoriasis Area and Severity Index (PASI) score, Overall Lesion Severity Scale (OLS), Physician's Assessment (PA), Psoriasis Symptom Assessment (PSA) and Safety and Tolerability Assessment (STA) (Lynda Sutton, 2001). The PASI score was calculated as the sum of the severity of the main symptoms multiplied by the numerical value of the area involved with various percentages of the 3 main body areas. The scoring was done depending on the area of the lesions. OLS was static assessment with 6 categories (clear, minimal, mild, moderate, severe, and very severe) based on the characteristics of plaque elevation, scaling, and erythema giving scoring from 1 (clear) to 6 (very severe). PA categorized the response to therapy of all clinical signs and symptoms of disease relative to baseline. Physicians used all available information for the assessment, including subjective information gathered from the patient and photographs taken at baseline. The categories were worse, unchanged, slight, fair, good, excellent, and cleared, giving score from 1 (cleared) to 7 (worse). The PSA was a measure of psoriasis-related eight cutaneous symptoms (hurt, burning or stinging, itching, bothered by water, irritated, sensitive, skin condition bled, scaling). The PSA included 2 subscales, one measuring the frequency of the 8 symptoms and the other assessing how troublesome or bothersome were the symptoms?

*2.8. Statistical analysis*

Data was expressed as mean ± standard deviation. Differences in the clinical characteristics between groups of patient treated with plain Acitretin gel and ActNLC gel were evaluated by $p$ value of <0.05, which was considered as significant.

## 3. Results and discussion

Preliminary batches of NLCs were prepared using solvent diffusion method to obtain blank NLCs (without drug). Prior to preparing the drug loaded NLCs, the possible factors influencing the formation of NLCs, size and polydispersity of NLCs were identified and optimized. The parameters studied were speed and time of stirring, type and amount of organic solvent and optimum lipid loading in 100 ml of dispersion. Extensive preformulation optimization studies indicated that, stirring speed of 1000 RPM with stirring time of 10 min and tetrahydrofuran:acetone in the ratio of 1:1 as an organic solvent resulted in desired particle size and polydispersity index for preparation of Acitretin loaded NLCs. Different solid lipids (Compritol 888, Precirol ATO 5, Dynasan 114 and Glyceryl monosterate) and liquid lipids (Oleic Acid, Labrafac Lipo WL 1349, Labrafil M 1944 CS and Capmul MCM) were evaluated for partioning and solubility of Acitretin and the one imparting higher partitioning and solubility was selected. Different amounts of solid lipid were tried to determine the required amount to prepare NLCs. It was found that at high solid lipid concentration of about 100 mg and more, relatively large NLCs were produced, whereas, low concentration of 70 mg produced smaller NLCs with good PI values. Based on the preformulation optimization studies, two major variables affecting the particle size of NLCs were identified. They were (1) amount of solid lipid, (2) amount of liquid lipid.

### 3.1. Factorial design

Using these two variables, 9 batches of Acitretin loaded NLCs were prepared by solvent diffusion method based on the $3^2$ factorial design. The real values and the transformed values for different batches of Acitretin loaded NLCs alongwith the MPS and EE are shown in Table 1. The results of the regression output and response of full model of Acitretin loaded NLCs are presented in Table 2. The mean particle size (dependent variable) of Acitretin loaded NLCs obtained at various levels of 2 independent variables ($X_1$ and $X_2$) were subjected to multiple regression analysis to yield full model second order polynomial equation. The MPS values showed a wide variation ranging from $112 \pm 18.7$ to $630 \pm 12.04$ nm, while EE values varied from $31 \pm 1.25\%$ to $63 \pm 1.54\%$. A substantially high EE was achieved in Acitretin loaded NLCs ($63 \pm 1.54\%$) at medium levels (0) of $X_1$ (70 mg) and $X_2$ (30 mg) in batch A5. The responses in the equation $Y_1$ and $Y_2$ are the quantitative effect of the formulation components on independent variables $X_1$ and $X_2$. The corresponding equations for the full model are:

$$Y_1 \ (\text{MPS}) = 231.6667 + 201.6667X_1 - 41.8333X_2 + 129X_{11} - 3.5X_{22} - 16X_1X_2 \quad (4)$$

$$Y_2 \ (\text{EE}) = 62.4444 + 7.3333X_1 - 2X_2 + 14.6667X_{11} - 6.6667X_{22} - 0.25X_1X_2 \quad (5)$$

The significance of each coefficient of Eqs. (4) and (5) was determined on the basis of $p$ values as listed in Table 2. Smaller the $p$ value, the more significant is the corresponding coefficient, implying that the amount of solid lipid and liquid lipid are significant. From Table 2, it is clear that the main effects of the independent variables ($X_1$, $X_{11}$, $X_2$, $X_{22}$) were the significant factors for prediction of both MPS and EE as their $p$ values are below 0.05 ($p < 0.05$), whereas the interaction term $X_1X_2$ has little predictive value as their $p$ values are above 0.05 ($p > 0.05$) (Jenning et al., 2000b,c,d). Model $F$ values for MPS and EE were found to be 79.11and 147.29, respectively, while their corresponding $F_{tab}$values were 3.44 and 4.86, respectively, which implies that the models are highly significant ($p$ value < 0.05). When the coefficients of the two independent variables in Eq. (4) were compared, the value for $X_1$ ($b_1 = 201.6667$) was found to be maximum and hence it was considered to be a major contributing factor affecting the particle size of the Acitretin loaded NLCs. It was indicated that the change in the ratio of Precirol ATO 5 and Oleic acid had significant effect on the particle size. Similarly, the value for $X_1$ ($b_1 = 7.333$) was also found to be maximum in Eq. (5) indicating that it was a major contributing variable for EE also. The goodness of fit of the model was checked by the determination coefficient ($R^2$). In this case, the values of the determination coefficients ($R^2$) for MPS (0.9925) and EE (0.9959) for full model indicated that over 98% of the total variations are explained by the model. The values of adjusted determination coefficients (adj $R^2$) were also very high (0.9799 for MPS and 0.9989 for EE) for the full model indicating a high significance of the model because $R^2$ value near to 1 signifies an excellent correlation between the independent variables. Thus, all the above considerations indicate that the model is highly significant and can effectively explain 99% variation around the mean value.

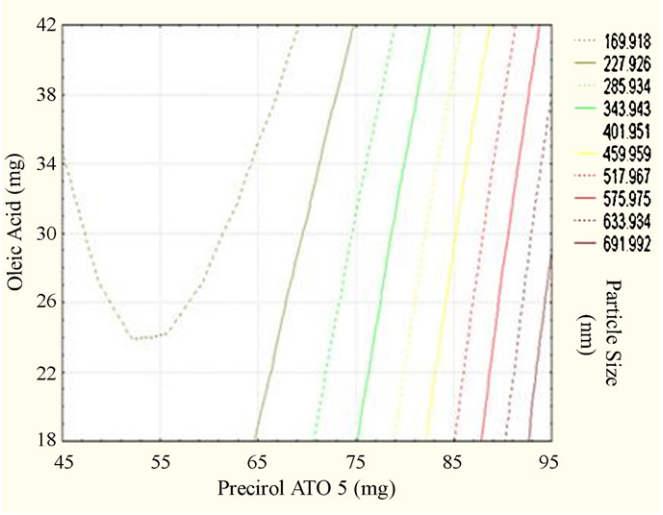



**Fig. 1.** Contour plot of Mean Particle Size (MPS) vs Amount of Precirol ATO 5 and Oleic acid.

### 3.2. Contour plots and surface response curves

The contour plot shows predicted particle size (MPS) values at different levels of Precirol ATO 5 and Oleic acid (Fig. 1). It can be seen that increase in Precirol ATO 5 increased, whereas Oleic acid decreased the MPS. The surface response curve also shows increased particle size with increasing amount of solid lipid, whereas increase in amount of liquid lipid led to little reduction in particle size (Fig. 2). The effect of oleic acid on the particle size was not significant but little variation in the particle size was observed, which can be seen in Table 1. The absence of curvature in the surface response curve indicates the insignificance of quadratic effects ($X_2{}^2$).

Fig. 3 reveals increase in EE when the amount of Precirol ATO 5 was increased up to 70 mg, later on increase in the EE can be obtained by increasing Precirol ATO 5 up to almost 85 mg with the fixed amount of oleic acid (30 mg) whereas, particle size was found to be increased beyond 70 mg. Similarly, EE was found to be increased when the amount of oleic acid (liquid lipid) was increased up to 30 mg, while decreased entrapment was observed beyond 30 mg of liquid lipid. This may be due to the fact that the lipid precipitation mechanism occurs during particle production. After homogenization, there is a mixture of drug and lipid in each droplet.

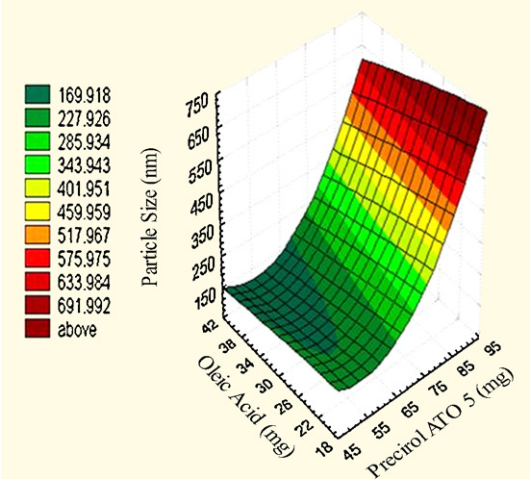



**Fig. 2.** Surface response curve of Mean Particle Size (MPS) vs Amount of Precirol ATO 5 and Oleic acid.

It is then cooled, which leads to the precipitation of lipid earlier than the drug which ultimately results in a drug-free core or at least a core with reduced drug content (Manchow et al., 2008). Thus, increase in the lipid load beyond certain extent (as optimized) causes the drug to be present in the outer shell and remain outside the NLC in the external aqueous phase leading to poor entrapment efficiency. This can be seen by the presence of curvature in the surface response curve for EE indicating the significance of quadratic terms ($X_1^2$ and $X_2^2$) (Fig. 4). Thus, it can be concluded that optimum responses can be attained when the amount of Precirol ATO 5 and Oleic acid varied between 50–70 mg and 20–30 mg, respectively.

### 3.3. Effect of the liquid lipid on particle size and zeta potential

Particle size of the Acitretin loaded NLCs was mainly affected by the Oleic acid content. It was found that increase in the Oleic acid content from 20, 30 to 40 wt%, resulted in decreased particle size. This may occur due to liquid lipid being excluded during the particle formation. As soon as the system is cooled, lipid starts solidifying and arranges itself as a nanoparticle, whereas liquid lipid because of its soft structure may remain outside or randomly distributed.

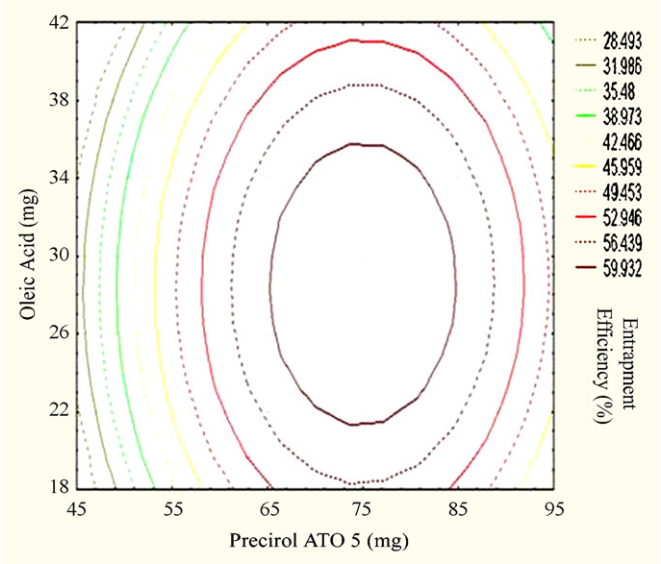



**Fig. 3.** Contour plot of entrapment efficiency vs amount of Precirol ATO 5 and Oleic acid.

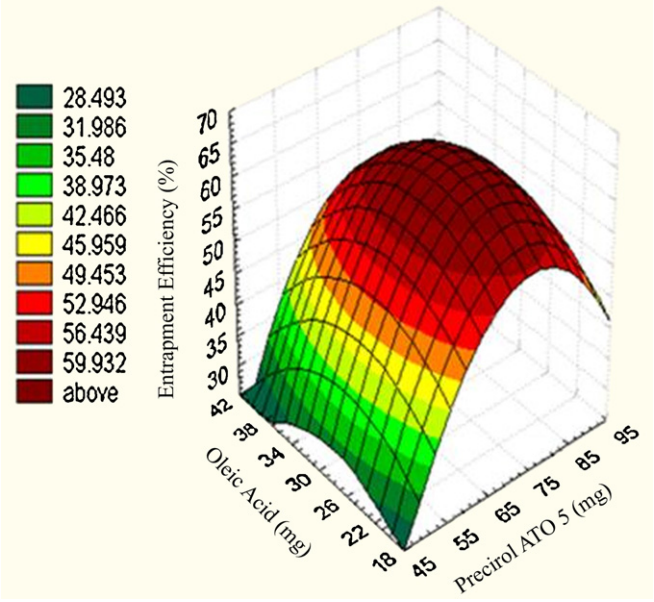



**Fig. 4.** Response surface plot of entrapment efficiency vs amount of Precirol ATO 5 and Oleic acid.

Polydispersity index of Acitretin loaded NLCs also decreased with increased Oleic acid content. The PI of the optimized batches was below 0.2, suggesting that the NLCs are monodispersed. Zeta potential is a key factor to evaluate the stability of colloidal dispersion (Komatsu et al., 1995). In general, particles could be dispersed stably when absolute value of zeta potential is above 30 mV due to the electric repulsion between particles (Müller et al., 2001). We found zeta potential values of the NLCs in the range of −30 to −35 mV with no influence of Oleic acid content in the NLC disperison. This implies that the ActNLCs prepared using solvent diffusion method would be a physically stable system.

### 3.4. Characterization of NLCs

#### 3.4.1. Morphology

TEM images of the NLCs confirmed that the particles were nearly spherical or oval in shape with narrow size distribution and were non aggregated (Fig. 5). The diameters of the particles observed

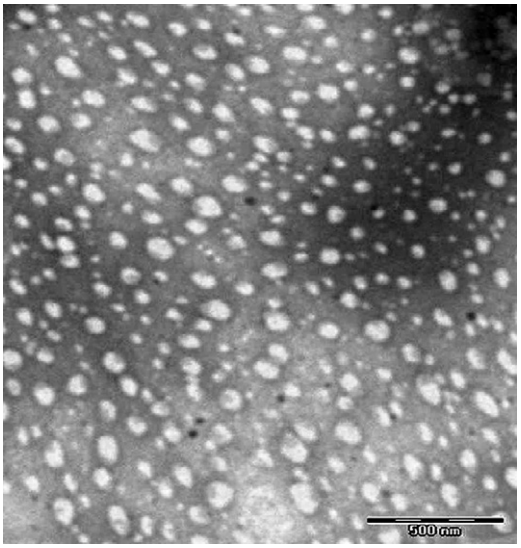



**Fig. 5.** TEM of the ActNLCs.

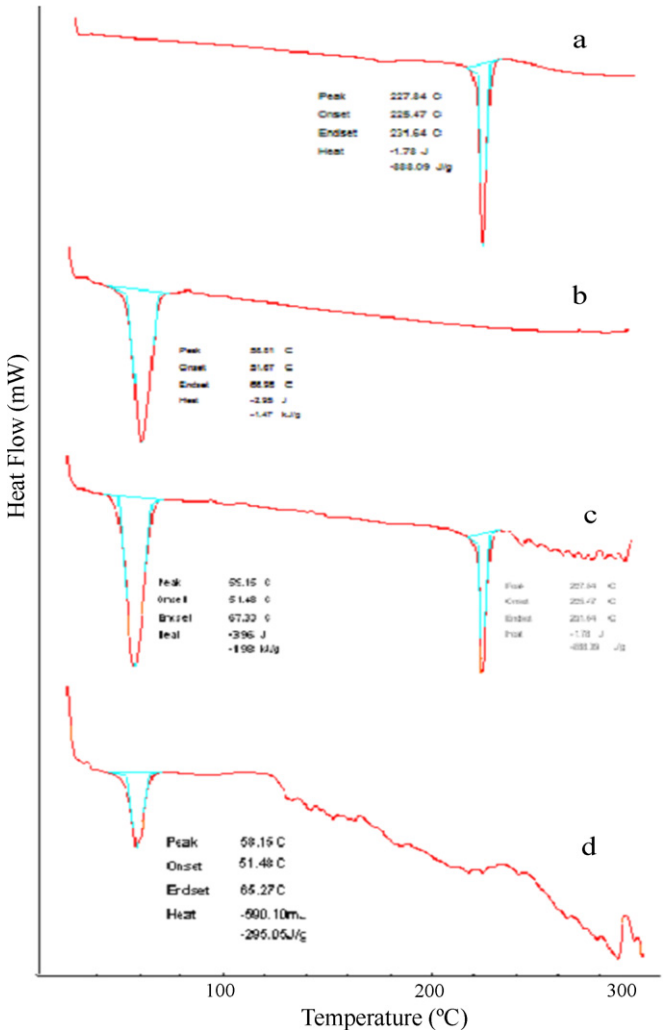



**Fig. 6.** DSC thermograms of a) Acitretin; b) Precirol ATO 5; c) Physical Mixture of Acitretin and Precirol ATO 5; d) ActNLC formulation.

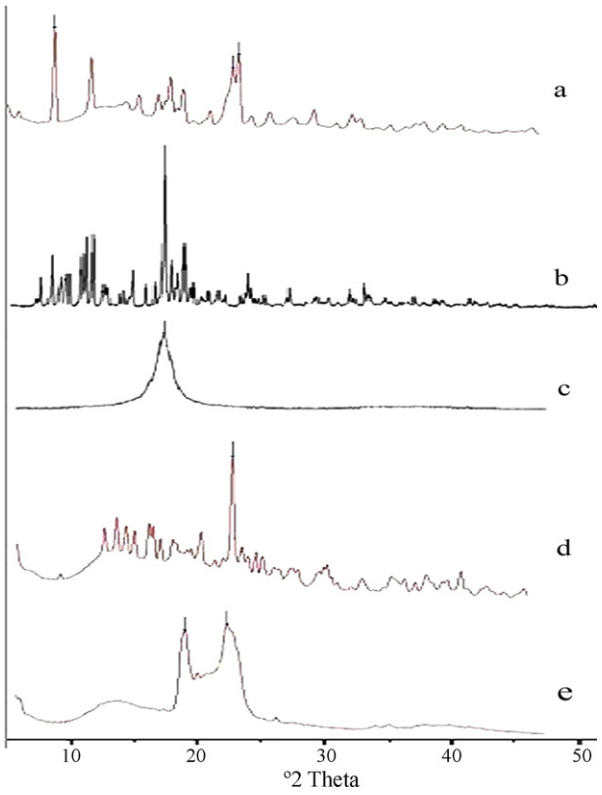



**Fig. 7 XRD of - a) Acitretin ; b) Trehalose ; c) Precirol ATO 5; d) Physical mixture of Precirol ATO 5 and Actretin; and e) Lyophilized ActNLC formulation.** .

in the micrographs are in good agreement with the data obtained from Malvern particle size analyzer (Table 1).

### 3.4.2. Differential scanning calorimetry

DSC gives the information regarding crystalline or amorphous nature of the samples using the fact that different lipid modifications possess different melting points. Fig. 6 shows DSC curves of pure Acitretin, Precirol ATO 5, physical mixture and lyophilized ActNLCs. Thermograms of bulk Acitretin and Precirol ATO 5 exhibited endothermic peak at 227.84 °C and 58.81 °C, respectively, corresponding to their melting temperatures (Fig. 6a and b). The peak for Acitretin was completely absent in lyophilized ActNLCs (Fig. 6d), while it was clearly evident in physical mixture of Acitretin (227.84 °C) and Precirol ATO 5 (58.81 °C) as shown in Fig. 6c. It has been reported that when the drug does not show its endothermic peak in the nanoparticulate formulations, it is said to be in the amorphous state (Sarmento et al., 2006; Reddy and Murthy, 2005). Hence, it could be concluded that the drug was present in the amorphous phase and may have been homogeneously dispersed in the NLCs.

### 3.4.3. X-ray diffraction

From the XRD data, it was clear that pure Acitretin showed highly crystalline nature with principal peak at $2\theta$ value of 9.005, whereas Precirol ATO 5 showed 100% intensity at d-value of 19.03 (Fig. 7a and b). In physical mixture, the crystalline peak for Acitretin was clearly evident (Fig. 7c), whereas the NLC formulation showed deformed peak for Acitretin, indicating its presence in an amorphous or molecular dispersion state (Fig. 7d). The XRD of lyophilized batch showed that there was some crystalline modification in the final formulation as there was slight shift in the major peaks of Acitretin and Precirol ATO 5. This is indicative of disordered crystalline structure of solid lipid due to liquid lipid. Moreover, XRD result of the cryoprotectant alone confirmed that the first peak in the NLC may be due to the cryoprotectant (Westesen et al., 1993).

## 3.5. Evaluation of Acitretin loaded NLCs

### 3.5.1. In vitro release studies

The cumulative percentage release of Acitretin from Acitretin suspension (ActS) and ActNLC were investigated *in vitro* over a period of 30 h. Each sample was analyzed in triplicate and release curves are shown in Fig. 8. It was indicated that ActS released almost 80% of the drug at the end of 30 h, while 46.29% release was observed from ActNLC which showed sustained and steady release over the entire period of study. ActNLCs showed biphasic drug release pattern with an initial sustained release phase for up to 10 h followed by a steady drug release phase. In the initial phase of up to 3 h, relatively slow release could be observed which can be attributed to the penetration of the aqueous diffusion medium into the hydrophobic lipid followed by slow dissolution and diffusion of the drug from the NLCs. Thereafter, the drug released from the lipid matrix by diffusion in fickian (1st order) pattern. After 10 h, the release rate was further reduced, probably due to the depletion of the drug from the matrix, leading to reduced concentration gradient. Therefore, the NLC exhibited initial sustained release phase for the first 10 h and subsequent steady state release of Acitretin (Pople and Singh, 2006). This kind of drug release pattern is of interest for dermal application in the view that initial sustained release

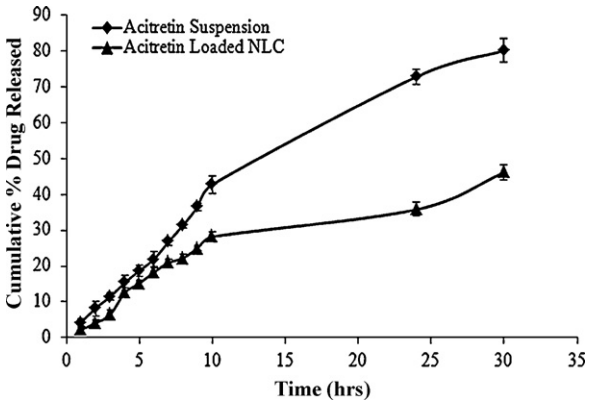
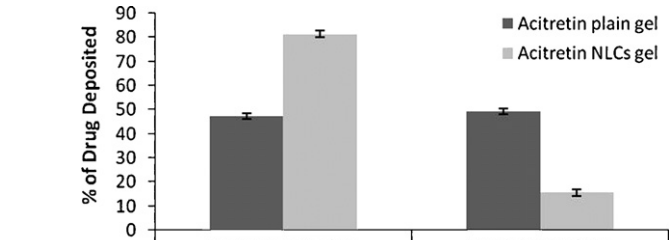



**Fig. 8.** Cumulative Percentage drug release from Act suspension and ActNLC in phosphate buffer pH 7.4 containing 3%w/v SLS.




| | %Drug retained in skin | %Drug in washing medium |
|---|---|---|
| Acitretin plain gel | 47.28 | 49.34 |
| Acitretin NLCs gel | 81.38 | 15.63 |

**Fig. 9.** *In vitro* skin deposition study of plain Act gel and ActNLCs gel.

improves the penetration of drug, while further steady release provides the drug over a prolonged period of time. Comparing the drug release from Act suspension, which showed ever increasing drug release up to 30 h with the Act release of 80.22 ± 3.40%, ACTNLC, presents a better delivery system for Acitretin.

The drug release data from the ActNLC was fitted into Korsemeyers Peppas model to find the release mechanism. Model fitting revealed that ActNLCs showed anomalous transport (non-fickian) mechanism for the release of drug based on the '*n*' value (0.911, which is $0.5 < n < 1$), whereas $R^2$ was found to be 0.98 (Jenning et al., 2000b,c,d).

### 3.5.2. *In vitro skin deposition studies*

One of the reasons to employ NLC approach for topical delivery of Act was the need of its dermal localization for enhancing the localized treatment of psoriasis and reducing its systemic toxicity. Hence *in vitro* skin deposition of plain Act gel and ActNLC gel was investigated using human cadaver skin and the results are shown in Fig. 9. The study revealed significantly higher deposition of Act from ActNLC gel in the skin (81.38 ± 1.23%) than the plain Act gel (47.28 ± 1.02%). Results of these studies supported the hypothesis that the incorporation of Acitretin into NLC enhances the drug deposition into HCS. The results are also in agreement with the reports that SLN and NLCs improve the dermal localization of several topical therapeutic agents (Maia et al., 2002; Liu et al., 2007).

### 3.6. *Clinical studies*

Results of the clinical study showed that, there was reduction in erythema followed by marked reduction in scaling, indicating moderate to excellent improvement in the disease symptoms from the ActNLC gel formulation. There was substantial reduction in PASI score from the initial level in the case of ActNLCs gel as compared to plain Act gel (Table 3). According to the Students *t*-test, there was significant difference between the two groups, with the % PASI improvement being significantly higher in ActNLC gel as compared to plain Act gel.

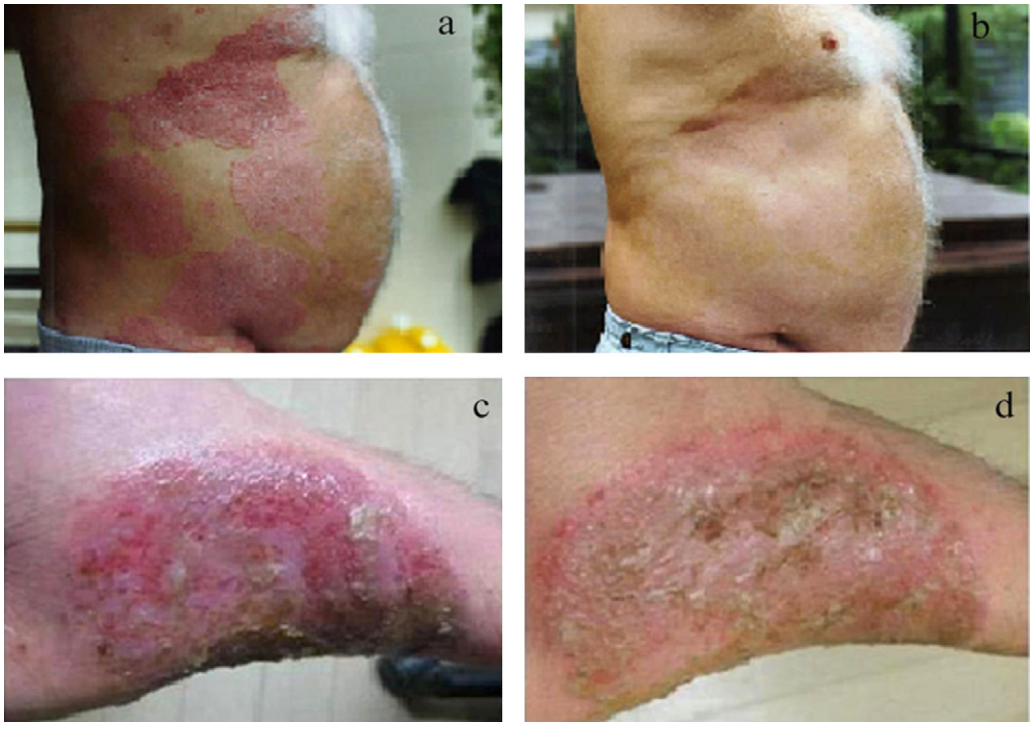



**Fig. 10.** Photographs of Psoriatic Patient before (a) and after (b) treatment with ActNLC gel. Photographs of Psoriatic Patient before (c) and after (d) treatment with Plain Act gel.

Significantly high number of patients treated with ActNLC gel showed an OLS rating of minimal or moderate after 4 weeks as compared to patients treated with plain Act gel, who showed rating of moderate to severe stage of Psoriasis (Table 3). Physician Assessment Score also indicated that patients treated with ActNLCs gel showed good to fair response, whereas in case of patients receiving plain Act gel, the responses were unchanged or only slightly changed (Table 3). In case of Psoriasis Symptom Assessment (PSA), the adverse symptoms were found to be mild with ActNLC gel as compared to plain Act gel (Table 3).

Thus, the outcome of the clinical studies have clearly demonstrated the impressive effects of the ActNLC gel applied topically in the treatment of palmoplanter type of psoriasis. Also, only 25% patients experienced mild adverse events, which were local in nature such as burning or irritation.There were no clinically significant changes in hematologic or clinical laboratory features in any patient from both the treatments.

The change in the disease status from baseline was consistent with changes in disease signs and symptoms. This can be seen from the physical changes captured in the photographs taken before and after the treatments of respective formulations. Fig. 10a and b shows the severity of lession in patients treated with ActNLC gel and plain Act gel, respectively. Results of Safety and Tolerability Assessment (STA) studies (Table 3) indicated that though the topical administration of Plain Act gel did not produce any major systemic side effects, the main topical side effect was irritation, which was very bothersome during the complete course of study. On the other hand, the patients treated with ActNLCs gel showed minor irritant effect, indicating that the disadvantages of Acitretin for topical administration can be overcome by formulating NLCs.

Thus, based on the various evaluation aspects of the clinical studies, we can conclude that once a day application of ActNLC gel could be superior in enhancing the patient compliance and management of symptoms of Psoriasis in comparison to twice daily application of Plain Act gel.

## 4. Conclusion

The formulation of ActNLC gel was optimized using a $3^2$ factorial experimental design, selecting the solid lipid and liquid lipid contents for achieving optimum particle size and maximum entrapment efficiency. The resultant NLCs were nearly spherical and smooth as seen under TEM. DSC and XRD study indicated that ActNLCs were present in amorphous or molecular dispersion state. *In vitro* release study of ActNLC showed biphasic drug release pattern with initial faster release up to 9–10 h which, may be due to uneven distribution of drug in the NLC during initial hours. Later on, ActNLC showed sustained release of drug for the entire period of study. *In vitro* skin deposition studies showed significantly higher ($p < 0.05$) deposition of Acitretin from ActNLC gel ($p < 0.05$) in the skin than Plain Act gel. Clinical findings indicated that ActNLC gel significantly improved therapeutic index in terms of PASI Score and PLS, PA, PSA and STA ratings.

The findings of this investigation conclusively demonstrate the promising role of Acitretin–NLCs gel in treating psoriasis patients. Although its use improved the therapeutic response, more benefits of the NLC based Acitretin gel were observed in terms of reduction in adverse effects of therapy, fostering better patient compliance. Thus, Acitretin–NLC gel may be considered as a supplementary to oral therapy, particularly in terminal stage of psoriasis treatment. However, the role of NLC based gel formulations may only be established after clinical evaluation of a large number of patients, with a special focus on the adverse symptoms of therapy.

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