A synergistic approach of adapalene-loaded nanostructured lipid carriers, and vitamin C co-administration for treating acne

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Abstract

The present study documents the fabrication and characterization of a topically applicable gel loaded with nanostructured lipid carriers (NLCs) of adapalene (ADA) and vitamin C (ascorbyl-6-palmitate [AP]). The NLCs were prepared by high pressure homogenization (HPH) method followed by incorporation into AP loaded gel. The fabricated system was characterized for size, polydispersity index, entrapment efficiency (EE) and in vitro drug release properties, and was further investigated for skin compliance, skin transport characteristics (skin permeation and bio-distribution), rheological behavior, texture profile analysis and anti-acne therapeutic potential against testosterone-induced acne in male Wistar rats. The NLC-based formulation improved targeting of the skin epidermal layer and reducing systemic penetration. The co-administration of vitamin C led to an adjunct effect in acne therapy in physiological conditions. In brief, the present results suggest the potential of NLCs as a novel carrier for the dermal delivery of ADA and also the synergistic effect of vitamin C in topical therapeutics.

Introduction

Acne vulgaris is a multifactorial disease associated with excessive sebum production by the sebaceous glands¹. Acne is supposed to be a trivial, self-limiting, cosmetic disorder, however, it has substantial effects on the quality of life, including anxiety, social withdrawal and depression, making its treatment important². The topical treatment of mild to moderate acne with all trans retinoic acid (RA) has been effective in treating acne³. The retinoids are natural or synthetic derivatives of vitamin A constituting the most effective comedolytic agents. They act by normalizing follicular keratinization, preventing the formation of new microcomedo, and also the synergistic effect of vitamin C in topical therapeutics.

Adapalene (ADA) is a new synthetic retinoid, having a great affinity for retinoic acid receptors (RAR) β and RAR γ, and is effective in the treatment of mild to moderate acne in humans. It has been seen to exert a powerful inhibitory effect on keratinocyte proliferation and differentiation, and manifests as strong as comedolytic activity⁷,⁸ clinically.

Vitamin C, a vital micronutrient carrying important metabolic functions, is sensitive to light and air and is despoiled by chemical and photochemical oxidation reactions⁹–¹¹. Ascorbic acid, because of numerous functions such as depigmentation, collagen synthesis and antioxidant activity, is widely used as an ingredient of anti-aging cosmetic products¹²,¹³. Formulations with L-ascorbic acid were found unstable due to the oxidation of L-ascorbic acid exposed to air. Therefore, ascorbyl-6-palmitate (AP), an esterified derivative of L-ascorbic acid, in topical formulations has been used to improve stability⁵. In addition, AP due to its antioxidant property, protects skin by neutralizing reactive oxygen species generated by exposure to sunlight and in biological systems, reduces both oxygen- and nitrogen-based free radicals¹⁴.

Over the years nanomaterials have been reported to be a promising tool for topical delivery. It presents obvious advantages over conventional formulations, such as reduced side effects, low irritancy and a controlled rate and extent of drug release into the defined skin stratum¹⁵,¹⁶. NLCs have materialized as the most rationalized carriers composed of nontoxic, bio-acceptable, biodegradable lipids components with a mean particle size between 50 and 1000 nm.
NLCs are being solid at both room and body temperatures. Moreover, the ability to form submicron particles, possibility of large-scale production, possible sterilization and circumvent toxicity problems are salient facets for parenteral administration offered by NLCs. Physical stability (several years), more flexibility in modulating the release of the drug and minimized chemical degradation of entrapped drug are very decisive issues that scores NLCs as an alternate colloidal drug delivery system to liposomes, micelles, emulsions and polymeric nanoparticles.

Recently several studies have been reported for the effective topical delivery of different kinds of anti-acne molecules. The various colloidal carriers like microemulsion, lipid-nanoparticles, solid lipid nanoparticles, liposomes and mixed vesicles were employed to either improve the physicochemical properties of drugs or to improve their preclinical efficacy. However, no studies have been reported on ADA-loaded NLCs co-administered with AP.

The current study was aimed at investigating the possibility of co-administration of encapsulated ADA and vitamin C (AP) in NLCs, within carbopol hydrogel in order to obtain a system possessing simultaneous anti-oxidative and prolonged anti-acne activity. Here, we demonstrate that the combination of ADA and AP (antioxidant) provided heightened/superior protection when compared with the ingredients individually. In addition, we studied and optimized the rheological behavior of the hydrogel for its intended topical use.

Materials and methods

Materials

The ADA was supplied as a kind gift by Glenmark Pharmaceuticals Ltd. (Nasik, India). Phospholipid (90 NG) was a generous gift from Lipoid, Ludwigshafen, Germany. Labrasol was obtained as a generous gift from Gattefosse, France. Tristearin, AP, Triton X-100 was procured from Sigma Aldrich (Mumbai, India). De-ionized and filtered water was used throughout this study.

Fabrication of ADA-loaded nanostructured lipid carriers (NLCs-A)

NLCs were prepared by hot high pressure homogenization (HPH) method reported elsewhere. In brief, to prepare 100 gm ADA-loaded NLCs s (NLCs-A); 100 mg of ADA was dissolved in a molten lipid phase containing the lipid mixture of 1% (w/v) tristearin & labrasol (4:1), 0.3% w/v phospholipid-90NG in 10 ml acetone–ethanol mixture (1:1; v/v). The aqueous phase consisted of 0.2% (w/v) Tween 80 solution in water (about 90 ml). The lipid phase and 50 ml of aqueous phase were heated to 70 °C for 1 min. The lipid and aqueous phase were then mixed in a beaker and stirred for few minutes. After that 100 g of hot water was added into this emulsion. Subsequently, this mixture was further sonicated using probe sonicator (Misonix-3000, Qsonica, LLC., Newtown, CT) at 3 Watts for 2–3 min. The rest of aqueous phase was then added into this mixture and subjected to HPH (Panda 2K Niro soavi, Homogenizer Systems, Derbyshire, UK) at 130 Barr for three cycles. Furthermore, the lipid dispersion was immediately cooled down to room temperature determination of entrapment efficiency (EE).

The NLCs-dispersion was poured into a cellulose dialysis bag (MWCO 10kDa) and was extensively dialyzed under magnetic stirring (50rpm) against double distilled water (DDW) under sink conditions for up to 10 min to remove un-entrapped drugs from the formulation. Samples were collected in HPLC vials and diluted with a solvent (Methanol and Dimethylformamide). The ADA was estimated by the HPLC method as reported earlier with slight modifications. Briefly, HPLC analysis was carried out on a Merck RP-8 column (250 mm × 4.6 mm particle size 5 μm; Mumbai, India). The mobile phase was acetonitrile–water (65:35 v/v; the pH was adjusted to 2.5 with ortho-phosphoric acid). Flow rate was kept 1.3 ml/min. The injection volume was 20 μL for all solutions, and a detection wavelength was set at 321 nm. The EE was calculated using the equation given below:

\[
\text{Entrapment Efficiency(%) = } \left( \frac{\text{Total amount of drug added}}{\text{Amount of drug in the collected sample}} \right) \times 100
\]

Fabrication of ADA-loaded NLCs gel (NLCs-A-gel) and ADA-loaded NLCs gel consisting ascorbyl-6-palmitate (NLCs-A-AP-gel)

A concentrated Carbopol® 934 gel base was prepared by dissolving carbopol® 934 into an aqueous solution of AP using 1% ethanol as the co-solvent. The NLCs-dispersion was incorporated into the concentrated gel base so that the final concentration of Carbopol® 934 and AP remained at 1% w/v and 15% w/w, respectively, and the gel was kept at room temperature to hydrate for 24 h. The resulting colloidal mix was neutralized with tri-ethanolamine to obtain an adequate semisolid gel matrix of pH 6.0. The NLCs-A-gel was also prepared the same way as above with the exception of the addition of deionized water instead of the aqueous solution of AP.

Characterization of NLCs-A and gel formulation

Particle size and zeta potential

The NLCs-A sample was well-dispersed in de-ionized water and measured for average particle size and polydispersity index (PDI) using a Zetasizer (PCS, Nano ZS90 Zetasizer, Malvern Instruments Corp, Worcestershire, UK) at a fixed angle of 90° at 25 ± 0.1 °C and for zeta potential in folded capillary cells using a laser Doppler anemometry Malvern Zetasizer (Worcestershire, UK) at 25 ± 0.1 °C and 15.24 V/cm.

Transmission electron microscopy (TEM)

The NLCs-A was characterized in terms of size and surface morphology by Transmission electron microscopy (TEM) using a Philips CM 10 electron microscope, with an accelerating voltage of 200 KV (Fei, Electron Optics, Hillsboro, OR). A drop of the sample was placed on a carbon coated copper grid leaving a thin film on the grid. Before the film dried, it was negatively stained with 1% phosphotungstic acid. Photomicrographs were taken at a suitable magnification.

Apparent viscosity and rheological behavior of the gel formulations

The apparent viscosity and rheological behavior of NLCs-gel and NLCs-A-AP-gel were determined by a dynamic rheometer (Anton Paar, Graz, Austria) using instrument software, Rheoplus, equipped with a cone and plate test geometry (cone diameter, 75 mm, cone angle 0.999°) at 25 °C. Samples were placed over the plate and parameters were adjusted as per manufacturer’s recommendations. The apparent viscosity of the NLCs-A &
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In vitro release study

The in vitro release of entrapped ADA from the NLCs-A, NLCs-A-gel and NLCs-A-AP-gel formulations as well as AP from the NLCs-A-AP-gel were estimated by employing dialysis tube diffusion techniques27. Five milliliters of the NLCs-A dispersion free from any un-entrapped drug and the amount weighed of the NLCs-A-gel and the NLCs-A-AP-gel consisting of an equivalent amount of ADA and AP in their respective formulations were individually kept in a dialysis membrane (MWCO 10–12kDa, Himedia, Mumbai, India). Dialysis membranes were tied at both ends and suspended in a separate beaker containing 20 ml, 80% v/v Methanol:DMF (50:1) in PBS (pH 5.6). The beakers were assembled in a continuous stirring mode with a magnetic stirrer at 100 rpm and at constant temperature, 32 ± 1°C. One milliliter of the sample was withdrawn intermittently and was replaced with the same volume of fresh solvent mixture in a receptor compartment28,29. Samples were analyzed to quantify ADA and AP by well-established HPLC methods as described in section 2.2.

Skin permeation study

The use of human skin and porcine ear skin was used as a relevant model for predicting transdermal permeation potential of drugs30–32. However, human skin and porcine ear skin were not easy to extract and available only in restricted amounts. Therefore, rat skin due to its greater permeation rates and easy extraction and available only in restricted amounts. The thick skin from the abdominal region of shaven rats was excised, rinsed with saline and the adherent subcutaneous fat was removed34. The prepared skin was surmounted on the Vertical Franz diffusion cell and the dermal side faced upwards into the donor compartment, and the dermal side faced downwards into the receptor compartment. The whole assembly was maintained at 32 ± 0.5°C using a recirculating water bath placed over a magnetic stirrer to constantly stir the medium filled in the receptor compartment. A plain drug solution (tincture), marketed adapalene gel formulation (ADAPEN gel, adapalene 0.1% w/w, INTAS, Ahmedabad, India), NLCs-A, NLCs-A-gel and NLCs-A-AP-gel (with an equivalent ADA to NLCs-A) were applied gently into the donor compartment. 0.5 ml of the sample from the receiver compartment was collected intermittently and replaced with the same volume of a PBS: methanol solution to uphold a constant volume throughout the study26. Samples were filtered through an aqueous 0.22 μm pore size cellulose membrane filter and the accumulated volumes of the ADA which permeated through the rat skins were analyzed. The whole experiment was carried out for 24 h.

Skin distribution study

The skin distribution study was carried out upon completion of the permeation experiment. Skin samples were removed carefully, scrapped to retrieve most of the adherent formulations, cleaned with lint-free cotton soaked in de-ionized water to remove any residual formulation and dried. Further, epidermal and dermal skin layers were manually separated using tweezers, cut into pieces, and homogenized in 5 ml methanol:DMF (50:1) to extract retained ADA and AP37. Samples were filtered through a 0.45 μm membrane filter and analyzed by HPLC. The accumulated permeated amounts of ADA and AP were calculated.

Testosterone induced acne model

Acne was induced by the application of testosterone in animals and further experiments were initiated. Briefly, male Wistar rats were divided into six groups of six animals each. Testosterone was applied daily on the shaved dorsal side of animals until the end of the study38. Group I received testosterone only, group II received testosterone and 0.1% ADA tincture, group III received testosterone and ADA-marketed gel, group IV received testosterone and NLCs-A-gel, group V received testosterone and NLCs-A-AP-gel and group VI received no treatment and served as a control for the study. All applications were made once-a-day consecutively for four weeks. The skin of animals was observed for the induction of any visible acne papules and/or other noticeable changes. The anti-acne potential of the formulation was reported as diminishing in the papule density (4 cm² skin area) by the fourth week of treatment compared to that of the group treated only with testosterone. The animals from all groups were euthanized after the fourth week of application, treated skin samples were excised, sectioned by microtome followed by paraffin embedding, and H & E staining was carried out on all sections. The processed skin sections were viewed under a microscope for changes in the number and size of sebaceous glands, and results were compared with those of the skin sections of untreated/control rats.

Stability study of NLCs-A and gel formulations

The physical and chemical stabilities of NLCs-A, NLCs-A gel and NLCs-A-AP-gel were concerned in short term observations of different attributes viz., possible changes in appearance like de-colouration, change in odours, gel consistency and appearance of drug crystals or precipitates. The formulations were evaluated at three different storage conditions, i.e. in refrigerated condition (RF; 5 ± 3°C), room temperature (RT; 25 ± 2°C/60 ± 5% RH) and elevated temperature (HT; 40 ± 3°C/75 ± 5% RH) over a period of three months in order to perceive clarity of formulation, particle size and zeta potential.

Statistical analysis

Statistical analysis of in vitro release data, skin permeation and distribution experiments of each formulation were performed three times, and data are expressed as mean ± SD with statistical analysis completed by one-way analysis of variance.
(ANOVA) with Tukey–Kramer multiple comparison post-tests using Graph Pad InStat™ software (GraphPad Software Inc., San Diego, CA). Statistical differences are denoted as \( p \leq 0.05 \) (NS = not significant), \( p \leq 0.01 \) (significant) and \( p \leq 0.001 \) (highly significant).

**Results and discussion**

**Fabrication of ADA-loaded nanostructured lipid carriers (NLCs-A), NLCs-A-gel, and NLCs-A-AP-gel**

As mentioned, a hot high pressure HPH technique was used to fabricate NLCs. The method involved emulsification of lipid phase followed by intense diffusion of the solvent across the lipid-solvent phase in an aqueous phase followed by evaporation of the solvent and subsequent cooling leading to enhanced rigidity of the lipid nanoparticles. The prepared NLCs were easily dispersed into a concentrated Carbopol® 934 gel to make 1% gel strength. The resulting gel was grizzled, made homomorphically viscous, and formed into acidic colloidal dispersion which upon neutralization with triethanolamine produced a white, homogenous, highly viscous gel. Furthermore, a NLCs-A-AP-gel was developed by incorporation of NLCs-A into a previously prepared concentrated Carbopol® 934 gel and AP mixture.

**Entrapment efficiency**

NLCs-A showed an entrapment efficiency (EE) of 87.29 ± 1.6%. The significantly higher EE may be attributed to their lipophilic nature as well as better compatibility between ADA and the lipid matrix of NLCs. The greater EE is suggestive of the formula and method of preparation. This may prove useful in reducing skin irritation and minimizing or providing no contact of the drug to the skin surface.

**Particle size and zeta potential**

Particle size is one of the most important characteristic features to be monitored in a nanometric system. An alteration of size extensively affects physical stability and bio-fate of the system39. The average size of the particles is also important with respect to EE and release pattern. The average size of all formulations was found 268.3 ± 2.5 with a narrow size distribution (PDI-0.218 ± 0.0012). The zeta potential was determined as a function of surface characteristic of particles and can be used to optimize the emulsion and suspensions formulations to predict their long-term stability and cellular demeanor for drug release40. The zeta potential of the NLCs-A dispersion was -16.35 ± 0.21 mV. A higher value of zeta potential is indicative of the stability of the dispersion. A low value approaching neutral demonstrates better permeability across bio-membranes and will prove beneficial while accumulating in skin layers41,42. TEM results showed that the NLCs are spherical in shape with smooth round edges. The results also suggestive of their nanometric size range and narrow size distribution (Figure 1).

**Rheological behavior and textural profile of gel formulations**

The gel formulation after attaining a particular phase and viscosity can be easily transported and stored at a given temperature. This is not subjected to any shear changes which might modify its viscosity, stability and structure. The apparent viscosity of NLCs-A-gel and NLCs-A-AP-gel was found to be 30 Pa and 9 Pa at 25 °C and a constant shear rate of 10 s⁻¹ (Figure 2a). The NLCs-A-AP-gel illustrated a lower apparent viscosity due to the humectant nature of AP. A rheological study demonstrated structured liquids with pronounced non-newtonian flow behavior of both gels43. Both gel formulations showed the same flow behavior at similar shear ranges (Figure 2b). The only difference was higher values of viscosity in NLCs-A-gel instead of NLCs-A-AP-gel at the same shear rate. This finding is in agreement with the fact that NLCs-A-gel had higher apparent viscosity compared to that seen with the NLCs-A-AP-gel. The value of flow behavior index obtained for both the gels was less than unity, which indicates a shear-thinning behavior of both gels44. The rheogram represents the value of viscosity obtained at varying shear rates as delineated in Figure 2(b). The result shows consistent decrease in the viscosity with increasing shear rate, suggesting that viscosity and shear rate are directly correlated.

The texture characterizations are in agreement with rheological results confirming the greater mechanical properties of gel formulations in terms of firmness, stickiness, hardness and compressibility (Figure 3). Both of the formulations showed a consequent slower recovery of system structure after compression. The greater elasticity of gels also explained good adhesive properties, which make them suitable semisolid systems, and prolonged drug residence time at the site of application and of course ease of extrusion from the tube which are the pre-requisites of any topical formulation16.

**In vitro drug release study**

NLCs-A, NLCs-A and NLCs-A-AP-gel showed a biphasic sustained release pattern of ADA for 48 h in vitro. The initial burst release of 45.57 ± 0.4%, 40.32 ± 0.7% and 39.82 ± 0.04% from NLCs-A, NLCs-A-gel and NLCs-A-AP-gel, respectively, after 8 h and then sustained release up to 48 h (Figure 4a and b). The initial burst release is probably due to either the rapid release of traces of drugs which might be bound/adsorbed onto the surface or presence of drug just underneath the stratum of NLCs. As is evident from the inset graph, significantly (\( n = 6; p \leq 0.05 \)) higher release of ADA from NLCs-A as compared to that from NLCs-gel and NLCs-A-AP-gel was observed at time points up to 48 h. The sustained release pattern of ADA over a period of 48 h could be attributed to diffusion of ADA through the lipid matrix of NLCs or slow degradation of the lipid matrix in the release medium. However, a substantial intermediate release rate of ADA from NLCs-A-AP-gel was observed in comparison to other NLCs formulations. This release is probably due to the intermediate viscosity of the vehicle which favors mobility of drug molecules to enrich the release medium. Similar results were obtained in the studies conducted by Jain et al. and Agrawal et al.16,45. Further, the release of AP from the NLCs-A-AP-gel was pretty fast in the beginning and almost 95% of AP was released after 8 h. The release pattern is probably due to the dilution of the gel.
matrix with the release medium and AP was readily soluble in the medium eventually diffusing into the bulk of the release medium.

Skin permeation studies

Skin uptake, skin-targeting potential of NLCs and permeation ability of ADA from NLCs formulations into rat skin was determined with Franz diffusion cells for up to 8 h (a clinical application time). 0.15% ADA tincture in Methanol: DMF was used as reference to evaluate the skin-targeting ability of NLCs-A, NLCs-A-gel, NLCs-A-AP-gel and marketed gel. It is evident from Figure 5 that the considerably less amount of ADA ($p < 0.001$) in receptor chambers for NLCs-A, NLCs-A-gel and NLCs-A-AP-gel was observed even after 8 h. However, the amount of ADA in the receptor chamber from reference tincture and the marketed gel showed a steady increase with an increase in time. Figure 5 indicated the inability of ADA from NLC-based formulations to penetrate the skin. Interestingly, it was observed that the amount of ADA permeated from the NLC-based formulations were considerably less than that obtained with plain ADA solution and marketed gel. The higher concentration of ADA in the receptor compartment of samples treated with tincture is due to rapid diffusion of drug molecules due to penetration enhancement properties of methanol. Moreover, evaporative loss of methanol may increase concentrations of ADA in tincture which, in turn, increases the concentration gradient of ADA. The present study obviates the importance of lipid matrix. It is well known fact that increases in the rate of permeation and the drug deposition is a function of lipid matrix used for the formulation of NLCs. It is therefore concluded that NLC-based formulations minimize or avoid systemic uptake of ADA when compared to that with tincture and the marketed gel, and to avoid or minimize systemic adverse side effects.

Skin distribution study

The skin deposition ability of NLCs-A, NLCs-A-gel and NLCs-A-AP-gel was studied with an objective to test their drug reservoir forming potential in skin. The results demonstrated that NLCs formulations deposited quantifiable amounts of ADA into the epidermis while a minimal ADA quantity was observed in the dermis (Figure 5). The substantial amount of ADA was retained in skin from NLC-based gels as compared to that calculated with tincture ($p < 0.001$) and the marketed gel ($p < 0.001$). Moreover, the amount of ADA deposited in the skin epidermis region from all NLCs formulations was not different, whereas very small

Figure 2. (a) Apparent viscosity of NLCs-A gel and NLCs-A-AP gel; (b) Flow curve graph of NLCs-A gel and NLCs-A-AP gel shows an increase in shear rate that first leads to an increase in gel viscosity followed by a decrease at the second point.

Figure 3. Textural behavior of NLCs-A gel and NLCs-A-AP gel.
amount of drug was deposited from the marketed formulations and drug solution. In dermis, however less amount of ADA deposited compared to epidermis region. The NLCs-gel formulation showed the highest drug deposition compared to ADA solution and marketed gel formulation. This difference in the drug deposition from nano-gel formulation and marketed formulation might be attributed to the small size of NLCs showing their merit in improving penetration ability of nanocarriers into skin. Higher lipophilicity of the dermal layer also circumscribe valuable partitioning of the hydrophobic drug. Therefore, drug penetration into skin layers may be achieved using NLCs. Further, the dermal retention of ADA was also attributed to the increased contact with the skin layer, occlusive effect and sustained release owing to the properties of NLCs. Results showed no effect of
extended viscosity of the gel on deposition of ADA into the skin in each investigated skin layer. The drug was entrapped within the solid lipid matrix, and the matrix in turn incorporated into carbopol gel adhered to the skin. This arrangement increased contact time of the formulation to the application site. The role of nonionic surfactants for the stabilization of NLCs at low concentration in enhancing drug penetration to an extent has been already reported\textsuperscript{48}. In case of AP, the NLC-gel showed maximum drug deposition in the epidermis and receptor, while fewer amounts were found in the dermis region. This increased penetration behavior of the AP from the formulation might be attributed to extended retention time of the formulation on the site of application, and skin hydration. The outcome of this skin distribution study delineates an advancement of a NLC-based drug delivery system, i.e. a competent topical drug delivery system with at least transdermal localization and reduced systemic access. Therefore, we claim enhanced ADA retention in skin is attributed to our novel carrier, their nano-metric size and bioadhesive properties.

**Testosterone induced acne model**

The microphotographs (Figure 6a–f) of skin sections from the diverse treatment groups show an induction of an induced acne model. The acne development after one week of topical application of testosterone (Figure 6a) and swelling of sebaceous glands is suggestive of successful induction of acne. Four week post-treatment, animals from groups II, III and IV showed a significant reduction in the number of swelled sebaceous glands which is an indication of the reduction in number of acne papules (Figure 6b–d). The papules (image not shown) and swelled sebaceous glands seen almost disappeared in the animals treated with optimized NLCs-A-AP-gel (Figure 6e) and histopathology images (which closely matched to that of saline control group, Figure 6f).

Mean (±SD) values of papule density in Group 1, Group 2, Group 3, Group 4 and Group 5 after four weeks of treatment, were found to be 53(±9), 26(±9), 20(±7), 14(±7) and 6(±3), respectively. The results unequivocally revealed the significant effectiveness of the NLCs-A-AP-gel to the marketed product ($p<0.001$), ADA tincture ($p<0.001$) and NLCs-A-gel ($p<0.001$). Thus, the nano-encapsulated ADA while co-administered with AP was found to be more efficacious and biocompatible vis-a-vis the other formulation tested.

**Stability study**

NLCs-A, NLCs-A gel and NLCs-A-AP-gel has illustrated the stability of formulation over the period of three months. No considerable variations in clarity, and phase separation and recrystallization were observed, demonstrating good physical stability of NLCs. Moreover, NLCs-A was found stable in centrifuge test, and the stability might inherit from the lethargic transition of dispersed lipid from metastable forms to the stable form in NLCs, low particles size, and steric effect of Tween 80 (Mumbai, India)\textsuperscript{49}. The minimal or non-significant degradation of ADA in NLCs and NLCs gel and AP in gel were seen with stable transparency for over three months.

**Conclusions**

The present study highlights a strategy to co-administer a specific drug combination (ADA and AP) of clinical relevance into a single dose regimen. Efforts have been made to obtain greater amounts of ADA loaded into NLCs. The NLCs showed higher skin-targeting potential in contrast to that seen with free ADA, and the adjuvant effect of antioxidant helped to aggravate the potential of ADA during the course of chronic therapy. The targeting potential of nanoparticles could be exploited further to improve the therapeutic efficacy of drug dosages to treat acne. Besides, testosterone induced acne model studies were carried out to better understand the advantage of antioxidant over an individual drug. In essence, the developed formulation strategy presents great potential leading to therapeutic efficacy, and safety profile of the combination. We therefore believe that the NLCs-A-AP-gel as illustrated in the current investigation could prove to be comparatively advantageous over NLCs-A-gel. These findings will put a future perspective into contributions to the enlargement of future clinical and basic studies of skin and thereby provide a
potential treatment strategy for skin disorders. This co-administration strategy may be an addition to acne therapeutic and interventional armory.

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Declaration of interest

The authors declare that they have no conflict of interest.

Research involving Animals

The authors declare that all animal experimental protocols were approved by the Institutional Animals Ethical Committee, Panjab University, Chandigarh, India (ref. letter no. IAEC/346 dated 11.02.2013).

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Supplementary material available online
Supplementary Table S1