ORIGINAL ARTICLE

Development of Tretinoin-Loaded Nanostructured Lipid Carrier and Hyaluronic Acid Topical Gel

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Abstract

Aims: The current study was focussed on the fabrication of nanostructered lipid carrier (NLC) of tretinoin (TRE) followed by its incorporation into hyaluronic acid (HA)-loaded carbapol 940 gel aiming to improve acne treatment. **Materials and Methods:** The TRE-NLC was fabricated using melt-emulsification and ultrasonication method. The fabricated NLC was studied for its particle size, zeta potential, polydispersity index, morphology, and entrapment efficiency of TRE. The gel containing HA and TRE-NLC were evaluated for pH, spreadability, extrudability, bloom strength, and viscosity. The *in vitro* release studies and *in vitro* occlusivity test for the formulated gel and marketed gel were carried out. **Results and Discussion:** The formulated gel showed better release of 86.32% when compared to marketed gel with 38.52% release in 8 h. Occlusion factor can be related to skin hydration based upon the ability to prevent water loss from the surface of the skin which was found to be 42.29% for the formulated gel and 4.76% for the marketed gel. **Conclusion:** The combination of TRE-loaded NLCs and HA gel may provide a more effective treatment for acne by diminishing the dryness than either TRE or HA gel alone.

Key words: Acne vulgaris, hyaluronic acid, nanostructured lipid carrier, topical gel, tretinoin

INTRODUCTION

Acne vulgaris is a common condition of pilosebaceous unit prevalent in major adolescent stage. In terms of severity, lesions for acne vulgaris include comedones, papules, pustules, cysts, and inflammatory nodules. Hyperseborrhea, microbial colonisation at the site of sebum production, hormonal dysregulation, interaction with neuropeptides, follicular hyperkeratinization, inflammation, and failure of the innate and adaptive immune systems are some of the factors that contribute to the emergence of acne.[1,2]

Many treatments have been developed for acne that ranges in severity from minor to severe. Such treatments include topical benzoyl peroxide, clindamycin, erythromycin, oral antibiotics such as minocycline, doxycycline or erythromycin, oral isotretinoin and topical retinoid treatment.[2] Among these treatments, topical application of retinoid has been a mainstay for the treatment of acne. Tretinoin (TRE) commonly named as all-trans retinoic acid belongs to first generation of retinoid (vitamin A), has been effectively used for anti-acne activity.[3] However, the dryness and peeling effect of skin has to be brought into consideration while using TRE topically.[4]

The nanotechnological-based carriers localize the drug substances in the dermal region, thereby minimizing the side effects and improving the delivery.[5] Their nanosize range ensures the penetration of drug substance deeper in the dermal layer. The nanostructered lipid carrier (NLC) comes under second-generation lipid carriers developed to address the drawbacks of solid lipid nanoparticle, a first-generation lipid carrier.[6] NLC comprises of solid lipid and liquid lipid in definite proportion and suitable surfactant (s) to emulsify the solid–liquid binary lipid.[7]

Hyaluronic acid (HA) or hyaluronan is known for its high moisture retention property in tissues and is described as

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highly hydrophilic in nature. HA is a naturally occurring polysaccharide present in the extracellular matrix in connective tissues, epithelial, and neural tissues.[8,9] Thus, developing the TRE-NLC along with HA gel facilitates the formulation, ensuring closer contact with the stratum corneum and subsides the dryness caused by TRE.

MATERIALS AND METHODS

Materials

TRE was purchased from Dhamtec Pharma and Consultants, Navi Mumbai. For the fabrication of NLC, oleic acid (Rankem chemicals, Haryana), cetyl alcohol (Loba chemie Pvt. Ltd, Mumbai), monegyl-CCTG (Mohini Organics Pvt. Ltd, Mumbai), and tween 80 (Loba chemie Pvt. Ltd, Mumbai) were used.

Methods

Screening of lipids and ratios of solid lipid to liquid lipid

Solid lipids (stearic acid and cetyl alcohol) were screened by adding TRE to the melted solid lipid and then subjected to congealing. Visual examinations for the solubility of the TRE in the lipids were done and the amount of TRE that the solid lipid could take up was measured using UV spectrophotometer. Liquid lipids (oleic acid and monegyl-CCTG) were screened based upon the relative drug solubility. The chosen solid lipid and liquid lipid were taken in 1:1 ratio in a test tube, melted, congealed and visually analyzed for separation of layers to examine the compatibility. Different ratios (90:10 to 10:90) of solid lipid to liquid lipid were examined and chosen based on their melting point.[7,10]

Preparation of TRE loaded NLC

The NLC was fabricated using melt-emulsification and ultrasonication method using different concentrations of lipids and surfactants, as shown in Table 1. The aqueous phase (water and tween 80) and organic phase (solid lipid, liquid lipid, and TRE) were taken in separate beakers, heated to 60°C and mixed well. Then, the aqueous mixture was added drop wise to the organic phase with continuous stirring on magnetic stirrer for 20 min maintained at 60°C. Obtained preemulsion was sonicated in Probe sonicator for 15 min (3 cycles with 10 s on cycle and 5 s off cycle) at 40% amplitude.[7,11]

Preparation of gel and loading of TRE-NLC

Appropriate amount of carbapol 940 and HA were made to swell in distilled water overnight. DMSO, propylene glycol, methyl paraben, and glycerine were accurately weighed as per Table 2 and added to the hydrated carbapol 940 and stirred until uniform mixture is formed. Then, the pH was adjusted to 6 using triethanolamine.[12-15] The NLC formulation with least particle size and polydispersity index (PDI) and zeta potential within the acceptable range (TF-4) were added to the prepared gel slowly with continuous stirring.

Characterization of TRE-NLC

Particle size, PDI and zeta potential

The sonicated TRE-NLC dispersions TF-1, TF-2, TF-3, TF-4 and TF-5 were diluted with distilled water as medium. The particle size and PDI were measured in Malvern zeta sizer which works on the principle of dynamic light scattering. Similar sample preparations were used for the measurement of zeta potential in the same instrument.[15]

Entrapment efficiency

The NLC dispersion with least particle size and PDI and zeta potential within the limit (TF-4) was selected for further studies. The entrapment efficiency was determined indirectly by measuring the amount of unentrapped drug. 1 mL of NLC dispersion was taken and subjected to centrifugation for 30 min at 10,000 rpm. The supernatant liquid obtained was diluted with methanol and analyzed spectrometrically for the measurement of unentrapped drug.[16,17]

Morphological study

The surface morphology of TRE-NLC (TF-4) was obtained using Scanning electron microscopy (SEM). One drop of the NLC dispersion was placed on a glass slide and the excess water was made to evaporate in room temperature for 24 h. Gold coating was done under vacuum and then analyzed under accelerating voltage of 15–20 kV.[18]

Table 1: Composition of tretinoin nanostructured lipid carrier (TRE-NLC)

Ingredients	TF-1 (w/v) (%)	TF-2 (w/v) (%)	TF-3 (w/v) (%)	TF-4 (w/v) (%)	TF-5 (w/v) (%)
Cetyl alcohol	3.5	1.4	1.4	3.5	2.1
Oleic acid	1.5	0.6	0.6	1.5	0.9
Tween 80	4	1	2	2	2
Tretinoin	0.05	0.05	0.05	0.05	0.05
Distilled water	q.s	q.s	q.s	q.s	q.s

TF: Tretinoin formulation, NLC: Nanostructered lipid carrier, TRE: Tretinoin

Table 2: Composition of gel

Ingredients	Quantity (w/v) (%)
Carbapol 940	0.5
Hyaluronic acid	0.2
Propylene glycol	30
Glycerine	1
Methyl paraben and propyl paraben	0.4
DMSO	6
Triethanolamine	q.s
Distilled water	q.s

DMSO: Dimethyl sulfoxide

Characterization of TRE-NLC gel

Viscosity

The rheology of the developed gel was determined by Brooke field viscometer. The spindle type-S63 was used and the angular velocity was increased from 5 to 100 rpm.[15]

Spreadability

The spreadabilty of the gel was determined by Texture Analyzer (TA.XT plus). Calibration was done followed by placing the sample on the cone sample holder. The upper cone probe moves towards the sample holder and then the spreadability is measured.[19]

Extrudability

In order to measure the extrudability, 100 g of gel was loaded into the load cell of TA and compression force was applied moving at a speed of 1 mm/s with a force of 10 g.[19]

Bloom strength

100 g of gel was taken in a container and placed beneath the probe of TA. 10 g of trigger force was applied to measure the bloom strength.[19]

Drug content

Appropriate amount of gel was taken and diluted with ethanol and pH 7.4 phosphate buffer saline mixture (Et-PBS) in the ratio 50:50. The amount of drug present in gel was determined using UV-visible spectrophotometer at 339 nm against Et-PBS as blank.[20,21]

In vitro drug release and drug release kinetics

The *in vitro* drug release study for the TRE-NLC loaded gel was done using dialysis bag diffusion method. The dialysis bag was soaked in Et-PBS overnight. The dialysis bag was filled with gel equivalent to 0.25 mg of TRE and tied at both ends, placed in a 250 mL beaker containing 100 mL of Et-PBS medium. The beaker was placed over magnetic stirrer maintained at 37°C. Sampling was taken at hourly intervals for 8 h with the replacement of fresh medium each time. The samples were analyzed at 339 nm using UV-visible spectrophotometer. The *in vitro* release studies were also performed for marketed TRE gel in similar conditions.[22-24] The drug release kinetics of TRE-NLC gel was estimated using DD solver considering different kinetic models like zero order, first-order, Higuchi model, Hixson-Crowell model, Korsmeyer-Peppas model and Weibull model.

In vitro occlusion test

The occlusive nature of the formulation plays a major role in skin hydration by forming a barrier over the skin. The occlusion property helps to prevent the water loss from the skin thus maintaining the skin hydration.[25,26] 100 mL beakers were filled with distilled water and covered with cellulose acetate filter paper and sealed. 200 mg of TRE-NLC gel and marketed gel was spread evenly over the filter paper in separate beakers and kept for incubation at 37°C with 50–55% RH for 48 h. The water loss due to evaporation can be determined by weighing the beakers after incubation. The occlusion factor can be determined by the formula:

Occlusion factor (F)=(A−B)/A × 100

A- Water loss from the filter paper without sample (blank)

B- Water loss from the filter paper with sample.

If F is 0, no occlusive effect is seen and 100 represents maximum occlusive effect.

RESULTS AND DISCUSSION

Screening of lipids and ratio of solid lipid to liquid lipid

The solid and liquid lipids selected for the preparation of NLC were cetyl alcohol and oleic acid based upon the solubility of TRE in the lipids and their ability to show homogeneity. Both the lipids showed no separation of layers indicating their compatibility. The solid lipid to liquid lipid ratio selected was 70:30 as the melting point of the lipid mixture was found to be 49°C which shows the capacity to remain in its solid consistency at room temperature.[7]

Characterization of TRE-NLC

Particle size, PDI and zeta potential

The particle size and PDI of the batch TF-4 was found to be 129.6 nm and 0.181, respectively as shown in Figure 1. The zeta potential of batch TF-4 was found to be −24.6 mV as shown in Figure 2.

Morphological study (SEM)

The morphology of TRE-NLC was found to be spherical in shape as shown in Figure 3.

Entrapment efficiency

The percentage drug entrapped in the formulation TF-4 was found to be 99.34%.

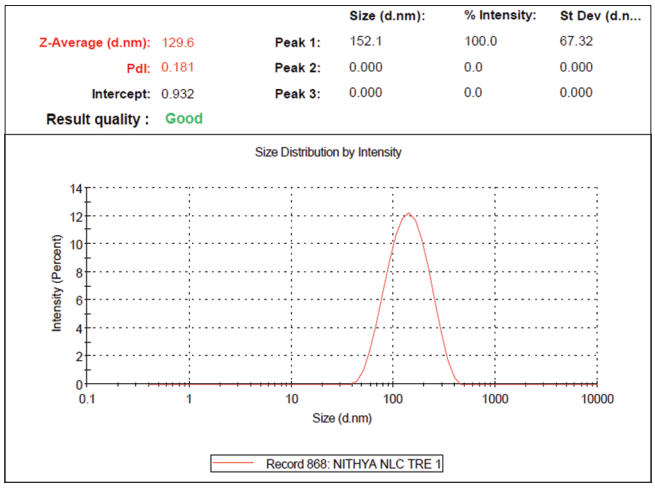

Figure 1: Particle size and polydispersity index of tretinoin nanostructured lipid carrier formulation TF-4

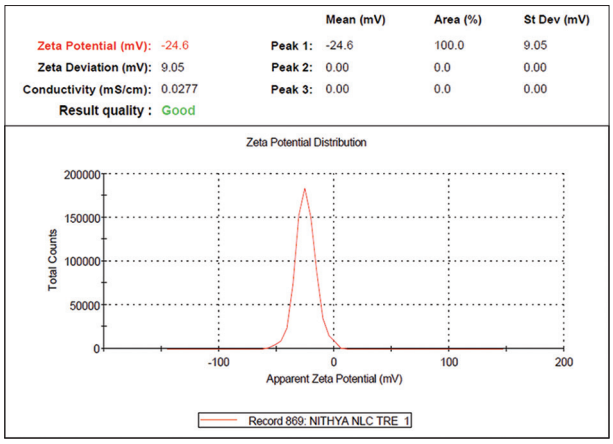

Figure 2: Zeta potential of tretinoin nanostructured lipid carrier formulation TF-4

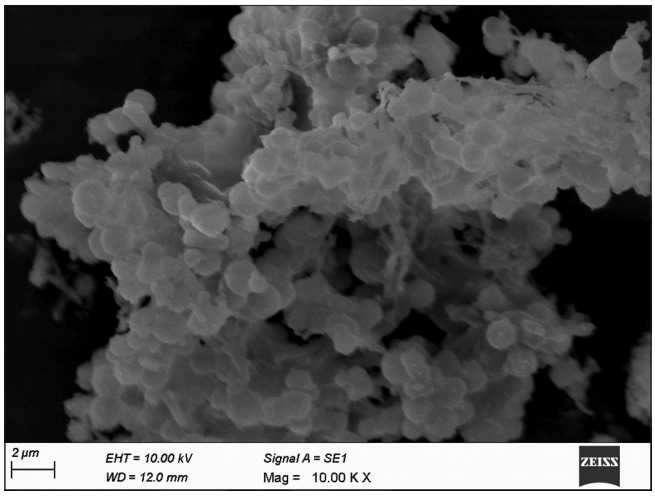

Figure 3: Scanning electron microscopy image of tretinoin nanostructured lipid carrier formulation TF-4

Characterization of gel

Viscosity

The viscosity of the gel decreases with the increase in shear rate which indicates that the gel follows non-Newtonian

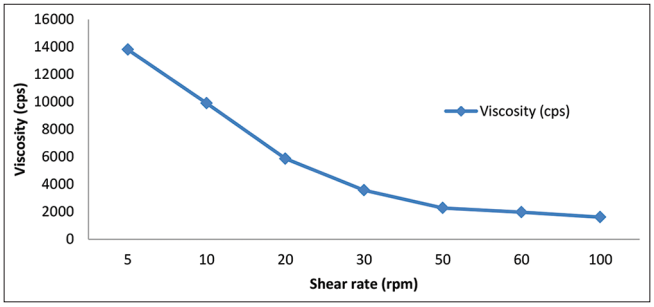

Figure 4: Rheological profile of tretinoin nanostructured lipid carrier gel

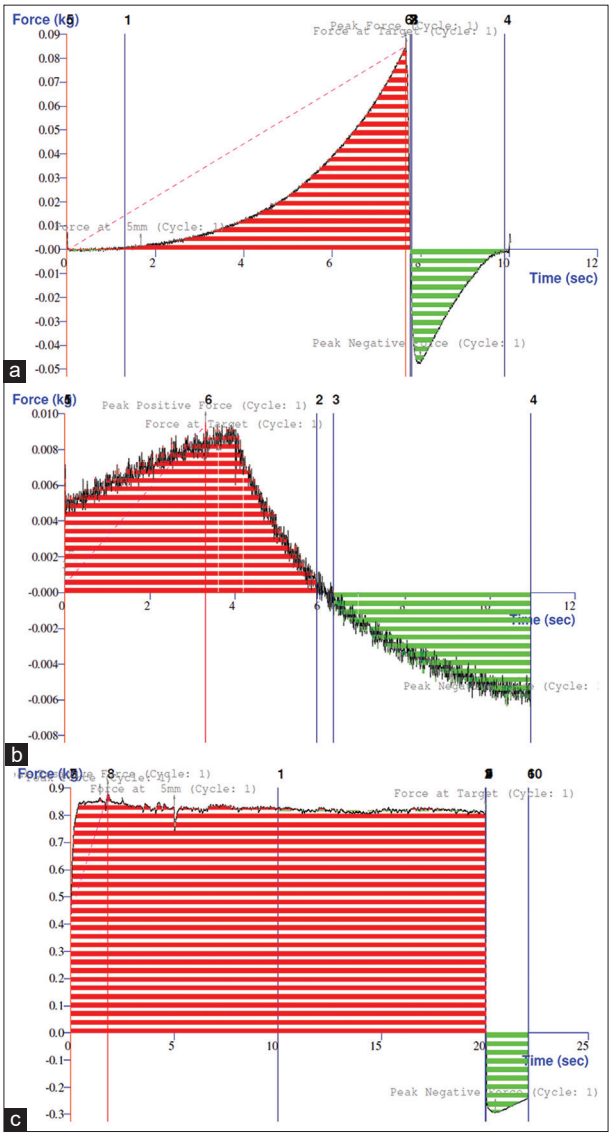

Figure 5: (a) Spreadability of tretinoin nanostructured lipid carrier (TRE-NLC) gel. (b) Bloom strength of TRE-NLC gel (c): Extrudability of TRE-NLC gel

Table 3: r^2 value for the *in vitro* release fitted in kinetic models for the gel

Zero order	First order	Higuchi model	Korsmeyer-peppas model	Hixson-crowell model	Weibull model
0.9549	0.8393	0.702	0.9858	0.8795	0.9893

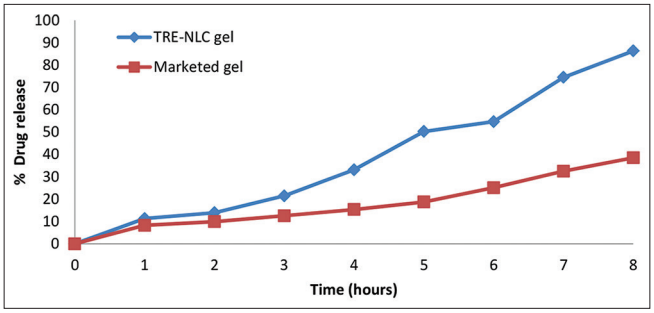

Figure 6: Comparison of *in vitro* release of tretinoin nanostructured lipid carrier gel and marketed gel

flow. The shear rate is plotted against viscosity as shown in Figure 4.

Spreadability, extrudability and bloom strength

The firmness and work of shear of the gel indicates the ability to spread over the skin. The firmness is indicated by the positive peak and work of shear is indicated by the negative peak. The firmness and work of shear was found to be 85 g and 164 g/sec as shown in [Figure 5a]. The bloom strength of the gel was found to be 0.462 g as shown in [Figure 5b] which suggests the force of resistance for penetration. Extrudability of the gel represents the force required to extrude the gel from a container which was found to be 818.059 g as shown in [Figure 5c].

Drug content, in vitro drug release and release kinetic studies

The amount of TRE present in the gel was found to be 99.98%. The TF-4 NLC formulation loaded into the gel showed release of 86.32% while the marketed TRE gel showed a release of 38.52% at the end of 8 h as shown in Figure 6. The increase in the release percentage of TRE from the formulated gel is influenced by the use of HA and glycerin. HA and glycerin are hydrophilic in nature and act as a permeation enhancer.[27,28] NLC aids in sustained release of TRE which helps to release the drug slowly. The *in vitro* drug release of the gel fitted into different kinetic models showed that the best fit model was Weibull model. The r^2 value was found to be 0.9893 as shown in Table 3.

In vitro occlusion test

The occlusion factor (F) for the TRE-NLC gel and marketed gel was found to be 42.29% and 4.76%, respectively. The lipids present in the TRE-NLC contribute to the occlusive effect which aids in preventing the loss of water, thereby helping in hydration while the marketed gel tends to have very low occlusive effect.

CONCLUSION

A topical gel containing HA was developed and then loaded with TRE-laden nanostructured lipid carrier prepared by melt-emulsification and ultrasonication method using different concentrations of lipids and surfactants. The TRE-NLC was evaluated for its particle size, PDI, zeta potential, entrapment efficiency and morphology. The NLC (TF-4) with the particle size of 129.6 nm was loaded into the gel and evaluated for its viscosity, spreadability, extrudability, bloom strength, drug content, *in vitro* drug release, drug release kinetics and *in vitro* occlusivity. The nano size range of NLC ensures deeper penetration and delivery of drug in the dermal layer. According to *in vitro* drug release profile, the drug is released slowly with 89.62% release at the end of 8 h. The HA added to the gel functions as a hydrating agent due to its ability to bind with large amount of water molecules. The occlusion effect produced by the gel ensures that the water loss from the skin surface can be diminished by the presence of lipids and HA.

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