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Solid lipid nanoparticles (SLN) of tretinoin: Potential in topical delivery

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Abstract

The objective of this investigation was to develop solid lipid nanoparticles (SLN) of tretinoin (TRE) with the help of facile and simple emulsification-solvent diffusion (ESD) technique and to evaluate the viability of an SLN based gel in improving topical delivery of TRE. The feasibility of fabricating SLN of TRE by the ESD method was successfully demonstrated in this investigation. The developed SLN were characterized for particle size, polydispersity index, entrapment efficiency of TRE and morphology. Studies were carried out to evaluate the ability of SLN in improving the photostability of TRE as compared to TRE in methanol. Encapsulation of TRE in SLN resulted in a significant improvement in its photostability in comparison to methanolic TRE solution and also prevented its isomerization. Furthermore, the skin irritation studies carried out on rabbits showed that SLN based TRE gel is significantly less irritating to skin as compared to marketed TRE cream and clearly indicated its potential in improving the skin tolerability of TRE. *In vitro* permeation studies through rat skin indicated that an SLN based TRE gel has permeation profile comparable to that of the marketed TRE cream.

Keywords: Tretinoin; Solid lipid nanoparticles (SLN); Emulsification-solvent diffusion; Photostability; SLN based gel; Draize patch test

1. Introduction

Tretinoin (TRE) or all-*trans* retinoic acid, a metabolite of Vitamin A has gained a great interest due to its multitude of physiological effects such as regulation of epithelial cell growth and differentiation, sebum production and collagen synthesis (Lucek and Colburn, 1985; Allen and Bloxham, 1989; Zouboulis, 2001). Due to these physiological effects, TRE is employed in the topical treatment of various proliferative and inflammatory skin diseases, such as psoriasis, acne, photoaging, epidermotropic T-cell lymphomas or epithelial skin cancer (Lucek and Colburn, 1985; Allen and Bloxham, 1989; Zouboulis, 2001). 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 may limit its incorporation in a suitable vehicle, while its poor photostability may render the topically applied drug ineffective. Furthermore, the topical application of TRE 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 (Lehman et al., 1990; Nighland et al., 2006). Thus, TRE represents a great challenge to the formulation scientists due to unique problems associated with its delivery. In view of this, researchers have explored the potential of various delivery strategies such as liposomes (Masini et al., 1993; Schäfer-Korting et al., 1994; Patel et al., 2000; Contreras et al., 2005), niosomes (Manconi et al., 2003) and inclusion complexes (Amidouche et al., 1994; Anadolu et al., 2004) in improving the problems associated with topical delivery of TRE. Amongst these approaches, liposomes have been investigated to the greatest extent by several investigators and their utility in reducing dermal side effects of TRE such as skin irritation, erythema, peeling and burning and in improving photostability has been successfully established (Schäfer-Korting et al., 1994; Patel et al., 2000; Ioele et al., 2005). However, the cost factor associated with liposomes and their inherent physical instability may limit their utility and commercialization.

Solid lipid nanoparticles (SLN) have emerged as an alternative to liposomes due to various advantages such as improved physical stability, low cost compared to phospholipids and ease of scale-up and manufacturing (Müller et al., 2000; Mehnert and Mader, 2001; Wissing and Müller, 2003). Moreover, their

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potential in epidermal targeting (Maia et al., 2002; Chen et al., 2006; Lopes et al., 2006; Liu et al., 2007), follicular delivery (Münster et al., 2005), controlled drug delivery (Müller et al., 2000) and photostability improvement of active pharmaceutical ingredients (Iannuccelli et al., 2006) has been very well established and is equivalent to liposomes. In view of this, exploring the potential of SLN in improving the topical delivery of TRE seems worthwhile. The main aim of our investigation was to develop and evaluate an SLN based TRE (0.05%, w/w) gel for topical delivery of TRE. The proposed TRE concentration is chosen on the basis of the marketed TRE formulations. Furthermore, in the present investigation, we aimed at fabricating SLN of TRE by using simple methods such as emulsification-solvent diffusion (ESD) and with easily available solid lipids such as glyceryl monostearate (GMS). The ability of SLN to improve photostability and skin tolerability of TRE was also investigated in the present study.

2. Materials and methods

2.1. Materials

Tretinoin (Shalaks Pharmaceuticals, New Delhi, India); glyceryl monostearate (Fine Organics Ltd., Mumbai, India); Compritol 888 ATO (Colorcon-Asia Ltd., Mumbai, India); Dynasan 116 (S. Zhaveri & Co., Mumbai, India); Cutina CBS (B.S. International, Mumbai, India); Epikuron 200 (Degussa GmbH, Düsseldorf, Germany); Xanthan Gum (Signet Chemicals, Mumbai, India); Carbopol® Ultrez 10, Carbopol® 940 and Carbopol® ETD 2020 (Noveon Chemicals, Mumbai, India) were received as gift samples. Mono- and Di-basic sodium phosphate, benzyl alcohol, Tween 80 (all AR grade) and Acetonitrile (HPLC grade) were purchased from S.D. fine chemicals (Mumbai, India). Retino-A® cream (Janssen-Cilag, India) was purchased from local market. All the excipients and reagents were used as received. Double distilled water was prepared freshly whenever required.

2.2. Solubility of TRE in solid lipids

One of the most important factors that determines the loading capacity of the drug in the lipid is the solubility of drug in melted lipid. However, equilibrium solubility studies cannot be carried out in this case. Hence, we used a modified method (Joshi and Patravale, 2006) to identify the solid lipid having better solubilization potential for TRE. GMS, Compritol 888 ATO, Dynasan 116 and Cutina CBS were screened for their potential to solubilize TRE. Briefly, 10 mg of TRE was taken in wide mouth screwcapped bottles covered externally with aluminium foil to prevent the photodegradation of TRE. The solid lipid was seperately heated above its melting point. This lipid melt was gradually added in portions to TRE with continuous stirring using cyclomixer. The amount of molten lipid required to solubilize the TRE was noted visually. The end point of the solubility study was the formation of clear, pale yellow solution of molten lipid. The solid lipid was selected on the basis of the solubilizing potential and the acceptability by topical, peroral and parenteral route.

2.3. Fabrication of SLN by emulsification-solvent diffusion (ESD) method

SLN were prepared by using method reported by Trotta et al. (2003) with slight modifications. Briefly, benzyl alcohol and water were mutually saturated at 55 ± 2 °C for 10 min in order to ensure initial thermodynamic equilibrium of both liquids. Typically, GMS, Epikuron 200 and TRE were dissolved in 2 g of water-saturated benzyl alcohol maintained at 55 °C. This organic phase was then emulsified at 55 °C with 15.75 g of benzyl alcohol-saturated aqueous solution containing 200 mg of Tween 80 using an over head stirrer (Remi, India) at 3000 rpm for 2 min. SLN were precipitated by quickly pouring this transient emulsion formed (20 g) into the aqueous phase (80 ml) containing a mixture of Tween 80 and Tween 20 maintained at 55 °C under over head stirring to extract the benzyl alcohol into the continuous phase. Stirring was continued for about 20 min until the dispersion cooled.

2.4. SLN characterization

2.4.1. Particle size determination

The average particle size and polydispersity index of the lipid particulate dispersions obtained via various experiments were determined in duplicate by the photon correlation spectroscopy (PCS; Beckman Coulter N4 plus, Wipro, India). Measurements were carried at an angle of 90° at 25 °C. Dispersions were diluted with double distilled water to ensure that the light scattering intensity was within the instrument's sensitivity range. Double distilled water was filtered through 0.45 μ membrane filters (Pall Life sciences, Mumbai) prior to particle size determination.

2.4.2. Entrapment efficiency (EE)

The entrapment efficiency (EE), which corresponds to the percentage of TRE encapsulated within and adsorbed on to the nanoparticles, was determined by measuring the concentration of free TRE in the dispersion medium (Joshi and Patravale, 2006; Pattani et al., 2006).

A known dilution of the SLN dispersion was prepared and 500 μl of it was transferred to the upper chamber of Nanosep® centrifuge tubes fitted with an ultrafilter (MWCO100KD, Pall Lifesciences, Mumbai, India). The Nanosep® was centrifuged at 14,000 rpm (Eltek TC 4100 D Research Centrifuge) for 40 min. The filtrate was analyzed for unencapsulated TRE at 344 nm by using validated UV-spectrophotometric method after suitable dilution.

The entrapment efficiency was calculated by the following equation:

$$\%\mathrm{EE} = \left[\frac{M_{\text{initial drug}} - M_{\text{free drug}}}{M_{\text{initial drug}}}\right] \times 100$$

where "$M_{\text{initial drug}}$" is the mass of initial drug used for the assay and the "$M_{\text{free drug}}$" is the mass of free drug detected in the supernatant after centrifugation of the aqueous dispersion.

2.4.3. Effect of lipid load on particle size of SLN and entrapment efficiency of TRE

To study the effect of lipid load or concentration on the particle size of SLN and entrapment efficiency of TRE, various amounts of GMS were used such that the percentage (w/w) of GMS in the final diluted emulsion was 1, 1.5 and 2%, respectively.

2.4.4. Scanning electron microscopy (SEM)

The morphology of the particles obtained by ESD method was examined. Samples were analyzed in the form of aqueous dispersion using Quanta 200 ESEM (FEI, USA) (magnification: 20,000×; accelerating voltage: 20.0 kV). Analysis was performed at 25 ± 2 °C.

2.5. UV analysis of TRE

For UV method, a standard solution of TRE (100 μg/ml) was prepared by dissolving accurately weighed quantity of TRE in methanol and working standards were prepared by dilution of this standard solution with methanol. The absorbance of the resulting solutions was recorded at 344 nm in a 10 mm quartz cell on a Shimadzu UV-1650 UV–vis double beam spectrophotometer (Shimadzu, Japan). The assay was linear ($r^2 = 0.999$; % CV = 1.12) in the concentration range 1–10 μg/ml. Sufficient care was taken to prevent the exposure of TRE to light.

2.6. Evaluation of photostability

The photostability of TRE entrapped in SLN was evaluated in comparison to the methanolic solution of TRE using the method suggested by Ioele et al. (2005). The photodegradation of TRE was monitored by recording its absorption spectra in the wavelength range of 200–500 nm in a 10 mm quartz cell on a Shimadzu UV-1650 PC UV–vis Spectrophotometer at the following conditions: scan speed—slow; time response 1 s; spectral band 1 nm. The software Shimadzu UV Probe (version 2.10) was used for spectral acquisition. Briefly, 10 ml of methanolic solution and SLN dispersion of TRE (concentration: 10 μg/ml) was exposed to natural sunlight and the UV spectra of both the samples were recorded just after preparation ($t = 0$) and at the following time intervals: 5, 30, 60, 90, 120 and 180 min, after suitable dilution with methanol. In case of SLN, baseline correction was done using a placebo dispersion diluted suitably with methanol to nullify any possible absorption arising from the solid lipid. Sufficient care was taken to maintain similar experimental conditions for both the samples, i.e. TRE in methanol and TRE in SLN.

2.7. Formulation of SLN based TRE gels

Various gelling agents namely, Xanthan Gum, Carbopol® Ultrez 10, Carbopol® 940 and Carbopol® ETD 2020 were evaluated for their ability to gel SLN dispersion of TRE (0.05%, w/w). The suitable gelling agent was selected on the basis of compatibility with nanoparticulate dispersion, feel and ease of spreadability.

2.8. Characterization of the SLN based TRE gel

2.8.1. Determination of drug content, pH and spreadability

For determination of drug content, about 1 g of the gel was weighed in a 100 ml volumetric flask and dissolved in methanol; it was diluted appropriately and analyzed on a Shimadzu UV-1650 PC UV–vis Spectrophotometer managed by Shimadzu UV probe version 2.10 at a λ-max of 344 nm. The spreadability of the gel was determined using the following technique: 0.5 g gel was placed within a circle of 1 cm diameter premarked on a glass plate over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5 min. The increase in the diameter due to spreading of the gels was noted. The pH of the 10% (w/w) gel was determined using Equip-tronic Digital pH meter Model EQ 610, standardized using pH 4.0 and 7.0 standard buffers before use.

2.8.2. Rheological studies on the gel

Brookefield Synchro-Lectric Viscometer (Model RVT) with helipath stand was used for rheological studies. The sample (30 g) was placed in a beaker and was allowed to equilibrate for 5 min before measuring the dial reading using a T-C spindle at 0.5, 1, 2.5, and 5 rpm. At each speed, the corresponding dial reading on the viscometer was noted. The spindle speed was successively lowered and the corresponding dial reading was noted. The measurements were carried in duplicate at ambient temperature. Direct multiplication of the dial readings with factors given in the Brookfield viscometer catalogue gave the viscosity in centipoises. The consistency index and flow index were calculated from the Powerlaw equation

$$\tau = Kr^n$$

where "τ" is the shear stress; "r" the shear rate; "K" the consistency index; "n" is the flow index.

Taking log of both sides,

$$\log \tau = \log K + n \log r$$

$$\text{Shear stress (dyn/cm}^2\text{)} = \text{Viscosity (mPa s)} \times \text{Rate of shear (s}^{-1}\text{)}$$

Thus, from the plot of log of shear stress versus log of shear rate, the slope of the plot representing flow index and antilog of the y-intercept indicating consistency index was calculated.

2.9. Skin-irritation testing (Draize patch test)

The irritation potential of the SLN based TRE gel in comparison to marketed TRE cream was evaluated by carrying out the Draize patch test on rabbits (Draize et al., 1944; Verneer, 1991; Joshi and Patravale, 2006). Animal care and handling throughout the experimental procedure were performed in accordance to the CPCSEA guidelines. The experimental protocol was approved by the Animal Ethical Committee of University Institute of Chemical Technology. White New Zealand rabbits weighing 2.5–3 kg were obtained from Nicholas Piramal Research Centre,

Mumbai, India and were acclimatized before the beginning of the study.

Animals were divided into four groups ($n = 3$) follows:

Group 1: No application (Control).
Group 2: Marketed formulation (Retino-A® cream containing 0.05% w/w TRE, Janssen-Cilag, India).
Group 3: SLN based gel without TRE (Placebo gel).
Group 4: SLN based gel containing TRE (0.05%, w/w).

The back of the rabbits were clipped free of hair, 24 h prior to the application of the formulations. Formulations, 0.5 g, were applied on the hair free skin of rabbits by uniform spreading within the area of 4 cm^2. The skin was observed for any visible change such as erythema (redness) at 24, 48 and 72 h after the application of various formulations. The mean erythemal scores were recorded (ranging from 0 to 4) depending on the degree of erythema as follows: no erythema = 0, slight erythema (barely perceptible-light pink) = 1, moderate erythema (dark pink) = 2, moderate to severe erythema (light red) = 3, and severe erythema (extreme redness) = 4 (Draize et al., 1944).

2.10. In vitro skin permeation studies

In vitro permeation of TRE from SLN based gel and marketed formulation (Retino-A® cream, Janssen-Cilag, India) was evaluated using hairless abdominal rat skin samples excised from animals aged about 3 months. The Wistar rats (weight range: 200–250 g) were obtained from Bombay Veterinary College, Mumbai, India.

The skin samples were mounted on modified Franz diffusion cells with a surface of 3.14 cm^2 and a receptor volume of 10 ml such that the dermal side of the skin was exposed to the receptor fluid and the stratum corneum remained in contact with the donor compartment. The receptor fluid (pH 7.4) consisted of a phosphate buffered physiological albumin solution which is described in the investigation reported by Contreras et al. (2005). TRE is freely soluble in this modified phosphate buffered solution despite its insolubility in plain phosphate buffer. Then, 0.45 g of the formulation (either marketed formulation or SLN based gel) was placed with a curved spatula in the donor compartment enabling a gel film to cover the entire skin surface evenly. Then the diffusion cells were covered with an aluminium foil to prevent light exposure. The temperature was maintained at 37.0 ± 0.1 °C. Sampling was done at 1, 4, 6, 8, and 12 h. At each point, 3.0 ml aliquots were drawn from the receiver compartment. Thereafter, an equivalent volume of receptor fluid was replaced to the receiver compartment. The concentration of TRE in receptor fluid was analyzed with the HPLC method described below. The total quantity of the drug (Q) that diffused to the receptor compartment in time (t) during the steady state and the flux at the steady state, J_s [μg/(cm^2 h)], was calculated using the linear portion of the correlation between the accumulated quantity of TRE that diffused through the skin by unit area and time. All the experiments were performed in triplicate.

2.11. HPLC analysis of TRE

The amount of TRE in the receptor compartment was determined by the reverse-phase HPLC method developed in house. The HPLC apparatus consisted of Jasco PU-2080 Plus Intelligent HPLC pump (Jasco, Japan) equipped with a Jasco UV-2075 Intelligent UV/vis detector (Jasco, Japan), a Rheodyne 7725 injector (Rheodyne, USA), a Jasco Borwin Chromatography Software (version 1.50) integrator software and a Hi-Q-Sil C_{18} (4.6 mm × 250 mm and 10 μm particle size) column. The mobile phase consisted of a mixture of methanol:acetonitrile:pH 6.8 phosphate buffer (65:20:15, v/v) at a flow rate of 1.2 ml/min that led to retention time of 6.5 min for when detection was carried out at 350 nm. The assay was linear ($r^2 = 0.999$; % CV = 1.22) in the concentration range 0.05–100 μg/ml with the lowest detection limit of 35 ng/ml of TRE. The method was validated in terms of accuracy (% CV = 1.31) and precision (% CV = 1.19).

2.12. Statistical analysis

Data obtained from skin permeation experiments were expressed as mean ± standard error (3 independent samples). The flux values of TRE and amount of TRE permeated at the end of 12 h from the SLN based gel and marketed cream were analyzed utilizing two-tailed paired 't' test (GraphPad InStat Demo Version). Differences were considered statistically significant at $P < 0.05$.

3. Results and discussion

3.1. Solubility of TRE in solid lipids

Solubility studies (Fig. 1) indicated that amongst the Compritol 888 ATO (Atomized glyceryl behenate), Cutina CBS (mixture of glyceryl stearate, ceteraryl alcohol, cetyl palmitate and cocoglyceride), Dynasan 116 (Tripalmitin) and GMS

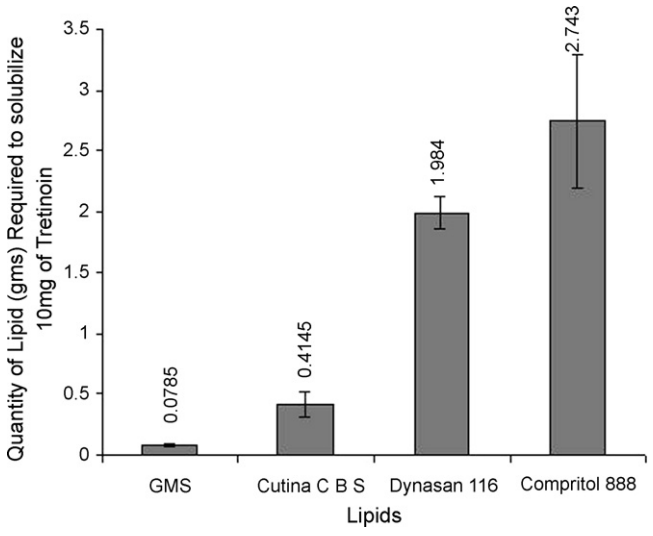

Fig. 1. Screening of solid lipids for their ability to solubilize TRE. Data expressed as mean ± S.D. ($n = 3$).

(glyceryl monostearate), GMS effectively solubilized TRE. The solubilizing potential coupled with already reported biocompatibility and acceptability of GMS for topical, peroral and parenteral route (Rowe et al., 2003a) has favored its selection for the present study.

3.2. Fabrication of SLN by emulsification-solvent diffusion (ESD) method

In the present investigation, benzyl alcohol was selected as a partially miscible solvent for the preparation of SLN due to its fairly good dermal acceptability as compared to other partially miscible solvents such as isobutyric acid and ethyl acetate. Moreover, benzyl alcohol has good solubilizing potential for GMS at 55 °C. The mutual saturation of benzyl alcohol and water was very critical for the preparation of SLN as the attempts to prepare SLN without saturation of benzyl alcohol and water were not successful and led to generation of microparticles (data not shown). The method of fabrication of SLN described herein, is based on the investigation reported by Trotta et al. (2003). However, they have reported fabrication of SLN of plain GMS (without any drug). In this investigation, we demonstrated suitability of the ESD method for encapsulation of hydrophobic drugs like TRE. The particle size observed in our investigation was almost in line with the particle size reported by Trotta et al. (2003) and is shown in Fig. 2. No efforts were made to remove benzyl alcohol and surfactants in the SLN dispersion, as all the excipients used in this study, at the concentration present in the dispersion, are acceptable for topical application (Rowe et al., 2003b). The benzyl acohol at the concentration present in the final dispersion has negligible solubilizing potential for GMS at the room temperature (data not shown) and is unlikely to affect SLN structure and integrity. Instead, it would act as a preservative for the SLN dispersion.

3.3. SLN characterization

The particle size data is depicted in Fig. 2. It is evident that the particle size and polydispersity index of SLN increase with

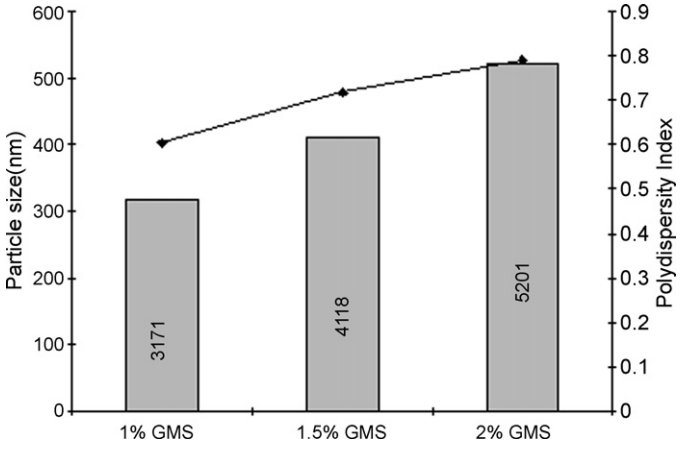

Fig. 2. Particle size and polydispersity index of TRE loaded SLN as a function of increasing GMS content. Particle size and polydispersity index expressed as mean ($n = 3$), where relative standard deviation was <5%.

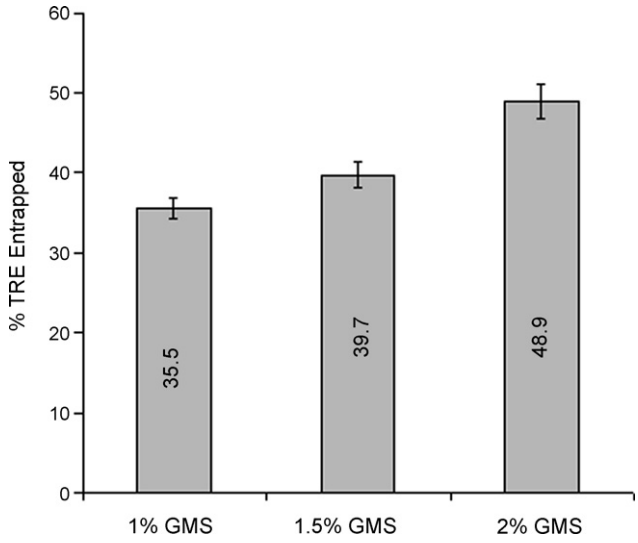

Fig. 3. Entrapment efficiency of TRE in SLN as a function of increasing GMS content. Data expressed as mean ± S.D. ($n = 3$).

the increase in the GMS content in the final dispersion. This could be due to the increased globule size of the primary emulsion with the increase in lipid content (or dispersed phase). It should be noted that although lipid content was increased in the primary emulsion, the stabilizer concentrations were kept constant. Hence, for the same concentration of stabilizers, there is likely to be increase in the globule size and polydispersity index of the primary emulsion with the increase in the lipid content or dispersed phase and the same is reflected in the particle size of SLN. The results of entrapment efficiency are shown in Fig. 3. Although, all the concentrations of the GMS used in this investigation are able to solubilize the target amount of TRE (50 mg), the encapsulation efficiency was not very high. This could be due to the partitioning of the TRE between GMS and benzyl alcohol. TRE has good solubility in benzyl alcohol (>50 mg/g at 55 °C) and hence it may get partitioned in the outer phase of the primary emulsion during the fabrication process. This is also supported by the observation that the increase in the lipid content (which reduces the partition of TRE in outer phase) leads to the increase in the entrapment efficiency but the increase in the entrapment is not proportional to the increase in the lipid content.

The SEM image (Fig. 4) revealed that the particle size was in nanometric range and that the particles had nearly spherical morphology.

3.4. Evaluation of photostability

The spectral curves recorded on the methanolic solution of TRE (10 μg/ml) at the various times of light exposure are shown in Fig. 5(a). The light exposure caused a sharp degradation in the first few minutes of irradiation with a contemporary shift of maximum peak from 344 to 340 nm indicating the isomerization of TRE. Rate of photodegradation of TRE slowed down after 5 min but the photodegradation process continued to occur further, leaving a small residual tretinoin concentration of ∼12%

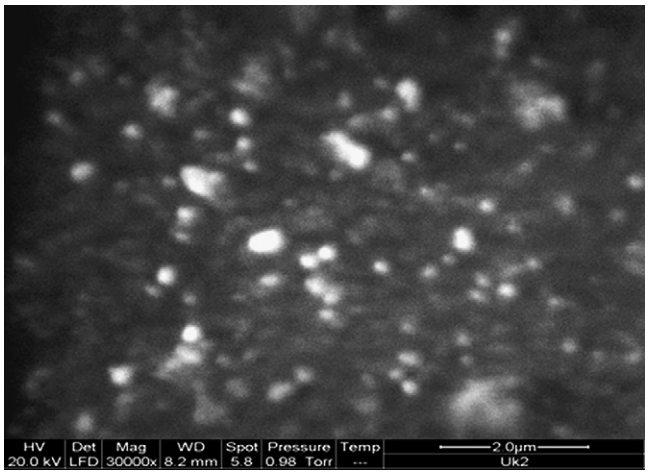

Fig. 4. SEM image of TRE loaded SLN.

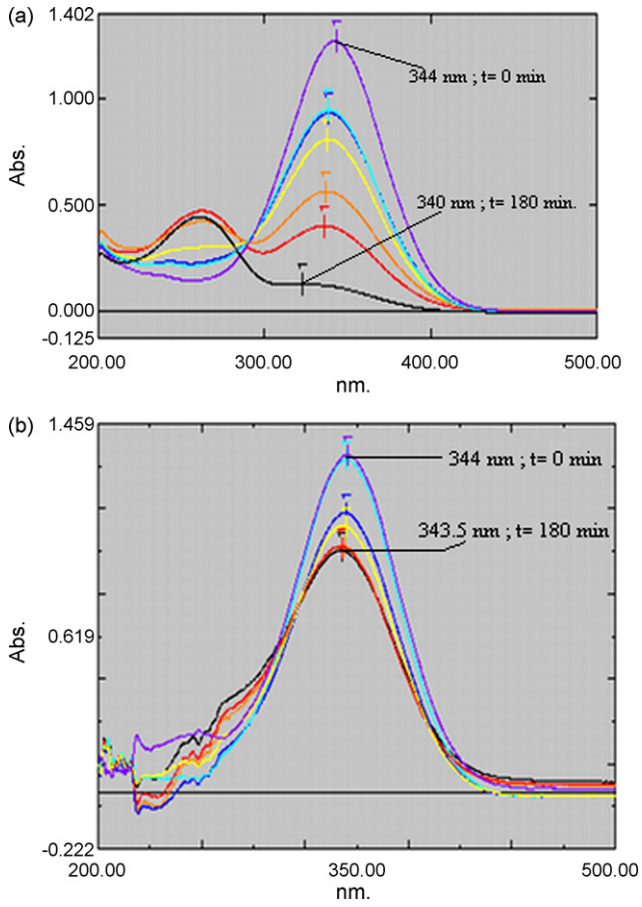

Fig. 5. Photodegradation of TRE after exposure to sunlight for different durations (a) methanolic solution of TRE; (b) SLN dispersion containing TRE. The reduction in absorbance values indicates the photodegradation.

0 min	5 min	30 min	60 min	90 min	120 min	180 min

at the end of 180 min. These observations demonstrate very high photolability of TRE and the results are in accordance with that of Ioele et al. (2005). Fig. 5(b) shows the spectral curves of TRE in SLN obtained after the sequential exposure times. As can be seen, the entrapment of TRE into SLN strongly reduced its photodegradation in comparison to the photodegradation of TRE in methanol. Furthermore, a very negligible shift of the maximum peak at 344 nm was observed indicating that TRE did not isomerize during the irradiation process. Also, a residual TRE concentration of ~71% was still present after irradiation for 180 min which was significantly higher in comparison to TRE concentration in methanolic solution at the end of 180 min ($P < 0.01$ when analyzed with two-tailed paired '*t*' test, GraphPad InStat Demo Version). The dramatic improvement in the photostability of TRE indicates that the drug is not just adsorbed on the SLN, rather a considerable amount of TRE is embedded in the SLN matrix. In nutshell, we can conclude that SLN exhibit a good potential to improve the photostability of TRE and can be employed as a valuable delivery strategy.

We understand that it would have been more appropriate to compare the photodegradation of TRE in SLN with the photodegradation of TRE in Retino-A® cream. However, such a comparison was not done as it was not possible to obtain freshly prepared Retino-A® cream to enable appropriate comparison with the freshly prepared SLN of TRE. But it is to be noted that the recent investigation carried out by Nighland et al. (2006) reports that marketed TRE formulation showed 81% degradation of TRE after 2 h of exposure to simulated solar UV irradiation.

3.5. Formulation of SLN based gel and characterization

Xanthan gum yielded gels with low viscosity and exhibited tackiness. Carbopol® Ultrez 10 and Carbopol® 940 resulted in the agglomeration of SLN. Carbopol® ETD 2020, at the concentration of 1% (w/w) was able to gel the SLN dispersion yielding gel with desired characteristics without inducing agglomeration of SLN. Hence, Carbopol ETD 2020 was selected for the preparation of SLN based TRE gel. Briefly, Carbopol ETD 2020, 1 g, was added to the 100 ml SLN dispersion containing TRE (theoretical TRE concentration = 0.05%, w/w) under overhead stirring at 800 rpm. Stirring was continued till Carbopol gets dispersed. The dispersion was neutralized using 50% (w/w) triethanolamine solution. The TRE content of the SLN based gel was found to be 97.2 ± 4.20% of the theoretical value (0.05%, w/w) and pH was found to be 6.8 which are in acceptable limits. Spreadability is an important property of topical formulation from patient compliance point of view. Application of the formulation on inflamed part would be more comfortable if the base spreads easily exhibiting maximum 'slip' and 'drag'. The diameter was found to be 5.8 cm which is indicative of good spreadability of the SLN based gel.

The results of various rheological parameters of SLN based TRE gel (gelled with Carbopol® ETD 2020) viz. viscosity, consistency index and flow index are listed in Table 1. The SLN based TRE gel (gelled with Carbopol® ETD 2020) was

Table 1
Evaluation of SLN based TRE gel

Evaluation parameters	Result
pH	6.8
Spreadability	5.8 cm
Viscosity (at 5 rpm)	88×10^5 mPa s
Consistency index	24.05×10^6 dyn/cm^2
Flow index	0.381

used further for the skin-irritation testing and *in vitro* skin permeation.

3.6. Primary skin irritation studies

One of the major disadvantages associated with the TRE therapy is skin irritation (erythema), which strongly limits its utility and acceptability by the patients. Ideally, the delivery system of TRE should be able to diminish or abolish these erythematic episodes. However, most of the currently marketed conventional dosage forms such as creams, lotions and gels are not able to reduce the irritation caused by topical application of TRE. It was hypothesized that encapsulation of TRE in SLN would reduce the contact of the acidic function (–COOH) of TRE (the triggering factor for the erythematic events) (Yamaguchi et al., 2005) with the stratum corneum thus resulting in reduced erythematic episodes. The results obtained from the primary skin irritation studies are listed in Table 2 and the actual photographs are depicted in Fig. 6(A)–(D). Draize patch test is a reliable method and the results obtained from this study can be linked to that obtained in humans. The skin-irritation studies indicated that SLN based TRE gel resulted in a considerably less irritation as compared to marketed TRE formulation (Retino-A® cream) after 24 h of application (Table 2 and Fig. 6). The irritation continued to increase even after 24 h in case of marketed TRE formulation whereas it did not increase in case of SLN based TRE gel (Table 2). Thus, SLN based gel demonstrated remarkable advantage over marketed formulation in improving the skin tolerability of TRE indicating their potential in improving patient acceptance and topical delivery of TRE.

Table 2
Mean erythemal scores observed for various TRE formulations obtained at the end of 24, 48 and 72 h

Formulation	Erythemal scores ($n = 3$)		
	24 h	48 h	72 h
Control (Group 1)	0	0	0
Marketed cream (Group 2)	3	4	4
SLN based gel without TRE (Group 3)	0	0	0
SLN based gel containing TRE (Group 4)	0	1	1

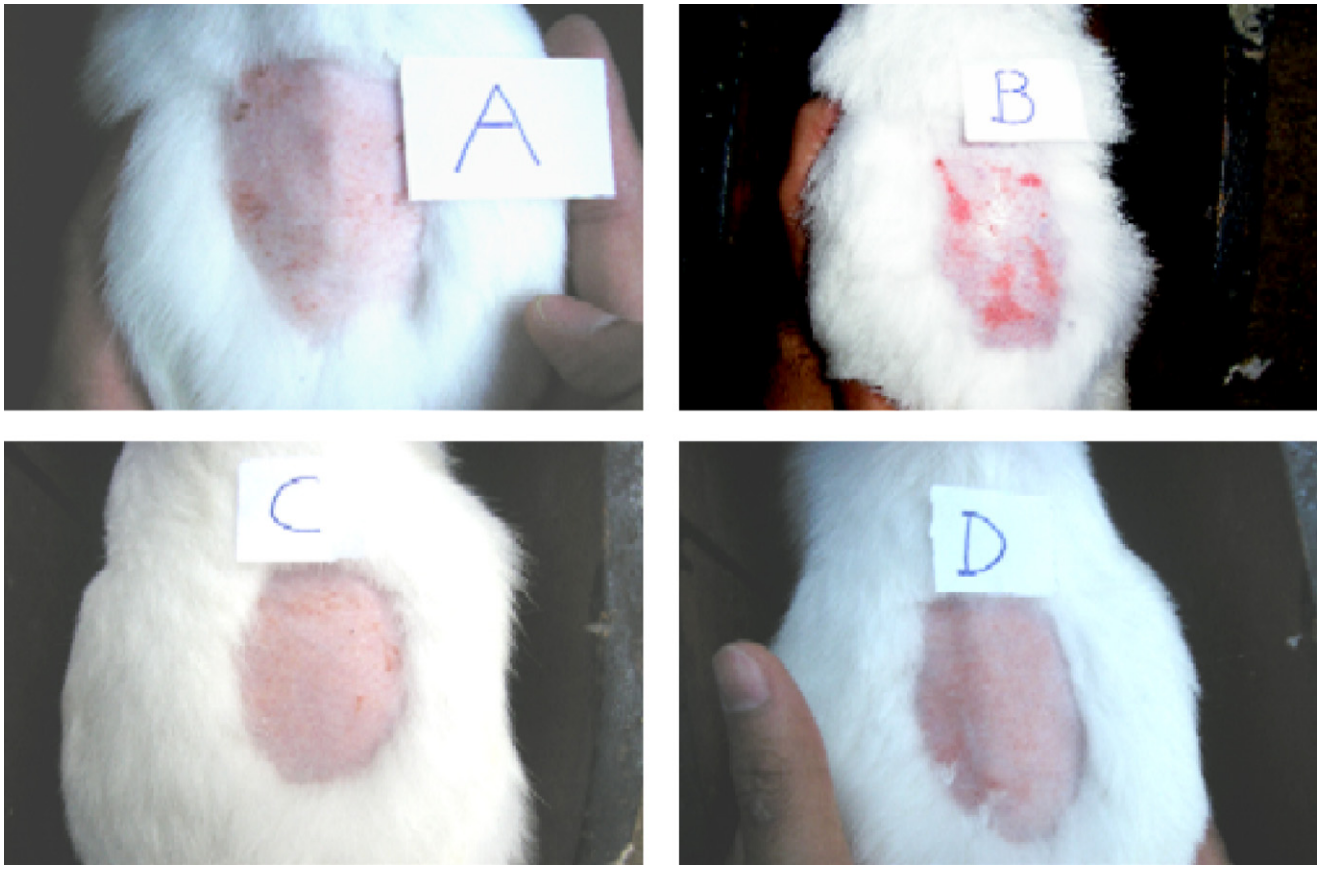

Fig. 6. Photographs of skin irritation studies carried out on New Zealand rabbits (A) control (no application); (B) marketed formulation (Retino-A® cream); (C) SLN based gel without TRE; (D) SLN based gel containing TRE (0.05%, w/w). Photographs have been taken after 24 h. Marketed TRE cream clearly shows erythemal lesions, which are not visible in SLN based TRE gel.

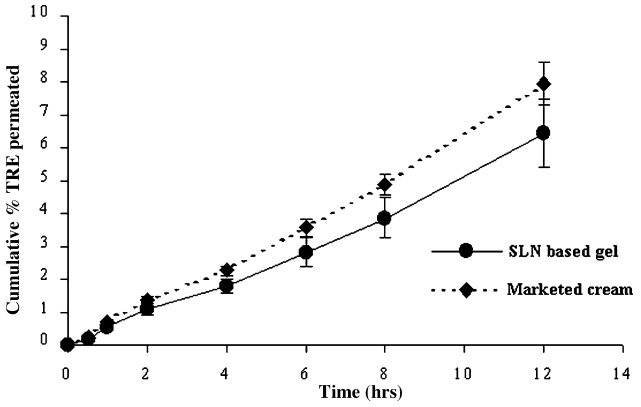

Fig. 7. *In vitro* permeation profile of TRE through rat skin from the SLN based gel and marketed cream. Data expressed as mean ± S.D. ($n = 3$).

3.7. In vitro skin permeation

The *in vitro* permeation of TRE through rat skin from SLN based gel and marketed cream (Retino-A®) was calculated in terms of mean cumulative amount diffused at each sampling time point during time period of 12 h (Fig. 7). The cumulative amount of TRE permeated (expressed as % dose) after 12 h from the SLN based gel and marketed formulation and TRE flux values are reported in Table 3. Moreover, plot of the amount of TRE permeated (from both the formulations) as a function of time, showed a linear relationship ($r^2 = 0.99$), thus indicating that TRE permeation followed pseudo-first order kinetics. Although, we did not attempt to establish the mechanism of TRE permeation through skin from SLN based gel, we believe that TRE might be getting transported in an encapsulated form (encapsulated in the SLN matrix). This hypothesis is also supported by the skin irritation studies carried out in rabbits. If TRE were being released from SLN before permeating through skin, then we would have observed significant irritation as observed in case of marketed TRE cream. While getting transported across the skin, SLN then probably expel TRE from SLN matrix as a consequence of polymorphic transitions occurring in the solid lipid. This phenomenon has been hypothesized in some investigations (Mei et al., 2003; Jain et al., 2005). SLN have been shown to improve the dermal localization of several topical therapeutic agents (Maia et al., 2002; Chen et al., 2006; Lopes et al., 2006; Liu et al., 2007). This was one of the reasons to employ SLN approach for topical delivery of TRE as its epidermal localization is highly desirable for enhancing the treatment of skin diseases such as psoriasis, acne, photoaging and epithelial skin cancer. Though the flux value of SLN based TRE gel is lesser than marketed TRE cream, the difference is not significant ($P > 0.05$). Similar observations have been reported in the recent literature (Sivaramakrishnan et al., 2004; Borgia et al., 2005). However, it should be noted that TRE flux value observed with the SLN based gel was almost equivalent to the flux values of TRE (through rat skin) reported in literature for TRE liposomal formulations (Contreras et al., 2005). Further studies would be focused on human cadaver skin and would be carried out for longer duration in order to get a proper insight into the potential of SLN based gel in TRE delivery.

Table 3
Mean values of cumulative TRE permeated at the end of 12 h and flux in marketed cream and SLN based gel

Formulation	Amount of TRE permeated at the end of 12 h (% of applied dose)	Flux value (ng/cm^2 h)
Marketed TRE cream	7.956 ± 0.651	77.7 ± 6.82
SLN based TRE gel	6.414 ± 1.031	75.6 ± 7.21

Data expressed as mean ± S.D. ($n = 3$); differences in the amount of TRE permeated and flux values are not statistically significant ($P > 0.05$).

4. Conclusion

SLN of TRE could be successfully fabricated with the help of simple and facile ESD method. Encapsulation of TRE in SLN resulted in dramatic improvement in its photostability as compared to TRE in methanol. The SLN based TRE gels of TRE could successfully be formulated. Draize patch testing indicated that the SLN based TRE gel resulted in remarkably less erythematic episodes as compared to currently marketed TRE cream. Thus, SLN based TRE gel can offer improved topical delivery of TRE as compared to existing conventional TRE formulations in terms of skin tolerability by patients and would be a viable alternative for them.

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