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Development of SLNs from natural lipids: Application to topical delivery of tretinoin

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ABSTRACT

The aim of this investigation was to develop solid lipid nanoparticles (SLNs) from indigenous, natural solid lipids by using a simple microemulsion technique. Furthermore, the aim was to characterize these SLNs and evaluate its potential in the topical delivery of a lipophilic drug, tretinoin (TRN). The developed SLNs were characterized for particle size, polydispersity index, entrapment efficiency of TRN and morphology. TRN-loaded SLN-based topical gels were formulated and the gels were evaluated comparatively with the commercial product with respect to primary skin irritation, *in vitro* occlusivity and skin permeation. The results of the study showed mean particle size <100 nm of the SLN dispersions with the novel lipids. Up to 46% of drug entrapment in the lipids was attained. Lesser skin irritancy, greater skin tolerance, occlusivity and slow drug release was observed with the developed TRN-loaded SLN-based gels than the commercial product. The research work could be concluded as successful production of SLNs using highly purified stearine fraction of natural solid lipids. The results of the characterization and evaluation established the safety for use, suitability and compatibility of indigenous natural lipids as a novel excipient.

1. Introduction

Recent advances in nanoparticulate systems for improved drug delivery display a great potential for the administration of exigent active molecules. Solid lipid nanoparticles (SLNs) have emerged as an alternative to other novel delivery approaches due to various advantages such as feasibility of incorporation of lipophilic and hydrophilic drugs, improved physical stability, low cost compared to liposomes and ease of scale-up and manufacturing. Moreover, the potential of SLNs in epidermal targeting, follicular delivery, controlled drug delivery, increased skin hydration due to greater occlusivity and photostability improvement of active pharmaceutical ingredients has been very well established (Mehnert and Mader, 2001; Muller et al., 2002; Shah et al., 2007). Solid lipid nanoparticles are colloidal carrier systems composed of a high melting point lipid/s as a solid core coated by surfactants. The term lipid in a broader sense includes triglycerides, partial glycerides, fatty acids, hard fats and waxes. A clear advantage of SLNs is the fact that the lipid matrix is made from physiological lipids which decreases the danger of acute and chronic toxicity. Highly purified natural solid lipids such as stearine fractions of fruit kernel are low cost alternative to the commercial lipids used for SLN production. This specialty material is derived from indigenous source, available in abundance and supplemented with essential bioactives. They are completely biodegradable. High saturated fatty acid–oleic acid–saturated fatty acid (SOS) content and exceptional high oxidation resistance of these fractions (Gunstone, 2005) would be beneficial for drug encapsulation efficiency and the drug stability upon the encapsulation, respectively. The main aim of this investigation was to develop SLNs from indigenous solid lipids by using a simple method such as microemulsion technique. Furthermore, the aim was to characterize these SLNs and evaluate its potential in the topical delivery system using a lipophilic drug model. Tretinoin (TRN), a metabolite of vitamin A has gained a great interest due to its multitude of physiological effects and employed in the topical treatment of various proliferative and inflammatory skin diseases such as psoriasis, acne, photoaging, epithelial skin cancer (Lucek and Colburn, 1985; Allen and Bloxham, 1989; Zouboulis, 2001). TRN is a choice of drug in the topical treatment of acne and period of topical administration for 6–8 weeks is recommended to have therapeutic effect (Andolsek, 1998). However, despite of all 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 TRN often leads to local irritation resulting in erythema, peeling and burning at the application site and increased

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susceptibility to sunlight, which often limits its acceptability by patients (Lehman et al., 1990; Nighland et al., 2006). All these suggest a strong rationale for the development of a suitable delivery system for tretinoin that would offer advantage like possible controlled drug release to reduce irritancy, increased drug stability and ease of large-scale production. The lipophilic drugs like, tretinoin can be efficiently incorporated in the lipid core of SLNs. The reported studies have shown that stearates have better solubilizing potential for TRN (Shah et al., 2007). Apart from skin occlusivity, the therapeutic effects of bioactives present in the natural solid lipids would also benefit for the skin repair and may circumvent the adverse effects of TRN observed (irritation and scaling) during the treatment. Bacteriostatic, antibacterial effect on *P. acnes* and anti-inflammatory action by these natural solid lipids could be synergistic with the treatment in prevention of septic and allied inflammatory conditions.

In view of this, exploring the potential of SLNs in improving the topical delivery of TRN seems worthwhile wherein; indigenous natural solid lipids were explored to offer their inherent merits to improve treatment efficacy and patient compliance. An alternative, lab-scale technique of microemulsion template (Gasco, 1997), was utilized for production of SLNs in the preparation of topical dosage form. In this investigation, SLN-based TRN (0.05%, w/w) gel for topical delivery of TRN was developed and evaluated. The proposed TRN concentration was chosen on the basis of the marketed TRE formulations.

2. Materials and methods

2.1. Materials

Refined, highly purified grade stearine fractions of fruit kernel fats were obtained as a gift samples from Charbhuja Trading Agencies & Pvt. Ltd., India. Tretinoin was a kind gift from Shalaks Pharmaceuticals Ltd., India. Cremophor RH 40 and Cremophor EL (BASF, Mumbai, India), Plurol Oleique CC 497, Labrasol and Gelucire 44/14 (Colorcon-Asia Pvt. Ltd., Mumbai, India) and Carbopol® Ultrez 10 (Noveon Inc., Mumbai, India) were obtained as a gift sample. Mono- and di-basic sodium phosphate, 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. Formulation development

Lab-scale technique of microemulsion template (Gasco, 1997) was adapted for the production of SLNs. Refined, highly purified grade stearine fractions from two indeginous natural solid lipids (MKSt and KFSt) were selected in the formulation development.

2.2.1. Screening of components (solubility studies)

The equilibrium solubility of TRN in MKSt and KFSt, surfactants and co-surfactants was determined. Briefly, excess amount of TRN was added to each screw-capped test tube wrapped with aluminium foil containing 1 ml of component. After sealing, the test tubes were shaken in an isothermal shaker ($37 \pm 1\,^{\circ}C$) for 72 h. After reaching equilibrium, each tube was centrifuged at 5000 rpm for 15 min and 0.5 ml clear supernatant layer was diluted suitably and analyzed by HPLC. The components were selected for further studies depending on the maximum drug solubilization in the surfactant/s and co-surfactant/s along with selected solid lipids.

2.2.2. HPLC analysis of TRE

The solubility of the TRN in various lipids and surfactants was determined by the reverse-phase HPLC method described by Shah et al. (2007). 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, U.S.A.), 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).

2.2.3. Construction of pseudoternary phase diagrams

The pseudo-ternary phase diagrams were constructed by titration of homogenous liquid mixtures of lipid, surfactant and co-surfactant with water (Djordjevic et al., 2004). Stearine (MKSt or KFSt) with Plurol Oleique CC 497 (Gattefossé, France) (1:1) was used as an oil phase, Cremophor RH 40 as a surfactant and Gelucire 44/14 was used as a co-surfactant. At K_m values 6, 3 and 1.5 (surfactant:co-surfactant ratio), the required quantities of lipid, surfactant and co-surfactant were warmed above the melting point of lipid (40–45 °C) and gently mixed to form a monophasic mixture varied from 9:1 to 1:9. These were slowly titrated with aliquots of distilled water (40–45 °C) and stirred for a sufficiently long time to attain equilibrium. The mixture was visually examined for transparency. After equilibrium was reached, the mixtures were further titrated with aliquots of distilled water until they showed the turbidity. Clear and isotropic samples were deemed to be within the microemulsion region. No attempts were made to completely identify the other regions of the phase diagrams. Based on the results, appropriate percentage of lipid, surfactant and co-surfactant was selected, correlated in the phase diagram and were used in the preparation of microemulsion.

2.2.4. Production of SLNs by microemulsion technique

TRN (50 mg) was solubilized in 3.5 g of solid lipid melt (MKSt or KFSt) (40–45 °C). A mixture of Cremophor RH 40 (3.6 g), Gelucire 44/14 (1.2 g) and Plurol Oleique CC 497 (3.5 g) was heated to the same temperature as the lipid phase and added with slow mixing to the drug–lipid melt. Finally, 8.2 g of distilled water (40–45 °C) was added with slow mixing. A transparent, thermodynamically stable gelled microemulsion system was formed. This microemulsion template (20 g) was then dispersed in five times volume of distilled water ($25 \pm 2\,^{\circ}C$) under slow stirring to form SLN dispersion. Stirring was continued for 20 min.

2.3. Characterization of SLN dispersions

2.3.1. Mean particle size and polydispersity index

The photon correlation spectroscopy (PCS) was used to assess mean particle size and polydispersity of the SLN dispersions. All measurements were performed in triplicate at a temperature of $20 \pm 2\,^{\circ}C$ and an angle of 90° to the incident beam. All data obtained were analyzed by Contin program on N4 Plus Submicron Particle Size Analyzer (Beckman Coulter, USA).

2.3.2. Drug entrapment efficiency

In the study, 500 μl of the TRN-loaded SLN dispersion was transferred to the upper chamber of Nanosep® centrifuge tubes fitted with an ultrafilter (MWCO100KD, Pall Life Sciences, India). The

Nanosep® was centrifuged at 13,500 rpm for 45 min. The filtrate was diluted appropriately and the content of TRN was determined by HPLC.

The entrapment efficiency was calculated by the following equation:

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

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.

2.3.3. Particle morphology

In the study, TRN-loaded SLN dispersion was dried on a carbon tape in a closed calcium chloride desiccator overnight and the sputtered with 50 nm of gold in an ion sputter for 300 s. Images were collected at an acceleration voltage of 15 kV using a back scattered electron detector on Hitachi S-4700 SEM (Hitachi Company, Japan). Analysis was performed at 25 ± 2 °C.

2.4. Formulation of SLN-based gels

Various gelling agents were evaluated for their ability to gel the SLN dispersions of TRN (0.05%, w/w). The suitable gelling agent was selected on the basis of compatibility with nanoparticulate dispersions, feel and ease of spreadability. For topical application, Carbopol® Ultrez 10 was found be suitable for gelling the TRN-loaded SLN dispersions. In the procedure, Carbopol® Ultrez 10 (at 1%, w/w) was dispersed in each SLN dispersion and then was neutralized with triethanolamine to yield TRN-loaded SLN-based gels.

2.5. Evaluation of TRN-loaded SLN-based gels

The gels prepared with SLN dispersions were labeled as Gel 1 (for MKSt lipid) and Gel 2 (for KFSt lipid). Both the gels were comparatively evaluated against marketed product (Retino-A®, TRN cream 0.05%, w/w, Johnson & Johnson, India).

2.5.1. Determination of drug content, pH and spreadability

For determination of drug content, about 1 g of the gel was weighed in a 100 ml amber volumetric flask and dissolved in methanol; it was diluted appropriately and analyzed by a HPLC method described earlier. The spreadability of test samples was determined using the following technique: 0.5 g test formulation was placed within a circle of 1 cm diameter pre-marked 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 test formulation was noted. The pH of 5% (w/w) aqueous dispersion of test sample was determined using Equip-tronic Digital pH meter Model EQ 610, standardized using pH 4.0 and 7.0 standard buffers before use.

2.5.2. Rheological studies

Brookefield Synchro-Lectric Viscometer (Model RVT) with helipath stand was used for rheological studies. The sample (50 g) was placed in a beaker and was allowed to equilibrate for 5 min before measuring the dial reading using a T-B 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 Power-law 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

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

$$\text{Shear stress}\,(\text{dyn/cm}^2) = \text{viscosity}\,(\text{mPa s}) \times \text{rate of shear}\,(\text{s}^{-1}).$$

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.

2.5.3. Primary skin irritation test

The comparative evaluation of skin irritation potential of SLN-based TRN gels with marketed cream was carried out using Draize patch test on rabbits (Draize et al., 1944; Verneer, 1991; Joshi and Patravale, 2006). The experimental protocol was approved by the Institutional Animal Ethical Committee (Approval No. UICT/PH/IAEC/0405/7). White New Zealand rabbits weighing 2.5–3 kg were acclimatized before the beginning of the study. Animals were divided into six groups (n = 3) follows:

- Group 1: No application (control).
- Group 2: Marketed formulation (Retino-A®).
- Group 3: SLN-based gel (with lipid MKSt) without TRN (Placebo gel 1).
- Group 4: SLN-based gel (with lipid MKSt) containing TRN (0.05%, w/w).
- Group 5: SLN-based gel (with lipid KFSt) without TRN (Placebo gel 2).
- Group 6: SLN-based gel (with lipid KFSt) containing TRN (0.05%, w/w).

The back of the rabbits were clipped free of hair, 24 h prior to 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) or oedema (swelling) after 24 h. To study the cumulative effect of repeated applications, the respective test sample was applied once daily for next 5 days on the same area of hair free skin. Evaluation was carried out by using the Draize scale (Draize et al., 1944). Scores between 0 and 4 were used to grade erythema and oedema which range from no response to a severe response.

2.5.4. In vitro skin occlusivity test

Efforts were made to study skin occlusivity and restoration function offered by the natural solid lipids in the developed gels. Prevention of water loss by the formulations was studied by *in vitro* occlusion test (de Vringer, 1992; Wissing et al., 2001). Here, the evaporation of water through a membrane was measured and the occlusion factor "F" was calculated. This test was performed with the drug-loaded SLN-based gels and marketed formulation. The test was performed by placing 25 g of distilled water in each beaker and closing the open end with Whatman glass microfibre filters (9.0 cm), on the upper surface of which 200 mg of the test formulation was evenly distributed. These beakers were then placed at 30 ± 2 °C/60 ± 5% RH for a period of 48 h. All formulations were tested in triplicate keeping all the condition constant. The occlusion factor "F" of the tested preparations was calculated according to the equation:

$$F = 100\left[\frac{A - B}{A}\right]$$

wherein, A is the water flux (percent water loss) through the uncovered filter and B the water flux (percent water loss) through the filter when covered by the tested preparation.

2.5.5. In vitro skin permeation studies

In vitro permeation of TRN from SLN-based gel and marketed product was evaluated using hairless abdominal rat skin samples excised from wistar rats (200–250 g). 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 buffer solution. 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 0.5, 1, 2, 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 TRN in receptor fluid was analyzed with the HPLC method as described. The total quantity of TRN that diffused to the receptor compartment in time during the steady state and the flux at the steady state was calculated using the linear portion of the correlation between the accumulated quantity of TRN that diffused through the skin by unit area and time. By determining the amount of TRN at various time intervals, the cumulative % of drug permeated versus time (h) graphs were plotted. Another graph of amount of TRN diffused per unit area (Q/A) versus time (h) was plotted. The amount of formulation remaining on the skin (donor compartment) was diluted suitably with mobile phase and amount of TRN was calculated by HPLC. To calculate the amount of drug deposited in the skin, the formulation remaining on the skin was wiped off gently. The skin was minced, transferred to a test tube and subjected to vortex for 15 min in 10 ml mobile phase. The resulting solution was filtered through 0.45 μm membrane and analyzed by HPLC. All the experiments were performed in triplicate.

The results were expressed as mean ± S.D. The significance of differences between the means was analyzed by student's t-test followed by Tukey's multiple comparison, wherever required. A probability of $p < 0.05$ was considered as significant.

3. Results and discussion

3.1. Formulation development

3.1.1. Screening of components (solubility studies)

TRN showed good solubility in the selected solid lipids, 18.58 ± 2.04 mg/ml in KFSt and 17.93 ± 1.89 mg/ml in MKSt. The solubility of TRN in Tween 80, Labrasol, Cremophor RH 40 and Cremophor EL was relatively higher, 23.08 ± 1.95, 22.66 ± 2.15, 20.23 ± 2.60 and 18.94 ± 2.26 mg/ml, respectively whereas TRN solubility in Gelucire 44/14 (Gattefosse, France) was 5.70 ± 1.88 mg/ml followed by in Plurol Oleique CC 497 (Gattefosse, France), 3.53 ± 1.93 mg/ml. Cremophor RH 40 was selected over the other surfactants because of its miscibility at higher ratio with MKSt and KFSt, sufficient drug solubilization capacity and lower melting point (16–26 °C) (preferred for making SLNs). Although, the drug solubility in Gelucire 44/14 was insufficient, Gelucire 44/14 plays an important role in reduction of mean particle size (Aungst et al., 1997). Hence, Gelucire 44/14 which is solid at room temperature (preferred for making SLNs) was opted for incorporation in the production of SLNs. The Plurol Oleique CC 497 showed least drug solubility but was important for miscibility of MKSt and KFSt with surfactant system.

3.1.2. Construction of pseudoternary phase diagrams

The area of microemulsion existence is depicted in phase Diagrams 1–3 by shaded color (Fig. 1). The area of microemulsion existence area for MKSt and KFSt at different K_m values was observed similar. In all the cases, initiation of microemulsion formation started as a clear viscous phase (gelled microemulsion) which further leads to formation of clear liquid microemulsion upon subsequent dilution with aqueous phase. Maximum area of microemulsion existence (greater gelled and microemulsion region) was obtained with $K_m = 3$. Though, GL region was greater with $K_m = 1.5$, the resultant microemulsion region was smaller than that of $K_m = 3$. This demonstrates limited water intake capacity of the gelled microemulsion with $K_m = 1.5$. Hence, the system with $K_m = 3$ was selected for further development of SLNs by the microemulsion template method. The optimized composition of developed microemulsion contained lipid phase (MKSt/KFSt) 17.5% (w/w), (Plurol Oleique CC 497) 17.5% (w/w), surfactant (Cremophor RH 40) 18.0% (w/w), co-surfactant (Gelucire 44/14) 6.0% (w/w) and aqueous phase 41.0% (w/w). SLNs could successfully be formed with the natural solid lipids (MKSt and KFSt) in a reproducible manner without any agglomeration.

3.2. Characterization of SLN dispersions

3.2.1. Mean particle size and polydispersity index

The results are listed in Table 1. The drug-loaded SLN dispersions showed marginal increase in mean particle size over the drug-free SLN dispersions. In all cases, mean particle size obtained was below 100 nm with polydispersity index less than 1.

3.2.2. Drug entrapment efficiency

The obtained TRN entrapment efficiency in the SLN dispersions was 45.28 ± 1.06% and 38.83 ± 2.47% for MKSt and KFSt, respectively. No attempts were made to correlate the effect of particle size on the drug loading ability.

3.2.3. Particle morphology

The SEM images (Fig. 2) revealed that the particle size was in nanometric range (≤100 nm) and the particles had spherical morphology.

3.3. Evaluation of TRN-loaded SLN-based gels

3.3.1. Determination of drug content, pH and spreadability

The TRN content of the SLN-based gels was found to be 98.1 ± 4.63% of the theoretical value (0.05%, w/w) and pH was found to be in acceptable limits. Spreadability is an important property of topical formulation from patient compliance point of view and indicated better spreadability of the SLN-based gels than the marketed formulation (Table 2).

3.3.2. Rheological studies

The developed gels from the TRN-loaded SLN dispersions were semi-transparent, pseudo-plastic in nature with superior product features, excellent spreadability and skin feel than the marketed. The comparative results of various parameters studied are listed in Table 2.

3.3.3. Primary skin irritation test

One of the major disadvantages associated with the TRN therapy is skin irritation (erythema), which strongly limits its utility and

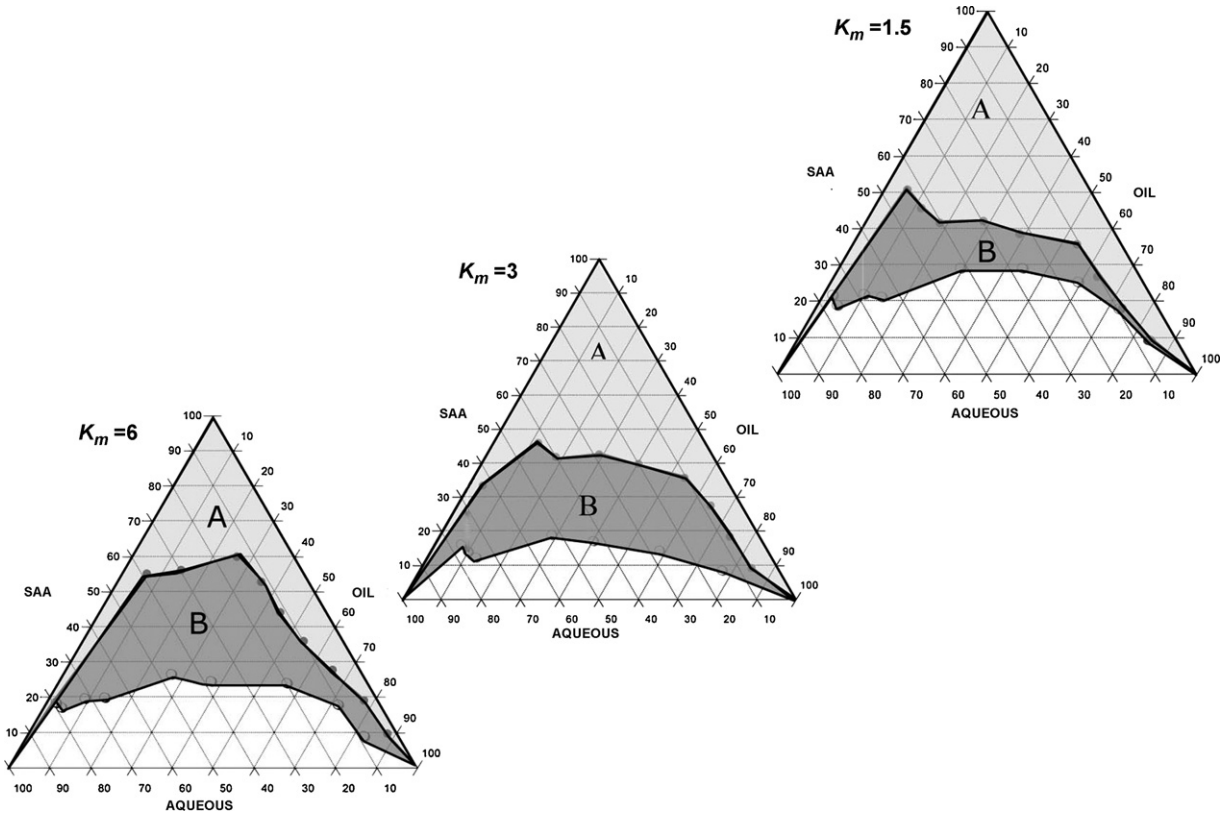

Fig. 1. Ternary phase diagrams at different K_m. (A) Gelled microemulsion existence region of the microemulsion template. (B) Total microemulsion existence region upon final dilution, comprising the area of A. Oily phase: MKSt/KFSt and Plurol Oleique CC 497 (1:1), SAA: surfactant (Cremophor RH 40) + co-surfactant (Gelucire 44/14) and Aqueous phase: distilled water. The shaded color region indicates microemulsion existence area.

acceptability by the patients. Ideally, the delivery system of TRN 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 TRN. 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 3. The skin-irritation studies indicated that SLN-based TRN gel resulted in a considerably less irritation as compared to marketed TRN formulation (Retino-A®) after 24 h of application. The irritation continued to increase even after 24 h in case of marketed TRN formulation whereas it did not increase in case of SLN-based TRN gels (Table 3). Thus, SLN-based gels demonstrated remarkable advantage over marketed formulation in improving the skin tolerability of TRN indicating their potential in improving patient acceptance and topical delivery of TRN. Cumulative applications of both the gels and respective placebos showed less skin irritancy (score = 1) and no scaling compared to marketed formulation (score = 3) on intact rabbit skin. In no case, oedema was seen. However, the marketed formulation showed well-defined erythema after 72 h which eventually increased with scaling at the end of 5 days.

The results of the primary skin irritation testing are even more promising as compared to our earlier investigation (Shah et al., 2007). In our earlier studies, we had used SLN gels based on a synthetic lipid (glyceryl monostearate) which showed signs of slight irritation after 48 h whereas in the present investigation, both the SLN-based gels did not show any signs of irritation up to 72 h. This clearly indicates the importance of the lipid matrix used in the current investigation. Thus, the natural lipids used in the present investigation are likely to be more advantageous as compared to synthetic lipids.

Table 1
Mean particle size and polydispersity index of SLN dispersions ($n = 3$).

Sample	Lipid phase	Mean particle diameter (nm ± S.D.), P.I.
Drug-free SLN dispersion	MKSt	46.5 ± 22.2, 0.404
Drug-loaded SLN dispersion	MKSt	52.0 ± 20.8, 0.448
Drug-free SLN dispersion	KFSt	60.7 ± 26.3, 0.760
Drug-loaded SLN dispersion	KFSt	65.7 ± 28.0, 0.653

P.I.: polydispersity index.

3.3.4. *In vitro skin occlusivity test*

The weight loss of water (water flux) is exclusively depending on the occlusivity of membrane offered by the formulations tested. The results of occlusivity are shown in Fig. 3. When values were compared to that of uncovered filter, both the TRN-loaded SLN-based gels showed significantly higher ($p < 0.001$) prevention of water loss than that of the marketed formulation. This enhanced *in vitro* occlusivity offered by lipids in the gels was similar for both the SLN-based gels. The better occlusivity of SLN-based gels could be attributed to solid state nature of lipidic components in the developed gels (clogging the micropores of filters, i.e. occlusivity) thus, preventing the water evaporation to a greater extent and comparatively larger quantity of an aqueous phase in the developed gels. The greater skin occlusivity offered by SLN-based gels would be helpful for mitigating the adverse reactions of the TRN therapy such as itching, dryness and scaling.

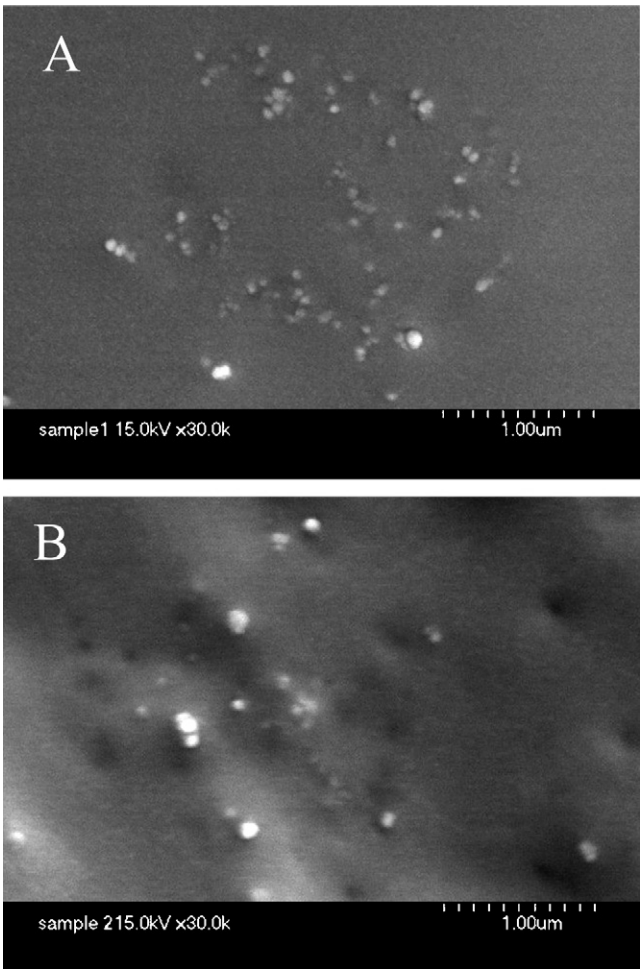

Fig. 2. SEM images of SLN dispersions: (A) dispersion made with MKSt lipid phase and (B) dispersion made with KFSt lipid phase.

3.3.5. *In vitro skin permeation studies*

The cumulative amount of TRN permeated, TRN retained in skin and TRN present in donor compartment (expressed as % dose) after 12 h was determined for the SLN-based gels and marketed formulation (Retino-A® cream). The results are depicted in Fig. 4. The flux values obtained for the various formulations are shown in Table 4. It is evident from Fig. 4 that SLN-based gels result in considerably less permeation of TRN as compared to that of the marketed formulation. Moreover, the flux values obtained with the SLN-based gels were significantly lower ($p < 0.001$) than that of the marketed formulation (Retino-A® cream). This observation is in accordance with the literature. However, the amount of TRN deposited in the skin for all the formulations was not different. Interestingly, it was observed that the flux values and the amount of TRN permeated from the SLN-based gels were considerably lower than that obtained with SLN-based gels reported in our earlier investigation (Shah et al.,

Table 3
Primary irritation index of various TRN formulations at the end of 24, 72 h and day 5 ($n = 3$)

Formulation	Irritation index		
	24 h	72 h	Day 5
Control (Group 1)	0	0	0
Marketed formulation (Group 2)	1	3	3
Gel 1 Placebo (Group 3)	0	0	0
Gel 1 (Group 4)	0	0	1
Gel 2 Placebo (Group 5)	0	0	0
Gel 2 (Group 6)	1	0	1

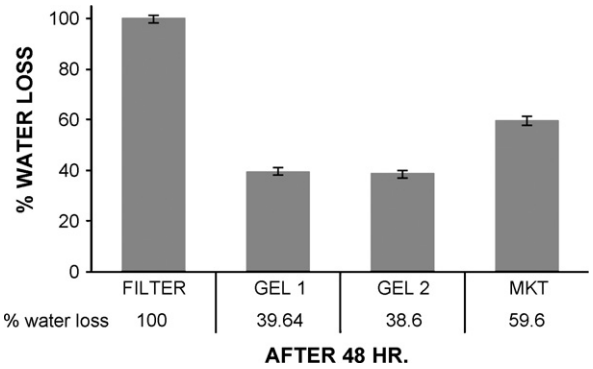

Fig. 3. Comparative *in vitro* occlusivity of various TRN formulations ($n = 3$).

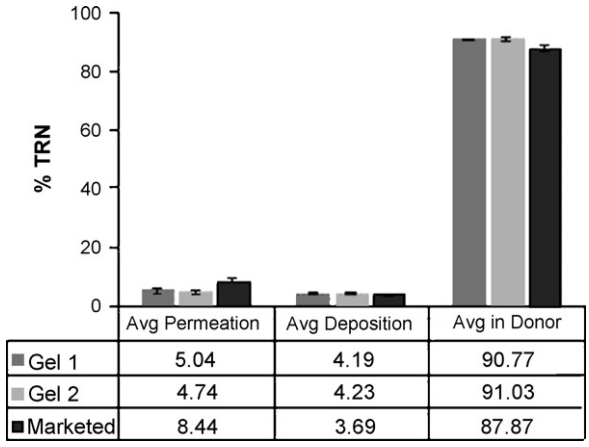

Fig. 4. Amount of TRN permeated and deposited through rat skin from various formulations at the end of 12 h ($n = 3$).

Table 4
Flux values of various TRN formulations ($n = 3$)

Formulation	Flux (ng/cm^2 h)
SLN Gel 1	37.7 ± 4.27[a]
SLN Gel 2	35.2 ± 4.15[a]
Marketed formulation	64.5 ± 6.63

[a] $p < 0.001$ as compared to the marketed formulation.

Table 2
Comparative evaluation of various parameters of various TRN formulations

Parameters	Results		
	Gel 1 (TRN-loaded SLN-based gel)	Gel 2 (TRN-loaded SLN-based gel)	Retino-A® (marketed formulation)
pH	6.78	6.83	5.72
Spreadability (cm)	7.4	7.1	5.8
Viscosity at 5 rpm (mPa s)	21.2×10^5	19.6×10^5	22.8×10^5
Consistency index	7025868	6471426	7490313
Flow index	0.256	0.264	0.249

2007). This clearly indicates the importance of the lipid matrix used in the present investigation. It is known fact that the rate of permeation and the drug deposition is a function of the solid lipid used for the formulation of SLN. In view of this, we believe that the SLN gels described in the current investigation could be more advantageous to the SLN gels described in our earlier investigation.

Our future studies would be concentrated upon the comparative evaluation of the clinical efficacy of the TRN-loaded SLN-based gels containing natural solid lipids and synthetic lipids.

4. Conclusion

The present research work could be concluded as successful production of SLNs using highly purified, natural solid lipids (stearine fractions) by microemulsion technique. The results of the characterization and evaluation established the suitability and compatibility of indigenous natural lipids as a novel excipient, hitherto unexplored in the novel drug delivery. Further, developed SLNs were meaningfully utilized for the topical delivery of lipophilic, anti-acne drug, TRN. Lesser skin irritancy, greater occlusivity and slow drug release that observed with the developed TRN-loaded SLN-based topical gels would be advantageous over the commercial product. Improved drug stability and encapsulation of TRN in the developed SLNs had overcome the adverse effects of TRN and would offer the efficacy in anti-acne treatment and patient compliance. Production of TRN-loaded SLNs with the novel solid lipids and its formulation as a topical gel could be a new, cost effective and commercially viable alternative to the commercial product.

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