

RESEARCH ARTICLE

Novel microemulsion-based gel formulation of tazarotene for therapy of acne

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Abstract

The objective of this study was to develop and evaluate a novel microemulsion based gel formulation containing tazarotene for targeted topical therapy of acne. Pseudoternary phase diagrams were constructed to obtain the concentration range of oil, surfactant, and co-surfactant for microemulsion formation. The optimized microemulsion formulation containing 0.05% tazarotene was formulated by spontaneous microemulsification method consisting of 10% Labrafac CC, mixed emulsifiers 15% Labrasol–Cremophor–RH 40 (1:1), 15% Capmul MCM, and 60% distilled water (w/w) as an external phase. All plain and tazarotene-loaded microemulsions were clear and showed physicochemical parameters for desired topical delivery and stability. The permeation profiles of tazarotene through rat skin from optimized microemulsion formulation followed the Higuchi model for controlled permeation. Microemulsion-based gel was prepared by incorporating Carbopol® 971P NF in optimized microemulsion formulation having suitable skin permeation rate and skin uptake. Microemulsion-based gel showed desired physicochemical parameters and demonstrated advantage over marketed formulation in improving the skin tolerability of tazarotene indicating its potential in improving its topical delivery. The developed microemulsion-based gel may be a potential drug delivery vehicle for targeted topical delivery of tazarotene in the treatment of acne.

Keywords

Acne, microemulsion, skin irritation study, skin retention study, tazarotene, topical drug delivery

History

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Introduction

Acne, a cutaneous disorder of multifactorial origin, is a disease whose cause and severity depends on relationship among hormones, keratinization, sebum, and bacteria. The objectives of acne therapy usually include controlling acne lesions, preventing scarring, and minimizing morbidity¹.

Tazarotene (TZR), 6-[2-(4,4-dimethylthiochroman- 6-yl)ethyl]-nicotinic acid ethyl ester, a member of a new generation of receptor selective synthetic retinoids, is used for the topical treatment of mild to moderate plaque psoriasis, acne vulgaris, and photoaging. Dermal safety studies have indicated that TZR did not show phototoxic or photoallergic potential^{1–3}. However, the course of treatment which is usually prolonged (weeks or months) may lead to adverse reactions like pruritus, burning/stinging, and erythema in a significant subset of users. These may often result in the interruption or discontinuation of the treatment regimen. Further, extremely low solubility limits TZR incorporation into an acceptable vehicle and its tolerability results in either discontinuation of treatment or poor compliance in patients^{2,3}. Therefore, it is prudent to improve the topical delivery and reduce

adverse effects of TZR using an appropriate carrier having desired skin targeting properties that may reduce the adverse reactions substantially and improve users' compliance to the novel regimen.

The conventional anti-acne formulations of TZR (gel, cream, aerosol, and foam) could be less efficacious due to insignificant skin penetration properties^{2,3}. In comparison, the novel drug delivery systems of recently available anti-acne formulations have potential to reduce the adverse effects without reducing the efficacy. Microemulsion (ME) offers a powerful way for drug delivery as colloidal drug carrier due to its versatility and favorable advantages^{4–7}. The application of ME vehicles to skin distributes the anti-acne agent(s) steadily and could reduce the irritancy. These systems have also shown to promote follicular targeting and thus creating high local concentrations of the active compound in the pilosebaceous unit and efficient penetration in hair follicles. Thus, microemulsions (MEs) could play a pivotal role in improving topical delivery of anti-acne agents by enhancing their dermal localization with concomitant reduction in their adverse effects. ME-based gels (MBGs) are known to provide faster drug release than conventional formulations^{6,7}. MBG was thus developed and evaluated using a suitable polymer that is capable of modifying the rheological behavior.

In order to find out innovative ways for administering TZR and assuaging their disadvantages, the present study investigated the development of ME-based gel containing TZR. It was

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hypothesized that the oily core present around TZR may result in the reduction of irritation when presented in the form of ME. The combination of effects namely equal and/or enhanced effectiveness, reduction in adverse reactions and regimen adherence may provide better platform of novel drug delivery with improved therapeutic efficacy. Thus, the present study focuses on novel formulation considerations, characterization, and evaluation of skin-targeting efficiency of TZR when loaded with MBG.

Materials and methods

Materials

TZR was procured on gratis from Dr. Reddy's Laboratory Ltd. (Andhra Pradesh, India). Labrafac CC, Labrasol, Plurol oleique (Gattefosse, Saint-Priest, France); Cremophor RH 40 (BASF India Ltd., Mumbai, India); Capmul MCM (Abitec Corporation, Janesville, WI); Carbopol®980P NF, Carbopol®971P NF, Carbopol®974P NF (Lubrizol Advanced Materials India Pvt. Ltd., Mumbai, India); Methocel E50 LV premium hydroxypropyl methylcellulose EP grade and Methocel K4M premium hydroxypropyl methylcellulose (Colorcon Asia Pvt. Ltd., Goa, India) were procured on gratis. Isopropyl myristate was purchased from National Chemicals (Vadodara, India). Ethanol was purchased from Baroda Chemical Ind. Ltd (Dabhoi, India). Double-distilled water was used throughout the study. All other chemicals and solvents were of analytical reagent grade and were used as received without further purification.

Methods

High performance thin-layer chromatography method and chromatographic conditions

The standard and formulation samples of TZR were spotted on pre-coated high-performance thin-layer chromatography (HPTLC) plates in the form of narrow bands of 6 mm length, with 10 mm from the bottom and the left margin and with 9 mm distance between two bands. Samples were applied using Linomat V autosprayer under the continuous drying stream of nitrogen gas at a constant application rate of 150 nL/s. Plates were developed using mobile phase consisting of toluene–methanol (9:1, v/v). Linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber equilibrated with the mobile phase. The optimized chamber saturation time for mobile phase was 20 min at $25 \pm 2^\circ\text{C}$. Ten milliliters of the mobile phase (5 mL in trough containing the plate and 5 mL in other trough) was used for each development and allowed to migrate a distance of 70 mm, which required 10 min. After development, the HPTLC plates were dried completely. Densitometric scanning was performed on Camag TLC Scanner III (Camag, Muttentz, Switzerland) in an absorbance mode and operated by winCATS Planar Chromatography version 1.3.4 (PerkinElmer Inc., Waltham, MA). The source of radiation utilized was deuterium lamp. The spots were analyzed at a wavelength of 327 nm. The slit dimensions used in the analysis were length and width of 5 mm and 0.45 mm, respectively, with a scanning rate of 20 mm/s. These were selected as recommended by the CAMAG TLC Scanner III Manual (Camag, Muttentz, Switzerland). It covers 70–90% of the application band length which in the present case is 6 mm. The monochromator bandwidth was set at 20 nm. Concentrations of compound chromatographed were determined from the intensity of diffusely reflected light and evaluated as peak areas against concentrations using linear regression equation. The method was validated for precision, recovery, repeatability, and robustness as per the International Conference on Harmonization Guidelines³.

Solubility determination

The solubility of TZR was determined in various oils, emulsifiers, and co-emulsifiers. In this experiment, excess amount of TZR was added in 1 mL of each excipient and mixed thoroughly using vortexer at ambient temperature. The mixed samples were kept for 72 h to allow equilibration. Then the samples were centrifuged at $5000 \times g$ for 30 min to separate the un-dissolved drug. Supernatants were diluted and analyzed by HPTLC method³ in order to estimate concentration of soluble TZR. Excipients in which TZR showed good or excellent solubility were chosen for preparing MEs⁷.

Construction of phase diagrams

The pseudo-ternary phase diagrams were constructed using the spontaneous microemulsification method at ambient temperature. The mixture of Labrasol (LAB) and Cremophor RH-40 (CRH-40) (1:1) was used as a mixed emulsifier. Three phase diagrams were prepared with the 1:1, 2:1, and 3:1 weight ratios of mixture of LAB/CRH-40 as emulsifier and Capmul MCM (Cap-MCM) as co-emulsifier (Emix), respectively. For each phase diagram at a specific emulsifier/co-emulsifier mixing ratio (Km), the ratio of Labrafac CC (Lab-CC) to the Emix were varied as 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. Spontaneous formation of ME was evaluated by the addition of known amount of water at once to the known amount of oil, emulsifier, and co-emulsifier with controlled stirring. The ease of formation of clear ME was taken as the criteria of spontaneity of microemulsification^{6,7}.

Preparation and characterization of microemulsions

Eight potential ME formulations, which were different from each other by water volume fraction, were selected and formulated at Km 1:1 by the spontaneous microemulsification method. The calculated amount of TZR (0.05%w/w) was added to the oily phase of ME and magnetically stirred until dissolved which was followed by adding Emix in a fixed proportion to produce clear mixture. Then a defined proportion of water was added and stirred to produce clear ME containing TZR^{6,7}.

The average droplet size and the polydispersity index (PDI) of ME were measured by photon correlation spectroscopy (PCS) with in-built Zetasizer (Nano ZS, Malvern Instruments, Malvern, UK) at 633 nm. Helium–neon gas laser having an intensity of 4 mW was the light source. The droplet size was calculated using the Stokes–Einstein relationship by Zetasizer software (Malvern Instruments, Malvern, UK). Electrophoretic mobility ($\mu\text{m/s}$) was measured using small volume disposable zeta cell and converted to zeta potential by in-built software using the Helmholtz–Smoluchowski equation. All determinations were made in triplicate. TEM was used to characterize the microstructure of TZR-loaded ME. The TEM images were obtained using a Tecnai G2 20 TEM (Philips, Holland, The Netherlands). TZR content in the formulation was assayed using HPTLC. The percent transmittance of the formulation was measured using a colorimeter (Digital Colorimeter, D-801, Photocon, New Delhi, India) at 570–590 nm. Isotropic nature of ME formulation was verified using cross-polarized light microscopy (Polarizing Microscope RPL-55 Series, Radical Instruments, Delhi, India). The pH value of the formulation was determined using a digital pH meter (HI 98107, Hanna Instruments, New Mumbai, India) which was standardized using pH 4 and 7 buffers before use. The electric conductivity of ME was measured using a conductivity meter (Equip-Tronics, EQ – 664, Mumbai, India) equipped with an inbuilt magnetic stirrer. The viscosity of ME was measured using a Brookfield Viscometer LVDV – IIIU (Brookfield Engineering LABS, Stoughton, MA) with spindle SC 18 at 100 rpm using interval of 30 s^{4–8}.

Preparation and characterization of MBG

Carbopol® 971P NF was selected as the gel matrix to prepare MBG. The oily phase (mixture of oil, emulsifier, and coemulsifier) of optimized batch-containing drug was then mixed with dispersion of Carbopol® 971P NF (2%w/w) in water. Then pH of the resultant dispersion was raised using triethanolamine (50% w/w in distilled water) to obtain MBG⁸.

For the 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 by the HPTLC method described earlier. 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 and the mean diameter was taken by repeating the experiment three times⁸. The pH of the 10% (w/w) gel was determined using a digital pH meter (HI 98107, Hanna Instruments, Mumbai, India), standardized using pH 4 and 7 buffers before use⁸. Brookfield Viscometer LVDV – IIIU (Brookfield Engineering LABS, Stoughton, MA) with the 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 triplicate at ambient temperature⁸.

Stability studies

The stability of ME formulations was studied through various parameters such as clarity, phase separation observation, droplet size determination, viscosity measurement, conductivity measurement, pH, and zeta potential determination for 6 months at room temperature. In order to estimate metastable systems, the selected ME vehicles were centrifuged (Remi Lab, Mumbai, India) at $5000 \times g$ for 30 min⁴⁻⁷. Beside the physicochemical properties, the chemical stability of the investigated drug in the vehicle plays a major role. Therefore, the drug content was analyzed at defined time intervals during the observation period of 6 months. During the observation period, the formulations were stored at room temperature in tubes to simulate patient usage conditions². During stability studies, the samples were taken at fixed time intervals.

Infrared study

The infrared (IR) spectra of TZR, unloaded ME, optimized TZR-loaded ME (TZR-ME), and TZR-loaded MBG (TZR-MBG) were taken using an IR spectrophotometer (Spectrum GX FT-IR, Perkin Elmer, Norwalk, CT). The unloaded ME, TZR-ME, and TZR-MBG were spread as a thin layer on potassium bromide cell and then scanned between 4000 and 400 cm⁻¹. The resulting IR spectra of TZR and unloaded ME were then compared with TZR-ME and TZR-MBG to detect any possible interaction between the drug and different components used⁵.

In vitro skin permeation study

The *in vitro* skin permeation study was carried out as per the guidelines proposed by Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA) and study protocols were approved by the Local Institutional Animal Ethics Committee (IAEC). The abdominal skins obtained from male Wistar rats weighing 230 ± 20 g (age, 6–8 weeks) were used for

in vitro permeation experiments of prepared formulations. After hair was shaved carefully with an electric clipper, the skin was excised from the abdominal region of each sacrificed rat and the subcutaneous fat and other extraneous tissues were removed without damaging the epidermal surface. The excised rat skin samples of almost the same thickness were rinsed with physiological saline^{7,9} and placed in refrigerator at 4°C prior to permeation experiments. The skin membranes were first hydrated for 30 min in buffer solution (pH 7.4) at room temperature to remove extraneous debris and leachable enzymes.

The permeation experiment was performed using Franz diffusion cell fitted with excised rat skin 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 effective permeation area was 3.14 cm² (20 mm diameter orifice) and the receptor compartment was filled with 12 mL of physiological saline:95% ethanol (7:3 v/v) for ensuring pseudo-sink condition by increasing the solubility of TZR in the receiving phase⁹. Ethanol was used in the receptor fluid to solubilize TZR. The temperature of the receiver chamber containing 12 mL of receptor media was controlled at $32 \pm 1^\circ\text{C}$ using circulating equibath (Model 8506, Medica Instrument Mfg. CO, Mumbai, India). Diffusion media was continuously stirred with a teflon-coated magnetic bar at a constant rate, in a way that the rat skin surface just flushes the diffusion fluid. The formulation (1 g) was gently placed in a donor chamber and then the diffusion cells were covered with an aluminum foil to prevent light exposure. At 1, 2, 4, 6, 8, 10, and 12 h aliquots of 1 mL sample were withdrawn from the receptor compartment and replaced immediately with an equivalent volume of receptor fluid. The concentration of TZR in receptor fluid was analyzed using the HPTLC method³. Each experiment was performed in triplicate. Cumulative corrections were made to obtain the total amount of TZR permeated at interval each time.

Data analysis and statistics

The permeation parameters such as flux and permeation coefficient were calculated using the equation $-J_{ss} = 1/A(dQ/dt)_{ss} = C_0 K_p$, where J_{ss} is the permeation rate at steady-state ($\mu\text{g}/\text{cm}^2/\text{h}$), A is the effective diffusion area (cm^2), $(dQ/dt)_{ss}$ is the amount of drug permeated through the skin per unit time at steady-state ($\mu\text{g}/\text{h}$), C_0 is the applied drug concentration in the donor compartment ($\mu\text{g}/\text{mL}$), and K_p is the permeability coefficient (cm/h). The average cumulative amount of drug permeated per unit surface area of the skin was plotted versus time. The permeation rate of TZR at steady-state (J_{ss} , $\mu\text{g}/\text{cm}^2/\text{h}$) through rat skin was calculated from the slope of linear portion of the cumulative amount permeated through the rat skin per unit area versus time plot. In order to obtain the permeability coefficient K_p (cm/h), the following equation was used $K_p = J_{ss}/C_0$ ⁶⁻⁸.

The data obtained in this study were expressed as the mean value $\pm$ SD. Statistical data were analyzed by one-way analysis of variance (ANOVA). A multiple comparison test was used to compare different formulations and $p < 0.05$ was considered to be significant.

Skin retention study

Skin retention study was performed in order to analyze the content of the drug in the skin. At the end of the *in vitro* skin permeation study, the skin samples were washed with water and methanol on both sides and carefully dried. Then a defined amount of methanol was added to each piece of skin¹⁰. The samples were vortexed for 10 min in order to extract its drug content and stirred overnight. The samples were analyzed by HPTLC³ after centrifugation.

Histopathological investigation of skin

The rat abdominal skin was mounted on the Franz diffusion cell. The optimized ME, MBG (1 g), and marketed gel were applied similar to the method of permeation study and the effects were compared against control. A piece of fresh excised untreated skin sample was used as control. The skin was fixed in 10% neutral formalin for 24 h and then cut vertically against the surface at the central region (4 mm width). Each section was dehydrated using graded solutions of ethanol and then embedded in paraffin wax. Tissues were divided into small pieces and stained with hematoxylin and eosin. The sections were observed under $100\times$ magnifications and photographed^{5–7}.

Skin-irritation testing (Draize patch test)

The irritation potential of the ME-based TZR gel in comparison with marketed TZR gel was evaluated by carrying out the Draize patch test on rabbits^{11,12}. Animal care and handling throughout the experimental procedure were performed in accordance with the CPCSEA guidelines. White New Zealand rabbits weighing 2.5–3 kg were acclimatized before the beginning of the study.

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

Group 1: No application (control).

Group 2: Marketed formulation (Tazret[®] gel containing 0.05% w/w TZR, Ranbaxy, Mumbai, India).

Group 3: MBG without TZR (placebo gel).

Group 4: MBG containing TZR (0.05%, w/w).

The back of the rabbits were clipped free of hair 24 h prior to the application of the formulations. 0.5 g formulations 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 erythema scores were recorded (ranging from 0 to 4) depending on the degree of erythema as follows: no erythema = 0, slight erythema (barely

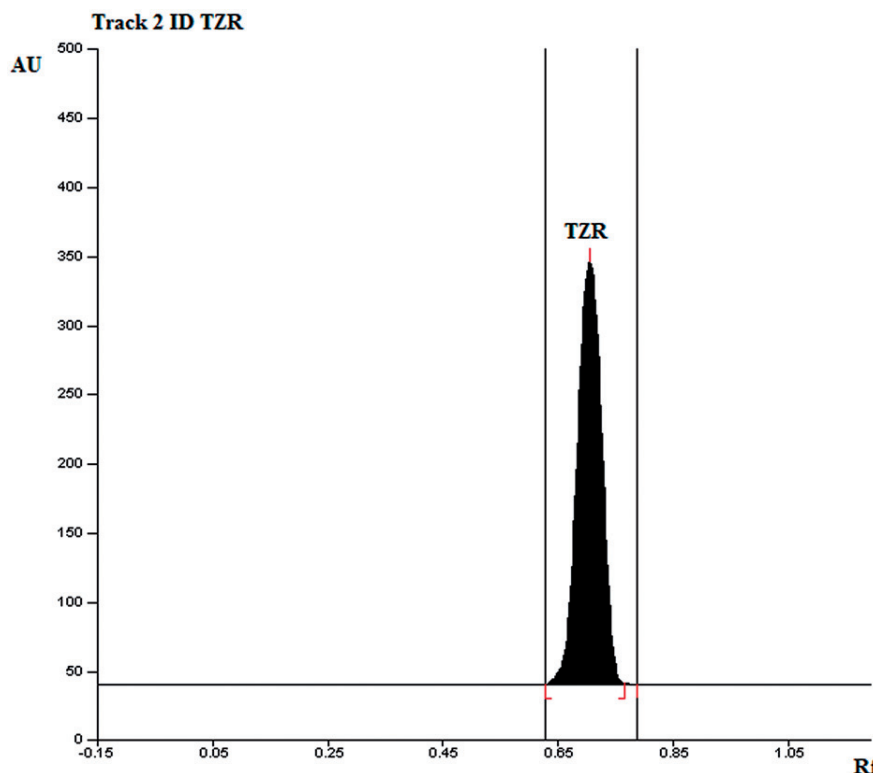
perceptible-light pink) = 1, moderate erythema (dark pink) = 2, moderate to severe erythema (light red) = 3, and severe erythema (extreme redness) = 4^{11,12}.

Results and discussion

The HPTLC separation was achieved on an aluminum-backed layer of silica gel 60F254 using toluene–methanol (9.0 + 1.0 v/v) as the mobile phase. Quantitation was achieved by densitometric analysis at 327 nm over the concentration range of 100–500 ng/mL³. The method was found to give a compact band for TZR ($R_f = 0.75 \pm 0.01$) (Figure 1). The linear regression analysis data for the calibration plots showed a good linear relationship with $r^2 = 0.9995$. The method was validated for precision, recovery, repeatability, and robustness as per the International Conference on Harmonization guidelines. The minimum detectable amount was found to be 18.59 ng/spot, whereas the limit of quantitation was found to be 56.34 ng/spot. Statistical analysis of the data showed that the method is precise, accurate, reproducible, robust, and specific for the analysis of TZR (Table 1).

The solubility of TZR was found highest in Lab-CC ($38.66 \pm 7.21\text{ mg/g}$), LAB ($80.17 \pm 8.17\text{ mg/g}$), and plulol oleique ($14.38 \pm 4.95\text{ mg/g}$) among the various oils, emulsifiers, and co-emulsifiers studied (Table 2). Lab-CC, a medium chain triglyceride used in variety of pharmaceutical formulations, is known to be solubilized with low emulsifier concentrations because of their smaller molecular volumes^{13,14}. LAB with a low potential to induce skin irritation and good penetration enhancing effects make it a suitable candidate for stabilizing ME vehicle. But as reported in the literature, the use of mixed emulsifiers increases the water solubilizing capacity and the HLB¹⁴. So CRH-40 was further selected as another emulsifier in spite of its low solubilizing capacity for drug as compared with other emulsifiers. During preliminary studies, it was observed that not Plulol oleique but only Cap-MCM could result in the formation of stable MEs with above-selected oil and a mixture of emulsifiers. So,

Figure 1. Chromatogram of standard tazarotene (300 ng/spot) using mobile phase toluene–methanol (9.0 + 1.0, v/v).



Cap-MCM was selected as a co-emulsifier for the preparation of MEs in spite of having low solubilizing capacity for TZR as compared with Plurol oleique as shown in Table 2. From these results, Lab-CC, LAB, CRH-40, and Cap-MCM were subsequently used as the oil phase, emulsifiers, and co-emulsifier for the formulation of MEs containing TZR in this study.

The pseudoternary phase behavior of the system of water, LAB, CRH-40, Cap-MCM, and Lab-CC was studied by constructing pseudoternary phase diagrams adopting a simple titration technique⁷. The phase boundary was determined by observing the changes of the sample appearance from turbid to transparent or from transparent to turbid based on visual identification. The mixture (blend) of emulsifiers changes the polarity of polar and apolar phases and also enhances the surfactant partitioning at the interface by increasing the stability of surfactant film. This will affect the packing parameter of the mixed emulsifiers in a manner that more water can be solubilized in the ME droplets. Therefore, the increased surfactant partitioning at the interface in the mixture of emulsifiers is synergistic. Huibers and Shah¹⁵ have also demonstrated a strong synergism in non-ionic mixture of emulsifiers similar to the findings in this study. The combined use of emulsifiers showed apparent advantages over the single use of surfactant; the ME region was greatly

increased in the phase diagram. As shown in Figures 2(M) and (N) and 3(O) the isotropic region of ME was increased when combination of emulsifiers [LAB + CRH-40 in Figure 3(O)] were used along with cosurfactant (Cap-MCM) as compared with single surfactant (CRH-40 in Figure 2(M) and LAB in Figure 2(N)). It is reported by Rhee¹⁶ that for CRH-40 in combination with LAB the ME existence area becomes enlarged as their weight ratio decreases. Same observations were made by us with the ME area reaching maximum at a weight ratio of 1:1. Hence, the ratio of emulsifiers (LAB: CRH-40) was fixed at 1:1. Addition of co-surfactant decreases the interfacial tension between water and the oil phase and reduces the spontaneous curvature leading to increased water solubilization. The co-surfactant penetrates into the interface and increases the flexibility of surfactant film, because the liquid crystalline phase is destabilized in favor of ME phase that even connects the aqueous with the oil phase¹⁶. The area of ME isotropic region changed in size with ratio of emulsifiers to co-surfactant (Km) in the order of: 1:1 > 2:1 > 3:1 (Figure 3). The maximum area of single phase ME region was obtained when ratio of Emix (LAB/CRH-40: Cap-MCM) was in the order of 1:1 (Km = 1) and so it was fixed at 1:1 for further studies. Further eight potential ME formulations, ME 1–ME 8 (Table 3) were selected to study the effects of formulation components on skin permeation of TZR. The entire drug-loaded ME formulations containing TZR were prepared in the same ratios and the proportion of TZR was kept constant at 0.05% w/w for all the batches.

Percutaneous penetration of a drug into and through the skin depends on its characteristics in an appropriate vehicle. Lab-CC is known penetration enhancer. LAB, CRH-40, and Cap-MCM are assumed to act as permeation enhancers by reducing the diffusional barrier of the stratum corneum¹⁴. So, in the present study, suitable vehicle in the form of ME comprising oil (Lab-CC), mixed emulsifiers (LAB and CRH-40), co-emulsifier (Cap-MCM), and aqueous phase was selected to alter the characteristics of TZR. The effect of composition of different ME vehicles and particle size on TZR permeation through the skin was evaluated using the excised Wistar rat skin as the model membrane. The permeation parameters of the tested ME formulations with various compositions are presented in Table 4. A steady increase of TZR in the receptor chamber with time (Figure 4) was observed due to reservoir effect of ME for TZR. The cumulative amount of TZR that had permeated through excised rat skin ($\mu\text{g}/\text{cm}^2$) was plotted as a function of time (hours). The steady state flux (J_{ss}) was obtained from the linear portion (2–8 h) of the graph. The flux in different compositions of MEs ranged from 0.0307 to 0.0508 $\mu\text{g}/\text{cm}^2/\text{h}$. This indicated that the permeation characters of TZR through the rat skin were significantly affected by ME formulations. As shown in Table 4 and Figure 4, the permeation of TZR was highest from ME 8 ($0.0508 \pm 0.00085 \mu\text{g}/\text{cm}^2/\text{h}$) and lowest from ME 1 ($0.0307 \pm 0.00176 \mu\text{g}/\text{cm}^2/\text{h}$). Statistical comparison of flux throughout 12 h showed that ME 8 provided fluxes higher than other ME formulations ($p < 0.001$ for ME 1, ME 2, ME 3, ME 5, and ME 6 and $p < 0.05$ for ME 7) except ME 4 ($p > 0.05$) having a flux value of $0.0473 \pm 0.00049 \mu\text{g}/\text{cm}^2/\text{h}$. Same observations were also made while considering permeability co-efficient (K_p) of different MEs with ME 8 having highest K_p ($1.02 \times 10^{-4} \pm 0.169 \times 10^{-5} \text{ cm}/\text{h}$) and ME 1 having lowest K_p ($0.613 \times 10^{-4} \pm 0.353 \times 10^{-5} \text{ cm}/\text{h}$). Thus, from Table 4, it was concluded that the permeation parameters of TZR from MEs were affected by the content of Emix, water, and oil.

It was found that the permeation rate was significantly increased by decreasing the content of Emix from 60% to 30% (w/w) at $K_m = 1$. The flux and K_p values of ME1–ME4 having 5% w/w content of Lab-CC was increased significantly ($p < 0.01$ for

Table 1. Analytical parameters and validation results of the determination of tazarotene using proposed HPTLC method.

Parameters	Results
Wavelength	327 nm
Linearity range	100–600 ng/band
Time of analysis	20 min/run
Migration distance	70 mm
Development	Ascending
Linearity	
Intercept	6506.47
Slope	16.74
Correlation coefficient	0.9995
Recovery (%)	99.44–100.11
Repeatability (RSD, %)	1.59–3.46
Precision (RSD, %)	
Interday	1.66–1.85
Intraday	1.21–1.49
Robustness (RSD, %)	1.29–1.91
LOD	18.59 ng/band
LOQ	56.34 ng/band
Specificity	Specific

Table 2. Solubility of tazarotene in various excipients used for microemulsion formulation.

Excipients	Solubility ^a
Oily phases	
Labrafil M 1944 (oleoyl polyoxylglycerides)	9.01 $\pm$ 3.11
Labrafac CC (caprylic/capric triglycerides)	38.66 $\pm$ 7.21
Captex 200 (propylene glycol dicaprylate/dicaprate)	20.45 $\pm$ 5.32
Captex 355 (glycerol caprylate caprate)	1.25 $\pm$ 0.51
Surfactants	
Labrasol (caprylocaproyl polyoxylglycerides)	80.17 $\pm$ 8.17
Tween (polysorbate) 80	75.47 $\pm$ 6.77
Cremophor RH 40 (polyoxyl 40 hydrogenated castor oil)	18.49 $\pm$ 3.19
Cosurfactants	
Plurol Oleique CC (polyglyceryl oleate)	14.38 $\pm$ 4.95
Capryol 90 (propylene glycol monocaprylate)	6.55 $\pm$ 1.04
Capmul MCM (glyceryl mono- and dicaprate)	11.61 $\pm$ 4.57
Propylene glycol	6.31 $\pm$ 2.46

^aData expressed as mg/g, mean $\pm$ SD, $n = 3$.

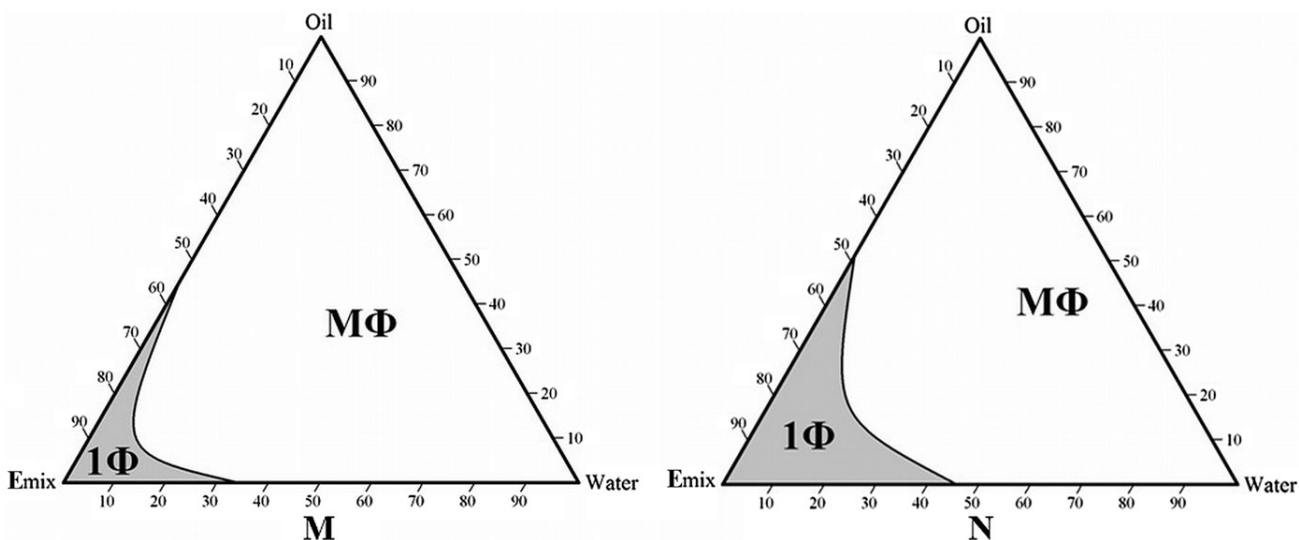


Figure 2. The pseudoternary phase diagram of the labrafac CC, cremophor RH 40/capmul MCM (Emix, 1:1), water (M) and labrafac CC, labrasol/capmul MCM (Emix, 1:1), water (N) at ambient temperature. The one phase region is designated by 1Φ and the multiphase region is designated by $M\Phi$.

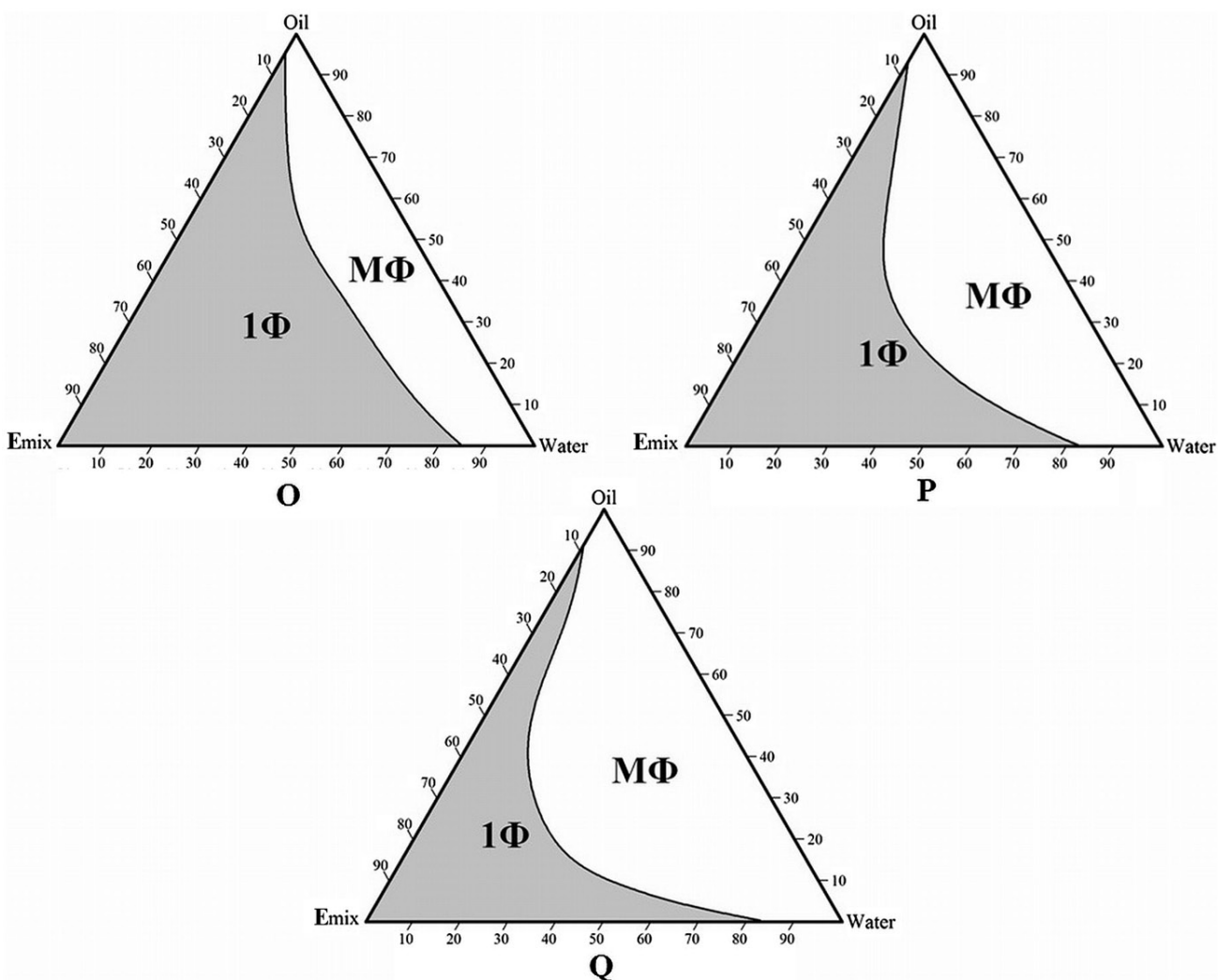


Figure 3. The pseudoternary phase diagrams of the labrafac CC, labrasol/cremophor RH 40 (1:1)/capmul MCM (Emix) water at the 1:1 (O), 2:1 (P), and 3:1 (Q) weight ratios of Emix (K_m) at ambient temperature. The one phase region is designated by 1Φ and the multiphase region is designated by $M\Phi$.

ME 1 and ME 2, $p < 0.05$ for ME 3 as compared with ME 4) as the content of Emix was decreased from 60% to 30% (w/w). The flux and K_p values of ME 5–ME 8 having 10% w/w content of Lab-CC were also increased significantly ($p < 0.01$ for ME 5 and ME 6, $p < 0.05$ for ME 7 as compared with ME 8) as the content of Emix was decreased from 60% to 30% (w/w). This may be due to the formation of viscous liquid crystalline gel at emulsifier–water interface in ME formulations with higher concentrations of emulsifiers as shown by the values of viscosity of ME formulations in Table 4. This leads to the decrease of thermodynamic activity and drug diffusion through the double layer ME to the receptor chamber¹⁷. The thermodynamic activity of drug in the formulation is a significant driving force for the release and penetration of drug into skin. The high saturation of drug in vehicle can result in high thermodynamic activity of drug in vehicle which can induce a high permeation rate¹⁸. As reported in the literature, ME systems have favorable solubilization behavior because of the combined effect of its hydrophilic and lipophilic components. So the solubilizing effect of TZR by ME may also have contributed to its increased permeation.

Further on increasing the oily phase from 5% to 10% (w/w), the flux value was increased showing a different mechanism from that of Emix. However, the difference in skin permeation rate of the drug from ME containing different amounts of oil (5% and 10%) was not significant ($p > 0.05$). It was assumed that increase in the number of internal phases with increase in content of oil might have resulted into the increased skin permeation rate of TZR^{17,19}. As shown in Table 4, skin permeation rate was significantly increased ($p < 0.01$ for ME 1 and ME 2, $p < 0.05$ for ME 3 as compared with ME 4) as the water content was increased from 35% to 65% (w/w) for the ME formulations containing 5% of oil. The flux value of ME 5 to ME 8 having 10% w/w content of Lab-CC was also increased significantly ($p < 0.01$

for ME 5 and ME 6, $p < 0.05$ for ME 7 as compared with ME8) as the content of water was increased from 30% to 60% (w/w). Thus, it was observed that there was a tight relationship between the hydration effect of the stratum corneum and the dermal permeation which is in accordance with observations made by Mohammed and Manoj¹⁹.

As observed from Table 4, the ME formulations with small particle size evidenced a higher permeation rate as compared with those having large particle size. The literature reports that small particle size makes a vehicle an excellent carrier for promoting percutaneous uptake of drug as the number of vesicles that can interact on a fixed area of stratum corneum would increase when the particle size decreases^{4–8}. The particle size of all the formulations was in nano range (19.05–85.36 nm), and thus providing a large surface area for transfer of TZR to skin due to very small droplet size.

The accumulative amount of TZR in skin was determined with an aim to formulate a vehicle with suitable penetration as well as uptake in the skin. MEs are believed to be in close contact of the skin lipid due to low interfacial tension values leading to faster permeation^{17,20,21}. As shown in Table 4, the amount of TZR retained in skin from MEs was found between $32.33 \pm 0.43 \mu\text{g}$ (6.4% of applied dose) and $46 \pm 0.55 \mu\text{g}$ (9.2% of the applied dose). The highest amount of TZR was retained in formulation ME 8 which also showed highest skin permeation rate. However, there was no significant ($p > 0.05$) linear correlation observed between skin content and flux. But it was found that ME 8 could significantly increase the skin content as compared with other formulations ($p < 0.01$ for ME 2, ME 3, ME 4, ME 5, and ME 6, and $p < 0.05$ for ME 7) except ME 1 ($p > 0.05$). This may be due to small particle diameter and controlled release of drug from ME¹⁰. The exact mechanism by which the uptake of drug is increased is still unclear and needs further investigation in future.

Thus, the ME formulation (ME 8) containing 10% Lab-CC, 15% LAB/CRH-40 (1/1) mixture, 15% Cap-MCM, and 60% aqueous phase (w/w) having appropriate skin permeation rate and skin uptake was considered as optimized formulation and was further characterized for physicochemical parameters. The physicochemical parameters of unloaded and drug loaded optimized ME are as shown in Table 5. For ME, zeta potential can be used to analyze the charge of the system. Zeta potential measurements of ME indicate that the interface is negatively charged. This charge may enhance the stability of the ME by causing double layer repulsion between droplets. The origin of interface charge may depend on the composition of oil, pH and electrolytes present in the water phase. ME formulations developed in this study exhibit negative zeta potential. This could be due to free fatty acids present in oil as well as ionic contaminants present in non-ionic surfactants which sometimes

Table 3. Compositions of the selected microemulsion formulations (% w/w).

Formulations	Labrafac-CC	Labrasol	Cremophor RH-40	Capmul-MCM	Water
ME1	5	15	15	30	35
ME2	5	12.5	12.5	25	45
ME3	5	10	10	20	55
ME4	5	7.5	7.5	15	65
ME5	10	15	15	30	30
ME6	10	12.5	12.5	25	40
ME7	10	10	10	20	50
ME8	10	7.5	7.5	15	60

Table 4. The permeation parameters, particle size and viscosity of tazarotene-loaded microemulsion formulations (mean $\pm$ SD, $n = 3$).

Microemulsion formulations	J_{ss} ($\mu\text{g}/\text{cm}^2/\text{h}$)	K_p (cm/h)	Q_{ret} (μg)	Particle size (nm)	PDI	Viscosity (cp)
ME 1	$0.0307 \pm 0.00176^*$	$0.613 \times 10^{-4} \pm 0.353 \times 10^{-5}$	$43.67 \pm 0.88^{\wedge}$	49.34 ± 2.69	0.341 ± 0.033	86 ± 0.07
ME 2	$0.0358 \pm 0.00156^*$	$0.736 \times 10^{-4} \pm 0.311 \times 10^{-5}$	$33.66 \pm 0.85^*$	50.11 ± 2.19	0.243 ± 0.037	53 ± 0.06
ME 3	$0.0391 \pm 0.00161^*$	$0.783 \times 10^{-4} \pm 0.312 \times 10^{-5}$	$36.00 \pm 0.57^*$	21.14 ± 1.32	0.143 ± 0.051	41 ± 0.03
ME 4	$0.0473 \pm 0.00049^{\wedge}$	$0.947 \times 10^{-4} \pm 0.982 \times 10^{-5}$	$38.00 \pm 0.62^*$	19.05 ± 1.75	0.139 ± 0.046	35 ± 0.02
ME 5	$0.0334 \pm 0.00075^*$	$0.669 \times 10^{-4} \pm 0.150 \times 10^{-5}$	$32.33 \pm 0.43^*$	85.36 ± 4.64	0.307 ± 0.065	95 ± 0.09
ME 6	$0.0368 \pm 0.00081^*$	$0.716 \times 10^{-4} \pm 0.156 \times 10^{-5}$	$35.00 \pm 0.71^*$	71.61 ± 2.87	0.267 ± 0.021	67 ± 0.05
ME 7	$0.0437 \pm 0.00112^{\#}$	$0.875 \times 10^{-4} \pm 0.220 \times 10^{-5}$	$41.66 \pm 0.87^{\#}$	31.72 ± 1.89	0.138 ± 0.056	46 ± 0.09
ME 8	0.0508 ± 0.00085	$1.020 \times 10^{-4} \pm 0.169 \times 10^{-5}$	46.00 ± 0.55	20.01 ± 1.28	0.117 ± 0.034	37 ± 0.05

* $p < 0.001$; # $p < 0.05$; $^{\wedge}p > 0.05$.

Figure 4. *In vitro* permeation profiles of tazarotene through rat skin from microemulsion formulations (mean $\pm$ SD, $n = 3$).

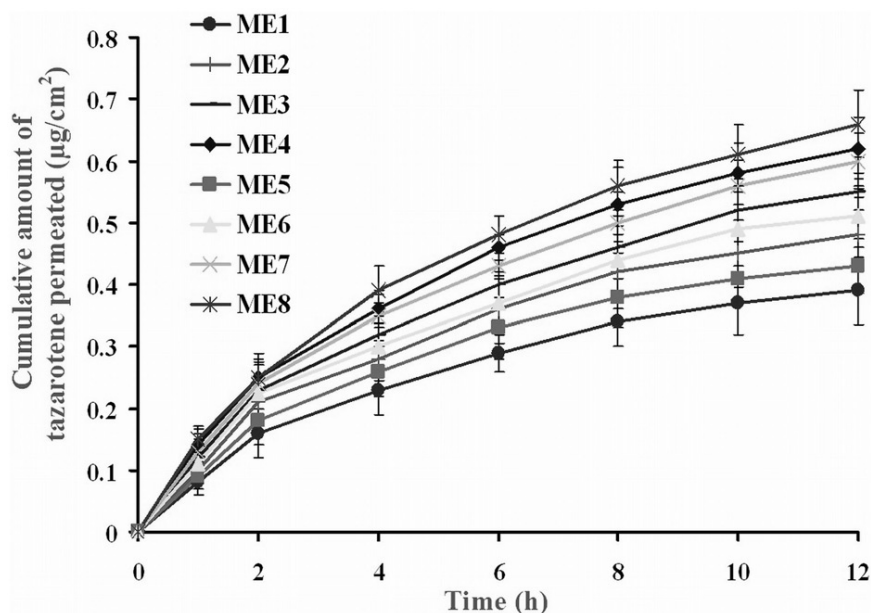


Table 5. Physicochemical characterization of optimized unloaded and tazarotene-loaded microemulsion formulation (freshly prepared and at the end of 6 months at room temperature) (mean $\pm$ SD, $n = 3$).

Parameters	ME		
	Unloaded	Drug loaded	Drug loaded after 6 months at room temperature
Zeta potential (mV)	-3.51 ± 0.27	-3.66 ± 0.23	-3.50 ± 0.22
Drug content (% w/w)	—	99.89 ± 2.66	99.79 ± 2.66
Percentage transmittance (% T)	99.17 ± 0.67	99.14 ± 0.52	99.29 ± 0.52
pH	6.72 ± 0.06	6.86 ± 0.02	6.89 ± 0.02
Conductivity (μ S/cm)	86.6 ± 0.4	315.7 ± 0.5	321.7 ± 0.8
Viscosity (cp)	34 ± 0.07	37 ± 0.05	38 ± 0.05
Particle size (nm)	22.14 ± 1.32	20.01 ± 1.28	22.08 ± 1.17
Polydispersity index	0.123 ± 0.037	0.117 ± 0.034	0.119 ± 0.042

are surface actives and thus contribute to the zeta potential¹⁸. The zeta potential of unloaded and drug-loaded ME formulations are shown in Table 5. It can be seen that the ME system is negatively charged and on the addition of drug the zeta potential values in the ME did not change significantly. TZR content of ME formulation was found to be $99.89 \pm 2.66\%$ w/w of the theoretical value (0.05% w/w). The % T values of unloaded and drug-loaded ME formulation were between 98 and 100% (Table 5) confirming its optical clarity. The pH value of unloaded and TZR-loaded ME formulation was 6.72 ± 0.06 and 6.86 ± 0.02 , respectively. It was observed that pH values were within the physiological range and the incorporated drug did not affect the optical texture or the pH values of the ME formulations. The average particle size of the plain and drug loaded ME was 22.14 ± 1.32 nm and 20.01 ± 1.28 nm, respectively. The particle size of drug-loaded ME was small as compared with unloaded ME, this may be due to the emulsified effect of the undissolved drug and reduction in surfactant mobility due to deposition of drug at the interface of ME²². All the formulations had droplets in the nano range which is very well evident from the low PDI values for both unloaded and drug-loaded vehicles. The PDI values of both formulations were very low in the range of 0.109 ± 0.017 – 0.123 ± 0.037 suggesting the higher uniformity of the droplet size within the

formulation^{4–6}. The TEM images of plain ME (Figure 5A) and drug-loaded ME (Figure 5B) revealed that the particle size was in the nanometric range and that particle had nearly spherical morphology without aggregation which is in accordance with particle size analysis. The samples were examined by ocular inspection in a cross polarizer for sample homogeneity and birefringence. All ME formulations appeared completely dark when observed under cross polarizer which confirmed its optically isotropic nature⁴. Electrical conductivity is a structure sensitive property and there are some studies reported about the systems of non-ionic emulsifiers. In present study, the conductivity measurements were done without incorporation of an electrolyte as it can lead to phase separation as reported in the literature¹⁶. The conductivity results obtained for unloaded and drug-loaded ME formulations (Table 5) showed that loading TZR and the addition of water phase into the formulation had no negative effects on system stability. When an unstable emulsion system and phase separation occurs, the conductivity values are greatly reduced²². However, the conductivity values for unloaded vehicle were about 2–3 factor lower than those of drug-loaded formulation. In the previous literature, it has been reported that the lowest conductivity plots extend to the largest droplet radii. The size of droplet for unloaded ME with low conductivity

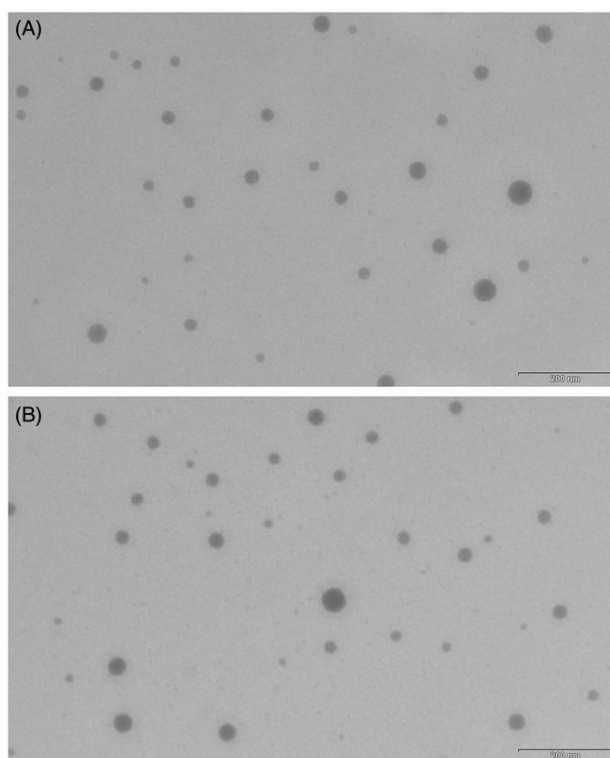


Figure 5. The TEM images of unloaded (A) and tazarotene-loaded (B) microemulsion formulations.

values was greater than those of drug-loaded formulation with higher conductivity values^{18,23}. The results of viscosity measurements indicate that the viscosity value of the drug-loaded ME was higher than the unloaded vehicle. Thus, the conductivity and viscosity studies suggest that the ME formulations are of o/w type.

Stability studies of the optimized ME formulation (ME 8) were carried out under stressed (centrifugation) and unstressed conditions by visual inspection as well as determination of physicochemical parameters such as particle size, polydispersity index, zeta potential, drug content, percentage transmittance, pH, conductivity, and viscosity. The ME showed good shelf stability showing no phase separation either cloudiness or any flocculation or precipitation for 6 months. These samples also showed no sign of phase separation under stress when subjected to centrifugation at $5000 \times g$ for 30 min. Thus centrifuge test showed that ME had good physical stability. All physicochemical parameters were determined at weekly interval for the first month and monthly interval for the subsequent months. However, no significant change in the physicochemical parameters was observed. The physicochemical parameters of ME formulation are shown in Table 5 at the end of 6 months.

One of the problems associated with the use of MEs for topical drug delivery is the difficulty of using these vehicles on the skin because of their fluidity^{17,19}. Hence, MBG of the optimized formulation (ME 8) was developed using a suitable polymer capable of modifying the rheological behavior. From various screened non-benzene grade Carbopol[®] polymers (Carbopol[®] 971P NF, Carbopol[®] 974P NF, and Carbopol[®] 980P NF), Carbopol[®] 971P NF was selected due to its compatibility with ME structure, feel, and ease of spreadability. It was observed that the order of the addition of Carbopol[®] 971P NF had no significant influence on the formation of MBG, but may influence the homogenized swelling of Carbopol[®] 971P NF. So for the

Table 6. Composition of microemulsion-based gel containing tazarotene.

Components	Content MBG
Tazarotene (% w/w)	0.05
Carbopol [®] 971P NF (% w/w)	2
Labrafac CC (% w/w)	10
Labrasol (% w/w)	7.5
Cremophor RH 40 (% w/w)	7.5
Capmul MCM (% w/w)	15
Water (g)	Quantity sufficient to produce 100 g of gel

subsequent study, MBG containing TZR was prepared by mixing the swollen gel matrix with the oily phase at 2% w/w concentration of Carbopol[®] 971P NF (Table 6). The TZR content of the MBG was found to $99.86 \pm 0.012\%$ w/w of the theoretical value (0.05% w/w). When a weighed quantity of gel was placed in between two glass plates of known weight, it spread uniformly to produce a circle, the diameter of this circle is related to spreadability of gel formulation. The larger is the diameter, the better is the spreadability¹⁷. The diameter of MBG was 7.5 ± 0.011 cm and that of marketed gel was found to be 6.7 ± 0.04 cm indicating better spreadability of MBG. This is because of the loose gel matrix nature of MBG formulation due to the presence of oil globules rather than the conventional gel matrix.

The pH of the gel was found to be 6.8 ± 0.03 indicating that it could result in less irritation to the skin. For a pharmaceutical or cosmetic product in order to be spread easily on the skin without running, it must be neither too fluid nor too viscous and show plastic or pseudoplastic rheological behavior²⁴. The MBG showed pseudoplastic behavior which facilitates the flow and improves the spreading characteristics of the formulation. The viscosity of MBG-Lab CC at 5 rpm was found to be $5.9 \times 10^3 \pm 0.6 \times 10^3$ cp. The MBG showed good shelf stability showing no phase separation or cloudiness for 6 months on visual inspection. The MBG was found to be stable at room temperature for 6 months with no significant change in the viscosity ($6.0 \times 10^3 \pm 0.8 \times 10^3$ cp), spreadability (7.3 ± 0.06 cm), pH (6.7 ± 0.05), and drug content ($99.89 \pm 0.043\%$ w/w).

The infrared spectra of TZR pure powder, plain ME, TZR-loaded ME, and MBG are as shown in Figure 6(A)–(D). TZR spectrum shows absorption bands at 3055 cm^{-1} due to aromatic C–H stretching and at 2908 cm^{-1} and 2961 cm^{-1} due to aliphatic C–H stretching. A absorption band at 2201 cm^{-1} corresponds to C≡C stretching of the di-substituted alkyne and prominent absorption band at 1719 cm^{-1} related to C=O stretching of ester. Band at 1585 cm^{-1} is due to aromatic C–C stretching. Absorption bands at 1365 cm^{-1} and 1448 cm^{-1} are due to aliphatic C–H bending. Other bands at 823 cm^{-1} and 777 cm^{-1} correspond to aromatic C–H bending. The IR spectrum of TZR-loaded ME and MBG is entirely different from TZR powder, while it closely resembles the spectrum of plain ME. The characteristic bands of TZR either have disappeared or few bands of TZR have reduced in intensity probably due to the restriction inside the formulation matrix. In IR spectrum of TZR-loaded ME and MBG no additional peak was observed which emphasized the absence of any possible interaction between the drug and formulation components used^{5,25–28}.

In order to assess the skin retention and penetration of TZR from MBG and marketed gel (Tazret[®]), the *in vitro* permeation ability was determined following the same procedure as described earlier for time period of 12 h (Table 7 and Figure 7). To understand the kinetics of permeation from these formulations, the data were treated according to zero-order (cumulative amount

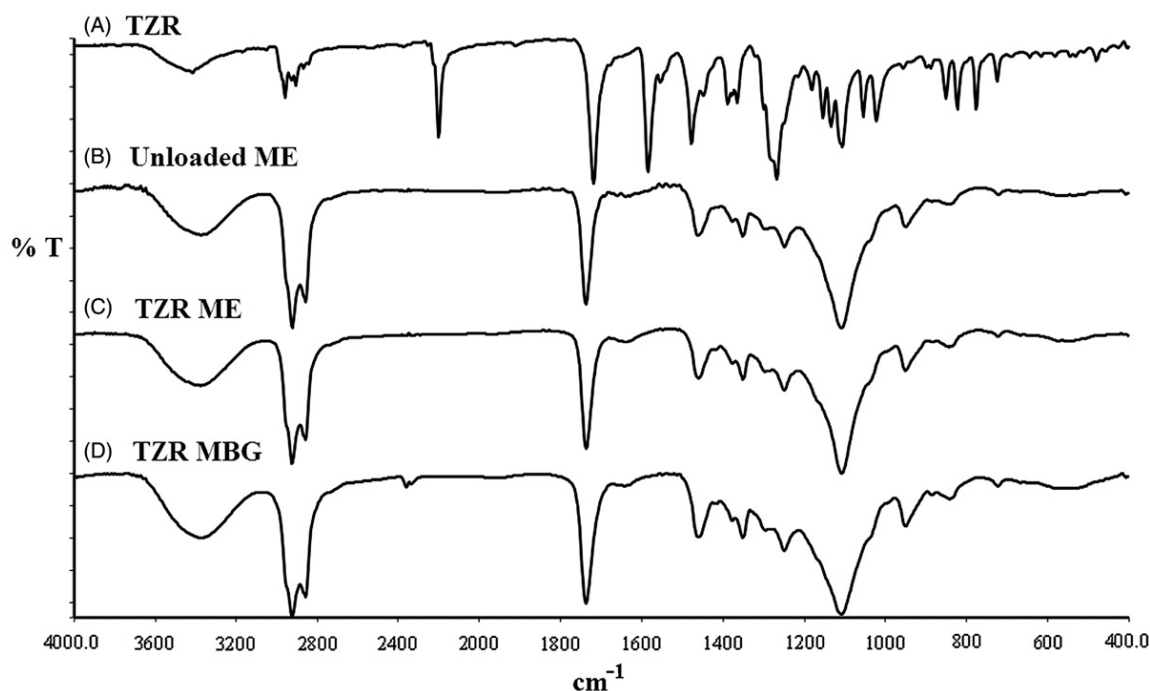


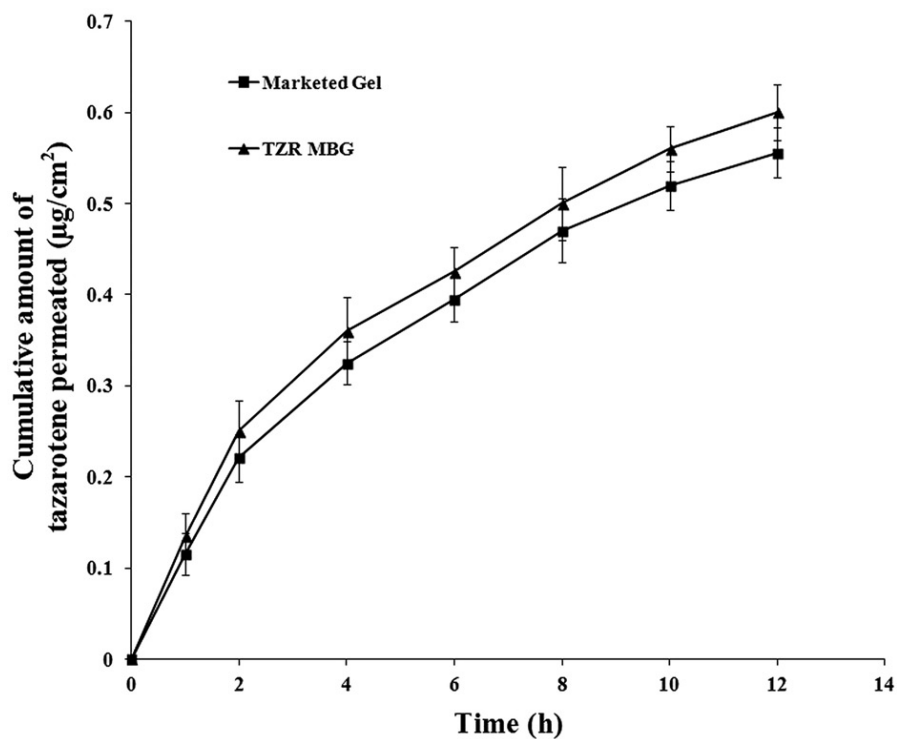
Figure 6. Infra red spectra of (A) tazarotene (TZR), (B) unloaded microemulsion, (C) tazarotene-loaded microemulsion (TZR-ME), and (D) tazarotene-loaded microemulsion-based gel (TZR-MBG).

Table 7. The permeation parameters of tazarotene-loaded microemulsion based gel and marketed gel (mean $\pm$ SD, $n = 3$).

Formulations	J_{ss} ($\mu\text{g}/\text{cm}^2/\text{h}$)	K_p (cm/h)	Q_{ret} (μg)
MBG	0.0413 ± 0.0004	$0.83 \times 10^{-4} \pm 0.1 \times 10^{-5}$	47.33 ± 0.82
Marketed gel	$0.0407 \pm 0.0002^*$	$0.81 \times 10^{-4} \pm 0.4 \times 10^{-5}^*$	$35.00 \pm 1.73^\#$

$^\#p < 0.05$; $^*p > 0.05$

Figure 7. *In vitro* permeation profiles of tazarotene through rat skin from marketed gel and microemulsion-based gel (MBG) (mean $\pm$ SD, $n = 3$).



of drug permeated versus time), first order (log of the cumulative amount of drug permeated versus time), and Higuchi (cumulative amount of drug permeated versus square root of time) equation. Moreover, the plot of cumulative amount of drug permeated versus square root of time showed a linear relationship for both MBG and marketed gel indicating that TZR followed Higuchi model for controlled permeation (Table 8).

The permeation profiles of TZR through rat skin from MBG and marketed gel are shown in Figure 7. The flux value for MBG ($0.0413 \pm 0.0004 \mu\text{g}/\text{cm}^2/\text{h}$) was found to be higher than that for marketed gel ($0.0407 \pm 0.0002 \mu\text{g}/\text{cm}^2/\text{h}$). Same observations were also made while considering permeability co-efficient (K_p) with MBG having higher value ($0.83 \times 10^{-4} \pm 0.1 \times 10^{-5} \text{ cm/h}$) than marketed gel ($0.81 \times 10^{-4} \pm 0.4 \times 10^{-5} \text{ cm/h}$). The difference in the flux and K_p values of marketed gel and MBG was not significant ($p > 0.05$). But slight higher flux and K_p values of MBG suggest that it might be able to enter the skin easily as compared with marketed gel with an advantage of low interfacial tension of the emulsifier film that ensures an excellent contact to the skin²⁹. The statistical comparison of flux and K_p values of MBG and its ME (ME 8) revealed that the permeation of drug from viscosized ME was lower as compared with fluid ME ($p < 0.05$). This might be attributed to its higher viscosity values that can further decrease permeation in the skin³⁰. Also the movement of droplets in case of MBG would be limited further limiting the diffusion of TZR dissolved in the droplets and slowing down its releasing rates. Therefore, it can be said that MBG might have an excellent ability for sustained release as compared with its ME formulation.

As shown in Table 7, the higher amount of TZR was retained in skin from MBG ($47.33 \pm 0.82 \mu\text{g}$, 9.5% of applied dose) and lower from marketed gel ($35.00 \pm 1.73 \mu\text{g}$, 7% of applied dose). The MBG could significantly ($p < 0.05$) increase the accumulative uptake of TZR in skin as compared with the marketed formulation. ME have been shown to improve the dermal localization of several topical therapeutic agents. This was one of the reasons to

employ ME approach for topical delivery of TZR as its epidermal localization is highly desirable for enhancing the treatment of skin diseases such as acne⁹.

The rat skin is a multilayered organ with many histological layers. The histology of excised rat skin in control and treated with optimized ME, MBG, and marketed gel is shown in Figure 8. The microscopic observations indicate that the optimized ME, MBG and marketed gel has no significant effect on the microscopic structure of the skin. The surface epithelium lining and the granular cellular structure of the skin were totally intact. No major changes in the ultra structure of skin morphology could be seen and the epithelial cells appeared mostly unchanged⁷.

One of the major disadvantages associated with the TZR therapy is skin irritation (erythema), which strongly limits its utility and acceptability by the patients. Ideally, the delivery system of TZR 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 TZR. It was hypothesized that encapsulation of TZR in ME would reduce the skin irritation^{11,12}. The results obtained from the primary skin irritation studies are listed in Table 9. Draize patch test is a reliable method and the results obtained from this study could be linked to that obtained in humans¹². The skin-irritation studies indicated that MBG containing TZR did not show any sign of skin irritation as compared to moderate erythema shown by marketed formulation (Tazret[®] gel) after 72 h of application (Table 9). Thus, MBG demonstrated advantage over marketed formulation in improving the skin tolerability of TZR indicating their potential in improving patient compliance and topical delivery of TZR.

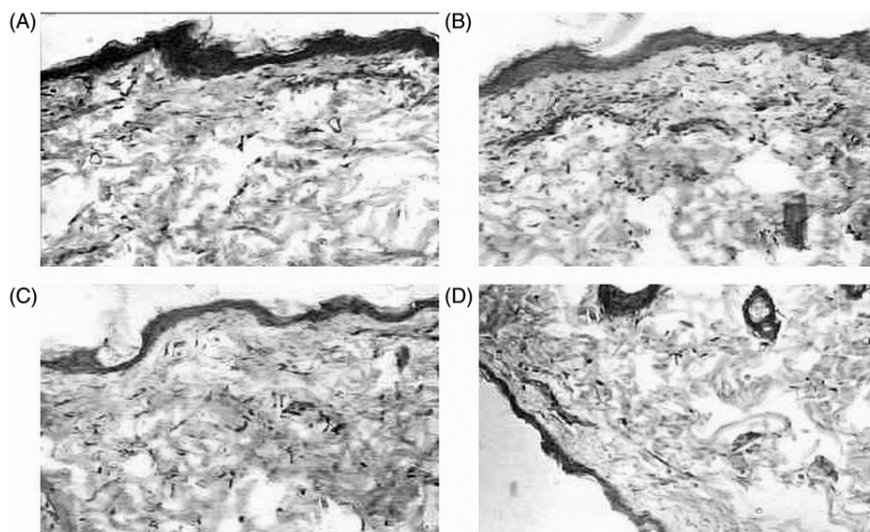
Table 8. The modeling parameters of tazarotene from marketed gel and microemulsion based gel (MBG) (mean $\pm$ SD, $n = 3$).

Formulation	Zero order R^2	First order R^2	Higuchi R^2
Marketed gel	0.9380	0.9384	0.9927
MBG	0.9261	0.9266	0.9942

Table 9. Mean erythmal scores for various tazarotene formulations obtained at the end of 24, 48, and 72h.

Formulations	Erythmal score ($n = 3$)		
	24 h	48 h	72 h
Control (Group 1)	0	0	0
Marketed gel (Group 2)	1	1	2
Microemulsion-based gel without tazarotene (Group 3)	0	0	0
Microemulsion-based gel containing tazarotene (Group 4)	0	0	0

Figure 8. Light microscopic photographs of *in vitro* histological study. (A) Control. (B) The rat skin treated with optimized microemulsion (TZR ME). (C) The rat skin treated with microemulsion based gel (MBG). (D) The rat skin treated with marketed gel.



Conclusion

Collectively, the results demonstrate that MBG may be a more promising approach for the topical delivery of TZR. It is assumed that MBG due to its appropriate physicochemical properties, high skin permeation ability, higher accumulative uptake in skin, and improved skin tolerability may be more suitable as a novel regime for topical administration of TZR. Nevertheless, significant work is yet to be conducted to elucidate the possible mechanism(s) of drug delivery into the skin. The clinical investigations with emphasis on desired therapeutic efficacy and favorable safety profile followed by pilot scale studies on manufacturing the product for commercial use also needs to be considered.

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Declaration of interest

The author(s) confirm that this article content has no conflicts of interest.

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