

Development and Characterization of Adapalene Loaded Microemulsion-Based Hydrogel for Acne Treatment

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ABSTRACT

Presently, acne is affecting globally many people (9.4%) and one of the leading causes of stress especially in youths. Adapalene (AD) has been applied successfully for acne treatment. However, adapalene drug is from BCS class II and exhibits less solubility in water (<1mg/mL) & causes skin irritation which leads to patient incompliance. The objective of the current study was design of microemulsion-based gel for topical delivery of adapalene. Solubility studies demonstrated that adapalene exhibit good solubility in Tween 80, propylene glycol, and oleic acid. The microemulsion showed globule size 248.4 ± 2 nm, PDI (0.251), and Zeta potential (-15.8 mV) confirming colloidal stability. The dilutability and freeze-thaw study of microemulsion showed no sign of phase separation. The microemulsion-based gel showed clarity, pH (5 ± 0.5), viscosity (64cps), and good spreadability (6.5 ± 0.6 cm) for topical application. In-vitro gel formation study showed that inclusion of polymer (Carbopol 940) maintained viscosity of microemulsion-based hydrogel for longer period of time. Dissolution study demonstrated that AD showed sustained release for 24h from gel as compared to microemulsion and drug suspension. Thus, it could be a prospective formulation for acne treatment via topical route which can improve patient compliance by reducing skin irritation.

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Introduction

Acne is affecting ~9.4% population worldwide and it is one of the eighth general condition in teenage (1). Acne problem arises due to the blockage of hair follicles by dead cells and oils. Acne can be of various types such as papules, seborrhoea, blackheads, comedons, nodules, and scar. Currently, variety of drugs available for acne treatment such as minocycline, clindamycin, retinoids, isotretinoin, etc (2). These drugs commonly administered by oral or topical route. In mild condition, topical application is the first choice, however, in severe situation oral or systemic treatment need to be given. Topical preparations are used for localized effects due to pharmaceutical penetration into the mucous membranes or deep skin layer at the application site. The key advantage of a topical delivery strategy is the capacity to distribute drugs more precisely to particular areas (3).

Adapalene (AD), is an antiacne drug, that has been proven advantageous in the treatment of acne (Figure 1) (4). Adapalene drug is from BCS class II and exhibits less solubility in aqueous phase (<1mg/mL). Moreover, adapalene cause photosensitivity, skin irritation, and less patient compliance when applied topically (5). Therefore, there is requirement of novel formulation to enhance the solubility of drug and reduce skin irritation caused by adapalene.

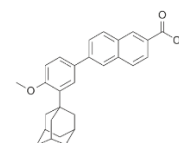


Figure 1. Chemical structure of adapalene

Several novel formulations have been studied for topical delivery of antiacne drugs viz. nanoparticles, nanoemulsion,

liposomes, SLNs, microemulsion, etc (6). Amongst them microemulsions are thermodynamically stable, clear, isotropic dispersion. They exhibit globule size in range of 5-200 nm. Microemulsions offer several advantages for topical delivery such as improvement of solubility of lipophilic molecules, hydration of stratum corneum resulting improvement of permeability and dermal flux (7). However, achievement of microemulsion stability for longer period of time is difficult due to less viscosity. This shortcoming can be resolved by formation of microemulsion-based gel by incorporating polymers such as Carbopol, HPMC, xanthum gum, etc (8).

Alam et al. developed microemulsion-based gel loaded with isotretinoin and erythromycin estolate for acne treatment (9). In a recent study conducted by Tambuwala et al. microemulsion-based gel containing azelaic acid and tea tree essential oil was designed for *Propionibacterium* and testosterone-induced acne treatment (10). Hu et al. designed levamisole microemulsion-based gel incorporating alginate-boric acid for transdermal delivery for high malleability (11). In another study, Patel et al. formulated tazarotene loaded microemulsion-based gel using Carbopol 971P for treatment of acne and showed prolonged release and good permeation of drug through gel (12).

In the current study we have developed adapalene loaded microemulsion-based gel by incorporating Carbopol 940 for topical delivery. To improve the solubility of adapalene and to decrease the skin irritation caused by adapalene, microemulsion-based gel was developed and evaluated for physicochemical properties of microemulsion, gel properties, and stability.

Abbreviations

ADAdapalene, PGPropylene glycol, SSurfactant, Cos Cosurfactant, ME Microemulsion, PDI Poly dispersity index.

Material and methods

Materials

Adapalene was kindly gifted by Abbott, Mumbai, India. Oleic acid, Polyoxyethylene-20 sorbitan monolaurate (Polysorbate 80), propylene glycol (PG) was obtained from Rankem, Avantor Ltd. India. Carbopol 940 was procured from Lubrizol, India. Sudan red was purchased from Anmol Colorants Global Pvt. Ltd, India. Analytical grade solvents and chemicals utilized in a study.

Methods

Solubility study of adapalene

The equilibrium solubility of adapalene in various ingredients of microemulsion (cosurfactants, surfactants, and oil) was assessed after addition of excessive amount of AD in 1mL each of ingredients in microcentrifuge tubes. Blends were kept for equilibration for 48h at 25°C with interim agitation. The equilibrated samples were allowed to centrifuge for 20 min at 15,000g. Then supernatant (0.5 mL) was diluted with tetrahydrofuran and AD was quantified by UV spectrophotometry at 234nm.

Preparation of pseudo-ternary phase diagram

The microemulsion region was found by designing pseudo-ternary phase diagrams by water titration method (13). The oleic acid as oil phase, Tween 80 as surfactant (S), and propylene glycol a cosurfactant (Cos) was selected. Phase diagrams were made using oleic acid and Tween 80/PG at varying ratios (1:1, 1:2, and 1:3) of S/Cos. The S/Cos mixture and the oil phase were then blended at weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. Under moderate stirring, these combinations were diluted drop-by-drop using double distilled water. Tridraw software 4.5 was used to plot the various regions (Informer Technologies, Inc. USA).

Preparation of microemulsion

The selected microemulsion containing oleic acid, Tween 80, and PG was loaded with adapalene (0.1 percent w/v). The mixture was titrated with distilled water and constantly stirred until solubilization of drug.

Development of microemulsion-based hydrogel

To optimize the microemulsion-based gel, Carbopol 940 at varying amount (1, 1.5, and 2% w/w) was incorporated as a polymer to improve the thickness of the microemulsion. Under stirring, the microemulsion (2mL) and polymer were progressively mixed together. Based on viscosity and transparency the final (%) concentration was chosen (14).

Characterization of microemulsion

Drug content

For drug content analysis of AD microemulsion 0.1 mL was diluted with methanol and centrifuged at 15,000 RPM for 10min. After that the supernatant was examined for drug content utilizing UV spectrophotometry at lambda max of 234 nm.

% Transmittance

The % transmittance of ME was carried out on UV spectrophotometry at 240 nm. Three readings were taken and mean was calculated.

Zeta Potential

The Zetasizer instrument (Malvern Instruments Ltd, UK) was utilized to examine the zeta potential. AD microemulsion was diluted by distilled water (1:10 & 1:100) and kept in a folded capillary cell for zeta potential analysis.

Particle size and polydispersity index analysis

The globule size was measured using particle size analyzer (Malvern Instruments Ltd, UK). The AD microemulsion was diluted in distilled water for the analysis (0.5 ml was diluted with 10 ml of purified water), and tested for globule size & PDI.

Dilutability and centrifugation

The microemulsions were diluted with deionized water in 1:100 and 1:10 ratios to check if microemulsion showed any indication of phase separation (15). Moreover, microemulsion

was centrifuged utilizing cold centrifuge (REMI R-8C 6000) for 30 mins at 15000 rpm and then examined for phase separation.

Freeze-thaw study

Freeze and thaw cycle was utilized to assess the microemulsion stability. The pre-concentrate of AD microemulsion was employed for 3-4 cycles of freeze-thaw. For freezing microemulsion was kept at -10°C for 24h, then allowed to thaw for 24h at room temperature (25°C). After that microemulsion was centrifuged at 10,000 rpm for 10 mins. Then formulation was visually observed for phase separation (16).

Characterization of AD-loaded microemulsion-based hydrogel

Viscosity

The viscosity of microemulsion-based gel was evaluated by a Brookfield viscometer (DV-II+pro). The viscosity of gel was measured by filling a 5ml beaker with 1ml of the gel and thickness was detected. The spindle was immersed in gel at 25°C and revolved at 50 & 100 RPM.

pH

The pH of the microemulsion-based gel was found out by an electronic calibrated pH metre. 1ml gel was taken and liquified using 10 ml of distilled water and pH of the gel was measured three times.

Spreadability

The spreadability study was conducted by reported method (17). The equipment made up of wooden block containing a pulley, two glass slides, and a scale was utilized. The excessive amount of gel was inserted within glass slides, and 20g weight was applied on the top slide for 5mins to maintain uniformity in thickness. Spreadability was measured by measuring the time in seconds required to detach both slides. The below mentioned formula was applied to calculate spreadability $S = (m \times l)/t$, where, S = spreadability; m = weight attached to higher slides; l = glass slide length, and t = time in seconds.

In vitro gel formation

In vitro gel formation was assessed by adding 500 µL of AD microemulsion and microemulsion-based gel containing Sudan red dye into a Petri plate at 37°C containing phosphate buffer (pH 5.5, 5 mL) (18). After formation of gel images were shot with a digital camera after 30 minutes and 24h.

Dissolution study

The Franz diffusion technique was applied to examine the release pattern of AD from AD solution, AD microemulsion, and gel (19). The dissolution media phosphate buffer (pH 5.5) and THF (6:4) was utilized. The cellophane membrane was measured to the desired size and then activated in buffer for 24 hours. After 24 hours, the cellophane membrane was placed under running distilled water and then kept in the Franz diffusion equipment. A weighed quantity of AD suspension, microemulsion, and microemulsion-based gel was taken in equivalent to the drug concentration (0.1%) and poured into the

apparatus from one end, while phosphate buffer + THF was kept into the acceptor compartment of the apparatus (6:4) (20mL). After that, the equipment was kept on a magnetic stirrer set at 400 rpm and 37°C. 1ml aliquots were removed at various time intervals (0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, & 24 hours) and were refreshed with fresh PBS each time. After that, the samples were diluted in the same solvent solution and examined using a UV spectrophotometer at 234 nm.

Stability study

The accelerated stability study of microemulsion and microemulsion-based gel was conducted as per ICH guidelines. The microemulsion and microemulsion-based hydrogel was kept at room temperature & accelerated conditions $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ RH for 6 months. The samples were withdrawn at an interval of one month and evaluated based on their physical appearance, drug content, particle size, and gelling property.

Results and Discussion

Solubility study of adapalene

Adapalene solubility was studied in various oils, surfactants, and cosurfactants. Amongst oils, adapalene revealed maximum solubility in oleic acid (56.23 ± 0.45 mg/mL), however, castor oil showed lowest solubility. Amid surfactants Tween 80 demonstrated highest solubility of adapalene (45.46 ± 0.35), whereas Span 80 showed less solubility. Amidst cosurfactants propylene glycol (PG) showed maximum solubility than other cosurfactants (Table 1). As suggested by the solubility studies, oleic acid was chosen as the oil, Tween 80 & PG was selected as the surfactant and co-surfactant, respectively.

Table 1. Solubility of adapalene in various ingredients of ME

	Ingredients	Solubility (mg/mL)
Oils	Oleic acid	56.23±0.45
	Coconut oil	15.05±0.25
	Tea tree oil	28.35±0.33
	Olive oil	9.98±0.55
	Castor oil	10.35±0.33
Surfactants	Tween 80	45.46±0.35
	Tween 20	40.45±0.45
	Span 20	3.35±0.5
Cosurfactants	Span 80	2.45±0.5
	Isopropyl alcohol	10.04±0.22
	Propylene glycol	35.05±0.45
	Polyethylene glycol 400	20.05±0.5

Pseudo-ternary phase diagram

Ternary phase diagrams were designed for identifying microemulsion area at various ratios of S/Cos 1:1, 2:1, and 3:1. Figure 2 indicates that composition of oleic acid and varied ratios of S/Cos demonstrated formation of microemulsion. Nevertheless, the S/Cos ratio at 1:2 demonstrated stability over 15 days after addition of adapalene as compared to other

combinations. Acharya et al. reported that microemulsion developed using same combination of S/Cos loaded with carbamazepine was found stable (20). Therefore, this combination was considered for further studies.

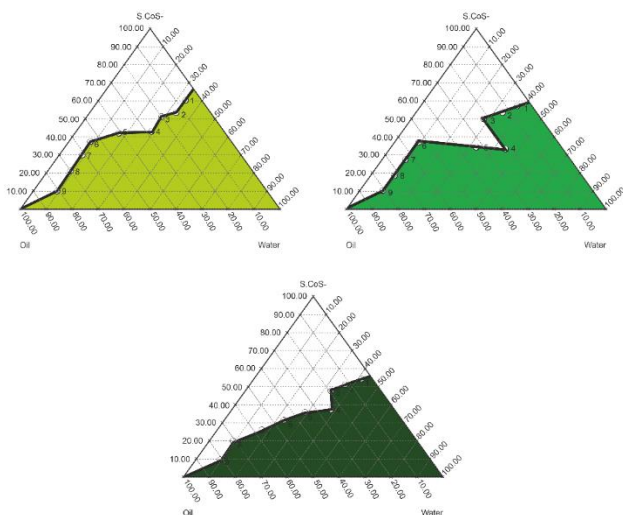


Figure 2. Pseudo ternary phase diagrams using oleic acid as oil and S: Cos at different ratios 1:1, 1:2, and 1:3

Adapalene loaded microemulsion

Microemulsion formed by using oleic acid and Tween 80/PG 1:2 S/Cos was found transparent (Table 2). Adapalene (0.1% w/v) was dissolved slowly in this combination. After complete solubilization, the mixture was titrated with distilled water with continuous stirring. Results showed that F4 batch showed highest drug loading (96.5±2 %) and found stable.

Table 2. Trial batches of microemulsion by water titration method (1:2)

Batch no.	% Oleic acid	% Smix [1:2]	% Water	Drug Loading (%)
F1	100	900	600	64.05 ±0.45
F2	200	800	500	68.10 ±0.19
F3	300	700	500	70.45±0.45
F4	400	600	700	96.5±2.0
F5	500	500	500	70.05±0.45
F6	600	400	400	62.33±0.23
F7	700	300	300	55.45±0.33
F8	800	200	200	48.66±0.22
F9	900	100	100	40.45±0.55

Development of microemulsion-based gel

To develop intact hydrogel, Carbopol 940 polymer was included in microemulsion at varied concentrations (1, 1.5, and 2%). The results suggested that 1% Carbopol 940 showed optimum stiffness with good viscosity and spreadability. Nevertheless, 1.5 and 2% Carbopol concentrations showed high viscosities and less spreadability (Table 3). For development of topical formulation, the formulation needs to be easily applied on the skin and spreadability is an important criterion (21). Thus, 1% Carbopol 940 concentration was chosen for gel development.

Table 3. Spreadability and viscosity of ME containing varying concentration of Carbopol 940

Carbopol 940 (%) in ME	Spreadability (cm)	Viscosity (cps)
1	6.5± 0.06	64
1.5	5.8± 0.02	70
2	4.5 ± 0.03	73

Characterization of microemulsion

Drug content and transparency

The optimized microemulsion (F4) showed (96.5 ±2 mg/ml) drug content and 93.95% transparency at 240 nm.

Zeta potential and particle size analysis

The microemulsion exhibit particle size 248.4±2 nm and PDI (0.251) suggesting uniformity. Zeta potential was found -15.8mV suggested good stability of the formulation (Figure 3). The negative charge of microemulsion is attributed to oleic acid which contains negatively charged functional groups (22). Studies suggested that zeta potential value less than ±30mV gives stable formulations (23).

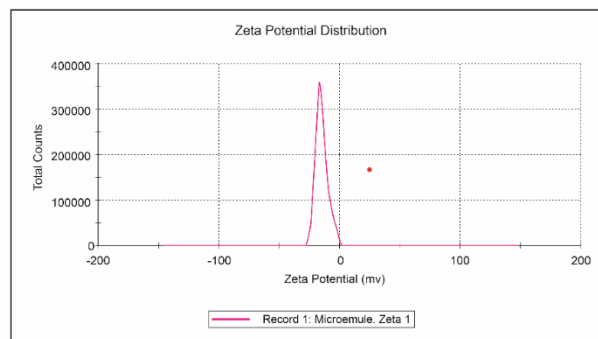


Figure 3. Zeta potential of AD loaded microemulsion

Dilutability & Centrifugation

The microemulsion was thinned by purified water in 1:10 and 1:100 ratios to check the stability of formulation. The microemulsion has not revealed any signal of phase separation

and found dilutable more than 100 times. Thus, the developed formulation showed good stability. Furthermore, after centrifugation at high speed there is no any indication of phase separation further confirmed stability of microemulsion.

Freeze-Thaw Study

Freeze-thaw study revealed homogeneity of microemulsion and is an important study of microemulsion to understand thermodynamic stability (24). No any sign of cracking, creaming, drug separation, and phase separation of microemulsion was observed. Thus, developed formulation showed thermodynamic stability.

Characterization of microemulsion-based gel Viscosity

The thickness of the microemulsion-based gel was found to be 64 cps. Carbopol 940 is a polymer which was added in microemulsion for imparting viscosity to the formulation. The viscosity of Carbopol 940 concentration (1%) was the optimum with good spreadability property.

pH

The pH of the developed formulation was found 5 ± 0.5 , which was found closer to the skin pH. Thus, formulation was found suitable for topical application and non-irritant to the skin.

Spreadability study

Spreadability of gel is described as the capability of gel to be spread easily on the skin surface. Moreover, spreadability is an essential parameter which needs to be considered in patient compliance for topical application (25). Greater is the region diameter by the gel, higher is the area covered by the gel. The spreadability of the optimized gel was found 6.5 ± 0.06 cm. The ideal range of good spreadability is considered ranging 5-7cm (26). As compared to other polymer concentrations (1.5 % and 2%) spreadability of 1% Carbopol 940 was found good exhibiting less time of spreading. The possible reason is that spreadability is inversely proportional to viscosity. Higher is the viscosity there is difficulty in spreading and less uniform layer get formed.

In Vitro Gel formation

In vitro gel formation was assessed by adding Sudan red dye to confirm instantaneous gelling of microemulsion in presence and absence of polymer in an aqueous phase. Microemulsion-based gel demonstrated low viscosity and rapidly spread with loss of stiffness over 30 min, however microemulsion containing polymer (Carbopol 940) formed a hydrogel with no evident spreading even after 4h (Figure 4). Carbopol 940 polymer is

known for hydrogel formation and presence of acrylic groups develops cross-linked structure at pH around 6-10 (27).

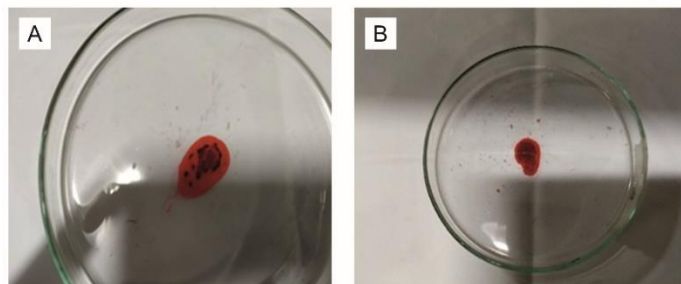


Figure 4. In-vitro gel formation of; A. Microemulsion; B. Microemulsion based gel

Dissolution Study

A Franz diffusion apparatus was utilized to check release pattern of AD. The dissolution study revealed that adapalene drug suspension showed 90% of drug release within 4h. However, ME demonstrated 90% drug release up to 8h suggesting sustained release of drug (Figure 5). Shukla et al. also mentioned that microemulsions provide sustained release of drugs via topical route (28). In another study, Shinde et al. designed repaglinide loaded microemulsion for sustained release of drug (29). Furthermore, microemulsion with Carbopol 940 polymer showed prolonged release up to 24h (Figure 5). The 3D network of hydrogel creates a barrier for adapalene release and due to this slow diffusion of drug from the swelled matrix system occurred. Thus, adapalene showed a prolonged release when incorporated into a gel system. In a similar type of study Thombre et al. reported prolonged release and high permeation of diclofenac sodium from microemulsion based gel using gelling agent carbopol (30).

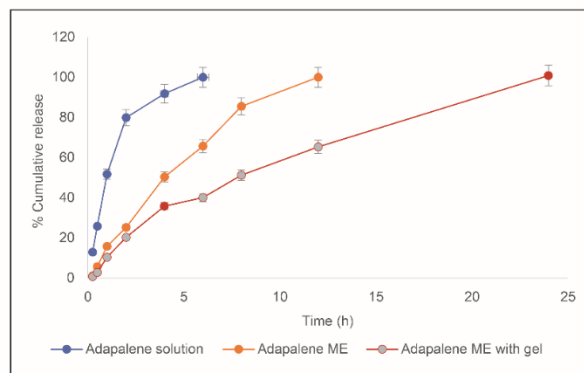


Figure 5. In-vitro release of adapalene from AD suspension, microemulsion, and microemulsion-based gel

Stability study

Table 4 displays stability testing of microemulsion and microemulsion-based hydrogel. At the end of six months, microemulsion revealed good appearance, drug content, and particle size. Microemulsion-based hydrogel was also found stable with good gelling property. There was no sign of drug precipitation and presence of particles. Thus, stability studies confirmed stability of formulation and good shelf-life.

Conclusion

The stable adapalene incorporated microemulsion-based gel was successfully developed with high drug loading. The microemulsion showed good % transmittance, particle size, PDI, zeta potential suggesting stability and uniform dispersion. Moreover dilutability, centrifugation, and freeze-thaw study confirmed stability of microemulsion. The microemulsion-based hydrogel demonstrated clarity, pH (~5), and free from particulates that is suitable for topical application. In-vitro gel formation demonstrated that after inclusion of polymer (Carbopol 940), gel was maintained due to improved viscosity. Furthermore, spreadability which is prerequisite property for topical gel was found good with 1% Carbopol 940 concentration and found easily spreadable covering large diameter (6.5±0.06 cm). *In vitro* dissolution studies showed sustained release of adapalene from microemulsion-based hydrogel for 24h as compared to microemulsion (8h) and adapalene suspension (4h). Therefore, developed formulation could improve solubilization of adapalene and reduce adapalene associated skin irritation. It could be a potential novel drug delivery system for acne treatment with high patient compliance. Nevertheless, preclinical studies are required to confirm the same.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Table 4. Stability study of microemulsion and microemulsion-based hydrogel

Microemulsion						
%	96.5±	95.1±	95±0.	96.1±	96±0.	95±0.3
Drug content	0.2	0.5	5	0.1	2	
Particle size (nm)	248.4 ±2 nm	249±0.5	250±0.5	249±1	250±0.5	251±0.3 nm
Microemulsion-based hydrogel						
%	96±0.	96±0.	96±0.	96±0.	96±0.	96±0.2
Drug content	2	2	2	2	2	
Gelling properties	++	++	++	++	++	++

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Storage conditions	25°C/60%RH condition			40±2°C/75% RH condition		
	Initial	3 months	6 months	Initial	3 months	6 months
Sampling time						

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