Original Article

EFFECT OF MENTHOL ON THE TRANSDERMAL PERMEATION OF ACECLOFENAC FROM MICROEMULSION FORMULATION

ABDUL BAQUEE AHMED[1*], GOURANGA DAS[2]

*[1]Department of Pharmaceutics, Girijananda Chowdhury Institute of Pharmaceutical Science, Hathkhowapara, Azara, Guwahati, Assam 781017, [2]Girijananda Chowdhury Institute of Pharmaceutical Science, Hathkhowapara, Azara, Guwahati, Assam 781017
Email: baquee1977@gmail.com

ABSTRACT

Objective: The aim of this investigation was to enhance the transdermal permeation of aceclofenac (ACF) from microemulsion formulation using menthol as a natural permeation enhancer.

Methods: Microemulsion containing 2% w/v of ACF was prepared by a titration method with different concentration of oil, surfactant and co-surfactant. The prepared microemulsion was evaluated for droplet size, viscosity, pH and *in vitro* skin permeation studies. Menthol at 3-8% w/w was added to the selected microemulsion formulation and their effect on skin permeation was evaluated across rat epidermis using modified Keshary-Chien diffusion cell. The Fourier transform infrared spectroscopy (FT-IR) was performed to understand the regulation action of menthol in the skin permeability barrier.

Results: The average droplet size of the microemulsion was found to be 89.4±2.12 to 175.2±3.10 nm. The transdermal flux of the microemulsion containing 8% w/w menthol showed 2.9 fold increases in transdermal flux of ACF compared with the formulation without menthol. Result of FT-IR studies showed decrease in peak height of the symmetric and asymmetric C-H stretching vibrations may be because of the extraction of the stratum corneum (SC) lipids and the alteration of the skin permeability barrier.

Conclusion: This result suggests that menthol significantly enhanced the transdermal permeation of ACF and may be an effective natural penetration enhancer for transdermal delivery of the drug.

Keywords: Menthol, Microemulsion, Transdermal flux, Surfactant, Co-surfactant

INTRODUCTION

Aceclofenac (ACF) (2-[(2, 6-dichlorophenyl) amine] phenylacetoxyacetic acid) is an orally effective non-steroidal anti-inflammatory drug (NSAID) of the phenylacetic acid group, which possesses remarkable anti-inflammatory, analgesic, and antipyretic properties. It is used in the treatment of osteoarthritis and inflammatory disease of the joints [1-2]. The most widely cited side-effect of NSAIDs includes, gastrointestinal ulcer, accompanied by anemia due to the bleeding, which is also true for ACF. In order to avoid the gastric irritation, minimize the systemic toxicity and achieve a better therapeutic effect, one promising method is to administer the drug via skin [3-4].

Microemulsion typically consists of oil, surfactant, co-surfactant and an aqueous phase, which is transparent, thermodynamically stable and has a droplet size<0.15 µm and does not have the tendency to coalesce [5-6]. Microemulsion has several advantages such as enhanced drug solubility, good thermodynamic stability, ease of manufacturing and enhancement effect on transdermal delivery over conventional formulation [7, 8]. Recently, more attention has focused on microemulsions for transdermal delivery of drugs such as meloxicam [9], diclofenac diethylamine [10], triptolide [11].

On the other hand, one of the greatest obstacles in the transdermal route of drug delivery is the stratum corneum, since it provides a rate limiting step for drug absorption across the skin [12]. A popular technique to enhance the percutaneous absorption is the use of penetration enhancers that reversibly perturb the permeability barrier of the stratum corneum [13]. The most commonly used penetration enhancers include organic solvents, fatty acids and alcohols, terpenes and azone derivatives.

An ideal penetration enhancer should be pharmacologically inactive and nonirritant for the skin, potent and cosmetically acceptable [14]. Menthol, a naturally occurring terpenes compound (as showed in fig. 1) isolated from *Mentha Piperita* is free from toxic effects and has been approved as a penetration enhancer in the transdermal delivery of several hydrophilic and lipophilic drugs [15-17].
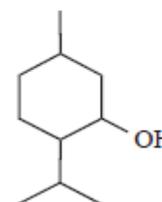

Fig. 1: Molecular structure of menthol

However, the penetration-enhancing characteristic of menthol on the percutaneous absorption of ACF was not yet tested. In present study, o/w microemulsion containing ACF have been developed to achieve maximum skin permeation rate of the drug through excised rat epidermis using menthol as a natural penetration enhancer.

MATERIALS AND METHODS

Materials

ACF was a gift sample from IPCA Pharmaceutical Pvt. Ltd. (India), castor oil, soybean oil, olive oil, oleic acid, polyoxyethylene sorbitan monolaurate (tween 20), polyoxyethylene sorbitan monooleate (tween 80), sorbitan monooleate (span 80), ethanol, and isopropyl alcohol (IPA) were purchased from Merck Chemicals (Mumbai, India). Menthol (Aldrich Chemical, Germany), sodium bromide (Loba Chemie Pvt. Ltd., Mumbai, India) and chloroform (Thomas Baker Chemicals Pvt. Ltd., Mumbai, India) were procured and used in this investigation. Water was purified by double distillation in a glass apparatus. All other chemicals and solvents were of analytical grade.

Methods

Screening of oils, surfactants, and co-surfactants for microemulsion

The solubility of ACF was investigated in various oils (castor oil, olive oil, soybean oil, and oleic acid), surfactants (Tween 20, Tween

80, Span 20) and co-surfactants (ethanol, IPA) to select a solvent system with good solubilizing capacity of ACF. An excess amount of ACF was added to 5 ml of each of the solvent and shaken at 25 °C for 72 h to attain equilibrium. The suspension was filtered through a 0.45 μm membrane filter and the concentration of ACF in the filtrate was determined by UV spectrophotometer at 273 nm.

Construction of pseudo-ternary phase diagrams and formulation of ACF microemulsion

The pseudo-ternary phase diagrams were constructed by installation of homogeneous liquid mixtures of oil, surfactant and co-surfactant with water at ambient temperature [18] at S/CoS ratio of 1:1, 2:1, 3:1 and 4:1 that resulted in large existent area of microemulsion containing ACF. The ratio of oil to mixture of S/CoS was varied from 9:1 to 1:9 and water was added drop by drop under continuous agitation. Usually with the addition of water to mixture of oils and S/CoS, the mixture become turbid at a certain point (beginning of phase inversion) and the turbid mixture again turned to clear indicating formation of O/W microemulsion. Further, 2-8% w/w menthol was added to the selected formulations and the skin permeation rate of ACF was also evaluated across rat epidermis using Keshary-Chien diffusion cell.

Characterization of microemulsion

Droplet size, pH and viscosity determination

The average droplet size of the microemulsions were measured by dynamic light scattering (DLS) using a zetasizer nano-S90 (Malvern Instruments, England). Light scattering was monitored at 25^0C at a 90^0 angle. The pH of the formulations was determined by using a pH meter (TOSHNIWAL, Model CL 54) at 25±1 °C. The viscosity of microemulsions was measured using a Brookfield rotational viscometer (LV2, Brookfield Inc., USA) equipped with the spindle no. 64 at 25^0 C.

In vitro skin permeation study

The abdominal hair of Wistar male albino rats (obtained from M/S Ghosh Enterprises, Kolkata, India and maintained under controlled conditions of temperature as well as humidity), weighing 150-200 g, was shaved using an electric razor after sacrificing with excess chloroform inhalation. The abdominal skin was surgically removed and adhering subcutaneous fat was carefully cleaned. The epidermis was then separated from dermis by soaking the full thickness skin in 2 M sodium bromide solution in water for 6-8 h [19]**.** The epidermis was thoroughly washed with water, dried at 25% RH, wrapped in aluminium foil and stored in freeze until further use. All experiments with animals were approved by the institutional animal ethics committee of Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS/IAEC/M. ph/2014/3) India.

For *in vitro* permeation studies, skins were allowed to hydrate for 1 h before being mounted on the Keshary-Chien diffusion cell with the stratum corneum (SC) with effective surface area 1.54 cm^2 facing the donor compartment. The receptor compartment was filled with 19.5 ml of pH 7.4 phosphate buffer and its temperature was maintained at 37±0.5 °C. 1 ml of microemulsion was applied on the SC side in the donor compartment and covered with aluminium foil to prevent drying out. 1 ml of receptor medium was withdrawn every hour for 8 h after the application and replaced with an equal volume of fresh phosphate buffer pH 7.4 equilibrated at 37±0.5 °C. The amount of drug permeated was determined spectrophotometrically at 273 nm.

Preparation of rat SC

The rat epidermis was incubated for 4 hr with 1% (w/v) trypsin solution in phosphate buffer pH 7.4 at 37 °C. The tissue was then smoothed out on a flat surface and the mushy epidermis was removed by rubbing with a moist, cotton-tipped applicator. The transparent SC so obtained was floated briefly on water, blotted dry, and used in the FT-IR studies [20-21].

FT-IR studies of rat SC

Earlier by Casal and Mantsch [22] have established that lipid extraction of SC can be observed with a decrease in the C–H stretching absorbance intensity of FT-IR spectra of SC. In the present study rat SC was treated with 3-8% menthol in 25:75 ratio of an ethanol-water co-solvent system for 24 h. The treated SC samples were vacuum-dried (650 mm of Hg) at 21±1 °C for 2 d and stored in a desiccators to remove the traces of menthol and ethanol [23-24]. The completely dried samples of the SC were then subjected to FT-IR (Bruker Alpha, Germany) study and spectra were recorded in the frequency range of 400 to 4000 cm^{-1}.

RESULTS AND DISCUSSION

Screening of oils and surfactants

The results of solubility studies of ACF showed variable solubility of ACF in oils, surfactant and co-surfactant. The solubility of ACF was found to be maximum in castor oil (23.23±0.13 mg/ml) followed by oleic acid (4.20±2.00 mg/ml), olive oil (1.45±0.06 mg/ml) and soy bean oil (1.07±0.13 mg/ml). Amongst the surfactants, tween 80 showed the maximum solubility (65.27±2.77 mg/ml) followed by tween 20 (10.52±1.90 mg/ml) and span 20 (6.24±0.34 mg/ml). Ethanol showed the highest solubility amongst the co-surfactants (84.56±1.43 mg/ml), followed by IPA (48.60±3.69 mg/ml). Based on solubility studies castor oil was chosen as oil phase, tween 80 and ethanol were chosen as surfactant and co-surfactant respectively to prepare microemulsion for ACF.

Optimization of microemulsion formulation

The phase diagram facilitated the determination of components concentration range for the existence of microemulsion. Fig. 2 showed the phase diagrams, constructed by ProSim ternary diagram software, version 1.0.3 to determine the optimum surfactant to co-surfactant ratio (S/CoS), for the formulation of O/W microemulsion consisting of castor oil as oil phase, tween 80 as a surfactant, ethanol as co-surfactant, and water. The S/CoS was varied as 1:1, 2:1, 3:1 and 4:1. With increases in S/CoS ratio, the existence area of O/W microemulsion becomes enlarged and reached maximum at 4:1 ratio. Based on this result, 4:1 ratio of S/CoS was selected to formulate ACF microemulsion. The detailed compositions of all formulations were showed in table 1.

Table 1: Composition of ACF microemulsion

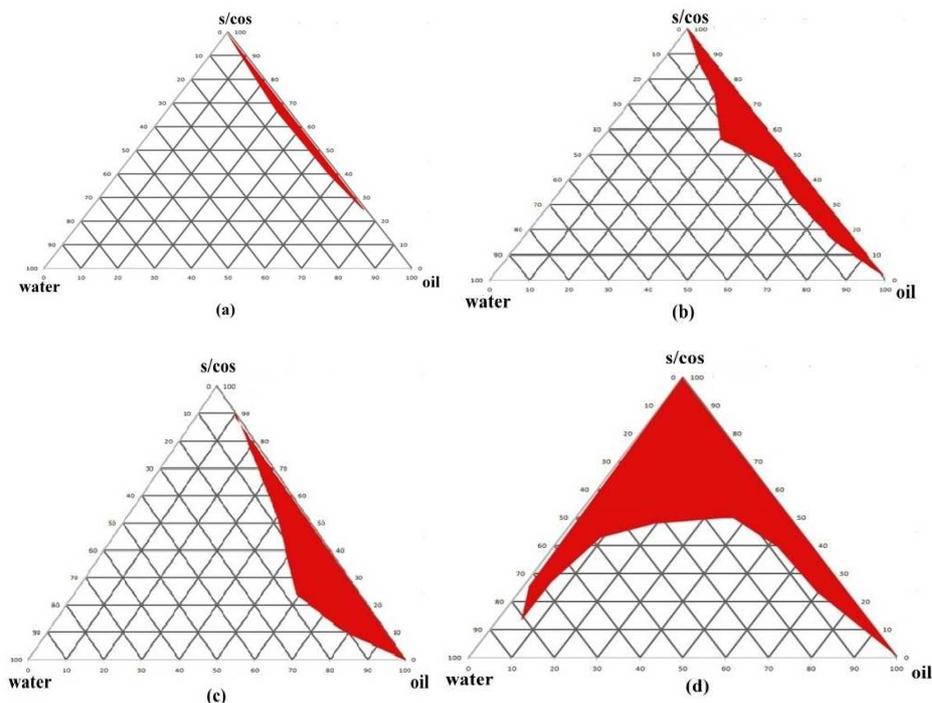| F. code	Components				
	ACF % w/w	castor oil	S/CoS (tween 80/ethanol)	water	Menthol % w/w
ME1	2	5	25	68	-
ME2	2	5	50	43	-
ME3	2	5	75	18	-
ME4	2	10	25	63	-
ME5	2	10	50	38	-
ME6	2	10	75	13	-
ME7	2	15	25	58	-
ME8	2	15	50	33	-
ME9	2	15	75	8	-
ME10	2	10	25	63	3
ME11	2	10	25	63	6
ME12	2	10	25	63	8

Fig. 2: Pseudo-ternary phase diagrams of microemulsion composed of castor oil, surfactant (tween 80), co-surfactant (ethanol) and water. (a) S/CoS, 1:1 (b) S/CoS, 2:1 (c) S/CoS, 3:1 (d) S/CoS, 4:1

Physicochemical evaluation of microemulsion

The results of the physical parameters of all formulations were presented in table 2. The average size of oil droplets of microemulsion formulations was found to be 89.4±2.12 to 175.2±3.10 nm. The pH of the formulations was range 6.2±0.16-6.7±0.40 and viscosity range 71-99 cP. The results of these study revealed that addition of 3-8% w/w menthol in the formulation did not significantly changes the physicochemical properties of the microemulsion.

Table 2: Physicochemical parameters of microemulsion formulations

Formulations	pH*	Particle size* (nm)	Polydispersity Index	Viscosity (cP)
ME1	6.2±0.16	105.3±1.05	0.310	73
ME2	6.3±0.05	151.2±2.15	0.255	74
ME3	6.5±0.22	101.6±1.25	0.343	71
ME4	6.4±0.06	170.7±1.03	0.230	82
ME5	6.3±0.32	102.6±3.05	0.310	84
ME6	6.6±0.05	93.5±1.50	0.255	87
ME7	6.4±0.22	166.1±2.52	0.343	94
ME8	6.7±0.40	152.3±1.15	0.230	98
ME9	6.5±0.13	89.4±2.12	0.310	92
ME10	6.3±0.23	157.4±3.05	0.324	87
ME11	6.4±0.08	166.1±1.09	0.338	92
ME12	6.5±0.18	175.2±3.10	0.343	99

*(mean±SD, n=3)

***In vitro* skin permeation study**

The skin permeation profiles of all microemulsion formulations without menthol (ME1 to ME9) are presented in fig. 3. The cumulative amount of ACF permeated through excised rat skins was plotted as a function of time. The slope and intercept of the linear portion of the plot were derived by regression. The permeation rate at steady-state (J_s, µg/cm^2/h) was calculated as the slope divided by the skin surface area. The intercept on the X-axis was taken as the lag time (T_L, h). The permeation parameters calculated from the profiles are presented in table 3.

Amongst the formulations tested, Formulation ME4 showed the highest permeation profile rate of ACF after 8h permeation study. The cumulated amount of skin permeation of ACF from this formulation was found to be 1492.33±10.43 µg/cm^2. The content of S/CoS mixture in formulation affected the skin permeation of ACF significantly (p<0.05). As the content of S/CoS mixture was increased from 25 to 75% in formulations (ME4 to ME6), the skin permeation rate of ACF was decreased (1492.33±10.43 to 1337.37±18.44µg/cm^2). The content of oil also played an important role in microemulsion formulation on the skin permeation of ACF as showed in the fig. 3. With decrease in oil content from 15% to 5% in formulations ME9, ME6, ME3 the skin permeation of ACF was increased and the cumulative drug permeated were found 1422.23±14.04, 1337.33±18.44µg/cm^2, 1249.33±22.36, µg/cm^2 respectively after 8 h, while the content of surfactant and co-surfactant mixture S/CoS (4:1) was fixed at 75%. This result suggested that, as the oil contents in the formulations decreased the water contents in the formulation increased which leads to hydrate skin and caused corneous cell to swell thus made drug channel wide, therefore with increasing amount of water content in the system the cumulative permeation amount was improved, this findings was supported with the previous study [25, 26].

Table 3: Permeation parameters of ACF from different formulations across rat epidermis

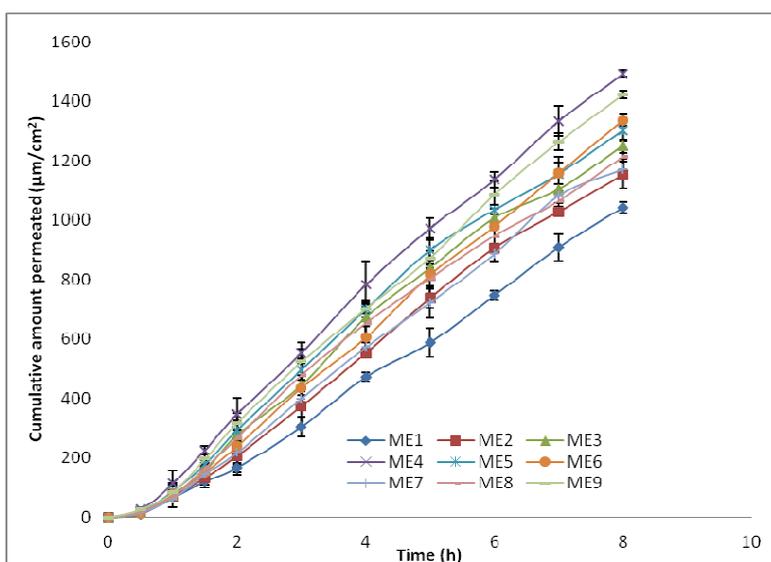| Formulations	Permeation parameters (mean±SD, n=3)	
	T_L (h)	J_S (µg/cm²/h)
ME1	0.86±0.23	130.41±3.12
ME2	0.77±0.16	144.16±5.04
ME3	0.68±0.31	156.17±3.92
ME4	0.59±0.13	186.54±6.20
ME5	0.73±0.24	162.79±3.30
ME6	0.69±0.32	167.16±5.32
ME7	0.81±0.22	146.54±3.70
ME8	0.71±0.33	151.54±4.23
ME9	0.64±0.62	177.79±3.55
ME10	0.68±0.14	311.54±14.80
ME11	0.64±0.23	424.04±5.29
ME12	0.53±0.21	540.79±15.05

Fig. 3: Permeation profiles of ACF through rat epidermis from microemulsions. Bar represents mean±SD (n=3)

Further, to improve the permeation rate of ACF from microemulsion formulations menthol was added at 3% w/w, 6% w/w and 8% w/w in ME10, ME11 and ME12 formulations respectively. The obtained permeation profiles were showed in fig. 4 and the permeation parameters calculated from profiles were presented in table 3. The permeation rate (transdermal flux) of ME10, ME11 and ME12 formulations were found to be 311.54±14.80, 424.04±5.29, 540.79±15.05 µg/cm²/h and a lag time 0.68±0.14, 0.64±0.23, and 0.53±0.21 h respectively, which revealed that formulation contained 8% w/w menthol (ME12) showed the most pronounced enhancing effect with 2.9 fold increased in the permeation rate of ACF as compared to control (ME4; without menthol).
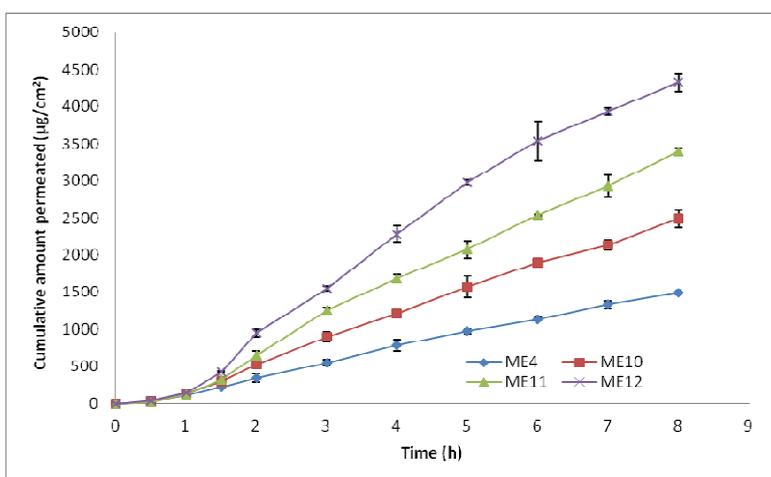

Fig. 4: Effect of menthol on transdermal permeation of ACF through rat epidermis from microemulsions. Bar represents mean±SD (n=3)

FT-IR Spectroscopy of rat SC

The FT-IR spectra of untreated rat SC showed characteristic absorption peaks (fig. 5A) at approximately 2852 cm^{-1} and 2921 cm^{-1} due to symmetric and asymmetric C-H stretching vibrations of lipid components [27-28] and at approximately 1750-1550 cm^{-1} due to C=O stretching vibrations of an intracellular protein [29]. The FT-IR study showed that the treatment of SC with 3-8% concentrations of menthol in 25:75 ratio of the ethanol-water solvent system did not produce a blue shift in the asymmetric and symmetric C-H stretching peak positions. However, they all showed a significant decrease in absorbance intensities for both asymmetric and symmetric C-H stretching absorbance in comparison with the SC treated with 25:75 ration of the ethanol-water solvent system considered as control

(table 4 and fig. 5B). 8% menthol produced a greater decrease in peak heights for C-H stretching absorbance (asymmetric, 55.06±0.007% and symmetric 53.88±0.006%) in comparison with the SC treated with 6% menthol (asymmetric, 48.41±0.008% and symmetric 40.29±0.007%) and 3% menthol (asymmetric, 44.62±0.004% and symmetric 36.40±0.004%). The decrease in peak height may be because of the extraction of the SC lipids and this result is in agreement with an earlier study reported by Goats and Knutson, 1994 [30]. It was also reported that terpenes increase the drug percutaneous permeation mainly by disrupting the intercellular packing of the SC lipids [31, 32]. Thus, the study suggests that extraction of the SC lipids by menthol at 3-8% w/w concentration was effective for enhancing the transdermal permeation of ACF from microemulsion drug reservoir.

Table 4: Peak height of asymmetric and symmetric C–H stretching absorbance of rat SC (mean±SD, n=3)

Rat's stratum corneum treated with	Asymmetric C–H stretching		Symmetric C–H stretching	
	Peak height	% Decrease in peak height[a]	Peak height	% Decrease in peak height[a]
Water	3.21±0.02	-------	2.18±0.04	-------
25:75 (v/v) ethanol–water solvent system	3.16±0.01	-------	2.06±0.01	-------
3% w/w menthol in 25:75 (v/v) ethanol–water solvent system	1.63±0.04	48.41±0.008	1.23±0.03	40.29±0.007
6% w/w menthol in 25:75 (v/v) ethanol–water solvent system	1.42±0.03	55.06±0.007	0.95±0.01	53.88±0.006
8% w/w menthol in 25:75 (v/v) ethanol–water solvent system	1.75±0.05	44.62±0.004	1.31±0.06	36.40±0.004

[a]%Decrease in peak height = (peak height from ethanol-water treated SC–peak height from enhancer treated SC)/peak height from ethanol-water treated SC × 100 [33].
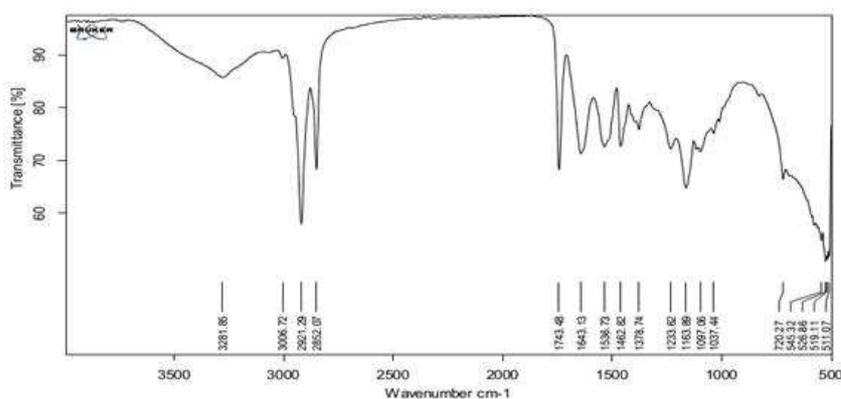
Fig. 5A: FT-IR spectra of rat SC
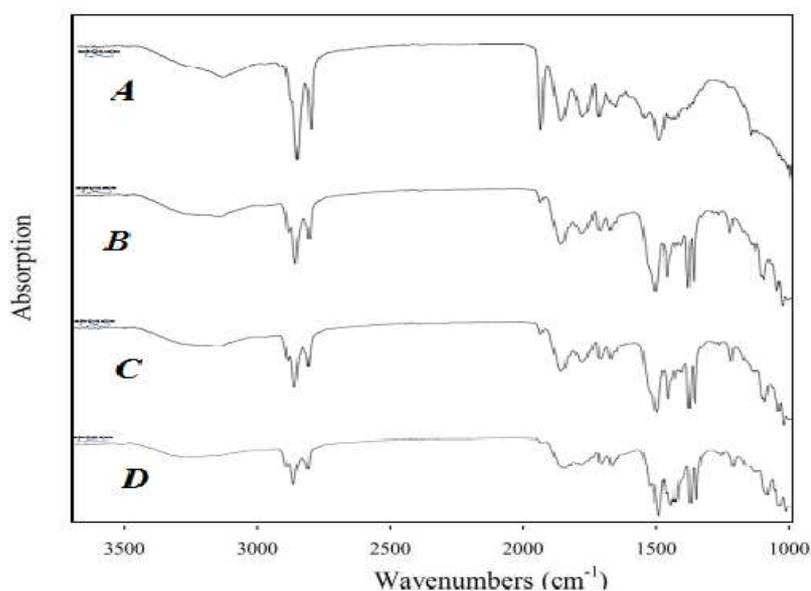
Fig. 5B: FT-IR spectra of rat SC treated with different concentration of menthol (A-without treatment, B-with 3% w/v menthol, C-with 6% w/v menthol, D-with 8% w/v menthol)

CONCLUSION

In conclusion, an O/W microemulsion containing 2% w/v of ACF was prepared successfully by a titration method with different concentration of oil, surfactant, and co-surfactant. The in vitro skin permeation studies showed that menthol could markedly enhance the transdermal permeation of ACF from microemulsion formulation. The average droplet size of the microemulsion was found to be 89.4±2.12 to 175.2±3.10 nm. The transdermal flux of the microemulsion containing 8% w/w menthol showed 2.9 fold increases in transdermal flux of ACF compared with the formulation without menthol. FT-IR studies revealed a decrease in peak height of the symmetric and asymmetric C-H stretching vibrations may be because of the extraction of the SC lipids and the alteration of the skin permeability barrier. This result suggests that menthol may be an effective natural penetration enhancer for transdermal delivery of ACF.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally

CONFLICTS OF INTERESTS

The authors declare that there is no conflict of interest

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