Enhancement of skin permeation and anti-inflammatory effect of indomethacin using microemulsion

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The objective of the present study was to investigate the potential of microemulsion formulations for transdermal delivery of indomethacin (IND). Microemulsion formulations with different surfactant:cosurfactant ratios (S_{mix}); F1-F6 (1:1, 2:2, 3:1, 4:1, 1:2, and 3:2) were prepared by the spontaneous emulsification method, and characterized for morphology, droplet size, and rheological characteristics. The *ex vivo* skin permeation studies were performed using Franz diffusion cell with rabbit skin as a permeation membrane. A significant increase in the steady-state flux (J_{ss}), permeability coefficient (K_p), and enhancement ratio (E_r) was observed in microemulsion formulations compared with the conventional IND gel. The anti-inflammatory effects of microemulsion formulations showed a significant increase in percent edema inhibition value after 4 hours. The optimized formulation showed a significant increase in the steady-state flux (J_{ss}) and permeability coefficient (K_p). The enhancement ratio (E_r) was found to be 8.939 in optimized formulation F1 compared with IND gel.

Key words: *Anti-inflammatory effect, indomethacin, microemulsion, permeability coefficient, surfactant:cosurfactant mixture*

INTRODUCTION

Microemulsions (NE) have received a growing attention as colloidal drug carriers for pharmaceutical applications. They typically consist of oil, surfactant, cosurfactant, and aqueous phase, which are transparent, thermodynamically stable with a droplet diameter usually within the range of 10-100 nm and do not have the tendency to coalesce.[1,2] Microemulsions have several advantages such as enhanced drug solubility, good thermodynamic stability, enhancing effect on transdermal ability over conventional formulation.[3-5] Moreover, microemulsions can accommodate both hydrophilic and lipophilic drugs.[6-8]

Indomethacin (IND) is a potent nonsteroidal anti-inflammatory drug with analgesic and antipyretic properties. Like other NSAIDs, the most common side effect of IND in oral dosage forms is gastrointestinal irritation. Thus, alternative routes of administration for these drugs are being currently investigated. Recently, more attention has been focused on microemulsions for transdermal delivery of drugs.[9-12]

The transdermal route has been known to eliminate oral gastrointestinal (GI) adverse effects and maintain the plasma drug level for longer period of time and suitable for long treatment of chronic disease. Recent studies have shown significant drug levels in deep tissues such as fascia, muscle, and synovium after topical application,[13,14] which is a desirable feature for the relief of local symptoms with low dose, thereby reducing systemic side effects.

Nanemulsions have also shown improved transdermal permeation of many drugs over the conventional topical formulations such as emulsions,[15] and gels.[16] The aqueous-phase titration or spontaneous emulsification method has been successfully investigated for the preparation of oil-in-water (o/w) microemulsions of many lipophilic drugs.[17,18] Several mechanisms have been proposed to explain the advantages of microemulsion for the transdermal delivery of drugs. First, the high solubility potential for drugs of the

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microemulsion system might increase thermodynamic activity toward the skin.[19] Second, ingredients of microemulsion , acting as permeation enhancers, might increase the flux of drug via skin.[20] Third, the permeation rate of the drug from microemulsion may be increased because the affinity of a drug to the internal phase could be modified easily to favor partitioning into stratum cornea.[21-23] Since microemulsions contain surfactant compounds in its composition, the application on the skin surface usually produces an increase in the membrane permeability facilitating transdermal transport.[24,25] The literature shows that NE can control release and bioavailability of many drug compounds.[26-28] In this study, an optimum topical microemulsion containing IND was developed after screening various oils to improve the drug solubility, the skin permeability, and the anti-inflammatory effect.

MATERIALS AND METHODS

IND was kindly supplied by Pharma Tech (Ireland). PEG-8 caprylic/capric glycerides (Labrasol®), diethylene glycol monoethyl ether (Transcutol® P), propylene glycol mono-laurate (Lauroglycol 90), Labrafil M 2155 CS, Labrafil M 1944 were kindly donated by Gattefosse, France. Isopropyl myristate (IPM), soya bean oil, and cotton seed oil (Sigma Chemical Co., St. Louise, USA). Acetonitrile, ethanol, and methanol used were of HPLC grade. Oleic acid, polyoxyethylene sorbitan ester (Tween 80, Tween 20), polyethylene glycol 600 were purchased from Sigma Chemical Co., USA. Commercial IND gel: Farcomethacin® 1% gel, batch no: 380 was purchased from Pharco Pharmaceuticals, Alexandria, Egypt. Cellulose membrane cut-off 12000D, Biogen, Belgium (Sigma Chemical Co., St. Louise, USA). All other chemical and solvents were of analytical reagent grade.

Rabbit ears were collected immediately after killing from a local slaughterhouse. The skin of rabbit was excised from freshly killed male New Zealand white rabbits for human alimentation.[29] The average thickness of the skin was 0.38 ± 0.06 mm.

Screening of oils and surfactants for microemulsion
In order to find out appropriate oils and surfactants that had good solubilizing capacity of IND and, thus, could be used as the oil phase and surfactants in microemulsion, the solubility of IND in various oils and surfactants were measured [Table 1]. Oils employed were soya bean oil, isopropyl myristate (IPM), labrafil 2155, labrafil 1944, Labrafac lipofile, and oleic acid. Surfactants employed were Tween 80, Tween 20, and Labrasol, and cosurfactant was Transcutol P. The solubility of IND in various oils and surfactants was determined using the shake flask method. Briefly, an excess amount of IND was placed in 2 ml of the vehicle in screw-capped glass vials. After sealing, the mixture was vortexed using a cyclomixer (Model 201, GFL, Germany) for 10 minutes in order to facilitate proper mixing of IND with the vehicles. Mixtures were shaken for 72

Table 1: Solubility of IND in various excipients ($n=3$)

Excipients	Solubility mean±SD (mg/g)	Excipients	Solubility mean±SD (mg/g)
Soya bean oil	13.87±2.65	Labrafac	27.34±5.83
Oleic acid	3.45±1.98	Labrasol	178.32±5.32
Isopropyl myristate	32.65±4.3	Propylene glycol	117.48±3.21
Labrafil 2155	10.23±1.87	Polyethylene glycol 400	107.34±3.69
Labrafil 1944	7.86±1.98	Transcutol P	185.52±4.57
Lauroglycol	8.28±2.09	Tween 80	110.65±3.82

hours in an isothermal shaker (GFL, Model 1083, Germany) maintained at $20 \pm 1°C$. Mixtures were centrifuged at 12,000 rpm for 15 minutes. The supernatant was filtered through a membrane filter (Nylon, 0.45 μm, Gelman, USA), and then probably diluted with methanol. The concentration of IND in the supernatant was determined by the high-performance liquid chromatography (HPLC) method as described below. Based on these results, appropriate oil, surfactant, and cosurfactant were selected and used in the preparation of microemulsions containing 1% IND. The effect of the mixture of surfactant and cosurfactant on the permeation of IND through excised rabbit skin was evaluated.

Construction of pseudo-ternary phase diagrams
Pseudo-ternary phase diagrams were constructed using a water titration method at ambient temperature to obtain the components and their concentration ranges that could result in a large existing area of microemulsion without the drug.[9] On the basis of the solubility studies, IPM was selected as an oil component and Labrasol as a surfactant, Transcutol P as a cosurfactant, in the ME systems. Six-phase diagrams were prepared at different surfactant/cosurfactant ratios (S_{mix}) of 4/1, 3/1, 2/1, 1/1, 1/2, and 3/2. For each phase diagram, oil and S_{mix} were combined in different weight ratios. The ratio of oil to the mixture of the surfactant and the cosurfactant was varied as 5, 10, 20, 30, 40, 50, 60%. The IND-containing formulations were prepared by dissolving 20 mg of the drug into 2 g of the oily mixtures. Water was added drop by drop using a micropipette. No heating was necessary during the preparation. However, the system was stirred using a magnetic stirrer to ensure a thorough mixing. The microemulsion regions were identified as transparent and isotropic mixtures. After each mixing, the sample was allowed to settle and its physical condition (clarity and flowability) was reviewed. The point at which the mixture became turbid or showed signs of phase separation was considered as the end point of the titration. If required, the sample was sonicated for 1-2 minutes to remove air bubbles and to enable a better visual examination. Mixtures that did not show a change in the meniscus after tilting to an angle of 90° were considered to be gels. After being equilibrated, the mixtures were assessed visually and determined as being microemulsions, crude emulsions, or gels. The concentrations of components were recorded in order to complete the

pseudo-ternary phase diagrams, and then the contents of oil, surfactant, cosurfactant, and water at appropriate weight ratios were selected based on these results. No attempt was made to distinguish between o/w, w/o, or bicontinuous-type microemulsions.

HPLC analysis of IND

The solubility of IND in various excipients was determined by a validated reverse-phase HPLC method. The HPLC apparatus (Shimadzu VP series) equipped with System controller (SCL-10 A VP, Shimadzu); UV-Vis Spectrophotometer detector (SPD-10 A VP, Shimadzu, Japan), a Rheodyne sample injector (Rheodyne, USA) with 50 μl sample loop and Bondapak C_{18} (4.6 mm (id) $\times$150 mm and 5-μm particle size) column. The mobile phase consisted of a mixture of acetonitrile:water (pH was adjusted to 3.2 with orthophosphoric acid) (60:40 v/v) at a flow rate of 1.5 ml/min that led to a retention time of 3.5 minutes when detection was carried out at 260 nm. The assay was linear (r^2=0.9996) in the concentration range 0.5-10 μg/ml with the lowest detection limit of 200 ng/ml of IND. The method was validated with respect to accuracy and inter- and intra-day precision as per ICH guidelines. The validation studies showed overall intra- and inter-day variations (RSD) of less than 3.1% and 8.2%, respectively. The percentage difference between amounts determined and spiked was considered to be a measure of accuracy. An accuracy of 98.7-112.0% was obtained for each of the analytes tested, and RSD was <9.8%.

Preparation of microemulsion formulations

From the constructed pseudo-ternary phase diagrams, different formulae were selected from the microemulsion region as described in Table 2, so that the drug could be incorporated into the oil phase. Exactly 1% w/w of IND, which was kept constant in all the selected formulations, was dissolved in the oil phase of the microemulsion formulation.

Characterization of the microemulsions
Viscosity measurements

The viscosity of emulsions was measured on a Brookfield R/S plus Rheometer equipped with Rheocalc V1.1 software (Brookfield Engineering Laboratories Inc. Massachusetts, USA). A C50- 1 cone- and plate-geometry was used with 50 mm diameter and cone of 1.0°. The temperature was controlled and kept constant at room temperature (20°C±0.2°C) using a model TC-500 thermostat Braive Instruments, Liege, Belgium). The volume of sample was 1 ml at 150 rpm.

Morphology of microemulsion

Morphology and structure of the microemulsion were studied using transmission electron microscopy, TEM (JEOL-1011, Electron Microscope, Japan) operating at 200 kV and capable of point-to-point resolution. To perform the TEM observation, a drop of sample was directly deposited and dried on the TEM copper grid, and then coated with carbon film under ambient conditions 22 (C). The samples were determined at 100 Kv.

Table 2: Composition of selected microemulsion formulations

Formulation code	S /Co S (S_{mix}) ratio	% w/w of components in microemulsion formulation			Oil: S_{mix} ratio
		Oil	S/Co S_{mix}	Water	
F1	1:1	43.2	43.2	13.6	1.0
F2	2:1	42.0	42.0	16	1.0
F3	3:1	33.9	50.0	16.1	0.678
F4	4:1	37.0	46.0	17	0.804
F5	1:2	41.6	41.6	16.8	1.0
F6	3:2	40.6	50.7	8.7	0.757

In vitro release studies

The release experiments employed the FDC-6 transdermal Diffusion Cell Drive Console (Logan Instrument Corp. NJ, USA). The apparatus consisted of clamped preconditioned synthetic membrane (cellulose; MW: 12,000) on to glass diffusion cell between donor and receptor compartments. The receptor compartment was filled with 12 ml of 20% alcohol in a 0.02 M phosphate buffer at pH 7.4.[30] The receptor solutions were magnetically stirred at 600 rpm throughout the experiment. The diffusion cell was maintained at 37°C using a recirculating water bath (Julabo, Model 105, Germany). The donor compartment was 1 g of prepared microemulsion containing IND (1% w/w), and the donor cap was covered with a parafilm and clamped. The sampling port was sealed with a parafilm to prevent the evaporation of the receptor medium. Five milliliter aliquots withdrawn from the receptor compartment at various intervals for 24 hours were filtered through 0.45 μm and IND was quantified using the HPLC method as described above. The receptor compartment was refilled with the same volume of fresh buffer solutions. Three replicates of each experiment were performed. Sink conditions were maintained in the receptor compartment during *in vitro* permeation studies.

In vitro skin permeation study
Preparation of skin samples

All animal procedures were conducted in accordance with approved institutional protocols. The rabbit ear model was adopted to monitor the skin delivery of a variety of drugs including lipophilic ones similar to our drug.[31] Full-thickness skin obtained from the inner side of freshly excised ears of six male rabbits, weighing 2-3 kg, was used. The average thickness of the skin was 0.28±0.06 mm. Skins were allowed to hydrate for 1 hour before being mounted on the Franz-type diffusion with the stratum corneum facing the donor compartment and the dermal side faced the receiver compartment.

Skin permeation studies

The extent and rate of skin permeation of IND from microemulsions of various compositions were determined using Franz diffusion cells fitted with excised rabbit skins. The effective diffusion area was 1.77 cm². The receiver medium constituted of 20% alcohol in a pH 7.4 phosphate buffer (0.02 M) was continuously stirred with a small magnetic bar and thermostated at 37±1°C, so that the skin surface temperature

was approximately at $32\pm1°C$. One gram of the formulation was placed on the skin surface in the donor compartment. After application of the test formulation on the donor side, 5 ml aliquots were collected from the receptor side at designated time intervals, for a 24-hour period, and replaced by the same volume of the fresh buffer to maintain a constant volume. The amount of IND in the receiver phase was assayed by HPLC. Each data point represents the average of three determinations.

Analysis of permeation data

Cumulative amount of drug (mg) penetrating the unit diffusion surface (cm^2) were plotted against time (hour). The *in vitro* skin permeation rate or flux (*J*) was calculated from the slope of the regression line fitted to the linear portion of the profile. Extrapolation of this line will intercept with the *x*-axis at a time equal to the lag time. The permeability coefficient, K_p, was estimated from the flux and donor drug concentration. Penetration enhancing activities compared with the conventional IND gel are expressed as the enhancement ratio (E_r).

The cumulative drug permeation (Q_t) was calculated from the following equation:

$$Q_t = V_r\,C_t \;+\; \sum_{i=0}^{t-1} V_s\,C_i \qquad (1)$$

where C_t is the drug concentration of the receiver solution at each sampling time, C_i the drug concentration of the *i*th sample, and V_r and V_s the volumes of the receiver solution and the sample, respectively, Data were expressed as the cumulative IND permeation per unit of the skin surface area, Q_t/S ($S=1.767$ cm^2). The steady-state fluxes (J_{ss} (2 – 10 h)) were calculated by linear regression interpolation of the experimental data at steady state (between 2 and 10 h):

Apparent permeability coefficients (K_p) were calculated according to the equation

$$K_p = J_{ss}\big/C_o \qquad (2)$$

where C_o is the drug concentration in the donor solution (1 $\times$ 10^4 $\mu g/cm^3$), while assuming that under sink conditions the drug concentration in the receiver is negligible compared to the drug in the donor.

The data are presented as means$\pm$SD obtained using 4-8 skin fragments from at least two animals.

The enhancement ratio (E_r) was calculated by dividing the J_{ss} of respective formulation with J_{ss} of control formulation by using the equation

$$E_r = \frac{J_{ss}\ of\ formulation}{J_{ss}\ of\ control} \qquad (3)$$

Anti-inflammatory effect on carrageenan-induced paw-edema in rats

Paw edema can be induced by murine carrageenan. Male Sprague-Dawley rats weighing 150-180 g were used for the experiments. The animal study protocol was reviewed and approved by the Animal Ethics Committee of the University of King Saud, Riyadh. All measurements were performed at $24\pm1°C$ in an air-conditioned room. The animals were randomly divided into eight groups of six rats each for administration. The rats of the first control group were treated with normal saline.[32] The other seven experimental groups received different topical formulations of IND microemulsions and the commercial IND gel. To induce local inflammation, 50 μl of 1% carrageenan (w/v) in saline was injected into the plantar surface of the left hind paw of the rats at time zero, using a 27-gauge needle coupled to a 100 ml Hamilton syringe. In the first experiment, 60 minutes later, 100 mg of IND microemulsion or IND gel was applied, nonocclusively, to the paws of the animals and spread gently. Animals were then housed in polypropylene cages with framed metal mesh on the floor to prevent absorption of applied products by sawdust. The animals were maintained without access to food and water during the experiment.

All experiments were carried out between 9.00 a.m. and 3.00 p.m. Measurements of foot volume were performed by the method described by[33] using water plethysmometer (LE 7500, Letica Scientific Instruments, Barcelona, Spain) before and 1, 2, 3, 4, and 5 hours after the injection of carrageenan into the planter region of the left hind paw. The degree of paw swelling was calculated as

$$\text{Swelling (\%)} = \frac{V_t - V_o}{V_o} \times 100 \qquad (4)$$

where V_t (ml) is the volume of the carrageenan-treated paw, V_o is that of the nontreated paw.

On the basis of equation (4), the percentage edema inhibition was calculated as

$$\text{Inhibition (\%)} = 1 - \frac{\substack{\text{\% Swelling of the}\\ \text{microemulsion-treated group}}}{\text{Swelling of the control group}} \times 100 \qquad (5)$$

Statistical analysis

Results expressed as mean$\pm$SD were assessed using the Kruskal-Wallis test. Individual differences between the different formulations of microemulsions were determined using a nonparametric *post hoc* test (Dunn's test) using graph Pad InStat Software - version 3.0 (Graph Pad Software, San Diego, CA, USA). Value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Screening components for microemulsion

The consideration for screening formulation of microemulsions usually involves the following: the formulation composition should be simple, safe, and compatible; it should possess good solubility; a large efficient region which should be found in pseudo ternary phase diagram, and have efficient droplet size after forming microemulsion.[34]

In order to screen appropriate solvents for the preparation of microemulsions, the solubility of IND in various oils and surfactants were measured and the results were shown in Table 1. The solubility of IND was highest in IPM followed by soyabean oil, Labrafil 2155, Labrafil 1944, Lauroglycol, and oleic acid. Previous reports indicated that the superior dermal flux appeared mainly due to the large solubilizing capacity of the microemulsions, which led to a larger concentration gradient toward the skin.[35] Isopropyl myristate had wide pharmaceutical applications owing to its good biological acceptance.[36,37] IPM was an excellent enhancer in transdermal delivery as previously reported,[38] and it was selected as the oil phase. Takahashi *et al*.[39] found that the skin permeation was inhibited as the affinity to vehicle become greater due to a slow release of the drug and/or a poor transfer from the vehicle to the skin. Li *et al*. had reported that the dermal drug permeation is influenced primarily by the solubility of drug in vehicle and the partition coefficient of skin/vehicle.[40,41] IND has the highest solubility in IPM; hence it was selected as the oil phase. IND also had a higher solubility in Labrasol (178.32 mg/g) and Transcutol P (185.52 mg/g) followed by propylene glycol (117.48 mg/g), and Tween 80 (110.65 mg/g). Cosurfactants can decrease interfacial tension between oil and water in microemulsion, adjust the flexibility of interfacial membrane and reduce the required amount of surfactant sometimes. The short-chain alcohols and Transcutol P were widely used as cosurfactant.[38,16] Transcutol P seems to be very attractive as a penetration enhancer due to its nontoxicity, biocompatibility with the skin, miscibiliity with polar and nonpolar solvents and optimal solubilizing properties for a number of drugs.[42] Therefore, Labrasol and Transcutol P were selected as surfactant and cosurfactant, respectively, for the phase study. The right blend of low and high hydrophilic lipophilic balance (HLB) surfactants leads to the formation of stable microemulsion formulations.[43] In this study, we selected Labrasol as a surfactant with an HLB value of 14, and Transcutol P with a low HLB value.[42]

### Characterization of the microemulsions

With the measurement of the transmission electron microscope, the optimized microemulsion vesicles appeared as a perfect round shape without aggregation [Figure 1]. The characteristics of microemulsions such as droplet size and viscosity measurements were given in Table 3. The parameters for physicochemical characters of the optimized formulations were as follows: 3.74-16.43 nm for the average size of all microemulsion vehicles particle size. All the droplets were found in the nanometer range which indicated the suitability of formulation for transdermal drug delivery. Polydispersity signifies the uniformity of droplet size within the formulation. The polydispersity value of the formulations was very low (<0.2) which indicated uniformity of droplet size within the formulation. The viscosity of the selected microemulsion was determined [Table 3]. The viscosity of formulation F1 (11.875 cP) was lower than that of other formulation; this difference was significant (P <0.05) compared with F2, F3,
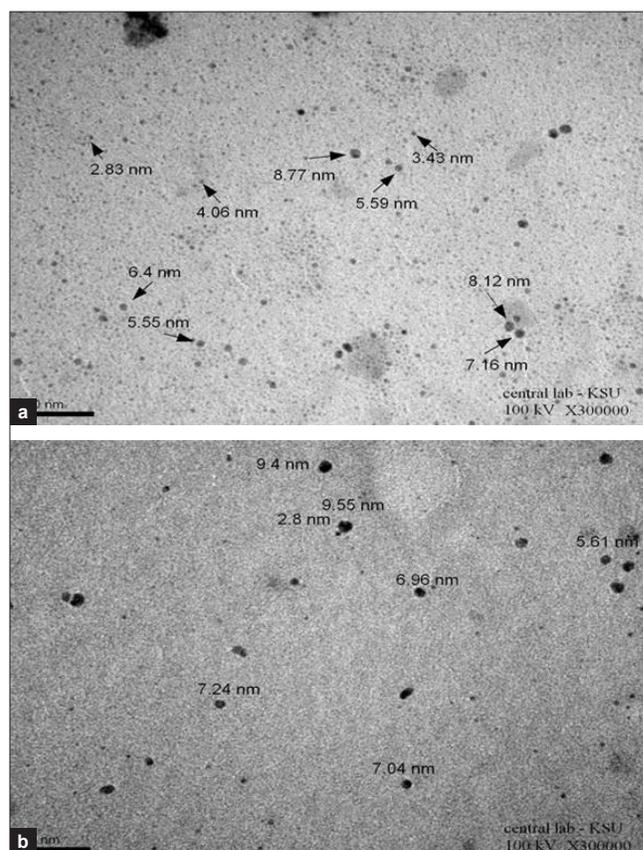
Figure 1 a, b: Transmission electron microscopic positive image of indomethacin microemulsion showing the size of some oil droplets (300,000×)

Table 3: The S/Co S (S$_{mix}$) ratios, average droplet size, polydispersity values, and viscosity of IND microemulsion formulations

Formulation code	F1	F2	F3	F4	F5	F6
Ratio S/Co S	1:1	2:1	3:1	4:1	1:2	3:2
Average droplet size (nm)	5.74±0.55	6.98±0.62	8.54±0.65	9.43±0.78	7.43±0.86	5.94±0.89
Polydispersity index	0.096	0.088	0.076	0.083	0.075	0.148
Viscosity (mPas)	11.875±4.1	23.451±2.9	26.116±2.9	33.361±2.8	12.534±3.7	18.598±3.98
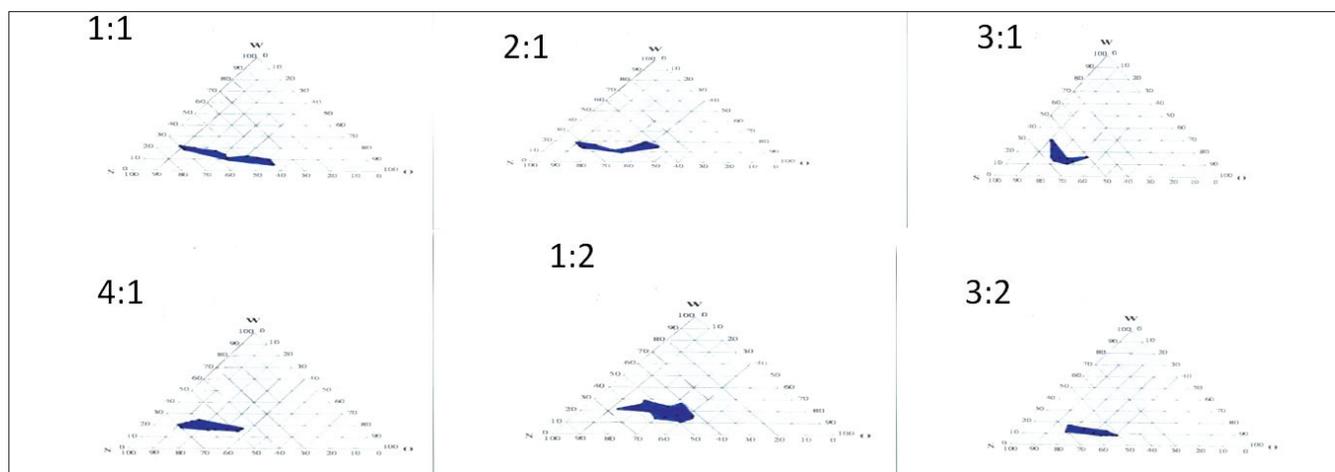
Figure 2: Pseudo-ternary phase diagrams of microemulsion composed of isopropyl myristate, surfactant (Labrasol), cosurfactant (Transcutol P), and water at different S_{mix}

F4, and F6. The viscosity of formulation F4 was highest (33.361 cP). Generally, it was observed that the viscosity of the microemulsion formulations was very low. Lower viscosity is one of the characteristics of microemulsion formulations.[4]

All microemulsion formulations were stable at ambient temperature in the presence or absence of IND. No changes of particle size, phase separation, and degradation of IND were observed during 6 months. The centrifuge tests showed that all microemulsion systems had good physical stability.

Construction of pseudo-ternary diagrams
The construction of pseudo-ternary phase diagrams was used to obtain appropriate concentration ranges of components in the areas of forming microemulsions. The pseudo-ternary phase diagrams of microemulsions composed of IPM, Labrasol, Transcutol P, and distilled water with various S_{mix} values were shown in Figure 2.
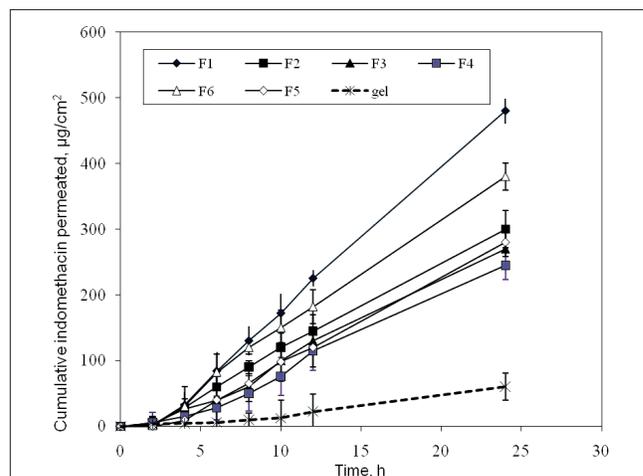
In vitro permeation study
Figure 3 shows the mean cumulative amount of IND released from microemulsion formulations through the cellulose membrane compared with the conventional IND gel. The figure showed a linear relationship as long as sink conditions were maintained, indicating nearly zero order release kinetics. The drug flux ranged from 21.212 μg/cm²/h (F4) to 46.796 μg/cm²/h (F6), while the drug permeated from IND gel lying in an immediate position (32.030 μg/cm²/h). Short initial lag times were observed, almost independent of microemulsion composition and/or drug flux variations, indicating that pseudo-steady state conditions were quickly achieved in all cases.[44]

Ex vivo permeation studies
The results of permeation experiments from the rabbit ear skin are shown in Figure 4. As expected, the drug penetration rate through excised rabbit ear skin was slower than that through artificial cellulose membrane and longer times were
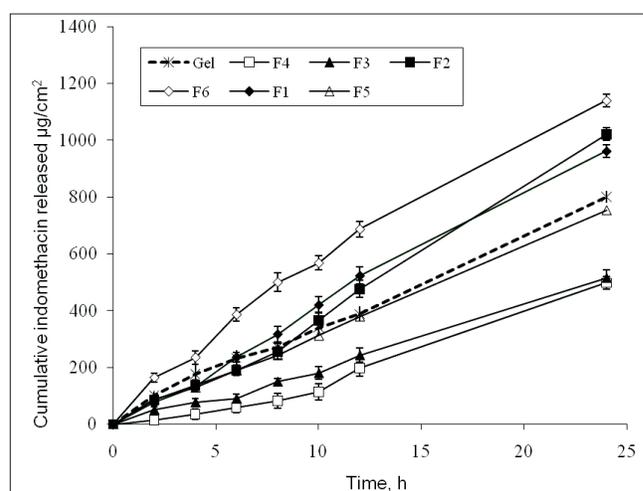
Figure 3: *In vitro* permeation profiles of indomethacin through cellulose membrane from 1% indomethacin microemulsion formulations with different S/Co S ratios

Figure 4: *In vitro* permeation profiles of indomethacin through excised rabbit skin from 1% indomethacin microemulsion formulations with different S/Co S ratios and indomethacin gel. Each point shows the means of three determinations±SD/

Table 4: Penetration flux, lag time, permeability coefficient, enhancement ratio, and correlation coefficient of regression analysis of release data according to the zero order model

Formulation code	Penetration flux J (µg/cm²/h)	Lag time (h)	Permeability coefficient K_p ×10² (cm/h)	Enhancement ratio**	Correlation coefficient R^2
F1, 1:1	22.608±3.45*	1.6±0.15	0.22±0.0003	8.939	0.9978
F2, 2;1	16.278±1.36*	2.2±0.23	0.16±0.0001	6.436	0.9938
F3, 3:1	11.819±3.56*	3.0±0.45	0.11±0.0003	4.673	0.9956
F4, 4:1	10.736±2.69*	3.5±0.32	0.10±0.0002	4.245	0.9877
F5, 1:2	12.355±1.05*	4.0±0.37	0.12±0.0001	4.885	0.9878
F6, 3:2	18.764±5.72*	2.0±0.28	0.18±0.0005	7.419	0.9974
Commercial IND gel (1%)	2.529±1.09	4.5±0.27	0.02±0.0001	-	-

*P <0.0001 compared with the commercial IND gel; **Enhancement ratio compared with the commercial IND gel

necessary to establish a uniform concentration gradient within the membrane and reach the quasi-stationary state. In fact, the early portion of all the permeation curves showed a more or less evident convexity to the time axis, typical of nonsteady state conditions, which was followed by an essentially linear profile. The permeability coefficient (K_p) and maximum flux (J_{ss}) of IND were calculated by fitting equations (1) and (2) to the permeated and accumulated amounts of drug versus time (<24 hours) [Table 4]. The correlation coefficient (r) of the fit ranged between 0.9878 and 0.9978. The mean permeability coefficient ranged between 0.22 × 10²/cm/h (F1) and 0.11 × 10²/cm/h (F4), the maximum flux ranged between 22.608 mg/cm²/h (F1) and 10.736 mg/cm²/h (F4). While the conventional IND gel showed 2.529 mg/cm²/h, 0.02 × 10²/cm/h, respectively. The rank of degree of permeation based on drug permeated at 24 hours [Figure 5] is F1 > F6 > F2 > F5 > F3 ≅ F4 IND gel.

From the permeation data, it was found that microemulsions could improve the skin permeation of IND over the commercial gel. As reported previously, the thermodynamic activity which can be described as viscosity is important for the permeation into skin. It is observed that the viscosity is inversely proportional with the flux of IND through rabbit skin. Increasing the viscosity results in lower flux (F4). Although F1 and F5 are comparable regarding the viscosity (11.875±4.1 and 12.534±3.7 mPas, respectively) F1 showed a significant increase in flux (22.608±3.45 and 12.355±1.05 μg/cm²/h, respectively). It is known that the viscosity of microemulsions is much lower than that of the gel, so the mobility of drug in microemulsions is more facile.[45] Furthermore, the microemulsions may affect the stratum corneum structure and reduce the diffusional barrier by acting as a permeation enhancer.[46-48] The amount of drug permeated at 24 hours ranged between 480.24 μg (F1) and 245.83 μg (F4), compared with 60 μg from the IND gel. The results showed a significant difference (P< 0.001). The high permeation rate of microemulsions might attribute to several factors. Firstly, the high concentration (1%) of IND in microemulsions resulted in high concentration gradient, which might be the main permeation mechanism of IND into the skin from these microemulsions. Microemulsions could act as drug reservoirs where drug is released from the inner
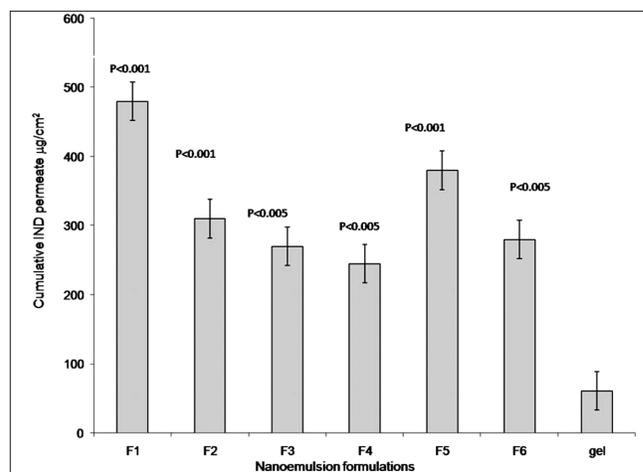

Figure 5: Cumulative indomethacin permeated through excised rabbit skin from 1% indomethacin microemulsion fromulations and indomethacin gel after 24 hours. The results are the mean±SD of three experiments. *P* values compared with the conventional indomethacin gel

phase to the outer phase and then further onto the skin.[19] Secondly, due to the small droplet size, some droplets may settle down to close contact with the skin and a large amount of inner IPM in microemulsions might penetrate into skins.[49] Transcutol P primarily acts as a cosolvent, promoting IND release from the dosage form by increasing the solubility. Therefore the concentration gradient of the drug in the solution was increased and favored the passage of larger quantities of the drug into the stratum corneum. Also, Transcutol P allows greater solubilization in the aqueous phase of the skin tissues.[50,51]

Anti-inflammatory study
Carrageenan-induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility.[52] The anti-inflammatory effect of IND microemulsion formulations and IND gel was assessed [Figure 6]. All formulations exhibited anti-inflammatory activity significantly greater than that in the IND gel group (P<0.05 and P<0.001). The increase in the paw volume
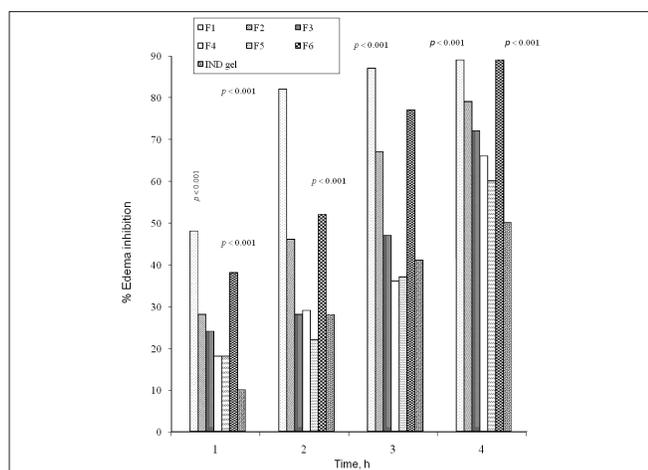
Figure 6: Anti-edema effect of indomethacin microemulsion and indomethacin gel as determined 1, 2, 3, and 4 hours after intraplantar carrageenan injection using rat paw swelling methods. Results are the mean±SD of six rats. *P* values compared with the conventional indomethacin gel

after 4 hours following carrageenan administration in the control (1.78±0.04 ml) and the microemulsion-treated groups ranged from 0.55±0. 01 to 0.96±0.05 ml, while the IND gel showed 1.16±0.05 ml increases in hind paw swelling. The anti-inflammatory activity was 48, 82, 87, and 98% and 10, 28, 41, and 50% at 1, 2, 3, and 4 hours, after administration of F1 microemulsion and IND gel, respectively. The enhanced anti-inflammatory effects of true microemulsions could be due to the enhanced permeation of indomethacin through the skin.[53]

CONCLUSION

Given the results of this study, it is clear that microemulsion formulation loaded with IND is potentially useful for permeation of IND in transdermal delivery. It is possible to conclude that transdermal administration of IND may be considered as an alternative to i.v. and p.o. administration to overcome its disadvantages. In this paper, the results suggested that the microemulsion played a role in the permeation-enhancing effect. Compared with commercial IND gel, the skin permeation ability of IND was significantly increased by microemulsions, which might result from the special characteristics of microemulsions. It is promising that the concentration of IND used to treat relative skin inflammatory conditions could be decreased due to the high permeation ability of IND microemulsion and side effects of IND might be reduced. Thus, this study suggests that, transdermal administration may be considered as an alternative noninvasive method for IND delivery to achieve rapid onset of its pharmacological effect.

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