



**Research Article**

## TRANSDERMAL DELIVERY OF CELECOXIB THROUGH RAT SKIN FROM VARIOUS MICROEMULSIONS

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**Abstract:** **Purpose:** This research was aimed to formulate and characterize and evaluation of in vitro skin permeability of celecoxib-loaded microemulsion systems as a topical delivery system of celecoxib for treatment of inflammation, rheumatoid arthritis, osteoarthritis and management of pain in these conditions. **Methods:** Celecoxib loaded microemulsions prepared by mixing of appropriate amount of surfactant including Tween 80 and Labrasol, co-surfactant such as Capryol 90 and oil phase including Oleic acid – Transcutol P (10:1 ratio). Physicochemical Characterization of selected Microemulsions such as, particle size, stability, viscosity, differential scanning calorimetry (DSC), scanning electron microscopy (SEM), surface tension, refractory index (RI), pH, drug release and *in vitro* skin permeability through rat skin using diffusion Franz cells were evaluated. **Results:** The mean droplets size of microemulsion formulation were in the range of 3.2 to 66.8 nm, and its refractory index (RI) and pH were 1.432 and 5.305, respectively. Viscosity range was 195.6- 335.2cps. Drug release profile showed that 26.94% of the drug released in the first 24 hours of experiment. In permeation studies the Jss and P parameters are significant with water percent in ME formulations, so that decrease in water percent is caused increase Jss and P parameters. Jss of ME 4 and Dapp in ME7 were 4.31times and 1.714 times higher than those of saturated water solution of celecoxib. **Conclusion:** In conclusion, the amount of components in ME formulation such as S/C ratio, water and, oil phase percentage in formulations have an essential role in the physicochemical properties and celecoxib permeability through rat skin..

**Keywords:** Celecoxib, Microemulsion, Phase Diagram, Transdermal Delivery, skin

### Introduction

Microemulsions are thermodynamically stable and low viscose mixtures of oil and water that have been stabilized with a surfactant and usually in combination with a cosurfactant. Microemulsions have shown several advantages for drug delivery such as; ease of preparation, perfect stability, increasing drug solubility, controlling drug delivery rate, improving bioavailability of hydrophilic and lipophilic drug through different delivery routes<sup>1</sup>.

Microemulsions were first observed by Schulman and Winsor in the 1950s<sup>2</sup>. Then, the term "microemulsions" has been used to describe multi-component systems comprising non-polar, aqueous, surfactant, and cosurfactant components. Conventional microemulsions can be classified oil-in-water, (o/w), water-in-oil (w/o) and bicontinuous phase microemulsions<sup>3</sup>. Some advantages offered by microemulsions include improvement in poorly drug solubility, enhancement of bioavailability, protection of the unstable drugs against environmental conditions and a long shelf life.

Celecoxib (CXB) is a selective cyclo-oxygenase-2 (COX-2) inhibitor used for treatment of rheumatoid arthritis and osteoarthritis. CXB has analgesic, antipyretic, and anti-inflammatory activity as a result of selective inhibition of the enzyme COX-2 and does not inhibit platelet aggregation<sup>4</sup>. In contrast with other non-steroidal anti-inflammatory drugs (NSAIDs) it has neither acute nor

chronic gastrointestinal toxicity. CXB is also used for treatment of colon cancer, ultraviolet (UV) light-induced skin cancer<sup>5</sup>, and breast cancer<sup>6</sup>.

Human skin is an important target site for the application of drugs. Permeation of drugs through the skin is the basis of transdermal delivery. Transdermal drug delivery is associated with some advantages such as controlled drug delivery, continuous drug delivery, first-pass intestinal and hepatic bypass, avoidance of the gastrointestinal irritation (which is common with oral medications), and facilitation of drug localization at target site<sup>7</sup>. Drug permeation across different skin layers is affected by various factors such as physicochemical properties of the drug, vehicle, and formulation components.

Although, the correlation between microemulsion structure and composition and successful topical and transdermal drug delivery is not fully explained but a few studies have presented knowledge on interaction of the inner structure of the microemulsion and drug penetration into the skin<sup>8,9</sup>.

The present study is an attempt to design various microemulsion formulations of celecoxib for topical and transdermal application. Here we have evaluated the in vitro permeation of celecoxib from microemulsion containing 1% celecoxib, then compared with aqueous saturated solution of celecoxib. Also an attempt was made to study the effect of

surfactant/cosurfactant mixing ratios, oil phase and water on the in vitro permeation of celecoxib using abdominal rat skin.

### Materials and Methods

Celecoxib was purchased from Exir company (Iran) and Diethylene glycol monoethyl ether (Transcutol P), Caprylocaproylmacroglycerides (Labrasol) and Capryol 90 (Propylene glycol monocaprylate) were obtained as gift samples (Gattefosse, Saint-Priest, France). Oleic acid, Tween 80 and propylene glycol (PG) were obtained from Merck (Germany). All chemicals and solvents were of analytical grade. Freshly double distilled water was used in the formulations. Minitab15 software was used for experimental design and the evaluation of the effect of variables on responses. Sigma plot11 software was applied for providing ternary phase diagrams.

### Celecoxib assay

The amount determination of Celecoxib was carried out by UV spectrophotometry (BioWaveII, WPA) at  $\lambda_{\max} = 252$  nm.

### Solubility of Celecoxib

The Solubility of celecoxib was determined in different oils (Isopropyl myristate, Oleic acid, Oleic acid + Transcutol P(10:1), Isopropyl myristate + Transcutol P(10:1)), surfactants (Tween 20, Labrasol, Tween 80) and co-surfactant (Propylene glycol, Polyethylene glycol 400, Capryol 90) by dissolving an excess amount of celecoxib in 3ml of oil, and other components using a stirrer at  $37 \pm 0.5$  °C for 24 h. The equilibrated samples were then centrifuged at 3000rpm for 15 min to remove undissolved drug, then the clear supernatant liquid was decanted. The solubility of celecoxib was measured by validated UV spectrophotometric method (Biochrom WPA Bioware) at 252 nm<sup>10</sup>.

### Pseudo-ternary phase diagram construction

To investigate concentration range of components for the existing boundary of microemulsion regions, pseudo-ternary phase diagrams were constructed using the water titration method. Three phase diagrams were prepared with the 2:1, 4:1, and 6:1 weight ratios of (Labrasol-Tween 80/Capryol 90) respectively. Oil phase (oleic acid + Transcutol-P)(10:1) and the surfactant/cosurfactant mixture were then mixed at the weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. These mixtures were diluted drop wise with double distilled water, under moderate agitation. The samples were classified as microemulsions when they appeared as clear liquids<sup>11,12</sup>.

### Preparation of celecoxib Microemulsions

Several parameters influence on final properties of microemulsions. Major variables take part in microemulsion properties includes surfactant/cosurfactant ratio (S/C), water percent (%w) and oil percent (% oil). Full factorial design was used concerning with 3 variables at 2 levels for formulations. Full factorial design was used concerning with 3 variables at 2 levels for formulations. Eight different formulations with low and high values of oil (5% and 10%),

water (20%, 30%), S/C ratio (6:1, 4:1) and Celecoxib(1%) were used for preparing of microemulsion formulations.

Various MEs were chosen from the pseudoternary phase diagrams with 4:1, and 6:1 weight ratio of (Labrasol + Tween 80)/Capryol 90. celecoxib was added to the mixtures of oil and S/C and then an suitable amount of double distilled water was added to the mixture drop by drop and the MEs containing celecoxib were formed by stirring the mixtures at ambient temperature<sup>12</sup>.

### Particle size measurements

The mean droplet size of samples was determined at 25 °C by SCATTER SCOPE 1 QUIDIX (South Korea).

### Viscosity Determination

The Viscosity of samples was measured at 25 °C with a Brookfield viscometer (DV-II+Pro Brookfield., USA) using spindle no. 34. in shear rate 50 rpm. Each measurement was performed in triplicate.

### Determination of pH

The pH value of microemulsion was obtained at 25 °C by pH meter (Mettler Toledo seven easy, Switzerland).

### Surface tension measurement

The surface tension of microemulsion was measured at 25 °C with a Torsion balance (WHITE ELEC Model NO. 83944E).

### Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was used to characterize microstructure of microemulsions. SEM of samples were recorded by LED 1455VP, Germany.

### Physical stability studies

Ten milliliter of drug-loaded microemulsion samples was stored at three temperatures (4, 25 and 40°C) for 2 months. During this period, samples were checked for any turbidity and coalescence. In addition, samples were centrifuged at 10000 rpm for 30 minutes for determination of physical instability such as phase separation and aggregation<sup>12</sup>.

### Differential scanning calorimetry (DSC)

DSC measurements were carried out by a Mettler Toledo DSC star system equipped with refrigerated cooling system (Hubert Tc45). Approximately, 10-15 mg of each microemulsion sample was weighted into hermetic aluminum pans and quickly sealed to prevent water evaporation. Simultaneously, an empty hermetically sealed pan was used as a reference. Microemulsion samples were kept in temperature ranging from 30°C to -50°C (scan rate: 10°C/min). All experiments were done at least in triplicate. In order to ensure accuracy and repeatability of data, DSC analyzer was calibrated and checked under the experiment conditions by indium standard<sup>12</sup>.

### Release study

Franz diffusion cells (area 3.4618 cm<sup>2</sup>) with a cellulose membrane were used to determine the release rate of celecoxib from different microemulsion formulations. The cellulose (molecular weight G12 000) membrane was

first hydrated in distilled water at 25 °C for 24 hours. The membrane was then clamped between the donor and receptor chambers of the cell diffusion. Diffusion cell was filled with 22 ml of methanol and phosphate buffer (pH =7.4)(2:1). The receptor medium was constantly stirred by externally driven magnetic beads at 300 rpm throughout the experiment. Celecoxib loaded microemulsion (5g) was accurately weighted and placed in donor compartment. At predetermined time intervals(0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 24h), a 2ml sample was removed from receptor for spectrophotometric determination and replaced immediately with an equal volume of fresh receptor medium. Samples were analyzed by UV visible spectrophotometer (BioWaveII,WPA) at 252 nm. The results were plotted as cumulative released drug percentage versus time<sup>13</sup>.

### Animal experiments

Male adult Wistar rats (weighing 250 - 300 g) and aged 10 - 12 weeks were purchased from Animals Laboratory, Jundishapur University of Medical Sciences, Ahvaz, Iran. The hair on the abdominal skin was removed with an electric clipper, taking care not to damage the skin. The rats were anaesthetized with ether prior to sacrificing them. Abdominal full-thickness skin was removed and any extraneous subcutaneous fats cleaned from the dorsal side using cooled pure acetone solution with 4 °C<sup>14</sup>. Whole skin thickness was determined using a digital micrometer (AAOC, France).

### In vitro permeation studies

Diffusion cells fabricated in-house and with an effective area of approximately 3.4618 cm<sup>2</sup>, were utilized for the permeation studies. Whole skin samples were placed between donor and receptor chambers of the cells with the stratum corneum side facing the donor compartment. Skin samples were hydrated prior to their being used. The donor phase was filled with a 5ml Celecoxib microemulsion sample while the receptor compartment was filled with 22 ml methanol : phosphate buffer (pH =7.4)(2:1). The diffusion cell was placed and clamped in a water bath 37 ± 0.5 °C placed on a magnetic stirrer with a heater.

The receptor chambers was stirred continuously with the help of magnetic bead at 300rpm. At predetermined time intervals( 0.5, 1, 2, 3,.....,72 h), a 2 ml of the receptor medium was withdrawn and immediately replaced with an equal volume of fresh Methanol and phosphate buffer mixture to maintain sink condition. The samples filtered and the permeated amount of celecoxib was analyzed by UV spectroscopy method at 252 nm<sup>15</sup>.

### Data analysis of skin permeation and statistics

The cumulative amount of Celecoxib permeated per unit skin area was calculated and plotted against time. The skin permeation rate at steady state ( Jss) was calculated from the linear portion of the slope of the permeation curve. The one-way analysis of variance (ANOVA) was used to see any significant differences and P < 0.05 was considered to be significant with 95% confidence intervals. All statistical analyses were conducted using SPSS software (version 16.0). All the experiments were repeated three times and data were expressed as the mean value ± SD.

Different permeability parameter was measured through permeation studies that includes flux(Jss) , permeability coefficient(P), lag time(T<sub>lag</sub>) and diffusivity coefficient(D).

Since the skin thickness ( h) does not show the real pathway for drug permeation then diffusivity coefficient is defined as appearance D. P and D parameters were calculated from equation( Jss=P.C<sub>0</sub>) and (D= h<sup>2</sup>/6T<sub>lag</sub>), respectively.

The Enhancement Ratio(ER) was calculated to find the relative enhancement in the permeability parameters amount of microemulsion formulations in respect of the control( drug saturated solution )permeability parameters. The enhancement ratio was estimated according to equation(Enhancement ratio (ER) =permeability parameter amount formulation/ permeability parameter amount control).

## Results and discussion

### Celecoxib solubility

To investigation ME system for transdermal delivery of Celecoxib suitable oil, surfactant and cosurfactant have to be chosen. since only the drug dissolved can permeate through the skin, the solubility of poorly water-soluble Celecoxib needs to be increased. The results of solubility of celecoxib are tabulated in Table 1. The maximum solubility of celecoxib was found in Oleic acid:Transcotol P (10:1) (6.494±0.125) as compared to other oils. In addition, the highest drug solubility of celecoxib in surfactants were found in Labrasol (2.352±0.152), and Tween 80 (1.15±0.3). Based on the solubility studies of celecoxib in oil, surfactant and co-surfactant and the preformulation studies, it was found that Oleic acid:Transcotol P, Labrasol, Tween 80 and Capryol 90 could be the most suitable combination for preparation of microemulsion.

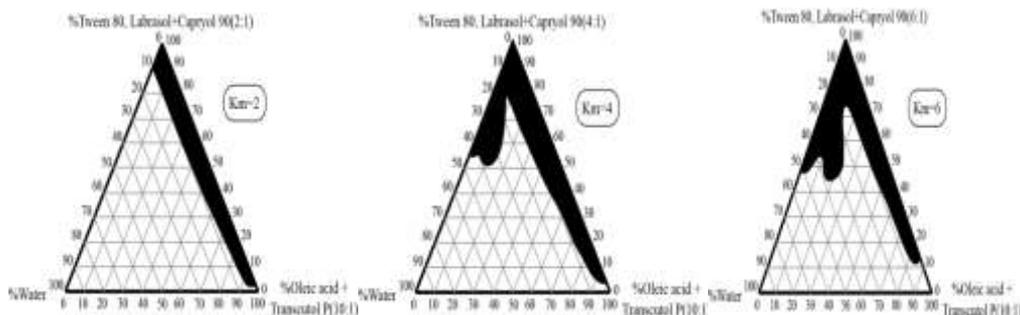
**Table 1. Solubility of celecoxib in different oils, surfactants and co-surfactants (mean±SD, n=3)**

Phase type	Excipient	Solubility(mg/ml)
Oil	Oleic acid	2.01 ± 0.1
	Isopropyl myristate	1.718 ± 0.07
	Oleic acid +Transcotol P(10:1)	6.494 ± 0.125
	Isopropyl myristate +Transcotol P(10:1)	5.7 ± 0.2
Surfactant	Tween 80	1.15 ± 0.3
	Labrasol	2.352 ± 0.152
	Tween 20	0.186 ± 0.05
Co-surfactant	Capryol 90	2.705 ± 0.01
	Propylen glycol	1.825 ± 0.1
	Polyethylen e glycol 400	2.163 ± 0.12

**Phase Studies**

The phase diagram system were composed of oil phase (oleic acid:Transcutol P (10:1)), surfactant( Tween 80-Labrasol) and cosurfactant( Capryol90). Oil, surfactant and cosurfactant were selected based on their drug solubility capacity, hydrophilic-lipophilic balance (HLB) values and ability of microemulsion formation. The phase diagram was used to determine the microemulsion zones. Three phase diagrams constructed at s/c of 2/1, 4/1 and 6/1 are presented in figure 1. The weight ratio of surfactant/cosurfactant is a critical and important parameter

affecting phase behaviors of microemulsion. Increase in microemulsion region with higher concentration of surfactant was reported. Microemulsions were formed at ambient temperature. The phase diagrams clearly indicated that microemulsion existence region increased with increase in the weight ratio of surfactant/cosurfactant and lead to presence of much more water amount in the microemulsion structure(Km=2-6). According to the full-factorial design, eight formulations were selected and examined *in vitro* skin permeability.



**Figure 1.** The pseudo-ternary phase diagrams of the oil-surfactant/cosurfactant mixture–water system at the 2:1, 4:1, and 6:1 weight ratio of Labrasol -Tween 80/ Capryol 90 at ambient temperature, dark area show microemulsions zone.

**Physicochemical Characterization of selected Microemulsions**

The mean particle size (PZ) of formulations was from 3 - 66 nm (Table 2). Particle size of free drug MEs and drug loaded MEs were determined and there was no significant difference observed in average particle size after loading the drug. The ME 5 formulation had the lowest average particle size  $3.2 \pm 0.3$  nm with polydispersity index

(PI) of  $0.372 \pm 0.017$  (Table 2). PI is a measure of particle homogeneity and it varies from 0.0 to 1.0. The closer to zero the PI value the more homogenous are the particles.

The PI indicated that ME formulations had narrow size distribution. Analysis of variance showed significant correlation between PZ and independent variable (S/C) ( $p < 0.05$ ) so that with increase in S/C ratio, PZ is increased.

**Table 2.** Compositions of Selected Microemulsions (% w/w), Particle Size and poly dispersity index (mean±SD, n=3)

Formulation	Factorial	S/C	% Oil	%(S/C)	% Water	Particle size(nm)	Poly dispersity index
ME-1	+++	6:1	10	60	30	53.7±3.5	0.383±0.028
ME-2	++-	6:1	10	70	20	6.5±0.9	0.386±0.011
ME-3	++	6:1	5	65	30	66.8±3.7	0.366±0.017
ME-4	+--	6:1	5	75	20	5.5±0.7	0.386±0.011
ME-5	---	4:1	5	75	20	3.2±0.3	0.372±0.017
ME-6	--+	4:1	5	65	30	8.1±1.1	0.385±0.011
ME-7	-+-	4:1	10	70	20	3.4±0.6	0.383±0.012
ME-8	-++	4:1	10	60	30	11.6±2.2	0.386±0.014

(High level= +, low level= -), S/C= surfactant/ cosurfactant ratio

The refractive index (RI) of the ME formulation was found 1.432 that is near to water phase which indicates ME formulations have water -in- oil structures(Table 3). Analysis of variance was showed that correlation between refractive index (RI) and independent variables are not significant ( $p > 0.05$ ).

The ME formulations had appropriate observed pH value ( $5.305 \pm 0.09$ ) that is best for topical application. Incorporation of celecoxib did not significantly affect the observed pH value of the ME formulations (Table 3).

The mean viscosity range of ME formulations were from  $195.6 \pm 0.98$  cps to  $335.2 \pm 1.54$  cps (Table 4). The highest viscosity belongs to ME-2 formulation. Multivariate regression was applied for the analysis of correlation between independent variables and MEs viscosity. It seems that viscosity of Celecoxib – loaded microemulsions enhanced with increase in the water percent in formulations ( $p < 0.05$ ).

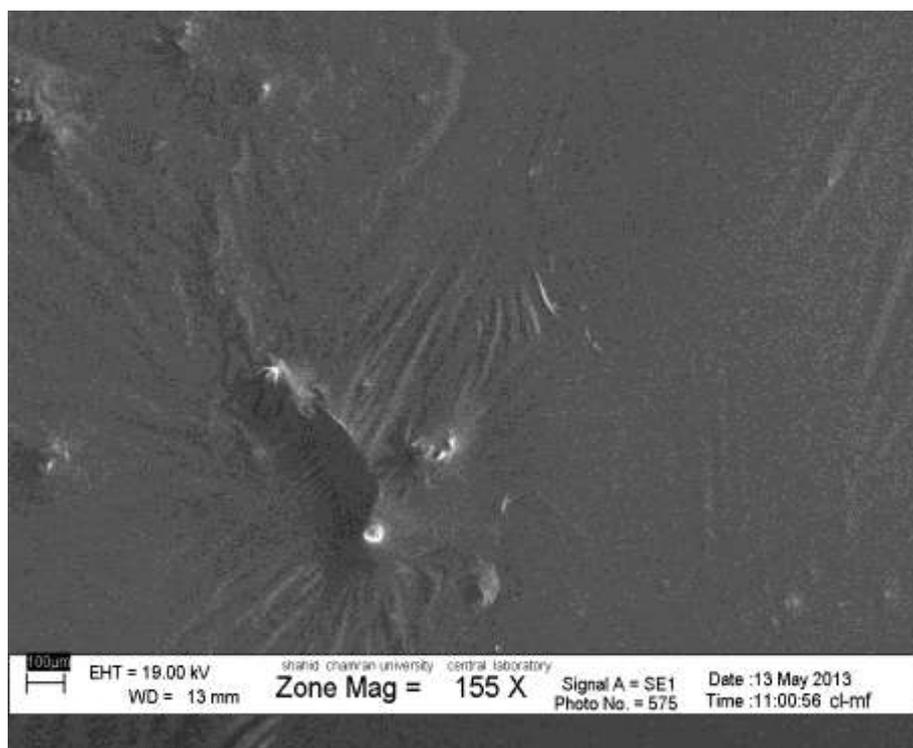
The mean surface tension of formulations was found between  $37.3 \pm 1.08$  to  $47.4 \pm 1.25$  dynes/cm (Table 4). The surface tension data implies oil-in-water microemulsions because surface tension amounts of MEs is

nearly to water phase surface tension. Figure 2 shows the

SEM image of ME-3 with lamellar structure.

**Table 3. pH, Refractive index, surface tension and viscosity of selected celecoxib microemulsions (mean  $\pm$  SD, n = 3)**

Formulation	pH	Refractive index	Surface tension(dyne/cm)	Viscosity(cps)
ME-1	5.04 $\pm$ 0.04	1.4288 $\pm$ 0.14	39.9 $\pm$ 1.18	314.3 $\pm$ 1.23
ME-2	5.44 $\pm$ 0.09	1.4392 $\pm$ 0.15	38.6 $\pm$ 1.21	335.2 $\pm$ 1.54
ME-3	5.37 $\pm$ 0.12	1.4291 $\pm$ 0.2	43.1 $\pm$ 1.19	210 $\pm$ 0.95
ME-4	5.68 $\pm$ 0.14	1.4418 $\pm$ 0.18	41.2 $\pm$ 1.27	244.4 $\pm$ 1.34
ME-5	5.22 $\pm$ 0.06	1.4273 $\pm$ 0.16	37.3 $\pm$ 1.08	222.8 $\pm$ 1.43
ME-6	5.39 $\pm$ 0.15	1.4377 $\pm$ 0.21	37.3 $\pm$ 1.08	195.6 $\pm$ 0.98
ME-7	5.26 $\pm$ 0.11	1.4330 $\pm$ 0.19	47.4 $\pm$ 1.25	234 $\pm$ 1.32
ME-8	5.04 $\pm$ 0.07	1.4270 $\pm$ 0.16	42.9 $\pm$ 1.2	253.1 $\pm$ 1.62



**Figure 2. SEM photographs of of ME-3 with lamellar structure**

Figure 3 shows the release profile of celecoxib ME formulations. The cumulative amount of celecoxib that had permeated through the cellulose membrane (%) was plotted as a function of time (hours). In this study, ME-3 and ME-2 have the highest and lowest accumulative release percent, respectively. Table 4 shows release percent and kinetic of release in celecoxib-loaded microemulsions. Multivariate regression was used for the analysis of correlation between independent variables and drug released from MEs. Analysis of variance is showed no significant correlation

between release percentage value of celecoxib and independent variables ( $p > 0.05$ ).

The release profile of MEs were calculated by fitting the experimental data to equations describing different kinetic models. Linear regression analyses were made for zero-order ( $Mt/M_0 = kt$ ), first-order ( $\ln(M_0 - Mt) = kt$ ), Higuchi ( $Mt/M_0 = (kt)^{1/2}$ ), Log Wagner, Linear wagner Second root of mass, Three-Seconds root of mass, Pepas and Weibul kinetics.

The amount of celecoxib released was varied between microemulsion carriers with different internal microstructure. Comparing the amounts of released

celecoxib after 24 hours indicates that (Figure 3) the slowest drug release was observed for ME -2 and the highest drug release was observed for ME-3.

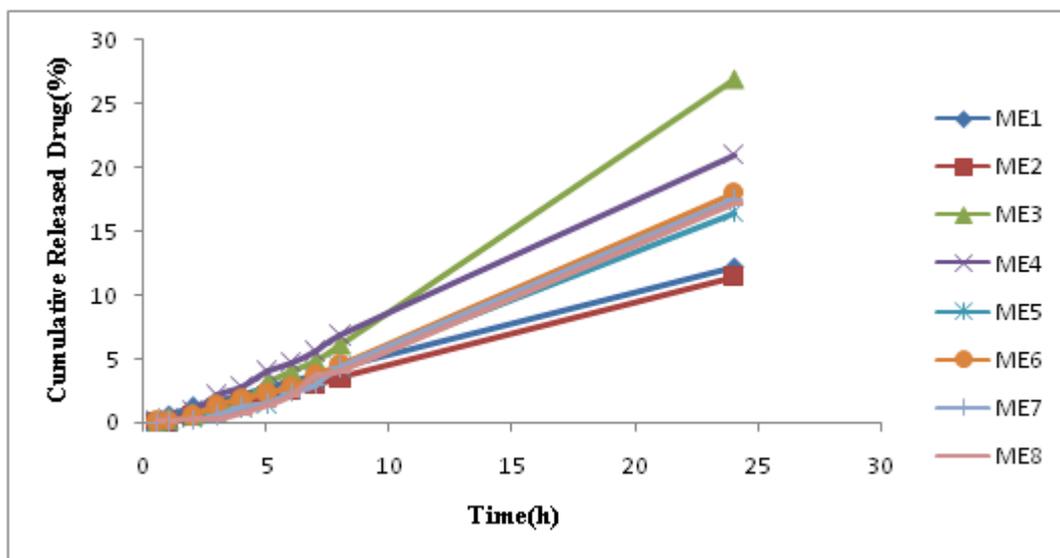


Figure 3. In vitro release profile of ME formulations of Celecoxib.

Table 4. Release kinetic models of celecoxib microemulsions (mean±SD, n=3)

Formulation	%Release	Kinetic model	R <sup>2</sup>	Slop	Intercept
ME-1	12.2157	Zero	0.9964	0.0052	-0.0011
ME-2	11.4784	Zero	0.9962	0.005	-0.0048
ME-3	26.9442	Weibul	0.9926	1.5607	-6.1082
ME-4	20.9693	Zero	0.9989	0.009	-0.0060
ME-5	16.4782	Weibul	0.9862	1.8160	-7.0763
ME-6	18.0183	Weibul	0.9944	1.4308	-6.069
ME-7	17.539	Log wagner	0.9938	0.7522	-3.3264
ME-8	17.2251	Zero	0.9791	0.0076	-0.0158

Figure 4 and Figure 5 are showed DSC cooling thermograms of celecoxib microemulsions. The thermal behaviour of water can be a useful and rapid means with which to understand the microstructure of microemulsions<sup>16</sup>. In this context, a small peak at very low temperatures (between -20 °C to -30<sup>0</sup>C) has been suggested to be either internal water or water that is interacting strongly with the surfactants<sup>12,17</sup>. When water is mixed in to a microemulsion system it can be either bound (interfacial) or free (bulk) water depending of its state in the system<sup>18</sup>. In cooling curves of the samples (ME1-ME5-ME6, ME7 and ME8), DSC thermogram showed one peak at around 0 to -5 °C that indicate the freezing of bulk water in these formulations. The other peak at around -10 to -20 °C belong to bound water freezing. In the systems where water is present in the continuous phase, a distinctive, large sharp peak appears at ca. -20°C, which indicates the freezing of supercooled water<sup>12,18</sup>.

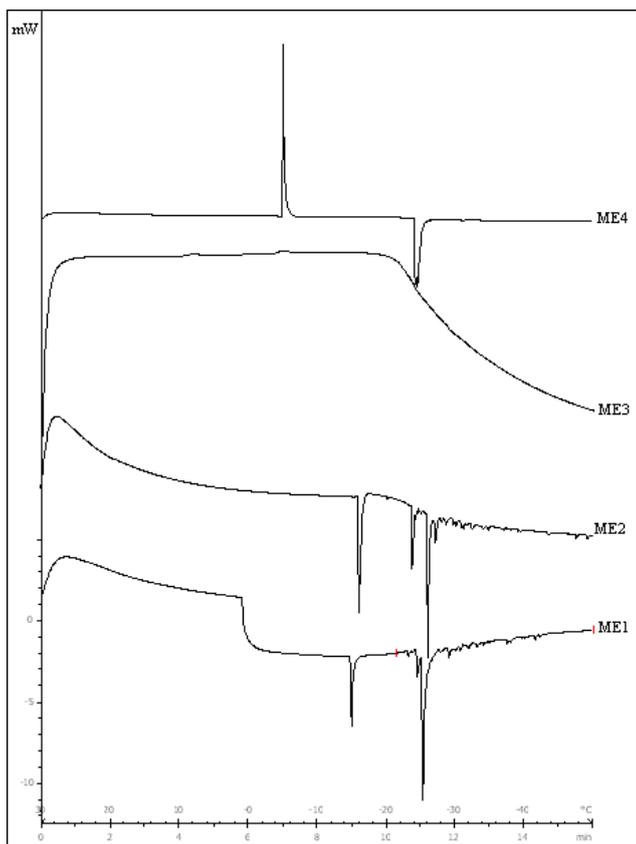


Figure 4. DSC cooling thermograms of ME-1 ,ME-2 ,ME-3 and ME-4 formulations

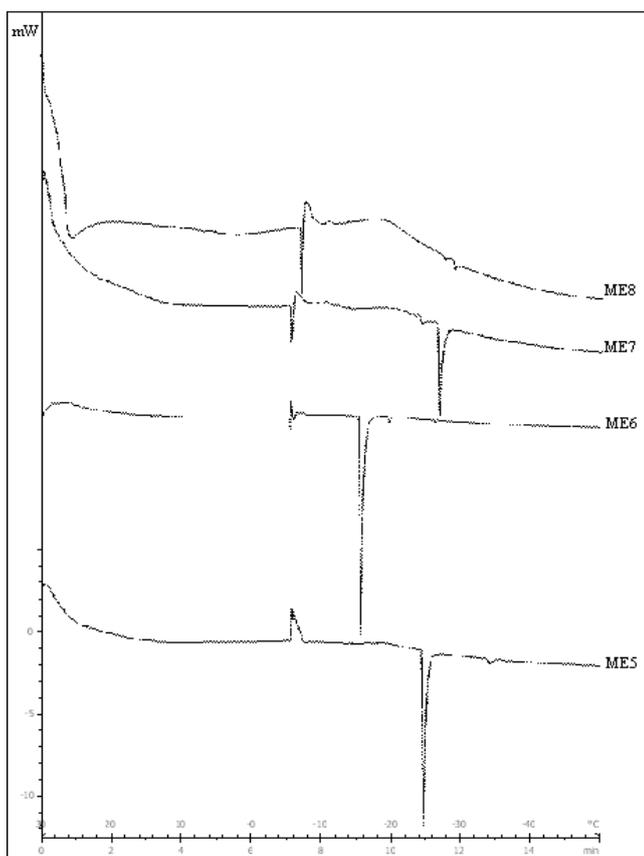


Figure 5. DSC cooling thermograms of ME-5 ,ME-6 ,ME-7 and ME-8 formulations.

The visual inspection experiment was performed for 2 months by drawing ME sample at weekly interval for the first month and monthly interval for the subsequent months. The visual observation showed no evidence of phase separation or any precipitation or flocculation.

These samples also revealed no sign of phase separation under stress when subjected to centrifugation at 10000rpm for 30 min. In addition, the centrifugation tests showed that microemulsions were remained homogenous without any phase separation throughout the test indicates good physical stability of both preparations.

#### In-vitro skin permeation studies

The permeability parameters of various microemulsions of Celecoxib are indicated in table 5. Figure 4 has showed Permeation profiles of Celecoxib through excised rat skins from various microemulsions.

Among the ME formulations tested, ME 4, which was composed of 1% Celecoxib, 5% oleic acid: transcutool P, 75% larasol-tween80/capryol 90(4:1) and 20% water, showed the highest skin permeability. The  $J_{ss}$  of celecoxib from ME 4 was  $0.0142 \pm 0.0061 \text{ mg cm}^{-2} \text{ h}^{-1}$ , 4.31times higher than those of the celecoxib saturated solution in water, which were  $0.0033 \pm 0.00028 \text{ mg cm}^{-2} \text{ h}^{-1}$ . The higher permeability rate of celecoxib from ME formulations is most probably due to the S/C amount and the oily phase, which act as penetration enhancers<sup>19</sup>. The enhancer can increase the transport of drug through skin by changing the diffusion or partitioning coefficient of drug<sup>20</sup>. The surfactant mixture content in the formulation affected the permeation rate significantly. As the composition of surfactant/cosurfactant mixture was increased from 60% (ME 1) to 75% (ME 4) at S/C = 6, the permeation rate of celecoxib increased approximately by 2.6 folds. The surfactants used, ie, Tween 80, have been found to act as permeation enhancers in different skin models<sup>16</sup>. Disruption of lipid structure and fluidization are the main modes of action exerted by oleic acid<sup>21</sup>. According to the results obtained from permeation studies, it was obvious that the maximum of P and  $T_{lag}$  parameters are belongs to ME 4 with  $0.0014 \text{ cm h}^{-1}$ , and ME 2, 18.18 h, respectively. The maximum of  $D_{app}$  parameter was obtained from ME 7 was  $0.00012 \pm 0.000088 \text{ cm}^2 \text{ h}^{-1}$ , 1.714 times higher than those of the celecoxib saturated solution in water, which were  $0.00007 \pm 0.0000027 \text{ cm}^2 \text{ h}^{-1}$ .

Multivariate regression was applied for the analysis of correlation between independent variables and MEs skin permeability parameters. Linear equations which shows for flux( $J_{ss}$ ),  $T_{lag}$ ,  $D_{app}$  and P are :

$$J_{ss} = 0.00821 + 0.000302(\% \text{ oil}) - 0.000469(\% \text{ w}) + 0.00211(\text{S/C}) \quad (\text{Equation 1})$$

$$P = 0.000911 + 0.000026(\% \text{ oil}) - 0.000050(\% \text{ w}) + 0.000215(\text{S/C}) \quad (\text{Equation 2})$$

$$D_{app} = 0.000117 - 0.000007(\% \text{ oil}) - 0.000000(\% \text{ w}) + 0.000004(\text{S/C}) \quad (\text{Equation 3})$$

$$T_{lag} = 11.4 + 0.661(\% \text{ oil}) + 0.118(\% \text{ w}) - 1.10(\text{S/C}) \quad (\text{Equation 4})$$

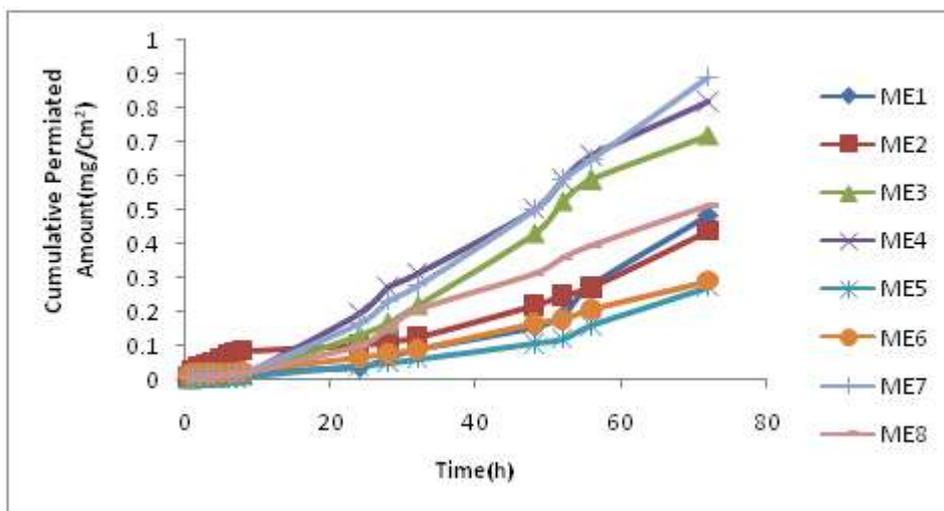


Figure 6. Permeation profiles of Celecoxib through excised rat skins from various microemulsions

Table 5. In vitro permeability Parameters of Celecoxibin aqueous saturated solution( control) and various ME formulations through excised rat skins(mean±SD, n=5)

Formulation	$J_{ss}$ (mg/cm <sup>2</sup> .h)	$T_{lag}$ (h)	$D_{app}$ (cm <sup>2</sup> /h)	P (cm/h)	ER <sub>flux</sub>	ER <sub>D</sub>
Control	0.0033±0.00028	5.765±0.098	0.00007±0.000027	0.825±0.007	-	-
ME-1	0.0055±0.00219	15.25±5.31	0.000065±0.000022	0.00056±0.00022	1.66±0.012	0.928±0.005
ME-2	0.0072±0.00078	18.18±2.48	0.000052±0.000062	0.00073±0.00079	2.181±0.021	0.742±0.0008
ME-3	0.0133±0.00057	15.69±0.86	0.000059±0.000033	0.0013±0.000058	4.03±0.05	0.368±0.0037
ME-4	0.0142±0.0061	11.96±1.82	0.00008±0.00001	0.0014±0.00061	4.31±0.008	0.843±0.034
ME-5	0.002 ±0.0006	10.4±7.047	0.00012±0.000098	0.00026±0.000064	0.606±0.0032	1.714±0.0044
ME-6	0.0047±0.0019	12.58±5.52	0.000082±0.000036	0.00048±0.00019	1.424±0.004	1.171±0.0011
ME-7	0.013±0.0084	9.85±7.098	0.00012±0.000088	0.0014±0.00085	3.939±0.017	1.714±0.005
ME-8	0.0132±0.0029	16.33±1.36	0.000057±0.000048	0.0013±0.0003	4±0.075	0.814±0.0004

Analysis of variance implies that correlation between independent variables and  $T_{lag}$  and  $D_{app}$  permeability parameters of Celecoxib formulations are not significant ( $p > 0.05$ ). On the other hand, analysis of variance shows significant correlation between independent variables and  $J_{ss}$  and P parameters of Celecoxib formulations ( $p < 0.05$ ). On the basis of Eq 1 and Eq 2, the correlation between independent variables and  $J_{ss}$  and P parameters are significant with water percent in ME formulations, so that decrease in water percent is caused increase  $J_{ss}$  and P parameters.

**Conclusion**

In the recent study established that physicochemical properties and in vitro skin permeation were dependent upon the content of water, oil and S/C ratio. Phase diagrams indicated more extensive microemulsion

zone with a rise in S/C ratio. Analysis of variance showed significant correlation between PZ and S/C ratio so that with increase in s/c ratio, PZ is increased. Also, It seems that viscosity of Celecoxib – loaded microemulsions enhanced with increase in the water percent in formulations.

In permeation studies the correlation between  $J_{ss}$  and P with water percent was significant and indirectly.  $J_{ss}$  of celecoxib of ME 4 and  $D_{app}$  in ME7 were 4.31 times and 1.714 times higher than those of saturated water solution of celecoxib. In conclusion, the amount of components in ME formulation have an essential role in the physicochemical properties and celecoxib permeability through rat skin.

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## References

1. Bagwe RP, Kanicky JR, Palla BJ, Patanjali PK, Shah DO. Improved drug delivery using microemulsion: rationale, recent progress, and new horizons. *Crit Rev Ther Drug* **2001**; 18: 77-140.
2. Winsor PA. Solvent Properties of Amphiphilic Compounds. London: Butherworth; **1954**.
3. Attwood D. Microemulsions. In: Kreuter J, editor. Colloidal Drug Delivery Systems. New York: Marcel Dekker; **1994**.P.31-71.
4. Baboota S, Faiyaz S, Ahuja A, Ali J, Shafiq S, and Ahmad S. Development And Validation Of A Stability-Indicating HPLC Method And Analysis Of Celecoxib (CXB) In Bulk Drug And Microemulsion Formulations. *ACTA Chromatographica* **2007**;18: 116-129.
5. S.M. Fisher, H.H. Lo, G.B. Gordon, K. Seibert, G. Kellof, R.A. Lubet, and C.J. Conti. *Mol. Carcinog.*, **1999**;25,:231
6. R.E. Harris, G.A. Alshafie, H. Asbou-Issa, and K Seibert, *Cancer Res.*, **2000**;60, 2101.
7. Hadgraft J, Lane ME. Skin permeation: The years of enlightenment. *Int J Pharm* **2005**; 305: 2-12.
8. Heuschkel S, Goebel A, Neubert RH. Microemulsion – modern colloidal carrier for dermal and transdermal drug delivery. *J Pharm Sci* **2008**; 97: 603-631.
9. Kreilgaard M. Influence of microemulsions on cutaneous drug delivery. *Adv Drug Del Rev* **2002**; 54: 77-98
10. Moghimipour E, Salimi A, Leis F. Preparation and Evaluation of tretinoin microemulsion based on pseudo-ternary phase diagram. *Adv Pharm Bull.* **2012**; 2(2): 141-7.
11. Wilk KA, Zielinska K, Hamerska-Dudra A, Jezierski A. Biocompatible microemulsions of dicephalicaldonamide-type surfactants: Formulation, structure and temperature influence. *J Colloid Interface Sci*, **2009**;334(1):87-95.
12. Salimi A, Sharif Makhmal Zadeh B , Moghimipour E. Preparation and Characterization of Cyanocobalamin (Vit B12) Microemulsion Properties and Structure for Topical and Transdermal Application. *Iran J Basic Med Sci*, **2013**:16(7):866-872.
13. Moghimipour E, Salimi A, Eftekhari S. Design and Characterization of Microemulsion Systems for Naproxen. *Adv Pharm Bull*, **2013**, 3(1), 63-71.
14. Sharif MakhmalZadehB ,Hasani MH.The Effect of Chemical and Physical Enhancers on Trolamine Salicylate Permeation through Rat Skin . *Tropical Journal of Pharmaceutical Research* **2010**: 9 (6): 541-548.
15. Sharif Makhmal Zadeh B, Yazdani M, Rezai S, Salimi E. The Effect of Chemical Enhancers on Tacrolimus Permeation through Rat Skin. *Journal of Pharmacy Research* **2012**:5(3):1309-1312.
16. Garti N, Aserin A, Tiunova I, Funun M. A DSC study of water behavior in wate- in – oil microemulsions stabilized by sucrose esters and butanol. *Colloids & Surface* **2000**; 120: 1-18.
17. Podlogar F, Gasperlin M, Tomsic M, Jamnik A, Rogac MB. Structural characterisation of water-tween 40/imwitor 308-isopropyl myristate microemulsions using different experimental methods. *Int J Pharm* **2004**;276(1-2):115-28.
18. Podlogar F, Bester Rogac M, Gasperlin M. The effect of internal structure of selected water-tween 40-imwitor 308-ipm microemulsions on ketoprofene release. *Int J Pharm* **2005**;302(1-2):68-77.
19. TrommerH,Neubert R.H.H. Overcoming the Stratum Corneum:The Modulation of Skin Penetration. *Skin Pharmacol Physiol.* **2006**:19:106–121.
20. Chen H, Mou D, Du D, Chang X, Zhu D, Liu J, et al. Hydrogelthickenedmicroemulsion for topical administration of drugmolecule at an extremely low concentration. *Int J Pharm.***2007**:341:78–84.
21. Okuyama H, Ikeda Y, Kasai S, Imamori K, Takayama K,Nagai T. Influenceof non-ionic surfactants, pH and propylene glycolon percutaneousabsorption of piroxicam from cataplasm. *Int J Pharm.***1999**;186: 141–148.