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Research Article

EFFECT OF FORMULATION COMPONENTS ON THE *IN VITRO* SKIN PERMEATION OF MICROEMULSION DRUG DELIVERY SYSTEM OF PIROXICAM

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Abstract: The aim of this research is to prepare , characterize and assessment of *in vitro* skin permeability of Piroxicam microemulsions through excised rat skin for pain relief. physicochemical drug properties , skin thickness, or formulation have role in drug transition from the skin surface to underlying tissues or to the systemic circulation. For this purpose, eight formulations made from oleic acid,tween 80,labrasol and propylene glycol were used at 0.3% of piroxicam to evaluate *in vitro* release through synthetic membrane and *ex vivo* permeation through rat skin using diffusion Franz cells. Phase diagrams indicated more extensive microemulsion region with a rise in S/C ratio. The release percent was enhanced with decrease in the oil percent. In permeation studies the correlation between Tlag and P with water percent was significant and indirectly. Jss of piroxicam of ME 3 and Dapp in ME7 were 1.8 times and 42.57 times higher than those of saturated water solution of piroxicam.
Key**words:** Piroxicam microemulsions, pain, synthetic membrane

Introduction

Human skin is an important target site for the application of drugs. Permeation of drugs through the skin is the basis of transdermal and topical delivery [1]. Drug permeation across different skin layers is affected by various factors such as physicochemical properties of the drug, vehicle, and formulation components. Transdermal drug delivery has some benefits such as controlled drug delivery, avoidance of first pass metabolism, continuous drug delivery , and facilitation of drug localization at target site [1].

Piroxicam is a potent nonsteroidal, anti-inflammatory, and analgesic agent used for treatment of acute and chronic rheumatoid arthritis. It is a water insoluble drug with an acidic pKa value of 5.3. It has a log *P* value of 1.8[2]. The rate and extent of drug permeation through the skin is influenced by the log P, size, hydrogen bonding ability, ionic strength, and physicochemical properties of the vehicle[3]. The correlation between the physicochemical properties of nonsteroidal anti-inflammatory drugs (NSAIDs) and the extent of their absorption from the skin has been proved by many examinations .These investigations have found that the log *P* value of NSAIDs seems to be the most important factor compared with their pKa value and molecular weight. Drugs with a log *P* value of around 2 are suggested to be good candidatesfor topical delivery. The enhancement of transdermal piroxicam permeation has been extensively examined[4]. Various techniques have been used, including ion pairing,[5] pretreatment of the skin with permeation enhancer,[6] inclusion into a carrier system with drug ionization, and application of permeation enhancement.

Microemulsions are thermodynamically stable, isotropic clear colloidal dispersion of oil, water and surfactant, frequently in combination with a cosurfactant which have high stability, ultra low interfacial tension, large interface area , low viscosity and ease of preparation[7].

Microemulsions (MEs) as colloidal carriers are one of the promising systems which have nowadays attracted the main interest in penetration enhancement because of their localized effect. Due to their special features, MEs offer several advantages for the pharmaceutical use such as improved drug solubilization for the hydrophilic and lipophilic drugs, enhancement of bioavailability, protection of the unstable drugs against environmental conditions and a long shelf life[8].

Microemulsions have many advantages for use as topical and transdermal drug delivery systems. Firstly, the main advantage of these colloidal systems is that a large amount of drug can be incorporated into the formulation due to the increased solubilization capacity, thereby enhancing thermodynamic activity in the skin which provides a large concentration gradient from the microemulsion vehicle to the skin. Secondly, the permeation rate of the drug can be enhanced by using a microemulsion system due to the synergistic effect of various components to enhance drug delivery across the skin. Thirdly, the main componentts, such as , oil phase , water phase, and surfactant-cosurfactant mixtures, can be combined synergistically to enhance drug flux[9]. Also it has been suggested that the surfactant and the oil in the microemulsion interact with the hard lipid bilayer structure and acts as a permeation enhancer[10]. Many studies have reported that ME formulations causes improved transdermal and dermal delivery properties, mainly in vitro [11] and in vivo [12].

The present study is an attempt to design various microemulsion formulations of piroxicam for topical and transdermal application. Here we have evaluated the in vitro permeation of piroxicam from microemulsion containing 0.3% piroxicam, then compared with aqueous saturated solution of piroxicam. Also an attempt was made to study the effect of surfactant/cosurfactant mixing ratios , oil phase and water on the in vitro permeation of piroxicam using abdominal rat skin.

Materials and Methods

Piroxicam was purchased from Sobhan daru Company (Tehran,Iran) Tween 80 and PG were obtained from Merck (Germany). Caprylocaproyl macrogoglycerides (Labrasol) and Labrafil M 1944 CS (oleoyl macrogol-6-glycerides) were obtained as gift samples (Gattefosse, Saint-Priest, France).

All other materials used were of analytical grade. Freshly double distilled water was used in the experiments. Sigma plot11 software was applied for providing tertiary phase diagrams.

Piroxicam assay

The quantitative determination of piroxicam was performed by UV spectrophotometry (BioWaveII,WPA) at λmax= 362 nm.

Solubility studies

The Solubility of piroxicam was investigated in different oils (oleic acid, isopropyl myristate), surfactants (Labrafil,Labrasol, Tween 80) and co-surfactant (Propylen glycol,) .An excess amount of piroxicam was added in 3ml of oil, and other components. The mixture was immersed in a water bath for 24 h at 37 ^{0}C and allowed to equilibrate. Then, suspension was centrifuged for 15 min at 3000rpm ,filtered, diluted with methanol and the dissolved drug measured by a validated UV spectrophotometric method at 362 nm [8].

Pseudo-ternary phase diagram construction

To investigate concentration area of components for the existing boundary of microemulsion spots, pseudo-ternary phase diagrams were constructed using the water titration method. Two phase diagrams were prepared with the 2:1 and 4:1 weight ratios of (Labrasol -Tween 80/ Propylen glycol). Oil phase (Oleic acid) 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 dropwise with double distilled water, under moderate agitation. The samples were classified as microemulsion when they appeared as clear liquids[8].

Preparation of piroxicam microemulsions

Several parameters influence on final properties of microemulsions. Full factorial design was used concerning with 3 variables at 2 levels for formulations. Major variables take part in microemulsion properties includes surfactant/cosurfactant ratio (S/C), oil percentage (% oil) and water percentage (%w). Eight different formulations with low and high amount of oil (5% and 20%), water (20%, 30%), S/C ratio (2:1, 4:1) and piroxicam (0.3%)were used for preparing of microemulsion formulations. (Table I). Piroxicam was added to the mixtures of oil and S/C and then an appropriate amount of distilled water was added to the mixture drop by drop and the MEs containing piroxicam were obtained by stirring the mixtures at ambient temperature. All MEs were stored at ambient temperature[7].

Scanning electron microscopy (SEM)

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

Zeta potential determination

Zeta potential of samples were measured by Zetasizer (Malvern instrument 1td ZEN3600, UK). Samples were placed in clear disposable zeta cells and results were recorded.

Particle size measurements

The average droplet size of samples was determined at 25 ℃ by SCATTER SCOPE 1 QUIDIX (South Korea) .

Determination of viscosity

The viscosity of microemulsions was measured at 25 °C with a Brookfield viscometer (DV-II+Pro Brookfield., USA) using spindle no. 34. With shear rate 100 rpm7.

Determination of pH

The pH value of microemolsions were determined 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).

Stability studies

The stability of microemulsions was evaluated regarding the temperature stability and centrifugation. Microemulsions were kept in different temperature ranges (4 °C, 25 °C and 37 °C) and observed for phase separation, flocculation or precipitation. Also, Microemulsions were centrifuged by high speed brushless centrifuge (MPV-350R, Poland) at 10000 rpm for 30 minute at 25 °C and checked for any change in their homogeneity.

Release study

Franz diffusion cells (area 3.4618 cm2) with a cellulose membrane were used to determine the release rate of piroxicam from different microemulsion formulations. The cellulose (molecular weight G12 000) membrane was first hydrated in the distilled water solution at 25 ^{0}C for 24 hours. The membrane was then fixed between the donor and receptor compartments of the cell.Diffusion cell was filled with 22 ml of phosphate buffer (pH = 7.4) . The receptor medium was constantly stirred by externally driven magnetic beads at 300 rpm throughout the experiment. Piroxicam microemulsion (5 g) was accurately weighted and placed in donor compartment. At predetermined time intervals (0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 24 h) ,a 2 ml sample

was removed from receptor for spectrophotometric determination and immediately replaced with an equal volume of fresh phosphate buffer. The samples filtered and released amount of piroxicam were determined by UV visible spectrophotometer (BioWaveII,WPA) at 362 nm. The results were plotted as cumulative released drug percent versus time[7].

Animal experiments

Male adult Wistar rats (weighing 150 - 170 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 anaesthesized 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 ^{0}C. Whole skin thickness was determineded using a digital micrometer (AAOC, France)[13].

In vitro skin permeation studies

In-vitro skin permeation studies were carried out using vertical glass diffusion cells fabricated in house with an effective diffusion area of approximately 3.46 cm^2. The volume of the receptor compartment was 22 ml .Whole skin sample, hydrated prior to use, was mounted between the donor and receptor compartments of the cell with the stratum corneum facing the donor medium.

Piroxicam microemulsion sample (5g), was in the donor compartment and the receptor cell was filled with phosphate buffer (pH 7.4). The diffusion cell was placed and clamped in a water bath 37 ± 0.5 ^{o}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,……,80 h), a 2 ml sample was withdrawn from the receptor medium and immediately replaced with an equivalent volume of fresh phosphate buffer to maintain sink condition. The samples filterd and the permeated amount of piroxicam was analyzed by UV spectroscopy method at 362 nm.

Data analysis of skin permeation and statistics

The cumulative amount of piroxicam 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 performed to see any significant differences and $P < 0.05$ was considered to be significant with 95% confidence intervals. All statistical analyses were conducted using SSPS 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_{lag}) and diffusivity coefficient(D). P and D parameters were calculated from equation($Jss=P.C_0$) and ($D= h^2/6T_{lag}$), respectively. Since the skin thickness (h) does not show the real pathway for drug permeation then diffusivity coefficient is defined as appearance D.

The Enhacement 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 parametert amount control)[14].

Results and discussion

Piroxicam solubility

To investigation ME system for transdermal delivery of piroxicam 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 piroxicam needs to be increased.To screen appropriate oil,surfactant and cosurfactant for preparation of microemulsion the solubility of piroxicam in different oils were determinedand the results are showed in table 1.In the recent study , The maximum solubility of piroxicam was found in oleic acid (13.2 ± 0.4) compared with other oils.In addition, the highest drug solubility of piroxicam in surfactants were found in Labrasol (9.36±0.6506), and Tween 80 (16.26±0.65) (Table 1). Based on the solubility studies of piroxicam in oil, surfactant and co-surfactant and preformulation studies, we found oleic acid, labrasol, tween 80 and propylene glycol could be the most appropriate combination for preparation of microemulsion.

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

Phase type	Excipient	Solubility (mg/ml)
oil	Oleic acid	13.2±0.4
	Isopropyl Myristat	9.7±0.556
surfactant	Tween 80	16.26±0.65
	Labrasol	9.36±0.651
	Labrafil M 1944CS	8.66±0.550
co-surfactant	Propylene glycol	7.1±0.655
	plurol oleique	3.26±0.305

Phase Studies

The phase diagram system were composed of oil phase (oleic acid) , surfactant(tween 80-labrasol) and cosurfactant(propylene glycol). 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. Two phase diagrams constructed at s/c of 2/1and 4/1 are presented in

Fig 1.The phase diagrams indicate that increase in s/c ratio to lead to a more wide microemulsion region and presence of much more water amount in the microemulsion structure .According to the full-factorial design , eight formulations were selected and examined *invitro* skin permeability.

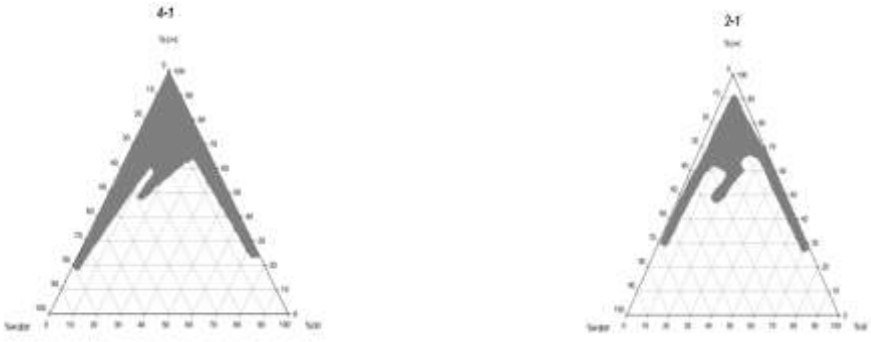

Figure 1. The pseudo-ternary phase diagrams of the oil-surfactant/cosurfactant mixture–water system at the 2:1and 4:1 weight ratio of labrasol -Tween 80/ Propylene glycol at ambient temperature, dark area show microemulsions zone.

Physicochemical Characterization of selected Microemulsions

The ME formulations had the mean droplet size in the range of 23-137 nm. Mean droplet size of ME without drug and drug loaded ME were determined and there was no significant difference observed in mean droplet size after loading the drug. The ME 4 formulation had the lowest average particle size 23.4±0.556 nm with polydispersity index (PI) of 0.360±0.010 (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 refractive index (RI) of the ME formulations were obtained 1.4 that is near to oil phase refractive index which indicates MEs formulations have water -in-oil structures. Analysis of variance is shown that correlation between RI and independent variables (s/c) so that with decrease in s/c ratio , RI is increased.

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

Formulation	Factorial	S/C	%Oil	%S+C	%water	Particle size(nm)	Polydispersity
ME-1	+ + +	4:1	20	50	30	32.86±0.850	0.361±0.011
ME-2	+ + -	4:1	20	60	20	137.13±0.808	0.360±0.007
ME-3	+ - +	4:1	5	65	30	45.26±0.971	0.362±0.010
ME-4	+ - -	4:1	5	75	20	23.4±0.556	0.360±0.010
ME-5	- - -	2:1	5	65	30	92.43±1.001	0.361±0.009
ME-6	- - +	2:1	5	75	20	88.4±0.655	0.361±0.008
ME-7	- + -	2:1	20	60	20	73.63±0.907	0.362±0.011
ME-8	- + +	2:1	20	50	30	77.7±0.6	0.360±0.009

The ME formulations had appropriate observed pH value (5.31 ± 0.3) that is good for topical application. Incorporation of piroxicam did not significantly affect the observed pH value of the ME formulations. Analysis of variance is shown that correlation between pH and independent variables (%W) so that with decrease in water percent , pH is increased (Table 3).

The ME formulations had the zeta potential average (-0.176 to+0.0876mv) (Table 3). The highest zeta potential belongs to ME-1 formulation and the lowest belong to ME-2. Analysis of variance is showed significant correlation between zeta potential average of piroxicam and water percent ($p<0.05$), it seems that zeta potential of piroxicam microemulsions enhanced with increase in the water percent in formulations.

The ME formulation had the average viscosity range (128.5±3.65cps to 521.13 ± 3.41cps) (Table 4). The highest viscosity belongs to ME-1 formulation with bicontinueous structure. Multivariate regression was showed no significant correlation between independent variables and MEs viscosity. The MEs formulation had the surface tension

average (41.16±1.04to 50.06±0.9dyne/cm) (Table 4).The surface tension data implies water –in-oil microemulsions because surface tension amounts of MEs is nearby to oil phase surface tension.

Table 3. pH, Refractive index, and Zeta potential of selected piroxicam microemulsions (mean ± SD, n = 3)

Formulation	pH	Refractive index	Zeta potential(mv)
ME-1	4.82±0.12	1.428±0.00086	0.0876±0.0012
ME-2	4.92±0.12	1.437±0.00065	-0.176±0.024
ME-3	5.31±0.16	1.4266±0.00045	-0.706±0.0018
ME-4	5.68±0.11	1.4373±0.00073	-0.349±0.0024
ME-5	5.57±0.11	1.4199±0.0005	-0.566±0.0015
ME-6	5.67±0.09	1.4333±0.00055	-0.277±0.0009
ME-7	5.2±0.07	1.4347±0.00045	-0.196±0.0017
ME-8	5.29±0.04	1.4298±0.00056	-0.214±0.0022

Table 4. Surface tension and viscosity of selected microemulsions (mean ± SD, n=3)

Formulation	Surface tension (dyne/cm)	Viscosity(cps)
ME-1	41.16±1.04	521.13±3.41
ME-2	42.56±1.30	326.53±2.65
ME-3	46.4±1.25	167.8±3.70
ME-4	43.2±0.91	193.46±2.15
ME-5	44.56±1.20	146±1.74
ME-6	42.9±1.25	128.5±3.65
ME-7	50.06±0.90	206.6±3.21
ME-8	42.26±1.12	206.1±2.16

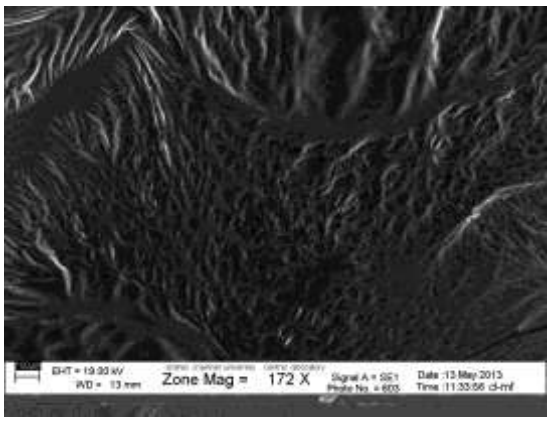

Figure 2. SEM photographs of of ME-3 with bicontinueous structure

Figure 2 shows the SEM image of ME-3 with reverse bicontinueous structure. Figure 3 shows the release profiles of microemulsions of piroxicam MEs formulations. The cumulative amount of piroxicam that had permeated through the cellulose membrane (%) was plotted as a function of time (hours). In this study, ME-3 and ME-1 have the highest and lowest accumulative release percent, respectively.Table 5 shows release percent and release kinetic models in piroxicam formulations. Multivariate regression was used for the analysis of correlation between independent variables and MEs release. Analysis of variance is showed significant correlation between release percentage value of piroxicam and oil percent ($p<0.05$). The percent of oil had more negative effect on accumulative release percent. Linear equation which shows all the main effects for accumulative release percent is:

%Release = 30.4+ 0.44(S/C)-0.629(%oil)-0.152(%water) - (Equation 1)

On the basis of Eq.1, it seems that release percent was enhanced with decrease in the oil percent. The release profile of MEs were calculated by fitting the experimental data to equations describing different kinetic models. Linear regression analysis 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, Weibul, Second root of mass, Three-Seconds root of mass, and Pepas kinetics.

The amount of released piroxicam is vary between microemulsion carriers with different internal microstructures. Comparing the amounts of released piroxicam after 24 hours as well as the release rate (Figure 3) the slowest release was observed for ME -1 and the highest release was observed for ME-3 with bicontinuous structure.

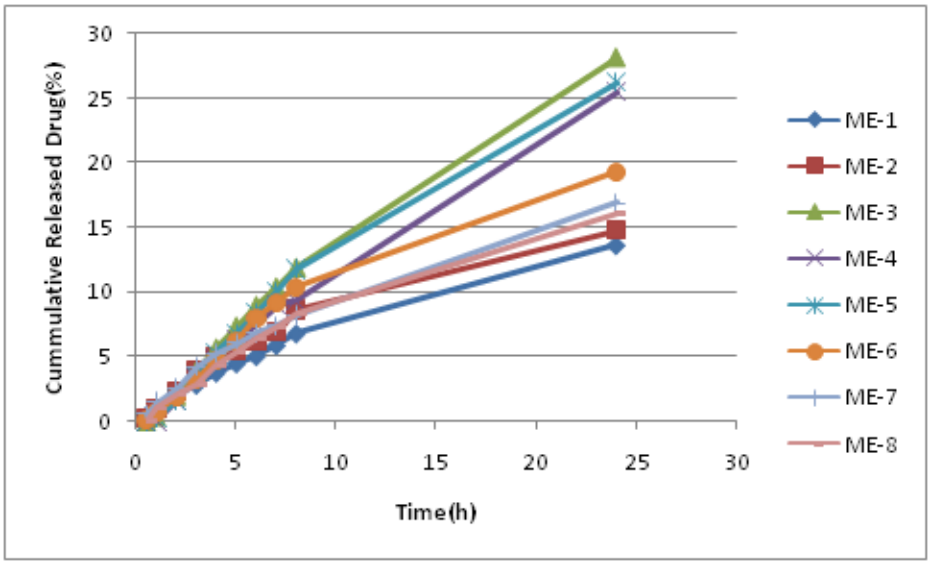

Figure 3. In vitro release profile of ME formulations of piroxicam

Table 5. release kinetic models of selected microemulsions(mean±SD, n=3)

Formulation	Kinetic model	R^2	Intercept	%Release
ME-1	log wagner	0.9986	-2.3218	13.639±0.63
ME-2	Higuchi	0.9920	-0.0244	14.701±1.89
ME-3	Higuchi	0.9924	-0.0846	28.162±2.029
ME-4	First	0.9952	0.0025	25.418±3.36
ME-5	Higuchi	0.9932	-0.0792	26.194±2.83
ME-6	Higuchi	0.9855	0.0486	19.270±2.23
ME-7	Higuchi	0.9968	-0.0269	16.903±3.46
ME-8	Higuchi	0.9938	-0.0321	16.062±1.0

The visual inspection test was performed for 3 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 flocculation or precipitation. These samples also revealed no sign of phase separation under stress when subjected to centrifugation at 10000 rpm for 30 min. The centrifugtion tests showed that microemulsions were remained homogenous without any phase separation throughout the experiment .

In-vitro permeation studies:

The permeability parameters of various microemulsions of piroxicam are indicated in table 6 . **Figure 4** has showed Permeation profiles of piroxicam through excised rat skins from microemulsions .

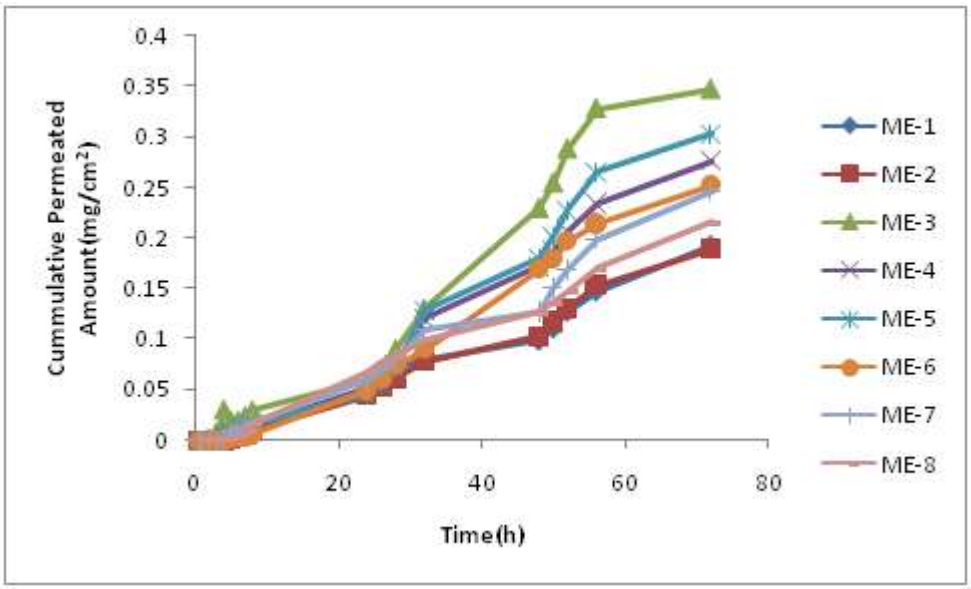

Figure 4 . Permeation profiles of piroxicam through excised rat skins from microemulsions with different ingredients.

Table 6. In vitro permeability Parametes of piroxicam in aqueous saturated solution(control) and various ME formulations through excised rat skins(mean±SD, n=5)

Formulation	$J_{ss(\ mg/cm^2.h)}$	T_{lag}(h)	$D_{app(cm^2/h)}$	P(cm/h)	ER_{flux}	ER_D
Control	0.0057± 0.000007	8.217±2.8	0.000109±0.00005	0.0394±0.0005	-	-
ME-1	0.002±0.001	4.5±2.12	0.0001±0.0000008	0.0006±0.001	0.347±0.004	1.7915±0.2435
ME-2	0.002±0.001	4±0.001	0.00021±0.001	0.0008±0.001	0.348±0.004	2.1908±0.759
ME-3	0.0103±0.005	18.18±3.97	0.00003±0.000007	0.0034±0.001	1.797±1.03	0.3783±0.2060
ME-4	0.0039±0.001	8.7±3.537	0.00012±0.0001	0.0015±0.0007	0.681±0.299	1.1485±0.70
ME-5	0.0074±0.007	17.6±2.041	0.00054±0.0007	0.00246±0.002	1.295±1.34	7.0041±2.705

ME-6	0.004±0.0002	13.59±1.05	0.00006±0.000004	0.0015±0.00007	0.861±0.047	0.6370±0.26
ME-7	0.002±0.0002	1.385±1.85	0.005±0.0071	0.0010±0.0001	0.4525±0.05	42.57±5.27
ME-8	0.003±0.0007	3.69±0.438	0.00012±0.00002	0.0011±0.0002	0.5921±0.13	1.963±0.45

Among the ME formulations tested, ME 3, which was composed of 0.3% piroxicam, 20% oleic acid, 60% larasol-tween80(1:1, w/w mixture) /propylene glycol(4:1) and 30% water, showed the highest permeation profile. The Jss of piroxicam from ME 3 was 0.01±0.005 mg cm^{-2} h^{-1}, 1.797times higher than those of the piroxicam saturated solution in water, which were 0.0057± 0.0000007mgcm^{-2} h^{-1} . The higher permeability rate of piroxicam from ME formulations is most probably due to the S/C amount and the oily phase, which act as penetration enhancers[15]. The enhancer can increase the transport of drug through skin by changing the diffusion or partitioning coefficient of drug [16]. The surfactant mixture content in the formulation affected the permeation rate significantly. As the composition of surfactant/cosurfactant mixture was decreased from 65% to 50% at S/C = 4, the permeation rate of piroxicam decreased approximately by 5-folds. The surfactants used, ie, Tween 80, have been found to act as permeation enhancers in different skin models[17]. Disruption of lipid structure and fluidization are the main modes of action exerted by oleic acid .According to the results obtained from permeation studies , it was obvious that the maximum of P and Tlag parameters are belongs to ME 3 with 0.0034 cmh^{-1}, 18.18 h .The maximum of D_{app} parameter was obtained from ME 7 was 0.005±0.0071cm^2h^{-1} ,42.57 times higher than those of the piroxicam saturated solution in water, which were 0.000109± 0.00003cm^2 h^{-1} . Figure 5 has showed Permeation profiles of piroxicam through excised rat skins from ME 3 and control .

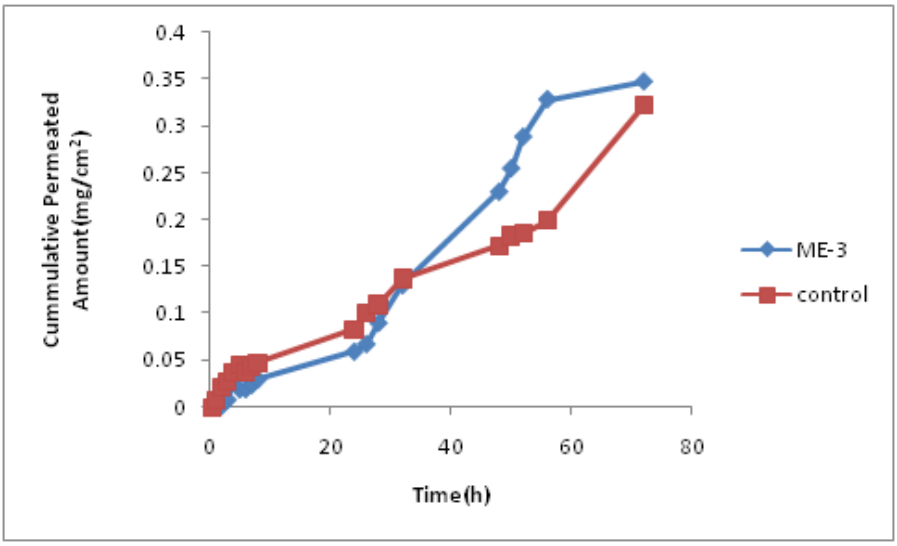

Figure 5. Permeation profile of piroxicam through rat skin from ME 3 formulation and control

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

jss = 0.0268 + 0.00154 %oil - 0.00273 %w + 0.0128 S/C (Equation 2)
D_{app} = 0.00056 - 0.000093 %oil + 0.000129 %w - 0.000595 S/C (Equation 3)
T_{lag} = 30.8 - 0.015 %oil - 1.11 %w + 2.04 S/C (Equation 4)
P = 0.00399 + 0.000003 %oil - 0.000136 %w + 0.000323 S/C (Equation 5)

Analysis of variance indicates that correlation between independent variables and Jss and D_{app} permeability parameters of piroxicam formulations are not significant (p>0.05) . On the other hand , analysis of variance shows significant correlation between independent variables and Tlag and P parameters of piroxicam

formulations($p<0.05$).On the basis of Eq4 and Eq5 , the correlation of between Tlag and P parameters are significant with water percent in ME formulations so that decrease in water percent is caused increase T_{lag} and P parameters. ER_{flux} for all the formulations was less than ER_D. This means that ME formulations affected on diffusion coefficient more than flux. The result indicates that ME formulations were increased.

Conclusion

In the recent study estabilished 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 region with a rise in S/C ratio. The release percent was enhanced with decrease in the oil percent. In permeation studies the correlation between Tlag and P with water percent was significant and indirectly. Jss of piroxicam of ME 3 and Dapp in ME7 were 1.8 times and 42.57 times higher than those of saturated water solution of piroxicam. In conclusion , the amount of components in ME formulation have an essential role in the physicochemical properties and piroxicam permeability through skin.

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