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PREPARATION AND CHARACTERIZATION OF NAPROXEN LOADED MICROEMULSION FORMULATIONS FOR DERMAL APPLICATION

Neslihan Üstündağ-Okur*, Mehmet Ali Ege, H. Yeşim Karasulu

Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Ege, 35100 Bornova, Izmir, Turkey

***Corresponding author e-mail:** neslihanustundag@yahoo.com

ABSTRACT

The aim of this study was to prepare new w/o microemulsions of naproxen (Np) for topical application and evaluate *in vitro* permeation of Np. The Pseudo-ternary phase diagrams were constructed for various microemulsions composed of isopropyl myristate (IPM) as oil phase, Span 80, Labrafil M, Labrasol, Cremophor EL as surfactants, ethanol as co-surfactant and water as aqueous phase. Finally, concentration of Np in all microemulsions was 10% (w/w). The physicochemical properties of microemulsions such as conductivity, droplet size, viscosity and pH were measured. The *in vitro* permeability of Np compared with commercial (C) and microemulsions. The permeation rates of Np from microemulsions were higher (1.2 times) than C gel formulation. Finally, according the histological examination of microemulsions did not show irritant effect on treated skin. In conclusion, the results of this study indicated that the microemulsions especially $M2_{Np}$ can be considered as potentially useful vehicles for topical application of Np.

KEYWORDS: histological evaluation, in vitro permeation, microemulsion, naproxen, topical application

INTRODUCTION

Microemulsions are homogeneous, transparent; thermodynamically stable dispersions optically isotropic liquid system of oil and water, stabilized by a suitable surfactant and cosurfactant [1-5]. Compared to conventional formulations microemulsions have some advantages such as improved drug solubility, simplicity of preparation, good thermodynamic stability and low viscosity, high drug loading ability, small droplet size and enhancing effect on transdermal capacity. There are numerous permeation enhancement mechanisms of microemulsions such as an increased concentration gradient and thermodynamic activity toward skin and permeation enhancement activity of the components of microemulsions [1,4,6-11]. Microemulsions provide another promising alternative for transdermal delivery of both hydrophilic and lipophilic drugs [5,11].
Naproxen (Np), (s)-6-methoxy-α-methyl-2-naphthalenenacetic acid (Figure 1), is a non-steroidal anti-inflammatory drug (NSAID) compound with analgesic and antipyretic effects, used for treatment of rheumatoid arthritis, osteoarthritis and traumatic conductions [12].
NSAIDs are most commonly used drugs to reduce inflammation and pain. Oral therapy of NSAIDs is very effective, but the clinical use is often inadequate because of their adverse effects such as irritation and ulceration of the gastro-intestinal mucosa. The eradication of these difficulties is possible by developing drug carriers so as to allow the dermal application of the drug [12-14].
The popularity of topical drug delivery systems is increasing, because of their advantages compared to more conventional treatments [15]. Topical drug delivery has many advantages over the oral route of administration: it avoids hepatic metabolism, the administration is easier and more convenient for the patient, and there is the possibility of immediate withdrawal of the treatment if necessary [16,17]. Compared to oral route, the dermal route of drug administration has the advantages of decreasing gastrointestinal side effects and drug degradation [18].

The goal of this study is to develop new Np loaded water in oil microemulsion formulations for dermal application and to evaluate physiologic characterization of microemulsions and *in vitro* permeation of Np. To confirm the dermal use of microemulsion formulations, the dorsal skins of rats treated with microemulsion were histologically examined and the findings compared to that of control rats treated with either the C gel formulation or serum physiologic (SP).

MATERIALS AND METHODS

Materials: Isopropyl myristate (IPM) and Cremophor-EL ((Polyoxyethylenglyceroltriricinoleat 35) were purchased from Sigma, Germany. Span 80 (Sorbitan monooleate) and ethanol were obtained from Merck, Germany. Labrafil-M and Labrasol (Glyceryl caprylate/caprateand PEG-8 caprylate/caprate) were gift from Gattefosse, France. Np was a gift from Deva Holding, Turkey. The cellulose membrane (molecular weight cut-off=12,000–14,000) was provided by Sartorius, Germany. All chemicals were used as analytical grade.

Microemulsion preparation: Pseudo-ternary phase diagrams were constructed to find out the existence range of microemulsions, by titration of a series of oil and surfactant/co-surfactant (S/Cos) mixtures with bidistilled water at 25 ± 2 ^{o}C. The boundaries of the microemulsion domains were determined for different values of the S/Cos (w/w) ratios. S/Cos weight ratios were varied as 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1 and 9:1. The oily mixtures were visually assessed and determined as microemulsions. Based on these pseudo-ternary phase diagrams, appropriate concentrations of oil, surfactants and cosurfactant were selected. All experiments replicated at least three times.

Microemulsions were prepared using IPM as oil phase, Span 80, Labrafil-M, Labrasol and Cremophor EL as surfactants, ethanol as co-surfactant and bidistilled water as aqueous phase. All microemulsion formulations were prepared in the same way.

After the microemulsion regions in the phase diagram were selected, two typical microemulsion vehicles (M1 and M2) were selected and prepared at different component ratios. Np was slowly incorporated into the microemulsion after microemulsion was prepared. After Np was entirely dissolved in the miroemulsion, the clear microemulsion-based formulation was obtained. The final concentration of Np in microemulsion systems ($M1_{Np}$ and $M2_{Np}$) was 10% (w/w) (100 mg/g) (Table 1). No phase change was noted after addition of Np.

Characterization of the microemulsion formulations: The microemulsions in the presence or absence of Np were analyzed for various physicochemical attributes. The viscosities of microemulsion formulations in the presence or absence of Np were measured at 25±2 ^{o}C using a viscosimeter (ULA spindle, Brookfield, USA). The pH of the microemulsion formulations in the presence or absence of Np were detected at 25±2 ^{o}C using a digital pH-meter (HI 221 – Mauritius). The refractive indexes of microemulsions in the presence or absence of Np were evaluated at 25±2 ^{o}C using a refractometer (Atago RX-7000 CX- Japan). The average droplet size and polydispersity index (PDI) of microemulsion formulations in the presence or absence of Np were studied by photon correlation spectroscopy (Nano ZS, Malvern Instruments, U.K.). The dynamic light scattering (DLS) experiments were performed on a Zetasizer Nano ZS (Malvern Instrument Laboratory, Malvern, U.K.) instrument employing a He–Ne laser operated at 4mW (λo = 633 nm), and a digital correlator.

Electrical conductivity of the microemulsions in the presence or absences of Np were studied at 25 ± 2 ^{o}C using a conductometer (Jenway 4071 – U.K.). Based on electrical conductivity, the phase systems of the microemulsions in the presence or absence of Np were determined. The experiments were carried out five times for each sample, and the results are presented as an average ± SD.

Transmission electron microscopy (TEM): The shape and surface morphology of microemulsion were examined by the transmission electron microscopy (TEM, FEI Tecnai G2 120kV, Netherlands). For observation by TEM, a drop of microemulsion formulation in the presence or absence of Np ($M1_{Np}$, $M2_{Np}$) (1 mL) was placed on 300 mesh copper electron microscopy grids and allowed to stand for 10 min after which any excess fluid was absorbed in a filter paper. Then the sample was quickly frozen at liquid nitrogen, followed by freeze-dried at - 55 °C. Before examination, one drop of 1% osmium tetrachloride was applied for fixation and allowed to dry for 5 min. Then the sample was quickly frozen at liquid nitrogen, followed by freeze-dried at - 55 °C again [19].

Stability of the microemulsion formulations: In the stability studies, the microemulsions in the presence or absence of Np were stored at 5±2, 25±2 and 40±2 °C for 12 months. After storage for 12 months the clarity, phase separation, concentration of Np were

investigated and the microemulsion formulations were also evaluated for changes in particle size, electrical conductivity, viscosity, pH and refractive index. The centrifuge tests were carried out at 12000 rpm at 25±2°C for 30 min to assess the physical stability of microemulsions [20]. In order to evaluate the stability during the temperature changes, formulations were frozen at −20±2°C for 30 min and thawed at 40±2°C for 15 min. after repeating this cycle for 4 times; the appearance of the formulations was examined. In the chemical stability tests, concentration of Np in the formulations was analyzed as spectrophotometrically at 5±1°C, 25±2°C and 40±2°C for up to 12 months.

***In vitro* permeation studies:** Diffusion cell was used to study the permeability of Np. The apparatus consisted of clamped preconditioned cellulose membrane (Sarttorius, Germany) onto glass diffusion cell between donor and receptor compartments. The cellulose membrane was first hydrated in a buffer solution at 20±1°C for 12h. Phosphate buffer pH 7.4 (10 mL, 600 rpm) was used in the receptor compartment. Temperature was maintained at 37±1 °C with the help of a circulating water bath. The donor compartment contained 500 mg microemulsion formulations (containing 50 mg Np) or C gel formulation (one gram gel contained 100 mg Np). Samples were filtered through a membrane filter (0.2 µm Nylon, Milipore Millex-GN) and the aliquots (1 mL) withdrawn at various intervals were immediately analyzed for drug concentration as spectrophotometrically (263 nm). Calibration and assay of Np was carried out using UV spectrophotometer at 263 nm. Each experiment replicated independently 5 times. Sink conditions were maintained in the receptor compartment during in vitro permeation studies.

Permeation data analysis: Average values of in vitro permeation data were calculated and cumulative amount of drug (mg cm^{-2}) permeated per unit surface area of the membrane was plotted versus time. The slope of the linear portion of the plot was calculated as flux (Jss) [21,22] and the permeability coefficient was calculated using Equation 1:

$$Kp = \frac{Jss}{Cv}$$

Where Kp is permeability coefficient and Cv is total amount of the drug.

Kinetic evaluation and determination of release mechanism: Kinetic evaluation of Np release from microemulsions and C gel formulation were estimated using a computer based kinetic program [23]. Zero-order, First-order and Higuchi kinetic models were used for evaluation and determination of the release mechanism [24,25].

Determination of Np release from microemulsions and C gel formulation were estimated [26] Equation 2:

$M_t / M_\infty = Kt^n$

M_t/M_∞; the fraction of drug released, t; released time, k; release rate constant and n is the release exponent. The n value is used to characterize different release mechanisms as given in Table 1 [27].

Histological studies of microemulsions: The experimental protocol was approved by the Local Animal Ethical Committee of Ege University, Faculty of Pharmacy (Approval No. 2008/3-1). Male wistar albino rats weighting 250±10 g were purchased from Experimental Animal Center of Ege University (Izmir, Turkey) for the histology studies. The dorsal part of the rat skin was carefully shaved. Rats were housed in a room maintained at 22 ± 1°C with an alternating 12 hour light-dark cycle. Animals had free access to pellet diet and water ad libitum. Microemulsion formulations, serum physiologic (SP) (Negative control) and C gel formulation (Positive control) were dermally applied on the dorsal skin of male wistar albino rat for 24 hours. 500 mg microemulsions, SP or C gel were applied for 24 hours on the excised skin. Rats were sacrificed by carbon dioxide gas 1 day later. The formulations were removed; dorsal side of rat skin were dissected and further processed for light microscopy. Each specimen was fixed in a 10 % formaline solution for approximately 24 h; then washed with tap water, dehydrated through an increasing ethanol series, immersed in xylene and were finally embedded in paraffine wax at 56°C. Paraffine blocks were cut serially 5µm using a rotary microtome (RM 2145, Leica Co., Nussloch Germany). Sections were stained with Hematoxylene & Eosine and examined by light microscope (Olympus BX-51, Japan).

Statistical data analysis: All permeation values were calculated for three independent experiments, and data are expressed as the mean value ± S.D (Standard Deviation). The statistical analysis were performed on the collected data , One way analysis of variance (ANOVA) was used to test the difference between the means of microemulsion formulations and C gel formulation permeation experiments. The results with $P < 0.05$ are considered statistically significant.

RESULTS AND DISCUSSION

Microemulsion preparation: The production of pseudo-ternary phase diagrams was used to determine the concentration range of components in

the presence range of microemulsion. The pseudo-ternary phase diagrams with various weight ratios of IPM, Labrafil M, Labrasol, Span 80, Cremophor EL, ethanol and distilled water were described in Figure 2. Microemulsions areas obtained using various S/Cos ratios.

Various microemulsion formulations (M1 and M2) were selected from gravity midpoint of the phase diagrams. Based on these diagrams, different microemulsions within the center of gravity region were prepared to evaluate the effects of microemulsion structure with various surfactants on the permeation skill. Compositions of the microemulsion formulations ($M1_{Np}$ and $M2_{Np}$) were given at Table 2.

Characterization of the microemulsion formulations: The physicochemical characterizations of microemulsions in the presence or absence of Np were measured and reported in Table 3. Incorporating the co-surfactant into the microemulsion resulted in a significant reduction in the viscosity of the formulations, with the flow changing to a simple Newtonian flow. Therefore, the viscosities of $M1_{NP}$ and $M2_{NP}$ were found to be 14.2±0.05 cP and 15.1±0.02 cP, respectively. The results indicated that the dynamic viscosity of the formulations has very low range. The average pH value of microemulsions ranged from 4.83±0.01 to 5.92±0.01. The incorporation of Np into M1 and M2 increased the pH values (Table 3). The pH of the microemulsion formulations was appropriate for topical delivery of Np. The average refractive index of microemulsions ranged from 1.409±0.001 to 1.415±0.001 (Table 3).

There was a robust relationship between the specific structure of the microemulsion systems and their electrical conductive behaviour [9]. The phase systems (o/w or w/o) of the microemulsions were determined by measuring the conductivity (Jenway 4071 – U.K.) of the microemulsions [6]. The results of electrical conductivimetry analysis showed that microemulsion was in the form of water-in-oil phase system, which could be regarded as appropriate for topical applications.

The droplet sizes of the microemulsion formulations were examined by photon correlation spectroscopy. In the absence of Np, the average droplet size of M1 and M2 was 1.813±0.050 nm and 1.809±0.030 nm, respectively. However, in the presence of Np, the average droplet sizes of $M1_{Np}$ and $M2_{Np}$ were 1.590±0.114 nm and 1.701±0.060 nm, respectively. In addition, the incorporation of Np into M1 and M2 resulted in a 0.3 to 0.1 nm decrease in the average droplet size (Table 3). These findings support a recent study that found the mean droplet size was decreased after loading the drug [28,29]. The polydispersity values demonstrated the homogeneity in the droplet size. All polydispersity values were smaller than 0.5. Therefore, these results indicate that the droplet size had high homogeneity.

Transmission electron microscopy (TEM): The shape and morphology of microemulsions by TEM analysis was shown in Figure 3. It showed the spherical shape and uniform droplet size of microemulsion. TEM image of $M1_{Np}$ and $M2_{Np}$ (Figure 3A and 3B) showed that the diameters of most particles were below 10 nm with narrow distribution.

Stability of the microemulsion formulations: The stability tests of all microemulsion formulations were completed at 5±1^{o}C, 25±2^{o}C and 40±2^{o}C for 12 months. The changes were not observed during 12 months in particle size, electrical conductivity, viscosity, pH, refractive index, phase separation and degradation of Np loaded microemulsions. The microemulsion vehicles were isotropic, transparent dispersions, and after centrifugation, no phase separation was observed. The centrifuge tests showed that all microemulsions had good physical stability. The formulations also remained stable after the temperature test, which indicated that their stability under different temperature conditions. Microemulsions which were kept at 5±1^{o}C, 25±2^{o}C and 40±2^{o}C showed no change in clarity and phase behaviour. The concentrations of Np in microemulsions were almost constant and no degradation was detected. The hydrolysis of Np in microemulsions was not determined during 12 months. The results showed that microemulsion formulations had good shelf stability and so microemulsions should be kept at 5±1 ^{o}C, 25±2 ^{o}C and 40±2 ^{o}C for 12 months.

***In vitro* permeation studies:** The cumulative amounts of Np through cellulose membrane from the microemulsions were evaluated and it was also found that the Np permeation rate values from the microemulsion formulations significantly higher than C formulation from cellulose membrane ($P<0.05$). The permeation parameters of microemulsions ($M1_{Np}$ and $M2_{Np}$) and C formulation were presented in Table 4. No significant difference was found between the releases rates of NP of microemulsion formulation that of C formulation ($P>0.05$). Statistical comparison of the flux throughout cellulose membrane for 8 hour showed that the microemulsions provided fluxes higher than the commercial formulation. In Figure 4, the rank order for in vitro release of Np from the three

formulations was found to be: $M2_{Np}$ > $M1_{Np}$ > C. $M2_{Np}$ microemulsion formulation with the highest permeation rate (1.207±0.022g/cm2/h) involved of IPM (2.135 g), Span 80 (0.570 g), Cremophor EL (0.228 g), Ethanol (5.586 g), bidistilled water (0.481 g) and Np (1 g). In a similar our previous study, when the microemulsion formulation contains Span 80 as a surfactant, the in vitro release of Np was determined highest permeation rate [30]. The microemulsions were able to reduce the interface tension between vehicle and skin because of their contact to the skin lipids, which resulted in faster permeation [31]. Moreover, the surfactant and cosurfactant in the microemulsions may reduce the diffusional barrier of the stratum corneum by acting as permeation enhancers [6]. In the present study, microemulsion formulations consisted of IPM as oil phase. IPM is an effective penetration enhancer that enables an extensive permeation [31-33]. Ethanol was shown to extract stratum corneum lipids and to disrupt barrier purpose, which improved the permeation of more hydrophilic drugs through the skin [6].

Kinetic evaluation: The mathematical parameters of microemulsion formulations and commercial formulation were presented in Table 5 and Table 6. The diffusional exponents (n) of Peppas equation are very low indicating that the drug release mechanism is non-Fickian diffusion (Table 6). This result is very normal for dissolutions of gels (C) and emulsions ($M1_{Np}$ – $M2_{Np}$) type dosage forms. In addition, first order kinetic is dominant for hydrophilic gels (C) and Higuchi for microemulsions. These kinetic results fit the results of Peppas analyses. Because above mentioned kinetic models related non-Fickian diffusion.

The model that gave higher 'r' value was considered as best fit model. The release of Np (in the $M1_{Np}$ formulation) across cellulose membrane followed Higuchi kinetics as its correlation coefficient (*r*=0.951) predominated over zero-order kinetics (*r*=0.923). This may indicate that the diffusion of Np in microemulsion matrix retards in the late stage of its dermatopharmacokinetics. This is consistent with Wang et al., who have suggested that the topical formulations could be followed Higuchi kinetics [34]. The release of Np (in the $M2_{Np}$ formulation) across cellulose membrane followed Zero Order kinetics as its correlation coefficient (*r*=0.981) predominated over Higuchi kinetics (*r*=0.979). This finding was in accordance with other reported works [33,35].

The diffusion mechanism of drug release was further confirmed by Peppas plots that showed fair linearity (r^2 values between 0.898 and 0.997), with slope values (n) less than 0.5, indicating that drug release mechanism of the formulations was diffusion controlled (Table 6).

Microemulsion histological evaluation: To understand the effect of microemulsion formulations on the skin irritation, the skin was pathologically investigated after use of formulations. The histology of excised dorsal rat skin in control and treated with microemulsions and C formulation after 24 hours is shown in Figure 5. In histological evaluation, epidermal liquefaction, dermal oedema was considered as the key criteria to distinguish the effect of Np. The microscopic observations indicate that the microemulsions have no severe effect on the microscopic structure of the skin. The surface epithelium lining and the granular cellular structure of the skin were totally intact. No main changes in the ultra-structure of skin morphology could be seen and the epithelial cells appeared mostly unchanged. Visible irritation was not observed after the application of SP (Group 3) (Figure 5D). No damage in the epidermal layers and no inflammation in the dermal layers were found in the skins applied with SP (negative control).

It was observed that there was no apparent change in skin morphology after the application of SF and $M2_{Np}$ (Figure 5D and 5B). In terms of epidermal disruption, when compared to the control (SF), C and $M1_{NP}$ formulations displayed low levels of disruption on the stratum corneum. Stratum corneum of the treated skin remained intact when applied $M2_{Np}$. In Group 2; the stratum corneum layer became thinner and subjacent layers remained after the application of the C formulation (Figure 5C). Regarding of dermal inflammation and oedema, compared to the SF group, the group treated with C displayed low grade of inflammation and oedema, at the same time moderate grade of inflammation and oedema obtained with $M1_{NP}$. No inflammation and oedema were observed in the group treated with $M2_{NP}$ (Table7).

IPM is widely used in cosmetics and topical formulations and is generally regarded as nontoxic. The irritation studies did not show visible irritation after application of $M1_{Np}$ - $M2_{Np.}$ Thus the developed microemulsions were considered to be safe for the use of dermal drug delivery. Hence this microemulsion system for the transdermal delivery of Np is viable. These histological findings were consistent with previous reports that compared to the control, stratum corneum of the treated skin remained intact [22,36].

CONCLUSIONS

Novel microemulsions containing Np were prepared with the aim of achieving maximum release through

the skin thus eliminating its gastrointestinal adverse effects. For the formulation of microemulsions containing Np, the proper components and their optimum concentration ranges were obtained by using pseudo-ternary phase diagrams. Thus a microemulsion was successfully prepared using IPM oil phase, Span 80, Labrafil-M, Labrasol and Cremophor EL as surfactants, ethanol as cosurfactant and water. The fluxes of microemulsions were better than C gel formulation in the in vitro release studies. In this study the findings revealed that microemulsion formulations which have been used as dermal delivery carriers for anti-inflammatory drugs among which $M2_{NP}$ is more suitable microemulsion formulation which has better permeation effect than C formulation. The release of NP fits well to the zero order and Higuchi model. Furthermore, irritation and histological studies clearly illustrated that the microemulsion formulations are safe for the skin. Therefore the microemulsion of NP was prepared to obtain improved patient compliance. The developed system displayed greater penetration, also enables the decrease in the number of applications of gels per day. The Np loaded microemulsion is advantageous for dermal use because it is well-tolerated in the skin and seemed to provide a higher degree of bioavailability. Finally, according to these results of the characterization, *in vitro* permeation and histology studies, microemulsion formulations were found to be appropriate vehicle for the dermal application of Np.

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AUTHOR DISCLOSURE STATEMENT

The authors reveal there is no conflict of interest regarding this manuscript.

Table 1: Diffusion exponent and solute release mechanism for cylindrical shape

Diffusion exponent (n)	Diffusion mechanism
0.45	Fickian diffusion
0.45 < n < 0.89	Anomalous (non-Fickian) diffusion
0.89	Case-II transport
n > 0.89	Super case-II transport

Table 2: Compositions and HLB values of the microemulsion formulations

Formulations	**M1 (w/o)**		**M2 (w/o)**	
	Formulation Components		**Formulation Components**	
HLB value	5.66		6.64	
	M1(%)(w/w)	**$M1_{Np}$ (g)**	**M2(%) (w/w)**	**$M2_{Np}$ (g)**
IPM	26.02	2.342	23.72	2.135
Labrafil-M	7.39	0.665	-	-
Labrasol	1.47	0.132	-	-
Span 80	-	-	6.33	0.570
Cremophor EL	-	-	2.53	0.228
Ethanol	62.09	5.588	62.07	5.586
Distilled water	3.03	0.273	5.34	0.481
Naproxen	-	1	-	1

Final concentration of Np in all microemulsion is 10 % (w/w).

Table 3: Pysicochemical characterizations of microemulsion formulations in the presence or absence of Np at 25 ^{o}C. Values are means of five determinations ± SD.

Codes	pH	Conductivity (µs/cm)	Viscosity (cP)	Droplet Size (nm)	Polydispersity index	Refractive index
M1	5.12 ± 0.02	21.1 ± 0.02	14.2 ± 0.05	1.813 ± 0.050	0275 ± 0.030	1.410 ± 0.001
M2	4.83 ± 0.01	24.8 ± 0.03	15.1 ± 0.02	1.809 ± 0.030	0.360 ± 0.030	1.409 ± 0.001
M1Np	5.92 ± 0.01	21.6 ± 0.03	14.2 ± 0.05	1.590 ± 0.114	0.396 ± 0.040	1.415 ± 0.001
M2Np	5.33 ± 0.02	25.9 ± 0.04	15.1 ± 0.02	1.701 ± 0.060	0.369 ± 0.029	1.411 ± 0.002

Table 4: The permeation parameters of the Np loaded microemulsions and commercial formulation. Values are means of five determinations ± SD.

Formulations	(mg/cm^2)	Jss ($mg/cm^2/h$)	D*10^{-7} (cm^2/h)
$M1_{Np}$	11.560±0.031	1.140±0.003	1.66±0.020
$M2_{Np}$	11.894±0.141	1.207±0.022	1.94±0.035
C	9.920±0.165	0.992±0.016	2.47±0.353

Table 5: The mathematical parameters of the Np loaded microemulsions and C formulation with in vitro permeation study

Kinetic Model	C		$M1_{Np}$		$M2_{Np}$	
	r2	K	r2	K	r2	K
First Order	0.9951	4.9256	0.9151	405.140	0.9330	161.4023
Higuchi	0.9894	22.271	0.9514	63.826	0.9790	25.9421
Zero Order	0.9483	11,395.94	0.9237	22,796.10	0.9819	12, 916.75

Table 6: Peppas values (n) of the Np loaded microemulsions and C formulation with in vitro permeation study

	C	$M1_{Np}$	$M2_{Np}$
r2	0.9979	0.8987	0.9734
n	0.4181	0.4067	0.3305
Log k	1.5848	1.7245	1.7076

Table 7: Epidermal disruption and Dermal inflammation and oedema degrees layers of the skin (-----: no effect; +: Weak; ++: Moderate; +++: Severe).

GROUPS		Disruption Degrees of Epidermal Layers					Inflammation and Oedema Degrees of Dermal Layers	
		Stratum corneum	Stratum lucidum	Stratum granulosum	Stratum spinosum	Stratum basale	Stratum papillare	Stratum reticulare
	$M1_{Np}$	+	-----	-----	-----	-----	++	++
Group I	**$M2_{Np}$**	-----	-----	-----	-----	-----	-----	-----
Group II	**C**	+	-----	-----	-----	-----	+	+
Group III	**SP**	-----	-----	-----	-----	-----	-----	-----

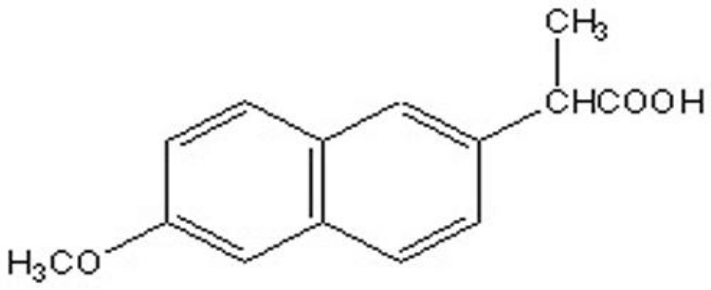
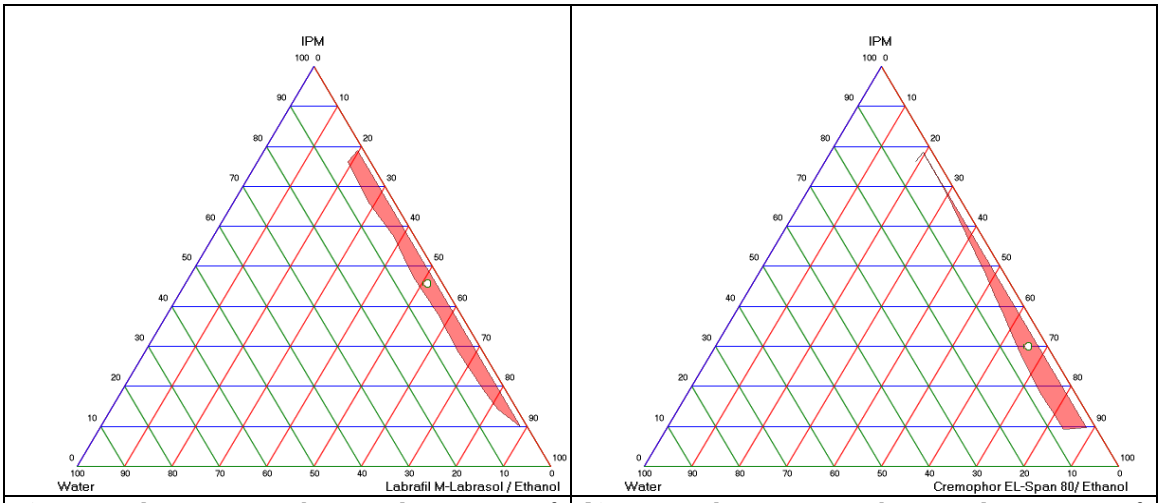


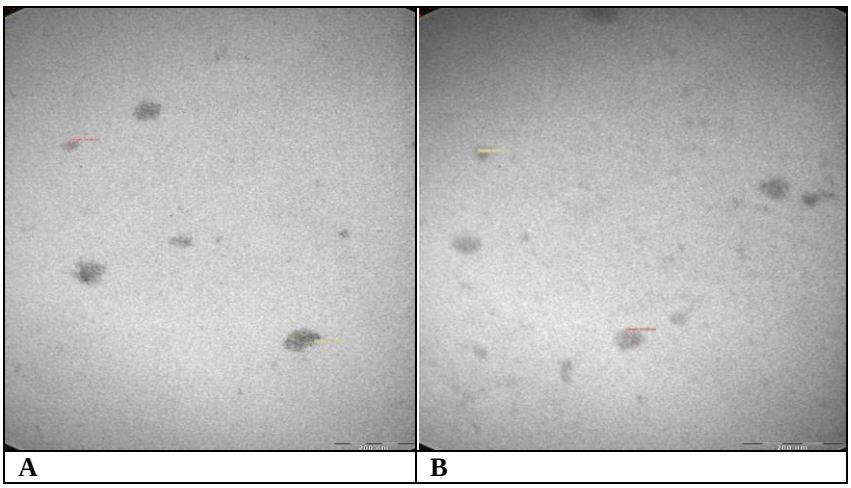
Figure 1: Chemical structure of naproxen



a) Pseudo-ternary phase diagrams of microemulsion composed of Labrafil M - Labrasol / Ethanol ratio 1:7 and distilled water

b) Pseudo-ternary phase diagrams of microemulsion composed of Cremophor EL - Span 80 / Ethanol ratio 1:7 and distilled water

Figure 2: Pseudo-ternary phase diagrams showing the w/o microemulsion regions at different surfactants



A **B**

Figure 3: Transmission electron microphotographs of microemulsion ($M1_{Np}$) (**A**) and ($M2_{Np}$) (**B**). Scale bar for image represents 200 nm; magnification × 87,000.

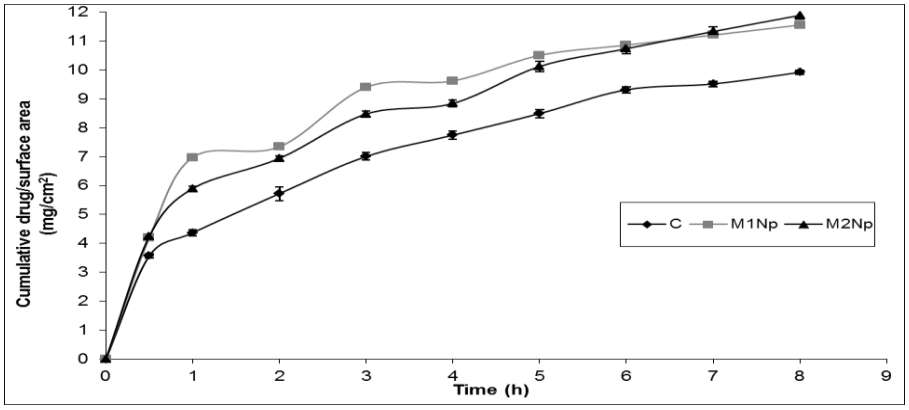


Figure 4: Permeation profiles of Np through cellulose membrane from microemulsion formulations and commercial formulation. Each point shows the average of five determinations, bars represents the ± SD. Standard deviations have been shown but in some cases they are too small to see clearly.

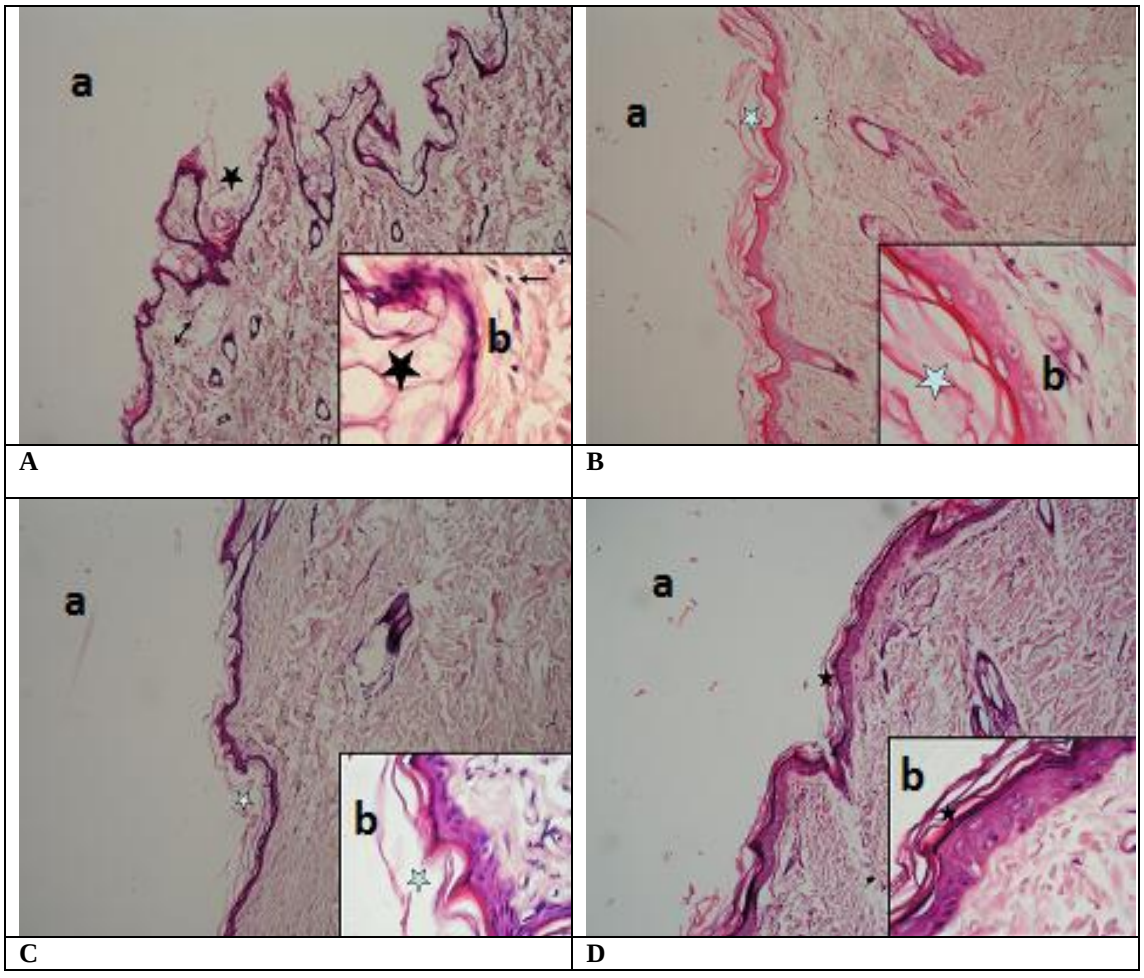


Figure 5: Microscopic images of treated rat skin with $M1_{Np}$ microemulsion (a), with $M2_{Np}$ microemulsion (b), with C (c) and with SP (d). a: 10 x (magnification value), b: 100 x, H&E (Hematoxylene and Eosin). Values are means of seven experiments ± SD.

☆ , stratum corneum of epidermis in the rat skin

⟶ , cells increased in dermis of the skin.

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