Comparison of Different Water/Oil Microemulsions Containing Diclofenac Sodium: Preparation, Characterization, Release Rate, and Skin Irritation Studies

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ABSTRACT

The aim of the present study was to make a comparison of the in vitro release rate of diclofenac sodium (DS) from microemulsion (M) vehicles containing soybean oil, nonionic surfactants (Brij 58 and Span 80), and different alcohols (ethanol [E], isopropyl alcohol [I], and propanol [P]) as cosurfactant. The optimum surfactant:cosurfactant (S:CoS) weight ratios and microemulsion areas were detected by the aid of phase diagrams. Three microemulsion formulations were selected, and their physicochemical properties were examined for the pH, viscosity, and conductivity. According to the release rate of DS, M prepared with P showed the significantly highest flux value (0.059 ± 0.018 mg/cm^2/h) among all formulations ($P < .05$). The conductivity results showed that DS-loaded microemulsions have higher conductivity values (18.8-20.2 microsiemens/cm) than unloaded formulations (16.9-17.9 microsiemens/cm), and loading DS into the formulation had no negative effect on system stability. Moreover, viscosity measurements were examined as a function of shear rate, and Newtonian fluid characterization was observed for each microemulsion system. All formulations had appropriate observed pH values varying from 6.70 to 6.85 for topical application. A skin irritation study was performed with microemulsions on human volunteers, and no visible reaction was observed with any of the formulations. In conclusion, M prepared with P may be a more appropriate formulation than the other 2 formulations studied as drug carrier for topical application.

KEYWORDS: Diclofenac sodium, microemulsion, release rate, regression model.

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INTRODUCTION

Microemulsions are clear, stable, isotropic mixtures of oil, water, and surfactant (S), frequently in combination with a cosurfactant (CoS).[1-3] Up to date microemulsions have been shown to be able to protect labile drug, control drug release, increase drug solubility, and reduce patient variability. Furthermore, it has proven possible to formulate preparations suitable for most routes of administration.[3,4] There are many studies on microemulsions as topical drug delivery vehicles, and it has been shown that microemulsion formulations have improved transdermal and dermal delivery properties.[5-16] In this study, non-ionic surfactants (Brij 58 and Span 80), which are also compatible with the other 3 classes of surfactants and retain this utility over a broad range of pH values, were used in preference, because they have minimal toxicity.[3,4] As the study aimed to examine the in vitro release of microemulsion (M) formulations, diclofenac sodium (DS) was selected as a model drug. DS is a nonsteroidal anti-inflammatory drug and is widely used clinically because of its strong analgesic, antipyretic, and anti-inflammatory effect.[17,18]

The aim of this study is to develop and compare appropriate microemulsion formulations as drug carriers for topical application and to demonstrate the release features of the formulations with a regression model. In addition, the physicochemical properties of selected microemulsions for the pH, viscosity, conductivity, droplet size distribution, and skin irritation were examined.

MATERIALS AND METHODS

Materials

Soybean oil and cellulose membrane were obtained from Sigma Chemical Co (St Louis, MO), and DS was kindly provided by Novartis Co (Istanbul, Turkey). Brij 58, Span 80, ethanol, isopropyl alcohol, and 1-propanol were purchased from Merck Co (Hohenbrunn, Germany). All chemicals used were of analytical grade.

Preparation of Water/Oil Microemulsions

Soybean oil was used as the oil phase, and Brij 58 and Span 80 were used as surfactants. Absolute ethanol, isopropyl

alcohol, and propanol were used as cosurfactants. To investigate the M formation regions, phase diagrams were constructed by titration of a series of S/CoS mixtures with distilled water at 25°C. The boundaries of the M domains were determined for different values of the S/CoS (wt:wt) ratios. S/CoS weight ratios were 1:1, 2:1, 3:1, 4:1, 5:1, and 6:1; and Span 80:Brij 58 weight ratio was 9:1 with hydrophilic-lipophilic balance (HLB) value of 5.44.

For the M formulation, surfactants were mixed and melted at 60°C, then added to the appropriate amount of soybean oil. CoS was added into this mixture, and the formulation was performed by titrating slowly with distilled water, while stirring mixture with a stirring bar using a magnetic stirrer (IKA Labortechnik, Staufen, Germany) (9 rpm) until turbidity was observed. Ideal S/CoS weight ratios and microemulsion areas were detected with the aid of phase diagrams drawn using a computer program developed in the Computer Center, Faculty of Pharmacy, University of Ege.[19] Three M formulations were selected using the gravity center of the microemulsion formation area.[20] DS was added into the microemulsions by stirring at the last stage. All formulations contain 1% (wt/wt) DS.

Selection of M Formulations for Further Studies

For further studies, 3 M formulations were selected based on pseudoternary phase diagrams. Microemulsions mentioned in Table 1 were chosen from the gravity center of the M formation area for studies such as in vitro release, conductivity, physicochemical property tests, and skin irritation.

Droplet Size Determinations

The particle size distribution and average droplet size of microemulsions were determined using Zeta Sizer 3000 HS_A (Malvern HPPS, UK).

In Vitro Drug Release Studies

Release studies were performed using vertical passive diffusion cells (area 6.15 cm^2), with a cellulose membrane. The cellulose (molecular weight <12 000) membrane was first hydrated in the buffer solution at 20°C for 24 hours. The membrane was then clamped between the donor and receptor compartments of the cells. The receptor solution was 20 mL of phosphate buffer pH 7.4, and it was maintained at 37°C ± 0.5°C using a thermostatic water bath (Variomag, Germany) and was magnetically stirred at 600 rpm throughout the experiment. The donor compartment contained 1 g sample.

The aliquots (0.7 mL) withdrawn at specified intervals from receptor compartment were then replaced by a fresh receptor solution. The samples were immediately analyzed directly for drug concentration spectrophotometrically at 277 nm. Three replicates of each experiment were performed.[21]

Table 1. The Contents of the Microemulsion Formulations Without Diclofenac Sodium*

Formulations	Oil (%)	Water (%)	Brij 58 (%)	Span80 (%)	Cosurfactant (%)
ME	34.6	4.5	5.2	46.9	8.7
MI	32.5	6.3	5.1	45.9	10.2
MP	27.2	10	5.2	47.1	10.5

* ME indicates microemulsion prepared with ethanol; MI, microemulsion prepared with isopropyl alcohol; and MP, microemulsion prepared with propanol.

Regression Model

For each microemulsion formulation, a curve estimation was performed, and changes of release rate against time were examined using least square linear regression analysis. Formulations were used in the regression model as dummy variables.[22]

Conductivity Measurements

The effect of the amount of water phase of microemulsions was monitored quantitatively by measuring the electrical conductivity. The water phase was added drop by drop into the mixture of oil phase (soybean oil-S-CoS), and after adding a drop of water, the electrical conductivity was measured using a conductometer WPA CM 35 (Cambridge, UK), at 20°C ± 0.2°C. Furthermore, the conductivity of all M formulations with and without DS was measured.

Viscosity Measurements

Viscosity measurements were performed at 25.0°C ± 0.1°C using a Brookfield digital viscometer-III rheometer V 3.3 HB (Middleboro, MA) (Spindle: SC4-21) at 200 rpm.

pH Measurements

The observed pH values of the samples were measured by a pH meter (Jenway 3040 Ion Analyze, Combined Glass Electrode, Mettler-Toledo, Greifensee, Switzerland), at 20°C ± 2°C.

Skin Irritation Study

Formulations were tested on the inner arms of 9 human volunteers for 8 hours. M samples (0.1 g) with or without DS were placed on a gauze dressing (1×1 cm^2) and then applied directly onto the skin of inner arms, fixed with stretch adhesive tape. There was at least 1-cm distance between

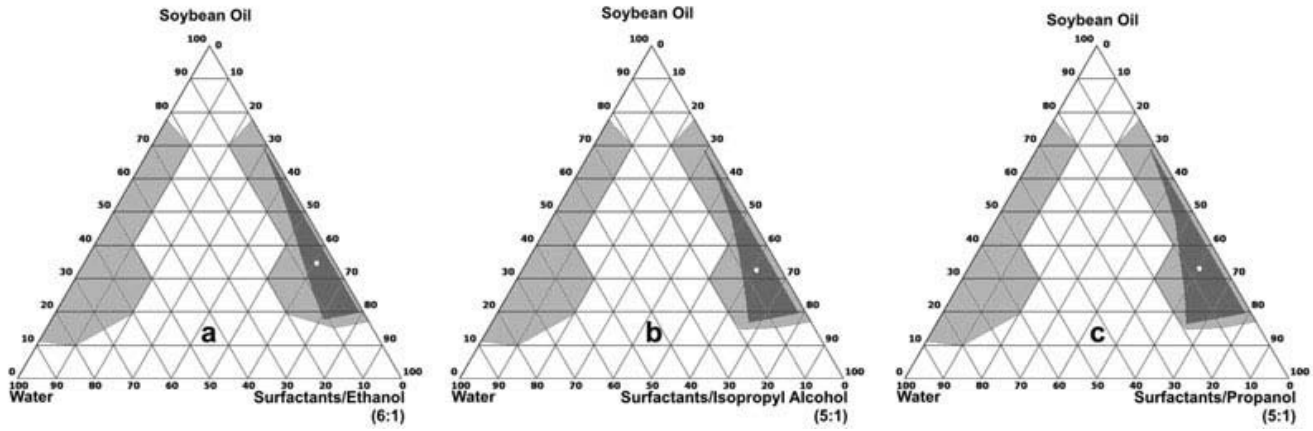

Figure 1. Pseudo-ternary phase diagrams of microemulsion systems: The dark gray areas belong to the transparent water/oil microemulsion systems and the light gray areas show the turbid states of the investigated systems.

application areas on each arm. After 8 hours, erythema was measured with Mexameter (n:8) (Courage+Khazaka Inst, Germany).[23] For control, the skin of inner arms was also measured before application of the formulations.

The results of skin irritation study were evaluated using repeated measures analysis of variance (ANOVA), with α: 0.05 ($P < .05$) taken as the level of significance.

Statistical Analysis

The differences in the results of in vitro release studies were evaluated using 1-way ANOVA followed by post hoc analysis (Duncan) for significance at $P < .05$.

Results and Discussion

The phase diagrams of the 3 selected systems are presented in Figure 1. It can be seen that the water/oil (w/o) M area of microemulsion prepared with P (MP) was slightly larger than the areas of M prepared with E (ME) and M prepared with I (MI). As shown in Table 1, MP can incorporate more water content into the formulation than MI or ME. This feature can be an advantage for loading water soluble drugs into the M.

The mean droplet diameters of microemulsions are shown on Table 2. The mean droplet size of M-loaded DS decreased slightly compared with the mean droplet size of M without the drug. Currently, it is not clear by which mechanism the droplet size was decreased. However, the 2 possible solutions are (1) a certain portion of the undissolved drug could have acted as an emulsifying agent by depositing drug particles at the interface of the M; or (2) by the deposition of the drug at the interface of the M, reduced motility of the S was thought to decrease the particle size of drug-loaded microemulsions as previously demonstrated.[2]

Figure 2 shows the release profiles of microemulsions of DS from the 3 M formulations. The cumulative amount of DS that had permeated through the cellulose membrane ($\mu g/cm^2$) was plotted as a function of time (hours) (Figure 2). It is possible to calculate the steady-state flux (J) obtained from the slope of linear portion (2-8 hours) of the graph.[24] In an earlier study, the release of diclofenac from gel dosage form through the synthetic membrane was examined, when plotting the penetration rate vs time, linear relationship was obtained.[25] The values of flux of DS through cellulose membrane from M formulations were shown in Table 3. When all formulations were evaluated according to the release rate of DS, MP showed the significantly highest flux value (0.059 $mg/cm^2/h$) among all formulations ($P < .05$). In a prior study performed with diclofenac diethylamine and cellulose membrane, it was observed that there is a strong correlation between the water phase concentration and the flux value of the drug. The maximum flux value of diclofenac diethylamine was obtained from w/o microemulsion containing the highest percentage of water.[15] In this study, MP has the highest water content and highest flux value of DS among

Table 2. The Particle Size Distributions of Water/Oil Microemulsions With/Without Diclofenac Sodium*

Formulations	ME	ME+DS	MI	MI+DS	MP	MP+DS
Mean diameter (nm)	13.3 ± 0.3	11.0 ± 0.4	11.7 ± 0.5	9.2 ± 0.1	11.6 ± 0.1	11.5 ± 0.5
Polydispersity index (PDI)	0.206 ± 0.110	0.210 ± 0.160	0.140 ± 0.108	0.155 ± 0.116	0.243 ± 0.112	0.270 ± 0.175

* ME indicates microemulsion prepared with ethanol; DS, diclofenac sodium; MI, microemulsion prepared with isopropyl alcohol; and MP, microemulsion prepared with propanol.

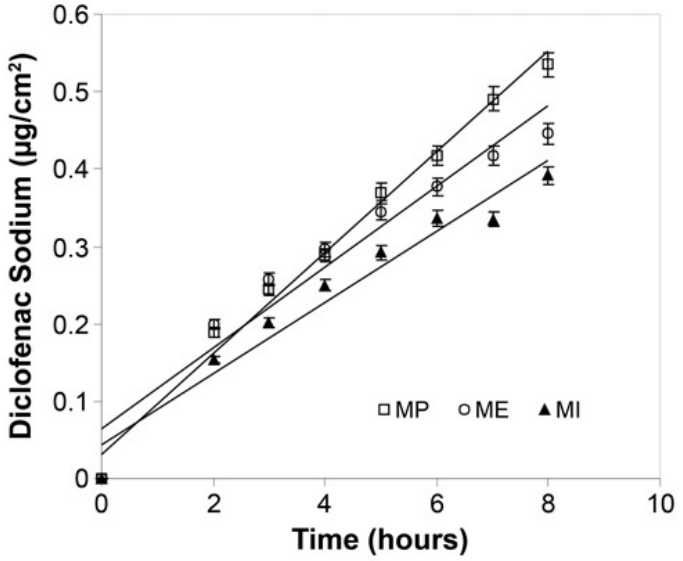

Figure 2. In vitro release profiles of diclofenac sodium through cellulose membrane from MP, ME, and MI. ME indicates microemulsion prepared with ethanol; MI, microemulsion prepared with isopropyl alcohol; and MP, microemulsion prepared with propanol.

the formulations studied and this fact supports the observation above (Table 3). This observation also agrees with the earlier study, which indicated that the release rate of diclofenac diethylammonium from the investigated systems depends significantly on the water volume fractions.[26]

In the beginning of release process of the formulations, MP has the highest release rate and ME has higher release rate than MI, which is the lowest. Later, the changes on the release rates of all formulations were similar ($P > .05$).

For each M formulation, curve estimation was performed and the changes of release rate against time were examined. The results of statistical analyses of curve estimation were used in order to develop regression models that have the best r^2. These results were considered to be quadratic (second degree) and cubic (third degree) effect of time, in addition to the linear effect of time. Then, 3 regression models were obtained for each formulation by using linear and quadratic effect of time. In former literature analyzed using a multiple linear regression model, drug permeability, was also modeled as a function of time.[25,27,28] Afterwards, 2 dummy variables[22] were selected (Equation 1) (Table 4) and 1 model was defined for 3 microemulsions (Equation 2). The calculated model showed good predictive abilities of release of DS from microemulsions through cellulose membrane. The r^2 of this defined model was found to be 90.4%.

$$y = a + bx + dummy\ variables \quad (1)$$

$$y(permeation) = 0.016 + [0.062^{*}hour] - [0.002^{*}hour^{2}] + 0.09^{*}[MP = 1] + 0.047^{*}[ME = 1] \quad (2)$$

As has been reported previously, in order to study electrical conduction of non-ionic microemulsions, a small amount of aqueous electrolyte must be added to provide the charges necessary for the charge transport.[29-31] However, the addition of salt, especially sodium chloride, can significantly affect the phase behaviors and structural properties of microemulsions,[4] and that even may result in phase separation. For this reason, in this study, the conductivity measurements were performed without deliberate incorporation of an electrolyte. With M formulations without water fraction, low conductivity values were obtained. It is known that autoprotolysis constants (K) for aliphatic alcohols are –logK = ~20 and for water, –logK = 14. Therefore, the solutions of alcohols are better conductors than water, and the conductivities of alcohols are also greatly increased by the presence of water.[32] The appropriate electrical conductivity values for the conductivity study were obtained with formulations without water and DS, primarily enabled by the presence of alcohols; then the electrical conductivity values of microemulsions with and without DS were compared. DS-loaded microemulsions clearly showed higher conductivity values than unloaded formulations. As shown in Table 5, the conductivity values of unloaded microemulsions vary between 16.9 and 17.9 μScm^{-1} and drug-loaded microemulsions vary between 18.8 and 20.2 μScm^{-1}. In the present study, the effect of the water phase on the amount of microemulsions was also evaluated. Electrical conductivity increases in drug-loaded formulations when the amount of water is increased. The titration process, performed by adding water, continued

Table 3. Release Rates of All Formulations Studied Through Cellulose Membrane*

Formulations	MP (± SD)	ME (± SD)	MI (± SD)
Flux ($mg/cm^2/h$)	0.059 ± 0.018	0.040 ± 0.002	0.038 ± 0.003

* ME indicates microemulsion prepared with ethanol; MI, microemulsion prepared with isopropyl alcohol; MP, microemulsion prepared with propanol; and SD, standard deviation.

Table 4. Dummy Variables That Were Taken for each Formulation*

Formulations	MP	ME	MI
Dummy variables	1	1	0
	0	1	0
	0	0	0

* ME indicates microemulsion prepared with ethanol; MI, microemulsion prepared with isopropyl alcohol; and MP, microemulsion prepared with propanol.

Table 5. The Conductivity, Viscosity, and Observed pH Values of Water/Oil Microemulsions With/Without Diclofenac Sodium*

	Unloaded Microemulsions			DS-Loaded Microemulsions		
Formulations	Conductivity σ (microsiemens/cm)	Viscosity ή (cps)	pH	Conductivity σ (microsiemens/cm)	Viscosity ή (cps)	pH
ME	16.9	87	6.70	18.8	176	6.70
MI	16.9	80	6.75	20.2	174	6.85
MP	17.9	90	6.75	18.8	194	6.70

* DS indicates diclofenac sodium; ME, microemulsion prepared with ethanol; MI, microemulsion prepared with isopropyl alcohol; and MP, microemulsion prepared with propanol.

until ~4.5% to 10% water content of the formulation. A linear increase was observed on the electrical conductivity of the drug-loaded microemulsions by adding water drop by drop (Figure 3). After certain percentages of water, conductivity values remain constant. As shown in Figure 3, MP, ME, and MI conductivity values remained constant after ~1%, 1.5%, and 4% water content, respectively.

The conductivity results obtained showed that loading DS and the addition of the appropriate amount of water phase into the formulation had no negative effects on system stability. When an unstable emulsion system and phase separation occurs, the conductivity values are greatly reduced.[33]

In previous literature, it has been reported that the lowest isoconductivity plots extend to the largest droplet radii.[33-35] Similarly, in this study, the size of the droplets for unloaded microemulsion with low conductivity values were greater than those of drug-loaded formulations with higher conductivity values.

Viscosity measurements were examined as a function of sheer rate, and Newtonian fluid characterization was observed for each drug-loaded microemulsion system (Figure 4). A similar characteristic was also found for unloaded microemulsions. As a result of viscosity measurements, it was observed that viscosity values of drug-loaded microemulsions were higher than the values of unloaded formulations (Table 5).

The M formulations with and without DS had appropriate observed pH values varying from 6.70 to 6.85 for topical application (Table 5). Incorporation of DS did not significantly affect the observed pH value of the microemulsions.

As the result of irritation study, no visible reaction was seen with any of the formulations after an 8-hour application on the inner arms of volunteers. The erythema values of formulations with and without DS were lower than control values, meaning that lightening of skin color did occur.

The results of the skin irritation study were analyzed according to repeated measures ANOVA. It was seen that the difference between erythema values of formulations and control was insignificant (P =.387). There was also no significant difference among the erythema values of any of the formulations (P > .05) (Table 6). Consequently, addition of DS and different cosurfactants in the formulations has no effect on skin irritation features of microemulsions. Detailed skin tests have to be performed on more volunteers before these formulations can be used for DS as a vehicle for topical application.

CONCLUSION

The results of in vitro release study, physicochemical property tests, and skin irritancy study showed that all 3 M formulations studied may be appropriate vehicles for topical

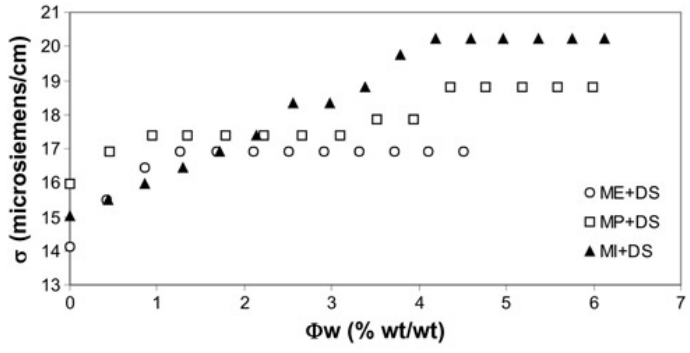

Figure 3. Electrical conductivity (σ) as a function of water phase volume fraction (%wt/wt) in the systems ME+DS, MP+DS, and MI+DS. ME indicates microemulsion prepared with ethanol; DS, diclofenac sodium; MP, microemulsion prepared with propanol; MI, microemulsion prepared with isopropyl alcohol.

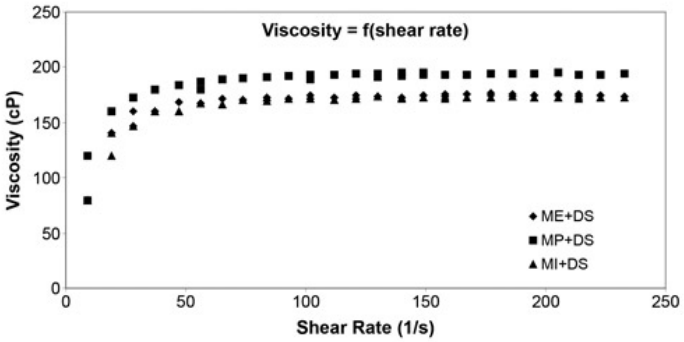

Figure 4. Viscosity (cP) of drug-loaded microemulsion systems (ME+DS, MP+DS, MI+DS) as a function of shear rate (1/s).

Table 6. Mean of Erythema Values of Microemulsion Formulations With/Without Diclofenac Sodium and Control*

Formulation	Number of Volunteers†	Mean of Erythema Values ± SEM
Control	9	208.2 ± 10.6
ME	9	177.3 ± 14.7
ME-DS	9	174.6 ± 13.6
MI	9	172.8 ± 21.1
MI-DS	9	179.1 ± 22.2
MP	9	189.6 ± 22.3
MP-DS	9	191.2 ± 15.1

* ME indicates microemulsion prepared with ethanol; DS, diclofenac sodium; MI, microemulsion prepared with isopropyl alcohol; and MP, microemulsion prepared with propanol.
† Inclusive age range of all volunteers was 22 to 49; male/female distribution was 1 male/8 females.

application; however, MP may be preferable because it has both the highest percentage of water for the increased facility of loading water soluble drugs and the highest flux value of the drug studied.

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