Transdermal Delivery of Diclofenac Sodium Through Rat Skin From Various Formulations

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ABSTRACT

The aim of this study was to evaluate and compare the in vitro and in vivo transdermal potential of w/o microemulsion (M) and gel (G) bases for diclofenac sodium (DS). The effect of dimethyl sulfoxide (DMSO) as a penetration enhancer was also examined when it was added to the M formulation. To study the in vitro potential of these formulations, permeation studies were performed with Franz diffusion cells using excised dorsal rat skin. To investigate their in vivo performance, a carrageenan-induced rat paw edema model was used. The commercial formulation of DS (C) was used as a reference formulation. The results of the in vitro permeation studies and the paw edema tests were analyzed by repeated-measures analysis of variance. The in vitro permeation studies found that M was superior to G and C and that adding DMSO to M increased the permeation rate. The permeability coefficients (Kp) of DS from M and M+DMSO were higher ($Kp = 4.9 \times 10^{-3} \pm 3.6 \times 10^{-4}$ cm/h and $5.3 \times 10^{-3} \pm 1.2 \times 10^{-3}$ cm/h, respectively) than the Kp of DS from C ($Kp = 2.7 \times 10^{-3} \pm 7.3 \times 10^{-4}$ cm/h) and G ($Kp = 4.5 \times 10^{-3} \pm 4.5 \times 10^{-5}$ cm/h). In the paw edema test, M showed the best permeation and effectiveness, and M+DMSO had nearly the same effect as M. The in vitro and in vivo studies showed that M could be a new, alternative dosage form for effective therapy.

KEYWORDS: microemulsion, diclofenac sodium, in vitro permeation, carrageenan-induced rat paw edema test.

INTRODUCTION

Transdermal drug delivery offers many important advantages. For instance, it is easy and painless, it protects the active compound from gastric enzymes, and it avoids the hepatic first-pass effect. Also, it is simple to terminate the therapy if any adverse or undesired effect occurs. But skin is a natural barrier, and only a few drugs can penetrate the skin easily and in sufficient quantities to be effective. Therefore, in recent years, numerous studies have been conducted in the area of penetration enhancement.[1,2] Penetration enhancers such as hydrogenated soybean phospholipids,[3] ethanol, alcohols with long carbon chains (C_8 to C_{14}), n-octanol and cyclic monoterpenes,[4,5] nonionic surfactants,[6] propylene glycol, and isopropyl myristate[4,5,7] have been used in many studies to increase the percutaneous absorption of drugs. Membranes from rats, mice, pigs, guinea pigs, snakes, rabbits, and humans as well as synthetic membranes have been used for these drug diffusion studies. Although human cadaver skin may be the first choice as a skin model for a study of a final product to be used in humans, it is not always easy to obtain, and rat skin is a commonly used substitute.[8,9] Al-Saidan et al showed that in vitro permeation studies using rat skin could provide information useful for manipulating the design of transdermal therapeutic system (TTS) patches so that the desired permeation of the drug across human skin would be achieved.[10] Therefore, we used rat skin as a model membrane for our permeation studies. Microemulsions containing the oil and aqueous phase, surfactant and cosurfactant (cos), are optically transparent mixtures with a very small droplet size (<140 nm).[11,12] Microemulsions have been increasing in popularity and garnering more attention in recent years, because they may enhance the transdermal absorption of drug molecules by increasing drug solubilities and modifying their partition coefficients.[13] A hydrogel base is used very often in topical formulations.[14,15] The hydrogel formulation was prepared and studied as a vehicle for its permeation potential.

DS is a nonsteroidal antirheumatic agent that has a potent anti-inflammatory effect, but it does not penetrate well through skin and cannot reach the effective concentration at the site of action after transdermal application.[4] For this reason, we wanted to suggest new, alternative dosage forms for transdermal application of DS. M and G formulations were developed and in vitro transdermal penetration of these formulations was compared with that of C. Furthermore, a pharmacodynamic study of DS was evaluated for its anti-inflammatory activity on a carrageenan-induced rat paw edema model for

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all formulations. This study aimed to both suggest a new, alternative dosage form for enhancing topical penetration of DS and to compare the study's formulations with the commercial formulation available, evaluating the potential for penetration and transdermal absorption.

Materials and Methods

Materials

Carbopol 940 was kindly supplied by Mustafa Nevzat Co (Istanbul, Turkey). Triethanolamine, soybean oil, and carrageenan were purchased from Sigma (St Louis, MO). DS was kindly provided by Novartis (Istanbul, Turkey). Brij 58, Span 80, isopropyl alcohol, and DMSO were purchased from Merck (Hohenbrunn, Germany). All chemicals used were analytical grade.

Preparation of Topical Formulations

G was prepared with Carbopol 940 (0.3%), triethanolamine (0.4%), ethanol, and distilled water (Table 1) using 2 mixtures. Mixture I was obtained by dispersing Carbopol 940 in a mixture of 25% distilled water and 18.75% ethanol. Mixture II was obtained by dissolving triethanolamine in a mixture of 6.25% ethanol and 49.3% distilled water. After complete hydration of Carbopol 940, mixture II was added drop by drop to mixture I by stirring with a mixer (IKA Labortechnik, Breen, Germany). The gelling process was completed, and G was obtained.

M (w/o) was prepared using soybean oil as the oily phase, Brij 58 and Span 80 as the surfactants, isopropyl alcohol as the cosurfactant, and distilled water as the aqueous phase (Table 1). For the M preparation, the surfactants were mixed and melted at 60°C, then added to soybean oil. Cos and distilled water were added to this mixture by stirring using a magnetic stirrer (IKA Labortechnik, Staufen, Germany). The surfactant-to-cosurfactant weight ratio was 5:1. Then, a transparent microemulsion was obtained. Droplet sizes of M were determined using a Zetasizer (Malvern HPPS, Malvern, UK). DMSO 10% (wt/wt) was added as an enhancer at the last stage (M+DMSO).

Table 1. Contents (% wt/wt) of the Microemulsion and Gel Formulations

Microemulsion		Gel	
Oil	32.5	Carbopol 940	0.3
Water	6.3	Triethanolamine	0.4
Brij 58	5.1	Ethanol	25.0
Span 80	45.9	Distilled water	74.3
Cosurfactant	10.2		

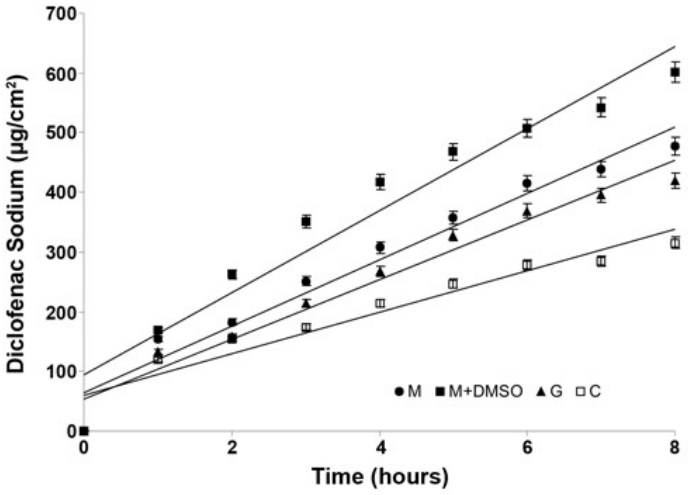

Figure 1. Permeation profiles of diclofenac sodium through rat skin from M, M+DMSO, G and C. Values are means of 3 determinations ± SD. M indicates microemulsion; DMSO, dimethyl sulfoxide; G, gel; C, commercial formulation.

One gram of Voltaren Emulgel (Novartis, Istanbul, Turkey), the commercial topical formulation, contains 11.6 mg of diclofenac diethyl ammonium (equivalent to 10 mg/g DS), isopropyl alcohol, propylene glycol, perfume, Cream 45, and other additives.

All prepared formulations and the C dosage form contained 1% (wt/wt) DS.

Assay of DS

A spectrophotometric analysis was used to determine DS permeation. First, 25 to 500 µL of stock solution (10 mg/10 mL, adjusted with phosphate buffer pH 7.4) was transferred by microsyringe into the 10 mL volumetric flasks. Then the volume was adjusted with phosphate buffer pH 7.4. The absorbances of the solutions were determined against a blank spectrophotometrically (Shimadzu UV-160A) at 277 nm. The aliquots of permeated formulations without DS were used as a blank. A calibration curve was then obtained ($Y = 34.1X - 0.603$, in which Y was concentration [µg/mL], X was absorbance, and r^2 was 0.999). The sensitivity was 2.5 to 50 µg/mL. The limit of detection was 7.5 µg/mL.

In Vitro Permeation Studies

Vertical Franz-type diffusion cells (PermeGear, Bethlehem, PA) with a diffusional surface area of 1.76 cm^2 were used to study the permeability of DS. The animal study protocol was reviewed and approved by the Ethics Committee at the Faculty of Pharmacy of Ege University. Skin samples were obtained from male Swiss albino rats weighing 140 to 160 g. After hair was shaven using a mechanical hair clipper, without damaging skin, a 5 × 5 cm patch of skin was excised

Table 2. Permeation Parameters of Diclofenac Sodium From Different Bases Through Rat Skin*

Formulation	Flux ± SD ($\mu g/cm^2/h$)	Kp ± SD (cm/h)
M	$4.9 \times 10^{-2} \pm 0.0040$	$4.9 \times 10^{-3} \pm 3.6 \times 10^{-4}$
M+DMSO	$5.3 \times 10^{-2} \pm 0.0120$	$5.3 \times 10^{-3} \pm 1.2 \times 10^{-3}$
Gel	$4.5 \times 10^{-2} \pm 0.0005$	$4.5 \times 10^{-3} \pm 4.5 \times 10^{-5}$
Commercial	$2.7 \times 10^{-2} \pm 0.0070$	$2.7 \times 10^{-3} \pm 7.3 \times 10^{-4}$

*Data are given as mean ± SD (n = 3). M indicates microemulsion; DMSO, dimethyl sulfoxide.

from the dorsal region of each sacrificed rat. The excised rat skins were stored at –80°C. The skin membranes were first hydrated for 30 minutes in the buffer solution (pH 7.4) at room temperature (23°C) to remove extraneous debris and leachable enzymes.[7] They were then placed between the donor and receptor compartments of the cells, with the dermal side in direct contact with the receptor medium. Approximately 20 mL of phosphate buffer (pH 7.4) was placed in the receptor compartment. Its temperature was maintained at 37 ± 0.5°C using a thermostatic water bath (Variomag, Munich, Germany), and it was stirred at 600 rpm throughout the experiment. The donor compartment contained 1 g of the sample. The aliquots (0.7 mL) were withdrawn at predetermined time intervals and then immediately analyzed spectrophotometrically at 277 nm against a blank prepared with the permeated formulation (M, M+DMSO, or G) without the drug.[16,17] The same amount of fresh buffer was added to the receptor compartment to replace what had been removed. Three replicates of each experiment were performed.

Determination of Drug Solubility

To determine the drug solubility, an excess amount of DS was added to distilled water. This suspension was stirred at room temperature for 24 hours with a magnetic stirrer. The sample was then filtered through a 0.45-μm cellulose acetate filter (Sartorius AG, Goettingen, Germany). The concentration of DS was determined spectrophotometrically at 277 nm.[18,19]

Determination of n-Octanol–Distilled Water Partition Coefficient

n-Octanol phases were saturated with distilled water for at least 24 hours before the experiment. A solution of DS (10^{-4}M) was prepared with distilled water. Then, 2 mL of this solution was transferred to 10-mL assay tubes containing 2 mL of the organic phase. The tubes were stoppered and agitated for 24 hours at room temperature. After centrifugation at 3500 U/min for 15 minutes, the concentration of the drug in the water phase was analyzed spectrophotometrically; the concentration of the drug in n-octanol was calculated from the difference between the initial and final concentrations in the water phase. Six replicates were used for the concentrations of n-octanol–distilled water solutions for partition coefficient calculations.[20] The same experiment was repeated using soybean oil as an organic phase.[19]

Anti-inflammatory Effect Test

The formulations of DS were evaluated for their anti-inflammatory activity on a carrageenan-induced rat paw edema model.[21,22] Inflammation was produced in the rats (Male, Wistar, weighing 200-250 g) using 100 μL of 1% carrageenan (wt/vol) in saline. This was injected into the plantar surface of the rats' left hind paw. To evaluate the topical anti-inflammatory activity of the formulations G, M, M+DMSO, and C, 4 groups of animals (n = 3) with carrageenan-induced paw edema were examined. Thirty minutes later, 100 μL of G, M, M+DMSO, or C was applied topically on the edematous paw. A fifth group of rats was used as a control (untreated). The increase in paw thickness was measured with the help of dial calipers before (time 0) and 1, 2, 3, 4, 5, and 6 hours after carrageenan administration. The percentage of paw thickness increase from time 0 was calculated.

Statistical Analysis

The results of permeation studies through rat skin were analyzed by repeated-measures analysis of variance (ANOVA).

Table 3. Means of Permeated Amounts of Diclofenac Sodium and Standard Deviations for All Formulations*

	Permeated Amount of DS ($\mu g/cm^2$) (± SD)			
Time (hour)	M	M+DMSO	Gel	Commercial Formulation
1	154.100 ± 2.0224	168.800 ± 3.0512	132.300 ± 4.2790	119.500 ± 2.3812
2	182.700 ± 2.9206	262.300 ± 2.8054	158.100 ± 3.3181	154.700 ± 4.3486
3	250.700 ± 2.6907	351.900 ± 4.6872	214.300 ± 5.1468	173.900 ± 4.8135
4	307.200 ± 3.9611	417.700 ± 3.5086	267.300 ± 6.3906	214.700 ± 7.4344
5	358.200 ± 6.1733	468.700 ± 2.6230	328.100 ± 6.4630	246.700 ± 6.4467
6	414.700 ± 4.6487	507.100 ± 2.7495	369.200 ± 5.6507	278.100 ± 3.2696
7	438.900 ± 4.9729	542.400 ± 6.0233	395.200 ± 5.5073	5.373 ± 2.4880
8	477.300 ± 5.9908	601.400 ± 9.8473	420.000 ± 5.3731	315.500 ± 4.1797

*Data are given as mean ± SD (n = 3). M indicates microemulsion; DMSO, dimethyl sulfoxide.

Table 4. Differences Among Formulations as to the Permeated Amount of DS for Each Hour*

Formulations	M+DMSO	M	Commercial Formulation	Gel
M + DMSO				
M	1, 2, 3, 4, 5, 6, 7, 8			
Commercial formulation	1, 2, 3, 4, 5, 6, 7, 8	1, 2, 3, 4, 5, 6, 7, 8		
Gel	1, 2, 3, 4, 5, 6, 7, 8	1, 2, 3, 4, 5, 6, 7, 8	1, 3, 4, 5, 6, 7, 8†	

*Numbers show significant difference ($P < .05$) at hours between 2 formulations crossed. M indicates microemulsion; DMSO, dimethyl sulfoxide.
†At all hours there was significant difference between the commercial formulation and the gel, except at hour 2.

Two different factors were examined. Factor 1 was the time repeated and was composed of 8 levels. Factor 2 was formed by 4 different formulations (M, M+DMSO, G, C).

The results of the paw edema test were evaluated according to repeated-measures ANOVA. Two different factors were compared. Factor 1 was the time and was composed of 6 levels (repeated factor). Factor 2 was the formulations and was composed of 5 levels (M, M+DMSO, G, C, control).

One-way ANOVA was used for further analysis, because there was significant interaction between 2 factors; 0.05 was taken as the level of significance. The Duncan test was also used as post hoc analysis in this study. Each value represents the mean ± SD ($n = 3$).

Results and Discussion

After addition of DS to M, no opalescence was observed and no significant changes were detected in the droplet sizes, indicating that these systems retained their stability when the drug was added. The viscosities of G and M were determined using a Brookfield digital viscometer-III Rheometer V 3.3 HB (Middleboro, MA) (Spindle: SC4-21) at 200 rpm and 25 ± 0.1°C as 1872 cps and 80 cps, respectively. The pHs of G and M were adjusted to 5.45 ± 0.1 and 6.75 ± 0.1, respectively, and physicochemical stability of the formulations was observed.

The mean droplet diameter of M prepared with isopropyl alcohol without DS was 11.7 ± 0.5 nm, and with DS the diameter was 9.19 ± 0.1 nm.[19] Park and Kim offered 2 explanations for the decrease in droplet size with the addition of the drug: (1) a certain portion of undissolved drug acts as an emulsifying agent by the deposition of drug particles at the interface of M, or (2) the deposition of drug at the interface of M causes reduced surfactant mobility, which decreases the particle size of drug-loaded microemulsions.[23]

The solubility of DS in distilled water has been previously found to be >9 mg/mL.[24] In this study, the solubility of DS in distilled water was 19.1 mg/mL. The partition coefficient of DS in n-octanol–distilled water and soybean oil–distilled water was calculated as 5.75 and 1.8, respectively. One gram of M in the donor compartment contains 10 mg DS. In the water phase (0.063 g) of M, ~10% of DS might be dissolved in conformity with the solubility studies. Judging from the partition coefficients of both n-octanol/distilled water and soybean oil/distilled water, the remaining DS may exist largely in the oil-surfactant region of w/o M in a dissolved form because of the high concentration of surfactants.

To calculate the permeation parameters of the Fick's law equation from the plot of penetrated amounts vs time, a graph was plotted (Figure 1). It is possible to calculate the steady-state flux (J) from the slope of the linear portion (2-8 hours) of the graph.[25,26]

The permeability coefficient (Kp) was calculated from the steady-state flux and the applied concentration in the donor compartment (C_{donor}) as follows:

$$K_p = J / C_{donor} \quad (1)$$

The flux and permeability coefficients of the formulations are given in Table 2. The results of permeation studies through rat skin were analyzed by repeated-measures ANOVA. According to the ANOVA, the interaction was significant between Factor 1 and Factor 2. There was a significant difference in the permeability rates among all formulations studied ($P < .05$). As to the results obtained using post hoc

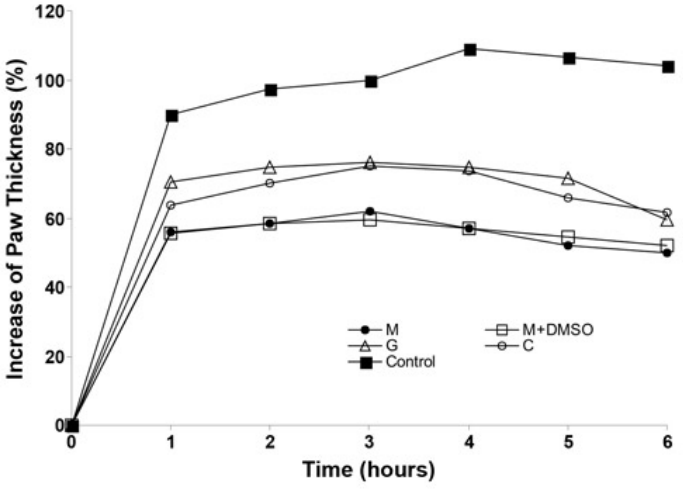

Figure 2. Percentage increase of paw thickness after subplantar injection of carrageenan. Values are means of 3 determinations ± SD. M indicates microemulsion; DMSO, dimethyl sulfoxide; G, gel; C, commercial formulation.

Table 5. Means of Paw Thickness Increase and SDs for All Formulations

	Increase of Paw Thickness % (±SD)*				
Time (hour)	M	M+DMSO	Gel	Commercial Formulation	Control
1	55.800 ± 1.7692	55.700 ± 1.4107	70.600 ± 1.8028	63.800 ± 1.9672	90.000 ± 2.8827
2	58.300 ± 1.2124	58.300 ± 0.7211	74.600 ± 0.7550	70.000 ± 2.8478	97.500 ± 0.7550
3	61.800 ± 1.4731	59.500 ± 0.8185	76.200 ± 0.7937	75.000 ± 0.9644	100.000 ± 1.8248
4	57.000 ± 1.5133	57.000 ± 1.5716	74.600 ± 0.8185	73.800 ± 0.9539	109.200 ± 1.4933
5	52.200 ± 0.7550	54.500 ± 1.9698	71.400 ± 1.3229	65.800 ± 1.2490	106.700 ± 1.4526
6	49.800 ± 1.7578	51.900 ± 0.9165	59.600 ± 3.6042	61.700 ± 1.6643	104.200 ± 1.0536

*Data are given as mean ± SD (n = 3). M indicates microemulsion; DMSO, dimethyl sulfoxide.

analysis (Duncan test), the differences among all pairwise comparisons of formulations were found to be significant. There was a significant difference among the formulations studied from the first hour; the rank order for in vitro percutaneous absorption of DS from the bases was M+DMSO > M > G > C (Figure 1; Table 3). This rank order held throughout the 8 hours. Pairwise comparisons of these formulations were done at each hour. At the second hour, the difference between C and G was not significant. All other pairs were found to be significantly different at all hours (Table 4). According to this order, the permeability rate of DS from M+DMSO was the highest ($Kp = 5.3 \times 10^{-3}$) and from C was the lowest ($Kp = 2.7 \times 10^{-3}$ cm/h) (Table 2). The higher permeability rate of DS from M is most probably due to the surfactants and the oily phase, which act as penetration enhancers to facilitate transdermal drug delivery.[27]

In this study, the flux values from M and M+DMSO were 4.9×10^{-2} and 5.3×10^{-2} μg/cm^2/h, respectively, yielding 1.8 and 2 times greater than that observed from C (2.7×10^{-2} μg/cm^2/h). As shown in Figure 1, addition of DMSO to M increased the in vitro permeation rate of DS. In a previous study, it was explained that DMSO interaction with the stratum corneum lipid alkyl chains resulted in decreased diffusion resistance of the barrier and increased drug penetration into the skin.[28]

Many different theories concerning the mechanism of action of penetrants have appeared in the literature. One of them attributes the penetrant effects of DMSO, dimethylformamide, and dimethylacetamide to their hygroscopic properties, which are said to increase the water content of the stratum corneum, thereby greatly increasing its permeability. Another attributes the effectiveness of penetration enhancers to their ability to lower the barrier properties of the stratum corneum by modifying its natural structure. Organic solvents like benzene, alcohol, and ether, which have been shown to enhance the penetration rate of both water-soluble and lipid-soluble substances, may act by removing the lipids from the stratum corneum. However, the action of hydrogen-bonding solvents like DMSO, dimethylformamide, and dimethylacetamide is attributed to membrane expansion and uniform increase in media diffusivity.

It is known that microemulsions have a great capacity to release drugs through the skin.[29] In this study, M showed a higher release capacity for DS than did G. The M structure may have allowed high drug mobility in the vehicle, which would translate into faster drug diffusion through the skin surface and thus a higher transdermal flux.[30]

It has been reported that carrageenan-induced edema can be divided into 2 phases. The first phase occurs throughout the first hour after carrageenan injection. It derives from the release of cytoplasmic enzymes and serotonin from mast cells and the increase of prostaglandin in the inflammatory area. The second phase occurs 3 to 5 hours after carrageenan injection. In this phase, the macrophages in carrageenan-insulted dermal tissue release interleukin-1 to induce accumulation of polymorphic nuclear cells into the inflammatory area. This then releases the lysosomal enzymes and active oxygen to destroy connective tissues and induce paw swelling.[31]

Table 6. Differences Among Formulations as to the Paw Thickness Increase for Each Hour*

Formulations	M+DMSO	M	Commercial Formulation	Gel	Control
M + DMSO					
M	3				
Commercial formulation	1, 2, 3, 4, 5, 6	1, 2, 3, 4, 5, 6			
Gel	1, 2, 3, 4, 5, 6	1, 2, 3, 4, 5, 6	1, 2, 5†		
Control	1, 2, 3, 4, 5, 6	1, 2, 3, 4, 5, 6	1, 2, 3, 4, 5, 6	1, 2, 3, 4, 5, 6	

*Numbers show significant difference ($P < .05$) at hours between the 2 formulations crossed. M indicates microemulsion; DMSO, dimethyl sulfoxide.
†At the first, second, and fifth hours there were significant differences between G and C.

In this study, the progress of the paw edema test was compatible with that found in the literature. Induction of acute inflammation in control rats resulted in a prominent increase in paw thickness throughout the first hour after intraplantar injection of carrageenan and reached a peak of inflammation after 4 hours (Figure 2).

The results of the paw edema test were evaluated using repeated-measures ANOVA, and the interaction was found to be significant between Factor 1 and Factor 2. This means that the paw edema differences among formulations for each hour were not similar (Table 5). The difference in the increase of paw thickness between hours was significant. Because of interaction between factor 1 and factor 2, formulations were compared for each hour using 1-way ANOVA. Homogeneity of variance, analyzed using the Levene test, was observed at all hours. The Duncan test, used as post hoc analysis, found that when all formulations were compared with the control, a significant difference was found. The differences in paw thickness increase among formulations against time are shown in Table 6 (Figure 2).

In vitro and in vivo studies were compared. According to both in vivo and in vitro studies, M showed the best permeation and effectiveness (Figures 1, 2). In in vitro studies, the permeation of M increased when DMSO was added (Figure 1), but in in vivo studies, M and M+DMSO had nearly the same effect.

In addition, in vitro studies demonstrated that G had better penetration than C, whereas in vivo studies showed that their effectiveness was nearly similar.

CONCLUSIONS

This study demonstrated that incorporating DS into M enhanced drug penetration through rat skin in vitro and in vivo (Figures 1 and 2). M containing DS may offer promise as an anti-inflammatory dosage form, ensuring more effective therapy, but additional extradermal tests and experiments should be performed before the formulation is used in humans.

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REFERENCES

1. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. *Eur J Pharm Sci.* 2001;14:101–114.

2. Moser K, Kriwet K, Naik A, Kalia YN, Guy RH. Passive skin penetration enhancement and its quantification in vitro. *Eur J Pharm Biopharm.* 2001;52:103–112.

3. Nishihata T, Kamada A, Sakai K, et al. Percutaneous absorption of diclofenac in rats and humans: aqueous gel formulation. *Int J Pharm.* 1988;46:1–7.

4. Parsaee S, Sarbolouki MN, Parnianpour M. In vitro release of diclofenac diethylammonium from lipid-based formulations. *Int J Pharm.* 2002;241:185–190.

5. Ho HO, Huang FC, Sokolaski TD, Sheu MT. The influence of cosolvents on the in-vitro percutaneous penetration of diclofenac sodium from a gel system. *J Pharm Pharmacol.* 1994;46:636–642.

6. Iwasa A, Irimoto K, Kasai S, Okuyama H, Nagai H. Effect of nonionic surfactants on percutaneous absorption of diclofenac sodium. *Yakuzaigaku.* 1991;51:16–21.

7. Santoyo S, Arellano A, Ygartua P, Martin C. Penetration enhancer effects on the in vitro percutaneous absorption of piroxicam through rat skin. *Int J Pharm.* 1995;117:219–224.

8. Nair VB, Panchagnula R. The effect of pretreatment with terpenes on transdermal iontophoretic delivery of arginine vasopressin. *Farmaco.* 2004;59:575–581.

9. Tokudome Y, Sugibayashi K. The synergic effects of various electrolytes and electroporation on the in vitro skin permeation of calcein. *J Control Release.* 2003;92:93–101.

10. Al-Saidan SM, Krishnaiah YSR, Chandrasekhar DV, et al. Formulation of an HPMC gel drug reservoir system with ethanol-water as a solvent system and limonene as a penetration enhancer for enhancing in vitro transdermal delivery of nicorandil. *Skin Pharmacol Physiol.* 2004;17:310–320.

11. Paul BK, Moulik SP. Microemulsions: an overview. *J Disper Sci Technol.* 1997;18:301–306.

12. Prince LM. Microemulsions. In: Lissant KJ, ed. *Emulsions and Emulsion Technology.* New York, NY: Marcel Dekker; 1974:125–178.

13. Delgado-Charro MB, Iglesias-Vilas G, Blanco-Mendez J, Lopez-Quintela MA, Marty JP, Guy RH. Delivery of a hydrophilic solute through the skin from novel microemulsion systems. *Eur J Pharm Biopharm.* 1997;43:37–42.

14. Celebi N, Kıslal O, Tarımcı N. The effect of β-cyclodextrin and penetration additives on the release of naproxen from ointment bases. *Pharmazie.* 1993;48:914–917.

15. Gupta P, Vermani K, Garg S. Hydrogels from controlled release to pH-responsive drug delivery. *Drug Discov Today.* 2002;7:569–579.

16. Dureja H, Tiwary AK, Gupta S. Simulation of skin permeability in chitosan membranes. *Int J Pharm.* 2001;213:193–198.

17. El Laithy HM, El-Shaboury KM. The development of Cutina lipogels and gel microemulsion for topical administration of fluconazole. *AAPS PharmSciTech.* 2002;3:E35.

18. Obata Y, Takayama K, Maitani Y, Machida Y, Nagai T. Effect of ethanol on skin permeation of nonionized and ionized diclofenac. *Int J Pharm.* 1993;89:191–198.

19. Kantarcı G, Özgüney I, Karasulu HY, Güneri T, Başdemir G. In vitro permeation of diclofenac sodium from novel microemulsion formulations through rabbit skin. *Drug Dev Res.* 2005;65:17–25.

20. Cordero JA, Alarcon L, Escribano E, Obach R, Domenech J. A comparative study of the transdermal penetration of a series of nonsteroidal antiinflammatory drugs. *J Pharm Sci.* 1997;86:503–508.

21. Winter CA, Risley EA, Nuss GV. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc Soc Exp Biol Med.* 1962;111:544–547.

22. Escribano E, Calpena AC, Queralt J, Obach R, Doménech J. Assessment of diclofenac permeation with different formulations: anti-inflammatory study of a selected formula. *Eur J Pharm Sci.* 2003;19:203–210.

23. Park KM, Kim CH. Preparation and evaluation of flurbiprofen-loaded microemulsion for parenteral delivery. *Int J Pharm.* 1999;181:173–179.

24. Adayeye CM, Li PK. Diclofenac sodium. In: Florey K, ed. *Analytical Profiles of Drug Substances.* New York: Academic Press; 1990:123–144.

25. Ferreira LAM, Seiller M, Grossiord JL, Marty JP, Wepierre J. Vehicle influence on in vitro release of glucose: w/o, w/o/w and o/w systems compared. *J Control Release.* 1995;33:349–356.

26. Panigrahi L, Pattnaik S, Ghosal SK. The effect of pH and organic ester penetration enhancers on skin permeation kinetics of terbutaline sulfate from pseudolatex-type transdermal delivery system through mouse and human cadaver skins. *AAPS PharmSciTech.* 2005;6: E167–E173.

27. Lawrence MJ, Rees GD. Microemulsion-based media as novel drug delivery systems. *Adv Drug Deliv Rev.* 2000;45:89–121.

28. Anigbogu ANC, Williams AC, Barry BW, Edwards HGM. Fourier transform raman spectroscopy of interactions between the penetration enhancer dimethyl sulfoxide and human stratum corneum. *Int J Pharm.* 1995;125:265–282.

29. Kreilgaard M. Influence of microemulsions on cutaneous drug delivery. *Adv Drug Deliv Rev.* 2002;54:S77–S98.

30. Kreilgaard M, Pedersen EJ, Jaroszewski JW. NMR characterisation and transdermal drug delivery potential of microemulsion systems. *J Control Release.* 2000;69:421–433.

31. Mei Z, Chen H, Weng T, Yang Y, Yang X. Solid lipid nanoparticle and microemulsion for topical delivery of triptolide. *Eur J Pharm Sci.* 2003;56:189–196.