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Enhanced Percutaneous Permeability of Diclofenac Using a New U-Type Dilutable Microemulsion

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Enhanced systemic absorption in vivo and percutaneous penetration in vitro was demonstrated after transdermal administration of diclofenac sodium formulated in U-type microemulsion. Diclofenac sodium was solubilized in a typical four-component system consisting of an oil, polyoxyethylene-10EO-oleyl alcohol (Brij 96V) as the surfactant, and 1-hexanol along water dilution line W46 (40 wt % surfactant and 60 wt % oil phase before water titration).Viscosity and small angle X-ray scattering measurements have evidenced bicontinuous structures within water fractions of 0.25 and 0.5 along the dilution line. Self-diffusion NMR studies showed that drug molecules accumulated in the interfacial film and, to some extent, dissolved in the oil. Relative to a commercial macro-emulsion cream (Voltaren® Emulgel®), microemulsions containing paraffin oil or isopropyl myristate increased the in vivo transdermal penetration rate of diclofenac by two order of magnitude, whereas the rat plasma levels were increased by one order of magnitude. The in vitro data obtained from excised rat skin were comparable to the in vivo results, but suffered from discrepancies from the ideal in vivo-in vitro correlation, which might be explained by optimal in vitro conditions of perfusion and hydration. It has also been found that when jojoba oil is formulated as the oil phase in the microemulsion, the penetration rate of the drug decreases significantly. Based on the three-dimensional structure of jojoba oil, the wax is presumed to prevent the drug from being freely diffused into the skin while migrating from the interfacial film into the continuous oil phase.

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Keywords microemulsion; transdermal drug delivery; percutaneous penetration; skin; diclofenac

INTRODUCTION

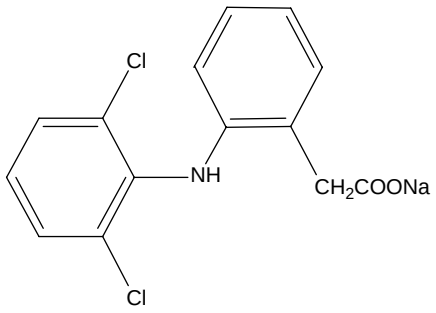
There has been a continuous interest during recent years in a search for new vehicle systems that could modify drug penetration into and through the skin. Many of the topical vehicles contain chemical enhancers and non-friendly solvents to achieve improved permeability (Walters, 1989). These vehicles usually result in various degrees of skin irritancy, especially when chronic treatments are required. Therefore, it is undoubtedly desirous to develop topical vehicles that do not necessitate chemical enhancers to facilitate drug penetration into and though the skin. There is a growing interest in microemulsions (MEs) for cosmetic and pharmaceutical applications, which is linked to their physicochemical properties—thermodynamic stability, spontaneous formation, clear appearance, low viscosity, and high solubilization capacity (Holmberg, 1998; Malmsten, 2002). A particularly attractive possibility is the use of microemulsions to enhance the efficacy of biologically active compounds by providing a readily controlled and stable medium for the solubilized components. Many studies have shown that microemulsion formulations possess improved transdermal and dermal delivery properties, mostly in vitro (Alvarez-Figueroa & Blanco-Mendez, 2001; Delgado-Charro et al., 1997; Dreher, Walde, Walther, & Wehrli, 1997; Kreilgaard, Pedersen, & Jaroszewski, 2000; Lee, Langer, & Shastri, 2003; Osborne, Ward, & O'Neill, 1991; Para, Coderch, Yuste, & de la Maza, 1997; Rhee,

Choi, Park, & Chi, 2001; Schmalfuss, Neubert, & Wohlrab, 1997; Trotta, Pattarino, & Gasco, 1996) and, in several cases, in vivo (Kemken, Ziegler, & Mueller, 1992; Kreilgaard, 2001; Kreilgaard et al., 2001; Sintov & Shapiro, 2004). In addition, microemulsions have been shown to stabilize and protect active substances, such as oxidation-sensitive vitamins (Chiu & Yang, 1992; Spiclin, Gasperlin, & Kmetec, 2001).

In this report we describe a new microemulsion system with a U-type phase diagram composed of a very large isotropic region starting from several oil/surfactant concentrates that can be progressively and continuously diluted with aqueous phase to the far corner of the phase diagram without any noticeable separation of the external phase. This special type of microemulsion system is actually a combination of ingredients for which the structures start from reverse micelles and swell with water to form W/O droplets that upon further addition of water will turn into bicontinuous domains into which the water and the oil are interwoven. Eventually, upon further addition of water, these biocontinuous domains will transform into O/W droplets. The uniqueness of this system is highlighted by the fact that a poorly-soluble drug can be loaded at very high loading capacities at the W/O interface and will remain solubilized even upon curvature inversion and phase transformation along with significant dilution effect. We have evaluated the transdermal drug delivery potential of the new microemulsion system using diclofenac sodium as the model drug. Diclofenac (Figure 1A) is a widely used, highly effective nonsteroidal anti-inflammatory agent (NSAID) in the management of acute conditions affecting soft tissue, such as tendus, bursa, and muscle. The topical application of diclofenac provides an alternative to the oral, rectal, and parenteral dosage forms, and is particularly suitable for musculoskeletal pain and inflammation of well-defined areas near the body surface. The concentrations of the drug in the systemic blood circulation following topical application are considerably lower than following other routes of administration. However, this route is associated with reduced risk of side effects, in particular, gastrointestinal adverse reactions.

A topical formulation, if properly designed to be locally effective (i.e., drug is highly absorbed into the peripheral blood in the site of action), may be beneficial in minimizing the inflammation process with a reduced risk to the patient. A topical composition of the commonly used Voltaren® Emulgel®, containing 1.16% diclofenac diethylammonium corresponding to 1% diclofenac sodium, is utilized for percutaneous treatment of the localized form of non-articular rheumatism and inflammations. Diclofenac compositions for topical application based on oil-water emulsion and also containing gel formers and lower alkanol ("emulgel") are described in US patent 4,917,886. Diclofenac is soluble in aqueous solutions as ionized salts and its penetration through the skin is dependent upon partition of the unionized form into the lipophilic phase of the Emulgel®. There are many published works attempting to challenge percutaneous penetration of diclofenac by various means (Arellano, Santoyo, Martin, & Ygartua, 1998; Bonina et al., 2001; Fini et al., 1999; Schwarz, Weisspapir, Shani, & Amselem, 1996; Takahashi et al., 2001). In our laboratory, we also have challenged this limitation by using the U-type dilutable microemulsion vehicle system containing diclofenac, and studied its properties and skin penetration enhancing ability. Our hypothesis is that the microemulsion system, by its virtue of solubilizing hydrophilic drug molecules in an oily environment, would facilitate the transdermal delivery of the drug and would increase its systemic absorption.

A. **Diclofenac sodium**



B. Jojoba wax

(Z,Z)-$CH_3(CH_2)_7CH{=}CH(CH_2)_mCOO(CH_2)_nCH{=}CH(CH_2)_7CH_3$

m = 7 (11%), 9 (71%), 11 (14%), 13 (1%)

n = 8 (1%), 10 (44%), 12 (45%), 14 (9%)

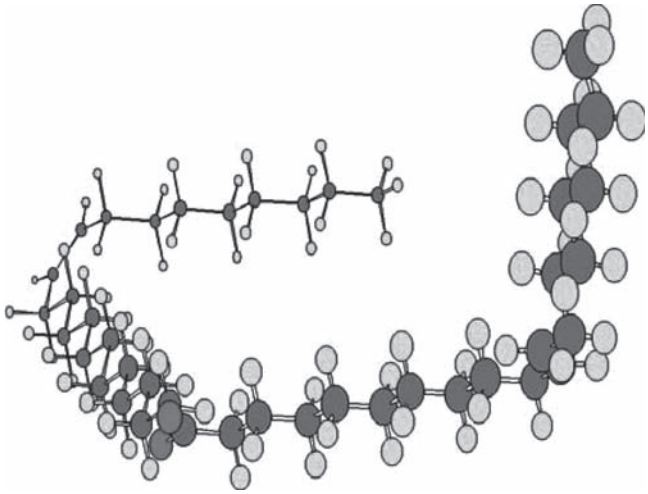


FIGURE 1. Chemical structures of (A) diclofenac sodium and (B) jojoba wax including 3-D illustration.

An additional objective of the present report was to examine jojoba oil as the oil phase in the microemulsion and its contribution to percutaneous drug penetration. Jojoba oil (Figure 1B) is a naturally golden liquid wax ester found in the seed of the jojoba plant. It is composed principally of 40 (and 42) carbon chain esters, which, in turn, are composed of monosaturated fatty acids and fatty alcohols of 20 (and 22) carbon chain length. Jojoba oil has a good skin moisturizing and softening capability, emolliency, and a high stability to oxidation reactions—properties that have introduced this oil as a preferred ingredient in present-day cosmetics. Formulations containing jojoba oil or its chemical derivatives have potential applications in other areas as well, including pharmaceuticals (topical applications) and lubrication (Schwarz et al., 1996; Shani, 1995; Wishniak, 1985). To the best of our knowledge, there are only a few publications that have demonstrated an enhanced efficacy of topical drugs by jojoba oil (El-Laithy & El-Shaboury, 2003; Schwarz et al., 1996).

MATERIALS AND METHODS

Materials

Diclofenac as sodium salt was obtained from Sigma (Rehovot, Israel). Polyoxyethylene-10EO-oleyl alcohol (Brij 96V) was purchased from ICI Specialty Chemicals (Essen, Germany). Jojoba oil with an iodine value of 80.9 was a gift from Jojoba Israel, Kibbutz Hatzerim, Israel, and it was used without further purification. The alcohols, 1-pentanol (CP) and 1-hexanol (purity ≥ 98%), were purchased from Fluka (Fluka Chemie AG CH-9471 Buchs). The water was double distilled.

Microemulsions and Phase Diagram

Sample Preparation

Pseudo-ternary phase diagrams were constructed in the following way: Mixtures of surfactant, oil, and alcohol (weight ratio of oil/alcohol was kept at 1:1) were titrated with water at room temperature to the solubilization limit, which was defined as the transition from the monophasic region to a polyphasic region or to a birefringent phase. The transition to a two-phase system could be detected visually, being marked by the appearance of cloudiness. Liquid crystal phases were identified using crossed polarizers.

Solubilization Parameters

Two suitable water-solubilization parameters were used in this work; the maximal amount of solubilized water, denoted W_m (Regev et al., 1996), and the total isotropic monophasic area, denoted A_T (Garti, Aserin, Ezrahi, & Wachtel, 1995). W_m was determined by titration of the oil phase with water (or water-diol mixture), as previously reported (Regev et al., 1996). Relative error was estimated as ± 2% for calculated W_m (wt %) and ± 0.5% for calculated A_T (%).

Solubilization of Diclofenac

Diclofenac sodium was solubilized to a final concentration of 1% (w/w) in preparations along dilution line W46 (composition of 40 wt% surfactant and 60 wt% oil phase—jojoba or paraffin oil/ hexanol at 1:1 wt ratio) (Figure 2). Diclofenac was added first to the oil while mixing and heating to 40°C. Afterwards, appropriate amounts of surfactant, cosurfactant, and water were gradually added to the oil phase. The resulted microemulsion was cooled to room temperature.

Viscosity Behavior

Microemulsions are Newtonian but viscosity is composition and structure-dependent. Viscosity behavior was examined using a CSL-50 R&D-grade rheometer (TA Instruments GmbH, Alzenau, Germany). Steady shear viscosity was determined. The samples were thermostated at 25°C using a model 9100 thermostat from PolyScience, Division of Preston Industries, Inc. (Niles, IL, USA).

SD-NMR Measurements

NMR measurements were performed at 25°C on a Bruker DRX-400 spectrometer with BGU gradient amplifier unit and a 5 mm BBI probe equipped with a z-gradient coil, providing a z-gradient strength (g) of up to 55 G cm^{-1}. The self-diffusion coefficients were determined using bipolar-pulsed field gradient stimulated spin-echo (BPFG-SSE). This work utilized bipolar gradient pulses as described by Wu et al. (Garti et al., 1995) for reduced eddy current ring down. Experiments were carried out by varying g and keeping all other timing parameters constant. The self-diffusion coefficient (D) is given by

$$I = I_0 \exp(-R(t) - (\gamma \delta G)^2 D(\Delta - (\delta/3)) \quad (1)$$

where I is the measured signal intensity, I_0 is the signal intensity for g = 0, δ is the gyro magnetic ratio for the ^{1}H nucleus, δ is the gradient pulse length, Δ is the time between the two gradients in the pulse sequence (and hence defines the diffusion time), and $R(t)$ is a constant that takes into account nuclear relaxation. Since $R(t)$ is a constant in our experiments, it may not be considered further. Typical experiments used a Δ of 100 ms, a δ of 8 ms, and g values from 1.7 to 32.3 G cm^{-1} in 32 steps.

SAXS Measurements

Small angle X-ray scattering (SAXS) measurements were performed using Ni-filtered Cu Kα radiation (0.154 nm) from a Philips sealed tube X-ray generator that operated at a power rating of up to 1.36 kW. X-radiation was further monochromated and collimated by means of a single Franks mirror and a series of slits and height limiters and measured by a linear position-sensitive detector. The samples were inserted into 1.5 mm quartz capillaries. The temperature was maintained at 25 ± 1°C.

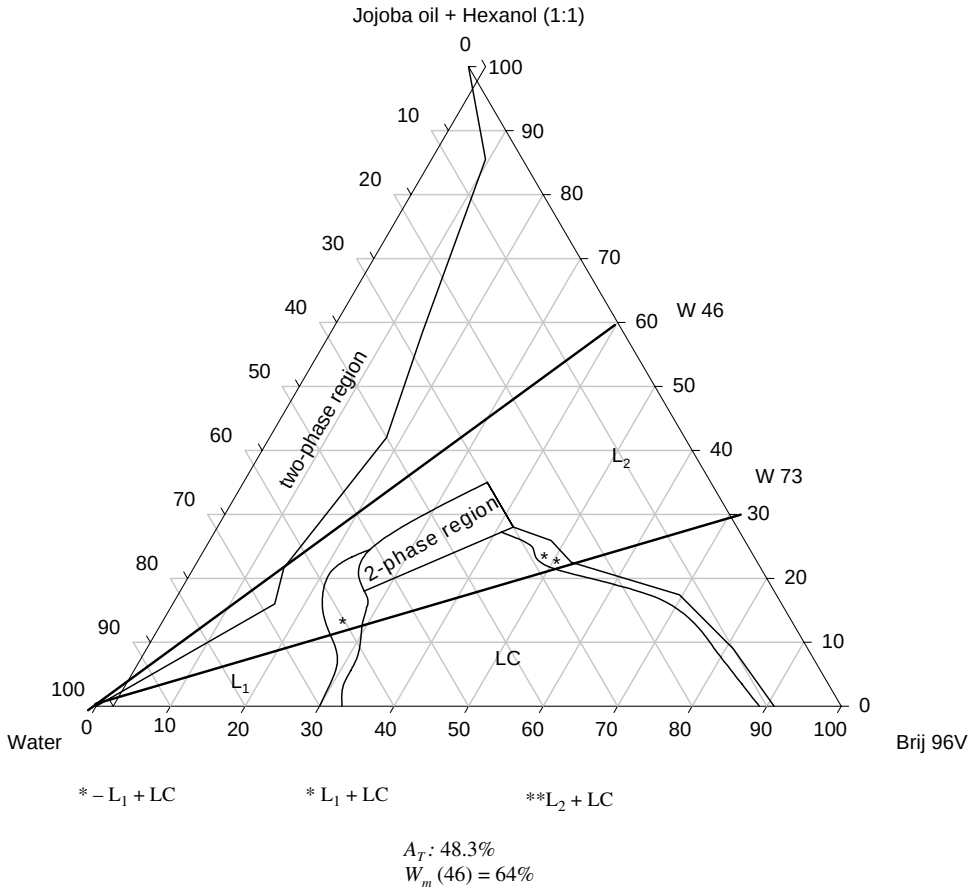


FIGURE 2. Ternary phase diagram of various combinations of water, jojoba wax/hexanol (oil phase including the cosurfactant), and polyoxyethylene-10EO-oleyl alcohol (Brij 96V, the surfactant). Water is titrated along dilution lines drawn from the oil + cosurfactant side of the triangle (0% water) to the water apex (100% water). For example, a dilution line starting from a composition of 40wt% surfactant and 60wt% oil phase (before the addition of water) is denoted as W46.

In-Vitro Skin Permeation of Diclofenac

The animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee, which complies to the Israeli Law of Human Care and Use of Laboratory Animals. The permeability of diclofenac through rat skin was measured in vitro with a Franz diffusion cell system (Crown Bioscientific, Inc., Clinton, NJ, USA). The diffusion area was 1.767 cm^2 (15 mm diameter orifice), and the receptor compartment volumes varied from 11 to 12 ml. The solutions in the receiver side were stirred by externally driven, Teflon-coated magnetic bars. Sprague-Dawley rats (males, 200–300g, Harlan Laboratories Ltd., Jerusalem, Israel, Rehovot, Israel) were sacrificed by aspiration of ethyl ether. The abdominal hair was clipped carefully and sections of full-thickness skin were excised from the fresh carcasses of animals. After subcutaneous fat was removed with a scalpel, the transepidermal water loss (TEWL) was measured before the skin sections were mounted in the diffusion cells. TEWL examinations were performed on skin pieces using Dermalab® Cortex Technology instrument, (Hadsund, Denemark) and only those pieces for which the TEWL levels were less than 10 $g/m^2/h$ were mounted in the diffusion cells, ready for testing. The skin was placed on the receiver chambers with the stratum corneum facing upwards, and then the donor chambers were clamped in place. The excess skin was trimmed off, and the receiver chamber, defined as the side facing the dermis, was filled with phosphate buffered saline (PBS, pH 7.4). After 30 minutes of skin washing at 37°C, the buffer was removed from the cells. Liquid microemulsions (see Table 1) or Voltaren® Emulgel® (Novartis) (0.5g) were applied on the skin, and the receiver chambers were filled with phosphate buffered saline (pH = 7.4). Samples (2 ml) were withdrawn from the receiver solution at predetermined time intervals, and the cells were replenished to their marked volumes with fresh buffer solution. Addition of solution to the receiver compartment was performed with great care to avoid trapping air beneath the dermis. The samples were taken into 1.5-ml amber vials and kept at -20°C until analyzed by HPLC.

Systemic Bioavailability of Topical Diclofenac Applied to Rats

The animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee, which complies to the

TABLE 1
Summary of Microemulsion Formulations Used for the Skin Penetration Studies

Microemulsion	Oil Phase	Surfactant	Co-Surfactant	Dilution Line	% Water
A1	Jojoba oil	Brij 96	Hexanol	W64	20
A2	Jojoba oil	Brij 96	Hexanol	W64	33
A3	Jojoba oil	Brij 96	Pentanol	W64	20
B1	Jojoba oil	Tween 60	Hexanol	W64	33
B2	Jojoba oil	Tween 60	Hexanol	W64	21
B3	Jojoba oil	Tween 80	Hexanol	W64	50
C1	Paraffin oil	Brij 96	Hexanol	W64	33
C2	IPM[1]	Brij 96	Hexanol	W64	33
D1	Decane	Brij 96	Hexanol	W64	33
D2	Dodecane	Brij 96	Hexanol	W64	33
D3	Tetradecane	Brij 96	Hexanol	W64	33

[1]IPM = Isopropyl myristate.

Israeli Law of Human Care and Use of Laboratory Animals, 1994 (also approved by the Division of Compliance, OPRR, OD of the US-NIH as a foreign institution with compliance to the standards for Human Care and Use of Laboratory Animals, approval: #A5060-01); the institution has adopted policies regarding animal care and use as outlined in the guide for the Care and Use of Laboratory Animals of the National Academy of Sciences, USA.

Male Sprague-Dawley rats (400–500g, Harlan Laboratories Ltd., Jerusalem, Israel) were anesthetized (15 mg/kg pentobarbital sodium *i.p.*) and were placed on their back. The hair on abdominal skin was trimmed off and TEWL was measured to check skin integrity, then it was washed gently with distilled water. Anesthesia was maintained with 0.1 ml pentobarbital (15mg/ml) along the experiment. Microemulsions (A2, C1, C2; see Table 1) containing diclofenac sodium (0.5ml) were applied on the skin surface (3.4 cm^2) in open containers glued to the skin by a silicon rubber. After 1, 2, 4, 6, and 8 hours of in vivo drug application, blood samples (0.5–0.7 ml) were taken from the tail vein into heparinized tubes. After separation, plasma samples were kept at –20°C until analyzed. Just before analyzing, 100 µl plasma were taken with 200 µl of perchloric acid solution (6%v/v), vortexed and centrifuged at 10,000 rpm for 5 minutes. The supernatant was transferred to a clean vial and injected into the HPLC as described below.

HPLC Analysis of Drug from Receiver Solutions and Plasma Samples

Aliquots of 20 ml from each sample were injected into the HPLC system, equipped with a prepacked C18 column (Lichrosphere 60 RP-select B, 5 mm, 125 × 4 mm). The detection of diclofenac was carried out at 275 nm. The samples were chromatographed using an isocratic mobile phase consisting of dibasic sodium phosphate (0.008M, pH 2.5) – methanol (1:3) at a flow rate of 1 ml/minute. Calibration curves (peak area versus drug concentration) were linear over the range 1–20 mg/ml. As a result of the sampling of large volumes from the receiver solution—and the replacement of these amounts with equal volumes of buffer—the receiver solution was constantly being diluted. Taking this process into account, the cumulative drug permeation (Q_t) was calculated from the following equation:

$$Q_t = V_r C_t + \sum_{i=0}^{t-1} V_s C_i \quad (2)$$

where C_t is the drug concentration of the receiver solution at each sampling time, C_i is the drug concentration of the i^{th} sample, and V_r and V_s are the volumes of the receiver solution and the sample, respectively. In vitro data were expressed as the cumulative diclofenac permeation per unit of skin surface area, Q_t/S ($S = 1.767\ cm^2$).

RESULTS AND DISCUSSION

General Characteristics of the Microemulsion

The unique progressively water-diluted microemulsions were constructed by well-balanced blends of oil, ethoxylated alcohol (Brij 96V), and medium chain alcohol as the cosurfactant (Shevachman, Shani, & Garti, 2004). A typical phase diagram for the system composed of jojoba oil, alcohol, water, and ethoxylated alcohol (Brij 96V) is shown in Figure 2. Detailed phase diagrams for the paraffin oils were not constructed but were examined along 46 dilution line (W46), in which a full dilution was ascertained with any one of the tested paraffin oils. The solubilization of diclofenac in a typical four-component system consisting of jojoba oil, hexanol, Brij 96V, and water was studied along water dilution line W46 (40 wt % surfactant and 60 wt % oil phase). The W46 line is the line along which maximum water was solubilized within the surfactant/oil phase (W*m* = 65%).

Viscosity measurements have proven to be a useful tool for elucidating secondary structural changes of the system. Samples examined along W46 dilution line exhibit Newtonian flow behavior (viscosity is independent of the shear rate). At first, viscosity increases gradually with increasing water content, but later two major deflection points are clearly seen. After a maximum value is reached at a water fraction (ϕ_w) of 0.25, the viscosity declines, and subsequently flattened out at $\phi_w = 0.50$. At low water contents the interactions between the dispersed particles are negligible and the microemulsion consists of isolated hard sphere-like globules of water dispersed in the continuous oil medium (Gradzielski & Hoffman, 1999). The initial rise in viscosity with increasing water content is attributable to the growth of dispersed droplets and increasing interdroplets interactions along with some structural deformation (spherical droplets turning into disc-like and warm-like droplets. The formation of clusters drawn from adjacent globules also is feasible. The decline in viscosity at $\phi_w > 0.25$ is related to the first structural change. From $\phi_w = 0.25$, the transition from a W/O microemulsion to bicontinuous structures starts to occur progressively and the water migrates to the outer phase. After 50% of water has been absorbed in the system, a second transition occurs corresponding to a bicontinuous transition to O/W microemulsion. Slowly, the droplets are increasingly diluted, the inter-droplets interactions decrease, and once high dilution is achieved spherical O/W droplets are formed. The transition to O/W microemulsion is manifested in a decrease in the viscosity.

The SAXS spectra of microemulsions exhibit a single broad maximum at $q \neq 0$, followed by a monotonic decrease of the scattering intensity $I(q)$ at large values of the wave vector amplitude $q[q = (4\pi/\lambda)\sin\theta$ where 2θ is the scattering angle and $\lambda = 1.54$ Å for Cu radiation]. The scattering pattern was fitted to the expression derived by Teubner and Strey (1987):

$$I(q) = \frac{1}{a_1 + c_1 q^2 + c_2 q^4} + b \tag{3}$$

where a_1, c_1, c_2, and b are constants. Such a functional form is convenient for the fitting of spectra.

Equation 3 corresponds to a real space correlation function of the form

$$\gamma(r) = \sin kr/kr\, e^{-r/\xi} \tag{4}$$

The correlation function describes a structure with periodicity $d(d = 2\pi/k)$ damped as a function of the correlation length ξ. d and ξ are related to the constants in Equation 3 by (Teubner & Strey, 1987):

$$d = \left[\frac{1}{2}\left(\frac{a_1}{c_2}\right)^{1/2} - \frac{c_1}{4c_2}\right]^{-1/2} \tag{5}$$

$$\xi = \left[\frac{1}{2}\left(\frac{a_1}{c_2}\right)^{1/2} + \frac{c_1}{4c_2}\right]^{-1/2} \tag{6}$$

From k and ξ, Chen and Chang (1992) derived an empirical parameter that characterizes bicontinuous microemulsions for ionic systems, $k\xi$. From experiments on ionic systems in the middle range with surfactant weight fractions of less than about 15%, it was found that $1.5 < k\xi < 2$. These values characterize disordered sponge-like bicontinuous structures. When surfactant concentration increases (as in our case), $k\xi$ can exceed the above limits, and then the structure is termed ordered bicontinuous, that is, alternating sheets of oil and water separated by surfactant film (Chen et al., 1991). It has been observed that the distance between micelles (d) increases in a monotonic manner with dilution. The correlation length increases as well. When water is located at the micellar core, the surfactant aggregates swell upon dilution, resulting in an increase in the order parameter (growth of ξ). When water is part of the bulk phase, additional dilution should reduce the order of the system and therefore ξ should decrease. The effect is clearly detected at water contents > 50%. Therefore, we can conclude that microstructural transition to O/W microemulsion occurs at this point. Graphs plotting $1/d$ versus water content (ϕ_w) can provide information about the dimensionality of swelling along the dilution line W64. Swelling with water can be described by (Cabos, Delord, & Marignan, 1988):

$$1/d = 1/d_0\,(1 - \phi_w) \tag{7}$$

where d denotes the periodicity and d_0 the thickness of the (surfactant + oil + alcohol) layer. If the swelling is one-dimensional, the graph of $1/d$ versus ϕ_w should be linear, with the intercept on the abscissa at $\phi_w = 1$. Isotropic systems that obey this equation have been characterized as "local lamellar structures." Such behavior is distinctive to dilution line W46 at $0.25 < \phi_w < 0.5$. The values calculated for $k\xi$ lie somewhat beyond this range (larger than 2), which means that ordered bicontinuous structures have been formed in this region. Similar behavior was obtained in an earlier study for a system composed of dodecane/pentanol/$C_{12}EO_8$ along dilution line W55 (Ezrahi, Wachtel, Aserin, & Garti, 1997). At $\phi_w > 0.5$ the system is no longer a bicontinuous microemulsion, and the microstructure of the system has undergone transformation to O/W microemulsion. These results are consistent with the viscosity measurements.

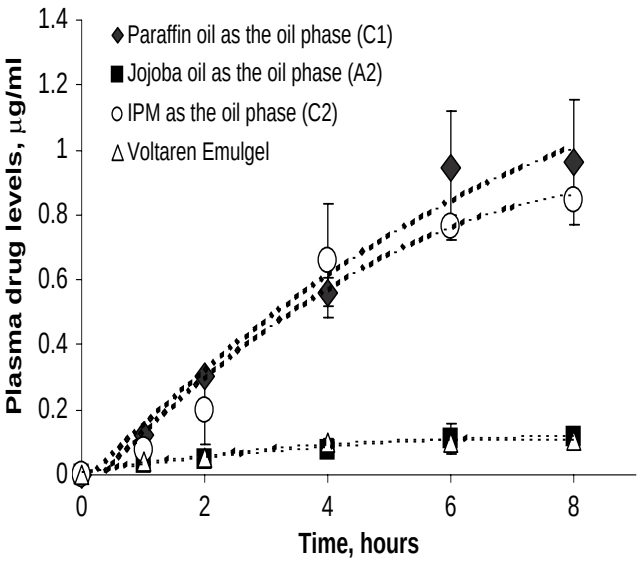
Effect of Diclofenac on the Microstructure of the Microemulsion

The effect of diclofenac sodium on the microemulsion's microstructure was examined using Self-Diffusion NMR technique. Self-diffusion coefficients of each component in the

microemulsion (composed of jojoba oil/hexanol/Brij 96V/ 33% water ([with and without diclofenac sodium]) were calculated. The diffusion coefficients of each component for the drug-free system when 33% of water included were: D_{Water} = 5.08×10^{-10} m^2/s, $D_{Oil} = 2.35 \times 10^{-11} m^2/s$, $D_{Surfactant} = 1.82 \times 10^{-11} m^2/s$ and $D_{Hexanol} = 8.36 \times 10^{-11} m^2/s$. When the same system (33% of water included) contained 1% diclofenac sodium salt, the following diffusion coefficients were obtained: D_{Water} = $6.44 \times 10^{-10} m^2/s$, $D_{Oil} = 1.35 \times 10^{-11} m^2/s$, $D_{Surfactant} = 1.13 \times 10^{-11} m^2/s$ and $D_{Hexanol} = 5.59 \times 10^{-11} m^2/s$. It can be clearly seen that the diffusion coefficient of water obtained in the presence of diclofenac is slightly larger than that obtained from the drug-free system. In contrast to the water diffusion coefficient, the value obtained for the oil and the surfactant decreased. From these important data it can be concluded that the drug molecules entered into the interface of the system ($D_{Surfactant}$ and $D_{Hexanol}$ decreased), accumulated in the interfacial film and, to some extent, dissolved in the oil (D_{Oil} also decreased).

Percutaneous Absorption of Diclofenac (In Vivo Studies)

The percutaneous absorption of diclofenac from the U-type dilutable microemulsions to the systemic circulation was examined in rats. Microemulsion consisting of jojoba oil/hexanol/Brij 96V/water was compared with similar microemulsions (i.e., same ingredients' ratio) containing paraffin oil/hexanol/Brij 96V/water and isopropyl myristate (IPM)/hexanol/Brij 96V/water. Voltaren Emulgel also was compared in this study as an example of diclofenac-containing macroemulsion formulation. It should be pointed out that the introduction of the commercial Emulgel into this study was for demonstrative purposes only without any consideration for the clinical implications of the therapeutic use of this drug. The effect of the oil constituent is demonstrated in Figure 3, showing that jojoba oil-containing microemulsion was as effective as the commercial emulsion by penetrating the drug at the same rate. In comparison, paraffin oil- and myristate ester-containing microemulsions increased the transdermal penetration rate of diclofenac by two orders of magnitude, whereas the plasma concentration was increased by one order of magnitude. Concerning the drug permeability aspect, the difference between the microemulsions may be simply attributed to the three-dimensional structure of jojoba wax (Figure 1B) as compared with the linear structures of paraffin oil and IPM. Although other properties of the microemulsion could influence the drug diffusion process, it may be reasonable to conceive that the steric configuration of jojoba wax restrains the diffusive motion of diclofenac in the vehicle, that is, through the interface and in the oil phase, on its way into the skin. More investigations should be performed to elucidate the exact penetration mechanism of drugs from the various microemulsions.



	C_{max}(mg·ml^{-1})	AUC (μg·ml^{-1}·h^{-1})
Paraffin oil - **C1**	0.962(± 0.191)	4.545 (± 0.615)
IPM - **C2**	0.845 (± 0.005)	4.067 (± 0.482)
Jojoba oil - **A2**	0.116 (± 0.031)	0.601 (± 0.107)
Voltaren Emulgel	0.106 (± 0.006)	0.558 (± 0.172)

FIGURE 3. Plasma diclofenac levels in rats after topical applications of three drug-containing microemulsions (all constructed on a same point over W64 dilution line) with three different oils, and a macro-emulsion exemplified by Voltaren® Emulgel® (Novartis).

Percutaneous Penetration of Diclofenac in Diffusion Cells (In Vitro Studies)

As shown in Figure 4, a significant difference in diclofenac penetration rates from the microemulsion containing jojoba oil and from the microemulsions containing paraffin oil or IPM also was noted in vitro. As in the in vivo studies, C1 and C2 microemulsions (Table 1) showed similar rates of percutaneous penetration through rat skin, while the same microemulsion composition but with jojoba wax as the oil phase (A2) resulted in a significant decrease in drug penetration through the skin (Figure 4). Unlike the in vivo results, the drug permeability in diffusion cells was higher with the jojoba wax than with the commercial macro-emulsion. In addition, the drug penetration rate using jojoba oil was lower only three fold than those obtained with mineral oil or IPM as the oil phase in the microemulsions. These discrepancies from an ideal in vivo-in vitro correlation was probably due to the optimal perfusion and

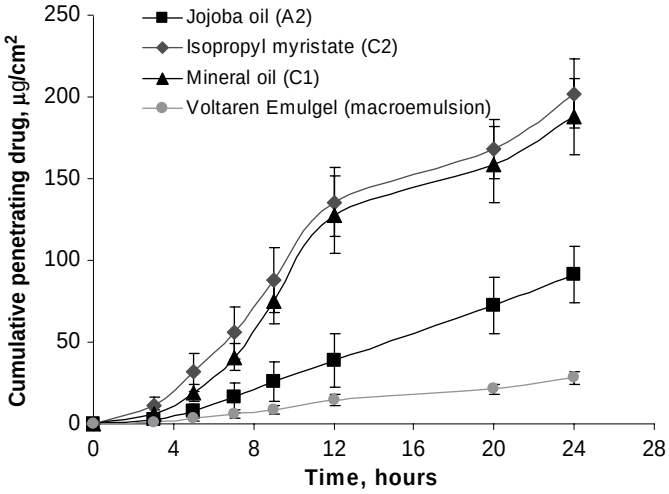


FIGURE 4. Percutaneous penetration of diclofenac using rat skin. In vitro testing of three drug-containing microemulsions (all constructed on a same point over W64 dilution line) with different oils as compared with a macro-emulsion exemplified by Voltaren® Emulgel® (Novartis).

hydration of the skin in the diffusion cells compared to the skin condition during an animal study.

The influence of the paraffin's chain length on the permeability of diclofenac was examined with C10, C12, and C14 chains. It is demonstrated in Figure 5 that no significant differences were found between the three chain lengths ($p > 0.05$). This may indicate that the length of the paraffin chain within the examined range does not serve as a main factor affecting the drug permeability. As mentioned above, it is presumed that the predominant factor is the spatial interactions occurring between of the oil and the diffusing drug through the interfacial membrane of the microemulsion.

The role of hexanol as an advantageous cosolvent has been previously described (Shevachman et al., 2004). Medium-chain alcohols such as butanol, pentanol, and hexanol have better solubility in oil but are relatively less soluble in water (compared to ethanol), and thus they penetrate to some extent into hydrophobic core and decrease its hydrophobicity (as cosolvents). In the present study, its additive value to the penetration process has been examined against a lower alcohol, pentanol. It can be seen in Figure 6 that diminishing of just one -CH_2 from the alkanol chain, used as a cosolvent, resulted in a significant reduction in the penetration rate of diclofenac. Long-chain alkanols, such as octanol and heptanol, do not form U-type microemulsions. The contribution of the non-ionic surfactant, polyoxyethylene-10EO-oleyl alcohol or Brij 96, to the skin penetration process of diclofenac was examined by comparison with the most commonly-used polysorbate surfactants. Figure 7 demonstrates that by using polysorbate 60 or 80, the microemulsion delivered the drug to the receiver side at lower rates and extents compared with the microemulsion consisting of Brij 96 as the surfactant. These findings regarding microemulsions with polysorbate 60 and 80 as the surfactants may provide the basis for elucidating the mechanism by which only certain microemulsions may be effective. It may occur that the substitution of polyoxyethylene-10EO-oleyl alcohol with polysorbates obstructs drug transport by rigidifying the interface between the water of the inner phase and the oil-continuous phase, resulting in a significant decrease in migration of diclofenac through the water channels within the droplet clusters. Interfacial rigidity may be formed by certain surfactants (e.g., polysorbates) due to strong hydrogen bonding interactions occurring between the bulky polar head groups in the interface as well as between the polar groups and drug molecules.

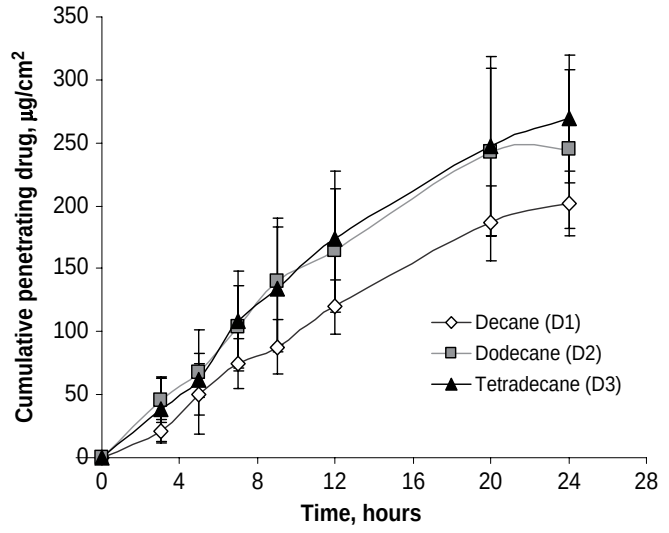


FIGURE 5. Percutaneous penetration of diclofenac using rat skin. Influence of paraffin chain lengths on drug permeation.

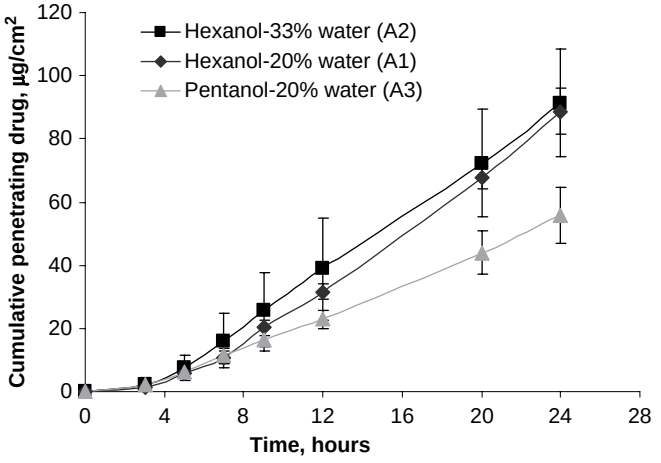


FIGURE 6. Percutaneous penetration of diclofenac using rat skin. Influence of the alkanol on drug permeation. A comparison was made between hexanol-containing microemulsions (at two water concentrations) and pentanol-containing microemulsion.

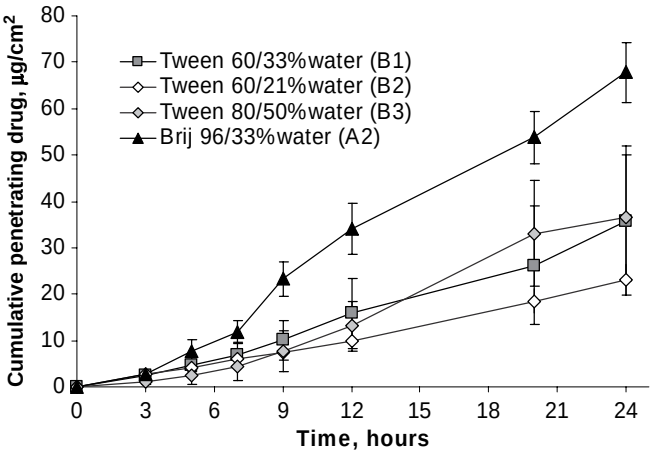


FIGURE 7. Percutaneous penetration of diclofenac using rat skin. Influence of the surfactant on penetration of drug through the skin.

CONCLUSION

It was demonstrated that skin permeation of diclofenac from microemulsion formulation based on polyoxyethylene-10EO-oleyl alcohol as the surfactant and hexanol as the cosurfactant was significantly higher than that from conventional cream formulation. It was shown that such formulations containing paraffin oils (C1, D1-D3) or isopropyl myristate (C2) increased the penetration of the drug through the skin compared with a macroemulsion cream. However, it has been found that jojoba wax as the oil phase in the microemulsion (A1-A2) did not contribute to drug permeation via the skin. It probably seems to prevent active molecules from being freely diffused into the skin. The explanation for this phenomenon might be based on the three-dimensional structure of jojoba oil. Thus, the non-linear configuration of the wax (as compared with paraffins and myristate ester) may result in delaying the diffusion of the drug while migrating from the interfacial film into the continuous oil phase and vice versa. It should be noted that a previous publication by Schwarz et al. (1996) has reported an improved anti-inflammatory activity following topical application of diclofenac in a O/W submicron emulsion based on jojoba oil. Our finding cannot be in contrast to the cited report from two reasons: (a) the plasma levels of diclofenac after the topical application of microemulsions may be much higher than that needed to obtain a similar anti-inflammatory effect in the animal model; and (b) the jojoba wax as the continuous phase of W/O microemulsions (or even in those having a bicontinuous structure) has more influence on drug penetration than formulations having the wax entrapped inside the droplets.

In summary, an appropriate combination of the oil, the cosurfactant, and the surfactant should be the major concern in microemulsion formulation for transdermal drug delivery and is definitely the success key of pharmaceutical development in this field.

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