Pharm Res (2011) 28:1683–1695
DOI 10.1007/s11095-011-0404-y

RESEARCH PAPER

Microemulsion Microstructure Influences the Skin Delivery of an Hydrophilic Drug

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Received: 19 November 2010 / Accepted: 21 February 2011 / Published online: 25 March 2011

ABSTRACT

Purpose We aimed to investigate the influence of microemulsion nanoscale organization as either oil-in-water droplets, water-in-oil droplets, or bicontinuous structures on skin delivery of drugs assisted by microemulsions.

Methods Three microemulsions of different microstructure, o/w, w/o, and bicontinuous at the skin temperature (32°C), having the same oil and water contents and containing the same ingredients were selected using the Kahlweit fish phase diagrams method. The microemulsions are quaternary mixtures of the Polysorbate 21 (Tween®21) and Sorbitan monolaurate (Span®20) surfactants, isononyl isononanoate oil and water. The microemulsion nanostructure was characterized by electrical conductivity, Pulsed Field Gradient Spin-Echo NMR and Small-Angle Neutron Scattering measurements. The Franz cell method was used to monitor skin absorption of caffeine loaded in microemulsions over 24 h exposure to excised pig skin.

Results Three microemulsions with the three structures were selected, keeping the same composition but the Tween®21/Span®20 ratio. The transdermal flux of caffeine was in the order aqueous solution ≈ w/o < bicontinuous < o/w microemulsion. The o/w microemulsion allows the permeation of 50% of the applied dose within 24 h.

Conclusions The structure of microemulsions is of relevance for skin absorption. The water-continuous structures allow faster transport of hydrophilic drugs.

KEY WORDS microemulsion · microstructure · percutaneous penetration · phase diagram · skin absorption

INTRODUCTION

Microemulsions are attractive drug carriers as modern drug delivery systems because of their high solubilization capacity and the possibility of controlling drug delivery rates. In particular, they have received considerable attention for topical use; recent review papers give an overview of the topic (1–4). It is recognized and demonstrated that permeation rates from microemulsions were significantly higher than from conventional emulsions or other vehicles (5–10).

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Microemulsions are defined as disperse systems containing at least oil, water and surfactant. On a microscopic level, microemulsions are structured into water-rich and oil-rich domains separated by an amphiphilic layer. The term *microemulsion*, introduced in 1943 by Hoar and Schulman (11), is misleading because it leaves aside the issue of the microstructure (12). Actually, the mixture of oil, water and surfactant leads to the formation of a wide variety of structures and phases easily recognized macroscopically. However, within the microemulsion regions, several internal structures can form: oily or water droplets and even bicontinuous structures. As the curvature of that film tends to zero, the droplets merge into an equilibrium bicontinuous structure made of two interconnected three-dimensional networks of water and oil. Such structures introduced by Scriven (13) are characterized by their higher amphiphilic character, lower interfacial tension and higher solubilizing properties because "bicontinuous" means they are likely to occur when similar amounts of oil and water are present. Whatever the structure of microemulsions, these dispersions are highly dynamic structures, undergoing continuous and spontaneous fluctuations with water- or oil-rich domain typically in the range of 1–100 nm.

Many physicochemical properties of microemulsion have been reported to influence the drug delivery to the skin. Several mechanistic hypotheses have been put forward. Kreilgaard (4) suggested that contact to the skin might be improved by the low interfacial tension between water and oil phases. Drug bioavailability is increased because both the low interfacial tension and the fluctuating oil–water interface facilitate materials transfer between both lipophilic and hydrophilic domains of the microemulsion and to the *stratum corneum*. The penetration enhancer effect of the excipients of the microemulsion has been reported (9). Lastly, the high drug loading capacity of microemulsions allows the administration of higher concentrations of poorly soluble drugs (14, 15). These physicochemical phenomena are particularly prominent in the case of bicontinuous microemulsions regarded as dispersions with very low interfacial tension, highly fluctuating interface and higher solubilising properties compared to globular microemulsions (16, 17).

On this basis, it is expected that the structure of the microemulsion is of relevance; one may presume that the percutaneous release from bicontinuous microemulsions is faster than from globular microemulsions. Since there are several parameters that influence skin absorption phenomena driven by microemulsions, it is quite difficult to decide which parameter is operating. The reports of the literature do not help so much in inferring the physico-chemical mechanisms, because several parameters were varied at the same time in most instances.

The present study aims at investigating the influence of the microemulsion microstructure on drug delivery to skin. For that purpose, the skin absorption of caffeine as a model drug was studied from microemulsions of the three different microstructures (o/w microemulsion, w/o microemulsion and bicontinuous). The experiment was designed such that the microemulsion microstructure was changed, while keeping all other parameters constant. The work started by the selection of three formulations of microemulsions having the same composition but only differing by their microstructure. Since pharmaceutical and cosmetic applications are intended, mild ingredients ensure low irritancy: fatty esters as oils and sorbitan esters as non-ionic surfactants. Phase diagrams were systematically drawn, and a detailed physico-chemical characterization by electrical conductivity, Pulsed Field Gradient Spin-Echo Nuclear Magnetic Resonance (PFGSE NMR) and Small-Angle Neutron Scattering (SANS) allowed checking against the microstructure. Finally, percutaneous penetration of caffeine from the three microemulsions was conducted *in vitro* on excised pig skin using the Franz cell method.

MATERIALS AND METHODS

Materials

Isononyl Isononanoate (DUB ININ) was a kind gift from Stéarinerie Dubois (Boulogne-Billancourt, France). Sorbitan monolaurate (Span®20 (HLB 8.6)) and Polysorbate 21 (Tween®21 (HLB 13.3)) were supplied by Croda (Trappes, France). De-ionized water of 18 $M\Omega.cm^{-1}$ resistivity was used throughout the work. Deuterated water 99.8% was purchased from Eurisotop (Saclay, France) for SANS and NMR experiments. Caffeine monohydrate was purchased from Sigma (Saint Quentin Fallavier, France).

Methods

The different microstructures of microemulsions are difficult to assess, because there is no well-established method of structural investigation. Phase diagrams allowed the selection of the three microemulsions, and the structure was checked by careful microscale characterization using small-angle neutron scattering for the microstructure, electrical conductivity and NMR self-diffusion measurements for the transport properties that give the large-scale topology of the structure.

Phase Diagrams

Phase diagrams were constructed using the Kahlweit fish representation (18) where the phase domains are reported with respect of the two variables: concentration of surfactant and temperature.

In order to study the effect of microstructure, three formulations of very similar composition and different microstructures were selected. The mixed surfactant system was made of a hydrophilic (Tween®21) and a hydrophobic (Span®20) surfactant (mixture of non-ionic surfactants compatible for the clinical use). The percentage of each surfactant was adjusted for obtaining different types of microemulsion (o/w, bicontinuous, and w/o) at 32°C (skin temperature) for a fixed oil/water ratio = 1/1. The microemulsions were prepared by mixing different components (oil, water and surfactant mixture) under magnetic stirring. Preparations were placed in ovens at different temperatures (from 10°C to 70°C with 5°C steps) for equilibration. Samples were sorted as monophasic (clear), diphasic with an oil excess phase (o/w microemulsion + oil), diphasic with a water excess phase (w/o microemulsion + water), or triphasic (bicontinuous microemulsion + oil + water). Observation of monophasic samples between crossed polars allowed distinguishing isotropic microemulsions and anisotropic lamellar mesophases that are birefringent.

Physico-Chemical Characterization

Polarizing light microscopy was first used to decide on optical isotropy, a basic feature of microemulsions. Microemulsions are isotropic, whereas lamellar phases are birefringent. The structures of microemulsions (o/w, bicontinuous and w/o) were determined by different methods: electrical conductivity (19, 20), Pulsed Field Gradient Spin-Echo Nuclear Magnetic Resonance (PFGSE NMR) (21, 22) and Small-Angle Neutron Scattering (SANS) (23).

Electrical Conductivity. Conductivity measurements do not only help to determine the nature of continuous phase of microemulsion, but allow also an estimation of the percolation threshold (19, 20), corresponding to the transformation from droplet-like to bicontinuous microemulsions as the droplets interconnect. Conductivity measurements were performed in triplicate using a CDM 210 Radiometer conductivity meter. Results are given as a function of temperature (±0.5°C) on samples in the one-phase domain. The electrode was dipped in the microemulsion sample until equilibrium was reached and reading became stable. The o/w microemulsions are conductive, while the w/o microemulsions are insulators. Thus, conductivity scans switched between two plateaus correspond to water-continuous and oil-continuous structures.

Pulsed Field Gradient Spin-Echo NMR (PFGSE NMR). The PFGSE NMR technique yields valuable information with respect to the microstructure of the system (21, 22). The measurements were made at the technical platform "Centre Commun de RMN" CCRMN, University of Lyon, Villeurbanne (France) (http://ccrmn.univ-lyon1.fr/). The ^{1}H 2D NMR DOSY spectra were collected at 32°C on a Bruker spectrometer Avance III working at 500 MHz. Samples were prepared by using D_2O in place of H_2O in order to use the deuterium NMR signal for locking the frequency to the magnetic field. Tubes of 3 mm diameter were used in order to suppress convection effects for measurement of the slowest diffusion coefficients. The standard Bruker sequence dstebpgp3s was used. Strong gradients of short duration were obtained using a trapezoidal shape for pulses (p30 = 5.12 ms, falling and raising times of 50 μs), and the diffusion delay was set at 200 ms. Data were processed with Topspin 2.3 and NmrNotebook 2.5 with the maximum number of iterations.

Small Angle Neutron Scattering (SANS). SANS measurements were performed at 32°C on the PAXE spectrometer at the Laboratoire Léon Brillouin (LLB) (24). The microemulsion samples were contained in quartz cuvettes of 0.2 mm optical path, giving 80% transmission that ensures favorable scattering conditions (negligible multiple scattering). Scattered intensity was collected on a 2D-detector; it was radially averaged, giving the standard scattering pattern of isotropic samples as a function of the modulus of the scattering vector $q = \frac{4\pi}{\lambda} sin\left(\frac{\theta}{2}\right)$ (momentum transfer) in the range 7×10^{-3} Å^{-1} $< q <$ 0.23 Å^{-1}. Data processing was made according to standard procedures available at the LLB as software packages (25).

Microemulsions have been prepared with the same chemical composition as used for percutaneous penetration experiments, but normal water was substituted by deuterated water.

Oil–Water Partition Coefficient of Caffeine. The shake flask method described in the NF T 20–043 AFNOR method (26) was followed. Briefly, the two solvents water and isononyl isononanoate were mutually saturated at the temperatures of the experiment. Then, equal amounts (5 g) of isononyl isononanoate and water containing 80 mg of caffeine were equilibrated at 32°C and 20°C for 24 h. The water and oil phases were separated by ultracentrifugation and analyzed for caffeine by HPLC. The partition coefficient P was calculated as the ratio of the caffeine concentrations expressed in mol/L. Experiments at each temperature were carried out in triplicate.

In Vitro Skin Penetration

Full-thickness skin was obtained from Landras et Pietrain pigs sacrificed at the Laboratoire de Pharmacologie (Faculty of Medicine, University of Lyon 1, France). The skin was cleaned up with water. The hairs were cut with an

electric cutter. The skin surface was shortly washed with 1% sodium dodecyl sulfate aqueous solution and rinsed with water. The thickness of each section (0.9±0.1 mm) was measured with a micrometer (Mitutoyo). The skin was mounted between the halves of vertical Franz-type diffusion cells, the *stratum corneum* facing the donor chamber containing the microemulsion where the caffeine is dissolved. The receptor solution which is in contact with the dermal side was composed of 0.9% NaCl aqueous solution. The solubility of caffeine was 25.16 mg.mL^{-1} in 0.9% sodium chloride aqueous solution at 32°C. Sink conditions were ensured. The receptor solution was continuously stirred with a small magnetic bar. The area available for diffusion was 2.54 cm^2. One gram of each formulation was uniformly spread on the skin surface. The diffusion cells were immersed in a water bath at 37°C, which maintained a constant temperature of the receptor fluid and the skin surface temperature at 32±1°C. The diffusion experiments were conducted in six replicates over 24 h with all the formulations (o/w, bicontinuous, w/o microemulsions, and the aqueous solution) containing the same concentration of caffeine (0.8%). The receptor fluid was withdrawn and refilled by fresh medium at 15 mn, 30 mn, 1 h, 1.5 h, 3 h, 4.5 h, 6 h, 7.5 h, 9 h, 15 h, 17 h, 19 h, 21 h and 24 h exposure times. The collected samples were filtered and analyzed by HPLC. After 24 h exposure, the Franz cells were dismantled and the distribution of caffeine was measured in the donor compartment and the different skin layers.

The *stratum corneum* was separated into 21 layers by tape-stripping using an adhesive tape D-Squame® (Monaderm, Monaco); caffeine was extracted out of the strips, and extracted samples were filtered and analyzed by HPLC. The viable epidermis was separated from the dermis by heat treatment in water at 60°C for 45 s. After separation, the epidermis and dermis were cut into pieces with a scalpel, collected in a vial with acetonitrile to extract caffeine from samples, and then they were filtered and analyzed by HPLC.

HPLC Analysis of Caffeine Content

The samples were analyzed for caffeine using liquid chromatography with a reverse phase column. The HPLC setup from Waters (St Quentin-en-Yvelines, France) was composed of a Waters 717 injector, a Waters 600 pump, a reverse phase column XTerra® MS C18 (3.9 mm× 150 mm, 5 µm) and a Waters 2996 photodiode array UV-detector working at 271 nm wavelength. The elution with water/acetonitrile/acetic acid (85:15:1 v/v) solvent at 1 mL.min^{-1} flow rate and 35°C gave a retention time of 6 min for caffeine. The calibration curve for quantitative analysis was linear up to 40 µg.mL^{-1}. Repeatibility was 1% and reproductibility was 2%, as assessed from a sample of the calibration curve (10 µg.mL^{-1}). The LOD value was 8 ng.mL^{-1} and the LOQ was 24 ng.mL^{-1}.

Data Analysis

Cumulative amounts of caffeine (µg.cm^{-2}) permeating through the skin were corrected to account for the previous sample removal and plotted against time. Permeability coefficient (P_S) and lag times were calculated using the pseudo-steady-state slopes from plots of cumulative permeation against time.

The standard error of the mean (SEM) of $n=6$ determinations was calculated. Statistical comparisons were made using the Student's *t*-test (two-sample assuming equal variances) and analysis of variance (ANOVA, single factor) with the level of significance at $p\leq 0.05$.

RESULTS

Formulation of Microemulsions

Methodology for Drawing Phase Diagrams

It is most usual to investigate the phase sequence of microemulsions described by Winsor (1948) (27) as a function of the hydrophilic character of the surfactant mixture using isothermal pseudo-ternary phase diagrams where the three pseudo-components are the water, oil, and surfactant mixture (Tween®21 + Span®20) as a third pseudo-component. In the present case, the hydrophilic character of the surfactant mixture is varied by selection of appropriate amounts of the hydrophilic and hydrophobic surfactants Tween®21 and Span®20. The method using pseudo-ternary phase diagrams is quite tedious and poorly informative. Indeed, experimental phase diagrams are distorted with respect to the theoretical ones, so that it is difficult to decide which type of Winsor phase behavior they correspond to. Additionally, they do not give information on the microstructure of microemulsions. Conversely, phase diagrams as a function of temperature provide such structural information because the temperature behavior of nonionic surfactants is well-known. Bicontinuous microemulsions are obtained at the phase inversion temperature (PIT); o/w microemulsions are found below the PIT, and w/o microemulsions are present above the PIT (28). A robust method for assessing the temperature-dependent phase behavior makes use of the fish diagrams introduced by Kahlweit (18). A vertical section at the constant oil/water mass ratio of 1 is selected through the Gibbs prism made of the pseudo-ternary phase diagrams as a function of temperature (Fig. 1). Kahlweit fish diagrams report the influence of temperature on samples containing equal masses of water and oil and increasing amounts of surfactant. This yields pseudo-binary

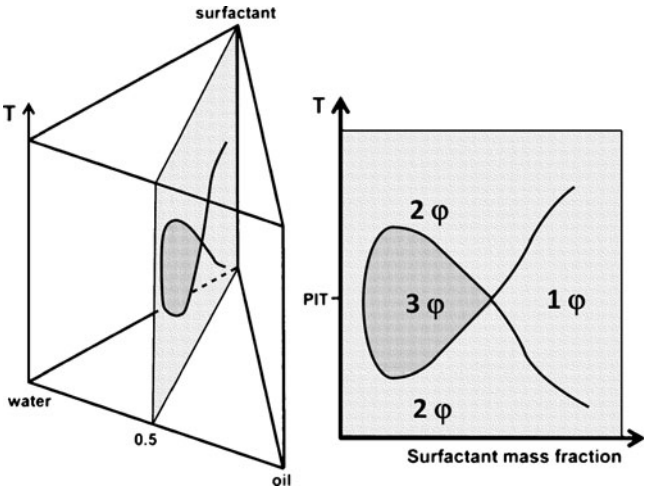

Fig. 1 Vertical section through the phase prism at a fixed water/oil mass ratio of 1/1, yielding a Kahlweit fish diagram showing the mono-, di- and triphasic domains in the temperature-surfactant content space.

phase diagrams showing monophasic, diphasic and triphasic domains in the temperature-surfactant concentration space. The concentration of mixed surfactant is expressed as its mass fraction in the sample. The overall shape of the diagram looks like a fish, where the body is the triphasic domain and the tail is the monophasic domain of microemulsions. The noticeable point of the Kahlweit fish diagram is the phase inversion point located at the link point between the tail and body of the fish, at the "phase inversion temperature" (PIT). The surfactant amount required to obtain a homogeneous single phase is minimum at this point, and the structure of the microemulsion is bicontinuous.

Kahlweit Fish Phase Diagrams

The Kalhweit fish diagrams were drawn for an oil/water weight ratio of 1/1 and different compositions of the Tween®21/Span®20 mixture (Fig. 2). The minimum concentration of mixed surfactant required for reaching a single phase system was ~25 wt% for all Tween®21/Span®20 ratios investigated. The phase inversion temperature was 43±1°C for microemulsion stabilized with pure Tween®21 (n=3); it decreased as the fraction of Span®20 increased. A plot of the PIT against the Span®20 content (Fig. 3) allowed the determination of the composition of the bicontinuous microemulsion having its PIT at 32±1°C.

Composition of the Samples for the Comparative Study

The Kahlweit phase diagrams allowed selecting the three microemulsions having almost the same composition but differing in their microstructure. According to the plot of Fig. 3, the bicontinuous microemulsion contains a Tween®21/Span®20 ratio of 90/10. The concentration of mixed surfactant should be above 25 wt%. O/w and w/o microemulsions are obtained by letting the Tween®21/Span®20 ratio depart away from the 90/10 composition. O/w microemulsion was obtained by lowering the Span®20 content to 0 wt%; w/o microemulsion was obtained by increasing the Span®20 content to 20 wt%. According to the phase diagrams, the minimum surfactant concentration required for the three samples being in the monophasic domain is 28 wt%. A mixed surfactant concentration of 30 wt% was selected for the final compositions that are reported in Table 1. It is worth noting that the three microemulsions contained the same ingredients and the same oil, water and surfactant mass fraction; they only differed by their Tween®21/Span®20 ratio. For percutaneous penetration studies the same amount of caffeine was dissolved in the various formulations; it was controlled by HPLC analysis.

Characterization of Microemulsions

Electrical Conductivity

In the same way as electrical conductivity allows identifying the continuous phase of emulsions, it allows assessment of

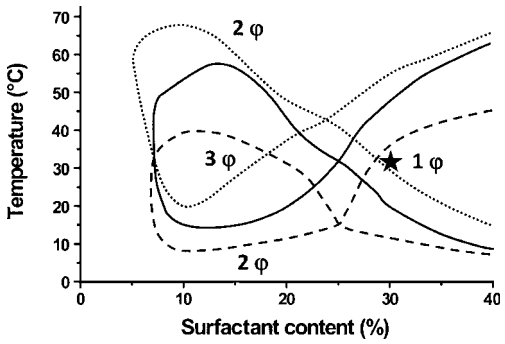

Fig. 2 Kalhweit fish diagrams for different Tween®21/Span®20 ratios: ..... : 100/0; — : 90/10; – – : 80/20. ★: Composition of the microemulsions used in this study with different Tween®21/Span®20 ratios.

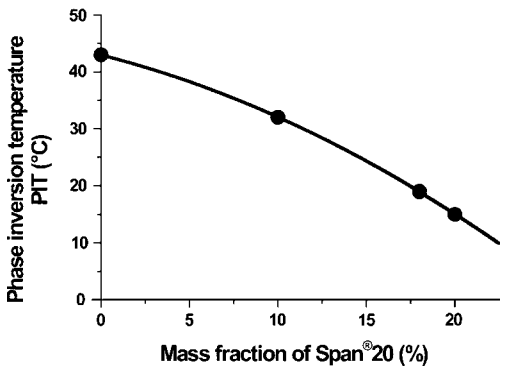

Fig. 3 Variation of phase inversion temperature as a function of the mass fraction of Span®20 in the total surfactant mixture. The *solid line* is a second degree polynomial fit to the data.

Table I Composition (wt%) of Investigated Formulations and Caffeine Content Measured by HPLC (Mean ± SEM; $n = 3$)

	O/W	Bicontinuous	W/O	Solution
Tween®21	30	27	24	–
Span®20	–	3	6	–
Isononyl isononanoate	35	35	35	–
Water	35	35	35	100
Caffeine content	0.763 ± 0.003	0.725 ± 0.001	0.727 ± 0.004	0.783 ± 0.003

the continuous domain of microemulsions. Indeed, water is conductive, whereas oil is insulating. Therefore, o/w microemulsions where water forms a continuous domain are conductive; w/o microemulsions are insulators. Transformation of a w/o into a bicontinuous microemulsion takes place through a percolation transition, which is easily detected by electrical conductivity measurements (19, 20). The percolation transition from an o/w to a bicontinuous microemulsion is less visible by means of a conductivity scan. Electrical conductivity has been measured as a function of temperature in the single phase domain of the three microemulsions ($n = 3$) (Fig. 4). The same sequence is observed for the three microemulsions: the high conductivity at low temperature indicates o/w microemulsion; the conductivity decreases as the o/w turns into a bicontinuous structure where the water mobility is restricted; finally, a low conductivity is reached as the w/o microemulsion is formed. This behavior suggests that the system undergoes a structural inversion from o/w to w/o microemulsion through the bicontinuous structure as the temperature increases. The three behaviors are similar; the conductivity drop is shifted with respect to temperature.

At the fixed temperature of 32°C, the microemulsion with a Tween®21/Span®20 ratio of 100/0 (sample A) has a high conductivity that remains constant with respect to temperature; this is characteristic of the o/w microemulsion structure. Similarly, the microemulsion with a Tween®21/Span®20 ratio of 80/20 (sample C) has a low and constant conductivity characteristic of the o/w structure. The behavior of the microemulsion with a Tween®21/Span®20 ratio of 90/10 (sample B) at 32°C is intermediate, and the conductivity varies as a function of temperature. This is the domain of the bicontinuous microemulsion. Conductivity scans with respect to temperature were not analyzed further for the determination of the percolation thresholds; this simply provides an experimental proof of the microstructures of the three microemulsions at 32°C as o/w, bicontinuous and w/o.

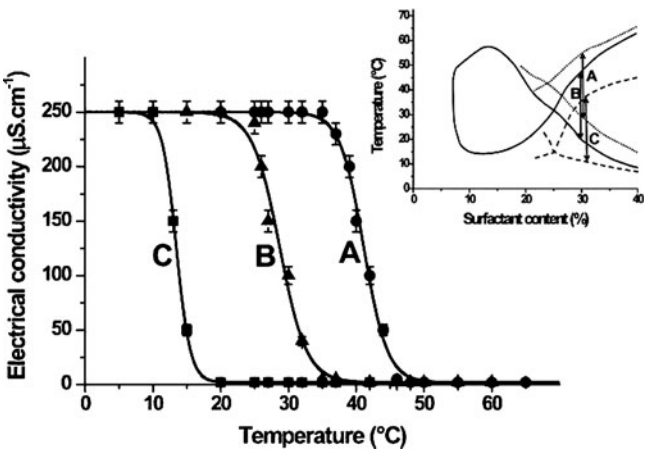

Fig. 4 Electrical conductivity of the system (Tween®21/Span®20/isononyl isononanoate/water) measured ($n = 3$) in the monophasic domains of microemulsion containing 30% mixed surfactant with different Tween®21/Span®20 ratios: A: 100/0; B: 90/10; C: 80/20.

Diffusion Coefficients

Measurements of the diffusion coefficients, D, of microemulsion components are often used in order to validate structural information. Fast diffusion is characteristic of free molecules (in solution), while slow diffusion indicates restricted mobility caused by binding to macromolecules or confinement inside droplets (21, 22).

The self-diffusion coefficients allow discriminating o/w, bicontinuous and w/o microemulsions. In a typical o/w microemulsion, diffusion coefficients sort in the order water > oil; in the bicontinuous structure, both diffusion coefficients of water and oil are quite similar; finally, the order is reversed in w/o microemulsion.

Pulsed Field Gradient Spin-Echo NMR (PFGSE NMR) was used to measure the diffusion coefficients of water, oil and surfactant. The 2D-NMR sequence DOSY gives self-diffusion data as a map in the ^{1}H NMR chemical shift-diffusion coefficient space. A correlation peak indicates the diffusion coefficient of the molecule corresponding to the NMR line. The DOSY maps of Fig. 5 illustrate the method. In sample A, the diffusion coefficient of water found at 4.8 ppm is smaller than that of oil found for the NMR lines between 0.8 and 1.6 ppm, at 2.0 and 4.1 ppm. Water has a restricted mobility compared to oil, showing that water is located inside the droplets, whereas oil is in the continuous domain. Therefore, sample A is a w/o microemulsion. The diffusion coefficient of water in sample B is faster than that of oil, showing the sample

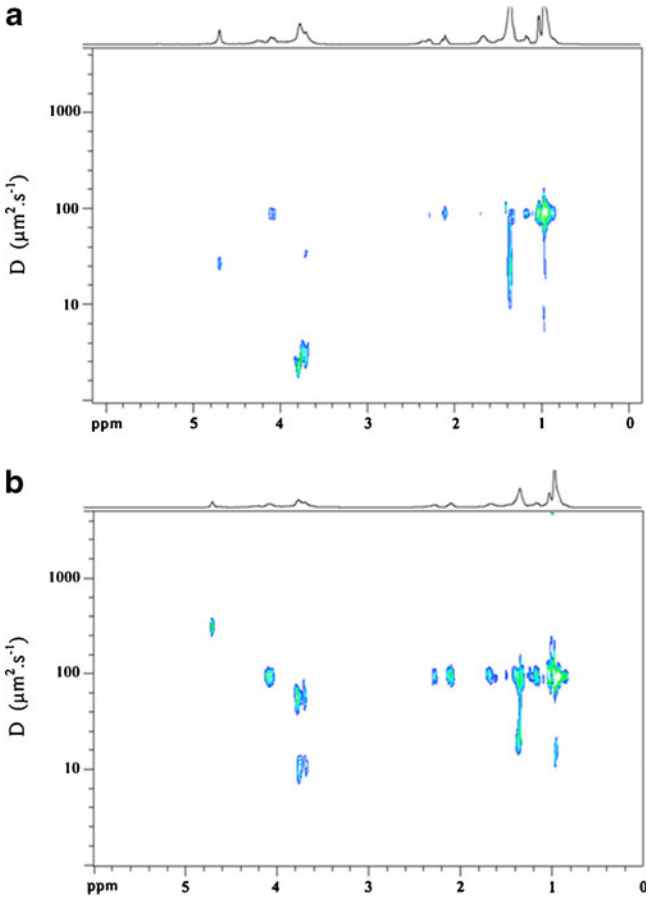

Fig. 5 ^{1}H DOSY 2D-NMR spectra of the w/o (**a**) and o/w microemulsion (**b**).

B is an o/w microemulsion. Notice that most of the lines of the oil and surfactants are not resolved; the line at 4.1 ppm is specific of the oil.

The structural inversion of the microemulsion (water/isononyl isononanoate/Tween®21: 35/35/30) was followed by diffusion measurements as a function of temperature (Fig. 6). The self-diffusion coefficient of water is faster than that of oil and surfactant at low temperature, showing that the structure is o/w. The diffusion of water molecules is slower as the temperature increases because the structure progressively turns from o/w droplets into bicontinuous. The bicontinuous structure is reached when the self-diffusion coefficients of water and oil are equal. At this stage, the phase inversion temperature (PIT = 43°C) is reached. Phase inversion into w/o droplets at higher temperatures is characterized by a faster diffusion for oil than for water molecules.

Table 2 summarizes the diffusion data of the three selected microemulsions. The self-diffusion coefficient of water in o/w microemulsion is 12 times higher than that of the w/o microemulsion. The diffusion of water in o/w microemulsion is 4 times faster than oil. The diffusion of oil is 4 times faster that water in w/o microemulsion. The self-diffusion coefficients of water and oil are similar in bicontinuous microemulsion.

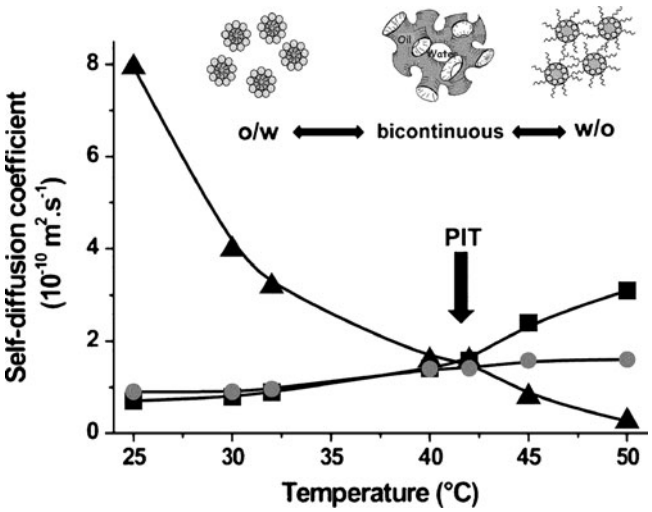

Fig. 6 Self-diffusion coefficients of the components of the water/isononyl isononanoate/Tween®21 (35/35/30) system as measured by means of PFGSE NMR: water (*filled triangle*); oil (*filled square*); Tween®21 (*filled circle*). The *arrow* marks the PIT.

Small-Angle Neutron Scattering (SANS)

SANS (23) was used in order to characterize the structure of the bicontinuous microemulsion at 32°C (Fig. 7). Such structure is difficult to describe because this is highly disordered; there are no droplets, and the interface undergoes strong fluctuations. The microemulsion structure was assessed with the classical structural model of Teubner-Strey (29) by means of two parameters: the mean domain size (of both water and oil domains), and the correlation length related to the length scale of the fluctuations of the interface. The correlation length is the statistical distance

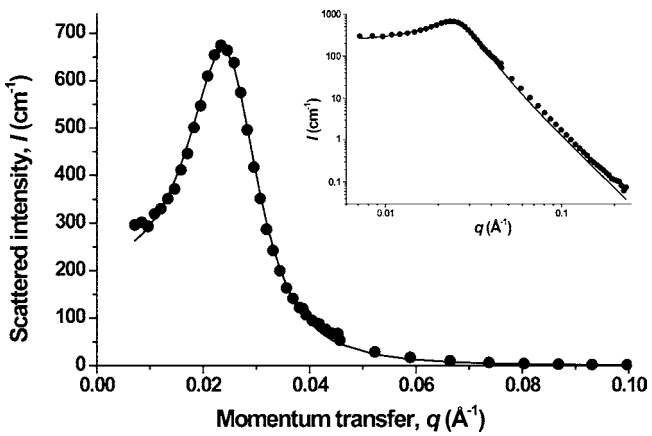

Fig. 7 SANS spectrum of bicontinuous microemulsion (Tween®21: Span®20 (90:10)/isononyl isononanoate/deuterated water) (30/35/35) at 32°C. *filled circle*: experimental; *solid line*: best fit of the Teubner-Strey model (28). The inset shows the same data in Log-Log scale.

Table 2 Self-Diffusion Coefficient of the Three Components, Oil, Water and Surfactant, for the Different Types of Microemulsions (o/w, Bicontinuous and w/o)

	Self-diffusion coefficient at 32°C ($10^{-10}m^2s^{-1}$)		
Type of microemulsion	Water	Oil	Surfactant
Oil/water	3.19	0.96	0.96
Bicontinuous	1.12	1.07	1.00
Water/oil	0.25	0.90	0.96

along which the interface is rigid. A short correlation length means that the interface is rigid over short distances only; it reveals a highly fluctuating interface. The scattered intensity shows a correlation peak in the low-q range and a decay of scattered intensity as q^{-4} in the high-q range. The peak is characteristic of a concentrated dispersion where the positions of the oil and water domains are correlated. The q^{-4} decay corresponds to the scattering of a sharp interface separating the oil and water domains (Porod's law) (23). A fit of the Teubner-Strey model to the experimental data gave the structural parameters. The characteristic domain size was $d=25.0$ nm, and the correlation length of the interface was $\xi=12.1$ nm. As for every bicontinuous structure of microemulsion, the characteristic domain size is larger than the correlation length. This shows the high flexibility of the interface required for the fluid disordered bicontinuous structure to be stable with respect to the rigid lamellar phase.

Partition of Caffeine between Water and Oil Domains

The partition coefficient between water and isononyl isononanoate has been measured at 20°C and 32°C in a diphasic system that did not contain surfactant. $P=0.025\pm0.001$ ($\mathrm{Log}P=-1.60\pm0.02$) at 20°C and $P=0.023\pm0.001$ ($\mathrm{Log}P=-1.64\pm0.02$) at 32°C. According to such low values, the major part of caffeine is solubilized in aqueous phase. The partition coefficient strongly depends on the oil type. The octanol-water partition coefficient of caffeine is close to 1 ($\mathrm{Log}P=-0.07$), which means that half the amount of caffeine is solubilized in the aqueous domains and half is in the oil domains for a 50:50 water/octanol system. The Log$P$ of the less polar isononyl isononanoate oil is lower, and it is even lower for the more hydrophobic silicone oil (30).

Percutaneous Penetration

Permeation Experiments

Comparative experiments of caffeine permeation were performed from o/w, bicontinuous, w/o microemulsions, and an aqueous solution. The cumulative amounts of released caffeine per unit area of skin ($\mu g.cm^{-1}$) were calculated from the permeation experiments (Fig. 8). A diffusion lag time was extracted from the curve by extrapolation of the linear part to the time axis. The slope yielded the pseudo-steady-state flux J_{SS} ($\mu g.cm^{-2}.h^{-1}$) (Table 3); it was calculated from the first part of the data between 1 h and 9 h. The permeability coefficient P_S ($cm.h^{-1}$) was calculated as $P_S = J_{SS}/C_0$, where C_0 was the overall concentration of caffeine in the donor compartment as explained in the data analysis section.

The o/w microemulsion generated a significant faster permeation of caffeine than the other formulations ($p<0.05$). After 24 h exposure, the permeated amount relative to applied dose reached 50% for the o/w microemulsion. Considering such high amounts reaching the receptor fluid, the experiment was conducted in finite dose conditions. Pseudo-steady-state fluxes were calculated from the linear portion of the permeation profile between the end of the lag time and the ninth hour of the experiment.

The caffeine amount delivered in the receptor fluid from the o/w microemulsion was always significantly higher compared to the solution and w/o microemulsion over 24 h. Compared to the bicontinuous microemulsion, the o/w microemulsion delivered significantly higher caffeine amount up to 9 h. After 15 h up to 24 h, the differences were not significant according to the t-test with $p<0.05$. A significant difference was observed again at 24 h, however. As an example, the amount of caffeine which permeated in the skin after 24 h exposure using o/w microemulsion (1,538 $\mu g.cm^{-2}$) is significantly larger than a bicontinuous (1,096 $\mu g.cm^{-2}$), w/o and caffeine solution (717 $\mu g.cm^{-2}$) (Fig. 8). The bicontinuous microemulsion is intermediate between o/w and w/o microemulsion but closer to the o/w microemulsion, especially after 9 h. The

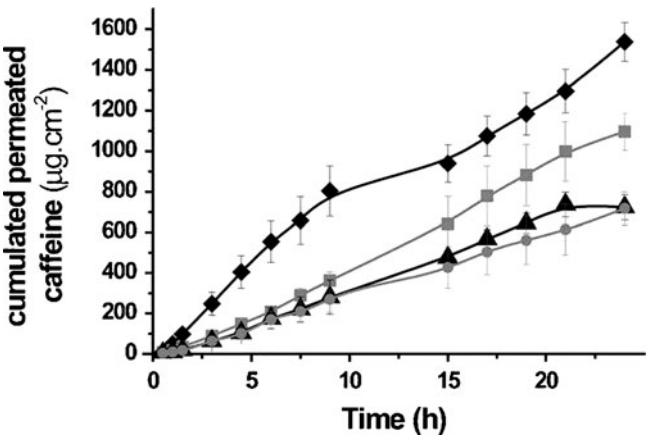

Fig. 8 Permeation profiles of caffeine ($\mu g.cm^{-2}$) from o/w microemulsion (*filled diamond*), bicontinuous microemulsion (*filled square*), w/o microemulsion (*filled triangle*) and caffeine solution (*filled circle*) over 24 h. Each point represents the mean ± SEM of $n=6$ experiments.

Table 3 Parameters of the Permeation Profiles of Caffeine (0.8 wt%) Through Pig Skin from Formulations: O/W Microemulsion, W/O Microemulsion, Bicontinuous Microemulsion and Caffeine Solution (Mean ± SEM of $n=6$ Determinations)

Formulations	Lag time (min)	Flux, Jss ($\mu g.cm^{-2}.h^{-1}$)	Permeability coefficient, P_S ($cm.h^{-1}$)
O/W	35 ± 8	99 ± 14	0.012 ± 0.002
W/O	52 ± 12	35 ± 10	0.004 ± 0.001
Bicontinuous	48 ± 7	43 ± 5	0.0053 ± 0.0006
Caffeine solution	57 ± 9	32 ± 10	0.004 ± 0.001

caffeine flux from the o/w microemulsion decreased from 99 to 54 $\mu g.cm^{-2}.h^{-1}$ because of the depletion of the donor compartment. Conversely, the caffeine flux from the bicontinuous microemulsion was constant (43 $\mu g.cm^{-2}.h^{-1}$) over 24 h exposure, so that the two permeation profiles come closer at large exposure times.

Whatever the experimental conditions, the higher flux (and permeability) values showed that the o/w microemulsion ($P_S=0.012$ $cm.h^{-1}$) significantly increased the caffeine permeation rate with respect to the bicontinuous microemulsion, w/o microemulsion and caffeine solution (Table 3). It was almost three-fold higher for o/w microemulsion than w/o microemulsion and caffeine solution.

Caffeine Distribution within the Skin Layers after 24 h Exposure

The caffeine distribution in the different layers of the skin was measured after 24 h exposure. The amount in the *stratum corneum* was measured by means of the tape stripping technique; the amounts in the viable epidermis and dermis were measured after they had been separated with hot water (Fig. 9). The major part of caffeine permeated to the receptor fluid (Table 4 and Fig. 9). According to the *t*-test with $p<0.05$, there was no significant difference of caffeine amounts stored in dermis, viable epidermis and *stratum corneum* between all formulations.

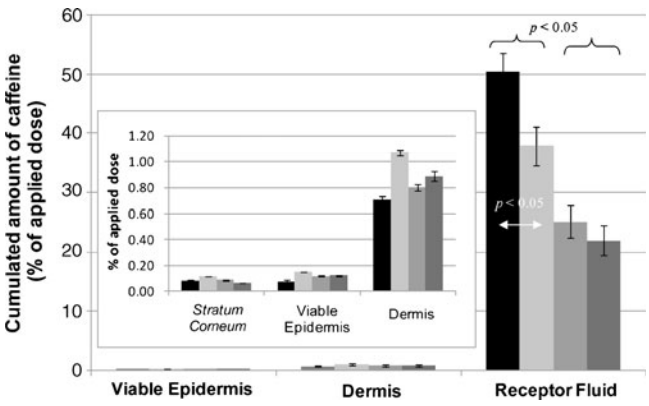

Fig. 9 Distribution of caffeine (% of applied dose) in the *stratum corneum*, viable epidermis, dermis and receptor fluid after 24 h exposure for all formulations (o/w, bicontinuous, w/o and solution) (mean ± SEM; $n=6$). Arrows mark the differences that were shown significant according to the ANOVA test.

DISCUSSION

Permeation of Caffeine Through the Skin: Enhancement by Microemulsions

Microemulsions attracted increasing attention for pharmaceutical formulations because they are well-known to enhance solubilization of drugs and to improve systemic and topical drug availability (1–4). We observed the same trend using caffeine-loaded microemulsions comparatively to classical vehicles such as emulsions or gels loaded with caffeine at the same concentration (5). In the present study, caffeine permeates the skin quite fast (Table 3, Fig. 8). Storage in skin layers was very low, since less than 2% of caffeine was recovered in skin layers from the different formulations, and no significant differences were observed between formulations (Table 4, Fig. 9). Only the amount of caffeine measured in the receptor fluid allows discriminating the different formulations as we previously observed in an earlier work (5). Microemulsions delivered caffeine faster than the aqueous solution. The lag times were shorter for all microemulsions than for the aqueous solution, but the difference was statistically significant in the case of the o/w microemulsion only (Table 3).

Caffeine permeation rates from microemulsion and aqueous solution cannot be compared in a straightforward way, however, because the concentrations of caffeine in the aqueous phase are different. More specifically, the chemical potential gradient of caffeine between the donor and receptor compartments matters better than the concentration. An equivalent way is to consider the activity instead of the concentration. The usual way to leave out of consideration the influence of concentration is to calculate the permeation coefficient defined as the ratio of the flux to the caffeine concentration. Indeed, the flux is proportional to the drug concentration for dilute delivery samples. The concentration of drug in the full sample is used in the standard definition of the permeability coefficient. However, the chemical potential of the drug is not directly related to the concentration in micro-heterogeneous systems such as microemulsions. Indeed, the drug experiences partition between the different solubilization sites of the microemulsion: water, oil and interface. In the case of a dispersed diphasic system (an emulsion), the chemical potential of the drug is the same in each phase, so that its thermodynamic

Table 4 Distribution of Caffeine in Skin Layers After 24 h Exposure (mean ± SEM of $n = 6$ Determinations)

	Caffeine concentration (% of applied dose)			
	O/W	Bicontinuous	W/O	Solution
Stratum corneum	0.08 ± 0.01	0.12 ± 0.02	0.09 ± 0.02	0.07 ± 0.02
Viable epidermis	0.08 ± 0.01	0.15 ± 0.03	0.12 ± 0.01	0.13 ± 0.03
Dermis	0.71 ± 0.07	1.1 ± 0.2	0.80 ± 0.14	0.89 ± 0.20
Receptor fluid	50.5 ± 3	38 ± 3	25 ± 3	22 ± 2

activity can be evaluated from the partition coefficient of the drug between the aqueous and oily phases. The same approach was applied in the present, although microemulsions are not diphasic systems: the activity of the drug is evaluated as the concentration in the aqueous domain. Since the measured partition coefficient between oil and water was $P = 0.023$ at 32°C, the fraction of caffeine in water was $\frac{1}{1+P\frac{\rho(\text{water})\ M(\text{oil})}{\rho(\text{oil})\ M(\text{water})}} = 97\%$ of the overall concentration distributed between equal masses of oil and water; the concentration of caffeine in the water domains of microemulsions was 1.56 wt%. Accordingly, the major part of caffeine is solubilized in the aqueous domains, so that the concentration in water was approximately twice the overall concentration. Indeed, caffeine is considered as a model hydrophilic compound for skin permeation experiments. It is also likely that caffeine solubilizes in the surfactant interfacial layer, which has been ignored in the present analysis. Assessment of the thermodynamic activity of caffeine is a complex issue that is beyond the scope of this paper, which is devoted to the influence of the microstructure of the microemulsions. Since the caffeine concentration is low, the thermodynamic activity in the aqueous solution is close to the concentration. The thermodynamic activity in microemulsions estimated from the partition between water and oil domains is the maximum activity of caffeine because the solubilization in the interface has been ignored. Indeed, the volume fraction occupied by the interfacial layer is quite high because of the large concentration of surfactant (30 wt%). A new permeation coefficient was calculated using the actual caffeine concentration in water instead of the overall concentration. This is a minimum permeation coefficient, since the way it has been calculated overestimated the caffeine concentration in water. The actual permeation coefficient that accounts for the activity of caffeine lies in between the two values given in Table 5.

Accounting for the activity of caffeine instead of the concentration changes the features of penetration enhancement by microemulsions with respect to the aqueous solution. It does not change the order with respect to microemulsion microstructure. Considering the minimum permeation coefficients, it remains that the o/w microemulsion significantly enhances the skin permeation, whereas there is no significant difference between the bicontinuous and aqueous solution, and the w/o significantly inhibits the caffeine permeation. It is concluded that a major contribution to the effect of microemulsion on skin absorption comes from the thermodynamic activity of drugs in the medium.

Possible Mechanisms of Enhancement: Role of the Microstructure

While the enhancement effect of microemulsion on drug permeation has been widely documented in the literature, studies on microemulsions have not been systematic and, therefore, did not allow definite mechanistic conclusions (4). This had prevented disclosure of relationships between physicochemical properties of microemulsions and drug delivery. In this context, the mechanisms by which microemulsions enhance drug bioavailability are still debated in the literature. Kreilgaard *et al.* cited the solubility properties as the driving force of enhancement. As the cutaneous drug delivery towards the skin is related to the concentration according to the Fick's laws of diffusion, the solubility potential of such systems is therefore an important factor. In this context, bicontinuous microemulsions that have excellent solubilizing properties should be appealing systems. The influence of the microstructure has been reported in the literature in few instances (31–34). The microemulsion structures were only characterized by electrical conductivity. Djordjevic *et al.* (31) focused their study on the comparison between microemulsions loaded with diclofenac diethylamine having almost the same

Table 5 Permeability Coefficient Calculated as $P_S = J_{SS}/C_0$ Using Either the Overall Concentration (0.8 wt%) or the Concentration in Water in Order to Account for the Thermodynamic Activity Coming from the Partition Between Water and Oil Domains

Formulations	Permeability coefficient, P_S ($cm.h^{-1}$)	
	$C_0 = 0.8$ wt%	$C_0 = C(\text{water})$
O/W	0.012 ± 0.002	0.0063 ± 0.001
W/O	0.004 ± 0.001	0.0022 ± 0.0006
Bicontinuous	0.0053 ± 0.0006	0.0028 ± 0.0003
Caffeine solution	0.004 ± 0.001	0.004 ± 0.001

composition but different microstructures. The release profiles measured through cellulose membrane showed fluxes in the order o/w > bicontinuous > w/o as in our present work. However, a highly non-linear release profile of the drug was observed for all microemulsions; a very fast initial permeation was followed by a slow steady-state regime where the flux could be measured. It is suggested that possible interaction of the ingredients of the formulation with the cellulose membrane clogged the membrane and stopped the fast initial diffusion. As a consequence, it was not clear what the fluxes measured at long times meant. Liu *et al.* (32) investigated the permeation through rat skin of the hydrophobic drug cyclosporin incorporated in microemulsions stabilized by AOT/Tween®85 mixed surfactant system. The bicontinuous microemulsion was shown more efficient, and it was claimed that the differences of permeation rates came from the different water contents. Here again, it is difficult to assess the origin of the phenomena because several physico-chemical parameters were varied at the same time. Yuan *et al.* (33) reported the faster permeation of lidocaine hydrophobic drug from the w/o microemulsion compared to the bicontinuous and o/w microemulsion; emphasis was given to the effect of hydrophilic and lipophilic linker (co-surfactants) to lecithin-based microemulsion. In spite of its favorable transport properties, the bicontinuous microstructure does not promote caffeine delivery compared to the globular microstructure.

Importance of Keeping Chemical Compositions Constant: Example of Water as Skin Permeation Enhancer of Hydrophilic Drugs

The effect of water on drug permeation has been reported in several instances. It is generally accepted that high water mass fraction has an enhancer effect on the diffusion of hydrophilic drugs. This observation was reported earlier by Osborne *et al.* for the transport of glucose (35) and more recently by Djordjevic (31), Changez *et al.* (36), Sintov *et al.* (6) and Kreilgaard *et al.* (9). The reverse effect has been observed in one instance, however (33). Accordingly, particular attention should be paid to the mass fractions of ingredients in the microemulsion before comparing drug release. The study by Kreilgaard *et al.* is very instructive to illustrate this point (9). The permeations of a lipophilic and a hydrophilic drug (lidocaine and prilocaine hydrochloride) were compared from o/w, bicontinuous, and w/o microemulsions. The microemulsions differed by their respective water, oil and surfactant mass fractions, but also by their cosurfactant/surfactant mass ratio. For both drugs the highest fluxes were obtained with the o/w microemulsion containing the highest amount of water (50 to 65%). The authors did not suggest the microstructure as a relevant enhancement parameter, but pointed out the influence of the mobility of the drug in the microemulsion. In the present study, the caffeine transdermal flux enhancement due to the o/w microstructure was not very high; a two-fold increase of the caffeine flux was observed only for the o/w microemulsion comparatively to the solution (Table 3). This result is far less than the 6-fold or 10-fold increase in prilocaine hydrochloride flux generated by o/w microemulsions (loaded at 13 or 14%) comparatively to a 2% Xylocain hydrogel (9). The difference between o/w and bicontinuous microemulsions is also less in our present work than in the previous by Kreilgaard *et al.* (9), where the o/w and bicontinuous microemulsions did not contain the same amounts of oil and water. Lee *et al.* (37) noticed an enhancement of drug permeation from the o/w microemulsion system of 17-fold for lidocaine free base, 30-fold for lidocaine HCl, 58-fold for estradiol, and 520-fold for diltiazem HCl. These results should be observed with caution because the microemulsions contained organic solvents and penetration enhancers, so that such large permeation enhancements might not be caused by the microemulsion microstructure.

It is stressed as a conclusion to this literature survey that the possible role of the microemulsion microstructure was often hidden in the various formulations tested by stronger influences of different mass fraction of water, oil and surfactant/cosurfactant. The work by Yuan *et al.* (33) gives an illustrative example showing how the variation of several physico-chemical parameters at the same time may be misleading. Thus, the permeation of lidocaine was found faster from the w/o microemulsion than from bicontinuous and o/w microemulsion, at variance with the conclusion of Lee *et al.* (37). The w/o and o/w microemulsions were prepared in a diphasic domain of the phase diagram, and single phase samples were obtained after the phase containing the microemulsion (either o/w or w/o) was separated from the excess phase (of either oil or water). The chemical compositions of the phase were totally different; in particular, the w/o microemulsion contained quite large amounts of the isopropyl myristate oil that is known to exhibit penetration enhancer properties. The effect of the microemulsion microstructure on skin release should have been undertaken under better controlled conditions such that the selected formulations have very close compositions and the same mass ratio of each component.

A Robust Methodology of Formulation for Microemulsions

A new formulation methodology was used in the present study, allowing for finding microemulsions with almost the same composition and a well-defined microstructure.

A systematic formulation work was required in order to find out a common single phase composition suitable for the investigation of the influence of the microemulsion microstructure. An efficient method to achieve such a goal was to draw Kahlweit fish diagrams. The water/isononyl isononanoate/Tween®21/Span®20 phase diagrams were investigated so as to get the phase inversion temperature at 32°C. The point at the intersection between the "fish tail" and the "fish body" is a measure of the efficiency of the surfactant system. The minimum amount of amphiphile required to mix equal amounts of water and isononyl isononanoate as a single phase microemulsion was 25 wt% at 32°C. The definite advantage of the Kahlweit diagram method is the robustness of the phase diagrams with respect to the formulation parameters, Tween®21/Span®20 ratio and temperature. It is established that the structure of the microemulsion is bicontinuous at the phase inversion temperature. Increasing the ratio of the hydrophilic surfactant Tween®21 turns the structure into o/w. Lowering the temperature causes the same effect. Increasing the ratio of the hydrophobic surfactant Span®20 or increasing the temperature turns the structure into w/o. Since it is not known how much the Tween®21/Span®20 should depart from the phase inversion composition, it is necessary to check against the microemulsion type using structural experimental methods (38).

Electrical conductivity and PFGSE NMR allowed for demonstrating the w/o, bicontinuous or o/w type of our microemulsion. The structure of the bicontinuous microemulsions was confirmed by SANS. In their investigation of the role of the microemulsion microstructure on the transdermal delivery of drugs, Djordjevic *et al.* (31) established the microemulsion structures from the value of the electrical conductivity of studied samples only. As a more precise characterization, Liu *et al.* (32) defined the structures from measurements of the percolation threshold of electrical conductivity. The structural characterization of o/w, bicontinuous, and w/o microemulsions by Yuan *et al.* (33) was only made through poorly informative viscosity and light scattering measurements along a dilution path in the phase diagrams; the most relevant experimental proof regarding microemulsion structure relies on pseudo-ternary phase diagrams and sections along the dilution path.

The second advantage of the Kahlweit fish diagram is that the intersection between the fish tail and the fish body corresponds to the microemulsion containing the minimum amount of surfactant. This allows for minimizing the surfactant content for better tolerance and minimum irritancy purpose. High surfactant concentrations have also been shown detrimental to the permeation of drug, probably because of a lower mobility of drug at high surfactant concentrations (1, 2, 6, 9, 39–41). As example, Rhee *et al.* (40) found an inverse correlation between surfactant content and ketoprofen transdermal flux. Chen *et al.* (41) found that triptolide-loaded microemulsions provided higher flux for lower amount of Tween®80 and propylene glycol. Finally, even if the chemical composition of the three microemulsions were very close, the Tween®21/Span®20 varied. The shorter lag times and enhanced permeation rates observed in the present case might also be caused by interactions of the Tween®21 and Span®20 surfactants with *stratum corneum* lipids, resulting in a penetration enhancer effect (42, 43). This point would deserve further investigations.

ACKNOWLEDGMENTS

This work was supported by a grant PHC Utique n°08G1106 for French-Tunisian cooperation.

REFERENCES

1. Heuschkel S, Goebel A, Neubert RHH. Microemulsions—Modern Colloidal carrier for dermal and transdermal drug delivery. J Pharm Sci. 2008;97:603–31.
2. Gupta S, Moulik SP. Biocompatible microemulsions and their prospective uses in drug delivery. J Pharm Sci. 2008;97:22–45.
3. Kogan A, Garti N. Microemulsions as transdermal drug delivery vehicles. Adv Colloid Interface Sci. 2006;123–126:369–85.
4. Kreilgaard M. Influence of microemulsions on cutaneous drug delivery. Adv Drug Deliv Rev. 2002;54:S77–98.
5. Bolzinger MA, Briançon S, Pelletier J, Fessi H, Chevalier Y. Percutaneous release of caffeine from microemulsion, emulsion and gel dosage forms. Eur J Pharm Biopharm. 2008;68:446–51.
6. Sintov AC, Shapiro L. New microemulsion vehicle facilitates percutaneous penetration *in vitro* and cutaneous drug bioavailability *in vivo*. J Control Release. 2004;95:173–83.
7. 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;19:203–10.
8. Kreilgaard M. Dermal pharmacokinetics of microemulsion formulations determined by *in vivo* microdialysis. Pharm Res. 2001;18:367–75.
9. Kreilgaard M, Pederson EJ, Jarozewski W. NMR characterization and transdermal drug delivery potential of microemulsion systems. J Control Release. 2000;69:421–33.
10. Bolzinger-Thevenin MA, Carduner C, Poelman MC. Bicontinuous sucrose ester microemulsion: a new vehicle for topical delivery of niflumic acid. Int J Pharm. 1998;176:39–45.
11. Hoar TP, Schulman JH. Transparent water-in-oil dispersions: the oleopathic hydro-micelle. Nature. 1943;152:102–3.
12. Schubert KV, Kaler EW. Nonionic microemulsions. Ber Bunsenges Phys Chem. 1996;100:190–205.
13. Scriven LE. Equilibrium bicontinuous structure. Nature. 1976;263:123–5.
14. Malcolmson C, Satra C, Kantaria S, Sidhu A, Lawrence MJ. Effect of oil on the level of solubilization of testosterone propionate into nonionic oil-in-water microemulsions. J Pharm Sci. 1998;87:109–16.
15. Malcolmson C, Lawrence MJ. A comparison between nonionic micelles and microemulsions as a means of incorporating the

poorly water soluble drug diazepam. J Pharm Pharmacol. 1990; Suppl 42:6P
16. Langevin D. Microemulsions - Interfacial aspects. Adv Colloid Interface Sci. 1991;34:583–95.
17. Shinoda K, Kunieda H, Arai T, Saijo H. Principles of attaining very large solubilization (microemulsion): inclusive understanding of the solubilization of oil and water in aqueous and hydrocarbon media. J Phys Chem. 1984;88:5126–9.
18. Kahlweit M, Strey R, Buse G. Microemulsions: a qualitative thermodynamic approach. J Phys Chem. 1990;94:3881–94.
19. Laguës M, Ober R, Taupin C. Study of structure and electrical conductivity in microemulsions: evidence for percolation mechanism and phase inversion. J Phys Lett. 1978;39:L487–91.
20. Laguës M, Sauterey C. Percolation transition in water in oil microemulsions. Electrical conductivity measurements. J Phys Chem. 1980;84:3503–8.
21. Stilbs P, Lindman B. NMR measurements on microemulsions. Prog Colloid Polym Sci. 1984;69:39–47.
22. Lindman B, Olsson U. Structure of microemulsions studied by NMR. Ber Bunsenges Phys Chem. 1996;100:344–63.
23. Chevalier Y, Zemb T. The structure of micelles and microemulsions. Rep Prog Phys. 1990;53:279–371.
24. Équipements expérimentaux du Laboratoire Léon Brillouin. LLB, CEN Saclay, F91191 Gif-sur-Yvette, France. (LLB is a CEA-CNRS laboratory). Available at: http://www-llb.cea.fr/index.html.
25. Cotton JP. Initial data treatment. In: Lindner P, Zemb Th, editors. Neutron, X-ray and light scattering. Introduction to an investigative tool for colloidal and polymeric systems. Amsterdam: North-Holland; 1991. p. 19.
26. NF T 20–043 AFNOR (1985). Chemical products for industrial use - Determination of partition coefficient - Shake flask method.
27. Winsor PA. Hydrotropy, solubilisation and related emulsification processes. Trans Faraday Soc. 1948;44:376–98.
28. Saito H, Shinoda K. The stability of w/o type emulsions as a function of temperature and of the hydrophilic chain length of the emulsifier. J Colloid Interface Sci. 1970;32:647–51.
29. Teubner M, Strey R. Origin of the scattering peak in microemulsions. J Chem Phys. 1987;87:3195–200.
30. Frelichowska J, Bolzinger M-A, Valour J-P, Mouaziz H, Pelletier J, Chevalier Y. Pickering w/o emulsions: drug release and topical delivery. Int J Pharm. 2009;368:7–15.
31. Djordjevic L, Primorac M, Stupar M. *In vitro* release of diclofenac diethylamine from caprylocaproyl macrogolglycerides based microemulsions. Int J Pharm. 2005;296:73–9.
32. Liu H, Li S, Wang Y, Han F, Dong Y. Bicontinuous water-AOT/Tween85-isopropyl myristate microemulsion: a new vehicle for transdermal delivery of cyclosporin A. Drug Dev Ind Pharm. 2006;32:549–57.
33. Yuan JS, Ansari M, Samaan M, Acosta EJ. Linker-based lecithin microemulsions for transdermal delivery of lidocaine. Int J Pharm. 2008;349:130–43.
34. Lee J, Lee Y, Kim J, Yoon M, Choi YW. Formulation of microemulsion systems for transdermal delivery of aceclofenac. Arch Pharm Res. 2005;28:1097–102.
35. Osborne DW, Ward AJI, O'Neill KJ. Microemulsions as topical drug delivery vehicles: *in-vitro* transdermal studies of a model hydrophilic drug. J Pharm Pharmacol. 1991;43:451–4.
36. Changez M, Varshney M, Chander J, Dinda AK. Effect of the composition of lecithin/*n*-propanol/isopropyl myristate/water microemulsions on barrier properties of mice skin for transdermal permeation of tetracaine hydrochloride: *in vitro*. Colloid Surf B Biointerfaces. 2006;50:18–25.
37. Lee PH, Langer R, Shastri VP. Novel microemulsion enhancer formulation for simultaneous transdermal delivery of hydrophilic and hydrophobic drugs. Pharm Res. 2003;20:264–9.
38. Kahlweit M, Stey R, Haase D, Kuneida H, Schmeling T, Faulhaber B, *et al.* How to study microemulsions. J Colloid Interface Sci. 1987;118:436–53.
39. Hua L, Weisan P, Jiayu L, Ying Z. Preparation, evaluation, and NMR characterization of vinpocetine microemulsion for transdermal delivery. Drug Dev Ind Pharm. 2004;30:657–66.
40. Rhee YS, Choi JG, Park ES, Chi SC. Transdermal delivery of ketoprofen using microemulsions. Int J Pharm. 2001;228:161–70.
41. Chen H, Chang X, Weng T, Zhao X, Gao Z, Yang Y, *et al.* A study of microemulsion systems for transdermal delivery of triptolide. J Control Release. 2004;98:427–36.
42. López A, Llinares F, Cortell C, Herráez M. Comparative enhancer effects of Span®20 with Tween®20 and Azone® on the *in vitro* percutaneous penetration of compounds with different lipophilicities. Int J Pharm. 2000;202:133–40.
43. Cappel MJ, Kreuter J. Effect of non-ionic surfactants on transdermal drug delivery: I. polysorbates. Int J Pharm. 1991;69:143–53.