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Influence of microemulsions on cutaneous drug delivery

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Abstract

In attempt to increase cutaneous drug delivery, microemulsion vehicles have been more and more frequently employed over recent years. Microemulsion formulations have been shown to be superior for both transdermal and dermal delivery of particularly lipophilic compounds, but also hydrophilic compounds appear to benefit from application in microemulsions compared to conventional vehicles, like hydrogels, emulsions and liposomes. The favourable drug delivery properties of microemulsions appear to mainly be attributed to the excellent solubility properties. However, the vehicles may also act as penetration enhancers depending on the oil/surfactant constituents, which involves a risk of inducing local irritancy. The correlation between microemulsion structure/composition and drug delivery potential is not yet fully elucidated. However, a few studies have indicated that the internal structure of microemulsions should allow free diffusion of the drug to optimise cutaneous delivery from these vehicles.

Keywords: Microemulsion; Drug delivery; Skin; Review; Solubility; Tolerability

Contents

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1. Introduction

The fundamentals of a successful pharmaceutical formulation are to enable delivery of the active substance to the target organ at therapeutically relevant levels, with negligible discomfort and side effects to the patient. In this respect, the route of administration is of major influence. Topical administration offers several attractions compared to the traditional routes. Transdermal drug delivery to the systemic circulation excels itself by avoidance of hepatic first-pass metabolism, potential of long-term controlled release with avoidance of the typical peak–trough plasma-profiles associated with frequent dosage regiments, ease of administration and possibility of immediate withdrawal of the treatment. However, the main indicator for topical administration is when the skin itself is the target organ. Despite the substantial potential of transdermal and dermal drug delivery, only relatively few drugs are yet commercially available as topical formulations. The main limitation lies in the barrier function of the skin, which is considered one of the most impermeable epithelia of the human body to exogenous substances. Therefore, the major challenge for topical formulations today is to provide a sufficient increase in drug penetration into the skin, without inducing significant irreversible alterations to the skin barrier function.

During the resent decades numerous studies have suggested that a novel vehicle described as 'microemulsions' has a potential of increasing cutaneous drug delivery of both hydrophilic and lipophilic drugs compared to conventional vehicles [1–8], and thereby fulfil the many promising aspects of the cutaneous route. The application of microemulsion vehicles for cutaneous drug delivery is becoming increasingly popular as the potential of these formulations is realised. During the last year alone, more than 11 papers have been published in this specific field. However, the correlation between components, compositions and structure of the microemulsions and increase in drug delivery rate is not yet fully elucidated.

The aim of this paper is to review the current literature with respect to the use of microemulsions for cutaneous drug delivery and to discuss the influence of microemulsion composition, components and structure on the drug delivery potential as well as the cutaneous tolerability of these vehicles.

2. Theory and properties of microemulsions

The theoretical existence of microscopic emulsion-like structures in a transparent mixtures of water, cationic soap (cetyl trimethyl ammonium bromide), oil phase and alcohol was first put forward by Hoar and Schulman in 1943 [9]. In 1959 these very small emulsion-like structures were confirmed by solidifying the oil phase through staining and visualised by electron microscopy by Schulman et al. [10], and therefore coined microemulsions by the authors.

A microemulsion is defined as a system of water, oil and surfactants, which is a transparent, single optically isotropic and thermodynamic stable liquid solution [11].

2.1. Formation

Depending on properties of the involved components, microemulsions can potentially appear over a wide range of oil–water–surfactant compositions. However, with given oil–water–surfactant components, microemulsions are usually only formed in narrow specific concentration ranges [12–16]. The region of existence is typically presented in pseudo-ternary phase diagrams, as ratios between oil, water and a fixed mixture of surfactant–co-surfactant (Fig. 1).

The primary determinant for the range of microemulsion formation is the physico-chemical properties of the aqueous phase, oil phase and surfactants. The physico-chemical interaction between the components is too complex to provide a functional general mathematical guideline for prediction of microemulsion formation as a function of component properties; however, a few essential conditions have been described by Schulman et al. [10]:

(1) the production of a very low interfacial tension at the water–oil interface;

(2) the formation of a highly fluid interfacial surfactant film;

(3) the penetration and association of the molecules of the oil phase with the interfacial surfactant film.

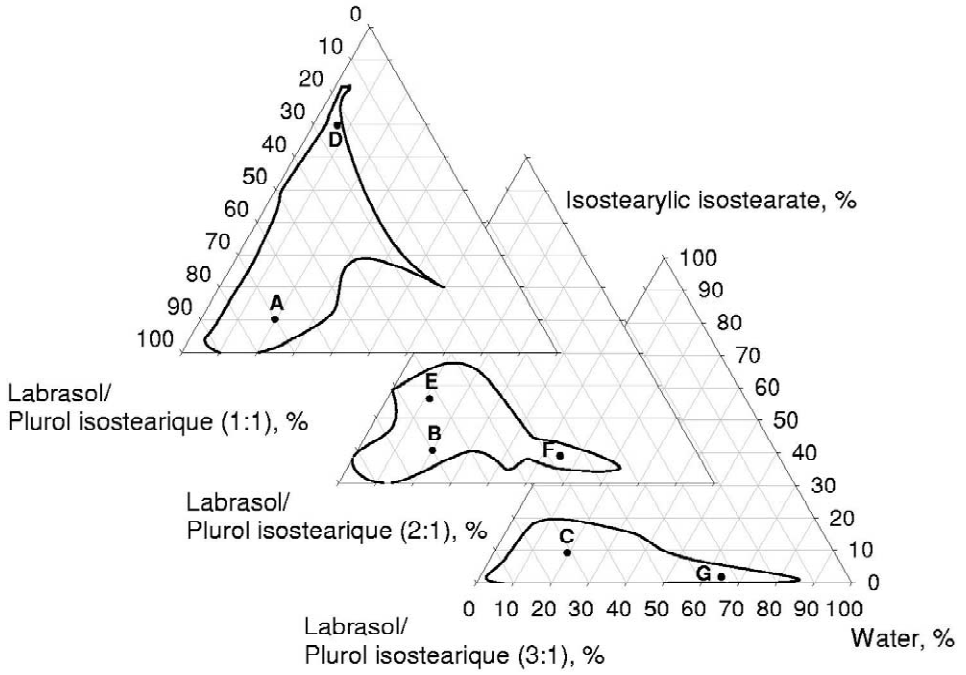

Fig. 1. Example of microemulsion region of existence in systems composed of isostearyle isostearate (oil), water, and various mixtures of Labrasol (surfactant) and Plurol Isostearique (co-surfactant); reproduced from Ref. [8]. Letters indicate the studied microemulsion compositions.

Lowering of the interfacial tension and fluidisation of the interfacial surfactant film, is usually done by introducing a short chain co-surfactant to the surfactant film. The introduction of a co-surfactant may also expand the field of existence for systems already capable of forming microemulsions without a co-surfactant [17,18], due to the more flexible interfacial film. If a single surfactant system is desired, the lipophilic chains of the surfactant should be sufficiently short, or contain fluidising groups (e.g., unsaturated bonds). To enable integration of the oil with the interfacial film, the size of the oil molecules should not be too large [19].

2.2. Structure

The mixture of oil, water and surfactants is able to form a wide variety of structures and phases. Besides microemulsions, structural examinations can reveal the existence of regular emulsions, anisotropic crystalline hexagonal or cubic phases, and lamellar structures depending on the ratio of the components. Most of these different phases and structures are easily recognised by simple visual inspection of the compositions due to their physical appearance (e.g., emulsions are nontransparent and phases separate after a while; lamellar structures and cubic phases are high viscous) or can be revealed by inspection with polarised light (crystalline phases), and thereby discerned from actual microemulsions.

However, even within the microemulsion regions, several different internal structures can form from the immiscible water and oil phase, and the interfacial surfactant film. The microemulsions structure is greatly influenced by the physico-chemical properties of the components used, and the ratios between the components. The term ‘micro-emulsions’ is somehow deceiving, though, as the structures often diverge from the static spherical droplet-shapes of regular emulsions. Microemulsions are dynamic systems in which the interface is continuously and spontaneously fluctuating [20]. In very dilute systems, with only a few percentage of oil or water phase, microemulsion structures may approach regu-

lar or reverse 'swollen micelle' droplet-like shapes [19]. However, in between these extremes, the microemulsion components typically form non-spherical aggregates, which may be more or less continuous in the phase with highest volume fraction [21–23]. For the majority of microemulsion systems, these aggregates fluently change into bicontinuous structures by titration with the phase of the lowest volume fraction, and through these structures, fluently invert to 'reversed' aggregates (Fig. 2). Thus, microemulsion systems do often not display emulsion-like behaviour with sudden inversion of the 'swollen micelle', and the emulsion terminology of characterising the systems as oil-in-water (o/w), or water-in-oil (w/o), is therefore in many situations not applicable to microemulsions.

Some microemulsion systems have though been suggested to display typical emulsion-like behaviour, forming small droplet-like 'swollen micelle' structures with a dispersed and a continuous phase [13,24–27]. By continuous addition of the dispersed phase to this system, the droplets may either swell and form other colloidal structures (typically regular macroemulsions) and thus lose the microemulsion structure and characteristics, or they may invert to reverse 'swollen micelles', changing the dispersed and continuous phase in the microemulsion. Separate regions of existence for o/w and w/o droplet-like microemulsion can also be observed for some systems [13,24,25]. The exact mechanism behind the structural formations and transitions, and the relation to the physico-chemical properties of the components is not yet well established. Most likely, the flexibility of the surfactant film is an important factor, which determines the possible structures and ways of structural transitions by changes in component ratios, for a given microemulsion system. A very rigid surfactant film, will likely result in droplet-like shapes and will not enable existence of bicontinuous structures. This will impede the range of existence, and microemulsions will only form in very narrow composition ranges. A more flexible surfactant film, will most likely enable the existence of several different structures like aggregates and bicontinuous structures, and therefore broaden the range of existence, enabling formation of microemulsion with a wide variety of compositions.

The internal structure of a microemulsion vehicle is very important for the diffusivity of the phases, and thereby also for the diffusion of a drug in the respective phases.

2.2.1. Characterisation

As the size of microemulsion aggregates (typically < 150 nm) is smaller than the wavelength of visible light, and the structures can be altered by changes in composition and temperature, direct examination of microemulsion structures (e.g., by light or electron microscopy) is very difficult. Therefore, indirect measurement techniques are often employed to obtain information about the internal structure. Due to the possible existence of non-uniform-shaped microemulsion aggregates and bicontinuous structures, previous methods of characterising emulsion structures such as electric conductivity or light scattering are often of limited value for microemulsion characterisation.

A good correlation has been demonstrated between the characteristics of the various microemulsion structures and the molecular mobility of the oil, water and surfactant phase, respectively [15,21–23,28–30]. The random movements of a molecule in an isotropic solution without thermal gradients, is termed self-diffusion. According to the Stoke–Einstein equation ($D = kT(6\pi\eta r)$, where D is the self-diffusion coefficient, η the viscosity of the medium and r the (macro-)molecular radius) the self-diffusion of a (macro-)molecule is dependent on the molecular size and shape and the molecule-solvent interactions. Stilbs and co-workers [15,21–23,28], have shown that these rules are also applicable to microemulsion systems. The self-diffusion of constituents in a microemulsion, relative to those of the neat components, can be related to the degree of freedom and molecular interaction of the phases, and can vary as much as 10^5 depending on the degree of structural encapsulation, providing a straightforward interpretation of the following cases.

(1) Micellar ('o/w') droplet structure: water diffusion will be rapid, in the same order of magnitude as that of neat water. Oil and surfactant diffusion will be slow and within the same order of magnitude.

(2) Inverted micellar ('w/o') droplet structure: oil diffusion will be rapid, in the same order of magnitude as that of neat oil. Water and surfactant

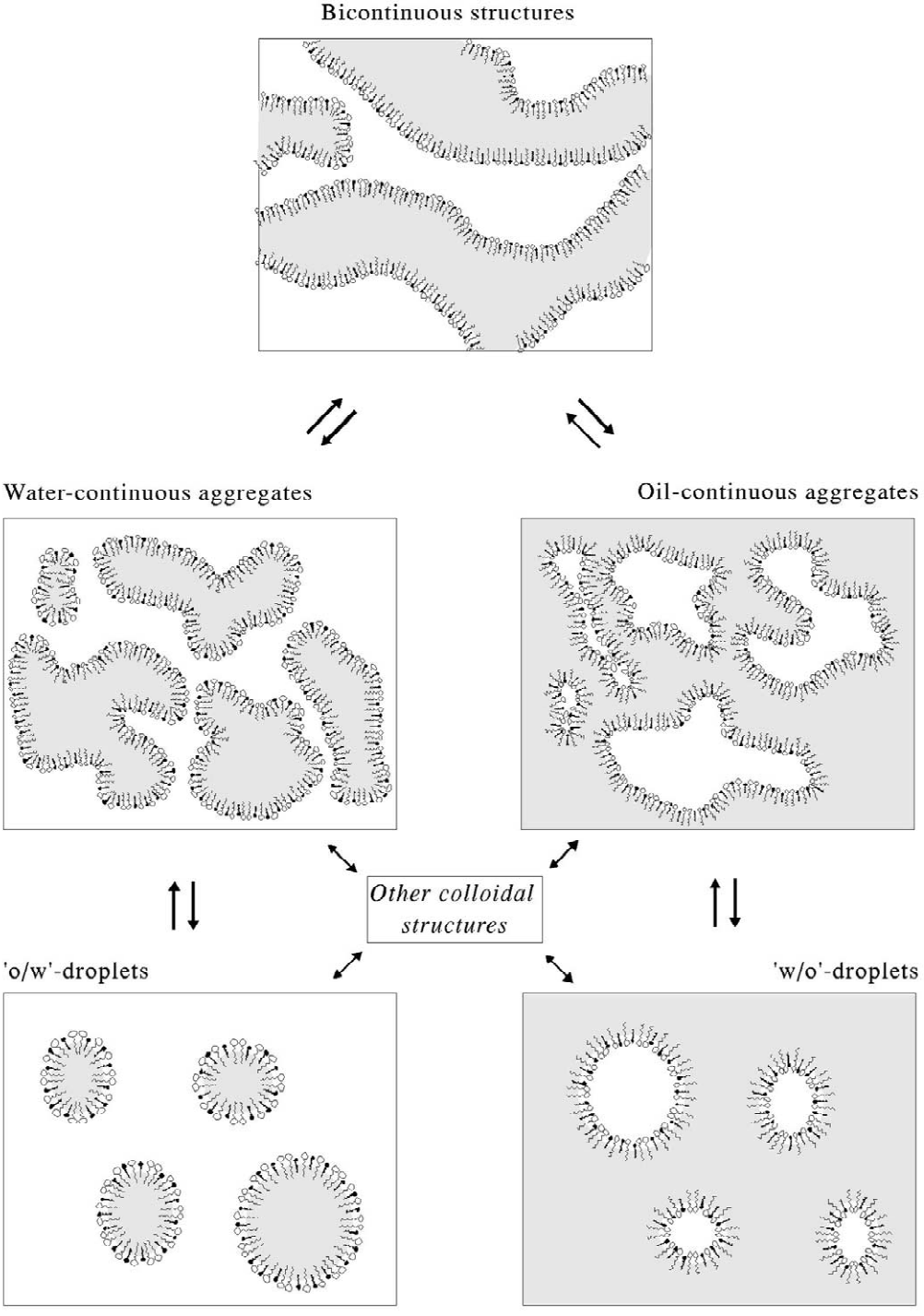

Fig. 2. Basic dynamic microemulsion structures formed by oil phase (grey), aqueous phase (white) and surfactant/co-surfactant interfacial film, and plausible transitions between the structures (indicated by arrows) by increase of oil fraction (clockwise from left to right) and water fraction (anti-clockwise from right to left), respectively.

diffusion will be slow and within the same order of magnitude.

(3) Bicontinuous structures: both water and oil diffusion will be rapid, only slightly slower than those of the neat liquids. Surfactant diffusion will be low due to the constitution of the interfacial film, but slightly higher than cases 1 and 2 as the interface is fluctuating and less restricted than in the micellar shapes.

Aggregates which are predominantly water or oil continuous will typically display self-diffusion coefficients like cases 1 and 2, respectively, but with slower diffusion of the 'continuous' phase and faster diffusion of the 'dispersed' phase, approaching case 3. Simple, nonstructured solutions will display rapid self-diffusion coefficients of all constituents. The above-mentioned three cases are ideal situations of the various microemulsion structures. As previously mentioned, microemulsions may form many intermediate structures, which will diverge from the ideal cases. Mixtures of different structures may coexist (e.g., droplets and aggregates), surfactants may fluctuate between the interfacial film and the aqueous–oil phase, and the oil or water molecules may be partly integrated into the surfactant film. In these situations, self-diffusion coefficients will be a weighted average of the diffusion within the different structures, and interpretation of the studies become more complex [15,20].

Measurement of self-diffusion coefficients can be performed by a number of nuclear magnetic resonance (NMR) spin-echo (SE) methods, neutron SE method, tracer techniques involving radioactive labelling of the compound, and transient optical grating methods [15]. Among the most prevalent is the ^{1}H Fourier transform pulsed field-gradient spin-echo (FT-PGSE) method. Self-diffusion coefficients are measured using a gradient spin-echo pulse sequence, modulating the amplitude of fixed-length gradient pulses, and diffusion of a molecule is directly related to the decay of the peak heights in the recorded ^{1}H NMR spectra, according to the following equation:

$$\ln(I_g/I_o) = -\gamma^2 d^2 G^2 (\Delta - d/3)]D \quad (1)$$

where I_g and I_o are intensities of NMR signal in the presence and absence of field gradient pulses, γ is the gyromagnetic constant for ^{1}H, d is the duration of the z gradient pulse, and Δ the time interval between the gradient pulses. While a technological description of this method is beyond the scope of this paper, further information is available in comprehensive reviews [15,28].

In pharmaceutical microemulsions, information about drug solubility and disposition within the phases, is also reflected in the self-diffusion coefficient of the drug relative to the respective constituents. Furthermore, drug disposition within the microemulsion structures, can be examined by measurement of NMR longitudinal (T_1) relaxation of the drug in the vehicle compared to that of the drug in the neat phases [8]. T_1 values basically provide information about the reorientational processes of a molecule, which reflects the environment of the substance.

2.3. Pharmaceutical considerations

2.3.1. Choice of microemulsion components

The choice of components for pharmaceutical microemulsions is often a balance between compounds, which are able to form microemulsions, are nontoxic and are able to fulfil the requirements of a good vehicle for optimal dermal absorption, i.e., high solubility of the drug of interest and a high thermodynamic activity of the drug. As the level of surfactants and oil phase in microemulsions typically is fairly high, it is important to consider the irritancy of these compounds. A lot of characterisation studies of microemulsions have been done with medium chain alcohols as co-surfactant [9,10,12,13,24,25] as they are acknowledged to form microemulsions with many surfactants and oils. However, they are generally acknowledged to induce skin irritation, and are therefore not suited for pharmaceutical microemulsions. Instead, several authors have focused on formulation of microemulsions with a single surfactant, e.g., lecithin [2,4,31] or *n*-alkyl polyoxyethylene ethers [32–34], which, however, impedes the region of existence and therefore limits the range of compositions and stability of the microemulsions. Alternative co-surfactants with better tolerability, e.g., Plurol Isostearique, have recently been introduced into topical microemulsion formulations, and have been demonstrated to provide extensive region

of existences with various surfactants and oils [3,8,17,35]. As a general guideline, nonionic and zwitterionic surfactants are less toxic than ionic ones.

If the drug of interest is lipophilic, the solvent properties of the oil should be compatible with the drug. Larger oil molecules, are generally able to dissolve lipophilic compounds of multiple sizes [19]. However, to enable the formation of microemulsions the importance of the penetration and association of the molecules of the oil phase with the interfacial surfactant film, which is governed by smaller oil molecules, must be recalled. A balance between these two properties in the choice of oil phase must therefore be found.

Depending on the physico-chemical properties of the constituents and composition, the stability of the microemulsion may be affected by the addition of buffers, electrolytes, preservatives, polymers and drugs, which may alter the microemulsion structure, and region/range of existence [29]. Microemulsion systems based on nonionic surfactants are generally less affected by additives and changes in pH than ionic surfactants.

Temperature usually also exerts an effect on the formation and region of existence of microemulsions, as the hydrophilic–lipophilic balance of the surfactants can change with temperature and destabilise the surfactant interface. Therefore, the stability of the final microemulsion formulation should always be examined within the temperature ranges of storage and application.

2.3.2. Formulation

In contrast to regular emulsions, microemulsion vehicles are formed spontaneously when admixing the appropriate quantities of the components, without requiring additional mechanical energy, and they are 'infinitely' physically stable due to their thermodynamic nature. Furthermore, they are transparent and have low viscosity (can principally be thickened by appropriate choice of noninteracting polymer [5,6,36,37], or gel forming surfactant [2,4,7,38], if desired), which facilitates filtration and visual inspection for particles. The characteristics of microemulsions make them very straightforward to prepare for pharmaceutical formulations, and the wide range of oil–water–surfactant compositions, which principally can form microemulsions, enable solubilisation of a wide range of both lipophilic and hydrophilic drugs—potentially even in the same vehicle.

3. Cutaneous drug delivery potential of topical microemulsion formulations

A substantial amount of individual reports of cutaneous drug delivery potential of topical microemulsion formulations has been published during the past decade [1–8,39–48], where the application of the vehicle has attracted increasing attention for pharmaceutical formulations. However, the studies have not been very systematic or consecutive, i.e., typically used microemulsion vehicles with diverging constituents and only implicated a few vehicles each. This has hampered general conclusions and parallels between microemulsion properties and drug delivery rate. Furthermore, the investigations have mainly been confined to in vitro studies using Franz-type diffusion cells, and only few in vivo studies have been performed to confirm the reported potential of increasing cutaneous drug delivery with these vehicles.

3.1. Solubility properties of microemulsions

Only the dissolved fraction of a drug in a vehicle can enter the skin, making solubility properties one of the initial objectives for a novel pharmaceutical formulation. Furthermore, as the cutaneous drug delivery rate of formulations is generally related to the concentration (activity) gradient of the drug towards the skin (provided that the affinity to the vehicle does not increase proportionally), the solubility potential of microemulsions may be an important factor in increasing skin absorption of drugs.

Generally, microemulsions have favourable solvent properties due to the potential incorporation of large fraction of lipophilic and/or hydrophilic phases. Furthermore, investigations have indicated that the unique structural organisation of the phases in microemulsions may contribute to additional solubility regions, increasing the load capacity of microemulsions, compared to nonstructured solutions containing the same fraction of the constituents [8,32,34,49]. In microemulsion systems consisting of

water, 2% (w/w) soybean oil and various volume fractions of nonionic surfactant (Brij 96), Malcolmson and Lawrence [49] found an increased solubility of three lipophilic model steroids (log $P = 3.9$–4.8) in the microemulsion systems, compared to similar micellar systems in the absence of the oil phase. Even though structural examinations of the microemulsions were not performed, the microemulsion systems were assumed to form 'o/w' droplet structures due to the low oil volume fraction. However, two other examined lipophilic steroids (log $P =$ 3.4 and 4.3) did not display significantly increased solubility in the microemulsion systems, compared to the micellar systems. The authors suggested the main site of solubilisation was in the lipophilic moiety of the micellar surfactant film, and that the solubility of the model drugs was only increased in the microemulsion vehicles, when the drug was additionally soluble in the neat soybean oil. This was confirmed in a later study [34], where the solubility of testosterone propionate in similar microemulsion and micellar systems, was examined with regards to different oil constituents. This study furthermore demonstrated that an increased solubility of the oil in the lipophilic moiety of the surfactant film, could lead to a competitive situation between the oil and the drug for the solubilisation sites and thereby lead to a decrease in overall drug solubility in the microemulsion vehicles, compared to that of the pure micelles.

Another investigation [8] has demonstrated an increase in solubility of both a lipophilic (22–58%) and a hydrophilic (20–36%) model drug in the studied microemulsion vehicles, compared to the solubility calculated from the additive solubility of the drugs in the respective neat constituents and their weight fractions in the microemulsion vehicles (Table 1). The increased solubility of the model drugs was suggested to be attributable to the additional solubilisation sites of the respective hydrophilic and lipophilic moiety of the formed surfactant interface film in the microemulsions.

This hypothesis was supported by assessment of NMR relaxation of the drugs in the vehicles, which indicated an association of the drug molecules with the surfactant film (most pronounced for the hydrophilic model drug).

3.2. In vitro investigations

The vast majority of drug delivery investigations with topical microemulsions have been done in vitro, using the classical Franz-type diffusion cells with various membranes. While this method actually determines percutaneous, rather than cutaneous drug delivery, a good indication of the cutaneous drug

Table 1
Actual measured (S_{mea}) and calculated (S_{calc}) solubility (from solubility in and weight fraction of the neat constituents) of lidocaine and prilocaine hydrochloride in seven microemulsion vehicles and constituents (Labrasol (Lab), Plurol Isostearique (PI), isostearylic isostearate (II)) at 24 °C

Microemulsion composition (water:II:Lab:PI%, w/w)	Lidocaine			Prilocaine hydrochloride		
	S_{mea} (%, w/w)	S_{calc} (%, w/w)	Increase (%)	S_{mea} (%, w/w)	S_{calc} (%, w/w)	Increase (%)
A (20:10:35:35)	26	18	44	6	5	20
B (20:10:47:23)	30	19	58	6	5	20
C (20:10:53:17)	27	19	42	6	5	20
D (7:70:11.5:11.5)	20	14	43	–	–	–
E (11:26:42:21)	28	19	47	4	3	33
F (55:8:25:12)	14	10	40	15	11	36
G (65:3:24:8)	11	9	22	16	12	33
Neat Lab	26	–	–	<1	–	–
Neat II	12	–	–	<1	–	–
Neat PI	22	–	–	2	–	–
Water	0.4	–	–	18.5	–	–

pH of saturated vehicles ranged from 7.7 to 8.6 for the lidocaine formulations and from 4.2 to 4.8 for prilocaine hydrochloride formulations. From Ref. [8].

delivery potential of microemulsions can be obtained from these studies. An overview of the studies is presented in Table 2.

Lecithins (phosphatidylcholines) have in several studies been used as surfactants for topical microemulsion vehicles [2,4,7,38]. These surfactants have the ability of forming microemulsions without co-surfactants in narrow composition ranges, dependent on the constituents, and are able to form gels at low water content, which is a practical feature for a

Table 2
Overview of cutaneous drug delivery studies with microemulsions in vitro

Drug	Microemulsion			Membrane/skin	Refs.
	Oil phase	Surfactants	Aqueous phase		
[^{3}H]H_2O	Octanol	Dioctyl sodium sulphosuccinate	Water	Human	[39,46]
8-Methoxsalen	IPM	Tween 80, Span 80, 1,2-octanediol	Water	Pig	[56]
Apomorphine hydrochloride	IPM, Decanol	Epikuran 200, 1,2-propanediol, benzyl alcohol	Water, Aerosil 200	Mouse	[37]
Diclofenac	IPP	Lecithin	Water	Human	[4]
	Diclofenac diethylamine	Lecithin	Water	Human	[1]
Diphenhydramine hydrochloride	IPM	Tween 80, Span 20	Water	Human	[40]
Felodipine	IPM, benzyl alcohol	Tween 20, taurodeoxycholate	Water, Transcutol, Carbopol	Mouse	[6]
Glucose	Octanol	Dioctyl sodium sulphosuccinate	Water	Human	[39]
Hematoporphyrin	IPM, decanol, hexadecanol, oleic acid, monoolein	Lecithin, sodium monohexylphosphate, benzyl alcohol	Water, PG	Mouse	[7]
Indomethacin	IPP	Lecithin	Water	Human	[4]
Ketoprofen	Triacetin, myvacet, oleic acid	Labrasol, Cremophor RH	Water	Rat	[55]
Lidocaine	Isostearylic isostearate	Labrasol, Plurol Isostearique	Water	Rat	[8]
Methotrexate	Decanol	Lecithin, benzyl alcohol Labrasol/Plurol	Water, PG	Mouse	[38]
	Ethyl oleate	Isostearique	Aq. 145 mM NaCl (pH 7.4)	Pig	[51]
	IPM	Tween 80, Span 80, 1,2-octanediol	Water	Pig	[51]
Nifedipine	Benzyl alcohol	Tween 20, taurodeoxycholate	Water, Transcutol, PG	Mouse	[5]
Prilocaine hydrochloride	Isostearylic isostearate	Labrasol, Plurol Isostearique	Water	Rat	[8]
Propanolol	IPM	Polysorbate 80	Water	Artificial	[62]
Prostaglandin E_1	Oleic acid	Labrasol, Plurol Oleique	Water[a]	Mouse	[41,42]
	Gelucire	Labrafac, Lauroglycol	Transcutol, water	Mouse	[42]
Sodium salicylate	IPM	Tween 21/81/85, bis-2-(ethylhexyl) sulphosuccinate	Water, gelatin	Pig	[36]
Sucrose	Ethyl oleate	Labrasol, Plurol Isostearique	Aq. 154 mM NaCl	Mouse	[3]

[a] A variety of seven different co-solvents was tested with the basic vehicle.

topical vehicle that is not applied in a sealed therapeutic system. Dreher et al. [4] compared a lecithin-based microemulsion gel to isopropyl palmitate (IPP) as vehicle for transdermal delivery of indomethacin and diclofenac. The microemulsion formulation was composed of 1.9 g soybean lecithin dissolved in 10 ml IPP (250 mM lecithin) and 135 μl of water in which the drugs were dissolved at half the saturation concentration. The final drug loads of the vehicles were 35 mg/ml in the microemulsion and, respectively, 1.5 and 0.1 mg/ml for indomethacin and diclofenac in IPP (saturated). The authors found a significant increase (3–6-fold) in flux through excised human skin for both drugs applied in the microemulsion vehicle compared to the neat oil vehicle. However, the IPP formulations displayed significantly higher permeability coefficients compared to the microemulsion, indicating an unfavourable partition coefficient for the microemulsion vehicle. These findings are probably attributable to the lower activity of the drugs in the microemulsion, which was formulated substantially below the saturation limit. Nevertheless, the study indicated a good potential of increasing transdermal drug delivery due to the higher drug loads in the microemulsion, which increased the concentration gradient towards the skin and thus overall steady-state flux.

Trotta and co-workers have in a series of studies investigated the transdermal flux through hairless mouse skin, of four model drugs (hematoporphyrin [7], nifedipine [5], felodipine [6] and methotrexate [38], respectively) applied in various microemulsion vehicles with alternating compositions compared to more simple vehicles. In the first study [7], the weight fraction (w/w) of the aqueous phase (water–propandiol, 18:8) and the surfactant phase (lecithin–hexylphosphate, 9:2–5) was kept relatively constant, while the co-surfactants–oil phase constituents (benzyl alcohol–isopropyl myristate (IPM)–decanol–hexadecanol–oleic acid–monoolein) and ratios (0–64) were altered in 10 different microemulsion vehicles. For all vehicles, a more than 10-fold increase in transdermal flux of hematoporphyrin was observed compared with an aqueous solution. The permeation rate of the drug from the microemulsion formulations was shown to be dependent on the oil phase constituents and ratios, with the formulation containing 50/50% IPM–decanol as oil phase providing the highest flux (23.5 ± 2.5 $\mu g/cm^2$) and the formulation with neat IPM as oil phase providing the lowest flux (4.5 ± 0.5 $\mu g/cm^2$). However, no clear trend was observed for the flux dependency of the various oils in regards to ratios of the different oil constituents or for example to physico-chemical characteristics of the oils. In a similar study [6], the effect of changing the ratio between benzyl alcohol (5–17%) and IPM (0–10%) as the oil phase in six microemulsions with relatively fixed weight fraction of aqueous phase (water–Transcutol, 40–48:14–16%) and surfactant phase (Tween 20–taurodeoxycholate 18–21:1%) on the transdermal flux of felodipine was investigated. All topical formulations were saturated with felodipine (2–6%, w/w) and gelled with Carbopol 940. The study demonstrated a larger transdermal flux of the drug, proportional to the volume fraction of benzyl alcohol incorporated into the microemulsion vehicle, which the authors suggested was due to an enhancement effect of the oil. However, upon closer examination of the results, the flux increase with larger benzyl alcohol content may also be attributable to the proportional solubility of felodipine in the microemulsions with benzyl alcohol content, increasing the concentration gradient towards the skin (assuming that the partition coefficient of the drug between the skin and vehicle was not proportionally reduced by addition of the oil). The individual effect of benzyl alcohol and Tween 20 in the fastest permeating microemulsion formulation was examined by application of two similar reference gels without the respective components. The permeation rate of felodipine was drastically decreased (30-fold) from the gel lacking benzyl alcohol (gel 1), and also somewhat decreased from the gel lacking Tween 20 (gel 2), suggesting an advantage of applying the microemulsion vehicle for transdermal drug delivery compared to a micellar and a water–oil mixture vehicle. However, compared to the investigations of the six microemulsion vehicles, the decreased flux of reference gel 1 was accompanied by a drastic reduction in solubility of felodipine in the formulation (from 6 to 0.06%), which may explain a part of the findings. Also a reduction in solubility was observed for gel 2 (0.9%).

In the third study of microemulsion vehicles composed of Tween 20, taurodeoxycholate, benzyl

alcohol (approximately 23:2:25%, w/w) and an aqueous phase (approximately 50%, w/w), the effect of changing the aqueous phase from water–Transcutol to water–propylene glycol (PG) (ratio 74:26) on the permeation rate of nifedipine (2%, w/w) was investigated [5]. The transdermal flux of the drug was reported to increase more than 2-fold by introducing Transcutol into the aqueous phase, compared to PG. PG is generally acknowledged as a good solubilising agent for lipophilic drugs in aqueous vehicles, and may also act as a penetration enhancer. However, large contents of the agent in a vehicle may substantially reduce the partition coefficient between the skin and vehicle for the drug, which can counteract the benefit of the increased concentration gradient by reducing the overall activity of the drug in the vehicle, and thereby actually decrease the transdermal flux [50]. Mean permeation rate of nifedipine from the Transcutol microemulsion was reported to be 62 times higher than a similar reference gel lacking benzyl alcohol and 37 times higher than a water–Transcutol vehicle saturated with benzyl alcohol, loaded with 0.6 and 0.5% nifedipine, respectively. The flux increase for the microemulsion formulation compared to the reference gels was substantially higher than what could be explained by the drug concentration increase alone, indicating that the microemulsion structure of the vehicle provided a further increase compared to the micellar and the simple solution. The microemulsion structures were suggested to be of the o/w-type, and a mean droplet size of 32 nm was reported according to assessments by the dynamic light scattering technique.

Finally, Trotta et al. have demonstrated that the transdermal delivery of methotrexate, a weakly acidic drug typically administered systemically for the treatment of psoriasis, could be increased up to 10-fold from a lecithin–water–PG–decanol–benzyl alcohol microemulsion compared to that from the water–PG solution alone [38], and 3-fold compared to an oil suspension. Also mean lag time was considerably decreased with the microemulsion vehicle. Various counter-ions were added to the microemulsion increased transdermal flux of the ionised methotrexate even further.

The use of microemulsions for iontophoretic drug delivery does not appear to provide any advantages compared to aqueous solutions. The use of microemulsion-based organogels with gelatin has been compared to aqueous solutions for transdermal iontophoresis delivery of sodium salicylate [36]. Transdermal salicylate flux was not discernible between the formulations without iontophoresis. Applying current through the skin, drug delivery from the aqueous formulation was enhanced more than from the gel. Also Alvarez-Figueroa and co-workers reported a decrease in transdermal iontophoretic delivery of methotrexate from a microemulsion vehicle [51] relative to that from an aqueous Hepes buffer [52], even though the microemulsions were superior to the aqueous solution in regards to rate of passive diffusion of the drug. Presumably, microemulsions hamper iontophoretic delivery due to decreased conductivity when incorporating oil and surfactants into the aqueous phase.

To investigate the influence of the water amount incorporated into a microemulsion vehicle on transdermal delivery rate of, respectively, radiolabelled water [39,46] and radiolabelled glucose [39], Osborne et al. formulated three vehicles consisting of a fixed ratio of dioctyl sodium sulphosuccinate and octanol (58:42) with, respectively, 15, 35 and 68% water incorporated. Octanol is generally regarded as highly irritating to the skin, which hampers the clinical relevance of the vehicle; however, the oil was employed to enable a wide range of water volume fractions in the microemulsion to comply with the aim of the investigations. The studies demonstrated large differences in permeation rates of water [39,46] and glucose [39], dependable on the water content of the vehicle. Transdermal glucose flux from the vehicles with 35 and 68% water was increased more than 30-fold, compared to neat water and the 15% water microemulsion with equal low glucose concentrations in the vehicles. Also transdermal water flux increased with increasing water content of the vehicles. An approximately 2-fold increase in water flux was observed from the microemulsion containing 68% water, compared to the one with 35% water, which correlated well with the corresponding increase in amount of tritiated water in the former (50 mCi $[^3H]H_2O$ per ml of water in the vehicles). However, a 6-fold increase in flux was observed for the 35% microemulsion compared to the one with 15%, which exceeded what could be

explained by the concentration increase alone. The authors suggested that the transdermal delivery of water and glucose from the microemulsion vehicles was generally enhanced due to the interaction of octanol with the stratum corneum; however, due to the low water content in the 15% water microemulsion, the hydrophilic compounds were closely associated with the surfactant film, which hampered release of the compounds from the vehicle and thus drastically decreased transdermal flux.

The influence of the colloidal structure of topical phospholipid formulations on the percutaneous permeation of diclofenac diethylamine has been studied by Kriwet and Müller-Goymann [1]. An elegant feature of this study was the use of the drug as the oil phase–co-surfactant in the vehicles, maximising thermodynamic activity of the drug in this phase. The structures of these systems formed ranged from liposomal dispersions via microemulsions to lamellar liquid crystals. Microemulsions were demonstrated to significantly increase transdermal drug delivery through excised human stratum corneum, compared to the other colloidal structures and an aqueous solution, both in terms of flux and permeation coefficient.

With an appropriate oil phase, a novel low irritant surfactant, Labrasol, has been demonstrated to form microemulsions with several non-alcohol co-surfactants, which diminishes the risk of cutaneous toxicological reactions by application of topical microemulsions, and thus increasing the pharmaceutical relevance of the vehicles.

Delgado-Charro et al. [3] studied a broad range of microemulsion vehicles, formed by Labrasol–Plurol Isostearique (3:1) as surfactant–co-surfactant at medium content levels (25–44%, v/v) using ethyl oleate as oil phase in volume fractions of 7.5–49% and a 154 mM sodium chloride aqueous solution (14–67.5%, v/v). The microemulsions were, by dynamic laser light scattering and by electric conductivity examinations, suggested to be either of w/o or o/w droplet-like structures, depending on the ratio between the water and oil phase; however, agglomerates and asymmetric structures were also indicated in four out of five of the microemulsions. Permeation rate of sucrose (20 mM in all vehicles) from the microemulsions through mouse skin was demonstrated to be substantially larger compared with an equivalent and a saturated (143 mM) aqueous solution. There was a slight trend for the 'o/w-microemulsions' to increase sucrose delivery more than their 'w/o microemulsion' counterparts; however, only permeation from one microemulsion formulation (with the least water content of the three 'o/w microemulsions') was statistically discernible from the others.

In a preliminary report [41] and a subsequent publication [42], Ho et al. investigated the effect of adding co-solvents to microemulsion vehicles on the transdermal flux of prostaglandin E_1 and its alkyl esters through hairless mouse skin. The two basic vehicles consisted of Transcutol–co-solvent–Labrasol–Plurol Oleique–oleic acid (28:19:19:28:4.5%, w/w) (microemulsion 1) and Transcutol–co-solvent–Labrafac–Lauroglycol–Gelucire (10:15:10:20:44.5%, w/w) (microemulsion 2), respectively. Generally, microemulsion 1 increased transdermal permeation of all the prostaglandins compared to microemulsion 2, independently of applied co-solvent, and permeation rates of prostaglandin E_1 was higher than its alkyl esters. Addition of water as co-solvent was found to provide the lowest transdermal flux and addition of PG the highest flux for prostaglandin E_1, which may be attributable to the penetration enhancing effect of PG [53,54]. However, the obtained flux values for the prostaglandins in the microemulsions were estimated by the authors to be insufficient for clinical purposes.

The transdermal flux of ketoprophen has been shown to vary more than 8-fold, depending on the oil incorporated into a Labrasol–Cremophor–water microemulsion with 3% drug load [55]. Furthermore, the study revealed that percutaneous ketoprophen delivery from the formulations was also greatly influenced by the fractional composition of the microemulsions constituents with an inverse correlation between surfactant content and transdermal flux.

Seven microemulsion vehicles with alternating compositions of Labrasol–Plurol Isostearique–isostearylic isostearate–water (Table 1) have been applied to compare transdermal flux of a lipophilic (lidocaine) and a hydrophilic (prilocaine hydrochloride) model drug through excised rat skin, to a commercially available regular o/w-emulsion (Xylocain® 5% cream, lidocaine, AstraZeneca), a hydrogel (Xylocain® 2% gel, lidocaine hydrochloride, AstraZeneca) and an o/w emulsion based on an eutectic mixture of the drugs (EMLA® 5%, lidocaine

and prilocaine, AstraZeneca) [8]. The applied microemulsion constituents were shown to enable a wide range of microemulsion compositions, which could be applied according to the desired drug solubility characteristics of the vehicle and optimised for high transdermal drug delivery rates. The seven investigated microemulsion vehicles were, by assessment of self-diffusion coefficients with FT-PGSE NMR, indicated to comprise water or oil continuous aggregates/droplets, or bicontinuous structures, respectively. The transdermal flux of lidocaine and prilocaine hydrochloride was demonstrated to vary substantially according to both the composition of the applied microemulsion vehicle, and the incorporated drug concentration relative to the saturation limit of each vehicle. The optimised microemulsions with drug loads near the saturation limit were found to increase the permeation rate of lidocaine up to 4-fold compared to the regular o/w emulsion and approximately 50% compared with EMLA. Similarly, the microemulsion vehicles were found to increase transdermal flux of the hydrophilic model drug up to 30 times compared to the hydrogel. The permeation coefficient of lidocaine from two of the microemulsions was, however, only slightly larger compared to that from the regular o/w emulsion, indicating that the microemulsion structure does not by itself offer a significant increase in permeation rate compared to a conventional o/w emulsion with equivalent drug load. Differences in partition coefficients between the vehicle and skin could also have influenced the results, though. The higher overall flux from the microemulsions therefore appears to be attributable to the high solubility of lidocaine in the vehicle (without a concurrent increase in overall vehicle affinity for the drug), creating an increased concentration gradient towards the skin. The permeation coefficient of prilocaine hydrochloride was generally increased from the microemulsions compared to lidocaine hydrochloride from the hydrogel. It was assumed that the permeation rates of the two hydrochloride salts of the anaesthetics were comparable, due to the very similar physico-chemical properties and permeation profiles of the base form of the drugs, which was demonstrated with EMLA. The differences in permeation rates of both drugs from the microemulsions were indicated to correlate with the mobility of the drugs in vehicles.

Both percutaneous and cutaneous delivery of 8-methoxsalen from six microemulsion vehicles with varying ratios between water–IPM–Tween 80, Span 80, 1,2-octanediol and corresponding neat oil, and water phase has been investigated [56]. With saturated vehicles, it was demonstrated that percutaneous delivery of 8-methoxsalen could be enhanced up to 5- and 8-fold compared to neat oil and water, respectively. The microemulsions with highest surfactant content increased drug permeation the most, indicating that the enhancement was mainly attributed to surfactant modulation of the skin barrier. The cutaneous drug delivery of the formulations correlated qualitatively well with the percutaneous results; however, the study indicated a larger difference (up to 33-fold) in dermal penetration between the microemulsions and the aqueous formulation.

Cutaneous penetration into defined tissue layers has been investigated by incubation of a finite dose microemulsion on excised human skin, followed by biopsy punctures and quantification of the active compound in stratum corneum–viable epidermis–dermis skin layers [40]. The three investigated microemulsion vehicles consisted of (1) a basic microemulsion composition of Tween 80–Span 20–IPM–water (7:13:74:5%, w/w) with 1% of a hydrophilic model drug (diphenhydramine hydrochloride), (2) the basic microemulsion with 2% cholesterol added and (3) the basic microemulsion with 5% oleic acid added. Incorporation of cholesterol into the microemulsion significantly enhanced the dermal delivery of the drug into all skin layers, and particularly into the stratum corneum layer. Interestingly, the addition of oleic acid, which is generally acknowledged as a penetration enhancer, into microemulsion 3, did not increase dermal delivery of the drug in any layers of the skin. The authors suggested the finding was due to the means of enhancement of the two additives. Cholesterol is believed to increase the hydrophilic domains in the stratum corneum, facilitating the passage of hydrophilic substances, while oleic acid is believed to alter the viscosity of the skin lipids, facilitating diffusion of lipophilic substances.

3.3. In vivo animal investigations

Also, in animals, the majority of topical microemulsion studies have focused on assessment of

Table 3
Overview of cutaneous drug delivery studies with microemulsions in vivo

Drug	Microemulsion			Species	Refs.
	Oil phase	Surfactants	Aqueous phase		
Bupranolol	IPP	Tween 85	–[a]	Rabbit	[43,44]
	IPP	Targat, glycerol monooleate	–[a]	Rabbit	[45]
	IPP	Tween 85, poloxamer 101	–[a]	Rabbit	[45]
Carazolol	IPP	Tween 85	–[a]	Rabbit	[44]
Hydrocortisone	IPM	Sucrose laurate, PG	Water	Human	[58]
	IPM	Targat, Plurololeat	Water	Human	[58]
Lidocaine	Isostearylic isostearate	Labrasol, Plurol Isostearique	Water	Rat	[48]
	Isostearylic isostearate	Labrasol, Plurol Isostearique	Water	Human	[47]
Methyl nicotinate	IPP	Lecithin	Water	Human	[2]
Piroxicam	IPM	Hexadecyltrimethylammonium bromide	Aq. phosphate buffer (pH 5.5)	Rat	[63]
Prilocaine hydrochloride	Isostearylic isostearate	Labrasol, Plurol Isostearique	Water	Rat	[48]
Timolol	IPP	Tween 85	–[a]	Rabbit	[44]

[a] 'Supersaturated' vehicle with water uptake during the study.

percutaneous drug delivery, with limited direct assessment of cutaneous drug delivery potential. These studies have been summarised in Table 3.

Several transdermal drug delivery studies, assessing drug levels in the systemic circulation, have been performed in rabbits by a research group consisting of Kemken et al. [43–45]. The group has mainly focused on the drug delivery properties of self-emulsifying 'supersaturated' microemulsion vehicles, consisting of a lipophilic model drug dissolved at near saturation limit in a oil–surfactant mixture, which upon occluded topical application theoretically transforms into a true microemulsion vehicle by uptake of water from the skin. During the water uptake, the solubility of the drug in the vehicle decreases, leading to 'supersaturation', which results in an increase in thermodynamic activity of the drug in the oil phase. The studies comprised indirect assessment of transdermal delivery of β-blockers from the microemulsions via pharmacodynamic response estimated by suppression of induced tachycardia. The basic reference vehicle consisted of approximately equal weight fractions (varied 5–10% according to the incorporated β-blocker, to enable formation of a microemulsion with all drugs) of Polysorbat 85 (Tween 85), Poloxamer 101 and IPP. In the first study [44], the effect of bupranolol and timolol following topical application in a pre-microemulsion vehicle and a matrix patch (Schwarz), respectively, was compared. The pre-microemulsion vehicle was demonstrated to increase transdermal delivery of both drugs compared to the matrix system, in terms of a faster increasing effect and higher maxima. The second study [43] involved transdermal delivery over 10 h of nine different β-blockers from the pre-microemulsion vehicle, with alternating concentrations (2.0, 5.0 and 7.7% bupranolol) and applied doses (0.5, 1 and 2 g bupranolol or carazolol). As expected, the effect seen after the administration of the bupranolol formulations was shown to increase with higher drug concentrations in the vehicle; however, no additional boost in permeation rate could be demonstrated for the 'supersaturated' vehicle, besides what could be ascribed the relatively higher drug load compared to the vehicles with sub-saturation level drug loads. Finally, Kemken et al. [45] have investigated the correlation between the effect and the saturation profile of bupranolol as a function of water addition to the pre-microemulsion reference vehicle, a pre-microemulsion composed of polyoxyethylene(20) glycerol monooleate (35%), Tegin (45%) and IPP

(29%), and the individual components of the reference vehicle. A qualitative correlation between the two parameters was observed in the study, indicating that the vehicles where the solubility of bupranolol decreased most rapidly by the addition of water would result in a higher degree of 'supersaturation' and thus higher transdermal flux. Bupranolol in neat IPP induced the most rapid effect, and obtained a maximum effect level similar to the reference vehicle, indicating that this vehicle would in fact be a more attractive solution for fast transdermal drug delivery, compared to the investigated microemulsion vehicles. However, IPP may have acted as a penetration enhancer, which by itself would induce the high permeation rate. The studies by Kemken et al. have indicated additional unique advantages of microemulsion formulations for cutaneous drug delivery, using the self-emulsifying properties of microemulsions, which occur without supplying external energy. It is general knowledge that water evaporation occurs continuously from the sweat glands in the skin. However, no evidence was provided for the actual degree of water uptake into the vehicle and transformation into microemulsions. Furthermore, the equilibration process for the formation of homogeneous microemulsions without accelerating the process (e.g., by stirring) is also time-dependent and may not be completed during the experiment, making the existence of actual microemulsions during the experiments somewhat controversial.

Comparable to the in vitro investigations [8], the dermal drug delivery potential of four optimised microemulsion vehicles with different weight fraction of the constituents (systems A, D, E and G formulated at near saturation limit, Table 1) has been examined in vivo in rats [48] and compared to Xylocain 5% cream, Xylocain 2% gel, and EMLA 5%, using lidocaine and prilocaine hydrochloride as model drugs. Apparent absorption rate and lag time of the drugs was assessed by microdialysis directly in the dermis of the skin. Similar to the in vitro study, large differences in absorption rate were observed (up to 4-fold), depending on the composition of the microemulsion vehicle, emphasising the importance of the internal microemulsion structure which is affected by the incorporated weight fraction of the constituents. All the microemulsion formulations were demonstrated to increase dermal delivery of lidocaine compared to the o/w emulsion cream, with a maximum of eight times faster absorption rate from microemulsion G with 9% drug load. This microemulsion vehicle was also demonstrated to be superior in cutaneous delivery of lidocaine compared to EMLA (3-fold higher absorption coefficient). EMLA is currently regarded as the vehicle providing the most rapid cutaneous absorption of lidocaine [57], due to the formation of an eutectic mixture of lidocaine and prilocaine, which lowers the melting point of the drugs and creates a neat oil phase of the drugs with maximum thermodynamic activity. Generally, the microemulsion vehicles with higher water content increased penetration rate the most, and the absolute vehicle saturation level of the lidocaine microemulsions did not appear to be the main determinant for the drug delivery rate. However, the absorption rate of lidocaine was reduced 35% by decreasing the drug load from 9 to 7.5% in microemulsion G, suggesting that the concentration gradient also influences drug delivery rate from microemulsions in vivo, within the limitations of drug diffusion in individual vehicles. However, lag time of lidocaine was not discernible between any of the formulations. Mean absorption rate of prilocaine hydrochloride was approximately doubled by the application in microemulsion G, relative to that of the lidocaine hydrochloride from Xylocain 2% gel. The penetration rates from the other microemulsions were not discernible from the hydrogel. However, mean lag time of prilocaine hydrochloride penetration into the dermis from all microemulsions, was demonstrated to decrease significantly, from 102 to 10–61 min, compared to the hydrogel. Cutaneous levels of the hydrophilic drugs were very low compared to lidocaine, indicating that while the presented microemulsions may provide a substantial increase in drug delivery rate compared with a conventional hydrogel, the therapeutic relevance is still fairly low unless the drug is very potent. Interestingly, the microemulsion composition, which provided the highest absorption rate of lidocaine, was also superior for the delivery of prilocaine hydrochloride. These findings could very well be due to the 3–6-fold higher drug level in this vehicle (14%) compared to the rest of the microemulsions, though.

3.4. Human investigations

In vitro and animal studies can provide very useful information and predictions about drug penetration and behaviour in man; however, the 'golden standard' for evaluation of pharmaceutical formulations is the actual assessment in humans. Presumably, due to the novelty of applying microemulsions for cutaneous drug delivery, and the frequent use of skin-irritating constituents in many of the early in vitro studies, very few human investigations have been reported.

Bonina et al. [2] have studied the dermal drug delivery properties of phospholipid-based vehicles with different colloidal structures, ranging from a microemulsion gel (soybean lecithin–IPP–water) to liposome suspensions (soybean lecithin–cholesterol 9:1%, w/w, and water). The vehicles were compared with an aqueous solution for cutaneous delivery of methyl nicotinate (0.5%, w/w, in all vehicles). Furthermore, the influence of incorporating the vehicles in various hydrophilic gels (Carbomer or carboxymethyl cellulose) on the penetration rate of the drug was evaluated. The formulations were applied topically for 30 min (only 15 min for the liquid formulations) under occlusion. Following removal of the applications, the decline of the methyl nicotinate-induced erythema was subsequently assessed over a 10 h period. Methyl nicotinate applied in the microemulsion gel was demonstrated to initially induce the most rapid and intense erythema of the evaluated formulations; however, the following decline of the effect was also substantially faster. The results indicated that the microemulsion vehicle provided the fastest penetration rate for the slightly lipophilic model drug, but the drug was not retained in the skin following penetration, which may be attributable to a disruption of the stratum corneum barrier enabling fast passage of the drug through the skin. This hypothesis was supported by a 3-h pretreatment experiment with the oil phase, which was demonstrated to substantially increase drug delivery for the aqueous solution. The enhancer effect of IPP has also been indicated by several other studies [4,45]. During the 10-h assessment period, the average effect of the liposome formulations was substantially larger than both the microemulsion and the aqueous solution–gels, and the effect was still significant at the endpoint of the experiment, indicating that these vehicles may enable incorporation of methyl nicotinate into the stratum corneum, forming a reservoir, which may be advantageous if prolonged release is desired.

Using a pharmacodynamic assessment method in terms of skin blanching, cutaneous delivery of hydrocortisone in either a water- or an oil-continuous microemulsion has been compared to an amphiphilic cream [58]. Both microemulsion formulations appeared to increase hydrocortisone penetration into the skin to a greater extent than the cream; however, the pharmacodynamic effect was counteracted by a hyperemic effect of the microemulsions, induced by skin irritation.

As a final evaluation of earlier in vitro [8] and in vivo studies in rats [48], the drug delivery potential of microemulsion system G (Table 1), using lidocaine (7.5%) as model drug, in comparison to a conventional o/w emulsion (Xylocain 5%) has been assessed in humans. The cutaneous bioequivalence of the formulations was evaluated in eight subjects, pharmacokinetically with the microdialysis technique, and pharmacodynamically by assessment of lidocaine-induced anaesthesia during application with von Frey hairs [59]. As indicated by the initial in vitro/in vivo studies, a substantial increase in absorption of lidocaine was found, when applied in the microemulsion vehicle compared to the o/w emulsion. The pharmacokinetic study demonstrated a four times larger total amount of lidocaine absorbed into the skin from the microemulsion relative to the o/w emulsion during a 4-h application period. The improved absorption was primarily shown to be attributable to a 3-fold increase in penetration rate into the dermis, but also mean lag time was significantly reduced from 110 to 87 min. The pharmacodynamic study indicated that the anaesthetic effect of lidocaine from the microemulsion formulation, estimated by area under the effect curve from the 4-h application period, was slightly larger than from the o/w emulsion. However, the results were not statistically discernible. It was suggested that the lack of differentiation in cutaneous drug delivery between the two vehicles in the pharmacodynamic study, could be explained by the low sensitivity of the assessment method and the low efficacy of the model drug.

3.5. Correlation between microemulsion characteristics and drug delivery potential

Numerous individual studies of structure, self-diffusion coefficients, formation and transdermal permeation of various microemulsion systems have been published. However, even though the importance of the microemulsion composition with given constituents and colloidal structure on the cutaneous drug delivery rate has been reported [1,2,8,39,46,48,51,55,56], little is known about the correlation between these parameters.

In a study investigating apparent mean droplet size of 'o/w'- and 'w/o'-like microemulsions and electric conductivity in five different microemulsion formulations with the same constituents, no significant influence of the assessed parameters on the transdermal flux of sucrose was observed [3]. Most of the microemulsion vehicles were, however, indicated to additionally contain agglomerates and asymmetry, which made the reported 'droplet' sizes controversial.

A hypothesis stating that a hydrophilic drug will not be available for percutaneous transport from a microemulsion unless water from the microemulsion is freely transported to the skin, was put forward by Osborne et al. [46] in 1988. A more recent study [8] has elaborated this hypothesis and suggests that the mobility of the actual drug in the vehicle is one of the main determinants of the cutaneous drug delivery potential of a microemulsion vehicle (with given constituents) that does not exhibit additional enhancer activities. Self-diffusion coefficients of the vehicle constituents and drug was assessed by FT-PGSE NMR, and the mobility of the drugs in the microemulsions was demonstrated to vary up to 15-fold for prilocaine hydrochloride and 3-fold for lidocaine, depending on the vehicle composition [8]. The differences in self-diffusion coefficients were indicated to be attributable to the internal structure of the microemulsion, in terms of the encapsulation degree of the phase in which the drug was mainly dissolved. Furthermore, the NMR relaxation study indicated that prilocaine hydrochloride to some extent adhered to the surfactant film (which became less pronounced at higher concentrations in the vehicles with low weight fraction surfactants), additionally hindering diffusion of the drug. Correlation of transdermal flux from the equally loaded microemulsions (5%) with drug self-diffusion values indicated a linear relationship for both lidocaine and prilocaine hydrochloride. This suggests that microemulsion structures allowing high drug mobility in the vehicle, translate into faster drug diffusion to the skin surface, and thus a higher transdermal flux. This compares well with the earlier studies performed by Osborne et al., where a correlation was found between water self-diffusion coefficients in three microemulsion vehicles and transdermal flux of both glucose [39] and water [39,46], assuming that glucose is primarily dissolved in the aqueous phase and the self-diffusion coefficient of glucose can be related to that of water.

The significant influence of drug diffusion in the vehicle on the transdermal drug delivery rate is also supported by in vitro flux investigations of diclofenac diethylamine from five phospholipid formulations with various colloidal structures, ranging from liposomal dispersions via microemulsions to lamellar liquid crystals [1]. The diffusion coefficient of the drug in the vehicles was assessed indirectly by estimating the release rate of the drug from the formulations through a non-rate-limiting silicone membrane and compared well with permeation rate of the drug through excised human stratum corneum.

3.5.1. Means of increasing cutaneous drug delivery

As described above, multiple factors may influence the drug delivery rate of a microemulsion formulation, depending on the characteristics of the applied constituents (i.e., physico-chemical properties and interaction with the skin) and the respective weight fractions incorporated into the vehicle. Generally, microemulsions have ultra-low interfacial tension, which, for ungelled microemulsions, ensures an excellent surface contact between the skin and the vehicle over the entire application area (i.e., within wrinkles and microscopic gaps). Furthermore, the low interfacial tension and the high content of both aqueous phase, lipophilic phase and surfactants in microemulsions, which are continuously and spontaneously fluctuating, facilitates transition of both lipophilic and hydrophilic drugs from the—typically relative hydrophilic—vehicle to the very lipophilic stratum corneum.

Two basic trends are observable from the reported

studies, which may further contribute to the favourable drug delivery properties of microemulsions, depending on the characteristics of the microemulsion constituents: high drug load capacity of the vehicle [4,6,8] and penetration enhancer effect [2,4,40,45,56].

While the permeability coefficients of drugs from microemulsion vehicles are often not substantially larger than from conventional vehicles with similar constituents, e.g., emulsions and neat oil phases [4,8], the overall transdermal flux is increased due to the larger concentration gradients, which are created by the ability of dissolving large amounts of both lipophilic and hydrophilic drugs in microemulsions, without a concurrent increase in vehicle affinity. This agrees well with the traditional comprehension of transdermal permeation rate from simple solutions according to Higuchi [60].

The microemulsions studied by our group (Table 1) [8] did not appear to increase cutaneous drug delivery by means of an enhancer effect. In fact, a general trend of decreasing cutaneous drug delivery was observed with increasing surfactant and oil content of the microemulsion formulations, for both the lipophilic and hydrophilic model drugs, indicating that the constituents of the vehicles did not increase drug delivery by means of an enhancer effect. Furthermore, 20 h pre-treatment of excised rat skin with a microemulsion vehicle did not significantly increase transdermal flux, additionally supporting the idea that the constituents did not act as enhancers.

Transdermal sucrose flux from microemulsion systems comprising Labrasol–Plurol Isostearique as surfactant system [3], showed a continuous increase in vitro during a 9-h sampling period, which the authors proposed was due to a perturbation of the skin by the microemulsions. However, this hypothesis was contrasted by similar transepidermal water loss values prior to and after a 3-h topical application period of the vehicle on human volunteers. The results may be explained by a non-equilibrated distribution of the drug in the skin or hydration state of the stratum corneum during the relatively short sampling period.

Transdermal delivery of ketoprophen from Labrasol–Cremophor–oleic acid–water microemulsions has been shown to increase with decreasing surfactant content of the vehicles and to be independent of the oil content [55]. Ketoprophen content was kept constant in the formulations (3%), which indicated that the drug delivery potential was correlated with increase in thermodynamic activity of the drug.

The potential enhancer effect of microemulsions is typically attributable to the individual constituents, i.e., the oil of surfactant phase, rather than the specific microemulsion structure. An enhancer effect of the frequently applied oil phase for microemulsions, IPP, has been indicated by several studies [2,4,45]. Fourier transform infrared spectroscopy examinations of stratum corneum following treatment with a lecithin–water–IPP microemulsion and neat IPP, indicated a substantial incorporation of IPP into the skin layer after 24 h, which enhanced drug delivery by disruption of the lipid organisation in the stratum corneum according to observations made by electron microscopy and differential scanning calorimetry [4].

Surfactants, which are able to function as enhancers, are believed to penetrate the skin mainly in their monomer form [54], and it is thus not likely that the micellar-like microemulsion structures are able to penetrate the skin intact [61]. However, as microemulsions are highly dynamic structures [20], it is plausible that monomer surfactants (or oil molecules) can diffuse to the skin surface and act as enhancers, either by disrupting the lipid structure of the stratum corneum, facilitating diffusion through the barrier phase, or by increasing the solubility of the drug in the skin, i.e., increasing the partition coefficient of the drug between the skin and the vehicle. It is likely that microemulsions, which enhance cutaneous drug delivery by disruption of the stratum corneum lipids, will result in an increase in both dermal and transdermal drug delivery, while the vehicles that act as enhancers by means of increasing the partition of the drug into the skin, will mainly yield an increase in dermal drug delivery.

The enhancer effect of the constituents is, however, also dependent on the favour of hydrophilic or lipophilic pathways for the drug through the stratum corneum. Schmalfuss et al. [40] demonstrated that the skin penetration of a hydrophilic model drug was only enhanced by the addition of cholesterol and not oleic acid to the microemulsion vehicle. While both additives are generally acknowledged to act as

penetration enhancers, cholesterol acts by increasing the hydrophilic domains and oleic acid facilitates diffusion through lipophilic domains in the stratum corneum.

The unique possibility for microemulsions to form super-saturated vehicles during application has been indicated by Kemken et al. [43–45], which may further add to the means of increasing cutaneous drug delivery with microemulsions.

However, as described above, the drug delivery potential of individual microemulsion vehicles is significantly influenced by the composition and internal structure of the microemulsion. While a microemulsion vehicle may exhibit excellent solubility of a drug and contain penetration enhancers, the full potential of the vehicle for high cutaneous drug delivery rates can be hampered by either diffusional hindrances of the drug (i.e., encapsulation of the solvent phase or adsorption to the surfactant film), unfavourable partitioning of the drug from the internal to the external phase, or unfavourable overall partition coefficient between the microemulsion and the skin. This has been demonstrated in a study [8] where the delivery rates of lidocaine were higher from the microemulsions with lower saturation level, due to the structural limitations for free drug diffusion in the other vehicles, independently of whether the vehicles contained equal drug loads or near saturated drug loads.

3.6. Tolerability studies of topically applied microemulsions

The potential risk of toxicological skin reactions from application of microemulsions is closely related to skin interaction properties of the constituents, and the use of penetration enhancers should therefore be minimised to ensure the therapeutic relevance of the vehicles. However, as microemulsions typically are composed of large amounts of surfactants and oil, it is particularly important to consider the potential skin irritation and toxicological reactions by topical application of these formulations.

Following 6 h pre-treatment of excised hairless mouse skin with a microemulsion composed of decanol–lecithin–hexylphosphate–benzyl alcohol–water–propandiol (54:8:5:6:18:8), Trotta et al. [7] did not observe an increase in transdermal flux of hematoporphyrin applied in a similar microemulsion vehicle, compared to no pre-treatment, indicating that the microemulsion did not induce barrier perturbation of the skin.

Dreher et al. [31] have studied the acute and cumulative irritation of neat IPP, a microemulsion gel (1.9 g soybean lecithin in 10 ml IPP and 135 μl water) and a lecithin–water liposome suspension. The acute irritancy of the vehicles was assessed by development of erythema, edema and infiltration in 151 subjects, following application of 40 μl in a large Finn chamber (14 mm i.d.) for 48 h. Thirty minutes and 1 day after removal of the application, respectively, a low irritancy of all applied vehicles was indicated (less than three persons showed signs of irritancy for each vehicle). During the cumulative study, 20 subjects were inspected every 24 h for development of erythema, scaling and fissuring, over a 21-day period. IT_{50} (irritation time for 50% of the test population), was estimated to be 13–17 days, and was significantly lower for the microemulsion gel and liposome vehicle compared to neat IPM, indicating that lecithin added to the irritancy of the vehicles. The authors concluded that all tested vehicles were suitable for clinical application, when the formulation was not applied to the same area of the skin for consecutive days. However, the very low volume of the applied vehicles over a large area may hamper the clinical relevance of the findings. In a later in vitro study using human skin, Dreher et al. [4] applied a 10-fold larger volume of similar vehicles over a smaller area (9 mm i.d.), and demonstrated a disruption of the stratum corneum lipids by IPP (both neat and from the microemulsion), 1 day after application.

The microemulsions studied by Lehmann et al. [58] (Table 3) was shown to induce skin irritation. After three times daily application of 50 μl to a 3-cm diameter area over 3 days, the water-continuous microemulsion (IPM–sucrose laurate–PG–water, 4:18:30:48%, w/w) significantly increased transepidermal water loss (TEWL) and produced dehydration compared with an untreated control area. Also the oil-continuous microemulsion (IPM–Targat®–Plurololeat®–water, 65:20:10:5%, w/w) significantly increased TEWL, but the skin hydration state was unchanged.

The potential barrier perturbation of microemul-

sions has also been investigated by a 20-h pre-treatment of excised rat skin with an unloaded microemulsion vehicle (1 ml) containing large amounts of surfactants (system 'A', Table 1) [8]. Compared to no pre-treatment and 20-h pre-treatment with neat water, no significant difference was observed in the permeation rate of prilocaine hydrochloride from a subsequently applied microemulsion formulation. The results from a human in vivo study, using a microemulsion vehicle based on the same surfactant system (Labrasol–Plurol Isostearique), additionally indicated that skin barrier function, evaluated by transepidermal water loss, was not affected by a 3-h application period [3]. Cumulative effects of the current surfactants–oil phase have not been studied, though.

4. Conclusions

Microemulsions can be formed with numerous different aqueous, surfactant and oil constituents, and, according to the properties of the applied constituents, microemulsions can exist over wide composition ranges. Due to the possible large concurrent implementation of oil and water in microemulsions, the vehicles have excellent solubility properties for both lipophilic and hydrophilic drugs. The specific structure of the surfactant interfacial film in microemulsions is indicated to substantially improve solubility properties over that of micellar solutions and neat constituents.

Topically applied microemulsions have been demonstrated to significantly increase the cutaneous absorption of both lipophilic and hydrophilic drugs, compared to conventional vehicles, e.g., aqueous solutions, neat oil phases, micellar solutions, emulsions and liposomes. The favourable drug delivery properties of microemulsions are indicated to be attributable to the large concentration gradients provided by the large drug solubility potential of the vehicles without concurrent increase in vehicle affinity for the drug, and/or to a potential penetration enhancer effect of the individual constituents. However, the maximum drug delivery rate potential of microemulsions with given constituents, has also been demonstrated to be dependent on the internal mobility of the drug in the vehicle, which is determined by the composition and internal structure of the microemulsion. The favourable cutaneous drug delivery and solvent properties, together with the ease of formulations and the 'infinite' physical stability of these unique oil–water–surfactant mixtures, makes microemulsions very promising vehicles for future topical formulations.

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