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Research paper

Dermal and transdermal targeting of dihydroavenanthramide D using enhancer molecules and novel microemulsions

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ABSTRACT

Specific accumulation of drugs in certain skin layers or in the blood circulation is the aim of (trans-)dermal targeting. As demonstrated previously, high dermal concentrations of the model drug dihydroavenanthramide D can be reached by the addition of 1,2-alkanediols as penetration enhancer to a conventional o/w cream. The focus of the present study is on an increased permeation by the choice of a modern colloidal drug carrier. Microemulsions based on a vegetable protein surfactant and 1,2-alkanediols as co-surfactant were developed. The respective pseudoternary phase diagrams revealed an increasing area of the optical isotropic phase with increasing chain length of the glycol (C3–C4–C5). Pentylene glycol-containing systems were characterized by electrical conductivity and differential scanning calorimetry indicating the presence of water-continuous microemulsions. Two selected formulations containing pentylene glycol and propylene glycol, respectively, were further investigated by TEM, conductivity, viscosity, and temperature stability. In the subsequently performed Franz type diffusion studies using full thickness human skin dihydroavenanthramide D was applied as model drug. Both formulations showed sufficient penetration into viable skin layers and particularly high permeation rates. Compared to the previously investigated glycol-containing cream, the microemulsions revealed a smaller fraction of the model drug within viable epidermis and dermis, but a strongly increased amount in the acceptor solution. Therefore, the formulations might find different application areas depending on needs concerning localization, beginning and duration of the drug effect.

1. Introduction

Dermal administration of drugs is performed for various destinations. Going from outside to inside the skin, it has to be differentiated between cleaning and protecting properties on the surface, keratolytic and moisturizing effects on the outermost skin layer, the stratum corneum, and drug interactions with pharmacological targets localized in the vital parts of the skin, which are viable epidermis and dermis. Furthermore, systemic bioavailability is the aim of transdermal delivery, which can be used to minimize the first pass metabolism in the liver or to avoid gastrointestinal side effects.

The intention of our research is to enlarge the spectrum of technological concepts to realize high drug concentrations either in the certain skin layers or in the blood circulation depending on the therapeutic needs. The focus of the present study is on permeation since we had previously demonstrated that addition of 1,2-alkanediols to a conventional oil-in-water cream significantly increased dermal availability of the applied model drug compared to the diol-free vehicle [1].

Again, dihydroavenanthramide D (DHAvD, M_w 285.3, Fig. 1) was used as model compound for physicochemical reasons. DHAvD is a synthetic analogue to naturally occurring avenanthramides – phenolic anthranilic acid amides present in oat. Its calculated log*P* is 3.34 ± 0.27 [2]. DHAvD is structurally very close to tranilast [*N*-(3′,4′-dimethoxycinnamonyl)anthranilic acid]. The latter is an anti-allergic, anti-inflammatory and analgesic drug. In addition to its oral administration in asthma and arthritis [3], it has been shown to be effective in topical treatment of skin diseases [4]. DHAvD itself was found to reduce histamine related skin disorders [5,6] and is used as an active ingredient in cosmetic products.

The choice for a suitable vehicle for transdermal delivery of DHAvD was made on microemulsions (MEs). In addition to their known enhanced penetration properties, they offer further advantages such as good solubilization capacity, ease of preparation and long-term stability. Basic components of these colloidal formulations are surfactant, co-surfactant, oil and water. When they are combined in an appropriate ratio optically isotropic, transparent, low-viscous and thermodynamic stable systems result [7]. In the literature, a lot of microemulsions are described; however, mostly

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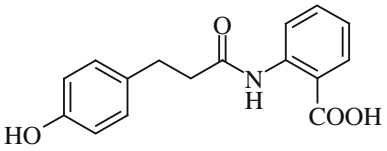

Fig. 1. Chemical structure of DHAvD.

a high surfactant amount is needed for the formation, which counteracts with the skin compatibility. Besides classical surfactants such as (PEGylated) fatty alcohols or polysorbates, amphiphiles from renewable raw materials came up in the development of colloidal carriers. In the 1990s the highly environmental and skin compatible alkyl polyglycosides were introduced. However, in order to develop customized drug carrier, a broader spectrum of such beneficial surfactant systems needs to be established. In the present study vegetable protein fatty acid condensates – a class of mild, ecologically friendly anionic surfactants that were already used in cosmetic products – were first applied to form microemulsions. According to the literature, addition of small amounts of acylated protein hydrolyzates to a formulation, which contains more strongly irritating surfactants, results in a disproportionately high improvement of skin compatibility [8]. Systematic studies on physicochemical properties of these surfactants are rare and limited to amino acid derivatives [9,10]. The product in use (Gluadin® WK) represents an acylation product of wheat protein hydrolyzate and coconut fatty acids with excellent physiological properties [11]. However, single reports on allergic reactions due to hydrolyzed wheat protein exist [12].

Low molecular weight alcohols can be used as co-surfactants in microemulsions. In general they influence curvature, packing and fluidity of the interfacial film [13]. Short chain alcohols in particular also show bulk effects by distribution between the microcompartments [14]. Since 1,2-alkanediols are less toxic compared to the respective alkanols, they were suggested for use in microemulsions [15,16]. 1,2-Pentylene glycol was pointed out to be a more effective co-solvent than propylene glycol [15]. Alany et al. investigated the effect of short chain alcohols and diols on the phase behavior of a quaternary system [17]. Whereas addition of propylene glycol led to the formation of a large liquid crystalline area, the use of 1,2-pentylene glycol and 1,2-hexylene glycol disrupted the packing of the surfactants and yielded larger microemulsion areas. In a more recent study 1,2-alkanediols (2, 3, 5, 6 or 8 C atoms) were tested for their irritation potential by means of HET-CAM [18]. The result was contradictive to previous reports: derivatives with carbon chain length 5–8 were found to be irritants. However, they were tested in an undiluted state for ophthalmic use. Besides their effectiveness as co-solvents in microemulsions, the enhancing effect of 1,2-pentylene glycol and 1,2-butylene glycol on DHAvD skin penetration as demonstrated in [1] argues for their use.

In the present study the development of novel microemulsion systems based on a wheat protein fatty acid condensate and different 1,2-alkanediols is described. The influence of the glycols' chain length on the phase behavior of the pseudoternary mixtures was investigated first and the system with the broadest microemulsion area was characterized by electrical conductivity and differential scanning calorimetry. Since our research is focussed on dermal drug administration, skin compatible microemulsions containing either propylene glycol or pentylene glycol were selected for further investigations. Their physicochemical properties were assessed in detail prior to Franz cell diffusion experiments on human skin. The penetration profiles of the microemulsions in general, the influence of the 1,2-alkanediols, and the comparison to the previously studied oil-in-water cream were of particular interest to gain information on dermal and transdermal drug targeting.

2. Materials and methods

2.1. Materials

Chemicals and reagents were obtained from the following commercial sources: dihydroavenanthramide D (DHAvD; 2-[[3-(4-hydroxyphenyl)-1-oxopropyl]amino]-benzoic acid), 1,2-pentylene glycol (Hydrolite-5, PeG) and 1,2-butylene glycol (BuG): courtesy of Symrise GmbH & Co. KG, Holzminden, Germany; propylene glycol (PrG): Caelo GmbH Hilden, Germany; Gluadin WK (sodium cocoyl hydrolyzed wheat protein, preserved, batch GR51017503): courtesy of Cognis GmbH & Co. KG, Duesseldorf, Germany; Synperonic PE/L 121 (Poloxamer 401): Uniqema, Bromborough, UK; Pelemol® BIP (eutectic mixture of butylphthalimide and isopropylphtalimide): courtesy of Phoenix Chemical Inc., Somerville, NJ, USA; buffer substances, formic acid: Merck, Darmstadt, Germany; undenaturated ethanol: BfB, Wittenberg, Germany. Water was of bidistilled quality. HPLC grade methanol was obtained from Baker, Deventer, The Netherlands.

2.2. Construction of phase diagrams

Pseudoternary phase diagrams were constructed at a formal surfactant mass ratio of Gluadin WK: Synperonic PE/L 121: glycol of 5:1:1.875. The applied batch of Gluadin WK contained 30.9% dry substance [19]. Therefore, the mass ratio was corrected for the amount of water and was finally $r_C = 1.545:1:1.875$. Systems were prepared by shaking Pelemol BIP, the surfactant mixture and water in the appropriate ratio. Ten percent increments were chosen for a rough orientation within the phase diagram and for precise assessment of the phase borders steps of 5% and 2.5% were used. The samples were left to equilibrate for 48 h and then characterized by visual inspection and polarized light microscopy (Zeiss Axiolab Pol, Carl Zeiss MicroImaging GmbH, Jena, Germany). Microemulsions were identified as transparent, low-viscous and optically isotropic mixtures. Fewer attempts were made to identify any other structural regions in the phase diagrams. In order to determine the size of the isotropic areas, a lattice with a graduation of 1/100 was applied on the phase diagram and the respective areas were enumerated.

2.3. Conductivity measurements

Electrical conductivity was measured using ProfiLab Conductivity Meter LF 597 equipped with a Standard-conductivity cell TetraCon® 325 having a cell constant of $0.475\ cm^{-1} \pm 1.5\%$ (WTW GmbH, Weilheim i. OB, Germany). Readings were taken at 22.4 °C after the value had remained constant for about 2 min.

2.4. Differential scanning calorimetry

DSC measurements were performed with a DSC 200 (Netzsch-Gerätebau GmbH, Selb, Germany). Accurately weighted samples (approx. 15 mg) were filled into 40 μL-aluminium pans which were then hermetically sealed to prevent evaporation of water. Thermograms were obtained by cooling from 40 °C to −60 °C at $10\ K\,min^{-1}$, equilibration at −60 °C for 5 min, and heating to 150 °C at $10\ K\,min^{-1}$ using an empty pan as reference. Nitrogen with a flow of $10\ mL\,min^{-1}$ was used as purge gas.

2.5. Viscosity measurements

Dynamic viscosity of the samples was determined using a rotational rheometer equipped with a Couette system cup at 25.0 ± 0.2 °C and 32 °C ± 0.2 °C (Fluids Spectrometer RFS II, Rheo-

metrics Scientific, Bensheim, Germany). A sample volume of 12 mL was required. At shear rates ranging from 0.1 to 160 s^{-1} shear stress was measured.

2.6. pH measurement

The pH value was measured by means of a pH meter type 1120 (Mettler-Toledo GmbH, Giessen, Germany).

2.7. FF-TEM

A small amount of the sample (PrG-ME and PeG-ME) was sandwiched between two copper profiles and frozen by plunging the sandwiches immediately into a liquid ethane/propane mixture (1:1 (V/V)) cooled by liquid nitrogen. Freeze-fracturing and replication were performed at −150 °C in a BAF 400T freeze-fracture device (BAL-TEC BAF 400T, Balzers, Liechtenstein) using a double replica stage. Pt(C) was evaporated onto fracture faces under 35° angle to a thickness of 2 nm. Perpendicular deposition of 20–30 nm pure carbon was made for mechanical stabilization. The replicas were placed on copper grids, cleaned with a chloroform/methanol mixture and examined in an EM902A electron microscope (Zeiss, Oberkochen, Germany).

2.8. Temperature stability

Test tubes with 2 mL of PrG-ME and PeG-ME, respectively, were incubated in a water bath in steps of 10 K between 30 and 70 °C for 6 h per temperature as well as in the refrigerator at 6 °C overnight. Samples were visually examined for changes in the appearance like turbidity or phase separation at the respective temperature and following shaking and re-adjustment of room temperature.

2.9. Saturation data

Since addition of DHAvD in excess to the microemulsions led to phase separation, the substance was added in small quantities to an exactly weighted amount of PeG-ME and PrG-ME, respectively. Following each addition, the test tube was shaken and visually observed for undissolved crystals. The concentration of DHAvD in the vehicles that just did not show precipitation was taken as saturation solubility. The determination was performed in triplicate.

2.10. Ex vivo penetration studies

Penetration experiments were carried out using Franz type diffusion cells (Crown Glass Company, Somerville, NJ, USA) under finite-dose conditions [20]. The studies were performed in triplicate using full thickness human skin samples from three different female patients (44, 61, and 68 years). Breast skin was obtained after cosmetic surgery. After cleaning with 0.9% sodium chloride solution and removal of the subcutaneous fat, the skin samples were stored at −20 °C until use. Before the experiments, the skin samples were thawed and placed onto filter gauze in the diffusion cells. The dermal side of the skin was in contact with the acceptor solution (phosphate buffered saline, pH 7.4, 20.0 mL) which was stirred continuously. A defined amount of the formulation (20 μL) was applied onto the skin surface (3.14 cm^2). After incubation (30 and 300 min) at 32 °C the remaining formulation was wiped by a cotton wool tip. Three punch biopsies (each 0.2827 cm^2) were excised from each skin sample and were cut in horizontal sections using a cryomicrotome (Jung, Heidelberg, Germany). Stratum corneum (SC) was removed by one 10 μm section, viable epidermis (EP) by 4 cuts à 20 μm, dermis sections 1–3 (DR 1–3) by 5 cuts à 40 μm and dermis 4 (DR 4) by 15 cuts à 40 μm. The remaining corium was also separated. The respective sections of the three punch biopsies coming from the same experiment were pooled to one sample to guarantee the detection of small drug amounts in the skin. The collected cuts were extracted with definite amounts of methanol (150 μL for SC – DR 3, 200 μL for DR 4 and corium). They were vortexed for 1.5 h at the beginning and for 1 h at the end of the extraction period of 22 h and were stored in a refrigerator in between. The supernatant was centrifuged for 10 min if necessary. The cotton wool tips were extracted for 8 h with 2.0 mL methanol. The amount of DHAvD in the different skin layers, in the cotton wool tips and in the undiluted acceptor solution was analyzed by HPLC. The mean recovery from all experiments (calculated from the skin biopsies, acceptor and cotton wool tip, but without the filter gauze) was 78.2 ± 16.0% of the applied dose.

2.11. Analytics

HPLC was performed on a HP 1100 (Agilent Technologies, Waldbronn, Germany) equipped with a vacuum degasser, a binary pump, an autosampler and a diodenarray detector. Ten microliters of each sample were loaded onto a YMC-Pack ODS-AQ column (150 × 4.0 i.d.; S-5 μm; 200 Å; YMC Europe, Dinslaken, Germany) and DHAvD was eluted by methanol/water/formic acid 60:40:0.1 (v/v) as mobile phase at a flow rate of 0.8 mL min^{-1} and a column temperature of 30 °C. Detection of the drug occurred at 224.4 nm with a reference wavelength at 360.1 nm. The limit of quantification was 0.05 μg mL^{-1}.

Standard solutions were prepared by adding a methanolic skin extract to the respective standards. They were injected in triplicate.

2.12. Statistics

Statistical significance was tested by one-way ANOVA followed by Tukey's multiple comparison. Differences were considered as significant at $p < 0.05$.

3. Results and discussion

3.1. Development of microemulsions and phase diagrams

Mixtures based on sodium cocoyl hydrolyzed wheat protein in combination with butylphthalimid/isopropylphthalimid (BIP) as lipophilic ingredient and water were prepared. The evaluation was limited by the low amount of active substance within the applied batch of protein surfactant (30.9% in an aqueous dispersion according to [19]). In order to prevent phase separation, the low HLB Poloxamer 401 was added. Pre-examinations revealed a mass ratio of 5:1 protein surfactant:poloxamer as beneficial to obtain microemulsions, which can be corrected for the amount of water in the protein surfactant batch. Thus, the corrected mass ratio, further referred to as r_C, was 1.545:1 and corresponds to a molar ratio of about 2.3:1. Since the addition of a co-surfactant supports the formation of microemulsions, different 1,2-alkanediols were further incorporated, namely propylene glycol (PrG), 1,2-butylene glycol (BuG) and 1,2-pentylene glycole (PeG). Finally, a ratio of protein surfactant:poloxamer:1,2-alkanediol of r_C = 1.545:1:1.875 turned out to be advantageous.

For a quantitative comparison of the influence of PrG, BuG and PeG on the position and dimension of the microemulsion area, pseudoternary phase diagrams were generated (see Fig. 2). The right part of the triangle had to be blanked due to the diluted state of the commercial protein surfactant product.

Mixtures of clear, low-viscous and optically isotropic appearance were obtained with all 1,2-alkanediols, however, the size of these areas differed significantly. Taking the available size of the triangle as 100%, the microemulsion region of the PrG-containing

phase diagram was 8.8%, the one with BuG was 11.4% and with PeG it was 16.2%. This increase in microemulsion area with increasing chain length of the 1,2-alkanediols might be due to more significant interfacial and / or bulk effects and is in accordance to findings of Alany et al. [17].

Liquid crystalline phases were not present, probably because of the lack of long range order packing surfactants.

3.2. Characterization of pseudoternary mixtures by electrical conductivity and DSC

The pseudoternary system that formed the largest microemulsion area was investigated in more detail since it offers a wide scope for stable, dermal applicable formulations.

Several mixtures along a line of constant surfactant:BIP mass ratio (76.6:23.4) and increasing water fraction have been prepared (see Fig. 2c). Detailed composition of the studied systems is given in Table 1. They were characterized by electrical conductivity and differential scanning calorimetry.

Electrical conductivity can be used to monitor structural changes within a microemulsion series. For example, the beginning interconnection of pre-existing isolated droplets by an increased water fraction can be detected. Clustering of the droplets which is accompanied by the formation of conductive paths at the so-called percolation threshold (φ_p) typically leads to a dramatic increase in conductivity [21]. In order to detect such changes, a sufficient amount of electrolyte is required which provides the charges to be transported [22,23]. In this work, an addition of electrolyte was unnecessary since the protein surfactant is anionic. On the other hand, a change in the composition of the microemulsions involved varying amounts of surfactants and, hence, a varying number of charge carriers. Therefore, the raw data had to be corrected by dividing electrical conductivity κ by the surfactant fraction (φ_s) resulting in the corrected value κ'.

The raw data of the electrical conductivity along the sampling path plotted vs. the amount of water followed a bell-shaped curve (not shown), but κ' increased exponentially as depicted in Fig. 3. A distinct inflection in the curve which would be representative for the percolation threshold did not occur. A more sensitive approach is the plot of the first derivate of κ ($\mathrm{d}\log\kappa/\mathrm{d}\varphi_w$) vs. water fraction φ_w [24]. The appearance of a maximum predicts the percolation threshold. As shown in the inset of Fig. 3 a beginning interconnection might be suggested. However, this tendency cannot be followed up since systems with a water fraction below 0.373 could not be prepared. Thus, due to the high water fraction and the high values of conductivity (around 1–8 mS cm^{-1}) the presence of a droplet like o/w structure is indicated for the whole isotropic area.

Conductivity experiments were repeated after 2 months of storage at room temperature. The values did not differ from the freshly prepared ones, which indicates the stability of the microemulsions.

Garti et al. first employed low-temperature differential scanning calorimetry (DSC) in order to investigate the water behavior in non-ionic microemulsions [25]. From size and position of the water freezing peak in the respective DSC cooling curve its physical state within the systems can be suggested. Solidification of water molecules that interact strongly with surfactants occurs at lower temperatures than of weaker interacting ones. Furthermore, differences in the enthalpy of freezing appear [26].

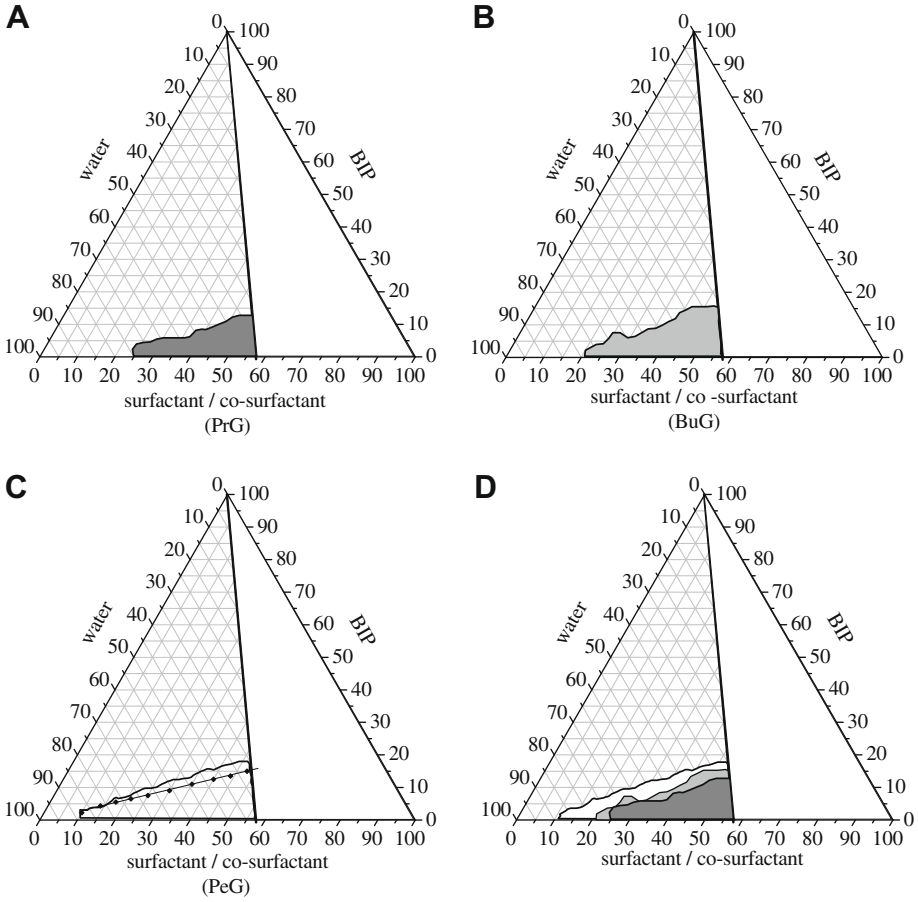

Fig. 2. Pseudoternary phase diagrams for the systems formulated with different co-surfactants. The isotropic areas are marked (A: propylene glycol (dark grey) B: butylene glycol (light grey), C: pentylene glycol (transparent/white) and D: combined figure). The line in (c) indicates the sampling path used for physicochemical characterization.

Table 1
Composition of the studied microemulsions along the sampling path in the pentylene glycol – containing phase diagram (% m/m) (Fig. 2c).

Water	87.25	81.7	77.0	72.5	67.5	61.0	54.0	47.5	42.5	37.3
Surfactant mixture (Gluadin/Poloxamer/PeG r_c = 1.545:1:1.875)	10.00	14.0	17.5	21.0	25.0	30.0	35.0	40.0	44.0	47.7
BIP	2.75	4.3	5.5	6.5	7.5	9.0	11.0	12.5	13.5	15.0

Similar to the experimental mode of Podlogar et al. [22,26] DSC cooling and heating curves of the pseudoternary systems along the sampling path as well as of the pure BIP, water and the surfactant mixture were recorded. Since the heating part did not offer further information, only the cooling curves are presented in Fig. 4.

In the surfactant blend consisting of the protein surfactant, poloxamer and PeG no freezing event was observable, although due to the use of an aqueous dispersion of the protein surfactant an amount of about 44% water was present. According to [26], the water molecules might interact strongly with the large amount of surfactants which shifts the freezing point towards low temperatures, probably out of the detected range, and also minimizes the freezing enthalpy. Moreover, PeG might contribute to this behavior. Water-soluble polyols act as cryoprotectants. This has been shown at least for propylene glycol [27] and 1,2-butylene glycol [28].

The cooling curves of all investigated pseudoternary mixtures show one exothermic peak. With increasing water fraction the peak shifts towards higher temperatures up to the sample with 77% water and the peak area increases linearly (not shown). Using an appropriate magnification, also the 37.3% water containing sample suggests this freezing event. The changes tend towards the freezing behavior of pure water as the reference measurement with its maximum at about −22 °C shows. Therefore, the exothermic event represents the freezing of supercooled water [22,29]. Considering the opposite direction, decreasing water fraction is accompanied by an increase in the surfactant amount which leads to an increased ratio of more strongly bound water molecules needed to hydrate the polar head groups. Moreover, the cryoprotective effect of PeG might be more influential. Correspondingly, a decrease in freezing temperature and transition enthalpy can be denoted as mentioned above and the occurrence of non-freezing water is probable [30]. Podlogar et al. interpreted the water peak which deviates in temperature from that of pure water but still shows a sharp form as less interacting water. On the other hand, a small peak at very low temperatures (below −30 °C) was suggested as internal water or water that interacts strongly with surfactants [22,26]. The latter might comply with the pseudoternary systems having less than 47.5% water since the peak does not show a sharp form any longer. These mixtures might indicate the beginning structural change towards bicontinuous microemulsions. Respective tendencies appeared in the electrical conductivity. Generally, the findings of both methods correlated and confirmed the presence of o/w microemulsions along the sampling path. However, the assumption about the existence of bicontinuous structures will have to be proven by non-invasive self-diffusion NMR. In the samples with a water content >80% interactions between water and surfactant are superimposed by the excess of free water, resulting in freezing temperatures comparable to pure water.

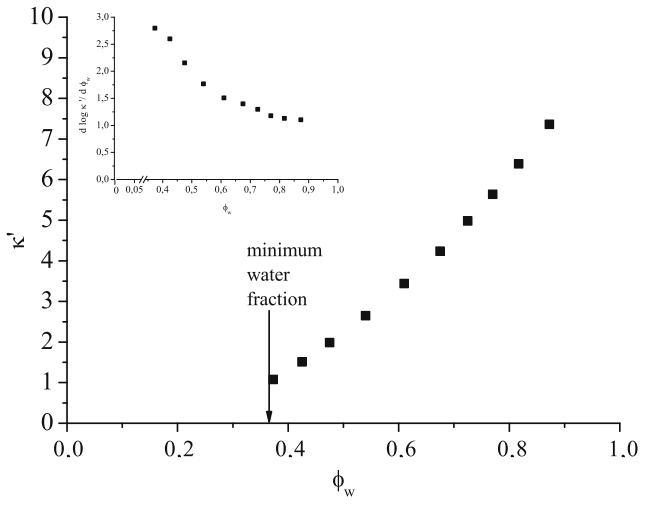

Fig. 3. Corrected electrical conductivity κ' as function of the water fraction φ_w in the microemulsions along the sampling path (see Fig. 2c and Table 1). Inset: plot of $d\log\kappa'/d\varphi$ versus φ_w.

The pure lipophilic component BIP did not show solidification between 40 and −60 °C which might result from the eutectic behavior of the alkylphthalimide mixture. Therefore, DSC is not suitable to characterize its state within the microemulsions. Finally, it has to be emphasized that DSC results should be handled with care because the method is invasive and the recorded signals may come from a sample possibly changed by the temperature regimen.

3.3. Characterization of the microemulsions used for the penetration study

o/w microemulsions revealed to be an excellent choice to enhance the penetration of a broad spectrum of drugs, even of others than (highly) lipophilic ones [7,31,32]. Moreover, they apparently exert no long-term influence on skin hydration [33] and their application seems to be more user-friendly compared to w/o microemulsions.

For the penetration study a propylene glycol-containing formulation (PrG-ME) and a pentylene glycol-containing one (PeG-ME) were chosen. Both are regarded as skin compatible due to the low amount of surfactants (less than 25% of Gluadin WK and Poloxamer). The detailed composition is given in Table 2. For reasons of stability, PrG-ME had to be prepared with 1.7% more surfactant. However, this difference is considered to be negligible regarding the comparability of the systems. Prior to the penetration studies, the microemulsions were characterized and compared by several physicochemical methods.

Polarizing light microscopy was employed to decide on optical isotropy. This typical feature of microemulsions could be confirmed since no birefringence was detected.

Temperature-dependent behavior determines usage properties of microemulsions. Therefore, it was tested on an organoleptic level by warming the microemulsions until phase separation occurred. The samples were observed at the respective temperatures and after re-adjustment of room temperature. PrG-ME remained in the initial microemulsion state up to 30 °C. From 40 to 50 °C the appearance changed from opalescent to turbid and at 60 °C the phases separated. Opalescence of PeG-ME occurred at 50 °C, but phase separation also became evident at 60 °C. However, all changes were reversible and the initial clear state reappeared rapidly after cooling to room temperature and shaking. Storage in the refrigerator at 6 °C did not cause any visible alterations. The results suggest that the microemulsions remain intact under several manufacturing and storage conditions and also when they are applied onto skin as far as warmth effects are concerned. The use of pentylene glycol as co-surfactant leads to a slightly less tempera-

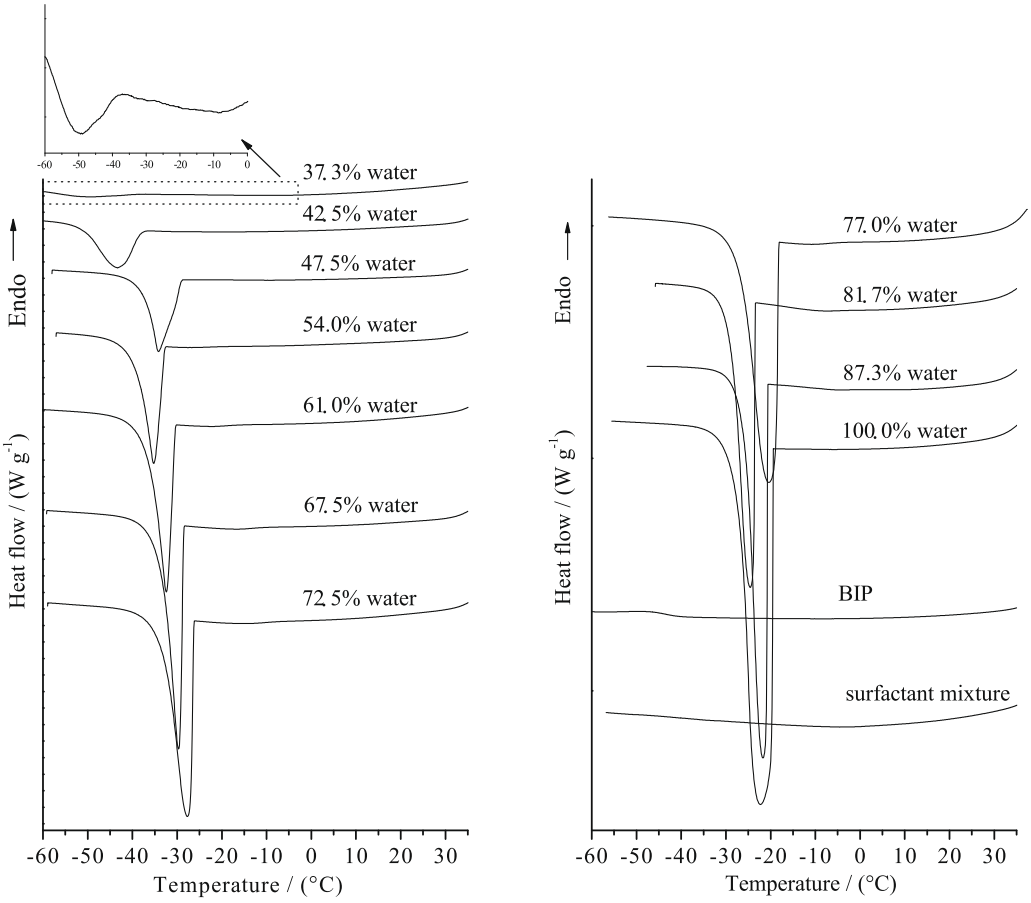

Fig. 4. DSC cooling curves of the microemulsions along the sampling path (see Fig. 2c and Table 1) as well as pure BIP, the surfactant mixture and water. The inset represents the magnified plot of the microemulsion with 37.3% water content.

ture sensitivity. This corresponds to the position of PeG-ME in the phase diagram, which is more distant to the borders of the ME-region than the respective PrG-ME.

Dynamic viscosity was determined at room temperature (25 °C) and at the temperature of the skin (32 °C). Both microemulsions showed Newtonian flow properties, which is typical for droplet-like microemulsions [21,34]. The viscosity was about 12 mPas for PrG-ME and about 20 mPas for PeG-ME at 25 °C. As expected, it decreased with increasing temperature. The differences were minor and constituted 2–4 mPas. The results are listed in Table 3.

Regarding electrical conductivity, similar values were obtained for both systems (Table 3). The slightly higher conductivity of PrG-ME certainly is a consequence of the likewise slightly higher amount of charge containing protein surfactant. Since the results are of the same magnitude like those coming from the detailed investigation along the sampling path in Fig. 2c, they were taken as indication for the existence of water-continuous microemulsions.

The pH value of about 8 is determined by the alkalescent protein surfactant.

PrG-ME and PeG-ME were examined by freeze-fracture transmission electron microscopy (FF-TEM). Both microemulsions showed a homogeneous microstructure and the observed impressions and elevations revealed a globular structure (Fig. 5). The estimated diameter of these spherical aggregates was 12–16 nm. However, the appearance of PrG-ME (Fig. 5B) was a little rougher; the globular structures occurred more frequently than in PeG-ME (Fig. 5A). Etching the fractured surfaces in order to obtain a more prominent relief was not possible, probably because of the high amount of non-aqueous compounds and the potentially high dispersity of the oil.

In summary, the selected formulations revealed as optically isotropic, thermodynamically stable droplet-like water-continuous microemulsions of low viscosity.

3.4. Penetration studies

The penetration behavior of the model drug DHAvD from the microemulsions in general, the effect of the two glycols – PrG and PeG – and the differences in drug distribution compared to

Table 2
Composition of PrG-ME and PeG-ME (% m/m) selected for the penetration studies.

	PrG-ME	PeG-ME
Gluadin WK $_c$	13.39	12.36
Poloxamer 401	8.67	8.00
BIP	7.50	7.50
Glycol	15.00 (PrG)	15.00 (PeG)
Water	55.44	57.14

Table 3
Dynamic viscosity, electrical conductivity, and pH value of PrG-ME and PeG-ME.

	Dynamic viscosity (mPa s)		Conductivity (mS cm^{-1})	pH value
	25 °C	32 °C		
PrG-ME	11.9	9.7	11.52	8.2
PeG-ME	19.7	16.1	11.23	8.0

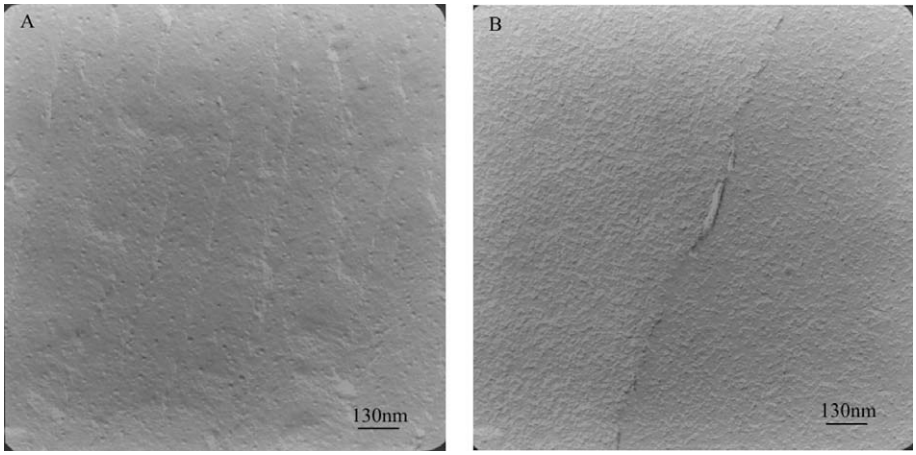

Fig. 5. Transmission electron micrographs of PeG-ME (A) and PrG-ME (B).

the conventional creams were evaluated. As in the creams, 0.2% (m/m) of DHAvD was dissolved in the microemulsions. Therewith, maximum solubilization capacity was not utilized since 3.04 ± 0.11% (m/m) DHAvD can be solubilized within PrG-ME and 3.68 ± 0.12% (m/m) in PeG-ME. The slightly higher level in the latter resulted from the higher solubilization capacity for DHAvD of pentylene glycol than that of propylene glycol [1].

Penetration experiments were performed in a finite-dose mode. Following 30 and 300 min of incubation, DHAvD content was analyzed within different skin compartments (stratum corneum, viable epidermis and dermis) as well as in the acceptor medium. Fig. 6 illustrates the results. Epidermis and dermis are summarized as viable skin layers. DHAvD is supposed to interfere with histamine related processes there. Generally, drug fraction (expressed as % of the applied dose) increased towards deeper skin layers including the acceptor solution and with the incubation time for both microemulsions. However, it should be noted that the thickness of the compartments increases from stratum corneum to dermis and, thus, the concentration of DHAvD shows an exponential decay from outside to inside the skin (see Fig. 7).

PeG-ME and PrG-ME showed a comparable distribution behavior within the skin. The amount of the model drug in the stratum corneum increased from nearly 1% at 30 min to about 3% at 300 min. In the viable skin layers the increase was from about 6% to 20% of the applied dose using PeG-ME, in the case of PrG-ME it was from 7% to 16%. Particularly high were the permeated quantities. Slight advantages of PrG-ME were noticeable at both incubation times. Around 24% DHAvD reached the acceptor within 30 min. The fraction increased up to 40% at 300 min. Administration of PeG-ME led to the permeation of 11% after 30 min and 30% after 300 min.

Transdermal delivery by microemulsions is a complex interaction between thermodynamic and structural effects in the vehicle as well as in the skin. Different partitioning processes, the drugs relative activity within the microcompartments and diffusion of single constituents have to be considered [35,36]. The studied microemulsions differ basically in the kind of glycol used as co-surfactant and co-solvent. Due to the influence of this ingredient on the position of the microemulsion in the phase diagram, PeG-ME is located more central within the respective phase area and PrG-ME is supposed to be closer to the border. One reason for the favored permeation by PrG-ME might be a higher thermodynamic activity resulting from the near maximum solubilization capacity of the system for lipophilic ingredients. Therefore, the driving force for diffusion might be higher than from PeG-ME.

Finally, the microemulsion's penetration results were compared to those obtained with conventional o/w creams as published in [1]. The cream used as comparator for PeG-ME was a Hydrophilic

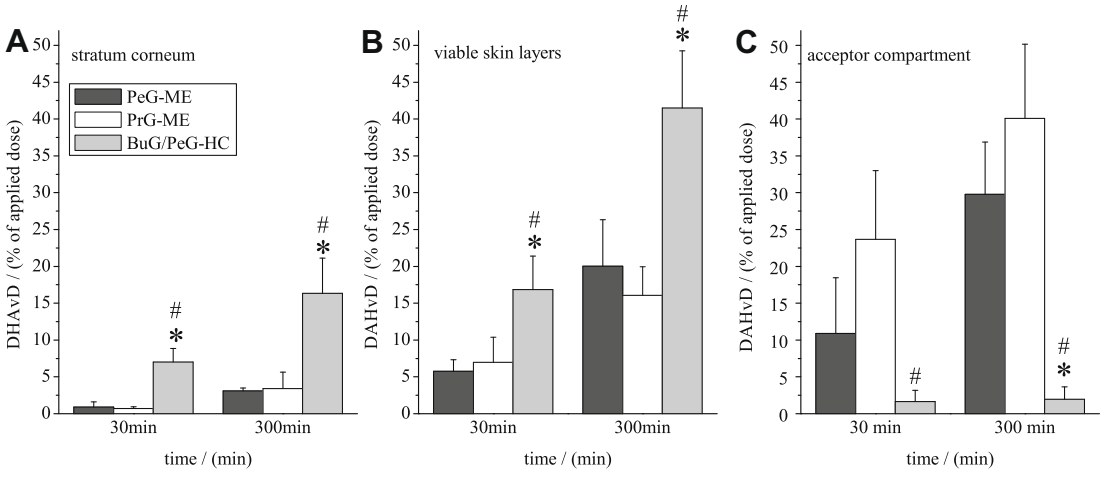

Fig. 6. Comparison of the relative amounts of DHAvD in the stratum corneum (A), viable skin layers (sum of viable epidermis and dermis) (B) and in the acceptor compartment (C) at 30 and 300 min following administration of PeG-ME, PrG-ME and BuG/PeG-HC (data given as mean ± standard deviation, $n = 3$; $^{*}p < 0.05$ vs. PeG-ME, $^{\#}p < 0.05$ vs. PrG-ME).

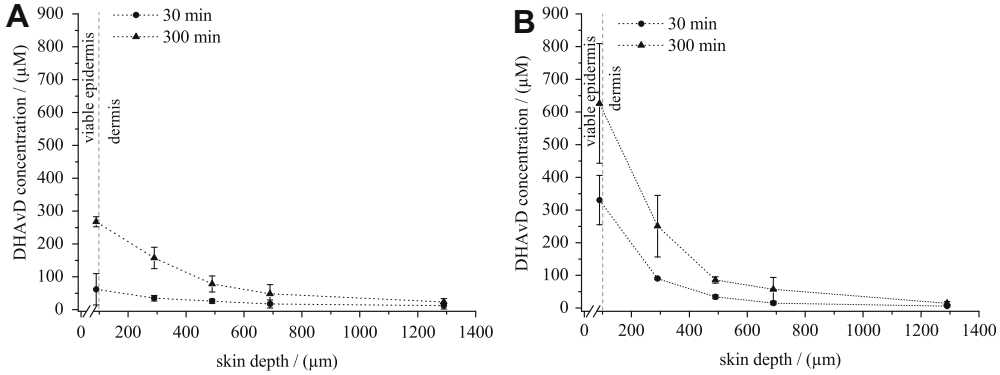

Fig. 7. Depth profiles of the DHAvD concentration within viable epidermis and dermis at 30 and 300 min following administration of PeG-ME (A) and BuG/PeG-HC (B) (data given as mean ± standard deviation, $n = 3$).

Cream according to the German Pharmacopoeia [37]. It was modified by the addition of 2% PeG and 2% BuG and is abbreviated as PeG/BuG-HC. In Fig. 6 the fraction of DHAvD within the viable skin layers and in the acceptor compartment is additionally presented for the administration of PeG/BuG-HC. While both types of vehicles show respectable drug diffusion, the distribution of DHAvD is opposed. Accumulation in the stratum corneum as found following administration of the cream was not observed in the case of the microemulsions. The figure reveals the significantly higher DHAvD penetration into the viable skin layers when the cream was applied. A more detailed view on the concentration gradient in viable epidermis and dermis is given in Fig. 7a for PeG-ME and in Fig. 7b for BuG/PeG-HC. On the other hand, permeation was considerably increased in the case of the microemulsion suggesting a distinct transdermal effect. The higher DHAvD mobility and the increased glycol fraction within the colloidal carrier compared to the semisolid cream might play an important role. If the results for the viable skin fraction and the acceptor are summed up for the single incubation times, they are comparable for the vehicles. Although it is not possible to estimate the drug concentration in the systemic circulation following topical administration *in vivo* directly from the drug amount in the acceptor fluid, this compartment is essential to consider since the drug molecules there have already passed the deeper skin layers and, hence, account for the systemic pharmacological effect.

In the literature, the high drug mobility in microemulsions owing to their fluctuating interfaces is discussed as reason for the penetration enhancement observed by these colloidal carriers. It results from a certain microstructure where enough free water is present and includes a specifically limited amount of surfactants to avoid unfavorable partitioning of the drug between vehicle and skin or even encapsulation [7,36,38].

It can be suggested that administration of the microemulsion leads to a fast and short local drug effect compared to the cream providing a delayed but prolonged action. The microemulsions are supposed to be promising vehicles for transdermal drug targeting, whereas the cream containing the glycols is useful for dermal delivery. However, this hypothesis has to be verified *in vivo*.

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