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Investigation of microemulsion microstructure and its impact on skin delivery of flufenamic acid

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ABSTRACT

Microemulsions are well known penetration enhancing delivery systems. Several properties are described that influence the transdermal delivery of active components. Therefore, this study aimed to characterize fluorosurfactant-based microemulsions and to assess the impact of formulation variables on the transdermal delivery of incorporated flufenamic acid. The microemulsion systems prepared in this study consisted of bistilled water, oleic acid, isopropanol as co-solvent, flufenamic acid as active ingredient and either Hexafor^{TM}670 (Hex) or Chemguard S-550-100 (Sin) as fluorosurfactant. Characterization was performed by a combination of techniques including electrical conductivity measurements, small-angle X-ray scattering (SAXS) and nuclear magnetic resonance (NMR) self-diffusion experiments. *In vitro* skin permeation experiments were performed with each prepared microemulsion using Franz type diffusion cells to correlate their present microstructure with their drug delivery to skin. Electrical conductivity increased with added water content. Consequently, the absence of a conductivity maximum as well as the NMR and SAXS data rather suggest O/W type microemulsions with spherical or rod-like microstructures. Skin permeation data revealed enhanced diffusion for Hex- and Sin-microemulsions if the shape of the structures was rather elongated than spherical implying that the shape of droplets had an essential impact on the skin permeation of flufenamic acid.

1. Introduction

Microemulsions (ME) generally consist of water, oil, surfactant and mostly a co-solvent. Contrary to (macro) emulsions, microemulsions are transparent and thermodynamically stable single-phase systems that demonstrate higher permeation rates than conventional emulsions or other vehicles (Lindman et al., 1999; Naoui et al., 2011).

Their enhancement of drug delivery to the skin is described by several properties such as the low interfacial tension between water and oil phase that may improve the contact to the skin. This low interfacial tension and the fluctuating oil-water interface facilitate component transfer between hydrophilic and lipophilic domains of the microemulsion and to the stratum corneum (Kreilgaard, 2002). Furthermore, also the microemulsion excipients can enhance penetration (Kaushik et al., 2010). Surfactants and alcohol, for instance, are well known components that are able to enhance permeation (Williams and Barry, 2012). Additionally, microemulsion formulations may increase cutaneous drug delivery due to their high solubility potential for lipophilic and hydrophilic drugs (Kreilgaard et al., 2000). Thereby higher concentrations of poorly soluble drugs can be administrated that create an increased concentration gradient towards skin (Kreilgaard et al., 2000).

In this context, especially the microemulsion microstructure is reported to be important for the rate of drug release (Podlogar et al., 2004). Macroscopically, microemulsions look like solutions, but internally a variety of structures can form. Therefore, the goal of this study was to characterize the microstructure of twelve fluorosurfactant-based microemulsions by physicochemical techniques and to correlate their present microstructure with their

Abbreviations: ME, microemulsion; NMR, nuclear magnetic resonance; SAXS, small angle X-ray scattering; Hex, fluorosurfactant Hexafor^{TM}670; Sin, fluorosurfactant Chemguard S-550-100.

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drug delivery to skin. For this purpose, the skin permeation behavior was assessed with the focus on several physicochemical parameters that may influence skin diffusion.

The microemulsion systems prepared in this study consisted of bistilled water, oleic acid, isopropanol as co-solvent, flufenamic acid as active ingredient and either Hexafor™670 (Hex) or Chemguard S-550-100 (Sin) as fluorosurfactant. The NSAID flufenamic acid represents an appropriate model drug for skin studies since it is dermally applied in commercially available products (Schwarz et al., 2012). Moreover, fluorine containing compounds were chosen as the ^{19}F isotope is a very sensitive NMR probe that is also suitable for self-diffusion studies (Hoeller et al., 2008). Fluorocarbon surfactants are surface active materials in which some or all hydrogen atoms in the hydrophobic moiety are replaced by fluorine atoms (Szymczyk, 2013). They have unique properties including chemical and biologic inertness, low surface tension, high density and the ability to dissolve large amounts of gases (Cui et al., 2003).

The initial selection of six microemulsions out of a variety of combinations with each fluorosurfactant was followed by a physicochemical characterization. Due to their small particle sizes and fluctuation interfaces, which makes a characterization highly challenging, different charaterization techniques were combined to achieve an appropriate characterization of ME microstructures (Zhang and Michniak-Kohn, 2011). While electrical conductivity measurements were conducted to assess structural changes from discontinuous to bicontinuous microemulsions, nuclear magnetic resonance (NMR) self-diffusion experiments additionally permitted to differentiate whether a discontinuous microemulsion is of W/O type or O/W type (Acharya and Hartley, 2012; Alany et al., 2001). Small-angle X-ray scattering (SAXS) studies were carried out to determine droplet size and droplet shape (Acharya and Hartley, 2012). Finally, *in vitro* skin permeation experiments were performed with each prepared microemulsion using Franz type diffusion cells and pig skin as model membrane.

2. Materials and methods

2.1. Materials

The fluorosurfactant Hexafor™ 670 (Hex), (a fluorinated ethoxylated pentaerythritol, with a hydrophilic/lipophilic balance; HLB of 13.6; average molecular weight of 1173 g/mol) was kindly donated by Maflon (Castelli Calepio, Italy). The fluorosurfactant Chemguard S-550-100 (Sin), (a perfluoroalkyl substituted polyether; HLB of 8.9; average molecular weight of 628–716 g/mol) was procured from Sintal Chemie GmbH (Weilrod, Germany). Oleic acid was purchased from Herba Chemosan (Vienna, Austria). Methanol, isopropyl alcohol, 1-octanesulfonic acid sodium salt and flufenamic acid were obtained from Sigma-Aldrich (Vienna, Austria). Abdominal porcine skin that was cut at a thickness of 700 μm with a dermatome (GB 228R, Aesculap) was bought from a local butcher and stored in a freezer at −18 °C for a maximum of 6 months. Methanol-d1 was obtained by Euriso-top (Gif sur Yvette, France).

2.2. Construction of pseudoternary phase diagrams

Varying amounts of the fluorosurfactants, either Hexafor™670 (Hex) or Chemguard S-550-100 (Sin), were mixed with the co-solvent isopropanol in the relation 1:1 (w/w), oleic acid as oil phase and distilled water were stirred overnight at room temperature to equilibrate (Table 1). Moreover, 1% (w/w) flufenamic acid as active component was incorporated. After equilibration, microemulsions were identified by visual observation and polarized light microscopy (Nikon GmbH, Düsseldorf, Germany). Transparent, isotropic mono-phase systems were assigned as colored area in phase diagrams. The pseudo-ternary phase diagrams were plotted by the recorded concentrations of components (Ge et al., 2014).

Table 1
Composition of the investigated microemulsions (ME) in % (w/w). Hex = fluorosurfactant Hexafor™670; Sin = fluorosurfactant Chemguard S-550-100. Furthermore, 1% flufenamic acid was incorporated into each formulation.

	Water	Surfactant	Isopropanol	Oleic acid
Hex 5w	5	30	30	35
Hex 10w	10	30	30	30
Hex 20w	20	30	30	20
Hex 25w	25	30	30	15
Hex 30w	30	30	30	10
Hex 35w	35	30	30	5
Sin 5w	5	30	30	35
Sin 6.5w	6.5	30	30	33.5
Sin 22.5w	22.5	30	30	17.5
Sin 25w	25	30	30	15
Sin 30w	30	30	30	10
Sin 35w	35	30	30	5

2.3. NMR self-diffusion experiments

Pulsed-gradient spin-echo (PGSE) NMR is an important tool for characterization of microemulsion structures (Kreilgaard et al., 2000). NMR experiments were performed on a BRUKER Avance III 600 MHz NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) operating at 600.13 MHz for ^{1}H and 564.69 MHz for ^{19}F. A 5 mm broad band observe probe including ^{19}F frequency on the broad band channel (SmartProbe™) with z axis gradient coil was used. For spectrometer stability reasons the H_2O phase in the microemulsions was replaced by D_2O. The diffusion coefficients at a constant temperature of 25 °C were derived from the signal attenuation in a series of stimulated echo spectra with increasing gradient amplitudes by fixed diffusion delay according to Wu et al. (1995). To overcome thermal convection effects the sample tubes were rotated. Typical experimental parameters were chosen as follows: diffusion time Δ between 100 and 300 ms, bipolar gradient pulse pair length δ between 2 and 3 ms, 32 data points by increasing the gradient amplitude from 5% to 95% of the maximum value of 50 G/cm. The processing and the analysis of the self-diffusion measurements was done within the Topspin Software, Version 3.0 and Dynamic Centre, Version 2.2.4 (Bruker BioSpin, GmbH). All experiments were repeated at least three times, the accuracy was ±4%.

2.4. Electrical-conductivity assessment

Along with other techniques conductivity studies can provide valuable information about the structure and phase behavior of microemulsions. Conductivity measurements were performed in triplicate using the conducting meter LF 521 (WTW) at 23 °C. The conductivity cell used was LTA 1 with glass/platinum electrode material and a cell constant of about 1 cm^{-1}. The application range was between 1 μS/cm and 20 mS/cm and a temperature range from −5 to 100 °C. The constant of the conductivity cell was calibrated using standard KCl solutions.

2.5. Small-angle X-ray scattering measurements

Small-angle X-ray (SAXS) scattering can be used to obtain information on droplet size, shape and morphology of microemulsions, arising from the electron density contrast between different phases. SAXS scattering patterns were collected using X-rays from a microfocus source (Incoatec IμS High Brilliance) and a 2D position sensitive detector (Vantec 2000, Bruker AXS). The sample-to-detector distance was chosen to cover a range of