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Research paper

Simultaneous analysis of skin penetration of surfactant and active drug from fluorosurfactant-based microemulsions

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ABSTRACT

The purpose of this study was to investigate the penetrated amount of the incorporated model drug diclofenac-sodium and of a fluorosurfactant as specific vehicle constituent of topically applied microemulsions at the same time. To this end, the penetration depth of each compound was elucidated through tape stripping studies by the simultaneous quantification of diclofenac-sodium and the fluorosurfactant from the same sample. A new approach was made by using the very sensitive and specific ^{19}F NMR (nuclear magnetic resonance) for quantification of the fluorinated vehicle component. The tape stripping experiments with the microemulsions showed an almost similar penetration velocity of diclofenac-sodium and fluorosurfactant, suggesting that the surfactant within the microemulsion-structure intensified the stratum corneum uptake of the incorporated active constituent. Moreover, ATR-FTIR studies on porcine ear skin revealed significant shifts of the CH_2 stretching absorbances, which are associated with an enhanced disorder of the SC lipids resulting in a decreased skin barrier function, after application of the microemulsions. However, the application of pure fluorosurfactant did not cause any shifts in the CH_2 stretching absorbances. It can be thereby concluded that the prepared microemulsions exerted specific effects on skin integrity resulting in a "push" of diclofenac-sodium penetration.

1. Introduction

The dermal and transdermal route offer several advantages compared to other routes of administration such as enhanced patient compliance, reduced first pass metabolism of drug and avoidance of gastric irritation. However, the poor permeability of the stratum corneum (SC), the outermost layer of the skin which tends to be the constrictive factor of penetration, often limits the possibilities for choosing the topical administration route [1,2]. The way substances penetrate into the skin strongly depends on their physicochemical properties and chemical structure, on the presence of penetration enhancers, on the employed vehicle systems and numerous other variables. To date, plenty of data dealing with the skin penetration of active substances is available. However, only little is known about the skin penetration of the applied vehicle compounds, such as lipids or surfactants.

Although many studies deal with vehicles in an indirect way for example by incorporating selected compounds into different formulations and in case of superior skin penetration they attribute this enhancement effect to the vehicle compounds like surfactants. Studies investigating the penetration of surfactants are scarce due to the difficult analytical access such as radioactive or fluorescent labelling.

The objective of the present study was to monitor the penetration route of the incorporated drug and the vehicle component at the same time. For this purpose microemulsion systems based on fluorosurfactants were developed and diclofenac-sodium (DS) was incorporated as model drug. After identification of microemulsions by microscopical and rheological studies their skin penetration behaviour was investigated by tape stripping experiments to examine whether synergies arise for the vehicle and the active component. The active component (DS) and the fluorosurfactants were directly quantified from the same strip by HPLC and

Abbreviations: ME, microemulsion; SC, stratum corneum; DS, diclofenac-sodium; FS, fluorosurfactant; HEX, Hexafor 670; SIN, Chemguard S-550-100.

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^{19}F NMR. The ^{19}F NMR is a highly sensitive and non-destructive analytical method that specifically quantifies fluorinated molecules. This method is an interference free method and works without radioactive labelling [3,4].

Microemulsions were chosen as colloidal vehicles because they are known to be efficient formulations for transdermal drug delivery [5]. Basically, microemulsions consist of water, oil, a surfactant and in most cases a co-solvent. Their components can clearly interact with SC intercellular lipids, which are considered to be the main pathway for the permeation of most drugs through the SC, facilitating drug transport across the barrier [6]. Besides, it has been suggested that microemulsion formulations may increase cutaneous drug delivery by means of high solubility potential for both lipophilic and hydrophilic drugs, which creates an increased concentration gradient towards the skin, and/or by using constituents with penetration enhancer activity [7,8]. Thus, microemulsions with the novel non-ionic ethoxylated fluorosurfactants Hexafor 670 (Hex) and Chemguard S-550-100 (Sin) were newly developed and prepared. The fluorinated surfactants in the microemulsions enabled us to follow the surfactant penetrating the layers of the stratum corneum.

Additionally, ATR-FTIR measurements were performed. The aim was to analyse possible interactions of the formulation constituents with SC lipids and to correlate these effects with their uptake in the SC. In particular, phase transitions of the SC lipids that may be the reason for an enhanced skin penetration were examined by detecting the shift of methylene (CH_2) bands. Therefore, the characteristic CH_2-stretching vibrations of pig ear skin that was incubated with a formulation were compared with the CH_2-stretching vibrations of the control.

2. Materials and methods

2.1. Materials

The fluorosurfactant Hexafor® 670 (Hex), according to company statements a fluorinated ethoxylated pentaerythritol, with a hydrophilic/lipophilic balance (HLB) of 13.6 and an average molecular weight of 1173 g/mol was kindly donated by Maflon (Castelli Calepio, Italy). The fluorosurfactant Chemguard S-550-100 (Sin), according to company statements a perfluoroalkyl substituted polyether, with a HLB of 8.9 and an 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 and diclofenac-sodium were obtained from Sigma–Aldrich (Vienna, Austria). Abdominal porcine skin was bought from a local butcher, cut at a thickness of 700 μm with a dermatom (GB 228R, Aesculap) and stored refrigerated at −18 °C for a maximum of 6 months. Methanol-d1 was obtained by Euriso-top (Gif sur Yvette, France). Acetonitrile was procured from Carl Roth (Graz, Austria).

2.2. Microemulsion development and characterization

Pseudoternary phase diagrams were constructed by mixing varying amounts of the fluorosurfactants with the co-solvent isopropanol in the relation 1:1 (w/w), oleic acid as oil phase and distilled water under magnetic stirring. After equilibration, the mixture was assessed by visual characterization and polarized light microscopy. Polarized light microscopy (Nikon GmbH, Germany) was performed to verify the isotropic nature of the developed microemulsions and to confirm the absence of liquid crystalline phases. Samples that remained transparent and homogenous after vortexing and did not interfere with plain polarized light were assigned as a monophasic area in phase diagram [2].

2.2.1. Rheology

In order to measure the viscosity of the drug-loaded MEs an MCR Modular Compact Rheometer 302 with a cone-plate measuring device of 50 mm diameter, employing a gap size of 1 mm (Anton Paar, Graz, Austria) was used. The temperature was maintained at 23 ± 0.2 °C. Flow curves were recorded with increasing and decreasing shear rates from 1 to 100 s^{-1} and vice versa. All measurements were carried out in triplicate.

2.2.2. Drug loaded microemulsions

The mixture containing 65% (w/w) surfactant/co-solvent, 10% (w/w) oleic acid and 25% (w/w) distilled water resulted in an isotropic microemulsion for both tested fluorosurfactants. This composition is marked with a dot in the pseudoternary phase diagrams (Fig. 1a and b). Based on these findings and a favourable

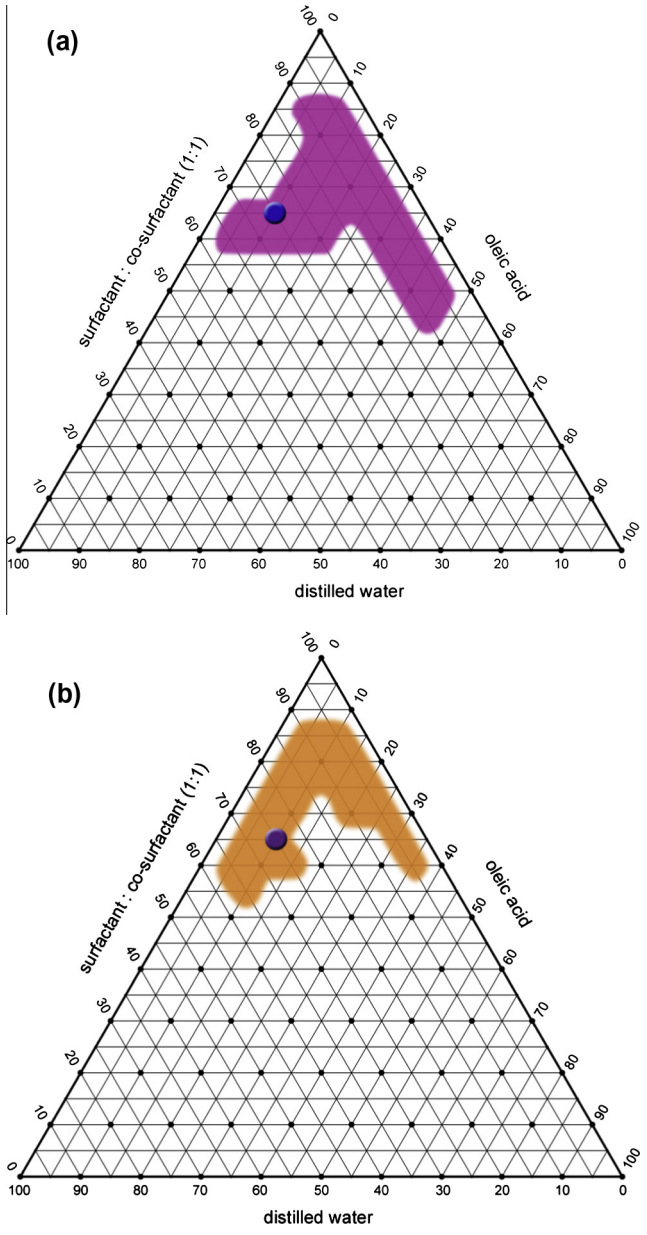

Fig. 1. Pseudoternary phase diagrams. The marked area labels the formation of isotropic microemulsions. The dot marks the composition which is used for the permeation and penetration studies of DS. (a) Isotropic microemulsions obtained with the fluorosurfactant Hexafor 670. (b) Isotropic microemulsions obtained with the fluorosurfactant Chemguard S-550-100. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)