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Natural surfactant-based topical vehicles for two model drugs: Influence of different lipophilic excipients on *in vitro*/*in vivo* skin performance

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ABSTRACT

This study focuses on the properties of topical vehicles based on alkylpolyglucoside natural surfactant-mixed emulsifier, cetearyl glucoside and cetearyl alcohol, in order to propose their use as "ready to use" pharmaceutical bases for a number of model drugs. We were interested to investigate how the alternative use of three lipophilic excipients (Ph. Eur. 6.0), differing in their polarity indexes (medium chain triglycerides (MG), decyl oleate (DO), and isopropyl myristate (IPM), respectively), affects the colloidal structure of the alkylpolyglucoside-based vehicles and *in vitro* permeation profiles of two model drugs: diclofenac sodium (DC) and caffeine (CF), both sparingly soluble in water. Finally, we aimed to evaluate the safety profile of such vehicles *in vitro* (acute skin irritation test using a cytotoxicity assay), comparing it with *in vivo* data obtained by the methods of skin bioengineering.

The results have shown that the emulsion vehicles consisted of a complex colloidal structure of lamellar liquid crystalline and lamellar gel crystalline type. Varying of lipophilic excipient influenced noteworthy variations in the colloidal structure demonstrated as different rheological profiles accompanied to the certain degree by different water distribution modes, but notably provoked by drug nature (an amphiphilic electrolyte drug vs. nonelectrolyte). *In vitro* permeation data obtained using ASC membranes in an infinite dose-type of experiment stressed the importance of the vehicle/solute interactions in case of small variation in formulation composition, asserting the drug properties in the first hours of permeation and rheological profile of the vehicles in the later phase of experiment as decisive factors. *In vitro* skin irritation test demonstrated a mild nature of the emulsifying wax and the absence of negative effects of used oil phases on cell viability in formulation concentrations correspondent to the therapeutic need. This result alongside with data obtained from *in vivo* study, could additionally promote investigated topical vehicles as prospective "ready to use" pharmaceutical bases.

1. Introduction

Emulsion systems used in dermopharmacy as drug carriers have to fulfill a number of requirements, e.g. acceptable physical stability, chemical inertness, satisfactory safety profile and drug delivery efficacy (cf. Refai and Müller-Goymann, 2002), reaching at the same time optimal sensory attributes (cohesiveness, spreadability, i.e. rub-out and rub-in and after-feel sensations. . .) (Smith et al., 2002). Most of them are based on traditional ionic or ethoxylated non-ionic emulsifiers or their mixtures with long chain fatty alcohols (so-called mixed emulsifiers). For example, European Pharmacopoeia 6.0 (Ph. Eur. 6.0) recognizes only two mixed emulsifiers, both of them of anionic type: cetostearyl alcohol (type A), emulsifying and cetostearyl alcohol (type B), emulsifying, the first one containing minimum 7% (w/w) of sodium cetostearyl sulphate (SCS), and the second one minimum 7% (w/w) of sodium lauryl sulphate (SLS). The latter surfactant is well established as cytotoxic marker chemical (OECD Draft Proposal for a New Guideline, 2008) and *in vivo* proved skin irritant (Fluhr et al., 2001). While vehicles based on these mixed emulsifiers meet general requirements for pharmaceutical bases, their use is definitely accompanied by adverse skin reactions (Bárány et al., 2000), or associated with unpleasing appearance and unacceptable skin feeling during application (Al-Bawab and Friberg, 2006). Consequently, overcoming the above problems is an important formulation task, which may be accomplished by adequate selection of an emulsifier system (Bárány et al., 2000; Williams and Barry, 2004).

In other words, to promote new simple topical vehicles based on so-called natural surfactant as prospective "ready to use" bases for a number of model drugs, a comprehensive study of their key properties has to be performed. This includes physicochemical characterization of colloidal structure and physical stability study

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of the variety of different formulations, investigation of the impact of those formulations on *in vitro* release/permeation and/or *in vivo* efficacy for model drugs, as well as an evaluation of the safety profiles of the formulations.

Therefore, this study focuses on the properties of vehicles based on alkylpolyglucoside natural surfactant-mixed emulsifier, cetearyl glucoside, combined with cetearyl alcohol, that is, an emulsifying wax, in order to propose their use as "ready to use" bases for a number of model drugs. Our previous studies presented a detailed physicochemical and *in vitro*/*in vivo* characterization of a range of vehicles (*placebo samples*), as well as the active samples containing hydrocortisone or urea as model drugs (Savic et al., 2004, 2007).

In this study, we were interested to investigate further how the alternative use of three lipophilic pharmaceutical excipients (Ph. Eur. 6.0), differing in their polarity indexes (medium chain triglycerides (MG), 21.3 mN/m; decyl oleate (DO), 18.7 mN/m and isopropyl myristate (IPM), 24.2 mN/m, respectively), alongside with two additional model drugs, affects the colloidal structure of the alkylpolyglucoside-based vehicles. Furthermore, we have assessed the *in vitro* permeation profiles of following model drugs: a salt of a weak acid (diclophenac sodium (DC), 1% (w/w)), known as an amphiphilic drug, and a weak base (caffeine (CF), 2% (w/w)), both sparingly soluble in water, aiming also to relate the physicochemical properties (water distribution mode and rheological behaviour) of the vehicles with their *in vitro* permeation through the reconstructed human skin models (artificial skin constructs, ASCs). In addition, an evaluation of the safety profiles of active samples *in vitro* was performed, using an alternative method for acute skin irritation test (a cytotoxicity assay) (Spielmann et al., 2007; Vinardell et al., 2008) and *in vivo* (test vehicles), employing the methods of skin bioengineering (Bárány, 2000b). *In vivo* parameters assessed prior and upon 24 h-treatment under occlusion, were: SC hydration (SCH) and transepidermal water loss (TEWL), as a measure of skin barrier properties and skin erythema index (EI), as an indicator of vehicle's irritant potential.

Overall, the study aim was to assess model topical vehicles formed by natural mixed emulsifier, varying in the lipophilic phase, as prospective pharmaceutical ("ready to use") bases for two representative drugs evaluating their colloidal structure and *in vitro/in vivo* skin performance.

2. Materials and methods

2.1. Materials

The alkylpolyglucoside mixed emulsifier – cetearyl glucoside and cetearyl alcohol – recently FDA certified as pharmaceutical excipient alkyl glucoside (Sepineo SE® 68, kindly donated by Seppic, France) was used in a fixed concentration of 7% (w/w) for the preparation of bases without active ingredient ("placebo") of three model creams, labeled as follows: MG-PL, a basic formulation with 17% (w/w) of medium chain triglycerides, DO-PL and IPM-PL with the same amounts of decyl oleate and isopropyl myristate, respectively, with addition of preserved double-distilled water up to 100% (w/w). All excipients were of pharmacopoeial quality (Ph. Eur. 6.0). Active samples contained dissolved model drugs: 1% (w/w) of diclofenac sodium (DC) (Merck, Germany) or 2% (w/w) of caffeine (CF) (Merck, Germany), respectively. Depending on the incorporated drug, active samples were assigned as: MG-DC, DO-DC and IPM-DC (with diclophenac sodium) or MG-CF, DO-CF and IPM-CF (with caffeine).

For the cell culture experiments, human dermal fibroblasts from the foreskin of newborns were used. These cells were obtained from Cascade Biologics (Mansfield, UK), cultured according to standard conditions and used from the third to the twelfth passage. Immortalised keratinocytes from the HaCaT-cell line (Human adult, low Calcium, elevated Temperature) were used according to a standard cell culture method during passages 68–84 (Freshney, 1994).

For cytotoxicity (MTT) experiments, 3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma, Steinheim, Germany), sodium dodecylsulphate (Acros, B-Geel), isopropanol (Riedel de Haën, Seelze, Germany), hydrochloric acid (Merck, Darmstadt, Germany) and freshly double distilled water were used.

2.2. Methods

2.2.1. Preparation of samples

Placebo samples (MG-PL, DO-PL and IPM-PL) were prepared by heating the emulsifier and oil at 70 °C in sealed glass vial, and adding the blend to the water phase, by stirring at constant temperature for 3 min (700 rpm), then 3 min at 500 rpm. Upon emulsification/solubilization, cooling was started whilst mixing at 500 rpm (1 min), then at 300 min to the room temperature. Active samples were manufactured by dissolving of the model drug in the hydrophilic phase of the system, using agitation and heating up to 70 °C. Test samples were stored for a week prior to the physicochemical investigation. For the *in vitro* permeation study, cytotoxicity assay and *in vivo* experiments, freshly prepared unpreserved samples were employed, after being stored for 48 h at 4 °C.

2.2.2. Microscopy

The mesomorphic structure of the samples was assessed with Leika (Germany) photomicroscope using cross-polarisers and a wavelength (λ) plate. Relevant images were digitalized using a camera attached to the microscope (Olympus DP12, Japan) and computer software (Olympus DP-Soft, version 3.2).

To perform a deeper insight into the colloidal structure of tested samples, a number of TEM micrographs (Leo 922, Leo D-Oberkochen, Germany) of sample replicas (made by the freeze-fracture technique) were taken. In a typical experiment, the samples were shock-frozen in melting nitrogen at 63 K between two flat gold holders. The frozen samples were fractured at 173 K in a BAF 400 instrument (Balzers, Wiesbaden, Germany) and then shadowed with platinum/carbon (2 nm) at 45° and with pure carbon at 90° for replica preparation. After cleaning with a chloroform-methanol mixture (1:1), the replicas on uncoated grids were fixed onto a sample holder, placed in the vacuum chamber of transmission electron microscope and viewed under a low vacuum at 200 kV.

2.2.3. Wide-angle X-ray diffraction (WAXD)

To obtain structural information on the test samples, short-range ordering was examined using WAXD measurements. Diffraction patterns were collected using an X-ray goniometer PW-1050/25 (Philips), coupled with a Xe-filled linear counter (Fuji, Japan). X-rays were produced by an X-ray generator PW-1730 (Philips) using a copper anode (anode current 25 mA; λ 0.154 nm, accelerating voltage 40 kV).

From diffraction angle theta (θ), the intermolecular distances were calculated according to the Bragg's law. For each sample measurements were performed twice.

2.2.4. Rheological measurements

Continuous and oscillatory measurements were performed in triplicate on all active samples, using CSR/CSS Rheometer (Bohlin Instruments, Pforzheim, Germany). The following conditions were used for all experiments: cone and plate measuring system (diameter 40 mm, angle 1°), with a sample thickness of 0.030 mm, at 20 ± 0.1 °C. During continual testing, a controlled shear rate procedure was applied (shear rate from 0.29 to 200 1/s and back, each stage lasting 120 s).