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Rapid communication

Self-assembled nanostructured aqueous dispersions as dermal delivery systems

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ABSTRACT

Due to their high interfacial area and capability of loading hydrophobic, hydrophilic and amphiphilic drugs, self-assembled nanoparticles are the subject of much attention in view of an application of these dispersions as carrier systems for a variety of different active ingredients. Therefore, the effect of the internal nanostructure of oil-loaded monoglyceride-based nanoparticles on the dermal delivery of diclofenac sodium was investigated. The different self-assembled phases of the nanostructured aqueous dispersions were characterized by small angle X-ray scattering (SAXS). The influence of the different phases ranging from cubic-bicontinuous, over hexagonal and cubic-micellar phases to emulsified microemulsions on the dermal delivery of the incorporated active was examined by Franz-type diffusion cell and *in vitro* tape stripping experiments on porcine skin. These studies revealed a dependency of the skin permeation of diclofenac sodium on the formulation's internal structure, which could be modified by varying the amount of *R*-(+)-limonene in the oil phase. A superiority of the emulsified microemulsion, possessing the highest amount of *R*-(+)-limonene, over cubic or hexagonal phases was evidenced in terms of dermal drug delivery.

1. Introduction

Monoglycerides are an interesting group of amphiphilic lipids that are able to self-assemble spontaneously in water to form various well-ordered nanostructures, which can be dispersed with energy input to isasomes – internally self-assembled particles – such as cubosomes, hexosomes and emulsified microemulsions (Yaghmur and Glatter, 2009). The latter are fascinating systems as the dispersed oil droplets in O/W emulsions or the kinetically stabilized internal W/O emulsion in double W/O/W emulsions are replaced by a thermodynamically stable inverted-type micro-emulsion (Yaghmur and Glatter, 2009). Due to their high interfacial area and capability of loading hydrophobic, hydrophilic and amphiphilic drugs, these systems are outstanding nanoparticulate carriers for a variety of different active ingredients (Yaghmur and Glatter, 2009; Salonen et al., 2007). Therefore, the effect of the internal nanostructure of oil-loaded monoglyceride-based nano-particles on the delivery of diclofenac sodium was investigated, since diclofenac is, among the group of topically used NSAIDs, proposed as a good candidate for anti-inflammatory and analgesic use (Goh and Lane, 2014).

The droplets of the nanostructured aqueous dispersions consisted mainly of monolinolein, Dimodan® U/J (Material no. 015312 (DU), donated by DANISCO A/S, Braband, Denmark). This material is a distilled monoglyceride mixture (mostly C_{18}-chains) comprising 96% monoglycerides with 62% linoleate and 25% oleate. The second lipid component, used to tune the internal structure, was the oil *R*-(+)-limonene, purchased from FLUKA Chemika (Buchs, Switzerland) (purity >96%). The triblock copolymer Pluronic® F127 (PEO_{99}–PPO_{67}–PEO_{99}, donated by BASF, Mount Olive, NJ, USA) was chosen as the stabilizer of the dispersions. The model drug diclofenac sodium was purchased from Sigma Aldrich (Vienna, Austria). The gelling agent agarose (technical grade, Sigma–Aldrich, Vienna, Austria) was used to tune the viscosity of the dispersions. All materials were used without further purification. Milli-Q water (Millipore, Merck KGaA, Darmstadt, Germany) was utilized as the solvent. All samples were a mixture of 2 g oil phase and 8 g water phase. The former was based on monolinolein with a fixed concentration of 5% diclofenac sodium and a variable amount of *R*-(+)-limonene. The water phase contained the fixed amount of 2% (0.16 g) F127. The water phase was added to the oil phase in a 20 ml vial. The mixture was sonicated using a high

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Table 1
Varying composition of the oil phase (the amount of the oil phase was kept at 20% in all formulations) and respective internal structure.

Formulation code	Composition of the oil phase (%)			Structure
	R-(+)-Limonene	Monolinolein	Diclofenac sodium	
Lim 0	0	95	5	Cubic-bicontinuous
Lim 10	10	85	5	Hexagonal
Lim 45	45	50	5	Cubic-micellar
Lim 60	60	35	5	Emulsified microemulsion

intensity ultrasonic processor (SY-LAB GmbH, Purkersdorf, Austria) for 5 min (1.5 s pulses interrupted by 0.5 s breaks) at 30% of the maximum power. The composition of the different samples prepared and studied is given in Table 1.

The different self-assembled phases of the nanostructured aqueous dispersions were characterized by small angle X-ray scattering (SAXS) at 25 °C.

For further experimental details of the SAXS experiments see Guillot et al. (2009). The different phases can be assigned by the peaks of the scattering curves according to the reflection laws for the space groups of the liquid crystalline phases or by the position of the broad correlation peak of the microemulsion (Yaghmur et al., 2006). In short, both cubic phases show several reflections, the hexagonal phase exhibits three peaks, while the microemulsion presents a strong correlation peak (see also Fig. 1a in Yaghmur et al. (2006).

The influence of the different phases on the dermal delivery of the incorporated active was examined by Franz-type diffusion cells using porcine abdominal skin as model membrane and *in vitro* tape stripping experiments on full-thickness porcine ear skin as previously reported (Hoppel et al., 2014).

Porcine abdominal skin with a thickness of 700 μm was used in the Franz-type diffusion cells with a permeation area of 0.95 cm^2 (Permegear, Hellertown, USA). The receptor compartment was filled with 2 ml of phosphate buffer (0.012 M, pH 7.4) containing 4% bovine serum albumin (BSA) and the diffusion cells were kept at 32 °C. An infinite dose of 500 mg cm^{-2} of formulation was applied onto the skin in the donor chamber. At least six parallel experiments were performed for each formulation ($n \geq 6$).

Each removed tape strip during the *in vitro* tape stripping experiments was at first analyzed for its amount of removed corneocytes by NIR densitometry (SquameScan® 850A, Heiland electronic GmbH, Wetzlar, Germany) and for its drug content by HPLC. Five parallel experiments for each formulation were performed ($n = 5$).

The quantification of diclofenac sodium was carried out by HPLC (PerkinElmer, USA) using a Nucleosil 100-5C18 column (250 mm × 4 mm, Macherey-Nagel, USA) plus pre-column (SS 8/4). The oven temperature was set at 50 °C. The mobile phase consisted of methanol/water/glacial acetic acid (80/20/1, v/v) and the flow rate was 0.7 ml min^{-1}. The detection wavelength was set at 245 nm. A calibration curve was calculated based on peak area measurements of diluted standard solutions ranging from 0.12 to 122.50 μg ml^{-1}. The obtained coefficient of determination was 0.9997. The limit of quantification was set at 0.12 μg ml^{-1}, while the limit of detection was found to be 0.06 μg ml^{-1}.

Results are expressed as mean values ± standard deviation (S.D.). Statistical data analyses were performed using the GraphPadPrism3 program (GraphPad Software, San Diego, USA). Effects of the various formulations on the dermal delivery of diclofenac sodium were evaluated using a one-way analysis of variance (ANOVA) with a post hoc Tukey test with $p < 0.05$ as level of significance.

By varying the amount of *R*-(+)-limonene of the oil phase, nanostructured aqueous dispersions with different internal structures were obtained (Table 1).

The SAXS results in Fig. 1 clearly show the structural dependence of the droplets resulting from the different *R*-(+)-limonene content. The *R*-(+)-limonene-free sample Lim 0 showed the signature of a bicontinuous cubic phase with symmetry Pn3m, 10% *R*-(+)-limonene in the oil phase (sample Lim 10) led to a hexagonal phase, at 45% *R*-(+)-limonene (sample Lim

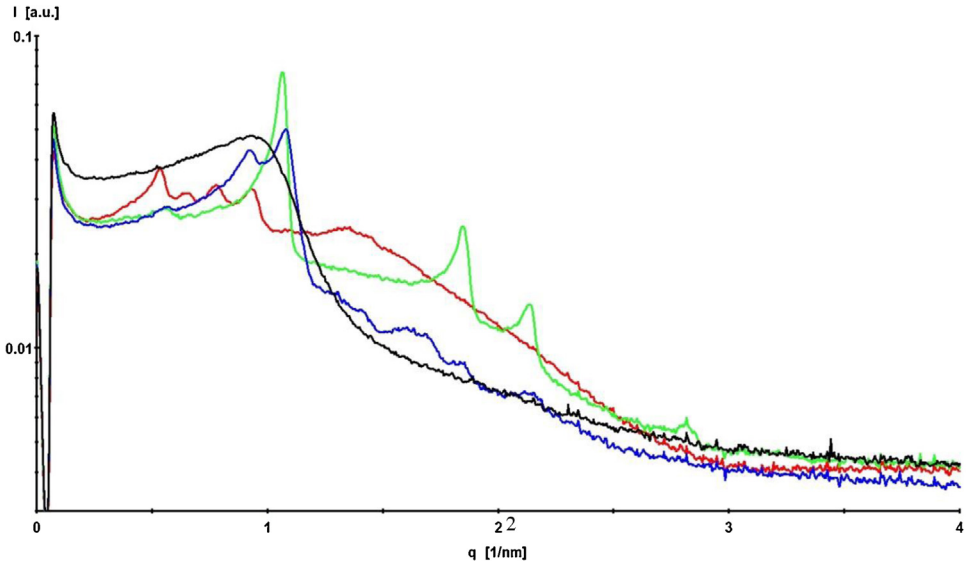

Fig. 1. SAXS results: the *R*-(+)-limonene-free sample Lim 0 (red curve) can be indicated as a bicontinuous cubic phase, sample Lim 10 (green curve) shows the signature a hexagonal phase, sample Lim 45 (blue curve) represents a micellar cubic phase and sample Lim 60 (black curve) is typical for a microemulsion. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)