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Topical delivery of acetyl hexapeptide-8 from different emulsions: Influence of emulsion composition and internal structure

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ABSTRACT

Acetyl hexapeptide-8 (AH-8) is a well-known component of anti-aging products and was recently explored as a promising topical treatment of blepharospasm. Although AH-8 appears in a variety of cosmetic products, its skin penetration is sparsely studied and controversially discussed. Therefore, the aim of the present study was to investigate the influence of the vehicle type on the AH-8 delivery to the skin. Besides skin permeation experiments with Franz type diffusion cells, the spatial distribution of AH-8 in the stratum corneum after a real in-use application was investigated by in vitro tape stripping on porcine ear skin. By applying LC–MS/MS for quantification of AH-8, we demonstrated that a multiple water-in-oil-in-water (W/O/W) emulsion can significantly increase penetration of AH-8 into porcine skin compared to simple O/W and W/O emulsions. The internal structure of the developed multiple emulsion was confirmed by electron microscopic investigations and NMR self diffusion studies. In general, a clear superiority of water-rich W/O/W and O/W emulsions over an oil-rich W/O emulsion in terms of dermal delivery of AH-8 was found. This enhanced delivery of AH-8 could be explained by an increased absorption of the water-rich emulsions into the skin, confirmed by combined ATR-FTIR and tape stripping experiments.

1. Introduction

Acetyl hexapeptide-8 (AH-8) is a topically applied hexapeptide that has a mechanism of action similar to botulinum neurotoxin (BoNT). It has been used effectively in anti-aging cosmetic applications and was recently explored as a promising treatment of blepharospasm (Lungu et al., 2013; Gorouhi and Maibach, 2009). Up to date, the only reliable effective treatment is a BoNT injection therapy with the disadvantages of significant costs, risks of side effects and discomfort (Lungu et al., 2013; Simpson et al., 2008).

However, topical delivery of hydrophilic macromolecules with a molecular mass >500 Da is challenging, due to the lipophilic nature of the skin's uppermost layer, the stratum corneum (Gorouhi and Maibach, 2009; Hadgraft and Lane, 2011; Choi et al., 2012). Up to date, the dermal delivery of large hydrophilic molecules such as peptides is sparsely studied. The diffusion through intercellular lipids as the classical pathway of transdermal permeation is mainly restricted to small lipophilic drugs. Therefore, delivery of hydrophilic molecules is attributed to two other pathways, namely to the transport through pores in the skin as well as through hair follicles and sweat ducts (Mitragotri, 2003; Hsu and Mitragotri, 2011). Many approaches for delivery of macromolecules through the skin, including chemical enhancers and physical delivery techniques, are restricted, due to skin toxicity, inconvenience or high production costs of sophisticated drug delivery systems (Choi et al., 2012).

Therefore, emulsions are still widely used dermal delivery systems (Otto et al., 2009). Water-rich oil-in-water (O/W) emulsions are most frequently used, due to their pleasant skin feel. Conversely, oil-rich water-in-oil (W/O) emulsions have lower cosmetic acceptance, caused by their greasy skin feel (Baran, 1998). More

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complicated multiple water-in-oil-in-water (W/O/W) emulsions are potential controlled release systems for dermal delivery of sensitive biologicals, such as peptides. The major benefit of multiple emulsions is a protection of the active, which can be encapsulated in the inner water droplets. However, their use is limited by lack of stability, caused by coalescence of the outer and inner droplets (Hoppel et al., 2014; Olivieri et al., 2003; Patravale and Mandawgade, 2008).

To this end, the applicability of different emulsions as dermal carrier systems for AH-8 was investigated in this study. Due to the skin irritation potential of conventional, synthetic surfactants and a recently increasing attention to the environment, all emulsions were based on a low concentration of natural origin and biodegradable emulsifiers (Savić et al., 2007; Mao et al., 2012).

The skin permeation of AH-8 from a multiple W/O/W emulsion was compared to simple O/W and W/O emulsions by Franz-type diffusion cell experiments. Furthermore, the developed multiple W/O/W emulsion was characterized in terms of stability, droplet size and rheological properties. The presence of the inner water phase in the multiple droplets was determined by electron microscopy and NMR self diffusion studies.

Interestingly, although AH-8 is widely used in anti-aging cosmetic formulations, detailed investigations regarding its skin penetration are still missing. In this study, the spatial distribution of AH-8 in the stratum corneum under real in-use conditions, namely after a short finite dose application of a practically relevant AH-8 concentration, was analyzed in detail. Furthermore, the stratum corneum penetration of the emulsions themselves and their influences on skin hydration were analyzed by combined ATR-FTIR and tape stripping experiments (Hathout et al., 2010; Hoppel et al., 2014).

2. Materials and methods

2.1. Materials

Acetyl hexapeptide-8 (Argireline® powder) was kindly provided by Lipotec GmbH (Hofheim-Wallau, Germany). Octyldodecanol & Octyldodecyl Xyloside & PEG-30 Dipolyhydroxystearate (Easynov®) was a gift from Seppic (Cologne, Germany). Sucrose stearates (Ryoto Sugar Ester® S-970 and S-1670) were kindly donated by Mitsubishi-Kagaku Foods Corporation (Tokyo, Japan). Isopropyl myristate (IPM) was procured from Herba Chemosan (Vienna, Austria) and neutral oil (Miglyol® 812) from Dr. Temt Laboratories (Vienna, Austria). Formic acid was obtained from Sigma Aldrich (Vienna, Austria).

2.2. Methods

2.2.1. Formulation preparation

The composition of the different formulations is given in Table 1. The multiple W/O/W emulsion was prepared by a one-step emulsification method (Hoppel et al., 2014). Briefly, both surfactants, Easynov® and sucrose stearate S-1670, were dissolved in the oil phase under stirring and heating. AH-8 was dissolved in distilled water at room temperature. Subsequently, the water phase was added to the oil phase under moderate stirring (750 rpm). Afterwards, the double emulsion was stirred for 20 min at 750 rpm.

The O/W emulsion was prepared by a method previously reported by our working group (Klang et al., 2011a). In short, sucrose stearate S-970 was dissolved in the oil phase under stirring at 50 °C. AH-8 was dissolved in distilled water at 40 °C. The aqueous phase was slowly added to the oil phase and after further stirring for 10 min, the formulation was treated with an ultraturrax (Omni 5000, Omni International, Kennesaw, USA) for 4 min at 2500 rpm.

The W/O emulsion was prepared by stirring the oil phase, consisting of neutral oil and Easynov®, and the water phase, consisting of AH-8 and distilled water, separately at 40 °C. Subsequently, the water phase was added to the oil phase and the formed W/O emulsion was further stirred for 10 min at 750 rpm.

2.2.2. Characterization of the developed multiple W/O/W emulsion

The multiple emulsion was observed immediately after preparation and monitored over a period of 14 weeks by macroscopic examination, in order to detect any instabilities such as creaming or phase separation.

The droplet size of the multiple emulsion was determined using a laser diffraction particle size analyzer (Mastersizer 3000, Malvern, Worcestershire, UK). The instrument was operated with the Hydro MV sample dispersion unit (Malvern, Worcestershire, UK) and software version 2.01. Particle size distribution was calculated according to the Mie theory. The samples were diluted with distilled water. Droplet sizes are presented as the D(v, 0.5). This parameter stands for the volume median diameter and marks the size where half of the particle size of the sample is above and half of the particle size of the sample is below this value. In addition, the D(v, 0.9) and D(v, 0.1) were analyzed. The span was automatically calculated. This value represents the width of the distribution based on the 10%, 50% and 90% quantile. The droplet size measurement was carried out over an observation period of 14 weeks. The developed formulation was prepared and analyzed in triplicate ($n = 3$). The formulations were stored at room temperature (25 ± 5 °C).

2.2.3. Skin permeation studies using Franz-type diffusion cells

In vitro skin permeation studies were performed using Franz-type diffusion cells (Permegear, Hellertown, USA). Porcine skin was cut with a dermatome (GB 228R, Aesculap, Center Valley, USA) set at 700 µm. The skin samples were stored at −24 °C for not longer than 6 months. Appropriately cut skin pieces were clamped between the donor and the receptor chamber of the diffusion cells having a permeation area of 0.95 cm^2. The diffusion cells were thermostatted at 32 °C to maintain skin surface temperature and were continuously stirred with magnetic bars. The acceptor compartment was filled with 2 ml of 0.1% aqueous formic acid. An

Table 1
Composition of the multiple W/O/W, O/W and W/O emulsions.

Excipients	Formulation code		
	W/O/W	O/W	W/O
	Formulation composition (%, w/w)		
Neutral oil	–	20	76
Isopropyl myristate	20	–	–
Distilled water	75.99	75.99	19.99
Octyldodecanol & Octyldodecyl Xyloside & PEG-30 Dipolyhydroxystearate (Easynov®)	1.5	–	4
S-1670	2.5	–	–
S-970	–	4	–
Acetyl hexapeptide-8 (AH-8)	0.01	0.01	0.01