Development and Production of Oil-in-Water Vehicles Sub-micron Emulsion the Dermal Application of Ectoin

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Introduction

Micro/sub-micron emulsion and related systems have received a lot of attention in the last years for their common use as vehicles for topical administration. These systems can be defined as fine emulsion dispersion with droplet sizes in the sub-micron range, and consisted of an aqueous phase, oily phase, and surfactants that are necessary for stabilizing the interface and forming the drops [1]. The main advantages of such systems are due to the high solubility as well as bioavailability potential for lipophilic and hydrophilic substance. Moreover, the emulsion ingredients (oil and surfactants) may enhance the absorption into the skin by interact with the lipids barrier of the stratum corneum to facilitate the molecule to the skin [2].

Ectoin (1,4,5,6-tetrahydro-2-methyl-4-pyrimidecarboxylic acid), chemically characterized as a heterocyclic amino acid, is one of the most common compatible solutes produced by halophilic bacteria as a protected mechanism against osmotic stress and dehydration at high temperatures [3].

In recent dermatological studies Ectoin was found to protect skin cells and cell biopolymers from external stress factors such as UV radiation, surfactants contact and heat [4]. It was found that the Ectoin protective effect was concentration dependant [5]. However, Ectoin as a very hydrophilic molecule has a very low ability to penetrate into the skin. Therefore, it is an advance to develop a topical vehicle to allow the application of high amount of Ectoin to the skin.

In the present study, 5% Ectoin were included to a formulation based on an oil-in-water (O/W) submicron-emulsion, with suggest for its topical administration to treat neurodermitic skin. Composing the o/w-formulations from pharmaceutically acceptable ingredients (oils and surfactants), with a low surfactants amount, may

reduce the limitations for use due to irritation and allow the possibility for a long-term skin application. Also, addition of known permeation enhancers was evaluated as potential application enhancers for Ectoin in the formulation.

For preparing the o/w emulsion, membrane emulsification method was examined. Membrane emulsification have received increase attention over the last decade, due to its main advantages of obtaining a uniform monodisperse emulsion with a narrow size distribution, at low energy input compared to emulsions prepared by the common methods as homogenizer and high-pressure homogenizer. In this process, a liquid is pressed through the membrane pores to form droplet at the permeate side of the membrane, that are then carried away by a continuous phase that flows across the membrane surface [6]. In comparison to conventional emulsification methods, the membrane emulsification may provided more control of the drop size and size distribution, which is influenced by membrane pore size, agitation speed, continuous phase viscosity, interfacial tension between the phases, as well as the chemistry of the formulation [7].
The formulation was prepared with the membrane emulsification method with the use of tubular Al_2O_3 ceramic membranes (Atech innovations, Gladbeck, Germany), and was compared to the common used emulsification methods such as homogenizer and high-pressure homogenizer. The droplet size properties of the different formulation have been evaluated. The efficiency of the formulations to bring Ectoin into porcine ear skin was assessed, and the addition of known permeation enhancers was evaluated as potential application enhancers for Ectoin in the formulation.

Material and methods

Materials:

Materials for the basic o/w formulation as described below were from Degussa, Germany.
Permeation enhancers were from Sigma, Germany.

Formulation ingredients by wt. %:

Oily phase: Dimethicone (0.05), Caprylic/capric triglyceride (6), Decyl oleate (6), Cetearyl Ethylhexanoate (5.5).

Surfactants: Glyceryl stearate and Cetech-20 (6), Stearyl alcohol (2).

Water phase: water (64.5), glycerine (3), Lactil®* (2), Ectoin (5).

(* Lactil® - sodium lactate, sodium PCA, glycine, fructose, urea, niacinamide, inositol, sodium benzoate, lactic acid.)

Emulsification methods for the formulation production:

Homogenizer:

The surfactants were mixed in the oily phase by stirring with magnetic stirrer at 60-65^{o}C, for about half-hour until complete dissolution and Ectoin was dissolved in the water phase. Than, the emulsification performed by mixing the phases of each formulation by homogenizer (DIAX 600, Heidolph, Germany) at 24000 rpm for 2 min.

High-pressure homogenizer:

The formulation was prepares as described above with the homogenizer. This time the formulation was mixed for a short time, and emulsification was completed by treatment with high-pressure homogenizer (EmulsiFlex-C5, Avestin, Canada) at 15,000 psi.

Membrane emulsification:

As can be seen in figure 1, the membrane emulsification system consisted of a membrane module, tubes connections, a pump and different vessels. A microporous Al_2O_3 membrane (Atech innovations, Gladbeck, Germany) that have countless pores, of a uniform pore size of 200nm was attached to the membrane module (MM). The water phase, which was put into vessel B1 was circulated through the membrane channel. The oily phase containing surfactants was places in reservoir (c). Both vessels were thermostated to 65°C. Different air pressure was applied on reservoir (c) to directed this phase towards the membrane. When a critical pressure was reached, emulsion particles were formed on the inner surface of the membrane.

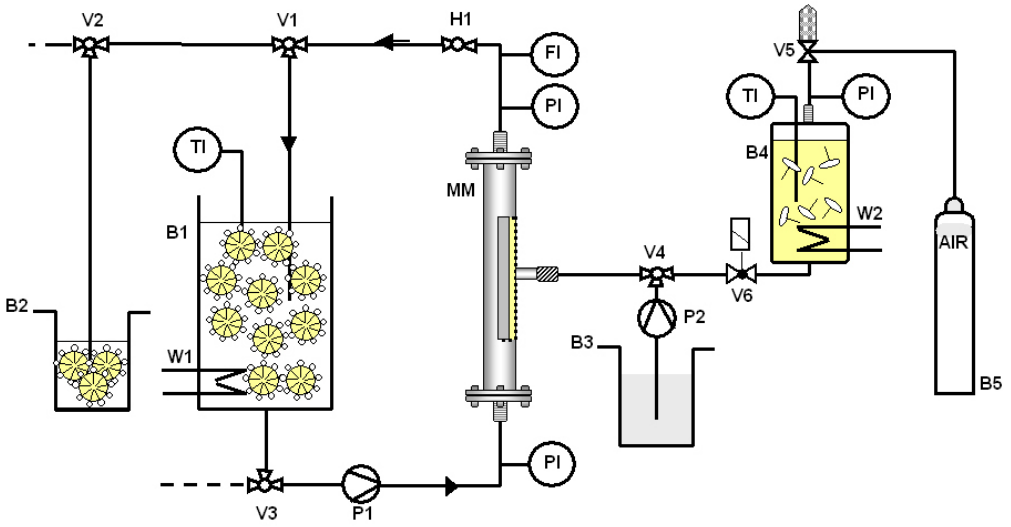

Figure 1: Schematic drawing of the membrane emulsification set up.

(H1) shut off valve, (V1, V2, V3, V4) three-way valve, (V5) pressure reducer, (V6) shut off valve, (W1, W2) heater, (MM) membrane module with ceramic membrane, (B1) vessel for the continuous phase, (B2) vessel for samples, (B3) vessel for cleaning agent solution, (B4) vessel disperse phase-oil phase, (B5) gas bottle, (P1) circulation pump, (P2) cleaning pump.

Formulation drop size analysis:

Drop size and distribution was measured by dynamic laser light scattering, at 25^{o}C using the Mastersizer (MSS 2000, Malvern, UK).

Skin penetration studies:

Permeation of Ectoin through cellulose acetate membrane (0,2 µm), and porcine skin, with and without stratum corneum were performed using Franz-diffusion cells with an effective permeation area of 1.767 cm^2 (Gauer Glass, Puttlingen, Germany). The formulations (250µl) were pipetted on the skin, into the donor compartment that was sealed with Parafilm to prevent evaporation. During the permeation studies, the diffusion cells were shaken at 120 rpm and thermostated to 37^{o}C.

In addition, Penetration of Ectoin into the skin was investigated in vitro, on porcine ear skin as described above. 24 h after applying the formulation on the skin, amount of Ectoin accumulated into the skin was examined following skin extraction in 1 ml ethanol and shaking at 130 rpm for 1.5 h at 60^{o}C. After removing tissue parts with centrifugation (400 rpm, 10 min) the supernatants were analysed for Ectoin by HPLC.

Ectoin HPLC analysis:

The Ectoin concentration determined by HPLC (pu-1580 Jasco), equipped with a column: CC 125X4 mm Nucleosil, 5 µm particle size, 100-5 NH2 (Macherey-Nagel, Düren, Germany). Samples were (20 µl) chromatographed using an isocratic mobile phase of acetonitrile and phosphate buffer (pH 7.4, 0,01 M) (70:30 v/v), flow rate of 1 ml/min and the detection wavelength was 210 nm.

Results and Discussion

Optimization of the membrane emulsification method

In order to optimize the formulation production method with the membrane system, different applied Trans Membrane Pressures (TMP) were examined.

The water continuous phase was flowing in a high velocity in order to achieve high Reynolds number Re for turbulent flow in the membrane channel.

The produced formulation had a similar mean drop size of 320-360 nm. As seen in figure 2, the TMP had an influence on the size distribution of the formulation drop size, and the narrowest distribution of 0.5 µm was achieved with TMP of 4.77×10^5 Pa. When the pressure was lower, a long time production of more than 2 h was necessary. Therefore, oil drops may aggregate during the long time circulated with the water phase. On the other hand, when pressure is too high, the dispersed phase may pass through the membrane as jet stream and the potential of coalescence of drops near the pores is increased. This leads to large drop size distribution of the produced emulsion.

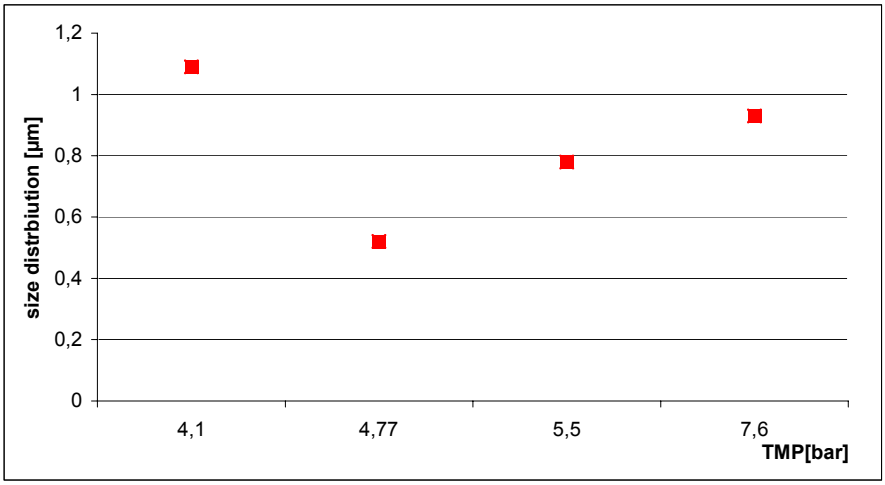

Figure2: Size distribution of o/w-formulation prepared with ceramic membrane with pore size 200nm, by using different Trans Membrane Pressure (TMP)

Formulations characterization

After optimizing the production process with the membrane system, this method was compared to the common emulsification methods by using homogenizer and high-pressure homogenizer.

The investigated emulsions were stable during storage at room temperature for at least 6 months. The characteristic drop size in figure 3 shows that the formulation prepared with homogenizer had a drop size of 670 nm with a high size distribution. In contrast, the high-pressure and the membrane formulations had a smaller drop size of 300-320 nm with a narrow size distribution. The formulation prepared with the membrane was characterized with the most narrow size distribution.

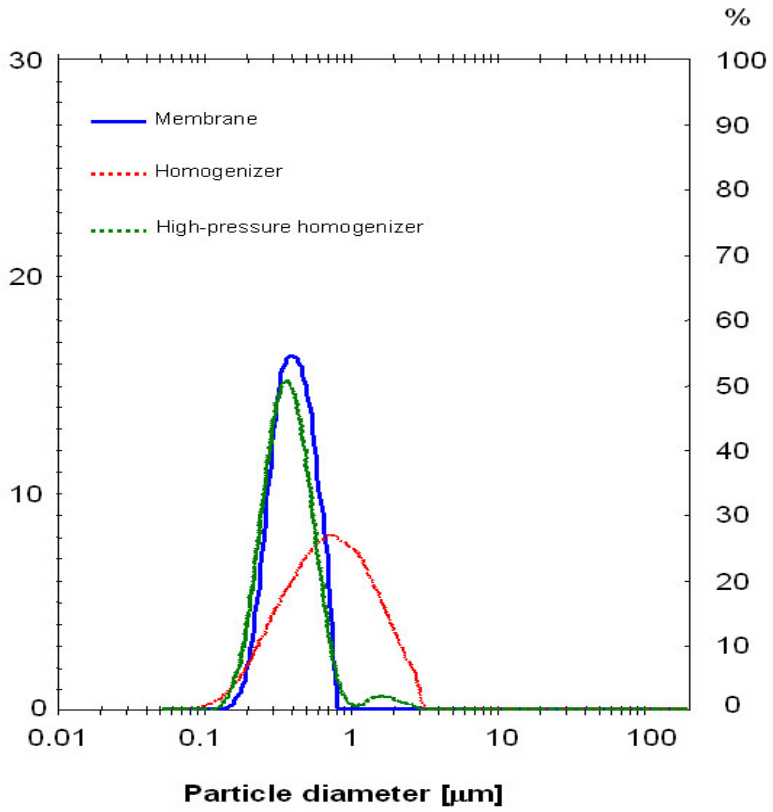

Figure 3: Drop size and size distribution of the formulations prepared with Homogenizer, High-Pressure Homogenizer and Membrane system.

Permeation studies

In order to establish a skin permeation model for the Ectoin, with similar properties as the human skin, the permeation of Ectoin in the basic formulation, prepared with homogenizer, was examined through cellulose acetate membrane, full thickness porcine ear skin (as a skin with intact barrier function) and skin without stratum corneum (as a skin with damaged barrier function).

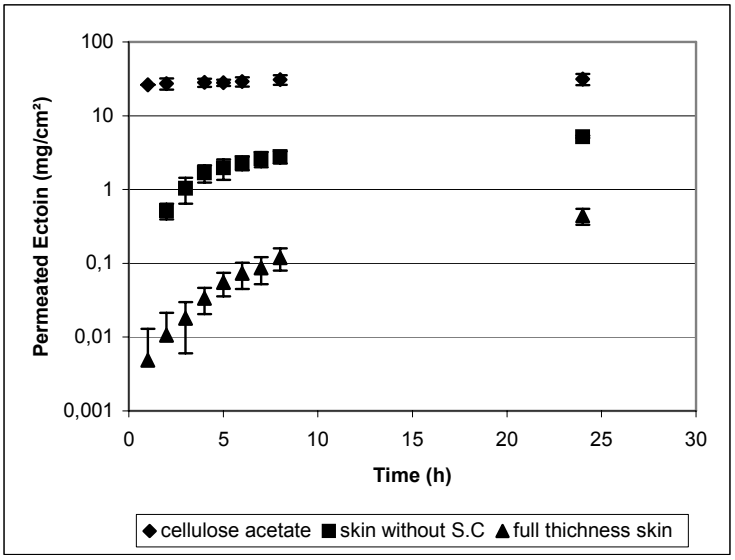

Figure 4: Permeation of Ectoin in the basic formulation (homogenizer) throgh cellulose acetate membrane, porcine skin without stratum corneum and full thickness skin.

As seen in figure 4, Ectoin as a hydrophilic molecule had a high permeability through the cellulose acetate membrane and the skin without the lipid barrier of the stratum corneum. In contrast, permeation through full thickness skin was limited by the lipid barrier of the stratum corneum. Therefore, it is decided to examine the permeation of Ectoin on and into porcine ear skin

The ability of the formulation prepared with different emulsification methods to apply the Ectoin into the skin

Penetration of Ectoin into full thickness porcine ear skin was dependant on the size of the oil drops in the formulation. Skin application of formulation prepared with high-pressure homogenizer and membrane emulsification with the smaller drop size of 300nm led to higher amount of Ectoin in the skin, in comparison to the lower amount of Ectoin in the skin with the homogenizer formulation.

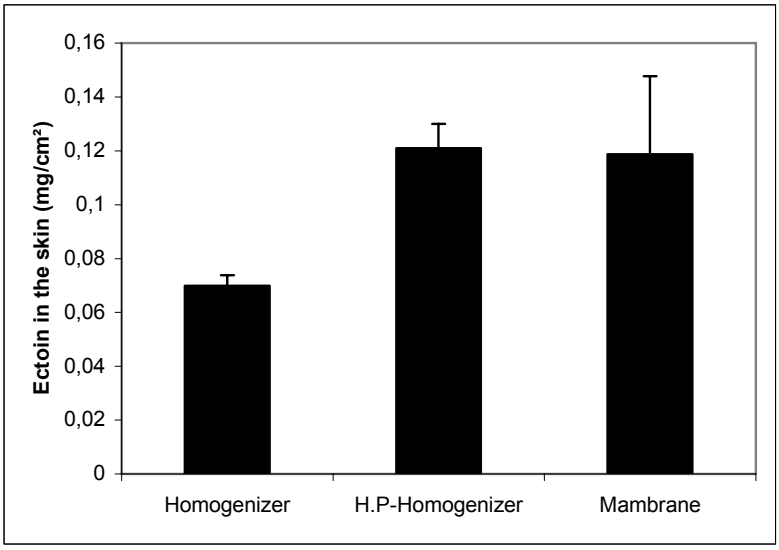

Figure 4: Penetration of Ectoin into the skin from the o/w formulation prepared with homogenizer, high-pressure homogenizer and membrane emulsification.

Effect of permeation enhancers addition

To promote the application of Ectoin to the skin, various known topical permeation enhancers: oleic acid, propylene glycol and cholesterol in a concentration of 2%, and decyl oleate or DMS oils were added to the basic S1-1 formulation by replacing the oil cetearyl ethylhexanoate. As shown in table 2, from the examined permeation enhancers, the addition of 2% of propylene glycol but mostly oleic acid and double amount of the oil decyl oleate (12%) gave the most pronounced enhancing effect on Ectoin permeation into the skin, almost 3-times more compared to the standard formulation without enhancer.

These permeation enhancers may fluids the lipid structure of the stratum corneum and by this increase the diffusion and partitioning of the molecule into the skin.

Table 1: Amount of Ectoin accumulated into porcine ear skin, 24 h following administration of S1-1 formulation containing different permeation enhancers

Addition of Permeation enhancer	**Ectoin penetrated into the skin (mg/cm²)**
Basic formulation	0.069 ± 0,003
+ 2% cholesterol	0.067 ± 0.017
+ 2% propylene glycol	0.100 ± 0.030
+ 2% oleic acid	0.160 ± 0.027
+ DMS oils	0.086 ± 0.004
+ decyl oleate	0.216 ± 0.014

Conclusions

Vehicles on a basic of o/w submicron emulsion were developed for the topical application of the hydrophilic molecule ectoin.

The formulation was prepared with different emulsification methods.

With the membrane emulsification process, o/w-submicron emulsion with drop size of 320nm and a narrow size distribution was gained by applying a trans membrane pressure of $4.77x10^5$ Pa.

The membrane emulsification and the high-pressure homogenizer achieved a formulation with the smallest drop size of 300nm. However, by producing with the membrane system a more narrow and uniform size distribution was reached.

Based upon results of Ectoin permeability into porcine ear skin, it was observed that the application of ectoin into the skin was depending on the drop size.

Both of the formulations prepared with membrane system and high-pressure homogenizer were more efficient in applying Ectoin into the skin than the formulation prepared by homogenizing.

In addition, the application of Ectoin into the skin was depended on the compounds of the formulation. Further enhancement of the Ectoin applied into the skin may be reached by the addition of oleic acid or propylene glycol to the formulation.

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