Research Communication

Effect of hydroxypropyl-β-cyclodextrin in fluid and semi-solid submicron emulsions on physiological skin parameters during regular in vivo application

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Abstract

OBJECTIVE: The aim of the present study was to evaluate the effect of hydroxypropyl-β-cyclodextrin (HP-β-CD) in cosmetic submicron emulsions and submicron emulsion gels on physiological skin parameters during regular application in a clinical set-up.

METHODS: Formulation morphology was investigated using cryo-transmission electron microscopy. Stability of the employed formulations was determined by photon correlation spectroscopy, measurement of pH and rheological properties. Effect on physiological skin parameters was evaluated during regular application over four weeks in a parallel group study ($n = 15$, healthy forearm skin) with a Corneometer, Sebumeter, skin-pH-Meter, Aquaflux and an Epsilon sensor. Confocal Raman spectroscopy was employed to monitor urea and NMF levels.

RESULTS: Both submicron emulsions and gels showed satisfying storage stability irrespective of cyclodextrin incorporation. No statistically significant effects on skin barrier function and any of the observed parameters were obtained, indicating good skin tolerability of all tested formulations.

CONCLUSION: Results suggest good skin tolerability of the developed cosmetic submicron emulsions and gels with HP-β-CD.

Résumé

OBJECTIF: Le but de la présente étude était d'évaluer l'effet de l'hydroxypropyl-β-cyclodextrine (HP-β-CD) dans les émulsions cosmétiques submicroniques et les gels d'émulsion submicronique sur les paramètres physiologiques de la peau lors d'une application régulière dans une configuration clinique.

MÉTHODES: La morphologie de la formulation a été étudiée en utilisant la microscopie électronique à transmission cryo. La stabilité des formulations employées a été déterminée par spectroscopie de corrélation de photons, mesure du pH et des propriétés rhéologiques. L'effet sur les paramètres physiologiques de la peau a été évalué lors d'une application régulière pendant quatre semaines dans une étude de groupe parallèle (n = 15, peau d'avant-bras saine) avec un cornéomètre, un sébumètre, un pH-mètre cutané, un Aquaflux et un capteur Epsilon. La spectroscopie Raman confocale a été utilisée pour surveiller les niveaux d'urée et de NMF.

RÉSULTATS: Les émulsions et les gels submicroniques ont montré une stabilité de stockage satisfaisante indépendamment de l'incorporation de cyclodextrine. Aucun effet statistiquement significatif sur la fonction de barrière cutanée et aucun des paramètres observés n'a été obtenu, indiquant une bonne tolérance cutanée de toutes les formulations testées.

CONCLUSION: Les résultats suggèrent une bonne tolérance cutanée des émulsions et des gels cosmétiques submicroniques développés avec HP-β-CD.

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Introduction

Cyclodextrins have been of interest for the pharmaceutical and cosmetic industry for many years [1–5]. Native cyclodextrins have been employed to improve physical stability of submicron emulsions for dermal delivery; skin permeation of incorporated drugs can be enhanced [6]. However, the clinical use of such submicron emulsions has not been investigated in the past regarding adverse effects. The aim of the present study was to evaluate the effect of a cyclodextrin as additive in cosmetic submicron emulsions on physiological skin parameters in vivo. A range of different β-cyclodextrins was evaluated; hydroxypropyl-β-cyclodextrin (HP-β-CD) led to the most promising results concerning stability and skin permeation of a lipophilic model compound (data not shown). Thus, HP-β-CD was chosen for the present clinical evaluation as excipient in cosmetic submicron emulsions.

Cyclodextrins can affect skin permeation [7–9]. While chemical enhancers may penetrate into the stratum corneum and disrupt lipid structures [10], cyclodextrins cannot penetrate into intact skin [11]. Extraction of lipids and weakening of barrier function have been described ex vivo [12,13], but were not confirmed in later

studies [14]. To exclude such effects for a later application, the skin compatibility of submicron emulsions with HP-β-CD in fluid and gel form was tested in a clinical set-up over 4 weeks.

Materials and methods

Materials

Lipoid S75 was kindly provided by Lipoid GmbH (Ludwigshafen, Germany). Medium chain triglycerides, Carbopol 940, trometamol and propylene glycol were purchased from Herba Chemosan Apotheker-AG (Vienna, Austria). Potassium sorbate and butylhydroxyanisole were obtained from Sigma Aldrich (St. Louis, USA). Kleptose HPB (hydroxypropyl-β-cyclodextrin) was a kind gift from Roquette Frères (Lestrem, France).

Preparation of formulations

The composition of the investigated formulations is given in Table 1. Medium chain triglycerides, propylene glycol and Lipoid S75 were stirred at 50°C, and then, butylhydroxyanisole was added. The water phase (distilled water, potassium sorbate, HP-β-CD) was brought to the same temperature. After adding the water phase to the oil phase, the resulting emulsion was pre-homogenized with an ultraturrax Omni 5000 (Omni International, USA, 4 min, 70 g). The emulsion was treated with a high-pressure homogenizer (Emulsiflex C3, Avestin, Canada, 1000 bar, 15 homogenization cycles). For gelification, Carbopol 940 was neutralized with an aqueous solution of trometamol and the fluid submicron emulsion was incorporated into the gel phase.

Characterization and stability of the employed formulations

All formulations were produced and characterized in triplicate. Fluid formulations were analysed by cryo TEM, and mean droplet size, polydispersity index (PDI) and pH were determined. For fluid submicron emulsions, stability monitoring was performed by monitoring particle size, PDI and pH. For semi-solid gels, rheological

Table 1 Composition of the investigated formulations in % w/w.

| | Formulation | | | |
Excipient	F_0	G_0	F_CD	G_CD
Lipoid S75	4	4	4	4
Medium chain triglycerides	20	20	20	20
Propylene glycol	3	3	3	3
Butylhydroxyanisole	0.1	0.1	0.1	0.1
Potassium sorbate	0.1	0.1	0.1	0.1
Kleptose® HPB	0	0	1	1
Distilled water	72.8	72.8	71.8	71.8
Gelation after high-pressure homogenization				
Carbopol® 940	-	0.25	-	0.25
Trometamol 10%	-	2.5	-	2.5
Submicron emulsion	-	97.25	-	97.25

All formulations were investigated for physicochemical stability. Test formulations for the in vivo study were the fluid F_CD and semi-solid G_CD containing HP-β-CD. The semi-solid G_0 served as control.
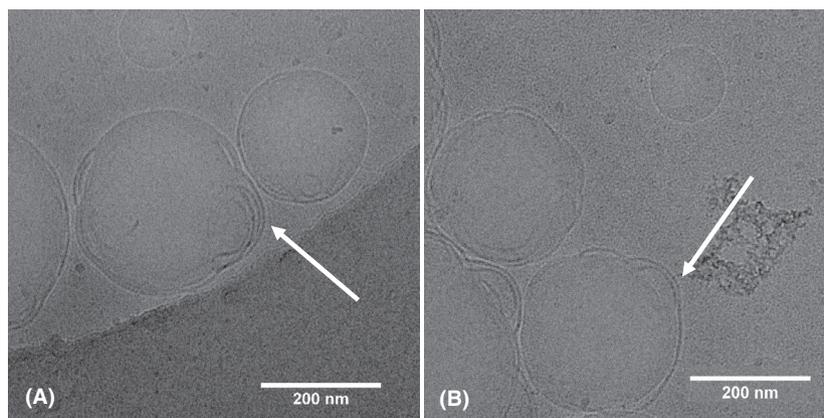
properties and pH were monitored to follow up on storage stability. All formulations were stored at +4°C. Measurements were performed in triplicate; results are expressed as mean values of $n = 3$ ±SD.

Cryo-transmission electron microscopy (TEM)

Formulation morphology was analysed by cryo TEM. Quantifoil (Großlöbichau, Germany) Cu 200 mesh R1.2/1.3 holy carbon grids were glow discharged for 1 min at −25 mA with a Bal-Tec (Balzers, Liechtenstein) SCD005 glow discharger and loaded into a Leica GP (Leica Microsystems, Vienna, Austria) grid plunger with the climate chamber set to 4° C and 75% relative humidity. Sample aliquots of 4 µl were applied to the carbon side of the grid and frontside blotted for 2 s (using the instrument's sensor function, no pre- or post-blotting incubation) with Whatman filter paper #1 (Little Chalfont, Great Britain). Grids were plunge frozen into liquid ethane at approximately −180°C for instant vitrification. Cryo-samples were transferred to a Glacios cryo-transmission microscope (Thermo Scientific, USA) equipped with a X-FEG and a Falcon3 direct electron detector (4096 × 4096 pixels). The microscope was operated in low-dose mode using the SerialEM software [15], and images were recorded digitally at a defocus of −4 to −8 µm in linear mode of the Falcon3 camera at a magnification of 45,000 resulting in a pixel size of 3.25 Å.

Mean droplet size, PDI and pH

A Zetasizer Nano ZS (Malvern, UK) was employed to determine the mean droplet size and PDI of formulations by photon correlation spectroscopy (PCS). Samples were diluted with freshly distilled water containing 0.01 mmol of sodium chloride (1:100 v/v). Measurements were performed in triplicate. To determine pH, a pH meter (Orion 420A, Bartelt, Austria) with an Orion ROSS micro pH electrode (8220BNWP) was employed.

Rheological properties

The dynamic viscosity of the formulations was determined employing an MCR Modular Compact Rheometer (Anton Paar, Graz, Austria) with a cone-plate measuring device (cone angle 2°, diameter 25 mm). Temperature was kept at 32 ± 0.2°C. Flow curves with increasing and decreasing shear rates from 1 to 100 s^{-1} and vice versa were recorded, and the dynamic viscosity at a shear rate of 10 s^{-1} was compared.

Effect of formulations on skin parameters: study design

Effect of the formulations on physiological skin parameters was evaluated in a parallel group study with fifteen healthy volunteers as approved by the Ethics Committee of the Medical University of Vienna (1724/2018) in accordance with the Declaration of Helsinki. Fifteen individuals of both sexes aged 23–30 years gave their informed written consent to participate. Exclusion criteria included skin lesions, tattoos and recent use of topical pharmaceuticals at the investigated skin site as well as chronic skin conditions. Participants were instructed not to use any other dermal products on their forearms for the duration of the study. Participants were randomly divided into three groups, each receiving one of the three formulations employed in the study, namely G_0 (gelled, control), G_CD and F_CD (gelled and fluid, both containing HP-β-CD).

Figure 1 Cryo TEM images showing the morphology of fluid submicron emulsions F_0 (A, control without hydroxypropyl-β-cyclodextrin) and F_CD (B, containing 1% of hydroxypropyl-β-cyclodextrin).

Participants were instructed to apply an amount of 0.15 g onto 60 cm^2 of the non-dominant volar forearm once daily before bedtime (2.5 mg/cm^2). The dominant volar forearm served as untreated control. Baseline values were determined at the beginning of the study. Skin parameter measurements were performed once weekly over four weeks. At least 12 h were kept between application and measurement. Participants were allowed to acclimatize for 15 min before measurements at 23.0 ± 1.3°C ambient temperature and 36.3 ± 8.6% relative humidity. One week after the last application recovery, values were determined.

Effect of formulations on skin parameters: biophysical analysis

Transepidermal water loss (TEWL) was determined after an acclimatization period of 15 min with a condenser-chamber device (AquaFlux AF200, Biox Ltd., London, UK). Skin hydration was assessed by measuring skin permittivity using a Corneometer CM 825 (Derma unit SSC3, Courage + Khazaka electronic GmbH, Germany, arbitrary units from 0 to 1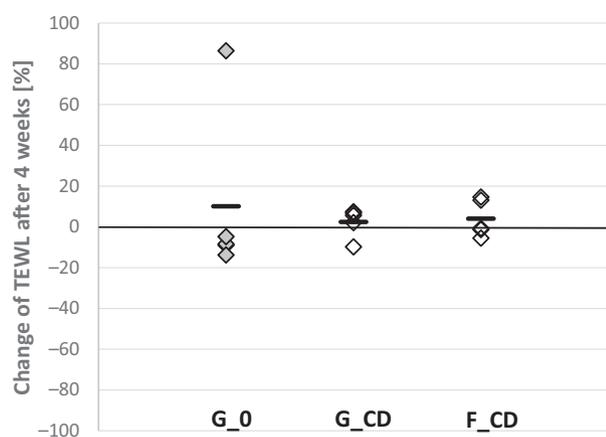20). A visual assessment of the skin hydration status was obtained by capacitive contact imaging (Epsilon E100 sensor, Biox Ltd., UK, skin permittivity values from 0 to 80, i.e. black to bright regions). Skin sebum and skin surface pH
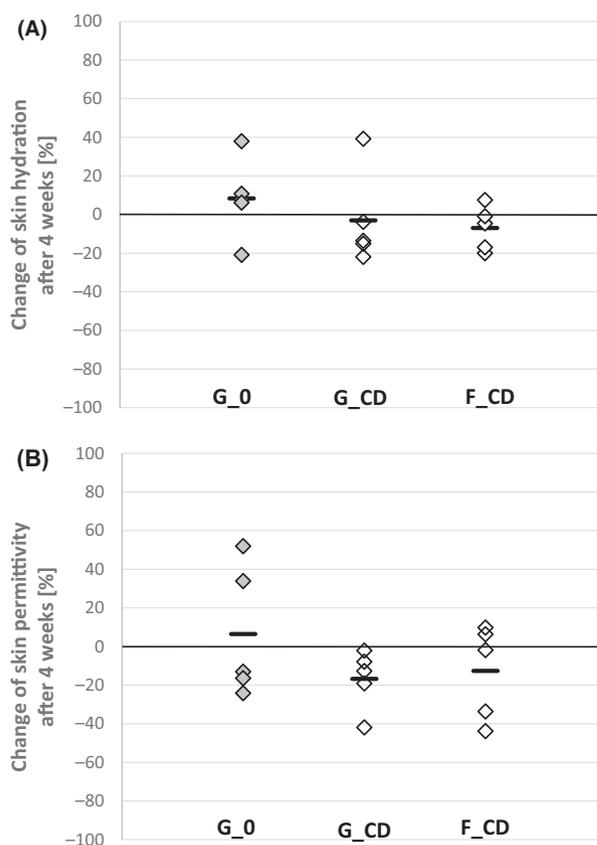

Figure 2 Change in TEWL after 4 weeks of daily application of control formulation G_0 and test formulations G_CD and F_CD. Changes are expressed in per cent (see Equation 1). Single values ($n = 5$) and the respective means are depicted.

Figure 3 Change in skin hydration (A) and skin permittivity (B) after four weeks of daily application of control formulation G_0 and test formulations G_CD and F_CD. Changes are expressed in per cent (see Equation 1). Single values ($n = 5$) and the respective means are depicted.
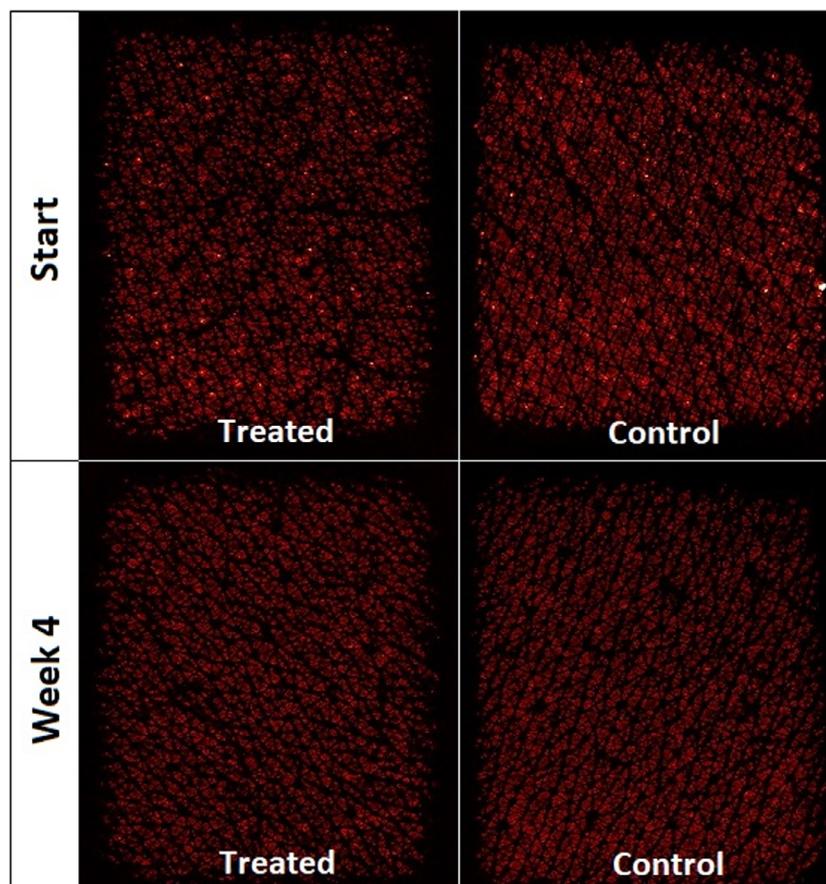
Figure 4 Representative example of capacitive sensor images (Epsilon, Biox Ltd., UK). The images show skin of a volunteer at the beginning of the study (above, non-dominant treated volar forearm on the left hand side, untreated control at the right hand side) and after 4 weeks of application of test formulation G_CD (below, non-dominant treated volar forearm on the left hand side, untreated control at the right hand side).

were measured with a Sebumeter (Derma unit SSC3, Courage + Khazaka electronic GmbH, Germany). All measurements were performed in triplicate at independent skin sites.

Confocal Raman spectroscopy (CRS)

Important skin parameters (NMF, urea and water content) were monitored by confocal Raman spectroscopy (gen2 SCA, River Diagnostics, The Netherlands, two incorporated lasers at 785 nm for the fingerprint region of 400–1800 cm^{-1} and 671 nm for the high wave number region of 2500–4000 cm^{-1}). Measurements were performed once weekly at least 12 h after treatment with the formulations. In the fingerprint region, measurements were performed in 2 μm depth increments up to a depth of 30 μm at a signal collection time of 5 s per spectrum. At least 5 measurements were performed on each volar forearm on random positions. Measurements in the high wave number region were conducted in 2 μm depth increments up to a depth of 40 μm at an exposure time of 2 s per spectrum. At least 3 measurements were performed on random positions of the designated area. The obtained spectra of each participant were averaged. Evaluation was performed with SkinTools 2.0 (River Diagnostics, The Netherlands using a non-restricted multiple least-square

fitting algorithm with keratin as an internal standard to cope for variations in signal intensity [16]. The section of 2–10 μm skin depth was analysed like previously described [17].

Calculation of skin parameter changes

To compare the effect of submicron emulsion on skin parameters considering control values (untreated dominant forearm), the following equation was used like previously described [18,19]:

$$\text{Skin parameter change}[\%] = \left(\frac{T_4/U_4}{T_0/U_0} - 1\right) \times 100 \qquad (1)$$

in which T_0 and U_0 are mean baseline values of treated and untreated forearm before the first application, whereas T_4 and U_4 are mean values of treated and untreated forearm after four weeks of application.

Statistical analysis

GraphPad Prism 3.0 (GraphPad Software, USA) was employed. Student's t-test was carried out to determine statistical differences.

Paired *t*-tests were performed in case of repeated measurements on the same individual. A probability of $P < 0.05$ was considered to show statistical significance.

Results and discussion

Formulation development and characterization

For the fluid emulsions, a curious droplet morphology was observed (Fig. 1, F_0 and F_CD) with oval bulges reminiscent of handles at the surfaces (white arrows). Similar structures have been reported [20]. The size distribution was in agreement with PCS data. After production, the fluid control submicron emulsions F_0 showed a mean droplet size of 177.4 ± 5.7 nm while the test formulation F_CD with HP-β-CD exhibited lower droplet sizes of 157.8 ± 3.9 nm ($P < 0.05$). After four weeks of storage, values of F_0 remained stable ($P > 0.05$) while an increase in droplet size was observed for F_CD (168.1 ± 4.8 nm, $P < 0.05$), indicating slightly higher droplet size growth. PDI values were below 0.2, indicating homogeneous droplet size distribution (0.103 ± 0.002 for F_0, 0.111 ± 0.002 for F_CD). Incorporation of HP-β-CD led to slightly higher PDI values ($P < 0.05$). After four weeks of storage, no significant changes occurred ($P > 0.05$).

All formulations exhibited pH values between 6.73 and 6.80. No significant changes after incorporation of HP-β-CD were observed between F_0 and F_CD or G_0 and G_CD ($P > 0.05$). As a result of pH adjustment with trometamol during the gelification process, the gels exhibited different pH when compared to submicron emulsions. After four weeks of storage, a decrease in pH was only observed in case of control submicron emulsion F_0 (from 6.73 ± 0.01 to 5.77 ± 0.25, $P < 0.05$). Better chemical stability of phospholipids in the other formulations might be ascribed to originally higher pH in gel formulations and/or presence of HP-β-CD, for example in F_CD. The incorporated HP-β-CD might prevent phospholipid hydrolysis or oxidation by forming inclusion complexes with the alkyl chains, thus improving chemical stability [21].

Characterization and stability testing of the submicron emulsion gels were confined to organoleptic, pH and rheological evaluation. Dynamic viscosity of submicron emulsion gels was comparable for G_0 and G_CD (4.28 ± 0.17 versus 4.47 ± 0.29 Pa.s at 10 s^{-1} at 32°C, $P > 0.05$) and remained stable during four weeks of storage ($P > 0.05$).

It can be summarized that both fluid submicron emulsions and corresponding gels exhibited adequate physicochemical stability.

Effect on physiological skin parameters

Participants described a pleasant skin feel, but unpleasant smell of formulations. This did not affect compliance, as confirmed by application diaries and gravimetric monitoring. Skin parameters were not affected in a negative way by either semi-solid control or fluid and semi-solid test formulations with HP-β-CD.

Transepidermal water loss values as a common indicator for impaired skin barrier function [22,23] were evaluated. The effect of the formulations on TEWL after four weeks is shown in Fig. 2. At the beginning of the study, TEWL values of 8.9 ± 1.8 g/cm^2/h were observed. After four weeks, mean values remained in the same range ($P > 0.05$). Mean TEWL values were well within physiological range [24].

The influence of the investigated formulations on skin hydration as determined by corneometry after four weeks is shown in Fig. 3 A. Values of 26.3 ± 4.5 a.u. were initially determined and remained stable during 4 weeks ($P > 0.05$). Minor trends were visible. Skin permittivity measurements confirmed these data (Fig. 3B). Trends towards decreased skin permittivity and thus decreased hydration for G_CD and F_CD group were more pronounced with this device (-16.8 ± 15.4 % for G_CD, -12.7 ± 24.5 % for F_CD, $P > 0.05$) as a result of larger measurement area and higher number of capacitive sensors [17]. High inter-individual variations were observed. A permittivity map of the skin surface is given in Fig. 4 (example from G_CD group). After 4 weeks, a mild decrease in brightness/ skin hydration could be observed for treated and control arm.

All volunteers exhibited low sebum (0–4 a.u., low number of sebaceous glands [25]), which remained constant over the treatment period ($P > 0.05$). Mean skin pH at the beginning of the study was 4.7 ± 0.5 – well within physiological range [26] – and remained stable during treatment ($P > 0.05$). Raman analysis was in line with these findings. NMF and urea content were not affected to a significant extent after the four week period for either of the formulations ($P > 0.05$). Similar trends as for corneometry and permittivity were visible since all parameters are related to cutaneous hydration [27]. An aspect worth mentioning is that daily application of formulations with relatively high water content is prone to cause dehydration. Beneficially, no such effects were observed.

In summary, the investigated formulations had no statistically significant effect, positive or negative, on any of the determined physiological skin parameters. Regarding skin hydration, consistent information was obtained with different techniques. No negative effects of the compound HP-β-CD were found, suggesting that any effects as penetration enhancer can be ascribed to solubilizing potential and effects within the formulation than to direct barrier impairment as a result of lipid extraction or interaction with other skin compounds. Results suggest good skin tolerability of the tested formulations and confirm the safety of the hydrophilic HP-β-CD for future use in cosmetic of pharmaceutical formulations.

Conclusion

Stable lecithin-based submicron emulsions and gels could be developed with 1% w/w HP-β-CD as additive. After daily application over four weeks, no statistically significant effects on physiological skin parameters were observed. No indication of lipid extraction and weakening of skin barrier function caused by HP-β-CD was observed.

Acknowledgement

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