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Vehicles based on a sugar surfactant: Colloidal structure and its impact on in vitro/in vivo hydrocortisone permeation

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Abstract

An emerging class of natural surfactants, named alkylpolyglucosides, which can form both, the thermotropic and the lyotropic liquid crystalline phases, were focused. The aim of the study was to integrate some physicochemical properties (characterised through the polarization and transmission electron microscopy, wide-angle X-ray diffraction, thermal analysis and rheology) of the three formulations based on cetearyl glucoside and cetearyl alcohol, with the in vitro (the artificial skin constructs) and in vivo bioavailability of hydrocortisone (HC), in comparison with a standard pharmacopoeial vehicle. The parameters measured in vivo were erythema index (an instrumental human skin blanching assay), transepidermal water loss (TEWL) and stratum corneum hydration. A complex colloidal structure of lamellar liquid crystalline and lamellar gel crystalline type was deduced for sugar surfactant-based vehicles. In dependence on surfactant/water/oil ratio, several thermodinamically variable fractions of water were predicted. Rheological profile of the vehicle appeared to influence the in vitro profile of permeation. A surplus of total amount of drug permeated in vitro from the alkylpolyglucoside-based vehicles coincided with the more pronounced increase of TEWL and less marked blanching action of HC from the selected alkylpolyglucoside-based vehicle tested in vivo, related to the pharmacopoeial one. These findings imply an enhanced delivery of HC from this vehicle and its putative penetration enhancing effect, probably dependent on specific distribution of the vehicle's inherent water.

Keywords: Alkylpolyglucosides; Lamellar gel phase/liquid crystals; Interlamellar water; In vitro/in vivo permeation; Hydrocortisone

1. Introduction

In local therapy, the drugs' diffusion/penetration abilities are governed by properties of the main skin barrier — stratum corneum (SC), and by composition and colloidal structure of the vehicle. The skin hydration level not only affects skin appearance or texture, but also affects its barrier properties, i.e. skin penetration/permeation coefficients for both, the polar and the non-polar actives. Actually, water is commonly called the most natural penetration enhancer (Williams and Barry, 2004).

A vehicle may affect the skin moisture content either through its lipid composition, in terms of an occlusive effect, or by a specific mode of water distribution within the system. The latter is connected to the microstructure of the vehicle, and could be achieved by adjusting a bulk/fixed ("depot") water ratio contained within it (Junginger, 1997; Eccleston et al., 2000; Makai et al., 2003).

The interlamelarlly fixed water, present in topical emulsions stabilized by lamellar gel crystalline and/or lamellar liquid crystalline phases, may serve as a formulation reservoir ("depot") for controlled skin hydration (Junginger, 1997). Besides the putative effects of other formulation ingredients on the SC structure, prolonged skin hydration may contribute to the penetration enhancing effect of a vehicle. In fact, the liquid crystalline structures affect the drug's solubility and chemical potential in both the vehicle and SC (Müller-Goymann and Frank, 1986; Farkas et al., 2000; Nesseem, 2001; Csóka et al., 2005). There is growing evidence on interactions between the solutes of different polarities and the lamellar liquid crystalline structures of vehicles, influencing the drug release and penetra-

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tion (Wilisch and Müller-Goymann, 1993; Carr et al., 1997; Brinon et al., 1999; Müller-Goymann and Alberg, 1999; Refai and Müller-Goymann, 2002; Brinkmann and Müller-Goymann, 2003). Some of these experiments considered in vitro permeation of hydrocortisone (HC) through the excised SC. However, the vehicles of pharmacopoeial quality with anionic mixed emulsifiers were used therein (Müller-Goymann and Alberg, 1999; Refai and Müller-Goymann, 2002; Brinkmann and Müller-Goymann, 2003). Although they may enhance drug penetration, the ionic and polyethoxylated non-ionic surfactants are frequently correlated with cutaneous adverse reactions (Ashton et al., 1992; Bárány et al., 2000). On the other hand, a novel class of so-called natural surfactants has been emerging in recent years. The investigation is particularly focused on sugar surfactants, named alkylpolyglucosides, which can form both, the thermotropic and the lyotropic liquid crystalline phases. In contrary to the ionic or ethoxylated non-ionic traditional surfactants, they are allegedly mild for skin, with large number of hydroxyl groups in the structure, which may provide an additional skin hydration (Stubenrauch, 2001).

In the previous study, a detailed physicochemical characterisation of binary mixtures and multiphase emulsions stabilized with an alkylpolyglucoside mixed emulsifier (cetearyl glucoside and cetearyl alcohol) was performed (Savic et al., 2005). It was intriguing to investigate further how the water distribution mode of vehicles based on this kind of emulsifiers affects the in vitro and in vivo bioavailability of a commonly used topical drug (HC). Therefore, the present study aimed to integrate the physicochemical properties of the three different cetearyl glucoside and cetearyl alcohol-based formulations with in vitro HC (1%, w/w) permeation through the artificial skin constructs (ASC). Finally, an in vivo comparison of HC permeation from a chosen cetearyl glucoside and cetearyl alcohol-based vehicle and a standard pharmacopoeial cream, based on a mixed non-ionic polyethoxylated emulsifier, was carried out on 10 female healthy volunteers. The parameters evaluated prior and upon 24 h treatment under occlusion were: SC hydration (SCH), transepidermal water loss (TEWL) as a measure of skin barrier properties, and a skin erythema index (a skin EI: Schwarb et al., 1999; Lehmann et al., 2001; Levin and Maibach, 2005). Since instrumental testing of corticosteroid-induced skin blanching was recommended as more suitable than subjective visual assessment (Adams and Singh, 1995), the intensity of HC-induced vasoconstriction was estimated by Mexameter® MX 18 (Courage + Khazaka, Germany), an EI meter based on determination of skin haemoglobin (Clarys et al., 2000; Wang et al., 2001). Such application is in accordance with the finding that the haemoglobin amount is a major determinant of the skin redness/blanching (Takiwaki et al., 2002).

2. Material and methods

2.1. Materials

An alkylpolyglucoside non-ionic emulsifier cetearyl glucoside and cetearyl alcohol (kindly provided by Seppic, France), in fixed concentration — 7% (w/w), was used for preparation of three model creams (PL1, PL2 and PL3). A coemulsifier, cetearyl alcohol (1.5%, w/w), was employed for PL3 sample. Due to their moderate polarity, medium chain triglycerides (Miglyol® 812, Hüls, Germany) were considered as a suitable oil phase for model creams, in the following contents: 20% (w/w) in samples PL1 and PL3, and 10% (w/w) in the sample PL2. The samples, properly preserved (0.5%, w/w; Euxyl K 300, Schülke&Mayr, Germany), were prepared with double-distilled water.

The pharmacopoeial non-ionic hydrophilic cream (NHC, DAB 1998), consisting of 10% cetearyl alcohol, 25% white petrolatum, 5% polysorbate 60, 10% glycerol and 50% water, served as a reference sample.

Active samples (PL1-, PL2-, PL3- and NHC-HC) contained 1% (w/w) of micronized HC (Synopharm, Germany), as a model drug.

2.2. Methods

2.2.1. Preparation of samples

Placebo vehicles (PL1–PL3) were prepared as previously described (Savic et al., 2005), whereas NHC was manufactured according to instructions of the German Pharmacopoeia DAB, 1998. Prepared samples were stored for a week prior to the physicochemical investigation.

In order to obtain homogeneous distribution within the creams, HC was suspended using a stirrer (Unguator, GAKO, Germany) at 1000 rpm for 2 min at room temperature. Active samples, left for 3 days to equilibrate, were used for further experiments.

2.2.2. Microscopy

Samples were examined microscopically in bright field and between crossed polarizers using the photomicroscope with λ plate (Zeiss, Type III, Oberkochen, Germany). In addition, transmission electron microscope (TEM) micrographs (Leo, Germany) of sample replicas (freeze fracture technique) were taken.

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

To obtain structural information on the 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.

2.2.4. Rheological measurements

Continual and oscillatory measurements, as well as yield value determination were performed with placebo samples (CSR/CSS Rheometer Bohlin Instruments, Germany).

The following conditions were used for all measurements in triplicate: cone and plate measuring system (diameter 40 mm, angle 1°), with thickness of sample of 0.030 mm, at 20 ± 0.1 °C.

During continual testing, controlled shear rate procedure was applied (shear rate 0.29–200 s^{-1} and back again to the start point, each stage lasting 120 s).

Yield value determination was carried out by continually applied controlled shear stress to the sample within the measuring range 0–100 Pa.

Oscillatory measurements were conducted in order to determine the linear viscoelastic region of the sample (amplitude sweep), at constant frequency of 1 Hz and amplitude sweep ramp from 0.5 to 100 Pa. A frequency sweep ramp from 0.1 to 10 Hz was performed at constant shear stress (6 Pa), which was within the previously marked linear viscoelastic region for all the samples. Yield stress value and apparent viscosities (η_{min} and η_{max}), storage (G') and loss (G'') modulus, as well as phase angle (δ), were employed for rheological characterisation of the investigated samples.

2.2.5. Thermogravimetric analysis (TG)

Aiming to differentiate between bulk and potentially fixed (interlamellar or "depot") water, thermogravimetric analysis was conducted using a TG 220 with disk station 5200 H (Seiko, Japan). The measurements (in triplicate) were performed with open aluminium pans in temperature range 20–100 °C, with a heating rate of 2 °C/min.

2.2.6. In vitro permeation studies through ASC

ASC was cultivated according to the previously described procedure (Specht et al., 1998; Winkler and Müller-Goymann, 2002). The dermis equivalent was produced using human dermal fibroblasts from newborn foreskin specimen. The dermis was covered with an epidermis equivalent employing keratinocytes from stable HaCaT-line.

The in vitro permeation studies were carried out with modified Franz cells ($n = 6$). The donor compartment was filled with the formulation (infinite dose), whereas the acceptor phase was phosphate buffer pH 7.4, permanently homogenized with rotating magnet (400 rpm). The acceptor compartments of the Franz cells were mounted in a water bath at 37 °C, thus the temperature of ASC membrane was 32 °C. Aliquots of 250 μl were taken over 30 h, and the HC assay was performed by high-performance liquid chromatography (HPLC, Waters, Eschborn, Germany), according to Refai and Müller-Goymann (2002).

Taking into account HC saturation concentrations (C_s) within different vehicles, flux of the drug (J, slope of the permeation curve in g/cm^2/s)), as well as the permeation- and per-sol coefficients (P and Z, respectively) were calculated according to Refai and Müller-Goymann (2002).

2.2.7. Determination of HC-C_s

A series of different HC dilutions within specified vehicles was prepared for each formulation and stored for 3 days to equilibrate. Afterwards, the samples were checked for presence of HC crystals, using the polarization microscope. The HC concentration at which crystals were firstly detected was taken as C_s for the given vehicle.

2.2.8. In vivo HC permeation — skin blanching test

In vivo permeation of HC was assessed through the skin blanching response, using EI measurements (Mexameter® MX 18) prior to and 60 min upon cessation of 24 h-occlusive treatment. Ten female volunteers (34.9 years, range 26–48) were included in assay (written informed consent). In parallel with the EI, TEWL (Tewameter® TM 210) and SCH (Corneometer® CM 825, Courage + Khazaka, Germany) were measured.

All subjects were with healthy skin without known allergy to any ingredient of the samples. Also, participants were instructed not to use any skin cleansing or skin care products at the test sites a week before the study and during the experiment. Subjects rested for 30 min before the measurement at room conditions (21 ± 1 °C and 50 ± 5% RH). A flexor side of each forearm was treated with placebo and the corresponding active sample (left arm: PL1 and PL1-HC; right arm: NHC and NHC-HC), using precisely delineated and marked cardboard ruler (with three empty spaces in the form of rectangles, 9 cm^2 each). The third site on each arm was left as non-treated control under (left arm, NCO) and without occlusion (right arm, NCWO). All occluded sites were covered with Parafilm® and, superficially, with cotton wraps. Samples were applied in quantities of 0.016 g/cm^2. All parameters were measured according to valid guidelines and documents (Adams and Singh, 1995; Fullerton et al., 1996; Berardesca, 1997; Clarys et al., 2000; Rogiers, 2001).

2.2.9. Statistical analysis

Whenever applicable, data are given as mean ± S.D. Parameters from in vivo experiment (EI, TEWL, SCH) are expressed as percent change, second versus first day, and plotted as vertical bars with medians, 25th and 75th percentile (10th and 90th percentile as error bars).

In vitro permeation data were compared by Student t-test for independent samples. In vivo effects (EI, TEWL, SCH) of placebo (PL1, NHC) and active samples (PL1-HC, NHC-HC) were compared mutually and related to non-treated controls, under and without occlusion (NCO and NCWO), using the Wilcoxon matched paired signed rank test. Statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Colloidal structure of vehicles

A lyotropic interaction of lamellar type was noticed in all three samples (PL1–L3) stabilized with the alkylpolyglucoside (Fig. 1). The structures observable at polarization micrographs are defined as distorted Maltese crosses, indicating the liquid crystalline or gel crystalline phase of lamellar type (Fairhust et al., 1998; Müller-Goymann, 2002).

A deeper insight into the structure of colloid systems is enabled by TEM micrographs (Fig. 2a and b) and WAXD profiles (Fig. 3a–c). In the cream sample PL1, a complex colloidal structure is clearly visible at droplets' surface and within continual phase of system (Fig. 2a), whereas Fig. 2b shows a planar arrangement of lamellar sheets. This kind of microstructure is comparable with the structure model described for the NHC

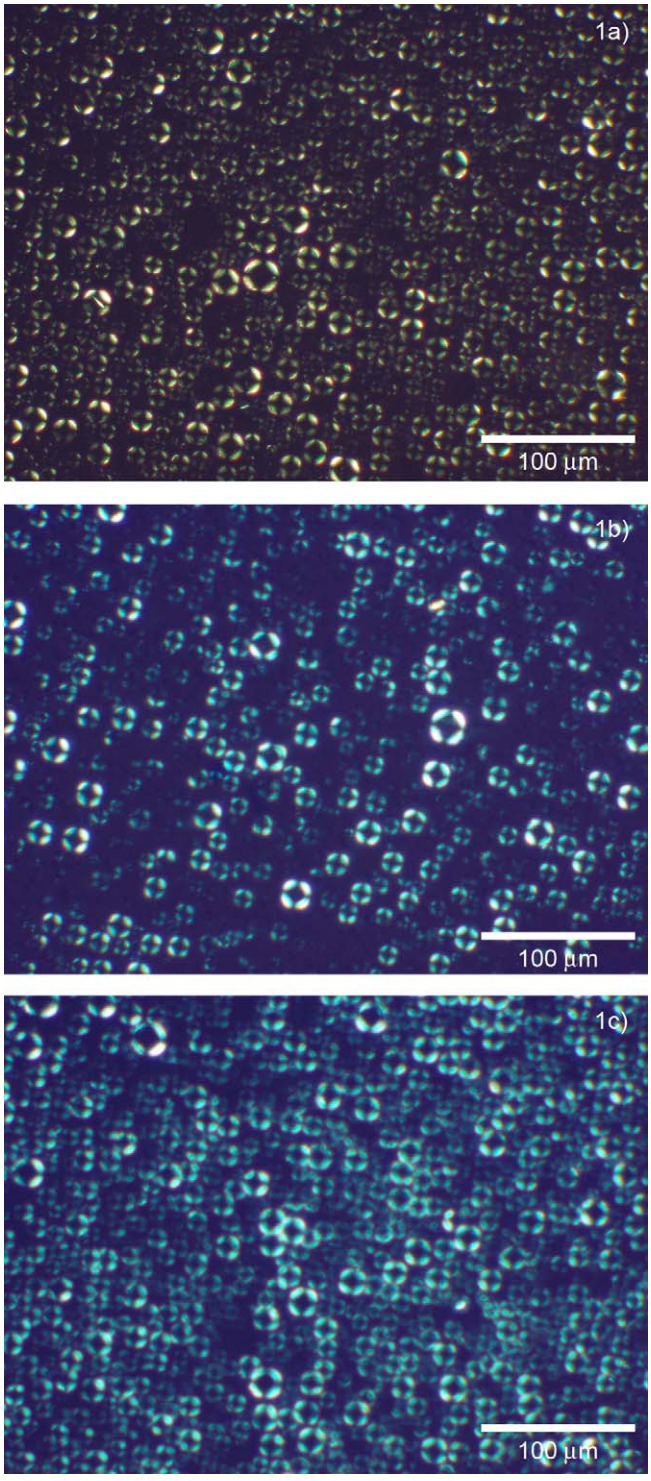

Fig. 1. Polarization micrographs of vehicles based on cetearyl glucoside and cetearyl alcohol: (a) PL1; (b) PL2; (c) PL3; bar 100 µm.

cream (Niedner and Ziegenmeyer, 1992). Generally, the two water fractions are observed in the vehicles stabilized with traditional ionic or ethoxylated non-ionic mixed emulsifiers: bulk (free) water and interlamellar water fixed between lamellae existing in gel crystalline state (Junginger, 1997; Eccleston et al., 2000). To this end, a similar stabilization mechanism could have been expected for vehicles based on sugar surfactants.

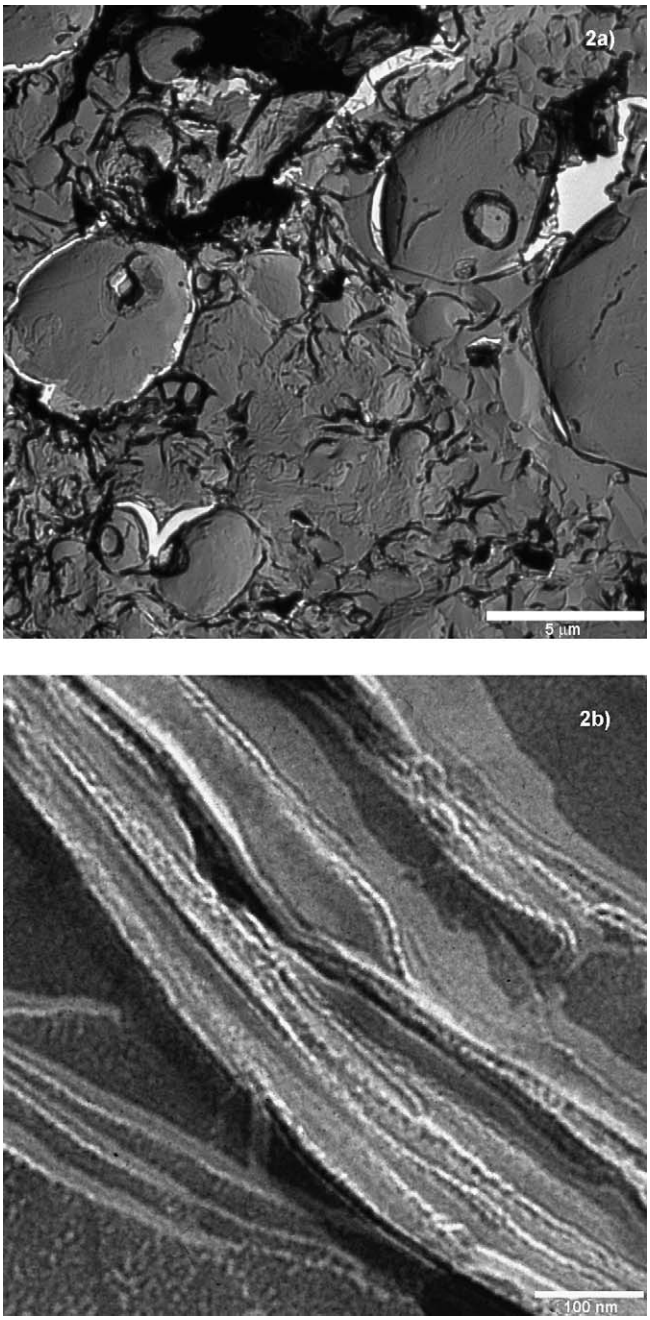

Fig. 2. TEM micrographs of the placebo sample PL1: (a) bar 5 µm; (b) bar 100 nm.

Nevertheless, the WAXD graph of the sample PL1 suggests a more complex structure. As it is well established, there are two main diffraction characteristics of hydrocarbon chains in liquid state: a diffuse halo in the wide-angle region with its centre at 0.45 nm, and the additional patterns within the small-angle region (Fairhust et al., 1998). Opposite to the liquid crystalline phase (L_α), the α-crystalline gel phase (L_β) is characterised by a single sharp reflection at 0.415–0.42 nm (Fairhust et al., 1998). The distinct single sharp reflections at 0.415 nm (sample PL1, Fig. 3a) and at 0.416 nm (samples PL2 and PL3, Fig. 3b and c) confirm the predominant presence of α-crystalline gel phase within these systems. However, overlapping of the single sharp

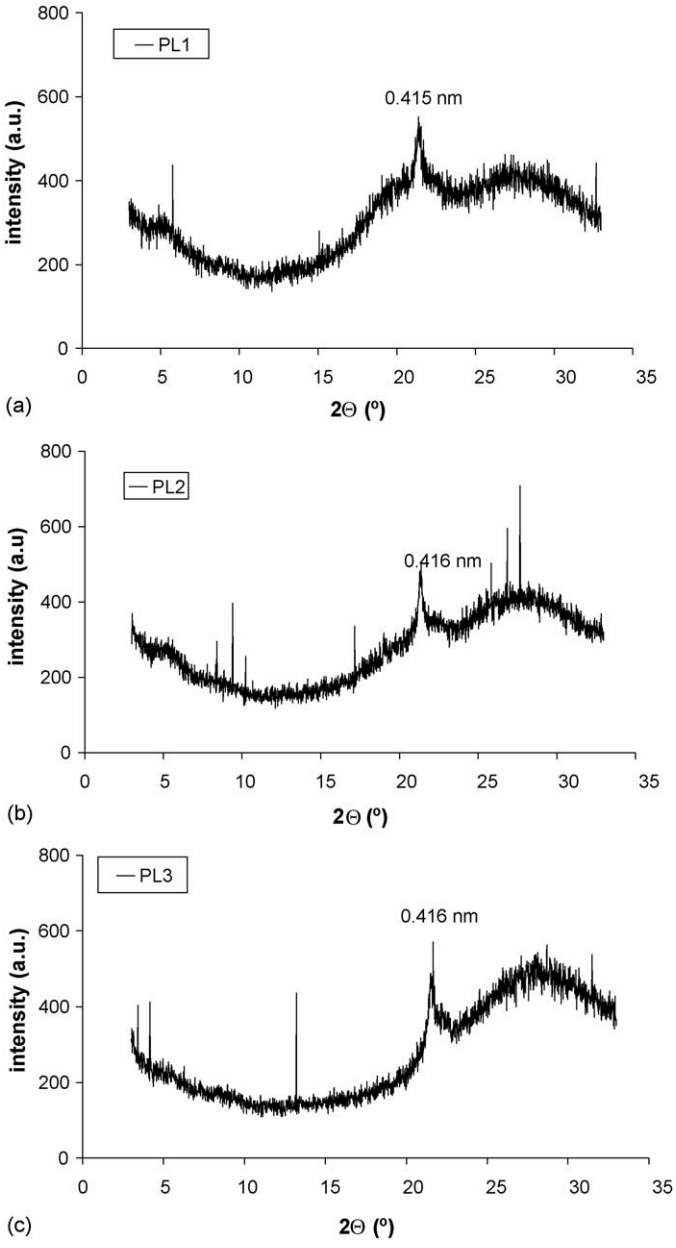

Fig. 3. WAXD patterns of vehicles based on cetearyl glucoside and cetearyl alcohol: (a) PL1; (b) PL2; (c) PL3.

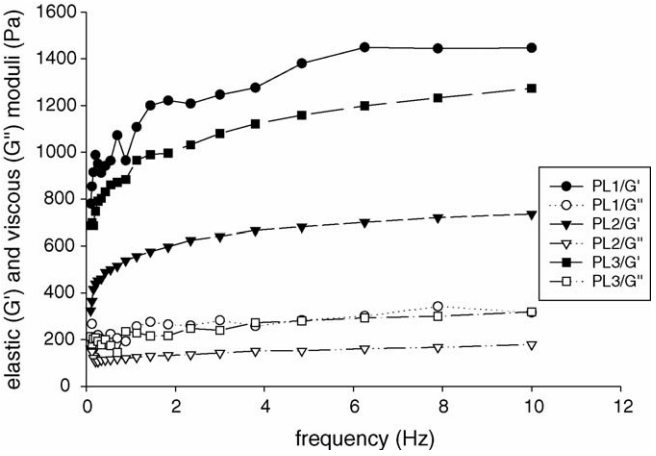

Fig. 4. Rheological oscillatory profiles (at frequency 0.1–10 Hz) of vehicles PL1, PL2 and PL3: G' and G'' modulus.

peak with the diffuse band, detected in the sample PL1, but not in two other samples, implies an equilibration of L_α and L_β lamellar phase.

These findings should be related to rheological measurements (Table 1, Fig. 4). In comparison with samples PL2 and PL3, the sample PL1 exerted the highest yield value, as a sign of the more pronounced elastic behaviour (Table 1). Indeed, the elastic modulus in the sample PL1 was half an order of magnitude (5.5-fold) greater than the viscous modulus, whereas in samples PL2, PL3 and NHC this ratio was 4.5, 4.2 and 2.3, respectively (Table 1). In accordance with Robles-Vasquez et al. (1993), the high viscoelasticity of the sample PL1 could be attributed to L_α. Hence, as already implied by WAXD measurements, this vehicle may exist as the equilibrium of L_β and L_α phases. It is obvious that a higher content of the oil phase of moderate polarity (20% in PL1 versus 10% in PL2) may contribute to phase transition from L_β to L_α state. This could have enlarged the solubilization capacity of the formulation, but such a potential has not been manifested through the respective C_s values (Table 3). On the other side, addition of a fatty amphiphile (cetearyl alcohol in PL3) may alter the mode of the molecule packaging within the lamellar sheets in favour of the gel crystalline phase. Besides the WAXD results, this supposition stems from a distinct decrease of elastic modulus (Table 1, Fig. 4) and a slight increase of apparent viscosities (Table 1) in the sample PL3, in comparison with the sample PL1. These subtle differences in structural organization of lamellae may affect water distribution within the vehicles and its thermodynamic properties.

A comparison of DTG (derivative TG) curve profiles for PL1 and NHC shows that water has evaporated from PL1 in several steps within the second and third temperature stage (50–70 and 70–100 °C, respectively) (Fig. 5, Table 2). Such DTG profile corresponds well with the findings of WAXD and rheological measurements and implies a complex microstructure, with more than two portions of water those were described in NHC (Niedner and Ziegenmeyer, 1992). PL1 lost 21.35% of weight in the first stage, then 39.47% in the second and 13.75% in the third stage (Fig. 5a, Table 2). Similarly, water evaporated in a discontinued manner from PL2 and PL3, but peaks were shifted towards lower temperatures and less percentages of water were lost within the last temperature stage (Table 2), suggesting that these samples have less interlamellarly fixed water disposable than the sample PL1. The DTG profile of NHC revealed the less marked steps of water loss (Fig. 5b), which could point at the less complex colloid structure. However, when analyzing the specified temperature ranges (Table 2), it is obvious that almost 90% of evaporated water (second and third evaporation stage) is fixed in some manner. It seems that NHC contains water interlamellarly fixed mostly between lamellae in gel crystalline state.

Based on the presented results of physicochemical characterisation, we could hypothesize that up to the four fractions of water, in variable proportions, could be expected to exist in

Table 1
Flow and oscillatory (at frequency of 1 Hz) parameters of vehicles based on cetearyl glucoside and cetearyl alcohol (PL1, PL2 and PL3) and of the pharmacopoeial vehicle (NHC)

Sample/placebo	η_{min} (Pa s) D 200 s^{-1}	η_{max} (Pa s) D 1.3 s^{-1}	Yield value (Pa)	δ (°)	G' (Pa)	G'' (Pa)
PL1	0.46 ± 0.06	12.46 ± 0.76	18.38 ± 0.98	13.0 ± 0.12	1407.70 ± 38.98	256.10 ± 19.23
PL2	0.33 ± 0.13	14.718 ± 0.79	5.64 ± 0.34	12.6 ± 0.18	555.93 ± 21.12	124.76 ± 10.08
PL3	0.52 ± 0.09	14.62 ± 0.27	7.15 ± 0.11	13.3 ± 0.09	965.89 ± 17.65	228.48 ± 11.07
NHC	1.48 ± 0.18	121.98 ± 7.98	91.89 ± 5.89	23 ± 0.56	12737 ± 109.91	5411.10 ± 67.91

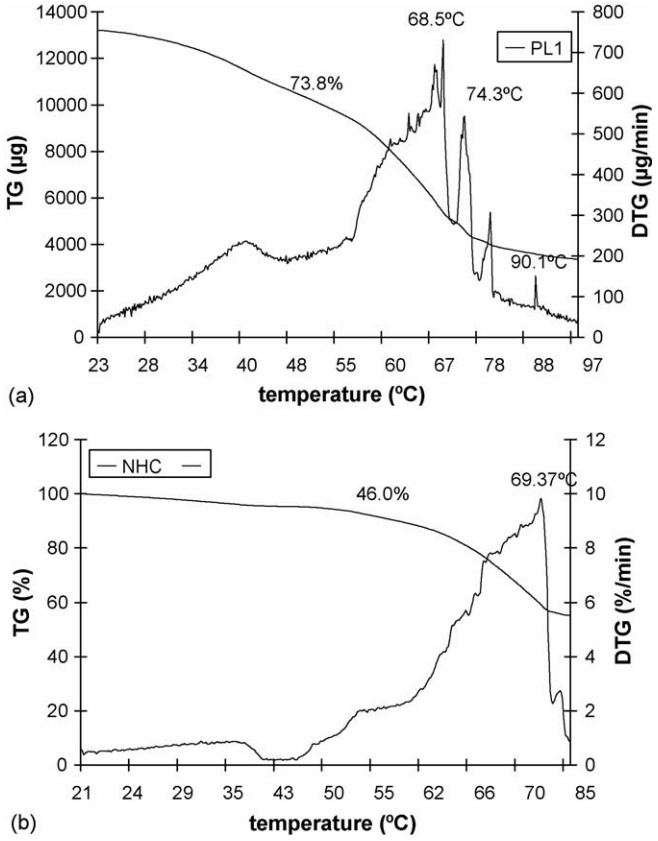

Fig. 5. TG and DTG-curve profiles of placebo samples: (a) PL1; (b) NHC.

vehicles based on the sugar surfactant: (1) free/bulk water, (2) water bonded within lipophilic gel phase ("secondary" water), (3) interlamellar water fixed between gel crystalline lipid bilayers (hydrophilic gel phase) and (4) water fixed between lamellae in liquid crystalline state. The last two fractions could be predominantly present in PL1, and in less percentages in PL2 and PL3. Moreover, some additional suppositions may arise: (1) as the oil content increases there are more viscoelastic lamellae within the vehicle, and (2) such kind of structure retards water evaporation, potentially providing prolonged skin hydration.

The distinct pattern of water distibution in vehicles based on the sugar surfactant, discerned by physicochemical characterisation, may impart bimodal influence on the drug delivery to the skin. It may affect the drug diffusion through the vehicle and/or govern skin hydration, changing its permeability. Both of these mechanisms could be significant for a drug's penetration/permeation profile.

Table 2
Percentage of partial weight loss (TG) over the specified temperature ranges for vehicles based on cetearyl glucoside and cetearyl alcohol (PL1, PL2 and PL3) and for the pharmacopoeial vehicle (NHC)

Sample	20–50 °C (%)	50–70 °C (%)	70–100 °C (%)
PL1	21.35 ± 1.12	39.47 ± 1.37	13.75 ± 0.78
PL2	21.62 ± 1.01	51.62 ± 1.28	10.11 ± 1.17
PL3	22.93 ± 1.03	44.11 ± 0.89	7.62 ± 0.29
NHC	5.18 ± 0.23	27.86 ± 0.54	11.79 ± 0.11

3.2. In vitro HC permeation through ASC

Results of HC permeation across ASC are given in Fig. 6 and in Table 3. The content of the oil phase did not influence HC permeation, i.e. there was no significant difference in HC permeated through ASC from PL1 and PL2 during the first 10 h, as well as after 24 h of permeation (Fig. 6). Accordingly, the corresponding values of flux and permeation coefficients were very close. In line with saturation concentrations of HC in these two samples (2.50×10^{-4} g/cm^3 in PL1 and 5.07×10^{-4} g/cm^3 in PL2, Table 3), the per-sol coefficient for the first sample was twice higher. Concerning PL3, a significant increase in the permeated drug in the first 6 h was recorded, compared to PL1 and PL2 (Fig. 6), and was accompanied by the higher flux and permeation coefficient (Table 3). Though non-significant, a trend of difference was kept throughout the experiment. On the other side, a maximum of HC permeation from the sample NHC was

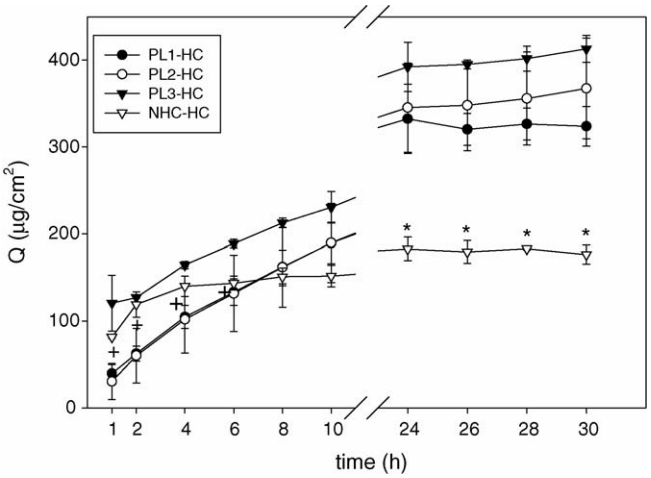

Fig. 6. In vitro permeation profiles of 1% (w/w) HC from different vehicles (PL1 vs. PL2 vs. PL3 vs. NHC) through ASC. Q = permeated amount/area (µg/cm^2). Bars = ±S.D. $^{+}p < 0.05$ PL1- and PL2-HC compared to PL3-HC; $^{*}p < 0.05$ NHC-HC compared to PL1-, PL2- and PL3-HC.

Table 3
Saturation concentration (C_s), flux (J), permeation (P) and per-sol (Z) coefficients of HC from 1% samples based on cetearyl glucoside and cetearyl alcohol (PL1-, PL2- and PL3-HC) and from the pharmacopoeial vehicle (NHC-HC)

Sample	C_s (g/cm^3) × 10^{-4}	J (g/cm^{2}s) × 10^{-9}	P (cm/s) × 10^{-7}	Z (cm/s) × 10^{-5}
PL1-HC	2.50	5.24 ± 0.42	5.25 ± 0.41	2.10 ± 0.24
PL2-HC	5.07	5.27 ± 0.52	5.19 ± 0.52	1.04 ± 0.18
PL3-HC	4.88	6.39 ± 0.71	6.56 ± 0.78	1.31 ± 0.15
NHC-HC	2.43	4.21 ± 0.34	4.32 ± 0.38	1.73 ± 0.19

nearly achieved after the 6th hour, whereas the drug continued to diffuse intensively from the alkylpolyglucoside-based vehicles. Thus, the cumulative quantity of HC permeated through ASC in 24 h was significantly higher for the alkylpolyglucoside-based vehicles (Fig. 6).

These results are only partially in line with recent findings on HC permeation through the excised human SC, which suggested that, in a suspension vehicle, the factors affecting drug release, such as vehicle composition, drug solubility in the base and its viscosity, do not play any role in HC permeation (Refai and Müller-Goymann, 2002). When relating our permeation results with the rheological findings, it appears that slight differences in rheological profiles are not of decisive influence in case of homologous formulations (PL1–PL3), at least in the later phases of permeation. The NHC, an o/w non-ionic cream as well, exerted a distinct rheological profile (higher yield value, higher storage as well as loss modulus, and higher viscosity under shear, Table 1) accompanied with specific permeation profile (Fig. 6). Thus, the rheological differences of formulations should be considered as important ones, at least in our settings. On the other side, in context of vehicle–solute interactions, the penetration rate of a topical drug should be dependent only on its thermodynamic activity within the vehicle, if specific penetration enhancement effects are absent (Bach and Lippold, 1998). Nevertheless, HC-C_s found in our samples (Table 3) could not consistently explain differences in permeation behaviour during the first 6 h, but do comply with the final quantities of drug permeated after 24–30 h (Fig. 6).

Considering the choice of the membrane, it is primarily dictated by purpose of the in vitro experiment. For instance, Cross et al. (2001), studying HC penetration, identified the silicone membrane as a simple predictive model to dissociate vehicle–solute effects on permeation from vehicle–skin interactions. Numerous studies established validity of ASC for in vitro quantification of skin permeation (Schmook et al., 2001; Winkler and Müller-Goymann, 2002; Ponec, 2002). ASC may provide reproducibility that is, due to inter- and intra-individual variations, hardly reached with excised human skin or SC. Although the same rank of the permeation profiles through ASC and human SC was recorded for some drugs, it is known that barrier properties of ASC are less developed (Schmook et al., 2001). Nevertheless, Ponec (2002) brings out that, despite deviations in tissue homeostasis and barrier properties, skin models provide a promising means for studying the effects of topically applied chemicals. Consequently, different HC in vitro permeation profiles from NHC and PL1 (Fig. 6) could be explained not only by the vehicle–solute (rheological and thermodynamic parameters), but also by the vehicle–ASC interactions. This supposition may explain higher total quantity of HC permeated from PL1 across ASC, after 24 h (Fig. 6). It implies certain penetration enhancer effect of the vehicle PL1, apart from putative controlled skin hydration. Such effect should be checked through an in vivo pharmacodynamic activity assessment (Schwarb et al., 1999; Lehmann et al., 2001).

3.3. In vivo HC penetration — skin blanching test

PL1 (with 7% of alkylpolyglucoside and 20% of Miglyol® 812), which has exhibited a satisfying rheological profile and the highest percentage of interlamellar water, was chosen for in vivo comparison with the pharmacopoeial cream. Changes of measured parameters (EI, TEWL, SCH) related to the basal values are given in Fig. 7a–c, respectively, whereas the corresponding p-values are shown in Table 4. Regarding the blanching effect, both active samples significantly decreased the EI, compared to NCO. However, when related to the corresponding vehicles, the vasoconstrictive effect of HC was of only borderline significance. This could be partly explained by clear tendencies of both vehicles to decrease EI (Fig. 7c and Table 4). Median values of EI changes for these two vehicles (−9% and −7%, respectively) correspond well with the result of Lehmann et al. (2001), given for an amphiphilic cream, which has changed the chromame-

Table 4
Statistical analysis (p-values of the Wilcoxon test) of in vivo parameters (transepidermal water loss (TEWL), stratum corneum hydration (SCH), erythema index (EI)) measured at sites treated with placebos based on cetearyl glucoside and cetearyl alcohol (PL1) or pharmacopoeial vehicle (NHC) and with the corresponding active samples (PL1- and NHC-HC), compared mutually and related to the non-treated control under occlusion (NCO)

	TEWL			SCH			EI		
	NCO	PL1	NHC	NCO	PL1	NHC	NCO	PL1	NHC
NCO	–	0.049	0.375	–	0.232	0.014	–	0.020	0.020
PL1-HC	0.020	0.375	–	0.027	0.010	–	0.004	0.084	–
NHC-HC	0.557	–	0.105	0.002	–	0.160	0.004	–	0.049

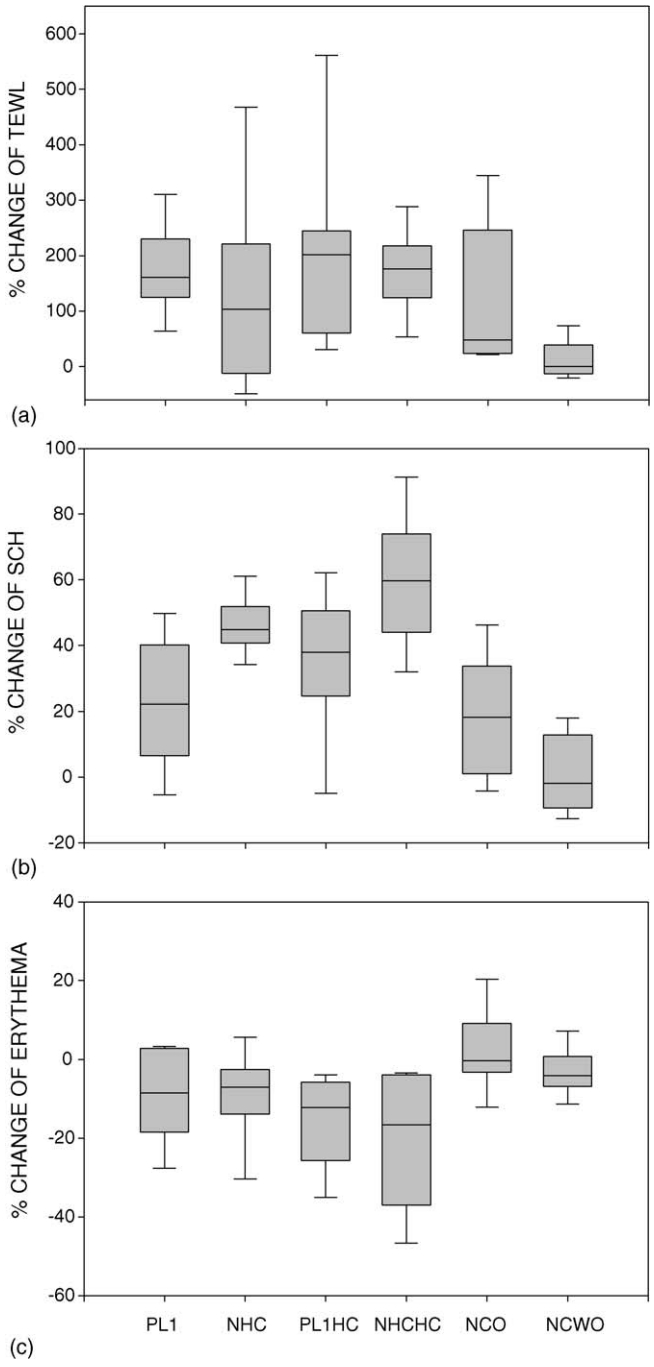

Fig. 7. Influence of placebo (PL1 and NHC) and active samples with 1% HC (PL1-HC, NHC-HC) on (a) TEWL, (b) SCH and (c) EI. Parameters are expressed as percent change second vs. first day, and plotted as vertical bars with medians, 25th and 75th percentile (10th and 90th percentile as error bars). The effects of placebo and active samples were compared mutually and related to NCO and NCWO, using the Wilcoxon test.

try score with a median of −11%. Somewhat less pronounced skin blanching produced by HC from PL1 (Fig. 7c) could be connected, at least partly, with a massive permeation of drug through the skin (in vitro results for ASC, after the 8th hour), and its shorter retention within the dermal layer. Namely, the maximum blanching effect for most of the topical corticosteroids is reached between the 10th and 18th hour after application (Haigh et al., 1997), suggesting that the observed HC effect after 24 h of occlusion was in its waning phase, particularly for PL1.

The TEWL values increased with all placebo and active samples as well as at NCO (Fig. 7a). As skin occlusion generally produces an increase of SCH and alterations of barrier permeability (Zhai and Maibach, 2001; Levin and Maibach, 2005), a certain increase of TEWL was expected. It is known that an increase in TEWL reflects the impairment of the skin barrier (Levin and Maibach, 2005). However, in our study, significance of the effect was reached only at sites treated with the placebo PL1 and the active PL1-HC. This corresponds well with significantly higher total quantity of HC permeated after 24 h across ASC from PL1-based cream. It is known that both TEWL and percutaneous absorption rates increase when the integrity of the SC barrier is compromised (Levin and Maibach, 2005). On the other side, SCH also increased at all treated sites as well as in non-treated occluded control, but significantly only in case of pharmacopoeial formulation NHC and its corresponding active sample (Fig. 7b). Presence of petrolatum and glycerol in NHC (25% and 10%, respectively) might have improved SCH (Lodén et al., 2001). It was shown that petrolatum can enter the human skin and even get incorporated within the intercellular lamellar bilayers of SC, thus assisting barrier integrity and skin hydration (Ghadially et al., 1992). A rapid improvement in barrier function exerted by petrolatum is mainly due to the interactions restricted to SC (Mao-Qiang et al., 1995). These findings underline our supposition that lack of significance of TEWL-increasing action at sites treated with NHC and NHC-HC is predominantly linked with the occlusive effect, either from the wrapping or from the non-polar lipophilic ingredient, petrolatum, itself. Also, this could be a part of explanation for lower total permeation of HC from the NHC-based cream across ASC. On the other hand, Gloor and Gehring (2003) found that an NHC formulation without glycerol produces significant increase of TEWL after a week of treatment. This part of evidence could underline a role of glycerol as a moisturizing and barrier repairing agent in topical formulations (Lodén et al., 2001). Taking into account the barrier repairing effects of petrolatum and glycerine, it appears that NHC has an ambivalent activity in terms of the vehicle's penetration-modulating effects.

Looking strictly at the in vivo results, both active samples produced a satisfying pharmacodynamic response, confirming percutaneous penetration of HC. However, the alterations in TEWL and SCH imply differential types of vehicle–skin interactions, respective of formulations' composition and physicochemical characteristics. The insignificant SCH improvement, gathered with a significant TEWL increase (PL1), could augment skin permeability for the actives to a greater degree than an opposite influence on these skin parameters (NHC). This difference in HC permeation profiles could be connected with the fact that SCH does not lead to an overall decrease in intercellular lipid order. Namely, it was suggested that hydration-induced disordering is limited to a small population of SC lipids (Gay et al., 1994). This is particularly interesting if it is known that HC belongs to a class of drugs diffusing mainly via lipid routs across the SC (Barry and Bennet, 1987).

In line with the previous statement, it seems that Miglyol® 812 from PL1 (20% of medium chain triglycerides), as moderately polar oil, influences the skin barrier, mostly through the interactions with the SC lipid bilayers, mainly in their hydrophobic parts (Suhonen et al., 1999), possibly contributing to HC permeation. This conclusion could be strengthen by results of a long-term in vivo study (Savic et al., 2004), which discerned a favourable hydration and barrier repair potential of vehicles based on the same sugar surfactant as in the present study, but with the more complex composition of the oil phase, including only 3% (w/w) of medium chain triglycerides. Hence, it is not highly probable that the sugar surfactant on its own undesirably affects the skin barrier. Nevertheless, a more focused study on the in vitro and in vivo interactions of commonly used pharmaceutical excipients, incorporated in vehicles based on sugar surfactants, is needed to be performed.

4. Conclusions

The present physicochemical characterisation of the three vehicles based on a sugar surfactant showed that these natural surfactants are promising tools in tailoring vehicles with complex colloidal structure of lamellar liquid crystalline and lamellar gel crystalline type. It appeared that, in dependence on surfactant/water/oil ratio, such a colloidal structure may harbour four thermodinamically variable fractions of water: (1) free/bulk water, (2) water bonded within lipophilic gel phase (“secondary” water), (3) interlamellar water fixed between gel crystalline lipid bilayers and (4) interlamellar water fixed between lipid layers in the liquid crystalline state. This feature, accompanied with a desirable rheological profile compared to a pharmacopoeial vehicle with traditional surfactants, may predict an eventual official appreciation of vehicles based on sugar surfactants.

The rheological profile of the vehicle and thermodynamic activity of HC as a model drug appeared to influence the in vitro profiles of permeation in an infinite dose approach. A surplus of total amount of drug permeated in vitro from the alkylpolyglucoside-based vehicles, related to the pharmacopoeial one, coincided with the more pronounced increase of TEWL and the less marked blanching action of HC from the selected vehicle (7% of the emulsifier and 20% of medium chain triglycerides) tested in vivo 24 h upon the sample application. These findings imply an enhanced delivery of HC from this vehicle and its putative penetration enhancing effect. The relation between the in vitro and in vivo permeation profiles of HC recommends use of ASC as a reliable tool for permeation studies, particularly in screening of novel types of vehicles.

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