Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmaceutics and Biopharmaceutics 60 (2005) 91–98
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Design of a phytosphingosine-containing, positively-charged nanoemulsion as a colloidal carrier system for dermal application of ceramides

Erol Yilmaz, Hans-Hubert Borchert*

Institute of Pharmacy, Free University of Berlin, Berlin, Germany

Received 24 September 2004; accepted in revised form 1 November 2004
Available online 13 February 2005

Abstract

Positively charged oil/water (o/w) nanoemulsions (PN) are effective vehicles to change the permeability of the skin. This study focused on the preparation and characterisation of phytosphingosine (PS) containing PN (PPN) which serve as colloidal carriers for the dermal application of ceramide IIIB (CIIIB) and the stratum corneum (SC) lipids (PPNSC) such as ceramide III (CIII), cholesterol, and palmitic acid. The investigations were conducted using appropriate emulsification and homogenisation processing conditions to optimise PPNSC with regard to droplet size, physical stability, and solubility of PS, CIII and CIIIB. A decrease in droplet size was observed through eight homogenisation cycles at a pressure of 500 bar and a temperature of 50 °C. Above these optimal values, an increase in droplet size was observed. PS and ceramides have low solubilities in oil and water. When Lipoid E-80® (LE80) was added to the oil phase, the solubility of PS and ceramides increased, indicating some interactions shown by DSC measurements. SC lipids and CIIIB could be successfully incorporated in PPN without producing any physical instability. The high stability of PPNSC is probably due to the presence of a hydrophilic (Tween 80) and a lipophilic surfactant (LE80), supported by the lipophilic cosurfactant PS, at the o/w interface. It was shown that PS was responsible for the positive charge and thus supported the high physical stability of PPNSC. This optimised emulsion was selected for further skin absorption evaluation.

Keywords: Positively charged oil/water nanoemulsion; Dermal application; Lipoid E-80®; Phytosphingosine; Ceramide; Stability of nanoemulsion

1. Introduction

Since all epithelial cells, including the skin, carry a negative charge on their surfaces, due to the presence of negatively charged residues of proteins in the outer membranes of the cells and active ion pumps, they are selective to positively charged solutes [1]. It has been shown that a positively charged nanoemulsion might enhance the permeability of a drug [2], making the development of topically applied positively charged nanoemulsions promising.

In this study, the droplets of the nanoemulsion exhibited their positive surface charge upon a physiological compound, phytosphingosine (PS). PS, a free sphingoid base (2*S*-amino-1, 3*S*, 4*R*-octadecanetriol) with a pK_b of approximately 9, is located at the oil/water interface due to its amphiphilic structure. Upon adjusting the pH lower 9, PS is protonated at the amino-group thus providing the positive charge of the nanodroplets. PS is naturally found in the human body and is present at high levels in the stratum corneum. Most of the published research on PS deals with the inhibition of microorganisms and its biological role as a natural anti-inflammatory agent [3–7]. Because of these properties, PS is considered to be part of the skin's natural defence system. Consequently, PS seems to have a wide range of product applications, which makes it an interesting candidate for topical use.

This investigation was focused on ceramide III (*N*-stearoyl-4-OH-sphinganine), the most abundant lipid in healthy human skin, and ceramide IIIB (*N*-oleoyl-4-OH-sphinganine), a related lipid not found in human skin.

* Corresponding author. Institute of Pharmacy, Free University of Berlin, Kelchstr. 31, 12169 Berlin, Germany. Tel.: +49 30 83850676; fax: +49 30 83850685.
E-mail address: hhb@zedat.fu-berlin.de (H.-H. Borchert).

0939-6411/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2004.11.009

As shown previously, both of these low-solubility ceramides are able to improve the water content + smoothness human skin upon topical application [8].

The main problem in the manufacturing of nanosized oil/water (o/w) emulsions is the physical instability, which appears as phase separation due to creaming (density differences), flocculation (aggregation through interparticle collision), coalescence (fusion of separate droplets), and Ostwald ripening (molecular diffusion degradation which depends on size, polydispersity and solubility of the dispersed phase in the continuous phase). Consequently, it is important to determine the optimal emulsification and homogenisation processing conditions required to obtain nanosized emulsions with high physical stability.

The objective of the present study was to develop and characterise a phytosphingosine containing positively charged oil/water nanoemulsion incorporating ceramides as a new type of dermal formulation prior to its evaluation in healthy human volunteers.

2. Materials and methods

2.1. Materials

Ceramide III (CIII), ceramide IIIB (CIIIB) and phytosphingosine (PS) were kindly provided by Degussa, Essen, Germany Lipoid E-80® (LE80) was obtained from Lipoid KG, Ludwigshafen, Germany, containing 81.5% phosphatidylcholine, 8.5% phosphatidylethanolamine, 2.3% lysophosphatidylcholine, and 2.7% sphingomyeline according to manufacturer's specifications. The cosmetic oil, Eutanol G (octyldodecanol), and the preservative potassium sorbate were purchased from Caelo, Caesar and Loretz GmbH, Hilden, Germany and conform to with European Pharmacopoeia specifications. The antioxidant D,L-α-tocopherol was supplied from Synopharm, Barsbüttel, Germany. Tween 80 (T80) was bought from Uniqema, Everberg, Belgium. All used ingredients were of pharmaceutical grade.

2.2. Emulsion preparation

Aqueous and oil phases were prepared separately. The aqueous phase containing T80, glycerol, potassium sorbate, and bidistilled water, was heated to 25, 50, 75 or 90 °C under slight stirring. PS, CIII and CIIIB were dissolved in Eutanol G above 100 °C and then cooled down to 75 °C. LE80, cholesterol, palmitic acid and α-tocopherol were dissolved in the oil phase and the temperature was set to 25, 50, 75 or 90 °C. At the adjusted temperature, the two phases were merged and prehomogenised with an Ultra-Turrax (Janke and Kunkel GmbH, Staufen, Germany) at 8000 rpm for 3 min, followed by further homogenisation using a high pressure homogeniser (Micron Lab 40, APV Systems, Germany). After rapid cooling to room temperature and adjusting pH (Table 1) with a pH meter (model pH 522, WTW, Germany), the nanoemulsions were filtered through a membrane filter (polytetra-fluorethylene filter, Sartorius AG Germany, pore size 1.2 μm), merged with nitrogen gas and characterised.

PS containing positively charged oil/water nanoemulsion incorporating CIIIB and stratum corneum lipids such as CIII, palmitic acid and cholesterol (Table 1) was prepared by varying the homogenisation parameters (Table 2) in order to optimise these parameters with respect to achieving nanosized emulsions with close size distributions for high physical stability.

The emulsification parameters were varied in order to provide electrostatic and/or steric stabilization to the droplets and improve the solubility of ceramides and phytosphingosine.

Table 1
Nanoemulsion used for optimising homogenisation and emulsification processing parameters

Compound	Content (w/w%)	Property
Oil phase		*Dispersed phase*
Eutanol G	**20**	Liquid lipid
Lipoid E-80	0.5, 1, 1.5, **2**, 3	Lipophilic surfactant
Phytosphingosine	0.2, 0.4, 0.5, **0.6**, 0.7, 0.8	Co-surfactant
Ceramide IIIB	0.1, **0.2**, 0.3, 0.5	Related stratum corneum lipid
Ceramide III	0.1, **0.2**, 0.3, 0.5	Stratum corneum lipid
Palmitic acid	0.1, **0.2**, 0.3, 0.5	Stratum corneum lipid
Cholesterol	0.1, **0.2**, 0.3, 0.5	Stratum corneum lipid
Vitamin E	**0.03**	Antioxidant
Water phase		*Continuous phase*
Tween 80	0.5, 1, 1.5, **2**, 3	Hydrophilic surfactant
Glycerol	**2.5**	Wetting agent
Potassium sorbate	**0.1**	Preservative
Water to	**100**	Bidistilled water
PH	5, **5.5**, 6, 6.5, 7, 8	

Bold numbers indicate the content of the compounds for optimising homogenisation and emulsification processing parameters.

Table 2
Homogenisation processing parameters of the high pressure homogeniser for optimisation of the nanoemulsion

Homogenisation processing parameters	
Homogenisation temperature (°C)	25, **50**, 75, 90
Homogenisation pressure (bar)	300, **500**, 700
Number of homogenisation cycles[a]	1, 3, 5, **8**, 10

Bold numbers indicate the processing parameters, which were kept constant, while the others were varied.

[a] The number of times that the emulsion was pressed through a gap by the piston of the homogeniser.

2.3. Emulsion characterisation

2.3.1. Particle size analysis

The mean droplet size and size distribution were determined by photon correlation spectroscopy with a Malvern Zetasizer 4 (Malvern Instruments, Worcestershire, UK) by diluting 10 μl of the nanoemulsion with 10 ml of an aqueous phase containing 0.1% Tween 80 and 1% glycerol 86–88%. The size distribution was represented by polydispersity index (PI) values PI values lower than 0.25 indicated a close size distribution providing good stability of nanoemulsions due to reduced Ostwald ripening [9].

2.3.2. Surface charge (zeta potential)

The surface charge was determined using a Zetasizer 4 (Malvern Instruments, Worcestershire, UK) by measuring the zeta potential (ZP) of the preparations. This was done by using an aqueous phase containing sodium chloride and hydrochloride and sodium hydroxide to adjust the conductivity (50 μS/cm) (conductivity meter model LF 537, WFT, Germany) and the pH (pH meter model pH 522, WTW, Germany), respectively. ZP characterises the surface charge of particles and thus it gives information about repulsive forces between particles and droplets. To obtain stable nanoemulsions by preventing flocculation and coalescence of the nanodroplets, ZP should usually reach a value above 30 mV [10].

2.3.3. Stability assessment

To reduce testing time, accelerated tests were conducted by storing the emulsions for 2 weeks at 50 °C. To evaluate the long term stability of the optimised emulsion, the zeta potential, the mean droplet size and the size distribution were monitored over a period of 6 months.

2.3.4. Electron cryo-microscopy

Droplets of the sample (5 μl) were applied to a perforated (1 μm hole diameter) carbon film covered 200 mesh grids (R1/4 batch of Quantifoil Micro Tools GmbH, Jena, Germany), which had been hydrophilised before use by 60 s plasma treatment at 8 W in a BALTEC MED 020 device (Baltec, Liechtenstein). The supernatant fluid was removed with a filter paper until an ultra-thin layer of the sample solution was obtained spanning the holes of the carbon film. The samples were immediately vitrified by propelling the grids into liquid ethane at its freezing point (90 K) operating a guillotine-like plunging device using the CEVS humidity and temperature controlled vitrification system (Institute of electron microscopy, FU Berlin, Germany). The vitrified samples were subsequently transferred under liquid nitrogen into a Philips CM12 transmission electron microscope (FEI Company, Oregon, USA) using a Gatan cryoholder (Gatan, Inc., California, USA) and stage (Model 626). Microscopy was carried out at 94 K sample temperature using the microscope's low dose protocol at a primary magnification of 58,300× and an accelerating voltage of 100 kV (LaB_6 illumination). The defocus was chosen in all cases to be 1.2 μm corresponding to a first zero of the phase contrast transfer function at 2.1 nm.

2.4. Thermal analysis

Differential scanning calorimetry (DSC) was conducted in order to characterise the interactions between CIII, CIIIB, PS and LE80. Samples were sealed in 40 μl aluminium pans, which were drilled with a fine hole in the lid and analysed using a DSC 821e (Mettler-Toledo, Giessen, Germany). The samples were preheated at 100 °C for 10 min. After being cooled they were reheated from 30 to 200 °C at a heating rate of 10 K/min. An empty aluminium pan served as reference. The oven was flushed with 80 ml N_2/min.

2.5. Solubility

The solubility of PS, CIII and CIIIB was determined by adding a fixed amount of PS or equimolar amounts of CIII and CIIIB (0.05% interval) to the oil phase of the positively charged nanoemulsion. Solubility was defined as the maximum PS and ceramide concentrations at which neither crystals, crystal needles, nor extreme fine strings were detected using a microscope with polarized light during 1 week (Axioskop, Carl Zeiss Jena GmbH, Jena, Germany).

2.6. Viscosity

The viscosity of the dispersed oil phase was measured using a falling ball-viscosimeter (Haake Technik GmbH, Vreden, Germany). The viscosity (η) was calculated by the following equation

$$\eta = t(\rho_1 - \rho_2)K$$

whereby t is the falling time, ρ_1 (2.392 g/ml) and ρ_2 (0.855 g/ml at 25 °C and 0.840 g/ml at 50 °C), measured with a density meter DMA 38 (Anton Paar GmbH, Graz, Austria) are the density of the ball and the dispersed oil phase, respectively, and K is the instrument constant (0.0586).

2.7. Statistical analysis

Data analysis was carried out with the software STATeasy. Statistically significant differences were determined using Student's t-test with $P < 0.05$.

3. Results and discussion

3.1. Homogenisation processing parameters

The influence of the homogenisation parameters on mean droplet size and size distribution of the phytosphingosine

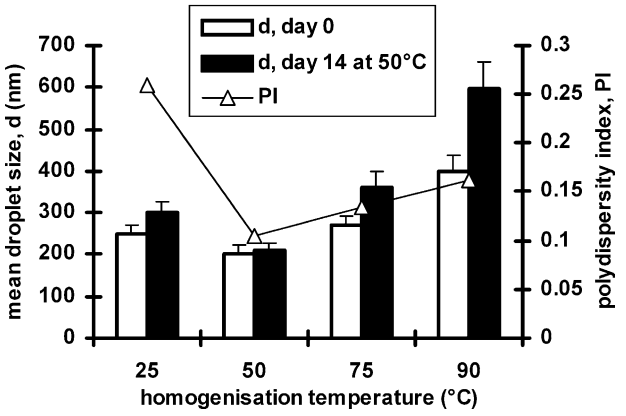

Fig. 1. Effect of homogenisation temperature on mean droplet size and polydispersity index (mean ± SD; $n = 5$).

(PS) containing positively charged oil/water nanoemulsion, which serves as colloidal carrier system for ceramide IIIB (CIIIB) and stratum corneum (SC) lipids (PPNSC), is shown in Figs. 1 and 2. In Fig. 1, it is apparent that the mean droplet size (210 ± 18 nm) is effectively reduced at 50 °C. Decreasing homogenisation energy input by reducing the temperature from 50 to 25 °C led to an increase in viscosity of the dispersed oil phase from 19 to 52 mPa s. Because of the lower energy input during the homogenisation process and the higher viscosity of the dispersed oil droplets, the mean droplet size and the polydispersity index value, measured immediately after production, increased from 210 ± 18 to 299 ± 29 nm and from 0.105 ± 0.019 to 0.260 ± 0.032, respectively, as described by Müller et al. [11]. Thus, a close size distribution could not be achieved, leading to higher extent of Ostwald ripening [9], and consequently, the stability of PPNSC, produced at 25 °C, was low after storage at 50 °C for 2 weeks.

The cloud point of Tween 80 (T80) in water is approximately at 70 [12], and 75 °C, the solubility of the hydrophilic surfactant decreased by dehydration, probably leading to leakage of T80 from the oil/water interface. In addition, the surface area and the interfacial tension

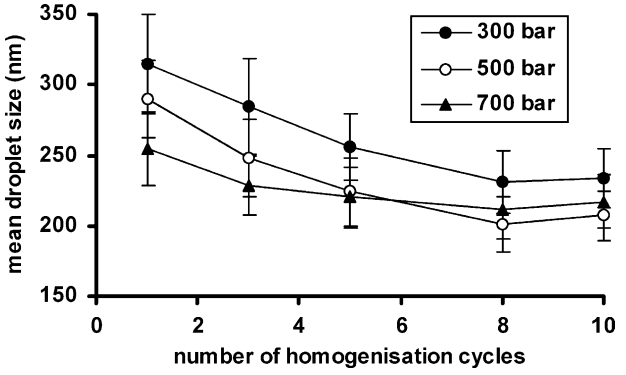

Fig. 2. Effect of number of homogenisation cycles and homogenisation pressure on mean droplet size (mean ± SD; $n = 5$).

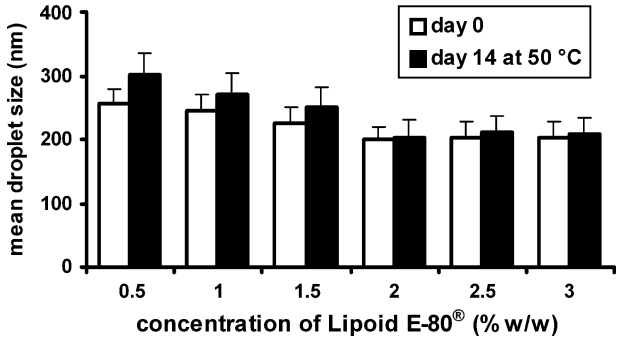

Fig. 3. Effect of Lipoid E-80® on mean droplet size; empty bars represent mean droplet size immediately after production and filled bars show mean droplet size after storage at 50 °C for 2 weeks (mean ± SD; $n = 5$).

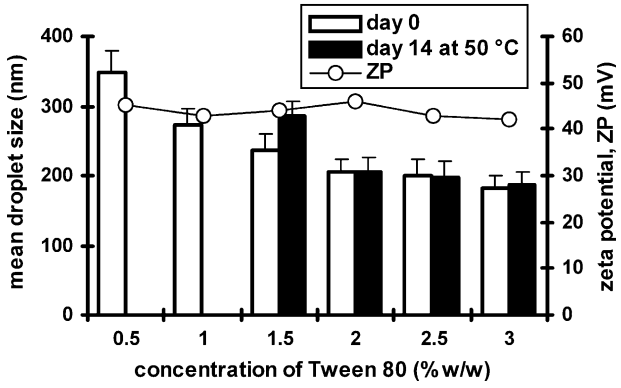

Fig. 4. Effect of Tween 80 on mean droplet size and zeta potential; empty bars represent mean droplet size immediately after production and filled bars show mean droplet size after storage at 50 °C for 2 weeks; if no bars are shown, phase separation occurred (mean ± SD; $n = 5$).

increased because of the high homogenisation energy input. These phenomena resulted in a higher extent of coalescence of oil droplets after their disruption by cavitation prior to coverage with surfactant [11,13,14].

The number of homogenisation cycles and the pressure also had a great influence on the mean droplet size and size

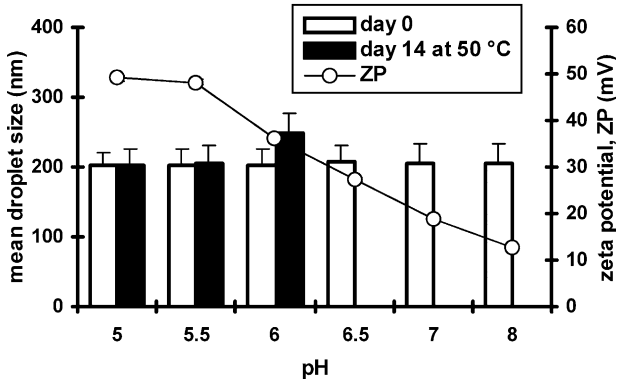

Fig. 5. Effect of pH on mean droplet size and zeta potential; empty bars represent mean droplet size immediately after production and filled bars show mean droplet size after storage at 50 °C for 2 weeks; if no bars are shown, phase separation occurred (mean ± SD; $n = 5$).

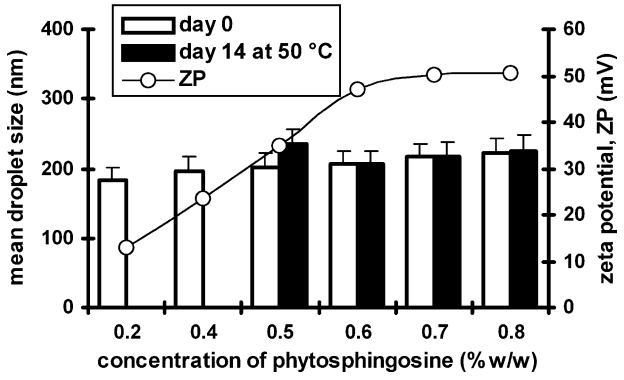

Fig. 6. Effect of phytosphingosine on mean droplet size and zeta potential; empty bars represent mean droplet size immediately after production and filled bars show mean droplet size after storage at 50 °C for 2 weeks; if no bars are shown, phase separation occurred (mean ± SD; $n = 5$).

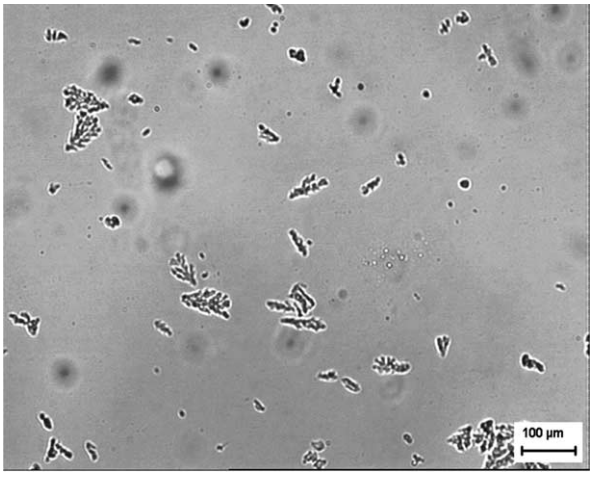

Fig. 7. Microscopic picture of the oil phase of PPNSC without ceramide IIIB and SC lipids containing 0.8% phytosphingosine after storage at room temperature for 1 week (magnification 20×).

distribution (Fig. 2). Upon increasing the pressure from 300 to 500 bar and the number of cycles from 1 to 8, a decrease in mean droplet size (315 ± 35 to 201 ± 19 nm) and polydispersity index 0.281 ± 0.013 to 0.105 ± 0.019 (not shown), occurred at a homogenisation temperature of 50 °C. However, a further increase to 700 bar and 10 cycles led to a slight increase in droplet size to 217 ± 19 nm, probably due to an increase in surface area and, thus, in interfacial tension. This increase in interfacial tension could not be compensated by the composition and the amount of surfactants at the o/w interface leading to coalescence of the nanodroplets [13–15].

3.2. Emulsification process parameters

The emulsions were prepared at the optimised processing conditions: 50 °C, 500 bar and eight homogenisation cycles. The effect of the lipophilic surfactant mixture, Lipoid E-80® (LE80), is shown in Fig. 3. Increasing concentrations from 0.5 up to 2% led to a decrease in mean droplet size from 256 ± 23 to 201 ± 20 nm. Further increases in LE80 concentration did not change the mean droplet size, indicating that at 2% LE-80 the o/w interface was maximally occupied with lipophilic surfactant. Accelerated stability tests at 50 °C for 2 weeks clearly indicated that at least 2% LE80 was necessary to obtain stable nanoemulsions.

The effect of the steric stabilizing hydrophilic surfactant T80 on mean droplet size and the stability is shown in Fig. 4. Increasing the T80 concentration from 0.5 to 3% led to a decrease in mean droplet size from 348 ± 31 to 202 ± 22 nm. At least 2% T80 was needed to obtain stable nanoemulsions indicating maximal occupation of the oil/water interface with T80. Below 2%, the nanoemulsions were unstable, resulting in coalescence and in phase separation. These results showed that T80 had a greater influence on the stability of the nanoemulsion than LE80.

The pH significantly affected surface charge, as determined by measuring zeta potential (ZP), and the stability (Fig. 5). The PPNSC was stable after stress only if the ZP values were at least +40 mV. When pH was decreased from 8 to 5, ZP values increased from +13 to +49 mV. Physiological skin pH values between 5 and 5.5 led to maximal ZP values of +49 and +47 mV, respectively, resulting in high physical stability.

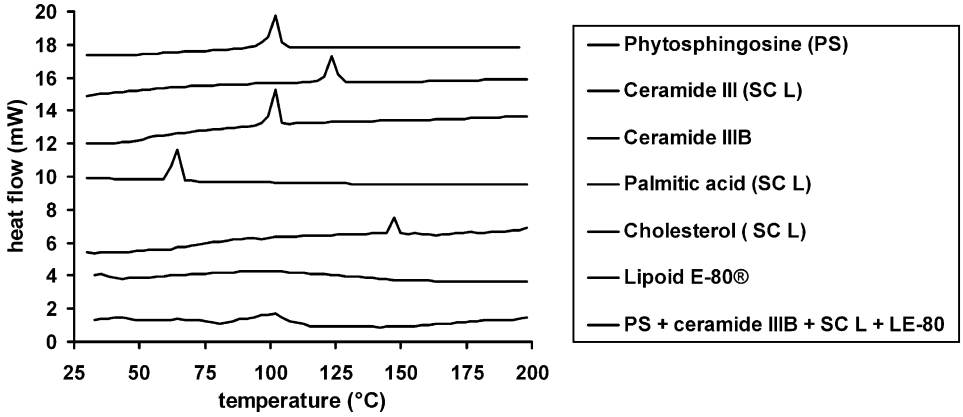

Fig. 8. DSC thermogram of phytosphingosine, ceramide IIIB, SC lipids (ceramide III, palmitic acid and cholesterol), Lipoid E-80®, and their mixture.

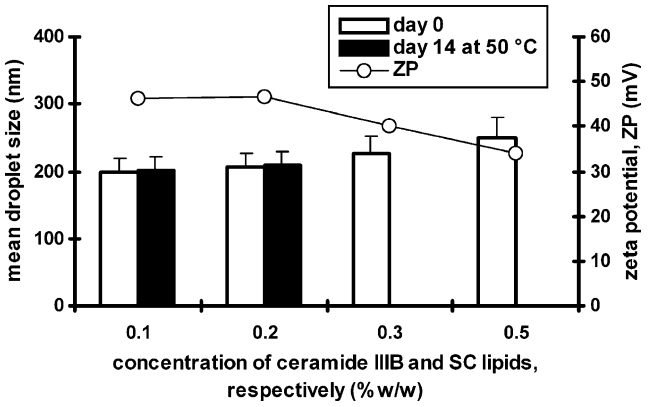

Fig. 9. Effect of ceramide IIIB and SC lipids on mean droplet size and zeta potential; empty bars represent mean droplet size immediately after production and filled bars show mean droplet size after storage at 50 °C for 2 weeks; if no bars are shown, phase separation occurred (mean ± SD; $n = 5$).

Fig. 6 shows the influence of PS on ZP. Increasing concentration of PS from 0.2 to 0.6% led to increase in ZP values from +13 to +47 mV, respectively, showing that PS was responsible for the positive charge and for the good stability. Consequently, stable nanoemulsions after stress were obtained at PS concentration of at least 0.6% leading to ZP values above +40 mV.

3.3. Solubility of phytosphingosine, ceramide III and ceramide IIIB

The solubility of PS in the oil phase of PPNSC without CIIIB and SC lipids was approximately 0.6%. Above this concentration crystals were detected after storage for 1 week at room temperature (Fig. 7) indicating the limit of PS solubility.

CIIIB and SC lipids such as CIII, cholesterol and palmitic acid were incorporated in an equimolar ratio because many studies have shown that the application of only one or two of these lipids to perturbed skin impeded rather than facilitated the rate of barrier repair expressed by an increase of transepidermal water loss (TEWL). In contrast, when all three lipids were applied together, normal rates of barrier repair occurred leading to decrease in TEWL [16,17].

The incorporation of ceramides is a technological challenge due to their very low solubility and their tendency to recrystallisation. The solubility could be improved by LE80. The DSC thermogram (Fig. 8) shows the influence of LE80 on the solubility of PS, CIII and CIIIB. PS, CIII, CIIIB, palmitic acid and cholesterol showed sharp endothermic peaks at 104, 123, 101, 64 and 147 °C, respectively. After heating and recooling LE80 showed no peak. When LE80 was mixed with PS, CIIIB and the SC lipids in the weight ratio 5:3:1:1:1:1 (LE80:PS:CIII:CIIIB:palmitic acid:cholesterol), there were no sharp peaks, only a slight broad peak at 100 °C indicating some interactions between LE80 and the lipids. This interaction led to an increase in the solubilities of CIII and CIIIB in the oil phase of PPNSC from 0.25 to approximately 1% (w/w), respectively, without any recrystallisation.

Consequently, up to 0.2% of CIIIB and of the SC lipids, respectively, could be incorporated in PPNSC remaining stable for at least 2 weeks at 50 °C (Fig. 9) without any crystal formation. (Fig. 10, left hand side). Above 0.2% the formation of needles or fine string-shaped crystals was observed after 3 days (Fig. 10, right hand side) due to the solubility limit of the ceramides. Additionally, when the lipid concentration was increased from 0.2 to 0.3%, ZP values decreased from 47 ± 1 to 40 ± 1 mV (Fig. 9) because ceramide IIIB and SC lipids, possessing also amphiphilic structures, might displace PS from the oil/water interface leading to smaller ZP values.

3.4. Long-term stability

Fig. 11 shows the long-term stability of PPNSC. The mean droplet size of 207 ± 19 nm, the PI value of

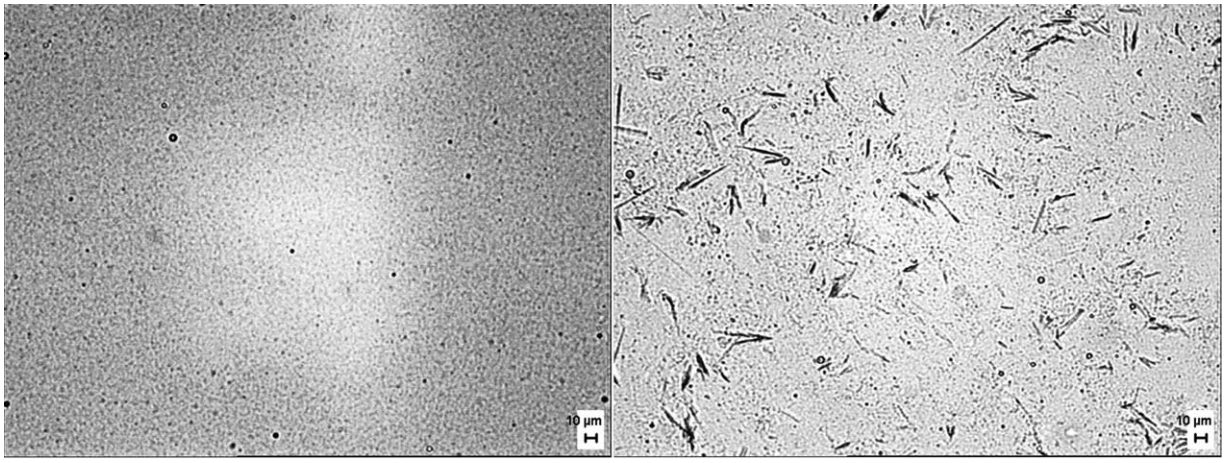

Fig. 10. Microscopic pictures of PPNSC containing 0.2 (left hand) and 0.3% (right hand) of ceramide III, ceramide IIIB, palmitic acid and cholesterol, respectively, after storage at room temperature for 3 days (magnification 10×).

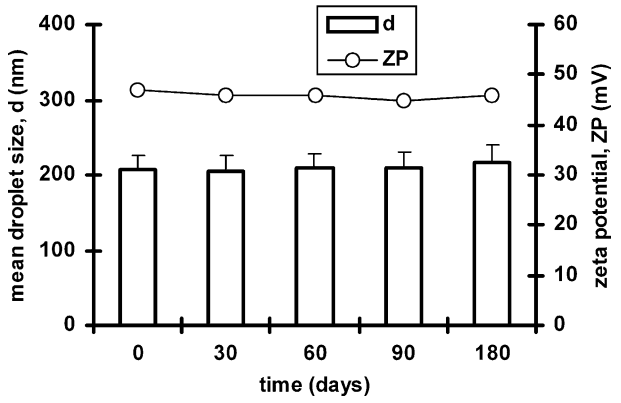

Fig. 11. Mean droplet size and zeta potential after storage of PPNSC over a period of 6 months (mean ± SD; $n = 5$).

0.100 ± 0.013 (not shown in the figure) and the ZP value of +47 ± 1 mV did not change significantly over a period of 6 months indicating the good resistance against Ostwald ripening, flocculation, creaming and coalescence.

3.5. Electron cryo-microscopy

To visualize the nanoemulsion, a cryo-microscopy picture was taken (Fig. 12). Here one can observe that the droplets are not uniformly spherical, but rather slightly deformed. Couvreur et al. [18] showed deformed, 'hand-handbag'-like structures of a positively charged nanoemulsion which is in good agreement with our results. This structure is probably due to the interaction of PS and LE80 at the oil/water interface. It was shown that the egg-phosphatidylcholine polar head groups of LE80 strongly interact with the positively charged stearylamine head groups in the electric double bilayer [19]. This interaction between two immiscible components such as LE80 and PS favours molecular rearrangements at the interface, which might be responsible for the deformed 'handbag'-like structures. Additionally, ceramides might influence these structures, which will be investigated.

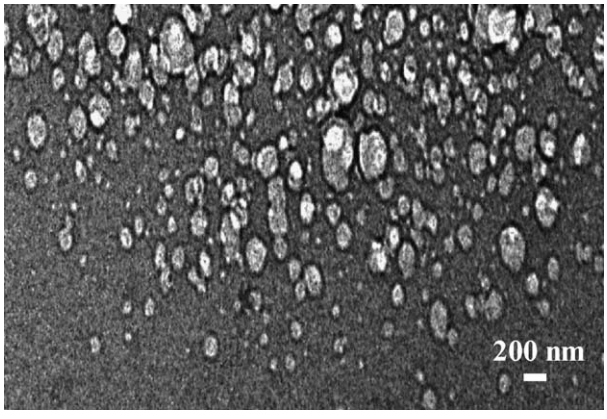

Fig. 12. Electron microscopic structure of the droplets in PPNSC.

4. Conclusion

A positively charged nanoemulsion containing ceramides which was stable for over 6 months was developed. The good stability was due to the positive surface charge of the nanodroplets, induced by phytosphingosine and pH, being responsible for repulsive forces, the close size distribution, preventing Ostwald ripening, and the surfactants, surrounding the oil/water interface and preventing collision of the droplets by a steric shield.

To deliver ceramides into the skin, they must be dissolved in the dosage form, which is a great challenge because of their low solubility. In this work, ceramides could be successfully incorporated in the positively charged nanoemulsion without any recrystallisation by adding Lipoid E-80®.

Due to the promising results of the present study, future studies are planned to evaluate the use of this formulation for the dermal delivery of ceramides.

Acknowledgements

We would like to thank Degussa (Essen, Germany) for providing ceramide III, ceramide IIIB and phytosphingosine. We are also thankful to the Institute of electron microscopy, Free University Berlin, Germany for taking the electron microscopy picture.

References

[1] Y. Rojanasakul, L.Y. Wang, M. Bhat, D.D. Glover, C.J. Malagna, J.K.H. Ma, The transport barrier of epithelia: a comparative study on membrane permeability and charge selectivity in the rabbit, Pharm. Res. 9 (1992) 1029–1034.
[2] M.P.Y. Piemi, D. Korner, S. Benita, J.-P. Marty, Positively and negatively charged submicron emulsions for enhanced topical delivery of antifungal drugs, J. Control. Release 58 (1999) 177–187.
[3] F. Wolf, C. Juestel, J. Schreiber, M. Klier, Sphingolipids as antimicrobial agents, German Patent 19602108 (1997).
[4] J.W.J. Lambers, H. Streekstra, Antimicrobial compositions for topical use, US Patent 6,147,118 (1998).
[5] C.-S. Park, J.-N. Kim, J.H. Jeong, Cosmetic preparations containing aqueous phytosphingosine solution, US Patent 6,403,111 (2002).
[6] C.-S. Park, J.-N. Kim, J.-N. Chong, Method for preparation and application of aqueous solution of phytosphingosine, Japan Patent 2001048848 (2001).
[7] P. Lersch, U. Schick, Choosing the right ingredients for cosmeceuticals, Special. Chem. Mag. 23 (2003) 30–31.
[8] J. W.J. Lambers, E.-L. Roehl, Topical application of ceramides, US Patent 6,001,375 (1999).
[9] M. Antonietti, K. Landfester, Polyreactions in miniemulsions, Prog. Polym. Sci. 27 (4) (2002) 627–809.
[10] R.H. Müller, Zetapotential und Partikelladung in der Laborpraxis, Band 37 Paperback APV, 1996.

[11] R.H. Müller, S. Benita, B. Böhm, Emulsions and Nanosuspensions for the Formulation of Poorly Soluble Drugs, Medpharm Scientific Publishers, Stuttgart, 1998.
[12] M. Jumaa, B.W. Müller, The stabilization of parenteral fat emulsion using non-ionic ABA copolymer surfactant, Int. J. Pharm. 174 (1998) 29–37.
[13] C.A. Coulaloglou, L.L. Tavlarides, Descriptions of interaction process in agitated liquid–liquid dispersions, Chem. Eng. Sci. 32 (1977) 1289–1297.
[14] R. Murahlidhar, D. Ramkrisham, Analysis of droplet coalescence in turbulent liquid–liquid dispersions, Ind. Chem. Fundam. 25 (1986) 554–560.
[15] M. Trotta, F. Pattarino, T. Ignoni, Stability of drug-carrier emulsions containing phosphatidylcholine mixtures, Eur. J. Pharm. Biopharm. 53 (2) (2002) 203–208.
[16] M. Mao-Qiang, P.M. Elias, K.R. Feingold, Fatty acids are required for epidermal permeability barrier homeostasis, J. Clin. Invest. 92 (1993) 791–798.
[17] M. Mao-Qiang, B.E. Brown, S. Wu, K.R. Feingold, P.M. Elias, Exogenous non-physiological vs. physiological lipids: divergent mechanisms for correction of permeability barrier dysfunction, Arch. Dermatol. 131 (1995) 809–815.
[18] H. Texeira, C. Dubernet, V. Rosilio, S. Benita, J. Lepault, I. Erk, P. Couvreur, New bicompartmental structures are observed when stearylamine is mixed with triglyceride emulsions, Pharm. Res. 17 (2000) 1329–1332.
[19] D. Korner, S. Benita, g. Albrecht, A. Baszkin, Surface properties of mixed phospholipid-stearylamine monolayers and their interaction with a non-ionic surfactant (poloxamer), Colloid. Surf. 3 (1994) 101–109.