International Journal of Pharmaceutics 383 (2010) 201–208

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Development of a positively charged prednicarbate nanoemulsion

Yücel Baspinar[a], Cornelia M. Keck[b,*], Hans-Hubert Borchert[a]

[a] Institute of Pharmacy, Free University of Berlin, Kelchstr. 31, 12169 Berlin, Germany
[b] Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia

ARTICLE INFO

Article history:
Received 5 May 2009
Received in revised form 29 August 2009
Accepted 3 September 2009
Available online 10 September 2009

Keywords:
Atopic dermatitis
Positively charged nanoemulsions
Phytosphingosine
Prednicarbate
High pressure homogenization
Chemical stability

ABSTRACT

A physically and chemically stable positively charged prednicarbate nanoemulsion was developed as a carrier system for the treatment of atopic dermatitis. Phytosphingosine was used to obtain the positive charge and also because of its supportive properties for the restoration of damaged skin. As production method high pressure homogenization was employed. The optimal concentrations of phytosphingosine, the oil phase, and the emulsifiers were investigated. The production was optimized by investigating the influence of homogenization cycles, homogenization pressure, production temperature and type of homogenizer with respect to particle size, physical stability of the emulsions and chemical stability of prednicarbate. From the results the best formulation and the most appropriate production parameters were identified. In addition it could be shown that during high pressure homogenization the drug is relocated from the inner oil phase of the emulsion towards the stabilizer layer, which could be shown by an increase in chemical stability of prednicarbate. The efficiency of incorporation is influenced by the energy input during homogenization (e.g. number of homogenization cycles) but also by the production temperature. It was found that the nanoemulsions should be produced at elevated temperatures, with low homogenization pressures but higher numbers of homogenization cycles (e.g. 300 bar and 10 cycles). The results prove that the efficiency of high pressure homogenization should not only be judged by investigating the particle size and the physical stability of the emulsions alone, but also by assessing the chemical stability of the incorporated drug.

1. Introduction

Atopic dermatitis means “inflammation of the skin”. It is characterized by temporarily inflamed skin areas, typically next to skin creases, e.g. wrists, the front of the elbows and the backs of knees. The causes of the skin inflammation are not yet fully identified, thus up today the only efficient therapy is the treatment of the symptoms (www.cks.library.nhs.uk, 2008). Symptoms are: dry skin, red and itchy skin, which is likely to be infected due to the invasion of bacteria, mainly *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The severity and duration of the inflammation varies from individual to individual. Mild atopic dermatitis is characterized by only one or two small patches of inflammation and a rare occurrence. In severe atopic dermatitis the inflammation areas cover many areas of the skin and the flare-ups can last several weeks or even longer. In such sincere cases of inflammation the application of corticosteroids or calcineurin inhibitors (e.g. Tacrolimus) is necessary to inhibit the acute inflammation. In the past years many attempts have been made to improve the therapy with corticosteroids (Callen et al., 2007; Fleischer, 2008). The aim was to reduce local and systemic undesired side effects, such as skin atrophy and hypothalamic–pituitary–adrenal (HPA) axis suppression, which limited the use of such drugs in the past. One successful outcome of this was a chemical derivate of prednisolone–prednicarbate (IUPAC name: (11β)-17-[(ethoxycarbonyl)oxy]-11-hydroxy-3,20-dioxopregna-1,4-dien-21-yl propionate). Up today prednicarbate (chemical structure Fig. 1A) was shown to possess the best benefit/risk ratio of all the corticosteroids being on the market (Gupta and Chow, 2004a,b).

In addition many efforts have been made to develop and to improve delivery vehicles of pharmaceutical actives for dermal application. Examples for pharmaceutical carriers are liposomes, drug nanocrystals, and lipid nanocarriers, such as solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) or nanoemulsions. The aim of each drug carrier is either to increase the chemical stability of the incorporated active, to improve the bioavailability of the drug and/or to target the drug to its desired site of action. With regard to dermal delivery the use of positively charged carriers is advantageous, because the positive charge promotes an intensive adsorption to the negatively charged skin. This increases the retention time and thus the bioavailability (Piemi et al., 1999).

Each of this carrier system has advantages. Nevertheless not every carrier can be used for every pharmaceutical active, because

* Corresponding author. Tel.: +49 30 83850631; fax: +49 30 83850616.
E-mail address: ck@ckc-berlin.de (C.M. Keck).

0378-5173/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2009.09.002

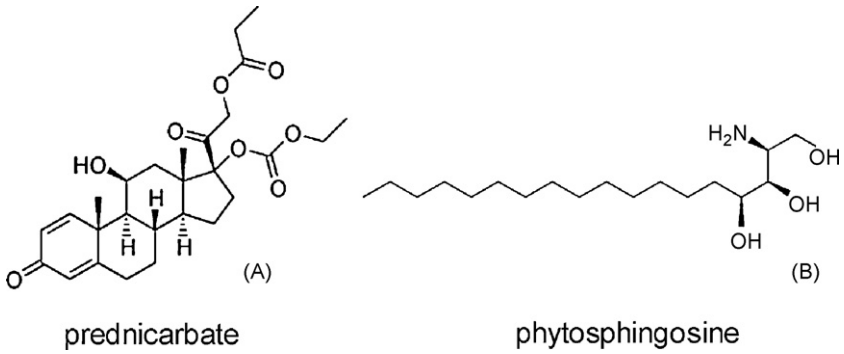

Fig. 1. Chemical structure of prednicarbate (A) and phytosphingosine (B), which is positively charged at a physiological skin pH of 5.5.

the physical properties (e.g. insufficient solubility) of the compound might be a limiting parameter for an effective incorporation. Further approaches to improve the dermal action of actives are the additional use of compounds which do not act as a drug in particular, but promote or accelerate the therapy by supplying additional benefits (Williams, 2000).

In case of atopic dermatitis, additional benefits could be an increase in skin hydration and skin elasticity and/or the prevention of inflammation due to bacteria. In this regard phytosphingosine seems to be a promising adjuvant.

Phytosphingosine is a compound of the mammalian stratum corneum. It indirectly influences a wide variety of cellular functions as, e.g. inflammation of the skin (Gupta et al., 1988) and takes part in the formation of ceramides, which are essential constituents of mammalian skin and are decreased in atopic dermatitis (Arikawa et al., 2002; Melnik, 2006; Wertz et al., 1987). Phytosphingosine (PS) increases skin hydration and skin elasticity (Yilmaz and Borchert, 2006) and possesses antimicrobial activity (Arikawa et al., 2002; Bibel et al., 1992; Melnik, 2006). PS (Fig. 1B) is a free sphingoid base with a pK_b of approximately 9, thus at a physiological skin pH of approximately 5.5 it is positively charged (Yilmaz and Borchert, 2005), which in theory should improve the absorption of pharmaceutical actives when formulated together.

The aim of this study was to develop and to formulate a nano drug carrier containing prednicarbate as active. Phytosphingosine was chosen as additive because of its skin beneficial properties and its positive charge. Nanoemulsions were chosen as carrier system, because preliminary studies revealed an insufficient solubility of PS in solid lipids, which limits the production of solid lipid nanoparticles.

In this study it was aimed to develop a physically but also chemically stable nanoemulsion. Firstly the optimal formulation was investigated by varying the concentration of the emulsifiers and the concentration of the oil phase. Secondly the influence of production parameters (e.g. production temperature, homogenization pressure, number of homogenization cycles) and different production lines on particle size and stability (physical and chemical) was investigated.

2. Materials and methods

2.1. Materials

Prednicarbate was purchased from mibe GmbH (Brehna, Germany) and phytosphingosine (2S-amino-1,3S,4R-octadecanetriol) was obtained from Degussa (Essen, Germany). As emulsifiers polysorbate 80 (Tween® 80, Uniquema, Everberg, Belgium) and a less purified egg lecithin (Lipoid E 80, Lipoid KG, Ludwigshafen, Germany) were chosen because of its known stabilizing effects for nanoemulsions (Yilmaz and Borchert, 2005). Eutanol® G (octyldodecanol, Caesar and Lorentz GmbH, Hilden, Germany) is a liquid wax and was used as oil phase, because in primary studies it was found to be the only lipophilic compound possessing a sufficient solubility for PS (Baspinar, 2009). As preservative, potassium sorbate (Caesar and Lorentz GmbH) was used and as antioxidant α-tocopherol (Synopharm, Barsbüttel, Germany) was added. Purified water was obtained using a MilliQ Plus (MilliQ, Schwalbach, Germany). All other chemicals used were of pharmaceutical grade and follow the specifications of the European Pharmacopeia.

2.2. Methods

2.2.1. Production of nanoemulsions

All nanoemulsions were produced by high pressure homogenization using an LAB 40 (APV Deutschland GmbH, Unna, Germany). Prior to homogenization the oil phase and the water phase were prepared separately.

The oil phase consisted of prednicarbate as active, PS, Lipoid E80, α-tocopherol and Eutanol as lipid base. It was produced by adding PS to the heated Eutanol (105–110 °C). The dispersion was kept at 105–110 °C and stirred with a magnetic stirrer until complete dissolution of PS was obtained. The solution was than cooled to 75 °C and the more heat sensitive compounds α-tocopherol and Lipoid E80 were added. Again the emulsion was kept at 75 °C and stirred until complete dissolution of these compounds was achieved. Afterwards the obtained oil phase was cooled down to the desired production temperature (25 or 50 °C, respectively). Finally prednicarbate was added and dissolved in the oil phase.

The water phase consisting of Tween 80, potassium sorbate and water, was obtained by dissolving the stabilizer and the preservative in the water, which was either kept at 25 °C or heated to 50 °C.

The pre-emulsion was obtained by adding the water phase to the oil phase, both being adjusted to the same temperature. Afterwards high speed stirring using an Ultra Turrax T25 (Janke and Kunkel GmbH, Staufen, Germany) was applied at 8000 rpm for 3 min. This pre-emulsion was than subjected to high pressure homogenization.

In the first part of the study various formulation parameters were checked, i.e. the influence of the concentration of PS, Tween 80, Lipoid and the oil phase. The concentration of either PS, Lipoid E80, Tween 80 or Eutanol was varied, whereas the other concentrations were kept constant. The base formulation was 0.25% (w/w) prednicarbate, 0.6% (w/w) PS, 2% (w/w) Lipoid E80 and Tween 80, respectively, 20% (w/w) Eutanol, 0.03% (w/w) vitamin E and 0.1% (w/w) potassium sorbate. In the first part of the study, the concentrations for PS are 0.3, 0.4, 0.5, 0.6 and 0.7% (w/w). The concentrations 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% (w/w) were explored for each of the stabilizers Lipoid E80 and Tween, respectively. Finally three different concentrations of Eutanol were studied (10, 15 and

Table 1
Overview of the formulations of nanoemulsions produced. The composition was kept constant; only the production parameters were varied, leading to a total of 24 emulsions.

Formulation	Production temperature 25 °C		Formulation	Production temperature 50 °C	
	HPH pressure	No. of cycles		HPH pressure	No. of cycles
1	300 bar	3 cycles	**13**	300 bar	3 cycles
2		5 cycles	**14**		5 cycles
3		8 cycles	**15**		8 cycles
4		10 cycles	**16**		10 cycles
5	500 bar	3 cycles	**17**	500 bar	3 cycles
6		5 cycles	**18**		5 cycles
7		8 cycles	**19**		8 cycles
8		10 cycles	**20**		10 cycles
9	700 bar	3 cycles	**21**	700 bar	3 cycles
10		5 cycles	**22**		5 cycles
11		8 cycles	**23**		8 cycles
12		10 cycles	**24**		10 cycles

20%, w/w). The resulting 20 formulations were produced at 50 °C applying 10 cycles and 300 bar.

In the second part the influence of production parameters was investigated. For this purpose the best formulation from the first part was chosen and subjected to different production parameters. The influence of the homogenization pressure was determined by applying 3 different homogenization pressures (300, 500 and 700 bar). The influence of the number of homogenization cycles was investigated by applying either 3, 5, 8 or 10 homogenization cycles, and finally the influence on the production temperature was analyzed by producing all the nanoemulsions from above at two different temperatures (25 and 50 °C). An overview of all the 24 emulsions produced in this second part is given in Table 1.

To investigate possible differences between different homogenizers the best formulation was used and was also produced using the homogenizer Emulsiflex C5 (Avestin, Ottawa, Canada) applying the optimal production parameters as described in part 2.

2.2.2. Characterization

2.2.2.1. Particle size analysis. The particle size of the freshly prepared emulsions was measured immediately after their production. The physical short and long term stabilities were investigated over a period of 1 year at two different storage temperatures (25 and 40 °C, respectively). In the stability study the size was measured 24 h after the production (d1) and then after 1 week, 2 weeks, 1 month, 2, 4, 6 and 12 months. The mean particle size was obtained by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). The technique yields a light intensity weighted mean diameter (*z*-average) and the polydispersity index (PI) as a measure for the width of the size distribution. A PI below 0.2 indicates a narrow size distribution. Dynamic light scattering has an upper detection limit of approx. 3 μm. Thus, to exclude the existence or the occurrence of larger emulsion droplets, low angle static light scattering (laser diffraction (LD), LS 230, Beckman-Coulter, Krefeld, Germany) with included PIDS technology was used. The LD results were analyzed using Mie theory with the optical parameters 1.456 (real refractive index) and 0.01 (imaginary refractive index). To further exclude the existence of larger droplets also light microscopy using an Ortophlan (Leitz, Germany) was employed (data not shown). The magnification used was 160-fold, because low magnifications give the fastest information whether larger droplets are present within the system or not (Keck, 2006).

2.2.2.2. Zeta potential. The surface charge of the emulsions was investigated by measuring the electrophoretic mobility (EM). All measurements were performed by adding 20 μl of the sample to 40 ml of purified water. The pH was measured and, if required, adjusted to a pH of 5.5 ± 0.1 using diluted HCl. The mixture was than adjusted to a conductivity of 50 μS/cm using a NaCl solution. The EM was measured using also the Zetasizer Nano ZS and the zeta potential was calculated by applying the Helmholtz–Smoluchowski equation (Malvern, 2002; Müller, 1996).

2.2.2.3. Chemical stability of prednicarbate. The chemical stability of prednicarbate was investigated for the most physically stable emulsions, using HPLC analysis. The concentration of prednicarbate was determined by the HPLC method described in the European Pharmacopeia. The HPLC system (Merck, Merck-Hitachi, Darmstadt, Germany) used consisted of a pump L 6200A with Interface D 6000, a UV/vis detector L-4500, an autosampler AS-2000A and a LiChroCART® column (125-4 mm) packed with LiChrospher® 100 RP-18, 5 μm, with a guard column RP-18 4.4 mm. The mobile phase consisted of acetonitril and water (ratio 5:6) and was filtered through a 0.45 μm polytetra-fluorethylene (PTFE) filter (Sartorius, Goettingen, Germany) prior analysis. For each measurement a sample volume of 20 μL was injected and the measurement was performed with a flow rate of 0.7 mL/min. The retention time of prednicarbate was approximately 17 min. The calibration curve was calculated by plotting the area under the curve vs. the prednicarbate concentration (c_{PC}), with a linearity from 1 to 100 μg/mL (AUC = 18424 c_{PC} + 1620, correlation coefficient r^2 = 0.9999).

3. Results and discussion

3.1. Formulation parameters

The influence of different concentrations of PS, Eutanol and stabilizer was investigated with respect to changes in size, size distribution (polydispersity index, PI), surface charge and the physical stability. The aim was to identify the formulation with a small particle size, a high positive surface charge and highest physical stability. All formulations produced revealed a small droplet size ranging from about 120 to 240 nm and a very narrow size distribution (PI range 0.06–0.16). Zeta potentials ranged from +41 mV to +60 mV, indicating a sufficient high positive charge for the aimed skin adhesion (Klang et al., 2000; Yilmaz and Borchert, 2006), as well as a good electrostatic stability (Malvern, 2002; Müller, 1996).

3.1.1. Variation of the concentration of phytosphingosine

A small, however, negligible increase in size was observed when the concentration of PS was increased. The increase in PS from 0.3 to 0.7% increased the size by only 12 nm and no significant trend was found for the size distribution (Fig. 2A, left). The zeta potential increased from +41 mV (0.3% PS) to +63 mV (0.7% PS), suggesting an increasing physical stability with an increasing concentration of PS (Fig. 2B). This expectation was not fully confirmed by the stability

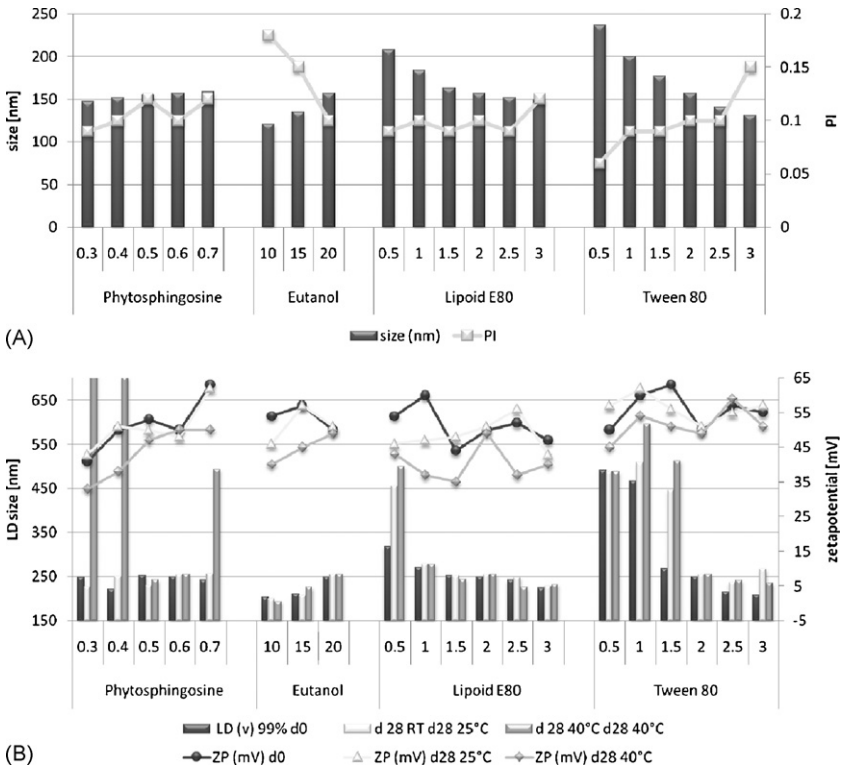

Fig. 2. Influence on droplet size (PCS data), particle size distribution (viewed as PI), zetapotential and short term stability due to variation of the formulation composition (variation of concentration of either Phytosphingosine, Eutanol, Lipoid E80 or Tween 80). (A) Influence on droplet size (PCS data) and particle size distribution (viewed as PI) due at day of production. (B) Influence on short term stability (LD data, viewed as $d(v)$ 99%) after 1 month of storage at either room temperature (RT) or 40 °C.

measurements. Only the formulations containing 0.5 and 0.6% were stable when stored at room temperature and 40 °C (Fig. 2B). Thus the best formulations were obtained with these PS concentrations. This result was also confirmed by zeta potential measurements. A decrease in the zeta potential for the emulsion stabilized with 0.7% PS was observed but was not seen for the emulsion stabilized with 0.6% PS.

3.1.2. Variation of the volume of the oil phase

In contrast to PS the increase of the Eutanol concentration led to a clear increase in size (from 121 to 167 nm) but to a decrease in PI (from 0.18 to 0.11, Fig. 2A). The result obtained is in line with the homogenization theory. A higher amount of the disperse phase will increase the final particle size when all the other formulation and homogenization parameters are kept constant. This is because a higher volume of the disperse phase requires more energy to break the emulsion droplets, hence if the energy input is kept constant an increase in the particle size is obtained (Jahnke, 2001). The decrease in the size distribution (decrease in PI) is observed, because a larger volume of the oil phase increases the shear stress and thus it promotes a more homogeneous size distribution.

The formulation containing 10% lipid phase was found to be most stable (Fig. 2B). Interestingly it was found that the formulation containing 20% lipid phase is more stable than the formulation containing only 15%, despite the zeta potential of the formulation containing 15% oil was highest directly after the production. When looking at the zeta potentials (ZP, Fig. 2B) after storage it turns out that only for the formulation containing 20% oil phase the ZP remains unchanged, whereas it was decreased for the formulations containing 15% stored at 40 °C as well as for both formulations (stored at 25 and 40 °C) containing 10% oil phase (Fig. 2B). The decrease in ZP indicates the chemical degradation of Lipoid E80 which is sensitive to oxidation. Formation of lysolecithin increases the number of negative charges (Müller et al., 1995) thus consequently reducing the positive zeta potential. The destabilizing effect was more pronounced at a small droplet size. Therefore it can be assumed that the driving force of this instability is due to the larger total surface of the smaller droplets, meaning the exposure of Lipoid E80 to oxygen and so lysolecithin formation is higher. Therefore the only stable formulation is the formulation containing 20% Eutanol. It does not possess the smallest size, but a constant zeta potential and unchanged size.

3.1.3. Variation of the concentration of stabilizers (Lipoid E80 and Tween 80)

The increase in concentration of the two stabilizers led to a decrease in size (Fig. 2A). The observed effects were most pronounced for Tween 80. While for Lipoid E80 the size decreased from 208 to 150 nm for Tween the size decreased from 237 to 131. No significant increase in PI could be observed for Lipoid 80. However, when the concentration of Tween 80 was increased the PI increased from 0.06 to 0.15. It is known that the concentration of stabilizer influences the final particle size of an emulsion. As a rule of thumb a ratio of oil phase: stabilizer of 5:3 will lead to the smallest droplet size (Jahnke, 2001). Nevertheless the addition of stabilizer also changes other parameters, e.g. the viscosity of the oil or the water phase which also can influence the homogenization results. Thus the observed increase in the size distribution (PI) might be related to this fact. A broad size distribution indicates physical instability. Therefore the optimal concentration of the stabilizers, leading to sufficient small droplets but in parallel to a narrow size distribution should be in the range between 1.5 and 2.5% (Fig. 2A).

The influence of the different concentrations of the two stabilizers on the physical stability revealed that the emulsions stabilized with 2% were most stable (Fig. 2B). All other concentrations showed either an increase in the size or non-constant zeta potentials. Based on these results, the formulation containing 0.6% phytosphingo-

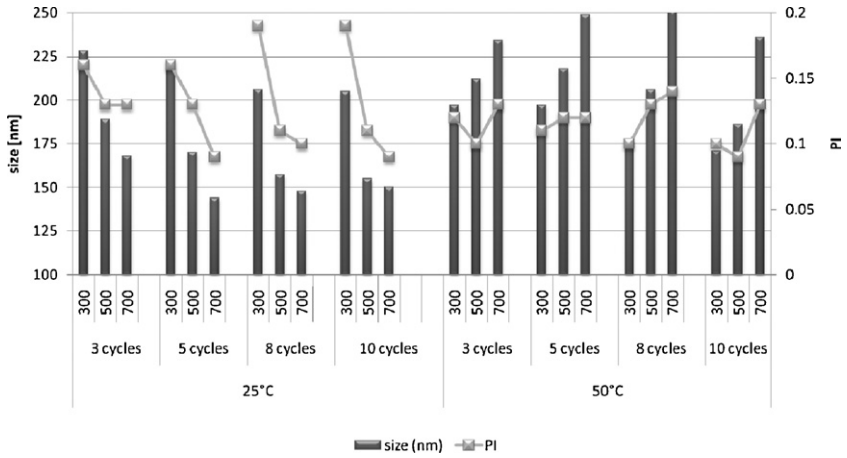

Fig. 3. Influence on droplet size (PCS data) and particle size distribution (viewed as PI), due to variation of the production parameters. Production at 25 °C leads to a decrease in size an size distribution with increasing homogenization cycles, the opposite trend is observed for the production at 50 °C (explanation cf. text).

sine, 20.0% Eutanol and 2.0% Lipoid E80 and Tween 80, respectively, was selected for further studies.

3.2. Influence of production parameters

In this part of the study the influence of production temperature, homogenization pressure, and number of homogenization cycles on size, physical stability of the emulsions and the chemical stability of prednicarbate were investigated.

3.2.1. Influence on particle size and size distribution

The best formulation of part 1 was homogenized at 25 and 50 °C and by varying the number of homogenization cycles and the homogenization pressure. The results reveal clear differences between the two different production temperatures. The production at 25 °C gave smaller particles and smaller polydispersity indices with an increasing homogenization pressure, whereas the production at 50 °C revealed the opposite. In this case it was found that the higher the homogenization pressure the larger was the particle size obtained (Fig. 3). With a production temperature of 25 °C the smallest particles were obtained at homogenization pressures of 700 bar, whereas for 50 °C production temperature the smallest sizes were obtained with a homogenization pressure of 300 bar. All over the smallest particles were obtained at production temperatures of 25 °C when applying 5 cycles and 700 bar (144 nm, PI 0.09).

Even when it seems to be controversial—the results are in agreement with the state of the art of high pressure homogenization. When looking at milk production, where high pressure homogenization is used to avoid creaming of the naturally larger fat droplets, it is suggested to use a two step homogenization process (Jahnke, 2001). The first homogenization step is used to comminute the fat droplets and a second homogenization step is used to separate agglomerates which were formed during the first homogenization step. The agglomeration or even coalescence of droplets after the first homogenization step is due to an insufficient coverage of stabilizer of the newly created surface, because the transport of stabilizer molecules to the new surface takes a certain time. Hence shortly after the homogenization the newly formed emulsion is sensitive to agglomeration and coalescence. This is due to a high level of energy and a not yet fully covered surface of the droplets. In the two step homogenization the time between the first and the second homogenization step is sufficient for the stabilizer to diffuse into the interface and to decrease the interfacial tension and thus to stabilize the emulsion droplets. The second homogenization step is than performed at low pressures (typically being approx. 15% of the first pressure applied (Jahnke, 2001)). This pressure is high enough to separate agglomerates, but does not further comminute the emulsion droplets, which would cause a further destabilization. In this study a one step homogenization process was used, therefore the emulsions tended to re-agglomerate and re-coalesce shortly after the homogenization. As the stability of an

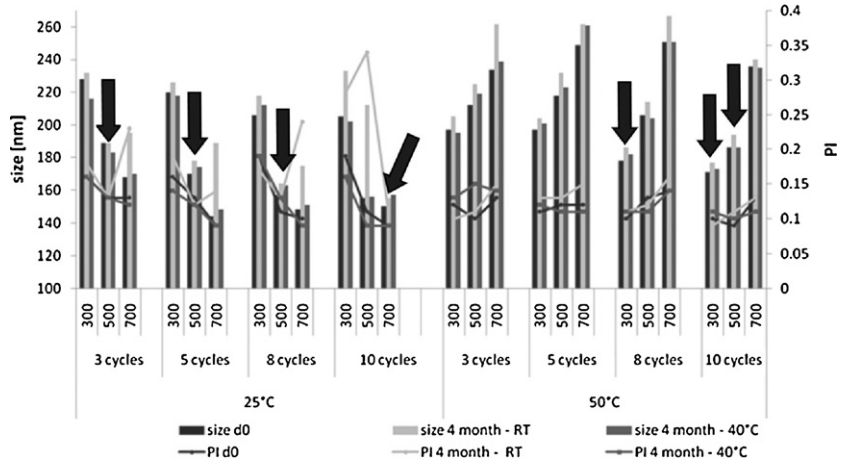

Fig. 4. Influence on physical stability (PCS data) due to variation of the production parameters. The most stable emulsions (indicated by black bold arrows) were chosen for further investigations.

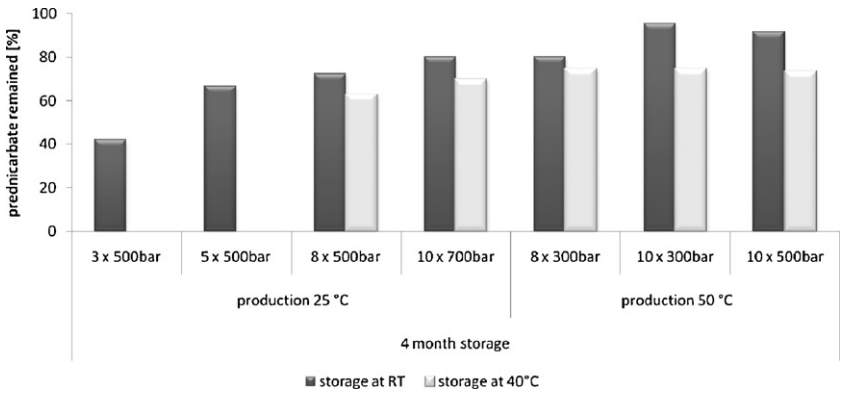

Fig. 5. Chemical stability of prednicarbate investigated for the physically stable emulsions. The highest chemical stability of prednicarbate is obtained with a production temperature of 50 °C, at low pressures and with 10 homogenization cycles.

emulsion also depends on the rigidity (microviscosity) of the emulsifier film formed, it can be assumed that the emulsions produced at 25 °C showed a higher microviscosity and thus the tendency of instability phenomena was decreased when compared to the emulsions produced at 50 °C. Therefore the effect of an increasing size with increasing homogenization pressures for the emulsions produced at 50 °C can be explained by an increasing energy input with an increasing homogenization pressure. This destabilizes the emulsions, leading to an increase in size with increasing pressure. In case of the emulsions produced at 25 °C the rigidity of the emulsifier film overwhelmed the destabilizing effect and avoided re-coalescence after the homogenization. Thus the increasing energy input with increasing homogenization pressures leads to a more effective comminution of the emulsion droplets.

3.2.2. Influence on physical stability

The physical stability was assessed by size measurements. The particle sizes obtained after 4 months of storage are shown in Fig. 4. It clearly shows that the physical stability of the emulsions stored at 40 °C is better than for the emulsions stored at room temperature. All emulsions stored at 40 °C are physically stable and no significant changes in size could be obtained, neither from DLS nor from LD measurements (Fig. 4). For the emulsions stored at room temperature significant differences were obtained. At a production temperature of 25 °C, the three emulsions produced with 500 bar and 3, 5 or 8 cycles and the emulsion produced with 700 bar and 10 cycles were most stable (Fig. 4, left, black arrows). For the emulsions produced at a temperature of 50 °C only the emulsions produced with 300 bar and 8 or 10 homogenization cycles and the emulsion produced with 500 bar and 10 cycles were stable (Fig. 4, right).

From the results obtained it is clear that optimal production parameters, e.g. number of homogenization cycles and homogenization pressure are temperature dependent. For a lower production temperature higher homogenization pressures were required (500 or 700 bar), than for the higher production temperature, at which a homogenization pressure of 300 bar was found to be sufficient.

3.2.3. Influence on chemical stability of prednicarbate

After 4 months of storage the chemical stability of prednicarbate was assessed for the most physically stable emulsions (cf. Section 3.2.1 and Fig. 4). The results of this study are shown in Fig. 5. The emulsions produced at 25 °C applying 500 bar clearly show an increasing stability of prednicarbate with an increasing number of homogenization cycles. The amount of non-degraded prednicarbate was 42% when 3 homogenization cycles were used, 67% when 5 cycles were applied, but 72% when 8 cycles were applied (Fig. 5). 80% of non-degraded prednicarbate was found in the emulsion produced at 25 °C applying 700 bar and 10 cycles. The same result was obtained for the emulsion produced at 50 °C applying 300 bar and 8 homogenization cycles. This again demonstrates that different production temperatures require different production parameters, to

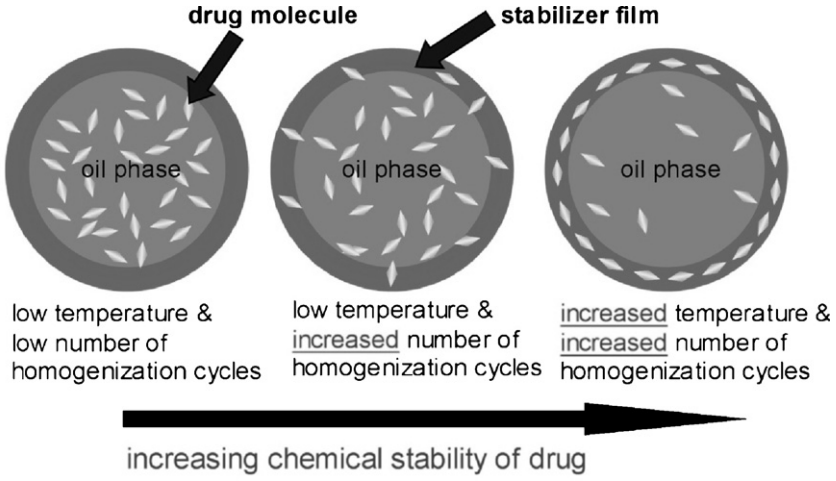

Fig. 6. Principle of chemical stabilization. (Left) Low temperature and low numbers of homogenization cycles lead to the location of the drug more towards the inner oil phase, the drug is not protected from chemical degradation. (Middle) An increasing temperature and more homogenization cylces allow the drug to partially diffuse into the stabilizer film, which leads to an increased chemical stability. (Right) The further increase in homogenization cycles further promotes the relocation of the drug into the stabilizer film, yielding maximal stability.

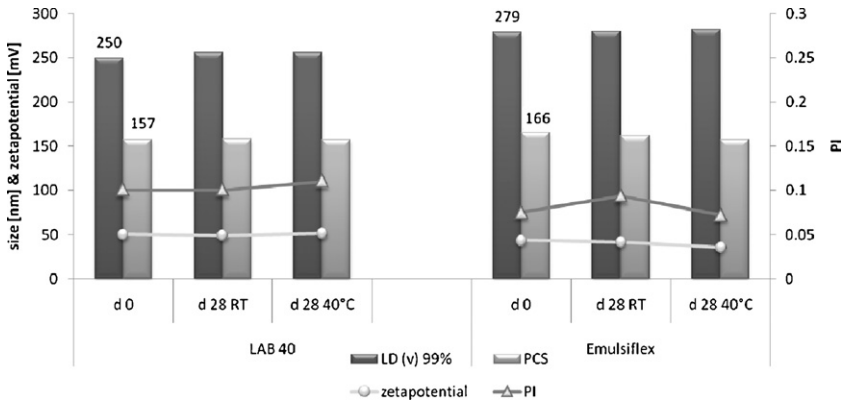

Fig. 7. Comparison of particle sizes obtained with different types of homogenizers. (Left) Lab 40. (Right) Emulsiflex C5 (given sizes: nm). The results can be judged as similar.

obtain similar results (in this case 80% chemical stability of prednicarbate). The highest stability (95% prednicarbate) was obtained for the emulsion produced at 50 °C, applying 300 bar and 10 cycles. For the emulsion produced at 50 °C with 500 bar and 10 cycles the chemical stability of prednicarbate was found to be 92% (Fig. 5).

The pronounced dependency of the chemical stability of prednicarbate on the production parameters indicates that the distribution of the drug within the emulsion droplets obviously differs for the different production parameters. It can be assumed that a high chemical stability of the drug incorporated is related to a localization of the drug within the stabilizer layer, whereas a low chemical stability of the drug is related to drug localization within the lipid phase of the emulsion droplet (Fig. 6). In previous work by Müller et al. it could be shown that high pressure homogenization increases the solubility of poorly water soluble drugs, when homogenized with lecithin based oil in water emulsions, e.g. Lipofundin (Müller, 2000). The process is known as SolEmuls technology and has been studied extensively (Akkar and Müller, 2003a,b; Akkar et al., 2004; Buttle, 2004; Müller et al., 2004). The increase in solubility via SolEmuls technology is due to the incorporation of the active into the lecithin layer of the emulsion during the homogenization process. Due to the localization of the drug within the lecithin layer a drug reservoir is formed, which means the drug is tightly associated with the drug nanocarrier (Junghanns et al., 2007). Processing a drug via SolEmuls technology is different to the process used in this study. In SolEmuls technology a drug crystal or drug nanocrystal is dispersed in the water phase of a readymade emulsion (e.g. Lipofundin or Intralipid) and the obtained dispersion is homogenized until a complete dissolution of the drug is obtained. In our case the drug was dissolved in the oil phase prior the homogenization process, however, the data indicate that also via this production process the drug seems to be incorporated into the lecithin layer of the emulsion. From the data it is suggested that a higher chemical stability of the drug – and thus a more efficient incorporation into the stabilizer film – is obtained with a high number of homogenization cycles and at elevated production temperatures. The latter is probably due to a less rigid stabilizer film, which allows the drug to diffuse easily between the stabilizer molecules. It was also found that the optimum was reached not at the highest homogenization pressure (700 bar) but at the lowest (300 bar).

To our best knowledge it was not reported before, that an increasing number of homogenization cycles can lead to an increasing chemical stability of the drug incorporated into an emulsion. To our opinion this is a very important finding, because in general drug loaded emulsions are considered to being not able to protect an active from chemical degradation (Schmidt, 2002). The incorporation of the drug into the stabilizer layer also means that the drug becomes closely associated to the drug carrier. This might not only be important for the chemical stability of the drug but also for dermal delivery, because for SLN it could be shown that an efficient epidermal targeting with corticosteroids can only be achieved if the drug is closely associated to the carrier (Santos Maia et al., 2002).

3.3. Comparison of homogenizers

The LAB40 requires a sample volume of 40 mL. This is a convenient sample volume for screening procedures, because a certain amount of sample is required for the standard stability measurements.

However, if only low quantities of drug are available (e.g. NCEs) or only low quantities are needed, a smaller sample volume would be beneficial. In case of the prednicarbate nanoemulsions in the future only small sample quantities will be required for the drug release or absorption studies. The Emulsiflex C5 only requires a minimum sample volume of 7 mL, making it an interesting alternative for the production of the nanoemulsions. However, the set up of the C5 homogenizer (e.g. design of homogenization chamber) is different to the LAB 40. Thus it was of interest to investigate if comparable results can also be achieved with the Emulsiflex. For this 10 g of the optimized formulation were homogenized at 50 °C with a pressure of 300 bar. The optimal homogenization time, leading to the smallest and most stable emulsion was found to be 5 min (data not shown). Shorter homogenization time resulted in less stable emulsions and higher homogenization times did not further improve the final particle size or the stability. The particle size obtained after 5 min of homogenization time and the short time stability (1 month) was compared to the results obtained with the LAB 40. The results are shown in Fig. 7. The data reveal practically identical results for both homogenizers. With PCS diameters of 157 and 166 nm (LAB 40 vs. Emulsiflex), the mean sizes are within the standard deviations of PCS measurements and the batch to batch variation of homogenization. In addition, the LD diameters $d(v)$ 99% are not different, considering the resolution of the method (Keck and Müller, 2008). Thus in the future smaller quantities of the developed nanoemulsion can be produced using the Emulsiflex C5.

To sum up: in this study a physically stable, positively charged nanoemulsion was developed. Due to its positive charge this formulation should lead to an improved adsorption to the skin when compared to negatively charged emulsions (Klang et al., 2000). Due to the achieved incorporation of prednicarbate into the lecithin layer of the emulsion the formulation should also show an altered release profile, when compared to conventional emulsions (Buttle, 2004) and a dermal drug targeting effect (Lombardi Borgia et al., 2007) upon dermal application. Thus, further studies will now investigate the release and the skin absorption of the optimized formulation.

4. Conclusion

The investigation of both, the formulation parameters and the production parameters, led to the conclusion that the optimal nanoemulsion formulation should contain 0.6% phytosphingosine, 20.0% Eutanol and 2.0% Lipoid E80 and Tween 80, respectively. The optimal production parameters are: a production temperature of 50 °C, a low homogenization pressure, e.g. 300 bar and 10 homogenization cycles. Identical results are obtained using either the LAB 40 or the Emulsiflex C5. Interestingly it was found that an increasing number of homogenization cycles and a higher production temperature increases the chemical stability of the drug incorporated. This is attributed to an enrichment of the drug in the surfactant layer. This finding is of importance, because it clearly demonstrates that an active might have different localizations within an emulsion, and that this location obviously can be influenced by the production parameters, e.g. number of homogenization cycles. Because it can be assumed that the location of a drug also influences the release and thus the bioavailability, the influence of different production techniques or parameters will be a subject for further investigations in the future. These findings show that the optimal formulation is not only characterized by the smallest particle size and the best stability, but also due to the fact that the smallest particles do not have the highest chemical stability. The findings might be exploited not only for emulsions but also for the production of solid lipid nanoparticles or nanostructured lipid carriers. Further studies will now investigate the release and the skin absorption of the optimized formulation.

References

Akkar, A., Müller, R.H., 2003a. Formulation of intravenous carbamazepine emulsions by SolEmuls technology. Eur. J. Pharm. Biopharm. 55, 305–312.

Akkar, A., Müller, R.H., 2003b. Intravenous itraconazole emulsions produced by SolEmuls technology. Eur. J. Pharm. Biopharm. 56, 29–36.

Akkar, A., Namsolleck, P., Blaut, M., Müller, R.H., 2004. Solubilizing poorly soluble antimycotic agents by emulsification via a solvent-free process. AAPS PharmSciTech 5, E24.

Arikawa, J., Ishibashi, M., Kawashima, M., Takagi, Y., Ichikawa, Y., Imokawa, G., 2002. Decreased levels of sphingosine, a natural antimicrobial agent, may be associated with vulnerability of the stratum corneum from patients with atopic dermatitis to colonization by *Staphylococcus aureus*. J. Invest. Dermatol. 119, 433–439.

Baspinar, Y., 2009. Nano- and microemulsions for topical application of poorly soluble immunosuppressives. PhD Thesis, Free University Berlin.

Bibel, D.J., Aly, R., Shinefield, H.R., 1992. Antimicrobial activity of sphingosines. J. Invest. Dermatol. 98, 269–273.

Buttle, I., 2004. O/W-emulsionen für die intravenöse Applikation von Arzneistoffen. PhD Thesis, Freie Universität, Berlin.

Callen, J., Chamlin, S., Eichenfield, L.F., Ellis, C., Girardi, M., Goldfarb, M., Hanifin, J., Lee, P., Margolis, D., Paller, A.S., Piacquadio, D., Peterson, W., Kaulback, K., Fennerty, M., Wintroub, B.U., 2007. A systematic review of the safety of topical therapies for atopic dermatitis. Br. J. Dermatol. 156, 203–221.

www.cks.library.nhs.uk, 2008. Eczema—atopic. NHS Clinical Knowledge Summaries.

Fleischer Jr., A.B., 2008. Diagnosis and management of common dermatoses in children: atopic, seborrheic, and contact dermatitis. Clin. Pediatr. (Phila.) 47, 332–346.

Gupta, A.K., Chow, M., 2004a. Prednicarbate (dermatop): a review. J. Drugs Dermatol. 3, 553–556.

Gupta, A.K., Chow, M., 2004b. A review of prednicarbate (Dermatop). Skin Ther. Lett. 9, 5–6, 9.

Gupta, A.K., Fisher, G.J., Elder, J.T., Nickoloff, B.J., Voorhees, J.J., 1988. Sphingosine inhibits phorbol ester-induced inflammation, ornithine decarboxylase activity, and activation of protein kinase C in mouse skin. J. Invest. Dermatol. 91, 486–491.

Jahnke, S., 2001. The theory of high-pressure homogenization. In: Müller, R.H., Böhm, B.H.L. (Eds.), Dispersion Techniques for Laboratory and Industrial Scale Processing. Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart.

Junghanns, J.U., Buttle, I., Müller, R.H., Araujo, I.B., Silva, A.K., Egito, E.S., Damasceno, B.P., 2007. SolEmuls technology: a way to overcome the drawback of parenteral administration of insoluble drugs. Pharm. Dev. Technol. 12, 437–445.

Keck, C.M., 2006. Cyclosporine nanosuspensions: optimised size characterisation & oral formulations. PhD Thesis, Free University Berlin.

Keck, C.M., Müller, R.H., 2008. Size analysis of submicron particles by laser diffractometry—90% of the published measurements are false. Int. J. Pharm. 355, 150–163.

Klang, S., Abdulrazik, M., Benita, S., 2000. Influence of emulsion droplet surface charge on indomethacin ocular tissue distribution. Pharm. Dev. Technol. 5, 521–532.

Lombardi Borgia, S., Schlupp, P., Mehnert, W., Schafer-Korting, M., 2007. In vitro skin absorption and drug release—a comparison of six commercial prednicarbate preparations for topical use. Eur. J. Pharm. Biopharm..

Malvern, 2002. Zeta Potential An Introduction in 30 Minutes. Technical Note, www.malvern.com.

Melnik, B., 2006. Disturbances of antimicrobial lipids in atopic dermatitis. J. Dtsch. Dermatol. Ges. 4, 114–123.

Müller, R.H., 1996. Zetapotential und Partikeladung in der Laborpraxis. Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart.

Müller, R.H., 2000. Dispersions for the formulation of slightly or poorly soluble agents. US Patent 7,060,285.

Müller, R.H., Peters, K., Becker, R., Kruss, B., 1995. Nanosuspensions for the i.v. administration of poorly soluble drugs—stability during sterilization and long-term storage. In: 22nd International Symposium of Controlled Release of Bioactive Materials, Washington, DC, pp. 574–575.

Müller, R.H., Schmidt, S., Buttle, I., Akkar, A., Schmitt, J., Bromer, S., 2004. SolEmuls-novel technology for the formulation of i.v. emulsions with poorly soluble drugs. Int. J. Pharm. 269, 293–302.

Piemi, M.P., Korner, D., Benita, S., MartyJp, 1999. Positively and negatively charged submicron emulsions for enhanced topical delivery of antifungal drugs. J. Control. Release 58, 177–187.

Santos Maia, C., Mehnert, W., Schaller, M., Korting, H.C., Gysler, A., Haberland, A., Schäfer-Korting, M., 2002. Drug targeting by solid lipid nanoparticles for dermal use. J. Drug Target 10, 489–495.

Schmidt, S., 2002. Parenteral o/w emulsions: drug incorporation and interaction with plasma proteins. PhD Thesis, Free University Berlin.

Wertz, P.W., Swartzendruber, D.C., Madison, K.C., Downing, D.T., 1987. Composition and morphology of epidermal cyst lipids. J. Invest. Dermatol. 89, 419–425.

Williams, H.C., 2000. Atopic Dermatitis: The Epidemiology, Causes and Prevention of Atopic Eczema. Cambridge University Press, Cambridge.

Yilmaz, E., Borchert, H.H., 2005. Design of a phytosphingosine-containing, positively-charged nanoemulsion as a colloidal carrier system for dermal application of ceramides. Eur. J. Pharm. Biopharm. 60, 91–98.

Yilmaz, E., Borchert, H.H., 2006. Effect of lipid-containing, positively charged nanoemulsions on skin hydration, elasticity and erythema—an in vivo study. Int. J. Pharm. 307, 232–238.