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Penetration and release studies of positively and negatively charged nanoemulsions—Is there a benefit of the positive charge?

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ABSTRACT

The surface of all tissues, including the stratum corneum, carries a negative charge. Following that fact it is assumed that a positively charged topical formulation could lead to an enhanced penetration because of an increased interaction with the negative charge of the membrane. The intention of this study is to prove an enhanced penetration of a positively charged nanoemulsion compared to a negatively charged nanoemulsion, both containing prednicarbate. The release and penetration of these nanoemulsions, produced with the high pressure homogenization method, were investigated. Regarding these results reveals that the release of the negatively charged formulation is higher compared to the positively charged nanoemulsion, while the penetration of the positively charged nanoemulsion is enhanced compared to the negatively charged formulation. The results of the investigated positively charged nanoemulsion containing prednicarbate show that its topical use could be advantageous for the therapy of atopic dermatitis, especially regarding phytosphingosine, which was responsible for the positive charge.

1. Introduction

Prednicarbate (PC) is a corticosteroid of the newer generation (Fig. 1A), which has as a "soft-steroid" an excellent benefit/risk relation (Gupta and Chow, 2004a,b). It is one of the most potent corticosteroids and does not cause any or just a little skin atrophy, even after repeated application. One of the indications of PC is atopic dermatitis, which means "inflamation of the skin". This inflammation, whose severity and duration varies from case to case, from mild to severe, is the result of skin-invasion with bacteria (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). It is necessary to counteract this inflamation by using topical corticosteroids, like PC, or other drugs like tacrolimus, a calcineurin inhibitor.

The symptoms of atopic dermatitis are red, itchy and dry skin. Dry skin and other skin disorders, which are characteristic for atopic dermatitis, are characterized by an impaired stratum corneum barrier function. Examining the straum corneum more closely one substance is remarkable among others, namely phytosphingosine (PS). PS (Fig. 1B) is present at high levels in the mammalian stratum corneum, increases its barrier function and is considered to be part of the skin's natural defence system (Wolf et al., 1997; Lambers and Streekstra, 1998; Park et al., 2002; Lersch and Schick, 2003). PS plays an important role in the formation of ceramides, influences a broad variety of cellular functions, like inflammation of the skin, and increases ceramide levels in and the barrier function of the stratum corneum after topical application (Gupta et al., 1988; Rawlings, 2003). Ceramides are essential constituents of mammalian skin and are decreased in the case of atopic dermatitis (Arikawa et al., 2002; Melnik, 2006; Wertz et al., 1987). The application of ceramide containing formulations on the impaired skin lead to an improved healing (Mao-Qiang et al., 1993, 1996; De Paepe et al., 2002; Chamlin et al., 2001). A further advantage of PS is its ability to increase the skin hydration and elasticity (Yilmaz and Borchert, 2006) and its antimicrobial activity (Arikawa et al., 2002; Bibel et al., 1992; Melnik, 2006). Although PS has several advantages, its very low solubility is a serious limiting factor for its topical use. That problem of low solubility could be solved by using Eutanol G at high temperatures (105–110 °C).

A low solubility and low bioavailability, chemical instability and an insufficient penetration are some of the problems which are limiting the topical application of many drugs. If the penetration of the drug into the skin is insufficient, the desired pharmalogical effect can be insufficient too. That low penetration of the drug can be solved either by increasing the drug concentration in the formulation or adding compounds, which do not act as a drug in particular, but can promote or accelerate the therapy and improve the dermal action of actives (Williams, 2000). Increasing the drug concentration leads to higher costs, especially for the manufacturer. Moreover, using penetration enhancer increases the risk of

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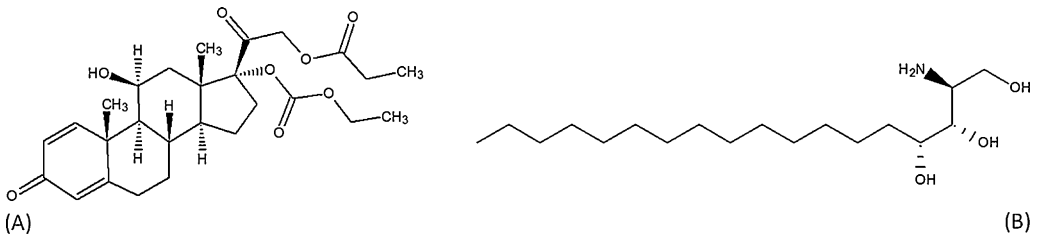

Fig. 1. Chemical structure of prednicarbate (A), which is positively charged at a physiological skin pH of 5.5, and of phytosphingosine (B).

undesired side effects. The problems, which are limiting the topical application of many drugs can be solved by using a recent topical drug delivery technology, namely nanocarriers, like nanoemulsions. In addition to a suitable nanoformulation, a positive charge could lead to an enhanced penetration of the applied drug into the skin. The positive charge of the produced nanoemulsion was caused by PS, which has in addition to causing the positive charge the before mentioned beneficial skin properties. It is known for many years that the surface of all tissues carries a negative charge (Rojanasakul et al., 1992) and that positively charged formulations causes an intensive adsorption to the negatively charged corneocytes of the stratum corneum, the main barrier of the human skin, which enhances the retention time and the bioavailability (Piemi et al., 1999). That is why using a positively charged formulations is a promising method for enhancing the penetration of a drug.

Wagner et al. (2001) investigated the interrelation of permeation and penetration parameters from in vitro experiments with human skin and the skin equivalents trypsin-isolated stratum corneum, heat-separated epidermis and reconstructed human skin (RHS). They concluded that an interrelation is existing for some parameters regarding the skin equivalents, but not for full-thickness skin. The relation between some parameters could not be successfully realized by using full-thickness skin. The influence of the dermis according to its changed polarity and the higher diffusion resistance is not neglectable and makes a correlation with data impossible. The barrier function in reconstructed epidermis and its resemblance to native human skin was examined by Ponec et al. (2001). Although reconstructed human epidermis shows similarities to native tissue, there are still significant differences in the stratum corneum function. Schmook et al. (2001) investigated the in vitro skin penetration of four topical dermatological drugs with widely varying polarities. They compared the penetration studies of human, pig and rat skin with the Graftskin™ LSE™ (living skin equivalent) and the Skinethic™ HRE (human reconstructed epidermis) models. They concluded that skin models cannot be regarded as generally useful for in vitro penetration studies. Due to the fact that skin models, like Epiderm™, Episkin® or SkinEthic®, so-called skin equivalents, RHS, reconstructed epidermis and reconstructed stratum corneum, are not equivalent to native skin the penetration of the positively charged PC containing nanoemulsion was investigated with excised human skin.

Here the circuit is complete between PC, atopic dermatitis, PS and nanoemulsion. The low solubility of PS could be enhanced by using nanoemulsions. PS caused a positive charge in this nanoemulsion, plays an important role for atopic dermatitis and could lead to an increased penetration of the drug into the skin.

In this comparative study the formulation properties like release, penetration and the stability of positively and negatively charged PC containing nanoemulsions were examined. The physically stable nanoemulsions were produced with the high pressure homogenization method, described more detailed in Jahnke (2001). The important factors, which destine the final properties of the formulation are the process parameter (homogenization temperature, homogenization pressure, number of homogenization cycles) and formulation parameter (amounts of the important formulation compounds). The development of the positively charged nanoemulsion used in this study was described in detail in a previous publication (Baspinar et al., 2010). The negatively charged nanoemulsion containing myristic acid was homogenized analogue to the positively charged nanoemulsion.

2. Materials and methods

2.1. Materials

PC was obtained from mibe GmbH (Brehna, Germany) and PS (2S-amino-1,3S,4R-octadecanetriol) was purchase from Degussa (Essen, Germany). As emulsifiers polysorbate 80 (Tween® 80, Uniquema, Everberg, Belgium) and a less purified egg lecithin (Lipoid E80, Lipoid KG, Ludwigshafen, Germany) were chosen because of its own stabilizing effects for nanoemulsions (Yilmaz and Borchert, 2005). Eutanol® G (octyldodecanol, Caesar and Lorenz 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). For the negatively charged nanoemulsions myristic acid was used (Carl Roth GmbH & Co, KG, Karlsruhe, Germany). As preservative, potassium sorbate (Caesar and Lorenz GmbH, Hilden, Germany) 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 the nanoemulsions

In a previous paper (Baspinar et al., 2010) the optimisation of the positively charged PC containing nanoemulsion was described detailed. The positively and the negatively charged nanoemulsions were produced by high pressure homogenization using an LAB 40 (APV Deutschland GmbH, Unna, Germany). The oil phase and the water phase were prepared separately before homogenized. The oil phase of the positively charged nanoemulsion consisted of PC as the drug, PS for the positive charge, Lipoid E80, α-tocopherol and Eutanol G as lipid base. In search of a suitable substance, that will cause the negative charge of the nanoemulsions, several fatty acids were tested (myristic acid, stearic acid, lauric acid and palmitic acid). PS was added to the heated Eutanol (105–110 °C). The dispersion was kept at 105–110 °C and stirred with a magnetic stirrer until PS was dissolved completely. Before adding and solving the more heat sensitive compounds α-tocopherol and Lipoid E80, the solution was cooled down to 75 °C. The obtained oil phase was cooled down to 50 °C before PC was added and dissolved in the oil phase. The water phase was obtained by dissolving the stabilizer Tween 80 and the preservative potassium sorbate in water, which was heated up to 50 °C. This water phase was added to the oil phase, adjusted to the

homogenization temperature of 50 °C and the pre-emulsion was obtained by using the high speed stirrer Ultra-Turrax T25 (Janke and Kunkel GmbH, Staufen, Germany) with 8000 rpm for 3 min at 50 °C and subjected afterwards to high pressure homogenization. After that the pH of this nanoemulsion was adjusted to 5.5 ± 0.1 using diluted HCl solution, because of the natural skin pH of approximately 5.5 on the one hand and causing the positive charge (pK_B of PS is approx. 9) on the other hand.

2.2.2. Characterization

2.2.2.1. Chemical stability of prednicarbate in the nanoemulsions. The chemical stability of PC in the nanoemulsions was investigated using the HPLC method described in the European Pharmacopeia by measuring the concentration of PC. 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 consisted of acetonitrile and water (ratio 5:6) and was filtered through a 0.45 μm polytetra-fluorethylene (PTFE) filter (Sartorius, Göttingen, Germany) prior analysis. A sample volume of 20 μl was injected for each measurement and performed with a flow rate of 0.7 ml/min. The retention time of PC was approximately 17 min. The calibration curve was calculated by plotting the area under the curve vs. the PC concentration (c_{PC}), with a linearity from 1 to 100 μg/ml (AUC = 18,424c_{PC} + 1620, correlation coefficient r^2 = 0.9999).

2.2.2.2. Physical stability of the nanoemulsions.

2.2.2.2.1. Particle size analysis. The particle size of the positively and negatively charged nanoemulsions was measured directly after their production (day 0). These nanoemulsions were stored at 25 ± 2 °C and 40 ± 2 °C over 12 months (positively charged nanoemulsion) and over 6 months (negatively charged nanoemulsion), respectively, and their long-term physicochemical stability was investigated by providing the measurements at the days 30, 90, 180 and 360, respectively. For measuring the mean particle size with dynamic light scattering (DLS) a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) was used. The DLS technique yields a light intensity weighted mean diameter (*z*-average) and the polydispersity index (PDI) as a degree for the width of the size distribution. A PDI below 0.2 indicates a narrow size distribution. DLS has an upper detection limit of approximately 3 μm. For excluding the existence or the occurrence of larger particles (emulsion droplets), low angle static light scattering (laser diffraction, LD, LS 230, Beckmann-Coulter, Krefeld, Germany), including Polarization Intensity Differential Scattering (PIDS) technology was used. Mie theory with the optical parameters 1.456 (real refractive index) and 0.01 (imaginary refractive index) were used for analysing the LD results.

2.2.2.2.2. Zeta potential. The surface charge of the nanoemulsions, expressed as zeta potential, was determined by measuring the electrophoretic mobility. 20 μl of the sample was added to 40 ml purified water and measured. A pH of 5.5 ± 0.1 is required for all measurements and is adjusted, if necessary, with diluted HCl. Prior to the measurement, the mixture was adjusted to a conductivity of 50 μS/cm using a NaCl solution. The zeta potential was calculated by applying the Helmholtz–Smoluchowski equation using the Zetasizer Nano ZS (Malvern, 2002; Müller, 1996).

2.2.2.2.3. Release. The release studies of both the positively and the negatively charged PC containing nanoemulsion were performed using Franz diffusion cells. Due to the fact that the used PC is practically insoluble in water, PBS as the acceptor fluid is a limiting factor. For this reason several ethanol–PBS mixtures (from 80:20 to 20:80) were tested as a possible acceptor fluid with respect to the solubility of PC. Using Nephrophan® (ORWO, Wolfen, Germany) membrane the Franz diffusion cells were filled with 7 ml of a ethanol–PBS mixture (1:1) and temperated for 1 h. 500 μl of the positively and negatively charged PC containing nanoemulsion were applicated on the Nephrophan® membrane. 500 μl samples were taken after 1, 2, 3, 6 and 24 h, replaced by freshly temperated ethanol–PBS mixture and analyzed for the drug concentration by HPLC (n = 5).

2.2.2.2.4. Penetration. The used excised skin came from Caucasian female patients aged from 18 to 55 years, who had undergone abdominal plastic surgery maximum 24 h ago. Using scalpel and scissors the subcutaneous fatty tissue was immediately removed after excision, then the blood was wiped off with PBS and patted dried with pulp. After that skin disks were punched out (diameter 30 mm) and stored in petri dishes with 1.5 ml Dulbecco's Modified Eagles Medium at 4 °C until the penetration experiments were finished within 72 h, in order to ensure the viability of the skin. The skin samples were carefully attached on Franz diffusion cells, filled with a ethanol–PBS mixture in equal parts (approximately 7 ml) and temperated for 1 h at 32 ± 1 °C using a water bath. 500 μl, equivalent to 1250 μg PC, of the positively and negatively charged nanoemulsion were applicated on excised human skin samples (n = 5) in order to investigate the penetration of PC. 300 μl samples were taken after 1, 2, 3, 6 and 24 h and replaced by freshly temperated ethanol–PBS mixture. After 24 h the residual nanoemulsion formulation was removed from the surface of the excised skin, washed carefully five times with 1 ml isopropanol, dried and the drug content was determined by HPLC. After that the skin samples were placed on dishes at −80 °C in order to cut the skin in layers using a cryomicrotome.

2.2.2.2.5. Extraction. Three different exctracting agents (ethanol, ethylacetate and a mixture of acetonitrile and water (6:4)) and extracting methods (ultrasonic bath, freezing with liquid nitrogen and a combination of ultrasonic bath and freezing with liquid nitrogen) were tested for the penetration studies.

3. Results and discussion

3.1. Production of the nanoemulsions

The optimization of the positively charged PC containing nanoemulsion was described detailed in Baspinar et al. (2010). The general procedure for producing nanoparticles with the high pressure homogenization method is described more detailed in Jahnke (2001). The positively and negatively charged nanoemulsions were produced at 50 °C, 300 bar and 10 homogenization cycles. The positively charged nanoemulsion was composed of 0.25% PC, 0.6% PS, 20% Eutanol, 2% Tween 80, 2% Lipoid E80, 0.03% α-tocopherol and 0.1% potassium sorbate. For the negatively charged formulation the fatty acids myristic acid, stearic acid, lauric acid and palmitic acid were tested. Stearic acid (1%) was not soluble in Eutanol G, while lauric acid and palmitic acid were soluble, but precipitated after 3 days. From the examined fatty acids only myristic acid was suitable for producing negatively charged nanoemulsions.

3.2. Characterization

3.2.1. Chemical stability of prednicarbate in the nanoemulsions

The concentration of PC was investigated at the day of the production for the negatively and positively charged nanoemulsions, but over a storage period of 12 months only for the positively charged nanoemulsion (Fig. 2). The PC concentration in the positively charged nanoemulsions decreased to 92% after storage at 25 ± 2 °C and to 83% after storage at 40 ± 2 °C, which are quite surprising results, especially for an O/W (nano-) emulsion. That decrease of the PC concentration is explainable with the chemical double ester structure of PC in combination with the O/W emulsion

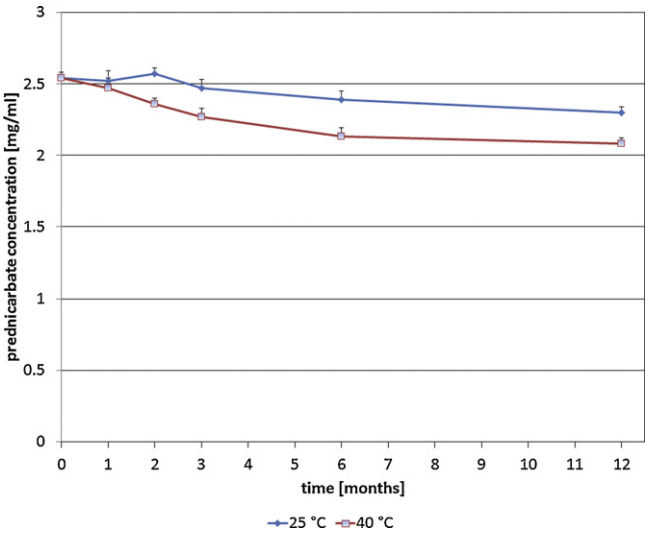

Fig. 2. Chemical stability of prednicarbate for the positively charged nanoemulsion after storage at 25 ± 2 °C and 40 ± 2 °C over a period of 12 months (mean ± SD; $n = 6$).

system, which leads to spontaneous, partial hydrolysis. However the chemical stability of the negatively charged nanoemulsion was just checked on the production date (day 0) and was not investigated further, because this formulation was produced just for the comparison of the release and penetration studies with the positively charged nanoemulsion.

In a previous study the relation between the homogenization parameter and the chemical stability was demonstrated (Baspinar et al., 2010). It could be shown, that increasing the number of homogenizing cycles and homogenization temperature increases the chemical stability of PC, until the best results, namely the highest concentration of the incorporated PC, was achieved with 50 °C, 10 cycles and 300 bar. The solubility of a poor water soluble drug could be increased be producing a lecithin based oil in water (O/W) emulsions (e.g. Lipofundin) with the high pressure homogenization method, known as the SolEmuls technology (Müller, 2000). There are several examples in the literature of incorporating poorly soluble drugs into a nanoemulsion resulting in stable formulations with the help of the SolEmuls technology (Akkar and Müller, 2003a,b; Akkar et al., 2004; Buttle, 2004; Müller et al., 2004; Junghanns et al., 2007). Although there are similarities between the SolEmuls technology and the method we have used here, the process of our study is different. For the SolEmuls technology as well as in our case the production was made by high pressure homogenization and the obtained formulation was an emulsion. While for the 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, in our case PC was dissolved in the oil phase prior the homogenization.

3.2.2. Physical stability of the nanoemulsions

3.2.2.1. Particle size analysis. The mean particle size of the positively charged nanoemulsion was 157 nm at the day of production (day 0), 166 nm and 158 nm after storage of 12 months at 25 ± 2 °C and 40 ± 2 °C, respectively (Fig. 3A). On day 0 the mean particle size of the negatively charged nanoemulsion was 136 nm, 141 nm and 150 nm after storage of 6 months at 25 ± 2 °C and at 40 ± 2 °C (Fig. 3B). That increase in the particle size of the negatively charged nanoemulsion shows a certain physical instability, which could be caused by an insufficient coverage of the particle surface with the surfactant. However, this physical instability was only observed for the negatively, but not for the positively charged nanoemulsion. Due to the fact that the only difference in the composition between the negatively and positively charged nanoemulsion is the agent which causes the charge of the formulation, that instability is most likely based on the function of this agent itself. The results of the physical stability investigations indicate that PS serves, due to its amphiphilic structure, partially as a (co-)surfactant and supports Lipoid E80 and Tween 80 by forming a mixed film at the o/w interface of the oil droplets. Very similar results were described by Elbaz et al. (1993) with stearylamine, phospholipids and poloxamer. The highest PDI of the positively and negatively charged nanoemulsions was 0.11 (Fig. 3), even after storage, which indicates a narrow distribution of the nanoemulsion droplets.

PS is a very poor soluble substance with distinctive solubility problems, thus is reflected in the bigger particles of the positively charged nanoemulsions compared to the negatively charged nanoemulsion containing myristic acid. Nevertheless the mean particle size and the polydispersity index of the positively and negatively charged nanoemulsions after storage are remarkably stable. The LD size (LD 99) of the positively and negatively charged nanoemulsions was consistently under 300 nm, even after storage (Fig. 4). That means 99% of the measured particles in the formulations are smaller than 300 nm.

3.2.2.2. Zeta potential. A basic rule signifies, that a formulation with a zetapotential value over 30 mV, independent from the positive or

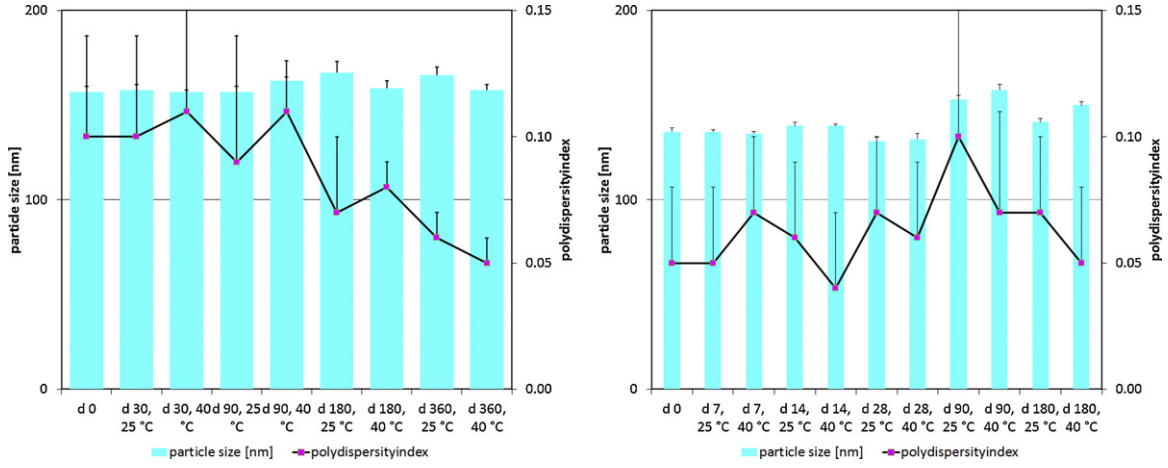

Fig. 3. The mean particle size (PCS) and the polydispersityindex of the positively (A) and negatively (B) charged prednicarbate containing nanoemulsion over a period of 12 months (A) and 6 months (B), respectively (mean ± SD; $n = 3$).

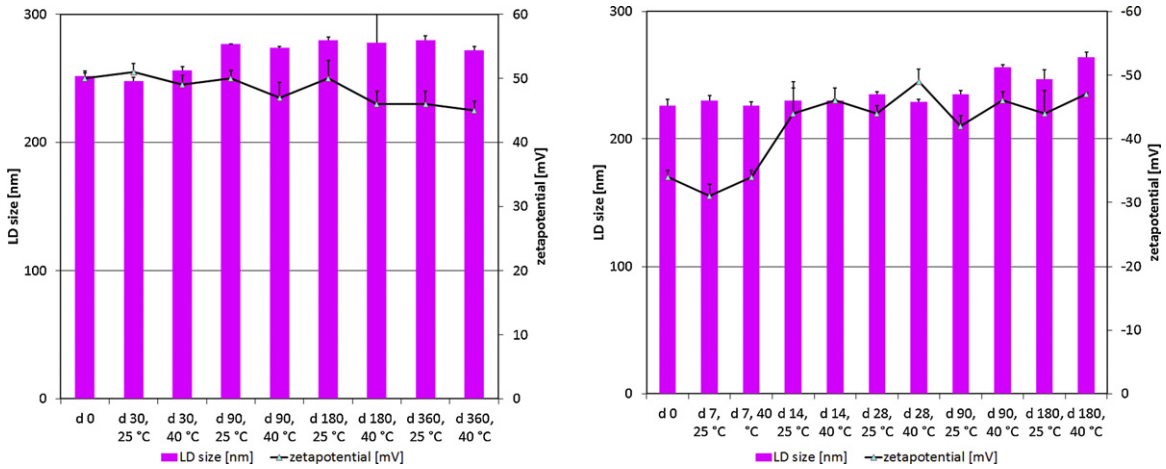

Fig. 4. The particle size distribution (LD) and the zetapotential of the positively (A) and negatively (B) charged prednicarbate containing nanoemulsion over a period of 12 months (A) and 6 months (B), respectively (mean ± SD; $n = 3$).

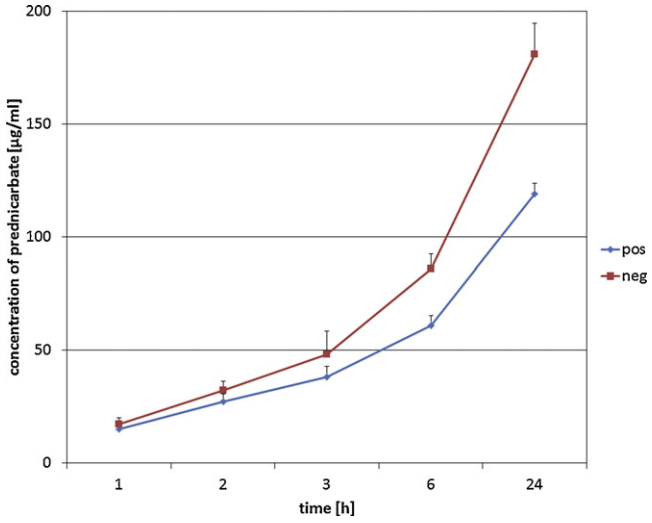

Fig. 5. Release of prednicarbate from the positively and negative charged nanoemulsion formulations (mean ± SD; $n = 5$).

negative prefix, is generally regarded as physically stable (Malvern, 2002; Müller, 1996).

3.2.2.3. Release. The release of PC from the negatively charged nanoemulsion was significantly ($p < 0.05$) higher compared to the positively charged nanoemulsion (Fig. 5). Regarding the particle size of the nanoemulsions (Fig. 3) reveals that the negatively charged formulation with the smaller particle size (136 nm) has a higher release compared to the positively charged formulation (157 nm). The smaller the particles are, the bigger is the surface and the solubility pressure of the particles. A similar result concerning the release of a positively charged submicron emulsion was also observed by Wang et al. (2006). They studied the release rate of nalbuphin and his two prodrugs in accordance to different surfactants. They found out that the release rate essentially depends from the co-surfactant. The formulation containing stearylamin, which caused a positive zetapotential, had the lowest release, which is concordant with our results. Summarizing the findings of Wang et al. (2006) and our results it can be concluded that the release is controlled, among others, by the particle size and probably by the (co-)surfactant, which is partially in our case PS.

3.2.2.4. Penetration. Prior to analysing the amounts of PC from the penetration studies, each skin was cut at −20 °C with a cryomicrotome into layers of 50–100 µm, in order to achieve the best possible extraction of the drug. The penetrated drug was extracted using the most suitable extraction agent (ethyleacetate) and the best extraction method (the combination of ultrasonic bath and freezing with liquid nitrogen) and was analysed by HPLC. Betamethasonevalerate was used as internal standard to check the recovery rate.

Regarding the released and penetrated amounts of PC, the penetration results become more interesting. The release of the negatively charged nanoemulsion is significantly higher (181 µg/ml after 24 h) compared to the positively charged nanoemulsion (119 µg/ml after 24 h). The penetration of PC from the positively charged nanoemulsion (18.4 ± 3.4 µg/ml after 24 h) is higher than the penetration rate of the negatively charged formulation (11.7 ± 2.5 µg/ml after 24 h). Comparing the penetration to release ratios of PC from the nanoemulsions reveals that approximately 6.5% of the negatively charged nanoemulsion (11.7 µg/ml to 181 µg/ml) and about 15.5% (18.4 µg/ml to 119 µg/ml) of the positively charged nanoemulsion penetrated into the skin.

These results support our postulation at the beginning of this paper that a positive charge of the nanoemulsion could have a benefit.

Generally, the smaller the particle size, the higher is the release, due to an increased surface of the particles and an increased solubility pressure of the particles. An enhanced penetration is expected for an adequate release. The results of our studies reveal the opposite effect. The negatively charged nanoemulsion, with a smaller particle size and higher release, has a decreased penetration compared to the positively charged nanoemulsion. The increased penetration of the positively charged nanoemulsion is explainable by an increased interaction and adsorption of the particles with the negatively charged corneocytes of the stratum corneum, the main barrier of the skin (Piemi et al., 1999; Song and Kim, 2006). These results show that there is a clear relation between the charge of the nanoemulsion and the skin penetration. After 24 h the residual formulation of the 5 excised skins was rinsed off with PBS and determined by HPLC. The recovery rate was about 90 ± 6% for the positively charged and 96 ± 3% for the negatively charged nanoemulsion. The samples taken after 1, 2, 3, 6 and 24 h from the acceptor compartment showed no detectable concentrations of PC. These results show that there is a clear penetration into the skin, but probably no detectable permeation through the skin.

4. Conclusion

Stable PC containing negatively and positively charged nanoemulsions were produced with high pressure homogenization method at 50 °C, 10 cycles and 300 bar. The positively charged nanoemulsion was composed of 0.25% PC, 0.6% PS, 20% Eutanol, 2% Tween 80, Lipoid E80, 0.03% α-tocopherol and 0.1% potassium sorbate. For the negatively charged nanoemulsion myristic acid was used instead of PS. These nanoemulsions were stored at 25 ± 2 °C and 40 ± 2 °C, respectively, and characterized during the storage time of 12 and 6 months, respectively, by measuring the PC concentration, the mean particle size, the PDI, the LD size, the zeta potential, the release and the penetration. Finally we could show that the physicochemically stable positively charged PC containing nanoemulsion had a decreased release, but an increased penetration of the drug into the excised human skin, compared to the negatively charged nanoemulsion, what we had postulated at the beginning of this paper. The higher release of the negatively charged nanoemulsion is explainable with the smaller particle size. The smaller particles of the negatively charged nanoemulsion are increasing during the storage at 40 ± 2 °C, which points a certain physical instability of the formulation. The increased penetration is explainable due to an enhanced interaction of the positively charged nanoemulsion with the negatively charged corneocytes of the stratum corneum, the main barrier of the skin. Beside this enhanced penetration, the incorporation of PS makes our developed formulation a promising alternative compared to the commonly used formulations. The decreased concentration of PS is associated with an impaired stratum corneum barrier function and dry skin, because of its role during the ceramid synthesis. Summarized it can be concluded that this positively charged nanoemulsion containing PC is a promising formulation for topical use with the indication atopic dermatitis.

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