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Research paper

The influence of solid lipid nanoparticles on skin hydration and viscoelasticity – in vivo study

Sylvia A. Wissing, Rainer H. Müller*

Department of Pharmaceutics, Biopharmaceutics and Biotechnology, The Free University of Berlin, Berlin, Germany

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Abstract

Effects on skin hydration and viscoelasticity are important criteria during the development of novel cosmetic formulations. This study focuses on the in vivo performance of a conventional o/w cream and of the same cream enriched with solid lipid nanoparticles (SLN). Influences on skin hydration and viscoelastic properties were investigated with validated devices (Corneometer® and Cutometer®). After an application period of 4 weeks, significant changes in skin hydration were detected for both formulations. The SLN-enriched cream was significantly more effective than the conventional cream (+24% for the cream and +31% for the SLN-cream). The viscoelastic parameters UF and UA/UF remained almost unchanged, which is attributed to the young age of the volunteers. At this stage, SLN represent a promising compound for hydrating new cosmetic formulations.

Keywords: Solid lipid nanoparticles; Skin hydration; Viscoelasticity; Cutometer; Corneometer

1. Introduction

For the development of new cosmetic products, important criteria are their positive effects on skin hydration and viscoelasticity [1]. These effects can be influenced by adequate vehicles or by active ingredients which are incorporated in a formulation [2].

The water content of the skin is 10–20% [2]. Skin hydration can be increased by occlusive topicals. Since many topical preparations have an undesirable esthetic appearance (e.g. petrolatum), the need for novel occlusives is rising.

The skin is a viscoelastic organ. Collagen and elastic fibers represent the elastic components [3,4], whereas friction between cells and collagen bundles and other components of the dermal matrix accounts for the viscous parts [5]. Also, the epidermis has a strong effect on frictional resistance of the skin, depending on its water and lipid content [6]. An increase in skin hydration and lipid content improves the viscous resistance against deformation.

Further internal and external factors influencing the biomechanical properties of the skin are gender, age, body region, genetic dispositions, exposure to UV radiation and diseases [3,7,8].

Solid lipid nanoparticles (SLN) have been introduced as a potent carrier system for various pharmaceutical drugs and cosmetic active ingredients [9–12]. They are capable of, e.g. protecting unstable active compounds from degradation [13,14] and releasing the actives in a controlled way [15–18]. It has also been shown that SLN have distinct occlusive properties in vitro depending on their size, crystalline status and lipid concentration [19,20]. This is due to the fact that SLN form an intact film upon drying thus decreasing water evaporation from the skin to the atmosphere.

In this study, the hydrating and viscoelasticity improving effects of an o/w cream containing SLN and of a conventional o/w cream were investigated in vivo after repetitive application for 28 days. The effects were monitored with well-established techniques, i.e. skin hydration was determined by the Corneometer® method [21,22] and viscoelastic measurements were performed with a Cutometer® in the strain-time-mode [23–25]. The

* Corresponding author. Department of Pharmaceutics, Biopharmaceutics and Biotechnology, The Free University of Berlin, Kelchstrasse 31, D-12169 Berlin, Germany. Tel.: +49-30-83850696; fax: +49-30-83850616.
E-mail address: mpharma@zedat.fu-berlin.de (R.H. Müller).

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investigated formulations did not contain active ingredients in order to visualize the effects of the pure vehicles. The aims of this study were the proof that both vehicles are effective within 28 days and the assessment whether and to which extent the SLN-containing formulation shows greater effects compared to the conventional cream.

2. Materials

The conventional cream was (formulation A) composed of Glyceryl stearate citrate, Hydrogenated coco glycerides, Tridecyl stearate, Tridecyl trimelliate, Depentaerythrityl hexacaprylate/hexacaprate, Octyl cocoate, *Camellia sinensis*, *Butyrospermum parkii* butter, Octyl methoxycinnamate, Myristyl alcohol, Cetyl alcohol, Carbomer, Glycerine, Titan dioxide, Phenoxyethanol, Methyl-, Ethyl-, Propyl- and Butylparaben and Methyldibromo glutaronitrile and was provided by Beiersdorf (Hamburg, Germany). Glycerol (Sigma, Germany), Carbopol 940 (BF Goodrich, Belgium), Tromethamine (Sigma, Germany) and MilliQ water (FU Berlin, Germany) were purchased. The SLN dispersion consisted of 10% cetyl palmitate (Cutina CP®, Henkel AG, Germany), 1.5% sucrose stearate S1670 (Mitsubishi-Kagaku Foods Korporation, Japan), and 88.5% MilliQ water (FU Berlin, Germany).

3. Methods

3.1. Production of SLN

SLN were produced by the hot homogenization technique on a homogenizer assembly consisting of a Lab 60 and a Gaulin 5.5. (APV Gaulin, Germany) [26,27]. The homogenizing temperature was 85°C, the main pressure was 500 bar, the holding pressure of the second homogenization valve was 50 bar and the product container was cooled with water at a temperature of 18°C. The feeding container was stirred using a dissolver disc at 500 rpm and the product container was stirred using a propeller stirrer at 500 rpm. One homogenization cycle was sufficient for the production of nanoparticles with a narrow size distribution.

3.2. Production of creams

Lipid phase and water phase were heated separately to 80°C, combined and cooled down to 38°C while stirring. At 38°C, the gelling agent (and the SLN dispersion in case of formulation B) was added before cooling down to room temperature while stirring. For the SLN-containing cream (formulation B), 40% of the water phase was replaced by SLN dispersion, i.e. formulation B contained a percentage of 4% SLN particles, additionally the oil content of the lipid phase was decreased by 4% in order to have identical lipid percentages in the two formulations.

3.3. Particle size measurements

Particle size was analyzed by laser diffraction (LD) using a LS230 (Coulter Electronics, Germany) with a measuring range from 40 nm to 2000 μm. This method was chosen because it can simultaneously detect nanoparticles from the SLN dispersion and microparticulate droplets in the o/w cream.

3.4. Thermal analysis

Differential scanning calorimetry (DSC) was performed in order to determine the integrity of the SLN after incorporation into the cream. Approximately 10 mg samples were filled in aluminum pans, sealed and analyzed using a DSC 821/700e (Mettler-Toledo, Germany). The samples were heated from 20 to 85°C at a heating rate of 5 K/min. An empty aluminum pan served as reference. The oven was flushed with 80 ml N_2/min.

3.5. Corneometer® measurements

Skin hydration was investigated using a Corneometer® CM 825 (Courage und Khazaka, Germany) which was mounted on a Multi Probe Adapter® MPA 5 (Courage und Khazaka, Germany). Capacitance changes depending almost solely upon the water content in the stratum corneum are detected and evaluated. Five measurements were performed in each testing area on the volar forearm. The results are given in 'arbitrary units' (arb. units). Statistical evaluation was performed with the Wilcoxon test ($\alpha = 5\%$).

3.6. Cutometer® measurements

The viscoelastic properties of the skin were investigated with a Cutometer® SEM 575 (Courage und Khazaka, Germany). The measuring principle is suction/elongation. An optical system detects the decrease of infrared light intensity depending on the distance the skin is being sucked into the probe. In this study, the strain-time-mode was applied. A probe with a 2 mm opening was used and a pressure of 400 mbar was applied in order to suck the skin into the probe. Each measurement consisted of five suction cycles (3 s of suction followed by 3 s of relaxation) and was performed in triplicate on the volar forearm. The absolute parameter UF (MD1 = maximum deformation during the first cycle) (mm) and the relative parameter UA/UF (relation between maximum deformation during the first cycle and back formation directly after the first cycle = gross elasticity) were evaluated. These parameters have been introduced to the literature by Aubert et al. [28] and Escoffier et al. [29].

4. Study design

The study was designed as a one-sided blind, placebo controlled study with intra-individual comparison of two formulations and two untreated test areas on the volar forearm, i.e. each forearm was virtually divided into two parts, one for the application of one formulation (left arm: A, right arm: B) and one control field per forearm. Twenty-five healthy Caucasian females (27 ± 4 years) with healthy skin, who signed informed consent used the two test formulations. The basic values were measured on three subsequent days immediately before the start of the study. For 28 days, the test formulations were applied twice daily on the forearms following the instruction leaflet. Approximately 0.2 ml product was applied each time; 12–16 h after the last application, the final values were measured. Before the measurements, the volunteers were accommodated in a controlled room at 21 ± 1°C and 40 ± 2% r.h. for 30 min. All volunteers finished the study, no drop-outs were present. The compliance was verified by weighing the samples before and after the study. The consumption was registered.

5. Results and discussion

Physically stable SLN with a narrow size distribution were produced and successfully incorporated into the o/w cream. Fig. 1 shows the size distributions of the nanoparticle dispersion, the o/w cream and the o/w cream containing SLN measured by LD one day after production.

The SLN dispersion is distributed completely in the nanometer range. About 99% of the particles are below 480 nm (Fig. 1, left). The cream formulations were diluted with water in a ratio of 1:100 before the measurements in order to prevent formation of aggregates. The size distribution of the pure cream formulation shows one homogeneous peak in the lower micrometer range (1–14 μm). The average particle size is 4.2 μm. Incorporation of SLN into the cream leads to a bimodal size distribution. Both peaks are clearly separated and correspond to the peaks of their respective formulations. The size distribution remained unchanged upon storage for up to 6 months [30] as has been shown for this formulation in the literature before [31].

Quantitative incorporation of solid state SLN and complete absence of SLN dissolution in the oil phase was further proven by DSC. The samples were heated above their melting point and the melting enthalpy was recorded. Fig. 2 shows the thermographs of pure SLN dispersion, pure o/w cream and SLN incorporated into o/w cream one day after production.

The cetyl palmitate SLN scan reveals the crystalline character of the nanoparticles. The o/w cream contains a partly crystalline surfactant, which melts well above cetyl palmitate. Incorporation of SLN into the cream leads to a visible decrease in the two melting points of approximately 2°C. However, at room temperature, the particles remain solid. Both peaks are well separated and the melting enthalpies can be evaluated. After standardization of the enthalpy values on the lipid content, the melting enthalpy in the SLN formulation is 91.0 J/g and after incorporation into the cream 89.8 J/g. After storage at room temperature for one month, the enthalpy values were 90.7 J/g (SLN) and 90.3 J/g (SLN-cream). Due to the fact that the melting enthalpies remain almost unchanged, quantitative incorporation and absence of dissolution of SLN is shown.

The actual study was performed in conformity to the original study design. No drop-outs were present in the study. No side-effects took place during the study period. The demographic data of the 25 female volunteers revealed

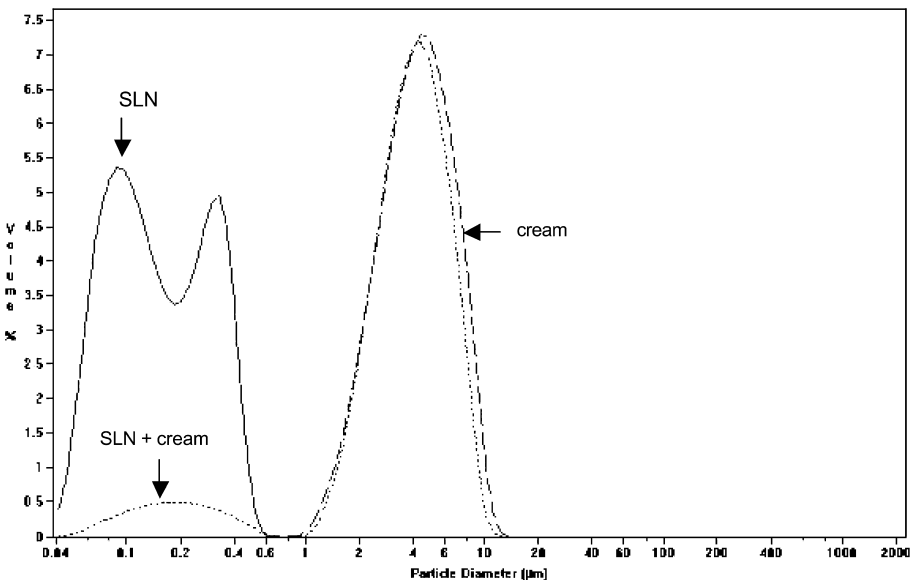

Fig. 1. Size distribution of SLN, o/w cream and SLN incorporated in o/w cream measured by laser diffraction.

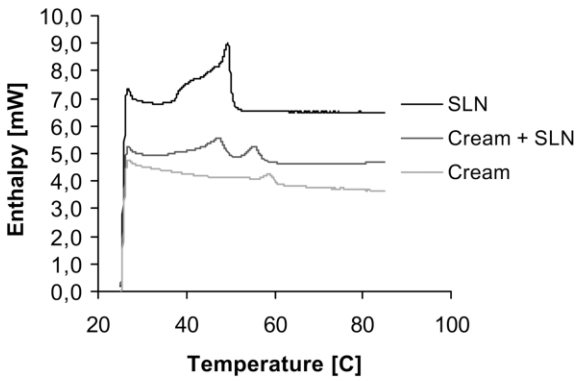

Fig. 2. DSC scans of SLN, o/w cream and SLN incorporated in o/w cream (20–85°C; 5°C/min).

an average age of 27 ± 4 years. Eight percent of the volunteers were smokers and all volunteers were university students at the time of the study. Therefore, the group was proportionally homogeneous.

Verification of the compliance showed that during the testing period, on an average 11.04 g of formulation A (conventional cream, SD 5.50) and 10.55 g of formulation B (cream with SLN, SD 6.36) were used. As expected, the distribution was wide-spread. Since the average weekly consumption was at least approximately 2 g, compliance was taken for granted. The amount used was similar for both formulations.

Tables 1 and 2 list the mean values, standard deviations, medians, minimal and maximal values of the raw data (Table 1) and the quotients with regard to untreated control (Table 2).

The initial Corneometer® values were similar (cf. Table 1). During the application period, formulation A increased the average skin hydration from 31.7 (arb. units) (min = 24.4, max = 41.7) to 39.7 (arb. units) (min = 30.2, max = 51.2). Application of formulation B for 28 days leads to an average increase in skin hydration from 31.7 (arb. units) (min = 25.1, max = 40.3) to 41.8 (arb. units) (min = 32.8, max = 52.6). The untreated controls remained almost unchanged, i.e. 31.7 (arb. units) on day 0 and 31.9 (arb. units) on day 28. Standard deviations of the raw data were between 4.3 and 4.9 (arb. units).

Relative inspection of the data with regard to the untreated control (Table 2) revealed an increase of the mean values after 28 days of 1.253 (arb. units) for formulation A (SD 0.013) and of 1.318 (arb. units) for formulation B (SD 0.007).

Variation analysis of the Corneometer® results is shown in Fig. 3. The results are calculated after Eq. (1)

$$\text{Parameter changes } (\%) = (\Sigma Q_{ti}/\Sigma Q_{0i} - 1)100, \quad (1)$$

where Q_{ti} is the quotient after application time t of 28 days for each volunteer i, and Q_{0i} is the quotient before application time for each volunteer i. Formulation A increased skin hydration by 24.14% and formulation B lead to an increase of 31.92%.

The Corneometer® results showed that both formulations increased the hydration of the skin. Formulation B is significantly more effective than formulation A (Wilcoxon test, $\alpha = 5\%$). One reason for this is the pronounced occlusivity of SLN formulations. The SLN formulation used in this study contains highly crystalline lipid nanoparticles made of the wax cetyl palmitate in a narrow size distribution in the lower nanometer range. It has been shown in vitro [19,20] that the occlusion factor of SLN depends strongly on crystallinity and size. SLN show the tendency to fuse forming a dense film after application to the skin. This effect is considered to be comparable to the film formation on coated tablets when using polymeric pseudolatices, e.g. Eudragit. The capillary forces of the nanometer pores between the SLN particles are contractive promoting fusion and dense film formation (Fig. 4, right). Large micrometer-sized particles or oil droplets (formulation A) form films with larger pores than 200 nm SLN (Fig. 4, upper left and right). Also, due to the liquid character of the droplets, water

Table 1
Descriptive statistics of the raw data

	Formulation	Day	Average	SD	Median	Minimum	Maximum
Corneometer	A	0	31.7	4.9	31.4	24.4	41.7
	B	0	31.7	4.7	31.0	25.1	40.3
	A	28	39.7	4.6	40.2	30.2	51.2
	B	28	41.8	4.4	41.3	32.8	52.6
	Control A	28	32.0	4.9	32.2	21.8	39.4
	Control B	28	31.7	4.3	31.8	25.2	41.5
Cutometer UF	A	0	0.40	0.05	0.38	0.32	0.48
	B	0	0.38	0.06	0.38	0.25	0.49
	A	28	0.43	0.06	0.43	0.30	0.55
	B	28	0.43	0.06	0.42	0.31	0.55
Cutometer UA/UF	A	0	0.84	0.10	0.87	0.57	0.96
	B	0	0.84	0.09	0.86	0.66	0.99
	A	28	0.93	0.07	0.96	0.76	1.00
	B	28	0.95	0.03	0.94	0.88	1.00

Table 2
Descriptive statistics of the quotients (with regard to untreated control)

	Formulation	Day	Average	SD	Median	Minimum	Maximum
Corneometer	A	28	1.25	0.01	1.28	1.24	1.27
	B	28	1.32	0.01	1.33	1.31	1.33
	Control A	28	1.01	0.06	1.03	0.89	0.94
	Control B	28	1.00	0.02	1.03	1.00	1.03
Cutometer UF	A	28	1.08	0.10	1.13	0.95	1.16
	B	28	1.14	0.06	1.11	1.24	1.12
Cutometer UA/UF	A	28	1.11	0.73	1.10	1.33	1.04
	B	28	1.13	0.36	1.09	1.32	1.01

evaporation through this film is even more likely to occur. Therefore, addition of SLN to a cream increases skin hydrating effects by different means.

The Cutometer® parameter UF (maximum deformation during the first cycle) gives information about the firmness of the skin. A lower amplitude correlates with firmer skin. During the application period, the parameter UF increased for formulation A from 0.40 mm (SD 0.05) to 0.43 mm (SD 0.06) and for formulation B from 0.38 mm (SD 0.06) to 0.43 mm (SD 0.06) (cf. Table 1).

The maximum amplitude during the first suction cycle of the Cutometer® increased slightly after application of the pure cream and remained almost unchanged after application of cream enriched with SLN. Therefore, the tightening effects on the skin are negligible. Also, it has to be remarked critically that the inter-individual differences of this absolute parameter were well-pronounced. In order to make valuable statements on effects which influence the viscoelasticity of the skin, it is useful to investigate relative parameters instead of absolute parameters.

The relative Cutometer® parameter UA/UF stands for the gross elasticity of the skin. The skin is 100% elastic if UA/UF reaches 1. If UA/UF = 1, the skin recovers completely directly after the first cycle. The initial values were similar (cf. Table 1). The application period of 28 days lead to an increase of UA/UF from 0.84 mm (SD 0.10) to 0.93 mm (SD 0.07) for formulation A and from 0.84 mm (SD 0.09) to 0.95 mm (SD 0.03) for formulation B (cf. Table 1). Investigation of the quotients with regard to the untreated control (Table 2) showed an increase of 1.11 mm for formulation A and 1.13 mm for formulation B. Both formulations increased the gross elasticity of the skin to a certain extent. However, there was no difference in effectiveness between the formulations, therefore no statistical analysis was performed. It has to be remarked that the volunteers had high initial gross elasticity values. Due to the fact that they were 27 ± 4 years old, they did not have so-called 'aged' skin. A further improvement of their skin elasticity would not be necessary or even desirable.

Using the chosen conditions, it was not possible to monitor an increase in elasticity and tightness on the skin. Other biomechanical devices or an older patient panel are needed to visualize these effects.

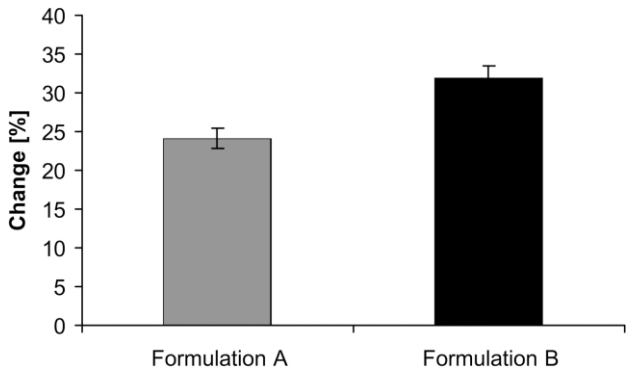

Fig. 3. Changes of Corneometer values after 28 days of application for formulation A (without SLN) and formulation B (with SLN).

6. Conclusion

It was possible to produce an SLN formulation which was physically stable after incorporation into a cosmetic o/w cream. The in vivo study showed that both formulations (pure cream and cream enriched with SLN) were able to significantly increase skin hydration within 4 weeks of treatment. Further, the formulation with additional SLN produced a distinctly higher increase of skin hydration compared to the conventional cream.

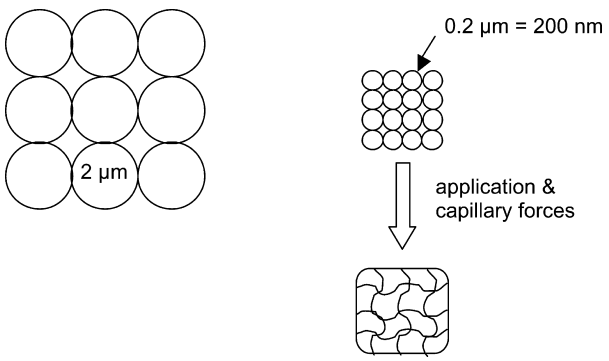

Fig. 4. Model for film formation of micro- (left) and nanoparticles (right) due to capillary forces after application to skin.

Due to the young age of the volunteers, effects upon the viscoelasticity of the skin were not detectable. No statement can be made regarding the influence of both test formulations on biomechanic skin properties.

Concluding, SLN represent a highly effective carrier for cosmetic creams which are intended to increase skin hydration.

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