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Stability and *In Vitro* Skin Compatibility of an Adapalene-Loaded Solid Lipid Microparticle Dispersion

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Abstract

A novel adapalene-loaded solid lipid microparticle (SLMA) dispersion composed of topically approved ingredients exhibited follicular penetration and a targeted drug release in sebum. In the present study, the stability regarding particle size, thermoanalytical properties, drug content, and pH value was tested after storage at 5°C, 23°C, and 40°C over 12 weeks. Additionally, a thermal cycling study was performed in order to consider storage temperature variations. The dermal compatibility was tested on a HaCaT monolayer in comparison to the Differin® cream in order to evaluate potential local side effects. The SLMA dispersion displayed a pronounced physical stability at 5°C including a constant particle size as well as melting point. Compared to that, the physical stability was limited at 40°C with significant particle growth and a significantly increased melting temperature, whereas the properties just slightly changed during storage at 23°C. The drug content and pH value remained the same. The skin compatibility test revealed a high cell viability of about 90% for all investigated dilutions of the SLMA dispersion probably due to the presence of glycerides and lecithin. On the contrary, the dilutions of the Differin® cream caused a significant decrease of the cell viability to approximately 11% based on its ingredients.

Keywords: Adapalene; Solid lipid microparticle dispersion; Stability; Skin compatibility; Inactive ingredient; Topical formulation.x

Abbreviations: Adapalene-Loaded Solid Lipid Microparticle (SLMA); 3-(4,5-dimethyl-2-thiazolyl) 2,5-diphenyl-2H-tetrazolium bromide (MTT); Dulbecco's Modified Eagle Medium (DMEM); Food and Drug Administration (FDA); Human adult low Calcium high Temperature (HaCaT); International Conference on Harmonisation (ICH); Laser Diffractometer (LD); Melting onset temperature (T_{onset}); Micellization onset temperature (T_{micell}); Poloxamer 407 (P407); Recrystallization onset temperature ($T_{recryst}$)

Introduction

A novel adapalene-loaded solid lipid microparticle (SLMA) dispersion composed of 13.93% hydrogenated palm oil, 5.97% purified lecithin, 0.1% adapalene, 12% poloxamer 407 (P407), 3% polyethylene glycol 12000, 0.2% potassium sorbate, 0.1% citric acid, and 64.7% water of double-distilled grade (all by weight) featured follicular penetration, erosion in sebum lipids and a superior drug release in sebum compared to the commercial cream formulation Differin® [1,2]. Further convenient attributes are the physical and chemical stability regarding particle size, melting behavior, and drug content at ambient storage conditions, and the utilization of safe inactive ingredients for dermal application. However, according to FDA and ICH guidelines storage at different as well as cycling temperatures is recommended in order to prove robust stability as well as the suitability for transportation [3,4]. Furthermore, even established ingredients such as isopropyl

myristate in topical vehicle compositions might decrease the viability of skin cells [5]. Therefore, the SLMA dispersion was subjected to a stability study at 5°C, 23°C, and 40°C over 12 weeks as well as a thermal cycling sequence including three cycles comprising alternating storage at 5°C and 40°C each for 2 days. Furthermore, the cell viability was compared between the SLMA dispersion and the Differin® cream on a HaCaT monolayer by performing an MTT test.

Materials and Methods

The SLMA dispersion was manufactured via a rotor-stator homogenization procedure by uniting both the aqueous and lipid phase, dispersing them with 16000 rpm for 3 min, and subsequent extrusion through a membrane all at 70°C. At each measuring point the mean particle size and particle size distribution comprising D_{10}, D_{50}, and D_{90} was determined with a laser diffractometer (LD, LS 13320, Beckman Coulter). The thermoanalytical data including the melting onset temperature (T_{onset}), recrystallization onset temperature ($T_{recryst}$), and the micellization onset temperature (T_{micell}) of P407 were determined with a DSC1 (Mettler Toledo). The drug content was measured with a high performance liquid chromatography system equipped with a LiChroCART® 250-4 Purospher® STAR RP-18 endcapped (5 µm) column (Merck) after extraction in a solvent mixture of 45% acetonitrile, 35% tetrahydrofuran, and 20% purified water (all by volume). The pH value was determined with an Inlab® solids polymer electrode (Mettler Toledo).

For the *in vitro* skin compatibility testing, 20.000 HaCaT cells were seeded into each well of a 96 well plate and incubated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum, 2mM l-glutamine, and antibiotics at 37°C with 5% CO_2 for 24 hours. After removal of the medium, the formulations were diluted to 1:10, 1:100, and 1:1000 in DMEM and incubated with the HaCaT monolayer under the mentioned conditions. Afterwards an MTT test was performed for cell viability determination compared to DMEM as positive and 0.1% Triton X-100 as negative control. The morphology of the keratinocytes was assessed with a microscope (IX50, Olympus).

Results and discussion

The SLMA dispersion showed an enhanced physical stability at 5°C since the particle size remained in the range of 3.5 to 3.7 µm, the particle size distribution was narrow between 1 and 10 µm (Fig. 1), and the T_{onset} of about 55°C and $T_{recryst}$ of approximately 28°C were nearly the same over 12 weeks (Table 1). Compared to that, a slight increase of the particle size from about 3.7 µm to 4.3 µm was detected at 23°C over 12 weeks which is in accordance with a previous report [1]. The same applies for the thermoanalytical data with a T_{onset} of approximately 56°C and a $T_{recryst}$ of about 28°C after 12 weeks. A significant increase and broadening of the particle size distribution was determined for samples stored at 40°C. The corresponding T_{onset} increased from 55°C to 57°C, whereas the $T_{recryst}$ was nearly constant. The T_{micell} of P407 in the aqueous phase decreased from about 13.4°C to less than 12°C in all cases, whereas the decrease was most significant at 40°C storage temperature over 12 weeks. A structural change could be excluded at 5°C since particles containing adapalene within their lipid matrix remained intact (**Figure 2**). However, the morphology was significantly altered after storage at 40°C which might be due to the preferential leakage of lecithin into the aqueous phase at elevated temperatures. An additional mode within the nanometer range was detected by LD in this case. The potential increase of the emulsifier mobility within the aqueous phase may be substantiated by the decreased T_{micell} of P407 which corresponds to an increased concentration of P407 in the outer phase. Due to the previously observed increase of the complex viscosity based on the liquefication of poloxamer 407 and the subsequent entanglement of the lipid particles the particles might be additionally stabilized at refrigerator temperature [2]. The chemical stability was ascertained for all storage temperatures since the drug content remained constant to 0.1%(w/w) adapalene and the pH value of approximately 5.4 was nearly the same over 12 weeks. The same chemical stability was detected within the thermal cycling study over 12 days in total. Regarding the particle size during thermal cycling, a particle growth to about 4.9 µm was detected which began after the first cycle, whereas the size distribution remained monomodal after storage at 5°C up to the second cycle but turned bimodal after each storage at 40°C throughout this study. The increase of the T_{onset} to about 57°C and the stability of the $T_{recryst}$ were also monitored within the thermal cycling study as for the storage at 40°C. The MTT test after incubation with the HaCaT monolayer revealed a cell viability of about 90% for all investigated dilutions which is consistent with the literature since hard fat and lecithin are associated with no negative impact on keratinocytes [6]. On the contrary, the dilutions of the Differin® cream caused a significant decrease of the cell viability to 11% in case of the highest concentration, whereas lower concentrations still featured minor values of 69% and 27%, respectively (**Figure 3**). The HaCaT cells displayed a similar morphology like the ones incubated with Triton X-100 (**Figure 4**). Critical inactive ingredients of the cream might be the PEG-derived emulsifier PEG-20-methyl glucose sesquistearate since cell toxicity depends on the surfactant type but not on the surface activity of the corresponding emulsifier [7]. Furthermore, preservatives such as methyl paraben may lead to a toxic effect since concentrations above 0.003% might cause a time-dependent decrease of the cell viability [8].

Conclusion

In conclusion, apart from the drug delivery potential of the novel SLMA dispersion its pronounced stability at 5°C and skin compatibility compared to a commercial topical formulation are additional expedient features. Thus, feasibility for transportation with certain limitations at elevated temperatures and improved local skin tolerability might be assumed. Both qualities are advantageous in topical retinoid therapy.

Acknowledgments

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Conflict of Interest

A national patent application claiming the novel pharmaceutical composition was filed under DE102013224627.7.

Table 1 Thermoanalytical data of the SLMA dispersion stored at 5°C, 23°C, and 40°C for 12 weeks.

	T_{onset} [ºC]			$T_{recryst}$ [ºC]			T_{micell} [ºC]		
Storage conditions	5ºC	23ºC	40ºC	5ºC	23ºC	40ºC	5ºC	23ºC	40ºC
1 day	55.3 ± 0.4	55.4 ± 0.4	55.7 ± 0.3	28.5 ± 0.1	28.5 ± 0.1	28.5 ± 0.1	13.1 ± 0.4	13.4 ± 0.3	13.4 ± 0.1
1 week	55.6 ± 0.1	56.2 ± 0.2	57.2 ± 0.1	28.1 ± 0.1	27.9 ± 0.1	28.0 ± 0.1	13.6 ± 0.2	12.6 ± 0.3	12.0 ± 0.1
4 weeks	55.5 ± 0.1	56.4 ± 0.2	57.6 ± 0.2	27.9 ± 0.1	27.7 ± 0.1	27.9 ± 0.1	12.6 ± 0.3	12.2 ± 0.1	11.1 ± 0.1
8 weeks	55.4 ± 0.1	56.8 ± 0.2	57.6 ± 0.1	27.3 ± 0.1	27.7 ± 0.1	27.9 ± 0.1	11.5 ± 0.3	11.9 ± 0.1	10.6 ± 0.2
12 weeks	55.6 ± 0.2	56.7 ± 0.1	57.6 ± 0.1	27.8 ± 0.1	27.8 ± 0.1	27.9 ± 0.1	11.5 ± 0.3	11.7 ± 0.1	9.7 ± 0.2

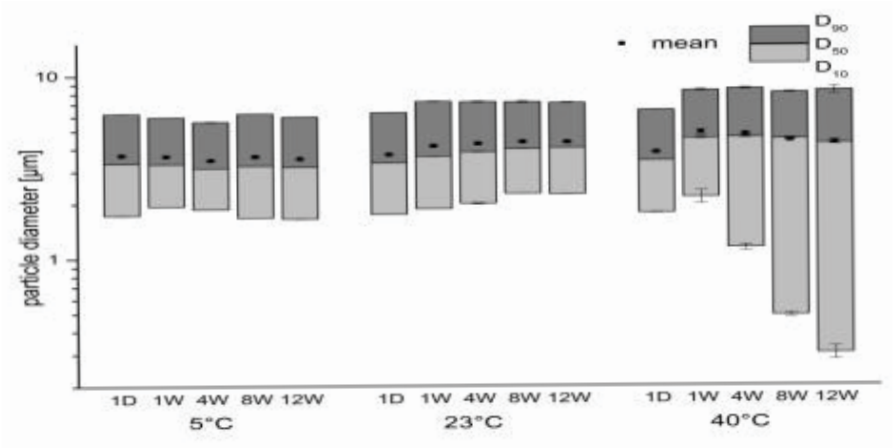

Figure 1 Mean particle size (mean) and particle size distribution (D_{10}, D_{50}, and D_{90}) after storage at 5°C, 23°C, and 40°C for 12 weeks.

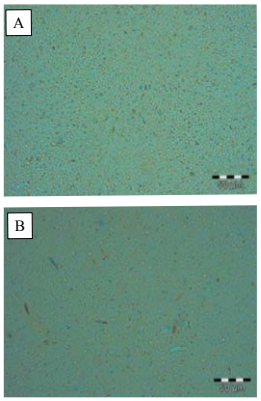

Figure 2 Exemplary micrographs of the SLMA dispersion after storage at 5°C (A) and 40°C (B) for 4 weeks.

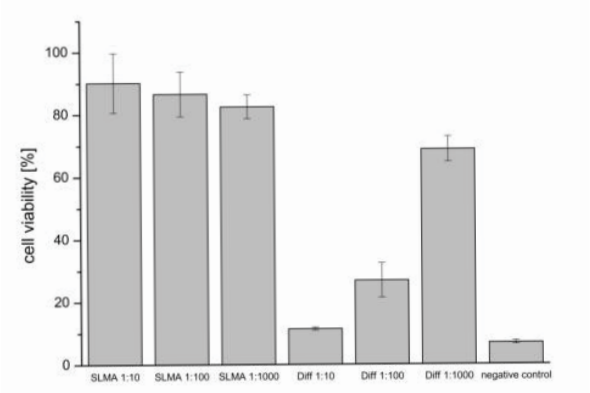

Figure 3 Cell viability of 1:10, 1:100, and 1:1000 dilutions of the SLMA dispersion and the Differin® cream (Diff).

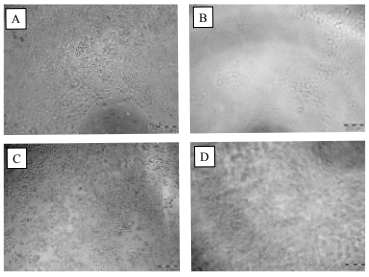

Figure 4 Micrographs of a HaCaT monolayer incubated with DMEM (A), Triton X-100 (B), a 1:10 dilution of the SLMA dispersion (C), and of the Differin® cream (D).

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