International Journal of Pharmaceutics 466 (2014) 122–132

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Development, formulation, and characterization of an adapalene-loaded solid lipid microparticle dispersion for follicular penetration

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ARTICLE INFO

Article history:
Received 20 January 2014
Received in revised form 26 February 2014
Accepted 28 February 2014
Available online 5 March 2014

Keywords:
Retinoid
Adapalene
Solid lipid microparticle dispersion
Poloxamer 407
Follicular penetration
Dermal application

ABSTRACT

The model retinoid adapalene was formulated in a novel solid lipid microparticle (SLM) dispersion as a topical drug delivery system for transport of the active pharmaceutical ingredient (API) into hair follicle orifices. The aims of the investigations were the solid-state characterization of the lipid matrix (LM) with wide angle X-ray diffraction (WAXD) and hot-stage light microscopy (HS), the design space analysis of the developed SLM dispersion with a Box–Behnken design, the stability study of the manufactured formulation for particle size with laser diffraction and polarization intensity differential scattering (LD/PIDS) and thermal behavior with differential scanning calorimetry (DSC), and the structure analysis of the SLM dispersion with light microscopy, transmission electron microscopy (TEM), and scanning electron microscopy (SEM). The formulation showed a constant mean particle size (MPS) of 4.2 μm over 24 weeks with a melting point of about 56 °C. The potential for dermal application was determined by a follicular penetration (FP) study with porcine ear skin and thermal analysis of the interaction with artificial human skin lipids like sebum and stratum corneum lipid mixture. The *in vitro* studies confirmed both the follicular penetration potential and a targeted erosion or dissolution of the particles in sebum.

1. Introduction

Retinoids are highly potent active pharmaceutical ingredients (APIs) that exhibit anti-inflammatory, keratolytic, and anti-seborrhoic effects by direct impact on target structures such as inflammation mediators, keratinocytes, and sebocytes (Zouboulis, 2001). For instance, adapalene inhibits the activity of polymorphonuclear leukocytes, the production of reactive oxygen species, the release of pro-inflammatory cytokines (Piérard et al., 2009), and the expression of the toll-like receptor-2, and decreases the expression of interleukin-10 (Tenaud et al., 2007). It suppresses the synthesis of suprabasal keratin, filaggrin, and transglutaminase (Asselineau et al., 1992). 10^{-6} M adapalene obliterates the proliferation of Sprague-Dawley rat sebocytes (Kim et al., 2000) and 10^{-7} M API abolishes the accumulation of sebum in differentiated hamster sebocytes (Sato et al., 2013).

The systemic administration of retinoids is to date efficient but causes severe damage to the unborn child including congenital defects of the craniofacial, cardiovascular, thymic, and the central nervous system, and spontaneous abortion (Collins and Mao, 1999). In contrast, the local bioavailability from topical application of common dosage forms like creams or gels is limited. Therefore, a sophisticated topical drug delivery system is needed imperatively and meets an unmet medical need. Known pharmaceutical formulations of adapalene are emulsions, lotions (Rolland et al., 1992), gels (Allec et al., 1997), microparticles (Rolland et al., 1993), microemulsions (Bhatia et al., 2013), and soy bean oil-cyclodextrin complexes (Trichard et al., 2008).

Follicular penetration (FP) is the transport and deposition of materials into the hair follicle. Particles in the nanometer range are considered as potential carriers for transfollicular delivery (Mittal et al., 2013; Meidan et al., 2005) or deep penetration to the hair root (Patzelt et al., 2011; Shim et al., 2004). Microparticles are regarded as efficient vehicle systems for FP to the orifice and infundibulum of the hair follicle and to the sebaceous gland (Rolland et al., 1993; Teichmann et al., 2006; Toll et al., 2004). Sebaceous glands secret sebum consisting of 45% glycerides, 25% wax esters, 15% squalene,

Abbreviations: API, Active pharmaceutical ingredient; DoE, Design of experiments; DSC, Differential scanning calorimetry; FF-TEM, Freeze-fracture transmission electron microscopy; FP, Follicular penetration; GRAS, Generally recognized as safe; HS, Hot-stage light microscopy; LD/PID, Slaser light diffraction with polarization intensity differential scattering; LM, Lipid matrix; P407, Poloxamer 407; PEG12000, Polyethylene glycol 12000; SEM, Scanning electron microscopy; SLM, Solid lipid microparticle; SLMA, Adapalene-loaded solid lipid microparticle; WAXD, Wide angle X-ray diffraction.

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http://dx.doi.org/10.1016/j.ijpharm.2014.02.050
0378-5173/

12% fatty acids, and 3% cholesterol and cholesterol esters (Lu et al., 2009) into the hair follicle shunt. Consequently, relevant target parameters for FP are a mean particle size (MPS) of about 5 μm, solid particles with a melting point above 40 °C, and lipophilic material that preferentially resembles the composition of sebum (Schaefer, 1993; Teeranachaideekul et al., 2007).

Sumian et al. (1999) and Mordon et al. (2003) described microparticles of nylon with a MPS of 5 μm for dye delivery into hair follicles of rats, Pitaksuteepong et al. (2007) prepared alginate/chitosan microparticles with artocarpin for transport into hair follicles of hamster flank organs, and Gelfuso et al. (2011) developed minoxidil-loaded chitosan microparticles with potential use for FP. Stecova et al. (2007) showed that lipid nanoparticles, a nanoemulsion, and lipid microspheres increase the absorption of cyproterone acetate into human skin *ex vivo*. However, the investigated lipid microspheres were physically instable showing aggregation after 7 days. Lipid nanoparticles for dermal application are used for several dermatological indications (Pardeike et al., 2009), *e.g.*, Muenster et al. (2005) showed a deep FP into human scalp skin of solid lipid nanoparticles and nanoemulsions with Nile Red and Padois et al. (2011) detected a similar skin penetration of solid lipid nanoparticles with minoxidil compared to commercial solutions.

However, a stable composition of solid lipid microparticles (SLMs) within a size range of 1–10 μm for the intended application has not been developed yet and is the objective of this study. Due to their simplicity and non-toxicity SLMs are appropriate for dermal application (Nnamani et al., 2013). The developed formulation is composed of excipients that provide generally recognized as safe (GRAS) status and are accepted for topical administration. The particles consist of hydrogenated palm oil as a glyceride of mainly palmitic and stearic acid, which resemble the acyl chain range of human sebum (Nordstrom et al., 1986). The incorporation of adapalene as the selected model retinoid API is arranged by the addition of lecithin as a lipophilic emulsifier avoiding any organic solvents. The unique particle size distribution is enabled by the utilization of poloxamer 407 (P407) as emulsifier and gelling agent. Poloxamer 407 is one of the most common polyethylene glycol-polypropylene glycol block-copolymers with many applications for dermal formulations (van Hemelrijck and Mueller-Goymann, 2011; Takahashi et al., 2002). Its gelation behavior is modified with polyethylene glycol 12000 (PEG12000), since high-molecular polyethylene glycol molecules form mixed micelles with P407 and cause gel melting of P407 solutions at high processing temperatures (Pandit and McGowan, 1998). A design of experiments (DoE), stability study, and microscopic analysis were employed to characterize the formulation in detail. The application-oriented performance of the dispersion for FP and interactions with skin lipid mixtures was checked in *ex vivo* and *in vitro* studies.

2. Materials and methods

2.1. Materials

Adapalene (Glenmark Generics, Mumbai, India) was chosen as the model API. Hydrogenated palm oil (Softisan® 154, Condea, Witten, Germany) and purified lecithin (Phospholipon® 90G, Lipoid, Ludwigshafen, Germany) formed the lipid matrix. Poloxamer 407 (Kolliphor® P407, BASF, Ludwigshafen, Germany), polyethylene glycol 12000 (Sigma–Aldrich, Seelze, Germany), potassium sorbate, and anhydrous citric acid (both Caelo, Hilden, Germany) were used for the preparation of the aqueous phase with water of double distilled quality. Squalene (Alfa Aesar, Karlsruhe, Germany), cetyl palmitate, olive oil (both Caelo, Hilden, Germany), oleyl oleate (Cognis, Monheim, Germany), palmitic acid (Hüls, Marl, Germany), coconut oil, oleic acid, cholesterol, cholesterol oleate (all Sigma–Aldrich, Seelze, Germany), ceramide IIIB (NP), and ceramide VI (AP) (both Franken Chemie, Wendelstein, Germany) were used as the components of the artificial skin lipid mixtures.

2.2. Preparation of lipid matrix (LM)

70% hydrogenated palm oil and 30% purified lecithin (all by weight) were stirred on a magnetic hot plate stirrer at 70 °C until a transparent and yellowish lipid melt was obtained. The solution was stirred at room temperature to solidification to generate the LM (Schubert et al., 2005). 99.5% LM was molten at 70 °C and 0.5% adapalene (all by weight) was added and stirred until a clear solution was generated. It was either stirred to solidification for solid-state characterization or subjected to further processing after the addition of the emulsifier solution to manufacture the dispersion.

2.3. Solid-state characterization

2.3.1. Hot-stage light microscopy (HS)

A small amount of recrystallized LMs with different concentrations of adapalene was monitored on an objective slide in a hot-stage device (FP 90 central processor, Mettler Toledo, Gießen, Germany) from 25 to 70 °C with a heating rate of 10 °C/min under the light microscope (Leica DM LM, Leica Microsystems GmbH, Wetzlar, Germany).

2.3.2. Wide angle X-ray diffraction (WAXD)

Recrystallized LMs with different concentrations of adapalene were subjected to WAXD. The X-ray generator PW3040/60 X'Pert PRO connected to the tube PW3373/00 DK 147726 Cu LFF copper anode delivered X-ray of a wavelength $\lambda = 0.1542$ nm at a high voltage of 40 kV and an anode current of 25 mA. The samples on the spinner PW3064 were scanned with the goniometer PW3050/60 MPD system from 3° to 45° in 0.015° steps and recorded with X'Pert Data Collector (all PANalytical, Almelo, Netherlands).

2.4. Preparation of solid lipid microparticle (SLMA and SLM) dispersion

An aqueous solution of 12% P407, 3% PEG12000, 0.2% potassium sorbate, and 0.1% anhydrous citric acid was added to 19.9% molten LM with 0.1% solubilized adapalene (all by weight) at 70 °C. Both phases were dispersed at 70 °C with 16,000 rpm for 3 min with an Ultra-Turrax® T25 digital (IKA, Staufen, Germany) and extruded once through a hydrophilic Nuclepore® membrane (Whatman, Maidstone, United Kingdom) with a pore size of 12 μm positioned in a pre-tempered Liposofast® basic (Avestin, Mannheim, Germany) at 70 °C into the final container. The formulation was left to stand at room temperature to obtain the crystallized solid lipid particles. Adapalene-loaded (SLMA) and -free SLM dispersions were prepared in triplicate for the stability study.

2.5. Statistical design of experiments (DoE)

A Box–Behnken design (Box and Behnken, 1960) was constructed in order to analyze the design space. Varied experimental factors were the weight percentage of LM and P407 as phase parameters and the dispersion rate and dispersion time as process parameters. The limits were set to 2 extreme and 1 central value encoded with −1, 0, and +1 given in Table 1. Other parameters as weight percentage of PEG12000, dispersion temperature, and cooling conditions were kept constant. The MPS and span were defined as the responses. The span was calculated as follows:

$$\text{span} = (D_{90} - D_{10})/D_{50}$$

The order of the experiments of the combinations of the parameters were randomized and evaluated with StatGraphics®