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Solid lipid nanoparticles suspension versus commercial solutions for dermal delivery of minoxidil

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ABSTRACT

Solid lipid nanoparticles have been reported as possible carrier for skin drug delivery. Solid lipid nanoparticles are produced from biocompatible and biodegradable lipids. Solid lipid nanoparticles made of semi-synthetic triglycerides stabilized with a mixture of polysorbate and sorbitan oleate were loaded with 5% of minoxidil. The prepared systems were characterized for particle size, pH and drug content. *Ex vivo* skin penetration studies were performed using Franz-type glass diffusion cells and pig ear skin. *Ex vivo* skin corrosion studies were realized with a method derived from the Corrositex® test. Solid lipid nanoparticles suspensions were compared to commercial solutions in terms of skin penetration and skin corrosion. Solid lipid nanoparticles suspensions have been shown as efficient as commercial solutions for skin penetration; and were non-corrosive while commercial solutions presented a corrosive potential. Solid lipid nanoparticles suspensions would constitute a promising formulation for hair loss treatment.

1. Introduction

Androgenic alopecia is a common form of hair loss in both men and women. Minoxidil, a pyridine-derivative, is widely used for the treatment of androgenic alopecia (Atrux-Tallau et al., 2009). Commercial products containing minoxidil are usually solutions with high percentage of ethyl alcohol and/or propylene glycol (Tata et al., 1995). Twice-daily applications are recommended as proper use. However repeated applications of high ethyl alcohol and/or propylene glycol content products lead to severe adverse effects (e.g., scalp dryness, irritation, burning, redness, allergic contact dermatitis) (Aronson, 2006; Pavithran, 1993; Wagner and Kenreigh, 2007). Since most of the products containing minoxidil available on the market consist of ethyl alcohol – propylene glycol – water solutions, new dermatological formulations free of organic solvents are needed to minimize adverse effects and optimize androgenic alopecia treatment. Recent work proposed liposomes or niosomes as alternative drug delivery systems. Mura et al. (2007) showed alcohol-free liposomal formulations potential as minoxidil topical delivery in hair loss treatment. Balakrishnan et al. (2009) suggested that niosomal formulations of minoxidil have a good potential for drug cutaneous targeting with regards to process variables. Mura et al. (2009) studied penetration enhancer-containing vesicles as carriers for cutaneous delivery of minoxidil and showed that association of soy Phosphatidylcholine and Transcutol® does not improve minoxidil diffusion through skin layers (Mura et al., 2011). Nanoparticles and microparticles have been largely developed for skin drug delivery. Skin provides a natural barrier against exogenous aggressions and particles penetration. However therapeutic nanoparticles and microparticles can be delivered in diseased skin and to hair follicles openings (Knorr et al., 2009; Lademann et al., 2007; Prow et al., 2011). Solid Lipid Nanoparticles (SLN) are composed of solid lipid cores (i.e., lipids solid at room temperature) stabilized by surfactant(s) in water suspension. The use of triglycerides in SLN formulations is an advantage in terms of toxicity (Traul et al., 2000). SLN are easily produced by melt emulsification followed by a homogenization step either with high pressure homogenizer or ultrasonic processor (Padois et al., 2009). SLN have been largely studied as potential carriers for topical drug application in skin diseases (Mehnert and Mäder, 2001; Müller et al., 2000,

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Table 1
SLN suspension composition.

Components	Content (g)
Minoxidil	5
Suppocire® NAI50	10
Montane® 80PHA	6
Montanox® 20PHA	4
Phosal® 50PG	4
Water	71

2002, 2007; Saupe and Rades, 2006; Schäfer-Korting et al., 2007; Souto and Doktorovová, 2009).

In the present work, a new SLN suspension containing 5% of minoxidil is proposed. Drug deposition in the different skin layers was determined *ex vivo* using Franz-type glass diffusion cells and compared to commercial products. Furthermore *ex vivo* skin corrosion studies were conducted in order to evaluate and compare the corrosive factor of new SLN suspension and commercial products. This paper focuses on the new SLN suspension potential as safe minoxidil carrier for androgenic alopecia treatment.

2. Materials and methods

2.1. Materials

Semi-synthetic triglycerides (Suppocire® NAI50) were kindly obtained from Gattefossé (Saint Priest, France). Polysorbate and sorbitan oleate (Montanox® 20PHA and Montane® 80PHA) were a gift from Seppic (Castres, France). Phosphatidylcholine (Phosal® 50PG) was obtained from Welding Pharma (Lyon, France). Minoxidil was a gift from Pierre Fabre (Ramonville Sainte Agne, France). Ovalbumin, NaCl, sulforhodamine B and dioctyl sulfosuccinate sodium salt were purchased from Sigma Aldrich (Saint Quentin Fallavier, France). Acetic acid, perchloric acid, nitric acid and methanol were purchased from Carlo Erba (Val de Reuil, France). All solvents were of analytical grade. Alopexy 5% (Pierre Fabre Dermatologie, Boulogne, France) and Minoxidil Bailleul 5% (Laboratoire Bailleul-Biorga, Villeneuve la Garenne, France) were purchased in a local pharmacy.

2.2. SLN suspension preparation

SLN suspension composition is reported in Table 1 (Padois et al., 2009). Commercial solutions compositions are reported in Table 2. SLN suspensions were prepared according to procedure described in the patent FR0952059 (Padois et al., 2009). Suppocire® NAI50, Montane® 80PHA, Montanox® 20PHA and Phosal® 50PG were mixed and heated at 40 ± 2 °C. Minoxidil was added to the mixture at 40 ± 2 °C under stirring at 100 rpm. Lipid–drug mixture was maintained under stirring at 40 ± 2 °C during 5 h. Water was heated at 40 ± 2 °C and added to the lipid–drug mixture under stirring at 500 rpm. Stirring was maintained during 1 h at 40 ± 2 °C. The obtained pre-emulsion was homogenized at the temperature of the melt using a high pressure homogenizer Emulsiflex® C5 (HHP) (Avestin GmbH, Mannheim, Germany) applying 100,000 kPa and three homogenization cycles.

Table 2
Commercial solutions compositions (g/100 mL).

Components	Alopexy 5%	Minoxidil Bailleul 5%
Minoxidil	5	5
Ethyl alcohol (96%)	25	58.6
Propylene glycol	50	20
Water	QS 100 mL	QS 100 mL

2.3. SLN suspension characterization

Photon correlation spectroscopy (PCS), performed using a Zetamaster® (Malvern Instruments, France), was used to assess the mean particle size (*z*-diameter) and the polydispersity index (PI). Data were fitted by the Contin method. Zeta potential (ξ) was measured as the particle electrophoretic mobility means of laser microelectrophoresis. A pH-meter Cyberscan® pH 110 (Eutech Instruments, Singapore) was used to determine the tested products pH. All measurements were made at 20 ± 2 °C.

2.4. Minoxidil content and drug loading

Minoxidil content (MC%) was determined by High Performance Liquid Chromatography (HPLC Agilent 1200 Series, Massy, France) directly by diluting the commercial solutions into the mobile phase or after disruption of SLN in the mobile phase. HPLC method was adapted from Asmus et al. (1984). Minoxidil content is expressed in gram of minoxidil per 100 g of sample. Analysis was carried out by reverse-phase absorption chromatography using a column Kinetex® (2.6 μm, 100 Å, C18, 4.6 mm × 100 mm) (Phenomenex, Le Pecq, France). The mobile phase was a mixture of water, methanol, acetic acid and dioctyl sulfosuccinate sodium salt (300 mL, 700 mL, 10 mL and 3 g, respectively). Mobile phase pH was adjusted to 3.0 with perchloric acid. The flow rate was 0.7 mL/min. The sample volume injected is 20 μL. Detection was performed at 254 nm. For concentrations from 1.56 to 100 μg/mL, chromatograms were linear with a factor correlation of 0.999. The method reproducibility was tested for 3 concentrations (1.56 μg/mL, 50 μg/mL and 100 μg/mL). The coefficient of variation was lower than 2.5%. The limit of quantification (LOQ) and the limit of detection (LOD) for chromatographic determination of minoxidil were calculated from the calibration curve (Eqs. (1) and (2)) (MacDougall and Crummett, 1980). LOQ was 0.37 μg/mL and LOD was 0.11 μg/mL.

$$\mathrm{LOQ} = 10 \times \left(\frac{\sigma b}{a}\right) \quad (1)$$

$$\mathrm{LOD} = 3 \times \left(\frac{\sigma b}{a}\right) \quad (2)$$

with a is the regression line gradient and σb is the intercept point deviation.

SLN drug loading (DL%) was expressed as the percentage of the amount of minoxidil initially used taking into account the water loss by evaporation during the SLN suspension preparation.

2.5. Ex vivo skin penetration studies

Pig ears were obtained from a local slaughterhouse. The skin was kept frozen until use. Before use, skin was briefly washed under tap water and full-thickness skin was removed from the dorsal side of the pig ear. Skin punches were used for *ex vivo* penetration studies carried out with Franz-type glass diffusion cells. These cells consist of two compartments with the skin clamped between the donor and receiver chambers, dermal side down. The receiver chamber was filled with a receptor phase consisting of water with 0.9% NaCl and 3% ovalbumin (Mura et al., 2007). The receptor phase was constantly stirred with a magnetic bar and thermostated at 37 ± 1 °C (resulting in a skin surface temperature of 32 ± 1 °C) throughout the experiments. A determined volume of studied product was placed in the donor chamber onto the stratum corneum of the porcine skin, in non-occlusive conditions. The diffusion area was 0.785 cm^2. At the end of the experiment (24 h), the skin surface was washed with 2 × 1 mL of distilled water to remove the residual donor sample. The receptor phase was removed and the Franz-type glass diffusion cells were dismantled. The skin