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Effect of drug solubility and lipid carrier on drug release from lipid nanoparticles for dermal delivery

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ABSTRACT

Lipid nanoparticles have gained increased interest in the field of dermal products because of various advantages such as improved drug absorption and controlled drug release. The main objective was to investigate the influence of drug solubility and type of lipid carrier on the *in vitro* drug release. Drugs of different solubilities in the release medium PBS pH 7.4 (dexamethasone: 0.1 mg/ml and diclofenac sodium: 5.0 mg/ml) and three different lipids (in which the drugs had the highest solubility), Gelucire® 50/13 (solid lipid, mp: 50 °C), Witepsol® S55 (solid lipid, mp: 33.5–35.5 °C) and Capryol® 90 (liquid lipid) were chosen. The lipid nanoparticles were prepared by high shear homogenization. All nanosuspensions were in the nanometer range (up to 400 nm) and the drug encapsulation efficiency was between 84% and 95%. The drug release was prolonged over 48 h without an initial burst release and was dependent on the lipid carrier. Formulations containing a higher amount of solid Gelucire® 50/13 released the drugs slower due to the high affinity of the drugs to this lipid product. Inclusion of the liquid lipid Capryol® 90 resulted in a less organized lipidic structures (softer particles) and therefore a faster drug release. Despite its higher water solubility, diclofenac was released slower than dexamethasone because of its higher solubility in the lipid carriers. DSC studies indicated a partial miscibility between the solid lipids and a good miscibility between the solid and liquid lipids. Primary studies using total internal reflection fluorescence (TIRF) microscopy indicated that it is possible to detect individual fluorescently labeled dexamethasone (DXM-F) molecules dissolved in the liquid lipid Capryol® 90. These studies will allow for the precise determination of the drug distribution within the lipid carrier, and the changes upon drug release. In conclusion, lipid carrier type and drug solubility in the lipid have a large influence on the *in vitro* drug release from lipid nanoparticles.

1. Introduction

The advantages of lipid nanoparticles include: flexibility in modulating the drug release [1–3], increased drug stability [4,5], high biocompatibility due to the good tolerability of the used lipids, encapsulation of both hydrophilic and lipophilic drugs, and a simple preparation process as well as scale up. Additional advantages regarding dermal application are: increased drug penetration due to their tendency to form an occlusive layer which increases the hydration/swelling of the stratum corneum [6–8], and the possibility for hair follicle targeting, thus exhibiting a depot effect and at the same time increasing drug penetration [9,10].

Different types/generations of lipid nanoparticles have been developed, fully characterized and compared regarding their crystalline structure and drug loading capacity [11–14]. The first generation, solid lipid nanoparticles (SLN), is composed of one solid lipid or blend of solid lipids, so that the lipid particles are in the solid state at both room and body temperature. The main challenge with SLN is the re-crystallization of the lipid after preparation (upon cooling) into a highly ordered crystalline structure, resulting in drug expulsion. For this reason, the drug loading is quite low. To overcome this problem, a liquid lipid (oil) was mixed with the solid lipid resulting in so-called nanostructured lipid carriers (NLC) [15]. It was assumed that the oil inside the particle would prevent re-crystallization and therefore, a higher drug loading and improved drug release could be obtained [11]. Depending on type and concentration of the liquid lipid, different types and structures of NLC can be obtained. With a complex mixture of up to 10 different solid and/or liquid lipids; a "chaotic" structure for improving drug loading and drug release even more than the NLC was claimed [16]. Polymorphic transitions of the solid lipids can occur during lipid

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nanoparticle preparation and storage. The more stable but also highly ordered beta structure will generally be obtained during storage. However, mixing of different lipids having different structures, and the use of certain surfactants can stabilize the less structured alpha modification resulting in a higher drug loading and an improved drug release [17].

The investigation of the nanostructures within the lipid nanoparticles dispersion is quite challenging. One new approach depends on single particle tracking (SPT) and super resolution based fluorescence microscopy techniques [18]. Traceable molecules are incorporated in the lipid phase and from their diffusion properties it can be inferred whether the molecules are distributed in the solid or liquid lipid phase and whether they are confined in nanocompartments.

For lipid nanoparticles intended for topical drug delivery, all excipients which are typically used for topical cosmetic and dermal products could be used: highly purified triglycerides, monoglycerides, complex glycerides mixture and hard fats. In addition to the lipid carrier, suitable surfactants are necessary to insure the preparation and the colloidal stability of the lipid nanosuspension. Surfactants could also affect chemical stability of the drug, drug release profile and bioavailability [19–21]. Gelucire® 50/13 is an amphiphilic lipid product that is generally regarded as safe (GRAS) and biocompatible. It has been mainly used for the preparation of solid dispersions to improve the solubility/bioavailability of poorly soluble drugs [22,23]. Because of its high hydrophilic-lipophilic balance (HLB = 13), it may act as stabilizer for lipid nanosuspension, thus, obviating the need of additional surfactants [24]. Drug release from lipid nanoparticles is complex and dependent on various formulation parameters and preparation conditions [25,26]. The release profile is usually characterized by an initial burst followed by prolonged drug release. The burst increased with increasing preparation temperature and increasing surfactant concentration. In the present study, surfactant-free lipid nanoparticles loaded with drugs having different aqueous solubility, namely dexamethasone (0.1 mg/ml) and diclofenac sodium (5 mg/ml) were investigated. It is assumed that the efficacy of the topical treatment with these drugs could be improved when formulated into lipid nanoparticles. Up to now, no comprehensive investigation comparing drug release from different types of lipid nanoparticles was performed. Therefore, the objective of this work was to investigate the influence of drug solubility and type of lipid carrier on the *in vitro* drug release.

2. Materials and methods

2.1. Materials

Dexamethasone (Fragron GmbH, Barsbüttel, Germany); diclofenac sodium, stearic and palmitic acid (Kolliwax® S) (BASF SE, Ludwigshafen, Germany); stearoyl macrogolglycerides (Gelucire® 50/13), glyceryl distearate (Precirol® ATO 5), propylene glycol monocaprylate (Capryol® 90) (Gattefossé GmbH, Bad Krozingen, Germany); hard fat (Witepsol® S55, Witepsol® E76), trimyristin (Dynasan® 114), hydrogenated coco-glycerides (Softisan® 142), glyceryl stearate (Imwitor® 900 K), caprylic/capric triglyceride (Miglyol® 812 N) (Cremer Oleo GmbH & Co. KG, Hamburg, Germany); ultrapure (Milli Q) water was used as an aqueous phase. All chemicals were used as received.

2.2. Drug solubility in the lipids

The solubility of the drugs in different lipids and their mixtures was determined gravimetrically. 3 g lipid was molten at 60 °C (temperature also used for the preparation of lipid nanoparticles) and the drug was added in certain amounts gradually (each time 1–2 mg for dexamethasone and 15–20 mg for diclofenac) and stirred in the melted lipid(s) until the lipid melt became turbid (saturation solubility exceeded). The stirring was maintained for an additional hour to ensure that no more drug was dissolved. The same procedure was repeated three times with a slower drug addition rate (0.5–1 mg for both drugs) shortly before the expected end-point, to ensure a more accurate determination of the drug solubility.

2.3. Preparation of lipid nanoparticles

Five lipid formulations (Table 1) were prepared using a high shear homogenization technique with a lipid content of 9–10% w/w (corresponds to lipid/water: 2.88 g/29.12 g and 3.2 g/28.8 g, respectively) and a drug loading of 0.75–3% w/w (based on lipid). The lipid(s) were weighed, mixed and melted together at 60 °C, the drug was then dissolved/dispersed in the lipid melt. Ultrapure water of the same temperature was then poured into the drug-lipid melt. The two phases were homogenized with an Ultra-Turrax for 1 min at 13,500 rpm followed by 3 min at 8000 rpm. Finally, the nanoemulsion was cooled to room temperature to solidify the lipid phase and thus, to obtain the lipid nanoparticle suspension.

2.4. Characterization of lipid nanosuspension

2.4.1. Mean particle size

The mean particle size of the lipid nanoparticles was determined using photon correlation spectroscopy (PCS) (Zetasizer® Nano ZS, Malvern Instruments GmbH, Herrenberg, Germany) equipped with a He-Ne-Laser (633 nm) at a backscattering angle of 173° and cell temperature of 25 °C. The lipid nanosuspension was diluted 1:10 (v/v) with ultrapure water. 10 runs were performed and thereof, the mean particle size (Z-average) and polydispersity index with standard deviation was calculated.

2.4.2. Drug entrapment efficiency

The entrapped drug in the lipid phase was determined by analyzing the drug amount in the aqueous phase and subtracting it from the total drug amount. The aqueous phase was separated from the lipid phase using centrifugal concentrator equipped with a filter of 10 kDa MWCO (Vivaspin® 500, VWR International GmbH, Darmstadt, Germany). 500 µl of the nanosuspension was filled into the concentrator tube and closed. The ultrafiltration was performed in a Hereaus™ Biofuge™ Stratos™ (Thermo Fisher Scientific, Waltham, Massachusetts, USA) for 4 h at 15.000 g and 10 °C. The drug content in the aqueous phase was quantified UV-spectrophotometrically at 242 nm. Possible drug adsorption to the filter was excluded, as the same procedure was performed with a pure drug solution. The percentage of drug encapsulated was obtained from the ratio of drug amount in the lipid phase to the total amount of drug in the nanosuspension. In fact, this method does not discriminate between drug which is entrapped and the one which is adsorbed onto the surface of nanoparticles.

2.4.3. In vitro drug release

In vitro drug release test was performed under sink/non-sink conditions. 1 ml of the nanosuspension was filled in a dialysis cell (MWCO 20 kDa, Float-A-Lyzer® G2) and immersed in a flask containing 50 ml (for sink condition), or 10 ml (for non-sink condition) phosphate buffer saline (PBS) pH 7.4. The flask was placed in an incubation shaker at 32 °C, 100 rpm. At predetermined time intervals, 1 ml of the release medium was withdrawn and replaced by freshly prepared PBS (pH 7.4). The samples were analyzed UV-spectrophotometrically (HP 8453, Agilent Technologies Deutschland GmbH, Waldbronn, Germany) at 242 nm.