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Research paper

Effect of lipid nanoparticle formulations on skin delivery of a lipophilic substance

Lucie Vidlářová [a], Jaroslav Hanuš [a], Martin Veselý [b], Pavel Ulbrich [c], František Štěpánek [a,*], Jarmila Zbytovská [b,d,*]

[a] Department of Chemical Engineering, University of Chemistry and Technology Prague, Technická 3, 166 28 Prague 6, Czech Republic
[b] Department of Organic Technology, University of Chemistry and Technology Prague, Technická 3, 166 28 Prague 6, Czech Republic
[c] Department of Biochemistry and Microbiology, University of Chemistry and Technology Prague, Technická 3, 166 28 Prague 6, Czech Republic
[d] Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Heyrovského 1203, 50005 Hradec Králové, Czech Republic

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ABSTRACT

The aim of this study was to follow the skin penetration of a model lipophilic compound (Nile red) delivered by nanoparticulate carriers, the so-called lipid nanocapsules. The nanocapsules consisting of an oil core stabilized by a mixture of surfactants were prepared by the phase inversion temperature method. Varying the particle composition (the oil/surfactant ratio) nanoparticles of different size were prepared and characterized. The penetration profile of Nile red delivered into the porcine skin by the nanoparticles compared to non-particulate samples was determined using fluorescence microscopy combined with a novel, statistically robust quantitative image analysis method. This study demonstrated that lipid nanoparticles promoted the skin penetration of encapsulated Nile red in comparison with all the non-particulate samples. Nile red delivered by the lipid-based nanoparticles was able to diffuse across the stratum corneum and partition itself uniformly in the epidermis. No relationship between Nile red penetration into the skin and the particle size was found. Moreover, the presence of sodium chloride in the water phase had a negative impact on the Nile red penetration into the skin. The results indicate that the physico-chemical circumstances of the nanoparticulate formulation play the major role in the penetration of lipophilic substances into the skin.

1. Introduction

Drug delivery via the skin, either for systemic or for local therapy, bears a lot of advantages such as avoiding the first-pass effect in the liver or the possibility to deliver the drug continuously. The main limitation for dermal and transdermal drug delivery resides however in the excellent barrier properties of the outermost skin layer, the stratum corneum [1,2]. Stratum corneum has an unique structure, which consists of flattened corneocytes surrounded by a lipid matrix [3,4] consisting predominantly of ceramides, fatty acids and cholesterol. This structural organization can be described as the so-called "brick and mortar model", first published by Michaels et al. in 1975 [5]. Later on, several models describing the arrangement of the intercellular lipid matrix have been proposed [6–8]. A current concept that the lipids are organized as stacked bilayers with fully extended ceramides has been suggested by Iwai et al. [9].

Numerous approaches have been investigated to enhance the skin permeation of drugs through the stratum corneum temporarily [10]. One of them is the application of nanoparticle systems [11]. Nanoparticles made from lipids such as solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), lipid nanocapsules and others are of major interest and their permeation enhancing effect and/or an improved uptake of drugs to specific skin layers was reported in many studies [12–17]. Thus, the application of lipid-based particles might facilitate the treatment of skin diseases by avoiding the unwanted effects.

Lipid nanocapsules were introduced as an alternative lipid based colloidal carrier [18]. In general, they consist of an oily liquid (triglyceride) core surrounded by an amphiphilic layer. Their preparation is based on a simple low-energy spontaneous emulsification method – the phase inversion temperature (PIT) method. This temperature cycling process is based on the changes in solubility of nonionic polyethoxylated surfactant whose affinity to the oil or aqueous phase differs depending on temperature. The

* Corresponding authors at: University of Chemistry and Technology Prague, Technická 3, 166 28 Prague 6, Czech Republic (J. Zbytovská and F. Štěpánek).
E-mail addresses: Frantisek.Stepanek@vscht.cz (F. Štěpánek), Jarmila.Zbytovska@vscht.cz (J. Zbytovská).

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PIT can be influenced by the composition of the water phase, e.g. the presence of sodium chloride [19–21].

Although nanoparticulate systems have been shown to be effective for the dermal and transdermal delivery, the exact mechanisms of their modes of action can be various and have not been completely elucidated by now. Many studies indicate that particles larger than 20 nm intensively accumulate in the skin adnexa [11,22,23]. The depth of follicular penetration depends on the particle size and formulation composition [24,25]. In other cases, the authors suggest that the individual components of the particulate formulation can act as permeation enhancers [26], that the particles show an occlusive effect [27] or that they specifically interact with the skin lipids [17,28] or skin cells [29,30]. The most controversial hypothesis probably is that the particles as themselves penetrate into the stratum corneum. Concerning the unimpaired skin barrier, it is generally accepted that such penetration can be possible for particles smaller than 10 nm [11,31]; there are, however, authors describing penetration into stratum corneum of even larger structures [11,32–34]. Anyway, various mechanisms of how nanoparticulate systems enhance penetration of substances into the skin have been proposed; the particular one depends on the type of the nanocarrier, its composition and size.

The aim of this study was to bring a deeper insight into the enhancing mechanism of lipid nanocapsules prepared by the phase-inversion method. Varying the lipid/surfactant mass ratio in the particular composition we were able to prepare particles of various sizes between 30 and 70 nm. We evaluated this series of nanoparticulate formulations as well as non-particulate samples concerning their effects on skin penetration of a lipophilic model permeant (Nile red). To quantify the studied effects, combination of confocal laser scanning microscopy and a unique image analysis method was used.

2. Materials and methods

2.1. Materials

Phospholipon 90G (soy phosphatidylcholine) and Crodamol GTCC (mixture of caprylic/capric acid triglycerides, $T_{mp} = -5$ °C) were kindly provided by Lipoid GmbH (Ludwigshafen, Germany) and Croda GmbH (Nettetal, Germany), respectively. Sodium chloride, Nile red (9-diethylamino-5-benzo[α]phenoxazinone; MW 318.4; LogP 3.8) and Kolliphor HS15 (polyethylene glycol-15-hydroxystearate) were purchased from Sigma-Aldrich. Deionized water (Aqual 25, 0.07 μS/cm) was used for all experiments.

2.2. Lipid nanoparticle preparation

The lipid nanoparticles were prepared by the phase inversion temperature process according to [18]. Firstly, all the components (Phospholipon, Crodamol, Kolliphor, deionized water, sodium chloride) were placed in a 20 ml glass vial, mixed for 5 min at 1400 rpm and heated to 80 °C at a rate of 3 °C/min. Three temperature cycles (200 min duration) in the range from 60 °C to 80 °C were then applied to reach the inversion from o/w to w/o emulsion. The emulsion type was detected by measuring the bulk conductivity with a conductivity meter SevenGo™ SG3 (Mettler Toledo, Switzerland). When the conductivity of the system was close to zero, the continuous phase was oil. Otherwise, high values of conductivity indicated that saline water was the continuous phase. Finally, rapid cooling with iced water of 0–2 °C was performed to obtain particles of nanometre size. The particles were kept in the saline water for further use. The range of particle composition (oil/lipid/surfactant ratio), salinity of the aqueous phase, and volume of the aqueous phase in which the lipids were dispersed, are summarized in Table 1. In the case of particles loaded with Nile red, the procedure was identical except that a Nile red solution in Crodamol (0.001 wt. %) was used instead of the pure oil.

2.3. Particle characterization

The particle size distribution and zeta potential were measured by dynamic light scattering with Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK) at 25 °C. All measurements were performed in triplicate. The volume-mean particle diameter and the polydispersity index were evaluated from the size distributions using the built-in General Purpose Analysis Model. To investigate their long-term stability, the particles were stored in glass vials at three different storage conditions (light at room temperature, dark at room temperature or dark at 4 °C) and their size distribution was measured at regular intervals over a 6-month period. The long-term stability was evaluated for two particle formulations (nos. 1 and 3) from Table 1. The transmission electron microscope JEM-1010 (JEOL Ltd., Tokyo, Japan) was used to visualize the size and structure of the lipid nanoparticles. Prior to the analysis, the particles were applied onto carbon-coated copper grids, stained by uranyl acetate and dried on air.

2.4. Skin penetration experiments

Full-thickness skin was isolated from the dorsal side of excised pig ears obtained from a local slaughterhouse. Subcutaneous tissue was removed and the skin samples were stored at a temperature of −20 °C for up to 3 months. Skin penetration experiments of Nile red loaded lipid nanoparticles were carried out on Franz diffusion cells as follows: the receptor compartment was filled with 10 mM phosphate buffer (pH 7.4) and the full-thickness porcine ear skin was mounted on the diffusion cell with stratum corneum facing up and dermis being in contact with the receptor medium. The diffusion cells with the skin were thermostated at 32 °C for 15 min, and the donor compartment was then filled with 200 μl of the donor samples described below and covered with a paraffin film to prevent evaporation of the samples. The assembled Franz diffusion cells were then placed to a thermostat at 32 °C for a period of 20 h. After the penetration test, the skin was removed from the Franz cells, washed in PBS buffer three times, gently dried with a cotton swab and kept frozen before sectioning. The frozen skin was then cut into 10 μm vertical slices using a CM-3050-S microtome (Leica Biosystems GmbH, Nussloch, Germany). Ten slices were taken per one skin sample and each experiment (i.e., each donor composition) was repeated on three different skin samples, resulting in 30 sections per donor formulation for further analysis.

The following formulations and reference samples were used as donor solutions for the skin penetration experiments: nanoparticle formulation nos. 1, 3 and 4 from Table 1 containing Nile red dissolved in the oil phase (Crodamol) were chosen based on their size and stability (cf. Section 3.1) as representative nanoparticle carriers. These three formulations made it possible to compare the effect of the phospholipid content on the skin penetration rate, as they contained either 0%, 1.5% or 3% of Phospholipon. Untreated skin was always used as a blank reference in order to compensate for any effect of autofluorescence during subsequent image analysis. The penetration profiles of Nile red delivered via nanoparticle-based carriers were compared with those of Nile red simply dissolved in oil (Crodamol) at the same concentration as in the nanoparticulate formulations. Two types of oil solutions were used – one containing only Nile red dissolved in Crodamol, and one containing also freely dissolved surfactants (Phospholipon and Kolliphor) along with Nile red in Crodamol. Finally, in order to elucidate an effect of salinity of the water phase on the skin penetration rate, additional four reference experiments were carried

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