Penetration and Distribution Studies of Sensitive Nanoparticles to the pH of the Skin: Targeting the Follicular Pathway

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ABSTRACT SUMMARY

The pH-sensitive nanoparticles (Nps) were loaded with Nile red (as a marker), prepared by a modification of emulsification-evaporation process and characterized by their size and charge. In vitro permeation studies, two photons and confocal microscopy studies using porcine ear and human abdominal skin showed that Nile red Nps targeted into the pilo sebaceous follicles dependent on time and in a greater rate than applying an emulsion (o/w) (Dermobras®) or a solution. NPs formulation represents a potential alternative to improve penetration through the skin, particularly into the hair follicles.

INTRODUCTION

There are several conventional formulations to treat affections in the follicular region; however, tolerability and safety are decisive steps to avoid its use. In addition, they have major limitations, such as poor penetration and uncontrolled drug release.¹ Recently, new strategies to enhance drug penetration through the skin have been proposed, such as nanoparticles which have been reported to be superior to conventional formulations, increasing efficacy, drug targeting and tolerability.²

The aim of this work was to evaluate the distribution and penetration of Nile red from Nps triggered for the pH of the skin, an emulsion (o/w) and a solution, in order to propose an option to improve the drug penetration through the skin, particularly in the follicular zone.

EXPERIMENTAL METHODS

Nps were prepared by a modification of the emulsification-evaporation process. The polymer (Eudragit E® 100) and the dye were dissolved in a water-saturated solvent (methyl ethyl ketone). This solution was emulsified with a solvent-saturated aqueous solution containing 5 % (w/v) of poly(vinyl alcohol). Finally, the organic solvent was removed by vacuum steam distillation at 33 °C and the dispersions were centrifuged at 35 000 rpm for 50 min, resuspending Nps with water (n=2).

Average particle size and Z potential analysis of the Nps were determined using a Zetasizer Malvern Systems (ZEN 3600, USA). Measurements were conducted at 25 °C. Z potential was determined with an electrical current of 150 V, using deionized water as dispersion medium (n=3).

In vitro permeation experiments were carried out for 1, 8 and 24 h (n=3) using human and porcine full-thickness skin (area=0.8 cm²) and vertical diffusion cells thermostated at 34 °C. The donor compartment was filled either with the Nps dispersion, an emulsion or an aqueous solution (water: polietilenglycol, 80:20) containing nile red (0.2 mg/ml). A blank for each contact time of permeation was used.

After the permeation studies, the skin was mounted on a glass slide with the stratum corneum side up, and immediately examined with a Zeiss 710 NLO Two-Photon Confocal Microscope (TPM, Carl Zeiss, Germany) in the follicular zone using a laser light (810 nm) focused by a W Plan-Apochromat 20x/1.0 DIC M27 75 mm objective lens. Two filters were used (494–549/567-674) to scan the skin autofluorescence (green) and Nile red (red). Then, xyz-sections were generated each 10 µm. Data processing and visualization out were analyzed by ImageJ software.

The skin samples from permeation studies were also analyzed using a Zeiss LSM 700 Confocal Microscope (Carl Zeiss, Germany). The tissues were treated with tragacanth gum (5%) to be frozen using liquid nitrogen. Then, these samples were cut at 320 µm and stained with Hoechst Blue dye solution. Images were visualized in a perpendicular position to the plane of the skin surface using an EC Plan-Neofluar 10x/0.30 M27 objective. Laser excitation wavelengths of 405 nm and 555 nm were used to scan Hoechst Blue (blue) and Nile red (red), respectively.

RESULTS AND DISCUSSION
Nile red loaded Nps showed a mean size of 560.6 ± 26.52 nm and an Z potential positive, high enough so as to provide a stable suspension (23±1.04), due to the cationic charge of Eudragit® E 100. By plotting the average of the fluorescence intensity taken against the z-slices depth, the emulsion (o/w) delivered the fluorophore in the uppermost layers during the first and the 8 h in a constant manner with a significant increase at 24 h, followed by the nanoparticle dispersion and finally by the solution (Fig. 1).

![Fluorescence intensity of Nile red found in the ear skin samples at 1, 8 and 24 h after the permeation studies.](image1)

**CONCLUSION**

In vitro permeation studies and confocal and two photon microscopy studies revealed that Nile red loaded Nps (of acceptable size and with a good physical stability) were found into the human and porcine skin, targeting the follicles and improving the penetration of the dye better than when the skin was treated with either the emulsion or the solution. Therefore, the pH-sensitive nanoparticles could be an excellent option to improve penetration of drugs through the hair follicles.

**REFERENCES**


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