An investigation of cutaneous delivery of lipoic acid loaded in liquid crystalline nanodispersion

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ABSTRACT SUMMARY
Lipoic acid (LA) loaded in liquid crystalline nanodispersion was developed and its in vitro skin permeation profile was assessed in a model of porcine ear skin. During the 8 h of experiment the liquid crystalline nanodispersion provided an enhancement of penetration and permeation of LA.

INTRODUCTION
Topical treatment of skin pathologies is achieved with nanocarriers formulations like liquid crystalline nanodispersions¹. With this delivery system it is possible to sustain and effectively release drugs into skin layers associated a lower risk of side effects. These systems combine the characteristic of nanometric size particles and the effect of monoolein, the structural lipid of the liquid crystal, which has known penetration enhancer property. These characteristics make liquid crystalline phase nanodispersion a potential carrier for dermatological drugs. Moreover, other advantage is related to drug protection against chemical degradation.

Lipoic acid (LA) is an ideal antioxidant for topical application. However, it is label and requires delivery systems to protect it against degradation and to deliver it into skin layers in order to have an efficient action. In this context, nanodispersion of liquid crystalline phase loaded with LA was assessed in terms of its penetration and permeation into/through the skin.

EXPERIMENTAL METHODS
Nanodispersions composed by monoolein: oleic acid: LA: aqueous phase (MO:OA:LA:AP, 8:2:0.4:89.6, w/w/w/w) were prepared excess of water by mixing MO (at 42°C), OA and 100 µg/mL LA (solution of 1 mg/mL LA in propylene glycol) and by adding aqueous phase composed by 30 mM phosphate buffer solution (pH 7.2) with Poloxamer® 1% w/v. After, the system was kept for 24 h to equilibrate the liquid crystalline phases, then vortex-mixed and sonicated in ice-bath for 2 min at 2 kHz at 200W and 20 kHz (Bandelin – HD 2200). The characterization of liquid crystalline nanodispersion (mean diameter, particle size distribution, zeta potential) was determined by dynamic light scattering using Zetasizer Nano ZS instrument (Malvern Instruments, Worcestershire, UK).

The in vitro penetration and percutaneous delivery were assessed in the model of porcine ear skin². Briefly, the skin from the outer surface of a freshly excised porcine ear was carefully dissected and dermatomized around 500 µm (Dermaton, Nouvag, Switzerland), then mounted in a Franz diffusion cell (diffusion area of 0.68 cm², Hanson Instruments, Chatsworth, CA), with stratum corneum facing the receptor compartment. The latter compartment was filled with 3 mL of 30 mM phosphate buffer solution (pH 7.2). The receptor phase was kept at 32±0.5°C and constantly stirred. Two hundred microliters of nanodispersion containing LA (n=3) was applied to the surface of the stratum corneum. A solution of 100 µg/mL LA in 30 mM phosphate buffer (pH 7.2) was used as control formulation (n=3). At 2, 4, 6, 8 h post-application, skin surfaces were thoroughly wiped with a cotton swab to remove excess formulation. To separate the stratum corneum (SC) from the remaining epidermis (EP) and dermis (D), skin sections were subjected to tape stripping (15 pieces of adhesive tape), the first one discarded and the others collected in 6 mL acetonitrile, vortex stirred for 2 min and bath sonicated for 15 min. The remaining (EP+D) was cut in small pieces, vortex mixed for 2min,
cutter tissue for 1 min, bath sonicated for 15 min and centrifugated at 1360 g for 5 min. The receptor phases were withdrawn at the time post-application. Finally, samples were filtered using a 0.45 µm membrane and assayed by the HPLC to quantify LA. LA was assayed by HPLC Shimadzu (Kioto, Japan), equipped with LC-AD pump, a model CTO-10A column oven, a model SCL-10A controller system, an electrochemical detector of glassy-carbon with amperometric detection at the high oxidation potential of 1.1V. Chromatographic separations were performed with a LiChrospher® 100 RP-18 (250 x 4 mm, 5 µm) with precolumn RP-18 (4 x 4 mm, 5 µm; Merck, Darmstadt, Germany) and the flow rate 0.8 mL/min. The mobile phase was 60% acetonitrile in 0.1 M sodium dihydrogenphosphate solution (pH 2.5). The running time was 10 min and injection volume 20 µL.

RESULTS AND DISCUSSION
The liquid crystalline nanodispersion loaded with LA had particle size in the range of 211.7 nm with a narrow size distribution (PI of 0.311) and zeta potential of -44.1 mV.

![Graph A](image1)

![Graph B](image2)

**Figure 1:** Time-course of the in vitro skin permeation and penetration of LA from liquid crystalline nanodispersion in the receptor phase (A) and skin layers (B)

Steady-state permeate flux (J) of LA was 0.269 µg. cm⁻².h⁻¹, obtained through of the slope of the linear regression (R² = 0.998). LA had its penetration increased at SC, epidermis and dermis at 2, 4 and 6 h showing that liquid crystalline nanodispersion was able to accumulate LA in the skin layers and to promote the cutaneous delivery of LA. Lower amount of LA retained into skin was observed at 8 h. This may be occurred due to high permeation rate of LA through the skin in the same time of post-application. Furthermore, none profile was observed with control formulation (buffer solution of LA), which did not allow detectable amounts of LA in the samples of *in vitro* experiment.

CONCLUSION
In summary, the present study showed that liquid crystalline nanodispersion significantly increased LA penetration into skin layers, and this penetration was dependent of the time of post-application (4 and 6 h showed to be more adequate for topical delivery), being a crucial characteristic to treatment of pathologies of skin.

REFERENCES

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