Liposomes as a topical carrier system for delivery of chlorogenic acid and Lychnophora salicifolia hydroethanolic extract: preparation, characterization and in vivo penetration evaluation

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ABSTRACT SUMMARY
Liposomes are promising systems that can carrier topically antioxidants compounds in order to protect human skin from UV radiation. We investigated the potential application of liposomes containing chlorogenic acid and Lychnophora salicifolia hydroethanolic extract as a topical carrier system for delivery these antioxidants. The results suggest that these liposomes can be successfully prepared and are able to increase the penetration of these antioxidants actives in vivo.

INTRODUCTION
Ultraviolet radiation (UVR) is a prominent initiator of reactive oxygen species (ROS) in skin, which can lead to the development of skin cancer and photoaging. Therefore, antioxidants compounds, such as polyphenols, in topical formulations have been suggested to protect the skin against UV-induced damage and skin ageing¹. Chlorogenic acid presents antioxidant, anti-inflammatory and anti-carcinogenic properties and is one of major polyphenolic compounds found in numerous plant species, including Lychnophora salicifolia Mart.². Nevertheless, suitable percutaneous absorption is known to be an essential requirement for satisfactory topically applied photoprotective agents.

Nanoparticulate carrier systems, such as liposomes, are promising delivery systems that can protect active substances against degradation while helping them to cross the barrier function imposed by the stratum corneum³. The aim of this study was to prepare liposomes containing chlorogenic acid and L. salicifolia hydroethanolic extract in order to investigate the potential application of liposomes as a topical carrier system for delivery these antioxidants.

EXPERIMENTAL METHODS
Liposomes were prepared by lipid film hydration method using phosphatidylcholine stabilized with 0.1% ascorbyl palmitate (Phospholipon® 90G, Lipoid GmbH, Germany) and tween 20 (4:1 w/w). The lipid film was hydrated with the appropriate volume of McIlvaine buffer (pH 5.0) containing or not 5.6mg/mL of chlorogenic acid or 33.6mg/mL of L. salicifolia hydroethanolic extract at room temperature. After complete lipid hydration and formation of liposomes, the vesicle dispersion was placed in a probe sonicator for ten 3 min cycles, until the dispersions were completely clear. Finally it was filtered through a disk filter (0.45µm). The characterization was performed by particle size distribution, polydispersity index and zeta potential determination, using Zetasizer Nano ZS 90 (Malvern Instruments, UK). The encapsulation efficiency (EE) was determined using a 50k Amicon® filter (Millipore, USA) to separate the free active substance by centrifugation at 8,000 rpm for 60 minutes and then measured by HPLC. EE was determined by the ratio [(amount of active used - amount of free active)/amount of active used] x 100. The prepared liposome dispersions were stored at 4, 40 and -20ºC for 45 days. For the determination of their stability were monitored the following parameters: particle size distribution and lipid peroxidation by thiobarbituric acid (TBA) test. For gel preparation, hydroxyethyl-cellulose (Natrosol®, Ashland, USA) was added slowly in a McIlvaine buffer solution (pH 5.0) or in the freshly prepared liposomal dispersion under constant stirring by a magnetic stirrer. In gel preparation without liposomes was added 0.5% (w/w) of chlorogenic acid or 2.5% (w/w) of extract. The skin penetration of active substances were assessed in hairless mice (HRS/J), thus 500mL of each gel formulation were applied on the back skin of each mouse. At 1 h post-application, the animals were killed with an overdose of carbon dioxide, and the treated skin area dissected, tape stripped and the amount of active in epidermis and dermis [E + D] determined by HPLC. All experiments were approval of the Ethic Commission for the Use of Animal of
RESULTS AND DISCUSSION
The initial particle size average of the liposome dispersions containing chlorogenic acid (AC) or not (BCO) was around 65nm and the zeta potential was around -2.0mV. For the liposome dispersions containing extract (ELS) the particle size average was around 115nm and the zeta potential was around -5.0mV. The polydispersity index for all prepared liposome dispersions was around 0.2.

Chlorogenic acid encapsulation efficiency was around 30%, whereas encapsulation efficiency of the extract was upper to 90%. In general, the liposome size did not change during 45 days storage neither at 4°C nor at 40°C (Figure 1). On the other hand, the storage at -20°C changed drastically the particle size in the first day of the stability test.

Figure 1. Particle size average during the storage at different temperatures.

Liposome dispersion containing actives (AC and ELS) showed lower amount of Malondialdehyde (MDA), a product of lipid peroxidation, in relation to free-actives liposome dispersion (BCO), confirming the antioxidant activity of these compounds. Results, expressed in nM MDA/mM lipid are shown in Figure 2.

Liposome systems incorporated in gel formulations significantly enhanced actives skin penetration 1h after application in vivo, when compared with gel formulations without liposome. Chlorogenic acid levels in [E+D] was approximately 0.75µg/cm². Concentration 2.5 times higher was detected when this polyphenol was incorporated into liposomes. After application of gel formulation with 2.5% (w/w) of extract on animals, no compound of the extract was detected in [E+D]. However, the application of gel formulation incorporated with extract into liposomes showed concentrations of 1.9, 5.7 and 0.4µg/cm³ of the vicenin-2, lychnopholic acid and chlorogenic acid, respectively, majority compounds of the extract.

CONCLUSION
The present study indicates that is possible prepare liposomes of chlorogenic acid and L. salicifolia hydroethanolic extract with success by lipid film hydration method and this system can be stable as to particle size and to lipid peroxidation when stored at 4°C for 45 days. This study also show that gel formulations with chlorogenic acid and L. salicifolia hydroethanolic extract into liposomes are able to increase the penetration of these antioxidants actives in the [E+D] of hairless mice 1h after the application, demonstrating the potential application of liposomes as a topical carrier system for delivery of these compounds.

REFERENCES

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