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Characterization and biocompatibility evaluation of cutaneous formulations containing lipid nanoparticles

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ABSTRACT

Nanostructured lipid carriers (NLC) are well-known systems that show effectiveness to improve skin hydration, being suggested for cosmetic and dermatological use. Nonetheless, NLC dispersions present low viscosity, which is non-attractive for cutaneous application. To circumvent this drawback, the dispersions can be gelled or incorporated in semisolid systems, increasing the final formulation consistency.

In this study, we prepared a hydrogel based on NLC containing vitamin E (HG-NLC_{VE}) and evaluated its suitability for cutaneous application. The experiments started with the HG-NLC_{VE} characterization (organoleptic analysis, accelerated stability, particle size, morphology, pH, texture and rheology). Afterwards, *in vitro* experiments were carried out, evaluating the formulation biocompatibility (MTT and Neutral Red) and irritant potential (Hen's egg test on the chorioallantoic membrane, HET-CAM) for cutaneous application. The results showed that the HG-NLC_{VE} has adequate features for skin application, is biocompatible and non-irritant. From this study, it was predicted the *in vivo* irritant potential of the developed formulation, avoiding the need to perform a high number of tests on human volunteers. Regarding vitamin E and NLC potential to improve skin hydration, we suggest that the HG-NLC_{VE} could be used in cosmetic (*e.g.* moisturizers and anti-aging) or dermatologic (*e.g.* xerosis and other skin disorders) products.

1. Introduction

Lipid nanoparticles, *i.e.* solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC), have been presented as superior carriers for the delivery of active ingredients and drugs, compared to other colloidal systems (*e.g.* liposomes, polymeric nanoparticles and nanoemulsions). Among the advantages of the former are the composition (physiological lipids and GRAS substances) and the lack of need to use organic solvents during production, which predicts absence of toxicity in the final formulations (Muller et al., 2002, 2011). Nevertheless, some natural polymers (*e.g.* chitosan) used to prepare polymeric nanoparticles might present similar benefits (Wang et al., 2011).

In this regard, several studies demonstrated that lipid nanoparticles formulations are safe for therapeutic and cosmetic uses. Nonetheless, when testing pharmaceutical formulations, the toxicity related to the drug molecules alone must be considered (Beloqui et al., 2016; Geszke-Moritz and Moritz, 2016; Puglia and Bonina, 2012; Silva et al., 2012c). Furthermore, some authors revealed concerns about the potential toxicity of using nanoparticles in cosmetics, related to the risk of occurring systemic absorption of the active ingredients, by means of the skin nanosized pores, wheat glands or hair follicles. Those are alternative penetration routes for nanoparticles that can transport active ingredients until the deepest skin layers, where are blood vessels, reaching systemic circulation. However, several studies indicate that there is no evidence of increasing cutaneous toxicity with particle size reduction, and that nanoparticles applied on the skin remain in the *stratum corneum* and/or in upper epidermis,

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being eliminated without systemic absorption (Nohynek and Dufour, 2012; Oliveira et al., 2016; Vogt et al., 2016).

Thereby, despite lipid nanoparticles are considered safe systems it is important to evaluate their suitability for cutaneous application, performing initially *in vitro* experiments, testing the biocompatibility and irritant potential. The biocompatibility of lipid nanoparticles formulations can be assessed by cell culture studies with keratinocytes (HaCa T cells), performing the (4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) bromide reduction and the neutral red (NR) uptake assays (Silva et al., 2011, 2012b, 2015; Wilson, 2014). In contrast, the irritant potential of cosmetic products is traditionally evaluated in animals, raising ethical concerns. To avoid these, efficient *in vitro* alternatives have been used, such as the HET-CAM (Hen's egg test on the chorioallantoic membrane) of fertilized chicken eggs (de Oliveira et al., 2016; Steiling et al., 1999).

If the results of *in vitro* experiments are satisfactory, *in vivo* tests should be carried out, performing clinical studies in human volunteers (Nasrollahi et al., 2016; Tichota et al., 2014). Nevertheless, according to the last revision of the Declaration of Helsinki, when developing a formulation, the number of tests in human volunteers should be minimum (General Assembly of the World Medical, 2014). These highlights the importance of performing preliminary *in vitro* and *ex vivo* studies predicting the *in vivo* effects of formulations.

Bearing this in mind, we evaluated the *in vitro* biocompatibility and irritation potential of a semisolid formulation based on lipid nanoparticles for dermatological or cosmetic application. In this study, we developed and characterized a hydrogel based on NLC containing vitamin E (HG-NLC$_{VE}$), a compound with antioxidant, protective and moisturizing properties (Nachbar and Korting, 1995; Pereira et al., 2016 Thiele and Ekanayake-Mudiyanselage, 2007).

2. Materials and methods

2.1. Materials

Precirol® ATO 5 (glyceryl palmitostearate) was acquired from Gattefossé (France), Cetrimide® was obtained from José M. Vaz Pereira, SA (Portugal), Tween® 80 (polysorbate 80) was purchased from Acofarma (Spain), alfa tocopheryl acetate (vitamin E) was provided by Acef (Italy). For the hydrogel preparation, the gelling agent PFC® (carbomer 2001) was provided by Guinama (Spain) and triethanolamine was purchased from Acofarma (Spain). The water used in all experiments was purified, obtained from a Direct-Q® Ultrapure Water Systems, Merck Millipore (Germany).

2.2. Methods

2.2.1. Preparation of NLC containing vitamin E (NLC$_{VE}$)

Table 1 shows the composition of the NLC containing vitamin E (NLC$_{VE}$) dispersion, which was prepared from the method previously employed by Silva et al. (Silva et al., 2011). Briefly, lipids and aqueous phase were melted and heated, respectively, at 5 °C–10 °C above the melting point of the solid lipid. Afterwards, the aqueous phase was added to the lipids and homogenized by high-speed stirring, using an Ultra-Turrax® T25 (IKA Labortechnik, Staufen, Germany), at 13500 rpm, for 5 min. The oil-in-water (O/A) emulsion obtained was placed under a probe sonicator (Vibro Cell VCX 130, 6 mm probe, Sonics & Materials, Newtown, CT, EUA), with a power-output amplitude of 70%, for 15 min, originating an O/W nanoemulsion, which was transferred to glass vials and cooled down, in an ice bath, forming the NLC.

Table 1
Composition (%, w/w) of the NLC containing vitamin E (NLC$_{VE}$) dispersion and hydrogel based on NLC containing vitamin E (HG-NLC$_{VE}$).

Materials (%, w/w)	NLC$_{VE}$	HG-NLC$_{VE}$
Precirol® ATO 5	7.0	–
Vitamin E	3.0	–
Tween® 80	2.5	–
Cetrimide®	0.5	–
Purified water	87.0	–
NLC$_{VE}$	–	99.5
Gelling agent PFC®	–	0.5
Triethanolamine	–	qs

2.2.2. Preparation of hydrogel based on NLC containing vitamin E (HG-NLC$_{VE}$)

The composition of the hydrogel based on NLC$_{VE}$ (HG-NLC$_{VE}$) is shown in Table 1 and was selected from a previous study (Tichota et al., 2014). For the hydrogel preparation, the gelling agent was milled in a porcelain mortar, the NLC$_{VE}$ dispersion was added, followed by neutralization with triethanolamine to acquire semisolid consistency.

2.2.3. Particle-size

Particle-size measurement was performed by Laser Diffractometry (LD), using a Mastersizer 3000 (Malvern, United Kingdom). Mie's theory model was employed with the following conditions: 1.4 for particle refractive index; 0.001 for particle absorption index; 1.33 for water dispersant refractive index. A volume distribution of 10%, 50%, and 90% was measured, which referred to particles with diameters equal or lower than the given values. The results presented are average values of five measurements (n = 5) plus standard deviation (SD).

To estimate the long-term stability of the NLC$_{VE}$ dispersion, the size was measured in the production day and after 7 months of storage at 20 ± 1 °C and 5 ± 1 °C.

2.2.4. Cryo-scanning electron microscopy (cryoSEM)

The structure of the NLC$_{VE}$ before and after incorporation in the hydrogel was observed by cryoSEM. For the experiments, samples were placed on metal stubs, immediately frozen with slush nitrogen, sublimated at −90 °C for 120 s, and coated with gold and palladium, by ion spray for 35 s and an electric current of 12 mA, under vacuum. Afterwards, samples were fractured and observed, at −150 °C, using SEM with X-Ray Microanalysis and CryoSEM experimental facilities (JSM-6301F; JEOL, Tokyo, Japan)/INCA Energy 350 (Oxford Instruments, Abingdon, UK)/ALTO 2500 (Gatan, Pleasanton, CA, USA).

2.2.5. Characterization of hydrogel based on NLC containing vitamin E (HG-NLC$_{VE}$)

2.2.5.1. Organoleptic analysis. Organoleptic analysis provides rapid assessment of formulations quality, since alterations in their appearance and/or homogeneity are indicative of poor raw materials quality or problems during production and storage. The organoleptic examination of the HG-NLC$_{VE}$ was performed on day 1 and after 7 months of storage at 20 ± 1 °C.

2.2.5.2. Accelerated stability. Accelerated stability by centrifugation was performed to estimate changes that may occur during the storage of the HG-NLC$_{VE}$, anticipating stability problems over time. The test was performed in triplicate with a centrifuge Eppendorf AG 5804 (Germany), adding 6 mL of sample to tube test and applying 2 cycles of 3000 rpm for 30 min. After the test, samples were examined to verify the presence/absence of phase separation, creaming or flocculation, which predict stability problems.