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Development of nanoemulsions for topical delivery of vitamin K1

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ABSTRACT

Vitamin K1 (VK1) is a natural and lipophilic compound currently used in dermatological formulations. In this work, nanoemulsions containing VK1 have been proposed to overcome some issues associated to semisolid VK1-incorporating formulations. The study has been focused on the design of a lipid-free aqueous formulation, easy to prepare and with low cost of production. Thus, a simply protocol, using a low-energy method, has been used to spontaneously form the nanoemulsions. The nanoemulsion composition has been optimized to improve its physical stability during storage in different conditions. Then, the possibility to administer VK1-containing nanoemulsions by nebulization without significant alteration of the formulation was tested. Moreover, the VK1 accumulation into the skin layers have been evaluated through permeation experiments on Franz cells, ATR-FITR analysis, confocal laser scanning microscopy (CLSM) observations, and high performance liquid chromatography (HPLC) analysis. The study demonstrated that NEs represent an interesting option for the commercial development of an aqueous spray formulation for the topical delivery of VK1.

1. Introduction

Vitamin K1 (2-methyl-3-phytyl-1,4-naphthoquinone; VK1) is a lipophilic molecule present in a wide variety of green species of vegetables (Pace, 1949). Many studies have evidenced many advantages of this natural compound in the prevention and resolution of different skin diseases (Lou et al., 1999; Lopes et al., 2007; Hemmati et al., 2014). In the clinical practice, the local administration of creams containing VK1 is used to prevent the occurrence of skin side effects, such as acneiform reactions after treatment of patients with anticancer drugs (Ocvirk, 2010; Pinta et al., 2014; Tan and Chan, 2009; Li and Perez-Soler, 2009 Pinta et al., 2014; Tan and Chan, 2009; Li and Perez-Soler, 2009).

Emulsion-based delivery systems, and in particular nanoemulsions, represent a promising tool for the topical and transdermal administrations of drugs (Sonneville-Aubrun et al., 2004). Nanoemulsions (NEs) consist of a fine oil/water dispersion with droplets having a 100–600 nm size (Nakajima et al., 1993, Nakajima, 1997), generally characterized by the presence of a hydrophilic surfactant and water–miscible solvent. O/w nanoemulsions have found increasing use as delivery systems due to their ability to encapsulate bioactive lipophilic components in cosmetic, personal care, functional food and pharmaceutical products (Saberi et al., 2013).

Nanoemulsions can be produced using different techniques, usually classified as high-energy methods and low-energy methods. On the other hand, the low-energy methods included several approaches such as spontaneous emulsification, phase inversion temperature (PIT), and phase inversion composition (PIC) methods (Anton et al., 2008). Compared to high-energy techniques, the use of low-energy methods had some advantages in terms of scale-up and industrial transfer, since no expensive equipments such as high pressure valve homogenizers or microfluidizers are required (McClements and Rao, 2011; Tadros et al., 2004). In addition, the possibility to use non-aggressive

Abbreviations: VK1, Vitamin K1; αTOC, α-tocopherol; $FeSCN_3$, iron thiocyanate; CH_3OH, methanol; CH_3CN, acetonitrile; $CHCl_3$, chloroform; CH_3CH_2OH or EtOH, ethanol; PE-CF, 1,2-dioleoyl-*sn*-*glycero*-3-phosphoethanolamine-*N*-carboxyfluorescein-ammonium salt; PI, polydispersity index; ζ, zeta potential; DI, deformation index; CLSM, confocal laser scanning microscopy; ATR-FTIR, attenuated total reflection Fourier transform infrared spectroscopy; SER, surfactant/emulsion ratio; O, oily phase; S, surfactant; W, water; UD, undetectable; NEs, nanoemulsions; BN, before nebulization; AN, after nebulization.

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preparation conditions can make these systems suitable to the delivery of labile molecules (Anton and Vandamme, 2009).

In the present study, we have investigated the possibility to develop nanoemulsions containing VK1 using a low-energy method and without lipid excipients. In an industrial standpoint, nanoemulsions are here proposed as a valid alternative, in terms of costs of production, to lipid-vesicles. Thus, we have prepared nanoemulsions by a spontaneous emulsification, at room temperature and using mild stirring conditions.

2. Materials and methods

2.1. Materials

Vitamin K1 (VK1), α-tocopherol (αTOC) and iron thiocyanate ($FeSCN_3$) were supplied by Sigma-Aldrich (St Louis, MO, USA). Analytical grade chloroform ($CHCl_3$) and ethanol (CH_3CH_2OH or EtOH) were purchased by Carlo Erba Reagents (Cornaredo, Italy). Polyoxyethilen-20-sorbitan-monooleate (tween 80) was provided by Farmalabor (Italy) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(carboxyfluoresceinammonium salt) (PE CF) was provided by Lipoid GmbH (Steinhausen, Switzerland).

2.2. Preparation of nanoemulsions

The nanoemulsions (NEs) were prepared using the procedure based on spontaneous emulsification, previously described by Anton and Vandamme (2009), with some modifications. Briefly, an oil phase composed of αTOC and VK1, the surfactant (tween 80) and the organic solvent (EtOH) was prepared. Then, the organic phase was slowly added using a syringe pump using a 50 μl/min flow rate (NE-300 "Just Infusion™", new era pump system Inc., NY USA) into an aqueous phase under magnetic stirring at 700 rpm and, finally, further stirred for 5 min at 1400 rpm. All the formulations have been stored at 4 °C in filled airtight vials and in absence of light. Different storage conditions were considered for the samples used in the stability studies.

2.3. Nanoemulsions size and zeta potential

Average diameter and size distribution of the obtained NE formulations were determined by photon correlation spectroscopy (PCS), while zeta potential (ζ) of NEs was measured by the Zetasizer Nano Z (Malvern, UK). For all experiments, 20 μl of the sample were diluted with water previously forced through a 0.22 μm filter water and analyzed by N5 (Beckman Coulter, USA). The results were expressed as NE mean diameter (nm) and polydispersity index (PI), and calculated as average of measures on three different batches of the same formulation.

2.4. Stability studies

Physical stability of NEs in time was evaluated on formulations prepared at increasing VK1 concentrations. Briefly, after preparation, each batch was stored at 4 °C and, at predetermined intervals, approximately 10 μl of the suspension were diluted with filtered distilled water and analyzed by PCS and ζ, as reported above.

The formulations were tested in different storage conditions as indicated by ICH guidelines (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use). In particular, NEs were incubated at different temperatures (4, 25 and 40 °C), in refrigerator (Hotpoint, Ariston, Italy) or in a laboratory oven (STF-F52Lt, Falc Instrument, Italy). All samples were kept under nitrogen atmosphere and protected from the light. At predetermined intervals, the samples were analyzed in terms of physical appearance, odor, NEs size and ζ. Results were averaged on three different batches.

2.5. NEs nebulization

NEs were also characterized before and after nebulization. To nebulize the formulations, a portable ultrasonic nebulizer (Eauté) kindly provided by Xenus, was used. Briefly, about 1 ml of the suspension, previously characterized for size, PI and ζ, was loaded into the device and nebulized. The aerosolized suspension was then collected in a 20 ml glass vial and characterized. The results were obtained by the average measurements of three different batches.

2.6. Skin penetration experiments

The penetration of VK1 in the skin and its transdermal delivery were assessed *ex vivo* using porcine ear skin. The porcine ears were kindly provided by a local slaughterhouse (Vendor Carni, Italy). All the experiments were performed on frozen-thawed skin stored at −20 °C and used for the experiments within 6 months. The part of the skin necessary for the experiments was removed from the external side of the pig ear. More in detail, the skin hair was preliminary removed and the skin was cut in suitable sized pieces (3 cm of diameter) with scissors as previously reported (Gillet et al., 2011). Only the epidermis, complete of the stratum corneum, and the dermis were used for Franz cells experiments. Thus, the thickness of the skin was measured by an electronic digital outside micrometer (Anhui Measuring Tools Company, China) and only samples with a thickness between 1.7 and 2.3 mm were considered for the permeation experiments. Then, the skin samples were mounted on the receptor compartment of Franz diffusion cells (Microglass Heim, Italy) assembly with the stratum corneum (SC) side facing upwards into the donor compartment. In the receptor medium (maximum capacity) seven milliliters of a mixture of PBS: EtOH (7:3 v/v) were added. A measured amount of NE was poured or nebulized into the donor compartment. To control the volume dispensed by the nebulizer, the device was loaded exactly with 1 ml of the formulations containing VK1 that was nebulized, until complete discharge of the device, directly into the donor chamber *VigorSkin K1*®. The concentration of all samples was adjusted so as to achieve the same VK1 amount (0.52 mg) in the donor compartment, while the diffusion area between the donor and the receptor compartments was 0.6 cm^2. The Franz cells were mounted on an H+P Variomag Labortechnik Telesystem (Germany) placed in a thermostatic bath Haake DC30 (Thermo Electron Corporation, Germany) under stirring at 600 rpm and at the temperature of 37 °C. At scheduled time points, aliquots of the receptor medium (700 μl) were withdrawn and replaced with the same amount of fresh medium. Each sample was filtered using 0.45 μm regenerated cellulose (RC) filters and the VK1 permeated in the donor compartment quantified. At the end of the experiments, skin surface was carefully washed with distilled water and dried with filter paper to remove the excess formulation and, subsequently, epidermis was separated from the dermis and the samples stored in plastic vials. The VK1 accumulated in the epidermis and dermis was extracted for 5 times with 1 ml of CH_3CN by bath sonicator (Branson 3510) for 30 min. The extracted solution was then filtered using 0.45 μm membranes and finally analyzed by HPLC to quantify the amount of VK1 accumulated in the different layers of the skin. The VK1 concentration was calculated with the help of a calibration curve ($r^2 = 0.999$) obtained with VK1 samples at a VK1 concentration from 50 to 0.5 μg/mL (LOD and LOQ of VK1 of 7.1×10^{-6} mg/L and 2.1×10^{-5} mg/L, respectively). The amount of VK1 accumulated in the different layers of the skin