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Combination of argan oil and phospholipids for the development of an effective liposome-like formulation able to improve skin hydration and allantoin dermal delivery

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ABSTRACT

Allantoin is traditionally employed in the treatment of skin ulcers and hypertrophic scars. In the present work, to improve its local deposition in the skin and deeper tissues, allantoin was incorporated in conventional liposomes and in new argan oil enriched liposomes. In both cases, obtained vesicles were unilamellar, as confirmed by cryo-TEM observation, but the addition of argan oil allowed a slight increase of the mean diameter (~130 nm versus ~85 nm). The formulations, especially those containing argan oil, favoured the allantoin accumulation in the skin, in particular in the dermis (~8.7 μg/cm^2), and its permeation through the skin (~33 μg/cm^2). The performances of vesicles as skin delivery systems were compared with those obtained by water dispersion of allantoin and the commercial gel, Sameplast®. Moreover, in this work, for the first time, the elastic and viscous moduli of the skin were measured, underlining the different hydrating/moisturizing effects of the formulations. The application of ARG liposomes seems to provide a softening and relaxing effect on the skin, thus facilitating the drug accumulation and passage into and trough it.

1. Introduction

Allantoin is the final product obtained from the oxidation/metabolism of uric acid metabolism of some vertebrates; it is also presents in different plants and currently can be easily chemically synthesized. In pharmaceutical and cosmetic fields, it is traditionally employed in the treatment of skin ulcers (Fu et al., 2006; Henderson, 1946) thanks to its capability to remove necrotic tissue promoting cell proliferation and skin epithelization (Durmus et al., 2012; Braga et al., 2012). It is also used as keratolytic agent in the treatment of the hypertrophic scar. Despite it being used in a wide range of pharmaceutical and cosmetic products for topical application, its skin penetration ability is very low due to its low log P ~3.14 and consequently, it beneficial effect is limited (Oliveira et al., 2014). A strategy to ameliorate its topical performances, prolonging the residence time at the action site and improving the local accumulation, may be the incorporation in liposome-like systems. In order to achieve this purpose, in the present work allantoin has been incorporate in liposomes and alternatively in liposomes enriched with argan oil (ARGliposomes). The addition of argan oil to liposomes was never previously tested and it is expected to improve the vesicle ability to modify stratum corneum lamellar assembly and its hydration favouring the allantoin skin delivery. Argan oil has been traditionally used in Morocco for centuries as a beauty oil or cosmetic ingredient, mainly for its ability to eliminate skin pimples as well as juvenile acne and to reduce dry skin matters and wrinkles (Guillaume and Charrouf, 2011a). It is mainly composed of acylglycerides (~99%), carotens, tocopherols, triterpene alcohols and xanthophylls (1%).

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Oleic and linoleic acids are the most abundant unsaturated fatty acids that significantly contribute to its favourable properties (Charrouf and Guillaume, 2008; Guillaume and Charrouf, 2011b).

The skin is the main barrier which avoid the passage of drugs topically applied and is formed by different layers: the stratum corneum, consisting of several layers of corneocytes (dead and flattened) embedded in a lipid-water lamellar matrix; the viable epidermis, consisting on living keratinocytes strongly joined with desmosomes junctions; the dermis, which is the support of the skin and it is formed by collagen and elastin fibres containing few fibroblasts; finally the hypodermis composed of fibroblasts, adipose cells, and macrophages. Due to its structure, the skin, in particular the stratum corneum, acts as main barrier, which controls the passage of foreign and endogenous molecules. The predominant pathway for drug passage through the skin is the paracellular way across the lipid domain between the epidermal cells, where the assembling of stratum corneum matrix and the hydration status of the skin, are key parameters which depend on human race, age, sex, skin type, anatomical location and humidity of the environment (Darlenski and Fluhr, 2012). Also cosmetic ointments and pharmaceutical preparations can affect the above mentioned properties as a function of formulation composition (Edwards and Marks, 1995; Esposito et al., 2007). In particular, nanocarriers, such as phospholipid vesicles and liposome-like systems due to their ability to deeply penetrate into the skin, are supposed to strongly modify such parameters (Castangia et al., 2015; Manca et al., 2016, 2015, 2014c, 2013a,b; Zaru et al., 2012).

Rheological study represents an innovative tool to evaluate the skin status, particularly its hydration and elasticity. Moreover, an adequate modelling to evaluate the viscoelastic properties of excised skin is of paramount interest in medical and cosmetic applications because it can aid to predict the modifications caused by topical preparations and their effect on its barrier function, providing an important support to select the most suitable formulations. Nevertheless its significance, actually few literatures reported detailed information regarding the rheological properties of excised skin and the effects of topical formulations. In this work, for the first time, we studied the rheological properties of excised skin and the influence of formulations in its behaviour. Due to the strong junctions and complementary structure of the main strata, the skin has been considered as a full and continuum layer thus microscopic properties of its components and modifications of the ordered structure may be reflected in its macroscopic viscoelastic behaviour.

In this work, allantoin liposomes were prepared and, as an alternative, ARGliposomes were formulated and characterized. Moreover, for the first time to our knowledge, the skin hydration effect of water, liposomal nanoformulations and Sameplast® gel was evaluated by rheological analyses and results were compared with those obtained performing *in vitro* allantoin permeation and penetration study.

2. Material and methods

2.1. Materials

Soy lecithin (SL) was purchased from Galeno (Prato, Italy). Allantoin (AL), argan oil (ARG) and all the other products were purchased from Sigma-Aldrich (Milan, Italy). Sameplast® gel (Savoma Medicinali s.p.a.) was purchased in a drugstore.

2.2. Vesicle preparation

Empty or drug-loaded vesicles were prepared by weighing soy lecithin (60 mg/ml), allantoin (10 mg/ml) and argan oil (5 mg/ml), when appropriate, in a glass test tube, adding water and leaving the samples one night at room temperature to facilitate the swelling of the phospholipids. The dispersions were then sonicated, 20 cycles (2 s ON and 2 s OFF) repeated 4 times, with a Soniprep 150 ultrasonic disintegrator (MSE Crowley, London, United Kingdom) at an amplitude of 15 μm. Dispersions were purified from the non-incorporated drug by dialyzing (Spectra/Por® membranes, 3 nm pore size; Spectrum Laboratories Inc., Rancho Dominguez, United States) them against water at 25 °C for 4 h (replacing the water once). Entrapment efficiency (EE) was expressed as the percentage of the drug amount found after dialysis versus that initially used. Allantoin content was determined by high performance liquid chromatography (HPLC) after disruption of the vesicles by dilution in methanol (1/100) and analysis of limpid solutions, was performed at 220 nm using a Thermo Scientific (Madrid, Spain) chromatograph. The column was a Waters C18, and the mobile phase was a mixture of methanol and water (5:95 v/v). The injection volume was 20 μl and the flow rate was 1 ml/min. A standard calibration curve (peak area of allantoin versus drug concentration) was built up by using standard solutions (range 1.0–0.01 mg/ml). Calibration graphs, plotted according to the linear regression analysis, gave a correlation coefficient value (R^2) of 0.999. The allantoin retention time was 2.7 min. The limit of detection was 2 ng/ml while the limit of quantification was 5 ng/ml.

2.3. Vesicle characterization

Vesicle formation and morphology were evaluated by cryo-TEM analysis. A thin film of each sample was formed on a holey carbon grid and vitrified by plunging (kept at 100% humidity and room temperature) into ethane, maintained at its melting point, using a Vitrobot (FEI Company, Eindhoven, The Netherlands). The vitreous films were transferred to a Tecnai F20 TEM (FEI Company), and the samples were observed in a low dose mode. Images were acquired at 200 kV at a temperature ~ -173 °C, using low-dose imaging conditions with a CCD Eagle camera (FEI Company).

The average diameter and polydispersity index (P.I.), of each sample, were determined by Photon Correlation Spectroscopy using a Zetasizer nano (Malvern Instrument, Worcestershire, United Kingdom). Zeta potential was estimated using the Zetasizer nano by means of the M3-PALS (Phase Analysis Light Scattering) technique. Before the analysis both liposomes and ARG liposomes (100 μl) were diluted with water (10 ml).

The stability of the vesicles was evaluated by using the optical analyser Turbiscan Ageing Station (Formulaction, L'Union, France) equipped with an ageing station with three thermo-regulated blocks for the storage of 54 samples. Turbiscan technology is based on Static Multiple Light Scattering for the analysis of concentrated dispersions (without mechanical stress or dilution). In our experiments, 10 ml of each sample were placed in a cylindrical glass cell and stored in the Turbiscan for 7 days at 25, 40 or 60 °C. The detection head was composed of a pulsed near-infrared light source (λ = 880 nm), two synchronous transmission (T) and back scattering (BS) detectors. The T detector receives the light, which crosses the sample (at 180° from the incident beam), while the BS detector receives the light scattered backwards by the sample (at 45° from the incident beam). The detection head scanned the entire height of the sample cell (65 mm longitude), acquiring T and BS each 40 μm. The measuring principle is based on the variation of the particle volume fraction (migration) or diameter (coalescence), resulting in a variation of BS and T signals. The stability of each sample was evaluated on the basis of the variation of back scattering (ΔBS). For a comparative evaluation between the different samples we exploited the Turbiscan Stability Index (TSI) computation, that provides a key number related to the