Colloids and Surfaces B: Biointerfaces 85 (2011) 182–188

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Structural characterization and *in vivo* evaluation of retinyl palmitate in non-ionic lamellar liquid crystalline system

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ARTICLE INFO

Article history:
Received 8 November 2010
Received in revised form 17 February 2011
Accepted 19 February 2011
Available online 1 March 2011

Keywords:
Liquid crystalline systems
Retinyl palmitate
SAXS
Rheological analysis
In vivo effectiveness

ABSTRACT

Carrier systems for lipophilic drugs, such as the liquid crystalline systems (LCS) have been extensively studied to improve effect and selectivity. Retinyl palmitate (RP) is widely used in pharmaceutical and cosmetics products to improve the skin elasticity. The aim of this study was the development, characterization and the *in vivo* effectiveness of RP in non-ionic LCS structures. LCS containing polyether functional siloxane as oil phase, silicon glycol copolymer as surfactant and water in the ratio 30:10:60, with and without RP were studied. The results of the polarized light microscopy, small-angle X-ray scattering and rheology analysis indicated the presence of typical LCS structures with lamellar arrangement. Regardless of the presence of RP, the rheological studies showed the pseudo plastic behavior of the systems. However, highest hysteresis area was verified when comparing the system in the presence and in the absence of RP. Stability study SAXS monitored, carried out up to 30 days in various storage temperature conditions ($25 \pm 2\,^{\circ}C$, $37 \pm 2\,^{\circ}C$ and $5 \pm 2\,^{\circ}C$) demonstrated the great structural stability of the LCS systems. The *in vivo* effectiveness analysis suggests that the RP-loaded LCS provided a significant reduction of the orbicular wrinkles in human volunteers ($P = 0.048$).

1. Introduction

Carrier systems for lipophilic drugs, such as the liquid crystalline systems (LCS) have been extensively studied to improve effect and selectivity [1–3]. It is known that the LCS can provide appropriated response for prolonged time, improving drug efficacy and reducing side effects [4–6]. These systems can be administered by different routes including ocular, oral, intraperitoneal, intramuscular, subcutaneous and cutaneous [7,8]. The development of new drug carrier systems based in the LCS structure has been a promising approach to increase and control the drug skin penetration [9–13]. Liquid crystalline phases are components mixtures that have mechanical properties of a liquid (fluidity) and optical characteristics of a crystal (optical anisotropy). Liquid crystalline systems are thermodynamically stable, thermotropic and lyotropic systems that can be stored for long periods without alterations [14–17]. Lyotropic liquid crystalline phases can be used as topical drug delivery systems because the high ability of drug solubilization, thermodynamic stability, a wide range of rheological properties and the high similarity with the intercellular lipid membranes of the skin [18–21]. Swarbrick and Siverly investigated the topical application of vehicles containing LCS and established that the percutaneous absorption of lipophilic drug model decreases significantly when the proportion of liquid crystalline phases increases above 5–10% [22]. LCS can control drug release because the low interfacial tension arising at oil–water interface [23,24]. The mechanism involves the progressive diffusion into the skin and to systemic circulation [25–27]. They can bring a considerable increase in the solubility of poorly or water insoluble drugs [28–31].

Appropriated methods of investigation and characterization of LCS are often used in drug development. Sophisticated techniques such as polarized light microscopy (PLM), ionic conductivity, rheology and small-angle X-ray scattering (SAXS) are available to achieve this goal [32].

The use of silicones in topical formulations is a world trend, due to their advantages like non-comedogenity, film formation, skin hydration, good skin feel and acceptance by the consumers. Thus, the association of LC and silicones allow for more stable and efficient formulations with adequate penetration, besides keeping the skin always moisturized [33,34].

Retinol, the form of vitamin A absorbed from animal food sources, is a yellow, fat-soluble substance. Since the pure alcohol form is unstable, the vitamin is found in tissues in a form of retinyl

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doi:10.1016/j.colsurfb.2011.02.027

ester. It is also commercially produced and administered as esters such as retinyl acetate or palmitate and is widely used as active substance in pharmaceutical topical formulations. RP improves the elasticity of the skin reducing up to 10% [35–37].

The aim of this study was the development, characterization, and *in vivo* effectiveness determination of the LCS containing RP. For this RP-unloaded and RP-loaded LCS, obtained from the combination of polyether functional siloxane as oil phase, silicon glycol copolymer as surfactant and water were used.

2. Material and methods

2.1. Material

Polyether functional siloxane (PFS), DC® 5329 (S) and silicon glycol copolymer (SGC), DC® 193 (O) were purchased from Dow Corning (Michigan, USA), retinyl palmitate (RP) 1,000,000 UI/g from Roche (Greenzach, Germany). The high purity water (W) from Millipore Milli-Q plus purification system was used throughout.

2.2. Methods

2.2.1. Formulations preparation

The samples were prepared by heating the mixture of O and S to 45 °C. The W was heated up to 40 °C was then carefully added under gentle and constant stirring until the mixture reaches at room temperature. The obtained systems containing different proportions of the components were characterized in a pseudo-ternary phase diagrams in order to describe the proportions of the components to form lamellar liquid crystalline systems (LCS). The proportions of each component were calculated from titrations of the binary mixtures of oil phase and surfactant with water. The transitions from opaque semisolid phase to clear liquid system (CL), clear viscous system (CV), viscous translucent system (TV), liquid emulsion system (LEM), and viscous emulsion (VEM) and phase separation (PS) were delimited. The diagrams were obtained from the mixtures RP-loaded (1%) and RP-unloaded.

2.2.2. Polarized light microscopy

A drop of the sample was placed in a glass slide that was covered with a cover slip and then analyzed under polarized light. A Motic Type 102 M Optical Microscope equipped with a digital camera was used to analyze several fields of each sample at room temperature. The isotropic or anisotropic behavior of the samples was noted. Photomicrographs were taken at 200× magnification.

2.2.3. Small-angle x-ray scattering (SAXS)

The nanometric structure of the phases was studied by (SAXS) measurements. Data was collected at the Synchrotron SAXS beam line of the National Laboratory of Synchrotron Light (LNLS, Campinas, Brazil), equipped with an asymmetrically cut and bent Si (1 1 1) monochromator ($\lambda = 1.608$ Å) that yields a horizontally focused beam. A vertical position-sensitive X-ray detector and a multichannel analyzer were used to record the SAXS intensity, $I(q)$, as a function of the modulus of the scattering vector q, $q = (4\pi/\lambda)\sin(\varepsilon/2)$, ε being the scattering angle. The parasitic scattering produced by slits was subtracted from the total scattering intensity.

2.2.4. Rheological analysis

The rheological analysis of formulations was carried out with a controlled-stress Carrimed CSL 100 rheometer (TA instruments) with plate–plate geometry. This geometry consists of two stainless steel plates of 2 cm diameter with a gap of 200 μm between the plates. Samples were carefully applied to the lower plate, ensuring that formulation shearing was minimized, and allowed to equilibrate for at least 3 min prior to analysis. The experiments were carried out with shear rates ($\dot{\gamma}$) in the range of 0.001–30 s^{-1}. The shear rate region used was selected on the basis of the strength of resistance to the applied stresses. The rheological measurements were performed on both the up and down curves. The data from the shear cycle were fitted to a power-law model, using Rheology Solutions Software (version Data V1.1.7, TA Instruments). All rheological determinations were carried out on all samples, at 25.0 ± 0.2 °C.

2.2.5. Wrinkles traces evaluation on the facial skin of human volunteers

This study was approved by the Research Ethics Committee of the Methodist University of Piracicaba, protocol 073/2005. The study was conducted on healthy women with white skin or light brown, aged between 30 and 45 years and with facial skin aging. It was selected 30 volunteers who did not use medicines for chronic use, except contraceptives, which were divided into three groups, each with ten volunteers. All volunteers were instructed to avoid the sun during the treatment period and guided to suspend the use of any product in the eyes periorbicular region one month before the beginning of the treatment. Terms of free consent were obtained from patients, who voluntarily participated after acknowledging the procedures of the study.

The first group used F1RP-unloaded to test de vehicle system, the second used F1 RP-loaded and the third was the control group (C) which did not use formulation. The volunteers were guided to apply fixed amount of the formulations 1×/day, at night, up to 30 days on the face skin including the eyelid region, through circular movements for 15 s. The entire volunteer's skin was previously photographed, using a CCD color microscope, Scope model. Reevaluation was carried out 30 days after the treatments.

For the calculation of the areas percentage with traces of wrinkles, the photographs were transferred to Corel Photo-Paint 8 software, the images divided in 266 areas of 1.2 cm^2, in images with 10× of magnification. The area with traces of wrinkles of the eye orbit region of each volunteer was calculated by planimetry for point's counting. [38] For the effectiveness study, comparisons between the treatment groups were performed using the analysis of variance followed Tukey test. The analysis was considered statistically significant for $P < 0.05$.

3. Results and discussion

The data of the pseudo-ternary phase diagram shows that it is possible to add a great volume of oil phase and limited volume of water phase maintaining the thermodynamic stability of systems. Clear and translucent regions are obtained in a wide range region of the pseudo-ternary phase diagram (Fig. 1). The analysis of the phase diagram regions in Fig. 1 shows a distinct transition from LCS to VEM, TV and CL; and from LEM, LCS and CL to PS. Independently on the proportions of the S and O a complete clear liquid phase (CL) exists with the aqueous phase proportions up to 70%, but with wide field in the low aqueous phase proportion and high oil phase contents. In the region where aqueous phase predominates and up to about 30% of the oil a phase separation region followed by LEM, VEM, LCS region and a CL system were observed with a progressive increase in the surfactant proportion. The formation of CL was favored in the region of oil phase dominance, but phase separation was verified when the aqueous phase was predominant. Two LCS regions separated by a translucent region (TV) were also found. The first region has been described in high surfactant proportions (above 50%), low proportions of aqueous (between 20 and 40%) and oil phases (up to 25%); and one second