Pharmaceutical nanotechnology

Physicochemical characterization of lycopene-loaded nanostructured lipid carrier formulations for topical administration

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A B S T R A C T

Nanostructured lipid carriers (NLC) are interesting delivery systems for enhancing the penetration of an active substance through the skin after topical administration. In the present study, lycopene was loaded into NLC, composed of Eumulgin® SG, orange wax and rice bran oil, using high pressure homogenization (HPH). Photon correlation spectroscopy analysis showed that the lycopene-loaded NLC had a size of 150–160 nm with a relatively small size distribution (PdI < 0.15). The entrapment efficiency of lycopene was found to be $100 \pm 0\%$ for all formulations. An in vitro release study of lycopene showed a biphasic release profile: a relatively fast release during the first 6 h followed by a sustained release during the next 18 h. An in vitro occlusion test showed that the occlusive properties of NLC increased with increasing lycopene loading. A free radical scavenging activity test of the NLC loaded with 50 mg% lycopene showed a Trolox equivalent antioxidant capacity value of $36.6 \pm 0.4\,\mu$M Trolox/mg NLC which is higher than that of the NLC base ($26.6 \pm 0.1\,\mu$M Trolox/mg NLC). The concentration of 50% antioxidant activity (IC_{50}) of the lycopene-loaded NLC was 14.1 ± 0.6 mg/mL, and lower than that of the formulation without lycopene (17.7 ± 0.4 mg/mL). The particle size, size distribution, and zeta potential of lycopene-loaded NLC stored at different temperatures of 4, 30, 40 °C for 120 days did not change in time, demonstrating an excellent colloidal stability of the systems. Chemical stability data indicated that the utilization of NLC increased the stability of lycopene and it was found that the degradation of lycopene was retarded when stored at low temperatures. In conclusion, NLC are attractive systems for the cutaneous delivery of lycopene.

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1. Introduction

Many colloidal carriers, such as liposomes (Allen and Cullis, 2013; Al-Jamal and Kostarelos, 2011; Barenholz, 2012), microemulsions (Chaiyana et al., 2013), and nanoparticles (Duncan and Gaspar, 2011; Lammers et al., 2011) have been studied as delivery systems for drugs to obtain improved therapeutic efficacy. Among them, nanostructured lipid carriers (NLC) have attracted increasing attention in the past decades as an alternative dosage form, because of their biocompatibility, biodegradability, high bioavailability, and good shelf-life. Moreover they protect chemically labile drugs from degradation and provide sustained release of the loaded active. Importantly, NLC can readily be produced at a large scale (Wissing et al., 2004). In the context of dermal drug delivery, there has been an intensified interest in in vitro test systems over the past few years. Such test systems are not only needed for the development of new pharmaceutical dosage forms and cosmetic products, but also to obtain information about the effect of drugs and/or excipients on the barrier function of the skin. Controlled drug release becomes important when the drug causes irritation of the skin at high concentrations and when the drug has to be delivered over a prolonged period of time (Souto et al., 2004). Moreover, penetration of active compounds into the human skin depends strongly on skin hydration which can be affected by occlusive compounds (Wissing and Muller, 2002). The application of occlusives prevents water evaporation from the skin into the atmosphere and thus water is retained in the skin. However, many commercially available occlusives have an unacceptable esthetic appearance (Barry, 1983) and consequently there is a need for innovative occlusive systems. It has been shown that solid lipid nanoparticles (SLN), which are structurally related to NLC, have better occlusive properties than conventional emulsions or microparticles and also a pleasant appearance (DeVringer, 1992). The extent of their occlusive properties depends on various factors, e.g., particle size and lipid concentration (Wissing et al., 2001). Both SLN and NLC are attractive carrier systems for topical products. However, NLC are preferred over SLN because of higher loading capacity of active compounds (Pardeike et al., 2009).

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Moreover, drug expulsion during storage is minimized or avoided by NLC (Muller et al., 2002).

Lycopene, which is present in tomato, watermelon, and pink grapefruit (Stahl et al., 2006; Stahl and Sies, 1996), is one of the most potent antioxidants. It has been shown that it functions as an anti-inflammatory, anti-cancer, and anti-mutagenic agent likely due to its high physical quenching rate for singlet oxygen (Giovannucci, 1999; Heber and Lu, 2002; Shi et al., 2003). However, its therapeutic potential is limited by some unfavorable properties. To mention, lycopene has a very low aqueous solubility and hardly diffuses through the epidermis when applied topically. The utilization of lipid nanoparticles for cutaneous delivery of lycopene is therefore an attractive option. Further, lycopene is very unstable and a suitable carrier system should retard its degradation.

The aim of the present study was therefore to investigate the suitability of NLC for loading, release, and stabilization of lycopene. The particles were characterized for size, charge, and stability, as well as in vitro release properties of NLC with different lycopene loadings. We also investigated the lycopene-loaded NLC for their occlusive properties and antioxidant activities.

2. Materials and methods

2.1. Materials

Orange wax was a kindly gift from Koster Keunen, LLC (Connecticut, USA). Rice bran oil was kindly provided by Surin bran oil Co., Ltd. (Bangkok, Thailand). Eumulgin SG (sodium stearoyl glutamate) was obtained from Cognis (Dusseldorf, Germany). Lycopene (90% pure), extracted from tomato, was purchased from Xian Guanyu Bio-technique Co., Ltd. (China). Ultra-pure water was prepared using a MilliQ Plus system (Millipore®, Schwalbach, Germany). Mineral oil was obtained from Sciencelab.com, Inc., Smith Rd., Houston, Texas. Propylene glycol was purchased from Lyondell Chemical Company (McKinney St., Houston, Texas, USA). 2,2′-Azinobis (3-ethylbenzothiazoline-6 sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Trolox® was purchased from Sigma, Steinheim, Germany. Potassium persulfate was purchased from BDH Chemicals Ltd., England.

2.2. Physicochemical properties of lycopene

The solubility of lycopene in certain oils and melted solid lipids as well as in water was tested in order to select the suitable lipids for the NLC preparations. An exact amount of 10.0 mg of lycopene powder was put into the beaker. Portions of about 0.5–2 mL of the oils or melted waxes were added and the mixtures were stirred for 5–10 min at 70 °C. This procedure was repeated until lycoprene was dissolved (visual inspection). Then, the beaker was weighed to get the exact weight of the added solvent. The solubility is expressed as mg of lycopene per g of the lipid solvent. DSC analysis was carried out using a Mettler DSC-1 apparatus (Mettler Toledo, Gieben, Switzerland). The melting temperature of the test samples was calculated by the STAR^e software. The instrument was calibrated with indium. Samples of accurate weight of 1–2 mg in a sealed pan were heated from 0 to 200 °C with a heating rate of 10 °C/min using an empty pan as a reference. Analysis was performed under a nitrogen gas purged at 80 mL/min.

2.3. Preparation of NLC

NLC was prepared according to the hot temperature high pressure homogenization (HPH) technique. In brief, to prepare 100 g lycopene-loaded NLC suspensions; 5, 25, or 50 mg of lycopene powder were separately dissolved in 5 g of melting lipid mixture (orange wax:rice oil = 9:1). For the aqueous phase, 1 g of Eumulgin®

SG was dissolved in water (about 90 mL). The oil phase and aqueous phase were separately heated to 75 °C for 1 min. Then, the oil and the aqueous phase were combined and vigorously stirred for 30 s. The weight of the mixture was adjusted with hot water to 100 g. The mixture was subsequently further mixed by using Ultra-Turrax T25 (Janke and Kunkel GmbH, Staufen, Germany) at high speed stirring of 12,000 rpm for 30 s to obtain a pre-emulsion. This hot pre-emulsion was then subjected to HPH (Micron LAB40, Homogenizer Systems, Germany) at 75 °C for 3 cycles. After that, the lipid dispersion was abruptly cooled down to room temperature to solidify the lipid droplet phase and obtain the aqueous NLC dispersions containing 5, 25, and 50 mg% lycopene.

2.4. Particle size measurements

Particle sizes of the NLC dispersions were analyzed using photon correlation spectroscopy (Zetasizer 4, Malvern Instruments, Herrenberg, Germany). A volume of 20 μL was diluted with 180 μL bidistilled water in order to eliminate multiple scattering. The samples were measured at an angle of 273°. The average diameter was calculated according to Stokes–Einstein after a curve fitting of the correlation function was done (Kato et al., 2012).

2.5. Zeta potential measurements

The zeta potential of the particles was measured by determining the electrophoretic mobility using the Malvern Zetasizer IV (Malvern Instruments, UK). A volume of 20 μL was diluted with 180 μL bidistilled water. Next, the samples were adjusted to a conductivity 50 μS/cm with sodium chloride solution (0.9% w/v). The pH was in the range 5.5–6.0, the electrophoretic mobility was measured at field strength of 20 V/cm.

2.6. Entrapment efficiency

The lycopene entrapment efficiency of the formulations was determined by an indirect method. A volume of 10 mL of the NLC sample was diluted with 40 mL of bidistilled water. The samples were centrifuged at 15,000 rpm for 30 min. The amount of lycopene in the supernatant was determined spectrophotometrically at 475 nm using a double-beam UV-2450 (Shimadzu, USA). The entrapment efficiency (EE) was calculated using the following equation.

$$EE(\%) = \frac{(W_i - W_f)}{W_i} \times 100$$

where "W_i" is the mass of initial added lycopene and "W_f" is the mass of lycopene detected in the supernatant after centrifugation of the formulations.

2.7. X-ray diffractometry

Lycopene-loaded NLC containing 50 mg% lycopene, lycopene powder, and the physical mixture of orange wax, rice oil, and lycopene powder were subjected to X-ray diffractometry (XRD) using a Philips PW1830 X-ray generator (Philips, Amedo, The Netherlands) with a copper anode (Cu-Ka radiation, 40 kV, 25 mA, $k = 0.15418$ nm), and using a Goniometer PW18120 as a detector. The measurements were analyzed at 2θ of 0.6–40.0°. The data were typically collected with a step width of 0.04° and a count time of 60 s.

2.8. Release study

A 'membrane free' release test model was developed to study the lycopene release profiles of the different NLC. Mineral oil was used as an acceptor medium. An aqueous NLC dispersion (1 mL of