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Development of a nanostructured lipid carrier formulation for increasing photo-stability and water solubility of Phenylethyl Resorcinol

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ABSTRACT

The Phenylethyl Resorcinol loaded nanostructured lipid carrier (PR-NLC) was developed by hot high-pressure homogenization method. The freshly prepared PR-NLC showed a spherical morphology under transmission electron microscope, and the particle size was 218.3 ± 9.2 nm. The value of the zeta potential of PR-NLC decreased from −30.2 ± 1.9 mV to −64.9 ± 1.3 mV when the dilution times reach 10. The loading amount of PR encapsulated in NLC was 2.94 ± 0.03%, and the average entrapment efficiencies of PR-NLC determinated by size exclusion chromatography and ultrafiltration were 90.2 ± 0.6% and 98.3 ± 0.3%. Lyophilization was proved feasible for the storage of NLC dispersion. Fourier transform infrared spectra (FTIR) was exploited to investigate the possible drug–lipid complex formation. Advancements in water solubility of PR were demonstrated by NLC using a contact angle measurement. The hemolysis percentage of the NLC was less than 1.3% in a certain range of concentration. In 90 days' storage, 88.6 ± 2.8% of PR remained unchanged in PR-NLC under natural daylight. In vitro release studies revealed a sustained drug release, and in vitro penetration studies showed an increase of retention amount of PR in the skin, when applying PR-NLC. Therefore, the NLC might be a potential delivery vehicle in cosmetic dermal products.

1. Introduction

Phenylethyl Resorcinol (4-(1-Phenylethyl)1,3-Benzenediol, PR) is a new lightening agent that has been found to have the ability to inhibit tyrosinase activity by Schmaus [1]. Tyrosinase is present in almost all types of organism and its main function is pigment biosynthesis [2]. In mammals, tyrosinase catalyzes oxidation of L-tyrosine and L-dopa to form melanin [3] which determines the color of mammalian skin and hair. Many investigations have delivered the unequivocal proof that Phenylethyl Resorcinol is one of the most potent lightening agents ever reported and is not due to cytotoxicity [1,3,4]. It has been demonstrated that the PR can reduce tyrosinase activity approximately 22 times more effectively than Kojic acid [3]. However, the application problems of PR lie in its light instability and poor water solubility. The poor water solubility may limit its absorption when used, while its low photo-stability may render the topically applied PR ineffective. Therefore, there is a need for appropriate delivery vehicles that can improve the photo-stability and water solubility of PR.

The nanostructured lipid carrier (NLC) is considered as the second generation of the lipid nanoparticles following the solid lipid nanoparticle (SLN) [5], which is widely studied and used in cosmetic and pharmaceutical fields [5–7]. This vehicle is produced by using a blend of solid lipids with liquid lipids as a lipid matrix, this blend also being solid at body temperature [6]. The imperfections and fluid domains in the lipid matrix of NLC show a higher loading capacity for many drugs than SLN and avoid/minimize potential expulsion of drug during the storage [8]. In recent years, the NLC has been intensively investigated in cosmetic dermal products because of many positive features have been reported after their application to the skin [6,8,9]. Due to the lipid matrix, the small particle size and related adhesive properties, the residence time of NLC on the skin is prolonged [8]. Enhancement of chemical stability of actives, controlled occlusion, increase in skin hydration, enhanced skin bioavailability of actives and skin targeting are also excellent cosmetic benefits of NLC [5,6,10].

At present, no basic investigation about possible solutions of application problems of PR is available. Therefore, the aim of this study was to develop a delivery vehicle for potential use of RP. For this, PR loaded NLC was prepared and studied for cosmetic solutions. The particle size, zeta potential, morphology, loading amount, entrapment efficiency, lyophilization, FTIR spectrum, and contact angle of the PR-NLC were characterized. A simple and fast HPLC method was developed to detect the PR. The physical and chemical stabilities of the PR-NLC were investigated during 90 days. The hemolysis was also investigated to evaluate its blood

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compatibility. Furthermore, the in vitro drug release and in vitro penetration were examined to elucidate the applicability of PR-NLC.

2. Materials and methods

2.1. Materials

Phenylethyl Resorcinol (PR) was provided by Symrise Co., Ltd. (Holzminden, Germany); Glycerin monostearate (GMS) and diglycerides (ACETEM), octyl and decyl glycerate (ODO) were purchased from Zhengtong Chemical Co., Ltd. (Henan, China); Behenyl Alcohol was provided by Better Chemical Co., Ltd. (Shanghai, China); Sympatens-O/100G (Trade Name) and Sympatens-AS/020G (Trade Name) were purchased from DKSH Co., Ltd. (Zurich, Switzerland); Methanol was provided by Merck (Darmstadt, Germany). This reagent was chromatographically pure and passed through 0.45 μm filter prior to use. Ultrapure water with conductivity of 18.2 MΩ cm was used in all the experiments. All other chemicals used were of analytic grade and commercially available products.

2.2. Preparation of PR-loaded NLC

The PR-loaded NLC dispersions were prepared by the hot high-pressure homogenization method as described elsewhere [5,6,10]. Briefly, solid lipids (2% GMS, 3% ACETEM, and 1.5% Behenyl Alcohol) were mixed with a high content of liquid lipid (5% ODO). The lipids were heated to a temperature 5–10 °C higher than its melting point (in case of Behenyl Alcohol to 70 °C). Then, PR (3%) was dissolved in the lipid melt, forming the lipid phase. In the hot state the lipids form one phase. Meanwhile, the aqueous phase (85.5%) containing 8.5% of surfactants (Sympatens-O/100G and Sympatens-AS/020G) was heated to the same temperature as the aqueous phase. More than 50 kinds of non-ionic surfactants were studied at different ratios to identify optimum stabilizer blend. The result indicated that combination of Sympatens-O/100G (8%) and Sympatens-AS/020G (0.5%) was optimal to prevent particle growth and stabilize the NLC system. Then, the aqueous phase was added to the lipid phase and emulsified by stirring at 600 rpm for 5 min and further dispersed using an Ultra-Turrax (FM200, FLUKO Technology, Germany) at 8000 rpm for 30 s, avoiding excessive foam formation. The obtained emulsion (generally called pre-emulsion) was homogenized by high pressure homogenizer (AH100D, ATS Engineering, Canada) using three homogenization cycles at 300 bar and 70 °C. Finally, the resulting dispersion was cooled at ambient conditions to room temperature. During the cooling process, phase separation occurred leading to the precipitation of small oil droplets. The oil droplets contained a high amount of PR and were incorporated into the solid matrix to obtain the PR-loaded NLC [5].

2.3. Particle size analysis

Analysis of the nanoparticle size and the polydispersity index (PDI) were performed by dynamic light scattering (DLS), also known as photon correlation spectroscopy (PCS), using a Malvern Zetasizer (ZS90, Malvern Instruments, UK). Prior to the measurements, all samples were diluted 200 times by ultrapure water to a weak opalescence [11]. The mean particle size (*z*-ave) and PDI values of the investigated samples were obtained by calculating the average of three measurements at 25 °C with a scattering angle of 90°.

In order to detect potential larger particles and oil droplets, static light scattering (SLS), also known as laser diffractometry (LD), was applied as additional characterization method using a Malvern Mastersizer (MS2000, Malvern Instruments, UK) with a measuring range up to 2000 μm [8,9], The LD data were analyzed using the Mie theory with the optical parameters 1.456 (real refractive index) and 0.01 (imaginary refractive index) [8]. The LD data obtained were evaluated using volume distribution as diameter values of D10, D50 and D90. The diameter values indicate the percentage of particles possessing a diameter equal or lower than the given size [12].

2.4. Zeta potential measurement

The zeta potential (ZP) was determined by the measurement of the electrophoretic mobility using a Malvern Zetasizer (ZS90, Malvern Instruments, UK). The field strength applied was 20 V/cm. The zeta potential was calculated using the Helmholtz–Smoluchowsky equation [13] at 25 °C. Disposable folded capillary cuvette was used for ZP measurement. Air bubbles, if any, were removed from the capillary before measurement [14].

2.5. Transmission electron microscopy (TEM) analysis

The shape and surface morphology of the PR-NLC were observed by transmission electron microscopy (TEM) (Tecnai G2 F20 S-TWIN, Fei, USA) using a negative-staining method [15]. Fresh prepared PR-NLC was diluted with ultrapure water and dropped on a copper grid. The sample was stained with 2% (w/v) phosphotungstic acid and air-dried for 5 min at room temperature after removing the excessive sample with filter paper. The air-dried samples were then examined under the TEM.

2.6. High Performance Liquid Chromatography (HPLC) measurement

The content of PR was analyzed by a High Performance Liquid Chromatography (PE200, Perkin Elmer, USA). The HPLC system was equipped with an Inertsil ODS-2 column, coupled with a PESciex AP13000 MS-MS apparatus operating in the multiple reaction monitor mode. The analytical chromatography column was a C18 column (250 mm × 4.6 mm, 5 μm) (Global, China). The injection volume was 20 μL; the mobile phase was methanol–water (70/30, v/v) at a flow rate of 1.2 mL/min; the wavelength was 254 nm; the column temperature was 35 °C.

2.7. Entrapment efficiency

The entrapment efficiency of PR-NLC was measured by using a Sephadex-G50 column (15 mm × 200 mm) [16] and ultrafiltration centrifugal filter tubes with a molecular weight cut-off of 30 kDa (Millipore, USA) [12,17]. The free PR was separated with size exclusion chromatography on a Sephadex G-50 column, and eluted with ultrapure water at a flow rate of 1.0 mL/min. In the ultrafiltration method, the PR-NLC suspension was added into ultrafiltration centrifugal filter tubes and centrifuged at 12,000 rpm for 2 h at 4 °C. The concentrations of PR in the PR-NLC (total Phenylethyl Resorcinol) and the ultrafiltrate (free Phenylethyl Resorcinol) were determined using HPLC as described before. The E.E. was calculated by the following equation:

$$\text{E.E.} = \frac{\text{Total amount of PR} - \text{Free amount of PR}}{\text{Total amount of PR}}$$

2.8. Lyophilization

The PR-NLC dispersion was lyophilized with and without cryoprotectant using a freeze dryer (LGJ-10, Four-ring science instrument, China). The NLC dispersion was pre-frozen at −20 °C for 4 h and subsequently lyophilized at a temperature of −40 °C