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PHENYLETHYL RESORCINOL LOADED IN LIPID NANO VESICLES: A PROMISING WHITENING COSMETIC INGREDIENT

[1]Feng, Y., [1]Lei, Y. and [2]Zhang, Q
[1]Department of Pharmacology, Jinan University, Guangzhou, China
[2]Department of Cell Biology, Jinan University, Guangzhou, China

Abstract: This study aimed to develop a delivery system using lipid nano vesicles (LNVs) for the whitening cosmetic ingredient phenylethyl resorcinol (PR) which has high tyrosinase inhibitory ability but suffers from poor water solubility and photostability. PR-loaded LNVs (PR-LNVs) were prepared and characterized for their physical and chemical features, including morphology, particle size, encapsulation efficiency, and stability. The PR-LNVs showed a homogeneous spherical morphology with a mean particle size of 132.4 nm and high encapsulation efficiency of 97.37%. Moreover, the PR-LNVs demonstrated sustained-release properties and physicochemical stability for at least three months. The results suggest the promising potential of PR-LNVs as a delivery vehicle for phenylethyl resorcinol in cosmetics. This study highlights the significance of nanocarrier technology in improving the efficacy of cosmetics and overcoming the limitations faced by poorly soluble and unstable cosmetic ingredients, such as PR. Furthermore, LNVs' small particle size, high encapsulation efficiency, good stability, and promotion of transdermal absorption suggest their potential as a delivery system for other active ingredients in cosmetics.

Keywords: lipid nano vesicles, phenylethyl resorcinol, delivery system, nanocarrier technology, sustained-release properties, physicochemical stability.

1. Introduction

Recently, as the improvement of living standards and the increasing interest in health and beauty, the demand for functional cosmetics that improve skin health and beauty is increasing rapidly [1]. Asian consumers demand more cosmetics, especially whitening cosmetics. The skin color of human is well known to be directly related to the amount of melanin in the epidermis [2]. Excessive production of melanin in the skin can induce hyperpigmentation disorders such as
Melasma, photopigmentation, age-related freckles, and post-inflammatory hyperpigmentation[3]. Therefore, skin-whitening products are mainly composed of substances that inhibit melanin synthesis. The synthesis of melanin is a complex process, in which tyrosinase is the key enzyme involved in melanin synthesis [4]. Therefore, inhibition of tyrosinase is the main strategy for whitening. Various tyrosinase inhibitors have been tested in cosmetics to prevent overproduction of melanin in the epidermis [5].
Phenylethyl Resorcinol (PR) possess effective tyrosinase inhibitory activity, and its tyrosinase inhibitory ability is 22 times that of kojic acid, a traditional tyrosinase inhibitor [6]. PR can significantly improve the

uneven skin color, reduce the synthesis and accumulation of melanin, and achieve the purpose of efficient whitening [7]. However, the application of PR in cosmetics is greatly limited by its weak light stability, poor solubility in water, easy chelation with metal ions, and discoloration under light conditions [8]. How to improve the stability and bioavailability of PR is a key issue for its application in cosmetics.

To overcome the limitations of PR, drug nanocarrier's technology is considered. Lipid nano vesicles (LNVs) are a potential drug delivery system due to high drug loading, biocompatibility, low toxicity, and ability to remain stable in most aqueous suspensions [9]. LNVs are a new type of nanocarrier with polymer nano capsule structure, which is an O/W type emulsion [10]. Traditional LNVs are generally composed of three components: water phase, oily phase and surfactant. However, due to the high irritability of surfactants, German company Lipiod developed a new type of LNVs using high biocompatibility phospholipids as the emulsifier of the system to replace synthetic surfactants. The improved LNVs have high biocompatibility and low skin irritation due to the use of naturally derived phospholipids [11].

In this study, PR-loaded lipid nano vesicles were prepared by high-pressure homogenization, and the relevant physical and chemical characterizations were detected. At the same time, the stability of PR-LNVs was investigated and evaluated for a period of three months.

2. Materials and methods

2.1 Materials

Phenylethyl Resorcinol (PR) was obtained from Shanghai Acmec Biochemical Co., Ltd. Lecithin was purchased from Macklin Biochemical Technology Co., Ltd. Tween®80 was purchased from MedChemExpress. Dipropylene glycol was supplied by Shanghai Jizhi Biochemical Technology Co., Ltd. Caprylic/capric triglyceride (GTCC) was obtained from

Shanghai Yuanye Bio-Technology Co., Ltd. Ultrafiltration centrifugal filter tubes were supplied by Beijing Solarbio Science & Technology Co., Ltd. Milli-Q water (NeoLab, Hangzhou, China) was used throughout the experiment. All chemicals were used as received without any modification.

2.2 Preparation of PR-LNVs

PR-LNVs were prepared by high-pressure homogenization method as follows: An oily phase: 6% lecithin was dissolved by stirring at room temperature using dipropylene glycol as an organic solvent, which was the oily phase A. Oily phase B was obtained by dissolving 3% Phenethyl Resorcinol using a liquid lipid (GTCC) at room temperature. An aqueous phase: An ultrapure aqueous solution containing 0.5% Tween® 80 and 40% glycerol was prepared. Subsequently, the oily phase B preheated to 70°C was added into the oily phase A, and the reaction was fully stirred at 1500rpm and 70°C in a water bath for 30 minutes. Finally, the colostrum samples were homogenized with a microfluidic high-pressure homogenizer at 1100 bar, and the number of homogenization cycles was 3 times. After the homogenized samples were cooled to room temperature, PR-NLVs were obtained.

2.3 Characterization of PR-LNVs

2.3.1 Particle Size, PDI, and Zeta Potential Analysis

The particle size, PDI, and zeta potential of the samples were determined by Zetasizer Nano ZS90 system (Malvern, Egham, U.K). The measurement samples for particle size and PDI were diluted 100-fold by ultrapure water, while the samples for zeta potential were diluted 10-fold.

All samples were measured with a scattering angle (θ) of 90 degrees at 25°C.

2.3.2 Drug Encapsulation Efficiency (EE) Analysis

The EE of PR-LNVs were determined by ultrafiltration centrifugation method. First, 0.5 mL of the sample was diluted to 2 mL with chromatographic methanol, and completely demulsified in a sonicator to release all

the PR in the PR-LNVs. After demulsification, the PR content was determined by HPLC, and this PR amount was designated as R_{total}. Then, 0.5 mL of PR-LNVs sample was added into ultrafiltration centrifugal filter tubes with a molecular weight cut-off of 30 kDa and centrifuged at 10,000 rpm for 1 hour at 4℃. After centrifugation, the ultrafiltration liquid was diluted 4-fold by ultrapure water, and the drug content in the obtained solution was detected under the same HPLC conditions. The amount of free PR was designated as R_{free}. The EE was calculated by the following formula:

$$EE\ \% = \frac{Rtotal - Rfree}{Rtotal} \times 100\ \%$$

2.3.3 Transmission Electron Microscopy (TEM) Analysis

Microscopic morphological structures of PR-LNVs were observed by TEM (JEOLTEM-1210). The sample was diluted 100-fold by ultrapure water and dropped on a copper grid. After natural drying, the excess liquid was absorbed with filter paper, and the microscopic morphology was observed under the TEM.

2.3.4 Atomic Force Microscopy (AFM) Analysis

The surface morphology of PR-LNVs was visualized by AFM (Bioscope Catalyst/ Multimode, Santa Barbara, CA, USA). The PR-LNVs sample was diluted 100-fold by ultrapure water and placed on a mica coverslip. After removing the excessive sample with filter paper, the sample was air-dried for 5 min at room temperature, and then investigated under the AFM.

2.3.5 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR spectra of the pure PR, the lyophilized blank LNVs, and the lyophilized PR-LNVs were detected by Fourier transform infrared spectrometer (VERTEX 70/70v FT-IR spectrometer, Germany) to detect the interaction between PR and the lipid molecules. The samples were attached to the infrared transparent crystal surface of the ATR accessory, and scanned 32 times in the range of 4000~400 cm^{-1} to record the infrared spectral information. Software (OPUS 8.5,
Germany) was used to analyze infrared spectral data.

2.3.6 In Vitro Drug Release Properties of PR-LNVs

To achieve controlled release and obtain prolonged-release information, the dialysis diffusion technique was used to investigate PR release from PR solution and PR-LNVs. Briefly, 7 mL of PR-LNVs samples and an equal concentration of PR standard solution were placed into a dialysis bag (molecular weight cut-off, 8000–14,000; Shanghai Acmec Biochemical Co., Ltd.). Then, the dialysis bag was placed into 300 mL release medium (PBS containing 1% Tween 80), which was stirred at 100 rpm with the temperature kept at 37℃. 1 mL of the release medium in the beaker was sampled at a preset time, and 1 mL of fresh release medium was supplemented at the same time. The collected samples were filtered by a 0.22 μm filter and their content of PR was determined by HPLC.

2.4 Long-term Storage Stability Investigation

Different batches of fresh PR-LNVs samples were prepared and stored at 4°C or 25°C under light conditions. Samples under different conditions were collected every month to determine the mean particle size and encapsulation efficiency. The entire long-term storage stability period was 3 months.

3. Results and Discussion

3.1 Characterization and analysis of PR-LNVs

In this study, the main formulation of PR-LNVs was obtained from 3% PR, 6% lecithin, 5% GTCC, and 40% glycerin. Lecithin acted as an emulsifier to emulsify. GTCC was chosen as a liquid lipid because of its good biocompatibility, spreadability, as well as skin moisturizing properties. The addition of glycerol can increase

the viscosity and stability of the system, and reduce the oxygen content in the system to reduce the oxidative degradation of lipids and active substances. After the formulation was determined, the mean particle size, PDI, zeta potential, encapsulation efficiency, physical morphology, and infrared spectrum of the prepared PRLNVs were characterized.

3.1.1 Morphology Analysis of PR-LNVs

Figure 1a showed that the color of undiluted PR-LNVs solution was dark milky white, mainly the color of PR, while the sample diluted 100 times had a bluish opalescent color, and the solution was uniform and transparent, indicating that the PR-LNVs were well dispersed in water. The morphology of PR-LNVs diluted 100 times was observed by TEM. PR-LNVs have regular single-cell spherical structure with uniform particle size (Figure 1b). The PR-LNVs were further viewed by AFM to predict the particle topology. Figure 1c showed that the size and distribution of the PR-LNVs were uniform. In addition, the 3-D graphs of PR-LNVs showed that their surfaces were smooth and uniform with gentle peak shapes (Figure 1d).

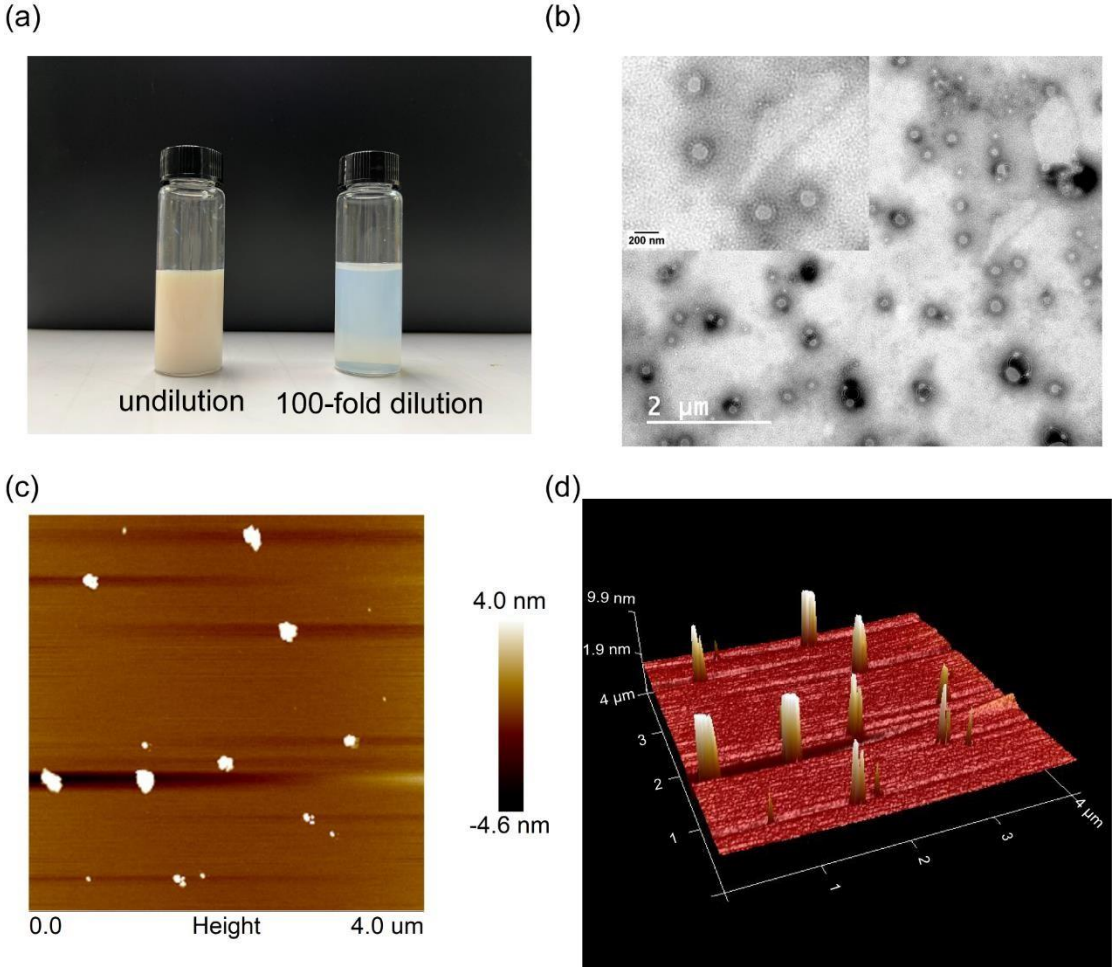

Figure 1. Physical morphology of PR-LNVs.

(a) The physical appearance of PR-LNVs. **(b)** Transmission electron microscopy (TEM) measurement of PR-LNVs. **(c)** 2-D atomic force microscopy (AFM) image of PR-LNVs showing deformation. **(d)** 3-D atomic force microscopy (AFM) image of PR-LNVs showing height.

3.1.2 Particle Size, PDI, Encapsulation Efficiency, and Zeta Potential

The particle size, PDI, encapsulation efficiency and zeta potential are important parameters for evaluating the nanosystem, which can directly reflect the stability of the system and the quality of nanoparticles. The mean particle size and PDI of the prepared PR-LNVs were 132.4 ± 1.04 nm and 0.137 ± 0.003, respectively

(Table 1). The zeta potential value was −43.9 ± 3.44 mV, which indicated that the system was stable. The high encapsulation efficiency (97.37 ± 6.14%) further indicated that PR-LNVs were efficiently load PR into nanocarriers. In addition, the particle size distribution of PR-LNVs is narrow, and this particle size (<200 nm) is favorable for transdermal absorption (Figure 2).

Table 1. Nanoparticle size, PDI, encapsulation efficiency, and zeta potential of Blank-LNVs and PR-LNVs (n = 3, mean value ± SD)

Group	**Blank-LNVs**	**PR-LNVs**
Size (nm)	43.93±0.87	132.4±1.04
Zeta potential (mV)	————	-43.9±3.44
PDI	0.215±0.017	0.137±0.003
EE (%)	————	97.37±6.14

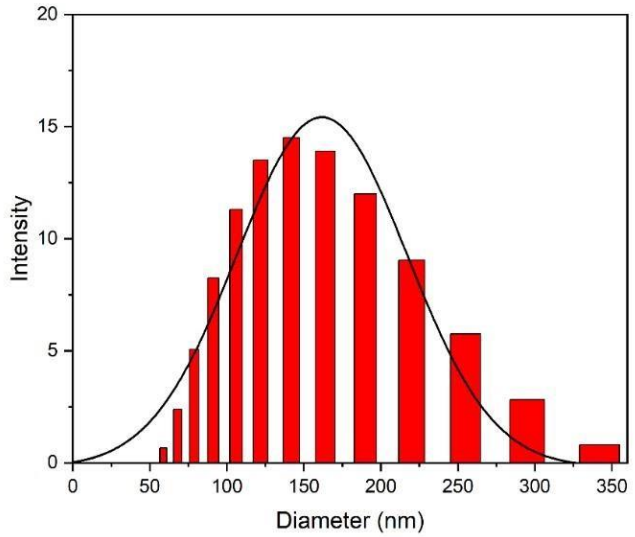

Figure 2. Particle size distribution of PR-LNVs

3.1.3 FTIR Analysis

To determine the possible interaction and complex formation between PR and lipids during the preparation of the PR-LNVs, FTIR spectroscopic studies were performed. Figure 3 showed that the FTIR spectrum of pure PR exhibited infrared characteristic ab sorption peaks at 1509, 1489, 1449, 1377, 1269, 1211, and 1157 cm^{-1}. However, these absorption peaks only appeared in pure PR drugs, not in Blank-LNVs and PR-LNVs. The FTIR spectra of Blank-LNVs and PR-LNVs were almost identical. In addition, we also measured the infrared spectrum of dipropylene glycol, lecithin, PR, and GTCC after physical mixing, and the results showed that there were characteristic infrared absorption peaks of PR at 1509, 1489, 1211, and 1157 cm^{-1}. These data indicated that PR was well encapsulated in LNVs, while simple physical mixing cannot form vesicle structures to load PR within.

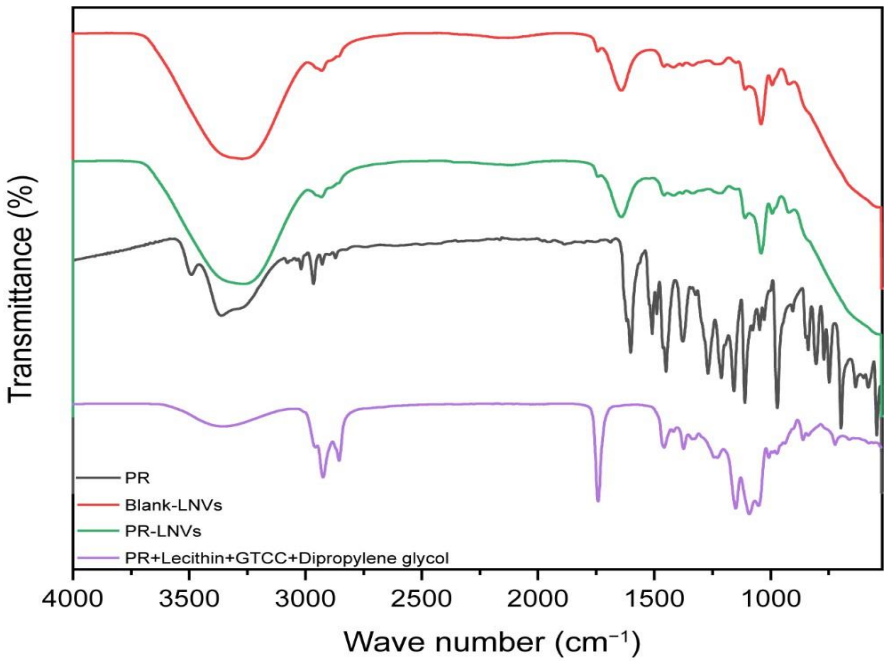

Figure 3. Fourier transform infrared spectra of PR, Blank-LNVs, and PR-LNVs

3.1.4 In Vitro Drug Release

Figure 4 showed the release profile of PR-LNVs and PR solution (in GTCC). Within 0~6 hours, the cumulative release of PR from PR-GTCC solution was 18.25 ± 1.68 %, while that of PRLNVs was 21.83 ± 3.92 %. Subsequently, the release rate of PR-LNVs slowed down and gradually became lower than that of the PR-GTCC solution after 10 h. This may be due to the increase of PR content in the release medium, which reducesd the osmotic pressure inside and outside the dialysis bag, and made it difficult for PR to be released from PR-LNVs, resulting in a decrease in its release rate. In the PR-GTCC solution, PR was in a free state, and the release was relatively easier. At 45 hours, the PR release in the PR-GTCC solution had reached a plateau, and its release rate was 96.63 ± 5.48 %, while the PR of the PR-LNVs at the same time point only released 55.81 ± 3.27 %. After 85 hours, the release of PR-LNVs reached the same level as the PR-GTCC solution. Compared with PR-GTCC solution, the release time of PR was prolonged after loading with PR-LNVs. These data proved that PR had a good sustainedrelease ability, which could release PR slowly to achieve long-term effect.

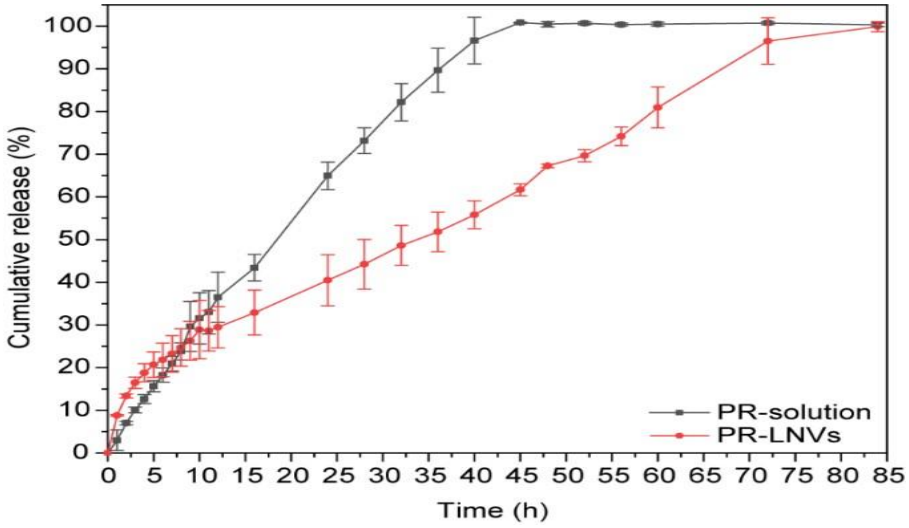

Figure 4. In vitro release curves of PR solution and PR-LNVs

3.2 Long-term Stability Test

For the long-term application of PR-LNVs, their storage stability was investigated. When PRLNVs samples were stored at 4°C or 25°C in the light for three months, their appearance and color did not change significantly, and there was no delamination and precipitation (Figure 5a). Moreover, the particle size and encapsulation efficiency of PR-LNVs did not change significantly (Figure 5b and 5c). These results demonstrated that the PR-LNVs had good stability within three months at 4°C and 25°C.

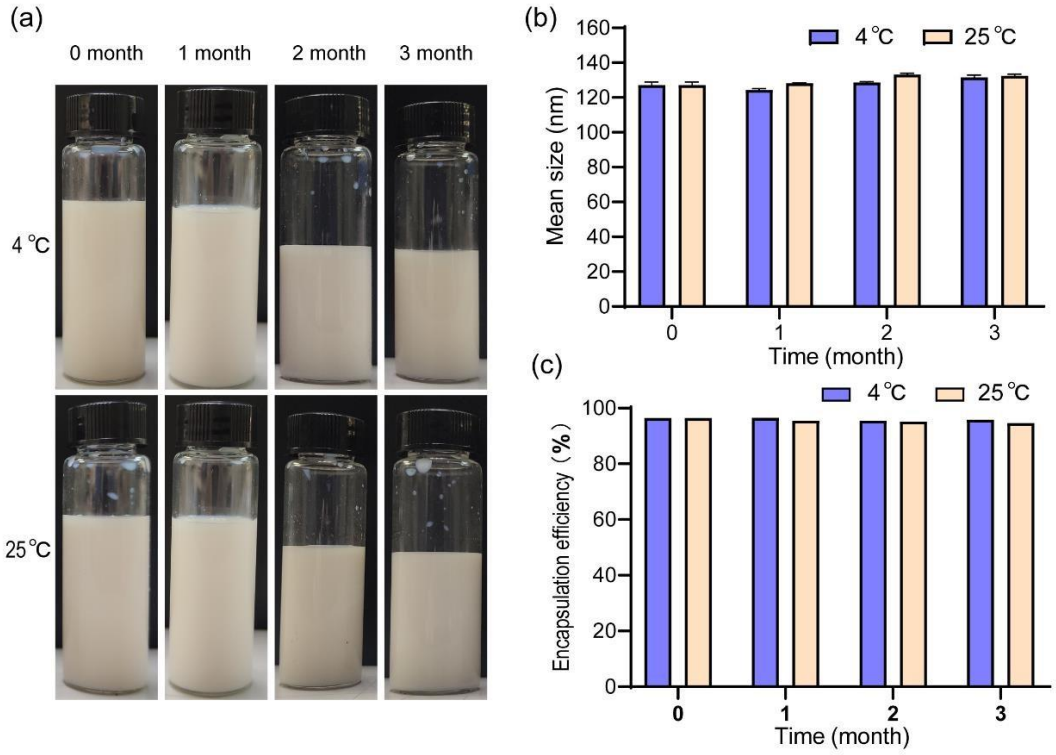

Figure 5. Changes in (a) appearance, (b) particle size and (c) encapsulation efficiency of PR-LNVs stored at 4 ℃ or 25 ℃ for 3 months

4. Conclusion

In this paper, high-pressure homogenization method had been used to prepare Phenylethyl Resorcinol Lipid Nano Vesicles (PR-LNVs). The freshly prepared PR-LNVs showed a spherical vesicles morphology with uniform size under TEM and AFM. This formulation had milky appearance with 132.4 ± 1.04 nm vesicular size, low PDI of 0.137 ± 0.003, high zeta potential of -43.9 ± 3.44 mV, and a PR encapsulation efficiency of 97.37 ± 6.14%. Infrared spectroscopy further verified that PR was encapsulated in lipid nanovesicles. In addition, it had good stability under storage condition at 4°C and 25°C for 3 months. The PR-LNVs was simple to prepare and could have good prospects in cosmetic.

References

Yeom S H, Gam D H, Kim J H, et al. Development of Ultrasound-Assisted Extraction to Produce Skin-Whitening and Anti-Wrinkle Substances from Safflower Seed[J]. Molecules, 2022, 27(4): 1296.

Liu J, Jiang R, Zhou J, et al. Salicylic acid in ginseng root alleviates skin hyperpigmentation disorders by inhibiting melanogenesis and melanosome transport[J]. Eur J Pharmacol, 2021, 910: 174458.

Le D D, Lee Y E, Lee M. Triterpenoids from the leaves of Osmanthus fragrans var. aurantiacus with their anti-melanogenesis and anti-tyrosinase activities[J]. Nat Prod Res, 2022: 1-7.

Liu S C, Sheu M L, Tsai Y C, et al. Attenuation of in vitro and in vivo melanin synthesis using a Chinese herbal medicine through the inhibition of tyrosinase activity[J]. Phytomedicine, 2022, 95: 153876.

Kim H, Choi H R, Kim D S, et al. Topical hypopigmenting agents for pigmentary disorders and their mechanisms of action[J]. Ann Dermatol, 2012, 24(1): 1-6.

Kim B S, Na Y G, Choi J H, et al. The Improvement of Skin Whitening of Phenylethyl Resorcinol by Nanostructured Lipid Carriers[J]. Nanomaterials (Basel), 2017, 7(9).

Xia H T, Y.; Huang, R.; Liang, J.; Ma, S.; Chen, D.; Feng, Y.; Lei, Y.; Zhang, Q.; Yang, Y.; Huang, Y. Nanoliposome Use to Improve the Stability of Phenylethyl Resorcinol and Serve as a Skin Penetration Enhancer for Skin Whitening[J]. Coatings, 2022, 12(3): 362.

Amnuaikit T, Limsuwan T, Khongkow P, et al. Vesicular carriers containing phenylethyl resorcinol for topical delivery system; liposomes, transfersomes and invasomes[J]. Asian J Pharm Sci, 2018, 13(5): 472-484.

Huynh N T, Passirani C, Saulnier P, et al. Lipid nanocapsules: a new platform for nanomedicine[J]. Int J Pharm, 2009, 379(2): 201-209.

Minkov I, Ivanova T, Panaiotov I, et al. Reorganization of lipid nanocapsules at air-water interface: Part 2. Properties of the formed surface film[J]. Colloids Surf B Biointerfaces, 2005, 44(4): 197-203.

Fang Q, Ma C, Xia Q. Preparation and evaluation of trans-resveratrol NanoSolve[J]. Integrated Ferroelectrics, 2016, 169(1): 7-14.