

The Preparation and Long-term Storage Stability of Carbomer-loaded Resveratrol Nanoethosomes Hydrogels

Zicong Wu^{1, a}, Duhong Xie^{2, b}, Wenliang Lu^{3, c}, Zhiping Wang^{3, d, *}

¹School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, China

²Health Career Academy of Changsha, Changsha 410100, China

³Tianjin Tasly Institute, Tianjin 300410, China

^a819530093@qq.com; ^bxdhong@163.com; ^cluwl@tasly.com; ^dwzping_jshb@126.com

*Corresponding author

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Abstract: This work aims to develop carbomer (934,0.25%) loaded resveratrol nanoethosomes hydrogel (Res-NH) as a novel dermal formulation. Res nanoethosomes were prepared by high pressure homogenization technique, applying 1500 bar up to 15 cycles. The long-term storage stability of Res-NH was studied after stored at room temperature for 90 days. The physicochemical characteristics of Res-NH were conducted and used to evaluate the stability. The results showed that the particle size(PS), polydispersity index(PDI) and zeta potential(ZP) were 101.9 ± 6.7 nm, 0.249 ± 0.121 and -19.91 ± 1.39 mV, respectively. After three months of storage, the Res-NH showed no significant changes in particle size and PDI except a minor change in ZP. These results demonstrate that particle size could be a promising formulation for enhanced pharmacological activity of Res and were stable at room temperature.

1. Introduction

Resveratrol (Res, Fig. 1), a major symbol ingredient in red grapes and peanuts^[1, 2]. Res was first isolated from the roots of white hellebore in 1940 in Japan, and later found in traditional Chinese medicine^[3]. It was initially characterized as a phytoalexin (substance produced by higher plants in response to attack by pathogens such as bacteria and fungi, or stress), and achieved notoriety in the scientific literature in 1992, when it was postulated as being responsible for the cardiac protective effects of wine (effect called "French paradox")^[4]. Since then, Res has been shown to exert a variety of pharmacological effects such as antioxidant, antidiabetes, anti-inflammatory and anti-cancer activities. Res is a natural compound currently under investigation due to its important biological anti-cancer properties, including effects on leukemia, skin, breast, lung gastric, colorectal, neuroblastoma, pancreatic and hepatoma cancers^[5-9].

However, Res is hardly water-soluble and its absorption *in vivo* is very poor after oral administration^[10]. A compound as a drug should have favorable absorption, distribution, metabolism, excretion and toxicity characteristics. To circumvent these pitfalls, nanomedicine have been proposed to deliver Res in the last few decades^[11-14]. Nanonization, the production of drug nanocrystals, is a common approach to overcome poor drug solubility in water. Nanonization produces drug particles in the sub-micron range via either bottom-up methods such as precipitation and self-assembly or top-down technologies such as milling and high pressure homogenization^[15, 16]. To stabilize nanocrystal formulations, hydrophilic polymers with or without surfactants are added to the nanocrystal suspensions. Nanonization dramatically increases the drug particle surface area, thereby enhancing the rate of dissolution. In addition, increase in saturation solubility can also occur as described by Ostwald-Freundlich's equation^[17]. Nanocrystal formulations have been reported to enhance the oral exposure by up to 60-fold, when compared to micronized formulation of the same drug substance^[18].

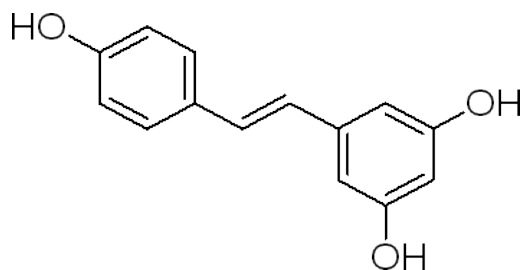


Fig.1 Chemical structure of Res

Several strategies have been used in order to overpass the stratum corneum barrier. Among other drug delivery systems, liposomes were used for topical drug delivery. Liposomes are typically hollow spheres surrounded by a lipid doubled layer. Once applied on skin surface, they only remain in the upper layer of the SC, acting as a drug reservoir. Thus, due to their unstable nature and poor skin permeability, they are only suitable for topical drug delivery. In order to overcome these limitations, novel lipid vesicles known as deformable or elastic (flexible) liposomes–ultradeformable vesicles (UDV) – were developed in the beginning of the 1990s. As UDV are more deformable than conventional liposomes, they have demonstrated a great ability to cross the intact skin and deliver the loaded drugs into the epidermis and dermis layers or even to the systemic circulation. Phospholipids, ethanol, bile salts, and many surfactants have been used for the preparation of these elastic vesicles.UDV present the advantages of being nontoxic and thermodynamically stable formulations. They have been used for dermal and transdermal delivery of many molecules including peptides and proteins. In addition, their production is relatively simple and easy to scale up. Currently, there are many types of UDV that have been successfully developed for both pharmaceuticals and cosmeceuticals, particularly transfersomes, ethosomes, and, more recently, transethosomes^[19-22].

Transfersomes are elastic nanovesicles essentially made of phospholipids and edge activators (EAs) like sodium cholate (NaCo), sodium deoxycholate, Span 60, Span 65, Span 80, Tween 20, Tween 60, Tween 80, and dipotassium glycyrrhizinate. This type of vesicle was firstly introduced in 1992 by Cevc (transfersomes, a trademark of IDEA AG, Munich, Germany), and it represents the first generation of UDV. The skin permeation and penetration of these elastic vesicles result from a synergic mechanism between the carrier properties and the permeation enhancement ability. Transfersomes can cross the skin layers by different mechanisms depending on their composition, in which these vesicles maintain their intact structure or fuse and mix with skin lipids. They can easily change their shape and cross the skin barrier due to the EA action in response to mechanical stress by relocating inside the vesicle to zones with smaller curvature, thus reducing the membrane elastic energy to a minimal level. Following this mechanism, transfersomes can easily squeeze through channels with one-tenth of the vesicles diameter, and cross the SC driven by an osmotic transdermal gradient. These elastic vesicles can only penetrate through skin layers under nonocclusive conditions in order to permit the excess water evaporation from the formulation and maintain this hydration gradient. Therefore, they pass in a nondiffusive way, which means that the penetration rate will not depend on the concentration gradient. Transfersomes also have the capability to protect the drug against rapid clearance to skin blood vessels and to promote the drug retention in the skin layers if needed.

Transfersomes have been studied as carriers for dermal or transdermal delivery for different drugs. However, one main disadvantage of these vesicles corresponds to the difficulty of loading hydrophobic drugs into the vesicles without compromising their deformability and elastic properties. In general, transfersomes have been proven superior to conventional gel-state and liquid-state vesicles as well as conventional liposomes in terms of enhancement of drug permeation and interactions with the human skin^[19].

Ethosomes are special type of UDV developed by Touitou et al in 1997^[20]. Due to their size (approximately 150–200nm) and high deformability, they are also referred to as elastic nanovesicles. Ethosomal systems are vesicles consisting essentially of phospholipids, water, and a high quantity

of ethanol. Phospholipids can be used at 0.5%–10% concentration range, and are obtained from natural semisynthetic and synthetic sources such as soybean and egg. Examples of phospholipids include phosphatidylethanolamine, phosphatidylinositol, phosphatidylcholine, and hydrogenated phosphatidylcholine. Ethanol can be used at 20%–45%, functioning as an efficient skin enhancer. This molecule interacts with the polar head group of the SC lipid molecules, leading to the reduction of the melting point of the SC lipids, thus increasing the fluidity of lipid bilayers and cell membrane permeability.

Nanoethosomes are special lipid vesicular carriers, constituting phospholipids, ethanol (relatively high concentration) and water. They can penetrate the skin and enhance the ability of drug transdermal absorption ^[23-25]. In this report, nanoethosomes loaded resveratrol nanoethosomes hydrogel (Res-NH) as a novel dermal formulation. Res nanoethosomes were prepared by high pressure homogenization technique, applying 1500 bar up to 15 cycles. The long-term storage stability of Res-NH was studied after stored at room temperature for 90 days. The physicochemical characteristics of Res-NH were conducted and used to evaluate the stability. The results showed that the particle size, polydispersity index (PDI) and zeta potential (ZP) were 101.9 ± 6.7 nm, 0.249 ± 0.121 and -19.91 ± 1.39 mV, respectively. After three months of storage, the Res-NH showed no significant changes in particle size and PDI except a minor change in ZP. These results demonstrate that particle size could be a promising formulation for enhanced pharmacological activity of Res and were stable at room temperature.

2. Materials and methods

2.1 Materials

Res form was purchased from Aladdin industrial corporation (Shanghai, China). Res standard was purchased from the National Institutes for food and drug Control ($\geq 98.0\%$). Carbomer 934P was purchased from Lubrizol Advanced Materials, Inc.

2.2 Preparation of the Res-NH

Res-nanoethosomes was prepared by ethanol injection method, Res of 0.1%, lecithin of 1% and Tween 80 of 0.2% were dissolved in absolute ethanol of 35%. The Res power was dispersed in the lipid solution using a constant temperature magnetic stirrer with speed of 800rpm at 60°C. In the continuous stirring, PBS of 65% was slowly injected into the above solution with the No. 5 injection needle, and the solution was further stirred for 30 min. After cooling, the PD-nanoethosomes solution was filtered through a 0.45µm microporous membrane. Then, the Res-NH was prepared by added the Res-nanoethosomes to the 0.25% carbomer 934 gel.

2.3 Characterization of the Res-NH

The particle size, polydispersity index (PDI), and Zeta potential measurements were performed on a Nano-ZS90 (Malvern Instruments Ltd., Malvern, UK) thermostated at 25 °C. The sample was diluted 50 times with bidistilled water before the measurements. All values were measured at an analysis angle of 90 °C in a 10-mm diameter cell. Each value reported is the average of three measurements.

2.4 Statistical analysis

Results were expressed as mean \pm standard deviation (SD). Student's t-test was used to compare the mean differences between samples using the statistical software SPSS version 16.0 (SPSS, Chicago). In all cases $P < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 Particle size analysis and Zeta potential of Res-NH

The mean particle size and PDI were measured immediately after the preparation of the nanoethosomes. The mean particle size with PDI 0.249 was 101.9nm (Fig. 2). The PDI is a measure of particles size distribution. The values less than 0.3 indicate a high degree of homogeneity in particle size and vice versa. The zeta potential was -19.91 mV (Fig. 3).

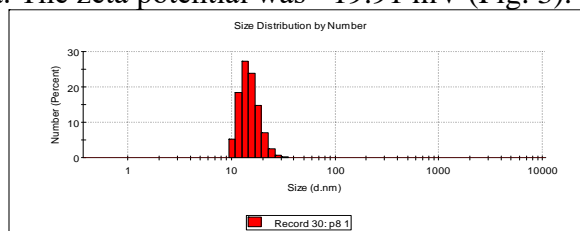


Fig. 2 The particles size of Res-NH

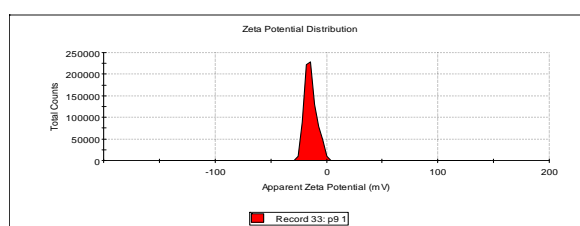


Fig. 3 The zeta potential of Res-NH

3.2 The physicochemical characteristics of Res-NH

The characteristics of Res-NH are shown in table 1. After three months of storage at room temperature, the mean PS and PDI of Res-NH display no significant differences except a little decrease in ZP, as compared with the fresh preparation.

Table 1 Characteristics of Res-NH (mean \pm SD, n = 6)

Time/month	PS	PDI	ZP
0	101.9 \pm 6.7	0.249 \pm 0.121	-19.91 \pm 1.39
1	102.1 \pm 5.9	0.250 \pm 0.131	-20.89 \pm 1.82
3	102.4 \pm 6.8	0.249 \pm 0.141	-21.04 \pm 1.63

4. Conclusions

These results demonstrate that Res-NH were stable at at room temperature and could be a promising formulation for enhanced pharmacological activity of Res. The nanoparticle size, polydispersity index and zeta potential have been used to evaluate the stability of nanoethosomes. In this study, Res-NH had good stability at room temperature. After three months of storage at room temperature, the suspension did not have agglomeration. Although a decrease in the zeta potential was observed, the zeta potential of the nanoparticles in the solution was still over -10 mV.

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