The Preparation and Long-term Storage Stability of Carbomer-loaded Puerarin Nanosuspension Hydrogels

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Abstract: This work aims to develop carbomer (934,0.25%) loaded Puerarin nanosuspension hydrogel (Pue-NH) as a novel dermal formulation. Pue nanosuspension were prepared by high pressure homogenization technique, applying 1500 bar up to 15 cycles. The long-term storage stability of Pue-NH was studied after stored at room temperature for 90 days. The physicochemical characteristics of Pue-NH were conducted and used to evaluate the stability. The results showed that the particle size, polydispersity index (PDI) and zeta potential (ZP) were 218.5 nm and −18.81 mV, respectively. After three months of storage, the Pue-NH showed no significant changes in particle size and PDI except a miner change in ZP. These results demonstrate that particle size could be a promising formulation for enhanced pharmacological activity of Pue and were stable at room temperature.

1. Introduction

Puerarin (Pue, Fig. 1) is a major active ingredient in the traditional Chinese medicine, Pueraria radix (Chinese name Gegen), which comes from the dried roots of *Pueraria lobata* (Willd.) Ohwi in accordance with Chinese Pharmacopeia. Pue is widely prescribed for patients with cardiocerebrovascular diseases in China. Pue has been reported to exhibit promising pharmacological activities including effects on hypertension [1], cerebral ischemia[2], myocardial ischemia[3], diabetes mellitus[4] and arteriosclerosis[5]. In recent decades, much focus has been put on its significant anti-cancer activities[6-8]. Despite the promising biological effects, owing to the short elimination half-life of Pue in human beings, intravenous administration of frequent and high doses may be needed, possibly leading to severe and acute side effects. Oral administration is the most preferred route regarding the conventional drug delivery system, especially for the treatment of chronic diseases[9]. However, Pue is hardly water-soluble (11 mM at 25 ^{0}C)[10], and its absorption in vivo is very poor after oral administration[11], which diminishes its therapeutic effects[12]. No oral formulations are currently available, thus, the clinical application of Pue is greatly restricted, and oral formulation with improved absorption of Pue is highly desired.

Numerous formulation approaches, such as cyclodextrin inclusion complex [13,14], microemulsion[15,16], nanoparticles[17-19], and nanosuspension[8], as well as chemically modified prodrugs, have been proposed to deliver Pue in the last few decades. Nanocrystal suspension, nanosuspension (NS) for short, is a carrier-free nanoparticle system containing only pure drug crystal and minimum surfactant and /or polymer for stabilization[20]. Reduction of particle size by nanocrystal technology to the nano-scale usually leads to a significant increase in drug solubility and dissolution rate with an obvious improvement in drug bioavailability[21]. A few techniques have been used to prepare drug loaded NS, including nanoprecipitation, pearl-milling, high speed homogenization, sonication, and high-pressure homogenization(HPH)[22]. Among these techniques, the HPH method with a high productivity and a lower level contamination which is favorable for

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implementation of industrial products has shown great superiority over other methods.

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 Puerarin nanosuspension hydrogel (Pue-NH) as a novel dermal formulation. Pue nanosuspension were prepared by high pressure homogenization technique, applying 1500 bar up to 15 cycles. The long-term storage stability of Pue-NH was studied after stored at room temperature for 90 days. The physicochemical characteristics of Pue-NH were conducted and used to evaluate the stability. The results showed that the particle size, polydispersity index (PDI) and zeta potential (ZP) were 96.1±4.5 nm, 0.121±0.027 and −17.31±1.67 mV, respectively. After three months of storage, the Res-NH showed no significant changes in particle size and PDI except a miner change in ZP. These results demonstrate that particle size could be a promising formulation for enhanced pharmacological activity of Pue and were stable at at room temperature.

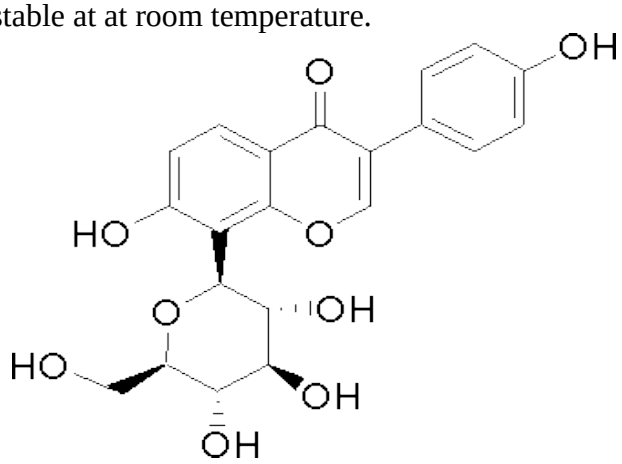

Fig.1 Chemical structure of puerarin.

2. Materials and Methods

2.1 Materials

Pue form was purchased from Aladdin industrial corporation (Shanghai, China). Pue standard was purchased from the National Institutes for food and drug Control (≥98.0%). Poloxamer 188 (P188, Lutrol® F68) was kindly donated from BASF (Ludwigshafen, Germany). Carbomer 934P was purchased from Lubrizol Advanced Materials, Inc.

2.2 Methods

2.2.1 Preparation of the Pue-NH

HPH technique was applied to prepare Pue-NS. Briefly, P188 of 1.0 % was dissolved in distilled water. The Pue powder of 0.5 % was dispersed in the aqueous surfactant solution using high speed homogenization 5000 rpm for 15 min (IKA T18 basic ULTRA-TURRAX®, Germany). Then the pre-mix was passed through a Lab HPH (APV-2000, Germany), 5 cycles were performed at 500 bar, and 15 cycles at 1500 bar. Then, the Pue-NH was prepared by added the Pue-NS to the 0.25% carbomer 934 gel.

2.2.2 Characterization of the Pue-NH

The particle size(PS), polydispersity index(PDI), and Zeta potential(ZD) measurements were performed on a Nano-ZS90 (Malvern Instruments Ltd., Malvern, UK) thermostated at 25 ^{0}C. The sample was diluted 50 times with bidistilled water before the measurements. All values were measured at an analysis angle of 90 ^{0}C in a 10-mm diameter cell. Each value reported is the average of three measurements.

2.2.3 Statistical analysis

Results were expressed as mean ± 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

3.1 Particle size analysis and Zeta potential of Pue-NH

The mean particle size and PDI were measured immediately after the preparation of the NH. The mean particle size with PDI 0.424 was 218.5 nm (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 of Pue-NH was −18.81 mV (Fig. 3).

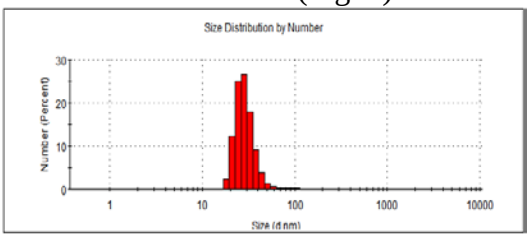

Fig. 2 The particles size of Pue-NH

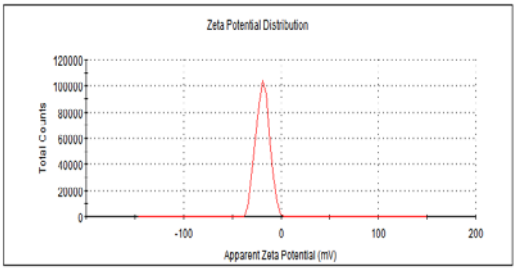

Fig. 3 The zeta potential of Pue-NH

3.2 The physicochemical characteristics of Pue-NH

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

Table 1 Characteristics of Pue-NH (mean±SD, n = 6)

Time/month	PS	PDI	ZP
0	218.5±7.1	0.424±0.121	−18.81±1.67
1	219.1±6.9	0.426±0.136	−18.89±1.84
3	220.2±6.8	0.449±0.141	−19.04±1.91

4. Conclution

These results demonstrate that Pue-NH were stable at at room temperature and could be a promising formulation for enhanced pharmacological activity of Pue. The nanoparticle size, polydispersity index and zeta potential have been used to evaluate the stability of nanoethosomes. In this study, Pue-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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