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The properties of bufadienolides-loaded nano-emulsion and submicro-emulsion during lyophilization

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Abstract

The aim of this study was to prepare two types of emulsions, bufadienolides-loaded nano-emulsion (BU-NE) and submicro-emulsion (BU-SE) which were separately prepared by ultrasonic-high-pressure homogenization (UHPH) and high-pressure homogenization (HPH) methods, and to try to stabilize the colloid systems by lyophilization. The lyoprotective effects of cryoprotectant carbohydrates during the freeze-thawing and freeze-drying cycles on the emulsions were investigated in detail. The lyophilized products were characterized with regard to their appearance and particle size by transmission electron microscopy (TEM), scanning electron microscopy (SEM), photon correlation spectroscopy (PCS) and zeta potential. The median diameter, polydispersity index (PI) and zeta potential of BU-NE and BU-SE were 43.5 nm versus 161.4 nm, 0.100 versus 0.143 and −19.7 to −26.2 mV versus −29.4 to −35.3 mV, respectively. With the same drug content (0.28 mg mL^{-1}) BU-SE exhibited a higher entrapment efficiency than BU-NE. The optimum cryoprotectant for BU-NE and BU-SE was maltose (20%) and trehalose (20%), respectively. The median diameters (95.7 and 168.5 nm) of the rehydrated BU-NE and BU-SE were slightly increased. For both of them, the bufadienolides entrapment efficiency was reduced whereas the drug content was not. The lyophilized BU-NE and BU-SE powders were stable over a period up to 3 months with no change in visual appearance, reconstitution ability, particle size distribution and drug concentration. This shows that freeze-drying could be a promising method to stabilize the emulsions.

Keywords: Bufadienolides; Nano-emulsion; Submicro-emulsion; Cryoprotectant; Lyophilization

1. Introduction

Over the last few decades, emulsions (Seki et al., 2004), liposomes (Maurer et al., 2001) and nanoparticles (De Chasteigner et al., 1996) have been attracting increasing attention as drug delivery systems for poorly water-soluble drugs because of their drug targeting effect and therapeutic benefit (Juliano, 1988). Commercially available formulations are still limited in number compared with other oral dosage forms. However, aggregation, fusion, phospholipid hydrolysis and leakage of the encapsulated drugs may occur during a long period of storage in an aqueous medium.

Freeze-drying appears as one of the most suitable methods to stabilize and improve the handling of colloidal systems (Seki et al., 1997; Franks, 1998), and this consists of nucleation, freezing and a subsequent sublimation process. The separation of water from a solution or a dispersion of given concentration in the form of a solid phase, ice, and the subsequent removal of water by vacuum sublimation, leaves the solutes or substrates in their anhydrous or almost anhydrous states. On the other hand, colloidal carriers are not easily lyophilized, due to several forms of damage, including drug leakage, fusion, and lateral phase separation. This fact has been discussed in many papers (Van Winden and Crommelin, 1999; Komatsu et al., 2001; Wang et al., 2006) mainly with regard to liposomes. The addition of sugars as cryoprotectants has been demonstrated to be effective in maintaining the initial formulation characteristics (Miyajima, 1997).

Toad venom, a traditional Chinese medicine, is prepared from the dried white secretion of the auricular glands and the skin glands of Chinese toads (*Bufo melanostictus Schneider* or *Bufo bufo gargarzinas Gantor*). The principal biological active components of toad venom are bufadienolides, a class of C-

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24 steriods with a characteristic α-pyrone ring at C-17 (Krenn and Kopp, 1998). The major bufadienolides from toad venom include bufalin (B), cinobufagin (C), and resibufogenin (R). Recently, bufadienolides have been shown to exert potent antitumor and analgesic activity (Hashimoto et al., 1997; Efferth et al., 2002; Yeh et al., 2003). In previous studies, bufadienolides were shown to be poorly water-soluble and exhibit pH-dependent distribution between aqueous phase and organic solvent, which make them excellent candidates for emulsions. In the present work, two kinds of o/w emulsions—nano-emulsions (NE) and submicro-emulsions (SE) were prepared by different methods. The drug was incorporated into the lipid phase or lecithin layer at the oil–water interface and the incorporated drug was sequestered on direct contacting with body fluids and tissues (Singh and Ravin, 1986).

In this study, the suitability of several cryoprotectant carbohydrates (trehalose, maltose, glucose, mannitol, sucrose, lactose and fructose) has been investigated to preserve the structure of NE and SE in comparison with an established freeze-drying process (Saleki-Gerhardt and Zografi, 1994). The particle size distribution (PSD) and zeta potential of samples both before and after lyophilization were assessed by photon correlation spectroscopy (PCS) and electrophoretic light scattering (ELS). The transmission electron microscope (TEM) photos of the emulsions before and after lyophilization and the scanning electron microscope (SEM) micrographs of the lyophilized products were also described.

2. Materials and methods

2.1. Materials

Bufadienolides were extracted from toad venom by staff at the Department of Pharmaceutics, Shenyang Pharmaceutical University, China. The bufadienolide mixture mainly consisted of bufalin (B), cinobufagin (C) and resibufogenin (R) in a ratio of 7:9:11.

Lipoid E-80® was purchased from Lipoid (Ludwigshafen, Germany). The Lipoid E-80®, according to manufacturer's specifications, consisted of 80% phosphatidylcholine, 8% phosphatidylethanolamine, 3.6% nonpolar lipids and about 2% sphingomyelin. Soybean oil was obtained from Tieling BeiYa Pharmaceutical Company (Liaoning, China). Pluronic F68 was obtained from BASF AG (Ludwigshafen, Germany). Sodium oleate, glycerin and Tween-80 were obtained from Dongshang Co., Ltd. (Shanghai, China), Suichang Glycerin Company (Zhejiang, China), Shenyu Medicine and Chemical Industry Company (Shanghai, China), respectively. Trehalose dihydrate was purchased from Sinozyme Biotechnology Co., Ltd. (Nanning, China). Mannitol, maltose, glucose, lactose, fructose and sucrose were purchased from Tianjin Bodi Chemicals Co., Ltd. (Tianjin, China). All other chemicals were of analytical or chromatographic grade.

2.2. Preparation of bufadienolides-loaded nano-emulsion (BU-NE)

BU-NE was prepared by an ultrasonic-high-pressure homogenization (UHPH) method. Bufadienolides were dissolved in soybean oil at 75 °C with magnetic stirring for 30 min, then the oil phase was filtered through a 0.45 μm membrane. Lipoid E-80®, Pluronic F68, Tween-80, glycerin and sodium oleate were dispersed in distilled water under the same conditions as the oil phase. Then, the oil phase was added dropwise to the water phase and stirred magnetically. A coarse emulsion was prepared by high shear mixing (FJ-200, Shanghai sample model factory, Shanghai, China) at 10,000 rpm for 10 min followed by ultrasonication at an intensity of 40 W, at 4 °C for 60 min using a high intensity ultrasonic processor (ultra-cell 750 W, Sonics Materials Inc., USA). Then, the volume was adjusted to 100 mL with double-distilled water and the pH was adjusted to 6.8 with 0.1 mol L^{-1} HCl. High-pressure homogenization was performed using a homogenization apparatus (NS10012K; Niro Soavi, Italy) at 40 °C applying 120 MPa for 8 cycles. BU-NE was sterilized by filtration through a 0.22 μm membrane and then transferred to vials, and stored at 4 °C. The drug-free NE was produced by the same procedure without drug incorporation.

2.3. Preparation of bufadienolides-loaded submicro-emulsion (BU-SE)

BU-SE was prepared using the high-pressure homogenization (HPH) method. The coarse emulsion was prepared by the same procedure as BU-NE. The differences were as follows: Lipoid E-80® was added to the filtered BU-loaded oil phase; the final BU-SE was obtained by passing the coarse emulsion directly through a high-pressure homogenizer without sonication; The homogenization conditions were 40 °C, and 70 MPa for 8 cycles; BU-SE in vials was sterilized on a water bath at 100 °C for 30 min and also stored at 4 °C. The basic formulae of BU-NE and BU-SE used in this study are shown in Table 1.

2.4. Freeze-thawing procedure

The BU-NE and BU-SE samples were diluted with high concentration of cryoprotectant solutions. Different cryoprotectants were added in increasing amounts to assess the optimum concentration (Table 2). Then samples containing cryoprotectant with

Table 1
The basic formulae of BU-NE and BU-SE

	Bufadienolides (mg mL^{-1})	Soybean oil (mg mL^{-1})	Lipoid E-80® (mg mL^{-1})	Pluronic F68 (mg mL^{-1})	Tween-80 (mg mL^{-1})	Sodium oleate (mg mL^{-1})	Glycerin (mg mL^{-1})
BU-NE	0.28	20	12	5	2	1	22.5
BU-SE	0.28	100	12	0	2	1	22.5