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Preparation of Liposome Particle of Atractylone by Supercritical Carbon Dioxide Process

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Abstract: The liposome particle entrapping atractylone extracted from Atractylodes macrocephala Koidz.was prepared and characterized. The liposome suspension of atractylone was formed by supercritical carbon dioxide (SC-CO_2) expansion process and was dried by vacuum freezing. The physicochemical properties of the liposome particle including microstructure, size, entrapment efficiency and drug loading content were measured. The liposome formation could be controlled by adjusting the processing conditions such as pressure, temperature of SC-CO_2 and mole fraction of ethanol in SC-CO_2 [x (CH_3CH_2OH)]. The entrapment efficiency, loading content, and average size of liposome particle were 83.1%, 5.1% and 506.5nm respectively under the optimum conditions of 30MPa，338K and x (CH_3CH_2OH) = 15%.The liposome particle presented good performance of redispersion to liposomal suspension. The physicochemical properties of liposome particle including entrapment efficiency, dissolution rate and stability complied with the provisions of Chinese pharmacopoeia. The results show the liposome particle can be used as an solid immediate for hepatic target of drugs.

Introduction

Atractylone, a cytotoxic component in essential oil extracted from Atractylodes macrocephala Koidz., can suppresse tumour growth through inhibition of tumour revascularization and tumour cell proliferation. Therefore, it can avoid hepatic immune dysfunction, protect liver cell and stimulate the rejuvenesce of the impaired liver cell [1, 2]. However, as a lipophilic substance, atractylone has poor solubility and low dissolution rate. To overcome these problem , preparation of a new target drug of atractylone is necessary. As a drug carrier, liposome particle is particularly suited for entrapping the lipophilic substance. It can increase the drug concentration in the target, reduce the toxicity and improve the therapeutic efficacy of drugs [3]. Under consideration of the properties of liposome particle, making the atractylone into liposome particle is practicable. It is expected the liposome particle of atractylone not only enhances solubility but also improves the efficiency of target delivery to hepatic cells of atractylone. Further, the liposome particle can be served as pharmaceutical intermediates to prepare the injection and solid oral formulations of hepatic target of drugs.

The preparation methods commonly used include thin film dispersion, melting, freeze-thaw, injection, reverse-phase evaporation method, etc [4]. However, the liposome particle made with the traditional methods has disadvantages such as low entrapment efficiency, low loading content and poor stability [5]. As a result, a new technology should be developed to improve the physicochemical activity and targeting property of liposome particle prepared by the Chinese traditional medicines [6].

As a new technology of preparing micro-composites, the rapid expansion of supercritical fluid solution technology (RESS) has been applied in the formation of micro-capsules and microspheres in the past decade [7-8]. According to the special properties of dissolving, expansion and precipitation, the supercritical CO_2 is particularly applicable to liposome particle formation. In this study, the solution of atractylone and liposome membrane materials contained phospholipid and cholesterol was introduced into the supercritical CO_2. After it was dissolved in the mixture of supercritical CO_2/ethanol, the solution was sprayed into a buffer through a coaxial nozzle to form liposome suspension by releasing the pressure of supercritical CO_2 rapidly. Then the physicochemical properties of collected liposome particle were assessed. It is expected this method can provide a new way to form liposome particle of Chinese medicines.

1 Experimental section

1.1 Materials

Atractylone [w (atractylone) > 90%], provided by Neptunus Corp., China, was used as the entrapped component. Atractylone [w (atractylone) > 99%], purchased from Wako Pure Chemical Industries, Ltd. of Osaka, Japan, was used as reference standard.

Phosphatidylcholine [PC, w (PC)> 95%] and cholesterol [w (cholesterol)> 95%], purchased from Shanghai Boao Biotechnology Co., Ltd, were used as liposomal membrane materials for liposome particle preparation.

CO_2 [w (CO_2)> 99.5%], supplied by Guangzhou Zhuo zheng Gas Co., Ltd., was used as solvent in RESS process.

Ethanol and n-hexane were purchased from Guangzhou Chemical Reagent Factory.TritonX-100, mannitol, disodium hydrogen phosphate and Sodium dihydrogen phosphate were purchased from Fu-chen Chemical Reagent Factory, Tianjin; Sephadex G-50 was purchased from Pharmacia Biotech (Uppsala, Sweden).All above materials were analytical reagent.

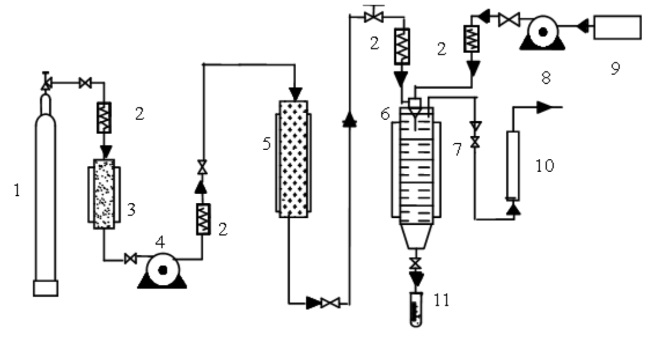
1.2 Apparatus

RESS apparatus was designed and made by the Modern Chemical Technology Laboratory, South China University of technology. It consisted of a stainless-steel reactor and a collector with coaxial injector. The reactor had a volume of 100 cm^3 and withstood a maximum pressure of 35 MPa. Apparatus temperature was controlled by a temperature controller jacket with circulating water. Pressure was controlled by a back-pressure regulator.

Atractylone was determined with Angient 6890 gas chromatography (Angient Tech. Ltd., USA). Liposome particle was dried by LGJ-10 lyophilizer (Xinzhi Biological Co., Ltd., Ningbo, China). The size distribution of liposome particle was measured by Malvem Zetasizer-3000 laser-particle size analyzers (Malvern Instruments Ltd., UK). The microstructure of the liposome particle was analyzed with Hitachis-3400N scanning electron microscope (Hitachi Ltd., Japan) and with JEM-1230 transmission electron microscope (Electronics Co., Ltd., Japan). Dissolution rate of atractylone liposome particle was determined with RC806 dissolving apparatus (Tianda Tianfa technology Co., Ltd., Tianjin, China).

1.3 Liposome particle preparation by supercritical CO_2

Fig. 1 was a schematic of the experimental apparatus used for liposome particle preparation. Atractylone was mixed with liposome membrane materials [m (PC): m (cholesterol) = 3:1] with a ratio of m (atractylone): m (membranes) = 1:20, then the mixture was dissolved in ethanol. The mixture solution was introduced into reactor 5.After CO_2 gas



1- Cylinder; 2- Heat exchanger; 3- Refrigerating machine; 4，8- Syringe pump; 5- Reactor;6- Coaxial Injector; 7- Collector, 9- Storage tank, 10- Rotameter; 11- Volumetric cylinder

Fig. 1 Flow diagram of liposome particle preparation by SC-CO_2

was cooled to liquid by the refrigerating machine 3, the liquid CO_2 was pumped into the reactor 5 with the syringe pump 4.The liposome membrane materials and atractylone dissolved in SC-CO_2 with ethanol used as a cosolvent at the set point of pressure and temperature. After phase equilibrium reached, the pressure was released rapidly. The dissolved liposome membrane materials and atractylone was mixed with the phosphate buffer solution (PBS) (pH = 7.2) brought with the syringe pump 4 via the coaxial nozzle 6. Atractylone was entrapped into liposome particle and dispersed in PBS solution. CO_2 was discharged to the air after it was measured with the rotameter. The liposome suspension was collected with a volumetric cylinder, and then filtered with a porous membrane to remove unentrapped liposome membrane materials. Finally, the liposome suspension was lyophilized with mannitol as a cryoprotectant.

1.4 Measurement of the total content of atractylone in the liposome particle

10 mg of atractylone liposome particle was broken with 10% of TritonX-100 in ethanol solution. Atractylone entrapped in liposome particle released to ethanol, and then it was determined by GC with the external standard method.GC conditions were set as follows: chromatographic column was HP-5 MS (30m × 250μm × 0.25μm); injection port temperature was 250 ℃, FID detector temperature was 280 ℃; column temperature was programmed as 80 ℃ (1 min) → (12 ℃ / min) → 250 ℃ (5min), flow rate of N_2 was 1.5mL /min.

1.5 Determination of entrapment efficiency and loading content

500 mg of atractylone liposome particle was dissolved in PBS solution to form liposomal suspension. 1 mL of liposomal suspension was eluted with PBS at a flow rate of 1 mL/min after it was loaded on the Sephadex G-50 column (2 × 80 cm) pre-saturated with blank liposomal suspension. The first fraction of about 15 mL was removed. The second fraction of about 40 mL containing unentrapped atractylone was collected. The unentrapped atractylone was extracted with n-hexane, finally it was measured by GC with the external standard method.

Entrapment efficiency and drug loading content of atractylone liposome particle were calculated with the following equations:

$$E\% = \left(\frac{m-n}{p}\right)\times 100\% \qquad (1)$$

$$D\% = \left(\frac{m-n}{q}\right)\times 100\% \qquad (2)$$

Where E was the entrapment efficiency (%), D was drug loading content (%), m was the total amount of atractylone in the liposome particle (g), n was the amount of unentrapped atractylone (g), p was the amount of atractylone added (g), and q was the amount of liposome particle (g).

1.6 Analysis of microstructure and size distribution of atractylone liposome particle

The freeze-dried liposome particle was taken photographs by SEM after it was sputtered with Au. The freeze-dried liposome particle was dispersed in PBS to form suspension. The liposomal suspension was stained with 1% of phosphorus acid after it was dropped on the copper wire mesh. a The microstructure of the liposome was observed and taken photographs by transmission electron microscope (TEM). The size distribution of the liposome particle was measured with laser particle size analyzer.

1.7 Determination of dissolution rate of atractylone liposome particle

Dissolution rate in vitro was determined according to dissolution testing methods afforded by Chinese pharmacopoeia with a dissolution apparatus [9]. The liposome particle sample was added respectively into artificial gastric juice (pH 1.2), distilled water (pH 5.8), and PBS (pH 6.8) at temperature of 37 ± 0.5 ℃. The solution was stirred at a speed of 100 r / min for 1 h and then extracted with n-hexane. Finally, 1 μL of organic phase was injected into GC to determine the amount of released essential oil.

2 Results and Discussion

2.1 Preparation parameters of SC-CO_2 pressure

Entrapment efficiency and drug loading content of atractylone liposome particle were measured under different pressure at 333 K and x (CH_3CH_2OH) = 15%. The results were shown in Fig. 2. Both entrapment efficiency and drug loading content of atractylone liposome particle were low at lower pressure. It was difficult to form a homogeneous mixture of phospholipids/ethanol/CO_2 because of the small solubility of phospholipids and cholesterol in SC-CO_2. When the pressure reached 20 MPa, the solubility of liposome membrane materials increased significantly, so that atractylone was easy to distribute into the phospholipid bilayers. With an increase in pressure, liposome membrane arranged disorderly so as to make more space available, and then atractylone could be embedded more effectively. Therefore, entrapment efficiency and loading content improved with the increase of pressure.

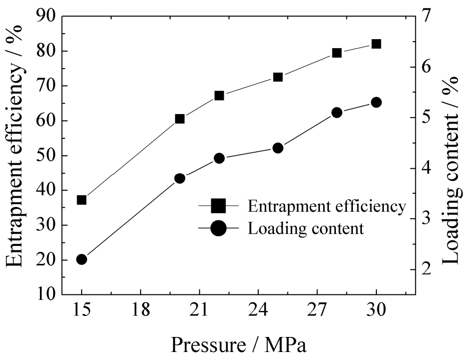


Fig. 2 Effect of pressure on liposome particle preparation

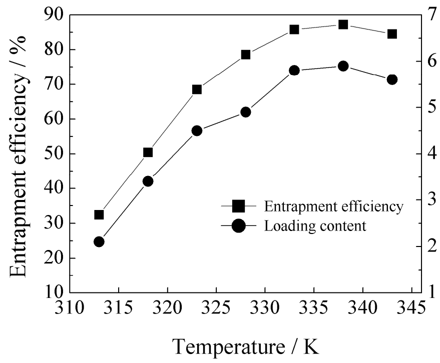


Fig. 3 Effect of temperature on liposome particle preparation

2.2 Preparation parameters of SC-CO_2 temperature

In order to discuss the effect of temperature, entrapment efficiency and drug loading content of atractylone, liposome particle was measured under different temperatures at 30 MPa and x (CH_3CH_2OH) = 15% .The result was shown in Fig. 3.

As shown in Fig. 3, both entrapment efficiency and drug loading content of atractylone liposome particle were low when the temperature was under 323 K which was the phase transition temperature (Tm) of phospholipids. At the temperature under Tm, the molecules of liposomal membrane arranged closely so that atractylone was difficult to be embedded into the liposome particle. Instead, when the temperature was above 323K, liposomal membrane fluidity increasing and embedded space expanding promoted the formation of liposome particle of atractylone. However, when the temperature continued to rise, the insoluble phospholipids gathered in liposome particle suspension, for the solubility of liposomal membrane materials would reduce with the decrease of the density of SC-CO_2. As a result, entrapment efficiency and drug loading content declined. According to the above analysis, both entrapment efficiency and drug loading content were highest at the optimum temperature of 338 K.

2.3 Preparation parameters of coslovent amount

Entrapment efficiency and loading content of atractylone liposome particle was measured under different x (CH_3CH_2OH) at 30MPa and 338K. The result was shown in Fig. 4.

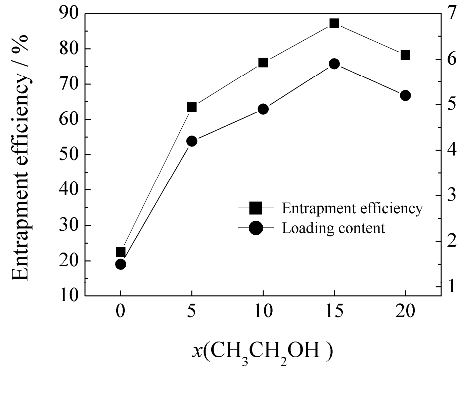


Fig. 4 Effect of cosolvent on liposome particle preparation

According to the Fig. 4, Entrapment efficiency and loading content of atractylone liposome particle were low due to the small solubility of phospholipid and cholesterol in the SC-CO_2 without cosolvent of ethanol. Adding ethanol enhanced the molecules interaction between phospholipids, cholesterol and SC-CO_2. The entrapment efficiency greatly improved as the solubility of liposomal membrane materials raised significantly with increasing x (CH_3CH_2OH). However, when x (CH_3CH_2OH) was higher than 15%, Entrapment efficiency and loading content decreased because of leakage of the liposomal membrane destroyed by the additional ethanol. This result showed that the optimum ethanol concentration which achieved the highest liposome trapping efficiency was x (CH_3CH_2OH) =15%.

2.4 Microstructure and size distribution of atractylone liposome particle

Freeze-dried liposome particle of atractylone was white powder. SEM photograph showed the liposome particle was in spherical or elliptical shape (Fig. 5). As shown in the TEM photograph (Fig. 6), the liposome particle took on grape-like vesicles in PBS. The average size of liposome particle was 506.5 nm. The properties showed the freeze-dried liposome particle has good performance of redispersion to liposomal suspension.

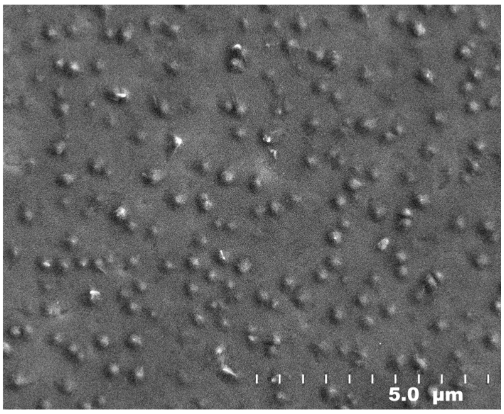


Fig. 5 SEM photograph of frozen liposome particle of atractylone

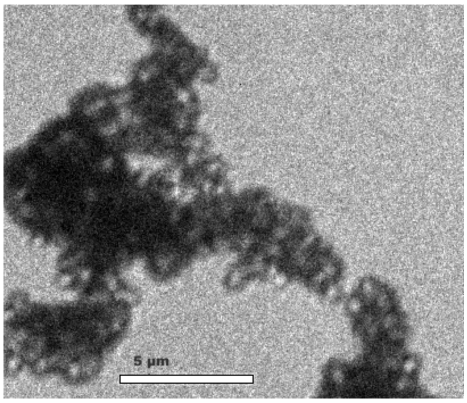


Fig. 6 TEM photph of frozen liposome particle of atractylonograe after redispersion

2.5 Dissolution rate of atractylone liposome particle

The dissolution rates of atractylone liposome particle were 91.5%、92.6%、94.5% in artificial gastric juice, distilled water, phosphate buffer respectively. The dissolution rate complied with the dissolution provisions of Chinese pharmacopoeia. It showed the bioavailability of atractylone improved greatly after liposome particle was formed.

2.6 Stability of atractylone liposome particle

The stability of atractylone liposome particle was studied at a constant temperature of 25±1℃ and a relative humidity of 75±5%. Entrapment efficiency, drug loading content and average size of atractylone liposome particle were examined in 1, 2, 3, 6 month. The results were shown in Tab. 1. Entrapment efficiency and drug loading content were unchanged obviously, while the size increased slightly. The stability of freeze-dried liposome particle met the regulations of solid intermediates in Chinese pharmacopoeiae.

Table 1 Stability of atractylone particle liposome

	Time (M)				
	0	1	2	3	6
Entrapment efficiency (%)	83.1	80.1	81.5	79.2	76.4
Loading content (%)	5.1	4.9	5.1	4.9	4.8
Average size (nm)	506.5	512.0	514.5	536.5	545.0

3 Conclusion

(1) The formation process of liposome particle could be controlled by adjusting parameters of supercritical CO_2 expansion. The entrapment efficiency and the loading content of atractylone liposome particle improved at pressures above 20 MPa, temperatures above 323 K and ethanol amounts above 5 %. Entrapment efficiency, loading content, and average size of liposome particle were 83.1 %, 5.1 % and 506.5nm respectively under conditions of 30MPa, 338K and x (CH_3CH_2OH) = 15%.

(2) The freeze-dried liposome particle could re-constructe liposomal suspension efficiently. The physicochemical properties including entrapment efficiency, dissolution rate and stability meet the standard of Chinese Pharmacopoeia. It is practicable to make atractylone into freeze-dried liposome particle powder, which can be used as an intermediate of the injection and other oral formulations.

Acknowledge

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