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Synthesis of highly dispersed nanoscaled CoQ_{10} liposome by supercritical fluid

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ABSTRACT

The nanoscaled CoQ_{10} liposome were synthesized by Rapid Expansion of Supercritical Solutions (RESS). Transmission electron microscopy (TEM) was used to characterize the particle size of CoQ_{10} liposome, and the UV spectrum was employed to evaluate the product's trapping efficiency. A series of CoQ_{10} liposome samples were synthesized by RESS under different pressures. The trapping efficiency of the CoQ_{10} liposome was up to 90% when the enduring pressure was at 200–300 bar, but the trapping efficiency was distinctly decreased when the enduring pressure is lower than 200 bar or higher than 300 bar. The high resolution TEM images exhibited that the particle size of CoQ_{10} liposome was only 20–40 nm, probably because CO_2 molecules act as the membranes between the CoQ_{10} liposome particles in this process, which prevent the agglomeration of liposome with each other.

1. Introduction

Coenzyme Q_{10} (CoQ_{10}) is very essential to the life processes for biological organisms, because the CoQ_{10} is one kind of important components for the electron/proton transport chains in the mitochondrias, which is necessary for the production of ATP [1–5]. Recently, the CoQ_{10} was used in many clinical fields such as the treatment of cardiomyopathy, diabetes, Parkinson's disease, Alzheimer disease and so on. Furthermore, CoQ_{10} could be also used in the health/cosmetic industry as one kind of nutritional supplements, because the CoQ_{10} exhibited amazing functions of anti-aging and anti-wrinkle due to its antioxidant property [2–5]. However, CoQ_{10} has a large molecular weight (863 Da) so that it is a poorly water-soluble substance which limits its bioavailability [3–5]. Therefore, several methods have been reported to improve its solubility and bioavailability [4–6]. Due to the similarities with natural cells, the strategy of liposome trapping should be a highly effective way to improve the CoQ_{10} absorption by human body [7,8].

The CoQ_{10} trapped by liposome could consequently provide a longer-lasting dosage of CoQ_{10} into the target tissues for the maximum effectiveness. Recently, many conventional methods have been developed to prepare CoQ_{10} liposome such as “bangham”, detergent depletion and injection methods [7–10]. However, most of these methods involved several kinds of toxic organic solvents (e.g. chloroform, ether or methanol) which are harmful to the environment and human body [9]. Additionally, liposome produced by these methods exhibited poor stability and broad distribution of particle size. Besides, all these methods are more complicated and not suitable for industrial-scale production [10].

Recently, preparation of liposome by supercritical fluids (SCFs) has attracted much attention. SCFs is neither a gas nor a liquid but possesses properties of both, and its density is liquid-like while its viscosity is gaseous [11]. The SCFs has been used widely in pharmaceutical industry. Since Frederiksen firstly reported the synthesis of liposome by SCFs in 1997, many related synthetic routes have been developed for liposome preparation [12,13]. Compared with conventional technologies, SCFs has several advantages such as environmental friendliness, nontoxic, inexpensive, mild operation temperature, and easy separation from liposome after procedures. Moreover, it is possible to achieve industrial-scale production under the conditions of current good manufacturing practice (cGMP) [14].

Consequently, several works reported the preparation of drug loaded liposome by SCFs. However, most previous works showed that the particle size of the liposome product was several hundred nanometers, and the size distribution was broad [15]. The liposome product with the particle size less than 100 nm has rarely been reported. Besides, preparation of CoQ_{10} liposome by SCFs has been very seldom reported yet.

In this paper, we reported an approach which could prepare highly dispersed nanoscaled CoQ_{10} liposome with small particle size (less than 100 nm) and high trapping efficiency by Rapid expansion of supercritical solution (RESS) without using toxic organic solvents. We also investigated the effect of pre-expansion

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0167-577X/

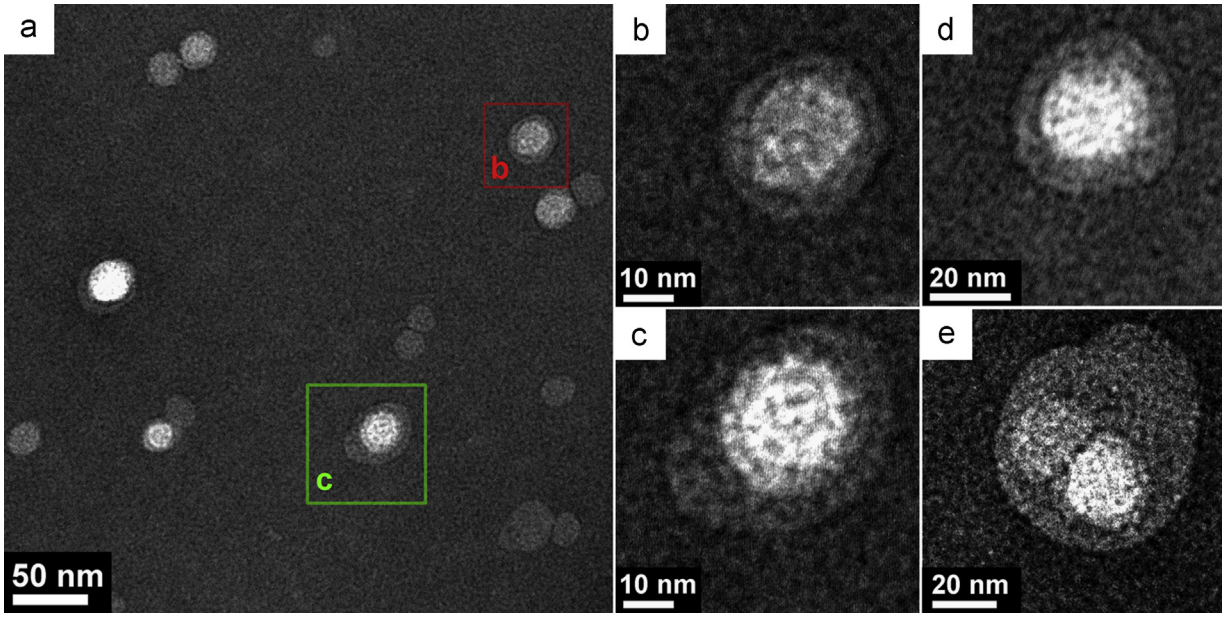

Fig. 1. TEM and HRTEM images of CoQ_{10} liposome prepared by RESS at 323 K and 200 bar.

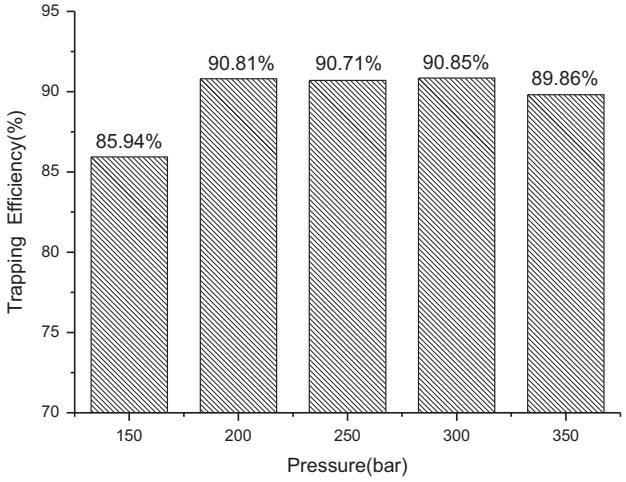

Fig. 2. Effects of pre-expansion pressures on the trapping efficiency of CoQ_{10} liposome.

pressure on the trapping efficiency of CoQ_{10} liposome. It was expected that this method could produce CoQ_{10} liposome continuously and could achieve pilot-scale production theoretically.

2. Experimental details

Supercritical apparatus with RESS function, manufactured by SEPAREX S.A. (France), was shown schematically in Fig. S1 (Electronic Supplementary material). In a typical procedure, CoQ_{10}, phosphatidylcholine and cholesterol were dissolved in ethanol and the solution was pumped into the reactor "A1" by the syringe pump. The temperature and pressure of reactor "A1" were maintained at 323 K and 200 bar, respectively. Consequently, the solution was further dissolved by the supercritical CO_2 fluid to form the final equilibrated mixture in "A1". Before RESS spray process, the phosphate-buffered saline (PBS) solution (pH 7.2, 0.01 mol/L) was added into the collector "A2". After 40 min of equilibration process in reactor "A1", the final equilibrated mixture was rapidly sprayed into the PBS solution in collector "A2" through RESS nozzle to form the dispersion. Finally, the final product of white liposomal dispersion was collected by a beaker and kept in refrigerator for further characterizations [16]. A series of products were obtained by adjusting the pressure of reactor "A1" from 150 to 350 bar. (150, 200, 250, 300 and 350 bar.)

The products were characterized by JEM-2010FEF transmission electron microscopy (TEM) and the images were taken with negative staining by using 1% (w/v) phosphotungstic acid (See details in Electronic Supplementary material). Details about the definition of trapping efficiency [13,14] and related method of measurement [17] were also elucidated in Electronic Supplementary material.

3. Results and discussion

Fig. 1 shows the TEM images of CoQ_{10} liposome nanoparticles prepared by RESS at 323 K and 200 bar. Fig. 1a exhibits the spherical morphology with smooth surface. The particle size of CoQ_{10} liposome ranges from 20 nm to 40 nm and exhibits a narrow particle size distribution, which is much smaller than that of earlier

reported products prepared by conventional methods [18, 19]. Fig. 1b–d shows that CoQ_{10} molecules are encapsulated in the liposome particles. The brighter cores of liposome should be the CoQ_{10} substance and the darker shells should correspond to the phospholipids' vesicles. A special liposome captured in Fig. 1e shows that two separated CoQ_{10} cores encapsulated in one liposome particle, which may be caused by the Sc-CO_2 acting as the membrane in the formation process of liposome.

The effect of pre-expansion pressure on the product's trapping efficiency was investigated in this work. A series of products were produced at 323 K for 40 min, under the pre-expansion pressures of 150, 200, 250, 300 and 350 bar via the RESS process. As shown in Fig. 2, the product of CoQ_{10} liposome obtained under 150 bar showed low trapping efficiency, probably because the phospholipids were not easily dissolved into ethanol and Sc-CO_2 while the pressure was less than 200 bar [16]. However, the trapping efficiencies of the products obtained under 200, 250 and 300 bar were greater than 90%, indicating that the solubility of liposomal materials in Sc-CO_2 increased significantly when the pressure was equal to or greater than 200 bar. Consequently, CoQ_{10} can be trapped into the phospholipid vesicles