Preparation of medical nanomaterials by ultrasonic irradiation

超音波照射による医療用ナノ材料創製

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1. Introduction

Liposomes have a closed vesicle structure consisting of phospholipid bilayers and are nanocarriers with a diameter of about 100 nm to 2000 nm. Effectiveness in the several fields such as injections, transdermal and oral administration and cosmetics are expected[1] because liposomes are highly biocompatible. In the conventional liposome preparation method (Bangham method[2]), liposomes are prepared by the steps of dissolving phospholipids in an organic solvent such as chloroform, forming a lipid film by vacuum drying, and preparing a vesicle structure by mechanical vibration. In this method, harmful organic solvents may remain in the liposomes, making it difficult to apply to the human body, which is a serious problem. Therefore, development of a novel liposome preparation method is desired that does not contain a harmful organic solvent in the formed liposome. It is reported that a liposome preparation method using carbon dioxide[3], which has low biotoxicity, instead of a harmful organic solvent. However, the solubility of phospholipids in carbon dioxide is low, so it has been considered difficult to prepare liposomes with a high yield by a method using carbon dioxide.

To solve these problems of the conventional method, the authors attempted a newly liposome preparation method by expanding the water-carbon dioxide nano-interface and inducing microphase separation by irradiating ultrasonic waves directly from the ultrasonic transducer into the system containing phospholipid, water and high pressure carbon dioxide as shown in **Fig. 1**. In this study, first, the morphology of liposomes prepared by the newly method is obsereved by TEM. In addition, the average particle size of the liposomes both the conventional method (Bangham method) and the newly method is compared. Finally, the deferences between liposome yield and average particle size due to operating factors (ultra-sonication time: 0 s to 250 s) are examined.

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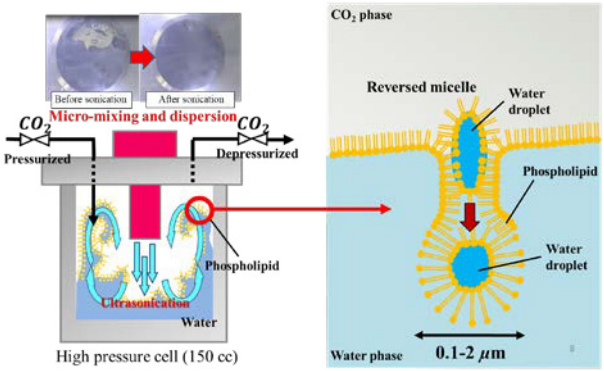

Fig. 1 Mechanism of liposome production by high pressure CO_2 with ultra-sonication method.

2. Experiments

Lecithin from soybean (> 97.0% purity) were purchased from FUJIFILM Wako Pure Chemicals Industries, Japan and used without further purification. Carbon dioxide (> 99 % purity) supplied from Fukuoka Oxygen Co. Ltd, Japan. Distilled water was deionized using a Milli-Q water purification system (Millipore Corp.) and used in the experiments.

A schematic diagram of the apparatus used for producing liposomes with carbon dioxide and direct ultra-sonication is shown in **Fig. 2**. Into the high pressure cell (inner volume, 150 mL; dimensions, 34 mm i.d. × 165 mm height; manufacturer, Toyo Koatsu Co. Ltd.) equipped with a titanium ultrasound horn, lecithin, ultra-pure water and carbon dioxide were placed and mixed with ultra-sonication (VC505; Sonic and Materials Inc., frequency: 20 kHz, maximum power: 500 W, amplitude: 18.3 μm, ultra-sonication time: 0 s to 250 s) at a pressure of 6.8 MPa and a temperature of 298 K. After depressurized, the particles in the high pressure cell were collected with the solution. The recovered solution was filtered (pore size; 0.45 μm) to remove unreacted solids and then analyzed. The morphology of liposomes was evaluated using transmission electron microscopy (TEM, JEOL JEM-2100F, Tokyo, Japan). The particle size distribution of liposomal suspensions was measured with dynamic light scattering and a particle size analyzer (DLS, Microtrac UPA 150, MicrotracBEL

Corp. Osaka, Japan) at room temperature. The liposome yield of the experiments was evaluated with following equaton:

Liposome yield (%) = (1- W_{remain} [g] / $W_{initial}$ [g]) × 100

where $W_{initial}$ is the initial phospholipid mass before the carbon dioxide treatment and W_{remain} is the mass of phospholipid remaining in the reactor after the carbon dioxide treatment.

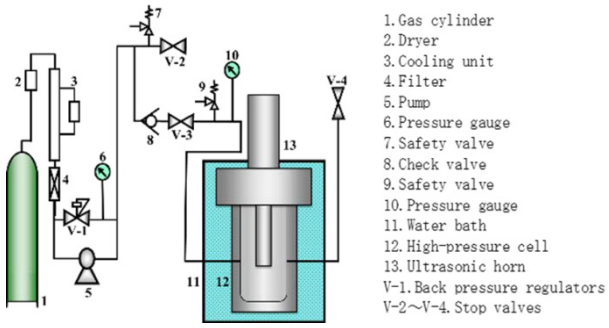

Fig. 2 Schematic diagram of the apparatus for liposome production by high pressure CO_2 with direct ultra-sonication.

3. Results and discussion

Fig. 3 shows the morphology of dried liposomes produced by high pressure carbon dioxide with ultra-sonication method observed by TEM (a) and the particle size distribution of liposomal suspensions measured by DLS (b), respectively. The morphology of the liposomes obtained by the novel liposome preparation method was confirmed to be spherical and nano-sized. The particle size distribution of the liposomal suspension was sharp and the average particle size was 144 nm. On the other hand, the average particle size of the liposomal suspension prepared by the conventional method (Bangham method) was 170 nm. These results reveal that preparing liposomes with a smaller average particle size than the conventional method (Bangham method) is successful using high pressure carbon dioxide with direct ultra-sonication.

Fig. 4 shows liposome yields and average particle sizes as a function of ultra-sonication time obtained from the high pressure carbon dioxide with direct ultra-sonication method. Liposome yields increased from 30 % to 66 % with increasing ultra-sonication time from 0 s to 75 s, respectively, whereas the average particle size decreased from 273 nm to 146 nm at increasing irradiation time from 0 s to 250 s, respectively. The decrease in liposome particle size with increasing ultra-sonication time indicates mechanical effects such as shock waves being formed during symmetric cavitation, or by microjets forming during asymmetric cavitation that cause physical disturbances that may reduce the particle size of the liposomes.

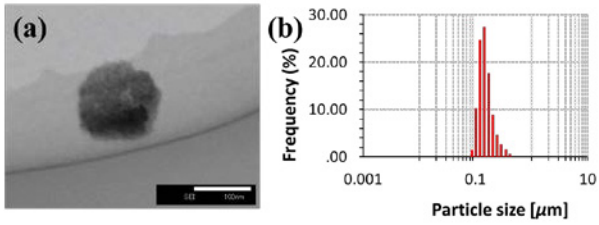

Fig. 3 TEM image (a) and particle size distribution (b) of liposomes produced by high pressure CO_2 with direct ultra-sonication.

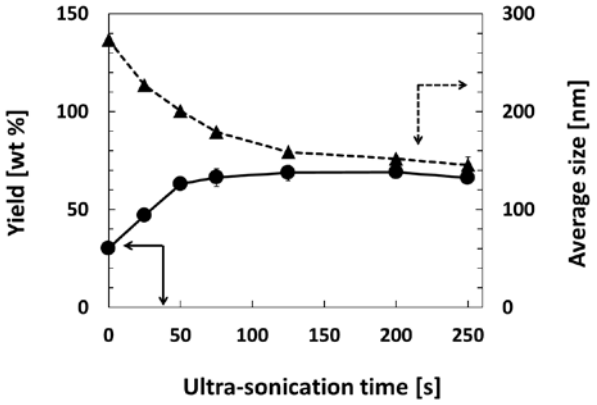

Fig. 4 Liposome yield (●) and average particle size (▲) as a function of ultrasonication time.

4. Conclusion

In this study, a newly liposome preparation method was attempted by expanding the water-carbon dioxide nano-interface and inducing microphase separation by irradiating ultrasonic waves directly from the ultrasonic transducer into the system containing phospholipid, water and high pressure carbon dioxide. The preparation of spherical nano-sized liposomes by the novel method was confirmed from the TEM image and the particle size distribution of the suspension. It was also clarified that the liposome yield increased and the average particle size of liposomes decreased with the increase of ultra-sonication time.

References

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