Continuous Production of Loposome Using Supercritical-Fluid-Based Technique

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Keywords: supercritical, liposome, micro-mixer, slug flow, flow process

INTRODUCTION

Liposome is recognized as an ideal carrier for water-soluble pharmaceutical compounds and intensively investigated for the last several decades. An isolated aqueous phase inside it can protect pharmaceuticals from outside and therefore, bioavailability of pharmaceuticals can be greatly improved by the encapsulation [1]. However, most of conventional production methods utilizes too much organic solvents and thus, additional proceses for the removal of these harmful solvents are necessary. Recently, supercritical carbon dioxide ($scCO_2$) has been gathering great attention as as alternative solvent because of its ideal properties for drug processing: mild critical temperature, non-toxicity either to human and environment and inexpensiveness [2]. However, these $scCO_2$-based process has not been optimized yet; most of them are batch processes and operating conditions are not elaborated.

In this research, we proposed novel $scCO_2$-based liposome production method utilizing micro-mixer and –channel. The micro-mixer and slug flow formed in the micro-channel can enhance mass transfer during the process and improve productivity. Additionally, this new process is designed to enable continuous production of loposome with contoled diameter.

EXPERIMENTAL

Timolol maleate (TM) was used as model drug in this work. Carbon dioxide was cooled and liquefied at -5 ℃ and then, pressurized to 10.0 MPa by a pump. TM aqueous solution and soy lecithin solution in ethyl acetate (EA) was sent by HPLC pumps. Pressurized carbon dioxide and lecithin / EA solution was mixed and heated up to 40 ℃ to form homogeneous supercritical phase. This supercritical phase and TM solution were mixed inside swirl-mixer. TM solution in supercritical phase will form small droplets by intensive mixing in the swirl mixer and stabilized by lecithin in the supercritical phase, which results in the formation of water in $scCO_2$ emulsion. At the next step, this emulsion and water will form slug flow in micro-channel and the droplets will be entrapped into this water phase. The mechanism of liposome formation is shown in Figure 1.

The size of the product was measured by dynamic light scattering method. Obtained liposome was observed by transmission electron microscope (TEM) by freeze fracture replica.

RESULTS and DISCUSSIONS

The size of liposome was found to be controllable by changing flow rate of TM solution (0.1 – 30.0 μm). This is because size of the TM droplets in the emulsion changes by changing the flow rate. Lecithin concentration in EA solution also affects the size of the product. If larger amount of lecithin is available in the supercritical phase, TM droplets will be surrounded by lecithin faster and thus it gets smaller. Therefore, the size of the product is also smaller. Figure 2 shows TEM image of the obtained liposome. Unilamellar liposome with thin lipid layer (around 8 nm) is confirmed in this image.

In conclusion, novel method for the liposome production proposed in this work could successfully prepare unilamellar liposome with tunable diameter. Additionally, continuous production and residual-solvent-free process was established by combining supercritical fluid with micro-devices.

REFERENCE

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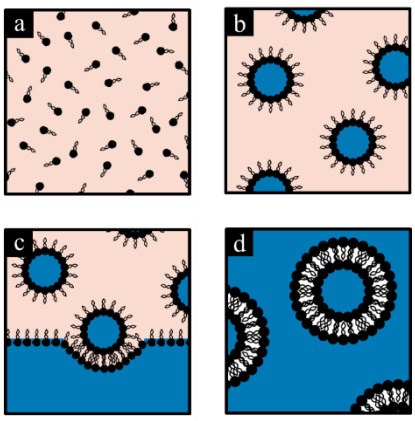

Figure 1. Liposome formation mechanism

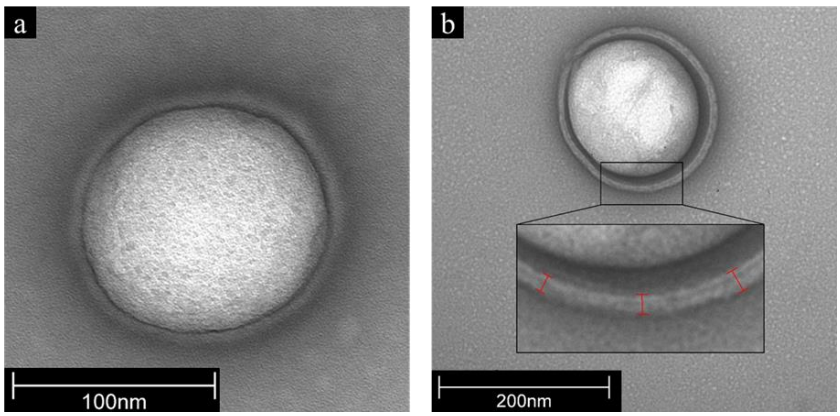

Figure 2. TEM image of liposome obtained in this work