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PREPARATION OF LIPOSOMES BY A CONTROLLED ASSEMBLY METHOD

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Abstract A method to prepare unilamellar liposomes is demonstrated, which contains two experimental steps. (a) Water in oil (W/O) droplets are formed by sonication. (b) The W/O droplets are passed through a monolayer at the oil/water interface due to gravity or centrifugation, and transformed into liposomes. The sizes of the liposomes are 100 ~ 500 nm in diameter. The bilayer of liposome is formed layer by layer, which provides an approach to prepare asymmetric liposomes.

INTRODUCTION

Liposome----self-assembling colloidal particle in which a lipid bilayer encapsulates a fraction of the surrounding aqueous medium -- has been attracting highly attentions from 70's in both scientific and technological areas.[1,2] Liposomes have been used as biological membrane models and drug carrier systems. In addition, liposomes or surfactant vesicles are considered useful in fabrication of molecular optical devices for their bilayer structure[3] and preparation of technologically important ultrafine particles as the sites and templates.[4]

Different methods of preparing liposomes are known, such as ultrasonic; rapid injection; gel filtration; dialysis; and reverse-phase evaporation.[5] Recently, we developed a method to prepare liposomes, which contains two experimental steps.[6] (a) A small amount of aqueous solution was added into a n-decane solution of lecithin and cholesterol. W/O droplets were formed by sonication. (b) W/O droplets were spread on the surface of a buffered saline, passed through a monolayer at the oil/water interface due to gravity and/or centrifugation, and finally transformed into liposomes in water phase.

METHODS AND EXPERIMENTS

The preparation method was described elsewhere in detail.[6] Figure 1 is a schematic drawing of the transformation of a W/O droplet into a liposome. The W/O droplet in the

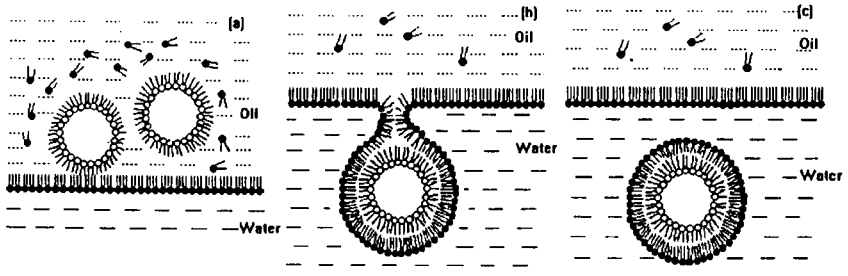

FIGURE 1 Formation process of liposomes by the controlled method.

oil phase moved close to the oil/water interface shown in Figure 1(a), and interacted with the hydrophobic tails of a monolayer in Figure 1(b). After the second monolayer had covered the surface of the water droplet, a liposome formed and entered into the water phase as shown in Figure 1(c).

RESULTS AND DISCUSSION

The size of lecithin liposome was measured by using a negative staining microscope. Liposomes prepared in a buffered solution were shown in Figure 2(a). The sizes were 100 ~ 500 nm in diameter. Liposomes containing a negative stain solution (2% ammonium molybdate tetrahydate solution) showed single-shelled circles in Figure 2(b). These single-shelled circles confirmed that the prepared liposomes were unilamellar other than multiple emulsion.

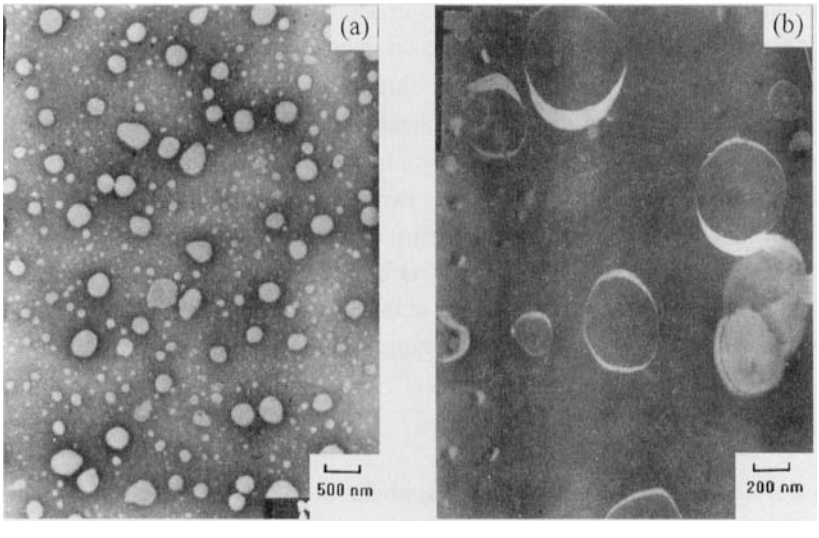

FIGURE 2 Negative staining micrographs of liposome preparation. (a) Liposome containing buffered saline. (b) Liposomes containing negative stain

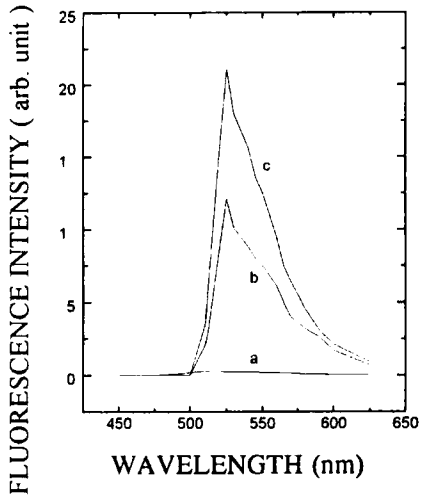

FIGURE 3 Fluorescent emission spectroscopy of liposome solution. (a) Liposomes with 100 mM 6-CF. (b) The liposomes disrupted by Triton X-100. (c) The same amount of 6-CF in water.

Fluorescent experiment was done to study the leakage and entrapment yield of the liposomes. 100mM aqueous solution of 6-carboxyfluorescein (6-CF) was used as an interior content of the liposomes. In dilute solution (under 30 μM) of 6-CF the fluorescent intensity is proportional to the number of the dye molecules. When the concentration is raised above about 10 mM, the intensity per molecule drops off rapidly due to self-quenching effect. The liposome containing 100 mM 6-CF solution emitted a very weak fluorescence as shown in curve a in Figure 3 (fluorescent intensity: 0.27, emission maximum: 512.3 nm). When the incorporated 6-CF was released and diluted into the bulk water by adding Triton X-100, the fluorescence increased rapidly as shown in curve b in Figure 3 (intensity: 12.1, emission maximum: 526 nm). This result confirmed strongly that 6-CF was enclosed in the liposomes without leakage during the preparation period. If the same amount of 6-CF was directly diluted into the same amount water without liposome encapsulation, the emitted fluorescent spectrum was shown in curve c in Figure 3 (intensity: 21.1, emission maximum: 526 nm). Since the concentrations of 6-CF in both curve b and c were below 30 μM, the quotient of their fluorescent intensities gave an entrapment yield. Entrapment yield reached 60% according to Figure 3. The loss might be due to Brown's motion which prevented the W/O emulsion from entering the water phase. Our recent studies found that the pH gradient across the bilayer could act as an energy source that drove the formation of liposomes at the oil/water interface. The pH gradient across bilayer can be controlled by adjusting the pH values of both the entrapped solution and the surrounding medium, which could increase both the entrapment yield and formation speed.

CONCLUSIONS

In this method liposomes can be directly prepared with the entrapped phase different from their suspension aqueous medium. Therefore, an additional purification process of liposomes solution, which is needed to remove the unentrapped materials in many other methods, is generally not required. On the other hand, the bilayer formation in liposomes is layer by layer. The inner layer is formed during preparation of W/O droplets and the outer formed by passing them through the monolayer at the oil/water interface. So it provides an opportunity to fabricate liposomes whose inner leaflet can be different from the outer one. Asymmetry in biological membrane[7] and liposome membrane[8] has been investigated, and becoming very promising in studying membrane functions, such as, energy and electron transfer reaction. In our group, lecithin/oleic acid vesicles, which were prepared by passing lecithin W/O droplets through an oleic acid monolayer at the oil/water interface, have been obtained. Further experiments are carried on to prove the asymmetric liposomes.

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