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SHORT COMMUNICATION

Investigation of PEG 6000/Tween 80/Span 80/H_2O niosome microstructure

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Abstract A stable niosome is prepared from Poly(ethylene glycol) [PEG] 6000/Tween 80/Span 80/H_2O lamellar liquid crystal. The niosome structures and properties are studied by the methods of negative-staining transmission electron microscopy and small angle X-ray diffraction. A new calculating method is first put forward to obtain the microstructure and layer number of the niosome membrane. The membrane thickness and layer number of the niosome are 8–22 nm and 1–3.5 in PEG 6000/Tween 80/Span 80/H_2O system.

Keywords Niosome · Preparation · Microstructure · Membrane · Layer number

Introduction

Vesicles are widely used not only as models for cell membranes but also as drug carriers to deliver to the targets in tumors and viruses [1–4]. They can be prepared spontaneously or by the addition of energy from phospholipids, nonionic surfactants and mixed cationic and anionic surfactant systems [5–11]. Vesicles are unilamellar or multilamellar spheroid structure composed of amphiphilic molecules [12, 13]. The membrane structure and layer number of vesicles are very important for the size, stability and application of the vesicles. Anthony Ryan and Bermudez had successfully obtained and calculated the membrane thickness of vesicles using small-angle X-ray scattering analysis and negative-staining transmission electron microscopy (TEM) analysis with the data in good agreement [14–16]. However, the membrane microstructure and the membrane layer number of vesicles have not been obtained.

Tween 80 and Span 80 are pharmaceutically acceptable, innocuous, non-ionic surfactants [17, 18]. Poly(ethylene glycol) [PEG], Tween 80 and Span 80 can be used to prepare highly stable niosome [19], which is provided with the hydrotrope-solubilization action to hydrophilic drug and hydrophobic drug. In the present work, a stable niosome is prepared from the PEG 6000/Tween 80/Span 80/H_2O lamellar liquid crystal. A new method is first put forward to calculate the membrane structure and layer number of the niosome from the film thickness of the fractured niosome in negatively stained TEM micrographs from the analogous microstructure of the lamellar liquid crystal.

Materials and methods

Materials PEG 6000 was bought from Shanghai Pudong Goulian Chemical Plant, Tween 80 from Shanghai Biological Engineering (99%) and Span 80 from Shanghai Commonage Pharmacy (99%). Doubly distilled and deionized water was used for the preparation of the solutions.

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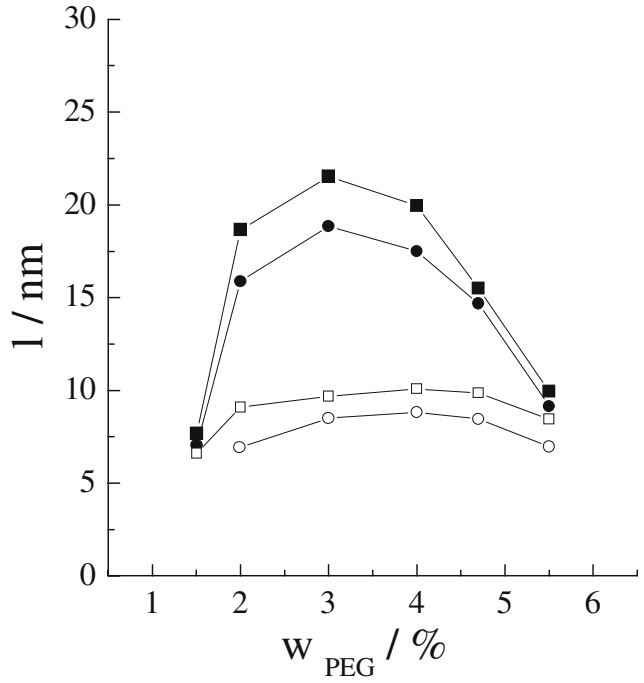

Fig. 1 Niosome thickness relates with PEG 6000 content. The *hollow squares* and *circles* correspond to Tween 80/PEG 6000/H_2O system. The *filled squares* and *circles* correspond to Tween 80/PEG 6000/Span 80/H_2O system. The *hollow* and *filled squares* correspond to 25 °C. The *hollow* and *filled circles* correspond to 37 °C

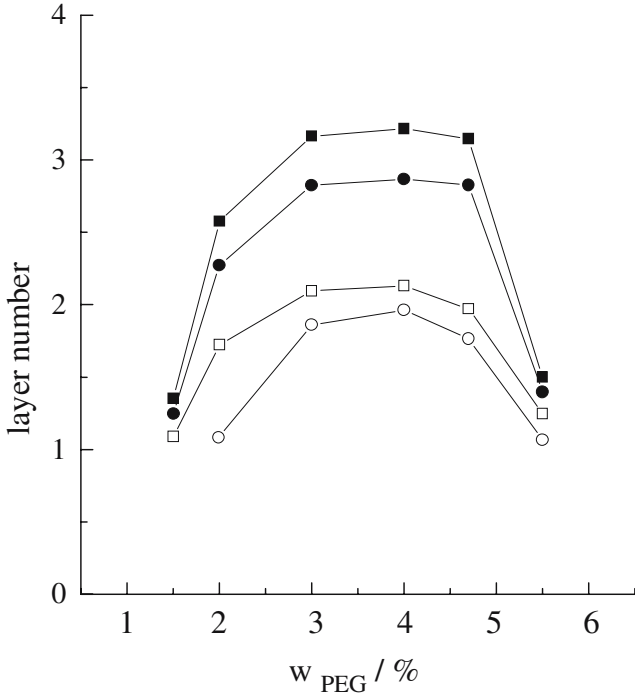

Fig. 3 Layer number of the niosome membrane relates with PEG 6000 content. The *hollow squares* and *circles* correspond to Tween 80/PEG 6000/H_2O system. The *filled squares* and *circles* correspond to Tween 80/PEG 6000/Span 80/H_2O system. The *hollow* and *filled squares* correspond to 25 °C. The *hollow* and *filled circles* correspond to 37 °C

Niosome preparation Tween 80, Span 80, PEG 6000 and water were vortex mixed at a certain weight fraction to prepare the lamellar liquid crystal. The lamellar liquid crystal was observed and validated by using polarizing microscope (×59, Shanghai Photology Apparatus) and small angle X-ray super speed diffractometer (Buruke D8, Buruke German). The niosome was obtained by sonicating the diluted solution (2% PEG+98% water) with the lamellar liquid crystal for 30 ~85 min [10]. The stability of niosome was studied by measuring the mean size distribution as a function of time after vesicle preparation using a computerized micrographs image analyzing meter (imaging microgram) and negative-staining TEM (NS-TEM).

Negative-staining TEM of niosome A drop of the sample with niosome was transferred into the copper mesh grids with the Formvar film. After the sample was adsorbed on the Formvar film about 15~20 min, the staining solution (example phosphor tungstenic acid) was dripped onto the Formvar film. The staining time was about 1~2 min. After drying the copper mesh grids, the size and morphology of the niosome were clearly observed by TECNAIR transmission electron microscopy (Philip Apparatus, USA) [14].

Small angle X-ray diffraction measurement Samples were placed in a special plane glass. Small angle X-ray diffraction (SAXD) was obtained by using an X-ray super speed diffractometer (Buruke D8, Buruke German) with a

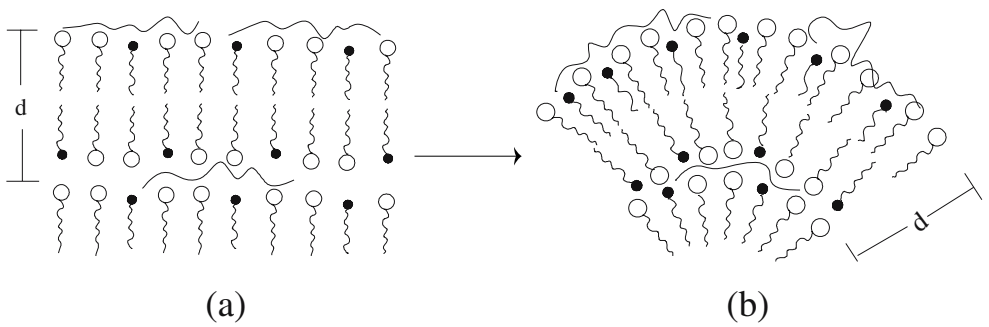

Fig. 2 Preparation of niosome from Tween 80/PEG 6000/Span 80/H_2O lamellar liquid crystal. (**a**) And (**b**) are the sketch maps of lamellar liquid crystal and niosome, respectively. ~, ~, and *symbols* are presented as PEG 6000, Tween 80 and Span 80, respectively

Ni filter and Cu radiation (λ=0.542 nm), tube voltage 40 kV and tube current 100 mA.

All measurements had been carried out at 25 °C.

Results and discussion

Vesicles can be observed by the imaging micrograph, freeze fracture transmission electron microscopy, scattering technique and NS-TEM [2–19]. The niosome membrane thickness, *l*, can be obtained from NS-TEM micrograph [12, 14] (Fig. 1). All of the niosome membrane thickness in this paper is an average value more than 20 niosomes. With the increase in PEG 6000 content, the membrane thickness increases. But when PEG 6000 content is more than about 5%, the membrane thickness decreases. PEG 6000 with long hydrophilic chain is mostly surrounded and adsorbed on the niosome, some inserts partially into the membrane phase of the niosome. These make the rigidity and stability of the niosome membrane enhance [19]. Alternately, more PEG 6000 can cause the fusion or disruption of the bilayer structure of the niosome. More hydrophilic PEG 6000 makes the hydrophilicity of the niosome increase and the thickness and the intensity of the membrane decrease until the structure with the ordered assemble is disrupted into O/W and W/O phases.

The interlayer space, *d*, of Tween 80/PEG 6000/Span 80/H_2O lamellar liquid crystal is directly determined by small-angle X-ray diffraction [20]. The niosome can be prepared by the addition of energy from the lamellar liquid crystal (Fig. 2). The niosome also is highly stable in Tween 80/PEG 6000/Span 80/H_2O system with a period of stability exceeding 180 days. The niosome from the lamellar liquid crystal may withhold the microstructure behaviors of the lamellar liquid crystal. The interlayer space of the niosome can be determined directly by SAXD curve of the niosome in high concentration. But in dilute concentration, supposing, the membrane microstructure of the niosome is provided with an analogous microstructure of the lamellar liquid crystal (Fig. 2). The interlayer space of the niosome can be inside extrapolated in the same contents of the niosome composes, which is from the relation of the interlayer space of the lamellar liquid crystal with the solvent content in the SAXD curve of the lamellar liquid crystal. The average layer number of the niosome can be approximately calculated from the thickness of the niosome membrane from NS-TEM and the interlayer space of the niosome.

Figure 3 shows the layer number varies with PEG 6000 content and temperature. From Fig. 3, the layer number is approximately in excess of 1, which indicates expressly that the niosome is, on the whole, not a unilamellar vesicle (ULV) but a multilamellar vesicle (MLV). Vesicle stability and membrane intensity with ULVs are inferior to those with MLVs. The Tween 80/PEG 6000/Span 80/H_2O niosome membrane with MLVs has superior intensity and stability.

Conclusion

The membrane thickness and layer number of the niosome are successfully obtained from the microstructure of the lamellar liquid crystal.

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