The Studies on the Aqueous Dispersed Particles Formed from Monoolein/Monopalmitin/Water Mixture

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The morphology and the microstructure of the dispersed particles, which were formed from monoolein/monopalmitin/water mixture, have been studied. The freeze-fracture TEM shows clearly that the aqueous dispersed particles have irregular cubic shapes. X-ray diffraction technique has been utilized to study the microstructure of the particles and it was found that these particles still retained the cubic character.

It is well known that the dispersions of cubic lipid-water phases can be used for development of controlled release formulations of biologically active agents in the field of drug delivery and for the encapsulation of enzymes in food technology. Colloidal particles of a reverse cubic phase with interior aqueous zones also provide certain advantages in technical applications compared to droplets of common oil-in-water emulsions.[1,2] It has been reported that the so-called cubosome particles were first prepared by mechanical fragmentation of the cubic lipid-water phase containing a liposomal dispersion composed of phosphatidyl choline-GMO-water.[3,4] Dispersions with moderate kinetic stability have been obtained. In order to differentiate from liposomes, these particles have been termed "cubosomes."[2,5,6] In the present work, our particles have been prepared differently from those of previous reports,[3–6] and we refer to the particles as "cubic particles."[7,8]

The aqueous dispersed cubic particles studied in this work were formed from monoolein (Danisco) and monopalmitin (Aldrich) mixture. At the ratio of 90:10 (wt:wt), monoolein and monopalmitin were completely dissolved in a small amount of ethanol to form a liquid precursor formulation. And then the cubic particles were prepared by dispersing this precursor in 5–2000 times excess water by vortexing. Since the internal structure of the cubic particle consists of lipid domain, water channel and interfacial region, cubic particles have been used to encapsulate amphiphilic, lipophilic, and protein drugs.[9,10]

The morphologies of the aqueous dispersed particles were investigated by using a freeze fracture apparatus (Eiko Model FD-2A) on a liquid nitrogen-cooled support and a transmission electron microscope (JEOL Model JEM-1200EX). The procedure has been described in previous reports.[11,12] In brief, a thin layer of the sample (20–30 μm) was placed on a thin copper holder and then rapidly quenched in liquid nitrogen. The frozen sample was fractured at −120 °C, in a high vacuum better than 10^{-5} Pa with the liquid nitrogen cooled knife in the freeze etching unit. Increasing the temperature to −95 °C and keeping the high vacuum, the fractured sample was left 10–15 min in order to evaporate the water adsorbed on the surface of the sample. Then decreasing the temperature to −120 °C again, the replication was done using unidirection shadowing at an angle of 45°, with platinum–carbon (Pt–C) and 1–10 nm of mean metal deposit. The replicas were washed with double distilled water and were observed in transmission electron microscope.

The microstructures of the aqueous dispersed particles were investigated by small angle X-ray diffraction technique. The samples were transferred to 1-mm diameter quartz capillary and sealed with epoxy. Copper Kα X-rays (1.542 Å) were produced using an X-ray generator (FL CU 4KE, Bruker, Germany) and operated at 40 kV and 45 mA. The exposure time was 2.5 h, and the sample-to-detector distance was 30 cm. X-ray diffraction (XRD) data was obtained by General Area Detector Diffraction System (GADDS, Bruker, Germany) at 25 °C.

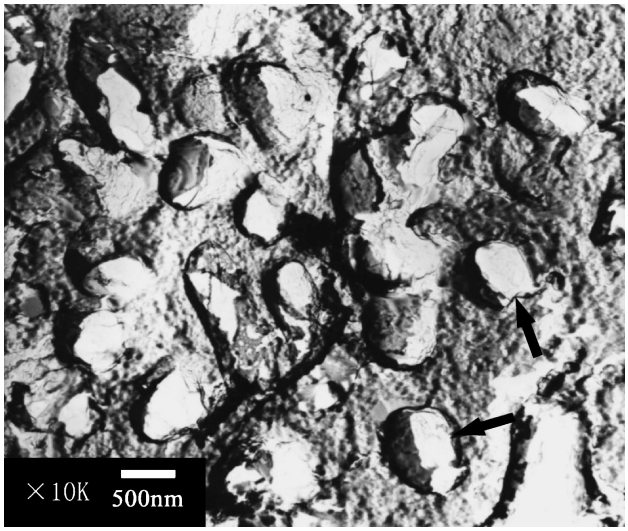

Figure 1. Photograph of freeze-fracture TEM (MP/MO = 10/90, wt/wt).

Gustafsson et al.[13,14] have studied the structures of the cubosomes (MO/F-127/H_2O) by the X-ray diffraction and by cryo-transmission electron microscopy (cryo-TEM). Good cryo-TEM images of the particle structure have been obtained and the outer shape and the inner periodicity have been observed in these images. Examination by means of cryo-transmission electron microscopy has revealed submicron particles of faceted morphology, showing an inner texture compatible with the expected periodicity from reverse bilayer cubic structure. The X-ray diffraction data have shown that the cubosomes have cubic feature when compared with the diffraction peaks so far reported. The correlation between the X-ray data and the texture has strongly suggested that the particles are made up of the reverse bilayer cubic structure. This observation has been in line with the suggestion that was put forward by Landh,[15] who claimed

that this cubic structure is the most easily dispersed.

Figure 1 is an image with a magnification 1×10^4 of the sample showing lots of irregular cubic particles, which are aqueous dispersions of MO/MP mixture. The dispersed particles are approximately 200–400 nm in width and 300–600 nm in length. Two irregular cubic particles are pointed out by the arrows. It also can be observed that some cubic particles are aggregated together, which makes it difficult to recognize their outer shapes. But the aggregated particles still retained the irregular cubic shape when they were dispersed into the water again.

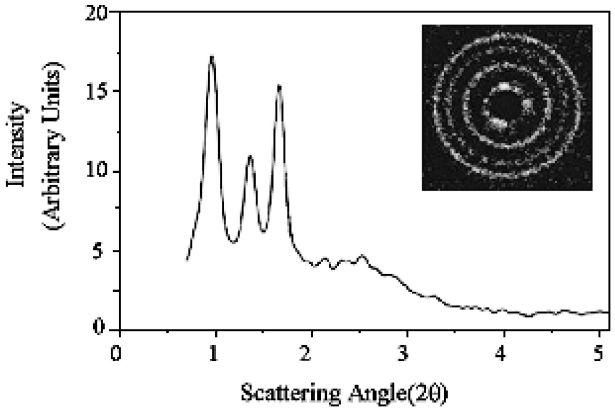

Figure 2. Small angle X-ray diffraction spectra of MO/MP system.

The microstructures of the dispersed particles prepared by the precursor method were investigated by small angle X-ray diffraction. The particles in the dispersion had to be concentrated by centrifugation to obtain clear XRD pattern. The concentrated particle systems were easily redispersed in water with a simple pipetting and retained original size distribution.[7] It indicated strongly that the centrifugation process did not cause a gross rearrangement of the structure of the cubic particles. The small angle X-ray diffraction in Figure 2 shows the repeat spacing ratio of $\sqrt{2}$:$\sqrt{4}$:$\sqrt{6}$. Therefore, it is highly probable that the internal structure of the cubic particle is a body-centered cubic, *Im3m* phase. Lattice parameter of the unit cell is calculated to be 13.14 ± 0.1 nm. The phase and the lattice parameter are very similar to those of cubic phase consisting of monoolein, F-127 and water[15] and cubosome.[13] This result suggests that the lipid particles with the cubic microstructure can be constructed by dispersing a precursor containing monoolein and monopalmitin in water without the aid of mechanical devices. Because of this structure, the cubic particles can encapsulate lipophilic drugs as well as hydrophilic drugs.

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