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The Aqueous Dispersions of Bicontinuous Cubic Phases Formed by Precursor Method

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Abstract: The morphologies and the microstructures of the dispersed particles of the cubic phase, which were formed by precursor method, were studied. The freeze-fracture TEM clearly showed 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. The sizes of the particles were measured by dynamic light scattering, and the results showed that the sizes of the dispersed particles were between 200 ~ 400 nm under different conditions.

Keywords: FF-TEM, X-ray diffraction, dynamic light scattering, aqueous dispersions, precursor.

It is well known that 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 in food technology for the encapsulation of enzymes. 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 in a three-phase region containing a liposomal dispersion, besides the cubic phase in the ternary system phosphatidyl choline-GMO-water[3, 4]. Dispersions with moderate kinetic stability have been obtained, in order to compare effects with liposomes; these particles have been termed "cubosomes"[2, 5-7]. In the present work, our particles have been prepared differently from those of previous reports[3-6], and the particles are simply prepared by the following procedure, monoolein (100 mg) is mixed homogeneously with 20 mg of pluronic F-127, then 280 μL of ethanol is added to form the particle precursor. The particles can be obtained by dispersing the precursor into the water by vortexing for several tens of seconds or just by shaking, we refer to the particles of this study as "cubic particles".

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

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been described in previous reports[8, 9]. 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.

Figure 1 is an image with a magnification 10^4 of the sample showing lots of irregular cubic particles, which were prepared by dispersing the precursor of MO / PF-127 / ethanol into the water. For the precursors with different compositions (same as the size measurement), similar photographs of FF-TEM were obtained and were not showed in this report. From **Figure 1**, it also can be seen that the dispersed particles are approximately 200-400 nm in width and 300-600 nm in length, the particles in the bottom right corner indicated by the arrow also have the irregular cubic shape. Some aggregated particles are also observed in the photograph and it is difficult to recognize their outer shape, but the aggregated particles still retained the irregular cubic shape when they were dispersed into the water again.

Figure 1 Photograph of freeze-fracture TEM (MO/PF-127=100/20 (w:w))

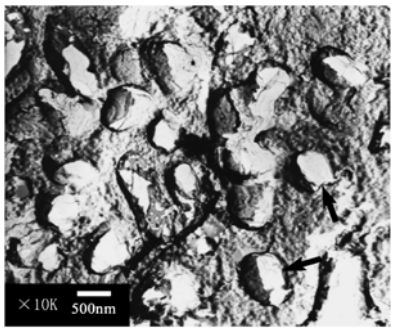
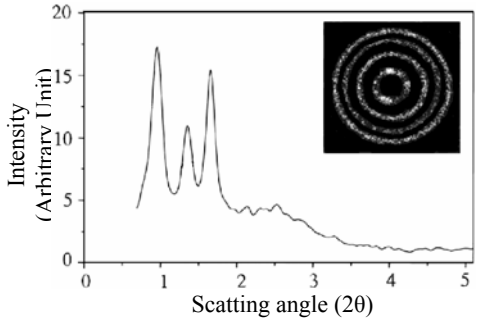


Figure 2 Small angle X-ray diffraction spectra of the concentrated cubic particles



The microstructures of the dispersed particles prepared by the precursor method were investigated by small angle X-ray diffraction (FL CU 4KE, Bruker, Germany). X-ray diffraction data was obtained by general area detector diffraction system (GADDS, Bruker, Germany) at 25°C. 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[10]. 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** showed the repeat spacing ratio of $\sqrt{2} : \sqrt{4} : \sqrt{6}$. Therefore, it was highly probable that the internal structure of the cubic particle was a body-centered cubic, *Im3m* phase. Lattice parameter of the unit cell was calculated to be 131.4±0.1 nm. The phase and the lattice parameter were very similar to those of cubic phase consisting of Monoolein, PF-127 and water[11] and cubosome[7]. This result suggested that the cubic particles with the cubic microstructure could be constructed by dispersing a precursor in water without the aid of mechanical devices. Because of this structure, the cubic particles can encapsulate lipophilic drugs as well as hydrophilic drugs.

The sizes of the dispersed particles were measured by dynamic light scattering, the results revealed that the sizes of the dispersed particles prepared by the precursor method were between 200 ~ 400 nm, corresponding to the results obtained from the FF-TEM. The effects of the amount of PF-127, monopalmitin (MP) and monomyristin (MM) in the precursor on the sizes of the cubic particles were also checked. From **Table 1**, it was seen that the sizes of the particles were slightly increased with the increasing amount of PF-127 in the precursor. Minoru Nakano[12] *et al.* have confirmed that with the increasing amount of PF-127 from 5% to 20% (wt), the cubic phase has changed the lattice structure of the cubic phase from *Pn3m* to *Im3m*. From the size measurement of the cubic particles, it also suggested that the amount of PF-127 in the precursor had an effect on the sizes and microstructures of the dispersed particles. From **Table 2**, it was seen that the sizes of the dispersed particles were about 300 nm when the amount of MP or MM in the precursor was changed from 5% to 20% (wt). The results did not have a

Table 1 The sizes of the cubic particles of MO/PF127/ethanol systems

MO：PF-127 (w:w)	Size (nm)
100：0	236.7±1.2
100：10	241.9±1.1
100：15	272.3±1.6
100：20	302.5±1.9

Table 2 The sizes of the cubic particles of MO/MM (MP) /PF127/ethanol systems

MO：PF-127：MM (w : w : w)	Size (nm)	MO：PF-127：MP (w : w : w)	Size (nm)
95：20： 5	286.0±1.9	95：20： 5	336.5±1.7
90：20：10	281.5±1.4	90：20：10	183.0±1.2
85：20：15	310.1±2.1	85：20：15	318.7±1.3
80：20：20	316.6±2.1	80：20：20	223.1±1.5

consistency trend, so the effects of lipids with different length of carbon chain on the cubic particles were not clear yet, and how the microstructure of the cubic particles changes with the addition of different lipids remained to be studied, especially how the values of the lattice parameter change with addition of different lipids. It should be very important for the cubic particles to be used as the drug delivery system.

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