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Polymerized Bicontinuous Cubic Nanoparticles (Cubosomes) from a Reactive Monoacylglycerol

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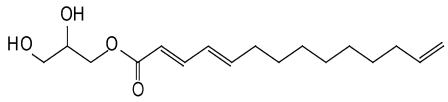
Biological and synthetic lipids can, under appropriate conditions of temperature and concentration, form hydrated nonlamellar assemblies such as inverted bicontinuous cubic (Q_{II}) and inverted hexagonal (H_{II}) phases.[1−3] Those inverted cubic phases which are bicontinuous with respect to the aqueous and the lipid regions can be considered as organic analogues of zeolites. If such cubic phases can be dispersed in water in the form of colloidal particles with a highly ordered internal structure, they are expected to be useful for a variety of applications, for example as drug carriers or bioreactors. Gustafsson et al. demonstrated that the cubic phases of monoglycerides/Poloxamer 407 ($PEO_{98}PPO_{67}PEO_{98}$)/water system can be dispersed into submicron particles, which they termed "cubosomes".[4,5] Technological applications of the lipid assemblies may be possible through stabilization of the nonlamellar architecture. It has been demonstrated that polymerization is an effective way to stabilize self-organized lipid phases,[6,7] including Q_{II}[8] and H_{II}[9,10] phases. The goal of this research is to stabilize cubosomes through suitable polymerization reactions, thereby expanding the temperature and concentration range over which they can be used. Here, the phase behavior of a polymerizable heterobifunctional monoacylglycerol (MAG), a readily synthesized lipid, has been studied. Certain compositions of MAG/water plus cross-linking monomers yield a bicontinuous cubic phase, which can be dispersed into cubosomes in water using Poloxamer 407. The polymerization of the hydrated MAG in the lipid region successfully stabilized the cubosome nanoparticles. The lipid used for these studies was 3-(2,4,13-(*E*,*E*)-tetradecatrienoyl)-*sn*-glycerol (**1**).



This novel heterobifunctional lipid was obtained from the acylation of 2,4,13-(*E*,*E*)-tetradecatrienoic acid with (*S*)-(+)-2,2-dimethyl-1,3-dioxalane-4-methanol, followed by deprotection of the isopropylidene protecting group. The 2,4,13-(*E*,*E*)-tetradecatrienoic acid was accessible in three steps from commercially available 9-decen-1-ol, which was oxidized to the corresponding aldehyde under Swern oxidation conditions. The Wittig−Horner reaction of this aldehyde with trimethyl 4-phosphonocrotonate gave methyl-2,4,13-tetradecatrienoate. Hydrolysis of this methyl ester using KOH/methanol affords the desired acid. The stereochemical purity was determined by ^{1}H NMR spectroscopy. The product mixture of (*E*,*E*) and (*E*,*Z*)-isomers were purified by urea inclusion complexation to provide the pure (*E*,*E*)-isomer.

The characteristic physical properties of the inverted bicontinuous cubic phase include a high viscosity and transparency to light, whereas the sample is totally dark when viewed between crossed polarizers. A sample of lipid **1** with 25 wt % water remained opaque after incubation at room temperature for a week. This is indicative of an anisotropic phase, most probably a lamellar liquid crystalline phase, due to the fact that lipid **1** does not have a large enough hydrophobic portion to attain sufficient curvature at the water−hydrocarbon interface, which is necessary for the change from lamellar to cubic structure.[11] A cross-linking monomer was introduced into the system, which localizes in the hydrocarbon region, to force the formation of cubic phase. Increasing amounts of this cross-linking monomer, divinyl benzene, was added to lipid **1** and the phase behavior of the mixtures studied. A hydrated 9/1 molar ratio mixture of lipid **1** and divinyl benzene at the concentration of 25 wt % water became clear after incubation at room temperature for 1 day. This isotropic character of the sample was confirmed by examination with cross-polarized light. The clear cubic gel was stable up to at least 45 °C.

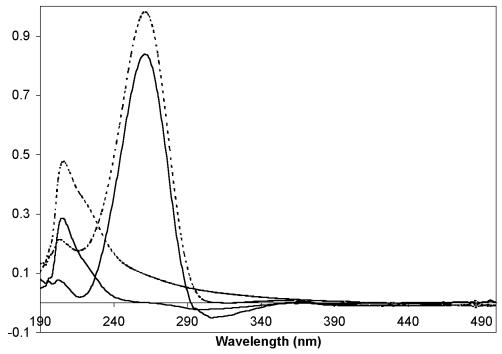


Figure 1. Absorption spectra of the cubosomes before and after polymerization. Regular lines show the absorption before and after sample exposure to UV light from a high-pressure Hg/Xe lamp. Dotted lines show absorption before and after redox polymerization.

The cubic gel was dispersed into nanoparticles by ultrasonication in the presence of Poloxamer 407 ($PEO_{98}PPO_{67}PEO_{98}$) as the dispersing agent. All dispersions had a water content of 95 wt %. The average diameter of the cubosomes was 300 nm as determined by quasi-elastic light scattering (QELS). The UV spectrum of the hydrated lipid shows the dienoyl absorption peak at 262 nm (ϵ 2.87 $\times$ 10^4 M^{-1} cm^{-1}). UV polymerization of the cubosomes was achieved by using 2 mol % of 4-(dimethylamino)benzophenone as a photoinitiator. A sample was irradiated at 20 °C with a high-pressure Hg/Xe lamp, resulting in essentially complete conversion of the dienoyl group within 3 h (Figure 1). A separate redox polymerization was performed at room temperature with a 1:1:1 molar ratio of [monomer]/$K_2S_2O_8$/$NaHSO_3$. This redox chemistry generates hydroxyl radicals, which can diffuse across the lipid layers and initiate the polymerization of both reactive groups regardless of the location. High conversion (95%) of the dienoyl group was achieved within 1 day (Figure 1). The nature of the resulting polymers, linear or cross-linked, was studied by particle dissolution

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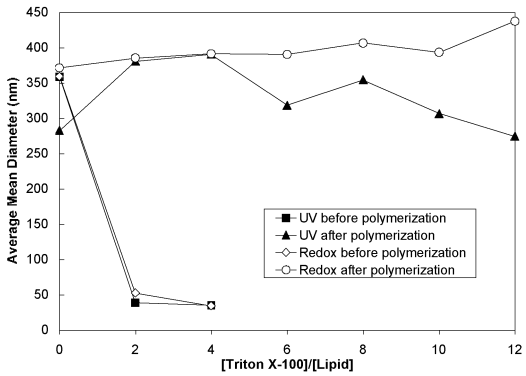

Figure 2. Stability test of polymerized cubosomes vs surfactant (Triton X-100). The average of mean diameters of various polymerized and unpolymerized cubosomes, determined by QELS is shown as a function of the molar ratio of Triton X-100 to lipid.

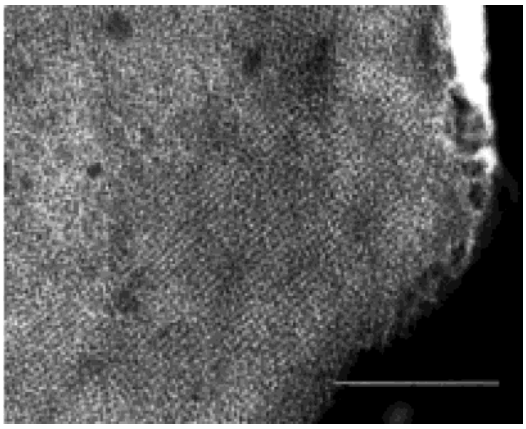

Figure 3. TEM of a portion of a cross-linked cubosome. The sample was stained with phosphotungstic acid to enhance the contrast between the lipid and water domains. The scale bar is 100 nm in length.

with the surfactant Triton X-100 (Figure 2). Surfactant-lysis of lipid particles yields mixed micelles that are much smaller than the particles; therefore, the dissolution of particles can be detected by QELS. Previous studies have demonstrated that cross-linked lipid vesicles were stable in the presence of excess surfactant, whereas unpolymerized or linearly polymerized vesicles are dissolved.[12,13]

As shown in Figure 2, 2−4 equiv of Triton X-100 per lipid was sufficient to significantly decrease the average of mean diameters of the suspended unpolymerized particles, indicating the dissolution of these cubosomes. The fact that polymerized cubosomes remain unchanged in size upon addition of up to 12 equiv of Triton X-100 clearly demonstrates the cross-linked nature of the structure in these materials. Transmission electron micrographs (TEM) of cubosomes in the presence of 7.4 wt % Poloxamer 407 are shown in Figure 3. The sample was diluted to a water content of 99 wt %, and negative stained with phosphotungstic acid. In the TEM the stained regions were ascribed to aqueous regions or channels. The apparent diameter of the channels in the micrograph was 3 ± 1 nm. To study the structure of the cubosome before and after polymerization, ^{2}H NMR spectra of cubosomes formed from partially deuterated **1** were obtained at room temperature in the presence of 7.4 wt % Poloxamer 407.[14] Provided the local environment of the lipid does not change significantly upon polymerization, a cubic phase should theoretically exhibit a characteristic sharp singlet peak, due to its isotropic nature.[15] An isotropic signal with a full-width at half-maximum of 0.5 ppm was obtained from cubosome sample prior to polymerization. UV polymerization of the NMR sample resulted in a progressive broadening of the original isotropic signal, an expected consequence of the decreased lateral diffusion of the polymerized lipids. This provides strong evidence that the cubosome structure is preserved upon polymerization.

In summary, we have demonstrated that stabilized bicontinuous cubic nanoparticles (stabilized cubosomes) can be readily prepared via cross-linking polymerization of cubosomes prepared from reactive amphiphiles. The ability to form stable biocompatible nanoparticles with interpenetrating water channels of high internal surface area provides opportunities for the sequestration and release of relatively large molecules from these novel nanoparticles. In this regard, Jeong et al. have recently shown that macromolecules of ca. 3 nm in diameter can diffuse in the water channels of the bicontinuous cubic phase formed from a MAG, such as monoolein as well as in those of a polymerizable MAG.[16]

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