

Effects of Sonication on the Lamellar Structures of L- α -Dipalmitoyl Phosphatidylcholine(DPPC)/Saccharide/Water Systems

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The effects of sonication, conducted prior to dehydration by heat drying, on the multilamellar vesicles of L- α -dipalmitoyl phosphatidylcholine (DPPC), DPPC/glucose, DPPC/trehalose or DPPC/maltose systems were examined by differential scanning calorimetry (DSC) and powder X-ray diffraction (XRD). The results were compared with those for the corresponding unsonicated and DPPC systems without saccharide. In the DPPC/glucose system, no clear differences between the unsonicated and sonicated systems were found because glucose did not prevent fusion of vesicles by dehydration. DSC showed one sharp peak at the gel-liquid crystal transition temperature (T_c) of 43 °C, indicating that glucose was distributed homogeneously between the DPPC bilayers of the vesicles. Subcells formed by hydrocarbon chains of DPPC changed from the hexagonal gel (L_{β}) to the hexagonal liquid crystal (L_{α}) form at T_c with an increase in temperature, essentially as noted for DPPC systems except for differences in T_c . In the DPPC/disaccharide system, the unsonicated and sonicated systems were clearly different. DSC and XRD of the unsonicated system consistently showed transition from a gel to a liquid crystal state over a wide temperature range, while for the sonicated system, there was only a sharp peak on the DSC curve. The thermal behavior of DPPC/disaccharide systems may be explained as follows. Although disaccharide is distributed homogeneously between the bilayers of multilamellar vesicles, interactions with DPPC depend on the surface curvature of the bilayer. Heating of multilamellar vesicles may possibly result in transition from a gel to a liquid crystal phase since multilamellar vesicles consist of many bilayers differing considerably in their surface curvature, in contrast to sonicated unilamellar vesicles which possess a definite curvature.

Key words DPPC; saccharide; sonication; gel-liquid crystal transition temperature

Various disaccharides prevent the fusion of vesicles and leakage of material present within vesicles during freeze-drying.^{1,2} Disaccharides, such as trehalose and maltose, effectively stabilize liposomes during freeze-drying.³⁻⁵ Glucose prevents the fusion of liposomes but not leakage of material from them while both are prevented by trehalose and maltose.⁶ These effects are thought to be due to the reduction in gel-liquid crystal transition temperature (T_c).⁷

The thermal behavior of a DPPC/saccharide mixture prepared by freeze-drying,^{3,6,8-10} air-drying¹¹ and heat-drying¹²⁻¹⁴ was examined by IR, differential scanning calorimetry (DSC) and powder X-ray diffraction (XRD). Differences in the thermal behavior were found to depend on whether the system consisted of unilamellar or multilamellar vesicles. In the case of unilamellar vesicles, T_c was 75 °C at the first scan but 24 °C at the second.⁸ This drop may be explained by the relationship between the lipid T_c and the glass transition temperature (T_g), of saccharides. Koster *et al.* noted that the lipid T_c in a lipid/saccharide mixture will fall provided it is less than the T_g of the saccharide.¹⁵ Crowe *et al.* considered that glass formation of the saccharide and its direct interaction with lipid was required for reduction in the lipid T_c in a lipid/saccharide mixture.¹¹ Multilamellar vesicles of heat-dried¹² and freeze-dried¹⁰ L- α -dipalmitoyl phosphatidylcholine (DPPC)/trehalose mixtures showed two peaks at 24 °C and 70 °C in the first and second scans. In a heat-dried DPPC/saccharide system involving multilamellar vesicles, DPPC/di or a trisaccharide system, two peaks were noted at 24 °C and 70 °C at a low water content but, for the DPPC/monosaccharide system, there was only one peak at 42 °C.¹³ By XRD, in multilamellar vesicles of a DPPC/trehalose mixture prepared by heat-drying, the transition from the gel (L_{β})

to liquid crystal (L_{α}) phase was found to occur between the two transition temperatures.¹⁴ Such a transition was not seen in the DPPC/monosaccharide system, although one endothermic peak was noted.

The stoichiometric relationship between trehalose and DPPC was determined using multilamellar DPPC/trehalose vesicles at various molar ratios, prepared by heat-drying under vacuum.¹² and showed a homogeneous distribution of saccharide between the bilayers. Crowe *et al.* reported that multilamellar vesicles always exhibit multiple transitions that are due to the inhomogeneous distribution of saccharide in the multilamellar vesicles.⁸ In the case of a lipid/saccharide mixture of multilamellar vesicles, the following questions need answered: does the saccharide distribute homogeneously between bilayers of multilamellar vesicle, why do successive transitions occur in the DPPC/disaccharide and why do such successive transitions not occur in the DPPC/monosaccharide. Multilamellar vesicles of heat-dried¹² and freeze-dried¹⁰ DPPC/trehalose mixtures showed two transition temperatures while unilamellar vesicles of the DPPC/trehalose system showed only one endothermic peak. The difference in the thermal behavior of these systems might markedly depend on the liposomal size of these systems.

To clarify the effect of liposomal size on the thermal behavior of the DPPC/saccharide system, the DPPC/saccharide system was sonicated. The effects of sonication conducted prior to dehydration by heat drying, on multilamellar vesicles of DPPC, were examined for unsonicated and sonicated DPPC, DPPC/glucose, DPPC/trehalose and DPPC/maltose systems by DSC and XRD. Whether saccharide is distributed homogeneously between the bilayers of multilamellar vesicles and successive transitions occur (described in Ref. 14),

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are matters to be discussed below based on a comparison of data for unsonicated and sonicated lipid/saccharide systems.

Experimental

Materials and Sample Preparation DPPC, glucose (GLU), trehalose (TRE) and maltose (MAL) were purchased from SIGMA and used without further purification. Water was purified using Milli-Q Labo (Millipore Ltd.).

DPPC (100 mg) in chloroform in a round-bottomed flask was dried at 20 °C by evaporation and dried further for 12 h at 60 °C under vacuum to obtain dried filmy DPPC. This film was hydrated with either 4 ml water or a saccharide solution at a molar ratio to DPPC of 2.6. Dispersion was equilibrated for 3 h at 60 °C with shaking. The dispersion was white and turbid, indicating multilamellar vesicle formation. Half the dispersion was transferred to a round-bottomed flask. One of the flasks was further equilibrated for 3 h at 60 °C with shaking while the other was sonicated under the same conditions in a bath sonicator Model 100Z (Kaijo Electric Co.). Sonication was carried out at 55 W. All unsonicated samples, with or without saccharide, were still white and turbid, while the sonicated samples became faintly opalescent or semitransparent pale blue, indicating that unilamellar vesicles had formed⁸⁾ and hence the vesicle particle size was smaller than that of the unsonicated particles. The unsonicated and sonicated samples are hereafter referred to as the 'unsonicated DPPC/saccharide dispersion' and the 'sonicated DPPC/saccharide dispersion,' respectively. Water in the samples was removed by evaporation at 45 °C and all samples were heated to 90 °C to obtain the powder and cooled to room temperature in air. Below, they are referred to as the 'sonicated DPPC/saccharide powder' and 'unsonicated DPPC/saccharide powder', respectively. The samples were transferred to an aluminum pan for DSC or a capillary for XRD and sealed immediately with a sealant or a flame. Water content of the powder was determined by the Karl Fischer method using a moisture meter, CA-06 (Mitsubishi Kasei Corp.).

Differential Scanning Calorimetry (DSC) T_c was computed from the onset temperature of the DSC curve, using a Rigaku DSC 8240D and TAS 200 thermal analysis system (Rigaku Corp.). The heating rate was 12 °C/h from 20 °C to 80 °C. The second scan was carried out immediately following the first, using Alumina as the reference.

Powder X-Ray Diffraction (XRD) XRD was recorded between 20 °C and 70 °C using a RINT 1400 X-ray diffractometer (Rigaku Corp.) at 60 kV and 200 mA, at 1°/min from a diffraction angle of 2.5° to 40° (2 θ) at various temperatures obtained using hot air. The temperature stability was ± 1 °C. Each X-ray capillary was 2.0 mm in internal diameter. Assuming that there was an asymmetric peak with a small peak at the lower angle side actually consisting of two symmetric peaks, peak fitting using the latter was carried out with Rigaku analysis system software, after background signals had been eliminated. The intensity, half-width, d-value and Gauss ratio (ratio of Gauss function to pseudo Voigt function) parameters were then determined. To measure the temperature change in each peak numerically, the d-value, half-width and relative intensity were used. The relative intensity was the ratio to the intensity of the higher angle peak at the lowest temperature.

Liposomal Size This parameter was found by dynamic light scattering using a Photal laser particle analyzer, Model ELS-800 (Otsuka Electronics), by means of which a particle diameter of 5 nm to 3 μ m can be determined. To measure the liposomal size of the unsonicated and sonicated DPPC dispersions, with and without saccharide, each dispersion was diluted with water purified by Milli-Q Labo. All measurements were performed at 25 °C. The rehydrated sonicated DPPC system, with and without saccharide, was produced by swelling a powdery sample in water for 10 min at 60 °C. The rehydrated sample is the "R-sonicated DPPC/saccharide system" in this study. Other R-sonicated DPPC/saccharide systems were sonicated for 10 sec at 60 °C subsequent to swelling for 10 min at 60 °C to ensure dispersion of DPPC or DPPC/saccharide powder in water, hereafter referred to as the "RS-sonicated DPPC/saccharide system."

Results

Thermal Behavior by DSC DSC endothermic curves for unsonicated and sonicated DPPC powders, with and without saccharide, are presented in Fig. 1. Table 1 shows the transition temperatures of unsonicated and sonicated DPPC/saccharide powders. The DSC thermograms in the first and second scannings were essentially the same (data not shown).

The T_c of the sonicated DPPC powder was 5.4 °C less than that of the unsonicated powder (Table 1) while the T_c of the two

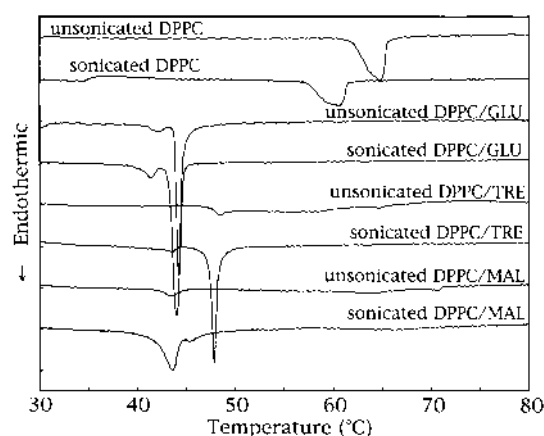


Fig. 1. DSC Curves of DPPC, DPPC/GLU, DPPC/TRE and DPPC/MAL Powders

The heating rate was 0.2 °C/min from 20 °C to 80 °C.

Table 1. Transition Temperature of the Unsonicated and Sonicated DPPC/Saccharide Powders

	System	Water content (%)	T_c (°C)	T_H (°C)
DPPC	Unsonicated	5.2	62.4	
	Sonicated	6.0	57.0	
DPPC/GLU	Unsonicated	6.5	43.8	
	Sonicated	6.2	43.2	
DPPC/TRE	Unsonicated	8.1	47.2	64.3
	Sonicated	8.3	47.4	
DPPC/MAL	Unsonicated	7.5	42.2	70.0
	Sonicated	7.8	41.8	

T_c is the gel-liquid crystal transition temperature. T_H is the higher transition temperature.

DPPC/water systems^{13,16)} was 64.5 °C at 4.8 wt% and 63 °C at 5.4% water. The values for the unsonicated DPPC powders were essentially the same as in the literature.^{13,16)} The difference in T_c might be due to the water content of the powders. Thus, the effects of sonication on the peak parameters was very slight although differences in T_c were evident.

The unsonicated and sonicated DPPC/GLU powders showed endothermic peaks at 43.8 °C and 43.2 °C, respectively (Table 1), although that was also a small peak on the lower temperature side. The origin of these small endothermic peaks was not determined. Compared with the DPPC systems without saccharide, GLU caused the transition temperature to fall by about 16 °C, while sonication had virtually no effect on the transition temperature.

For unsonicated DPPC/TRE powder, small endothermic peaks at 47.2 °C and 64.3 °C were noted (Table 1), and the base-line had changed (Fig. 1). These findings were consistent with those for the heat-dried DPPC/trehalose mixture.¹⁴⁾ The sonicated DPPC/TRE powder showed a relatively sharp endothermic peak (47.4 °C) at the same lower temperature as the peak for the unsonicated DPPC/TRE powder (Table 1). Thus, in the case of DPPC/TRE powder, sonication clearly effects thermal behavior.

The thermal behavior of the unsonicated DPPC/MAL

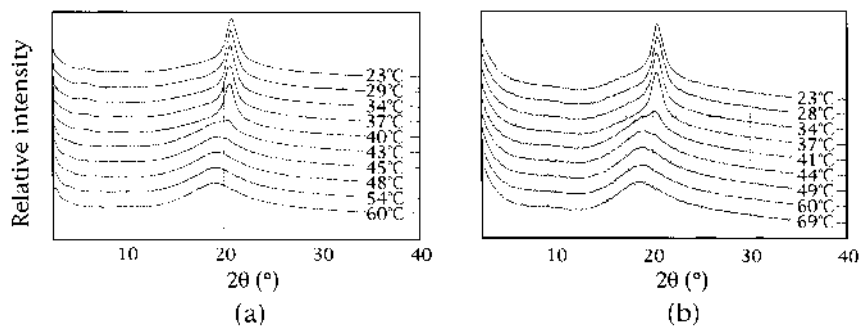


Fig. 2. Temperature Change in XRD of (a) Unsonicated and (b) Sonicated DPPC/GLU Powders

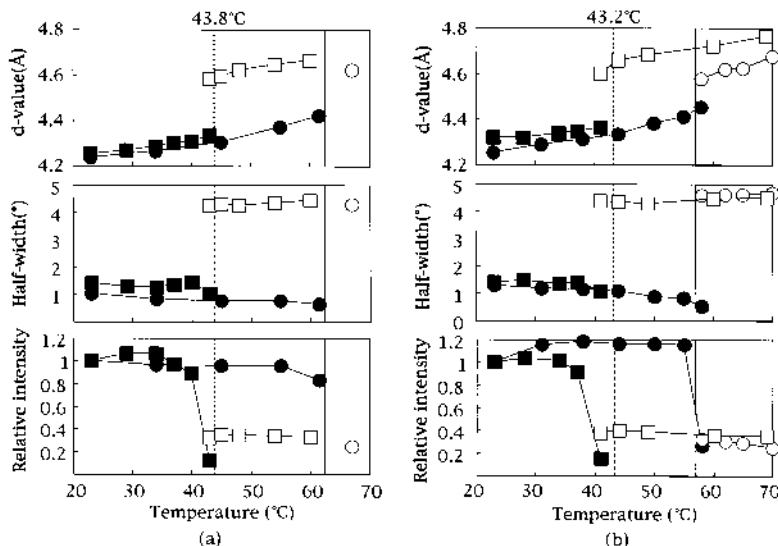


Fig. 3. Peak Parameters of (a) Unsonicated and (b) Sonicated DPPC/GLU Powders

Squares and circles indicate DPPC/GLU and DPPC powders, respectively. Dotted lines indicate the T_c of the DPPC/GLU powder obtained from DSC. Solid lines indicate the T_c of the DPPC powder. \square , peak parameter of lower angle peak; \blacksquare , peak parameter of higher angle peak; \bullet , peak parameter in gel phase of DPPC powder; \circ , peak parameter in liquid crystal phase of DPPC powder.

powder was similar to that of the unsonicated DPPC/TRE powder except for the transition temperature. For the sonicated DPPC/MAL powder, a sharp lower temperature transition was seen as for DPPC/TRE powder. Sonication, thus, also clearly has an effect on the transition temperature of DPPC/MAL powder.

Phase Behavior by XRD XRD of DPPC/Glucose Powder: Figure 2 shows the temperature change in the XRD of unsonicated and sonicated DPPC/GLU powder. The DPPC/GLU for each showed a symmetric diffraction peak at 4.2 Å ($2\theta=21^\circ$) and 23°C, which slightly shifted to a lower 2θ angle with an increase in temperature. The peak became a broad symmetric diffraction peak at 4.6 Å ($2\theta=19^\circ$) at 45°C and 44°C, respectively, for the two powders and a symmetric diffraction peak at 4.2 Å and 4.6 Å indicated the gel phase with a hexagonal subcell (L_β) and a liquid crystal phase (L_α), respectively. The asymmetric diffraction peak at 43°C for unsonicated powder and 41°C for sonicated powder indicated the gel and liquid crystal phases coexisted in the powder. The temperature change in XRD of unsonicated and sonicated DPPC powders was similar to that of the unsonicated and sonicated DPPC/GLU except for the transition temperature from the gel to the liquid crystal phase. Above 70°C, for the unsonicated DPPC powder, and 62°C, for the sonicated

DPPC powder, these systems were in the liquid crystal phase. The change in the subcell of the unsonicated DPPC powder was consistent with that reported previously for the heat-dried DPPC system.¹⁴⁾

Figure 3 shows the temperature change in XRD parameters of the two DPPC/GLU powders (\blacksquare and \square). The transition temperature based on DSC is shown by the dotted line and temperature change in XRD parameters of the two DPPC powders, without saccharide, is also shown in Fig. 3 (\circ and \bullet). DPPC data without saccharide were plotted for comparison as the solid line. It is known that the d-value and half-width increase discontinuously and the relative intensity decreases discontinuously at the gel-to-liquid crystal transition temperature.¹⁴⁾ For either DPPC powder, the endothermic peak deriving from DSC represents the T_c since d-value and half-width increased and the relative intensity decreased discontinuously at this endothermic peak. Compared with the DPPC powders, it was concluded that 43.8°C and 43.2°C for the DPPC/GLU powders were the T_c of DPPC and the gel phase existing below T_c and the liquid crystal phase above T_c . Subcells of unsonicated and sonicated DPPC/GLU powder changed from the hexagonal (L_β) to the extended hexagonal (L_α) form. Sonication had virtually no effect on the changes in peak parameters.

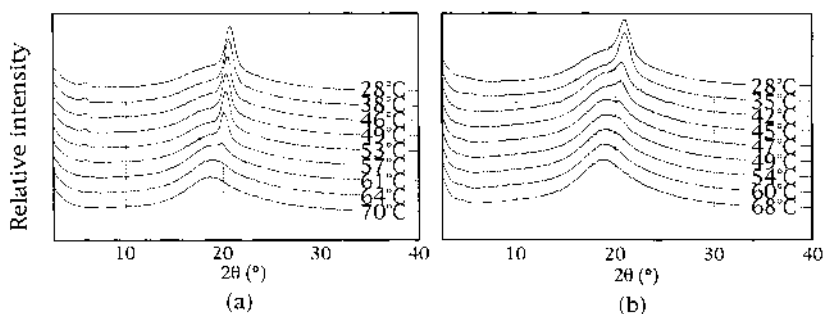


Fig. 4. Temperature Change in XRD of (a) Unsonicated and (b) Sonicated DPPC/TRE Powders

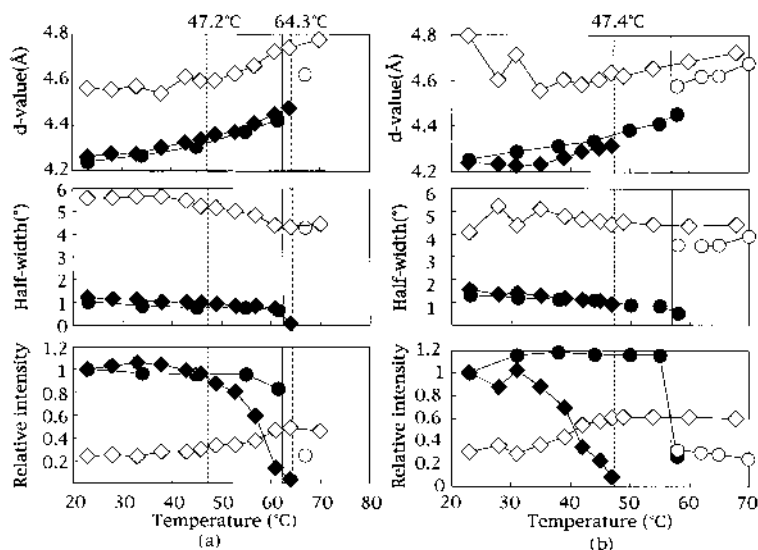


Fig. 5. Peak Parameters of (a) Unsonicated and (b) Sonicated DPPC/TRE Powders

Rhombi and circles indicate DPPC/TRE and DPPC powders, respectively. Dotted lines indicate the T_c of the DPPC/TRE powder obtained from DSC. Solid lines indicate the T_c of the DPPC powder. ◇, peak parameter of lower angle peak; ◆, peak parameter of higher angle peak; ●, peak parameter in gel phase of DPPC powder; ○, peak parameter in liquid crystal phase of DPPC powder.

XRD of DPPC/Trehalose Powder: Figure 4 shows the XRD at various temperatures for unsonicated and sonicated DPPC/TRE powder. The XRD for each showed an asymmetric peak with a broad peak at the lower angle side at room temperature. Above 64 °C and 49 °C, in the case of the unsonicated and sonicated powders, a broad symmetric peak could be seen, indicating that both powders were in the L_{α} phase.

Figure 5 shows the change in XRD parameters of the two powders. The transition temperature based on DSC appears as a dotted line. The temperature change in d-value and the half-width of the higher angle peak (◆ in Fig. 5) were consistent with that of the gel phase of the DPPC powder (● in Fig. 5). The d-value and half-width of the lower angle peak (◇ in Fig. 5) were slightly greater but the differences were relatively small compared with those of the liquid crystal phase of the DPPC powder (○ in Fig. 5). This indicates that the DPPC/TRE powder is in the liquid crystal phase (L_{α}), above 64.3 °C for the unsonicated powder and 47.4 °C for the sonicated powders. The transition temperature of 64.3 °C and 47.4 °C, for unsonicated and sonicated powders is therefore the T_c since the d-value and half-width of the higher angle peak disappeared at this temperature. The gel and liquid crystal phases, thus, coexist in the unsonicated and sonicated

DPPC/TRE powders below 64.3 and 47.4 °C, respectively. The relative intensity of the lower angle peak increased gradually then reached a plateau constant above 64.3 °C for the unsonicated powder and 47.4 °C for the sonicated powder. Sonication, consequently, causes a reduction of 17 °C in the temperature of transition to L_{α} . The present data for unsonicated powder are in agreement with those for the heat-dried DPPC/TRE system reported previously,¹⁴⁾ in which two endothermic peaks were observed and the transition from gel-to-liquid crystal phases was found to take place between the two transition temperatures. The transition from the gel-to-liquid crystal phase should occur between these temperatures (47.2 and 64.3 °C) for unsonicated DPPC/TRE powder. Subcells of unsonicated and sonicated DPPC/TRE powders changed from the non-hexagonal to the extended hexagonal (L_{α}) form.¹³⁾ Thus, sonication has an effect on the change in these parameters.

XRD of DPPC/Maltose Powder: Figure 6 shows the XRD at various temperatures of the unsonicated and sonicated DPPC/MAL powder, and it was noted that there was an asymmetric peak with a broad peak at the lower angle side at room temperature. Above 70 °C and 47 °C, for unsonicated and sonicated powder, respectively, a broad symmetric peak was noted, indicating the two powders to be in L_{α} .

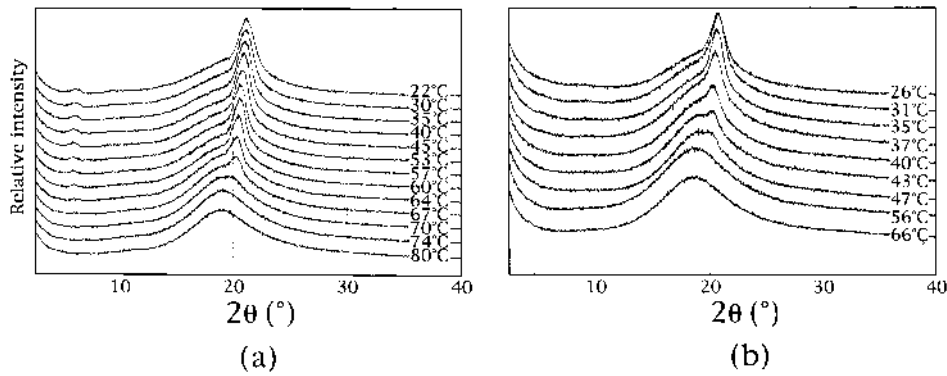


Fig. 6. Temperature Change in XRD of (a) Unsonicated and (b) Sonicated DPPC/MAL Powders

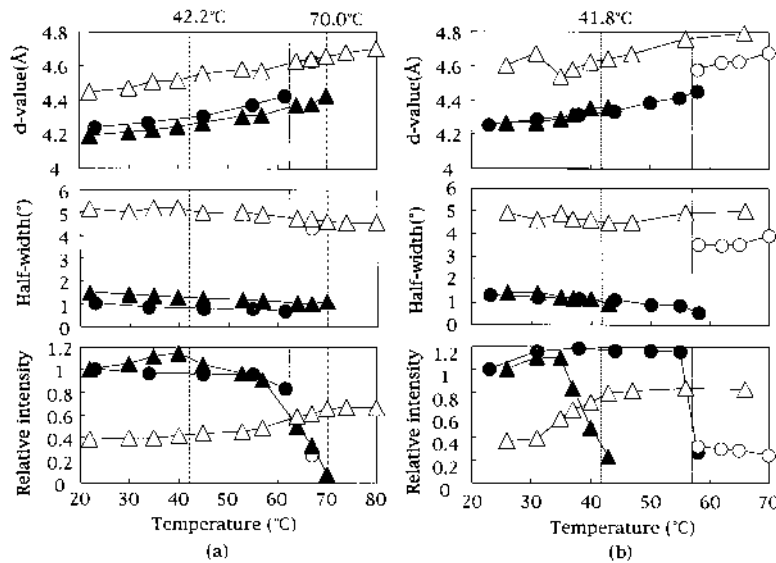


Fig. 7. Peak Parameters of (a) Unsonicated and (b) Sonicated DPPC/MAL Powders

Triangles and circles indicate DPPC/MAL and DPPC powders, respectively. Dotted lines indicate the T_c of the DPPC/MAL powder obtained from DSC. Solid lines indicate the T_c of the DPPC powder. Δ , peak parameter of lower angle peak; \blacktriangle , peak parameter of higher angle peak; \bullet , peak parameter in gel phase of DPPC powder; \circ , peak parameter in liquid crystal phase of DPPC powder.

Figure 7 shows the change in XRD parameters of the two powders. The transition temperatures based on DSC are indicated by the dotted lines. The transition temperature T_c was 70.0 °C for the unsonicated and 41.8 °C for the sonicated DPPC/MAL powder. Sonication, thus, reduced the T_c by 28 °C. The relative intensity of the lower angle peak increased gradually then remained constant above 70.0 °C for the unsonicated and 41.8 °C for the sonicated powder. The data for unsonicated powder were consistent with those of the heat-dried DPPC/MAL system reported previously.¹⁴⁾ The transition from the gel to liquid crystal phase should occur between the two transition temperatures (42.2 and 70.0 °C) for unsonicated DPPC/MAL powder, as noted for unsonicated DPPC/TRE powder. The subcells of unsonicated and sonicated DPPC/MAL powder changed from the non-hexagonal to the extended hexagonal (L_{α}) form,¹³⁾ thus showing that sonication has an effect on the change in parameters.

Liposomal Size To clarify the liposomal features of dried powder, the liposomal size of the dispersion before drying and after rehydrating the dried powder was examined. Figure 8 shows the size distribution in liposome weight, with

and without saccharide. The mean diameter and particle width are shown in Table 2. The value of the former for unsonicated DPPC, DPPC/GLU, DPPC/TRE and DPPC/MAL was 29.7, 35.7, 7.79 and 44.0 μm , respectively (Table 2-1). The cumulative weight (%) less than 1 μm for unsonicated DPPC, DPPC/GLU, DPPC/TRE and DPPC/MAL was 0, 0, 68.5 and 0.9%, respectively, (Fig. 8-A1-D1) indicating that TRE produces vesicles smaller than other saccharides.

The size distribution of the unsonicated DPPC and DPPC/saccharide dispersion was the same as that noted for rehydrated unsonicated samples (data not shown).

The particle size in the R-sonicated DPPC system was 1.51 and 44.0 μm (Table 2-A3), this (Fig. 8-A3) being the same as that observed (Fig. 8-A1) for the unsonicated DPPC dispersion (1.75 and 29.9 μm in diameter). The similarity between the unsonicated dispersion (Fig. 8-A1) and the R-sonicated system (Fig. 8-A3) suggests that dehydration causes fusion of the vesicles in the absence of saccharide. The similarity in the R-sonicated (Fig. 8-A3) and RS-sonicated systems (Fig. 8-A4) indicates that sonication for 10 seconds at 60 °C in the R-sonicated DPPC system causes hardly any change in the particle distribution.

Table 2. Mean Diameter (μm) of Size Distribution

No.	System	DPPC (A)	DPPC/GLU (B)	DPPC/TRE (C)	DPPC/MAL (D)
1	Unsonicated dispersion	1.75 [0.26]	1.66 [0.35]	0.832 [0.358]	1.36 [0.39]
		29.9 [6.3] (29.7 [6.7])	43.8 [5.8] (35.7 [1.7])	41.7 [7.2] (7.79 [15.6])	46.1 [4.9] (44.0 [10.6])
2	Sonicated dispersion	0.089 [0.010]	0.015 [0.003]	0.051 [0.011]	0.020 [0.003]
		0.203 [0.037]	0.047 [0.019]	0.315 [0.080]	0.060 [0.027]
		0.442 [0.10]	0.277 [0.10]	5.93 [0.63]	0.525 [0.24]
		1.20 [0.062] (0.27 [0.16])	5.18 [0.64] (0.229 [0.82])	0.797 [1.8]	0.303 [0.30]
3	R-Sonicated system	1.51 [0.31]	1.30 [0.34]	0.071 [0.042]	0.044 [0.006]
		44.0 [6.3]	45.8 [5.4]	1.28 [0.26]	0.600 [0.11]
		44.9 [6.6]	44.5 [9.1]	44.5 [5.8] (42.7 [10])	4.26 [1.4]
					25.5 [9.1] (16.9 [13])
4	RS-Sonicated system	1.03 [0.26]	0.502 [0.076]	0.044 [0.007]	0.049 [0.013]
		3.31 [0.58]	1.89 [0.53]	0.861 [0.16]	0.178 [0.062]
		47.7 [4.4]	48.2 [4.2]	3.88 [0.85]	1.93 [0.31]
		45.3 [10]	38.6 [19]	2.52 [1.9]	32.4 [4.1] (20.0 [15])

Shown in Fig. 8, of the unsonicated (1) and sonicated (2) DPPC/saccharide dispersions and R-sonicated (3) and RS-sonicated (4) DPPC/saccharide systems. The width of the distribution is in brackets and the total mean diameters are in parenthesis.

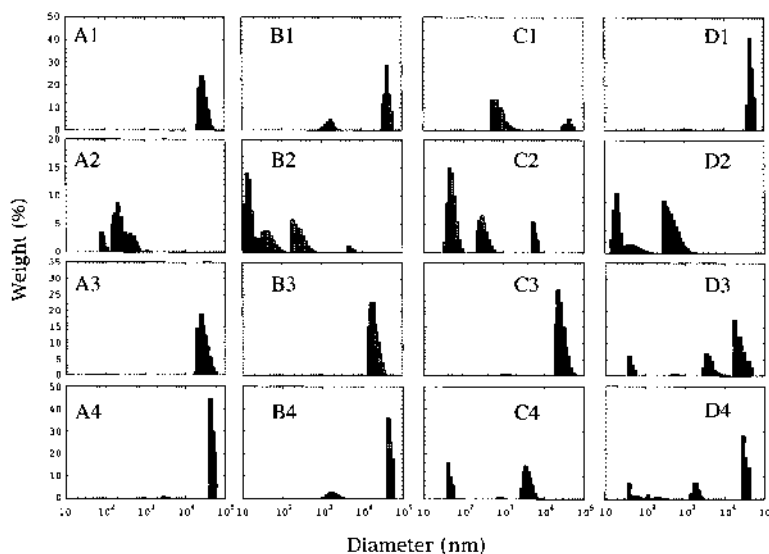


Fig. 8. Weight-Averaged Size Distributions of the Unsonicated (1) and Sonicated (2) Dispersions, with and without Saccharide, and R-Sonicated (3) and RS-Sonicated (4) Systems, with and without Saccharide

A, B, C and D are the DPPC, DPPC/GLU, DPPC/TRE and DPPC/MAL systems, respectively. Symbols correspond to those in Table 2.

The particle size in the RS-sonicated DPPC/GLU system was 0.502, 1.89 and 48.2 μm and this distribution (Fig. 8-B4) was essentially the same as that (Fig. 8-B1) for the unsonicated DPPC/GLU dispersion (1.66 μm and 43.8 μm in diameter). GLU, thus, does not prevent fusion of the vesicles during heat-drying.

The mean diameter (42.7 μm) in the R-sonicated DPPC/TRE system (Fig. 8-C3) differed from that (7.79 μm) for the unsonicated DPPC/TRE dispersion (Fig. 8-C1). Sonication in the R-sonicated DPPC/TRE system clearly causes a change in size distribution (from Fig. 8-C3 to Fig. 8-C4). By this light sonication, the size distribution approached that of the sonicated dispersion (Fig. 8-C2) for which peaks at

0.051, 0.315 and 5.93 μm in diameter were observed. It, thus, follows that TRE prevents the fusion of vesicles during heat-drying.

The particle size in the R-sonicated DPPC/MAL system (Table 2-D3), for which peaks at 0.044, 0.600, 4.26 and 25.5 μm were noted, differed from that for the unsonicated (1.36 and 46.1 μm in diameter) and the sonicated (0.020, 0.060 and 0.525 μm in diameter) DPPC/MAL dispersion. Sonication of the R-sonicated DPPC/MAL system led to no significant change in particle distribution and, accordingly, the sonicated sample is dispersed by swelling in the DPPC/MAL system. The similarity to the rehydrated systems (Fig. 8-D3 and D4) suggests that MAL partially prevents the fusion of vesicles

during heat-drying.

Discussion

In the DPPC/water system without saccharide, dehydration such as caused by freeze-drying causes unilamellar liposomes to become multilamellar ones due to fusion.¹⁷⁾ Comparing the R- and RS-sonicated DPPC systems (Fig. 8-A3 and A4) with the DPPC dispersion (Fig. 8-A1), heat-drying appears to cause fusion of the liposomes. Comparing unsonicated and sonicated DPPC powder, subcells composed of hydrocarbon chains of DPPC were noted to change at T_c from the hexagonal subcell (L_{β}) to the extended hexagonal subcell (L_{α}), which was also observed in the dried DPPC system.¹⁴⁾ In the hydrated DPPC/water system,¹⁸⁾ a non-hexagonal (L_{β}) to hexagonal (L_{α}) change was seen with an increase in temperature, with the transition to the hexagonal subcell (P_{β}) form intervening. The change in subcell form of the dried DPPC system was not the same as that noted for hydrated DPPC.

The size distribution in the RS-sonicated DPPC/GLU system (38.6 μm , B4 in Table 2 and Fig. 8-B4) was essentially the same as that for the unsonicated DPPC/GLU dispersion (35.7 μm , B1 in Table 2 and Fig. 8-B1) and, thus, GLU would be unlikely to prevent fusion of the liposomes. The prevention by GLU of DPPC liposome fusion during heat-drying differs from that during freeze-drying since GLU prevents fusion during freeze-drying.⁶⁾ The sharp endothermic peaks in DSC for unsonicated and sonicated powders indicate that GLU is distributed homogeneously between the bilayers of multilamellar vesicles. Otherwise, DPPC which does not interact with saccharide would cause other transitions at higher temperatures in contrast to DPPC which interacts with saccharide. The reason for this is that, when the DPPC and DPPC/GLU systems each have a water content less than 20%, the T_c of the DPPC system exceeds that of the DPPC/GLU system (see Fig. 2 in Ref. 13). No endothermic peaks for this were observed in the present study (see Fig. 1).

TRE prevents the fusion of small vesicles during freeze-drying.⁶⁾ The size distribution in the RS-sonicated DPPC/TRE system (2.52 μm , C4 in Table 2 and Fig. 8-C4) is basically the same as that for the sonicated DPPC/TRE dispersion (0.797 μm , C2 in Table 2 and Fig. 8-C2), suggesting that TRE prevents liposome fusion. TRE prevented the fusion of vesicles during heat-drying although the method of heat-drying differs from that for freeze-drying.

It is possible that there may be inhomogeneous distribution in the present system during sample preparation, since Gruner *et al.* reported that sucrose is likely to be excluded from multilamellar vesicles¹⁹⁾ and also that plurilamellar vesicles, *i.e.*, multilamellar vesicles prepared in a way different from that for conventional multilamellar vesicles, are well distributed throughout the solute itself. Trehalose has not yet to be shown to be excluded from multilamellar vesicles such as sucrose.

The stoichiometric relationship between DPPC in bilayers and TRE between bilayers was clarified using samples (multilamellar vesicles) at various molar ratios (TRE/DPPC).¹²⁾ Had TRE not been distributed homogeneously between bilayers, this relationship could not have been determined. Thus, in the present system, TRE may be considered to be

distributed homogeneously in vesicles. The effect of TRE on bilayer of vesicle differ from that on another.

In the case of homogeneous TRE distribution in vesicles, an explanation is needed for the broad endothermic peak in the DSC of unsonicated DPPC/TRE. In multilamellar vesicles, many bilayers are present, each with its own surface curvature. Should TRE be distributed homogeneously between the bilayers, it would interact with the bilayers in a manner peculiar to each surface curvature. Consequently, there would be many slightly different modes of interaction. Heat would alter these modes with a consequent transition of the gel to the liquid crystal phase. This explains the X-ray diffraction and DSC results for the unsonicated DPPC/TRE system, in which there is a transition from the gel to the liquid crystal phase between the two endothermic peaks.

Okamura *et al.* noted that the ¹⁴N-NMR spectra of hydrated DPPC liposomes depend markedly on the surface curvature and temperature.²⁰⁾ Liposomes with a diameter of 55 nm showed very broad signals below T_c , which became sharp with an increase in temperature. At more than 100 nm, no signals were detected and they concluded that the rotational motion of choline N⁺ in bilayers of hydrated DPPC liposomes is restricted in liposomes with a diameter more than 100 nm. Strauss has pointed out that only vesicles smaller than 80–100 nm give high-resolution NMR spectra in hydrated egg PC.²¹⁾ Restriction of the head group motion is probably relaxed in vesicles less than 100 nm in diameter and, thus, the vesicles would interact strongly with saccharide. Relaxed restriction of the head group of DPPC would, thus, cause liposomes of any diameter less than 100 nm to interact with TRE in the same manner. In sonicated DPPC/TRE dispersion, the weight % of liposomes with a diameter less than 100 nm was found to be 60% and, accordingly, such liposomes would give rise to sharp endothermic peaks (Fig. 1).

A phase diagram of DPPC/saccharide multilamellar vesicles could be roughly divided into two portions based on thermal behavior; for monosaccharide and for di- and trisaccharide, respectively.¹³⁾ The latter case showed two transitions which were sensitive to the water content at low water concentrations. For the former, one transition was observed whose temperature was largely constant. Disaccharide interacts with DPPC by hydrogen bonding¹¹⁾ and hydrogen bonding is strongly dependent on the orientation of the hydroxyl group. Consequently, the transition temperature of DPPC with disaccharide is sensitive to the water content at low water concentrations.^{12,13)} A change in the surface curvature may readily influence the interactions between disaccharide and DPPC since a change in the surface curvature will alter the orientation of the oxygen atoms of the surface phosphate groups.

Interactions between monosaccharide and DPPC were shown in this study to be affected by hydrophobic interactions, using the hydrophobic-rich side of the pyranose ring.²²⁾ A monosaccharide may interact hydrophilically with DPPC and by hydrogen bonding at the same time.²³⁾ Hydrophobic interactions between the pyranose ring of glucose and the choline methyl group were found to obey the molecular mechanism for a DPPC/glucose mixture model without water.²⁴⁾ This indicates that interactions between monosaccharide and phospholipid are independent of the surface cur-

vature.

For cases of a small weight % of liposomes with a diameter less than 100 nm, there are very broad peaks at the higher temperature and this supports the above suggestion that such liposomes cause sharp endothermic peaks.

The temperature change of DPPC hydrocarbon chains (Fig. 7) and the thermal behavior (Fig. 1) of unsonicated DPPC/MAL powder were essentially the same as for unsonicated DPPC/TRE powder although differences in transition temperatures were noted, as reported previously.¹⁴ The endothermic peak at 41.8 °C for sonicated DPPC/MAL powder was sharper than that for unsonicated DPPC/MAL powder, but the very broad peak at higher temperature remained. A comparison of the size distributions for the RS-sonicated DPPC/MAL system (Fig. 8-D4) and the sonicated DPPC/MAL dispersion (Fig. 8-D2) indicated that MAL partially prevented the fusion of liposomes during heat-drying. The weight % of sonicated DPPC/MAL dispersion was 44%, and so less than the 60% for the sonicated DPPC/TRE dispersion. This partial prevention of fusion in the DPPC/MAL system causes a reduction in the weight % (44%) of liposomes less than 100 nm in diameter and this, in turn, may give rise to a very broad peak (sonicated DPPC/MAL powder in Fig. 1) at a higher T_c . Disaccharide may have a similar effect on the phospholipid membrane but the number of liposomes less than 100 nm in diameter would cause the DPPC/disaccharide powders to behave in a slightly fashion.

Conclusions

Sonication on the DPPC and DPPC/GLU systems had virtually no effect on thermal behavior while, in the case of the DPPC/disaccharide system, sonication clearly did have an effect. Disaccharide (TRE and MAL) prevents the fusion of DPPC vesicles during dehydration by heat-drying while GLU does not. Saccharide, regardless of type, is distributed homogeneously between the bilayers of DPPC in vesicles. The interactions of disaccharide with bilayers of DPPC depend on the vesicle surface curvature. There are, thus, many modes of interaction, each differing slightly and by applying heat to DPPC with various interaction modes, the transition from gel to liquid crystal state occurs continuously. For GLU, there is no such dependence on surface curvature.

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