Small-Angle Neutron Scattering Study of Lipid Phase Diagrams by the Contrast Variation Method†

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ABSTRACT: A small-angle neutron scattering (SANS) study of slightly sonicated liposomes of binary lipid mixtures is presented. It is demonstrated that the neutron scattering of lipid lamellae may be analyzed in terms of the Kratky–Porod model of scattering by two-dimensional systems. The contrast variation technique may thus be applied in order to study the structure and phase diagrams of lipid layers not disturbed by heavy sonication. The thickness of isolated bilayers is measured, and molar volumes of pure lipid phases are determined. Mixtures of deuterated dimyristoylphosphatidylcholine with (1) protonated dipalmitoylphosphatidylcholine and (2) protonated distearoylphosphatidylcholine, respectively, are studied. Excess volumes of lipid mixtures are determined by the contrast variation. For the first mixture positive excess volumes of +86 Å^3 in the crystalline phase (5 °C) and of +49 Å^3 in the fluid phase (35 °C) are obtained. These large positive excess volumes are interpreted in terms of free volume creation at the interface between the monolayers, which indicates that the polar head groups are rather fixed with respect to the lipid–water interface. We show that the phase boundaries at a given temperature may be determined by performing contrast variation experiments for two mixtures with different initial composition. Good agreement with existing experimental data is observed for the first mixture. A miscibility gap is established in the crystalline state of the second mixture. A most interesting result is the finding of an immiscibility in the fluid state. This is interpreted in terms of critical concentration fluctuations caused by the critical demixing point of the solid-state miscibility gap hidden below the liquidus line.

The thermodynamic properties of lipid mixtures are a central point of interest in membrane research. This interest originates in the question of whether lateral phase separation together with selective lipid–protein interaction plays an essential role in the process of self-organization of biological membranes. Many methods have been applied to establish phase diagrams of lipid mixtures since the pioneering work of Ladbrooke & Chapman (1969). Most techniques are based on the analysis of cooling and/or heating curves either by thermodynamic (Wilkinson & Nagle, 1979; Mabrey & Sturtevant, 1976; Albrecht et al., 1981) or spectroscopic techniques (Shimshik & McConnell, 1973). A main drawback of these methods is that it is very difficult to observe phase boundaries in fluid or crystalline mixtures. The freeze etching electron microscopy is a valuable equilibrium technique for crystalline mixtures. Due to the fast lateral diffusion, however, difficulties arise in fluid systems. In the present work we applied the small-angle neutron scattering (SANS)[1] technique to study lateral phase separation of binary lipid mixtures in thermal equilibrium. In order to minimize the disturbance of the lipid structure by a high curvature, we studied slightly sonicated liposome preparations with a high population of thin-walled systems. It is demonstrated for the first time that the contrast variation method can be applied to such lipid layers by analyzing the scattering curves in terms of the Kratky–Porod model of small-angle scattering by quasi-two-dimensional systems. The method allows determination of phase boundaries, observation of critical concentration fluctuations, and measurement of excess volumes and bilayer thicknesses. The successful application of the contrast variation technique is a first step toward the application of the SANS technique (1) to study the lateral organization of lipid components in demixed lipid mixtures and (2) to evaluate the structure of proteins in reconstituted model membranes (Osborne et al., 1978). A detailed description of the method is published elsewhere (Knoll et al., 1981).

Experimental Procedures

(A) Materials and Preparation. DMPC,[1] DPPC, and DSPC were obtained from R. Berchtold, Biochemisches Laber (Bern, Switzerland). DMPC-d_{54} with perdeuterated hydrocarbon chains was synthesized by H. Vogel and H. H. Füldner (MPI für Biophysikalische Chemie, Göttingen, West Germany). All lipids gave single spots on a TLC plate. The H_2O was purified with a Millipore filter (pH 5) while D_2O (Uvasol pH 5) was purchased from Merck (Darmstadt, West Germany). According to density measurements its purity is better than 99.8% D_2O. For experiments at pH 9, a buffer containing 0.041 M $Na_2B_4O_7$, 0.037 M H_3BO_3, and 0.009 M NaCl was used.

For each measurement a fresh liposome preparation containing a final lipid concentration of 10 mg of lipid/mL of the H_2O/D_2O solvent was prepared as follows: 2 mg of the lipid mixtures was dissolved in $CHCl_3$. A thin lipid film was deposited on the wall of a glass flask by solvent evaporation. The film was dried in vacuo to remove traces of solvents. The lipid was taken up by adding the appropriate H_2O/D_2O mixture and by heating above the transition temperature of the highest melting component. The flask was shaken for several minutes and was then put in a bath sonifier for 1 min. A milky dispersion was obtained which was stable for several hours. Special care was taken that all samples were prepared in the same way.

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[1] Abbreviations used: DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; SANS, small-angle neutron scattering; TLC, thin-layer chromatography.

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According to the freeze–fracture micrographs taken from the samples, most liposomes had diameters ranging between 0.1 and 1 μm. Since the solubilities of the lecithins in the solvent used for spreading the lipid films do not differ appreciably and since the liposomes are rather large, a nonhomogeneous lipid distribution between the liposomes can be excluded. Due to the large curvature of the liposomes the lipids are also expected to be equally distributed between opposing monolayers. The equal lipid distribution between both the monolayers and the vesicles is clearly demonstrated in Figure 2 for the system DMPC-d_{54}/DPPC which exhibits unlimited miscibility both in the fluid and in the solid state. Prior to the scattering experiments the samples which were stored for several hours at 4 °C were kept for 1–2 h at the desired temperature in order to allow for a complete equilibrium distribution of the lipids.

For the scattering experiments, the dispersions were transferred into quartz cells (Hellma, Müllheim, West Germany) with 1-mm path length. In order to avoid D_2O exchange with the atmosphere, we sealed the cells with Teflon foils. For the temperature control, a thermostated sample holder was used. Scattering data were taken with the D11 camera at the Institut Laue-Langevin in Grenoble, France (Ibel, 1976). The neutron wavelength was 10 Å, and the sample–detector distance was 10 m. This corresponds to a range of momentum transfer of $\kappa = 2.5 \times 10^{-3}$–$2.2 \times 10^{-2}$ Å^{-1}.

(*B*) *Analysis of Scattering Data.* The neutron scattering of aqueous solutions (dispersions) is described in terms of the so-called scattering length density $\rho(\mathbf{r})$ (Jacrot, 1976). For the contrast variation technique it is convenient to write (Stuhrmann, 1976)

$$\rho(\mathbf{r}) = \bar{\rho}\rho_c(\mathbf{r}) + \rho_F(\mathbf{r}) \quad (1)$$

$\bar{\rho} = \bar{\rho}_p - \rho_s$ is the difference of the average scattering length density of the particle ($\bar{\rho}_p$) and of the solvent (ρ_s), $\rho_F(\mathbf{r})$ is a fluctuation term describing the internal structure of the particle, and $\rho_c(\mathbf{r})$ is a hard core shape function with $\rho_c(\mathbf{r}) = 1$ inside the particle and $\rho_c(\mathbf{r}) = 0$ in the solution. For three-dimensional particles the average scattering length density difference $\bar{\rho}$ is related to the scattering intensity, $I(0)$, at zero scattering angle and the particle volume, V_c (Stuhrmann, 1976), according to

$$I(0) = (\bar{\rho}V_c)^2 \quad (2)$$

For a solution of (monodisperse or polydisperse) particles with identical average scattering density, $\bar{\rho}_p$, a plot of $[I(0)]^{1/2}$ vs. ρ_s (e.g., the H_2O/D_2O ratio) should yield a straight line. For $\rho_s = \bar{\rho}_p$ the scattering intensity $I(0)$ becomes zero. If the solution contains particles with different $\bar{\rho}_p$ values, the matching of the solute and solvent scattering densities is impossible. This is the basis for the evaluation of phase diagrams described below.

For particles with lateral dimensions large compared to their thickness (quasi-two-dimensional systems), the scattering intensity $I(\kappa)$ at very small scattering angles is given by a modified Guinier's law (Kratky & Porod, 1948; Porod, 1948)

$$I(\kappa) = I_d(0)(1/\kappa^2)e^{-R_d^2\kappa^2} \quad (3)$$

R_d (corresponds to the radius of gyration in Guinier's law and is related to the thickness, d, of the particle by $d = R_d(12)^{1/2}$. If $\log [\kappa^2 I(\kappa)]$ is plotted as a function of κ^2 (Kratky–Porod plot), a straight line with a slope of $-R_d^2$ is expected. According to Pilz and co-workers (Pilz et al., 1970), this modified Guinier's law is also valid for curved lamellae.

For lamellar systems with homogeneous scattering length density the intensity at $\kappa \to 0$ is again proportional to the solvent scattering density ρ_s since in analogy to eq 2 it is

$$I_d(0) \propto (\bar{\rho}d)^2 \quad (4)$$

The average scattering length density of the lamella is obtained from the matching point, where $I_d(0) = 0$, by taking scattering curves for different H_2O/D_2O ratios. Such a series of measurements is called a contrast variation experiment in the following.

For two-dimensional systems which exhibit lateral phase separation, the scattering of the particles cannot be matched to zero by adjusting the H_2O/D_2O ratio. Only a minimum value of $I(\kappa)$ at $\kappa = 0$ is observed. The phase boundaries are determined as follows (Knoll et al., 1981). Consider a binary mixture of lipid components A and B with an initial mole fraction x_A of the component A. Assume that it has separated into two phases with the mole fractions x_1 and x_2 of the component A and which have scattering densities $\bar{\rho}_1$ and $\bar{\rho}_2$, respectively. The zero-angle scattering density is then

$$I_d(0,\rho_s) = \frac{x_2 - x_A}{x_2 - x_1}(\bar{\rho}_1 - \rho_s)^2 + \frac{x_A - x_1}{x_2 - x_1}(\bar{\rho}_2 - \rho_s)^2 \quad (5)$$

$I_d(0)$ is a quadratic function of the solvent scattering density, $\bar{\rho}_s$, with a minimum at $\rho_s = \rho_s^*$. The minimal average scattering density is $\bar{\rho}_{min}$. This value is related to ρ_s^*, $\bar{\rho}_1$, and $\bar{\rho}_2$ according to

$$\bar{\rho}_{min}^2 = (\rho_s^* - \bar{\rho}_2)(\bar{\rho}_1 - \rho_s^*) \quad (6)$$

$\bar{\rho}_{min}$ and ρ_s^* are measurable quantities. If two pairs of values are measured in two separate contrast variation experiments (cf. Figure 3) for two different initial concentrations x_A and x_A', $\bar{\rho}_1$ and $\bar{\rho}_2$ can be determined from eq 6, while x_1 and x_2 are obtained from the minimum of $I_d(0,\rho_s)$ according to eq 5. In principle, the complete phase diagram may be determined by a series of such experiments. The procedure is considerably simplified if part of the phase diagram is known from other experiments.

Results

Figure 1a shows Kratky–Porod plots ($\log [\kappa^2 I(\kappa)]$ vs. κ^2 plots) of crystalline DMPC-d_{54} liposomes in different H_2O/D_2O mixtures in the region of very low scattering angles. In the region $\kappa^2 = 1 \times 10^{-4}$–$5 \times 10^{-4}$ Å^{-2} straight lines are observed for all solvent contrasts. The slopes are identical within experimental error. The interceptions of the straight lines with the ordinate yield extrapolated values of zero-angle scattering intensities $I_d(0)$.

In Figure 1b, $[I_d(0)]^{1/2}$ is plotted as a function of the volume percentage of D_2O for a temperature above and below the lipid chain melting transition (T_m = 17.3 °C for DMPC-d_{54}). Straight lines are obtained as expected for homogeneous systems according to eq 4.

Figure 1c shows the variation of the square root of the extrapolated scattering intensity with solvent contrast for protonated DMPC, deuterated DMPC-d_{54}, and a 1:1 mixture of both lipids. The temperature was adjusted so that all lamellae are in a fluid state. In all cases straight lines are observed, that is, matching is possible. This is clearly expected for a mixture of isotopically substituted molecules. The matching point of DMPC is exactly found. In order to verify the miscibility of the 1:1 mixture, we performed more experiments for the mixtures than for the pure lipids.

Figure 2 shows an example of a mixture (DMPC-d_{54} and DPPC) which exhibits phase separation. The phase diagram for these lipids is given in Figure 2a. The mixture is expected to be miscible both in the fluid and in the crystalline state while it exhibits lateral separation into a fluid and a crystalline phase

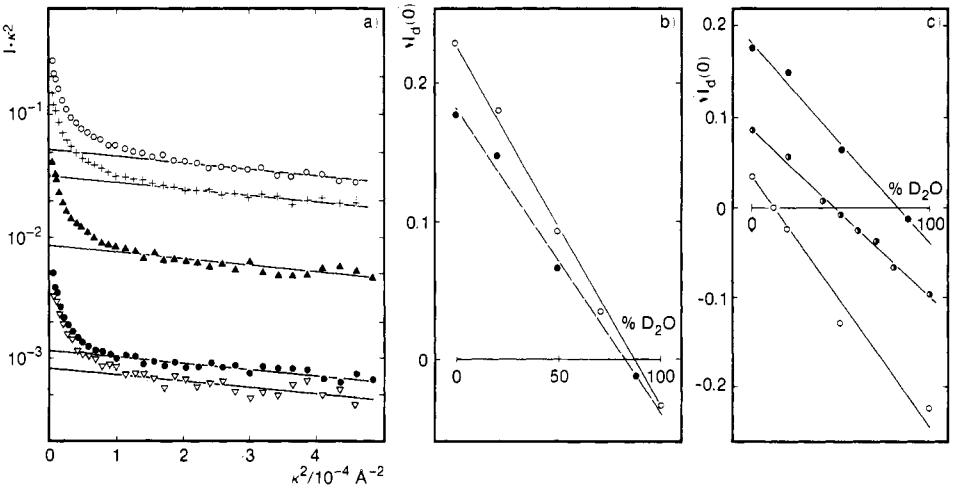

FIGURE 1: (a) Kratky–Porod plots of scattering intensity for slightly sonicated liposomes of DMPC-d_{54} at 5 °C observed at different H_2O/D_2O ratios: (○) 0%, (+) 20%, (▲) 50%, (▽) 70%, and (●) 100% D_2O, respectively. $I_d(0)$ is obtained by extrapolating the straight lines to $\kappa = 0$. (b) Square root of zero-angle scattering intensity, $[I_d(0)]^{1/2}$, plotted as a function of the solvent D_2O content (volume %) for a temperature above (●, 22 °C) and below (○, 5 °C) the chain melting transition of DMPC-d_{54} (T_m = 17.3 °C). (c) Plot of $[I_d(0)]^{1/2}$ for liposomes of protonated DMPC (○), the same lipid with deuterated chains, DMPC-d_{54} (●), and a 1:1 mixture of both lipids (◑) as a function of the solvent contrast. All lipids are in the fluid state.

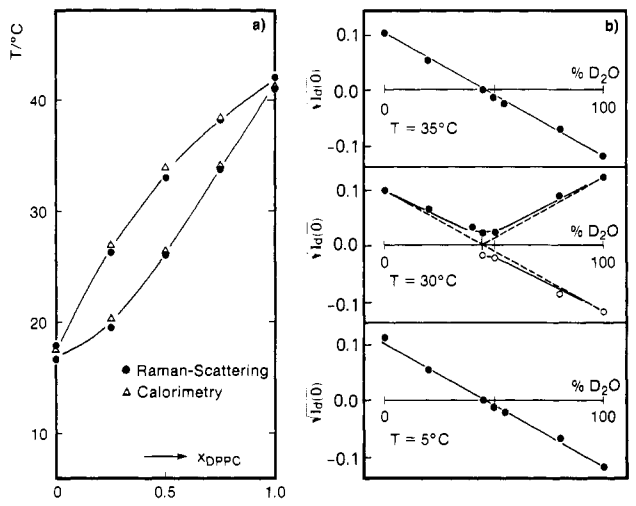

FIGURE 2: (a) Phase diagram of a mixture of deuterated DMPC-d_{54} and protonated DPPC as determined by Raman spectroscopy and calorimetry; (b) plot of $[I_d(0)]^{1/2}$ vs. D_2O content for a 1:1 mixture of DMPC-d_{54} and DPPC for a temperature above (35 °C), below (5 °C), and within (30 °C) the cigarlike phase diagram. The drawn parabolic curve has been calculated by using eq 5 and 6. The values of the molar fractions of DPPC in the two phases (x_1 = 0.37 and x_2 = 0.63) were taken from (a).

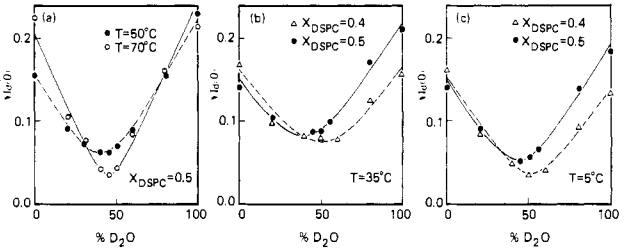

FIGURE 3: Plot of $[I_d(0)]^{1/2}$ as a function of D_2O content for mixtures of DMPC-d_{54} with protonated DSPC: (a) above the liquidus line (50 and 70 °C); (b) within the coexistence region (35 °C); (c) below the solidus line (5 °C). The compositions of the mixtures are indicated in the figures.

within the cigarlike phase diagram. The results of the contrast variation experiments for these three possible situations are summarized in Figure 2b. Above and below the coexistence region, $[I_d(0)]^{1/2}$ is a linear function of the D_2O content which shows that the mixtures are clearly homogeneous. A parabolic curve is observed for a temperature within the coexistence region. The latter is a clear indication that the lipid lamellae exhibit lateral phase separation.

Figure 3 shows the result of three contrast variation experiments for mixtures of protonated DSPC and DMPC-d_{54} at a temperature well above the liquidus line (50 and 70 °C, Figure 3a), within the coexistence region (35 °C, Figure 3b), and below the solidus line (5 °C, Figure 3c). For 5 and 35 °C, plots for two mixtures of different DSPC concentrations are presented from which the phase boundaries were determined. For all temperatures the data can be fitted with parabolic curves. This is clearly expected for 35 °C where the mixture decomposes into a fluid and a solid phase. It is also not surprising that mixtures of two lipids differing in chain length by more than four CH_2 groups exhibit immiscibility in the crystalline state. However, the appearance of parabolic curves above the liquidus line is a clear indication of nonrandom lipid distribution even in the fluid state. The experiment was repeated several times with different preparations. According to Figure 3a the minimum $[I_d(0)]^{1/2}$ is considerably lower at 70 than at 50 °C, that is, the mixture becomes more miscible with increasing temperature. This provides strong evidence that the apparent immiscibility is not due to nonrandom lipid distribution between different liposomes or between two opposing monolayers.

Discussion

According to Figure 1, the small-angle scattering of slightly sonicated liposomes may be analyzed successfully in terms of the Kratky–Porod scattering theory of two-dimensional particles. Plots of log $[I(\kappa) \times \kappa^2]$ vs. κ^2 yield straight lines in the low-angle region over an extended region of κ^2 values (10^{-4} Å$^{-2} < \kappa^2 < 5 \times 10^{-4}$ Å^{-2}). At higher angles, $\kappa^2 = 6 \times 10^{-3}$–$12 \times 10^{-3}$ Å^{-2}, Bragg reflections are observed which correspond to the periodicity of the stacked bilayers in the direction normal to the membrane plane (Tardieu et al., 1973; Büldt et al., 1978). The Bragg peak vanishes for single-shelled vesicles obtained after 15 min of sonification (Knoll et al., 1981). In a Guinier plot (plot of log $[I(\kappa)]$ vs. κ^2) no straight line is observable in the whole κ^2 region studied ($0 \leq \kappa^2 \leq 10^{-3}$ Å^{-2}). From the slopes of the straight lines of the Kratky–Porod plots an apparent thickness of the lamellea of $d^* = 125$ Å is obtained. This corresponds to liposomes with shells composed of two to three bilayers.[2]

The most important finding is that the slopes of the straight lines (or the thickness d) do not depend on the solvent contrast. It is for this reason that the contrast variation method can be applied to liposomes. This point is discussed in more detail in a forthcoming paper. In the following we present some important results obtained from the contrast variation experiments described in the preceding section.

(*a*) *Molar Volumes.* The molar volumes of pure lipids are obtained as follows. First, the average scattering density, $\bar{\rho}_p$, of the lipid is determined by searching the matching point. The molecular volume, V, is then obtained from the definition of the scattering length density

$$\bar{\rho}_p = \frac{1}{V}\sum_i b_i \qquad (7)$$

b_i are the scattering lengths of the nuclei which are given in the literature (Jacrot, 1976). Because of the large difference of the b_i values of D and H, the degree of deuteration of the lipid must be known accurately in order to obtain reliable values of the molar volumes. In a separate experiment the degree of deuteration of the DMPC-d_{54} used in our experiments was determined to 96.2% (Knoll, 1981). With this result one obtains from the values of the scattering length density in the crystalline state (5 °C) of $\bar{\rho}_p = 5.47 \times 10^{10}$ cm^{-2} and in the fluid state (22 °C) of $\bar{\rho}_p = 5.19 \times 10^{10}$ cm^{-2} molar volumes of $V = 1044$ Å^3 at 5 °C and of $V = 1102$ Å^3 at 22 °C. The volume change in this temperature region is then $\Delta V = 58$ Å^3. The corresponding value measured by densitometry for protonated DMPC is $\Delta V = 56$ Å^3 (Nagle & Wilkinson, 1978).

(*b*) *Excess Volumes.* The contrast variation technique allows detection of nonideal behavior of lipid mixtures in a direct way and determination of excess volumes. For a nonideal mixture, the measured scattering density, $\bar{\rho}_p$, obtained from the matching point ($\bar{\rho}_p = \rho_s$*) deviates from the value, ρ_p^{id}, of the corresponding ideal mixture. ρ_p^{id} may be calculated from the scattering densities of the pure components. The excess volume is then obtained (Orwoll & Flory, 1967) according to

$$V_E = \frac{\bar{\rho}_A V_A x_1 + \bar{\rho}_B V_B (1 - x_1)}{\bar{\rho}_p} - [x_1 V_A + (1 - x_1) V_B] \qquad (8)$$

$\bar{\rho}_A$ and $\bar{\rho}_B$ are the scattering densities of the pure components. V_A and V_B are the corresponding molar volumes and x_1 and $(1 - x_1)$ the molar fractions.

For the mixture of DPPC and DMPC-d_{54} studied in Figure 2 one obtains positive excess volumes: $V_E = +86$ Å^3 at 5 °C and $V_E = +49$ Å^3 at 35 °C. Previously published excess volume data (Knoll et al., 1981) were calculated by assuming for DMPC-d_{54} a complete deuteration of the hydrocarbon chains. Recently, a combination of mass and scattering length densitometry allowed us to determine the degree of deuteration of our lipid samples to 96.2% (Knoll, 1981). Although $\bar{\rho}_p$ can be determined to an accuracy of 3%, the excess volumes can only be estimated since they are obtained as a difference of two large quantities. The large positive excess volumes are in contrast to the findings for *n*-alkanes where small volume contractions of <1% have been observed (Mnyukh, 1960; Orwoll & Flory, 1967). However, preliminary density measurements by W. Knoll yielded also a positive excess volumes of about 2–3% for the DPPC/DMPC-d_{54} mixture. Wilkinson & Nagle (1979) reported for the DMPC/DSPC system a positive excess volume of 0.3%. A volume expansion for lipid layers may be understood by assuming that the lipid head groups are rather fixed with respect to the plane of the polar interface. This would lead to a free volume at the interface between the opposing monolayers. The molar volumes of DMPC and DPPC differ by 120 Å^3. For a 1:1 mixture of the two components, one would thus expect an excess volume of $V_E \simeq 60$ Å^3 which is of the right order of magnitude. Similar high positive excess volumes were observed for alkanes, alcohols, or fatty acids intercalated between silicate layers (Lagaly, 1976). In these layers the head groups are fixed with respect to the silicate surface. Above the chain melting transition the molecules may move as freely in the plane of the layers as the lipid molecules in the bilayer plane, and the two systems may thus well be comparable.

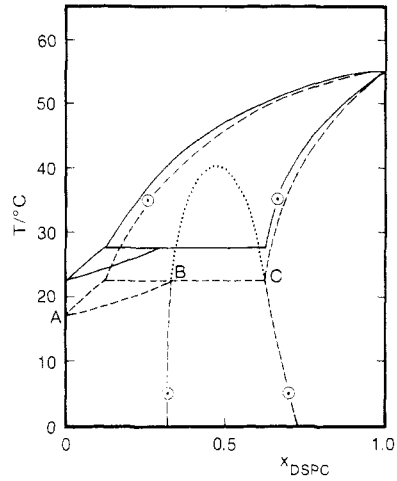

FIGURE 4: (Full line) Phase diagram of DSPC/DMPC mixture as obtained by Raman spectroscopy (Knoll, 1978), density measurements (Wilkinson & Nagle, 1979), and calorimetry (Phillips et al., 1970; Mabrey & Sturtevant, 1976; Jacobs et al., 1977; van Dijck et al., 1977). (Dashed curve) Phase diagram of DSPC/DMPC-d_{54} mixture as obtained by shifting the phase lines of the protonated lipid mixture to lower temperatures in such a way that the transition temperature at the left ordinate coincides with the main transition of DMPC-d_{54} ($T_m = 17.3$ °C). As described under Discussion, the mixture may exhibit peritectic behavior. Also shown are the phase boundaries for 5 and for 35 °C as determined by SANS measurements (⊙).

(*c*) *Phase Diagram of DMPC-d_{54}/DSPC Mixtures.* This system has been studied extensively as a prototype of a mixture of components with identical head groups but which differ in chain length by four CH_2 groups. The phase diagram of the protonated lipids as obtained by combining the results of different authors (Phillips et al., 1970; Shimshick & McConnell, 1973; Mabrey & Sturtevant, 1976; Jacobs et al., 1977; van Dijck et al., 1977; Knoll, 1978; Wilkinson & Nagle, 1979) is given in Figure 4 (full line). The corresponding phase boundaries of the mixture with DMPC-d_{54} may be obtained approximately by shifting the transition temperature of this lipid from $T_m = 23$ °C to $T_m = 17.3$ °C, the chain melting transition of the deuterated compound. An essential point is the form of the solidus line at low DSPC concentrations. As Figure 4 shows, it exhibits an ascending slope between A and B while a horizontal deflection is observed between B and C. Strong evidence for a horizontal BC line is provided by the experiments reported by several groups (Phillips et al., 1970; Mabrey & Sturtevant, 1976; van Dijck et al., 1977; Jacobs et al., 1977). The horizontal deflection strongly suggests a miscibility gap below the solidus line. This is indeed verified in a direct way by the finding that the 1:1 mixture exhibits nonmiscibility at 5 °C. The position of the phase boundaries of the DMPC-d_{54}/DSPC mixture has been determined for 5

[2] For sonicated single-shelled vesicles of DMPC at 37 °C a thickness of $d = 41$ Å is observed.

and 35 °C as described above. For this purpose contrast variation experiments were performed for two mixtures with initial DSPC concentrations of $x_A = 0.5$ and $x_A' = 0.4$. The DSPC concentrations at the phase boundaries thus determined were $x_1 = 0.32$ and $x_2 = 0.70$ for 5 °C while $x_1 = 0.25$ and $x_2 = 0.67$ were found for 35 °C. The latter result is in good agreement with the values of x_1 and x_2 estimated from the phase diagram of the protonated lipid mixture. This shows that phase boundaries may indeed be determined by the proposed contrast variation technique in a reliable way.

The appearance of a miscibility gap and the fact that the solidus line exhibits an ascending slope between A and B also suggest that the mixture exhibits peritectic behavior. A closer inspection of the published results (Mabrey & Sturtevant, 1976; Knoll, 1978) shows that the liquidus line exhibits indeed a dip near the temperature of the horizontal slope BC. A peritectic behavior is also suggested by the large difference in the transition temperatures of the two lipids.

The most surprising result is the finding that the mixture cannot be masked above the liquidus line. Since the neutron scattering from individual lipid molecules does not contribute to the small-angle scattering region, the finite contrast at the minimum of the $[I_d(0)]^{1/2}$ vs. ρ_s curve must be due to a large-scale heterogeneity in the lateral distribution of the lipids. Two explanations are possible. First the heterogeneity could be due to a lateral phase separation. The second more possible explanation is that it is due to critical concentration fluctuations. The two nearly vertical phase lines below the solidus line could be extrapolated into the coexistence region as indicated by a dotted line in Figure 4. The latent critical demixing point hidden within the coexistence region could then well lead to critical concentration fluctuations above the liquidus line. Fluctuations caused by hidden critical demixing points have been reported for metallic systems. A well-studied system is "Hydrogen in Tantalum" (Bauer et al., 1977). The strong decrease in the minimum value of $[I_d(0)]^{1/2}$ at increasing temperature (cf. Figure 3a) provides good evidence for the explanation of the nonmiscibility in terms of critical concentration fluctuations.

Critical concentration fluctuations may also explain nuclear magnetic resonance studies of a 1:1 mixture of DPPC and dielaidoylphosphatidylcholine by Brulet & McConnell (1976). These authors observed a much stronger or "earlier" onset of lateral phase separation when the liquidus line is approached from the fluid phase than expected from theoretical considerations. These findings were explained in terms of the formation of crystalline nuclei within the fluid phase. The experiments could also be explained in terms of critical concentration fluctuations in the fluid phase.

Conclusion

In the present work we demonstrated that the small-angle neutron scattering of thin-walled liposomes may be analyzed on the basis of the Kratky–Porod model. Although the thicknesses of the liposome walls vary, the contrast variation technique may be applied for structural studies. This allows the measurement of molar volumes and excess volumes of mixtures and the determination of phase diagrams of binary lipid alloys. For single-walled vesicles obtained by sonication the bilayer thickness may be measured from the slopes of the log $[I_d(0)]$ vs. κ^2 plots.

The SANS technique has a number of outstanding advantages. It is a disturbless technique. Compared to the X-ray method a large contrast may be achieved by partial deuteration. Since the neutron scattering is a fast process, it also allows detection of short-lived structural features with lifetimes of $\sim 10^{-12}$ s. These advantages compensate for the fact that the SANS technique is a rather expensive method.

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