

Redetermination of the Pressure Dependence of the Lipid Bilayer Phase Transition[†]

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ABSTRACT: The effect of pressure on the phase transition temperature for the dipalmitoyllecithin bilayer was redetermined by following the volume change accompanying the transition. These measurements were carried out isothermally with the transition from the ordered to the disordered phase induced by decreasing the pressure. This contrasts with our previous measurements which were carried out at constant pressure and increasing temperature. The transition at every temperature was sharp and confirmed our previous observation

that the volume change associated with the transition (0.033 mL g⁻¹) is invariant with pressure. However, our present measurements, in contrast to our previous results, indicate that dP_m/dT_m at all pressures is in agreement with the 1 atm value of $\Delta H/T_m\Delta V$ within experimental error where T_m and P_m are the temperature and pressure of the phase transition, respectively. These results, which are now in agreement with all other known pressure data, indicate that the entropy change associated with the transition is invariant with pressure.

In a previous publication (Srinivasan et al., 1974), the effect of pressure on the transition temperature for dipalmitoyllecithin (DPL)¹ was reported in considerable detail. The results indicated a $\Delta\bar{V}$ that was independent of pressure up to 100 bar and its magnitude (0.032 mL g⁻¹) was in good agreement with Nagle's very precise measurements (Nagle, 1973b). However, dP_m/dT_m (where T_m and P_m are the temperature and pressure of the phase transition, respectively) decreased with increasing pressure, indicating that the transition occurred with a steadily decreasing entropy change but a constant volume change. This result could not be explained, particularly when compared with Nagle's general theory (Nagle, 1973a), and became most suspect when compared with the unpublished data of McColl as indicated in an addition to the proof of the previous paper. McColl found both $\Delta\bar{V}$ and dP_m/dT_m invariant with pressure, a result far easier to understand on a molecular basis than our result.

Since our measurements were carried out with considerable care by a method with sufficient precision to detect the difference found, we felt we should investigate our method thoroughly. Although we cannot indicate with certainty the cause of what we now consider errors in our previous measurements, we have been able to identify possible sources of error and have redesigned the measurements to reduce them as much as possible. Briefly, the measurements were carried out at constant temperature rather than at constant pressure, the pressure vessel was completely immersed in the constant temperature oil bath, the pressure range was extended to 270 bar, and a 5% rather than a 2% dispersion of DPL was employed.

Experimental Section

The volumometer used for all the measurements has been described in detail in our previous publication (Srinivasan et al., 1974) and further details will appear in another publication from this laboratory (Srinivasan and Kay, 1977). It is sufficient

to point out here that volume changes were detected by means of a moving piston, the position of which was determined by an electrical contact system. Under normal operating conditions, the piston moved in a downward direction when the pressure was decreased or the temperature increased. The volumometer contained about 32 mL of sample. Air bubbles were eliminated by filling under vacuum. The previous volume calibration, based on data for water at 25 °C and pressures up to 1 kbar (Kell and Whalley, 1965), were used. The reproducibility was 0.02% in the total volume with a detectability of 2×10^{-3} mL. The correction in the calibration due to the expansibility of steel for the small temperature range used here was only 0.01% and was ignored. The same was true of the compressibility correction which amounts to less than 0.02% for pressures up to 300 bar.

In the previous experiment, the volumometer was immersed in the constant temperature oil bath up to the level of its pressure bearing steel cap. Since the room temperature was 15–20 °C below the bath temperature, the possibility existed for thermal gradients in the pressurizing oil within the volumometer. Independent measurements indicated that this was indeed the case. Consequently, all measurements reported here were carried out with the pressure vessel completely immersed in the oil bath. The oil bath was regulated to 0.01 °C and the absolute temperature determined with a calibrated Beckman thermometer.

The compressing fluid was Marcol 90 oil. The pressure to the nearest bar was determined by means of a Heise gauge, full scale 400 atm. In order to extend the transition pressure to 270 atm, it was necessary to expose the sample to pressures as high as 400 atm but no deterioration in the sample was detected due to this high pressure; i.e., the reproducibility remained unchanged.

Owing to the low thermal conductivity of stainless steel and the thick walls of the pressure vessel, 2 h was required for thermal equilibrium after any significant change in temperature of the oil bath. It is possible that operating at constant pressure and changing the temperature to observe the phase transition could have introduced some error due to lack of thermal equilibrium. Also, changing the temperature required constant adjustment of the pressure. Consequently, it was decided that operating at constant temperature and changing

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¹ Abbreviations used: DPL, 1,2-dipalmitoyllecithin; DML, 1,2-L-dimyristoyllecithin; ESR, electron spin resonance.

TABLE I: Transition Point (T_m , P_m) and the Corresponding Change in Volume.

T_m ($^{\circ}\text{C}$)	P_m (atm)		$10^3 \Delta \bar{V}$ (mL g^{-1})
	Series A	Series B	
(41.35) ^a	1		(36 ± 2)
42.51	50		
43.19		82 ± 3	33 ± 3
43.52	92		
44.15		126 ± 3	33 ± 3
44.53	137		
45.13		167 ± 3	33 ± 3
45.51	184		
46.49	218		
47.50	264		

^a See Nagle (1973b).

pressure would be an improvement in the technique. Steady-state conditions were obtained about 20 min after a change in the pressure of 10 atm. In order to eliminate small amounts of trapped air bubbles, the system was precompressed to 200 atm until a constant 1 atm reading was obtained. The measurement of the volume was continued at regularly decreasing pressures until well past the transition into the disordered phase.

The DPL was obtained from Calbiochem. A 5% by weight dispersion was made by shaking the white powder in conductivity grade water ($\kappa_0 = 1 \times 10^{-7} \Omega^{-1} \text{cm}^{-1}$, pH 7) at a temperature above the transition.

Results and Discussion

Two separate series of runs were carried out using different samples of DPL. In the first (series A), runs at six different temperatures and at pressures between 50 and 264 atm produced the data recorded in Table I. The transition pressure P_m was selected as the pressure of initial increase in the volume above the baseline after the volume data had been corrected for the compressibility and expansibility of pure water (Kell and Whalley, 1965). This value of the pressure was selected because the initial rise was sharp and a simple extrapolation permits P_m to be determined precisely. This was originally demonstrated by Nagle (Nagle, 1973b) who showed that the initial transition from the ordered phase is extremely sharp and independent of DPL concentration, whereas the final transition into the completely disordered phase is gradual. This latter fact made it difficult to select the midpoint of the curve (the coexistence pressure) as has been done by other investigators (Trudell et al., 1974; McColl, 1974, private communication). Unfortunately in filling the cell under vacuum for this series of runs, some of the DPL dispersion was lost and had to be replaced with pure water. This left the exact weight of DPL in the volumometer unknown and consequently measurement of the exact $\Delta \bar{V}$ for the transition was not possible. However, this did not affect the determination of the transition pressure at each temperature.

The results for series B are plotted in Figure 1 and recorded in Table I. Our average value, $\Delta \bar{V} = 0.033 \pm 0.03 \text{ mL g}^{-1}$ agrees reasonably well with recent precise measurements which will report $\Delta \bar{V} = 0.036 \text{ mL g}^{-1}$ (Nagle and Wilkenson, 1977, private communication). The sharp transition into the completely ordered phase is evident at the bottom of each of the three plots. The more gradual transition into the disordered phase is also evident in Figure 1. In our previous results, the lack of this gradual change in volume as the pure disordered phase was approached was a matter of much concern and could

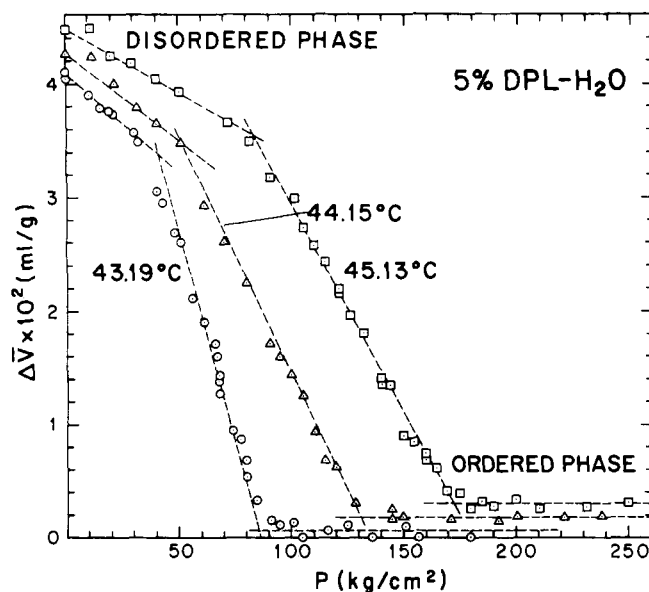


FIGURE 1: The effect of reducing the pressure on the specific volume of DPL at various temperatures as the DPL undergoes the transition from the ordered to disordered phase. The volume changes due to the water have been subtracted from the measured volumes. The experiments were carried out by compressing the samples to the highest pressure and recording the volumes as the pressure was decreased.

not be explained. One different feature of our results when compared with the data of others is the continuous increase in volume after the transition into the completely disordered phase. This is not evident in the data of Nagle nor those of Trudell. However, Nagle's data were carried out at 1 atm only and Trudell and co-workers did not measure the volume change due to the transition. It would appear that the disordered phase is considerably more compressible than the ordered phase. Slopes of the initial portion of the curves in Figure 1 indicate a compressibility for the disordered phase of DPL of approximately $0.1 \text{ mL mol}^{-1} \text{ atm}^{-1}$.

In Figure 2 are plotted the values of the phase transition pressure for various transition temperatures. The Clapeyron slope is given by $dP_m/dT_m = (41.3 \times 10^3) \Delta H / (T_m \Delta \bar{V}) \text{ atm deg}^{-1}$ where ΔH is in kcal mol^{-1} , T_m is in K, and $\Delta \bar{V}$ is in mL mol^{-1} . The 1-atm value of this slope ($43.5 \text{ atm deg}^{-1}$) is given by the solid line in Figure 2 using what we consider the most accurate data now available, namely, $\Delta H = 8.74 \text{ kcal mol}^{-1}$ (Mabrey and Sturtevant, 1976), $\Delta \bar{V} = 0.036 \text{ mL g}^{-1}$ (Nagle and Wilkenson, 1977, private communication), $T_m = 314.5 \text{ K}$ (Nagle, 1973b), molecular weight DPL, 734.1. The dotted lines indicate the maximum limit of error resulting primarily from the uncertainty in $\Delta \bar{V}$. As can be seen, all known data lie very close to the theoretical limiting slope, except for our previous datum point at 100 atm, a result that is obviously in error. Each of the investigations of dP_m/dT_m used different methods. McColl used an adiabatic compression method and Trudell used a spin label ESR method. It appears that the evidence is overwhelming that dP_m/dT_m is independent of pressure up to at least 200 atm and in excellent agreement with our present best estimate of $\Delta H/T_m \Delta \bar{V}$ for 1 atm. No significance should be given to the slight decrease in dP_m/dT_m above 200 atm since experimental errors could easily account for such small changes. If a straight line is passed through the points for each worker shown in Figure 2, the estimates of dP_m/dT_m obtained

² In our previous publication (Srinivasan et al., 1974), the constant in this equation was quoted incorrectly as 0.0413.

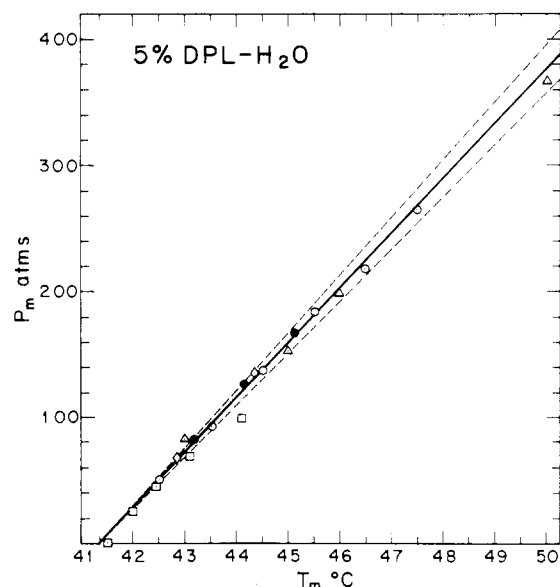


FIGURE 2: Phase diagram for the DPL phase transition. The solid line was calculated from the best values known for the quantities in $\Delta H/(T_m \Delta V)$. The dotted curves indicate the probable error owing to an uncertainty of almost 10% in ΔV . (○) This work, series A. (●) This work, series B. (◻) Srinivasan et al. (1974). (Δ) McColl (1974, personal communication). (◊) Trudell et al. (1974).

are 44 ± 1 (McColl, 1974, private communication), 45 ± 3 (Trudell et al., 1974), and 43.7 ± 1.5 (this work). All are in good agreement with the 1-atm result of $43.5 \text{ atm deg}^{-1}$. The results shown in Figure 2 could be a good indication of the correctness of the present data used here for 1 atm. The fact

that $\Delta \bar{V}$ reported in Table I does not change with pressure therefore indicates that ΔS the entropy change associated with the transition must also be invariant with pressure.

It is interesting to note that a light scattering investigation (DeSmedt et al., 1975) of very dilute aqueous dispersions of vesicles of L-dimyristoyllecithin (DML) (0.1% by weight) at pressures up to 1000 atm indicated that dP_m/dT_m for the C_{14} lecithin (molecular weight 678) was also independent of pressure with $dP_m/dT_m = 49 \text{ atm deg}^{-1}$.

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