Volume behaviour of dipalmitoylphosphatidylcholine bilayer membrane: pressure perturbation calorimetry and densitometry

T Izumikawa¹, Y Nambu¹, K Fujishige¹, M Goto², N Tamai², H Matsuki², S Kaneshina²

¹Graduate School of Advanced Technology and Science, The University of Tokushima, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan
²Department of Life System, Institute of Technology and Science, The University of Tokushima, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan

E-mail: matsuki@bio.tokushima-u.ac.jp

Abstract. Pressure perturbation calorimetry (PPC) is a relatively new technique based on a commercially available calorimeter which enables us to determine the isobaric coefficient of the thermal expansion of a solute for aqueous polymer solutions and colloidal dispersions. We applied the PPC to the investigation on the volume behaviour of dipalmitoylphosphatidylcholine (DPPC) bilayer membrane and compared the obtained results of the expansivity $D_2$ and the apparent molar volume $M_2$ of DPPC in its bilayer membrane with those from a conventional densitometry. The temperature dependence of $M_2$ from the PPC was in good agreement with that from the densitometry. Both techniques revealed that the $D_2$ value changes with the phase state of the DPPC bilayer membrane in the order of ripple gel ($P_{E}'$) phase $\rightarrow$ liquid crystalline ($L_{D}$) phase $\rightarrow$ lamellar gel ($L_{E}'$) phase. From the dependence, the change of $\varphi_2$ at the main transition from the $P_{E}'$ phase to the $L_{D}$ phase was estimated to be $22.7 \pm 2.62$ cm$^3$ mol$^{-1}$ from the PPC and at 24.0 cm$^3$ mol$^{-1}$ from the densitometry, which are comparable with 25.4 cm$^3$ mol$^{-1}$ previously obtained by the calculation based on the Clapeyron equation. On the other hand, the change of $\varphi_2$ at the pretransition from the $L_{E}'$ phase to the $P_{E}'$ phase could not be determined with sufficient reliability. This may be due to the small change in $M_2$ at the pretransition (less than 0.5% of $M_2$ at the temperature).

1. Introduction

A phospholipid, known as a major constituent of biomembranes, is an amphiphilic molecule, which is usually composed of a polar headgroup and two hydrocarbon chains attached to a glycerol backbone. This amphiphilic nature allows them to form a self-assembled bilayer membrane in the presence of excess water. Artificial phospholipid bilayer membranes have long been utilized as models of biomembranes for investigating fundamental structure and properties of biomembranes, because they have essentially similar architecture to the real biomembrane. Dipalmitoylphosphatidylcholine (DPPC) is the most frequently used phospholipid in the mimetic membrane studies [1], and consequently the structural change of its membrane with the variation of environmental conditions,
such as temperature $T$ and pressure $P$, has been firmly established by X-ray diffraction, nuclear magnetic resonance, differential scanning calorimetry and so forth. At ambient pressure, the DPPC bilayer membrane undergoes three types of the structural changes, generally regarded as phase transitions, with increasing temperature: the subtransition from the subgel ($L_{c}$) phase to the lamellar gel ($L_{E}'$) phase at 21.2 °C [2], the pretransition from the $L_{E}'$ phase to the ripple gel ($P_{E}'$) phase at 34.3 °C [3], and finally the main transition from the $P_{E}'$ phase to the liquid crystalline ($L_{D}$) phase at 42.0 °C [3]. It is also known that the interdigitated structure, one of non-bilayer structure, is formed in a finite temperature range at high pressures above ca. 100 MPa [4,5].

The investigation on the volume behaviour of phospholipid bilayer membranes is also important for better understanding of the physicochemical properties of biomembranes. Volume $V$ of a substance, well defined as a fundamental parameter in thermodynamics, is a macroscopic physical quantity which reflects its microscopic structure and molecular interactions. For several typical phospholipids including DPPC, the volume behaviour of their bilayer membranes has been already clarified by means of densitometry and dilatometry [6,7]. These techniques have long been employed as major experimental techniques for directly determining the $V$ value. However, these techniques generally require severely precise control of the temperature (on the order of mK) for obtaining reliable data. Because of this practical difficulty, very few studies on the volume behaviour of phospholipid bilayer membranes have so far been reported, and consequently, our knowledge concerning their volume properties is rather limited.

Recently, a novel calorimeter has been commercially developed by MicroCal [8] and it allows us to investigate the volume properties for aqueous polymer solutions and colloidal dispersions. This type of calorimeter can measure the heat transferred into (or from) a sample solution during the course of an isothermal small pressure change, and so this technique is generally called pressure perturbation calorimetry (PPC). The $\Delta q$ value can be related to the isobaric thermal expansion coefficient $\alpha$ by a thermodynamic equation, which means that the $\alpha$ value can be obtained from the PPC measurement. Although a similar method based on the same thermodynamic principle has been known as piezothermal technique for more than 30 years [9], the commercial availability of the calorimeter has made the method more familiar to us, as is evident from the fact that the application of the PPC technique to various systems [10,11] has been reported during the last decade. Aqueous dispersions of phospholipid vesicles are also the systems to which the PPC can be suitably applied, but it is unfortunate that there are still few reports on the application of the PPC to the phospholipid bilayer membranes except for several earlier pioneering works [12–13]. We started the research on the volume behaviour of phospholipid bilayer membranes by utilizing the PPC technique. In this paper we report the volume behaviour of the DPPC bilayer membrane revealed by the PPC and the density measurements. The aim of this paper is to confirm the validity of the PPC experiment by comparing the result from the PPC with that from the conventional densitometry as a first step of our future research using the PPC technique.

2. Theory for PPC Technique

According to thermodynamics, heat quantity $dq$ transferred into or from an isolated system during a reversible process of an infinitesimal pressure change $dP$ at a constant temperature $T$ is expressed as follows:

$$\left( \frac{\partial q}{\partial P} \right)_T = -\alpha TV$$  \hspace{1cm} (1)

Here, $V$ denotes the volume of the system and $\alpha$ represents the thermal expansivity, which is defined as

$$\alpha = \frac{1}{V} \left( \frac{\partial V}{\partial T} \right)_P$$  \hspace{1cm} (2)
When the system is a binary solution composed of \( n_1 \) moles of a solvent (component 1) and \( n_2 \) moles of a solute (component 2), \( V \), or its total volume, can be written using the molar volume of the solvent \( v_1^o \) and the apparent molar volume of the solute \( M_2 \) as follows:

\[
V = n_1 v_1^o + n_2 M_2
\]

Therefore, Eq. (1) can be rewritten as follows:

\[
\left( \frac{\partial q}{\partial P} \right)_T = -T(n_1 v_1^o + n_2 M_2 \alpha_1 + n_2 \phi_2 \alpha_2)
\]

where \( \alpha_1 \) and \( \alpha_2 \) are the thermal expansion coefficients defined against \( v_1^o \) and \( \phi_2 \), respectively. For the pure solvent of the same volume \( V \), a similar relation

\[
\left( \frac{\partial q_1^o}{\partial P} \right)_T = -\alpha_1 T(n_1 v_1^o + n_2 \phi_2)
\]

holds, so subtracting Eq. (5) from Eq. (4) yields the following equation.

\[
\left( \frac{\partial (q_1^o - q_2^o)}{\partial P} \right)_T = -(\alpha_2 - \alpha_1) T n_2 \phi_2
\]

For experimental heat quantities of \( \Delta q_1^o \) and \( \Delta q_2 \) with a small finite pressure change \( \Delta P \), we finally obtain the following expression.

\[
\Delta q = \Delta q_1^o - \Delta q_2 = -(\alpha_2 - \alpha_1) T c_2 \phi_2 \Delta P
\]

\[
\Rightarrow \alpha_2 = \alpha_1 - \frac{\Delta q}{T c_2 \phi_2 \Delta P}
\]

where \( c_2 \) means the molar concentration of the binary solution (i.e., \( n_2 = c_2 V \)).

3. Experimental

A synthetic phospholipid, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) was purchased from Sigma-Aldrich Co. (Alabaster, AL), and used without further purification. A weighed amount of lipid powder was suspended in an appropriate amount of double distilled water by vortexing at room temperature for a few minutes and subsequently sonicating at about 50 °C for 1–4 hours to obtain a homogeneously translucent aqueous dispersion of the DPPC multilamellar vesicle with a desired lipid concentration. The lipid concentration was adjusted to 6.81 mmol kg \(^{-1} \) (= 5.0 mg mL \(^{-1} \)) for the PPC measurement and to 6.0 mmol kg \(^{-1} \) for the densitometry.

In the PPC measurement, heat quantity \( \Delta q \) during the course of a pressure change \( \Delta P \) of 60 psi (= 0.41 MPa) was recorded at every 0.2 °C in the temperature range of 20–60 °C for the aqueous dispersion of the DPPC vesicle using a VP-DSC calorimeter (MicroCal, Northampton, MA) equipped with an accessory for the pressure perturbation measurement. The volume of the sample cell was 0.52 mL. The pressure was supplied by a compressed gas cylinder of nitrogen connected to the PPC accessory. We used 0.9604 mL g \(^{-1} \) (at 36.0 °C) as the value of the partial specific volume. The \( \Delta q \) data were analyzed using attached software Origin 7.0 (Lightstone Corp., Tokyo) to obtain the \( \alpha_2 \) values of DPPC in the aqueous dispersion.

The density of the aqueous dispersion of the DPPC vesicle was measured with a Digital Density Meter DMA 60 (Anton Paar, Graz, Austria) and a Density Measuring Cell DMA 602 (Anton Paar, Graz, Austria) at various temperatures in the range of 20–45 °C. Throughout the measurement, the temperature of the sample solution in the cell was monitored by a D632 Thermal mk Referencer (Takara Thermistor Instruments Co. Ltd., Yokohama, Japan) with a resolution of 0.001 °C and controlled within the fluctuation of ± 0.005 °C by circulating water the temperature of which was controlled with a Precise Temperature Control Unit (YTS, Tokushima, Japan). Two apparatus constants were determined by measuring the densities of water and air at varying temperature within the same temperature range. The \( \phi_2 \) value was calculated using the following equation:
where \( m, M, \rho \) and \( \rho_0 \) represent the molality of DPPC, molar mass of DPPC, the density of the aqueous DPPC dispersion and the density of water, respectively.

4. Results and Discussion

Figure 1 shows the temperature dependence of the \( \alpha_2 \) value obtained from the PPC measurement for the aqueous dispersion of the DPPC vesicle. Similar result has been reported by Wang and Epand [13]. Hereafter we call this type of curve (i.e., \( \alpha_2 \) vs. \( T \) curve) “PPC curve”. In the PPC curve, a first-order phase transition is detected as a peak due to the discontinuous volume change at the transition, as observed in a conventional DSC thermogram where a transition can be similarly recognized as an endothermic or exothermic peak. For the DPPC bilayer membrane, a quite sharp peak was clearly observed at ca. 42 °C, as shown in Fig. 1-A, which indicates that the DPPC bilayer membrane underwent the main transition at the temperature. However, the pretransition was not clearly detected as a peak from the PPC measurements (Fig. 1-B) although the DSC measurement using the same calorimeter can reveal also the pretransition as a small endothermic peak appearing around 34 °C. This may be due to the small volume change at the pretransition (less than 0.5% of apparent molar volume of DPPC) or due to the overlap of the large main-transition peak. This fact is also consistent with the previous report [13] in which only the main transition has been dealt with in regard to the analysis of the transition volume based on the PPC data.

In a DSC thermogram, the area of a peak corresponds to the difference in enthalpy between the states below and above the peak temperature. Therefore, the transition enthalpy \( \Delta H \) can be evaluated by integrating the peak after subtracting the baseline. A general PPC curve, on the other hand, represents the relation between \( \alpha_2 \) and \( T \), and so when a peak is found there the information about the volume change of a solute \( \Delta \alpha_2 \) associated with the transition can be obtained from the peak area. With regard to the analysis of the peak area, theoretical basis is given by the following equation:

\[
\int_{T_o}^{T_c} \alpha_2 dT = \ln \frac{\varphi_2(T_c)}{\varphi_2(T_o)} \approx \frac{\Delta \varphi_2}{\varphi_2(T_o)} = \left( \varphi_2(T_c) - \varphi_2(T_o) \right)
\]

where \( T_o \) and \( T_c \) correspond to the onset and completion temperatures of the peak, respectively. It should be noted that for an aqueous system of phospholipid vesicle dispersion, the apparent molar volume of the phospholipid can be regarded as approximately equal to its partial molar volume. The above equation is, to be sure, mathematically correct as long as \( \Delta \varphi_2 \) is sufficiently small relative to \( \varphi_2(T_o) \), but it is not consistent with the actual analysis of the peak area on a computer in that there is no term corresponding to the baseline subtraction in Eq. (8). This means that the integral in the left-hand

![Figure 1](image_url)
side of Eq. (8) is not equivalent to the peak area. Therefore, it is logically inappropriate to estimate the $\Delta \phi_2$ value by substituting the numerical result from the computer analysis of the peak area into Eq. (8) without taking account of the contribution of the baseline subtraction, even though the magnitude of the error caused by ignoring the baseline subtraction is trivial. To avoid this logical disagreement, we adopted an alternative method to estimate the $\Delta \phi_2$ value, which is described later.

Figure 2 shows a plot of $\phi_2$ versus $T$ for the DPPC bilayer membrane, where the open circles and the solid line, respectively, indicate the results obtained from densitometry and by applying the following recurrence relation to the PPC data.

$$
\phi_2(T_i) = \phi_2(T_{i-1})[\alpha_2(T_{i-1})\Delta T + 1]
$$

Here, $T_i$ is defined as $T_i = T_0 + i\Delta T$, and $\Delta T (= 0.2 \, ^\circ\text{C}$ in this study) corresponds to the temperature interval between two consecutive PPC scans. We selected 36.0 $^\circ\text{C}$ as $T_0$ and used 705.0 cm$^3$ mol$^{-1}$ as $\phi_2(T_0)$, which is the value obtained from the densitometry. It should be also noted that $i$ is not a natural number but an integer. This means that $i$ can take a negative value when the temperatures at which the PPC scans were carried out are below $T_0$ of 36.0 $^\circ\text{C}$. As is clear from Fig. 2, the $\phi_2$ values calculated by applying Eq. (9) to the PPC data (i.e., the solid line in Fig. 2) are in good agreement with those from the densitometry. This agreement indicates the reliability of the PPC experiment in its application to aqueous systems of phospholipid vesicle dispersions, as already proved for other aqueous systems including a pure water [8,14]. Looking at the detail of the plot very carefully, however, we notice that there is a very slight but definite deviation between the results from the PPC and the density measurements in the lower temperature region, though the deviation is so small as to be regarded as experimental error. This difference is probably due to the fact that the PPC scans were not able to detect the pretransition as a clear peak.

**Figure 2.** Apparent molar volume $\phi_2$ of DPPC versus temperature curve. A solid line and open circles represent the results from PPC and density measurements, respectively.

| Table 1. Transition volume $\Delta \phi_2$ for DPPC bilayer membrane. |
|---------------------------------|----------------|----------------|
|                                 | $\Delta \phi_2$ / cm$^3$ mol$^{-1}$ | $P_0 / P_0'$ |
| --------------------------------|---------------------------------|---------------|
| PPC                            | n. d.                           | 22.7 ± 2.62   |
| Densitometry                   | 2.5                             | 24.0          |
| Clapeyron Eq.$^a$              | 1.9                             | 25.4          |
| a) data from Ref. [3].         |                                 |               |
Finally, we summarize the volume properties of the DPPC bilayer membrane revealed by the PPC and the density measurements. As for the estimation of the $\Delta \Phi_2$ value from the PPC result, we have not adopt the conventional procedure based on Eq. (8) and the area of the peak found in the PPC curve because it is, in a strict sense, not appropriate for the reason as described already above. Instead, we determined the $\Delta \Phi_2$ value from the abrupt change of $\Phi_2$ at the main-transition temperature in the $\Phi_2$ versus $T$ curve. This method was applied also to the result from the densitometry. The $\Delta \Phi_2$ values of the main transition obtained from the PPC and the density measurements are 22.7 ± 2.62 cm$^3$ mol$^{-1}$ and 24.0 cm$^3$ mol$^{-1}$, respectively, which are in good agreement with our previous result of 25.4 cm$^3$ mol$^{-1}$ [3] obtained by applying the Clapeyron equation to the thermodynamic data of the main transitions. Regarding the pretransition, on the other hand, the PPC measurement could not determine the $\Delta \Phi_2$ value because the abrupt change of $\Phi_2$ is not discernible at the transition temperature in the $\Phi_2$–$T$ curve. All these values are listed in Table 1. In Table 2, the $\alpha_2$ values of DPPC in its bilayer membrane for each bilayer phase state, namely $L'_{p^1}$, $P'_{p^1}$ and $L_{\alpha}$ phases, are listed together with several literature values. From the densitometry, the $\alpha_2$ value for each phase was determined as a slope of ln $\Phi_2$ vs $T$ curve (i.e., $(\partial \ln \Phi_2/\partial T)_H$) in the temperature range corresponding to each phase. The $\alpha_2$ value to the $P'_{p^1}$ phase from the densitometry is slightly higher than those from the other measurements, but except for this there is good agreement in these $\alpha_2$ data, which demonstrates that the $\alpha_2$ value for the $P'_{p^1}$ phase is higher than those for the other phases. This may indicate that the DPPC bilayer membrane in the $P'_{p^1}$ phase state has the most thermally unstable structure due to the undulation of the bilayer surface (i.e., ripple structure) as compared to those in the $L'_{p^1}$ and the $L_{\alpha}$ phase states.

| Table 2. Thermal expansion coefficient $\alpha_2$ of DPPC in its bilayer membrane. |
|-------------------------------|-------------------------------|-------------------------------|
| $10^3 \alpha_2 / K^{-1}$  | $10^3 \alpha_2 / K^{-1}$  | $10^3 \alpha_2 / K^{-1}$  |
|-------------------------------|-------------------------------|-------------------------------|
| PPC                           | PPC                           | PPC                           |
| $L'_{p^1}$                     | $P'_{p^1}$                     | $L_{\alpha}$                  |
| 0.87–1.02                     | 1.37–1.44                     | 1.10–1.15                     |
| Densitometry                  | Densitometry                  | Densitometry                  |
| 1.02                          | 1.89                          | 1.26                          |
| PPC                           | PPC                           | PPC                           |
| 1.14                          | 1.39                          | 1.1                           | [13]|
| Densitometry                  | Densitometry                  | Densitometry                  |
| 0.95 ± 0.1                    | 1.35 ± 0.1                    | 1.00 ± 0.1                    | [15]|
| Densitometry                  | Densitometry                  | Densitometry                  |
| 0.9 ± 0.07                    | 1.4 ± 0.15                    | 0.94 ± 0.07                   | [16]|

5. Conclusions
We have revealed the volume properties of the DPPC bilayer membrane by means of the PPC and the density measurements. The $\Delta \Phi_2$ value for the main transition was estimated to be 22.7 ± 2.62 cm$^3$ mol$^{-1}$ from the PPC and 24.0 cm$^3$ mol$^{-1}$ from the densitometry. These values were consistent with our previous result obtained from the calculation using a thermodynamic relation. The $\alpha_2$ values for the $L'_{p^1}$, $P'_{p^1}$ and $L_{\alpha}$ phases were obtained, respectively, from the PPC and from the densitometry. The $\alpha_2$ values for each phase from both kinds of measurements were in good agreement with each other and also with the literature values except that the $\alpha_2$ value for the $P'_{p^1}$ phase from the densitometry was slightly larger than those from the other kinds of measurements. All the agreements confirmed in this study strongly support the reliability and accuracy of the result from the PPC experiment. However, the PPC is not always superior to other techniques; that is, in this study the PPC measurement could not detect the pretransition as a clear peak, so that the $\Delta \Phi_2$ value for the pretransition was not clarified by the PPC. This may be due to the small change in $\Phi_2$ at the pretransition or due to the overlap of the large peak of $\alpha_2$ at the main-transition temperature in the PPC curve.
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