High-pressure dilatometer

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A high-pressure dilatometer capable of measuring volume changes of 2×10^{-6} cm^3 is described. Fractional volume changes of 1 part in 10^4 can be measured over a temperature range of -30–90 °C, and a pressure range of 1 bar (0.1 MPa)–3 kbar (300 MPa). The dilatometer is constructed out of a commercial high-pressure valve and a compact home-built pressure sensor. The system was specially designed to study lipid-water biological systems. Preliminary studies on the lipid dioleoylphosphatidylethanolamine (DOPE) revealed the existence of phases not previously reported for this lipid.

I. INTRODUCTION

During the course of structural investigations of lipid-water lyotropic liquid crystal systems, it was determined that x-ray diffraction studies could not determine all the structural parameters of interest in low-dimensional liquid-crystalline systems. For example, while the x-ray data were of sufficient quality to measure the electron density distribution in the unit cells from both the one-dimensional lamellar L_α and the two-dimensional inverted hexagonal H_{II} phases,[1,2] a lack of knowledge of the structure of the liquid crystal in all three directions meant that important structural parameters such as the area per head-group and the lipid molecular volume could not both be determined from the x-ray data alone. A high-pressure dilatometer was constructed to provide data complementary to the x-ray measurements to allow determination of the missing parameters, as well as to determine additional thermodynamic parameters of interest, such as the enthalpy and entropy of transition, as a function of temperature and pressure.

II. HIGH-PRESSURE DILATOMETRY

Measurements of changes in specific volumes provide a sensitive method to monitor phase changes. It is therefore not surprising that many implementations of designs for dilatometers working at ambient pressure have been described.[3] Pioneering work by Nagle's group[4] has shown that dilatometry can be fruitfully applied to biological systems. However, the construction of *high-pressure* dilatometers has received less attention, although a number of instruments capable of obtaining *P-V-T* (pressure-volume-temperature) data have been described in the literature,[3,5] using approaches different from the method of controlled volume variation described here.

A. Design criteria

The design of a high-pressure dilatometer for studying biological materials is determined by the unique difficulties associated with aqueous solutions. Changes in volumes in biological samples are dominated by effects of the bulk compressibility of water. Extracting the contributions from dispersed biomolecules therefore imposes constraints on the sensitivity of the system.

(1) *Resolution of the instrument:* Biologically interesting lipid-water phase transitions include the well-known lamellar melting transition which is accompanied by correspondingly large changes in enthalpy, volume, and other extensive thermodynamic quantities, as well as weaker first-order nonlamellar transitions which are associated with much smaller changes in the extensive variables. Typical volume changes associated with nonlamellar phase transitions at ambient pressure are thought to be around 10^{-3} ml/g. Measurement of the small changes in transition volumes in nonlamellar transitions to within 10% requires that the system be capable of measuring volume changes of 1 part in 10^4. This sensitivity may also be sufficient to measure volume changes accompanying the denaturation reaction in some protein solutions which are on the order of $\sim0.1\%$.[6,7]

(2) *Precision of pressure control:* Both the pressure and temperature of the sample have to be controlled in order to be able to measure the volume changes to high accuracy. The typical bulk compressibility $B=-(1/V)(\Delta V/\Delta P)$ of aqueous materials is $\sim5\times10^{-5}$ bar^{-1} (5×10^{-4} MPa^{-1}). A fluctuation in the pressure of the system of 10 bar (1 MPa) leads therefore to fractional volume changes of $\sim5\times10^{-4}$. For studies conducted at 1 bar (0.1 MPa), Wilkinson and Nagle[4] used the ambient atmosphere to maintain the pressure to an accuracy of 0.1 bar (0.01 MPa) and were able to measure fractional volume changes of better than 10^{-5}. For high-pressure studies, controlling pressure to within this accuracy at pressures of 2 kbar (200 MPa) poses a considerable challenge. The system reported here is capable of measuring and controlling the pressure to about 3 bar (0.3 MPa), which corresponds to measuring fractional volume changes of $\sim10^{-4}$. This is adequate for the detection of volume changes in most phase transitions of interest.

(3) *Precision of temperature control:* The thermal expansion of water at room temperature is $\sim2\times10^{-4}$ K^{-1}, so controlling the temperature to within ~50 mK at room

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temperature is sufficient to measure fractional volume changes of $\sim 10^{-5}$. The temperature control system used in this experiment was capable of controlling the sample temperature to within 10 mK.

(4) *Additional requirements:* In order to measure volume changes across phase transitions, which in lyotropic (multicomponent) membrane systems are not always sharp, some thought has to be given to consistent definitions of locating phase transitions and selecting points on the curves from which to measure ΔV. The main *systematic* error in determining volume and enthalpy changes is in the procedure for choosing baselines before and after the transition.

III. EXPERIMENT

Most conventional dilatometers measure volume changes at fixed pressures, where the expanding sample pushes a piston attached to a position sensor. At high pressure such systems suffer from excessive friction at the seals of the high-pressure feedthroughs for the piston, which ultimately limits the resolution of the instrument. In our design, pressure is measured as a function of controlled volume changes, and so the friction problem is bypassed.

A. Apparatus

Figure 1 is a schematic of the dilatometer, which consists of a modified commercial right-angle high-pressure valve (30-12HF4-HT, HIP, Erie, PA). These valves have a hardened steel piston with a diameter of 0.1980 ± 0.0005 in. (5.029 ± 0.013 mm). The precision of the piston diameter was verified by disassembling several valves and measuring the diameters with a digital micrometer. The valve was modified in four ways: (i) The bottom opening of the valve was enlarged from 0.125 in. (0.316 cm) to 0.25 in. (0.63 cm) to allow easy loading of the sample capsule. (ii) The valve piston was shortened by 0.75 in. (1.90 cm) to allow a larger sample cell volume. Sample capsules with a maximum volume of 500 μl could then be placed inside. (iii) The handle of the valve was removed and was replaced by a 4 in. (10.1 cm) belt driven gear (Berg, East Rockaway, NY) which was coupled to a stepper motor driver assembly.

The position of the valve piston is controlled by a computer driven stepper motor (4SHG-120A-56S, Airpax, Cheshire, CT). The position of the piston, and thus the cell volume, can be measured by a position transducer, which is a linearly variable differential transformer (S5-200AG, LVDT, Sensotec Inc., Columbus, OH). By advancing and retracting the valve piston, the sample inside the valve will be pressurized or depressurized. Thus the piston is used for both pressure generation and for volume measurements, allowing for a more compact cell design. This pressure change is measured by a homemade low volume pressure sensor connected to the valve. The temperature of the chamber is also sensed and regulated by two commercial Platinum RTDs (Omega Engineering, Stamford, CT), which are interfaced to the computer. An automated *P-V-T* diagram of the sample can then be generated via appropriate software.

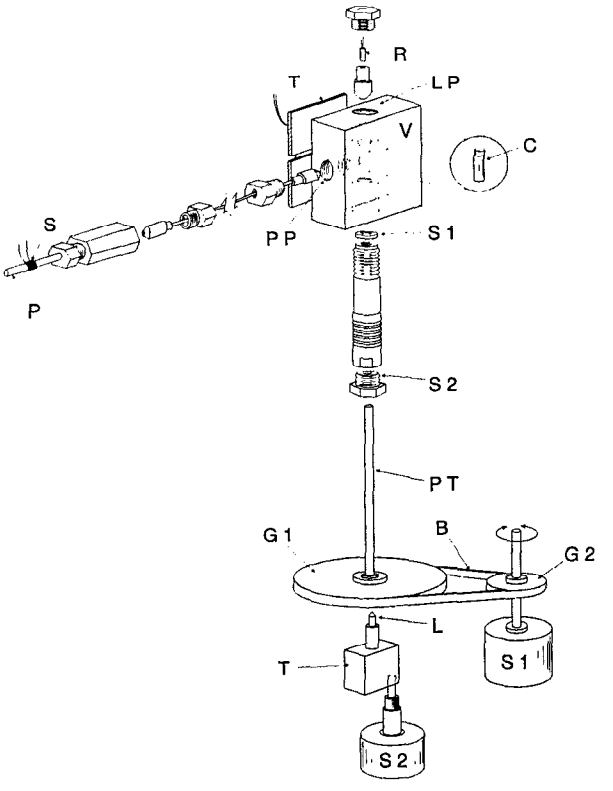

FIG. 1. Schematic of the high-pressure dilatometer. A modified high-pressure–high-temperature valve is used as the sample cell. *V* is the valve body. *S*1 is the first seal which connects the valve body to the elongated valve stem. *S*2 seals the nonrotating valve piston, PT, against high pressure. LP is used as the loading port; the RTD temperature sensor, *R*, is mounted in the sealing plug of this port. The temperature of the dilatometer is controlled by the thermoelectrics, *T*. The RTD temperature sensor and the thermoelectrics are controlled by a single board computer. The second port, PP, is connected to the pressure sensor, *P*. A strain gauge *S* is attached to the pressure sensor. The strain gauge is read by the pressure controller single board computer which controls the pressure by either advancing or retracting the piston, PT, via the gear *G*1 affixed on the piston stem. The gear *G*1 is connected to a smaller gear *G*2 by a toothed driving belt *B*. The gear *G*2 is coupled to a stepper motor/reducer assembly, *S*1, which is directly controlled by the pressure controller. The position of the piston is measured by a LVDT, *L*. The output of the LVDT is amplified by a lock-in amplifier which is read by an *XT* computer. The LVDT is mounted on a translation stage, *T*. The translation stage position is controlled by a second stepper motor, *S*2, which is used to reset the LVDT when necessary.

B. Sample loading

Lipid-water samples are prepared by mixing known weights of lipid and water. A Cahn 29 Electrobalance (Cahn Instruments Inc., Cerritos, CA) is used to weigh the sample to within 10 μg. The sample is placed in a sample capsule which is a thin-walled steel cylinder 1-in. (2.54 cm) long, 0.22-in. (0.56 cm) diameter, with a wall thickness of 0.005 in. (0.1 mm). This cylinder is sealed with a close fitting Delrin cap. A 0.06-in. (0.2 mm) hole is drilled through the Delrin cap and a thin piece of Teflon (PTFE) tape is stretched over the hole and the cap is inserted in the cylinder, thereby securing the tape. The thin Teflon tape transmits pressure and prevents mixing of the sample and mercury. The capsule is then placed cap-side down into the valve. Mercury is used as pressurizing me-

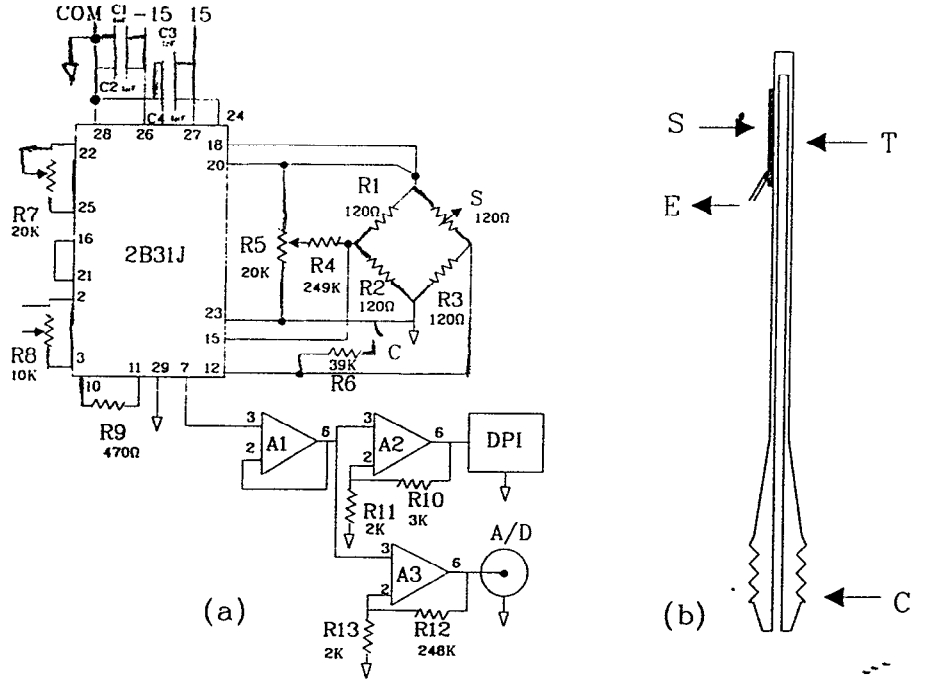

FIG. 2. Design of low volume pressure sensor. (a) A circuit diagram of the pressure sensor amplifier built for the dilatometer. *S* is the strain gauge (120 Ω) which is epoxy bonded to the pressure sensor. *R*1, *R*2, and *R*3 are identical strain gauges (120 Ω) used to balance the bridge which are in close proximity but are not bonded to the sensor. 2B31J is a hybrid amplifier chip. This chip provides bridge excitation voltage which can be adjusted by R7, a 20-K adjustable resistor. A gain of 200 is programed with resistor R9 (470 Ω). Resistor R8 (10 K) provides fine gain control. Offset is adjusted by the R4 (249 K), R5 (20 K) resistor combination. *C* is a shut calibration switch associated with a shut calibration resistor R6 (39 K). *A*1 (OP27) is an op-amp and buffers the output of the amplifier chip. *A*2 (OP27) with resistors R10 (3 K) and R11 (2 K) amplifies the signal 1.5 times. This yields a 2000 bar to 2-V conversion. This voltage is fed to an LCD display digital panel meter, DPI. *A*3 (OP27) with resistors R10 (248 K), R11 (2 K) amplifies the buffered signal by 125 times and outputs the signal to the Octagon single board computer *A/D* input via a coaxial connection. 2B31J input voltage isolation capacitors have the values: *C*1, *C*3 (1000 pF) and *C*2, *C*4 (1 μF). (b) A schematic drawing of the low-volume high-pressure sensor. *T* is a high-pressure steel tube thinned down to a wall thickness of 0.0275 in. (0.7 mm) for sensitivity. Standard 1/4 in. (0.63 cm) cone seal threads are machined on one end of the tube. A strain gauge *S* is attached to the tube using epoxy and is connected to the electrical amplification circuit *E*. There is a close fitting steel rod placed inside the steel tube to minimize dead volume (not shown).

dium because of its low compressibility and because it does not react with the lipid sample. Since mercury also has higher density, the sample is trapped securely inside the capsule.

C. Displacement driver and transducer

As stated earlier, the pressure change in this system is generated by advancing or retracting the piston of the modified high-pressure valve. The piston stem is driven by a computer controlled stepper motor-gear system to generate a smooth and controlled displacement. The gear system was designed for a reduction ratio of 1×10^5:1 so that one step of the stepper motor coupled though the gear chain corresponds to 1×10^{-5} of a rotation of the valve stem. Due to friction and backlash the smallest displacement which can be reliably generated is about 0.1 μm. Given the valve piston diameter of 0.198 in. (5.029 mm), this corresponds to a volume change of 1 nl. The stepper motor system was used for convenience because motors and controllers were readily available in our lab. In retrospect, considering the large torques required, a servo motor would have been a much better choice.

A linear variable displacement transducer (LVDT) (Sensotec, Columbus, OH) is used to measure the linear displacement of the valve stem. The LVDT is excited by 3-V_{rms}, 5-kHz ac signal and its response is amplified by a lock-in amplifier (Model 9502, Ortec, Brookdale, England). The output of the amplifier is interfaced to the control computer. The LVDT is mounted onto a translation stage which is itself coupled to a second stepper motor. The second stepper motor is computer controlled such that it will automatically advance or retract the LVDT stage to re-zero the LVDT after a full scale translation. This allows us to increase the dynamic range of the position measurement to 10^5 with a resolution of 0.1 μm.

D. Pressure transducer

Precision pressure measurement is crucial to accurate dilatometry. Commercial pressure transducers typically have a large a dead volume. Since measurement sensitivity is inversely proportional to system volume, the measurement sensitivity is compromised. We have constructed a strain gauge type pressure sensor with a dead volume less than 10 μl [Fig. 2(b)]. The pressure gauge is constructed out of a 0.25-in. (0.63 cm) diameter, 3-in. (7.6 cm) long high-pressure steel tubing (Vascomax, Latrobe, PA). A 0.125-in. (0.32 cm) hole is drilled 2.5 in. (6.3 cm) into the tubing. A standard high-pressure cone seal and thread are

cut on the open end of the tubing.[8] The last 2 in. (5.1 cm) of the tubing wall is then thinned down to 0.18 in. (0.46 cm). In order to minimize inelastic creep of the tube under high pressure, it is essential that the yield point of the steel is much higher than the maximum pressure to which the tube will be subjected. A typical rule of thumb is that the yield strength should be at least 20 times the maximum pressure. The yield strength of the Vascomax steel can be increased to 300 kpsi (about 2 GPa) by heat treatment. A strain gauge (Omega Engineering, Stamford, CT) is glued to the thinned tube longitudinally. Three additional free strain gauges are used to complete a standard bridge circuit [Fig. 2(a)]. All strain gauges are in close proximity to one another to minimize the effect of temperature fluctuations. The bridge is excited by a 3-V dc signal and the bridge response is amplified and output to computer interface electronics. Both the excitation and preamplification are handled by a single chip transducer amplifier (2B31J, Analog Devices, Norwood, MA). After a gain of 100 at the preamplifier, the signal is fed into an op-amp chain consisting of a buffer and another op-amp in a noninverting amplifier configuration with a gain of 200, resulting in a total gain of about 20 000. At this gain a 1-bar variation in pressure corresponds to a voltage change of 5 mV. The transducer is found to have a resolution of 3 bar (0.3 MPa) and an absolute accuracy of 40 bar (4 MPa) at the highest pressures used, which is comparable to the specifications of most commercial pressure sensors in this pressure range.

E. Temperature sensors and control

Following the now common practice in temperature control of cryogenic systems, the temperature sensor that monitors the sample temperature is distinct from the sensor that is used to control the temperature. To ensure temperature stability, the sample cell is installed in an evacuated sample chamber, equipped with vacuum feedthroughs for electrical and high-pressure connections. In addition, the stem of the HP valve is fed through the center of the base via a 1-in. vacuum feed through. The sample stage is attached to the side of the valve opposite to the mounting angle. This whole sample chamber is supported by two steel plates. Since the displacement transducer stage is mounted onto these steel plates, it is essential that the thermal expansion of the supporting steel plate matches the expansion coefficient of the valve stem. To accomplish this goal, these steel plates feature a two section construction where each section is made of steel with different thermal expansion coefficients (S.S. 304 with and Ph 17-4). The lengths of the individual sections are chosen to be 11 and $5\frac{7}{8}$ in., respectively. The ratio of these lengths is such that the combined thermal coefficient of the composite support plate matches the thermal coefficient of the valve stem. These supports are then bolted to an aluminum base plate which is placed in a sand box for vibration isolation.

IV. DATA ACQUISITION SYSTEM

All parameters of the dilatometer are under software control. This allows complex scan sequence to be programmed such that the P-V-T diagram of a system can be automatically mapped. An IBM XT computer serves as the central controller of this system. The actual control of both system volume, LVDT sensor rezeroing and temperature is delegated to two controllers each based on a single board computer (SBC) (SBS-2300, Octagon System Corporation, Westminster, CO). One SBC controller handles temperature control. The stepper motor for the displacement driver and the LVDT positioning driver are connected to the second SBC controller. Digital signals are used to control each of the stepper motor drivers. For pressure control, the controller reads the output of the pressure sensor via channel 0 of its analog I/O and turns on the stepper driver in the appropriate direction until the desired pressure is reached. The control of the LVDT is partially dictated by the IBM XT itself. When the XT detects that the LVDT lock-in amplifier approaches full scale, the XT alerts the SBC controller which stops the scan and repositions the LVDT until its output is reset. The dilatometer then resumes the scan. The output of the temperature, pressure, and position sensors are communicated to the XT via 12-bit A/D channels on a multifunction board (Lab Master from Tecmar Inc., Cleveland OH) plugged into the XT bus. The time-averaged data are stored into computer memory during a scan at user predefined intervals. The communication between the PC and the Octagon controller is accomplished via the digital port of the Tecmar board in the PC and the digital ports of the Octagon computers.

V. DATA COLLECTION AND ANALYSIS

The dilatometer is designed to operate in a pressure scan mode at constant temperature. After the sample is loaded and temperature has reached equilibrium, the sample volume is compressed or dilated continuously, and the resulting pressure change is measured. This sample can also operate in a temperature scan mode, but due to the significant backlash in the gear chain, the uncertainty in position measurement is much larger. The typical scan rate of the sample is about 1 bar every 10 s, so a typical scan from 100 bar (10 MPa) to 2000 bar (200 MPa) takes about 4 h. We found that the dilatometer is able to detect specific volume changes of about 1×10^{-4} ml/g. For a typical lipid sample sample with specific volume of 1 ml/g, this corresponds to a resolution of 1 part in 10^4.

A. Calibration

Since we are measuring the change in pressure as a function of total system volume change, it is necessary to perform calibration runs to subtract out the volume change contributions from the rest of the system in order to measure the true volume of the sample. Further, additional calibration is need to convert the LVDT voltage measurement to an actual volume measurement. The following sequence of experiments is performed. First, pressure scans at a series of temperatures is performed with the sample cell containing a known weight of the sample mixed with water. Second, the same series of scans is performed with a sample cell containing water alone. Third, this same series

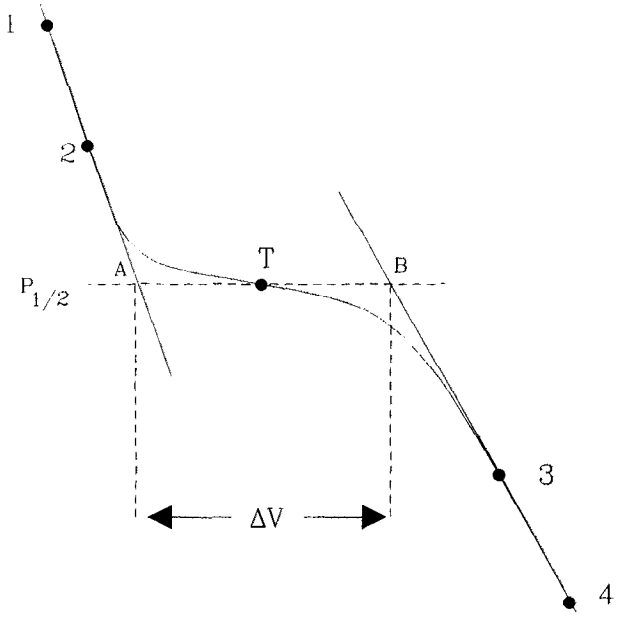

FIG. 3. Wilkinson–Nagle inflection method. Shown is a schematic of the method used for reconstructing volume changes in phase transitions which is based on the work in Ref. 4.

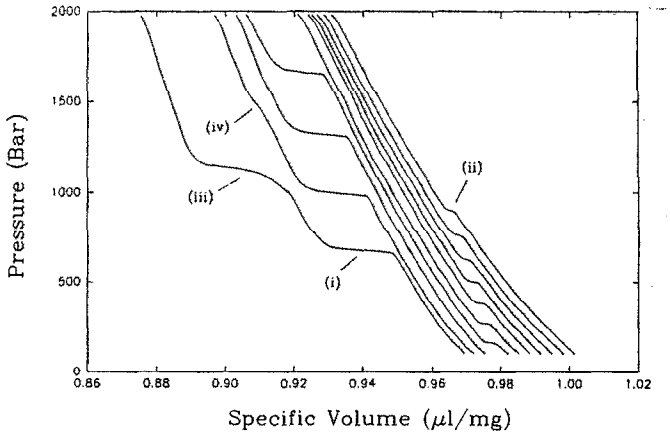

FIG. 4. Isotherms of the P-V-T diagram for DOPE-water system. Solid lines indicate experimental data, obtained using a sample with 290 mg of DOPE and 227 mg of water. At this concentration, the system is at "excess water" at all temperatures and pressures studied. Isotherms at 7.7, 12.3, 16.9, 21.7, 26.6, 31.6, 36.5, 41.5, 46.6, and 51.8 °C (from left to right) are shown. The phase transitions indicated are (i) the melting or lamellar liquid-crystalline-gel phase transition L_α–L_β; (ii) the inverted hexagonal to lamellar transition H_{II}–L_α. Other transitions indicated by (iii)–(iv) are gel–gel phase transitions not reported for DOPE water before, but are presumed to be similar to reported transitions in other lipid-water systems (Ref. 10).

of scans is performed yet another time with the sample cell filled with half mercury and half water. Since the specific volumes of both water and mercury as a function of temperature and pressure are tabulated in the literature,[9] the difference between the second and third set of scans yields a conversion factor between measured LVDT voltage output and the actual volume. Since we are looking at the difference of the two sets of scans, the contribution from the rest of the system is automatically cancelled out. With the LVDT voltage to volume conversion factor known, we can deduce from the difference of the first and second set of scans the difference between the specific volumes of lipid and water. One can then calculate the volume of lipid. Of course, in all these calculations, we have made the assumption that the specific volumes of lipid and water are additive, which is not exactly correct. Narayan *et al.*[2] have measured the volume change when water molecules are transferred from the bulk phase to the inverted hexagonal lipid-water phase. From their measurements deviations from additivity amount to fractional volume changes of less than $\sim 1 \times 10^{-5}$ which is much smaller than the resolution of our measurements.

B. Estimating ΔV in phase transitions

Many interesting transitions in biological multicomponent systems exhibit a marked degree of hysteresis and coexistence of low- and high-pressure phases. The hysteresis is due to the extremely sluggish nature of the phase transitions and is not due to instrumental artifacts. Under these conditions, a systematic procedure for picking the transition pressure and the transition volume is needed. Two different procedures were tried (Fig. 3).

Wilkinson–Nagle method: Shown in Fig. 3 is a schematic of the method employed which is similar to Wilkinson and Nagle's approach.[4] The point of greatest inflection is picked as the half-way point in the transition. A horizontal line ($\overline{AB}$, Fig. 3) through this point determines a half-way pressure $P_{1/2}$. Straight lines ($\overline{A21}$ and $\overline{B34}$) are fit to the data just before and after the transition and the volume change is read off.

Equal area method: An alternative procedure was to draw a horizontal isobaric line at a point where the area enclosed by the curve above the line is equal to the area enclosed by the curve below the line. This point determines the half-way point in the transition, and the volume change is read off as before.

Only slight differences are seen in the results obtained from these two methods and all results shown here employ the first approach.

C. Some results

Figure 4 shows some representative scans of specific volumes as a function of pressure for the lipid DOPE-water system at various temperatures. Both the main and the inverted hexagonal transition are clearly observable. In addition, we also found several additional transitions in the gel phase region which were not reported in the x-ray diffraction literature on DOPE water before. In several other lipid-water systems as a function of pressure, there have been reports of many gel–gel phase transitions.[5,10] The volume changes for the melting transition at 1 bar (0.1 MPa) for DOPE-water systems are in good agreement with the work of Wilkinson and Nagle on other lipids.[4] So far as we are aware, no one has reported the volume change $\Delta V_{L_\alpha - H_{II}}$ accompanying the pressure-induced inverted hexagonal to lamellar phase transition. It is interesting to

note that this last volume decreases as a function of pressure.

ACKNOWLEDGMENTS

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[1] D. C. Turner and S. M. Gruner, Biochemistry **31**, 1340 (1992).
[2] O. Narayan, P. T. C. So, D. C. Turner, S. M. Gruner, M. W. Tate, and E. Shyamsunder, Phys. Rev. A **42**, 7479 (1990).
[3] B. S. Hsiao, M. T. Shaw, and E. T. Samulski, Rev. Sci. Instrum. **58**, 1009 (1987).
[4] D. A. Wilkinson and J. F. Nagle, Anal. Biochem. **84**, 263 (1978).
[5] S. Utoh and T. Takemura, Jpn. J. Appl. Phys. **24**, 1404 (1985).
[6] P. L. Privalov, Adv. Protein Chem. **33**, 167 (1979).
[7] A. Zipp and W. Kauzmann, Biochem. **12**, 4217 (1973).
[8] V. C. D. Dawson, in *High Pressure Technology* edited by I. L. Spain and J. Paauwe (Marcel Dekker, New York, 1977), chap. 7.
[9] D. E. Gray, ed., *American Institute of Physics Handbook*, 3rd ed., (McGraw-Hill, New York, 1992).
[10] P. T. T. Wong, Ann. Rev. Biophys. Bioeng. **13**, 1 (1984).