BRIEF COMMUNICATION

CHANGE IN VOLUME MAGNETIC SUSCEPTIBILITY AT THE PHASE TRANSITION OF DIPALMITOYLPHOSPHATIDYLCHOLINE

R. K. KUNZE, JR., J. T. HO, AND E. P. DAY, *Department of Physics, State University of New York at Buffalo, Amherst, New York 14260 U.S.A.*

ABSTRACT We have observed a change at 41.4°C in the relative volume magnetic susceptibility of an aqueous dispersion containing 13 wt% multilamellar dipalmitoylphosphatidylcholine (DPPC) vesicles. The magnitude of the change is consistent with the known density change of the phospholipid bilayer and the assumption that the mass susceptibility of the system is constant through the transition. The superconducting susceptometer used in this study of the sharp transition of DPPC will be able to detect 1% changes in bilayer density for 10 wt% dispersions even when the transition is as broad as 10°. Therefore this new instrument can be used to follow changes in transition temperature and transition width of phospholipid vesicles under various experimental conditions.

INTRODUCTION

We have detected the density change at the main transition of the dipalmitoylphosphatidylcholine (DPPC) bilayer by measuring the relative volume susceptibility of a 13 wt% aqueous dispersion of multilamellar vesicles using a superconducting susceptometer. Measurements of the change in density (1) at the main phase transition of DPPC have been important along with calorimetry (2) in establishing the thermodynamic parameters of this transition. These studies along with x-ray measurements (3) have established that the main transition of DPPC is an order-disorder transition of the hydrocarbon tails which go from the ordered all-*trans* state below the transition to the disordered high temperature state containing many gauche conformations. This transition involves a net increase in the volume occupied by the hydrocarbon tails. We are interested in using the density change at this bilayer phase transition as a means of identifying the temperature and the width of the phase transition of various phospholipids under different experimental conditions in our studies of vesicle fusion (4, 5, 6).

Volume magnetic susceptibility (κ) measurements can be used to detect density (ρ) changes if the mass susceptibility (χ) is a constant because $\kappa = \chi\rho$. The results we report here for the change in the relative volume magnetic susceptibility at the main transition of DPPC are consistent with the assumption that the mass susceptibility of DPPC does not change at the main transition.

This is the first measurement of the change in relative volume susceptibility at the bilayer phase transition of an aqueous phospholipid dispersion. Preliminary measurements of the relative mass susceptibility of this same system have previously been performed (7). Absolute susceptibility measurements of the phospholipid bilayer have not been reported. Our results give a good indication of the developing sensitivity of superconducting susceptometers which are still several orders of magnitude short of their ultimate capability (8). The present results for the sharp transition of DPPC indicate that our superconducting susceptometer will be able to detect 1% changes in bilayer density for 10 wt% dispersions even when the change occurs over a temperature range as broad as 10°.

A preliminary account of this work has previously been reported (9).

METHODS

The buffer consisted of 0.1 M NaCl, 2.0 mM N-Tris-(hydroxymethyl) methyl-2-amino ethane sulfonic acid, 2.0 mM L-histidine, and 0.1 mM EDTA in twice-distilled water. The pH was adjusted to 7.4 with NaOH. The buffer was pumped briefly while boiling to remove dissolved oxygen and then stored under argon until needed. Synthetic DPPC (200 mg) received from Calbiochem (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) in powder form was dissolved in 2 ml of chloroform and then evaporated to dryness in a sample preparation tube. Degassed buffer (1.5 ml) was next added under flowing argon. The multilamellar vesicle dispersion was formed above the 41°C phase transition of DPPC by alternately immersing the sealed preparation tube for 30 s in a 65°C bath then shaking on a vortex mixer for 30 s. This heat-then-shake cycle was repeated until a clear dispersion resulted. The

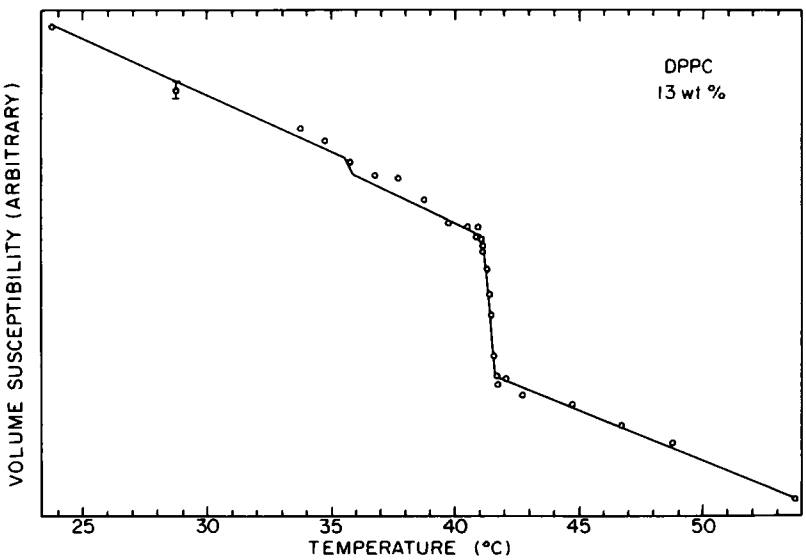

FIGURE 1. Relative volume magnetic susceptibility vs. temperature of an aqueous dispersion of multilamellar DPPC vesicles. Circles (o) are experimental data for a 13 wt% aqueous dispersion of DPPC vesibles with the quartz sample holder subtracted. The quartz sample holder signal subtracted at each temperature was found by measuring the peak insertion signal of the empty holder at the extreme temperatures and interpolating. The error bar at 29°C is the standard error of the mean and is representative of the uncertainty of all of the data. The nearly vertical line at the transition is a least squares fit to the data at the transition. The lines in the wings of the transition were calculated as described in the text.

preparation was next pumped briefly to prevent bubble formation during the ensuing susceptibility measurements. Finally it was transferred under flowing argon quickly and gently to the quartz nuclear magnetic resonance (NMR) sample tube which was capped and sealed.

The sample height was 7.3 cm in the 5-mm OD quartz sample tube before the susceptibility measurements began and 6.0 cm at the completion of the susceptibility measurements 1 wk later. The concentration change associated with this settling occurred slowly compared with the 1 d time interval of a single heating or cooling run.

The susceptometer is a prototype commercial version (10) of similar instruments described in the literature (8, 11). Its performance is based on the sensitivity of a superconducting quantum interference device (SQUID) and the stability of a persistent mode superconducting magnet.

The susceptometer was run continuously for months while these and other precision measurements were made. The magnetic field was set to 0.125 T and allowed to settle for 3 wk before these measurements. Field stability was checked by repeated measurement of a standard quartz sample. The worst case change in field over an entire heating or cooling run caused a total change of less than one half of an error bar at one temperature on Figs. 1 and 2.

Volume susceptibility measurements were made by detecting the change in SQUID detector output as the sample was moved from outside the detection region into the position of maximum signal. The liquid sample was made long enough that its meniscus was well outside the detection region when the

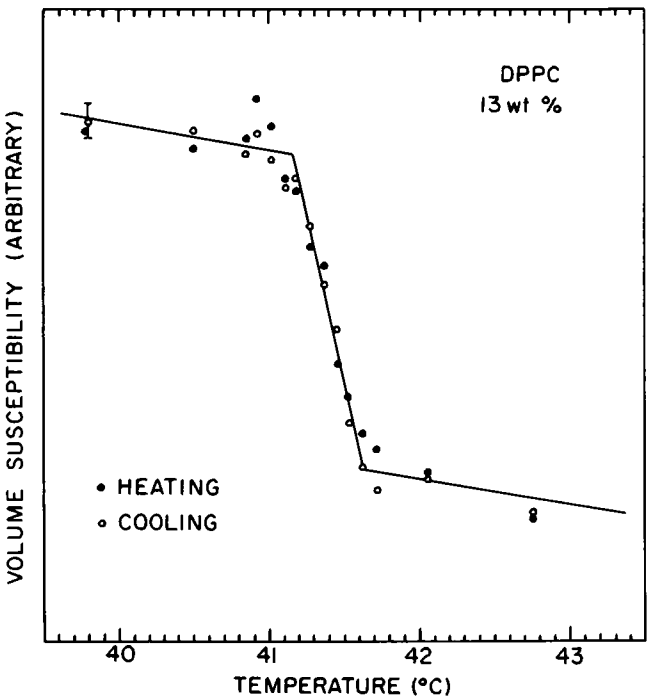

FIGURE 2. Relative volume magnetic susceptibility vs. temperature of an aqueous dispersion of multilamellar DPPC vesicles. Circles are experimental data for a 13 wt% aqueous dispersion of DPPC separately heated (•) and cooled (o) through the transition. The quartz sample holder signal subtracted at each temperature was found by measuring the peak insertion signal of the empty holder at the extreme temperatures and interpolating. The error bar at 39.8°C is the standard error of the mean and is representative of the uncertainty in all the data. The nearly vertical line at the transition is a least squares fit to the data at the transition. The lines in the wings of the transition were calculated as described in the text.

peak signal was detected. Therefore the changes at the meniscus with temperature were not detected and the signal was a direct measure of the sample's volume susceptibility.

The insertion region of the susceptometer has a temperature gradient of 1.5°C. Therefore the sample was continuously cycled over this insertion region at the data collection rate throughout a heating or cooling run to ensure uniform sample temperature during data collection. The flow of helium gas through this sample region was maintained at the same rate as during the temperature calibration of the susceptometer with a thermocouple. The quality of our data in the transition region (see Fig. 2) indicates an effective temperature gradient within the measured DPPC sample of ~0.1°C.

Liquid helium was transferred to the main dewar of the susceptometer, if required, a minimum of ½ h before a heating or cooling run. It was not transferred during a run.

Separate measurements over the temperature range from 26° to 55°C were taken on the empty quartz sample tube, on the sample tube filled with buffer, and on the sample tube filled with the vesicle preparation. Typically, ten round trip insertions taking 6 min each were made at each temperature with one or two of these being rejected due to the occurrence of a magnetic flux jump.

The error bars of Figs. 1 and 2 indicate the standard error of the mean, namely, S E = $\sigma/\sqrt{n}$, where σ is the standard deviation and n is the number of insertions at that temperature.

RESULTS

Fig. 1 shows the relative volume magnetic susceptibility vs. temperature in a single heating run from 24° to 54°C for a 13 wt% dispersion of multilamellar DPPC vesicles. The circles are the experimental data for the aqueous dispersion of vesicles with the quartz sample holder having been subtracted. The quartz sample holder signal subtracted at each temperature was found by measuring the peak insertion signal of the empty holder at the extreme temperatures and interpolating. Fig. 2 shows similar results for separate heating and cooling runs through the phase transition region. The overall slope in the data of Fig. 1 is primarily due to changes with temperature in the volume susceptibility of the buffer. A small part of this overall slope is due to changes with temperature in the volume susceptibility of the bilayer. The dramatic step change at 41.4°C seen in the data of Figs. 1 and 2 is due to the change in volume susceptibility of the bilayer at the phase transition.

The data were analyzed for consistency with the density measurements of Nagle and Wilkinson (NW) (1). Three separate linear least squares fits to the data below, at, and above the transition were used to determine the end points of the transition and the change of signal at the transition. The nearly vertical straight lines through the main transition in Figs. 1 and 2 are least squares fits to the data at the transition. The least squares fits to the wings of the transition are not shown in the figures. From the observed change in signal at the phase transition and using NW's value of 0.037 for $\Delta\rho/\rho$ at the phase transition, the concentration of DPPC in the sample was calculated to be 12.7 ± 0.4 wt%. Using this value for the DPPC concentration, NW's values for the thermal expansivity of DPPC,[1] and our volume susceptibility data for the buffer; we calculated the expected temperature dependence of the volume susceptibility of the vesicle sample. These are the lines drawn through the wings of the transition in both Figs. 1 and 2. Although the calculated curve of Fig. 1 shows the pretransition contained in NW's data, our susceptibility data is of insufficient precision to

[1]The thermal expansivity of DPPC was taken to be 83×10^{-5} ml/g below the main transition and 50×10^{-5} ml/g above the main transition.

resolve the pretransition. The good agreement between the calculated curve and the data shown in both figures indicates that our data are consistent with those of NW.

Using the NW criterion that the transition width ΔT be determined by the minimum temperature change corresponding to one-half the total volume change, we get $\Delta T = 0.26°C$, which is almost twice NW's reported $\Delta T = 0.15°C$ for DPPC. Our larger ΔT is due to a temperature gradient of 0.1°C in the sample caused by the temperature gradient in the sample region of the susceptometer. The mid-point of the transition we observe (41.40 ± 0.05°C) is consistent with the data of NW.

The signal to noise of the data in Figs. 1 and 2 indicates our susceptometer will be able to detect the 1% density changes at phospholipid phase transitions in 10 wt% dispersions even when the changes occur over a temperature range as broad as 10°.

This experiment indicates the feasibility of studying the conformation of diamagnetic biopolymers in aqueous solution through precision susceptibility measurements. The technique requires comparable sample material (200 mg) and comparable data collection time (10 h) to the dilatometry measurements of NW. Our ability to detect a relative volume change of 0.02% when the mass susceptibility is constant gives us slightly less sensitivity than that of NW. Improved precision could be achieved by using less water. Moreover, the present technique offers the potential advantage of providing unique information. The temperature dependence of the mass diamagnetic susceptibility of pure water has been determined with great precision (12). It is unusually large compared with other liquids (13) and is not understood though it bears a striking similarity to the temperature dependence of water's electric susceptibility (14). Further study of the temperature dependence of water's diamagnetism as a function of ionic strength and in comparison with other liquids may establish a correlation between the temperature dependent diamagnetism of water and the structure of water. Precision studies of the mass susceptibility changes at the phase transition of a concentrated phospholipid dispersion would then be of interest in establishing the effect of the bilayer on the structure of water.

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