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Laboratories and Demonstrations

Transition State Thermodynamics of Lipid Bilayers Characterized by Differential Scanning Calorimetry.

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The simplicity and relatively low cost of DSC instruments have made DSC readily accessible to most researchers and schools.

The laboratory experiment presented in this paper focuses on using differential scanning calorimetry to determine the calorimetric enthalpy and cooperativity of the gel to liquid crystalline phase transition in hydrated lipid bilayers as a function of cholesterol content. The procedure and analysis are appropriate for junior- and senior-level physics and biophysical chemistry courses in the undergraduate curriculum. The laboratory is used to emphasize the use of thermodynamic data to obtain information about structure–function relationships in biological systems. The experimental results are directly related to the authors' ongoing research in lipid bilayer structure characterization and applications of hydrated lipid bilayers as model systems for the interpretation of MRI contrast. The laboratory is easily modified to study the effects of other conditions, such as degree of hydration, pH, and composition, on the thermodynamic behavior of lipid bilayers.

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Introduction

Differential scanning calorimetry (DSC) is a technique commonly used for determining the thermal properties of a variety of materials including biologically relevant systems. The simplicity and relatively low cost of DSC instruments have made DSC readily accessible to most researchers and schools. The main applications of DSC include (a) determination of the effect of composition, hydration, pH, and solvent, on the phase-transition temperatures and enthalpies of biological membranes and pharmaceuticals; (b) thermal characterization of complex processes, such as the denaturation of proteins; and (c) specific heat measurements in the glass-transition of polymers.

Thermodynamics is a topic covered in undergraduate physics and chemistry courses. The calorimeter can provide a tool for a multidisciplinary laboratory experience in which concepts from a thermodynamics course can be examined experimentally. Laboratory experiments using calorimetry to study the denaturation of proteins have been presented [1, 2]. In particular, the paper by Chowdry and Leharne [1] presents an excellent DSC simulation experiment. We describe an empirical laboratory designed to use DSC for both a senior-level biophysical chemistry course and a junior-level physics laboratory course. The students experience a “real” biophysical experiment by determining the effect of cholesterol composition on the phase behavior of hydrated phospholipid bilayers, in particular, phosphatidylcholine. The thermal properties of lipids, which are a major component of biomembranes, have been characterized by DSC for several decades, making this system one that can easily be referenced. Students determine the transition temperatures and both the calorimetric and van’t Hoff enthalpies, and from this information can also determine the size of the cooperative unit. Students can prepare samples, acquire the DSC data, and analyze their set of experimental results in two 3-hour laboratory sessions.

Phase Transitions in Biological Systems

The elegant and precise secondary and tertiary structures of biological membranes, proteins, and polynucleotides are critical to performing their specific biological functions. These structures are stabilized by large collections of intra- and intermolecular hydrogen bonding and van der Waals interactions. Changes in the biological environment, such as alterations in temperature, pH, or solvent, may disrupt

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these relatively weak forces, leading to significant alterations in structure, and hence in function.

It is well known that the stability of these structures is very sensitive to temperature, and that changes in fluidity and permeability of membranes, denaturation of proteins, and the folding of helices occur within very narrow temperature ranges [3–5]. Long-range interactions, such that the state of one region of the structure affects the state of a neighboring or distant region, cause the physical properties to change within narrow temperature regions. The extent to which there is interdependence between regions reflects the stability and the cooperativity within the structure associated with transitions between states.

Biological membranes consist predominantly of lipid bilayers with proteins and sterols imbedded in this structure. Hydrophobic bonding stabilizes the orientation of the hydrocarbon tails toward the interior of the bilayer. The hydrophilic polar head-groups are oriented toward the surrounding aqueous phase. Biological membranes have varied roles in biological function. Cellular membranes (e.g., plasma and mitochondrial membranes) define the cell and its compartments, and serve as selectively permeable barriers to ionic, nonionic, polar, and nonpolar substances. Localization of chemical processes and physical processes in cells depends on the selective passage of compounds and ions through the cellular membranes. Myelin sheaths are membranes that surround the axons of brain cells and enhance signal conduction between neurons.

Hydrated phospholipids are used extensively as model systems to study membrane properties. The lipids assemble into bilayers, or lamella (**L**), characterizing the long-range order of the system [3, 6]. The physical characteristics of the bilayer are related to the short-range conformation of the hydrocarbon chains in the bilayer, which can be characterized as ordered (gel, β), or disordered (fluid, α). The characteristic transition between the gel and fluid lamellar states occurs when the energy added is sufficient to overcome the van der Waals interactions between the hydrocarbon chains, increasing rotational mobility around carbon–carbon bonds allowing them to assume more random, disordered, fluidlike conformations.

Studies of the physical properties above and below the transition temperature and during the thermal transition are important in understanding structure–function relationships. The effects of composition, solvent, and pH affect the energy,

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cooperativity, and transition temperature, and provide insight into the functional importance of the structure. The energy associated with the transition between states, and the temperature range over which the transition occurs, provide information about the stability and the cooperativity within the structure.

Thermodynamics of Bilayer Phase Transitions

The gel and liquid crystalline states of lipids depend on the existence of the large aggregates of lipid molecules in the ordered bilayer structure. As the aggregate gets smaller, there is less long-range order in the system. In the extreme case of one lipid molecule, one cannot consider a gel or liquid crystalline state. In the aggregate bilayer, the extent of long-range interactions determines the degree of cooperativity in the gel to liquid crystalline transition, the limit of cooperativity being all the molecules of the bilayer going between states together at a single temperature. Due to heterogeneity and the existence of discrete domains in the bilayers, the size of the cooperative unit tends to be less than the number of lipid molecules defining the bilayer. Nonetheless, these properties of long-range order and interactions leading to cooperativity are characteristic of phases and phase transitions [7].

The thermodynamic model for the phase transitions in hydrated lipid bilayers generally starts with the initial assumption of a two-state system, that is, a molecule or region of a molecule within the structure can be in only one state or another [4, 5]. Considering the thermal transition from the gel state to the liquid crystalline state, there is an enthalpy associated with the energy added as heat to overcome the intermolecular van der Waals forces between the lipid molecules. This is commonly determined using calorimetric techniques, and is usually measured as the enthalpy per gram of lipid (or per mole of lipid) for the transition between states, ΔH_{cal}, the calorimetric enthalpy.

The cooperativity of the gel to liquid crystalline transition describes the extent, as a function of temperature, to which the transition has occurred within the bilayer. The fraction of the bilayer in the liquid crystalline phase, Θ, can be described thermodynamically with an apparent equilibrium between the two states,

$$\text{gel} \rightleftharpoons \text{liquid crystalline}$$

$$K_{app} = \frac{c_{lc}}{c_{gel}} \qquad (1)$$

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$$\Theta = \frac{c_{lc}}{c_{lc} + c_{gel}} \qquad K_{app} = \frac{\Theta}{1-\Theta} \tag{2}$$

(The concentrations, c_{lc} and c_{gel}, have been normalized to the appropriate standard state.) As the temperature increases, there will be a shift in the fraction of sample in the liquid crystalline state, dictated by the temperature dependence of the equilibrium constant, K_{app}. A transition with a high degree of cooperativity means that Θ changes from very small to very large over a very narrow temperature range, and infers a very strong temperature dependence to the equilibrium constant. The temperature range of the gel to liquid crystalline transition in lipid bilayers is narrow, but finite [3, 8, 9], which supports a significant degree of cooperativity.

The degree of cooperativity can be determined from the temperature dependence of the extent of reaction, Θ, which is determined by the temperature dependence of the apparent equilibrium constant, K_{app}. The temperature dependence of K_{app} can be expressed using the van't Hoff expression [4, 10] defining an apparent enthalpy, $\Delta H_{van't\ Hoff}$,

$$\frac{d \ln K_{app}}{dT} = \frac{\Delta H_{van't\ Hoff}}{RT^2} \tag{3}$$

where R is the gas constant, and T is the temperature in kelvins. A cooperative transition means that the temperature dependence of K_{app} is very strong and the apparent, or van't Hoff, enthalpy will be large. Following the elegant theory developed for cooperative transitions, it has been shown that the van't Hoff enthalpy can be related to the size of the cooperative unit (*c.u.*). In particular, in the region of the midpoint of the transition the ratio of the van't Hoff enthalpy to the calorimetric enthalpy is equal to the *c.u.* [5, 11].

$$\frac{\Delta H_{van't\ Hoff}}{\Delta H_{cal}} = c.u. \tag{4}$$

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Intuitively, $\Delta H_{van't\ Hoff}$ reflects the enthalpy associated with the transition of the entire cooperative unit. ΔH_{cal} is the enthalpy associated with overcoming the intermolecular forces between molecules, or intersegmental forces within molecules.

Cooperativity is expected in hydrated macromolecular systems characterized by a macroscopic extended network of homogeneous intrasegmental and intermolecular forces. In proteins, *c.u.* is usually about one, which infers a high degree of cooperativity, with the whole protein molecule as the cooperative unit [4]. In lipid bilayers, *c.u.* can be as large as 1000, which demonstrates a high degree of cooperativity, where 1000 lipid molecules define the cooperative unit [8, 9].

Differential Scanning Calorimetry

Calorimetric methods have been used extensively to study the energetics involved in lipid phase transitions [12, 13]. DSC is used in this laboratory exercise to determine the temperature, calorimetric enthalpy (ΔH_{cal}), and cooperative unit of the gel to liquid phase transitions in hydrated phospholipid bilayers. The output of the calorimeter is the difference in energy, transferred as heat, required to maintain the sample and reference at the same temperature. As the temperature is raised, enough energy becomes available as heat to overcome the intermolecular forces between the hydrocarbon chains of neighboring lipid molecules. As this phase transition in the sample occurs, the energy will be absorbed by the sample at a higher rate than the reference to maintain the two at the same temperature, and therefore this process is endothermic. The amount of power absorbed will depend on the fraction of sample undergoing the transition at a particular temperature. This will be a maximum when $K_{app} = 1$, or at the midpoint of the transition. The standard output of the calorimeter can be used directly to determine T_m, ΔH_{cal}, and $\Delta H_{van't\ Hoff}$.

Calorimetric Enthalpy

The actual output of the calorimeter is the differential power between that supplied to the sample and to the reference as a function of time as the temperature of the system is scanned. This can be used to calculate the enthalpy of the transition by converting the ordinate from power to specific heat capacity, and converting the abscissa into temperature, using the following equations,

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$$C_{p,\,diff} = \frac{P_{diff}}{s.r. \times m}$$
$$T = s.r. \times t \qquad (5)$$

where $C_{p,diff}$ is the differential heat capacity (or heat capacity of the sample), P_{diff} is the differential power output of the calorimeter, *s.r.* is the scan rate in K sec^{-1}, m is the mass of the sample, T is the temperature in K, and t is the time in seconds. Example DSC curves for hydrated dipalmitoylphosphatidylcholine (DPPC) are presented in Figure 1. The enthalpy, ΔH_{cal}, is the energy transferred as heat at constant pressure, which is the area under the curve of heat capacity as a function of temperature,

$$\Delta H_{cal} = q_p = \int C_{p,\,diff}(T)dT \qquad (6)$$

Transition Temperature

The gel to liquid crystalline transition of lipid membranes is referred to as “melting” in analogy to solid–liquid phase transitions. For an isothermal transition, such as occurs for water, the melting temperature, T_m, is defined by a single temperature. For bilayer transitions, which occur over a temperature range, one definition for the melting point, T_m, is the midpoint of the transition where $K_{app} = 1$.

van’t Hoff Enthalpy and Cooperativity

The van’t Hoff enthalpy, $\Delta H_{van't\ Hoff}$, is derived from the temperature dependence of the apparent equilibrium constant for the phase transition (eq 3). Although the derivation of the mathematical relationship is quite involved, it is not difficult to understand that K_{app}, which defines the extent of transition, will depend on both (a) the thermodynamics of each elementary step involving the enthalpy to overcome the van der Waals interactions between individual lipid molecules, and (b) the thermodynamics of the transition of the cooperative unit [5, 11]. From eqs 2 and 3, an expression has been derived relating $\Delta H_{van't\ Hoff}$ to the temperature dependence of Θ,

$$\frac{d \ln K_{app}}{dT} = \frac{1}{\Theta(1-\Theta)}\frac{d\Theta}{dT} = \frac{\Delta H_{van't\ Hoff}}{RT^2} \qquad (7)$$

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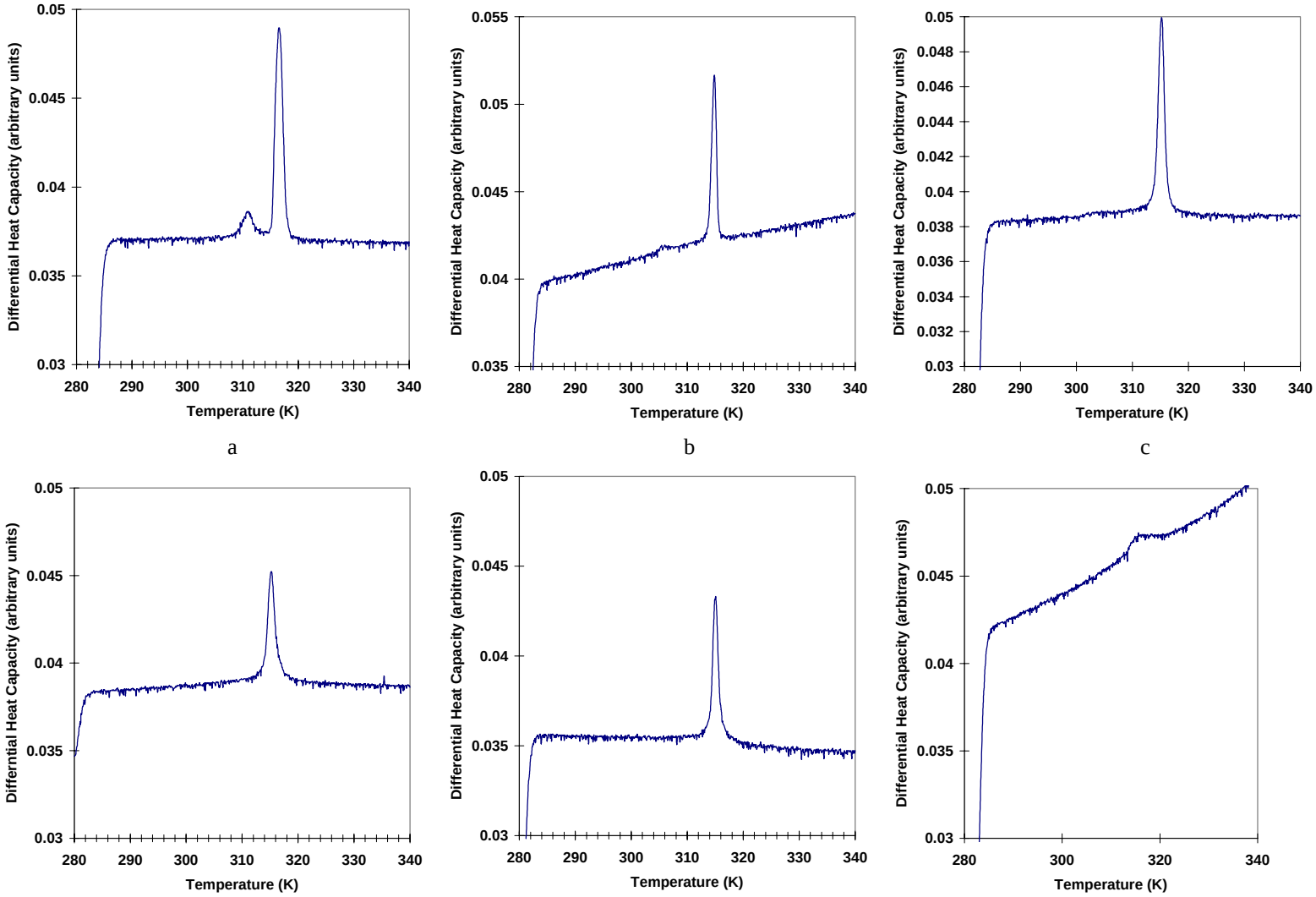

FIGURE 1. STUDENT-COLLECTED DIFFERENTIAL SCANNING CALORIMETRY DATA. THE DSC DATA ARE PLOTTED AS DIFFERENTIAL HEAT CAPACITY (IN ARBITRARY UNITS) VS TEMPERATURE FOR DIPALMITOYLPHOSPHATIDYLCHOLINE LIPID BILAYERS AS A FUNCTION OF CHOLESTEROL CONTENT. ALL SAMPLES WERE HYDRATED TO 50% BY WEIGHT WITH WATER. (A) 0 MOL % CHOLESTEROL. (B) 2.5 MOL % CHOLESTEROL. (C) 5 MOL % CHOLESTEROL. (D) 10 MOL % CHOLESTEROL. (E) 15 MOL% CHOLESTEROL. (F) 25 MOL % CHOLESTEROL.

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The extent of reaction as a function of temperature, $\Theta(T)$, can be determined directly from the fractional extent of the heat capacity curve taken as the ratio of the area from the initial temperature T_i, to T, over the area of the whole curve from T_i to T_f [9],

$$\Theta(T) = \left(\int_{T_i}^{T} C_{p,\,diff}(T)\,dT \right) \Big/ \left(\int_{T_i}^{T_f} C_{p,\,diff}(T)\,dT \right) \tag{8}$$

From eq 7, the slope of $\Theta(T)$ versus T can be used to calculate $\Delta H_{van't\ Hoff}$.

Following a statistical mechanical derivation, it has been shown that specifically at the *midpoint of the transition*, the slope of $\Theta(T)$ at T_m is directly related to the calorimetric enthalpy and the size of the cooperative unit [11, 14],

$$\left.\frac{d\Theta}{dT}\right|_{T_m} = \left(\frac{c.u.}{4}\right)\frac{\Delta H_{cal}}{RT_m^2} \tag{9}$$

Combining eqs 7 and 9, and $\Theta = 1/2$ at T_m, gives eq 4 (reproduced here as eq 10),

$$c.u. = \frac{\Delta H_{van't\ Hoff,\,m}}{\Delta H_{cal}} \tag{10}$$

Experimental Procedure

The experimental procedure describes using DSC to follow the thermodynamic affects of cholesterol concentration on the phase behavior of hydrated dipalmitoylphosphatidylcholine (DPPC) bilayers. Pure DPPC hydrated to 50% w/w undergoes two phase transitions. The first is called a pretransition, and it occurs at about 35 °C. This pretransition corresponds to a transition from $\mathbf{L}_\beta$ (lamellar gel phase) to $\mathbf{P}_\beta$ (rippled gel phase). The gel ($\mathbf{P}_\beta$) to liquid ($\mathbf{L}_\alpha$) phase transition (the main or chain-melting transition) occurs at about 42 °C. Incorporation of cholesterol into DPPC bilayers lowers the enthalpy and cooperativity of the phase transition [13]. The rigid cholesterol molecule incorporates into the hydrocarbon region, lying parallel to the lipid molecules. The cholesterol acts a spacer in the bilayer, separating the choline head-groups and lowering the van der Waals interactions between the hydrocarbon chains. A disordering effect is introduced in the gel phase, and an ordering effect into

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the liquid phase, creating a new phase denoted β [13, 15]. In general, the incorporation of cholesterol into the lipid bilayer affects permeability, fluidity, and hydration of the bilayer, properties clearly essential to the function of the system.

The lipid sample preparation and DSC data acquisition can be accomplished within a 3-hour laboratory period. We use a rotating laboratory schedule so that only one group is using the instrument at a time to maximize the hands-on participation in the experiments. The students work in groups of two. One lab partner takes responsibility for the sample preparation, while the other learns how to run the calorimeter and begins acquiring the reference data. The data analysis is done outside of lab or during a second laboratory period.

Sample Preparation

The two lipid samples described in this procedure are pure dipalmitoylphosphatidylcholine hydrated to 50% w/w with H_2O, and dipalmitoylphosphatidylcholine with 1–30 mol% cholesterol hydrated to 50% w/w with H_2O. The lipid and cholesterol were purchased from Avanti Polar Lipids (Alabaster, Alabama), and used without further purification.

To prepare the pure DPPC bilayer sample, a mass of approximately 5 mg of lipid was analytically transferred to the DSC sample pan. Assuming a water density of 1 g/mL, the appropriate mass of water was added volumetrically directly to the pan using a syringe. The pan was sealed and used directly in the differential scanning calorimeter.

To prepare the DPPC–cholesterol sample, the cholesterol was first incorporated into the DPPC by dissolving the appropriate quantities of DPPC and cholesterol in a small volume of chloroform. This was achieved by transferring approximately 20 mg of DPPC to a ~2-mL plastic conical vial. An appropriate volume of the cholesterol stock solution was added to the DPPC to attain the chosen mole percent composition. The cholesterol was provided to the students as a 20.4 mg cholesterol/mL solution in 2:1 chloroform–methanol. Approximately 0.5 mL chloroform was then added, and the solution was mixed. The bulk of the chloroform was evaporated using a stream of nitrogen. A perforated cap was placed on the vial, and the sample was further dried in a vacuum desiccator. Approximately 5 mg of the dried DPPC–cholesterol sample was transferred analytically to a DSC pan, and the appropriate mass of water was added to provide a 50% w/w sample.

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TABLE 1. Heating Parameters.

Sample	Initial Temperature	Final Temperature	Heating rate
Indium reference	400 K	450 K	5 K/min
Cyclohexane reference	260 K	290 K	5 K/min
Blank	280 K	340 K	5 K/min
Hydrated DPPC	280 K	340 K	5 K/min
Hydrated DPPC–cholesterol	280 K	340 K	5 K/min

Based on our experience, the student results for the pure DPPC sample are consistent with the literature, suggesting reproducible and accurate sample preparation. The DPPC–cholesterol sample preparation is more problematic. This is most likely due to the cholesterol stock solvent's volatility and/or the transfer of the stock solution to the lipid vial leading to uncertainty in the actual cholesterol content. In order to provide confidence for the instructor testing the procedure, the concentration of cholesterol could be checked using standard chromatography methods such as HPLC.

Data Collection

Data were collected on a Perkin-Elmer DSC-2 (Perkin-Elmer Corp., Norwalk, CT). The calorimeter is interfaced to an IBM PS/2 through an A/D board (Computer Boards, Inc., Mansfield, MA). Data were collected under software control with a program called CONTROL, supplied by Computer Boards. An empty pan was used in the reference cell, and the sample was placed into the sample cell. Each group ran two standards, indium and cyclohexane, for temperature and enthalpy calibration (Table 1). A blank scan (empty pan) was also recorded for baseline correction. Heating curves only were recorded.

Heating curves were run twice for the DPPC and DPPC–cholesterol samples, again to allow for hydration and sample homogeneity. The total time to acquire all the DSC scans is about 75–80 minutes, with another 30 minutes needed to allow for sample changes and resetting of the instrument.

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The scatter in the student data may in part be a result of sample inhomogeneity. To minimize this problem, the lipid samples, once sealed in the DSC pans, could be allowed to sit in a water bath at about 60 °C until needed. This would allow for more efficient hydration and equilibration of the lipid and, if using other lipid systems, disruption of metastable states that may initially form.

Data Analysis

Phase-transition temperatures

The temperature of the sample does not change during the transition for isothermal phase transitions, which occur for the pure reference substances indium and cyclohexane. The output of the calorimeter shows a transition with a finite width as a result of the thermal lag of the system. The transition temperature, T_m, is taken as the temperature at which the transition is first detected. For the lipid gel to liquid crystalline transitions, which are expected to occur over a broad temperature range, the transition temperature is taken as the midpoint of the transition. To accurately determine this temperature, the thermal lag of the system should be taken into account [16]. We have not taken this into account in the analysis presented here.

Phase-transition Enthalpies

The area under the DSC curve is proportional to the calorimetric enthalpy for the lipid samples. To avoid calibrating the ordinate, we have used the known enthalpy of the indium standard as a reference. If phase transitions for any two substances, *X* and *Y*, are measured on the same calorimeter, the transition enthalpies are related by

$$\frac{A_x}{\Delta H_x \cdot m_x} = \frac{A_y}{\Delta H_y \cdot m_y} \tag{11}$$

where *A* is the area under the phase transition peak, *m* is the mass of the substance, and ΔH is the phase-transition enthalpy. Knowing the phase-transition enthalpy for indium, the phase transition enthalpy for the lipid samples can be calculated by rearranging the above equation,

$$\Delta H_{lipid} = \frac{A_{lipid} \cdot m_{indium} \cdot \Delta H_{indium}}{A_{indium} \cdot m_{lipid}} \tag{12}$$

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ΔH_{indium} is 6.80 cal/gm.

The areas under the curves were calculated using either the program ORIGINS™ (MicroCal, Inc., Northhampton, Massachusetts) or Microsoft Excel. A straight-line baseline correction was used in both programs. This has been shown to introduce little error into the area analysis when the difference in heat capacity between the two phases is small [1]. The integration in Excel was based on a simple point-by-point trapezoidal integration of the peak areas.

Cooperativity

The size of the cooperative unit in the hydrated lipid bilayers is calculated based on eqs 6–10. $\Theta(T)$ is calculated again using trapezoidal integration in Excel. The slope of Θ versus T at T_m gives the van't Hoff enthalpy at T_m, $\Delta H_{van't\ Hoff,m}$. The size of the cooperative unit is calculated from $\Delta H_{van't\ Hoff,\ m}$ and ΔH_{cal} based on eq 10.

Results and Comments

Class data for the calorimetric enthalpies determined for dipalmitoylphosphatidylcholine 50% w/w in water are presented in Figure 2. The average ΔH_{cal} for the main gel to liquid crystalline transition is 7.6 ± 0.5 kcal/(mol DPPC). The transition is centered at the T_m of 316.3 K. Literature values for the main transition in aqueous dispersions of DPPC range from 7.7 kcal/(mol DPPC) to 8.7 kcal/(mol DPPC), with T_m about 314.5 K [12, 13]. The discrepancy in the transition temperature most likely results from our relatively rapid scan rate. The class data for the effects of the addition of cholesterol to the bilayer clearly show the expected decrease in the ΔH_{cal} for the main gel to liquid crystalline transition (Figure 2b). The values compare reasonably with those in the literature [13], though there is a significant amount of scatter. We believe the scatter is due primarily to the lack of precision in the sample preparation, specifically to the addition of the cholesterol stock solution to the dry lipid. Also, the samples with high concentrations of cholesterol had transitions that were very broad, making the determination of the DSC peak area difficult and therefore prone to error. Even with the scatter, the trend upon the addition of cholesterol is apparent and can be used to demonstrate the effect upon the bilayer thermodynamics.

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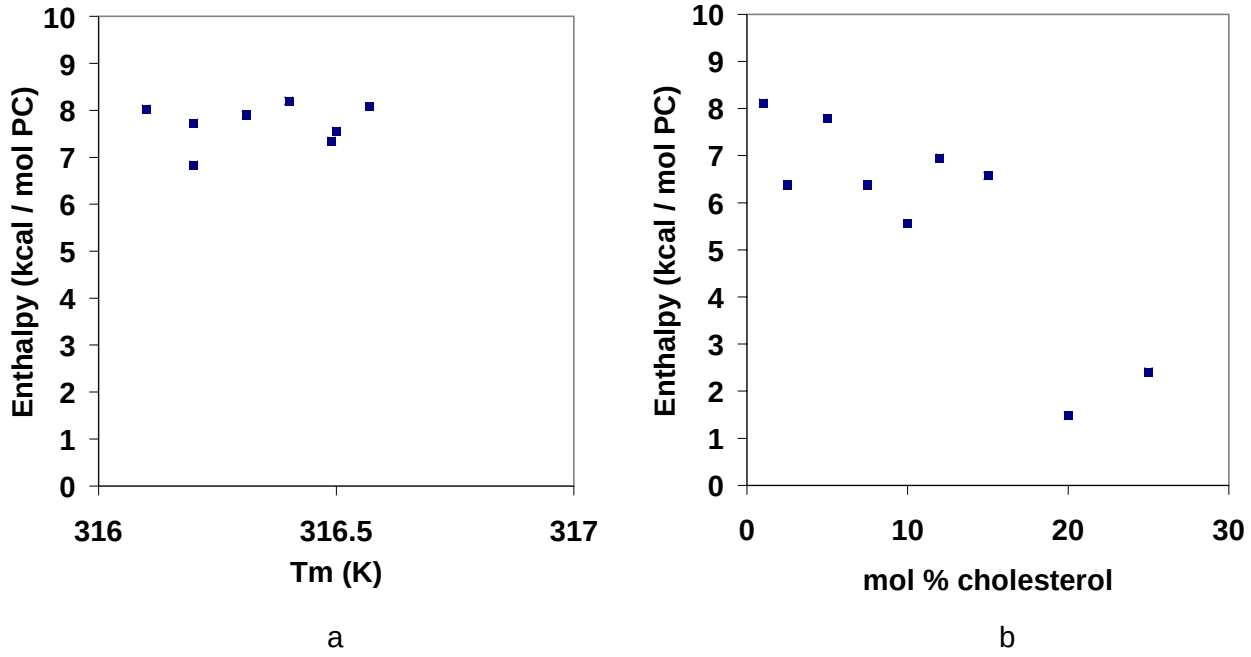

FIGURE 2: STUDENT DETERMINED CALORIMETRIC ENTHALPY DATA, $\Delta H_{cal,}$ FOR THE MAIN GEL TO LIQUID CRYSTALLINE PHASE TRANSITION IN HYDRATED DIPALMITOYLPHOSPHATIDYLCHOLINE BILAYERS. A) COMPILED DATA FOR ΔH_{cal} VERSUS TRANSITION TEMPERATURE, $T_{m.}$ B) COMPILED DATA FOR ΔH_{cal} VERSUS CHOLESTEROL CONTENT IN MOL % CHOLESTEROL.

The size of the cooperativity unit for the hydrated DPPC samples for the main gel to liquid crystalline transition ranged between 40 and 80. This is significantly lower than values reported in the literature [8, 9]. We associate the discrepancy with our relatively rapid scan rate, which was 10 to 50 times faster than those used by these investigators. Rapid scan rates result in broadened peaks and therefore low values of $\Delta H_{van't\ Hoff}$. Slower scan rates would be possible if the laboratory periods were extended, or if the data were collected over several successive laboratory periods. Also, we have used the lipid samples directly from the manufacturer without further purification. Any impurities present in the sample will effect the cooperativity. As inferred earlier, we also presume that the sample homogeneity would be improved by allowing the hydrated lipids to equilibrate for a period of time above the phase transition temperature.

The size of the cooperativity unit for the hydrated DPPC/cholesterol samples was calculated to be around 60–80 for all samples. There was no apparent correlation showing a decrease in cooperativity as the cholesterol content increased. The absence

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of an observable decrease in the size of the cooperativity unit is consistent with the literature [13]. As cholesterol is added, it incorporates nonuniformly into the bilayer, resulting in cholesterol-rich and cholesterol-poor domains. The DSC peak is a superposition of the gel to liquid crystalline transition peaks for these different domains. Though the cholesterol-rich region shows a decrease in cooperativity, the superimposed peak does not show a clear increase in peak width due to the contribution of the cholesterol-poor domain.

Student Discussion

This laboratory allows for a broad range of discussion . We have asked our students to address the proposed role of cholesterol in membranes [13, 17, 18], and to relate their results from this laboratory to these effects. One of the points we encourage them to recognize is the correlation of the decrease in the gel to liquid crystalline phase transition enthalpy with the disappearance of the phase transition as cholesterol is added to the bilayer. The students should also recognize that the endothermic peak corresponding to the gel to liquid crystalline transition is broadened and asymmetric in samples with high cholesterol content. They should interpret the asymmetry of the peak as resolvable into several components, which correspond to two different lipid domains. The better students may even suggest that the two different domains consist of one cholesterol-rich lipid domain and one cholesterol-poor domain.

Another area of discussion relates to the size of the cooperative unit (*c.u.*) and the definition of discrete phases. Pure substances, proteins in aqueous solution, and lipids in hydrated bilayers are all characterized as existing in characteristic phases depending on the temperature and composition of the system. In the pure substances indium and cyclohexane, the individual molecules are small and therefore there must be a large degree of cooperativity for discrete phases to exist. In hydrated lipid bilayers, the individual lipid molecules are relatively small. Discrete phases exists as a result of the cooperativity between on the order of 100 to 1000 lipid molecules within the bilayer. The fact that the *c.u.* is discrete in size may be used to introduce the concept of domains within the system. The c.u. is about one for proteins, which implies a highly cooperative transition where the protein molecule is large enough that a discrete phase is defined by a single molecule.

We envision a potential extension of this laboratory to the parallel characterization of the behavior of water in hydrated lipid systems using nuclear magnetic resonance and

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relaxation techniques. The properties of water are coupled to the lipid bilayer properties through water–macromolecular interactions in the hydration layer. These interactions make it feasible for water to be used a probe of hydrated macromolecular systems, and are the basis of generating contrast in MRI. By studying both the lipid and water phases of the system, the students would have the opportunity appreciate how the effects of composition on the properties of lipid membranes also lead to effects on water properties.

The laboratory provides a good example of using a state-of-the-art technique, DSC, to measure thermodynamic data that can be analyzed to address phase stability and structure–function relationships in biological systems. It can be easily modified to address effects of solvent, degree of hydration, pH, lipid composition, and purity on the phase behavior of lipid bilayers.

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