Characterizing the Gel to Liquid Crystal Transition in Lipid-Bilayer Model Systems

T. M. Koyama, C. R. Stevens, E. J. Borda, K. J. Grobe, and D. A. Cleary*

Department of Chemistry, Gonzaga University, Spokane, Washington 99258, cleary@gonzaga.edu

Abstract: A laboratory exercise that introduces differential scanning calorimetry (DSC) is presented. In order to connect this basic physical chemistry technique with an application in the biological sciences, students investigated the thermodynamic properties of lipid-bilayer model compounds. DSC was used to characterize the gel-to-liquid-crystal transition for three phosphatidylcholines of varying acyl chain length. The laboratory exercise is suitable only for students with some background in physical chemistry and biochemistry.

Introduction

As part of our ACS certified chemistry major, we offer a biochemistry emphasis [1]. This option is popular with students, and the majority of our majors complete it. In an effort to make the advanced laboratory component of the Chemistry major more relevant for students interested in biochemistry, we have modified several of our traditional laboratory experiments. For example, we recently reported how we use glycolysis as a vehicle for studying kinetics [2]. In this paper we describe a laboratory exercise where the gel-to-liquid-crystal transition of lipid-bilayer model systems is used to study basic thermodynamics.

Laboratory exercises designed to study thermodynamic principles are often tedious and difficult to execute. Modern instrumentation has alleviated some of the drudgery, but advanced chemistry laboratory courses continue to emphasize spectroscopy and synthesis. With support from the National Science Foundation Instrument and Laboratory Improvement (NSF-ILI) program, we acquired a differential scanning calorimeter. This instrument conveniently measures the heat capacity of a sample (relative to a reference sample) as a function of temperature. Differential scanning calorimetry (DSC) is used to characterize phase transitions with respect to such thermodynamic parameters as transition temperature, enthalpy of transition, entropy of transition, and glass transition temperature. It is used extensively in the polymer industry to characterize the thermal properties of plastics. In this laboratory exercise it is used to characterize the gel-to-liquid-crystal phase transition which occurs in liposomes composed of phosphatidylcholines.

Phosphatidylcholines are used as model systems for cell membranes because they will form bilayer structures. The phosphatidylcholine molecules consist of a polar head group connected to two long acyl chains. In water, these molecules form bilayers where the polar heads form the exterior surface. The structure, fluidity, transport properties, surface tension, and a host of other properties can be systematically studied with such model systems with the goal of understanding the more complicated cell membrane itself. These studies have relevance from topics as wide ranging as premature infant care to reindeer legs [3, 4]. The goal of this experiment is to introduce a standard physical chemistry experimental technique, differential scanning calorimetry, and show how this technique can be applied to a biological problem.

In order to determine some of the parameters that affect the transition temperature and the width of the transition, this exercise was performed with samples of varying acyl carbon chain length, samples containing varying levels of an impurity, and different scan rates for a given sample. For a molecular level description of the phase transition, students must return to their biology or biochemistry course material. The structure of lipid bilayers, liposomes, and the nature of the phase transition is covered in any standard biology or biochemistry text. The emphasis in this exercise is on the physical chemistry. A similar laboratory exercise devoid of any biochemical connection could be constructed where, instead of liposomes, students studied the phase behavior of a material like benzoic acid or tin. With the laboratory exercise presented here, however, we feel that a strong connection can be shown between basic physical chemistry and biochemistry. We are interested in making this connection because the majority of our chemistry majors express an interest in biochemistry and take upper division biochemistry courses.

The liposomes used in this experiment were all made from phosphatidylcholines. The phosphatidylcholines differed by the number of carbon atoms in the acyl chain. The acyl carbon chains all contained an even number of carbons and no multiple bonds. These compounds are insoluble in water, but they form aqueous suspensions of liposomes under the proper circumstances [5]. The gel-to-liquid-crystal transition involves a partial melting of the acyl chains while the spherical liposomes remain intact. In the liquid crystal state, the acyl chains become mobile within the hydrophobic region of the liposome while the anchoring of the polar head groups prevent complete freedom of movement.

In this laboratory exercise, three lipid-bilayer model systems were examined: L-α-dimyristoylphosphatidylcholine (DMPC), L-α-dipalmitoylphosphatidylcholine (DPPC), and L-α-distearoylphosphatidylcholine (DSPC). In addition, the gel-to-liquid-crystal transition of DSPC *intercalated* with cholesterol was examined. Intercalated is used here in the biological sense of inserting a guest molecule (or ion) into a void or interstice of a host complex. From a physical chemistry perspective, the cholesterol can be view as a solute and the liposome viewed as the solvent; hence, the interpretation of the effect of

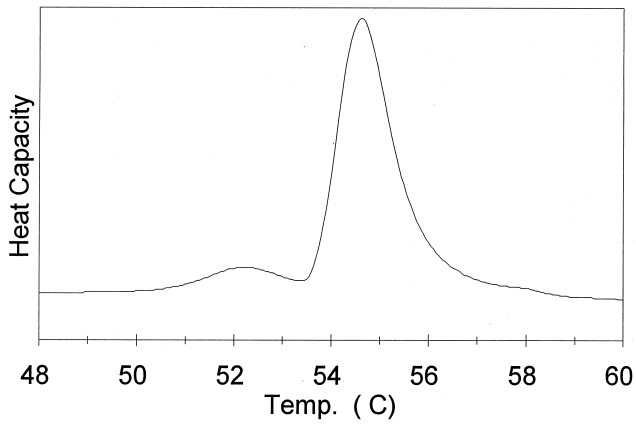

Figure 1. DSC thermogram of DSPC. The heating rate is 5 °C min^{-1}, and the mass of the lipid is 766 micrograms.

Table 1. Transition Temperature for the Gel-to-Liquid-Crystal Transition of Lipid-Bilayer Model Systems

Lipid	T_{trans}, °C (measured)	T_{trans}, °C[a]
DMPC (14:0)[b]	23	24
DPPC (16:0)	42	42
DSPC (18:0)	54	54

[a]From ref 6. [b]Number of carbons in the acyl chain: number of double bonds.

cholesterol on the gel-to-liquid-crystal phase transition can follow a standard colligative property argument.

High purity indium was also used in this experiment. It was used to calibrate the temperature of the calorimeter. In addition to serving as a convenient temperature standard, the indium provided a measure of how a "simple" melting is characterized by differential scanning calorimetry. This is important for students who have no experience characterizing a phase transition beyond using a MEL-TEMP. With indium, students can determine how an instrumental parameter, such as heating rate, effects the observed DSC data. These determinations are best made on a system with a narrow, well-defined phase transition, such as the melting point of high purity indium.

Experimental Section

The differential scanning calorimeter was a Perkin-Elmer DSC 7 interfaced to a PE NELSON 1020 TA computer. Large Volume Capsules (Perkin-Elmer part no. 0319-0218) DSC sample pans were used to prevent evaporation of the solvent (water) as the samples were heated. The pans are designed to hold 364 psia, the equilibrium vapor pressure of water at 225 °C. The phosphatidylcholines and cholesterol were purchased from Sigma (all 99+% purity). The high purity indium was provided by Perkin-Elmer.

The lipid-bilayer samples were prepared using a procedure similar to the one reported by Oritz et al. [6]. All the pure lipid samples were prepared in the following manner. First, a MOPS (3-(*N*-morpholino)propanesulfonic acid, 0.0536 g) solution was prepared using deionized water (15 mL). Dilute NaOH (*aq*) was used to bring the pH of the solution to 6.98. This solution was then diluted to 25 mL with deionized water. Approximately 1 mg of lipid was added to approximately 25 μL of MOPS. The lipid did not dissolve in the MOPS. Each sample was then placed in an ultrasonic cleaner in order to cause the formation of liposomes. Sonication lasted about 30 min. After sonication, the sample was a milky-white suspension. For DSC analysis, 15 μL of each lipid suspension were placed in a Large Volume Container DSC sample pan. The pan was sealed using an O-ring as described in the manufacturer's instructions.

The cholesterol-intercalated liposome was prepared in a slightly different manner. When cholesterol was simply added to the lipid and MOPS prior to sonication, no intercalation occurred. As an alternative synthetic route, the lipid (DSPC, 8.6 mg) and cholesterol (1.1 mg) were dissolved in chloroform. The chloroform was evaporated under a stream of nitrogen gas for 10 min. The sample was further dried under vacuum for 20 hours at 70 °C. This lipid/cholesterol mixture (3.4 mg) was added to MOPS (80 μL) and sonicated like the pure lipid samples. The result was a milky-white suspension.

DSC data were collected from 10 °C to 90 °C with scan rate of 5 °C min^{-1}. Ice water was added to the liquid-nitrogen reservoir of the calorimeter in order to operate at temperatures below 25 °C. Each data set was analyzed in terms of the onset temperature for the transition. This is conveniently done with the software. The width of the transition was also determined by manipulating a cursor on the display screen. This width is reported here as FWHM, the **f**ull-**w**idth at **h**alf **m**aximum of the transition peak. In principal, the enthalpy of the transition can be determined by integrating the transition peak. This would be straightforward for a simple system like pure indium; however, for reasons discussed below, the area under the transition peak of the phosphatidylcholines is more difficult to interpret.

Results and Discussion

The DSC thermogram for DSPC is shown in Figure 1. (In this Figure, endothermic events result in a positive (upward) deviation from the baseline, and exothermic events result in a negative (downward) deviation from the baseline.) The large peak at 55 °C corresponds to the gel-to-liquid-crystal transition [7]. The smaller peak, called the pretransition, is due to a rearrangement of the individual lipid molecules within the liposome bilayer [8]. The DPPC and DMPC DSC thermograms were similar to the one shown in Figure 1 except the peaks occurred at lower temperatures. The scan rate used for these thermograms was chosen as a compromise between signal-to-noise ratio and resolution. High scan rates give high signal-to-noise ratios, but they also broaden peaks. Low scan rates are used for resolving narrow peaks, but one suffers decreased peak height.

The DSC thermogram for DSPC intercalated with cholesterol is shown in Figure 2. The pretransition was not observed, and the transition temperature decreased with respect to the pure DSPC. In addition, the transition occurred over a broader temperature range than did the pure DSPC. The mass of lipid (766 μg of DSPC and 637 μg of intercalated DSPC) was determined from the mass of sample load into the DSC pan and the known percent weight of lipid and water used to prepare the liposomes. When samples were prepared with higher concentrations of cholesterol, the transition was either completely eliminated or so broad as to be beyond detection. These data are not shown.

Reporting the enthalpy of the phase transition presents a special challenge when working with lipid bilayers and liposomes. While it is true that the area under the peaks in Figures 1 and 2 is the enthalpy of the phase transition, it must be remembered that the liposomes are suspended in water, which is not participating in the phase transition. Moreover, the number of phosphatidylcholine molecules contained in a given liposome is unknown and likely to contain a range of values. To address this problem, the *cooperative unit* has been proposed [9–11]. The details of this approach are outside of the scope of this laboratory exercise. The reader is referred to

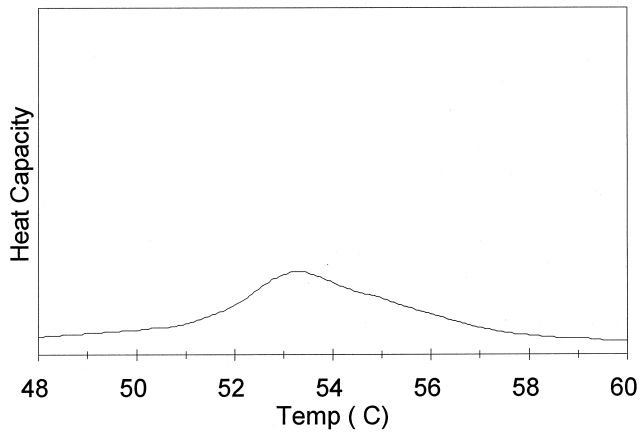

Figure 2. DSC thermogram of DSPC intercalated with cholesterol. The heating rate is 5 °C min^{-1}, and mass of the intercalated lipid is 637 micrograms.

Table 2. Transition Temperature Data for DSPC Intercalated DSPC and Indium

Sample	T_{trans}, °C	FWHM[a], °C
DMPC	54	1.37
DPPC/Cholesterol	51	3.71
Indium	156	0.63

[a] Full width at half maximum, determined manually using a screen cursor.

several references for details [12–16]. While this approach has been used repeatedly for the past 25 years, its validity has recently been called into question [17, 18]. As an interesting review of thermodynamics and how this argument is related to the validity of the cooperative unit determination, the reader is referred to the discussion in the literature generated by reference 17 [19, 20]. In our laboratory exercise, where the emphasis is on experimental physical chemistry, we chose not to pursue the cooperative unit analysis. As a biochemistry or biophysical laboratory exercise, such a modification might be appropriate.

When the liposome composed of DSPC was intercalated with cholesterol, the transition temperature of the gel-to-liquid-crystal transition was reduced, and the width of the transition was significantly increased. These results are summarized in Table 2.

These results can be interpreted by analogy to colligative properties. The freezing point depression for an ideal dilute solution is expressed as:

$$\Delta T_{fp} = \frac{-x_b R\left(T^*_{fp}\right)^2}{\Delta \bar{H}_{fus,A}} \Delta T$$

where x_b is the mole fraction of solute, R is the gas constant, T^*_{fp} is the freezing point of the pure solvent, and $\Delta H_{fus,A}$ is the molar heat of fusion for the pure solvent [21]. The cholesterol can be viewed as the "solute" and the hydrophobic region of the liposomes as the "solvent." The presence of the solute is observed to lower the temperature at which the solvent undergoes a phase transition from a more-ordered to a less-ordered state. The change in width of the transition, which can be measured quantitatively, is consistent with what students

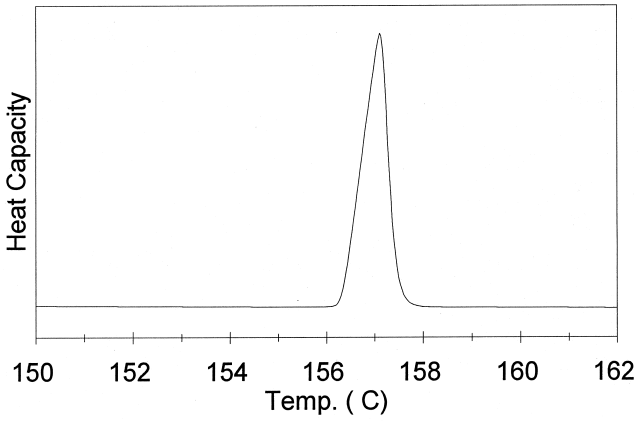

Figure 3. DSC thermogram of high purity indium. The heating rate is 5 °C min^{-1}, and the mass of indium is 5.225 mg.

Table 3. Melting Point of Unbranched Alkanes

Compound	No. of Carbons	T_{mp} (°C)
Tetradecane	14	5.88
Hexadecane	16	18.36
Octadecane	18	28.13

observed in organic chemistry laboratory courses where melting point ranges where determined semiquantitatively and related to the purity of the melting sample. This broadening of the transition can also be interpreted using a two-component phase diagram [22].

The decrease in the transition temperature as the number of carbons in the acyl group decreases is consistent with what one would expect from consideration of the normal melting points of unbranched alkanes. The alkane melting points, taken from the NIST website (http://webbook.nist.gov/chemistry/), are summarized in Table 3.

With both the alkanes and the lipid bilayers, the transition temperature increases with carbon chain length; however, the magnitude of the increase decreases with increasing carbon chain length in both cases. These trends serve to highlight the melting nature of the gel-to-liquid-crystal transition observed in the lipid bilayers. In normal alkane melting, the van der Waals interactions among the individual molecules must be partially overcome to allow the molecules to move about in the liquid state. In the liposome, the same van der Waals interactions among the acyl chains must be overcome in order for the chains to adopt a fluid configuration.

In order to calibrate the temperature of the scanning calorimeter, a sample of high purity indium was tested. The DSC thermogram of this standard is shown in Figure 3. The heat capacity of a sample diverges as it undergoes a first-order phase transition (such as melting) because there is no change in temperature when a finite amount of heat is added. Recall the definition of heat capacity is $C = dq/dT$. The finite width of the endothermic peak in Figure 3 gives a measure of the instrumental broadening caused by the noninfinitesimal heating rate. When the peak width in Figure 1 is compared to the indium melting shown in Figure 3, one can see that the gel-to-liquid-crystal transition is not as sharp as the melting of pure indium. This is a result of nonuniform liposome size and the presence of water in the liposomes. A similar broadening would be observed for the indium sample if an impurity such as lead was added.

When the DSPC was intercalated with cholesterol, two important effects on the gel-to-liquid-crystal transition were observed. First, the transition temperature decreased as would be expected from a standard freezing-point-depression argument. Second, the width of the transition substantially increased. This width of the transition, beyond that due to instrumental effects discussed above, is indicative of the purity of the sample. The width of the high purity indium is listed in Table 2 for comparison. As cholesterol is added to the lipid-bilayer sample, the purity of the lipid bilayer is decreased and a resulting increase in the width of the transition is observed. Students are introduced to this idea early in their chemistry training when they learn to measure melting points in general or organic chemistry: the sharpness of the transition is related to the purity of the sample. DSC provides a graphic and quantitative measure of what had previously been a qualitative observation.

Cholesterol was chosen as the impurity because of its importance in the composition of cell membranes. As already stated, lipid bilayers, like the ones used in this exercise, are often used to model the more complex system of a cell membrane. If enough cholesterol is intercalated into the lipid bilayer, the transition will broaden beyond detection [6]. This has important consequences for the rigidity and fluidity of cell membranes.

Student response to this laboratory has been positive. It has both a preparative and analytical component. Students like that the DSC analysis can be repeated many times on a given sample. This is particularly important for establishing the importance of scan rate in scanning calorimetry. Students also liked the connection between this laboratory exercise and their extensive lecture work in classical thermodynamics.

Conclusion

Lipid bilayers were used to introduce a standard physical chemistry technique, differential scanning calorimetry. The interpretation of the data relied on basic thermodynamics including ideal solutions and phase transitions. The transition temperature was determined for the gel-to-liquid-crystal transition for a series of pure lipid-bilayers and for a cholesterol-intercalated lipid bilayer. The results were compared to published data for melting of unbranched alkanes with the same number of carbon atoms as the acyl group in the lipid bilayers. This experiment could be modified for a biochemistry or biophysical laboratory by including an analysis of the cooperative unit and by considering other sample parameters such as degree of saturation in the acyl chains, the nature of the polar head group, and the degree of hydration in the lipid bilayers.

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