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Thermal Properties of Some Lipid Components of Cell Membranes

Lihua Wei

THERMAL PROPERTIES OF SOME LIPID COMPONENTS OF CELL MEMBRANES

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In Partial Fulfillment

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Master of Science

by

Lihua Wei

April 1992

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THERMAL PROPERTIES OF SOME LIPID COMPONENTS OF CELL MEMBRANES

Recommended *April 29, 1992*
                       (date)

David R. Hartman
Director of Thesis

Weiping Pan

Warter K Houston

Approved *April 30, 1992*
                  (date)

Elmer Gray
Dean of the Graduate College

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THERMAL PROPERTIES OF LIPID COMPONENTS OF CELL MEMBRANES

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Directed by: Dr. David R. Hartman, Dr. Wei Ping Pan
                Dr. Martin R. Houston, Department of Biology

Department of Chemistry   Western Kentucky University

Phospholipids are the major structural components of the lipid portion of biological membranes. The study of the thermal properties of these phospholipid provides a systematic understanding of the relationships between the chemical structures and functional properties of phospholipid molecules in artificial and biological membranes. This study examined the thermal behavior of phospholipids in the solid state using DSC and TG coupled with FTIR. The phospholipids were found to have phase transitions below the melting point. Egg-yolk lecithin had four transitions below its melting point; sphingomeylin had two phase transitions below its melting point. Phosphatidylethanolamine and phosphatidylserine had complex phase transitions. The water content of the lipids affected the phase transition. In this study, TG provided a quicker, an easier and more accurate way to find the percentage of water in lipids. Most of the phospholipids existed in the hydrated state, and

different lipids were associated with different amounts of water. One mole of egg-yolk lecithin was associated with one to two moles of water. One mole of phosphatidylethanolamine contained two moles of water. Phosphatidylserine was a monohydrate, and one mole of sphingomeylin associated with two moles of water, a dihydrate.

INTRODUCTION

Phospholipids are the major structural components of the lipid portion of biological membranes (1). They give the membrane properties such as fluidity, mechanical stability, and selective permeability (2). Each membrane type usually contains a variety of phospholipid classes. In most mammalian membranes the diacyl-L-phosphatidylcholines (lecithins) are the most numerous phospholipids. In the plasma membrane, most of the sphingomyelin and phosphatidylcholine are in the outer portion of the membrane, while phosphatidylethanolamine and phosphatidylserine are contained in the inner portion of the membrane (3). Figure 1 shows the basic structures of phosphatidylcholine, phosphatidyLethanolamine, phosphatidylserine, sphingomyelin and cholesterol. The study of the thermal properties of these phospholipids may result in a systematic understanding of the relationships between the chemical structures and functional properties of phospholipid molecules in artificial and biological membranes. The physical properties of a series of pure synthetic phosphatidylcholines and phosphatidylethanolamines have been studied by a variety of physical methods including X-ray diffraction,

Figure 1. Structures of phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingomyelin and cholesterol
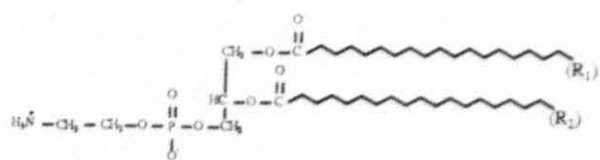
Phosphatidylethanolamine
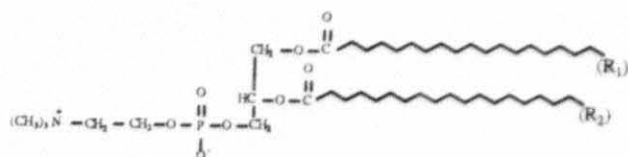
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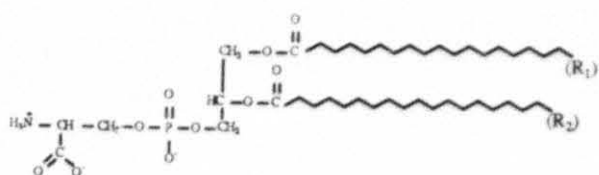
Phosphatidylserine
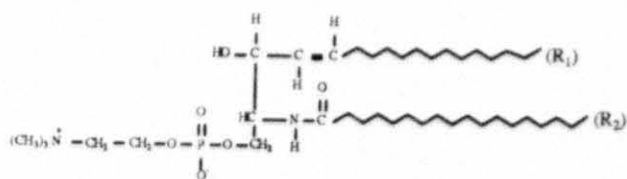
Sphingomyelin
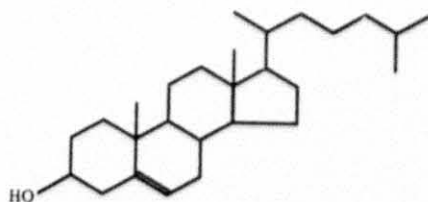
Cholesterol

thermal analysis and spectroscopic techniques by Chapman in the 1970s (4).

Polymorphic behavior is prevalent in lipids. Almost every compound examined exhibited polymorphism of fatty acids and their derivatives, glycerides, and lipids in general (5). The production of various polymorphic forms of long-chain compounds depended upon temperature, hydrocarbon chain length, method of crystallization, solvent from which crystallization was effected, purity, and other parameters. Many substances, such as, soaps and phospholipids, do not undergo a direct transition on heating from a crystalline form to a liquid. A number of states intermediate between a crystal and liquid are found to exist: these states have been variously called mesomorphic (6):

Crystalline <==> Condis Crystal <==> Plastic Crystal <==> Liquid Crystal <==> Liquid

Condis crystal and plastic crystal are more closely related to crystals.

These molecules have several phase transitions for several reasons. There are long fatty acid chains in the structures of these phospholipids. In long-chain compounds, the chains have different packing forms at different temperatures. The different forms can be observed in a microscope when the temperature is increased. Long chain compounds generally crystallize so that the molecules are

arranged in layers. The hydrocarbon chains, usually in the extended trans configuration for unsaturated portions of chains, are packed with the layers planes, and may be either vertical or tilted to these planes. Hence several different polymorphic forms are possible as a result of this tilting. The relative packing of the hydrocarbon chains is also subject to variation; such variation gives rise to another source of polymorphic behavior. Phospholipids containing complicated ionic or polar regions might exhibit polymorphism resulting from another source. The existence of a particular polymorph often depends completely on the presence or absence of impurities. In a crystalline lipid the hydrocarbon chains arrange themselves to allow the highest possible van der Waals interaction. Since slight changes in the mutual orientation of neighboring chains makes little difference to the van der Waals interaction energy, a large number of structures with minor differences of lattice energy can exist as several different polymorphic forms. On heating molecules such as the phospholipids, an endothermic transition is observed at a temperature well below the true melting point of the material. At this transition a change of state occurs from the crystalline to the liquid-crystalline, or mesomorphic states. In these mesomorphic states the hydrocarbon chains have a conformation similar to that observed in liquid hydrocarbons while the polar moieties of the molecules hold the structure together in some particular manner. Melting of the hydrocarbon chains thus

occurs at this crystal to liquid-crystal transition.

The study of the thermal properties and phase transitions of the synthetic phosphatidylcholines and phosphatidylethanolamines in the solid state has been investigated (7). The synthetic phosphatidyl-choline exists in the form of monohydrate and has three phase transitions below the melting point (7). The study of the thermal properties and phase transitions of the natural sources of phospholipids in solid states has been investigated in less detail. The natural sources of phospholipids contain a distribution of fatty acid chain lengths and varying degrees of unsaturation.

The heat flows at the transition points can be measured by differential scanning calorimetry (DSC) (8). In this technique, heat is supplied to the sample (and to a relevant reference) so that the temperature increases at a constant rate with time. As long as the specific heat of the sample remains constant, the rate of supply of heat to the sample will be constant too, but when the temperature of an endothermic phase change is reached, the heat supply must be increased in order to maintain the linear rate of temperature increase. It is the difference in the energy supplied to the sample and to the reference (e.g., an empty sample container or, for solutions, an identical quantity of the solvent) which is actually recorded, and the optimum temperature for the phase change is the temperature at which the trace first leaves the base line. The peaks can be integrated to provide a measure of the energy absorbed or

yielded by the sample at the phase transition. The DSC measures enthalpy change in a particular sample, when under fixed conditions, it is heated or cooled. This method detects the heat lost (exothermic) or the heat gained (endothermic) by chemical reactions or physical state changes occurring during the thermal process.

A number of investigators have analyzed phospholipids and biological membranes with DSC. Some of them have examined the membrane phase transitions which are responsible for imbibitional damage in dry pollen (9). Differential scanning calorimetry has been used to analyze the effects of carbohydrates on the membranes in anhydrobiotic organism (10). The thermal behavior of a mixture of dry carbohydrates such as trehalose, sucrose, fucose, and pure dry individual lipid, such as, dipalmitoyl-phosphatidylcholine (DPPC) has been examined in order to elucidate the mechanism by which some of these carbohydrates preserve structural and functional integrity of dry membranes. They found that in the presence of trehalose, dry dipalmitoyl phosphatidylcholine (DPPC) had a transition temperature similar to that of the fully hydrated lipid. Hence trehalose specificity may be an important factor in the ability of this molecule to stabilize dry membranes in anhydrobiotic organisms. Infrared analysis suggested that the mechanism or interaction between the carbohydrate and lipid involves hydrogen bonding between hydroxyl groups on the carbohydrate and the phosphate polar group of the phospholipid (10).

Phospholipids in aquous solution have been examined using DSC in recent years. Brauner and Mendelsohn have used the technique of Fourier Transform Infrared (FT-IR) spectroscopy coupled with differential scanning calorimetry (DSC) toward elucidation of changes in both the dynamics and thermodynamics of lipids and proteins that occur upon their mutual interaction (11). The phase of the binary mixtures of phosphatidylethanolamines (PE) with phosphatidylcholines (PC) have been determined by using high-sensitivity differential scanning calorimetry. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) constitute the major glycerolphospholipid species found in the membranes of most animal cells. The PE/PC balance in natural membranes may be an important determinant of their function. It is important to characterize the thermodynamics of mixing of PE and PC species. Silvius results demonstrated that calorimetry can be useful in determining accurate phase diagrams for lipid mixtures of this type (12). They have studied the "unnatural" phospholipid analogues with modified polar headgroups and/or backbone structure by DSC. They found that the thermodynamic characteristics of the "classical" hydrated-gel-to-liquid-crystalline phase transition often appear surprisingly insensitive to the nature of both the polar head group and the backbone moieties(13).

Thermogravimetric analysis (TG) measures the mass change as function of temperature (or time) in a given atmosphere. TG can

detect the thermal stability. The TG curve provides quantitative information on weight change processes, and enables the stoichiometry of a reaction to be followed directly (14). A TG curve plots percent of weight (Y axis) versus temperature (X axis). A differential of the thermogravimetric (DTG) analysis plots the rate of weight loss against the temperature.

An FTIR can be interfaced with the TG. As decomposition occurs in the TG, the gaseous products from the sample are passed to the Fourier Transform Infrared spectrometer (FTIR). Other possible forms of coupling include gas chromatography (GC) and mass spectrometry (MS) which can be linked to either the TG, or the DSC (15). The gaseous products formed from the decomposition of the sample can be more closely analyzed to understand the chemical decompositions which are occurring.

Chapman has examined loss of water from the phospholipid samples by using heating in the oven and a balance (4). Some investigators have done polymer analysis by using TG. Not many investigators have examined phospholipids and biological membranes using TG.

The dynamic and mechanical properties of membranes play important roles in membrane function. Each class of lipid plays a fundamental role. How they behave in different environments seems to be a very important thing to study. The thermal behavior of lipids is one area of interest. The basic structure of phospholipids

consists of a polar group and a hydrocarbon portion. The thermal properties of pure synthetic phospholipids have been studied. The polar group and hydrocarbon portion of phospholipid molecules were modified in different ways and then checked for thermal behavior. More recently studies have demonstrated that under suitable conditions, many synthetic phospholipids form a solid phase other than the classical "gel" phases, "subgel" phase, and interdigitated phases. Whatever other investegators have studied, the aim is to understand the natural phospholipids and membranes very well. In this research, the thermal behavior of phospholipids from natural sources was examined and the behavior of a mixture of cholesterol with various phospholipids determined. The decomposition products produced during heating were also examined using TG-FTIR.

EXPERIMENTAL

A. Sample Preparation

The four pure lipids used for study were: phosphatidyl-choline(PC), phosphatidylethanolamine(PE), phosphatidylserine(PS), sphingomyelin(SM). They are naturally occurring phospholipids. Phosphatidylcholine and sphingomyelin were purchased from SIGMA and phosphatidylethanolamine and phosphatidylserine were purchased from ICN Pharmaceuticals, Inc. These four samples were powders. For comparison purposes, two other pure samples of phosphatidylethanolamine and phosphatidylserine were used. They were purchased from ICN Pharmaceuticals, Inc. These two samples were in a chloroform solution. They were dried under nitrogen and in the oven under vacuum at room temperature overnight. All the samples were analyzed by DSC and TG as described in the next section.

To study the effects of cholesterol on the thermal properties of phospholipids, four sample mixtures were used. Each mixture contained one phospholipid with cholesterol at 1:1 ratio. Cholesterol was purchased from Matheson Coleman & Bell. In the process of preparing the mixed lipid samples, several different

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solvents (ether, acetone, methanol, and benzene) were tested. Phospholipids and cholesterol are non-polar organic compounds and dissolve in non-polar organic solvents. Two milligrams of each sample (PC, PE, PS, SM, and cholesterol) were placed in separate test tubes and tested for solubitity in 0.5ml of each solvent (ether, acetone, methanol, and benzene). The sample solution was shaken. Some of the solutions were cloudy indicating low solubility, whereas the others were clear (completely soluble). Benzene was chosen as the best solvent since the sample lipids all formed clear solutions with it as indicated in Table 1.

Table 1.   Solubility of phospholipids in different organic solvents

	PC	PE	PS	SM	Chol
Ether	+	+	+	+	++
Acetone	+	+	+	+	+
MeOH	+	+	+	+	+
Benzene	++	++	++	++	++

"+" partially soluble; "++" completely soluble.

Equal amounts of PC and cholesterol were weighed; each was dissolved in about 1.5ml benzene and then mixed. The benzene was evaporated in a rotoevaporator with water aspiration at room temperature. The mixture was dried under vacuum overnight (about 12 hours) at room temperature. The same procedure was used to prepare mixed samples containing PE, PS, or SM with cholesterol. The mixed samples were stored in the freezer until they were analyzed. The mixed lipids were analyzed by DSC as described in the next section.

Preparation of phospholipids in aqueous solution followed the method described by Barenholtz (19). Dipalmitoylphosphatidylcholine (DPPC), 3.7mg was dissolved in 5ml 50 mM KCl solution by incubating in hot water ($65-70^0C$) under nitrogen for 20 minutes then immersing in ice bath (0^0C) for 30 minutes. The freeze-thaw cycle was repeated three times. A DPPC solution was prepared, a liposome. The sample was stored at 4^0C.

B. Instrument Operation

The experiments were performed on a DuPont 910 -- differential scanning calorimeter (DSC) and a DuPont 951 thermogravimetric analyzer (TG) which was connected with a Perkin Elmer 1650 Fourier Transform Infrared spectrometry (FTIR) (see Figure 2) (20).

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Figure 2. Schematic diagram of the TG-FTIR instrument
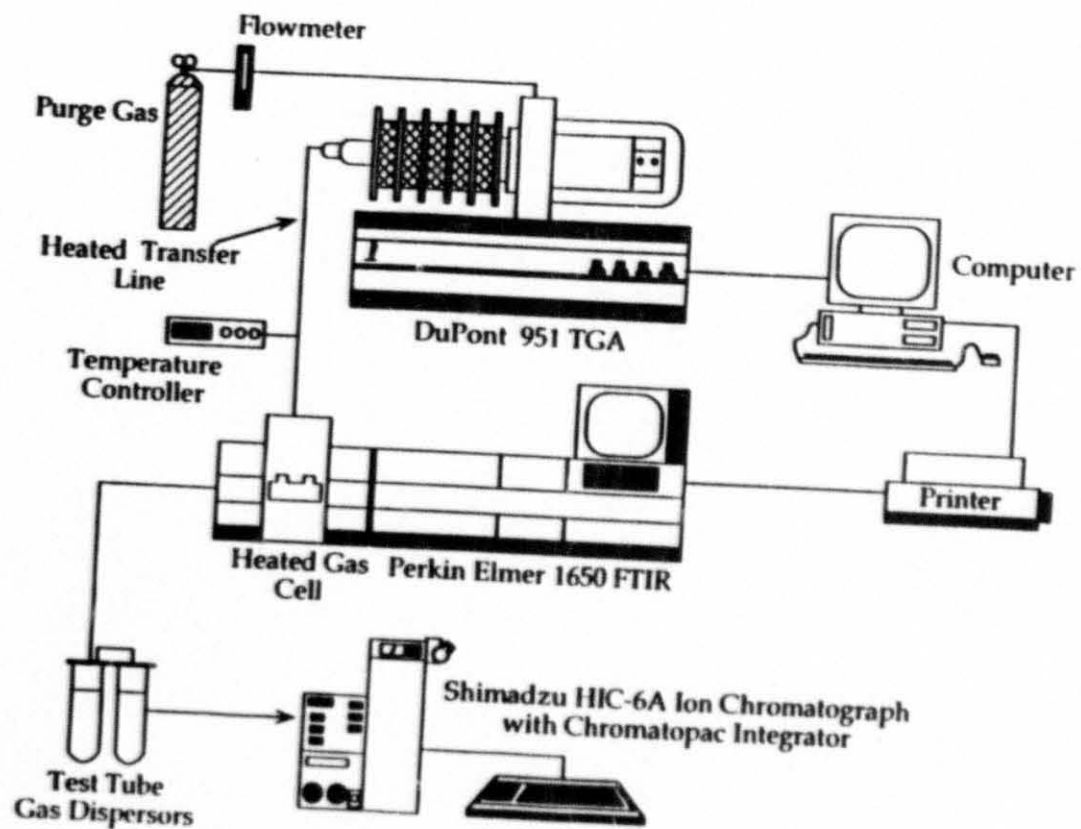
The analytical procedure for studying the thermal properties of the lipids by DSC was as follows: different sample sizes of approximately 10mg were analyzed. The sample was placed in a preweighed aluminum pan, weighed and then placed in the DSC instrument cell. An empty aluminum pan was used as the reference. The sample was heated from room temperature to 500^0C under nitrogen gas. A continuous flow of nitrogen gas was maintained through the thermal analysis. For all the experiments, a flow rate of 50ml/min was used. We tried different scanning rates for the study. Based on the reference (19), we tried 15^0C/hour which was too slow. We ran the samples at 1^0C/min and 5^0C/min; no difference in DSC curves were observed for the two different scanning rates. In order to prevent a loss of detail in the DSC scan we choose 5^0C/min as scanning rate. Exactly the same operating conditions were used for all samples.

The analytical procedure for studying the decomposition of lipids by TG-FTIR was as follows: about 10mg (different sample sizes) of a phospholipid sample was placed in a platinum sample pan on a microbalance in the TG and heated employing the temperature programmed furnace in the TG. The sample was heated at 5^0C/min from ambient temperature ($\sim 25^0C$) to 200^0C. The lipid samples (PE, PS, PC and SM) were heated under N_2 with a flow rate of 50ml/min. While the TG experiment was in progress, an FTIR spectrum was scanned at 10^0C increments. For the FTIR data, a teflon tube

connected the outlet valve of the TG to the inlet valve of the gas cell on the FTIR. This allowed for the continuous flow of gas from the TG through the FTIR. The gas cell was 100mm X 25.4mm. The total time lag for gas to pass through the cell and the heated transfer line was calculated to be 67 seconds (20). The temperature of the lipids on the sample pan was determined by the thermal detector in the TG. The weight loss was determined by the microbalance in the TG. T_{max} and weight loss were determined from TG plots of mass versus temperature recorded during the experiments.

C. Experimental Technique

Because the phospholipid samples were not stable in air, we analyzed the sample as quickly as possible after removal from the freezer. During the thermogravimetric analysis, as soon as the sample was introduced, the system (DSC and TG) was held at approximately ambient temperature for 15 minutes. This was sufficient time for the system to be purged of all air.

RESULTS AND DISCUSSION

A. DSC on phospholipds:

1. Phosphatidylcholine (PC)

Figure 3 shows a typical DSC curve for PC in nitrogen. This curve shows four endothermic peaks : one at 80^0C, a second at 107^0C, a third at 121^0C and the fourth one is at 157^0C. Comparing our DSC data with the data obtained by Chapman (16), egg-yolk lecithin (PC) and 1,2-distearoyl-phosphatidylcholine have similar phase transitions except egg-yolk lecithin has the extra phase transition at 107^0C. Egg-yolk lecithin undergoes four observable polymorphic changes when heated from ambient temperature to 200^0C in nitrogen. For such processes the change in entropy ΔS equals q_{rev}/T, where q_{rev} is the heat absorbed or latent heat and T is the temperature (^0K). For a first order transition at constant pressure the free energy change is zero so that the latent heat becomes equal to the enthalpy change ΔH and hence we can calculate the entropy change (ΔS) for each structure phase transition.

$$\Delta S = \Delta H/T$$

where, ΔH = Specific Enthalpy Change; T = Temperature at Maximum Peak (^0K). Table 2 shows the transition temperature, enthalpies and

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Figure 3. DSC curve for phosphatidylcholine in nitrogen

Sample: PC
Size: 5.3000 mg
Method: WEI
Comment: 5C/MIN TO 200 IN N2 50ML/MIN

DSC

File: A:WEI09.10
Operator: WEI
Run Date: 12-Oct-91 22:53

73.06°C
166.6J/g

79.96°C

104.93°C
3.879J/g

107.45°C

124.28°C
116.44°C
5.922J/g

151.86°C
27.35J/g

156.61°C

Heat Flow (W/g)

Temperature (°C)

General V4.1C DuPont 2000
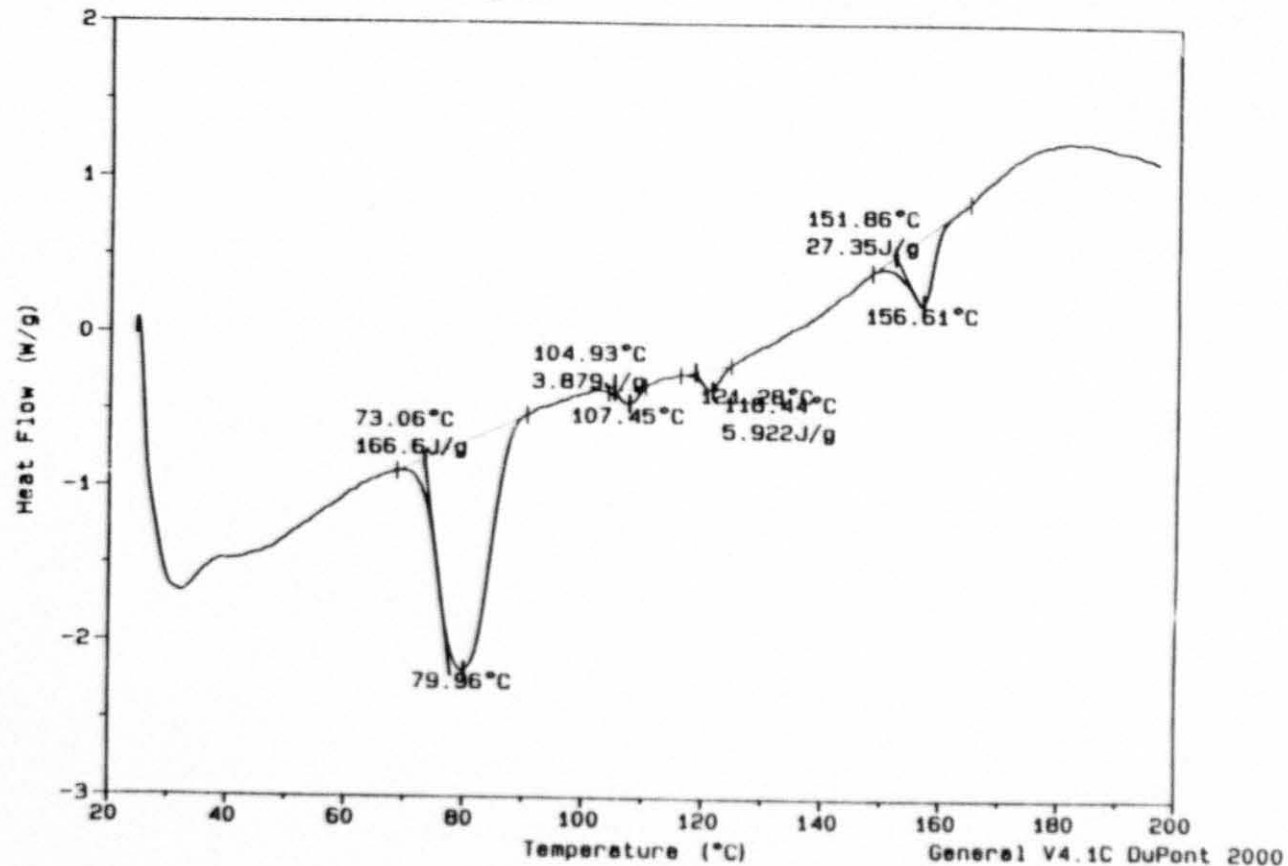
entropies for phosphatidylcholine. Using a microscope Chapman observed phase transitions at 72-78^{0}C, 118^{0}C and 149^{0}C for 1,2-distearal-phosphatidylcholine (DSPC). In our results, we found phase transitions at 80^{0}C, 107^{0}C, 121^{0}C and 157^{0}C. Chapman found that the DSPC changed from an anisotropic crystalline solid to an anisotropic fluid liquid crystal at T1, 72-78^{0}C. Using DSC, with egg-yolk lecithin, we observed a large ΔS at T1. The change from an anisotropic, crystalline solid to an anisotropic fluid liquid crystal is causing a large change in ΔS, 336.1J/molK. At T3, 118 ^{0}C, Chapman observed a change in DSPC from an anisotropic, fluid liquid crystal to an isotropic, viscous liquid crystal. At T3, 121^{0}C, we observed a small ΔS, 10.70J/molK. At T4, 149^{0}C, Chapman observed a change in DSPC from an isotropic, viscous liquid crystal to an anisotropic, fluid liquid crystal. At T4, 157^{0}C, we observed a somewhat larger ΔS of 45.30J/molK. Using DSC, we observed an extra phase transition at T2, 107^{0}C, with a small ΔS, 7.27J/molK. The large ΔS at T1 may be partly due to water loss. The maximum rate of water loss observed using TG occured between 60^{0}C and 89^{0}C (Figure 17).

Table 2. The transition temperature, enthalpy changes and entropy changes of egg-yolk lecithin

| Transition	Temperature(^{0}C)	ΔH(KJ/mol)	ΔS(J/molK)
T1	80	118.8	336.1
T2	107	2.77	7.27
T3	121	4.22	10.70
T4	157	19.50	45.30

Figure 4 shows a DSC curve for phosphatidylcholine crystallized from benzene in nitrogen. The peak at 80^0C has shifted to 95^0C and the peak at 157^0C has shifted to 165^0C. There is a big difference of ΔS between phosphatidylcholine and phosphatidylcholine crystallized from benzene (see Table 3). The enthalpy change for T1 is much lower than 118.8KJ/mol. It can be seen that the entropy change of phosphatidylcholine is much more than that of phosphatidylcholine crystallized with benzene at T1. This shows that much more energy was required to transpire from an ordered state to a state of disorder and therefore exhibiting more freedom. This indicates that phosphatidylcholine crystallized with benzene is more ordered than phosphatidylcholine at T1. Most of it may be in a viscous liquid crystal, isotropic state, instead of an anisotropic crystal state. According to the data from Table 3 and comparing the result of phosphatidylcholine, we can deduce that at T1, T2, and T3, the sample is isotropic, viscous liquid crystal; only at T4, the sample is anisotropic, fluid liquid crystal. The difference of shifting to a higher temperature at T1 and T4 might be caused by the different water content in these two samples. The water content is higher in phosphatidylcholine than in phosphatidylcholine crystallized from benzene. Because we used benzene to dissolve the sample. In order to get rid of benzene, we put the sample in the oven under vacuum over night. In this case, some amount of water was

Figure 4. DSC curve for egg-yolk lecithin crystallized from benzene in nitrogen

Sample: PC (TREATED WITH BENZENE)   DSC   File: A:WEI09.06
Size: 8.8000 mg   Operator: WEI
Method: WEI   Run Date: 11-Oct-91 16:47
Comment: 5C/MIN TO 200 IN N2 50ML/MIN

General V4.1C DuPont 2000
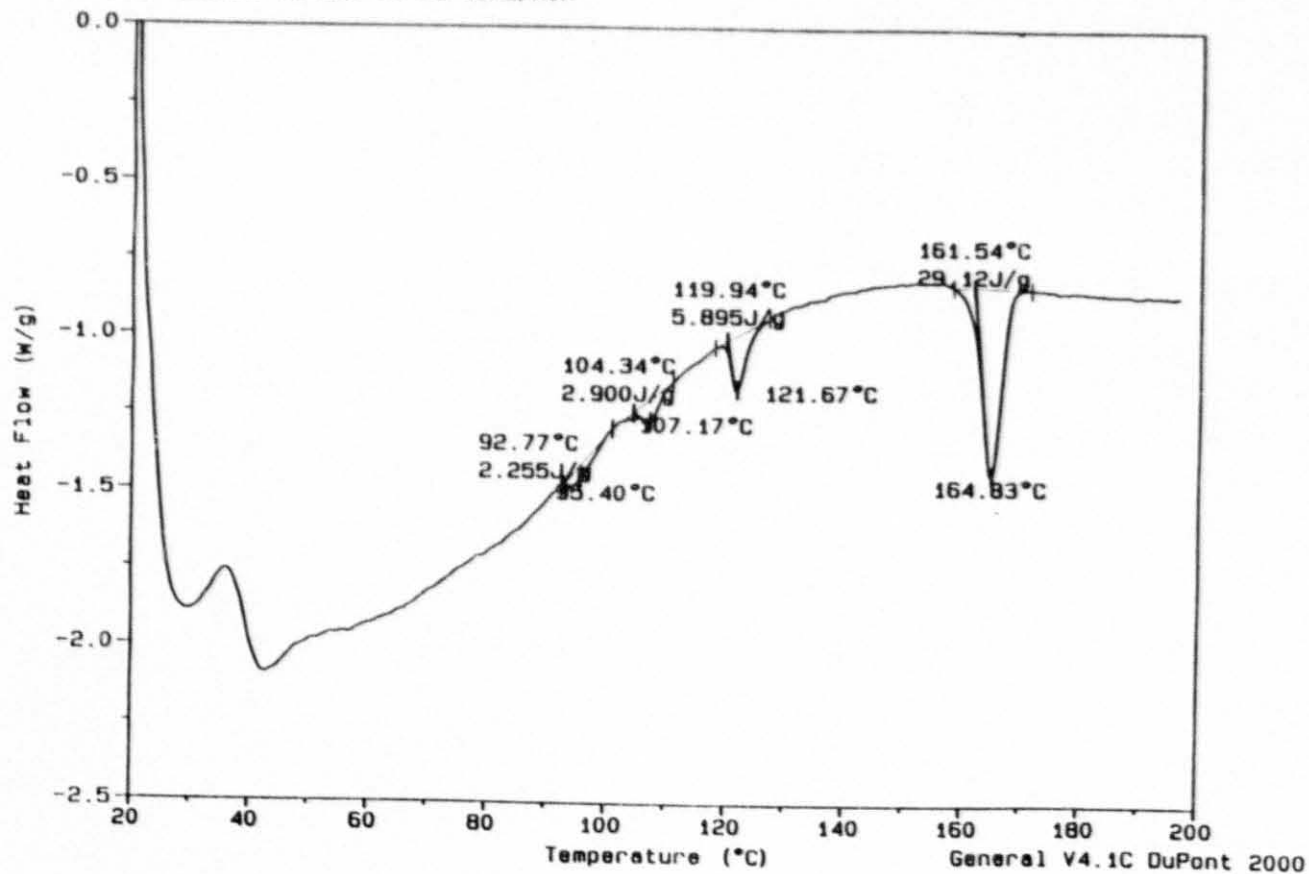
removed. The smaller ΔS at T1 may be due to a lower water content also. Other investigators also found that the temperature of the main transition varies depending upon the actual water content of the sample (4). Considering the differences between these two kinds of samples, we also can imply that the solvent altered the crystal forms. The phase transitions at 107^0C and 121^0C were not affected much.

Figure 5 is the DSC curve for PC from another source (different lot number). It is similar to that of PC crystallized from benzene. The melting point of phosphatidylcholine is 226^0C which is 4^0C lower than that (230^0C) of 1,2-diacylphosphatidylcholine which is characterized by a marked increase in the fluidity of the sample (4). Table 4 shows the transition temperature, enthalpy changes and entropy changes for this sample. This sample was probably crystallized from a different solvent.

Table 3. The transition temperature, enthalpy changes and entropy changes of egg-yolk lecithin crystallized from benzene

Transition	Temperature(^{0}C)	ΔH(KJ/mol)	ΔS(J/molK)
T1	95	1.61	4.36
T2	107	2.07	5.44
T3	122	4.20	10.63
T4	165	20.76	47.34

Figure 5.   DSC curve for egg-yolk lecithic from different lot number in nitrogen

Sample: PC (FROM DIFFERENT LOT NUMBER)
Size: 12.1000 mg
Method: WEI
Comment: 5C/MIN TO 300 IN N2 50ML/MIN

DSC

File: A: WEI09.33
Operator: WEI
Run Date: 14-Feb-92 23:03

General V4.1C DuPont 2000
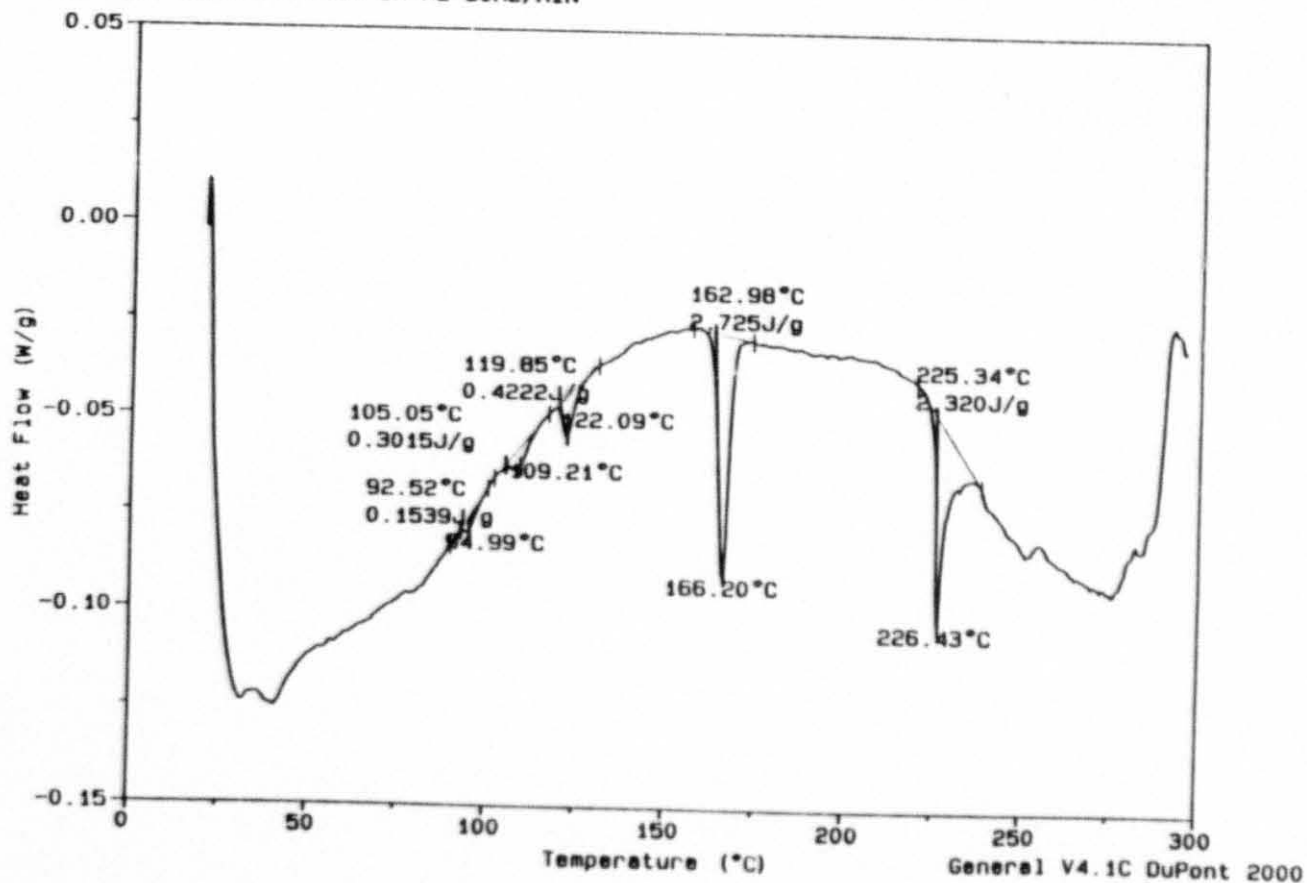
Table 4. The transition temperature, enthalpy changes and entropy changes of egg-yolk lecithin from a different lot number

Transition	Temperature(^{0}C)	ΔH(KJ/mol)	ΔS(J/molK)
T1	95	0.11	0.30
T2	109	0.22	0.56
T3	122	0.30	0.76
T4	166	1.94	4.42

Figure 6.   Comparison among the data for three different samples

Legend:
EY: Egg-Yolk Lecithic
EYC: Crystallized Egg-Yolk Lecithic
EYD: Different Lot No. Egg-Yolk Lecithic

T1, T2, T3, T4
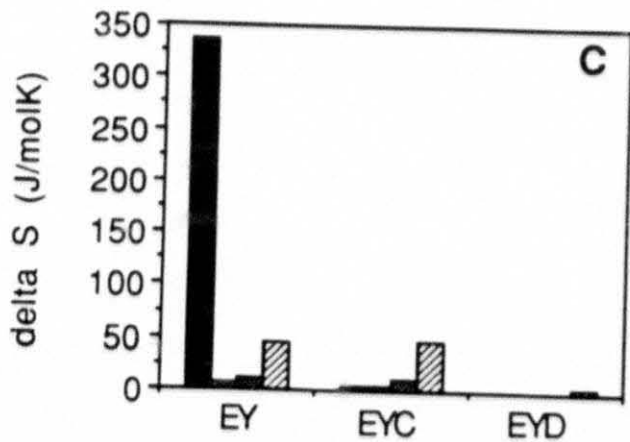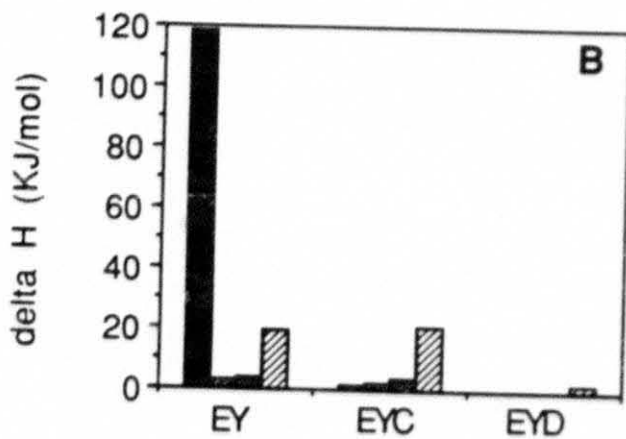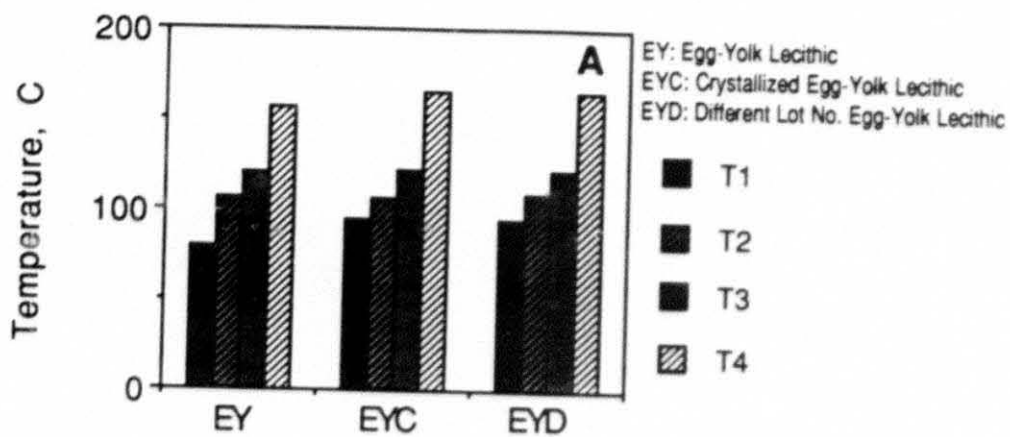
From figure 3, 4 and 5, the peaks at 157^0C, 164^0C and 166^0C have large peak areas which corresponds to great enthalpy changes. They seemed to be caused by melting of the hydrocarbon chain portion of the molecule. Figure 6 shows the comparison among these three samples which are the same compounds but from different sources. Obviously transitions at T2 (107^0C) and T3 (121^0C) are not affected much. The transitions at T1 (80^0C shifted to 95^0C) and T4 (157^0C shifted to 165^0C) may be affected by a different water content. For phosphatidylcholine and phosphatidylcholine crystallized from benzene ΔS values did not changed too much at T2, T3, and T4 ; the ΔS value at T1 was changed greatly. Comparing the ΔS values for phosphatidylcholine from a different lot number with those for the other two samples, the values of phosphatidylcholine from a different lot number were found to be about one tenth of those for the other two samples.

2. Phosphatidylethanolamine (PE)

Figure 7 shows a typical DSC curve for phosphatidylethanolamine in nitrogen. This curve shows a broad peak at 185^0C which is probably the melting point. The melting point of pure phosphatidylethanolamine is 197^0C (7). This sample apparently was not pure. The peaks at 235^0C, 245^0C, 340^0C and 435^0C show decomposition. Figure 8 shows a DSC curve for a sample of phosphatidylethanolamine which was more pure. The peak at 191^0C

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Figure 7.   DSC curve for phosphatidylethanolamine (less pure) in nitrogen

Sample: PE (LESS PURE)
Size: 10.4000 mg
Method: WEI
Comment: 5C/MIN TO 500C IN N2 50ML/MIN

DSC

File: A:WEI09.38
Operator: WEI
Run Date: 1-Mar-92 22:54

184.91°C
234.55°C 245.17°C
340.26°C
435.21°C

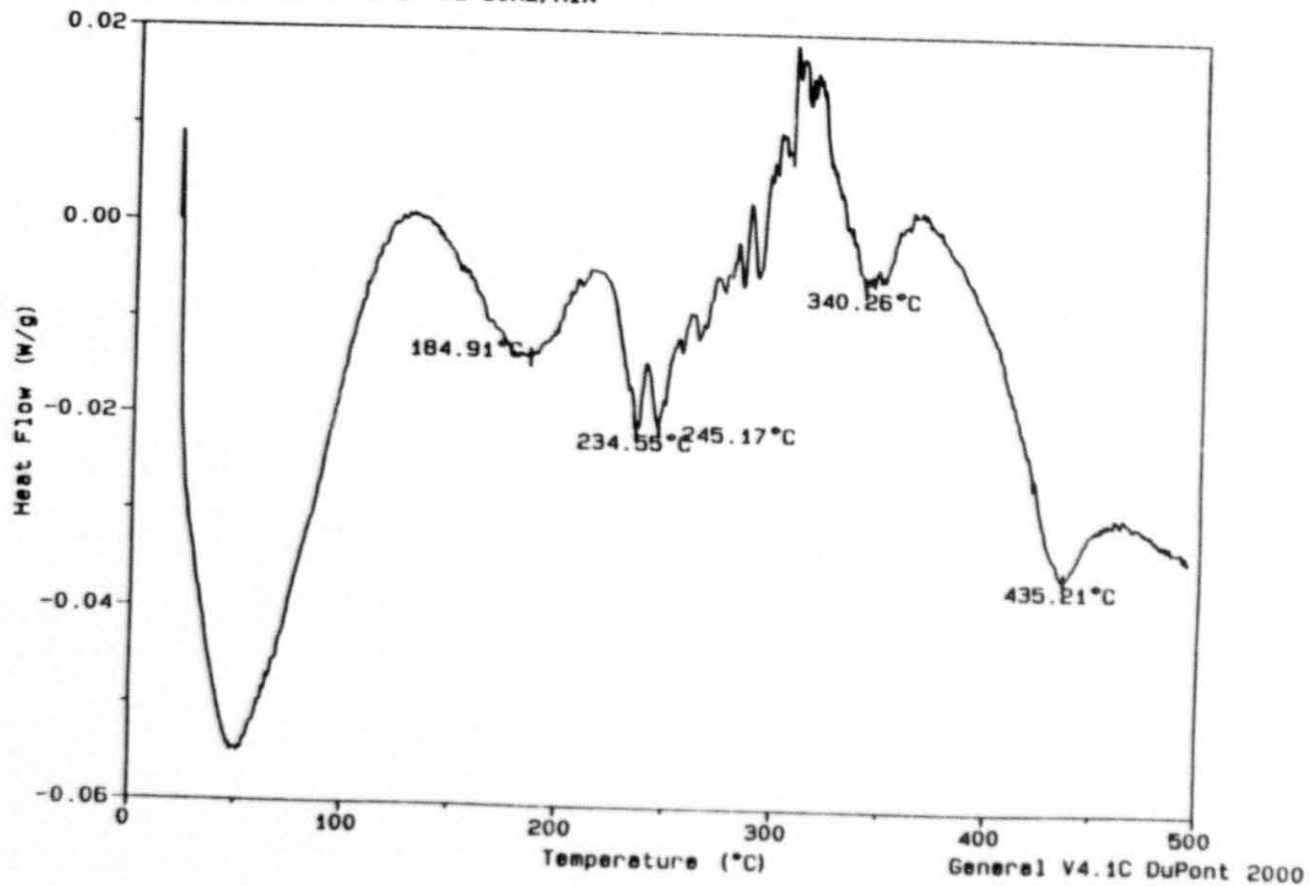Heat Flow (W/g)
Temperature (°C)
General V4.1C DuPont 2000

Figure 8.   DSC curve for phosphatidylethanolamine (more pure) in nitrogen

Sample: PE(PURE FROM CHCL3)
Size: 4.0000 mg
Method: WEI
Comment: 5C/MIN TO 500C IN N2 50ML/MIN

DSC

File: A:WEIO9.34
Operator: WEI
Run Date: 15-Feb-92 01:40

General V4.1C DuPont 2000

Figure 9. DSC curve for phosphatidylserine (less pure) in nitrogen

Sample: PS (LESS PURE)
Size: 9.3000 mg
Method: WEI
Comment: 5C/MIN TO 500C IN N2 50ML/MIN

DSC

File: A: WEI09.39
Operator: WEI
Run Date: 2-Mar-92 00:57

154.58°C

150.06°C
1.756J/g

178.28°C

356.22°C
1.767J/g

377.72°C

71.87°C
1.457J/g

238.24°C

74.76°C

Heat Flow (W/g)

Temperature (°C)

General V4.1C DuPont 2000

Figure 10. DSC curve for phosphatidylserine (more pure) in nitrogen

Sample: PS (PURE FROM CHCL3)
Size: 5.0000 mg
Method: WEI
Comment: 5C/MIN TO 500C IN N2 50ML/MIN

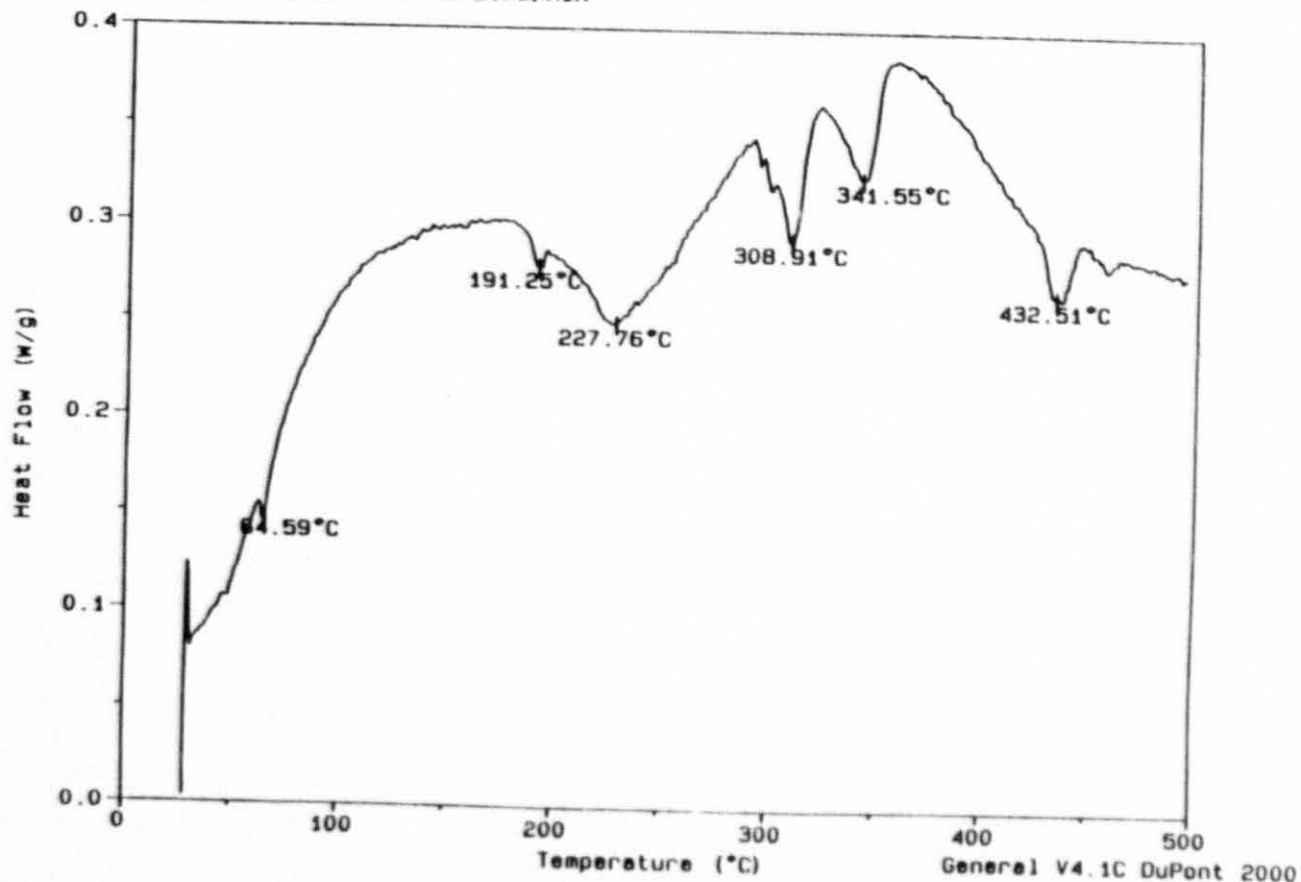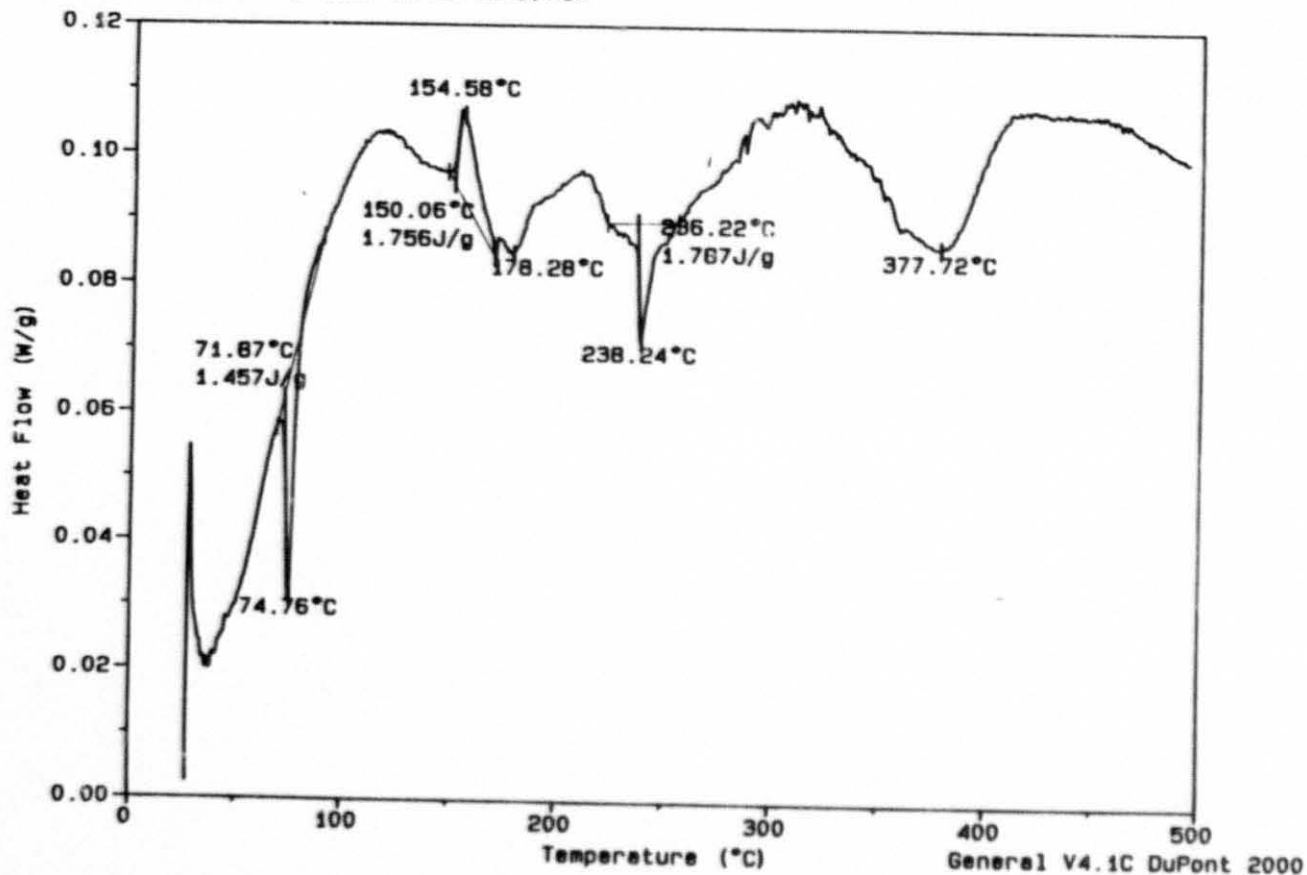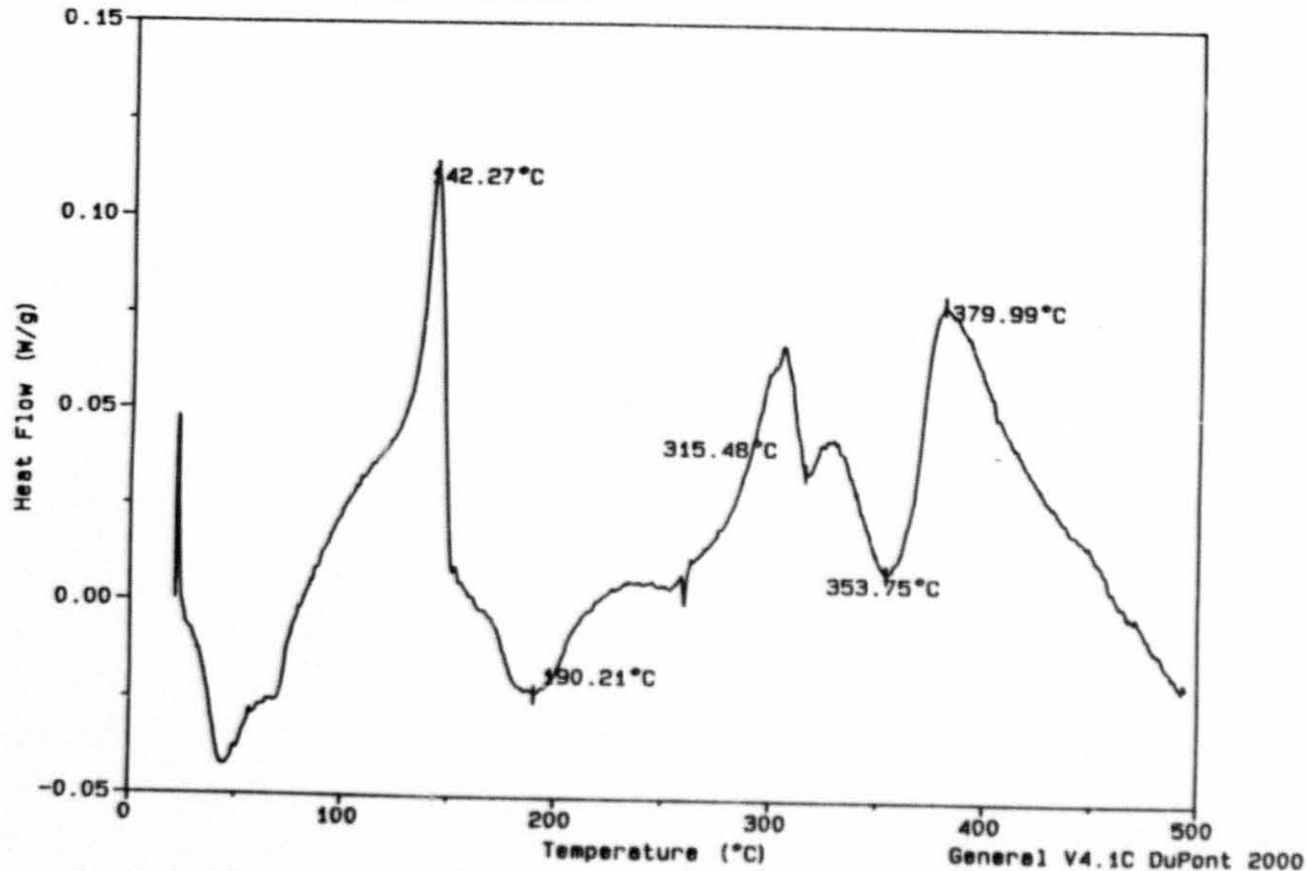DSC

File: A: WEI09.35
Operator: WEI
Run Date: 15-Feb-92 04:22

is probably the melting point peak. These two DSC curves are quite different.

3. Phosphatidylserine (PS)

Figure 9 shows a typical DSC curve for phosphatidylserine in nitrogen. The melting point of phosphatidylserine is 190^0C (4). On the DSC curve, the peak at 178^0C is probably the melting point peak. Figure 10 shows a DSC curve for a more pure sample of phosphatidylserine in Nitrogen. The broad peak at 190^0C is probably the melting point peak. The two DSC graphs are quite different. The peak at 142^0C is a exothermal peak which may show the decomposition or recrystallization. Some investigators have reported that the melting point of the phosphatidylserine depended upon the rate of heating. Decomposition has been shown to occur below the melting point (4).

4. Sphingomyelin (SM)

Figure 11 shows a typical DSC curve for sphingomyelin in nitrogen. This curve shows two sharp endothermic peaks at 88^0C and 130^0C and one broad endothermic peak at 219^0C which is the melting point of sphingomyelin. It is 10^0C lower than the value obtained by Chapman (4). The rest of the peaks show the decomposition. The peak at 130^0C has a large area. This indicates the transition change at this temperature requires great thermal

energy. This is probably caused by melting of the hydrocarbon chains portion of the molecule. Table 5 shows the transition temperature, enthalpies, and entropies of SM.

Figure 11. DSC curve for sphingomyelin in nitrogen

DSC

Sample: SM
Size: 13.8000 mg
Method: WEI
Comment: 5C/MIN TO 500C IN N2 50ML/MIN

File: A:WEIO9.37
Operator: WEI
Run Date: 15-Feb-92 08:28

General V4.1C DuPont 2000
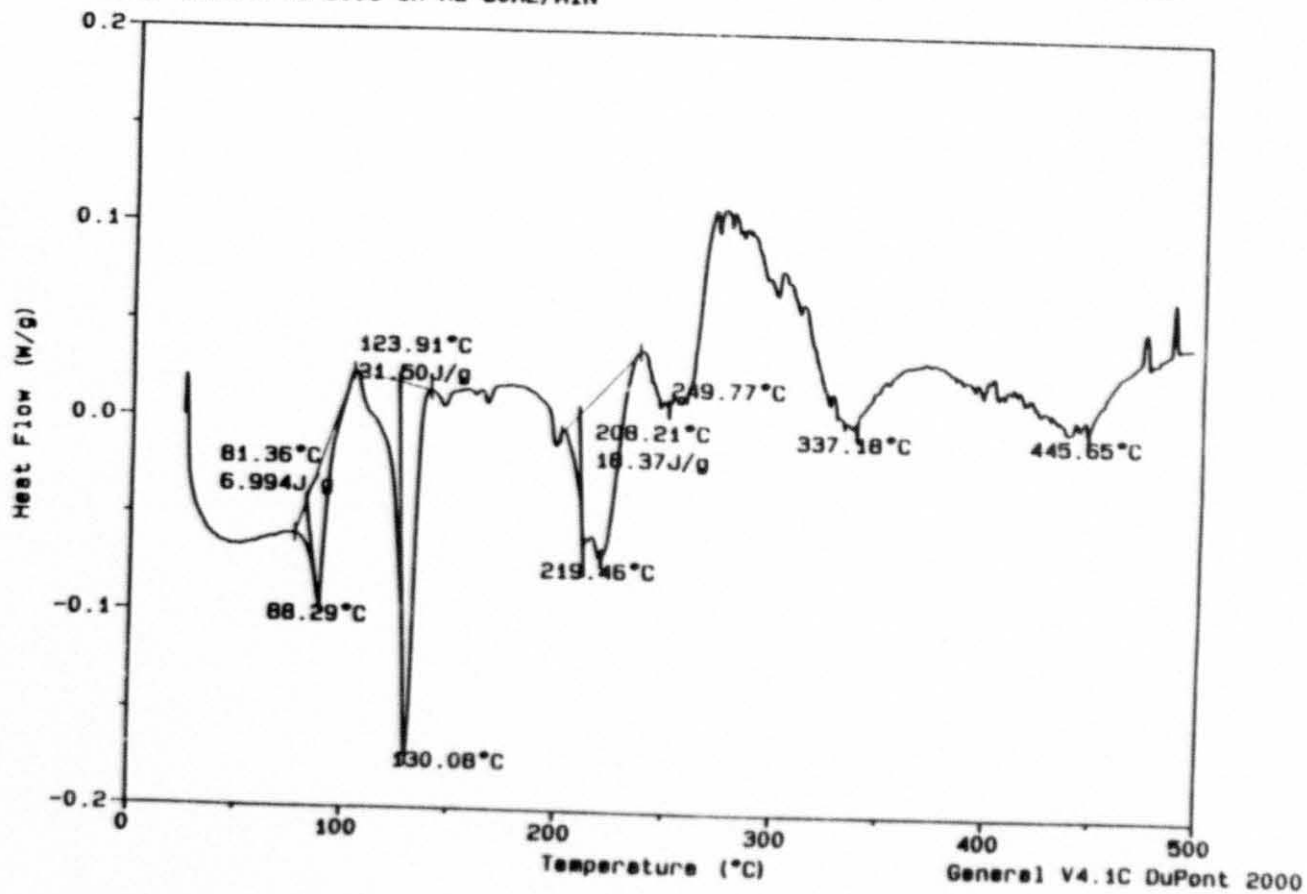
Table 5. The transition temperature, enthalpy changes and entropy changes of sphingomeylin

Transition	Temperature(^{0}C)	ΔH(KJ/mol)	ΔS(J/molK)
T1	88	5.23	14.47
T2	130	16.04	39.75

According to the data in Table 5, the sample is probably changing from anisotropic, fluid liquid crystal to an isotropic, viscous liquid since we observed small ΔS values for this change in PC at T1. At T2., a somewhat larger ΔS would indicate a change from an isotropic, viscous liquid state to an anisotropic, fluid liquid crystal.

It was necessary to test to see whether or not reproducibility could be obtained. Table 6 shows two different runs using phosphatidylcholine and sphingomyelin. These two runs involved the same experimental conditions with different sample sizes. It shows very good reproducibility.

Table 6. The transition temperature of egg-yolk lecithin and sphingomeylin at different runs

Phosphatidylcholine

Mass(mg)	Peak 1(^{0}C)	Peak 2(^{0}C)	Peak3(^{0}C)	Peak4(^{0}C)
12.1	109.4	122.1	166.2	226.4
6.4	110.3	122.3	165.8	225.6

Sphingomyelin

13.8	88.3	130.1	219.1	253.0
7.3	88.5	126.7	219.9	253.5

B. DSC on phospholipids in aquous solution:

We tried to examine phase transitions for phosphalipids in aqueous solutions. No phase transitions were observed for these solutions. The DuPont 910 DSC was not suitable for the study of phosphalipids in aquous solution. The Perkin Elmer DSC has been used to study the phosphalipids in aqueous solutions, primarily because it can hold a larger sample container (~500ul).

C. DSC on the mixture of cholesterol and phospholipids:

1. Cholesterol

Figure 12 shows a DSC curve for cholesterol. The peak at 142^0C is the melting point peak. The melting point of cholesterol is 142^0C shown in the CRC handbook (18). The broad peak at 50^0C is a phase transition peak.

2. Mixture of Phosphatidylcholine with Cholesterol

Figure 13 shows the DSC curve for a mixture of phosphatidylcholine with cholesterol. The phase transition shifted from 50^0C to 58^0C and the melting point elevated from 142^0 to 147^0C. It is not close to the melting point of PC, 226^0C (figure 5).

3. Mixture of Phosphatidylethanolamine with Cholesterol

Figure 14 shows a DSC curve for a mixture of phosphatidyl-

ethanolamine and cholesterol. They seemed to have formed a eutectic mixture with a melting point (130°C) below that of cholesterol 142°C and PE 184°C (Figure 6).

4.   Mixture of Phosphatidylserine with Cholesterol

Figure 15 shows a DSC curve for a mixture of phosphatidylserine and cholesterol. They apparently formed a eutectic mixture with a melting point (132°C) below that of cholesterol 142°C and PS 178°C (Figure 9).

5.   Mixture of Sphingomeylin with Cholesterol

Figure 16 shows a DSC curve for a mixture of sphingomeylin and cholesterol. Again they seemed to have formed a eutectic mixture with a melting point (106°C) below that of cholesterol 142°C and SM 219°C.

Figure 12. DSC curve for cholesterol in nitrogen

Sample: CHOLESTEROL
Size: 7.1000 mg
Method: WEI
Comment: 5C/MIN TO 200 IN N2 50ML/MIN

DSC

File: A:WEI09.08
Operator: WEI
Run Date: 11-Oct-91 22:00
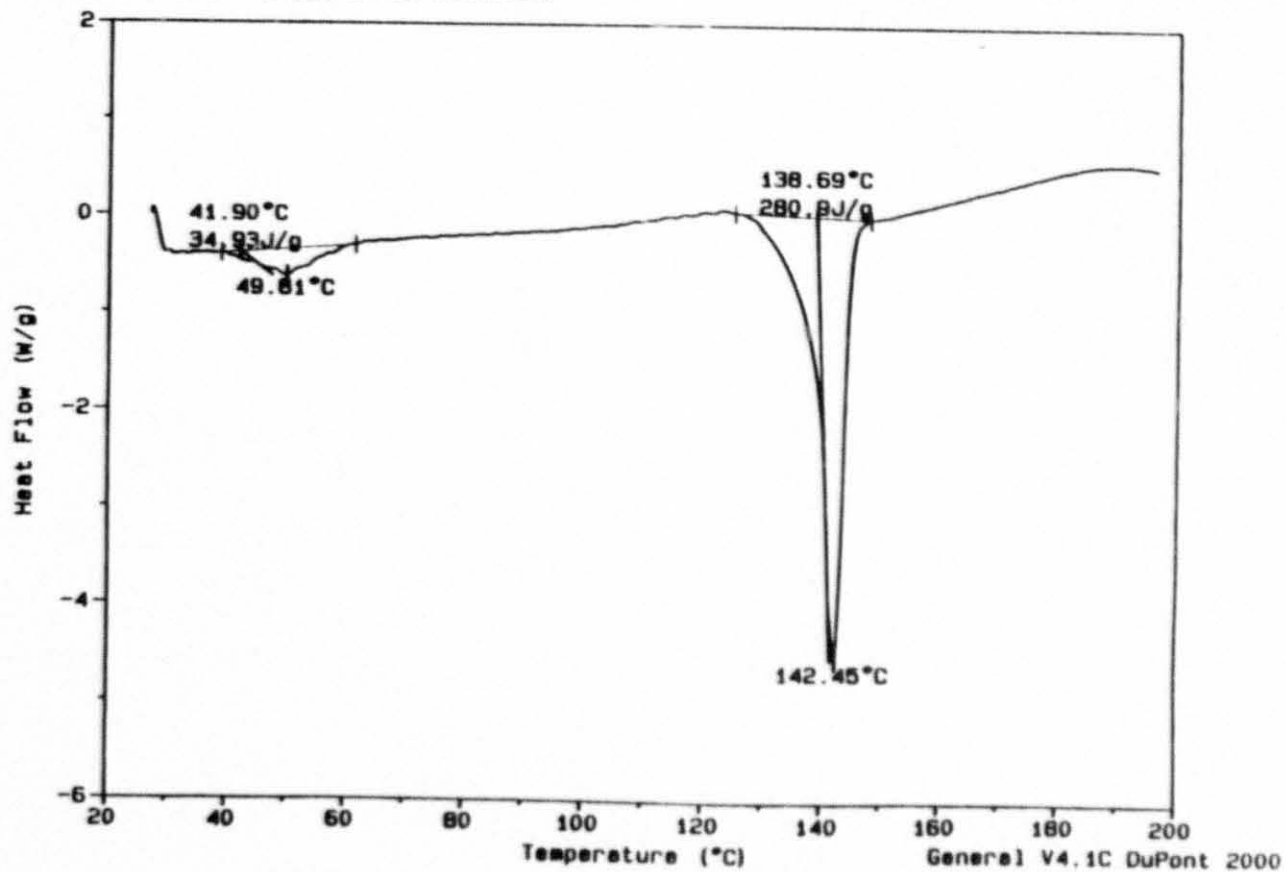
General V4.1C DuPont 2000

Figure 13.   DSC curve for a mixture of egg-yolk lecithin with cholesterol in nitrogen

Sample: PC+CHOL (1:1)
Size: 6.5000 mg
Method: WEI
Comment: 5C/MIN TO 200 IN N2 50ML/MIN

DSC

File: A:WEI09.18
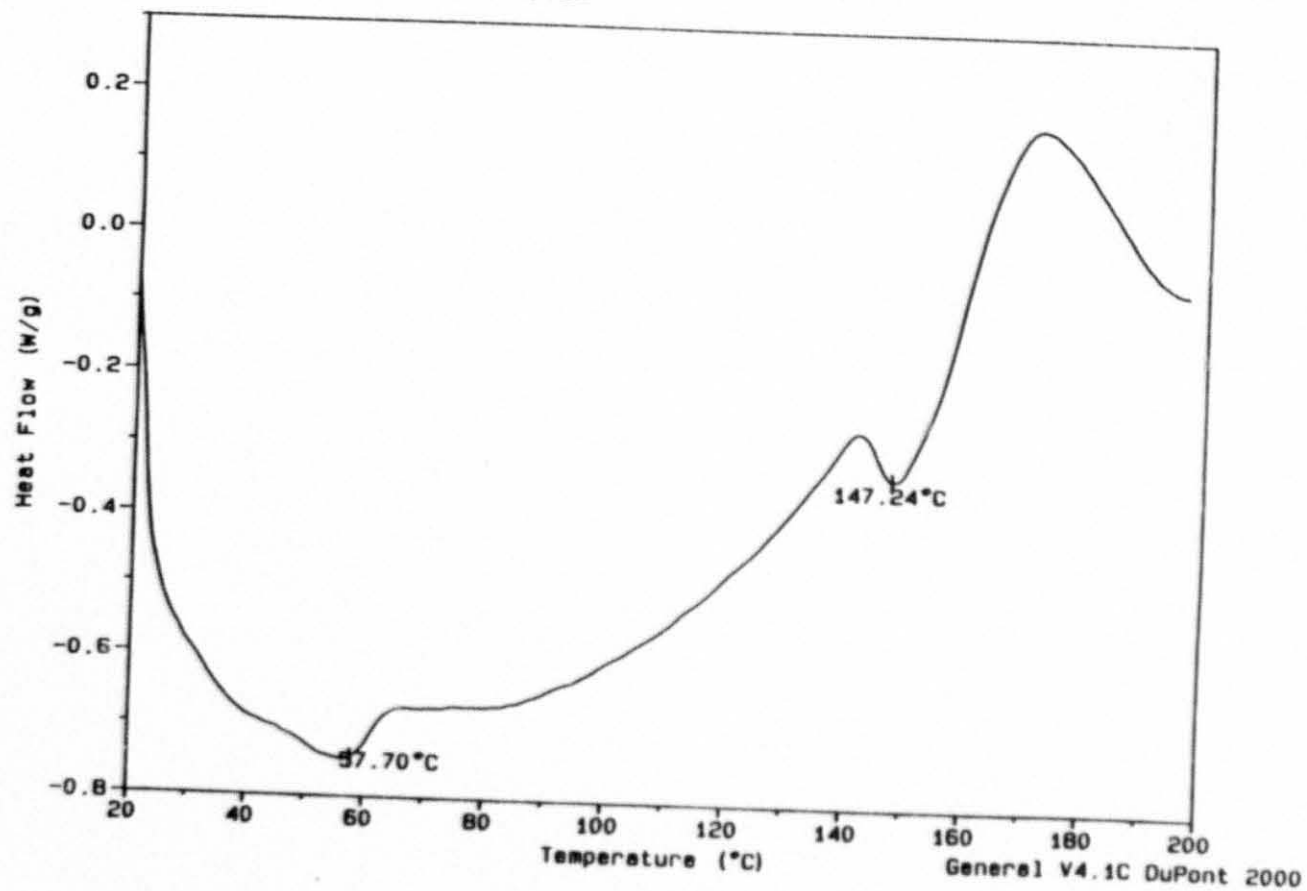Operator: WEI
Run Date: 17-Oct-91 15:24

General V4.1C DuPont 2000

Figure 14.   DSC curve for a mixture of phosphatidylethanolamine with cholesterol in nitrogen

Sample: PE + CHOL (1:1)   DSC   File: A:WEIO9.14
Size: 7.2000 mg   Operator: WEI
Method: WEI   Run Date: 15-Oct-91 22:10
Comment: 5C/MIN TO 200 IN N₂ 60ML/MIN

146.00°C

130.18°C

Heat Flow (W/g)
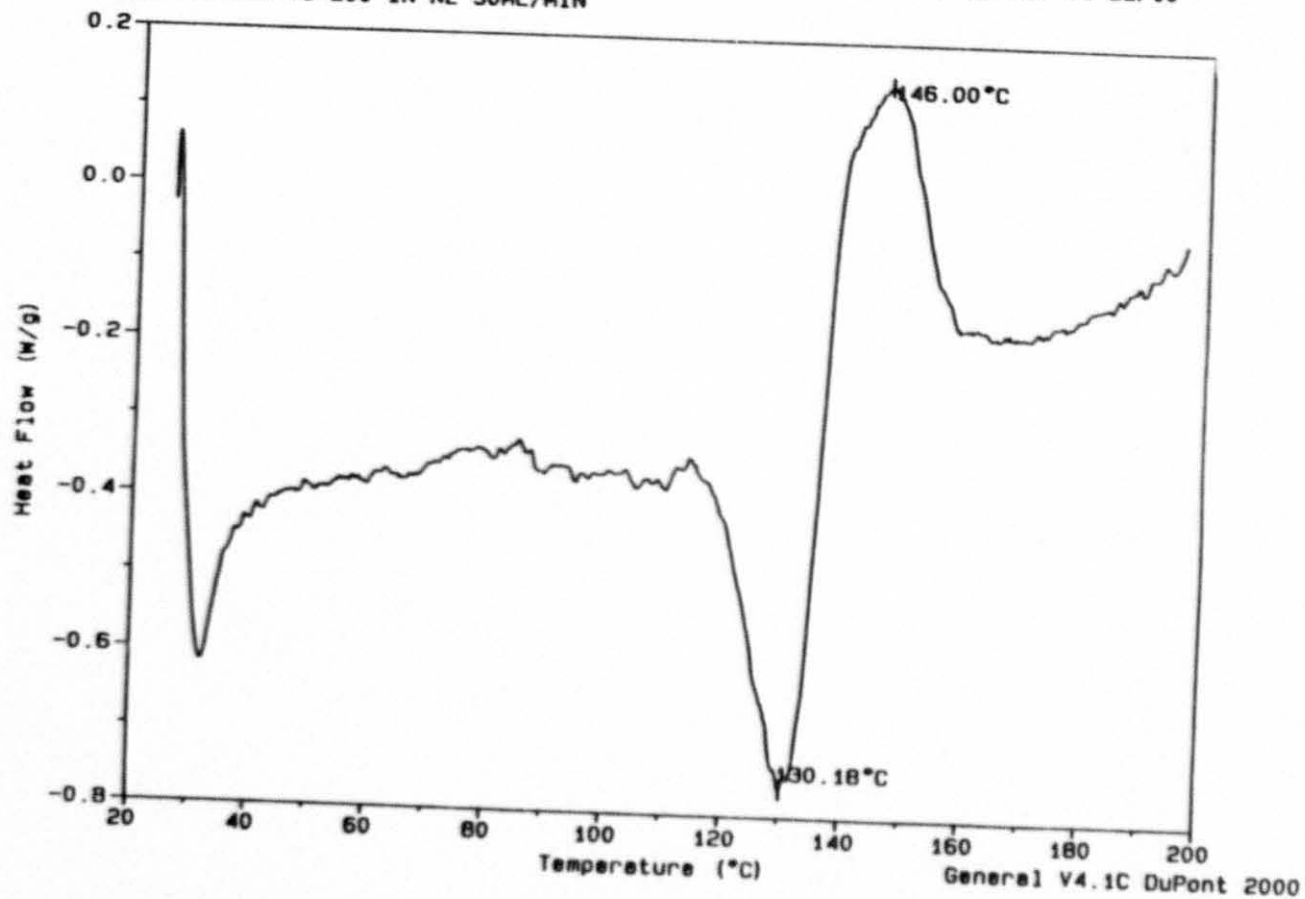Temperature (°C)

General V4.1C DuPont 2000

Figure 15.   DSC curve for a mixture of phosphatidylserine with cholesterol in nitrogen

DSC

Sample: PS + CHOL (1:1)
Size: 7.9000 mg
Method: WEI
Comment: 5C/MIN TO 200 IN N2 50ML/MIN

File: A:WEI09.13
Operator: WEI
Run Date: 15-Oct-91 21:01
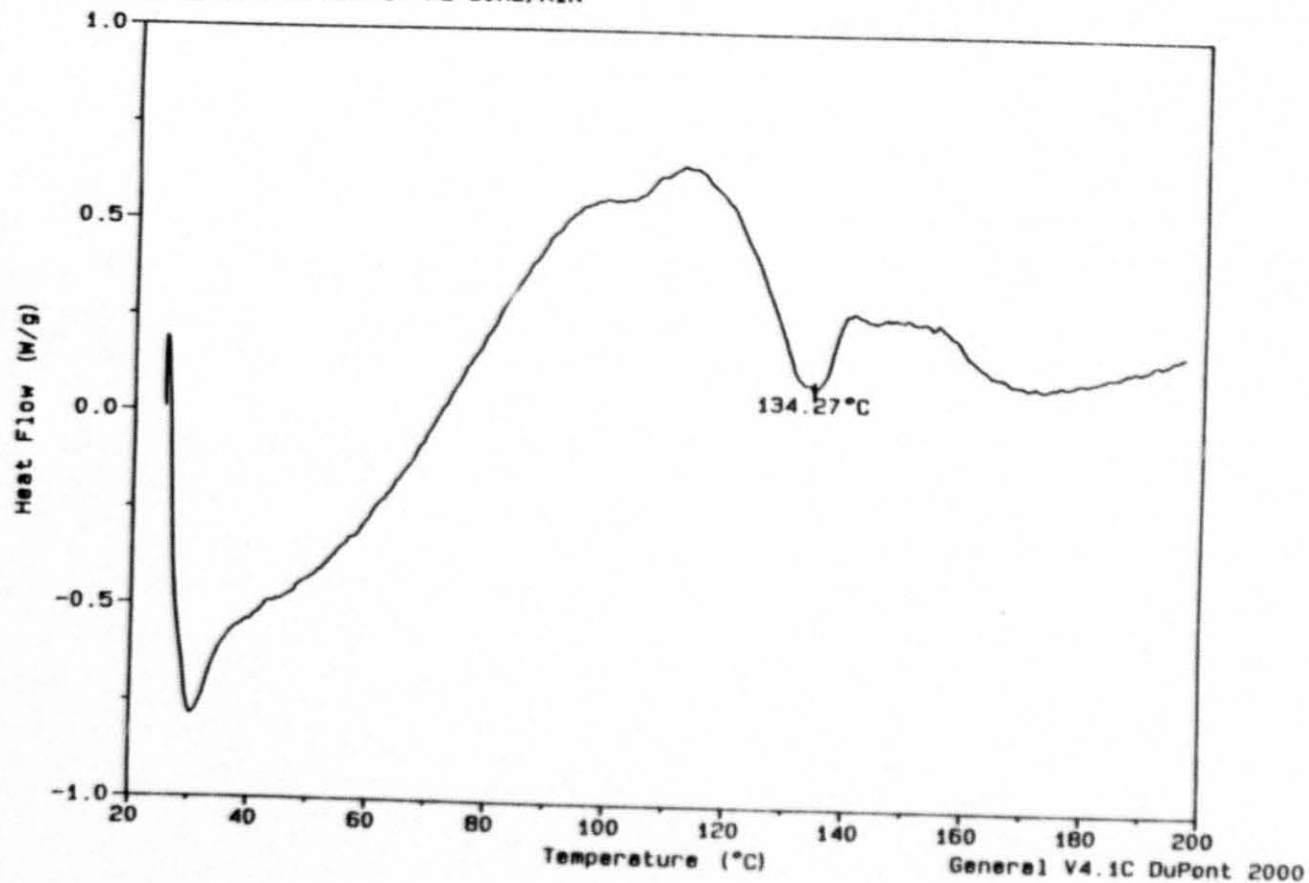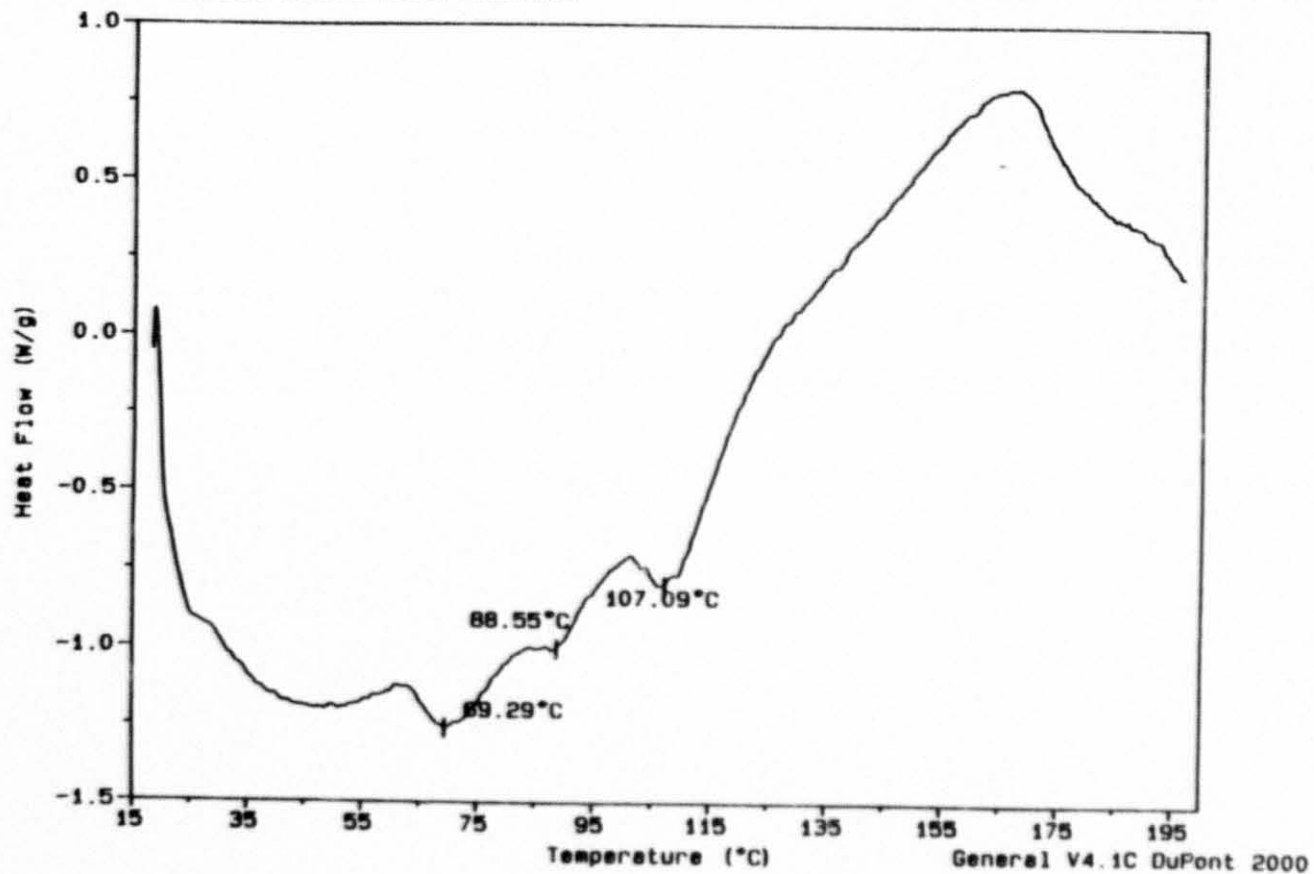
134.27°C

General V4.1C DuPont 2000

Figure 16.   DSC curve for a mixture of sphingomeylin with cholesterol in nitrogen

Sample: SM + CHOL (1:1)
Size: 4.3000 mg
Method: WEI
Comment: 5C/MIN TO 200 IN N2 50ML/MIN

File: A:WEI09.15
Operator: WEI
Run Date: 16-Oct-91 19:46

General V4.1C DuPont 2000

88.55°C
107.09°C
69.29°C

Heat Flow (W/g)

Temperature (°C)

D. TG-FTIR on phospholipids:

1. Phosphatidylcholine (PC)

The TG curve of phosphatidylcholine (Figure 17) shows a weight loss of 2.5% below 90^0C and about 4% at constant weight. On the TG curve (Figure 17), the maximum rate of weight loss is at 89^0C. When the temperature is above 130^0C, the weight stays constant to 200^0C. Figure 18 shows the TG-FTIR spectrum of phosphatidylcholine at 30^0C in nitrogen. There is no peak. Figure 18 shows the TG-FTIR spectrum of PC at 100^0C. There is a broad peak at $1523cm^{-1}$ which is a water peak. The FTIR indicated that the weight loss was water lost.

For this study, the average molecular weight of egg-yolk lecithin was calculated using composition data from Marsh and Phil. (18). It is 713. The percent water for a monohydrate would be $18/(713+18)$ = $0.025 \times 100\%$ = 2.5%. The percent water for a dihydrate would be $36/(713+36)$ = $0.048 \times 100\%$ = 4.8%. So we can say phosphatidylcholine has more water than a monohydrate but less than a dihydrate. Chapman (4) found that DSPC existed in the form of a monohydrate.

It was found that using TG-FTIR was quicker than Chapman's method of using a drying oven. The TG-FTIR required about 30 minutes while Chapman's study required 4 hours. On Figure 19, there is CO_2 peak at $2333cm^{-1}$. At this temperature, no decomposition

Figure 17.   TG curve for egg-yolk lecithin in nitrogen

Sample: PC
Size: 13.7820 mg
Method: WEI
Comment: 5C/MIN TO 200 IN N2 50ML/MIN

TGA
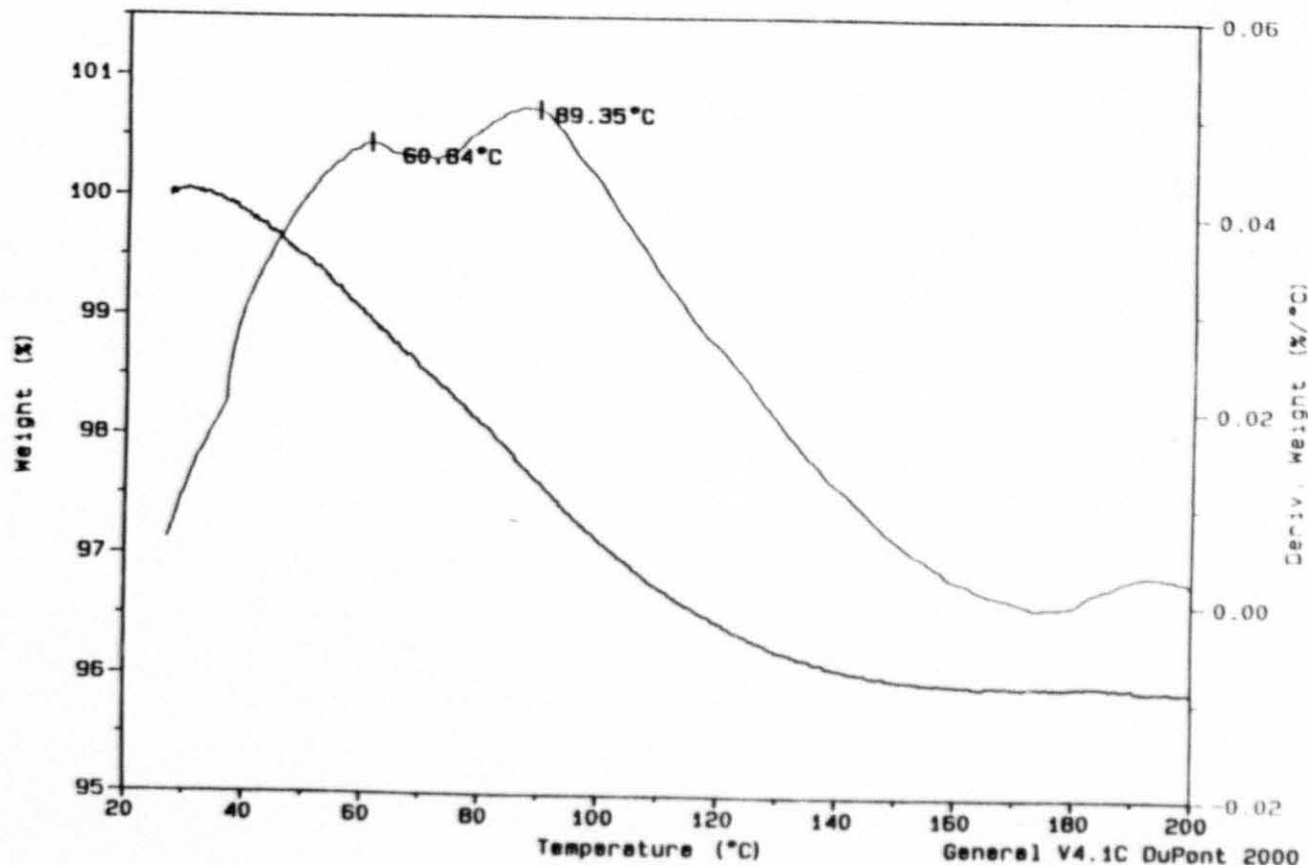
File: A:WEI09.03
Operator: WEI
Run Date: 23-Sep-91 19:18

60.84°C

89.35°C

Weight (%)

Deriv. Weight (%/°C)

Temperature (°C)

General V4.1C DuPont 2000

48

Figure 18. TG-FTIR spectrum for egg-yolk lecithin in nitrogen at 30°C

PERKIN ELMER

100.92
%T

95.00
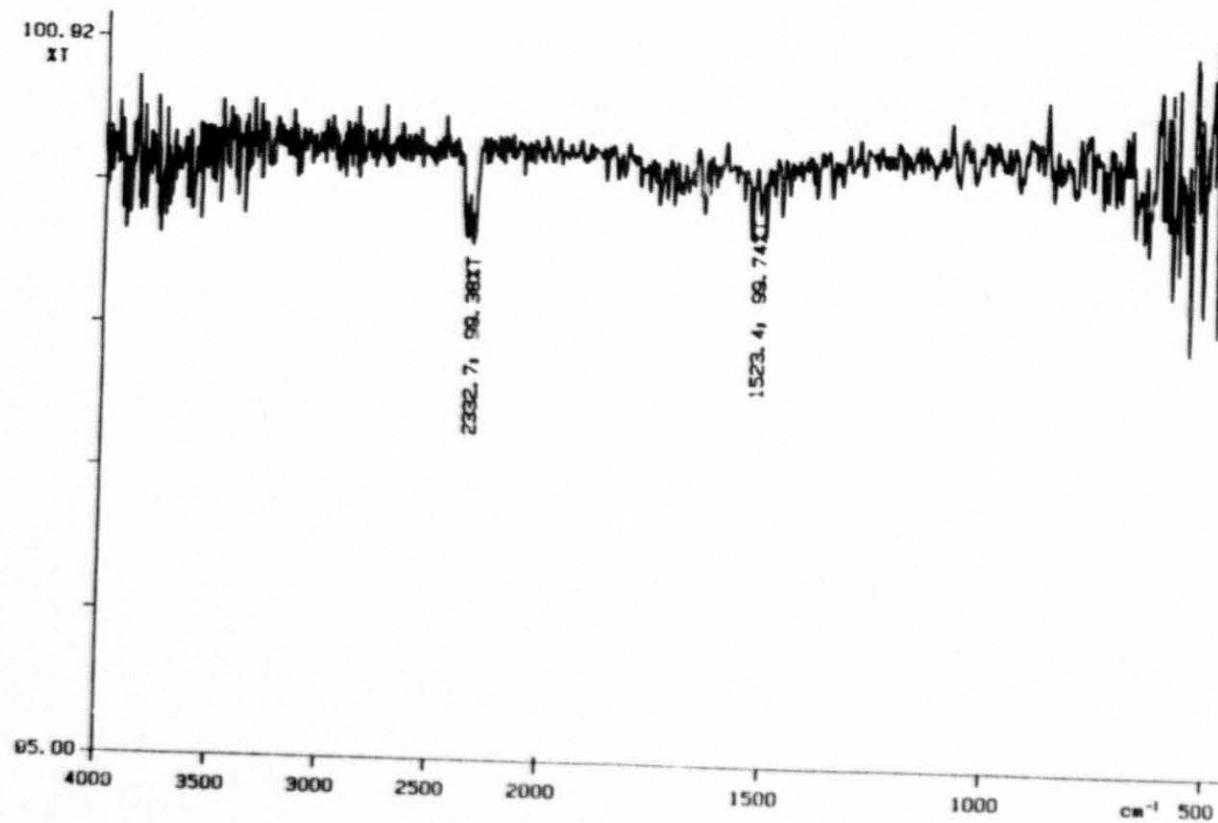
4000   3500   3000   2500   2000   1500   1000   cm⁻¹ 500
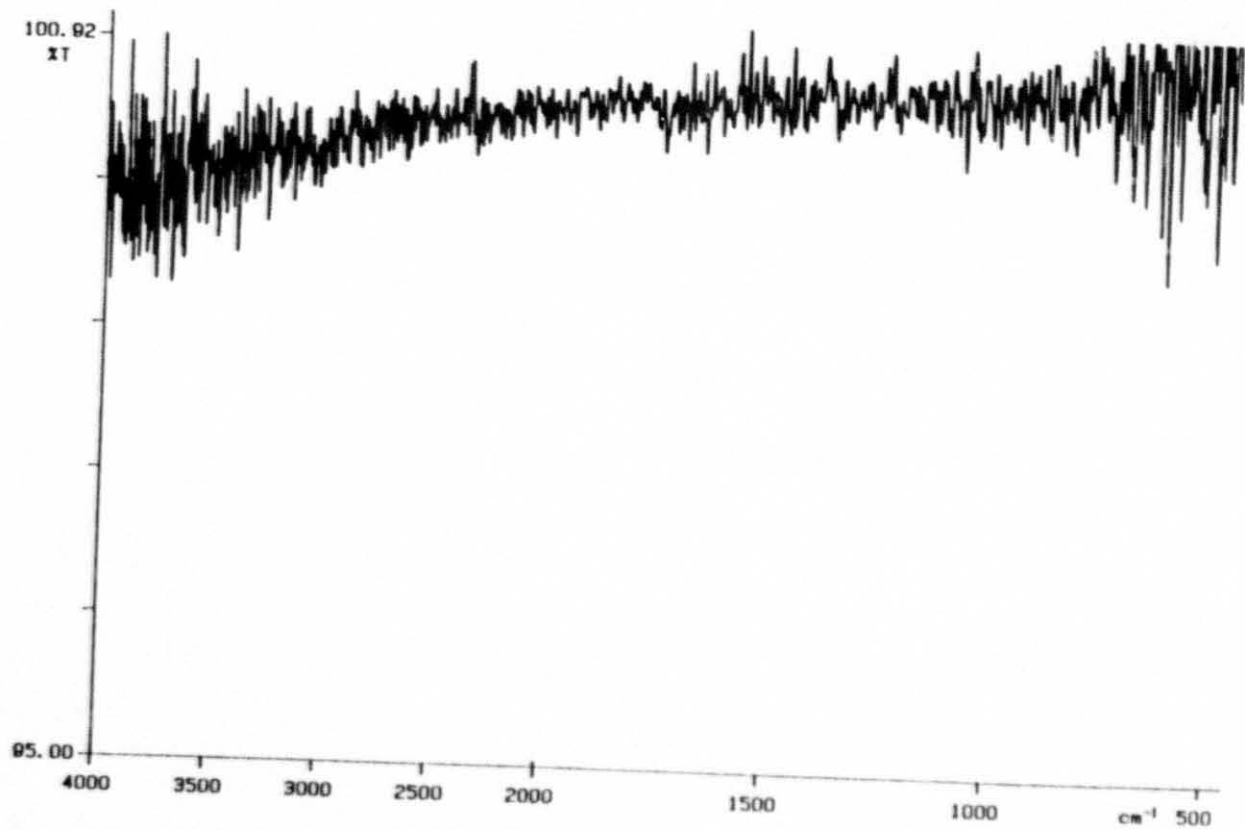
91/09/23 10:21 dbd
30c: 1 scan, 4.0cm-1, ext

Figure 19. TG-FTIR spectrum for egg-yolk lecithin in nitrogen at 100^{0}C

PERKIN ELMER

100.92
%T

2332.7, 99.38%T

1523.4, 99.74%T

95.00

4000   3500   3000   2500   2000   1500   1000   cm⁻¹ 500

91/09/23 18:38 dbd
100c; 4 scans, 4.0cm-1, ext.

should happen. The CO_2 was propably absorbed in the sample and was released during the heating process.

2. phosphatidylethanolamine (PE)

The TG (Figure 20) and FTIR data (Figure 21, and Figure 22), indicated only moisture was given off under 90°C. The weight loss was about 5%, showing that phosphatidylethanolamine is probably a dihydrate. The calculated average molecular weight of phosphatidylethanolamine for this study is 725 (18). The percent water for a dihydrate is $36/(725+36) = 0.047 \times 100\% = 4.7\%$. Figure 20 shows the TG-FTIR spectrum of phosphatidylethanolamine at 22°C in nitrogen. No peaks were showing on the spectrum. Figure 21 shows the TG-FTIR spectrum of phosphatidylethanolamine at 90°C in nitrogen. There is a broad peak at 1523cm-1 which is a water peak. These data prove that phosphatidylethanolamine is releasing water.

3. phosphatidylserine (PS)

The TG curve (Figure 23) shows two peaks. One is at 59°C which T_{max1} and the other is at 156°C which is T_{max2}. T_{max1} shows the loss of water, while T_{max2} probably shows decomposition. If we also consider the TG-FTIR data (see Figure 24 and Figure 25) at these temperatures, we see that there is H_2O given off below 90°C. The weight loss is about 2.5%, so phosphatidylserine is probably a monohydrate. The calculated average molecular weight of

phosphatidylserine for this study is 764 (18). The percent of water is $18/(764+18) = 0.023 \times 100\% = 2.3\%$ with one mole of phosphatidylserine with one mole of water. The TG-FTIR (see Figure 26) shows that the decomposition that occurs at T_{max2} results in CO_2 gas being given off at this temperature.

4. sphingomeylin (SM)

The TG curve (Figure 27) in nitrogen shows a weight loss of about 5% at about 115^0C. The FTIR data (Figure 28 and Figure 29) shows there is H_2O with a peak at $1551cm^{-1}$. It can be deduced that there is moisture given off on heating indicating that sphingomeylin is dihydrate. Some CO_2 was also released (Figure 29), this was propbably absorbed in the sample. The calculated average molecular weight of sphingomeylin for this study is 746 using the data from the reference (18). Assuming one mole sphingomeylin combine with two moles water, the percent of water would be $2 \times 18/(746+36) = 0.046 \times 100\% = 4.6\%$. So sphingomeylin is probably a dihydrate.

Figure 20.   TG curve for phosphatidylethanolamine

Sample: PE
Size: 9.4040 mg
Method: WEI
Comment: 5C/MIN TO 300C IN N2 50ML/MIN

TGA
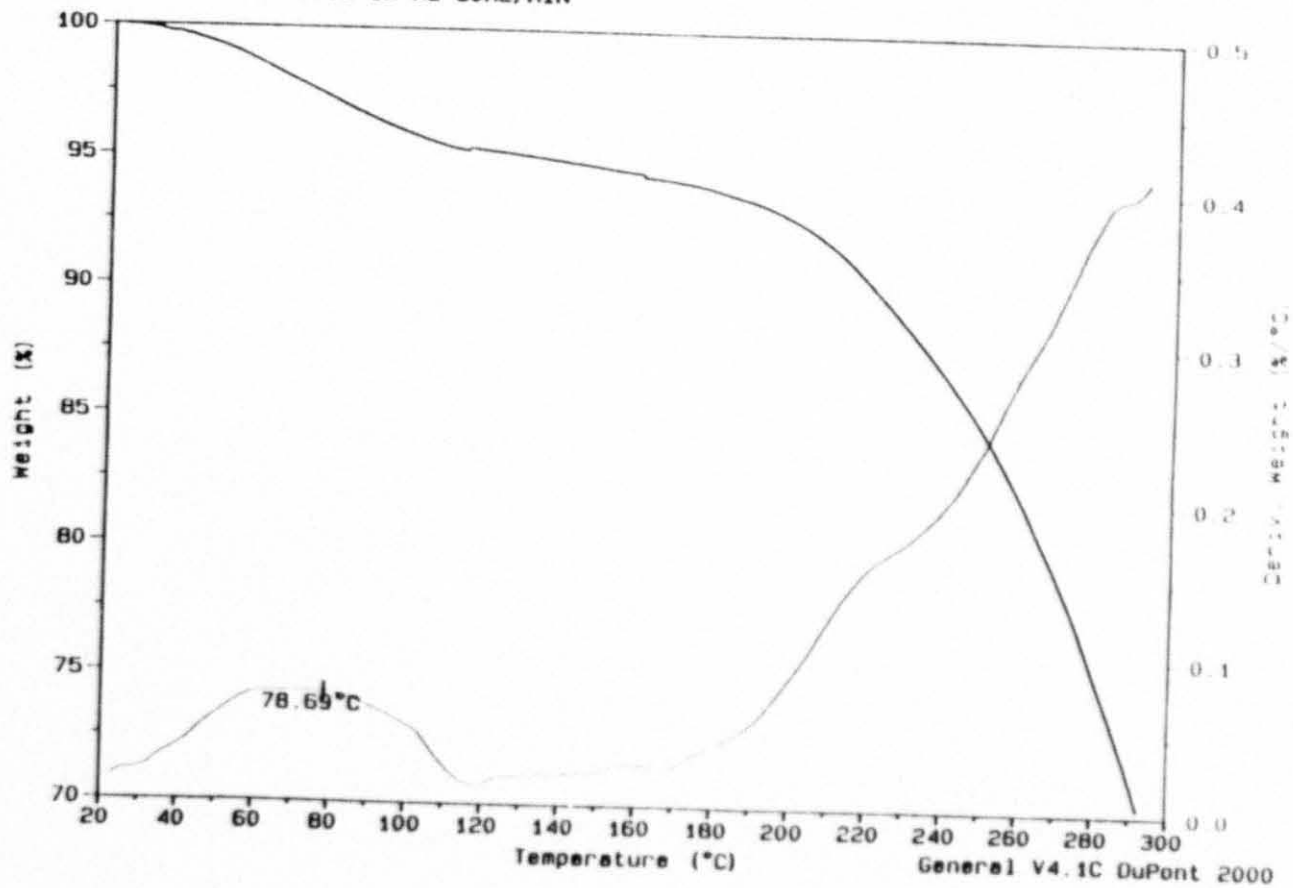
File: A:WEI09.32
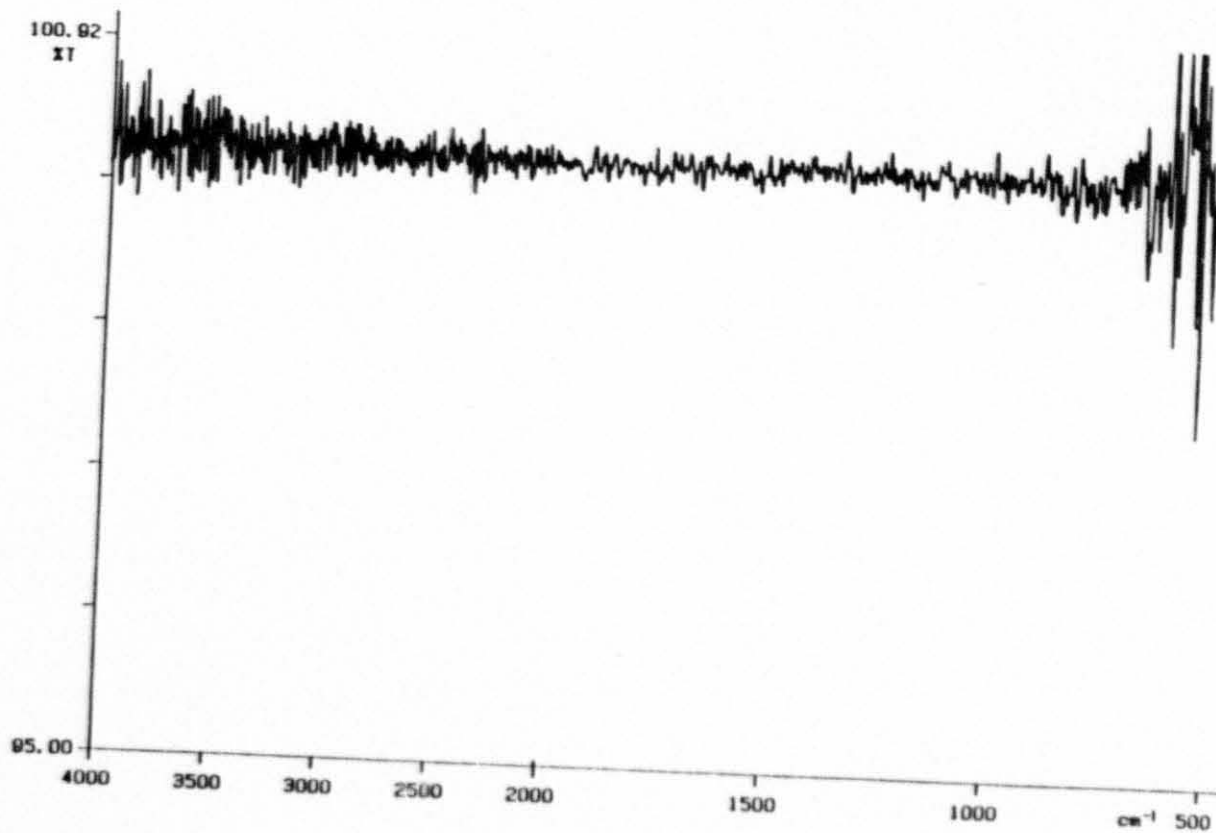Operator: WEI
Run Date: 8-Dec-91 14:24

78.69°C

Weight (%)

Temperature (°C)

General V4.1C DuPont 2000

53

Figure 21. TG-FTIR spectrum for phosphatidylethanolamine in nitrogen at 22°C

PERKIN ELMER

100.92
%T

95.00

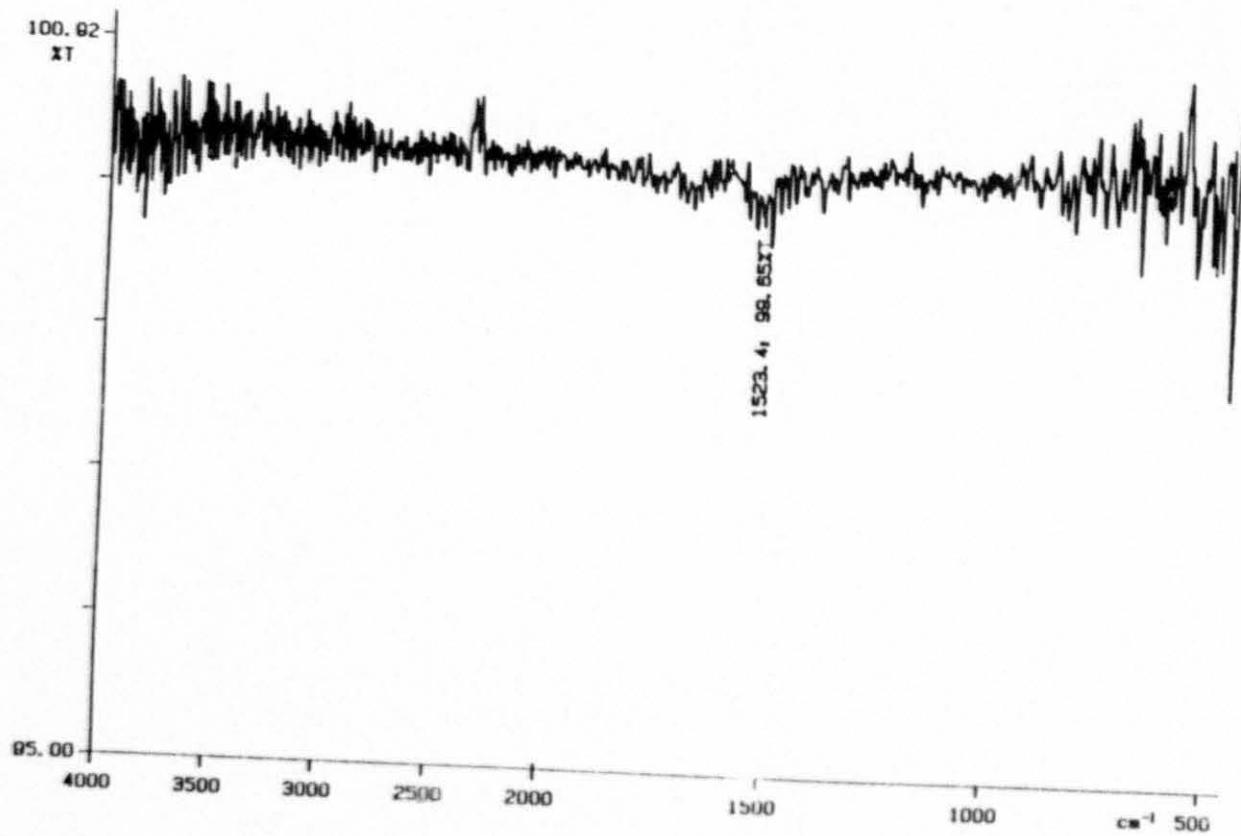4000   3500   3000   2500   2000   1500   1000   cm⁻¹ 500

91/12/05 16:27 dbd
pel: 4 scans, 4.0cm-1, ext
21.80

Figure 22. TG-FTIR spectrum for phosphatidylethanolamine in nitrogen at 90^{0}C

PERKIN ELMER

100.02
%T

1523.4, 99.65%T

95.00

4000   3500   3000   2500   2000   1500   1000   cm⁻¹ 500

91/12/05 16:41 dbd
pe8, 4 scans, 4.0cm-1, ext
80

Figure 23. TG curve for phosphatidylserine in nitrogen

Sample: PS
Size: 4.0350 mg
Method: WEI
Comment: 5C/MIN TO 200 IN N2 50ML/MIN

TGA

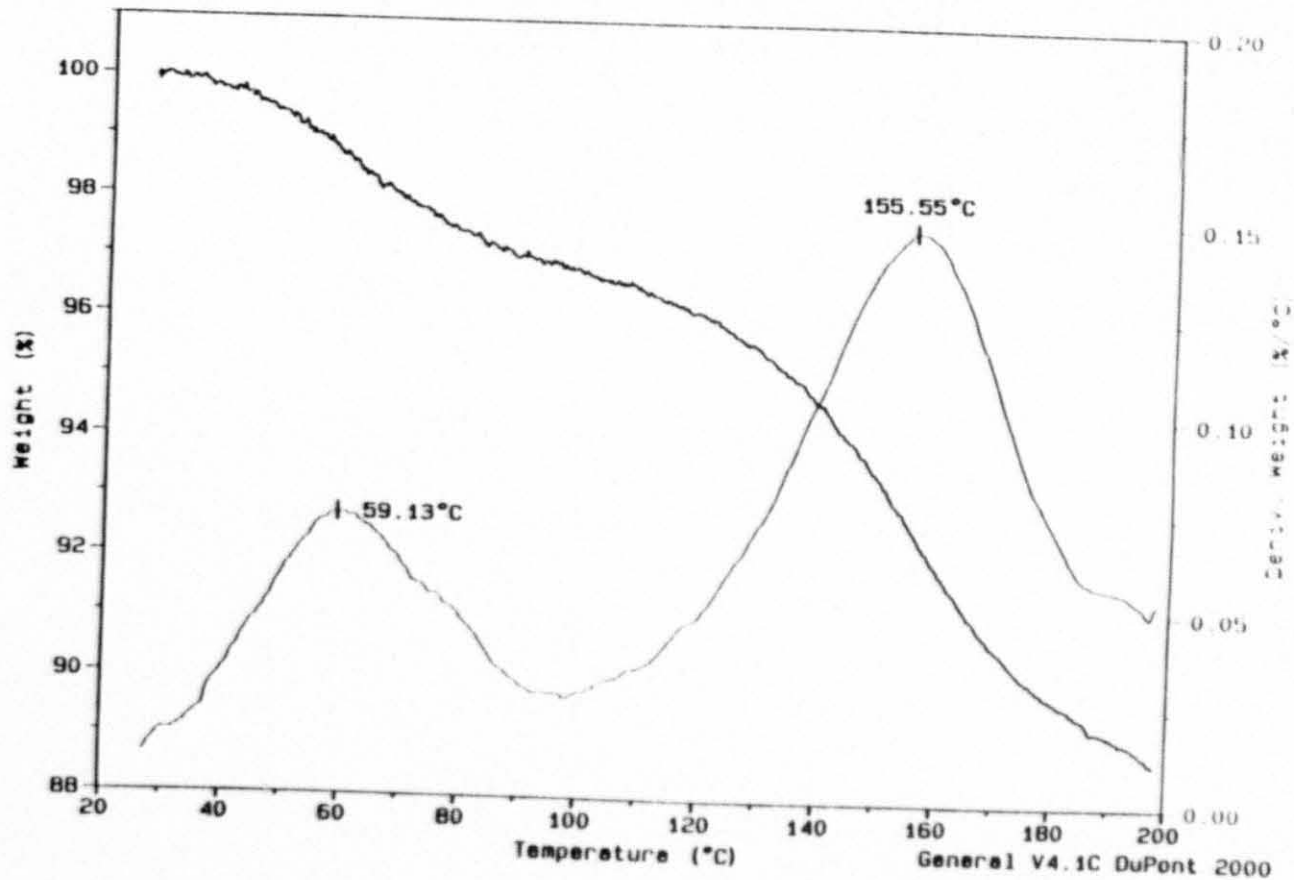File: A:WEI09.04
Operator: WEI
Run Date: 23-Sep-91 21:11
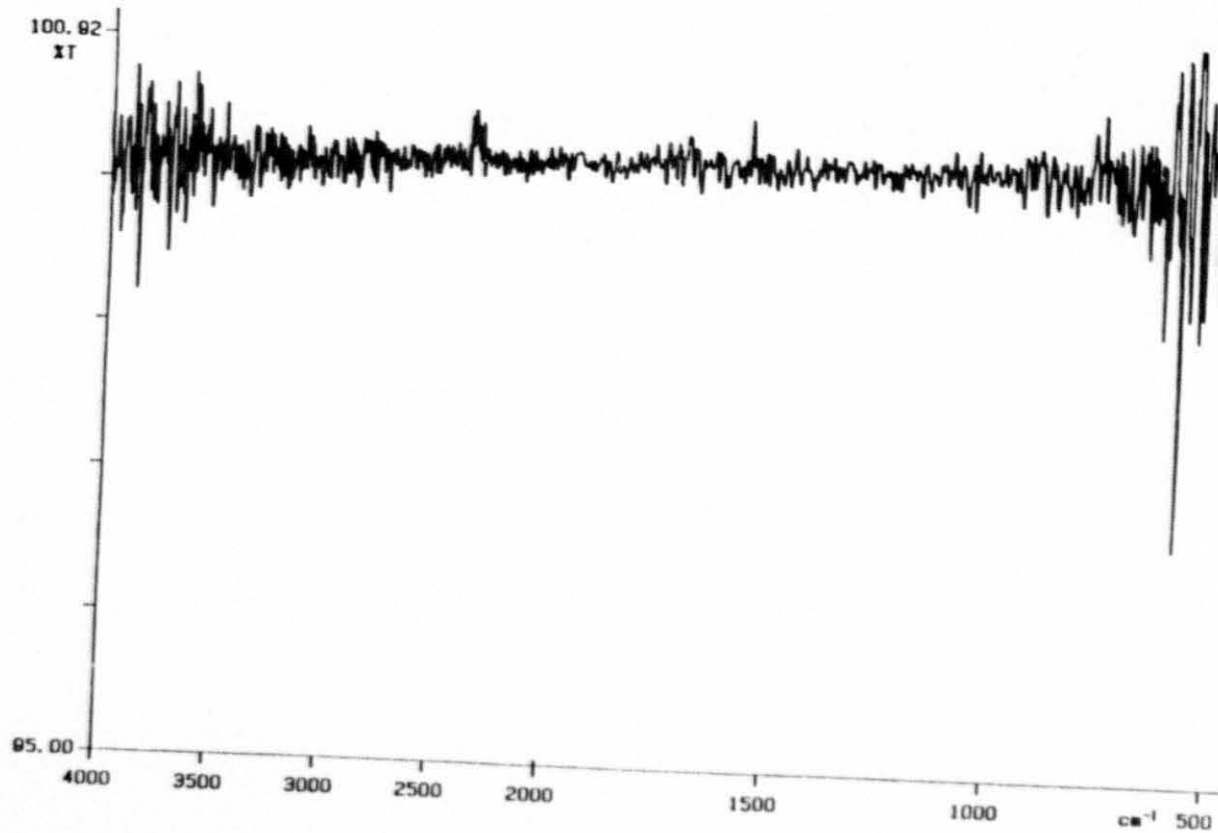
General V4.1C DuPont 2000

56

Figure 24. TG-FTIR spectrum for phosphatidylserine in nitrogen at 34°C

PERKIN ELMER

100.02
%T

95.00

4000   3500   3000   2500   2000   1500   1000   cm⁻¹ 500
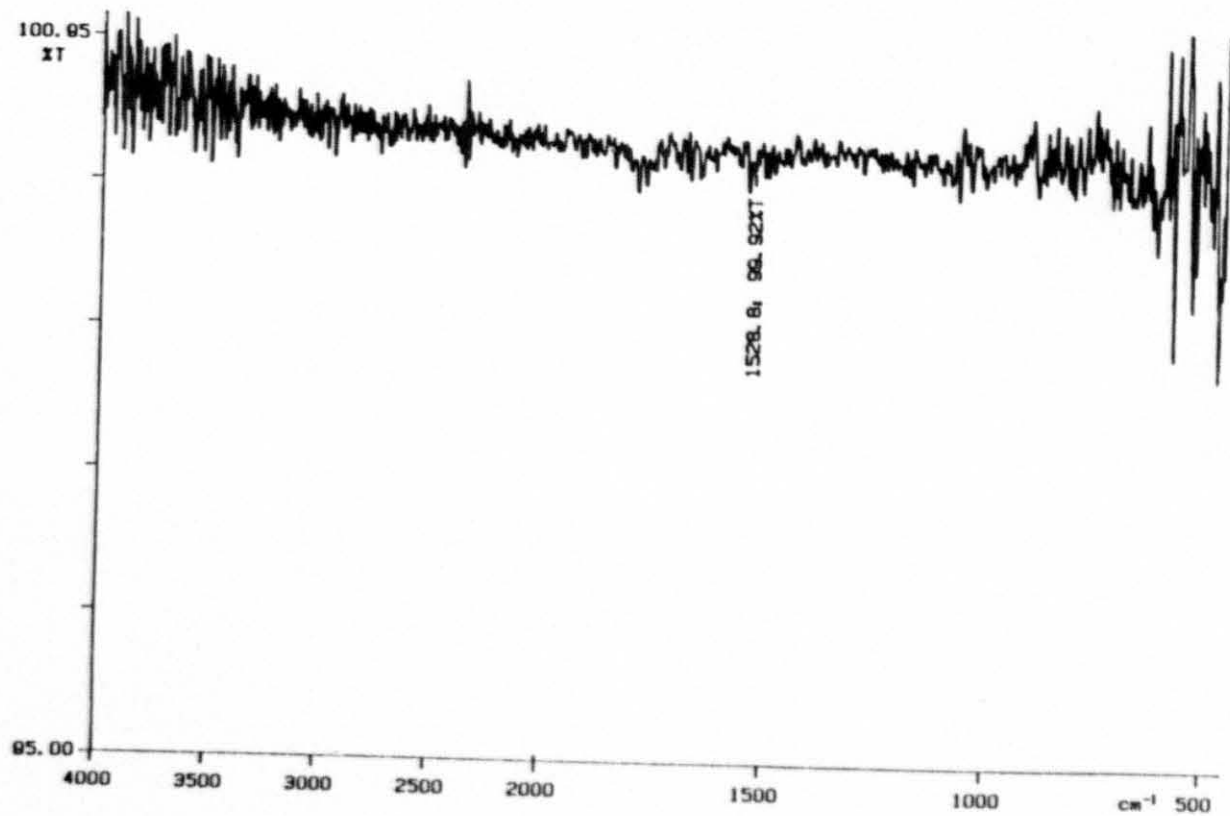
91/09/23 20:18 dbd
34a: 4 scans, 4.0cm-1, ext

Figure 25.   TG-FTIR spectrum for phosphatidylserine in nitrogen at 90°C

PERKIN ELMER

100.05
%T

1528.8, 99.92%T

95.00

4000   3500   3000   2500   2000   1500   1000   cm⁻¹ 500
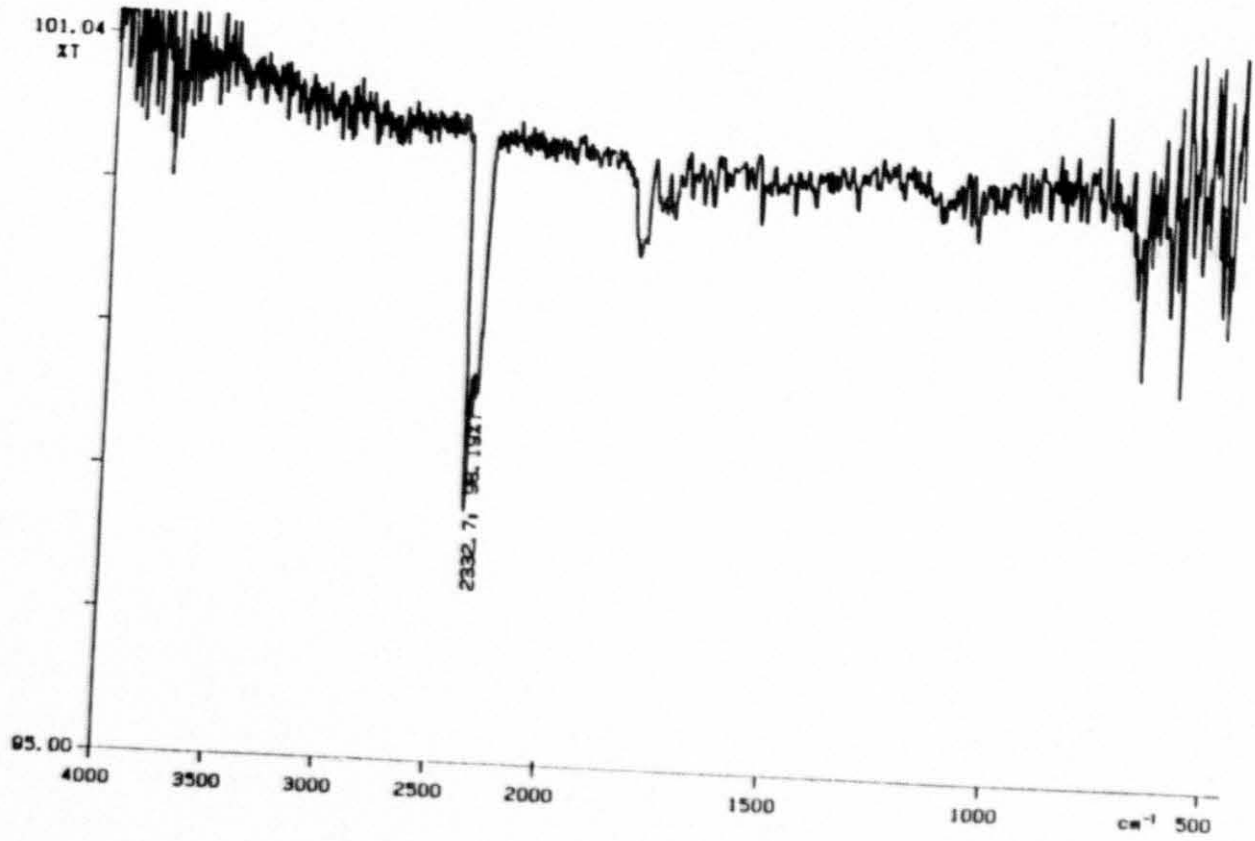
91/09/23 20:29 dbd
80a: 4 scans, 4.0cm-1, ext

58

Figure 26. TG-FTIR spectrum for phosphatidylserine in nitrogen at 160^{0}C

PERKIN ELMER

101.04
%T

2332.7, 96.197

95.00

4000   3500   3000   2500   2000   1500   1000   cm⁻¹ 500

91/09/23 20:43 dbd
180a: 4 scans, 4.0cm-1, ext

Figure 27. TG curve for sphingomeylin in nitrogen
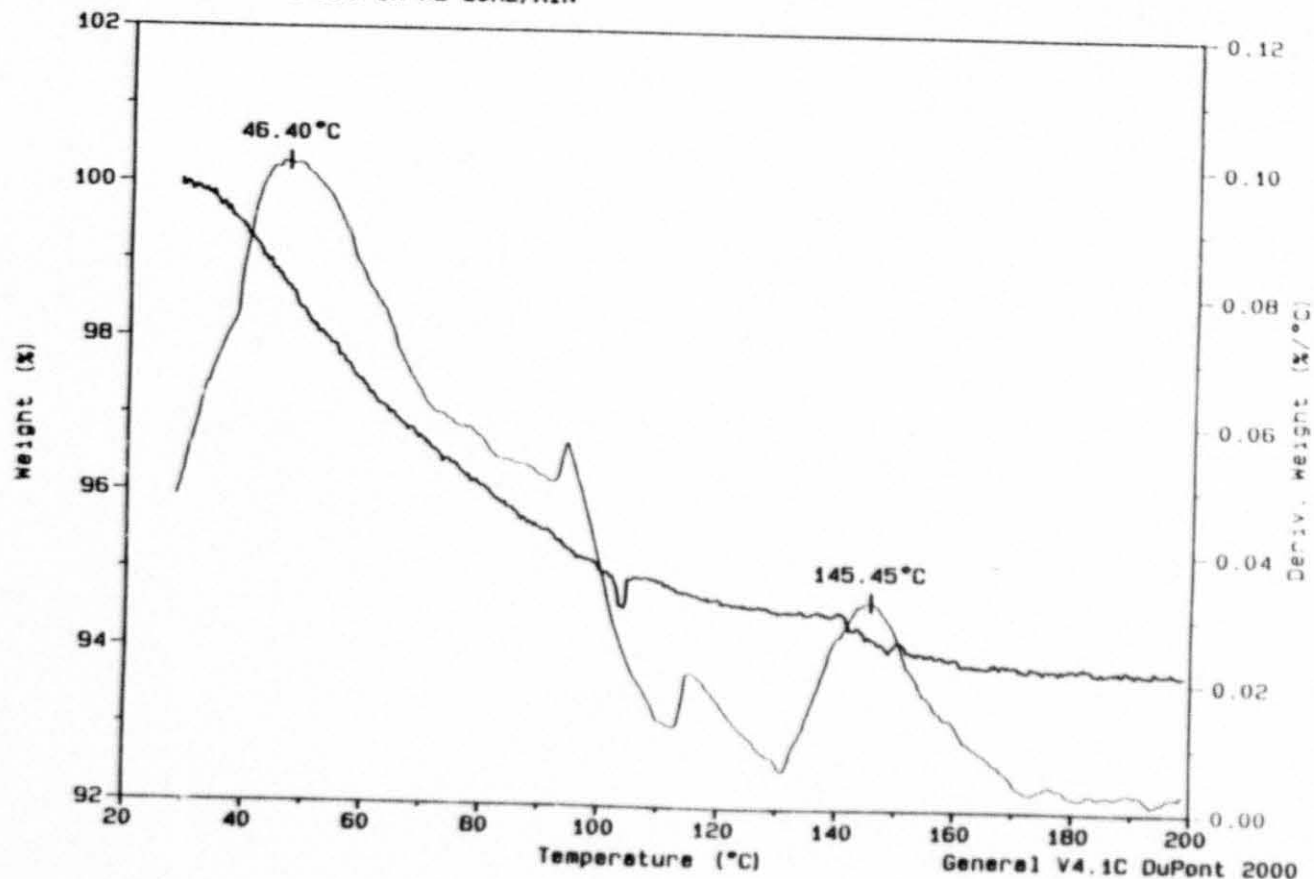
Figure 28.   TG-FTIR spectrum for sphingomeylin in nitrogen at 29°C

PERKIN ELMER

100.95
%T

95.00

4000   3500   3000   2500   2000   1500   1000   cm⁻¹ 500
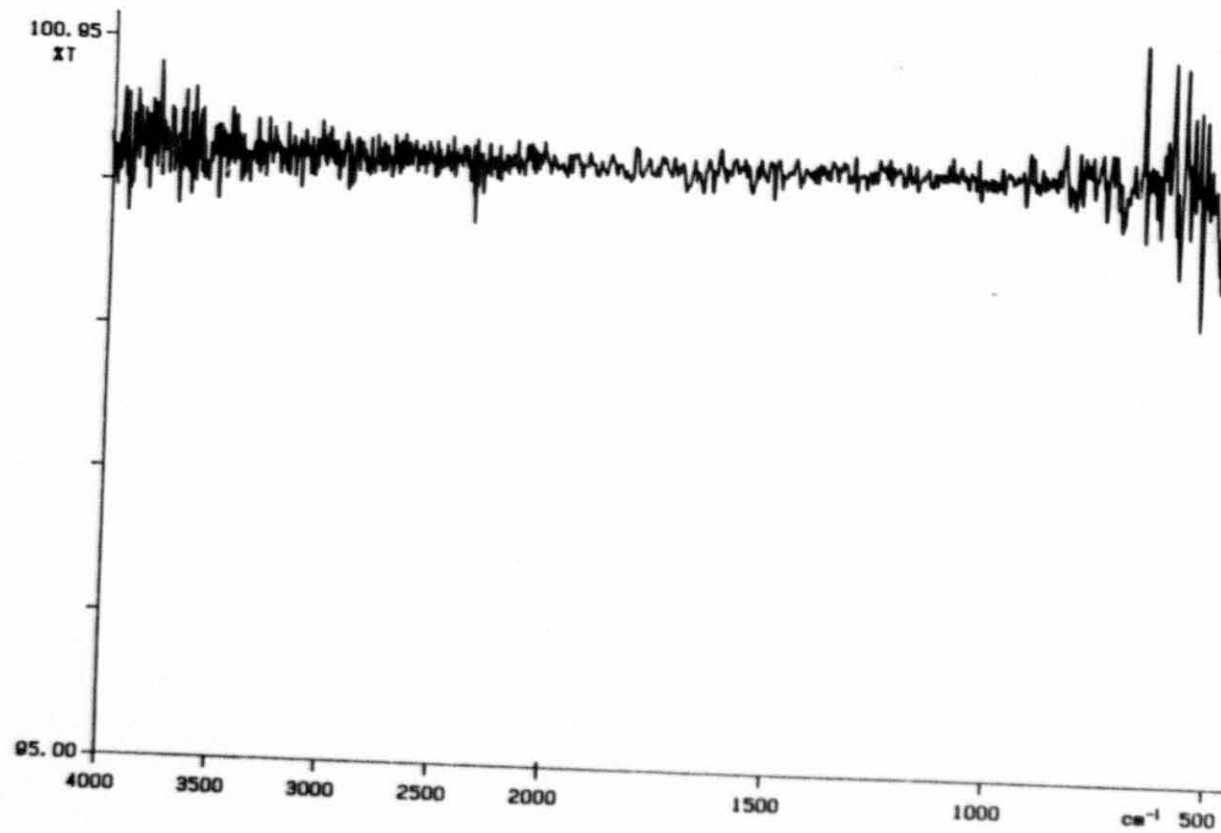
91/12/05 18:45 dbd
em1, 4 scans, 4.0cm-1, ext
29
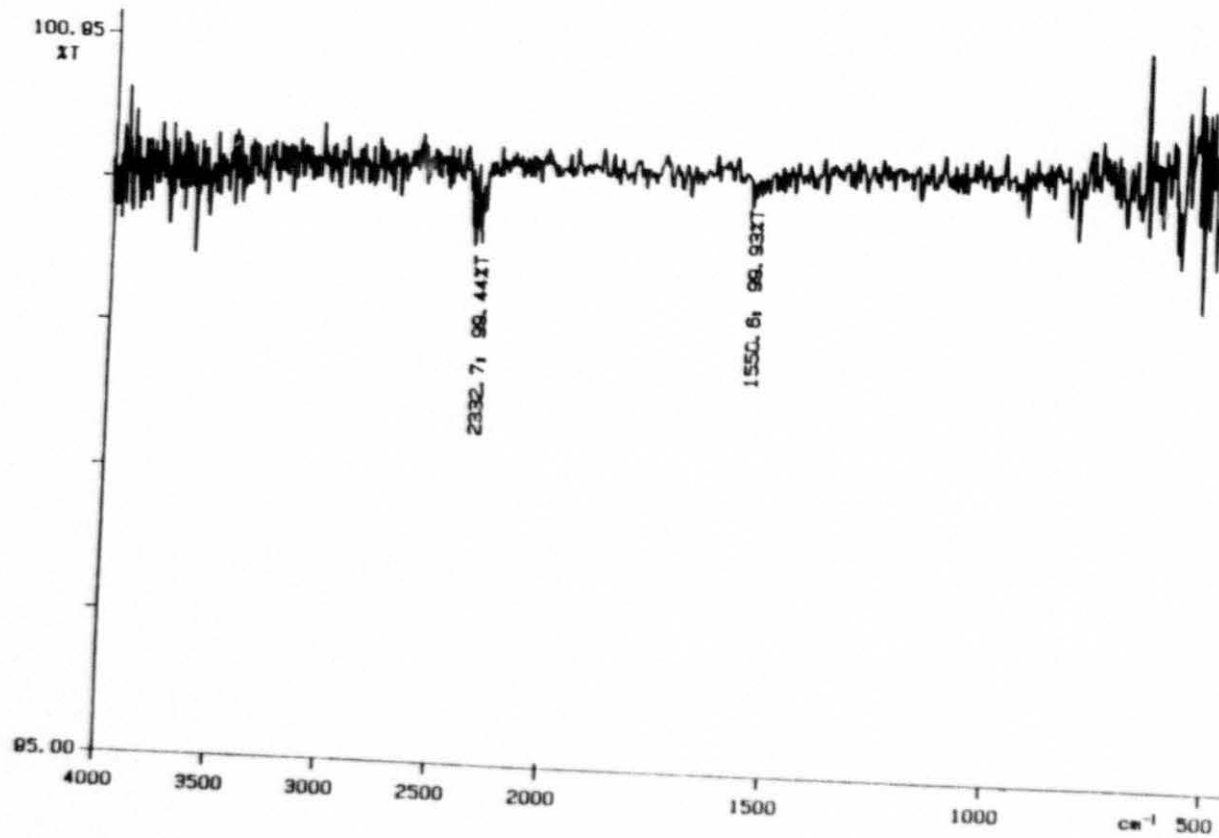
Figure 29.   TG-FTIR spectrum for sphingomeylin in nitrogen at 90°C

PERKIN ELMER

100.85
%T

2332.7, 98.44%T

1550.6, 98.93%T

4000   3500   3000   2500   2000   1500   1000   cm⁻¹ 500

85.00

91/12/05 18:57 dbd
em7: 4 scans, 4.0cm-1. ext
80

CONCLUSIONS

A. DSC on Phospholipids

1. Phosphatidylcholine (Egg-Yolk Lecithin)

Four phase transitions were observed below the melting point. ΔS values were calculated from DSC data. Through ΔS values, the different states which the sample existed at different transition temperatures could be estimated. The water content of PC was found to affect the temperature of transitions and the ΔS at transition 1.

2. Sphingomeylin

Sphingomeylin has not been examined by DSC before. Sphingomeylin was found to have two phase transitions below its melting point. ΔS values were also calculated for sphingomeylin. Sphingomeylin exists in different states at these two phase transition temperatures.

3. Phosphatidylethanolamine and Phosphatidylserine

The data were inconclusive for these two samples. These samples had complex changes during the heating process. Also, sampls were not sufficiently pure.

B. TG-FTIR on Phospholipids

TG provided a quicker, easier and more accurate way to find the percentage of water in lipids.

1. Phosphatidylcholine (Egg-Yolk Lecithin)

Egg-yolk phosphatidylcholine associated with one to two moles of water per mole.

2. Phosphatidylethanolamine

One mole of phosphatidylethanolamine associated with two moles of water.

3. Phosphatidylserine

Phosphatidylserine was observed to be in a monohydrate state. It decomposed as the temperature approached $160^{\circ}C$.

4. Sphingomeylin

One molecule of sphingomeylin was found to contain two molecules of water.

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