Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl16

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To cite this article: Bernard J. Bulkin & N. Krishnamachari (1973): Vibrational Spectra of Liquid Crystals. VII. Infrared Spectra of Lecithin—Phosphatidyl Serine—Water Systems, Molecular Crystals and Liquid Crystals, 24:1-2, 53-58

To link to this article: http://dx.doi.org/10.1080/15421407308083388

Molecular Crystals and Liquid Crystals. 1973. Vol. 24, pp. 53–58
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Vibrational Spectra of Liquid Crystals. VII. Infrared Spectra of Lecithin–Phosphatidyl Serine–Water Systems†

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Received December 18, 1972

Abstract—Infrared spectroscopic studies of a mixed phospholipid–water system can be used to construct the phase diagram for this system. The results show that a small amount of phosphatidyl serine (PS) causes an inordinately large depression in the liquid crystal phase transition temperature. Results at higher concentrations of PS are also presented.

Phospholipids play an important structural and functional role in biological membranes. The important phospholipids are (1) Lecithins or phosphatidyl cholines (PC), (2) phosphatidyl ethanolamines, (3) phosphatidyl serines and inositols, and (4) sphingomyelins. In 1964 it was shown(2) that when a fully saturated phospholipid is heated, the fine structure in the infrared (IR) spectrum is replaced by diffuse bands, resembling those of long chain hydrocarbons in the liquid state. This type of change in the IR is attributed to the so-called liquid crystalline phase transition which occurs well below the capillary melting point. A number of physical techniques,(3–7) have been employed to study not only the crystalline to liquid crystalline phase transitions but also other mesomorphic phase transitions in phospholipid–water gels.

Earlier results(6a,8) from IR and Raman spectroscopy indicated that molecular vibrations can be a sensitive probe of order in the fatty acid chains of phospholipids. Infrared and Raman spectroscopy are found to be important tools because of their ability to probe all

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† Presented at the Fourth International Liquid Crystal Conference, Kent State University, August 21–25, 1972.

structural groups of phospholipid bilayer systems without introducing any chemical perturbation. At the same time they require only small amounts of sample.

In a previous study, it was shown that the peak absorbance of an infrared band at 1470 cm^{-1} was a good probe of hydrocarbon chain fluidity. When this intensity (relative to another band in the spectrum) is plotted as a function of temperature for phospholipid-water gels, sigmoidal curves are obtained from which transition temperatures can be measured.

The potential importance of the presence of anionic phospholipids in membranes has been recognized for some time.(9) The important anionic phospholipids are phosphatidyl serines and phosphatidyl inositols. It is found that in mitochondria membranes phosphatidyl serines constitute about 0.5% of the total phospholipid. Such membranes behave as cation exchangers for calcium, so they may provide negatively charged sites for the cation exchange. This also suggests an important role for the phosphatidyl serines in active transport, despite the fact that they are present in minute amount in relation to the total phospholipid content of the membranes.

In the present work we investigate the influence of the presence of an anionic phospholipid (phosphatidyl serine) on the thermal phase transition of the mixture of phospholipid-water (20% water). Infrared spectra (600–4000 cm^{-1}) of such mixtures with varying ratios of phosphatidyl serine (PS) : lecithin (PC) are reported as a function of temperature.

1. Experimental

Phospholipids All phospholipids used were chromatographically pure (tlc). Lecithin was obtained from Mann Research and was tlc pure L-2-3-Dihexadecyl glycerine -1-phosphoryl choline. Phosphatidyl serine (Bovine) chromatographically pure was obtained from SUPELCO as a solution in benzene (25 mg/ml). The molecular weight was approx. 788 and $\%P = 3.93$.

Infrared Spectra IR spectra were obtained on a Perkin–Elmer Model 521 dual grating spectrometer. The instrument was run at a spectral slit width of ca. 1 cm^{-1}. The sample was a film about 10μ thick, held between two IRtran plates.

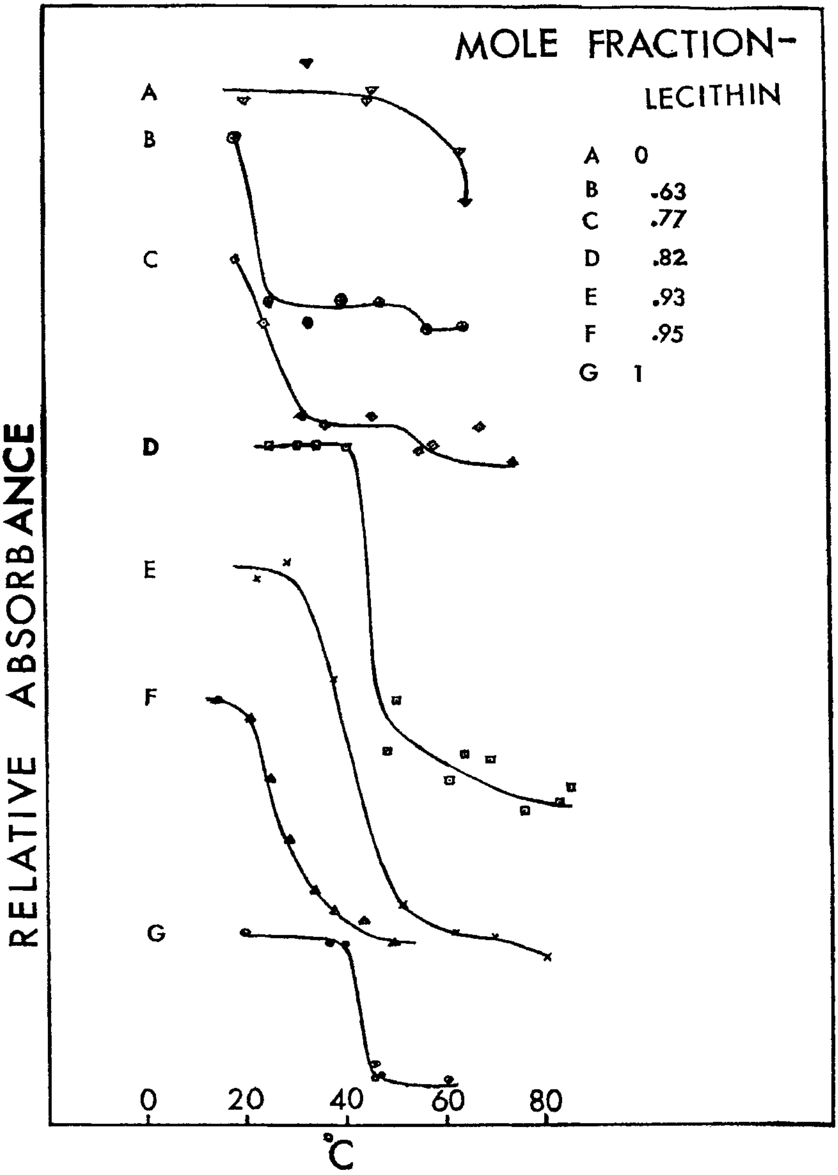

Figure 1. Relative absorbance of infrared band at 1470 cm^{-1} vs. temperature of PC-PS-H_2O gels containing varying mole fractions of PC.

A Barnes Engineering Model VTC-1 variable temperature chamber was used to obtain spectra as a function of temperature Spectra were obtained from − 20 to 80° with proper care taken not to destroy the gel. Temperatures were recorded using a thermocouple and potentiometer. In the plots shown in Fig. 1, the computed uncertainty in a given absorbance ratio is ± 0.005, using propagation of errors theory. The estimated uncertainty in the temperature is ± 1°.

A sonifier cell disruptor by Heat Systems—Ultrasonics Inc. was used for the preparation of PS-PC-water gels. Sonication for not more than 5 seconds was sufficient. The sonication was done at room temperature.

2. Results

Figure 1 shows the plot of the peak intensity of the 1470 cm^{-1} band (methylene deformation vibration), relative to that of another band in the spectrum, as a function of temperature in ternary mixtures

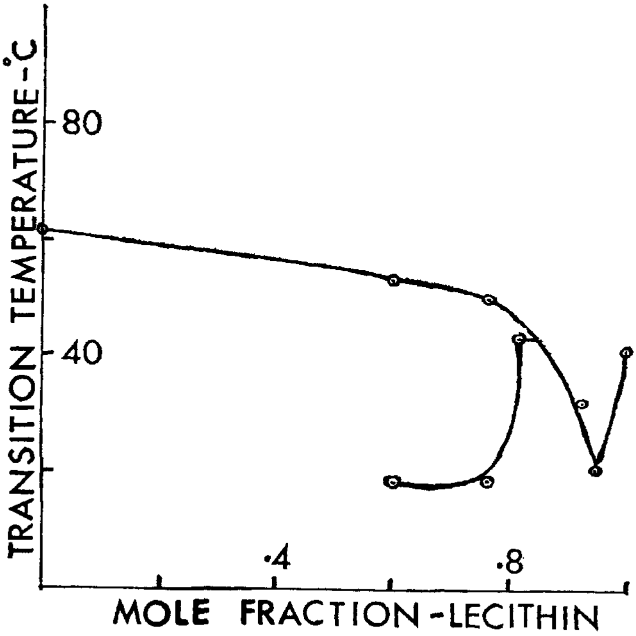

Figure 2. Data of Fig. 1 plotted as a phase diagram for the PC-PS-H_2O system.

PS-PC-water. At low mole fractions (up to 0.05) the PS depresses the transition temperature from the PC-water value of 41 °C. Above this eutectic point, the increase in PS mole fraction causes a continuing rise in the transition temperature. At 0.23 and 0.37 mole fraction of PC one can observe two transitions, one at low temperature (approx. 20°) and another at higher temperature (approx. 50°).

In Fig. 2 a plot of transition temperature versus mole fraction of PC is given. Both the eutectic point and the peritectic-like higher PC concentration behavior are clearly seen in this figure.

A temperature plot was taken with decreasing temperature starting at a temperature above the PC-water gel phase transition. A small hysteresis effect was observed (about 3°). This type of experiment was not performed with the PC-PS-water gel.

3. Discussion

From Fig. 1F it is clear that even the presence of a small amount of phosphatidyl serine (0.05 mole fraction) depresses the transition temperature by about 20° from that of a pure PC-water gel. DTA of a PC-water mixture was run from 10–70 °C. From this curve the ΔH_f was found to be 16.38 cal/g (12.02 kcal/mole). Assuming ideal solution behavior a freezing point depression calculation was carried out for a PC-PS water mixture in which the PS mole fraction was 0.05. The depression in transition temperature by that calculation was found to be ~ 1 °.

It is of interest to compare this effect on the PC-water transition temperature of an added ionic phospholipid, to that produced by cholesterol. The latter broadens the transition to a very wide temperature range, and also shifts the transition to lower temperature.

As the mole fraction of PS increases from 0.05 to 0.18 the transition temperature increases from 20° to 43°. Above this point, however, there appear to be two transitions. This suggests that at large concentration of anionic phospholipid there may be more than two mesomorphic phases existing. The apparent peritectic point near 0.23 mole fraction of PS indicates the presence of an unstable PS-PC-H_2O compound in the higher concentration region of the system. Ohki has proposed the existence of such phases in biomembranes in the past.(10)

A phase diagram obtained by plotting transition temperature *vs.* mole fraction gives a good picture of the influence of the presence of PS in PC-water mixtures. No experiments were done with mixtures containing more than 0.37 mole fraction PS as all the PS bearing membranes contain much smaller amounts.

Acknowledgement

The support of the American Cancer Society Grant BC-21b is gratefully acknowledged.

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