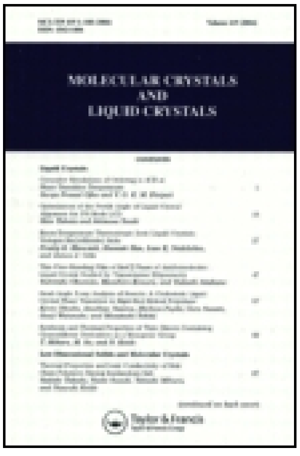

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SANS INVESTIGATION OF THE PRESSURE- AND TEMPERATURE-DEPENDENT STRUCTURE OF THE BILE SALT/LECITHIN SYSTEM

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Abstract We used small angle neutron scattering to investigate the pressure and temperature dependent particle structure in mixed colloids of egg yolk phosphatidylcholine with the bile salt, glycocholate in D_2O. The concentrations and compositions of the mixtures, at ambient temperature and pressure, corresponded to the mixed vesicle region of the isotropic (*I*) phase. Upon increasing the hydrostatic pressure at ambient temperature, interesting morphological changes were observed. At a pressure of 6.9 MPa, the system is best described by a coexistence of the mixed, spherical single bilayer vesicle, stacked lamellar and stacked ribbon phases. At higher pressures, the system transformed to a stacked ribbon phase. Analysis of scattering data as a function of temperature, at ambient pressure, revealed that a vesicle phase remained stable with the average spherical radius decreasing as the temperature was increased from 15 to 40 °C.

INTRODUCTION

In recent years, there has been considerable interest into the stability of bilayer vesicles formed by mixed surfactant systems[1]. Safran and coworkers[1], considering the curvature free energy, showed that, while bilayer vesicles composed of a single surfactant are in general unstable with respect to flat lamellae, mixed surfactant bilayer vesicles can be stable. With the introduction of a co-surfactant, the composition of each monolayer of the bilayer provides an additional degree of freedom, giving rise to the stability. A compositional change within a layer then (which may be induced by changes in external pressure or temperature) can result in particle instability. The stable shapes of closed fluid lipid-bilayer membranes have been modeled by a number of different groups[2,3]. From the classic work of Young and Laplace, a pressure gradient, Δp, should exist across a curved interface. For the special case of a spherical vesicle of average curvature, R^{-1}, the Young-Laplace (Y-L) equation gives $\Delta p = 2\gamma/R$, where γ is the surface tension. We see then, that when $\Delta p = 0$, a transition from the vesicle state to a state of zero average curvature should occur. Since γ is known to be temperature dependent and further that the composition of a mixed surfactant vesicle may change with temperature, varying the temperature (T) and external hydrostatic pressure (P) should both have profound effects on vesicle stability. In order to elucidate the role of P and T in determining stability criteria for vesicles in mixed surfactant systems, we performed small-angle neutron scattering (SANS) measurements on particle morphology as a function of pressure and temperature of bile salt (glycocholate)/egg yolk phosphatidylcholine (EYPC) mixtures in the region of the isotropic (*I*) phase where mixed, spherical single bilayer vesicles (SLV's) are present [4,5].

Particles formed in aqueous solutions of bile salts and EYPC, while addressing fundamental issues of particle self-assembly in mixed colloids, are of additional interest to the medical community as models for the structure and action of bile.

MATERIALS AND METHODS

Purified EYPC (Sigma, type VIII-E) and Sodium glycocholate (Sigma Chemical Co.) were dissolved in ethanol and mixed in the molar ratio (L/BS) of 0.8. After vacuum drying, the preparation was dissolved in a D_2O buffer containing 0.15 M NaCl with tris buffer (pH 7.5) to make a stock solution containing 50 mg/ml total lipid concentration. The samples (2 mg/ml) were prepared by further dilution with D_2O buffer, sealed under nitrogen in vials and incubated, at the measurement temperature for ~ 2 days in the dark.

SANS measurements were performed at the Intense Pulsed Neutron Source at Argonne National Laboratory, employing the Small-Angle Neutron Diffractometer (SAD). Scattering data were acquired using the time-of-flight method[6]. In order to interpret the data, a nonlinear least squares procedure was used to fit the measured lineshapes to the expected scattering of different model systems.

RESULTS

SANS measurements of the glycocholate/EYPC system as a function of pressure (T = 22 °C) are shown in Fig. 1. At atmospheric pressure (Fig. 1a), the data is consistent with the presence of mixed SLV's, as anticipated from the phase diagram[4]. The solid line in the figure is the result of a nonlinear least squares fit of the data to the particle form factor for an SLV with outer radius, R = 152 Å and thickness, t = 35 Å. Deviations of the model from the measured data are likely due to polydispersity.

As the pressure was increased, dramatic changes were seen in the scattering curves. Fig. 1b shows the result at P = 6.9 MPa. As indicated by the dashed line in the figure, at low values of Q, the curve falls off as Q^{-3}. The appearance of a Bragg peak, centered at Q

= 0.12 $Å^{-1}$, corresponding to EYPC bilayer thickness, suggests the presence of lamellar order and thus a transition from the isotropic, mixed vesicle phase to an ordered, lamellar phase. Further increase of the hydrostatic pressure to P = 51.8 MPa brought additional morphological changes as can be seen in Fig. 1c. While the Bragg peak remains, the intensity at low-Q now falls off as Q^{-2}, suggesting a change in the fundamental unit comprising the lamellar phase.

Modeling of the lamellar phase observed at 51.8 MPa as stacks of extended sheets or disks failed to accurately describe our data, as the modeled intensity falls of as Q^{-4} at low Q. However, a stacked array of ribbon-like particles is in excellent agreement with the

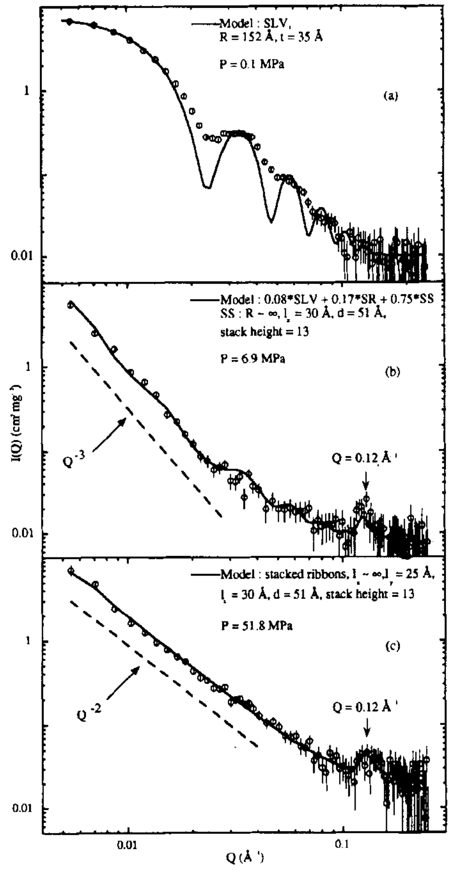

Figure 1 SANS measurements of the glycocholate/EYPC system (L/BS = 0.8, 2 mg/ml) as a function of pressure : (a) P = 0.1 MPa, solid line is the calculated scattering curve for a spherical vesicle, R = 152 Å, t = 35 Å, (b) P = 6.9 MPa, model is a weighted linear combination of the SLV, stacked sheet & stacked ribbon models (c) P = 51.8 MPa, solid line is the expected scattering from a stacked ribbon phase.

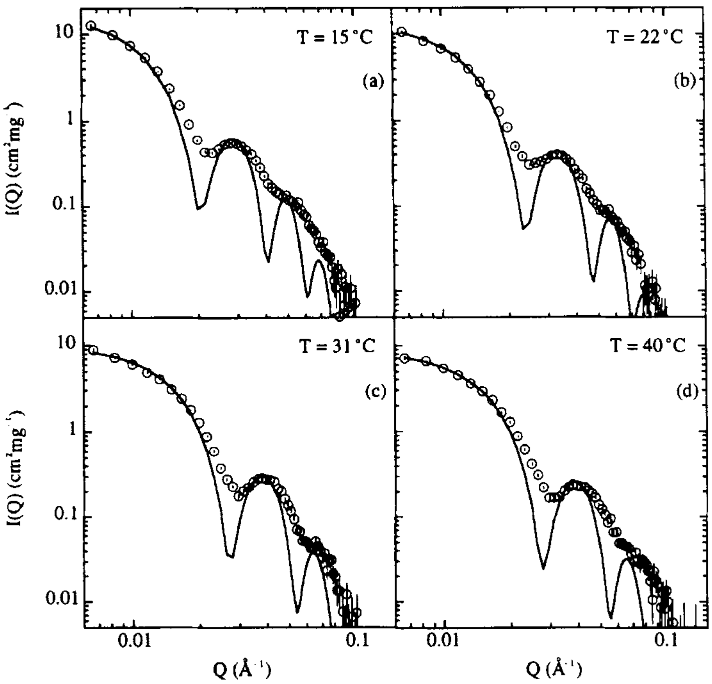

Figure 2 SANS measurements as a function of temperature. The solid lines are the expected scattering curves from SLV's : (a) R = 184 Å, t = 60 Å, (b) R = 162 Å, t = 60 Å, (c) R = 144 Å, t = 60 Å, (d) R = 141 Å, t = 59 Å.

measured data as indicated by the solid line in figure 1c. The data at P = 6.9 MPa (Fig. 1b), however, is best described by a coexistence of the SLV, stacked sheet and stacked ribbon phases, with the stacked sheet phase being the predominant one at this pressure.

Results of SANS measurements of the glycocholate/EYPC system as a function of temperature are shown in Fig. 2. At all temperatures, the data is consistent with the presence of mixed SLV's. As the temperature was increased, the vesicles were seen to decrease in size. This behavior is evidenced by the shift, to higher Q- values, of the characteristic maxima and the decrease in the differential cross section at low-Q. The solid lines in Fig. 2 are the results of least squares fits to the particle form factor for spherical vesicles of R = 184, 162, 144 and 141 Å, respectively.

DISCUSSION

The observed lamellar phase at 6.9 MPa indicates collapse of the spherical SLV's. This observation may be understood in terms of the Y-L equation. According to this interpretation, we exceeded the internal pressure of the vesicles, forcing them to collapse into stacked sheets, upon increasing the external hydrostatic pressure to 6.9 MPa.

Further increases in the pressure above 6.9 MPa may result in a redistribution of the bile salt from the interior of the bilayer to the edges, thus stabilizing the ribbon phase by shielding the EYPC tails from contact with water. The anisotropic form of the ribbon phase indicates a strong disposition of the bile salt to associate with the EYPC tail as opposed to being in solution. Such a pressure-driven re-arrangement implies a considerable volume change associated with the removal of bile salt from the bilayer interior to the bilayer edge, such a process is consistent with the molar ratio of glycocholate to EYPC and we are working on a thermodynamic model to explain these observations.

The decrease of the vesicle radius with increasing temperature, is ascribed to removal of bile salt to the bulk phase by increased bile salt solubility with temperature. This observation is similar to the trend found in this system upon dilution, where the decrease in R is likely driven by mass action[5] giving rise to the same overall change in the composition of the bilayer.

CONCLUSION

As the hydrostatic pressure was increased, a transition from the isotropic, mixed vesicle phase of the glycocholate/EYPC system to an ordered ribbon phase was observed. With increasing temperature, the vesicles were seen to decrease in size.

REFERENCES

1. S. A. Safran, P. A. Pincus, D. Andelman and F. C. MacKintosh, Phys. Rev., A43, 1071 (1991).
2. O. Zhong-can and W. Helfrich, Phys. Rev., A39, 5280 (1989).
3. L. Miao, B. Fourcade, M. Raos, M. Wortis and R. K. Zia, Phys. Rev., A43, 6843 (1991).
4. R. P. Hjelm, P. Thiyagarajan and H. Alkan-Onyuksel, J. Phys. Chem., 96, 8653 (1992), and references therein.
5. R. P. Hjelm, H. Alkan and P. Thiyagarajan, Mol. Cryst. Liq. Cryst., 180A, 155 (1990).
6. R. P. Hjelm, J. Appl. Cryst., 21, 618 (1988).