Chapter 4. Small Angle X-Ray and Neutron Scattering — Its Application to Supramolecular Solutions

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4.1 Solvent Effects on Phase Separation in Micellar Solutions

Project Staff

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In previous years our group has used small angle neutron scattering (SANS) technique to determine the structure and extent of growth and polydispersity of zwitterionic micelles formed from short chain lecithins in aqueous (D_2O) solution. The main finding was that the species with 6 carbons in each fatty acyl chain ($[C_6]_2$-PC) formed monodisperse ellipsoidal micelles that did not grow as the lipid concentration was increased whereas the longer chain species ($[C_6C_7]$-PC, $[C_7]_2$-PC, $[C_6C_8]$-PC and $[C_7C_8]$-PC) formed rod-like micelles that did grow as more lipids were added to the aqueous solution. A thermodynamic model of micellar formation and growth (frequently called the ladder model) was successfully applied to the analysis of the SANS data. From this model, we were able to extract the aggregation number of the minimum size

micelle, N_o; the free energy change, Δ, upon assembling the N_o monomers into an aggregate; and the free energy change, δ, upon inserting an additional monomer into a micelle of the minimum size or larger. From these three parameters we can construct a fourth important thermodynamic parameter, namely, the free energy advantage of growth, $\Delta\mu = \Delta - N_o\delta$.

Our recent studies have focused on the lecithin species with 8 carbons in each fatty acyl chain ($[C_8]_2$-PC). Unlike its shorter chain homologues, this lecithin species exhibits liquid-liquid phase separation with an upper consolute temperature, T_c, when it is dispersed in water; in H_2O, $T_c = 45°C$ and in D_2O, $T_c = 60°C$. We have found that the consolute temperature can be dramatically lowered by the addition of a small amount of urea to the lipid/water mixture. For example, in a 1 Molar aqueous Urea solution T_c is suppressed below 0 degrees C.

Figure 1 shows our measurements of the cloud point curve for the system $[C_8]_2$-PC/H_2O with different amounts of urea. We should mention that since the urea concentration in the coexisting (equilibrium)

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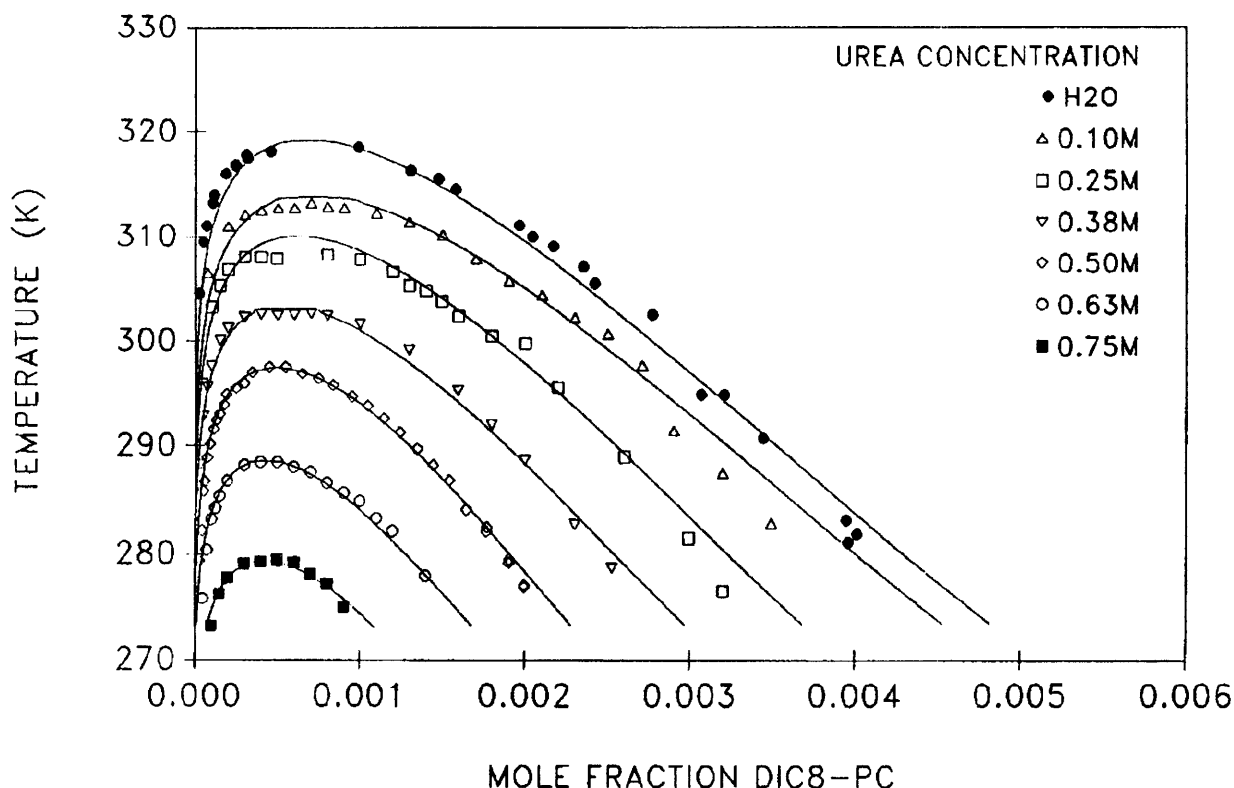[2] Tsingua University, Department of Biological Sciences, Beiging, China.

Figure 1. Cloud point curves for the system dioctanoylphosphatidylcholine/H_2O with different amounts of added urea. The symbols correspond to the experimental points and the solid lines correspond to the theoretical fits.

phases at a number of temperatures was found to be the same (via colorimetric assay of nitrogen content), we have regarded the ternary system $[C_8]_2$-PC/H_2O/urea as a pseudo-binary system when the field variable, urea concentration, is fixed. These cloud point curves were analyzed with a recent theory of phase separation in a micellar solution.[3] This theory relates the phase separation phenomena to the free energy advantage of growth, $\Delta\mu$, the ratio of lipid molecular volume to water molecular volume, γ, and the mean field interaction between lipid monomers, C. By fitting our measured cloud point curves with the theoretically generated coexistence curves we were able to extract $\Delta\mu$, γ, and C as a function of urea concentration.

The main conclusion is that as more urea was added, Δ and C decreased but γ increased. The decrease in $\Delta\mu$ can be attributed to a decrease in the hydrophobic interaction brought on by the addition of urea. The decline of C occurs because the addition of urea increases the static dielectric constant of the aqueous solution. And the increase in γ may be due to the fact that some urea molecules are attached to the hydrophyllic part of the lipid molecule and thus increase its effective molecular volume in water. Presently we are undertaking SANS experiments on the system $[C_8]_2$-PC/D_2O/urea. Our preliminary results indicate that the value of $\Delta\mu$ extracted from the SANS data is consistent with that extracted from the appropriate $[C_8]_2$-PC/D_2O/urea cloud point curve.

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[3] D. Blankschtein, G.M. Thurston, and G.B. Benedek, *Phys. Rev. Lett.* 54:955 (1985).

4.2 Protein - Surfactant Interactions and Protein Denaturation

Project Staff

Professor Sow-Hsin Chen, Professor Nan-Ming Zhao, Xuan-Hui Guo

Although it has been common practice in protein chemistry to determine molecular weights of proteins by performing electrophoresis of protein/SDS complexes in polyacrylamide gels, the precise under-standing of the basic principle of SDS gel-electrophoresis at the molecular level is still lacking. The goal of our project is to under-stand protein-surfactant interactions and to elucidate the resultant structure.

Small angle neutron scattering (SANS) was used to study the structure of protein-surfactant complexes. The proteins with molecular weights in the range of 24,000 to 68,000, namely, trypsinogen, ovalbumin and bovine serum albumin were studied. The anionic surfactant used was sodium dodecylsulfate (SDS). Absolute intensities of SANS distribution were analysed and fitted by two models: a fractal model and a polymer-like model. In the former model, we can extract from the data parameters such as the fractal dimension D, the correlation length ξ , and micelle size R. In the latter model, we obtain the fractal dimension D, the radius of gyration of the unfolded protein R_g, numbers of micelles in the unfolded chain N, and the micellar size R. Both models fit the data successfully and suggest that the protein-SDS complexes have pearl necklace-like structure under the present values of pH and ionic strength. It is also found that the interaction between protein and SDS is pH and ionic strength-dependent, the detail of the structure of the complexes may be dependent on these factors.
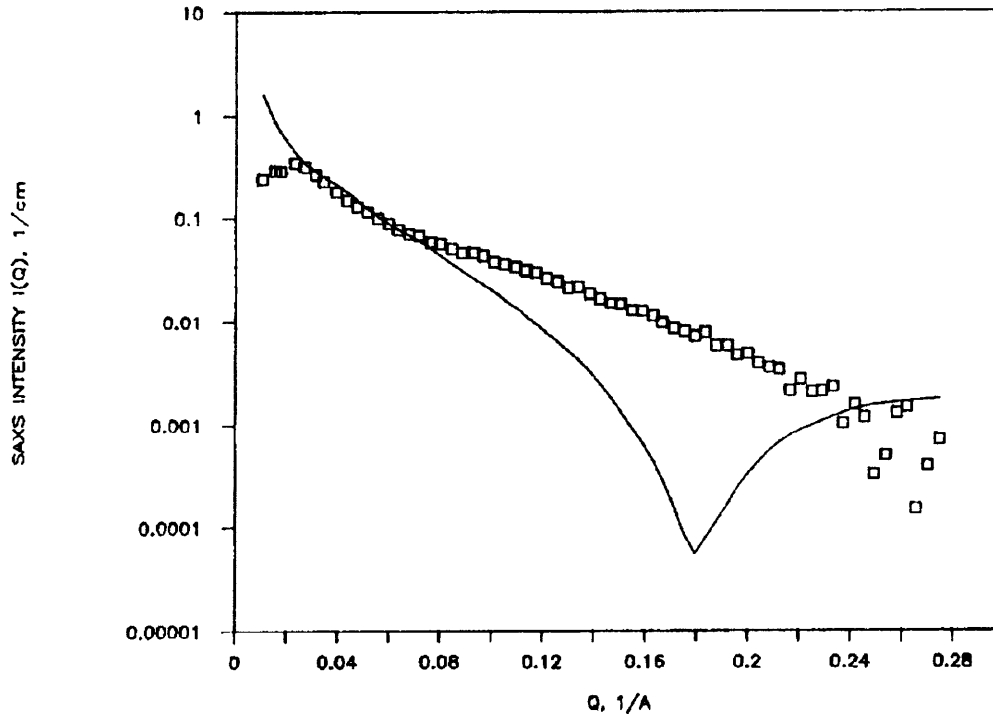

Figure 2. A log scale comparison of the calculated scattering intensity (solid line) and the experimental SAXS data (symbols) for the case that DNA concentration is 2.5 mg/ml, no salt is added and the counterion is TL+. The counterion distribution is P-B analytical solution outside the DNA region (r > 10Å) only.

4.3 Ion Distribution and Solubilization in Reverse Micelles

Project Staff

Professor Sow-Hsin Chen, Bruce Carvalho, Vivian Leung

This project concerns the thermodynamics of the preferential solubilization of multivalent cations into the interior of reverse microemulsions. The results of this project will be used to understand the removal of metal ions from a waste stream by microemulsions. The thermodynamics of ion solubilization depends on the distribution of ions in the water core of the reverse microemulsion.

The procedure to determine the ion distribution has recently been established. We assume that a fraction of the counterions in the water core are localized at the surfactant head groups; the concentration of the remaining free ions varies with the electrostatic potential in the water core which can be expressed as solution of the Poisson-Boltzmann (P-B) equation. We have used this theoretical ion distribution to calculate the angular distribution of scattered x-ray intensities which are then compared with the experimental small angle x-ray scattering data. The preliminary results indicate that only a small fraction (30%) of the counterions are free in the water core of the microemulsion.

4.4 Ion Distribution around DNA Molecules

Project Staff

Professor Sow-Hsin Chen, Szu-Li Chang

Nucleic acids are highly charged molecules and electrostatic interactions play an important role in many aspects of their structure and function. It has also become clear that electrical force is an important component of protein-DNA and drug-DNA interactions and

for this reason the study of the distribution of ions around DNA has received much attention in recent years.

There are several methods to calculate the ion distribution around DNA. The more primitive of these methods have considered only the radial distribution of ions and have modeled the DNA as a uniformly charged cylinder. This type of calculation has neglected the discontinuity of the dielectric constant at the surface of the DNA. This simplified approach has two types of solutions: 1) the analytical solution of the Poisson-Boltzmann (P-B) equation; 2) a solution based on the more precise Monte Carlo (MC) method. Both types of calculations use Coulomb's law for the electrostatic interaction.

A set of small angle x-ray scattering (SAXS) experiments on DNA-water-salt mixtures has been performed. We use the P-B analytical solution, which assumes that counterions cannot penetrate into the cylindrical region occupied by the DNA, as the counterion distribution to calculate a theoretical scattering intensity, $I(Q)$. The theoretical (solid line) and experimental (square) $I(Q)$ vs. scattering vector, Q, are shown in figure 2. It is clear that the simple P-B result does not fit the data.

We have also used a counterion distribution, which is assumed to be flat and non-zero inside the region occupied by DNA double helices and is given by the P-B solution outside this region, to get the scattering intensity plotted in figure 3. Because this latter approach better fits the data, we believe that some counterions do penetrate into the major and minor grooves of the DNA molecule. A Monte Carlo program for this simple DNA model with simple Coulomb inter-ionic potentials has also been developed and tested for several cases. The result showed that the radius and valence of the counterion alters the ion distribution significantly. We can calculate $I(Q)$ from the counterion distributions predicted by the P-B solution and the MC stimulation.
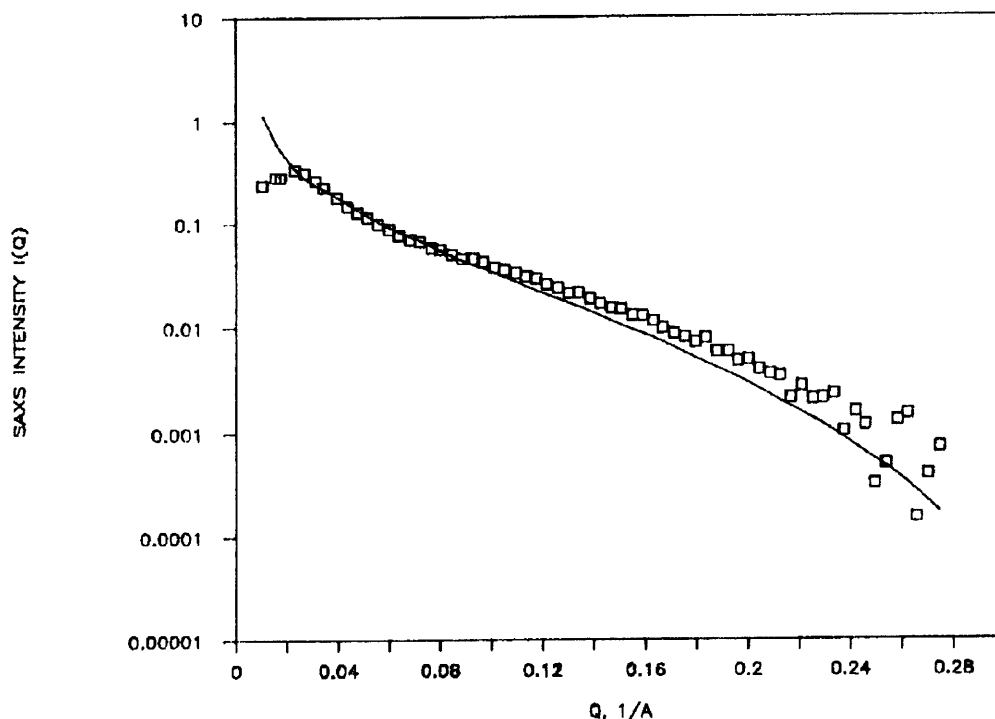
Figure 3. A log scale comparison of the calculated scattering intensity (solid line) and the experimental SAXS data (symbols) for the case same as in figure 2. The counterion concentration in the DNA core ($\lambda < 10\text{Å}$ region) is assumed to be flat and given by the value of P-B solution at $\lambda = 10\text{Å}$. The ion distribution outside the DNA region ($\lambda > 10\text{Å}$) is P-B solution.

4.5 Photon Correlation Spectroscopy and Its Application: Critical Phenomena in a Microemulsion

Project Staff

Professor Sow-Hsin Chen

Extensive light scattering measurements, including the intensity, turbidity and linewidth, on a three component microemulsion system consisting of mixtures of water, decane and a surfactant AOT have been made. The critical and several off-critical mixtures have been studied along the constant microemulsion droplet volume fraction lines in the one-phase region, over a very large temperature range. In the vicinity of the lower phase separation temperature, T_p,

the intensity data are very well accounted for by the standard theory of critical binary fluids using a single value for the short range correlation length, $\xi_0 = (13.5\pm1.5)\text{Å}$. By combining a mode-coupling theory, that includes the background effects, and a linear model equation of state applicable in the critical region, we have been able to fit the dynamic light scattering data using a Debye cutoff length, q_D^{-1}, which is equal to the constant average diameter of microemulsion droplets. Furthermore, we find clear evidence for a crossover from critical to single particle behavior in both static and dynamic light scattering data. A crossover temperature, T_x, has been identified at which $q_D\xi(T_x) = 1$. Analyses of the dynamic light scattering data show that q_D, which can only be measured far away from T_p, in fact, plays a decisive role in controlling the critical dynamics in the whole temperature range.

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