Chapter 3. Small Angle X-Ray and Neutron Scattering — Its Application to Supramolecular Solutions

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3.1 Interlayer Diffusion in Langmuir-Blodgett Films

Project Staff

Bruce L. Carvalho, Professor Sow-Hsin Chen

The Langmuir-Blodgett film deposition technique has recently been used to fabricate a variety of dense electronic and nonlinear optics devices on a single crystal silicon surface. An important technological problem arises from the use of this technique: the interdiffusion of polymer and surfactant molecules between multilayers in Langmuir-Blodgett films directly affects the stability of these films (as a function of temperature and age).

We have recently used the neutron surface specular reflection technique to study the dynamics of interdiffusion of polymer and surfactant molecules in Langmuir-Blodgett films. With this new technique, we can obtain the normal surface density profile at a spatial resolution of 10 Å. By varying the time-temperature annealing history, we observed the interdiffusion in a stepwise fashion, also with a spatial resolution of 10 Å.

Figures 1a and 1b illustrate examples of our measurements. The system shown consists of 5.5 repeat units of deuterated surfactant/protonated optically active polymer, each component having approximately 25 Å thick layers. The first three peaks of the reflectivity curve come from an interference pattern of the total film thickness (about 270 Å); the fourth peak is a Bragg reflection from a lattice spacing of 53 Å. We measured this sample twice: immediately after we made it (figure 1a), and after it was annealed at 60°C for 10 minutes (figure 1b). A significant finding was that the interdiffusion of polymer and surfactant molecules during the annealing process completely destroyed the Bragg peak. This is a striking example of mutual diffusion at a length scale of about 10 Å, a distance scale which is difficult to measure by any other means in a hydrocarbon system.

In future experiments, we plan to increase the number of repeat units in the film,
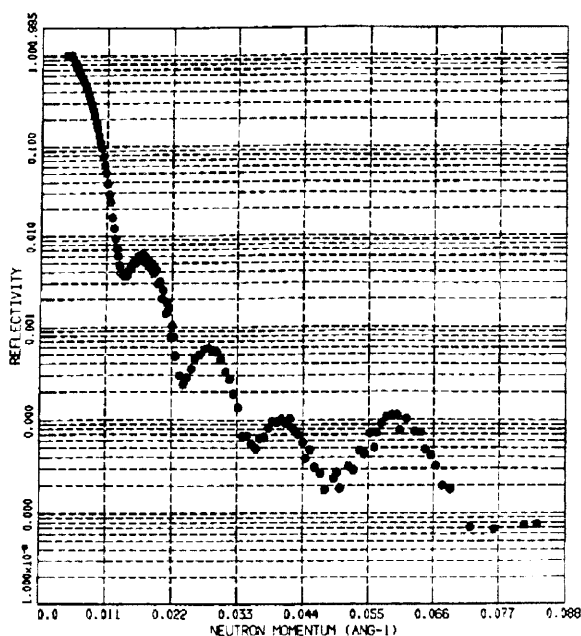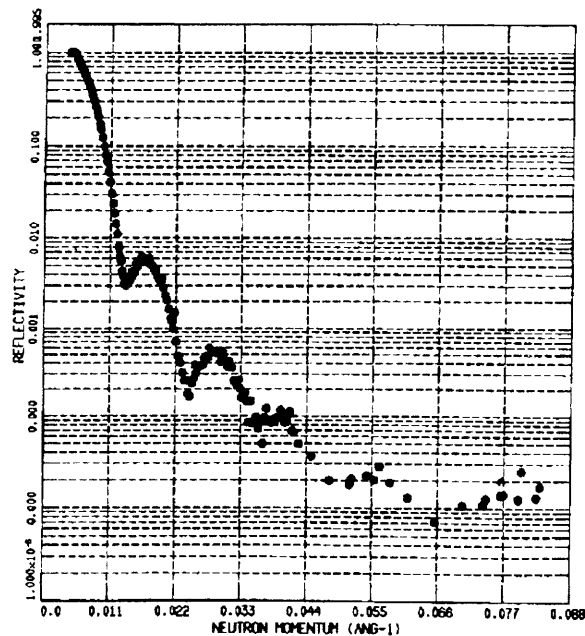
Figure 1a. Reflectivity vs. momentum transfer for the system 5 (deuterated cadmium arachidate - PECH polymer) - deuterated cadmium arachidate - silicon substrate, before annealing.

Figure 1b. Reflectivity vs. momentum transfer for the same system as figure 1a but after annealing, 10 minutes at 60°C.

enhance the Bragg peak, and possibly observe intermediate intensities before the Bragg peak is destroyed. We then plan to extract the interdiffusion coefficient directly from the decay of the Bragg peak in time, as has been done in artificially modulated metallic thin film systems. In addition, we plan to perform a series of experiments on bilayer samples in which layers of deuterated surfactant are stacked on top of a layer of protonated polymer. We will anneal these samples, extracting the interdiffusion coefficient from the concentration profile of deuterium across the surfactant-polymer interface.

3.2 Thermodynamics of Protein/Surfactant Complex in Aqueous Solution and Reptation Mechanism for Protein/SDS Polyacylamide Gel Electrophorosis

Project Staff

Xuan-Hui Guo, Professor Sow-Hsin Chen

During the past few years, we have studied the structure of bovine serum albumin (BSA)/sodium dodecylsulfate (SDS) complexes in solutions by small angle neutron scattering (SANS). From the quantitative interpretation of SANS intensity distributions, we concluded that BSA/SDS complexes in high ionic strength solution were most likely flexible polymer-like objects. Currently, we are exploring the consequences of our SANS studies. We recently discovered two significant phenomena: (1) polymer-like phase separation of the BSA/SDS complexes in solution; and (2) reptation behavior of the complexes migrating through a gel during electrophoresis.

Pertaining to the first phenomenon, we successfully discovered the specific conditions (pH $\leq$ 5.0, ionic strength = 0.6M) under which the complexes undergo polymer-like phase separation in an accessible temperature range. We determined the cloud-point curve (CPC) of the complexes in solution. Then, we carried out the light scattering along an isotherm above CPC in the one-phase region to approximately determine the critical volume fraction, which we found to
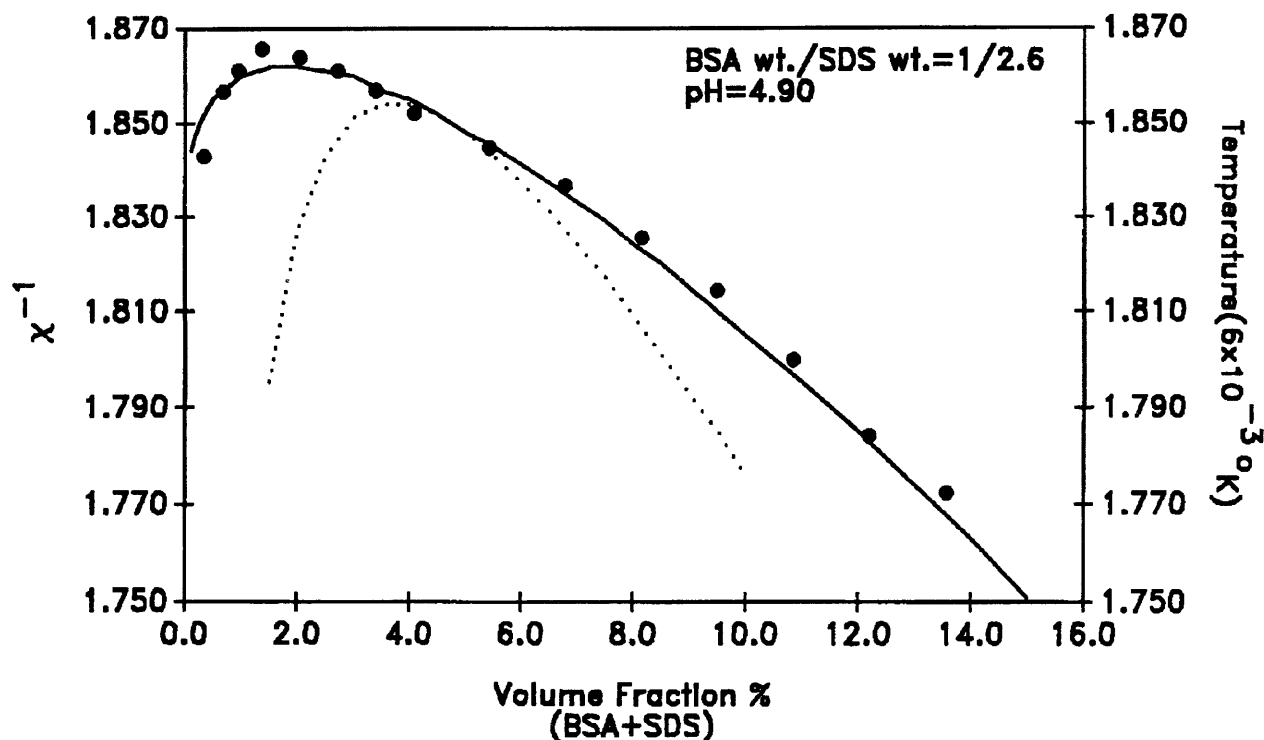
Figure 2. Comparison of the experimental cloud-point curve (CPC) with the theoretical CPC calculated using the generalized Flory-Huggins theory for polydisperse polymers in solution. The filled circles are the experimental cloud-point temperature scaled by the factor 6×10^{-3} and vs. ϕ_s curve. The solid line is the theoretical χ^{-1} calculated using a polydispersity index $Z = 1$ and an average effective chain length $\lambda_1 = 676$. The dashed line is the spinodal line corresponding to the CPC.

be about 4.5 percent. The critical point was located on the right branch of the CPC similar to synthetic polydisperse polymers.

To calculate the theoretical CPC, we applied the generalized Flory-Huggins (F.-H.) theory for polydisperse polymers. This theory was in strikingly good agreement with our experiment. Thus, our thermodynamic analyses also establish that the BSA/SDS complexes in solution are polymer-like objects. We are particularly interested in further exploring the complete tenary phase diagram of the protein/SDS/H₂O system and in more precisely locating the critical point.

Evidence accumulated from our SANS studies and phase separation study strongly supports the view that protein/SDS complexes in solution are polymer-like objects. We propose a reptation mechanism for the explanation of the migration of the pro-

tein/SDS complexes in polyacrylamide gel during electrophoresis. According to our model, the polymer-like object undergoes one-dimensional worm-like motions in a tube formed by the consecutive pores housing the molecule in the three-dimensional mesh of the gel. Electrophoretic data taken from four independent experiments confirms our conjecture. We discovered a power-law, namely mobility, that is inversely proportional to protein molecular weight within a broad range of molecular weights. The exact physical mechanism underlying this technique is not clearly understood, even though it is popular in protein chemistry and engineering. Further investigation of the influence of various parameters, such as electric field, ionic strength, temperature and gel concentration on mobility would increase our fundamental understanding of, and possibly improve, this popular biochemical technique.

3.3 Phase Separation in a Lipid/Water/Urea Micellar System

Project Staff

Bruce L. Carvalho, John Chen, Professor Sow-Hsin Chen

We have found that the upper consolute temperature of the dioctanoyl-phosphatidylcholine-water micellar system can be dramatically lowered by adding a small amount of urea. We found the lowering of the consolute temperature to be a linear function of urea concentration. After determining the cloud point curves for this micellar system at several urea concentrations in both water and heavy water, we analyzed the system in terms of a recent thermodynamic theory of micellar phase separation proposed by Blankschtein, Thurston and Benedek. This theory connects the phase separation phenomena to the micellar growth and polydispersity and the monomer-monomer interaction in the single phase region.

We performed three separate measurements on the dioctanoylphosphatidylcholine-heavy water [urea] = 1 Molar micellar system in the single phase region. We measured: (1) the temperature dependence of the critical micellar concentration; (2) small angle neutron scattering intensity distributions at dilute concentrations; and (3) static light scattering over a wide range of concentrations. We also measured the cloud point curve for this particular system. We made consistent analysis of these four measurements using the ladder model of micellar growth and this recent theory of micellar phase separation. We can explain the experimental data from these four measurements with the same set of model parameters.

3.4 Ion Distribution and Solubilization in Reverse Micelles

Project Staff

Bruce L. Carvalho, Vivian Leung, Professor Sow-Hsin Chen

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

We recently established a procedure for determining the ion distribution. 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, comparing them with the experimental small angle x-ray scattering data. The preliminary results indicate that only a small fraction (30 percent) of the counterions are free in the water core of the microemulsion.

3.5 Ion Distribution around DNA Molecules

Project Staff

Szu-Li Chang, Professor Sow-Hsin Chen

Nucleic acids are highly charged molecules, and electrostatic interactions play an important role in many aspects of their structure and function. Electrical force is an important component of protein-DNA and drug-DNA interactions. For this reason, the study of the distribution of ions around DNA has received considerable attention in recent years.

In our project, we used small angle x-ray scattering (SAXS) to measure counterion distribution around the persistence length DNA molecules in aqueous solutions both with and without salts. We chose heavy metal counterions such as Tl^+ and Ba^{++} to enhance the counterion contribution to the scattering intensity (by a factor of Z^2), resulting in a measurable effect. Figure 3a shows the SAXS intensity distribution $I(Q)$ vs. Q in a semilog plot. This sample contains 2.5 mg/ml of thallium-DNA without added
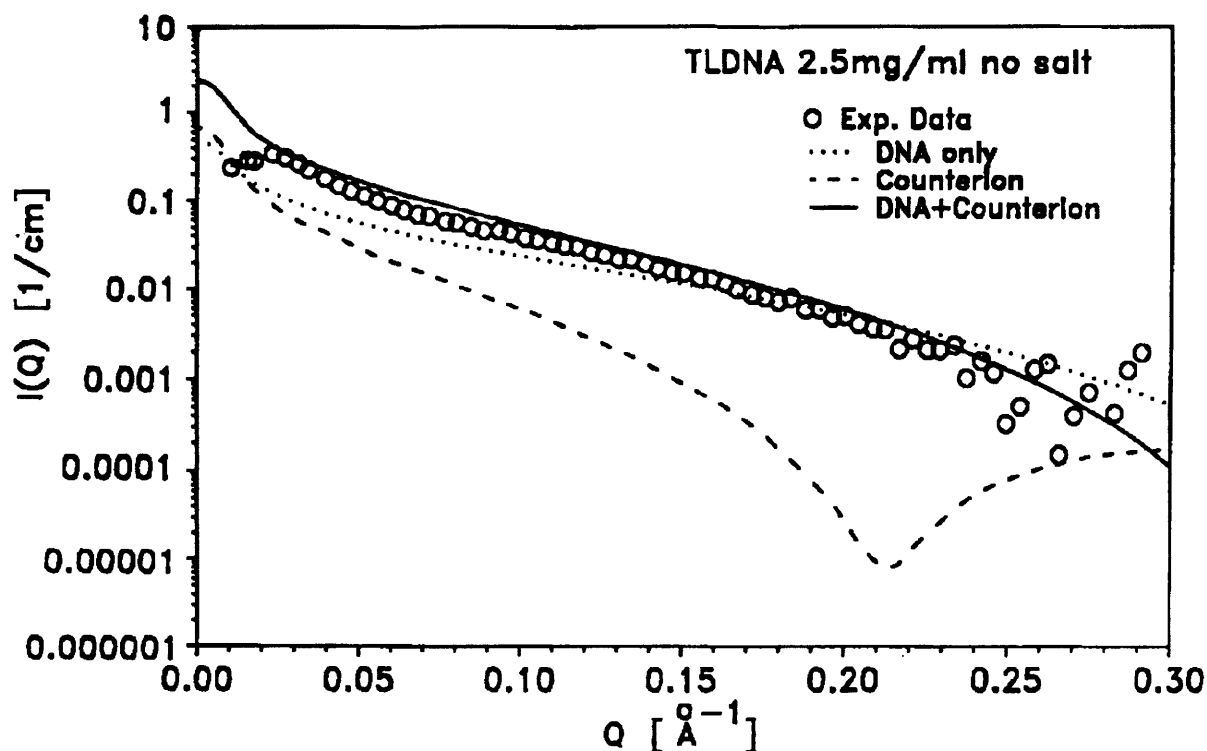
Figure 3a. SAXS intensity distribution I(Q) vs. Q in a semilog plot. The sample contains 2.5 mg/ml of persistence length DNA. The counterion has been exchanged to Tl^+ by an extensive dialysis. There is a residual TlCl on the order of 0.1 mM in the solution. The dotted line represents the contribution from the DNA core only, while the dashed line that from the counterions. The solid line represents the total calculated scattering intensity. Note, the solid line is not the sum of the dotted line and the dashed line because contribution from the DNA core and from the counterions do not add incoherently.

salt. The open circles represent the experimental data, the solid line represents the theoretical calculation. The dotted line represents the partial contribution of the DNA only, and the dashed line those of the counterions. The figure shows that, without taking into account the contribution of counterions, there would be poor agreement between the experiment and the theory. Figure 3b depicts the scattering length densities used to calculate the intensity. We calculated the intensities shown in figure 3a by assuming that 10 percent of the counterions are inside the DNA core, neutralizing 10 percent of the phosphate groups. If we exclude the counterions from the DNA core completely, the computation would not agree as well quantatively with the experiment. We found similar agreement for the case of 4.2 mg/ml of thallium-DNA in a solution containing 10 mM of TlCl and 3.7 mg/ml of

barium-DNA in a solution containing 5 mM of Ba_2Cl. The slight discrepancy between the experiment and the calculation in the region $Q \leq 0.03$ Å^{-1} is due to the small DNA-DNA correlation effect at finite concentrations.

3.6 Structural Study of Vesicles Formed from a New Bolaamphiphile

Project Staff

Pierandrea Lo Nostro, Professor Sow-Hsin Chen

Amphiphilic molecules with one polar head group and two aliphatic chains can form vesicles (or liposomes) in water. Vesicles are sealed structures formed by one bilayer of amphiphiles that separate an inner aqueous compartment from the external medium. The
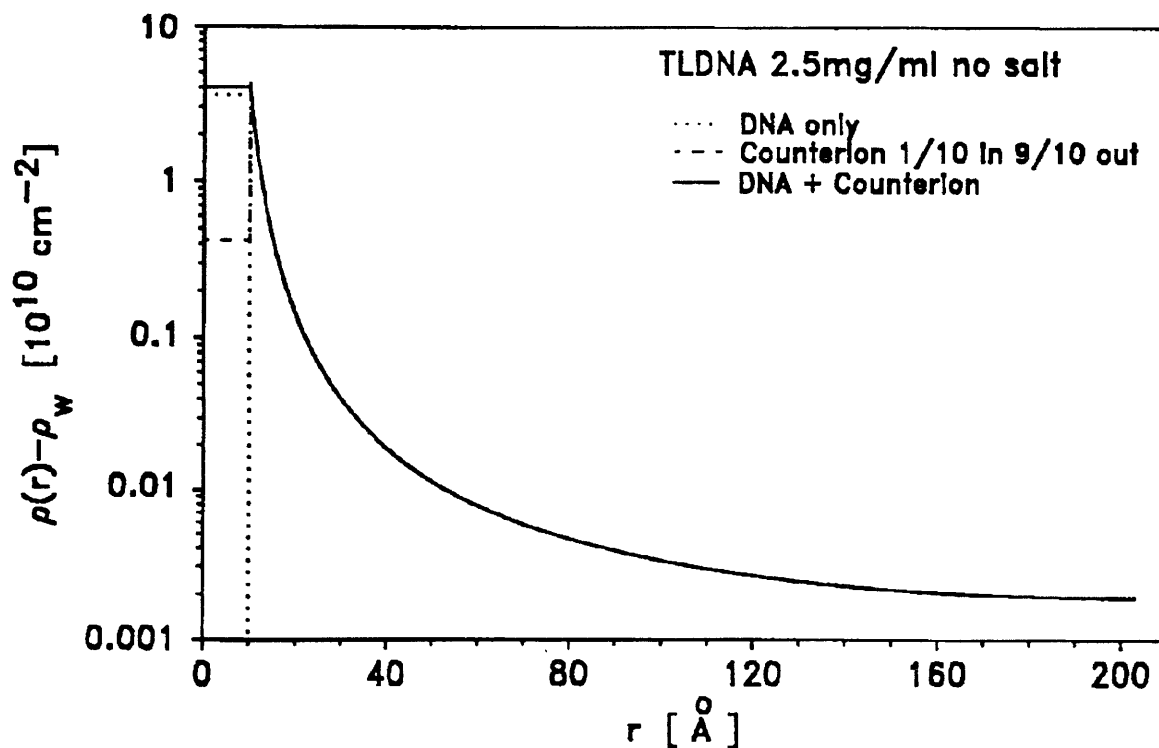
Figure 3b. The three net scattering length densities used to compute the corresponding three curves in figure 3a.

thickness of this bilayer is usually 4–5 nm. For this reason, vesicles constitute a very realistic model for biological membranes and an interesting system for entrapping and releasing large molecules (pollutants, nucleic acids or drugs), and for stereochemical centers of reactions.

We investigated aggregation properties of a new bolaform amphiphile, the (R,S)3,25,(26)-bis(β-D-glucophyranosylthio)-1,6,23,28-tetraoxacyclotetracontan-2,5,24,27-tetrone, which has never been studied. The Department of Chemistry at the University of Florence, Italy, first synthesized this amphiphile. A "bolaamphiphile" (or "bipolar lipid") is an amphiphilic compound containing two hydrophilic head groups connected by the hydrocarbon core and forming monolayered lipid membranes (MLM). Every molecule provides the two requested head groups facing the outer and the inner aqueous compartments. These bolaamphiphilic vesicles show some different and interesting properties from the usual bilayered membranes. For instance, the vesicles are thinner (2.5 nm) and do not fuse. If the two

polar head groups are different in charge, chemical composition or size, we can produce asymmetric vesicles, an interesting model for vectorial membranes. Bolaamphiphiles exist in nature, forming monolayered membranes in Archaebacteria, the primordial microorganisms living in volcanoes and oceans in very drastic environmental conditions (T > 90 degrees C, high pressure, high salt concentration).

We sonicated and ultracentrifugated this bolaform compound in aqueous dispersions to investigate vesicular properties through scanning electron microscopy (SEM) and dynamic light-scattering. Observing the formation of vesicles with a diameter between 200 and 400 nm, we found the same value for the diameter both with SEM and with light-scattering (within experimental errors).

We also investigated the stability of the vesicles over time, temperature and addition of a co-surfactant, dioctanoyl-phosphatidylcholine (diC$_8$-PC). Vesicles were very stable even several weeks after the preparation. We found that their

hydrodynamic diameter increases with temperature (from 18 to 50 degrees C), probably because of the increased softness of vesicles at higher temperatures. The addition of different amounts of diC_8-PC (2.5 percent, 20 percent, and 40 percent) did not affect the stability or size of vesicles.

Calculations according to Nagarajan's theory show that this bolaform compound spontaneously aggregates in planar monolayers or in very large structures, but produces vesicles only upon sonication.

3.7 Photon Correlation Spectroscopy and Its Applications: Dynamic Transition at the Percolation Threshold of a Three-Component Microemulsion

Project Staff

Professor Sow-Hsin Chen, Jacque Rouch, Piero Tartaglia

We have performed an extensive series of dynamic light scattering experiments on a three-component water-in-oil microemulsion consisting of AOT(surfactant)/water/decane, in the isotropic one-phase region of the phase diagram (covering a range of volume fractions ϕ of the dispersed phase from 0.1 to 0.7 and temperatures T from 15 to 50°C). Previous studies have established a well-defined electrical percolation locus, extending from the cloud-point curve near T = 40°C and ϕ = 0.098, down to 22°C and ϕ = 0.70. The microemulsion droplet density time correlation function has a stretched exponential form for ϕ > 0.30. The exponent β changes continuously as a function of temperature at a given volume fraction and shows a maximum close to unity at the percolation temperature. The relaxation time, as a function of temperature, also shows an abrupt change of slope at the percolation threshold. A physical interpretation of the results involves a theoretical model for the dynamics of percolated cluster of microemulsion droplets.

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