Structure and Flow in Surfactant Solutions

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Chapter 5

Microstructure of Complex Fluids by Electron Microscopy

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Understanding the relationship between molecular organization and macroscopic properties of emulsions, lyotropic and thermotropic liquid crystals, colloidal dispersions, and other such "complex fluids" is generally a first step towards understanding rheology. As the trend in both science and technology is toward dilute, mixed surfactant solutions, light scattering, X-ray diffraction, and various spectroscopy techniques are not sufficient to determine complex fluid microstructure. Modern rapid-freezing methods followed by freeze-fracture or cryoelectron microscopy are much better suited to visualize the three-dimensional structure of the aggregates that make up the dispersion, while simultaneously revealing particle orientation and distribution. The direct structural information obtained by microscopy requires no model-dependent interpretation as is the case in scattering or spectroscopy data. However, the loss of ambiguity in interpretation can be more than made up for by artifacts of sample preparation.

Most attempts at understanding the rheology of surfactant solutions and complex fluids begin with a more or less detailed model of molecular organization and interactions in solution (1-5). Light, X-ray, and neutron scattering, NMR spectroscopy, fluorescence quenching, flow birefringence, and rheological techniques have provided a wealth of information on the implications of surfactant microstructure (6-20). However, especially in dilute or mixed surfactant systems, the microstructure is often so unexpected as to make it difficult to construct a model; or worse, a model built on simple structural concepts leads to erroneous interpretation of experimental results (21,22). Electron microscopy is becoming a necessary complement to these techniques as the structural information provided is model independent. Information at all relevant length scales, from 1 nm to 100 μm is available in the same experiment, allowing not only the structural characterization of the individual surfactant aggregate, but the organization of the aggregates in solution.

Two methods have evolved, each with specific benefits, to image surfactant solutions with electron microscopy; they are direct imaging of the frozen, hydrated material in the electron microscope, also known as cryo-TEM, and freeze-fracture replication (23, 24). Although these techniques have been applied to microstructured
fluids for more than three decades (25-31), they are only now coming into wider acceptance in the complex fluids community. This reluctance to embrace microscopy is undoubtedly due to the many possible artifacts associated with the non-equilibrium aspects of rapid freezing (24,32-37) or staining and drying (38-48) procedures.

In many early investigations, the cooling rates employed were too low to prevent structural rearrangements caused by crystal formation and phase separation. If the solvent phase crystallizes as distinct grains, the advancing crystal front expels any dispersed or dissolved species to the grain boundaries, thereby destroying the structure and spatial organization of the material. More than a decade ago, in reviewing the state of electron microscopy as applied to colloidal dispersions, Menold, Lütte, and Kaiser (36) concluded that solvent crystallization caused by slow cooling usually led to unacceptable distortions, segregation, and reorganization of dispersed particles. The general consensus at that time was that, although intuitively appealing, electron microscopy was not a valid means of determining fluid microstructure due to freezing artifacts. However, in more recent times microscopy has been essential to settling long-standing questions such as the bicontinuous structure of oil-water-surfactant microemulsions (49,50), the structure of thermotropic blue phase (51-53) and twisted smectic liquid crystals (54), the organization of bilayers in dilute lamellar and isotropic L3 phases (55-57), the organization of worm-like (58-63) and disc micelles (58,64), vesicle-micelle transitions (65-67), vesicle-lamellar phase transitions (68-70), the microstructure of lyotropic smectic and nematic phases (64,71-75), and aqueous polymer gels (76,77).

The obvious alternatives to microscopy for detailed structural characterization in more ordered lyotropic and thermotropic liquid crystals have been light, X-ray, and neutron scattering (20,86,87). Although each of these techniques have specific limitations, most samples can be examined in the fluid state without any special precautions. Unfortunately, the price of simplified sample preparation is increased difficulty in data interpretation. There are three principal problems in interpreting scattering data; the first is the averaging of structure over a macroscopic scattering volume, the second is the difficulty in uniquely inverting the Fourier scattering pattern to determine real space structure, and the third is the limited spatial dimensions available from a particular experiment.

Typical scattering experiments are constructed so that information is collected and averaged over a macroscopic volume (from mm$^3$ to cm$^3$) which contains a large number of individual particles, domains, etc. This averaging can be useful to assigning overall structural features and symmetries, but the detailed characteristics of the microstructure are averaged out. These characteristics, which may include structural features such as the number and distribution of polymer crosslinks or entanglements (76,77), membrane geometry and topology (49,50,72-75), liquid crystalline defects (26,51-54,88), particle shape and size polydispersity (66-71), grain and domain boundaries (89,90,91), can be important, even dominant factors in macroscopic physical phenomena. For instance, the importance of dislocations and grain boundaries in the deformation of crystalline solids is well documented (92).

The more limiting feature of scattering data is that the information provided is indirect. A scattering experiment can be described mathematically as the Fourier transform of some density, atomic number, etc. distribution in the sample (87,93). To understand the experiments, the researcher has two options. The first is to uniquely invert and interpret the experimentally limited amount of Fourier data, a difficult if not impossible task. The second option is to invoke a mathematical model of the anticipated intra- and inter- particle structures and distributions and compare the theoretical scattering from the model with the experimental data. Although this second option is used almost exclusively, the physical limitations and assumptions inherent to the model can bias the data. Often, several conflicting models, and even unphysical models, can account for the same experimental data (93). An important example of this has been in investigations of middle phase microemulsions. Scattering patterns
are not detailed enough to distinguish between several proposed structural models due to the polydispersity and random structures of the microemulsions (20); however freeze-fracture images by Jahn and Strey (49,50) clearly show that the microemulsion consists of interconnected, bicontinuous oil and water domains as originally proposed by Scriven (94).

Structure on both an average and discrete basis can be seen simultaneously and directly with electron microscopy. For freeze-fracture, image contrast is generated by simple mass-thickness contrast (24). For direct imaging, the thin samples typically prepared are nearly pure phase objects and the dominant contrast is phase contrast (23). In both cases, it is possible to relate the structures observed in the images to the structures in the sample with a minimum of modeling or interpretation. It is usually much easier to interpret the real space structure from an image than from scattering data in Fourier space; however, an added benefit of the image is that the Fourier space information can be readily obtained from the image by either optical diffraction from the TEM negative, or by modern digital image analysis (24).

The third benefit of microscopy is that structural studies of colloidal systems often need to cover a wide range of length scales simultaneously to determine the details of the particles making up the dispersion and their three-dimensional orientation and distribution in the suspending fluid. This is perhaps the greatest benefit of either freeze-fracture or cryo-TEM. In a given image, information is available at length scales from the resolution limit of about 2 nm to roughly 100 microns, or about 6 decades in length scale. For a typical scattering experiment, information can only be obtained over 2 – 3 decades in length scale.

The main drawback to TEM investigations of microstructured fluids has been the fear that freezing-induced artifacts cause unacceptable changes in the chemical or physical state of the sample, leading to uncertainty and error in the interpretation of the TEM images. Recent advances in the science and art of sample freezing, including the jet-freezing technique pioneered by Müller, Meister, and Moor (95), and controlled plunge freezing of thin sandwich samples (96) have allowed for rapid advances in the applications of freeze-fracture to microstructured fluid systems. With cryo-TEM, it has been shown that water and other simple liquids can be frozen quickly enough to prevent crystallization (80,97). Studies of blue phase liquid crystals have shown that phases stable over as little as 0.5°C can be captured by rapid freezing (52,53).

With the expanding possibilities for investigations of microstructured fluids via high resolution microscopy, this review attempts to present the state of the art of freeze-fracture and cryo-TEM as it is applied to surfactants. We will present what we believe to be the necessary and sufficient conditions for reliable sample freezing, a discussion of fracture in frozen liquids, the limitation and resolution of replication, and a discussion of image resolution in freeze-fracture and cryoelectron microscopy.

Experimental Methods

Rapid Freezing. The most important step in any TEM investigation is the initial rapid freezing or quench step. The goal of rapid freezing is to remove heat at such a rate that 1) the details of the individual particle structure are retained and 2) the distribution and orientation of the particles are not disturbed. For most microstructured fluid systems, the second criterion is much more difficult to achieve and usually requires that the solvent or continuous phase be vitrified. In freeze-fracture, vitrification generally is taken to mean that the solvent phase contains no recognizable crystals larger than the typical resolution of the images. In cryo-TEM, it is easy to see if vitrification has occurred by examining the diffraction pattern of the solvent phase. Simple plunge freezing into liquid propane or ethane has been sufficient to vitrify water films up to about 50 – 200 microns in thickness.

However, if the solvent phase does crystallize, dispersed particles and solutes are swept to the crystalline grain boundaries and information on the original orientation
and distribution of the particles in the solvent is lost (see Figure 1). The distribution of the colloidal particles reflects the crystallization behavior of the solvent rather than any of the original properties of the system. Theoretical estimates of the cooling rates necessary to vitrify water range from $10^4$ to $10^{10}$ K/sec (98), although experimentally, the minimum cooling rate appears to be in the range of $10^4$ to $10^5$ K/sec (97).

For freeze-fracturing a broad variety of liquid samples of any viscosity such as suspensions, solutions, and emulsions, the most practical method of rapid freezing is sandwiching the sample in a rigid container that is then contacted with a liquid cryogen. A wide variety of sample holders have appeared in the literature (99); however, the most commonly used are variations on the "copper sandwich" holders developed by Gulik-Krzywicki (100) and commercialized by Balzers (BUO-12-056T and variations; Hudson, New Hampshire). Typically, from 0.1 – 0.5 ml of sample liquid is pipetted onto one of the planchettes, then a second planchet is used to spread the liquid to form a thin, 10 – 50 micron thick film. A variant of this sample holder with an annular opening in the top planchette is used by Jahn and Strey (50). By far the most common method of rapid freezing is immersing the sample sandwich into a liquid cryogen, typically with a spring-loaded device to increase the relative specimen-cryogen velocity (50,101).

For cryo-TEM, the sample liquid is spread as a thin film (<0.15 μm) on polymer coated electron microscope grids prior to plunge freezing (23). The most difficult part of sample preparation for cryo-TEM is to ensure that the sample is thin enough (<150 nm) for electrons to pass through the sample. A small droplet of sample is placed on a grid, then blotted to form a thin film. This entire procedure is done within a temperature and humidity controlled chamber to eliminate evaporation of the solvent (23). Practice is required to form the thin films, and the high shear associated with the thinning process can create or destroy certain microstructures. This technique produces higher cooling rates due to the thinner samples than the freeze-fracture samples, although it is limited to fairly dilute, low viscosity (<10 cp) materials.

Freezing a multicomponent, structured fluid sample sandwiched between metal sheets in a liquid cryogen is a complicated heat transfer process that involves convection at the cryogen-sandwich boundary, conduction through the sandwich, and conduction and possible phase changes, crystallization, etc. within the fluid specimen (101). A simplified model of cooling shows that the cooling rate of samples is limited by convection from the cryogen to the specimen surface (101,102). The important criterion is that the Biot modulus of the specimen, $h d^2 k$ is $<< 1$; (see (102) for discussion) $h$ is the heat transfer coefficient from the cryogen to the sample, $d$ is the sample thickness, and $k$ is the average sample thermal conductivity; for a typical freeze-fracture sample of thickness 100 – 200 microns, the Biot number, $B_i$, is 0.05 to 0.5. For the thinner cryoelectron samples, $B_i$ is even smaller. An important physical consequence of convection limited cooling ($B_i < 1$) is that the temperature is spatially uniform within the sample during freezing (102). In this approximation, the cooling rate of a sample of area $A$, volume $V$, heat capacity $C_p$, and density $\rho$, is:

$$\frac{dT}{dt} = -\frac{A}{V} h(T - T_C) \frac{1}{\rho C_p}$$

(1)

$T$ is the sample temperature and $T_C$ is the liquid cryogen temperature. Experimental measurements of the average cooling rate of a wide variety of specimens using various cryogens can be correlated using this simplified model (101). The cooling rate is proportional to the ratio of specimen surface area to volume, and inversely proportional to the thermal density, $\rho C_p$, of the sample, which varies little between the typical materials encountered in complex fluids. Surprisingly, the cooling rate is independent of the sample thermal conductivity, and hence, virtually all samples of the same characteristic dimensions freeze at the same rate.
Optimization of the freezing process: transfer coefficient, while minimizing T of cryogen and the velocity at which amount of cryogen boiling enhances if results in the formation of a vapor film rate of heat transfer (103). Liquid nitrogen should be avoided for this reason. The propane cooled to near its freezing point and orientation of dispersed macromolecules controlled plunge freezing in liquid propane impossible to say if the water surrounds microcrystalline (see Figure 1).

Many of the phases of interest in above or below room temperature. The controlled chamber be coupled to have been used to observe temperate nematic micellar phases (64) and phase temperature and humidity controlled equilibrated prior to plunge freezing. Temperature jump experiments by focus film sample (79, 80). Jahn and Streit examine "middle-phase" microemulsions stable over less than 1°C have been res by freeze-fracture (51-53), provided that nucleation and growth. Thermotrope quenched from above 100°C for freeze

Because the chemical and physical optimized for rapid freezing by chemic the structure, a judicious choice of s between success and failure. As an importance to successful images, it is to affect crystallization. Crystallization of a critical size, then the grow liquids, nucleation occurs heterogeneously often the colloidal particles we wish to more important quantity to minimize t crystal growth velocity is proportional of fusion, β, and the fraction, f, of acco steric constraints involved in pa configuration), and inversely proportion

\[ T_m \] is the equilibrium melting point. than are viscous effects in Equation ((CH)\textsubscript{2})\textsubscript{2}CH(CH)\textsubscript{2}CH)\textsubscript{3} freeze at a straight chain n-hexane (CH\textsubscript{3} CH restrictions imposed by the branchi Cyclohexane, on the other hand, adopt that is easy to pack into a crystalline like many of the physical properties of th

Figure 1a. Freeze-Fracture TEM image of poorly frozen dispersion of tobacco mosaic virus in water. The slow cooling rate allows the formation of characteristic ice patches, surrounded by particles forced to boundaries between crystals.

Figure 1b. Freeze-fracture TEM image of well-frozen sample of the same dispersion as Figure 1a. The orientation and distribution of the nematically aligned specimen is identical to room temperature material.
Optimization of the freezing process can be achieved by maximizing $h$, the heat transfer coefficient, while minimizing $T_C$, the cryogen melting point, by proper choice of cryogen and the velocity at which the cryogen contacts the sample. A limited amount of cryogen boiling enhances the heat transfer coefficient; too much boiling results in the formation of a vapor film around the sample that drastically reduces the rate of heat transfer (103). Liquid nitrogen, and other cryogens at their boiling points should be avoided for this reason. The best practical cryogen appears to be ethane or propane cooled to near its freezing point by liquid nitrogen (98-103). The distribution and orientation of dispersed macromolecules in water has been preserved using both controlled plunge freezing in liquid propane and propane jet freezing, although it is impossible to say if the water surrounding the colloidal particles is amorphous or microcrystalline (see Figure 1).

Many of the phases of interest in the study of microstructured fluids are either above or below room temperature. This requires that a temperature and environment controlled chamber be coupled to the freezing apparatus. Sealed, two stage ovens have been used to observe temperature dependent phase transitions in lyotropic nematic micellar phases (64) and phospholipids (105). Bellare (23) has constructed a temperature and humidity controlled cell in which cryo-TEM samples can be equilibrated prior to plunge freezing. This chamber has been modified to do temperature jump experiments by focusing a high intensity mercury lamp onto the thin film sample (79,80). Jahn and Strey (49,50) have used a similar configuration to examine "middle-phase" microemulsions. Thermotropic liquid crystalline phases stable over less than 1°C have been resolved using quick freezing techniques followed by freeze-fracture (51-53), provided that the equilibrium phase transition occurs by nucleation and growth. Thermotropic smectic phases have been successfully quenched from above 100°C for freeze-fracture investigation (54).

Because the chemical and physical properties of a microstructured fluid cannot be optimized for rapid freezing by chemical or physical cryoprotectants without changing the structure, a judicious choice of systems to investigate usually is the difference between success and failure. As avoiding solvent crystallization is of primary importance to successful images, it is useful to understand the solvent properties that affect crystallization. Crystallization consists of two steps, nucleation of small crystals of a critical size, then the growth of these crystals. Except for extremely pure liquids, nucleation occurs heterogeneously at insoluble impurities; such impurities are often the colloidal particles we wish to study. The crystal growth velocity, $v$, is the more important quantity to minimize by appropriate choice of sample properties. The crystal growth velocity is proportional to the degree of supercooling, $\Delta T$, the entropy of fusion, $\beta$, and the fraction, $f$, of acceptable sites on the interface (which reflects the steric constraints involved in packing solvent molecules into a different configuration), and inversely proportional to the solvent viscosity, $\mu$:

$$v \propto \frac{f \beta \Delta T}{\mu T_m}$$

(2)

$T_m$ is the equilibrium melting point. Steric effects, are generally more pronounced than are viscous effects in Equation 2. Branched hydrocarbons such as isohexane ((CH$_3$)$_2$CH(CH$_2$)$_2$CH$_3$) freeze at a much lower temperature ($-153.7$°C) than does straight chain n-hexane (CH$_3$ (CH$_2$)$_4$CH$_3$) ($-94$°C), indicating that the steric restrictions imposed by the branching make crystallization much more difficult. Cyclohexane, on the other hand, adopts a fairly rigid conformation in the liquid phase that is easy to pack into a crystalline lattice and freezes at about 6°C (106). Clearly, as many of the physical properties of these solvents are similar, to optimize the system for rapid freezing, isohexane, which is sterically hindered, is a much better choice than cyclohexane, which crystallizes readily. In aqueous solutions, salts, solutes and macromolecules that tend to disrupt water structure by hydrogen bonding can hinder
crystallization. For instance, ice formation in polyacrylamide gels is suppressed to below -17°C (107).

**The Freeze-fracture Technique.** Once frozen successfully, cryo-TEM specimens are inserted directly into the TEM via a "cryo-transfer holder" which keeps the sample temperature below -140°C, the recrystallization temperature of water (23). The original sample is examined in the TEM, which presents opportunities and problems that are discussed in the next section. In the freeze-fracture technique, the sample is replaced by a metal "replica" that is compatible with the TEM environment. The fracture, etching, and replication steps of the freeze-fracture technique are carried out at low temperature and high to ultrahigh vacuum. Typically, the "copper sandwich" samples are loaded under liquid nitrogen into a hinged brass block fracture stage. The fracture stage has sufficient thermal mass that the specimens do not heat up significantly during the brief time they are exposed to air during transfer into a vacuum chamber. The fracture stage is clamped to a temperature controlled coldfinger within the vacuum chamber. The specimen stage fractures the sandwiches on opening; a sharp, quick break is preferable to a long, steady pull for reasons discussed below. The stress on the specimen is primarily tensile.

Griffith (110) proposed that, even prior to fracture, a brittle material contains a population of small cracks at impurities, boundaries, and other imperfections. When placed under sufficient stress, one or more of these cracks spreads into a brittle fracture, thereby decreasing the elastic energy at the expense of increasing the surface area, and hence the surface energy of the material. A crack will spread when the decrease in elastic energy is at least equal to the energy required to create the new crack surface. The surface energy can be thought of as the product of the new surface area created and the specific energy per unit area of the fracture surface. The Griffith theory states that the fracture will follow the path of least resistance (smallest specific surface energy or smallest molecular cohesion) provided that the fracture area created is not too large. In most two or more phase colloidal dispersions, the fracture surface propagates along the interface between the two phases, usually at particle-solvent boundaries (see Figures 1,2). Apparently, solvent-solvent cohesion and particle-particle cohesion are larger than solvent-particle cohesion. Alternately, small differences in the volume contraction on cooling between the solvent phase and the particles could lead to debonding prior to fracture, or to the formation of cracks at the particle-solvent interface. In either case, the weak zone appears to be at the interface and interpretation of freeze-fracture images is greatly simplified. Branton (111) has shown that the weak zone in bilayer membranes is along the hydrocarbon interior of the membrane; this also appears to be true for lyotropic lamellar phases in general.

**Etching.** The controlled sublimation of the solvent, known as etching, can be used to enhance the topographic variations in a fractured, microstructured fluid. However, removing too much of the solvent can alter the apparent location and distribution of dispersed particles, hide evidence of crystallization induced reorganization, and make the replicas difficult to pick up and clean. If the dispersed phase is entangled and self-supporting, as are polymer solutions and gels, or bicontinuous L3 or microemulsion phases, a limited amount of etching can bring out the network structure (76,77) (see Figure 2). The important parameter in etching a sample is temperature. The sublimation pressure, hence the sublimation rate, is set once the sublimation temperature is fixed. The sublimation rate, S, in nanometers per second from a surface under vacuum can be obtained from gas kinetic theory:

$$S = \frac{P_s - P_v}{\rho_c} \left( \frac{M_k}{2\pi RT} \right)^{1/2} \times 10^7$$  \hspace{1cm} (3)
Figure 2. Freeze-fracture image of L₃ phase of cetyltrimethylammonium bromide-sodium 1-octane sulfonate-water mixture. The sample has been etched to bring out the relief between the water (W) and membrane (M) bicontinuous structures.
in which $P_s$ is the saturation vapor pressure and $P_v$ the background pressure of the sublimating phase in dynes/cm$^2$, $M_e$ is the molecular weight (gr/mole) and $p_c$ (gr/cm$^3$) is the density of the sublimating phase. $R$ is the gas constant, and $T$ is the sublimation temperature (K). The saturation vapor pressure for most solvents can be found in general engineering handbooks. If the background pressure of the solvent is greater than the vapor pressure at the temperature chosen, material will condense from the vacuum onto the sample, obscuring surface details. Hence, care is necessary to understand the relative composition of the residual gases in the vacuum chamber.

**Replication.** The goal in the replication process is to reproduce the fracture surface as accurately as possible with an electron opaque shadowing layer backed by a continuous, electron transparent, backing layer. The resolution in freeze-fracture electron microscopy is limited by imperfections in replication. Ideally, the evaporated metal atoms, which are usually a mixture of platinum and carbon, stick exactly where they land and form a structureless layer. However, the surface energy of the metal layer is much higher than that of the original fracture surface of water or hydrocarbons; hence, the metal film does not spread or "wet" the surface, but aggregates into small droplets (112). The aggregates grow in size, eventually merging with neighboring aggregates to form a continuous film. For most electron microscopy applications, the evaporation is stopped prior to the formation of a continuous metal layer. In our lab, about 1.5 nm of platinum carbon followed by about 15 nm of carbon backing gives optimum results. For proper interpretation of the replica, the sample material must be completely removed from the replica before viewing. In our lab, the cleaning method of Fetter and Costello (113) has always given the best results.

**Contrast Mechanisms in Electron Micrographs**

The contrast mechanism, that is, how the final image is related to the original microstructure, is quite different for freeze-fracture and cryo-TEM, as should be expected from the quite different chemical and physical characteristics of the specimens. An electron micrograph is the intensity distribution of an incident plane electron wave transmitted through the specimen and detected at the viewing plane. The plane of the specimen is denoted by $r = (x,y)$ and $z$ is the direction of the electron beam. Electrons can be scattered, absorbed, or phase shifted by the sample, all of which lead to contrast in the final image that must be related to structural features in the specimen.

The phase shift and absorption are generally described separately. In traversing a specimen of local thickness $t(r)$ and local potential distribution $V(r,z)$, the incident electron wavefunction, $\Psi_i$, is altered:

$$\Psi_t = \Psi_i \exp \{-i\eta(r)\} \quad (4)$$

corresponding to a phase shift of $\eta(r)$ in the transmitted wave, $\Psi_t$, relative to the incident wave. $\eta(r)$ is related to the local column average potential in the specimen:

$$\eta(r) = \sigma \int_0^t V(r,z) \, dz \quad (5)$$

$\sigma$ is a constant $= \pi/\lambda E_0$ for electrons of wavelength $\lambda$, and energy $E_0$ (114).

The incident wave is also scattered within the specimen; that part of the wave that scatters outside the objective aperture of the microscope will not be available for image formation and can be considered absorbed. Electrons are scattered elastically by the Coulomb potential and inelastically by plasmon and inner shell ionizations. The decrease in amplitude of the incident wave can be described as (114, 115):
\[ \Psi_t = \Psi_i \exp \{-S_p(E_0, \alpha) \rho(r) t(r)\} \]  
(6)

in which \( S_p(E_0, \alpha) \) is dependent only on the electron energy and the aperture used (hence on the microscope and its operating conditions (114)) and \( \rho(r) \) is the local, column average mass density within the specimen:

\[ \rho(r) = \int_0^t \rho(r, z) \, dz \]
(7)

\( S_p \rho t \) can be considered the absorption of the specimen; it is directly proportional to the product of the mass and thickness of the sample. In general, the incident wave will undergo both a phase shift and absorption such that the transmitted amplitude is:

\[ \Psi_t = \Psi_i \exp \{ i\eta(r) - S_p(E_0, \alpha) \rho(r) t(r)\} \]
(8)

Specimens are classified as phase objects if the \( \eta \) term dominates the exponent, or as mass-thickness objects if the \( \rho \) term dominates. For a typical cryo-TEM sample, the small density differences make the specimen a phase object, and the primary contrast mechanism is phase contrast. For freeze-fracture replicas, the platinum film scatters electrons very strongly, and the specimens are mass-thickness dominated. Hence, the information obtained from the two techniques is complementary.

**Cryo-TEM and the Weak Phase Object Approximation.** A further approximation known as the "weak phase object" is often used to help relate the sample features to the image. If \( \eta(r) \ll 1 \), and density variations can be neglected, the linearized form of Equation 8 is:

\[ \Psi_t = \Psi_i [1 - i\eta(r)] \]
(9)

The first term in the brackets corresponds to the unscattered beam, and the remaining terms to the scattered beam. (What is actually more important to the contrast are variations from the mean phase shift \( \langle \eta \rangle - \eta(r) \); hence, even though the absolute density or phase shift might be large, if the variance is small, the object can be considered a weak phase object.) However, detectors do not record the amplitude and phase of an image, but its intensity. Hence, a weak phase object would not have any contrast in an ideal image because

\[ |\Psi_t|^2 = |1 - i\eta(r)|^2 = 1 \]
(10)

where \( \eta^2 \) terms have been neglected in keeping with the weak phase object approximation. Phase contrast can only be realized in an image by further changing the relationship of the scattered beam with respect to the unscattered beam. In optical microscopy, a phase plate is introduced at the focal plane. This plate produces a phase shift of \( \pi/2 \) between the scattered and unscattered beams resulting in a transmitted beam amplitude of:

\[ \Psi_t = \Psi_i [1 - i\eta(r) \exp(-i\pi/2)], \]
(11)

the image intensity, \( |\Psi_t|^2 = 1 - 2\eta(r) \), and the image contrast is directly related to the projected potential of the specimen. However, phase plates are generally unavailable for electron microscopes and phase contrast is generated by the combined effects of apertures, lens defects and defocusing the image, which can be calculated using the "transfer theory" of imaging (114-118).

TEM images are complicated by the interaction of the transmitted beam amplitude with the "microscope transfer function" that describes the combined effects of imperfections in the electron optics and defocusing (116,117). The effect of apertures, aberrations and defocus are lumped into the "point spread function," \( P(r) \), which can be thought of as the distortion induced by the microscope on the image amplitude and phase of an ideal point object. The real image amplitude, \( \Psi_{\text{real}} \), is then
related to the ideal image amplitude, \( \Psi_{\text{ideal}} \), by a convolution of the ideal image amplitude with the point spread function (the ideal amplitude is given by Equation 9):

\[
\Psi_{\text{real}} = \Psi_{\text{ideal}} \ast P(r)
\]  

(12)

where the convolution of two functions \( f(r) \) and \( g(r) \) is defined in the usual way:

\[
f(r) \ast g(r) = \int_{-\infty}^{\infty} f(r') \ g(r - r') \ dr'
\]  

(13)

It is simpler and more conventional to use the two-dimensional Fourier space representation of both the point spread function and the image amplitude to describe these effects. \( S(q) \) is defined as follows:

\[
S(q) = \int_{-\infty}^{\infty} \Psi_{\text{u}}(r) \ exp \ (-2\pi i \ r \cdot q)) \ dr = \Psi_{\text{u}}[\delta(q) - iA(q)]
\]  

(14)

\( A(q) \) is the two-dimensional Fourier transform of \( \eta(r) \); \( \delta(q) \) is the Dirac delta function and represents the unscattered beam. It is simpler to calculate the contributions of apertures, aberrations, and defocus to the Fourier transform of \( P(r) \), which is known as the transfer function or \( T(q) \). Each imperfection contributes an optical path difference between the actual and ideal waves. These path differences, when integrated over the lens area yields the transfer function, which describes the phase change in the incident wave induced by the imperfections (II4). Spherical aberration, defocus and axial astigmatism contribute to the transfer function:

\[
T(q) = \exp \ [-i\phi(q)]B(q)
\]  

(15)

where the phase shift \( -\phi(q) \) introduced by the lens imperfections is given by:

\[
\phi(q) = \frac{\pi}{2} \left[ C_s \lambda^3 q^4 + 2\Delta f \lambda q^2 - 2\lambda C_a(q_x^2 - q_y^2) \right]
\]  

(16)

in which \( \Delta f \) is the defocus (positive for overfocus or a strongly excited lens), \( C_s \) is the coefficient of spherical aberration and \( C_a \) is the astigmatism coefficient of the objective lens. \( B(q) \) is the aperture function, \( B(q) = 1 \) for \( q_0 < q_0 \), and zero everywhere else, where \( q_0 \) is the acceptance angle of the objective aperture.

The maximum spatial frequency \( q_{\text{max}} = \alpha_0 / \lambda \) determines the best resolution that can be achieved in the image. For example, if \( \alpha_0 \) is .01 rad (a typical 40 \( \mu \)m aperture for an objective lens of focal length 2 mm), \( q_{\text{max}} = 2.7 \text{ nm}^{-1} \) for 100 kV electrons and the resolution in the image is 1/\( q_{\text{max}} \) or .37 nm. The real image amplitude, \( \Psi_{\text{real}} \) is calculated from the inverse Fourier transform of \( S_i(q) = S(q)T(q) \):

\[
S_i(q) = [\delta(q) \ exp[-i\phi(q)]B(q)] - i[A(q)\cos[\phi(q)]B(q)] - [A(q)\sin[\phi(q)]B(q)];
\]  

(17)

\[
\Psi_{\text{real}} = 1 - i[\eta(r) * F(r)] - [\eta(r) * G(r)]
\]  

(18)

where * is the convolution integral as before (Equation 13) and \( F(r) \) and \( G(r) \) are the inverse Fourier transforms of \( \cos[\phi(q)]B(q) \) and \( \sin[\phi(q)]B(q) \) respectively. The convolutions in Equation 18 describe the blurring effect of the microscope lens aberrations on the projected potential of the specimen, \( \eta(r) \). The image intensity is:

\[
|\Psi_{\text{real}}|^2 = 1 - 2\eta(r) * G(r)
\]  

(19)

where the squared terms are neglected, consistent with the original weak phase object approximation. Equation 19 shows that the image intensity is linearly related to \( \eta(r) \), and the image represents a distorted version of the structure of the specimen due to the lens aberrations and defocus. If the image contrast is defined as
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\begin{equation}
C(r) = \frac{|\Psi_i|^2 \cdot |\Psi_{\text{real}}|^2}{|\Psi_i|^2}
\end{equation}

where $\Psi_i$ is the incident wave amplitude, then

\begin{equation}
C(r) = 2\eta(r) * G(r).
\end{equation}

An ideal microscope (for weak phase specimens) has no aperture ($B(q) = 1$) and introduces a uniform phase shift of $\phi = \pi/2$ and the ideal image contrast is:

\begin{equation}
C_{\text{ideal}}(r) = 2\eta(r) = 2\sigma \int_0^t V(r,z) \, dz.
\end{equation}

An ideal image of a weak phase object is the projected atomic potential of the specimen.

The behavior of the transfer function over the $q$ range resolved in the image determines the image. Of the factors that influence $\phi(q)$, two are under the control of the microscopist - defocus and astigmatism. In any modern microscope, small lenses are available to minimize the effects of astigmatism; hence the third term in Equation 16 can be neglected. What the microscopist does is try to maximize $\sin[\phi(q)]B(q)$ for the $q$ range in the specimen by adjusting the degree of defocus. Images are never recorded overfocus, because the spherical aberration and defocus terms are additive and cause rapid oscillations in $\sin[\phi(q)]$ (see Equation 16). The defocus where $\sin[\phi(q)] = 1$ over as large a range as possible is known as the optimum defocus. Although the transfer function for a typical 100 kV microscope is optimized for $\Delta f = 100$ nm, low frequency (low resolution) information up to $2 \text{ nm}^{-1}$ ($> 0.5$ nm in real space) is transferred to the image with a relatively small amplitude. For cryo-TEM, radiation damage to the specimen limits the useful magnification to about 20,000 times, and the smallest features of interest such as micelles, bilayers, etc. are often 3-10 nm in extent and much larger underfocus conditions are needed to bring out this information. The approximate defocus required to optimize phase contrast for a particular resolution can be calculated from Equation 16. Provided $q$ is small, the defocus term in Equation 16 is significantly greater than the spherical aberration term. Thus for a spatial frequency $q$ the transfer function can be optimized by choosing the $\Delta f$ value that makes $\sin[\phi(q)] = 1$, or $\phi = \pi/2$, that is

\begin{equation}
\Delta f = r^2/2\lambda
\end{equation}

Hence, for a typical rod micelle of diameter 5 nm, with $\lambda = 3.7$ pm for 100 kV electrons, $\Delta f = 3.4$ $\mu$m. A drawback to these large defocus values is that $\sin[\phi(q)]$ oscillates widely for higher resolution information in the image, making this information unreliable. That is, an artificial granularity is created by the oscillations in the transfer function at these large defocus values. The net effect is that even a featureless substrate can appear to have a great deal of texture when imaged under these conditions. Care must be taken in distinguishing structural features such as spherical micellar aggregates from the background granularity. This is further complicated by radiation damage to the specimen by the electron beam that can also generate small differences in thickness or potential that can appear to be real microstructure. A general rule for imaging surfactant microstructure is that rod-like micelles and bilayers are relatively easy to visualize due to their large extent in two dimensions, while spherical aggregates and micelles, especially less than 10 nm, are much harder to distinguish from the background, and should be examined with caution (see Figures 3, 4).

**Mass-Thickness Contrast from Freeze-fracture Replicas.** The three-dimensional contours of the fracture surface intuitively appear to give rise to the contrast variations in a freeze-fracture micrograph. However, the surface itself is not
Figure 3. Sequence of cryo-TEM images of a dilute dispersion of 7 nm diameter silica particles. The images are of the same area, but taken at different amounts of defocus, from +4 μm overfocus to -4 μm underfocus. The image taken at focus (F) is essentially featureless, consistent with the weak phase object approximation. However, distinct features emerge at higher defocus and coarsen with increasing defocus. The arrows at top and bottom show the contrast reversal common to phase contrast imaging. It is difficult in any of the images to unambiguously determine the location of the silica particles relative to the background graininess of the image. Spherical micelles and other small aggregates would be equally difficult to distinguish.
Figure 4. Cryo-TEM image of wormy vesicle phase of dimyristoylphosphatidylincholine and geraniol in water. In this image the bilayers are simple to see as they form the undulating, intertangled network of vesicles. P marks the holey polymer grid upon which the sample is suspended.

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Structure and Flow in Surfactant Solutions
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examined in the microscope, rather a metal shadowed replica of the surface. If the height (relative to some origin) of the fracture surface can be represented as a single valued function, f(r), the metal thickness, \( t_m(r) \), at that point in an unidirectionally shadowed replica, is given by (114):

\[
t_m(r) = d + f(r - a) - f(r),
\]

(24)

d is the thickness of metal deposited on a horizontal, flat surface, and a is the horizontal projection of the shadowing metal thickness in the direction of the evaporating metal beam. This expression is only meaningful if \( t_m(r) \geq 0 \); the metal film thickness can never be negative. (The regions where Equation 24 is negative correspond to the areas of the specimen where there is no metal film.) If the metal is deposited normal to the fracture surface, a is zero and no thickness variations are present in the metal film, and hence no contrast variations are developed in the image. This is the case for the carbon reinforcing film used to strengthen freeze-fracture replicas. In a typical experiment, the total metal thickness, \( \Delta \), deposited on an oscillating quartz crystal monitor normal to the shadowing direction, and \( \theta \), the shadow angle from the horizontal, are measured. In these terms:

\[
d = \Delta \sin \theta, \quad |a| = \Delta \cos \theta
\]

(25)

Fourier analysis of the thickness distribution is the most accurate method of determining the fracture surface contour, f(r) from a freeze-fracture electron micrograph. The Fourier transform of \( t_m(r) \) gives \( T_m(q) \):

\[
T_m(q) = d\delta(q) - [1 - \exp(-2\pi q \cdot a)] F(q)
\]

(26)

in which \( F(q) \) is the Fourier transform of the surface profile f(r). From Equation 26, \( T_m(q) \) is zero along the line \( q \cdot a = 0 \); in physical terms, there is no information about fracture surface contours along the direction perpendicular to the shadow direction (114). An optical diffractionogram of the freeze-fracture negative shows the line of zero information as a dark zone; this the most accurate determination of the shadowing direction. The fracture surface, f(r), can be determined from the inverse transform of:

\[
F(q) = \frac{d\delta(q) - T_m(q)}{[1 - \exp(-2\pi q \cdot a)]}
\]

(27)

for \( q \), \( q \cdot a \neq 0 \).

Although Equations 24–27 are exact, they do not provide much insight into the relationship between surface contours and image contrast. A more intuitive description can be obtained by a linear approximation to Equation 24. If the shadowing direction is along the x direction of a Cartesian coordinate system, r can be written as an ordered pair \((x,y)\) and f(r) is the z coordinate corresponding to \((x,y)\): \( z = f(x,y) \). With these assumptions, Equation 24 becomes:

\[
t_m(x,y) = \Delta \sin \theta + f((x - \Delta \cos \theta),y) - f(x,y)
\]

(28)

Equation 25 was used to replace d and a. If Equation 28 is expanded in a Taylor series,

\[
f((x - \Delta \cos \theta),y) = f(x,y) - \Delta \cos \theta \frac{\partial f}{\partial x} + O[(\Delta \cos \theta)^2 \frac{\partial^2 f}{\partial x^2}]
\]

(29)

If only linear terms are considered:

\[
t_m(x,y) = \Delta \sin \theta + (f(x,y) - \Delta \cos \theta \frac{\partial f}{\partial x}) - f(x,y)
\]

(30a)

\[
t_m(x,y) = \Delta (\sin \theta - \cos \theta \frac{\partial f}{\partial x})
\]

(30b)
In this approximation, the metal thickness profile is simply related to gradients in the fracture surface profile along the shadow directions. Once the metal thickness distribution is known along the shadow direction, Equation 30b can be integrated to give the fracture surface profile directly \((119, 120)\). The error in this approximation can be appreciable at areas where the slope of the fracture surface changes rapidly (where \(\partial^2 h/\partial x^2\) is large).

Image contrast for replicas depends on differential scattering of electrons from regions of the specimen with differing \(t_m(x, y)\). For a composite replica made up of \(n\) layers of different materials, Equation 8 becomes:

\[
\Psi_i = \Psi_i \exp \left( \sum_{i=1}^{n} -S_p \rho_i(x, y) \right) \tag{31}
\]

For electron image film, there exists an approximate relationship between the number of electrons striking the film, \(N\), and the optical density, \(D\):

\[
D = D_{\text{max}} C N \tag{32}
\]

\(D_{\text{max}}\) is the saturation density of the film and \(C\) is a constant that depends on the characteristics of the film and developer. Combining Equations 12 and 13 gives the relation between the density of the negative and the film thickness:

\[
D \approx D_{\text{max}} C \Psi_i \exp \left( \sum_{i=1}^{n} -S_p \rho_i(r) \right) \tag{33}
\]

At first glance, Equation 33 seems to have an inordinate number of parameters that are difficult to measure. However, if ratios of densities, rather than the absolute densities are considered, the result is considerably simplified. In an image of an area of the replica devoid of metal with only the carbon backing layer, the optical density of the negative is:

\[
D_c = D_{\text{max}} C \Psi_i \exp (-S_p \rho_c) \tag{34}
\]

At a point \(r\), the metal layer thickness is given by \(t_m(r)\) as given in Equation 24, in addition to the carbon layer thickness, \(t_c\), which is uniform over the entire replica. Taking the ratio of the density at the point \(r\) to the reference carbon film density gives:

\[
\frac{D_m(r)}{D_c} = \frac{D_{\text{max}} C \Psi_i \exp (-S_p \rho_c - S_p \rho_m(r))}{D_{\text{max}} C \Psi_i \exp (-S_p \rho_c)} = \exp (-S_p \rho_m(r)) \tag{35}
\]

and only a single parameter is required to relate the metal thickness to the optical density. Therefore, a single negative contains all the information necessary to determine the fracture surface profile. The carbon density, \(D_c\), is the maximum density recorded on the negative. This simple relationship of metal thickness to optical density, when combined with Equations 27 or 30b, show that there is a direct relationship between the fracture surface profile and the images recorded with the electron microscope. Absolute heights might be determined directly from the electron microscope images; however, recent advances in imaging freeze-fracture replicas with the scanning tunneling microscope allow for a much simpler and direct determination of the three-dimensional fracture profiles with better resolution \((123, 124)\).

**Conclusions**

The early disappointments of TEM investigations of microstructured fluids have undoubtedly limited the number of colloid and materials scientists using this very promising experimental technique. These early investigations were plagued by slow cooling rates that led to unacceptable distortions of fluid structure. With the major
advances in ultra-rapid freezing, such problems have now been eliminated. The benefits of direct visualization of fluid structure with near molecular resolution are only slowly being realized. As more investigators come to understand the potential of being able to study the real-space structure of complex dispersions, emulsions, gels, and solutions, the pace of progress will accelerate. Freeze-fracture electron and cryo-TEM microscopy have made it possible to see the three-dimensional structure and organization in a wide range of systems that have previously thwarted analysis; the future promises to be equally bright.

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