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Two new and rapid approaches for studying the phase properties of cosmetic lipids and oils

MARTIN CAFFREY and MARIA T. BYWATER, *Department of Chemistry, The Ohio State University, 120 W. 18th Avenue, Columbus, OH 43210.*

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Synopsis

Lipids and oils, integral components of cosmetic products, exist in a number of intermediate physical states, or mesomorphic phases, between the crystalline solid and isotropic liquid. The stability of these phases depends on temperature and composition, and each lipid's pattern of dependency is conveniently described in the form of an isobaric (constant pressure) temperature-composition phase diagram. Such diagrams are of great utility in guiding the choice of formulation ingredients for inclusion in cosmetic products and for obviating such manufacturing problems as caking and immiscibility. This paper describes two new and related methods of collecting mesomorphic phase information which are less time-consuming and more efficient than conventional techniques. By incorporating a range of conditions into each sample preparation—a temperature gradient in the first method and a lyotrope gradient in the second—and utilizing a new method of phase identification called time-resolved x-ray diffraction, the time required to collect phase information for a complete diagram is reduced to minutes. Temperature-composition phase diagrams constructed using these methods compare well with those constructed by conventional means.

INTRODUCTION

Lipids and oils are integral components of many cosmetic products, and an understanding of a particular lipid's properties is essential in designing products and efficient processing procedures. However, lipids and oils exhibit some unconventional properties which can make their behavior unpredictable.

Lipids can exist in a number of intermediate phase states, or mesomorphs, between the crystalline solid and the isotropic liquid that result from differences in the way the lipid and solvent (usually water) molecules arrange themselves in three-dimensional space. These molecular arrangements combine elements of order as found in crystals and disorder as found in liquids. As such, the phases constitute what might be considered a fourth state of matter: the so-called liquid crystalline state. Although most lipids display the same basic mesomorphic phases, each individual species exhibits its own pattern of dependency on temperature and composition. The same holds true for mixtures

of lipids. It is this complex dependence on temperature and composition which makes lipid behavior difficult to predict.

Lipid phase diagrams conveniently summarize these temperature-composition dependencies (see Figures 1,2,3). With temperature on the ordinate (y-axis) and composition on the abscissa (x-axis), the diagrams indicate the phases that exist over the entire range

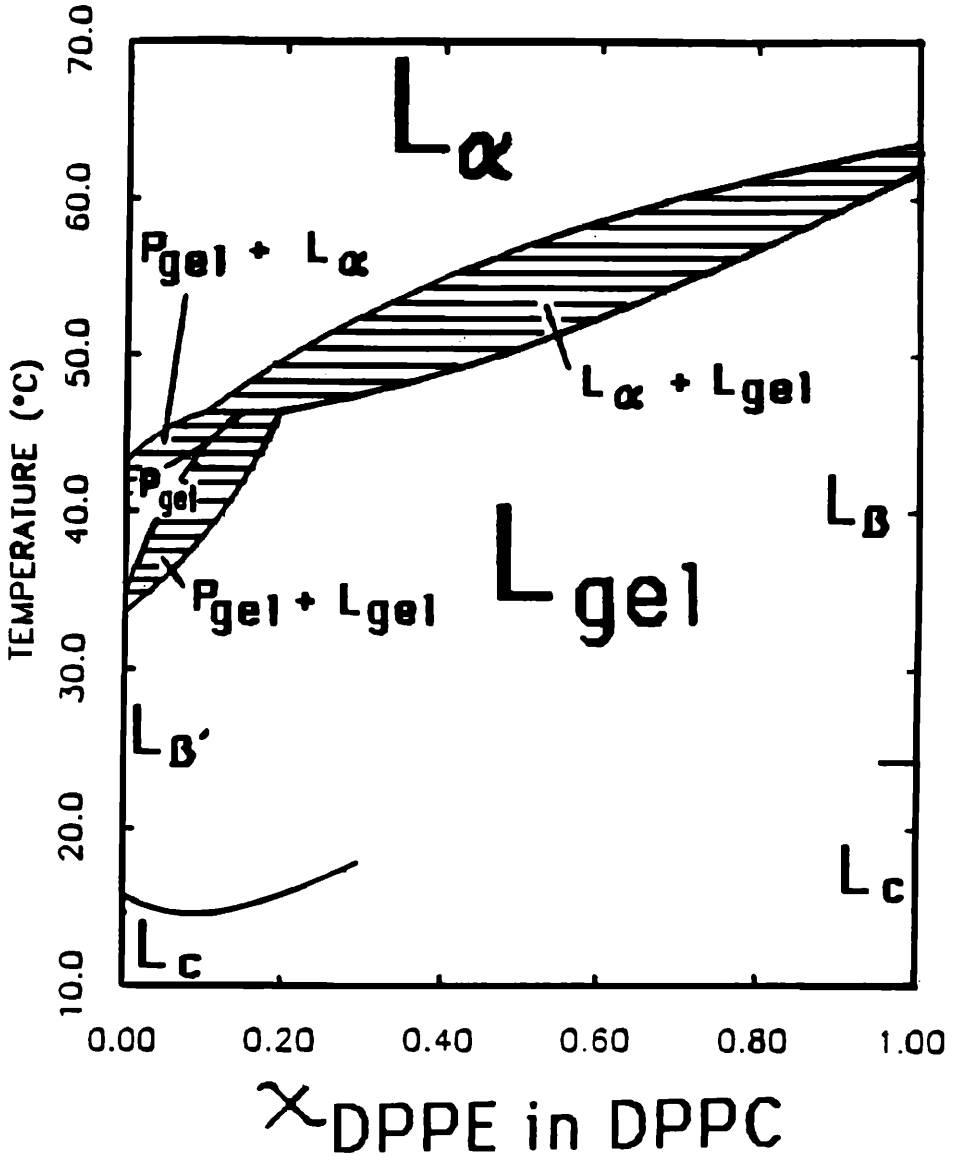

Figure 1. Temperature-composition isobaric phase diagram for the fully hydrated dipalmitoylphosphatidylcholine/dipalmitoylphosphatidylethanolamine system constructed using the temperature gradient method. The notation used is that of Luzzati (8) and is as follows: L_c, lamellar crystalline (also referred to as the subgel phase); $L_{\beta'}$, lamellar gel phase with hydrocarbon chains tilted with respect to the bilayer normal; P_{gel}, ripple phase; L_α, lamellar liquid crystal phase.

of conditions. Vertical lines in the diagram, which show the phases that exist for a sample of particular composition over a range of temperature, are referred to as isopleths. Horizontal lines, which show the phases that exist for a range of compositions at a particular temperature, are referred to as isotherms.

In the traditional approach to collecting lipid mesomorphic phase data, a series of samples encompassing the entire range of composition to be analyzed are raised in a step-wise manner to progressively higher temperatures at each of which the phase is identified. This is referred to as the equilibrium, isoplethal method. It is isoplethal because it involves moving up isopleths in the phase diagram and is an equilibrium method because samples of fixed composition are equilibrated at discrete temperatures so that they represent particular combinations of temperature and composition—single points in a phase diagram.

The equilibrium, isoplethal method has a number of drawbacks (1). First of all, it is time-consuming. A number of samples must be raised to progressively higher temperatures and equilibrated for a time at each temperature before being separately analyzed for phase type. Secondly, vertical boundaries are difficult to isolate. And thirdly, because only a limited number of samples representing isolated points in the diagram are

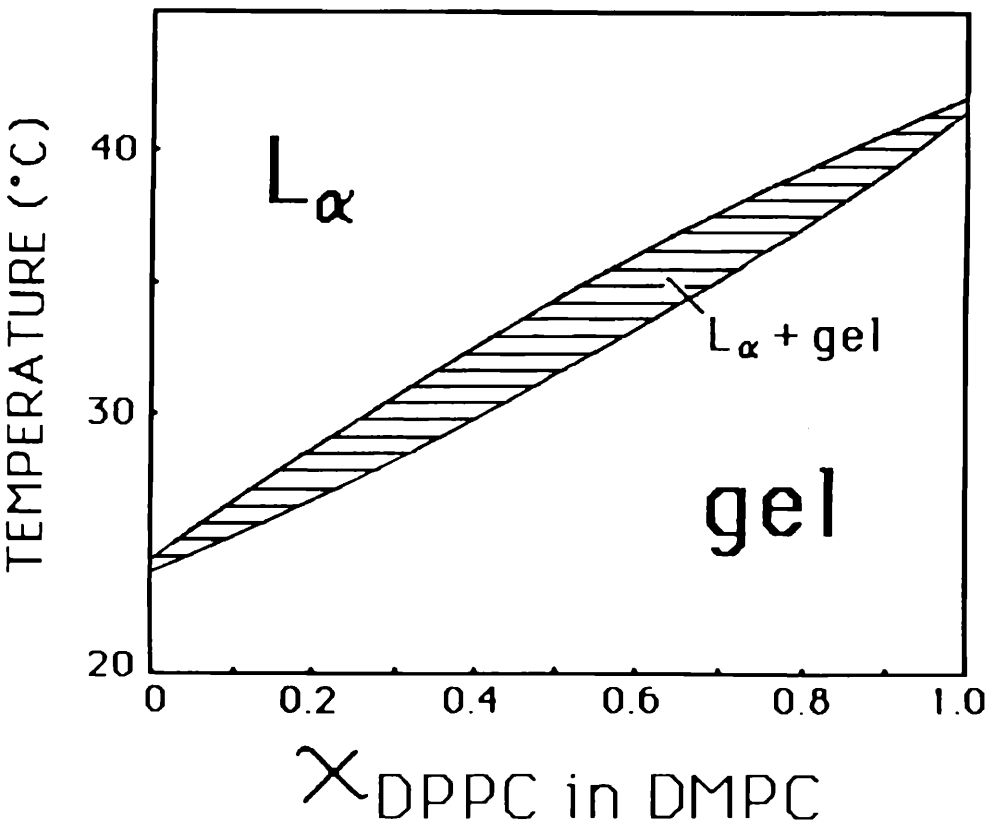

Figure 2. Temperature-composition phase diagram for the fully hydrated dipalmitoylphosphatidylcholine/dimyristoylphosphatidylcholine system constructed using the temperature gradient method. See legend to Figure 1 for notation.

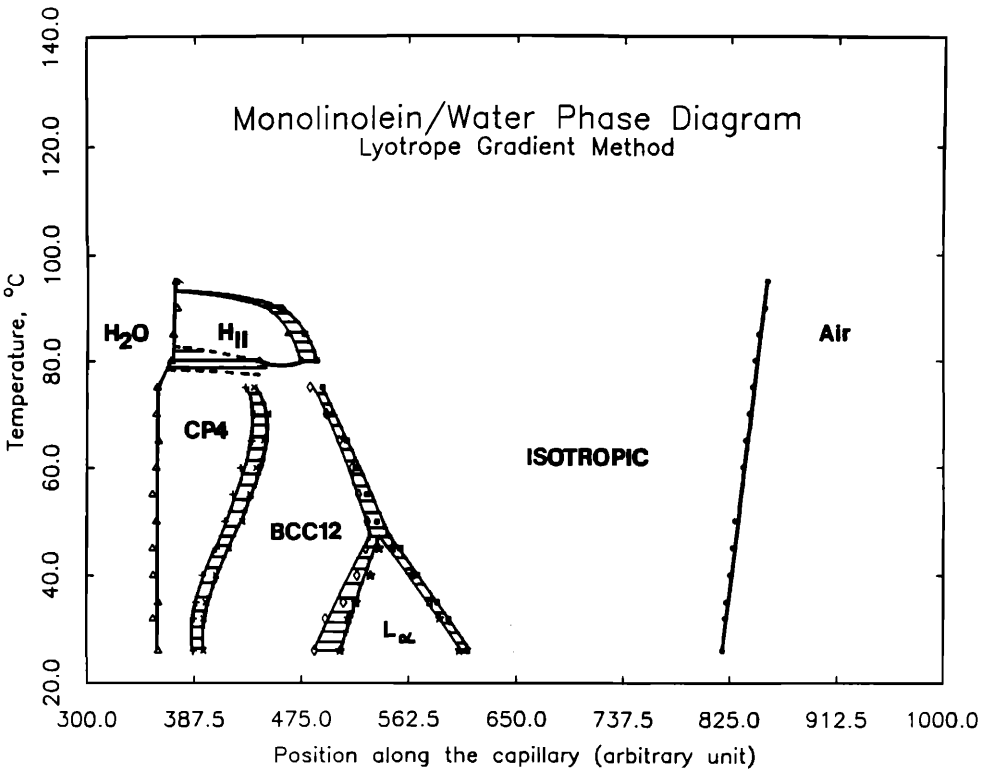

Figure 3. Temperature-composition isobaric phase diagram for the monolinolein/water system constructed using the lyotrope gradient method. See legend to Figure 1 for notation, H_{II}, inverted hexagonal phase; CP4, cubic, primitive (space group 4, Pn3m, Pn3); BCC12, cubic, body-centered (space group 12, Ia3d); isotropic, fluid isotropic or mel.

analyzed it is possible that phases which exist over a small range of conditions will go undetected.

DISCUSSION

THE THEORY BEHIND THE METHODS

To overcome the limitations of conventional equilibrium techniques, two new approaches have been developed to expedite lipid phase data acquisition (2,3). The underlying principle in both methods is that a continuous range of conditions, a gradient of either temperature or composition, is incorporated into each sample. The samples are placed in x-ray capillary tubes, and the gradients are established along their lengths. Thus, each sample represents not a single point in a phase diagram but an entire line.

In the first approach, a temperature gradient is imposed on samples of constant composition so that each sample represents a vertical line, or isopleth, in the corresponding phase diagram. In the second approach, the sample contains a solvent gradient and is raised to progressively higher temperatures. At each temperature the sample represents

a horizontal line, or isotherm, in the phase diagram. These techniques are referred to, respectively, as the temperature gradient and lyotrope gradient methods.

The sample preparations create the potential for more efficient phase data collection, but making the most of this potential requires a means of rapid phase identification and characterization. In this regard, x-ray diffraction is a particularly powerful technique because it provides *direct* structural information. Since each molecular arrangement characteristic of a given mesomorphic phase generates a unique diffraction pattern, diffraction data provide the means to positively identify phases in single phase regions, to determine the proportion of each phase type in multiphase regions, and to structurally characterize each phase. However, the low photon flux of conventional x-ray sources necessitates long (hours to days) exposure times to produce satisfactory diffraction patterns.

This time limitation can be overcome with synchrotron x-radiation when used in conjunction with a suitable two-dimensional live-time x-ray imaging device. This combination dramatically decreases the time required to record a diffraction pattern. To analyze samples in both the temperature and lyotrope gradient methods, capillaries are passed through a synchrotron-derived x-ray beam while a video camera records the image-intensified diffraction pattern in live-time continuously along the length of the sample in a process called time-resolved x-ray diffraction (TRXRD) (4–6). A schematic illustration of the TRXRD apparatus and the associated equipment used in making the diffraction measurements is shown in Figure 4. Synchrotron-derived monochromatic x-radiation enters the experimental hutch and passes through a delimiting collimator. A translation stage positioned perpendicular to the x-ray beam moves the sample capillary tube through the beam. The diffraction pattern generated at each point along the sample is amplified in the image intensifier tube—basically a modified night scope—which enhances the pattern so that it can be recorded on video tape using a video

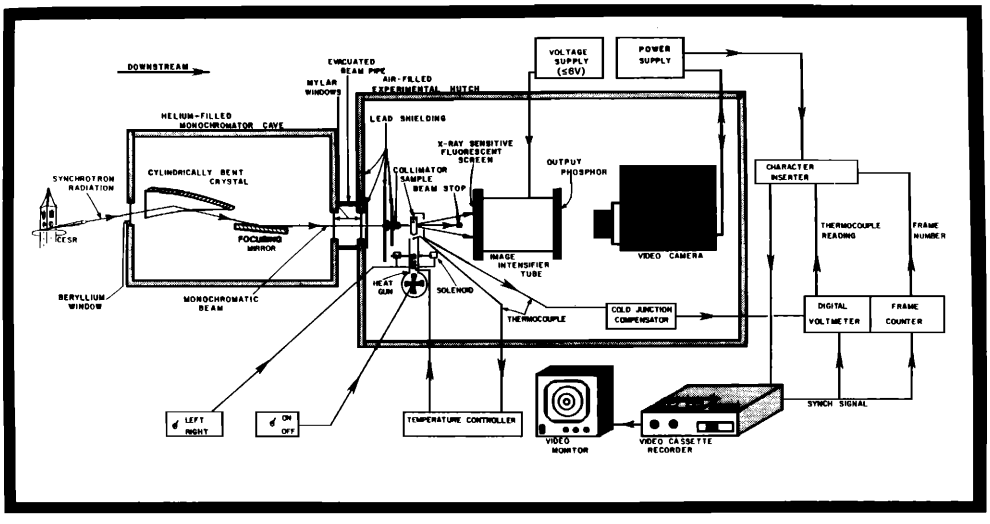

Figure 4. Schematic of the experimental arrangement at the Cornell High Energy Synchrotron Source for making time-resolved x-ray diffraction measurements.

camera. The output of the camera feeds into a video cassette recorder and a monitor. With this system it is possible to record diffraction information in live-time continuously along the length of the sample capillary.

The diffraction patterns indicate the phases present in the sample and the relative amount of each phase in multiphase regions. By recording capillary position along with the changing diffraction pattern on video tape, phase boundaries, signified by changes in the diffraction pattern, are assigned to precise points in the sample. In the temperatures gradient method, each point corresponds to a specified temperature, and in the lyotrope gradient method, to a particular lyotrope concentration. To collect phase information for the entire diagram, the procedure is repeated with samples representing different lines—either isopleths or isotherms.

THE TEMPERATURE GRADIENT METHOD

In this method, samples represent isopleths in the corresponding phase diagram. To collect data for an entire diagram, a number of sample capillaries encompassing the range of compositions to be analyzed are subjected to the same temperature gradient and analyzed by TRXRD.

The temperature gradient is supported on a metal rod. A regulatable heater at one end of the rod and a circulating water bath at the other end create a linear gradient which is monitored by using thermocouples positioned at intervals along its length. Sample capillaries are placed lengthwise around the perimeter of the rod with a thermal compound used to promote good thermal contact (Figure 5) and equilibrated so that the gradient is communicated into the samples.

The gradient rod, mounted on a goniometer and attached to the translation stage of the TRXRD apparatus, is rotated to bring the first sample in line with the x-ray beam. As the translation moves the capillary through the x-ray beam, the video system records the diffraction pattern continuously along its length. A single scan takes about 20–30 sec to complete. After each scan, the rod is rotated like a Colt revolver to bring the next

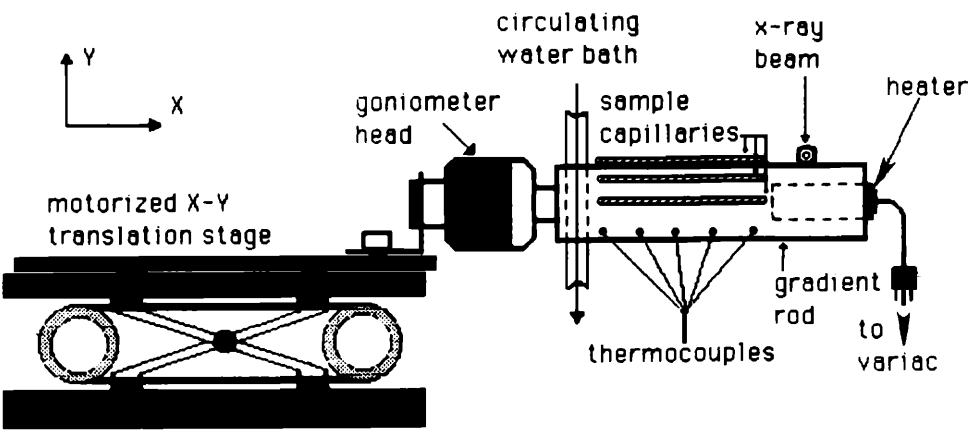

Figure 5. Schematic of the experimental arrangement used in making live-time x-ray diffraction measurements with the temperature gradient apparatus.

capillary in line with the x-ray beam, and the process is repeated. The entire process of data collection requires approximately 30 min.

A linear potentiometer attached to the translation stage generates positional information which is recorded on the videotape along with the diffraction information. This positional information is later correlated with temperature so that the diffraction pattern, and therefore the phases type associated with each point along the length of the capillary, is recorded as a function of position and thus temperature.

The positions of the various phase boundaries along each isopleth are plotted against temperature and composition from which the corresponding phase diagram is obtained. Phase diagrams constructed for fully hydrated mixtures of dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylethanolamine (DPPE) (Figure 1) and mixtures of DPPC and dimyristoylphosphatidylcholine (DMPC) (Figure 2) using the temperature gradient method correspond closely to diagrams obtained for these same systems using conventional so-called equilibrium methods, although the phase coexistence regions determined with the temperature gradient method are generally narrower.

In the present experimental set-up, a small temperature gradient exists across the width of the capillary because of the difference in temperature between the gradient rod surface and the surrounding air. This gradient may cause a widening of phase boundaries, but in the systems studied thus far, the effect was minimal. Encasing the gradient apparatus in an evacuated chamber will eliminate this problem.

THE LYOTROPE GRADIENT METHOD

Samples prepared for use with this method represent isotherms in the corresponding temperature-composition phase diagram. In contrast to the temperature gradient method in which a sample of constant composition varies in temperature along its length, the lipid sample in this method contains a concentration gradient along its length and is scanned while maintained at a constant temperature. In addition, this method requires but a single sample. Analyzing the sample capillary at progressively higher temperatures results in a videotape record of the entire body of phase information, each scan representing a single isotherm in the phase diagram.

To prepare a sample, the lipid or liquid crystal of interest, preferably in liquid form, and the lyotrope are brought into contact in an x-ray capillary tube, and the capillary is incubated until mutual diffusion of lyotrope and lipid results in a gradient of sufficient length. The sample then has an increasing lyotrope concentration along the length of the capillary from pure lipid at one end to "pure" solvent at the other (Figure 6). To collect phase information for the entire diagram, this single capillary is subjected to TRXRD analysis at progressively higher temperatures using a temperature-regulated forced-air device for temperature control. Data collection by this method is usually complete in one hour.

As in the temperature gradient method, a linear potentiometer attached to the translation stage generates positional information which is recorded simultaneously with the changing diffraction pattern on video tape. However, in this case, position correlates with composition rather than temperature. Because a method has not yet been developed to measure absolute lyotrope concentration nondestructively and noninvasively

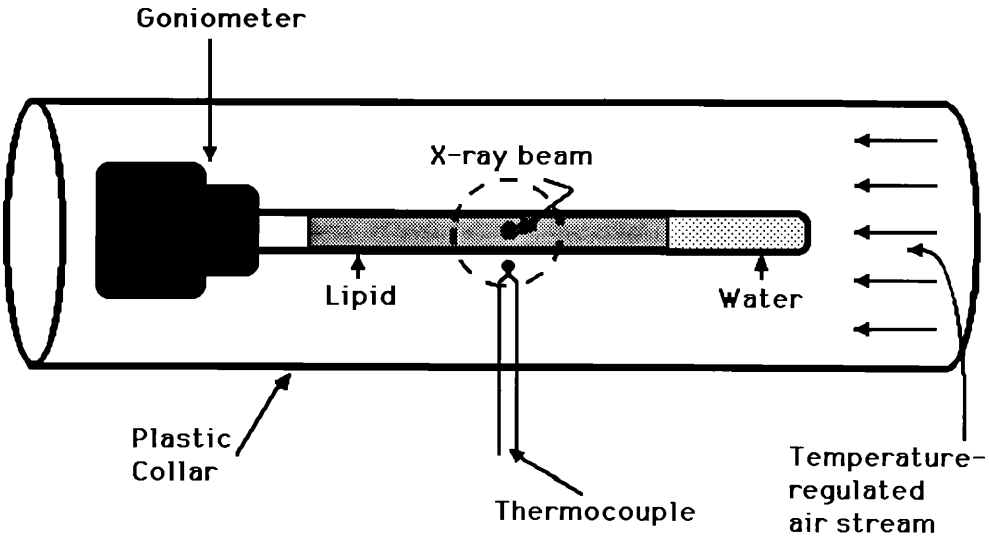

Figure 6. Schematic of the experimental arrangement used in making time-resolved x-ray diffraction measurements with the lyotrope gradient apparatus.

along the length of the gradient, phases are reported in terms of position in the capillary. Despite this shortcoming, diagrams created with this method possess the same general form as those produced with conventional, so-called equilibrium methods. We show the phase diagram for monolinolein in water as an example (Figure 3).

EVALUATION OF METHODS

The two new methods just described offer several advantages over conventional equilibrium methods:

- They facilitate rapid data collection by combining sample preparations that incorporate a range of conditions with a method of rapid phase identification and structural characterization.
- They are efficient. Because data collection is continuous along a temperature or composition gradient, the possibility that phases which exist in a narrow temperature or composition range will go undetected is eliminated.
- The gradients are flexible. The temperature range in the first method can be expanded or contracted by simply and rapidly adjusting the limiting temperatures on either end of the gradient rod. In the lyotrope gradient method, narrow phase fields can be extended by incubating the sample for a longer period of time or by adjusting the amount of lyotrope used.
- They utilize x-ray diffraction. X-ray diffraction allows *direct* qualitative and quantitative phase characterization—even in multiphase regions—and no potentially perturbing additives or molecular labels are needed. Although the high photon flux of synchrotron radiation is potentially damaging to the sample (7), particular parts need only be exposed to the beam for a short period of time and, as a result, radiation damage is not a problem with this method.

- They have general applicability. Both methods apply to a wide range of materials including liquid crystals, polymers, and nonaqueous solvents.

There are some limitations to these methods which must be addressed:

- They require access to a high-intensity x-radiation source of the type available at a synchrotron radiation facility.
- They require a large amount of sample. However, this disadvantage is offset by the fact that both methods are nondestructive and the samples can be recovered for reuse. Furthermore, the lyotrope gradient method in its present configuration requires only a single sample representing about 40 mg lipid to construct a complete phase diagram.
- Lyotrope gradient samples ideally should be fluid.
- Since both methods are based upon x-ray diffraction, difficulties are encountered in distinguishing liquid phases.
- Phase diagrams are meant to indicate phase behavior at equilibrium. In these methods, a non-equilibrium or steady-state approach is used to obtain equilibrium information. Although this theoretically is a point of contention, experimental evidence shows that phase diagrams created with these methods agree with and extend the information contained in phase diagrams produced with conventional equilibrium methods.

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