A lyotrope gradient method for liquid crystal temperature–composition–mesomorph diagram construction using time-resolved x-ray diffraction

Martin Caffrey
Department of Chemistry, The Ohio State University, Columbus, Ohio 43210

ABSTRACT A new method for rapidly constructing isobaric temperature–composition–mesomorph (T-C) diagrams is described. The method involves establishing a lyotrope concentration gradient in a liquid crystal lengthwise in an x-ray capillary tube. At a fixed temperature such a sample corresponds to an isotherm in the corresponding isobaric T-C diagram. The concentration gradient is conveniently established by bringing the two components into contact in the capillary and allowing limited diffusion of one component into the other. Phase boundaries are located and phases are identified and structurally characterized continuously along the length of the capillary using time-resolved x-ray diffraction. Repeating the measurement on the same sample at a series of temperatures in the range of interest completes that T-C diagram. The method has been used to construct the T-C diagram for detergent/water and lipid/water binary and ternary systems in the 20–120°C range. They agree well with and extend the results obtained by conventional methods.

INTRODUCTION

A knowledge of the conditions governing lyotropic mesomorphism and phase stability requires an understanding of liquid crystal–liquid crystal and lyotrope (solvent)–liquid crystal interactions and how these depend on the underlying chemistry. Such information is conveniently and concisely summarized in an isobaric temperature–composition phase diagram. Of the many physical techniques available for phase diagram construction, x-ray diffraction is particularly powerful because it provides direct phase identification both in single- and multiple-phase regions and facilitates the location of phase boundaries. However, data acquisition using conventional x-ray sources is laborious and time-consuming because of low photon flux. The advent of synchrotron x-radiation with its extreme brightness enables phase identification and structural characterization at unprecedented rates.

In seeking to take advantage of this rapid data output, a new method for temperature–composition–mesomorph (T-C) diagram construction was developed. The method utilizes time-resolved x-ray diffraction (TRXRD [1–6]) measurements on liquid crystal samples contained in capillaries and upon which a lyotrope concentration gradient has been imposed. The essential features of the method are illustrated in Fig. 1 for a binary lipid/water system. The lyotrope concentration gradient is established by bringing the two pure components into contact in the capillary and allowing limited diffusion of one component into the other until a gradient of sufficient length forms. At a fixed temperature, such a sample represents an isotherm in the corresponding T-C diagram with the different phases and phase boundaries localized at different points along the length of the capillary. Recording the two-dimensional diffraction pattern in live-time while the capillary is moved through the x-ray beam enables phase boundaries to be localized and individual phases to be identified on the basis of the pattern itself. Repeating this measurement at a series of fixed temperatures in the range of interest completes the T-C diagram. Because a continuous lyotrope concentration gradient is employed, there is no need for multiple samples of differing concentration, thereby greatly reducing data collection time. Furthermore, data to complete the phase diagram can be collected using just one sample. Since two-dimensional diffraction patterns are recorded, analysis problems that might arise due to preferential sample orientation are obviated.

The lyotropic gradient method was developed for use with biologically important lipids with water or aqueous salt solutions as the lyotrope. However, since the majority of lipids exhibit liquid crystalline mesomorphism and are lyotropic for the most part, the method finds general applicability in the broad domain of lyotropic liquid crystals. This encompasses such diverse materials as polymeric liquid crystals, detergents, fats, and oils. Naturally, the method can be used with a variety of lyotropes, although water (^1H_2O and ^2H_2O) was used exclusively in this study.

MATERIALS AND METHODS

Materials

Monoacylglycerides were purchased from Nu-Chek-Prep (Elysian, MN). The detergent tetraoxyethyleneglycol-decyl 2,3-dihydroxypropyl

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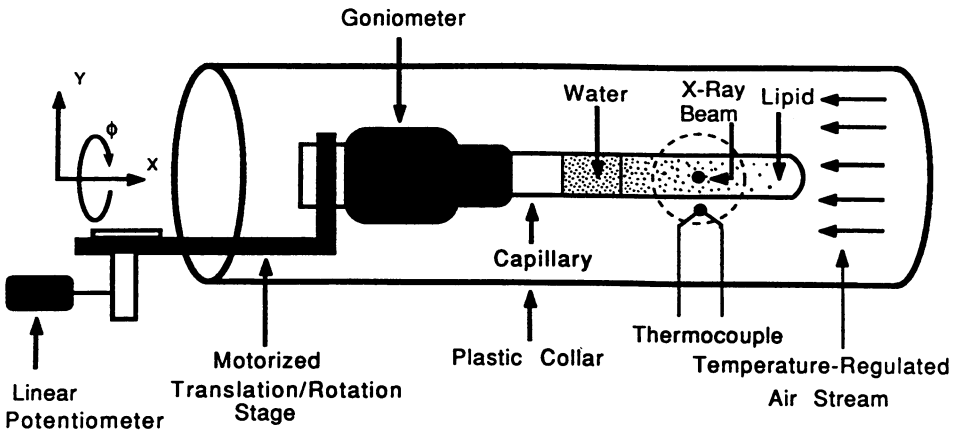


FIGURE 1 Schematic diagram of the experimental arrangement for time-resolved x-ray diffraction from lyotrope gradient samples. See text for details.

ether ($C_{10}E_4G$) was a gift from R. G. Laughlin (Procter & Gamble Co., Cincinnati, OH). Lipids and detergent are reported to be >99% pure and were used without further purification. Water was purified by using a Milli-Q (Millipore Corp., Bedford, MA) system.

Sample preparation

Melted liquid crystal samples and water were sequentially centrifuged into 1-mm diam quartz x-ray capillaries (Charles Supper Co., Natick, MA) by using low speed on a clinical centrifuge (model 1528E, International Equipment Co., Needham Heights, MA). Capillaries were then flame- and epoxy-sealed (Hardman Inc., Belleville, NJ). Gradients were established by incubating the samples above the melting point of the pure liquid crystal or lipid mixture in a temperature-controlled oven for several days. The actual incubation temperatures used are indicated in the legend to Fig. 2.

X-Ray diffraction

The time-resolved x-ray diffraction method and apparatus has been previously described (5). Briefly, focused, monochromatic (1.56 Å) radiation on the A1 line at the Cornell High Energy Synchrotron Source was used as the x-ray source with the storage ring operating at 5–5.5 GeV and 20–50 mA of electron beam current. This provided approximately 2–5 × 10^{10} photon/s through a 0.3-mm diam collimator. Essential components of the time-resolved system include a homemade low-angle x-ray diffraction camera, a two-dimensional live-time x-ray imaging device, a character generator interfaced to a digital volt meter (thermometer, linear potentiometer), an electronic clock and a video camera, recorder, and monitor (5). Quantitative diffracted intensity and position information was obtained by digital processing of the live-time diffraction patterns recorded on video tape as previously described (5). Calibration of the video system was effected by recording standard diffraction patterns using TRXRD and statically on x-ray sensitive film (Kodak DEF5). The x-ray capillary containing the lyotrope gradient sample was positioned in the x-ray beam by using a goniometer mount as depicted in Fig. 1. Computer-controlled horizontal (X), vertical (Y), and rotational (ϕ) adjustment of the sample was provided by a Huber rotation state mounted on an X-Y translation device. Horizontal position was encoded on video tape using a linear potentiometer. Translation speeds of 0.1–1 mm/s were used throughout these measurements. Temperature was controlled by a forced-air crystal heating/cooling apparatus (FTS Systems, Inc., Stone Ridge, NY) and was measured by using a thermocouple positioned next to the intersection of the x-ray beam and the capillary. Damage during the course of a typical measurement was examined for in the case of the monoolein system using thin-layer chromatography. No significant lipid breakdown was observed. It is expected that radiation damage which is a problem in many lipid systems (7) is minimized in this case because the sample is continuously translated through the x-ray beam during measurement. In a single traverse at a translation speed of 0.3 mm/s total exposure at any point in the sample amounts to 1 s using a 0.3 mm collimator.

RESULTS AND DISCUSSION

To demonstrate the method, isobaric T-C diagrams for a detergent/water and a number of neutral lipid/water binary and ternary systems were constructed using the new lyotrope gradient technique. The lipids used include the following monoacylglycerides: monoolein (C18:1c9), monoelaidin (C18:1t9), monolinolein (C18:2c9,12), monolinolenin (C18:3c9,12,15), and monoerucin (C22:1c13). The detergent is tetraoxyethylene glycol decyl 2,3-dihydroxypropyl ether ($C_{10}E_4G$). The T-C diagrams are quite complex containing micellar, fluid isotropic (FI), normal or reversed hexagonal, lamellar/smectic liquid crystalline (L_α), and a variety of cubic-phase fields. Where possible the data so obtained compared favorably and extended that available in the literature (8–12) with monoerucin as a notable exception. In this latter case Lutton (12) had demonstrated the presence of a FI and a lamellar phase field. In the present study extensive hexagonal and cubic phases, in addition to the lamellar and FI phase, were consistently observed. The origin of this disparate behavior is not immediately obvious.

The $C_{10}E_4G$/water T-C diagram was constructed in the temperature range 25–70°C (Fig. 2 H). The sequence of

phases observed at low temperature is FI, L_α, cubic-body centered (space group 12, Ia3d [BCC12]), normal hexagonal, and micellar in the direction of increasing water concentration. At higher temperatures, the BCC12 followed by the hexagonal and finally the L_α phases are lost. The open symbols in this figure demonstrate the repeatability of the measurement after an initial heating of the sample to 69°C. The data for constructing this diagram were collected, analyzed, and plotted in a total of 90 min. It is important to emphasize that, although data were collected continuously along each isotherm in all of these figures, for the sake of clarity only the position of the boundaries are plotted.

In a lyotrope gradient system such as this the miscibility gap or coexistence region disappears. Coexistence in a binary T-C phase diagram represents a region where the coexisting phases are in equilibrium. Thus, the chemical potential of the common components is the same in the two phases, and the region of coexistence along an isotherm in a T-C phase diagram collapses to a single point along the corresponding lyotrope gradient isotherm. The plots in Fig. 2 indicate regions of coexistence because this is what was observed experimentally. However, since an x-ray beam with a diameter of 0.3 mm was used in these measurements, coexistence widths of this order could result from finite beam size effects.

The low-angle d-spacings of the various phases along the 26°C isotherm in the monolinolein/H_2O gradient are shown in Fig 2 I. While discrete locations on this isotherm were chosen for data acquisition, again it is emphasized that data were recorded and are available continuously along the length of the gradient. Phase boundaries are apparent as defined breaks or changes in slope in the d-spacing curves. Within single phase regions, d-spacing consistently rises toward the high water concentration end of the gradient as expected of a lyotropic liquid crystal. Interestingly, the scattering angle of the diffuse, low-angle FI peak decreases in this direction along the gradient. At phase boundaries, the relationship between unit cell dimensions of "coexisting" phases can be ascertained. Among other things this plot serves to illustrate the enormous information density of the lyotrope gradient method.

As noted above the lyotrope concentration gradient is established by bringing the two components into contact in the capillary and allowing limited diffusion of one into the other until a gradient of sufficient length is established. Since the various phases that develop at a fixed temperature depend on water concentration as dictated by the phase diagram, the movement of water into the lipid can be conveniently tracked using the TRXRD method. The progress curve for the hydration process occurring in monoolein was so determined by recording the diffraction pattern along the length of the capillary at various times after initial contact. The data in Fig. 3 demonstrate that at time zero and 28°C, the lipid is entirely FI (undercooled). As time elapses and water migrates into the lipid the relative amount of FI decreases accompanied by the emergence and growth of the L_α, BCC12, and cubic-primitive (space group 4, Pn3m, Pn3[CP4]) phases. Since there is an excess of water available, the sample would eventually convert entirely to the CP4 phase (see Fig. 2 *A*). What immediately emerges from the progress curve in Fig. 3 is that passive equilibration of the monoolein cubic phases with water is extraordinarily slow. As will be described elsewhere, a computer algorithm has been developed to determine the mutual diffusion coefficient for water in this contiguous series of monoolein mesomorphs. The algorithm searches for diffusion coefficients such that the position in time of the calculated and experimentally determined boundaries coincide. The diffusion coefficients are used in defining optimum conditions for the preparation of specific mesomorphs and phase sequences and provide insights into mesomorphic phase transition mechanisms and kinetics.

The present lyotrope gradient method has a number of optical counterparts wherein mesomophic data are collected using either polarized light microscopy (PLM [13, 14]) or transmission interferometry (TI [10]). In all cases, the means for establishing the lyotrope gradient relates back to the so-called penetration or contact technique (13). This method involves flooding with water or a solution a dry specimen held between a microscope slide and a coverslip and following the lyotrope diffusion at a fixed temperature by PLM or TI. The various mesomorphs form and are recognized as concentric rings of defined textures or refractive index (and, occasionally, relative viscosity) around the specimen with the gradient extending from low to high lyotrope concentration in the direction away from the center of the specimen. In this way, the sequence of mesomorphs along the lyotrope gradient is determined at a fixed temperature. In an extension of the contact method Hakemi et al. (14) determined the diffusion coefficients of liquid crystal phase boundaries in surfactant solutions by following the space-time dependence of phase boundary lines.

The advantages of the new lyotrope gradient method of T-C diagram construction are many and varied. To begin with, it is an intuitively simple method wherein the sample, at a fixed temperature, corresponds to an isotherm in the corresponding phase diagram. The continuous nature of the method essentially eliminates the possibility of missing phase fields that exist over narrow ranges of lyotrope concentration. The manner in which the gradient is established in the course of these measurements lends itself nicely to a determination of lyotrope transport properties. Furthermore, the method is applicable to a wide range of lyotropic liquid crystalline materi-

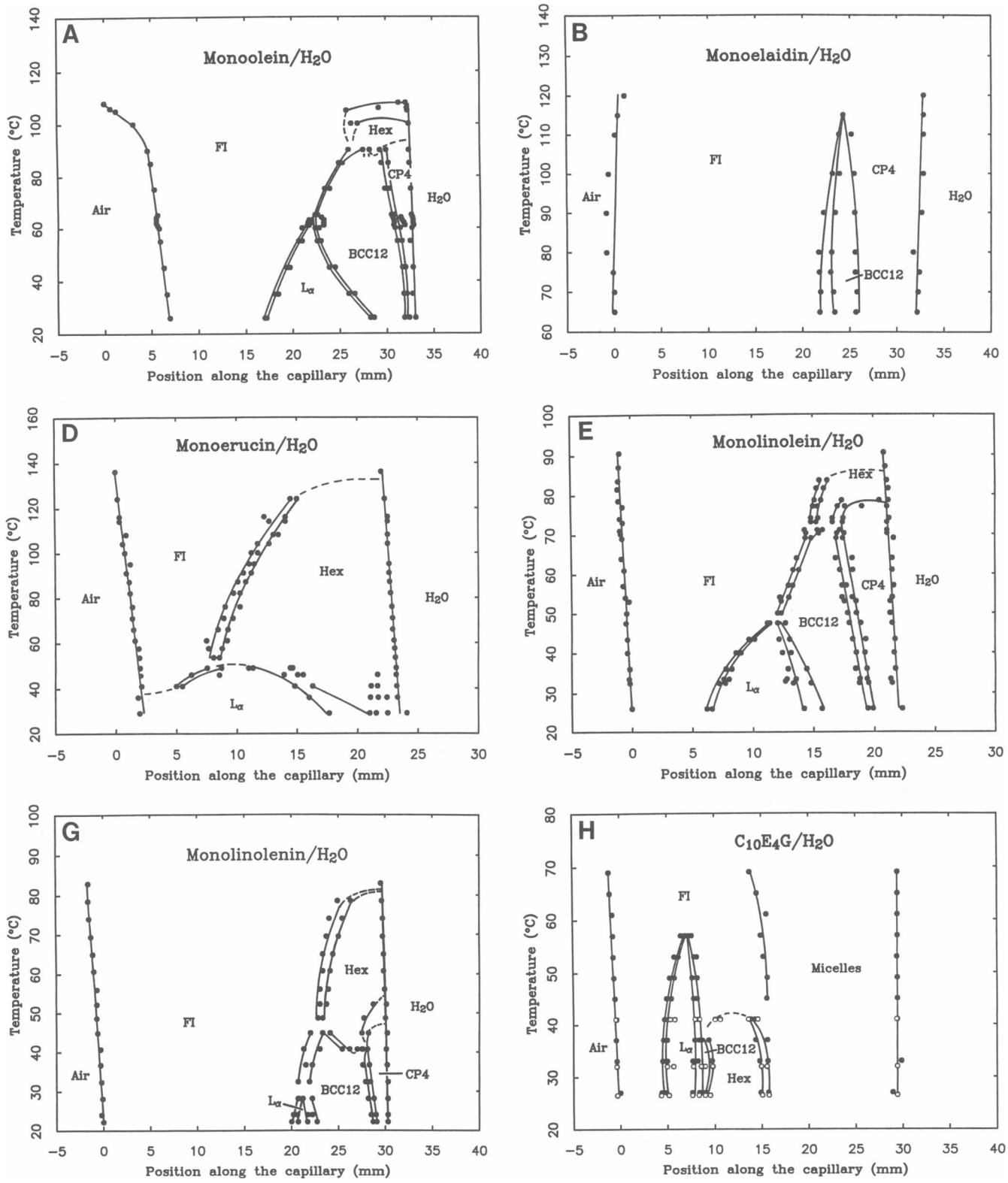
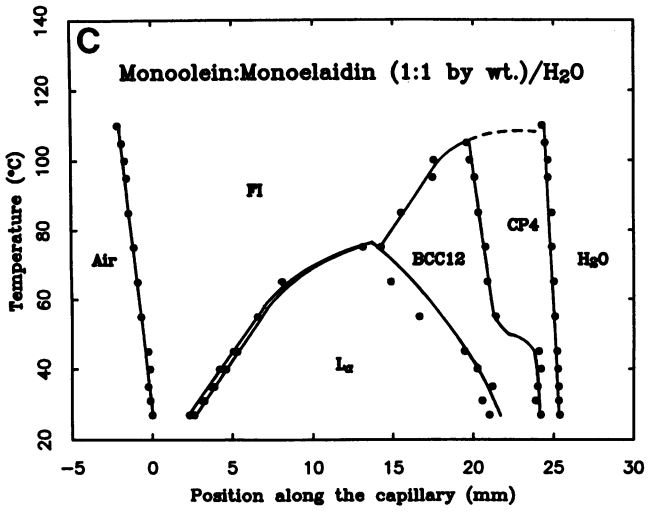
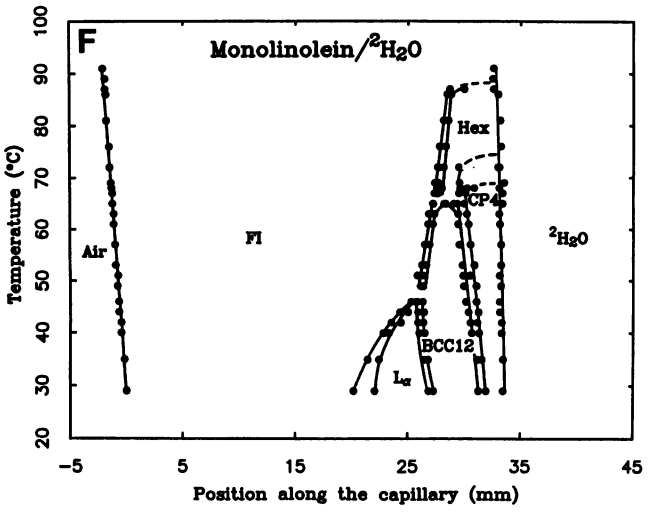
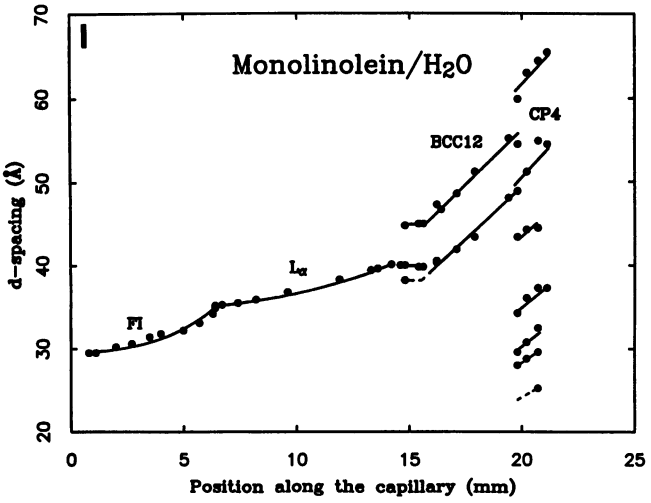


FIGURE 2 T-C diagrams for (*A*) monoolein/water, (*B*) monoelaidin/water, (*C*) monoelaidin:monoolein (1:1 by weight)/water, (*D*) monoerucin/water, (*E*) monolinolein/water, (*F*) monolinolein/deuterated water, (*G*) monolinolenin/water, and (*H*) $C_{10}E_4G$/water constructed using the lyotrope gradient method. Phases were identified and onset and completion of phase transitions were determined by visual inspection of the low-angle diffraction data on a video monitor in combination with static recordings on x-ray film. It is emphasized that for each isotherm phase determination has been made continuously along the length of the gradient and that for the sake of clarity only phase boundaries are shown. Unlabeled regions indicate that phase coexistence was observed. Shown in (I) are the d-spacings of the various low-angle reflections (L_α[001]; BCC12[211], [220]; CP4[110], [111], [200], [211], [220], [321]) recorded in live-time along the 26°C isotherm of the monolinolein/water system (*E*), illustrating the enormous information density of this method. The incubation temperatures used for establishing lyotrope gradients are as follows: 20–25°C (*A*, with monoolein in the undercooled state), 60–65°C (*B*, *C*), 55°C (*D*), and 20–25°C (*E–H*).







als. The fact that TRXRD is used to provide phase information offers many additional advantages such as (*a*) direct phase identification and quantitation in single and multiphase regions, (*b*) phase boundary location, (*c*) structural characterization in the various phases along a given isotherm continuously as a function of lyotrope concentration (Fig 2 *I*), (*d*) elimination of artifacts due to nonrandom sample orientation, (*e*) elimination of the need for additives or labels, (*f*) the features that it is nondestructive and the sample can be recovered for reuse, and (*g*) extreme rapidity of data collection.

On the negative side, the lyotrope gradient method is dependent on having access to items of high technology, not the least of which are a synchrotron x-ray source, a two-dimensional x-ray imaging device, video equipment, and an image processor. There is no doubt that the method could be used with conventional x-ray sources and detectors especially in the case of the more slowly diffusing systems. However, the time required to collect data of sufficient quality increases by at least two orders of magnitude, and instead of being continuous, measurements, of necessity, would be stepped along the length of the gradient. Since the samples are up to 3 cm long, it is necessary to have uniform temperature control along the length of the capillary. Concentration gradients are most easily established with a fluid liquid crystal. The method is really an approach-to-equilibrium rather than a true equilibrium method. The empirical observation is that it works. T-C diagrams obtained by this method are in excellent agreement with phase diagrams obtained by other so-called equilibrium methods. The fact that lyotrope diffusion is sufficiently slow relative to data acquisition time provides significant latitude in this regard. A

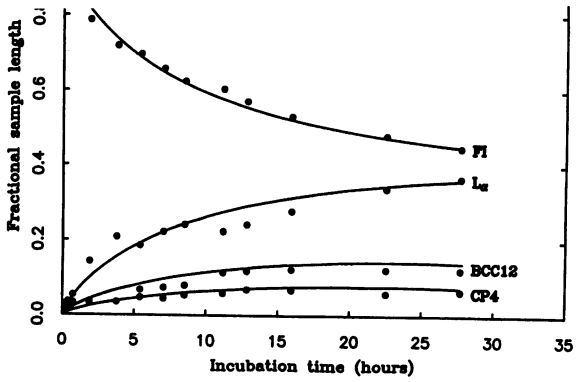


FIGURE 3 Fractional mesomorphic phase length along the lyotrope gradient as a function of incubation time at 28°C after initial contact of water (in excess) and monoolein. Total length of lipid in the 1-mm diam capillary was 15–16 mm. Sample preparation and experimental conditions are described under Materials and Methods.

major shortcoming of the method is that lyotrope concentration along the gradient is not known and must be established by independent means. Ideally, such a measure should be made simultaneously with the collection of diffraction data. To this end, infrared and Raman micro techniques are being investigated. As a result of this shortcoming, the T-C plots in their present form must not be referred to as phase diagrams in the conventional sense. Finally, since the method relies on x-ray diffraction, liquid–liquid phase immiscibility regions are resolved with difficulty.

In summary, a new approach-to-equilibrium method is described that combines a lyotrope gradient and TRXRD for isobaric T-C diagram construction at unprecedented rates. The method has been used to construct T-C diagrams for a number of water-containing binary and ternary liquid crystal systems that agree with and extend data obtained by other methods. Advantages of the new method include rapid data acquisition rates, direct phase identification, quantitation, and structural characterization continuously as a function of lyotrope concentration in single- phase regions. The method is nonperturbing, nondestructive, insensitive to orientation artifacts, and applicable to a range of lyotropic liquid crystals. However, the method does require access to a high-intensity, monochromatic x-ray source, to suitable data collection and analysis equipment, and to an independent determination of lyotrope concentration along the gradient.

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REFERENCES

1. Caffrey, M., and F. S. Hing. 1987. A temperature-gradient method for lipid phase diagram construction using time-resolved x-ray diffraction. *Biophys. J.* 51:37–46.
2. Caffrey, M., and D. H. Bilderback. 1983. Real-time x-ray diffraction using synchrotron radiation: system characterization and applications. *Nucl. Instrum. Methods.* 208:495–510.
3. Caffrey, M., and D. H. Bilderback. 1984. Kinetics of the main phase transition of hydrated lecithin monitored by real-time x-ray diffraction. *Biophys. J.* 45:627–631.
4. Caffrey, M., and M. Bywater. 1988. Two new and rapid approaches for studying the phase properties of cosmetic lipids and oils. *J. Soc. Cosmet. Chem.* 39:159–167.
5. Caffrey, M. 1987. Kinetics and mechanisms of transitions involving the lamellar, cubic, inverted hexagonal and fluid isotropic phases of hydrated monoacylglycerides monitored by time-resolved x-ray diffraction. *Biochemistry.* 26:6349–6363.
6. Caffrey, M. 1987. A lyotrope gradient method for lipid mesomorphic phase diagram construction using time-resolved x-ray diffraction. *Biophys. J.* 51:444*a*. (Abstr.)
7. Caffrey, M. 1984. X-Radiation damage of hydrated lecithin membranes detected by real-time x-ray diffraction using wiggler-enhanced synchrotron radiation as the ionizing radiation source. *Nucl. Instrum. Methods.* 222:329–338.
8. Hyde, S. T., Andersson, S., Ericsson, B., and K. Larsson. 1984. A cubic structure consisting of a lipid bilayer forming an infinite periodic minimum surface of the gyroid type in the glycerol-monooleat-water system. *Z. Krist.* 168:213–219.
9. Krog, N. J., Riison, T. H., and K. Larsson. 1985. *In* Encyclopedia of Emulsion Technology. Volume 2. P. Becher, editor. Marcel Dekker Inc., New York. 321–365.
10. Laughlin, R. G., and R. L. Munyon. 1987. Diffusive interfacial transport: a new approach to phase studies. *J. Phys. Chem.* 91:3299–3305.
11. Caffrey, M. 1985. Kinetics and mechanism of the lamellar gel/lamellar liquid crystal and lamellar/inverted hexagonal phase transition in phosphatidylethanolamine: a real-time x-ray diffraction study using synchrotron radiation. *Biochemistry.* 24:4826–4844.
12. Lutton, E. S. 1965. Phase behavior of aqueous systems of monoglycerides. *J. Am. Oil Chem. Soc.* 42:1068–1070.
13. Lawrence, A. S. C. 1961. Polar interaction in detergency, *In* Surface Activity and Detergency. K. Durham, editor. Macmillan & Co., Ltd., London. 158–192.
14. Hakemi, H., Varanasi, P. P., and N. Tcheurekdjian. 1987. Diffusion studies in liquid crystalline phases of surfactant solutions: A new mass-transport approach. *J. Phys. Chem.* 91:102–125.