Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl16

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Version of record first published: 15 Sep 2010.

To cite this article: R. R. Balmbra , D. A. B. Bucknall & J. S. Clunie (1970): Mesomorphic Phase Structure in the Potassium Oleate + Water System, Molecular Crystals and Liquid Crystals, 11:2, 173-186

To link to this article: http://dx.doi.org/10.1080/15421407008083510

Molecular Crystals and Liquid Crystals. 1970. Vol. 11, pp. 173–186

Printed in Great Britain

Mesomorphic Phase Structure in the Potassium Oleate + Water System

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Received July 13, 1970

Abstract—Polarization microscopy, low-angle X-ray diffraction and electron microscopy have been used to examine the lyotropic mesomorphic phases which occur at room temperature in the potassium oleate + water system. Low-angle X-ray diffraction has confirmed the occurrence of neat, complex hexagonal, rectangular and middle mesomorphic phases, with increasing water content in the system at 22 °C (295 K). Direct visual confirmation of the structures of these different mesomorphic phases has been obtained by transmission electron microscopy using the specimen preparative technique of osmium tetroxide fixation, followed by thin sectioning.

1. Introduction

Electron microscopy has been successful in providing direct visual confirmation of the structures of neat (lamellar) and middle (hexagonal) phase,[1] the two most commonly occurring anisotropic mesomorphic phases in surface-active agent + water systems.[2] The most versatile specimen preparative technique for the investigation of lyotropic mesomorphic phase structure by electron microscopy has been osmium tetroxide fixation of unsaturated surfactant + water systems, followed by thin sectioning. Some initial experiments using this technique have provided clear confirmation of the cross-sectional structure of neat phase and middle phase in the potassium oleate + water system.[1,3,4] However, between neat phase and middle phase in this system there exists a distinct anisotropic mesomorphic region and, within this intermediate region, low-angle X-ray diffraction[5,6] has indicated the occurrence of two other mesomorphic phases—" complex hexagonal " phase and " rec-

tangular " phase, so-called because of the symmetry revealed by their respective X-ray diffraction patterns.

In this paper we present confirmatory electron micrographs for the structures of complex hexagonal phase and rectangular phase obtained by applying the osmium tetroxide fixation and sectioning technique to the potassium oleate + water system. This system is particularly suited for such a study since all the lyotropic mesomorphic phases exist at room temperature and the intermediate mesomorphic region extends over a relatively wide concentration range compared to other known systems, e.g. sodium linolenate + water.[7]

2. Experimental

(A) Materials

Oleic acid (Fluka AG) was shown to contain at least 99% 9-octadecenoic acid by G.L.C. and T.L.C. of the methyl ester. Potassium oleate was prepared by dissolving oleic acid in ethanol and titrating with aqueous potassium hydroxide to pH 9. The resulting clear solution was evaporated using a freeze-drying method, and finally dried over phosphorus pentoxide to yield the product as a white powder.

(B) Phase Diagram

A complete binary phase diagram for the potassium oleate + water system could not be constructed by the usual optical method[8] alone because of difficulties in distinguishing textures in the two phase mixtures which predominate between 53 and 70% surface-active agent. Approximate phase boundaries in the intermediate region between neat phase and middle phase were located using low-angle X-ray diffraction.

(C) X-ray Diffraction

Low-angle X-ray diffraction patterns were recorded at room temperature (~22 °C (295 K)) using a flat-plate vacuum camera with pin-hole collimation[8] and copper Kα radiation (λ = 1.542 Å

(0.1542 nm)). Mesomorphic samples were sealed in Lindemann glass capillaries.

(D) Electron Microscopy

Specimens of each mesomorphic phase were equilibrated isopiestically over sulphuric acid solutions of known vapour pressure for a period of 3–4 weeks at 25 °C (298 K). Introduction of osmium tetroxide vapour into the equilibration vessel resulted in fixation of the mesomorphic phase samples over a period of 24 hours. The fixed material was partially insoluble in water and after dehydration in a graded series of acetone solutions was embedded in Araldite in the usual way.[9] Thin sections of the embedded material were cut using a Porter-Blum MT – 1 ultra-microtome and examined on a " holey " carbon film support in a Siemens Elmiskop I at 80 kV using a 50 μm objective aperture and magnifications up to 80,000 ×. All micrographs were selected from through-focal series in order to obtain the in-focus plate. This procedure is necessary because contrast reversal in the micrograph can occur simply by defocusing the periodic images being examined.[10]

With some sections, contrast was enhanced by using a supplementary staining technique (OTO staining[11]). This entailed treatment of the osmium tetroxide fixed sections with a 1% aqueous solution of thiocarbohydrazide followed by further exposure to osmium tetroxide vapour. In this post-staining method, the initial thin sections were picked up on uncoated nickel grids instead of copper grids bearing a " holey " carbon film support.

3. Results

(A) Phase Diagram

The phase diagram for the potassium oleate + water system is shown in Fig. 1, attention having been focused on the lyotropic mesomorphic phase regions. At 25 °C (298 K), the temperature of the osmium tetroxide fixation experiments, middle phase is separated from neat phase by an anisotropic intermediate mesomorphic zone which exists over the concentration range 53–70% surface-active agent. This isothermal section differs somewhat from that reported

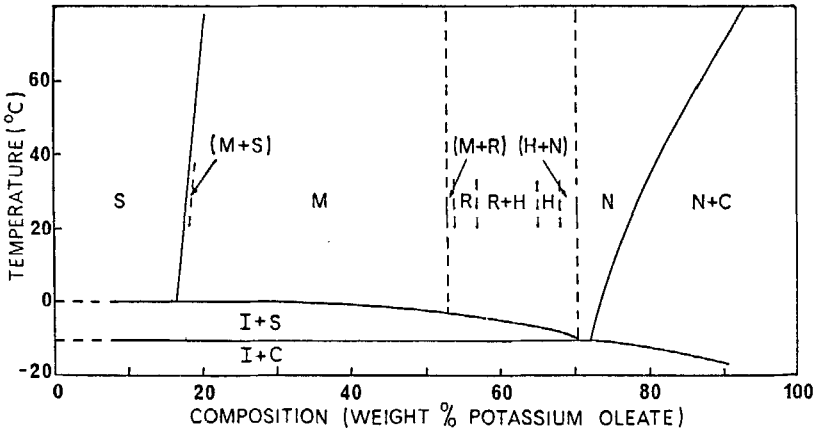

Figure 1. Partial phase diagram for potassium oleate + water. S: isotropic solution, M: middle phase, R: rectangular phase, H: complex hexagonal phase, N: neat phase, C: crystalline solid, I: Ice; —— experimental boundary; - - - - interpolated boundary.

by Ekwall *et al.*[12] and by Luzzati *et al.*[5,6] but, like these workers, we encountered extensive two phase regions within the intermediate mesomorphic zone. This made precise location of phase boundaries within this composition range difficult but, like Luzzati *et al.*,[5,6] we recognize the occurrence of both complex hexagonal phase and rectangular phase; Ekwall *et al.*,[12] on the other hand, have recently reported only the occurrence of rectangular phase in the intermediate region at 20 °C (293 K).

(B) X-ray Diffraction

The variation of principal low-angle X-ray spacing with composition across the various mesomorphic phases at 22 °C (295 K) is given in Fig. 2. Both neat phase and middle phase gave the simple conventional diffraction patterns expected from these phases,[2,4,6] showing up to three orders of diffraction which could be readily measured and indexed. The measured X-ray periodicities (Fig. 2) agree well with those of Luzzati *et al.*[5]

By comparison, the low-angle X-ray diffraction patterns from the intermediate mesomorphic zone were complex. The recorded reflections tended to vary irregularly in number (from 4 to 8), in relative intensity and in degree of preferred orientation. Most

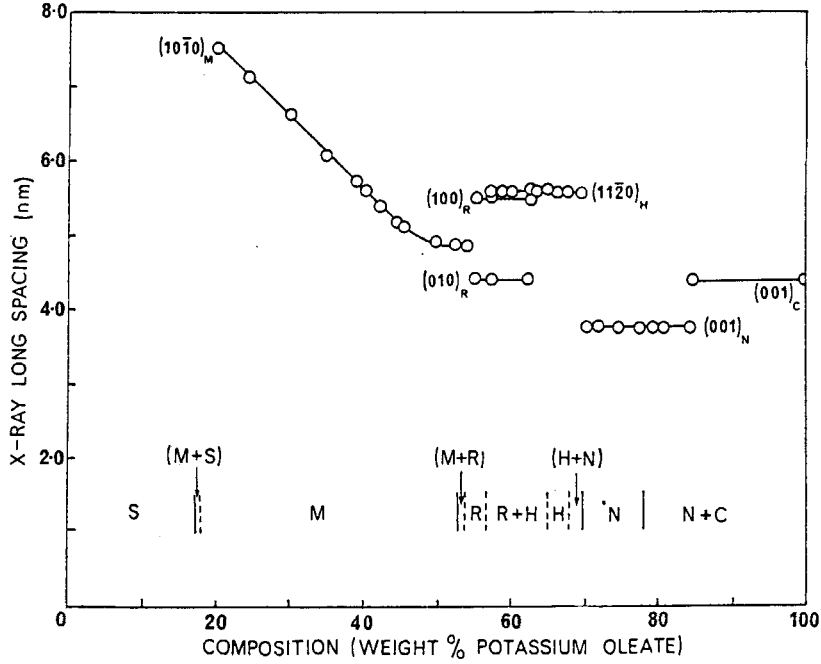

Figure 2. X-ray long spacing as a function of composition for potassium oleate + water at ~22 °C (295 K) 1 nm = 10 Å.

samples were mixtures of mesomorphic phases which proved difficult to separate by centrifugation at 1500 g at room temperature. Despite complications deriving from the occurrence of mixtures, diffraction patterns have been obtained consistent with the presence of a complex hexagonal phase and a rectangular phase in the composition range between neat phase and middle phase in the potassium oleate + water system. Complex hexagonal phase is characterized by up to six orders of reflection of the (hkiO) type indicating that this phase is based on a two dimensional hexagonal lattice structure. On the other hand, rectangular phase is characterized by first and second orders from two sets of reflections of the type (hOO) and (OkO), suggesting a two dimensional orthorhombic lattice structure.[5] In Table 1 we record structural data for these intermediate phases.

It may be observed (Fig. 1) that on the concentration scale at 22 °C (295 K) the ranges of existence of both complex hexagonal phase and rectangular phase, as homogeneous phases, are very

TABLE 1

Phase	% Surfactant	X-ray data (nm)	E.M. data (nm)
Middle	45	$a = 5.9$	5.2
	35	$a = 7.0$	6.2
Rectangular	55	$d_{100} = 5.55$ (a)	5.6
		$d_{010} = 4.38$ (b)	4.6
Complex hexagonal	58	$a = 11.2$	10.8
Neat	75	$d_{001} = 3.8$	3.0

narrow, and most of the intermediate region consists of mixtures of these phases.

(C) Electron Microscopy

Thin sections from the osmium tetroxide fixed neat phase specimens (70–78% potassium oleate) showed a regular lamellar structure (Fig. 3) with a periodicity (3.0 nm) slightly lower than that obtained by X-ray diffraction (3.8 nm). However, the contrast in these sections was poorer than might have been expected, even after fixing at higher temperatures (~50 °C (323 K)), and while OTO staining did make some slight improvement the best results were obtained using sodium oleate rather than potassium oleate. In the sodium oleate experiments, neat phase compositions containing 70% surfactant were fixed by osmium tetroxide vapour at ~60 °C(333 K) because of the relatively high Krafft boundary in the sodium oleate + water system.[6,13]

Osmium tetroxide fixed middle phase specimens gave sections with extensive areas showing a two dimensional hexagonal array of units with circular cross-section (Fig. 4). In the in-focus plate the hexagonal array appears as white circular units set in an electron dense matrix. For an original middle phase composition of 45% potassium oleate, the interparticle spacing in the electron micrograph (a = 5.2 nm) is in reasonable agreement with X-ray spacing before fixation (a = 5.9 nm). These results may be compared with an electron micrograph spacing of a = 6.2 nm and an X-ray spacing of a = 7.05 nm for an original middle phase composition of 35% potassium oleate. Osmium tetroxide fixation of middle phase samples containing 25% potassium oleate, however, yielded only disrupted structures

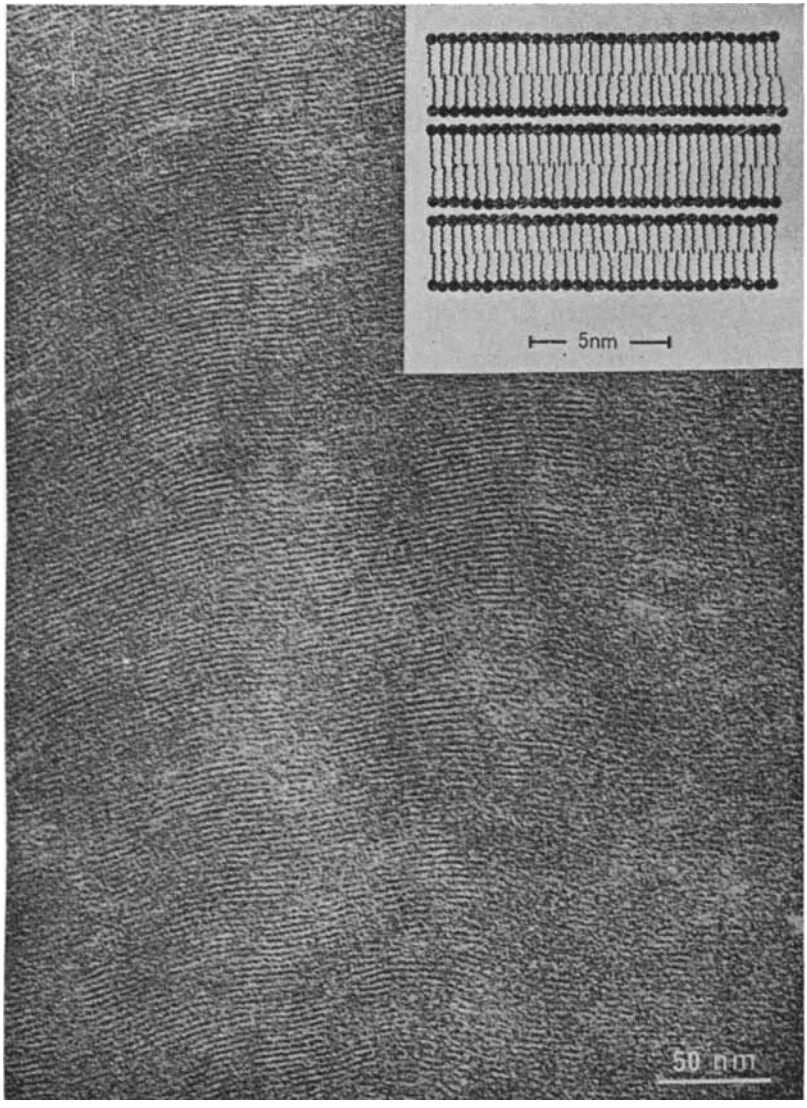

Figure 3. Thin section of osmium tetroxide fixed neat phase from potassium oleate + water system. Inset: model of neat phase structure in cross-section.

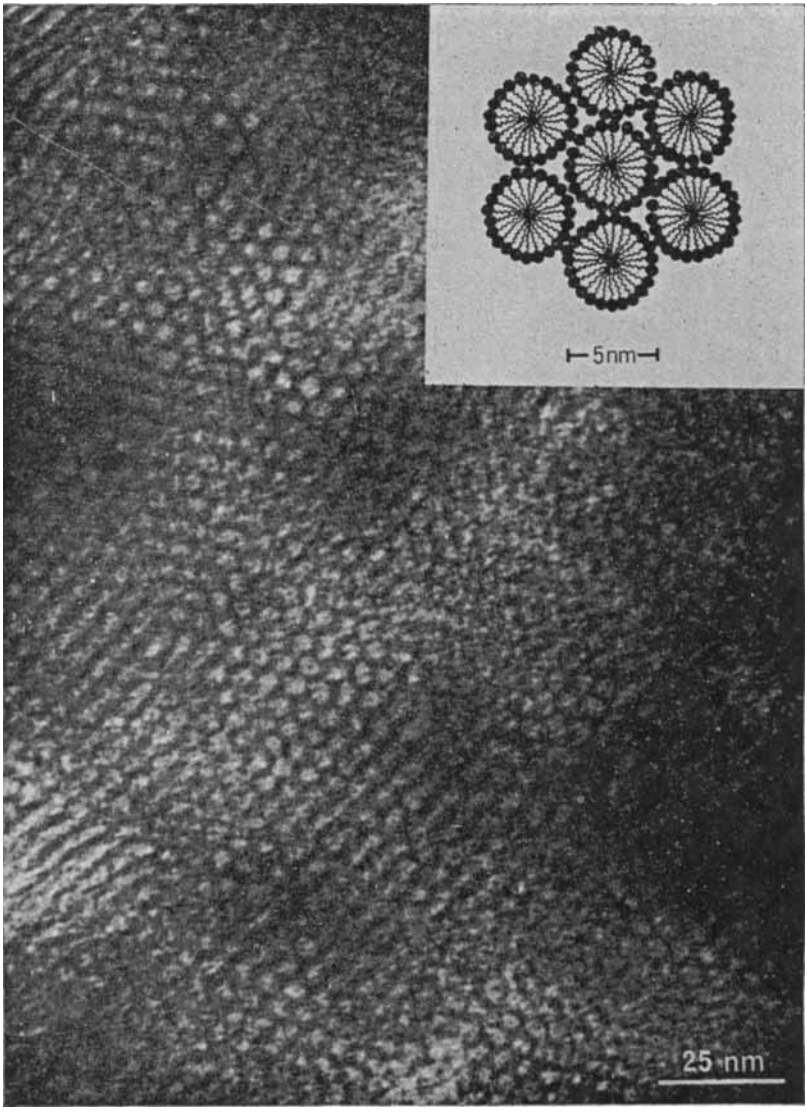

Figure 4. Thin section of osmium tetroxide fixed middle phase from potassium oleate + water system. Inset: model of middle phase structure in cross section.

when viewed in the electron microscope. An additional feature in middle phase sections was the existence of areas showing an unconvoluted lamellar structure with a periodicity of 4.5 nm. This periodicity is much greater than that obtained from neat phase and represents a longitudinal section of middle phase ($d = \sqrt{3}a/2$).

Several osmium tetroxide fixed samples from the intermediate mesomorphic region in the potassium oleate + water system have been examined as thin sections. From the concentration range 52–65% potassium oleate electron micrographs have been obtained (Fig. 5) which show an extensive hexagonal array of electron dense rings (interparticle spacing, a = 10.0–11.1 nm). These annular features have the dimensions expected from low-angle X-ray diffraction (a = 11.2 nm), and represent the transverse section of complex hexagonal phase i.e. hexagonally packed tubular units.[5] Several electron micrographs from this composition range also showed patches of complex hexagonal phase (a = 10.5 nm) grossly segregated from patches of middle phase (a = 5.2 nm). Other electron micrographs from the composition range 57–63% potassium oleate frequently showed patches of complex hexagonal phase in admixture with areas showing a reticular structure strongly resembling that proposed for rectangular phase, viz. an electron dense cross-banded structure (Fig. 6). The two sets of dark bands run at right angles to each other and it may be noted that the banded appearance is generally more prominent in one direction than in the other. Measurements of the two-dimensional rectangular lattice have yielded spacings of a = 5.6 nm and b = 4.6 nm which compare favourably with the low-angle X-ray diffraction data (Table 1).

4. Discussion

The electron micrographs (Figs. 3 and 4) confirming the structures of neat phase and middle phase in cross-section are conventional types and have been discussed before.[1,3] One surprising feature is the relatively poor contrast shown by neat phase sections compared to middle phase sections in the potassium oleate + water system. Although the amount of water present in the sample may be a contributory factor—for example, anhydrous potassium oleate is not satisfactorily fixed by osmium tetroxide vapour—the nature of the

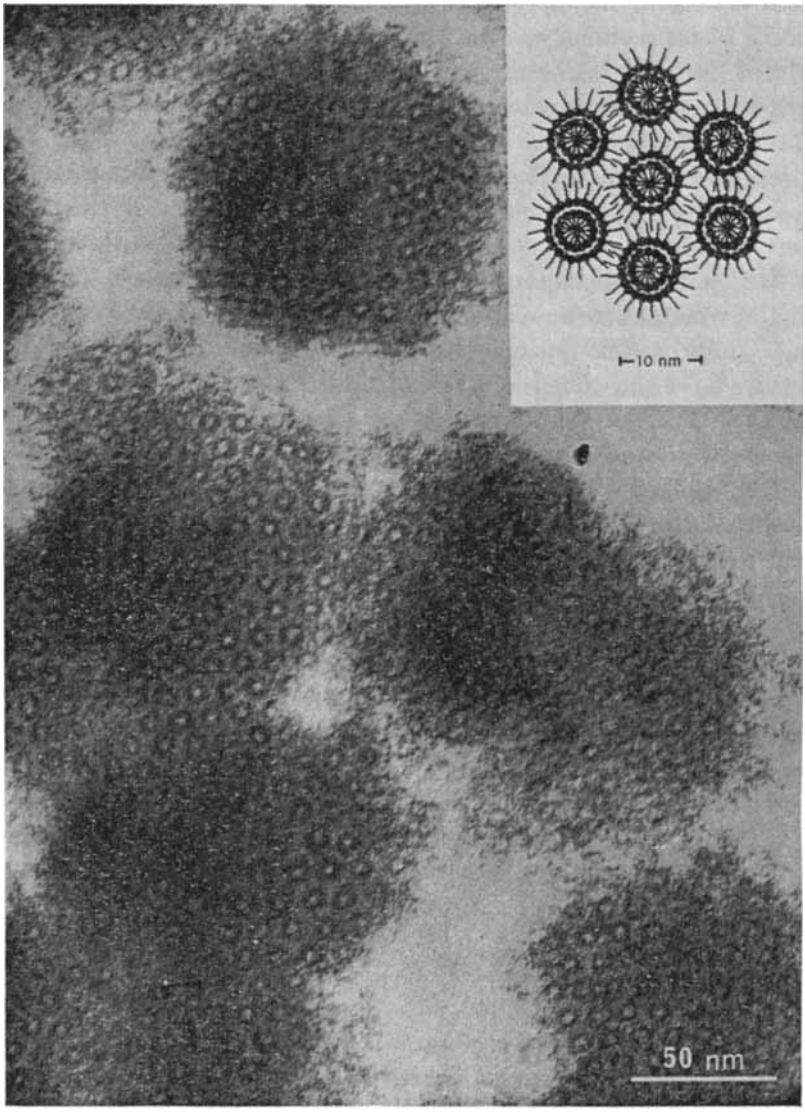

Figure 5. Thin section of osmium tetroxide fixed complex hexagonal phase from potassium oleate + water system. Inset: model of complex hexagonal phase structure in cross section.

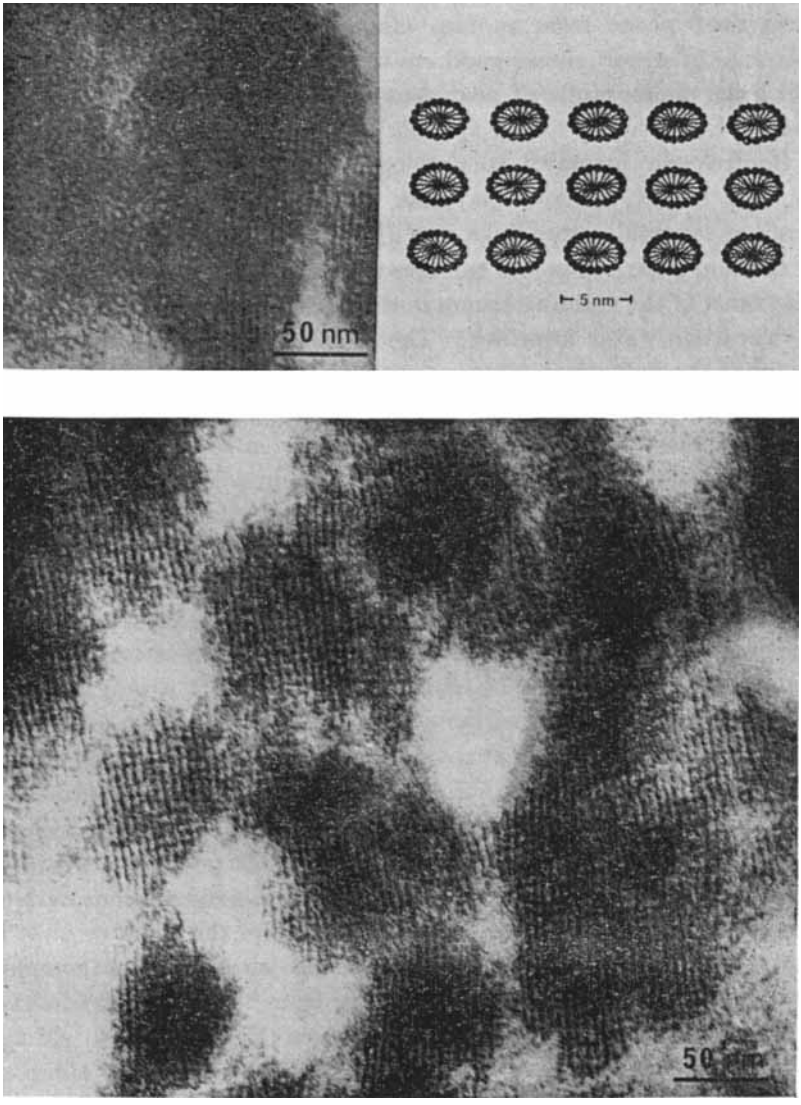

Figure 6. Thin section of osmium tetroxide fixed rectangular phase from potassium oleate + water system. Inset (left): osmium tetroxide fixed thin section showing complex hexagonal phase and rectangular phase in close proximity. Inset (right): model of rectangular phase structure in cross section.

cation appears to be the important factor since the osmium tetroxide fixed neat phase from sodium oleate, which contains equivalent amounts of water, shows good contrast in its electron micrographs. The best micrographs of neat phase have been obtained from the sodium linolenate + water system.[1]

Controversy exists[14] concerning the origin of the contrast in osmium tetroxide fixed material. Although it is accepted that osmium tetroxide cross-links the unsaturated alkyl chains situated in the non-polar regions of the mesomorphic structure, it is believed that most of the staining occurs in the polar head-group region at the hydrocarbon/water interface. The in-focus micrograph for middle phase in the potassium oleate + water system—an hexagonal array of white dots on a dark background—agrees with this interpretation since this phase consists of a two dimensional lattice array of rod-like micellar units.[2] The correctness of this interpretation has been confirmed by osmium tetroxide fixation studies in the dodecyl triallylammonium bromide $[C_{12}H_{25}N^{+}(CH_2CH{=}CH_2)_3Br^{-}]$ + water system where the unsaturation is present in the head-group region and not in the non-polar hydrocarbon chain. In this system, only one mesomorphic phase—middle phase—exists, occurring over the concentration range 58–87% surfactant at 298 K. A middle phase sample containing 70% surfactant has been successfully fixed with osmium tetroxide vapour at room temperature. The electron micrographs from the resulting thin sections show the same contrast as that for middle phase in the potassium oleate + water system (Fig. 4). There now seems little doubt that in the potassium oleate + water system the contrast in osmium tetroxide fixed sections derives principally from the polar head-group region of the structure. It is therefore possible to deduce the correct structures of complex hexagonal phase and rectangular phase from the observed contrast in the electron micrographs of these phases (Figs. 5 and 6). Of the two possible model structures tentatively proposed[2,5] for complex hexagonal phase on the basis of X-ray diffraction data, only one is compatible with the contrast shown in the electron micrographs, viz. a two dimensional array of tubular units composed of water both filled with and surrounded by hydrocarbon (Fig. 5). Consistent with this model is the observation of a lamellar structure of varying interlayer spacing (5.8 to 6.4 nm) which is sometimes associated with

the annular features, and which possibly represents various longitudinal sections of the structure.

Because the intermediate mesomorphic zone in the potassium oleate + water system consists mainly of two phase co-existence regions, it is perhaps not surprising that many electron micrographs from this region show mixtures of phases. For example, micrographs have been obtained clearly showing regions of complex hexagonal phase and middle phase in the same field of view, and in Fig. 6 we see regions of complex hexagonal structure adjacent to an area showing a structure resembling that of the proposed rectangular phase. The rectangular lattice structure, though less well-defined than the complex hexagonal structure, is a distinct feature which cannot be ascribed to any oblique section of any other known mesomorphic phase. Nor can it be attributed to a Moiré pattern caused by super-imposed layered structures, since the rectangular structure was frequently observed over large areas with the bands of fixed spacing always running at right angles to each other. Moreover, the symmetry and dimensions of the cross-banded structure (Fig. 6) agree with the low-angle X-ray diffraction data (Table 1), and with the proposal that rectangular phase is based on a two dimensional orthorhombic lattice. An earlier claim by Eins[15] to have provided electron microscopical evidence for rectangular phase is in error because the published micrograph shows an overall hexagonal symmetry and probably represents a distorted aspect of middle phase structure in cross-section. The present micrographs clearly show the rectangular symmetry and support the tentative proposals of Luzzati *et al.*[5] that rectangular phase consists of a two dimensional orthorhombic lattice array of indefinitely long parallel ribbons of surfactant. The nature of the cross section of the structural units in rectangular phase remains uncertain because a blurring of particle shape occurs when working near the practical resolution limit.[16] For rectangular phase, the structural units may be either ellipsoidal or rectangular[5] in cross section.

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