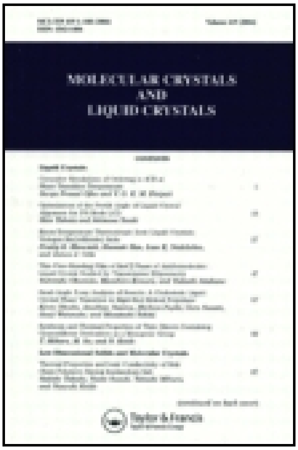

Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl16

Electron Microscopy of Mesomorphic Structures of Aqueous Lipid Phases. I. The System Potassium Oleate/ Water

Siegfried Eins [a]

[a] Zentralinstitut für Mikrobiologie und experimentelle Therapie der Deutschen Akademie der Wissenschaften zu, Berlin, DDR 69, Jena

Version of record first published: 15 Sep 2010.

To cite this article: Siegfried Eins (1970): Electron Microscopy of Mesomorphic Structures of Aqueous Lipid Phases. I. The System Potassium Oleate/Water, Molecular Crystals and Liquid Crystals, 11:2, 119-132

To link to this article: http://dx.doi.org/10.1080/15421407008083506

Molecular Crystals and Liquid Crystals. 1970. Vol. 11, pp. 119–132

Printed in Great Britain

Electron Microscopy of Mesomorphic Structures of Aqueous Lipid Phases. I. The System Potassium Oleate/Water

SIEGFRIED EINS

Zentralinstitut für Mikrobiologie und experimentelle Therapie
der Deutschen Akademie der Wissenschaften zu Berlin
DDR 69 Jena

Received November 10, 1969; *and in revised form June* 15, 1970

Abstract—Mesomorphic lyotropic phases, including the gel state of a special soap-water system (potassium oleate), have been studied by electron microscopy over the whole concentration range. Pictures obtained are interpreted in terms of known model structures.

Hexagonal, lamellar and rectangular phases are demonstrated. Structural parameters of these phases are measured at room temperature and as a function of concentration. Equations are given for calculating transition concentrations from measured values.

The investigation shows that in spite of rigorous preparation techniques results generally are in good agreement with those obtained by X-ray diffraction method. This is of interest for interpretation of ultrastructure of biological specimens in terms of structural parameters of naturally occurring lipids.

1. Introduction

Before studying structures of some special lipid-water systems of biological interest(1) by electron microscopy influence of preparation techniques of samples of well-known soap-water systems should be established in order to reduce artifacts. The system potassium oleate-water was chosen, since extensive X-ray studies by Luzzati *et al.*(2,3,4) have been done on structural parameters of various mesomorphic phases under different conditions. Potassium oleate contains a double bond in its oleic acid residue which favours fixation by osmium tetroxide. Its chain length is comparable to those of lipids of biological membranes. It forms lyotropic mesomorphic phases at room temperature. This is important as autoxidation processes diminish the value of the results obtained.

Electron microscopic data which are available are limited to special conditions.(5,6,7,8) They do not allow one to draw conclusions about the equivalence of the results from X-ray and electron microscopic techniques. We attempt to do this by a systematic investigation of the dependence of structural parameters on conditions of the system used.

2. Materials and Methods

Potassium oleate was prepared by neutralizing oleic acid (Fluka) with potassium hydroxide in ethanolic solution. The reaction was controlled by the colour change of phenolphthalein. The precipitated soap was dried to a constant weight in a refrigerator under vacuum or a nitrogen atmosphere. An aliquot of this was dissolved in a surplus of water and dried to the desired concentration in the absence of air. Thereafter the system was stored for about one hour at room temperature to establish equilibrium.(5) The last procedure was carried out in the same polyethylene capsules (BEEM) in which further steps in the preparation of the sample were to take place. The following steps were employed in sample preparation:

(a) Fixation by vapour of an osmium tetroxide crystal in the closed capsule (requires 5 to 20 minutes);

(b) Dehydration in graded concentrations (each concentration 20 minutes, 3-fold changes) by use of acetone or ethanol with propylene oxide. Because of the small water content of the specimen starting concentration generally was 90 or 100% in terms of oleate;

(c) Embedding in methacrylate, Vestopal, Epon, or Araldite according to methods generally used(9);

(d) Ultra-thin sectioning was performed by a Porter-Blum Microtome (Ivan Sorvall, Inc.) or an Ultratome I (LKB). No section staining was necessary.

Electron micrographs were taken with a Siemens Elmiskop I provided with a cooling device at apertures of 200 and 50 μm for the condenser and the objective, respectively. The electronic mangification in the range of 80000-fold was repeatedly and carefully calibrated, using different methods (replicas of diffraction gratings, and test objects in combination with internal markers.(9,10) Structures were measured on optical enlargements (3 ×) by a filar micrometer using a stereomicroscope SM 20 (VEB Carl Zeiss Jena) with 6 × magnification. Each point in the diagrams is the mean value of 20 to 100 single measurements. The standard deviations of the measured periods are in no case greater than ± 2.0 Å. Section deformation caused by the cutting process was eliminated by mounting the section on the microscope stage in such a way that direction of sectioning was known on the photographic plate. Only structural parameters of the undeformed direction were measured.

3. Results and Discussion

A. HEXAGONAL PHASE

In accordance with X-ray studies(2,3) (Fig. 1) we observed a hexagonal structure (Fig. 2) at lower concentrations (30–60%). It consists of hexagonally arranged circles or hexagons, which show a remarkably higher contrast at the periphery than in the interior. This is in agreement with present ideas about the deposition of contrasting material after osmium tetroxide fixation.(5)

The hexagonal period is strongly dependent on soap/water ratio adjusted in the system (Fig. 3). Deviations of our results from those of the X-ray studies are tolerable (Fig. 3), but it is difficult to coordinate this difference to a special preparation step. Contrary to the periods, however, diameters of the inner contrastless parts of the structural units remain approximately constant. This is in accordance with the prediction(2,3,4,11) that thickness of water shells between single cylinders increases as dilution rises. The agreement of the measured diameters with those calculated from X-ray measurements (37 Å(4)) is less good than for the periods, particularly at low soap concentrations (Fig. 3). Reasons for this fact may be: (a) Standard deviations of the diameters are greater than those of the periods (Fig. 3). This is obvious in view of the fact that an oblique position of the cylinders to the plane of the section leads to a smearing effect of contrast within the visible projection of the cylinder. The influence of this effect on the period is only very small, as long as the structure remains visible. (b) Calculations for the X-ray

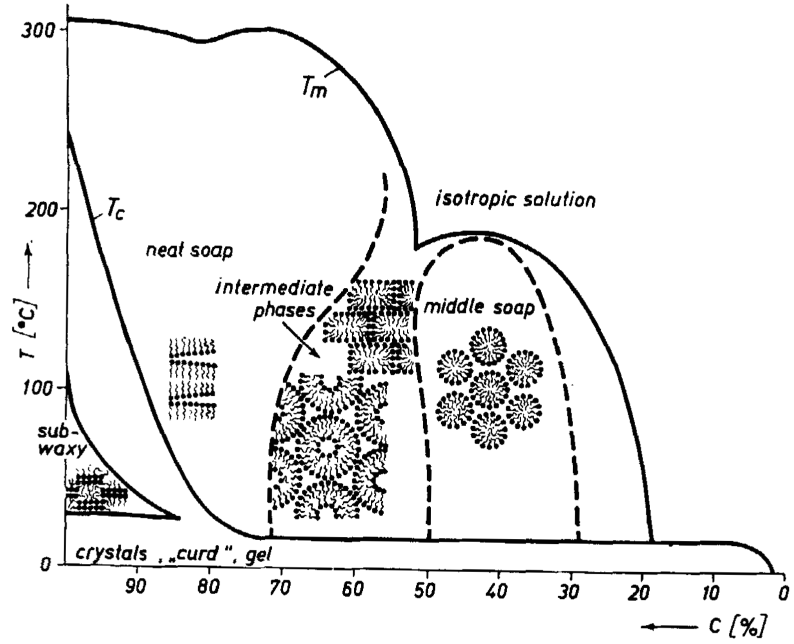

Figure 1. Schematic drawing of a soap phase diagram and structure of some phases. The boundaries for the main phases of the system studied are 30–60% for the hexagonal phase and 71–82% for the lamellar phase at the 20 °C-isotherm.

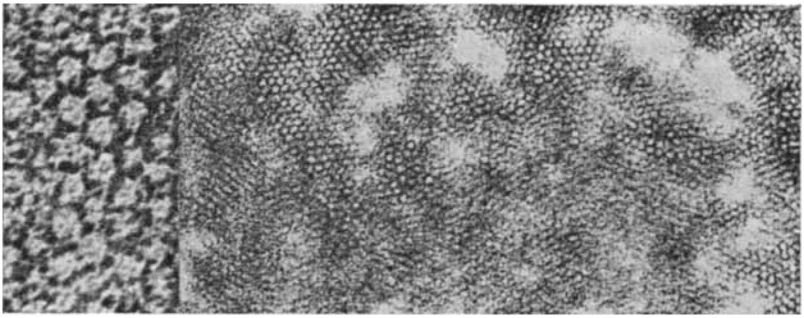

Figure 2. Electron micrograph of the hexagonal phase of potassium oleate. c = 47%, methacrylate embedding, 953 000 : 1 and 229 000 : 1, respectively.

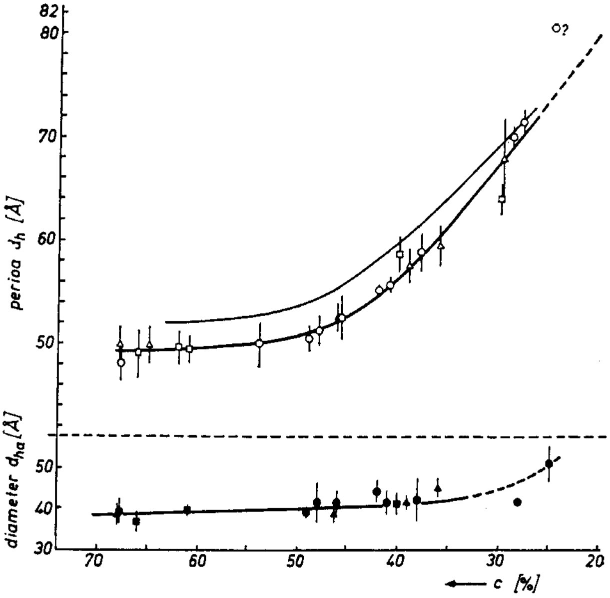

Figure 3. Hexagonal phase. Period d_h and diameter of the low-contrast interior d_{ha} of the cylinders (different scale). The various symbols stand for single embedding media (□—methacrylate, △—Vestopal W, ○—Epon and Araldite). The weak line is taken from Husson *et al.*(4) for comparison.

values(2,11) were done assuming a constant specific volume of the paraffin part of the structure, reaching up to the middle of the carbonyl bond, whereas the electron microscopically visible contrast is located more at the periphery of the structural elements. Possibly the discrepancy observed in d_{ha} values is due to those differences.

The hexagonal structure is accompanied by a seemingly lamellar one (Fig. 2), which is due to special projections of the hexagonal pattern with a direction of view perpendicular or oblique to the cylinder axis.(5,12) This is demonstrated in Fig. 4, where two planes of the image parallel to the cylinder axis are indicated leading to the two possible lamellar patterns with periods d_1 and d_2. One of the most impressive criteria for distinguishing seemingly lamellar periods from true ones are their constant ratios to the hexagonal periods d_h, independent of cutting angle. The correlated periods d_h, d_1 and d_2 vary in the same manner dependent on the cutting angle (Fig. 5).

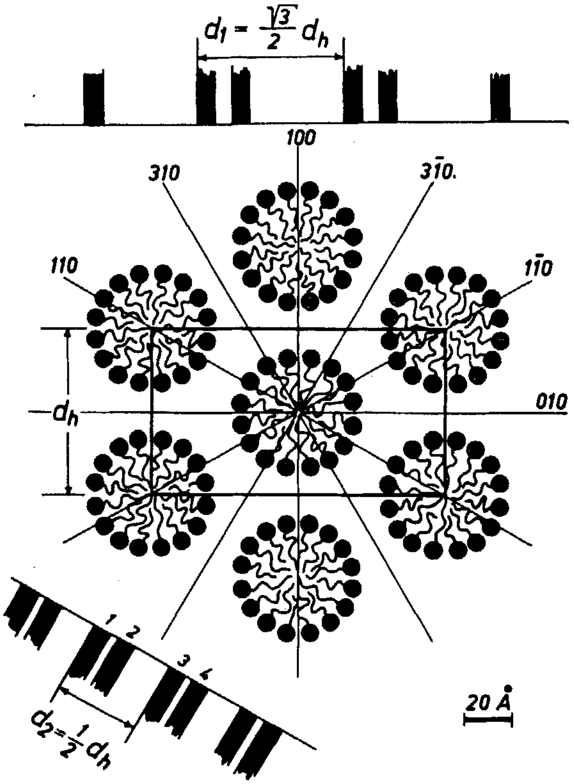

Figure 4. Model of hexagonal structure. Cross-sections and favoured directions of view which lead to lamellar appearances.

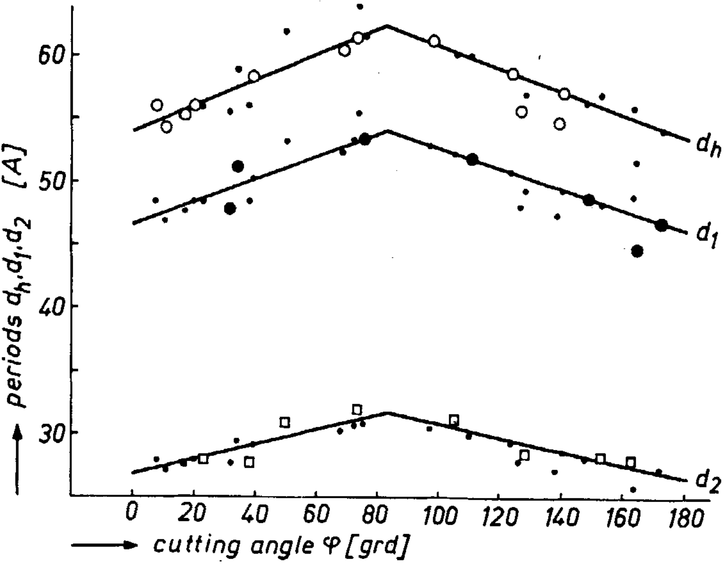

Figure 5. Constant ratio of hexagonal to seemingly lamellar periods on the same plate. Small points are calculated from the other two parameters by formulae given in Fig. 4.

B. Lamellar Phase

According to existing phase diagrams(3,4,8,13,14) this structure at ordinary temperatures covers only a small soap concentration range between 70 and 80%. The concentration range may be broader, if the phase does not exist as a single-phase system, but in equilibrium with one or two other phases, which is frequently observable.(2) Assuming that contrasting substances after osmium tetroxide fixation are accumulated in the aqueous and polar regions(5) of the known 1-dimensional lattice,(2,3,4,15) a structure of alternating dark and light bands is to be expected.

Figure 6 shows such a lamellar structure. Beside this we find regions with less pronounced regularity. This is similar to the hexagonal phase and is either due to obliquely sectioned lamellae or to some decomposition in the course of preparation.

The lamellar period within the small concentration range of this phase remains constant at 36.9 Å (Fig. 7), a value being quasi

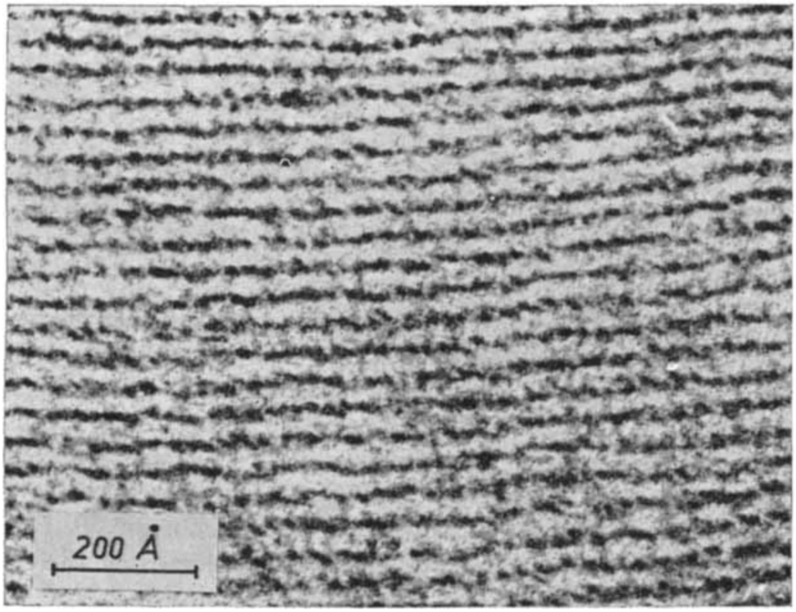

Figure 6. Electron micrograph of the lamellar phase of potassium oleate. $c = 76\%$, methacrylate embedding, 950 000 : 1.

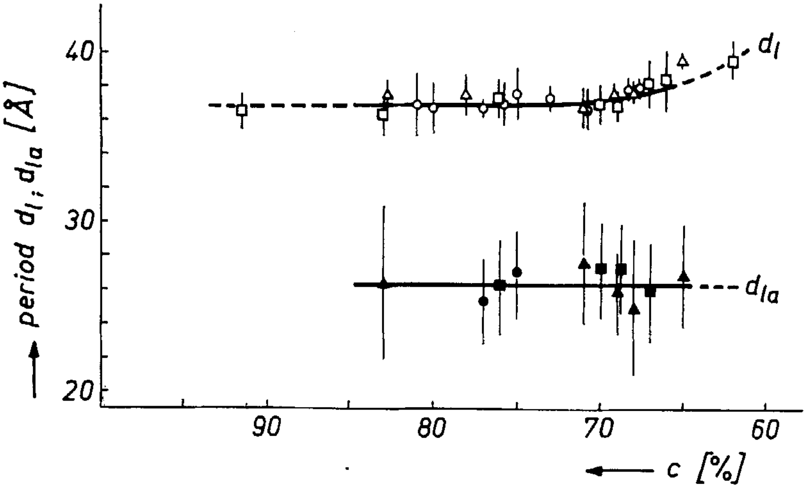

Figure 7. Lamellar phase. Period d_l (upper curve) and width of the low-contrast region d_{la}. Symbols as in Fig. 3.

identical with that of Husson *et al.*(4) using X-ray diffraction techniques under the same conditions of sample preparation. Again regions free of blackening are measurable with a lower accuracy than is possible with periods (Fig. 7). The average value amounts to 26.3 Å. Contrary to X-ray studies on some saturated soap-water systems(2,3,4,11) no concentration dependence has been observed in our experiments.

C. INTERMEDIATE PHASES

It is assumed by Luzzati *et al.*(2,3,4,11) that several new phases in the concentration range between hexagonal and lamellar phases may exist. The reality and structure of these phases are not generally accepted(16,17,18) because of difficulties arising from interpretation of X-ray diagrams having only a few diffraction lines from several coexisting phases. Recently the reality of the proposed models for these new phases have been questioned by Luzzati himself.(19) Direct visualization of these structures by electron microscopy therefore may contribute to solving this question.

Assumed structures are (with increasing concentration): (a) deformed hexagonal, (b) rectangular, (c) complex-hexagonal, and (d) cubic. Structures (a) and (d) do not exist in the system potassium/oleate water(2) and we have confirmed this.

Pictures with a rectangular arrangement (structure (b)) were found by us several times(20) in the concentration range given for this phase by Luzzati *et al.*(2) We wish to mention(20) a structural difference to the model proposed by these authors. Instead of the rhombic lattice postulated by them we found a rhombic face centered one, in which the single structural elements (quaders) were mutually displaced like the bricks in a wall. The dimensions of the elements (a = 54.2 ± 1.9 Å and b = 40.9 ± 1.7 Å, at a concentration of 65%) agree well with X-ray data.(3)

The efforts to demonstrate the structure of complex-hexagonal phase was less successful. We never found a somewhat extended region with the expected structure (a hexagonal pattern of circles, each of which contains a dark spot (*cf.* Fig. 1)), though single elements of this type were present. In her paper on aqueous phases of sodium linolenate Husson(19) discusses the instability of this phase during the time interval necessary for a single X-ray diffraction experiment. It may be that the instability is responsible for our failure to see this structure in our system.

D. GEL STRUCTURE

Gel structure of soaps was first investigated seriously in 1966. Using X-ray diffraction technique Vincent and Skoulios(22,23) found a lamellar structure in some saturated soaps which contrary to the other mesomorphic phases contains the fatty acid residues in a stiff configuration. The single chains show mutual overlap resulting in a hydro-carbon layer only one molecule thick.

This characteristic feature was found in our preparations below the T_c-curve as indicated in Fig. 1. At high lipid concentrations the period amounts to 30.0 Å, whereas the contrastless middle zone has an average thickness of only 21.4 Å (Fig. 8). The double bond in the paraffin chain of our system does not alter the rules found by Vincent and Skoulios(22,23).

It is interesting that in preparations as used in our experiments the gel state is not a uniform phase, or it has not reached structural equilibrium. In addition to typical lamellar gel structures we observed spindle-shaped designs with high contrast (Fig. 9); these designs frequently occur in equilibrium with the true lamellar phase. The width of the spindles is correlated to the amount of water present in the system. Therefore it seems that they are water-containing zones of the structure. Using different preparation methods, the possibility of an artifact produced by orientation of the specimen

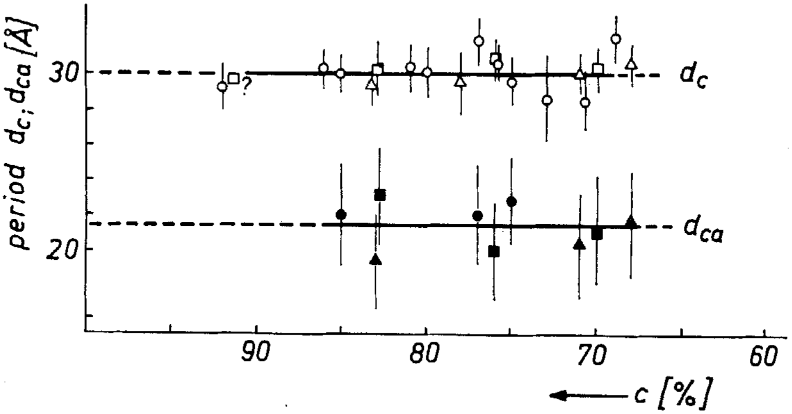

Figure 8. Period d_c (upper curve) and thickness of the contrastless part d_{ca} of lamellar gel structure. Symbols as in Fig. 3.

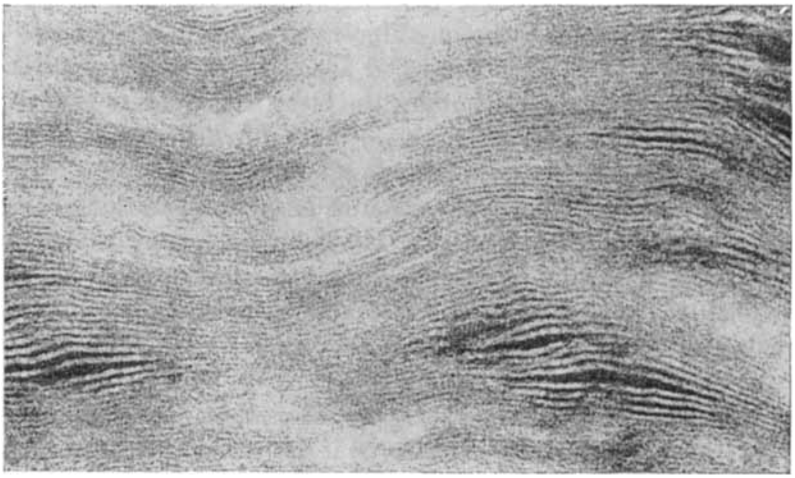

Figure 9. Electron micrograph of potassium oleate gel. $c = 81\%$, Vestopal embedding, 240 000 : 1.

by the surface of the support, by polymerization or by cutting could be excluded.(24)

The same appearances as described above for the gel phase at high concentrations (to the left of the ascending branch of the T_c-curve in Fig. 1 and at a smaller degree in equilibrium with the lamellar phase) may be observed over an extended concentration range on ageing the soap; the melting point goes to higher temperatures. Therefore at room temperature the T_c-curve is above the working range in the phase diagram at all concentrations and the systems are in the gel region.

At high dilutions the gel is able to take up considerable amounts of water. Thereby the period increases.(22,23) This effect was confirmed only qualitatively in our investigation. The exact frontiers of the gel phase were not detected, neither by Vincent and Skoulios,(22,23) nor by us. Our interest concerned mainly the liquid paraffin phases.

The gel state holds a position between crystalline and mesomorphic soaps. The chains are stiff, but packing is relatively loose.(22,23) The last point is of importance for fixation by osmium tetroxide, which requires special steric conditions; these are realized, for example, in the mesomorphic system with liquid paraffin chains.(25) For the same reason the waxy structure of water-free soaps are not demonstrable by the technique used. As pointed out by Skoulios *et al.*(26) polar groups are in a crystalline state in these phases and reaction with osmium tetroxide cannot take place.

E. MECHANISM OF PHASE TRANSITIONS

At present there is no possibility to predict the structures and transition ranges of a given amphiphile-water system.(7) A first trial in this direction was done by Parsegian,(27) who calculated the free energies of electrostatic charge for water-hydrocarbon interactions at an interface. The results explain the existence of hexagonal and lamellar structures, each being found at one side of a transition concentration. Intermediate phase transitions could not be explained. We were confronted with the same difficulty in predicting the transition concentrations on the basis of volume relations of the corresponding phase structures. It is possible, however, to calculate the transition concentrations for other phases.

The concentration of a special component of a given system may be written as:

$$c = \frac{m_L}{m_L + m_P} = \frac{\rho_L V_L}{\rho_L V_L + \rho_P V_P},$$

where m, ρ and V are mass, density and volume, respectively, and the indices L and P mean lipid and polar parts of corresponding structures. For the hexagonal phase we can write

$$c_h = \frac{\rho_L d_{ha}^2}{\rho_L d_{ha}^2 + \rho_P (d_h^2 - d_{ha}^2)} = \frac{d_{ha}^2 \rho_L}{d_h^2 \rho_P - d_{ha}^2 (\rho_P - \rho_L)}.$$

Inserting into this equation the smallest observable parameters of the hexagonal phase, we find a relation for transition concentration to the next phase:

$$c_h^{\max} = \frac{d_{ha}^2 \rho_L}{d_h^{\min 2} \rho_P - d_{ha}^2 (\rho_P - \rho_L)}.$$

Using the calculated value of the partial specific volume of the oleate residue (0.96 $\mathrm{cm}^3\,\mathrm{g}^{-1}$(4)) and a value of $\rho_P = 1.13\ \mathrm{g\,cm}^{-3}$ (from the calculated density of a potassium hydroxide solution(4)), we find with our results $d_h^{\min} = 49.5$ Å and $d_{ha} = 38.0$ Å

$$c_h^{\max} = 0.60.$$

This value agrees well with the concentration determined experimentally for the transition from hexagonal to intermediate phases.(3,4) Corresponding calculations were done for the transition from lamellar phase to the gel of potassium oleate and, in addition, for the system potassium palmitate-water at 100 °C using the results of Husson and co-workers,(4) which were the bases of Parsegian's work.(27) In all cases the agreement of calculated and observed transition concentrations is very good (Table 1). This is also true for special phospholipid-water system with other structural characteristics, as will be reported later.(1)

These results indicate that volume relations play an important role in the configuration and transformation of liquid crystalline structures and that the colloidal elements are not deformed in these cases. We did not try to give any predictions concerning the range of the existence of intermediate phases because of the inaccuracy of structural parameters, especially of the apolar part.

TABLE 1 Calculated Transition Concentrations of a Given Phase with known Structural Parameters

Object	Structure	Parameters (Å) Periods	Parameters (Å) Low contrast regions	Densities (g cm^{-3}) ρ_L	Densities (g cm^{-3}) ρ_P	Transition concentrations obs.	Transition concentrations calc.
KC 18: 1	hexagonal	49.5	38.0	1.04	1.13	0.60	0.60
KC 18: 1	lamellar	37.0	29.5	1.04	1.15	0.78	0.79
KC 16: 0	hexagonal	49.0	38.0	0.97	1.13	0.54	0.56

Acknowledgements

My thanks are due to Prof. M. Girbardt for stimulating this work, to Mrs. Martin, Mrs. Fritsche, Mrs. Quittkatt, Mr. Grünzig, Mr. Hädrich and Mrs. Wachsmuth for preparative and technical assistance and to the Forschungsgemeinschaft of the German Academy of Sciences at Berlin under the tenure of which this work was done.

The data given in this manuscript are based in part upon the thesis submitted by the author in partial fulfillment for the degree of Dr. sc. nat., Friedrich Schiller University of Jena, 1969.$^{(24)}$

REFERENCES

1. Eins, S., in preparation.
2. Luzzati, V., Mustacchi, H., Skoulios, A. E. and Husson, F., *Acta Cryst.* **13**, 660 (1960).
3. Luzzati, V. and Husson, F., *J. Cell Biol.* **12**, 202 (1962).
4. Husson, F., Mustacchi, H. and Luzzati, V., *Acta Cryst.* **13**, 668 (1960).
5. Stoeckenius, W., *J. Cell Biol.* **12**, 221 (1962).
6. Stoeckenius, W., *Symp. Internat. Soc. Cell Biol.* **1**, 349 (1962).
7. Corkill, J. M. and Goodman, J. F., *Adv. Colloid Interface Sci.* **2**, 298 (1969).
8. Bucknall, D. A. B., Clunie, J. S. and Goodman, J. F., *Molecular Cryst. and Liquid Cryst.* **7**, 215 (1969).
9. Glauert, A. M., in *Techniques for Electron Microscopy*, Ed. D. H. Kay, Blackwell Scientific Publications, Oxford 1965.
10. Sjöstrand, F. S., *Electron Microscopy of Cells and Tissues*, Academic Press, New York and London, 1967, pp. 362–365.
11. Luzzati, V., Mustacchi, H. and Skoulios, A., *Disc. Faraday Soc.* **25, 43** (1958).
12. Eins, S., *Stud. Biophys.* **1**, 391 (1966).

13. McBain, J. W. and Elford, W. J., *J. Chem. Soc.* 421 (1926).
14. McBain, J. W. and Sierichs, W. C., *J. Amer. Oil Chem. Soc.* **25,** 221 (1948).
15. Stauff, J., *Kolloid-Z.* **89,** 224 (1939).
16. Spier, H. L., *Fette, Seifen, Anstrichmittel* **67,** 943 (1965).
17. Clunie, J. S., Corkill, J. M. and Goodman, J. F., *Proc. Roy. Soc.* **285A,** 520 (1965).
18. Luzzati, V., Tardieu, A., Gulik-Krcywicki, T. *et al.*, *Nature* **220,** 485 (1968).
19. Luzzati, V., in *Biological Membranes*, Ed. D. Chapman, Academic Press, London and New York 1968, pp. 71–123.
20. Eins, S., *Naturwissensch.* **53,** 551 (1966).
21. Husson, F., *Compt. rend. hebd. Séances Ac. Sc.* **253,** 2948 (1961).
22. Vincent, J. M. and Skoulios, A. E., *Acta Cryst.* **20,** 432 (1966).
23. Vincent, J. M. and Skoulios, A. E., *Acta Cryst.* **20,** 441 (1966).
24. Eins, S., Dissertation, Jena 1969.
25. Eins, S., in preparation.
26. Skoulios, A. E. and Luzzati, V., *Acta Cryst.* **14,** 278 (1961).
27. Parsegian, V. A., *Trans. Faraday Soc.* **62,** 848 (1966).