

X-Ray study of structural changes in the liquid crystal system AOT / N-Heptane-Water depending on the concentration of components

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Abstract. The work investigated the structural changes of the multicomponent liquid crystal system sodium salt of di- (2-ethylhexyl) ester of sulfosuccinic acid (AOT) –n-heptane – water depending on the concentration of AOT in a mixed solution by X-ray diffraction. The studies were carried out in a wide concentration range from 27 to 92% AOT in a mixed solvent with an initial n-heptane / water ratio of 5.8. The studies were carried out with a gradual decrease in the n-heptane / water ratio to the content of bound water in the system and an increase in the concentration of AOT in the solution to 92%. The mesomorphism of the liquid crystal system is established depending on the content of the concentration of the components and the region of the compact liquid crystal lamellar “smooth” phase for modeling biological membranes is established.

Keywords: x-ray graphics, lyotropic liquid cryztall, models of biomembranes, lamellar phase

1. Introduction

All existing studies of the structure of biomembranes have led to the conclusion that most of the phospholipid molecules in them are in the form of bilayers, the functional properties of which are determined by their liquid crystal structure [1–4]. When lipid molecules are hydrated, lyotropic mesomorphism is manifested [5–7]. Numerous studies also show that lyotropic liquid crystals are typical representatives of biological membranes, and the legitimacy of using the latter as models for organizing biomembranes has been proven. For a complete understanding of the diversity of phase states and conditions of phase transitions, the phase states of the phospholipid–water system, depending on their concentration, have been largely clarified [8–11]. Phase diagrams of lipid–water mixtures depending on concentration and temperature have been studied for a wide range of soaps and membrane lipids [12]. Biomembrane studies have shown that membrane functions can be controlled by environmental changes. Direct relationship between membrane function and phase state can be proven.

In connection with the increasing use of lyotropic mesophases in the study of the structure and function of biomembranes, it is of interest to study models of biomembranes consisting of an aerosol–mixed solvent system based on an amphiphilic compound of the sodium salt of di- (2-ethylhexyl) ether of sulfosuccinic cyst (SAS), n-heptane and water ... The latter necessitates the study of the structure and nature of molecular rearrangements in lyotropic mesophases both under external influences (including the action of non-mesogenic components) and at the stage of relaxation and formation of stable structures.

To solve the main problem of this work in the aspect of the analysis of structural rearrangements underlying the shifts from the side of the amorphous crystalline properties of model membranes, we used the method of X-ray diffraction at small and large angles.

2. Experiment

The long-range ordering and the sizes of the diffracting cell of the VLC are determined using X-ray diffraction. However, X-ray structural studies of VFA require the solution of a number of technical and methodological problems. The samples were taken on X-ray units URS-60 and URS-2 with cameras of the KRON, RKSO type intended for shooting on a flat film, which were modified to study small-angle scattering at a distance of the samples from the films $0.05 \div 0.1$ m. In this case, the cameras make it possible to obtain reflections in the range of angles from 1 to 45° . A schematic representation of the camera is shown in Fig. 1.

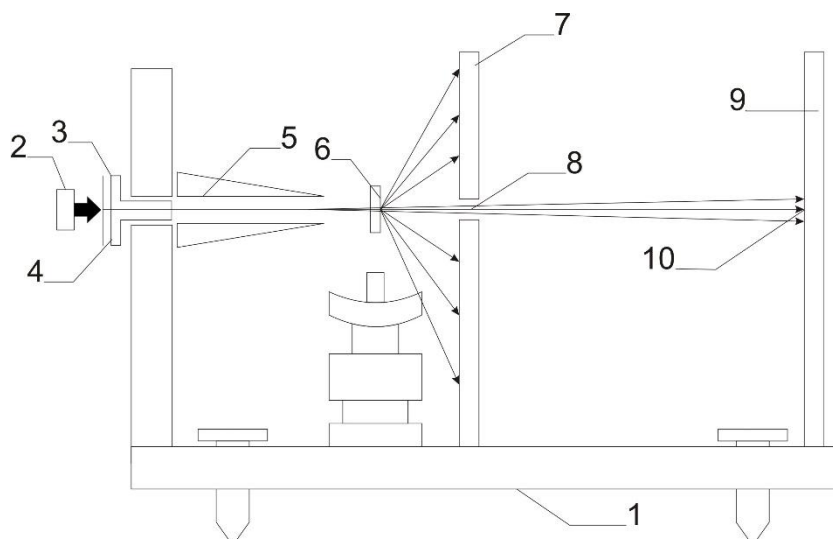


Fig. 1. Schematic picture of the camera.

X-rays emerging from the focus of the X-ray tube 2 pass through a nickel filter 3, aperture 4 and a collimator 5 and fall on the sample 6. The diameter of the aperture of the diaphragm is 1×10^{-4} m, the length of the collimator is 8×10^{-2} m, the diameter of its hole at the exit is 1×10^{-4} m. This collimation provides reasonably good accuracy. The beams diffracted at large angles are recorded on an X-ray film placed in a cassette 7. The latter has a hole 8 in the middle, through which the transmitted rays are fixed on a film placed in a cassette 9. The primary beam is absorbed by an absorber 10. The modified camera makes it possible to register reflections within angles $1 \div 45^\circ$ (low angle part is $1 \div 5^\circ$). We used tubes of types BSV-24, BSV-11 with a Cu anti-cathode, emitting radiation in the region of relatively long waves. Copper $K\alpha$ lines were separated using nickel filters 0.015 mm thick. Anode voltage 40 kV, anode current 20 mA. The exposure time was chosen for 4 hours. Films of the RTG-B type from Primax Berlin Germany were used. To prepare the sample, thin-walled quartz capillaries (made in Germany) with a wall thickness of 0.01 mm and a diameter of $0.4 \div 1$ mm were used. Absorption of rays and the appearance of a background on X-ray diffraction patterns when using these capillaries is practically absent. Our redesigned X-ray diffraction method will provide information on the size, shape, and compactness of the arrangement of colloidal formations.

3. Samples

In this work, the structures of mesophases obtained on the basis of Aerosol-OT (AOT) / n-heptane and water have been studied. We used 98% AOT from Sigma-Aldrich, commercial name – Dioctylsulfosuccinate sodium salt ($C_{20}H_{37}NaO_7S$), analysis (according to USP) – purity 97. Used n-heptane from Macrochem (Holland).

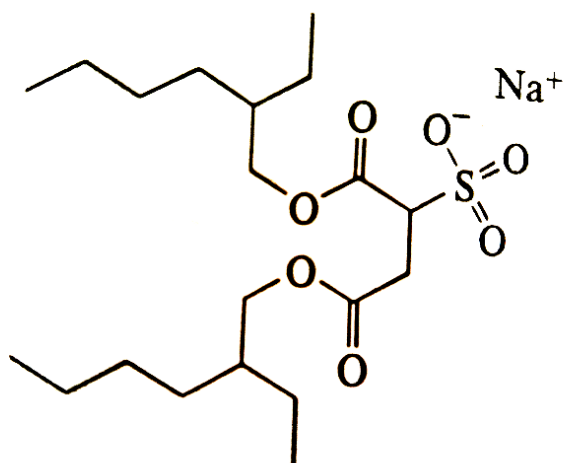


Fig. 2. Structural formula of the AOT molecule.

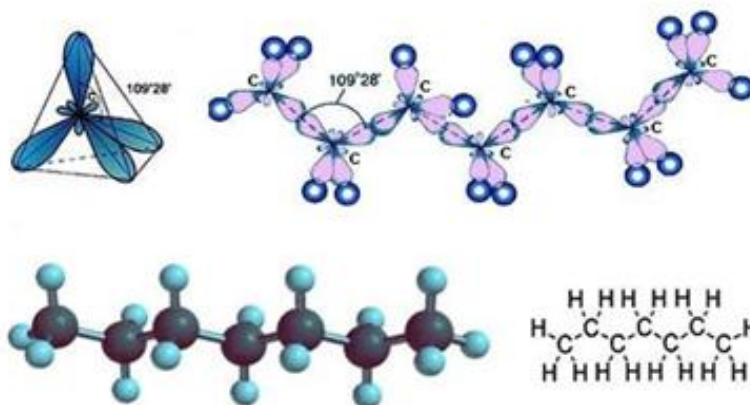


Fig. 3. Zigzag structure of the N-heptan molecule.

The solutions were prepared by dissolving AOT in n-heptane and then adding an appropriate amount of water.

Samples for X-ray diffraction were prepared according to the following procedure: heat treatment was carried out – before the X-ray diffraction patterns were taken, the sample was kept at a temperature of 50° C for half an hour, after which an isotropic melt of the corresponding concentration was introduced into the capillary, hermetically sealed on both sides, and cooled to room temperature. The finished sample was examined immediately after processing, as well as at regular intervals. The orientation of the samples was carried out using mechanical tension. X-ray diffraction patterns of stretched concentrated samples made in the form of cylinders with a diameter of 0.4÷1.0mm were taken without a capillary. Orientation of concentrated aqueous solutions was carried out by drawing the solution into a capillary. To prepare the samples, we used quartz capillaries with a wall thickness of 1×10^{-5} m and a diameter of 0.4×10^{-3} to 1×10^{-3} m.

4. Results

A solution of the liquid crystal system was prepared at a ratio of the mixed solvent n-heptane / water = 5.8. The structural rearrangements of the AOT–n-heptane – water system was investigated depending on the concentration of AOT in a mixed solvent. Were removed samples with concentrations from 30% to 92% and “dry” solutions, which were obtained by openly long–

term storage of the solution. “Dry” samples were taken without a capillary. The X-ray diffraction patterns obtained reflections in the form of uniform circles, indicating the presence in the sample of randomly spaced relative to each other domains, inside which AOT molecules are arranged in an orderly manner, forming liquid crystal mesophases [13–15]. Fig. 3 shows X-ray diffraction patterns of samples at different concentrations of AOT in a mixed solvent, where C_s is the concentration of the solvent, C is the concentration of AOT. Starting from low AOT concentrations (30%) in the mixed solvent, a halo appears on the X-ray diffraction patterns with a separating outer boundary corresponding to interplanar distances in the range from $24 \div 80$ Å (Fig. 3a, Fig. 3b). With an increase in the concentration of AOT (50%), the interval of interplanar distances narrows. With a further increase in concentration, a clear reflection with an interplanar distance of $20 \div 21$ Å and a more diffuse reflection with an interplanar distance in the range of $33 \div 36$ Å are separated in the X-ray diffraction patterns (Fig. 3d, e, f). The stability of the structure and the presence of long-range order are found. Long-range ordering and the size of the diffracting cell of the lyotropic liquid crystal were determined using X-ray diffraction.

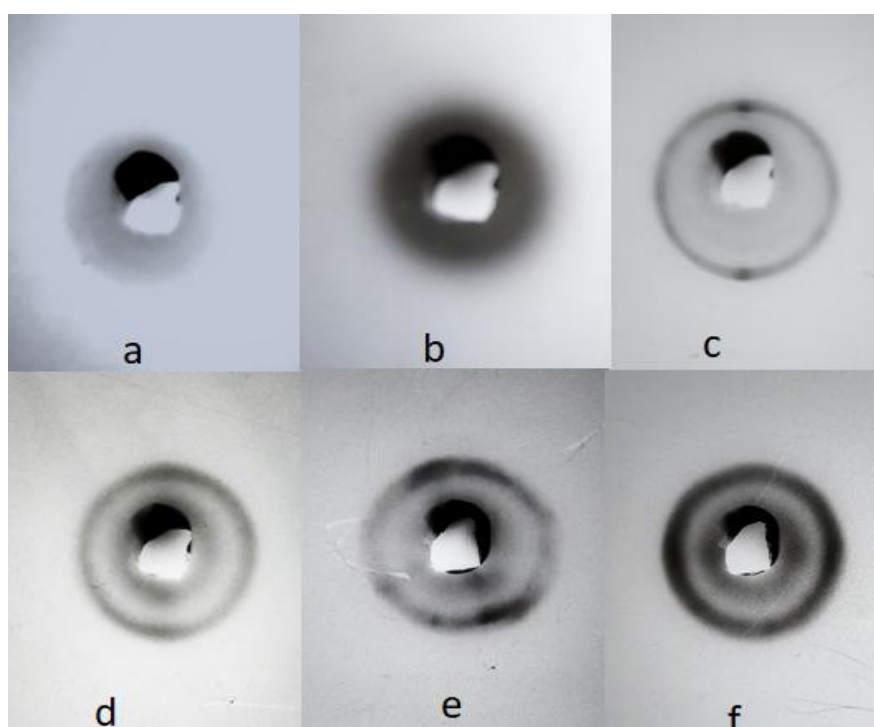


Fig. 4. X-ray diffraction patterns of the AOT–n-heptane–water sample a : $C_s/C = 2.3$; b – $C_s/C = 1.1$; c – $C_s/C = 0.54$; d– $C_s/C = 0.28$; e – $C_s/C = 0.18$, f – $C_s/C = 0.08$.

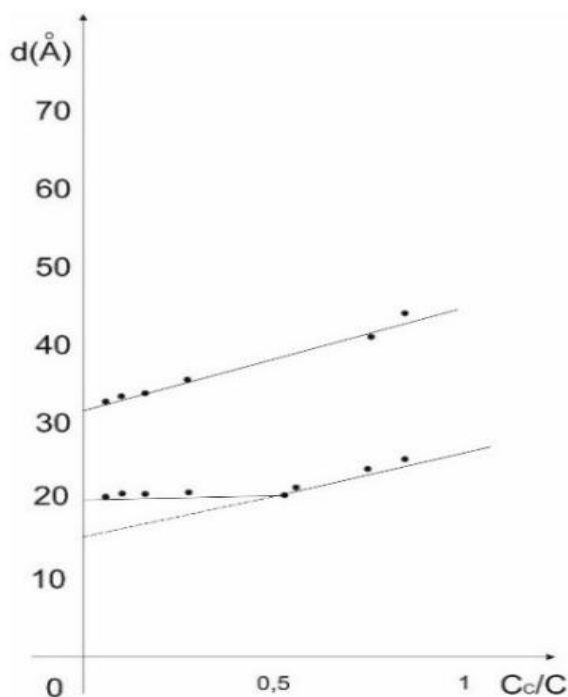
In the presence of forced mechanical tension – (pulling the sample into the capillary), there is a mutual orientation of the domains. X-ray diffraction patterns of samples of oriented domains are shown in Fig. 4 e.

Interplanar distances and relative intensities at different concentrations of AOT solutions are given in Table 1 [16].

Based on the data obtained, the dependence of the interplanar distance d on the ratio of water concentration (C_s) to the concentration of AOT (C) was constructed (Fig. 5).

Table 1. Interplanar distances and relative intensities of the system AOT–n–heptane–water.

| N | Concentration AOT % | C _s /C | Interplanar distans d in Å | Relative intensity |
|----|---------------------|-------------------|----------------------------|--------------------|
| 1 | 30 | 2,3 | 24 ÷ 80 | 5 |
| 2 | 47 | 1,1 | 24 ÷ 80 | 8 |
| 3 | 49 | 1,04 | 4,6; 24 ÷ 60 | 2; 6 |
| 4 | 53 | 0,88 | 4,6; 44; 23 ÷ 55 | 2; 7; 6 |
| 5 | 65 | 0,54 | 20,6; 20 ÷ 45 | 7 ; 4 |
| 6 | 66 | 0,51 | 20,56 | 8 |
| 7 | 78 | 0.28 | 20,6 ; 36,3 | 8; 6 |
| 8 | 85 | 0.18 | 20.56; 34,5 | 8; 6 |
| 9 | 90 | 0.1 | 20.64 ; 34,3 | 9 ; 7 |
| 10 | 92 | 0.08 | 20,7 ; 34,2 | 10; 8 |

**Fig. 5.** Dependence of the interplanar distance d on the ratio of the solution concentration (C_s) to the concentration of AOT (C).

5. Discussion

AOT molecules have high amphiphilic properties, as a result of which they form micellar solutions and liquid crystalline mesophases. Due to the amphiphilicity of AOT molecules, their lyotropic mesomorphism is manifested. The peculiarities of their behavior in a mixed solvent are determined by the balance of hydrophilic and hydrophobic interactions, which provide, on the one hand, the maximum contact of the polar groups of molecules with water, and, on the other hand, the contact of hydrocarbon sites mainly with each other and with the organic solvent n–heptane. At low AOT concentrations in a mixed solvent, inverted micelles are formed due to the larger amount

of n-heptane relative to water in the solution. From a monomer solution at a critical micelle concentration (CMC), spherical micelles with a polar core are formed, formed by hydrophilic head groups of AOT molecules, surrounded by a hydrophobic layer of alkyl chains "floating" in an organic solvent [17–19]. CCM for AOT in most organic solvents is $0.5 \div 3.0$ mM [20–23].

With an increase in the concentration of AOT, starting from 30% in a mixed solvent n-heptane – water, spherical micelles in the system become compressed ellipsoids, and with a further increase in the concentration, they become elongated in the form of layers with different thicknesses in an inhomogeneous solution within the boundaries of interplanar distances from 24 Å up to 80 Å. With an increase in the concentration of the system against the background of a dark halo near the primary beam, the detection of circles can be explained by the formation of lamellar layers in the system, alternating from water near the polar groups of AOT molecules and hydrocarbon chains with an organic solvent. An analysis of the registration data revealed that, over time, a "smooth" lamellar lyotropic-liquid-crystalline phase with intradomain lamellar structures appears in the AOT-mixed solvent sample; there is an alternation of parallel AOT layers of mono- (L_2) and bimolecular (L_1) thickness and water layers. Thus, in the lyotropic mesophase, at higher AOT concentrations, a liquid crystal structure and the presence of long-range order are found. However, the system is not homogeneous. Simultaneously, lamellae of monomolecular thickness coexist, starting with interplanar distances of 24 Å, and lamellae of bimolecular thickness, up to the limits of the possibility of experimenting interplanar distances of 80 Å. The reflex corresponding to the lamellar structure of the monomolecular thickness of 24 Å is located at the outer boundary of the dark halo, the corresponding interplanar distance of which decreases with increasing concentration of AOT, and the reflex becomes sharper and thinner. Correspondingly, the interplanar distance of the lamella of bimolecular thickness also decreases. Starting from a concentration of 65% AOT in a mixed solution, the interplanar distance of the lamella of monomolecular thickness remains constant at 20–21 Å, varying within 1 Å, and the second diffuse reflection is 33–36 Å. Extrapolating the straight line d from Cs/C to zero content of the mixed solvent, according to the data in Fig. 5, the thickness of the lamella of monomolecular thickness was determined: $d_l = 16$ Å, which corresponds to the length of one chain of the AOT molecule, inside the layer of which there are two chains together with an organic solvent immersed in each other. Comparison of the thickness of the lamella with half the length of the AOT molecule of the trans configuration, calculated by the formula [24].

$$l = 1.265n_c + 3\text{Å} \quad (1)$$

(n_c – is the number of hydrocarbon atoms in the AOT molecule, 3 Å the length of the polar head) and equal to 16.5 Å, suggests that the AOT molecules in lamellae of monomolecular thickness are immersed in each other. The difference $d - d_l = d_w$ represents the minimum thickness of the interlamellar water layer, i.e. the amount of water bound to the surface of polar groups of AOT molecules. This value is 4.5 Å (Fig. 4). As can be seen from the figure, at high AOT concentrations, the interplanar distances remain unchanged. At $C_s/C \leq 0.54$, they do not depend on the concentration of the mixed solvent. This indicates that "bound" water (hydrated water) remains in the interlamellar space, while in the organic space the chains of AOT and n-heptane are immersed in each other with a certain amount of n-heptane.

According to the method developed by Luzati, it is possible to determine the changes in the interplanar distances (d) of small-angle reflections on the concentration of the amphiphilic substance [24]. For the lamellar phase, this formula has the following form:

$$d = d_l \left(1 + \frac{pC_s}{p_s C} \right) \quad (2)$$

where p and p_s are the density of lamella and mixed solvent, C and C_s are the concentration of AOT and mixed solvent. Equation (2) makes it possible to determine the density of the lamella

without hydrated water, which is a measure of the structural organization of the hydrophobic core of the lamella. From the slope of the straight line d from C_s / C for the density of the AOT lamella, it was obtained at $C_s / C = 0.3$ $p = 0.883p_w = 0.9169 \text{ g / cm}^3$; at $C_s / C = 0.255$ $p = 0.9804p_w = 1.0787 \text{ g / cm}^3$, where $p_w = 1.1003 \text{ g / cm}^3$ [24].

Since the measure of the compactness of the arrangement of AOT molecules in the lamella, in addition to the density, is also the specific surface area S , i.e. the value of the area on the surface of the lamella falling on the polar group of one AOT molecule, then, having determined the values of the density p and the thickness of the lamella d_l , we obtain for the average interfacial area per polar group of one molecule the values for a 75% solution $S = 100.67 \text{ \AA}^2$, and for 80 % solution $S = 85.57 \text{ \AA}^2$.

The presence of uniform circles on the X-ray diffraction patterns indicates a chaotic arrangement of domains in the sample. But on the roentgenograms, crescent-shaped circles are also found (Fig. 4e). This occurs in the presence of mechanical forced stretching or when the sample is pulled into the capillary. It can be assumed that there is an orientation of the domains, and hence of the lamellae. The angle of inclination of the axis of the molecule can also be determined directly from the analysis of the intensity distribution relative to the equator (the equatorial direction is taken to be the direction parallel to the lamellar plane of the multilayers) in the X-ray diffraction pattern from the oriented samples along the intensity clumps (sickles).

Thus, replacing the water of the AOT–water system with a mixed solvent *n*-heptane–water, the concentration interval of lyotropic mesomorphism in the system was established. A liquid crystalline “smooth” phase is formed by coexisting lamellae of monomolecular thickness (L_2) with a hydrated water layer between the layers and a bimolecular thickness (L_1) with an excess of organic *n*-heptane. The structural parameters that determine the compactness of the liquid crystal state are revealed.

References

- [1] A.G. Petrov, Proc. 5 Winter Sch. Michatowice **1** (1979) 297.
- [2] S.E. Friberg, B. Thundathil, J.J. Stoffen, Science **205** (1979) 607.
- [3] K. Arnold, H. Frischleder, G. Klose, Phase Numwandlungen in Phospholipid Model Membrans (Wiss Z. Karl-Marx-Univ., Leipzig, 1976).
- [4] A. Wieslander, Y. Ulmius, G. Lindblom, K. Fontel, Biochem. Biophys. Acta **512** (1978) 241.
- [5] L. Powere, Biophys. J. **20** (1977) 137.
- [6] J. Schnakenberg, Surface Sci. Lect. Jut. Course **2** (1974) 69.
- [7] P.A. Winsor, Mol. Cryst. and Liquid Cryst. **12** (1971) 141.
- [8] J.L. Mateul, D.M. Suller, A. Tardieu, K.T. Gulik, V. Luzzati, J. Mol. Biol. **85** (1974) 249.
- [9] H.M. Gottlieb, Eares D.E., Biophys. J. **14** (1974) 335.
- [10] W. Albon, Y.M. Startevant, Proc. Nat. Acad. Sci. USA **78** (1978) 2258.
- [11] B. Pulman, I.M. Saran, Int. J. Quantum Chem. **9** (1975) 71.
- [12] W. Lesslauer, Acta Crystallografica **13–30** (1974) 1932.
- [13] P.L. Folgnier, T.E. Thompson, D.Y. Barenholz, D. Lichtenberg, Biochemistry **22** (1983) 1670.
- [14] R.P. Rand, S. Sengupta, Biochim. et Biophys. Acta **255** (1972) 484.
- [15] D.L. Dorest, Biochim. et Biophys. Acta, Biomembranes **203** (1987), 319.
- [16] P.B. Hitchcock, R. Mason, J. Mol. Biol. **94** (1975) 297.
- [17] A.R. Sarkisyan, G.A. Shahinyan, Sh.A. Markaryan, J. Physical Chemistry **88** (2014) 859.
- [18] Sh.A. Markaryan, A.R. Sarkisyan, G.A. Shahinyan, J. Physical Chemistry **89** (2015) 1606.
- [19] G.A. Shahinyan, H.R. Sargsyan, A.M. Kolker, S.A. Markaryan, Chem. J. of Armenia **69** (2016) 407.
- [20] J.J. Silber, Adv. Colloid Interface Sci. **82** (1999) 189.
- [21] J.L. Geick P. Bednarek, J. Mol. Struct. **555** (2000) 227.
- [22] A. Mohammad, S. Hena, Chromatography **25** (2004) 111.
- [23] S. Abel, F. Sterpone, S. Bandyopadhyay, M. Marchi, J. Phys. Chem. B **108** (2004) 19458.
- [24] V. Luzzati, D. Chapman, X-ray Diffraction Studies of Lipid-Water Systems in Biological Membranes (N.Y., 1968).