

Investigation of structural changes in the liquid crystal system AOT / n–heptane–water under the influence of vitamin E by the method of X–ray analysis

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Abstract. Structural rearrangements of the lyotropic liquid–crystal system sodium salt of di–(2–ethylhexyl) ester of sulfosuccinic acid (AOT) –n–heptane – water under the influence of vitamin E were studied by X–ray diffraction. The studies were carried out in the concentration range from 50 to 80% AOT+E in a mixed solvent n–heptane/water. The studies were carried out with a gradual increase in the concentration of vitamin E in the system and with an increase in the concentration of AOT + E in a solution of n–heptane/water. It has been established that, in the model liquid crystal system under study, vitamin E penetrating into the structure of the liquid crystal phase AOT–n–heptane–water leads to structural rearrangements and compactization of the molecular packing of the “smooth” phase.

Keywords: x–ray graphics, lyotropic liquid crystal, models of biomembranes, lamellar phase

1. Introduction

With the expansion of research in the field of the molecular structure of biological complexes, there was an urgent need to deepen the understanding of the principles of organization of biological liquid crystal structures. There are deep grounds for the great role of the liquid–crystal state in biological phenomena. This is primarily due to the dynamic capabilities of the structure while maintaining its internal order. This principle is important for understanding the essence of not only physiological processes, but also the occurrence of pathological situations. In light of these solutions, one of the most important problems is the problem of the structure and function of biomembranes. Studies of biomembranes have shown that membrane functions can be controlled by changes in the environment. A direct relationship between membrane function and phase state can be proven. A number of pathological conditions are based on changes in the properties of cell membranes caused by both environmental factors and internal functional disorders. Changes in the properties of biomembranes are based on structural changes, rearrangements of liquid–crystal states, and mesomorphism [1–3]. Numerous physiologically active substances, medicines, vitamins, etc. are taken to regulate the structure and function of biomembranes. [4–6]. Together with polyunsaturated fatty acids, vitamin E is incorporated into cell membranes, protecting them from oxidation more effectively than other antioxidants and protecting the outer shell of the cell from free radicals. Vitamin E is a powerful antioxidant that protects cells from the damaging effects of oxygen. As an antioxidant, vitamin E reduces the risk of developing a number of diseases, strengthens the immune system, and protects body cells. However, scientists are currently trying to establish all the detailed effects that vitamin E has on the human body [7–9]. It has been established that the prooxidant activity is due to vitamin E [10], while the antioxidant effect of vitamin E depends on its localization in the membrane [9]. In this circumstance, it is of interest to study structural changes in biological membrane models under the influence of vitamin E [11, 12]. Numerous studies have shown that lyotropic liquid crystals are typical representatives of biological membranes, and the validity of using the latter as models for

the organization of biomembranes has been proven [13–16]. One of the approaches to the study of such problems was developed in the works of Markaryan S.A. and others, where surfactants were used that form reverse micelles in water [17–19].

Using lyotropic mesophases in the study of the structure and function of biomembranes, in this work, we studied models of biomembranes based on the Aerosol(AOT)–*n*-heptane–water mixed solvent system under the influence of vitamin E. The latter necessitates studying the structure and nature of molecular rearrangements in lyotropic mesophases as 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 crystal properties of model membranes under the influence of vitamin E, the method of X–ray diffraction at small and large angles was used.

2. Experiment

The long–range ordering and the dimensions of the diffracting LLC cell were determined using X–ray diffraction. However, X–ray diffraction studies of VFAs require the solution of a number of technical and methodological problems. The samples were taken on X–ray installations URS–60 and URS–2 with cameras of the KRON, RKSO type designed for shooting X–ray diffraction at large angles on a flat film, which were modified to study small–angle scattering at sample distances from films of $0.05 \div 0, 1$ m. Redesigned, while the cameras make it possible to obtain reflexes in the range of angles from 1 to 45° .

We used tubes of the BSV–24 and BSV–11 types with an anticathode made of Cu, which gives radiation in the region of comparatively long waves. Copper $K\alpha$ lines were isolated using nickel filters with a thickness of 1.5×10^{-3} mm. Anode voltage 40 kV, anode current 20 mA. The exposure time was chosen 4 hours. Films of the RTG–B type from Primax Berlin Germany were used. To prepare the sample, thin–walled quartz capillaries (manufactured by Germany) with a wall thickness of 1×10^{-5} m and a diameter of 0.4×10^{-3} to 1×10^{-3} m were used. The absorption of rays and the appearance of the background on radiographs when using these capillaries is practically absent. The method of X–ray diffraction that we have reconstructed will allow us to obtain information about the size, shape, and compactness of the location of colloidal formations.

3. Samples

In this work, the structures of mesophases obtained on the basis of Aerosol–OT(AOT)/*n*-heptane–water – vitamin E were studied. We used 98% AOT from the Sigma–Aldrich company, the production name is Dioctylsulfosuccinate sodiumsalt, the formula is $C_{20}H_{37}NaO_7S$, analysis (according to USP) – purity 97.0 – 103.0%.

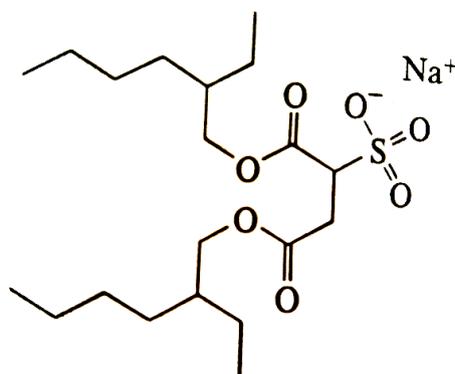


Fig. 1. Structural formula of the AOT molecule.

The used n-heptane was produced by Macrochem (Holland). Gross formula – C_7H_{16} .

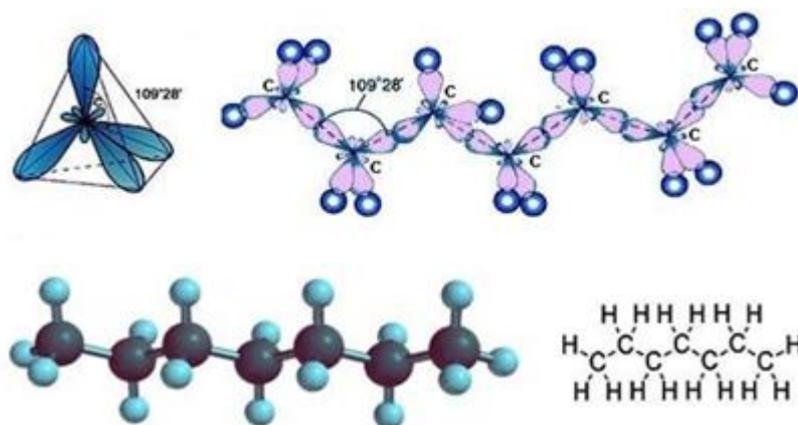


Fig. 2. Structural formula of the heptane molecule.

Solutions were prepared by dissolving AOT in n-heptane and then adding the appropriate amount of water, followed by vitamin E (α -tocopherol).

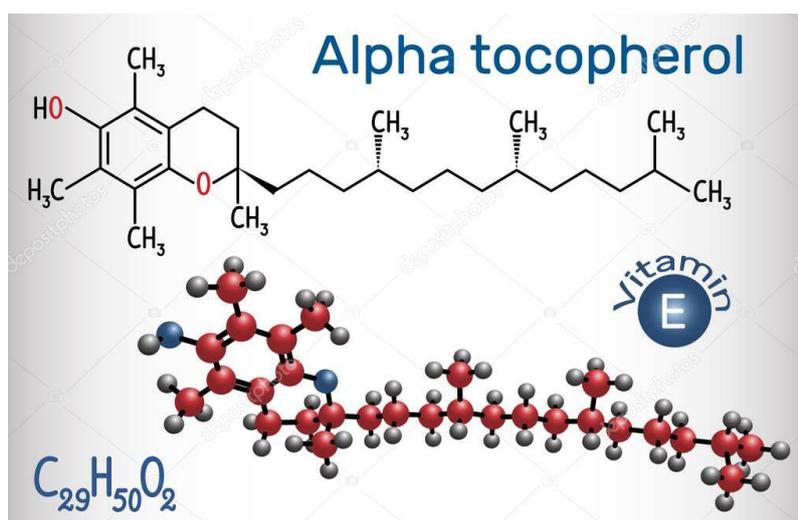


Fig. 3. Structural formula of α -tocopherol molecule.

Samples for X-ray diffraction were prepared according to the following procedure: heat treatment was carried out – before taking X-ray patterns, the sample was kept at a temperature of $40^{\circ}C$ for half an hour, after which an isotropic melt of the appropriate 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 certain intervals.

4. Results

The liquid-crystal system AOT+E-n-heptane-water was studied depending on the concentration of AOT+E in a mixed solvent. A mixed solvent was prepared at a ratio of n-heptane/water=1. Samples were taken with concentrations of AOT + E from 46% to 90% and “dry” solutions, which were obtained during open long-term keeping of the solution. “Dry” samples were taken without a capillary.

Reflexes were obtained on X-ray patterns, which determine the liquid-crystal structure of lipid molecules, which have uniform circumferences. Samples of the AOT+E-n-heptane-water system were studied with a content of 4.6% vitamin E in terms of the AOT ratio. Starting from low concentrations of the AOT+E system (46%) in a mixed solvent, a halo and a weak diffuse reflection at small angles appear on the X-ray patterns. With an increase in the concentration of AOT+E (53%), the reflection becomes granular, but its intensity remains weak (Fig. 4a). At a concentration of 56%, the grains become large (Fig. 4b). With a further increase in the concentration, AOT+E are separated and two distinct reflections are formed near the primary beam at small angles (Fig. 4c). As the concentration of AOT+E increases, the intensity of the second reflection increases and is clearly expressed at a concentration of 82% (Fig. 4d).

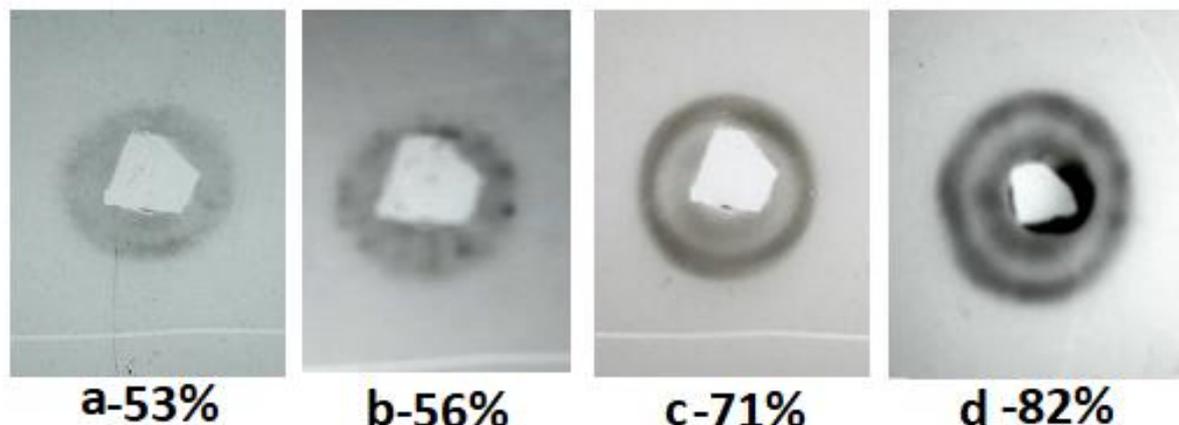


Fig. 4. X-ray diffraction patterns of AOT+E-n-heptane-water sample depending on concentration a – 53%, b – 56%, c – 71%, d – 82%.

Table 1 shows the values of interplanar distances at different AOT+E concentrations in a mixed solvent, where C_p is the solvent concentration, C is the AOT+E concentration. The relative intensities of the reflections revealed on the X-ray patterns were also determined.

Table 1. Interplanar distances and relative intensities of the AOT+E-n-heptane-water system.

N	Concentration AOT %	C_p/C	Interplanar distance d (Å)	Relative intensity
1	46	1,17	33	2
2	51	0,96	31	3
3	53	0,89	29	2
4	56	0,79	28	4
5	61	0,6	27	6
6	71	0,4	25; 30,5	7; 5
7	76	0,31	23,5; 31	8; 6
8	82	0,2	22; 30	9; 7
9	90	0,1	21,5; 30,5	9; 8

Changes in structural characteristics at different concentrations of vitamin E in the 46% and 63% AOT+E systems were also studied in the work.

Table 2. Interplanar distances and relative intensities of 46% concentrations of the AOT+E-n-heptane-water system depending on the concentration of vitamin E.

N	Concentration Vitamin E in%	Interplanar distance d (Å)	Relative intensity
1	1	4,6; 24 ÷ 60	2; 3
2	2,7	28 ÷ 60	3
3	3	28 ÷ 60	5
4	3,5	28 ÷ 40	5
5	4	35	5
6	4,6	33	6

Table 3. Interplanar distances and relative intensities of 63% concentration of the AOT+E-n-heptane-water system depending on the concentration of vitamin E.

N	Concentration Vitamin E in%	Interplanar distance d (Å)	Relative intensity
1	1	21	6
2	2,7	21,3	7
3	3,5	23	8
4	4,5	24,5; 30	9; 6
5	4,7	26; 30,5	9; 8

Based on the data obtained, the dependence of the interplanar distance d on the ratio of water concentration (C_p) to the concentration of AOT + E (C) was plotted (Fig. 5).

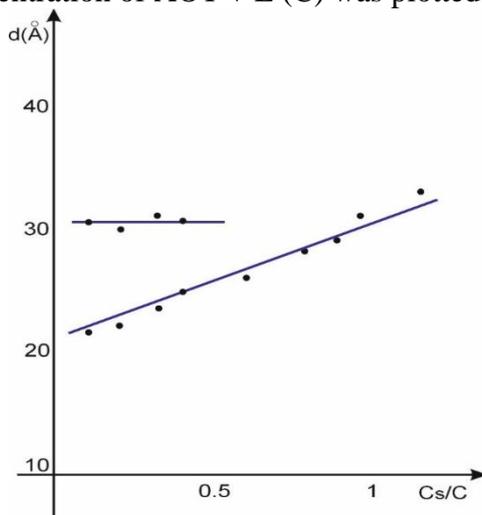


Fig. 5. Dependence of the interplanar distance d on the ratio of the concentration of the mixed solvent (C_p) to the concentration of AOT + E (C) at a content of 4.6% vitamin E in relation to AOT

5. Discussion

Due to the high amphiphilic properties of AOT, they form liquid-crystalline mesophases, where lyotropic mesomorphism is manifested. The features of their behavior in a mixed solvent are determined by the balance of hydrophilic and hydrophobic interactions, which ensure, 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.

AOT in a mixed solvent n-heptane – water at much higher concentrations forms a lyotropic lamellar mesophase (53%), where a liquid crystal structure and the presence of a long-range order are detected. However, the system is not homogeneous. Initially, only lamellae of bimolecular thickness (44 Å) are formed [20]. With a further increase in the concentration in the lyotropic

mesophase, at higher AOT concentrations, a “smooth” liquid–crystalline mesophase is revealed. Simultaneously, lamellae of monomolecular and bimolecular thicknesses coexist.

The influence of vitamin E on the AOT system in the mixed solvent *n*-heptane – water 4.6% leads to an increase in the rate of formation of the liquid crystalline mesophase at significantly low concentrations of AOT + E in the mixed solvent. Starting from a concentration of 46%, a weak reflex is separated, which is characteristic of the lamellar structure of lipid molecules. With increasing concentration, the interplanar spacing of the layered structure decreases. At concentrations of 50–55%, the identified circles on the X–ray patterns have a granular structure (Fig. 4a). This is explained by the fact that, in solution, spherical micelles, with an increase in concentration, turn into compressed ellipsoids, which, being attached to each other, stretch in large domains in the form of layers of different thicknesses. A further increase in concentration (by evaporation of the solution) leads to enlargement of the domains, due to the fact that in an inhomogeneous solution, wandering free molecules, being compacted, gather in formed aggregates (in domains) increasing their size (Fig. 4b). Over time, the ordering of the structure increases and the size of the domains decreases. This is evidenced by the appearance of homogeneous circles on X–ray patterns over time and with a further increase in concentration (Fig. 4c). However, a second reflex of weak intensity also appears on the radiographs. At high concentrations, the intensity of the second reflection increases (Fig. 4e). From the graph shown in Fig. 5, it can be seen that, compared with the results of studies of this system without vitamin E [20], with increasing concentration, the interplanar distance for lamellae of monomolecular thickness decreases and remains constant for lamellae of bimolecular thickness.

Studies of structural rearrangements of the AOT+E-*n*-heptane–water system on the dependence of the concentration of vitamin E in a 46% solution showed that a gradual increase in the concentration of vitamin E from 1 to 4.6% promotes the formation of a “smooth” phase when without vitamin E this phase is formed only at a concentration of 65% solution. The effect of vitamin E (from 1 to 4.7%) on a 63% solution of the AOT+E-*n*-heptane–water system leads to an increase in interplanar distances compared to the system without vitamin E.

From the above data and the above discussions, it can be assumed that vitamin E is incorporated into lipid layers, increases the structural packing and compactness of the liquid–crystalline “smooth” phase, thereby being incorporated into cell membranes, and protects the outer shell of the cell. Since vitamin E is fat soluble, it retains moisture and can be expected to promote cell renewal.

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