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Lithocholic acid derivative in the presence of dimethyl sulfoxide: Morphology and phase transitions

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ABSTRACT

We report on the properties of a new organogelator, which is an ether derivative of lithocholic acid (70PhOLCA). The correctness of the chemical structure and purity of 70PhOLCA was confirmed by thin layer chromatography, proton nuclear magnetic resonance (¹H NMR) spectroscopy, elemental analysis (EA) and infrared spectroscopy (IR). Phase transition temperatures and enthalpies of the gel were obtained by differential scanning calorimetry (DSC) and polarizing optical microscopy (POM). Changes in the vibrational spectra depending on the temperature modifications were studied using the technique of FTIR Spectroscopy with 2D correlation analysis. The small angle neutron scattering method (SANS) was used to determine the morphology and internal structure of the investigated system. It was found that the substance forms a non-transparent stable gel with a spherulite organization at the macro-scale level. The morphology of the self-assemblies and internal structure at the nano-scale level are quite different with variation of the temperature in the Gel phase. A further increase in temperature leads to the formation of the sol phase again. It turns out that the temperature of the gel-sol transition changes significantly with the concentration of 70PhOLCA. The results of the DSC and SANS measurements indicated the reversible behavior of the Gel-SolGel transition with hysteresis on the temperature during heating and cooling.

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1. Introduction

Over recent years an interest in supramolecular gels based on LMOG's (low molecular mass organic gelators) has been increasing, as these substances can constitute an alternative biomaterial to polymer gels. Since 1990 the number of publications on gels has increased from near five to at least five hundred papers per year [1]. The potential applications of LMOG's arise from the fact that they are sensitive to physical stimuli, such as temperature, light, ultrasound or chemical stimuli: metal ions or anions [2]. LMOG's are used in drug delivery, three-dimensional cell culture [3], tissue engineering and regenerative medicine or photoelectronics [4].

Both natural and synthetic substances may be used as organogelators. They may be applied as three-dimensional (3D) networks formed from derivatives of peptides [5,6], saccharides [7], urea's [8], or as natural nucleobases hydrogels [9–11].

Sterols – natural solid alcohols – are also commonly used for the synthesis of hydro- and organogelators. Their chemical structure is based on a saturated tetracyclic hydrocarbon perhydrocyclopen-

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http://dx.doi.org/10.1016/j.tca.2016.09.013 0040-6031/© 2016 Elsevier B.V. All rights reserved. tanophenanthrene system, usually known as a steroid or sterane nucleus [12]. The steroid system has an elongated shape and is sufficiently stiff. Conformational changes within the ring under the influence of a solvent are not possible. LMOG's based on sterols are most often formed as long, thread-like structures that can easily immobilized solvent molecules. Additionally, thanks to the presence of one or more hydroxyl groups, they can readily form hydrogen bonds with water or organic proton acceptor solvents.

A series of azo- and polyhydroxy derivatives forming the gels has been characterized in the literature. Some of them can even consist of the di-sterane system [13]. Steroids are substances that are prevalent in nature and they contain easy-to-access hydroxyl groups. They can easily form a hydrogen bond with the proton acceptor molecules.

Latest papers report on self-assembly LMOG's with 2,3dihydroxycholestane steroids [14]. As it was described in these papers, the presence of two hydroxyl groups in the molecule should facilitate the formation of hydrogen bond connections. This makes them ideal as organogelators.

During our studies it was found that only one isomer with transdiaxial hydroxy groups was able to create a hydrogel with the proposed solvents. Moreover, the presence of the gel was observed only in chloride solvents or benzene or DMSO. The phenomenon





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Fig. 1. Scheme of synthesis of 4-heptyloxyphenylo-lithocholic acid ether - 70PhOLCA.

of self-assembly after dissolving this derivative alkyl alcohol and higher alkanes did not occur. This indicates that the type of a solvent and the conformation of the asymmetric center at which the hydroxyl group is substituted have a significant influence on the gelation process.

In recent years a new group of ionic and neutral sterols derivatives containing basic amino groups has been described [15]. An ionic derivative forms the Gel phase only with chloride benzene. Dietoxyamino-derivatives are able to self-assembly in chloride benzene or in a solvent with an addition of water, but not in pure water.

A special group of sterols are bile acids, which are characterized by the presence of one or more hydroxyl groups in the sterane ring. The presence of the carboxyl group further enhances the ability of these substances to form intermolecular hydrogen bonds. The main representative of this homologous series is lithocholic acid (LCA) [16].

It is known that a molecule of LCA can easily form hydrogen bonds with proton donor-acceptor solvents. This feature makes the dimethyl sulfoxide (DMSO) is an ideal solvent. It has the ability to form hydrogen bonds thanks to the presence of the carbonyl oxygen and acidic protons in the methyl group. Moreover, in small concentrations it is non-toxic to cells [17].

In this work we present a new derivative of LCA, 4-heptyloxyphenyloxylithocholic acid ether (70PhOLCA) as an organogelator in deuterated dimethyl sulfoxide (DMSO-*d*₆). Five calibration solutions with different 70PhOLCA concentrations in DMSO-*d*6 were prepared for checking the gelation degree. The resulting gels were characterized by differential scanning calorimetry (DSC), transmitted light intensity (TLI), polarized optical microscopy (POM), small angle neutron scattering (SANS) and the technique of two-dimensional Fourier transform infrared correlation spectroscopy (2D-FTIR).

2. Material and methods

2.1. Synthesis

The synthesis of the organogelator – 4-heptyloxyphenylolithocholic acid ether (70PhOLCA) – was carried out starting with lithocholic acid (acronym–LCA) (99.8% purity), which was purchased from Sigma–Aldrich Company. Phase behavior of this compound was described in the literature [16]. The first step of the synthesis was the esterification reaction of lithocholic acid using dried methanol (Fig. 1). LCA (Fig. 1A) had been previously dried over phosphorus oxide (P_2O_5). The methanol was a reaction medium and a solvent. The methanol of purity 99,8% was purchased from Sigma–Aldrich Company. Methanol was further dried by addition of magnesium turnings and distillation of the protection from moisture. 3 g LCA was dissolved in 45 ml anhydrous methanol. The reaction was stirred for about 15 h at a temperature of 35-40 °C and was monitored by thin layer chromatography (TLC). The eluate was a mixture of methylene chloride: acetone in a ratio of 9:1. After the reaction, the methanol was removed by rotary evaporation. The methyl ester of lithocholic acid (Fig. 1B) was isolated using column chromatography.

The next step was etherification of the hydroxyl group of the ester (B) using 4-heptyloxy-4'- hydroxybenzene (OHC₆H₄OC₇H₁₅) in Mitsunobu reaction [18]. The product was purified using column chromatography (eluate dichloromethane). The resulting ether was hydrolyzed in a mixture of water-ethanol (1:1) at 40–45 °C for 10 h. The hydrolysis was monitored by thin layer chromatography. The crude 4-heptyloxyphenylolithocholic acid 70PhOLCA (Fig. 1C) was purified using column chromatography (eluate dichloromethane–methanol 95:5) and by crystallization from ethyl acetate (three times) to constant melting point, T_m = 128.7 °C.

The purity of 70PhOLCA was denoted by TLC (R_f =0.738, CH₂Cl₂:CH₃OH 9.5:0.5) and spectroscopy method ¹H NMR, ¹³C NMR (Varian 400 MR), Fig. 2.

The correctness of the structure of 7OPhOLCA was verified by ¹H NMR, ¹³C NMR, IR and elemental analysis: ¹H NMR (400 MHz, CDCl₃ δ H 7.26 ppm, 25 °C, TMS): δ 6.82–6.84 (d, 2H, –Ph orto to –O–Ph), δ 6.81–6.79 (d, 2H, –Ph orto to –O-steroid), δ 4.46 (s, 1H, O–CH–, ring A), δ 3.90–3.87 (t, 2H, –CH₂- α to COOH), δ 2.44–2.36 (m, 1H, steroid), δ 2.29–2.22 (m, 1H, –steroid), δ 2.17–1.99 (m, 2H, –CH₂-, β to –OPh), δ 1.98–1.71 (m, 9H, steroid), δ 1.58 –1.25 (m, 19H, aliphatic and steroid), δ 0.93–0.87 (m, 6H, –CH₂- aliphatic), δ 0.98 (s, 3H, –CH₃ steroid), δ 0.93–0.87 (m, 6H, –CH₃, steroid), δ 0.66 (s, 3H, –CH₃, aliphatic). ¹³C NMR (400 MHz, CDCl₃ δ C 77.00 ppm, 25 °C): δ 120.04 (<u>COO</u>), 153.23 (ArC–O-steroid), 151.53 (ArC–O–R), 117.62 (Ar2<u>C</u>, orto to O-steroid), 115.26 (Ar2<u>C</u>, orto to O–R), 73.27 (<u>C</u>–O, ring A steroid), 68.53 (aliph.-C-O–R), 56.62 (steroid),



Fig. 2. NMR spectrum of 70PhOLCA, (a) ¹H NMR, (b) ¹³C NMR.

ringC–<u>C</u>(H)-ringD), 55.95 (steroid, ringD–<u>C</u>–R₁), 42.77 (steroid, ringC–<u>C</u>(CH₃)-ringD), 40.23–12.06 (25<u>C</u> steroid and aliphatic). **IR**: υ OH– 3415.69 cm⁻¹; asymmetric υ CH– 2929.72 cm⁻¹; symmetric υ CH– 2859.09 cm⁻¹; υ C=O 1705.12 cm⁻¹; υ C=C 1508.16 cm⁻¹;

C–O–H in plane 1448.46 cm⁻¹ – 1383.16 cm⁻¹; δ O–H 1227.68 cm⁻¹; υ C–O 1035.62 cm⁻¹. **EA:** C-77.94%, H-10.02% (calculated: C-78.09%, H-10.11%)

2.2. Samples preparation

For SANS experiment the gel samples were prepared by dissolving white crystalline powder of 70PhOLCA at room temperature in deuterated DMSO-d₆ with concentration: "S1"–0.005 g/ml, "S2"–0.01 g/ml, "S3"–0.015 g/ml, "S4"-0.02 g/ml and "S5"-0.025 g/ml. DMSO-d₆, isotopic purity 99,8% was purchased from Deutero GmbH Company. A deuterated solvent is used in order to enhance the neutron contrast and therefore the sensitivity of the SANS technique. For better homogenization the solutions were mixed using a shaker at temperature T = 60 °C and cooled to 20 °C. The samples for SANS experiments were placed in quartz cells as shown in Fig. 3 and incubated at room temperature for 12 h. A non-transparent, stable gel was observed in the concentration above 0.015 g/ml. In this publication we present the results of optical, spectroscopy and calorimetry experiments for the samples in the concentrations of 0.015 g/ml and 0.025 g/ml.

2.3. Experimental techniques

DSC measurements at the rate of 5deg/min were performed using a DSC 822^e Mettler Toledo Star System calorimeter. Calibration of the DSC822^e Mettler Toledo Star^e System was performed using indium. The results of the indium check are within the tolerance limits defined by Mettler Toledo: Onset temperature: 156.6 °C (\pm 0.3 °C), our results 156.3 °C; Heat flow 28.45 J/g (\pm 0.6 J/g), our results 27.89J/g, Our calibrations correspond to the requirements of the Mettler Toledo Star^e Thermal Analysis System (User Handbook, Instruction of the Mettler Toledo).

IR spectra of the gel materials (concentration 0.015 g/ml) were recorded using a FTIR Nicolet Magna 760 spectrometer. The measurements were performed with a minimum of 64 co-added scans at a resolution of 1 cm⁻¹. For temperature-dependent FTIR measurements the Linkam temperature controller was used, operating at $\pm 2^{\circ}$ /min. The temperatures were stabilized and kept constant while taking FTIR scans. Baseline correction was performed using the PeakFit v4.12 [19]. The distribution of peaks on FTIR scans was mathematically described by fitting Gauss functions to the data. The number of peaks, peak centers and band shapes was assigned before fitting. The heights, areas and widths (full-width at half-maximum, FWHM) were allowed to vary during fitting. The sample of the gel at room temperature was placed between the ZnSe windows. During heating and cooling, the spectra were taken at 30 °C, 40 °C, 42 °C, 44°C, 46°C, 48°C, 50°C, 52°C, 54°C, 56°C, 58°C. The implementation of spectrum below room temperature was not possible for reasons related to the apparatus.

Small Angle Neutron Scattering investigations were performed at YuMO time-of-flight spectrometer at the IBR-2 pulsed reactor (Dubna, Moscow region, Russia). The studied samples were placed in 1 mm thick quartz cells (Hellma, Germany). During data collection the samples were kept in the temperature controlled holder $(\pm 0.2 \,^{\circ}\text{C})$ connected to the liquid thermostat (Lauda, Germany). Standard data acquisition time per sample was 30 min. Two ring wire He³-detectors [20] at distances of 4 m and 13 m from the sample position were used in our experiment. The scattered intensity (differential cross section per sample volume) was registered as a function of the momentum transfer modulus $q = (4\pi/\lambda) \sin(\theta/2)$, where θ is the scattering angle and λ is the incident neutron wavelength. An incident neutron beam distribution provides an available wavelength range of $0.5 \div 8$ Å, which corresponds to momentum transfer range q of $0.007 \div 0.5 \text{ Å}^{-1}$. The raw data treatment was done by the SAS program [21]. The measured SANS spectra were converted to the absolute scale by normalization to the incoherent scattering cross section of a standard vanadium sample. Additionally the measured spectra were corrected taking into account scattering from the setup and empty cells, as well as the background. The final small-angle neutron scattering curves are presented in the absolute scale with background subtraction [22]. The analysis of the SANS spectra was performed by the models proposed in the next sections using SasView software [23]. The morphology of the samples at the macro-scale was monitored using an optical microscope OLYMPUS SZX – ILLK 200 with max magnification of 6.3 x (OLYMPUS OPTICAL CO (EUROPA) GMBH, Hamburg, Germany).

3. Result and discussion

3.1. DSC measurements

For the organogelator samples with the concentration of 0.015 g/ml (mass 14.02 mg) two full cycles of heating and cooling at a rate of 5°/min were performed. The second cycle is shown in Fig. 4. The phase transition between the Crystal and Gel phases was observed during heating in the range of temperature: from $T_0 = -5.25 \text{ °C}$ to $T_e = 6.32 \text{ °C}$ with a peak temperature $T_p = 4.72 \text{ °C}$ (T_o , T_e —onset and endset temperature) with a high value of enthalpy amounting to $\Delta H = 61.21$ /g.

Additionally, we have observed transitions with small enthalpy in the Gel phase. These transitions will be discussed in detail the next section. Here, we indicate the physical parameters of the transitions Gel 1 (G1) \rightarrow Gel 2 (G2) and Gel 2 (G2) \rightarrow Gel 3 (G3) only.

The transition from G1 to G2 occurred at 28.42 °C (T_o)-34.92 °C (T_e), (Δ H=0.20J/g). The second transition from G2 to G3 was observed above $39.82 \degree C (T_0) (\Delta H = 0.03 J/g)$. The phase transition to Sol was not observed. The substance did not decompose, while heating up to 60°C. The cooling cycle was performed immediately after the heating cycle. A minor change heat was observed at 18.58 °C (Δ H = 0.21J/g). It was probably characterized by the transition from Sol to G3. This means that the sample was going to at least Sol state during heating. However, the heat of this transition was not high enough for its registration by DSC method. G3 appeared in the range of about 7.50 °C. Then, at 14.99 °C the transition to G2 (Δ H = 0.08]/g) was observed. A suitable rate of measurement made it possible to observe of the transition from G2 to G1 phase at 11.46 °C (Δ H = 0.09J/g). Crystallization of the substance was identified due to an exothermic peak in the range from $T_0 = -5.37 \degree C$ to $T_e = 7.53 \circ C (\Delta H = 81.90 J/g).$

In order to verify the effect of the concentration of the gel on the phase transition temperatures shifts, the DSC experiment for a sample with the concentration of 0.025 g/ml (26.21 mg) was conducted at a rate of 5deg/min. During heating an endothermic peak of melting Crystal phase to G1 phase at $T_p = 4.37 \degree C (1 \text{ cycle}, \Delta H = 52.99 \text{J/g})$, 4.62 °C (2 cycle, $\Delta H = 56.74 \text{J/g}$) and 4.78 °C (3 cycle, $\Delta H = 57.93 \text{J/g}$) was observed. Unfortunately, the transitions G1 \rightarrow G2 and G2 \rightarrow G3 were not registered in DSC measurements for this sample. That is the reason to make SANS experiment.

The greatest change in the phase transition temperature during the heating cycle was observed for the phase transition from Gel 3 to Sol phase. For a sample with the concentration of 0.015 g/ml the phase transition occurred in the range of temperatures $39.82 \,^{\circ}\text{C}-43.08 \,^{\circ}\text{C}$. A precise determination of this temperature for sample "3" was not possible, which was due to the fact that this transition was not observed using DSC method. For sample "5" (concentration of 0.025 g/ml) the temperature of phase transition from Gel 3 to Sol phase was characterized by an exothermic peak in a temperature range from $T_0 = 28.75 \,^{\circ}\text{C}$ to $T_e = 35.27 \,^{\circ}\text{C}$ ($\Delta\text{H} = 1.29$]/g).



Fig. 3. Solutions of gel 70Ph0LCA in DMSO-d₆ in concentrations: 1–0.005 g/ml; 2–0.010 g/ml; 3–0.015 g/ml; 4–0.020 g/ml; 5–0.025 g/ml at room temperature.



Fig. 4. Thermograms of full cycle heating and cooling for 7OPhOLCA in DMSO-*d*₆ (0.015 g/ml), in the range of $-20 \,^{\circ}$ C to $60 \,^{\circ}$ C with a rate of 5deg/min for a sample with mass 14.02 mg.



Fig. 5. SANS curves (symbols) with fits (lines) for 7OPhOLCA in DMSO- d_6 at concentrations C = 0.005, 0.010, 0.015, 0.020 and 0.025 g/ml at T = 10 °C after single heating-cooling cycle. The dashed lines indicate a border between regions of low and high q-values; q₁ and q₂ correspond to the positions of the Bragg peaks maxima. The OM images were obtained for samples incubated in quartz cells at 10 °C during 12 h. Inset: variation of the correlation length L and exponent m with concentration.

3.2. Small angle neutron scattering (SANS)

The images of the 7OPhOLCA in DMSO- d_6 with C \geq 0.010 g/ml (shown in Fig. 5) are obtained using OM indicating the spherulite organization with a radius of about several millimeters at room temperature. The similar structure has been found in gels based on LMOG's. CNC (5 α -cholestan-3 β -yl N-(2-naphthyl) carbamate)/n-alkane sols, when cooled rapidly to 0 °C and incubated there, are known to yield gels with self-assembled fibrillar networks in the form of highly branched, small fibers in spherulitic aggregates [24,25]. The formation of the tubular spherulites in an aqueous solution at pH=7.5 has been observed in self-assembling lithocholic acid [26].

3.2.1. Morphology and internal structure

The morphology and internal structure of self-assemblies at the nano-scale was performed by small angle neutron scattering. The total SANS intensity I(q) is a sum of three contributions

$$I(q) = I(q)_{L} + I(q)_{S} + I_{Incoherent}$$
(1)

The first term in Eq. (1) describes the scattering from large self-assemblies and corresponds to the region at low q values $(q < \sim 0.1 \text{ Å}^{-1})$, where the shape of the investigated samples can be obtained. In this region the scattering cross-section does not depend on the particles interaction. The fractal dimension D can be determined in this region using the Porod regime $(\log I(q) vs. \log q)$ [27]. In this case the scattering intensity is determined by a power law:

$$I(q)_L \sim q^{-n} \tag{2}$$

where n is a power-law (Porod) exponent, which gives information assemblies at the length scale of \sim 100–900 Å. It should be noted about the morphology of the self- that the size of large selfassemblies is apparently quite bigger than the d-space which can be determined from the available q region by SANS method. The n value is related to a surface fractal dimension as $D_s = 6 - n$, when n varies from 3 to 4 or the mass fractal dimension as $D_m = n$, when n has a values of 1-3 [28]. The D_s = 3 indicates the surface with maximum roughness, while at $D_s = 2$ a Porod scattering $(I(q) \sim q^{-4})$ exhibits a perfectly smooth surface and can be interpreted in our case as a scattering from self-assemblies with a sharp interface to the surrounding solvent. The fractal dimension D_s less than 2 indicates that the surface is diffuse. The second term $I(q)_S$ in Eq. (1) originates from small structures at the length scale \sim 100–10 Å and characterizes the interactions between small self-assemblies or individual molecules and solution. Ornstein-Zernike (Lorentzian) equation is commonly used to describe the solution scattering from self-assembled hydrogels [29,30]:

$$I(q)_{S} = C/[1 + (qL)^{m}]$$
 (3)

where m is a Lorentzian exponent, L is a correlation length and C is a constant. Then the SANS data can be analyzed using a simple function:

$$I(q) = A/q^{n} + C/[1 + (qL)^{m}] + B$$
(4)

In Eq. (4), B is a q-independent (mostly incoherent) scattering background.

The SANS spectra for 70PhOLCA in DMSO- d_6 at concentrations C = 0.005, 0.010, 0.015, 0.020 and 0.025 g/ml at T = 10 °C in a double log scale (log I(q) vs. log q) are presented in Fig. 5. The scattering at high q region is characterized by two Bragg peaks with positions of the maxima in the relation q₁:q₂ = 1:2, indicating the lamellar structure of the system. The corresponding inter-lamellar distance can be calculated as $d_N = N 2\pi/q_N$. This is confirmed by the result of [31] where the lamellar structure of lithocholic acids (0.1 wt%)



Fig. 6. Variation of the SANS spectra with temperature increase for 70PhOLCA in DMSO- d_6 at C=0.015 g/ml. Inset: SANS spectra (circles) for S3 at T=20 °C during cooling; red line is the result of the SANS spectra fitting by model M2; green dashed line is the SANS data analysis using the model M3; blue dashed line shows the contribution of the scattering by the cylinders (Eq. (7)). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

recrystallized from DMSO/H2O (1:9) at the molecular length scale was observed by atomic force microscopy and wide angle X-ray scattering.

In order to analyze the measured SANS data, the following model (M1) is used

$$I(q) = A/q^{n} + C_{1}/[1 + (|q-q_{1}|L)^{m}] + C_{2}/[1 + (|q-q_{2}|L)^{m}] + B(5)$$

where A, n, m, C_1 , C_2 , L, q_{N1} and B are all floating parameters in the nonlinear least squares fits to the SANS data.

Analysis of the SANS data in the low q region specified the Porod exponent n = 2.4 and n = 4 for 7OPhOLCA in DMSO- d_6 with C = 0.005 g/ml and C > 0.010 g/ml, respectively. It means that the morphology of the self-assemblies changes with the increasing concentration up to 0.010 g/ml only. The self-assemblies exhibit a sharp interface to the surrounding solvent while the studied system has the spherulite organization at the macro-scale. Nevertheless all investigated samples demonstrate a short-range order. The inter-lamellar spacing decreases with the increasing concentration: 50.2 ± 0.5 Å (S1), 45.9 ± 0.1 Å (S2) and 44.5 ± 0.1 Å (S3–S5). It is remarkable that the computed end-to-end distance of the 70PhOLCA molecule (l_m) in optimized geometry equals 25.6Å (MOPAC2016, PM7). The value of 50.2 Å is approximately of a doubled molecule length. Thus it can be assumed that the lamellar structure is formed by the repetition of the dimers elongated orthogonally to the plane of the layer as it was discussed in details in the case of bile acids [31]. The statement about the dimer associations is based on the nature of the 70PhOLCA molecules that make hydrogen bonds between the COOH groups only as compared to the LCA molecules. In lithocholic acid the COOH group of one molecule may associate itself in hydrogen bonding with the COOH (predominantly) or OH groups of another LCA molecule [26,31]. Decreasing of the d-value for samples S2–S5 induced by the increasing concentration can be interpreted as the interdigitation, perhaps combined with molecular tilting with respect to the lamellar planes. At the same time the coherent length increases from 34.5 ± 5.8 Å to 79.2 ± 10.5 Å, while the exponent m decreases from 3.7 ± 0.1 Å to 2.1 ± 0.4 (shown in Fig. 5).



Fig. 7. Optical microscopy image for 70PhOLCA in DMSO- d_6 at concentration C = 0.025 g/ml (A) after preparation, (B) after 4 days (inset: max. magnification of $6.3 \times$ for the crystallization center) and (C) after 14 days.



Fig. 8. SANS curves for 7OPhOLCA in DMSO-d₆ with concentrations C = 0.015, 0.020 and 0.025 g/ml at T=20 °C after preparation (*left*) and after 14 days (*right*).



Fig. 9. Diagram of the Sol-Gel transition in depending on the temperature and sample concentration for (A) heating and (B) cooling.

Table 1

The variation of the parameters Porod exponent (n), Lorenzian exponent (m), correlation length (L), radius (r_c) and length (l_c) of the 7OPhOLCA molecule with temperature during heating (\uparrow) and cooling (\downarrow). The data are obtained for 7OPhOLCA in DMSO- d_6 with C = 0.015 g/ml (S3) and C = 0.025 g/ml (S5) using Models M1, M2 and M3 for analysis the SANS spectra: M1 (Eq. (5)) for G1 state, M2 (Eq. (4)) and M3 (Eq. (9)) for G2, G3 and Sol state.

T (°C)		n		m		L, Å			n		l _c , Å		r _c , Å	
		S3	S5	S3	S5	S3	S5		S3	S5	S3	S5	S3	S5
10 ↓	M1	3.9(0.1)	3.9(0.1)	2.5(0.3)	2.1(0.4)	64.1(4.9)	79.2(6.9)							
20 ↑	M1	3.7(0.1)	3.9(0.1)	1.5(0.3)	1.5(0.2)	83.2(5.3)	82.2(7.1)							
20	M1		3.9(0.1)		1.8(0.3)		30.7(4.2)							
\downarrow	M2	3.5(0.6)		2.9(0.4)		5.6(0.6)		M3	3.6(0.5)		24.5(0.8)		6.6(0.6)	
25 ↑	M1	3.8(0.1)	3.8(0.1)	1.9(0.4)	1.4(0.2)	23.4(2.7)	58.9(4.8)							
25 ↓	M2	3.8(0.3)	3.8(0.1)	3.6(0.4)	3.5(0.3)	5.6(0.3)	5.7(0.4)	M3	3.9(0.6)	3.8(0.5)	24.6 (0.7)	25.0(0.8)	7.1(0.6)	8.3(0.6)
35 ↑	M2	3.5(0.4)	3.1(0.3)	3.6(0.3)	3.8(0.2)	5.6(0.4)	5.7(0.3)	M3	3.5(0.3)	3.1(0.6)	24.1(0.9)	24.3(1.2)	6.9(0.7)	8.4(0.8)
35 ↓	M2	3.7(0.5)	3.5(0.2)	3.6(0.4)	3.4(0.5)	5.4(0.3)	5.9(0.4)	M3	3.8(0.5)	3.6(0.4)	24.2 (0.6)	25.3(0.8)	7.1(0.6)	8.5(0.9)
45 ↑	M2	3.2(0.5)	3.2(0.2)	3.4(0.3)	3.2(0.3)	5.5(0.7)	5.6(0.4)	M3	3.3(0.4)	3.3(0.3)	23.8(0.5)	24.0(0.9)	7.1(0.9)	8.2(0.7)
45 ↓	M2	2.8(0.3)	3.3(0.3)	3.4(0.4)	3.3(0.4)	5.3(0.6)	5.8(0.5)	M3	2.9(0.4)	3.5(0.4)	23.8(0.9)	24.7(1.0)	7.0(0.3)	8.7(0.9)
55 ↑	M2	3.1(0.3)	2.6(0.1)	3.4(0.2)	3.4(0.3)	5.2 (0.4)	5.6(0.5)	M3	3.1(0.3)	2.6(0.2)	23.6(0.7)	24.6(0.8)	6.9 (0.7)	7.7(0.8)
55 ↓	M2	2.1(0.8)	2.4(0.2)	3.2(0.3)	3.7(0.4)	5.3 (0.7)	5.6(0.4)	M3	2.3(0.4)	2.3(0.3)	23.8(0.4)	23.9(0.8)	7.1(0.6)	7.3(0.9)
65 ↑	M2	2.4(0.3)	2.4(0.3)	3.7(0.4)	3.4(0.2)	5.1(0.3)	5.7(0.4)	M3	2.8(0.4)	2.5(0.2)	23.6(0.9)	23.0(1.1)	7.1(0.5)	9.1(0.7)

3.2.2. Effect of temperature on the SANS spectra

The lamellar structure has been observed for initially prepared samples S2–S5 at T=20 °C and has been preserved up to T=25 °C.

However, the increasing temperature destroys the short-range ordering and induces the changes in the self-assemblies morphology. It should be noted that the sample with concentration 0.005 g/ml (S1) does not show the lamellar structure in the whole range of the investigated temperatures ($20 \circ C \rightarrow 65 \circ C \rightarrow 20 \circ C$). The behavior of the samples S2–S5 with a change in the temperature was identical and for this reason we discussed the SANS data for S3 only in the present article. The variation of the SANS spectra with the increasing temperature for 70PhOLCA in DMSO-*d*₆ with C=0.015 g/ml is presented in Fig. 6. The parameters n, m and L for S3 at 20 °C and 25 °C were obtained using model M1 (Eq. (5)) and presented in Table 1. The expected decrease in the correlation length from 83.2 ± 5.3 Å to 23.4 ± 2.7 Å and increase in the inter-lamellar spacing from 41.6 ± 0.3 Å at T = 20 °C to 44.5 ± 0.1 Å at T = $25 \circ C$ were observed. A further increase in the temperature up to 35 °C led to the destruction of spherulite organization of the large self-assemblies at the macro level, as it was seen with the naked eye, and it was accompanied with rebuilding of the internal structure. In this case Eq. (4) was used for SANS data analysis. The fitted parameters n, m and L are collected in Table 1. The 70PhOLCA in DMSO- d_6 forms self-assemblies with n and m values in range 3–4 (surface fractal) for two scales investigated by SANS with no significant reduction of the correlation length from 5.6 Å to 5.1 Å in the temperature range 35–65 °C. It should be noted that the morphology change of the large self-assemblies occurs in the temperature range 55–65 °C. The mass fractal (n = 2.4) was observed at T = 65 °C.

A different model can be used for SANS data analysis (Fig. 6) at temperatures higher than 25 °C. In the first approximation the 70PhOLCA molecule is a cylinder with a length (l_c) and a radius of cross section (r_c). In this case we expect that the cylinder structure makes a significant contribution to the scattering in high q region. Then the second term in Eq. (4) can be replaced by the scattering intensity function for right circular cylinders with uniform scattering length density [23,32]. The form factor P(q) is normalized by the particle volume V_c:

$$P(q) = \frac{\text{scale}}{V_c} f^2(q) + bkg$$
(7)

where

$$f(q) = 2(\Delta \rho) V_{c} \frac{\sin(q l_{c} \cos \alpha/2)}{q l_{c} \cos \alpha/2} \times \frac{J_{1}(q r_{c} \sin \alpha)}{q r_{c} \sin \alpha}$$
(8)

In Eq. (8), α is the angle between the axis of the cylinder and the q-vector, $\Delta\rho$ (contrast) is the scattering length density difference between the scatterer ($\bar{\rho}$) and the solvent (ρ_s) and J₁ is the first order Bessel function. Finally, Eq. (4) can be rewritten as:

$$I(q) = A/q^{n} + (D/V_{c})f^{2}(q) + B,$$
(9)

where D is a scale factor. The values obtained for relevant parameters (n, lc and rc) from the nonlinear least squares fits of model M3 (Eq. (9)) for the SANS spectra of 70Ph0LCA (0.015 g/ml) in DMSO-d6 solution are summarized in Table 1. The scattering from self-assemblies is described by the same term A/qⁿ for both models (M2 and M3). As a result the values of n coincide with a good accuracy for both models at all investigated temperatures. For the M3 model the average cylinder length of 23.7 Å (with a nonsignificant change of \sim 1 Å with variation of T) and radius of 6.9 Å are obtained within the fit errors. As it was mentioned before, the computed length of the 70PhOLCA molecule is 25.6 Å. The difference between l_c and l_m values can be easily explained. The length of the 70PhOLCA molecule was obtained for a crystal structure when all CH groups demonstrated a trans-conformation. Increasing the temperature induces a decrease in the molecule length, which is due to the trans-gosh-isomerisation and translational motions involving many bonds. It can be concluded that the total scattering from the solution 70PhOLCA in DMSO-d6 is a sum of the scattering from the self-assemblies in the low q-region and the scattering from the individual molecules in the high q-region. Thus the both models (M2 and M3) can be used successfully to analyze the SANS spectra for



Fig. 10. The full-width at half-maximum (FWHM) as a function of temperature of deformation vibrations (δ CH) in the range of temperature from 30 °C to 60 °C for sample "3". Blue–Gel phase, red–Sol. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

solution 70PhOLC/DMSO-d6 as depicted for S3 at $T = 20 \degree C$ (shown in Fig. 6). The SANS spectra indicate two Bragg peaks at temperature $10 \degree C$ (Fig. 5). It means that the formation of the self-assemblies with the lamellar internal structure is a reversible process with a hysteresis on the temperature during heating and cooling.

It is remarkable that the gel-sol-gel transition for S5 has been observed in the range of temperatures 45–55 °C and the formation of the lamellar phase startds at ~25 °C. The variation of the parameters: Porod exponent (n), Lorenzian exponent (m), correlation length (L), radius (r_c) and length (l_c) for 70PhOLCA in DMSO- d_6 with C = 0.015 g/ml (S3) and C = 0.025 g/ml (S5) with temperature during heating (\uparrow) and cooling (\downarrow) are summarized in Table 1.

Three areas G1, G2 and G3 on the DSC diagram (Fig. 4) constitute a conditional split of the gel state and can be interpreted as the states when the internal structure or morphology of the selfassemblies at one of the hierarchical levels is changed. G1 is the state with a lamellar internal structure, while the G2 and the G3 are the states with a non-lamellar internal structure. The $G1 \rightarrow G2$ transition was observed in the temperature range of 25–35 °C for S3. The difference between the morphology of G2 and G3 states cannot be distinguished by SANS method within the available size range. The fitting parameters n and m indicate the scattering from surface fractal (see data in Table 1). Additionally a further increase in the temperature leads to the sol formation (55–65 °C for S3), as has been observed with the naked eye and characterized by the mass fractal (n = 2.4) of the self-assemblies at $T = 65 \circ C$. The cooling process from 60 to 10°C, accompanied with the transition of Sol \rightarrow G3 in the range of temperatures from 45 °C to 35 °C and transition G2 \rightarrow G1, finishes at T = 10 °C.

3.2.3. *Time stability effect*

The Gel state of the samples S3–S5 was stable for a long time. However, it should be noted that the density of spherulites increases while its size decreases with time at the macro- scale, as it is illustrated in Fig. 7 for 70PhOLCA (0.025 g/ml) in DMSO- d_6 at room temperature. The splitting of the lamellae becomes barely visible. The SANS has been used to investigate the morphology of the self-assemblies and the internal structure of the samples S3–S5 at the nano-scale.

The fitting of the SANS curves by Eq. (5) indicates the morphology change in the self-assemblies for 70PhOLCA from the surface fractal (n=3.7 for S3, n=3.9 for S4 and S5) after the preparation of the sample (Fig. 8, left) to the diffusive fractal structure



C: Lamellar form of G3-Sol (44°C-56°C)

D: Sol (52°C-56°C)

Fig. 11. Two-dimensional correlation spectroscopy (2D-IR) asynchronous spectrum for 70PhOLCA/DMSO-d₆, during heating cycle, in the range of temperature 30 °C–58 °C.

 $(n = 4.2 \pm 0.1)$ for samples kept at room temperature for 2 weeks (Fig. 8, right).

The analysis of the SANS spectra in high q-region indicates that there is scattering from the mass fractal ($m \approx 1.5 \pm 0.2$) with a lamellar structure for both experimental series. However, the interlamellar distance increases up to 43.6 ± 0.1 Å with time, while the fresh-prepared samples have the d-value of 41.6 ± 0.3 Å.

3.2.4. Sol-Gel transition

The Gel–Sol transition for 7OPhOLCA in DMSO- d_6 at the concentrations of C=0.005, 0.010, 0.015, 0.020 and 0.025 g/ml was investigated in a wide range of temperatures (Fig. 9). The results from data analysis at low q values are presented in Fig. 9, A for heating and in Fig. 9, B for cooling, respectively. The values in the figures correspond to the slopes of the SANS curves obtained from the equation (1). The diagrams were simplified for better understanding (shown in Fig. 9, A and in Fig. 9, B). The purple area corresponds to the objects with a smooth surface, the red area—to the surface fractal, the green area—to the lamellas and the blue area—to the disordered system. The hatchings mark the area where the slope of the curves is not defined since the statistics of the experimental data were poor. The Sol–Gel transition observed by SANS corresponds to the transformation of the system from the ordered into the disordered state (blue area in Fig. 9) and it takes place is present in all investigated samples.

The analysis of the experimental data suggests that the transition temperature (T_t) decreases with the increasing sample concentration during heating, for example, the $T_t = 52 \circ C$ for 7OPhOLCA in DMSO- d_6 at the concentrations of 0.010 and 0.015 g/ml. At the same time T_t is equal to 34.8 °C for C = 0.025 g/mi and this value is concordat with the DSC data (Table 2).

The decreasing of the T_t values comes as no surprise to us. It is well known that an increase in the concentration of the sulfoxides, such as dimethyl sulfoxide and diethyl sulfoxide, leads to an increase in the temperature of the main phase transition of the model phospholipid membranes DPPC, as was discussed in detail in [33] and in the references in this paper.

The temperature of the Sol–Gel transition does not change significantly in the concentration range of $0.010 \text{ g/ml} \le C \le 0.020 \text{ g/ml}$, as shown in Fig. 9, B. The T_t increases from 18 °C to 29 °C with the increasing sample concentration up to 0.025 g/ml.

Summarizing, it can be concluded that small angle neutron scattering yields satisfactory results related to the determination of the morphology of the 7OPhOLCA self-assemblies with varying temperature and concentration in the available q region. The SANS technique can be used successfully for investigation of the internal structure of the scattering objects. Nevertheless, a combination

Table 2

The values of temperatures, enthalpies and entropies for morphology and phase transitions within the 7OPhOLCA in DMSO-*d*6 during heating and cooling in the range of $-20 \,^{\circ}\text{C} - 60 \,^{\circ}\text{C}$; T [°C], Δ H [J/g], Δ S [J/g K] (T_p-peak temperature, $\pm 0.3 \,^{\circ}\text{C}$; Δ H $-\pm 0.6$ J/g). The measurements 1 day after preparation of the sample. Δ H^{*} concern exclusively to the very low enthalpy of phase transition determined by DSC although they are considerably lower than the accuracy of the DSC method. (Air static). G1, G2, G3–Gel phases.

Type of transition	HEATING CYCLE								
		0.015 g/ml DSC 5°/min 14.02 mg	SANS	FTIR	0.025 g/ml DSC 2°/min 26.21 mg	SANS			
Cr-G1	T _p ΔH ΔS	4.7 61.2 0.22	Х	Х	4.4 53.0 0.20	Х			
G1-G2	$T_p \\ \Delta H^* \\ \Delta S$	32.9 0.2 0.00065	Х	Х	X X X	25			
G2-G3	$T_p \Delta H^* \Delta S$	42.3 0.03 0.00010	45	42	X X X	28			
G3–Sol	T _p ΔH ΔS	X X X	52	50	33.6 1.3 0.0042	35			

Type of transition COOLING CYCLE

		0.015 g/ml DSC 5°/min 14.02 mg	SANS	FTIR	0.025 g/ml DSC 2°/min 26.21 mg	SANS
Sol-G3	T _p ΔH [*] ΔS	18.6 0.2 0.00072	18	Х	X X X	29
G3-G2	$T_p \\ \Delta H^* \\ \Delta S$	15.0 0.08 0.00028	15	Х	X X X	21
G2-G1	$T_p \\ \Delta H^* \\ \Delta S$	11.5 0.09 0.00032	11	Х	15.3 1.2 0.0041	18
G1–Cr	T_p ΔH ΔS	-6.4 81.9 0.29	Х	Х	-4.9 69.6 0.25	Х

of different methods should be used for getting credible information about the internal structure of the objects at the nano-scale level.

3.3. Infrared spectroscopy (FT-IR)

SANS measurements provide information about the morphology of individual phases, but do not allow us to determine the correlation between the molecules during the phase transition. The infrared spectroscopy measurements and two-dimensional correlation spectroscopy analysis were performed to confirm the phase transition temperatures and to determine—which functional groups were involved in the transition.

The positions, intensities and shapes of the bands are dependent on the molecular interactions and for this reason attempts were made to determine the phase transitions temperatures, as their registration was not possible using DSC method.

For the gel sample (concentration 0.015 g/ml) at $30 \,^\circ\text{C}$ the bands occur in the FT-IR pattern at the positions (a–asymmetric; s–symmetric, ν –stretching, δ –deformation): $\nu(O-H)$ 3690–3111 cm⁻¹; $\nu_a(C-H)$ 3066–2770 cm⁻¹; $\nu_s(C-H)$ 2700–2334 cm⁻¹; $\nu_a(C-D)$ 2254 cm⁻¹; $\nu_s(C-D)$ 2135 cm⁻¹; $\nu(C=O)$ 1706 cm⁻¹; $\nu(C=C)$ 1541 cm⁻¹; $\delta(C-H)$ 1447 cm⁻¹; $\nu(C-O)$ 1314–1185 cm⁻¹; $\nu(S=O)$ 1048–997 cm⁻¹; $\delta(C-H, C-D)$ 830–720 cm⁻¹. The location of the functional groups of DMSO- d_6 was determined on the basis of the literature [34].

Analysis of full-width at half-maximum (FWHM) was made on the basis of deformation vibrations of the C–H group (1447 cm^{-1}) (Fig. 10.). It was found that the temperatures of phase transitions from the G1 \rightarrow G2 and G2 \rightarrow G3 in the Gel state (blue in Fig. 10.) correspond to temperatures of this transition obtained with the DSC method. Additionally, the interpretation of the spectra of FWHM in the function of temperature allowed us to determine the phase temperature of the G3–Sol transition at about 52 °C.

The phase transitions temperatures observed by the FTIR spectroscopy are in good agreement with the results of previous calorimetric studies.

3.3.1. FTIR spectroscopy with 2D correlation analysis

Systematic changes within the vibrational spectra as changes of temperature can be studied using two-dimensional correlation spectroscopy (2D-IR). It is a mathematical tool used to assess specific interactions of molecules within two phases. It is possible to define relationships between the dynamics of various fragments of molecules in the function of temperature. The definitions of synchronous and asynchronous 2D-IR spectra are given in the literature [35–38].

Increasing the temperature of the Gel 2 has a significant effect on the symmetric and asymmetric stretching vibration: ν C–H, ν C–D, ν O–H and ν S=O (Fig. 11).

The transition from the fractal form G2 to the lamellar form of the G3 phase is followed by the reorientation of the C–H group, which is specifically against the reorientation of the S=O (1048–997 cm⁻¹) (Fig. 11B). The reorientation of the hydroxyl group was not observed. This indicates that in this phase transition the hydrogen bonds involving the hydroxyl group probably have a smaller share.

Higher dynamics of the hydroxyl group is accompanied by the transition from the Gel 3 form to the Sol phase. According to the Noda's rule, during this transition it is first the hydroxyl group that undergoes reorientation, followed by C–H and C–D bonds (Fig. 11C). The C–D bond has the priority of reorientation in this transition before the C–H bond. This means that when temperature increases there are changes in the hydrogen bonding occurring between the molecules 70PhOLCA and DMSO. Probably a portion of the hydrogen bonds tears off, which results in the release of molecules of the compact lamellar structure (Gel 3). The formation of Sol is especially characterized by dynamic movements of the hydroxyl, carbonyl and thionyl group (Fig. 11D). An increase in the temperature causes firstly the reorientation of hydroxyl group and then multiple bonds, in particular C=O and S=O. It is preceded by the reorientation of C–H and C–D bonds.

As a summary of the morphology and phase transition investigations, in Table 2 we present the data obtained with the use of different methods: infrared spectroscopy, neutron scattering and scanning calorimetry. Thus, we can conclude that both an increase as well as a decrease in the temperature can induce the morphology and phase transitions. In the present work we have observed 3 phase states (Crystal, Gel and Sol) and 3 morphology states in the Gel phase (G1, G2 and G3). Gel 1 corresponds to the objects with a smooth surface, G2–surface fractal, and G3–lamellas in solution.

4. Conclusions

- (1) Five samples of organogelator 70PhOLCA in DMSO- d_6 with different concentration levels were prepared. The substance heated from room temperature up to the 60 °C did not undergo decomposition. It was found that the phase transition occurred between Gel and Sol. Three morphology states in the Gel phase were found using the DSC method. The temperatures of the transitions during heating for the sample with the concentration of 0.015 g/ml were determined: Cr G1 (4.7 °C), G1–G2 (32.9 °C). G2–G3 (42.3 °C), G3 Sol (the temperature was not possible to register) and during cooling: Sol G3 (18.6 °C), G3–G2 (15.0 °C), G2–G1 (11.5 °C), G1–Cr (-6.4 °C).
- (2) Small angle neutron scattering yields satisfactory results on the determination of the morphology of the 7OPhOLCA selfassemblies with varying temperature and concentration in the available q region. Three areas G1, G2 and G3 on the DSC diagram (Fig. 4) constitute a conditional split of the Gel state and can be interpreted as the states when the internal structure or morphology of the self-assemblies at one of the hierarchical levels is changed. G1 is the state with a lamellar internal structure, while G2 and G3 are states with a non-lamellar internal structure. The G1 \rightarrow G2 transition was observed in the temperature range of 25–35 °C for S3. The difference between the morphology of G2 and G3 states cannot be distinguished by SANS method within the available size range.

The fitting parameters n and m indicate the scattering from surface fractal (see data in Table 1). Additionally, further increase in the temperature leads to the sol formation (55–65 °C for S3), as has been observed with the naked eye, and is characterized by the mass fractal (n=2.4) of the self-assemblies at T=65 °C. The cooling process from 60 to 10 °C accompanied with the transition of Sol \rightarrow G3 in the range of temperatures from 45 °C to 35 °C and the transition of G2 \rightarrow G1 finishes at T=10 °C.

The SANS technique can be used successfully for investigation of the internal structure of the scattering objects.

(3) Analysis of mid-infrared spectra during heating showed that the temperature strongly influenced hydrogen bonds in the fractal form of the fractal Gel. This is demonstrated by a significant decrease in the intensity of stretching bands of the hydroxyl group $(3690-3111 \text{ cm}^{-1})$. The reorientation of the hydroxyl group does not take part in the morphology transition to the lamellar form of the Gel. However, it has a significant impact on the system during the transition to Sol. Some hydrogen bonds were broken, and the molecules released from the lamellar structure could freely float in the solvent.

(4) Comprehensive methods presented in this work allowed us to draw conclusions about the reversible morphology and phase transitions for all investigated samples.

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