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The Effect of Lysophosphatidylcholine and Phosphatidylglycerol on Lecithin Polymer-Like Micelles

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Abstract—The effect of phosphatidylglycerol and lysophosphatidylcholine on the formation, properties, and intermolecular interactions in polymer-like (cylindrical) micelles that arose in nonaqueous lecithin solutions upon the addition of trace amounts of water is studied by the methods of dynamic rheology and IR spectroscopy. It is established that micellar aggregates of lecithin are not changed markedly in the presence of the first phospholipid but are disintegrated into smaller aggregates upon the addition of second phospholipid. The IR spectroscopic studies demonstrate that phosphatidylglycerol and lysophosphatidylcholine do not significantly change intermolecular interactions in polymer-like micelles. Their effect on the structure of micellar aggregates is associated with the variations in the shape of molecules.

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INTRODUCTION

Polymer-like micelles are formed upon the addition of trace amounts of water to organic lecithin solutions. In this case, reverse spherical micelles are transformed into extended cylindrical aggregates [1–5]. As was shown in [6–8], water plays the key role in the micelle stabilization. Its molecules bind phosphate groups of lecithin adjacent molecules via hydrogen bonds by "bridging mechanism". The entanglement of long flexible micelles leads to the formation of three-dimensional network in the solution bulk that causes sharp increase in viscosity typical of polymer solutions or gels. Therefore, they are usually called organogels, while lecithin cylindrical aggregates, whose properties and behavior resemble polymer macromolecules, are named polymer-like micelles.

The purity of lecithin and the composition of fatty acid residues in its molecule are of prime importance for the formation of polymer-like micelles. It already was mentioned in our first publication [9] that the organogel is formed only from natural phospholipid of high purity. The study performed in [10] with a number of synthetic substances demonstrated that the gelation is observed only in the case of lecithin with hydrocarbon chains consisting of more than 12 carbon atoms, one of which contains double bond. The formation of polymer-like micelles was not observed when using lecithin preparations with saturated fatty acid residues. Organogels are usually prepared from natural (soybean and egg) lecithins containing unsaturated hydrocarbon chains [11]. Their hydrogenation, during which double bonds are eliminated, leads to the loss of the ability to gelate [12].

Usually, lecithin organogels are extremely sensitive to the addition of other substances [9]. The formation and properties of lecithin polymer-like micelles in the presence of only carbohydrate surfactants have been systematically studied to date [13–15], although the problem seems to be very important. It was shown that the effect of surfactants is determined by the set of factors. The shape of molecules and the ability of polar groups to form hydrogen bonds are of great significance [6, 12]. If hydrogen bonds are not formed, the incorporation of surfactant molecules into polymer-like micelles leads to their decomposition. Such effect was mentioned in [16] when studying organogels with added substance containing poly(ethylene glycol) chain.

Note that, in the natural lecithins, various impurities are present that can significantly affect the formation and properties of organogels. It was noted already in the first publication [1] that the gelation did not occur in nonaqueous solutions of lecithin with a low degree of purity. To prepare organogels, it is necessary to use lecithin containing no less than 95% of main substance. However, the formation of polymer-like micelles was observed for the preparation containing about 40 wt % of lecithin [17]. When the mixture contains 25 wt % each of lecithin, phosphatidylethanolamine, and phosphatidylinositol which, as lecithin, belong to the same class of phospholipids, only spherical micelles are formed [18]. These facts state that impurities can both prevent or promote the gelation, depending probably on the type and concentration of impurity.

† Deceased.

The character of the action of impurities on the formation and properties of organogels has not so far been studied. To our knowledge, there are only three publications where this effect was considered. For example, it was shown [19] that β-carotene provitamin exerts strong destabilizing effect on lecithin polymer-like micelles suppressing their formation at a concentration of 0.1 wt %. Mixtures of lecithin and phosphatidylethanolamine were studied in [5, 8]. The latter phospholipid represents the major components of biological membranes [20] and is one of the main phospholipid impurities. It was shown that the addition of phosphatidylethanolamine markedly affects the organogel properties; however, in contrast to β-carotene, its action is exhibited at a concentration of several percents.

This work continues our studies on the effect of phospholipids that are present in lecithin preparations as impurities on the formation of polymer-like micelles. The results reported in this work were obtained with phosphatidylglycerol (PG) and lysophosphatidylcholine (LPC) whose action on lecithin organogels has not been studied earlier. These substances are present in biological membranes together with lecithin and, correspondingly, are separated together [11]. In addition, LPC is of interest as a lecithin derivative with one fatty acid residue. Therefore, LPC contains one, not two, hydrocarbon chains. This explains substantial difference in the geometry of molecules of these two phospholipids that makes it possible to consider its role in the formation of polymer-like micelles.

MATERIALS AND METHODS

Materials

Soybean lecithin, Epikuron 200, was supplied by Lukas Meyer (Germany). The content of ground substance is no less than 99.5%. Soybean lysophosphatidylcholine (LPC content ≥ 97.5%) and sodium phosphatidylglycerol (PG content ≥ 98.0%) were granted by Lipoid (Germany). All studied phospholipids were not purified additionally. Their structural formulas are shown in Fig. 1.

n-Decane, pure grade, was used as an organic solvent. Distilled water was prepared by the standard procedure.

Preparation of Organogels

Organogels were prepared by the dissolution of weighed amounts of lecithin, modifying substances, and water in *n*-decane. After the addition of all components, solutions were mixed with a magnetic stirrer for 3–5 h and then left to stand for 3–5 days until the equilibrium was established. The content of water is given in water : lecithin molar ratio n_w; concentration of additives, as additive : lecithin mass ratio.

Scaling dependences of rheological parameters were determined by studying a series of organogels prepared by the dilution of initial concentrated solutions with given water : lecithin : additive ratios. After the addition of calculated amount of *n*-decane, organogels were mixed with a magnetic stirrer for 3–5 h and then left to stand for 3–5 days until the equilibrium was established.

For rheological measurements, we used mixtures with total concentrations of lecithin and modifying component ranging from 100 to 650 mg per 1 ml of *n*-decane. The IR spectroscopic studies were performed with solutions containing 50 mg/ml of lecithin.

Methods of Study

Rheological characteristics were measured in the regime of low-amplitude oscillations on a Rotovisco RT 20 shear-controlled rheometer (Haake, Germany). Oscillation frequency varied within 100–0.001 Hz range. Measuring cell had a cone-and-plate geometry; cone diameter was 60 mm, the angle of cone inclination was 1°. Minimal gap in automatic regime was 0.052 mm. Measurements were performed at 25.00 ± 0.05°C controlled by a K 20 thermostat (Haake, Germany). To decrease solvent evaporation during measurements, a special chamber was used. Frequency dependences of complex viscosity $|\eta^*|$ and shear moduli G' and G'' obtained during rheological measurements were used to determine zero-shear viscosity η_0, plateau modulus G_0, and terminal relaxation time τ_t in accordance with the procedures described in [21–23].

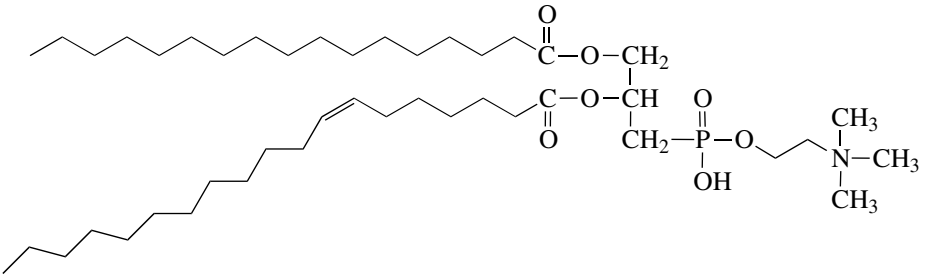
Absorption spectra of organogels were recorded at room temperature on a Nicolet Protégé 460 FTIR spectrometer (US) within the 650–4000 cm^{-1} region (the resolution is 1 cm^{-1}, the range of frequency measurement is 0.25 cm^{-1}). Cell thickness was 0.25 mm and windows were made of sodium chloride. Residue factor was determined using the absorption band of *n*-decane at a frequency of 894 cm^{-1}. Spectra were processed using software OMNIC to the IR spectrometer [8].

EXPERIMENTAL

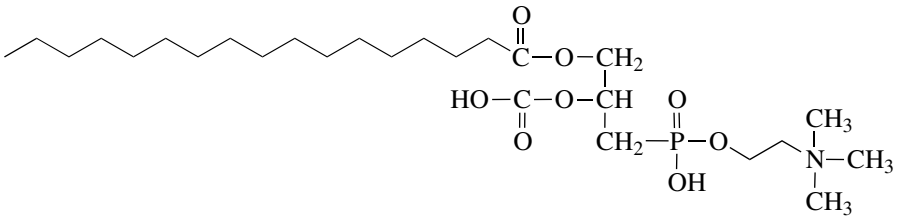
Rheological Studies

Figures 2a and 2b demonstrate normalized values of zero-shear viscosity η_0 and plateau modulus G_0, respectively, as functions of PG and LPC content in lecithin organogel with water : lecithin molar ratio equals 2.6. As can be seen, the addition of PG does not virtually affect the η_0 and G_0 values. A substantial decrease in the values of rheological parameters is observed upon the addition of LPC. At 0.7 wt % LPC concentration, η_0 (Fig. 2a) and G_0 (Fig. 2b) decrease by two and one decimal orders of magnitude, respectively.

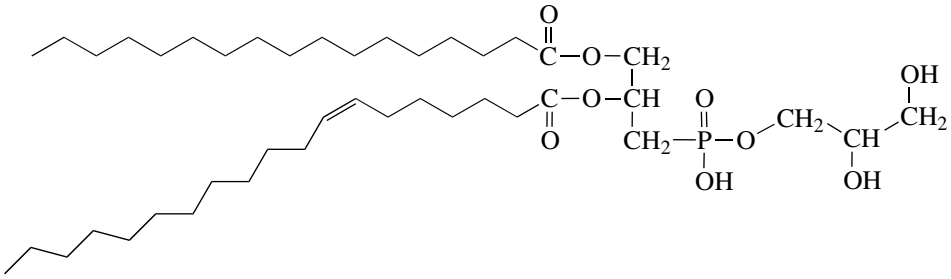
Figure 3 presents the dependences of terminal relaxation time τ_t on the phospholipid's content in organogel. As in the previous case, LPC causes a considerable (to one order of magnitude at a concentration of 0.7 wt %) decrease in τ_t, and the addition of PG slightly decreases τ_t.



Lecithin



Lysophosphatidylcholine



Phosphatidylglycerol

Fig. 1. Structural formulas of employed phospholipids.

Significant changes in rheological parameters indicate considerable rearrangement of lecithin micelles upon the addition of LPC.

As was shown in [9, 21, 24], the formation mechanism of polymer-like micelles and their type can be established by analyzing scaling dependences of rheological parameters. Organogels containing 0.3 wt % LPC and 5 wt % PG were studied at water : lecithin molar ratios equal to 2 and 3. Such a choice of n_w values is based on our previous studies [8, 21] where it was shown that, at molar ratios mentioned above, linear and branched polymer-like micelles, respectively, were formed. It was of interest to consider the action of modifying phospholipid additives on the aggregates with different structures.

The exponents of concentration dependences of zero-shear viscosity, plateau modulus, and terminal relaxation time are summarized in Table 1. These exponents were obtained by processing experimental data using the best fits as described earlier [15, 21]. Only final results, but not corresponding plots, are reported. Theoretical values of exponents [21, 25, 26] are also listed in Table 1.

It follows from the comparison of exponents (Table 1) expected for the corresponding models of linear and branched polymer-like micelles and those obtained from the analysis of experimental data for organogel free of modifying phospholipids that, at $n_w = 2$ and 3, exponent values are close to theoretical ones for linear and for branched micelles, respectively. This agrees with our previous results [8, 21]. In practice, the addition of PG to lecithin organogel does not change the character of scaling dependences. This allows us to conclude that this phospholipid does not alter the formation mechanism of lecithin polymer-like micelles and their rearrangement.

Considerable deviations of exponent values from theoretical ones are observed for organogels containing LPC (Table 1). This testifies that the formation mechanism of lecithin polymer-like micelles is changed in the presence of LPC. Note that the obtained exponents of scaling dependences for organogels containing LPC are

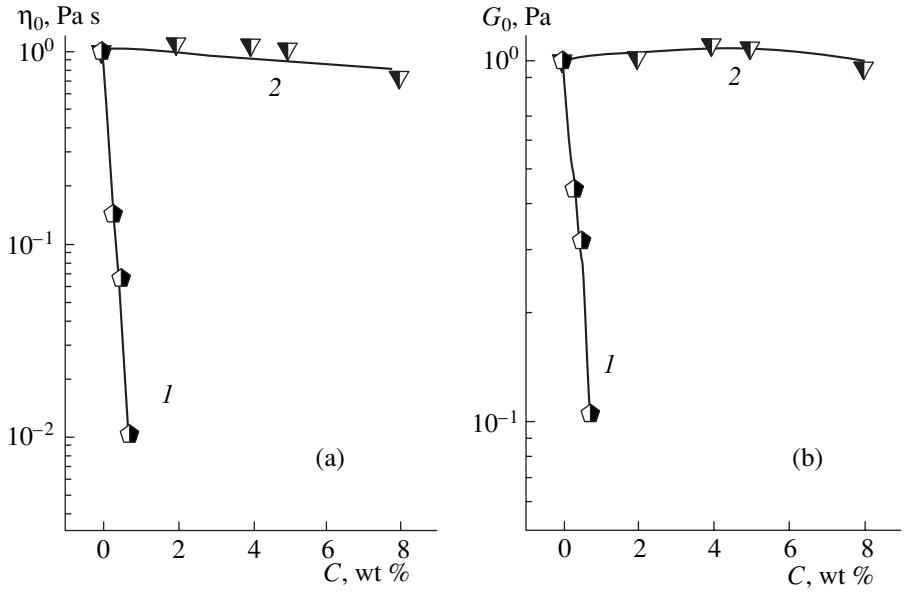


Fig. 2. Dependences of the normalized values of (a) zero-shear viscosity and (b) plateau modulus on concentration of (*1*) LPC and (*2*) PG. Total concentrations of phospholipids in lecithin solutions containing LPC and PG are 300 and 200 mg/ml, respectively. Molar water : lecithin ratio is 2.6.

not consistent with any proposed models of polymer-like micelles.

IR Spectroscopic Studies

The IR spectroscopy was applied to elucidate the effect of LPC and PG additives on intermolecular interactions in lecithin polymer-like micelles. The analysis was performed using absorption bands assigned to ν(OH), ν(C=O), and $\nu_{as}(PO_4)$ stretching vibrations, as was proposed in [7, 8]. The assignment of bands was done in accordance with [27, 28]. The results are shown in Table 2. Note that water : lecithin molar ratio in organogels containing LPC was equal to 2.2; in organogels containing PG, to 2.3. This causes some differences in the position of absorption band maxima in the IR spectra, because stretching vibrations to a large extent depend on the hydration of polar portion of phospholipid [6, 8].

According to results obtained (Table 2), the addition of LPC and PG is accompanied by a slight increase in the frequency of the maximum of ν(OH) band. This band corresponds to the vibrations of OH groups of lecithin phosphate residue and water molecules. The ν(OH) value does not exceed 3390 cm^{-1}. Because the frequency value for free water (3420 cm^{-1}, [29, 30]) is not achieved in the considered systems, this means that

Table 1. Theoretical and experimental values of exponents of concentration dependences of η_0, G_0, and τ_t

Theory			
parameter / model	η_0	G_0	τ_t
Linear micelles	3.5	2.25	1.25
Branched micelles	2.5	2.25	0.25

Experimental data						
parameter / system	$n_w = 2$			$n_w = 3$		
	η_0	G_0	τ_t	η_0	G_0	τ_t
Unmodified organogel	3.9 ± 0.1	2.5 ± 0.1	1.5 ± 0.1	2.7 ± 0.3	2.2 ± 0.3	0.2 ± 0.1
0.3 wt % of LPC	6.9 ± 0.2	3.1 ± 0.1	3.3 ± 0.4	3.5 ± 0.7	2.9 ± 0.3	–
5 wt % of PG	3.8 ± 0.4	2.3 ± 0.2	1.5 ± 0.2	2.5 ± 0.2	2.0 ± 0.1	0.4 ± 0.1

the addition of LPC and PG does not lead to the water separation in organogels. As in the absence of modifying additives, water molecules are bound with phospholipid functional groups.

The frequency of the maximum of ν(C=O) vibration band in organogel remains unchanged in the case of added LPC and increases by 0.6 cm^{-1} in the case of PG. The latter testifies the weakening of interactions between C=O group and H_2O that can be explained by a decrease in the number of water molecules in the region of carbonyl groups due to their partial transfer to the glycerol residue in PG.

The analysis of the position of the maximum of $\nu_{as}(PO_4)$ band in the IR spectra of lecithin (Table 2) demonstrates that LPC addition leads to the shift of this band toward low-frequency region by 0.5 cm^{-1}; PG addition, toward high-frequency region by 0.6 cm^{-1}. Asymmetric vibrations of phosphate group characterize its interactions and the formation of hydrogen bonds with water molecules. An increase in the number of such bonds is accompanied by the bathochromic shift, which take place upon the addition of LPC to organogel. This is indicative of enhanced interactions between H_2O and PO_4 groups of phospholipid molecules. The addition of PG to organogel results in the opposite effect. Possibly, this can be explained by the competition between OH groups in glycerol residue and PO_4 groups for the binding of water.

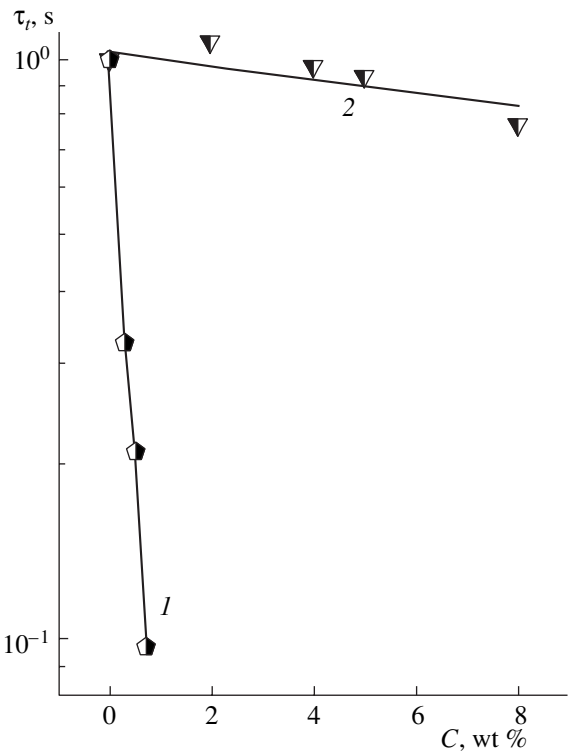


Fig. 3. Dependences of the normalized values of terminal relaxation time on the concentration of (*1*) LPC and (*2*) PG. Experimental conditions are the same as shown in Fig. 2.

DISCUSSION

Rheological studies of lecithin organogels containing LPC and PG demonstrated that modifying additives affect differently their mechanical properties and the dynamics of polymer-like micelles (Figs. 2, 3). If the addition of first substance leads to a significant decrease (up to several orders of magnitudes) of static viscosity, plateau modulus, and terminal relaxation time, the addition of PG causes only small decrease of the values of these parameters. In addition, the influence of LPC is displayed when its concentration is less than 1 wt %, whereas the influence of PG is observed at concentrations higher than 3 wt %.

According to the theory of polymer solutions [31–33], static viscosity is proportional to the molecular mass of a substance; plateau modulus, to the number of contacts between macromolecules in network structure. Terminal relaxation time also depends on the molecular mass, however, in a more complex manner. Analogous notions are applicable to polymer-like micelles [21, 34]. Therefore, sharp decrease of η_0, G_0, and τ_t observed upon the addition of LPC indicates the decomposition of micellar aggregates and network structure of organogel. The addition of PG does not cause significant changes in rheological parameters. This means that the incorporation of this phospholipid to lecithin micelles does not change their structural organization.

Analogous conclusions follow from the analysis of scaling dependences of rheological parameters (Table 1). The addition of PG to organogels containing both linear and branched polymer-like micelles does not affect the exponents of concentration dependences. In other words, any noticeable rearrangement of micelle structure does not occur upon the incorporation of this phospholipid. Significant changes in the character of scaling dependences take place upon the addition of LPC to organogel. In this case, the exponents change in a manner satisfying neither of the proposed models of polymer-like micelles. This result implies that the incorporation of LPC into lecithin micellar aggregates leads to

Table 2. The values of frequencies (cm^{-1}) of the maxima of absorption bands assigned to functional groups of lecithin

Phospholipid, wt %	ν(OH)	ν(C=O)	$\nu_{as}(PO_4)$
LPC			
0	3376.3	1739.0	1245.3
0.2	3377.3	1739.0	1244.9
0.7	3379.2	1739.0	1244.8
PG			
0	3376.3	1738.3	1241.3
5	3390.0	1738.9	1241.9

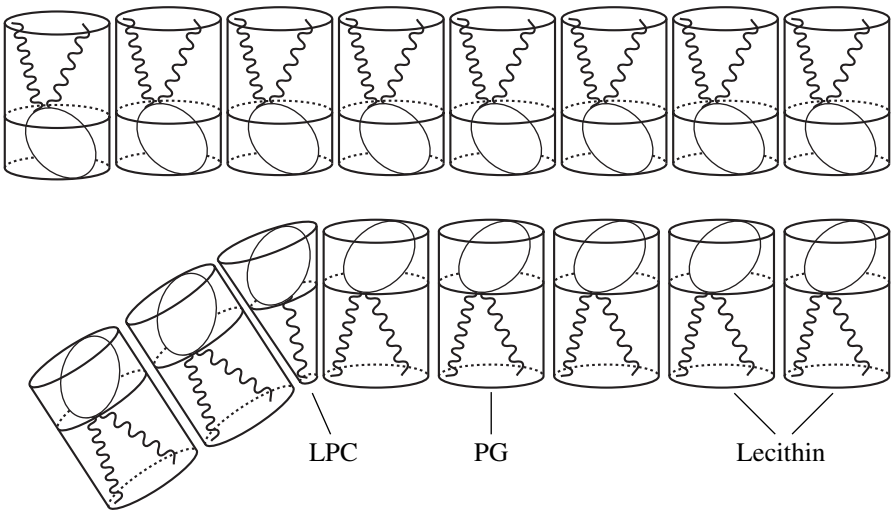


Fig. 4. Schematic representation of lecithin polymer-like micelle with incorporated molecules of LPC and PG. See the text for explanation.

the fundamental rearrangement of their structure. Most likely, this rearrangement consists in the decomposition of polymer-like micelles into shorter rodlike aggregates whose rheological behavior is described within the framework of other theoretical approaches [35, 36].

According to the results of IR spectroscopic study, LPC (whose addition leads to the decomposition of aggregates) facilitates the formation of hydrogen bonds between phosphate groups and water molecules which are responsible for the binding of lecithin molecules by bridging and for the stabilization of micellar aggregates [6, 8]. The magnitude of observed effect determined by the shift of the frequency maximum of $\nu_{as}(PO_4)$ vibration band in the IR spectrum (Table 2) was small that manifests the changes in intermolecular interactions which do not have any significance. This can be explained by the fact that polar parts of LPC and lecithin (Fig. 1) are identical. Two phospholipids differ only in their nonpolar fragments. Lecithin contains two hydrocarbon radicals, while LPC has only one. As a result, their molecules have different shapes. Therefore, it is reasonable to relate the influence of LPC on polymer-like micelles described in this paper with the differences in the molecular geometry of modifying additive and lecithin.

The self-organization of surfactants in solutions and the type of forming structures can be conveniently considered within the framework of the Israelachvili theory [37, 38] that is based on the molecule shape. Lecithin has the shape of truncated cone that is explained by a slight increase in the cross-sectional area of nonpolar region compared to polar one [39]. Therefore, in nonaqueous media, lecithin forms reverse spherical micelles [5, 11]. As the hydration proceeds, the shape of molecule changes from conical to cylindrical due to the leveling of cross-sectional areas of nonpolar and hydrated regions. Changes in molecular geometry lead to the transitions: reverse spherical micelles $\longrightarrow$ polymer-like micelles $\longrightarrow$ lamellar L_α-phase that was confirmed by the experimental studies of the phase behavior of lecithin–water–decane system [40]. Polymer-like micelles act as an analog of hexagonal phase; they exist within a fairly narrow range of water : lecithin molar ratio. As was shown in [9], lecithin organogel begins to be formed at $n_w = 1.0$–1.5 and decomposes at $n_w = 5.2$–5.5. In the phase diagram, a very narrow region of organogel existence corresponds to this range (see [40]). This fact demonstrates that the formation of polymer-like micelles is characterized by the high sensitivity to the molecular geometry that varies with the hydration of the polar part of lecithin molecule. In this case, the effect of modifying additives on organogel studied in this work becomes understandable.

Unlike lecithin, the LPC molecule has only one hydrocarbon chain (Fig. 1). Hence, the cross section of nonpolar region is twice as small. According to estimates made in [41], dimensionless packing parameters for LPC and lecithin with saturated fatty acid residues are 0.4 and 0.74, respectively. Differences in molecular geometry are displayed in their self-organization. LPC, having the shape of inverse cone, tends to form direct micelles in aqueous solutions; lecithin, lamellar L_α-phase [5, 11, 42, 43]. The addition of LPC to lecithin-containing systems results in the decomposition of its bilayer structures [37, 41, 42, 44–46]. This effect is owing to the changes in the curvature of their surfaces. The effect of LPC on lecithin polymer-like micelles can be explained similarly. This is shown schematically in Fig. 4. The incorporation of cone-like LPC molecule into the row composed of lecithin cylindrical micelles leads to the appearance of cavities owing to the differences in cross-sectional areas of nonpolar and polar portions. This can be interpreted as the appearance of structural defects that causes the micelle decomposition.

Lecithin and PG, whose molecules are similar in shapes, do not differ significantly in their properties. They are rather well compatible with each other and both form lamellar L_α-phase [45, 47]. Therefore, as is shown in Fig. 4, the incorporation of PG into lecithin polymer-like micelles does not lead to significant distortion of the structure. As a result, upon the addition of PG, rheological parameters of organogel remain practically unchanged (Figs. 2, 3; Table 1).

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