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ORIGINAL ARTICLE

Self-Organizing Structures of Phosphatidylcholine in Nonaqueous Solvents: Tailoring Gel-like Systems

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Abstract   In this paper, we investigated the role played by solvent type and additives (water [W] and citric acid [CA]) on the self-assembly of phosphatidylcholine (PtdCho) into gels. Soybean lecithin (L) served as the PtdCho source used in this study. Lecithin was combined with different oils: hexadecane (HEX), sunflower oil, and medium-chain triacylglycerol to explore its ability to form organogels. Among the solvents, only HEX was able to form a translucent self-sustainable gel with lecithin. It was found that the lower water solubility and viscosity of HEX favored gel formation. Small-angle X-ray scattering revealed different structures arisen from lecithin organization depending on the organic medium type. In addition, the gel properties of the L-HEX binary system were also tailored through inclusion of the "primers" W or *CA*. Oscillatory rheological behavior of organogels was effectively described by a single relaxation-time Maxwell model, with good fitting at low and intermediate frequencies and with deviations at higher frequencies, indicating the presence of reverse wormlike micelles. Organogels were thermoreversible and, upon CA addition, a substantial increase in zero-shear viscosity was observed. Incorporation of W led to a similar microstructure to that obtained only with L, whereas CA promoted a different ordering of PtdCho assemblies.

Formation and properties of PtdCho-based organogels were dictated by using different oil types or by changing the polarity of the medium with the incorporation of primers. A better understanding of phospholipid-based nonlamellar mesophases in organic solvent offers the prospect of adapting the gel properties to the desired application and functionality.

Introduction

Organogels (OG) are systems formed by a three-dimensional network capable of holding liquid oil, forming a viscous system. Different gelator (G) molecules have been studied for oil structuring purposes. Supramolecular assembly of gelators can be categorized into crystalline particles such as waxes, self-assembled structures of amphiphilic molecules (reverse micelles, tubules, *etc*.), self-assembled structures of polymers or polymer strands and miscellaneous structures (colloidal particles and emulsion droplets) (Co and Marangoni, 2012; Patel and Dewettinck, 2016). In general, gelators must be relatively insoluble in the solvent to promote gelation; but interactions should also occur between their soluble portion and the oil moieties (Co and Marangoni, 2012). For instance, gels formed from lecithin reverse micelles have gained much attention because of their property to undergo a phase transition from solution to viscous OG (Gaudino et al., 2019; Vierros and Sammalkorpi, 2015).

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Lecithin is a ubiquitous natural phospholipid present in cell membranes and it is widely used due to its biocompatibility and zwitterionic characteristic. Usually, the major component of lecithin is phosphatidylcholine (PtdCho). This ingredient has been employed in the pharmaceutical, cosmetic, and food industries (Patel and Dewettinck, 2016; Raghavan, 2009). The polar head group of PtdCho is composed of a positively charged choline group attached to a negatively charged phosphate group. This moiety is bounded to glycerol and two fatty acyl groups (to provide lipophilicity), such as palmitic, stearic, oleic, and linoleic acids. PtdCho molecules can self-assemble when present above the critical micelle concentration (CMC), forming either spherical or ellipsoidal reverse micelles when dispersed in oily phase or bilayers (lamellar phase) (Cautela et al., 2017; Shchipunov, 2001; Walde et al., 1990). Above the CMC of lecithin, a viscoelastic structure can be formed by establishment of a transient network of entangled micelles (Dreiss, 2007; Schurtenberger et al., 1989). The gel-inducing capacity of lecithin is related to phosphatidylcholine content, since it increases the viscoelastic properties of the systems (Scartazzini and Luisi, 1988).

A key step in the formation of lecithin OG is the addition of a compound, referred to as a primer, which promotes the longitudinal assembly of the colloidal mixture of dispersed micelles to form a coherent structure. For instance, a molecule of water tends to bind stoichiometrically to two adjacent lecithin hydrophilic head groups (phosphate group) *via* hydrogen bonds, thereby reducing the interface curvature and promoting network formation (Imai et al., 2013; Raut et al., 2012; Shchipunov, 2001). In addition, the quaternary ammonium group of choline or other polar groups (e.g., carboxylic acids) may bind to lecithin phosphate groups through electrostatic interactions (Imai et al., 2013).

Thus, when considering the self-assembly of lecithin, the empirical packing parameter can be employed to describe the preferred molecular assembly in different conditions, including the choice of solvent. The packing parameter (P), calculated from the model of Israelachvili (2011), is described by Eq. (1).

$$P = \frac{V}{a_0 l_c} \qquad (1)$$

where V is the volume of hydrophobic group, a_0 the optimum head group area, and l_c the chain length of hydrophobic group.

Reverse micelles formation occurs when $P > 1$. Under this condition, V is large, which is equivalent to the surfactant forming a truncated cone shape (Correa et al., 2012). Both chain volume and chain length set limits on how the fluid chains can pack together, showing the direct influence of solvent. Moreover, the network organization is dependent on the adequate hydration of the phospholipids.

Gelation of lecithin is observed at proper water content, observed by a sharp increase in the viscosity of the system. However, when the amount of water exceeds a maximum value for OG formation (W_{cr}), there is a drop in viscosity and phase separation takes place (Angelico et al., 2004; Scartazzini and Luisi, 1988; Schurtenberger et al., 1989). Therefore, formation of lecithin OG depends directly on physicochemical conditions that are influenced by primer addition and solvent properties.

In view of that, two approaches for lecithin OG were investigated: (i) lecithin assembly in different types of nonpolar solvents (oils) and (ii) addition of molecules –primers – to the binary system possibly affecting lecithin network formation and properties. The behavior of lecithin in binary systems was studied by applying different organic phases: hexadecane (HEX) as a linear alkane hydrocarbon model, and a long- and medium-chain triacylglycerol (MCT), also differing in degree of saturation. Furthermore, changes in the behavior of lecithin-HEX systems were determined when trace-amounts of primer (water or organic acid) were incorporated into the systems. The effect of lecithin concentration was studied. In this sense, it is of interest to verify how the evaluated variables modified and modulated the formation and properties of OG by investigating OG's structural, rheological, and thermal behaviors.

Experimental

Materials

Soybean lecithin (L) Phospholipon® 90G (96.9 wt.% phosphatidylcholine- PtdCho, 1.1 wt.% lysophosphatidylcholine) was purchased from Lipoid GmbH (Ludwigshafen, Germany) and used without further purification. The fatty acid composition of lecithin was approximately 62 wt.% linoleic acid (18:2), 15 wt.% palmitic acid (16:0), 12 wt.% oleic acid (18:1), 5 wt.% linolenic acid (18:3) and 3 wt. % stearic acid (18:0). Due to its high PtdCho content, we refer to lecithin as "phosphatidylcholine" throughout the paper. Experiments were performed with oils having very different molecular structures: hydrocarbon (HEX) as model oil, and triacylglycerols (sunflower oil [SFO] and medium chain triacylglycerol [MCT]). HEX ($C_{16}H_{34}$) (Sigma Aldrich, Saint Louis, MO, USA), SFO (Bunge Alimentos S.A., Gaspar, Brazil), and a MCT, Delios V (MCT) kindly donated by Cognis (Monheim am Rhein, Germany) were used as oils. Citric acid ($C_8H_8O_7$) and methanol (analytical grade) were purchased from Synth (Diadema, Brazil). Fatty acid composition of SFO and MCT, and properties of all three oils are given in Table 1.

Table 1 Water solubility, viscosity, density, and average molecular weight for solvents (for HEX, SFO and MCT) and prevailing fatty acid composition for oils

Organic solvent	Water solubility (mg/g)	Viscosity (mPa.s)	Density (g/cm³)[a]	Molecular weight (g/mol)	Prevailing fatty acid composition[b] (%)
HEX	0.05 ± 0.01	3.24 ± 0.01	0.7698	226.4	–
SFO	1.08 ± 0.13	55.82 ± 0.07	0.9138	879.0	16:0 (5.22); 18:1 (41.35); 18:2 (47.32)
MCT	2.45 ± 0.05	24.11 ± 0.01	0.9414	554.0	8:0 (55.16); 10:0 (43.55)

8:0 caprylic acid, 10:0 capric acid, 16:0 palmitic acid, 18:1 oleic acid and 18:2 linoleic acid. Viscosity and density measured at 25 °C.

[a]Standard deviation < 0.0001.

[b]Standard deviation < 0.4.

Methods

Preparation of Samples

Lecithin-based systems were prepared by mixing lecithin and oil (HEX, SFO, or MCT). The samples were placed in a 50 mL beaker ($\phi = 42$ mm) and heated at 90 ± 4 °C for approximately 1 h under mild agitation (200–300 rpm) using a magnetic stirrer until a clear solution was formed. The samples were cooled at 25 ± 2 °C and quiescent conditions. Three different lecithin concentrations (10, 20 and 30 wt.%) were studied to produce OG, referred to as L10, L20, and L30, respectively. Water (W) or citric acid (CA) was added to the mixture (solvent + lecithin) to tailor gel properties. These formulations were identified as L10W, L20W, L30W for water and L10CA, L20CA, and L30CA for CA addition. The molar ratio of primer to phosphatidylcholine (W_0-[H_2O]/[L] or [CA]/[L]) was 1.0 for water and 3.3 for organic acid, defined as the amount necessary to form self-sustainable gels (visual appearance: tilt test). The water was added during the homogenization of the mixture. The amount of water reported includes only water added to the formulations and not water contained in soybean lecithin (<1.5 wt.%, according to the supplier). Lecithin generally contains 0.7–2.3 wt.% moisture (water/lecithin ratio of 0.3–1.0 mol/mol), depending on production and storage conditions (Angelico et al., 2005). For CA addition, L and CA were previously dissolved in methanol to obtain 400 mM and 300 mM stock solutions, respectively, as described by Imai et al. (2013). Mixtures of these solutions were prepared to obtain appropriate lecithin/organic acid ratio. Subsequently, methanol was removed by drying the samples in an oven at 70 ± 3 °C for at least 24 h.

Solvent Characterization

The water content of the different oil phases was measured using a coulometer (Karl Fischer Titrator, model 831- Metrohm, Herisau, Switzerland). Samples were prepared by mixing an equal mass ratio water and oil for 1 h using a magnetic stirrer. The samples were equilibrated for at least 3 days, to ensure saturation of the oil phase, before measurements were taken (Sawalha et al., 2012). The density of the solvents was measured in a Digital Density Meter (Anton Paar, Graz, Austria) at 25 °C.

Rheological measurements were performed in a stress-controlled rheometer (TA Instruments, New Castle, DE, USA). Flow curves of oils were obtained using double concentric cylinders with double gap (rotor outer radius of 17.53 mm, rotor inner radius of 16.02 mm, stator inner radius of 15.10 mm and cylinder immersed height of 58 mm). The shear rate varied between 0 and 300 s^{-1} and the flow curves were obtained in three sequential flow steps: up-down-up cycles. Data obtained from the curve for the third flow step were fitted to the Newton's Law of Viscosity. All measurements were performed at 25 °C in triplicate.

The fatty acid composition of SFO and MCT was determined according to the method described by Hartman and Lago (1973). The analysis was performed on fatty acid methyl esters obtained *via* transesterification of oils by gas chromatography using a capillary column (DB-23 from Agilent (Santa Clara, CA, USA), 60 m long, 0.25 mm internal diameter, 0.25 mm film thickness) with the following conditions: oven temperature: 110 °C (5 min hold), increase from 110 °C to 215 °C (5 °C/min), 215 °C (24 min hold); flame ionization detector temperature of 280 °C; injector temperature of 250 °C; helium carrier gas at a, split ratio of 1:50; and injection volume of 1 mL. Chromatographic peaks were identified based on retention times in comparison with fatty acid methyl ester standards.

Small-Angle X-Ray Scattering

Structural information of lecithin in different solvents was obtained from small-angle X-ray scattering measurements,

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which were performed at the Small-Angle X-Ray Scattering (SAXS) 1 beamline at Brazilian Synchrotron Light Laboratory (LNLS/CNPEM, Campinas, Brazil). Experiments were performed using an 8 keV beam energy ($\lambda = 1.55$ Å) and a sample-to-detector distance of 1010.5 mm. The measured scattered intensity was normalized for the beam intensity and absorption from the sample. The data were obtained as plots of the SAXS intensity, I(Q), as a function of the modulus of the scattering vector $Q = 4\pi \sin(\theta)/\lambda$, where 2θ is the scattering angle and λ is the beam wavelength.

Each SAXS analysis corresponds to a data collection time of 100 s. All I(Q) data were corrected (subtraction) for a background scattering from the Kapton tape for gel-like samples and mica window for the liquid holder device. Scattered intensity (I(Q)) can be modeled according to I(Q) = F(Q).S(Q), where F(Q) is the form factor, describing the form of structure (i.e., lecithin micelles) and S(Q) is the structure factor, which reflects how the structure are organized in the space (i.e., disposition of structures).

Polarized Light Microscopy

Microstructure was checked through a crossed polarizer using an optical microscope (model Multizoom AZ100, Nikon Co., Tokyo, Japan) and the images were recorded by a DS-Ri1 camera (Nikon). A drop of sample collected just after gel preparation was gently placed between a cover slip and a glass slide to be examined after 24 h of storage at room temperature under polarized light. At least 10 images were taken for each sample slide.

Rheological Measurements

Dynamic shear rheology was performed on a stress-controlled rheometer (MCR301, Anton Paar, UK). Measurements were performed in triplicate using cone-plate geometry (50 mm, 2° angle, truncation 208 μm). A Peltier-based temperature control device was used for setting the temperature. Dynamic rheological measurements were performed within the linear viscoelastic region (LVR) previously defined by stress sweep measurements.

Complex (G^*), elastic (G'), and viscous (G'') moduli were evaluated using a frequency sweep ranging from 0.01 to 10 Hz at different temperatures (20, 23, 25, 27 and 30 °C). The Maxwell model was fitted to the data (G' and G'' versus frequency), considering a single-stress relaxation process according to Eqs. (2) and (3).

$$G' = \frac{\omega^2 \tau_R^2}{1 + \omega^2 \tau_R^2} G_0 \qquad (2)$$

$$G'' = \frac{\omega \tau_R}{1 + \omega^2 \tau_R^2} G_0 \qquad (3)$$

where G_0 is the maximum modulus and represents the G' value in the high-frequency (ω) region (frequency limit). The relaxation time, τ_R, is estimated as the inverse of frequency at G' and G'' crossover point ($1/\omega_c$). The zero-shear viscosity (η_0) can be estimated by Eq. (4) showing its dependency of both network structure and dynamic behavior.

$$\eta_0 = G_0 \tau_R \qquad (4)$$

Temperature sweeps were carried out at a fixed frequency of 1 Hz. Three sweeps (cooling–heating–cooling) were performed between 90 °C and 25 °C at a rate of 5 °C/min. The sol–gel temperature ($T_{sol-gel}$) was defined in the G' and G'' crossover point during the cooling ramp, while the gel–sol phase transition temperature ($T_{gel-sol}$) was recorded from the viscous and elastic moduli crossover point in the heating step.

Results and Discussion

Role of Solvent Type on Ability of Lecithin to Form Gel-like Systems

Gel Formation

Figure 1 shows the influence of oil type on lecithin OG formation at different phospholipid concentrations. Self-sustainable systems were formed only with HEX as the oil in the studied conditions. Indeed, lecithin has been previously described in the literature to form an efficient binary system with hydrocarbons such as HEX, hexane, and decane
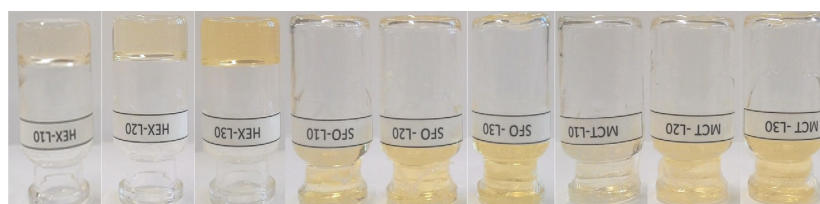

Fig 1 Visual appearance of systems containing HEX, SFO, and MCT as solvents and different soybean lecithin concentration (10, 20 and 30 wt.%) as gelator

(Palazzo, 2013; Raut et al., 2012). However, a notable increase of viscosity was observed for SFO as oil, whereas for MCT the system showed the lowest viscosity.

The oils are quite diverse in their properties (Table 1). It is likely that the oils will strongly influence the ability to form OG from lecithin-based 3D networks. HEX is a linear hydrocarbon with 16 carbons, SFO mostly comprised of unsaturated fatty acid chain with 18 carbons (bended chains) while MCT is fully saturated and comprised medium saturated chain fatty acids (8–10). HEX was investigated as a surrogate nonpolar solvent with lower water solubility value (0.05 mg/g at 25 °C) followed by SFO (1.08 mg/g) and medium chain triacylglycerol (2.45 mg/g), respectively (Table 1). Several reports are in agreement that an increase of solvent polarity (e.g., MCT > SFO > HEX) is deleterious to gel formation (Liu et al., 2013; Sawalha et al., 2012).

Furthermore, the viscosity during OG formation is also affected by the oil type. HEX has lower viscosity than MCT and SFO (around 7 and 17 times at 25 °C, respectively Table 1), which suggests that HEX would more readily support lecithin self-assembly as wormlike micelles. Low viscosity is associated with the ease of self-packing for gelators to form a three-dimensional network. Therefore, a more viscous solvent might result in a diffusion-limited assembling process, producing a weaker gel or the absence of gel.

The presence of unsaturation in SFO is anticipated to possess low lecithin solubility, thereby providing more suitable conditions for gel formation than with MCT (composed of saturated fatty acids), in which the solubility of

PtdCho would be much higher. An oil's unsaturated fatty acid content can impact both gel formation and properties. A positive effect of unsaturation is the greater degree of conformational freedom provided by double bonds and a higher molar volume of the oil, which would facilitate the formation of a greater number of inter-gelator connections thus producing gel-like structures (Gravelle et al., 2016; Laredo et al., 2011).

The possible steric hindrance of the hydrophobic tails of lecithin might affect the critical packing parameter (P) according differences in solvent solubility. The hydrocarbon chain length will affect penetration tendencies of oils into the lipophilic portion of gelator. Among the two triacylglycerol oils, SFO has similar chain length and degree of unsaturation as lecithin, suggesting high compatibility between gelator and oil, whilst PtdCho is more soluble in MCT due to shorter oil chain length. Even subtle differences in lipophilic tail architecture are reported to affect gelling capability through its impact on the preferential packing geometry of the gelator (Shrestha et al., 2010).

Small-Angle X-Ray Scattering

SAXS measurements showed that the oil type impacted the lecithin self-assembly systems that were formed. The scattering profile of systems with the same concentration of lecithin (20 wt.%) in different solvents is shown in Fig. 2. For the lecithin in HEX system (curve [1]), no clear diffraction peak was observed, indicating the absence of hexagonal or cubic liquid crystalline morphology. Whether the intensity exhibits Q^{-1} dependence at the lower Q values (Guinier region), it
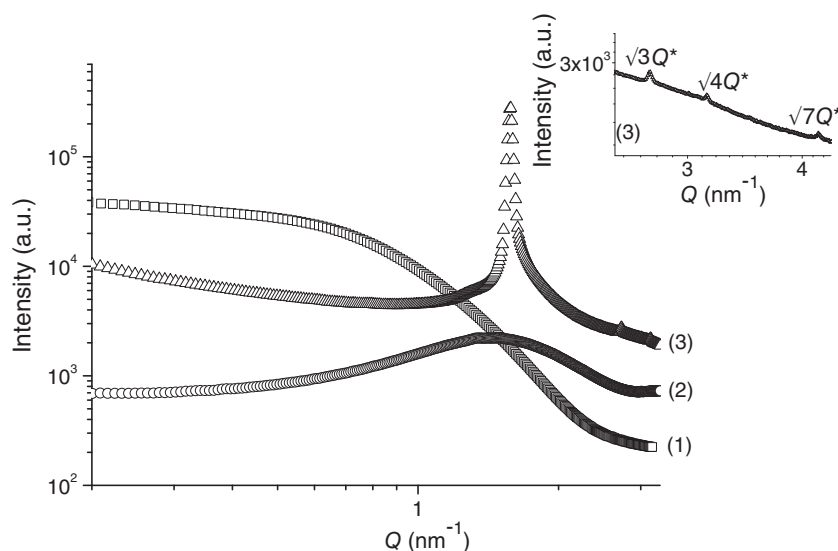

Fig 2 Small-angle x-ray scattering (SAXS) data for lecithin in the presence of different solvents: (1) HEX, (2) MCT and (3) SFO corresponding to 20 wt.% of lecithin in the samples. The curves were shifted vertically for better visualization using a factor of 10^4 and 10^{-2} for HEX and MCT, respectively. Inset figure shows Bragg peaks at high Q values for L with SFO system
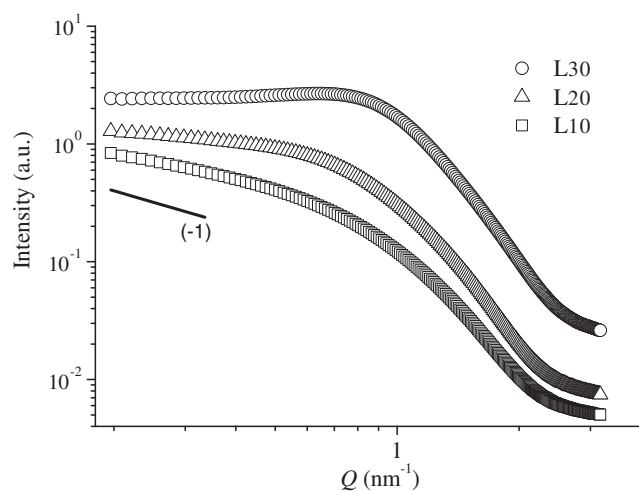
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Fig 3 Experimental X-ray scattered intensities, I(Q), of systems with different concentrations of lecithin (10, 20 and 30 wt.%) in hexadecane. The curves were shifted vertically for better visualization using a factor of 6, 10, and 30 for 10, 20, and 30 wt.% of lecithin, respectively. Solid line represents Q^{-1} slope

can be an indication of wormlike micelle presence in the system (Shrestha et al., 2011). However, due to high lecithin concentration (as discussed below) it would be necessary to have access to lower Q values than those accessed on our experiment in order to make quantitative statement. Lecithin molecules in SFO showed peaks at 1.58; 2.73; 3.15; and 4.17 nm^{-1} (Fig. 2 curve [3] inset). These peak positions are consistent with a two-dimensional (inverse) hexagonal array (H$_2$) (Campo et al., 2011; Chung et al., 2016). Long rods can pack into hexagonal structures, reducing the solubility of lecithin. For MCT (Fig. 2 curve [2]), the notable peak at 1.6 nm^{-1} can be related to a structure factor (S[Q]). The high packing of the building block structures responsible for the structure factor behavior hides the low Q intensity associated with its form factor, making difficult to state the shape and size of reverse building block structure. In any way, it is clear that different structures from lecithin in nanoscale were formed depending on the solvent type.

The gelator concentration may also infer in the SAXS profile. Figure 3 shows the SAXS intensity for lecithin systems at different concentrations of PtdCho in HEX (10, 20 and 30 wt.%). All systems showed no defined diffraction peak, which is indicative of the absence of hexagonal or cubic liquid crystalline morphology. As aforementioned, Q^{-1} intensity slope at low Q values would suggest wormlike micelles presence. The low Q slope can only be seen for L10 sample, since as Q approaches zero the increase of lecithin concentration led to a decrease in power law slope, which is related with strengthening between adjoining reverse wormlike micelles (Hashizaki et al., 2009).

The structure factor (S[Q]) gives structural information related to intermicellar organization (such as distances and volume fraction) that are mainly significant only at higher lecithin concentrations. The effect of this organization in the scattering profile is easily seen in Fig. 3 for L30 sample due to the presence of a broad peak located close to $Q = 0.9$ nm^{-1} and the decreases of I(Q) as Q approaches zero. The increase in lecithin concentration would enhance the reverse micelle network, improving overall mechanical properties of this gel.

Tuning HEX-L OG Properties: Addition of Primers

Microscale Domain: Polarized Light Microscopy

Although OG formed from binary (lecithin and HEX) or ternary (lecithin, HEX, and water or CA) mixtures were optically transparent, their analysis under crossed polarized light contributes to additional microstructural information (Tung et al., 2008). Usually lecithin-based gels are transparent and isotropic under quiescent conditions; however, they show birefringence when moved (Hashizaki et al., 2012). Birefringence represents a nonequilibrium arrangement of lecithin OG (transient network) since the gels became disordered and lost their structure when placed on glass slides. Birefringence, which appears as bright structures in the micrographs of Fig. 4, can arise from spontaneous alignment/organization of structures in the medium (Tung et al., 2008). In general, higher lecithin concentration led to a more densely packed structure. The arrangement at microscale reveals a pattern inconsistent with that expected for lecithin reverse wormlike micelles, depending on the primer added. The structures observed for OG with water as primer were similar (streaks with coherent structure) to those obtained with pure lecithin (Fig. 4a, b), although citric acid seemed to induce slight changes in the microstructure (Fig. 4c). In fact, micrographs of the latter contain small Maltese crosses that differ in their dimensions (Fig. 4c). The Maltese cross pattern is indicative of lamellar phases, and was found throughout all systems at different sizes and amounts. The information provided by polarized microscopy is not strictly conclusive; moreover, the Maltese cross signature can indicate the presence of spherulites (i.e., radially oriented microcrystals). This arrangement has been found in polymer solutions, starch granules, thermotropic liquid crystals, and mineral aggregates (Angelico et al., 2004).

Nevertheless, it should be noted that even the systems with no water added, they actually were not absolutely water free, since lecithin contains a trace amount of water (as described above). This may explain the similar patterns observed in Figs. 4a, b with Maltese crosses and intertwined streaks. In addition, if the water content in lecithin
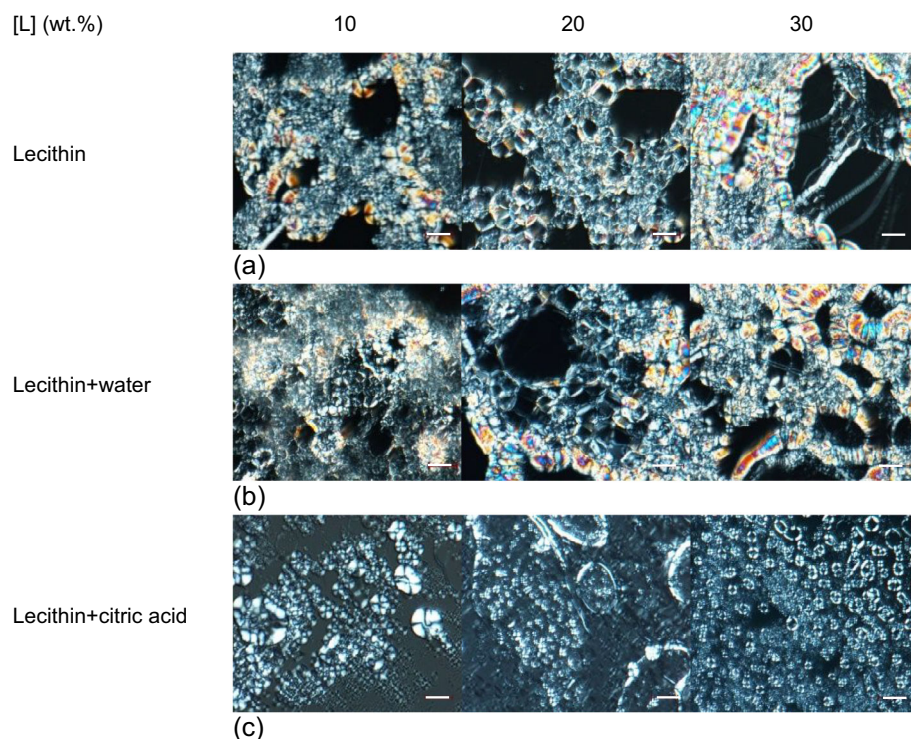
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Fig 4 Polarized light microscopy of HEX-based gels corresponding to (**a**) binary systems: L10; L20; L30; (**b**) with water addition: L10W; L20W; L30W and (**c**) with citric acid addition: L10CA; L20 CA; L30CA. The molar ratio of [W]/[L] and [CA]/[L] was 1.0 and 3.3, respectively. Scale = 50 μm

OG exceeds W_{cr}, a liquid crystalline phase forms, which may coexist with the OG (Angelico et al., 2004).

Rheological Characterization: Isothermal and Nonisothermal Oscillatory Measurements

According to literature on the rheology of wormlike semi-diluted micelles, they behave as living polymers (Cautela et al., 2017). All lecithin-based systems with HEX showed a viscoelastic behavior typical of physical networks as shown in Fig. 5. At high frequency (or short time scale), G' was greater than G'', suggesting the predominance of elastic behavior (Fig. 5). In contrast, at low frequency values (or long time scale), the samples exhibited predominantly viscous behavior, where G'' was greater than G', for which G'' corresponds to the energy dissipated under flow conditions (Fischer and Rehage, 1997; Schurtenberger et al., 1989; Shrestha et al., 2011; Tung et al., 2008). In addition, at high frequencies, G' approaches a constant (*plateau*) value (G_0). The observation that $G'' > G'$ over long observation times (low frequencies), suggests that gel structures can no longer withstand the applied deformation; thus, the system is prone to undergo flow.

Therefore, a transient network occurs for the L-HEX binary system rather than a true gel because rheological properties exhibited the remarkable frequency dependence described above (Lee et al., 2010). In addition, increased lecithin concentration led to higher G' and G'' moduli values, indicating greater overall resistance to deformation of the material. The Maxwell model was fit to the data, and good agreement between the experimental data and the model occurred at low frequencies, with a pronounced disagreement at high frequencies for G'', a trend that was expected for these samples and indicates the presence of lecithin wormlike micelle (Fig. 5b) (Cautela et al., 2017; Granek and Cates, 1992). The good fitting of the Maxwell model at low and medium frequencies may also indicate the presence of intertwined micelles (Cates and Candau, 1990; Hashizaki et al., 2009; Imai et al., 2013; Njauw et al., 2013). However, the deviation between the model and data at high frequencies is explained by the relaxation mode of the reverse wormlike micelles, which do not strictly follow the single Maxwell model. The disagreement is probably due to a faster time for relaxation related to micro-Brownian motion (Imai et al., 2013). Thus, rheological data have indicated the formation of a supramolecular structure, in which lecithin assemblies are constantly breaking, recombining, and reforming through a dynamic physical mechanism (Cates and Candau, 1990; Cautela et al., 2017; Njauw et al., 2013).

Figure 6 shows the plateau modulus, G_0, zero shear viscosity, η_o, and relaxation time, τ_R, derived from the Maxwell model fit to rheology data *versus* L concentration for the L-HEX binary system in the presence and absence of the
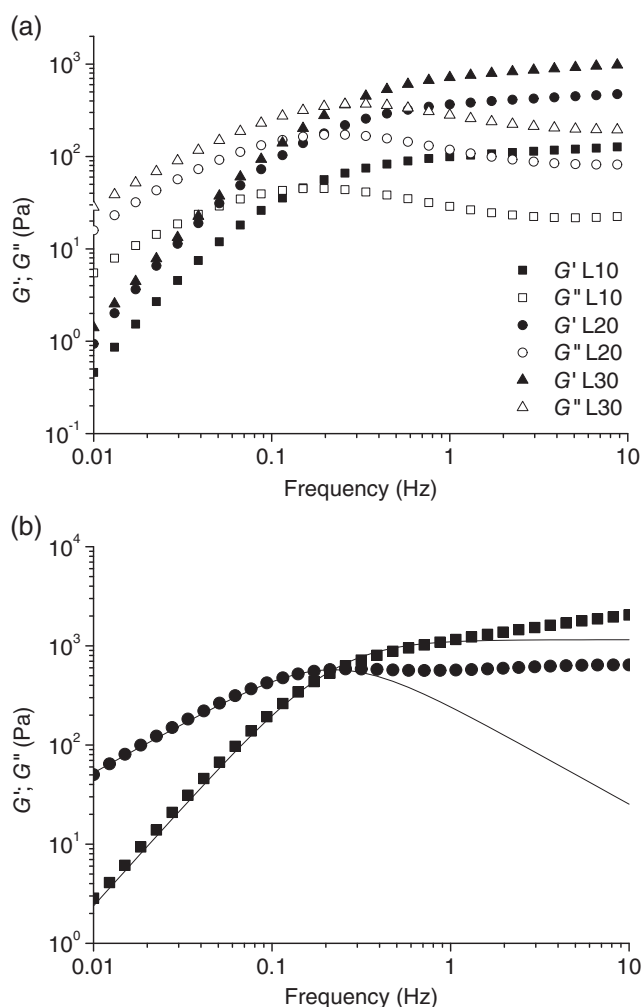
(a)

(b)

Fig 5 Representative plot of storage (G') and loss (G'') moduli as function of angular frequency (0.01–10 Hz) at 25 °C at different lecithin concentrations (**a**). Storage (G': ■) and loss (G'': ●) moduli represent experimental data points, and (**b**) the adjusted solid lines are the Maxwell model fitted for L20W sample

(a)

(b)

(c)

Fig 6 Rheological parameters (**a**) G_0; (**b**) τ_R and (**c**) η_0 *versus* lecithin concentration with no primer, W or CA addition with HEX. The molar ratio of [W]/[L] and [CA]/[L] was 1.0 and 3.3, respectively. Parameters were obtained from the fitting of the Maxwell model to the rheological measurements data

primers W and *CA*. With increasing L concentration, both G_0 and η_0 increased, demonstrating the improvement of elastic and viscous properties. The parameter G_0 is associated with the network strength, which is related to the formation of entanglements by lecithin structures (Shrestha et al., 2011). The high number of reverse micelles may be causing the increase of viscosity. Figure 6 also shows that G_0 and η_0 tend to increase with addition of primers and the highest values were achieved with CA incorporation. Thus, comparing the effects of water and CA primers, the latter led to higher values of viscosity (Fig. 6c), although the increase of viscosity according to increment of lecithin concentration with CA addition was less pronounced than response of viscosity when different lecithin concentrations were combined with water.

Relaxation time (τ_R), which reflects the relationship between viscous and elastic properties, increased as lecithin
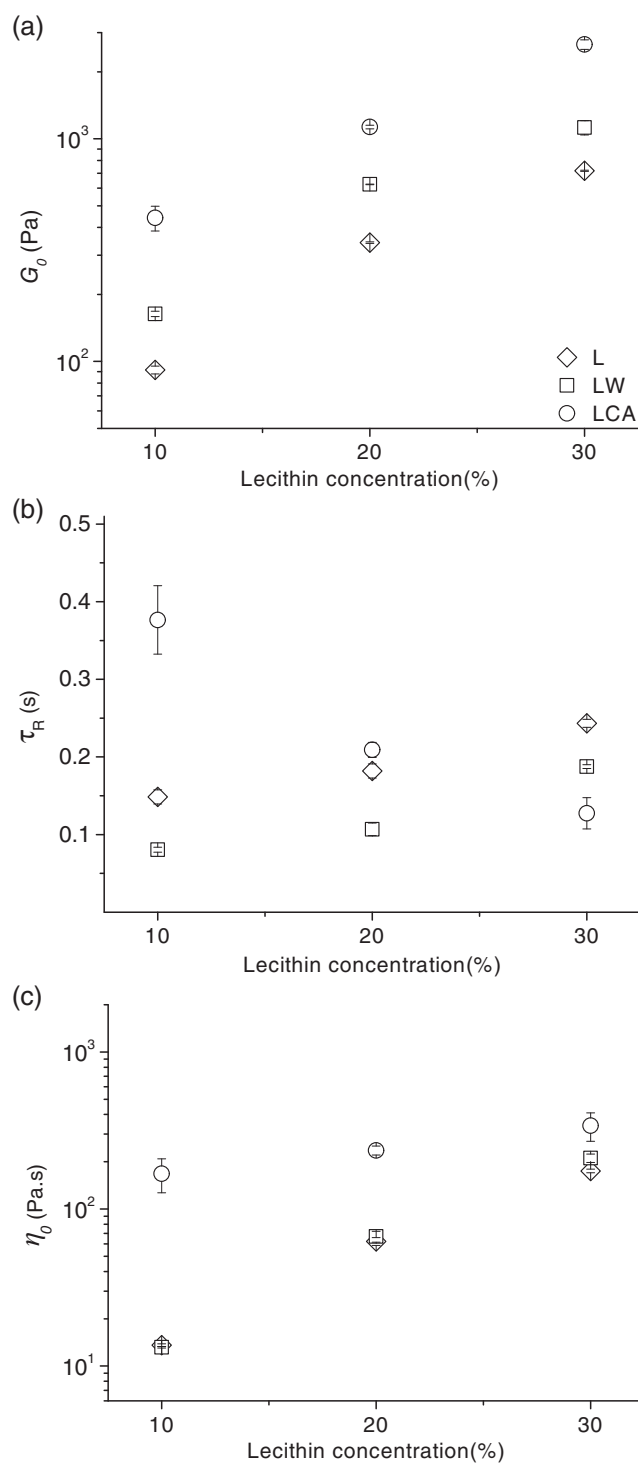
concentration increased in the absence and presence of water. Conversely, τ_R decreased with L concentration in the presence of *CA*. This can be explained by the smaller

magnitude for the difference of the viscous and elastic properties (η_o and G_0) for samples with CA. In addition, the relaxation mode of lecithin OG is complex since it may show a mixture of entities possessing various relaxation times (Shchipunov et al., 2001), which could be even more complex with the presence of primers.

The large polarity difference between the oil and primers (water or CA) may enhance structure organization, thereby improving gel strength (G_0) compared to systems containing only L and oil. In this sense, the addition of primers induced a positive effect on the gel strength, reinforcing the gel structure. Moreover, interactions such as hydrogen bonding between lecithin head groups and primers are very important to explain the greater structure organization (Lin et al., 2016; Njauw et al., 2013).

The thermoresponsive behavior of lecithin OG was evaluated using dynamic oscillatory-shear measurements at different temperatures (frequency sweeps under isothermal conditions). Higher temperatures shifted the crossover frequency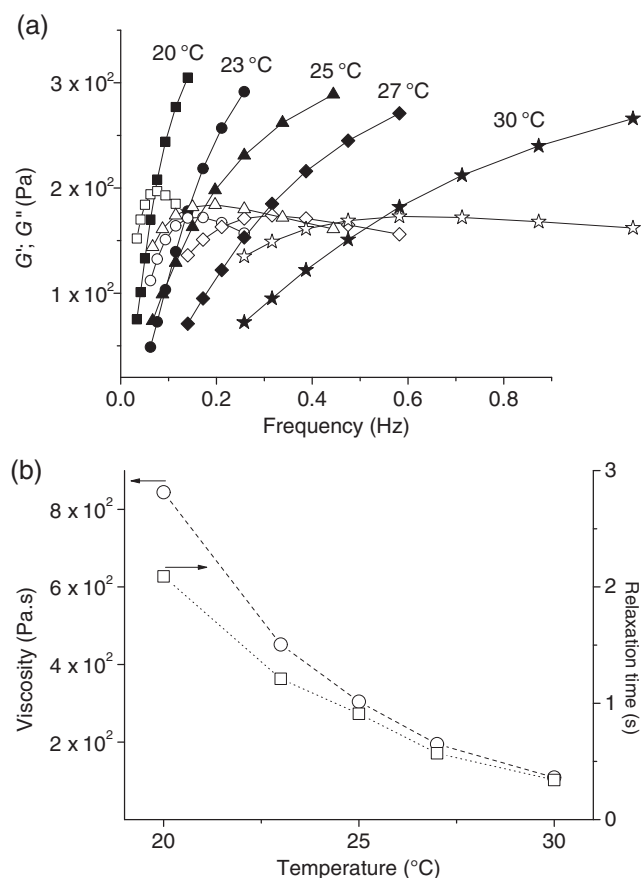 to higher values (Fig. 7a), indicating an increase of surfactant solubility in oil, i.e., increasing *Van der Waals* interactions between lipophilic moieties (hydrocarbon chain) of L and the oil (Shrestha et al., 2011). Therefore, there was an increase of oil penetration into the surfactant's fatty acyl chains, consistent with an increase of P, which is not favorable to wormlike micelle formation (Shrestha et al., 2011; Tung et al., 2007; Tung et al., 2008). Moreover, for inverted spherical micelles to transform into cylinders, P must decrease. The increase of crossover frequency with an increase of temperature corresponds to a decrease of relaxation time (Fig. 7b). Physically, a decrease of relaxation time means physically that when a mechanical disturbance is applied, the sample reaches equilibrium faster, and this can be associated with a less ordered structure.

Furthermore, an increase of gel modulus with decreasing temperature has been previously observed for other gel-like systems. The reduction of temperature leads to an entropy decrease that facilitates attractive forces such as hydrogen bonding and *Van der Waals* forces between gelator moieties during gel formation (Palazzo, 2013; Rafe et al., 2013). It is clear that an increase of temperature decreased η_o of gel as depicted in Fig. 7b. The viscosity decreased almost 8-fold (e.g., from 844.19 Pa.s at 20 °C to 108.84 Pa.s at 30 °C for L20), indicating a strong effect of the temperature on the self-assembly of lecithin in organic solvents. Thus, temperature sweeps were performed from 25 to 90 °C. G^* increased as T decreased (Fig. 8). Two transition temperatures are usually identified from the temperature sweep data for OG: sol–gel and gel–sol transitions as presented in Table 2. Gel–sol transition temperatures determined during the heating stage ($T_{gel-sol}$) ranged from 31.5 ± 0.8 °C to 41.3 ± 1.2 °C (Table 2) and nearly identical for the cooling stage (35.3 ± 0.8 °C to 45.3 ± 1.4 °C), indicating

Fig 7 Effect of temperature on the dynamic rheological behavior of lecithin gels (L20). Storage (G' as full symbols) and loss modulus (G'' as empty symbols) at different temperatures: 20 °C (■, □), 23 °C (●, ○), 25 °C (△, ▲), 27 °C (◆, ◇) and 30 °C (☆, ★) (a) and (b) the zero shear viscosity, η_o, (○) and relaxation time, τ_R, (□) *versus* temperature
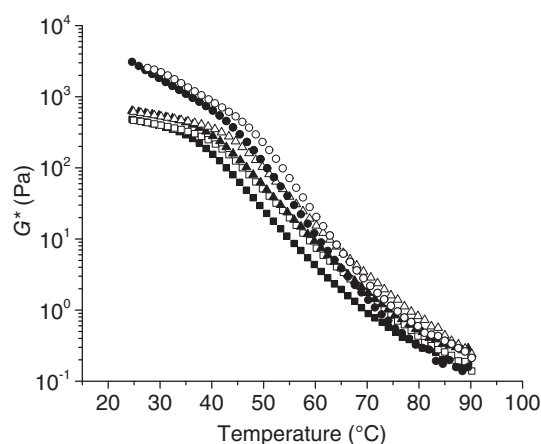
Fig 8 Representative temperature sweeps for lecithin organogels produced with 20 wt.% of lecithin considering complex moduli G^* (Pa) with f of 1 Hz and γ of 1%. For systems with lecithin only (□, ■), water inclusion (△, ▲), and citric acid (○, ●) addition. Cooling (full symbols) and heating (empty symbols) sweeps

Table 2 Sol–gel ($T_{sol\text{-}gel}$) and gel–sol ($T_{gel\text{-}sol}$) transition temperatures of lecithin-based OG with HEX

Sample	$T_{gel\text{-}sol}$ (°C)	$T_{sol\text{-}gel}$ (°C)
L10	38.4 ± 0.7	41.9 ± 0.9
L20	38.7 ± 2.1	41.7 ± 2.1
L30	40.4 ± 0.9	44.4 ± 1.2
L10W	35.7 ± 0.7	39.5 ± 0.9
L20W	39.7 ± 0.7	42.9 ± 0.8
L30W	39.6 ± 1.1	43.6 ± 0.7
L10CA	31.5 ± 0.8	35.3 ± 0.8
L20CA	40.8 ± 1.5	45.3 ± 1.4
L30CA	41.3 ± 1.2	44.4 ± 1.2

reversibility for phase transitions. The G^* *versus* T profile for heating and cooling stages were similar, showing the typical thermal reversibility observed for OG with only a minor hysteresis (Lescanne et al., 2003). Sol–gel and gel–sol phase transition temperatures seemed to be related to lecithin concentration, more strongly in the presence of primers. However, results did not indicate a strong difference between the primers added to the system (either W or CA). The thermoreversibility displayed by these gels was due to thermodynamic aspects governing miscibility (solvent/gelator) and the intrinsic weakness of OG formed through physical bonds (Liu et al., 2013).

Although the gelator-gelator interactions are the main driving force for self-assembly of amphiphilic molecules, proper gelator-solvent affinity is also important for the preparation of OG. In this sense, the interactions between (i) oil and gelator fatty acyl chains (solubility); (ii) polar moieties of gelators and (iii) gelator head groups and primers, were important to govern gel formation and properties.

Conclusions

Chemical composition, polarity, and viscosity of the organic solvents are important parameters to consider in the formation of lecithin-based OG. Through SAXS measurements, different structures from lecithin self-assembly were observed as a result of the influence of medium characteristics. Only for the HEX-L binary system, typical reverse worm-like micelles were observed *via* SAXS, which is in agreement with the formation of a structured (gel-like) system. The strategy of changing the environment by adding water or CA to control geometry packing was evaluated. Rheological properties of gels (elastic modulus and zero-shear viscosity) were improved either by increasing lecithin concentration or by adding primers. In general, systems containing CA showed greater elastic properties compared to those containing water. OG were thermoreversible, with a slight hysteresis observed between cooling and heating stages. Some differences in gels' microstructure were observed depending on how lecithin molecules could organize and/or rearrange. These findings express the concept of "tunable OG" related to reverse micellar geometry, in which phospholipid tail architecture, concentration, and temperature act as parameters to control the structure of lecithin-based OG. The understanding of L-HEX binary system can be useful for with more complex systems such as those based on vegetable oils. Moreover, the use of lecithin as gelator represents an important application as it is a natural and versatile ingredient.

Conflict of Interest The authors have declared no conflict of interest.

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