Ternary Phase Diagram of Soybean Phosphatidylcholine–Water–Soybean Oil and Its Application to the Water Degumming Process

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ABSTRACT: Crude soybean oil contains phospholipids (2.5 wt%) that must be removed from oil during processing. A common method is the water-degumming process. A ternary phase diagram of soybean oil–water–soybean PC, a major component of phospholipids, was established. From this diagram, phase transitions and compositions of phases can be determined. A theoretical model describing the relationship between aggregation curvature and the amount of water added is presented to explain the phase transitions. The amount of water absorption by the lamellar phase should be larger than the critical value of 34 wt% based on the total weight of water and PC. Below this critical amount, phospholipids tend to form liposomes. Above the critical point and below the saturation point, larger aggregates of particles form and can be easily separated. When more water is added to reach the water adsorption limit, about 40 wt% based on the total weight of water and PC, a phase transition boundary is observed, beyond which a third phase, water, appears and the particle size falls dramatically. In between the critical line predicted by the model and the water adsorption saturation line observed experimentally, there is an operation window on the ternary phase diagram for the water-degumming process.

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Part of the soybean oil refining process is the removal of phospholipids from the crude oil (1), and the most widely practiced process is water degumming (2–4). The phospholipids in the crude oil, most of which are diacyl surfactants that form bilayer structures in aqueous solutions, are a mixture of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidic acid (PA), phosphatidylinositol (PI) and a small amount of the monoacyl phospholipids: lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE) (5,6). In the water degumming process, the partially oil-soluble phospholipids in the crude oil are transformed into oil-insoluble lamellar liquid crystals (gum) by absorption of added water (1). These oil-insoluble liquid crystals have higher density and can aggregate into bigger particles, which can be separated from oil by centrifugation.

The separated gums, also called lecithin, contain lipids entrained with an equal amount of water. The dried lecithin contains approximately 30 wt% neutral soybean oil (TAG) and 44 wt% polar phospholipids. The percent composition of oil-free lecithin is PC 24, PE 20, PI 10, PA 5; the remaining materials are lysophospholipids, complex glycolipids, carbohydrates, and other minor components (7,8). The amphiphilic nature of phospholipids controls the degumming process: Although partially soluble in oil, in the presence of water phospholipids always tend to form monolayers at the water–oil interface to lower the interaction energy associated with hydrophilic and hydrophobic parts of phospholipid molecules. Their acyl chain lengths can range from 16 to 20 carbons, mostly with 18 carbons with one or two unsaturated bonds (1). In this study, to establish a model phase diagram, we chose one of the predominant phospholipids, PC, water, and soybean oil. A ternary phase diagram of the PC–water–soybean oil system is needed to establish the relationships among the components and consequently to optimize important parameters such as the amount of added water in the degumming process. Because of the chemical similarity of other components of phospholipids, their phase behaviors can be studied in a similar way.

The current industrial practice in degumming has been summarized as follows (9): "The amount of water used in the industrial practice to hydrate the phospholipids during the degumming step is critical. Usually the amount of water added is 75% of the phospholipid content of the oil. The phospholipid content of soybean oil varies between 1.5 to 3 wt% of the oil, which requires roughly 1 to 2 wt% of water. It is known in the industry if too little water is added, the gums phase will be dark and viscous and the oil phase will be hazy due to the residual phospholipids. If too much water is added three phases will develop making the degumming operation difficult and also results in greater oil loss." The goal of this paper is to relate current practice to the phase diagram and colloidal behavior of lecithin, oil, and water systems.

EXPERIMENTAL PROCEDURES

Theoretical model. Two major factors in the process of separating phospholipids from the oil are: (i) the density differ-

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ence between lamellar particles and the oil, and (ii) the size distribution of gum particles. How these two factors affect the process can be understood by Stokes' Law:

$$V_c = \frac{d^2(\rho_P - \rho_L)}{18\eta} \cdot ng \quad [1]$$

where V_c is the sedimentation velocity, d is particle diameter, ρ_P is particle density, ρ_L is the density of the continuous phase, η is the viscosity of the continuous phase, g is gravitational acceleration, and n is related to the rotational velocity of the centrifuge. The density difference between the particles and the continuous phase, $(\rho_P - \rho_L)$, is almost constant: ~0.169 g/cm^3 for lamellar particles in soybean oil at room temperature. As shown in Equation 1, V_C is proportional to d^2, i.e., larger particle sizes result in a better degumming process.

In an excess oil phase, bilayers or lamellar phases may be present in a dispersed form or exist as flocculants in the oil. The formation of the bilayer structures in the oil depends on the water concentration, phospholipid concentration, and the packing parameter of the phospholipid. In the ternary phase diagram of the PC–water–soybean oil system reported in the next section, both dispersed and flocculated particles can exist depending on the amount of water in the lamellar phase. To explain this observation, a theoretical model that relates packing parameter N_S and the aggregation properties of self-assembled structures can be adopted (10), where

$$N_S = \frac{V_L}{d_L \cdot a} \quad [2]$$

In Equation 2, a is the head group area, V_L is the volume of the hydrocarbon chain or chains, which can be assumed to be fluid and incompressible, and d_L is the extended hydrocarbon chain length that can be calculated from Equation 3:

$$d_L = 0.154 + 0.1265 n_c \text{ nm} \quad [3]$$

where n_c is the number of carbons per hydrocarbon chain. The value 0.154 nm in this equation comes from the subtraction of half the bond length of the first atom excluded in the hydrocarbon core (0.06 nm) from the van der Waals radius of the terminal methyl group (0.21 nm). The value 0.1265 nm is the carbon–carbon bond length (0.154 nm) projected onto the direction of the chain for all-*trans* configuration. The hydrocarbon volume can be calculated using the following equation:

$$V_L = 0.027(n_c + n_{\text{Me}}) \text{ nm}^3 \quad [4]$$

where n_{Me} is the number of methyl groups, and the value 0.027 is the volume of the hydrocarbon core of saturated hydrocarbon chain (10). It has been shown that for lipids N_S determines the type of self-assembled structures formed: spherical micelles when $N_S \leq 1/3$, nonspherical micelles when $1/3 < N_S \leq 1/2$, vesicles or bilayers with a normal outer layer when $1/2 < N_S < 1$, and vesicles or bilayers with an inverted outer layer when $N_S > 1$ (10,11). The kinds of structures that phospholipids form, "normal" or "inverted," are directly related to particle size of the lamellar phase. In the model to be presented, the packing geometry of PC within an aggregate is considered, and the experimentally measured d-spacing values are used to determine the favorable structures that PC forms in the ternary system based on the above criteria.

Materials and sample preparation. Soybean PC (purity >95%) was obtained from Avanti Polar Lipids, Inc. (Alabaster, AL) and used without further treatment. Refined, bleached, and deodorized soybean oil (purity >99.9%) was obtained from Cargill Inc. (Minneapolis, MN). Water was double distilled and deionized. The soybean PC–water–soybean oil samples were prepared by mixing them with small magnetic bars in 7-mL sealed glass tubes (1 cm in diameter) submerged in a water bath with precise temperature control (±0.1°C).

Phase identification. Glass tubes, containing samples, were submerged in a water bath and placed between two crossed polarizing films (Bausch & Lomb, Rochester, NY), and a strong light source was aligned behind the water bath to transmit light for the examination of birefringence in the samples. A laser beam was used to detect the solution's turbidity. The samples were allowed sufficient time to reach equilibrium. Turbidity, viscosity, birefringence, and several other criteria reported by Lang and Morgan (12) were used to identify the phases and phase boundaries: (i) a two-phase boundary was indicated by the onset or disappearance of turbidity; and (ii) hexagonal or lamellar liquid crystal phases resulted in birefringence under polarized light. The hysteresis on the phase transition for temperatures reached from below and above was observed to be smaller than 0.4°C, and the phase boundary was determined by averaging the upper and the lower boundaries (12). Further phase identification was performed with small angle X-ray scattering.

Small angle X-ray scattering experiments were performed on a modified Kratky camera from Anton Paar KG (Graz, Austria) equipped with an extended flight tube and a movable beam stop. The X-ray generator had a rotating anode (Rotaflex model RU-200B; Rigaku Corp., Japan) operating at 10 kW with a copper target. The K_α wavelength of 1.54 Å was selected by means of Nichol filters. The energy window on a model MBRAUN OED-100-M 10-cm linear position sensitive detector (Innovative Technology, Inc., Newburyport, MA) was set to accept only the scattering photons with energy close to 1.54 Å. The Kratky linear collimation produced a 15×0.13 mm^2 X-ray area on the sample that was sealed in a 1.5-mm diameter glass capillary (Charles Super Co., Natick, MA). The sample-to-detector distance was set at 68.2 cm. The detectable wave vector $\mathbf{q}$ range was from 0.02 to 0.3 Å^{-1}, where $\mathbf{q} = (4\pi/\lambda)\sin(\theta/2)$ and θ is the scattering angle. The scattering data accumulation time varied between 30 and 240 min, depending on the scattering intensity of the sample.

Particle size measurements. Light microscopy with a post-image processing technique was used to image the liquid crystal phases in the systems and to analyze the particle sizes. A Nikon Optiphot-Pol microscope (Tokyo, Japan) was used to image samples on glass slides under the crossed polariza-

tion mode. Particle sizes were measured by averaging the particle sizes from several micrographs of the same sample using a built-in macro program in a digital image acquisition/processing software Metaphorph (Universal Imaging Corporation, Downingtown, PA).

RESULTS AND DISCUSSION

Experimental observations. A ternary phase diagram of the PC–water–soybean oil system at room temperature was constructed (Fig. 1). In this phase diagram, only one liquid crystal region was observed—a lamellar liquid crystal phase. This lamellar liquid crystal phase existed at the corner of high PC concentration region. There was water uptake by this lamellar phase, and the water absorption by PC can reach as high as 40 wt%. At water concentrations higher than that, either a three-phase or a two-phase region appeared. The oil uptake of this lamellar phase was 12 wt%, presumably one of the reasons that oil is lost in the degumming process. From the PC–water binary part, two phases exist—a lamellar phase dispersed in an aqueous solution at high water concentration (13,14). On the PC–oil binary side, a one-phase region, the L_2 phase, is observed at high oil concentration. This L_2 phase is very sensitive to water content. Addition of only 0.05 mL of water to the L_2 phase of several milliliters can cause phase separation into the lamellar phase and oil. The maximal water uptake of oil that contains phospholipids is marked by the two-phase to three-phase transition line.

The phase diagram (Fig. 1) provides insight into the degumming process. During the water degumming process, both chemical composition and phases change. The crude oil, which is marked on the phase diagram, contains a mixture of 2–3 wt% phospholipids. Upon addition of water to the system along the dashed line, crude oil–water changes the compositions of phases and induces phase transitions in the system. When the system contains almost no water or very little water, the phospholipids are dissolved in the oil and the solution is a clear phase, marked L_2 in the phase diagram. After water is added to the system, phase separation occurs, and phospholipids aggregate into lamellar liquid crystal by taking up water. As the amount of added water increases, the water uptake of the lamellar liquid crystal increases until a water adsorption saturation point is reached that is right on the phase transition boundary. Beyond the phase boundary, another phase, water, is observed. This water phase complicates the degumming process. The amount of water added to the crude oil is critical for the successful removal of phospholipids from the oil (2), and it is known in industrial practice that the amount of added water should be equal to or less than the amount of phospholipids in the crude oil. This can be understood from the ternary phase diagram. If the water added is more than the amount of phospholipids, the phases for the degumming process are three, of which the third phase is almost pure water. This would cause the extra problem—removing water from the oil. Another problem resulting from excess water addition is the dramatic decrease in particle size, as discussed below.

The particle size found is directly related to the water absorption of PC. In this study, a solution with 8.0 wt% PC in the soybean oil was prepared, and the particle sizes were measured as various amounts of water were added to the solution, as marked on the dotted line in Figure 2. The average particle size increased as the amount of water added approached the water adsorption saturation value and decreased once the amount of water added passed this value. Table 1 shows the particle size measurement results from light microscopy images for different amounts of added water: 1:4, 1:2, 2:3, and 1:1 water-to-PC volume ratios. Representative micrographs of the four samples are shown in Figure 3. A needle-like morphology with small dimensions was observed in sample A, and many individually dispersed liposomes were

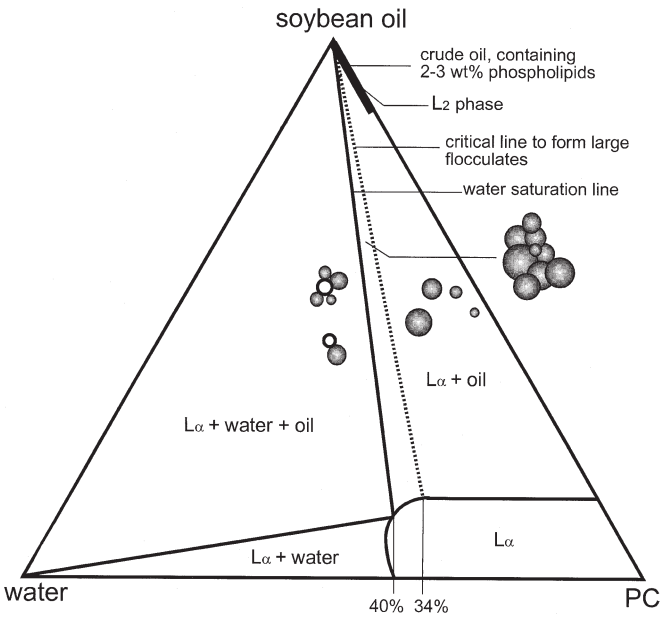

FIG. 1. Room temperature ternary phase diagram of soybean oil–water–soybean PC. The dotted line is the critical line above which large flocculates can form.

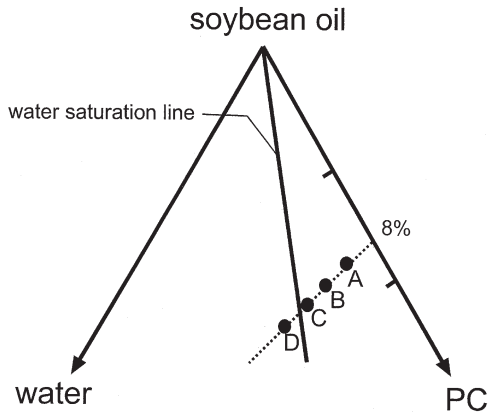

FIG. 2. The particle size and microstructure of the PC aggregation in oil were studied for marked points in the partial phase diagram. The water–PC ratio for A, B, C, and D are 1:4, 1:2, 2:3, and 1:1, respectively.

TABLE 1
Compositions of the Four Samples (A–D) and Particle Sizes Determined by Light Microscopy

Sample	Water/PC vol. ratios	Particle size (μm)	SD (μm)
A	1:4	2.3	2.1
B	1:2	12.2	8.9
C	2:3	84.0	49.5
D	1:1	7.5	6.0

observed in sample B. Sample C consisted mainly of large aggregate-like particles, and sample D consisted of particles similar to those in C but with much smaller dimensions.

Theoretical explanations. In the oil–PC–water system, N_S is an important factor in determining the spontaneous structure of vesicles, or the preferred outer layer of liposomes (11). If N_S is greater than 1, the inverse structure, which can be dispersed in the oil, is preferred for PC molecules due to their small head groups and large tail groups (15–18). If N_S is less than 1, normal-structured vesicles result. These normal-curved liposomes have high interfacial energy, and as a result, they are not stable and tend to aggregate into bigger flocculates in the oil to reduce the interfacial area.

To calculate N_S, the parameters in Equation 2 need to be determined. d_L was found to be 2.436 nm based on Equation 3, for an average chain length of 18 carbons. Because of oil swelling of the unsaturated bonds in the hydrocarbon chains and the splaying effect of the two neighboring tails, the tail volume for PC is nearly tripled (9,18). By adapting a factor of 2.9 to Equation 4, V_L was calculated to be 1.4877 nm^3. The effective head area of a PC molecule in lamellar phase increases with increasing water concentration and can be calculated from Equation 5 (13):

$$a = \frac{V}{(D/2)\phi_l} \qquad [5]$$

where ϕ_l is the lipids volume concentration, D is the lamellar repeat spacing, which can be measured by small-angle X-ray scattering, and V is the molecular volume of a lipid molecule, including the hydrocarbon tail volume and the head group. V can be calculated by using the following equation:

$$V = \frac{M}{N}\frac{1}{\rho}10^{24}\ \text{Å}^3 \qquad [6]$$

where M is the M.W. of soybean PC, 758.07; N is Avogadro's number; and ρ is the PC density, 0.985 g/cm^3, by experimental measurement.

Below the saturation point of water adsorption in the lamellar phase, D increases and ϕ_l decreases as the water concentration increases. The measured D values for samples E–H are shown in Table 2. The overall effect for $D\phi_l$ is a decrease. The molecular volume of the PC molecule is constant and is equal to 1278 Å^3. Thus, by increasing water concentration, the effective head group area is enlarged: The hydrophilic head group takes up more water and swells. Other reports show that hydratable phospholipids can have larger head group areas, compared to nonhydratable ones, and tend to form bigger particles in oil (20,21). The calculated head group areas and packing parameters for samples E–H are shown in Table 2 (note that the compositions of samples E and H are the same as those of samples A and C in Table 1, respectively). From the N_S values, we see that an inverse structure ($N_S > 1$) is preferred for samples E, F, and G, and a normal structure ($N_S < 1$) is preferred for sample H. In this model, the limit for stable liposome formation in oil is that N_S equals 1. If the amount of water addition is small enough that an inverse structure is favored, the stable PC liposomes can exist in the oil as individual particles. When more water is added to further increase the head group area of PC, N_S becomes less than 1, and the normal structure is favored. The

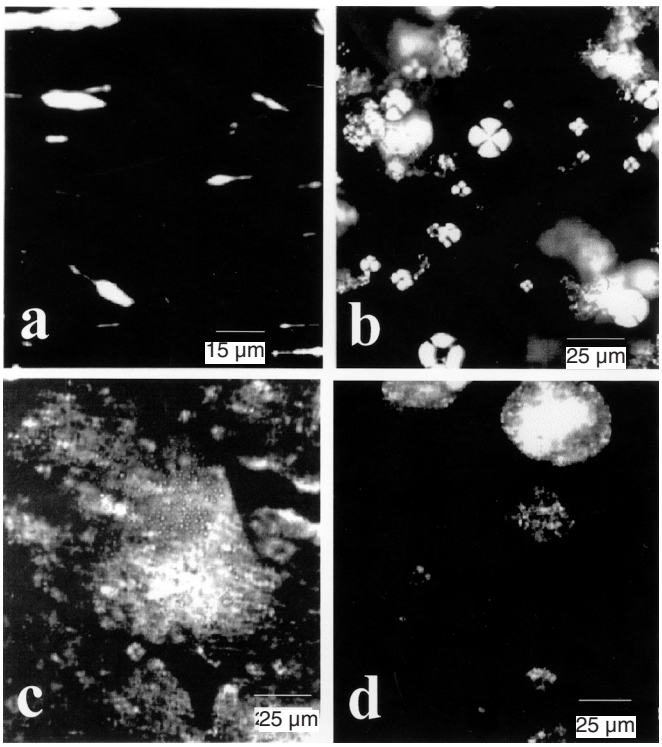

FIG. 3. Light microscopy images of samples listed in Table 1. From sample A to sample C the particle size increases (Figs. 3a to 3c), and the particle size falls in sample D (Fig. 3d).

TABLE 2
Compositions and *d*-Spacings Measured by Small Angle X-ray Scattering of the Four Samples (E–G) Chosen to Calculate Their Packing Parameters in Lamellar Phase and to Predict Their Preferred Structures in Oil

Sample[a]	PC vol. fraction based on water and PC (%)	Lamellar spacing D (Å)	Head group area (Å^2)	Packing parameter N_S	Preferred structure
E	80	52.64	60.17	1.07	Inverse
F	75	54.48	62.02	1.04	Inverse
G	70	57.08	63.42	1.02	Inverse
H	60	62.40	67.68	0.95	Normal

[a]Sample E has the same composition as sample A, and sample G has the same composition as sample C in Table 1.

high interfacial energy of these normally curved liposomes in oil drives the formation of large aggregations of the liposomes to reduce the total interfacial area. Above the saturation point of water adsorption in the lamellar phase, the lamellar phase can no longer take up water. Excess water penetrates into large aggregates of liposomes, accumulating around the particles and eventually coming out as a free phase. This free water phase increases the free energy of the system dramatically. To reduce the free energy of the system, the water droplets tend to be surrounded by the vesicles or liposomes, resulting in a breakup up of large flocculates. Graphical illustrations of the above descriptions are shown in Figure 4.

Better explanations of microscopic observations can now be drawn. At very low water concentration, below the tail melting water concentration at room temperature, PC exists as a rigid bilayer structure, which aggregates into needle-like structures (Fig. 3a). At a slightly higher water concentration, the head groups are swelled, the tails melt, and the bilayer structure becomes softer and can be packed. Liposomes form and disperse in oil, and the size of these liposomes depends on the PC concentration. The greater the packing parameter, the higher the tendency of the liposomes to disperse individually in the oil. Figure 3b shows that larger liposomes exist in sample B whose water/PC ratio is 1:2. As water concentration increases further, the head group area becomes even larger, and the packing parameter falls below 1—the "normal" structures form. These normal structures are not stable in the oil-continuous phase and have a strong tendency to flocculate, as in sample C, whose predicted N_S is 0.95 (Fig. 3c). When the water added exceeds the adsorption saturation point, the free water phase forms and the free energy of the system increases. As a consequence, the large flocculates break, resulting in smaller particles as shown in Figure 3d. The water content in sample D is 50 wt%, based on water and PC.

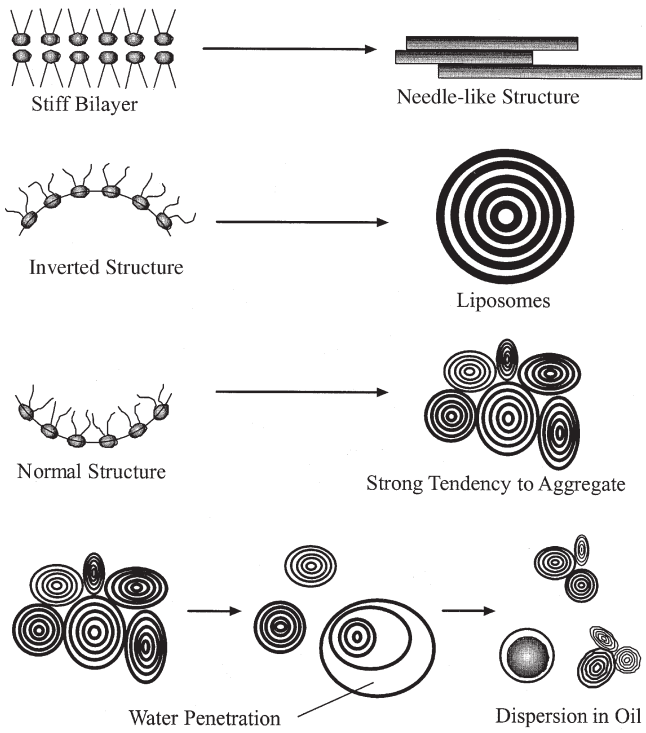

FIG. 4. Graphical illustrations to explain the mechanism of particle formation and size change. At very low water concentration, phospholipids still exist in a gel state, and the tails are not melted. The lamellar particle formed is rigid. At higher water concentration, their tails are melted, and stable inverted liposomes form and disperse in oil. At even higher concentration, the normal structure is preferred in oil, and these inverted liposomes are not stable and flocculate into large particles. At a water concentration higher than 40 wt%, the excess water forms a new phase, and the large flocculates break down into smaller particles.

Process optimization. To remove phospholipids successfully from crude oil by water degumming, the particle size of the aggregates of the lamellar phase must be large enough, which, in turn, requires a packing parameter of phospholipids in the lamellar structure less than 1, as demonstrated above. When the packing parameter of phospholipids in the lamellar structure is greater than 1, inverted liposomes form and are stable in the continuous oil phase, resulting in difficulties in separation. At a packing parameter equal to 1, the water-to-PC ratio is calculated to be 34 wt% based on water and PC; thus, the amount of water added must be greater than this value for large flocculates of normal structure liposomes to form. On the other hand, these large flocculates begin to break into smaller ones as the amount of water exceeds the adsorption saturation point by the lamellar phase and exist as a new phase in the system, indicated by the two- to three-phase transition line in the ternary phase diagram. Beyond this boundary, it is not only harder to separate the smaller particles but also more complicated to remove the water phase. Therefore, the amount of water added should not exceed 40 wt%, based on water and PC. The process operation window should be between 34 and 40 wt% added water based on water and PC, and an optimal amount of water in the degumming process should be close to 40 wt%. However, in industrial practice, due to the variation of the phospholipid content and chemical heterogeneity of the phospholipid mixture (PC, PE, PA, PI, etc.) in the crude oil, the amount of water added during the degumming will vary from this window but will be close.

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