

# Accounts of Materials & Surface Research

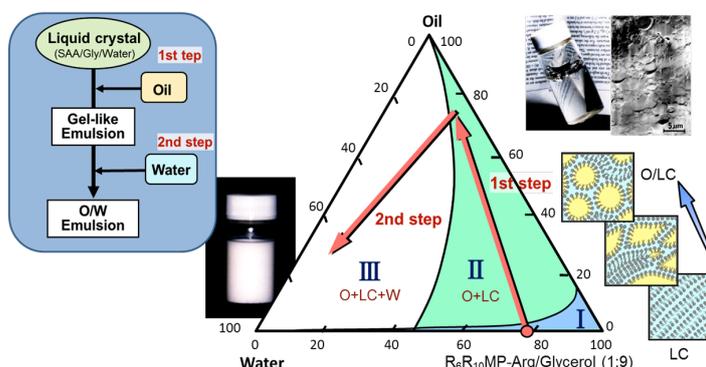
## Liquid Crystal and $\alpha$ -gel-Based Emulsion and Soft Gel formulations

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Significance and application of molecular assemblies, liquid crystal and  $\alpha$ -gel, for the preparation of emulsion and lipid dispersion formulations are explained. Unique formulations, soft gel, micro gel dispersion and multilamellar emulsion, were produced using the stable  $\alpha$ -gels obtained from L-arginine salt of long-chain monoalkyl phosphate. The stabilization of  $\alpha$ -gel was achieved by the contribution of L-arginine as the counterion of monoalkyl phosphate; the dissociation of three functional groups that induces the electrostatic interaction between the neighboring molecules, and the bulkiness that suppresses the precipitation of  $\beta$ - or  $\gamma$ -crystals.

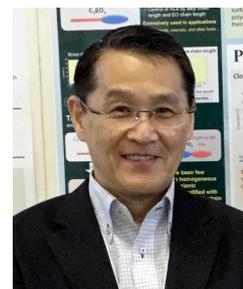
Lamellar liquid crystal of  $\beta$ -branch-type L-arginine long-chain monoalkyl phosphate was applied to the preparation of gel-like oil-in-liquid crystal (O/LC) emulsions of high internal phase ratios, and fine O/W emulsions. This liquid crystal emulsification was available for a wide variety of oils of various polarities, and even for silicone oils and perfluoropolyethers. From the phase behavior during emulsification and analysis of the dynamic behavior of the liquid crystal membrane, it is concluded that the unique property of liquid crystal emulsification is attributed to the independence of liquid crystal membrane as a phase against oil and water.



Process of liquid crystal emulsification indicated in phase diagram.

**Keyword:** Liquid crystal,  $\alpha$ -gel, Emulsion, Monoalkyl Phosphate, Phase behavior

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# Liquid Crystal and $\alpha$ -gel-Based Emulsion and Soft Gel formulations

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## 1. Introduction

Emulsions are widely used in practical systems including cosmetics, pharmaceuticals, food, paint, etc., since they can coexist mutually insoluble oil (oil soluble materials), water (water soluble materials) in an arbitrary ratio. However, they are thermodynamically unstable system, and the state and stability vary greatly depending on the emulsification procedure and environmental conditions during preparation. We often hear the trouble such as the following: "It's not possible to prepare an emulsion of the wished state." "Selection of suitable emulsifier and optimum emulsification condition is difficult and time consuming." "Stability and the viscoelastic behavior is uncontrollable." Therefore, an emulsion is said to be complex and cumbersome system.

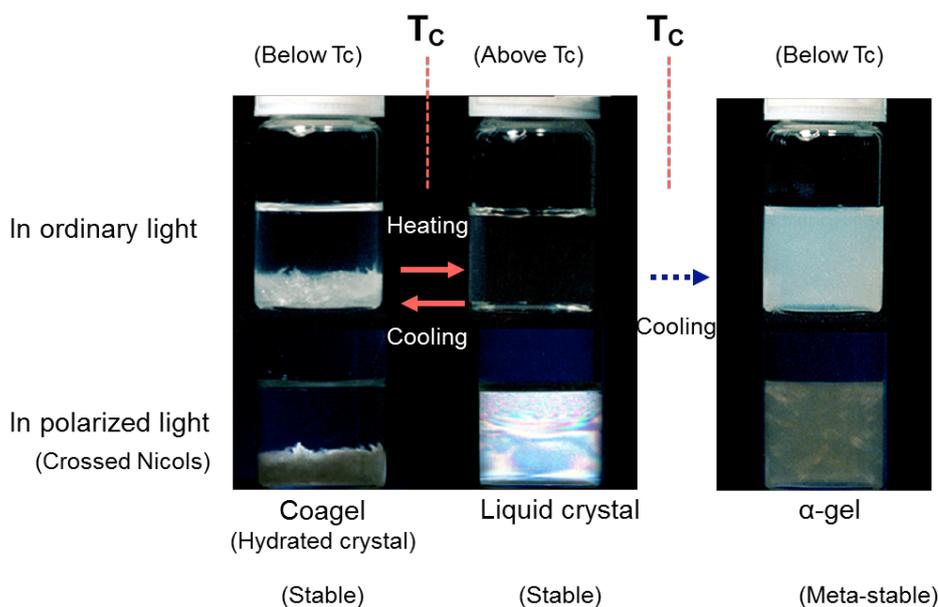
On the other hand, it is known that the stability of an emulsion remarkably improved and the viscoelastic behavior varies greatly by formation of infinite molecular assemblies such as liquid crystal or  $\alpha$ -gel.<sup>1,2</sup> Further, use of liquid crystal or surfactant phase (D-phase) make possible to produce fine emulsions and emulsions of high internal phase ratio.<sup>3-5</sup>

In this paper, the generation of characteristic emulsions and lipid dispersions using liquid crystal and  $\alpha$ -gel

formed with L-arginine long-chain mono-alkyl phosphate (linear and  $\beta$ -branched type)<sup>6,7</sup> will be explained. The mechanism of liquid crystal emulsification and significance of molecular assembly for the practical emulsion and lipid dispersion systems are also discussed.

## 2. Formation and the characterization of lyotropic liquid crystal and $\alpha$ -gel

Amphiphilic molecules possessing hydrophilic group and a lipophilic group within a molecule show the self-organizing property induced by hydrophobic interactions and form molecular assemblies.<sup>8</sup> Micelles or liquid crystals being the typical molecular assembly are formed only when the hydrophobic portion is in liquid state. Surfactants possessing solid hydrophobic chain cannot form molecular assemblies like micelles and liquid crystals without heating above the gel-liquid crystal transition point,  $T_C$ . Figure 1 shows the appearances of a surfactant / water system observed at temperatures above and below the  $T_C$ . The upper photographs are the appearances observed under ordinary light, whereas lower photographs are those observed under crossed polarizers. Though the optical anisotropic substances, crystals, liquid crystals and gels, appear to be glowing bright, the isotropic substances such as water and micellar solutions



**Figure 1.** Appearances of a surfactant / water system observed at above and below the phase transition temperature,  $T_c$

remain dark. The hydrated crystals, coagel, appear as precipitate and coexist with water are observed in the left hand side of Figure 1.

When the coagel is heated above the  $T_c$ , it changes to a liquid crystal, which is slightly viscous transparent phase retaining a large amount of water within the hydrophilic moiety, and shows optical anisotropy. In the transition of coagel to liquid crystal, relatively large endothermal peak induced by the melting of hydrophobic chains is detected by the differential scanning calorimetry (DSC) measurement. The coagel-liquid crystal transition is reversible, and the water held in the liquid crystal is released at the temperature below the  $T_c$ , to separate into two phases of hydrated crystals and water. On the other hand, translucent gel state may remain without releasing the interlayer water when it is cooled below the  $T_c$  in case of certain compounds (Figure 1 the right hand side).

This state is called as  $\alpha$ -gel. Since an  $\alpha$ -gel is generally thermodynamically metastable, it changes to a coagel by releasing water with time passage. However, sometimes there happens that the gel state maintained for a long time depending on the storage conditions.

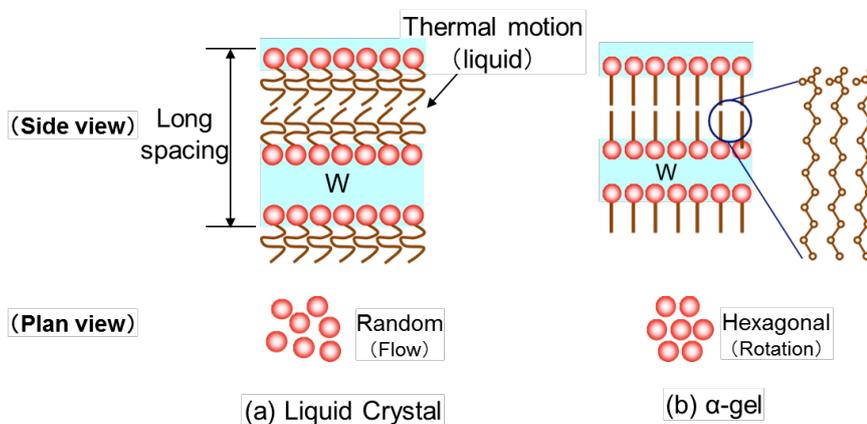
The formation and structure of a liquid crystal relate to the hydrophilic-lipophilic balance and concentration, besides the geometric factors of amphiphilic molecules. Liquid crystals formed by amphiphilic molecules with solvent (mainly water) addition are called as lyotropic liquid crystals. The association structures and their schematic models, optical textures observed by polarizing microscopy, and ratios of interlayer spacing (Bragg distance) of typical lyotropic liquid crystals are summarized in Table 1. Four typical structures including hexagonal, lamellar, cubic and reverse hexagonal can be identified as main liquid crystal structure.

	Association structure		Symbol	Optical texture	Bragg spacing ratio
Hydrophilic	Cubic		I <sub>1</sub>	Isotropic	1: $\sqrt{3/4} : \sqrt{3/8} : \sqrt{3/11}$ (BCC) 1: $\sqrt{1/2} : \sqrt{1/3} : 1/2$ (FCC)
	Hexagonal		H <sub>1</sub>		1: $1/\sqrt{3} : 1/\sqrt{4} : 1/\sqrt{7}$
	Cubic		V <sub>1</sub>	Isotropic	1: $\sqrt{3/4} : \sqrt{3/8} : \sqrt{3/11}$
Lipophilic	Lamellar		L $\alpha$		1: 1/2 : 1/3 : 1/4
	Cubic		V <sub>2</sub>	Isotropic	1: $\sqrt{3/4} : \sqrt{3/8} : \sqrt{3/11}$
	Reversed hexagonal		H <sub>2</sub>		1: $1/\sqrt{3} : 1/\sqrt{4} : 1/\sqrt{7}$
	Cubic		I <sub>2</sub>	Isotropic	1: $\sqrt{3/4} : \sqrt{3/8} : \sqrt{3/11}$ (BCC) 1: $\sqrt{1/2} : \sqrt{1/3} : 1/2$ (FCC)

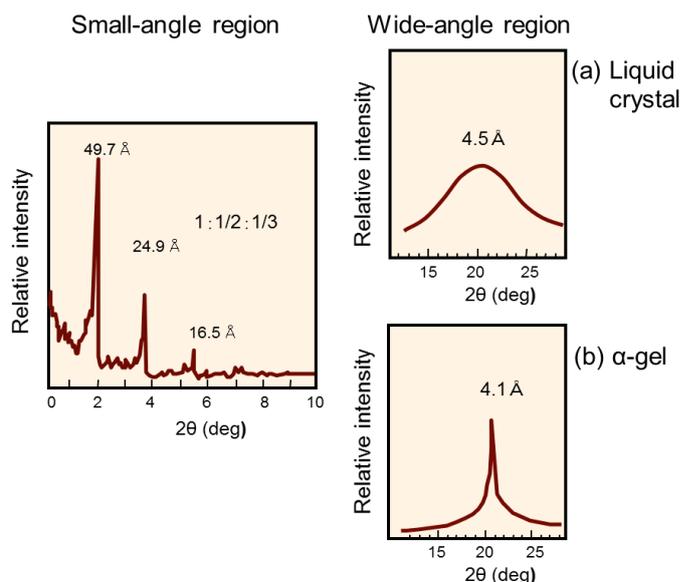
**Table 1.** Structures, optical textures and interlayer spacing ratios of typical lyotropic liquid crystals.

Schematic models indicating the state of liquid crystal and  $\alpha$ -gel are shown in Figure 2. The hydrophobic chains of Liquid crystal constituent molecule are in liquid state due to the thermal motion and do not show any ordered structure, whereas hydrogen bonding between hydrophilic groups are maintained. The characteristic of "long-distance orderliness and short-distance

disorderliness" is the definition of liquid crystalline state. When a liquid crystal is cooled to a temperature below the  $T_C$ ,  $\alpha$ -gel often appears. An  $\alpha$ -gel is the layered crystal retaining a large amount of water in the hydrophilic moiety, and the constituent molecules have rotational freedom in each layer.<sup>9</sup> Differences in liquid crystal and  $\alpha$ -gel is hard to determine by visually, but



**Figure 2.** Schematic model of liquid crystal and  $\alpha$ -gel.



**Figure 3.** X-ray diffraction pattern of liquid crystal and  $\alpha$ -gel.

can be confirmed from the wide-angle X-ray diffraction pattern (Figure 3). Association structures are determined from the ratio of interlayer spacing of small-angle X-ray scattering, and the state of hydrophobic chains is confirmed from the wide-angle X-ray diffraction pattern.<sup>10-12</sup> A diffuse halo detected at the diffraction angle around  $2\theta=20^\circ$  ( $d=4.5\text{\AA}$ ) in wide-angle X-ray diffraction of liquid crystal indicates that the lipophilic group is in liquid state owing to the thermal motion. Whereas, the sharp single peak at  $21.5^\circ$  ( $d=4.1\text{\AA}$ ) in wide-angle X-ray diffraction of  $\alpha$ -gel indicates that the hydrophobic chains in solid state are arranged hexagonally. An  $\alpha$ -gel holding a large amount of water in the hydrophilic portion of the layered crystal, does not form a variety of association structures as liquid crystal.

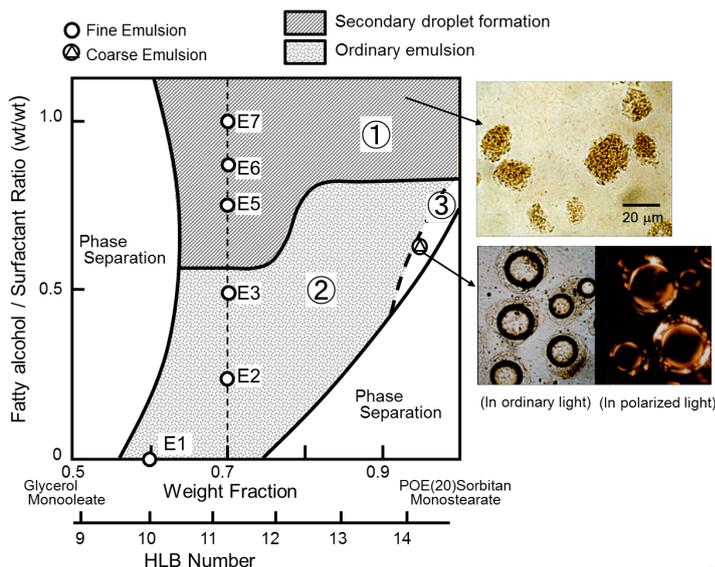
### 3. Significance of liquid crystal and $\alpha$ -gel formation in emulsion and dispersion systems

#### 3-1 Presence of molecular assembly in emulsions

The presence of liquid crystalline meso-phases in emulsions has already been recognized in 1960's. In addition, in 1970's, S. Friberg and co-workers showed that the stability of O/W emulsions are significantly improved by the presence of the liquid crystalline phase, from the phase study of surfactant / oil / water system.<sup>1, 13</sup>

With respect to the practical system, fatty alcohols, such as hexadecanol and octadecanol are often used to O/W emulsions as the bodying agents, which improve the stability

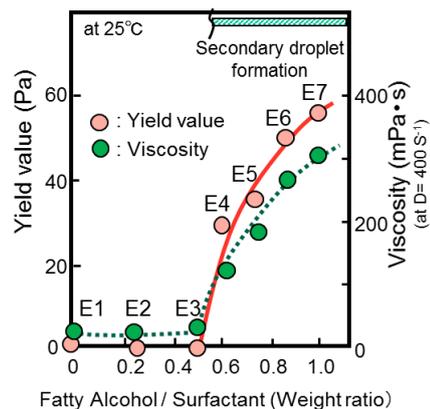
and enhance the viscosity and the consistency.<sup>14-16</sup> Figure 4 shows the state of emulsions composed of nonionic surfactant / fatty alcohol / liquid paraffin / water in the weight ratio of 4 / 0 - 4 / 24 / 72 - 68. They were prepared by varying the fatty alcohol content and HLB number of mixed surfactant. O/W emulsions were obtained within the region surrounded by solid lines. Emulsions formed in the region ① were semi-solid (cream) whereas fluid emulsions were formed in the region ②. Though the appearance and the microscopic image of these emulsions were identical, emulsions obtained in the region ① formed the secondary droplets, aggregates of emulsion droplets.<sup>17</sup> They were observed by microscopy of diluted emulsion with water as shown in the upper picture of Figure 4. In Figure 5, the yield value and viscosity (apparent viscosity at shear rate  $400\text{ s}^{-1}$ ) corresponding to the emulsion E1 - E7 in Figure 4 were plotted as a function of fatty alcohol content. It is obvious that the yield value and viscosity



**Figure 4.** State of emulsions composed of nonionic surfactant / fatty alcohol / liquid paraffin / water.

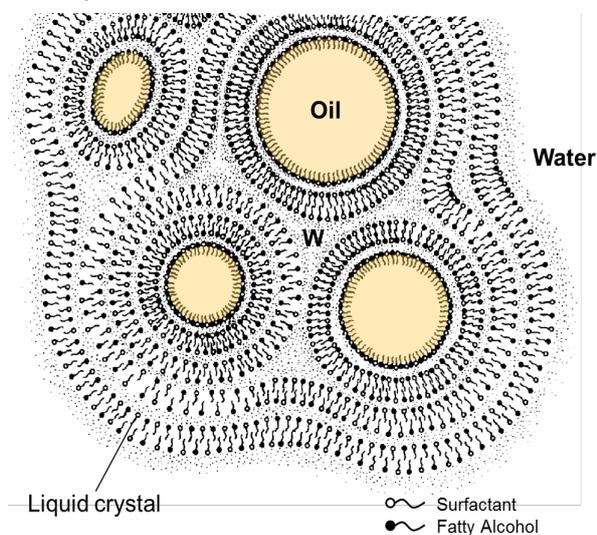
increased remarkably by presence of secondary droplet formed when fatty alcohol / surfactant ratio exceed more than certain ratio. The secondary droplet emulsion showed excellent stability against creaming and coalescence.

Though a coarse emulsion was formed in the region ③ where the HLB is not optimum, it showed relatively high yield value and good stability. As shown in the lower pictures of Figure 4, the emulsion possessed double structure in which oil droplets were surrounded by translucent optically anisotropic substance. When an emulsion of region ① was treated by ultracentrifugation with 20,000 G, it was separated to three layers of condensed emulsion, water and lamellar liquid crystal. Based on this fact, the model shown in Figure 6 could be considered.<sup>17</sup> The excellent stability of the secondary droplet emulsion against coalescence and creaming is attributed to the formation of molecular assembly surrounding the emulsion droplets as well as in the



**Figure 5.** Change in rheology properties of emulsions with secondary droplet formation.

continuous phase. The molecular assembly formed in an emulsion has been confirmed liquid crystal or  $\alpha$ -gel depending upon the character of surfactant and the fatty alcohol / surfactant ratio.



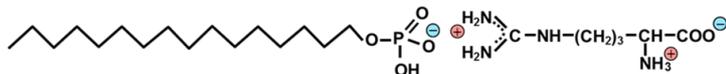
**Figure 6.** Schematic illustration of an O/W emulsion stabilized with a lamellar liquid crystalline phase

### 3-2 Stability and characterization of $\alpha$ -gel

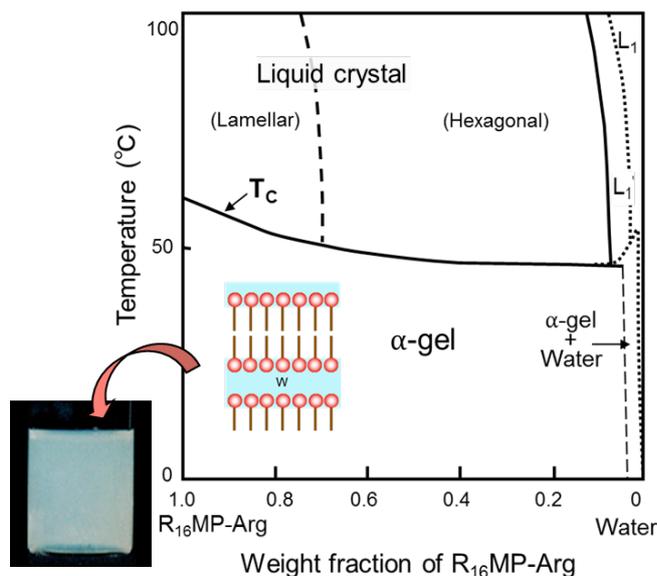
Since in general an  $\alpha$ -gel is metastable, it gradually precipitates as  $\beta$ - or  $\gamma$ -crystal within an emulsion with time and causes a

significant change in the viscosity and the deterioration of appearance. Therefore, careful adjustment of the polar lipid composition and the combination with surfactants is necessary when it is applied. Under these circumstances, it was found that the L-arginine salt of long-chain monoalkyl phosphate forms a stable  $\alpha$ -gel in quite wide temperature and concentration range below the phase transition temperature ( $T_C$ ) and applied to the preparation of emulsions and  $\alpha$ -gel formulations.<sup>18, 19</sup>

Figure 7 shows the phase diagram of L-arginine monohexadecyl phosphate ( $R_{16}$ MP-Arg) / water system.  $R_{16}$ MP-Arg (Scheme 1) shows a peculiar behavior that it forms a stable  $\alpha$ -gel in the entire region below the  $T_C$  (about 53 °C).<sup>6</sup> The preparation of an  $\alpha$ -gel is generally



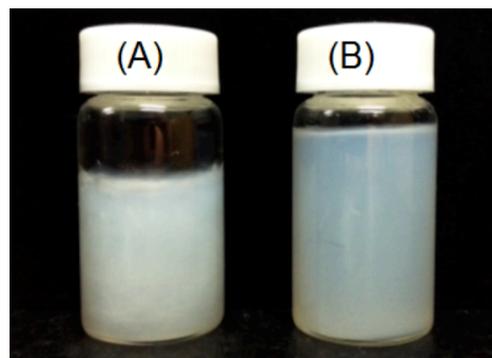
**Scheme 1.** L-Arginine hexadecyl phosphate ( $R_{16}$ MP-Arg).



**Figure 7.** Phase diagram of L-arginine hexadecyl phosphate / water system.

performed by heating the mixture of amphiphilic materials and water at above the  $T_C$  to form the liquid crystal, and followed by cooling it below the  $T_C$ . The  $\alpha$ -gel of  $R_{16}$ MP-Arg / water system was also obtained with the identical process. It is noteworthy that the  $\alpha$ -gel of  $R_{16}$ MP-Arg was formed spontaneously even in the condition below the  $T_C$  by swelling water without heating it above the  $T_C$ . From this point, the  $\alpha$ -gel of  $R_{16}$ MP-Arg / water system was concluded to be thermodynamically stable.

The unique behavior of  $R_{16}$ MP-Arg as to form the thermodynamically stable  $\alpha$  gel in wide temperature and concentration ranges was also reflected in the mixed system with polar lipid like fatty alcohol. Figure 8 shows the appearance of  $\alpha$ -gels of  $R_{16}$ MP-Arg / hexadecanol / water (3 / 3 / 94 in weight ratio) and  $R_{16}$ MP-Arg / hexadecanol / monohexadecyl glyceryl ether / water (3 / 3 / 1 / 93 in weight ratio) systems. They were prepared at above



(A):  $R_{16}$ MP-Arg /  $R_{16}$ OH / Water  
(weight ratio : 3 / 3 / 94)

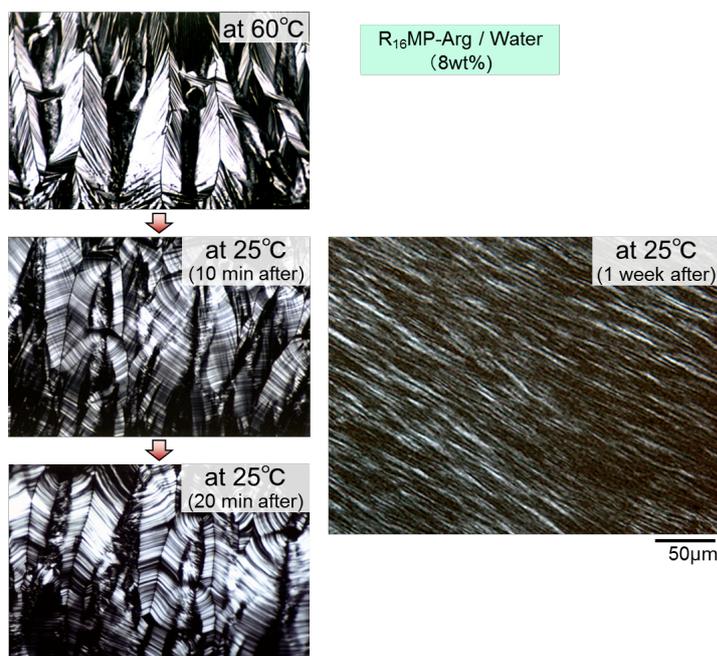
(B):  $R_{16}$ MP-Arg /  $R_{16}$ OH /  $R_{16}$ GE / Water  
(weight ratio : 3 / 3 / 1 / 93)

$R_{16}$ OH : Hexadecanol,  
 $R_{16}$ GE : Monohexadecyl glyceryl ether

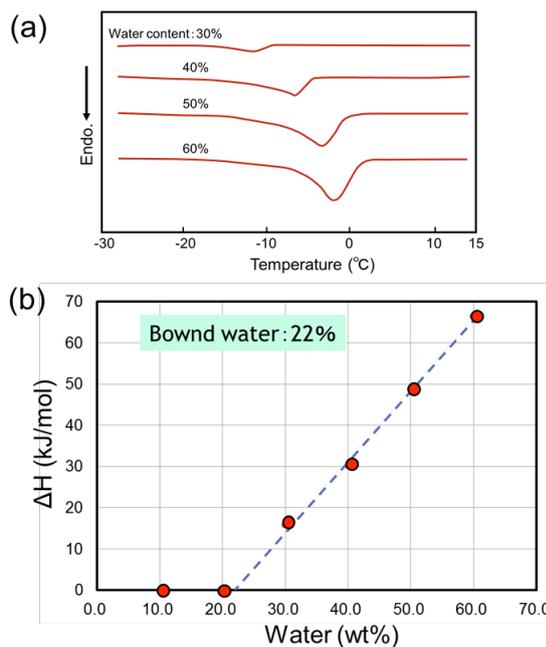
**Figure 8.**  $\alpha$ -gels formed with  $R_{16}$ MP-Arg / polar lipid / water systems.

the melting point of solid polar lipids and stored at room temperature for three years. The  $\alpha$ -gels of these mixed lipid system containing polar lipid crystals of high melting point have been maintained without causing crystal precipitation. Fatty alcohol is the optimal polar lipid, which forms stable  $\alpha$ -gel in combination with R<sub>16</sub>MP-Arg. For example, hexadecanol / R<sub>16</sub>MP-Arg mixed system produces stable  $\alpha$ -gel at any ratio within the molar ratio from 6 / 1 to 0 / 1.

Figure 9 shows the change in optical texture of R<sub>16</sub>MP-Arg / water system stored below the T<sub>C</sub>. When the liquid crystal is cooled to below the T<sub>C</sub>, stripe pattern appeared within the arrow feather-like angular texture peculiar to the hexagonal liquid crystal. It changed to full stripe texture reflecting the layered structure by one-week storage. The layered structure has been confirmed by small-angle X-ray scattering. Figure 10 shows the DSC



**Figure 9.** Change in optical texture of R<sub>16</sub>MP-Arg / Water system stored below the T<sub>C</sub>, 53°C.



**Figure 10.** DSC measurement of R<sub>16</sub>MP-Arg / water systems of various concentrations.

(a) DSC curves, (b) Plot of the melting enthalpy ( $\Delta H$ ) against the water content.

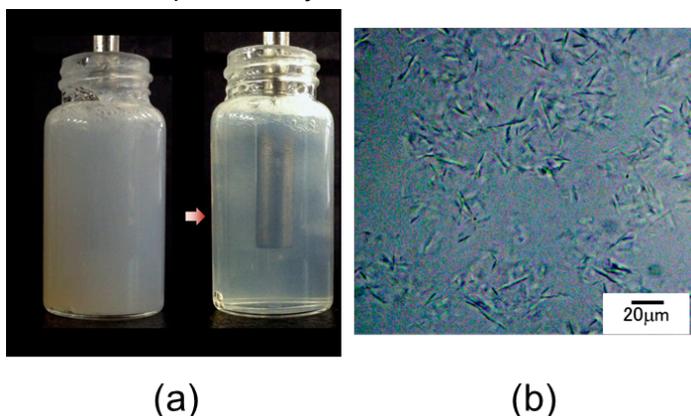
measurement of R<sub>16</sub>MP-Arg / water systems of various concentrations. Bound water content of  $\alpha$ -gel was obtained from the extrapolation of the melting enthalpy ( $\Delta H$ ) plotted against the water content (Figure 10-b). The  $\alpha$ -gel of R<sub>16</sub>MP-Arg / water was found to retain 22 % of water as bound water.

When considering the reason that R<sub>16</sub>MP-Arg forms stable  $\alpha$ -gel at the temperature below the T<sub>c</sub>, the bulkiness and dissociation state of L-arginine molecule as counter ion is thought to exert a big influence. L-arginine takes four different dissociation states by pH. Since the pH value of equimolar neutralizing R<sub>16</sub>MP-Arg / water system is approximately 6, being in the intermediate of pK<sub>1</sub> (2.17) and pK<sub>2</sub> (9.04), three functional

groups are in dissociation state as shown Scheme 1. Of these three dissociation groups, guanidyl group of  $\omega$ -position is the most strong base, and therefore being attracted to the close of the phosphate group as a direct counterion of  $R_{16}MP$ .<sup>20</sup> On the other hand, amino and carboxyl groups of amino acid residue in  $\alpha$  position dissociate to form zwitterion. Therefore, it is considered that the strong intermolecular interaction between the neighboring molecules promotes the formation of infinite molecular assemblies like liquid crystal or  $\alpha$ -gel, while the bulkiness of L-arginine as the counterion suppresses the precipitation of  $\beta$ - or  $\gamma$ -crystals.

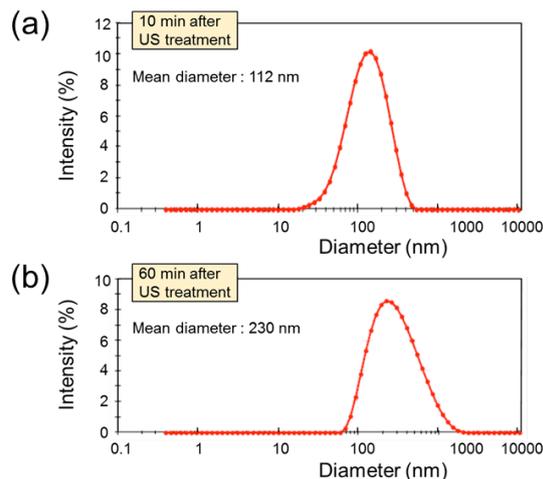
### 3-3 Preparation of fine $\alpha$ -gel dispersion and multilamellar emulsion

An  $\alpha$ -gel is semi-solid soft gel which retains a large amount of water within the hydrophilic portion, and shows pseudo-plastic flow as the rheological property reflecting the layered structure. Since it shows excellent spreadability with unique texture different from an emulsion, it will be applicable as the new-type formulation in practical systems.



**Figure 11.** Sonication of an  $\alpha$ -gel.

- (a) Change in the appearance by sonication.  
 (b) Microscopic image of sonicated  $\alpha$ -gel



**Figure 12.** Particle size distribution curves of sonicated  $\alpha$ -gel.

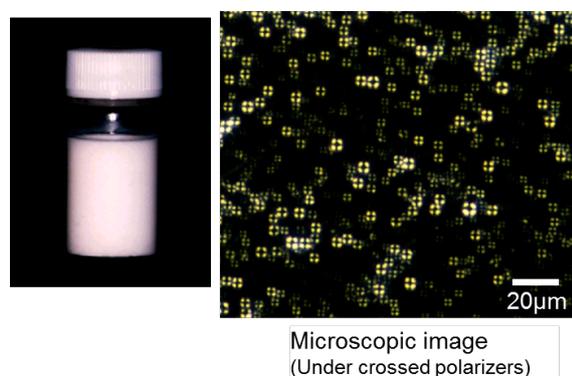
- (a) After 10 minute. (b) After 60 minute.

The  $\alpha$ -gel or two-phase system of  $\alpha$ -gel + water, formed from  $R_{16}MP$ -Arg as the main component, changed to the translucent sol or soft gel by sonication at 20 kHz for about 7-8 min. The change in appearance of sonicated  $\alpha$  gel system, and the particle size distribution curve measured by the laser scattering is shown in Figure 11 and 12. The bluish translucent appearances of the sol or gel caused by Rayleigh scattering suggest the generation of colloidal dispersion of  $\alpha$ -gel.

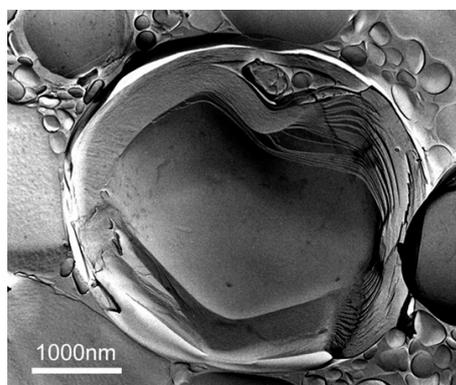
Dispersion of fine fibrous particles was recognized by optical microscopic observation (Figure 11-b). The mean particle size immediately after sonication was about 112 nm. Although the mean diameter increased to about 230 nm after 1 hour, it has been maintained unchanged as translucent sol or gel state for more than two years. A report about the superior skin moisturizing effect and barrier function of the  $\alpha$ -gel formulation made from pseudo-ceramide and

polar lipid has been made.<sup>21</sup> Soft gel or fine dispersion, which is made from  $\alpha$ -gel, can be expected as a new formulation base for cosmetics and pharmaceuticals.

When R16MP-Arg was used as an emulsifier, stable semi-solid emulsions in which  $\alpha$ -gel structure is formed in the continuous phase as well as around the emulsion droplet were generated. The application of old but very power-full nascent soap emulsification technique; where aqueous phase dissolving L-arginine is added to the oil phase containing monohexadecyl phosphate under stirring, makes possible to produce fine emulsions which involve polar lipids or solid fats hard to emulsified. Figure 13 shows the appearance and polarizing photomicrograph of an emulsion rich in



**Figure 13.** Appearance and polarizing microscopic image of lipid emulsion.



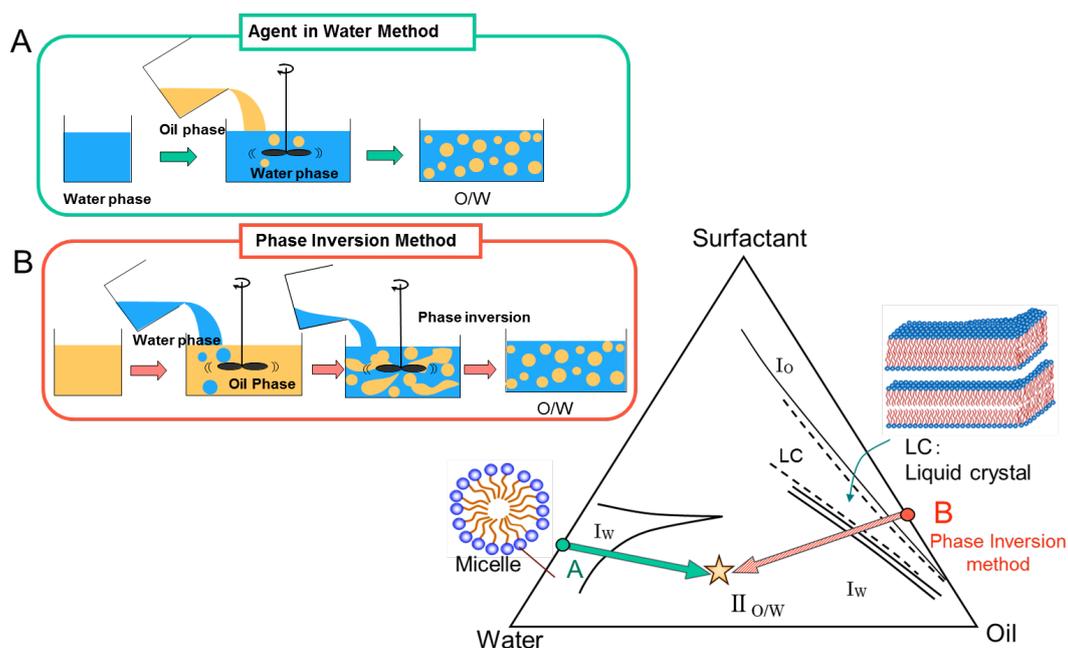
**Figure 14.** Electron microscopic image (Cryo-SEM) of lipid emulsion

polar lipids produced with this method. Each emulsion droplet shows optical texture of Maltese cross. From the electron microscopy of Cryo-CEM, the emulsion was confirmed to be a multilamellar emulsion in which each droplet possesses concentric lamellar structure (Figure 14). Multilamellar emulsion possessing strong water retention ability exhibits excellent skin moisturizing effect when it is applied as the base of cosmetic and pharmaceutical emulsions.<sup>22, 23</sup>

#### 4. Liquid crystal emulsification

##### 4-1 Contribution of the liquid crystal to the generation of emulsions

We often experience that the state of emulsions is quite different according to the preparation process, even though being the same composition. This is the difficulty of emulsification, which produces thermodynamically unstable dispersion systems. Figure 15 describes the illustrations of two different emulsification methods, “agent-in-oil method” and “phase inversion method”.<sup>24</sup> The arrows, A and B, in the phase diagram of surfactant / water / oil system indicate the emulsification processes to produce the O/W emulsion of the same composition marked as star (☆). In agent-in-water method (A), the oil phase is added to the aqueous phase dissolving surfactant to generate an O/W emulsion. On the other hand, in the phase inversion method (B), the water phase is poured to the oil phase in which surfactant is dissolved or dispersed. Generally, it is known that finer droplets are formed by phase inversion method compared with agent-in-water method.

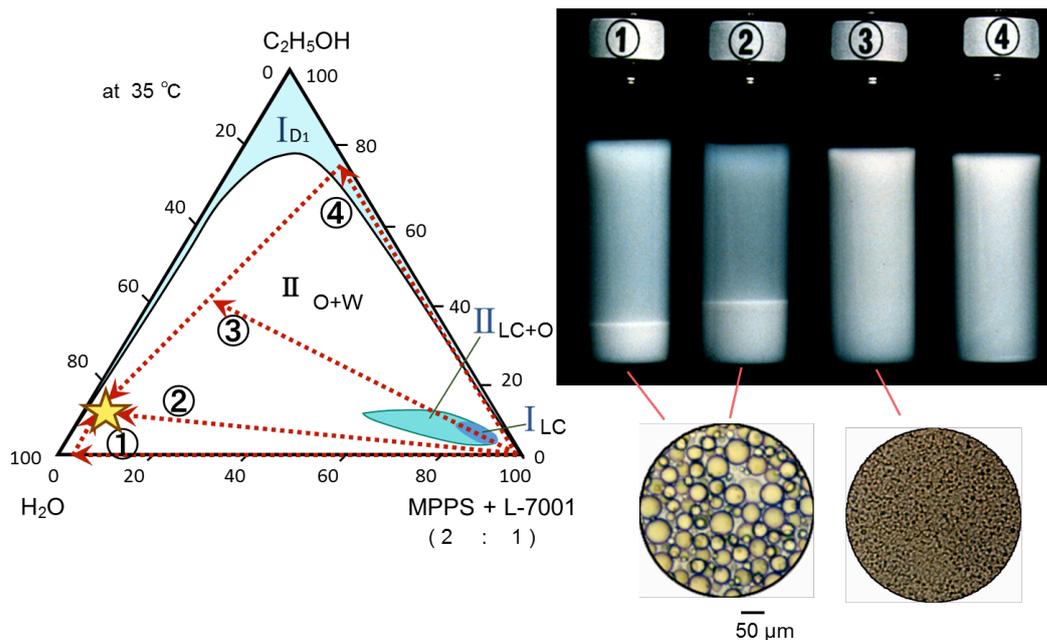


**Figure 15.** Illustrations of two different emulsification methods and the emulsification pathways described in the phase diagram.

The emulsification starts from the end of arrows respectively, and the composition of the system varies along the arrows and finally reaches the composition of the star (☆). The state of resulting emulsion is affected by the phase state of the region in which the arrows are passed through. The arrow A corresponding to the emulsification method A, enters two-phase region of oil and water directly, after passing through the micellar region (Figure 15). Whereas, the arrow B, indicating the emulsification method B, passes lamellar liquid crystal region and two-phase region of liquid crystal and oil, before entering two-phase region of oil and water where the emulsion is formed. It has been reported that the oil-water interfacial tension decreases remarkably by the appearances of D-phase, infinite aggregates of surfactant molecule, at the hydrophilic-lipophilic balanced condition in the nonionic surfactant / oil / water system.<sup>25, 26</sup> Therefore, the process B,

which passes through the region where infinite molecular assembly is formed, generates finer emulsion droplets, even though the same stirring power (mechanical energy) is applied.

As an actual example, differences in the state of the emulsions composed of nonionic surfactant (POE·POP dimethylpolysiloxane), oil (methyl phenyl polysiloxane), ethanol and water by the preparation processes are compared in Figure 16. The point marked with a star (☆) in the triangle phase diagram corresponds to the composition of the low-viscosity emulsion composed of silicone oil (2 wt%), surfactant (1 wt%), ethanol (12 wt%) and water (85 wt%). Four different emulsification processes, ① - ④, are indicated with arrows. In spite of being the same composition, the state of these emulsions is very different. Of these four emulsification processes, only the method ③, which passes through the



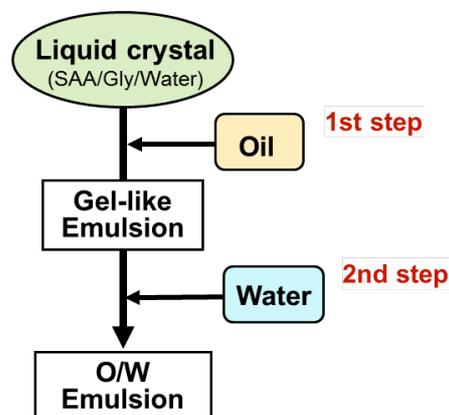
**Figure 16.** Emulsification processes indicated in a phase diagram, and state of emulsions after storage at RT for one month.

liquid crystal region and the two-phase region of the liquid crystal + oil in the phase diagram, produced a fine emulsion of excellent stability.<sup>27</sup> Effective emulsification is achieved by selecting such optimum conditions.

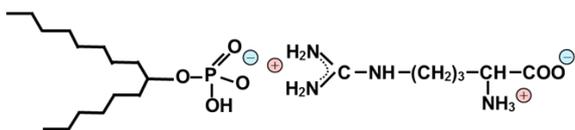
**4-2 O/LC gel emulsion and fine three-phase emulsion generated by liquid crystal emulsification**

Finding the optimum conditions for liquid crystal formation in the emulsification pathway by investigating different variables such as temperature, concentration and combination of each component is time consuming. To avoid these complicated processes, an emulsification method using a liquid crystal was developed.<sup>28</sup> The liquid crystal emulsification is performed in two steps as shown in the flow diagram of Figure 17. In this method, an oil phase is added directly to a lamellar liquid crystalline phase, which is prepared from a surfactant and a portion of the water phase, under

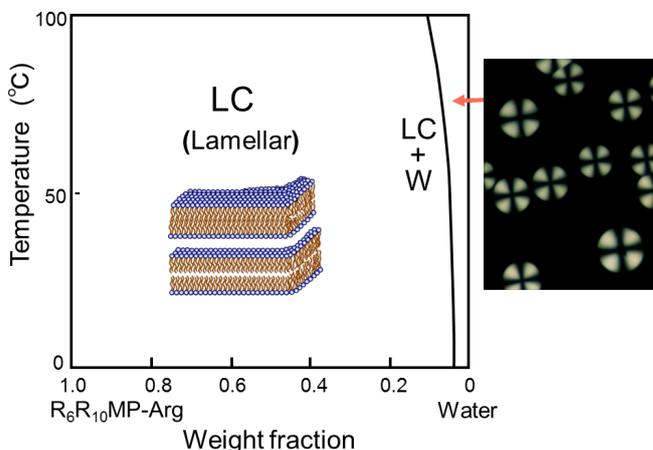
stirring to produce gel-like phase. Then it is diluted with water to produce an O/W emulsion. The key to this emulsification is selecting a surfactant which easily forms a lamellar liquid crystalline phase. In general, it is known that hydrophilic-lipophilic balanced di-alkyl type surfactants, such as  $\beta$ -branched L-arginine hexyldecyl phosphate (Scheme 2), tend to form lamellar liquid crystals. Figure 18 shows the phase diagram of L-arginine hexyldecyl phosphate ( $R_6R_{10}MP\text{-Arg}$ ) / water system.



**Figure 17.** Process of liquid crystal emulsification.



**Scheme 2.** L-Arginine hexadecyl phosphate ( $R_{16}MP\text{-Arg}$ ).

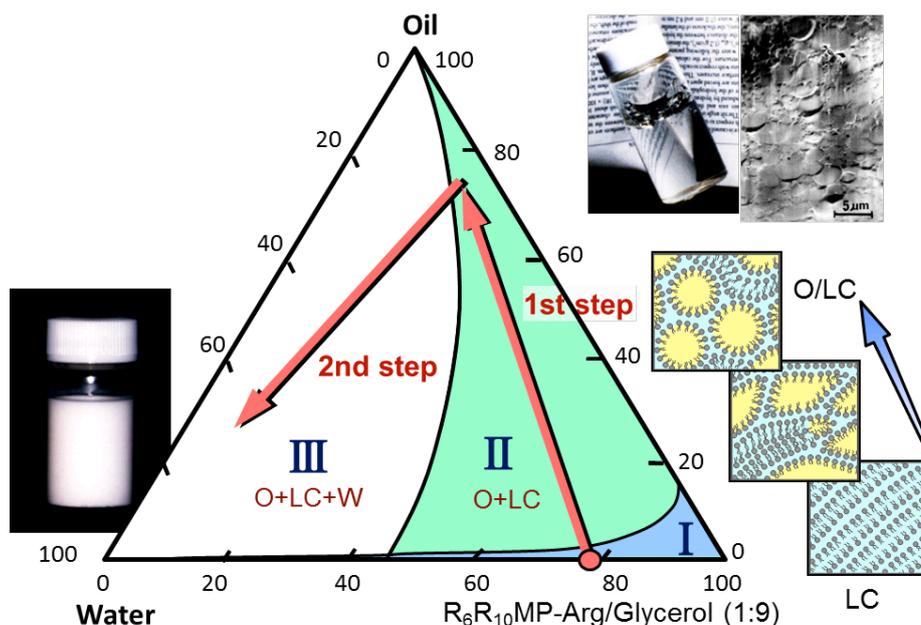


**Figure 18.** Phase diagram of  $\beta$ -branched L-arginine hexyldecyl phosphate ( $R_6R_{10}MP\text{-Arg}$ ) / water system.

Since the phase transition temperature is quite low due to the branched alkyl chain, the major portion of the phase diagram is occupied by lamellar liquid crystalline phase. Lamellar liquid crystal is maintained as a dispersion of concentric lamella even

in dilute systems. Surfactants possessing such characteristic are suitable for liquid crystal emulsification. When a portion of the water in the liquid crystal is replaced with trivalent or higher valent polyols such as glycerol, the oil phase is easily dispersed and retained in the liquid crystal phase.

The change in phase state during emulsification is recognized by tracing the two arrows indicated in the pseudo-ternary phase diagram of  $R_6R_{10}MP\text{-Arg}$  / Glycerol / water / oil system (Figure 19). The emulsification starts from a point in the one-phase liquid crystal region in the first step. The composition moves toward the oil apex to form a clear gel. Since the one-phase liquid crystal region is so small, the arrow representing the emulsification process enters into the two-phase region of liquid crystal and oil, with addition of a small amount of oil in the initial stage. The pictures in the upper right

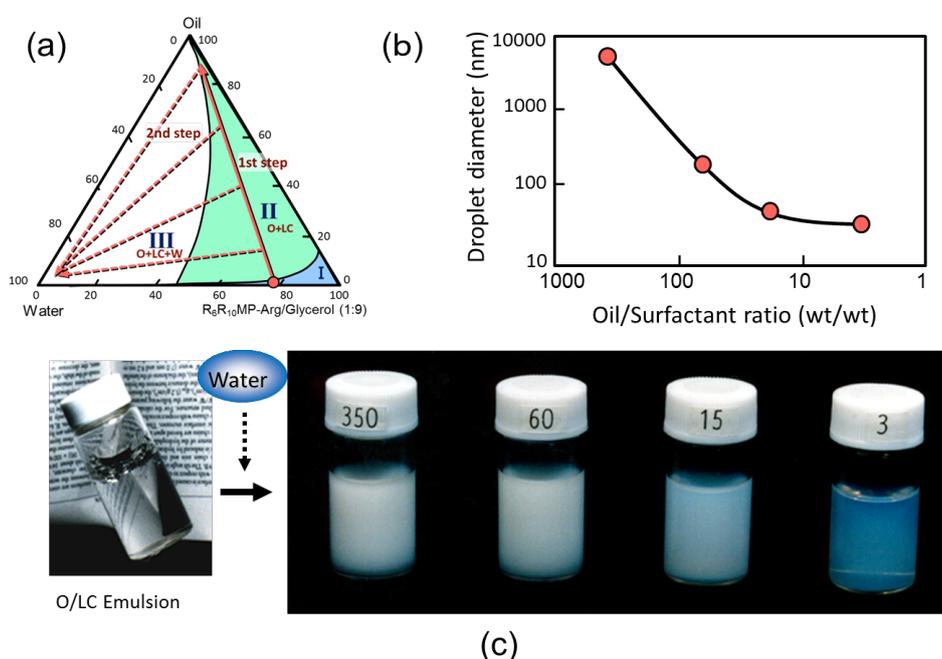


**Figure 19.** Process of liquid crystal emulsification indicated with arrows in phase diagram.

of Figure 19 show the appearance and cryo-SEM image of the gel formed in the first step. Though the appearance is transparent and looks homogeneous, closely packed oil droplets are observed. From these results, it is concluded that the gel-like phase is an oil-in-liquid crystal (O/LC) emulsion, in which oil droplets are dispersed and retained within the liquid crystal, as shown by the schematic illustration in the lower right of Figure 19. The transparent appearance of the gel is due to the close refractive indexes of the oil and the liquid crystalline phase composed of water, glycerol and surfactant. The small one-phase solubilization area suggests that there is little interaction between the oil and the liquid crystalline phase. In the second step of the emulsification, water is poured to the gel-like O/LC emulsions to generate an O/W emulsion. In

this step, gentle stirring with relatively rapid water addition is available as compared with the first step. When tracing the change in the phase state along the arrow of the second step in Figure 19, it enters to the 3-phase region of O + LC + W from the 2-phase region of O + LC. Therefore a three-phase emulsion in which emulsion droplets are surrounded by liquid crystal and dispersed in water phase is produced.<sup>28</sup> Since the liquid crystalline shell protects emulsion droplets, the three-phase emulsion shows excellent stability against coalescence.

Emulsification processes, mean droplet diameters, and appearances of emulsions with various oil contents are indicated in (a), (b), (c) of Figure 20 respectively. Numerical values shown in the cap of the sample bottle in the photograph (c) is an oil / surfactant weight ratio. Since surfactant



**Figure 20.** Formation of macro- to nano-emulsions by liquid crystal emulsification.

- (a) Emulsification processes of different oil content
- (b) Relation between oil / surfactant ratio and mean droplet diameter
- (c) Appearance of emulsions

molecules orient at oil / water interface effectively in liquid crystal emulsification, the droplet diameters of emulsions depend mainly on the oil / surfactant ratio and the efficiency of stirring during the formation of O/LC emulsions. Nanoemulsions with a translucent appearance, as well as ordinary macroemulsions can be obtained in the identical manner with this emulsification.

### 4.3 Emulsification mechanism analyzed from the dynamic behavior of liquid crystal membrane

Liquid crystal emulsification is suitable for a wide variety of oils including hydrocarbons, ester oils, triglycerides, and even silicone oils and perfluoropolyethers (Table 2).<sup>29, 30</sup> This fact suggests that this emulsification is independent of the hydrophilic-lipophilic balance required of oils, and therefore it will save a lot of time that needed for adjust the

Oil	O/LC Gel	O/W Emulsion
<b>《Nonpolar》</b>		
Squalane	○	○
Liquid paraffin	○	○
<b>《Polar》</b>		
Octyldodecyl myristate	○	○
Isopropyl palmitate	○	○
Tri(2-ethylhexyl) glyceride	○	○
Di(2-ethylhexyl) glyceride	○	○
<b>《Others》</b>		
Dimethylpolysiloxane	○	○
Perfluoropolyether	○	○

○ : Possible    × : Not possible

Table 2. Availability of liquid crystal emulsification to various oils.

optimal condition of emulsification. Figure 21 shows the oil retention capacity of 1.0 ml of lamellar liquid crystals composed of R<sub>6</sub>R<sub>10</sub>MP-Arg (10wt%) / glycerol / water systems. Gel-like O/LC emulsions are formed with oils of different atomic species, squalane as hydrocarbon, dimethylpolysiloxane (DMPS) as silicone oil, and

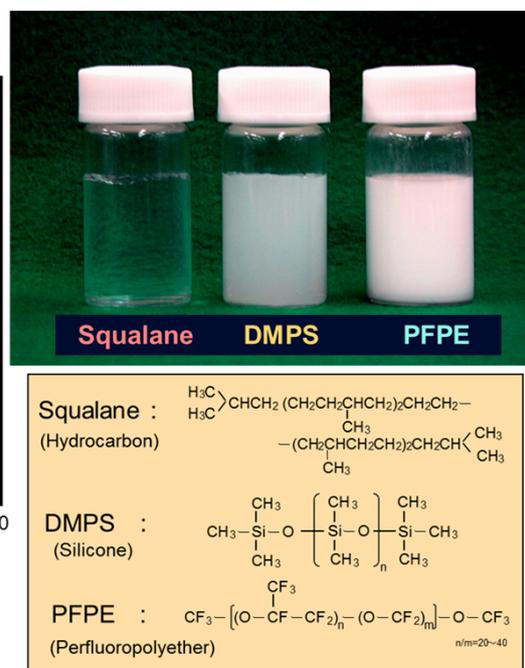
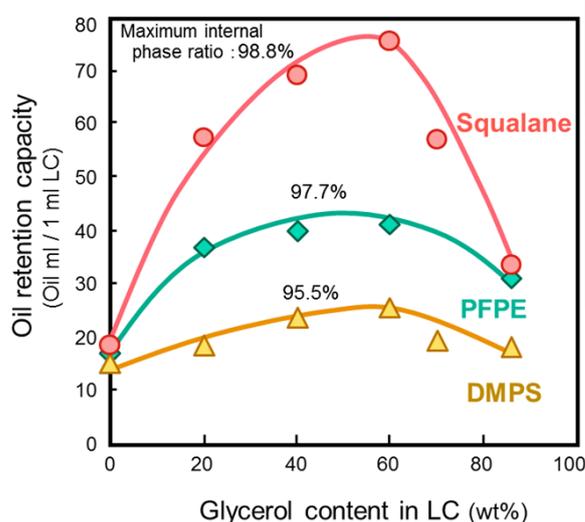


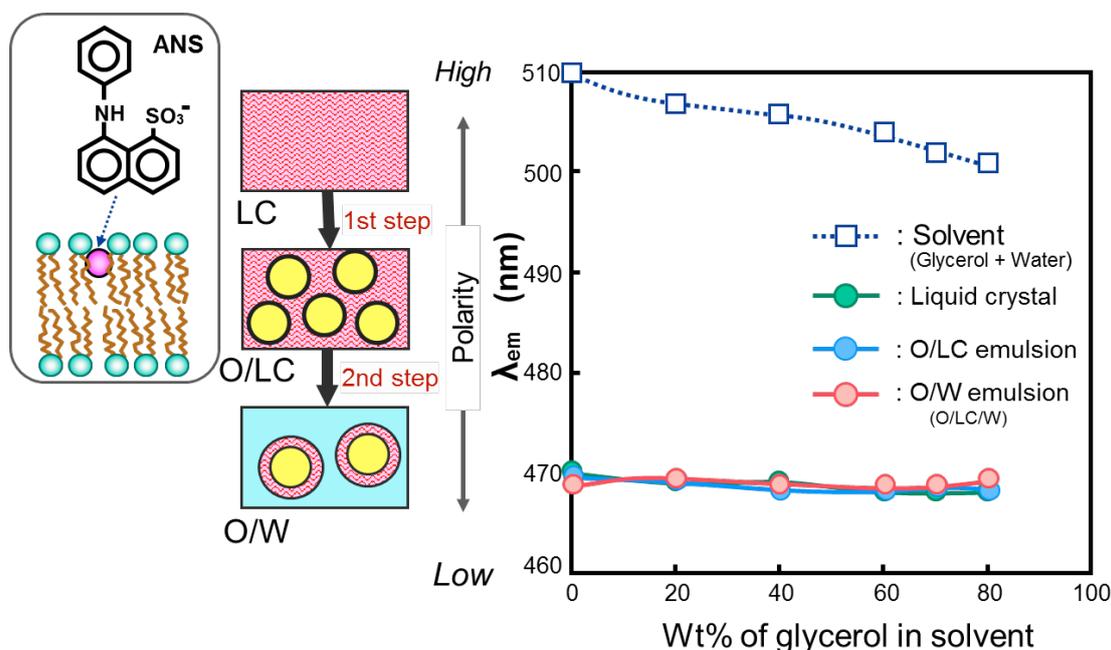
Figure 21. Oil retention ability of liquid crystals and the appearance of O/LC emulsions of various oils.

LC : R<sub>6</sub>R<sub>10</sub>MP-Arg (10 wt%) / glycerol / water systems

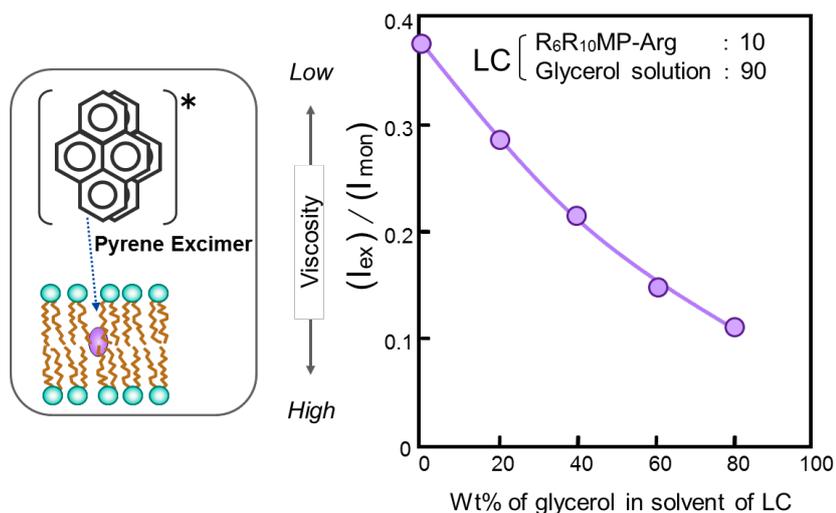
perfluoropolyether (PFPE) as fluorine oil. Since the specific gravities of the oils are significantly different, the vertical axis is shown with the volume of oils being retained in the O/LC emulsions. The appearances of gels vary from transparent to turbid white according to the differences in the reflective index, but they are all O/LC emulsions.

To clarify the reason for these unique properties of liquid crystal emulsification, the dynamic behavior of the liquid crystalline membrane was analyzed by fluorometry<sup>31</sup> and spin probe method of electron spin resonance (ESR)<sup>32-36</sup> using probes with a specific orientation in the liquid crystalline membrane. Figure 22 shows the changes in maximum emission,  $\lambda_{em}$ , of the fluorescent probe 1-anilino-8-naphthalene sulfonic acid (ANS). Since ANS tends to orient itself towards the polar group of the liquid crystalline membrane, the environmental

change around the probe is detectable. The  $\lambda_{em}$  values decreased gradually with increasing glycerol content in solvent systems of glycerol and water. On the other hand, they were much lower and remained constant in liquid crystalline systems. This suggests that no structural changes in the molecular assembly took place around the probe. The identical measurement on the squalane emulsion system was performed to estimate the interaction between the liquid crystal constituent molecule and oils. The  $\lambda_{em}$  values were identical to those of liquid crystalline systems even after introduction of oil into the liquid crystal. The same result was also obtained from three-phase emulsions generated by the second step of the emulsification (Figure 22). This suggests that there is no definite influence on membrane fluidity following oil addition and that the character of the liquid crystalline membrane is maintained in O/LC and O/W three-phase emulsion



**Figure 22.** Analysis of interaction between LC constituent molecule and oil by using fluorescence probe, ANS.



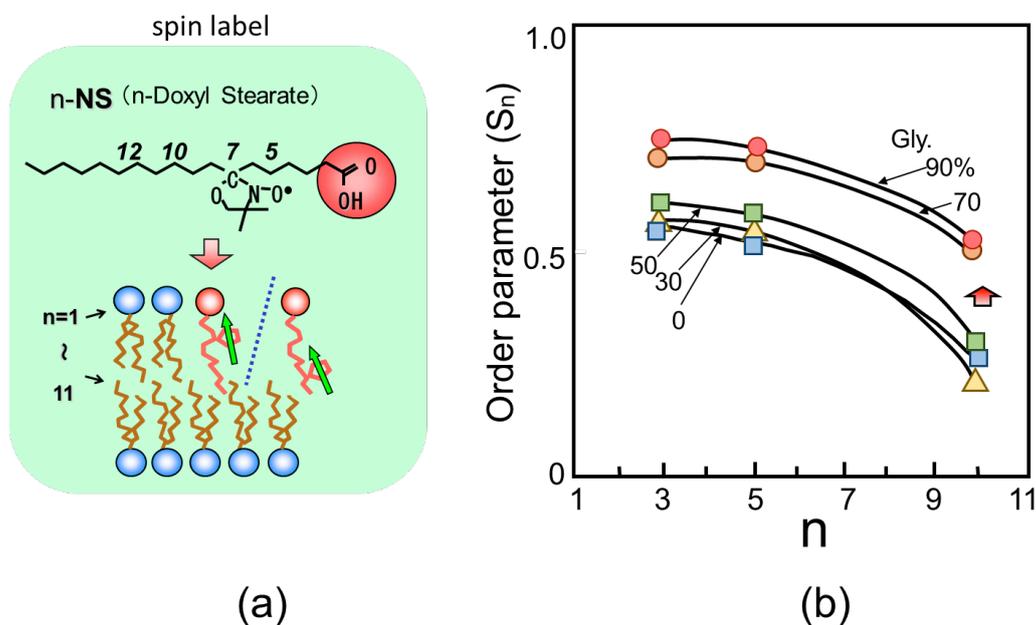
**Figure 23.** Analysis of local viscosity of liquid crystal membrane with glycerol content by using fluorescence probe, pyrene.

systems.

Using another fluorescent probe, pyrene, the viscosity of the hydrophobic part of the liquid crystalline membrane was estimated. Pyrene tends to form excimers by molecular collision when the environmental viscosity around the probe is low. The

excimer / monomer fluorescence intensity ratio, which is the index of molecular motility, decreased markedly with glycerol content (Figure 23). This suggests that the motility of the hydrophobic chains in the liquid crystal structure decreased.

To clarify the dynamic behavior of the liquid



**Figure 24.** Electron spin resonance (ESR) analysis of liquid crystals of various glycerol content using fatty acid spin labels.

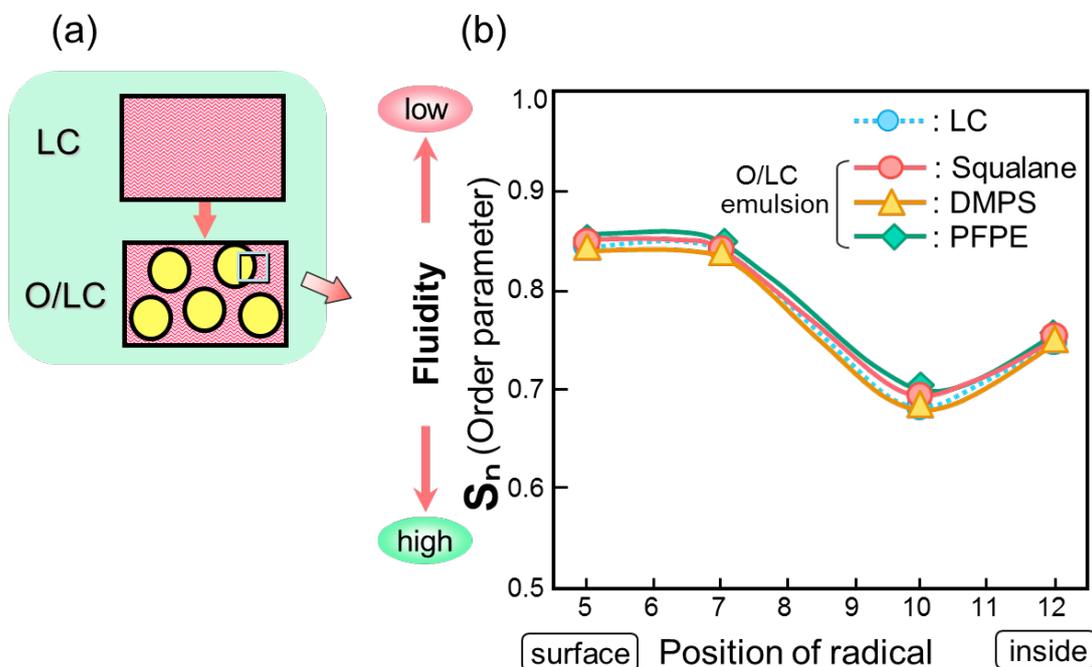
(a) Fatty acid spin label located in liquid crystal membrane.

(b) Changes in order parameter as a function of n, position of radical in spin labels, for liquid crystals of different glycerol content.

crystalline membrane more precisely, the spin probe method of ESR was used. Using a series of fatty acid type spin labels of various nitroxide radical position as shown in Figure 24-a, the local mobility of the alkyl chains in liquid crystal membrane can be analyzed. In Figure 24-b, the order parameter  $S_n$ , which shows the rigidity of the membrane, was plotted as a function of "n" indicating the position of the nitroxide radical away from the hydrophilic end.  $S_n$  has a value from 0 to 1, and the closer to 1 means the mobility around the probe is suppressed.  $S_n$  values decreased with increasing in n for all the liquid crystals of different glycerol content. This is due to the promotion of molecular mobility with increasing the distance from the polar group. Glycerol did not affect the  $S_n$  value of liquid crystals until 50 wt% in

concentration, whereas  $S_n$  value remarkably increased when the glycerol content exceeded 50 wt%. That is, high concentration glycerol enhances the intermolecular interactions of surfactant molecules by hydrogen bond, and thus stabilizing the gel structure retaining high amount of oil.

Figure 25 shows the effect of oil addition to the liquid crystal membrane from the viewpoint of changes in  $S_n$  values. Interestingly,  $S_n$  curve of O/LC emulsion systems obtained by adding various oils to a liquid crystal were exactly same as the  $S_n$  curve in the liquid crystal system. This means that the state of liquid crystal membrane is maintained intact in spite of the addition of a variety of oils, and suggests that the liquid crystal membrane exists as an independent phase against oil



**Figure 25.** Analysis of changes in dynamic behavior of liquid crystal membrane with the addition of various oils.

(a) Schematic model of O/LC emulsion

(b) Changes in order parameter,  $S_n$ , in O/LC emulsions

and water. The unique property of liquid crystal emulsification, which is available for a wide variety of oils, is derived from the independence of liquid crystal membrane as a phase.

## 5. Conclusion

This paper is the compilation of my research results concerning the application of molecular assemblies, liquid crystal and  $\alpha$ -gel, for the generation and stabilization of emulsions and lipid dispersion. The formulations, such as multilamellar emulsion, O/LC emulsion and soft gel have been applied to the practical systems including cosmetics and pharmaceuticals, and exhibit various performances like excellent skin moisturizing ability, high protection effect and good spreadability while keeping the long-term stability. In addition to the generation and stabilization technology of these distinctive emulsion and lipid dispersion formulations, the action mechanism of molecular assemblies underlying the technology was discussed. I hope this paper will be helpful for the researchers and engineers who concern the emulsion and dispersion systems.

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