Phase Behavior and Formation of O/W Nano-Emulsion in Vegetable Oil/ Mixture of Polyglycerol Polyricinoleate and Polyglycerin Fatty Acid Ester/Water Systems

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Abstract: It is reported that mixing polyglycerol polyricinoleate (PGPR) and polyglycerol laurilester has a great emulsifying capacity, and consequently fine oil-in-water (o/w) emulsions can be formed. However, the role of PGPR is not clear. The objective of this research is to investigate the phase behavior of vegetable oil/ mixture of PGPR and polyglycerol fatty acid ester/water systems, and to clarify the role of PGPR in making a fine emulsion. Phase diagrams were constructed to elucidate the optimal process for preparing fine emulsions. In all the systems examined in this study, the phases, including the liquid crystal phase (L_c) and sponge phase (L_3), spread widely in the phase diagrams. We examined droplet size of the emulsions prepared from each phase and found that o/w nano-emulsions with droplet sizes as small as 50 nm were formed by emulsifying either from a single L_3 phase or a two-phase region, $L_c + L_3$. These results indicate that a sponge phase L_3 or liquid crystal phase L_c or both is necessary to form an o/w nano-emulsion whose average droplet diameter is less than 50 nm for PGPR and polyglycerin fatty acid ester mixtures used as surfactant.

Key words: PGPR, nano-emulsion, phase diagram, sponge phase, liquid crystal

1 INTRODUCTION

Nano-emulsions are emulsions with droplet sizes typical in the range of 20–200 nm. Due to their small droplet size, they may appear transparent or translucent to the naked eye¹. Since the very small droplet size causes a substantial reduction in the buoyancy and gravitational forces acting on the droplets, Brownian motion may be sufficient to overcome these effects. As a result, creaming and sedimentation during storage may be reduced or eliminated. The small droplet size also aids in preventing flocculation of the droplets².

The application of nano-emulsion technology in the food, medical and pharmaceutical industries has received great attention from the scientific community. The technology is particularly suited to the fabrication of encapsulating systems for functional compounds because it prevents degradation and improves bioavailability³.

External energy is required to form nano-emulsions since it is a non-equilibrium system. The methods of pre-

paring nano-emulsions can be categorized into two types: physical and chemical. Physical methods involve an intensive energy input using a high-shear stirrer or a high-pressure homogenizer. Chemical methods, such as liquid-crystal emulsification⁴, surfactant phase emulsification^{5,6} and phase inversion emulsification^{7–9}, use spontaneous phase transitions that are driven by thermodynamic effects. Chemical methods thus allow for the preparation of nano-emulsions at a constant temperature with minimal energy input¹⁰. However, the preparation of nano-emulsions from food ingredients such as vegetable oil using low-energy emulsification methods has rarely been reported^{11–13}.

On the other hand, it is reported that a mixture of polyglycerol polyricinoleate (PGPR) and polyglycerol laurilester has a great emulsifying capacity, and consequently, fine oil-in-water (o/w) emulsions can be formed¹⁴. PGPR, a non-ionic surfactant, is allowed as a food additive in many countries and is a powerful lipophile emulsifier, which can be used to obtain stable water-in-oil (w/o) and water-in-oil-

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in-water (w/o/w) emulsions^{15, 16)}. The high emulsifying properties of PGPR are attributed to the excellent water-binding capacity of the long hydrophilic polyglycerol chain. It has been reported that when used at concentrations $>2\%$, w/o and w/o/w emulsions attain remarkable stability¹⁷⁾. In the food industry, PGPR is as effective as lecithin in lowering the viscosity of chocolate to improve its molding properties, with the best effects obtained from PGPR/lecithin mixtures¹⁸⁾. However, there have been few reports of PGPR being used to prepare o/w emulsions without the patent¹⁴⁾. The role of PGPR in making a fine emulsion for a mixture system with polyglycerol fatty acid ester is also not clear.

The objective of this research is to investigate the phase behavior of vegetable oil/mixture of PGPR and polyglycerol fatty acid ester/water systems, and to clarify the emulsification mechanism of PGPR for the mixture systems.

2 EXPERIMENTAL PROCEDURES

2.1 Materials

PGPR, (commercial name SY-Glyster CRS-75), decaglycerol monolaurilester (commercial name SY-Glyster ML-750), hexaglycerol monolaurilester (commercial name SY-Glyster ML-500) and decaglycerol monomyristilester (commercial name SY-Glyster MM-750) were purchased from Sakamoto Yakuin Kogyo Co. Vegetable oil was purchased from Nisshin Oil Co. All materials were used without further purification. Purified water was prepared using an E-pure system (Dubuque, USA).

We used a mixture of PGPR with polyglycerol fatty acid ester (ratio 1:1) in this study. The mixtures of PGPR and decaglycerol monolaurilester, PGPR and hexaglycerol monolaurilester, and PGPR and decaglycerol monomyristilester are abbreviated as surfactant DLP, surfactant HLP and surfactant DMP, respectively.

2.2 Methods

2.2.1 Determination of the phase diagram

Test tubes containing mixtures with the desired compositions were repeatedly shaken using a vortex mixer and then stored at 25°C . The formation of liquid crystals was confirmed using polarizing plates placed on either side of the test tube. If a uniform liquid crystalline phase was formed, the light passed through the plates due to the birefringence of the liquid crystal. Detailed phases were identified using polarized light microscopy (OLYMPUS, BH-2) and small-angle X-ray scattering (SAXS) techniques.

2.2.2 Small-angle X-ray scattering

SAXS measurements were performed on a Nano-Viewer SAXS instrument (Rigaku Co., Tokyo, Japan) equipped with a PILATUS at an applied voltage and filament current of 40 kV and 30 mA, respectively.

2.2.3 Preparation of an O/W emulsion

The emulsifier mixtures previously described, water and vegetable oil were mixed at the desired weight ratios to prepare mixtures before emulsification. Water (7 g) was then added dropwise to 3 g of the mixture before emulsification with stirring using a mechanical stirrer at 300 rpm and 25°C to prepare an o/w emulsion.

2.2.4 Measurement of the particle-size distribution

Particle-size distribution was determined using a dynamic light scattering device (FPER-1000; Otsuka Electronics, Osaka, Japan).

3 RESULTS AND DISCUSSION

3.1 Phase behavior

The phase diagram for the vegetable oil/surfactant DLP/water system (system A) at 25°C is shown in Fig. 1. Figure 2 shows the polarized microphotographs, and SAXS patterns of mixtures of compositions (a) and (b) described in Fig. 1.

The sponge phase (L_3) is often stated to be flow birefringent¹⁹⁾, and it is reported that the SAXS pattern obtained from L_3 indicates a broad peak²⁰⁾. The sample represented by composition (a) in Fig. 1 was a visually transparent isotropic phase and did not show birefringence under polar-

Fig. 1 Phase diagram of system A (vegetable oil/surfactant DLP/water system) at 25°C . L_3 , L_c , W_m and O indicate a sponge phase, a liquid crystal phase, a micellar phase and an oil phase, respectively. $L_c + L_3$ indicates a two-phase equilibrium consisting of a liquid crystal phase and a sponge phase. $W_m + O$ and $L_3 + W_m + O$ indicate two- and three-phase equilibrium of W_m and $L_3 + W_m$ with an expelled oil phase, respectively.

Fig. 2 Polarized microscopic images and SAXS patterns of samples consisting of 10.0 wt%, 80.0 wt%, and 10.0 wt% (a); 10.0 wt%, 75.0 wt%, and 15.0 wt% (b) of vegetable oil, surfactant DLP, and water, respectively. Compositions (a) and (b) are indicated in Fig. 1 using the corresponding letters in parentheses.

ization microscopy (Fig. 2(a)) or between crossed polarizes in a static state (Fig. 3(A)). However, this sample showed flow birefringence between crossed polarizes in a flow state (Fig. 3(B)). Consequently, this phase was identified as L_3 . For system A (vegetable oil/surfactant DLP/water), L_3 could be seen at surfactant DLP and water content above 40 wt% and below 15 wt%, respectively.

From the micrograph shown in Fig. 2(b), the samples represented by composition (b) in Fig. 1 were determined to form a liquid crystalline (L_c) phase. In addition, when these samples flowed between crossed polarizes, the samples showed birefringence more intensively. Consequently, we determined that these phases are a two-phase region coexisting with L_c and L_3 . The scattering peaks for sample (b) in Fig. 1 were broad. These scattering peaks indicate the presence of a type of liquid crystal. We presume that the type of liquid crystal is not cubic liquid crystal, which does not show birefringence under polarization microscopy²¹⁾. However, the type of liquid crystal, for example, lamellar liquid crystal and hexagonal liquid

Fig. 3 Photographs of sample represented by composition (a) in Fig. 1 (vegetable oil: 10 wt%, surfactant DLP: 80 wt%, water: 10 wt%) between crossed polarizes, in static state (A) and in flow state (B).

crystal, could not be determined since the SAXS profile for sample (b) showed one broad peak and one narrow peak. At least two narrow peaks are necessary to determine the type of liquid crystal (Fig. 2b). Even though we could not confidently identify the type of liquid crystal in these phases, for system A, a region with two coexisting phases, L_c and L_3 , appeared when the content of surfactant DLP and water was below 85 wt% and below 45 wt%, respectively. When the content of surfactant DLP and water was below 55 wt% and below 65 wt%, respectively, a three-phase region with L_3 , micelle solution and an oil phase ($L_3 + Wm + O$) could be seen. At surfactant DLP content less than 35 wt%, a two-phase region ($Wm + O$) was confirmed.

The phase diagram for the vegetable oil/surfactant HLP/water system (system B) at 25°C is shown in Fig. 4. Figure 5 shows polarized microscopic images and SAXS patterns of the mixtures (a), (b) and (c) shown in Fig. 4. From the micrograph shown in Fig. 5(a), (b) and (c), the samples represented by composition (a), (b) and (c) in Fig. 4 were determined to form an L_c phase. The SAXS pattern obtained from samples (a) and (c) showed 3 peaks. We considered the first peak to be L_3 , because these peaks were broad. The scattering peak ratio of the secondary peak to the third peak for both (a) and (c) shown in Fig. 5 were

Fig. 4 Phase diagram of system B (vegetable oil/surfactant HLP/water system) at 25°C. L_3 , L_c , La , Wm and O indicate a sponge phase, a liquid crystal phase, a lamellar phase, a micellar phase and an oil phase, respectively. $L_c + L_3$ and $L_3 + La$ indicate a two-phase equilibrium consisting of a liquid crystal phase and a sponge phase, a sponge phase and a lamellar phase, respectively. $Wm + O$ and $L_3 + Wm + O$ indicate two- and three-phase equilibrium of Wm and $L_3 + Wm$ with an expelled oil phase, respectively.

1:1/2, and the micrograph in Fig. 5(a) and (c) shows a typical extinction-cross texture for a lamellar phase (La)¹⁹. Thus, it is considered that a lamellar liquid crystal was formed in these regions. Consequently, (a) and (c) shown in Fig. 4 were identified as $L_3 + La$. On the other hand, the SAXS pattern obtained from sample (b) shown in Fig. 5 did not correspond to any liquid crystalline structure, for example, lamellar liquid crystal or hexagonal liquid crystal. In addition, the flow birefringence between crossed polarizers was also observed for sample (b), which overlaps with the birefringence of liquid crystal. We think that this phase is $L_c + L_3$.

The inter-bilayer spacing $d = \lambda/2\sin\theta$ of the lamella structure of sample (a) in Fig. 4 was 6.53 nm and that of sample (c) was 17.3 nm. The composition of sample (a) was 1.0:7.5:1.5 (oil:surfactant:water) and that of sample (c) was 1.0:5.5:4.0 (oil:surfactant:water). It is clear that the inter-bilayer spacing for lamella structure increased with an increase in the content of water. It is considered that the distance between the hydrophilic plates for the lamella structure increased due to the increasing number of water molecules.

For system B, $L_c + L_3$ spread toward the low surfactant HLP content region. Moreover, there were two regions with La in $L_c + L_3$ (dotted line in Fig. 4). At a vegetable oil content below 15 wt%, one region of $L_3 + La$ was observed when the content of surfactant HLP and water was approximately 70–85 wt% and 15–30 wt%, respectively, and the other region was observed when the content of surfactant HLP and water was approximately 40–55 wt% and 45–60 wt%, respectively. At a vegetable oil content below 60 wt%, three phases with $L_3 + Wm + O$ was observed when the content of surfactant HLP and water was approximately 25–35 wt% and 15–85 wt%, respectively. When surfactant HLP content was below about 15–25 wt%, $Wm + O$ was confirmed.

For system B, the region of $L_c + L_3$ is much wider than that of system A on the phase diagram. It is considered that the hexaglycerol monolaurilester (HGML) in system B tended to orient rather than the decaglycerol monolaurilester (DGML) in system A because the hydrophilic group of HGML was smaller than that of DGML.

As shown in Fig. 6, the phase diagram for vegetable oil/surfactant DMP/water system (system C) was similar to that for system A (Fig. 1). All phases were determined by polarized microscopic images, observation of test tubes containing samples between polarizing plates and SAXS measurement, the same as the methods described above. L_3 could be seen at surfactant DMP and water content above about 40 wt% and below about 10 wt%, respectively. A region with two coexisting phases, L_c and L_3 , appeared when the content of surfactant DMP and water were 90 wt% and below 60 wt%, respectively. In this system, the type of liquid crystal for L_c could not be identified, similar to the

Fig. 5 Polarized microscopic images and SAXS patterns of samples consisting of 10.0 wt%, 75.0 wt%, and 15.0 wt% (a); 10.0 wt%, 65.0 wt%, and 25.0 wt% (b); 10.0 wt%, 50.0 wt%, and 40.0 wt% (c) of vegetable oil, surfactant HLP, and water, respectively. Compositions (a), (b), and (c) are indicated in Fig. 4 using the corresponding letters in parentheses.

case as described above for system A. When the content of surfactant DMP and water was below 45 wt% and below 60 wt%, respectively, a three-phase region with $L_3 + W_m + O$ could be seen. At surfactant DMP content of less than 40 wt%, $W_m + O$ was formed.

For system C, the region of $L_c + L_3$ is slightly wider than that of system A on the phase diagram. We expect that decaglycerol monomyristilester (DGMM) in system C is slightly more hydrophobic than DGML in system A because the fatty acid chain of DGMM(C14) is longer than that of

Fig. 6 Phase diagram of system C (vegetable oil/surfactant DMP/water system) at 25°C. L_3 , L_c , W_m and O indicate a sponge phase, a liquid crystal phase, a micellar phase and an oil phase, respectively. $L_c + L_3$ indicates a two-phase equilibrium consisting of a liquid crystal phase and a sponge phase. $W_m + O$ and $L_3 + W_m + O$ indicate two- and three-phase equilibrium of W_m and $L_3 + W_m$ with an expelled oil phase, respectively.

DGML(C12).

3.2 Preparation of nano-emulsion

An o/w emulsion was prepared as described in the experimental section (2.2.3). The average droplet diameter of the emulsion depended highly on the phase type, suggesting that the physicochemical properties of the phase critically affected the properties of the resulting emulsion.

Table 1 shows the phase type and weight composition of the samples before emulsification and the average droplet diameter of the o/w emulsions prepared from the samples as described in the experimental section (2.2.3). The UM in Table 1 means the samples could not be used to determine the droplet size of the emulsion because the w/o emulsion was contained in the sample or the oil spread on the sample.

Figure 7 shows a photograph of the o/w emulsions prepared from samples I to VIII indicated in Table 1.

In system A, containing the 1.0 ratio of oil (Table 1 system A-a) and a water ratio of 0.5–3.5, the phase type of the mixtures before emulsification was L_3 or $L_c + L_3$, the average droplet diameter of the prepared o/w emulsions was about 30 nm, and the emulsions were bluish and translucent (Fig. 7 System A I–VII). When the phase type of the mixture before emulsification changed to $L_3 + W_m + O$ or $W_m + O$, the droplet diameter of the prepared emulsions was more than about 200 nm, and the emulsions were

milky white (Fig. 7 System A VIII). The droplet diameter and appearance changed remarkably even though the content of the samples was not so different. For the 4.0 ratio of oil (Table 1 system A-b) and a water ratio of 0.5–1.5, the average diameter was 100 nm to 130 nm even in high oil content and the phase types before emulsification were also L_3 or $L_c + L_3$. On the other hand, good o/w emulsion could not be prepared from the region of $L_3 + W_m + O$ in system A-b.

In system B-a, containing the 1.0 ratio of oil and a water ratio of 0.5–6.0, the phase type of the mixtures before emulsification was $L_c + L_3$ or $L_3 + L_a$, the average droplet diameter of the prepared o/w emulsions was less than 30 nm, and most of the emulsions were transparent (Fig. 7 System B). The average droplet diameter of the o/w emulsions prepared from the $L_3 + W_m + O$ and $W_m + O$ regions was 46.1 nm and 892.7 nm, respectively. It was considered that $L_3 + W_m + O$ in system B-a was the border between $L_c + L_3$ and $W_m + O$, and thus the average droplet diameter of the o/w emulsions prepared from this sample was smaller than 100 nm. For the 4.0 ratio of oil (Table 1 system B-b) and a water ratio of 0.5–2.5, o/w nano-emulsion with droplet sizes as low as 100 nm were formed even in high oil content and the phase type before emulsification was $L_c + L_3$. When the phase type changed to $L_3 + W_m + O$ or $W_m + O$, the droplet diameter of prepared emulsion was more than 250 nm. For system B, the o/w nano-emulsion could be obtained from a wider range than system A.

In system C, containing the 1.0 ratio of oil (Table 1 system C-a) and a water ratio of 0.5–4.5, the average droplet diameter of the prepared o/w emulsions was 35–45 nm, the phase type of the mixtures before emulsification was L_3 or $L_c + L_3$, and the prepared emulsion was bluish milky white (Fig. 7 System C). For the 4.0 ratio of oil (Table 1 system C-b), even though the phase type before emulsification was L_3 or $L_c + L_3$, a fine emulsion was not prepared in some cases, i.e., the oil:surfactant:water ratio was 4.0:5.5:0.5 or 4.0:5.0:1.0. On the other hand, when the o/w nano-emulsion was prepared, the oil:surfactant:water ratio was 1.0:7.5:1.5. We presume that the oil was a continuous phase in the former case and the water was a continuous phase in the latter case.

The results in this paper indicate that a sponge phase L_3 or a liquid crystal phase L_c or both is necessary to form an o/w nano-emulsion whose average droplet diameter is less than 50 nm for PGPR and polyglycerin fatty acid ester mixtures used as surfactant.

Miyanoishi *et al.*²¹⁾ reported that nano-scale droplets about 100 nm were obtained to cross a lamellar phase for a vegetable oil/decaglycerol monolaurilester (DGML)/40 wt% sucrose solution system and a sponge phase for a vegetable oil/polyoxyethylene sorbitan monooleate/40 wt% sucrose solution system along the emulsification path. These results are similar to our observation in the present study.

Table 1 Phase type and weight composition of the samples before emulsification in each system and the average droplet diameter of the o/w emulsions prepared from the samples before emulsification.

System of diagram	Weight ratio			Phase type	Average droplet diameter (nm)	Sample number
	Oil	Surfactant	Water			
system A	1.0	8.5	0.5	L ₃	22.6	I
	1.0	8.0	1.0	L ₃	25.7	II
	1.0	7.5	1.5	Lc + L ₃	25.8	III
	1.0	7.0	2.0	Lc + L ₃	28.1	IV
	1.0	6.5	2.5	Lc + L ₃	27.0	V
	1.0	6.0	3.0	Lc + L ₃	31.0	VI
	1.0	5.5	3.5	Lc + L ₃	29.9	VII
	1.0	5.0	4.0	L ₃ + Wm + O	198.6	VIII
	1.0	4.5	4.5	L ₃ + Wm + O	316.8	
	1.0	3.0	6.0	Wm + O	396.1	
	4.0	5.5	0.5	L ₃	102.3	
	4.0	5.0	1.0	Lc + L ₃	114.4	
	4.0	4.5	1.5	Lc + L ₃	132.5	
	4.0	4.0	2.0	L ₃ + Wm + O	UM*	
	4.0	3.5	2.5	L ₃ + Wm + O	UM*	
	4.0	3.0	3.0	L ₃ + Wm + O	UM*	
system B	1.0	8.5	0.5	Lc + L ₃	32.2	I
	1.0	8.0	1.0	Lc + L ₃	28.9	II
	1.0	7.5	1.5	L ₃ + La	28.0	III
	1.0	7.0	2.0	L ₃ + La	17.8	IV
	1.0	6.5	2.5	Lc + L ₃	17.7	V
	1.0	6.0	3.0	Lc + L ₃	22.8	VI
	1.0	5.5	3.5	Lc + L ₃	20.1	VII
	1.0	5.0	4.0	Lc + L ₃	22.9	VIII
	1.0	4.5	4.5	L ₃ + La	29.3	
	1.0	4.0	5.0	L ₃ + La	27.4	
	1.0	3.5	5.5	Lc + L ₃	27.9	
	1.0	3.0	6.0	Lc + L ₃	29.9	
	1.0	2.5	6.5	L ₃ + Wm + O	46.1	
	1.0	2.0	7.0	Wm + O	892.7	
	4.0	5.5	0.5	Lc + L ₃	50.8	
	4.0	5.0	1.0	Lc + L ₃	61.3	
	4.0	4.5	1.5	Lc + L ₃	68.3	
system C	4.0	4.0	2.0	Lc + L ₃	78.5	
	4.0	3.5	2.5	Lc + L ₃	100.5	
	4.0	3.0	3.0	L ₃ + Wm + O	257.9	
	4.0	2.5	3.5	Wm + O	622.6	
	4.0	2.0	4.0	Wm + O	837.3	
	1.0	8.5	0.5	L ₃	37.2	I
	1.0	8.0	1.0	L ₃	40.5	II
	1.0	7.5	1.5	Lc + L ₃	38.7	III
	1.0	7.0	2.0	Lc + L ₃	35.9	IV
	1.0	6.5	2.5	Lc + L ₃	44.1	V
	1.0	6.0	3.0	Lc + L ₃	39.4	VI
	1.0	5.5	3.5	Lc + L ₃	40.6	VII
	1.0	5.0	4.0	Lc + L ₃	37.3	VIII
	1.0	4.5	4.5	Lc + L ₃	41.7	
	1.0	4.0	5.0	Wm + O	1244.6	
	4.0	5.5	0.5	L ₃	UM*	
	4.0	5.0	1.0	Lc + L ₃	UM*	
	4.0	4.5	1.5	Lc + L ₃	126.0	
	4.0	4.0	2.0	L ₃ + Wm + O	UM*	

* UM : unmeasurable level

Fig. 7 Photographs of the o/w emulsions prepared using the method described in 2.2.3. The sample number (I–VIII) refers to the number noted in Table 1.

On the other hand, the droplet size of emulsions prepared from the system with PGPR in the present study was almost half that compared with those for the system with single DGML²¹⁾. This is considered to be because a part of the lamellar structure changed into a sponge structure by mixing PGPR with DGML, and that structure change is advantageous for the formation of an o/w nano-emulsion.

4 CONCLUSION

Phase diagrams of vegetable oil/mixture of PGPR and polyglycerin fatty acid ester/water systems were constructed to clarify the mechanism of o/w fine emulsion formation with PGPR. In all the systems examined in this study, the

phase, including the liquid crystal phase (L_c) and sponge phase (L_3), spread widely in the phase diagrams. The droplet size of the emulsions prepared from each phase was examined and it was found that an o/w nano-emulsion with droplet sizes as small as 50 nm were formed by emulsifying either from a single L_3 phase or a two-phase region, $L_c + L_3$. These results indicate that a sponge phase L_3 or a liquid crystal phase L_c or both is necessary to form an o/w nano-emulsion whose average droplet diameter is less than 50 nm for PGPR and polyglycerin fatty acid ester mixtures used as surfactant.

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