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Dispersion Stability of Phytoglycogen in Water/Phytoglycogen/Various Nonionic Surfactant Systems: Effect of Hydrophile–Lipophile Balance (HLB) of Nonionic Surfactants

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The phase behavior was investigated in water/phytoglycogen/water-soluble nonionic surfactant (poly(oxyethylene) type surfactant, C_mEO_n) systems, showing that phytoglycogen is precipitated by the addition of those surfactants. Therefore, the effect of the hydrophile–lipophile balance (HLB) on the dispersion stability of phytoglycogen (DSP) was assessed.

At constant-temperature, phytoglycogen in a lower HLB surfactant system dispersed more stably than that in a higher HLB surfactant system. With raising the temperature, the DSP in the higher HLB surfactant system was improved, whereas phytoglycogen became to disperse unstably in a low-HLB surfactant ($C_{12}EO_7$) system. In the $C_{12}EO_9$ and $C_{12}EO_{10}$ systems, the DSP was improved over a range of lower temperatures, whereas phytoglycogen became to disperse unstably over a range of higher temperatures. We proposed a schematic representation on the phase behavior in water/phytoglycogen/nonionic surfactant systems. It was clarified that DSP in phytoglycogen and surfactant-coexisting systems depends on the HLB and the aggregation number of each surfactant. Furthermore, the characteristics of surfactant micelles in water were evaluated macroscopically from the investigation.

Amylopectin and glycogen are reserve polysaccharides in plants and animals, respectively, and also represent the (1→4)(1→6)-linked α-D-glucans.[1,2] The latter disperses well in water, while the former, i.e., a major component of starch, does not dissolve in it. Glycogen is a highly branched spherical polymer comprising α-D-glucose, and forms a molecular colloid in water; the solution shows opalescence. Furthermore, water-soluble (1→4)(1→6)-linked α-D-glucans are included in some plants, such as sweet corn, and other sources. Since such polysaccharides have a similar structure to that of glycogen, those from plants are called phytoglycogens.[3—6] Phytoglycogen is expected to be widely applied for practical use instead of animal glycogen in the future.

Water-soluble polymers are often used in mixtures with surfactants having a structure containing both hydrophilic and lipophilic groups in a molecule. Since surfactants aggregate in several solvents, water-soluble polymers and water-insoluble substances can be mixed by stable surfactant aggregates. Furthermore, it has been reported that various surfactants modify the dispersion conditions of the polymers in aqueous solution.[7] Therefore, it is necessary to understand the interaction between polymers and surfactants from both applied and fundamental points of view.

In the food industry, the interaction between starch and a surfactant has been investigated by many workers, due to improvements of the starch properties;[8,9] however, only a few investigations have been carried out concerning the interaction between glycogen or phytoglycogen and a surfactant. In biological chemistry, this is very important because both glycogen and various surfactants are found in the human body. In colloidal chemistry, it is also an important theme because glycogen and phytoglycogen are highly branched and the conformation is spherical; also, since it is rigid compared with linear polymers, it seems that the characteristics of surfactant micelles in water can be evaluated through the interaction of those with glycogen or phytoglycogen.

In a previous paper[10] we reported that the dispersion stability of phytoglycogen (DSP) in aqueous solution is closely related to the degree of branching, i.e., the numbers of (1→6)-α-D-glucosidic linkages. Thus, we have attempted to understand the interaction between phytoglycogen and a water-soluble nonionic surfactant (icosaethylene glycol monododecyl ether, $C_{12}EO_{20}$), and found that phytoglycogen is precipitated by the addition of a nonionic surfactant. In both phytoglycogen and surfactant-coexisting systems, it was assumed that DSP was dominated by the hydration powers of surfactant micelles, determined by the hydrophile–lipophile balance (HLB) of each surfactant.

In this study, the phase behavior was investigated in a

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water/phytoglycogen/$C_{12}EO_{20}$ system in detail. Then, the effect of HLB on DSP was assessed by using various nonionic surfactants (poly(oxyethylene) surfactant, C_mEO_n); also, we proposed a schematic representation on the phase behavior in water/phytoglycogen/nonionic surfactant systems. Furthermore, the characteristics of surfactant micelles in water were evaluated through the interaction of those with phytoglycogen.

Experimental

Materials. Phytoglycogen was extracted from sweet corn kernels (cv. Golden Cross Bantam, 40 days after pollination). Dried sweet corn kernels were ground, and subsequently homogenized with three weights of a $HClO_4$ aqueous solution (the $HClO_4$ content in total weight was 5%(w/w)) at 25 °C for 5 min. The details were described in a previous paper.[10)]

Various types of nonionic surfactants (poly(oxyethylene) type, $C_mH_{2m+1}(OC_2H_4)_nOH$ (C_mEO_n) and $C_{27}H_{45}(OC_2H_4)_{20}OH$ ($CSEO_{20}$)), were obtained from Nihon Emulsion Co., Ltd. Descriptions of the surfactants used in this study are given in Table 1. The lipophilic-group and the hydrophilic-group indicate the average of hydrocarbon (C) and ethylene oxide (EO) chain length, respectively. The HLB numbers were calculated by Griffin's method.[11,12)] CP means a cloud point.[13—15)] The surfactants used in this experiment dissolve in water and form an aqueous micelle at room temperature. These surfactants were used without purification. Distilled deionized-water was used.

Procedures. Various fractions of water, phytoglycogen, and surfactant were sealed in test tubes. A series of test tubes kept in a thermostat were shaken well and left at constant temperature for 1 h. Subsequently, the phase equilibrium was determined by observing the precipitation.

The transmittance of aqueous solutions was measured in order to determine the degree of agglomeration and the concentration of phytoglycogen particles. Transmittance measurements were carried out in a double-beam spectrophotometer (Shimadzu Co., Ltd., UV-2100) at 600 nm. A series of test tubes kept in the thermostat were shaken well and left at constant temperature for 1 h. After the samples were shaken well again, the transmittance of stirred solutions was measured. The samples in which precipitates were observed, were immediately centrifuged at 5000 rpm for 5 min. After the precipitates were separated by centrifugation, the transmittance of supernatant solutions was measured. Control solutions were prepared by means of substituting water for the phytoglycogen or surfactant. The transmittance of those solutions containing only phytoglycogen or surfactant was also measured under the same conditions.

The separated precipitates were washed with ethanol and acetone, and dried in a water bath at 90 °C. The yields were determined by weighing the dried phytoglycogen; then, the transmittance of the fractionated phytoglycogen aqueous solutions (0.5 wt%) was measured.

Results and Discussion

Phase Diagram of a Water/Phytoglycogen/$C_{12}EO_{20}$ System. Figure 1 shows the phase diagram in a water/phytoglycogen/$C_{12}EO_{20}$ system at 25 °C. In this Fig. 1, the ratio of the concentration of phytoglycogen relative to the phytoglycogen and surfactant, W_p, is plotted vertically, and the sum of the concentration of the phytoglycogen and surfactant in this system, X, is plotted horizontally. All of the concentrations are given in weight fractions. The phase behavior was classified into two regions, I and II based on the precipitation of phytoglycogen. The precipitates of phytoglycogen were observed in region II (two-phase region),

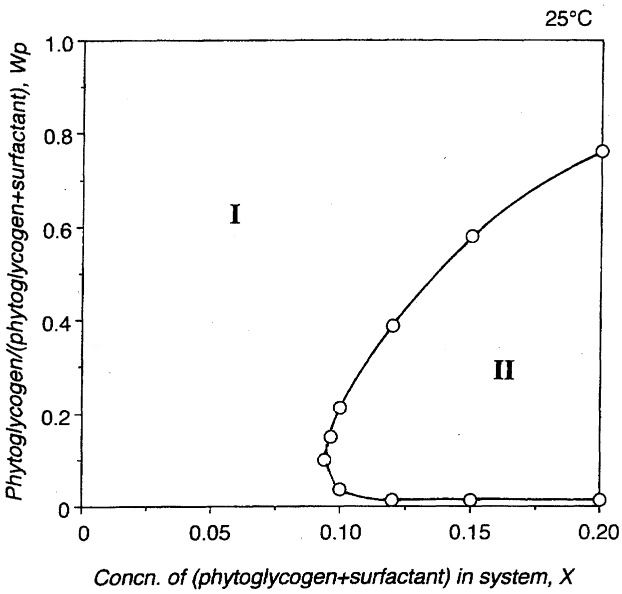

Fig. 1. The phase diagram of a water/phytoglycogen/ $C_{12}EO_{20}$ system as a function of (phytoglycogen + surfactant) concentration in system, X, at 25 °C. I and II indicate one- and two-phase regions, respectively. All the concentrations are given in weight fractions.

Table 1. Descriptions of Surfactants

Surfactant	Lipophilic-group	Hydrophilic-group	HLB number	CP (°C)
EMALEX-707	12	7	12.5	58.5[a)]
EMALEX-709	12	9	13.6	84[b)]
EMALEX-710	12	10	14.1	88[c)]
EMALEX-720	12	20	16.5	> 100
EMALEX-730	12	30	17.5	> 100
EMALEX-750	12	50	18.4	> 100
EMALEX-120	16	20	15.7	> 100
EMALEX-BHA20	22	20	14.6	> 100
EMALEX-CS20	Cholesterol	20	13.9	> 100

a) Data from Ref. 13. b) Data of $C_{12}EO_{9.4}$ from Ref. 14. c) Data from Ref. 15.

while no precipitates were observed in region I (one-phase region). In a dilute solution ($X < 0.09$) the precipitates were not observed, though they began to appear at $X = 0.10$ at a limited W_p ratio, near to 0.10. With increasing X concentration, the possible range of W_p for the precipitation increased remarkably. Even in a high-X solution, phytoglycogen was not precipitated in the high-W_p region. Phytoglycogen and this surfactant each well disperse stably as a molecular and micellar colloid in water at 25 °C, respectively. Furthermore, phytoglycogen is not precipitated at high temperature, e.g., 90 °C, in the absence of a surfactant. However, precipitates were observed under the coexisting conditions of phytoglycogen and the nonionic surfactant in aqueous solution.

Transmittance and Yield Measurements in a Water/Phytoglycogen/$C_{12}EO_{20}$ System. The turbidity of an aqueous solution of phytoglycogen expresses the dispersion conditions of such molecules, i.e., the size and degree of agglomeration of the dispersoid etc., because the solution is a molecular colloidal dispersion system and shows opalescence.[10)]

Figure 2 shows the variation in the transmittance of the aqueous solution as a function of W_p. X is fixed at 0.15 (w/w). The transmittance of controls including only phytoglycogen or the surfactant is also shown in this Fig. 2. In the case of phytoglycogen (surfactant) controls, the horizontal axis indicates the composition at which water is substituted for the surfactant (phytoglycogen). As the result of controls, surfactant micelles scarcely interfered with the penetration of the rays at 600 nm, whereas the transmittance of phytoglycogen controls decreased with increasing the phytoglycogen concentration. In the case of stirred solutions, there is a slight possibility that the size of the micelles is varied in the presence of phytoglycogen; however, the turbidity of the solutions most likely depended on the concentration and degree of agglomeration of the phytoglycogen particles.

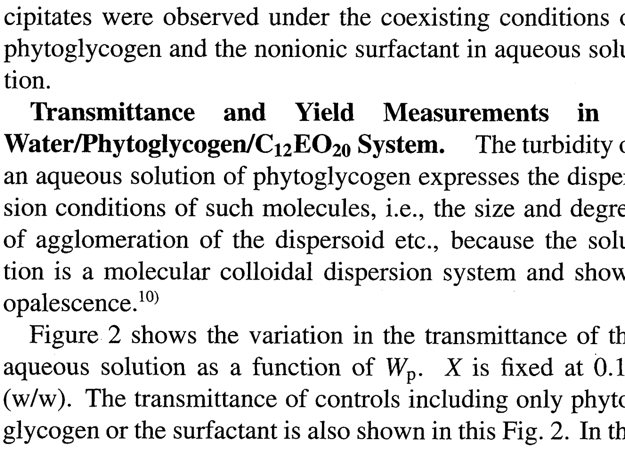

Fig. 2. The transmittance of the aqueous solutions of a water/phytoglycogen/$C_{12}EO_{20}$ system as a function of phytoglycogen/(phytoglycogen+surfactant), W_p, at 25 °C. The concentration of (phytoglycogen + surfactant) in system, X, is fixed at 0.15 (w/w). In the case of phytoglycogen (surfactant) controls, the horizontal axis indicates the composition at which water is substituted for surfactant (phytoglycogen). All the concentrations are given in weight fractions. (◆); stirred solution, (+); supernatant after centrifugation, (○); control (phytoglycogen), (●); control ($C_{12}EO_{20}$).

The transmittance of the controls increased along with a decrease in the concentration of phytoglycogen, whereas that of stirred solutions decreased monotonically with decreasing W_p (increasing relative surfactant concentration); also, the solutions in the two-phase region hardly admitted the rays at 600 nm. The transmittance of supernatant solutions after centrifugation increased remarkably with decreasing W_p (increasing relative surfactant concentration) below the boundary between one- and two-phase regions. It seemed that the major portion of phytoglycogen precipitated below $W_p = 0.30$ because the transmittance was nearly equal to the value of the surfactant controls. With decreasing W_p (increasing relative surfactant concentration), it was revealed that phytoglycogen started to disperse more unstably, and then precipitated below the boundary due to the agglomeration of particles.

The precipitate after centrifugation was washed and dried, and the yield of phytoglycogen was determined, as shown in Table 2. X was fixed at 0.15 (w/w). With decreasing W_p (increasing relative surfactant concentration), the amounts of the precipitates increased and the major portion of phytoglycogen precipitated below $W_p = 0.30$, e.g., 91% at $W_p = 0.1$. These results were in good agreement with the results given in Fig. 2. After dried phytoglycogen was dissolved with water, the transmittance of aqueous solutions (0.5 wt%) was measured, as also shown in Table 2. The transmittance increased along with decreasing W_p. Especially, the transmittance with phytoglycogen obtained at $W_p = 0.50$ was no more than 27%. This ratio of the solution corresponded to the transmittance in, approximately, an aqueous solution with 4 wt% phytoglycogen according to the results of control measurements.

Phytoglycogen is a mixture having a distribution of the degree of branching and the molecular size. In a previous study[10)] we investigated the effect of the salt concentration on DSP, and reported that phytoglycogen can be fractionated by the salt concentration gradient, and that the turbidity of an aqueous solution of phytoglycogen dispersing unstably is larger than that dispersing stably. The turbidity of an aqueous solution of phytoglycogen precipitated with a low surfactant

Table 2. Yield and Transmittance of Phytoglycogen after Precipitation

W_p	0.10	0.20	0.30	0.40	0.50
Yield (%)	91	90	86	68	17
Transmittance (%)	74	74	72	68	27

The concentration of (phytoglycogen + surfactant) in system, X, is fixed at 0.15 (w/w).

concentration was larger than that precipitated with a high concentration; therefore, it was demonstrated that the phytoglycogen that precipitated with low W_p (high surfactant concentration) dispersed more stably in water than that with high W_p (low surfactant concentration). As a result, phase behavior was realized in a water/phytoglycogen/$C_{12}EO_{20}$ system.

Effect of HLB on DSP in Various Surfactant Systems at Constant Temperature. Surfactant molecules aggregate in several solvents above the critical micelle concentration (CMC). Surfactants form various aggregates, e.g., micelle, microemulsion, and liquid crystal etc.,[16,17] and the HLB of each surfactant is the most important factor in determining the shape of the aggregates.[18] Griffin introduced a semiempirical scale, i.e., the HLB number, based on the stability of the emulsion.[11,12] The HLB number calculated by Griffin's equation is also given in Table 1. At constant temperature, the hydrogen bonding between the hydrophilic groups of the surfactant and water become stronger with increasing the HLB number under constant C or EO chain length; therefore, it seemed that the HLB of each surfactant would influence the DSP.

Figure 3 shows phase diagrams of water/phytoglycogen/$C_{12}EO_7$, $C_{12}EO_{20}$, $C_{12}EO_{30}$, and $C_{12}EO_{50}$ systems at 25 °C. Along with an increase in the EO chain length, the boundary between one- and two-phase regions shifted to a small-X region. When the hydrocarbon chain length of the surfactant is fixed, the HLB number increases along with increasing the EO chain length, as shown in Table 1. Therefore, the increment in the HLB number of the surfactant may promote the precipitation of phytoglycogen. However, the $C_{12}EO_7$ system showed a similar boundary to the $C_{12}EO_{20}$ system. It seemed that the other properties of each surfactant also affected the DSP.

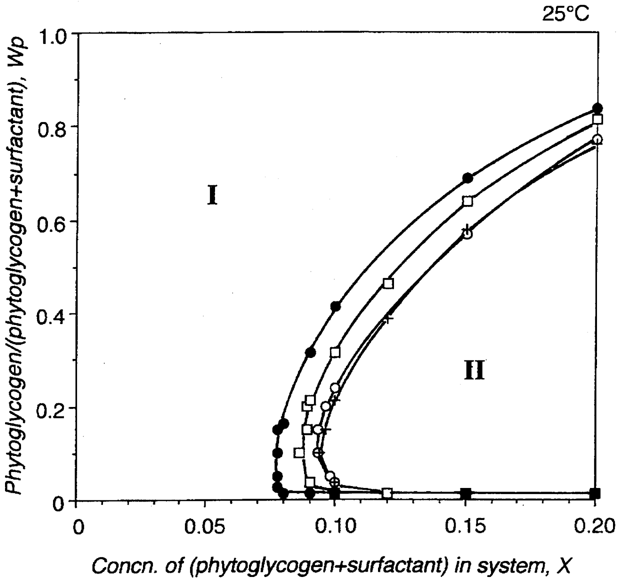

Fig. 3. The phase diagrams of water/phytoglycogen/$C_{12}EO_7$ (○), $C_{12}EO_{20}$ (+), $C_{12}EO_{30}$ (□), and $C_{12}EO_{50}$ (●) systems as a function of (phytoglycogen + surfactant) concentration, X, at 25 °C. I and II indicate one- and two-phase regions, respectively. All the concentrations are given in weight fractions.

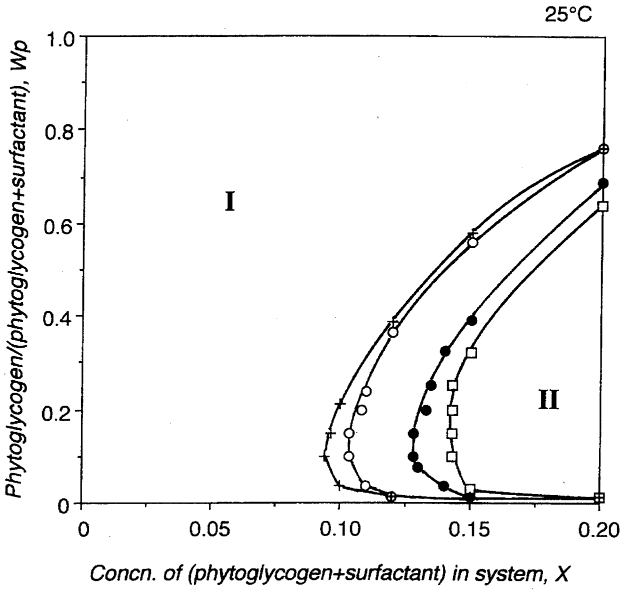

Fig. 4. The phase diagrams of water/phytoglycogen/$C_{12}EO_{20}$ (+), $C_{16}EO_{20}$ (○), $C_{22}EO_{20}$ (●), and $CSEO_{20}$ (□) systems as a function of (phytoglycogen + surfactant) concentration, X, at 25 °C.

Phase diagrams of the water/phytoglycogen/$C_{12}EO_{20}$, $C_{16}EO_{20}$, $C_{22}EO_{20}$, and $CSEO_{20}$ systems are shown in Fig. 4. As increasing C chain length, the boundary between the one- and two-phase regions monotonically shifted to the large-X region. Since the HLB number decreases along with increasing the C chain length under constant EO chain length, the DSP was improved with decreasing the HLB number. This result was in good agreement with that in Fig. 3. The boundary of the $CSEO_{20}$ system also shifted to a higher concentration than that of the $C_{22}EO_{20}$ system. Although the lipophilic-group of $CSEO_{20}$ is not linear, different from that of the other surfactants, the above rule concerning the HLB number was applied.

For the precipitation process of phytoglycogen, we first considered complex formation between a nonionic surfactant and phytoglycogen. When an ionic surfactant like sodium dodecyl sulfate (SDS) was added to a nonionic polymer solution, the surfactant started to bind with the polymer below CMC, and modified the solubility of the polymer in water.[19—21] Phytoglycogen also forms a complex with ionic surfactants; consequently, the complex presents many interesting properties: e.g., a liquid–liquid phase-separation region is observed in the higher W_p region.[22] In contrast, the regions and the other properties were not observed in nonionic surfactant systems. It therefore seems that phytoglycogen particles and the surfactant micelles coexist in water without binding in the one-phase region. In the two-phase region, since phytoglycogen tended to be precipitated more easily with the addition of a higher HLB surfactant, dehydration of the surface of phytoglycogen particles due to increasing the hydration power of the surfactant micelle may lead to the precipitation of phytoglycogen.

Temperature Dependence on DSP in Water/Phytogly-

cogen/Nonionic Surfactant Systems. It is well known that a nonionic surfactant changes from hydrophilic to lipophilic as the temperature rises, because the hydrogen bonding between the hydrophilic groups of the surfactant and water becomes weaker. Since phytoglycogen seemed to be precipitated due to dehydration of the surface of the particles, it was inferred that DSP would also be affected by the temperature.

Figure 5 shows phase diagrams of water/phytoglycogen/$C_{12}EO_{20}$, $C_{12}EO_{30}$, and $C_{12}EO_{50}$ systems as a function of the temperature. W_p is plotted horizontally, and X is fixed at 0.15 (w/w). The concentration of the boundary was independent of the temperature in the $C_{12}EO_{50}$ system with the highest EO chain length, but shifted to the lower side with increasing temperature in the $C_{12}EO_{20}$ and $C_{12}EO_{30}$ systems. The variation tended to increase with decreasing EO chain length. It seemed that the dehydration of surfactant micelles improved DSP in an aqueous solution. It was also noted that the micelles of lower HLB surfactant were dehydrated more easily than those of a higher HLB surfactant.

Figure 6 shows phase diagrams of water/phytoglycogen/$C_{12}EO_{20}$, $C_{16}EO_{20}$, $C_{22}EO_{20}$, and $CSEO_{20}$ systems as a function of the temperature. DSP was also improved with increasing temperature in these systems, and the changes of DSP in lower HLB systems were larger than those in higher HLB systems, as with the results given in Fig. 5. The two-phase region disappeared at 42.5 °C in the $CSEO_{20}$ system. Since the molar concentration of $CSEO_{20}$ was smaller than that of other systems, the two-phase region seemed to disappear.

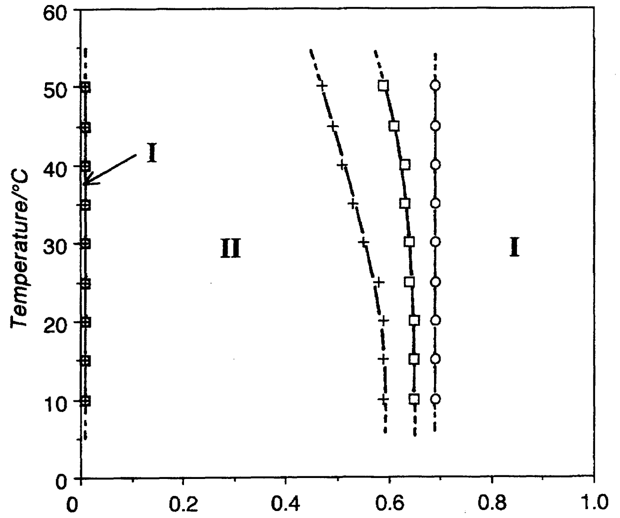

Fig. 5. The phase diagrams of water/phytoglycogen/$C_{12}EO_{20}$ (+), $C_{12}EO_{30}$ (□), and $C_{12}EO_{50}$ (o) systems as a function of temperature. Phytoglycogen/(phytoglycogen + surfactant), W_p, is plotted horizontally. The concentration of (phytoglycogen + surfactant) in system, X, is fixed at 0.15 (w/w). All the concentrations are given in weight fractions.

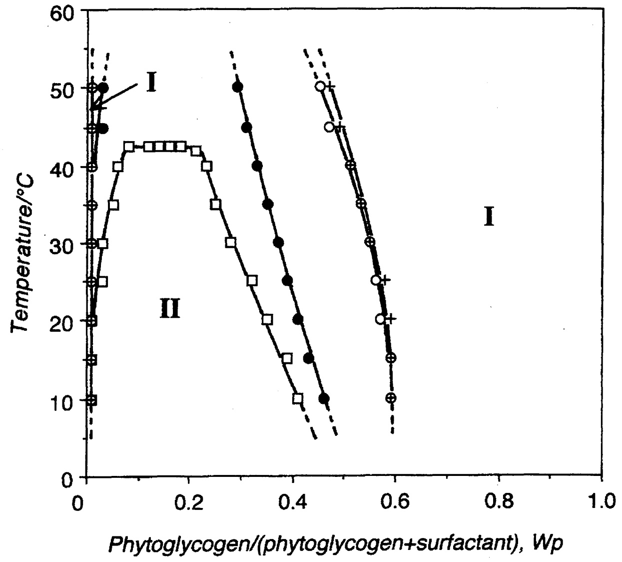

Fig. 6. The phase diagrams of water/phytoglycogen/$C_{12}EO_{20}$ (+), $C_{16}EO_{20}$ (o), $C_{22}EO_{20}$ (•), and $CSEO_{20}$ (□) systems as a function of temperature. The concentration of (phytoglycogen + surfactant) in system, X, is fixed at 0.15 (w/w).

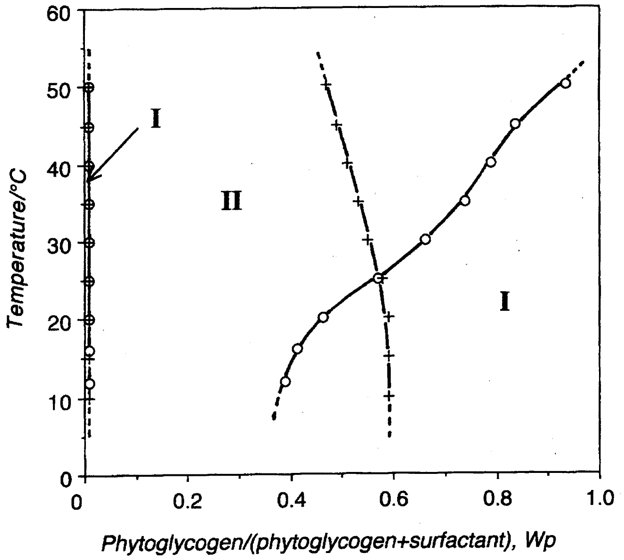

Fig. 7. The phase diagrams of water/phytoglycogen/$C_{12}EO_7$ (o) and $C_{12}EO_{20}$ (+) systems as a function of temperature. The concentration of (phytoglycogen + surfactant) in system, X, is fixed at 0.15 (w/w).

Figure 7 shows phase diagrams of the water/phytoglycogen/$C_{12}EO_7$ and $C_{12}EO_{20}$ systems. A peculiar change in the shape of the boundary was observed in $C_{12}EO_7$ system; namely, phytoglycogen became to disperse more unstably with increasing temperature. As the temperature rises, it is known that the aggregation number of a nonionic surfactant becomes larger. With further increasing the temperature, the number drastically increases above a certain temperature. An abrupt increase in the aggregation number in water appears up to a temperature called the cloud point (CP). Above CP, the surfactant solution becomes cloudy, because the surfactant aggregates are separated from water. In general, the CP tends to increase with increasing the HLB number if the C or EO chain length is fixed.[23] For example, the aggregation numbers of $C_{12}EO_6$ are 400, 1400, and 4000 at 25, 35, and 45

°C, respectively,[24] and the CP of the surfactant is 52 °C.[25]

As the results in Fig. 7 show, it seems that the aggregation number of the micelles affected the DSP because the CP of commercial $C_{12}EO_7$ is 58.5 °C,[13] and the aggregation number of the surfactant seems to increase drastically in the range of these temperatures in Fig. 7. With increasing the size of the surfactant micelles, the micelles should start to restrict the molecular motion of phytoglycogen; therefore, it seems that phytoglycogen was precipitated more easily with increasing the aggregation number. In Fig. 3, the $C_{12}EO_7$ system shows a similar boundary to the $C_{12}EO_{20}$ system. Although the HLB number of the $C_{12}EO_7$ is smaller than that of $C_{12}EO_{20}$, increasing the aggregation number may promote a precipitation of phytoglycogen, as shown in Fig. 7. Since a different phase behavior was observed between the $C_{12}EO_7$ and other systems, it was suggested that the DSP depends not only on the HLB, but also on the aggregation number of each surfactant.

Different Factors Affecting the DSP under Coexisting Conditions of Molecular and Micellar Colloids. Figure 8 shows phase diagrams of the water/phytoglycogen/$C_{12}EO_9$, $C_{12}EO_{10}$, $C_{12}EO_{20}$, and $CSEO_{20}$ systems as a function of the temperature. The HLB numbers of $C_{12}EO_9$, $C_{12}EO_{10}$, $C_{12}EO_{20}$, and $CSEO_{20}$ are 13.6, 14.1, 16.5, and 13.9, respectively. With increasing the temperature, DSP in the $C_{12}EO_9$ and $C_{12}EO_{10}$ systems was improved at a lower temperature compared with the $C_{12}EO_{20}$ system, whereas the turning points were observed and phytoglycogen started to disperse more unstably with increasing temperature above these points. The temperature of the point in the $C_{12}EO_{10}$ system was higher than that in the $C_{12}EO_9$ system. The temperature of the turning points increased under constant C chain length with increasing the HLB number. These results show the same tendency as CP or the phase inversion temperature (PIT) proposed by Shinoda.[26] Since the CPs of $C_{12}EO_{9.4}$ and $C_{12}EO_{10}$ are 84 and 88 °C, respectively,[14,15] it seems that the surfactant aggregation number began to increase drastically around the temperature of the turning point. Furthermore, the point at which the two-phase region disappeared in the $CSEO_{20}$ system existed between the turning points in the $C_{12}EO_9$ and $C_{12}EO_{10}$ systems. The HLB number in $CSEO_{20}$ is 13.9, and exists between the numbers of $C_{12}EO_9$ and $C_{12}EO_{10}$. However, the CP of the $CSEO_{20}$ system is somewhat above 100 °C due to the different shape of the lipophilic moiety of the surfactant, compared with other surfactants having a constant C chain length. Therefore, it can be presumed that the temperature at which the aggregation number starts to increase drastically in the $CSEO_{20}$ system exists above that of the $C_{12}EO_{10}$ system. Consequently, it was suggested that the two-phase region would appear again upon further increasing the temperature. From all of the results, it was clarified that DSP below the turning point is mainly related to the HLB of each surfactant, and that DSP above that is mainly related to the aggregation number of each surfactant.

Schematic Representation of the Phase Behavior in Water/Phytoglycogen/Nonionic Surfactant Systems. In

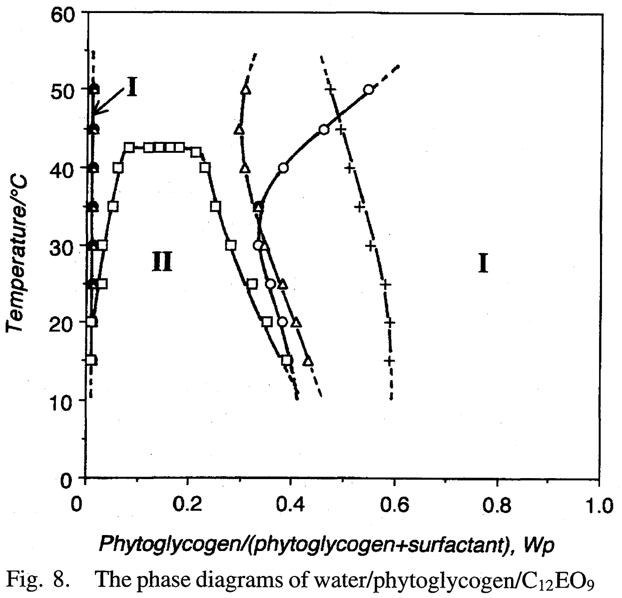

Fig. 8. The phase diagrams of water/phytoglycogen/$C_{12}EO_9$ (○), $C_{12}EO_{10}$ (△), $C_{12}EO_{20}$ (+), and $CSEO_{20}$ (□) systems as a function of temperature. The concentration of (phytoglycogen + surfactant) in system, X, is fixed at 0.15 (w/w).

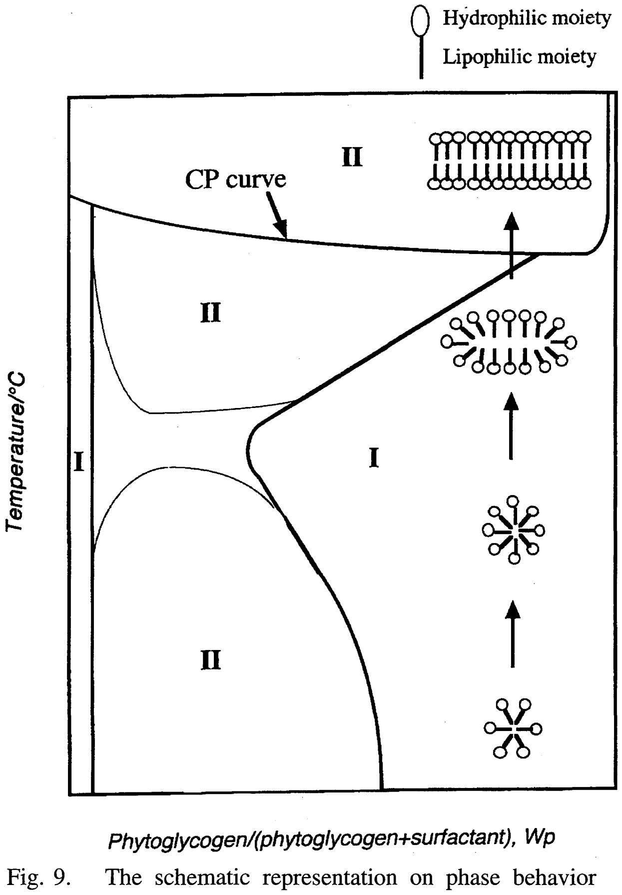

Fig. 9. The schematic representation on phase behavior in water/phytoglycogen/nonionic surfactant systems as a function of temperature. Phytoglycogen/(phytoglycogen + surfactant), W_p, is plotted horizontally. The concentration of (phytoglycogen + surfactant) in system, X, is fixed.

this section we discuss the effect of HLB on DSP in phytoglycogen and nonionic surfactant-coexisting systems. Figure 9 shows a schematic representation in the water/phytoglycogen/nonionic surfactant systems as a function of the temperature. Phytoglycogen is precipitated along with the addition of a nonionic surfactant at a lower temperature. With increasing the temperature, the W_p values on the boundary between the one- and two-phase regions decrease, and the variation of the values in a lower HLB surfactant system is larger than those in a higher HLB surfactant system. It seems that the dehydration of surfactant micelles improves DSP in an aqueous solution. At a certain temperature, a turning point appears or the two-phase region disappears. If the C chain length is fixed, the larger is the HLB number of the surfactant, the higher is the temperature of the turning point. With further increasing the temperature up to CP, phytoglycogen starts to disperse more unstably, because the aggregation number of the surfactants increases drastically. Even in a system in which the two-phase region disappears at lower temperature, it is inferred that a two-phase region again appears with increasing in the temperature. Above the CP curve, surfactant aggregates are separated from the water phase; consequently, it is suggested that phytoglycogen disperses in water again and a distinct two-phase (phytoglycogen aqueous solution + surfactant phase) would be observed. From all of the results, it is apparent that DSP at a lower temperature is mainly related to HLB, and DSP at higher temperature is mainly related to the aggregation number.

In this study we investigated the interaction between phytoglycogen and various surfactants in aqueous solution in terms of the phase behavior in water/phytoglycogen/various nonionic surfactant systems. It was clarified that phytoglycogen is precipitated due to the addition of various nonionic surfactants, and that DSP in water mainly depends on the HLB number and the aggregation number of each surfactant. Furthermore, it was confirmed that the characteristics of surfactant micelles in water can be evaluated by the simple method described above. The phase equilibrium of the water/mixed surfactants/hydrocarbon system is complicated because the monomeric solubility of nonionic surfactant in oil is usually high, and that of a lipophilic nonionic surfactant is usually higher than that of a hydrophilic nonionic surfactant.[27,28] However, the values of a nonionic surfactant having a long hydrocarbon chain length ($\geq C_{12}$) in water are very low, and usually negligible. Although the surfactants used in this study were of commercial grade, and having a chain length distribution, it was demonstrated that the characteristics of the micelles in water can be evaluated macroscopically from the experimental results of this investigation.

Conclusions. Phytoglycogen is precipitated by various nonionic surfactants. With increasing relative surfactant concentration, phytoglycogen starts to disperse more unstably, and then precipitates due to the agglomeration of particles. The dispersion stability of phytoglycogen (DSP) tends to be improved with the addition of a lower HLB surfactant at constant temperature. With increasing the temperature, DSP is improved in a higher HLB surfactant system, and the improvement increases with decreasing the HLB number, whereas phytoglycogen starts to disperse unstably in the $C_{12}EO_7$ system. The two-phase region disappears in the $CSEO_{20}$ system, and the turning points of the W_p values appear in the $C_{12}EO_9$ and $C_{12}EO_{10}$ systems. The temperature of the turning points increases under constant C chain length with increasing the HLB number. DSP is mainly related to HLB and the aggregation number of each surfactant.

References

1) D. J. Manners, *Carbohydr. Polym.*, **11**, 87 (1989).
2) D. J. Manners, *Carbohydr. Polym.*, **16**, 37 (1991).
3) D. L. Morris and C. T. Morris, *J. Biol. Chem.*, **130**, 535 (1939).
4) C. D. Boyer and K. C. Liu, *Phytochemistry*, **22**, 2513 (1983).
5) N. Inouchi, D. V. Glover, and H. Fuwa, *Starch/Stärke*, **39**, 259 (1987).
6) S.-H. Yun and N. K. Matheson, *Carbohydr. Res.*, **243**, 307 (1993).
7) S. Saito, in "Nonionic Surfactants-Physical Chemistry," ed by M. J. Schick, Surfactant Science Series Vol. 23, Marcel Dekker, New York (1987), pp. 881—926.
8) A.-C. Eliasson, *Carbohydr. Polym.*, **6**, 463 (1986).
9) A.-C. Eliasson and H. R. Kim, *J. Rheol.*, **39**, 1519 (1995).
10) K. Tateishi and A. Nakano, *Biosci. Biotechnol. Biochem.*, **61**, 455 (1997).
11) W. C. Griffin, *J. Soc. Cosmet. Chem.*, **1**, 311 (1949).
12) W. C. Griffin, *J. Soc. Cosmet. Chem.*, **5**, 249 (1954).
13) H. Scott, *J. Pharm. Sci.*, **58**, 1443 (1969).
14) T. Kuwamura, in "Structure/Performance Relationships in Surfactants," ed by M. J. Rosen, ACS Symp. Series 253, American Chemical Society, Washington, D.C. (1984), p. 32.
15) A. N. Wrigley, F. D. Smith, and A. J. Stirton, *J. Am. Oil Chem. Soc.*, **34**, 39 (1957).
16) H. Kunieda, K. Nakamura, and D. F. Evans, *J. Am. Chem. Soc.*, **113**, 1051 (1991).
17) H. Kunieda and M. Yamagata, *Colloid Polym. Sci.*, **271**, 997 (1993).
18) H. Kunieda and K. Shinoda, *J. Colloid Interface Sci.*, **107**, 107 (1985).
19) A. Carlsson, G. Karlström, and B. Lindman, *Langmuir*, **2**, 536 (1986).
20) A. Carlsson, G. Karlström, B. Lindman, and O. Stenberg, *Colloid Polym. Sci.*, **266**, 1031 (1988).
21) S. Suto and I. Iwasawa, *J. Polym. Sci., Part-A-1*, **31**, 1599 (1993).
22) A. Nakano, R. Irie, and K. Tateishi, *Biosci. Biotechnol. Biochem.*, in press.
23) D. J. Mitchell, G. J. T. Tiddy, L. Waring, T. Bostock, and M. P. McDonald, *J. Chem. Soc., Faraday Trans. 1*, **79**, 975 (1983).
24) R. R. Balmbra, J. S. Clunie, J. M. Corkill, and J. F. Goodman, *J. Chem. Soc., Faraday Trans. 1*, **58**, 1661 (1962).
25) A. W. Cohen and M. J. Rosen, *J. Am. Oil Chem. Soc.*, **58**, 1062 (1981).
26) K. Shinoda and H. Saito, *J. Colloid Interface Sci.*, **30**, 258 (1969).
27) H. Kunieda and M. Yamagata, *Langmuir*, **9**, 3345 (1993).
28) H. Kunieda, A. Nakano, and M. Akimaru, *J. Colloid Interface Sci.*, **170**, 78 (1995).