Effect of Freeze–Thaw Treatment on the Precipitation of Octyl β -D-Galactoside Hemihydrate Crystal from the Aqueous Solution

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Abstract: Cryogenic treatment, like the freeze–thaw process, has been reported to be effective in modifying the physicochemical properties of polymeric hydrogels. However, not much attention has been paid to this process in terms of the precipitation of surfactant–water systems. In this study, two effective cryogenic methodologies were successfully reported to alter the physicochemical properties of a precipitate of an octyl β -D-galactoside (Oct-Gal)–water system. First, hyperrapid cooling (i.e., cooling at 30°C/min) was found to be an effective type of cryogenic treatment: the phase transition temperature (T_K) and enthalpy at the phase transition (ΔH_K) between the crystal-dispersed phase and the sol (micelle) phase significantly decreased. In addition, cryogenic treatment in the presence of electrolytes, such as NaCl, NaBr, and CsCl, was effective even in the absence of the hyperrapid cooling condition. The hyperrapid cooling or the addition of certain electrolytes was considered to prevent the precipitation of the Oct-Gal hemihydrate crystals prior to the complete freezing of ice and the electrolyte/ice eutectic. Hence, the size of the aggregated crystals prepared by the above-mentioned effective cryogenic treatments seemed to be decreased compared with that of the normal precipitated crystals, thereby changing T_K and ΔH_K . Thus, two basic methodologies for the modification of the physicochemical properties of the crystal-dispersed phase of surfactant–water systems are discussed.

Key words: octyl β -D-galactoside, *n*-alkyl glycoside, freeze–thaw, crystal-dispersed phase, cooling rate, electrolyte, space group transition

1 INTRODUCTION

Surfactants play an important role in the development of various application fields. Cost-effective, nonionic *n*-alkyl glycosides (Alkyl-Glys), which are renewable, biodegradable, and environmentally friendly, have various applications in food, cosmetics, and pharmaceuticals. Numerous fundamental studies on the toxicologically harmless and physicochemical properties of Alkyl-Glys–water systems, including lyotropic liquid crystalline (LC) phases as well as colloidal assembly systems, have ensured their performance at temperatures greater than subzero^{1–6)}. On the basis of the ability to form lamellar vesicles, they can be used as drug carriers^{7, 8)}. They are also used as a ubiquitous

tool in biochemical and biophysical studies of membrane proteins. For instance, some Alkyl-Glys micelles can be used as membrane-mimetic media⁹⁾. The lamellar gel and crystal phases formed by alkyl polyglucoside (APG)-type Alkyl-Glys have also attracted attention because of their structuring of viscoelastic networks¹⁰⁾. Such gel or crystal phases give the continuous phase its structure, contributing to the immobilization of species, the reduction of flocculation, and the coalescence of other colloidal assemblies such as emulsions.

The phase behavior of Alkyl-Glys–water systems at subzero temperatures has also recently attracted attention^{11–15)}. Currently, several Alkyl-Glys–water systems

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exhibit glass transitions without precipitation of Alkyl-Glys crystals even below the melting point or solubility curves in the temperature–concentration phase diagram of a water system^{11–13, 15}. These systems form a glassy phase under supercooling conditions in extremely high-concentration systems as well as under supersaturated conditions in an unfrozen matrix in which Alkyl-Glys is substantially concentrated by the freezing of water (solvent) at subzero temperatures. The stabilization of alkyl β -D-glucosides (Alkyl-Glus) for water-soluble proteins during the freeze–thaw process has been reported to be strongly related to the surface-active properties of the Alkyl-Glus^{15, 16}, whereas their bifunctional characteristic that satisfies the glass-forming properties and the surface activity are potentially related to their function of stabilizing therapeutic proteins during the freeze-drying process^{15, 17}. Thus, the importance of fundamental investigations on physicochemical properties and the availability of Alkyl-Gly at the subzero region have been somewhat demonstrated.

Meanwhile, the freeze–thaw or freeze-concentration protocol has been widely used for polymeric compound systems to modify various hydrogel systems^{18–21}. Upon freezing of the water, heterophase systems comprising solid ice and some unfrozen phases called “unfrozen liquid microphases,” in which the solutes are highly concentrated, are obtained. Dispersed ice becomes a physical disturbance; hence, a unique polymeric gel system called “cryogel,” which exhibits an unusual heterophase macroporous morphology and modified physicochemical characteristics, is obtained^{18–21}. A similar effect has been reported for the freeze-concentration protocol for the modification of a hydrogel system comprising a low-molecular-weight FmocFF system²². Because the lamellar gel or crystalline phases of Alkyl-Glys have important pharmaceutical applications¹⁰, the exploration of a cryogenic treatment to control such phase behaviors of the Alkyl-Gly–water system can further enhance its usability. This idea motivated us to investigate the conditions required to carry out an effective cryogenic treatment of the semi-solid phase.

n-Alkyl D-galactoside (Alkyl-Gal) consisting of D-galactose and a 1-alcohol is a representative family of the Alkyl-Glys system. The physicochemical properties of Alkyl-Gals in aqueous systems at temperatures above subzero have been widely investigated for both α - and β -anomers^{7, 23–28}. In the case of a longer alkyl chain, Kiwada et al. have reported the formation of vesicles based on Alkyl-Gals that exhibited higher affinity for the liver, specifically in the binding of hepatocytes through specific galactose interactions, than vesicles based on glucosides⁷. Hence, the Alkyl-Gals can be used in certain pharmaceutical applications that differ from those of Alkyl-Glus in the form of a semi-solid colloidal assembly. For instance, a porous polymer scaffold functionalized by galactose has been reported to

Chart 1 Chemical structure of Oct-Gal.

improve the 3D growth of hepatocyte-based cells²⁹; thus, the self-organized colloidal assembly of amphiphilic galactose may be similarly used without the need to functionalize the polymer scaffold. Because Alkyl-Gals have simple compositions that do not require significant modification, and given the background of many reports of recent advances in the enzymatic preparative procedures for Alkyl-Gals^{30–32}, they are a promising candidate among Alkyl-Glys to be used as unique functional materials.

Our group has recently verified that the crystal-dispersed phase, or the so-called coagel, precipitates from an octyl β -D-galactoside (Oct-Gal: Chart 1)–water system, leaving behind an aqueous micelle solution³³. In this phase, the water-dispersed crystals comprising three-dimensional (3D) fiber-like crystals covered a considerable amount of water. The system exhibits a phase-transition temperature (T_K) greater than 30°C between the crystalline dispersed phase and the sol (micelle) phases during heating. Given the resemblance between the crystal-dispersed phase in the Oct-Gal–water system and a typical hydrogel system with respect to their 3D semicrystalline networks, cryogenic treatment is also expected to become an effective methodology to control the phase behavior of the Oct-Gal–water system. Furthermore, the crystal-dispersed phase of Oct-Gal was found to be macroscopically homogeneous at a low concentration (2.5 wt. % surfactant concentration), whereas those phases from aqueous octyl α -D-glucoside and dodecyl β -D-glucoside solutions formed inhomogeneous states. Such performance of Oct-Gal toward a macroscopically homogeneous matrix was expected to provide detailed insights into the effect of ice formation on the precipitation of the crystal-dispersed phase from an Alkyl-Glys–water system, whereas the inhomogeneous matrix formed by other Alkyl-Glys was thought to be too complex to clarify the phase behavior controlled by cryogenic treatment.

This work aimed to explore the cryogenic treatment conditions that enable control of the phase behavior of the Oct-Gal–water system. To this end, we examined the effect of cryogenic protocols on the physicochemical properties of the crystal-dispersed phase formed from aqueous Oct-Gal solutions. In this regard, two effective methodologies to modify the physicochemical properties of the pre-

precipitates of Oct-Gal hemihydrate crystals—hyperrapid cooling and electrolyte addition—were verified. In addition, detailed behavior of precipitation via cryogenic treatment was investigated by simultaneous X-ray diffraction and differential scanning calorimetry (XRD-DSC) measurements.

2 EXPERIMENTAL PROCEDURES

2.1 Materials

Oct-Gal was prepared in a manner similar to the method reported previously by our group³³⁾ and used after drying under vacuum at 60°C for 2 h. Water and electrolytes purchased from Wako Pure Chemical Industries Ltd. were used as received. The sample solutions were prepared by the dissolution of prescribed amounts of Oct-Gal and electrolyte in water at a bath temperature of 50°C. The Oct-Gal and electrolyte concentrations were expressed as weight percentage (wt.%) of Oct-Gal (e.g., Oct-Gal in g/(Oct-Gal + H₂O) in g) and weight molar percentage (mol/kg) for the electrolytes (e.g., electrolyte in moles/H₂O in kg).

2.2 Measurements

The freeze–thaw behavior of Oct-Gal–electrolyte–water systems was examined by DSC and XRD-DSC simultaneously measurement. For DSC measurements, DSC-8500 (PerkinElmer, Inc.) and DSC-60 (Shimadzu Corp.) systems were used. The former and latter were used to confirm the effect of the hyperrapid cooling at a temperature of 30°C/min and for other measurements, respectively. Hermetically sealed aluminum pans were used in the above instruments. The cooling and heating rates for each measurement were stated in the caption of each of the figures shown. For XRD-DSC measurements, an XRD-DSCII (Rigaku Corp.)^{11, 13, 14, 34, 35)}, combining a RINT-Ultima III unit and a Thermo Plus 2 DSC (Rigaku Corp.), was used. Measurements were carried out at 2θ values of 1°–30° using Cu K α radiation (1.54 Å, 40 kV, and 40 mA) at a scan rate of 30°/min (($2\theta/\omega$)/min). The sample was measured under N₂ with a square nonhermetical aluminum container. To analyze the full width at half maximum (FWHM) of the diffraction peak, XRD profile fitting was carried out by Gaussian peak adjustment using the Origin 6.1 data analysis software (OriginLab Corp.).

3 RESULTS & DISCUSSION

3.1 Effect of the Cooling Rate on the Physicochemical Properties of the Precipitate Formed during Cryogenic Treatment

The cooling rate was reported to be an important parameter for effectively performing cryogenic treatment for polymeric cryogels. Effective cryogenic treatment is not

possible if hydrogelation occurs before ice freezing is completed. However, some systems can overcome the prevention of hydrogelation prior to ice freezing via the increase in the cooling rate²¹⁾. Based on these studies, the cryogenic protocol in a binary system was first examined at different cooling rates, for example, 10°C/min and 30°C/min.

Figure 1 shows the heating DSC thermograms of a 10 wt.% Oct-Gal–water binary system after cooling the sample (sol) to –50°C at a rate of (a) 10°C/min and (b) 30°C/min. In these heating thermograms, high-intensity endothermic peaks were observed at around 0°C, corresponding to the melting of ice (m.i.), and an endothermic peak was observed at a higher temperature, corresponding to the phase transition between the dispersed-crystal and sol phases. At a cooling rate of 10°C/min, T_{K1} corresponding to normal T_K was observed at around 31°C (Fig. 1(a)), which is comparable to that obtained without ice freezing, indicative of no significant effect on the physicochemical properties of the resultant crystal-dispersed phase by cryogenic treatment in this time. Alternatively, the appearance of such an endothermic peak indicates that the Oct-Gal–water binary system exhibited a higher crystallization (precipitation) tendency during the freeze–thawing process than Oct-Glu–water binary systems, where no crystallization occurred under similar experimental conditions^{13, 15)}. Also, in the case of anhydrous LC phase, Oct-Gal tended to crystallize even under rapid cooling conditions³⁶⁾, whereas the anhydrous Oct-Glu LC phase readily exhibited a glass transition^{12, 15)}. Therefore, the high precipitation tendency of the Oct-Gal during the freeze–thawing process is consistent with this behavior.

Fig. 1 DSC thermograms showing the thawing behavior of 10 wt.% Oct-Gal–water binary mixtures after the system was frozen by cooling at (a) 10°C/min and (b) 30°C/min. Each measurement was performed using the same mass of the sample in the sol state.

On the other hand, for the hyperrapid cooling rate at 30°C/min, the endothermic peak was observed at a temperature less than T_{K1} (Fig. 1(b)). This reduced temperature was designated as T_{K2} in the thermogram. In thermogram (b), the total phase transition enthalpy (ΔH_K) at around T_{K1} and T_{K2} was 18.3 mJ/mol; this value is remarkably less than that observed in thermogram (a), 24.9 mJ/mol. As the same sample mass (same sample) was used for both measurements, these differences originated from the cryogenic effect utilizing hyperrapid cooling. The reason for which only the hyperrapid cooling rate can accomplish the effective cryogenic treatment is crucial to explain for whether precipitation occurs before the freezing of ice.

Previously, our group reported that the precipitation of Oct-Gal from an aqueous Oct-Gal solution starts within 1 min when the solution is stored at 4°C³³. Even though the cooling rate was relatively high (i.e., 10°C/min), precipitation can start before the freezing of ice because of such an inherent property; water started to freeze at around -20°C during cooling, indicative of the passage of 2.4 min after the system is cooled at temperatures less than 4°C at 10°C/min. On the other hand, the precipitation of Oct-Gal crystals might be avoided by the hyperrapid cooling at 30°C/min prior to the freezing of ice because even 1 min is not required to cool the sample from 4°C to -20°C at the cooling rate. Hence, the reduction of T_K and ΔH_K compared to the normal values must be observed because of the effective cryogenic treatment. However, notably, a small endothermic peak was observed at around 31°C even for the heating thermogram obtained by the hyperrapid cooling, indicating that not all of the crystal-dispersed phases are formed under the effective cryogenic treatment.

3.2 Effect of Electrolyte Addition on the Physicochemical Properties of the Precipitate Formed during the Cryogenic Treatment

Electrolytes are involved in many food processing methods and biological systems. With respect to applications, understanding the effect of electrolytes on the cryogenic treatment is important. Hence, the addition of electrolytes during cryogenic treatment has been reported to lead to substantial changes in the gel properties of poly (vinyl alcohol) (PVA)^{37, 38}, which possesses multiple hydroxyl substituents, similar to sugar molecules. In addition, in the case of a natural sugar such as D-glucose, both salting-out and salting-in effects by NaCl were observed, whereas only the salting-out effect was confirmed for KCl³⁹. That is, the addition of a certain type of electrolyte can drastically change the phase-transition behavior of Alkyl-Gal during the freeze-thawing process.

In a preliminary study, the addition of NaCl was confirmed to prolong the stability of the sol state of an Oct-Gal-water system at 4°C. Although rapid precipitation within 1 min has been observed in the absence of NaCl³³, it

can be inhibited for at least 35 min in the presence of a 1.0 M NaCl aqueous solution under the same conditions. Such elongation of the induction period for the precipitation of the Oct-Gal crystal was expected to be crucial to prevent rapid precipitation during cooling. Therefore, the effect of cryogenic treatment on the precipitation in the presence of NaCl is investigated.

Figure 2(a) shows the heating DSC thermograms of 10 wt.% Oct-Gal-NaCl-water ternary mixtures at different NaCl concentrations, in addition to the thermogram of a 10 wt.% Oct-Gal-water binary mixture for comparison. Each sample was cooled at 13°C/min to form ice prior to the heating measurement. In the thermogram, the melting of ice (m.i.) and NaCl dihydrate (electrolyte)/ice eutectic (m.e.) was observed. Similar to that observed in Fig. 1(a), T_{K1} was observed in the thermogram for a binary mixture (Fig. 2(a), 0.0 mol/kg NaCl concentration). However, in the DSC thermograms of ternary mixtures (Fig. 2(a), from 0.2 to 2.5 mol/kg NaCl concentrations), T_{K2} was observed at a lower temperature of around 21°C, and ΔH_K decreased to approximately 0.6 times the value obtained without cryogenic treatment (Table 1). Notably, no endothermic peak was observed at around 31°C (Fig. 2(a), from 0.2 to 2.5 mol/kg NaCl concentrations), while a small endothermic peak was observed under the hyperrapid cooling condition (Fig. 1), indicating that the addition of NaCl possibly aids in the more effective cryogenic treatment compared with that of hyperrapid cooling (30°C/min) without NaCl.

Then, the effect of the initial states, that is, precipitate or no precipitate (sol phase), on the phase behavior was compared using cooling-heating thermograms (Fig. 2(b)). Here, thermogram (i) was recorded for a sample in the initial sol state, while thermogram (ii) was recorded for a sample in the precipitated state prior to the measurement. At the phase transition in thermogram (i), T_K and ΔH_K decreased, whereas these variables in the thermogram (ii) did not decrease, clearly indicating that the freeze-thaw treatment of the sample in the supersaturated sol state is crucial. In addition, when the exothermic peak corresponding to the eutectic crystallization of NaCl dihydrate/ice (f.e.) was compared for both thermograms, the exothermic peak clearly broadened for the sample in the initial sol system (i), whereas a sharp exothermic peak was observed for the sample in the initial precipitated system (ii). From these results, the sol system under the unfrozen matrix is reasonably concentrated by ice freezing, and the eutectic crystallization can be prevented because of the increased viscosity of the concentrated unfrozen matrix and the deep freezing-point depression with the increase in the Oct-Gal concentration. On the other hand, the initially precipitated system did not prevent the eutectic formation (f.e.) in such a manner as a majority of the Oct-Gal molecules are in the crystal state.

Subsequently, the effect of electrolytes on the cooling-

Fig. 2 DSC thermograms showing the (a) thawing behavior of 10 wt.% Oct-Gal–NaCl–water ternary mixtures after the system was frozen by cooling at 13°C/min with the variation in the NaCl concentrations and (b) freeze–thaw behavior of 10 wt.% Oct-Gal–2.0 mol/kg NaCl–water ternary systems using (i) sol and (ii) crystal-dispersed phase states. In (a), each of the NaCl concentrations was shown in the figure.

Table 1 Onset and offset temperatures (T_K) and transition enthalpies (ΔH_K) of the crystal-dispersed phase–sol transition prepared by the freeze–thaw treatment of the sol samples with cooling at 13°C/min in the absence and presence of electrolytes.

Electrolyte	conc. in mol/kg	T_K in °C	ΔH_K in kJ/mol
None	0.0	28.5–33.5	25.6 ± 1.0
NaCl	0.5	18.0–24.0	14.7 ± 3.2
NaBr	0.5	17.0–24.0	18.4 ± 5.0
CsCl	0.5	22.5–26.0	16.3 ± 0.8

heating thermograms was examined. Interestingly, the electrolytes were also crucial to induce a decrease in ΔH_K and T_K (Fig. 3). In short, the addition of KCl and RbCl did not significantly affect ΔH_K and T_K , whereas the addition of CsCl and NaBr decreased T_K and ΔH_K , similar to that of NaCl (Table 1), whereas even precipitation was not observed in the NaI system under the same conditions. With respect to the exothermic peaks corresponding to the eutectic crystallization (f.e.), a sharper exothermic peak was apparently observed in the thermograms for those in KCl and RbCl systems at a temperature greater than -33°C , whereas a broader peak was confirmed at a temperature less than -39°C for those in CsCl, NaBr, and NaI systems; each temperature was discussed using the peak top temperature of f.e. These results indicated that the system

that is subjected to effective cryogenic treatment does not appear to precipitate before the freezing of the system. In addition, precipitation at a temperature less than -39°C might be significant to induce a decrease of ΔH_K and T_K . On the other hand, possibly because of the excellent prevention of the precipitation of Oct-Gal, even the precipitation during the freeze–thaw process did not occur in the NaI system. The influence of added electrolytes cannot be easily explained, as indicated in previous reports on PVA cryogels³⁷⁾. The occurrence of a specific interaction between Oct-Gal molecules or assemblies observed in the present study might be one explanation. Alternatively, because the addition of electrolytes into a water system changes its viscosity, the difference in viscosity can alter the crystallization behavior of Oct-Gal during the freeze–thaw process. The finding that the addition of KCl can decrease the viscosity when water viscosity is high but increase the viscosity when the water viscosity is low is highly surprising⁴⁰⁾. If a similar decrease in viscosity occurred in the present system as occurred in the Oct-Gal system, then the KCl system might promote Oct-Gal crystallization. Such intrinsic behavior of electrolytes in a water system can induce a different effect.

3.3 Study on the Phase Behavior of the Oct-Gal–NaCl–Water System during the Freeze–Thaw Process

XRD–DSC measurements were simultaneously carried out to understand the detailed freeze–thaw behavior of the

Fig. 3 DSC thermograms showing the thawing behavior of 10 wt.% Oct-Gal-electrolyte-water mixtures after the system was frozen by cooling at $13^{\circ}\text{C}/\text{min}$. Each type of electrolyte and the corresponding concentration are shown in the figure.

Oct-Gal-electrolyte-water ternary mixture, where effective cryogenic treatment was accomplished, and a decrease of T_K and ΔH_K was observed. The Oct-Gal-2.0 mol/kg NaCl-water system was selected as a representative system. Figure 4 shows the result obtained from the simultaneous XRD-DSC measurement of the freeze-thaw behavior of the system. The right and left figures show the DSC thermogram as a function of time and the XRD patterns corresponding to each measurement time, respectively. The solution was initially solidified by storage at room temperature prior to measurement. Throughout the analysis, the thermal protocol was carried out as depicted by the temperature curve in the figures. That is, (I) the sample was heated to 42°C to completely dissolve the precipitated Oct-Gal crystals, (II) cooled to -57.5°C to form ice and NaCl dihydrate/ice eutectic in the system, followed by (III) heating to 41.5°C . Each of the diffraction peaks of hexagonal ice (i in Fig. 4, left) and NaCl dihydrate (e in Fig. 4, left)

was identified from the data reported in previous studies for the crystal structural analysis of hexagonal ice⁴¹⁾ and NaCl dihydrate⁴²⁾, respectively. For the Oct-Gal hemihydrate crystal, the lattice constants and space group were identified from the data obtained by grazing incidence X-ray diffraction analysis using submicrometer-thick Oct-Gal hemihydrate crystal films³⁶⁾.

As the initial state was the precipitated state, diffraction peaks at low and high diffraction angles were clearly observed at 0 min (Fig. 4). Interestingly, the magnification of the XRD pattern between 2.5° and 4.0° revealed the clear presence of a diffraction at around 3.18° in the XRD profile for a 10 wt.% Oct-Gal-2.0 mol/kg NaCl-water system (Fig. 5, lower). Previously, the X-ray analysis using crystalline films and the powder of the Oct-Gal hemihydrate crystal³⁶⁾ suggested a $P2_12_12_1$ space group for the crystal, and in the case of such a space group, the (010) reflection cannot be obtained because of the extinction law. In addi-

Fig. 4 Simultaneous XRD-DSC measurement of the freeze–thaw process of 10 wt.% of the Oct-Gal–2.0 mol/kg NaCl–water system. The sample was (I) heated to 42°C, (II) cooled to -57.5°C , and (III) then heated to 41.5°C. In the XRD profile, diffraction peaks from hexagonal ice and NaCl dihydrate were assigned as (i) and (e), respectively.

Fig. 5 XRD profiles of 10 wt.% of the Oct-Gal–2.0 mol/kg NaCl–water system, which are prepared with (upper) and without (lower) cryogenic treatment. (a) is the overall picture and (b) is the enlarged one around low diffraction angles. In (b), the dotted line is the fitting curve.

tion, such a diffraction peak corresponding to the (010) plane was not clearly distinguished in a study previously reported for a 10 wt.% Oct-Gal–water system³³⁾ in the absence of an electrolyte. A space phase transition in the orthorhombic crystal systems was assumed to occur. A

certain protein crystal has been reported to exhibit space group transition in orthorhombic crystal systems by cryo-annealing⁴³⁾. However, in this case, the addition of electrolyte seemed to induce space group transition. Specific interactions between Oct-Gal and the electrolyte, which

induce the delay of precipitation, were observed, which was discussed in Section 3.2. Alternatively, because Oct-Gal forms a fiber structure with a high aspect ratio in aqueous media³³⁾, a disordered domain, which induces such a transition, may exist in the dispersed crystal. However, in the current situation, a more detailed study is required to draw a conclusion. Meanwhile, because of the correspondence of the XRD diffractions from the (200) and (210) planes, the principle crystal structure of the precipitated Oct-Gal in the system must be similar to those of the powder state. Hence, it is reasonable to consider the crystal as hemihydrate crystals.

In the initial heating process of the crystal-dispersed phase sample, the diffraction peaks assigned as the crystals disappeared after the system completely attained the sol state ((i) in Fig. 4). During the subsequent cooling of the sol system, the ice formation (f.i.) and eutectic formation (f.e.) were observed in the Oct-Gal–NaCl–water system ((ii) and (iii) in Fig. 4). At this time, the low-angle diffraction peak corresponding to the Oct-Gal molecular assembly was observed at around 3.3° at -30°C , indicative of the formation of the Oct-Gal ordering structure, indicating that the freeze-concentration by both ice and eutectic on Oct-Gal leads to a phase transition from the isotropic micelle solution. Here, the ordered molecular assembly must be the LC phase as the broad peak at the wide-angle diffraction reflected the absence of the ordered packing of alkyl chains. In addition, the formation of the LC phase from the micelle solution by freeze-concentration was observed in the Oct-Glu–NaCl–water system^{14, 15)}.

Next, in the subsequent heating process, no significant changes at the diffraction peaks were detected until the start of eutectic melting. Prior to the main melting of the eutectic (m.e.), the low diffraction peak at around 3.0° became sharper, and broad diffraction peaks in a wide range were observed after the exothermic peak was observed. Characteristic diffraction peaks at around 3.4° for the (200) plane and 4.7° for the (210) plane at that temperature corresponded to the diffraction for the Oct-Gal hemihydrate crystal³⁶⁾, indicative of the precipitation of the Oct-Gal hemihydrate crystals accompanied by exothermic heat ((iv) in Fig. 4). Thus, the precipitation utilizing cryogenic treatment was strongly indicated. The diffraction peaks did not distinctly change during the melting of eutectic (m.e.) and ice (m.i.) at temperatures ranging from -20 to 0°C ((v) and (vi) in Fig. 4), indicating that the Oct-Gal hemihydrate crystal-dispersed phase persists after the complete thawing of ice. Later, the diffraction peaks corresponding to the phase disappeared with the endothermic peak ((vii) in Fig. 4) around 20°C (T_{K2}), clearly demonstrating that the endothermic peak observed at around 20°C in the heating DSC thermogram corresponds to the phase transition between the Oct-Gal hemihydrate dispersed-crystal phase and the sol phase. From these

results, the crystal-dispersed phase of the Oct-Gal hemihydrate crystal exhibiting a phase transition at T_{K2} is formed prior to the main melting of the eutectic, and the importance of cryogenic treatment including freezing and thawing is recognized.

3.4 Discussion Related to the Cryogenic Effect on the Precipitation of the Oct-Gal Crystal from the Oct-Gal–NaCl–Water System

The principle crystal structure of precipitated Oct-Gal prepared by the effective cryogenic treatment appeared to be similar to that prepared without the freezing of ice because of the correspondence of the XRD diffractions from the (200) and (210) planes (Fig. 5). Therefore, differences in the physicochemical properties, including T_{K} and ΔH_{K} , result from those of the physical states of the dispersed crystals rather than the modification of the crystal structure.

Actually, distinct differences were observed in the FWHMs between each of the XRD profiles obtained with and without cryogenic treatment. Without the freeze–thaw protocol, a relatively sharp diffraction peak was observed at 3.5° with an FWHM of 0.116° (Fig. 5, lower). On the other hand, for the XRD profile obtained by the freeze–thaw protocol, the corresponding diffraction peak was apparently broader (FWHM: 0.214°) (Fig. 5(b), upper). Similarly, the diffraction peaks at wide diffraction angles greater than 20° were also broader in case of the XRD profile obtained by the freeze–thaw protocol compared to the diffraction peaks for the XRD profile without the freeze–thaw protocol. From the Scherrer equation⁴⁴⁾, the average crystallite size L is expressed as follows;

$$L = K\lambda / (\beta \cdot \cos \theta), \quad (1)$$

where λ is the X-ray wavelength in nanometers (nm), β is the FWHM corresponding to a small crystallite size in radians, and K is a constant related to the shape of the crystallite. According to the small FWHM and the Scherrer equation, the precipitated Oct-Gal exhibits lower crystallinity by the cryogenic treatment in the presence of NaCl. Thus, the decrease of ΔT_{K} and ΔH_{K} is possibly related to the low crystallinity of the precipitate.

For the Alkyl-Glys–electrolyte–water system, rapid cooling has been reported to enable the formation of a glassy state in the unfrozen phase after the sufficient freezing of ice and eutectic for other sugar–surfactant–water systems^{11, 13, 15)}. Typically, molecular rearrangement is effectively inhibited in the glassy state because of the cessation of the translational and rotational motions. Considering from the similar situation for Oct-Gal–electrolyte–water system, it must be difficult for the electrolytes present at the glycolipid interlayer to diffuse inside the matrix. Hence, after the evolution of the Oct-Gal crystals, the crystallization of Oct-Gal molecules can be partially prevented by the

presence of electrolytes. On the other hand, even in the absence of electrolytes, the hyperrapid cooling may avoid the crystallization of Oct-Gal at higher temperature and induced small crystallites under considerable freeze-concentrated state with low crystallinity, decreasing ΔT_K and ΔH_K . In addition to the effective freeze-concentration before precipitation, whether the specific interaction between Oct-Gal and electrolytes and/or differences in the viscosities between each of the Oct-Gal–electrolyte–water systems may determine whether the effective cryogenic treatment can be accomplished. However, further detailed studies in terms of the specific interactions and viscosity of the ternary systems are further needed to reach conclusions.

4 CONCLUSIONS

In this study, two effective cryogenic methodologies for changing the physicochemical properties of the precipitated Oct-Gal crystals from Oct-Gal–water mixtures were revealed: hyperrapid cooling at 30°C/min and freeze–thaw treatment with the addition of appropriate electrolytes. In the latter case, a hyperrapid cooling rate was not required to induce the modification of the physicochemical properties of the coagel of Oct-Gal–water mixtures. In both cases, complete freezing prior to the precipitation of the Oct-Gal hemihydrate was thought to be important. As a majority of the practical formulations are prepared in multi-ingredient systems, including various electrolytes, the novel inspection verified in this study in terms of the combinatorial use can be significant for the development of surfactant-based crystalline materials. Although the dissolving temperature of Oct-Gal crystals obtained in this work by an effective cryogenic treatment was lower than 25°C and its usability is low, increasing the dissolving temperature by using Alkyl-Gal with a longer alkyl chain length and the adaptation of the effective cryogenic treatment established in this work may lead to the construction of a self-organized scaffold with various bio-related applications (e.g., 3D growth of hepatocyte-based cells), as reported for polymeric cryogels.

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References

- 1) von Rybinski, W.; Hill, K. Alkyl polyglycosides—properties and applications of a new class of surfactants. *Angew. Chem. Int. Ed.* **37**, 1328–1345 (1998).
- 2) Kocher, K.; Wiegand, H.-J. Toxicology and dermatology. in *Nonionic surfactants, alkyl polyglucosides* (Balzer, D.; Lüders, H. eds.), Surfactant science series 91, Marcel Dekker, Inc., pp. 365–383 (2000).
- 3) Claessono, P.M.; Kjellin, U.R.M. Sugar Surfactants. in *Encyclopedia of surface and colloid science second edition* (Somasundaran, P. ed.), Vol. 8, CRC press, Taylor & Francis Group, pp. 5831–5845 (2006).
- 4) Hill, K.; LeHen-Ferrenbach, C. Sugar-based surfactants for consumer products and technical applications. in *Sugar-based surfactants: Fundamentals and applications* (Ruiz, C.C. ed.) Surfactant science series 143, CRC press, Taylor & Francis Group, pp. 1–20 (2009).
- 5) Geetha, D.; Tyagi, R. Alkyl poly glucosides (APGs) surfactants and their properties: a review. *Tenside Surf. Det.* **49**, 417–427 (2012).
- 6) Smulek, W.; Kaczorek, E.; Hricovíniová, Z. Alkyl xylosides: physico-chemical properties and influence on environmental bacteria cells. *J. Surfact. Deterg.* **20**, 1269–1279 (2017).
- 7) Kiwada, H.; Niumura, H.; Fujisaki, Y.; Yamada, S.; Kato, Y. Application of synthetic alkyl glycoside vesicles as drug carriers. I. preparation and physical properties. *Chem. Pharm. Bull.* **33**, 753–759 (1985).
- 8) Aripin, N.F.K.; Park, J.W.; Park, H.J. Preparation of vesicle drug carrier from palm oil- and palm kernel oil-based glycosides. *Colloids Surf. B* **95**, 144–153 (2012).
- 9) Hutchison, J.M.; Lu, Z.; Li, G.C.; Travis, B.; Mittal, R.; Deatherage, C.L.; Sanders, C.R. Dodecyl- β -melibioside detergent micelles as a medium for membrane proteins. *Biochemistry* **56**, 5481–5484 (2017).
- 10) Savić, S.; Tamburić, S.; Savić, M.M. From conventional towards new – natural surfactants in drug delivery systems design: current status and perspectives. *Expert Opin. Drug Deliv.* **7**, 353–369 (2010).
- 11) Ogawa, S.; Osanai, S. Inhibition effect of sugar-based amphiphiles on eutectic formation in the freezing–thawing process of aqueous NaCl solution. *Cryobiology* **54**, 173–180 (2007).
- 12) Ogawa, S.; Asakura, K.; Osanai, S. Glass transition behavior of octyl β -D-glucoside and octyl β -D-thiogluconate/water binary mixtures. *Carbohydr. Res.* **345**, 2534–2541 (2010).
- 13) Ogawa, S.; Asakura, K.; Osanai, S. Freezing and melting behavior of an octyl β -D-glucoside–water binary system – inhibitory effect of octyl β -D-glucoside on ice crystal formation. *Phys. Chem. Chem. Phys.* **14**, 16312–16320 (2012).
- 14) Ogawa, S.; Asakura, K.; Osanai, S. Lyotropic behavior of a mono-tailed glycolipid assembly during solidifica-

- tion and melting of electrolyte/ice eutectic systems. *Chem. Pharm. Bull.* **62**, 1180-1184 (2014).
- 15) Ogawa, S. Phase behavior of *n*-alkyl glucosides in the arid system—recent developments and experimental notes. in *Glucosides, Sources, Applications, and New Research* (Boyd, I. ed.), Nova Science Publishers, Inc., pp. 63-98 (2017).
 - 16) Ogawa, S.; Kawai, R.; Koga, M.; Asakura, K.; Takahashi, I.; Osanai, S. Degree of maintenance of lactate dehydrogenase (LDH) activity during freeze/thaw process highly depends on hydrophobic chain length of synthetic mono-tailed glycolipid stabilizer. *Cryobiology and Cryotechnology* **61**, 89-94 (2015).
 - 17) Ogawa, S.; Kawai, R.; Koga, M.; Asakura, K.; Takahashi, I.; Osanai, S. Oligosaccharide-based surfactant/citric acid buffer system stabilizes lactate dehydrogenase during freeze-drying and storage without the addition of natural sugar. *J. Oleo Sci.* **65**, 525-532 (2016).
 - 18) Lozinsky, V.I.; Galaev, I.Y.; Plieva, F.M.; Savina, I.N.; Jungvid, H.; Mattiasson, B. Polymeric cryogels as promising materials of biotechnological interest. *Trends Biotechnol.* **21**, 445-451 (2003).
 - 19) Gutierrez, M.C.; Ferer, M.L.; Francisco del, M. Ice-templated materials: sophisticated structures exhibiting enhanced functionalities obtained after unidirectional freezing and ice-segregation-induced self-assembly. *Chem. Mater.* **20**, 634-648 (2008).
 - 20) Kirsebom, H.; Mattiasson, B. Cryostructuration as a tool for preparing highly porous polymer materials. *Polym. Chem.* **2**, 1059-1062 (2011).
 - 21) Lozinsky, V.I.; A brief history of polymeric cryogels. in *Polymeric cryogels: macroporous gels with remarkable properties* (Okay, O. ed.), Advances in Polymer Science book series (Polymer, vol. 263), Springer International Publishing AG, pp. 1-48 (2014).
 - 22) Berillo, D.; Mattiasson, B.; Galaev, I.Y.; Kirsebom, H. Formation of macroporous self-assembled hydrogels through cryogelation of Fmoc-Phe-Phe. *J. Colloid Interface Sci.* **368**, 226-230 (2012).
 - 23) Kiwada, H.; Niimura, H. Kato, Y. Tissue distribution and pharmacokinetic evaluation of the targeting efficiency of synthetic alkyl glycoside vesicles. *Chem. Pharm. Bull.* **33**, 2475-2482 (1985).
 - 24) Matsumura, S.; Imai, K.; Yoshikawa, S.; Kawada, K.; Uchibori, T. Surface activities, biodegradability and antimicrobial properties of *n*-alkyl glucosides, mannosides and galactosides. *J. Am. Oil Chem. Soc.* **67**, 996-1001 (1990).
 - 25) Sakya, P.; Seddon, J.M. Thermotropic and lyotropic phase behaviour of monoalkyl glycoaideds. *Liq. Cryst.* **23**, 409-424 (1997).
 - 26) Hashim, R.; Mirzadeh, S.M.; Heidelberg, T. Minamikawa, H.; Yoshiaki, T.; Sugimura, A. A reevaluation of the epimeric and anomeric relationship of glucosides and galactosides in thermotropic liquid crystal self-assemblies. *Carbohydr. Res.* **346**, 2948-2956 (2011).
 - 27) Chen, G.; Li, Z.; Chen, L.; Ji, S.; Shen, W. Synthesis and properties of alkyl β -D-galactopyranoside. *J. Surfact. Deterg.* **19**, 1095-1105 (2016).
 - 28) Chen, G.; Li, Z.; Chen, L.; Ji, S.; Shen, W. Synthesis and properties of alkyl α -D-galactopyranoside. *J. Disper. Sci. Tech.* **38**, 506-514 (2017).
 - 29) Hayward, A.S.; Eissa, A.M.; Maltman, D.J.; Sano, N.; Przyborski, S.A.; Cameron, N.R. Galactose-functionalized polyHIPE scaffolds for use in routine three dimensional culture of mammalian hepatocytes. *Bio-macromolecules* **14**, 4271-4277 (2013).
 - 30) Hronoská, H.; Mastihuba, V.; Tokošová, S.; Rosenberg, M. Semicontinual synthesis of alkyl galactosides using β -galactosidase entrapped in polyvinylalcohol hydrogel. *Biocatal. Biotransform.* **34**, 219-225 (2016).
 - 31) Vera, C.; Guerrero, C.; Wilson, L.; Illanes, A. Synthesis of butyl- β -D-galactoside with commercial β -galactosides. *Food Bioprod. Process* **103**, 66-75 (2017).
 - 32) Yang, J.; Pérez, B.; Anankanbil, S.; Li, J.; Gao, R.; Guo, Z. Enhanced synthesis of alkyl galactopyranoside by *Thermotoga naphthophila* β -galactosidase catalyzed transglycosylation: kinetic insight of a functionalized ionic liquid-mediated system. *ACS Sustainable Chem. Eng.* **5**, 2006-2014 (2017).
 - 33) Ogawa, S.; Koga, M.; Asakura, K.; Takahashi, I.; Osanai, S. Coagel prepared from aqueous octyl β -D-galactoside solution. *J. Surfact. Deterg.* **20**, 255-261 (2017).
 - 34) Ariei, T.; Kishi, A. A new simultaneous apparatus for X-ray diffractometry and differential scanning calorimetry (XRD-DSC). *Thermochim. Acta* **325**, 151-156 (1999).
 - 35) Thanatukorn, P.; Kajiwar, K.; Murase, N.; Franks, F. Freeze-thaw behavior of aqueous glucose solutions—the crystallization of cubic ice. *Phys. Chem. Chem. Phys.* **10**, 5452-5458 (2008).
 - 36) Ogawa, S.; Ozaki, Y.; Takahashi, I. Structural insights into solid-to-solid phase transition and modulated crystal formation in octyl- β -D-galactoside crystals. *ChemPhysChem* **17**, 2808-2812 (2016).
 - 37) Lozinsky, V.I.; Domotenko, L.V.; Zubov, A.L.; Simenel, I.A. Study of cryostructuration of polymer systems. XII. Poly(vinyl alcohol) cryogels: influence of low-molecular electrolytes. *J. Appl. Polym. Sci.* **61**, 1991-1998 (1996).
 - 38) Pačachia, S.; Florea, C.; Friedrich, C.; Thomann, Y. Tailoring of poly(vinyl alcohol) cryogels properties by salts addition. *Express Polym. Lett.* **3**, 320-331 (2009).
 - 39) Kajiwar, K.; Franks, F. Solubilities in the ternary system alkali metal halide–glucose–water. *J. Solution*

- Chem.* **33**, 157-167 (2004).
- 40) Yanaihara, Y.; Kobayashi, H.; Abe, S.; Kawai, K.; Kajiwara, K. Effects of potassium ions on the viscosities in the potassium chloride-glucose-water ternary system. *Cryobiology and Cryotechnology*, **63**, 29-32 (2017).
- 41) Klewe, B.; Pedersen, B. The crystal structures of sodium chloride dihydrate. *Acta Cryst.* **B30**, 2363-2371 (1974).
- 42) Barnes, W.H. The crystal structure of ice between 0°C and -183°C . *Proc. R. Soc. Lond. A* **125**, 670-693 (1929).
- 43) Pinard, M.A.; Kurian, J.J.; Aggarwal, M.; Agbandje-McKenna, M.; McKenna, R. Cryoannealing-induced space-group transition of crystals of the carbonic anhydrase psCA3. *Acta Crystallogr. F Struct. Biol. Commun.* **72**, 573-577 (2016).
- 44) Patterson, A.L. The Scherrer formula for X-ray particle size determination. *Phys. Rev.* **56**, 978-982 (1939).
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