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Freeze Drying of Pharmaceuticals.[1] Eutectic Temperature and Collapse Temperature of Solute Matrix upon Freeze Drying of Three-Component Systems

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For the model solution of the parenteral formulation, four ternary solutions were used: NaCl–KCl–H_2O with 10% of total solutes, NaCl–mannitol–H_2O, NaCl–glycine–H_2O and mannitol–sorbitol–H_2O with 5%.

Measurements were carried out on the electrical resistance and the temperature of the sample solution during cooling and rewarming, and the change of the macroscopic appearance of the frozen sample during freeze drying was observed.

The eutectic temperature T_e and the collapse temperature T_c of the solute matrix were derived from above measurements and observation.

The mechanism of the collapse during freeze drying was discussed in relation to the state of frozen sample and T_c was classified in three types: the first was related to T_e, the second to a glass transition temperature T_g and the third was the temperature below which the gross matrix of crystallized solute persisted even in the presence of a small amount of solution among crystallized solutes and ice crystals.

From the properties of supercooled aqueous solution, it was found that the solution which did not exhibit eutectic crystallization could be freeze-dried if the temperature of sample was controlled below T_c. It was also suggested that the difficulty of freeze drying of the solution with low T_c could be improved by addition of a suitable solute.

The eutectic and the supercooling phenomena of pharmaceutical solution afford useful informations to the production of qualified freeze dried injectables. The method suitable to freezing and the processing temperature suitable to drying are suggested by the low temperature behavior of solution. In this connection, the eutectic temperature T_e and the collapse temperature T_c of the solute matrix upon freeze drying of various binary solutions have been measured in the previous paper.[3]

Since suitable substances are usually added in the practical parenteral formulation to secure the stability and the effectiveness of a drug, the influenc of such additives on the freeze drying characteristics should be taken into consideration.

There are some instances that the solution can not be freeze-dried due to its low T_e or T_c, or a tendency to supercooling, and that the dried material becomes shrunk during or after dryring with the lapse of time. In these cases, it is necessary to improve the nature of the solution with addition of a third component.

The present work was attempted to examine the properties of simplified three-component systems of NaCl–KCl–H_2O, NaCl–mannitol–H_2O, NaCl–glycine–H_2O and mannitol–sorbitol–H_2O.

The study regarding binary systems[3] showed that aqueous solutions of NaCl, KCl, mannitol and glycine exhibited the eutectic behavior during freezing, but that sorbitol solution did not show any eutectic crystallization and produced the concentrated amorphous mixture filling the space between ice crystals. NaCl is used for the adjustment of tonicity of the par-

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[1] Preceding paper: K. Ito, *Chem. Pharm. Bull.* (Tokyo), **18**, 1519 (1970).

[2] Location: *Narihira 5-6-9, Sumida-ku, Tokyo.*

[3] K. Ito, *Chem. Pharm. Bull.* (Tokyo), **18**, 1509 (1970).

enteral solution. Mannitol[4] is added sometimes as a carrier or a diluent to the freeze dried pharmaceuticals.

The examination of mixed systems is important not only for the practical purpose but for the basic study of the boundary region between liquid and solid.

Experimental

Materials——NaCl, KCl, D-mannitol, D-sorbitol and glycine were of the guaranteed reagent grade. Test solutions were prepared with fresh distilled water.

Apparatus——The freeze drying machine, the electrical resistance indicator and the recorder for the resistance and the temperature were the sames as described in the previous paper.[3]

Procedure——Solutions containing 10% (w/v) of total solutes for NaCl–KCl–H_2O and 5% (w/v) for other systems were employed.

The measurements were carried out on the electrical resistance and the temperature of test solution during cooling and rewarming. The solutions were cooled to about −30° in one and a half hour, then being rewarmed gradually. When the sufficient crystallization was not obtained, cooling was applied for a longer period.

The macroscopic appearance of frozen sample during freeze drying was observed as follows. Each 2 ml of solution was filled in ampoules and small glass vessels and frozen on the shelf inside the freeze drying machine. The sample frozen to about −50° was then freeze–dried until the sublimation front was established 1 to 3 mm beneath the upper surface of the sample. On elevating the temperature of the sample gradually, the observation was made on the macroscopic appearance of the drying sample, especially of the region near the sublimation front. At a certain range of temperature the front became dimmed, and then the collapsing of the solute matrix was observed. Thus obtained temperature of the collapse upon freeze drying was reproducible.

Result and Discussion

NaCl-KCl-H_2O

Fig. 1 shows the change in electrical resistance of the frozen sample measured during rewarming. From a point of inflection of the curve, the eutectic temperature T_e was

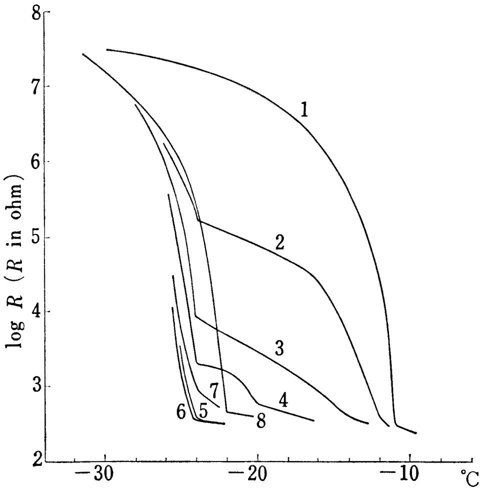

Fig. 1. Electrical Resistance of NaCl–KCl–H_2O during Rewarming

concentrations of NaCl and KCl, 1: 0%, 10%; 2: 0.1%, 9.9%, 3: 1%, 9%; 4: 2%, 8%; 5: 4%, 6%; 6: 6%, 4%; 7: 8%, 2%; 8: 10%, 0%

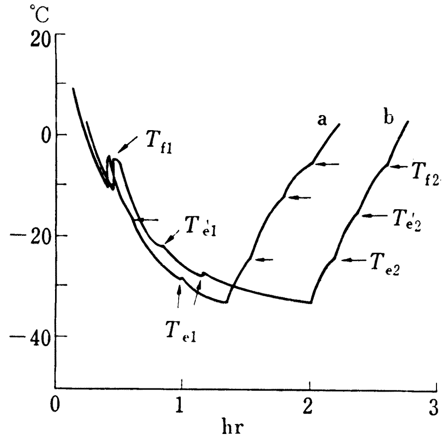

Fig. 2. Examples of Temperature–Time Curve of NaCl–KCl–H_2O during Cooling and Rewarming

concentrations of NaCl and KCl, a: 2%, 8%; b: 4%; 6%; T_f: freezing temperature, T_e: ternary eutectic temperature, T_e': temperature corresponding to the point J in Fig. 4

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[4] a) G. Pancrazio, M. Vitali and E. Marchese, *Farmaco* (Pavia), *Ed. Prat.*, **12**, 417 (1957) from *CA*, **52**, 10498 h (1958); b) P. Deluca and L. Lachman, *J. Pharm. Sci.*, **54**, 617 (1965); c) A.P. MacKenzie, *Bull. Parent. Drug Assoc.*, **20**, 101 (1966).

obtained, as described in the previous paper³⁾; those of NaCl and KCl were —22° and —11° respectively and that of the ternary mixtures was —24°.

Fig. 2 depicts the examples of the temperature change of sample. The points of inflection of the temperature curve during rewarming also gave the temperature which corresponded to the phase transition.

The temperatures, at which the dimming and the collapsing at the sublimation front of sample upon freeze drying occurred, were also obtained. These critical temperatures are plotted against the composition in Fig. 3.

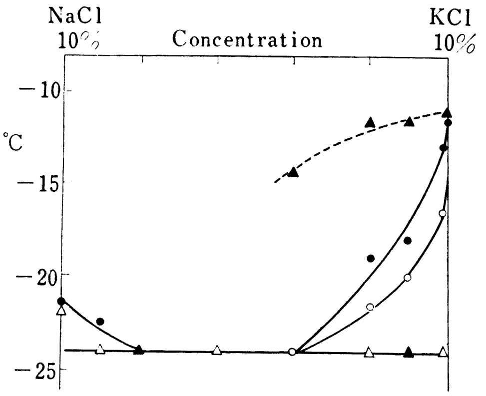

Fig. 3. Eutectic Temperature and Collapse Temperature of NaCl–KCl–H_2O (Total Solute: 10%)

○: temperature at which the sublimation front became dimmed
●: temperature at which the collapse was observed
△: temperature at which the ellectrical resistance inflected during rewarming
▲: temperature at inflecttion points of temperature-time curve during rewarming
········ : corresponds to curve k_3K in Fig. 4

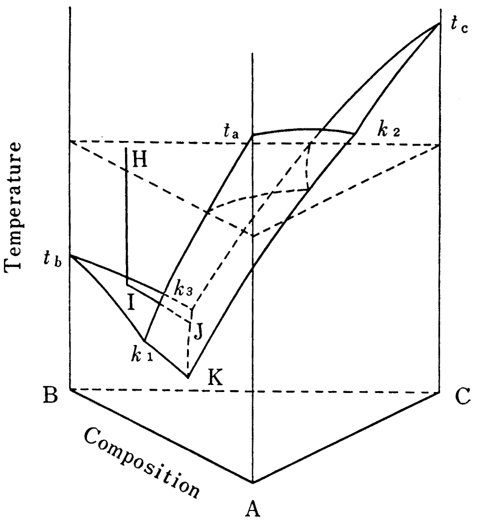

Fig. 4. Phase Diagram of a Ternary System A-B-C consisting of Solid and Liquid Phases only

k_i: binary eutectic points, K: ternary eutectic point, t_a, t_b, t_c: melting points of A, B and C

For the study of the ternary solid–liquid equilibria, a triangular prism is usually employed for the graphic representation of system. Fig. 4 is a model phase diagram where the solid phases are pure components, k_1 and K are the binary and the ternary eutectic points respectively, and t_a, t_b and t_c the melting points of pure components. The curve k_1K slopes inwards and downwards, and indicates the varying composition of the ternary liquid phase with which a mixture of solid A and B are in equilibrium. At the point K, the three solid components are in equilibrium with the liquid phase, and the point represents the lowest temperature attainable with these components. If the solution at the point H is cooled and the phase change occurs in equilibrium, the first solid B appears at the point I which is on the curved surface $t_bk_1Kk_3$, and then the composition of the solution moves along the curve IJ. On further cooling solid C also begins to separate at the point J and the composition of the remaining solution moves along the curve JK. Eventually the temperature of K is reached at which the solution solidified completely to give the ternary eutectic mixture of A, B and C. The phase diagram of NaCl–KCl–H_2O is expressed by a similar phase diagram to Fig. 4. The invarient points given by the literature⁵⁾ are —11.1° for KCl+ice, —21.85° for $NaCl \cdot 2H_2O$ +ice, —23.7° for $NaCl \cdot 2H_2O$+KCl+ice and —2.35° for $NaCl \cdot 2H_2O$+NaCl+KCl. The

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5) "International Critical Table," Vol. IV, McGraw-Hill Book Co., Inc., New York, N.Y., 1928, p. 314.

ternary eutectic point from Fig. 3 is −24° agreeing well with the above value in the literature.

The dashed line in Fig. 3, which was given from the rewarming curve of the temperature of sample, corresponds to the curve k_3K in Fig. 4.

The liquid phase appeared in a frozen sample during freeze drying would cause the solute matrix to collapse. But the macroscopic collapse was not always observed at the ternary T_e. It depends upon the quantities fo the solute solid and the solution filling in the space among ice crystals. If the volume of the solution in a frozen sample is very small, a gross feature of the solute matrix remains though a very short-range or microscopic collapse might occur during drying. On further elevating the temperature of this sample, however, the volume of the solution increases and the collapsing becomes apparently observed. This seems to be the reason why T_c of NaCl-KCl-H_2O became higher than the ternary T_e with the decrease in the concentration of NaCl as shown in Fig. 3.

NaCl-Mannitol-H_2O and NaCl-Glycine-H_2O

The electrical resistance during rewarming of NaCl–mannitol–H_2O is shown in Fig. 5. The pretreatment of the solution was cooling to about −30° in one and a half hour. The binary solutions of mannitol and NaCl were eutectically crystallized easily during cooling and produced sharp drops in the electrical resistance during rewarming as shown by the curves No. 1 and 8 of Fig. 5. But the cooling condition above mentioned was insufficient for the ternary mixtures to be crystallized well. The electrical resistance curve, No. 5 of Fig. 5, especially, did not show any sharp change which seemed to correspond to the eutectic crystallization. In order to obtain the ternary T_e from the curves, the test solutions were subjected to further cooling before rewarming. The examples are illustrated in Fig. 6, where the curve No. 2, for example, means that the solution was frozen to −32° in 2 hours and then held at −32° for 5 hours. The characteristic temperature was given as −23° from the point of inflection of the curves.

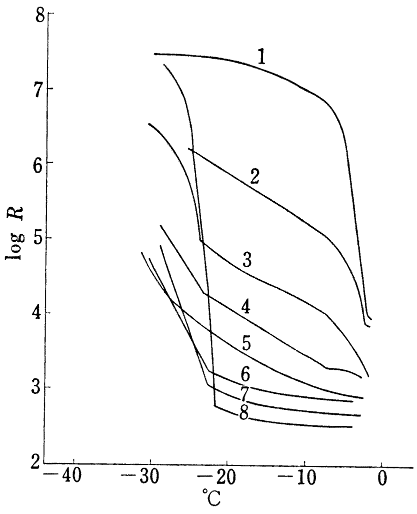

Fig. 5. Electrical Resistance of NaCl–Mannitol–H_2O during Rewarming

concentrations of NaCl and mannitol, 1: 0%, 5%; 2: 0.1%, 4.9%; 3: 0.5%, 4.5%; 4: 1%, 4%; 5: 2%, 3%; 6: 3%; 2%; 7: 4%, 1%; 8: 5%, 0%

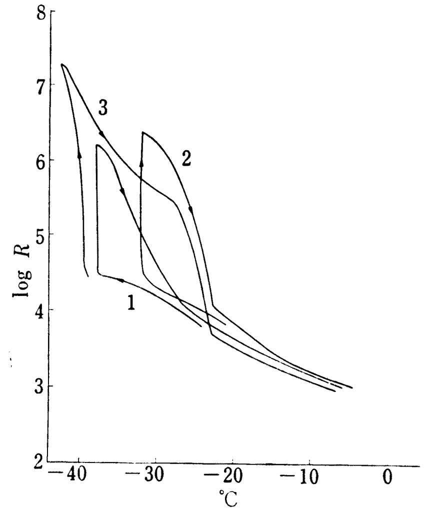

Fig. 6. Effect of Cooling on Electrical Resistance of Solution consisting of 2% NaCl and 3% Mannitol

1: from room temp. to −38° in 30 min, 2: to −32° in 2 hr and −32° for 5 hrs, 3: to −43° in 30 min and −43° for 1.5 hr

In case of NaCl-glycine-H_2O, the solutions also suffered supercooling and the same kind of resistance curves as Fig. 5 was obtained. Rewarming of the sufficiently frozen solution, however, gave the remarkable drop in the resistance which is shown in Fig. 7.

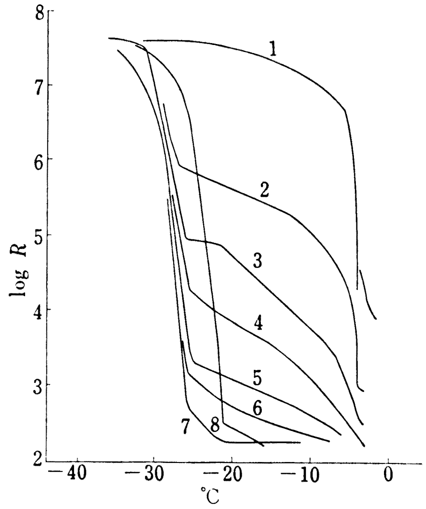

Fig. 7. Electrical Resistance during Rewarming of Sufficiently Frozen NaCl-Glycine-H_2O

concentrations of NaCl and glycine, 1: 0%, 5%; 2: 0.1%, 4.9%; 3: 0.5%, 4.5%; 4: 1%, 4%; 5: 2%, 3%, 6: 3%; 2%; 7: 4%, 1%; 8: 5%, 0%

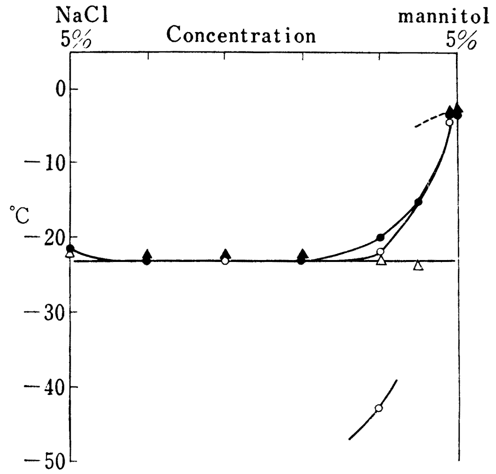

Fig. 8. Eutectic Temperature and Collapse Temperature of NaCl-Mannitol-H_2O (Total Solute: 5%)

symbols as in Fig. 3

Solutions (2% NaCl, 3% mannitol) and (3%, 2%) had other collapse temps. below −50°, but their precise temps. could not be ascertained.

The macroscopic appearance of the sample during freeze drying was also observed to obtain the collapse temperature. These data are plotted against the composition in Fig. 8 for NaCl-mannitol-H_2O and Fig. 9 for NaCl-glycine-H_2O.

When the solution composed of 1% NaCl and 4% mannitol was freeze-dried, the first collapsing during rewarming occurred at −42° to −43°, which was far below the ternary T_e. This seemed due to the supercooling of the solution. On further drying, the collapsing proceeded while the eutectic crystallization progressed with the elevation of temperature and with the lapse of time. Consequently the normal drying took place again after some period. The second collapsing was observed at −20°, just above the ternary T_e. The freeze drying of solutions of 2% NaCl and 3% mannitol, and 3% NaCl and 2% mannitol gave two T_c respectively, one was below −50° and the other was −23° or T_e. The precise value of the former T_c could not be ascertained because the temperature below −50° was beyond the capacity of the machine.

The samples of NaCl-glycine-H_2O also seemed to have T_c below −50°.

Mannitol-Sorbitol-H_2O

The shape of the electrical resistance curve during rewarming changed little with way of cooling. The resistance, as shown in Fig. 10, decreased gradually with the elevation of temperature, and a sudden large increase occurred at about −2°. Same phenomenon had been observed in the previous work[3] on such binary systems, as mannitol, glucose and lactose solutions. Deluca, *et al.*[6] also noticed this phenomenon on some electrolyte solutions

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[6] P. Deluca and L. Lachman, *J. Pharm. Sci.*, **54**, 1411 (1965).

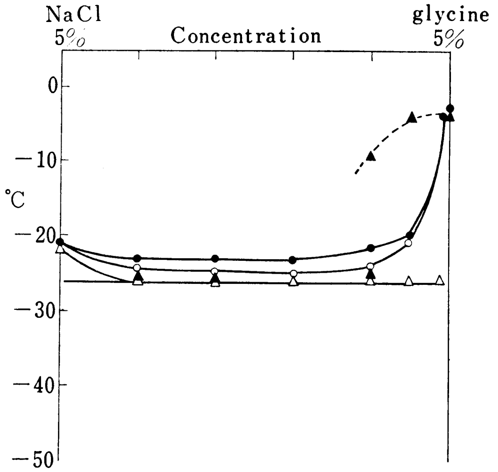

Fig. 9. Eutectic Temperature and Collapse Temperature of NaCl-Glycine-H_2O (Total Solute: 5%)

symbols as in Fig. 3
Solutions (1% NaCl, 4% glycine), (2%, 3%) and (3%, 2%) had other collapse temps. below −48°, but their precise temps. could not be ascertained.

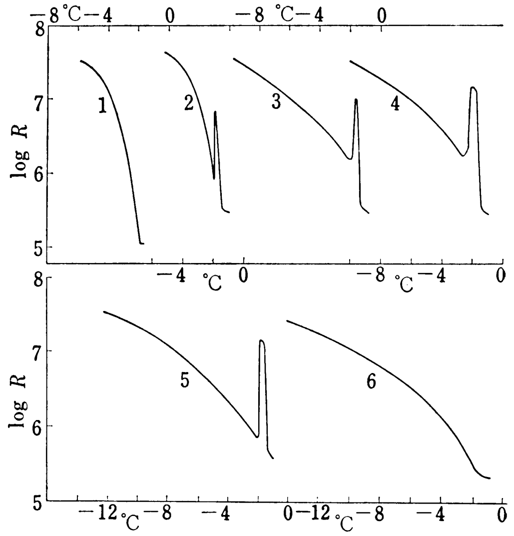

Fig. 10. Electrical Resistance of Mannitol-Sorbitol-H_2O during Rewarming

concentrations of mannitol and sorbitol, 1: 5%, 0%; 2: 4%, 1%; 3: 3%, 2%; 4: 2%, 3%; 5: 1%, 4%; 6: 0%, 5%

between T_e and the freezing point. This phenomenon has not been analyzed yet and further discussion must wait more examinations.

The resistance curve of the ternary mixture, except a sudden increase at about −2°, have no such sharp change as those of NaCl-mannitol-H_2O and NaCl-glycine-H_2O. Any indication of the eutectic transition also was not found in the curve of the temperature change of the sample during rewarming.

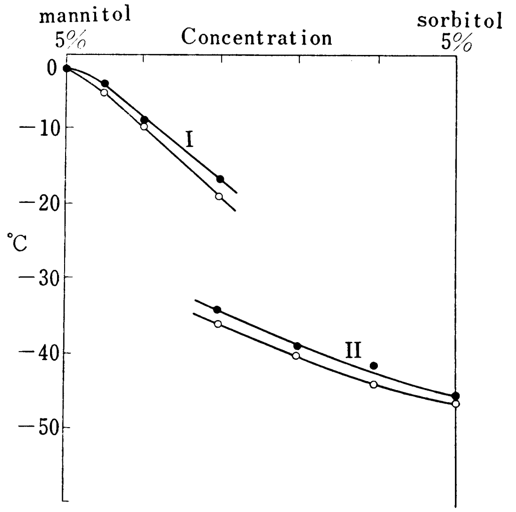

Fig. 11. Collapse Temperature of Mannitol-Sorbitol-H_2O (Total Solute: 5%)

symbols as in Fig. 3

The collapsing of the frozen sample upon freeze drying observed and the data are illustrated in Fig. 11.

When the test solution of 3% mannitol and 2% sorbitol was cooled to about −55° in one hour and then freeze-dried, the first collapsing occurred at about −34°. And on further drying, the collapsing stopped at about −30° and the normal drying began again. The second collapsing was observed at about −17°. On the solutions with other compositions, however, only one T_c was observed for each solution. The data of T_c, plotted in Fig. 11, are expressed in two curves, the upper curve I and the lower II.

Since the sorbitol solution is supercooled, the ternary T_e for mannitol-sorbitol-H_2O was not derived. When the concentration of sorbitol was larger than 3% in this ternary system, mannitol also became supercooled and did not exhibit the crystallization. T_c of the lower curve II, therefore, indicates the collapse of the supercooled mixtures between ice crystals, where both solutes remain in the amorphous state.

When the concentration of sorbitol was smaller than 3%, however, mannitol could be crystallized. T_c of the upper curve is, therefore, due to the persistence of the gross feature of the mannitol matrix.

General Discussion of Collapse Temperature T_c

The liquid appeared in a frozen sample causes the solute matrix collapse during freeze drying. But the mechanism of the collapsing differs according to whether the liquid is originated from the melting of the eutectic crystals or the softening of supercooled amorphous mixtures.

In Relation to Eutectic Temperature T_e——From a equilibrium phase diagram of a three-component system, two solutes and water, a ternary eutectic temperature T_e is given as the invarient temperature where three solid components can exist with the solution. The collapse of the eutectically crystallized sample should be observed at T_e of the sample. Experimental data of T_c of most test solutions were found at or near T_e of the sample. But T_c became higher as the concentration of one solute increases, which is illustrated in Fig. 3, 8 and 9. The reason of this phenomenon has been discussed already. This fact have a practically important meaning for freeze drying, in other words, it is possible to raise the apparent T_c of the solution by adding a suitable amount of the substance which has higher T_e such as mannitol and glycine.

In Relation to Glass Transition Temperature T_g——In the previous paper[3] the author had found that the binary solution had T_c characteristic of the solute even when the solution did not exhibit the eutectic crystallization. The collapsing of the supercooled ternary solution was also found in this work. Experimental values of these temperatures were reproducible and was in a very narrow range of the temperature, in 2° at most. The equilibrium phase diagram can not give any explanation for the existence of T_c of the supercooled sample.

The freezing leaves the concentrated supercooled solution between ice crystals, which becomes more viscous as the temperature falls and finally loses its fluidity. If the existence of T_c is attributed to fluidity of the supercooled solution, a very large increase in viscosity must occur at a narrow range of the temperature where supercooled liquid becomes solid. This transition is quite different from such phase transition as the melting or the freezing. The mechanical and thermodynamic properties change discontinuously at the melting point, but at T_c of the supercooled sample the properties seem to change continuously though inflectively. These features of T_c correspond well to those of a glass transition temperature T_g.

Many works have been made on glass transition phenomena of the macromolecular substance. The existence of T_g of the smaller molecule was demonstrated recently by Seki, *et al.*[7] Seki stated in his review[8] that the glassy state must be distinguished from the supercooled liquid at T_g. Specific heat, volume and other properties are not a single linear function of temperature but have a point of inflection at T_g.

Since the glass transition is not a equilibrium phase change, T_g may depend on the time scale of the determination. But every experimental value of T_g is said to agree well with each other, and it has been proved theoretically that the effect of the time scale of the usual determination of T_g is small.

No literature is found on the glass transition of the supercooled sample of aqueous solution, but it is reasonable to suppose the existence of T_g and the close relation between T_c and T_g of the aqueous supercooled sample.

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7) *a*) M. Sugisaki, K. Adachi, H. Suga and S. Seki, *Bull. Chem. Soc. Japan*, **41**, 593 (1968); *b*) K. Adachi, H. Suga and S. Seki, *ibid.*, **41**, 1073 (1968); *c*) N. Onodera, H. Suga, and S. Seki, *ibid.*, **41**, 2222 (1968); *d*) M. Sugisaki, H. Suga and S. Seki, *ibid.*, **41**, 2586 (1968); *e*) M. Sugisaki, H. Suga and S. Seki, *ibid.*, **41**, 2591 (1968); *f*) K. Adachi, H. Suga and S. Seki, *ibid.*, **43**, 1916 (1970).

8) S. Seki, *Kagaku To Kogyo* (Tokyo), **23**, 498 (1970).

It is also supposed that T_g depends on the concentration. But T_c of the supercooled binary aqueous sample was constant over a wide range of the concentration. Sucrose solution[3] of 5 to 50%, for example, had T_c at —25°. This fact seems to indicate that the concentration of the supercooled amorphous mixture between ice crystals below T_c is almost constant irrespective of the initial concentration of the solution. In the early stage of cooling in usual freeze drying, the binary solution is frozen along the freezing point lowering curve. Since the remaining solution between ice crystals becomes more concentrated and viscous with the lowering of the temperature, successive crystallization of ice may be suppressed, and the temperature and the concentration of solution may deviate largely from the equilibrium curve. The viscous solution between ice crystals is converted into supercooled amorphous solid at T_g, which should have almost constant concentration according to the above finding. If the amorphous solid with any arbitrary concentration could be prepared, it would be possible to change T_c with the concentration on the binary solution.

The ternary solutions in this work had the concentration-dependent T_c. When the highly viscous supercooled mixture between ice crystals is formed without any separation of the solute solid, the reason of the concentration-dependent T_c of the ternary solution can be understood by the explanation of the deviation from the curve IJ in Fig. 4 which is depend on the initial concentration of the sample. The lower curve II in Fig. 11 for mannitol–sorbitol–H_2O is the example of this kind of T_c.

In Relation to Temperature having a Different Meaning from both Eutectic and Glass Transition Temperatures——When one solute can be crystallized but the other can not, this is the case of mannitol–sorbitol–H_2O whose T_c is expressed by the upper curve I in Fig. 11, T_c is also changeable with the initial composition. The solution formed among ice crystals and the separated solid probably causes the microscopic short-range collapse of solute matrix during freeze drying at and above T_c of the supercooled solution. But the apparent collapse temperature T_c obtained from the macroscopic appearance change is far higher than the estimated T_c due to the persistence of the crystallized solute matrix. Since the volume ratio of the solution and the separated solute solid in a frozen sample is a major factor for the apparent T_c, the observed temperature becomes concentration-dependent.

It is understood from this result that the solution, which does not exhibit the eutectic crystallization and has very low T_c, can be freeze-dried with less difficulty at higher temperature by addition of sufficient quantities fo suitable solute such as mannitol and glycine.

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