[Chem. Pharm. Bull. 18(8)1509—1518(1970)] UDC 615.011.4 : 615.014.413

Freeze Drying of Pharmaceuticals. On the Change in the Macroscopic Appearance during Freezing and the Critical Temperature necessary for Freeze Drying[1]

Koji Ito

Pharmaceutical Research Laboratory, Research Laboratories, Daiichi Seiyaku Co., Ltd.[2]

(Received November 10, 1969)

The low temperature behavior of solutions of simple substances and pharmaceuticals was studied. The authors found noticeable changes in the macroscopic appearance of solutions during freezing of some substances which exhibited the eutectic behavior.

Appearance changes were classified into four types: I) no change, II) slight increase in opacity, III) very noticeable formation of clear white spots due to eutectic crystallization with very slow rate of growth of spots, and IV) formation of spots with high rate of growth.

The eutectic temperatures (T_e) were obtained from the temperature at which spots faded or disappeared.

The collapsing temperatures (T_c) were also obtained from the observation during freeze drying, of the temperatures above which the frozen layer of the solution became puffed or collapsed.

The electrical resistances of the specimens were measured during cooling and rewarming.

These data were compared with each other to discuss the meaning of the changes in appearance during freezing and drying as follows. The formation of spots was due to crowds of eutectic crystal structures. T_c was observed at T_e for the substances which exhibited the eutectic behavior, and T_c for the substances which froze solid but in super-cooled or super-eutectic states was supposed to be the temperature above which the frozen solution lost solidity.

The freeze drying technique is one of the most useful ways to dry very unstable substances and much attention has been focussed on the freeze dried injectable pharmaceuticals in recent years.

Freeze drying techniques are now used to dry many kinds of materials, especially biological substances. Foods, in particular, are processed on a large scale. It seems that the approach has been almost always empirical and that conditions which yielded good dried products have been found mostly by trial and error.

To achieve a successful drying, the solution is frozen such that no liquid phase remains in the specimen; the temperature of the specimen must, moreover, be low enough during drying to prevent any melting but high enough to permit drying in a short time.

Much work has been done on the effects of freezing on biological systems, and the structure of frozen solutions has been studied using such techniques as differential thermal analysis,[3] electrical resistivity[3a,4] and microscopy.[5] But further studies are needed before a full discussion of the low temperature behavior of solutions can be given.

1) A part of the work was presented at the 89th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1969.

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

3) *a*) L.R. Rey, "Recent Research in Freezing and Drying," ed. by A.S. Parkes and A.U. Smith, Blackwell, Oxford, 1960, p. 40; *b*) L.R. Rey and M-C. Bastien, "Freeze-drying of Foods," Nat. Acad. Sci., Nat. Res. Council, 1962, p. 25.

4) *a*) O.V.St. Whitelock (ed.), "Freezing and Llrying of Biological Materials," *Ann. N. Y. Acad. Sci.*, **85**, 501–734 (1960); *b*) P.P Deluca and L. Lachman, *J. Pharm. Sci.*, **54**, 617 (1965); *c*) L. Lachman, P.P. Deluca and R. Withneth, *ibid.*, **54**, 1342 (1965); *d*) P. Deluca, L. Lachman and W. Yost, *ibid.*, **54**, 1348 (1965); *e*) P. Deluca and L. Lachman, *ibid.*, **54**, 1411 (1965).

5) *a*) B. Luyet, "Recent Research in Freezing and Drying," ed. by A.S. Parkes and A.U. Smith, Blackwell, Oxford, 1960, p. 3; *b*) G. Rapatz and B. Luyet, *Biodynamica*, **7**, 346 (1957); *c*) A.P. MacKenzie, *ibid.*, **9**, 214 (1964); *d*) T. Nei, *Low Temp. Sci., Ser.* **B**, **23**, 149, 157, 163 (1965).

Most studies of freezing and freeze drying have been made with special reference to biological substances and foods; very little information has been given on the same process with special reference to pharmaceuticals.

The author found that a solution showed a distinct change of macroscopic appearance through the eutectic phase formation during freezing, giving much information on such matters as the eutectic temperature and the degree of super-eutectic behavior which might be useful to controlling the freeze drying process.

Although microscopic observations of ice formation during freezing and thawing processes have been reported by several authors,[5] few reports have been published on the change in macroscopic appearance of a frozen system.

In the present work changes in macroscopic appearance were observed in solutions of pharmaceuticals during freezing and freeze drying. Solutions were classified into four groups on the basis of change in appearance, while the super-eutectic behavior during freezing and rewarming, and the eutectic temperatures were obtained. The collapsing temperatures of the frozen matrices were also obtained during freeze drying. These data were compared with the results obtained from the electrical resistivity measurements.

Experimental

Materials

All the materials used were of reagent grade, J.P. or U.S.P. grade and were listed in Table II. Test solutions were prepared with distilled water.

Apparatus

The freeze drying machine used[6] was of the maximum drying capacity of 1.5 liters water per one cycle. The drying chamber was 400 mm in diameter and 418 mm in height and had two shelves. The shelves were both cooled and heated, as required. The lowest temperature attainable on the shelf was $-55°$; that of the cold trap was $-60°$. The chamber was evacuated to pressures of the order of 10^{-3} mmHg by the vacuum pump with a capacity for removing 200 liters per minute. A Geissler's tube and a Pirani gauge were used to indicate the degree of vacuum. The temperatures of shelves, cold trap and specimen and the degree of vacuum were recorded by a six pen recorder[7] with ranges of $-70°$ to $+70°$ and 0 to 500 μHg.

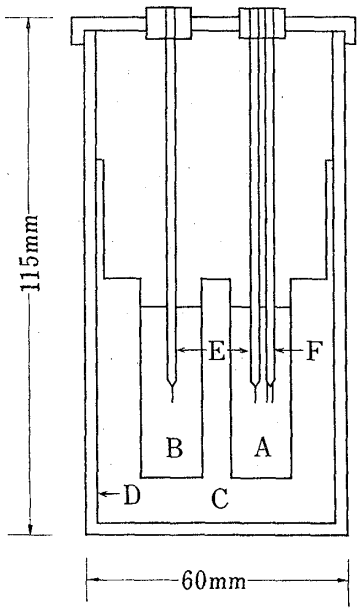


Fig. 1. Stainless-steel Cell and Plastics Buffer for Measuring Electrical Resistance

A: sample solution
B: standard solution (H_2O)
C: cell
D: plastic thermic buffer
E: thermocouple
F: electrode

The macroscopic changes in appearance were observed in a plastic cell of $1 \times 95 \times 70$ mm in size, a test tube and an ampoule in a cold bath attached to the freeze drying machine.

The electrical resistance of the specimen was measured by a Resistance Indicator[8] capable of measuring to 30 M ohms. Fig. 1 illustrates a cell and a thermic buffer. The cell was cooled and rewarmed in the cold bath in a range of room temperature to $-60°$, both the resistance and the temperature being recorded.

All the temperature measurements obtained were corrected by reference to a standard thermometer.

Procedures——a) Observation of the Macroscopic Change of Appearance during Freezing: The sample was cooled and rewarmed in the cold bath or on the shelf of the freeze drying machine. Observation was carried out through the process. When a remarkable change due to the formation of the eutectic structure was observed, the cooling was stopped and the sample was allowed to rewarm gradually. The eutectic temperature was obtained from the sample's behavior during the rewarming process.

6) Type R_2CL-15MB, Kyowa Vac. Engineering Co., Ltd., Tokyo.
7) Type ER6-90ZML 6-23/123, Yokokawa Electric Work Inc., Tokyo.
8) Model ER-1, Kyowa Vac. Engineering Co., Ltd., Tokyo.

b) Observation of the Change of Appearance during Freeze Drying: The solution was filled in an ampoule or a small glass vessel and frozen on the shelf inside the machine. An electric torch was used to light the sample through a window pane of the chamber to make the observation easier during the drying process. The sample, sufficiently frozen, was dried for about one hour until a sublimation front was established 1 to 3 mm beneath the free surface. A relatively distinct difference of appearance was observed between a dried porous mass and a frozen layer. Then the sample was heated more than needed for a proper drying by controlling the temperature of the shelf. On elevating the temperature there was a temperature at which the frozen layer was collapsed. The collapsing temperature was measured by a thermocouple inserted in the layer.

c) Electrical Resistence Measurement: About 6 ml of a solution was filled into the cell, and the thermocouple and electrode assembly was inserted. The cell was cooled enough in a cold bath and then allowed to be warmed gradually. The cell covered by the plastic thermic buffer was cooled at the rate of about 15° per 30 min, while the warming rate, under the same buffer, was about 30° per 30 min. The rates without the buffer were about 60° and 45° per 30 min for cooling and warming, respectively. The resistance and the temperature were recorded throughout the cycle. From the recorded chart given by the multi-pen recorder, the resistance was plotted against the temperature and the shape of the curve was examined. A graph of the temperature change with the lapse of time was also obtained from the same recorded chart.

Result and Discussion

The Change in Macroscopic Appearance during Freezing

On cooling solutions, ice crystals appeared at first to spread and grow throughout the cell. On further cooling after the apparently opaque ice matrix was formed, the macroscopic changes in appearance were observed as follows: I) No change; II) Slight increase in opacity but not a sharp change; III) The formation of very noticeable clear white spots which were dark opaque in transmitted light and distinguished clearly from the light opaque layer around them (the rate of growth of spots was very low and, during rewarming, spots vanished in a very narrow range of temperature); IV) Formation of the spot just as type III, but with very high growth rate. These are summarized in Table I.

Table I. Types of Change in Macroscopic Appearance during Cooling and Rewarming

Type	When cooling	When rewarming
I	no change	no change
II	slight increase in opacity	slight decrease in opacity
III	appearance of white spots with slow rate of growth	disappearance of spots at a certain temperature
IV	appearance of spots with high rate of growth	disappearance of spots at a certain temperature

Fig. 2 is the schematic expression of the growth of spots. Fig. 3 shows examples of types III and IV.

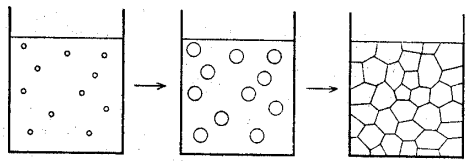
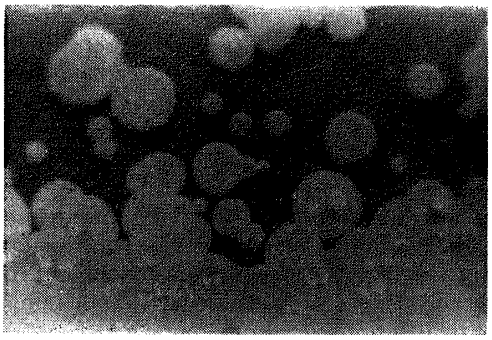


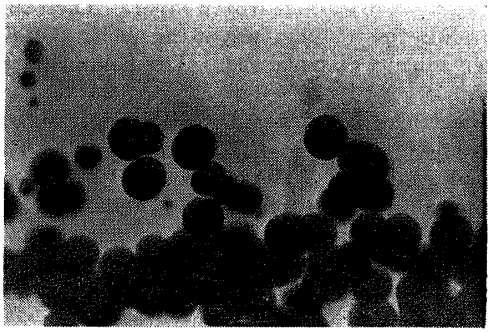
Fig. 2. Growth of Spots observed in a Thin Cell

The frozen samples of NaCl, KCl and acetic acid solutions showed many densely opaque spots, which grew to cover the whole samples in several minutes in the thin cell. But in the case of GABA the growth of such spots was observed only to a small extent even when the sample was held at a temperature between −20° and −40° for half an hour. The growing speed of the spot was greatly influenced by the temperature of the cold bath.

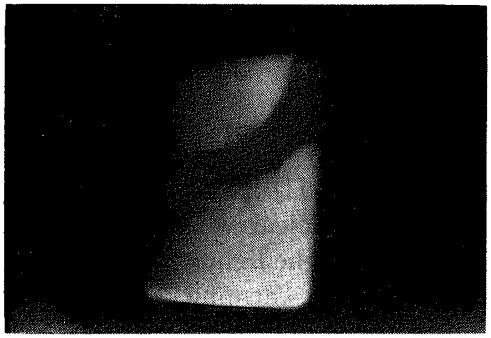
When the frozen sample was warmed, the spot faded or disappeared at a certain temperature which was characteristic of the solute. The temperatures are shown in Table II, which



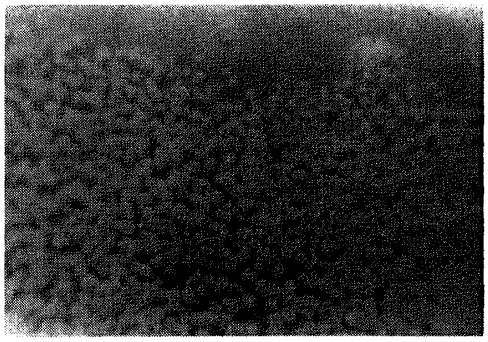
3-1



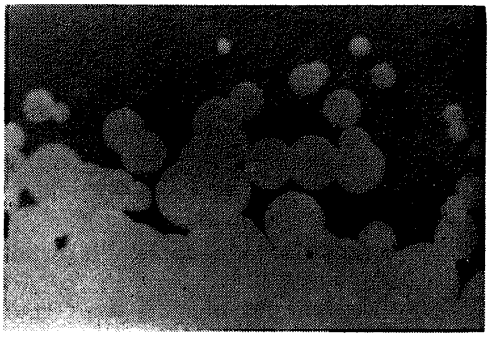
3-2



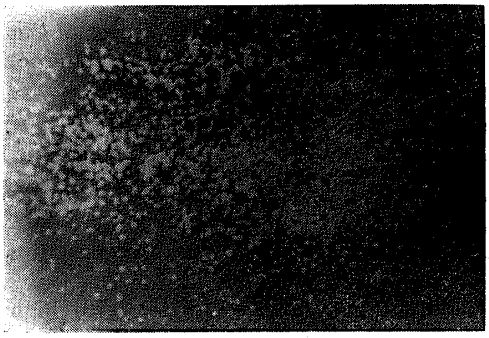
3-3



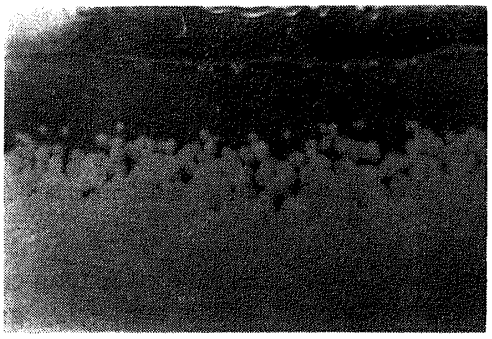
3-4



3-5



3-6



3-7

Fig. 3. Pictures of Frozen Samples which formed Spots

3-1: 10% NaCl solution in a thin plastic cell in a reflected light
3-2: same sample in a transmitted light
3-3: same sampe in a 5 ml ampoule
3-4: 10% KCl solution
3-5: 10% acetic acid solution
3-6: 10% α-alanine solution
3-7: 5% pyridoxine hydrochloride solution
magnif.: about × 1

were in good agreement with the eutectic or cryohydrate temperature (T_e) from the literature[9] in the cases of NaCl, KCl and NaOH.

Table II. Critical Temperatures

A	B	C	D (T_e)	E (T_c)	F
Sucrose 10%	−25°a	I		−25°	−13.9°d, −32°f, −32°g
5, 25, 50%		I		−25°	
Glucose 10%	−25°a	I		−38°——40°	−41°f, −40°g
Lactose 10%	−19°a	I		−18°——19°	−31°g
Mannitol 10%	−6°a	I		− 2°—— 4°	− 1.0°d
Sorbitol 10%	−21°a	I		−41°——42°	−57°g
Xylose 10%	−30°a	I		−43°——44°	
Dextran					
low mol. wt. 10%	−10°a	I		5%, −2°	−9°——10.5°g
high mol. wt. 10%	−24°——26°a	I		5%, −3°	
Gelatin 5%		II			−11°f, − 8°g
PVP m. wt.					
3×10^4 10%	−25°a	II			−24°f, −23°g
PEG 6000 10%	−15°a	I		− 9°——10°	−70°e, −65°f, −13°g
NaCl 1%	−21°——22°b	IV			
10%	−21°——22°bc	IV	−21°——22°	−21°——22°	−21.6°, −21.3°d
KCl 10%	−10°——11°bc	IV	−10°——11°	−10°——11°	−10.7°, −11.1°d
NaOH 10%	−28°b	III	−28°		−28°d
Acetic acid 10%	−26°——30°b	IV	−26°——27°	−26°——27°	−26.4°d
Citric acid 10%	<−46°a	I		<−50°	−12.2°d
Thiamine hydrochloride 10%	−50°a	I			
Thiamine mononitrate 1%	−15°a	I		−5°	
Pyridoxine hydrochloride 10%	− 3°—— 5°b	IV	− 3°—— 4°	−3°	
Ascorbic acid 5%	−38°a	I		−36°——37°	
10%	−44°a	I		−36°——37°	
Na Ascorbate 5, 10%	−42°a	I		−30°——33°	
Nicotinamide 10%	− 3°—— 8°b	IV	− 4°—— 5°	− 3°—— 4°	
Ca Pantothenate 10%	−30°a	I		−18°——19°	
IHMS 5%[a]	−36°a	II		−28°——30°	
Acetamide 10%	−25°——30°bc	IV	−25°	−25°	
Na Barbital 10%	− 4°—— 8°b	IV	− 4°	− 4°	
Glycine 10%	−16°a	I		− 3°	
α-Alanine 10%		IV	− 2°—— 3°	− 2°—— 3°	
β-Alanine 10%	−12°——13°bc	IV	−13°——14°	−13°	
Arginine 10%	−42°a	I		−33°——35°	
EACA 10%[b]	−14°——16°bc	III	−15°	−15°	
GABA 10%[c]	−16°——20°bc	IV	−18°——20°	−18°——20°	
Cl-GABA 10%[d]	− 1°—— 2°b	III	−2°		

A: substances
B: temperatures obtained from electrical resistance during rewarming, at max. resistance-a, at max. change-b, and from eutectic halt-c.
C: types of macroscopic appearance change during freezing
D: fading temperatures of spot during rewarming
E: collapsing temperatures during freeze drying
F: temperatures from literatures, eutectic temperature-d[9] and-e,[14] recrystallization temperature-f,[12] and collapsing temperature-g[13]
a) Na isonicotinyl hydrazide methanesulfonate
b) ε-amino caproic acid
c) γ-amino butylic acid
d) α-chloro-γ-amino butylic acid

9) "International Critical Table," Vol. I, II, IV, McGraw-Hill Book Co., Inc., New York, N. Y., 1926, 1927, 1928.

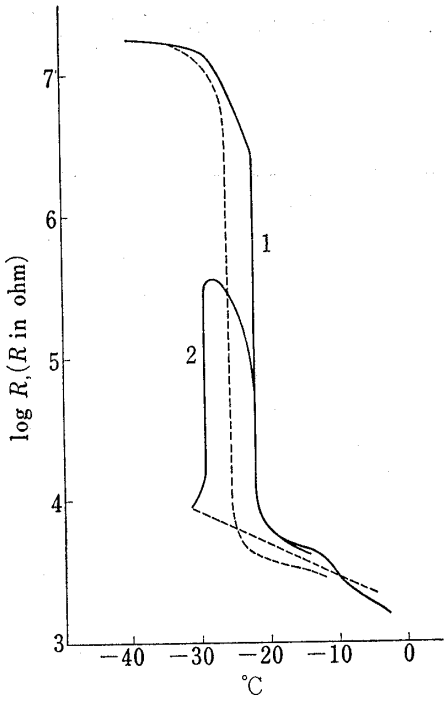


Fig. 4. Variation of Electrical Resistance of 1% NaCl Solution

——: rewarming, ------: cooling
1: with thermic buffer (slow processing),
2: without the buffer (fast processing)

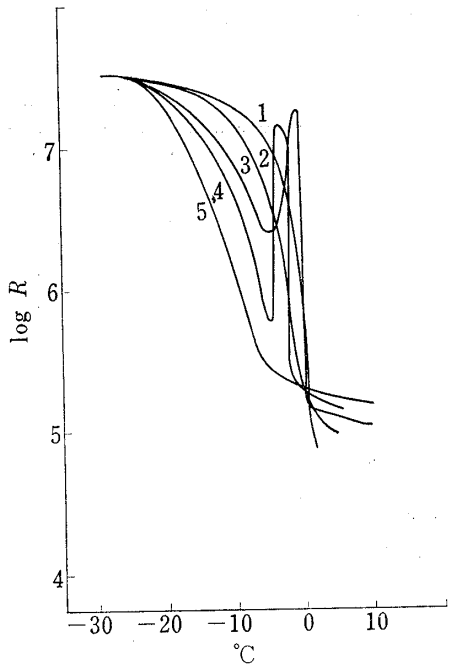


Fig. 5. Variation of Electrical Resistance of Sucrose Solution during Rewarming

1:1%, 2:5%, 3:10%, 4:25%, 5:50%

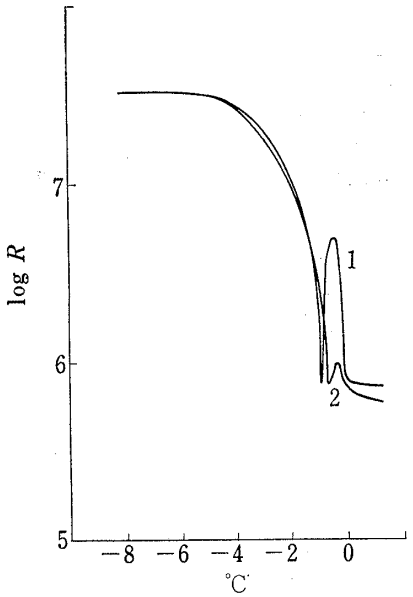


Fig. 6. Variation of Electrical Resistance of Mannitol Solution during Rewarming

1:5%, 2:10%

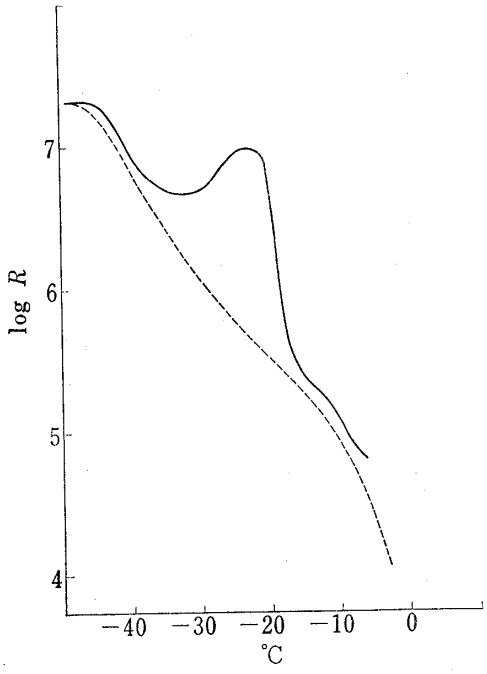


Fig. 7. Variation of Electrical Resistance of 10% γ-Amino Butylic Acid(GABA) Solution

——: rewarming, ------: cooling

The Electrical Resistance Measurement

The typical curves are shown in Fig. 4,5,6 and 7. The resistance of NaCl sample, in Fig. 4, changed sharply in a narrow temperature range. The eutectic temperature was

obtained from a sudden decrease in resistance in a rewarming curve, corresponding to a melting point of the cyrohydrate. Similar curves were obtained in cases of other strong electrolyte solutions. But of weak and non-electrolytes, the resistance changed rather slowly.

Some sucrose solutions in Fig. 5 showed remarkable increases of the resistance near 0° during rewarming. Similar curves were obtained in cases of mannitol, sorbitol, glucose and lactose solutions. Deluca, *et al.*[4e] also noticed this kind of phenomenon between T_e and a freezing point on solutions of NaCl, NaBr, NaI, KCl, KBr and KI. Any noticeable macroscopic change of appearance was not observed in this range of temperature. This might be due to the recrystallization of ice, but further discussion must await more examination.

The warming curve of 10% GABA in Fig. 7 shows the characteristic resistance increase from about −30° and then the relatively sharp drop from −20°. The formation and fading out of clear densely opaque spots also took place in the same region. The shape of the curve was much affected by the warming rate, for example, no resistance maximum was observed when the processing was very fast. This means that the characteristic resistance increase is due to the formation of a new phase or eutectic crystals and that T_e is obtained from the decrease in the resistance.

Critical temperatures are listed in the second column in Table II. For the substances which did not give such a characteristic curve as NaCl or GABA, temperatures at the maximum resistance are listed.

The Temperature Change with the Lapse of Time

Using the same chart in which the electrical resistance and the temperature were recorded, we obtained the change in temperature of the specimen as a function of time. Fig. 8 depicts one of the results acquired from a NaCl solution. The curve demonstrates points of inflection or plateau regions which are due to endothermic or exothermic phenomena caused by the phase change. The T_e of the NaCl solution was obtained from the eutectic halt at T_{e2} of the warming curve. The points of inflection or plateau regions became indistinct when the solution was too dilute or the processing was very fast. Data are listed in the second column of the Table II.

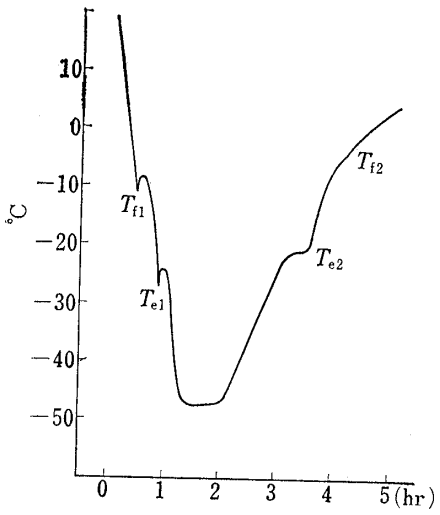


Fig. 8. Temperature-Time Curve of 10 % NaCl Solution during Cooling and Rewarming

T_f: freezing temp., T_e: eutectic temp.

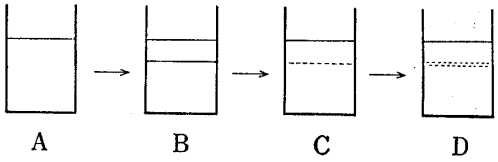


Fig. 9. Schematic Appearance Change of a Sample at Collapsing Temperature during Freeze Drying

A: frozen sample before drying
B: Normal freeze drying, a sublimation front is 1 to 3 mm beneath a surface.
C: dimming a sublimation front or collapsing
D: collapsing and puffing

The Collapsing during Freeze Drying

In all cases examined, puffiing or collapsing of the frozen layer was observed during freeze drying when the temperature of the sample exceeded a certain value (T_c) which was

characteristic of the solute. The change in appearance is shown schematically in Fig. 9.

The temperature T_e was reproducible. To observe the temperature distribution in the frozen layer, the location of a thermocouple was changed several times in the case of glucose. T_e of glucose was always in $-38°$ to $-40°$ and the temperature gradient in the frozen layer seemed to be small. Nei[10] and Suezawa[11] also found that the temperature distribution in the frozen layer was small during drying, though the geometry of the sample, the location of the crack, the nature of the container and so forth were each considered to have some influence on the temperature distribution.

Data of T_e are listed in the fifth column of Table II.

For the substances classified to types III and IV of the appearance change during freezing, the critical temperatures obtained from the fading of spots and from the resistance measurements are found to agree quite well with collapsing temperatures. But the temperatures obtained from the maximum resistance for substances of types I and II are not always in agreement with collapsing temperatures.

In the last column of Table II, recrystallization temperature and collapsing temperature from the literature,[9,12-14] are listed according to the reference.

Luyet[12] measured the devitrification temperature, or the temperature of abrupt recrystallization (T_r) upon gradual rewarming where the solution had been cooled so rapidly in isopentane at $-150°$ that it remained transparent. He found that the temperature at which the frozen transparent solution became opaque with the formation of ice was characteristic of each solute. MacKenzie[13] pointed out in his review that some of the collapsing temperatures were close to T_r and that T_r was often twenty or thirty degrees lower than the equilibrium freezing point of the solution. Data on glucose cited in his review were $-41°$ and $-40°$ for T_r and T_e, respectively. Nei[5d] also observed the recrystallization of glucose solution photomicroscopically, and found it was a gradual process ranging approximately from $-40°$ to $-10°$ upon rewarming.

The collapsing and the recrystallization seem to be closely correlated with changes in the visco-elastic properties of solutions.

In case of PEG 6000 there was a great difference between T_r and T_e. This difference has since been attributed to the crystallization of the PEG upon freeze-drying, the same substance remaining amorphous in the frozen state in the absence of an applied vacuum.[15]

General Discussion on the Appearance Change during Freezing and Drying

Densely opaque spots observed macroscopically were, no doubt, due to the formation of finely grained eutectic crystal mixtures.

Cooling the solutions classified as types III and IV, ice is formed, the solution becoming concentrated. And even when the temperature falls below T_e there still exists a solution in a super-eutectic state dispersed within the ice network. Evidently, many solute crystal nuclei must become effective or arise in the solution below T_e to cause the observed growth of very fine crystals at the proper range of temperature. These little crystals may cause the surrounding super-eutectic solution to crystallize, with the progressive crystallization of the entire system. The spots observed with the naked eye to increase the opacity of the sample in reflected light grow, most probably, by repeated surface nucleation. During rewarming the spots fade out at T_e, which corresponds to the melting temperature of the eutec-

10) T. Arakawa and T. Nei, *Low Temp. Sci.*, Ser. B, **19**, 43 (1961).
11) Y. Suezawa and S. Kawamura, *Kagaku Kogaku*, **22**, 258 (1958).
12) B.J. Luyet, *J. Phys. Chem.*, **43**, 881 (1939).
13) A.P. MacKenzei, *Bull. Parent. Drug. Assoc.*, **20**, 101 (1966).
14) "Bussie Zyosu," Maruzen Co., Ltd. Tokyo, 1964.
15) A.P. MacKenzie, personal communication.

tic crystal mixture. This is supported by the sudden drop in the electrical resistance, the eutectic halt on the rewarming curve and the collapsing during freeze drying.

Substances of types I and II did not produce the sharp change such as the eutectic transition, but had the critical temperature or collapsing temperature T_c during freeze drying. Cooling these solutions, ice is formed in the same general way, the solution becoming more concentrated with the decrease of temperature. The concentrated supercooled solution may become more viscous and finally in a certain temperature range, undergo a large increase in viscosity and loss in fluidity. This kind of solidified, supercooled liquid is easily obtained in concentrated solutions, for examples, of sucrose and gelatin. About 3M sucrose solution does not produce any ice at any temperature but becomes solidified with sufficient decrease in temperature.

When the frozen solution is very concentrated, ice crystals formed may be separated from each other and surrounded by more concentrated supercooled solution. But when more dilute solutions are frozen, just as in this study, ice crystals come in contact or, at least, interdigitate with each other and approach very closely indeed the free surface of the sample as in Fig. 10. When this sample is freeze dried below the temperature at which the solution exists in a solidified supercooled form, ice crystals are removed by direct sublimation and the residual supercooled matrix maintains its rigidity. Water in the supercooled layer may move to the cavity by molecular diffusion resulting in a drying rate much lower than that of the eutectic-containing sample.

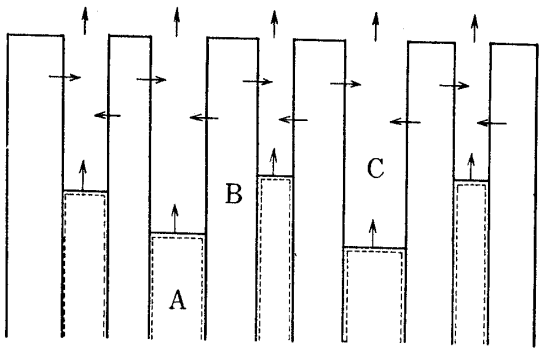


Fig. 10. A Schematic Cross Section of a Sample classified to Types I and II during Freeze Drying

A: ice crystal
B: solidified supercooled solution
C: cavity made by sublimation of ice
→ direction of water vapor

The collapsing temperature is therefore the temperature at which a large decrease in viscosity appears and the supercooled solution loses the solidity. This explanation seems to be most probable for the collapsing of substances of types I and II.

During a usual gradual freezing, just as in this study, the concentration of the residual solution probably changes according to the ideal relationship between the equilibrium freezing point and the concentration. The concentration of the residual solution below the freezing point may therefore be expressed quite accurately as a function of the temperature. So the temperature at which the solution loses fluidity may be settled characteristically of the solute independently of the initial concentration of the solution.

The freeze dried 10% sucrose solution was found to shrink readily for several hours or even a day when it was opened to air of 50 to 70% relative humidities, which were all lower than the usual critical relative humidity of sucrose (about 81.5% RH). The rate of shrinkage became very great at higher temperatures. This may be one proof not only of a porous but of an amorphous state of freeze dried sucrose.

The abrupt recrystallization temperatures found by Luyet[12] seem to be the temperatures above which ice begins to crystallize at measureable rates due to decreases in viscosity of amorphous components in frozen solutions.

The measurement of the electrical resistance of a solution during freezing or freeze drying is one of the most useful and convenient methods of observing whether the solution is crystallized or not, especially for such substances as simple salts (Fig. 4) and GABA (Fig. 6). The resistance curve of the substances classified as types I and II by changes in appearance

does not produce such a characteristic value as T_e, but the resistance observed must be reflected by the state of the frozen sample. So the temperature at the maximum resistance become an important point as one of the critical values, though the resistance is affected a little by the way of cooling or rewarming. The temperatures where the resistance is a maximum in Table II are identical with or lower than the collapsing temperature with some exceptions.

When it is intended that some substances are to be freeze dried, it is very important to find out first whether the solution becomes easily supercooled or not, what cooling process is best to promote a complete freezing, how the temperature of the specimen must be controlled during drying and so forth.

In a practical preparation, the solution usually contains more than one solute and the low temperature behavior must be more complicated. However, the measurements examined in this report should be very useful even for such a practical reparation.

Acknowledgement The author wishes to express his deep gratitude to Dr. Alan P. Mac Kenzie of American Foundation For Biological Research, Prof. Tokio Nei of The Institute of Low Temperature Science, Hokkaido University, and Dr. Tsuneji Nagai of Faculty of Pharmaceutical Sciences, University of Tokyo for their helpful suggestions and discussions. Thanks are also due to Dr. M. Shimizu, Director of the Laboratories, Dr. T. Naito, Director of this Laboratory and Mr. S. Kaga for kind encouragement and for permission to publish this work.