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Freeze Drying of Pharmaceuticals.[1] On the Macroscopic Appearance of Frozen and Dried Samples in Connection with the Growth of the Eutectic Crystals[2]

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In order to get useful information regarding the freeze drying process, macroscopic appearance during freezing and drying were observed on solutions of amino acids exhibiting eutectic behavior.

The eutectic crystal structures were observed to take the form of white, densely opaque spots. The velocity of the eutectic crystallization was roughly estimated from the increase of the spots' size and number. The electrical resistance of the solution during freezing was also measured.

There existed an optimum temperature range to promote the eutectic crystallization for each solute.

Freeze drying of these substances produced very characteristic surfaces and interiors leaving behind the solute crystal portion of the eutectic crystal mixtures.

The dried sample had a more concentrated shell, namely, considerable differences in concentration occured between the surface layer and the bulk.

Many solutions change easily to supercooled or supereutectic states during freezing. For freeze drying of these solutions the freezing process should be well controlled so that the crystallization proceeds as fast and as completely as possible. When the solution is not sufficiently crystallized, or not solidified, the product after freeze drying possesses a collapsed form.

Within the literature related to freeze drying, no report is found on eutectic crystal structures or on the relation between the freeze drying process and the morphological property of a dried mass, though there are many reports[4] on the growth and elaboration of the ice crystals.

The author found that a solution showed a remarkable macroscopic change of appearance when eutectic crystallization took place and that the observation of such an appearance change was useful in estimating the rate of the crystallization.[1] The author also observed that the characteristic surfaces of the dried masses of some substances originated with the structure of the eutectic crystal system.

In the present work the effect of manner of freezing on the forms of the eutectic crystallization and the dried sample was investigated by observing the change in macroscopic appearance of solutions of some amino acids, which demonstrated low eutectic crystallization rates.

1) Preceding paper: K. Ito, *Chem. Pharm. Bull.* (Tokyo), **18**, 1509 (1970).
2) A part of the work was presented at the 89th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April, 1969.
3) Location: *Narihira 5-6-9, Sumida-ku, Tokyo.*
4) *a*) B.J. Luyet, *J. Phys. Chem.*, **43**, 881 (1939); *b*) B. Luyet, "Recent Research in Freezing and Drying," ed. by A.S. Parkes and A.U. Smith, Blackwell, Oxford, 1960, p. 3; *c*) T. Nei, *Low Temp. Sci., Ser. B*, 23, 149, 157, 163 (1965).

Experimental

Materials——All the materials used were of reagent or pharmaceutical grade. The following substances will be abbreviated as: ε-amino caproic acid: EACA; γ-amino butylic acid: GABA; α-chloro-γ-amino butylic acid: Cl-GABA.

Apparatus——The freeze drying machine, the electrical resistance indicator and the six pen recorder were the sames as described in the previous paper.[1)]

Procedure——About 50 ml of a test solution was put in a Petri dish of 9 cm in diameter and cooled on the shelf of the freeze drying machine. The direct macroscopic observation of the sample surface and the measurement of the electrical resistance were carried out throughout the process by changing the rate of rewarming or by repeating the cooling and rewarming. The appearance of the freeze dried sample was also observed.

Result and Discussion

Observaiotn during Freezing

During freezing of solutions of EACA, GABA and Cl–GABA, ice crystals were formed first, the remaining solution becoming more concentrated. Then the solutions became lightly opaque and the entire surface seemed to be covered with ice crystals. On further processing, however, white, densely opaque spots appeared as shown in Fig. 1 and increased in number and size. This notable change in appearance was attributed to the formation of eutectic crystal structures.[1)]

The temperature and appearance of samples during the process are shown in Fig. 2, 3 and 4, in which "a" indicates appearance of the first detectable spot, "b" apparent area covered by spots and average size of spots (area in %, size in mm) and "c" disappearance of spots, which gives the eutectic temperature. The above observations shown in the Figures were carried out by watching the surface of the frozen sample. Each Figure denoted as (1), (2), (3) and (4) were obtained under four experimental conditions by combining cooling and rewarming. The frozen samples were sometimes taken out from the cooling shelf of the machine and cut to observe the interior. The same kind of spots was also found in the frozen solution. Fig. 5 is an example of the cross section of frozen 10% Cl–GABA. Even when the temperature was between −40° and −50°, spots appeared in EACA and GABA solu-

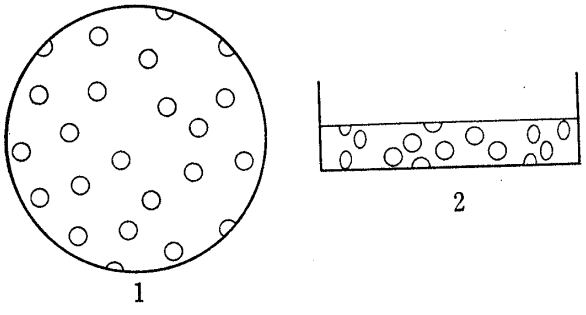

Fig. 1. Densely Opaque Spots appeared during Cooling Process of EACA, GABA and Cl–GABA Solutions in a Petri Dish

1: upper surface, 2: side

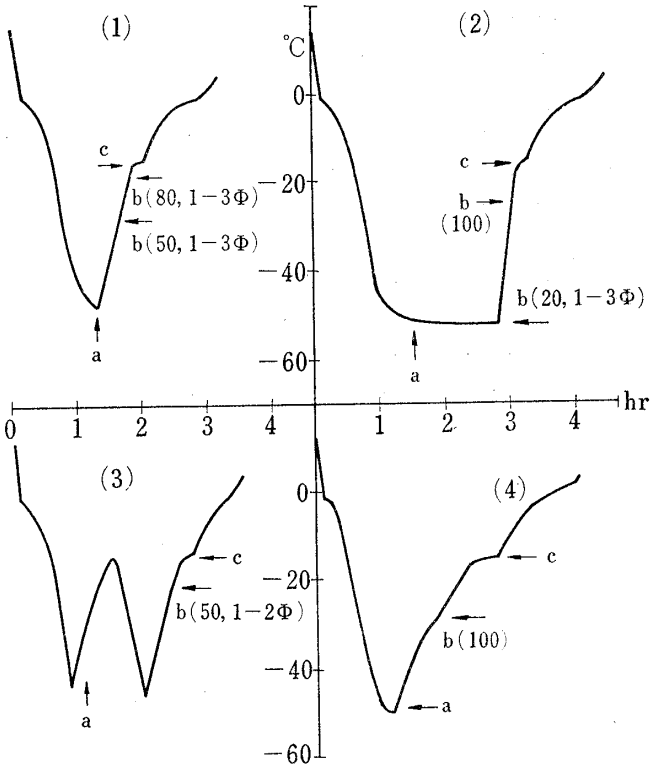

Fig. 2. Freezing Curves and Appearance Changes of 10% EACA Solution

a: appearance of a first spot, b: apparent area covered by spots and size of spots (% and mm in size), c: disappearance of spots

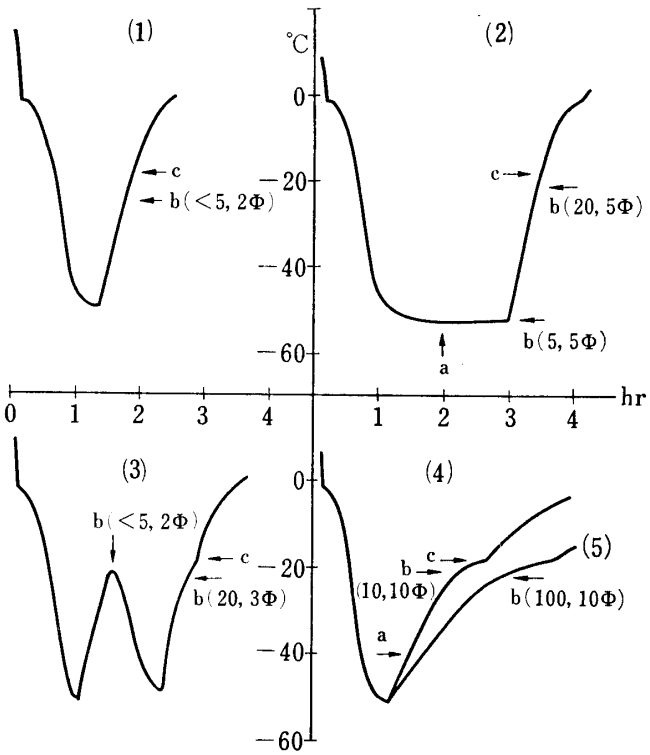

Fig. 3. Freezing Curves and Appearance Changes of 10% GABA Solution

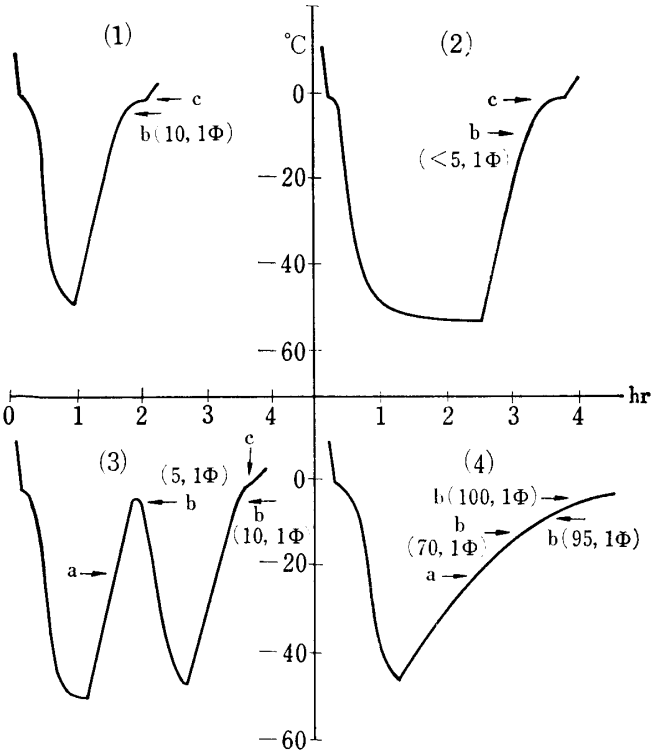

Fig. 4. Freezing Curves and Appearance Changes of 10% Cl-GABA Solution

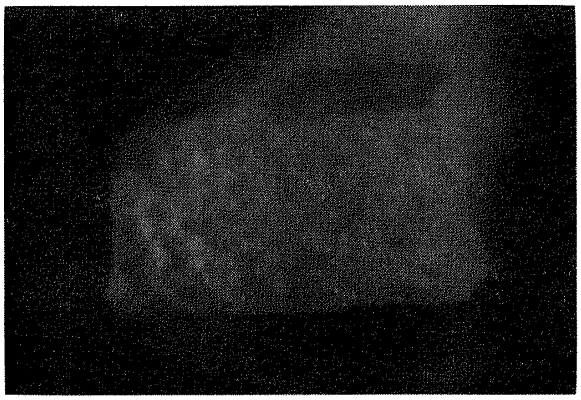

Fig. 5. Densely Opaque Spots on the Cross Section of Frozen 10% Cl-GABA

magnif. ×1

tions, but the rates of their growth were very small. The optimum temperature ranges to promote the eutectic crystallization were between 5° and 15° below the eutectic temperatures, as shown in Table I.

The electrical resistances of the frozen solutions during rewarming of each process are shown in Figs. 6, 7 and 8. The resistance was influenced by the state of the frozen solutions as discussed in the previous paper.[1)]

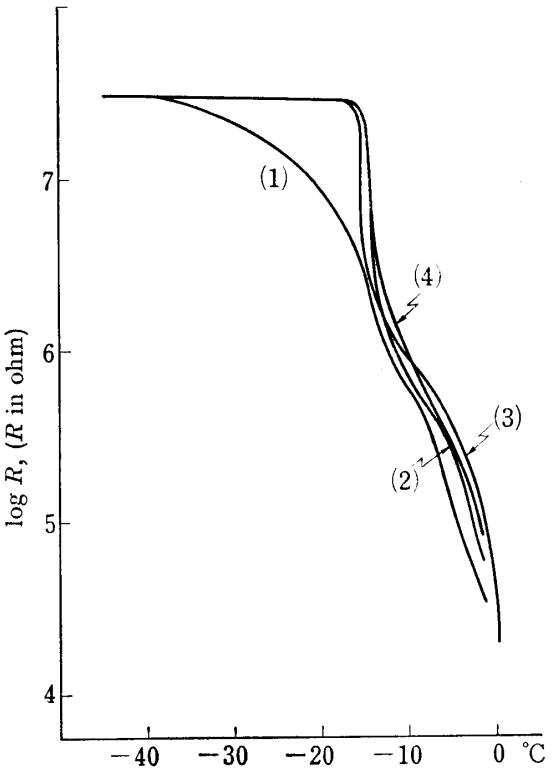

Fig. 6. Variation of Electrical Resistance of 10% EACA Solution

( ): indicates each process same as in Fig. 2

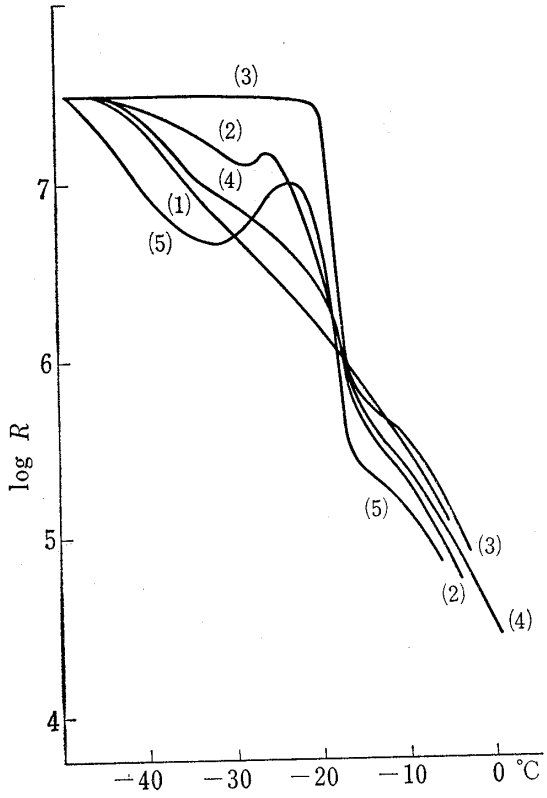

Fig. 7. Variation of Electrical Resistance of 10% GABA Solution

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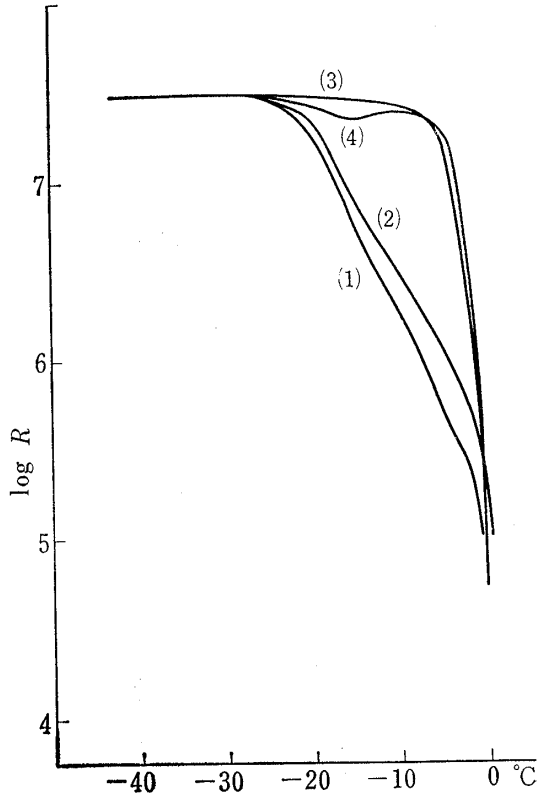


Fig. 8. Variation of Electrical Resistance of 10% Cl-GABA Solution

( ): same as in Fig. 4

Table I. Eutectic Temperature T_e and the Optimum Temperature Range to Promote the Eutectic Crystallization

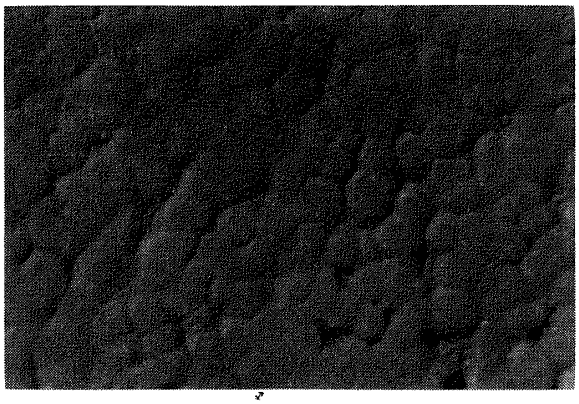
Substance	T_e	Temp. range
EACA	−15°	−20°——30°
GABA	−18°——20°	−20°——30°
Cl-GABA	−2°	− 5°——20°

In the processes (2), (3) and (4) of 10% EACA, for example, large differences existed, one from another, in the macroscopic appearance, while no noticeable difference was observed in the resistance curves. This seemed due to the fact that the resistance indicator recorded the resistance of the solution near the electrode itself. Therefore, it was considered that the resistance measurement did not always give the precise estimation of the rate of the crystallization of the whole solution.

The rate of the eutectic crystallization roughly estimated by the observation of the macroscopic appearnce became maximum in a certain range of the degree of supercooling. The tendency to crystallization increased with the degree of supersaturation, *i.e.* the extent to which the supereutectic state was entired, but it was evident that the viscosity of the solution increased rapidly in a certain temperature range. The cooling must be, therefore, carried out in the proper temperature range for each solute to promote the eutectic crystallization.

Observation of the Dried Sample

All freeze dried samples of EACA and Cl-GABA had very characteristic surfaces and interiors as shown in Fig. 9.



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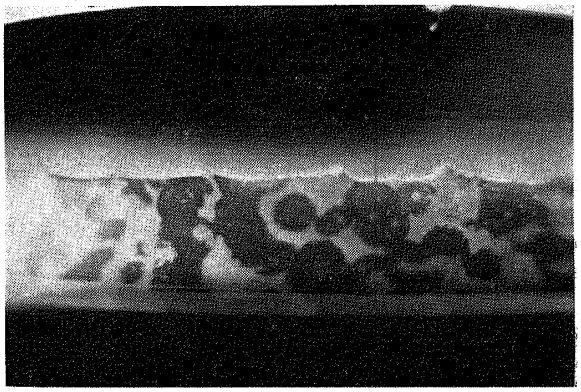

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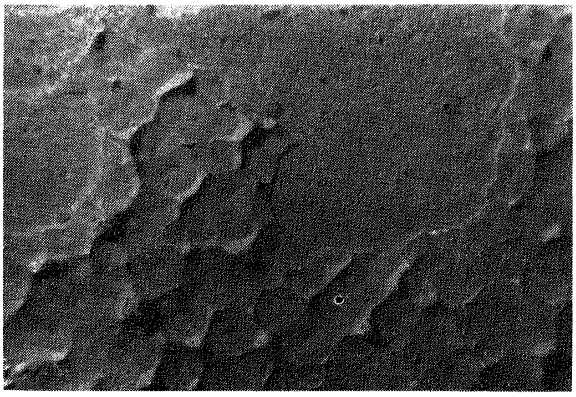

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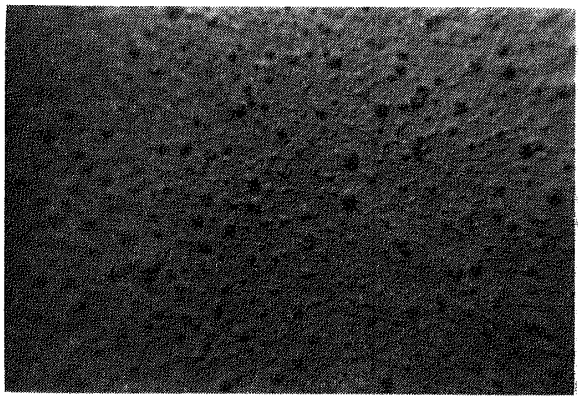

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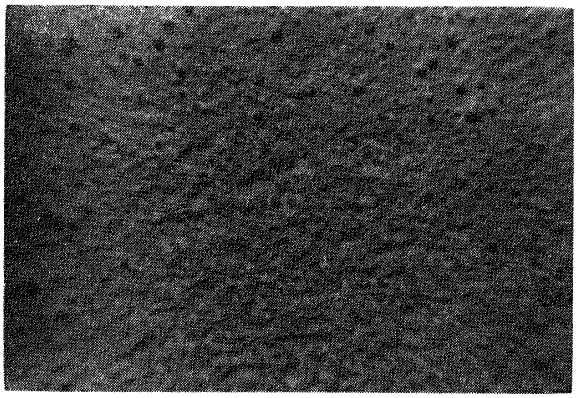

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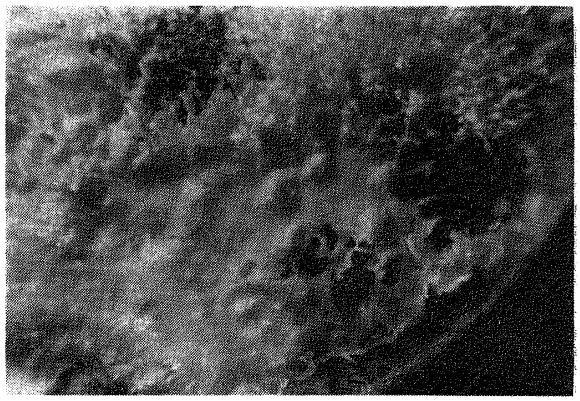

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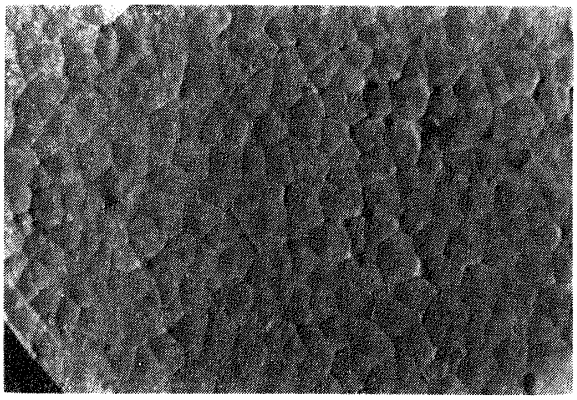

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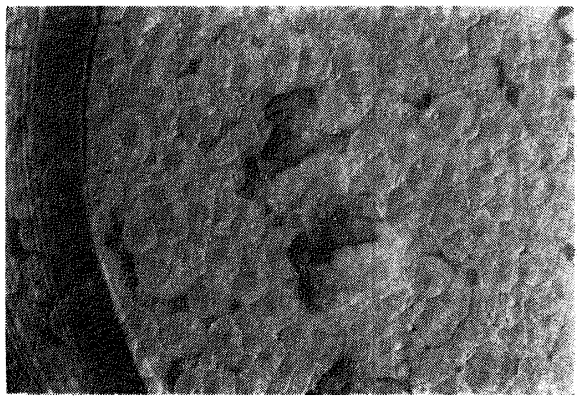

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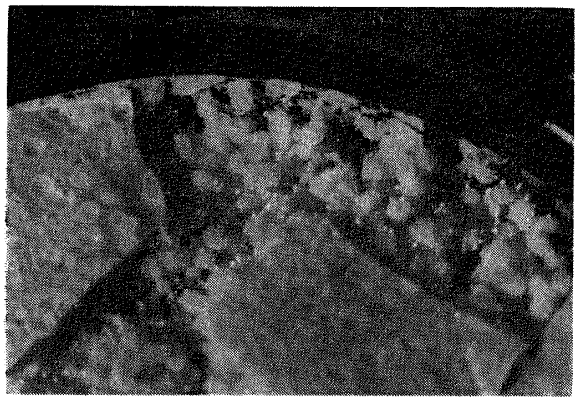

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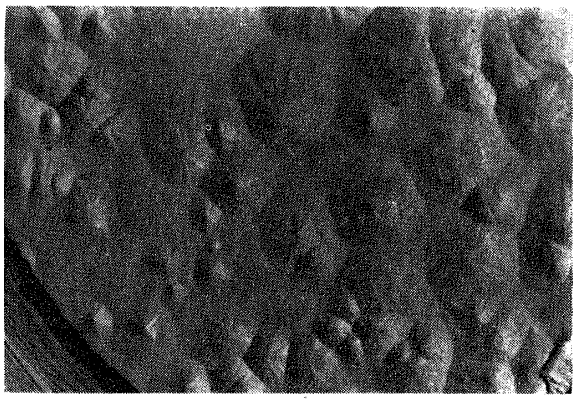

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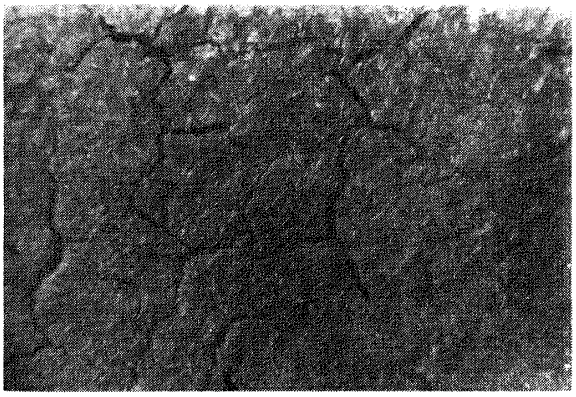

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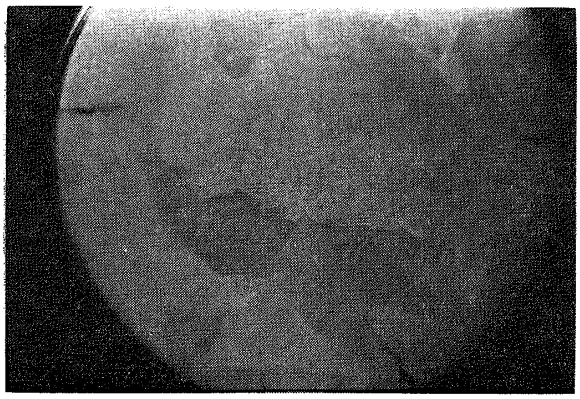

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Fig. 9. Pictures of Freeze Dried Samples in a Petri Dish

ε-amino caproic acid (EACA, 10%)
1: upper surface of well crystallized, 2: side, 3: surface having a shell, 4: surface leaving the figure of puffing, 5: surface leaving the figure of collapsing, 6: collapsed
α-chloro-γ-amino butyric acid (Cl–GABA, 5, 10%)
7: 10%, surface of well crystallized, 8 and 9: 5%, surface and interior of half crystallized
KCl (10%) 10: surface
pyridoxine hydrochloride (10%) 11: surface
sucrose (10%) 12: surface, 13: lower surface
magnif.: about ×1

The temperature change of 10% EACA, for example, during freeze drying are shown in Fig. 10 in which A represents the process for Pictures 1, 2 and 3, and B for 4 and 5 in Fig. 9. In process A drying was started after all the surfaces of the frozen solution were covered by the deep opaque spots representing the eutectic crystal. But in process B drying was initiated before the eutectic crystallization was finished. Spots observed during freezing were 3 to 4 mm in diameter for case A, 1 mm for case B. The temperature of the cold trap and the shelf during drying were −55° and 20° respectively and the degree of vacuum of the drying chamber was 20 to 30g μHg.

Picture 3 shows the sample which had the surface shell. The concentration of the shell of 10% EACA estimated roughly from the apparent specific gravity was about 5 times larger than that of the bulk. The same kind of characteristic surfaces and shell were observed also in KCl and pyridoxine hydrochloride preparations, which were in the group of solutions

readily undergoing eutectic crystallization. Although the surface had so distinctive appearnce, such clear figures were not observed below the surface shell of a well crystallized sample, as shown in Picture 3.

In the process of freezing the solution, the white densely opaque spots appeared after the ice network had spread through the sample. Then the propagation of the eutectic crystallization was observed as a change in the spots in size and number. The total volume of the sample might increase a little with the ice crystal formation because ice had a larger specific volume. Then the remaining solution might be pushed upward through the ice network and the eutectic crystal structures already formed. Thus the solute was considered to be more concentrated in the surface layer on the average, than in the bulk. The formation of the eutectic in spots in this layer also caused the surrounding solution to be pushed up, lifting the border region between spots higher, until, finally, all solution was crystallized out. This would explain the formation of the characteristic surface and the dense surface shell of the dried sample.

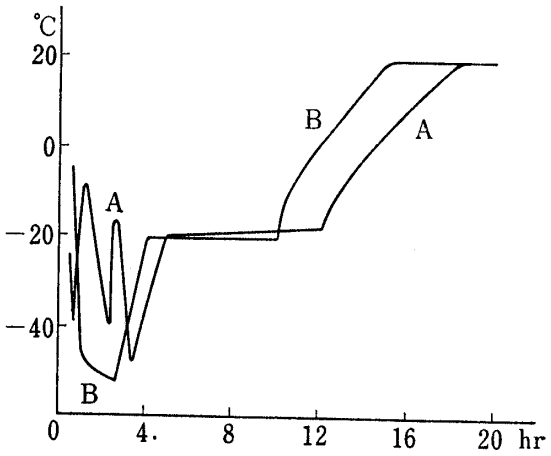

Fig. 10. Temperature Curves of 10% EACA during Freezing and Freeze Drying

temp. of shelf during drying: 20°
temp. of cold trap: −55°
degree of vacuum: 20—30 μHg

When drying or evacuation was begun before the crystallization was completed, the remaining "supereutectic" solution became puffed or collapsed. The surface leaving figures of puffing and collapsing can be observed in Pictures 4 and 5, respectively. Pictures 8 and 9 of Cl-GABA are examples of the round skeletons of eutectic crystallized portion, around which many little collapsed masses of dried "supereutectic" solution are attached.

GABA solution did not produce the dried mass with such characteristic figures as EACA and Cl-GABA, but with collapsed figures. This was due to the difficulty of obtaining a completely crystallized solution.

Pictures 12 and 13 show the upper surface and the back, respectively, of a quantity of 10% sucrose freeze dried in a Petri dish. Eutectic behavior was not exhibited. The ice pattern and cracks by shrinkage observed are due to the structure of the frozen sucrose solution which are composed only of ice and solidified supercooled solution, as discussed in the previous paper.[1)]

Although the experiments were carried out on several substances only, it was clear that the structure of the frozen solution could be well preserved in each dried product examined.

Further detailed examination from the microscopic standpoint would, most probably, help to confirm the phenomena observed by studying the macroscopic appearance, but the method applied in this study is considered to offer a very simple and useful way of furthering both the practical and fundamental study of freeze drying.

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