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Effect of the Rate of Cooling upon the Rate of Drying and the Residual Moisture Content of Specimens in Freeze-Drying*

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Abstract

In the freeze-drying of solutions, it was found that the rate of cooling in primary freezing affected the rate of drying and the residual moisture content; dehydration proceeds more rapidly in the slowly frozen material during the primary phase of drying, but more rapidly in the quickly frozen material during the final stage. The ultimate residual moisture content is independent of the initial freezing velocity. These effects of the rate of cooling have been interpreted from the morphological point of view.

I. Introduction

With reference to the freeze-drying of biological substances, the effect of the rate of initial freezing (the first step in the process) upon the viability of living cells after freeze-drying has previously been reported[1)]. The effect of initial freezing rate upon the drying process and on the residual moisture content of freeze-dried solutions has, however, not yet been elucidated.

It is very important in large-scale production by freeze-drying to enhance the rate of drying. The residual moisture content of the dried material is a measure of the closeness with which the end point of drying has been approached; at the same time the residual moisture content affects the preservability of the material during storage after drying[2)].

In the studies here presented, the effects of the rate of cooling on both

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the rate of drying and the residual moisture content of solutions was investigated.

II. Materials and Methods

1. *Materials.*

Rabbit serum, 5% and 10% gelatin solutions, 20% pepton solution and 5% skim milk solution with 5% added glucose were used as materials to be freeze-dried.

2. *Freeze-drying procedure.*

0.3 ml of the material was used for the measurement of the rate of drying and 0.5 or 1.0 ml was used for the measurement of the residual moisture content. The materials were put in specially made containers of celluloid film or in weighing bottles and either frozen rapidly at a rate of about 100°C/min by immersion in liquid nitrogen or frozen slowly at a rate of about 1°C/min by placing first in a cold room at −30°C and, thereafter, in liquid nitrogen in which cooling to −196°C was completed. After freezing, the materials were transferred to containers which were then connected to manifolds and drying was started. Drying apparatus consisted of manifolds, cold trap using liquid nitrogen, vacuum gauge and rotary pump. The containers were exposed to the air at room temperature throughout the drying process.

3. *Measurement of the rate of drying.*

A self-recording vacuum torsion balance[3)] was employed for recording the changes in weight of the material during drying. Rates of drying were obtained from the dehydration-time curves illustrated in the recording paper.

4. *Measurement of residual moisture content.*

Residual moisture content of the dried materials was measured by ABDERHALDEN's method with a micro-balance; calculations employed the following equation:

$$\text{Moisture content} = \frac{A - B}{B} \times 100$$

where A stands for the initial weight of the dried material and B for the weight after 3 hours' desiccation at 60°C under vacuum of 10^{-5}mmHg.

III. Results

1. *Velocity of freeze-drying.*

As shown in Figs. 1 and 2, the rates of drying, measured from the drying

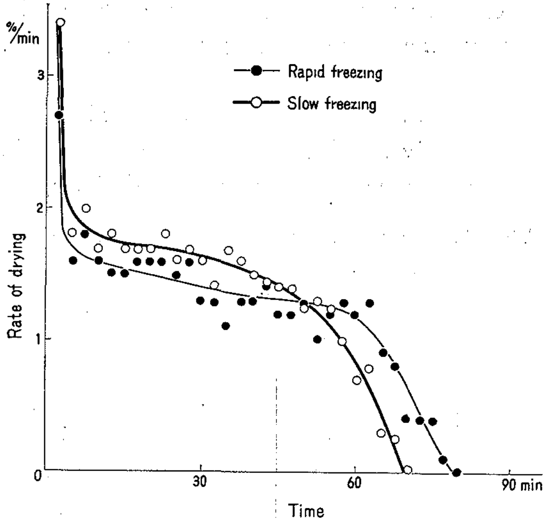

Fig. 1. Drying rate-time curves of rabbit serum

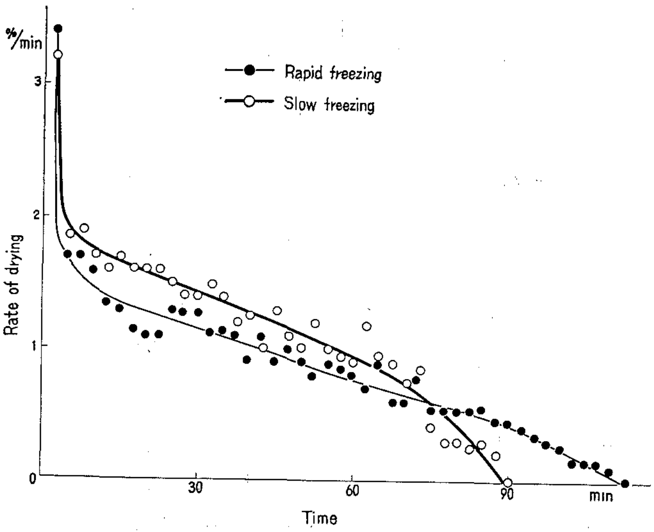

Fig. 2. Drying rate-time curves of 5% gelatin solution

curves obtained by the vacuum torsion balance, during the removal of the majority of the water present, were higher in the cases of the slowly frozen material.

2. *Residual moisture content of the dried materials.*

Residual moisture contents of the dried materials, after ordinary freeze-drying experiments of 9 hours duration, were in each case larger in the slowly frozen material than in the corresponding rapidly frozen ones, as shown in Table 1.

As the drying time was prolonged, however, differences in the average drying rate became gradually less for a given material. Table 2 shows changes in the residual moisture content in such prolonged drying.

Table 1. Effect of the rate of cooling on the residual moisture content of dried materials

Materials		Freezing	Residual moisture content*		Ratio (rapid/slow)
			Exp. 1	Exp. 2	
5% glucose-5%skim milk	1.0 ml	rapid	3.2 %	4.4 %	2/2.3
		slow	5.6	11.8	
20% pepton	0.5 ml	rapid	4.0	4.7	1/1.2
		slow	4.5	6.2	
Rabbit serum	1.0 ml	rapid	0.3	0.2	1/2.1
		slow	0.5	0.5	
10% gelatin	0.5 ml	rapid	0.2	—	1/10.5
		slow	2.1	—	

* Average in 6 cases

Table 2. Changes in the residual moisture content in prolonged drying

Materials		Freezing	Residual moisture content	
			After 9 hours drying	After 14 hours drying
10% gelatin	0.5 ml	rapid	0.2 %	0.2 %
		slow	2.1	0.9
Rabbit serum	1.0 ml	rapid	0.1	0.1
		slow	0.7	0.3

When dehydration at 60°C under a vacuum of 10^{-5}mmHg was applied for 3 hours after primary drying, the dry weight were the same for rapidly and for slowly frozen samples.

3. *The whole process of drying.*

The results obtained from such experiments as described above suggest that two drying curves crossing each other at one point could be drawn as shown in Fig. 3.

The existence of two such curves was actually verified by measuring the residual water in samples taken at intervals throughout the process of drying, as shown in Table 3.

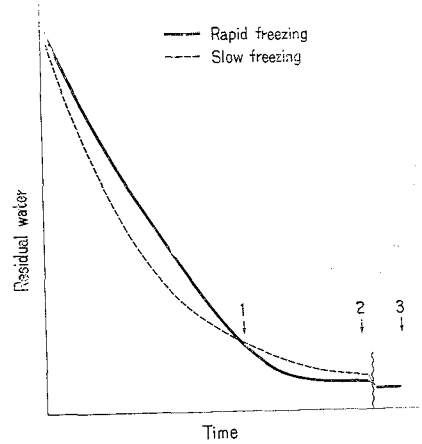

Fig. 3. The schema of the whole process of drying. 1, 2 and 3 (arrows) indicate the end points of sublimation of ice, dehydration by a rotary pump and dehydration by a diffusion pump, respectively.

Table 3. Effect of the rate of cooling on the change in water content during drying process

Material		Freezing	Water content (Residual water content/Total water content)				
			After				
			1.5 hrs	3 hrs	4 hrs	5.5 hrs	7 hrs
Rabbit serum	1.0 ml	rapid	50.2 %	7.8	1.5	0.1	0.0
		slow	48.8	4.8	1.9	0.2	0.1

IV. Discussion

From the results obtained, it was noted that dehydration of the slowly frozen samples was faster than that of the corresponding rapidly frozen ones throughout the sublimation of the majority of ice in the material, whereas the extent of dehydration in the former was surpassed by that in the latter during the further removal of water contained in the concentrated materials, that is, during the so-called secondary drying. If the drying was advanced further yet, however, the residual moisture contents tended to the same final value.

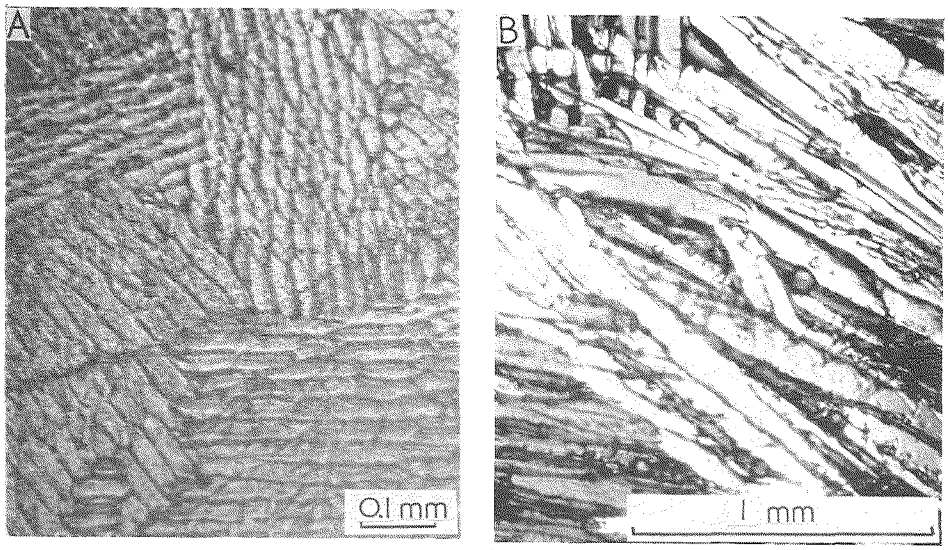

Fig. 4. Morphological patterns of serum in the frozen state.
A, rapid freezing, × 100; B, slow freezing, × 40

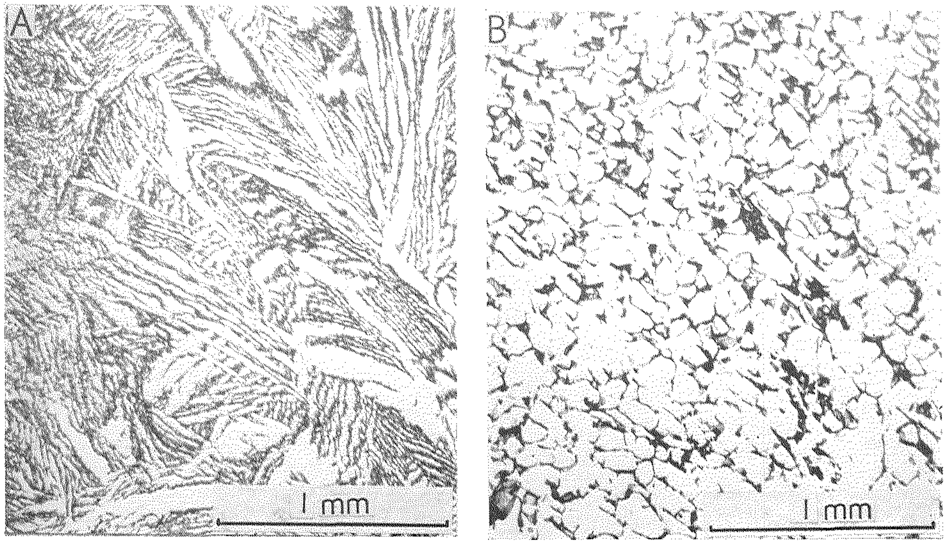

Fig. 5. Morphological patterns of serum dried from the frozen state.
A, rapid freezing, × 30; B, slow freezing, × 30

It is reasonable to approach the interpretation of such drying processes from the morphological point of view. In slow freezing, ice particles formed in the material grow to a considerable size and show mostly longitudinal arrangement. On the contrary, in rapid freezing, the ice particles are more numerous but small, and each particle may be isolated from others by concentrated solute phase. Previous observation of specimens, thin-sectioned in the frozen state[4] and observation of specimens, paraffin-embedded and thin-sectioned after drying in the present experiments, provided evidence of such morphological patterns of ice formation as shown in Figs. 4 and 5.

From the date described above, it is supposed that drying proceeds faster in the slowly frozen materials (large and ranged ice particles) than in the rapidly frozen ones (small and disconnected ice particles) during the sublimation of ice, but, at the next stage (during the removal of water contained in the concentrated solute phase) the order of rates is reversed because of hindrance due to the layers of those concentrated solute phase which are wider in the slowly frozen than in the rapidly frozen materials. Finally, the residual moisture contents reach exactly equal values, as one might logically expect.

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