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Mechanisms of Deterioration of Nutrients

by Marcus Karel and James M. Flink

Department of Nutrition and Food Science

Massachusetts Institute of Technology

Cambridge, Massachusetts 02139

ANNUAL REPORT - PHASE 1

NASA/MSC CONTRACT No. 9-12485

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I. General Introduction

Phase I of this contract has been devoted to initiating studies which will lead to development of methods by which freeze-dried foods of improved quality will be produced. To achieve the aims of the contract, experimental evaluations were begun in the following specific areas:

1) The retention of characteristic flavor of freeze-dried food liquids (carbohydrate solutions) has been previously demonstrated to depend on the structure of the dry material. The applicability of these theories to flavor retention in a solid non-carbohydrate polymer system was demonstrated.

2) Mass transfer factors which influence the flavor quality of frozen material prior to drying were evaluated.

3) Studies on the formation of structures during the freeze-drying process have been initiated using an optical microscope having a specially designed freeze-drying stage.

4) The extent to which organoleptic qualities deteriorate during the second (desorption) period of freeze-drying, has been determined and improved procedures developed.

5) The influence of water activity during storage on the retention of model flavor materials has been evaluated for a solid non-carbohydrate polymer system.

6) A freeze dried food of high quality whose processing is based on results obtained in the areas discussed above has been prepared, evaluated and samples are being provided to NASA/MSC, as provided for in the contract schedule for Phase I.

The first year has been specifically devoted according to the contract schedule to the following topics:

1) Kinetics and mechanisms of mass transfer factors affecting the loss of volatiles from frozen material immediately prior to freeze drying.

2) Separation of components during freezing to give the structure of freeze dried material is characterized by optical microscopy using a freeze-drying stage and data evaluated in regards to its influence on flavor retention, in freeze-dried food.

3) Recently developed flavor retention theories have been evaluated for applicability to solid model systems resembling fruits.

4) Evaluations necessary to optimize the quality (including nutritional value) of freeze-dried food by control of heating during the desorption period of dehydration have been determined.

5) Water activity effects on flavor stability during storage of freeze-dried foods has been evaluated.

6) 5 lbs (dry weight) of a freeze-dried food processed by utilization of appropriate considerations of advanced technologies developed in this study, is being delivered to NASA/MSC.

Since the first year involves the initiation of several lines of study which will be progressively unified during subsequent phases, it was felt that a most logical presentation of the accomplishment of Phase I is as separate sections which follow.

II) Volatile Transport in Frozen Aqueous Solution

III) The Freeze-Drying Microscope

IV) Browning of Dried Model Systems Heated by Radiation

V) Volatile Retention during Freeze-Drying of the Polar, Non-Carbohydrate Polymer Polyvinyl Pyrrolidone

VI) Development of a Food Product

Summary of results of Phase I is present as the last section VII.

II. Volatile Transport in Frozen Aqueous Solution

II.A. Introduction

The study on the loss of volatile organic compounds from aqueous solutions held in the frozen state has been prepared as a Master of Science thesis by Mr. Denis Lambert. Two papers have been written and submitted for publication to the scientific journal, "Cryobiology". As these two journal articles and the abstract from the Master of Science thesis give a concise and complete presentation of the work in this area of the study, this section of the annual report will be comprised of the thesis abstract and copies of these papers.

THESIS ABSTRACT

VOLATILE TRANSPORT IN FROZEN AQUEOUS SYSTEMS

by Denis Lambert

Submitted to the Department of Nutrition and Food Science on August 14, 1972, in partial fulfillment of the requirements for the degree of Master of Science

ABSTRACT

The factors affecting the loss of butanol from frozen aqueous solution during storage were investigated and a mechanism was derived to explain the partial retention of butanol.

Frozen aqueous solutions containing n-Butanol and distilled water were equilibrated over activated charcoal in a chamber held at constant sub-freezing temperature. A fraction of the initial butanol was lost. The remainder was retained throughout equilibration provided that the water content of the samples stayed constant. The loss of the latter fraction was linearly related to the water loss.

It was found that the amount of butanol lost:

1. increased with the equilibration temperature
2. was directly proportional to the initial butanol concentration
3. increased with decreasing freezing rate
4. was independent of the sample thickness and proportional to the sample surface area.

Removal of a surface layer of the frozen samples and subsequent equilibration of the remainder of the samples showed that the loss of butanol was a surface phenomenon.

The porosity of ice to the transport of butanol was also demonstrated. Uptake of butanol by pure ice showed that butanol vapors seem to be able to be adsorbed on to the surface and pore spaces of ice.

Three types of ice-butanol interactions sites were postulated to explain the butanol retention mechanism:

1. Adsorption of pure butanol in the surface layer of the samples resulting from phase separation during freezing or from the uptake of butanol vapors.

This butanol fraction can be desorbed and thus is available for loss during equilibration.

2. Concentration of butanol in the unfrozen interdendritic phase. This fraction can not be lost without the breakdown of the interdendritic structure and the simultaneous loss of water.
3. Location of butanol in the pore and cracks interaction sites of the ice resulting from the uptake of butanol vapors. During freezing of aqueous butanol solutions these pore spaces are free of butanol.

Thesis Supervisor: Dr. James M. Flink

Title: Assistant Professor of Food Engineering

Volatile Transport in Frozen Aqueous Solutions (1): Development of a Mechanism[1]

DENNIS LAMBERT*, JAMES FLINK and MARCUS KAREL

Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

*Present Address: 21 Rue de Bourgogne, 75-Paris-7, France

INTRODUCTION

Changes in flavor of frozen foods can occur during frozen storage due to the loss or uptake of volatiles (6). This requires the transport of organic volatiles in the frozen foods since it is these compounds which comprise much of the product flavor. There is a lack of quantitative information in the literature on this aspect of flavor change during frozen storage; most studies in this area relate to the development of off flavors due to chemical reactions in the frozen state.

In extending work on volatile retention in freeze-dried solutions, Steijvers (11) and Gejl-Hansen (5) showed that a certain amount of organic volatile was lost during frozen storage of aqueous malto-dextrin solutions.

Properties of ice and ice-solute interactions should strongly influence the transport behavior of volatiles in frozen materials. A limited amount of information is available regarding the interaction of ice and volatile materials. It has been shown that ice placed in the presence of vapors acts as an absorbant. Mechanistic interpretations vary. For example, nitrogen is said to be retained by physical adsorption (2), while gas hydrate formation inside the ice is considered responsible for uptake of n-alkanes (8); carbon dioxide at low vapor

pressure is surface adsorbed, though at higher vapor pressures, formation of a carbon dioxide gas hydrate results in sizable CO_2 uptake (1).

Soldano and Ward (10) tested the ability of ice to remove iodine vapors from an air stream. A large amount of the iodine being passed through a bed of ice cubes was retained by the ice; it could be partially removed, however, by subsequent air stripping. The presence of pores and micro-passages in ice and frozen solutions has been suggested by El-Sherbieny (4) to explain observed oxygen permeability.

The loss or uptake of volatile from frozen aqueous solutions will be related to conditions resulting from the freezing. During freezing of aqueous solutions, water is removed from solutions and transformed into ice crystals, solutes being rejected from the growing ice crystals into the unfrozen phase where they undergo concentration (7). As a result, the distribution of volatile in a frozen aqueous solution is different from the initially uniform aqueous solution.

Taborsky (12), studying solute redistribution following freezing of aqueous solutions, showed that due to the rejection of solute by the growing ice crystals, a relatively large amount of solute is found in the part of aqueous solutions frozen last.

Constitutional supercooling, a consequence of both rejection of solute and thermal behavior associated with fast freezing (3), can result in entrapment of solute as cellular structures in the ice crystals (9).

This study examines volatile loss during frozen storage of aqueous solutions and presents a mechanism by which the observed behavior is related to freezing phenomena associated with aqueous solutions.

EXPERIMENTAL TECHNIQUES

Sample Preparation--The aqueous solution was n-butanol (0.8% wt/wt) in distilled water. Aliquots of the solution were pipetted into plastic petri dishes (Falcon Plastics, Oxnard, Calif.) with a very flat bottom which gave the samples uniform thickness.

Samples were weighed, placed on a chilled surface at constant temperature, and frozen under conditions of unidirectional heat transfer. The chilled surface was an aluminum plate immersed to just below its upper surface in liquid nitrogen. The average freezing time was about 5 min.

Upon complete freezing the samples were placed in a glass desiccator for equilibration over activated charcoal. An amount of cocoanut charcoal (Fisher Scientific Company, Fair Lawn, N.Y. 6-14 mesh) with excess sorption capacity

was present in the desiccator. The desiccator was held at atmospheric pressure in a thermoregulated cooling bath and had a special cover cooled by continuously circulating refrigerant liquid (Figure 1). The fan circulated the air, insuring constant temperature conditions and accelerating the diffusion of butanol vapors from the frozen samples to the activated charcoal. The samples were placed between two layers of crushed ice to maintain a constant water content in the samples.

After equilibration duplicate samples were removed from the desiccator, immediately weighed, and thawed by placing the sample containers in a water bath. Several thawing temperatures were investigated and 25°C was found to be optimum with respect to the minimizing loss of butanol after a freeze-thaw cycle.

Analysis--Sample weights before and after equilibration were used to determine water loss. Contribution of butanol lost during equilibration to weight change could be neglected since total butanol content was low compared to the potential water loss.

Butanol retention was determined by injecting aliquots of the thawed solution into a gas chromatograph (Varian Aerograph, Wilkens Instrument and Research, Inc., Walnut Creek, Calif.), equipped with a flame ionization detector. A standard solution of known butanol concentration

corresponding to the sample concentration before equilibration was prepared and injected into the chromatograph for each analysis. Butanol retention is generally expressed as the ratio of the final and initial peak areas. The variability of the analytical procedure is 3%, and of the overall experimentation 5-10% depending on conditions.

RESULTS AND DISCUSSION

Butanol retention in frozen samples equilibrated over activated charcoal as a function of time is shown in Figure 2. The short-term experiment indicates that the loss of butanol is a gradual process taking place within 24 hr. The long-term experiment confirms that after the initial loss of butanol, there is no further loss. This proves that only a fraction of the total volatile is available for loss; the other fraction is retained by the frozen sample throughout storage. The difference in butanol retention is due to different equilibration temperatures.

To assure that the cessation of loss of butanol was not due to saturation of the charcoal, samples were equilibrated at -10°C in a desiccator, then removed and placed along with newly prepared samples in a second desiccator containing fresh charcoal.

Figure 3 indicates that cessation of butanol loss is not due to the saturation of the charcoal by butanol

vapor, as renewed equilibration over fresh charcoal does not promote further loss. Neither is the cessation of butanol loss due to the saturation of charcoal by water vapors as charcoal in the second desiccator had ample time to equilibrate with water vapor from the crushed ice. The freshly prepared samples lost 25% of their butanol, whereas the previously equilibrated samples lost no further butanol. Another experiment showed that the amount of butanol lost from the frozen samples was apparently independent of the presence or absence of charcoal.

Replacing the special cooling cover with one without cooling gives considerable loss of water from the samples due to a sizable temperature gradient inside the desiccator. The effect of water loss on butanol retention is shown in Figure 4. Experiment 1 had two layers of samples in a non-cooling cover desiccator having one layer of crushed ice. The water content of the lower level of samples stayed constant for 44 hr during which time the retained butanol dropped to 80% of the initial amount. After 44 hr, when these samples started to loose water, the butanol content dropped further. The upper level of samples simultaneously lost butanol and water throughout the equilibration period.

Experiment 2 was conducted in a cooling cover desiccator with one sample layer between two layers of crushed ice. Within 1 day the butanol retention dropped

to 81% and remained there for an additional 2 days equilibration; the water retention being constantly 100%.

These experiments show two distinct mechanisms for the loss of butanol:

1) During storage of frozen aqueous solutions at a fixed temperature, an amount of butanol is lost from frozen aqueous solutions during storage without any change in the water content. This can be called water-independent butanol loss.

2) When water is lost from the frozen samples, butanol is lost simultaneously. Once the water-independent butanol loss has occurred, all subsequent butanol loss is linearly related to the water loss. This loss of butanol can be prevented by maintaining a constant sample water content. Both mechanisms can take place simultaneously.

Since the cessation of butanol loss at fixed water content is not due to the saturation of the activated charcoal, this phenomenon must be related to structural aspects of the frozen aqueous solution which allows only a fixed fraction of volatile to be lost. To study the porosity of ice to the transport of butanol, frozen samples were covered with a layer of ice and equilibrated over activated charcoal. The results (Table 1) indicate that a layer of pure ice does not constitute a barrier to the loss of butanol. Thus, pure ice must have a porous

structure which allows transport of butanol. This confirms El-Sherbieny's conclusions (4).

After equilibration, separation of the two layers (using a scalpel) permitted measurement of the distribution of butanol. Table 1 shows that the retention in the bottom layer is the same as samples having the same thickness but with no upper layer of ice. From the approximately 16% butanol lost from the lower layer, 6% is retained by the upper layer of ice; the remainder being transported through the ice. This indicates that the samples have sufficient porosity so that if butanol has the potential for loss throughout the sample, the ice cake will not prevent this loss.

The effect of the sample thickness on butanol loss is shown in Table 2. For a given sample area the amount of butanol lost is approximately the same for the three thicknesses investigated. For all samples the amount of butanol lost per unit area does not seem to be greatly affected by the sample thickness. Therefore, despite the porosity of ice to the transport of butanol, it seems that butanol loss does not occur throughout the thickness of the sample, but rather from a surface layer.

By assuming that all butanol loss comes from a surface layer of concentration equal to the initial solution, the thickness of this hypothetical "surface

layer" is calculated to be approximately 0.5 mm. The calculation also shows that the "surface layer" thickness is not affected by the total sample thickness or the surface area of the samples.

To verify this result, approximately 0.5 mm was scraped from the free surface of samples having two different thicknesses. The butanol retention of the remainder of the sample was analysed both before and after equilibration.

Table 3 shows that:

1) Butanol retention of the layer remaining after scraping (but without equilibration) is approximately equal to the butanol retention of equilibrated unscraped samples.

2) For scraped samples, equilibration does not cause additional butanol loss.

3) Surface scraping results are independent of sample thickness.

Using the data in Table 3 the butanol concentration in the scraped layer is calculated to be twice the butanol concentration of the solution.

These results show that all the butanol loss takes place from a surface layer which is *less than* 0.5 mm thick. This was calculated by assuming that all the butanol present in this layer is lost upon equilibration

and it has the same concentration as the initial solution concentration prior to freezing. However, the real surface layer from which the butanol is lost probably has a thickness much smaller than 0.5 mm and a butanol concentration higher than twice the initial concentration.

It appears that the loss of butanol during equilibration is not due to the diffusion of the butanol located throughout the entire thickness of the sample (despite the porosity of ice to the transport of butanol). The fraction of the butanol located in the bulk of the sample appears to be strongly entrapped. Furthermore, the concentrated surface layer from which the butanol is lost exists in the samples immediately after freezing and can not be attributed to butanol movement and accumulation at the surface during equilibration.

The butanol retention curve as a function of equilibration time (Figure 2) is similar to the desorption curve obtained for the case of ice cubes having absorbed iodine vapors and exposed to a flowing air stream (10). Only a fraction of the iodine vapors absorbed by the ice cubes could be removed by subsequent air stripping.

An experiment similar to that conducted by Soldano and Ward has been carried out with the evacuation of pure ice samples which had sorbed butanol vapors. It was found that 70% of the butanol present on the samples was

removed by a 5 min evacuation. Evacuation for an additional 5 min gave little additional loss.

This experiment demonstrated that only a fraction of the vapors taken up by pure ice is easily desorbed. Since the porosity of ice to the transport of volatile has been clearly demonstrated, it is very likely that: 1) A fraction of the volatile taken up diffuses from the surface into the ice where it is either trapped or only slowly removed during subsequent evacuation; 2) Another fraction is adsorbed on the surface and is readily desorbed.

CONCLUSION

To interpret our results, three types of ice-butanol interaction are postulated:

1) *Butanol in the porous structure of the sample "surface layer"*--A fraction of the butanol, located at the sample surface, is available for loss. Vapors adsorbed on the ice surface exhibit similar behavior. During freezing a fraction of the initial butanol is rejected to the free surface of the samples and undergoes phase separation during the last stages of the freezing. Pure butanol is thus separated from the freezing aqueous solution and *acts* as if adsorbed in the porous structure of the surface layer. The butanol-containing porous strucure is in effective communication with the environment

so that the pure butanol is available for loss without any loss of water.

2) *Entrapment of butanol in the interdendritic phase*--A fraction of the initial butanol is concentrated in the interdendritic unfrozen phase. This fraction is strongly entrapped by the frozen sample and is retained throughout equilibration despite the porosity of ice to the transport of butanol. This fraction can not be lost without the breakdown of the dendritic structure and simultaneous loss of water.

3) *Butanol located in pores and cracks*--Butanol diffuses into pores and cracks of the ice during the uptake of butanol vapors. Subsequent evacuation results in the corresponding diffusion of butanol from the pores and cracks. This results in the time dependence of the loss of sorbed vapors. During freezing when butanol separates, these pore spaces (which probably are the pathways by which butanol is transported through pure ice) are free of butanol.

SUMMARY

Frozen aqueous butanol solutions are equilibrated at constant subzero temperature over activated charcoal. A fraction of the butanol is lost within 24 hr, the remainder being retained for over 350 hr. The retained butanol is lost only with the simultaneous loss of water. Pure ice is demonstrated to be permeable to the transport of butanol. Based on experiments which remove the free surface, the butanol loss that is independent of water loss, is shown to originate from a surface layer postulated to form during freezing of the solution.

Three types of butanol-ice interactions are postulated:

1) Butanol in the surface layer

2) Butanol entrapped in interdendritic spaces

3) Butanol present in pores and cracks following sorption from the vapor state.

FOOTNOTE

[1]This work was supported in part by The National Aeronautics and Space Administration/Manned Spacecraft Center Contract No. 9-12485.

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TABLE 1

DEMONSTRATION OF POROSITY OF ICE TO BUTANOL TRANSPORT: LOSS OF BUTANOL FROM LAYERED SAMPLES

	Butanol Content (% of total initial butanol)
Time Dependence	
Equilibration time (hours)	
0	99
22	94
95	91
119	92
167	91
Distribution Evaluation	
	Initial / Final
Top layer	0 / 6
Bottom layer	100 / 84
Loss (by difference)	-- / 10

TABLE 2

EFFECT OF SAMPLE THICKNESS AND SURFACE TO VOLUME RATIO ON THE LOSS OF BUTANOL

Amount of solution (ml)	Thickness (mm)	Area (cm^2)	Butanol Retention (%)	Butanol loss (g)	Butanol loss per unit area (g/cm^2)
6	6.6	9.1	92	0.0039	0.00043
4	4.4	9.1	86	0.0045	0.00049
2	2.2	9.1	80	0.0032	0.00035
4	2.0	20	79	0.0067	0.00033

TABLE 3

BUTANOL RETENTION OF SCRAPED AND UNSCRAPED SAMPLES

	Butanol Retention (%)	
	Scraped Samples	Unscraped Samples
Sample Thickness=2.2 mm		
After Freezing	77	98
After Equilibration	77	80
Sample Thickness=6.6 mm		
After Freezing	90	99
After Equilibration	89	92

FIGURE LEGENDS

Figure 1

Desiccator with the Cooling Cover.

Figure 2

Time Dependence of the Loss of Butanol.

Figure 3

Evaluation of Sorptive Capacity of Activated Charcoal.

Figure 4

Effect of Water Loss on Butanol Loss.

Figure II-1

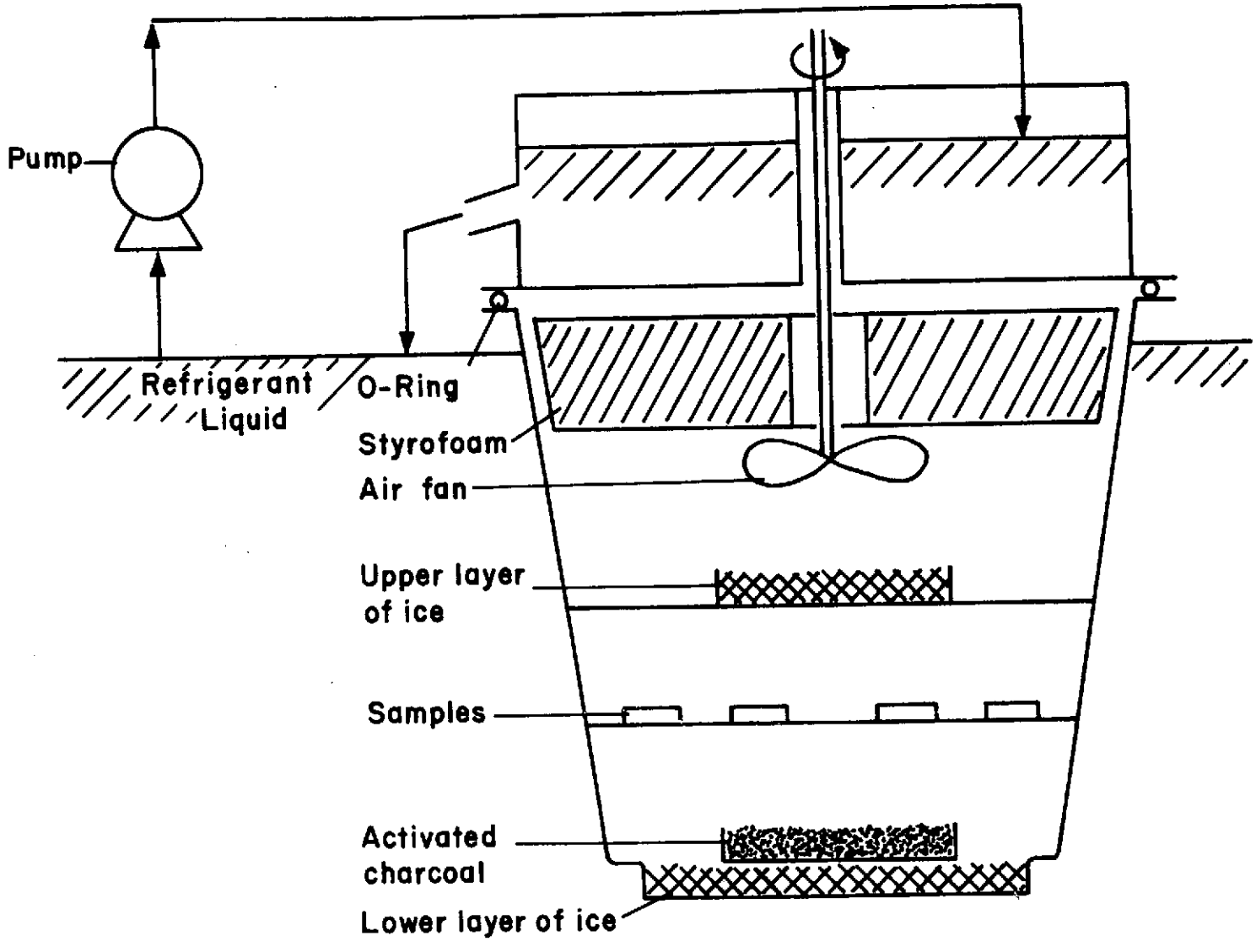

Pump
Refrigerant
Liquid
O-Ring
Styrofoam
Air fan
Upper layer
of ice
Samples
Activated
charcoal
Lower layer of ice

Figure II-2

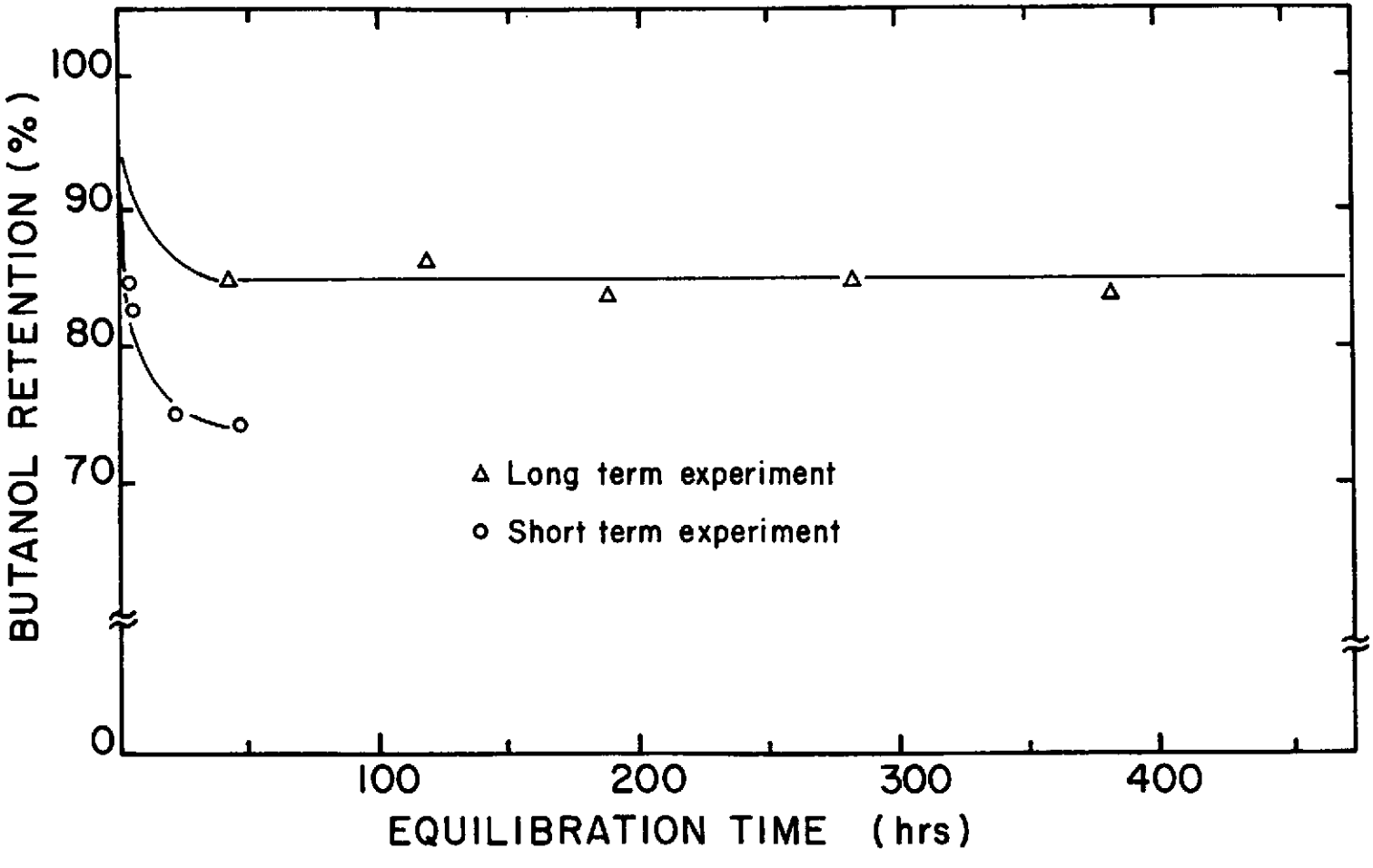

BUTANOL RETENTION (%)
100
90
80
70
0
100
200
300
400
EQUILIBRATION TIME (hrs)
Long term experiment
Short term experiment

Figure II-3

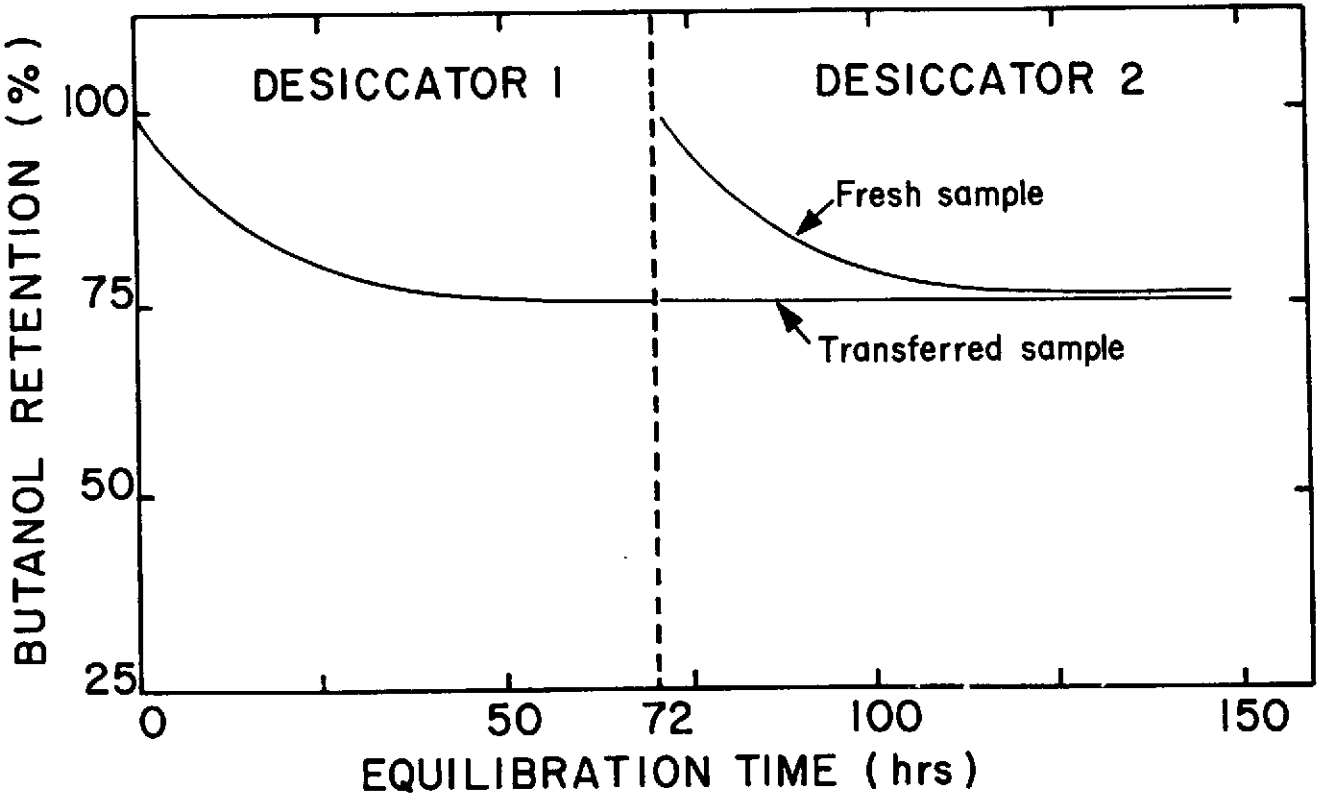
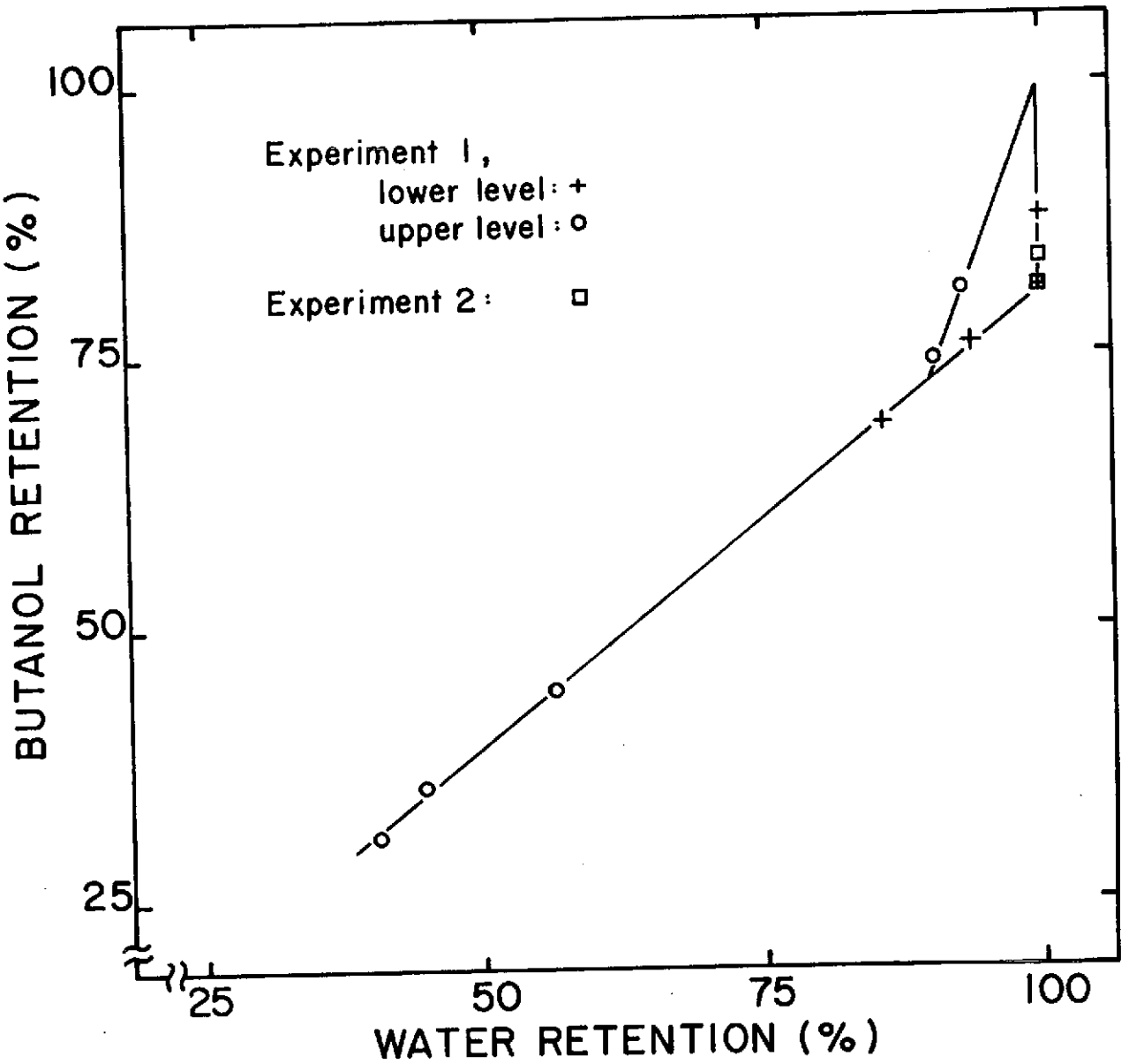

DESICCATOR 1
DESICCATOR 2
Fresh sample
Transferred sample
BUTANOL RETENTION (%)
100
75
50
25
0
50
72
100
150
EQUILIBRATION TIME (hrs)

Figure II-4


Experiment 1,
lower level: +
upper level: o
Experiment 2: □
BUTANOL RETENTION (%)
100
75
50
25
25
50
75
100
WATER RETENTION (%)

Volatile Transport in Frozen Aqueous Solutions (2): Influence of System Parameters[1]

DENNIS LAMBERT*, JAMES FLINK and MARCUS KAREL

Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

*Present Address: 21 Rue de Bourgogne, 75-Paris-7, France

INTRODUCTION

Freezing prolongs storage life of foods by preventing microbiological and chemical deterioration. The flavor quality often is inferior to that of fresh foods, due to the loss of volatiles. Even at subfreezing temperatures, some flavor volatiles have high vapor pressures and can be lost during frozen storage (3).

Steijvers (6) studied the loss of alcohols from frozen aqueous malto-dextrin solutions. He found that a fraction of the alcohol is available for loss and that the amount of alcohol lost increased with an increase of the frozen storage temperature. Gejl-Hansen (2), measuring the loss of hexanal from frozen aqueous malto-dextrin solutions, found similar results.

Recently, Lambert *et al* (4) studied the loss of n-butanol from frozen aqueous solutions equilibrated over activated charcoal in the presence of air. The demonstrated results are similar to Steijvers and Gejl-Hansen in the absence of malto-dextrin. They postulated that the fraction of butanol lost during frozen storage corresponds to pure butanol separated from the aqueous solutions during freezing and located at the last frozen part of the sample, the free surface layer. The other fraction of butanol, retained by the frozen samples, was assumed to be concentrated in the

interdendritic unfrozen phase during freezing.

As a consequence of the above mechanism, system parameters which influence the redistribution and phase separation of solutes during freezing of aqueous solutions can be expected to influence the butanol retention during frozen storage of aqueous solutions.

As an ice crystal grows there is rejection of solute from the moving interface into either the bulk or interdendritic liquid which leads to solute redistribution during freezing. Taborsky (7) has studied the redistribution of solute and found that the degree of non-uniformity after freezing is a function of the solute itself and its initial concentration. Entrapment of solute by the ice phase due to constitutional supercooling is a consequence of both solute rejection and thermal behavior associated with fast freezing (1).

As a result of the rejection and entrapment of solute by the ice phase upon freezing, volatile retention during storage of frozen aqueous solutions should be dependent on the volatile initial concentration and on the freezing conditions.

The objective of this work is to study the effect of the system parameters on the retention of n-butanol from frozen aqueous solutions equilibrated over activated charcoal at constant temperature and in the presence of air.

EXPERIMENTAL TECHNIQUES

The detailed experimental techniques have already been presented (4). The loss of n-butanol from thin slabs of frozen aqueous solution (initial concentration 0.8%) was measured during storage over activated charcoal in desiccators held in a cooling bath at the desired sub-zero temperature. Samples were removed from the desiccators at regular intervals, thawed and analysed for butanol (gas chromatographically) and water (gravimetrically).

RESULTS AND DISCUSSION

When frozen solutions consisting of n-butanol and distilled water are equilibrated over activated charcoal at sub-freezing temperatures, a certain fraction of the butanol is lost. A fraction is retained throughout storage, provided that the water content stays constant. Evidence that the fraction of butanol is located in a surface layer of the sample and exhibits a behavior similar to vapors adsorbed on ice has been reported (4).

Fundamental to the presentation is the time dependence of the loss of butanol. It has been shown that the loss of butanol is a gradual process, equilibration taking place over a period of 24 hr. Following this initial loss, the butanol content remained constant for a period of over 350 hr [Figure 2, (4)].

The influence of the equilibration temperature is shown in Figure 1. The amount of butanol lost increases with an increase of the equilibration temperature, confrming Steijvers and Gejl-Hansen's results. If a surface layer mechanism is considered to describe the loss of butanol, the effect of equilibration temperature on butanol retention must be related to the effect of temperature on ice butanol surface layer structure interrelationships. It may be that the extent of communication between the surface layer and the environment will depend on the equilibration temperature. Vapor pressure effects will also lead to increased loss at higher temperatures.

Following the water independent butanol loss, further butanol loss is linearly related to water losses. This fraction of butanol can not be lost without breakdown of the dendritic structure and simultaneous loss of water. The effect of the equilibration temperature on the loss of butanol due to the loss of water is shown in Figure 2. The ratio of the butanol loss to the water loss increases with increasing equilibration temperature. Thus, as the temperature increases, both the water-independent butanol loss and the water-dependent butanol loss increase. The influence of freezing rate on butanol loss was investigated for two freezing temperatures, -10°C and -196°C (liquid

nitrogen). The samples were equilibrated over activated charcoal at -10°C in both cases. Results are shown in Figure 3. The loss of butanol is higher for slowly frozen samples.

During freezing of aqueous solutions, solutes are rejected from the growing ice crystals into the unfrozen liquid. Entrapment of solute by the growing ice crystals will depend on the freezing rate for a given initial solute concentration. During slow freezing, more butanol is rejected from the ice-liquid interface and thus more butanol is available for phase separation at the sample surface layer during the last stages of freezing. Conversely, fast freezing promotes solute entrapment by the ice phase and less butanol is available for phase separation. Thus fast freezing will result in a lower butanol concentration in the surface layer and lower loss.

The effect of butanol concentration was studied using two sets of samples, one three times more concentrated (2.37%, wt/wt) than normally used (0.80%, wt/wt). The results (Table 1) show that the butanol retentions (expressed as percentage of the initial butanol content) were the same for both samples, indicating that the amount of butanol lost was directly proportional to the initial concentration.

During freezing, the butanol is moved from the growing ice crystals into the unfrozen phase. It must be assumed

that for the concentrations studied, no preferential solute entrapment by the ice phase occurs for either concentration. In this case, the amount of butanol rejected into the unfrozen solution is proportional to the initial concentration and so also the amount of butanol available for phase separation at the sample surface layer during the last stages of freezing. This then determines the subsequent loss during equilibration.

The effect of sample thickness on the loss of butanol has been reported earlier [Table 2, (4)]. The *amount* of butanol lost during frozen storage was shown to be approximately the same for the three thicknesses investigated. This was postulated to result from the fact that the same solute redistribution effect takes place during freezing, independent of the sample thickness.

Rohatgi and Adams (5) found that the freezing time of samples, subjected to undirectional freezing conditions, is directly proportional to the square of the sample thickness. It might be considered that thicker samples should lose more butanol, due to a lower freezing rate. For the sample thickness and freezing conditions utilized, the sample thickness does not significantly affect the freezing rate or solute entrapment by the ice phase.

These results are consistent with the mechanism previously presented. We postulated that, due to freezing,

a fraction of the initial butanol (which is lost upon equilibration) is rejected from the ice crystal interface and undergoes phase separation during the last stages of the freezing. Pure butanol is thus separated from the freezing aqueous solution and is in effect entrapped or sorbed in a porous structure of the sample surface layer. The other fraction of the initial butanol is postulated to be concentrated in the interdendritic unfrozen liquid. This fraction is strongly entrapped by the frozen samples and is retained throughout equilibration.

SUMMARY

Frozen aqueous butanol solutions are evaluated for the influence of sample preparation and equilibration conditions on the loss of butanol.

It is shown that an increase of equilibration temperature results in increased butanol loss, both water-independent and water-dependent. The freezing rate will influence the equilibration retention level, with faster freezing resulting in smaller butanol loss. An increase in butanol concentration gave the same percentage butanol loss, i.e. the amount of butanol lost was directly proportional to the initial concentration.

FOOTNOTE

[1]This work was supported in part by The National Aeronautics and Space Administration/Manned Spacecraft Center Contract No. 9-12485.

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TABLE 1

EFFECT OF SAMPLE CONCENTRATION ON THE LOSS OF BUTANOL

Equilibration Time (hours)	Butanol Retention (%)	
	0.80% solution	2.37% solution
3	89	89
25	83	84
49	75	82
117	82	78
141	79	84
166		78
Average	80%	81%

FIGURE LEGENDS

Figure 1

Effect of Equilibration Temperature on Butanol Retention.

Figure 2

Effect of Equilibration Temperature on Water and Butanol Retention.

Figure 3

Effect of Freezing Rate on the Loss of Butanol.

Figure II-1

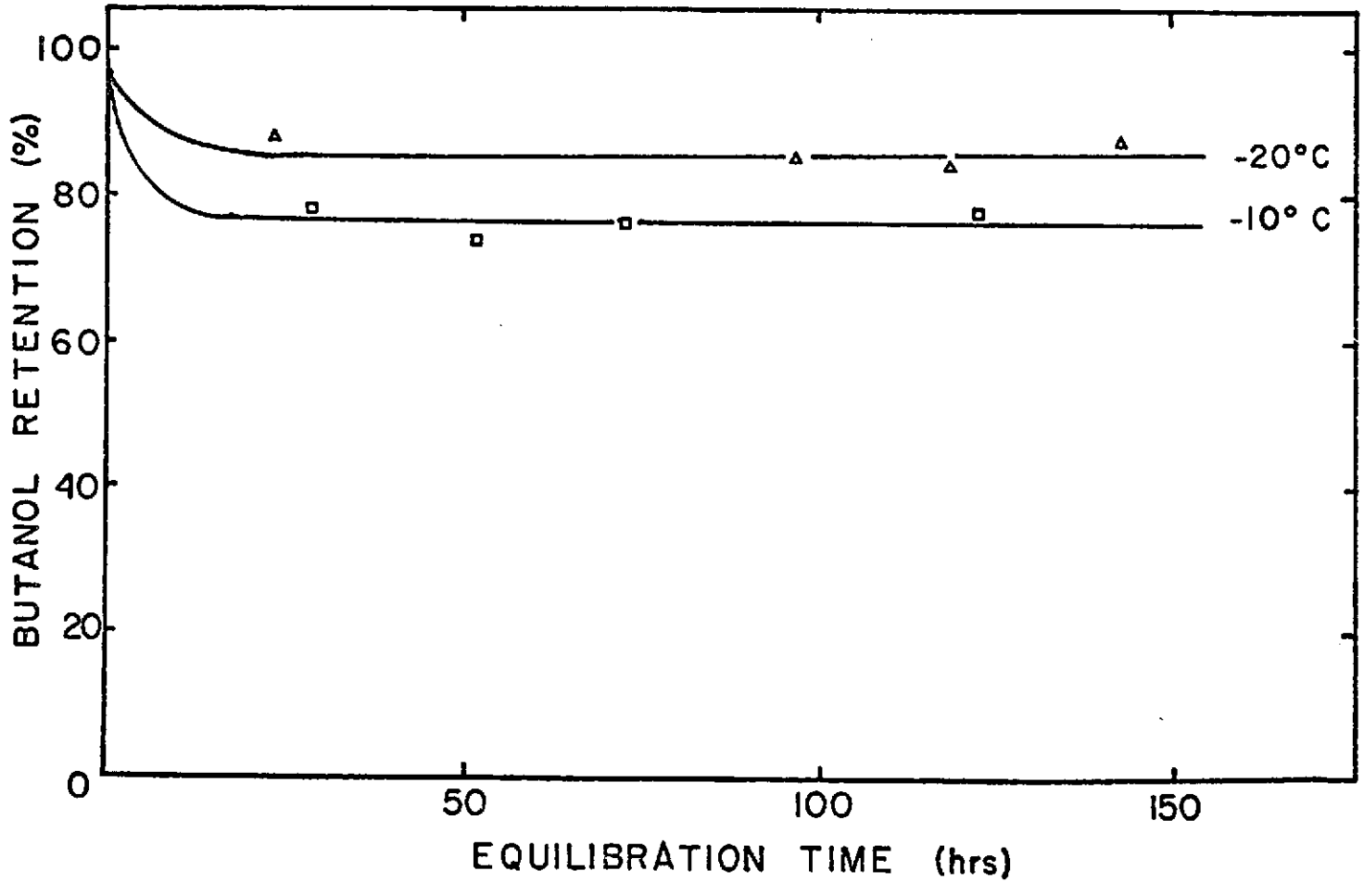

100
80
60
40
20
0
BUTANOL RETENTION (%)
50
100
150
EQUILIBRATION TIME (hrs)
-20°C
-10° C

Figure II-2

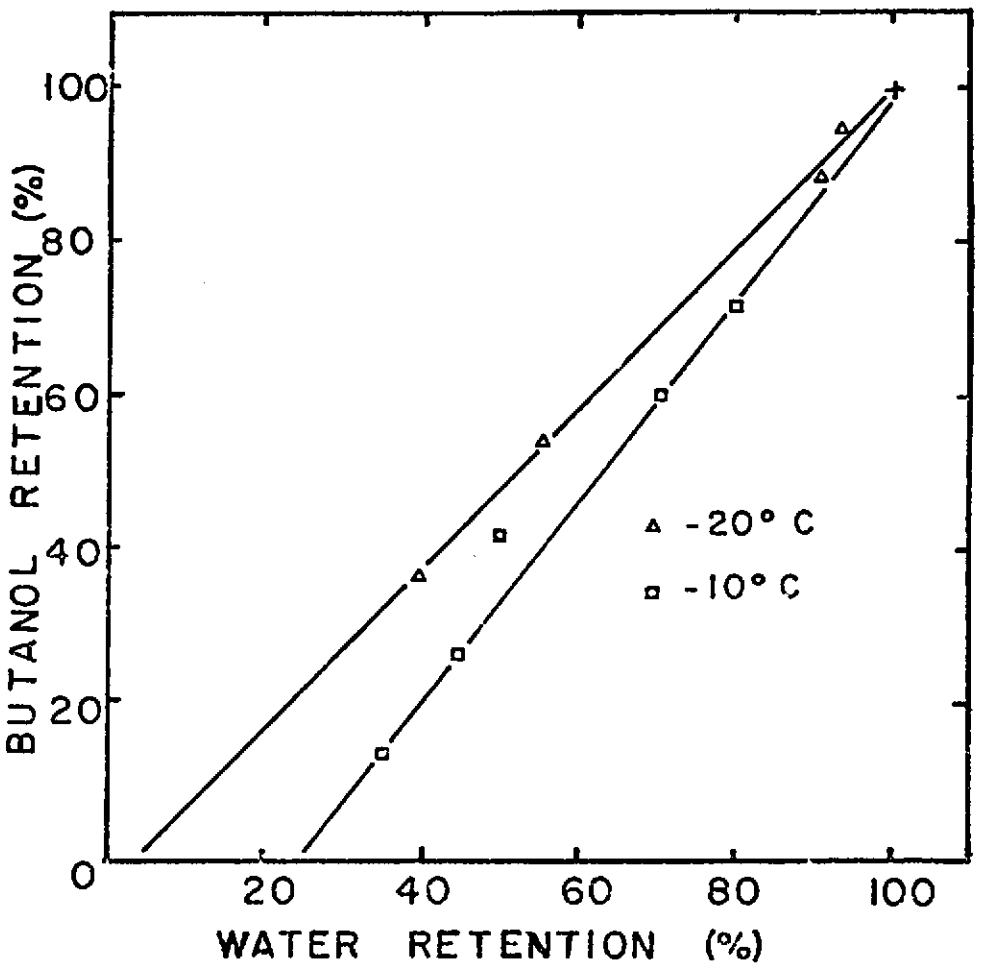
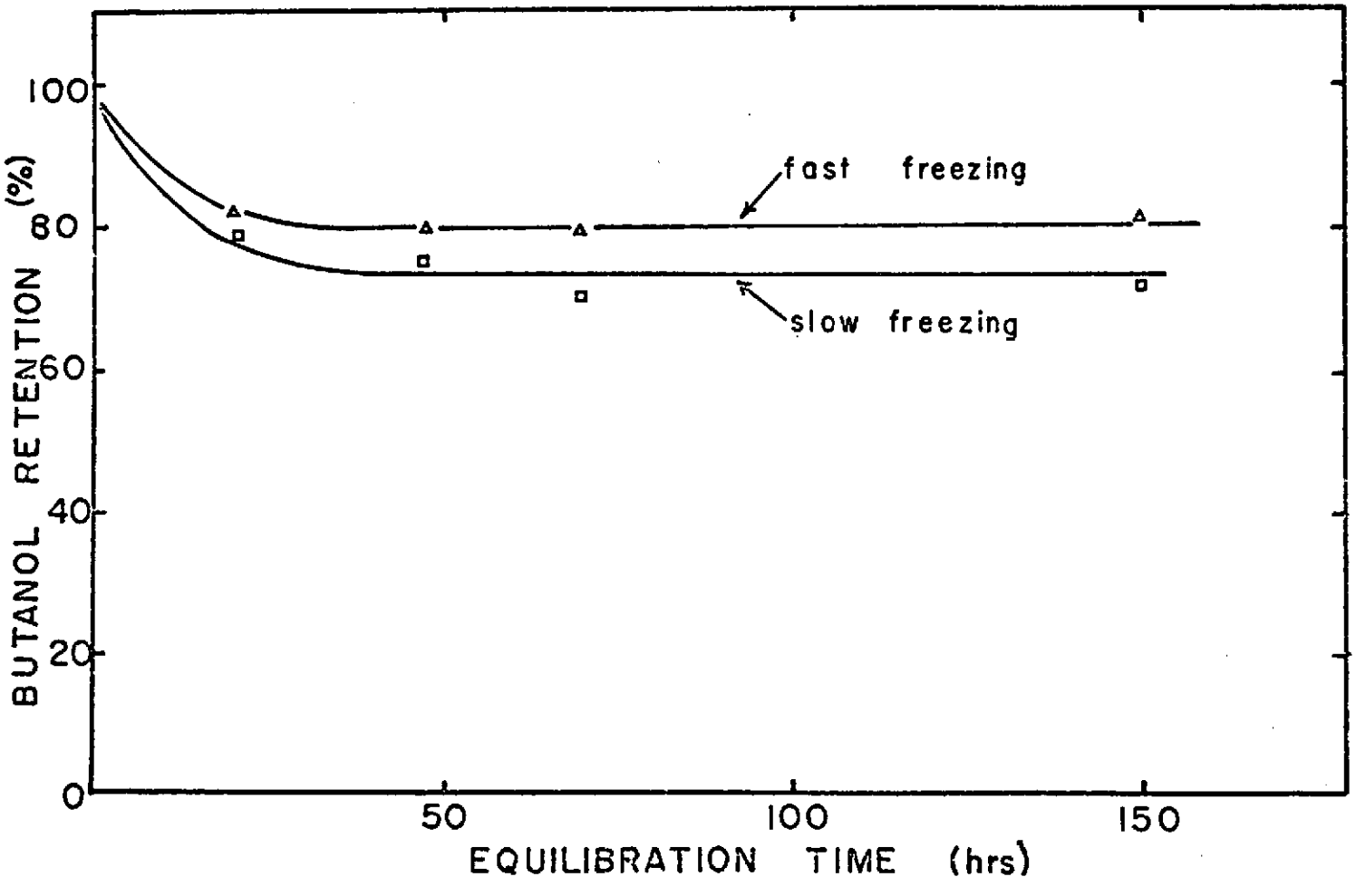


Figure II-3


100
80
60
40
20
0
BUTANOL RETENTION (%)
fast freezing
slow freezing
50
100
150
EQUILIBRATION TIME (hrs)

III. The Freeze-Drying Microscope

III.A. Introduction

Aqueous solutions of organic solutes are the basic fluids of biological systems. The behavior of these solutions during lowering of the temperature to a level at which the solvent, water, starts to crystallize as ice is important in determining changes which will occur in these systems. The continuation of this freezing phenomenon, with the increased conversion of liquid water to ice, results in the eventual solidification of the aqueous solution as a relatively complex system of ice crystals, concentrated solute phase or phases and perhaps even pure solute phases.

For simple systems the interrelationships among these various components are presented in the form of phase diagrams. Complete phase diagrams for complex, aqueous solutions containing organic solutes have generally not been experimentally determined. To date the major interest in the freezing of aqueous solutions has centered almost completely around the crystallization of the water molecules and the subsequent differences in ice species which result. The importance of considering the presence of the solute species and their participation in the freezing process is now becoming apparent.

III.B. Literature

III.B.1. Freeze-drying microscope systems

Recent work illustrated the value of microscopic techniques when qualitative and semi-quantitative information

is needed on freeze-dried systems (Flink and Gejl-Hansen, 1972). Standard analytical techniques, based on the use of the microscope have been developed in many fields, most notably biology, microbiology, chemistry, mineralogy, metallurgy and material science. (Gejl-Hansen, 1972). A short review article by Lyne and Moss (1971) gives particular examples (with references) of microscope utilization in these areas.

Interest in the field of cryobiology, the study of the changes accompanying the freezing of biological cells and cell constituents, resulted in the development of various freezing (and sometimes also freeze-drying) microscope stages so that the test systems could be observed continuously during the experimental processes (McCrone and O'Bradovic, 1956; MacKenzie, 1964; Chauffard, 1971; Freedman et al, 1972). Many studies have been reported using this type of equipment, but they have dealt almost exclusively with investigations on the conditions which affect the development and behavior of the ice phase, (Luyet, 1968). Recently, the use of a freezing and freeze-drying microscope for the study of solidification of organic mixtures and aqueous solutions of inorganic salts has been reported (Rey et al, 1966). In some instances these freezing investigations have been a part of overall studies into mechanisms characterizing the freezing and freeze-drying of aqueous solutions.

III.B.2. Volatile Retention During Freeze-Drying

In a continuation of studies on the desorption of volatile organic constituents from aqueous carbohydrate systems during freeze-drying (Flink and Karel 1969, 1970a, 1970b), microscopic analysis of the freeze-dried material showed changes which had taken place in the system during the freezing and freeze-drying (Flink and Gejl-Hansen, 1972). Most notable were the partitioning of the carbohydrate non-volatile solute (Malto-dextrin) into two fractions, and the appearance of the volatile organic constituent (hexanal) as liquid droplets. Quantitative gas chromatographic evaluation of the concentration of the volatile organic species remaining in the carbohydrate matrix following freeze-drying agreed with concentrations calculated from the micrographs assuming that all the retained volatile is present as the liquid droplets. It could therefore be concluded that the formation of the liquid droplets and their partitioning into carbohydrate rich phase were very important factors in accounting for the retention of the volatile organic compound during freeze-drying. It has not been demonstrated during which part of the freeze-drying process the partitioning occurs, but it can be presumed that the solute separations happen in the course of the freezing. It has been shown that the freeze-drying process parameters themselves do exert an influence on the retention of organic volatiles; however, the freezing conditions are usually noted to be of primary importance provided that

the subsequent freeze-drying occurs without melting or structural collapse (Flink and Karel, 1970b; Flink and Labuza, 1972; Rulkens and Thijssen, 1972; Bellows, 1971; Ettrup-Petersen et al, 1973).

III.C. The Freeze-Drying Microscope

A freeze-drying microscope, which is capable of being used for continuous observation of the freeze-drying sample at magnifications up to 600x has been developed for use in investigating the separation of components of aqueous solutions during freezing which will provide needed information in the areas of organic solute concentration during freezing and organic volatile retention during freeze-drying. This high magnification has been shown to be essential when it is desired to investigate the formation and separation of droplets within the non-volatile solute matrix.

At present, the freeze-drying microscope consists of three systems which can be considered independently. These are:

1) The microscope system
2) The freeze-drying system
3) The data acquisition systems.

III.C.1. The microscope system

The freeze-drying microscope is based on Olympus Model EH microscope body. This microscope is a modular design and though basically intended for biological use is easily adapted for use in almost any mode such as light

field, phase contrast, transmitted illumination, incident illumination, polarized light etc. Choice of this microscope gave an economical instrument which was adaptable to changing analytical requirements.

The microscope is equipped with the following special purpose options:

A) Trinocular head giving either binocular visual or monocular photographic or video observations

B) Wide field eyepieces (15x) and a flat field photographic eyepiece (15x)

C) In addition to the normal dry objectives of 4x, 10x, and 60x,and 40x objective having a working distance of approximately 1.3 mm.
This means that the distance between the sample and front surface of the objective lens will be 1.3 mm, a very important consideration with the design of the allowable dimension of the freeze-drying chamber.

D) A polarizing filter set is available. The analyzer is permanently mounted within the trinocular head while the rotatable polarizer is mounted below the specimen stage when crystallinity determinations are to be made.

E) An illumination system which can be regulated in intensity and focused to give Kohler's illumination.

F) The microscope sits on a special vibration damping support. This support rests on a specially constructed heavy weight table having individually adjustable legs for leveling purposes.

III.C.2. The Freeze-Drying System

The freeze-drying system consists of a freezing and freeze-drying microscope stage specially designed and constructed in this laboratory for use with the Olympus microscope, and the associated support equipment.

a) The freeze-drying chamber is a vacuum-tight optical system which is capable of being refrigerated (Fig. III-1). Three glass windows provide for sample holding (2) and vacuum seals (1,3). These are set into recesses milled into an aluminum block. The block also contains an internal path for the flow of chilled refrigerant (4,5) as well as a vacuum passage to the lower vacuum chamber (6). The glass sample holder has a large contact area with the aluminum block at the refrigerated end, giving a large heat transfer surface. It is sometimes necessary to prepare several sample holders before finding one giving good thermal contact. A gap is left between the sample holder and one wall of the block so that an open path is available for the flow of air and water vapor to the lower chamber and out the vacuum line. The lower window is semi-permanently sealed to the block with Apiezon putty. The upper window, a 0.17 mm thick cover glass (45 x 50 mm) rests on a rubber O-ring (7) which is held in a circular shape with an aluminum ring (8). The total thickness of O-ring and cover slip is less than 1.3 mm, allowing use of the long working distance objective.

Chips of a cover slip are placed at the extremities

of the sample holder to support a cover slip for the sample at an approximate distance of either 170 μm (1 chip) or 340 μm (two chips). (Fig. III-2). This enables control of the sample thickness which is optically important.

b) A dry ice-alcohol refrigeration system is used to provide refrigerating capacity for freezing and temperature maintenance during freeze drying. (Fig. III-3). A bottom emptying flask maintains a flooded suction head on the centrifugal pump. Fluid is pumped through a copper coil immersed in a dry ice-alcohol bath and then to the freeze-dryer, before returning to the suction line reservoir. Temperature can be regulated either by on-off cycles of the pump, or by control of the pump speed.

c) Vacuum - A single stage rotary oil vacuum pump is used in conjunction with a $CaSO_4$ desiccant vapor trap to remove fixed gases and water vapor from the freeze-drying chamber.

d) Condensation prevention system - Dried compressed air is gently blown across the upper cover slip window to prevent condensation of environmental water vapor. The lower glass surface is within an insulation system and does not suffer condensation problems.

e) Insulation - A carved, balsa wood container is used for thermal insulation. This container is attached to the motion controls of the microscope stage to give scanning ability of the freeze-drying sample.

III.C.3. Data Acquisition Systems

Evaluation of freezing and freeze-drying experiments requires the measurement of temperatures and pressures as well as documentation of visual observations. The following systems supply this information:

a) Temperatures are measured by insertion of a microthermocouple junction into the sample. The junction diameter is 125 μm. The thin thermocouple wires (50 μm) are passed between the O-ring and aluminum block of the freeze-dryer without loss of vacuum. Readout is with either meter, recorder or a temperature controller monitor circuit.

b) The system pressure is measured on the vacuum line using a thermocouple type vacuum gauge having a range of 0-20 torr.

c) Photographic records of typical visual observations are made using a Polaroid ED-10 microscope camera. Photographs supplied with this report were made with this camera.

d) To improve observational capabilities a closed circuit television system with video recording capabilities is being tested prior to installation. Initial tests demonstrated many advantages of this system. Permanent, publishable records will be made by photographing the television monitor.

III.D. Methods of operation

1) Preparation of model system

An aqueous solution is prepared according to a standardized procedure. This is especially important when utilizing components of limited solubility. The model system is either held at preparation temperature or chilled to 0°C by holding in crushed ice.

2) Preparation of microscope equipment

The dry ice-alcohol cooling system is prepared and the microscope stage connected to the cooling system. The freezing stage may be precooled to 0°C at this time, if desired. Dehumidified air sweeps the stage to prevent condensation of water vapor.

3) Sample freezing

The cooling system flow is adjusted to give the desired freezing conditions at the microscope stage. Freezing progress is followed either visually or photographically.

4) Subsequent steps prior to freeze-drying

Upon the completion of the first freezing analysis, the sample can either be freeze-dried or thawed and subjected to further freezing analysis. The latter is of interest when studying the resolubilization of the organic constituents or the influence of freezing history on subsequent freezing and freeze-drying behavior. Thawing is accomplished by stopping the coolant flow or additionally removing the heat absorbent from the lamp of the microscope optical system.

5) Sample freeze-drying

Upon completion of the final freezing analysis, the chamber is evacuated and the frozen sample is freeze-dried. Due to the small sample dimension, relatively rapid sublimation of the ice occurs. Following drying the material can further be analyzed by more standard procedures as described by Flink and Gejl-Hansen (1972).

III.E. Results and Discussion

The scope of the experimental results obtained to date can be divided into (1) system characterization and evaluation and (2) initial stages in the study of freezing and freeze drying of aqueous solutions containing volatile organic compounds. The results section will be accordingly divided, with discussion only accompanying the data relating to the study on volatile compounds.

III.E.I. Freezing behavior

Freezing of water and aqueous maltodextrin solutions (10%) from room temperature generally occurs in 2-10 minutes. Samples have been frozen both as droplets at 1/2 to 2 mm thickness or slabs under a cover glass of 0.15 to 0.40 mm thickness. Temperature measurements indicated that the maltodextrin solution commences freezing at approximately -5°C and is completed at -7°C.

Fast freezing is characterized by solidification of the sample in less than 20 seconds (initial appearance of ice to complete solidification). The ice structure appears

as plates or sheets without fine structure. (Fig. III-10).

Slow freezing of the sample is obtained by operating at a reduced rate of cooling. The sample closest to the chilled surface will not slow freeze. Thus slow freezing is characterized by a fast freezing of a small part of the sample closest to the chilled surface followed by dendritic growth of ice crystals over a period of 1/2 to 10 minutes (Fig. III-5). Some samples which have been slow frozen in the form of thin slabs undergo dendritic crystallization in two layers, the bottom of the sample crystallizing first as disordered dendrites (Fig. III-9); the upper region crystallizing later as ordered dendrites (Fig. III-8).

III.E.2. Freeze-drying behavior

Freeze-drying is initiated by evacuation of the microscope freeze-dryer chamber to pressure levels between 0.3 to 1 torr. The flow rate of coolant is slowed at the same time. Freeze-drying fronts (the moving interface between frozen and dried regions) recede into the sample from all four sides. Samples of an approximate size 1 cm x 1 cm and thickness of 0.155 to 0.3 mm under a cover slip require between 25 to 80 minutes to freeze dry.

Separate freeze-drying fronts are observed in each of the different ice crystal orientations (and thus solute matrix orientations) in the sample by selective focusing of the microscope (Fig. III-8, III-9). The fronts are observed to be not completely planar, with small variations

occuring within crystal bundles having the same orientation, and larger variation between locations in different orientations in the vertical plane (Fig. III-6, III-7).

Sample temperature during freezing and freeze-drying is shown for thick drops (Fig. III-14). More temperature variability is noted during the freeze-drying of thin samples, perhaps relecting sudden changes in mass transfer resistances. With the passage of the interface past the thermocouple junction, temperature reading tended to oscillate. The oscillations ended when the junction was totally in the dry layer. While it is not certain yet if this behavior is normal it is possible that this is due to the relative sizes of the interface region and the thermocouple junction, and sudden changes in the balance of heat and mass transfer resistance in the interface region.

III.E.3. Freezing and freeze-drying of aqueous solutions containing volatile organic compounds

Aqueous solutions of maltodextrin (10% w/v) and hexanol (0.3% w/v) are being utilized in the freeze-drying microscope for studying the phenomena associated with freezing and freeze-drying which are responsible for retention of the volatile organic compounds in the dried material. It is observed that the initial solution contains some low number of droplets prior to cooling. During the freezing process, it is noted that the hexanol solubility limit is exceeded and many droplets of hexanol

liquid appear. These droplets of hexanol are moved by both temperature gradients and fluid flow associated with the growth of ice crystals. Figure III-4 portrays the path taken by a hexanol droplet during the concentration steps associated with freezing which results finally in the entrappment of the hexanol droplet in the interstitial solute matrix consisting of eutectic maltodextrin solution. Figure III-10 shows droplets at the grain boundaries of a completely frozen maltodextrin sample. These entrapped volatile remain stationary during freeze-drying and are found in the dry amorphous matrix in the form of droplets of an average diameter of 2 microns. The droplets are observed through the thickness of the sample (Figs. III-11, III-12 and III-13).

Experiments conducted at hexanol concentrations above (0.8% w/v) and below (0.1% w/v) that noted above showed similar behavior.

The appearance of liquid droplets of alcohols in freeze-dried aqueous malto-dextrin solutions has been related to the solubility of the volatile alcohols (Flink and Gejl-Hansen, 1972). Furthermore the influence of molecular size, solubility and concentration on retention of the volatile following freeze-drying has been demonstrated by Flink and Karel (1969, 1970a). A 0.5% (w/v) solution of a more soluble n-alcohol, n-butanol, revealed the same behavior, though the droplets formed during freezing are much smaller in size, making them more difficult to observe.

While the experimental results obtained to date with the freeze-drying microscope must be considered preliminary in nature, it appears obvious that for a number of typical volatile organic compounds of limited aqueous solubility, retention in freeze-drying is in the form of liquid droplets which are for the most part formed during cooling and freezing and entrapped in the interstitial matrix after freezing. These droplets of volatile compounds are locked into the dry material following the freeze-drying step.

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Les lyophilsations complexes in "Advances in Freeze-drying" (L. Rey, ed) Hermann, Paris.

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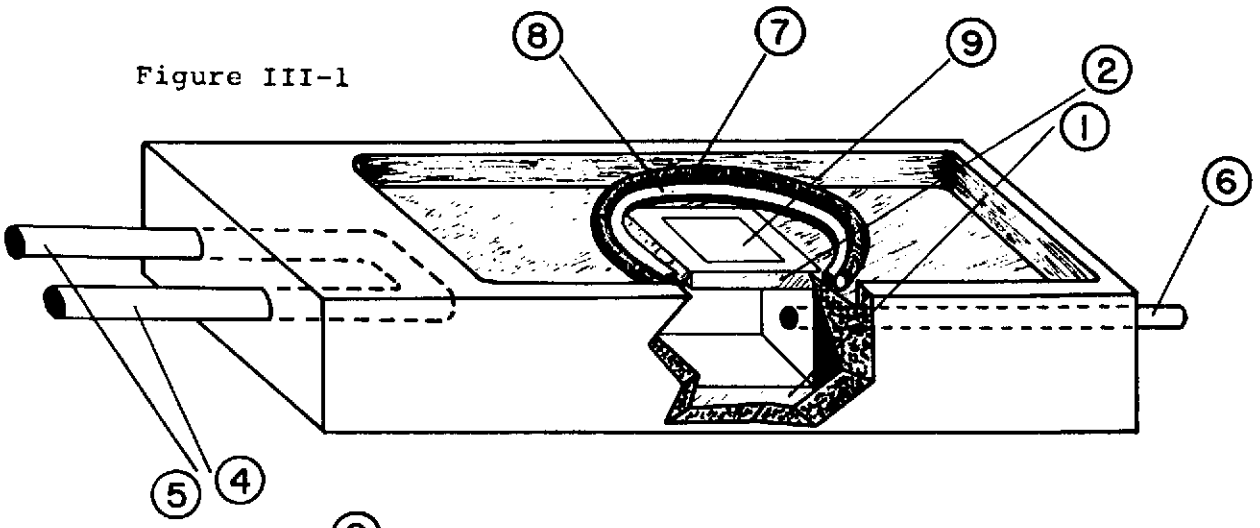
Figures

Figure III-1



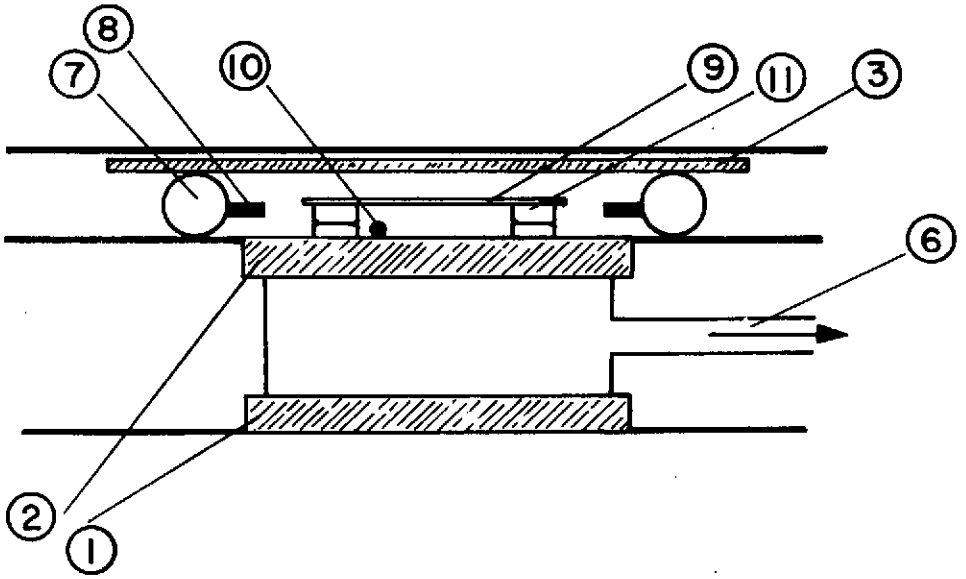


Figure III-2

① ② GLASS WINDOWS

③ GLASS COVER SLIP

④ ⑤ COOLING INLET AND OUTLET

⑥ VACUUM PASSAGE

⑦ RUBBER O-RING

⑧ ALUMINUM RING

⑨ SAMPLE COVER SLIP

⑩ THERMOCOUPLE JUNCTION

⑪ GLASS CHIPS

F

Figure III-3

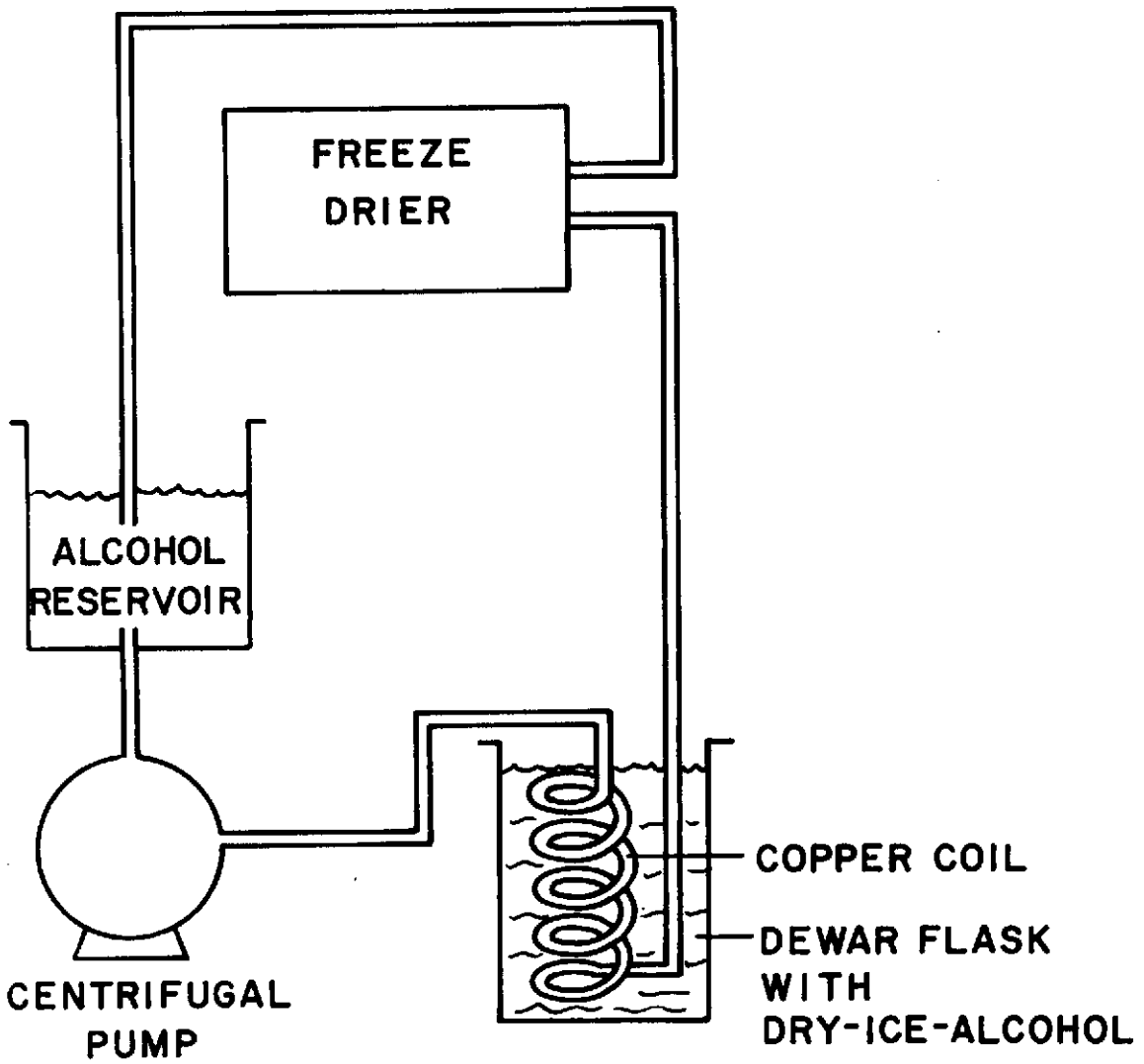
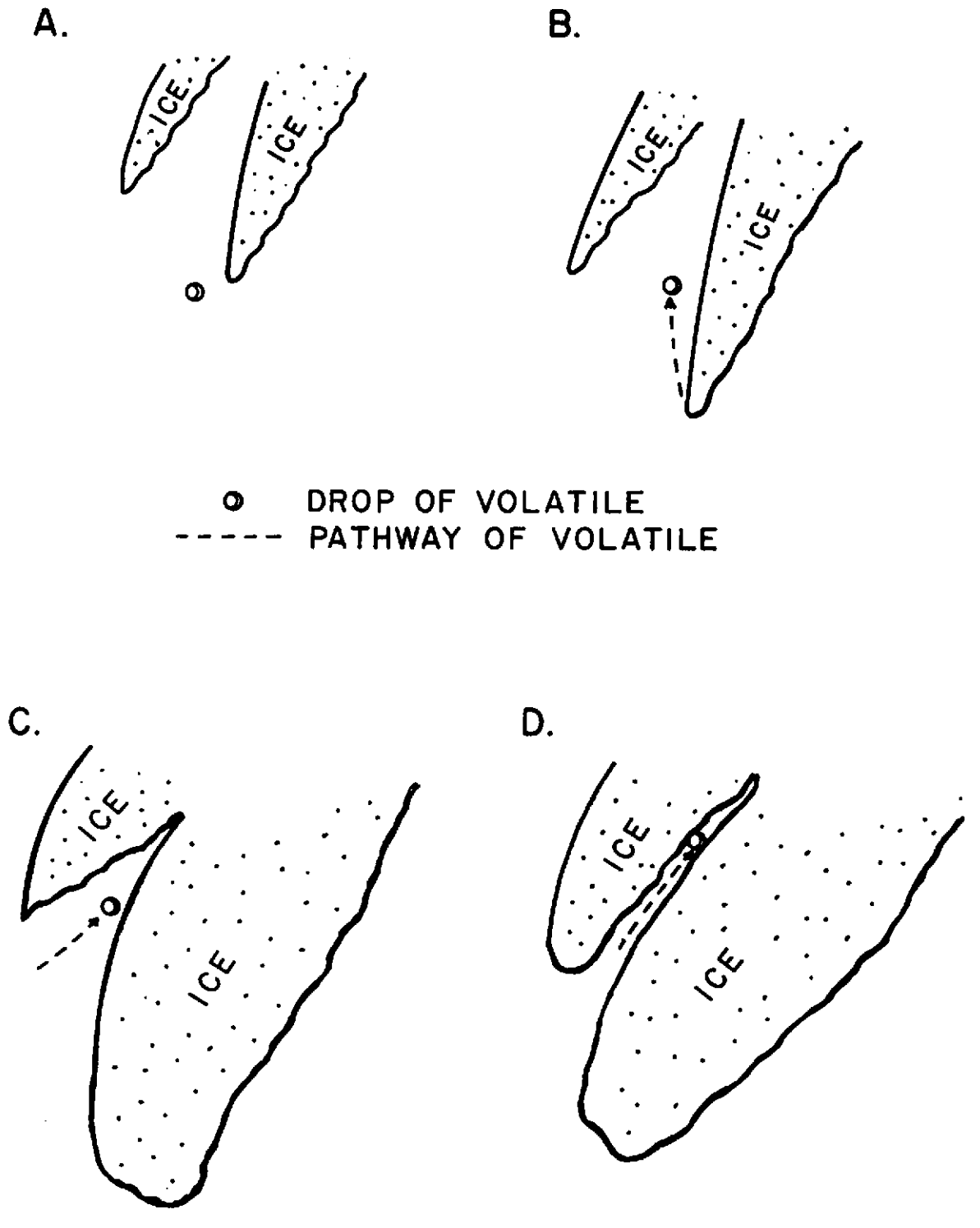

FREEZE
DRIER
ALCOHOL
RESERVOIR
COPPER COIL
DEWAR FLASK
WITH
DRY-ICE-ALCOHOL
CENTRIFUGAL
PUMP

Figure III-4


A.
ICE
ICE
B.
ICE
ICE
DROP OF VOLATILE
PATHWAY OF VOLATILE
C.
ICE
ICE
D.
ICE
ICE

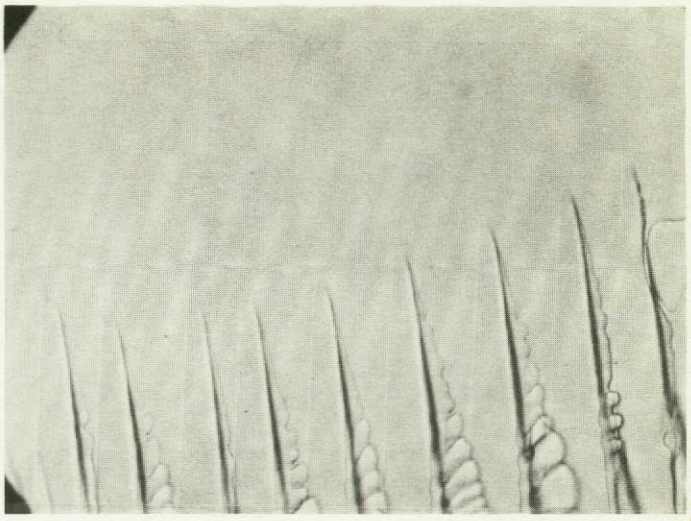


Figure III-5

100μ

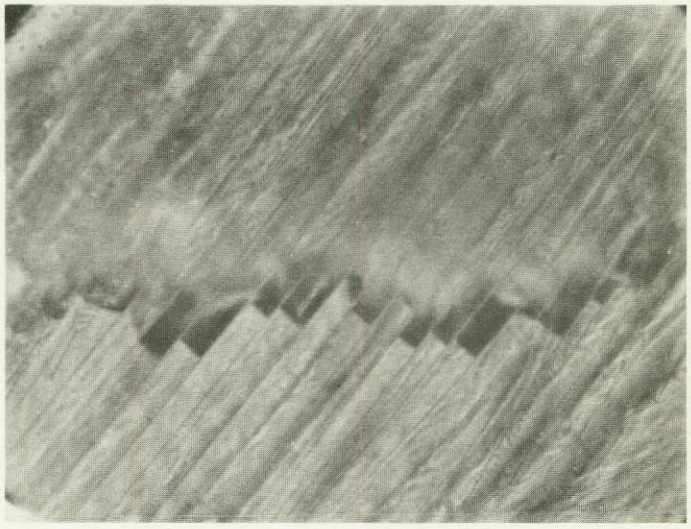


Figure III-6

100μ

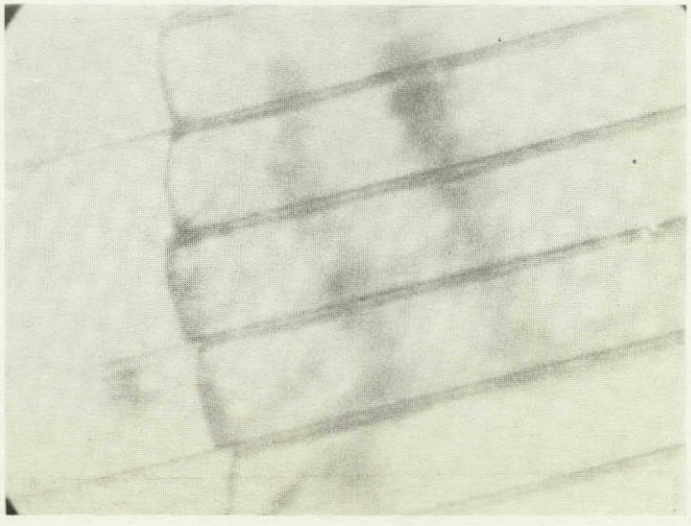


Figure III-7

10μ

Figure III-8

100μ

Figure III-9

100μ

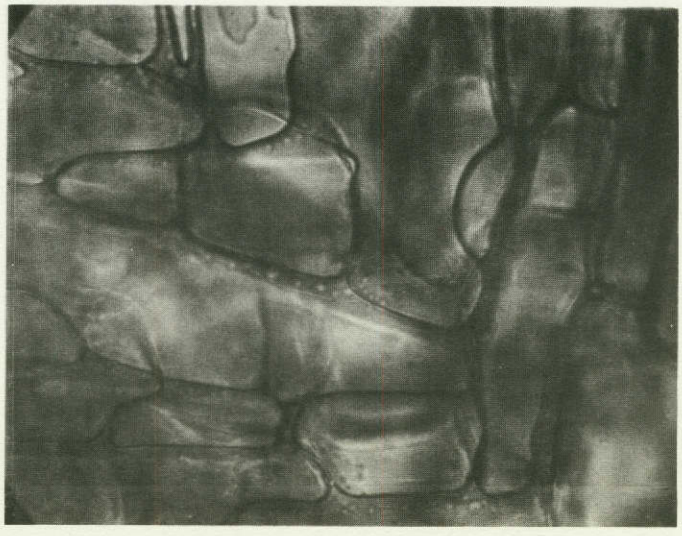


Figure III-10

10μ

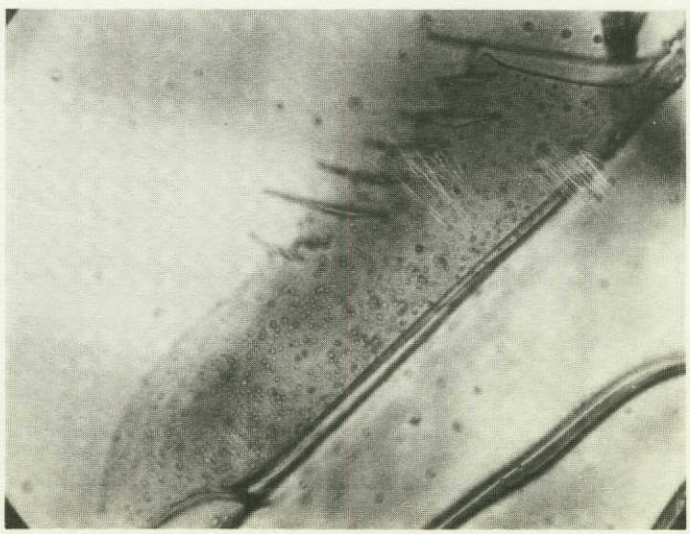
Figure III-11

100μ

Figure III-12

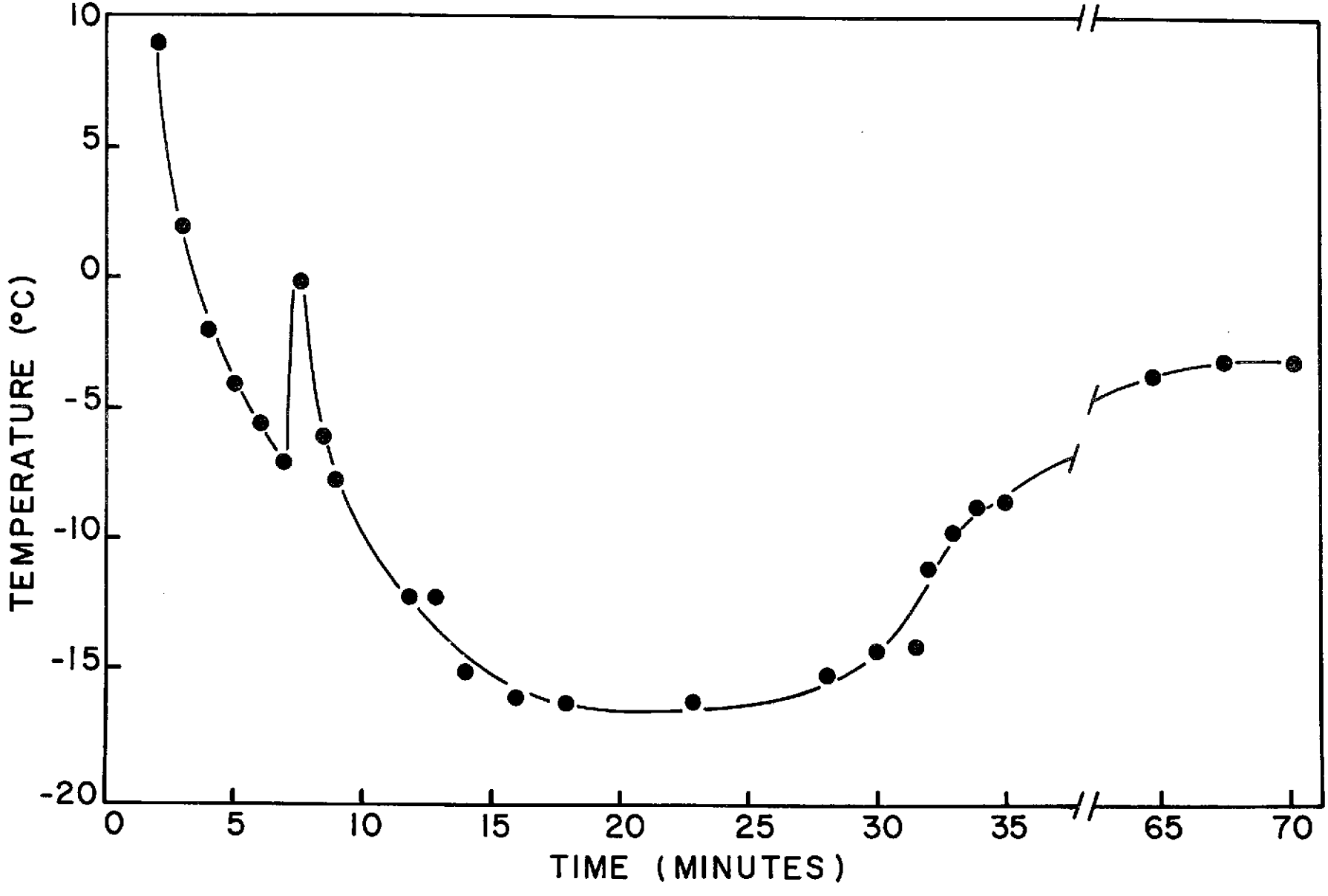
100μ

Figure III-13



10μ

Figure III-14



IV. Browning of Dried Model Systems Heated by Radiation

IV. A. Introduction

The preparation of high quality freeze-dried foods requires control of the total freeze-drying process. One step of freeze-drying in which the product is increasingly susceptible to nutritional and organoleptic deterioration is the second (desorption) period. During this time the product temperature rises and chemical changes, particularly non-enzymatic browning, can occur. Processing conditions must be chosen such that deleterious reactions will not have sufficient time to act at the temperatures attained in the essentially dry material. Thus, it is essential to develop temperature-time relationships of the non-enzymatic browning reaction during heating of food systems under conditions of vacuum and radiant heat transfer.

Most dehydrated foods, and practically all intermediate moisture foods are subject to non-enzymatic browning. The water-dependence of this reaction is well known and it invariably shows a maximum reaction rate at intermediate moistures. Our recent research in this area (Eichner and Karel, 1972; Labuza et al. 1970) indicates that these effects are due to the dual role of water which acts as solvent, but is also a product of the reaction and hence inhibitory. At low water activity the limiting factor is lack of adequate mobility, therefore addition of water, which adds solvent power, promotes the reaction. At high water contents, however the dilution of reactants by water, and the product-inhibition of condensation

steps by water predominate, and water strongly inhibits the browning. The exact position of browning maxima depends on specific products, but generally concentrated liquids (for instance unfrozen fruit concentrates) and intermediate moisture foods (for instance fillings, and so called evaporated fruit such as prunes) are in the range of moisture content most susceptible to browning. Similar behavior of water was observed in other chemical reactions in foods. In lipid oxidation water has a double role as "mobilizer" of catalysts and inhibitors, and as modifier of catalytic or inhibitory activities of various substances present in food, including metals (Heidelbaugh and Karel, 1970). The role of water in imparting mobility has been recently given the name of "mobility catalyzer" by Chirgadze and Owsepyan (1972), who studied peptide structure rearrangements.

During the secondary drying (desorption) process in freeze drying the food materials are often in a temperature and moisture content range, which allows rapid browning. Relatively little work, however, has been devoted to the systematic study of browning in this period, and in general the information regarding the influence of time and temperature during freeze-drying on deteriorative reactions is limited. Kluge and Heiss (1967) have measured rates of browning of glucose-glycine model systems supported on cellulose powder. Using an analogy to thermal sterilization processing, they develop simplified expressions

for the extent of browning as a function of temperature and relative humidity, and use this to calculate the times at which specific levels of browning will be reached. The lowest moisture content for this study was 1.5%. Tolerances of some protein materials to heating (but not necessarily during freeze-drying) have been reviewed by Bender (1966)(1972). He notes that Greaves, Morgan and Lovern (1938) report that casein heating at 100-110°C shows no degradation, while heating to 120°C for 8 hours is equivalent to 2 hours at 130°C. Further, the damage at a fixed temperature is proportional to time. Bender further notes that moisture content is important, with dry foods suffering little protein damage. Relating work by De Grost (1963) on comparative drying methods, little damage during freeze-drying was reported for the proteins of beef, fish or other protein-rich products.

Holmes (1970) studied flavors produced by non-enzymatic browning reactions during heating of model substances at elevated temperatures, and the destruction of amino acids during this reaction. Most of his work, however, was conducted at temperatures in excess of those typical in secondary drying, and does not include water content as a variable. This study, therefore, while of great value in showing scope and complexity of the reaction does not provide enough data for prediction of quality losses during drying.

Brown color formation during the air drying of white potatoes was evaluated by Hendel et al (1955). They found linear relationships for the brown color formation as a function of time, though their lowest water content was 4.9%.

Most other studies on the temperature-time tolerance of freeze-dried food relates to storage stability. These are generally concerned with lower temperatures and longer reaction times than present in the freeze-drying process. Typical papers (Karel and Nickerson, 1964; Draudt and Huang, 1966) show the same relationships as observed during drying; higher temperatures giving more damage, though higher system stability at very low moisture contents.

Recent work in our laboratories also indicates the possible utilization of data obtained at several constant temperatures and humidities for calculation of reaction progress in dynamic situations, (Mizrahi et al, 1970a, 1970b) in which moisture content was increasing slowly due to infiltration of water from the environment, and due to water produced by the reaction itself. In the secondary drying period both temperature and moisture are changing, hence here too a dynamic situation will have to be considered.

IV.B. Experimental methods

The basic experimental procedures used for studying the browning of model food systems in vacuum heating is given below. Slight variations in preparation and analysis were used for the sucrose and glucose based systems. Additionally as noted below, visual assessment of browning was utilized with the sucrose-citric acid systems as the spectrophotometric tests was not sufficiently sensitive.

1) Basic procedures

The model systems were prepared by mixing the components in a high speed blender. The mixed systems were weighed into petri dishes as a thin layer (~1.5 mm), frozen by placing on a cold temperature plate at -196°C (liquid nitrogen) and freeze-dried for 2 days with the heating platens at ambient temperature and a chamber pressure of less than 100 millitorr. Upon removal from the freeze-drier samples were stored in evacuated desiccators over $CaSO_4$ until used.

The samples were heated in an equilibrated vacuum oven by placing the petri dishes on a single unheated shelf. The walls of the vacuum oven were the radiant heat sources. All samples to be processed were placed into the oven at the initial time, and as desired, samples were removed for analysis.

2) Analysis of glucose containing samples

Browning was measured by the following procedure:

a) The sample cake was crushed and mixed in the petri dish.

b) An amount of powder between 1.2 to 1.5 gms was weighed into an erlenmeyer flask.

c) An amount of water corresponding to 10 ml/0.3 gm powder was added to the flask.

d) The flask was shaken for 1 1/2 hours.

e) The contents were allowed to settle for 1/2 hour.

f) The liquid was decanted onto tared centrifuge tubes and weighed.

g) The solution was centrifuged for 20 minutes.

h) The absorbance of supernate was measured at 400 mμ using water as a blank. (Initial tests using water and extracts from unheated samples as blanks showed equivalence in trends and only small differences in absolute values).

i) The brown color is reported as optical density per 0.3 grams of solids.

3) Analysis of high-concentration systems

The brown color of the high-concentration sucrose solutions was measured by the following procedure:

a) The crushed weighed sample cake was dissolved in 26 ml of water.

b) This slurry was then filtered using No 576 filter paper to remove the avicel, leaving a clear solution.

c) The absorbance was measured at 400 mμ using the extract from an untreated sample as a blank

d) The brown color was reported as optical density per gram of solids.

4) Visual tests

Sucrose-containing samples having low optical densities were evaluated using standard triangle test procedures. Two equally heated samples and one unheated sample, or two unheated samples and one heated sample were presented to laboratory personnel. Each participant was asked to identify the odd sample with respect to color. The answers were tabulated as to the number of correct identifications, and the results evaluated for significance at the 95% level using a summed binomial distribution table.

IV.C. Results and Discussion

Several model systems were evaluated, prior to choosing one for an in-depth study. The brown color formation obtained when heating these systems is given in Table (IV-1). All the trends observable from the results presented in Table (IV-1) are more clearly demonstrated with the chosen model system and will be discussed then. It will be of interest however to note that no browning occurs in Avicel (microcrystalline cellulose) and very little browning occurs after 4 hours at 80°C when glucose and Avicel are in a mixture.

1) The model system chosen for studies on browning during the desorption phase of freeze-drying consisted of Avicel (3.3%), which served as an inert support for the reactive components, glucose (8.3%) and glycine (8.3%).

The brown color formation due to heating of this model system in a bacuum is shown as a function of time for temperatures from 55°C to 80°C in Figure (IV-1). The rate of browning which is measured from the slopes of the lines in Figure (IV-1), is presented as a function of temperature in Figure (IV-2).

Figure (IV-3) presents a plot of the log of the browning rate vs the recipricol of the absolute temperature. From the slope of this line, the energy of activation for browning due to radiant heating in vacuo is calculated to be 19 Kcal/mole. It is easily seen that the browning reaction is very sensitive to heating temperature and time, with a small increase in temperature above 70°C giving a sizable increase in browning rate. It can further be noted that there exist time-temperature combinations for the equivalent extent of brown color formation. Thus, for example, if 0.200 O.D. units/0.3 gms is acceptable for a product, the system can be processed for either 12 hours at 55°C, or 3 1/2 hours at 66°C or 40 minutes at 75°C.

It thus appears that the concept of absolute limits to dry layer temperatures during freeze-drying should be reconsidered to allow for time factors.

2) The effects of sample geometry on browning of the model system was investigated using sample thickness as the parameter of interest. Samples with thicknesses of about 1-2 mm and 6-7 mm were used. The thicker samples enabled a cross-sectional visualization of the browning

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progress with time. With the thicker samples it is observed that brown color formation begins at the free surface and progresses into the sample. A relatively sharp boundary exists between severely browned and lightly browned regions, probably refecting the dual effects of temperature sensitivity of the browning process, and conduction-heat transfer limitations within the freeze-dried sample. Figure (IV-4) shows the extent of browning as a function of time for the thick and thin samples. It is seen that thickness has only a small effect. These slight differences probably reflect the effect of thickness on interval heat transfer and local mass average temperatures.

3) Results of initial experiments presented in Table (IV-1) indicated that the extent of browning is a function of the concentration of the various components of the model system, including the inert support. With constant concentrations of glucose and glycine (each 8.3% in the solution prior to freeze-drying), avicel concentration in the range of 1-5% (in the initial solution) showed little effect on the rate of brown color formation, when compared to the respective curves for browning of 3.3% avicel systems at 65°C and 75°C, Figure (IV-5). The slightly earlier initiation of brown color formation for the 5% avicel system may be related to somewhat higher conduction heat transfer and resultant higher mass average temperature at any given time. As noted above the browning rates however are essentially equal once browning is initiated.

4) The concentrations of the reactant species, glucose and glycine, were varied, while maintaining the initial solution at a constant total concentration (19.3% w/w). It is seen in Figure (IV-6) that browning decreased greatly when the glucose/glycine ratio was decreased, indicative that glucose is the limiting reactant.

An increase in the glucose concentration to 14% (i.e. a decrease in the glycine concentration to 2%) showed no difference in brown color formation when compared to the samples which contain each at 8.3%.

5) Visual triangle test studies on the high concentration sucrose systems were conducted at 45,53,60 and 70°C for samples heated 1,2,3 or 4 hours. The results indicated that the samples heated for all times at 45 and 53°C could not be differentiated from the unheated control sample, and that all samples heated at 60 and 70°C could be differentiated from the unheated control. While it cannot be conclusively demonstrated, it appears that the browning rate must begin to rise sharply somewhere in the range of 53 to 60°C.

6) Studies to date on the browning of freeze-dried apple slices during radiant heating in a vacuum indicate that the apple slices can tolerate relatively high temperatures (70°C) for long periods of time (3 hrs) without noticeable brown color formation.

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Table I: Browning of various dry model systems when radiantly heated in a vacuum

Model System Composition[a]			Temperature (°C)	Brown Color[b] (OD/0.3 gm solids) Time (hrs) 1/2	1	2	4
25% Avicel			80	-	-	-	0.000
1% Avicel	1% Ascorbic Acid		60	-	-	-	0.000
1% Avicel	1% Ascorbic Acid		80	0.031	0.028	0.030	0.098
1% Avicel	1% Glucose		60	-	-	-	0.000
1% Avicel	1% Glucose		80	0.010	0.060	0.046	0.013
1% Avicel	1/2% Glucose	1/2% Lysine	60	-	-	-	0.000
3/4% Avicel	3/4% Glucose	3/4% Lysine	80	0.039	0.238	0.535	0.680
3/4% Avicel	3/4% Glucose	3/4% Glycine	80	0.241	0.400	1.200	1.500
"	"	3/4% Glycine[c]	80	0.087	0.850	1.500	3.100
"	"	"	60	0.000	0.012	0.029	0.119
"	"	"	40	0.000	0.000	0.000	0.000
5% Avicel	5% Glucose	5% Glycine	40	no browning			
"	"	"	80	0.141	0.700	0.850	1.480
6% Avicel	14% Glucose	14% Glycine	40	0.000	0.000	0.000	0.000
"	"	"	60	0.131	0.140	0.167	0.170
"	"	"	80	0.227	0.532	0.600	1.500
8% Avicel	8% Glucose	8% Glycine	40	no browning			
"	"	"	80	0.150	0.760	0.760	1.200
5% Avicel	75% Sucrose	2.5% Citric Acid	45	-	-	-	0.002
"	"	"	60	-	0.001	-	0.001
"	"	"	80	-	0.001	0.002	0.010
"	"	"	100	-	0.015	0.030	0.180

a) Percentage composition refers to initial aqueous solution prior to freeze-drying
b) Absorbance
c) Stirred while freezing

Figures

Figure IV-1

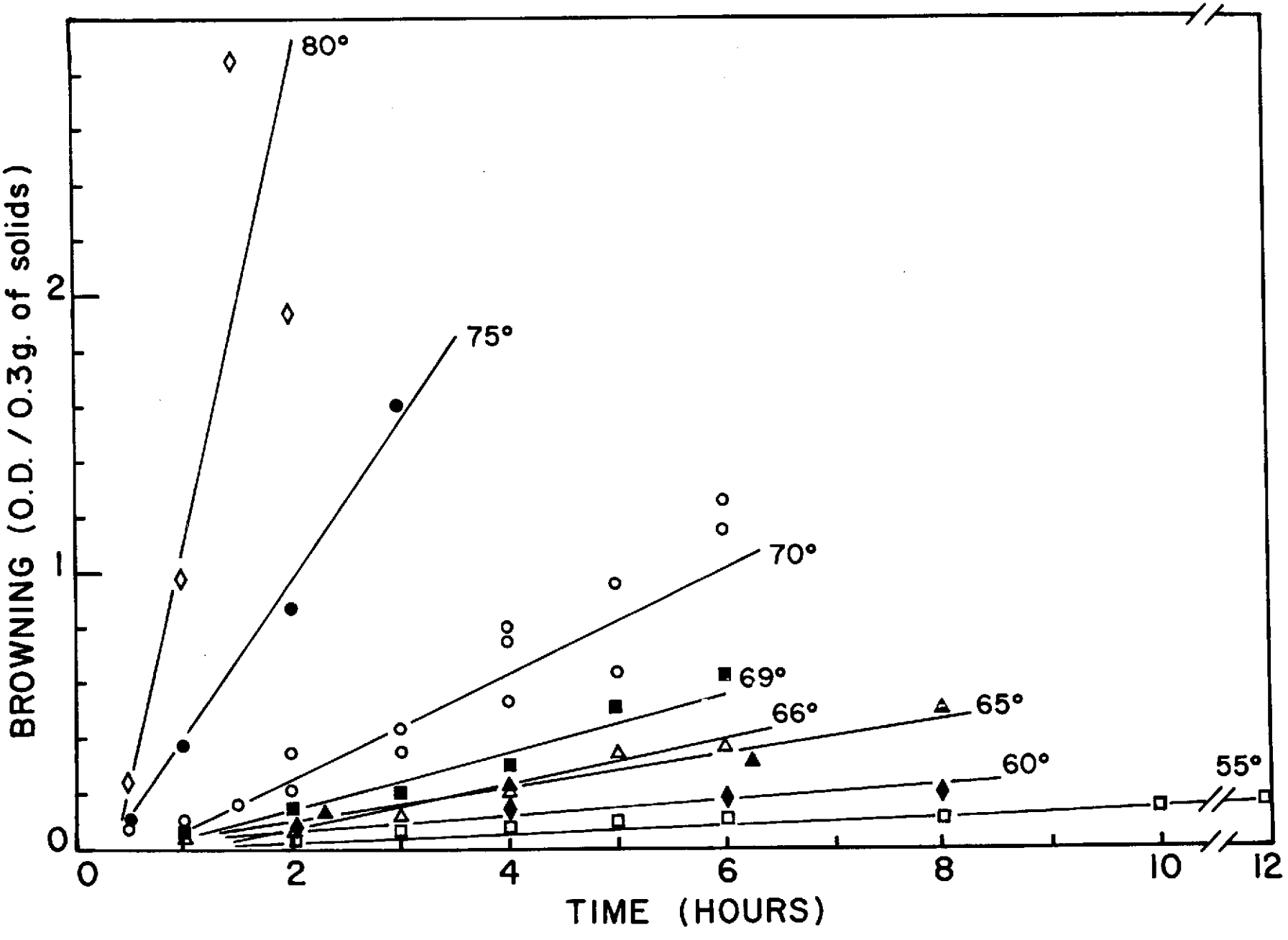

80°
75°
70°
69°
66°
65°
60°
55°
BROWNING (O.D. / 0.3g. of solids)
0
1
2
0
2
4
6
8
10
12
TIME (HOURS)

Figure IV-2

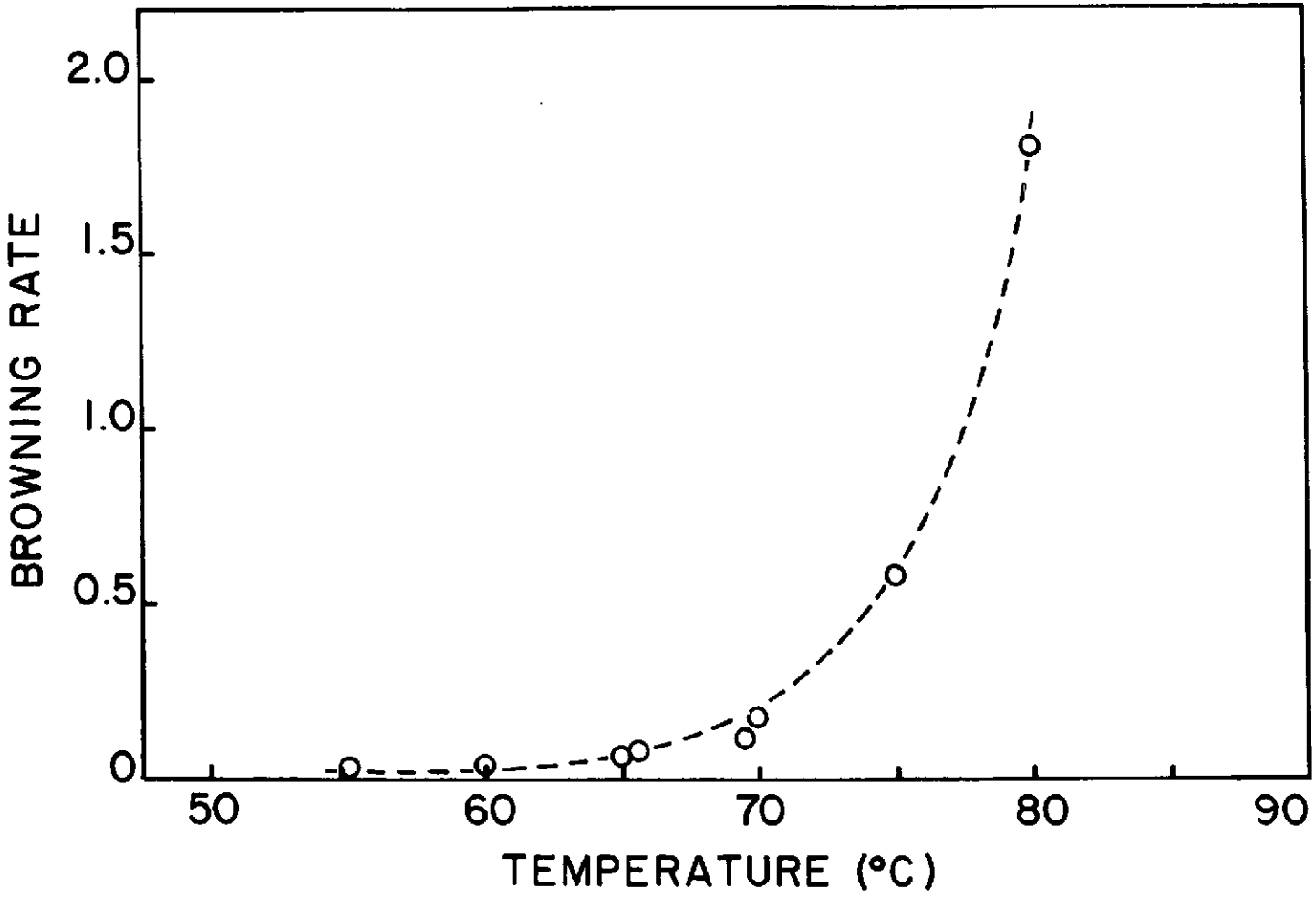


Figure IV-3

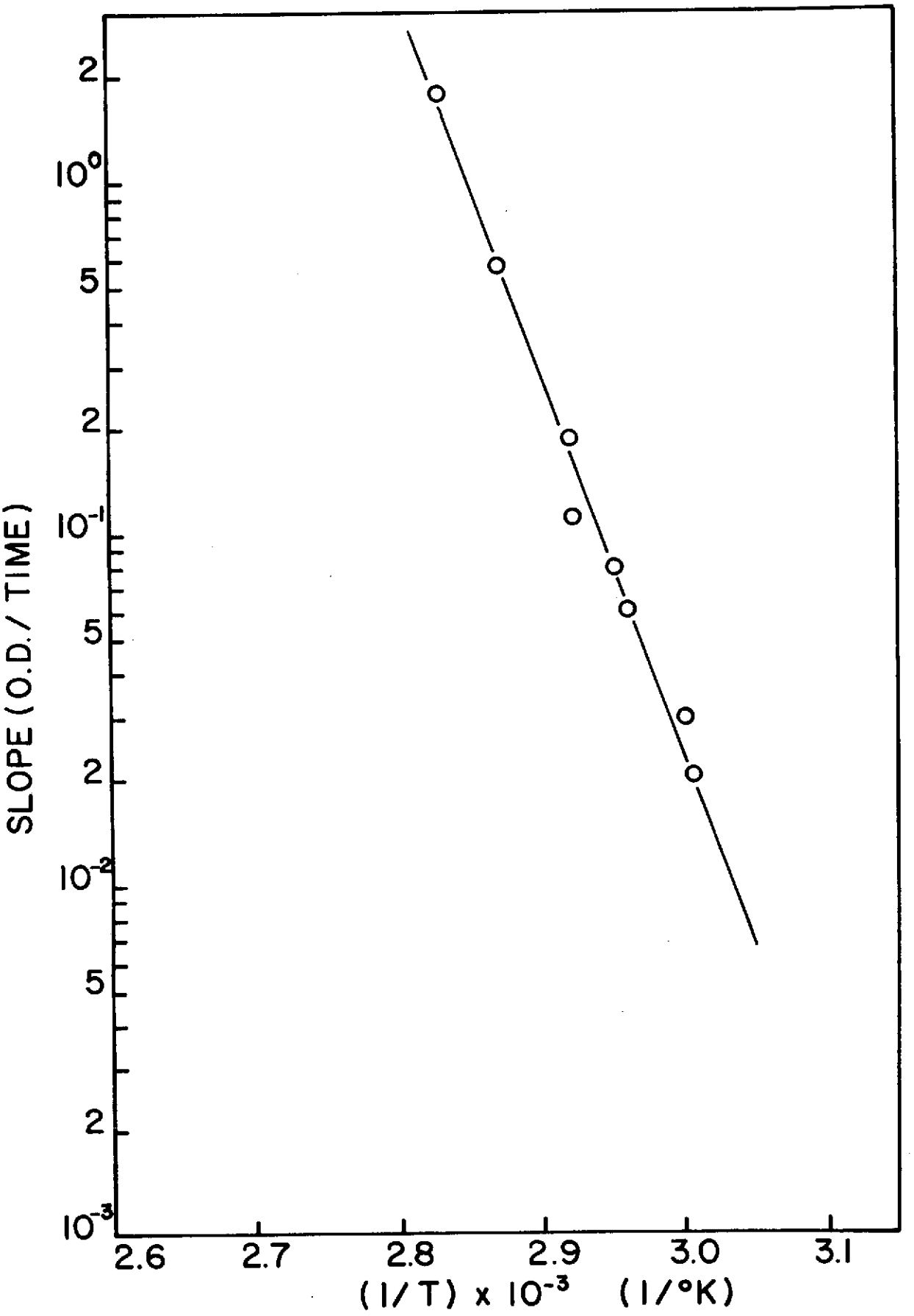

SLOPE (O.D./ TIME)
2
10^0
5
2
10^-1
5
2
10^-2
5
2
10^-3
2.6
2.7
2.8
2.9
3.0
3.1
(1/T) x 10^-3 (1/°K)

Figure IV-4

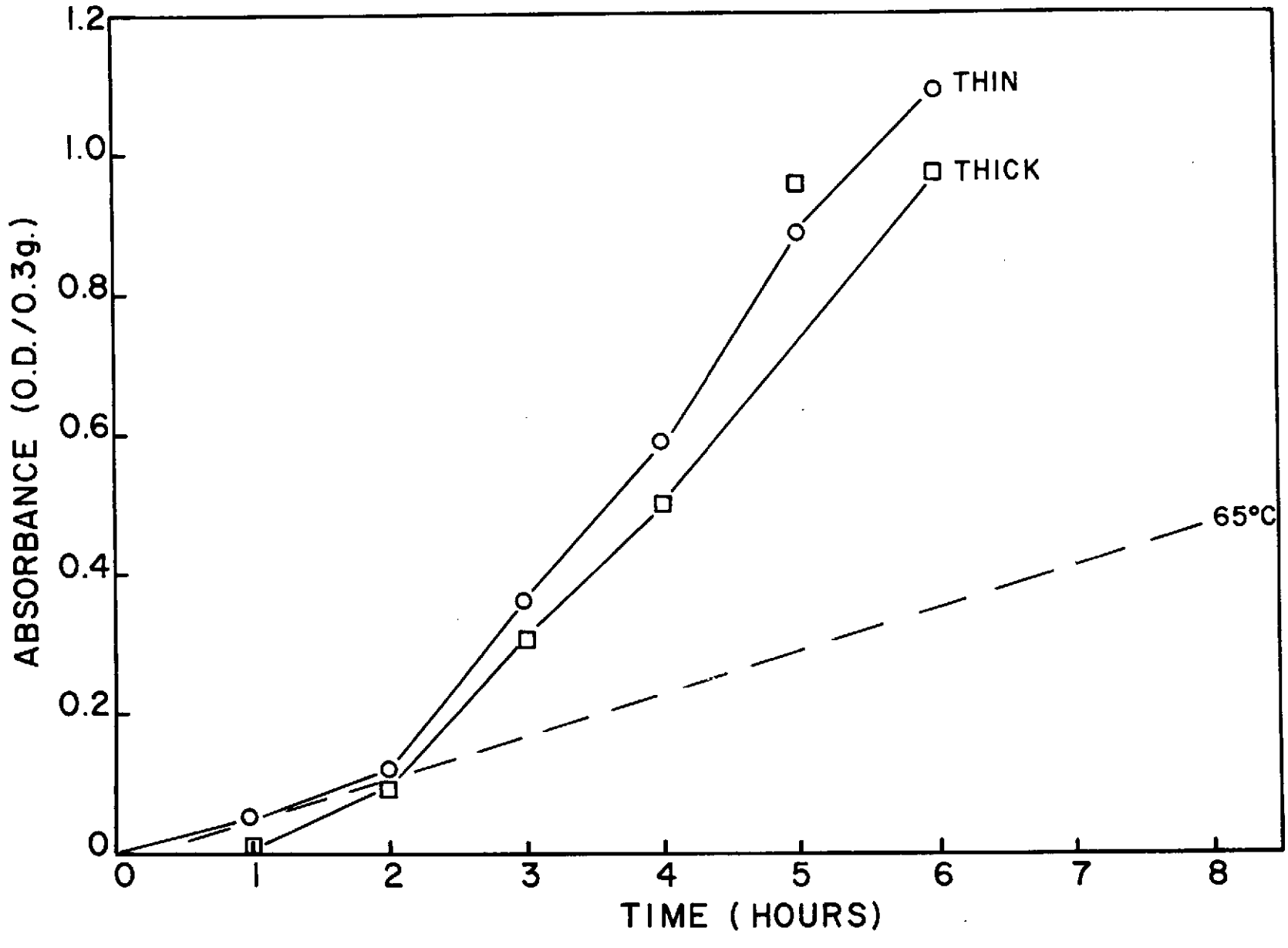

1.2
1.0
0.8
0.6
0.4
0.2
0
ABSORBANCE (O.D./0.3g.)
THIN
THICK
65°C
0
1
2
3
4
5
6
7
8
TIME (HOURS)

Figure IV-5

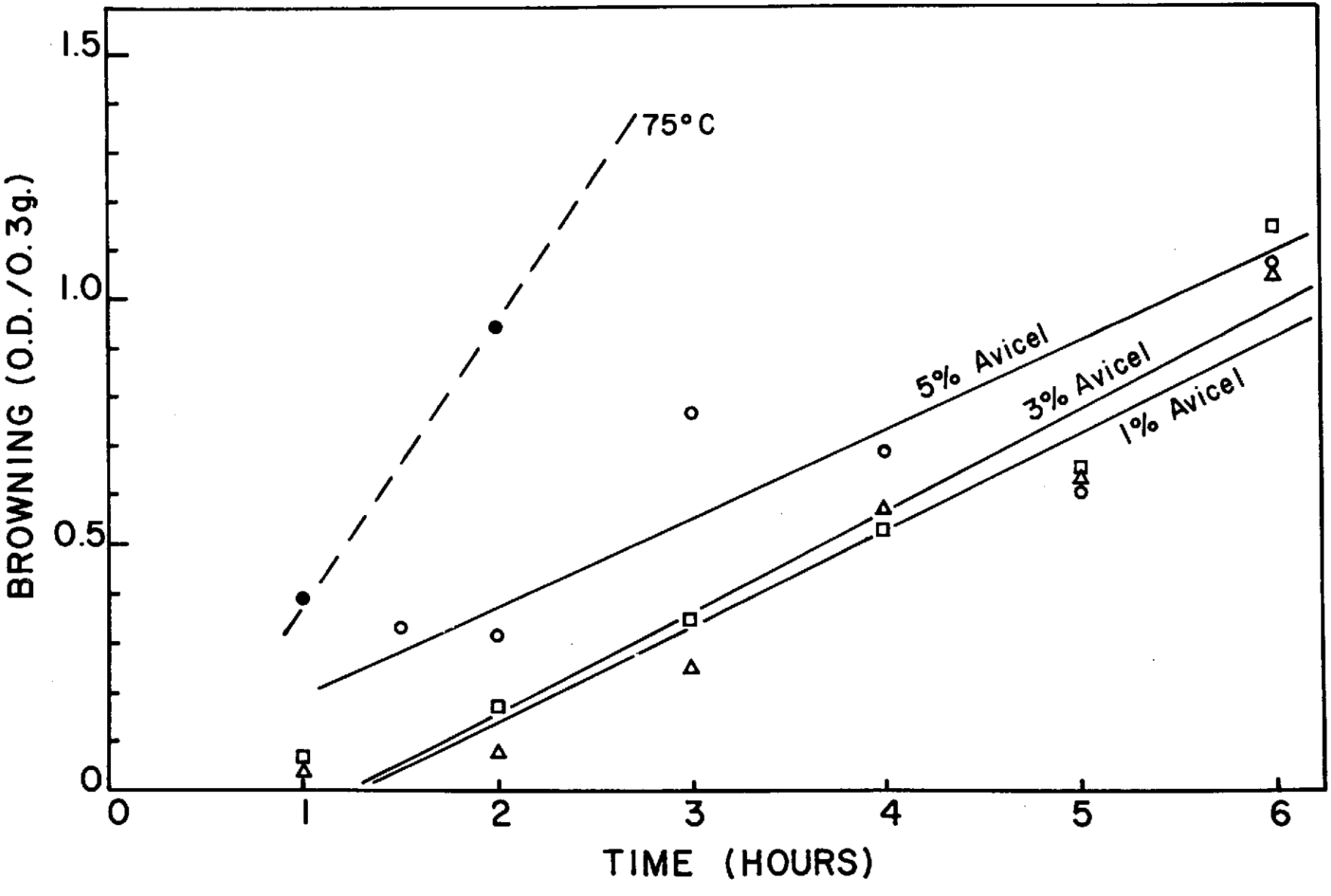
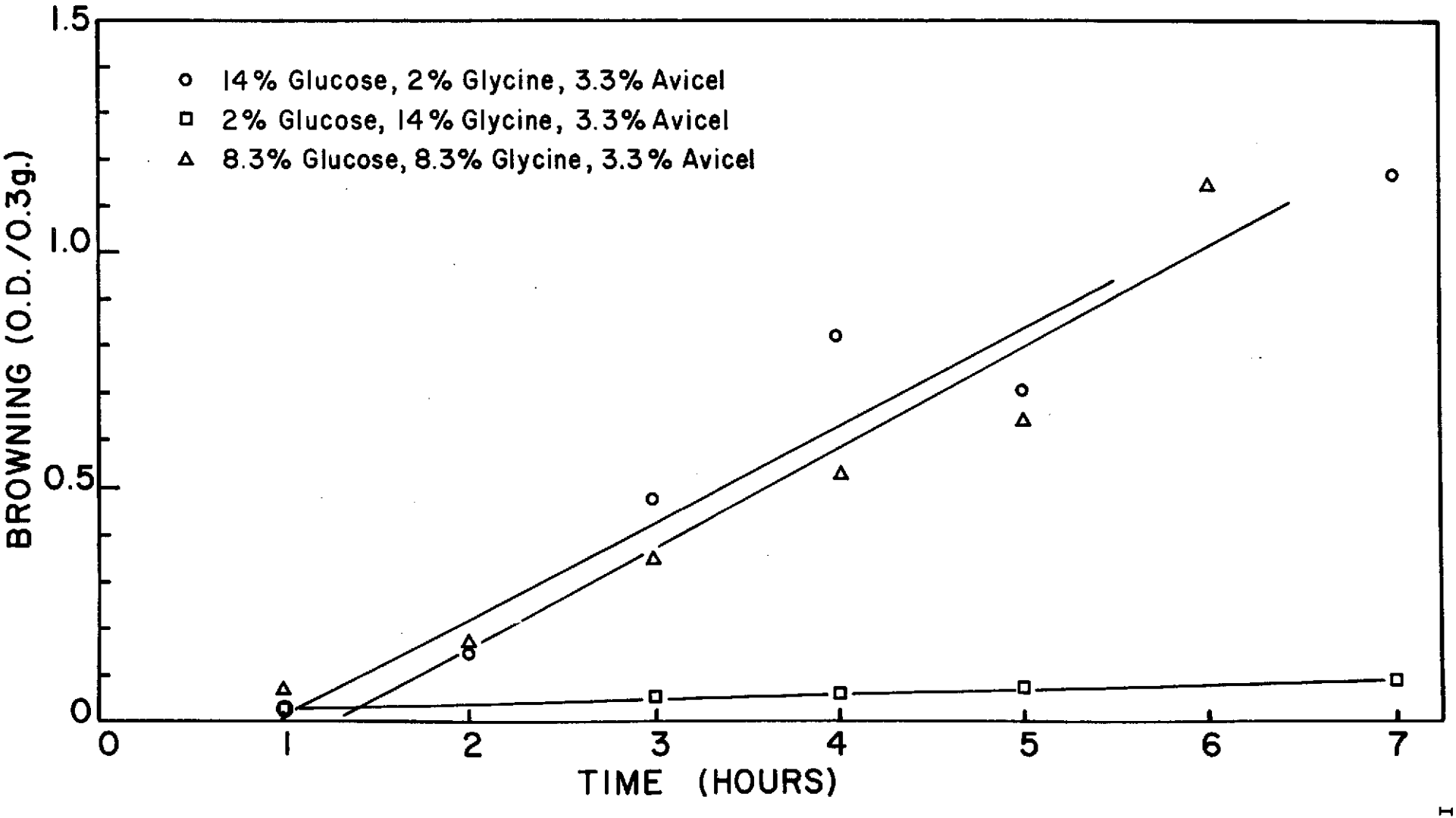

BROWNING (O.D./O.3g.)
1.5
1.0
0.5
0
75°C
5% Avicel
3% Avicel
1% Avicel
0
1
2
3
4
5
6
TIME (HOURS)

Figure IV-6


14% Glucose, 2% Glycine, 3.3% Avicel
2% Glucose, 14% Glycine, 3.3% Avicel
8.3% Glucose, 8.3% Glycine, 3.3% Avicel
BROWNING (O.D./0.3g.)
1.5
1.0
0.5
0
0
1
2
3
4
5
6
7
TIME (HOURS)

V. Volatile Retention during Freeze Drying of the Polar, Non-Carbohydrate Polymer-Polyvinyl Pyrrolidone.

V.A. Introduction

Previous work on carbohydrate systems showed that flavor retention could be explained on the basis of the microregion theory of Flink and Karel, and that this theory could serve as a basis for optimization of process parameters. It seemed important to extend the work to systems other than carbohydrates, and the polymer PVP and C^{14}-labeled n-propanol were the subject of extensive studies in Phase I. This work resulted in preparation of two research papers. The first of these form section V.B. and has been accepted for publication in J. Food Sci. The second forms section V.C. and has been submitted to J. Food Sci.

Studies on Mechanisms of Retention of Volatile in Freeze-Dried Food Models: The System PVP-n-Propanol

Jorge Chirife*, Marcus Karel, and James Flink
Department of Nutrition and Food Science
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Running Title: Volatile Retention in Freeze-Dried PVP

* Present Address: Departamento de Industrias,
Facultad de Ciencias Exactas y Naturales
Buenos Aires, Argentina

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SUMMARY

Retention of C^{14}-labeled n-propanol was studied in a freeze-dried system containing polyvinylpyrrolidone (PVP). Variables affecting retention during freeze drying were: initial concentrations of PVP and n-propanol, rate of freezing, and sample thickness. Rehumidification above the BET monolayer value resulted in losses of propanol which increased with increasing water content.

Behavior of the model system based on PVP is consistent with the "microregion volatile entrapment theory". The PVP system (and perhaps polymeric carbohydrates) differ from low molecular weight carbohydrates: water sorption below the BET monolayer is reduced by entrapped volatile, and absolute level of retention is lower.

INTRODUCTION

Food materials are generally freeze dehydrated by placing the frozen food in a high vacuum environment. Contrary to what one might expect, volatile compounds of different vapor pressures have similar retentions. The retention of the organic volatiles is based largely on the properties of the solute which forms the amorphous matrix of the freeze-dried solid.

It is believed that the retention of organic volatiles results from surface adsorption of the volatile on the dry layer of the freeze-drying sample (Rey and Bastien, 1962) or from an entrapment mechanism which immobilizes the volatile compounds within the amorphous solute matrix (Flink and Karel, 1970a; Thijssen, 1971; Thijssen and Rulkens, 1969; Chandrasekaran and King, 1971).

The physical aspects by which the volatile is entrapped within the amorphous solute matrix are only partially understood. Two mechanisms, selective diffusion (Thijssen, 1971; King, 1971; King, 1970a; Rulkens and Thijssen, 1972; Thijssen and Rulkens, 1968) and microregions (Flink and Karel, 1970a, 1972) perhaps represent macro- and microviews of the same basic phenoma. The selective diffusion concept utilizes diffusional analysis on the system components to develop mathematical expressions by which volatile retention behavior during freeze-drying can be predicted. Phenomonologically, as noted by King

(1970b): "A simple diffusion mechanism is not in itself sufficient." Structural aspects of the microregion concept allow qualitative explanation of behavior both during freeze-drying and during humidification of the dried material. Flink and Karel (1970a) postulated that crystallization of water during freezing and other concentration processes result in the formation of "microregions" containing highly concentrated solutions of carbohydrates and volatile organic compounds. As the local moisture content within the microregion decreases, first due to freezing and then to freeze drying, molecular association occurs; in the case of carbohydrates, this association is caused by H-bonds. The structure of the microregion, as well as its permeability to organic compounds and water, depends upon local moisture content. As the moisture content decreases, the ease of loss of the organic compound decreases until at some critical moisture there is no further loss (Flink and Karel, 1970a, b; 1972). The size of the microregions is small, since grinding and evacuation of the dry material do not release any volatile. Recently the size of the volatile entrapments has been shown to vary with, among other things, the solubility of the organic volatile in the aqueous solution. Retained hexanal (about 1 g hexanal per 100 g maltodextrin) freeze-dried from an aqueous 20% maltodextrin solution appeared in the optical microscope within the amorphous solute matrix as 2- to 6-μm droplets (Flink and Gejl-Hansen,

1972). Concurring evaluations have been made with the scanning electron microscope. The addition of sufficient water to the dry material will cause volatile loss, the extent of which depends on the amount of water added and the particular solute matrix. Water influences volatile loss according to changes in the matrix in the amorphous state.

It seems important to extend the applicability of above concepts to systems other than carbohydrates; therefore, we have initiated work on polymers, proteins, and on selected foods.

In this study we are presenting results which characterize the retention of n-propanol in a model system based on polyvinylpyrrolidone (PVP), which is a polar, water-soluble polymer containing polar groups different from those of polysaccharides but similar to proteins. PVP does not, however, manifest the complexity of interactions that occurs in proteins. C^{14}-labeled n-propanol enables us to study low concentrations, both in the presence and absence of other compounds, which can potentially interfere with conventional analyses.

EXPERIMENTAL

Model system preparation

The model system consisted of a water-soluble polymer

(PVP), C^{14}-labeled n-propanol, and water. The model system was prepared by dissolving the desired amount of PVP in water and adding n-propanol. Five ml aliquots of the solution were pipetted into 50 ml Erlenmeyer flasks, frozen as specified below and then freeze dried for 48 hr at room temperature and at a chamber pressure of less than 100 μm in a Virtis freeze drier (Model 10-MRTR). No partial melting or collapse was noted in the dried material following removal from the freeze-drier. The volume of solution per flask and resultant sample thickness were varied in some experiments as noted under Results and Discussion. In most experiments the composition was fixed as the following initial concentration expressed in weight percent: PVP 20%, n-propanol 1%, water 79%. In several experiments, the effect of changing concentrations was studied; the changed compositions are noted under Results and Discussion.

Samples were frozen as slabs in the flasks by one of two methods: fast freezing was accomplished by immersion of flasks in liquid nitrogen; slow freezing refers to placing the stoppered flasks in still air at -40°C.

PVP

Polyvinylpyrrolidone K-30 (Molecular weight 40,000) was obtained from Matheson, Coleman, and Bell (East Rutherford, New Jersey).

N-Propanol

Reagent grade n-propanol was mixed with C^{14}-labeled

n-propanol to give the desired specific radioactivity. The radioactive propanol was obtained from International Chemical and Nuclear Corporation in Irving, California.

Humidification Experiments

In several experiments freeze-dried PVP-propanol systems were humidified by placing tared and weighed flasks in vacuum desiccators containing saturated salt solutions, which maintained the desired constant relative humidities.

N-Propanol Analysis

The n-propanol content was determined by measuring the radioactivity of the samples with a liquid scintillation counter.

The dried samples of PVP were dissolved in water (to 10% solution); 1 ml of this solution was added to 10 ml of water-miscible scintillator (2,5-diphenyloxazole 1 g, naphtalene 100 g, dioxane to 1,000 ml volume) in the counting vial, and the resulting solution was counted with a liquid scintillation counter (Nuclear Chicago Corp., 720 series). The counting efficiency (measured with a C^{14} toluene standard) was 77.5%; no correction by quenching was found to be neccessary.

Statistical evaluation of variation due to freeze drying and analysis

Ten identical samples of the model system were

prepared, frozen, freeze dried, and analyzed by liquid scintillation counting. The variation coefficient determined for the ten samples gives a measure of the range of significance for the overall process (freeze drying and analysis). The results were:

Fast frozen samples: n-propanol content 0.49 g/100 PVP ± 3.5%
Slow frozen samples: " " 1.2 g/100 PVP ± 9.0%

Water sorption isotherm

The water sorption isotherm for PVP in the absence of entrapped propanol and selected values of sorption of water in the presence of entrapped propanol were obtained by placing tared weighed samples in vacuum desiccators over constant humidity solutions and weighing periodically until equilibrium was established.

RESULTS AND DISCUSSION

As discussed previously, we conceive the retention of volatiles in freeze-dried solutions to be caused by the formation of microregions during freezing or other concentration processes preceeding the final drying step. During dehydration the microregions are stabilized by formation of a matrix which becomes completely impermeable to organic volatiles but not to water when the local moisture content drops to a critical level.

In the case of carbohydrates, the matrix is stabilized by hydrogen bonds and the critical moisture content tends to occur at the BET monolayer value (Flink and Karel, 1972).

Process conditions affect the formation of microregions and, consequently, the extent of retention. Freezing rate, for instance, is of considerable importance: slow rates promote volatile retention in microregions (Flink *et al.*, 1970b; Flink and Labuza, 1972).

Table I presents a comparison between retention of propanol in freeze-dried PVP systems and retentions observed previously in freeze-dried carbohydrates. In both types of systems slow freezing resulted in higher retention than rapid freezing. The fraction retained was considerably higher at low absolute concentrations of the alcohol. Finally, the results show that both polymeric systems (PVP and Dextran) retained less alcohol than the low molecular weight carbohydrates. All of the above findings are compatible with the microregion concept: slow freezing, which allows diffusion of solute from the freezing front, results in fewer, larger, more concentrated microregions, which are less permeable than those created by rapid freezing (Flink and Karel, 1970b). Similar conclusions regarding larger regions of concentrated solute phase resulting from slow freezing were reached by King (1970). The reduced mobility

of the polymers, as compared to the low molecular weight carbohydrates, also retards formation of impermeable microregions which entrap the volatiles; this is the reason that Dextran-10 and PVP retain less alcohol under the conditions of our experiments.

We also observed a consistent effect of concentration of alcohol. When solids content is kept approximately constant, relative retention decreases with increasing alcohol concentration. The use of C^{14}-labeled n-propanol allowed for a wide range of concentrations; results obtained are shown in Figure 1. They are consistent with results obtained previously with carbohydrates, which showed that retention expressed in absolute amounts (as weight of volatile/weight of solid) increases nonlinearly with concentration, resulting in the relative retention showing the decrease noted above. This behavior reflects a saturation of microregion entrapment capacity (Flink and Karel, 1970b). The microregion theory predicts that increasing the solid concentration increases volatile retention up to a limiting concentration which depends on the type of solid and type and amount of volatile. Figure 2 shows results obtained with the PVP-n-propanol system compared with results published for other systems. There is a general agreement on shape of curves obtained.

Thickness of sample is also a factor in retention.

Figure 3 presents the retention of n-propanol (initial concentration 1%) in freeze-dried PVP (initial concentration 20%). The retention decreases with increasing thickness as predicted by the theory advanced previously, primarily because the more rapid drying and steep moisture gradients in thin samples decrease the time during which the moisture content in the microregions is high enough to permit volatile escape (Flink and Karel, 1970b).

The most convincing evidence for the existence of water-sensitive volatile-entrapping microregions in carbohydrate systems was obtained through humidification experiments, in which water vapor sorption above a critical moisture level resulted in structural changes and consequent volatile release (Flink and Karel, 1972; Karel and Flink, 1972). Figure 4 presents results of humidification experiments using PVP. In the corresponding experiment, PVP solutions (20% solids, 1% n-propanol) were freeze dried under standard conditions (slow freezing, ambient freeze-drying temperature). These conditions resulted in the retention of 1.2 g n-propanol per 100 g of PVP. The freeze-dried systems were then exposed to different relative humidities, and water uptake and volatile loss were measured as a function of time. At 11% R.H. we can note a small loss of propanol from the samples. The amount of this loss is comparable to the experimental error; however, it is possible that a part of this measured loss is real, perhaps resulting

from surface adsorption of the propanol or from imperfect microregions communicating with the free surface. The loss increases as the moisture contents increase with rising humidity. It should be noted that even in the case of exposure to higher humidity, sizable volatile loss does not commence until sufficient water is absorbed to begin disrupting the PVP structure. Thus, in the case of exposure to 32% relative humidity, complete humidification is achieved in about four hours, and the volatile loss appears to become significant at about the same time. Extensive tests have confirmed that the BET monolayer value, which for PVP is about 12.5 g water/100 g solids occurring at an equilibrium relative humidity of about 30%, seems to mark the initiation of volatile release due to microregion disruption.

The PVP system is similar therefore to the carbohydrate system in its sensitivity to water. At high humidities, however, there is a new constant level of retention of volatile in carbohydrate systems after an initial loss following attainment of the new moisture equilibrium, whereas with PVP we have observed a very slow, continuing loss at high humidities. These differences may be due to the reduced mobility in the polymer which results in sluggish approach to the new equilibrium value, or to the greater sensitivity of polar bonds in PVP (as compared with carbohydrates) to continuing disruption in the

presence of limited amounts of water.

Water and polar volatiles can compete for polar sites in polymers and other solids (Lauer and Ayer, 1957; Bell and Breuer, 1971; Fogiel and Heller, 1966; De Boer, 1968). In the present study we have obtained indications that entrapped n-propanol competes with water for sorbing sites in PVP microregions. Figure 5 shows the sorption isotherm for propanol-free freeze-dried PVP. This isotherm was independent of the method of freezing. Table 2 compares the water sorption in the presence and absence of propanol, in which a small but significant reduction in water sorption occurs.

In studies of water sorption of PVP (Dole and Fuller, 1950; Jellinek *et al.*, 1968), it is considered that the monolayer of water is held by the carbonyl groups. We calculated the BET monolayer and found it to be 12.5% (dry basis) occurring at about 30% relative humidity. This corresponds to 0.77 moles of water per mole of CO groups, in reasonable agreement with Dole and Fuller, who found that approximately 1 mole of water is bound per mole of PVP repeating units.

Some deductions can be made comparing the water sorption by dried PVP with and without n-propanol. Table 2 shows that at a relative humidity of 11%, the sorption of water is lowered for the samples with n-propanol by 11.5 moles H_2O/mole PVP. This could be considered as

evidence that the n-propanol is sorbed in the PVP, thus occupying some of the available sites for water sorption.

The amount of n-propanol retained at 11% R.H. is about 1.1 g/100 PVP, which is equal to 7.4 molesof n-propanol/mole PVP. Thus, 7.4 moles of n-propanol are apparently accommodated in the sites which held 11.5 moles of water. The preferential adsorption of the alcohol at low water contents is probably due to the alcohol's inability to be desorbed until the water content is sufficiently high to cause partial microregion disruption and alcohol release.

We are currently trying to determine how much of this effect is due to selective impermeability of the microregions, and whether chemisorption plays a part.

It is conceivable that within the microregions the organic molecules are in one of the following states:

a) adsorbed on sites which can be competitively occupied by water

b) adsorbed on specific sites, but do not reduce the total sorption capacity for water

C) entrapped as condensed aggregates, such as droplets, in which most of the molecules do not saturate the internal surfaces of the entrapping solids.

Flink and Gejl-Hansen (1972) showed that at least some

B

volatiles are entrapped in maltodextrin in the form of fairly large droplets (up to several microns in size) as in "c" above.

Flink and Karel (1970a) have reported that in some freeze-dried carbohydrates, the presence of entrapped organic volatiles does not significantly affect the water sorption by these solids, indicating that the volatile was probably in a state other than "a" above.

In light of the results obtained in this study with PVP, the sorption isotherm data of Flink (1970) were reevaluated. While the maltose samples definitely show no effect of retained volatile on the water sorption, a possible effect similar to that reported here is noted for the *tert*-butanol retained by the polymeric carbohydrate Dextran-10. The number of samples utilized by Flink did not allow the same statistical accuracy as was achieved in this study; thus, the behavior observed in the reevaluation cannot definitely be deemed significant.

In summary, the results presented above lead us to the conclusion that the behavior of a model system based on PVP is consistent with the "microregion volatile entrapment theory", which was formulated on the basis of work with carbohydrates. The PVP system (and perhaps polymeric carbohydrates) shows differences from low molecular weight carbohydrates: an apparent reduction in water sorption below the BET monolayer due to

entrapped volatile, and a lower absolute level of volatile retention.

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Table 1. Effect of freezing rate on retention of propanol by model systems.

Solid	Initial Conc.(%)	Volatile	Initial Conc.(%)	Retention of Volatile (%) Fast Freezing	Slow Freezing
PVP	20.0	n-propanol	1.0	9.8	24.0
PVP	20.0	n-propanol	0.01	-	58.0
Glucose	18.8	n-propanol	0.75	47.8	-
Glucose	18.8	2-propanol	0.75	52.8	-
Maltose	18.8	2-propanol	0.75	67.6	87.5
Dextran-10	18.8	n-propanol	0.75	4.2	-
Dextran-10	18.8	2-propanol	0.75	7.5	-
Dextran-10	20.0	2-propanol	0.01	56	88

Table 2. Effect of retained n-propanol on water sorption by PVP.

Equilibrium relative humidity	Water content (% dry basis) without propanol	with propanol	% Difference	Coefficient of Variation (%)	n-propanol retained moles per mole of PVP	Apparent decrease in water sorption moles/mole PVP
11	5.50	4.98	9.5	1.2	7.4	11.5
32	13.60	13.30	2.2	0.58	4.0	6.7
52	21.40	21.14	1.2	-	1.6	5.8

FIGURE LEGENDS

Figure 1. Effect of initial n-propanol content on the retention during freeze-drying of PVP solutions. (20% PVP)

Figure 2. Effect of concentration of solids on volatile retention during freeze drying.

A. Maltodextrin: n-propanol
   initial n-propanol content: volatile to solid ratio constant at 1:100 (Rulkens and Thijssen, 1972)

B. Maltose: butanol
   initial butanol content: 1% in initial solution (Flink and Karel, 1970b)

C. Earle salt and glucose: acetone
   initial acetone content: 10% in initial solution (Rey and Bastien, 1952)

D. PVP: n-propanol
   present study
   initial n-propanol content: 1% in initial solution

E. Glucose: acetone
   initial acetone content: 2.5% in initial solution (Flink and Karel, 1970b)

Figure 3. Effect of sample thickness on n-propanol retention during freeze-drying of PVP solutions.

Figure 4. Loss of n-propanol and fractional water uptake from freeze-dried PVP humidified to specified relative humidities at 5°C.

Initial n-propanol retention = 1.2 g/100 g PVP

Equilibrium water content at 32% R.H. =

100% fractional water uptake = 13 g/100 g PVP

Figure 5. Water sorption isotherm for freeze-dried PVP at 25°C.

Figure V-1

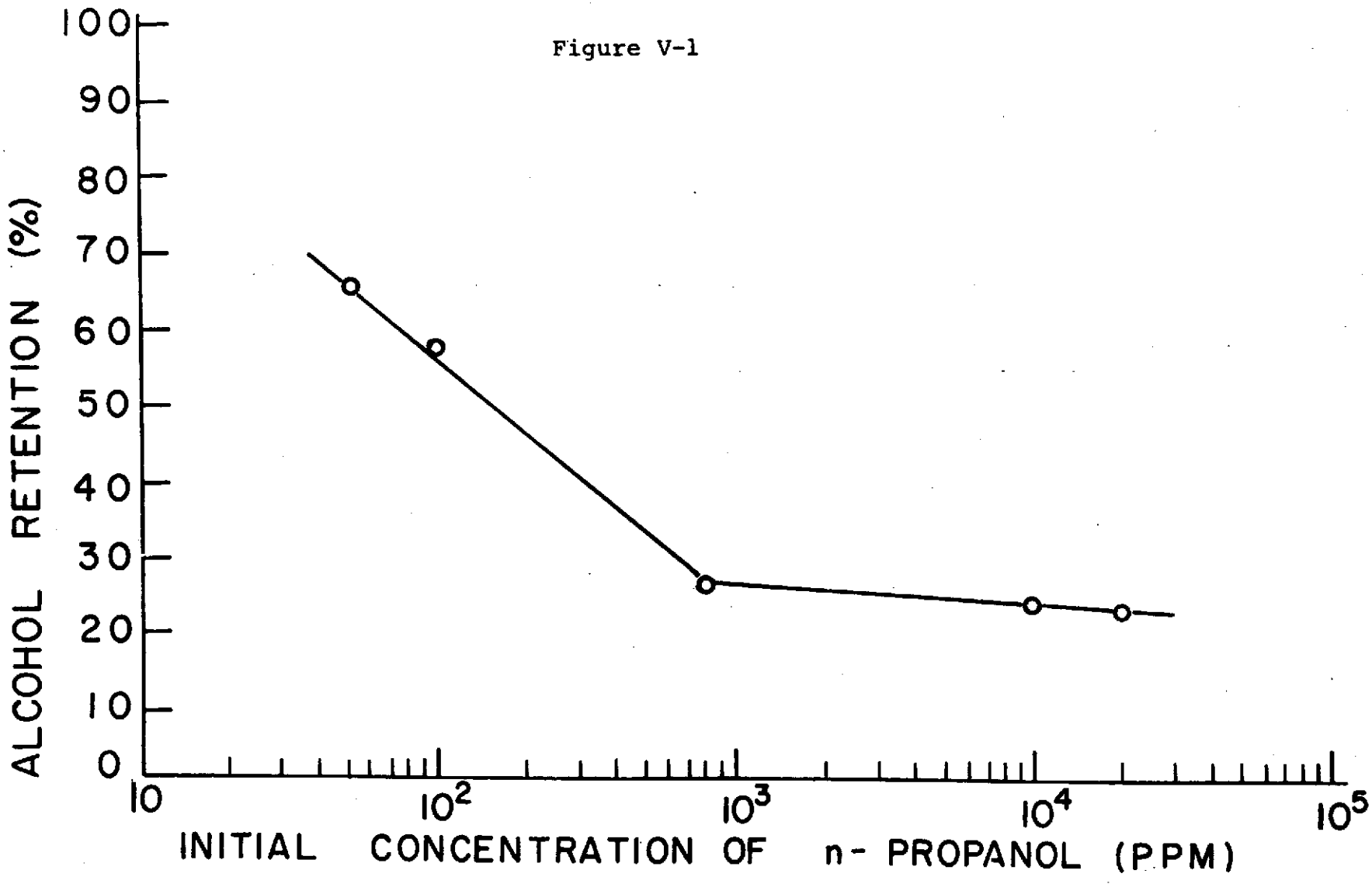

ALCOHOL RETENTION (%)
100
90
80
70
60
50
40
30
20
10
0
10
10^2
10^3
10^4
10^5
INITIAL CONCENTRATION OF n- PROPANOL (PPM)

Figure V-2

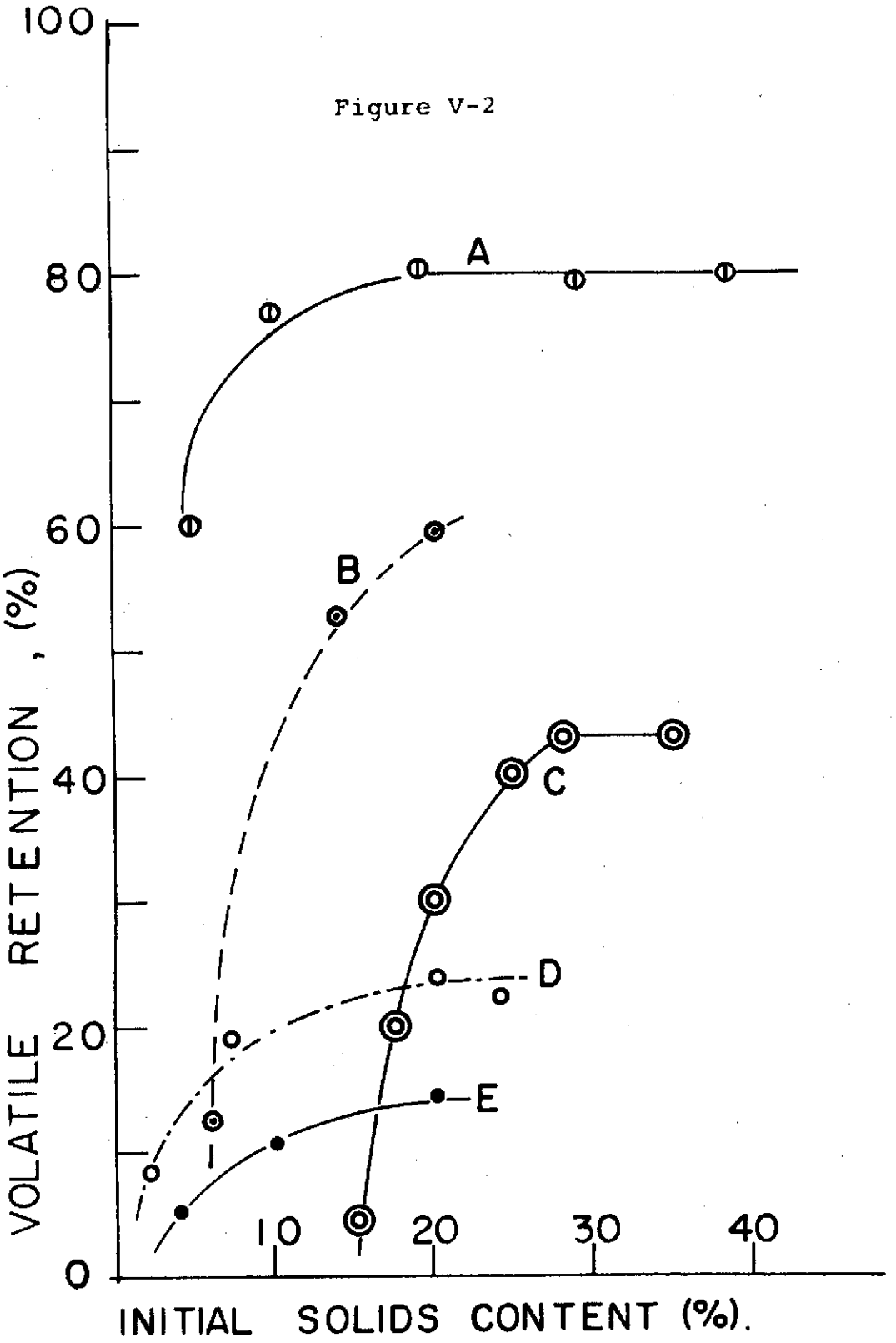

100
80
60
40
20
0
VOLATILE RETENTION , (%)
A
B
C
D
E
10
20
30
40
INITIAL SOLIDS CONTENT (%).

Figure V-3

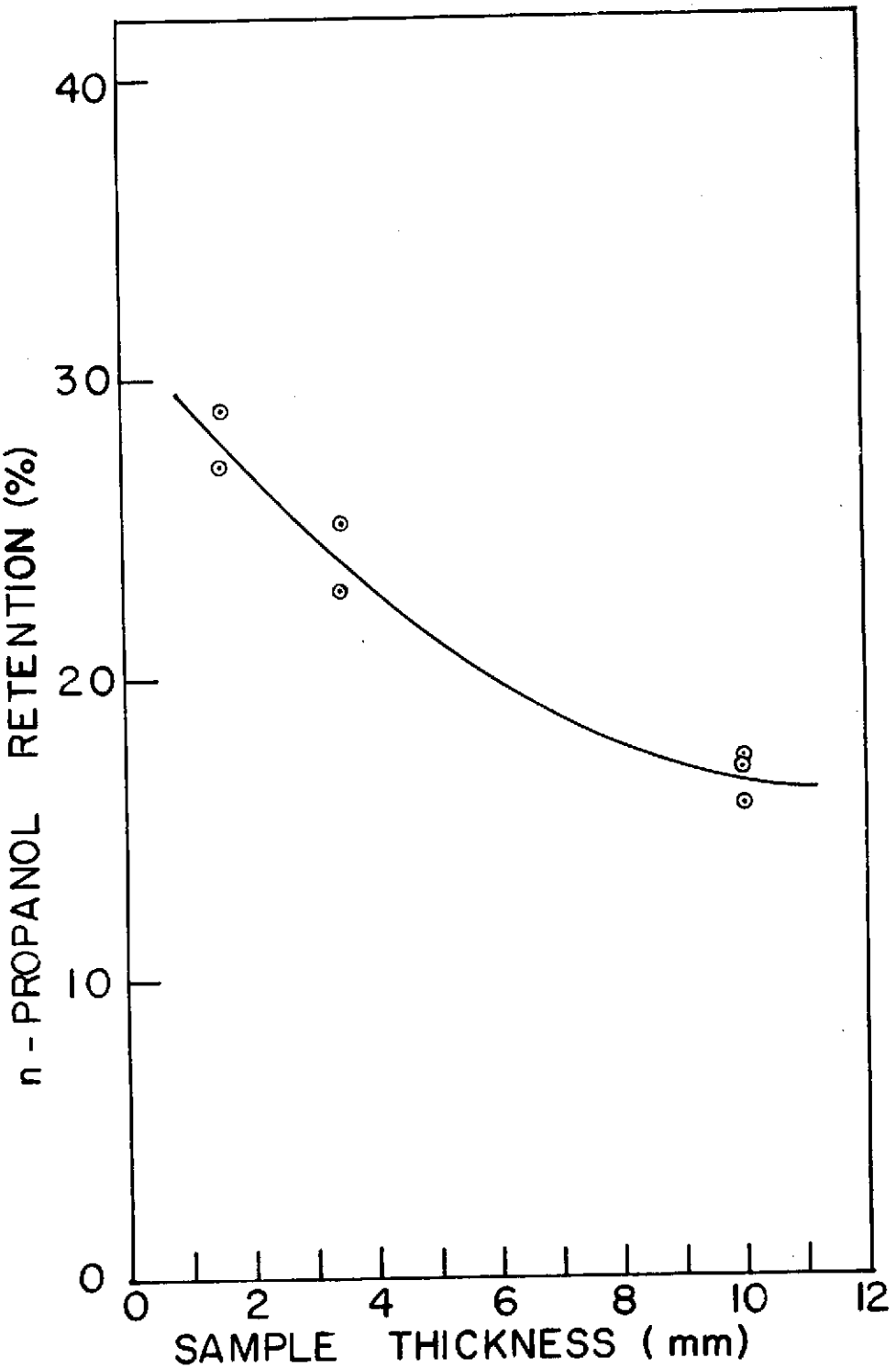

40
30
20
10
0
n - PROPANOL RETENTION (%)
0
2
4
6
8
10
12
SAMPLE THICKNESS (mm)

Figure V-4

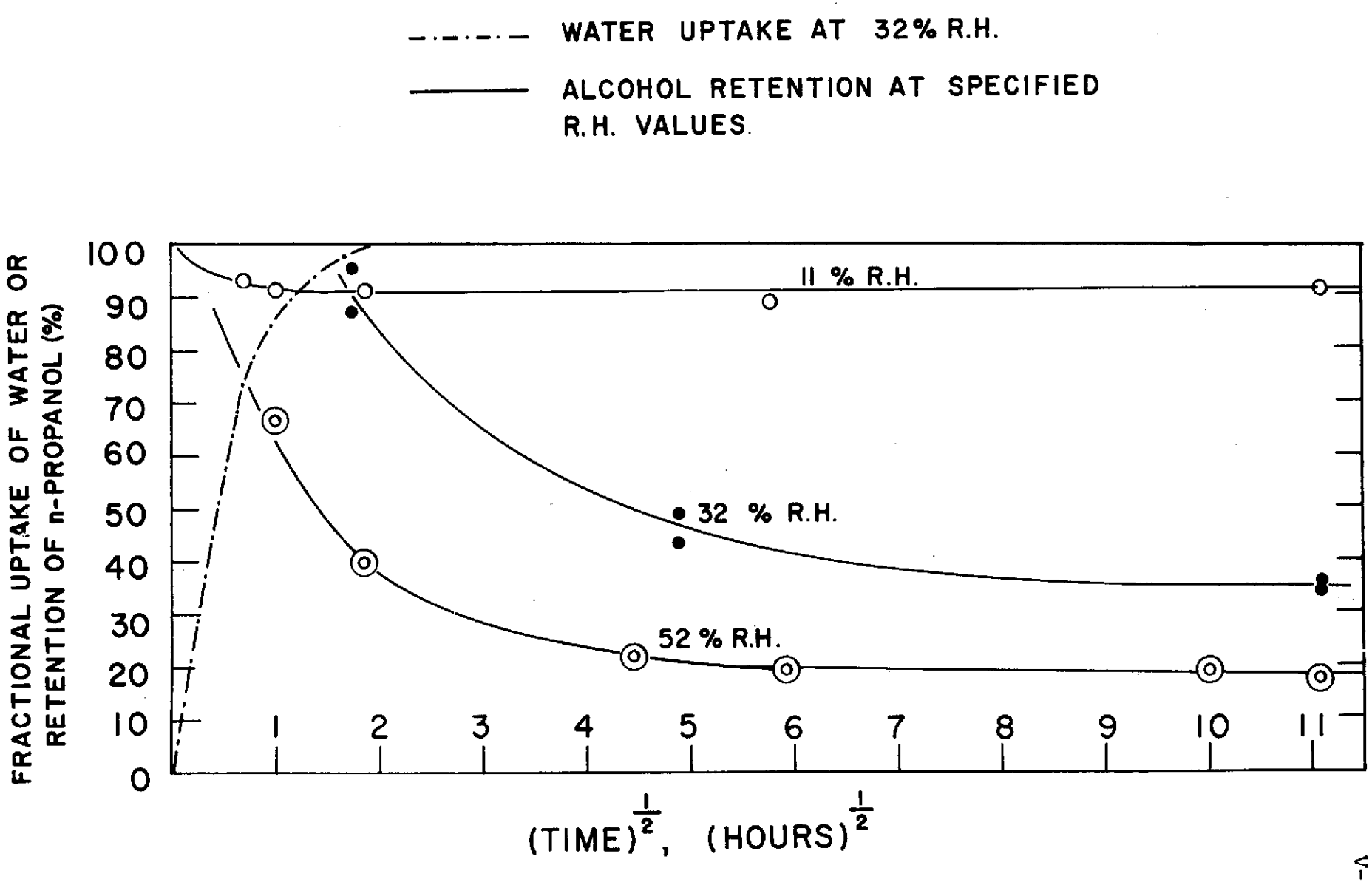
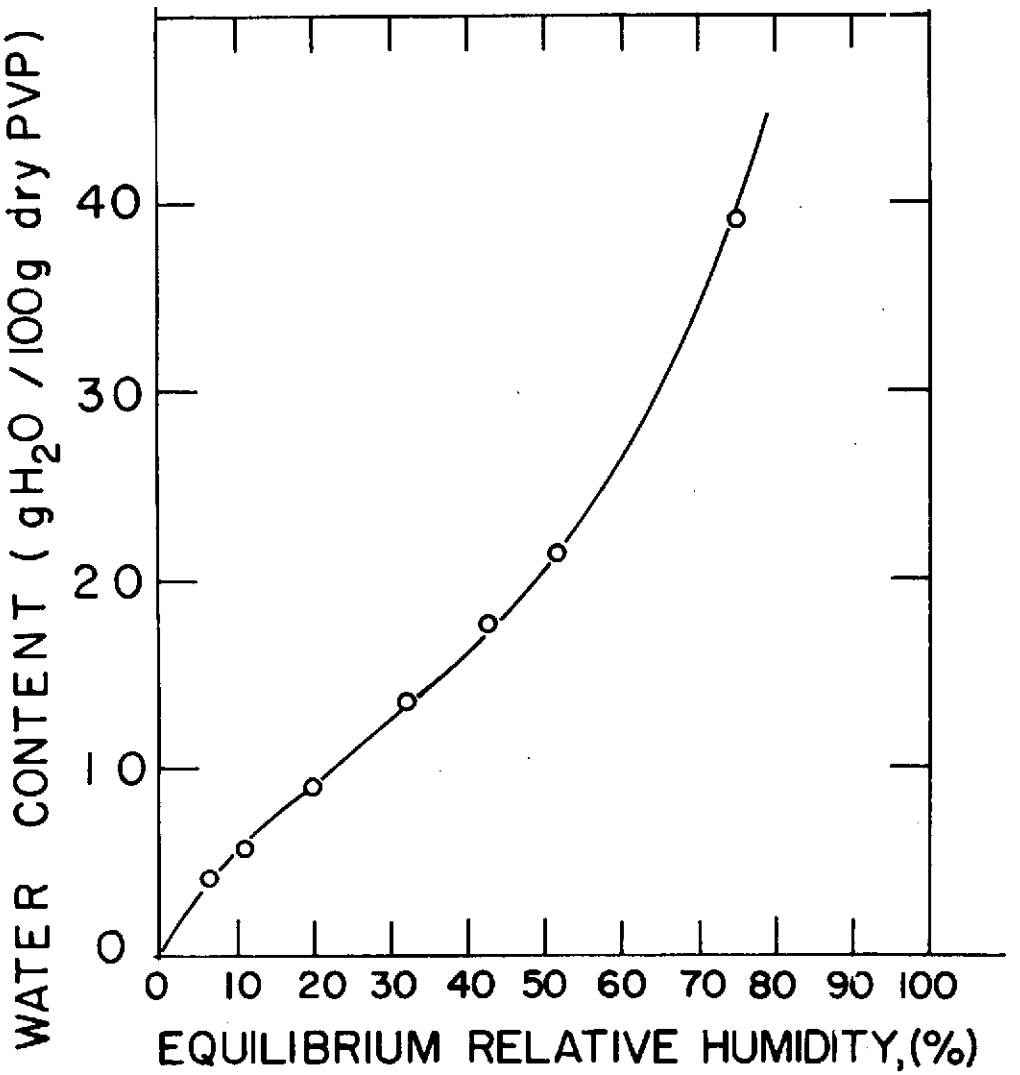

WATER UPTAKE AT 32% R.H.
ALCOHOL RETENTION AT SPECIFIED R.H. VALUES.
FRACTIONAL UPTAKE OF WATER OR RETENTION OF n-PROPANOL (%)
100
90
80
70
60
50
40
30
20
10
0
11 % R.H.
32 % R.H.
52 % R.H.
1
2
3
4
5
6
7
8
9
10
11
(TIME)$^{\frac{1}{2}}$, (HOURS)$^{\frac{1}{2}}$

Figure V-5


WATER CONTENT (gH2O / 100g dry PVP)
0
10
20
30
40
0 10 20 30 40 50 60 70 80 90 100
EQUILIBRIUM RELATIVE HUMIDITY,(%)

Contribution of Adsorption to Volatile Retention in a Freeze-Dried Food Model Containing PVP

Jorge Chirife* and Marcus Karel
Department of Nutrition and Food Science
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

* Present address: Departmento de Industrias
Facultad de Ciencias Exactas y Naturales
Buenos Aires, Argentina

Summary - Retention of ^{14}C-labeled n-propanol was studied in a freeze-dried system containing polyvinylpyrrolidone (PVP). The major fraction of n-propanol retained after freeze drying of aqueous solutions of PVP and propanol is held by entrapment in microregions. Some n-propanol can also be entrapped by the polymeric aggregates of PVP after sorption of the alcohol from the vapor phase, even in the absence of plasticizing action of water, apparently because of the ability of propanol to act as solvent for PVP.

In addition to entrapment, there is a small but significant contribution of adsorption to n-propanol retention in freeze drying which could not be determined exactly. However, dry layer adsorption experiments on rapidly frozen PVP show readsorption amounting to approximately 10% of the total retention.

INTRODUCTION

In a previous paper we reported that in a freeze-dried system containing PVP (polyvinylpyrrolidone) and n-propanol the retention of most of the alcohol could be explained by entrapment of the alcohol in microregions formed by PVP (Chirife et al., 1972). There appeared to be a competition for water sorbing sites by the entrapped propanol, resulting in reduction of water sorbing capacity at levels below the BET monolayer value.

The ability of various polar sites in hydrophilic polymers to adsorb either water or alcohol has also been observed by Le Maguer (1972) and Fogiel et al. (1966).

In the present study we show the extent to which adsorption is important in the retention of n-propanol in freeze-dried PVP solutions.

MATERIALS AND METHODS

Model system preparation

The model system consisted of a water-soluble polymer (PVP), ^{14}C-labeled n-propanol, and water.

The system was prepared by dissolving the desired amount of PVP in water and adding n-propanol. Five-ml aliquots of the solution were pipetted into 50-ml

Erlenmeyer flasks, frozen as specified below, and then freeze dried at room temperature and at a chamber pressure of less than 100 μ for 48 hr in a Virtis freeze drier (Model 10-MRTR).

The volume of solution per flask and resultant sample thickness varied in some experiments, as noted under Results and Discussion.

The composition of the system was fixed as the following initial concentration expressed in weight percent: PVP 20%, n-propanol 1%, water 79%.

Samples were frozen by one of two methods: rapid freezing was accomplished by immersion of flasks in liquid nitrogen; slow freezing by placing the stoppered flasks in still air at -40°C.

PVP

Polyvinylpyrrolidone K-30 (molecular weight 40,000) was obtained from Matheson, Coleman, and Bell (East Rutherford, New Jersey).

N-Propanol

Reagent grade n-propanol was mixed with ^{14}C-labeled n-propanol to give the desired specific radioactivity. The radioactive propanol was obtained from International and Nuclear Corporation in Irving, California.

Humidification Experiments

In several experiments freeze-dried PVP-n-propanol systems were humidified by placing tared and weighed

flasks in vacuum desiccators containing saturated salt solutions, which maintained the desired constant relative humidities.

N-Propanol Analysis

The n-propanol content was determined by measuring the radioactivity of the samples with a liquid scintillation counter.

The dried samples of PVP were dissolved in water (to 10% solution); 1 ml of this solution was added to 10 ml of water-miscible scintillator (2,5-diphenyloxazole 1 g, naphtalene 100 g, dioxane to 1,000 ml volume) in the counting vial, and the resulting so.ution was counted with a liquid scintillation counter (Nuclear Chicago Corp., 720 series).

N-Propanol Adsorption

Some experiments on sorption of n-propanol in freeze-dried PVP were carried out as shown in Fig. 1. The sample was maintained at 24°C by circulating heated air from a blower operated by a temperature control. The activity of the n-propanol vapor was regulated by controlling the temperature in the cold bath.

RESULTS AND DISCUSSION

Retention of n-propanol after freeze drying solutions of PVP and n-propanol depends on freezing rate, sample

thickness, drying conditions, and the concentration of PVP and propanol (Chirife et al., 1972). Under typical drying conditons for 20% PVP, 1% n-propanol solution, the retention was 0.49 g n-propanol/100 g PVP in rapidly frozen samples and 1.2 g/100 g in slowly frozen systems. Most of the retained alcohol was entrapped internally in microregions, as evidenced by lack of desorption in the absence of structural disruption by water (Chirife et al., 1972).

There appeared to be indications, however, that part of the alcohol may be retained after freeze drying by strong adsorption. Relative contribution of adsorption is likely to be most pronounced in rapidly frozen systems in which the microregion development is less complete than in slow frozen systems, especially in the case of polymers which have a low mobility, and in which entrapment is more difficult.

Fig. 2 shows the n-propanol content in freeze-dried PVP after humidification to 11% and 52% RH. In the PVP system the BET monolayer value for water, at which microregion disruption begins to occur, corresponds to 30% RH (Chirife et al., 1972). The loss at 11% RH therefore may be due to desorption of the adsorbed fraction; relative contribution is greater in rapidly-frozen PVP than in slowly-frozen PVP, even though the absolute losses at 11% RH are comparable (0.11 g/100 g PVP for

slow frozen and 0.15 g/110 g for rapidly frozen).

Fast and slow frozen freeze-dried PVP-n-propanol systems were kept at very low humidity by placing samples in evacuated desiccators containing activated charcoal and calcium sulfate at 37°C and 50°C with the loss of n-propanol determined. Results are shown in Fig. 3. In all cases there is a loss asymptotically approaching a value dependent on temperature. These losses are likely to correspond to the portion of total alcohol which is held by "adsorption irreversible by freeze drying" but reversible by desorption at a higher temperature. The remaining n-propanol is held in microregions which are impermeable until disrupted by treatment with water vapor or possibly other structure-disrupting treatments (polar solvents, very high temperature). The fractional contribution of adsorption is larger for rapidly frozen than for slowly frozen samples. However, the absolute losses do not differ much: at 50°C and 93 hr 0.20 g n-propanol/100 g PVP are lost in slowly frozen samples, and 0.19 g/100 g PVP in rapidly frozen samples.

In order to confirm that a portion of the total propanol retained after freeze drying of PVP is capable of being held by adsorption, we conducted adsorption-desorption experiments. Freeze-dried samples of PVP containing no propanol were placed in a vacuum oven at 95-100°C for 72

hr to remove the last traces of water. Adsorption of n-propanol was then carried out at room temperature (24°C) by exposing the samples to the alcohol vapor at propanol activities of 0.20 or 0.11. The samples were then transferred to a vacuum desiccator containing activated charcoal and calcium sulfate; desorption of n-propanol was followed with time at either 25°C or 37°C.

Fig. 4 shows the decrease of adsorbed amount with time. It can be seen that, following an initial rapid rate of desorption, the removal of n-propanol continues very slowly and appears to approach a constant retention level; this level decreases with increase in temperature (curves B and D).

The interpretation of this phenomenon could be simply made on the basis of the energies of activation of desorption (Bliznakov et al., 1966). The removal of each successive portion of alcohol is more difficult due to the increase of the energy barrier, until a value is reached of the energy of desorption at which the rate of the process becomes practically zero. This will determine the irreversible amount of adsorbed n-propanol.

However, that the situation is not so simple is clearly illustrated by curve C in Fig. 4, which corresponds to a sample adsorbed initially at a lower vapor activity (0.11) than that for curve B (0.20) and even more dramatically in Fig. 5 which shows desorption of n-propanol

after exposure to saturated vapor of the alcohol up to an adsorption of about 12%. The amount of retained n-propanol depends on the initial amount adsorbed and on the activity of the sorbate. Even at the relatively low vapor activitites in Fig. 4 n-propanol is capable of penetration into the PVP structure and subsequent entrapment.

This fact makes difficult to assign the total retention observed in adsorption experiments to a true adsorption process. This is also confirmed by the results showed by curve E (Fig. 4) corresponding to the desorption of adsorbed n-heptanol: the retained amount is practically zero. Heptanol does not readily dissolve PVP, in contrast to n-propanol.

The retention of strongly adsorbed alcohol in the polar PVP in preference to water would be difficult to accept in view of the relative polarities of water and propanol. However, our experiments in the PVP system showed that there is water retention in addition to n-propanol retention:

Twenty samples of 20% PVP solutions (3.5 mm thickness) without volatile were freeze dried in standard conditions for 48 hr. The samples were analyzed gravimetrically (24 hr, vacuum oven at 95-100°C) for residual moisture content. No significant difference was found between slow and fast frozen samples. The "water retention" was found to be 0.66 $\pm$ 0.11 g water/100 g PVP.

For fast frozen samples the retention of n-propanol was 0.49 g n-propanol/100 g PVP. On molar basis the retention of water alone is much higher than the total retention of the n-propanol, which also included the alcohol entrapped in microregions. The values for water are: 14.7 moles/mole PVP; for n-propanol they are 3.27 moles propanol/mole PVP.

Desorption of previously adsorbed water was studied in the following experiment:

Samples of PVP freeze-dried without n-propanol were placed in a vacuum oven at 95-100°C for a period of 24 hr. The dry weight was recorded. The samples were placed in a vacuum desiccator over a constant humidity solution until they reached an equilibrium moisture content of 13.5 g water/100 g PVP. The samples were then removed and desorbed by evacuation over a bed of calcium sulfate. Fig. 4, curve A shows the decrease of the adsorbed amount (on molar basis) of water with time. It can be seen that below about 0.039 moles water/100 g PVP (~0.71 g water/100 g PVP) there is almost no decrease with time. This amount of adsorbed water is close to the water content retained after freeze drying (0.66 $\pm$ 0.11 g water/100 g PVP). This strong semipermanent adsorption responsible for part of the retention is referred to as irreversible adsorption, which is defined as the situation occuring when there is adsorbed material

which cannot be removed by evacuation at the temperature at which the adsorption was carried out (Clark-Monks et al., 1970).

Additional evidence for adsorption as a mechanism for part of retention in the PVP systems was obtained from experiments with layered systems. Samples were prepared by rapidly freezing alternate layers of a solution containing no volatile and layers of a solution containing the volatile. Each layer was completely frozen before the next layer was added. After the standard cycle of freeze drying (48 hr) the layers were separated for individual analysis. In some samples the layers were separated by thin brass mesh to avoid any "contamination" between layers. In other samples, an already freeze-dried layer of PVP with n-propanol was placed on the frozen layer containing the volatile.

Good agreement was found among all the samples: it was observed that the amount of n-propanol adsorbed in the dry layers was about 10% of the total retention found in the layers originally containing the volatile. The absolute amounts were 0.047-0.061 g n-propanol/100 g PVP.

We can now explain the observed results by the hypothesis that, in addition to the bulk of the retained alcohol which is entrapped in impermeable microregions, a portion of the retained alcohol and a portion of the

water remaining after freeze drying are held by adsorption outside the impermeable microregions.

The situation in which two vapors are in competition for sites capable of adsorbing either vapor can be approximated by equations (1) and (2) derived from the Langmuir isotherm, provided that the assumption is made that there is no heterogeneity of the solid surface, and no opening of additional sites or adsorbate interactions during adsorption:

$$n_w = \frac{N_w b_w p_w}{1 + b_w p_w + b_a p_a} \quad (1)$$

$$n_a = \frac{N_a b_a p_a}{1 + b_a p_a + b_w p_w} \quad (2)$$

where N_w = number of sites available for water sorption

N_a = number of sites available for alcohol sorption

p_a = partial pressure of alcohol

p_w = partial pressure of water

n_a = number of sites occupied by alcohol

n_w = number of sites occupied by water

b_a, b_w = constants

The above equations show that only when

$$b_w p_w + b_a p_a << 1$$

is the adsorption of the two vapors independent of one another.

The experimental observations of competition by sorption sites in the PVP between water and n-propanol (Chirife et al., 1972) and the observed losses of alcohol at low relative humidities (Fig. 2) can be explained qualitatively through the set of equations (1) and (2). However, these equations would have to be modified to give quantitative information, since adsorption of polar vapors on polar polymers does involve surface heterogeneity, interactions between vapors, as well as changes in number of available sites during sorption (Le Maguer, 1972). Perhaps this could be partially accounted for through the study of Ross and Oliver (1964) who devised a means of allowing quantitatively both for intermolecular attraction and for surface heterogeneity at the same time.

Our results confirm that the total amount of alcohol remaining in the freeze-dried PVP solutions is composed of a fraction entrapped in impermeable microregions, and a fraction adsorbed, in competition with water, in locations from which it can be desorbed, albeit slowly.

In slow frozen samples the entrapped fraction accounts for most of the retained n-propanol and the effect of processing variables are explainable entirely by the microregions theory (Flink and Karel, 1970b).

In the case of fast-frozen PVP, however, the adsorbed

fractions is sufficiently large to cause some deviations from behavior attributable entirely to entrapment.

According to the microregions theory (Flink and Karel, 1970b), retention decreases with increasing thickness primarily because the more rapid drying and steep moisture gradients in thin samples decrease the time during which the moisture content in the microregions is high enough to permit volatile escape. Fig. 6 presents the retention of n-propanol in fast and slow frozen PVP as a function of thickness of sample. In fast frozen PVP the retention is independent of the thickness of the sample. This observation may be due to a combination of adsorption and entrapment, since at the end of the drying period desorption will be less complete in thicker samples than in the thinner samples.

Adsorption and desorption of organic volatiles on food components has been studied by other investigators. Rey and Bastien (1962) interpreted their observations of acetone retention in a freeze-dried model containing glucose as an indication of sorption of the acetone by the dry layer. However, it was later demonstrated by Menting et al. (1967) and Flink et al. (1970) that, at least for systems containing carbohydrates and alcohols or ketones, the observed retention during freeze drying could not be attributed to adsorption in the dry portion of the freeze-dried material, and adsorption as well as

desorption required humidification of the carbohydrates above the BET monolayer value.

Issenberg et al. (1968) and Le Maguer (1972) studied volatile adsorption by cellulose, but failed to relate the observed sorption to any amounts retained in freeze drying.

Maier (1969, 1970, 1971, and 1972) studied the binding of various volatile organic compounds to foods and food components. He found strong interactions between aliphatic amines and acidic polysaccharides, which resulted in irreversible sorption. Reversible sorption occured with neutral polysaccharides. In contrast to alcohols, the amines were capable of penetration into polymeric aggregates even in the absence of water. Strong amine-pectin interactions with acidic polysaccharides were observed also by Gray and Roberts (1970). Maier (1972) reported on sorption of ketones on a number of dry and humidified substrates including: coffee, milk, potato flakes, fruit powders, zein, cellulosic materials, pectins, starches, alginic acid, casein, agar, and egg albumin.

He observed that sorption of ketones usually required either the presence of water to allow ketone penetration into polymeric aggregates, or presence of fat in which the ketones apparently dissolve. In absence of water there was some sorption of acetone on zein, starch, and pectin. The adsorption was irreversible. Infrared measurements confirmed that adsorption was due to

(ketone)-C=O···HO-(polymer) hydrogen bonds.

Binding of alcohols to the peptide CO groups of poly-L-proline was demonstrated by the shift of the OH stretching vibration of the alcohols (Strassmair et al., 1971). (PVP)-C=O···HO-(alcohol) bonds are probably involved in the PVP-n-propanol interactions. In the PVP-n-propanol system, however, some of the propanol adsorbed is apparently held irreversibly with respect to desorption under freeze-drying conditions; however, n-heptanol which is not a solvent for PVP is readily desorbed after adsorption in the dry state.

In conclusion, we showed that in rapidly frozen, freeze-dried polymer solutions, adsorption may contribute significantly to the total amount of volatile retained after freeze drying. Our results do not allow us to determine the exact amount held by adsorption rather than entrapment, but in most of the cases we studied, the major fraction is that held by entrapment in microregions.

Further studies on more complex systems are underway.

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FIGURE LEGENDS

Fig. 1. Apparatus for study of n-propanol adsorption on PVP.

Fig. 2. Retention of n-propanol in freeze-dried PVP humidified to 11% and 52% RH.

Fig. 3. Retention of n-propanol in freeze-dried PVP during desorption at 37°C and 50°C.

A. Slowly frozen, desorption at 37°C

B. Slowly frozen, desorption at 50°C

C. Rapidly frozen, desorption at 37°C

D. Rapidly frozen, desorption at 50°C

N-propanol content after freeze drying:

A and B: 1.2 g/100 g PVP

C and D: 0.49 g/100 g PVP

Fig. 4. Desorption of water and of alcohols after adsorption on freeze-dried PVP

A. Water, desorption at 25°C

B. N-propanol (adsorption at propanol activity of 0.2) desorption at 25°C

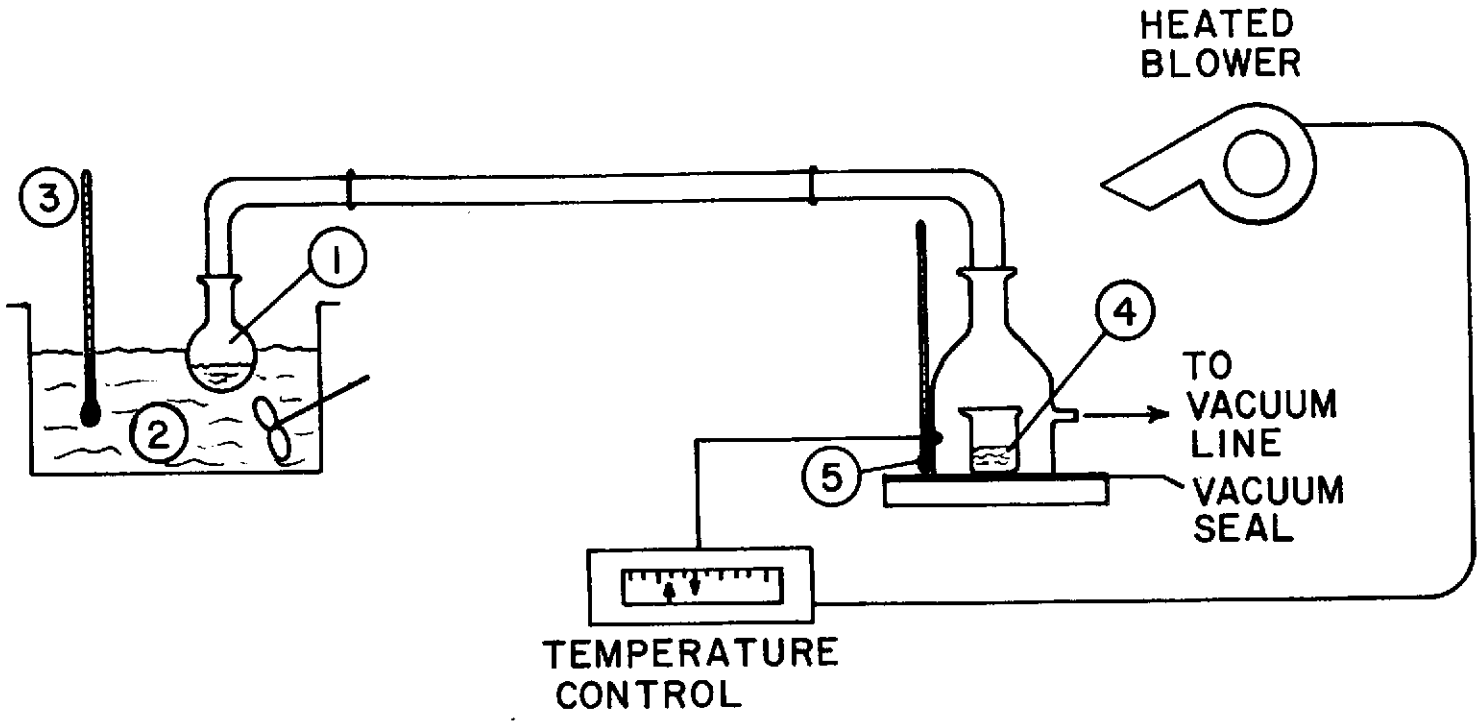
C. N-propanol (adsorption at propanol activity of 0.11) desorption at 37°C

D. N-heptanol, desorption at 25°C

Fig. 5. Desorption of n-propanol adsorbed by dry PVP by exposure to saturated vapor.

Fig. 6. Effects of freezing rate and of sample thickness on retention of n-propanol in freeze-dried PVP.

Figure V-1



(1) n-PROPANOL

(2) CONSTANT TEMPERATURE BATH

(3) THERMOMETER

(4) SAMPLE

(5) THERMOMETER

Figure V-2

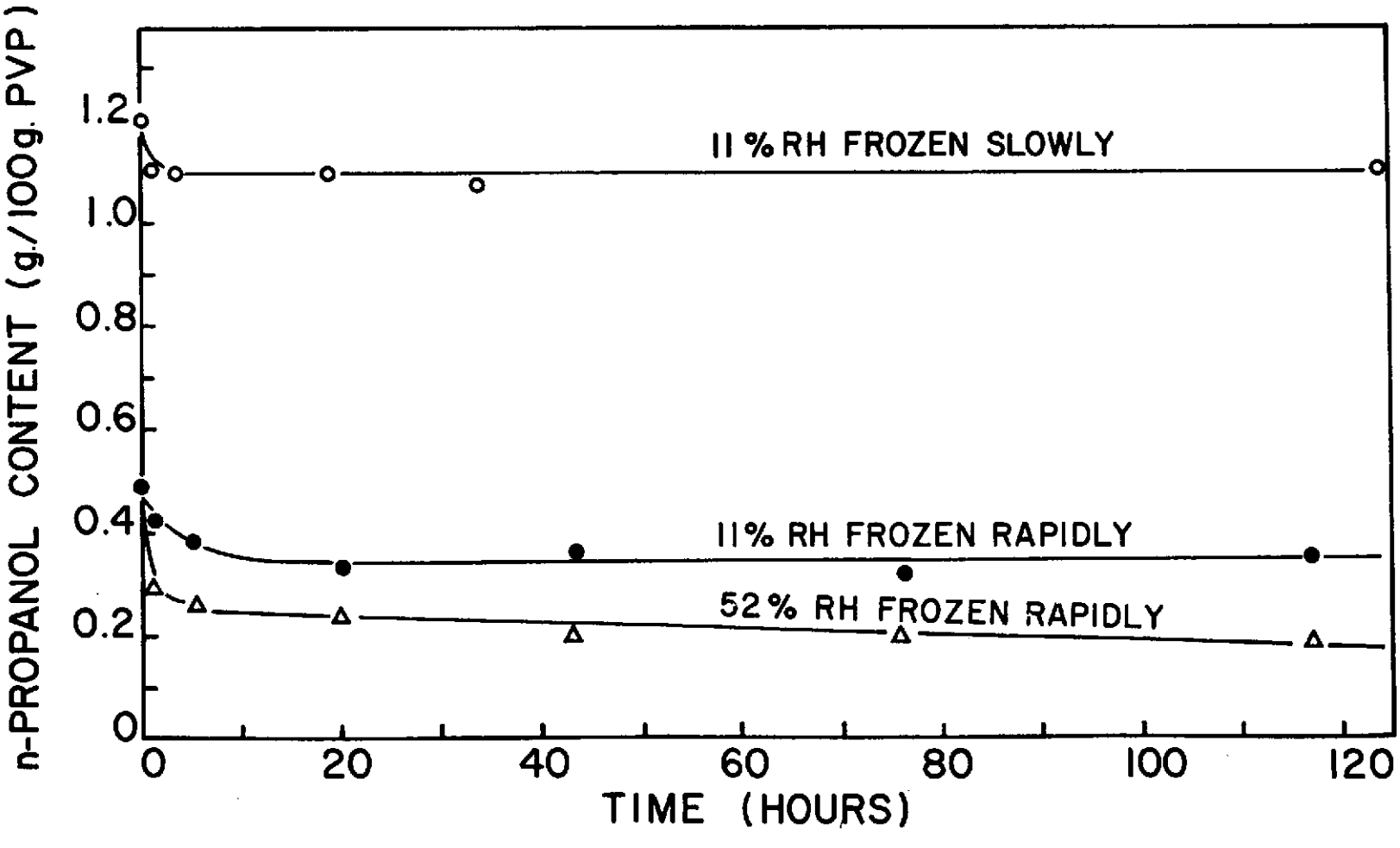

n-PROPANOL CONTENT (g./100g. PVP)
1.2
1.0
0.8
0.6
0.4
0.2
0
11% RH FROZEN SLOWLY
11% RH FROZEN RAPIDLY
52% RH FROZEN RAPIDLY
0
20
40
60
80
100
120
TIME (HOURS)

Figure V-3

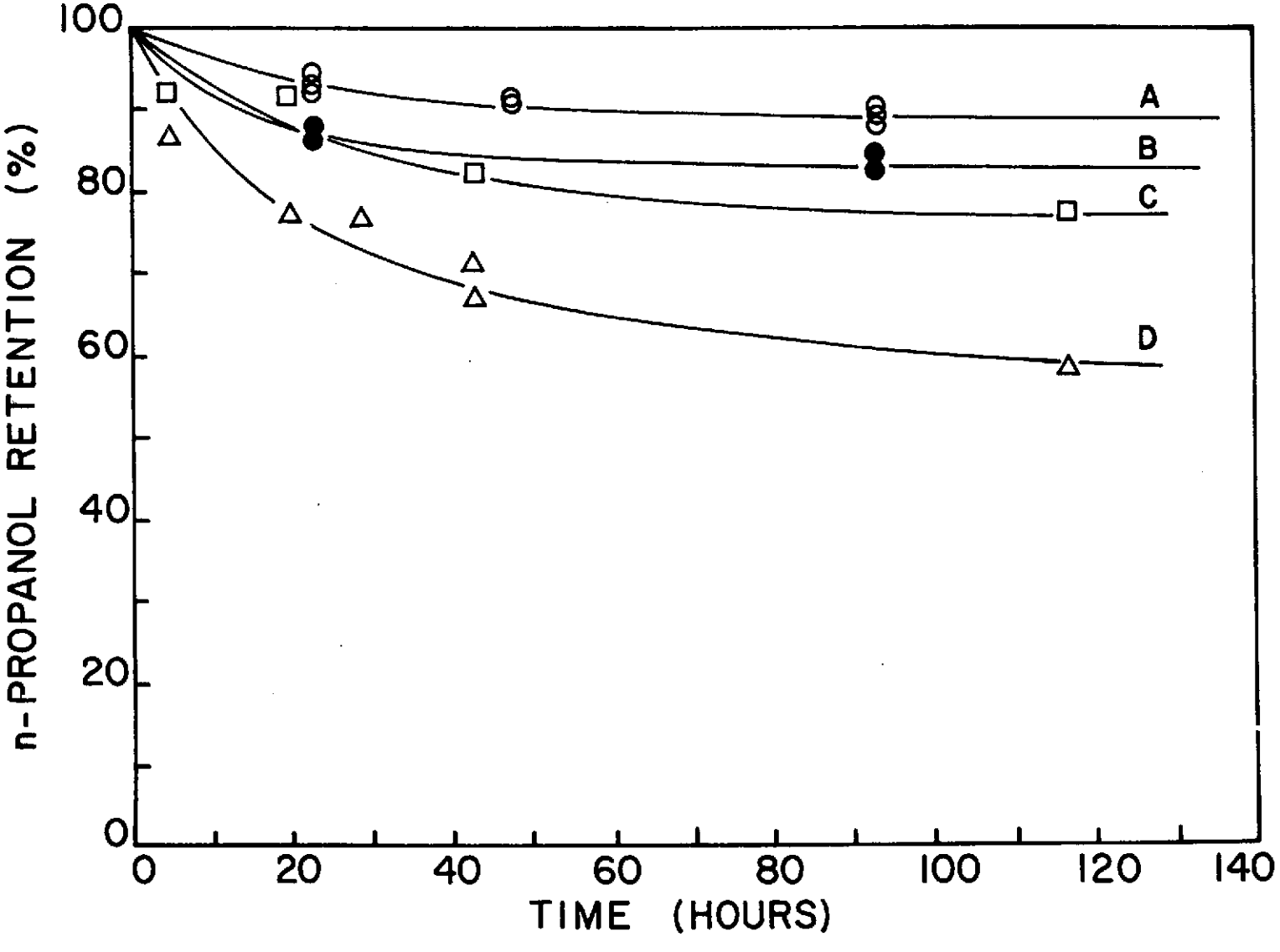

100
80
60
40
20
0
n-PROPANOL RETENTION (%)
0
20
40
60
80
100
120
140
TIME (HOURS)
A
B
C
D

Figure V-4

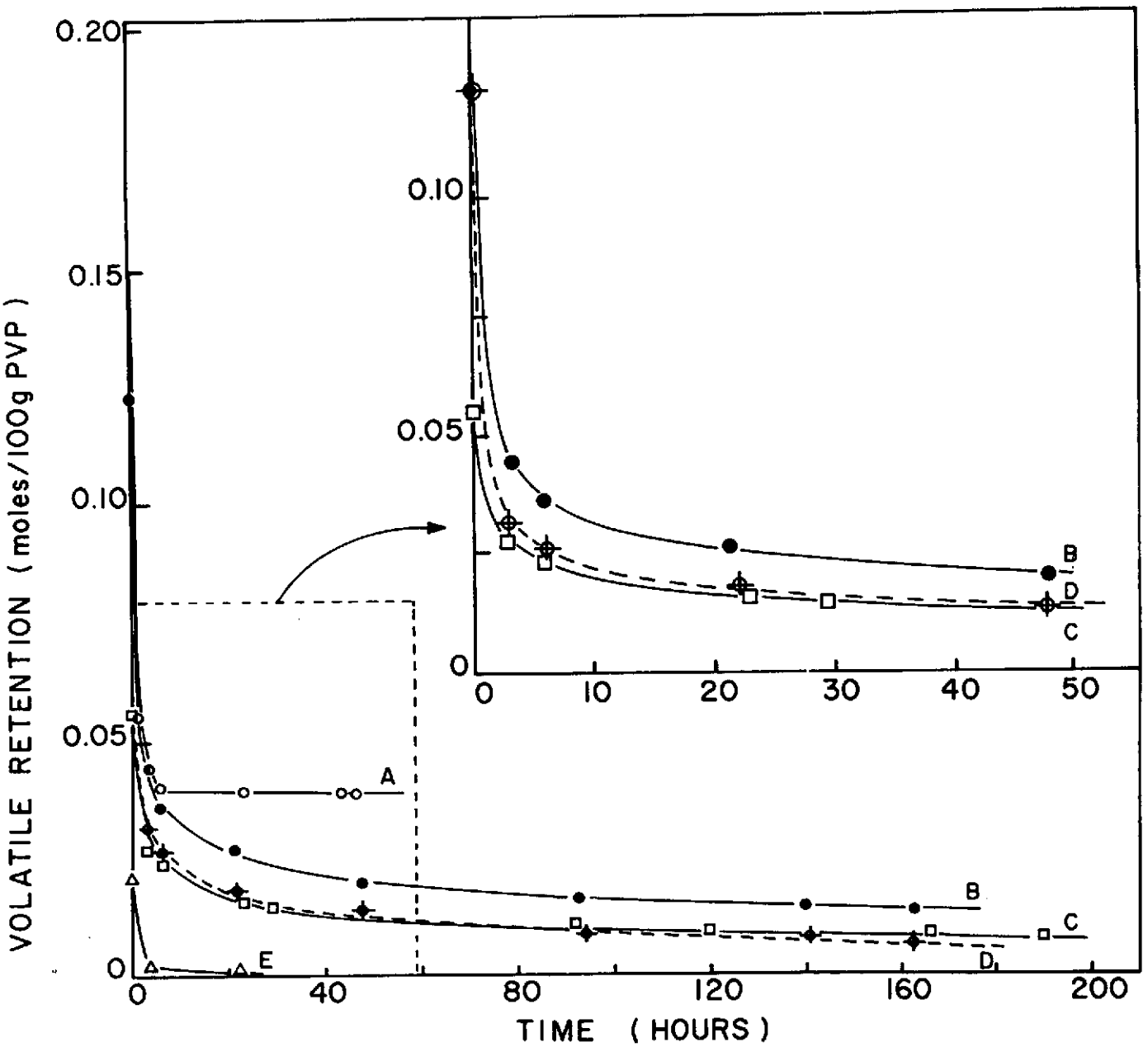

VOLATILE RETENTION (moles/100g PVP)
TIME (HOURS)
0.20
0.15
0.10
0.05
0
0
40
80
120
160
200
A
B
C
D
E
0.10
0.05
0
0
10
20
30
40
50
B
D
C

Figure V-5

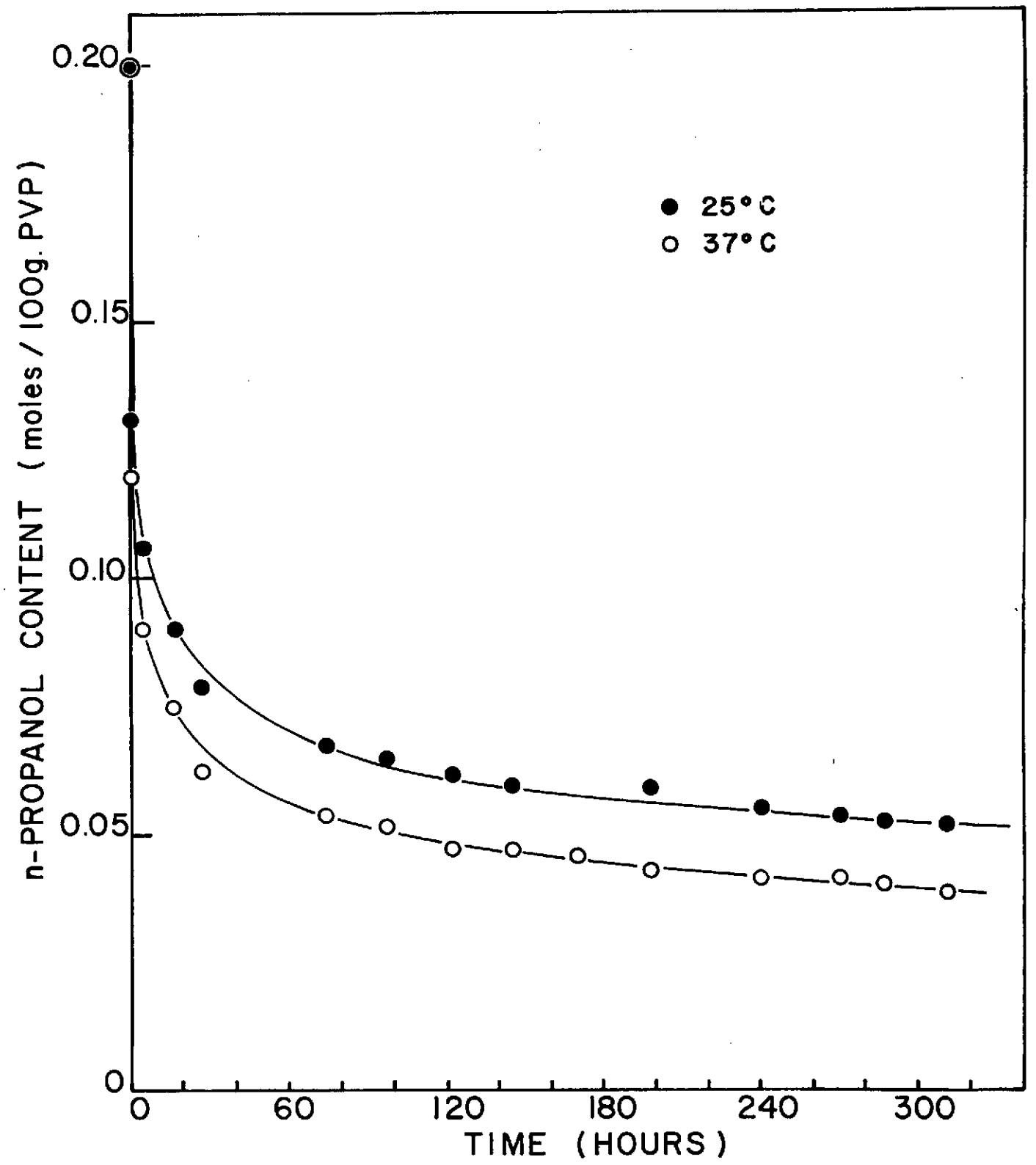
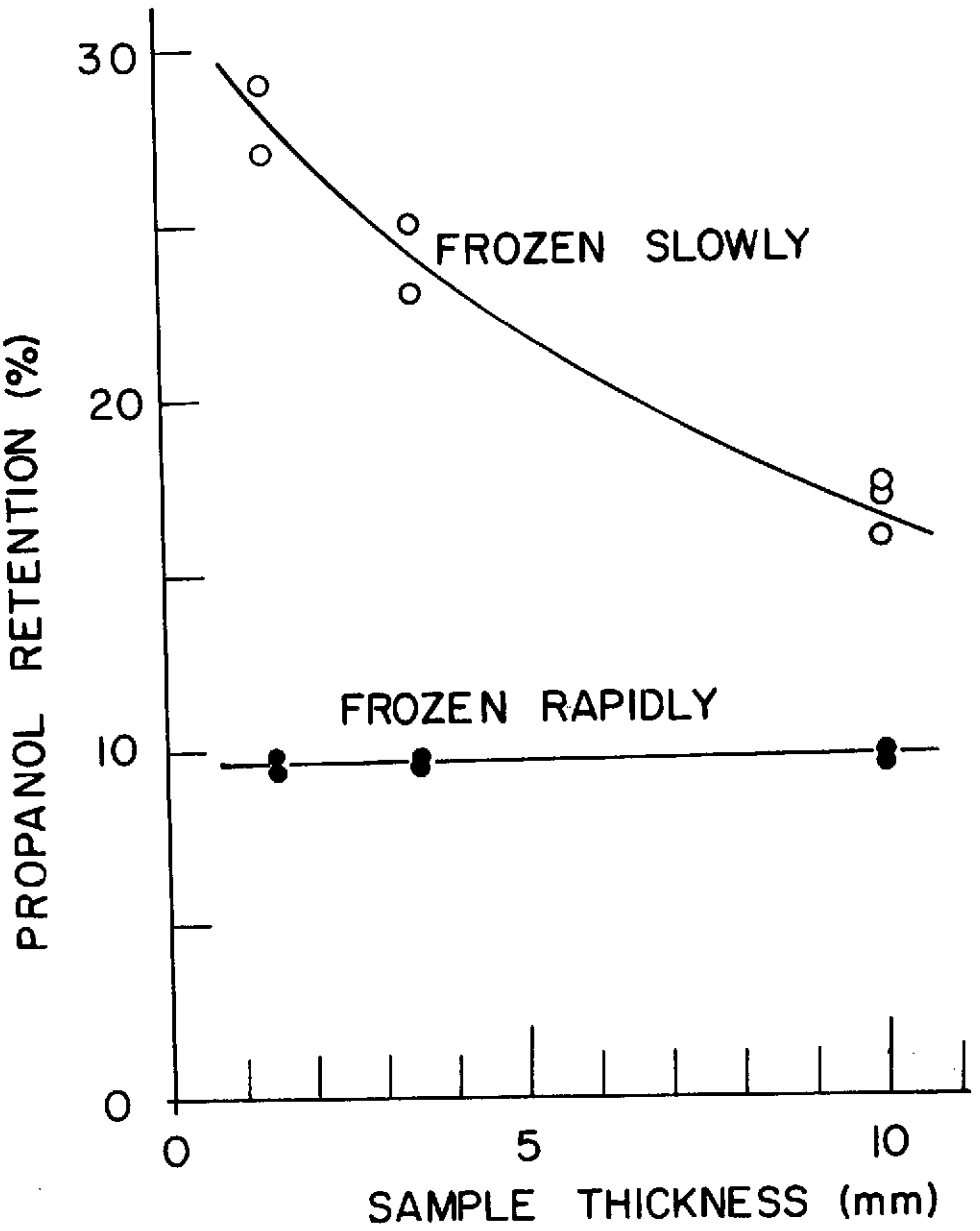

0.20
0.15
0.10
0.05
0
n-PROPANOL CONTENT (moles / 100g. PVP)
25° C
37° C
0
60
120
180
240
300
TIME (HOURS)

Figure V-6


30
20
10
0
PROPANOL RETENTION (%)
FROZEN SLOWLY
FROZEN RAPIDLY
0
5
10
SAMPLE THICKNESS (mm)

VI. Development of a Food Product

VI.A. Introduction

Studies on the utilization of improvements in freeze-dehydration processing suggested by research conducted in Phase I of this project was directed to the development of dehydrated fruit products. Our research has shown that the process variables of greatest influence on quality retention are freezing rate and initial solids content. Studies on a method for increasing the initial solids concentration has been based on sucrose immersion prior to freeze-drying. This method of increasing the solids concentration has been conducted at two temperatures and for two apple varieties. One variety of apple has been evaluated for the influence of freezing rate and initial solids content.

Organoleptic evaluations are being conducted for the response of taste and texture of the dried and rehydrated products to the changes of the various processing conditions, most specifically, solids content and freezing rate.

VI.B. Fruit products and processing

Three raw materials have been given preliminary evaluations; peaches, melon and apples. At the time when more complete evaluations could begin, the only raw material readily available was apples, and thus the remaining studies were conducted with apple slices. It is intended to renew the studies of other fruits as they become readily available again.

Apples are washed, peeled and cored and then cut into slices approximately 1/4" thick. The weighed slices are immersed in an agitated 60% aqueous sucrose solution which also contains 0.5% ascorbic acid and 0.12% malic acid. The immersion in the sucrose solution results in a partial dehydration of the apple slices through osmotic transport of water from the slices and the increase of sugar content in the apples due to diffusive transport of sucrose. Both these mass transfers contribute to the desired goal of increased initial solids content prior to freeze-drying. Experience has shown that immersion between 3 and 5 hours gives desirable changes in the initial solids content, an increase from 13% in the raw apples to 35% in the treated apples.

Following the immersion treatment, the samples are frozen according to the desired method (either a -30°C cold room or periodic immersion in liquid nitorgen), and then freeze-dried. Until now freeze-drying has been conducted with limited radiational heat transfer at a chamber pressure of approximately 100 microns. The dried product is stored in glass jars while awaiting evaluation.

VI.C. Organoleptic evaluations

Two major evaluations have been conducted using the dehydrated material as the final product. Organoleptic evaluations of the rehydrated material are currently in progress for products produced using methods giving good dehydrated products. The process variable being evaluated for their influence on the organoleptic properties of taste

and texture is given below:

Test 1: Apples

A) MacIntosh apples; sucrose immersion at 25°C (M)

B) MacIntosh apples; " " " 45°C (MH)

C) Cortland apples; " " " 25°C (C)

D) Cortland apples; " " " 45°C (CH)

Test 2: MacIntosh apples

A) Normal solids (13%); slow freezing (-30°C room) (NS)

B) Normal solids (13%); fast freezing (LN_2) (NF)

C) Increased solids (35%); slow freezing (-30°C room) (IS)

D) Increased solids (35%); fast freezing (LN_2) (IF)

Test 1 and Test 2 were based on suggested methods from "Methods for Sensory Evaluation of Food" (E. Larmond, Canadian Dept. of Agriculture, Publ. 1284, 1970) and the results of Test 1 were often not statistically significant but still we feel that they were indicative of panel preferences. The panel size was increased for Test 2 to give better statistical information.

Test 1: Comparison tests for texture and taste showed that the room temperature sucrose immersion gave the preferred product for both varieties of apples (M/MH-7/1; C/CH-7/1). When comparing all four process variables 6 of the 9 panelists chose M as the best (1 chose MH and 2 chose CH). When giving numerical values to first choice through fourth, the average order of preference

was M,CH,C,MH, though difference tests using a 6 point scale (excellent to very ppor) had M and/or C much superior to MH or CH. The difference between M and C was negligible.

Test 2: Since MacIntosh apples are more readily available, they were the variety chosen for Test 2. Here a larger sample size was utilized and statistically significant differences were obtained. In difference tests the following observations were made.

A. Taste

1) IS differed from NS and IF differed from NF

2) IS differed from IF

B. Texture

1) IS differed from NF and NS

Preference tests were made between 3 groupings which give conclusive evidence for the superiority of slowly frozen, immersion treated apples.

1) 12 of 12 judges prefer IS over IF

2) 8 of 12 judges prefer NF over NS

3) 12 of 12 judges prefer IS over NS

Tests are continuing on the evaluation of the rehydrated apple slices, and next spring and summer the evaluations will be expanded to include other fruits, as they are available.

VII. Summary of Results

1) Losses of n-butanol from its frozen aqueous solution can be characterized as water-independent and water-dependent.

2) During unidirectional freezing, a butanol rich layer forms at the sample surface. It is the loss of this butanol during equilibration which is independent of water loss.

3) Butanol is easily transported through a cake of bulk ice.

4) Bulk ice can sorb butanol vapors on the free surface and inner pore spaces.

5) The water-independent butanol loss (absolute amount) increased with increased equilibration temperature, decreased freezing rate, increased initial butanol concentration and increased sample surface area.

6) A fraction of the butanol, which is entrapped within the ice dendritic structure during freezing is lost only with simultaneous loss of water.

7) A freeze-drying microscope having high magnification capability has been developed.

8) Preliminary studies indicate that n-alcohols of intermediate solubility exist as droplets and/or solution in aqueous media. Cooling of the solution results in increased droplet formation.

9) Droplets are pushed by growing ice dendrites until they are entrapped in the carbohydrate solute matrix. Freeze-drying stabilizes the matrix and the entrapment

of the volatile.

10) Similar phenomenological behavior has been noted for n-butanol and n-hexanol, the n-hexanol being tested at concentrations from 0.1% to 0.8%.

11) Browning of a glucose-glycine-Avicel model system showed a time-temperature tolerance with a sizable increase in browning rate at temperatures in excess of 70°C.

12) The energy of activation for the browning reaction was 19 Kcal/mole.

13) Sample geometry gave only a very minimal effect probably related to heat transfer consideration. Dark brown color formation was observed to progress as a front through the sample.

14) Changes in the inert support concentration exhibited only small effects on browning.

15) Varying the glucose-glycine ratio showed that the browning reaction is limited by the glucose concentration.

16) PVP was used as a model for polymeric substances containing the -C=O group, to complement results obtained previously with carbohydrate systems, and formed a suitable model for polymeric solids.

17) The bulk of the retention of n-propanol in freeze dried PVP showed behavior consistent with the microregion theory of Flink and Karel, and confirmed the general validity of the following guidelines for maximal retention:

a) Absolute retention increase with concentration of volatile, but the fractional retention decreases with volatile concentration.

b) Retention increases with increasing solids content.

c) Slow freezing increases retention over fast freezing.

d) Retention increases with decreasing sample thickness.

e) Absorption of water in amounts exceeding the monolayer value results in disruption of micro-regions and invvolatile loss. This loss increases with increasing water uptake.

f) Disruption may also be achieved, and volatile released, by absorption of vapors others than water, provided that the matrix material is soluble in them. Thus PVP microregicns can be disrupted by substantial sorption of propanol.

18) In contrast to carbohydrate systems in which the contribution of adsorption to total retention is minimal, in the fast frozen PVP-propanol system adsorption appears to account for a small but significant fraction of total retention (∿10%).