This is to certify that the

dissertation entitled

THE FORMATION OF DIFRUCTOSE DIANHYDRIDES AND THEIR IMPACT ON FRUCTOSE CRYSTALLIZATION

presented by

Yi-ding Chu

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Agricultural Engineering

Kris A. Bruglund
Major professor

Date June 15, 1988

THE FORMATION OF DIFRUCTOSE DIANHYDRIDES AND THEIR IMPACT ON FRUCTOSE CRYSTALLIZATION

By

Yi-ding Chu

A DISSERTATION

Submitted to

Michigan State University

in partial fullfilment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

in

Agricultural Engineering

Department of Agricultural Engineering

1988

ABSTRACT

THE FORMATION OF DIFRUCTOSE DIANHYDRISES AND THEIR IMPACT ON FRUCTOSE CRYSTALLIZATION

By

Yi-ding Chu

Fructose undergoes dehydration reactions and forms difructose dianhydrides *in situ* during crystallization. Difructose dianhydrides have been proposed as a cause in the decrease of overall yields of fructose crystallization. In this study, the kinetics of difructose dianhydrides formation under industrial crystallizations and the effect of difructose dianhydrides on the crystal growth of anhydrous fructose from aqueous solution were investigated.

The kinetics of difructose dianhydrides formation were determined by HPLC analysis. Four difructose dianhydrides were detected. A second-order irreversible kinetic model was proposed for the reaction. The extent of reaction increased with increasing temperature and decreasing pH value of the solution. The amounts of two of the difructose dianhydrides stopped increasing when the fructose concentration was 70% or less. On the other hand, the formation of all difructose dianhydrides were retarded when the initial fructose concentration was higher than 80%.

The kinetics of fructose crystal growth in the presence of difructose dianhydrides were studied using photomicroscopic contact nucleation techniques. Difructose

dianhydrides were found to cause inhibition of crystal growth. Since the molecular structures of difructose dianhydrides are similar to that of fructose, the decrease of crystal growth is probably caused by the incorporation of these impurities or adsorption to the crystal face which would accept fructose molecules in the orientation that existed in the difructose dianhydride.

ACKNOWLEDEGMENTS

The author is deeply indebted to her major professor, Dr. Kris A. Berglund for his guidance, understanding and encouragement throughout this work.

Appreciations are also expressed to Drs. Eric Grulke, Pericles Markakis, and James Steffe for their helpful suggestions and as the members of the guidance commitee.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1

CHAPTER 2

LIST OF FIGURES

INTRODUCTION

Fructose, also called levulose or fruit sugar, has great potential use as a natural sweetener due to its wide abundance and high sweetness. Pure crystalline fructose has until recently remained a noncommercial product because of the expense involved in its production. Earlier methods of fructose isolation from either hydrolyzed sucrose or hydrolyzed fructose polymers were not economically competitive with the very low price for sucrose processed from sugar cane or sugar beets. The large scale industrial production of fructose became possible when ion-exchange techniques were developed to separate the glucose and fructose components in aqueous solutions of inverted sucrose or a mixture obtained by glucose isomerase conversion of glucose to fructose. In 1988, the wholesale price of commercial crystalline fructose in the United States is about $2.2/Kg. At the retail level, crystalline fructose still costs about 10 times as much as sucrose. This is mainly because the crystallization step in its production is very time-consuming and requires careful control of process conditions.

The physico-chemical properties of fructose are unfavorable to crystallization. It is highly soluble in

water resulting in a very viscous saturated solution. It tends to crystallize as hemihydrate and/or dihydrate phases rather than the anhydrous form under centain conditions. These hydrate compounds melt near room temperature which makes them unacceptable as products. Fructose also undergoes a complex mutarotation reaction in aqueous solution with three tautomeric forms existing in significant amounts.

In addition to physico-chemical properties, the presence of impurities in fructose syrup may retard crystal growth. These imputities include glucose and difructose dianhydrides which may exist in the fructose syrup in substantial amounts. Residual glucose is often found after the ion-exchange enrichment of fructose syrup. Difructose dianhydrides are formed in situ under industrial crystallization conditions and have been reported to decrease the overall yield in fructose crystallization.

The objectives of this research are:

(1) to study the kinetics of difructose dianhydides formation under fructose crystallization conditions;

(2) to determine the effect of difructose dianhydrides on the growth of fructose crystals.

The results of this study will not only provide information necessary to the control of fructose crystallization, but also contribute to the basic understanding of fructose chemistry as well as the effect of imputities on the growth of crystals.

CHAPTER I

LITERATURE REVIEW

1.1. Crystallization

1.1.1. Introduction

Crystallization is one of the oldest unit operation used for production of solid material in the chemical industry. It has the advantage of yielding an end product that has good flow, handling, and packaging characteristics, and also attractive appearance. Major concerns in production of crystalline product include control of uniformity, or crystal size distribution, and purity.

The use of crystallization as a purification and separation process has extended far outside the traditional chemical industry. For example, the recent advances in microelectronics have been made possible in part because of the ability to grow single crystals of precisely controlled composition and structural perfection. Thus, basic understanding of the crystallization process is necessary not only for the design and control of industrial crystallizer, but also for advances in materials science [1].

Any crystallization operation can be considered to be comprised of three basic steps: achievement of

supersaturation, formation of crystal nuclei, and growth of the crystals. All three processes may occur simutaneously in different regions of the crystallization unit and control the quality and uniformity of the end product [2].

1.1.2. Supersaturation

Supersaturation is defined as the presence of more solute in solution than would exist at equilibrium. The condition of equilibrium is called the solubility of a particular solute. A typical solubility diagram is shown in Figure 1.1. Three regions exist in the solubility diagram [2]:

(1) stable zone (undersaturated including solubility curve) where no nucleation or growth occurs;

(2) metastable zone (supersaturated) where growth may occur but nucleation does not;

(3) labile zone (supersaturated) where both nucleation and growth occur.

The boundary between labile and metastable zones is not well defined.

Supersaturation can be achieved by cooling (corresponding to line AC' in Figure 1.1), evaporation (line AC) or a combination of cooling and evaporation (curve AC"). The choice of method depends on the compounds to be crystallized. Cooling is employed to create supersaturation for compounds having a steep solubility curve, while evaporation is used to achieve supersaturation for compounds

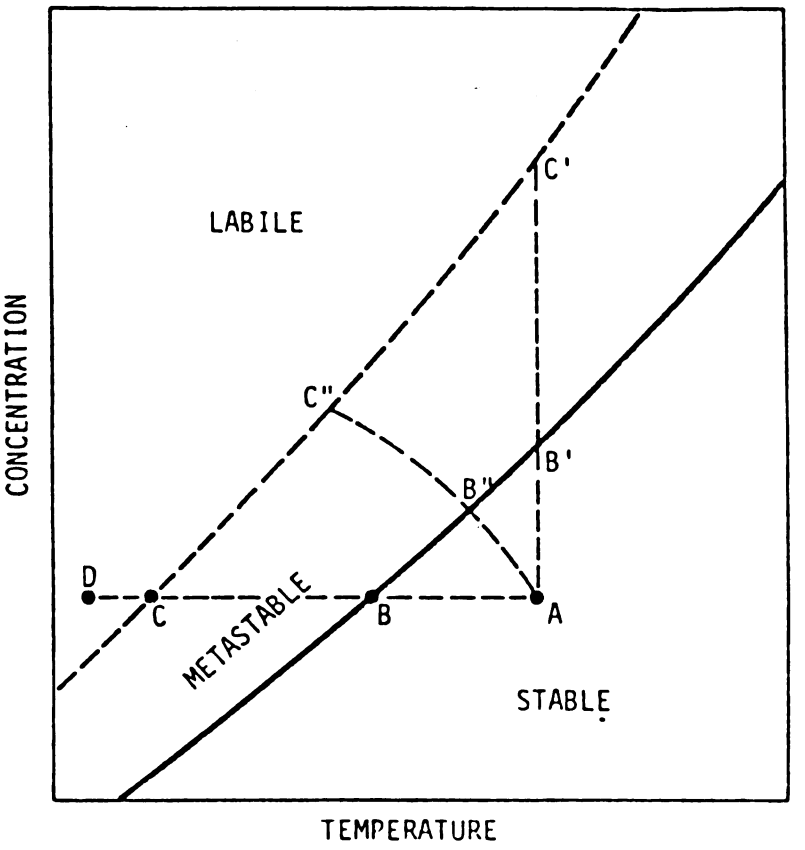


Figure 1.1 Typical solubility plot.
(from Mullin [2])

having a flat solubility curve [3].

The most common expressions of supersaturation are the concentration driving force ΔC, the supersaturation ratio S and relative supersaturation s defined as follows.

$$\Delta C = C - C^* \quad (1)$$

$$S = C/C^* \quad (2)$$

$$s = \Delta C/C^* = S-1 \quad (3)$$

where C is solution concentration and C^* is saturation concentration. Another often used expression is supercooling ΔT defined as

$$\Delta T = T^* - T \quad (4)$$

[2].

1.1.3. Nucleation

Nucleation is the formation of new solid phase. It is generally classfied as primary and secondary based on the presence of growing crystals in the solution [4].

a. Primary nucleation

Primary nucleation occurs in systems that do not contain crystals. There are two types of primary nucleation: homogeneous and heterogeneous. In homogeneous systems there is no foreign substance and the formation of nuclei is due to supersaturation only [2]. The mechanism is generally believed to be a series of bimolecular reactions:

$$A + A = A_2$$

$$A_2 + A = A_3$$

$$\vdots$$

$$A_{n-1} + A = A_n$$

The overall free energy of these aggregates goes through a maximum at some critical size which is inversely proportional to the logarithm of the solution supersaturation [5]. Aggregates larger than the critical size will decrease their free energy by further growth. Thus stable nuclei are formed and grow to macroscopic crystals.

The rate of nucleation is given by the equation

$$B = C_1 \exp\left[\frac{-C_2 \gamma_s^3}{T^3 (\ln S)^2}\right] \qquad (5)$$

where B is the number of nuclei formed per unit time per unit volume, C_1 and C_2 are constants, γ_s is interfacial tension, T is temperature, and S is supersaturation. Homogeneous nucleation is therefore dominant only at high supersaturation with the rate negligible at low supersaturations [1]. Most primary nucleation that occurs in practice is likely to be heterogeneous, i.e. induced by surfaces of foreign substances. The mechanism is unclear, yet it is thought to be the result of a local ordering process brought about by interactions across the interface [6].

b. Secondary nucleation

Secondary nucleation requires the presence of growing crystals in solution. It occurs at much lower supersaturation as compared to that in primary nucleation. In general most industrial crystallization processes operate under conditions favoring secondary nucleation [4].

Secondary nuclei can originate from different sources as discussed by Botsaris [4] and Jancic and Grootscholten [3]. The most important types of secondary nucleation are summarized in the following:

(1) initial breeding - when a crystal whose surface contains tiny crystallites is introduced into the solution, the crystallites fall off and form nuclei;

(2) needle breeding - when dendrites are formed on the surface of a crystal growing at high supersaturation, the dendrites may break off to form nuclei;

(3) fluid shear - when shearing action occurs at the crystal surface where certain ordered aggregated of solute molecule are attached, the aggregates may be removed and develop into new crystals;

(4) contact nucleation - when the surface of a growing crystal contacts the wall of the crystallizer, impeller or another crystal, nucleation occurs.

Contact nucleation has been more widely studied than other categories and is suggested to be the most significant secondary nucleation in most industrial crystallization [1]. There are two explanations for this phenomenon. The first possibility is that the contact results in microattrition of the crystal surface [7]. The second explanation involves the removal of an absorbed layer of solute molecules at the crystal surface [8].

The rate of secondary nucleation depends on three factors: supersaturation, degree of agitation ,and

suspension density. Empirical expressions which employ power law relations are generally used to model the kinetics of secondary nucleation [1].

1.1.4. Crystal growth

The growth of crystals from solution involves two steps. The solute is first transported from the bulk of solution to the vicinity of the crystal surface, then it is adsorbed and incorporated into the crystal lattice. The latter stage is often referred to as the surface integration process [3].

a. Mass transfer limited growth model

In some instances, the crystal growth is limited by the mass transfer of solute. The growth model usually used for this case is

$$G = k_C A \Delta C \qquad (6)$$

where G is crystal growth rate, k_C is mass transfer coefficient, A is surface area, and ΔC is the concentration difference between the solid surface and the bulk of solution [2].

b. Surface integration limited growth models

The mechanism of surface integration is depicted in Figure 1.2. Instead of being flat, a growing crystal surface has steps and the step may be incomplete, having one or more kinks. A growth unit which is adsorbed on the surface will diffuse across the surface looking for a favorable site to incorporate into the lattice. The most favorable site is a

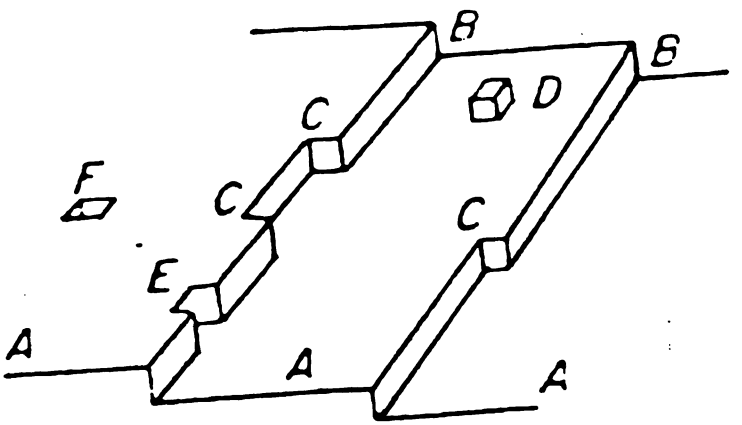


Figure 1.2 Surface of a growing crystal showing (A) flat surfaces (B) steps (C) kinks (D) surface-adsorbed growth unit (E) edge vacancies and (F) surface vacancies. (from Mullin [2])

kink followed by a step. Growth occurs when the units fill out the steps causing them to move across the surface [9]. Growth models are differentiated by the source of steps as follows.

(1) Two-dimensional growth models

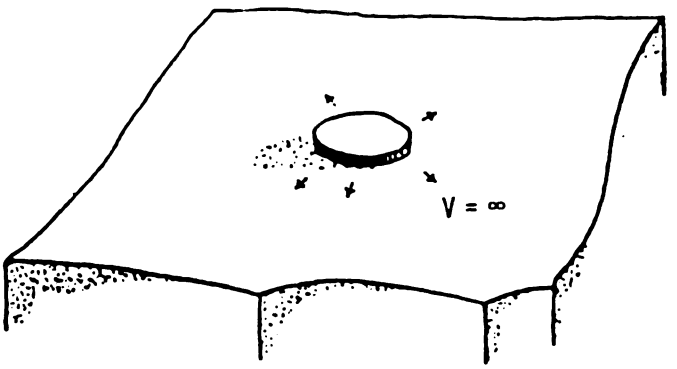
A new step could be created on a flat crystal surface by surface nucleation provided that the supersaturation is large enough. Ohara and Reid [10] discussed three models of this type as shown in Figure 1.3. They differ from each other in the relative rates of formation and growth (i.e. spreading) of the two dimensional nuclei. The mononuclear model applies for the case of infinite fast spreading; the polynuclear model corresponds to the case of near-zero spreading velocity. The birth and spread model describes cases between the two extremes, i.e. finite rate of both nuclei formation and spreading, and nuclei can be formed on unfinished layers. The general form of two-dimensional growth models is given by

$$G = A_1 s^p \exp(A_2/s) \qquad (7)$$

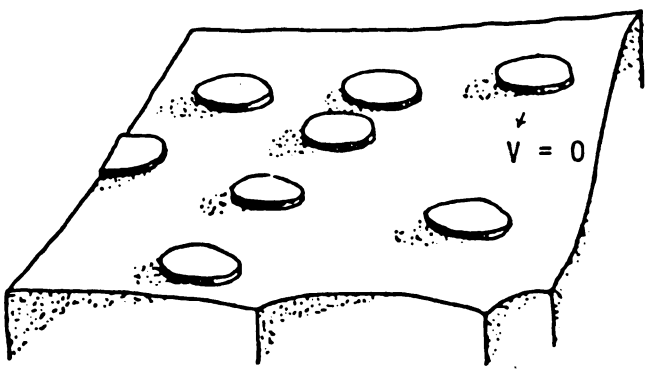
where G is growth rate, A_1 and A_2 are constants, and s is supersaturation. The exponent p = 1/2, 3/2 and 6/5 for mononuclear, polynuclear and birth and spread model respectively.

(2) Burton-Cabrera-Frank (BCF) model

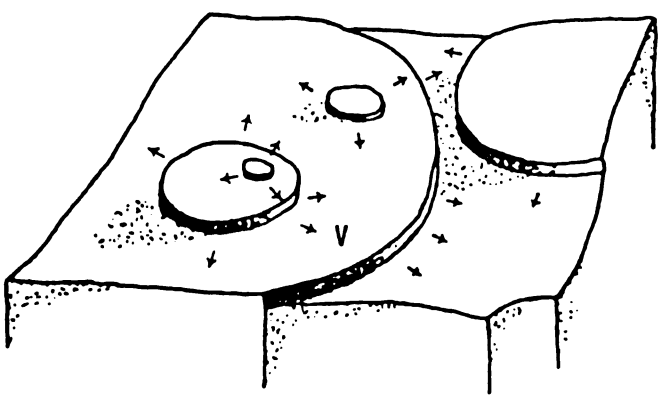
Burton et al. [11] developed a model explaining growth at low supersaturations which do not allow the formation of nuclei as required in the two-dimensional growth model.



(a) Mononuclear Model



(b) Polynuclear Model



(c) Birth and Spread Model

Figure 1.3 Two-dimensional nucleation models. (from Ohara and Reid [10])

Here a self-perpetuating step is provided by the screw dislocation on the surface. Figure 1.4 shows the development of a growth spiral. The mathematical form of this model is

$$G = C\ (s^2/s_c)\ \tanh(s_c/s) \qquad (8)$$

where G is growth rate, C is constant, s is supersaturation, and s_c is a critical supersaturation. For $s << s_c$, i.e. at low supersaturation, this reduces to

$$G = C\ (s^2/s_c) \qquad (9)$$

while for $s >> s_c$ at high supersaturation,

$$G = C\ s \qquad (10)$$

Since the constants in those models are not known, the simple power law equation

$$G = a\ s^b \qquad (11)$$

is generally used to correlate growth rate data [1]. This equation represents the two limiting cases of the BCF model (equations (9) and (10)) and is a good approximation in the intermediate regime when $1 < b < 2$.

c. Growth rate dispersion

When exposed to constant conditions of supersaturation, temperature, and hydrodynamics different crystals of the same material usually grow at different rates. This phenomenon is known as growth rate dispersion [12]. Two possible mechanisms have been proposed:

(1) random fluctuation (RF) model - the growth of crystal fluctuates during the course of time [13];

(2) constant crystal growth (CCG) model - the growth rate of each crystal is constant, yet the rate varies from

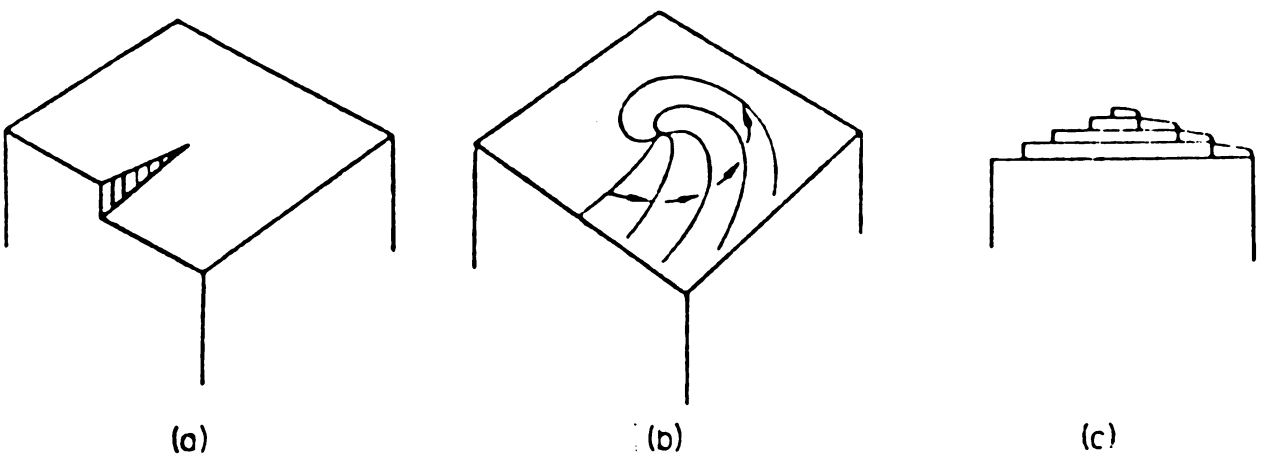


Figure 1.4 Development of a growth spiral starting from a screw dislocation: (a) screw dislocation (b) growth spiral, top view (c) growth spiral, side view (from Mullin [2]).

crystal to crystal [14].

Observations on single crystals for shorter periods (at least a few hours) generally support the CCG model. Since the fast-growing crystals will increase in size more rapidly than slow-growing, the larger crystals are more likely to have faster growth rates, thus size-dependent growth has been incorrectly employed to interprete experimental growth data and model the crystal size distribution [1].

1.1.5. Impurities in crystal growth

Most industrial crystallization processes take place in the presence of some impurities. An impurity can exert a profound effect not only on the nucleation and growth kinetics, but also on the purity and morphology of the product.

The influence of impurities is generally classified into four groups as dicussed by Mullin [2]:

(1) impurities which exert a dilution effect, retard diffusion and thus the growth rate;

(2) impurities which alter the properties of solution or the solubility of solute, by either promoting or retarding the formation of the more ordered species necessary for nucleation and growth;

(3) impurities which is adsorbed to the crystal surface and interferes with the growth by presenting steric or energetic barriers to the subsquent addition of growth units. The Langmuir adsorption isotherm has been employed in modelling this type of impurity effect and fairly good

correlation have been reported by Blitznakov and Kirkova [15];

(4) impurities which are built into the crystals. Impurities can be incorporated into crystal lattice if there is some degree of structural similarity or they can enter a growing crystal through the inclusion of liquid. Inclusions may be formed when dendritic (needle) growth is followed by a filling in process that emtraps fluid in pockets. Growth instabilities which result in depressions in the crystal surface may also cause liquid inclusion. In general large crystals and/or fast growth are more likely to form inclusions [3].

The effect of impurities on crystal habit modification has been the focus of recent studies in crystallization. The habit of a crystal is defined as the type of external shape which results from the different rates of growth of the various faces. The growth of a given face is governed by the crystal sturcture as well as the environmental conditions including solvent, impurities, supersaturation and temperature [2]. An impurity which affects the growth of specific faces can be used to modify the habit and produce crystals of desired properties for washing, centrifugation/filtration, handling and subsequent use. This has been referred to as "tailor-made impurities" in the literature. Davey et al. [16] studied the growth of urea crystals in the presence of biuret, which is the dimer of urea formed during its synthesis reaction. When grown from

pure aqueous solutions urea crystallizes as long needles with a length to breadth ratio exceeding 50:1. The presence of biuret results in crystals with a much smaller length to breadth ratio. Such crystals are easier to separate and handling. Black et al. [17] and Weissbuch et al. [18] both reported the crystallization of racemic mixtures of amino acids in the presence of chiral additives, e.g. a different amino acid. The habit of crystals were modified because the additives were adsorbed only onto surfaces of similar chirality.

1.2. Fructose

1.2.1. Occurrence

Fructose, also called levulose or fruit sugar, is one of the most common natural sugars. It is found in the free form in almost all sweet fruits and vegetables. About 50% of the dry matter of honey is fructose. The fructose contents of some common foods is presented in Table 1.1 [19].

Fructose is also found in the polymer form, as is the case with inulin, the storage polysaccharide in dahlia and artichoke tubers. Levan is another fructosan which occurs in some bacteria. In addition, fructose as well as glucose constitutes the abundant disaccharide, sucrose [20].

1.2.2. Usage of crystalline fructose

The rising interest in using sugars other than the conventional sucrose results from:

Table 1.1 Average fructose content in common foods

Fruit	Fructose content (g/100g of edible portion	% total carbohydrate	% total solid as fructose
Honey	40.5	82.3	48.9
Banana	5.9	22.2	39.0
Apple	5.9	14.5	38.0
Grape	6.5	15.7	35.5
Pear	5.6	15.3	33.3
Cherry	5.4	17.4	27.3
Strawberry	2.1	8.4	21.1
Blueberry	3.3	15.3	19.5
Grapefruit	2.3	10.6	19.5
Orange	2.6	12.2	18.3
Blackberry	2.7	12.9	17.7

(1) diabetics must avoid sucrose in their diet;

(2) sucrose-containing snacks have been proven harmful to the teeth;

(3) the synthetic sweeteners, e.g. saccharin, have a bitter aftertaste, and the safety of using synthetic sweeteners has been questioned [21].

Fructose meets many of the requirements when a substitute for sucrose is called for.

(1) It is a natural sugar. It has not shown any toxic properties. Reasonable intake of fructose has not been found to cause side-effects, such as diarrhea, which is typical of the sugar alcohols [22].

(2) Fructose metabolism is insulin-independent and it is tolerated better than sucrose or glucose by diabetics [23].

(3) Fructose causes less formation of dental plaque than does sucrose [21].

(4) It is the sweetest natural sugar [19]. Table 1.2 shows the relative sweetness of some sweetners [24]. The caloric saving provided by its greater sweetness (i.e. less intake) make fructose a desirable sweetener for dietetic foods.

1.2.3. Manufacture

Pure crystalline fructose has until recently remained a noncommercial product because of the expense involved in its production. Earlier methods of fructose isolation from either hydrolyzed sucrose or hydrolyzed fructose polymers, e.g. inulin, were not economically competitive with the very

Table 1.2 Relative sweetness of some sugars and noncaloric sweeteners

Sugar	Relative sweetness
Sucrose	1.0
Glucose	0.5
Fructose	1.7[a]
Lactose	0.2
Saccharin	400
Sodium cyclamate	30
Aspartame	180
Monellin	2000

[a]1.8 in Doty and Vanninen [19].

low price for sucrose processed from sugar cane or sugar beets [25]. The large scale industrial production of fructose became possible when ion-exchange techniques were developed to separate the glucose and fructose components in aqueous solution of inverted sucrose. The calcium form of a sulfonated-polystyrene exchange resin is employed in the separation step [22]. More recent technologies also involve ion-exchange separation of fructose from glucose in a mixture obtained by the isomerization of glucose by means of immobilized glucose isomerase [26]. Another procedure uses glucose-2-oxidase to oxidize glucose. The glucosone is then selectively hydrogenated to fructose [25].

In 1988, the wholesale price of commercial crystalline fructose in the United States is ca $2.2/Kg. At the retail level, crystalline fructose still cost about 10 times as much as sucrose. This is mainly because the crystallization step in its production is very time-consuming and requires careful control of process conditions [28].

Crystalline fructose can be obtained by crystallizing from aqueous alcohol solutions. Lauer et al. [29] proposed a process for crystallization of fructose from methanol. The employment of solvents is undesirable from the economic point of view for both solvent addition and the later solvent removal. Kusch et al. [30] reported a process wherein a fructose syrup of 95% at a pH of 3.5 - 8.0 is concentrated by vacuum evaporation to 2 - 5% water and cooled to 60 - 85 ^{o}C. Pure crystalline fructose is added as

seeds and the whole mass is solidified. The solid is then ground to achieve a crystalline product. In a subsequent design, Forsberg et al. [31] presented another process for crystallization from seeded aqueous solution. The key to this process is pH adjustment. A pH of 4.5 - 5.5 is found to be the most favorable range. Another method presented by Yamauchi [32] involves seeding and then concentrating or cooling the solution while maintaining the sugar concentration and temperature in the liquid phase within a carefully defined range. Dwivedi and Raniwala [28] presented a seeded process wherein large pellets are formed which must be ground to the proper size.

1.2.4. Properties

a. Solubility

Fructose is the most water-soluble of all sugars [21]. The solubilites of fructose, glucose and sucrose in water are shown in Table 1.3. Fructose is also soluble in ethanol up to 6.71 g/100 ml at 18 ^{o}C [19].

b. Density

The density of fructose-water solutions at different temperatures and concentrations is given in Table 1.4 [33].

c. Viscosity

The viscosity of saturated and supersaturated aqueous fructose solutions is much higher than that of other sugars due to its high solubility. Table 1.5 lists the viscosity data reported by Watanabe [34].

Table 1.3 Solubilities of some sugars in water

Temperature (°C)	Fructose (wt%)	Sucrose (wt%)	Glucose (wt%)
20	79.0	66.6	47.1
30	81.5	68.2	54.6
40	84.3	70.0	61.8
50	87.2	72.0	70.9
60	90.3	74.2	-

(fructose & sucrose : Vanninen and Dotty [22];
glucose : Dotty and Vanninen [19])

Table 1.4 Density of aqueous fructose solution

Temperature (°C)	Concentration (wt %)			
	50%	60%	70%	80%
10	1.2357	1.2927	-	-
20	.2301	.2860	1.3452	1.4068
30	.2242	.2792	.3378	.3990
40	.2176	.2721	.3303	.3915
50	.2106	.2647	.3227	.3832
60	.2036	.2574	.3145	.3744
70	.1962	.2498	.3062	.3661
80	.1890	.2415	.2979	.3584
90	.1824	.2343	.2907	.3502

Table 1.5 Viscosity of aqueous fructose solution

Concentration (wt %)	Temperature (°C)		
	25	40	60
30	2.6	1.8	1.1
50	8.5	5.2	2.8
60	24	13	5.2
70	170	39	18
80	-	-	92
90	-	-	2100

(unit: c.p.)

d. Diffusion coefficient

The diffusion coefficient of fructose in concentrated aqueous solutions is not available in the literature. Uedaira and Uedaira [35] reported an equation for dilute solutions at 25 ^{o}C:

$$D = (7.002 - 0.813\ C)\times10^{-6} \quad (cm^2/s) \quad (12)$$

The applicable concentration range is C < 3%.

e. Phase diagram

Fructose is most often crystallized from aqueous solution as an anhydrous phase. However, fructose hemihydrates and dihydrates form rapidly in the vicinity of room temperature or lower. The phase diagram of D-Fructose-water system reported by Young et al. [36] is shown in Figure 1.5.

Fructose dihydrate is the stable phase and crystallizes at conditions of below 19.9 ^{o}C and 79.4%. Although the hemihydrate form is the stable form only in the range from 19.9 to 21.4 ^{o}C, it frequently has formed spontaneously at 24 to 26 ^{o}C and its transformation to anhydrous form is quite slow. Below 19.9 ^{o}C it usually changes to dihydrate form if it is crushed or vigorously stirred in a saturated solution. Once the transformation is initiated, it proceeds rapidly [36]. The melting points of anhydrous, hemihydrate and dihydrate crystal are 102-104, 68 and 21.3 ^{o}C respectively [36,37,38].

f. Refractive index

Refractive index of fructose solution at two temperatures is given in Table 1.6 [33].

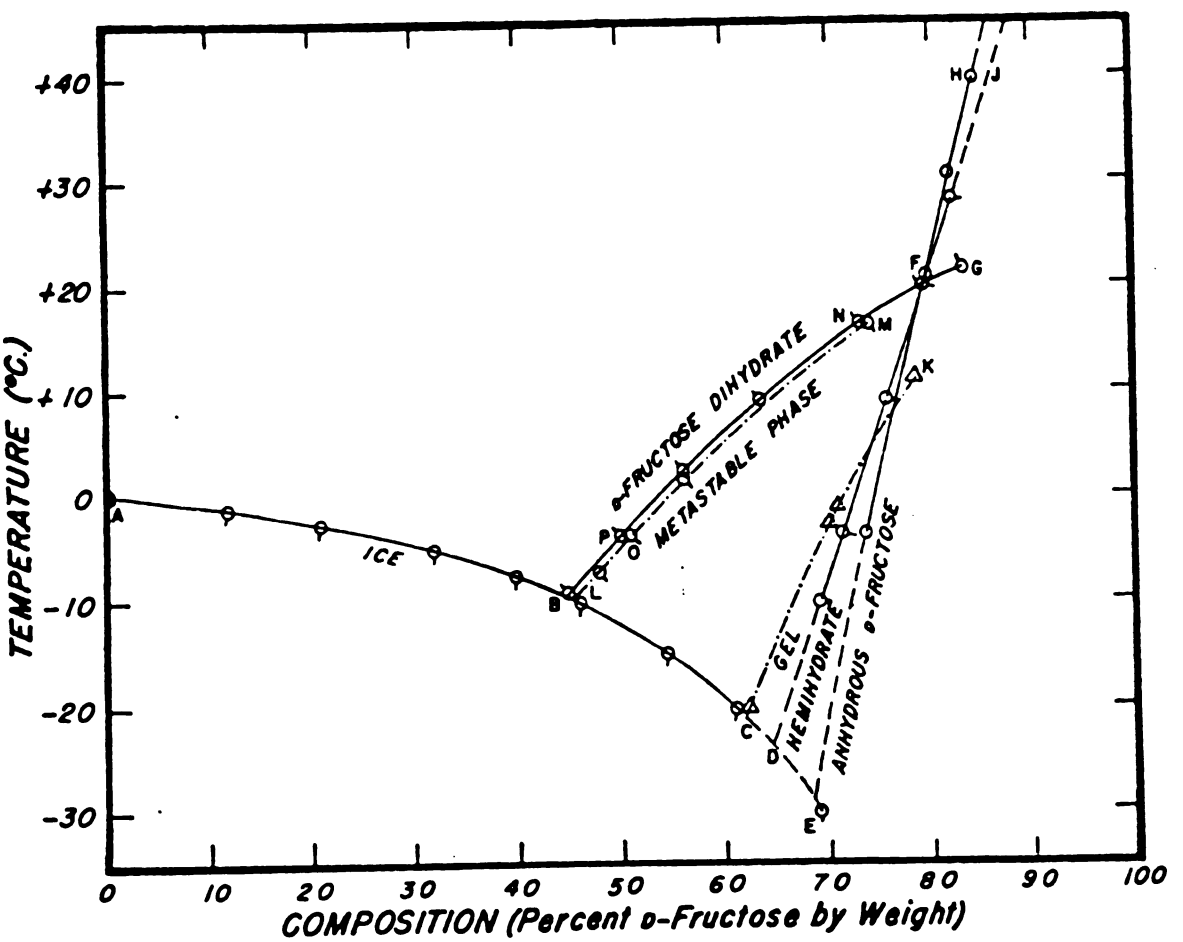


Figure 1.5 D-Fructose-water phase diagram: ♀ ice, o anhydrous D-fructose, o- D-fructose hemihydrate, ♉ D-fructose dihydrate, ♌ metastable phase, ▲ gel. (from Young et al. [36])

Table 1.6 Refractive Index of aqueous fructose solution

Concentration (wt %)	Temperature (^{o}C)	
	20	25
0	1.33300	1.33252
11.855	.34043	.34985
19.098	.36185	.36115
30.912	.38197	.38104
40.906	.40035	.39932
50.212	.41856	.41758
60.488	.44013	.43894
69.166	.45964	.45854
75.242	.47524	.47399
82.321	.49062	.48945

g. Tautomeric equilibrium

Crystalline anhydrous fructose has the configuration of β-D-pyranose. In solution, fructose establishes an equilibrium state of its various tautomers as shown in Figure 1.6. The influences of temperature and concentration on the tautomeric equilibrium has been investigated by Hyvonen et al. [39]. It is found that the proportion of furanose forms increases with temperature, while concentration does not change the equilibrium significantly (Table 1.7).

h. Optical rotation

The specific rotation for β-D-fructopyranose is -131^{o}. Because no crystalline form of the other tautomers has been obtained, specific rotation values for them are calculated from tautomeric equilibrium data and inconsistency is found among different sources. For fructose solution, the generally accepted specific rotation is -91^{o} at 23 ^{o}C [40].

i. Hygroscopicity

Fructose is a very hygroscopic sugar. The rate of water absorption by fructose is higher than that of glucose and sucrose as shown in Table 1.8 [41].

j. Crystal morphology

β-D-fructopyranose crystallizes in the rhombic bisphenoidal class of the orthorhombic system with a:b:c = 0.801:1:0.907; space group $P2_12_12_1$. Figure 1.7 shows the crystal and different development of crystallographic faces [42,43].

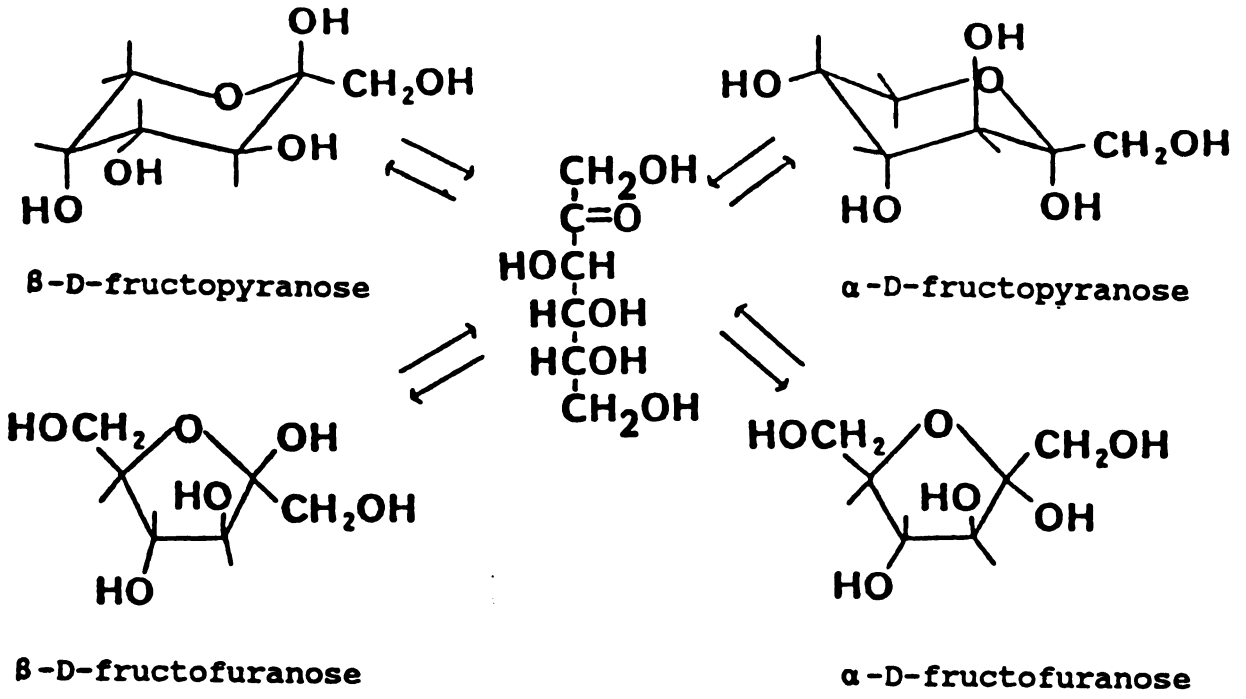


Figure 1.6 Possible tautomeric forms of D-fructose in solution.

Table 1.7 Tautomeric equilibria of D-fructose solutions.

Temperature (^{o}C)	Concentration (wt%)	α-furanose (%)	β-furanose (%)	β-pyranose (%)
23	20	6	21	73
23	50	4	21	75
23	80	5	21	74
0	20	4	18	78
22	20	6	21	73
67	20	8	28	64
77	20	12	31	57

Table 1.8 Percentage of water absorbed by some sugars from moist air

Sugar	Relative humidity at 20 °C		
	60%, 1 hr	60%, 9days	100%, 25days
Sucrose	0.04	0.03	18.4
Glucose	0.07	0.07	14.5
Fructose	0.28	0.63	73.4
Maltose	5.05	5.1	-

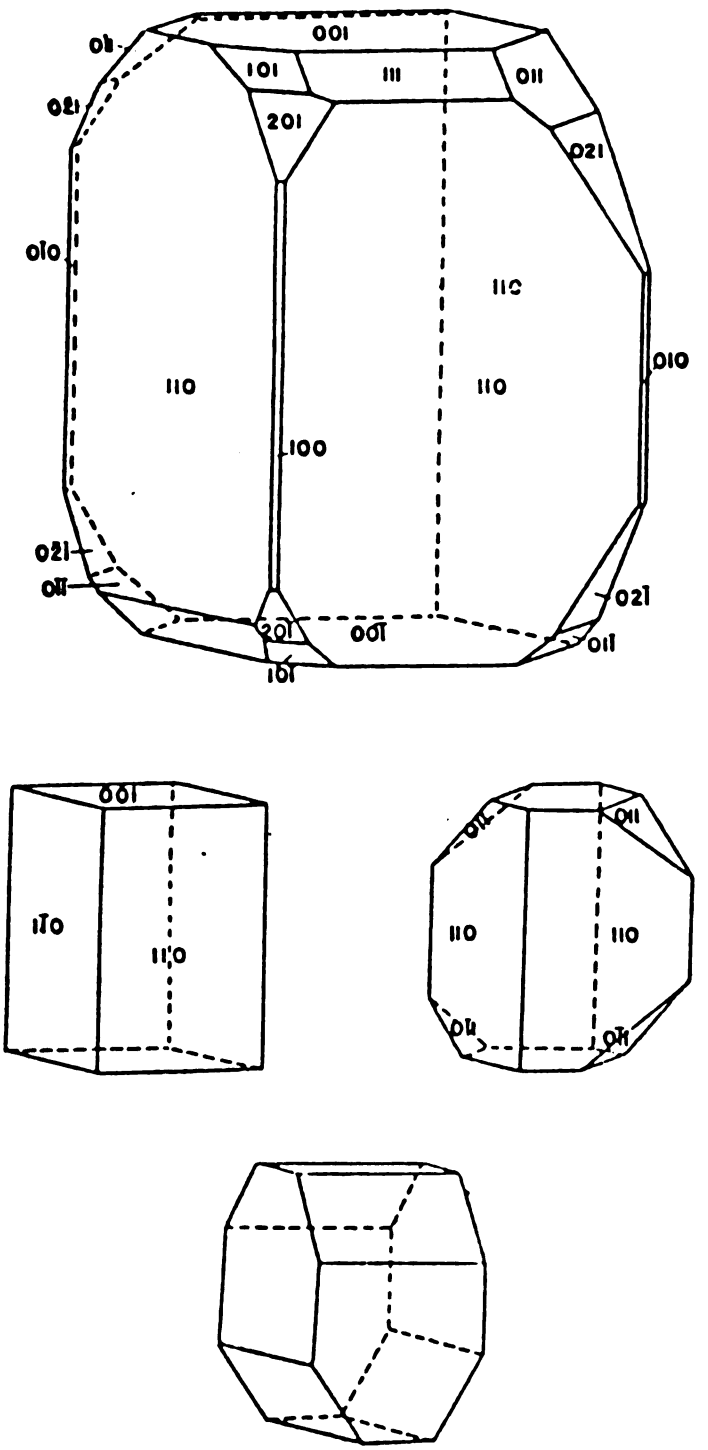


Figure 1.7 Fructose crystal showing different development of faces. (from Bates [42])

k. Crystal growth kinetics

The growth kinetics of anhydrous fructose crystals from aqueous solution have been studied by Shiau and Berglund [44]. Crystals were formed by contact nucleation and the growth was described by the Constant Crystal Growth model, i.e. each crystal grows at constant rate while different crystals have different growht rates.

The kinetic model is given by

$$G = 1.47 \times 10^{7} \exp(-6120/RT)\ s^{1.25} \qquad (13)$$

where G is growth rate in μm/hr, R is gas constant in cal/g-mole ^{o}K, T is temperature in ^{o}K, and s is relative supersaturation. The variance of the growth rate distribution was approximated by

$$\sigma_G^2 = 0.165\ G^{1.35} \qquad (14)$$

1.3. Difructose Dianhydrides

Fructose dehydrates in acid solution and/or on prolonged heating and forms a series of non-reducing, dimeric dianhydrides. The dehydration-dimerization produces an extremely stable central 1,4-dioxane ring [45].

1.3.1. Structure and properties

Difructose dianhydrides differ in that

(1) the acetal rings of fructose moieties may be both pyranoid, mixed, or both furanoid;

(2) the dioxane ring may be formed by dehydration at

different carbon atoms of each fructose unit;

(3) additional anomerism is produced by the asymmetry of the second carbon atoms of each fructose unit.

The first difructose dianhydride was isolated by Pictet and Chavan [46]. It was later shown to be di-D-fructopyranose-1,2′:2,1′-dianhydride and known as diheterolevulosan I. The other three compounds in these series, diheterolevulosan II - IV, were found by Wolfrom and coworkers [47,48]. These compounds have at least one moiety of fructose in pyranoid structure. The name "levulosan" is generally used for this type of difructose dianhydride. A second series, known as difructose anhydride I - III, was reported by Jackson and coworkers [49,50]. In this series both fructose moieties are in furanoid form. In a more recent study Defaye et al. [51] added a new difructose dianhydride to the list. Details about the structure and properties of these eight difructose dianhydrides are summarized in Table 1.9. The structures of some of these compounds are also shown in Figure 1.8.

2. Analysis

The separation of different difructose dianhydrides is generally achieved by chromatographic techniques. McDonald [52] developed paper chromatograph of diheterolevulosan I, II and difructose anhydride I - III. Hilton [45] used a carbon-celite column to prepare diheterolevulosan I - IV. The TLC and HPLC analyses of diheterolevulosan I and II were reported by Velasco and Dowing [53] and Hamada et al. [54]

Table 1.9 Known difructose dianhydrides

fructose moiety		linkage	trivial name		$[a]_D$	m.p.
ring	anomeric C					
pyr-pyr	β, α	1,2:2,1	diheterolevulosan	I	-44°	266
fur	β, α	1,2:2,1		II	-40°	261
fur	β, β	1,2:2,1		III	-179°	257
pyr	β, β	1,2:2,1		IV	-309°	240
pyr	β, β	1,2:2,3	-		-58.5°	206
fur-fur	α, β	1,2:2,1	difructose anhydride	I	26.9°	164
fur	-	1,2:2,4		II	13.9°	198
fur	α, β	1,2:2,3		III	135.6°	162

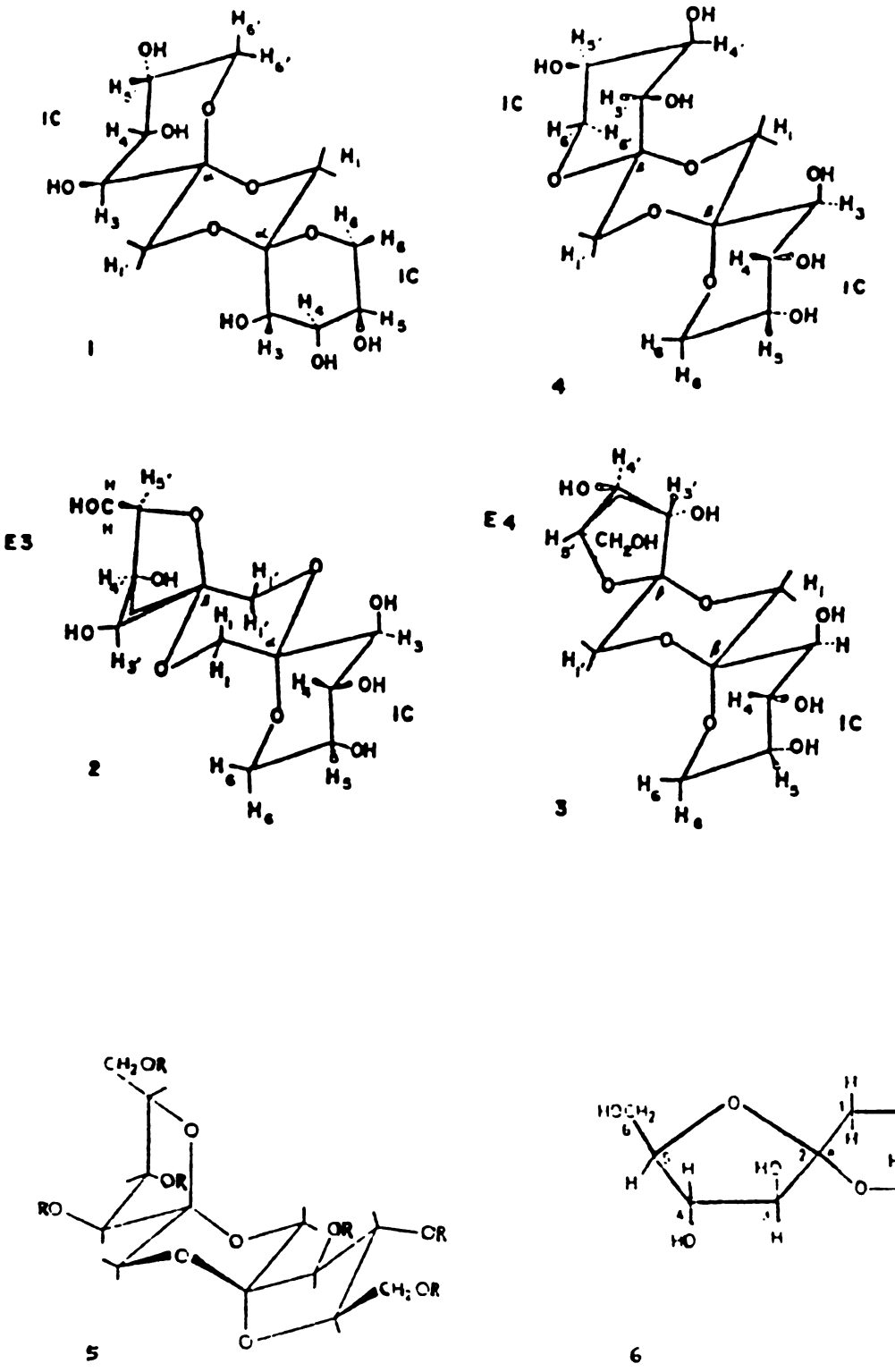


Figure 1.8 Structures of some difructose dianhydrides: 1. diheterolevulosan I, 2. diheterolevulosan II, 3. diheterolevulosan III, 4. diheterolevulosan IV, 5. difructose anhydride I, 6. difructose anhydride III.

respectively. Defaye et al. [51] analyzed the methylated derivatives of six dianhydrides on a GC system.

The structure of these dianhydrides were first examined by periodate oxidation to determine the form of fructose rings [47]. Later studies have applied NMR and X-ray to analyze the configuration and the ring conformation.

The conformation of diheterolevulosan I - IV and the new dianhydride have been determined by ^{1}H-NMR analysis of their hexa-acetate derivatives; their anomeric configuration was assigned by ^{13}C-NMR studies [55,56,51]. Structure of difructose anhydride I was elucidated from ^{1}H-NMR spectra of its 6,6'-dideoxytetra-O-acetyl derivative [57]. Configuration of difructose anhydride III was determined by ^{13}C-NMR [58] and the conformation was established from X-ray analysis of its crystal [59].

3. Effect on fructose crystallization

Difructose dianhydrides have been reported to form <u>in situ</u> under industrial crystallization conditions. It is suggested that they are inhibitors for the growth of fructose crystals because their presence decreases the overall yields as illustrated in Figure 1.9 [31].

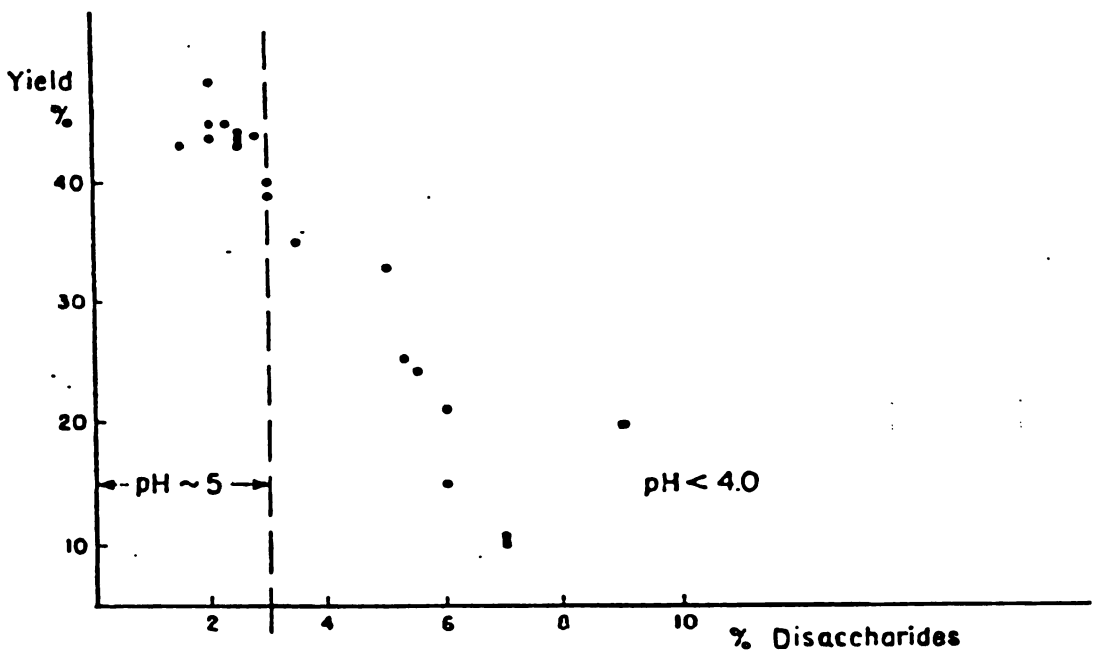


Figure 1.9 The effect of difructose dianhydrides on yield of fructose crystallization.
(from Forsberg et al. [31])

1.4. References

1. J. Garside, Chem. Engng. Sci. 40, 3 (1985).

2. J.W. Mullin, Crystallization (Butterworth & Co Ltd, London, 1972).

3. S.J. Jancic and P.A.M. Grootscholten, Industrial Crystallization (Delft University Press, Delft, Holland, 1984).

4. G.D. Botsaris, in Industrial Crystallization, J.W. Mullin Ed. (Plenum Press, New York, 1976) p. 3.

5. A.G. Walton, in Nucleation, A.C. Zettlemoyer Ed. (Marcel Dekker, Inc., New York, 1969) p. 225.

6. A.G. Walton, The Formatin and Properties of Precipitates (Interscience Publishers, New York, 1967).

7. J. Garside and M.A. Larson, J. Crystal Growth 43, 694 (1978).

8. W. Drost-Hansen, Ind. Eng. Chem. 61, 10 (1969).

9. W. Kossel, Annln Phys. 21, 457 (1934).

10. M. Ohara and R.C. Reid, Modeling Crystal Growth Rates from Solution (Prentice Hall, Inc., Englewood Cliffs, NJ, 1973).

11. W.K. Burton, N. Cabrera and F.C. Frank, Phil. Trans. Roy. Soc. 243, 299 (1951).

12. E.T. White and P.G. Wright, Chem. Engng. Prog. Sym. Ser. 67, 81 (1971).

13. A.D. Randolph and E.T. White, Chem. Engng. Sci. 32, 1067 (1977).

14. M.A. Larson, E.T. White, K.A. Ramanarayanan and K.A. Berglund, AIChE J. 31, 90 (1985).

15. G. Blitznakov and E. Kirkova, E., Krist. Tech. 4, 331 (1969).

16. R. Davey, W. Fila and J. Garside, J. Crystal Growth 79, 607 (1986).

17. S.N. Black, R.H. Davey and M. Halcrow, J. Crystal Growth 79, 765 (1986).

18. I. Weissbuch, L.J.W. Shimon, E.M. Landau, R. Popovitz-Biro, Z. Berkovitch-Yellin, L. Addadi, M. Lahav and L. Leiserowitz, Pure App. chem. 58, 947 (1986).

19. T.E. Dotty and E. Vanninen, Food Technol. 11 (1975) 34.

20. R.S. Shallenberger and G.G. Birch, Sugar Chemistry (The AVI Publishing Co., Inc. Wesport, CN, 1975).

21. L. Hyvonen and P. Koivistoinen, in Nutritive Sweeteners G.G. Birch and K.J. Parker Ed. (Applied Science Publishers, New york, 1982) p. 133.

22. E. Vanninen and T. Dotty, Sugar: Science and Technology (Applied Science Publishers, New York, 1979) p. 311.

23. L. Sestoft, Nutrition Update 1, 39 (1983).

24. A.L. Lehninger, Principles of Biochemistry (Worth Publishers, Inc., New York, 1982).

25. G.N. Bollenback, in Kirk-Othmer Encyclopedia of Chemical Technology (John Wiley & Sons, New York, 1983) p. 944.

26. W.P. Chen, Process Biochem. 15, 36 (1980).

27. Chemical Marketing Reporter, May 9, 1988.

28. B.K. Dwivedi and S.K. Raniwala, U.S. Patent no. 4,199,373 (1980).

29. K. Lauer, P. Stephan and G. Stoeck, U.S. Patent no. 3,607,392 (1971).

30. T. Kusch, W. Gosewinkel and G. Stoeck, U.S. Patent No. 3,513,023 (1970).

31. K.H. Forsberg, L. Hamalainen, A.J. Melaja and J.J. Virtanen, U.S. Patent no. 4,199,373 (1975).

32. T. Yamauchi, U.S. Patent No. 3,928,062 (1975).

33. J. Timmermans, The Physico-chemical Contants of Binary Systems in Concentrated Solutions (Interscience Publishers, New York, 1960).

34. T. Watanabe, Seito Gijutsu Kenkyu Kaishi 28, 70 (1978).

35. H. Uedaria and H. Uedaria, J. Phys. Chem. 74, 2211 (1970).

36. F.E. Young, F.T. Jones and H.J. Lewis, J. Phys. Chem. 56, 1093 (1952).

37. F.T. Jones, F.E. Young and D.R. Balck, Anal. Chem. 25, 649 (1953).

38. F.E. Young, F.T. Jones and H.J. Lewis, J. Phys. Chem. 56, 738 (1952).

39. L. Hyvonen, P. Varo and P. Koivistoninen, J. Food Sci. 42, 652 (1977).

40. R.S. Shallenberger, Pure Appl. Chem. 50, 1409 (1978).

41. J.E. Hodge and E.M. Osman, in Principles of Food Science Part I: Food Chemistry, O.R. Fennema Ed. (Marcel Dekker, Inc. New York, 1976) p. 88.

42. F.J. Bates, Polarimetry, Saccharimetry and the Sugars (United States Government Printing Office, Washington, 1942).

43. A.N. Winchell, The Optical Properties of Organic Compounds, 2nd ed. (Academic Press, Inc., New York, 1954).

44. L.D. Shiau and K.A. Berglund, AIChE J. 33, 1028 (1987).

45. H.W. Hilton, in Methods in Carbohydrate Chemistry, Vol II, R.L. Whistler and M.L. Wolfrom Ed. (Academic Press, Inc, New York, 1963) p. 199.

46. A. Pictet and J. Chavan, Helv. Chim. Acta. 9, 809 (1926).

47. W.L. Wolform and M.G. Blair, J. Am. Chem. Soc. 70, 2406 (1948).

48. W.L. Wolfrom, H.W. Hilton and W.W. Binkley, J. Am. Chem. Soc. 74, 2867 (1952).

49. R.F. Jackson and S.M. Goergen, Bur. Stand. J. Res. 3, 27 (1929).

50. R.F. Jackson and E.J. McDonald, Bur. Stand. J. Res. 6, 709(1931)

51. J. Defaye, A. Gadelle and C. Pedersen, Carbohydr. Res. 136, 53 (1985).

52. E.J. McDonald and B.K. Goss, Anal. Chem. 24, 422 (1952).

53. V.S. Velasco and J.F. Dowling, U.S. Agri. Res. Serv. South. Reg., [Rep.], ARS-S-88, 138 (1975).

54. K. Hamada, H. Yoshihara, G. Suzakamo and O. Hiroake, Bull. Chem. Soc. Jpn. 57, 307 (1984).

55. R.W. Binkley, W.W. Binkley and A.A. Grey, Carbohyd. Res. 28, 365 (1973).

56. R.W. Binkley, W.W. Binkley and B. Wickberg, Carbohyd. Res. 36, 196 (1974).

57. R.U. Lemieux and R. Nagarajan, Can. J. Chem. 42, 1270 (1964).

58. T. Uchiyama, Carbohyd. Res. 101, 138 (1982).

59. T. Taniguchi and T. Uchiyama, Carbohyd. Res. 107, 255 (1982).

CHAPTER 2*

KINETICS OF DIFRUCTOSE DIANHYDRIDES FORMATION UNDER FRUCTOSE CRYSTALLIZATION CONDITIONS

2.1. Abstract

Fructose undergoes dehydration reactions and forms difructose dianhydrides in situ during crystallization. These difructose dianhydrides can be incorporated into fructose crystals and inhibit the crystal growth. In this study, the kinetics of difructose dianhydrides formation under industrial crystallization conditions were investigated.

Four difructose dianhydrides were detected by HPLC analysis. A second-order irreversible kinetic model was proposed for the reaction. The extent of reaction increased with increasing temperature and decreasing pH value of the solution. The amount of two of the difructose dianhydrides stopped increasing when the fructose concentration was about 70% or less. On the other hand, the formation of all dimers were retarded when the initial fructose concentration was higher than 80%.

---

*This chapter contains a paper submitted to "Biotechnology & Bioengineering".

2.2. Introduction

Fructose is one of the most abundant monosaccharides and is known to be the sweetest natural sugar [1]. Crystalline fructose has not been widely available due to its high cost resulting from a major processing difficulty in the crystallization step of fructose from aqueous solutions [2].

Fructose undergoes a complex mutarotation reaction in aqueous solution with three tautomeric forms, β-pyranose, β-furanose and α-furanose, existing in measurable amounts at equilibrium. The reaction is shown in Figure 2.1 and the equilibrium composition of aqueous solutions at different temperatures and concentrations are given in Table 2.1[3]. Fructose undergoes dehydration reactions in acid solution and/or on protracted heating. Unlike aldoses which generally form monomolecular anhydrides, fructose forms difructose dianhydrides possessing a central 1,4-dioxane ring as shown in Figure 2.2.

Difructose dianhydrides have been suggested to be inhibitors of fructose crystal growth. Forsberg et al. [4] have reported that difructose dianhydrides were formed <u>in situ</u> under industrial crystallization conditions and caused a decrease in the overall yields of fructose crystallization. Chu et al. [5] found that difructose dianhydrides inhibit fructose growth. The inhibition is probably caused by the incorporation of these compounds in

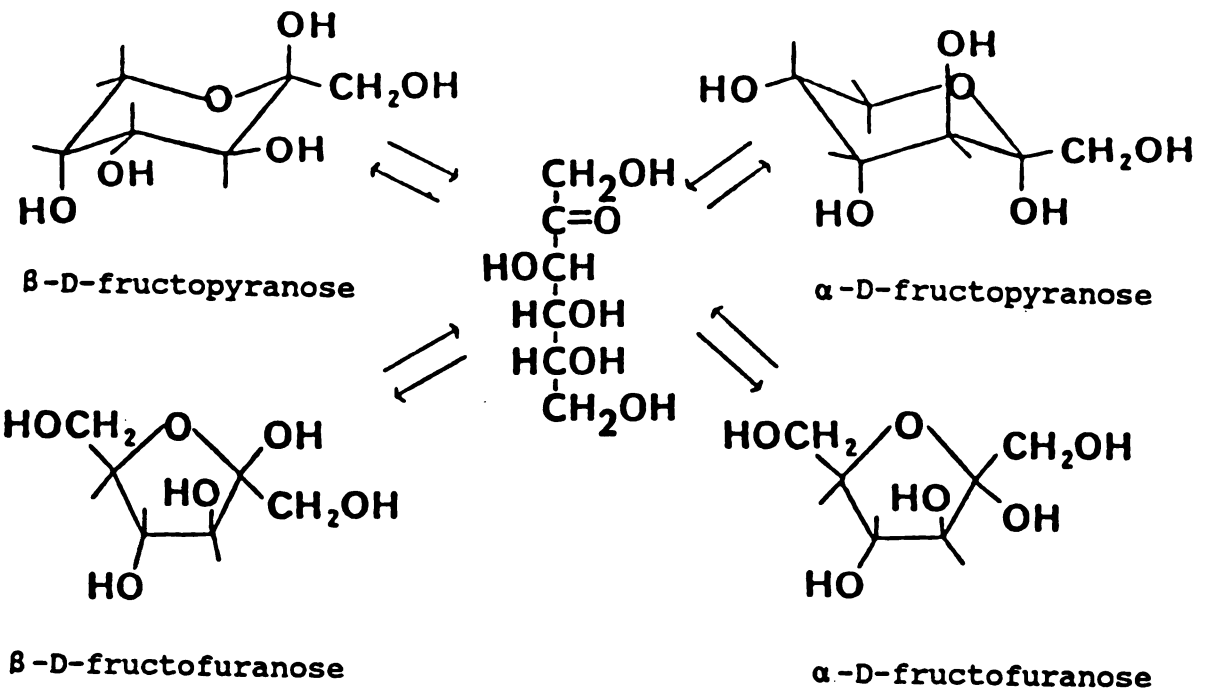


Figure 2.1 Possible tautomers of D-fructose in solution.

Table 2.1 Tautomeric equilibria of D-fructose solutions.

Temperature (°C)	Concentration (wt %)	α-furanose (%)	β-furanose (%)	β-pyranose (%)
23	20	6	21	73
23	50	4	21	75
23	80	5	21	74
0	20	4	18	78
22	20	6	21	73
67	20	8	28	64
77	20	12	31	57

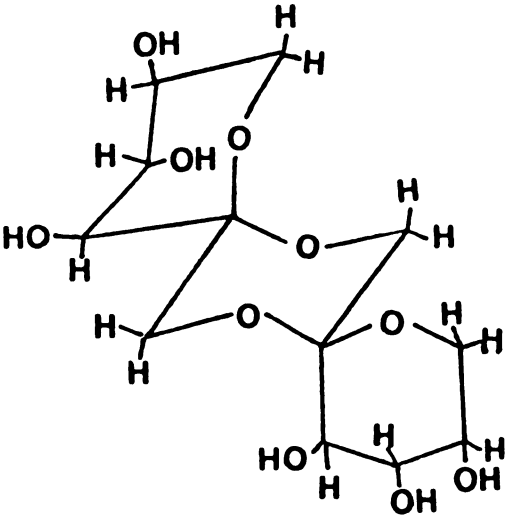


Figure 2.2 Diheterolevulosan I.

the surface of crystal due to the structural similarity of difructose dianhydrides to fructose.

The formation of difructose dianhydrides was first reported by Pictet and Chavan [6] in a study of the action of concentrated hydrochloric acid on fructose; the compound studied was later known as diheterolevulosan I. Wolfrom and Blair [7] isolated diheterolevulosan II on heating concentrated aqueous fructose solution. Diheterolevulosan III and IV were later found by Wolfrom and coworkers [8] and Wickberg [9]. A second set of difructose dianhydrides has been isolated mainly from syrup of hydrolyzed inulin, which is a polyfructofuranose. Difructose anhydride I was reported by Jackson and Goergen [10] and Irvine and Stevenson [11] simultaneously, and difructose anhydride II and III were prepared by Jackson and McDonald [12]. The term "levulosan" was applied to those compounds with at least one pyranoid structure, while the set named "anhydride" possess furanoid forms only. Defaye et al. [13] have recently reported a new difructose dianhydride, thus there are at least eight difructose dianhydrides in the literature. Their sturctures differ in the configuration of each fructose moiety and the linkage between them as summarized in Table 2.2.

Most reports regarding difructose dianhydrides deal with the isolation and structure elucidation of these compounds. The kinetic information about their formation is not available in the literature. The objective of this work

Table 2.2 Known difructose dianhydrides.

fructose moiety		linkage	trivial name	
ring	anomeric C			
pyr-pyr	β, α	1,2:2,1	diheterolevulosan	I
fur	β, α	1,2:2,1		II
fur	β, β	1,2:2,1		III
pyr	β, β	1,2:2,1		IV
pyr	β, β	1,2:2,3	-	
fur-fur	α, β	1,2:2,1	difructose anhydride	I
fur	-	1,2:2,4		II
fur	α, β	1,2:2,3		III

is to study the kinetics of difructose dianhydrides formation under fructose crystallization conditions.

2.3. Experimental

Fructose solutions were prepared by adding fructose to hydrochloric acid solutions of desired pH. Reagent grade fructose and double-distilled water were used. The reaction was carried out in a 250 ml flask filled with 250 g solution. Temperature was controlled by a water bath with a Haake constant temperature circulator model E3 and a Curtin Matheson Scientific 244-793 magnetic stirrer was used to agitate the solution.

Samples were taken at intervals and further reaction was minimized by immediate dilution and refrigeration. About 2 g aliquot was taken and diluted with water in a 50 ml volumetric flask. The concentrations of difructose dianhydrides and residual fructose were analyzed on a Biorad Carbohydrate Analysis HPLC system, which was equipped with a model 1770 refractive index detector and a HP 3392A integrator. The column used was a 4.0x250 mm Bio-Sil Amino-5S. The mobile solvent was acetonitrile/water with a volume ratio of 70/30 and a flow rate of 1.0 ml/min. Samples of 5 µl were injected and eluted at room temperature. An external standard composed of fructose and sucrose was used to calibrate the analyses.

Some of the final reaction solutions were analyzed by GC/MS spectrometry after silylation by TRI-SIL 'Z' purchased

from Pierce (trimethylsilylimidazole in dry pyridine, 1.5 meq/ml). The trimethylsilyl derivatives were analyzed on a HP 5985 GC/MS system with a electron multiplier detector with the ionization potential at 70 eV. The column was a 6 ft 3% OV-225 and the temperature program was 140-200 ^{o}C (3 ^{o}/min).

2.4. Results and Discussion

The crystallization conditions of fructose manufacture usually involve a temperature range of 30 to 60 ^{o}C and the pH value of solution is controlled between 4 and 6. The growth rate of fructose crystals is so low that the crystallization step usually occurs in times on the order of days [2,4,14,15]. Thus the reaction temperatures of 30, 40, 50, and 60 ^{o}C and pH values of 2.65, 4.35 and 5.90 were chosen to study the effect of temperature and pH on the formation of difructose dianhydrides. The fructose concentrations were 10% less than the saturated values and the reaction was monitored for two weeks.

Since difructose dianhydrides are not available commercially and sucrose has been reported to have a retention time very close to difructose dianhydrides in a TLC and a GC systems [16], it was used to calibrate the relative amounts of difructose dianhydrides. Four peaks were found in both HPLC and GC analysis. The typical chromatograms are shown in Figures 2.3 and 2.4 respectively. Since difructose dianhydrides are non-reducing dimers, the

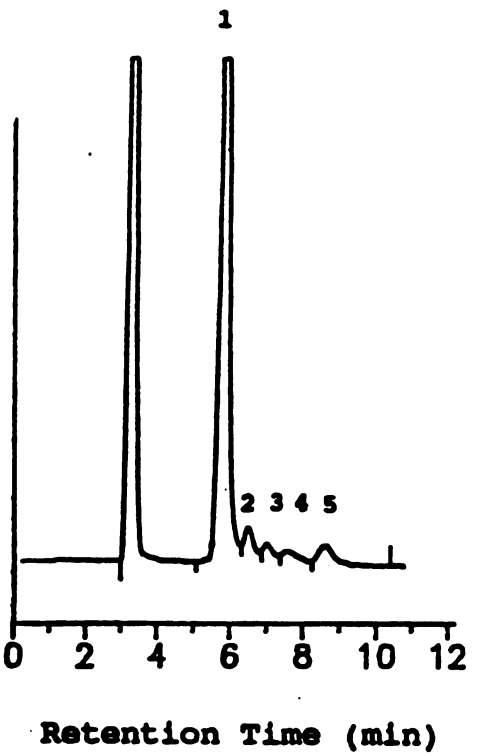


Figure 2.3 HPLC analysis of difructose dianhydrides. 1. Fructose 2. diheterolevulosan II 3. diheterolevulosan I 4. diheterolevulosan III 5. diheterolevulosan IV.

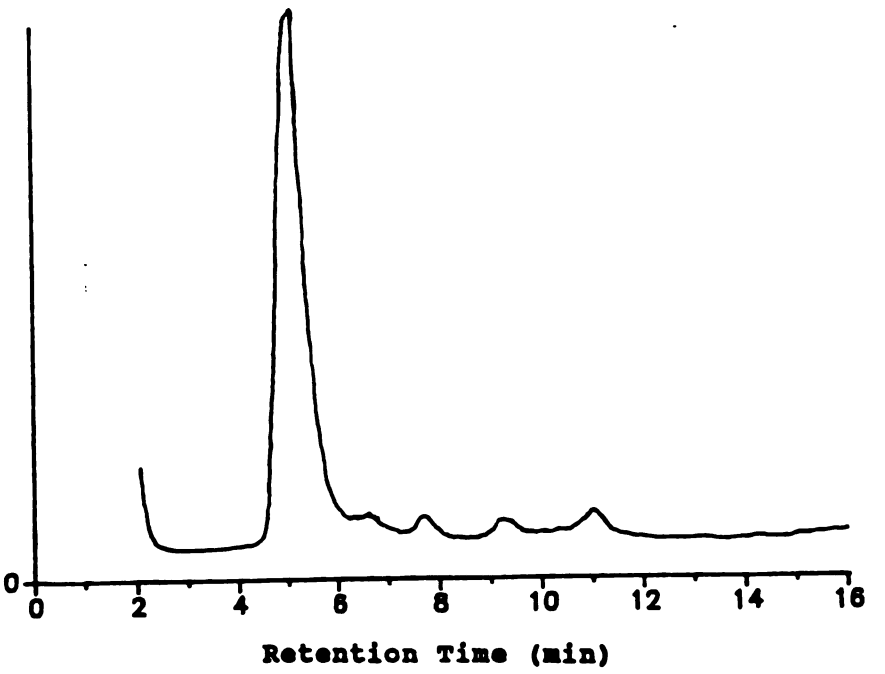


Figure 2.4 GC analysis of difructose dianhydrides.

chance of having anomeric isomers overlapped in the same peak does not exist and it is reasonable to assume that there were only four compounds formed in the reaction. Hereafter they are designated as D1, D2, D3 ,and D4 in order of increasing retention time.

Hamada et al. [17] reported a method of synthesizing diheterolevulosan I and II with a ratio of 1 to 2.5. Their procedure was followed and analyzed by the HPLC system for the retention times of diheterolevulosan I and II. It was found that compound D1 has the retention time of diheterolevulosan II and D2 has the retention time of diheterolevulosan I. The other two compounds are believed to be diheterolevulosan III and IV because they possess at least one β-pyranose moiety, which is the most abundant species in aqueous solution, and might be formed in significant amounts. Diheterolevulosan IV might appear faster and/or in larger amount than diheterolevulosan III since it is composed of two β-pyranoses, thus the pudative assignments for D3 and D4 are diheterolevulosan III and IV. The set of difructose anhydrides is less likely to be produced from fructose solutions because they consist of the less abundant furanoid structures only. This assumption is supported by the report of Hilton [18], in which difructose dianhydrides were prepared from acid treatment of 100 g fructose and the amount of difructose anhydrides formed was less than 5 g while that of diheterolevulosans was about 35 g.

The formation of difructose dianhydrides at 60 ^{o}C and a

pH of 2.65 is shown in Figure 2.5. It was found that D3 and D4 were formed first yet their concentration did not increase after a certain period of time. On the other hand, D1 and D2 were formed later and the extent of formation increased throughout the two weeks reaction duration. For an initial fructose concentration of 70 to 80%, the total amount of difructose dianhydrides formed in two weeks was 10% or less with D3 and D4 being 1.5 to 3.5%.

Since the difructose dianhydrides formed are stable in acidic solutions [19], an irreversible reaction is assumed and a second order kinetic model is proposed as following

$$\begin{array}{rll} & \xrightarrow{k_1} & D1 \\ 2\ Fructose & \xrightarrow{k_2} & D2 \\ & \xrightarrow{k_3} & D3 \\ & \xrightarrow{k_4} & D4 \end{array} \qquad (1)$$

where k_i is reaction rate constant, or

$$2\ Fructose \xrightarrow{K} Difructose\ dianhydrides \qquad (2)$$

where $K = \sum k_i$. Thus the rate of reaction is given by

$$-dC/dt = KC^2 \qquad (3)$$

where C is fructose concentration and t is time, which on integration yields

$$1/C - 1/C_o = Kt \qquad (4)$$

where C_o is initial fructose concentration. The model was tested graphically by plotting the reciprocal of fructose concentration versus time. Figure 2.6 shows the plot at 60 ^oC as an example. It was found that a two section broken line

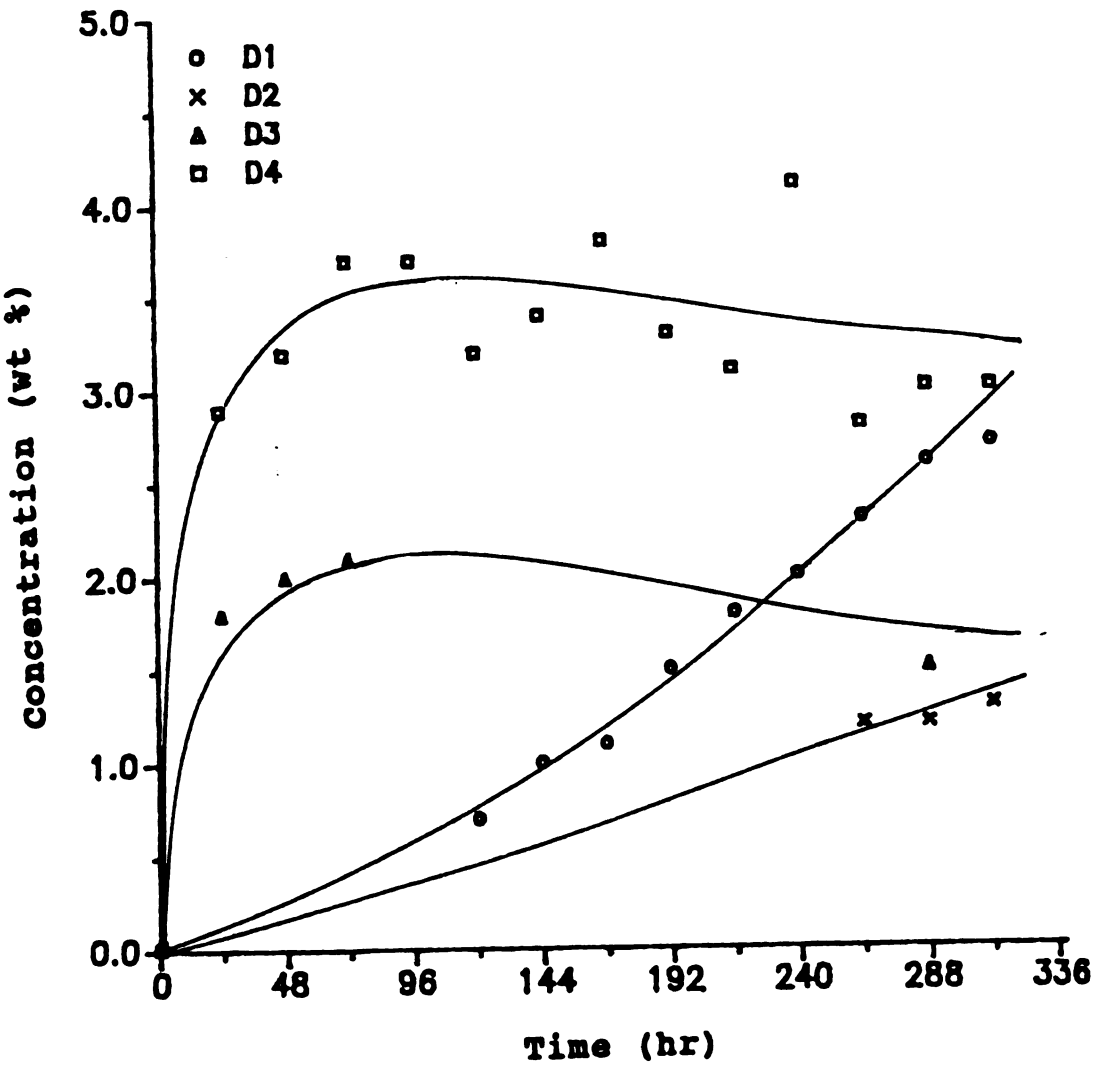


Figure 2.5 The formation of difructose dianhydrides at 60 ^{o}C and pH 2.65 with a initial fructose concentration of 80.3%.

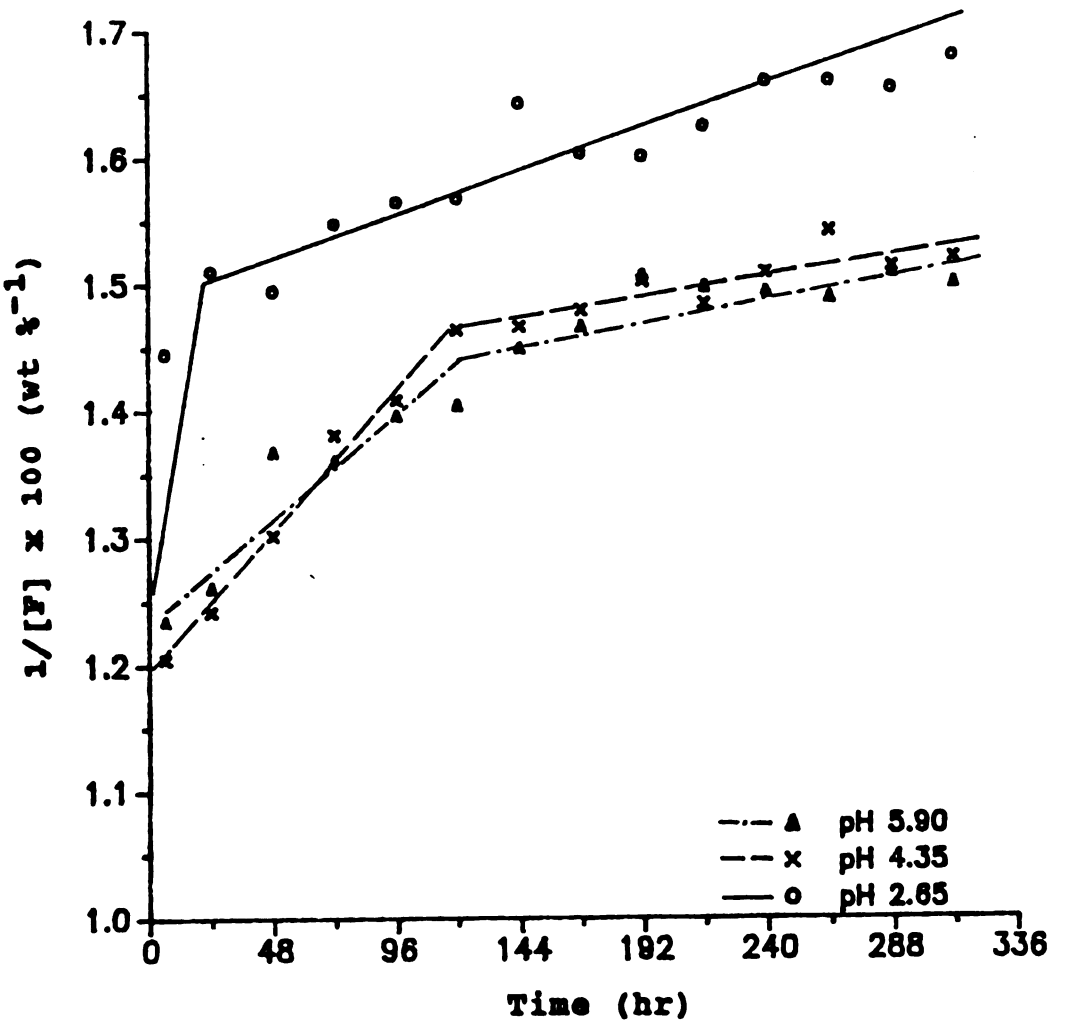


Figure 2.6 Second-order kinetic plot of difructose dianhydrides formation at 60 °C.

fitted the data best for all pH values at 50 and 60 ^{o}C. Data of 30 and 40 ^{o}C were fitted by single straight line. The slope, which is the reaction rate constant, was evaluated for each straight line section with the results summarized in Table 2.3.

2.4.1. Effect of pH

The tendency that D3 and D4 formed first yet leveled off after a certain period of time was observed for all pH values. The amount of difructose dianhydrides formed was increased by lowering the pH values of the solutions. However, this acid-accelerating effect was demonstrated only in the first reaction section. The reaction rate constants of the second section appeared to be pH-independent as were shown in Table 2.3 previously.

2.4.2. Effect of temperature

The formation of difructose dianhydrides showed no qualitative difference at temperatures in the range of 30 to 60 ^{o}C. The extent of reaction increased with increasing temperature. Figure 2.7 shows the Arrhenius plot for the first reaction section. It was found that the data could be fitted by straight lines, thus the Arrhenius equation was applied to describe the relationship between temperature and the rate constant. Since the slopes were similar for all pH values, it was assumed that the mechanism of reaction does not change within the pH range studied. Rate constant data of all pH values were pooled to evaluate the activation

Table 2.3 Second-order rate constants of difructose dianhydrides formation reaction

Rate constant	Temperature (°C)	Initial concentration (wt %)	pH		
			2.65	4.35	5.90
K_1	30	71.6	.00026	-	.00004
	40	74.4	.00022	.00024	.00026
	50	76.9	.00221	.00100	.00107
	60	80.3	.00353	.00239	.00185
K_2	30	71.6	*	-	*
	40	74.4	*	*	*
	50	76.9	.00024	.00025	.00038
	60	86.3	.00059	.00045	.00048

(K in units of $wt\%^{-1}hr^{-1}$)
*Data were fitted by single straight line with the slope being K_1.

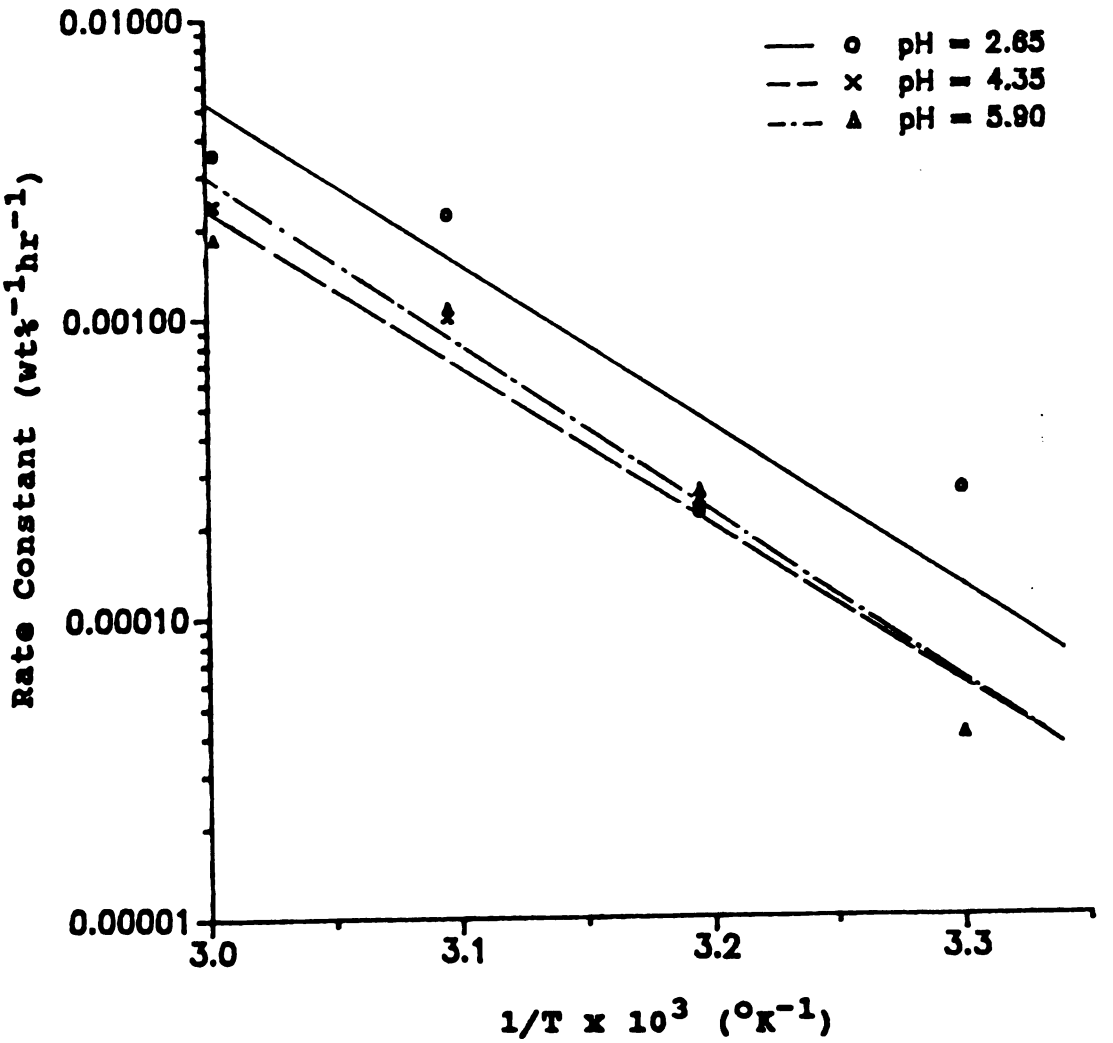


Figure 2.7 The Arrhenius plot of the first reaction section of difructose dianhydrides formation.

energy with the effect of pH being included in the preexponential factor. The resulting equation is

$$K_1 = K_{1,o} \exp(-23300/RT) \quad (5)$$

where R is ideal gas constant in units of cal/g-mole ^oK, T is temperature in units of ^oK, and $K_{1,o} = 8.34x10^{12}$, $4.69x10^{12}$ and $3.87x10^{12}$ $(wt\%)^{-1}hr^{-1}$ for pH = 2.65, 4.35 and 5.90 respectively.

Similar analysis were applied to rate constant data of the second reaction section, although there were only two data points for each pH values due to the limits of fructose solubility and the concentration dependence of the reaction. The resulting equation is

$$K_2 = K_{2,o} \exp(-12400/RT) \quad (6)$$

where $K_{2,o} = 8.58x10^4$, $7.69x10^4$ and $6.75x10^4$ $(wt\%)^{-1}hr^{-1}$ for pH = 2.65, 4.35 and 5.90 respectively.

The activation energy of a reverse reaction, acid hydrolysis of some disaccharides, has been reported ranging from 25 to 40 Kcal/g-mole [20]. The activation energy for this dehydration reaction is 23.3 Kcal for the first reaction section and 12.4 Kcal for the second reaction section, which are in the same order of magnitude but less than that of the hydrolysis reaction.

2.4.3. Effect of concentration

By comparing plots of dimer formation and fructose consumption (reciprocal of fructose concentration versus time), it was noticed that the point at which the

concentration of D3 and D4 levels off coincided with the break point of the two section reaction. This phenomenon appears for all temperature and pH values, thus it suggests that only the formation of D1 and D2 may account for the second section of reaction, while all four dimers are produced in the first section.

It was also noticed that the fructose concentration at the break point was about 70% or less independent of temperature and pH values. Further experiments were performed with initial fructose concentaration being 75, 70 and 65% at 60 ^{o}C and a pH of 2.65. The result of fructose consumption is shown in Figure 2.8 along with the data of 80.3% from a previous experiment. In all three cases the fructose concentration fell to 70% or less within one day and thereafter a linear relationship was found for each set of data. The slopes of these three lines are similar and close to that of the second reaction section in the case of 80.3%. Table 2.4 lists the slope, or second order rate constant, of data with different initial fructose concentration. The formation of fructose dimers is illustrated in Figure 2.9 and it is confirmed that the concentrations of D3 and D4 will reach a certain value and stop increasing when the fructose concentration is about 70% or less.

The molar ratio of water to fructose in the reaction solution was calculated and the result of reactions at 50 ^{o}C is shown in Table 2.5 as an example. It was found that the

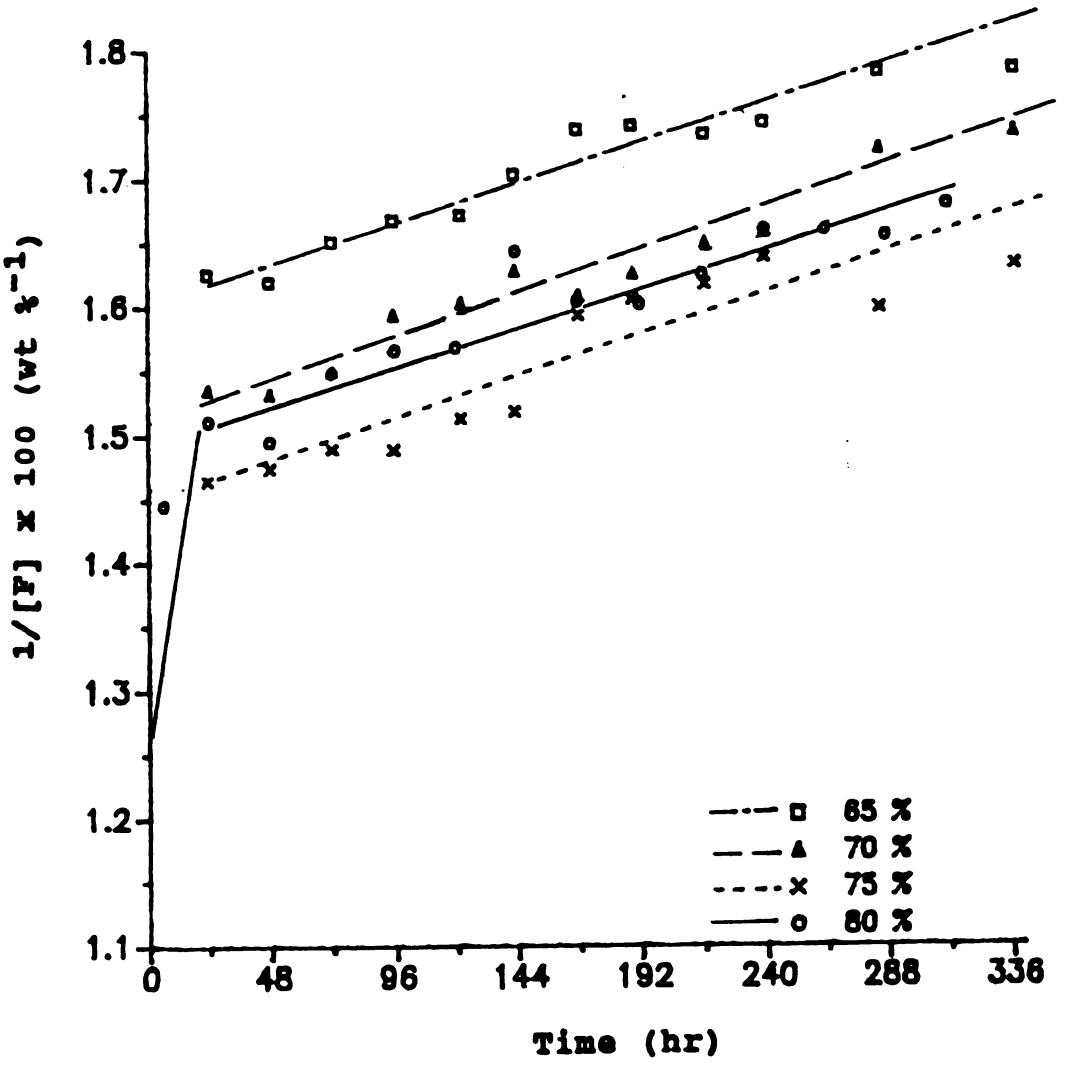


Figure 2.8 Second-order kinetic plot of difructose dianhydrides formation at 60°C and pH 2.65 with different initial fructose concentration.

Table 2.4 Second-order rate constant of difructose dianhydrides formation reaction at 60 °C and pH 2.65

Initial concentration (wt %)	Rate constant (K_2)*
80.3	.00059
75.0	.00062
70.0	.00066
65.0	.00056

(K in units of $wt\%^{-1}hr^{-1}$)
*Data were fitted by single straight line.

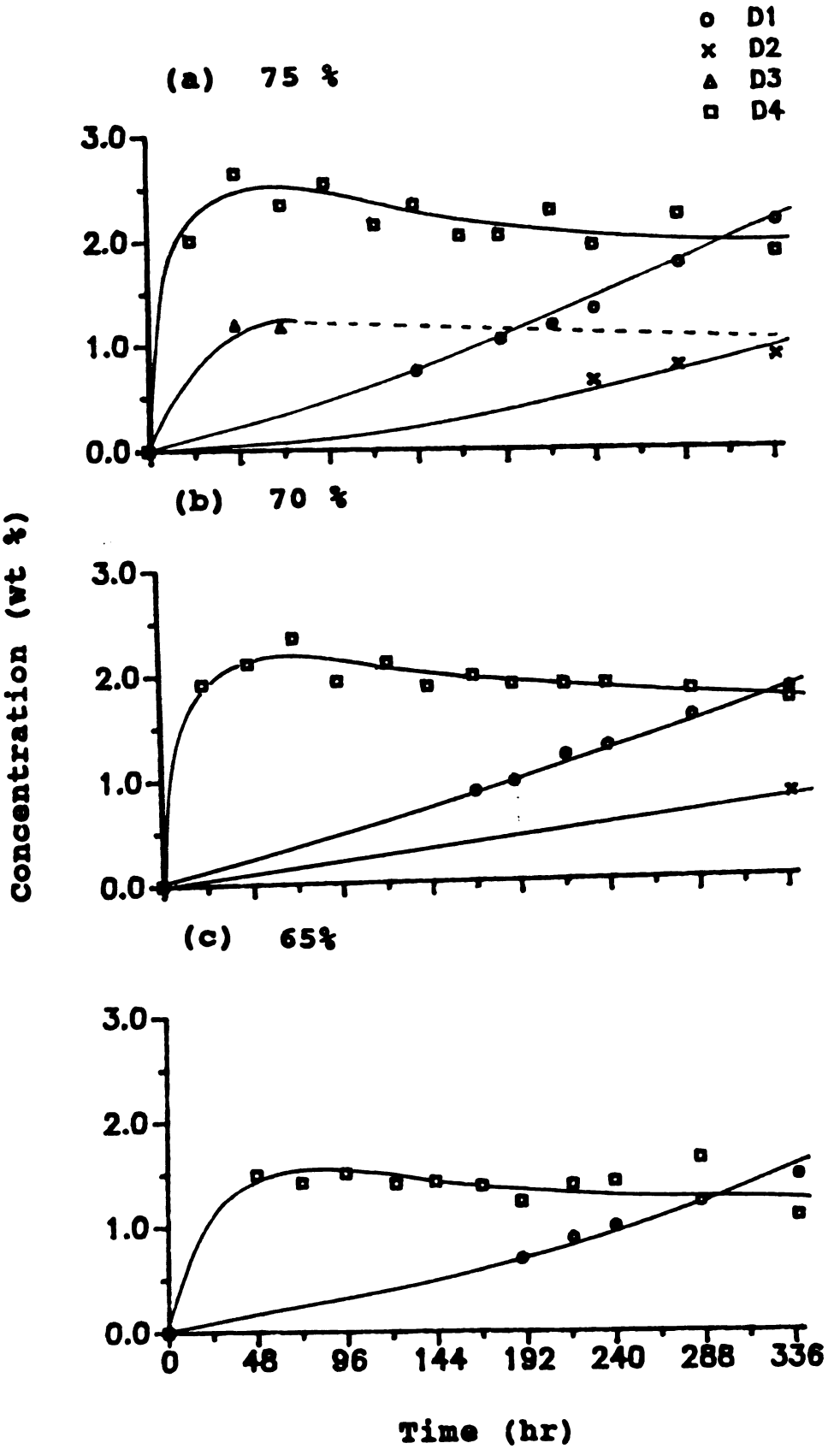


Figure 2.9 The formation of difructose dianhydrides at 60 ^{o}C and pH 2.65 with different initial fructose concentration.

Table 2.5 Molar ratio of water to fructose in reaction solutions at 50 °C

Reaction time (hr)	pH		
	2.65	4.35	5.90
6	3.3	3.0	2.7
24	3.9	3.2	3.0
48	4.2	3.5	3.0
72	4.2	3.6	3.4
96	4.3	4.0	3.9
120	4.4	4.1	3.9
144	4.5	-	4.1
168	4.4	4.4	4.4
192	4.3	4.5	4.2
216	4.4	4.7	4.6
240	4.5	4.5	4.8
264	4.7	4.4	4.3
288	4.3	4.1	4.2
312	4.4	4.5	4.3
336	4.5	4.1	4.6

ratio was four or higher when the concentration of D3 and D4 stop increasing. This suggests that the solvation of fructose molecules may affect the formation of difructose dianhydrides. Hyvonen and coworkers [3] have studied the effect of concentration on fructose mutarotation in aqueous solution and reported that the proportion of fructose tautomers did not change much within the concentration range of 20 to 80% as shown previously in Table 2.1. Thus the equilibrium composition of solution could not account for the concentration effect of this reaction.

The reactions at 60 ^{o}C, a pH value of 5.90 and initial concentrations of 84, 88 and 92% were also studied to determine whether concentration in this range has any influence on the formation of difructose dianhydrides. The results for fructose consumption are shown in Figure 2.10 which also includes data of 80.3% from a previous experiment. Since the fructose concentrations were higher than 70% at the end of two weeks, the slopes from linear fits were compared with that of the first reaction section in data of 80.3%. It was found that the slope decreased as the initial fructose concentration increased with the values listed in Table 2.6. The formation of different dimers is shown in Figure 2.11. The amounts of D1 and D2 cannot be determined separately for some data points, thus the sum of these two compounds was used in Figure 2.11c. It appears that in this concentration range the formation of dimers was retarded by high fructose concentration. The effect of

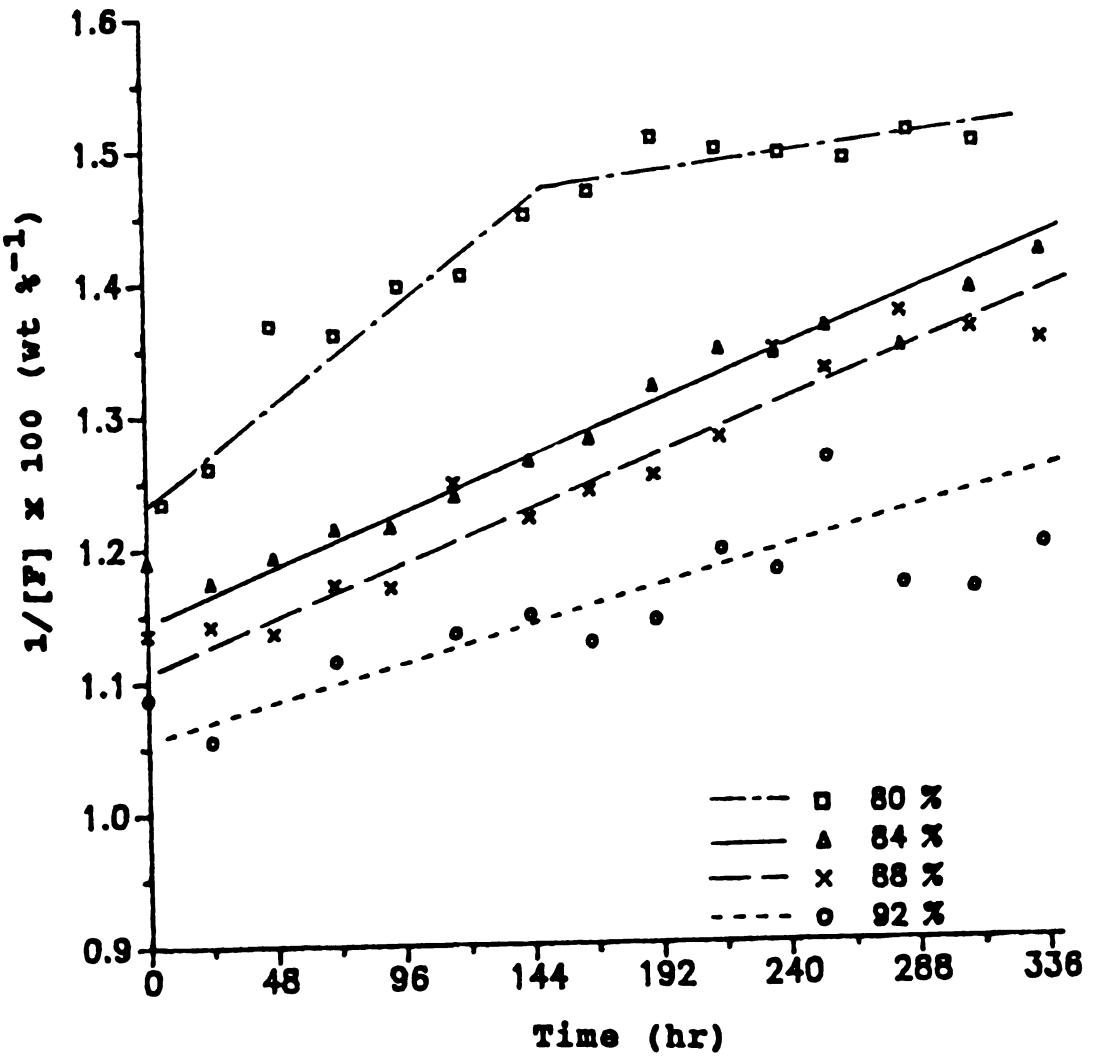


Figure 2.10 Second-order kinetic plot of difructose dianhydrides formation at 60°C and pH 5.90 with different initial fructose concentration.

Table 2.6 The effect of fructose concentration on the reaction rate of difructose dianhydrides formation at 60 ^{o}C and pH 5.90

Initial concentration (wt %)	Rate constant (K_1)*
80.3	.00185
84.0	.00073
88.0	.00078
92.0	.00039

(K in units of $wt\%^{-1}hr^{-1}$)
*Data were fitted by single straight line.

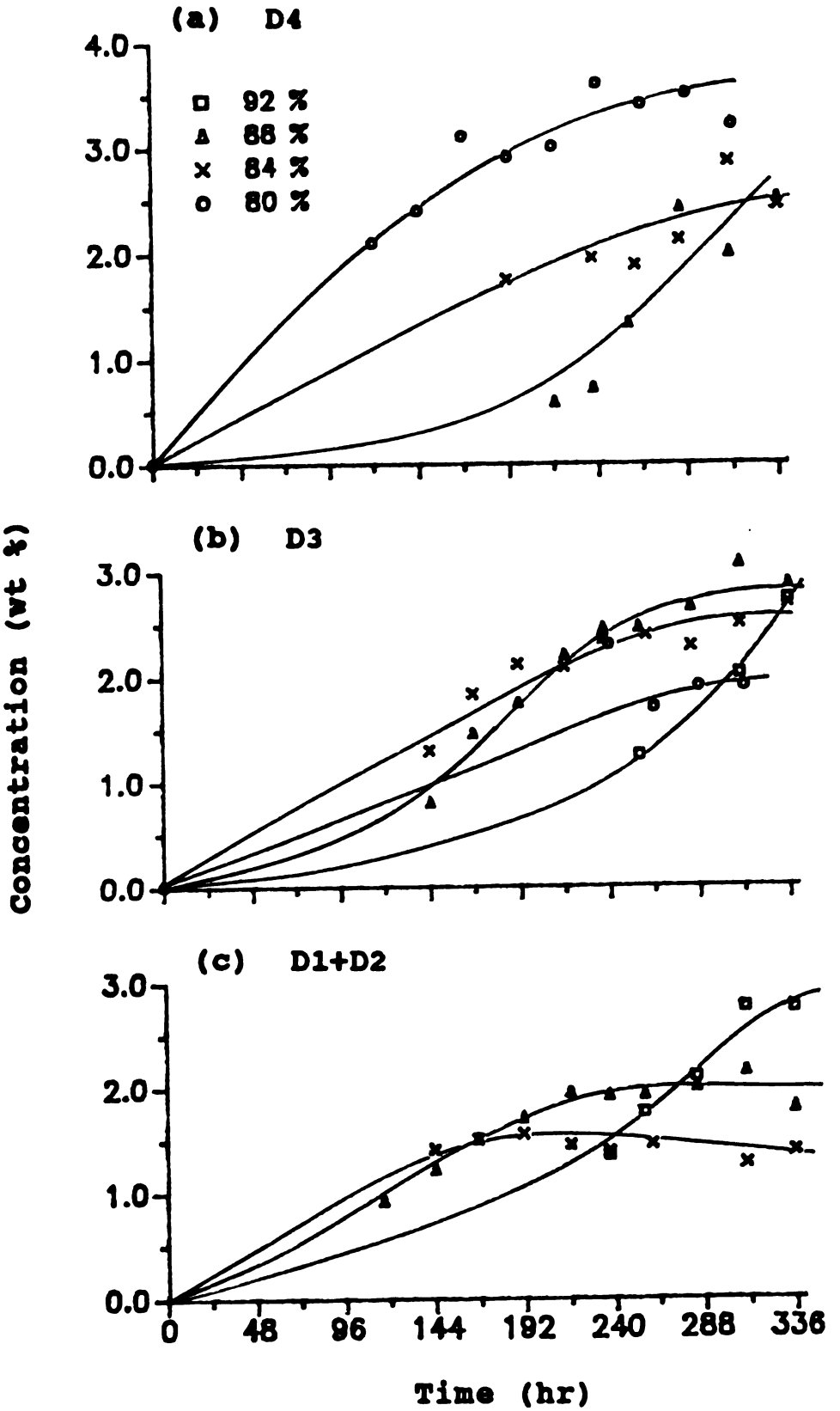


Figure 2.11 The effect of initial fructose concentration on the formation of (a) D4 (b) D3 (c) D1+D2 at 60^{o}C and pH 5.90.

concentration on the formation of D4 was greatest, wherein the amount of D4 formed decreased as the initial fructose concentration increased from 80 to 92%. The formation of D3 and D1+D2 were less affected with the retardation effect demonstrated only at initial stage of reaction when the fructose concentration was still high.

Watanabe [21] has reported the viscosity of aqueous fructose solution increases an order of magnitude as the concentration goes from 70 to 90% at 60 ^{o}C. Table 2.7 lists the viscosity values of concentration ranging from 30 to 90%. Although agitation was provided for these highly viscous solutions, the extent of micromixing might not be significant and the reactant molecules probably encountered each other through molecular diffusion. The conditions of diffusion control for reaction in solution have been discussed by Moore [22]. For a bimolecular reaction, the diffusion-limiting second-order rate constant is proportional to the diffusivity of reactant molecules. With typical diffusivity value of 10^{-5} cm^2s^{-1}, diffusion control will take over from reaction (collision) control at about K = 10^9 $(mol/l)^{-1}s^{-1}$. The diffusivity of fructose in dilute aqueous solution has been reported by Uedaira and Uedaira [23] at 25 ^{o}C and for concentration lower than 3%; the diffusivity is given as

$$D = (7.002-.813C)x10^{-6} \quad (cm^2s^{-1}) \qquad (7)$$

where C is concentration in units of wt%. The diffusivity data for highly concentrated solutions are not available in

Table 2.7 Viscosity of fructose solutions at 60 °C

Concentration (wt %)	Viscosity (c.p.)
30	1.1
50	2.8
60	5.2
70	18
80	92
90	2100

the literature. The second-order rate constant found for the dimer formation reaction is about 10^{-3} $(wt\%)^{-1}hr^{-1}$, or 0.5 in terms of $(mol/l)^{-1}s^{-1}$ approximately. Although the diffusion-limiting rate constant under crystallization conditions will be smaller than 10^{9} due to the low diffusivity value in highly viscous solution, the reaction rate still seems to be too slow to be limited by diffusion. However, the rate of mutarotation is relatively fast (in times on the order of minites [1]) and is more probably subjected to diffusion control as compared to the rate of dimer formation reaction. Thus the high concentration may retard fructose murarotation, which will affect the composition of solution and then the formation of difructose dianhydrides. Measurements of fructose diffusivity and mutarotation rate in saturated and supersaturated aqueous solution will be needed to prove this assumption.

2.5. Conclusions

1. Fructose underwent dehydration reactions and formed four difructose dianhydrides under industrial crystallization conditions.

2. The kinetics of difructose dianhydrides formation reaction was modelled employing a second-order irreversible rate equation. The extent of reaction increased with increasing temperature and decreasing pH value of the solution.

3. The amounts of two of the four difructose dianhydrides stopped increasing when the fructose concentration was about 70% or less. This is probably caused by the effect of solvation of fructose molecules.

4. The formation of all four dimers was retarded when the initial fructose concentration was higher than 80%. The rate of fructose mutarotation may be limited by diffusion in highly concentrated solution, thus affects the formation of difructose dianhydrides.

2.6. References

1. R.S. Shallenberger, Pure Appl. Chem. 50, 1409 (1978).

2. B.K. Dwivedi and S.K. Raniwala, U.S. Patent no. 4,199,373 (1980).

3. L. Hyvonen, P. Varo and P. Koivistoninen, J. Food Sci. 42, 652 (1977).

4. K.H. Forsberg, L. Hamalainen, A.J. Melaja and J.J. Virtanen, U.S. Patent no. 4,199,373 (1975).

5. Y.D. Chu, L.D. Shiau and K.A. Berglund, "Effect of impurities on crystal growth in fructose crystallization", submitted to J. Crystal Growth (1988).

6. A. Pictet and J. Chavan, Helv. Chim. Acta. 9, 809 (1926).

7. W.L. Wolform and M.G. Blair, J. Am. Chem. Soc. 70, 2406 (1948).

8. W.L. Wolfrom, H.W. Hilton and W.W. Binkley, J. Am. Chem. Soc. 74, 2867 (1952).

9. B. Wickberg, Acta Chem. Scand. 8, 436 (1954).

10. R.F. Jackson and S.M. Goergen, Bur. Stand. J. Res. 3, 27 (1929).

11. J.C. Irvine and J.W. Steveson, J. Am. Chem. Soc. 51, 2197 (1929).

12. R.F. Jackson and E.J. McDonald, Bur. Stand. J. Res. 6, 709 (1931).

13. J. Defaye, A. Gadelle and C. Pedersen, Carbohydr. Res. 136, 53 (1985).

14. T. Kusch, W. Gosewinkel and G. Stoeck, U.S. Patent No. 3,513,023 (1970).

15. T. Yamauchi, U.S. Patent No. 3,928,062 (1975).

16. V.S. Velasco and J.F. Dowling, U.S. Agri. Res. Serv. South. Reg., [Rep.], ARS-S-88, 138 (1975).

17. K. Hamada, H. Yoshihara, G. Suzukamo and O. Hiroaki, Bull. chem. Soc. Jpn., 57, 307 (1984).

18. H.W. Hilton, in Methods in Carbohydrate Chemistry, Vol II, R.L. Whistler and M.L. Wolfrom Ed. (Academic Press, New York, 1963) p. 199.

19. R.S. Shallenberger, Advanced Sugar Chemistry (The AVI Publishing Company, Inc. Westport, CN, 1982) p. 216.

20. J.H. Pazur, in The Carbohydrates, 2nd Edition, Vol IIA, W. Pigman and D. Horton Ed. (Academic Press, New York, 1970) p. 76.

21. T. Watanabe, Seito Gijutsu Kenkyu Kaishi 28, 70 (1978).

22. W.J. Moore, Physical Chemistry (Prentice-Hall, Inc., Englewood Cliffs, NJ, 1972) p. 405.

23. H. Uedaria and H. Uedaria, J. Phys. Chem. 74, 2211 (1970).

CHAPTER 3*

EFFECTS OF GLUCOSE AND DIFRUCTOSE DIANHYDRIDES ON CRYSTAL GROWTH IN FRUCTOSE CRYSTALLIZATION

3.1. Abstract

The influence of impurities on the crystallization of anhydrous fructose from aqueous solution was studied. The growth kinetics of fructose crystals in the fructose-water-glucose and fructose-water-difructose dianhydrides systems were investigated using photomicroscopic contact nucleation techniques. Glucose is the major impurity likely to be present in fructose syrup formed during corn wet milling, while several difructose dianhydrides are formed *in situ* under crystallization conditions and have been proposed as a cause in the decrease of overall yields.

Both sets of impurities were found to cause inhibition of crystal growth, but the mechanisms responsible in each case are different. It was found that the presence of glucose increases the solubility of fructose in water and thus lowers the supersaturation of the solution. This is

---

*This chapter contains a paper submitted to "Journal of Crystal Growth". In this paper, the effects of glucose and difructose dianhydrides on fructose crystal growth were studied. The author is responsible for the part related to difructose dianhydrides.

probably the main effect responsible for the decrease of crystal growth. Since the molecular structures of difructose dianhydrides are similar to that of fructose, they are probably "tailor-made" impurities. The decrease of crystal growth is probably caused by the incorporation of these impurities into or adsorption to the crystal surface which would accept fructose molecules in the orientation that existed in the difructose dianhydride.

3.2. Introduction

The monosaccharide D-fructose has great potential use as a natural sweetener due to its wide abundance and high sweetness (1.8 times that of sucrose). Unlike other sweeteners such as sucrose, the crystallization of fructose is very difficult due to unfavorable physico-chemical properties [1]. Fructose is much more soluble in water as compared to sucrose resulting in a highly viscous saturated solution. For example, 100 g of water can dissolve 662 g of fructose at 50 ^{o}C and the viscosity of this saturated solution is about 4000 c.p. [2]. On the other hand, only 260 g of sucrose can be dissolved in 100 g of water with a resulting solution viscosity of 102 c.p. [3]. Fructose tends to crystallize as hemihydrate and/or dihydrate forms rather than the anhydrous form under certain conditions. These hydrate compounds melt near room temperature which makes them unacceptable as products [4,5]. As a reducing sugar, fructose undergoes mutarotation in solution as

depicted in Figure 3.1, but the only configuration of fructose in the crystalline state is β-pyranose [6]. β-Furanose and α-furanose are also found in significant amounts in aqueous solution with the equilibrium compositions of fructose solutions at different temperatures and concentrations given in Table 3.1 [7].

In addition to physico-chemical properties, the presence of impurities in fructose syrup may retard crystal growth. These impurities include glucose and difructose dianhydrides which may exist in the fructose syrup in substantial amounts. Residual glucose is often found after glucose isomerase conversion of glucose to fructose and subsequent ion exchange enrichment [8]. Difructose dianhydrides are formed in situ under industrial crystallization conditions and have been reported to decrease the overall yield in fructose crystallization [9].

The growth kinetics of fructose crystals from the pure fructose-water system have been studied by Shiau and Berglund [10]. It was found that the growth of fructose crystals was size-independent with growth rate dispersion among different crystals. The Constant Crystal Growth model was employed and linear growth rates were evaluated from plots of size versus time. The dependence of growth rate on supersaturation and temperature was described by a power law model and Arrhenius relation. The mean growth rate was expressed as

$$G = A \exp(-E/RT) S^n \qquad (1)$$

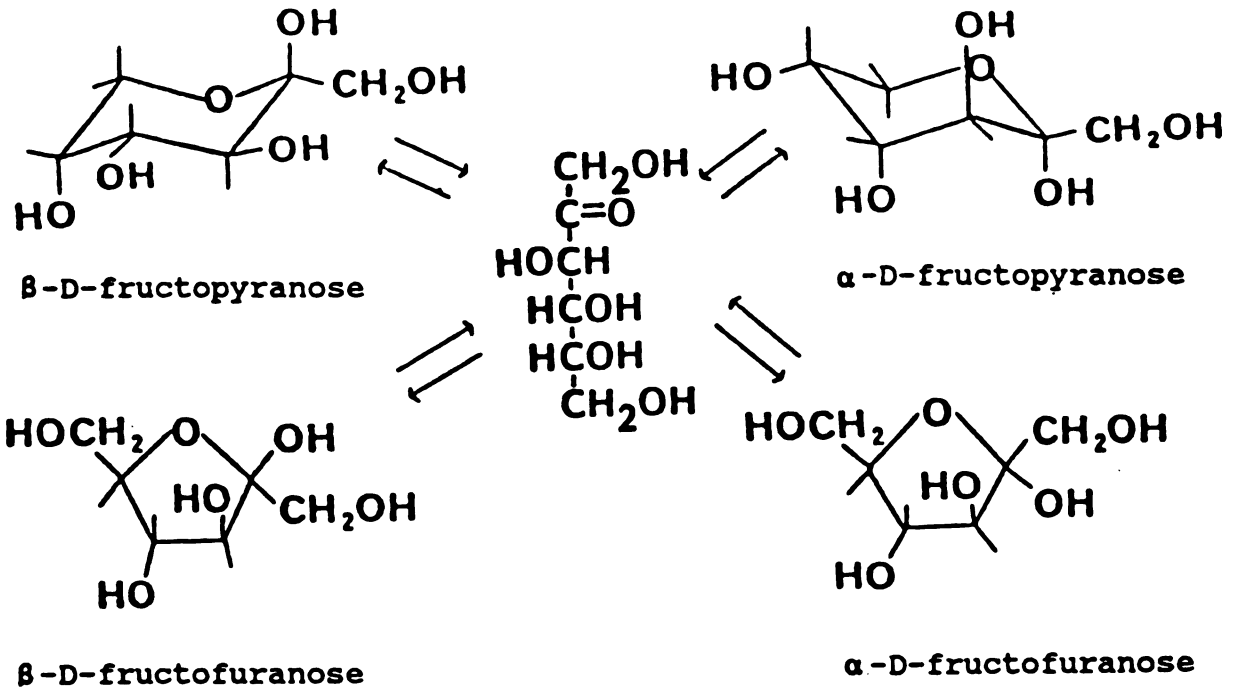


Figure 3.1 Possible tautomers of D-fructose in solution.

Table 3.1 Tautomeric equilibria of D-fructose solutions.

Temperature (°C)	Concentration (wt %)	α-furanose (%)	β-furanose (%)	β-pyranose (%)
23	20	6	21	73
23	50	4	21	75
23	80	5	21	74
0	20	4	18	78
22	20	6	21	73
67	20	8	28	64
77	20	12	31	57

where $A = 1.5 \times 10^7$ μm/hr, $E = 6100$ cal/g-mole, and $n = 1.3$. The variance of the growth rate distribution was approximated by the following power law model:

$$\sigma_G^2 = aG^b \qquad (2)$$

where $a = 0.17\ (\mu m/hr)^{0.65}$ and $b = 1.4$.

The objective of this study is to determine the effects of glucose and difructose dianhydrides on the growth of anhydrous fructose crystals under conditions likely to exist in commercial crystallization.

3.3. Experimental

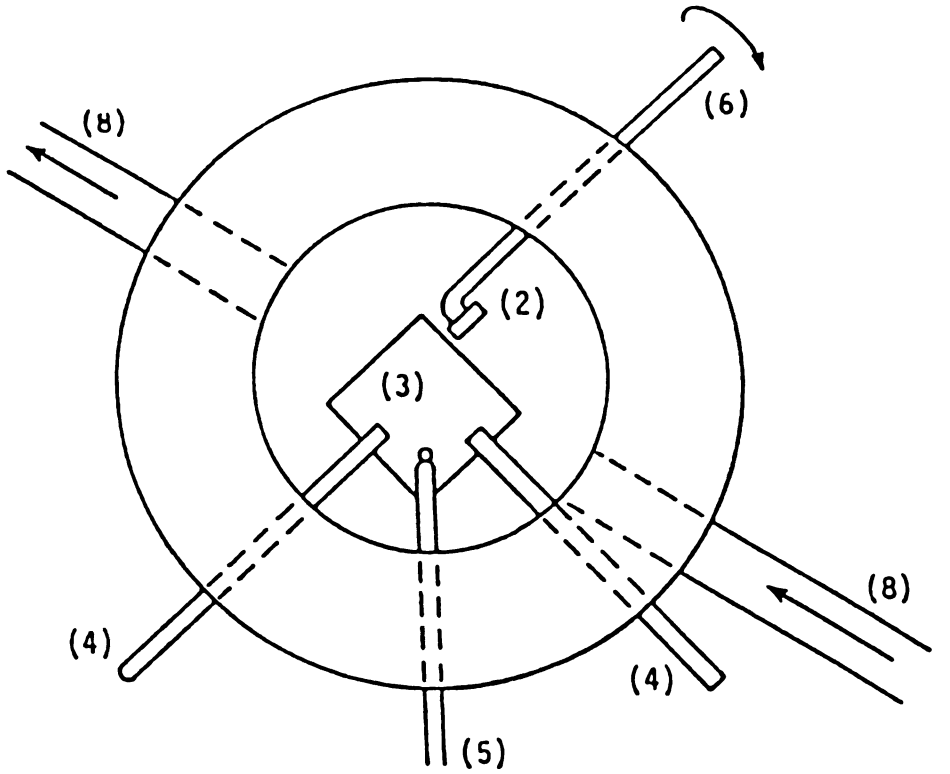
Solutions containing glucose were prepared by adding glucose to pure fructose solutions. The concentration of impurity was expressed as ratio of impurity to water (I/W). Solutions containing difructose dianhydrides were prepared initially by stirring an 80% pure fructose solution at 70 ^oC for a certain period of time to generate difructose dianhydrides. Extra fructose was then added to obtain supersaturated solutions at the desired temperature. Solubility data published by the National Bureau of Standards [11] were used to calculate supersaturation in the pure system. Since the influence of impurities on fructose solubility is not clear, the same solubility as in the pure system was used to calculate supersaturation in the impure systems.

The concentration of difructose dianhydrides was

determined by a Biorad Carbohydrate Analysis HPLC system, which was equipped with a Bio-Sil Amino 5S column and a refractive index detector. The mobile solvent was acetonitrile/water with a volume ratio of 70/30 and a flow rate of 1.0 ml/min. Samples were eluted at room temperature.

Contact nucleation experiments were conducted to study crystal growth rates. The experimental apparatus and technique described by Shanks and Berglund [12] for the sucrose-water system was used in this work with the growth cell shown in Figure 3.2. Here the growth rates of crystals in a stagnant cell were studied; since the aqueous fructose solutions were highly viscous, stirring is unlikely to have an effect on mass transfer. Contact nuclei were created by sliding the parent crystal along a glass plate in the growth cell with the resulting growth of nuclei monitored photomicroscopically. Photographs were subsequently analyzed by an image analyzer to determine the area of each crystal and the equivalent circular diameter was taken as the characteristic size. The experimental conditions for the fructose-glucose and fructose-difructose dianhydrides systems are listed in Tables 3.2 and 3.3.

Fructose crystals growing in the presence of glucose or difructose dianhydrides were collected after completion of the growth experiments. The adhering mother liquor was washed from the surface by saturated alcohol solution. The crystals were dissolved and the solution was analyzed for



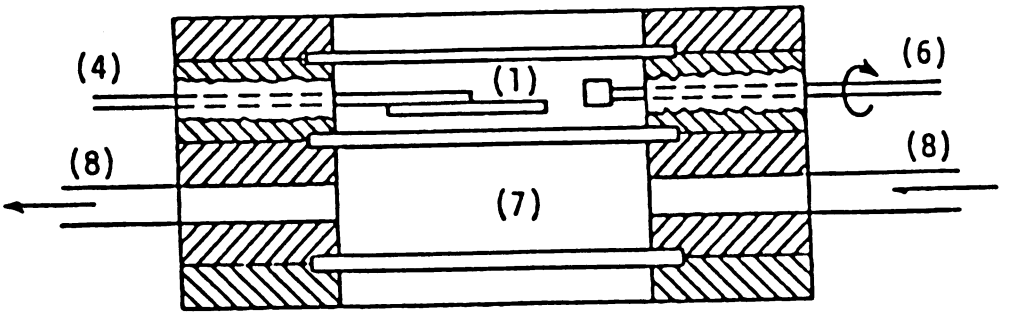


Figure 3.2 Schematic diagram of nucleation cell with the features:
(1) chamber containing solution; (2) parent crystal; (3) glass cover slip where parent crystal is slid; (4) support rods for glass cover slip; (5) thermistor; (6) movable rod holding parent crystal; (7) chamber containing constant temperature water; and (8) water inlet and outlet.

Table 3.2 Conditions of crystal growth experiments for fructose-water-glucose system at 40 °C.

Exp	Impurity/Water ratio (I/W)	Supercooling (°C)	No. of nuclei analyzed
1	0.05	1	12
2	0.05	3	13
3	0.05	5	15
4	0.05	7	11
5	0.05	9	14
6	0.3	1	13
7	0.3	3	12
8	0.3	5	11
9	0.3	7	15
10	0.3	9	12
11	0.3	11	13
12	0.6	5	14
13	0.6	7	13
14	0.6	9	13
15	0.6	11	16
16	0.9	5	18
17	0.9	7	16
18	0.9	9	12
19	0.9	11	15

Table 3.3 Conditions of crystal growth experiments for fructose-water-difructose dianhydrides system.

Exp	Temperature (°C)	Supersaturation $(C-C_e)/C_e$	Difructose dianhydrides (wt%) total	D_1+D_2	D_3	D_4	No. of nuclei analyzed
1	30	.053	1.6	.6	.6	.4	21
2	30	.055	1.2	.5	.4	.3	19
3	30	.068	1.2	.5	.5	.2	13
4	40	.039	3.0	.9	1.3	.8	13
5	40	.048	3.3	1.2	1.3	.8	10
6	40	.041	3.2	1.1	1.2	1.0	14
7	40	.021	4.5	.8	1.5	2.2	12
8	40	.045	4.6	.8	1.5	2.3	12
9	40	.045	5.5	1.3	1.9	2.3	25
10	50	.028	4.4	1.8	1.7	.9	14
11	50	.049	4.4	1.8	1.7	.9	12

Note: D_1, D_2, D_3, D_4 represents diheterolevulosan II, I, III, IV respectively.

the presence of impurities by the HPLC technique described above.

3.4. Results and Discussion

Figure 3.3 shows examples of size versus time plots for several crystals grown in the presence of impurity. It was demonstrated that the growth rate of a single crystal was constant while different crystals had different growth rates. The phenomena of size-independent growth and growth rate dispersion were observed for all impurity concentrations in both the glucose and the difructose dianhydrides systems. Thus, the Constant Crystal Growth model used in the pure system was also employed in the impure systems.

3.4.1. Effect of glucose on fructose crystal growth

From a plot of size versus time, the linear growth rate was evaluated for each single crystal. The mean growth rate and the variance of the growth rate distribution were then calculated for each level of supersaturation at 40 ^{o}C.

The relationship between mean growth rate and supersaturation at different values of impurity/water (I/W) ratio is shown in Figure 3.4. The mean growth rate was decreased to 25 to 50% of that in the pure system by the presence of glucose at concentrations ranging from 0.05 to 0.9 in terms of I/W ratio. This can be described by the following equation

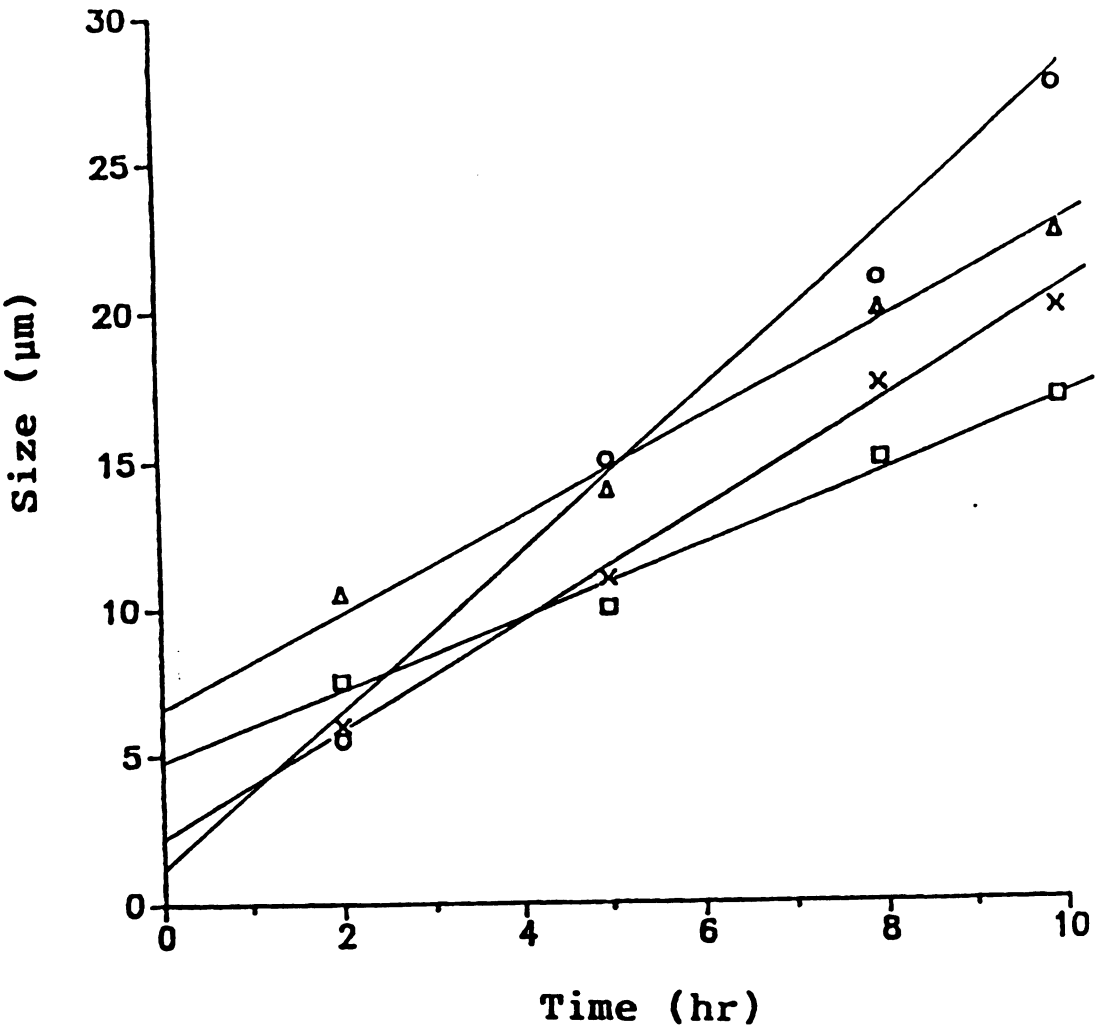


Figure 3.3 Size versus time plot of fructose contact nuclei grown in the presence of glucose. Temperature = 40 ^{o}C, supercooling = 5 ^{o}C and impurity/water ratio = 0.3. Each line represents a single crystal.

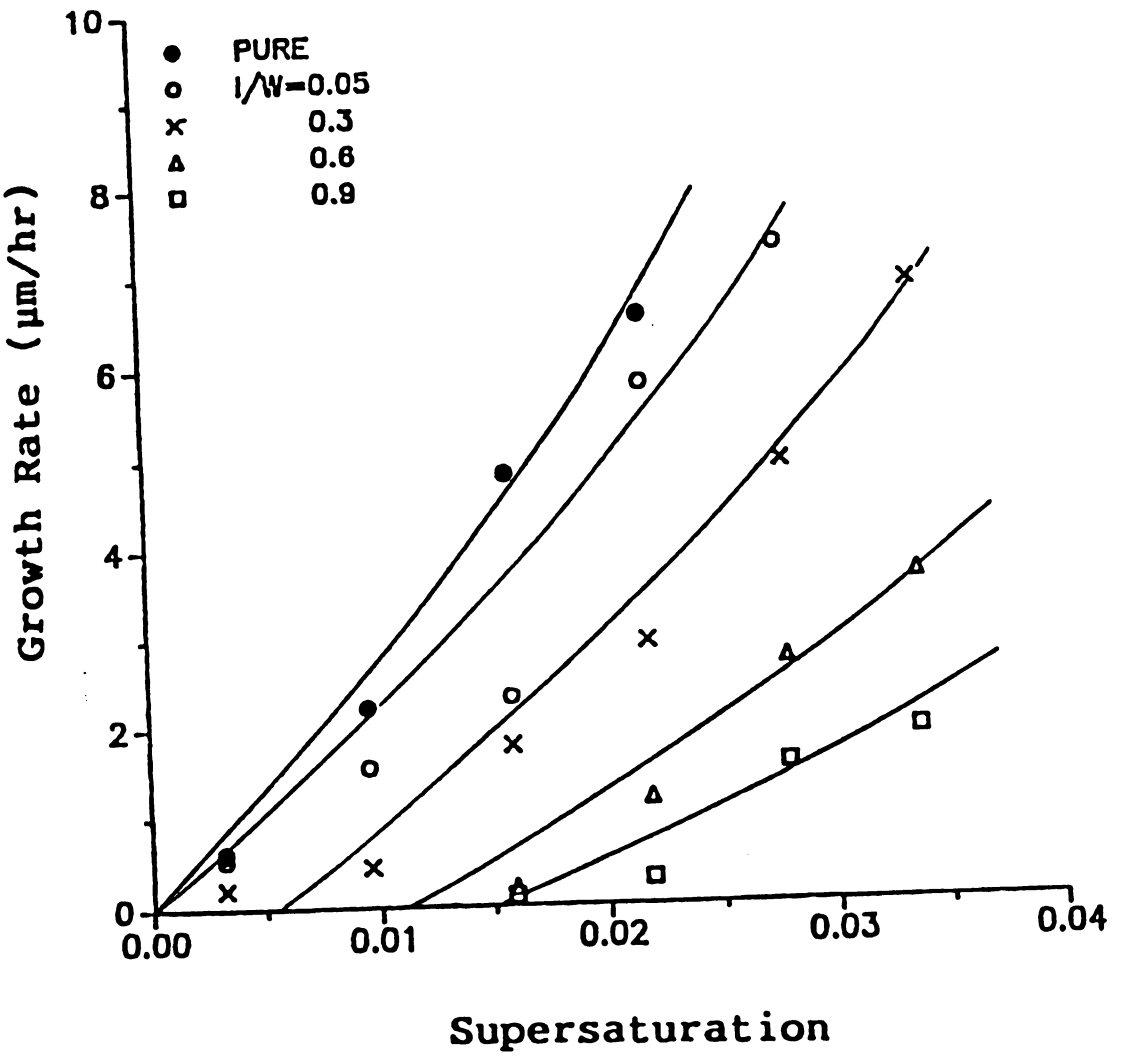


Figure 3.4 The influence of glucose on the relationship between mean growth rate and supersaturation at 40 ^{o}C.

$$G = A \exp(-E/RT)(S-2.7\times10^{-4}(I/W)^{0.89})^{n} \exp(-0.0098I/W) \quad (3)$$

Here A, E and n are assumed to be the same as in the pure fructose system. The estimation of parameters is summarized in Table 3.4.

It was observed that nuclei generated in a solution of high supersaturation would dissolve at a temperature lower than the supposed saturation temperature of the solution. Nuclei were generated and grew only when supersaturation reached a critical value which increased as impurity/water ratio increased. This phenomenon suggests that solubility of fructose increases with an increase of glucose concentration. In the equation of mean growth rate, the term $2.7\times10^{-4}(I/W)^{0.89}$ is subtracted from S to account for this increase of solubility. Besides the solubility change, the presence of glucose could also affect volume diffusion in solution and/or surface integration of crystal molecule. The need for the inclusion of exp(-0.0098I/W) in the model may represent these effects, but the scatter of the data precludes any definitive comment to be made.

Figure 3.5 shows the variance of the growth rate distribution at 40 ^{o}C. A power law model was used to fit the data resulting in following equation

$$\sigma_G^2 = 0.16G^{1.4} \quad (4)$$

The statistics pf regression is summarized in Table 3.5. No significant difference of the variance of growth rate distribution was found between pure and glucose impurity systems.

Table 3.4. Summary of parameter estimation for growth rate data

Data	Model	parameters*			Residual sum of squares $(\mu m/hr)^2$
		a	b	c	
Glucose	1. $G=Aexp(-E/RT)S^n$	-	-	-	383.58
	2. $G=Aexp(-E/RT)(S-a(I/W)^b)^n$	4.5E-4	0.85	-	11.90
	3. $G=Aexp(-E/RT)(S-a(I/W)^b)^n$ $exp(-cI/W)$ (equation (3))	2.7E-4	0.89	9.8E-3	3.39
Difructose dianhydrides	1. $G=Aexp(-E/RT)S^n$	-	-	-	2274.35
	2. $G=Aexp(-E/RT)S^n exp(-aI/W)$ (equation (5))	-23	-	-	9.67

*Nonlinear parameters were estimated by minimizing residual sum of squares.

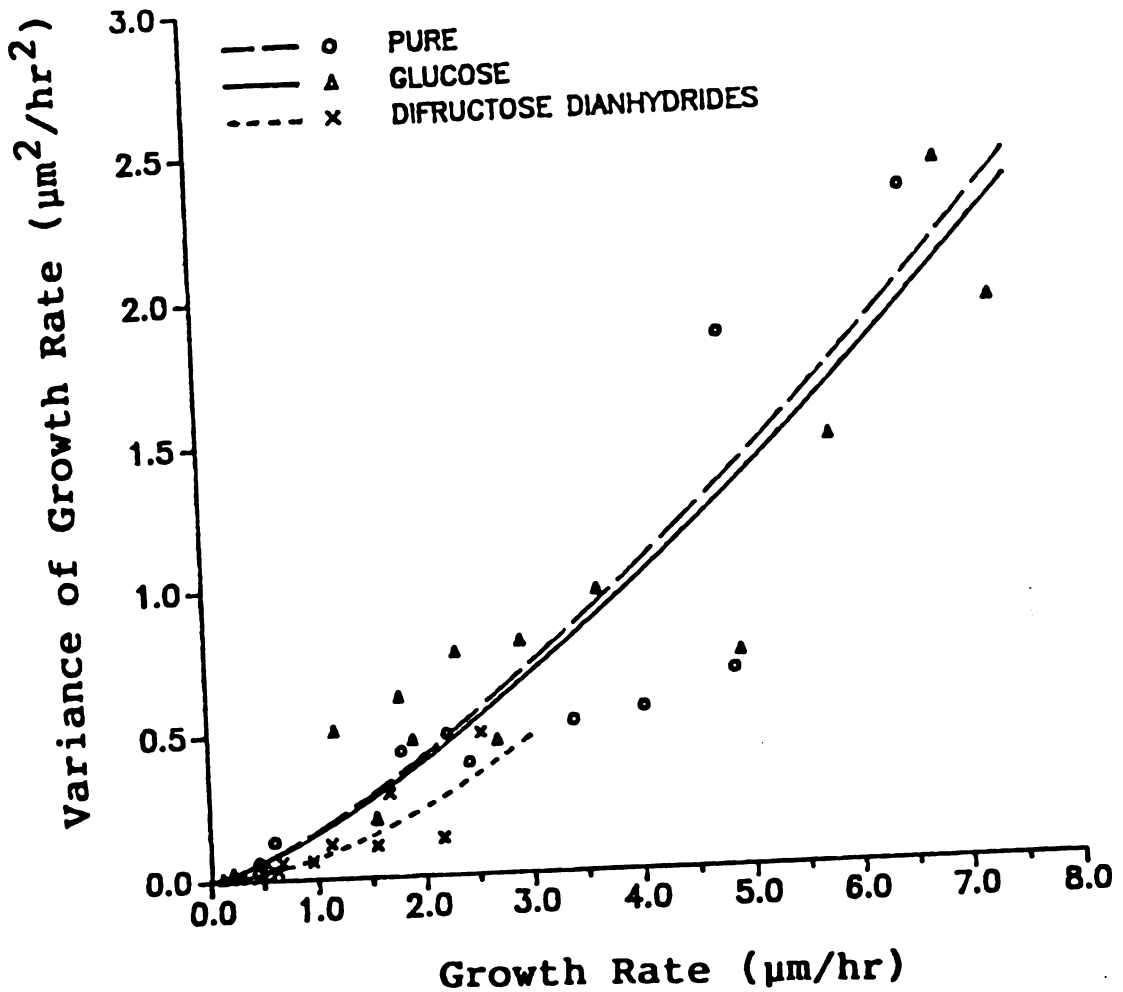


Figure 3.5 The influence of impurities on the variance of growth rate.

Table 3.5. Summary of statistics of regression equations (4) and (6): $s_G^2 = aG^b$ *

Equation	Correlation coefficient	parameters ± standard error	
		lna	b
(4)	0.95	-1.86±0.28	1.4±0.1
(6)	0.89	-2.64±0.18	1.7±0.1

*Linear parameters were estimated by ordinary least square method after logarithmic transformation of the equation.

3.4.2. Effect of difructose dianhydrides on fructose crystal growth

Fructose undergoes irreversible dehydration reactions in acid solution or at high temperature and forms several difructose dianhydrides. These dianhydrides possess a central 1,4-dioxane ring as shown in Figure 3.6 with at least eight difructose dianhydrides having been reported [13,14]. Their structures differ in the configuration of each fructose moiety and the linkage between them, as listed in Table 3.6.

Since the rates of crystal growth are so low the crystallization step in fructose manufacture requires time on the order of days. The temperature range involved is 30 to 60 ^{o}C and pH is controlled between 4 and 6 [5,9,15,16]. Under these conditions, four difructose dianhydrides, diheterolevulosan I - IV, were found by HPLC analysis with a typical chromatogram shown in Figure 3.7. The appearance of these four dimers is expected since all of them have at least one b-fructopyranose, which is the major form of fructose in solution as shown previously in Table 3.1. When kept at 60 ^{o}C for two weeks, an 80% fructose solution with a pH value of 4.35 formed about 6% dianhydrides in total.

Since difructose dianhydrides are not commercially available, the preparation of supersaturated solution for studying the dianhydrides impurities was different from that used for glucose. Thus, the supersaturation and impurity concentration could not be similarly controlled for the two

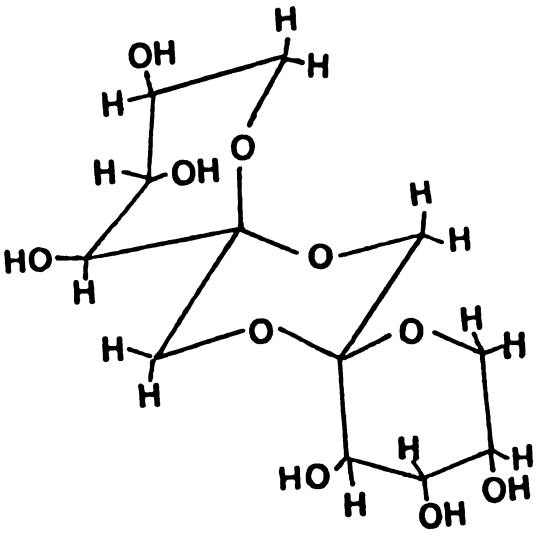


Figure 3.6 Diheterolevulosan I.

Table 3.4 Known difructose dianhydrides.

fructose moiety		linkage	trivial name	
ring	anomeric C			
pyr-pyr	β, α	1,2:2,1	diheterolevulosan	I
fur	β, α	1,2:2,1		II
fur	β, β	1,2:2,1		III
pyr	β, β	1,2:2,1		IV
pyr	β, β	1,2:2,3	-	
fur-fur	α, β	1,2:2,1	difructose anhydride	I
fur	-	1,2:2,4		II
fur	α, β	1,2:2,3		III

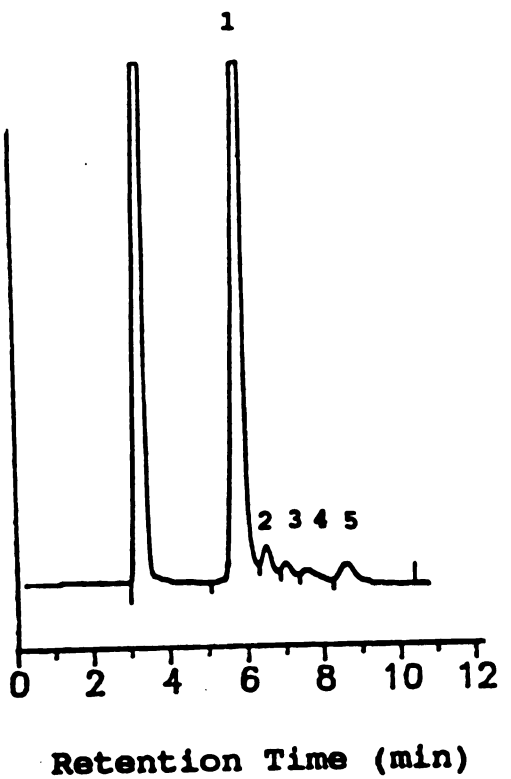


Figure 3.7 HPLC analysis of difructose dianhydrides. 1. Fructose 2. diheterolevulosan II 3. diheterolevulosan I 4. diheterolevulosan III 5. diheterolevulosan IV.

sets of impurities.

Growth rate was normalized by division by the growth rate of the pure system so that the effect of impurity could be evaluated from data of different supersaturation and temperature levels. Figure 3.8 shows the relationship between normalized mean growth rate and impurity/water ratio (I/W). The effect of difructose dianhydrides on crystal growth inhibition was greater than that of glucose with a rapid decrease in growth rate occurring over a narrow range of impurity concentration. The growth rate was only one tenth of that in the pure system at an impurity/water ratio of 0.1, or 0.006 in terms of mole fraction of total solid. The following equation is applied to fit the data:

$$G = A \exp(-E/RT) S^n \exp(-9.9I/W) \qquad (5)$$

Parameters A, E and n are the same as in the pure system. The estimation of parameters is summarized in Table 3.4. The impurity (I) used was the total amount of difructose dianhydrides. Individual dianhydride levels were studied, but no significant difference among the dianhydrides was demonstrated by the experimental data.

The variance of the growth rate distribution is shown in Figure 3.5 along with that of pure and glucose impurity systems. The equation representing the variance data is

$$s_G^2 = 0.071 G^{1.7} \qquad (6)$$

The statistics of regression is summarized in Table 3.5. Growth rate dispersion appears to be less in the difructose dianhydrides impurity system, but the exponents in the

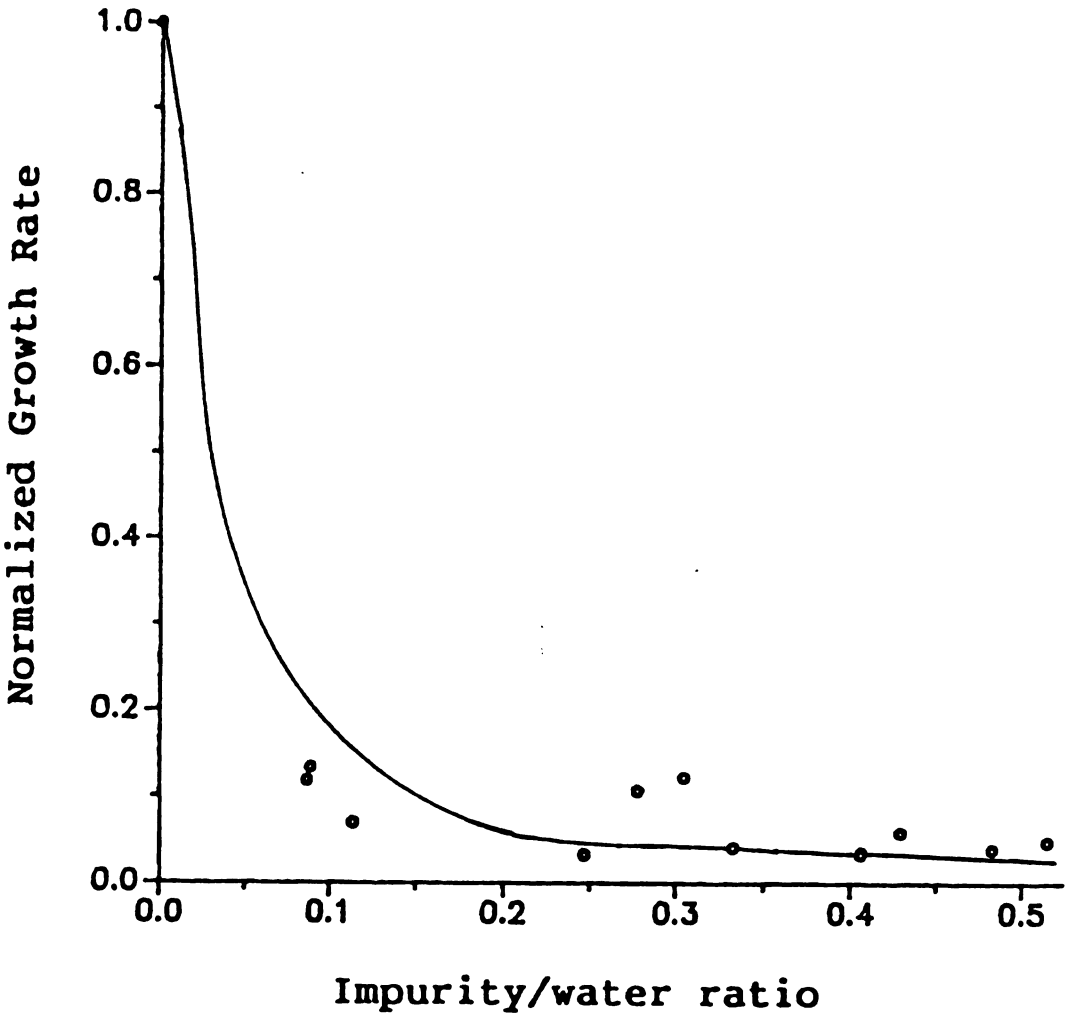


Figure 3.8 The effect of difructose dianhydrides on normalized mean growth rate.

variance equations of the two impure systems were compared with that of the pure system by a t-test and no significant difference was demonstrated in both cases (P = 0.05). However, it should not be concluded by the comparison alone because the range of growth rate data in dianhydrides system is much smaller than those of the pure and glucose impurity systems.

A small amount of difructose dianhydrides (< 1 %) was found by HPLC analysis in fructose crystals grown from solution containing these impurities (Figure 3.9). Crystals grown in the presence of glucose were also analyzed; however, glucose was not detected. The appearance of impurities in the crystal may be caused by inclusion of mother liquor as well as incorporation of impurity molecules into lattice of the growing crystal. The amount of mother liquor inclusion should be negligible because fructose crystals grow very slowly in the presence of impurities (0.5 to 2.0 μm/hr in difructose dianhydrides impurity system) and is further supported by the absence of glucose in crystals. Thus, it is suggested that molecules of difructose dianhydrides were incorporated into the fructose crystal.

Difructose dianhydides consist of two fructose moieties and thus possess some of the structural characteristics of fructose molecules. This suggests that the possible mechanism of growth retardation occurring in the difructose dianhydrides impurity system is the so-called "tailor-made crystal growth inhibition", which means the structural

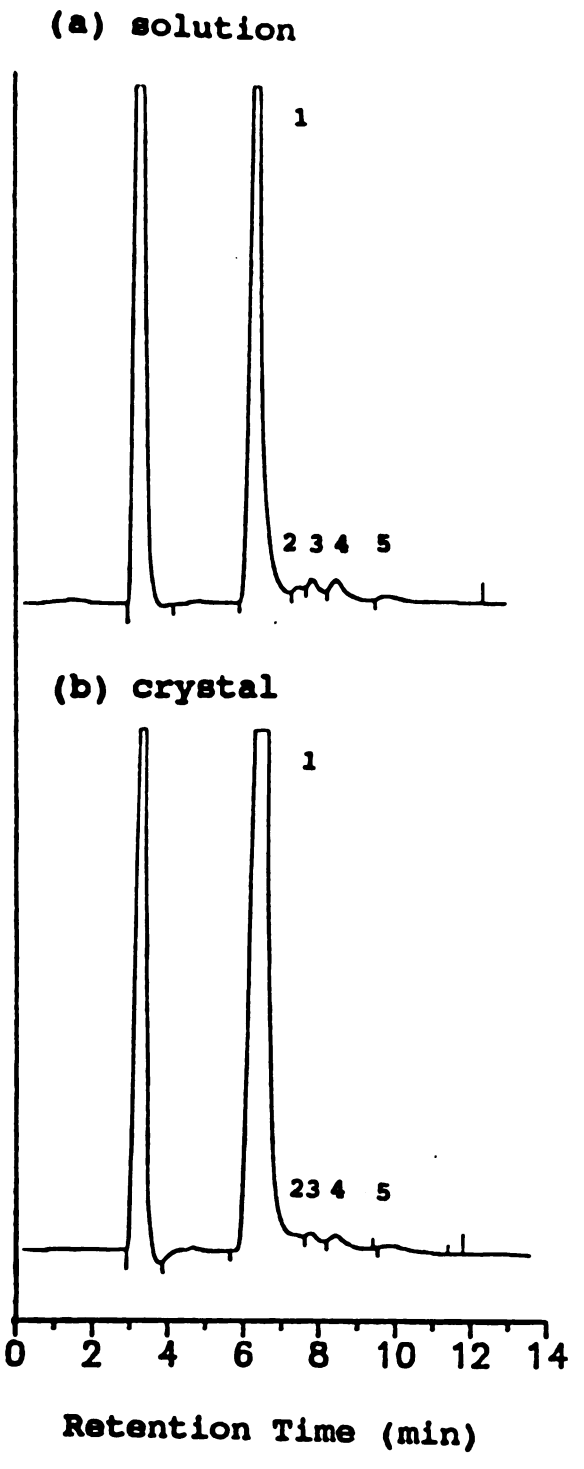


Figure 3.9 HPLC analysis of difructose dianhydrides incorporation. 1. Fructose 2. diheterolevulosan II 3. diheterolevulosan I 4. diheterolevulosan III 5. diheterolevulosan IV.

similarity of the impurity and crystal molecules leads to incorporation of impurity into the growing surface. This will disrupt the bonding sequences in the crystal and interfere with subsequent incorporation of crystal molecules [17,18]. Based on HPLC analysis, the incorporation of difructose dianhydrides had no preference among the four dianhydrides. The effects of growth inhibition exerted by different dianhydrides were also found to be similar. Since all of these four dimers have at least one moiety of β-fructopyranose, which is the form of crystalline fructose, all of them are expected to be occluded into the crystal and retard the growth of crystal similarly.

The morphologies of fructose crystals growing from the pure and two impure systems were compared and no significant difference was found. Since the sizes of crystals studied were less than 50 mm, differences in crystal habit probably could not be detected, and further observation on larger crystals is necessary to show the effect of these impurities on crystal habit modification.

3.5. Conclusions

1. The growth rate of fructose crystals in the presence of impurities was size-independent, with growth rate dispersion among different crystals.

2. Growth inhibition was observed in both impure systems. At the same impurity level, the mean growth rate

in the presence of difructose dianhydrides was lower than that in the presence of glucose.

3. The growth inhibition is probably caused by an increase of solubility by glucose. Difructose dianhydrides, on the other hand, appear to be incorporated into the crystal, thus inhibiting subsequent surface integration of fructose molecules.

4. The effects of growth inhibition exerted by different difructose dianhydrides were similar, probably because they have the same structural characteristics of crystalline fructose molecules.

Notation

A = frequency factor, μm/hr

a,b = correlation constants

C = concentration, g fructose/100 g solution

C_e = saturation concentration, g fructose/100 g solution

E = activation energy, cal/g-mole

G = mean linear crystal growth rate, μm/hr

I = impurity, g

n = growth rate order

R = ideal gas constant, 1.987 cal/g-mole ^{o}K

S = supersaturation, $(C-C_e)/C_e$

T = temperature, ^{o}K

W = water, g

Greek Symbol

σ_G^2 = variance of growth rate distribution, $\mu m^2/hr^2$

3.6. References

1. G.N. Bollenback, Kirk-Othmer Encyclopedia of Chemical Technology (John Wiley & Sons, New York, 1983).

2. T. Watanabe, Seito Gijutsu Kenkyu Kaishi 28 (1978) 70.

3. J. Timmermans, The Physico-chemical Contants of Binary Systems in Concentrated Solutions (Interscience Publishers, New York, 1960).

4. F.E. Young, F.T. Jones and H.J. Lewis, J. Phys. Chem. 56 (1952) 1093.

5. T. Yamauchi, U.S. Patent No. 3,928,062 (1975).

6. T.E. Dotty and E. Vanninen, Food Technol. 11 (1975) 34.

7. L. Hyvonen, P. Varo and P. Koivistoninen, J. Food Sci. 42 (1977) 652.

8. E. Vanninen and T. Dotty, Sugar: Science and Technology (Applied Science Publishers, London, 1979).

9. K.H. Forsberg, L. Hamalainen, A.J. Melaja and J.J. Virtanen, U.S. Patent no. 4,199,373 (1975).

10. L.D. Shiau and K.A. Berglund, AIChE J. 33 (1987) 1028.

11. F.J. Bates, Polarimetry, Saccharimetry and the Sugars (United States Government Printing Office, Washington, 1942).

12. B.H. Shanks and K.A. Berglund, AIChE J. 31 (1985) 152.

13. R.S. Shallenberger, Advanced Sugar Chemistry (The AVI Publishing Company, Inc. Westport, CN, 1982).

14. J. Defaye, A. Gadelle and C. Pedersen, Carbohydr. Res. 136 (1985) 53.

15. T. Kusch, W. Gosewinkel and G. Stoeck, U.S. Patent No. 3,513,023 (1970).

16. B.K. Dwivedi and S.K. Raniwala, U.S. Patent no. 4,199,373 (1980).

17. S.N. Black, R.H. Davey and M. Halcrow, J. Crystal Growth 79 (1986) 765.

18. R. Davey, W. Fila and J. Garside, J. Crystal Growth 79 (1986) 607.

SUMMARY AND CONCLUSIONS

The kinetics of difructose dianhydrides formation under industrial crystallization conditions and the effect of difructose dianhydrides on the crystal growth of anhydrous fructose from aqueous solution were investigated.

The kinetics of difructose dianhydrides formation were determined by HPLC analysis. Conclusions are:

1. Fructose underwent dehydration reactions and formed four difructose dianhydrides under industrial crystallization conditions.
2. The kinetics of difructose dianhydrides formation reaction was modelled employing a second-order irreversible rate equation. The extent of reaction increased with increasing temperature and decreasing pH value of the solution.
3. The amounts of two of the four difructose dianhydrides stopped increasing when the fructose concentration was about 70% or less. This is probably caused by the effect of solvation of fructose molecules.
4. The formation of all four dimers was retarded when the initial fructose concentration was higher than 80%. The rate of fructose mutarotation may be limited by diffusion in highly concentrated solution, thus affects the formation of difructose dianhydrides.

The kinetics of fructose crystal growth in the presence of difructose dianhydrides were studied using photomicroscopic contact nucleation techniques. Conclusions are:

1. The growth rate of fructose crystals in the presence of difructose dianhydrides was size-independent, with growth rate dispersion among different crystals.

2. The presence of difructose dianhydrides caused inhibition of fructose crystal growth.

3. Difructose dianhydrides appeared to be incorporated into the crystal, thus inhibiting subsquent surface integration of fructose molecules.

4. The effects of growth inhibition exerted by different difructose dianhydrides were similar, probably because they have the same structural characteristics of crystalline fructose molecules.

RECOMMENDATIONS

1. The diffusivity of fructose in aqueous solution under crystallization conditions should be measured to understand the transport of fructose molecules and the kinetics of both chemical reaction and crystallization.

2. The kinetics and equilibrium of fructose mutarotation should be investigated to determine the actual composition of solution under crystallization conditions.

3. The solvation of fructose molecules at different concentrations and its relationship to the formation of difructose dianhydrides should be studied.

4. The packing arrangement of fructose molecules in the crystal should be studied to understand the incorporation of difructose dianhydrides in the surface of the growing crystal.

5. Single crystal growth experiments should be performed to determine the growth rates of different crystallographic faces and the effect of difructose dianhydrides on the growth rates.

6. A mathematical model for crystal growth under industrial crystallization conditions should be developed employing the kinetics of crystal growth in the presence of difructose dianhydrides and the kinetics of the formation of difructose dianhydrides.

APPENDICES

APPENDIX A

METHODS AND MATERIALS

Kinetics of Difructose Dianhydrides Formation Reaction

1. Reaction

Experiments were conducted at 30, 40, 50 and 60 ^{o}C. Hydrochloric acid solutions of pH 2.65, 4.35 and 5.90 were first prepared. Fructose was added to these solutions to get the desired concentrations, which were 10% less than the solubility of fructose at the reaction temperature. Analytical grade reagents and distilled-deionized water were used.

250 g fructose solution was added to a 250 ml flask. The flasks were put into a water bath controlled by a Haake constant temperature circulator model E3. The solutions were agitated by a Curtin Matheson Scientific 244-793 magnetic stirrers for a period of two weeks.

About 2 g samples were taken on a daily basis. After weighting they were diluted immediately with water in a 50 ml volumetric flask and then refrigerated to minimize further reaction.

2. HPLC analysis

The sample solutions were filtered through a Millipore

Millex-HA disc filter of size 0.45μm and then analyzed on a Biorad Carbohydrate Analysis HPLC system. The system consists of a Model 1330 pump, a Model 1770 refractive index detetor and a Model 3392A integrator. The column was a 250x4.0 mm Bio-Sil Amino-5S. A 30x4.6 mm guard column which had the same packing material was installed in front of the analysis column. Samples of 5 μl were injected and eluted at room temperature with 70% (v/v) acetonitrile. The eluant was filtered and degassed with a Millipore All Glass Filter Apparatus using a 0.5 μm Fluoropore membrane.

The attenuation range of the detector was set at 16X ($16x4x10^{-6}$ RIU/FS). The peak controls of integrator were set as width = 0.16 min, area rejection = 0 area count (1 area count = 0.125 mV-sec) and threshold = 9 ($2^{9}x1.25x10^{-4}$ mV). The analysis was calibrated by external standard method. The standard solution was a mixture of fructose and sucrose with a concentration of 1.0 g/100 ml for each sugar. The response factor of sucrose was used to calibrate the amounts of difructose dianhydrides. The sample solutions were further diluted with water for the determination of residual fructose concentration if the initial analysis indicated that it was out of the range of column capacity.

The concentrations of dianhydrides were based on peak area. The concentrations of residual fructose was based on peak height. The concentration data were transformed from g/100 ml to wt% using the data of sampling weight and dilution ratio.

3. GC analysis

A 2 g of sample was diluted with water in a 50 ml flask. 0.5 ml of the resulting solution was lyophilized and then dissolved in 1 ml TRI-SIL 'Z' purchased from Pierce (trimethylsilylimidazole in dry pyridine, 1.5 meq/ml).

The GC/MS analysis was performed by the Mass Spectrometry Facility in Department of Biochemistry, Michigan State University. The spectra were taken by a HP 5985 GC/MS system which was equipped with electron multiplier detector with the ionization potential at 70 eV. The column was a 6 ft 3% OV-225 and the temperature program was 140 - 200 ^{o}C (3 ^{o}/min).

Effect of Difructose Dianhydrides on Fructose Crystal Growth

1. Supersaturated solution

Crystal growth experiments were conducted at 30, 40 and 50 ^{o}C. An 80% fructose solution was first stirred at 70 ^{o}C for 24 or 48 hr to generate difructose dianhydrides. A large excess of fructose was added to the resulting solution and stirred for at least 24 hr at different temperatures to provide the desired degree of supersaturation. The solutions were filtered through a nylon mesh of 15 μm and then kept at the previous temperature (used in dissolving fructose) until they were free of crystals when checked by a microscope with 200X magnification. The concentration of fructose and difructose dianhydrides were analyzed on the HPLC system described earlier in this section.

2. Contact nucleation

A parent crystal of size of approximately 2 mm was glued to the movable rod with epoxy. Solution was added to the cell and heated at a temperature of 10 ^{o}C higher than the experimental temperature for 30 min to dissolve any nuclei that may have been generated in the solution and to remove surface irregularities of the parent crystal. The solution was brought to the desired experimental temperature and the parent crystal was slid across the glass plate to generate contact nuclei. Photographs were taken at intervals and the experiment was stopped after the crystal sizes reached approximately 40 μm. The magnification of the microscope was 200X; a 50x2 μm scale was photographed and used to calibrate the analysis.

The raw data obtained from each experiment consisted of a series of slides, which were analyzed on a Joyce-Loebl Magiscan 2 image analyzer to determine the area of each crystal. The equivalent circular diameter was taken as the characteristic size and linear growth rate was evaluated by ordinary least square estimation.

APPENDIX B

ORIGINAL DATA

Kinetics of Difructose Dianhydrides Formation Reaction

Concentration (wt%) vs. Time (hr)
T: Temperature, IC: Initial concentration, F: Fructose

Table B1. T = 60 C, pH = 2.65, IC = 80.3%

Time	F	D1	D2	D3	D4
6	69.17	0.00	0.00	0.00	0.00
24	66.19	0.00	0.00	1.84	2.90
48	66.88	0.00	0.00	2.05	3.16
72	64.60	0.00	0.00	2.05	3.72
96	63.94	0.00	0.00	0.00	3.70
120	63.77	0.69	0.00	0.00	3.19
144	60.94	0.96	0.00	0.00	3.35
168	62.36	1.15	0.00	0.00	3.80
192	62.46	1.46	0.00	0.00	3.26
216	61.56	1.84	0.00	0.00	3.10
240	60.31	1.95	0.00	0.00	4.14
264	60.31	2.34	1.16	0.00	2.84
288	60.47	2.55	1.24	1.45	3.00
312	59.58	2.71	1.26	0.00	2.98

Table B2. T = 60 C, pH = 4.35, IC = 80.3%

Time	F	D1	D2	D3	D4
6	82.95	0.00	0.00	0.00	0.00
24	80.51	0.00	0.00	0.00	0.00
48	76.77	0.00	0.00	0.00	0.00
72	72.35	0.00	0.00	0.00	0.00
96	70.97	0.00	0.00	0.00	0.00
120	68.26	0.00	0.00	1.87	2.42
144	68.19	0.00	0.00	1.81	2.97
168	67.59	0.00	0.00	0.00	3.21
192	66.58	0.00	0.00	0.00	3.09
216	67.44	0.00	0.00	1.90	3.52
240	66.30	0.00	0.00	1.99	3.28
264	64.88	0.00	0.00	1.91	3.40
288	66.15	0.00	0.00	1.76	3.70
312	65.75	0.53	0.00	1.87	3.44

Table B3. T = 60 C, pH = 5.90, IC = 80.3%

Time	F	D1	D2	D3	D4
6	80.96	0.00	0.00	0.00	0.00
24	79.32	0.00	0.00	0.00	0.00
48	73.14	0.00	0.00	0.00	0.00
72	73.52	0.00	0.00	0.00	0.00
96	71.64	0.00	0.00	0.00	0.00
120	71.24	0.00	0.00	0.00	2.15
144	69.00	0.00	0.00	0.00	2.37
168	68.24	0.00	0.00	0.00	3.10
192	66.38	0.00	0.00	0.00	2.95
216	66.77	0.00	0.00	0.00	3.01
240	66.97	0.00	0.00	2.34	3.64
264	67.23	0.00	0.00	1.74	3.44
288	66.29	0.00	0.00	1.92	3.45
312	66.71	0.00	0.00	1.92	3.18

Table B4. T = 50, pH = 2.65, IC = 76.9%

Time	F	D1	D2	D3	D4
6	75.15	0.00	0.00	0.00	0.00
24	72.03	0.00	0.00	0.00	0.00
48	70.15	0.00	0.00	0.00	0.00
72	68.64	0.00	0.00	0.00	2.42
96	68.41	0.00	0.00	0.00	2.37
120	67.50	0.00	0.00	0.00	2.50
144	67.45	0.00	0.00	0.00	2.13
168	67.50	0.00	0.00	0.00	2.91
192	68.00	0.00	0.00	0.00	3.09
216	67.59	0.00	0.00	0.00	2.74
240	66.84	0.00	0.00	0.00	2.88
264	65.93	0.00	0.00	0.00	3.09
288	66.94	0.00	0.00	1.49	2.78
312	66.51	0.00	0.00	1.53	3.01
336	65.79	0.00	0.00	1.52	2.94

Table B5. T = 50 C, pH = 4.35, IC = 76.9%

Time	F	D1	D2	D3	D4
6	76.96	0.00	0.00	0.00	0.00
24	76.03	0.00	0.00	0.00	0.00
48	74.02	0.00	0.00	0.00	0.00
72	73.32	0.00	0.00	0.00	0.00
96	71.62	0.00	0.00	0.00	0.00
120	70.91	0.00	0.00	0.00	0.00
144	-	0.00	0.00	0.00	0.00
168	69.54	0.00	0.00	0.00	0.00
192	68.80	0.00	0.00	0.00	0.00
216	68.10	0.00	0.00	0.00	0.00
240	67.72	0.00	0.00	0.00	1.91
264	68.20	0.00	0.00	0.00	2.12
288	68.14	0.00	0.00	1.41	2.39
312	66.27	0.00	0.00	1.38	2.46
336	67.86	0.00	0.00	1.40	2.64

Table B6. T = 50 C, pH = 5.90, IC = 76.9%

Time	F	D1	D2	D3	D4
6	78.69	0.00	0.00	0.00	0.00
24	77.20	0.00	0.00	0.00	0.00
48	76.68	0.00	0.00	0.00	0.00
72	74.60	0.00	0.00	0.00	0.00
96	72.23	0.00	0.00	0.00	0.00
120	71.88	0.00	0.00	0.00	0.00
144	70.85	0.00	0.00	0.00	0.00
168	69.55	0.00	0.00	0.00	0.00
192	69.38	0.00	0.00	0.00	1.71
216	68.55	0.00	0.00	0.00	0.00
240	67.66	0.00	0.00	0.00	0.00
264	68.23	0.00	0.00	0.00	2.24
288	67.99	0.00	0.00	1.30	2.35
312	67.23	0.00	0.00	1.37	2.38
336	66.90	0.00	0.00	0.00	2.54

Table B7. T = 40 C, pH = 2.65, IC = 74.4%

Time	F	D1	D2	D3	D4
25	75.09	0.00	0.00	0.00	0.00
48	74.51	0.00	0.00	0.00	0.00
72	73.16	0.00	0.00	0.00	0.00
96	73.04	0.00	0.00	0.00	0.00
120	70.25	0.00	0.00	0.00	0.00
146	71.27	0.00	0.00	1.07	0.00
168	72.61	0.00	0.00	1.23	1.40
192	72.17	0.00	0.00	1.28	1.50
218	72.00	0.00	0.00	1.17	1.53
239	70.99	0.00	0.00	1.29	1.44
264	71.71	0.00	0.00	1.19	1.62
289	71.16	0.00	0.00	1.16	1.77
313	70.66	0.00	0.00	1.15	1.90
337	70.36	0.00	0.00	1.09	1.97

Table B8. T = 40 C, pH = 4.35, IC = 74.4%

Time	F	D1	D2	D3	D4
25	75.99	0.00	0.00	0.00	0.00
48	77.62	0.00	0.00	0.00	0.00
72	76.53	0.00	0.00	0.00	0.00
96	75.49	0.00	0.00	0.00	0.00
120	75.66	0.00	0.00	0.00	0.00
146	75.31	0.00	0.00	0.00	0.00
168	71.48	0.00	0.00	0.00	0.00
192	74.73	0.00	0.00	0.00	0.00
218	74.18	0.00	0.00	0.00	0.00
239	72.47	0.00	0.00	0.00	0.00
264	73.30	0.00	0.00	0.00	0.00
289	73.20	0.00	0.00	0.00	0.00
313	73.79	0.00	0.00	0.00	0.00
337	72.37	0.00	0.00	0.00	0.00

Table B9. T = 40 C, pH = 5.90, IC = 74.4%

Time	F	D1	D2	D3	D4
25	78.46	0.00	0.00	0.00	0.00
48	73.33	0.00	0.00	0.00	0.00
72	76.06	0.00	0.00	0.00	0.00
96	75.40	0.00	0.00	0.00	0.00
120	76.25	0.00	0.00	0.00	0.00
146	75.37	0.00	0.00	0.00	0.00
168	75.07	0.00	0.00	0.00	0.00
192	74.46	0.00	0.00	0.00	0.00
218	73.73	0.00	0.00	0.00	0.00
239	73.57	0.00	0.00	0.00	0.00
264	73.39	0.00	0.00	0.00	0.00
289	73.06	0.00	0.00	0.00	0.00
313	72.42	0.00	0.00	0.00	0.00
337	71.46	0.00	0.00	0.00	0.00

Table B10. T = 30 C, pH = 2.65, IC = 71.7%

Time	F	D1	D2	D3	D4
24	73.54	0.00	0.00	0.00	0.00
48	72.99	0.00	0.00	0.00	0.00
72	72.08	0.00	0.00	0.00	0.00
98	69.48	0.00	0.00	0.00	0.00
120	71.23	0.00	0.00	0.00	0.00
144	70.68	0.00	0.00	0.00	0.00
170	69.30	0.00	0.00	0.00	0.00
191	69.76	0.00	0.00	0.00	0.00
216	69.35	0.00	0.00	0.00	0.00
241	69.14	0.00	0.00	0.00	0.00
265	69.66	0.00	0.00	0.00	0.00
289	68.83	0.00	0.00	0.00	0.00
311	68.74	0.00	0.00	0.00	0.00
338	70.26	0.00	0.00	0.00	0.00

Table B11. T = 30 C, pH = 5.90, IC = 71.7%

Time	F	D1	D2	D3	D4
24	72.41	0.00	0.00	0.00	0.00
48	74.63	0.00	0.00	0.00	0.00
72	74.56	0.00	0.00	0.00	0.00
98	74.29	0.00	0.00	0.00	0.00
120	74.97	0.00	0.00	0.00	0.00
144	74.49	0.00	0.00	0.00	0.00
170	75.45	0.00	0.00	0.00	0.00
191	74.85	0.00	0.00	0.00	0.00
216	74.64	0.00	0.00	0.00	0.00
241	75.16	0.00	0.00	0.00	0.00
265	74.88	0.00	0.00	0.00	0.00
289	75.03	0.00	0.00	0.00	0.00
311	74.20	0.00	0.00	0.00	0.00
338	73.00	0.00	0.00	0.00	0.00

Table B12. T = 60 C, pH = 2.65, IC = 75%

Time	F	D1	D2	D3	D4
24	68.27	0.00	0.00	0.00	2.01
48	67.82	0.00	0.00	1.20	2.65
72	67.13	0.00	0.00	1.18	2.35
96	67.18	0.00	0.00	0.00	2.55
122	66.12	0.00	0.00	0.00	2.15
144	65.89	0.74	0.00	0.00	2.34
168	62.81	0.00	0.00	0.00	2.04
189	62.33	1.04	0.00	0.00	2.04
217	61.89	1.18	0.00	0.00	2.28
240	61.09	1.33	0.64	0.00	1.94
286	62.64	1.76	0.78	0.00	2.23
338	61.33	2.16	0.87	0.00	1.86

Table B13. T = 60 C, pH = 2.65, IC = 70.0%

Time	F	D1	D2	D3	D4
24	65.15	0.00	0.00	0.00	1.91
48	65.29	0.00	0.00	0.00	2.10
72	64.56	0.00	0.00	0.00	2.34
96	62.78	0.00	0.00	0.00	1.92
122	62.44	0.00	0.00	0.00	2.09
144	61.48	0.00	0.00	0.00	1.85
168	63.13	0.85	0.00	0.00	1.99
168	61.37	0.00	0.00	0.00	1.91
189	61.57	0.94	0.00	0.00	1.86
217	60.70	1.18	0.00	0.00	1.85
240	60.38	1.26	0.00	0.00	1.85
286	58.10	1.53	0.00	0.00	1.78
338	57.66	1.77	0.78	0.00	1.67

Table B14. T = 60 C, pH = 2.65, IC = 65.0%

Time	F	D1	D2	D3	D4
24	61.52	0.00	0.00	0.00	0.00
48	61.78	0.00	0.00	0.00	1.50
72	60.58	0.00	0.00	0.00	1.42
96	59.98	0.00	0.00	0.00	1.51
122	59.85	0.00	0.00	0.00	1.41
144	58.74	0.00	0.00	0.00	1.43
168	57.57	0.00	0.00	0.00	1.39
189	57.47	0.69	0.00	0.00	1.24
217	57.71	0.89	0.00	0.00	1.40
240	57.40	1.00	0.00	0.00	1.43
286	56.16	1.23	0.00	0.00	1.65
338	56.10	1.48	0.00	0.00	1.09

Table B15. T = 60 C, pH = 5.90, IC = 92.0%

Time	F	D1	D2	D3	D4
24	94.77	0.00	0.00	0.00	0.00
71	89.70	0.00	0.00	0.00	0.00
116	88.10	0.00	0.00	0.00	0.00
144	87.05	0.00	0.00	0.00	0.00
167	88.67	0.00	0.00	0.00	0.00
191	87.46	0.00	0.00	0.00	0.00
216	83.73	0.00	0.00	0.00	0.00
237	84.75	0.00	1.35	0.00	0.00
256	79.16	0.00	1.76	1.25	0.00
284	85.68	0.00	2.10	0.00	0.00
310	85.98	0.00	2.76	2.02	0.00
336	83.55	0.00	2.75	2.72	0.00

Table B16. T = 60 C, pH = 5.90, IC = 88.0%

Time	F	D1	D2	D3	D4
24	89.11	0.00	0.00	0.00	0.00
48	88.87	0.00	0.00	0.00	0.00
71	87.27	0.00	0.00	0.00	0.00
92	86.31	0.00	0.00	0.00	0.00
116	80.02	0.00	0.92	0.00	0.00
144	82.03	0.00	1.22	0.80	0.00
167	80.57	0.00	1.51	1.46	0.00
191	79.89	0.00	1.71	1.75	0.00
216	78.18	0.00	1.95	2.20	0.58
237	80.21	0.00	1.92	2.45	0.72
256	79.84	0.50	1.43	2.46	1.33
284	76.69	0.00	2.00	2.65	2.42
310	77.43	0.00	2.15	3.06	1.99
336	76.03	0.45	1.34	2.86	2.50

Table B17. T = 60 C, pH = 5.90, IC = 84.0%

Time	F	D1	D2	D3	D4
24	85.20	0.00	0.00	0.00	0.00
48	83.78	0.00	0.00	0.00	0.00
71	82.40	0.00	0.00	0.00	0.00
92	82.31	0.00	0.00	0.00	0.00
117	80.82	0.00	0.00	0.00	0.00
144	79.11	1.41	0.00	1.30	0.00
167	78.08	1.51	0.00	1.84	0.00
191	75.83	1.56	0.00	2.12	1.75
216	74.32	1.45	0.00	2.09	0.00
237	74.51	1.39	0.00	2.35	1.95
260	73.39	1.46	0.00	2.39	1.88
284	74.34	0.00	0.00	2.28	2.12
310	72.01	1.27	0.00	2.49	2.85
336	70.57	1.39	0.00	2.68	2.44

Effect of Difructose Dianhydrides on Fructose Crystal Growth

Crystal size (μm) vs. Time (hr)
(note: Time is listed in descending order in the following tables.)
T: Temperature, S: Supersaturation, D: Difructose dianhydrides

Table B18. T = 30 C, S = 0.053, D = 1.6%

No.	24.25	22.	20.	16.	12.	10.	8.	6.	4.hr
1	29.18	26.78	23.56	21.30	19.33	13.88	10.70	9.79	7.35
2	35.20	31.69	29.03	25.43	18.30	17.24	13.72	11.69	8.53
3	29.40	27.28	24.52	20.21	12.01	12.85	10.75	9.27	5.94
4	16.53	16.79	15.28	12.33	14.43	10.34	8.06	8.40	6.88
5	24.25	23.04	21.22	24.16	14.73	13.60	10.28	9.45	7.99
6	29.52	27.39	26.76	17.40	18.06	17.62	13.56	11.35	9.96
7	19.77	20.43	17.02	15.67	13.36	11.59	9.56	8.72	7.27
8	41.16	38.46	35.91	29.81	25.81	22.19	19.61	15.92	12.94
9	37.55	33.07	31.22	26.07	23.47	19.41	17.37	15.32	13.80
10	21.25	20.46	20.16	16.96	14.80	12.19	9.21	8.06	6.12
11	24.09	22.82	19.80	16.96	22.06	21.84	8.33	6.88	6.47
12	19.86	16.92	17.75	14.16	12.24	9.09	6.73	7.42	5.25
13	32.95	33.00	29.18	26.45	13.07	11.97	10.18	12.90	11.01
14	30.07	28.14	25.44	19.63	14.47	12.42	9.45	6.72	7.99
15	23.28	22.11	20.43	18.89	15.03	12.46	11.45	7.11	7.27
16	25.92	22.36	22.09	17.28	12.90	11.78	9.39	7.27	5.55
17	23.51	23.28	19.49	15.50	12.19	11.59	9.15	9.09	6.30
18	21.22	19.27	17.44	13.15	11.69	10.39	9.96	6.72	5.45
19	36.17	34.36	32.35	27.83	21.56	18.60	16.12	13.36	10.81
20	23.49	21.17	19.72	17.99	13.88	11.87	9.62	7.78	7.42
21	20.08	18.83	15.78	13.36	9.68	9.45	7.12	6.03	4.06

Table B19. T = 30 C, S = 0.055, D = 1.2%

No.	14.	12.	10.	8.	6.
22	22.32	22.07	20.14	18.44	15.91
23	27.89	23.53	20.07	17.09	12.53
24	27.88	25.01	20.71	17.88	14.67
25	26.15	22.65	19.65	15.97	13.49
26	34.63	32.03	26.22	22.87	18.76
27	27.84	27.18	26.74	22.19	19.03
28	34.23	25.40	22.07	17.30	13.80
29	37.32	32.80	28.29	22.48	19.25
30	22.42	21.41	17.65	16.40	13.21
31	25.52	20.07	16.17	11.85	7.06
32	19.34	18.36	15.05	14.13	11.65
33	23.95	22.15	17.72	15.59	11.69
34	33.05	27.68	22.71	17.41	14.67
35	18.93	16.87	16.31	12.57	9.56
36	38.22	33.79	29.88	24.96	19.79
37	33.35	30.31	25.40	20.55	15.68
38	18.74	17.06	15.44	11.29	9.56
39	16.23	15.79	13.76	10.22	8.04
40	28.60	25.81	24.03	19.63	15.05

Table B20. T = 30 C, S = 0.068, D = 1.2%

No.	10.	9.	8.	7.	6.	5.	4.	3.	2.
41	29.14	26.69	23.96	22.66	18.39	16.46	13.28	10.01	8.06
42	35.97	34.33	29.92	27.93	26.82	23.96	19.69	16.46	12.33
43	36.87	35.73	32.89	31.17	30.16	26.32	22.06	20.43	17.69
44	39.83	37.90	36.10	36.17	34.22	31.03	27.25	25.30	20.46
45	35.42	33.50	30.14	26.90	25.19	21.17	16.56	13.64	9.21
46	28.49	25.43	23.06	20.57	19.24	15.99	15.74	10.91	8.40
47	35.88	33.37	28.20	26.84	22.36	18.33	13.96	9.39	6.12
48	34.17	32.30	29.37	25.92	24.55	19.27	17.99	12.46	9.96
49	26.34	21.99	21.30	19.66	16.22	15.32	13.28	9.50	7.04
50	20.70	19.27	17.81	17.44	15.78	15.06	11.64	9.73	6.72
51	31.71	30.65	27.96	25.54	21.99	18.95	15.88	13.80	10.23
52	31.20	31.66	28.82	26.82	22.85	20.35	18.27	15.53	12.51
53	15.20	13.56	11.25	10.81	10.34	10.35	9.50	6.80	5.55

Table B21. T = 40 C, S = 0.039, D = 3.0%

No.	40.	36.	32.	28.	24.	21.	17.	13.	9.	7.
54	27.05	26.15	25.50	22.74	21.19	22.50	18.43	15.80	12.84	11.85
55	26.36	24.23	22.16	20.69	18.86	16.33	12.97	9.66	10.21	8.15
56	17.33	16.04	13.60	14.67	14.50	13.73	14.27	10.77	9.62	7.89
57	36.58	33.19	30.99	29.36	27.40	26.42	22.83	21.29	18.66	16.81
58	22.52	22.46	19.10	16.43	17.18	14.35	12.27	10.62	10.58	9.08
59	19.63	20.59	18.16	16.09	15.26	14.73	9.88	10.09	7.62	6.68
60	20.07	18.30	16.71	13.23	13.60	13.48	13.16	10.25	7.28	6.92
61	17.88	15.35	13.29	12.77	11.49	12.10	10.09	8.15	6.92	6.92
62	21.51	17.95	17.69	14.41	11.82	12.54	11.00	9.40	7.34	7.39
63	26.99	26.32	23.91	21.33	19.06	13.54	11.12	10.21	9.66	8.99
64	22.65	22.24	20.77	17.78	17.40	17.85	16.35	13.66	10.58	8.84
65	27.63	23.33	22.93	20.53	19.73	19.02	16.83	14.70	11.53	9.31
66	20.06	20.07	17.54	16.02	14.24	15.48	13.63	11.85	8.70	6.42

Table B22. T = 40 C, S = 0.048, D = 3.3%

No.	12.	10.	8.25	6.	5.	4.	3.
67	28.03	23.90	20.87	16.35	13.51	11.99	8.50
68	27.02	24.33	19.43	13.42	11.96	10.77	7.94
69	34.40	25.91	21.09	16.86	13.00	10.54	8.30
70	26.88	21.60	17.78	14.38	12.20	9.57	7.22
71	29.42	23.45	19.60	13.19	11.31	9.17	7.22
72	29.73	27.33	20.40	14.98	13.45	11.38	7.78
73	28.15	25.61	23.74	16.68	12.41	10.85	9.12
74	20.61	17.25	14.50	11.64	8.45	6.86	7.62
75	24.47	22.91	20.30	15.91	13.10	7.78	7.99
76	21.45	20.09	16.63	12.57	9.96	7.99	7.39

Table B23. T = 40 C, S = 0.041, D = 3.2%

No.	14.	12.	10.	8.	6.	4.	2.
77	31.21	26.88	22.16	19.69	14.76	11.56	6.74
78	23.49	18.23	14.79	12.57	10.17	8.20	6.35
79	23.95	23.74	19.41	17.83	12.84	9.31	7.10
80	26.91	22.52	19.98	17.33	12.84	10.21	6.42
81	31.25	27.54	21.21	20.34	15.59	10.97	8.55
82	18.99	14.67	13.54	12.71	12.24	8.30	6.98
83	22.93	19.96	17.28	13.26	11.11	8.30	5.35
84	30.51	24.40	21.56	17.40	13.16	9.49	7.56
85	15.64	14.64	11.45	10.54	9.62	8.10	7.34
86	19.43	20.13	17.35	15.01	10.54	8.05	8.15
87	27.94	26.85	20.59	17.90	14.24	11.00	8.75
88	26.80	24.98	22.89	19.56	15.37	11.16	-
89	29.16	23.90	19.37	19.58	16.91	12.24	-
90	26.13	25.35	22.31	14.93	12.61	7.67	-

Table B24. T = 40 C, S = .021, D = 4.5%

No.	48.	42.	34.5	30.	23.83	20.5
91	20.66	19.77	17.28	14.45	12.38	11.04
92	21.56	18.76	17.28	16.51	12.86	7.87
93	16.54	15.79	13.28	11.41	8.91	8.76
94	25.07	22.52	20.57	18.19	16.37	14.98
95	18.11	16.11	12.50	11.41	9.42	7.81
96	21.02	19.08	16.68	11.97	11.89	11.93
97	19.37	17.62	14.39	13.76	11.21	11.17
98	10.12	10.66	9.37	7.45	6.86	5.85
99	9.99	10.17	10.04	7.87	6.08	4.90
100	11.97	11.13	9.90	8.65	7.26	7.75
101	10.44	7.98	6.93	6.59	6.93	5.60
102	7.69	7.06	8.16	8.27	6.86	6.30

Table B25. T = 40 C, S = 0.045, D = 4.6%

No.	48.	44.	40.	36.	32.	28.	24.	20.	16.	12.	8.
103	34.53	34.76	31.18	30.19	27.82	24.59	22.29	18.73	16.56	13.91	11.31
104	43.63	39.65	36.61	32.71	30.18	27.10	21.35	20.18	17.35	13.32	11.31
105	23.25	20.55	19.02	17.23	16.22	14.70	14.15	12.03	9.71	10.29	7.04
106	24.85	21.97	21.17	18.93	18.73	14.09	11.60	11.04	9.17	7.73	5.27
107	25.78	23.04	20.93	19.94	19.21	15.40	15.04	12.34	11.38	9.79	5.19
108	31.02	28.07	25.95	24.93	23.61	19.97	19.37	14.79	12.64	6.74	5.94
109	22.65	21.53	21.01	19.65	17.47	15.53	12.67	11.64	10.97	8.30	8.05
110	36.60	34.51	30.14	28.25	25.95	22.89	21.86	19.13	16.17	14.09	10.05
111	31.06	28.50	27.00	27.53	25.35	22.10	18.27	17.45	14.50	7.04	4.76
112	23.47	22.35	21.47	18.95	18.02	15.45	13.32	13.48	11.78	9.31	6.61
113	19.15	18.36	15.04	15.26	14.56	15.32	10.66	10.66	11.12	10.81	8.94
114	28.56	27.62	25.36	24.16	21.68	19.48	17.18	14.82	11.92	11.67	8.30

Table B26. T = 40 C, S = 0.045, D = 5.5%

No.	13.67	12.	10.	8.	6.	4.
115	18.64	16.68	13.49	12.90	8.32	9.56
116	13.66	11.69	10.35	9.71	10.83	8.04
117	23.86	22.38	19.27	17.49	17.14	13.86
118	10.13	10.49	7.13	8.65	6.45	4.99
119	13.39	10.88	9.37	9.12	6.93	5.93
120	11.25	9.22	7.06	7.51	5.85	5.60
121	10.04	7.69	8.16	6.80	5.52	4.30
122	7.51	6.30	6.52	3.96	5.44	4.40
123	12.57	11.86	8.04	8.32	7.45	6.30
124	13.21	11.29	7.32	9.47	7.81	6.00
125	12.57	10.83	7.81	9.17	6.80	6.52
126	12.35	10.31	7.63	8.81	6.73	7.45
127	11.53	8.60	8.10	7.69	7.45	5.44
128	13.83	11.57	9.66	8.86	7.26	6.45
129	12.53	10.49	9.42	7.81	6.23	5.69
130	12.72	11.00	9.56	8.86	8.04	4.99
131	12.72	12.46	9.07	8.32	7.69	7.63
132	11.13	9.12	10.40	8.38	6.73	6.00
133	13.90	12.79	11.85	9.27	8.65	6.59
134	12.27	10.66	10.79	8.10	8.32	6.23
135	12.04	11.25	10.22	9.52	7.00	6.59
136	13.56	11.04	11.85	8.43	9.02	6.08
137	13.11	10.62	10.26	8.21	10.79	8.27
138	13.28	12.68	11.93	9.80	6.93	6.08
139	12.97	11.25	9.12	8.86	7.06	5.26

Table B27. T = 50 C, S = 0.028, D = 4.4%

No.	21.83	20.16	15.67	14.	12.	10.	8.	6.	4.	2.
140	12.01	12.28	11.01	10.70	8.78	9.09	9.56	7.49	7.27	6.47
141	13.11	12.55	12.24	11.72	10.44	10.34	9.62	7.99	9.33	5.84
142	14.20	14.66	10.70	10.96	11.30	7.85	7.85	7.49	10.28	5.55
143	25.94	24.61	20.30	20.08	18.42	16.46	12.90	13.02	10.23	7.99
144	24.18	23.14	20.30	19.47	18.18	15.28	13.60	12.77	9.79	8.33
145	24.61	23.11	21.30	19.41	18.83	15.28	13.60	10.70	9.73	7.71
146	26.11	24.16	22.56	21.58	18.42	16.63	15.88	13.32	11.35	7.99
147	27.49	28.45	18.33	18.39	15.50	15.02	12.64	11.45	10.34	6.96
148	22.24	22.92	16.86	17.18	16.79	13.76	13.36	10.07	7.78	8.33
149	23.93	22.85	21.20	16.02	14.73	13.48	15.88	10.07	7.04	8.97
150	17.15	17.96	17.24	21.69	19.61	17.96	11.78	11.40	9.67	5.65
151	16.79	16.76	14.08	11.97	12.64	10.34	10.34	8.13	7.78	7.12
152	22.68	21.81	18.39	17.50	15.46	13.96	11.59	11.21	7.04	5.94
153	12.64	12.33	12.01	10.75	8.78	6.55	7.35	7.49	6.96	5.65

Table B28. T = 50 C, S = 0.049, D = 4.4%

No.	21.83	19.83	18.	16.	14.16	13.	12.	11.	10.	9.	8.
154	31.50	29.33	28.14	24.48	24.84	21.84	21.20	19.61	19.01	18.27	14.50
155	30.94	26.69	25.86	24.09	19.63	19.88	18.42	16.63	14.47	13.88	12.06
156	40.78	37.78	36.69	31.68	30.00	27.61	26.65	25.17	22.24	21.46	14.31
157	33.27	29.20	27.73	26.55	23.06	23.30	21.20	19.06	18.36	17.18	18.89
158	23.61	24.09	21.71	20.16	18.74	17.40	17.47	17.18	15.67	14.39	11.30
159	33.25	32.92	29.18	25.92	22.85	21.76	21.53	20.91	18.80	18.42	15.78
160	27.41	25.79	24.09	21.71	20.62	20.05	17.15	16.96	15.39	15.57	12.28
161	15.53	16.56	15.35	13.15	11.69	10.07	8.59	8.91	9.27	9.39	7.71
162	19.01	16.63	17.87	16.09	13.69	12.64	11.97	11.78	9.68	9.69	9.15
163	27.47	25.19	24.75	20.08	21.28	18.89	17.81	16.82	14.77	15.95	13.48
164	24.55	22.80	23.04	20.51	18.77	17.78	17.28	15.17	15.10	13.11	11.64
165	23.70	22.04	21.74	18.15	17.02	15.57	15.21	13.72	13.96	10.55	8.59