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Full Report of the Research Project

Alcoholic Crystallization of High Fructose Syrup

By Assistant Professor Dr. Adrian Flood

Suranaree University of Technology

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Abstract

Project Code: RSA/17/2540

Project Title: Alcoholic Crystallization of High Fructose Syrup

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Project Period: 2540 – 2543

The properties of high fructose syrups in aqueous ethanolic solutions, and the crystallization of the two solutes present in high fructose syrups, fructose and glucose, was studied to determine if it is possible to separate these solutes without the need for chromatographic separation. Physical and thermodynamic properties of both mixtures of high fructose syrups and ethanol, and mixtures of fructose, glucose, ethanol, and water (an essentially identical solution, but without the problem of microbial storage damaging the experiments) were determined. The project also investigated the crystal growth of both fructose and glucose in experimental and theoretical studies. It was found that the yields of both fructose and glucose were very good in aqueous ethanolic solutions, much higher than could be achieved by cooling crystallization from aqueous solutions. It was also found that the two solutes could be seaparated by a salting out crystallization by ethanol, under the condition that one solute was seeded (usually glucose) and one solute was allowed to nucleate. This allows the solutes to be separated via size separation with sieves. The principal problem with the method is the very low growth rates of the sugars, of the order of 0.1 micrometer/minute, which is much lower than comparable growth rates in aqueous solutions.

A number of theoretical studies into the effect of mutarotation on crystallization, and on the actual thermodynamic driving force for the ethanolic crystallization were also performed.

Keywords: salting out crystallization, high fructose syrups, physical and thermodynamic properties

Contents

1. Introduction

High Fructose Syrups (HFS) are commercial sweeteners produced worldwide through enzymatic conversion of starch. The type of starch used in the process depends on the agricultural output of the region in which the syrup is produced. HFS is produced primarily from tapioca starch in Thailand, while in North America it is produced mainly from corn starch. Essentially any type of starch can be used to produce similar HFS products. The conversion process first reduces starch to (mainly) glucose , and then partially converts glucose to fructose, the latter reaction being limited to around fifty percent by an equilibrium conversion. The formation of fructose in the syrup is particularly advantageous, as fructose is significantly sweeter than sucrose, while glucose is less sweet than sucrose. HFS is used in the soft-drink, canning, packaged food, and baking industries as a sweetener. Solid fructose is produced either in the amorphous form, or through crystallization of high purity fructose syrups (that have had the glucose removed through chromatography). Crystalline fructose is sweeter than either crystalline sucrose (common table sugar), and any high fructose syrup. Crystalline fructose is currently prepared for low calorie and dietic products, and a range of specialty products, at prices that make it competitive with sucrose.

The production and use of HFS (and to a lesser extent, crystalline fructose) is increasing rapidly, particularly in North America and Europe. This production has limited the growth of sugar production since the first HFS products in the 1980's, and the overall trend for sugar market growth has been poor over this time. This situation is illustrated in figure 1.1, showing HFS and sugar consumption in the United States over recent years.

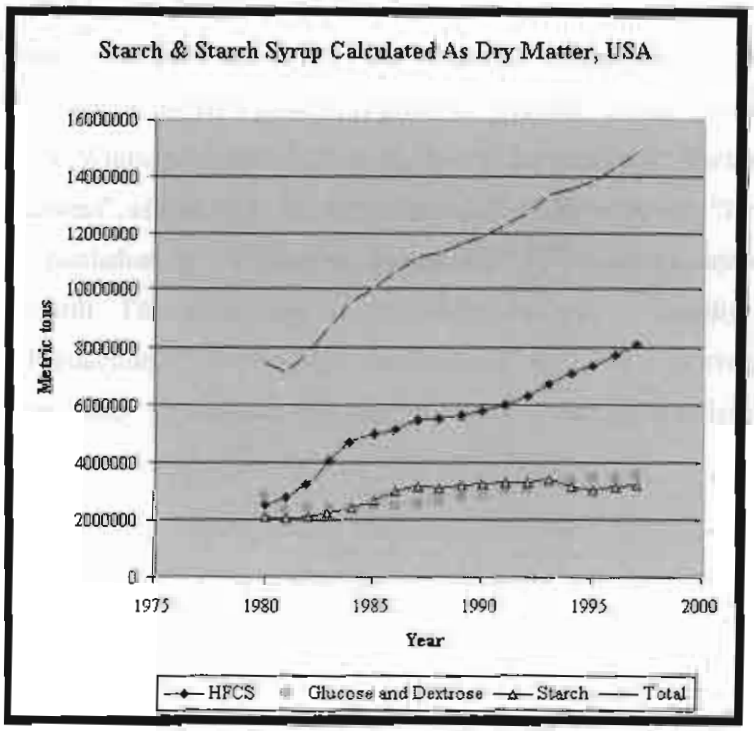

Figure 1.1 Sugar and HFS consumption in the United States, 1990 – 2002. (Source; United States Department of Agriculture).

The composition of the basic high fructose syrup product (HFS-42) is approximately 53 percent glucose, 42 percent fructose on a dry weight basis, with the residual solids being higher molecular weight carbohydrates. The syrup is 71 weight percent solids, but due to the high solubility of glucose and fructose it is stable to crystallization. Syrups of higher fructose concentration (50, 80, 90, and 95 percent fructose on a dry weight basis) are made by chromatographic separation and blending, but these products are more expensive than HFS-42 due to the difficulty and expense of the chromatographic step. The current Thai producer of high fructose syrups imports the required chromatographic resin from France in order to achieve the separation, and this is a considerable cost to the factory. The flowsheet for HFS manufacture (similar to what is being used in Thailand) is presented in figure 1.2. If crystalline fructose is to be produced, the HFS-90 is used as a feedstock for aqueous or aqueous-ethanolic crystallization.

The difficulty in modeling the crystallization of any reducing sugar (including fructose and glucose) is that the sugar occurs in many forms in solution (anomers or tautomers), but that only one of these forms is stable as a crystal phase under a given set of conditions. The crystallization process is therefore a two-step process; the first step is the crystallization of the stable form, and the second is the replacement of this form via a reversible equilibrium reaction with the other forms in solution (Flood et al., 1996b). The reaction which replenishes the crystallizing form is known as the mutarotation reaction, because the reaction causes variations in the optical rotation of the solution. This reaction – crystallization scheme requires not only knowledge of crystal growth rates and equilibrium, but also mutarotation reaction rates and equilibrium.

Several book chapters have been devoted to the industrial production of HFS and crystalline fructose. Excellent discussion on the HFS process is given in "Fructose Syrup: Production, Properties, and Applications" by J. S. White, published in "Starch Hydrolysis Products: Worldwide Technology, Production, and Applications", edited by F. W. Schenck and R. E. Hebeda, and "High Fructose Corn Syrup" by J. E. Long, published in "Alternative Sweeteners" (2nd Edition), edited by L. O'Brien Nabors and R. C. Gelardi. The production of crystalline fructose is equally well covered in "Crystalline Fructose: Production, Properties, and Applications" by L. M. Hanover, published in the Schenck and Hebeda text, and "Crystalline Fructose" by T. F. Osberger, published in the O'Brien Nabor and Gelardi text.

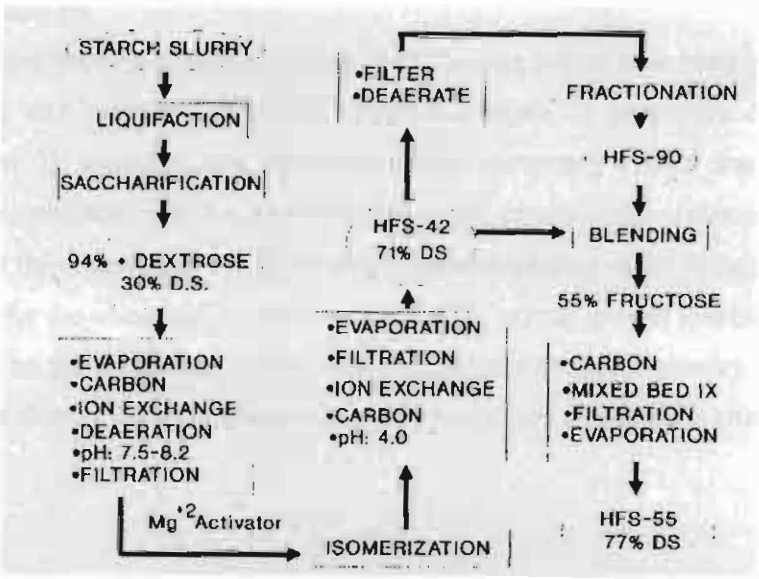

Figure 1.2 The flowsheet for manufacture of high fructose syrups. (From J. S. White, 1992).

There is significant potential to improve the productivity and economics of the process in two ways. The first is to produce crystalline fructose, rather than HFS, since this is a significantly higher value commodity. The second is to replace the chromatographic separation of fructose and glucose with separation via crystallization, which would also achieve the first objective, and also produce crystalline glucose as a by-product. The objective of the current study is to evaluate the possibilities of separating the two sugars via crystallization, and to determine rates, equilibrium, and yield of this process.

The system under study is interesting from the view of crystallization for several reasons. It may be seen as a two solute system (difficult for crystallization systems already), however both fructose and glucose appear in solution in the guise of several forms, fructose as three anomers (α-D-fructofuranose, β-D-fructofuranose, and β-D-fructopyranose), and glucose as two anomers (α-D-glucopyranose andβ-D-glucopyranose). The two glucose anomers, and the three fructose tautomers interchange via the mutarotation reaction, and when one component form is extracted from solution through crystallization, some of the other forms of the same sugar will convert to this form to maintain the mutarotational equilibrium. The mutarotation reaction is thus crucial for maintaining the crystallization rate, and may be rate controlling for either fructose crystallization, or for glucose crystallization. The β-D-fructopyranose form is the only form of fructose that can crystallize (as an anhydrous crystal), and (for the range of temperatures in the present study) α-D-glucopyranose is the only form of glucose that can crystallize, although this may crystallize as either an anhydrous crystal or a monohydrate, depending on ethanol content in the solvent, and temperature. The system is also interesting as an example as a drowning out crystallization (using ethanol), and because of the very

high solubilities in the system (particularly at low ethanol contents) that make the crystallization particularly challenging.

Thus to achieve the objective of the study, the following points have been studied (and discussed in the following text; review of pertinent literature (chapter 2), properties of HFS syrup-ethanol mixtures (chapter 3), solubility and properties of the quaternary system fructose-glucose-ethanol-water, which is representative of the mixtures undergoing crystallization (chapter 4), potential crystal product yields in the system (chapter 5), thermodynamic modeling of the system (chapter 6), kinetics and equilibrium for the mutarotation reaction (chapter 7), crystal growth kinetics (chapter 8), and the effect of activity on the crystal growth rate equation (chapter 9). Some primary conclusions are made from the study in chapter 10, and outcomes from the project are discussed in chapter 11.

2. Literature Review

2.1 Background of High Fructose Syrup and Crystalline Fructose Production

The concept of producing syrups having a high proportion of fructose has been considered for more than a century. Early efforts attempted to produce these syrups though conversion of sucrose (either by enzyme of acid hydrolysis) into a mixture of glucose and fructose, or by the hydrolysis of inulin. These processes did not produce competitively priced products, the former because the main competitor (sucrose) was used as a feedstock, and the latter due to the cost of the feedstock (usually Jerusalem artichokes) and the recovery.

The major advance that led to the commercial viability of HFS was the development of immobilized glucose (or in some cases xylose) isomerases, which enabled a significant fraction of the glucose in glucose syrups to be converted to fructose. The first commerical processes were in the United States, and commenced operation in the late 1960s. The process involved conversion of corn starch to glucose syrup using the enzymes α-amylase and glucoamylase, and then partial isomerization to fructose using imobilized glucose isomerase or xylose isomerase, using magnesium as an activator. The isomerization is an equilibrium reaction, with an equilibrium at approximately fifty percent conversion; the time required for the equilibrium conversion was found to be unacceptably high, so the reaction was stopped at approximately 42 – 45 percent conversion, thus producing HFS-42. The system is illustrated in the first part of figure 1.2.

The second major development of the HFS process was the development of chromatographic fractionation techniques to separate glucose and fructose. HFS-42 is slightly less sweet than crystalline sucrose, and was therefore unable to compete in some applications where cost was not the main criteria. The fractionation of fructose and glucose can be achieved using the calcium form of a cation-exchange resin in a moving bed chromatography system. Chromatography allowed the production of relatively high purity fructose syrups (equal to, or greater than ninety percent), which could be blended with HFS-42 to produce fifty-five percent fructose syrups (HFS-55), which were comparable to sucrose in sweetness. The chromatographic separation is reasonably expensive, but only a small amount of high purity syrup is required to blend with HFS-42 to produce HFS-55; thus HFS-55 could be produced at a cost lower than that of sucrose. Only very small amounts of high purity syrups were sold due to their high cost; the main objective was to produce them for blending. This process was first used in the United States in 1982, and allowed HFS to dominate the very important soft drink market in the United States. Properties of the major types of high fructose syrups are given in the following table from White (1992).

Table 2.1 Standard physical and chemical properties of high fructose syrups. Values are taken from technical data sheets supplied with the product.

	HFS Product Type (Number refers to approximate percent fructose)				
Property	42	55	80	90	95
Composition of sugars (%)					
Glucose (Dextrose)	53	41	18	9	4
Fructose	42	55	80	90	95
Larger sugars	5	4	2	1	1
Solids (%)	71	77	77	77	77
Moisture (%)	29	23	23	23	23
pH	4	4	3.5	3.5	3.5
Ash (%)	0.03	0.03	0.03	0.03	0.03
Colour, RBU max	25	25	35	35	35
Sweetness Relative to sucrose	92	99		106	
Density g/mL at 20 °C	1.346	1.384	1.384	1.407	1.385
Viscosity cP At 27 °C	160	800	600		575

The 42 % fructose syrups have a lower solids content than the higher syrups because glucose is much less soluble than fructose, and this syrup would crystallize at 77 weight percent solids. Fructose is much sweeter than sucrose, which is itself more sweet than glucose; this means that as the fructose content increases the sweetness of the syrup increases also. Glucose solutions are more viscous than fructose solutions, so the trend is for the viscosity to decrease as the amount of fructose increases. The viscosity of the 42 % syrup is much lower than the others, however, because it has a lower solids (sugars) content.

2.2 Overview of Industrial Crystallization from High Fructose Syrups

Fructose in the crystalline form is the sweetest naturally occurring sugar. The crystalline form is significantly sweeter than even the purest high fructose syrups, because the crystal form (β-D-fructopyranose) is sweeter than the two other forms that occur with it in the liquid phase (β-D-fructofuranose and α-D-fructofuranose). A second benefit of crystalline fructose is that it can compete with sucrose in applications that require a free flowing, solid product, in particular in domestic applications. The major limitations to the production of crystalline fructose are the high cost of the

feed stream (very high purity high fructose syrups produced from the fractionation process), and the difficulty of producing crystal product from the high solubility, high viscosity solution. Even so, by the 1990 crystalline fructose could be produced for around $0.36/lb (Osberger, 1991). This price is more expensive than sucrose, but is still competitive; the higher sweetness of crystalline fructose means that only around half the amount of fructose is required to produce the same taste response as sucrose.

The first time that crystalline fructose was produced on a large scale was in the late 1960s. Several European countries produced fructose – glucose mixtures from the inversion of sucrose. These mixtures were then separated by ion-exclusion, and the purified fructose stream was then crystallized from alcoholic solutions (Germany and France) or aqueous solutions (Finland). The difficulty of the separation and crystallization processes meant that the process required one week to produce a batch. The product was used in specialty applications, but could not compete with sucrose; it was necessarily more expensive since it used sucrose as a feed stock, and the processing was very difficult.

The first industrial production of crystalline fructose from high fructose syrups was at an American Xyrofin plant in 1981. The process used a crystallization process similar to that used by the Finns, but used high fructose as a feedstock. Processing required approximately five days (Osberger, 1991). Large scale manufacture of crystalline fructose was also began by very large corn starch / sweetener companies such as A. E. Staley and Archer Daniel Midlands. Typical physical properties of commercial crystalline fructose have been compiled from technical data sheets by Osberger, and are shown in Table 2.2.

Table 2.2 Physical Properties of commercial crystalline fructose (Osberger, 1991).

Appearance	White crystalline powder forming anhydrous needle-shaped crystals
Empirical formula	$C_6H_{12}O_6$
Molecular weight	180.16
Melting point	102 – 105 °C
Density	1.60 g/mL
Bulk density	0.8 g/mL
Caloric value	3.7 cal/g

Loss on drying (at 70 °C for 4 hr in a vacuum)	Less than 0.2 %
Residue on ignition	Less than 0.5 %
Heavy metals	Less than 5 ppm
Arsenic	Less than 1 ppm
Chloride	Less than 0.018 %
Sulfate	Less than 0.025 %
Calcium and Magnesium	Less than 0.005 %
Hydroxymethylfurfural	Less than 50 ppm
Glucose	Less than 0.1 %
Assay (dry basis)	98.0 – 102.0 %
pH in aqueous solution (0.1 g/mL)	5.0 – 7.0

The crystallization of fructose is extremely difficult even when the feed stream is relatively pure. This is due largely to the very high solubility of fructose, and to the extreme viscosities of supersaturated aqueous solutions of fructose. Early research focused on ethanolic crystallization of very pure fructose syrups (Bates, 1942), as the solubility of fructose in ethanol is relatively low (around 6.5 g fructose/100 g ethanol compared to a solubility in aqueous solutions of more than 400 g fructose/100 g of water). The reduced solubility also has the effect of greatly reducing the viscosity of the supersaturated solutions, and thus greatly simplifying the process mixing. (The reduction in viscosity can be around three orders of magnitude according to the study of Flood et al., 1996a). There is also some problems with the ethanolic process, partly due to formation of impurities, and partly due to the requirement to recover the (rather expensive) ethanol solvent in order to make the process economically viable. Currently both the aqueous and ethanolic processes are being used in various plants.

A typical crystallization process may use a concentrated, purified fructose syrup at 60 – 80 °C and moderate pH, which is mixed with ethanol in a ratio between 1:3 to 3:1 parts by weight. Decomposition of fructose is lower at lower temperatures, so the optimum is probably closer to 65 °C. This mixture is seeded with anhydrous crystalline fructose, possibly a fraction of the product from a previous batch. Supersaturation can be achieved through three processes, (a) cooling of the feed to around 25 °C, (b) concentrating the mixture by evaporation of water at a reduced temperature, (c) using both a reduction in temperature, and a low pressure. Crystallization may be done in batches, or in continuous systems, but for batch systems the batch time varies between around 10 hours and 180

hours, depending on the specifics of the process. A series of crystallization steps may be required to achieve the specified product purity, with downstream crystallization units tending to produce a better product.

After the final crystallization unit, the product must be centrifuged to separate the crystals from the mother liquor (a very difficult operation when the viscosity is extremely high), and then dried, conditioned, screened (for size classification), and packaged. An example crystallization (from an Archer Daniels Midland Patent) is shown in figure 2.1.

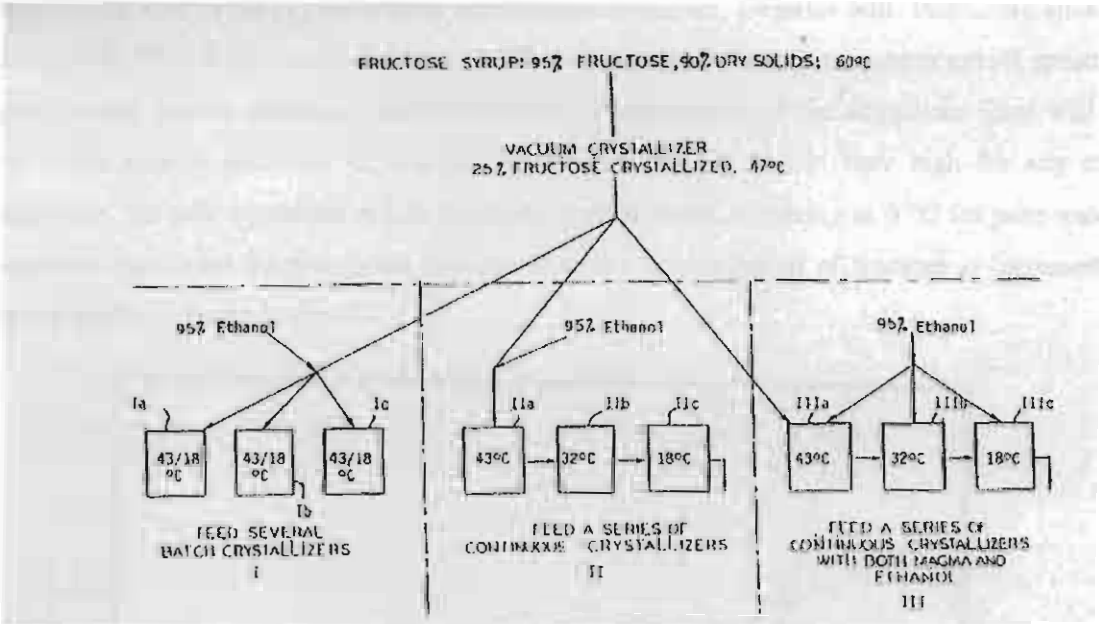

Figure 2.1 Ethanolic crystallization of fructose from high concentration, high purity HFS. (Archer Daniels Midland).

2.3 Solubility of Fructose and Glucose in Pure Solutions

The solubility of the crystallizing components is of critical importance in crystallization operations. It will determine the minimum concentration of solute possible in the crystallization unit (thereby determining viscosity and other processing parameters), the solute which will crystallize out first (in multiple solute systems), and the maximum yield of the process in cases where solvent evaporation cannot be performed.

The solubility of pure aqueous solutions of fructose, and pure aqueous solutions of glucose has been studied extensively, including complete phase diagrams. The solubility of fructose in ethanol – water solvents, and glucose in ethanol – water solvents have been studied by a very limited number of researchers, but some useful information has been obtained, while solubility in the system fructose – glucose – water has only been determined at one temperature (30 °C) in a relatively old study (Kelly, 1954). Solubility in the four-component system fructose – glucose – ethanol – water (representative

of the main components found in aqueous ethanolic crystallization of high fructose syrups) has not been studied until this work.

The phase diagram of aqueous solutions of fructose was studied by Young et al., in 1952. No significant further work has been published since this date, despite some uncertainties in this diagram. At high fructose concentrations, anhydrous D-fructose will crystallize at temperatures above 21 °C, and as the temperature decreases below this value, D-fructose dihydrate, and then D-fructose hemihydrate will form. It is usual in crystallization that anhydrous crystal forms occur at high temperatures, and as the crystallization temperature decreases, hydrates with increasing amounts of water occur. The most crucial information on the diagram is that a concentration of greater than eighty weight percent fructose is required before crystallization of the anhydrous form will occur. This is the highest solubility of any of the common sugars, and is very high for any class of compounds. Ice will crystallize at low fructose concentrations, occurring at 0 °C for pure water, and displaying significant freezing point depression as the concentration of fructose is increased. The phase diagram is shown in figure 2.2.

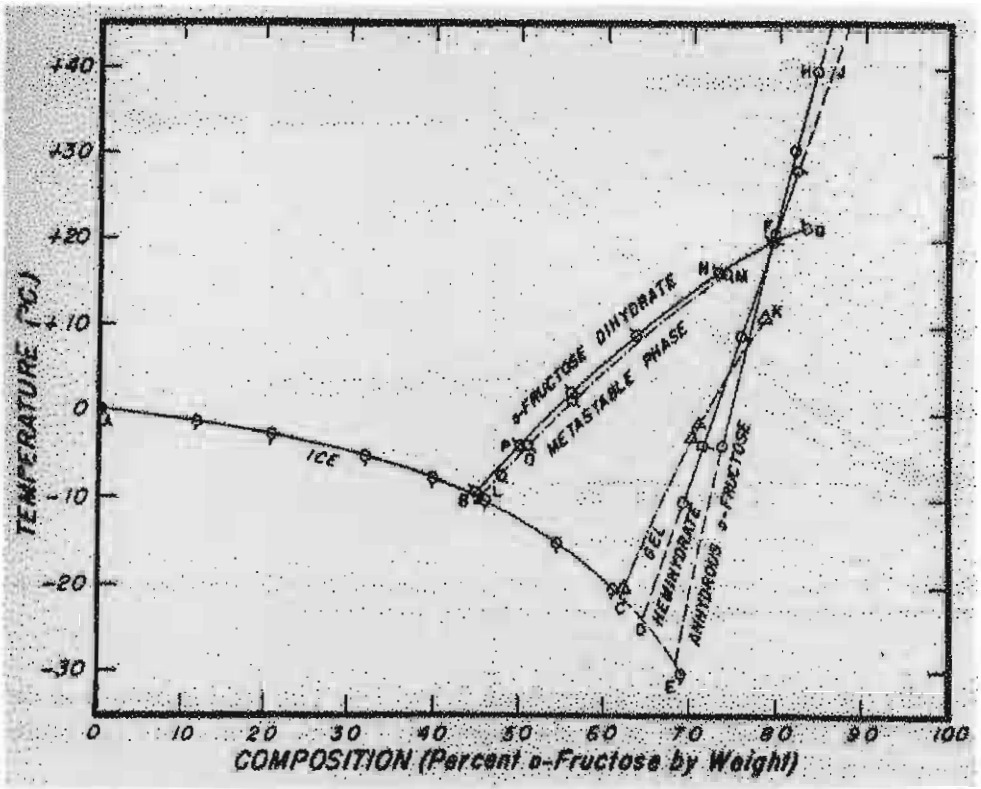

Figure 2.2 Phase diagram for D-fructose – water. (Young et al., 1952).

The phase diagram of aqueous solutions of glucose has been studied primarily by Jackson and Silsbee (1922) and more recently by Young (1957). The two sets of data have some discrepancies, but

tend to agree over most of the temperature range. Any of three crystal forms may be present, depending on the temperature, with anhydrous β-glucose occurring at the highest temperatures, anhydrous α-glucose at moderate temperatures, and α-glucose monohydrate occurring at lower temperatures. The two studies do not entirely agree on the phase transition temperatures, but the most important transition (anhydrous α-glucose to α-glucose monohydrate) is now accepted to occur at 55 – 56 °C. The phase diagram is shown in figure 2.3.

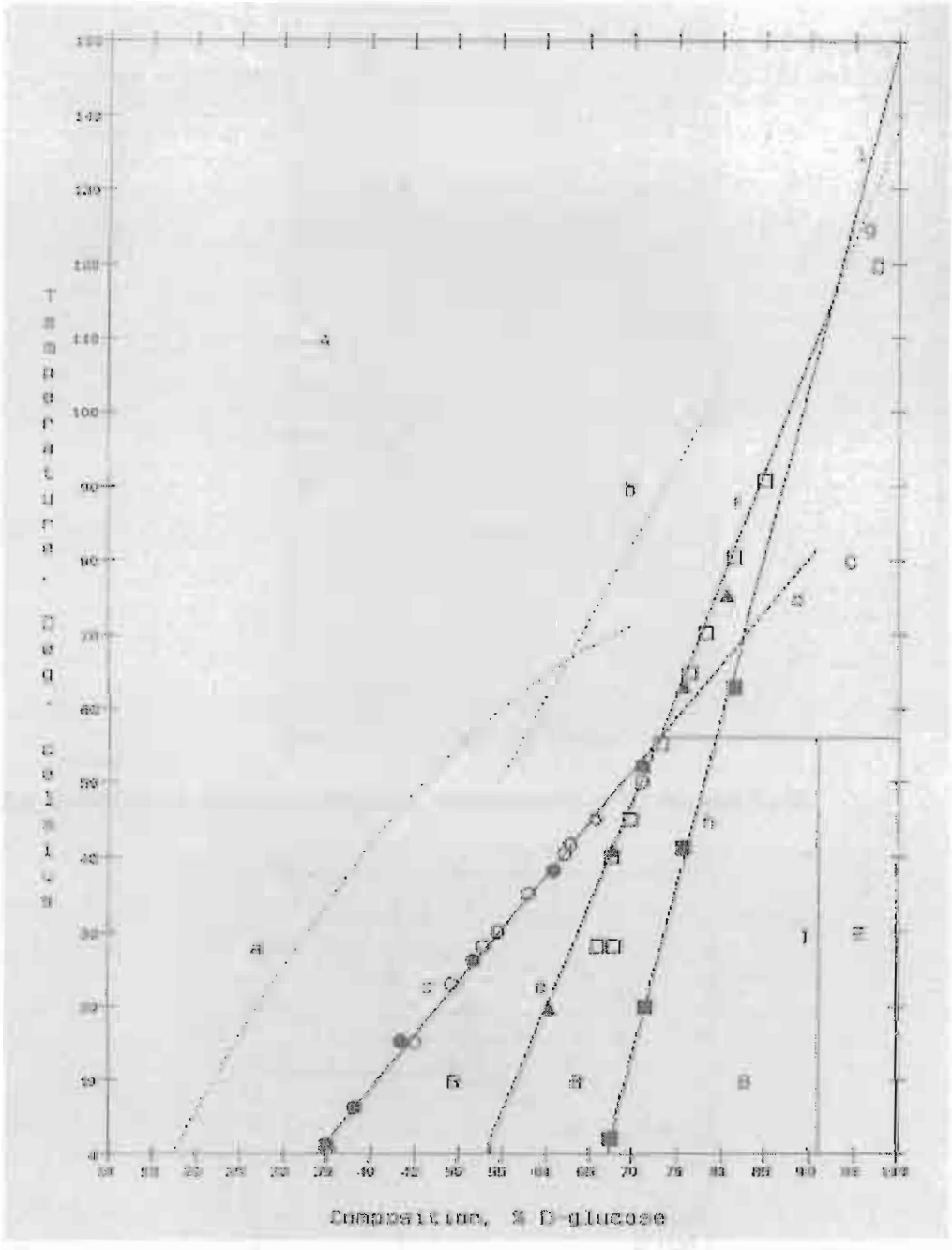

Figure 2.3 Phase diagram for D-glucose – water (from Mulvihill, 1992). ● represent α-monohydrate, ■ α-anhydrous. Filled symbols from Jackson and Silsbee (1922); Open symbols from Young (1957).

The solubility of fructose and glucose in solutions of ethanol and water have been published only comparatively recently, despite their obvious industrial significance. Solubility in the system fructose – ethanol – water (Flood et al., 1996c) is shown in the traditional format of three component solubility diagrams in figure 2.4, and as a solvent basis diagram at 30, 40, and 50 °C (Flood et al., 1996a) in figure 2.5. Solubility is strongly influenced by both temperature and solvent composition, with relatively low solubilities possible at lower temperatures in solutions of nearly pure ethanol.

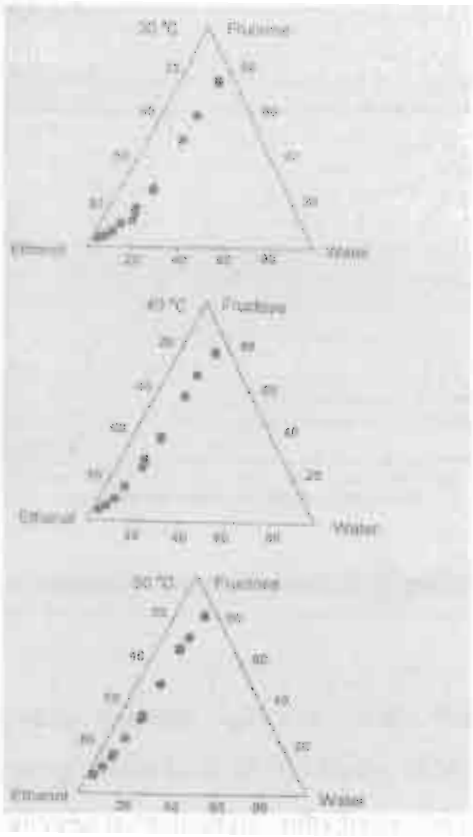

Figure 2.4 Solubility of fructose in ethanol – water solvents at 30, 40, and 50 °C.

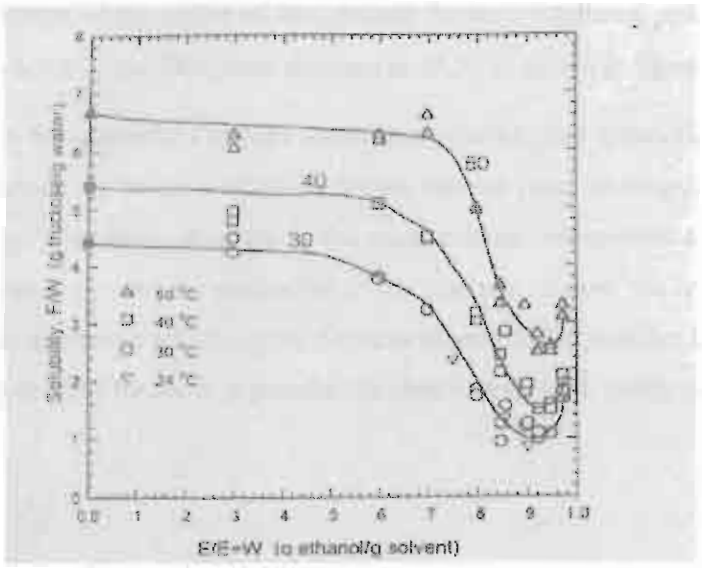

Figure 2.5 Solubility of fructose in ethanol – water (ternary diagram format).

The solubility of glucose in ethanol – water solutions has measured by two groups (Bockstanz et al., 1989; Peres and Macedo, 1996). The two sets of data were performed at different temperatures (30 °C for the former, 40 and 60 °C for the latter), but the two sets are consistent in terms of agreement with the binary glucose – water data, and trends as the ethanol composition in the solvent is increased. The effects of temperature and solvent composition are similar to that seen for fructose, although the solubility of glucose is lower than that of fructose at all conditions. The data of Peres and Macedo are shown in figure 2.6.

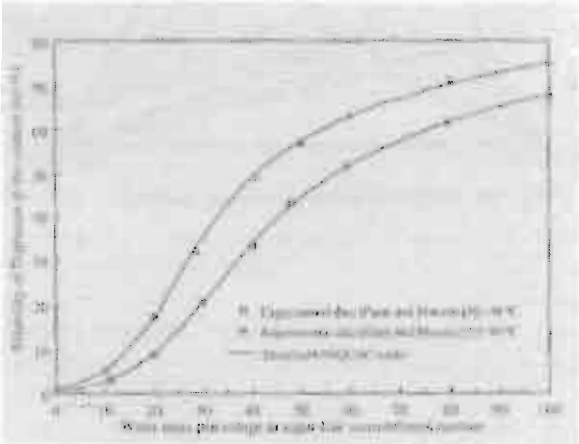

Figure 2.6 Solubility of glucose in ethanol – water solutions at 40 and 60 °C.

The last of the ternary systems, fructose – glucose – water was the first studied, but also is the least studied, with data only being available at 30 °C (Kelly, 1954). The two solute system displays three different crystal types (glucose monohydrate, anhydrous glucose, and anhydrous fructose), and therefore also two invariant points. The solubility in the system where fructose crystallizes is significantly higher than where either of the glucose forms crystallizes, although both components still have quite high solubilities. The phase diagram at 30 °C is shown in figure 2.7.

The solubility in the quaternary system which best characterizes ethanolic crystallization of high fructose syrups, fructose – glucose – ethanol – water, has not been investigated at all, up to the time of the present study. This phase diagram is the most critical information in order to estimate the viability of the proposed process for separation of fructose and glucose via crystallization. The phase diagram allows us to determine which crystal phase or phases will crystallize under a given condition, what are maximum driving forces it is possible to obtain, and what yields we may expect from the process.

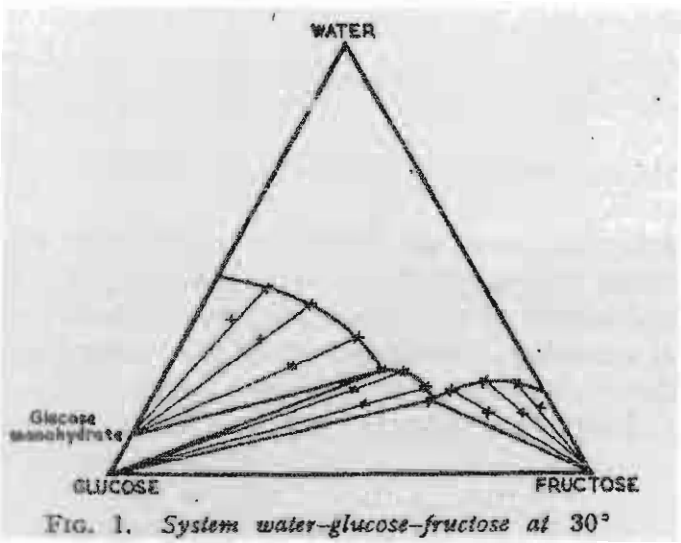

FIG. 1. *System water–glucose–fructose at 30°*

Figure 2.7 Solubility in the system fructose – glucose – water at 30 °C.

2.4 Crystal Growth Rates of Fructose and Glucose in Pure Solutions

Crystal growth rates of fructose from aqueous solution were measured by the group of Berglund in the late 1980s (Shiau and Berglund, 1987; Chu et al., 1989). Experiments were preformed in a small contact nucleation cell. The crystal growth was measured at 30, 40, and 50 °C, and at between 1 and 7 °C subcooling (from which the concentration driving force can bet determined). The study was conducted at very low relative supersaturation (0.004 to 0.02), and very high viscosities (in the range of 2000 mPa.s) due to the very high solubility of fructose in aqueous solution.

As is typical in this type of results, there is some scatter of the data, but two conclusions can be made; the crystal growth rate depends on the supersaturation to an order slightly greater than one, and the growth rate is higher at higher temperatures. The authors correlated their data with an Arrhenius model, obtaining the result $G = 0.00397\exp(-25.6/RT)S^{1.25}$, where G is the average growth rate, R is the gas constant, T is the crystallization temperature (in Kelvin), and S is the relative supersaturation, defined by $(C - C^*)/C^*$. The measured crystal growth rate data is shown in figure 2.8.

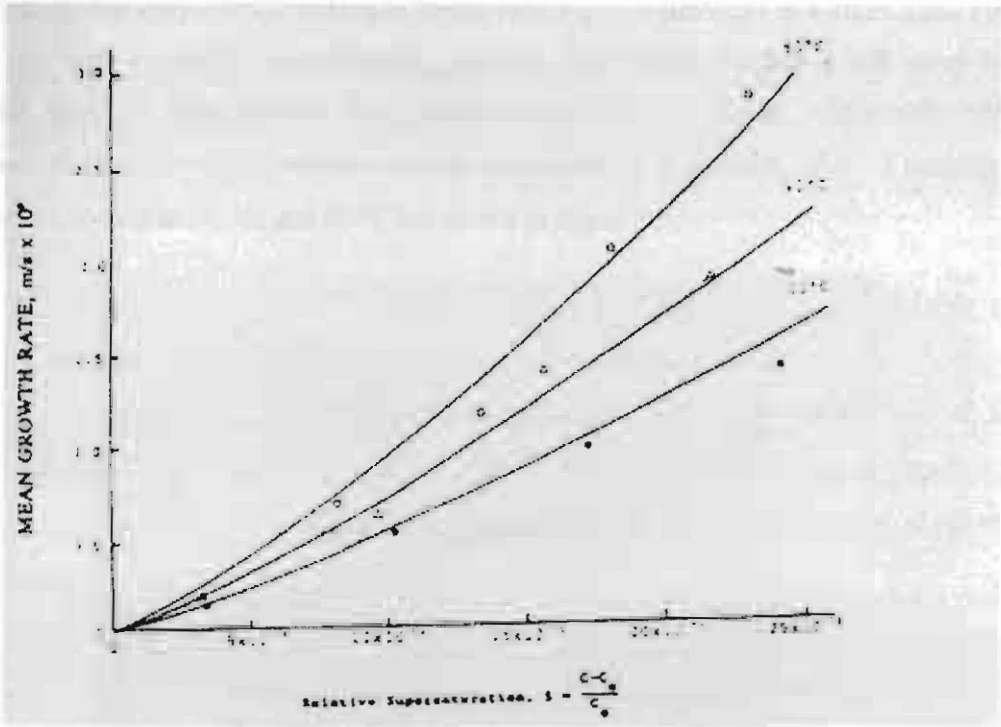

Figure 2.8 Mean crystal growth rates vs relative supersaturation for the growth of fructose from solutions.

Fructose crystal growth from aqueous- ethanol solutions has been studied by Johns et al., (1990), Addai-Mensah (1996) and Flood et al., (1996c and 2000), the last studies being probably the most accurate. Flood et al. (1996c) showed that the rate of mutarotation was partially rate controlling in crystal growth of fructose from aqueous – ethanol solutions, due to the extreme reduction in the rate of mutarotation when ethanol is added to the solution. This shows that it is more accurate to define driving force in terms of the concentration of the crystallizing tautomer, rather than overall fructose. The main drawback of this method is the difficulty in determining tautomeric concentrations rather than total fructose concentration. The technique for this measurement requires a fast derivatisation of the mixture to the trimethylsilyl derivatives followed by gas chromatography of the derivatives. The method is rather complex and expensive.

The highest growth rate measured in aqueous ethanolic solutions was in excess of 6 μm/min (at the early stages of the experiment) which is significantly larger than the highest growth rate in aqueous solution (less than 0.2 μm/min). The difference is mostly due to the much larger driving forces possible in aqueous ethanolic solutions, which is due to much lower solubilities. The reduction in the viscosity of the crystallizing solution also makes the crystallization substantially easier.

The crystal growth in aqueous ethanolic solutions is unusual in that early crystal growth rates are very high, but very quickly reduce to lower values (1 – 2 μm/min) in a short time, even though the driving force has stayed approximately constant. The reason for this is still unresolved. After the initial time of high growth, the growth rates follow a linear relationship with respect to supersaturation ($G = k_g S$), with growth rate constants (k_g) of the order of 2 – 3 μm/min. Examples of growth rate data at 24, 30, and 40 °C are shown in figure 2.9.

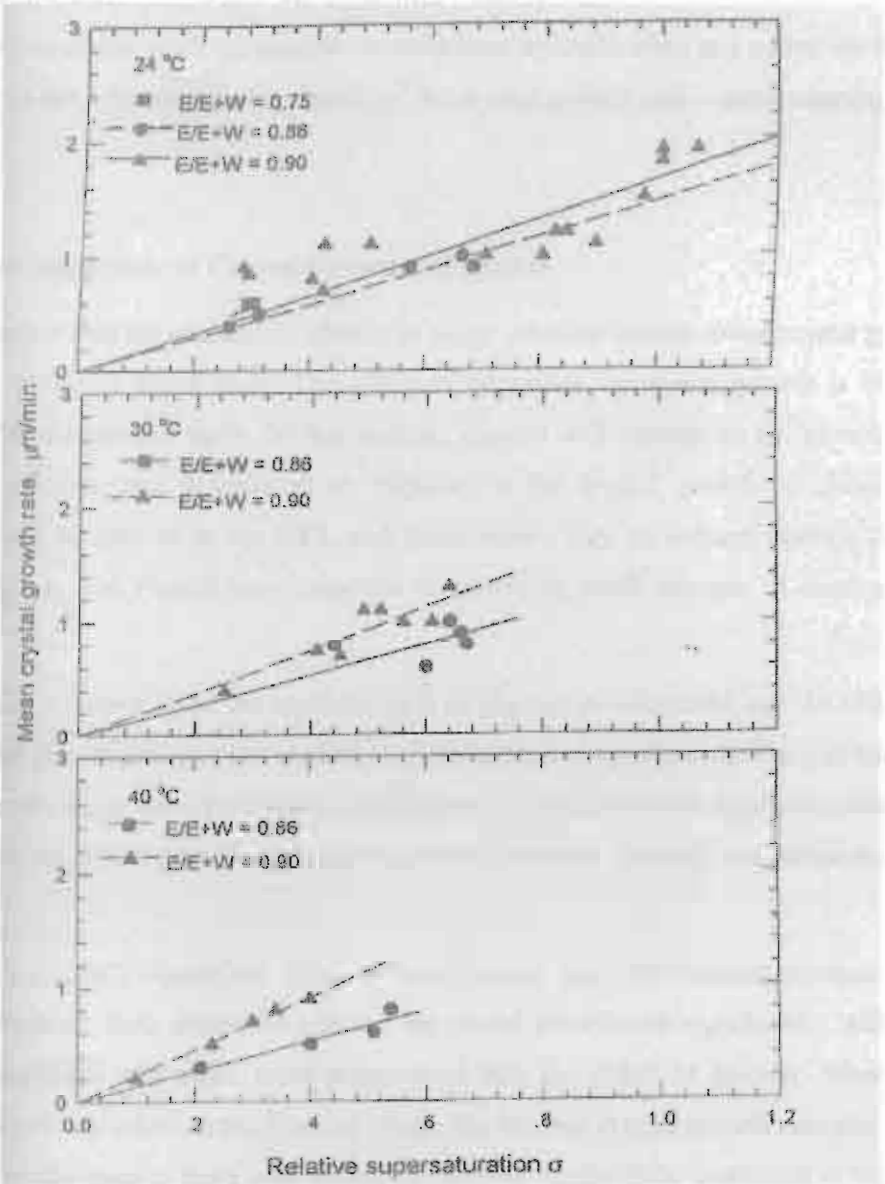

Figure 2.9 Mean crystal growth rates of fructose from aqueous ethanolic solutions at 24, 30, and 40 °C. E/S is the ratio of ethanol to total solvent.

The crystallization of glucose has mainly been studied using the anhydrous form, including the work of Kraus and Nyvlt (1993, 1994a,b,c) and Elankovan and Berglund (1987). The study of Elankovan and Berglund is unusual, in that they claim to measure growth rates of anhydrous glucose at 314 K, which is well inside the limit of alpha glucose crystallization, although it is possible that this limit depends on the total glucose concentration, and therefore the driving force used in the study. The present study does not concern the growth rate of anhydrous glucose, because we expect to operate at temperature ranges that will crystallize monohydrate.

The only available work on glucose monohydrate crystallization is a patent by Edwards (1980), This patent is not very specific on details of the crystal growth rate – supersaturation curve for the system.

2.5 Effect of Impurities on Crystal Growth of Fructose

It is possible that the addition of ethanol to sugar solutions causes lower crystal growth, based on the earlier studies of Flood et al. The effect of impurities on crystal growth is likely to be very important for the present study. In this system, glucose will operate as an impurity in the crystal growth of fructose, and fructose as an impurity in the crystal growth of glucose. Some other impurities will be present in the HFS, and some others may be formed through the effect of the ethanol addition. The ethanol may cause the formation of small amounts of dianhydride species in particular.

Not much is known about the crystallization of glucose monohydrate, and the effect of impurities on the crystal growth has not been studied at all (or at least not published). We will concentrate on the effect of impurities on the crystallization of fructose, for which limited data is available. The effect of impurities on the crystal growth of glucose is probably similar, although not identical to, the effect for fructose.

Chu et al., (1987) studied the effect of both glucose and difructose dianhydrides on the crystal growth of fructose. Both impurities affected the crystal growth rate significantly, although the effect of the dianhydrides was much more pronounced than the effect of glucose. When 0.05 to 0.9 g glucose/g water was added to the fructose syrup, the fructose crystal growth rate was reduced to 25 – 50 percent of the growth from pure solutions. Adding dianhydride impurities at the level of 0.1 g impurity/g water reduced the fructose crystal growth rate to only ten percent of the pure solution growth rate. The authors concluded that glucose inhibited the growth rate through increasing the solubility of fructose, whereas the growth inhibition caused by difructose dianhydrides is due to impurities being incorporated into the crystal, and thus inhibiting surface integration of fructose molecules. It is possible that glucose also has the effect of increasing the viscosity of the solution, thus decreasing mass transfer of fructose to the surface of the crystal.

dehydration to difructose dianhydrides, however none of these compounds was found in ethanolic crystallization from pure fructose solutions (Flood et al., 1996b), and so they are not expected to be a problem in the current study. However the current study has glucose concentrations much higher than 0.9 g glucose/g water, and inhibition by glucose is expected to be relatively severe. Although there is no experimental evidence of it, it is also likely that fructose will show a similar inhibitive effect on the crystal growth rate of glucose.

3. Properties of High Fructose Syrup + Ethanol Mixtures

The properties of high fructose syrups are mostly well known, however in the present study we will use ethanolic crystallizations, and thus properties of mixtures of ethanol and high fructose syrups are very important. The parameters required for the following crystallizations studies were the solubility of the solutions, viscosity, density, and refractive index. Mixing different quantities of HFS-42 with ethanol quickly showed that there was no region of immiscibility, which would have complicated crystallization studies considerably. Vigorous mixing over a period of time was required to completely dissolve the HFS in ethanol however.

3.1 Experimental Procedures

HPLC Technique for determination of fructose and glucose concentrations

Chromatography standards were produced from A.R. grade D-fructose (Merck), R.P.E. grade anhydrous D(+)glucose(Carlo Erba), analysis grade anhydrous ethanol (Carlo Erba), and HPLC grade filtered, distilled water (Carlo Erba). 1 mM sulfuric acid (prepared from concentrated sulfuric acid) was used as a mobile phase when the Aminex HPX-87H column was used, and HPLC grade water (Carlo Erba) was used as a mobile phase when the Aminex HPX-87C column was used.

Sugar standards were produced at 0.45, 0.9, and 1.35 g sugar (both glucose and fructose)/25 mL aqueous solution. Each standard contained both a glucose standard and a fructose standard. Ethanol standards were prepared at 17.5, 20.0, and 22.5 g ethanol/25 mL standard, which was expected to be approximately the concentration that would be found in the solubility data. These standards were used to determine a calibration for the HPLC technique.

After sampling from the saturated solution (or from the re-dissolved crystal phase) the sample was filtered through a 0.45 μm pore size polytetrafluroethylene HPLC filter (Biorad, US). The sample was degassed for five minutes in a shaken vessel, and then injected into the HPLC using the following technique.

In general, the HPLC technique used an Aminex HPX-87C (Biorad, US) column (250.0 mm × 4.0 mm) with a Microguard C cartridge as a guard column. HPLC grade water (Carlo Erba) was used as a mobile phase, with an injection volume of 25 μL, and a flow rate of 0.6 mL/min. The column was run under ambient conditions, and detection was performed using UV detection at 193 nm. Samples were tested two to three times to determine the reproducibility of the data.

The determination of sugar concentrations was found to be adequate using this technique (less than five percent relative error), and the fructose and glucose peaks were adequately baseline separated. The ethanol determinations were not sufficiently accurate, however, and hence ethanol concentration was determined solely by the amount of ethanol added to the solution, as ethanol does not appear in the crystal phase.

Crystallization Technique for Solubility Determination

Crystallization to equilibrium conditions was performed on a range of samples and ethanol contents to determine solubility in the system. Materials used were as previous, with the reagent fructose and glucose being used only to provide crystal seed to commence the crystallizations, and ethanol used for salting out the sugars. HFS-55 (Chao Khun Agro Products, Bangkok) was used as the HFS feedstock for the determinations. The specifications of this product are identical to those given in the literature review section of the report.

Samples of the HFS-55 and ethanol of the desired solvent composition were prepared in 100 mL Schott bottles. The solutions were put into a constant temperature shaking bath at either 30 or 40 °C. In seeded experiments a small amount of either crystalline glucose or crystalline fructose was added to the bottle to induce crystallization of that solute. The bottle was sealed and shaken until the solution had a constant refractive index over a period of several hours. This indicated that the solution had reached a constant composition.

When equilibrium was reached the solution and crystals were separated, and the crystals dried and re-dissolved at a known concentration. Both the liquid and crystal phases were analyzed by HPLC.

This method was used for ethanol/HFS-55 mass ratios of 4:1, 5:1, 6:1, 8:1, and 9:1 at 30 and 40 °C. Under each condition the experiment was either seeded with fructose, glucose, or left unseeded. Several experiments were performed under each condition in order to determine the reproducibility of the method.

Viscosity Measurement

Samples taken during the course of other experiments were also used to determine solution viscosity, using a Haake falling ball viscometer, with a temperature jacket connected to a constant temperature bath. Temperatures were controlled to within 0.1 °C.

Experiments were commenced at least fifteen minutes after any temperature change in the jacket, or after the sample was added to the system, in order to allow the sample to reach the required temperature. After this time, the cylinder of the falling ball viscometer was inverted, and the time for the #1 ball to travel between the first, second, and third lines was measured. (The first distance is exactly the same as the second, however a more accurate measurement is achieved by recording both times independently). The experiment was repeated a minimum of five times in order to verify the reproducibility of the result.

After each determination the cylinder was washed with distilled water to avoid cross sample contamination. Before the next determination the cylinder was rinsed with the new sample to avoid dilution.

Density Measurements

Density bottles of 10.0 mL were used for density determinations. Bottles were calibrated against pure distilled water. Before analysis, the bottles were washed with distilled water and dried for eight hours at 105 °C. Samples at 30 °C were added to the bottles until the capillary on the cap was filled. The bottles were then weighed to 0.1 mg on an electronic balance.

Refractive Index Determination

An Atago 2T Abbe refractometer with a temperature controlled lens (attached to a constant temperature bath) and temperature sensor was used to measure refractive index of the samples prepared. Temperature was constant to within 0.1 °C. The refractive index of each sample was measured to four decimal places, and each sample was replicated three times. After each determination the lens was cleaned with distilled water (to avoid contamination and to protect the lens), and the lens then dried with a lens tissue to avoid dilution of subsequent samples.

Crystal Phase Determination

Supersaturated solutions were made based on the results of the solubility work. (The supersaturated region for a particular solvent composition is the region below the solubility line in this figure). Points were chosen on either side of possible invariant points. Solutions were produced at 60 °C to dissolve the solute completely. After dissolution the samples were cooled to 40 °C, and seeded with a small amount of all likely preferred crystalline phases. Seeding was performed because sugar solutions have very large metastable regions and primary nucleation was unlikely at 20 °C subcooling. Seeding also resulted in large sized crystals, suitable for easy separation from solution.

Product crystals were vacuum filtered and then dried at 70 °C for 24 h, and then stored over silica gel. Higher temperatures were not used due to the melting point of glucose monohydrate (83 °C) and the decomposition temperature of fructose (~ 75 °C). It is believed that drying at this temperature could not alter the crystal phase from glucose monohydrate to anhydrous glucose. After drying the crystal phase was determined using X-ray diffraction (XRD). XRD was performed on a Bruker D5005 diffractometer using a copper anode. The 2θ range was 5 to 60°, using a step of 0.020°, and a step time of 0.6 s. In all cases there was excellent agreement between the intensity spectrum of the unknown and the intensity spectrum of the related compound in the XRD library.

3.2 Results

The results are presented in four sections, viscosity, density, refractive index, and solubility. Where solutions result from both mixing and crystallization, results will be presented based on whether the crystallization was seeded with fructose, seeded with glucose, or unseeded.

Viscosity of Solutions of HFS-55 and Ethanol

Figure 3.1 shows the viscosity of different mixtures of HFS-55 and ethanol (not necessarily saturated) at 30, 40, and 50 °C. The graph shows how moderate amounts of ethanol can significantly reduce the viscosity of the HFS solution. At low ethanol concentrations the viscosity is in the range of 100 – 300 cP, whereas at high ethanol concentrations the viscosity is in the range of 1 to 2 cP. This displays the effect of ethanol reducing the viscosity of the crystallizing solutions. It is also noticeable that the viscosity is lower at higher temperatures, with the highest viscosity occurring at 30 °C. (Remember that most of these solutions are not saturated, so the carbohydrate concentration is not a function of temperature. If the solutions were saturated the higher temperature solutions would have higher carbohydrate concentrations, and possibly also higher viscosities).

Figure 3.2 shows the viscosity of saturated solutions of HFS-55 and ethanol. These points were performed at high ratios of ethanol:HFS-55 because the solutions will be undersaturated at low ethanol compositions. Saturation was reached either by seeding with fructose or with glucose, with both techniques resulting in approximately the same final solution viscosity, despite possible small differences in concentrations of fructose and glucose. The viscosities in saturated solutions formed by the addition of ethanol (in the range of 1 – 2 cP) can be compared to the viscosity of the HFS-55, quoted as 900 cP at 27 °C in the technical specifications.

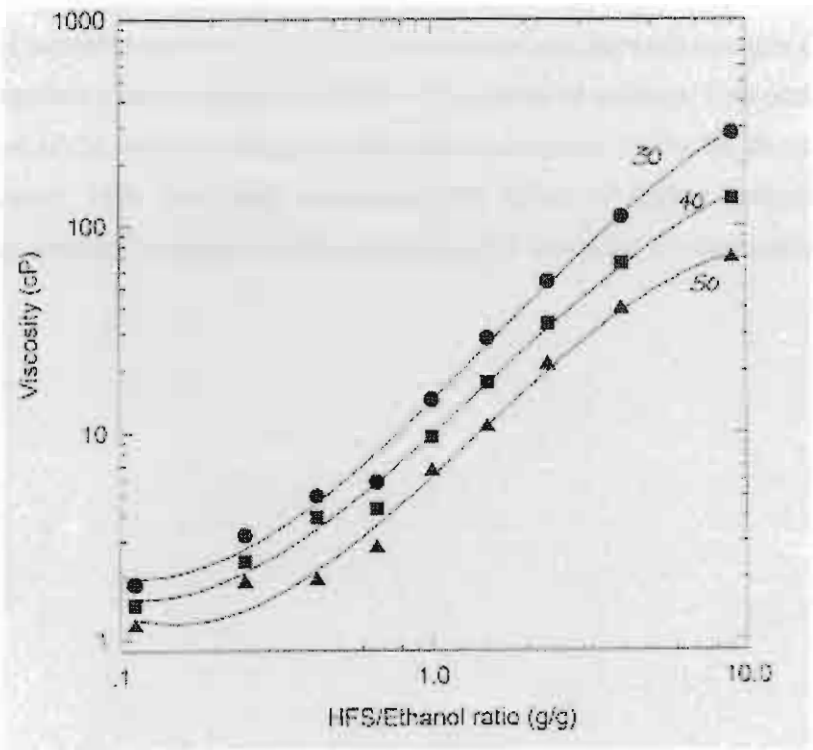

Figure 3.1 Viscosity of unsaturated mixtures of HFS-55 and ethanol.

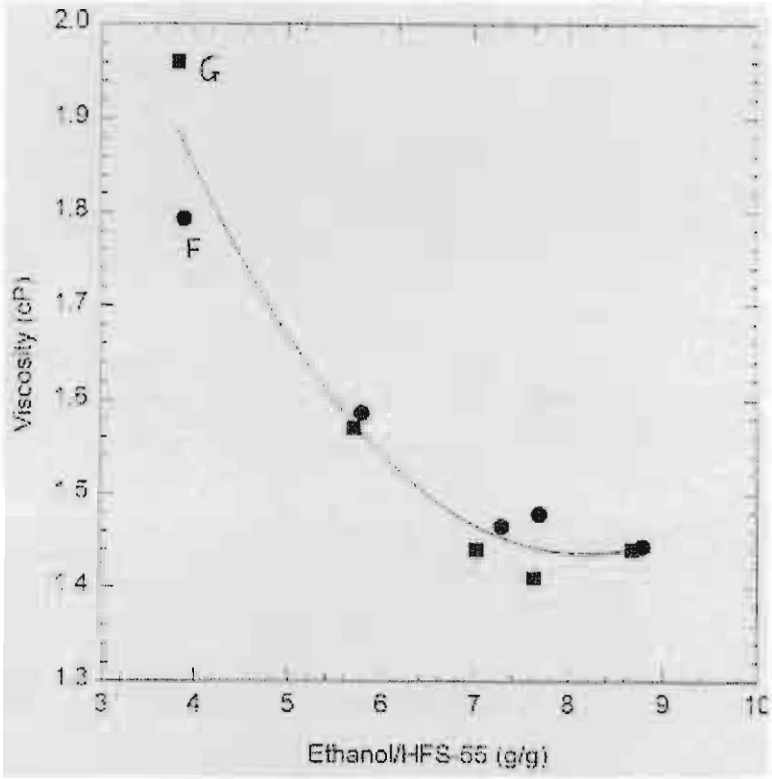

Figure 3.2 Viscosity of saturated mixtures of HFS-55 and ethanol.

The viscosity of saturated solutions of HFS-55 and ethanol also depends strongly on temperature, with increasing temperature increasing the viscosity of the saturated solution. This occurs because the carbohydrate content of the solution at higher temperatures increases due to the increasing solubility at higher temperatures. This more than cancels out the effect of higher temperature lowering viscosity, assuming constant composition. Viscosity data for unseeded crystallizations is shown in figure 3.3

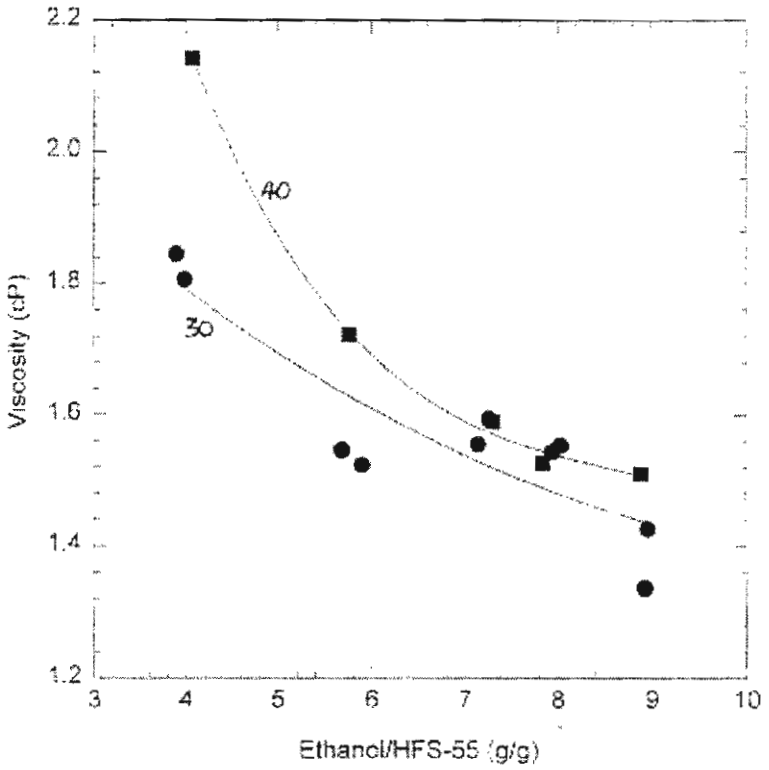

Figure 3.3 The effect of temperature on the viscosity of saturated mixtures of HFS-55 and ethanol.

Refractive Index of Solutions of HFS-55 and ethanol

Refractive index is commonly used in crystallization of carbohydrates to provide a measurement of the total carbohydrate composition. The current study aimed to determine whether it could be used to determine total sugar content, or more optimistically, the content of both fructose and glucose independently. Even if RI could only be used for total sugar content, it is a useful indicator of the progress of the crystallization.

Figure 3.4 shows the refractive index of unsaturated solutions of HFS-55 and ethanol at 30, 40, and 50 °C. At constant composition the refractive index is highest at 30 °C and lowest at 50 °C, which agrees with the general rule that RI should vary proportionately with density. The curves for the three temperatures are essentially parallel, with an offset of about 0.004 RI units per 10 °C temperature change. At 30 °C the refractive index reaches limits of approximately 1.45 at very low ethanol contents, and 1.36 at very high ethanol contents.

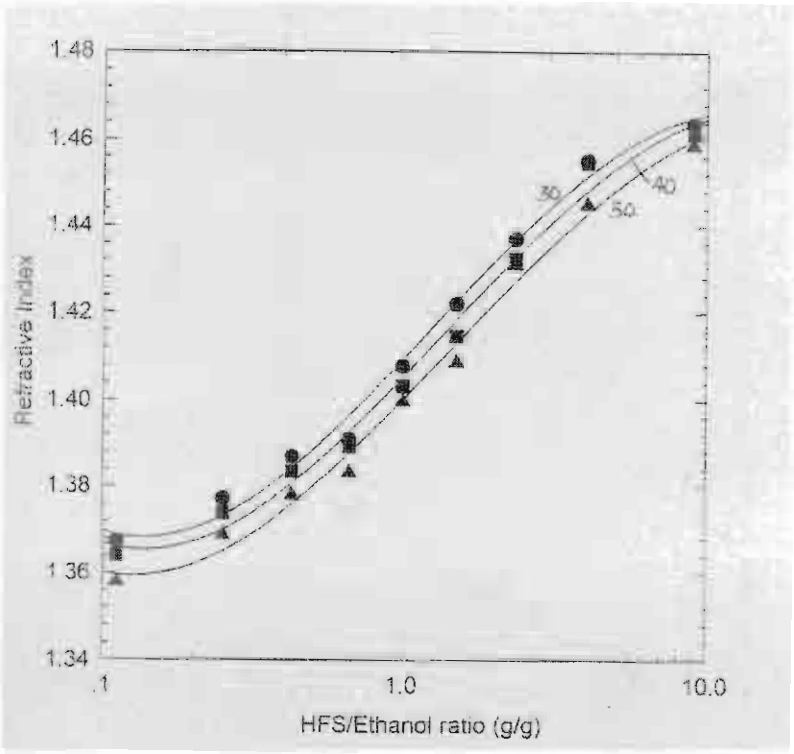

Figure 3.4 Refractive index of unsaturated mixtures of HFS-55 and ethanol at 30 – 50 °C.

Figure 3.5 shows the refractive index of saturated solutions of HFS-55 and ethanol at 30 °C. The two sets of data are for fructose seeded and glucose seeded solutions. The type of seed does not have any significant effect on the refractive index of the solution, despite probably having some effect on the proportions of glucose and fructose in the solution. The lack of any such effect is most likely due to the fact that the refractive indexes of fructose and glucose are quite similar compared to either ethanol or water. Higher carbohydrate compositions at higher temperatures lead to higher refractive index at higher temperature.

Figure 3.6 shows the effect of temperature on the refractive index of saturated solutions of HFS-55 and ethanol. Both sets of data points are for unseeded solutions that have reached equilibrium. The temperature has a significant effect, due to variation in the solubility of glucose and fructose at equilibrium. Higher carbohydrate compositions at higher temperatures lead to higher refractive index at higher temperature.

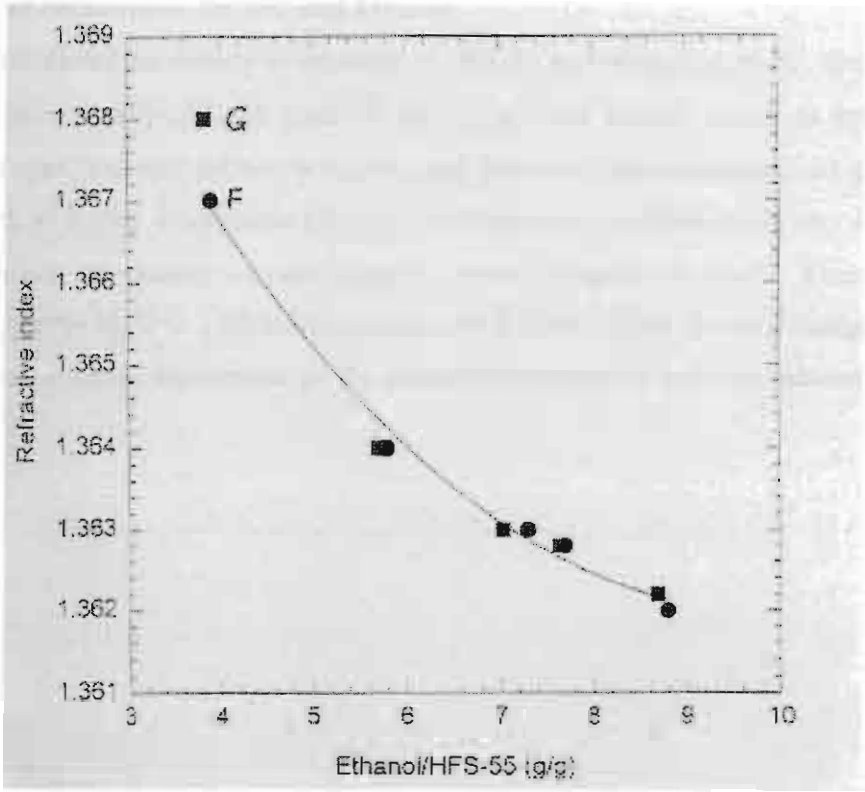

Figure 3.5 Refractive index of saturated solutions of HFS-55 and ethanol at 30 °C.

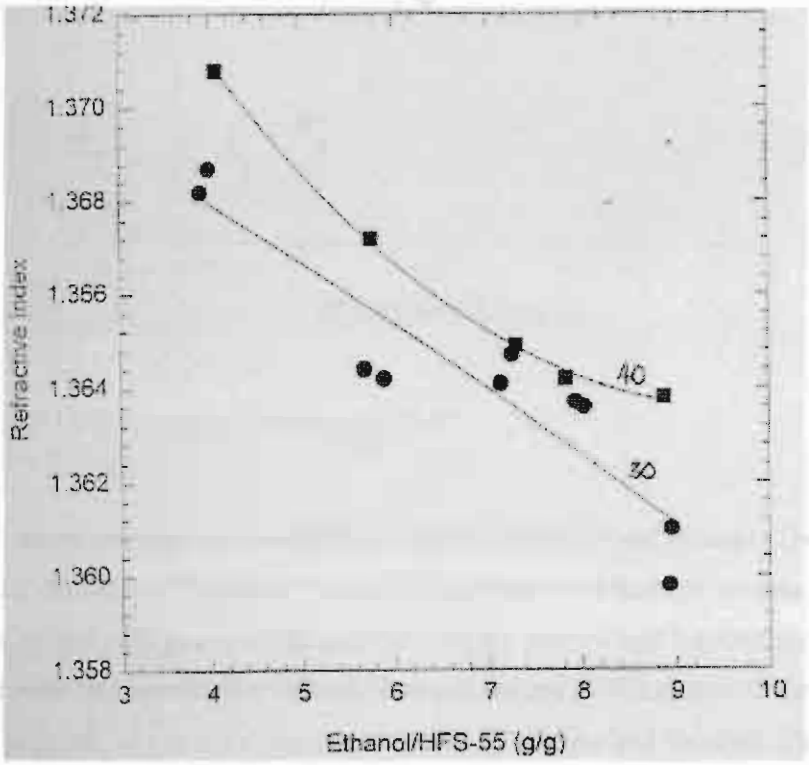

Figure 3.6 The effect of temperature on the refractive index of saturated solutions.

Density of Solutions of HFS-55 and Ethanol

Figure 3.7 shows the density of mixtures of HFS-55 and ethanol at 30 °C. The specification of HFS-55 gives a density of 1.38 g/mL at 20 °C, and the density which is approached as the HFS/ethanol ratio becomes infinite is slightly less than this (approximately 1.32 g/mL) due to the lower density at higher temperature (30 °C). The density of solutions with very large amounts of ethanol approach the density of pure ethanol, around 0.8 g/mL at 30 °C. There is an apparent discontinuity in the slope at a HFS/ethanol ratio of 0.8. This may be due to a change in the structure of the ethanol – water interactions as the amount of ethanol in solution increases past a certain amount.

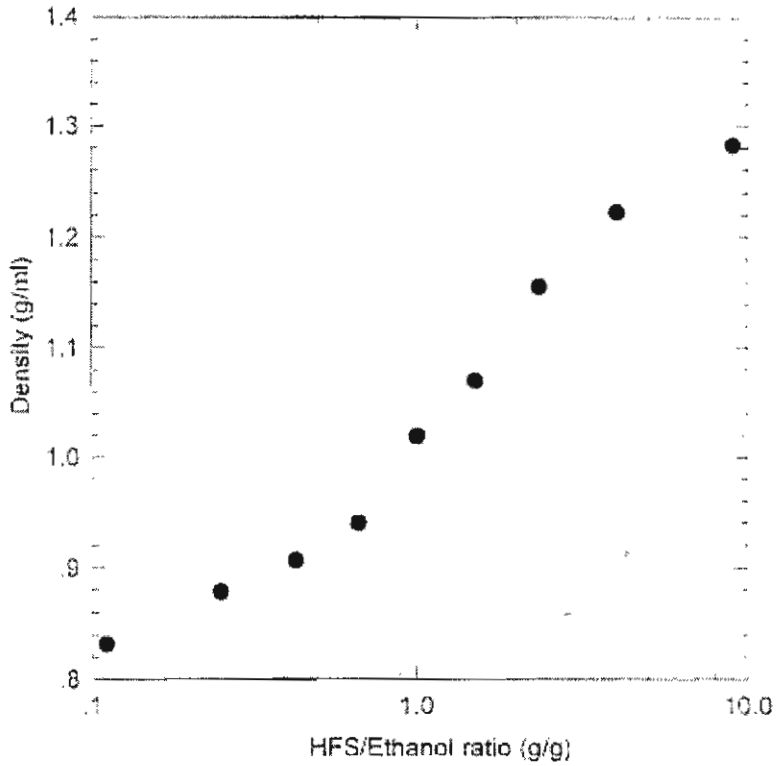

Figure 3.7 Density of HFS-55 ethanol solutions at 30 °C.

Figure 3.8 shows densities of saturated solutions of HFS-55 and ethanol. There is a significant difference in the densities of those solutions reaching saturation through seeding with fructose, and those that were seeded with glucose. The densities of pure glucose and fructose are almost identical at 1.6 g/mL, so it must be assumed that these differences are due to differences in the total carbohydrate content of the solution, not to differences in the ratio of glucose and fructose. The difference in the total carbohydrate loads is most likely due to differences in the solubilities of the two sugars, fructose having a much higher solubility than glucose. Thus, solutions seed with fructose, and therefore

having a greater crystallization of fructose and less fructose remaining in solution, may have a lower total carbohydrate content, and therefore a lower density.

Figure 3.9 shows the density of solutions of HFS-55 and ethanol saturated at 30 and 40 °C. (Saturation was reached at 30 and 40 °C , but all density measurements were performed at 30 °C; the solutions did not crystallize due to the short time period between saturation and measurement, and the large metastable region for the two solutes). The density in the two cases is significantly different, because the solutions saturated at 40 °C have much higher carbohydrate solubilities than those saturated at 30 °C.

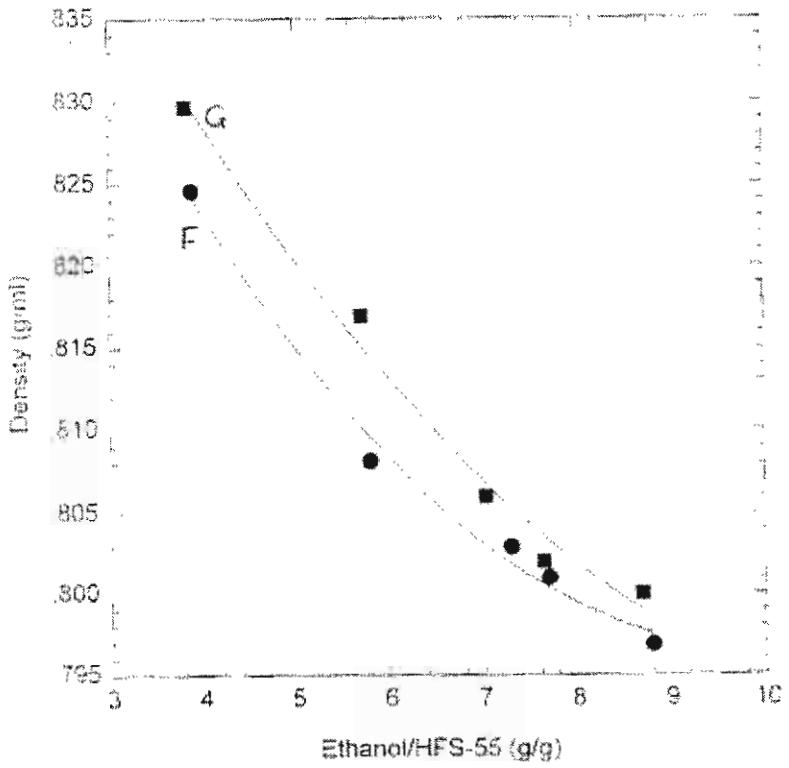

Figure 3.8 Density of saturated solutions of HFS-55 and ethanol.

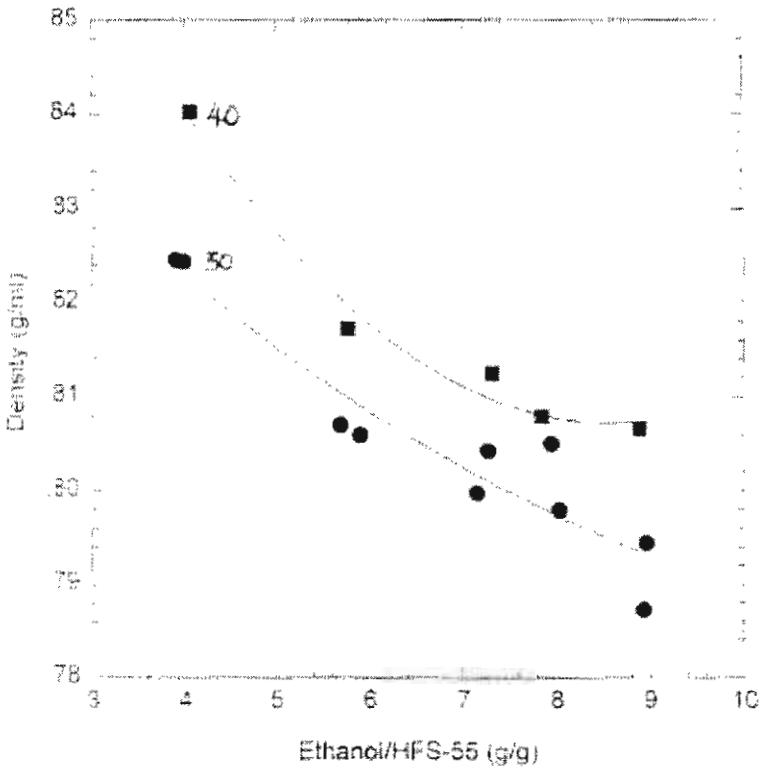

Figure 3.9 Density of solutions of HFS-55 and ethanol saturated at 30 and 40 °C.

Solubility and Crystal Yields for HFS-55 – Ethanol Mixtures

One of the most important parameters in any crystallization is the solubility. It is particularly important for systems that can not undergo evaporative crystallization, because in this case it will limit the yield of the process. The system under study is not suitable for evaporative crystallization due to the high solubilities, and the problems with fructose decomposition at temperatures much exceeding 65 °C. In this case yields were determined by collecting, filtering, drying, and weighing the crystals produced during the time the solution was reaching the solubility limit. The maximum possible yield of crystals per g of HFS-55 is 0.77g, due to the solids content of HFS-55, assuming that anhydrous glucose crystallizes. If the monohydrate form of glucose crystallizes the yield might be higher than this, as the crystals will also remove water from the solution.

The crystal yields may depend on how the crystallization was undertaken, i.e. fructose seeded, glucose seeded, or unseeded, and also on the solvent composition. E/(E + W) is the solvent composition, g ethanol/ g total solvent, including the water contained in the high fructose syrup. Results for the three cases are given in Table 3.1 for 30 °C and Table 3.2 for 40 °C.

Table 3.1 Crystalline Yields from HFS-55 – ethanol mixtures at 30 °C.

Solvent content E/(E+W)	Crystal Yield (g crystal/g HFS-55)		
	Fructose seed	Glucose seed	Unseeded
0.947	0.56	0.63	0.50
0.964	0.60	0.64	0.49
0.973	0.70	0.68	0.56
0.976	0.78	0.72	0.53

Table 3.2 Crystalline Yields from HFS-55 – ethanol mixtures at 40 °C.

Solvent content E/(E+W)	Crystal Yield (g crystal/g HFS-55)		
	Fructose seed	Glucose seed	Unseeded
0.947	0.39	0.46	0.26
0.964	0.47	0.61	0.33
0.973	0.62	0.66	0.43
0.976	0.70	0.70	0.50

The crystal yields are quite high, which is promising, however clearly both sugars are crystallizing out simultaneously whenever the yield is higher than the fraction of an individual sugar. Unseeded solutions have lower yields than either of the seeded cases, and it appears this is due to reaching a metastable limit where one of the solutes does not crystallize even though it is supersaturated. This is very promising, because it suggests that we can separate the two forms by seeding one, while being within the metastable zone for the second solute, and thus having no crystallization of the second solute.

3.3 Discussion and Conclusions

The results of the crystallization studies to determine yields proved that ethanolic crystallization from these syrups is not only possible, but is potentially a high yield process. Differences in yields between different seeding regimes show that one (and probably both) of the solutes is in its metastable limit in some cases, and may not crystallize unless seed of that solute is added.

Collection of other property data also went very well. Viscosity data shows low viscosities, suitable for easy agitation, and good mass transfer for the crystallization process. Refractive index and density vary strongly with the process parameters, and are suitable for following the solution concentration throughout the crystallization as a monitoring tool. It is unlikely that the combination of

RI and density could be used to determine the independent concentrations of the two solutes; it is likely that HPLC is the only suitable technique for this.

The main difficulty presented at this stage was the unstable nature of the high fructose syrups, which tend to decompose relatively quickly at ambient temperatures, but even at relatively low temperature. This may be due to residual enzymatic activity, or due to the solutions becoming more acidic over time. This resulted in results that were less reproducible than hoped, and supplies could not be easily obtained regularly because of the distance to the factory. For this reason it was decided to study the representative system fructose – glucose – ethanol – water. The first three of these components make up more than 95 percent of the high fructose syrup, and with ethanol, the components make up more than 99 percent of the solution likely to be found in an industrial ethanolic crystallization. The components of this system may be purchased either as pure solvents, or in the crystalline form, and solutions prepared when the need arises. This will limit problems with solution degradation.

4. Properties of the System Fructose + Glucose + Ethanol + Water

The system fructose – glucose – ethanol – water is characteristic of those that would be found in ethanolic crystallization of high fructose syrups. At the high ethanol contents required for effective reduction of the solubility, these four components make up more than 99.5 percent of the crystallization solution. Because crystallization studies would be undertaken with this model system, the solubility/phase diagram, and other important properties were measured.

4.1 Experimental Procedures

Chemicals D-(−)-fructose, D-(+)-glucose anhydrous, (both ACS – for analysis), and ethanol (99.9 % v/v, for analysis) were obtained from Carlo Erba Reagenti (Milan) and used without further purification.

Experimental Procedures The solubility of fructose and glucose in solutions of ethanol and water was measured at 30 and 40 °C, with the solutions maintained at the desired temperature with an uncertainty of 0.2 °C. All determinations were made in sealed glass Schott bottles into which a known quantity of ethanol + water (of desired concentration) and a known quantity of anhydrous fructose were added. The ethanol concentration of the ethanol + water solution was known to an accuracy of 0.1 mg/g of solution. An amount of fructose was dissolved in the bottles, with the exact amount varying between bottles such that the experiments covered a range of points between the previously published systems glucose + ethanol + water (Peres and Macedo, 1996) and fructose + ethanol + water (Flood et al., 1996a). An amount of anhydrous crystalline glucose sufficient to achieve at least 50 % excess of glucose over the amount needed for saturation was added to each bottle, and the bottles were shaken in an orbital shaking bath at 100 rpm and 30 °C until saturation was reached. After 24 hr, the refractive index of the liquid was determined every 6 hr to determine if saturation was reached. Saturation was complete within 7 – 10 days for all determinations.

Sugar concentrations were determined by a chromatographic technique, similar to that described in section 3. It was found that if samples were left to stand over several days a reaction product was formed, which tended to decrease the sugar concentrations. For this reason samples of approximately 1 mL were weighed to 0.1 mg, and then dried to remove the ethanol, and reweighed to 0.1 mg. This allowed the removal of ethanol while maintaining known amounts of original sample. These dried samples were diluted to 1 g solids / 100 mL solution by the addition of a known (to 0.1 mg) amount of distilled water. This resulted in concentrations suitable for HPLC determination.

Diluted samples were filtered through a 0.45 μm filter, and then a 2 μL sample was injected onto a 250 mm × 4 mm HPX 87C column (Biorad, Bangkok) using a water mobile phase at 0.3 mL/min. The column temperature was 80 °C. Detection was performed by a diode array detector measuring UV at 193 nm. The uncertainty (95 % probable error) in the concentration determinations, including the dilution and the HPLC, was 0.002 g of glucose/g of solution and 0.003 g fructose/g of solution.

Duplicate solubility determinations showed that the uncertainty (95 % probable error) in the solubility measurements was 0.005 g of sugar/g of solution for both fructose and glucose. Uncertainties in other variables, such as bath temperature, solvent composition, or saturation point, may be responsible for duplicate bottles having larger uncertainties than seen in the concentration measurement alone.

Refractive index was measured for solvent compositions of 40, 60, and 80 mass % ethanol, and solute compositions of 1:1, 1:2, and 2:1. The total solute concentrations were chosen so that a range of data points up to approximately the saturation condition was measured. Duplicate refractive index determinations were made on an Abbe refractometer with temperature control to 0.1 °C. The precision of the refractive index was 0.0005 refractive index units.

Solution viscosity was measured in duplicate in a falling ball viscometer (Haake) with the sample viscosity determining the ball that was used in the determination. The viscometer was jacketed, and water from a constant temperature bath kept the visometer temperature constant to within 0.1 °C. The solutions had viscosities in the range 1 to 1000 mPa.s, and hence balls 1 (2.4 $g.cm^{-3}$, 15.81 mm) and 4 (8.13 $g.cm^{-3}$, 15.2 mm) were used. These balls were calibrated against sugar solutions of known viscosity. The time period used in the viscosity determination was the average of eight measurements of the time required for the ball to travel the required distance. The error of the viscosity measurements is expected to be with 3 %.

Solution density was measured in triplicate at 25 °C using 10 cm^3 density determination bottles weighed to 0.1 mg. The uncertainty (95 % probable error) was 0.0003 $g.cm^{-3}$.

4.2 Results

The solubility of fructose and glucose in ethanol + water are plotted as ternary diagrams in figure 4.1 (30 °C) and figure 4.2 (40 °C). Since there are four components in the system it is not easy to illustrate the data on a two dimensional plot. The illustration is simplified by having one axis as total solvent (water + ethanol), with the lines on the plot depicting constant solvent composition (in the case of this study 40, 60, and 80 mass % ethanol). The data for the system fructose + glucose + water at 30 °C (equivalent to a 0 mass % ethanol line), which also appear on the 30 °C plot, were taken from the study of Kelly (1954). Kelly only took measurements at 30 °C, so this data does not exist for 40 °C.

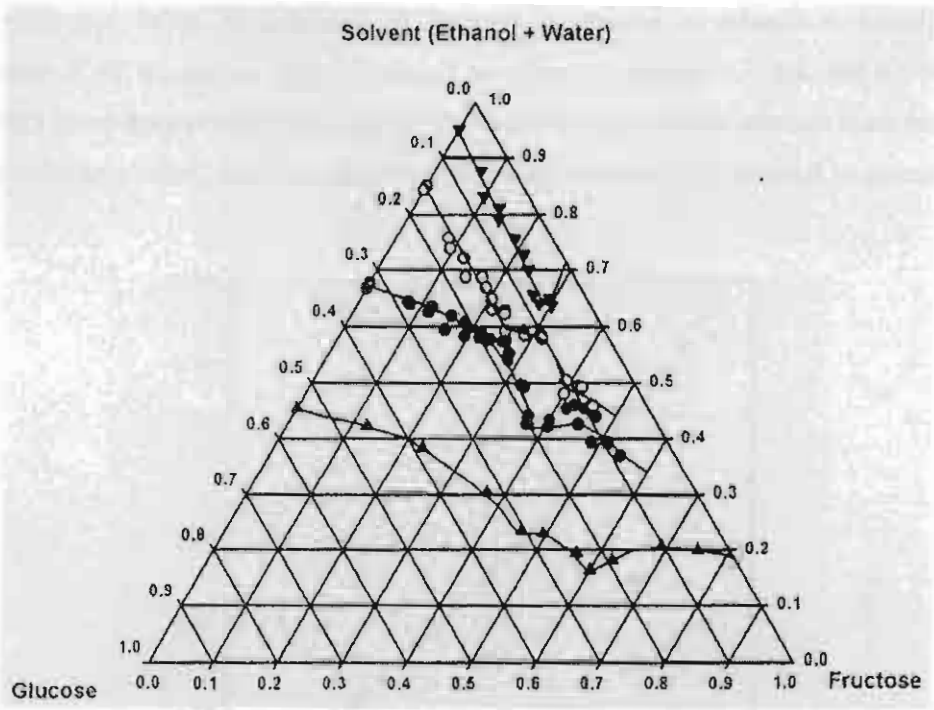

Figure 4.1 Solubility for the system fructose + glucose + ethanol + water at 30 °C.

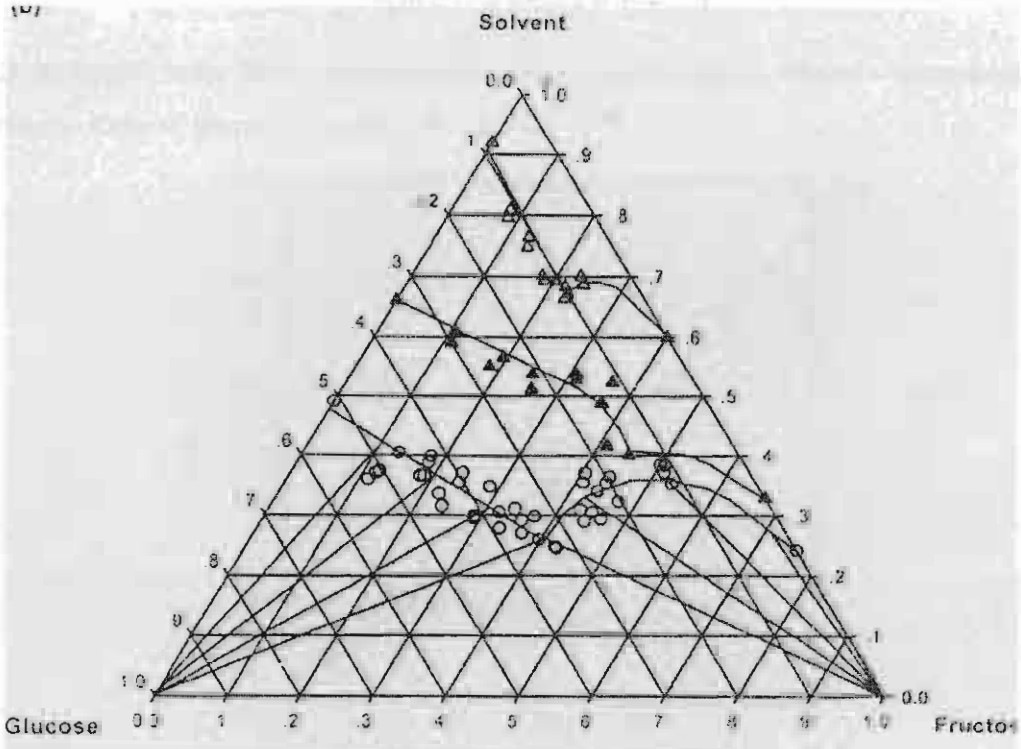

Figure 4.2 Solubility in the system fructose + glucose + ethanol + water at 40 °C.