CARBOHYDRATE
RESEARCH

ELSEVIER

Carbohydrate Research 288 (1996) 45–56

# Mutarotation of D-fructose in aqueous–ethanolic solutions and its influence on crystallisation [1]

Adrian E. Flood, Michael R. Johns *, Edward T. White

*Department of Chemical Engineering, University of Queensland, Brisbane, Qld 4072, Australia*

Received 18 December 1995; accepted 27 March 1996

## Abstract

The mutarotation of D-fructose in aqueous–ethanolic solutions was studied between 24 and 50 °C. The ethanol:water mass ratios of the solvent were 3:1, 6:1, and 9:1. The principal tautomers found in the solution were β-D-fructopyranose, β-D-fructofuranose, and α-D-fructofuranose, as is found with aqueous solutions of D-fructose. The furanose tautomers were more strongly favoured in the aqueous–ethanolic solutions, comprising approximately 60% of the fructose in solution for the range of solvent conditions studied, in comparison to 27% in aqueous solution at 24 °C. The rate of mutarotation from β-D-fructopyranose to the furanose tautomers was fivefold slower in solutions of ethanol:water ratio of 9:1 than in aqueous solutions. The tautomeric composition of D-fructose in aqueous–ethanolic solutions was shown to affect the crystallisation of the sugar, due to the slow rate of mutarotation of furanose forms to β-D-fructopyranose. © 1996 Elsevier Science Ltd.

*Keywords:* Mutarotation; Crystallisation; D-Fructose

## 1. Introduction

Large quantities of the monosaccharide, D-fructose, are produced for use as a sweetener. Aqueous crystallisation of fructose is difficult due to the very high solubility

---

* Corresponding author.

[1] Abbreviations and definitions. A, Acyclic tautomer. $C_i$, Concentration of species i in solution. [Fr], Total fructose concentration in solution. [Fr$^*$]. Saturation concentration of fructose in solution. $k$, Rate constant of β-D-fructopyranose to other tautomers. $k'$, Rate constant of other tautomers to β-D-fructopyranose. $k_i$, Rate constant of species i to acyclic form. $k'_i$, Rate constant of acyclic form to species i. $k_{ij}$, Rate constant of species i to species j. $K$, Equilibrium constant. $K_F$, Furanose equilibrium constant. $t$, Time. $x_A$, Fraction of fructose in form A. α-fur, α-D-fructofuranose. α-pyr, α-D-fructopyranose. β-fur, β-D-fructofuranose. β-pyr, β-D-fructopyranose. ξ, Reduced rate constant.

0008-6215/96/$15.00 © 1996 Elsevier Science Ltd.
*PII* S0008-6215(96)00099-7

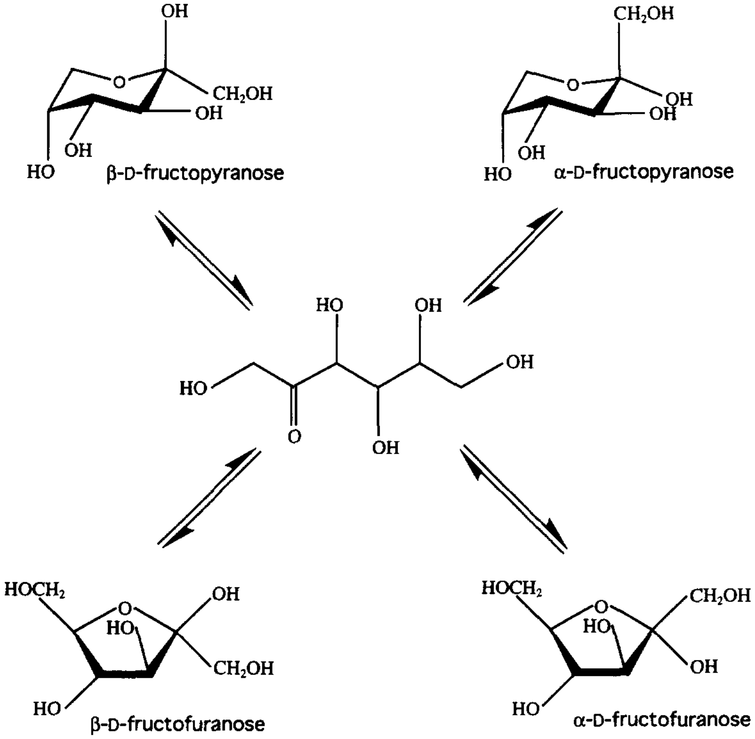

Fig. 1. Mutarotation of D-fructose [4,5].

of fructose in water. Crystallisation processes [1,2] have used the addition of lower alcohols, including ethanol, to concentrated syrups to decrease fructose solubility and solution viscosity [3], thus enhancing the crystallisation process.

Crystalline fructose consists solely of the anhydrous β-D-fructopyranose form. In solution, however, fructose can exist as any of five tautomeric forms, which interconvert through the acyclic keto form [4,5] as shown in Fig. 1. The β-D-fructopyranose form is the most plentiful in aqueous solutions, with the two furanose forms also being present in quantifiable amounts (Table 1). The remaining two tautomers are present only in trace amounts.

The mutarotation of D-fructose in aqueous solutions has been studied by several different researchers using polarimetry [6], gas–liquid chromatography (GLC) [4,5,7], and $^{13}C$ nuclear magnetic resonance ($^{13}C$ NMR) [8]. Most of these studies attempted only to find the equilibrium composition of the tautomers in water at different temperatures, although the studies of Shallenberger [4] and Cockman et al. [5] included kinetic studies. Unfortunately, β-D-fructopyranose is the only tautomer readily available in pure form. This has meant that, although the specific rotation of β-D-fructopyranose is well known, the specific rotations of α-D-fructopyranose and the acyclic form are unknown, while those of the α-D-fructofuranose and β-D-fructofuranose forms have been estimated only with a large degree of uncertainty [4].

Table 1
Tautomeric composition of aqueous solutions of D-fructose at equilibrium

| Author | Temp (°C) | α-fur | β-fur | α-pyr | β-pyr | Keto | Method |
|---|---|---|---|---|---|---|---|
| Hyvönen [6] | 22 | – [a] | 31 | – | 69 | – | Polarimetry |
| Hyvönen [8] | 22 | – | 31 | – | 69 | – | GLC |
| Angyal and Bethell [7] | 27 | 4 | 21 | Tr [b] | 75 | – | $^{13}$C NMR |
| Angyal and Bethell [7] | 30 | 5 | 23 | Tr | 72 | – | $^{13}$C NMR |
| Shallenberger [4] | 20 | 4 | 20 | – | 76 | – | GLC |
| Cockman et al. [5] | 25 | 5 | 22 | < 1 | 72 | < 1 | GLC |

[a] – Indicates the tautomer was not detected in solution.
[b] Tr indicates the tautomer was detected in trace amounts.

The lack of information on the specific rotation of the tautomers and the complex nature of fructose mutarotation has meant that the interpretation of polarimetric data is especially difficult for this system. Consequently, the use of GLC [4,5] and NMR [8] techniques appears to give the most consistent equilibrium tautomer compositions in aqueous solutions. The results of an earlier study using polarimetry [6] are largely in error due to the assumption of a two-component mixture.

It has been suggested that the rapid (first) phase of fructose mutarotation after dissolution of the β-D-fructopyranose crystal in water is predominantly a β-pyranose–β-furanose interconversion, and that subsequent mutarotation involves mainly α–β interconversions for the two ring forms [5], although data presented for fructose mutarotation at 25 °C appear not to agree with this representation. It seems more likely [4] that furanose–furanose rate kinetics are sufficiently rapid compared to pyranose–furanose kinetics that the two furanose forms are always in equilibrium with each other.

Published research on mutarotation rates in non-aqueous solutions is very limited. Verstraeten [9] used polarimetry to show that ethanolic solutions had reduced rates of mutarotation and lower concentrations of the β-D-fructopyranose tautomer at equilibrium, although neither of these effects was quantified. The equilibrium composition of D-fructose in dimethyl sulfoxide [10] and in pyridine [4] demonstrate a markedly reduced proportion of β-D-fructopyranose and an increase in the proportion of β-D-fructofuranose and α-D-fructofuranose tautomers.

The rate of mutarotation may affect the crystallisation rate of sugars [11]. As the mutarotation rates of fructose in aqueous–ethanolic solutions are important for the interpretation and modelling of the crystallisation kinetics of the system, the aim of the research described in this paper was to examine the effect of mutarotation on the crystallisation of D-fructose in aqueous–ethanolic solutions.

## 2. Experimental

*Reagents.*—Solutions were prepared from vacuum-dried USP grade anhydrous D-fructose (A.E. Staley, Decatur, IL), anhydrous absolute ethanol (CSR, Sydney, Australia), and water, which had been treated by reverse osmosis and activated carbon.

*Mutarotation experiments.*—Solvent (282.0 g) of a particular ethanol:water mass ratio was prepared using ethanol and water weighed to within ±0.01 g. The solvent was brought to temperature in a closed 1-L stirred glass vessel held in a water bath maintained at constant temperature to within 0.1 °C. D-Fructose (18.0 g) was added to the solvent and rapidly dissolved to give a 6% by weight fructose solution. Duplicate 3-μL samples were periodically taken from the vessel with a gas-tight syringe (SGE, Melbourne, Australia) and immediately injected through the septum of a glass vial containing 1 mL of refrigerated derivatisation mixture to halt the mutarotation process. The mixture was shaken vigorously and held at 4 °C until analysed.

*Tautomer analysis.*—The tautomeric composition of fructose solutions was determined by GLC analysis of $Me_4Si$ derivatives after the method of Nikolov and Reilly [12]. The derivatising mixture was prepared by adding 0.1 mL of trimethylsilylimidazole (Pierce, Rockford, IL) to 0.9 mL of anhydrous pyridine (Ajax, Sydney, Australia), which had been dried previously over anhydrous $Na_2SO_4$. The mixture was sealed in a 2-mL septum-capped vial, and refrigerated until use. After derivatisation a 3-μL sample of the mixture was injected into a Perkin–Elmer Autosystem gas chromatograph equipped with an FID detector and using a 0.25 mm i.d. × 30 m fused silica capillary column (J&W Scientific, Folsom, CA) coated with a 0.25-μm thickness of DB-5. The operating conditions were as follows: helium carrier gas at 1.0 mL/min; split ratio 12:1; column temperature of 190 °C; injector at 250 °C; and FID at 280 °C. Duplicate injections of each sample were analysed.

*Mass spectrometry.*—Mass spectra were collected using a Perkin–Elmer QMASS-910 benchtop quadrupole mass spectrometer, with a source temperature of 190 °C, a source voltage of 70 eV, and a vacuum of $10^{-6}$ torr. The data was collected in full-scan acquisition mode. The column and operating conditions were the same as used for tautomer analysis.

*Crystallisation experiments.*—Seeded batch crystallisation experiments were carried out isothermally at 24 °C in a 1-L sealed, agitated glass vessel at an ethanol:water mass ratio of 9:1, according to the method of Johns et al. [13]. Seed crystals were prepared by ball-milling anhydrous D-fructose and retaining a 38–45-μm wet-sieved fraction. Samples were taken from the crystalliser for size analysis, total fructose concentration, and tautomer concentration determination. Total fructose concentration was determined gravimetrically [14]. Crystals were sized in fructose-saturated absolute ethanol, using a Malvern Mastersizer/E (Malvern, Malvern, England) with a 300-mm focal length lens. Crystal growth rates were calculated from these data by the method of Johns et al. [13].

## 3. Results and discussion

*Tautomer identification.*—A typical chromatograph of the silylated tautomers of fructose is shown in Fig. 2. Peaks a and b exhibited a large 217 $m/z$ mass fragment on their mass spectra which is characteristic of furanose forms [15], and the same identification as Cockman et al. [5] was used for these peaks. Peaks c and d were determined to be pyranose forms through the appearance of the large 204 $m/z$ mass fragment on their mass spectra [15]. The identity of peak c was confirmed as β-D-

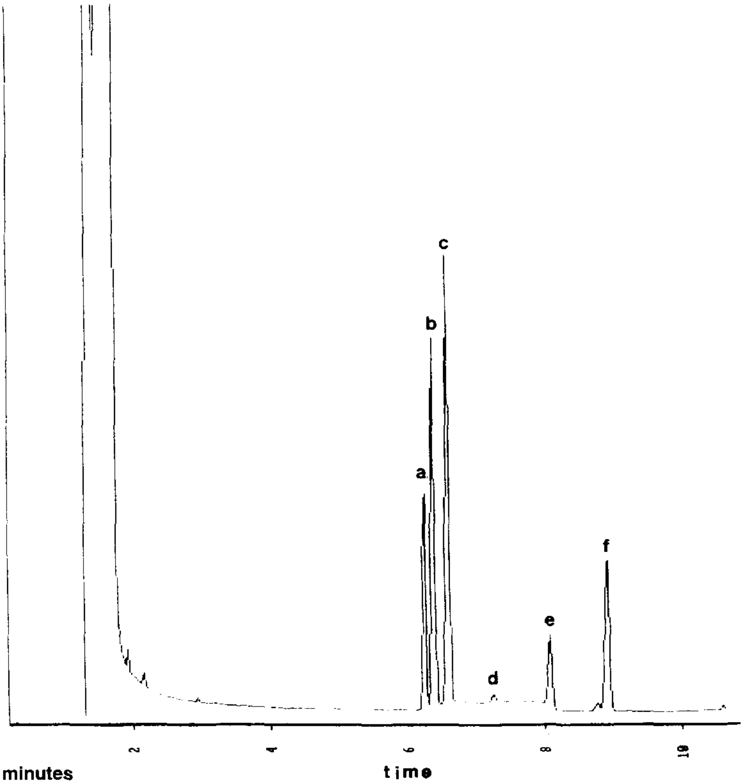

Fig. 2. Gas–liquid chromatogram of the trimethylsilylated derivatives of D-fructose. a = α-Furanose, b = β-furanose, c = β-pyranose, d = α-pyranose, e = acyclic tautomer.

fructopyranose through the silylation of crystalline fructose. The 204, 217, 306, and 319 $m/z$ mass fragments in the mass spectra of peak e identified it as the open-chain tautomer. Peak f on the chromatogram appears to be a non-carbohydrate artefact of the experimental technique. It contained neither the 204 or 217 $m/z$ mass fragments apparent in silylated fructose forms, although a 73 mass fragment does suggest that it is silylated. The chromatographic method was calibrated using a silylated aqueous solution of fructose at 20 °C, using the composition data of Shallenberger [4].

*Mutarotation equilibria.*—The mutarotation of fructose was studied at 24, 30, 40, and 50 °C, with ethanol:water mass ratios in the solvent of 3:1, 6:1, and 9:1. The effect of fructose concentration was not considered in this study as sugar concentration has been shown to have little effect on equilibrium tautomer concentration [6] or mutarotation rate [16]. This range of conditions represents the most likely conditions for industrial crystallisation. A typical time course of the mutarotation of β-D-fructopyranose dissolved in an ethanol–water mixture is shown in Fig. 3. Considerable time was required to achieve tautomeric equilibrium at all conditions tried. Final equilibrium values for the proportion of each tautomer in solution are given in Table 2. Percentages are considered accurate to ±2%.

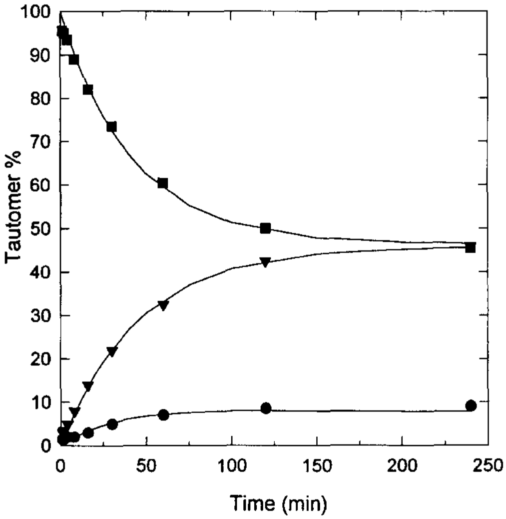

Fig. 3. Mutarotation of D-fructose at 24 °C and an ethanol:water mass ratio of 6.0:1.0. ● α-Furanose, ▼ β-furanose, ■ β-pyranose.

In ethanol–water solutions the β-D-fructopyranose (β-pyr) and β-D-fructofuranose (β-fur) tautomers are the major forms present at equilibrium under all conditions, with the α-D-fructofuranose form (α-fur) also present. The concentrations of the α-D-fructopyranose and the open-chain form were negligible. The concentration ratio of α-D-fructofuranose to β-D-fructofuranose was constant with time at a single set of conditions, within the experimental error of the technique (±2%). This suggests that the α-fur-

Table 2
Equilibrium composition of fructose in aqueous–ethanolic solutions

| Ethanol:water | Temp (°C) | Per cent | | |
|---|---|---|---|---|
| | | β-pyr | β-fur | α-fur |
| 3.0:1.0 | 24 | 40 | 50 | 10 |
| | 30 | 39 | 55 | 6 |
| | 40 | 40 | 53 | 7 |
| | 50 | 39 | 49 | 12 |
| 6.0:1.0 | 24 | 46 | 46 | 8 |
| | 30 | 39 | 55 | 6 |
| | 40 | 35 | 59 | 6 |
| | 50 | 38 | 50 | 12 |
| 9.0:1.0 | 24 | 38 | 57 | 5 |
| | 30 | 38 | 56 | 6 |
| | 40 | 34 | 60 | 6 |
| | 50 | 39 | 48 | 13 |

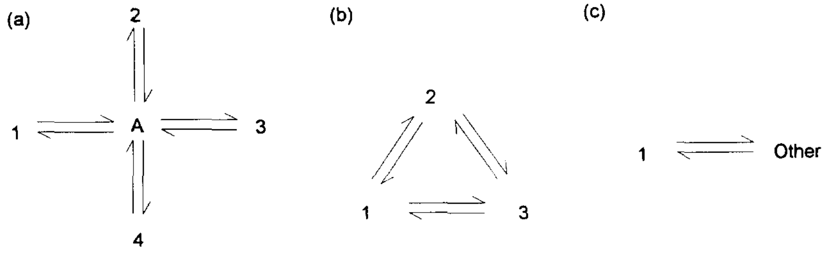

Fig. 4. Mutarotation schemes for D-fructose. 1 = β-Pyranose, 2 = β-furanose, 3 = α-furanose, 4 = α-pyranose, and A = acyclic tautomer. (a) Full mutarotation scheme. (b) Three-tautomer model. (c) Reduced model.

anose–β-furanose transformation is much quicker than that between the pyranose–furanose forms, in agreement with results of Shallenberger in water solutions [4].

*Kinetic model for fructose mutarotation.*—The interconversion between the five tautomers is considered to take place through the acyclic keto form as illustrated in Fig. 4. This corresponds to Fig. 4a, where β-pyr, β-fur, α-fur, and α-pyr will be referred to as 1, 2, 3, and 4, respectively, and the acyclic form as A. It is assumed that the interconversion reactions follow reversible first order kinetics where the rate constants $k_i$ refers to the reaction of the species i (i = 1, 2, 3, 4) to the acyclic form A, while $k'_i$ refers to the reverse reaction A → i.

The mutarotation kinetics may then be expressed as,

$$-\frac{dC_i}{dt} = k_i C_i - k'_i C_A \ (i = 1,4)$$

$$\frac{dC_A}{dt} = \sum_{i=1}^{4} k_i C_i - C_A \sum_{i=1}^{4} k'_i$$

If the concentration of any species is very much smaller than the others, the model may be simplified. In this case the acyclic keto form, (A) and the α-pyr form (4) are found to be at very low levels, i.e., $C_A$, $C_4 \ll C_1$, $C_2$, $C_3$, hence,

$$\frac{dC_A}{dt} \simeq 0; \text{ hence, } C_A = \sum_{i=1}^{4} k_i C_i / \sum_{i=1}^{4} k'_i$$

$$\frac{dC_4}{dt} \simeq 0; \text{ hence, } C_4 = k'_4 C_A / k_4$$

Substituting these two equations into the first, the kinetic relations become,

$$-\frac{dC_i}{dt} = k_i C_i - \xi_i \sum_{i=1}^{3} k_i C_i \ (i = 1,3)$$

where

$$\xi_i = k'_i / \sum_{i=1}^{3} k'_i \ (i = 1,3)$$

This is equivalent to a three-tautomer model (Fig. 4b), which has six rate constants, $k_{ij}$, where $k_{ij}$ applies to the reaction i → j. These link to the above rate constants by,

$$k_{ij} = \xi_i k_i$$

Further, if the interconversion kinetics between the two furanose forms (2 and 3) is very fast (i.e., $k_{23}$, $k_{32} \gg k_{12}$, $k_{21}$, $k_{13}$, $k_{31}$), it follows that the kinetics are effectively those of a simple two-tautomer system, 1 → others, (Fig. 4c), where others represents the other fructose species (largely the furanoses in equilibrium). Taking the forward and reverse rate constants for the effective binary system as $k$ and $k'$, it follows that,

$$k = k_{12} + k_{13}$$

$$k' = (k_{32}k_{21} + k_{23}k_{31})/(k_{32} + k_{23}) = (k_{21} + K_F k_{31})/(1 + K_F)$$

where $K_F = k_{23}/k_{32}$ is the equilibrium constant for the β-fur to α-fur (2 → 3) interconversion.

The results allow the complex mutarotation scheme described in Fig. 1 to be reduced to the binary scheme, β-D-fructopyranose–α- and β-furanoses, and two equilibrium constants, $K$ and $K_F$, defined by:

$$K = \frac{x_{\beta\text{-pyr}}}{x_{\alpha\text{- and }\beta\text{-fur}}}$$

$$K_F = \frac{x_{\alpha\text{-fur}}}{x_{\beta\text{-fur}}}$$

*Effect of temperature and ethanol:water ratio.*—The effects of temperature and solvent composition on the equilibrium constants are shown in Fig. 5. The values of the equilibrium constants $K$ and $K_F$ were found to be constant within experimental error

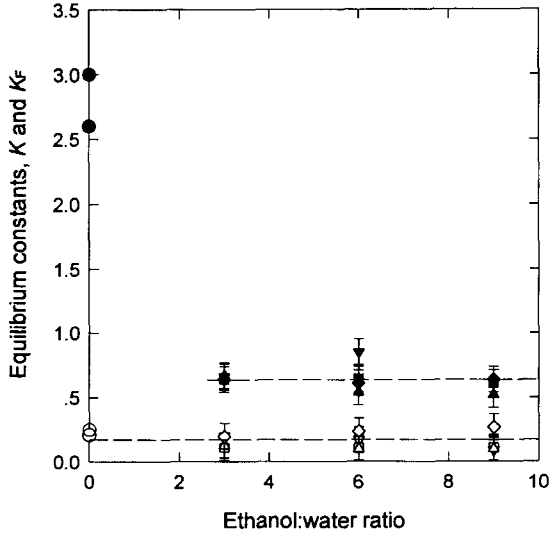

Fig. 5. Equilibrium constants for fructose tautomers at ▼ 24, ■ 30, ▲, and ◆ 50 °C. Hollow symbols are $K_F$, filled symbols are $K$. Shallenberger [4] and Cockman et al. [5].

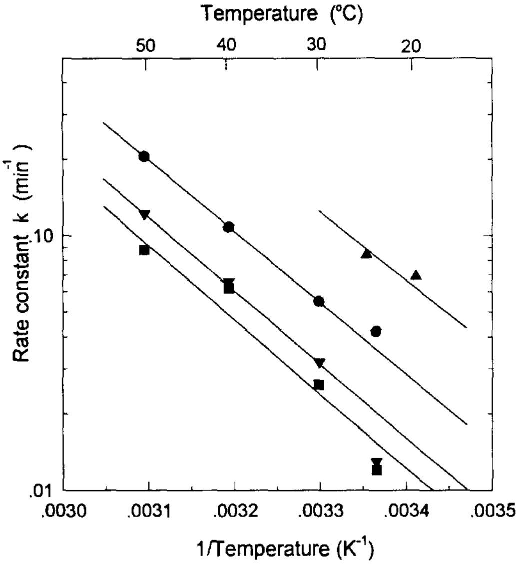

Fig. 6. Effect of temperature and ethanol:water ratio on the fructose mutarotation rate constant. ▲ Aqueous solution [4,5], ● ethanol:water = 3:1, ▼ ethanol:water = 6:1, ■ ethanol:water = 9:1.

(Fig. 5) over the range of temperatures and solvent compositions studied, although the value of $K$ was significantly less in the presence of ethanol than in water solutions.

In aqueous–ethanolic solutions, the furanose forms are relatively more stable than in aqueous solutions, although the ratio of the two furanose forms remains constant irrespective of the solvent composition. The higher proportion of the β-pyranose form in aqueous solution with respect to ethanolic mixtures is considered to be due to the preferential stabilisation of this fructose tautomer structure by the tridymite structure of water [17], the ordered nature of which is broken by the addition of small amounts of another solvent [10]. This also explains why the pyranose:furanose ratio is essentially constant over the ethanol composition range studied.

*Rates of mutarotation.*—The binary mutarotation scheme allows the rate of the mutarotation reaction to be adequately described by the rate constant for the conversion of β-D-fructopyranose to the furanose forms ($k$).

Values for the reaction rate constant, $k$, are plotted in Fig. 6 for the temperatures and ethanol:water ratios studied. The value of the rate constant at any ethanol:water ratio follows an Arrhenius relationship with respect to temperature. The activation energy of mutarotation is not affected by the solvent composition and has a value of 53.0 (±5) kJ/mol. The mutarotation rate constants for the forward ($k$), and reverse ($k'$) reactions are given in Table 3.

At any temperature the rate of mutarotation decreases as the concentration of ethanol in the solvent increases, which confirms previous qualitative observations by Verstraeten [9]. Note that the mutarotation rate constant in the most alcoholic sample is about one-fifth of that in water.

Table 3
Rate constants for mutarotation of fructose in aqueous–ethanolic solutions

| Ethanol:water | Temp (°C) | $k$ (min$^{-1}$) | $k'$ (min$^{-1}$) |
|---|---|---|---|
| 3.0:1.0 | 24 | 0.042 | 0.028 |
| | 30 | 0.055 | 0.035 |
| | 40 | 0.108 | 0.072 |
| | 50 | 0.205 | 0.131 |
| 6.0:1.0 | 24 | 0.013 | 0.011 |
| | 30 | 0.032 | 0.020 |
| | 40 | 0.066 | 0.036 |
| | 50 | 0.122 | 0.075 |
| 9.0:1.0 | 24 | 0.012 | 0.007 |
| | 30 | 0.026 | 0.016 |
| | 40 | 0.062 | 0.032 |
| | 50 | 0.088 | 0.056 |

*Effect of mutarotation on fructose crystallisation.*—Two batch crystallisations were performed in aqueous–ethanolic solutions under identical conditions, except that one experiment was initiated by adding seed crystals immediately after dissolving crystalline β-D-fructopyranose (when the β-D-fructopyranose form is in excess in solution), whereas the other was seeded with crystal only after the fructose tautomers present in solution had reached their equilibrium composition. Two observed initial crystal growth rates from the non-equilibrium mixture were significantly higher than those from the equilibrium mixture at the same supersaturation (defined as the difference between the solution fructose concentration [Fr], and the solubility limit of fructose [Fr$^*$] under the experimental conditions), as shown in Fig. 7. The reason may be the higher concentration of the β-D-fructopyranose tautomer, since the total fructose concentration in both vessels was equal. This is evidence that the rate of fructose crystallisation may be dependent on the concentration of the β-D-fructopyranose tautomer being crystallised and not on the total fructose concentration in solution.

This finding is significant when the crystallisation of carbohydrates having tautomers such as fructose is studied. During the above crystallisation the rate of mutarotation of D-fructose in the aqueous–ethanolic solutions was sufficiently slow that the relative proportion of the β-D-fructopyranose tautomer in solution was depleted by its crystallisation to a value less than the tautomer equilibrium value. This is shown in Fig. 8 where the measured fraction of β-D-fructopyranose in solution is shown during a further batch crystallisation. Although not shown in the figure, the tautomeric composition of the solution returned towards the final equilibrium value only when the crystal mass deposition rate, due to the low value of supersaturation, fell below the rate of mutarotation of furanose forms to the β-D-fructopyranose tautomer.

Fig. 8 also shows the β-D-fructopyranose in solution during the crystallisation, using a model incorporating the rate of crystallisation (with the crystal growth rate having a power law dependence on supersaturation) and the mutarotation kinetics described earlier. The model shows reasonable agreement with the experimental results.

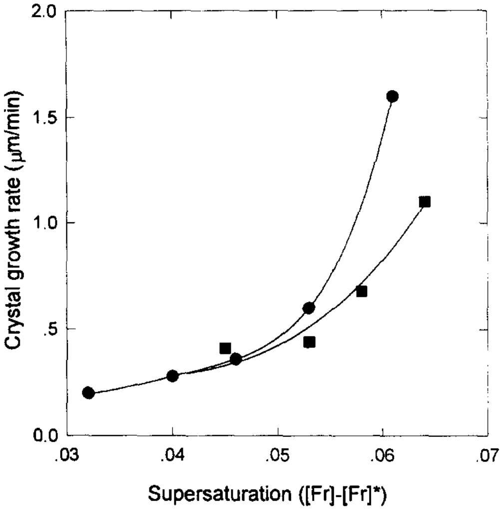

Fig. 7. Crystal growth rate of D-fructose in aqueous–ethanolic solutions at 24 °C. ● Seeded while β-D-fructopyranose is in excess. ■ Seeded at tautomeric equilibrium.

The possibility that the mutarotation rate might impede the rate of crystallisation of carbohydrates has been raised previously, particularly in the case of lactose [11], in which the less prevalent of the two lactose tautomers present in aqueous solution is crystallised. Our results demonstrate that this phenomenon seems to occur for fructose crystallised rapidly from ethanol–water mixtures. Work is proceeding in this laboratory on further study of this phenomenon.

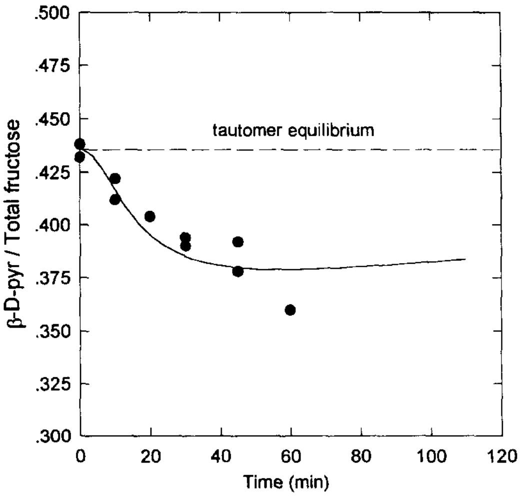

Fig. 8. Fraction of D-fructose in solution in β-D-fructopyranose form, at ethanol:water = 9:1 and 24 °C. ● Experimental values. (———) Model. (– – –) Equilibrium values.

## Acknowledgements

The authors thank Professor Peter Reilly (Department of Chemical Engineering, Iowa State University) for his assistance with gas–liquid chromatography, the interpretation of the mass spectra data, and his constructive criticism of the manuscript. Staff of the PEAC laboratory (University of Queensland) are acknowledged for their help with gas–liquid chromatography analyses. This work was funded by the Australian Research Council grant A89130370.

## References

[1] J.C. Mahoney, U.S. Patent 2 357 838 (1944); *Chem. Abstr.*, 39 (1945) 636.
[2] G.A. Day, European Patent 0 156 571 A2 (1985); *Chem. Abstr.*, 104 (1985) 70617w.
[3] A. Flood, J. Addai-Mensah, M.R. Johns, and E.T. White, *J. Chem. Eng. Data* (in press).
[4] R.S. Shallenberger, *Pure Appl. Chem.*, 50 (1978) 1409–1420.
[5] M. Cockman, D.G. Kubler, A.S. Oswald, and L. Wilson, *J. Carbohydr. Chem.*, 6 (1987) 181–201.
[6] L. Hyvönen, P. Varo, and P. Koivistoinen, *J. Food Sci.*, 42 (1977) 652–653.
[7] L. Hyvönen, P. Varo, and P. Koivistoinen, *J. Food Sci.*, 42 (1977) 654–656.
[8] S.J. Angyal and G.S. Bethell, *Aust. J. Chem.*, 29 (1976) 1249–1265.
[9] L.M.J. Verstraeten, *Adv. Carbohydr. Chem.*, 22 (1967) 230–289.
[10] P. Dais and A.S. Perlin, *Carbohydr. Res.*, 169 (1987) 159–169.
[11] A. Van Hook, *Crystallization Theory and Practice*, Reinhold Publishing Co, New York, 1961, p 253.
[12] Z.L. Nikolov and P.J. Reilly, *J. Chromatogr.*, 254 (1983) 157–162.
[13] M.R. Johns, R. Judge, and E.T. White, *A.C.S. Symp. Ser.*, 438 (1990) 199–209.
[14] Standard Laboratory Manual for Australian Sugar Mills, Vol. 2, 1991, method 19.
[15] H.C. Curtius, M. Müller, and J.A. Völlmin, *J. Chromatogr.*, 37 (1968) 216–224.
[16] G. Haase and T.A. Nickerson, *J. Dairy Sci.*, 49 (1966) 127–132.
[17] M.A. Kabayama and D. Patterson, *Can. J. Chem.*, 36 (1958) 563–573.