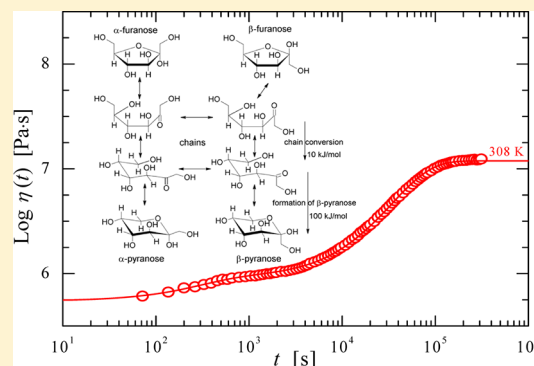


Rheological Study of Mutarotation of Fructose in Anhydrous State

Yangyang Wang,[†] Patryk Włodarczyk,[‡] Alexei P. Sokolov,^{†,§} and Marian Paluch^{*,||}[†]Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States[‡]Institute of Non-Ferrous Metals, ul. Sowinskiego 5, 44-100 Gliwice, Poland[§]Department of Chemistry, University of Tennessee, Knoxville, Tennessee 37996, United States^{||}Institute of Physics, University of Silesia, ul. Uniwersytecka 4, 40-007 Katowice, Poland

ABSTRACT: Rheological measurement was employed to study the mutarotation of D-fructose in anhydrous state. By monitoring the evolution of shear viscosity with time, rate constants for mutarotation were estimated, and two different stages of this reaction were identified. One of the mutarotation stages is rapid and has a low activation energy, whereas the other is much slower and has a much higher activation energy. Possible conversions corresponding to these two phases are discussed. This work demonstrates that, in addition to the routine techniques such polarimetry and gas–liquid chromatography, rheological measurement can be used as an alternative method to continuously monitor the mutarotation of sugars.



■ INTRODUCTION

A complicated and fascinating aspect of carbohydrate chemistry is that sugars can be found in different cyclic forms. Fructose, for example, exists in four ring forms (Figure 1): α -pyranose, β -pyranose, α -furanose, and β -furanose. The pyranose and furanose forms differ by the number of carbon atoms in the ring, whereas the α and β forms refer to structures with

different positions of the hydroxyl group on the anomeric carbon (in the case of D-fructose, it is the second carbon atom). These cyclic tautomers can transform from one to another, depending on the temperature and chemical environment. Such isomerization reactions are called mutarotation. This transformation is complicated, as it is composed of two sequential chemical reactions. The first reaction is related to the opening of the ring, and the second one is the formation of a different ring. The open-chain structure is the intermediate form between the two stages.

Mutarotation of sugars in solutions has been extensively studied by several different experimental techniques,^{1–4} including polarimetry, gas–liquid chromatography, and nuclear magnetic resonance. On the other hand, much less attention has been paid to the mutarotation in anhydrous state. Recent attacks on this problem include studies by temperature-modulated scanning calorimetry,^{5,6} dielectric spectroscopy,^{7–10} PVT measurements,¹¹ and Raman spectroscopy.^{12,13} Despite these efforts, there is still a number of fundamental issues that need to be firmly addressed.

Rheological measurement can provide a fast and precise way to continuously monitor the change of material properties and is commonly used as both online and offline characterization tools in a wide variety of industry applications.^{14,15} Vulcanization of rubbers and curing of epoxy resins, for example, are typically monitored by measuring the change of shear modulus or viscosity with time. In theory, rheological probe can be applied in any system where the change of chemical

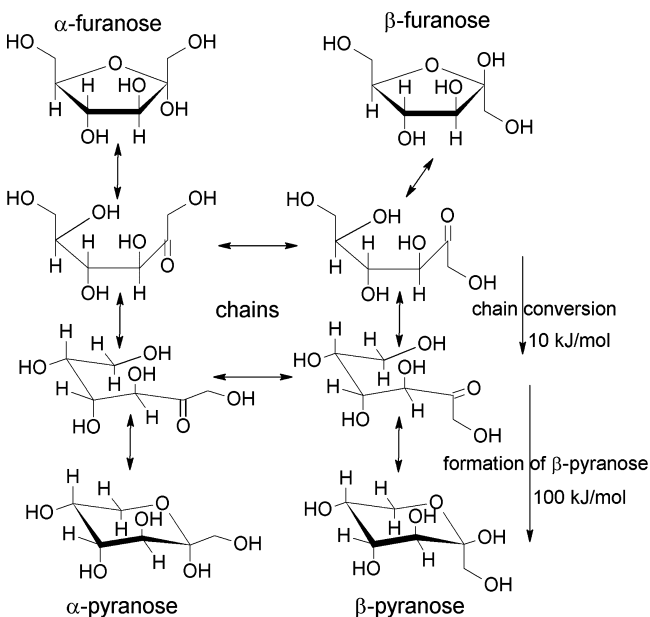


Figure 1. Structure of the four cyclic tautomers and chains of D-fructose.

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composition is accompanied by a change of mechanical properties. From the application's point of view, rheological tests are conceptually intuitive, easy to set up, and can provide direct information about mechanical properties that are often important to process and quality control.

In this work, we demonstrate the rheological measurement can be used to study the mutarotation of D-fructose in anhydrous state. Kinetic parameters have been obtained from the evolution of shear viscosity, and two different stages of mutarotation mechanism have been identified. The result of this study agrees reasonably well with the earlier investigation using dielectric spectroscopy.⁷ Although gas–liquid chromatography and polarimetry have been routine techniques to monitor mutarotation, rheological measurement can be an alternative and supplementary choice.

EXPERIMENTAL SECTION

Anhydrous D-fructose ($\geq 99\%$) was purchased from Sigma-Aldrich and used as received. The rheological measurements of D-fructose were carried out on an AR-2000ex rheometer (TA Instruments) equipped with an Environmental Test Chamber. Nitrogen was used as the oven gas source to avoid sample degradation at high temperature. In each test, fructose was sandwiched between 8 mm parallel plates and melted at 390 K for 2 min. The sample was subsequently quenched to the desired experimental temperature using liquid nitrogen cooling. After temperature stabilization, a constant stress of 1 kPa was applied, and the viscosity of the sample was recorded as a function of time.

RESULTS AND DISCUSSION

The crystalline D-fructose consists mainly of the β -pyranose form. Mutarotation of the β -pyranose to the other three forms (α -pyranose, α -furanose, and β -furanose) takes place upon melting of the D-fructose crystals. As the melting process occurs at high temperature (390 K), the equilibration process should be completed within 2 min. Quenching the sample causes destabilization of the equilibrium achieved at the melting point, and a new equilibrium that is specific to the new thermodynamic conditions has to be established. Because each of the four cyclic forms has different molecular mobility at a given temperature, the change of chemical composition of fructose can be monitored by measuring the evolution of shear viscosity.

Figure 2 presents the time-dependent shear viscosity of fructose at five different temperatures. In all cases, the shear viscosity increases monotonically with time and reaches a steady state at long time. This implies that the new low-temperature mixture of tautomers, that is, α -pyranose, β -pyranose, α -furanose, and β -furanose, on average has higher viscosity than the high temperature mixture achieved at melting point. Our earlier study indicates that the final mixture has a higher glass transition temperature than the initial one. This explains the increase of the shear viscosity during the mutarotation.

Six-membered rings such as pyranoses are typically the most stable forms of sugars. As a result, the concentration of five-membered furanoses in liquid sugar samples is usually less than 30% at ambient pressure and temperature. D-Fructose is a carbohydrate, which has a relatively large population of furanoses in the liquid state (up to 30% at ambient temperature and pressure). As the temperature becomes higher, the internal

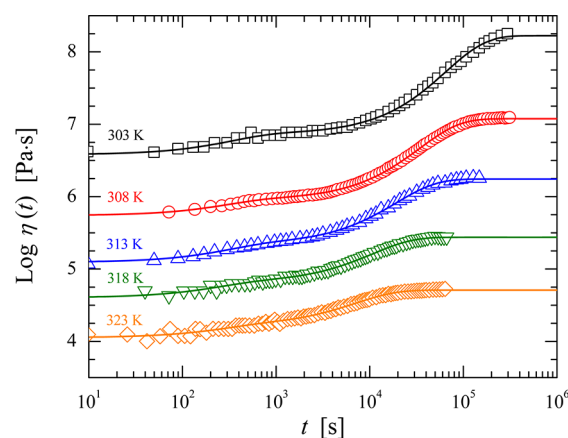


Figure 2. Evolution of shear viscosity with time. Solid curves: Fits of experimental data with the double-exponential function (eq 1).

energy of molecules is higher, and the difference in concentration between different cyclic forms decreases, which can be explained on the basis of the Boltzmann distribution. This leads to the conclusion that there is a larger population of furanoses in the high temperature melt, than in the quenched and equilibrated sample. Moreover, the fact that glass transition temperature rises when the quenched sample is equilibrated can be connected to the rising concentration of pyranoses. It means that in the case of D-fructose, pyranoses have a higher glass transition temperature than that of furanoses.

The second noticeable feature of the transient shear viscosity curves is that they all exhibit a two-step growth: a small step at short time and a much more pronounced step at long time. This suggests that the mutarotation of fructose in solid state consists of two phases. To extract kinetic parameters from the rheological data, the transient viscosity curves are fitted by the superposition of two exponential functions:

$$\log \eta(t) = \log \eta_1 + (\log \eta_2 - \log \eta_1)[1 - \exp(-k_1 t)] + (\log \eta_3 - \log \eta_2)[1 - \exp(-k_2 t)] \quad (1)$$

where k_1 and k_2 are characteristic rate constants, and $\log \eta_1$, $\log \eta_2$, and $\log \eta_3$ are viscosity constants. Note that $\log \eta_1$ represents the viscosity of the freshly quenched sample, $\log \eta_2$ is the viscosity of the intermediate mixture, and $\log \eta_3$ stands for the viscosity of the final mixture.

The proposed fitting function is based on three assumptions. The first is the so-called log–linear empirical mixing rule of viscosity (Arrhenius rule).^{16–19} It states the logarithmic viscosity of a mixture is the sum of the weighted logarithmic viscosity of each component:

$$\log \eta = \sum x_i \log \eta_i \quad (2)$$

where x_i and η_i are the molar fraction and viscosity of each component, respectively. Although this mixing rule is purely empirical, it has been proved to be useful in many small-molecule and polymeric mixtures. The second assumption behind the fitting function is that both the fast and the slow conversions follow first-order kinetics, that is, exponential time dependence. This cannot be strictly true, because of the complex nature of the multiphase and multipathway mutarotation reaction. However, both earlier investigations and the present study suggest that exponential functions can effectively and adequately describe the kinetics of D-fructose mutarotation.

Last, to simplify the mathematical treatment, it is further assumed that the rate constants are not affected by the change of viscosity during mutarotation, and they are significantly different (i.e., the two conversions are well separated in time). Otherwise, a slightly more complicated equation should be used.

The solid curves in Figure 2 are the fits of transient viscosity by the two-exponential function. At all five temperatures, the experimental data can be satisfactorily described by the proposed fitting function. The corresponding fit parameters are summarized in Table 1.

Table 1. Fitting Parameters of Transient Viscosity during D-Fructose Mutarotation by Equation 1

T [K]	k_1 [s^{-1}]	k_2 [s^{-1}]	$\log \eta_1$ [Pa·s]	$\log \eta_2 - \log \eta_1$ [Pa·s]	$\log \eta_3 - \log \eta_2$ [Pa·s]
303	3.09×10^{-3}	1.55×10^{-5}	6.582	0.279	1.362
308	3.50×10^{-3}	2.96×10^{-5}	5.740	0.215	1.122
313	3.30×10^{-3}	5.25×10^{-5}	5.094	0.247	0.900
318	4.32×10^{-3}	9.38×10^{-5}	4.605	0.206	0.629
323	3.77×10^{-3}	1.42×10^{-4}	4.053	0.164	0.491

The temperature dependence of rate constants is shown in Figure 3. Herein, the data from the earlier dielectric study are

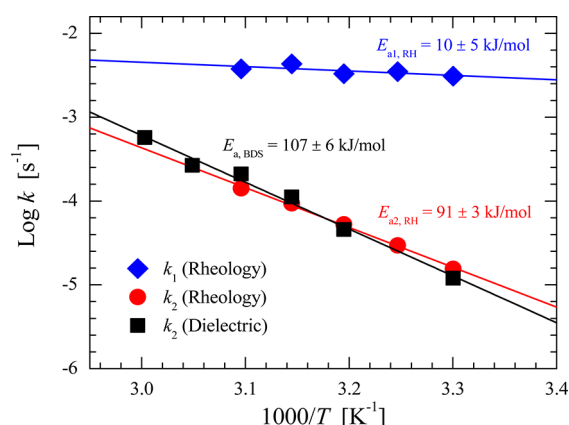


Figure 3. Comparison of rate constants determined from rheological and dielectric measurements. Solid lines: Arrhenius fits of the experimental data. Blue \blacklozenge : Rate constant of the fast conversion in rheological measurement. Red \bullet : Rate constant of the slow conversion in rheological measurement. \blacksquare : Rate constant of the slow conversion in dielectric measurement.

included as a comparison. The rate constants of both the fast and the slow processes decrease with decreasing temperature and can be well described by the Arrhenius equation:

$$\log k = \log k_0 - E_a/2.303RT \quad (3)$$

where E_a is the activation energy, and R is the gas constant.

The fast process has a low activation energy of 10 ± 5 kJ/mol, whereas the slow process has a much higher activation energy, about 91 ± 3 kJ/mol. The dielectric measurement reveals a slightly higher E_a (107 ± 6 kJ/mol) for the slow process than does the rheological measurement.⁷ This difference might be related to the different thermal history of the sample in the two experimental setups.

Furthermore, the viscosity of the initial, intermediate, and the final furanose–pyranose mixture can be extracted from the

fitting function. As mentioned earlier, $\log \eta_1$ represents the viscosity of the freshly quenched sample, $\log \eta_2$ is the viscosity of the intermediate mixture, and $\log \eta_3$ is the viscosity of the final mixture. The obtained viscosities are plotted as a function of $1000/T$ in Figure 4. The viscosities of the initial and the final

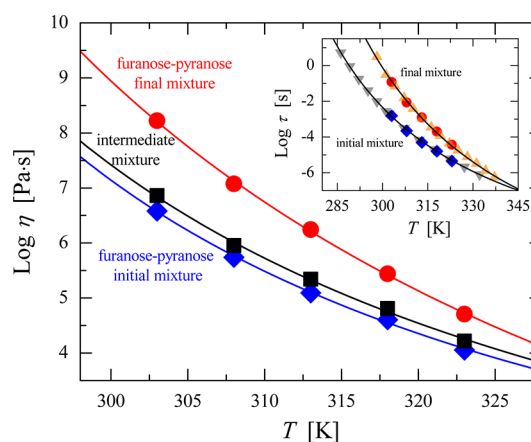


Figure 4. Temperature dependence of shear viscosity of the initial, intermediate, and final furanose–pyranose mixtures. Inset: Comparison of temperature dependence of structural relaxation time from rheological and dielectric measurement. Red \bullet : Final furanose–pyranose mixture (rheological). \blacksquare : Intermediate mixture (rheological). Blue \blacklozenge : Initial pyranose–furanose mixture (rheological). Orange \blacktriangle : Final mixture (dielectric). Gray \blacktriangledown : Initial mixture (dielectric). The concentration of β -pyranose is presumably higher in the final mixture.

furanose–pyranose mixture clearly have different temperature dependence, which should be related to the different glass transition temperatures of the two mixtures.

The viscosity data can be converted into structural relaxation time τ , by using the Maxwell relation, $\eta = G\tau$, with G being the shear modulus of the sample. The latter was used as a temperature-independent parameter with the value estimated by matching the dielectric and viscosity relaxation times at a single temperature. In the inset of Figure 4, the structural relaxation times from viscosity and dielectric measurement are compared, and good agreement between the two methods has been found. The temperature dependence of relaxation time can be fitted with the Vogel–Fulcher–Tammann (VFT) equation.^{20–22} Extrapolating τ to 100 s yields a glass transition temperature (T_g) of 282 K for the freshly quenched mixture and a T_g of 293 K for the final mixture. Figure 4 proves that the glass transition temperatures of different forms of fructose are different. This could be the origin of discrepancy of T_g values reported in the literature.^{23,24} It should be noted that to collapse the viscosity and dielectric structural relaxation time, different moduli are used in the calculation from the Maxwell relation: 2.51 GPa for the freshly quenched sample, and 1.38 GPa for the final mixture. This should be expected, as the chemical structures of the initial and final mixtures are different. Using the obtained T_g and VFT fitting parameters, one can further calculate the change of fragility of the sample.²⁵ The fragilities of the initial and final mixtures are 94 and 98 respectively, indicating that the sample with decreasing number of pyranose form becomes more fragile.

Last, we need to answer the following important question: What is the nature of the two stages in the mutarotation of anhydrous fructose? To reiterate, the rheological measurements have revealed that the mutarotation of anhydrous D-fructose

consists of two phases: a rapid and temperature-insensitive step with an activation energy around 10 ± 5 kJ/mol, and a much slower step with an activation energy of approximately 91 ± 3 kJ/mol.

Interestingly, earlier studies of mutarotation of fructose in water have also indicated a complicated mechanism.^{2,3,26} In particular, Shallenberger has pointed out the differences between the α - β and the furanose-pyranose transformation.² However, such an α - β conversion cannot be related to the fast process in our case, as the activation energy of conversion is only 10 kJ/mol. Both types of conversion, α - β (pyranose-pyranose) and pyranose-furanose, must go through the stage of open chain. This means that in both cases the covalent bonds in rings need to be broken. In general, the low activation energy can be attributed only to the conformational interconversion, not chemical reactions. In our specific case, the change of sugar conformation can influence the average viscosity of the sample. In the equilibrated sample, all conformations are also in equilibrium, but once the sample falls out of equilibrium upon quenching, the conformation ratios are disturbed. This can be explained on the basis of a single mutarotation path (Figure 1). In the pyranose-to-furanose transformation, D-fructose exists in three different chemical forms. The first one, pyranose, is a six-membered ring, the second one is the open-chain structure, and the third one is furanose, that is, a five-membered ring. The chain structure, a product of the pyranose decay, attains a conformation that can be characterized as pyranose-similar. The chain needs to be converted to the form that is furanose-similar before it can be closed to yield the furanose ring. Because the chain conformations are unequilibrated, the change of conformation can be reflected in the evolution of viscosity during the rheological measurement.

The change of viscosity during the change of chain conformation is relatively small, as compared to the dramatic change during the main stage when a different ring is formed. The major mutarotation stage, that is, the ring-to-chain or chain-to-ring conversion, is the origin of the slow rheological process with an activation energy equal to 91 kJ/mol. It can be related to the formation of the most stable pyranosidic form (from the chain) or to the decay of furanoses. The slowest process limits the rate of reaction; thus theoretical examination of energies of decay of furanoses and formation of pyranoses could be crucial for understanding which phenomenon is observed during the rheological or dielectric measurements. In an earlier article,¹¹ it is shown that the formation of β -pyranose within the framework of internal mechanism has a higher activation energy than the decay of furanoses (165 vs 150 kJ/mol). Therefore, it is very probable that the exponential kinetic curve represents the formation of β -pyranose from the chain tautomer. The proposed mutarotation mechanism, however, needs to be confirmed by future studies. It is worth noting that the mutarotations of fructose in anhydrous state and in solutions presumably have different mechanisms and follow different kinetic pathways. In ethanol-water mixtures, the activation energy of pyranose-furanose conversion is about 53 ± 5 kJ/mol,³ whereas in our case (anhydrous state), the activation energy is almost twice higher (91 kJ/mol). We emphasize that these values should not be directly compared, because in the solution case the transformations actually run in the opposite direction. The dissolution of D-fructose in water or water-ethanol mixture leads to the decay of crystalline β -pyranosidic rings and formation of furanoses. As the β -pyranose

decay probably has the highest activation energy, it would be the main process observed in solution.

CONCLUSIONS

In summary, mutarotation of anhydrous D-fructose has been studied in the temperature range of 303–323 K, and two stages of the reaction have been revealed. The fast process has a low activation energy of 10 ± 5 kJ/mol and is presumably related to the conversion of the conformations of a chain form, which is an intermediate product between the two main states. The slow process has a much higher activation energy of 91 ± 3 kJ/mol and corresponds to the formation of the new ring (probably the most stable pyranosidic ring) after the conformation change of the intermediate product. The result of this study is in reasonable agreement with our earlier dielectric spectroscopy studies and suggests the mutarotations of fructose in solutions and anhydrous state have different kinetic pathways.

The present study demonstrates that rheological measurement can be used to monitor the mutarotation of sugars. As compared to other techniques, rheological tests are inexpensive, conceptually intuitive, technically less demanding, and can be easily integrated in industrial production. It should be noted that the power of rheological measurement is not limited to the study of mutarotation. In principle, it can be applied to a wide variety of materials, where the change of chemical composition is accompanied by a change in viscosity.

AUTHOR INFORMATION

Corresponding Author

*E-mail: marian.paluch@us.edu.pl.

Notes

The authors declare no competing financial interest.

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