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Chromatographic Separations in Sugar Analysis and Processes

Kinetic study of the mutarotation of D-glucose in concentrated aqueous solution by gas-liquid chromatography

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Abstract

Growth is the most important step of industrial seeded crystallisation. The overall laws that govern α-D-glucose monohydrate crystal growth should depend upon mutarotation. This parameter could become a limiting factor, as it is the case for diffusion or the integration of molecules at the surface of crystal. Consequently, it was considered essential to obtain additional information on mutarotation kinetics, under temperature and concentration conditions close to solution saturation. Composition of D-glucose concentrated solutions was studied, using GC procedure after trimethylsilylation in pyridine. This is commonly used to obtain directly the anomeric composition of α and β-D-glucopyranose in solution. Moreover, minor components, such as D-glucofuranose, can be evidenced. GC has one main and obvious advantage over polarimetric measurement as it can be applied to turbid solutions, which is the case for concentrated syrups. Admitting that mutarotation is a first order kinetics reaction, it was found that concentration has no significant effect on the rate constant for mutarotation, whereas increasing temperature enhanced the mutarotation rate of D-glucose solution. Results show that the proportion of the α-anomer at equilibrium increases slightly with both saturation and temperature, whatever the anomer (α or β) in the original solution. These results agree with previous studies by Kraus and Nyvlt, who have established kinetic parameters of mutarotation at higher temperatures.

1. Introduction

Crystallisation of α-D-glucose monohydrate proves to be a slow process, which depends on different parameters. Amid these parameters, nucleation, transport of D-glucose molecules to the growing nuclei, adsorption and orientation on the crystal surface, desorption and dissipation of energy , are important to know together with mutarotation. Both temperature and concentration have an influence on the relative growth rate of crystals, because of the saturation limit. As D-glucose concentration in solution is increased, the interactions between individual sugar molecules become more significant, until crystallisation takes place. Moreover, with regard to the crystallising species α-D-glucose, the transformation in solution also includes mutarotation. It is known that the crystallisation of α-D-glucose monohydrate proceeds very slowly and its rate is even reduced by the presence of other sugars (Nesterova, Shterman, & Sapranov, 1988). Little is known, however, of how it is influenced by mutarotation, a characteristic property of D-glucose. When α-D-glucopyranose or β-D-glucopyranose crystals are dissolved in water, the fresh solution has the optical properties of the initial anomer. Each of these solutions, however, will change with time as the mutarotation reaction proceeds, until equilibrium between the alpha and beta forms is attained. As soon as α-D-glucose, crystallises, the solution becomes less concentrated in this anomer. Consequently, the equilibrium will be displaced along the growth of crystals.

Therefore, it was considered essential to obtain additional information on factors affecting the mutarotation of D-glucose. D-glucose shows relatively simple mutarotation behaviour, since the pyranose form of the sugar predominates in solution. The composition of mixture of anomers has been delineated extensively (Angyal, 1984). Sweeley, Bentley and Makita (1963) used GC techniques in their study of the maturational equilibrium of D-glucose.

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The effect of temperature and concentration on the tautomeric equilibrium was studied by Hyvonen, Varo and Koivistoinen (1977a, b). Only a limited number of papers dealt with the kinetics of mutarotation and most of work on rate constant took place at low concentration. (Maslov & Martsinovskaya, 1980; Perelygin, Krylskii, & Gulyuk, 1991). The aim of this paper is to describe D-glucose mutarotation under conditions comparable to that met in crystals production; i.e. at elevated temperatures and concentrations around saturation concentration. Applying of GC helps in determining the kinetic conditions of mutarotation of D-glucose. The results may provide explanation for the varying growth rate of α-D-glucose monohydrate. Likewise, the preponderance of β-anomer may be used to interpret the morphology of α-D-glucose monohydrate obtained after crystallisation.

2. Materials and methods

2.1. Choice of silylating reagent

The process of mutarotation of D-glucose anomers in water has been studied by gas chromatographic techniques since 1958 (Pigman & Isbell, 1968). In analytical chemistry, silylation has been used since the late fifties in gas chromatography and mass spectroscopy for the derivatization of a wide variety of products and functional groups. Silylation of a polar compound results in reduced polarity, enhanced volatility and increased thermal stability. The trimethylsilyl group is the most popular and versatile silyl group for these purposes, and a variety of trimethylsilyl agents with different properties (e.g. volatility, silylation by-product, reactivity, and selectivity) have been developed.

In this paper, effects of temperature and concentration on the mutarotation kinetics have been studied, using the gas chromatographic procedure based on Dutton's (1969) work. N-(trimethylsilyl) imidazole (TMSIM) and N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) were used. The trimethylsilylation has been carried out in pyridine solution. TMSIM is one of the most powerful silylating agents for hydroxyl group. Its selectivity against aliphatic amines together with its high reactivity makes TMSIM a widely used silylating agent especially for analytical purpose. The silylation procedure works normally with pyridine. It is mostly used without a catalyst, nevertheless, the silylation rate can be increased in our case by addition of BSTFA which is the most commonly used trimethylsilylating agent currently used for the analytical derivatisation of all classes of compounds.

Because of its polar nature, BSTFA, like BSA [N, O-bis(trimethylsilyl)acetamide] is able to act as its own solvent but the use of solvent of different polarity can influence its TMS donor strength (Gehrke & Patel, 1970). BSTFA has two main advantages over BSA in gas chromatography. BSTFA and its by-products are more volatile than BSA and so cause less interference in chromatograms. The presence of fluorine atoms results in less fouling of flame ionisation detectors by deposits of silica .

2.2. Experimental procedure

Anhydrous α-D-glucose (Roquette) or β-D-glucose (Sigma) were dissolved in distilled water equilibrated at 30, 35, 40 and 45°C. The experimented concentrations of D-glucose correspond to the following degrees of saturation of α-D-glucose monohydrate 0.55, 0.7, 0.8 and 0.9. It corresponds to the mass concentrations given in Table 1.

After dissolution, 1 μl of the above solution and 50 μl of anhydrous pyridine have been added in a 2-ml vial. Silylation was accomplished with 100 μl of BSTFA and 50 μl of TMSIM. Before injection, the solution was heated 20 min at 70°C to complete silylation. The sugar was analysed on a Varian Series 2800 chromatograph with a flame ionisation detector and a packed column DB-1 (30–0.32–0.25). The oven temperature is programmed between 150 and 230°C (7°C/min) and the split is 50ml/min, with a flow rate of 40 ml/min.

3. Results and discussion

3.1. Minor component

Chromatograms of D-glucose derivatives are reported in Fig. 1. They are directly informative on the proportions of α and β anomers. Nevertheless, minor peaks are also observed. The first experiment allows following of the proportion of unknown component during 4 h, until equilibrium is reached. Results are shown in Fig. 2. As may be observed, the third minor product does not change with time and corresponds to less than 1% of total at equilibrium. This peak was not taken into account for the calculation of the anomeric composition

Table 1
Concentrations (% m/m) of D-glucose solutions corresponding to different saturation ratios σ and temperatures

	$\sigma = 0.55$	$\sigma = 0.7$	$\sigma = 0.8$	$\sigma = 0.9$
30°C	40	46	49	52.2
35°C	43.8	49.8	53.1	56
40°C	50	53.8	57.1	60
45°C	52.1	58.2	61.4	62.4

of D-glucose solution. As reported by Bentley and Botlock (1967), such peaks are assumed to be due to incomplete silylation. They were found to represent glucose anhydrides formed during the silylation procedure. It is very likely that such minor components correspond to D-glucofuranoses. The predominant anomers (α-D-glucopyranose and β-D-glucopyranose) are effectively converted to each other via an open chain to reach the equilibrium. The alpha and beta forms have similar stability but the aldehyde form is less stable (Yamabe, 1999). From the literature, and whatever the method, it may be observed that only 1% or less of D-glucofuranose is found at equilibrium (Table 2).

3.2. Anomeric equilibrium of D-glucose

The influence of temperature on the composition of D-glucose solutions was studied in the range of 30–45°C varying saturation ratio. GC results are reported in Table 2. The results show that the proportion of the α-anomer in the equilibrium mixture increases slightly with increasing both concentration and temperature. These results are in good agreement with Hyvonen, Varo and Koivistoinen (1977a, b), who described the influence of temperature on the tautomeric equilibria of D-glucose and determined the anomeric composition at 5, 20 and 80°C

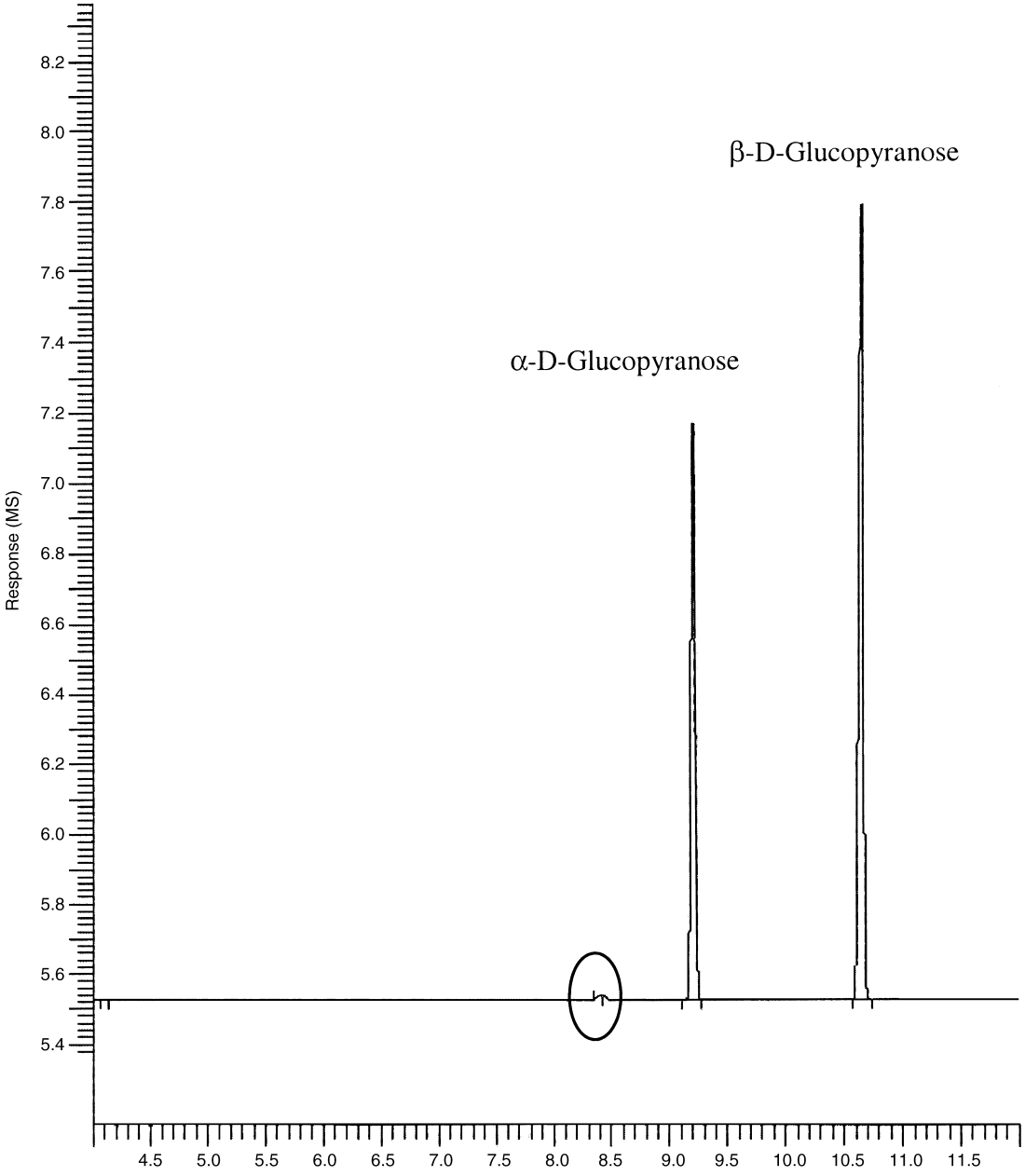

Fig. 1. Gas-liquid chromatograph of the TMSI-D-glucose tautomers (conditions of experiment are T = 30°C, C = 46% (w/w) whcih corresponds to saturation degree σ = 0.55).

Results reported in Table 3 were obtained from a freshly prepared β-D-glucose solution. We observed that the effect of temperature and concentration on the anomeric equilibrium obtained with a fresh solution of α-D-glucose are fairly equal to those obtained with a fresh solution of β-D-glucose. The average results of anomers concentrations obtained from these two solutions are summarised in Table 4. Finally, we can say that working in concentrated solution at high temperature is on the side of the alpha anomer.

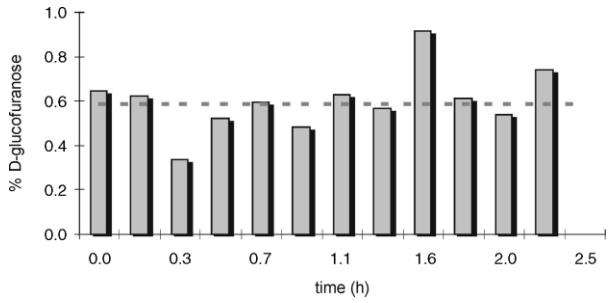

Fig. 2. Evolution of unknown peak (assigned to D-glucofuranose) during nutarotation (▒ average).

3.3. Mutarotation rate

Kinetic studies were performed at 30, 35, 40 and 45°C in a freshly prepared solution with α or β-D-glucose, at a low saturation ratio: $\sigma = 0.55$. These experiments were repeated at higher concentrations corresponding to the following saturation ratios: $\sigma = 0.7$, 0.8 and 0.9. It was observed that the higher the solution concentration, the slower the dissolution of α-D-glucopyranose crystals. This refers to a lack of precision in the determination of kinetic rates during the first hours of mutarotation (Fig. 3). Moreover, the presence of such high standard deviation in the reported proportions of anomers (Table 4) is very likely due to the difference in solubility

Table 2
Composition of D-glucose solution at equilibrium studied by different methods and authors

Ref.	Method	α-D-glucopyranose	β-D-glucopyranose	D-glucofuranose	Open chain
Lemieux and Stevens (1966)	NMR	36	64		
Angyal and Pickels (1972)	NMR	38	62	<	< 0.1%
Los, Simpson, and Wiesner (1956)	Polarography				0.03%
Hyvonen et al. (1977b)	NMR	39	63	< 1%	
Hyvonen et al. (1977a)	GC	38	62	< 1%	

Table 3
Composition of D-glucose in aqueous solutions at anomeric equilibrium obtained by GLC of the trimethylsilylethers at different concentrations and temperatures

σ	Temperature and percentage of the anomers at equilibrium[a]							
	30°C		35°C		40°C		45°C	
	α-D-Glc	β-D-Glc	α-D-Glc	β-D-Glc	α-D-Glc	β-D-Glc	α-D-Glc	β-D-Glc
0.55	39.11	60.89	39.84	60.16	40.13	59.87	40.91	59.09
0.7	40.17	59.83	40.43	59.57	40.66	59.34	41.36	58.64
0.8	40.11	59.89	40.73	59.27	40.86	59.14	41.95	58.05
0.9	40.02	59.98	41.04	58.96	41.24	58.76	41.77	58.23

[a] σ, Saturation ratio (corresponding concentrations at the different temperatures are given in Table 1).

Table 4
Comparison of the anomeric equilibrium of α and β-D-glucose freshly prepared solutions

σ	Temperature and percentage of the anomers at equilibrium[a]											
	30°C			35°C			40°C			45°C		
	α-D-Glc	S.D.	C.V.	α-D-Glc	S.D.	C.V.	α-D-Glc	S.D.	C.V.	α-D-Glc	S.D.	C.V.
0.55	38.87	1.07	2.76	39.90	0.09	0.23	40.36	0.33	0.82	41.01	0.14	0.34
0.7	40.05	0.16	0.42	40.40	0.03	0.08	40.64	0.03	0.07	41.42	0.08	0.20
0.8	40.38	0.38	0.96	39.74	1.39	3.50	40.94	0.12	0.29	41.9	0.07	0.16
0.9	39.93	0.12	0.30	40.95	0.12	0.31	–	–	–	–	–	–

[a] σ, Saturation ratio (corresponding concentrations at the different temperatures are given in Table 1). α-D-G, proportion of α-anomer in the equilibrated solution (%); S.D., standard deviation; C.V, coefficient of variation.

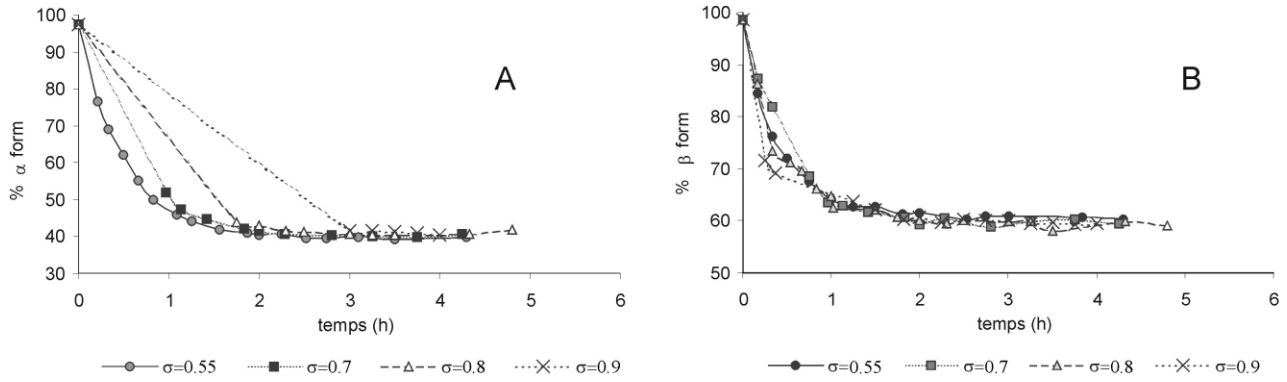

Fig. 3. Evolution of anomet proporation in aqueous solution of D-glucose (A: the dissolved crystals are that of α-anomet; B: the dissolved crystals are that of β-anomer) during mutarotation at different concentrations (expressed as saturation ratio σ) and T = 30°C.

Table 5
Mutarotation rate constants (k_α and k_β) based on GC peaks areas of derivatised β-D-glucopyranose[a]

σ	30°C		35°C		40°C		45°C	
	k_α	k_β	k_α	k_β	k_α	k_β	k_α	k_β
0.55	1.441	0.926	1.541	1.021	1.853	1.242	2.422	1.677
0.7	1.163	0.787	1.379	0.936	1.482	1.016	2.321	1.632
0.8	1.258	0.849	1.922	1.321	1.947	1.345	2.786	2.013
0.9	1.209	0.807	1.527	1.063	1.502	1.085	2.975	2.134

Table 6
Mutarotation average rate constants (k_α and k_β) and mutarotation equilibrium constant (k_m) as a function of temperature

	Temperature of kinetic study							
	30°C		35°C		40°C		45°C	
	k_α	k_β	k_α	k_β	k_α	k_β	k_α	k_β
K (h^{-1})	1.266	0.842	1.592	1.085	1.696	1.172	2.626	1.864
S.D.	0.123	0.061	0.232	0.166	0.239	0.149	0.307	1.248
C.V.	0.097	0.073	0.146	0.153	0.141	0.127	0.117	0.133
K_m	1.503		1.467		1.447		1.409	

of anomers, resulting from anomer specific intermolecular interactions. Therefore, it was decided to only report the rate constants determined using freshly prepared β-D-glucopyranose solution.

Using specific peaks for each of the anomers, their concentration as a function of time was derived from the evolving chromatographic profiles. Monitoring the relative concentration of each anomer, it is possible to determine the rate constant of mutarotation based on the experimental data within a range of 4 h.

The mutarotation of D-glucose may be considered a reversible mono-molecular reaction of first order, and the rate constants are k_α and k_β. At the beginning of the dissolution of β-D-glucose only, this anomer is present in the solution, and only during the experiment does the concentration gradually change with k_β as velocity constant. Likewise, the concentration of α-D-glucose will change with kα until equilibrium is reached. Kinetic constants (h^{-1}) of mutarotation in both directions are listed in Table 5.

The rate constant k_α is systematically higher than k_β. Observation of results show that concentration has no significant effect on the velocity constant for mutarotation. That is why we can determine the average value of k for each temperature and the ratio of kinetic constants can be substituted by the equilibrium constant of mutarotation K_m (Table 6). A temperature effect is also reported. Increasing temperature enhanced the mutarotation rate of D-glucose in aqueous solution. Comparing our results with that of Kraus and Nyvlt (1994) allows extension of the temperature range from 30 to 70°C. In this domain we have crystallization of α-D-glucose monohydrate (< 48°C) and that of anhydrous α-D-glucose (> 48°C). The trend (increase with temperature

of k_α) is observed for our results as for Kraus and Nyvlt (1994).

4. Conclusion

A kinetic study of the mutarotation reaction of D-glucopyranose in concentrated aqueous solutions can be performed using gas chromatography after derivatisation. Direct determination of the proportion of both anomers is possible. Traces of other forms, very likely, D-glucofuranose is detected by this method.

One of several other advantages associated with this method is that it can be applied to solutions which are very turbid, moreover difficult to analyse by polarimetry, which is the case for concentrated syrup. GLC results indicate that increasing both the D-glucose concentration and temperature slightly increased the proportion of the α-anomer at equilibrium. On the other hand, we can conclude that concentration has no significant effect on the rate constant of mutarotation, whereas increasing temperature enhanced the mutarotation rate of D-glucose in solution.

In all cases, we obtained the anomeric equilibrium after only 4 h. According to Arkipovitch and Petruschevskii (1970), for low concentrated solution, it appears that mutarotation cannot be a kinetic limiting factor of α-D-glucose crystal growth. However, because of the known equatorial conformation of β-D-glucose and of the strong structure of water around this solute (Kabayama & Patterson, 1958), it is possible to predict that the dehydration step, before crystallisation, becomes very slow. The equatorial hydroxyl groups of β-D-glucose will be more strongly hydrated than the axial groups. Consequently, the preponderance of β-D-glucose in solution can explain the slow rate of growth of α-D-glucose monohydrate crystals.

References

Angyal, S. J. (1984). The composition of reducing sugar in solution. *Advances in Carbohydrate Chemistry and Biochemistry, 42*, 15–68.

Angyal, S. J., & Pickels, V. A. (1972). Equilibria between pyranoses and furanoses. II-Aldoses. *Aust. J. Chem., 25*, 1695–1710.

Arkipovitch, N. A., & Petrushevskii, V. V. (1970). Effect of mutarotation on the rate of crystallization of hydrated glucose. *Sakh. Prom-st, 2*, 84–85.

Bentley, R., & Botlock, N. (1967). A gas chromatographic method analysis of anomeric carbohydrates and for determination of mutarotation coefficients. *Anal. Biochem., 20*, 312–320.

Dutton, G. S. (1973). Applications of gas-liquid chromayography to carbohydrates : Part 1. *Advances in Carbohydrate Chemistry and Biochemistry, 28*, 11–160.

Gherke, C. W., & Patel, A. B. (1970). Effect of solvents on derivatization using bis(trimethylsilyl)trifluoroacetamide. *Journal of Chromatography, 53*, 201.

Hyvonen, L., Varo, P., & Koivistoinen, P. (1977a). Tautomeric equilibria of D-glucose and D-fructose: Gas-liquid chromatographic measurements. *Journal of Food Science, 42*, 654–656.

Hyvonen, L., Varo, P., & Koivistoinen, P. (1977b). Tautomeric equilibria of D-glucose and D-fructose: NMR spectroscopic measurement. *Journal of Food Science, 42*, 657–659.

Kabayama, M. A., & Patterson, D. (1958). The thermodynamics of mutarotation of some sugar. II-Theoretical considerations. *Canadian Journal of Chemistry, 36*, 563–573.

Kraus, J., & Nyvlt, J. (1994). Crystallization of anhydrous glucose. I-Mutarotation rate and solid-liquid phase equilibria. *Zuckerind, 119*, 24–29.

Lemieux, R. U., & Stevens, J. D. (1966). The proton magnetic resonance spectra and tautomeric equilibria of aldoses in deuterium oxide. *Canad. J. Chem., 44*, 3249-262, .

Los, J. M., Simpson, L. B., & Wiesner, K. (1956). The kinetic of mutarotation of D-glucose with consideration of an intermediate free-aldehyde form. *J. Amer. Chem. Soc., 78*, 1564–1568.

Maslov, I. A., & Martsinovskaya, E. G. (1980). Effects of choice of anomer on the kinetics of mutarotation of D-glucopyranose in aqueous solution. *Russ. J. Phys. Chem., 54*, 4583-584, .

Nesterova, I. N., Shterman, V. S., & Sapranov, A. R. (1988). Procedures for shortening the time required for crystallization of glucose hydrate. *Pishch. Prom-st, 11*, 23–24.

Perelygin, Y. M., Krylskii, D. V., & Gulyuk, N. G. (1991). Effect of mutarotation on the kinetics of mass crystallization of glucose. *Izv.Vyssh.Uchebn., Zaved.Pishch.Technol., 4–6*, 36–39.

Pigman, W., & Isbell, H. S. (1968). Mutarotation of sugar in solution. Part 1: History, basic kinetics and composition of sugar solutions. *Advances in Carbohydrate Chemistry and Biochemistry, 23*, 11–57.

Sweeley, C. C., Bentley, R., & Makita, M. (1963). Gas-Liquid Chromatography of trimethylsilyl derivatives of sugar and related substances. *J. Amer. Chem. Soc., 85*, 2497–2500.

Yamabe, S. (1999). Theoretical study of mutarotation of glucose. *Journal of Organic Chemistry, 64*, 4519–4524.