

High Pressure Kinetics of Glucose Mutarotation Studied by Optical Rotation

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This paper describes an apparatus allowing continuous determinations of optical rotation of solutions at variable pressures, and its application to the study of mutarotation of glucose. The specific optical rotation of the components was found to be pressure independent in preliminary measurements.

With the apparatus the kinetics of the general acid, general base catalyzed mutarotation of glucose has been studied at 25 °C, 1–1200 atm (1 atm = 101.325 kPa), in the pH range where it is virtually independent of pH.

The observed rate constant $k = k_a + k_b$ increases with pressure while the equilibrium constant $K_{eq} = k_a/k_b$, is nearly unchanged from the 1.75 found at 1 atm.

From the experimental results the individual rate constants k_a and k_b and the corresponding activation volumes ΔV_i^\ddagger and ΔV_{-1}^\ddagger can be calculated. The values obtained, -11.7 and -10.8 cm³/mol, are discussed in relation to the reaction mechanism.

The first paper in the field of high pressure chemistry was published in 1892. In this paper, Röntgen¹ described a retarding effect on the acid catalyzed inversion of sucrose at elevated pressures. However, because of engineering difficulties, it is only during the last fifteen years that the influence of pressure on the rate of chemical reactions has begun to be investigated intensively. Today observation of the influence of pressure on the rate constant is a valuable tool in the study of the mechanism of reactions. In this paper an apparatus allowing continuous measurements of optical rotation as a function of pressure up to 1500 bar is described. The apparatus is of general interest since it allows, in principle, the study of any reaction accompanied by a change in optical rotation. The reaction investigated here

is the mutarotation of glucose. Since the discovery by Dubrunfaut² that the optical rotation of glucose dissolved in water changes with time, approaching a final value, the reaction has been so intensively studied that even a tabulation of the literature would be too extensive. References to papers before 1942 may be found in Bates.³ In particular the classical paper of Brønsted and Guggenheim⁴ may be mentioned. For a discussion of the different proposals for the mechanism of the reaction see Swain.⁵

The reaction consists of an interconversion of the α - and β -form of glucopyranose. Although the reaction is generally catalyzed by acids and bases, it is independent of pH over a wide range from 2.5 to 7.⁶ By performing the experiments near the middle of this range, the reaction is made insensitive to such changes in pH as could be the result of pressure effects on the various acid constants. In this way a change of rate with pressure may be assigned to the rate constants alone, but not to catalyst concentrations.

A condition for using optical rotation in pressure assays as a measurement of concentration is that the specific optical rotation should not be altered with pressure. In the case of glucose one can evaluate the optical rotation of the starting form by extrapolating to zero time. The initial rotation at the pressure P , evaluated from a series of measurements at this pressure should then be identical with the zero time rotation at 1 bar when divided by the relative solution volume at P . Further support for the independence of the specific optical rotation of pressure was obtained from a series of measurements on sucrose.

EXPERIMENTAL

Pressure generator. In a two-stage process a pre-set pressure from 600 to 10 000 bar can be delivered and maintained by the generator. The connection tube to the measuring cell is a stainless steel tube 10 mm O.D., 2 mm I.D. The pressure-transmitting medium is a mixture of 50 % white spirit and 50 % DialaC. A detailed description has been given in a previous paper.⁷

Pressure measurements. The high pressure is read from a Bourdon manometer on which is mounted a sensor contact serving as an on-off regulator. This manometer is calibrated against another Bourdon manometer (NOVA, 0–4 kbar) guaranteed to within 1 %.

Measuring cell. In a previous paper⁸ a thermostated high pressure cell for spectrophotometric measurements has been described. As can be seen from Fig. 1 the cell we used for optical rotation has a somewhat similar construction. However, sapphire windows cannot be used in this case, since they become birefringent with elevated pressure. Several

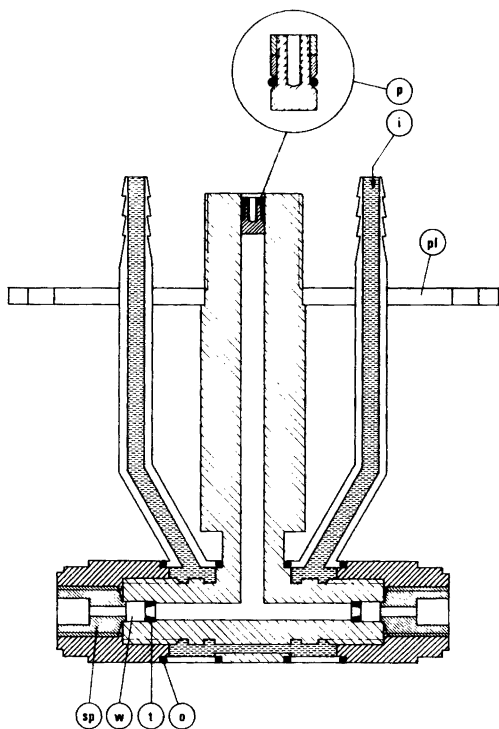


Fig. 1. Measuring cell for optical rotation at high pressure. p: Floating piston, i: Inlet for thermostat water, pl: Plate for fixation of the cell, sp: Steel plug supporting the window, w: Window, t: Teflon guide ring, o: O-ring water seal.

different materials have been investigated for the purpose, among others quartz and different sorts of glass. For the assays here described Schotter Glas K5 was used. Before and after each run the optical rotation of the cell filled with water was determined at several pressures. In some cases a slight optical rotation (0–0.3°) owing to the birefringency, was observed which remained constant until the next mounting of the cell. The light path between the two glasses is approximately 6.50 cm but has to be determined after each polishing by measurements of a calibration solution.

Detection equipment. The cell was built to fit a Zeiss polarimeter allowing readings with an accuracy of 0.005°. The wave length used was 546 nm. The temperature in the cell was 25.00 ± 0.05 °C.

A very accurate fixation of the cell in the light path was an indispensable necessity since the double refraction caused by a slight tilting ruined the measurements.

Procedure. Acetate buffer pH 4.75, 0.005 M with respect to sodium acetate and 0.005 M with respect to acetic acid. Sugars: 10 g in 100 ml solution.

In a typical experiment 10 g of the sugar was placed in a volumetric flask at 25 °C. The buffer at the correct temperature was added with agitation. Zero time was defined as the time when half the amount of buffer was added. After transferring the reaction mixture to the measuring cell, thermostated at 25 °C, and application of the desired pressure, the readings were started, usually 6–7 min after mixing.

RESULTS

With a linear relation between concentration of solutes and optical rotation α , a first-order reaction in solution will give rise to the following dependence of α on t :

$$\alpha(t) = \alpha(\infty) + [\alpha(0) - \alpha(\infty)] \exp(-kt)$$

Neither the usual method of plotting $\ln[\alpha(t) - \alpha(\infty)]$ vs. t [which rests heavily on $\alpha(\infty)$] nor Guggenheim's method (which aims mainly at calculating k) are entirely satisfactory for the determination of $\alpha(\infty)$. Instead, a nonlinear least-squares method was adopted, which rests on minimizing the sum

$$s = \sum_j [\alpha_j - \alpha(t_j)]^2$$

where α_j is the j 'th measurement taken at t_j , and $\alpha(t_j)$ is the value calculated from the above functional relationship. s depends on the choice of the

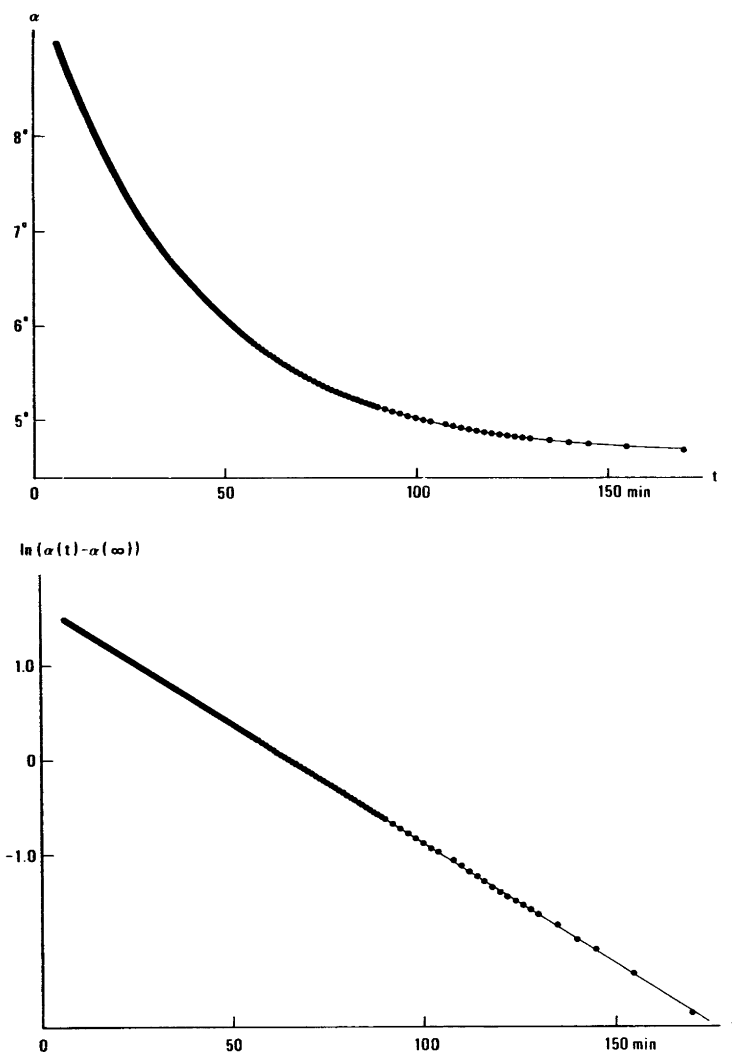


Fig. 2. Experimental and calculated data for a typical mutarotation run, starting with α -glucose, at 1 bar. a. Plot of experimental data (α_j, t_j); the curve is based on computer calculated parameters. b. Plot of $\ln[\alpha_j - \alpha(\infty)]$ vs. t using the same data set. Both $\alpha(0)$, $\alpha(\infty)$ and k were calculated using the procedure described.

parameters $\alpha(0)$, $\alpha(\infty)$, and k , and it is possible in a series of iterations to approach a local minimum of s systematically by varying the three parameters. A succinct description is given in Ref. (9). The method has several advantages: all measurements are used with equal weight, all three parameters are treated on equal footing and a maximum of precision is obtained. An Algol program was written and used for the calculations. Fig. 2a shows a plot of both

observed α_j 's and a curve based on the calculated parameters for a typical run. A plot of $\ln[\alpha(t) - \alpha(\infty)]$ vs. t based on the same set of data and a computer calculated value of $\alpha(\infty)$ is shown in Fig. 2b.

Calculations in the remainder of the paper are based on the assumption that the optical rotation due to a solute is proportional to the volume concentration with a proportionality factor indepen-

Table 1. Experimental data on optical rotation of sucrose at 25 °C. Last row gives the ratio optical rotation/volume concentration.

<i>P</i> /bar	1	510	705	900	1100
$\alpha(25\text{ °C}, 546\text{ nm})/\text{degree}$	5.260	5.368	5.413	5.459	5.481
<i>c</i> /(g/100 cm ³)	10	10.220	10.298	10.374	10.449
α/c	0.5260	0.5252	0.5256	0.5262	0.5246

Table 2. Kinetic experiments with α -glucose (α -G) and β -glucose (β -G) at 25 °C. *k* is the rate constant determined experimentally, *k_f* and *k_b* the calculated forward and backward rate constants.

<i>P</i> /bar	Reactant	$\alpha(\infty)/\text{degree}$	<i>k</i> /min ⁻¹	<i>K_{eq}</i>	<i>k_f</i> /min ⁻¹	<i>k_b</i> /min ⁻¹
1	α -G	4.62	0.02485	1.739	0.01578	0.00907
1	α -G	4.63	0.02522	1.730	0.01598	0.00924
1	β -G	4.66	0.02558	1.704	0.01612	0.00946
705	α -G	4.77	0.03630	1.729	0.02300	0.01330
705	β -G	4.70	0.03612	1.791	0.02318	0.01294
705	β -G	4.75	0.03505	1.746	0.02229	0.01276
1145	α -G	4.91	0.04182	1.676	0.02619	0.01563
1145	α -G	4.74	0.04344	1.824	0.02806	0.01538
1145	β -G	4.66	0.04209	1.899	0.02757	0.01452

dent of pressure in the range examined. It is not possible to test this hypothesis accurately with α -glucose and β -glucose since both mutarotate upon dissolution in water. Instead we have chosen sucrose which has a constant optical rotation in aqueous solution. The results of such a test are seen in Table 1 which gives the optical rotation at 25 °C, 546 nm when the cell is filled with a sucrose solution, the volume concentration of which is 10 g per 100 cm³ at 1 bar. Its value at higher pressures may be estimated from Grindley and Lind's¹⁰ values of the specific volume of water as a function of pressure, assuming the solution to behave like pure water. By fitting a fourth degree polynomial to their values, $V(P)/V(1\text{ bar})$ was calculated at the pressures of the experiments, and the resulting sucrose volume concentrations are given in Table 1. Inspection of the last row shows that the ratio optical rotation/volume concentration of sucrose is independent of pressure in the range considered. We take this as sufficient evidence that the same applied to α -glucose and β -glucose at elevated pressure.

The results of the kinetic experiments are summarized in Table 2. Both α -glucose and β -glucose have been used as reactants; the choice is indicated

in the second column. The third and fourth columns give $\alpha(\infty)$ and the rate constant *k* (based on natural logarithms) as calculated from the method of least squares. It will be noted that, at a given pressure, both are independent of the choice of starting material. The equilibrium constant *K_{eq}* of the reaction, listed in the fifth column, is calculated from $\alpha(\infty)$ as follows. If the volume concentration of α -glucose and of β -glucose at equilibrium are called *c_α* and *c_β* respectively, then

$$\alpha^\circ c_\alpha + \beta^\circ c_\beta = \alpha(\infty)$$

where α° and β° are the pressure-independent proportionality factors referred to above. By dissolving one or the other compound, following the mutarotation at 1 bar in the cell, and extrapolating back to the moment of dissolution, the values were found to be $\alpha^\circ = 9.89^\circ/(10\text{ g}/100\text{ cm}^3)$ and $\beta^\circ = 1.59^\circ/(10\text{ g}/100\text{ cm}^3)$ respectively. Furthermore, at any pressure *P*,

$$c_\alpha + c_\beta = 10\text{ g}/100\text{ cm}^3/[V(P)/V(1\text{ bar})]$$

From these two equations, *c_α*, *c_β* and *K_{eq}* = *c_β*/*c_α* may be found. It will be seen that *K_{eq}* does not change much with pressure. Finally, the last two

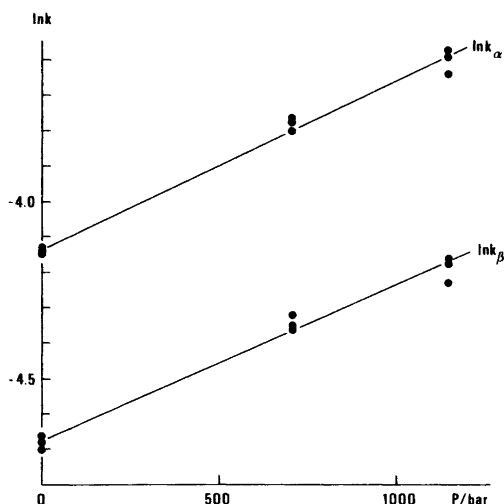


Fig. 3. Plot of $\ln k_\alpha$ and $\ln k_\beta$ vs. P .

columns contain the calculated values of k_α and k_β found from

$$k_\alpha + k_\beta = k \text{ and } k_\alpha/k_\beta = K_{\text{eq}}$$

In Fig. 3 values of $\ln k_\alpha$ and $\ln k_\beta$ are plotted against P . The relationship is close to being linear with slopes 0.472 kbar^{-1} and 0.437 kbar^{-1} based on least squares. This corresponds to the activation volumes:

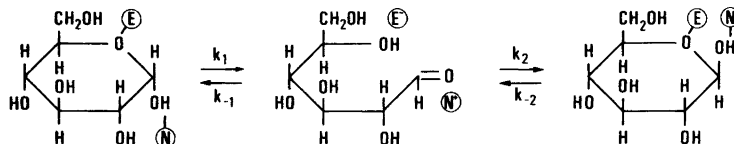
$$\Delta V_1^\ddagger = -11.7 \text{ cm}^3/\text{mol},$$

$$\Delta V_{-1}^\ddagger = -10.8 \text{ cm}^3/\text{mol}$$

for the forward and backward processes.

DISCUSSION

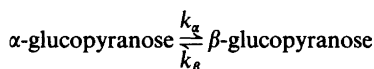
Mutarotation of sugars are reactions of the type: pyranose(1) \leftrightarrow pyranose(2) or: pyranose \leftrightarrow furanose or a combination of both. For glucose in water it is well established that the reaction consists in the



Scheme 1.

interconversion of α -glucopyranose to β -glucopyranose. A very small amount of the free aldehyde serves as an intermediate in the reaction. Los *et al.*¹¹ have determined the concentration of the free aldehyde in the reaction to be 0.0026 % of the total amount of glucose (for 0.665 M glucose). The reaction is summarized in Scheme 1, where E and N symbolize an electrophilic and a nucleophilic compound, respectively. In this scheme the rate constants of aldehyde formation k_1 and k_{-2} must be smaller than the closure rate constants k_{-1} and k_2 by three to four orders of magnitude in order to account for the low aldehyde concentration.¹¹

In the relevant literature, the mutarotation reaction of glucose is usually described as:



where the reaction proceeds to equilibrium with the observed rate constants $k = k_\alpha + k_\beta$. The presentation of the results in this paper is in keeping with this, since the final parameters, listed in Table 2 are k_α and k_β . Analysis of the four-constant reaction scheme, with application of the steady state principle to the aldehyde, leads to the same kinetics as that of the simple scheme with

$$k_\alpha = \frac{k_2}{k_{-1} + k_2} k_1; \quad k_\beta = \frac{k_{-1}}{k_{-1} + k_2} k_{-2}$$

and the equilibrium constant

$$K_{\text{eq}} = \frac{k_1 k_2}{k_{-1} k_{-2}}$$

Therefore, the observed parameters k_α and k_β may not be identified with the constants k_1 and k_{-2} . The problem of assigning the observed influence of pressure on k_α and k_β to the four rate constants may be viewed in the following way. Since the constants k_{-1} and k_2 both refer to closure of the

same open aldehyde molecule, it is probable that they will change with pressure in much the same way. If this is so, the fractions

$$\frac{k_2}{k_{-1} + k_2} \text{ and } \frac{k_{-1}}{k_{-1} + k_2}$$

will be almost insensitive to pressure and

$$\frac{\partial \ln k_a}{\partial P} \approx \frac{\partial \ln k_1}{\partial P}, \quad \frac{\partial \ln k_b}{\partial P} \approx \frac{\partial \ln k_{-2}}{\partial P}$$

Further it may be argued that the most probable explanation of the experimental fact that

$$\frac{\partial \ln K_{eq}}{\partial P} \approx 0$$

is that:

$$\frac{\partial \ln (k_1/k_{-2})}{\partial P} \approx 0 \text{ and } \frac{\partial \ln (k_{-1}/k_2)}{\partial P} \approx 0$$

In aqueous solution, the reaction is catalyzed by strong acids and bases, but the reaction is not only dependent on c_{H^+} and c_{OH^-} , since undissociated acids and anions of weak bases have a catalytic effect.⁴ It is believed that the mechanism of reaction is a concerted attack by a nucleophilic and an electrophilic reagent as shown above. If the binding of E to the oxygen is independent of the simultaneous binding of N to the OH group and *vice versa*, the first-order rate constant

$$k = \frac{v}{[\text{glucose}]}$$

is a sum of products of the present nucleophilic and electrophilic reagents.^{5a} In the case of acetate buffer the sum of products of concentrations is:

$$k_0[H_2O]^2 + k_I[H_2O][Ac^-] + k_{II}[H_2O][HAc] + k_{III}[Ac^-][HAc] + k_{IV}[H_2O][H^+] + k_V[H_2O][OH^-] + k_{VI}[HAc][OH^-] + k_{VII}[Ac^-][H^+] + k_{VIII}[OH^-][H^+]$$

The k_0 term represents the case where water molecules act as both N and E. In the weak acetate buffer used in our assays, the k_0 term is responsible for nearly 95% of the experimental rate constant, and the next term $k_I[H_2O][Ac^-]$ contributes most of the rest. Even if some doubt has been raised as to

the general validity of this model^{5b} we may safely assert that the rate constant observed in our conditions corresponds almost exclusively to a case of H_2O catalysis, with little or no contributions from other compounds.

The rate constant depends on pressure according to the following relation:

$$\frac{\partial \ln k}{\partial P} = -\Delta V^\ddagger (RT)^{-1}$$

where ΔV^\ddagger may be interpreted as the difference in volume between the activated complex and the reactants from which it is formed. In the case considered here the activated complex is formed by linking two molecules of water to the pyranose structure and subsequent opening of the ring structure. The overall effect of forming two new linkages, of nearly the same length as ordinary chemical bonds, and of extending one existing bond, is shrinkage. The sign of the activation volumes is in agreement with this. As for its magnitude, experience shows that the bond breaking/making is generally accompanied by volume changes of the order of $10 \text{ cm}^3/\text{mol}$ (see, e.g., Le Noble¹²) so that one would expect an overall volume change of about $-10 \text{ cm}^3/\text{mol}$ in the present case. In view of this the present data seem to agree well with the reaction model described.

REFERENCES

1. Röntgen, W. C. *Ann. Phys.* 45 (1892) 98.
2. Dubrunfaut, A. P. *C.R. Acad. Sci.* 23 (1846) 38.
3. Bates, F. J. *Polarimetry, Saccharimetry and the Sugars, Natl. Bur. Stand. C 440* (1942).
4. Brønsted, J. N. and Guggenheim, E. A. *J. Am. Soc.* 49 (1927) 2554.
5. a. Swain, C. G. *J. Am. Chem. Soc.* 72 (1950) 4578; b. Swain, C. G., Di Milo, A. J. and Cordner, J. P. *Ibid.* 80 (1958) 5983.
6. Isbell, H. S. and Pigman, W. W. *Natl. Bur. Stand.* 20 (1938) 773.
7. Grønlund, F. and Andersen, B. *Acta Chem. Scand.* 23 (1969) 2452.
8. Andersen, B. and Broe, P. E. *Acta Chem. Scand.* 26 (1972) 3691.
9. Shoemaker, D. P., Garland, C. W. and Steinfield, J. L. *Experiments in Physical Chemistry*, 3rd. Ed., McGraw-Hill, New York 1974, p. 44.
10. Grindley, T. and Lind, J. E. *J. Chem. Phys.* 54 (1971) 3983.
11. Los, J. M. and Wiesner, K. *J. Am. Chem. Soc.* 75 (1953) 6346; Los, J. M., Simpson, L. B. and Wiesner, K. *Ibid.* 78 (1956) 1564.
12. Le Noble, W. J. *Prog. Phys. Org. Chem.* 5 (1967) 207.

Received October 16, 1978.