BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 46, 2107—2111 (1973)

Polarographic Study of Monosaccharides in Unbuffered Solution and Its Application to the Determination of the Mutarotation Rate Constants

Tokuji IKEDA and Mitsugi SENDA
Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606

(Received November 8, 1972)

The polarographic behavior of monosaccharides in unbuffered neutral solution was investigated. The limiting current was ascertained to be an autocatalytic current which could be caused by the production of hydroxyl ion at the electrode surface. Equations for the characteristic limiting current and for the shape of current-potential curve were derived by use of the reaction layer concept. Experimental results for D-glucose, D-galactose, and D-xylose were fairly well expressed by these equations. The wave can also be applied to the determination of the mutarotation rate constants of monosaccharides, the values obtained (D-glucose: $0.53\times10^{-3}\,\text{s}^{-1}$, D-galactose: $0.54\times10^{-3}\,\text{s}^{-1}$, and D-xylose: $1.31\times10^{-3}\,\text{s}^{-1}$) being in fairly good agreement with those obtained by the polarimetric method (D-glucose: $0.4\times10^{-3}\,\text{s}^{-1}$, D-galactose: $0.5\times10^{-3}\,\text{s}^{-1}$, and D-xylose: $1.3\times10^{-3}\,\text{s}^{-1}$).

The polarographic behavior of monosaccharides in buffered solutions has been investigated by a number of workers.[1–4] The limiting current of the wave in buffered solutions has a kinetic character; it depends on the pH as well as the concentration of buffer salts. The mutarotation rate constants of monosaccharides have been determined by detailed analysis of the polarographic limiting currents.[5]

Little attention has been paid to their polarographic behavior in unbuffered solutions. We have observed that in unbuffered solutions the polarographic wave of monosaccharide gives a limiting current, which seems to be an autocatalytic current. The present investigation was undertaken to elucidate the characteristic behavior of the polarographic wave of monosaccharide in unbuffered media. Application of the wave to the determination of the mutarotation rate constants of monosaccharide has also been studied.

Experimental

Materials. Commercial D-glucose, D-galactose and D-xylose of reagent grade were used. Pure α-D-glucose: $[\alpha]^{25}_{\text{D}}=+112$ (c 4 in water), mp 146 °C, was supplied by Tokai Togyo Co., Ltd. Pure α-D-galactose[6] and α-D-xylose[7] were prepared by recrystallization from ethanol and water, respectively; α-D-galactose: $[\alpha]^{25}_{\text{D}}=+150$ (c 4 in water), mp 167 °C, α-D-xylose: $[\alpha]^{25}_{\text{D}}=+94$ (c 1 in water), mp 145 °C. Commercial potassium chloride and tetraethyl ammonium iodide (TEAI) of reagent grade were used as supporting electrolytes. The latter was used after recrystallization from methanol.

---

[1] K. Wiesner, *Collect. Czech. Chem. Commun.*, **12**, 64 (1947).

[2] P. Delahay and J. E. Strasner, *J. Amer. Chem. Soc.*, **74**, 893 (1952).

[3] W. G. Overend, A. R. Peacocke, and J. B. Smith, *J. Chem. Soc.*, **1961**, 3487.

[4] B. Capon, A. A. Levy, and W. G. Overend, *Carbohyd. Res.*, **5**, 93 (1967).

[5] J. M. Los, L. B. Simpson, and K. Wiesner, *J. Amer. Chem. Soc.*, **78**, 1564 (1957).

[6] M. L. Wolfrom and A. Tompson, "Method in Carbohydrate Chemistry," Vol. 1, ed. by R. L. Whistler and M. L. Wolfrom, Academic Press, New York and London (1962), p. 120.

[7] K. Anno and N. Seno, "Jikken Kagaku Koza," Vol. 23, ed. by S. Akabori and S. Funahashi, (in Japanese) Maruzen, Tokyo (1957), p. 336.

Apparatus. Polarograms were recorded with a Yanagimoto PE 21-TB2S polarograph connected to a Yokogawa 3077 X-Y recorder. Polarimetry was carried out with a Yanagimoto polarimeter OR-20.

Measurements were carried out with an H-type cell. A saturated calomel electrode (SCE) was used as reference electrode. In order to minimize accumulation of hydroxyl ions in the close neighbourhood of the electrode, which could be caused by unsufficient stirring of the neighbouring solutions after the detachment of the individual mercury drops, a pencil-sharpened capillary (tip outside-diameter 1 mm) was used. The capillary characteristics of the dropping mercury electrode measured in 0.1 M potassium chloride (open circuit) at a mercury reservoir height of 52.5 cm were $m=1.306\ \mathrm{mg\ s^{-1}}$ and $t=5.65\ \mathrm{s}$. All experiments were carried out at 25± 0.05 °C. The procedure for measuring the polarographic current has been described previously.[8]

Theory

Limiting Current. According to Los, Simpson and Wiesner,[5] the strictly rate-controlled limiting current $\bar{i}_l$ of reaction scheme (1)

$$\alpha\text{-pyranose} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \gamma(\text{reducible form}) \underset{k_2}{\overset{k_{-2}}{\rightleftarrows}} \beta\text{-pyranose} \tag{1}$$

is given by

$$\bar{i}_l = nF\bar{q}\bar{\mu}(k_1C_\alpha + k_2C_\beta) \tag{2}$$

$$\bar{\mu} = (D_s/(k_{-1}+k_{-2}))^{1/2}, \tag{3}$$

where n denotes the number of electrons consumed in the electrolysis per one molecule of monosaccharide, F Faraday constant, $\bar{q}$ the mean electrode surface area, D_s the diffusion coefficient of monosaccharide, C_α and C_β the bulk concentration of α- and β-pyranose, respectively, and $\bar{\mu}$ the mean thickness of the reaction layer. Equation (2) was later derived by one of us (M.S.)[9] and Paldus and Koutecký[10] on the basis of a rigorous mathematical procedure.

The rate constants k_i (i=1, 2, −1, and −2) of Eq. (1) depend on the concentrations (activities) of acid and base catalysts. In unbuffered aqueous solutions, they are generally given by[11,12]

$$k_i = k_{i,w} + k_{i,H}(\mathrm{H^+}) + k_{i,OH}(\mathrm{OH^-}), \tag{4}$$

where $k_{i,w}$ represents the catalytic constant of water, $k_{i,H}$ and $k_{i,OH}$ the catalytic coefficients of the catalysts indicated by subscripts, and the symbols in brackets the concentrations (activities) of the catalysts, $\mathrm{H^+}$ and $\mathrm{OH^-}$ being hydrogen ion and hydroxyl ion, respectively.

In the reaction layer the solution is basic because of the production of hydroxyl ions by the electro-reduction of monosaccharide at the electrode surface. For example, a rough estimation (see below) shows pH is 9.8 at the electrode when 0.006 M D-xylose solution is reduced at d.m.e. in unbuffered solution. The catalytic effect of hydrogen ion can be neglected in such a basic solution. The first term on the right hand side of Eq. (4), $k_{i,w}$, can also be neglected in comparison with the third term. Accordingly, the rate constants k_i (i=1, 2, −1, and −2), in the reaction layer are eventually expressed, in the first approximation, by

$$k_i = k_{i,OH}(\mathrm{OH^-})^\circ, \tag{5}$$

where $(\mathrm{OH^-})^\circ$ represents the mean concentration of hydroxyl ions at the electrode surface, which may be assumed to be approximately constant throughout the reaction layer due to the thinness of the layer.

Since hydroxyl ions diffuse into the solution and the concentration of hydroxyl ion is practically zero in the bulk solution, the limiting current of monosaccharide is related to the concentration of hydroxyl ions at the electrode surface, with the aid of the Ilković equation[13]

$$\bar{i}_l = \bar{\kappa}_{OH}(\mathrm{OH^-})^\circ, \tag{6}$$

where $\bar{\kappa}_{OH}$ denotes the Ilković constant for hydroxyl ion.

Substitution of Eqs. (5) and (6) into Eq. (2) gives

$$\bar{i}_l = 1.322\bar{\kappa}_s(D_s/D_{OH})^{1/2}\tau(k_{-1,OH}+k_{-2,OH})^{-1} \times (k_{1,OH}C_\alpha + k_{2,OH}C_\beta)^2, \tag{7}$$

where $\bar{\kappa}_s$ denotes Ilković constant for the hypothetical diffusion current for monosaccharide with $n=2(\bar{\kappa}_s = 2(D_s/D_{OH})^{1/2}\bar{\kappa}_{OH})$, D_{OH} the diffusion coefficient of hydroxyl ion and τ the drop time. For the solution of equilibrated mixture of monosaccharide, Eq. (7) is reduced to

$$\bar{i}_l = 1.322\bar{\kappa}_s(D_s/D_{OH})^{1/2}\tau(k_{1,OH}+k_{2,OH}K)^2(C_{\alpha,eq}+C_{\beta,eq})^2 \times (k_{-1,OH}+k_{-2,OH})^{-1}(1+K)^{-2} \tag{7'}$$

where $C_{\alpha,eq}$ and $C_{\beta,eq}$ represent the equilibrated concentrations of α- and β-pyranose, respectively, and K the equilibrium constant defined by $K=C_{\beta,eq}/C_{\alpha,eq}$. The sum $C_{\alpha,eq}+C_{\beta,eq}$ is equal to the total concentration of monosaccharide because the concentration of the intermediate γ-form is negligibly small.

Electrode reaction of the same mechanism as that described above was proposed for the electro-reduction of formaldehyde in unbuffered solution by Brdička[13] and a mathematically rigorous solution of the process was given by Koutecký.[14] The result requires that the numerical coefficient of Eq. (7′), 1.322, should be replaced by 0.966. The value was adopted in the following.

Current Potential Curve. Since the electroactive intermediate γ-form is formed from α- and β-pyranose, the current at a point of the kinetic wave of monosaccharide can be given by

$$\bar{i} = nF\bar{q}\bar{\mu}(k_1C_\alpha^\circ + k_2C_\beta^\circ - (k_{-1}+k_{-2})C_\gamma^\circ), \tag{8}$$

where C_α°, C_β°, and C_γ° represent the mean concentration of α-, β-, and γ-form, respectively, in the reaction layer. Provided that the limiting current has a strictly kinetic character as given by Eq. (2), there is no significant depletion of α- and β-pyranose in the reaction layer. Thus substitution of Eq. (2) in Eq. (8) gives

---

[8] T. Ikeda and M. Senda, This Bulletin, **46**, 1650 (1973).

[9] M. Senda, *Rev. Polarogr.* (Kyoto), **6**, 95 (1958).

[10] J. Paldus and J. Koutecký, *Collect. Czech. Chem. Commun.*, **23**, 376 (1958).

[11] H. S. Isbell and W. Pigman, "Advances in Carbohydrate Chemistry and Biochemistry," Vol. 24, ed. by M. L. Wolfrom and R. S. Tipson, Academic Press. London (1969), p. 13.

[12] B. Capon, *Chem. Rev.*, **69**, 407 (1969).

[13] a) R. Brdička, *Collect. Czech. Chem. Commun.*, **20**, 387 (1955). b) R. Brdička, *Z. Electrochem.*, **59**, 787 (1955).

[14] J. Koutecký, *Collect. Czech. Chem. Commun.*, **21**, 652 (1956).

$$\bar{i} = \bar{i}_l - nF\bar{q}\bar{\mu}(k_{-1}+k_{-2})C_\gamma^\circ \tag{8'}$$

Based on the theory of slow discharge, the current intensity can be given by

$$\bar{i} = nF\bar{q}k_{el} \exp\left(\left(-\alpha_a n_a F/RT\right)(E-E_0)\right)C_\gamma^\circ, \tag{9}$$

where k_{el} represents the standard rate constant at the standard potential E_0, $\alpha_a n_a$ the product of the transfer coefficient and the number of electrons in discharge process, R the gas constant and T the absolute temperature. Other symbols were defined above.

In unbuffered solutions the current intensity can also be related to the concentration of hydroxyl ion at the electrode surface by

$$\bar{i} = \bar{\kappa}_{OH}(OH^-)^\circ \tag{6'}$$

Substituting the appropriate values from Eqs. (5), (6′), and (9) into Eq. (8′), we obtain after rearrangement

$$E = E_0 + \frac{RT}{\alpha_a n_a F}\ln\left[\frac{k_{el}(\bar{\kappa}_{OH})^{1/2}}{(D_s(k_{-1,OH}+k_{-2,OH}))^{1/2}}\right] + \frac{RT}{\alpha_a n_a F}\ln\left[\frac{\bar{i}_l-\bar{i}}{\bar{i}^{3/2}}\right], \tag{10}$$

here it is assumed that Eq. (5) holds.

Results and Discussion

Experiments were carried out for several monosaccharides (D-glucose, D-galactose, and D-xylose) in unbuffered solutions of 0.1 M potassium chloride.

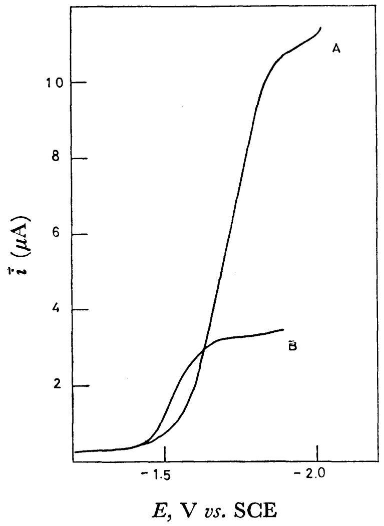

Fig. 1. Polarogram of 0.06M D-xylose in 0.1M potassium chloride A, 0.2M ammonia buffer pH 8.59 B.

Curve A in Fig. 1 shows an example of the polarographic wave of D-xylose in unbuffered neutral solution. The wave is very large in comparison with that of D-xylose of the same concentration (curve B in Fig. 1) in buffered solution of 0.2 M ammonia at pH 8.59. Similar large waves were observed for all other monosaccharides studied. The value of $\bar{i}_l/\bar{i}_d$ is shown in Table 1, $\bar{i}_d$ being the hypothetical diffusion current of monosaccharide. The limiting current $\bar{i}_l$ is still small enough to be considered as strictly kinetic-controlled in the concentration ranges studied. Thus the use of Eq. (2) in deriving the theoretical equations is justified.

TABLE 1. DEPENDENCE OF THE LIMITING CURRENT $\bar{i}_l$ ON THE HEIGHT OF MERCURY RESERVOIR h (Current sens 0.2 μA/cm Temp. 25 °C)

	D-Xyl.(10mM)[a]		D-Glu.(60mM)[b]		D-Gal.(14mM)[c]	
h (cm)	$\bar{i}_l$ (cm)	$\bar{i}_l\,(h^{1/2})$	$\bar{i}_l$ (cm)	$\bar{i}_l\,(h^{1/2})$	$\bar{i}_l$ (cm)	$\bar{i}_l(h^{1/2})$
42.5	5.16	34	4.26	28	4.51	29
52.5	4.80	35	4.16	30	4.25	31
62.5	4.45	35	3.87	31	4.10	32

at h=52.5 cm a) $\bar{i}_l/\bar{i}_d=2.1\times10^{-2}$ b) $\bar{i}_l/\bar{i}_d=3.0\times10^{-3}$ c) $\bar{i}_l/\bar{i}_d=1.3\times10^{-2}$

It is expected from Eq. (7′) that $\bar{i}_l$ is approximately proportional to $m^{2/3}\tau^{7/6}$. Since m is proportional to the height of mercury reservoir h, and τ is inversely proportional to h, $\bar{i}_l$ is eventually proportional to $h^{-1/2}$. Experimental results (Table 1) show that $\bar{i}_l$ decreases with increasing height of mercury reservoir and is approximately proportional to $h^{-1/2}$.

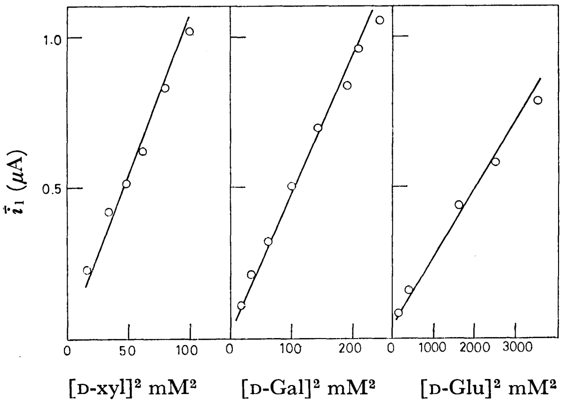

Fig. 2. Relationship between the limiting current $\bar{i}_l$ and the square of the concentration of monosaccharides.

Figure 2 shows the plot of $\bar{i}_l$ against the square of the monosaccharide concentration $(C_\alpha+C_\beta)^2$. In accordance with theoretical consideration, all the data in Fig. 2 are distributed along a straight line, deviating downward from the line at higher concentrations of monosaccharides. A similar deviation from linearity was also observed for the limiting current of formaldehyde in unbuffered solutions by Brdička,[13] who attributed the deviation to the streaming of the solution in the neighbourhood of the electrode.

The concentration of hydroxyl ions in the reaction layer $(OH^-)^\circ$ can be calculated with the aid of the Ilković equation (6). Even at the lowest concentration studied (0.04 M D-xylose) $\bar{i}_l$ reached 0.23 μA. Thus $(OH^-)^\circ$ may be estimated as 3.6×10^{-5} M or more, where $D_{OH}=5.23\times10^{-5}$ cm^2 s^{-1} was used.[15] In view of the numerical values of the rate constants,[8] *i.e.* $k_{1,W}=2.4\times10^{-3}$ s^{-1} and $k_{1,OH}=1.2\times10^3$, the catalytic effect of water may be considered to be of secondary significance under the present experimental conditions. Thus the use of Eq. (5) in place of Eq. (4) is justified.

The mean thickness of the reaction layer $\bar{\mu}$, which

---

[15] J. Heyrovský and J. Kůta, "Principle of Polarography," Academic Press, New York and London (1966), p. 105.

is now given by $\bar{\mu}=(D_{xy}/(k_{-1,OH}(OH^-)^o+k_{-2,OH}(OH^-)^o))^{1/2}$, is calculated to be 9.3×10^{-4} cm when $(OH^-)^o=3.6\times10^{-5}$ M, D_{xy}[16] $=6.7\times10^{-6}$ cm^2 s^{-1}, $k_{-1,OH}=8.4\times10^4$ s^{-1} l mol^{-1}, and $k_{-2,OH}=11.5\times10^4$ s^{-1} l mol^{-1}. The numerical value of the rate constants used were obtained in the present study (Table 3). The magnitude of $\bar{\mu}$ is less than one twentieth of the thickness of the mean diffusion layer for hydroxyl ion, ($\delta\simeq2.3\times10^{-2}$ cm). This is acceptable.

Similar arguments can also be applied to D-glucose and D-galactose.

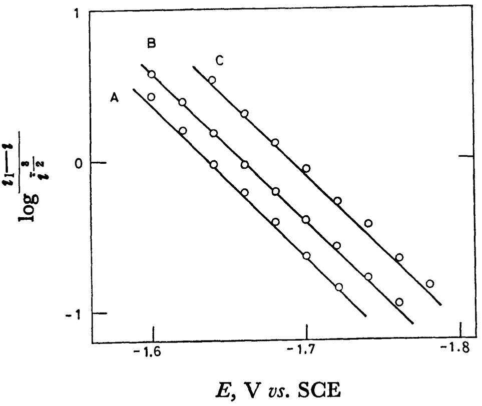

Fig. 3. Log$((\bar{i}_1-i)/\bar{i}^{3/2})$ *vs.* E plot for the polarographic current of D-xylose(8 mM) A, D-glucose(40 mM) B, D-galactose (12 mM) C, in 0.1M potassium chloride. Reciplocal slope of the solid lines: 100 mV.

Figure 3 shows the plot of log$[({}_1\bar{i}-\bar{i})/\bar{i}^{3/2}]$ against E. The experimental points lie on a straight line, as expected from Eq. (10) with a reciprocal slope 2.303 $RT/\alpha_a n_a F\sim100$ mV per log unit.

The general solution of the current-potential curve of the kinetic wave in buffered solutions has been given by Koutecký.[17] Rewriting his result for the current-potential curve of monosaccharide in buffered solutions, we obtain

$$E=E_0+\frac{RT}{\alpha_a n_a F}\ln\left[k_{el}(D_s(k_{-1}+k_{-2}))^{-1/2}\right]+\frac{RT}{\alpha_a n_a F}\ln\left[\frac{\bar{i}_l-\bar{i}}{\bar{i}}\right] \tag{11}$$

Figure 4 shows the plot of log$[(\bar{i}_l-\bar{i})/\bar{i}]$ *vs.* E for the polarographic current of monosaccharides in buffered solution of pH 8.56. As expected from Eq. (11), a straight line was obtained. The value of the slope 2.303 $RT/\alpha_a n_a F$ was again *ca.* 100 mV; this is reasonable provided that the discharge process of monosaccharides is identical both in buffered and in unbuffered solutions.

From Eq. (10) we obtain

$$E_{1/2}=E_0+\frac{RT}{\alpha_a n_a F}\ln\left[\frac{k_{el}(\bar{\kappa}_{OH})^{1/2}}{(D_s(k_{-1,OH}+k_{-2,OH}))^{1/2}}\right]-\frac{RT}{\alpha_a n_a F}\ln\left[\frac{\bar{i}_l}{2}\right]^{1/2} \tag{12}$$

for the half-wave potential of the polaragraphic wave in unbuffered solutions. Figure 5 shows the plot of

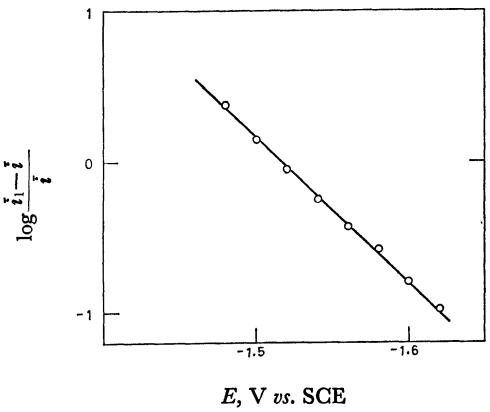

Fig. 4. Log$((\bar{i}_1-\bar{i})/\bar{i})$ *vs.* E plot for the polarographic current of D-xylose (0.06 M) in ammonia buffer pH 8.56. Reciprocal slope of the solid line: 100 mV.

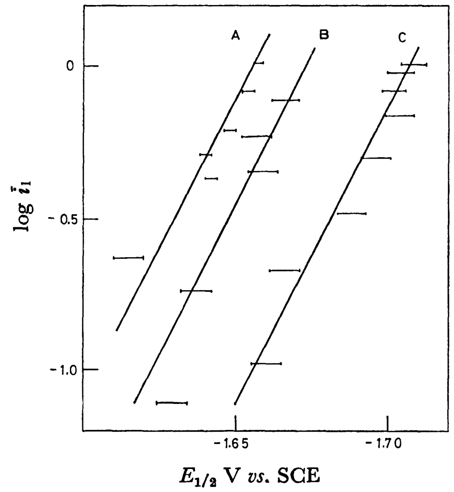

Fig. 5. Log$(\bar{i}_1)$ *vs.* $E_{1/2}$ plot for the polarographic current of D-xylose A, D-glucose B, and D-galactose C, in 0.1M potassium chloride. Reciprocal slope of the solid lines: -50 mV.

log $\bar{i}_l$ against $E_{1/2}$. As expected from Eq. (12), the data are distributed along a straight line with a reciprocal slope $RT/2\alpha_a n_a F\sim-50$ mV. The fact that the values of $\alpha_a n_a$ are nearly identical for all monosaccharides investigated (Figs. 3 and 4) suggests the similarity of the discharge process for these monosaccharides.

In conclusion, the current-potential relationship of the monosaccharide is fairly well reproduced by Eq. (10). It is to be noted that in deriving Eq. (10), k_{el} is assumed to be independent of the concentrations of acid and/or base at the electrode surface. However, there is a reason to believe that the discharge process should depend on acid and/or base.

When TEAI was used as a supporting electrolyte, the half-wave potential of the monosaccharide appeared in more negative potential than in KCl solution. The reciprocal slope of the plot of log$[(\bar{i}_l-\bar{i})/\bar{i}^{3/2}]$ against E became 200 mV or even greater. A similar effect was observed[3,4] when tetramethylammonium phosphate was used as the supporting electrolyte. This effect may be attributed to the adsorption of the quarternary

---

[16] L. Friedman and P. G. Carpenter, *J. Amer. Chem. Soc.*, **61**, 1745 (1939).

[17] J. Koutecký, *Collect. Czech. Chem. Commun.*, **20**, 116 (1955).

ammonium cation on the electrode surface,[18] which results in the retardation of the electro-reduction of monosaccharide.

Determination of the Mutarotation Rate Constant

When a weighed amount of a monosaccharide in pure α-pyranose form was dissolved in 0.1 M potassium chloride, the limiting current decreased with time and reached a constant value corresponding to the value for the equilibrated mixture of the monosaccharide. This behavior is very much similar to that in buffered solutions and the mutarotation rate constants may be obtained by analyzing the limiting current with the aid of Eq. (7) as a function of time.

When we start from α-pyranose, the change of concentrations of α- and β-pyranose with time is given as follows,[5]

$$\begin{aligned} C_\alpha &= C_{\alpha,\mathrm{eq}}(1+K\mathrm{e}^{-k_{\mathrm{o,w}}t}) \\ C_\beta &= C_{\beta,\mathrm{eq}}(1-\mathrm{e}^{-k_{\mathrm{o,w}}t}), \end{aligned} \tag{13}$$

where $k_{\mathrm{o,w}}$ is the overall mutarotation constant of water and is defined by four individual rate constants as follows.

$$k_{\mathrm{o,w}} = (k_{1,\mathrm{w}}k_{-2,\mathrm{w}}+k_{-1,\mathrm{w}}k_{2,\mathrm{w}})/(k_{-1,\mathrm{w}}+k_{-2,\mathrm{w}}) \tag{14}$$

Substitution of Eq. (13) into Eq. (7) gives

$$\begin{aligned} \bar{i}_1(t)^{1/2} = {} & (0.966\bar{\kappa}_{\mathrm{s}}(D_{\mathrm{s}}/D_{\mathrm{OH}})^{1/2}\tau)^{1/2}(k_{-1,\mathrm{OH}}+k_{-2,\mathrm{OH}})^{-1/2} \\ & \times(1+K)^{-1}(C_{\alpha,\mathrm{eq}}+C_{\beta,\mathrm{eq}})((k_{1,\mathrm{OH}}+Kk_{2,\mathrm{OH}}) \\ & +K(k_{1,\mathrm{OH}}-k_{2,\mathrm{OH}})\mathrm{e}^{-k_{\mathrm{o,w}}t}) \end{aligned} \tag{15}$$

Subtraction of the expression corresponding with Eq. (15) at time $t+\Delta t$ and taking logarithms leads to the result

$$\begin{aligned} \ln[\bar{i}_1(t)^{1/2}-\bar{i}_1(t+\Delta t)^{1/2}] = {} & \ln[(0.966\bar{\kappa}_{\mathrm{s}}(D_{\mathrm{s}}/D_{\mathrm{OH}})^{1/2}\tau)^{1/2} \\ & \times(k_{-1,\mathrm{OH}}+k_{-2,\mathrm{OH}})^{-1/2}(1+K)^{-1}K(k_{1,\mathrm{OH}}-k_{2,\mathrm{OH}}) \\ & \times(C_{\alpha,\mathrm{eq}}+C_{\beta,\mathrm{eq}})(1-\mathrm{e}^{-k_{\mathrm{o,w}}\Delta t})] - k_{\mathrm{o,w}}t \end{aligned} \tag{16}$$

Plotting $\log[\bar{i}_1(t)^{1/2}-\bar{i}_1(t+\Delta t)^{1/2}]$ with constant Δt *vs.* t, according to the method of Guggenheim,[19] should give a straight line with slope $-k_{\mathrm{o,w}}$, and

$$\begin{aligned} \text{intercept} = {} & \ln[(0.966\bar{\kappa}_{\mathrm{s}}(D_{\mathrm{s}}/D_{\mathrm{OH}})^{1/2}\tau)^{1/2} \\ & \times(k_{-1,\mathrm{OH}}+k_{-2,\mathrm{OH}})^{-1/2}(1+K)^{-1}K(k_{1,\mathrm{OH}}-k_{2,\mathrm{OH}}) \\ & \times(C_{\alpha,\mathrm{eq}}+C_{\beta,\mathrm{eq}})(1-\mathrm{e}^{-k_{\mathrm{o,w}}\Delta t})] \end{aligned} \tag{17}$$

At $t\to\infty$, Eq. (15) is reduced to

$$\begin{aligned} \bar{i}_1(t\to\infty)^{1/2} = {} & (0.966\bar{\kappa}_{\mathrm{s}}(D_{\mathrm{s}}/D_{\mathrm{OH}})^{1/2}\tau)^{1/2} \\ & \times(k_{-1,\mathrm{OH}}+k_{-2,\mathrm{OH}})^{-1/2}(1+K)^{-1}(C_{\alpha,\mathrm{eq}}+C_{\beta,\mathrm{eq}}) \\ & \times(k_{1,\mathrm{OH}}+Kk_{2,\mathrm{OH}}) \end{aligned} \tag{18}$$

Namely, the overall catalytic rate constant $k_{\mathrm{o,w}}$ is determined from the slope of the Guggenheim plot. Furthermore, if the overall catalytic rate coefficient of hydroxyl ion $k_{\mathrm{o,OH}}$ and the equilibrium constant K are known, Eqs. (17) and (18) in combination with the following two equations

$$k_{\mathrm{o,OH}} = (k_{1,\mathrm{OH}}k_{-2,\mathrm{OH}}+k_{-1,\mathrm{OH}}k_{2,\mathrm{OH}})/(k_{-1,\mathrm{OH}}+k_{-2,\mathrm{OH}}) \tag{19}$$

$$K = C_{\beta,\mathrm{eq}}/C_{\alpha,\mathrm{eq}} = k_{1,\mathrm{OH}}k_{-2,\mathrm{OH}}/k_{-1,\mathrm{OH}}k_{2,\mathrm{OH}} \tag{20}$$

can be solved with respect to four individual rate coefficients $k_{1,\mathrm{OH}}$, $k_{2,\mathrm{OH}}$, $k_{-1,\mathrm{OH}}$, and $k_{-2,\mathrm{OH}}$.

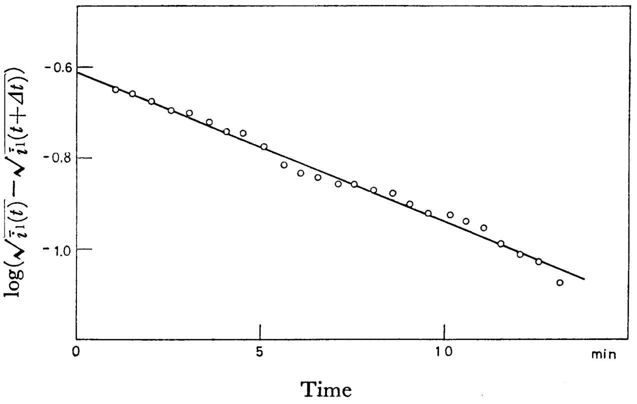

Fig. 6. Analysis of $\bar{i}_1$ *vs.* time curve of (α—β) D-xylose in 0.1M potassium chloride solution. $\Delta t=15$ min.

An example of the Guggenheim plot according to Eq. (16) is shown in Fig. 6. The values of $k_{\mathrm{o,w}}$ determined by the present method and by the polarimetric method are given in Table 2. The results are in good accordance with each other.

TABLE 2. THE OVERALL MUTAROTATION RATE CONSTANT $k_{\mathrm{o,w}}$ IN 0.1M KCl SOLUTION (Temp. 25 °C)

	$k_{\mathrm{o,w}}$ ($\mathrm{s^{-1}}$)	
	Polarography	Polarimetry
D-Xylose	1.31×10^{-3}	1.3×10^{-3}
D-Glucose	0.53×10^{-3}	0.4×10^{-3}
D-Galactose	0.54×10^{-3}	0.5×10^{-3}

TABLE 3. THE FORWARD AND BACKWARD RATE COEFFICIENTS $k_{i,\mathrm{OH}}$ (i=1,2, −1, and −2) (Temp. 25 °C)

	$k_{1,\mathrm{OH}}$	$k_{2,\mathrm{OH}}$	$k_{-1,\mathrm{OH}}$	$k_{-2,\mathrm{OH}}$
		($\mathrm{l\ mol^{-1}\ s^{-1}}$)		
D-Xylose	8.2×10^{2}	6.0×10^{2}	8.4×10^{4}	11.5×10^{4}
D-Glucose	11.6×10	6.5×10	9.1×10^{4}	8.9×10^{4}

Table 3 shows the individual rate coefficients of hydroxyl ion for D-xylose and D-glucose, $k_{\mathrm{o,OH}}=8\times10^{2}\ \mathrm{s^{-1}\ l\ mol^{-1}}$ (D-xylose),[8] $9\times10\ \mathrm{s^{-1}\ l\ mol^{-1}}$ (D-glucose),[11] D_{xy}[16]$=6.71\times10^{-6}\ \mathrm{cm^2\ s^{-1}}$, D_{glu}[16]$=6.65\times10^{-6}\ \mathrm{cm^2\ s^{-1}}$, K_{xy}[20]$=1.87$ and K_{glu}[20]$=1.74$ being used. The forward rate coefficients $k_{1,\mathrm{OH}}$ and $k_{2,\mathrm{OH}}$ for D-xylose are in fair agreement with those obtained from the analysis of the polarographic currents in buffered media.[8] The ratio of the concentration of the intermediate γ-form $C_{\gamma,\mathrm{eq}}$ to that of α- and β-pyranose form, $C_{\alpha,\mathrm{eq}}+C_{\beta,\mathrm{eq}}$, was $3\times10^{-1}\%$ for D-xylose and $4\times10^{-2}\%$ for D-glucose. The results are also in the same order as those obtained from the experiments in buffered media.[8]

The authors thank Tokai Togyo Co., Ltd. for supplying α-D-glucose.

---

[18] Ref. 15), p. 301.

[19] E. A. Guggenheim, *Phil. Mag.*, **2**, 538 (1926).

[20] W. Pigman and H. S. Isbell, "Advances in Carbohydrate Chemistry," Vol. 23, ed. by M. L. Wolfrom and R. S. Tipson, Academic Press, New York and London (1968), p. 11.