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Determination of Glucose by Means of Sodium Chlorite

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The oxidation of several aldoses with acid sodium chlorite solution was investigated in order to obtain a method for the determination of aldehyde groups in sugars and their polymers.

The kinetics of the oxidation of glucose and cellobiose were studied over the experimental ranges 3.4 to 4.4 pH, 30° to 65° C, 0.005- to 0.15-*M* sodium chlorite, and 0.00006- to 0.0016-*M* aldose, both by the determination of the change in chlorite concentration by iodometric titration and by the photometric measurement of ClO_2 formed. The rate of oxidation was found to be approximately first order with respect to aldose and chlorous acid. The measurement of the oxidation was complicated by the second-order decomposition of chlorous acid. This decomposition was corrected for by a calibration curve and an approximate formula derived from the reaction kinetics. Use of either of these corrections gave experimental values for glucose within a few percent of the theoretical value.

Melibiose, maltose, and lactose were oxidized at about the same rate as glucose and cellobiose. Nonreducing sugars and sugar acids were not appreciably oxidized under the experimental conditions used.

I. Introduction

During a study of the photochemical degradation of papers it was desired to measure small changes in functional group content, such as aldehyde and carboxyl, resulting from irradiation.

Various methods that have been proposed for the estimation of aldehyde in cellulose are the hypoiodite method [1, 2, 3, 4, 5, 6, 7],[2] the dye absorption method of Geiger and Wissler [8], the acid permanganate method of Hiller and Pacsu [9], modifications of the Kiliani reaction by Frampton et al. [10] and Yundt [11], and the mercaptalation method of Wolfrom and coworkers [12]. Isbell [13] has used the Kiliani reaction in connection with radioactive tracer techniques to determine the reducing end groups in dextrans. The hydroxylamine method of Gladding and Purves determines total carbonyl [14].

Most of the above methods use an alkaline medium or are not sensitive enough for many purposes. It has been shown that oxidized celluloses may be extensively changed in alkaline media [15, 16, 17, 18]. In the permanganate method of Hiller and Pacsu, an acidic medium is employed, but this method had been severely criticized by Meller [19] and by Husemann [20]. Attempts in the Bureau's Paper Section to obtain quantitative oxidation of several simple sugars, using the permanganate method, were not satisfactory. For example, glucose, cellobiose, galactose, and arabinose were oxidized 10, 11, 12, and 24 percent, respectively, calculated from the $KMnO_4$ reduced.

Chlorite in acid solution as a reagent for the determination of aldehyde in cellulose was suggested by the work of Jeanes and Isbell [21], who reported that the aldehyde in sugars is oxidized by acid chlorite solutions at room temperature to carboxyl with very little side reaction. Ketoses, polyhydroxy alcohols, and aldonic acids were attacked only after many days' treatment with chlorite solutions. The oxidation rates increased with a decrease in pH, thus indicating that chlorous acid was the oxidant. These workers suggested that the main reaction corresponded approximately to the equation

$$RCHO+3HClO_2 \rightarrow RCOOH+2ClO_2+HCl+H_2O. \quad (1)$$

They showed that the study of the reaction system was complicated by the spontaneous decomposition of chlorites in acid solution and that chlorine dioxide reacts slowly or not at all with aldoses.

The present paper deals with the stoichiometry and kinetics of the reaction of chlorite with glucose and, to a lesser extent, cellobiose. Some data on melibiose, maltose, and lactose are also given. This work was undertaken preliminary to a study of the determination of the aldehyde content of cellulose.

2. Chemistry and Kinetics of Decomposition of Acidic Chlorite Solutions

The chemistry of chlorite has been investigated by Holst [22], Taylor, et al. [23, 24], Jeanes and Isbell [21], and Barnett [25]. Chlorites are most useful as oxidants in acid solution, but a study of the kinetics of the reactions is complicated by the instability of chlorous acid. The decomposition of chlorous acid is a function of pH, temperature, concentration, ionic strength, and impurities that may act as catalysts.

The net equation for the decomposition (or dismutation) of chlorous acid has been shown by Barnett [25] and confirmed by Taylor et al. [23] to conform approximately to the equation

$$4HClO_2 \rightarrow 2ClO_2+HClO_3+HCl+H_2O. \quad (2)$$

Barnett [25] has shown that the decomposition is second order, and this was confirmed in the present investigation, using the differential method.

Figure 1 is a plot of decomposition versus time of a 0.005 *M* solution of sodium chlorite at a pH of 3.52

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[2] Figures in brackets indicate the literature references at the end of this paper.

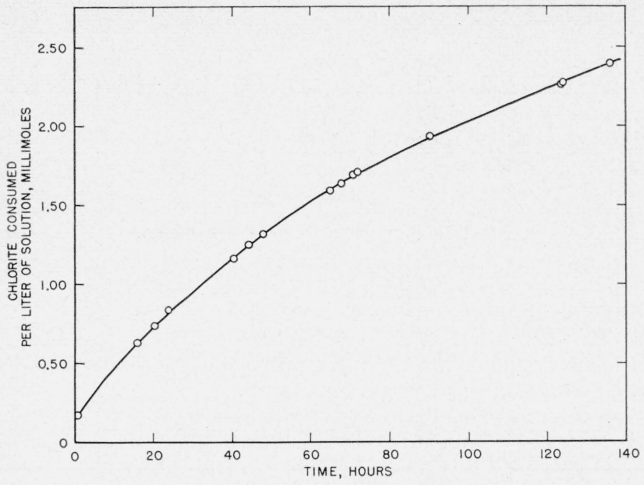

FIGURE 1. *Decomposition of chlorous acid with time.*
0.005-*M* sodium chlorite buffered to pH 3.52 at 40° C.

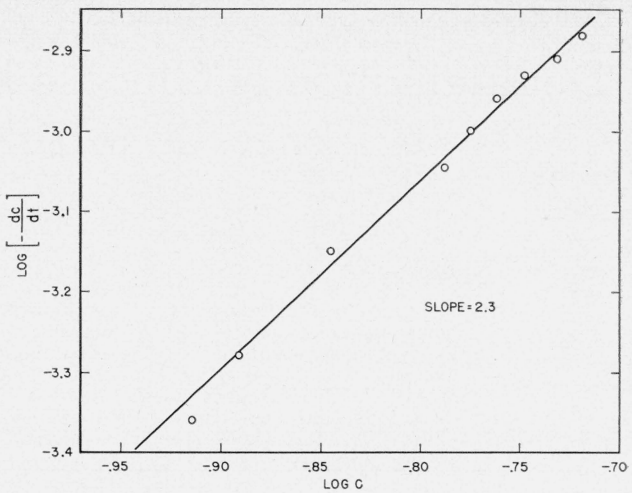

FIGURE 2. *Order of decomposition of $HClO_2$ by the differential method.*
0.005-*M* sodium chlorite buffered to pH 3.52 at 40° C.

and an ionic strength of 0.10. The chlorite concentration was determined by iodometric titration. A plot of the logarithm of the rate of decomposition against the logarithm of the chlorite concentration (fig. 2) gives a straight line with a slope of 2.3, the latter indicating the order of the decomposition with respect to chlorite.

The rate expression for the decomposition of acid chlorite solutions at constant pH may be written

$$-\frac{dC}{dt}=kC^2, \tag{3}$$

where C is the chlorite concentration at any time t. If chlorous acid is the active substance in the decomposition of acid chlorite solutions, eq (3) may be modified by expressing the concentration of chlorous acid in terms of $[H^+]$, C, and K_{HClO_2}, the dissociation constant of chlorous acid. From the equation for the dissociation of chlorous acid, $HClO_2 \rightleftharpoons ClO_2^- + H^+$, it follows that

$$[HClO_2]=\frac{[H^+]\,C}{[H^+]+K_{HClO_2}}=hC.$$

Then

$$-\frac{dC}{dt}=k_D h^2 C^2, \tag{4}$$

where k_D is the rate constant for the decomposition. Values of k_D were obtained by plotting $1/C$ against t in the decomposition experiments. The average of the values obtained was 0.67 ± 0.05 liter mole^{-1} sec^{-1}. These data were obtained at chlorite concentrations of 0.005 and 0.01 millimolar, a temperature of 40° C, and pH values of 3.40, 3.52, 3.70, and 4.00. The initial concentration of chlorite was obtained by extrapolating the plot of $1/C$ against t to zero. This was necessary because of an immediate reaction upon addition of chlorite to the acid reaction system, presumably with impurities in the reagents. This "initial decomposition," averaging about 1.5 percent of the total chlorite, may be reduced considerably by recrystallizing the sodium chlorite and sodium acetate, the latter a component of the buffer system.

As indicated by eq (1) and (2), acids are produced in the decomposition of chlorous acid and in oxidation of aldose by chlorous acid. Therefore, it is necessary to keep the solutions well buffered to minimize changes in acidity with time. Barnett showed, in a comparison of rates, that it is also necessary to keep the ionic strength essentially constant.

Buffer systems consisting of sodium acetate and acetic acid were prepared that effectively covered the range of hydrogen-ion concentration used in this study. Buffer data are given in table 1. The ionic strength was kept constant, and the composition of the buffers, to give the desired pH, was calculated from the Debye-Huckel form of the mass-action equation [26]. These buffers had sufficient capacity to prevent changes in acidity great enough to be measured with a pH meter.

TABLE 1. *Sodium acetate-acetic acid buffers*

Temperature	Glacial acetic acid per liter of solution [a] to obtain the following pH values [b]		
	pH=3.40	pH=3.52	pH=3.70
°C	*g*	*g*	*g*
40	418.6	315.4	209.6
50	453.4	------	218.2

[a] All buffers contained 55.0 g of reagent-quality sodium acetate, $NaC_2H_3O_2 \cdot 3H_2O$ per liter.
[b] The pH values apply after the buffers have been diluted 1:4 by addition to the analytical mixtures.

Ferric ion, found in traces in most reagents, catalyzes the decomposition of chlorous acid. As little as 0.005 mg of iron as ferric ion caused considerable decomposition of chlorite solutions. The addition of a small quantity of sodium oxalate to complex the iron was found to lower k_D, the rate constant for the decomposition, to 0.55 liter mole^{-1} sec^{-1} at 40° C.

Sodium oxalate was used in all the determinations of sugars by the iodometric titration except for the data in table 4. It was not used in the determinations in which chlorine dioxide was measured photometrically, since sodium oxalate appeared to react to a slight extent with ClO_2 under the conditions of these experiments.

3. Kinetics of the Reaction of Glucose and Cellobiose With Acid Chlorite Solutions

The quantity of aldose oxidized may be estimated photometrically from the chlorine dioxide produced or iodometrically from the chlorite reacted.

3.1. Studies Based on the Iodometric Determination of Chlorite

As the ClO_2 formed in the decomposition of chlorous acid and in the oxidation of aldose interferes with the iodometric determination of chlorite, it must be removed by aeration before titrating.

There is considerable decomposition of $HClO_2$ during the oxidation of sugars such as glucose and cellobiose. Hence it is necessary to run "controls" along with the "test solutions." The control solution is identical in compositions with a test solution, except for aldose. In a rate experiment the control curve is subtracted from the test solution curve to obtain the net curve, the latter representing the approximate amount of chlorite consumed in the oxidation of aldose.

Data for the oxidation of glucose and cellobiose are plotted in figures 3 and 4, respectively. Figure 5 is a semilog plot of percent chlorite remaining against time for an experiment with a sevenfold excess of glucose. This plot indicates a first-order reaction with respect to chlorite up to about 50-percent consumption of chlorite.

Figures 6 and 7 are semilog plots of unoxidized glucose and cellobiose (in percent) against time in the presence of excess chlorite. The data for these plots are the same as used for figures 3 and 4. The straight lines obtained indicate that the reactions were first order with respect to glucose and cellobiose.

The percentage of aldose at any time t was determined from the curves in figures 3 and 4 by assuming that the maximum of the net (test solution minus control) curves represented complete oxidation.

It is now possible to write a rate expression for the oxidation of aldose at a constant pH in terms of chlorite reacted as

$$-\frac{dC}{dt}=kAC, \tag{5}$$

where A is the concentration of aldose and C the concentration of chlorite at any time t. Assuming that chlorous acid is the oxidant, the rate expression may be written by analogy with eq (4), as

$$-\frac{dC}{dt}=k_A hAC. \tag{6}$$

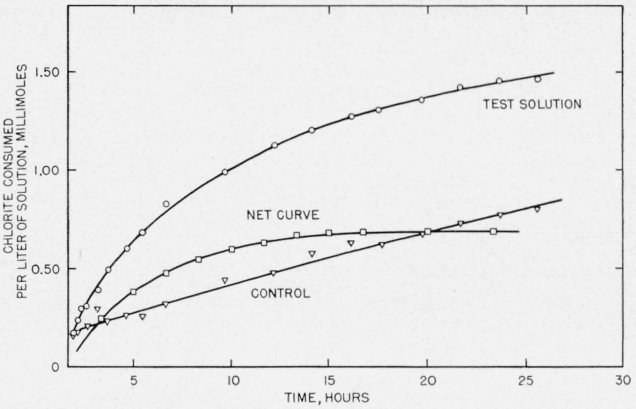

FIGURE 3. *Oxidation of glucose with chlorous acid.*
0.25 millimolar glucose in 0.005-*M* sodium chlorite buffered to pH 3.52 at 40° C.

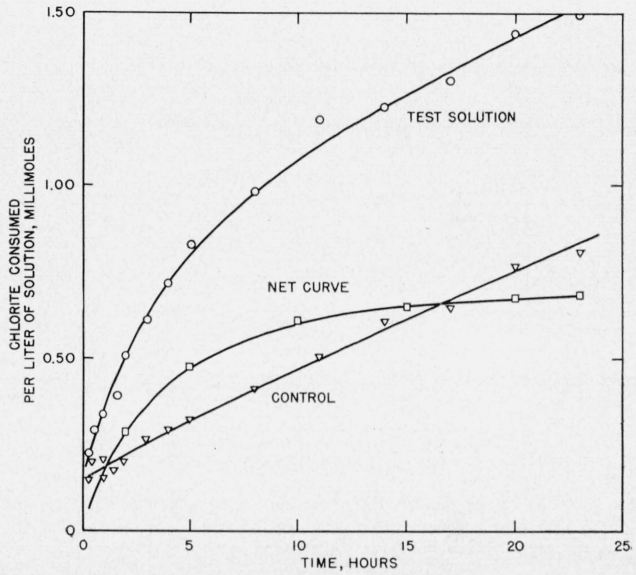

FIGURE 4. *Oxidation of cellobiose with chlorous acid.*
0.25 millimolar cellobiose in 0.005-*M* sodium chlorite buffered to pH 3.52 at 40° C.

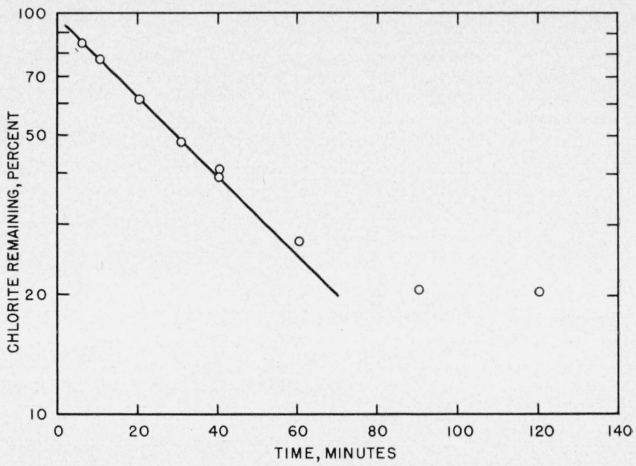

FIGURE 5. *Reaction of excess glucose with chlorous acid; determination of order with respect to chlorite by the integration method.*
0.005-*M* sodium chlorite buffered to pH 3.52 at 40° C. 0.0125-*M* glucose.

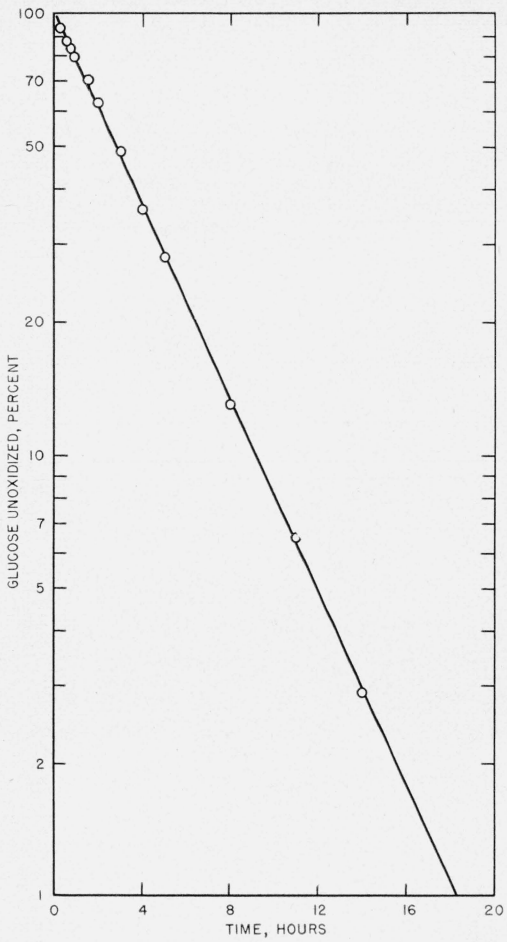

FIGURE 6. *Reaction of 0.01 millimole of glucose with excess chlorous acid; determination of order with respect to glucose by the integration method.*

0.005-*M* sodium chlorite buffered to pH 3.52 at 40° C.

The complete equation for the consumption of chlorite in a solution containing aldose may now be written

$$-\frac{dC}{dt}=k_A hAC+k_D h^2C^2. \tag{7}$$

It is possible to calculate approximate values for k_A. With a large excess of chlorite, the average value of the chlorite concentration is taken as a constant. Therefore the rate equation becomes

$$-\frac{dA}{dt}=k_A hAC, \tag{8}$$

and integrating:

$$\log A=\frac{k_A hC}{2.303}t+\text{const.} \tag{9}$$

If log A is plotted against t, then the slope m of the line is $-k_A hC/2.0303$ and

$$k_A=\frac{-2.303m}{hC}. \tag{10}$$

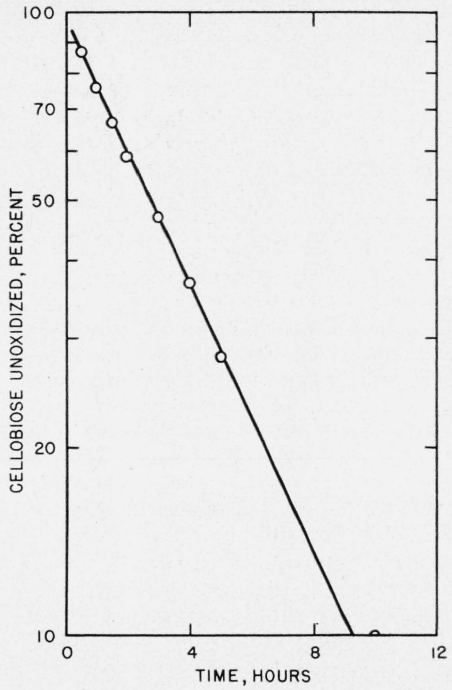

FIGURE 7. *Reaction of 0.01 millimole of cellobiose with chlorous acid; determination of order with respect to cellobiose by the integration method.*

0.005-*M* sodium chlorite buffered to pH 3.52 at 40° C.

Using values of 0.0042 M for C and -0.0000306 for m from figure 6, and calculating h at 40° C and a pH of 3.52 as 0.026,[3] $k_A=0.64$ liter mole^{-1} sec^{-1}.

Similarly, with a large excess of glucose, A is essentially constant, and

$$k_A=-\frac{2.303m}{hA}. \tag{11}$$

From figure 7, using values of 0.026 for h, 0.012 M for glucose concentration and $m=-0.000167$, $k_A=1.23$ liters mole^{-1} sec^{-1}.

It must be emphasized that this approach leaves much to be desired, and it is not surprising that the values for k_A do not show better agreement. Possible side reactions that are relatively unimportant when the chlorite is in excess may become important when the aldose is in excess, or vice versa. The combination of decomposition of chlorite and consumption of chlorite by aldose represents a decrease in chlorite concentration to about 70 percent of the initial value, and it is the average of this rather large change in chlorite concentration that is treated as a constant in eq (10).

The value of C in the control is always higher than the value of C in the test solution, as chlorite is consumed in the oxidation of aldose in the latter solution. Therefore, the decomposition of chlorite in the con-

[3] The value of K_{HClO_2} used in calculating h was calculated from the work of Barnett [25] as 0.0107 at 50° C and 0.0113 at 40° C at an ionic strength of 0.11.

trol is always greater than in the test solution, and a simple subtraction of curves, as in figures 3 and 4, represents an overcorrection for decomposition.

One approach to this problem is to prepare a calibration curve for each aldose, relating millimoles of chlorite consumed by the aldose (control minus test solution) to millimoles of aldose. This procedure automatically takes into account (1) the fact that the exact stoichiometric equations for the oxidation of aldose by chlorous acid are unknown, (2) the fact that "slow" and "fast" aldoses require slightly different quantities of chlorous acid for oxidation, and (3) the difference in amount of spontaneous decomposition in the control and in the oxidation.

The data for the calibration curve for glucose, figure 8, are given in table 2. Table 3 contains data showing the accuracy with which glucose can be determined by using the calibration curve.

TABLE 2. *Data for glucose calibration curve* [a]

Glucose added	$NaClO_2$ consumed at 20 hr: control minus test solution	Glucose added	$NaClO_2$ consumed at 20 hr: control minus test solution
Millimoles/ 40 ml	*Millimoles/ 40 ml*	*Millimoles/ 40 ml*	*Millimoles/ 40 ml*
0.001	0.0028	0.020	0.0526
.002	.0055	.025	.0652
.003	.0083	.030	.0768
.004	.0109	.035	.0880
.005	.0140	.040	.0986
.005	.0144	.045	.1072
.010	.0273	.050	.1153
.015	.0402		

[a] The first five values in the table are the average of triplicate determinations at 20 hr. The other values are taken from rate curves for the specific concentration of glucose.

TABLE 3. *Determination of glucose, using a calibration curve*

Glucose added		Glucose found [a]		Error
Millimoles	*mg*	*Millimoles*	*mg*	%
0.0010	0.180	0.0011	0.198	+10
.0030	.540	.0031	.558	+3
.0050	.90	.0050	.90	0
.0150	2.70	.0153	2.75	+2
.0300	5.40	.0293	5.28	−2
.0450	8.10	.0444	7.99	−1

[a] Each value reported in the table represents the average of triplicates.

Another approach to the problem of difference in amount of decomposition in the control and in the oxidation is to assume that eq (1) correctly represents the stoichiometry of the reaction of chlorous acid with "slow" sugars and derive an analytical expression that one can use for calculating glucose. This involves the integration of eq (7).

Differential equations describing systems of simultaneous reactions of higher than first-order reaction kinetics are not in general integrable in a closed form [27]. Therefore, an exact analytical expression for the aldose concentration as a function of the rate constants, chlorite concentration, and time, cannot be obtained. However, an expression relating the aldose concentration to the concentrations of chlorite in the test and control solutions can be derived if certain approximations are made.

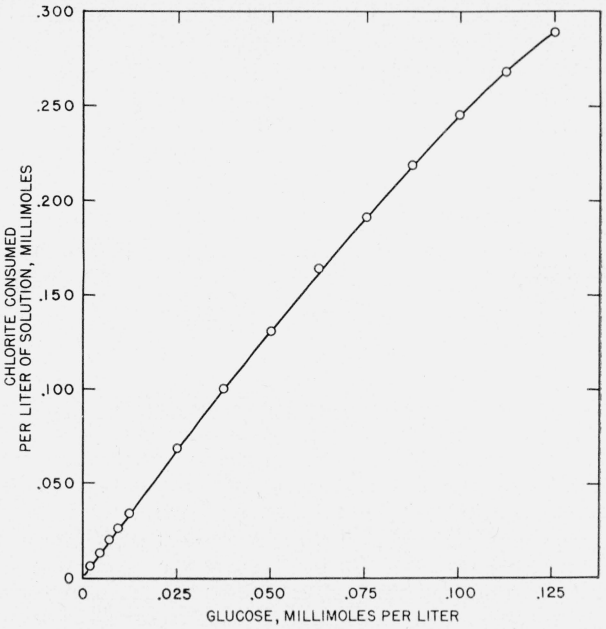

FIGURE 8. *Calibration curve for glucose.*

Assuming the stoichiometry of eq (1), the aldose concentration in the test solution (A_T) at any instant is given by

$$A_T = A_0 - \tfrac{1}{3}(C_0 - C_T - D_T), \tag{12}$$

where A_0 is the initial aldose concentration, C_0 is the initial chlorite concentration, C_T, the chlorite concentration in the test solution at time t, and D_T, the change in concentration of chlorite in the test solution due to decomposition. D_T cannot be measured directly. If C_c is the concentration of chlorite in the control solution at time t, then eq (4) may be integrated to obtain

$$C_0 - C_c = k_D h^2 C_0 C_c t. \tag{13}$$

Over any small time increment Δt, the change in concentration of chlorite in the test solution due to decomposition, ΔD_T, is approximately given from eq (4) by

$$\Delta D_T \cong k_D h^2 \overline{C}_T^2 \Delta t, \tag{14}$$

where $\overline{C}_T$ is an average value of the chlorite concentration in the test solution over the time interval. If it is assumed that eq (14) may be applied over the time interval $\Delta t = t$, and that $\overline{C}_T^2$ may be represented by the geometric mean, then

$$D_T \cong k_D h^2 C_0 C_T t. \tag{15}$$

Division of eq (15) by eq (13) leads to the relation

$$D_T \cong (C_0 - C_c)\frac{C_T}{C_c}, \tag{16}$$

which expresses the decomposition in the test solution in terms of chlorite concentrations.

When the aldose oxidation is essentially complete, $A_T=0$; so substituting the expression for D_T from eq (16) into eq (12) and solving for A_0

$$A_0=\frac{C_0}{3}\left(1-\frac{C_T}{C_c}\right). \tag{17}$$

If titration volumes are substituted for concentrations,

$$\text{Millimoles of aldose}=\frac{V_0N}{12}\left(1-\frac{V_T}{V_c}\right), \tag{18}[4]$$

where V_0, V_T, and V_c are the milliliters of the thiosulfate of normality N equivalent to the chlorite in the control initially, in the test solution at the completion of the reaction, and in the control at the completion of the reaction, respectively.

Equation (18) was tested by substituting the data from rate experiments; the results are given in table 4. Considering the assumptions made in deriving the equation, the small quantities of sugar determined and the range of experimental conditions, the results are quite acceptable. Although eq (18) cannot be considered as accurate as a calibration curve, it is extremely useful in that it can be used over a wide range of experimental conditions, while a calibration curve applies only to the set of experimental conditions for which it was determined.

TABLE 4. *Analytical results for glucose and cellobiose calculated from eq (18)*

Experiment	pH	Temperature	$NaClO_2$	Glucose [a]	Time for completion	Error
		°C	*moles/liter*	*Millimoles/liter*	*hr*	
1a[b]	3.70	40	0.01	1.80	29	+1
1b	3.70	40	.01	2.40	39	+1
2a	3.70	40	.01	0.12	11	−2
2b	3.70	40	.01	.24	11	−2
2c	3.70	40	.01	.48	13	−1
3a	3.70	40	.005	.12	20	−1
3b	3.70	40	.005	.18	20	0
3c	3.70	40	.005	.24	21	−1
4	3.70	40	.005	.25	25	−2
6	3.70	40	.005	.06	18	0
7	3.70	40	.005	.06	18	−6
8	3.70	40	.005	.06	18	−7
9	3.70	40	.005	.06	(c)	−6[d]
10	3.70	40	.005	[e].06	18	−1
11	3.52	40	.005	[e].25	14	−2[f]
12	3.52	40	.005	.25	14	−1
13	3.52	40	.005	.25	14	+1[f]
14	3.40	40	.005	.25	13	−1
15a	3.40	40	.005	.06	11	−3
15b	3.40	40	.005	.12	12	−1
16	3.70	50	.005	.06	9	−2
17	3.40	50	.005	.06	4	0
18	4.00	50	.005	.06	(c)	−6[d]

[a] NBS Standard Sample. The weighed amount was large enough to permit a weighing accuracy of 0.1 percent, and aliquot portions were taken from the solutions made therefrom. The other sugars were kindly furnished by H. S. Isbell.

[b] Experiments with same numbers but different letters had same V_c values. More than one oxidation run with one control.

[c] Incomplete.

[d] The curves for 9 and 18 were still rising rapidly, and the oxidation was obviously incomplete.

[e] Cellobiose.

[f] Curves were rising slightly at 14 hr.

[4] A similar derivation leading to this expression was given by Herbert F. Launer and Yoshio Tomimatsu in a paper presented before the Carbohydrate Division of the American Chemical Society in Chicago, September 8, 1953. This has been submitted for publication in Analytical Chemistry as a contribution from Western Regional Research Laboratory, U. S. Department of Agriculture.

3.2. Studies Based on the Photometric Determination of Chlorine Dioxide

From eq (1) it is apparent that aldoses may also be determined by measuring the concentration of the colored gas, ClO_2, photometrically when clear solutions are available. As in the iodometric method, the amount of aldose oxidized can be determined either from a calibration curve or from an analytical expression in which the quantities in eq (17) are expressed in terms of chlorine doxide.

$$2A_0=(ClO_2)_A=(ClO_2)_T-(ClO_2)_D \tag{19}$$

$$C_T=C_0-1.5(ClO_2)_A-2(ClO_2)_D \tag{20}$$

$$C_c=C_0-2(ClO_2)_c, \tag{21}$$

wherein $(ClO_2)_A$ is the chlorine-dioxide concentration arising from the oxidation of the aldose, and $(ClO_2)_D$ is that resulting from chlorous-acid decomposition. $(ClO_2)_T$ and $(ClO_2)_c$ are final concentrations in the test solution with aldose, and in the control solution without aldose, respectively, and are experimentally measurable, whereas those with subscripts A and D are not measurable and must be eliminated algebraically.

By combining eq (17), (19), (20), and (21), an expression for aldose in terms of chlorine dioxide and initial chlorite, in moles per liter, is obtained:

$$A_0=\frac{C_0}{2}\left[\frac{(ClO_2)_T-(ClO_2)_c}{C_0-1.5(ClO_2)_c}\right]. \tag{22}$$

Inasmuch as $(ClO_2)_c$ is small relative to C_0, eq (22) is insensitive to C_0 values.

The concentration of ClO_2 evolved in the test and control solutions was determined by measuring the transmission of light of 436-mμ wavelength[5] through glass-stoppered transmission cells with optically flat windows in which the reactions took place.

The simple abridged spectrophotometer previously described [28] was used in a modified form. The ranges of temperature, pH, and concentrations of chlorite and aldose used are given in table 5. Sodium-chlorite solution was added last to buffered solutions with or without aldose in volumetric flasks. A portion was then transferred by pressure through a drawn-out delivery tube to the transmission cell, and another portion was analyzed iodometrically for initial chlorite concentration, C_0. The mixing and transferring could be done with only small loss of ClO_2 by working at temperatures considerably below

[5] Obtained by use of a G. E. model H1 mercury-vapor lamp, using Corning filters 3389 and 5113 and an infrared filter.

the reaction temperatures. The filled cell was then placed in a constant-temperature water bath; it was found that the solution inside the cell attained the temperature of the bath in approximately 3 min.

TABLE 5. *Determination of glucose and cellobiose, using the photometric method*

Experiment	pH[a]	Temperature	Time for completion	Initial concentrations of reactants		ClO_2 produced		Aldose calculated from eq (22)
				$NaClO_2$	Glucose	In test solution	In control solution	
		°C	min	Millimoles/liter	Millimoles/liter	Millimoles/liter	Millimoles/liter	Percentage of amount added
1	3.6	30	[b] 181	73	1.56	5.33	2.62	[c] (92.0)
2	3.8	30	[b] 227	73	1.56	5.12	2.34	(93.9)
3	4.0	30	[b] 350	73	1.56	4.01	1.13	(95.2)
4	4.0	40	260	73	1.56	5.22	2.35	96.8
5	4.2	40	[b] 236	73	1.56	4.16	1.36	(92.6)
6	4.2	45	113	73	1.56	3.96	1.12	93.6
7	4.2	45	190	73	1.56	4.50	1.90	87.1
8	4.2	50	80	73	1.56	3.95	1.50	81.3
9	4.2	50	129	73	1.56	4.96	1.92	101.9
10	4.4	50	200	73	1.56	4.87	1.90	99.4
11	3.8	30	110	146	1.56	6.97	4.25	96.1
12	3.8	45	140	37	0.78	2.55	1.17	92.9
13	4.4	60	75	73	.80	3.13	1.85	82.1
14	4.2	60	70	38	.20	1.31	0.92	101.2
15	4.2	55	101	38	.40	2.03	1.27	100.0
16	4.0	55	182	25	.30	1.60	1.03	101.6
17	4.0	60	109	25	.30	1.52	0.94	102.5
18	4.0	65	109	18	.30	1.46	.87	106.2
19	4.0	65	60	40	.30	2.29	1.82	85.0
20	4.0	65	110	20	.30	1.57	1.01	101.0
21	4.0	65	205	10	.15	0.948	0.672	102.3
22	4.0	65	205	10	.075	.836	.672	122.0
23	4.0	65	180	10	.15	.930	.670	96.7
24	4.0	65	180	10	.075	.790	.670	88.7
25	4.0	65	210	10	[d] .15	.91	.69	82.0
26	4.0	65	150	20	[d] .30	1.87	1.32	101.6
Mean of values within the range 90.0 to 110.0%								[e] 99.6
Mean of all values								[e] 96.5

[a] Acetate buffers were used throughout.
[b] Incomplete.
[c] The values in parentheses refer to experiments known to be incomplete and were not included in the means.
[d] Cellobiose.
[e] The correction for chlorine dioxide decomposition would raise the means by 1.0 percent (see text).

Transmittance values were converted into concentrations by the use of Beer's law:

$$(ClO_2),\ \text{moles per liter} = 0.00853 \log \frac{T_{H_2O}}{T_{\text{solution}}}. \qquad (23)$$

The constant was calculated for a 10.00-mm cell thickness at a temperature of 65.0° C. At 25.0° C the constant was 0.00873, a difference of about 2 percent, allowing fairly accurate interpolation when required. Chlorine dioxide was found to follow Beer's law, with a standard deviation of individual values of 0.31 percent over the range of interest, 0.00032 to 0.0089 *M*. This was determined by measuring transmissions of fresh solutions of ClO_2 at known concentrations in buffer solution. ClO_2 was generated by adding warm 6-*N* sulfuric acid to 0.7-*M* sodium chlorite and washed by passing the gas through several portions of water. ClO_2 concentrations were determined iodometrically.

If desired, the analyst can measure the transmittance of the test cell in terms of that of the control cell by setting the transmittance scale to 100 percent when the control cell is in the beam. This gives the transmittancy of the *difference* in ClO_2 concentrations. Equations (22) and (23) may be combined to give

$$A_0 = -0.00427 \frac{C_0}{C_0 - 1.5(ClO_2)_c} \log \frac{T_T}{T_c}. \qquad (24)$$

The value for $(ClO_2)_c$ must then be determined separately with eq (23), but the requirements for its accuracy are much less than for the ratio T_T/T_c.

Equation (22) was tested under experimental conditions that varied with respect to pH, temperature, and concentrations of reactants and products. The ClO_2 concentrations were taken from time-concentration curves at points indicating completion of reaction, excepting experiments 1, 2, 3, and 5 in table 5, which were discontinued before the curves had reached a maximum aldose value.

Although the aldose values calculated from eq (22), presented in table 5, show considerable variation, much more than that of aldose values determined by the volumetric technique, the mean values by the two techniques are in essential agreement and thus appear to confirm eq (1) with respect to the ratio 1 aldose:$2ClO_2$. The variations do not appear to be inherently greater in the photometric than in the volumetric method. They probably arise from the fact that in the photometric work, results were taken from time-concentration curves, each representing one reaction mixture, and thus, any rate discrepancy affected the entire curve. In the volumetric method, however, each point represented a separate reaction mixture and individual rate errors tended to be averaged out by the drawing of a smooth curve through the points. The same result was, of course, achieved in the photometric method by averaging the values from the individual experiments of table 5.

The aldose values of table 5 (column 9) tend to be low because of ClO_2 decomposition, shown by Bray [29] to occur in aqueous solution. This was confirmed photometrically for the present experimental conditions. Using the time-concentration curves shown in figure 9, it is possible to correct the ClO_2 values of table 5 for decomposition, raising the aldose values by an average of 1.0 percent. These corrections are maximum at high temperature and acidity and low aldose.

A factor that tends to limit the sensitivity of the photometric technique is the occurrence of the "initial immediate decomposition" of the reagent. This effect, which was discussed previously, occurs whenever the chlorite solution is added to the acidic buffer, as seen from the immediate development of yellow color. The amount of ClO_2 thus produced, before the decomposition or oxidation occurred to an appreciable extent, averaged 0.20 millimole/liter,

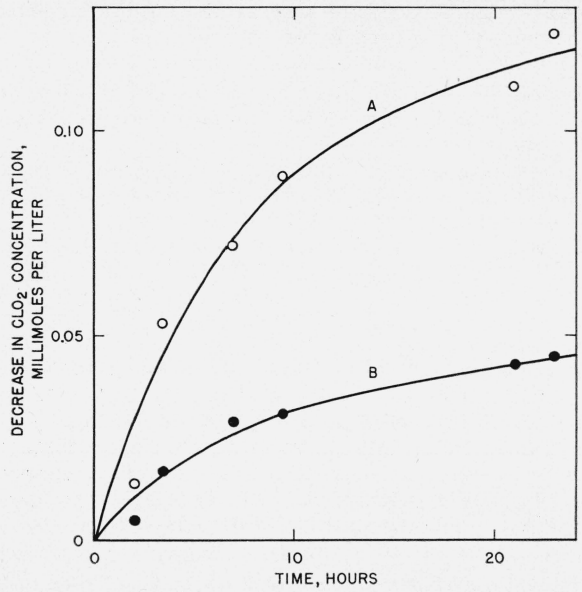

FIGURE 9. *Decomposition of ClO_2 under typical conditions.*

pH 3.7 (acetic acid-acetate buffer) 40° C, initial ClO_2 concentration for (curve A) 2.92 and for (curve B) 0.53 millimole per liter. Reaction was carried out in transmission cells and ClO_2 measured photometrically.

and appeared to be due to reaction of the reagent with impurities. Its value was taken in each experiment as the zero-time intercept of the decomposition curves and was thus accounted for in the ClO_2 values of table 5. Application of the photometric technique to very low aldose quantities would require the careful control or evaluation of this factor.

4. Determination of Melibiose, Maltose, and Lactose

Melibiose, maltose, and lactose are oxidized at about the same rate as glucose and cellobiose. Table 6 contains some analytical data on these sugars, using the iodometric method. These data are probably typical of the accuracy one could expect in the determination of small quantities of any particular sugar by this method without further standardization of conditions for it. The curves for lactose and maltose were quite smooth, but the data for melibiose were very erratic.

Results obtained with calcium lactobionate shown in table 7 suggest that lactose is hydrolyzed to a slight extent.

TABLE 6. *Determination [a] of maltose, lactose, and melibiose*

Calculated by—	Time	Error for—		
		Maltose	Lactose	Melibiose
	hr	%	%	%
Equation (18)	12	−1	−1	[b]
	18	−1	+7	
	24	+3	+10	
Glucose calibration curve	20	−4	+1	−10
Net curve: control minus test solution	20	−12	−6	−19

[a] Reactions were carried out in liter wash bottles and 25-ml aliquots taken at intervals. 0.25 millimole aldose per liter, 0.005-*M* $NaClO_2$, pH=3.52, t=40° C.
[b] Data too erratic to warrant calculation.

5. Action of Chlorous Acid in Nonreducing Sugars and Sugar Acids

Information on the action of chlorous acid on nonreducing sugars, sugar acids, salts, and lactones is given in table 7.

The data, in agreement with the work of Jeanes and Isbell [21], indicate that these compounds are oxidized little or not at all for the experimental conditions indicated.

TABLE 7. *Reaction of chlorous acid with lactones, salts of sugar acids, sugar acids, raffinose and sucrose*

0.005-*M* $NaClO_2$, pH=3.52, t=40°

Compound	Millimoles per liter	Extent of oxidation
		Percent
Rhamnomic lactone	0.25	Negligible
Do	1.25	Do.
Mannonic lactone	1.25	Do.
Calcium lactobionate	1.25	3
Calcium melibionate	1.25	Negligible
Gluconic acid	0.25	Do.
Raffinose	.25	Do.
Sucrose	14.6	2
Do	1.25	2

6. Recommended Procedure for Volumetric Method

The procedure recommended below is specific. As indicated by the range of conditions in table 4, one may use many different combinations of values for the pH, time, dilution, concentrations of aldose and chlorous acid, temperature, etc., to satisfy particular requirements.

This procedure was not applied to the photometric method. Higher chlorite concentrations may be used in that method to decrease the time for completion of the reaction as it does not depend upon the measurement of the change in chlorite concentration. The shorter reaction time is preferable in the photometric determination as it minimizes error introduced by the decomposition of chlorine dioxide.

Experimental conditions:	
Temperature	40° ±0.1 deg C
pH	3.52
Chlorite concentration	5.0 millimolar
Aldose	Not more than 0.50 millimolar
Sodium oxalate	125 mg/liter
Total volume	40 ml
Composition of test solution:	
Buffer [6]	10 ml
Sodium chlorite, 0.04 *M*	5 ml
Water and aldose solution to make a total volume of	40 ml
Composition of control:	10 ml
Buffer [6]	10 ml
Sodium chlorite, 0.04 *M*	5 ml
Water	25 ml

Glass-stoppered flasks of 250-ml capacity are recommended. The sodium chlorite solution is added last, and immediately thereafter the flask is placed in the constant-temperature bath. At the proper time the flask is removed from the bath

[6] The buffer composition is given in table 1. The sodium oxalate is added to the buffer solution.

and fitted with a short piece of tubing with a standard taper joint. Twenty-five to 50 ml of water is added and one or two bubblers, made by drawing about 8-mm tubing to about 1 mm at the end, are inserted. After bubbling the solution for 10 min with CO_2, N_2, or air, 50 ml of 10 percent KI and 5 ml of 6-*N* HCl are added and the liberated iodine titrated with 0.025 *N* thiosulfate. Under these conditions, 0.01 millimole of glucose is oxidized in about 20 hours.

Rate data may be obtained in the above manner, or aliquots may be taken from larger batches of solution, mixed in the same proportions as above. In the latter case, it is preferable to use wash bottles and blow out some solution into a graduated cylinder from which an aliquot may be pipetted. It must be remembered that ClO_2 is poisonous, and a water aspirator should be used when pipetting solutions containing ClO_2. The ClO_2 should be bubbled out of the above solutions under a hood.

The aldose may be estimated by eq (18), or by referring to a calibration curve.

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