

**STUDY OF THE CARBOHYDRATE PEELING AND  
STOPPING REACTIONS UNDER THE CONDITIONS  
OF OXYGEN-ALKALI PULPING**

**Project 3265**

**Report Three  
A Progress Report  
to**

**MEMBERS OF THE INSTITUTE OF PAPER CHEMISTRY**

**July 14, 1977**

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

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SUMMARY

The importance of this research in clarifying ambiguous results in the literature has been described and attributed to analytical uncertainties. Some of the difficulties analyzing for reaction products by GLC have been described and overcome. Inositol has been shown to be inert to oxygen and alkali and therefore suitable as an internal standard.

Oxygen analysis has been shown to be unaffected by the presence of cellobiose or its degradation products. Measurement of oxygen consumed during the reaction showed that about 3 to 4 moles of oxygen reacted with one mole of cellobiose and that a great excess of oxygen remained in solution after the destruction of cellobiose.

Reactions between dissolved oxygen and a carbohydrate substrate have been carried out in solutions of varying pH and at different temperatures, the experimental conditions being such that mass transfer from a gaseous phase was not a factor. The extent of oxidation (yield of glucosylaldonic acids formed from cellobiose) ranged from 6 to 23%. The bulk of the cellobiose was destroyed by the peeling reaction. The oxidation was favored, relative to peeling, by high pH, low temperatures, and a slow heat-up period at the beginning of the reaction. No glucosylaldonic acids were observed when nitrogen was used instead of oxygen.

Reactions run at 100°C showed two effects. First, the rate of reaction tended to level out due to the formation of glucosylaldonic acids. The yield of these acids was about 15% for a high pH system (sodium hydroxide)

and about 5% for a lower pH (sodium carbonate). Secondly, the rate of reaction in the initial phases was much slower for an oxygen system than for a nitrogen system, due again to the formation of the alkali-stable glucosylaldonic acids.

When the reaction temperature was increased to 130°C, the yield of glucosylaldonic acids in a sodium hydroxide-oxygen system was about 6-8%. When the two solutions were mixed at room temperature and then brought to temperature within 30 seconds, this heat-up period increased the yield of glucosylaldonic acids to about 23%. The possibility of an induction period has been considered.

When sodium carbonate was used instead of sodium hydroxide, the reaction system with a 30-second heat-up period gave about half the yield of glucosylaldonic acids (10.5%) showing the effect of lower pH. This same pH effect was shown for reactions carried out in a digester with either sodium hydroxide or sodium carbonate for a heat-up period of 90 minutes. However, the use of a heat-up period with sodium carbonate at a higher temperature seems to be more beneficial (10.5% at 130°C) than the use of a lower temperature with no heat-up period (5% at 100°C).

#### OBJECTIVE

This project is one of the supporting projects currently active at the Institute, which have their general objectives aimed at providing the required information for the successful development of a commercial oxygen-alkali process for pulping wood. The purpose of this project is to study the relative rates of peeling of glucose end groups present in oligosaccharides in comparison with the oxidation stopping reaction of the same groups in a system composed of oxygen and alkali or in buffered solutions of lower pH. This information is necessary to ensure that carbohydrate loss is kept to a minimum and that the yield of an actual pulping process will be optimum.

## INTRODUCTION

Previous reports on this project have described the assembly and choice of reactor materials (1), and later an improved reactor design, a comparison of gas and liquid chromatographic techniques and a satisfactory method of oxygen analysis (2). This report continues the development of analytical techniques, develops the objective more fully, and locates the parameters of greatest importance to the project.

The tendency for the oxidized end groups to stabilize cellulose against "peeling" had led to attempts to apply this modification to pulping technology; unfortunately, with indifferent success when oxygen is employed as an oxidant (3). Since some of the possible oxidized end groups are thought to be relatively unstable in hot alkali (4), the anomalous behavior reported after attempted pulp stabilization may result from the reactivities of the different types of terminal acids formed during the pretreatment stages. Knowledge of the relative stabilities of these end groups, as well as conditions necessary for their formation, are necessary to attain the goals of this project.

The problems to be solved were as follows: (a) to prepare solutions of dissolved oxygen under pressure in various alkaline or buffer solutions, (b) to analyze these solutions for oxygen content, (c) to maintain the oxygen in solution while reacting carbohydrates at various times and temperatures, and finally, (d) to analyze the resulting solutions for the extent of reaction by either oxidation or peeling. These problems have been alleviated sufficiently to permit an investigation of the major goals of the project.

In this report, details of reactions carried out in a flow reactor are given, which show how dissolved oxygen reacts with solutions of carbohydrates.

The experiments are done in a reaction system that is isolated from any external oxygen supply, such as an oxygen atmosphere ordinarily employed in these reactions. There is no gaseous phase; rather a simple mixing of two aqueous solutions initiates the reaction. The concentration of dissolved oxygen is about ten times (molar basis) that of the cellobiose, so that an excess of oxygen is always present during the reaction. Control experiments have shown that the dissolved oxygen will remain in solution during the course of the reaction and that the calculated excess of oxygen is present. Thus, there is no problem of mass transfer of gaseous oxygen into the aqueous system. To our knowledge, this is the first time that such an isolated system containing dissolved oxygen has been operated successfully at the relatively high temperatures and pressures needed to allow direct comparison to pulping reactions.

Because the reaction in the flow reactor is started by rapidly mixing two solutions already maintained at the reaction temperature, there is no period of "heat-up" of the reaction solution. In contrast, heat-up periods of 20 to 90 minutes are frequently required to bring the contents of conventional laboratory digesters to reaction temperature. Also, since the reaction is stopped rapidly, there is no loss of products due to continued reaction during the cooling period. Therefore, it is possible to study the effect of temperatures on these reactions in an unambiguous manner for the first time.

The light mass and thin walls of the coil reactor allow rapid heat transfer, so that a heat-up period of a few seconds can be achieved and the extent of unwanted degradation is kept to a minimum. This behavior makes possible multistage temperature treatments to simulate some types of industrial practice. This same behavior makes it difficult for us to reproduce the oxidations run in conventional laboratory digesters reported in the literature where exact heat-up periods are often not given.

Since the solubility of oxygen in aqueous solutions is rather low, on the order of 300 mg/liter, reactions were carried out with equally low concentrations of carbohydrate, to ensure an excess of oxygen in the reactions studied. The molar ratio of oxygen to cellobiose was about 10 to 1, and the mass ratio about 1 to 1. The alkali, either sodium hydroxide or sodium carbonate, is present in much higher concentration. The oxygen has to compete with this greater amount of alkaline reagent for cellobiose in the parallel reactions culminating in the various cellobiose degradation products as shown in Fig. 1. The success of this project resides in the nature of the effect of different external experimental parameters upon those competing parallel reactions.

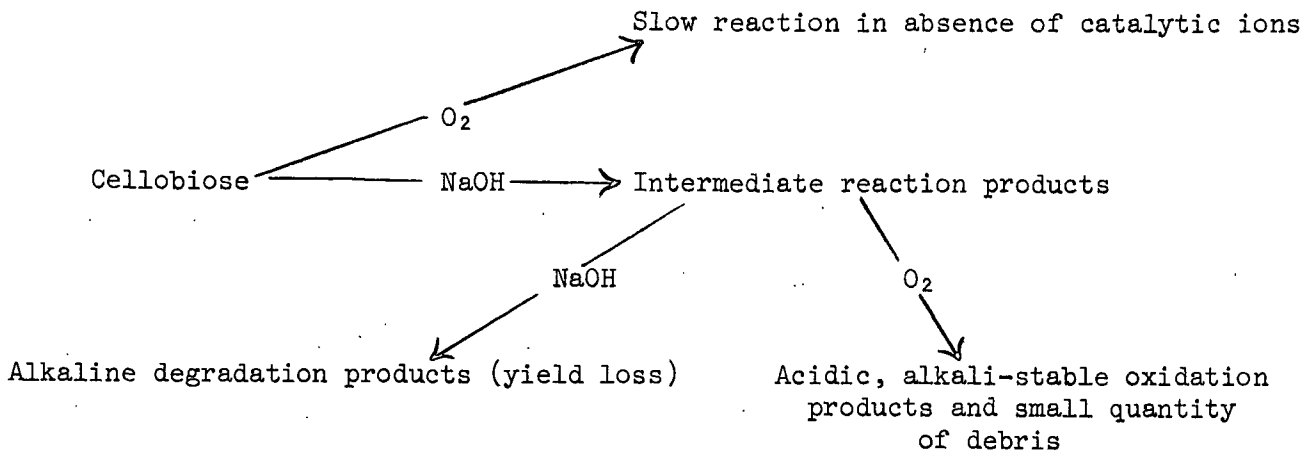


Figure 1. The Competition of Caustic and Oxygen for Cellobiose

Finally, it should be pointed out that, while the present investigation involves an attempt to discover the parameters of the oxygen-alkali cellobiose system, the quantitative relations of these parameters have not yet been determined but will be the subject of a final report.

## CONCLUSIONS

Recent activity in this project can be summarized as follows:

1. Preliminary oxidations of cellobiose with oxygen have confirmed the dependence of degradation upon time of reaction and it has been shown that the initial rate of degradation is dependent upon the pH of the reaction. The ultimate degradation does not depend upon pH in a simple manner, but is dependent upon the formation of alkali-stable glucosylaldonic acids. Thus, the ultimate degradation of glycosidic bonds in many instances is greater in carbonate solution than it is in caustic solutions.

2. The formation of glucosylaldonic acids is a result of competing reactions of alkali and oxygen for cellobiose or its reaction products. An initial reaction with alkali is probably necessary to provide satisfactory reactive intermediates. If these intermediates can be oxidized to glucosylaldonic acids by oxygen more readily than they can be degraded by elimination reactions to useless products, then stabilization of polysaccharide end groups to alkali is possible. Preliminary results suggest oxygen pressure and sodium hydroxide concentration will be the most acceptable parameters controlling these reactions.

3. Additional uncertainties in the experimental procedure for the evaluation of the oxidation of cellobiose by oxygen have been eliminated when it was shown that the quantity of dissolved oxygen was greatly in excess of that consumed by the experimentally acceptable quantity of cellobiose. Also, it was shown that this quantity of disaccharide did not interfere significantly with the Winkler method of oxygen analysis. Suitable internal standards were considered, and inositol was demonstrated to be unaffected by the alkaline oxygen reagent under the most strenuous conditions contemplated for this research.

4. The separation of cellobiose, cellobiitol, and possibly cellobiulose from the glucosylaldonic acids by conventional GLC techniques could not be accomplished. Removal of the glucosylaldonic acids from the reaction mixture was easily accomplished, but their recovery from the ion exchange resins was very difficult. It was decided to eliminate the cellobiose remaining by chemical degradation and analyze only for the alkali-resistant major acidic components. This course of action requires the elimination of lactone formation during sample recovery and such procedures are now being developed.

5. Besides the above-mentioned analytical task, it will be necessary to locate and identify the pertinent, major acidic products detected by the quantitative GLC procedure. Glucosyl gluconic acid and glucosyl arabinonic acid have already been prepared and glucosylmannonic acid and glucosylerythronic acid are partially synthesized. These acids will be used as authentic controls to locate reaction products and to demonstrate the stability of the controls to the conditions of the cellobiose degradation.

Future activity will center on determining the effect of various experimental parameters such as time, temperature, oxygen pressure, alkalinity and pH, and catalytic influences on the formation of alkali-stable products. Once this is accomplished, work on the project will terminate.

## BACKGROUND

Oxygen is relatively inert to many organic substances. The slow decomposition of newsprint and other paper products in air suggests oxygen does react with woody substances, especially lignin. Alkali, needed to promote the reaction with lignin, also brings about an interaction of a carbohydrate end group with oxygen to form a relatively stable acidic end unit. It can be conjectured that in the alkaline system the carbonyl group of the carbohydrate end unit is converted to enediols or to dicarbonyl intermediates, which might react rapidly with dissolved oxygen, resulting in the loss of one or more carbon atoms. Either or both of these intermediates have been proposed to react with oxygen. Since in many instances they give very similar degradation products (5-12), as suggested in Fig. 2, it is difficult to distinguish between these pathways. It is not likely that the carbonyl group of the reducing end of a polysaccharide will react directly with oxygen initially. With the passage of time, as the peroxide level builds up and induction periods are eliminated, it is likely these intermediate peroxides derived from the reduction of oxygen will react with carbonyl groups by the mechanisms suggested by Isbell, et al. (13). This aspect of the degradation will not be considered further in this report.

Other alkaline rearrangements of the carbohydrate material can also occur, such as rearrangement of cellobiose to the ketose isomer cellobiulose, and subsequent formation of a 2,3-enediol can occur before reaction of the intermediates with oxygen. An outline of the hypothesized partial degradation of cellobiose in alkali alone is given in Fig. 3 and illustrates the many possible intermediates produced from cellobiose by alkali which can react with oxygen.

It is obvious from Fig. 3 that oxygen must oxidize the intermediates at an early stage of the alkaline degradation in order to avoid the peeling of

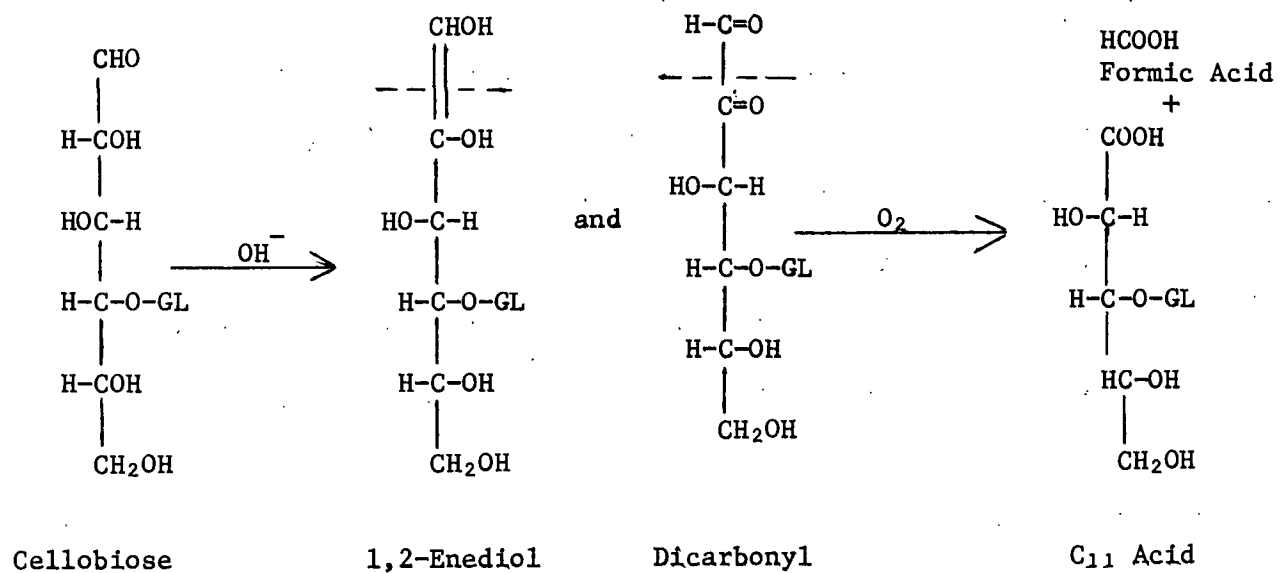


Figure 2. Oxidation of Cellobiose With Loss of One Carbon Atom

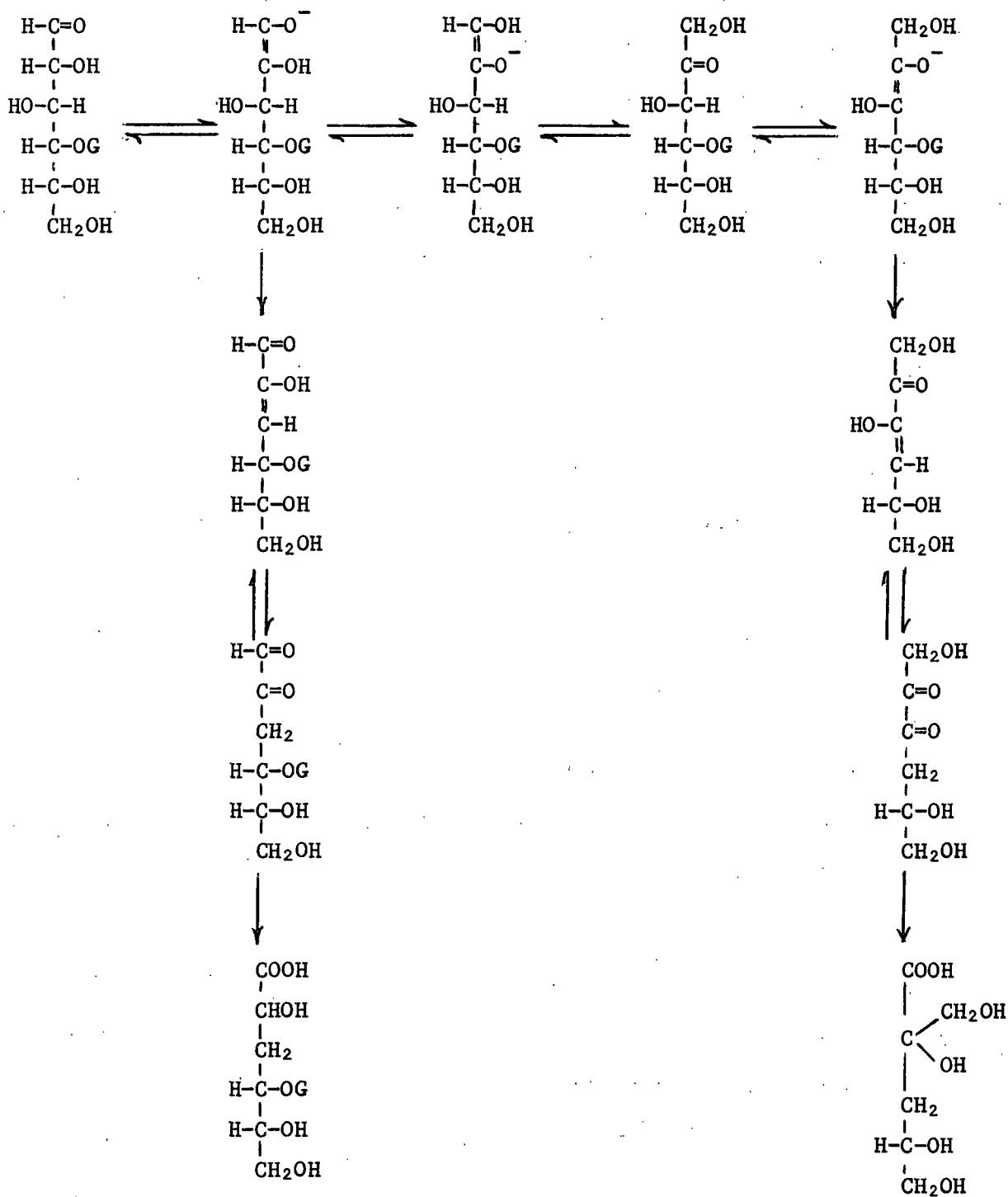


Figure 3. Partial Mechanism of Alkaline Degradation of Cellobiose (16)

the modified terminal unit from the polysaccharide. In the case of cellobiose, the oxidation may involve the degradation of a glucosyl-glucosone intermediate, or a reaction with an enediol by the conventional oxidative mechanisms as suggested earlier. A schematic representation of the main degradation routes of glucosyl-glucosone in oxygen-alkali is given by Lindberg and Theander (11). A complex mixture of C<sub>12</sub>, C<sub>11</sub>, and C<sub>10</sub> acids (collectively called glucosylaldonic acids in this report) can be theoretically-obtained from these two conjectured reaction mechanisms, and the identification and characterization of all these acids presents a formidable task beyond the scope of this project. The most likely four C<sub>12</sub>, three C<sub>11</sub>, and two C<sub>10</sub> acids expected if the intermediates in Fig. 3 are oxidized by oxygen or peroxide are shown in Fig. 4. The acids present in major amounts will have to be identified and measured in a quantitative manner for the success of this project. It is not anticipated that C<sub>12</sub> to C<sub>9</sub> dicarboxylic acids will be encountered in these investigations and that unsaturated and keto acid derivatives are equally unlikely.

Many studies of the oxygen oxidation of cellobiose and other carbohydrates have been reported in the literature (10-21) and many of the reaction products have been identified. Of particular interest here are the glucosylaldonic acids which can give resistance to further degradation. These experiments frequently employ relatively long reaction times, involving a heating-up period, and the minimum reported reaction time at maximum temperature is 5 minutes. Sometimes the reactions involving the peeling or oxidation of cellobiose are completed during the heat-up period before maximum temperature is reached. This ambiguity clouds the interpretation of some experimental results reported in the literature.

In an early publication, Hardegger, *et al.* (17), optimized the formation of glycosyl arabinonic acids by oxidizing the corresponding C<sub>12</sub> disaccharides

with oxygen in Ba(OH)<sub>2</sub> solutions at room temperature. The conditions were chosen for manipulative convenience and the glucosylaldonic acid was isolated in about 14% yield after crystallization as a brucine salt.

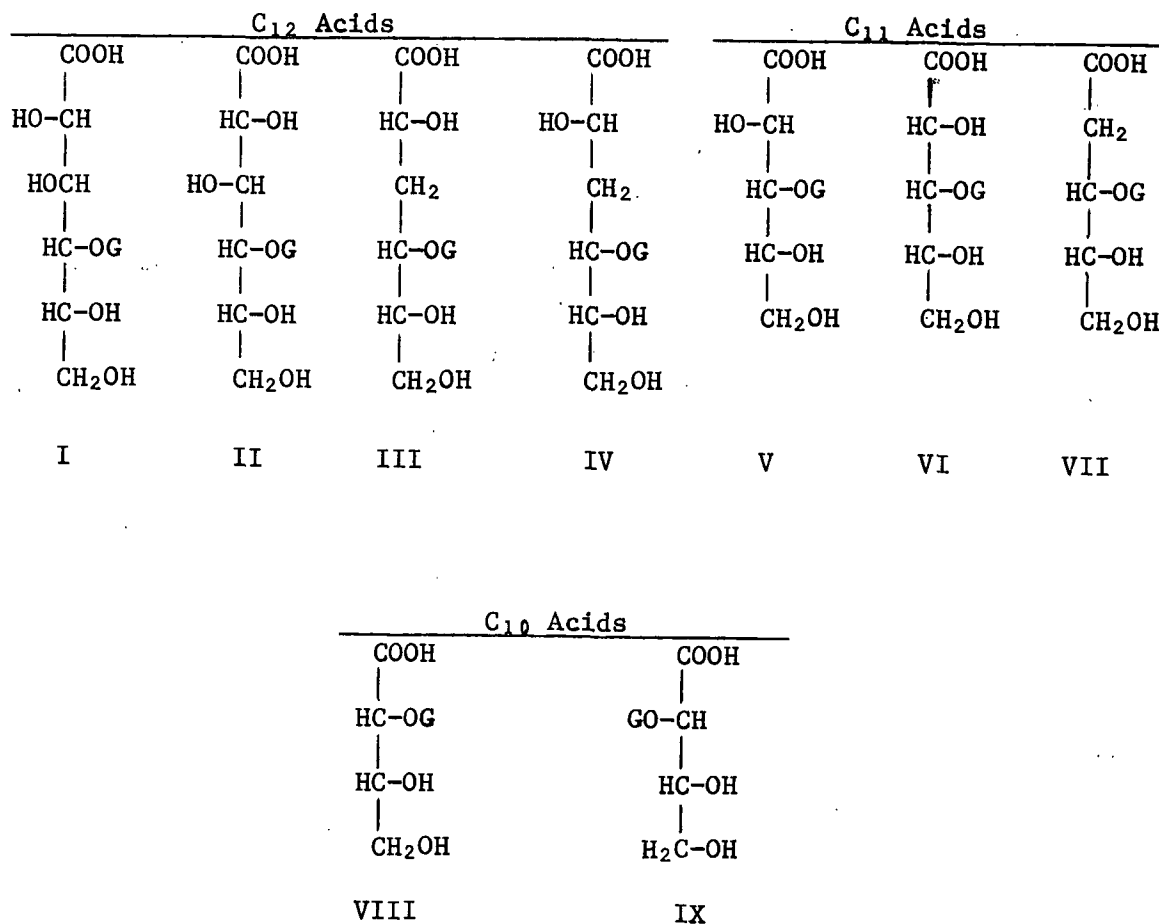


Figure 4. Disaccharide Acids Potentially Available from Cellobiose Oxidized by Oxygen in Caustic Solutions

Rowell, et al. (16) compared the rate of peeling and oxidation of cellobiose during oxygen-alkali degradation. They calculated the relative yields of isosaccharinic acid (as the main product of peeling) and of the unseparated mixture of glucosylaldonic acids at the end of an alkali-oxygen reaction carried out at 25, 50, and 100°C for varying periods of time in sodium hydroxide. Qualitative analysis of the reaction mixture showed the major disaccharide acids were 3-O-β-D-glucopyranosyl-D-arabinonic acid, 2-O-β-D-glucopyranonyl-D-erythronic acid and

4-O-β-D-glucopyranosyl-D-mannonic acid. The total yield of these acids depended upon the presence of oxygen, type of base, and the concentration of hydroxide ion as shown in Table I. By comparing Rowell's work with Hardegger's research, it is likely that the reaction product is predominantly glucosylarabinonic acid when the oxidation is carried out in the presence of barium hydroxide and at lower temperatures.

TABLE I

THE ALKALINE DEGRADATION OF CELLOBIOSE IN NITROGEN, AIR, AND OXYGEN (16)

Temperature, °C	Atmosphere	Time of Reaction	(0.04N) Base	Combined Yield of Glucosylaldonic Acids <sup>a</sup>
25	N <sub>2</sub>	48	Ba(OH) <sub>2</sub>	0
25	Air	48	"	9.1
25	O <sub>2</sub>	48	"	27.1
50	N <sub>2</sub>	6	Ba(OH) <sub>2</sub>	0
50	Air	6	"	3.4
50	O <sub>2</sub>	6	"	9.9
100	Air	2	Ba(OH) <sub>2</sub>	1.5
25	Air	48	NaOH	2.4
50	Air	6	"	1.7
100	Air	2	"	0

<sup>a</sup>These acids were not analyzed separately.

At a later date, Malinen (12) and Malinen and Sjostrom (18-21) reacted cellobiose at temperatures from 50 to 120° with oxygen and alkali. Their experimental conditions required a 20-minute heat-up period before reaching reaction temperatures. They found that the combined yield of glucosylaldonic acids (C<sub>12</sub> to C<sub>10</sub>) were proportional to (a) the pressure of oxygen and (b) the pH of the system, and inversely proportional to (c) the temperature and (d) to the length of reaction time in a complicated manner. They attributed the effect of reaction

time to the fact that certain of the glucosylaldonic acids were unstable when exposed for periods of 1 to 2 hours to either alkali or alkali-oxygen at elevated temperatures (19). In the fragmentation of cellobiose to C<sub>11</sub> to C<sub>10</sub> acids, the carbon atoms are lost as formic acid and glycolic acids, respectively. Malinen and Sjostrom (18-21) reported appreciable amounts of these low molecular weight "monomeric" acids. However, they did not differentiate between the several glucosylaldonic acids, i.e., to the extent of C<sub>12</sub>, C<sub>11</sub>, or C<sub>10</sub> acid formation. They also reported appreciable amounts of glyceric acids; a C<sub>3</sub> acid which would be formed from the oxidation of a glucose intermediate after the removal of the terminal glucose fragment of this cellobiose.

In their second publication, Malinen, *et al.* (19) demonstrated that cellobionic acid, cellobiitol, and glucosyl erythronic acid were stable to 1% NaOH at 135°C in the absence of oxygen, whereas glucosyl arabinonic acid was completely destroyed in 2 hours. The degradation of the acids was not appreciably altered by the presence of oxygen. Thus, they concluded that the degradation of the arabinonic acid end groups of cellulose may not be particularly severe during oxygen bleaching because of the low temperatures and alkalinities generally employed, but it probably will become very significant at higher levels of these parameters. These results imply that terminal arabinonic acid units of modified cellulose would not render it completely stable to alkali during the kraft pulping process. This behavior of certain terminal acids in cellulose may help explain the increase in destabilization of oxygen pretreated wood meal with temperature described by Karna and Sarkanen (3) as compared to pretreated hydro-celluloses if preferential oxidation occurred in one instance and not in the other.

The data of Kolmodin and Samuelson (15) and others (11,14,20,22), shown in Table II, suggest that the ratio of the aldonic acids remaining on cellulose after reaction with oxygen may be altered. They studied the oxidation of various celluloses with oxygen and alkali and found that the aldonic acid end groups liberated after acid hydrolysis were primarily the C<sub>5</sub> acid, arabinonic acid, the C<sub>4</sub> acid, erythronic acid, and the C<sub>6</sub> acid, mannonic acid. The first two would correspond to the C<sub>11</sub> and C<sub>10</sub> acids in the case of cellobiose, while the hexonic acid correspond to the C<sub>12</sub> acids obtained after the degradation of cellobiose by various researchers. It is apparent from the table that the oxygen-alkali bleaching of pulp or hydrocellulose, relatively free of lignin for various periods of time, gives oxidized end groups at one end of the cellulosic chains that differs considerably from those detected in cellulose that had been oxidized under more strenuous conditions in the presence of a lignin derivative. It is, however, not known whether this difference reflects the longer reaction time (which would tend to substitute stable terminal acids for unstable acids along with greater yield loss) or the catalytic presence of lignin (and its attendant transition metal ions) in the degradative mechanisms leading to acid formation. The table does demonstrate that different acidic end group combinations can be achieved experimentally, and some of these combinations of acids are thought to be more resistant to alkaline and possibly oxygen degradation than other combinations.

It is interesting to speculate that little, if any, acidic end groups, characteristic of the alkaline rearrangements terminating the peeling reaction, have been detected after oxygen oxidation of cellulose. The data in Table II indicate 3 to 6% of a metasaccharinic acid. These acids might not be formed because of the more rapid formation of alkali resistant oxygen-derived end units. On the other hand, that type of acid (such as Acids III and IV in Fig. 4) might be unstable to oxygen-alkali combinations. This degradation might be analogous



to the unexplained relative instability of methyl 2-deoxy- $\beta$ -D-glucopyranoside (compared to methyl  $\beta$ -D-glucoside) to oxygen and alkali at 120°C observed by McCloskey, *et al.* (23).

The smaller "monomeric" acid products formed as a result of the oxidative removal of end groups from cellulose and cellobiose include glucoisosaccharinic acid, formic acid, glycolic acid, as well as glyceric, lactic, butyric, 3,4-dihydroxy butyric and 3-deoxy pentonic acids (15,20,24). Some of these acids can be formed by the destruction of a glucopyranosyl ring. Other products formed include hydrogen peroxide (23,26) and organic peroxides in smaller quantities (23,27).

## RESULTS AND DISCUSSION

### ANALYSIS OF REACTION PRODUCTS

It is unfortunate that no technique exists in the literature for the analysis of the expected acidic mixtures from the oxidation of cellobiose with oxygen. The researches of Rowell (16), although quantitative in many other respects, did not separate the glucosylaldonic acids by their cumbersome "Auto-analyser" technique. The more rapid GLC procedures employed by Malinen and Sjoström (13,18,19) were not carried out in a quantitative manner and the important acidic products were most often reported as ratios of the acids detected on the chromatogram. In only one instance was it possible to calculate the yield of total glucosylaldonic acid with respect to cellobiose from their data. The individual glucosylaldonic acids were not analyzed because of the confused GLC pattern produced as a result of lactone formation. Their results were further complicated by the nonquantitative responses due to the difficulty of silylating the acids in a pyridine solvent. By retaining the acids as an ammonium salt and by silylating the products in DMSO solution, these previous difficulties have been overcome.

Preliminary experiments were carried out to follow the reaction of cellobiose with oxygen and alkali in the most efficient manner possible. GLC techniques were chosen and the silyl derivatives of cellobiose and glucose could be resolved into their  $\alpha$ - and  $\beta$ -anomers as shown in Fig. 5. Also shown in Fig. 5 is the GLC pattern after the complete degradation of cellobiose by refluxing it in NaOH for 10 minutes. The low molecular weight "monomeric" acids have mobilities closer to those of glucose and the resolution of this fraction will not be attempted. One difficulty encountered in the analysis of the oxidation products of cellobiose is the close similarity of the mobilities of cellobiose

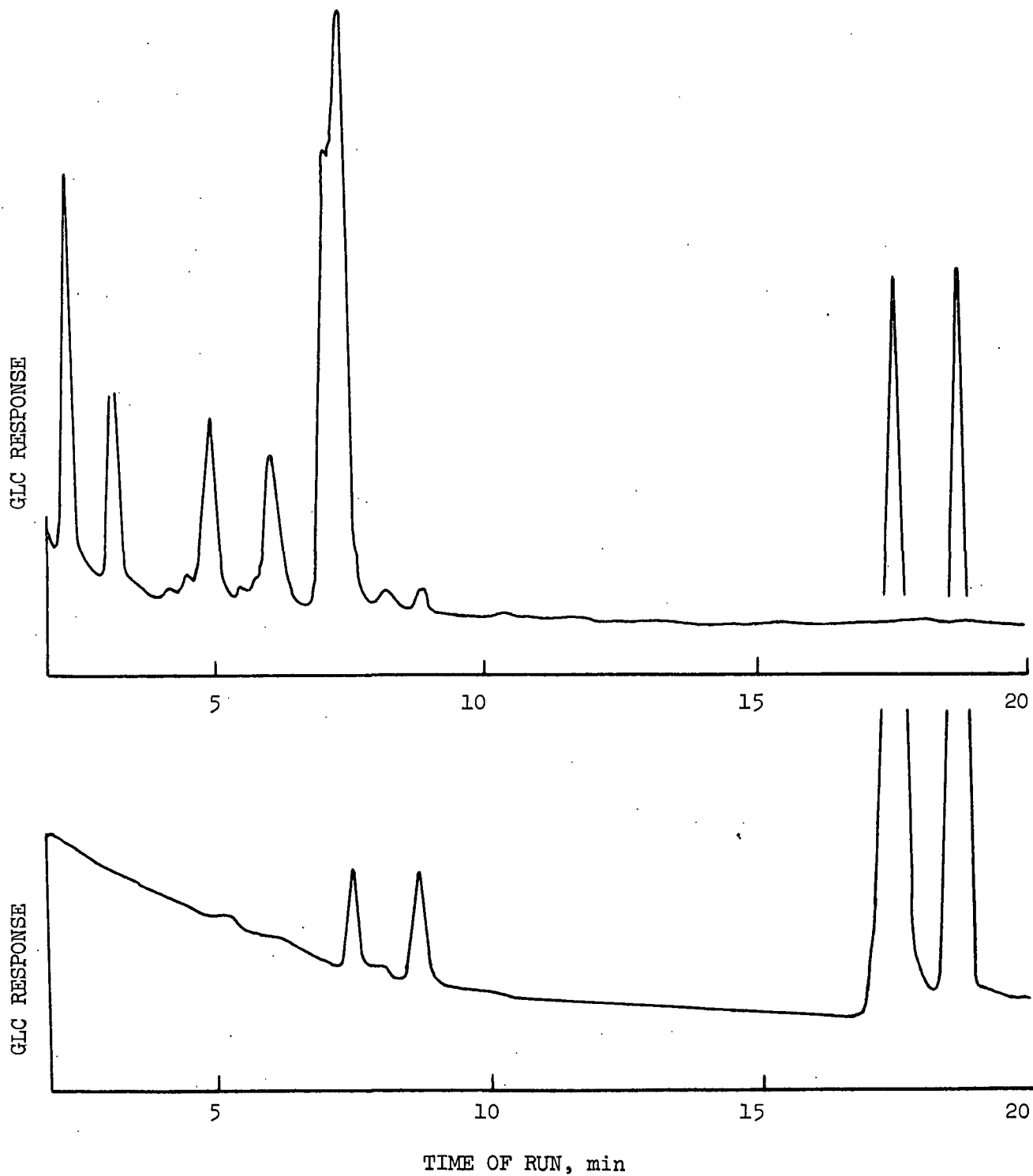


Figure 5. Chromatograms of Glucose and Cellobiose Silyl Derivatives (Below) and the Products from the Complete Degradation of Cellobiose with Alkali (Above)

and the glucosylaldonic acids as shown in Fig. 6. It is difficult to analyze such patterns quantitatively because of the overlapping of their different response factors. Attempts were then made to separate the acidic components from the nonacidic using exchange resins, such as MB-3. This permitted a separation of cellobiose, but the glucosylaldonic acids could be removed from the resins only with difficulty. The conversion of cellobiose to cellobiitol did not help greatly, since considerable GLC overlap still existed (see Fig. 6 and 7) and the removal of the glucosylaldonic acids from the ion exchange resin was in no way affected by these changes. Since the object of this research is not the degradation of cellobiose, but the formation and analysis of glucosylaldonic acids, some type of specific chemical destruction of residual cellobiose will be carried similar to that described previously (28). This will eliminate the formation of hold-up in the ion exchange resin.

As reported earlier (28), the change in concentration of the reaction products will be followed by comparing the areas (and responses) of the glucosylaldonic acids to those of an appropriate (inert to oxygen) standard. Several such standards were considered, such as inositol and glucoheptonic acid, shown in Fig. 7. Inositol was chosen because it migrates to a region in our GLC relatively clear of both disaccharide acids and other low molecular weight debris formed during the reaction and because it does not form lactones.

An internal standard stable to the reaction conditions makes the quantitative recovery of the reaction solution from our high pressure oxygen reactor unnecessary. The stability of inositol was established by comparing the areas of the GLC pattern after reaction with alkali and oxygen under different conditions. Note that quantitative recovery of all reaction solutions from the reactor is necessary for the success of this test. The data in Table III demonstrate that

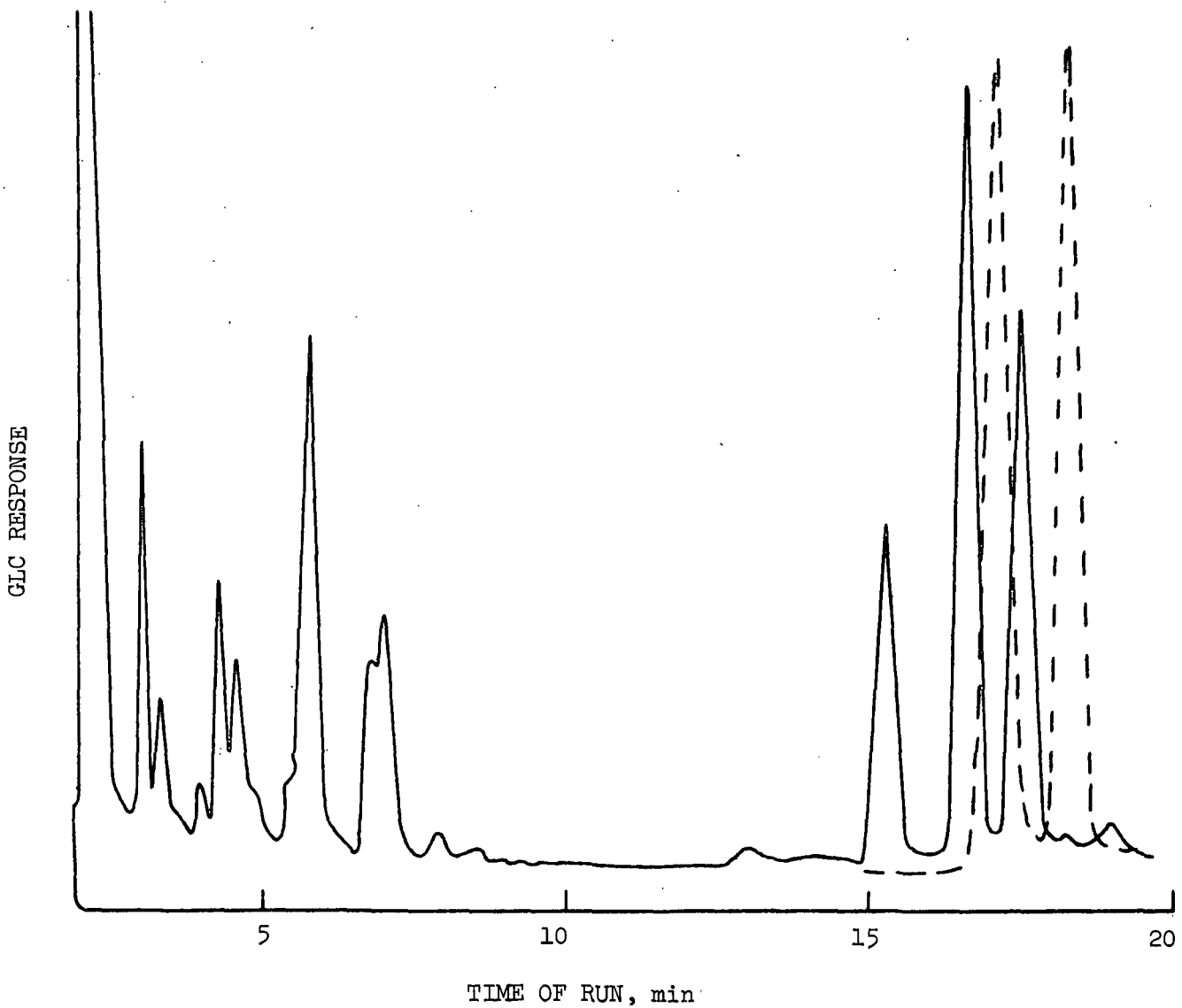


Figure 6. Chromatogram of Disaccharide Acids Derived from the Complete Oxidation of Cellobiiose and the Cellobiiose Pattern (Dotted Lines) if Run at the Same Time

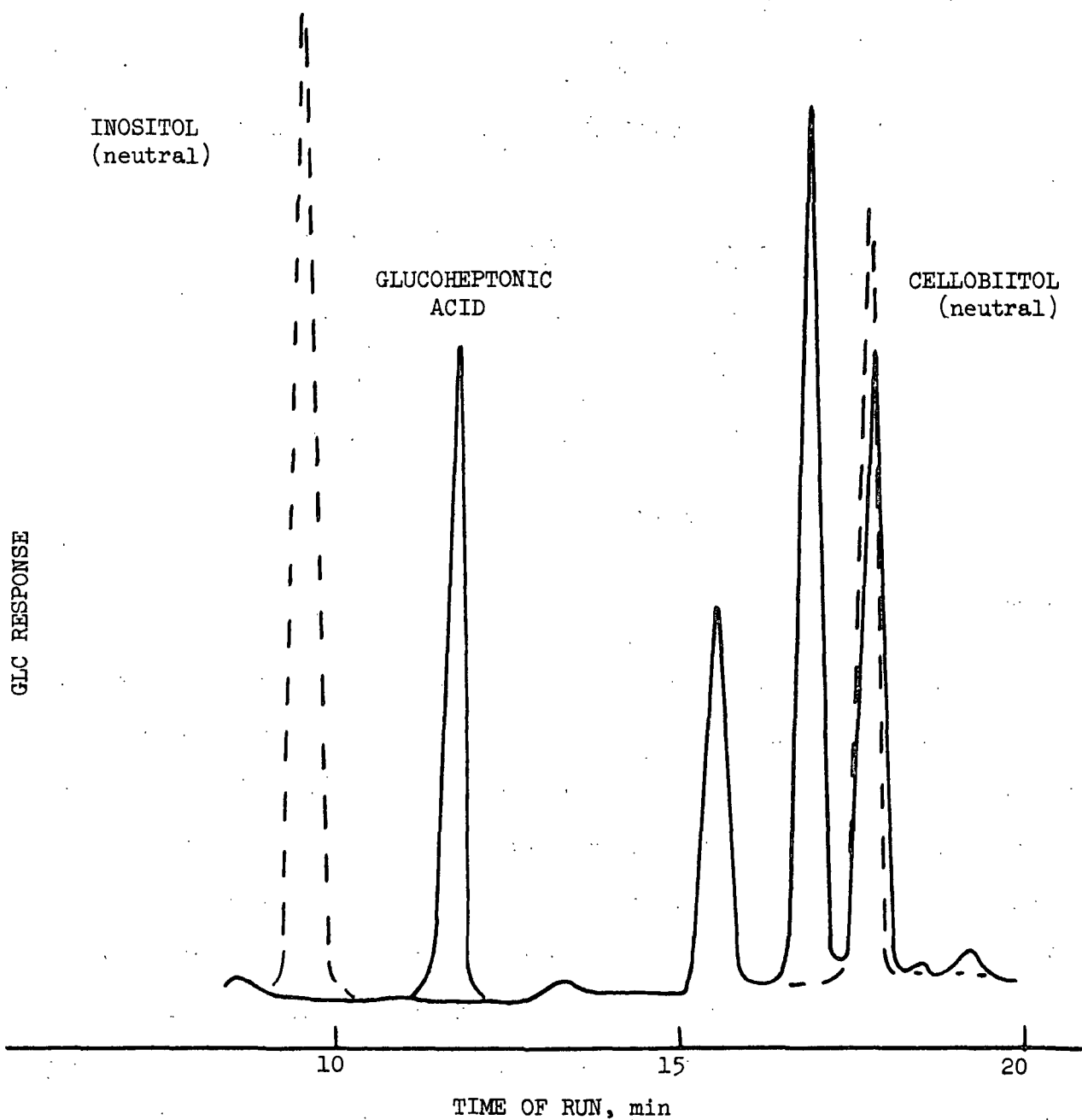


Figure 7. A Portion of a Chromatogram Illustrating the Mobilities of Internal Standards, Cellobiitol and "Disaccharide Acids"

inositol is resistant to oxidation by oxygen in 0.36N NaOH for at least 10 minutes at 130°C. The deviation of the areas from the average is that to be expected from the experimental manipulations involved in the test.

TABLE III

THE REACTION OF INOSITOL WITH 0.36N NaOH AT 130°C  
AND OXYGEN UNDER VARIOUS CONDITIONS

Time at 25°C	Time at 40°C	Time at 130°C	Inositol Response, g
0	0	0	0.0199
60	0	10	0.0219
0	30	10	0.0205
0	60	10	0.0207
0	120	10	0.0209

Average = 0.0208 ± 0.0011 g

#### THE ANALYSIS OF OXYGEN

The concentration of oxygen during these reactions must be in excess or at least known if kinetic significance is to be attached to the anticipated analytical results. Since the Winkler method of analysis was adopted for our purposes when such a design was not considered (19), it was feared that the unreacted cellobiose might consume the oxidized states of manganese (illustrated schematically in Fig. 8) that form the basis of the test. This behavior would therefore interfere with oxygen analysis and give low results. The data shown in Table IV illustrate that the test is not greatly affected by the quantity of cellobiose proposed for use in these tests. Other data included in the table show that the organic material in the spent liquor from a sodium carbonate-oxygen delignification of a hardwood prepared from Project 3264 also does not greatly interfere with the analysis of oxygen by the Winkler procedure. The difference of this test from the blank in each instance is within experimental

error. The slight positive response in one instance may be due to the presence of additional dissolved oxygen in the spent liquor although additional tests would be necessary to prove this conjecture.

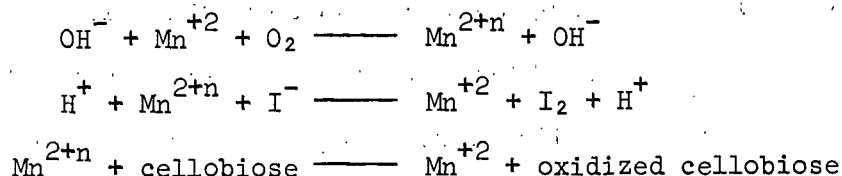


Figure 8. A Schematic Representation of the Chemistry of the Winkler Process and Possible Interferences

TABLE IV

THE EFFECT OF ORGANIC COMPOUNDS ON THE WINKLER OXYGEN ANALYSIS PROCESS<sup>a</sup>

Organic Material Added to Test Analysis	Amount Added	Blank <sup>b</sup> (no additive)	Run <sup>b</sup> (with additive)
Cellobiose	2 mg	38.1	37.2
Spent liquor from reactor (no flushing)	2 mg solids	36.7	37.4
Spent liquor from reactor (residual O <sub>2</sub> flushed out with N <sub>2</sub> )	2 mg solids	39.6	38.4

<sup>a</sup>All data given in ml of 0.025N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> used to titrate a standard reactor sample of 20.9 ml dissolved oxygen.

<sup>b</sup>Average of triplicate analysis.

OXYGEN CONSUMPTION DURING COMPLETE CELLOBIOSE OXIDATION

The next series of experiments involved the measurement of the quantity of oxygen consumed during a conjectured oxidation of cellobiose with oxygen and alkali at 130°C. The first experiment involved the oxidation of 3 mg of dissolved

oxygen with 3 mg of cellobiose (10:1 molar ratio) in NaOH at 130°C for 10 minutes. During this interval, the cellobiose is completely destroyed, leaving the gluco-sylaldonic acids which gave the GLC pattern similar to that shown in Fig. 6 previously. Only 0.5 mg of oxygen was consumed and this is outside the experimental error of the technique (see Table V). The disruption of the Winkler test by 2 mg of cellobiose described earlier gave an apparent loss of 0.16 mg of oxygen (see Table IV) and was considered to be within experimental error.

TABLE V

OXYGEN CONSUMED DURING AN ALKALI-OXYGEN REACTION WITH CELLOBIOSE

	Conventional	Swamped with Cellobiose
Weight of cellobiose in test, mg	3.0	31
Winkler determination, mg O <sub>2</sub> originally	3.0	3.0
Millimoles oxygen/millimoles cellobiose	10.0	1.0
Oxygen left after reaction, mg	2.50	0.48
Oxygen consumed, mg	0.50	2.52
Oxygen consumed, millimoles	0.016	0.079
Cellobiose originally present, millimoles	0.009	0.091
Moles oxygen reacting/mole cellobiose	1.76	0.87
Cellobiose oxidized, %	100	25

Another experiment was conducted in which 31 mg of cellobiose was reacted with 3 mg of oxygen. This gives an approximate 1:1 molar mixture of reactants. More oxygen was consumed as was anticipated. At present it is impossible to calculate the exact stoichiometry of any of these reactions. Even simple glucose, an intermediate in the peeling reaction, will also react with oxygen in an equally complex manner. It can be stated that one equivalent of oxygen (16-g

atoms) would be required to oxidize a  $C_{12}$  aldehyde to a  $C_{12}$  acid, and that two equivalents (or 1 mole) of oxygen would be required to oxidize it to a  $C_{11}$  acid and formic acid with the breaking of a double bond. The formation of a  $C_{10}$  acid would require 1.5 moles of oxygen per mole cellobiose.

#### INITIAL REACTIONS OF CELLOBIOSE IN ALKALINE SOLUTIONS WITH OXYGEN

It is known that cellobiose in NaOH is degraded more rapidly in the presence of oxygen than it is in the presence of nitrogen (16). The effect of sodium carbonate solutions upon such a reaction was tested with two kinetic runs made at  $100^{\circ}C$  for varying periods of time. The results, shown in Fig. 9, illustrate the analytical problems involved because unreacted cellobiose was not separated from the glucosylaldonic acids of similar mobilities during chromatography (see Fig. 6 for the interactions involved). The ratio of the areas of total disaccharide components to that of the starting concentration of cellobiose was therefore plotted against reactor time, and the plots are not inconsistent with apparent first-order behavior. The greater yield (or slower-reaction) in the presence of oxygen is due to the formation of the glycosylaldonic acids, while the lesser yield, in the case of the nitrogen atmosphere, was due to the lack of those acids as stable reaction products. Samples of the reaction mixtures have been saved for the confirming analysis of the acidic components themselves to be conducted at a future date.

The behavior of the reaction products during this first test can be further rationalized as follows: As Rowell (16) has suggested, if we confine ourselves to a simplified scheme of an alkali-oxygen system for disaccharides, then there are two primary reactions, the peeling of the disaccharide and the oxidation of the disaccharide to several more or less alkali-stable disaccharide acids (3). Such a system is illustrated in Fig. 10, where the overall reaction

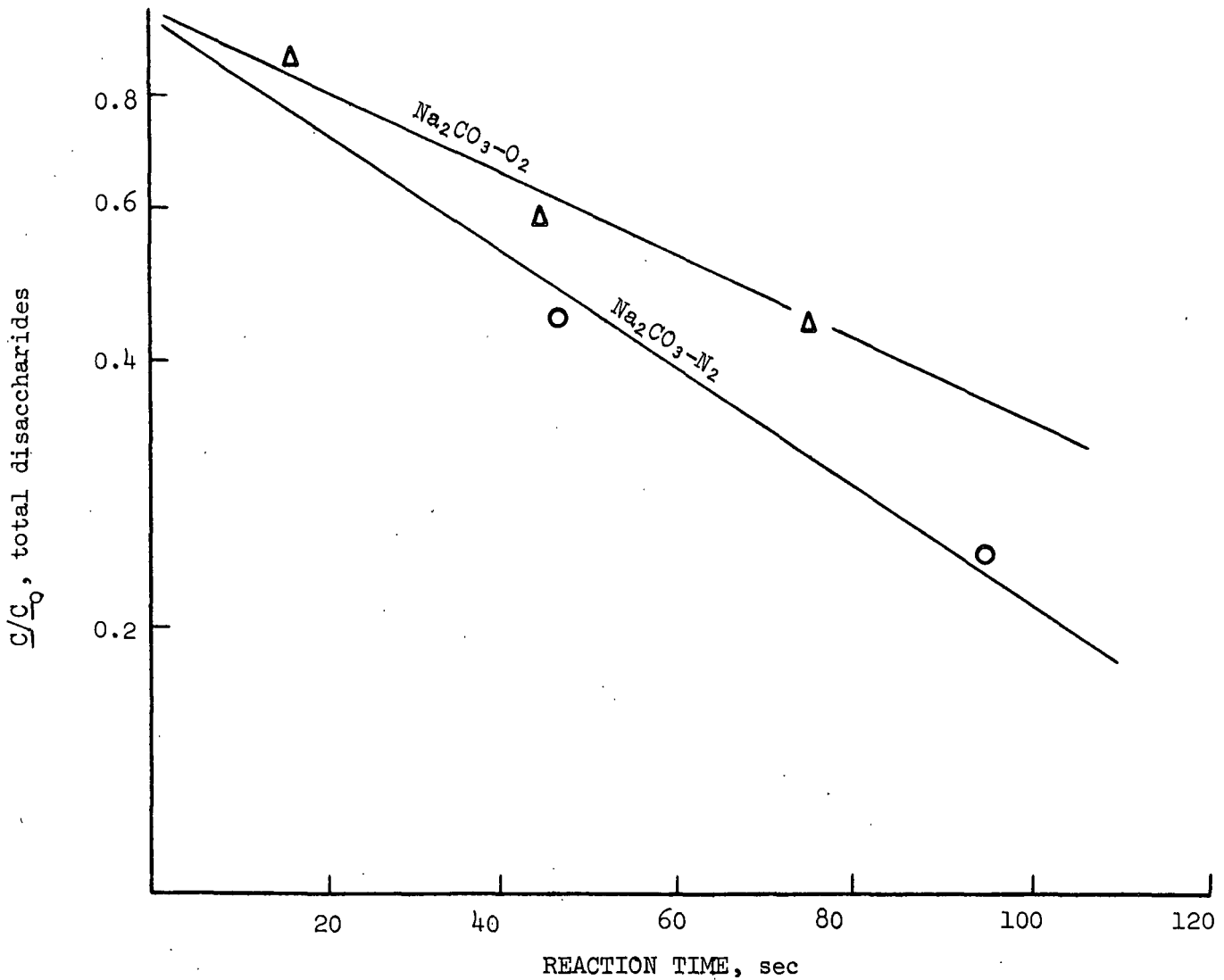


Figure 9. First-order Kinetic Plot of Disappearance of Total Disaccharide Components in the Reaction of 0.00044M Cellobiose with 0.005M Dissolved Oxygen in 0.15M Sodium Carbonate at 100°C

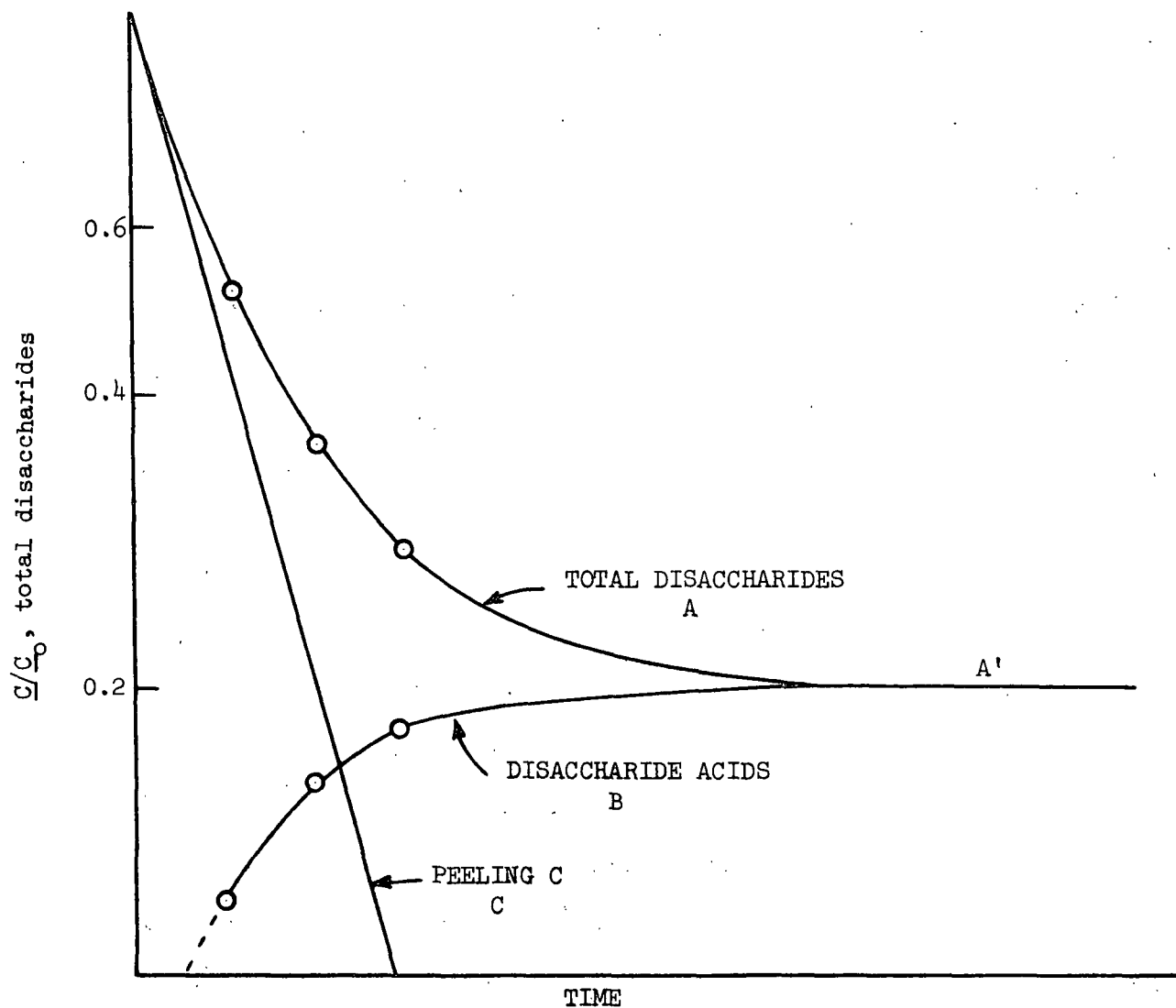


Figure 10. Idealized Plot of Rate of Oxidation, Compared to Peeling

or loss of disaccharide components (Curve A) levels off after a certain time due to the formation of alkali-stable glucosylaldonic acidic products. Also, it can be seen that this rate of loss of total disaccharide components is initially much slower than that observed in an alkali-nitrogen system where only peeling occurs (Curve C) and the proportion of alkali-stable acids formed is much less.

This scheme ignores any oxidation of fragments containing six carbons or less, and for the purpose of our chromatographic manipulation it simplifies analysis greatly. In this report we have analyzed for (a) total disaccharides in some kinetic runs (Curve A) and (b) for disaccharide acids only after Curve A have leveled off (Region A') in other runs. So far we have not analyzed for the rates of formation of disaccharide acids (Curve B). The separation of these acids from the unreacted disaccharides is involved, but feasible and will be employed.

For additional orienting purposes, three kinetic runs were carried out at 100°C with solutions of cellobiose and oxygen at different pH levels. Since Malinen (12) and Malinen and Sojstrom (18) had varied the pH by varying the sodium hydroxide concentration of their reaction systems (with all the attendant concentration problems), it was decided to control the pH of these reactions with various combinations of sodium hydroxide and carbon dioxide, both to give better pH control and to approximate conditions that might be encountered in actual pulping processes. As anticipated, the initial rate of loss of total disaccharide component reflected the effect of increasing pH, as shown in Fig. 11 and 12. After an interval, the loss of disaccharide components from the reaction of cellobiose with NaOH stops due to the depletion of cellobiose and to the formation of alkali resistant glucosylaldonic acids. Although the rate of loss of disaccharide components is initially less in sodium carbonate solution compared to the rate in sodium hydroxide solution, this rate does not stop as a result of the

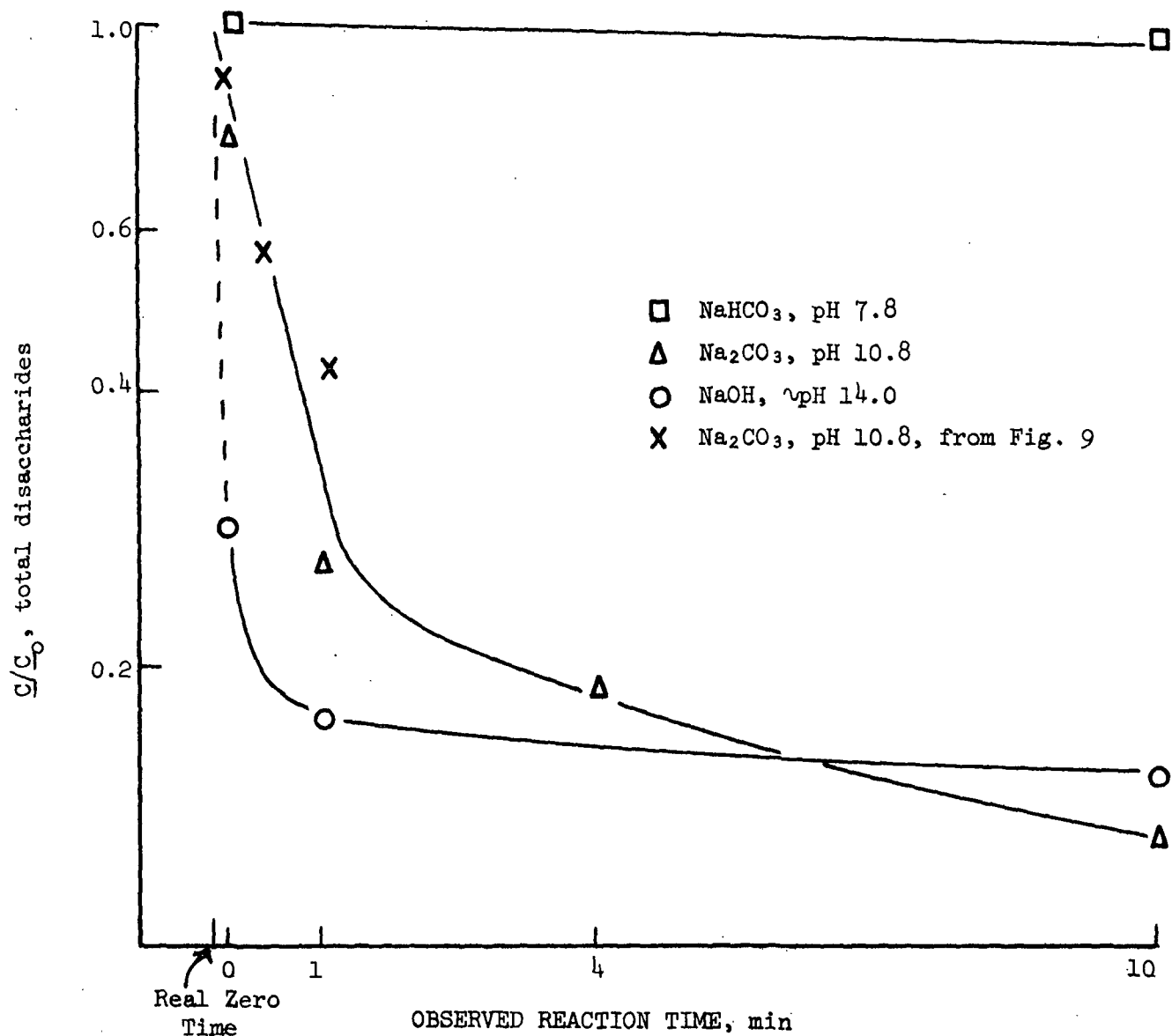


Figure 11. The Reaction of Cellobiose with Oxygen at 100°C in the Presence of Different Buffers, 0.375N

formation of alkali resistant glucosylaldonic acids. Thus, the rate of degradation of cellobiose after 4 minutes reaction with oxygen is greater in carbonate solution than it is in sodium hydroxide. This state of affairs may reflect the lack of a suitable oxidizable intermediate as a result of the interaction of cellobiose and carbonates (lower pH), or less likely, the possibility that carbonate radicals can contribute to this oxidation of the acids in a manner analogous to their suspected interaction with glycosidic bonds (29).

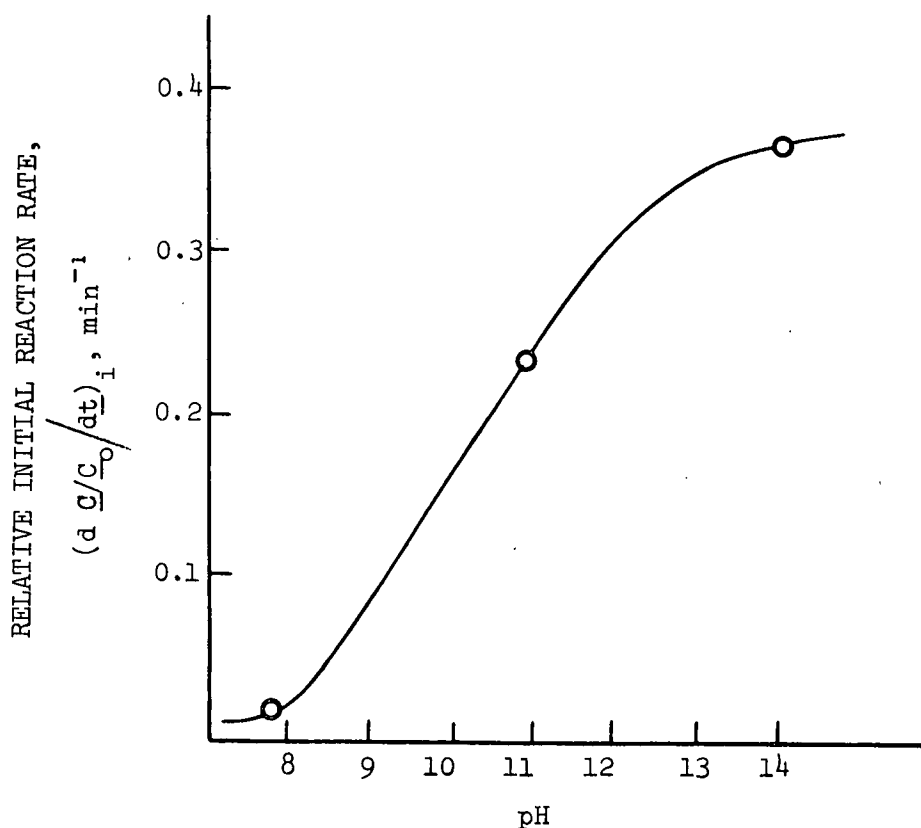


Figure 12. The Initial Rate of Degradation of Cellobiose at 100°C by Oxygen as a Function of pH

### The Effect of Temperature

The effect of temperature on the degradation of cellobiose by oxygen was determined by comparing the previous results at 100°C with a reaction carried out at 130°C. The data was summarized in Table VI and they confirm the conclusions of Rowell (16) and others (12,18,19) that the degradation is markedly increased by increasing temperatures. Also included in the table are the yields of glucosylaldonic acids obtained by Rowell (16) and Malinen and Sjostrom (18) under somewhat similar circumstances. The lack of acid detected by Rowell (16) may be due to the very poor solubility of oxygen under atmospheric conditions at 100°C and the resulting preponderance of competing alkaline degradation reactions. The significantly greater yield of glucosylaldonic acids [calculated by us from Malinen and Sjostrom's data (18)] was initially surprising, and a plot of yield against maximum reaction temperatures, shown in Fig. 13, indicated unusual behavior. However, the insulating qualities of the Teflon lining of Malinen's batch reactor could result in the reactor temperature never reaching the bath temperature of 120°C. The half life of cellobiose in 1N sodium hydroxide is given in Table VII, and the yields at 30 seconds and 20 minutes suggest that even in 1/4N NaOH all the cellobiose was either oxidized by oxygen or degraded by alkali long before the maximum reaction temperature had been reached.

An attempt was next made to duplicate the results of Malinen and Sjostrom in our laboratory using a similar Teflon-lined batch reactor, originally designed by Sinkey (27). The results shown in Table VIII indicate significant agreement between the two oxidations conducted in the batch reactors. In our experiments, the oxidation was terminated after 90 minutes when reaction temperature (120°C) was reached.

TABLE VI

YIELD OF TOTAL GLUCOSYLALDONIC ACIDS AFTER OXYGEN ALKALI  
DEGRADATIONS OF CELLOBIOSE

Alkali Concentration, <u>N</u>	Oxygen, ppm	Cellobiose, <u>M</u>	Temp., °C	Heat-up Period, min	Time of Reaction, min	Yield of Glucosyl- aldonic Acid, %
0.375 (NaOH)	175	0.00044	100	0	10	15
0.375 (Na <sub>2</sub> CO <sub>3</sub> )	175	0.00044	100	0	10	<5
0.375 (NaOH)	175	0.00044	130	0	10	6-8
0.25 (NaOH)	? <sup>a</sup>	0.03	120	~20 <sup>b</sup> →60	30?	17
0.04 (NaOH)	1 atm <sup>b</sup>		100	?	120	0

<sup>a</sup>From the data of Malinen (18), a 20-min heat-up period was claimed (in the absence of a Teflon lining); maximum temperature was probably never achieved due to the insulating properties of Teflon. Applied O<sub>2</sub> pressure was 9 Kp/cm<sup>2</sup>.

<sup>b</sup>From the data of Rowell (16), oxygen was continuously bubbled through the reaction flask, but the solubility of oxygen is at a minimum at 100°C.

TABLE VII

HEAT-UP TIME RELATIVE TO REACTION TIME

Temperature, °C	Half-life of Cellobiose <sup>a</sup> in <u>N</u> NaOH	Percent Cellobiose Reacted in 30 sec	20 min
25	30 hr	0.0014	1.8
60	500 sec	3.1	87.5
100	10 sec	87.5	~100
120	2 sec	~100	~100
130	0.5 sec	~100	~100

<sup>a</sup>See Reference (29).

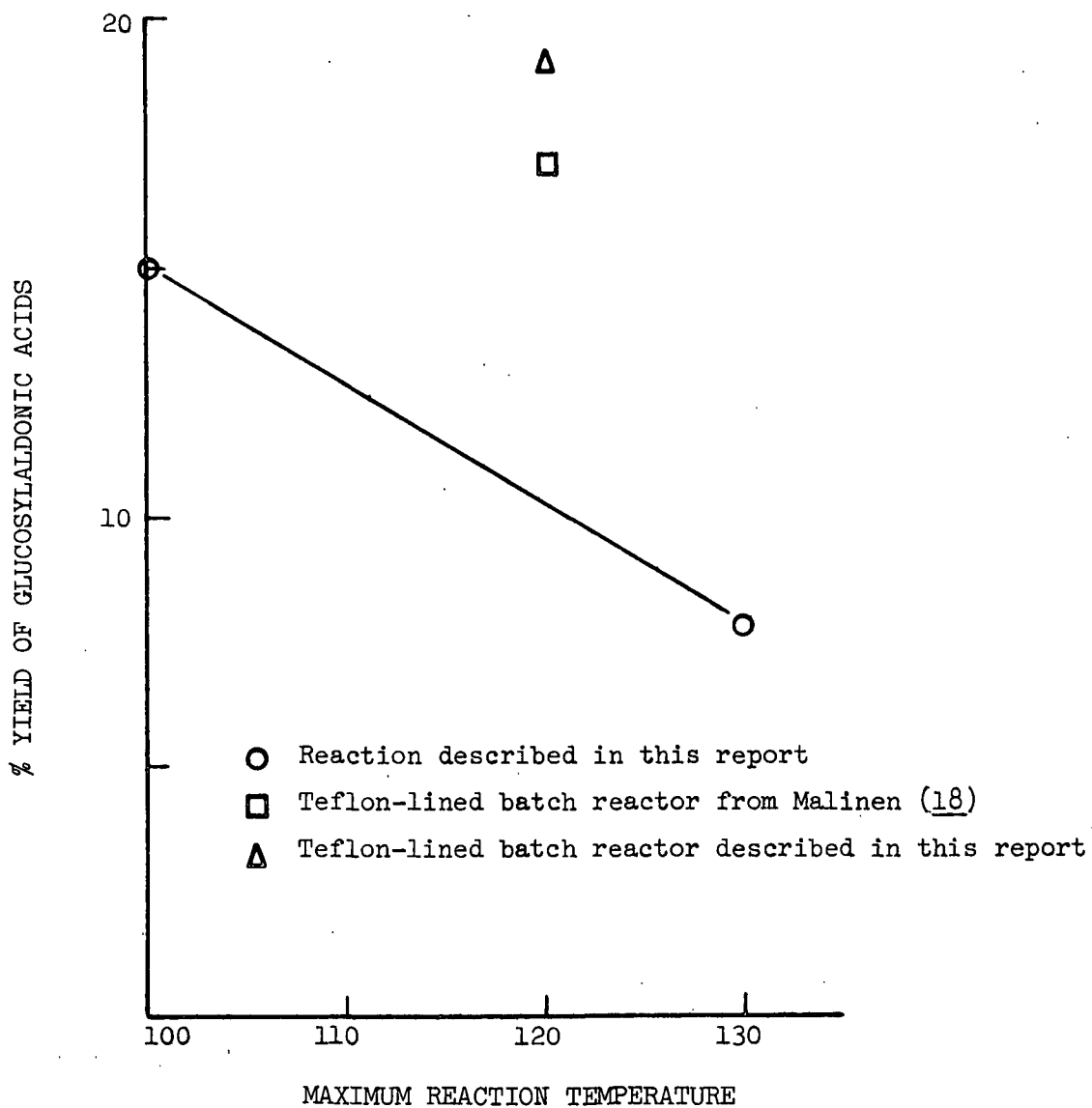


Figure 13. The Apparent Relationship Between Yield of Glucosylaldonic Acid and Maximum Reaction Temperature During the Reaction of Cellobiose with Oxygen and NaOH

TABLE VIII

YIELD OF TOTAL GLUCOSYLALDONIC ACIDS AFTER OXYGEN ALKALI  
DEGRADATIONS OF CELLOBIOSE

Alkali Concentration, <u>N</u>	Oxygen, ppm	Cellobiose, <u>M</u>	Maximum Temp., °C	Heat-up Period, min	Time of Reaction at Temp., min	Yield of Glucosyl- aldonic Acid, %
0.25 (NaOH)	-- <sup>a,b</sup>	0.03	120	20→60	?	17
0.25 (NaOH)	70 <sup>b,c</sup>	0.03	120	90	0	19
0.75 (NaOH)	350	0.00044	130	0.5	10	23
0.75 (Na <sub>2</sub> CO <sub>3</sub> )	350	0.00044	130	0.5	10	10

<sup>a</sup>From the data of Malinen (18). A 60-min reaction was carried out. Applied O<sub>2</sub> pressure was 9 Kp/cm<sup>2</sup>.

<sup>b</sup>Digester run with supporting oxygen atmosphere.

<sup>c</sup>Based on data provided by Hearne (30) from the behavior of a similar reactor.

These results with the Teflon-lined reactor are indicative of the problems this type of equipment gives in obtaining reliable data in systems where major reactions occur (and are completed) before the test solutions ever reach maximum temperature. This is one of the reasons this present research has been carried out in the flow reactor which eliminates these difficulties.

An attempt was also made to simulate the effect of the heat-up period encountered in batch reactors in our isothermal reactor. The mechanical restraints of the reactor at that time limited the pretreatment to 30 seconds before the maximum temperature of 130°C was achieved. It was not possible to dilute the alkali at that time, and the resulting compromises, shown in Table VIII, make it difficult to provide an unambiguous interpretation. It is felt that the significantly greater yield of 23% for caustic and 10% for carbonate solutions over the previous results of 15% and less than 5%, respectively, are due to the effect of increases in both chemical and oxygen concentrations. The 30 second pretreatment probably had little relative influence.

#### FUTURE RESEARCH

It is planned to study the relationship of glucosylaldonic acid production, from the reaction of cellobiose with oxygen, to such parameters as time, temperature, pH and oxygen concentration. The catalytic influence of certain metal ions will be briefly examined if time permits. Statistical techniques are to be employed which allow the effect of the parameters on the oxidation of cellobiose to be determined with a minimum of effort. Optimization of a particular cellobiose oxidation will not be attempted. The development of the quantitative measure of glucosylaldonic acid is presently underway.

### EXPERIMENTAL

The flow reactor has been slightly altered since a description of it was given in the first report on this project (1). The major change involved moving the quench inlet from a positive position following the reaction coil to a position in front of the coil so that the quench will drive the reaction solution into the receiver. This change gave improved reproducibility. The small fraction of time required for complete mixing of the quench in the receiver is considered unimportant for reaction times greater than one minute. The operation of the reactor was described in previous reports (1,2,29,31).

The reactor may be operated in two modes: (a) a continuous operation, where the reaction time is inversely proportional to the flow rate through a reaction coil; and (b) an intermittent operation, where the reaction solution is mixed rapidly, but maintained motionless in the reaction coil for a period of several minutes. This adaptation is useful where an appreciable heat-up time is necessary to duplicate a batch system. Tests with thermocouples have shown that heat transfer at 100°C is almost complete for reactions in 30 seconds, and as a result, two minutes have been used as a standard heating interval for reactants for isothermal reactions. The rate of heating can be controlled only by controlling the rate at which this bath is heated.

#### A FLOW REACTOR IN CONTINUOUS OPERATION

This is the normal mode for which this reactor was designed, and this has been discussed in detail in earlier reports (28-30). The two reaction solutions are heated in heating coils for about 2 minutes to bring them to the desired temperature. (The coils are heated empty for 15 minutes first to insure that they and the nickel mixer housings are equilibrated with the oil bath.)

The two solutions are then pushed into a mixer and the resulting reaction solution driven through the reaction coil at a given rate; quench reagent is added as the reaction solution reaches the end of the coil. The quench reagent (usually room temperature boric acid) lowers the pH and the temperature and the reaction is, thus, rapidly stopped.

The quenched solution is collected in a bottle in a pressure chamber (nitrogen) which avoids effervesence and serves to keep the dissolved oxygen in solution, this also prevents the aqueous solutions from boiling at temperatures above 100°C.

#### A FLOW REACTOR IN INTERMITTENT OPERATION

Here the two heated solutions are pushed into a reaction coil at a rapid rate to insure good mixing. The reaction solution is left in the coil for a given period of time, then pushed out of the coil by displacing with fresh solution from the heating coils. The movement of the liquid must be adjusted so that only a portion of the reaction solution is removed, and that there is no mixing with fresh unreacted solution entering the reaction coil. The use of only a portion of the reaction solution is necessary as there is a chance of diffusion of liquid from the heating coil through the mixer into the reaction system and, thus, affecting the kinetic history of the portion of the reaction solution.

#### THE FLOW REACTOR OPERATED AS AN "ISOTHERMAL DIGESTER"

This is also an intermittent operation. The two solutions are mixed at room temperature and then pushed into a single heating coil connected to a mixer and quench syringe, placed in the oil bath. This is shown in Fig. 14.

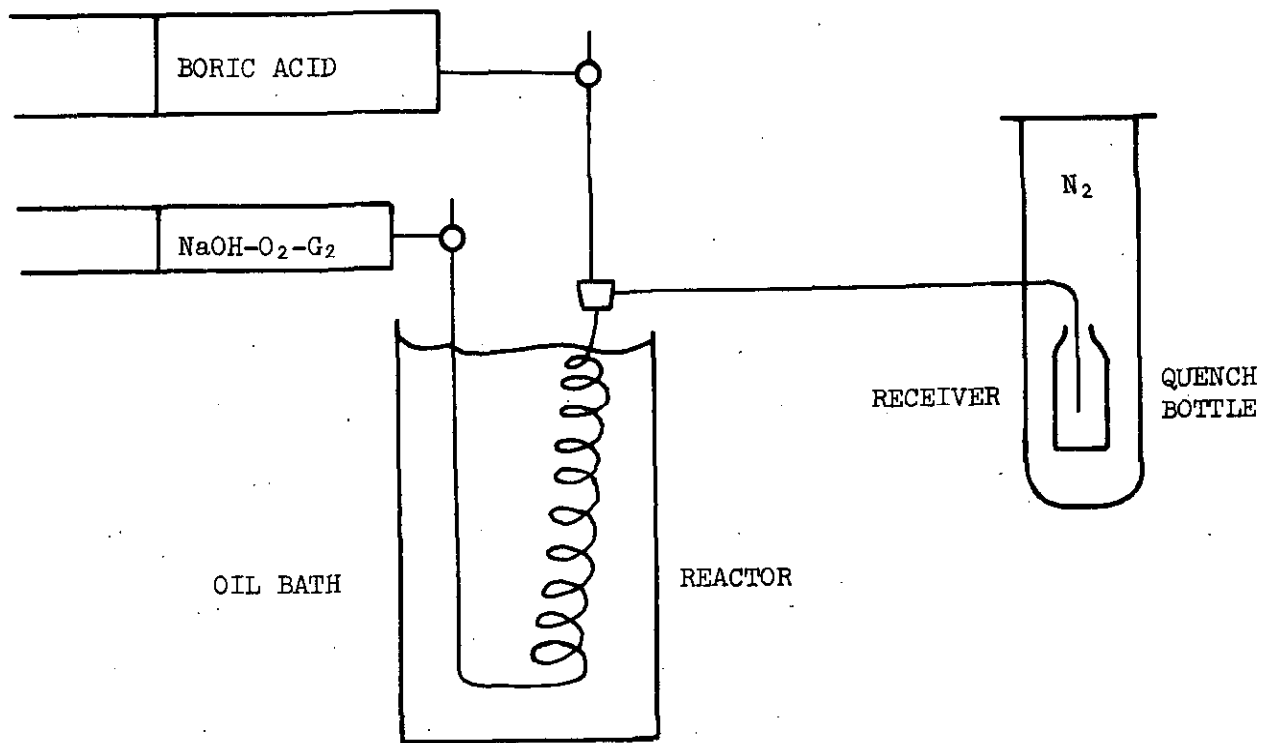


Figure 14. Intermittent Mode of Flow Reactor

The solution warms up to temperature rapidly as it enters the heated coil. After a given time in the coil, the solution is pushed out through the mixer and quenched.

#### OPERATION OF A LABORATORY DIGESTER

The laboratory digester is a small 1-liter container, fitted with a Teflon lining (9). A run contained 250 ml of solution, and the amount of cellobiose was 2.5 g. The contents were stirred with a magnetic stirrer. The digester was brought to 100 psig oxygen at room temperature, and then immersed in an oil bath at 120°C. After an interval temperature of 120°C was reached, the digester was immediately removed and cooled, and the contents worked up in the usual manner.

#### PREPARATION OF CARBOHYDRATE SOLUTIONS

In most of the runs an aqueous solution of 300 mg cellobiose per liter was prepared, with the addition of 50 mg of inositol as an internal standard. The concentration of cellobiose is about 0.00088 molar. During subsequent gas chromatographic analyses, the relative areas of the inositol and the combined disaccharide peaks were compared, to calculate the change in the latter with time. The solutions were made up fresh each day to avoid any bacterial attack.

In the batch digester runs, with long heat-up times, inositol was not added as an internal standard initially, as it was felt that the long reaction time might affect the standard adversely. The short reaction and heat-up times in the flow reactor are not harmful to inositol.

#### HANDLING OF DISSOLVED OXYGEN IN THE FLOW REACTOR

Dissolved oxygen was prepared for kinetic runs in the usual manner by spraying a given aqueous solution into pressurized oxygen (1,2) and then analyzing

the liquor by the modified Winkler method. A 20.7 ml aliquot of this solution was drawn into one of the mixing syringes and then pushed into a heating coil for the normal operation of the flow reactor.

The oxygen chamber was maintained at a pressure of 20 psig, which resulted in a dissolved oxygen content of about 300-350 ppm. The pressure of the nitrogen chamber at the quench end of the flow reactor was maintained at 250 psig to keep the oxygen in solution.

For some yet unknown reason, analyses for dissolved oxygen in sodium carbonate solution were very erratic, in contrast to the analyses obtained for sodium hydroxide solutions. This agreed with previous results (1,2).

#### WORK-UP QUENCHED SAMPLES

The samples, about 116 ml volume, after being quenched with boric acid, had a pH in the region of 9.5. They were stirred with IR-120 cation exchange resin (resin regenerated with 2N HCl and washed with water to a pH of 4.5). The pH of the solutions dropped to about 3.7 due to the presence of both boric acid and saccharinic acids.

The solutions were each filtered through paper, and concentrated in vacuo at 60°C to dryness in a 500 ml flask. The bulky white residue was treated with about 100 ml of methanol and the concentration repeated to remove boric acid as methyl borate. The small amount of organic residue was then dissolved in a little water and transferred to a 50 ml flask and this solution concentrated again to dryness.

The residue in the small flask was carefully dissolved in about 0.5 ml water. This solution was transferred in four equal aliquots to 6 ml vials for

concentration before silylation. This transfer was done with the aid of a disposable transfer pipet; this pipet has a fine tip that forms about 40 drops per ml. The 0.5 ml, equivalent to about 20 drops, was divided dropwise into the four vials. Then about 0.25 ml water was added to the flask and the solution again divided into the four vials.

The several vials were then carefully concentrated to dryness over  $P_2O_5$  in a vacuum desiccator. With care, this can be done without bumping and takes about an hour. On the assumption that the original quench solution contained about 2 mg of organic material (original cellobiose substrate), each vial will contain about 500  $\mu$ g of material.

After the residues have dried, they are pumped down hard over  $P_2O_5$  for about an hour to remove the last traces of moisture. Then a given vial is treated with 500  $\mu$ l each of dimethyl sulfoxide and Tri-Sil Concentrate (14), the vial capped with Teflon disks and crimped aluminum seals, and shaken overnight. The next day the caps are removed and the contents transferred with a transfer pipet to 1-ml reacti-Vials, and 10  $\mu$ l of the upper layer injected in the gas chromatograph.

While dividing the quench sample into 1/4 aliquots before silylation reduces the size of the sample that is injected into the gas chromatograph, this procedure does provide spare samples that can be analyzed in case one is lost through a mishap. The silylated samples are usually stable for only a day or so after preparation, and it is best to inject them on the gas chromatograph as promptly as possible. They can be stored in a desiccator in a refrigerator without too much degradation, if a fresh Teflon-lined disk is placed in the cap. A disk that has already been pierced by a microsyringe should be replaced before cold storage.

Because of the small size of the sample obtained from the flow reactor, all of the quench solution must be worked up for subsequent silylation. This is in contrast to earlier work with the flow reactor for peeling only, where large samples of carbohydrate substrate could be used, and only a portion of the quenched sample used. Here, with the low concentration of dissolved oxygen present, the concentration of cellobiose must be kept at the same low level to insure an excess of oxygen as a reactant.

#### CHROMATOGRAPHIC IDENTIFICATION OF DISACCHARIDES AND DISACCHARIDE ACIDS

In Table IX are listed the retention times for the peaks of the trimethylsilyl ethers of some of the major compounds to be investigated in this study. It can be seen that the parent sugars generally give two peaks, for the  $\alpha$ - and  $\beta$ -anomers, and that the salt of the acid derived from a given sugar gives a peak that is slightly slower (a larger retention time). The acids give a broad spectrum of peaks related to lactone formation. On the other hand, the sugar alcohol, obtained by borohydride reduction, has a single peak that is faster than the acid peak.

At present we have not tried to separate mixtures of disaccharides and the related acids. As can be seen from the table, there is too much overlapping of peaks. We have explored briefly the reduction of such a mixture by sodium borohydride, with the hope that we could separate the two C<sub>12</sub> alcohols (retention time at 18.0 to 18.1 min) from the several sugar acids.

From our reaction mixtures we have obtained a mixture of acid peaks, as shown in Fig. 6. These peaks range in retention time from 19.0 to 15.5 min and notably the peak at 19 min, that for cellobionic acid, is very small. The pattern of these peaks varies from run to run, and we will have to explore this

more in the future. At present we have been determining only the total area of these peaks, relative to the internal standard inositol (retention time 9.8 min), and attempting to get the maximum yield of disaccharide acids as such.

TABLE IX

RETENTION TIMES FOR VARIOUS DISACCHARIDES AND DERIVATIVES  
(AS THE TRIMETHYL SILYL ETHERS)

Parent Sugar	Retention Time on OV-17 Column, min <sup>a</sup>		
	Parent Sugar <sup>b</sup>	Acid <sup>d</sup>	Alcohol
Cellobiose	17.9	19.1	18.1
	19.0		
Glucosyl-mannose	17.0	--	18.0
	17.8		
Glucosyl-fructose	--	--	18.0 <sup>c</sup>
Glucosyl-arabinose (C <sub>11</sub> sugar)	16.4	17.9	--
	17.0		
Glucose	8.0		
	9.2		
Inositol (internal standard)	9.8		

<sup>a</sup>The chromatography was done on a 6-ft x 1/8-inch OV-17 column, programmed from 130°C to 250°C at 6°/min.

<sup>b</sup>The two peaks for the parent sugar represent the  $\alpha$ - and  $\beta$ -anomers.

<sup>c</sup>The alcohol derived from the reduction of glucosyl-fructose is a mixture of glucosyl-mannitol and cellobiitol.

<sup>d</sup>Potassium salt.

Cellobionic acid was prepared by oxidation of cellobiose with iodine and potassium hydroxide in methanol (15). The potassium salt precipitates from the alcohol solution and is purified by further precipitation.

The C<sub>11</sub> acid, glucosylarabinonic acid, was prepared by degradation of cellobiose oxime to the C<sub>11</sub> sugar (31) via the nitrile acetate, and then oxidation as above to the C<sub>11</sub> acid. An alternative method, involving the oxidation of cellobiose in barium hydroxide solution with oxygen at room temperature has also been employed (17).

Two other acids, glucosylmannonic and glucosylerythronic acid, are also being prepared by conventional techniques for use as controls in this research.

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