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**The Effect of Oxygen and Anthraquinone on
the Alkaline Depolymerization of Amylose**

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THE EFFECT OF OXYGEN AND ANTHRAQUINONE ON
THE ALKALINE DEPOLYMERIZATION OF AMYLOSE

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TABLE OF CONTENTS

| | Page |
|--|------|
| SUMMARY | 1 |
| INTRODUCTION | 3 |
| Perspective | 3 |
| Carbohydrate Degradation in Alkali | 5 |
| General Aspects | 5 |
| Alternative Hypotheses | 7 |
| Effect of Oxygen | 11 |
| Effect of Anthraquinone | 12 |
| THESIS OBJECTIVES | 14 |
| APPROACH | 15 |
| Selection of Substrate | 15 |
| Reaction Conditions | 17 |
| Data Treatment and Analysis | 17 |
| RESULTS AND DISCUSSION | 20 |
| Alkaline Reactions | 20 |
| Reactions in Oxygen-Alkali | 25 |
| Reactions with Addition of Anthraquinone | 33 |
| CONCLUSIONS | 37 |
| EXPERIMENTAL | 39 |
| Solutions and Reagents | 39 |
| Sodium Hydroxide Stock Solution | 39 |
| Amylose | 41 |
| Anhydrous Pyridine | 42 |
| Water | 42 |
| Methyl N-phenyl Carbamate | 42 |
| Reactor Operation | 43 |

| | Page |
|---|------|
| Preparation of Reaction Alkali | 43 |
| Loading | 43 |
| Sampling | 44 |
| Analysis of Reaction Samples | 45 |
| Amylose Precipitation | 45 |
| Amylose Derivatization | 45 |
| Amylose Concentration | 48 |
| ACKNOWLEDGMENTS | 53 |
| LITERATURE CITED | 54 |
| APPENDIX I. DERIVATION OF THE RATE EQUATION FOR DEPOLYMERIZATION VIA RANDOM CHAIN CLEAVAGE | 58 |
| APPENDIX II. EXCLUSION CHROMATOGRAPHY | 60 |
| APPENDIX III. THE REACTOR SYSTEM | 77 |
| APPENDIX IV. TABULATED DATA | 80 |
| APPENDIX V. EVALUATION OF METHODS FOR DETERMINATION OF AMYLOSE CONCEN- TRATION | 84 |

SUMMARY

The alkaline depolymerization of polysaccharides is generally believed to occur via stepwise elimination of monomers from the reducing end and via random internal scission of the chain. Mechanisms for these reactions have been postulated on the basis of a vast number of studies on mono- and disaccharide model compounds. A review of the literature indicates that predictions based on the model compounds are not always true for polysaccharides. This has encouraged postulation of alternative hypotheses about the degradation processes to improve the classical theories.

The validity of such postulates is addressed in the present research in which the homogeneous alkaline degradation of purified potato amylose was studied in terms of yield loss and shifts in the molecular weight distribution with respect to reaction time. These data allowed the modes of depolymerization to be identified and characterized. The reactions were run in a Teflon-lined autoclave. In all reactions the amylose concentration was 1% (wt./vol.), the liquor was 1.0M purified sodium hydroxide, and the total pressure, 1.14 MPa (150 psig). The reaction variables were temperature (80 and 100°C), atmosphere (nitrogen and oxygen) and anthraquinone addition (0 and 5% wt./wt. based on amylose).

For anaerobic conditions, the results were consistent with the classical theory of alkaline degradation. There was no measurable random cleavage of polymer chains, while 290 and 400 glucose units were lost per molecule via the peeling mechanism at 80 and 100°C, respectively. Thus, the chemical stabilization of reducing ends was more important at lower temperatures. This contradicts the results of an earlier study [Haas, Hrutfiord and Sarkanen, J. Appl. Polymer Sci. 11, 587 (1967)] which had concluded that the extent of chemical stabilization instead increases with temperature. That work, however, was based on a

kinetic model which assumed that hydrocellulose can be physically stabilized due to inaccessibility in the crystalline regions. Present data further show that the single-chain theory of amylose degradation is incorrect. This theory [Ziderman and Bel-Ayche, J. Appl. Polymer Sci. 22, 1151 (1978)] assumes that reacting polymer molecules are totally eliminated in the form of monomeric products and predicts that the molecular weight distribution remains constant. It was found, however, that in all reactions, the molecular weight distribution shifted uniformly toward lower molecular weights, in a fashion consistent with classical concepts.

Degradation of amylose in oxygen-alkali verified that oxygen affects the relative rates of the two modes of alkaline depolymerization. Oxygen provides additional pathways which terminate the peeling reaction, so that the average peeling length at 100°C was only about 5-8% (i.e., 20-30 glucose units) of what it was in the absence of oxygen. Furthermore, oxygen enhanced random chain cleavage and caused a large continuous shift in the amylose molecular weight distribution toward lower molecular weights. The polydispersity of the amylose decreased during the reaction, i.e., the distribution curves became narrower.

Anthraquinone affected the amylose degradation in a manner similar to molecular oxygen. The additive stabilized the amylose against peeling yield losses under nitrogen as well as oxygen atmospheres. The average peeling length in the presence of anthraquinone was estimated to be about 20-30 units under nitrogen and 10-15 units under oxygen. In both atmospheres, random chain cleavage was important. It is proposed that the anthraquinone induced random cleavage of the amylose molecules. A similar reaction could be responsible for the observed lower viscosities of soda-anthraquinone pulps relative to soda pulps.

INTRODUCTION

PERSPECTIVE

The cellulose fibers in the sheet on which these words are printed have probably been exposed to a high pH environment more than once during the manufacturing of the paper. The sulfate or kraft process has long been the dominant pulping method for wood, and alkaline extractions are important steps in practically every bleach plant today. In addition, recent efforts aimed at reducing the cost of controlling the environmental impact of pulp mills have resulted in innovations such as oxygen-alkali bleaching and soda-anthraquinone pulping. Thus, one can safely assume that processes with high pH will be prevalent for many more decades to come. Alkaline treatments do, however, have disadvantages, such as significant carbohydrate degradation and loss of yield, which are associated with many of the above processes.

Such large-scale technical applications have encouraged much research to be devoted to the study of reactions of carbohydrates in alkali and how their loss and deterioration might be prevented. The main features of the alkaline reactions are now well known and widely accepted. Much uncertainty remains, however, about the molecular mechanisms and the details of the reactions, and further studies are needed to conclusively distinguish between viable and incorrect hypotheses.

It was the primary purpose of this investigation to clarify some unresolved aspects of the degradation of polymeric carbohydrates in alkali under anaerobic and oxidizing conditions. Comparative analyses of the experiments should demonstrate the effects of molecular oxygen and/or anthraquinone on the alkaline reactions. The program was designed to provide new quantitative information which would characterize the depolymerization. Improved knowledge of these areas of

chemistry will aid the development of new technologies better suited to the needs of the future.

CARBOHYDRATE DEGRADATION IN ALKALI

GENERAL ASPECTS

Polymers degrade via random cleavage of the chain, stepwise elimination of monomers from the end of the molecule ("peeling") or via a combination of the two processes (1). Both peeling and chain cleavage are potential reactions of polysaccharides in alkali although the specific conditions determine their relative significance. The reactions are schematically described in Fig. 1 as they apply to cellulose or amylose.

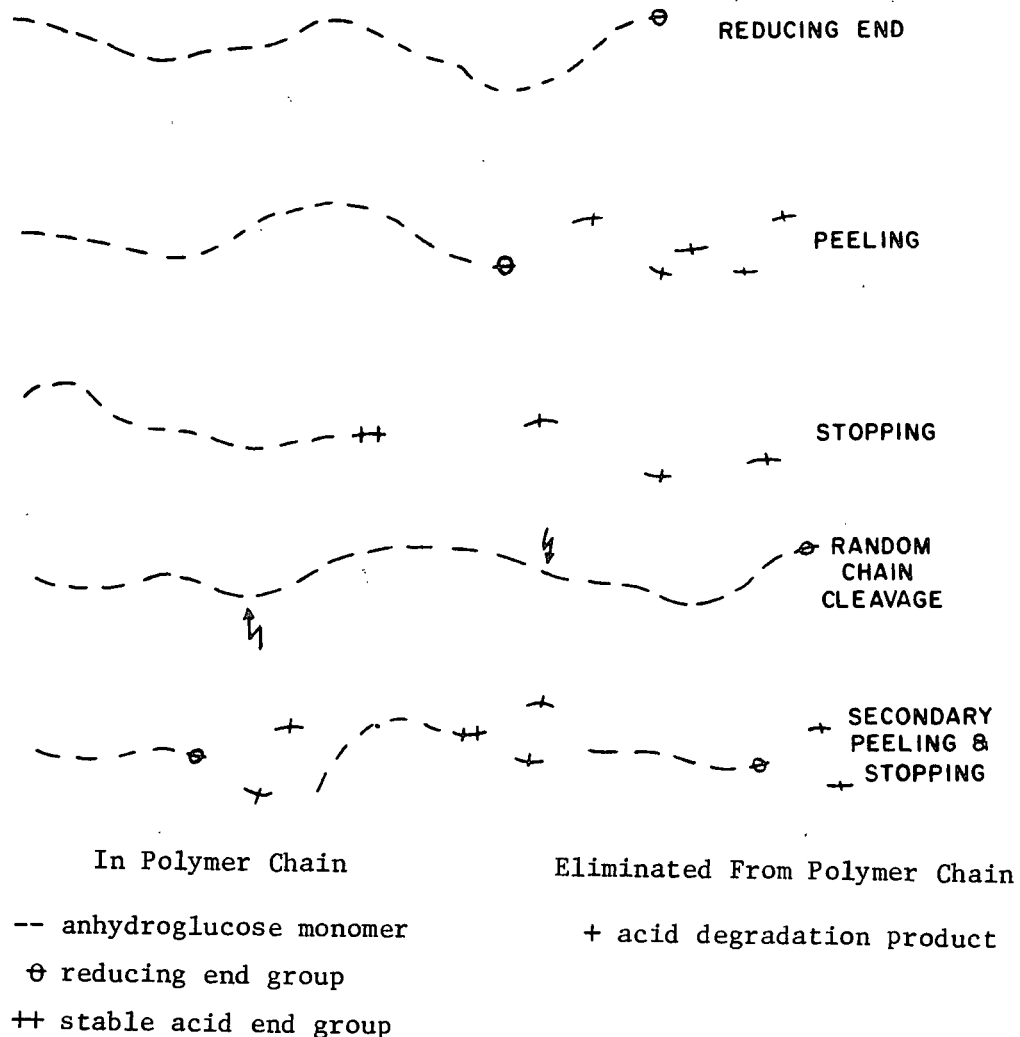


Figure 1. Features of Cellulose or Amylose Degradation in Alkali

The peeling reaction is propagated only from the reducing end of the molecule (\ominus , Fig. 1). Peeling proceeds probably already at room temperature, but does not go on indefinitely in which case the loss of polysaccharide would be total. A competing mechanism ("stopping") rearranges the reducing group into an alkali-stable acid end group ($\oplus\oplus$, Fig. 1), and terminates the peeling process. Generally the eliminated glucose unit (\oplus , Fig. 1) has been envisioned as forming isomeric 3-deoxy-2-C-hydroxymethyl-pentonic (isosaccharinic) acids while the stabilized groups ($\oplus\oplus$, Fig. 1) are thought to be isomeric 3-deoxy-hexonic (metasaccharinic) acids (2,3). It has been shown that although these are the predominant products, large amounts of other acids (e.g., 2-C-methyl-glyceric acid and aldonic acids) are formed as well. These reactions have been reviewed by Sjöström (4).

The average number of monomers eliminated from the end of a molecule before the peeling reaction is terminated at that site is called the number average kinetic chain length (\bar{l}) or simply the peeling chain length. It is defined by the ratio of the probability that the reducing end group will be eliminated, to the probability that it will rearrange into a stable metasaccharinic acid. This can also be expressed as the ratio of the peeling rate to the stopping rate.

The peeling length can easily be measured when no chain cleavage or secondary peeling are occurring. The peeling causes a yield loss which tends to level off when all reducing ends gradually become stabilized. That yield loss multiplied by the original average degree of polymerization ($\overline{DP}_{n,0}$) gives the average DP of the part of the chain which was lost via peeling or the peeling chain length (Fig. 2). When both chain cleavage and secondary peeling reactions are operating the estimation of peeling length is difficult, since both yield and DP are affected by the two interdependent reactions.

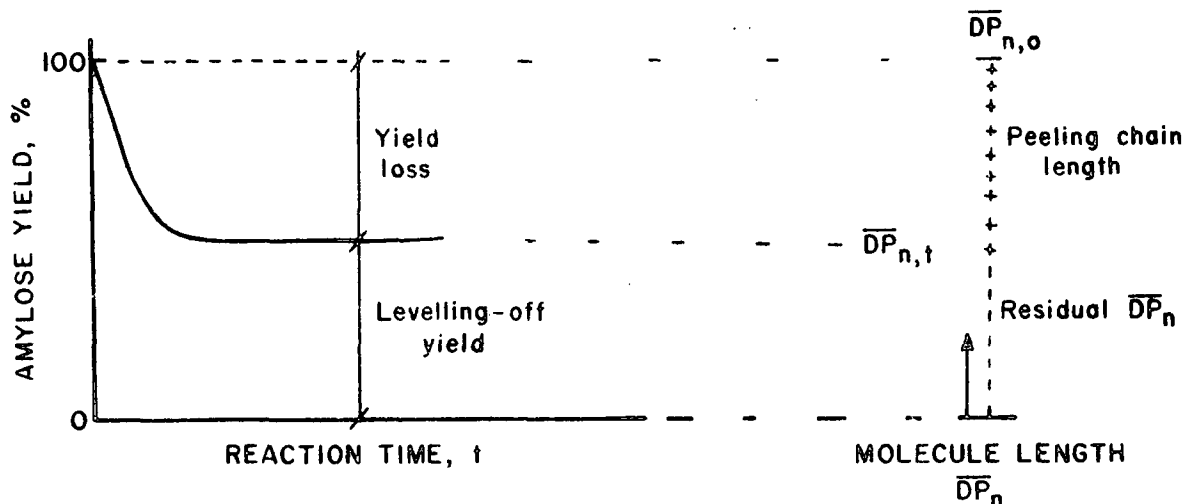


Figure 2. The Relationship Between the Amylose Yield Loss and the Peeling Chain Length During Alkaline Depolymerization. The Residual \overline{DP}_n (%) is Defined as $100 \cdot \overline{DP}_{n,t} / \overline{DP}_{n,o}$

Random chain cleavage in alkali is only important when the temperature is 140°C or higher (3,5). The reaction causes a large and rapid reduction of DP but contributes only indirectly to the yield loss by creating sites for secondary peeling. The rate of chain cleavage is directly proportional to the number of glycosidic links in the system (1,6). The reaction is of first order and described by Eq. (1).

$$\ln (1 - 1/\overline{DP}_{n,o}) - \ln (1 - 1/\overline{DP}_{n,t}) = k_{cc} \times t \quad (1)$$

For moderate levels of degradation, Eq. (1) reduces to Eq. (2). The derivation of the equations is given in Appendix I.

$$1/\overline{DP}_{n,t} - 1/\overline{DP}_{n,o} = k_{cc} \times t \quad (2)$$

ALTERNATIVE HYPOTHESES

Peeling, stopping and random chain cleavage are the three major alkaline reactions of polysaccharides. Together they represent the classical theory of

degradation and explain adequately most depolymerization phenomena. Some unresolved aspects of the process indicate that it is in reality more complex; e.g., it is not known to what extent the physical structure and the degree of crystallinity affect chemical reactivity. Three modifications (7-11) which were proposed as refinements of the classical degradation theory are discussed in the following sections.

Influence of Physical Structure

It is well known that cellulose exhibits partly crystalline properties (3) as revealed by X-ray diffraction, spectrometry, and other methods. However, attempts to measure the proportions of crystalline and amorphous regions give results which vary widely, depending on which analytical technique is used. Thus, chemical accessibility cannot be directly deduced from the estimated degree of crystallinity of the cellulose. The ratio of ordered to disordered regions probably influences the rate of chemical reactions, including those in alkaline media, but disagreement remains as to the extent. Sarkanen and coworkers (7) assumed that the crystalline, ordered regions of a hydrocellulose were essentially inert toward alkali and that reducing end groups bordering such regions were stable and did not propagate the peeling reaction. After kinetically analyzing the alkaline degradation of hydrocellulose, they concluded on the basis of kinetic analyses that chemical stopping via formation of metasaccharinic acid end groups increases with temperature and is practically insignificant at temperatures below 170°C. The major part of cellulose stabilization was thought to be due to physical inaccessibility in the crystallites.

These results were later challenged by Samuelson and Johansson (12) who, in similar experiments, found that about 80% of the molecules had some form of carboxylic acid end group derived from a chemical stopping reaction and that this

reaction was important even during the earliest part of the cook. There was no evidence of unreacted reducing end groups.

This unresolved conflict suggested that a homogeneous reaction system should be employed in this work, since it was designed specifically to study the rates of the respective chemical reactions and how they were influenced by the reaction conditions. Results derived from degradations of cellulose could potentially become difficult to interpret unambiguously, since they might depend, to some unknown degree, on the heterogeneous nature of the substrate.

The Di-ion Theory of Stopping

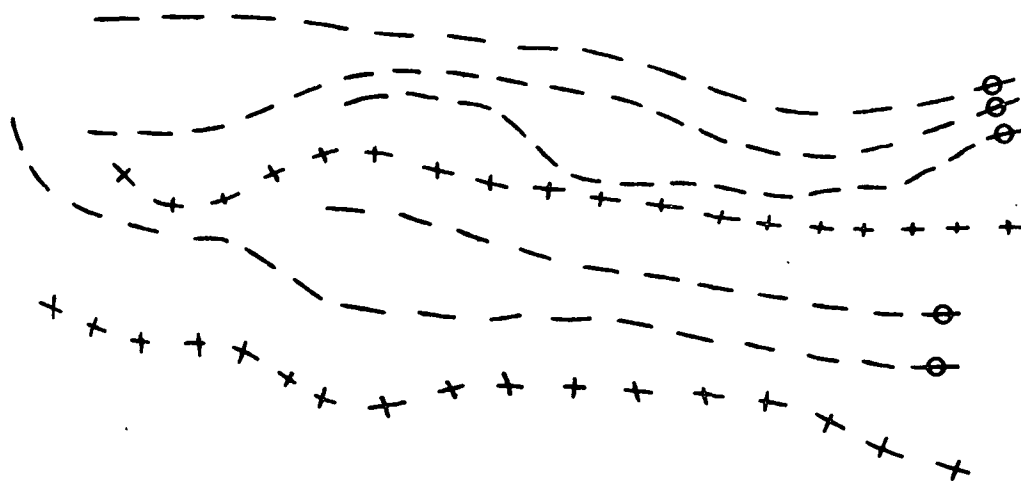
Lai and Sarkanen studied the anaerobic alkaline degradation of amylose in homogeneous phase (8,9) and found the average peeling length to be about 300 glucose units under conditions which would eliminate approximately 70 units from cellulose. This difference was interpreted as support for the hypothesis (7) that physical stopping is primarily responsible for the stabilization of cellulose. On the other hand, the amylose reactions (8) indicated that the chemical stopping reaction was relatively more competitive at lower temperatures, which directly opposed the conclusions of the previous study (7). Possible reasons for this difference were not discussed.

The level of degradation of amylose was found to be strongly dependent on pH, and it was concluded that the pH must be 13 or higher for the chemical stopping reaction to be significant (8,9). This was explained in terms of a doubly ionized reducing end group as being a necessary intermediate of the rearrangement to metasaccharinic acid.

The validity of the di-ion postulate was not tested in this work, but the data (8) were used for comparison with the results of this study.

The Single Chain Theory

Another modern amendment to the classical carbohydrate degradation theory is called "the single chain hypothesis" or total unzipping, and was postulated by Ziderman and Bel-Ayche (10,11) as an extension of Lai's study (8). The theory states, without elaborating on how it is possible, that the ionization and peeling of the original reducing end is slow and rate-determining while the peeling of consecutive units is at least 1000 times as fast. Since the stopping reaction is also slow, the overall result would be that the polysaccharide molecules would either entirely disappear as monomeric degradation products (+, Fig. 3) ("total unzipping") or remain entirely unreacted (Fig. 3). The consequence of this would be that while the yield would decrease steadily, the original molecular weight distribution would not change, unless one assumes a relationship between the peeling tendency of the original reducing group and the DP of the molecule it is attached to.



Legend: See Figure 1.

Figure 3. Schematic Representation of Total Unzipping According to the Single Chain Theory

The results of this thesis directly address the validity of the single chain hypothesis.

EFFECT OF OXYGEN

The peeling, stopping, and random chain cleavage reactions, which characterize the alkaline degradation, also proceed when molecular oxygen is present, but their mechanisms and rates relative to each other change. Stabilization toward the peeling reaction becomes much more competitive because the reducing end can be oxidized to stable aldonic acids via new pathways. The β -elimination mechanism of the peeling process is presumably not affected by oxygen. Thus the portion of the molecule lost via peeling is decreased by oxygen. Malinen (13) studied the oxygen-alkali degradation of hydrocellulose at 120°C and estimated the average kinetic chain length to be 10-50 units. This was significantly longer than what Malinen predicted based on his studies of mono- and oligomeric model compounds (13).

Oxygen effectively accelerates the chain cleavage reaction, causing a rapid loss of DP and viscosity (2). This detrimental side effect has been a major obstacle to implementation of commercial oxygen bleaching, limiting its use to a prebleaching stage followed by a conventional bleaching sequence. The mechanism of the oxidative chain cleavage reaction is not well understood. Generally, it is envisioned as a free radical reaction in which a keto-group is introduced at the 2-, 3-, or 6-position of a glucose unit (14). This makes β -elimination of the polysaccharide chain residue at C1 or C4 possible. The newly created reducing end is then available for secondary peeling degradation.

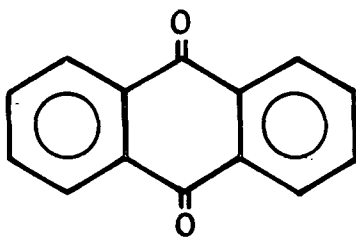
The reactions of polysaccharides in oxygen-alkali are strongly influenced by catalytic amounts of metal ions (15). It is therefore important to eliminate

traces of such contaminants in the reagents used in oxygen-alkali experiments and to perform the reactions in vessels of inert materials, e.g., Teflon.

EFFECT OF ANTHRAQUINONE

The effectiveness with which anthraquinone (AQ) and related compounds catalyze alkaline pulping of wood is well proven, verified, and documented (16-18). With an addition of 0.1% AQ wt./wt. on wood, the rates of lignin fragmentation and carbohydrate stabilization are significantly accelerated during the cook.

ANTHRAQUINONE (AQ)



The AQ additive is believed to function as a redox catalyst (18). AQ is reduced to anthrahydroquinone (AHQ) in reactions which oxidize reducing polysaccharide end groups to aldonic acids. Presumably, AQ is then regenerated from AHQ when the reduced form reacts with lignin, promoting its fragmentation and preventing the condensation of the fragments (18). Alkaline degradation products of carbohydrates are also able to react with AQ (19,20), but the mechanisms are unknown. Considerable controversy remains regarding the influence of oxygen on the activity of AQ additives (21-25), and the details of the reaction mechanisms have not been conclusively proven.

The increase in pulping selectivity offered by AQ is the reason for industry-wide interest in using AQ in alkaline cooking liquors. The main benefits of the

process, which make it economically attractive, are savings in wood and energy. Yield gains of 2-3% are attainable (17,26), while the pulp quality in general can be kept comparable to that of the previous, AQ-free cooking condition. Similarly, the energy consumption is reduced, since the H-factor can be lowered 10-15% by reducing the cooking temperature 10°C and the cooking time by as much as 25 minutes (17). The use of AQ will be particularly rewarding in mills which are limited by their digester capacity.

THESIS OBJECTIVE

In the area of oxygen-alkali reactions, the major part of the literature deals with characterization of low molecular weight degradation products from monomeric model compounds, or with optimization of pilot-scale bleaching of commercial pulps. The present study was intended to bridge the gap between these areas by analyzing the polymer fraction of a pure polysaccharide system for changes as functions of reaction time, an area where little quantitative information is available.

The objectives of this study were to improve our understanding of the depolymerization of polysaccharides in alkaline media and of how this process is influenced by an oxygen atmosphere and by a pulping catalyst, such as anthraquinone. The investigation was designed to resolve some of the uncertainty associated with recent postulates in the literature about the mechanisms of alkaline depolymerization.

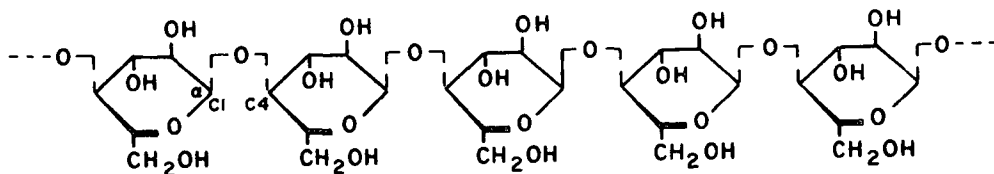
Alkaline degradations were performed at two temperatures, under nitrogen and oxygen atmospheres, and with and without anthraquinone. The combined determination of yield and molecular weight was used to characterize the depolymerization and determine the relative importance of peeling and random chain cleavage under different conditions.

APPROACH

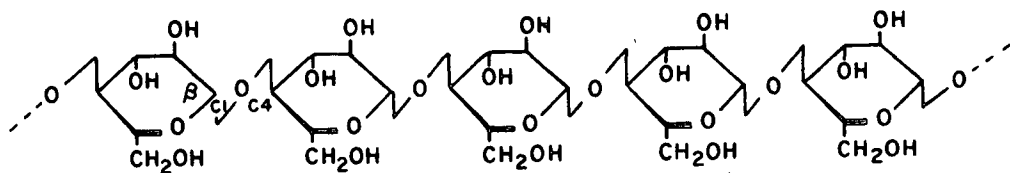
SELECTION OF SUBSTRATE

Amylose was chosen as the polysaccharide substrate to be used in this study because, chemically, it closely resembles wood polysaccharides and because it dissolves quickly and homogeneously in alkali. In alkaline solution, the amylose is believed to exist as an extended, random coil (27,28) in which all monomers of the molecule are equally accessible to chemical reagents. Thus the degradation is a homogeneous process, and the experimentally observed extent of the degradation can be attributed solely to the rates of the chemical reactions and their equilibria.

An additional benefit of using a soluble polysaccharide was that samples of the polymer dissolved in the cooking liquor could be withdrawn while the reactor remained at reaction pressure and temperature.



STARCH AMYLOSE



CELLULOSE

This study was designed to follow the changes in yield and molecular weight distribution of the polysaccharide, and the amylose selected for the study was well suited for this purpose. Its molecular weight distribution was uniform and low enough to fall entirely within the limits of the universal calibration of the exclusion chromatography columns (Appendix II, p. 60). Simultaneously, it was high enough to be representative of wood polysaccharides and to permit separate identification of the peeling and chain cleavage modes of depolymerization.

Information gained from the study of an α -linked polysaccharide will, to a large extent, be applicable to β -linked analogs as well. Corbett and Kenner showed in their early work on the peeling reaction that maltose (α) and cellobiose (β) degrade in nearly identical manner (29). It has also been reported that alkaline peeling of amylose and cellulose are very similar (30). The reactions yielded the same degradation acids in similar proportions, and the properties of the residual alkali-stable polymers indicated that the substrates had undergone the same modifications (30).

The rate of anaerobic alkaline random cleavage of glycosidic bonds is dependent on the configuration of the anomeric carbon (3). The reaction conditions in this study were sufficiently mild, however, so that such alkaline random cleavage would not contribute significantly to the degradation.

The degradation of α - and β -linked methyl glycosides in oxygen-alkali at 120°C was studied by Malinen (31). The two isomeric methyl glucosides reacted at the same rate and yielded essentially the same distribution of products, which indicated that the orientation of the glycosidic bond is not important under oxidative conditions.

To the best of my knowledge, the present study was the first concerned with the fundamental aspects of oxygen-alkali degradation of an α -linked polysaccharide. Neither could any information be found in the literature about the possible interaction between α -glycosidic bonds and anthraquinone.

REACTION CONDITIONS

The reactions were carried out in a Teflon-lined autoclave to avoid catalysis of the oxygen-alkali reactions by metal surfaces (15). The reagents were prepared and placed in the reactor under a nitrogen atmosphere. The amylose (1% wt./vol., alkali basis) was kept in a Teflon capsule until the NaOH solution (1.0M) had reached the desired reaction temperature (80 or 100°C). Pressurization of the reactor (total pressure ca. 1.14 MPa = 165 psia) released the amylose into the alkali to start the reaction. The reactor system is described in detail in Appendix III.

Anaerobic degradation conditions were created by pressurizing the reactor with prepurified nitrogen. The reactor was pressurized with oxygen for oxygen-alkali reactions. Anthraquinone (5% wt./wt., amylose basis) was added to the alkali in the reactor.

Although the reactions were monitored for up to about 20 hr, most samples were taken during the first 5-7 hr. when the major part of the degradation occurred.

DATA TREATMENT AND ANALYSIS

The simultaneous measurement of material loss and molecular weight distribution of a degrading polymer can be used to analyze the ways in which it depolymerizes. Particularly, the data provide means to differentiate between random chain cleavage and stepwise peeling from one end. Such information for the alkaline degradation

of amylose would not only supplement the literature but also provide a direct test of the validity of the single chain theory of degradation.

The diagram in Fig. 4 illustrates how the characterization of the depolymerization is done. The number average DP of the starting material ($\overline{DP}_{n,o}$) and the \overline{DP}_n of each sample ($\overline{DP}_{n,t}$) are calculated from the molecular weight distributions. The residual \overline{DP}_n , expressed in percent, is then defined as $100 \times \overline{DP}_{n,t} / \overline{DP}_{n,o}$. Yield, on the ordinate axis, is defined as the ratio of the mass of remaining polymeric amylose to the originally present mass of amylose.

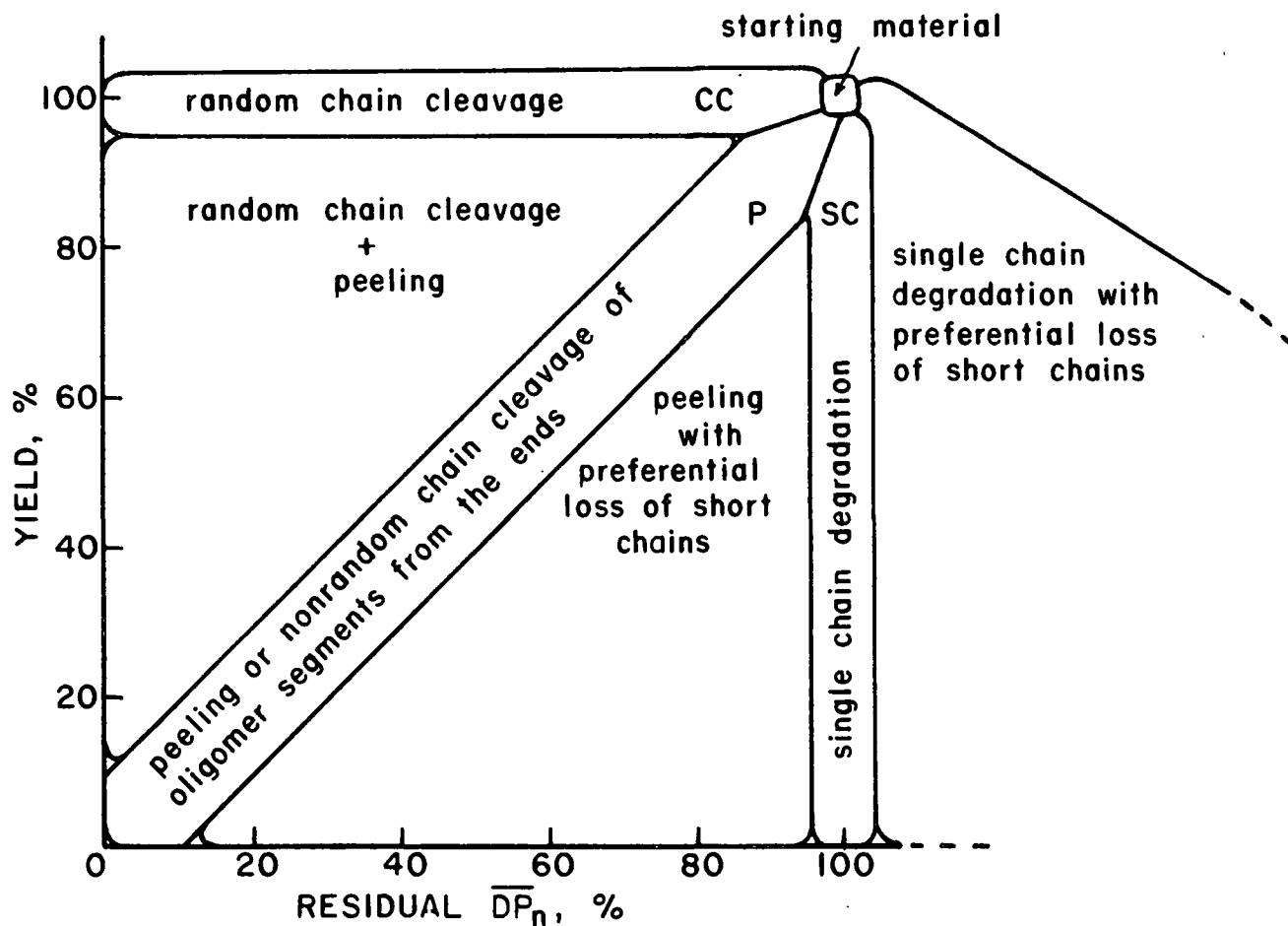


Figure 4. The Different Modes of Depolymerization Are Represented by Different Areas in a Graph Where Amylose Yield is Plotted vs. Residual \overline{DP}_n

The original polymer is, of course, represented by the point 100 - 100. Each sample taken from a reaction is described by a point in the diagram, and the reaction, as will be exemplified later, is then represented by the line or arc connecting those individual points. The position of the reaction curve relative to the axes determines which type of degradation is typical for the reaction. For example, exclusive random chain cleavage, with no loss of oligomers as degradation products to the liquor or to the atmosphere by volatilization, retains the entire yield, with drastic reductions in molecular weight (area CC in Fig. 4). Similarly, pure peeling follows along the diagonal of the diagram (area P in Fig. 4). If nonrandom chain cleavage of the chains would produce oligomer segments from the ends and be lost to the liquor, the reaction curve would also fall in the peeling area. Loss of entire molecules regardless of their original size, i.e., single chain type degradation, would be represented by a vertical line down from the starting point (area SC in Fig. 4). Reactions in which two types of degradation proceed simultaneously fall in areas between the respective component areas.

RESULTS AND DISCUSSION

ALKALINE REACTIONS

Amylose was degraded in the absence of oxygen at 80 and 100°C. Yield and number average DP were determined as functions of reaction time. The values are listed in Tables IV and VII in Appendix IV. The rate of degradation was significant at both temperatures, and the yield loss levelled off after 2-5 hr. Plots of residual \overline{DP}_n and yield versus reaction time (Fig. 5) practically coincide at both temperatures and confirm that random cleavage of amylose chains is not important under anaerobic conditions. The yield curves are also shown as solid lines in Fig. 6. The corresponding yield curves from the study of Lai and Sarkanen (8,9) are included for comparison (dashed lines). The peeling lengths for the two reactions were estimated as previously described (p. 6). Since the original \overline{DP}_n was ca. 630, the leveling-off yield losses of 45 and 65% correspond to peeling lengths of 290 and 400 monomer units, respectively. The absolute values of those lengths may be dependent on the origin of the amylose and on other experimental factors, but the leveling-off yield loss, i.e., the peeling length, did clearly increase with temperature.

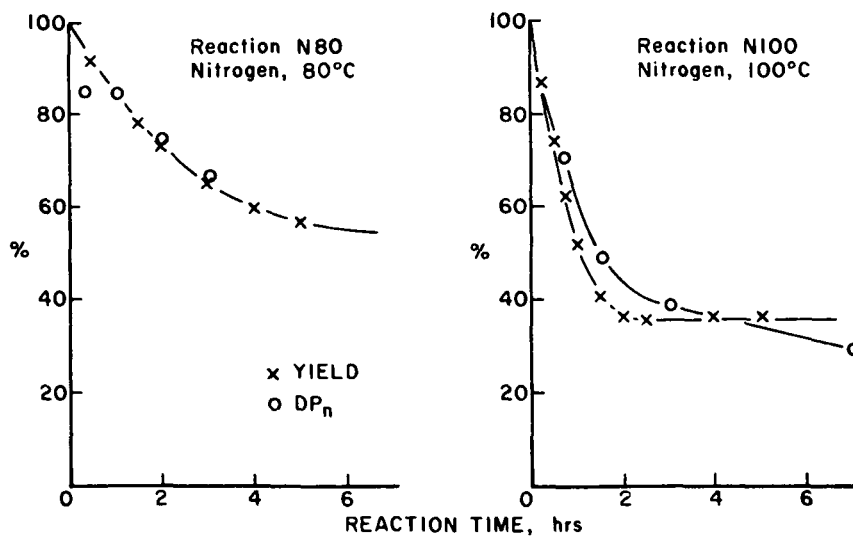


Figure 5. The Amylose Yield and Residual \overline{DP}_n as Functions of Reaction Time in Anaerobic Alkali. The Graphs Indicate that Chain Cleavage is not Important

Reanalysis of the data from Lai's investigation (8) showed the same trend for temperatures between 56 and 118°C. The amylose yield curves for conditions most similar to those used in this work are shown as dashed lines in Fig. 6. The reported leveling-off yields correspond to peeling lengths of 205 and 368 units.

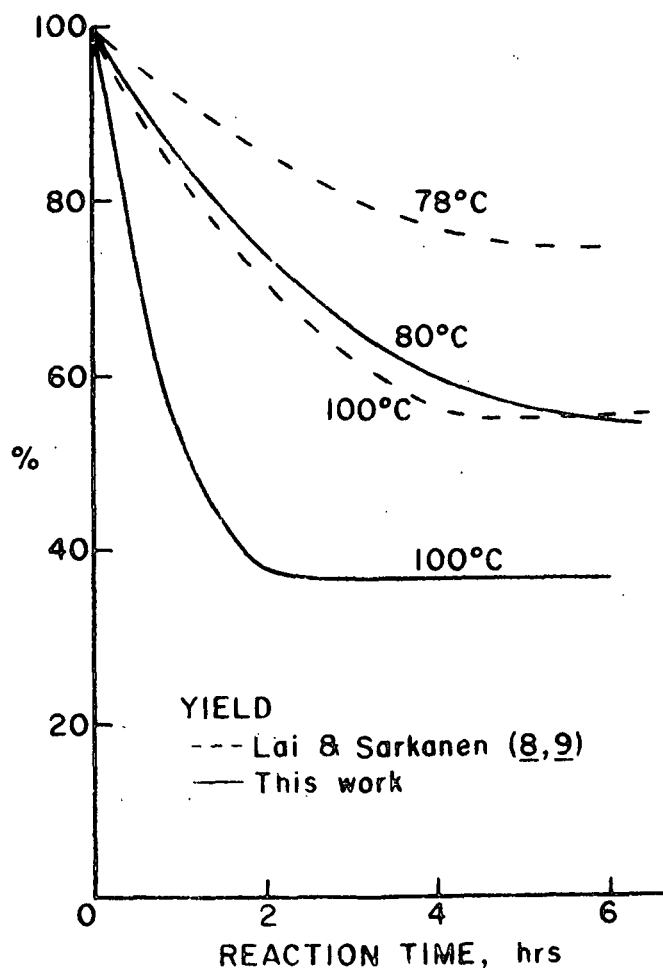


Figure 6. Amylose Yield During Anaerobic Alkaline Degradation

The fact that the leveling-off yield loss and the peeling lengths increase with temperature can be explained in terms of reduced competitiveness of the stopping reaction at higher temperatures. This is contrary to the conclusions

that were reached by assuming the existence of a physical stopping mechanism for stabilization of cellulose (7). That theory predicts that the chemical stopping reaction should increase in significance with temperature. This inconsistency suggests that the kinetic model of Sarkanen and coworkers (7) was incorrect. The current data support earlier criticism of the physical stopping theory (12).

The amylose degradation in this work was more severe than that reported in the literature. This apparent disagreement may be caused by the different techniques used in initiating the reactions and supports that above conclusion about the effect of temperature on the stopping reaction. In the earlier study (8), amylose was dissolved in alkali at room temperature and the solution was heated to reaction temperature. During the lower temperatures of the heating-up period, the stopping reaction would be relatively more important, causing additional stabilization of end groups and thus less opportunity for subsequent peeling and consequently a higher final yield and lower reaction rate. At a higher temperature, the heating-up time would be longer, and the effect would be more pronounced, as was illustrated in Fig. 6.

The molecular weight distribution of the amylose changed continuously during alkaline degradation. Data for the 80°C anaerobic reaction (Fig. 7) show that the shape of the distribution remains the same, while the entire curve is shifted toward lower molecular weights. At the time when the yield curve levels out, the shifts in the distribution become negligible. This indicates that all molecules have been stabilized by the stopping reaction, rendering the system essentially inert.

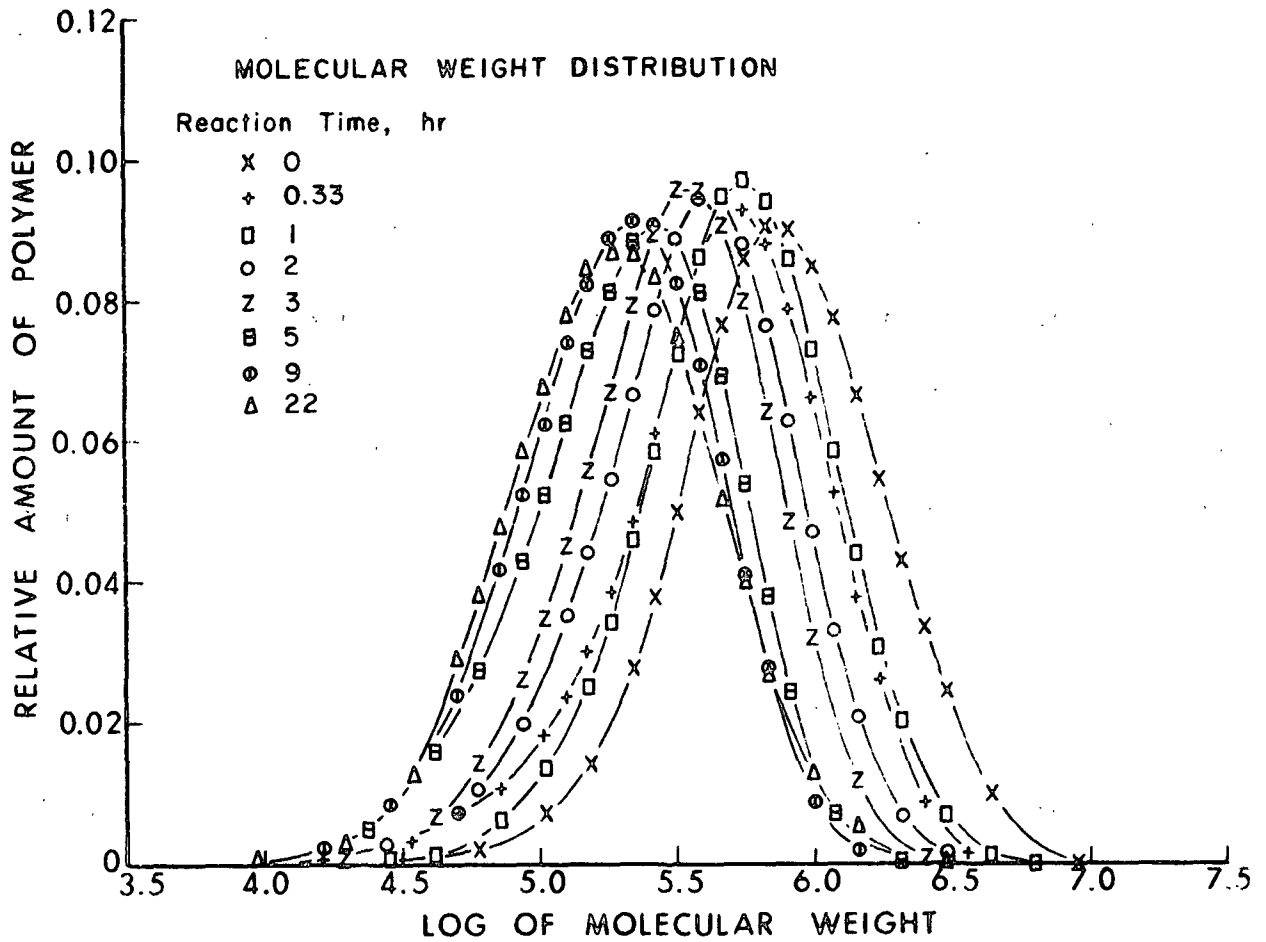


Figure 7. The Molecular Weight Distribution of Amylose During Alkaline Degradation at 80°C

The analyses of the changes in the molecular weight distribution with respect to reaction time under anaerobic conditions provide a direct test of the validity of the so-called "single chain theory of degradation," which was described earlier (p. 10,11).

The molecular weight distributions of the amylose shifted toward lower molecular weights during degradations at both anaerobic and oxidative conditions. This observation, exemplified in Fig. 7, immediately contradicts the single chain

theory (10,11), which predicts a constant molecular weight distribution during a reaction. The experimentally determined shifts cannot be explained in terms of a preferential loss of polymers of certain DP values, since all portions of the distribution curves shift practically the same amount. However, the results are consistent with the classical peeling-stopping reaction system.

The method of separating the different modes of depolymerization by plotting yield versus residual \overline{DP}_n is helpful in evaluating the single chain theory. The locus of a single chain type degradation is clearly separated from the areas representing classical peeling and chain cleavage (Fig. 4) and from experimentally determined curves for alkaline reactions (Fig. 8). Similarly, as will be discussed later, the data for reactions in the presence of oxygen or anthraquinone were not consistent with single chain kinetics (Fig. 14).

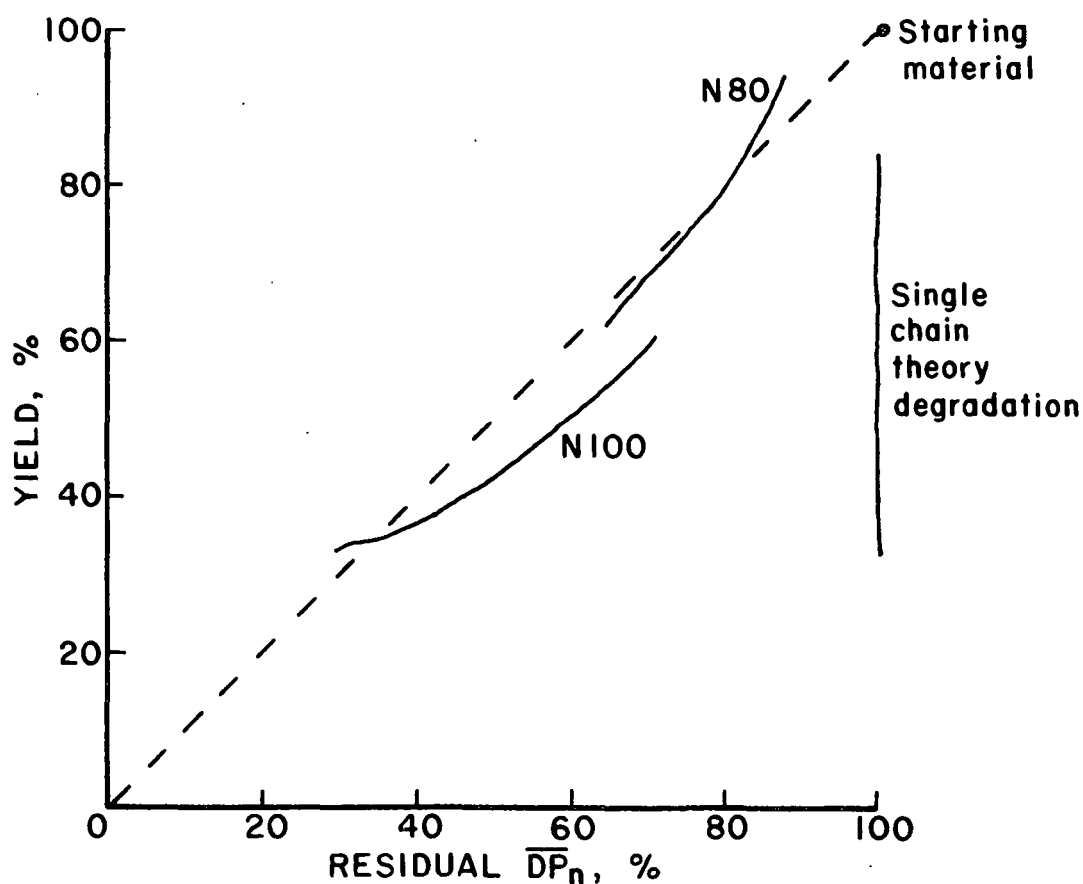


Figure 8. Comparison Between Experimental Data from Alkaline Degradation and the Single Chain Theory Prediction

Thus one may conclude that the single chain theory does not describe the alkaline degradation of amylose. However, this work does not exclude the possibility that polysaccharides of relatively low DP (ca. 100 or less) may degrade in a way resembling the single chain theory. The classical peeling reaction is, in effect, a single chain type process when the original \overline{DP}_n of the polymer is of the same order as, or is shorter than, the average peeling chain length. This has been shown experimentally by Johansson and Samuelson (12), who demonstrated that the \overline{DP}_n of a hydrocellulose increased from 189 to about 240 during alkaline degradation. The increase was due to the total loss of a large fraction of short molecules. It was estimated that 170 glucose units (including those of totally dissolved molecules) were brought into solution per each stabilized acid end group.

REACTIONS IN OXYGEN-ALKALI

Degradation of amylose was also performed in oxygen-alkali. The reactor was pressurized with oxygen, while all other conditions were kept the same as in the anaerobic reactions. Figure 9 shows the yield and residual \overline{DP}_n curves for reaction at 80 and 100°C. The values are also listed in Tables V and VII in Appendix IV.

At both temperatures the yield losses were much smaller than those typical of the oxygen-free reactions. In fact, at 80°C there was no significant loss of amylose for the first 5 hours. This was a surprising degree of stability in oxygen-alkali, compared to the rate of degradation in the absence of oxygen (Fig. 5) and was first thought to be an induction period similar to the induction periods often encountered in oxygen-alkali reaction systems (32,33). DP measurements showed, however, that the \overline{DP}_n dropped continuously from the start

of the reaction. Thus the lack of significant yield loss was not due to an induction period, but to a shift in the relative importance of the depolymerization reactions. Stabilization of polysaccharides toward peeling and random cleavage of polymer chains are both greatly enhanced by the presence of oxygen. This becomes clear from the yield versus residual \overline{DP}_n curves for the oxygen-alkali reactions (Fig. 10). The presence of oxygen moves the curves from the area of pure peeling to those of pure chain cleavage and chain cleavage with a minor contribution from peeling (cf. Fig. 4).

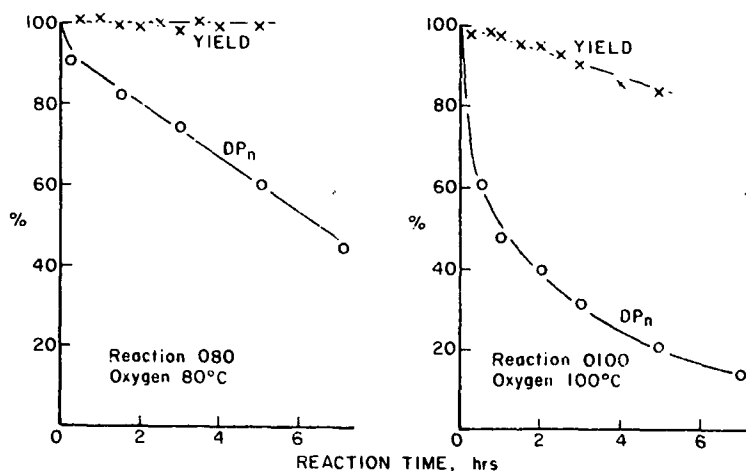


Figure 9. The Amylose Yield and Residual \overline{DP}_n as Functions of Reaction Time in Oxygen-alkali

The extensive random chain cleavage depolymerization, which is typical of oxygen-alkali degradation, leads to a rapid shift from higher to lower molecular weights in the molecular weight distributions (Fig. 11). The chain cleavage and secondary peeling reactions prevent the distributions from becoming constant and stationary, as they do in the absence of oxygen (Fig. 7). The distribution curves become increasingly narrow, and the polydispersity decreases. It is

characteristic for depolymerization via random chain cleavage to proceed with decreasing polydispersity which approaches 2.0, to form a so-called "most probable" or "Flory-type" distribution (34,35). The peeling reaction is probably also contributing to the narrowing of the distribution. As discussed on pages 23-25, it is to be expected that some of the chain fragments, whose DP is shorter than the average kinetic chain length, will be entirely degraded to monomeric products. Thus the distributions are not likely to have any significant amounts of material in the lowest range of molecular weights. The specific range, applicable to Fig. 11, depends on the peeling length in oxygen-alkali at 100 C. This parameter is estimated as follows.

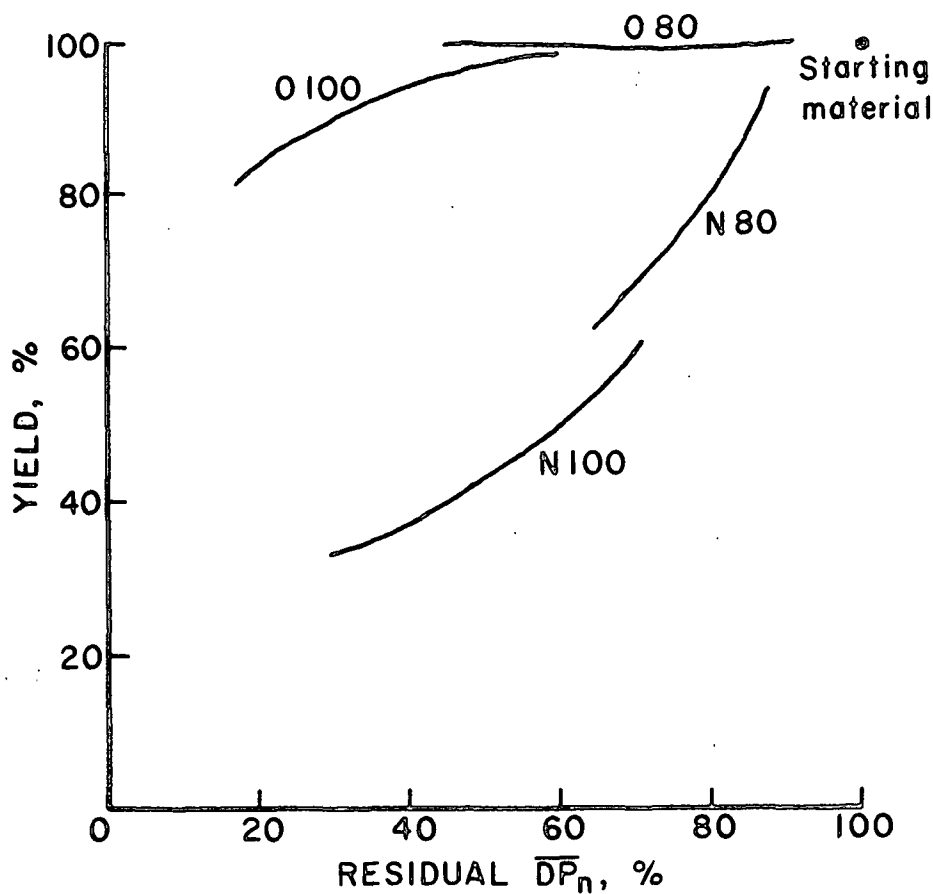


Figure 10. Comparison Between Alkaline Degradation Under Nitrogen and Oxygen. Oxygen Enhances Depolymerization via Random Chain Cleavage

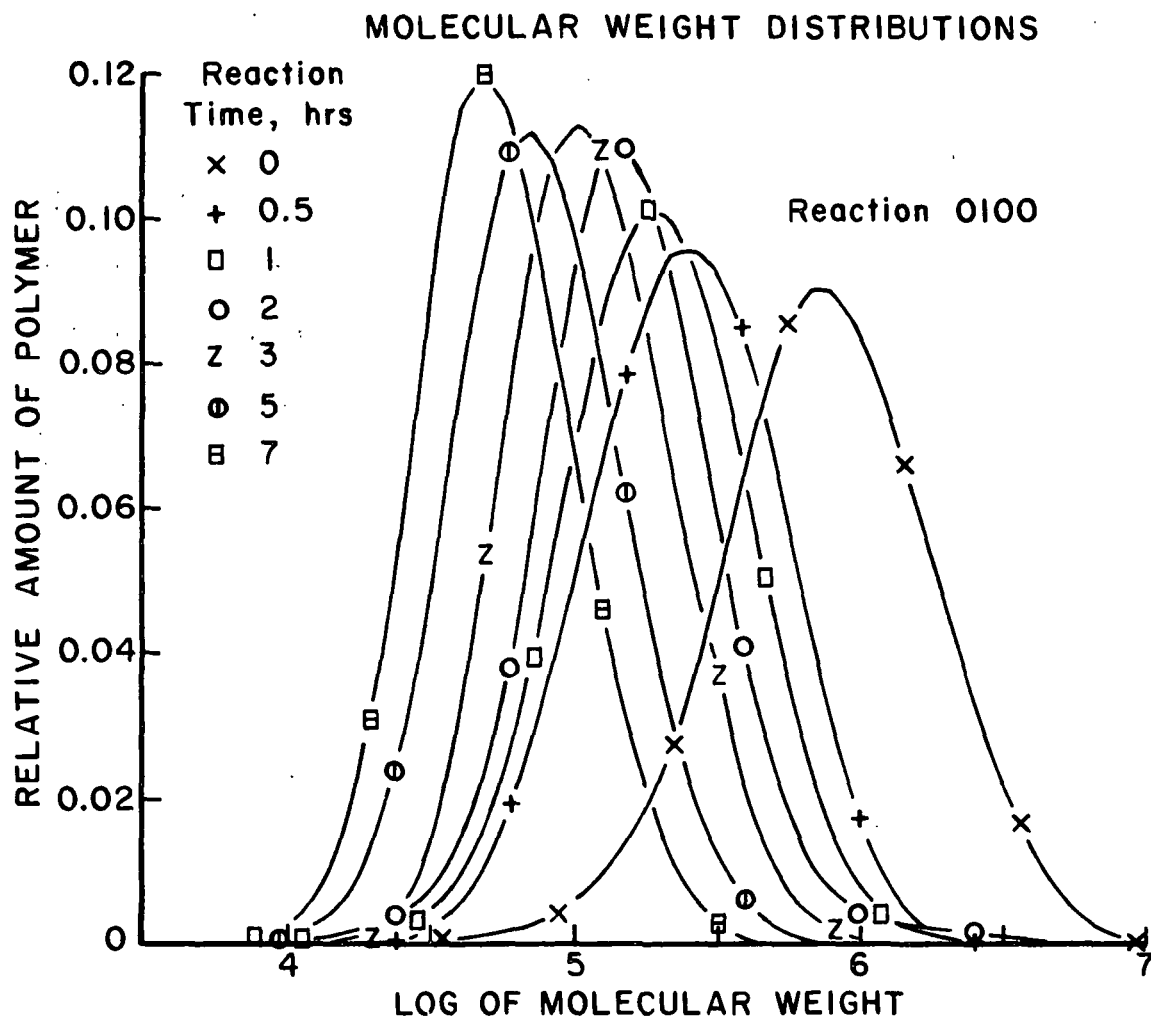


Figure 11. The Molecular Weight Distribution of Amylose During Oxygen-alkali Degradation at 100°C

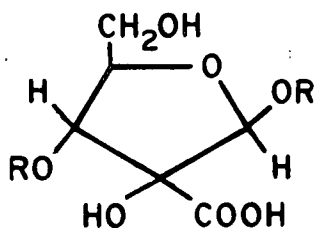
The complex interdependence between the peeling and the chain cleavage reactions makes separate measurement of them very difficult. However, the fact that only random chain cleavage was important in the oxygen-alkali reaction at 80 C (Fig. 9), presented a possibility for estimating the average chain length lost via peeling in the 100 C reaction.

The rate constant for chain cleavage at 80 C was calculated according to Eq. (2), which applies to exclusive random chain cleavage. From a plot of the inverse of experimentally determined \overline{DP}_n values (Table I) versus reaction time

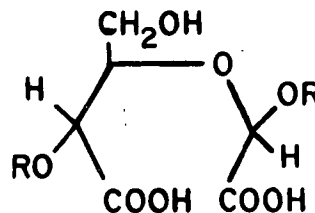
(Fig. 12), the rate constant was calculated to be $6.08 \times 10^{-8} \text{ s}^{-1}$ (line CC80, Fig. 12). McCloskey (36) estimated the activation energy for glycosidic bond cleavage in oxygen-alkali to be 21 kcal/mole under conditions similar to those used in this work. This activation energy was used to predict the rate of amylose chain cleavage at 100°C . According to the Arrhenius' equation, it should be 5.0 times faster than at 80°C , corresponding to a rate constant of $30.4 \times 10^{-8} \text{ s}^{-1}$ (line CC100, Fig. 12). Furthermore, the experimentally determined $\overline{\text{DP}}_n$ values for degradation at 100°C (Table I) were plotted with their linear approximation (line T100) in Fig. 12. The actual $\overline{\text{DP}}_n$ values were significantly smaller than predicted solely on the basis of chain cleavage. This difference represents the peeling contribution to the depolymerization and can be interpreted as the average length of each chain lost via peeling. The peeling length was 20-30 monomer units (Table I), which is only 5-8% of the corresponding length in anaerobic alkali. Such a relatively short peeling length is consistent with the small yield loss of the oxygen-alkali reactions (Fig. 9) and also agrees with the estimate of Malinen and Sjöström (13) that the peeling length for hydrocellulose at 120°C in oxygen-alkali is of the order of 10-50 glucose units. The agreement between reactions with the amylose and the hydrocellulose (13) systems suggests that physical structure, at the most, has a subordinate influence on the degradation rate of the carbohydrate. It also supports the assumption that differences in degradation between α - and β -linked saccharides in oxygen-alkali systems are small (31).

Based on their studies of degradations of monomeric glycosides in oxygen-alkali, Ericsson et al. (37) and Malinen and Sjöström (31) have proposed dicarboxylic acid and carboxy-furanoside structures as potentially stable intermediates in the chain cleavage mechanisms.

2C-carboxy-furanoside



Dicarboxylic acid



The presence of such groups in oxygen-alkali treated polyglucans has, however, not been verified. In this work, amylose recovered after extensive degradation (yield ca. 80%, residual \overline{DP}_n 14%) was analyzed by ^{13}C -NMR and gave a spectrum practically identical with that of the starting material and of the amylose spectra in the literature (38). The sensitivity of the spectrometer to functions of low concentration was estimated by observing the signals from the reducing end group carbon atoms of an amylose with \overline{DP}_n of 18. They were barely distinguishable above the noise, indicating that functions appearing less frequently than on approximately every 20th glucose unit could not be expected to be seen in the spectrum.

Nevertheless, peripheral evidence was found which indicated that oxidative modifications of the amylose did occur. Under anaerobic conditions, the amylose concentration in the liquor, as determined from the absorbance of the amylose-iodine complex (39) agreed with the primary determination by enzymatic assay of glucose after total hydrolysis. In the case of degradation under oxygen at 100°C , however, the iodine analysis gave a much lower value, and the discrepancy increased with reaction time. The error is not primarily due to the oxygen-induced DP reduction, since amylose yields at similar DP levels in anaerobic

cooks were accurately determined by the iodine complexing method. Similar observations about the influence of the presence of molecular oxygen during the alkaline degradation on the iodine binding capacity (IBC) of the reacted amylose were made by Hollo, Szejtli and Laszlo (40), but they offered no speculation about possible causes.

TABLE I
 DP_n VALUES FOR THE ESTIMATION OF AVERAGE PEELING LENGTH
 IN OXYGEN-ALKALI (FIGURE 12)

| Reaction Time, hours | Experimental DP_n | | Calculated DP_n | | Peeling Length CC100 - T100 |
|-------------------------|---------------------|-------|-------------------|-------|--------------------------------|
| | 80°C | 100°C | T100 ^a | CC100 | |
| 0 | 630 | 630 | ND ^b | ND | ND |
| 0.25 | 568 | ND | ND | ND | ND |
| 0.5 | ND | 376 | 445 | 469 | 24 |
| 1 | ND | 298 | 344 | 374 | 30 |
| 1.5 | 515 | ND | 280 | 310 | 30 |
| 2 | ND | 244 | 237 | 265 | 28 |
| 3 | 465 | 195 | 180 | 206 | 26 |
| 5 | 381 | 124 | 122 | 142 | 20 |
| 7 | 284 | 91 | 92 | 109 | 17 |

^aLinear approximation.

^bND = not determined.

The blue amylose-iodine complex is characterized as a regular, helical structure (28) in which polyiodide ions (I_5^-) (41) are captured inside the helices. Banks and Greenwood (27) have shown how the formation of the complex depends on the DP of the amylose. Their data are listed in Table II.

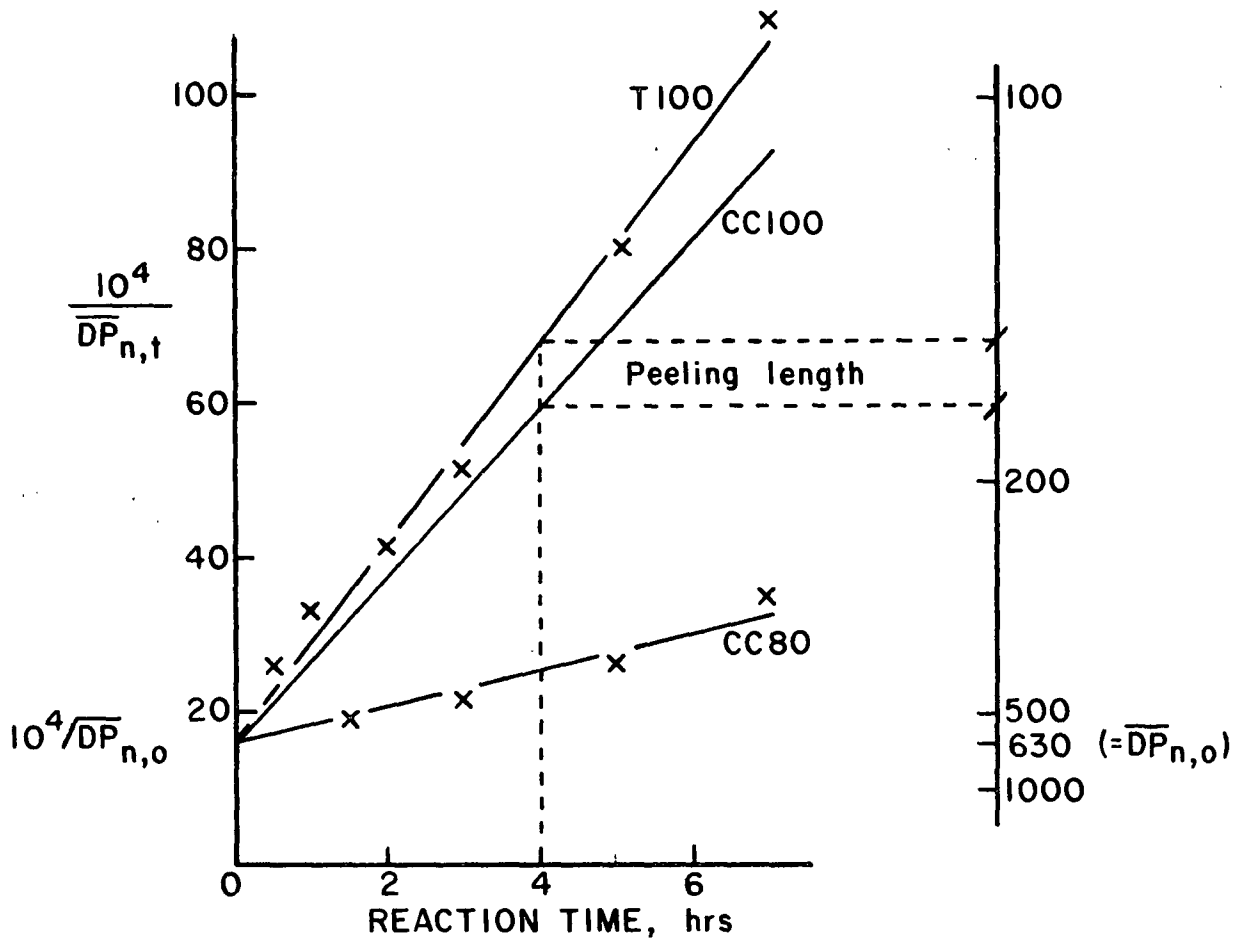


Figure 12. The Inverse of \overline{DP}_n of Amylose Degraded in Oxygen-alkali as Functions of Reaction Time

$\overline{DP}_{n,0}$ - number average DP of starting material (= 630)

CC80 - observed depolymerization at 80 C

CC100 - calculated chain cleavage depolymerization at 100 C

T100 - linear approximation of observed depolymerization at 100 C

TABLE II

THE IODINE BINDING CAPACITY (IBC, IN mg BOUND IODINE PER 100 mg POLYSACCHARIDE) OF AMYLOSE FRACTIONS OF DIFFERENT \overline{DP}_n (27)

| | | | | | | | | | | |
|-------------------|----|----|-----|-----|------|------|------|------|------|------|
| \overline{DP}_n | 22 | 29 | 31 | 36 | 51 | 93 | 105 | 134 | 260 | 1500 |
| IBC | 0 | 0 | 1.3 | 3.6 | 11.1 | 16.2 | 16.4 | 17.3 | 19.3 | 19.5 |

One possible explanation for the comparatively low complexing tendency of oxygen-alkali degraded amylose may be that molecular oxygen introduces disruptions in the polysaccharide chain, by formation of the internal dicarboxylic acid (via intragluco-sidic cleavage) or carboxy-furanoside functions. The oxidized groups would alter the flexibility of the amylose chain, which in turn would prevent long range order in the helices. The amylose complex is envisioned as normally existing as a deformed helix (also referred to as a wormlike coil) (27), while the disruptions introduced by the oxygen in alkali in essence would transform the complex to the structure of the interrupted helix model (27). The effective DP, i.e., the distance between such kinks of altered chain stiffness, might well be as low as 30, when no complex would form, while simultaneously the true DP of the oxidized amylose was of the order of 300-500. The number of oxidized internal glucose units would be small compared to the number of normal monomers, which would explain why it was not possible to find spectrometric evidence for their presence.

REACTIONS WITH ADDITION OF ANTHRAQUINONE (AQ)

Alkaline degradations of amylose under nitrogen and oxygen at 100°C were also performed in the presence of AQ. The amount of additive, 5% wt./wt. amylose basis, was considerably larger than the 0.1% often used in wood pulping experiments (16). This dosage was chosen to maximize the effect of AQ under the

anaerobic condition, where AQ could be expected to be rapidly reduced to AHQ, without any possibility of becoming reoxidized to AQ. In wood pulping systems, this reoxidation is thought to occur via reactions with lignin fragments (18).

The yield and \overline{DP}_n data for the AQ reactions are listed in Tables VI and VII in Appendix IV and are shown graphically in Fig. 13. The presence of AQ resulted in dramatic stabilization of the amylose. At least 80% of the polyglucan lost in alkali alone (Fig. 5) was retained by the addition of AQ to the alkali. The yield loss was about the same as that of the AQ-free oxygen-alkali reaction (Fig. 9). AQ had a less pronounced influence on the amylose degradation under oxygen atmosphere. The yield loss was essentially the same when the degradation took place in the presence of both oxygen and AQ (reaction O-AQ-100) as when either reagent was used alone (reactions N-AQ-100 and O-100).

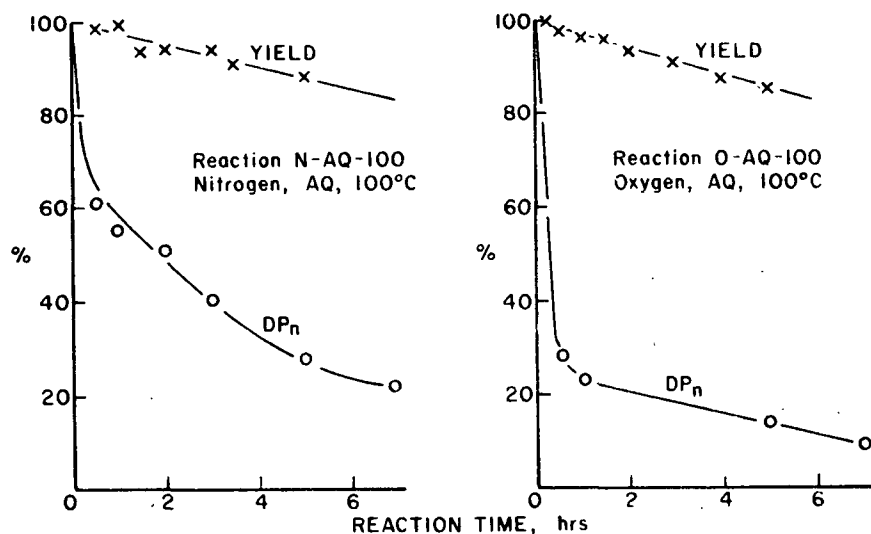


Figure 13. The Amylose Yield and Residual \overline{DP}_n as Functions of Reaction Time in Alkali and Oxygen-alkali with Addition of AQ

The residual \overline{DP}_n decreased much more rapidly than the yield in the AQ reactions (Fig. 13). This indicated that random cleavage of the amylose chains was brought about by the additive. This change in the mode of depolymerization

relative to AQ-free conditions is also depicted in Fig. 14. The addition of AQ made alkaline degradation similar to that occurring in oxygen-alkali. AQ also enhanced the chain cleavage reaction under oxygen (O-AQ-100), decreasing the residual \overline{DP}_n by about 50% compared to the AQ-free oxygen-alkali reaction (O-100, Fig. 14). Thus, there must have been a proportionately greater number of reducing ends available to the peeling reaction. Consequently, a relatively higher yield loss should have been expected. The fact that the yield loss instead was essentially the same for oxygen-alkali degradation with and without AQ shows that the AQ does contribute to the stabilization of reducing end groups in the presence of oxygen. As a crude estimate, the average peeling chain length under oxygen with AQ added must have been about one half of that in the absence of AQ, i.e., 10-15 units, to compensate for the approximately double number of sites available to peeling.

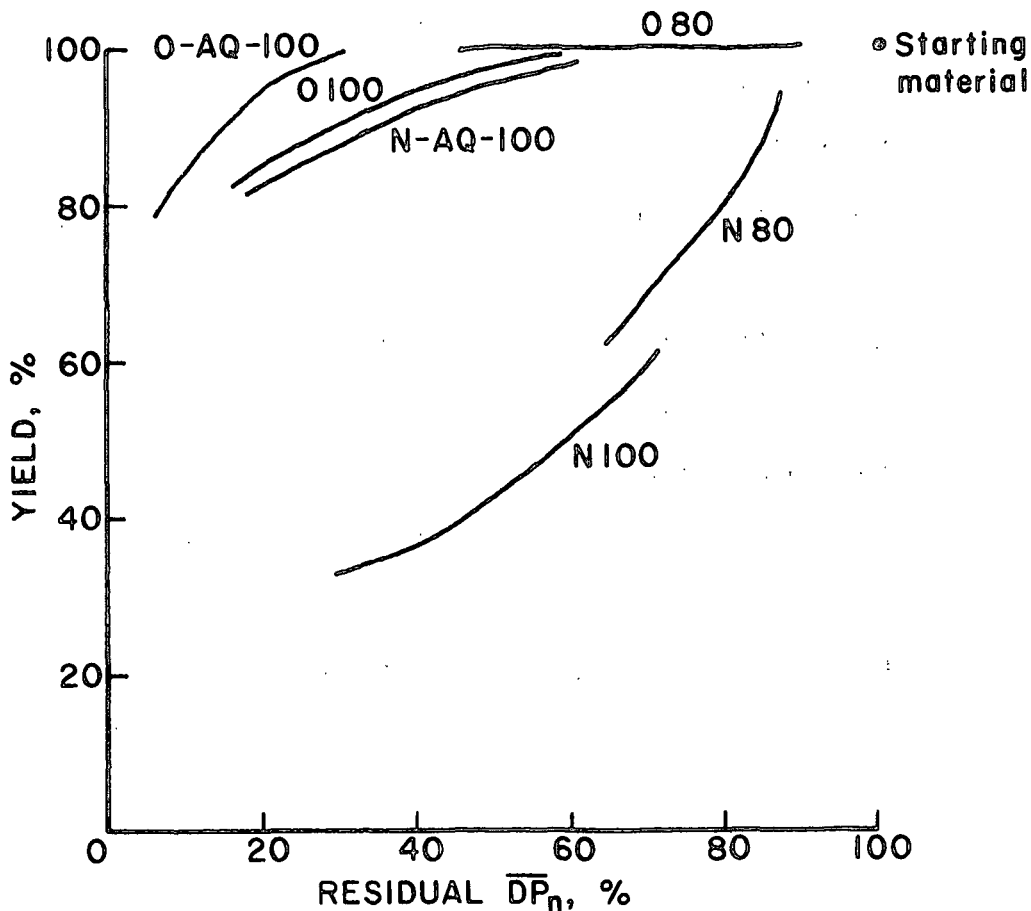


Figure 14. Comparison Between the Alkaline Degradation of Amylose Under Different Conditions, Showing the Influence of Oxygen and Anthraquinone

The discovery of a reaction path by which AQ can induce cleavage of glycosidic bonds in amylose may also have implications for AQ pulping of wood. Small reductions in viscosity (20,42) and tear strength (43) relative to reference pulps produced without AQ have been reported. This detrimental side effect was attributed at least in part to increased stabilization and retention of hemicelluloses caused by the presence of AQ. Now, the present results suggest the possibility that AQ also directly shortens cellulose chains via random chain cleavage attack. Such a reaction is consistent without present understanding of the mechanism by which AQ reacts in alkaline pulping systems (44).

In anaerobic AQ-alkali cooks, the liquor develops a deep red color which is taken as qualitative evidence that AQ has been reduced by the carbohydrate to form soluble AHQ-ions or -ion radicals (18). The red color did develop in the reactions in this work but vanished unexpectedly after about the first hour. The liquor then remained pale yellow. AQ (oxidized form) is known to react with acid degradation products of carbohydrates (19,20) and the disappearance of the red color indicated that the reduced forms (AHQ) are also able to do so. The structure of the reactive products could alternatively be similar to aromatic lignin fragments. Highly colored, phenolic compounds are known to be formed in small amounts during alkaline degradation of glucose and xylose (45). Several substituted acetophenones capable of forming quinone-methide structures were reported. These are also likely to be formed during degradation of amylose (45). Brunow and coworkers (46) have shown that AHQ does react with such quinonemethides. Thus, it seems probable that AHQ had the opportunity to participate in secondary reactions, leading to the loss of the red color.

CONCLUSIONS

It is proposed that the alkaline depolymerization of amylose proceeds, under the conditions studied in this work, according to classical theory, i.e., via exclusive stepwise elimination of monomers from the reducing end of the polysaccharide molecule. The average kinetic chain lengths lost via peeling were determined to be 290 and 400 glucose units at 80 and 100°C, respectively. Thus, the stabilization reaction (stopping) is more important at lower temperatures. Complete degradation of polymer molecules via total unzipping, as postulated in the single chain theory (10,11), is not consistent with the present data which indicate a continual shift in the molecular weight distribution toward lower values during reaction. The theory may be true in the special case of amylose substrates with a \overline{DP}_n of about 100 or less, but it is not valid as a general rule.

When the amylose is reacted in alkali under oxygen pressure, the depolymerization occurs via both endwise peeling and random cleavage of the polymer chain. Stabilization of reducing end groups is favored, leading to a relatively small yield loss. The average peeling length lost per each reducing site under oxygen at 100°C was only 5-10% of the corresponding length under anaerobic conditions. The random chain cleavage reaction caused a large continuous shift in the molecular weight distribution toward lower molecular weights.

The addition of anthraquinone to the alkaline medium stabilizes the amylose against peeling yield loss under both nitrogen and oxygen atmospheres. Anthraquinone is also able to induce cleavage of glycosidic bonds of a homogeneously dissolved polysaccharide, since the reduction of the residual \overline{DP}_n of amylose was significantly larger than the yield loss. This not previously reported

reaction path is proposed to be partly responsible for the often observed lower viscosities and tear strengths of soda-AQ derived pulps (20,42). Such a reaction is consistent with our present understanding of the redox cycle theory of anthraquinone activity in alkaline pulping systems (18,44).

EXPERIMENTAL.

SOLUTIONS AND REAGENTS

SODIUM HYDROXIDE STOCK SOLUTION

Reagent-grade sodium hydroxide pellets normally contain on the order of 1-10 ppm of iron, nickel and other transition metals. Even at these low concentrations, the metals can significantly affect the reactions of carbohydrates in oxygen-saturated alkali (15,32). The reduction-extraction process of Reiner and Poe (47) was utilized to remove such trace contamination of metals.

Glassware* used in handling the alkali during purification was cleaned by soaking it in strong nitric acid (ca. 10M) and then rinsing it thoroughly with triply-distilled water.

Reagent-grade sodium hydroxide (0.9 kg) and freshly boiled triply distilled water (2 liter) were mixed in a 3-liter round-bottom flask. About 0.2 g of 10% palladium on carbon was wetted with triply-distilled water and added to the alkali solution. The complexing agent, phenyl-2-pyridyl-ketoxime (1.5 g), was dissolved in a minimum amount of hot ethanol and added to the solution in the flask. The mixture was stirred and heated to 100°C while hydrogen gas was bubbled through a Teflon tube (0.75 mm ID) into the solution for about 4 hours. The mixture was allowed to cool to about 40°C and was filtered through a glass fiber filter (Reeve Angel 934 AH) on a sintered glass filter (porosity M). Each liter of filtrate was extracted with three 50-mL portions of ethanol:isopentanol (1:3). The alcohol phase attained a deep brown-reddish color, indicating that

* It is preferable to use all polyethylene or Teflon-coated utensils in order to avoid contact between concentrated alkali and glass surfaces. This will help eliminate dissolution of silicone compounds from the glass.

organic metal ion complexes had been extracted from the aqueous phase. Each liter of the extracted alkaline solution was then placed in a 1-liter Teflon bottle fitted with a length of Teflon tubing (0.75 mm ID) reaching to the bottom of the bottle. Phenyl-2-pyridyl-ketoxime (0.5 g) was dissolved in hot ethanol (ca. 4 mL) and added to the solution. It was then placed in an oven at 100°C, and nitrogen was bubbled through the solution overnight. The solution was allowed to cool and again was extracted with three 50-mL portions of ethanol:isopentanol (1:3). The solution was returned to the oven (100°C), and nitrogen was bubbled through it overnight.

The alkali was transferred to a paraffin-lined, Teflon-stoppered glass bottle under a nitrogen atmosphere. This stock solution was then tested by UV spectroscopy for remaining organic complexing agent and by gas chromatography for traces of extraction alcohols. Both tests were negative and the solution considered pure and ready for use in the degradation experiments. The paraffin-lined bottle was kept in a glove bag filled with nitrogen. The concentrations of transition metals in the stock alkali, after purification, were determined by inductively coupled argon plasma emission spectroscopy (48). The following metals were present in quantities less than 1 ppm: Cr, Fe, and Mg and the following, at levels less than 0.05 ppm: Cd, Co, Cu, Mn, Ni, and Zn (48).

The alkali concentration of the purified solution was determined by titration with a potassium acid phthalate standard (49). Dried potassium acid phthalate (0.7-0.9 g) was weighed into each of four 250-mL Erlenmeyer flasks and dissolved in distilled water (50-75 mL). Stock sodium hydroxide solution (1.3547 g/mL) was diluted volumetrically (100:1) and used to titrate the potassium acid phthalate

to the phenolphthalein end point. The concentration of the stock solution was 33.75% wt./wt. or $11.43 \pm 0.03M$.

AMYLOSE (50,51)

Amylose (50 g) was dissolved in reagent grade dimethyl sulfoxide (1 liter) with vigorous stirring and heating on a steam bath. When the amylose was completely dissolved, the solution was transferred to a 3-liter beaker on a heavy duty magnetic stirrer. Absolute ethanol (700 mL) was added slowly from a dropping-funnel to the agitated solution, and a precipitate formed which did not redissolve. The supernatant and the precipitate were separated by centrifugation. The precipitate was thoroughly washed with ethanol and diethyl ether on a glass filter. The mother liquor was returned to the 3-liter beaker and more ethanol (50 mL) was added dropwise to precipitate another fraction of amylose. The fractionations were repeated until the amylose had been recovered completely. All the fractions were dried in a vacuum oven overnight and then weighed.

Some of the amylose fractions were lumpy and smelled of solvent. Soxhlet extraction with diethyl ether proved to be necessary to completely remove all the dimethyl sulfoxide and ethanol. The fractions were first washed twice with ethanol in a centrifuge bottle, and then any lumps were broken up in a mortar and pestle. The amylose was then placed in a sintered glass container in a large (2 inch ID) Soxhlet extractor and extracted with anhydrous diethyl ether for about 8 hours. [Note: It was necessary to often replace the boiling flask solution with fresh ether; otherwise the DMSO enrichment led to a boiling point increase and a higher temperature in the boiling flask causing violent flashing when the extractor emptied itself.] Once during the extraction, the process was interrupted and the amylose was reground in a mortar and pestle. After extraction, the amylose was dried in a vacuum oven at room temperature. When

the ether odor was gone, the oven temperature was raised to 40°C. The resulting amylose fractions were fine, odor-free powders whose weights remained constant during storage over phosphorous pentoxide.

ANHYDROUS PYRIDINE (52)

Barium oxide (ca. 25 g) was added to analytical reagent grade pyridine (ca. 2 liters) in a 3-liter round bottom flask. The mixture was refluxed for 5 hours and then distilled through a Vigreux column at atmospheric pressure with exclusion of moisture. The first 250 mL of distillate were discarded, after which the distillate boiling at 114°C was collected in glass bottles which had been dried in a vacuum oven. The bottles were filled completely and sealed with Teflon-coated ground glass stoppers and parafilm.

WATER

The water used in the degradation reactions and the analyses was triply distilled. Deionized, distilled water was obtained from a commercial Corning ag-11 Pyrex laboratory still. The second distillation was made from a solution of 0.02% potassium permanganate and 0.05% potassium hydroxide to remove organic impurities. The final distillation was additive-free. The specific conductance of the water was normally below $1.0 \times 10^{-6} \text{ ohm}^{-1} \text{ cm}^{-1}$.

METHYL N-PHENYL CARBAMATE

The use of methyl N-phenyl carbamate in exclusion chromatography is discussed in Appendix II.

Anhydrous methanol (5.0 mL \approx 110 mmole) was added to a 25-mL Erlenmeyer flask in an ice bath. Phenyl isocyanate (8.1 mL \approx 75 mmole) was slowly added to

the cold methanol. The exothermic reaction heated the solution, and when the solution was cooled, crystals formed, filling the flask. The material was recrystallized twice from petroleum ether (b.p. 60-110°C) to give crystals (10.1 g) with a melting point of 47.3-47.7°C.

REACTOR OPERATION

PREPARATION OF REACTION ALKALI

Cooking liquor for each reaction was prepared by dilution of the stock sodium hydroxide solution. Triply distilled water (ca. 700 mL) in a Teflon bottle was placed in an oven at 105°C. Prepurified nitrogen was bubbled through the water via a Teflon tube for 8-10 hours to remove dissolved gases. The bottle was transferred to the nitrogen glove bag where the stock alkali, the reactor with its Teflon lining and the reactor cover were stored. When the water had cooled, it and the stock alkali (11.43M) were measured out in volumetric cylinders (e.g. 44 mL stock alkali and 456 mL triply distilled water) to make 1.0M alkali for the reactions. A sample (5.0 mL) was saved for a control titration of the alkali concentration. Enough reaction alkali was placed in the Teflon lining to make the preweighed amylose in the capsule give a concentration of 1% wt./vol. alkali basis. Unused alkali was stored in the Teflon bottle under nitrogen in the glove bag.

LOADING

The reactor system is described in detail in Appendix III.

Fractionated and purified amylose (ca. 2.5 g) of known dryness (ca. 96%) was weighed analytically into the Teflon capsule. The filled, closed capsule,

the Teflon lining and the reactor with its cover were placed in the nitrogen glove bag, where the alkali solutions were stored. After thorough flushing with nitrogen, the 1.0M reaction alkali was measured by volumetric cylinders into the Teflon reactor lining. The amount of 1.0M reaction alkali required to make the cooking liquor 1% wt./vol. in amylose was calculated from the oven-dry weight of amylose in the capsule. The capsule containing the amylose was attached to the reactor cover (Fig. 23, Appendix III) and the cover was placed on top of the Teflon lining in the steel reactor. The reactor was sealed and the assembly was removed from the glove bag. The pressurizing and sampling valves were closed. The pneumatic magnetic stirrer was started and the reactor was immersed in the heated oil bath. The thermocouple was switched on and the warm-up sequence monitored on a recorder. When the reactor temperature was within one or two degrees of the desired reaction temperature, the reaction could be started, since pressurization always increased the temperature by about that amount. The gas regulator valve was adjusted to about 50 psig and the pressure line valve was opened to release the capsule contents into the liquor and start the reaction. The total reactor pressure was then regulated to 1.14 MPa (150 psig) and kept at that level until the reaction was terminated. The viscosity of the liquor increased significantly when the amylose dissolved, lowering the stirring rate and the pitch of the sound of the pneumatic turbine.

SAMPLING

Samples for amylose yield (ca. 3 mL) and molecular weight distribution (15-20 mL) analysis were withdrawn at appropriate times by opening the sampling line valve and collecting the liquor in a vial. The small volume of liquor in the sample line from the previous sample was purged before the samples were taken.

ANALYSIS OF REACTION SAMPLES

AMYLOSE PRECIPITATION

Samples (15-20 mL) withdrawn to recover the partially degraded polymeric amylose were neutralized with hydrochloric acid (2.2M) and then poured into ethanol (150-200 mL). It was important to remove water from the amylose to prevent subsequent hornification. This was done by solvent exchange. Precipitated amylose was washed repeatedly with absolute ethanol and then with anhydrous ether. The amylose was filtered from the ether suspension and dried under vacuum over phosphorous pentoxide. The weight of the dried amylose in conjunction with the original sample volume was used to estimate the amylose yield.

A test comparing the recovery of amylose by ultrafiltration and by precipitation was performed. Figure 15 shows the molecular weight distributions of degraded amylose samples which were recovered from the same liquor by ultrafiltration with Amicon filters DM-5 and PM-10 and by precipitation. Unfortunately, the two filtrations could not be carried to the same degree of solvent exchange due to filter plugging. The microsolite removal efficiency was 60% with DM-5 and 90% with PM-10. The finer filter (DM-5) did retain somewhat more of the low molecular weight material, although it is clear that differences between the methods are only minor. Precipitation was practically equivalent with ultrafiltration using a PM-10 filter with sufficiently high flow rate. Amylose recovery by precipitation was selected for use in this work, since ultrafiltration did not offer any advantage over precipitation and required more time and labor.

AMYLOSE DERIVATIZATION

Before amylose samples could be analyzed for their molecular weight distribution, they had to be derivatized to a compound which was compatible with the

exclusion columns, the eluting solvent and the detector system. Percarbanilation with phenyl isocyanate in anhydrous pyridine had been successfully applied in studies of cellulose (53) and was adopted for this work. Details about the use of amylose tricarbanilate in exclusion chromatography are given in Appendix II.

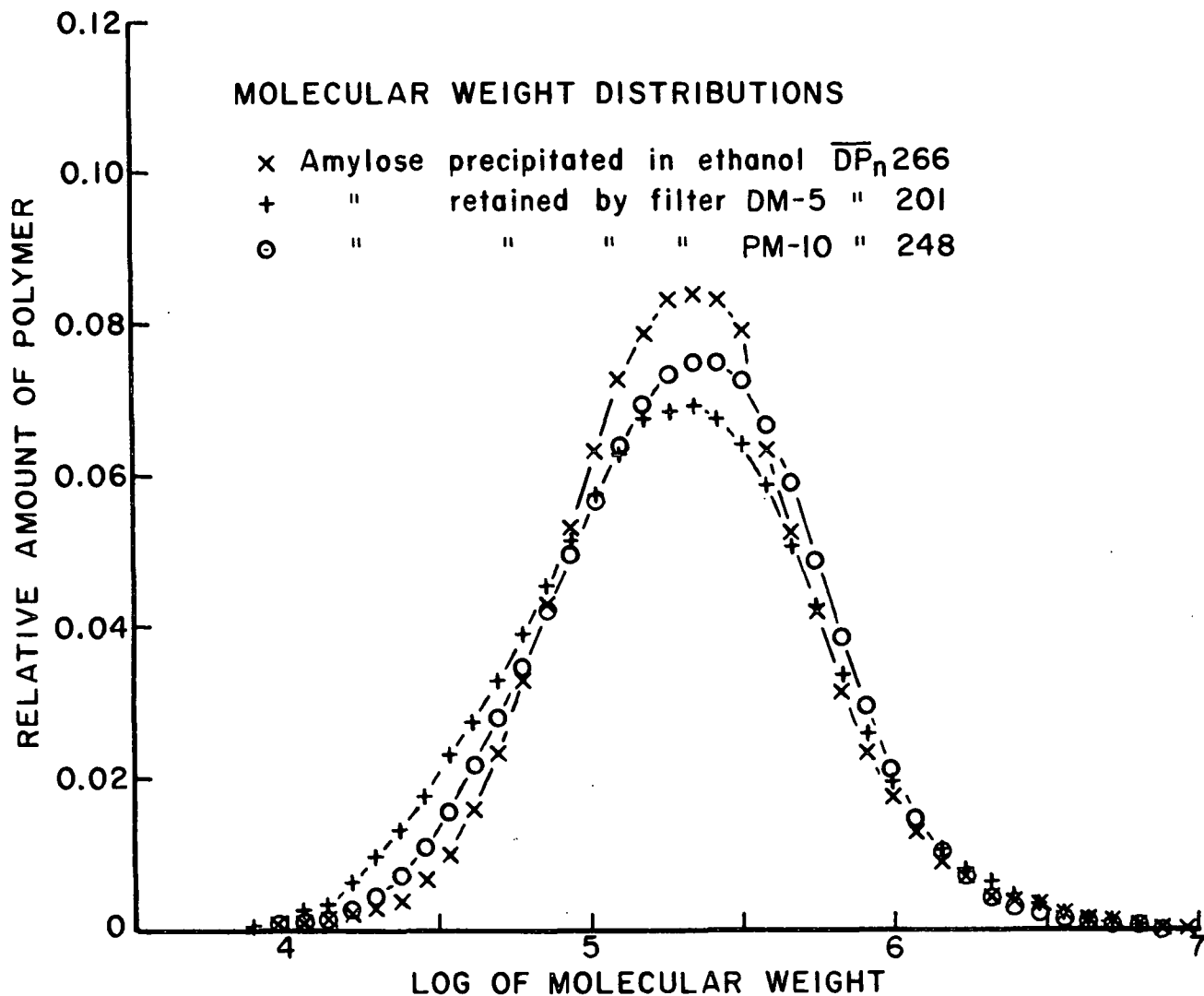


Figure 15. Comparison Between Amylose Recovery by Precipitation and Ultrafiltration

Anhydrous pyridine (90 mL) was added to the dried amylose (ca. 200 mg) in a 4-oz narrow-neck bottle. Phenyl isocyanate (18 mL) was added to the mixture and the bottle was sealed with a Teflon-lined cap. Two of the reaction bottles were snugly fitted inside a stainless steel laboratory digester by placing wads of absorbent cotton between the bottles. The digesters were rotated at ca. 3 rpm in an oil bath at 80°C for 48 hours. The digesters were then removed and cooled with cold water, and the bottles were removed and allowed to cool. Methanol (8.3 mL) was added to each bottle to react with excess phenyl isocyanate. The clear dark brown solution was filtered through a glass fiber filter on a coarse, sintered-glass filter. The filtrate was slowly added from a 250 mL separatory funnel to a well agitated solution of acetic acid (5 mL) in methanol (ca. 400 mL). The precipitated white polymer was allowed to settle and the yellow supernatant was decanted. The polymer was transferred to a centrifuge bottle and washed twice with dilute acid (ca. 5 mL acetic acid in 250 mL water) and once with water (250 mL). The polymer was then freeze-dried from water.

Schroeder et al. (53) showed that carbanilation of cellulose could not be performed, as literature sources suggested (54,55), at 110°C without significant depolymerization. Nondestructive and reproducible derivatization was achieved at 80°C.

An experiment was performed to test whether amylose was more reactive and perhaps suffered degradation even at the lower derivatization temperature. The molecular weight distributions of samples of varying carbanilation time at 80°C were essentially identical, and the calculated \overline{DP}_n values were nearly constant between 10 and 62 hours (Fig. 16). Carbanilations were run for 48 hours throughout this work, but this time could probably be reduced, if necessary, to 15 hours or "overnight".

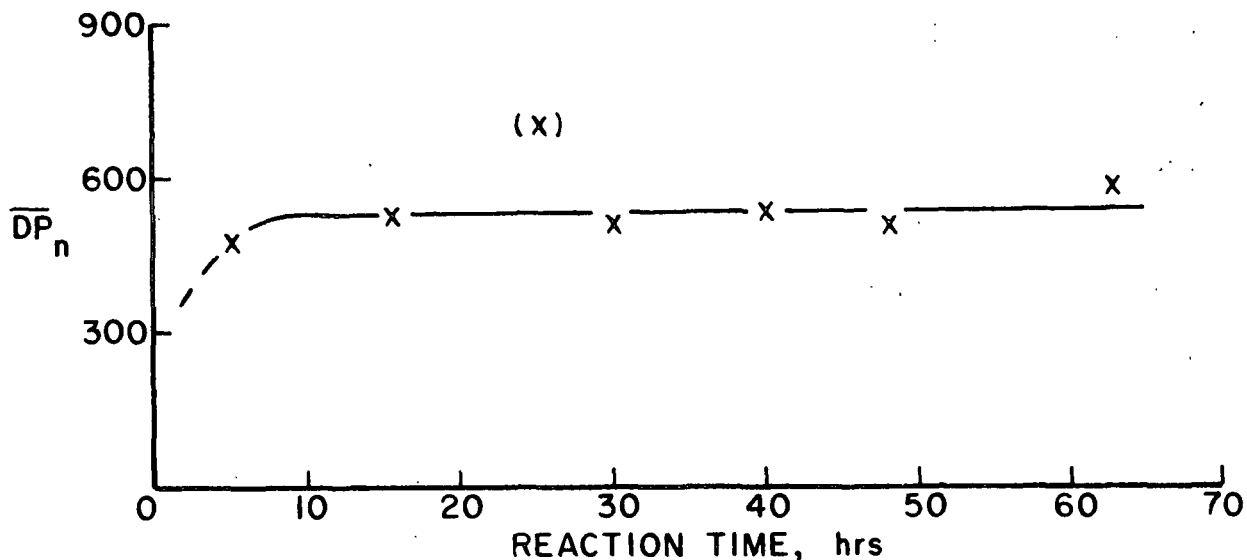


Figure 16. Apparent Number Average Degree of Polymerization of Amylose-tricarbanilate as a Function of Carbanilation Time

AMYLOSE CONCENTRATION

Four different procedures to measure the concentration of amylose in the cooking liquor were tested for their accuracy and efficiency. The results are described in Appendix V. The best overall method was the enzymatic determination of glucose, derived from the amylose after total hydrolysis. The yield data tabulated in Appendix IV are based on this method.

The method based on the formation of a blue complex between polyiodide ions and amylose (39,41) was also used initially. It agreed well with enzymatic determinations for modest degradation under anaerobic conditions. The complex formation is, however, not a singular function of amylose concentration, but is also influenced by the molecular weight distribution of the amylose and of other poorly understood factors. Thus, this method gives erroneous results for degradations of amylose in oxygen-alkali.

Enzymatic Method

Amylose hydrolysis. — Reaction solution (1.0 mL) was placed in a test tube (125 x 15 mm) and hydrochloric acid (1.0 mL; 2.2M) was added. Each tube was covered loosely with a piece of aluminum foil and placed in a rack in a pressure cooker (Sears Kenmore, 4 quart) which contained boiling water about 5 cm deep. The cooker was sealed and heated to reach equilibrium pressure and temperature (ca. 120°C). The tubes were removed from the cooker after 1 hour. The solution in each tube was brought to nearly neutral pH with a few drops of 1M sodium hydroxide. It was then transferred quantitatively to a 10-mL volumetric flask and diluted to volume with a neutral TRIS-buffer. Independent tests showed that there was no significant reversion of glucose to oligomers during hydrolysis for 30-90 minutes under these conditions.

Buffer solution. — A TRIS-buffer of pH 7.6 was prepared according to Colowick and Kaplan (56). Trishydroxymethylamino methane ("TRIS", 6.0674 g) was dissolved in triply distilled water (250 mL). This solution (62.5 mL; 0.2M) and 0.2M HCl (48 mL) were then mixed together and diluted to 250 mL with triply distilled water.

Glucose assay. — One enzyme vial (Sigma Chemical Company, St. Louis, MO; Stock no. 15-10) was opened and 31 mL of triply distilled water was added to dissolve the contents. The UV-spectrometer (Perkin-Elmer 576 ST) was balanced at 340 nm with water (3.0 mL) in both the sample and the reference cells. A sample (20.0 µL) of the hydrolysis solution was added to the water in the sample cell and the solution was mixed by gentle inversions. The absorbance (A_{blank}) was normally ca. 0.000. The cell contents were then discarded. Pure enzyme solution (3.0 mL) was then placed in the rinsed sample cell and its absorbance (A_{initial}) was recorded. This value was normally 0.01-0.03. Hydrolysis solution (20.0 µL) was then added to the enzyme solution in the cell. The absorbance

(A_{final}) of the solution was recorded at equilibrium, which normally was reached after 3-5 minutes. The equilibrium absorbance was in the range 0.2-0.6.

The relationship between absorbance and glucose concentration is described by Eq. (3):

$$\text{Glucose (mg/dL)} = \Delta A \times \frac{V_c \times M_g \times 100}{\epsilon_z \times V_s} = \Delta A \times 437.4 \quad (3)$$

where $\Delta A = A_{\text{final}} - A_{\text{initial}} - A_{\text{blank}}$

$V_c = 3.02 \text{ mL} = \text{volume of liquid in sample cell}$

$M_g = 0.18016 \text{ mg} = \text{weight of } 1 \text{ } \mu\text{mole of glucose}$

100 = conversion of mg/mL to mg/dL

$\epsilon_z = 6.22 = \text{absorbance at } 340 \text{ nm of a solution containing } 1 \text{ } \mu\text{mole of the enzyme NADPH per mL}$

and $V_s = 20.0 \text{ } \mu\text{L} = \text{volume of test specimen.}$

Further details about this method, including references, are given in the supplier manuals (57,58).

Yield curve correction. — Most amylose yield curves derived in this way extrapolated well to a zero-time yield of 100%. Due primarily to amylose sticking to the Teflon capsule surfaces and to difficulties in measuring the original dryness of the amylose accurately, however, some curves extrapolated to zero-time yields of 85-90%. In such cases a corrected yield curve was established. Each calculated yield value was divided by the apparent zero-time yield, to give a new set of data points which did extrapolate to 100%.

Iodine Complexing ("Blue Value" Method) (39,41)

This method, which was adopted from earlier studies of alkaline degradation of amylose (8,10,40), proved to be reliable only for modest depolymerization under

anaerobic conditions. Speculations about why it fails in oxygen-alkali systems are given in the Discussion section (p. 29-33).

A reaction sample (1.5 mL) was added to a 25-mL volumetric flask containing triply distilled water (ca. 15 mL) and 1M HCl (1.5 mL). The solution was diluted to volume with triply distilled water and thoroughly mixed. A sample of this solution (1.0 mL) was transferred to a 50-mL volumetric flask containing triply distilled water (ca. 30 mL). Potassium hydrogen tartrate crystals (70-100 mg), serving as a buffer (39), and polyiodide solution (0.5 mL; consisting of 2 mg/mL iodine and 20 mg/mL potassium iodide in water) were added to the solution which was then diluted to volume with water. The absorbance (A_{680}) of the blue solution was measured at 680 nm after ca. 20 minutes. The reference cell contained a yellow solution prepared as described above, with the exception of the amylose solution.

The absorbance is directly proportional to the amylose concentration as long as the absorptivity of the complex (the "Blue Value" of the amylose) is constant. This requires that the \overline{DP}_n does not fall significantly below ca. 100 (27) and that no other factors impede the formation of the complex. Eq. (4) defines "Blue Value" according to Gilbert and Spragg (39).

$$BV = \frac{4 \times A_{680}}{c} \quad (4)$$

Here BV = "Blue Value" (a measure of complex absorptivity)

A_{680} = absorbance at 680 nm

and c = amylose concentration in the cell in mg/dL.

The amylose concentrations were calculated as shown in the following sample computation. A solution of the starting amylose was prepared to simulate zero-time

cooking liquor (ca. 100 mg amylose in 10.0 mL 1.0M NaOH). This solution was analyzed as described above. The absorptivity or "Blue Value" of the amylose-iodine complex was calculated from the known amount of amylose and the absorbance, according to Eq. (4). As an example, 132.5 mg amylose per 10 mL alkali gave, after dilution, an amylose concentration in the blue solution of 1.59 mg/dL and its absorbance was measured to be 0.561. The corresponding "Blue Value" was then 1.411, and was assumed constant for the modest levels of degradation studied. Thus, the unknown concentrations in the original, undiluted reaction liquor were calculated from Eq. (4) by insertion of $BV = 1.411$ and appropriate dilution factors. An absorbance of 0.423 would, in this case, correspond to 10.0 mg/mL or an amylose yield of 100%.

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* * *

Jag känner den största tacksamhet gentemot mina föräldrar. De har alltid givit sitt helhjärtade stöd under min utbildning, trots att de aldrig gavs motsvarande tillfällen. Deras uppmuntran var särskilt värdefull då det tycktes mej att jag i stället fått alltför mycket av det goda.

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APPENDIX I

DERIVATION OF THE RATE EQUATION FOR DEPOLYMERIZATION via
RANDOM CHAIN CLEAVAGE

A good method to study the random hydrolytic cleavage of glycosidic bonds in polysaccharides involves measurement of molecular weight or degree of polymerization (6). It is a potentially very accurate method due to its sensitivity. As an example, at a chain length of 1000 units, the cleavage of one bond corresponds to an extent of reaction of only 0.1%, while the measured variable (DP) has decreased by 50% from 1000 to 500.

Assume that a polysaccharide system contains a total of $n(t)$ glycosidic bonds and A monomer units (constant) at an average DP of $P(t)$. The chain cleavage reaction is first order with respect to reactive bonds.

$$- \frac{dn}{dt} = k \times n \quad (5)$$

The number of glycosidic bonds per molecule is always one less than the DP of the molecule. Hence, for the entire system it follows that,

$$\frac{\text{number of bonds}}{\text{number of molecules}} = \frac{n}{A/P} = P - 1$$

$$n = (A/P) \times (P-1) = A - A/P$$

Substituting for n in Eq. (5):

$$- \frac{d(A-A/P)}{dt} = k \times (A-A/P)$$

Integrating by separation:

$$- \int \frac{d(A-A/P)}{A - A/P} = \int k dt$$

$$\int_{P_0}^{P_t} \ln(A - A/P) = \int_0^t k \times t$$

$$-\ln(A - A/P_t) + \ln(A - A/P_0) = kt - k \times 0$$

$$\ln A + \ln(1 - 1/P_0) - \ln A - \ln(1 - 1/P_t) = kt$$

$$\ln(1 - 1/P_0) - \ln(1 - 1/P_t) = kt \tag{6}$$

Eq. (6) is the same as Eq. (1) in the Introduction section.

In practice, P_0 and P_t are often large, i.e., the extent of reaction is modest. Then a series expansion of $\ln x$ [e.g., Eq. (7)]

$$\ln(1 + x) = \sum_n (-1)^n x \frac{1}{n} x^n = x - \frac{1}{2}x^2 + \frac{x^3}{3} - \dots \quad -1 < x \leq 1 \tag{7}$$

can be used to simplify Eq. (6) to give Eq. (8) which is identical to Eq. (2) in the Introduction section.

$$1/P_t - 1/P_0 = k \times t \tag{8}$$

APPENDIX II

EXCLUSION CHROMATOGRAPHY

INTRODUCTION

The procedure for measuring DP averages and molecular weight distributions of amylose was based on size (sometimes referred to as steric) exclusion chromatography (SEC), which is the modern high pressure version of gel permeation chromatography (GPC) (59). The principle of separation is the same for both methods. The polydisperse polymer sample is eluted in a good solvent through a chemically inert microporous column packing. There the smaller molecules are delayed more than larger ones by being able to flow with the solvent into a greater portion of the pores.

The theory behind the technique is thoroughly described in recent monographs (59-61) and journals (62,63).

CALIBRATION OF COLUMNS

Well characterized, monodisperse samples of amylose tricarbanilate of different molecular weights are difficult to obtain. Therefore, the columns were calibrated according to the universal calibration concept (53,60,63). This method is based on an assumption, which is valid for many polymers (60), that the elution time or volume is directly related to the hydrodynamic volume of the polymeric species in solution. Thus, under the same experimental conditions, all polymers having the same hydrodynamic volume will be eluted with the same velocity, i.e., they will have the same elution volume.

The hydrodynamic volume of the polymer is proportional to the product of its intrinsic viscosity $[\eta]$ and its molecular weight, M . The Mark-Houwink equation

[Eq. (9)] relates the intrinsic viscosity to the molecular weight.

$$[\eta] = K \times M^{\alpha} \quad (9)$$

The constants K and α are characteristic for each polymer-solvent combination. Multiplying Eq. (9) by the polymer's molecular weight yields Eq. (10), which expresses the polymer's hydrodynamic volume

$$[\eta] \times M = K \times M^{(\alpha + 1)} \quad (10)$$

Thus, exclusion chromatograms obtained for amylose tricarbanilate (ATC) can be analyzed on the basis of a column calibration made with another polymer, e.g., polystyrene (PS), through Eq. (11),

$$K_{ATC} \times M_{ATC}^{(1 + \alpha_{ATC})} = K_{PS} \times M_{PS}^{(1 + \alpha_{PS})} \quad (11)$$

which relates molecular weights of the same hydrodynamic volume.

A number of commercial, nearly monodisperse polystyrene standards were analyzed. The elution volume (i) at the apex of each peak was assumed to be representative of the nominal molecular weight of the particular standard ($M_{i,PS}$). All elution volumes were measured relative to the internal reference, methyl N-phenyl carbamate. This yielded the calibration curve shown in Fig. 17. A regression line was calculated for the linear interval of the calibration curve and the molecular weight of polystyrene associated with each discrete mL of relative elution volume (i) was interpolated. This data was read into the computer memory from a permanent data file [LCCAL2(F49), p. 74], before each computer analysis of chromatograms.

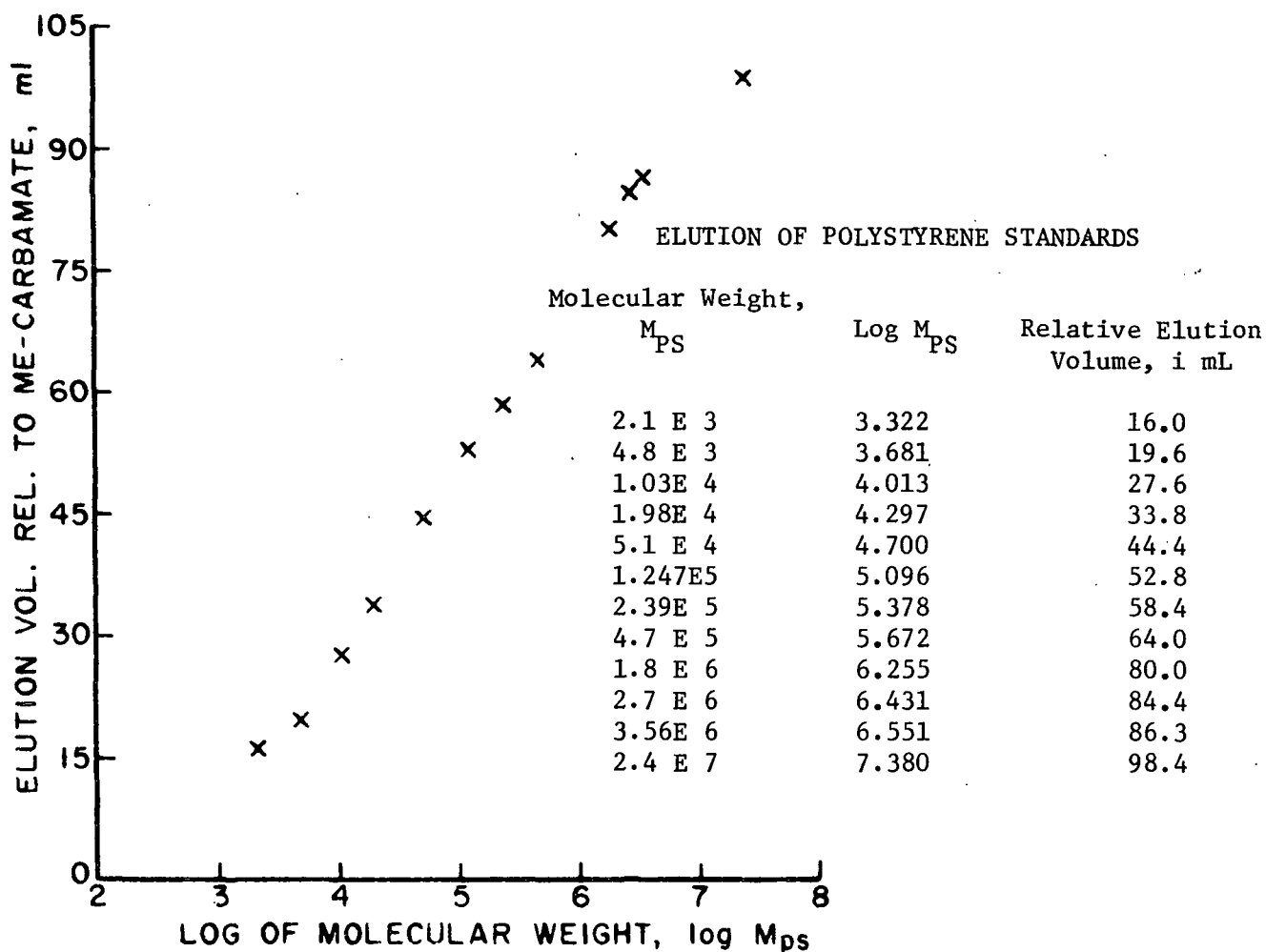


Figure 17. Relative Elution Volume as a Function of Log Molecular Weight of the Polystyrene Standards

Using the polystyrene data, the Mark-Houwink constants and Eq. (11), the computer first calculated a new calibration curve, which applied to amylose tricarbanilate polymers. This transformation [Eq. (12)] requires that the K and

$$M_{i,ATC} = (K_{PS}/K_{ATC})^{1/(\alpha_{ATC} + 1)} \times M_{i,PS}^{(\alpha_{PS} + 1)/(\alpha_{ATC} + 1)} \quad (12)$$

α constants are known for both polystyrene and amylose carbanilate in the particular solvent used, which in this work was tetrahydrofuran (THF). The new calibration curve was stored in the computer in the form of pairs of numbers: molecular weight (M_i) and relative elution volume (i). This table was used to calculate the number, viscosity, and weight averages for entire distributions according to their respective definitions [Eq. (13-15)],

$$\bar{M}_n = \sum h_i / \sum (h_i / M_i) \quad (13)$$

$$\bar{M}_v = (\sum (h_i \times M_i^\alpha) / \sum h_i)^{1/\alpha} \quad (14)$$

$$\bar{M}_w = \sum (h_i \times M_i) / \sum h_i \quad (15)$$

where h_i is the ordinate height of the chromatogram above the baseline measured at the relative elution volume i . Increments between measured heights were 2.0 mL throughout this work.

MARK-HOUWINK CONSTANTS

The values of the K and α constants for amylose tricarbanilate in tetrahydrofuran were not available in the literature and attempts to measure them at another laboratory were fruitless (66). Published data (Table III) show that the constants do not vary greatly between other good solvents and, therefore, the dioxane values were used. A recent investigation of the behavior of amylose tricarbanilates in dioxane (64) indicated that the Mark-Houwink constants are difficult to measure and are not accurately known. Interestingly, it also showed that the Mark-Houwink Eq. (9) does not apply strictly in the molecular weight region below DP 150-200 (Fig. 18). Thus, K and α are really functions of M_i and the assumption that they are true constants will introduce a small systematic error in all distributions which have a significant portion in the lower molecular weight range. Most of the amylose samples analyzed in this work belonged to this category.

TABLE III

THE MARK-HOUWINK CONSTANTS FOR AMYLOSE
TRICARBANILATE IN VARIOUS SOLVENTS

| Solvent | K, cm ³ /g | α | Source |
|----------|-----------------------|----------|--------|
| Dioxane | 2.36×10^{-3} | 0.88 | (64) |
| Dioxane | 9.06×10^{-4} | 0.92 | (65) |
| Pyridine | 5.89×10^{-4} | 0.92 | (65) |
| Acetone | 8.14×10^{-4} | 0.90 | (65) |

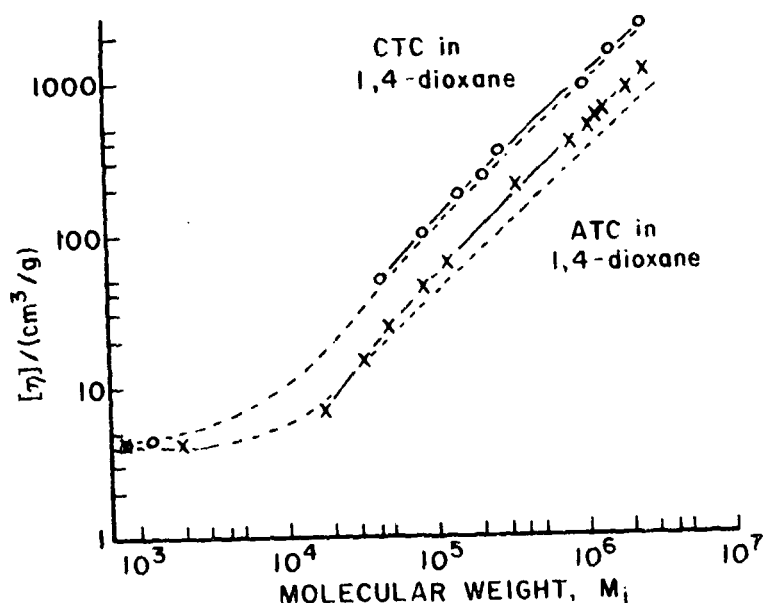


Figure 18. Intrinsic Viscosity of Cellulose (CTC) and Amylose Tricarbanilate (ATC) as Functions of Molecular Weight (64).

In conclusion, the number average DP values calculated from the molecular weight distributions in this work are of the correct magnitude, although they are based on perhaps slightly incorrect Mark-Houwink constants. It should be noted that the discussion of experimental results is based on differences or ratios between \overline{DP}_n values rather than on absolute numbers and this tends to negate the influence of a constant deviation due to a systematic error in the universal calibration of the columns.

EQUIPMENT AND OPERATION

The chromatographic analysis of amylose tricarbanilates was performed according to a procedure developed at The Institute of Paper Chemistry (53). A schematic of the equipment used is shown in Fig. 19. A Waters Associates 6000 A solvent delivery system pumped the tetrahydrofuran solvent from a reservoir via a sample injection valve to a set of five Styragel columns (Waters Associates, 7.8 mm ID x (61 + 61) cm). There was one column each of the permeabilities 10^3A , 10^4A , 10^5A , 10^6A , and 10^7A . A Perkin-Elmer LC-55 B spectrophotometer equipped with a flow cell and operated at 235 nm served as the detector. The measured absorbance was registered continuously on a Perkin-Elmer 023 recorder. The system was located in a room with controlled conditions at 73°F (23°C).

OPERATION

The amylose tricarbanilate sample (ca. 20 mg) was dissolved in distilled tetrahydrofuran (8-13 mL) to give solutions in the concentration range 0.1-0.25% wt./vol. Samples with lower polydispersity required a lower concentration for the chromatogram not to go off scale on the recorder. The reference compound, methyl N-phenyl carbamate (p. 42-43) (ca. 75 μ L of a solution prepared from 110.5 mg dissolved in 10 mL tetrahydrofuran), was added to each carbanilate solution. Most samples already contained some of the carbamate, which was formed when the excess phenyl isocyanate had been quenched with methanol during the derivatization process (p. 45-47).

The injected sample volume was 200 μ L and each chromatogram was eluted for about 2 hours at a flow rate of 2.0 mL/min. During every run, the column system was monitored for leaks by collecting the eluent in a graduated cylinder and comparing the volume of eluted solvent to that predicted from the elapsed time.

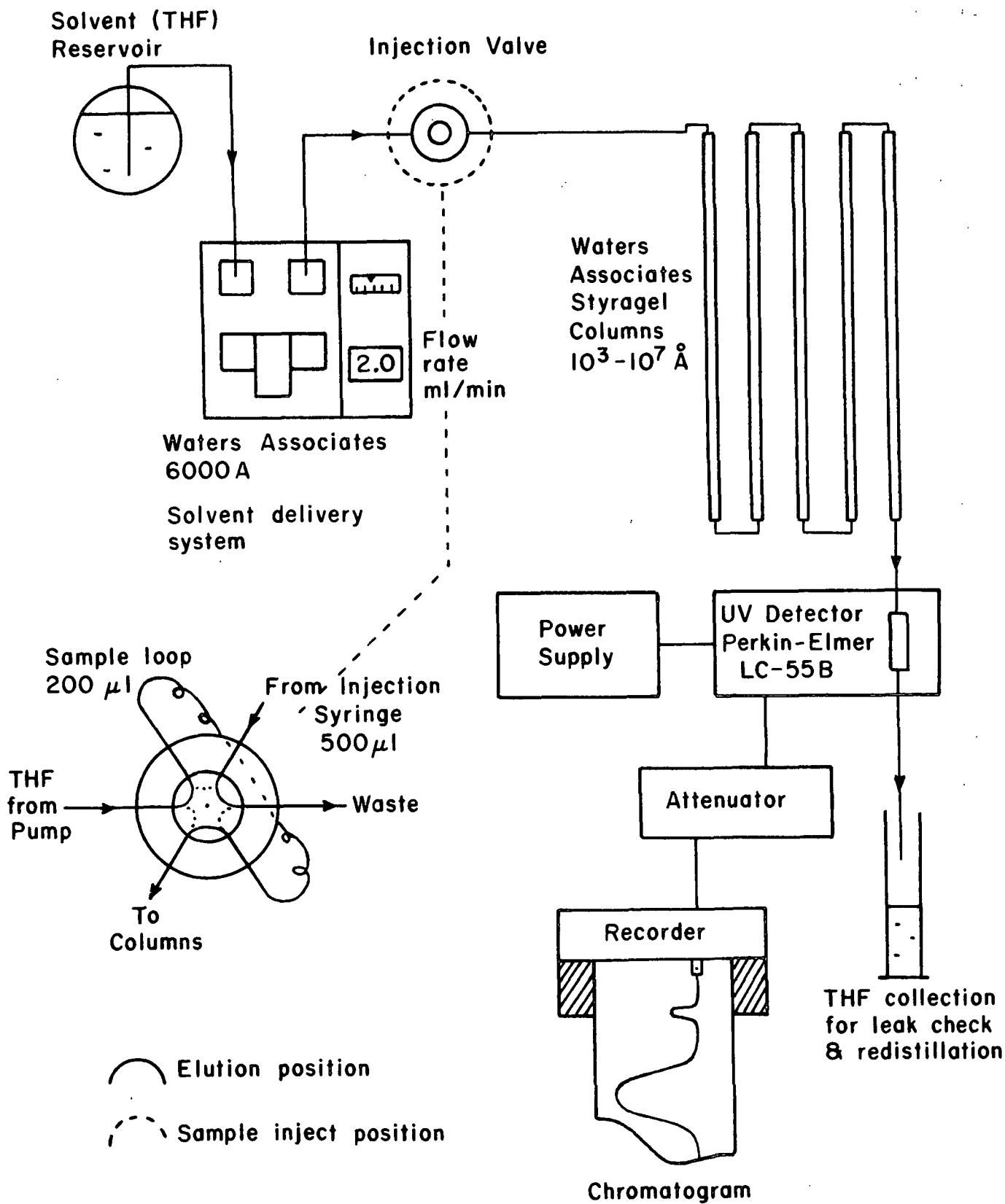
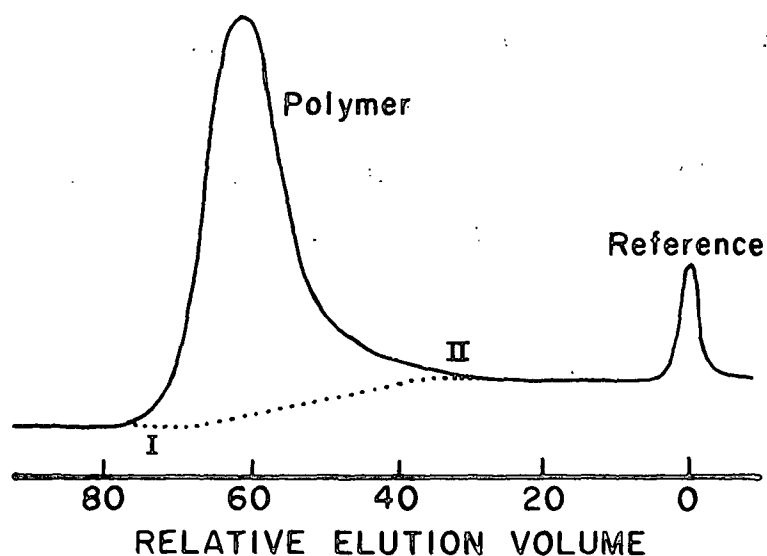


Figure 19. Schematic Diagram of the Exclusion Chromatography System Used to Measure Molecular Weight Distributions

Usually the two agreed to within 2% and leaks were rare. The eluent was recovered and purified by refluxing it with lithium aluminum hydride and then fractionally distilling it through a 40 cm column packed with 5 mm Raschig rings.

ANALYSIS OF CHROMATOGRAMS

The chromatograms were measured manually and entered in digital form into the computer. The elution volumes of the polymer peak were counted relative to the carbamate reference peak. First a base line was established by extending the horizontal portions of the curve at I and II below, for a short distance (ca. 6 mL).



These horizontal lines were then connected to give the sigmoidal base line (.....), in an attempt to give proper emphasis to both the high and the low ends of the distribution. The base line drift was normally small and is exaggerated in the figure above for the sake of clarity. The height of the distribution curve in mm above the base line was then measured at increments of 2.0 mL relative elution volume, i.e., either odd or even integer volumes. One zero height was reported

each for the high and the low end of the distribution. The relative elution volumes associated with these zeroes were entered into the computer as MAXV and MINV.

COMPUTER PROGRAMS

The computer programs which were developed to perform the analysis of the exclusion chromatograms are listed on pages 71-73. The flow chart in Fig. 20 outlines the main steps in the computations.

First a column calibration curve is calculated based on the universal calibration concept. With known Mark-Houwink constants, the program is very flexible and can directly be applied to any polymer which is chemically compatible with the system. The program then reads one chromatogram at a time and computes its molecular weight averages and their ratios. The program also normalizes the curve, by reassigning ordinate heights on a relative basis to make their sum equal unity, and, if requested, plots it in this form. Examples of a general input data file and output of results are listed on pages 74-76.

Band broadening, due to longitudinal diffusion in the columns, was neglected in the chromatogram analysis for two reasons. Mathematical corrections are extremely complex and have been shown not to be necessary for practical work with polymers of a polydispersity greater than 2 (60) which are analyzed on modern high efficiency columns.

Computer analyses of the chromatograms were run in batch mode from a card reader. The digital form of each chromatogram was punched on cards which were placed in the /DATA section of the deck. A typical card deck for a run on the Institute's computer system is shown in Fig. 21. Following each set of chromatogram cards was a plotter control card containing the control variables KPLO3,

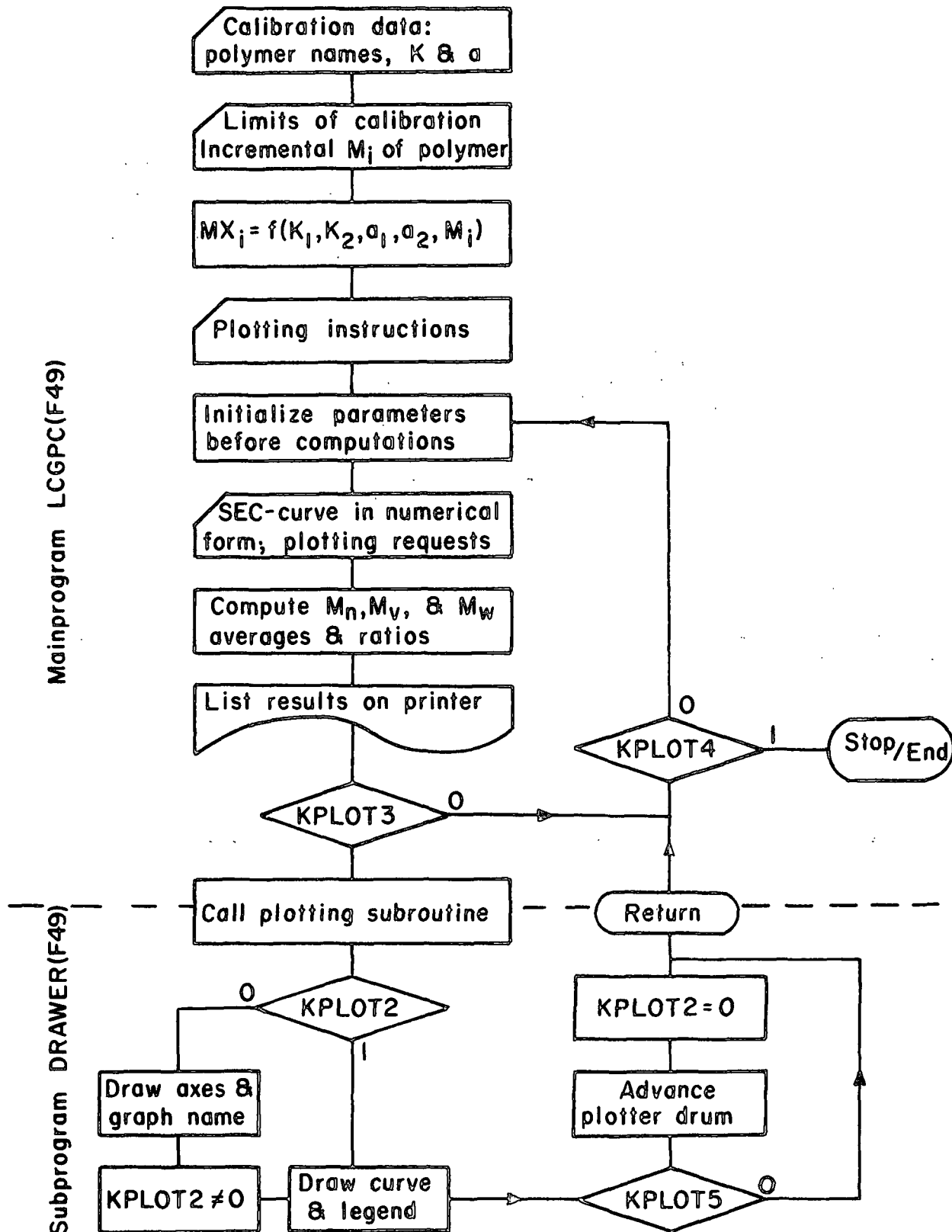


Figure 20. Flow Chart for the Computer Analysis of Exclusion Chromatograms

KPLOT4, and KPLOT5. Their use is explained in the flow chart (Fig. 20) and in the program listing (p. 72). Blank cards were used as plotter control cards to generate a complete printout of results without plots of normalized curves.

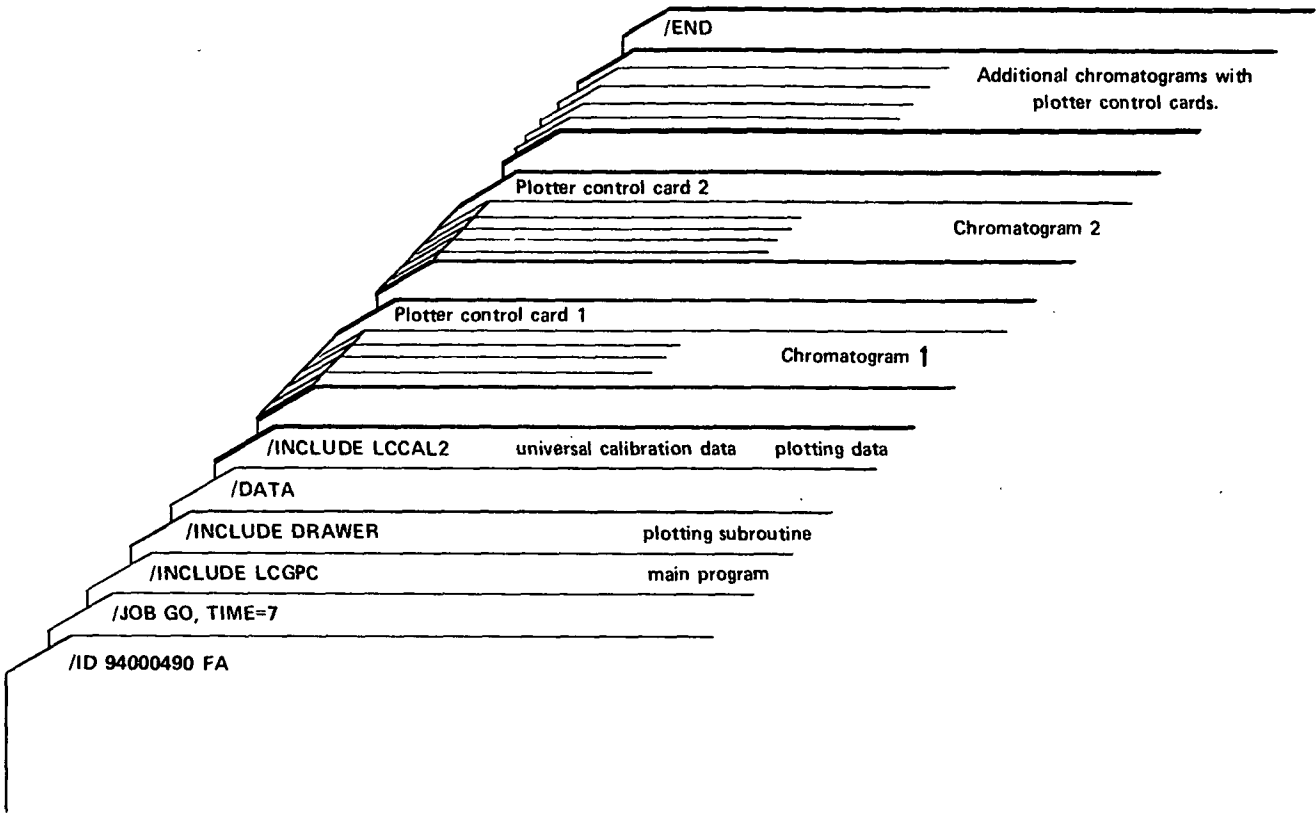


Figure 21. A Card Deck for Computer Analysis of Exclusion Chromatograms

Main Program LCGPC(F49)

```

C      MOLECULAR WEIGHT DISTRIBUTIONS.
C      *****
C      THIS IS PROGRAM LCGPC(F49)
C      FIRST SECTION DEALS WITH A UNIVERSAL CALIBRATION OF THE GPC-COLUMNS.
C      POLCAL IS THE CALIBRATION POLYMER (COMMONLY POLYSTYRENE) AND
C      POLYX IS THE POLYMER UNDER STUDY. FOR ALL ELUTION VOLUMES,
C      (BETWEEN VLO AND VHI) THE MOLECULAR WEIGHT OF POLCAL IS READ IN
C      AND THE CORRESPONDING FIGURE FOR POLYX IS COMPUTED ACCORDING TO THE
C      MARK-HOUWINK-SAKURADA EQUATION.
C
C      DIMENSION MPS(130), MX(130), POLYX(7), SAMPLE(14), Y(130),
C      *YP(60), X2(60), POLCAL(3), R(3,3), MRES(3), DP(3), HEAD(10),
C      *NAMY(10), NAMX(10), KAR(16)
C      INTEGER VLJ, VHI, Y
C      REAL KPS, KX, MPS, MX, MWMONO, MRES
C
C      READ IN MARK-HOUWINK DATA.
C
C      READ (5,1) POLYX, MWMONO, POLCAL
C      1 FORMAT (7A4,F8.2,3A4)
C      READ (5,2) KPS,APS
C      READ (5,2) KX,AX
C      2 FORMAT (2E10.3)
C      WRITE (6,3) POLCAL, KPS, APS, POLYX, KX, AX
C      3 FORMAT (///,*****VALUES OF MARK-HOUWINK CONSTANTS*****,//,°FOR°
C      °, 3A4,16X, ° K =°, ° 1PE10.3,° AND ALFA = °,OPF07.3,//
C      °,°FOR°, 7A4, ° K =°,1PE10.3,° AND ALFA = °,
C      °OPF07.3,///)
C
C      VLO AND VHI ARE THE REL. ELUTION VOLUMES BETWEEN WHICH STANDARD POLY-
C      STYRENES HAVE BEEN ELUTED.
C
C      READ (5,11) VLJ, VHI
C      11 FORMAT (10I7)
C      READ (5,4) (MPS(J),J=VLO,VHI)
C      4 FORMAT (10E7.2)
C      C1 = ( KPS/KX ) ** ( 1. / ( AX + 1. ) )
C      C2 = ( APS + 1. ) / ( AX + 1. )
C
C      OPTIONAL LISTING OF CALIBRATION DATA.
C
C      WRITE (6,5) POLCAL, POLYX
C      5 FORMAT (°1°,1X,°REL. ELUTION VOL., ML°, 2X,°MOL. WEIGHT OF °
C      °, 3A4, ° 2X,°MOL. WEIGHT OF°,7A4,03X,° = DP°,07X,
C      °LV M°,/)
C      DO 100 J=VLJ,VHI
C      MX(J) = C1 * MPS(J) ** C2
C      DPJ = MX(J) / MWMONO
C      ALNJ = ALOG(MX(J))
C      WRITE (6,6) J,MPS(J), MX(J), DPJ, ALNJ
C      6 FORMAT (113,12X,1PE15.4,15X,1PE15.4,26X,OPF7.0,01X,F14.5)
C      100 CONTINUE
C
C      THIS SECTION MAKES DATA AVAILABLE FOR SUBSEQUENT PLOTTING.
C      NAMY, NAMX - ARE THE HEADINGS TO BE PRINTED ON THE Y- AND X-AXES.
C      HEAD - IS THE NAME OF THE GRAPH.
C      XLO, XHI, YLO, YHI - ARE THE LIMITING VALUES OF THE AXES.
C      KAR(J) - A SET OF NUMBERS FOR PLOTTING CHARACTER CONTROL.
C
C      READ (5,16) NAMY,NAMX,HEAD
C      16 FORMAT (10A4)
C      READ (5,4) XLO, XHI, YLO, YHI
C      READ (5,11) (KAR(J),J=1,16)
C      KPLOT2 = 0
C
C      THIS SECTION ANALYZES ONE LC-GPC CURVE AT A TIME AND COMPUTES THE
C      DIFFERENT MOLECULAR WEIGHT AVERAGES.
C
C      88 SH = 0.
C      SHM = 0.
C      SHMA = 0.
C      SHOM = 0.
C      RCHK = 0
C      YPCHK = 0.
C      READ (5,8) SAMPLE
C      8 FORMAT (14A4)
C      WRITE (6,12) SAMPLE
C      12 FORMAT (°1°,14A4,//,5X,°LIMITS OF ELUTION VOLUME, ML.°)
C
C      MINV AND MAXV ARE THE ELUTION VOLUMES ASSOCIATED WITH THE LOWEST
C      HEIGHTS ON BOTH SIDES OF THE PEAK. THEY MAY OR MAY NOT BE ZERO.
C      Y(J) IS THE HEIGHT OF THE LC-CURVE IN MM AT REL. ELUTION VOLUME J.

```

(continued)

(continued)

```

C
C MINV AND MAXV ARE THE ELUTION VOLUMES ASSOCIATED WITH THE LOWEST
C HEIGHTS ON BOTH SIDES OF THE PEAK. THEY MAY OR MAY NOT BE ZERO.
C Y(J) IS THE HEIGHT OF THE GPC-CURVE IN MM AT REL. ELUTION VOLUME J.
C
  READ (5,11) MINV, MAXV
  WRITE (6,18) MINV,MAXV
  18 FORMAT ('+',35X,I4,' AND ',I4,'//,4X,' HEIGHTS OF GPC-CURVE',/)
  READ (5,11) (Y(J),J=MINV,MAXV,2)
  WRITE (6,11) (Y(J),J=MINV,MAXV,2)
  READ (5,11) KPLJ3, KPLJ4, KPLJ5
C
C *****PLOTTING CONTROLS***** (ONE CARD AFTER EACH CHROM.)
C KPLJ3 - 0 IF PLOTTING IS NOT DESIRED, 1 IF PLOT IS REQUESTED.
C KPLJ4 - 0 IF MORE DATASETS ARE FOLLOWING, 1 IF THIS SET IS THE LAST.
C KPLJ5 - 0 IF MORE CURVES WILL GO INTO THIS PLOT, 1 IF THIS IS THE
C          LAST CURVE TO BE DRAWN IN THE CURRENT COORDINATE SYSTEM.
  DO 110 I=MINV,MAXV,2
  SH = SH + FLJAT(Y(I))
  SHM = SHM + FLJAT(Y(I)) * MX(I)
  SHMA = SHMA + FLJAT(Y(I)) * MX(I) ** AX
  SHOM = SHOM + FLJAT(Y(I)) / MX(I)
  110 CONTINUE
C
C DO-LOOP 120 NORMALIZES THE CURVE FOR SUBSEQUENT PLOTTING.
C
  N = (MAXV - MINV) / 2 + 1
  I = MINV
  DO 120 J=1,N
  XP(J) = ALJG10(MX(I))
  XP(J) = MX(I) / MWMQND
  YP(J) = (FLJAT(Y(I)) / SH) * (1.E6/MX(I))
  YP(J) = FLJAT(Y(I)) / SH
C THIS SECTION COMPUTES THE DP ASSOCIATED WITH PEAK MAXIMUMJM (DPMAX).
  IF(KCHK) 51,51,52
  51 ZP = (YP(J) - YPCHK)
  IF(ZP) 53,52,52
  53 KCHK = 1
  DPMAX = MX(I-2) / MWMQND
  52 YPCHK = YP(J)
C
C OPTIONAL LISTING OF CURVE POINTS.
C
  WRITE (6,2) YP(J), XP(J)
  I = I + 2
  120 CONTINUE
C
C COMPUTATION OF MOLECULAR WEIGHTS. 1 - NUMBER, 2 - VISCOSITY, 3 - WEIGHT
C THE MATRIX R CONTAINS THE POLYDISPERSITY RATIOS AND INVERSES.
C
  MRES(1)=SH/SHOM
  MRES(2)=(SHMA/SH)**(1./AX)
  MRES(3)=SHM/SH
  DO 130 K=1,3
  DP(K) = MRES(K) / MWMQND
  DO 130 L=1,3
  R(K,L) = MRES(L) / MRES(K)
  130 CONTINUE
C
C LISTING OF RESULTS.
C
  WRITE (6,13) SH,SHM, SHMA, SHOM
  13 FORMAT ('//, 'SUM H(I) = ',11X,1PE10.4,'//, 'SUM H(I) * M(I) = ',
  *6X,1PE10.4,'//, 'SUM H(I) * M(I) ** AX = ',1PE10.4,'//,
  *'SUM H(I) / M(I) = ',06X,1PE10.4)
  WRITE (6,17) DPMAX
  17 FORMAT (' DP AT PEAK MAX. =',07X,F5.0)
  WRITE (6,15)
  15 FORMAT ('//,31X,'NUMBER', 07X,' VISCOSITY', 07X,' WEIGHT',
  */,31X,'*****', 07X,' *****', 07X,' *****',/)
  WRITE (6,14) MRES, DP, R
  14 FORMAT ('MOLECULAR WEIGHT AVERAGE ',02X,03(1PE12.4,3X),//
  * ' DEGREE OF POLYMERIZATION ',03(OPF09.0,6X),
  *//, 42X,'**MW-RATIOS**', /, 'NUMBER AVE. VS. ',13X,
  *03(F09.4,6X)//, ' VISCOSITY AVE. VS. ',09X,03(F09.4,6X)//,
  * ' WEIGHT AVE. VS. ',12X,03(F09.4,6X),//)
  IF(KPLJ3) 44,45,44
C
C PELLE - IS A SUBROUTINE (IN DRAWER(F49)) WHICH HANDLES THE
C PLOTTING.
C
  44 CALL PELLE(XP, YP, N,XLO,XHI,YLO,YHI,NAMX,NAMY,HEAD,SAMPLE,
  *KPLJ2,KPLJ3,KPLJ4)
  45 IF(KPLJ4) 55,88,55
  55 CALL EXIT
  END

```

Subprogram DRAWER(F49)

CFTC LIST

C SUBROUTINE PELLE HANDLES THE CALCOMP-PLOTTING OF DATA FROM MAIN
C PROGRAM LCGPC(F49) FOR ANALYSIS OF EXCLUSION CHROMATOGRAPHY CURVES.

C SUBROUTINE PELLE(XP, YP, N, XLO, XHI, YLO, YHI, NAMX, NAME, HEAD,
*SAMPLE, KPLJT2, KPLDT5, KAR)
C DIMENSION NAME(10), NAMX(10), HEAD(10), XP(60), YP(60),
*SAMPLE(14), KAR(16)

C XLEN AND YLEN ARE THE LENGTHS OF THE AXES IN THE PLOT.

C XLEN = 10.0
C YLEN = 10.0
C DELX = (XHI-XLO)/XLEN
C DELY = (YHI-YLO)/YLEN

C POINTS N+1 AND N+2 MUST BE DEFINED IN THIS WAY TO MAKE SUBR. LINE RUN.

C XP(N+1) = XLO
C XP(N+2) = DELX
C YP(N+1) = YLO
C YP(N+2) = DELY

C KPLDT2 = 0 CALLS FOR DRAWING OF AXES.

C IF(KPLDT2) 90, 91, 90
91 CALL ITLZ
CALL DPT(1, 4)
CALL PLOT(0., -11., 3)
CALL PLOT(0., -10.5, -3)
CALL AXIS(0., 0., NAME, 40, YLEN, 90., YLO, DELY)
CALL AXIS(0., 0., NAMX, -40, XLEN, 0., XLO, DELX)

C YLA, YLH AND YLB POSITION THE LEGEND ON THE PLOT.

C YLA = YLEN - 1.5
C YLH = YLA + 0.5
CALL SYMBOL(0.6, YLH, 0.14, HEAD, 0., 30)
90 KPLDT1 = KPLDT2 + 1
C YLA = YLA - 0.25
C YLB = YLA + 0.03
CALL LINE(XP, YP, N, 1, 5, KPLDT1)
CALL DRAW(0.9, YLB, 0., KAR(KPLDT1), 1., 0., 0.42)
CALL SYMBOL(1.1, YLA, 0.14, SAMPLE, 0., 30)
CALL PLOT(0., 0., 3)
KPLDT2 = KPLDT2 + 1
IF(KPLDT5) 94, 93, 94
94 CALL PLOT(9.5, 0., -3)
KPLDT2 = 0
CALL FINAL
93 RETURN
END

General input data file ICCAL2(F49).

AMYLOSE-TRI-CARBANILATE 519.00 POLYSTYRENE
1.1790E-2 0.74E00
0.9060E-3 0.92E00
10 130
1.854E32.047E32.260E32.495E32.755E33.042E33.358E33.708E34.093E34.519E3
4.990E35.509E36.082E36.715E37.413E38.185E39.036E39.977E31.101E41.216E4
1.343E41.482E41.637E41.807E41.995E42.202E42.432E42.685E42.964E43.272E4
3.613E43.989E44.404E44.862E45.368E45.926E46.543E47.224E47.976E48.805E4
9.722E41.073E51.185E51.308E51.444E51.595E51.761E51.944E52.146E52.369E5
2.616E52.888E53.189E53.520E53.887E54.291E54.738E55.231E55.775E56.376E5
7.039E57.772E58.580E59.473E51.046E61.155E61.275E61.408E61.554E61.716E6
1.894E62.091E62.309E62.549E62.814E63.107E63.430E63.787E64.182E64.617E6
5.097E65.627E66.213E66.859E67.573E68.361E69.231E61.019E71.125E71.242E7
1.372E71.514E71.672E71.846E72.038E72.250E72.484E72.742E73.028E73.343E7
3.691E74.075E74.499E74.967E75.483E76.054E76.684E77.379E78.147E78.995E7
9.931E71.096E81.211E81.336E81.476E81.629E81.799E81.986E82.192E82.420E8
2.672E8
RELATIVE AMOUNT OF POLYMER
LOG. OF MOLECULAR WEIGHT
MOLECULAR WEIGHT DISTRIBUTION
3.50E0 7.50E0 0.00E0 0.12E0
170 183 175 176 181 182 185 171 172 173
174 177 178 179 180 184

Example of results output for one chromatogram.

05-2 790310

LIMITS OF ELUTION VOLUME, ML. 20 AND 88

HEIGHTS OF GPC-CURVE

| | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1 | 1 | 2 | 4 | 5 | 8 | 12 | 19 | 31 | 51 |
| 78 | 118 | 158 | 196 | 221 | 225 | 214 | 189 | 155 | 119 |
| 84 | 56 | 36 | 21 | 13 | 9 | 7 | 5 | 4 | 4 |
| 3 | 2 | 2 | 1 | 0 | | | | | |

SUM H(I) = 2.0540E 03
 SUM H(I) * M(I) = 4.7321E 08
 SUM H(I) * M(I) ** AX = 1.7135E 08
 SUM H(I) / M(I) = 1.6227E-02
 DP AT PEAK MAX. = 312.

| | NUMBER ***** | VISCOSITY ***** | WEIGHT ***** |
|--------------------------|-----------------|--------------------|-----------------|
| MOLECULAR WEIGHT AVERAGE | 1.2658E 05 | 2.2348E 05 | 2.3039E 05 |
| DEGREE OF POLYMERIZATION | 244. | 431. | 444. |

MW-RATIOS

| | | | |
|--------------------|--------|--------|--------|
| NUMBER AVE. VS. | 1.0000 | 0.5664 | 0.5494 |
| VISCOSITY AVE. VS. | 1.7655 | 1.0000 | 0.9700 |
| WEIGHT AVE. VS. | 1.8201 | 1.0309 | 1.0000 |

APPENDIX III
THE REACTOR SYSTEM

The degradations were performed in a reactor which was a modification of a design used in several previous investigations at The Institute of Paper Chemistry (14,32,67). An extensive description of the reactor and its accessories is given by Sinkey (32).

The key features of the original reactor design were retained: all Teflon surfaces, devices for sampling during the reaction, and internal magnetic stirring. In addition, the reactor was equipped with a chromel-constantan thermocouple in a Teflon sleeve and an internal Teflon capsule to keep the reagents separated during the heat-up period. Crozier (67) described a similar injection device in great detail. The sampling line contained a loop which was kept in an ice bath to provide initial quenching of the sample.

The reactor is shown in Fig. 22. The cover assembly is shown in more detail in Fig. 23. The Teflon capsule to hold the amylose was screwed into the socket of the gas pressurizing line. A small hinge of Teflon tubing kept the cap of the capsule from falling into the reactor and interfering with the stirring magnet. The internal volume of the capsule was 15.7 mL.

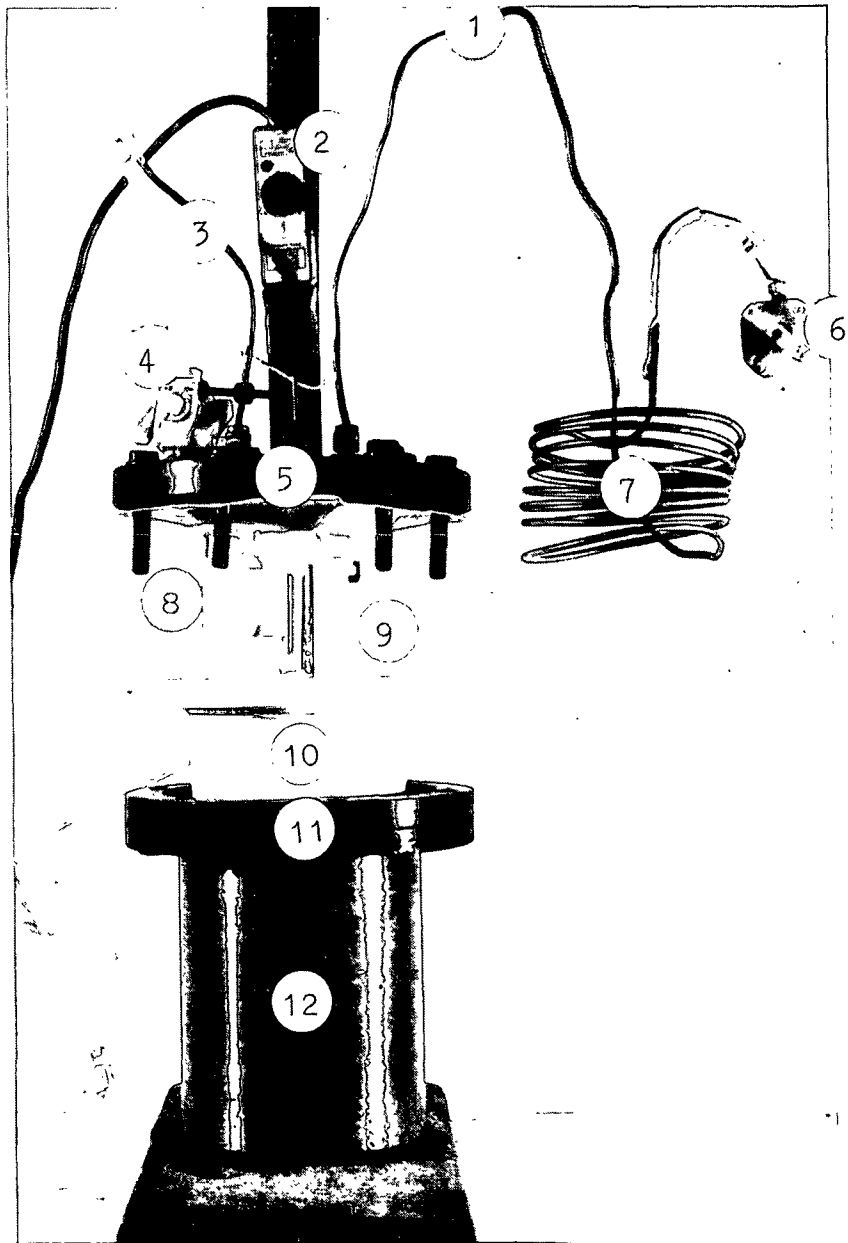


Figure 22. The Reactor

- | | |
|---|---|
| 1. Sampling line | 7. Cooling coil for quenching of sample |
| 2. Connector and electronic zero point reference for thermocouple | 8. Teflon capsule for amylose |
| 3. Pressurizing line | 9. Sampling outlet |
| 4. Pressurizing line valve | 10. Teflon insert |
| 5. Reactor cover | 11. Brass sleeve |
| 6. Sampling line valve | 12. Stainless steel autoclave |

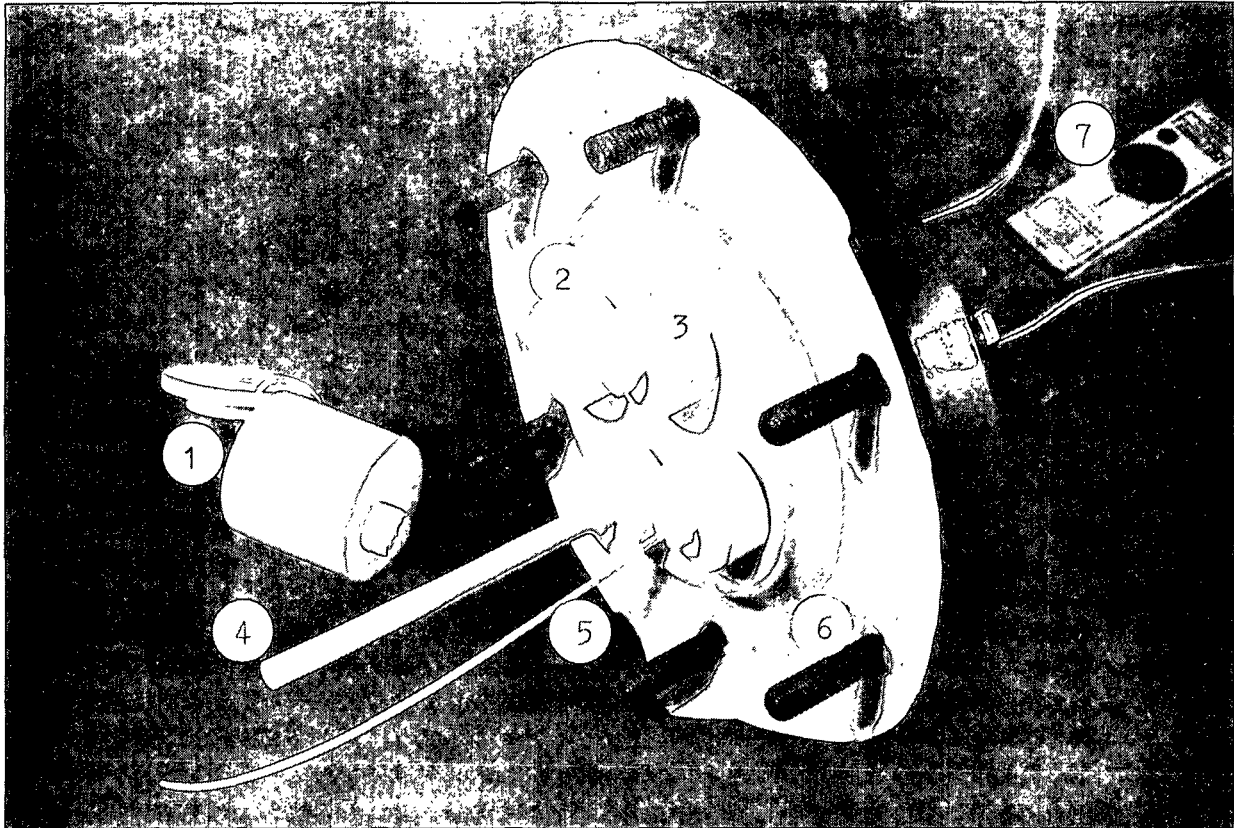


Figure 23. The Reactor Cover Assembly

1. Teflon capsule with cap
2. Pressurizing line inlet with socket for capsule
3. Extra inlet/outlet port
4. Chromel-constantan thermocouple in Teflon sleeve
5. Sampling line
6. Teflon gasket
7. Connector and electronic zero point reference for thermocouple

APPENDIX IV
TABULATED DATA

TABLE IV

AMYLOSE YIELDS IN PERCENT DURING ALKALINE DEGRADATION UNDER NITROGEN

Determinations made according to the enzymatic method (p. 49)

| Reaction Time, hours | Reaction Identifier and Temperature | |
|-------------------------|-------------------------------------|---|
| | N80, 80°C | N100, 100°C |
| 0.1 | 71.6 | -- ^b (-- ^b) ^a |
| 0.25 | 90.0 | 86.6 (82.4) |
| 0.5 | 91.1 | 74.9 (73.1) |
| 0.75 | -- ^b | 62.4 (62.1) |
| 1.00 | 84.4 | 52.0 (52.7) |
| 1.5 | 77.4 | 40.7 (39.5) |
| 2.00 | 73.8 | 36.2 (37.0) |
| 2.5 | 76.5 | 35.5 (-- ^b) |
| 3.0 | 65.2 | 38.3 (36.1) |
| 4.0 | 59.7 | 36.4 (36.1) |
| 5.0 | 56.8 | 36.4 (-- ^b) |
| 6.0 | 54.5 | 38.0 (-- ^b) |
| 7.0 | 53.4 | 37.7 (37.4) |

^aYields in parenthesis were determined by the iodine-complexing method (p. 50-51).

^bNot determined.

APPENDIX IV (continued)

TABLE V

AMYLOSE YIELDS IN PERCENT DURING ALKALINE DEGRADATION UNDER OXYGEN

Determinations made according to the enzymatic method (p. 49)

| Reaction time, hours | Reaction Identifier and Temperature | |
|-------------------------|-------------------------------------|------------------|
| | 080, 80°C | 0100, 100°C |
| 0.1 | 102.0 | --- ^a |
| 0.25 | 103.2 | 100.8; 97.7 |
| 0.5 | 100.4 | 95.1 |
| 0.75 | --- ^a | 98.6 |
| 1.0 | 101.1 | 95.0; 98.2; 97.5 |
| 1.5 | 99.3 | 95.6 |
| 2.0 | 98.7 | 95.0 |
| 2.5 | 100.2 | 92.7 |
| 3.0 | 97.8 | 90.2 |
| 3.5 | 100.5 | --- ^a |
| 4.0 | 99.1 | 85.2 |
| 5.0 | 99.5 | 83.7 |

^aNot determined.

APPENDIX IV (continued)

TABLE VI

AMYLOSE YIELDS IN PERCENT DURING ALKALINE DEGRADATION AT 100°C
IN THE PRESENCE OF ANTHRAQUINONE

Determinations made according to the enzymatic method (p. 49)

| Reaction time, hours | Reaction Identifier and Atmosphere | |
|-------------------------|------------------------------------|------------------|
| | N-AQ-100; Nitrogen | O-AQ-100; Oxygen |
| 0.25 | 90.1 | 100.1 |
| 0.5 | 98.9 | 98.0 |
| 1.0 | 99.9 | 96.9 |
| 1.5 | 93.6 | 96.2 |
| 2.0 | 94.3 | 93.1 |
| 3.0 | 94.3 | 91.1 |
| 3.5 | 91.7 | -- ^a |
| 4.0 | 94.1 | 87.5 |
| 5.0 | 88.2 | 85.6 |
| 6.0 | 83.1 | -- ^a |
| 7.0 | 82.0 | 80.5 |
| 8.0 | 81.5 | -- ^a |
| 9.0 | 78.3 | -- ^a |
| 20.0 | 74.0 | 61.1 |

^aNot determined.

APPENDIX IV (continued)

TABLE VII

NUMBER AVERAGE DEGREE OF POLYMERIZATION (\overline{DP}_n) OF THE AMYLOSE DURING ALKALINE DEGRADATION UNDER DIFFERENT CONDITIONS

Original \overline{DP}_n : 630

| Reaction time, hours | Reaction Identifier | | | | | |
|-------------------------|---------------------|------|-----|------|----------|----------|
| | N80 | N100 | 080 | 0100 | N-AQ-100 | 0-AQ-100 |
| 0.25 | | | 568 | | | |
| 0.33 | 535 | | | | | |
| 0.5 | | | | 376 | 385 | 177 |
| 0.75 | | 447 | | | | |
| 1.0 | | | | 298 | 347 | 148 |
| 1.5 | | 303 | 515 | | | |
| 2.0 | 469 | | | 244 | 320 | |
| 3.0 | 419 | 244 | 465 | 195 | 252 | |
| 5.0 | 276 | | 381 | 124 | 174 | 87 |
| 7.0 | | 186 | 284 | 91 | 138 | 58 |
| 20.0 | | | | | | 34 |

APPENDIX V

EVALUATION OF METHODS FOR DETERMINATION OF AMYLOSE CONCENTRATION BACKGROUND

Initially, analyses of amylose degradations resulted in yield curves of low accuracy. Initial yield data from oxygen-alkali reactions proved later to be totally incorrect. Thus, it became necessary to evaluate a number of possible procedures for measurement of amylose concentration in solution. It was hoped that the substudy would provide a method which was rapid, convenient, accurate, and insensitive to the presence of the products of amylose degradation. Ideally, it would also shed some light on why a particular method failed under certain circumstances.

POTENTIAL METHODS

Gravimetric analysis of recovered amylose would be the most direct way to estimate amylose yield. It offers the additional advantage that the recovered polysaccharide can be used in other analyses. When small samples must be utilized in the determination, the method suffers from large relative errors and poor precision.

Amylose forms a blue-colored complex in the presence of polyiodide ions, and the absorbance of such solutions has been utilized to measure amylose concentrations in other studies of alkaline degradation of amylose (8,10,40). It was known that the molar absorptivity of the complex is a function of the DP of the amylose (27, 50), but it was not clear to what extent this would influence the method's applicability to this work. Iodine complexing was the main method used to determine amylose concentration in the beginning of this study.

A number of indirect methods to determine amylose concentration are based on the measurement of the glucose liberated from total hydrolysis of the amylose.

Reducing sugars have been successfully measured quantitatively (68) by reacting them with triphenyltetrazolium chloride to form a stoichiometric amount of a red formazan dye. Despite much development work, the method could not be made sufficiently accurate. Its problems were, at least in part, caused by the ability of degradation products from oxygen-alkali reactions to react with the tetrazolium salt. The method may serve well as a qualitative test in a production environment, but is not likely to be adaptable for analytical work.

Quantitative gas chromatographic analysis of sugars is a routine technique (33,69) but requires substantial processing of the hydrolyzate. Its tediousness makes it undesirable for a large test series. However, it has nearly perfect selectivity and it is the preferred method when several types of monosaccharides must be measured simultaneously, such as in wood composition analyses. It offers no particular advantage when applied to systems containing only glucose.

Glucose is routinely determined in medical analysis of body fluids. A variety of enzymatic methods are used for their simplicity, speed, and specificity. The one chosen for this study is based on a two-step reaction generating the co-enzyme nicotinamide adenine dinucleotide phosphate in reduced form (NADPH) which is easily measured spectrometrically (57,58). The method is insensitive to contamination by substances such as fructose and mannose, and citric, ascorbic and oxalic acids (58). These and related compounds were likely to be present in significant amounts in the alkali-degraded samples.

EXPERIMENTAL

Amylose was degraded in oxygen-alkali at 100°C. Liquor was withdrawn after 1 and 4 hours reaction time. Both samples were subjected to multiple tests with the four methods to be evaluated. The results are listed in Table VIII.

TABLE VIII

COMPARISON OF DIFFERENT METHODS TO MEASURE AMYLOSE YIELD. AVERAGE YIELDS IN PERCENT AND STANDARD DEVIATIONS FOR 4 OR 5 DETERMINATIONS

| Liquor Sample; reaction time | Precipitation- Gravimetry | Method | | | Gas | | |
|---------------------------------|------------------------------|-------------------|------------------|---|------------------|---|-----------------|
| | | Iodine Complex | Enzymatic | | Chromatography | | |
| | | | HCl ^a | H ₂ SO ₄ ^a | HCl ^a | H ₂ SO ₄ ^a | |
| 1 hour | Av.: | 98.6 | 71.4 | 100.5 | 91.6 | 87.5 | 93.3 |
| | S. dev.: | 4.2 | 0.49 | 2.2 | 1.8 | 3.4 | 5.5 |
| 4 hours | Av.: | 94.8 | 38.0 | 96.1 | 89.2 | 81.7 | -- ^b |
| | A. dev.: | 6.8 | 0.51 | 3.3 | -- ^b | -- ^b | -- ^b |

^a Acid used for hydrolysis.

^b Not determined due to insufficient data.

RESULTS

Gravimetry — The method of weighing the precipitated amylose gave results of the correct magnitude, but it was not very accurate and had a relatively large standard deviation. The main problems contributing to the data scatter appeared to be an irreproducible filtration rate and the hygroscopicity of the amylose and the filter paper. It did not become clear if the method is dependent on the \overline{DP}_n of the amylose.

Iodine complexing — The agreement between duplicate tests with this method was outstanding and the standard deviation was small. It was, however, the only method which implied a large and rapid loss of amylose. Apparently, it could

not be assumed that the "Blue Value" (p. 51-52) of amylose remained constant during degradation in oxygen-alkali. Since the method had been proven reliable for modest degradation under anaerobic conditions, it was concluded that the observed change in the absorptivity was indicative of modifications of the amylose molecules caused by the presence of molecular oxygen during the alkaline reaction. Possible explanations for this effect of oxygen are discussed on p. 29-33.

Enzymatic method.— The glucose concentration indicated by the enzymatic determination agreed well with the gravimetric results. The standard deviation of the enzymatic method shown in Table VIII was smaller than that of the gravimetric and chromatographic methods. Because of the short sample preparation time, it was possible to run multiple tests on a large number of samples, although the method proved so reproducible in actual use that duplicate measurements rarely were necessary.

Gas chromatography — The results provided by gas chromatography were of the same magnitude as those of gravimetry and enzyme spectrometry. The reproducibility between duplicate injections of the same solution was excellent, while duplicate liquor sample treatment and glucose derivatization was poor. Significant improvement in this area could probably have been achieved, but the method held no apparent advantage over the enzymatic procedure which offered greater accuracy and a relatively small investment in sample preparation. Experimental details for gas chromatographic analysis of acetylated alditols, as performed in this study, can be found elsewhere (69).

CONCLUSIONS

The method based on iodine-complexing was the only one which gave an erroneous value of the amylose concentration. The enzymatic determination of glucose

after total hydrolysis was chosen as the best method and was applied to all subsequent reactions. The amylose was hydrolyzed with hydrochloric acid since, in agreement with the literature (50,70), it was more effective than sulfuric acid of similar normality.