DEVELOPMENT OF A QUANTITATIVE METHOD FOR THE ANALYSIS OF NEUTRAL SACCHARIDES IN WOOD PULP USING ANALYTICAL PYROLYSIS

JOHN KELLY, B.Sc., G.R.S.C.

Development of a Quantitative Method for the Analysis of Neutral Saccharides in Wood Pulp using Analytical Pyrolysis

by

John Kelly, B.Sc., G.R.S.C.

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Memorial University of Newfoundland

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ABSTRACT

Traditional methods for the determination of the neutral saccharide composition of complex biomaterials such as wood pulp are difficult and time-consuming. By comparison analytical pyrolysis techniques offer the advantages of speed and simplicity. Under the appropriate conditions, pyrolysis yields products that retain the stereoconfiguration of the parent neutral saccharide (the anhydrosugars). The objective of this investigation was to develop a method to quantitate the neutral saccharides in wood pulp using on-line pyrolysis - gas chromatography (Py-GC). Initial efforts focussed on maximizing the yields of anhydrosugars from isolated polysaccharides resembling those found in wood. Factors such as pyrolysis temperature and cation-exchange were investigated. Next, a variety of wood pulps of differing composition and origin were investigated by Py-GC. The numerous pyrolyzates produced by these pulps were characterized by Py-GC-MS using both electron impact (EI) and chemical ionisation (CI). Finally, Py-GC methods were developed to quantify the saccharide compositions of the pulps. Comparisons were made with results obtained by acid hydrolysis - derivatization / gas chromatography. Two on-line pyrolyzers, the CDS Pyroprobe 120® and the SGE Pyrojector®, were compared as to their suitability for quantitative analysis.

DEDICATION

This thesis is dedicated to my wife Ann and to my parents, Vincent and Mary Kelly.

ACKNOWLEDGEMENTS

I wish to express my appreciation to my supervisors, Dr. Bob Helleur and Dr. Michael Mackey, for their encouragement, guidance and advice. I would also like to thank Dr. Brian Gregory and Ms. Marion Baggs for their help with the Chemical Ionization Mass Spectrometry. Finally, the financial support of the School of Graduate Studies in the form of a fellowship, and of the Department of Chemistry, is gratefully acknowledged.

GLOSSARY

A.H.	Acid Hydrolysis - Derivatization / Gas Chromatography
CI	Chemical Ionization
CIMS	Chemical Ionization Mass Spectrometry
C.V.	Coefficient of Variation
DP_n	Degree of Polymerization
EI	Electron Impact Ionization
EIMS	Electron Impact Ionization Mass Spectrometry
EIC	Extracted Ion Chromatogram
FID	Flame Ionization Mass Spectrometry
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
MC	Microcrystalline Cellulose
N.S.P.	Noranda Sulfite Pulp
PA	Proton Affinity
Py	Pyrolysis
Py-GC	Pyrolysis - Gas Chromatography
Py-MS	Pyrolysis - Mass Spectrometry
TFA	Trifluoroacetic Acid

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CHAPTER 1: INTRODUCTION

1.1. Pyrolysis - Gas Chromatography

1.1.1. Analytical Pyrolysis

Pyrolysis can be described as a chemical degradation reaction that is induced by thermal energy alone (1). Analytical pyrolysis can be further defined as the characterization of a material or a chemical process by the instrumental analysis of it's pyrolysis products (1).

Though the first scientific use of analytical pyrolysis was documented in 1860 (2), it was not until the 1940's that pyrolysis was used in combination with modern analytical instrumentation. At this time a number of reports were made of the use of mass spectrometry to characterize the pyrolyzates of synthetic polymers (3,4,5). In 1952, Zemany used pyrolysis - mass spectrometry (Py-MS) to characterize large molecular weight biopolymers such as albumin and pepsin (6). His results were significant in that characteristic pyrolysis profiles could be reproducibly obtained for these compounds. The first truely integrated pyrolysis - mass spectrometric instrument was described in 1953 (7).

The introduction of gas-liquid chromatography (GC) in 1952 (8) rapidly led to the first reports of it's use in combination with pyrolysis (9,10,11). Pyrolysis - gas chromatography (Py-GC) quickly overshadowed pyrolysis - mass spectrometry as the technique of choice due to it's ability to separate volatile pyrolyzates prior to detection, and because pyrolyzers could be easily integrated with GC instruments. In addition, mass spectrometry was very expensive and difficult to use. The recent development of rugged open tubular capillary columns greatly enhanced the resolving power of gas chromatography, and has simplified the interfacing of GC to mass spectrometers. Complex

pyrolyzate mixtures can now be resolved and unambigously identified by pyrolysis - capillary column gas chromatography - mass spectrometry (Py-GC-MS).

The technique of Py-GC, in all its forms, continues to be the dominant method in analytical pyrolysis. However, Py-MS has steadily increased in popularity because of it's ability to detect larger molecular weight pyrolyzates that are unable to pass through a GC column. The developments in analytical pyrolysis instrumentation have been reviewed on a number of occasions (12,13,17,18).

1.1.2. Types of Pyrolyzers

The majority of pyrolyzers used in combination with GC fall into three categories: resistively heated filaments, inductively heated (Curie - point) filaments, and continuously heated microfurnaces. The first two categories are also classified as pulse pyrolyzers. Each of the above types was developed early in the evolution of analytical pyrolysis and the basic features have changed little since (14,15,16).

Resistively heated pyrolyzers are usually composed of platinum filaments or ribbons. Soluble samples can be easily applied to the metal surface. An electric current is then passed through the wire, which in turn heats and pyrolyzes the sample. Insoluble samples can be pyrolyzed by placing them in the centre of a coiled filament with the aid of a quartz boat or tube. Modern resistively heated pyrolyzers, such as the Pyroprobe® (Fig. 2.1) and the Pyrola®, are designed to tightly control important parameters such as the final pyrolysis temperature and temperature rise time.

Inductively heated pyrolyzers operate on a different principle. A sample is applied to the surface of a ferromagnetic wire which is then placed in the centre of a high frequency induction coil.

A magnetic flux is initiated, which in turn induces eddy currents in the wire's surface. The filament is heated rapidly until a temperature (Curie point) is reached at which the wire becomes paramagnetic and absorbs no more energy. This temperature will be maintained as long as the rf field is applied to the wire. The Curie point can be varied from approximately 300 to 1100°C by altering the ferromagnetic alloy composition of the filament. For insoluble samples, boats, tubes, and v-shaped ribbons can be used. Curie - point pyrolyzers are characterised by a very rapid temperature rise time but are inflexible in that the final temperature is determined by the composition of the wire.

Microfurnace pyrolyzers are simpler in design than the previous two categories. They usually consist of a quartz tube surrounded by a temperature-regulated microfurnace. The tube is constantly swept by an inert gas (usually the carrier gas for the GC). Samples are either dropped into the pyrolysis zone (vertical furnace), or are placed in a boat which is then moved into the heated region (horizontal furnace). Soluble samples can also be coated on a wire which is then introduced into the pyrolyzer. Though microfurnace pyrolyzers can give pyrolysis profiles similar to those obtained using a pulse pyrolyzer, irreproducibility can be a serious problem. This is most often due to slow temperature rise times and secondary reactions occurring as the pyrolyzates are swept through the large heated zone.

1.1.3. Factors Affecting Pyrolysis

There are many parameters that must be tightly controlled in order to guarantee reproducible pyrolysis. Many of these parameters have been extensively documented (13,19,20,21). It is agreed that a pyrolyzer must be able to reproduce the same temperature-time profile and that, in most cases, a temperature rise time in the order of milliseconds is most desirable. In addition, samples must be

homogenous, small in mass (5-100 µg), and have a large surface to volume ratio. Compliance with these recommendations should minimize the problems of thermal gradients and secondary reactions occurring within the sample.

Other factors are important in Py-GC. For example, a high carrier gas velocity within the pyrolyzer is required to ensure the fast removal of volatile pyrolyzates from the pyrolysis zone. This must be done in order to prevent secondary reactions which lower the yields of the primary, high mass products. However, if the gas flow is too fast it may affect the temperature-time profile. Cold spots between the pyrolysis zone and the GC column are another potential problem. The regions in between these two zones must be kept sufficiently warm in order to prevent the condensation of the higher molecular weight pyrolyzates.

Analytical pyrolysis continues to suffer from the mistaken belief that it is irreproducible. It has been demonstrated that even a continuously heated microfurnace can reproduce pyrolyzate ratios for synthetic co-polymers in the order of 0.45% relative standard deviation (22). Results such as this demonstrate that a high degree of precision can be achieved provided the pyrolysis process is optimised and rigidly controlled.

However, interlaboratory reproducibility has been a serious problem with both Py-GC and Py-MS. Unfortunately the development of analytical pyrolysis has not been well coordinated. This has resulted in a large variety of instruments and practices. Comparative interlaboratory trials have been carried out for both Py-GC (23-28) and Py-MS (29). Initial qualitative and quantitative comparisons for PY-GC were poor, especially for complex polymers (23-25). However, the later trials set stricter guidelines for all steps in the analysis and this resulted in significant improvements

in interlaboratory precision for both peak intensity and retention time (28). It can be concluded that as long as the variables are controlled, different pyrolyzer systems can produce similar results.

1.1.4. Applications of Analytical Pyrolysis

Organic polymers, whether synthetic or biological, are difficult to analyze by more conventional analytical techniques because of their poor solubility and/or complexity. However, pyrolysis breaks down polymer molecules into smaller, more easily identifiable fragments which are frequently characteristic of the parent molecule. It is in this area that analytical pyrolysis has made it's most significant contributions. In addition to those given below, there are many examples of the different applications of analytical pyrolysis to be found in the Journal of Analytical and Applied Pyrolysis. Furthermore, a number of comprehensive review texts and articles are available (13,30,31,32).

Analytical pyrolysis has important industrial and forensic applications in the characterization of synthetic polymers and rubbers (33,34,35). It is also used in the analysis of geological and environmental materials such as coals (36), kerogens (37), soils (38), and sediments (39). In food chemistry, the technique has been used to investigate the pyrolytic reactions that occur during some cooking processes (40). Analytical pyrolysis has also been used to differentiate between microorganisms (41). Finally, it has been used to characterise biopolymers such as proteins (42), polysaccharides (43), lignins (44), and , as well as intact biological systems such as wood (45) and spruce needles (46).

1.2. Wood Pulps

1.2.1. Composition of Wood

Wood is the major supportive tissue in trees and other higher order plants. It is also responsible for the upward movement of water and nutrients from the roots to the leaves and upper parts of the plant. From a chemical point of view, 90 - 95% of the dry weight of wood can be accounted for by three polymer classes: cellulose, hemicellulose, and lignin (47). Inorganic ash and extractives, such as waxes, resins, proteins, gums, etc., make up the remainder of the dry weight.

Cellulose is composed entirely of 1,4-linked ß-D-glucopyranose and accounts for 40-50% of the dry weight of wood (Fig. 1.1). It is a linear polymer with a number average degree of polymerization (DP_n) of 9,000 - 10,000 (48). Because of it's abundance and commercial importance it has been extensively investigated (48,49). The individual molecules are aggregated into microscopic structures called microfibrils and these form the primary lattice work of the cell walls (47).

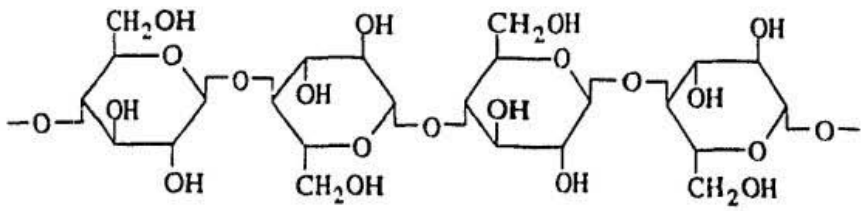


Figure 1.1: The Structure of Cellulose.

The hemicelluloses are mixtures of short chain polysaccharides (DP_n = 100-200). They are closely associated with cellulose and lignin and it is believed that they act as a binding matrix for these two insoluble polymers. Fully 25-35% of dry wood is hemicellulose. The structures of the major softwood hemicelluloses are outlined in Fig. 1.2.

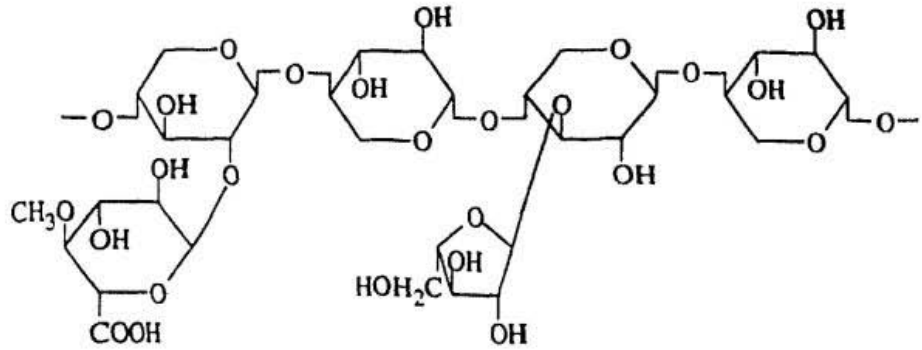
Lignins are three dimensional, polyphenolic macromolecules that are distributed throughout the cell wall and the middle lamella (intercellular region). Lignin is incorporated into the wall during the maturation of the cell and gives it strength and rigidity (47). The three lignin precursors are listed in Fig. 1.3 (48). The monomeric units are randomly linked via ether and carbon - carbon bonds. Therefore there is no typical repeating unit as there are for the polysaccharides.

1.2.2. Differences in Chemical Composition Between Hardwoods (Angiosperms) and Softwoods (Gymnosperms).

As a general rule, hardwoods and softwoods differ in their hemicellulose and lignin compositions. Softwood hemicellulose is commonly a 2:1 mixture of galactoglucomannans and arabinoglucuronoxylans. Hardwood hemicellulose is largely composed of glucuronoxylans with small quantities of glucomannans (48,49). In addition, hardwood xylans are heavily acetylated at the C2 or C3 ring positions.

Softwood lignins are derived almost entirely from coniferyl alcohol (guaiacol) precursors, whereas hardwood lignins also contain syringyl propane units (i.e. derived from sinapyl alcohol). Derivatives of *p*-coumaryl alcohol are found in minor amounts in all lignins.

a)



b)

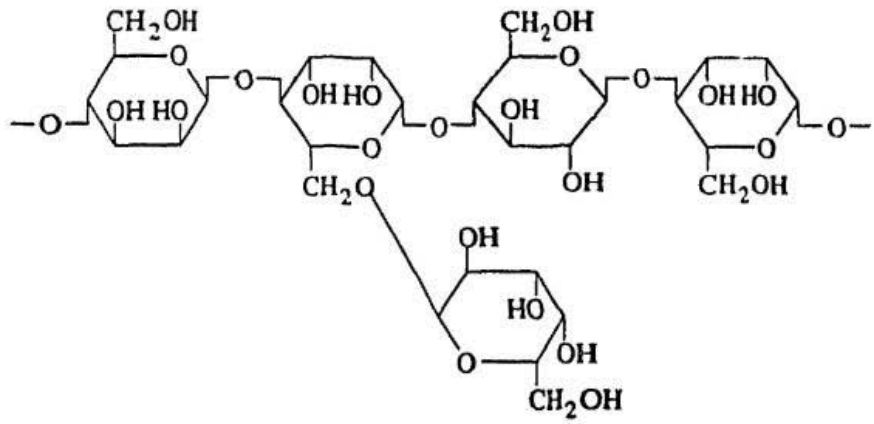
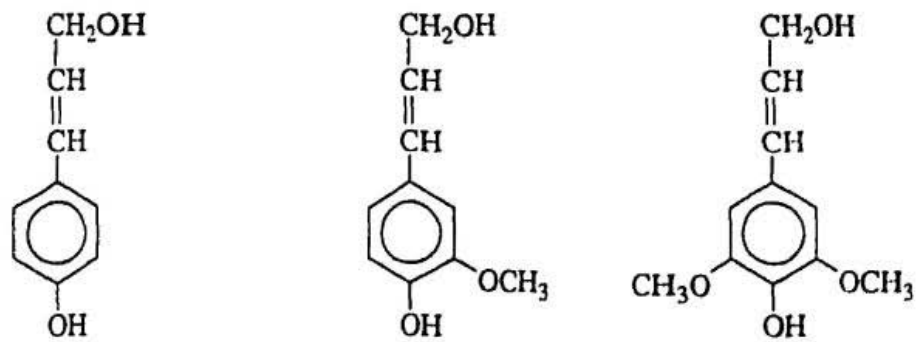


Figure 1.2: The Structures of the Major Hemicelluloses, a), Arabinoglucuronoxylan (Softwood) and b), Galactoglucomannan (Softwood).



p-Coumaryl Alcohol Coniferyl alcohol Sinapyl Alcohol

Figure 1.3: The Lignin Precursors.

1.2.3. The Pulping of Wood

Pulping is the process by which wood is reduced to fibers. The choice of pulping process will depend on a number of factors including the end use of the pulp, the tree species to be exploited, and the relative costs of the various processes. The most widely used processes fall into two categories: mechanical and chemical pulping.

Mechanical pulps are obtained by abrading the fibers from the wood. This is done either by pressing debarked logs against a revolving grindstone or feeding wood chips between two rotating, grooved disks (50). The former process normally yields a pulp with short fibres and a lot of fines. The latter process is called refining and produces a more homogenous pulp. These pulps are used to make paper with a high opacity. An important advantage of the mechanical pulping is that there is little wastage (i.e., pulp yields are 90% or greater) and there is no toxic effluent. However, these mechanical processes require a large energy input. In addition, paper made exclusively of mechanical pulp tears easily and yellows quickly when exposed to light. Generally, mechanical pulps undergo further treatment and may be mixed with the longer fibres of chemical pulp in order to strengthen the final product (51).

Chemical pulping processes release the fibres by chemically attacking the encrusting lignin polymer. There are two predominant processes: kraft and sulfite pulping. In the kraft process wood chips are usually cooked for 1-2 hours at 170-180°C in a solution of NaOH and Na_2S (52). The proposed lignin depolymerization reactions are described in Figure 1.4 (53). Kraft fibres are long, dark and strong and are used to produce paper bags and boxes. Bleached kraft pulp is used for fine paper, dissolving pulps for the polymer industry, and for white packaging board. In a typical

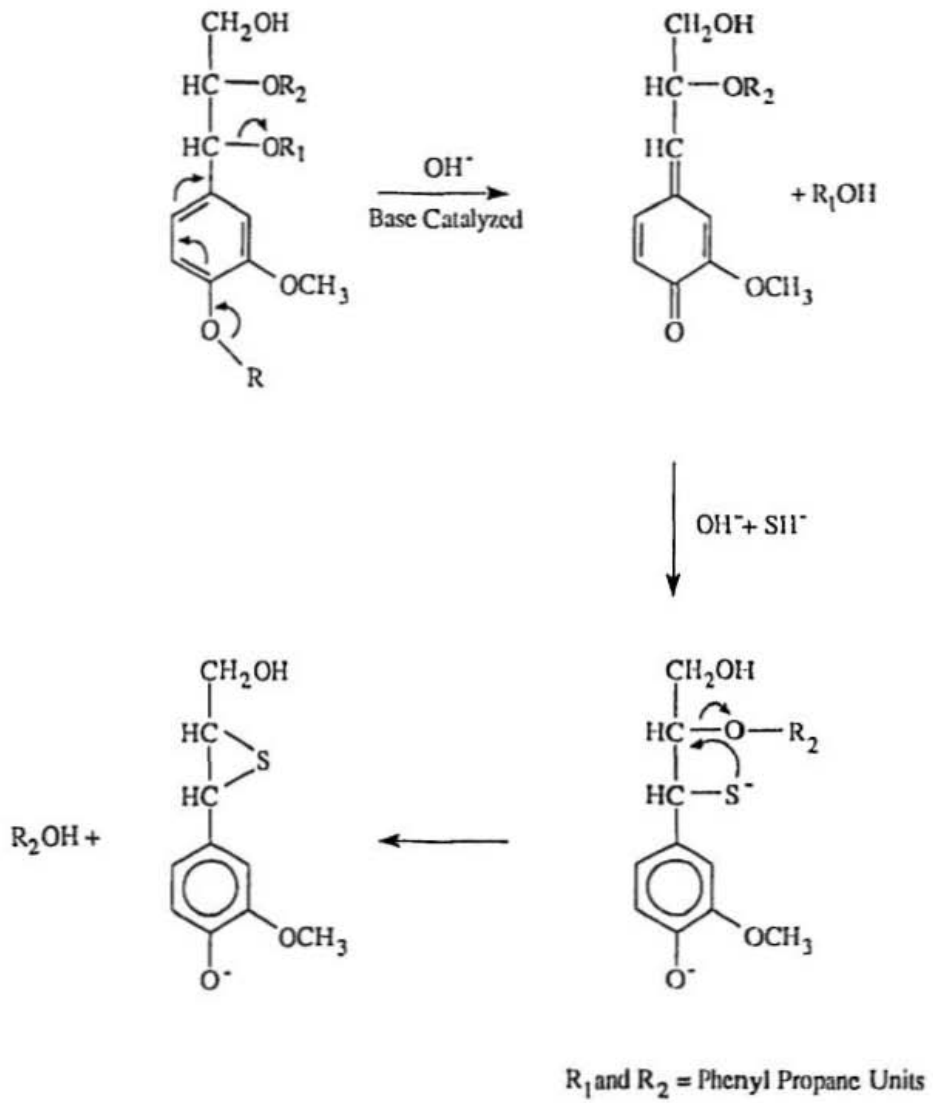


Figure 1.4: Example of the Lignin Degradation Mechanisms in Kraft Pulping (53).

kraft pulp, 80% of the lignin, 50% of the hemicellulose (especially the glucomannans), and 10% of the cellulose are removed during cooking. In addition, the acetyl groups and pendant sidechains of the hemicelluloses are quickly lost.

The sulfite process is, in fact, a number of pulping processes that cover the whole pH range, from sulfur dioxide solutions (pH = 1) to sodium sulfite / sodium hydroxide solutions (pH = 13). No one reaction scheme can describe the lignin depolymerization processes that occur during cooking, though the alpha carbon of the phenyl propane unit seems to be the site of initial attack in most cases. A possible reaction scheme for neutral sulfite (pH 6-10) pulping is described in Figure 1.5 (54). At either pH extreme lignin removal and hemicellulose degradation is extensive. Xylans are

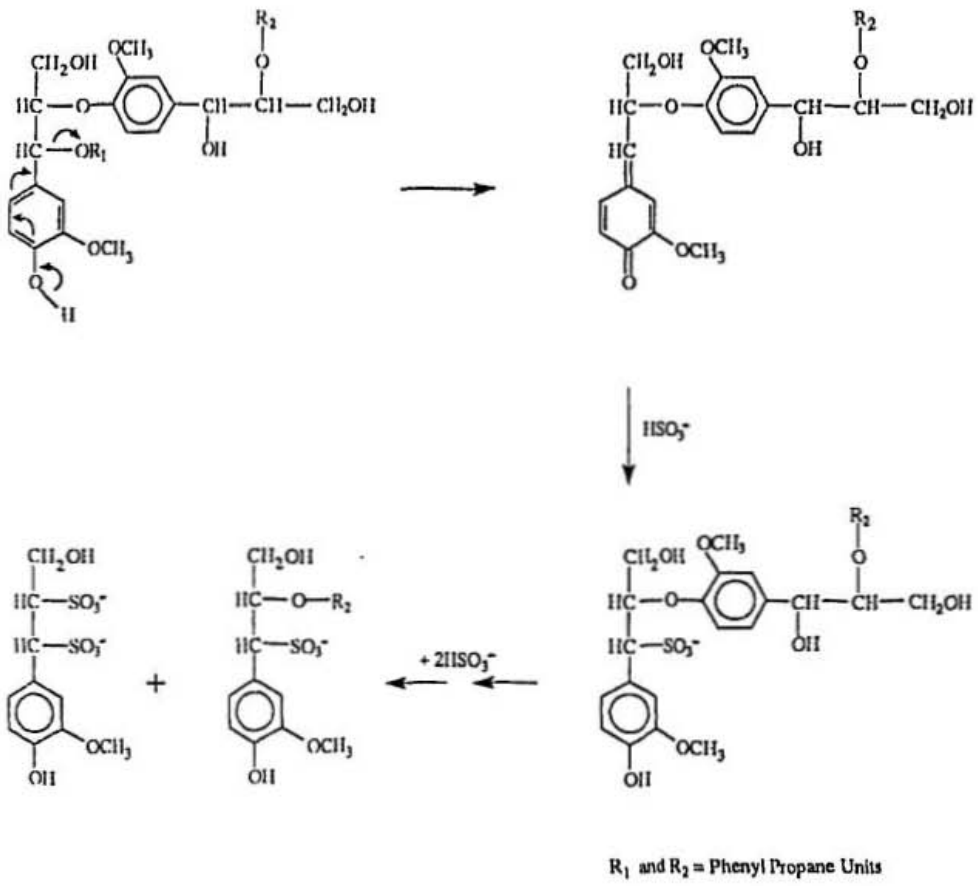


Figure 1.5: Example of the Lignin Degradation Mechanisms in Neutral Sulfite Pulping (54).

preferentially lost at low pH whereas the mannans are easier to remove in alkaline processes. Pulp yields tend to be higher (75 - 90%) for mid-range processes but mechanical defribation may be needed to completely separate the fibres. Because of the flexibilty of the sulfite process, pulps with a wide variety of characteristics and end uses can be produced. For example, acid sulfite pulps (low pH) are used for dissolving pulps and intermediate pH pulps (pH 4-10) can be used for newsprint, paper, and corrugated board. Finally, the recently developed alkaline process (pH 11-14) produces a pulp similar to kraft pulp but without the noxious odours associated with the latter process (54).

There are many processes that do not fit neatly into either of the above categories. For example, many processes use an initial chemical soak to soften the lignin prior to grinding or refining (chemimechanical). Other processes use heat to achieve the same effect (thermomechanical). Steam-explosion pulp is produced when wood that has been treated with steam (with or without chemicals) at high pressures is suddenly depressurised. Finally, there are the recently developed solvent pulping processes. A good example would be the Alcell® ethyl alcohol pulping process (55). Wood chips are cooked in an alcohol-water mixture at 200°C. Delignification is enhanced by the conversion of the hemicellulose acetyl groups into acetic acid.

1.3. Traditional Methods of Wood Pulp Carbohydrate Analysis

1.3.1. Non-Specific Saccharide Analysis

The following are examples of commonly used methods for characterizing wood and pulps that do not distinguish between individual monosaccharides present in the sample. The total carbohydrate content is determined by removing the lignin. This can be achieved by treating the sample with chlorine gas and 2-aminoethanol until a white residue, called holocellulose, remains (56).

Inevitably, some of the hemicellulose will be lost during this treatment.

The cellulose content can be determined in a number of ways. For example, Cross and Bevan cellulose is obtained by chlorination of the wood sample, followed by washing with 2% Na_2SO_3 and 3% SO_2, and finally by boiling in Na_2SO_3 solution (57). Alpha cellulose content is determined by treating holocellulose with 17.5% NaOH (58). This procedure removes most, though not all, of the hemicelluloses.

The pentose content, xylose and arabinose, is determined by boiling the sample in 3.85 N HCl with some NaCl (59). These sugars form furfural which is then separated by distillation. Its concentration is determined colorimetrically with orcinol-$FeCl_3$ reagent. The uronic acid content can be determined by generating, and subsequently analyzing for, CO_2 from its carboxylic acid moieties. This is done by boiling the sample in 12% HCl (60). Alternatively, colorimetric analysis can be used (61,62).

1.3.2. Monosaccharide Analysis

The wood polysaccharides are reduced to their constituent monosaccharides by acid hydrolysis. The most commonly used method is Saeman hydrolysis (63). The wood sample is treated with 72% H_2SO_4 at 30°C for 1 hour to swell it. The acid is then diluted to 1 M and the solution is refluxed for 2 to 5 hours. The insoluble material remaining is referred to as Klason lignin, even though it may contain as much as 15 % carbohydrate.

Trifluoroacetic acid (TFA) is also used to hydrolyze wood and wood pulps (64,65,66). It is

a volatile acid which facilitates it's removal after the hydrolysis step. In addition, anhydrous TFA is a good solvent for cellulose which can aid in it's hydrolysis. Fengel et al. have developed a hydrolysis procedure specifically for lignocellulosics (65). The sample is soaked overnight in anhydrous TFA at room temperature, followed by refluxing for 1 hour. The solution is then diluted to 80% TFA and refluxed for 15 minutes. Finally the solution is diluted to 30% TFA and refluxed for 2 hours. In another investigation, Paice et al. hydrolyzed lignocellulosic samples in different concentrations of TFA at 100°C in sealed, evacuated vials (66). They found that the best results were obtained for 2 hours in 80% TFA.

In general these methods are effective at releasing the neutral monosaccharides from the samples. However, little or no uronic acid is detected. In addition, some of the monosaccharides are acid labile (e.g., arabinose and xylose) and care must be taken not to destroy them. This must be balanced with the fact that cellulose requires an aggressive hydrolysis.

Traditionally, the monosaccharides were separated by paper chromatography (63). The individual monosaccharides could then be quantified using, for example, the Nelson-Somogyi colorimetric assay for reducing sugars (67). However, methods based on high performance liquid chromatography (HPLC) or gas chromatography (GC) are now dominant.

The most successful HPLC methods for monosaccharide separation use ion-exchange resins. However, in many cases size exclusion is the principal separatory mechanism. For example, Paice et al. (66) used a HPX-85 lead ion exchange column with deionised water as mobile phase. The disaccharide cellobiose was the first analyte to be eluted. More recently, a method for the separation of wood hydrolyzates based on true ion exchange principles was published (68).

Because saccharides do not contain chromophores or fluorophores, refractive index is the most commonly used detector in HPLC. However, it has poor sensitivity (0.1 µg/µl) detection limit). By comparison, pulsed amperometric detection is quite sensitive (0.1 ng/µl) detection limit) and was used in the investigation of the wood hydrolysates by Edwards et al. (68). Alternatively, the saccharides can be derivatized to increase their UV or fluorescense sensitivity.

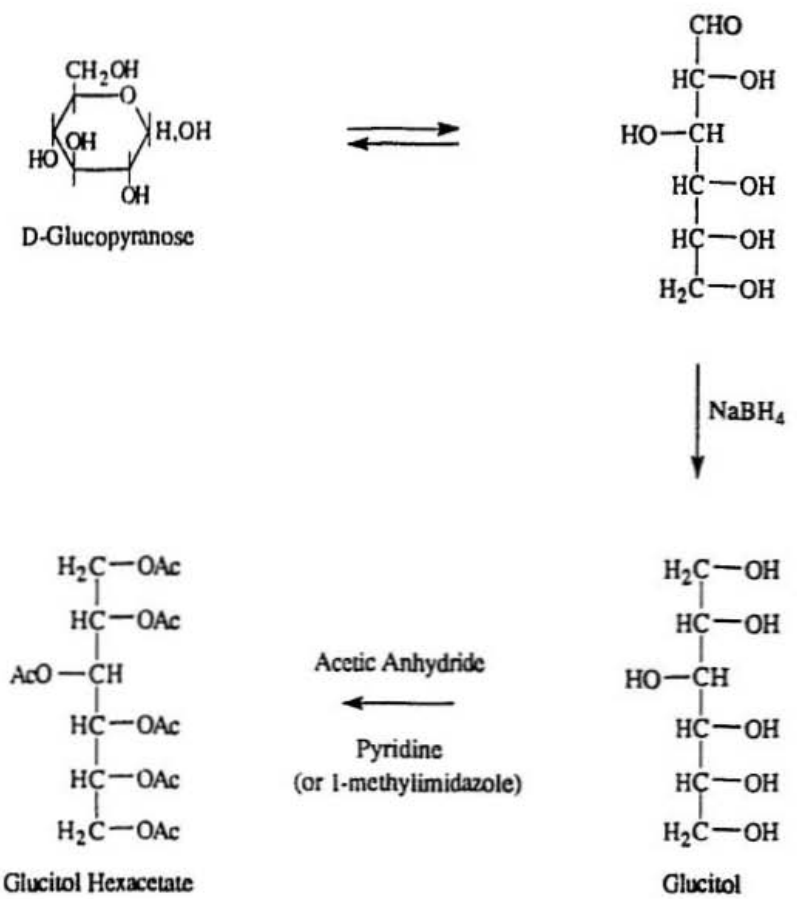


Figure 1.6: Alditol Acetate Derivatization. Conversion of D-Glucopyranose into Glucitol Hexacetate.

Mono- and oligosaccharides are involatile and therefore must be derivatized if a GC method is adopted. Alditol acetate derivitization is the procedure most widely used and is illustrated in Fig. 1.6. Though this method is relatively laborious, only one acetate product is produced per saccharide species. This is not the case with other procedures such as trimethylsilyation. In their discussion of alditol acetate preparation and analysis, Fox et al. (69) reviewed the columns and stationary phases used to separate alditol acetate compounds. Capillary columns offer superior resolution and peak shape with respect to packed columns. In addition, the more polar the stationary phase the better the resolution between alditol acetates within the same class (i.e., pentoses, hexoses, etc.). The flame ionization detector is most commonly used and is sensitive enough for most purposes. However, samples with a complex matrix may require the use of a more selective detector. In these cases selective ion monitoring with a mass spectrometer would be the appropriate detector.

1.4. Pyrolysis of Carbohydrates

From an analytical point of view, pyrolysis offers an alternative method of characterizing these frequently intractible polymers. However, there has also been a great deal of interest in applied pyrolysis for the production of useful chemicals from carbohydrate-containing biomass (70).

1.4.1. Carbohydrate Pyrolysis Mechanisms

The pyrolysis of polysaccharides, and even simple monosaccharides, can produce a wide variety of products and unravelling all the potential reaction mechanisms has proved no simple task. For the sake of simplicity and because of the widespread interest in its properties, cellulose has been the focus of attention for most researchers.

Shafizadeh divided the pyrolysis of cellulose into three distinctive, temperature related pathways (71). Low temperature pyrolysis (ambient to 300°C) slowly yields char, water, CO_2 and CO. Intermediate temperature pyrolysis (300-600°C) produces mainly 1,6-anhydro-ß-D-glucopyranose and other related compounds. Above 600°C gasification of the polymer occurs. In many instances all three processes may occur during the course of pyrolysis. Other parameters, such as the time-temperature profile, the presence of inorganic impurities, and the rate of removal of the pyrolyzates from the heated zone, can have a significant effect on the nature and distribution of products.

The principle product of fast pyrolysis is 1,6-anhydro-ß-D-glucopyranose (levoglucosan), **I**. Though the true reaction mechanism has not been determined, a number of theories have been proposed. Golova suggested that pyrolysis is initiated by homolytic fission at glycosidic bond sites dispersed throughout the cellulose molecule (72). The polymer is then rapidly unzipped via a self-propagating radical rearrangement to form levoglucosan (Fig. 1.7).

Most researchers discount this in favour of a heterolytic fission mechanism. Essig et al. (73) proposed that breakage of the glycosidic bond produces a resonance stabilised carbocation which then undergoes an intramolecular addition (Fig. 1.8). The process is propagated by the continual loss of the levoglucosan end unit.

However, pyrolysis of cellulose also produces 1,6-anhydro-ß-D-glucofuranose, **II**, at approximately 10% the yield of levoglucosan, and neither of the above mechanisms can account for its formation. Shafizadeh et al. (74) have proposed the generation of a number of intermediate anhydrosugars via the nucleophilic displacement of the glycosidic bond by one of the ring hydroxyl groups (Fig. 1.9). One of these, 1,4-anhydro-∝-D-glucopyranose, **III**, could then rearrange to form

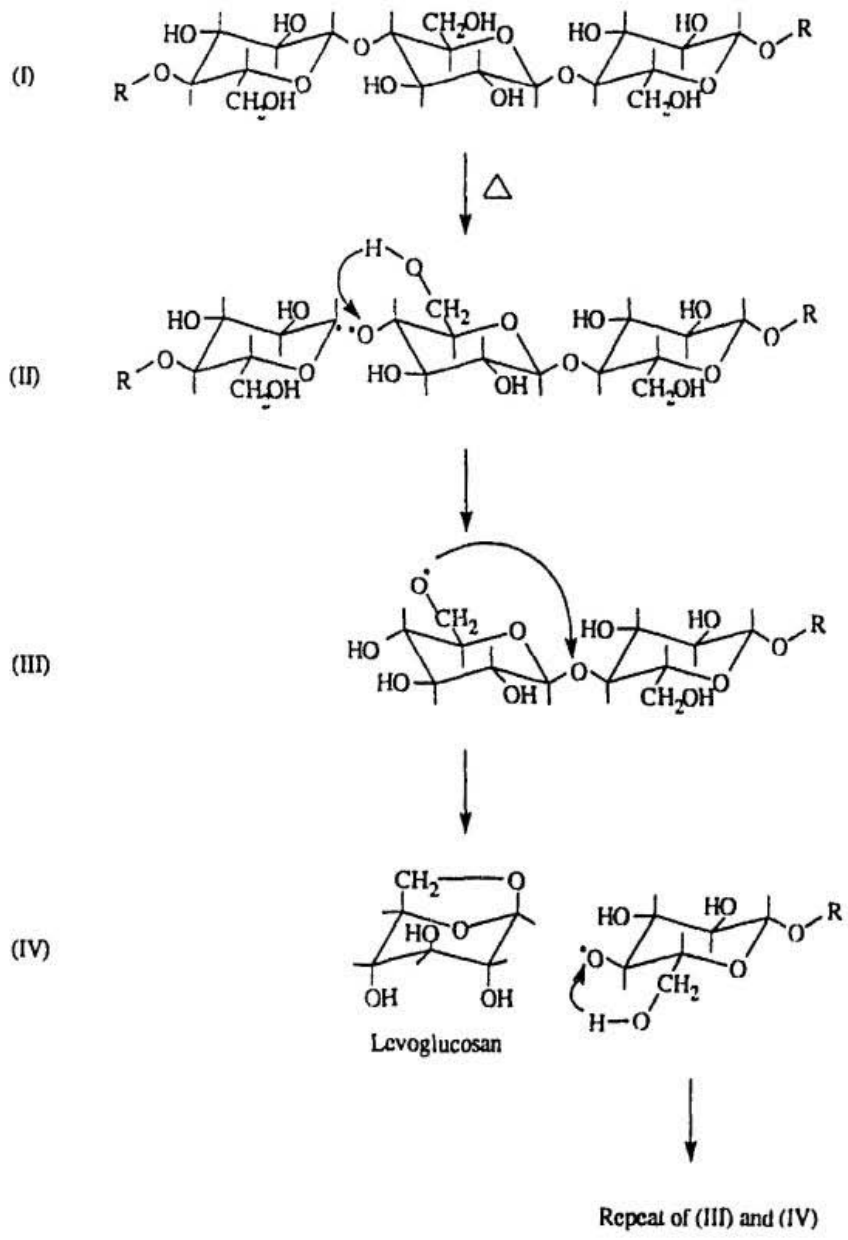


Figure 1.7: Homolytic Fission of Cellulose (72).

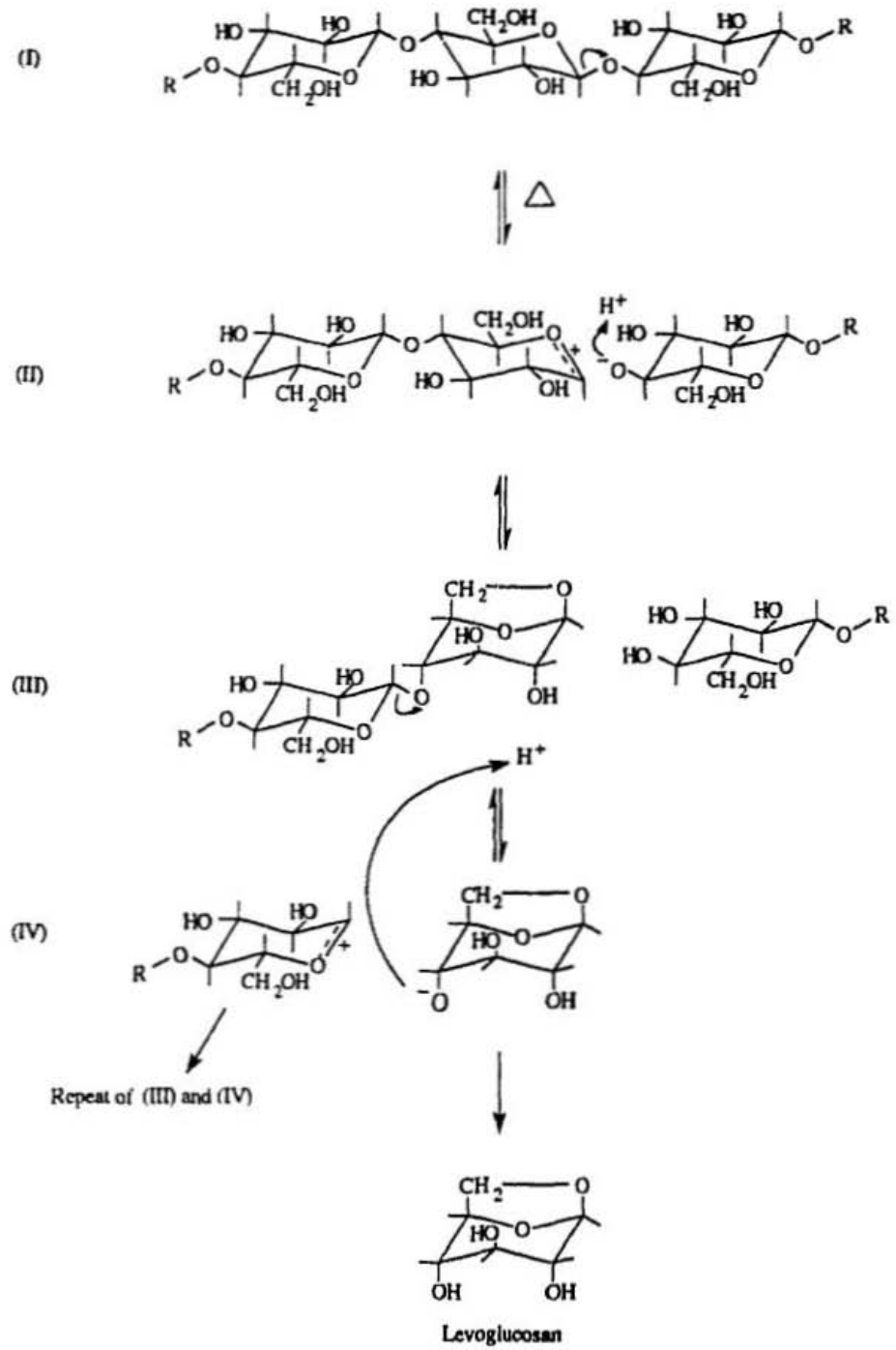


Figure 1.8: Heterolytic Fission of Cellulose (73).

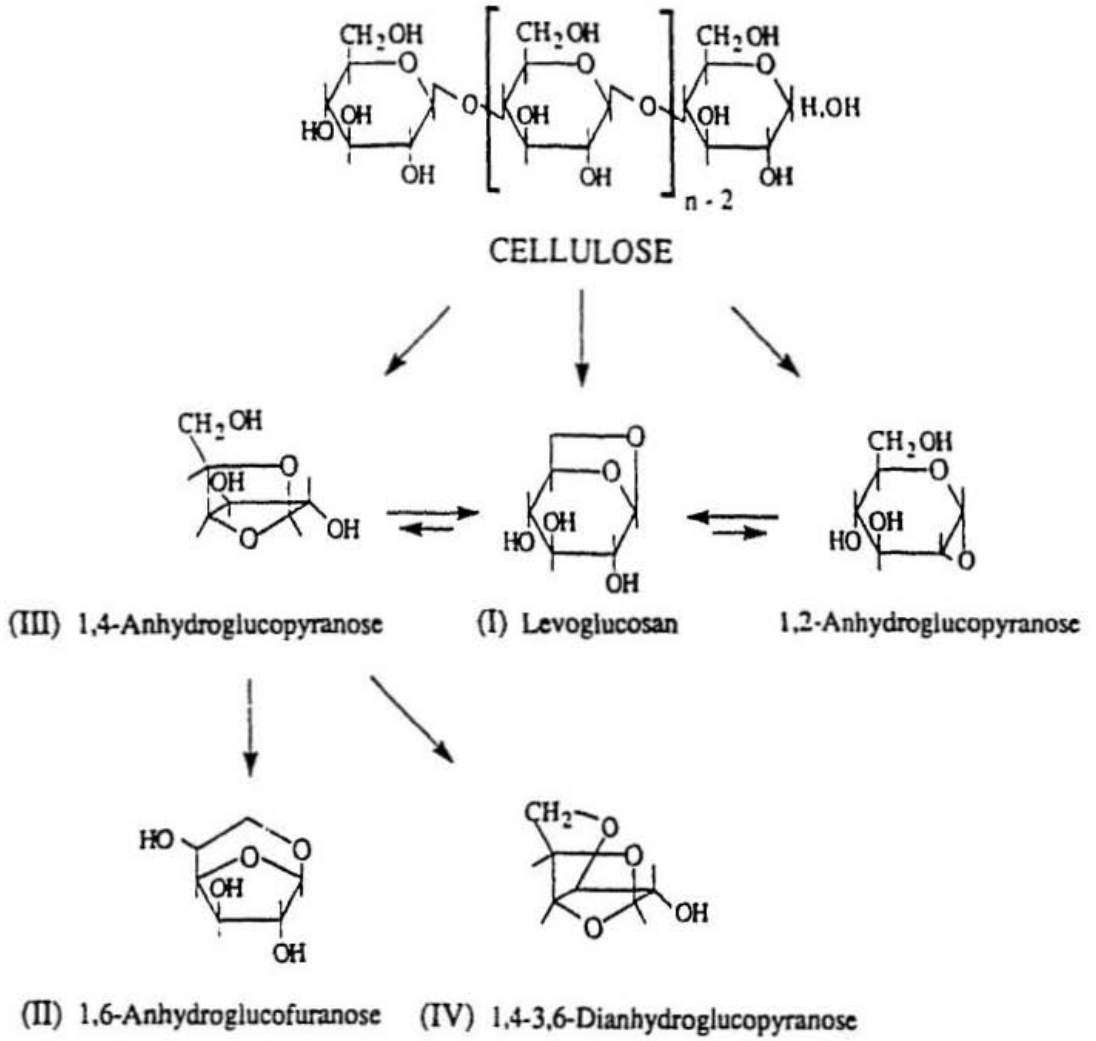


Figure 1.9: Pyrolytic Pathway for the Formation of 1,6-Anhydroglucofuranose (74).

either levoglucosan, I, or 1,6-anhydro-ß-D-glucofuranose, II.

A large number of smaller, non-specific compounds can be formed during pyrolysis. Indeed, Pouwels et al. pyrolyzed microcrystalline cellulose by curie-point-GC-MS and detected 96 pyrolyzates

(75). Many of the larger pyrolyzates, such as 1,4:3,6-dianhydro-∝-D-glucopyranose, **IV** ,5-hydroxymethyl-2-furaldehyde, **V**, and 2-furaldehyde, **VI**, are characteristic products of cellulose pyrolysis and Shafizadeh (71) has proposed that these products are formed by the degradation of levoglucosan and the intermediate anhydrosugars (Fig. 1.10). The smaller products are formed by a variety of multistep pathways involving the rupture of the pyranose ring. They may originate from the pyranose units in the polymer chain or from the anhydrosugars, and a number of mechanisms have been proposed (75,76).

1.4.2. Matrix Influences

In addition to those parameters discussed in section 1.1.3, the composition of the matrix can have a profound influence on the nature of the pyrolysis products. For example, it has been shown that acids enhance the formation of levoglucosenone from cellulose at the expense of levoglucosan (77).

The presence of inorganic ash is especially important in the pyrolysis of carbohydrates. For example, Essig et al. found that as little as 0.05% NaCl (w/w) can reduce levoglucosan yields from 55% to 9% (78). There was a corresponding increase in the yields of char and light volatiles.

1.4.3. The Specificity of Anhydrosugars

Anhydrosugars are the only chromatographable pyrolyzates that retain the original stereoconfiguration of the parent saccharide. Budgell et al. (79) demonstrated that unique anhydrosugars could be produced for a variety of hexoses and pentoses, and they could be resolved

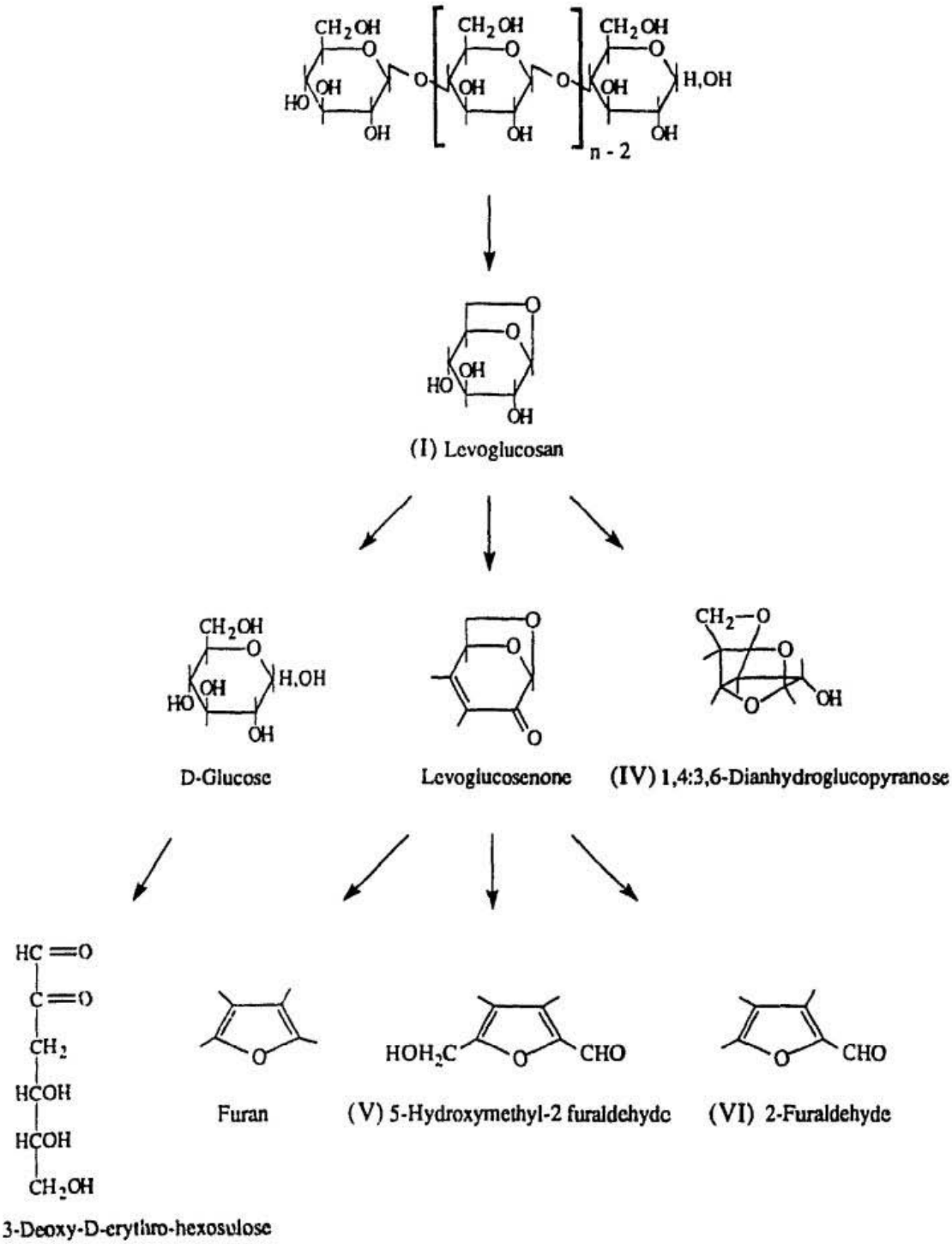


Figure 1.10: Formation of Non-Specific Carbohydrate Pyrolyzates (71).

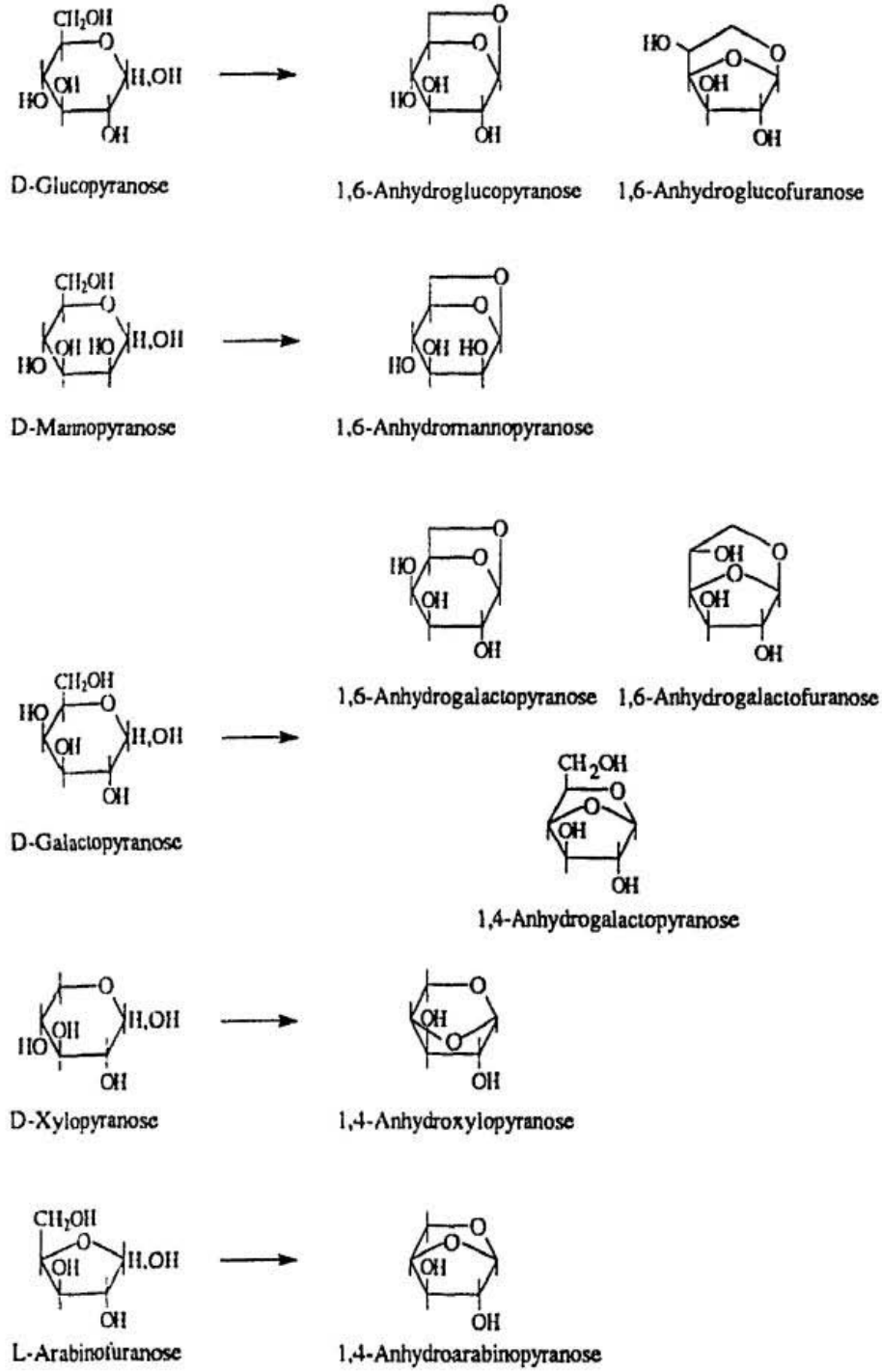


Figure 1.11: The Saccharides Found in Wood and The Anhydrosugars They Form Upon Pyrolysis.

using polar phase capillary gas chromatography. This same system was used to characterize the saccharide composition of a number of homo- and heteropolysaccharides (80,43). Interestingly, electron impact mass spectrometry could distinguish between different classes of anhydrosugars (i.e., 1,6-anhydropyranoses and 1,6-anhydrofuranoses) but not between anhydrosugars within the same class (i.e., 1,6-anhydroglucopyranose and 1,6-anhydromannopyranose). The saccharides encountered in this study and the anhydrosugars they produce when pyrolyzed are illustrated in Figure 1.11.

1.5. Objectives of This Study

The objective of this work was to determine the usefullness of on-line Py-GC (-MS) as a qualitative and quantitative tool for the investigation of the saccharide composition of wood pulps. Traditional methods of saccharide analysis are long and arduous and do not lend themselves to routine use. By comparison, Py-GC offers the advantages of speed and simplicity. Moreover, it has been shown that unique pyrolyzates can be produced for neutral saccharides (79).

Prior to this investigation, a number of pyrolyzers were investigated as to their suitability for carbohydrate analysis. The instruments were judged on criteria such as ease of sample weighing and loading, reproducibility of pyrolysis, and effectiveness of pyrolyzate transfer. Where possible, hardware modifications were made to meet the above demands. Those pyrolyzers that were found to be most suitable are described in section 2.5.

Initial experiments focussed on the pyrolysis of isolated polysaccharides that were representative of the different carbohydrate classes found in wood. The influence of parameters, such as the pyrolysis temperature, inorganic ash and its removal, and ion exchange, were investigated.

Next, the saccharide compositions of a number of wood pulps were characterized by Py-GC. The pulps chosen were both hardwoods and softwoods and were manufactured by a number of different processes. Where necessary, pyrolyzates were identified by Py-GC-MS using both electron impact (EI) and chemical ionization (CI). Here again, the influence of a number of parameters such as methods of ash removal, ion-exchange and suspension pH were investigated. Based on these results, optimized pre-treatment procedures for pulps of differing chemical composition were developed.

Finally, analytical approaches were evolved whereby the saccharide composition of the pulps was quantified by Py-GC. Comparisons were made with results obtained by classical methods of analysis.

CHAPTER 2: EXPERIMENTAL

2.1. Materials

2.1.1. Chemicals

Trifluoroacetic acid was purchased from Sigma Chemicals (St. Louis, Mo.). Analar grade hydrochloric, sulfuric, nitric and acetic acids, and sodium sulfite were purchased from BDH (Toronto Ont.). Certified ACS grade pyridine, methanol, chloroform, dichloromethane, and tetrahydrofuran were purchased from Fisher Scientific (Halifax, N.S.). Sodium borohydride, acetic anhydride, and 1-methylimidazole were purchased from Aldrich (Milwaukee, WI.). All metal chlorides and the Amberlite® ion-exchange resin (H^+ form) were purchased from Canlab (Mississauga, Ont.).

2.1.2. Carbohydrates and Wood Pulps

Amylose, ∝-cellulose, locust bean gum, arabinogalactan, xylan from oat spelts, 1,6 anhydro-ß-D-glucopyranose, and all monosaccharides were purchased from Sigma Chemicals (St. Louis, Mo.). Standard alditol acetate mixtures were purchased from Pierce Chemicals (Rockford, IL). Microcrystalline cellulose (TLC grade) was obtained from J.T. Baker Chemical Company (NJ.). Avicel was obtained from FMC Corporation (Philadelphia, PA). The anhydrosugar standards, 1,4-Anhydroxylopyranose, 1,4-anhydroarabinopyranose, 1,6-anhydromannopyranose, 1,6-anhydrogalactopyranose, and 1,6-anhydroglucofuranose were donated by Dr. A.S. Perlin, McGill University.

The black spruce and birch mechanical pulps and spruce kraft pulps were a gift from Dr. P. Whiting, Abitibi-Price, Ont. The black spruce sulfite pulps were donated by Mr. Gordon Broderick,

Forest Technologies Laboratory, Noranda Technologies Centre, PQ. The aspen steam-explosion pulp was obtained from Dr. B.V. Kokta, CRPP, Université du Quebec à Trois Rivières. The spruce thermal mechanical pulp and the spruce milled wood lignin were a gift from Dr. M. Kleen, Swedish Pulp and Paper Insitute, Stockholm. The birch organosolv pulp was obtained from Mr. J. Moisey, Alcell Co., Newcastle, N.B.

2.2. Hydrolysis / Alditol Acetate Derivatization Procedures

2.2.1. Isolated Polysaccharides

Polysaccharides were hydrolyzed in a manner similar to that described by Honda et al. (81). Samples (5-10mg) were suspended in 2 M trifluoroacetic acid (TFA) in 15 mL round bottomed flasks. The flasks were stoppered after purging with nitrogen for a few minutes. This was done in order to prevent degradation of the monosaccharides by oxygen during hydrolysis. The samples were then placed in a 100°C oven for six hours. After cooling, the samples were evaporated to dryness on a rotary evaporator. A small aliquot of methanol (2 mL) was added to each flask and the hydrolyzates were evaporated to dryness once again. This procedure was repeated until the odour of TFA could no longer be detected (usually three times).

The monosaccharides were analyzed as their corresponding alditol acetates. The hydrolyzates were dissolved in water (3mL) and 1-2 mg of $NaBH_4$ (a reducing agent) was added to each flask. The samples were allowed to stand at room temperature for one hour, at which time the excess borohydride was neutralised by the addition of a few drops of 50% acetic acid. The solutions were evaporated to dryness and the borate was removed from the samples by evaporating three times with small aliquots of 4:1 methanol / acetic acid.

Acetic anhydride (1 mL) and dry pyridine (1 mL) were added to the flasks, which were then stoppered and incubated at 100°C for one hour. After the addition of water (3 mL) the derivatized solutions were evaporated to dryness. Evaporation was repeated three times after the addition of small quantities of methanol (2-3 mL). The derivatized hydrolyzates were dissolved in chloroform (1 mL) and the insoluble material was removed by passage through a glass wool filter. The round bottomed flask was rinsed with 0.5 mL of chloroform which was also filtered and added to the original solution. The chloroform was removed under a stream of nitrogen and the dried derivatives were stored in a freezer until analysis.

2.2.2. Wood Pulps

The hydrolysis procedure followed was that described by Fengel and Wegener for cellulose-containing material with a high lignin content (65). Pulp samples were extracted for four to six hours with an ethanol/benzene (1:1) mixture to remove lipids, waxes, etc. The dried pulps (15-30 mg) were suspended overnight in 5g of anhydrous TFA. After refluxing for one hour, the acid was diluted to 80% with deionised water and refluxed a second time for twenty minutes. The samples were further diluted to 30% and refluxed again for one hour. After filtration through glass wool the solutions were evaporated to dryness on a rotary evaporator. The residual TFA was removed by repeated evaporation with methanol until the hydrolyzates were odour free.

Allose, the internal standard, was accurately weighed into each flask (2-3 mg), and the samples was dissolved in water (3 ml). The hydrolyzates were reduced by the addition of sodium borohydride (5-6 mg). After one hour the residual borohydride was neutralised by the addition of a few drops of 50% aqueous acetic acid. The samples were then evaporated to dryness. The

acetylation procedure is based on that described by Blakeney et al. (82). The reduced saccharides were acetylated at room temperature with acetic anhydride (2 ml) using 1-methylimidazole (0.2 ml) as catalyst. After 10 minutes, water (5 ml) was added to each flask and the samples were allowed to sit until cool. The samples were transferred to 25 ml screw cap test tubes, the reaction flasks were rinsed twice with small aliquots of water, and the rinses were added to the original solutions. The solutions were extracted three times with dichloromethane (2 ml). The dichloromethane extracts were pooled, evaporated to dryness under nitrogen, and stored in a freezer until analysis.

2.3. GC Analysis of Derivatives

The alditol acetate derivatives of the isolated polysaccharide hydrolyzates were dissolved in 0.5 ml of chloroform and analyzed using a Varian 3700 gas chromatograph (Georgetown, ONT.) equipped with J & W DB-225 capillary column (30 m x 0.225 mm, 0.25 μm film thickness, Chromatographic Specialties, Brockville, ONT.) and a flame ionisation detector. The injection port and detector were maintained at 250°C and the oven temperature program was as follows: 200°C for 2 min., 3°Cmin^{-1} until 235°C, hold for 25 min. The column flow was 1 ml/min. of helium with a split ratio of 1:30. The individual alditol acetates were identified by comparing their retention times with those of commercially available standards.

The alditol acetate derivatives isolated from the pulp samples, however, gave unacceptable chromatograms on the DB-225 phase column, most probably due to deterioration in column performance. These samples were separated instead on a CPSIL-19 column (30 m x 0.25 mm, 1.2 μm thickness, Chrompack Canada, Blenheim, Ont.) under similar conditions. Quantification of the individual saccharide derivatives in the pulp samples was made by comparing their peak areas with

that of the internal standard allose. The relative detector response for each monosaccaride derivative with respect to that of the internal standard, allositol acetate, was determined previously.

2.4. Sample Preparation for Pyrolysis

2.4.1. Proton Exchange of Isolated Polysaccharides

Because of the diverse nature of the carbohydrates under investigation no one method for metal ion removal suited all. Water soluble polysaccharides were treated using a strong cation exchange resin (Amberlite H^+ form). An 0.5% w/v solution was usually prepared, the resin was added in a ratio of 10:1 and stirred for 4 hours. The resin was then allowed to settle and the treated solution was decanted. The polysaccharides were recovered from solution either by lyophilisation or, if possible, by precipitation with a non-solvent such as tetrahydrofuran. In the latter process, finely divided product was obtained when the polysaccharide solutions were added slowly to at least twice the volume of non-solvent while vigorously agitating the mixture with a high shear homogeniser. The polysaccharides were then collected by filtration, air-dried to remove most of the volatile solvent and finally oven-dried at 75°C for eight hours.

Water-insoluble carbohydrates such as cellulose were proton-exchanged most effectively by suspending 0.5g of sample in 100 ml of 0.1 N HCl for 4 hours. The sample was maintained in suspension by gentle stirring with a magnetic stir bar. Amylose was proton-exchanged in this manner using 0.1 N HCl in 50 % aqueous ethanol. The solids were then collected by filtration, washed with 200 ml of deionised water (50% aqueous ethanol for amylose) and oven-dried at 75°C for eight hours.

2.4.2. Proton Exchange of Wood Pulps

After some initial pyrolysis investigations, the most suitable method was found to be the following. Pulps were deionised by suspending 0.5g of the dried material in 100 ml of 0.1 N HCl for 4 hours. The samples were then collected by filtration, washed with deionised water (200 ml) to remove any residual acid and oven-dried for 8 hours at 50°C.

2.4.3. pH Adjustment of Acid-Washed Wood Pulps

For the study of the influence of suspension pH on the anhydrosugar yield the procedure described in the last section was modified to include another step. After acid-washing and filtration, the samples were resuspended for 15 minutes in a solution whose pH was adjusted using 0.1 N HCl and NH_4OH solutions. The samples were collected by filtration, washed with a very small quantity of water (20 ml), and oven-dried at 50°C. The final acidity of the isolated samples was not measured.

2.4.4. Cation Exchange of Sulfite Pulps

Sulfite-treated pulps were first suspended in 0.1 N HCl for 2 hours, collected by filtration and washed with a small quantity of deionised water (50 ml). The pulps were then suspended for 2 hours in a 0.1 N solution of the cation under investigation. The chloride salts were used in all cases. The pulps were then filtered, washed with deionised water (500 ml) and dried overnight at 50°C.

2.5. Description of Pyrolyzers

Initial experiments focussed on developing a reproducible and easy to use on-line pyrolysis - GC method. Two types of pyrolyzers were found to most suitable and they are described in the following sections. A third pyrolyzer, the Packard Model 891 Curie Point Pyrolyzer, was also investigated. However, while soluble samples could be coated on the curie point wires, it proved impossible to satisfactorily apply insoluble samples such as wood pulp fibres. Furthermore, with regard to reproducibility and anhydrosugar yield, the resulting pyrograms were inferior to those produced by the other two pyrolyzers. Because of these problems, this pyrolyzer was not used in the following investigations.

2.5.2. CDS Pyroprobe 120

The Pyroprobe 120 (Chemical Data Systems, Oxford, Pennsylvania) consists of a probe fitted with a platinum coil (Fig. 2.1). The sample is placed in a quartz tube fitted with a porous quartz plug which is then inserted into the centre of the coil. The coil is heated resistively to a preset temperature and the pyrolysates are swept by the carrier gas from the tube into the interface oven and finally into the GC injection port (gas flow rate = 13.7 mL/min.). The interface oven is maintained at 250°C in order to prevent condensation of the pyrolysate. The interface design was modified so that the effluent from the pyrolyser passed through a 22 guage needle before entering the injection port (Fig. 2.1). Previously, the pyrolyser was attached to the GC injection port by a 5 cm long, stainless steel, wide-bore tube. The reproducibility between runs was poor, most probably due to incomplete mixing of the pyrolysate with the carrier gas. Also, because the tube was unheated, there was the strong possibility of pyrolyzate condensation on the internal surfaces of the tube. By comparison, the

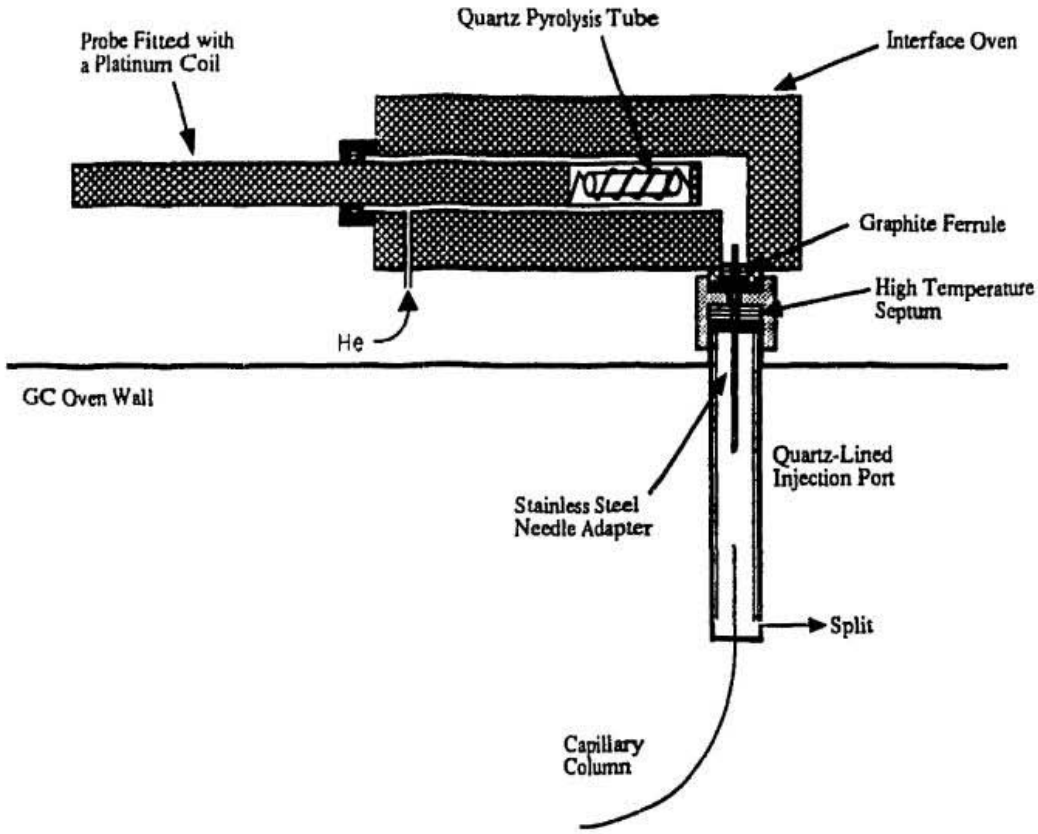


Figure 2.1: CDS Pyroprobe 120[®].

modified design has a very small dead volume and is shorter (1.5 cm in length). Repeated pyrolysis of isolated polysaccharides, such as microcrystalline cellulose, was found to give anhydrosugar yields that vary by 2 % or less.

The samples, usually 100 -150 µg, were weighed into the quartz tube using a Perkin Elmer

Autobalance AD-2Z. The tube was fitted with a small quantity of quartz wool in order to facilitate sample handling. Unless stated otherwise the samples were pyrolyzed at 500°C for 20 seconds and no temperature ramp was used (i.e., maximum heating rate).

2.5.3. SGE Pyrojector®

The Pyrojector (Scientific Glass Engineering, Austin, Texas) consists of a continuously heated furnace lined with a quartz tube (Fig. 2.2). Samples are introduced into the pyrolyzer through a normal GC injection port using a solids injector. The septum is pre-drilled to allow easy passage of the injector barrel. The pyrolyzates are swept into the GC injection port by the carrier gas through a needle interface similar to that described in the previous section (ie., total gas flow rate = 13.7 mL/min.).

For quantitative analysis it is necessary to determine the weight of the sample before pyrolysis, and this proved difficult to do using a solids injector. After some investigation, the most suitible weighing procedure was found to be the following: The injector was disassembled, and the injector barrel was weighed on the Autobalance AD-2Z. The sample (100-150 µg) was introduced into the barrel, which was then reweighed. After reassembly the sample was injected into the pyrolyzer. The pyrolysis temperature was 500°C, unless stated otherwise.

The solids injector supplied with the Pyrojector was found to be suitable for the introduction of most isolated polysaccharides into the pyrolyzer. However, the internal diameter of the injector barrel was too small to accomodate large, fibrous samples such as wood pulps. Therefore, an injector with a larger bore barrel (2 mm, internal diameter) was fabricated. The bore through the injection

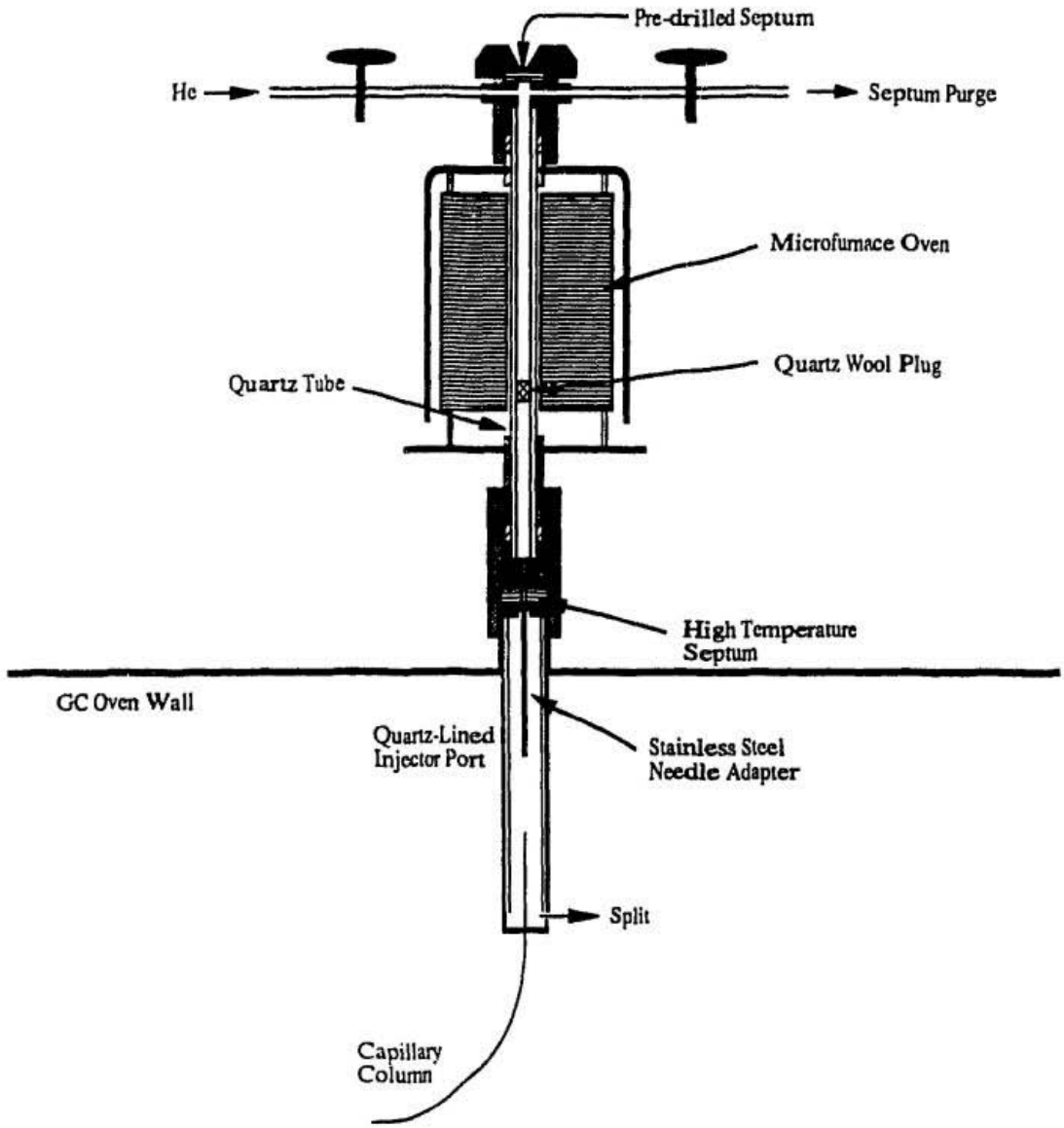


Figure 2.2: SGE Pyrojector[8].

head assembly of the pyrolyser was widened to facilitate the larger injector. The new injector worked well for the pulp samples and the minor modifications did not affect the performance of the pyrolyzer.

2.6. Pyrolysis - Gas Chromatography

Each of the pyrolyzers discussed above was attached to the injection port of a Varian 3700 Gas Chromatograph equipped with a flame ionisation detector and a J & W DB-1701 fused silica capillary column (1 μm thickness, 30 M x 0.329 mm, Chromatographic Specialties, Brockville, ONT.). Poor resolution of the anhydrosugar products was obtained if this column or its equivalent was not used. The injection port and detector were maintained at 270°C. The temperature program settings were as follows: 100°C, hold for 2 minutes, increase 5°Cmin^{-1} until 260°C, hold for 5 minutes. The split was measured at 10.5:1 with a column flow rate of 1.2 mlmin^{-1}. The chromatograms were acquired using a Spectra-Physics SP4290 integrator linked, via a Labnet interface, with a Tandy 1200 HD personal computer, and controlled by a Spectra-Physics Winner® data system (San Jose, California).

2.7. Pyrolysis - Gas Chromatography - Mass Spectrometry

2.7.1. Electron Impact Ionization

Pyrolysis-Gas Chromatography-Mass Spectrometry using electron impact ionization, Py-GC-MS(EI), was carried out by attaching the Pyrojector to the injection port of a Hewlett Packard Model 5790A GC/MSD equipped with a HP 5970A Workstation. The GC was fitted with a normal bore J & W DB-1701 column (0.255 mm x 30 m, 0.5 μm thickness). Many of the pyrograms, especially

the wood pulps, contain thirty or more peaks. The increased resolution obtained by using a narrower bore column facilitated the identification of these peaks. The injection port and MS inlet line were maintained at 270°C. The oven temperature program and split flow rate were as described in section 2.6. The column flow rate was 1 mL/min. The electron impact ionization voltage was 70 eV. The mass spectrometer was scanned from 30 to 250 a.m.u.

2.7.2. Chemical Ionization

The Pyrojector was interfaced with a Varian 3700 GC fitted with a normal bore J & W DB-1701 (0.255 mm x 30 m, 0.5 μm thickness). The end of the capillary column was introduced into the combination EI/CI source of a VG 7070HS double focussing mass spectrometer equipped with a DS 2035 data system. The chromatographic conditions were identical to those described in section 2.7. Ammonia was used as the reagent gas and the ion source pressure was 6×10^{-5} mbar. The temperatures of the column inlet and ion source were 270°C and 200°C respectively. The source was used in the CI mode and the ionization voltage was 100 eV. The mass spectrometer was scanned from 60 to 250 a.m.u. at 1 s per decade.

2.8. Inorganic Analysis

2.8.1. Ashing

The ash content of the polysaccharides and wood pulps was determined using the ASTM procedure for ashing wood (83). The sample (1-2 g) was placed in a preweighed porcelain crucible and was weighed to the nearest 0.1 mg. The crucible was then placed in an oven at 105°C for one hour. After cooling in a dessicator for 30 minutes the crucible + sample was weighed. This

procedure was repeated until the weight remained constant to within 0.1 mg. The final weight measurement is used to determined the true dry weight of the sample. The crucible was then placed on the lip of a 600°C muffle furnace and the contents were allowed to ignite slowly. After 10 minutes, when the majority of the combustible material has been removed, the crucible is moved to the centre of the furnace, which is then closed. After one hour the crucibles are removed, cooled in a dessicator, and weighed. This procedure is repeated until the weight is constant to within 0.1 mg. At every transfer step the crucible is covered with a lid in order to prevent the loss of sample. The ash content is determined as a percentage of the dry weight.

2.8.2. The Determination of Iron in the Acid-washed, Fe^{2+}-Exchanged, Sulfite-Treated Pulps

The ashed samples from the Fe^{2+}-exchanged, sulfite pulps were dissolved in 2 mL of ultrapure HNO_3 with gentle heating if necessary. The solutions were then diluted to 50 mL. The iron concentration was determined using a Perkin-Elmer 2380 atomic absorption spectrophotometer which was set to monitor the absorption at 248 nm. The instrument was calibrated with a commercial standard solution (1-10 ppm). Samples with off-scale absorbances were diluted 1/10 until in range.

2.8.3. Sulfur Content Analysis of the Sulfite Pulps by X-Ray Fluorescence

The sulfite / sulfonic acid content of the sulfite pulps was measured indirectly by determining their sulfur content using an ARL 8420+ wavelength dispersive X-ray fluorescence spectrometer. Though methods do exist for determining the reducible sulfur content in pulps and paper (84,85), they are laborious and sulfite containing samples are not necessarily quantitatively reduced. By comparison X-ray fluorescence spectrometry is fast and accurate, and is routinely used to quantify

the major elemental composition of a wide variety of solid and liquid samples.

Standards were prepared by mixing finely ground Na_2SO_3 with a non-sulfite treated pulp (< 0.1% sulfur). Standard and sample pulps were milled to less than 60 mesh with a Tekmar A-10 analytical mill prior to analysis. The spectrometer was equipped with a Rh anode X-ray tube source operated at 30 kV and 100 mA, and an argon flow proportional counter detector. The sulfur $K_{\alpha 1,2}$ line ($2\Theta = 110.68°$) signal was measured for 20 seconds for each sample. All measurements were background subtracted ($2\Theta = 114.00°$).

CHAPTER 3: PYROLYSIS OF ISOLATED POLYSACCHARIDES

3.1. Glucans

3.1.1. Microcrystalline Cellulose

Microcrystalline cellulose (MC) is a very pure, particulate form of cellulose. It is isolated from ∝-cellulose after intensive mechanical and acid treatment (86). It has a low molecular weight (i.e., degree of polymerization, DP_n, of 200). MC from two different sources were investigated: Avicel® PH-101 from FMC and TLC grade MC from Baker. Here, as with all the following polysaccharides, the samples were pyrolyzed using the CDS Pyroprobe®.

Figure 3.1(a) is the pyrogram obtained by pyrolyzing Avicel MC at 600°C. The pyrolyzates were identified either by comparison of their retention times with those of authentic standards or by their EI mass spectra. Table 3.1 lists only the important carbohydrate pyrolyzates. Further discussion on the identification of pyrolyzates can be found in section 4.4. The optimum pyrolysis temperature for levoglucosan production was found to be between 550 and 600°C. The Avicel MC pyrogram is remarkable for its simplicity, especially when compared with that obtained for the same cellulose by Pouwels et al. (76) who used Curie-Point Py-GC analysis. Levoglucosan, **73**, dominates the pyrogram and 1,6-anhydro-glucofuranose, **85**, is also present, though in smaller quantities. By comparing the detector response with that of pure levoglucosan standard it has been determined that 51.6% of the pyranose units in the cellulose sample were converted into the two anhydrosugar products (Table 3.2). It was observed that an oily, non-volatile residue condensed at the end of the pyrolysis tube and its presence would account for much of the remaining pyrolyzate. No char remained in the tube after pyrolysis.

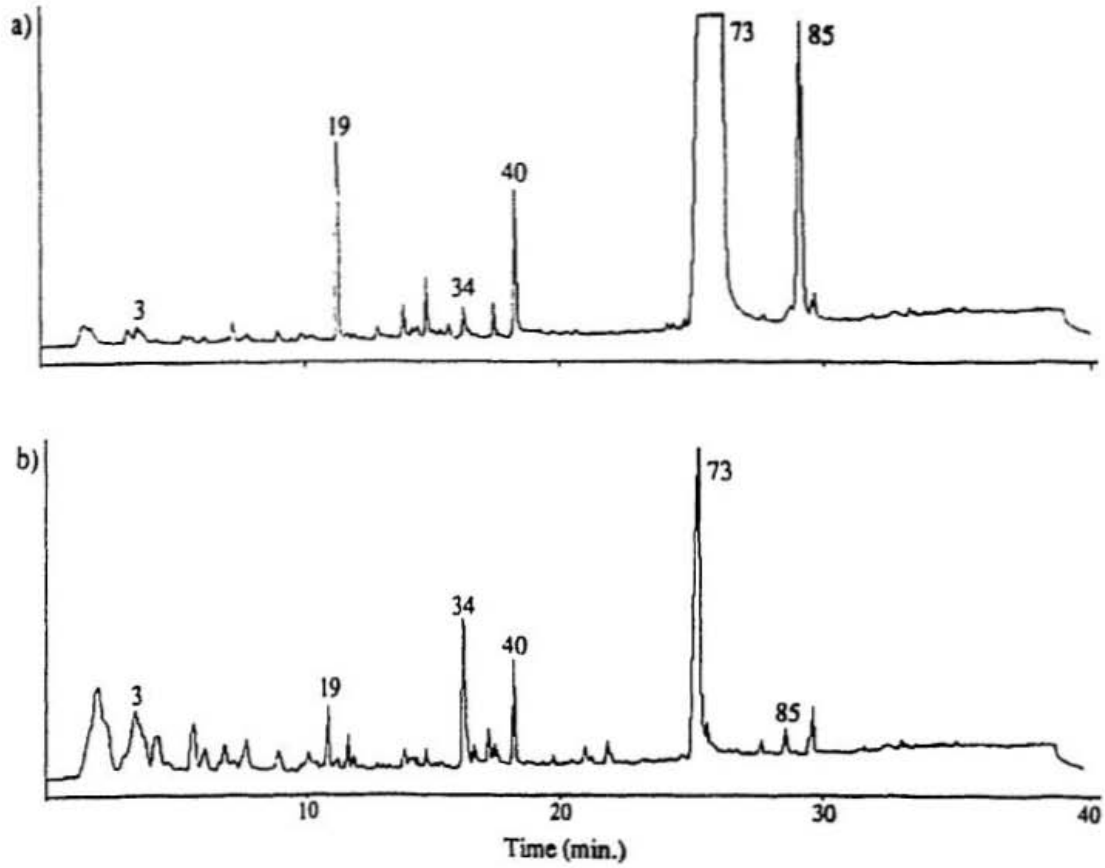


Figure 3.1: Pyrograms of (a) Avicel Microcrystalline Cellulose and (b) Baker MC treated with 0.05 M KCl.

Baker microcrystalline cellulose gives a pyrogram similar to that obtained for Avicel. However, the anhydrosugar yield is significantly lower (36.1%). One possible explanation for this result is that Baker MC contains a small quantity of inorganic material (0.07% ash) whereas Avicel contains almost none. After undergoing a mild acid wash as described in section 2.4.1, the % ash was reduced to near zero and the anhydrosugar yield for Baker MC rose to 51.0%, which is very close to that of Avicel MC (Table 3.2). Further acid treatment of either sample failed to increase the yield of anhydrosugars. This yield seems to represent the maximum obtainable for a pure

Table 3.1: Identification of the Important Carbohydrates Pyrolyzates Observed in the Pyrograms of the Isolated Polysaccharides (see Section 4.4).

Peak No.	Pyrolyzate Identification
3	2-Furaldehyde
8	4-Hydroxy-5,6-dihydro-(2H)-pyran-2-one
19	Levoglucosenone
31	1,4-Anhydroarabinopyranose
34	5-Hydroxymethyl-2-furaldehyde
40	1,4-Dideoxy-D-glycero-hex-1-eno-pyranos-3-ulose
42	1,4-Anhydroxylopyranose
60	1,6-Anhydrogalactopyranose
65	1,6-Anhydromannopyranose
70	1,4-Anhydrogalactopyranose
73	1,6-Anhydroglucopyranose (Levoglucosan)
84	1,6-Anhydrogalactofuranose
85	1,6-Anhydroglucofuranose

polysaccharide under the specified experimental conditions. Moreover, this result is very reproducible which is important if quantitative results are to be obtained from Py-GC.

Though this is not conclusive proof, it does seem to suggest that, even at low % ash content, ash removal is very important for the optimum and reproducible production of anhydrosugars. When acid-washed Baker MC was treated with 0.05 M KCl the anhydrosugar yield was greatly reduced (Fig. 3.1b). In addition, a large quantity of char remained in the pyrolysis tube. This result agrees with the findings of other researchers, that alkali metals catalyze the char-forming reactions during pyrolysis (78,87).

Table 3.2: Yields of the Glucose Anhydrosugars for the Glucans.

Glucan	Treatment	Peak Area / Microgram[1]	% Yield[2]	% Ash
Avicel MC	None	25149	51.6	0.00
"	Acid-Washed	25318	51.9	0.00
Baker MC	None	17610	36.1	0.07
"	Acid-Washed	24894	51.0	0.00
Amylose	None	5275	10.8	1.22
"	Acid-Washed	24460	50.2	0.00

1 : Measured as the sum of the peak areas of both levoglucosan and 1,6-anhydroglucofuranose divided by the weight of the sample (assuming 100% glucose). Average of duplicate runs.

2 : Calculated as the percentage conversion of the glucose units into anhydrosugars. Pure 1,6-anhydroglucopyranose was used as standard.

3.1.2. Amylose

Amylose is an ∝-1-4 linked glucan found in many higher order plants. This sample was isolated from potato and has a DP_n of approximately 3200 (88). Pyrolysis of the untreated sample gives a pyrogram (Fig. 3.2a) very different from that of Avicel MC. The small molecular weight pyrolyzates are dominant and the anhydrosugar yield is very low (Table 3.2). However, following treatment with 0.1 N HCl in 50% aqueous ethanol as described in section 2.4.1, the pyrogram (Fig. 3.2b) is very similar to those of the ashless microcrystalline celluloses. More importantly, the anhydrosugar yield increases to 50.2%. Here also there is a clear relationship between the removal of the inorganic materials and the increase in the production of anhydrosugars (Table 3.2).

These results suggest that the anhydrosugar yields from the glucans are not dependent on source, degree of polymerization, or anomeric configuration. It has been proposed that the initial reaction at the onset of pyrolysis is a reduction in DP_n of the polysaccharide to about 200 (73,90). This would explain why the longer polymer chain length in amylose does not affect the pyrolysis. The fact that the different anomeric linkage seems to have no effect lends credence to reaction mechanisms such as that described in Figure 1.8 (73). In this mechanism the pyranose unit that eventually forms the anhydrosugar does not retain the glycosidic oxygen. Therefore the original anomeric configuration is irrelevant.

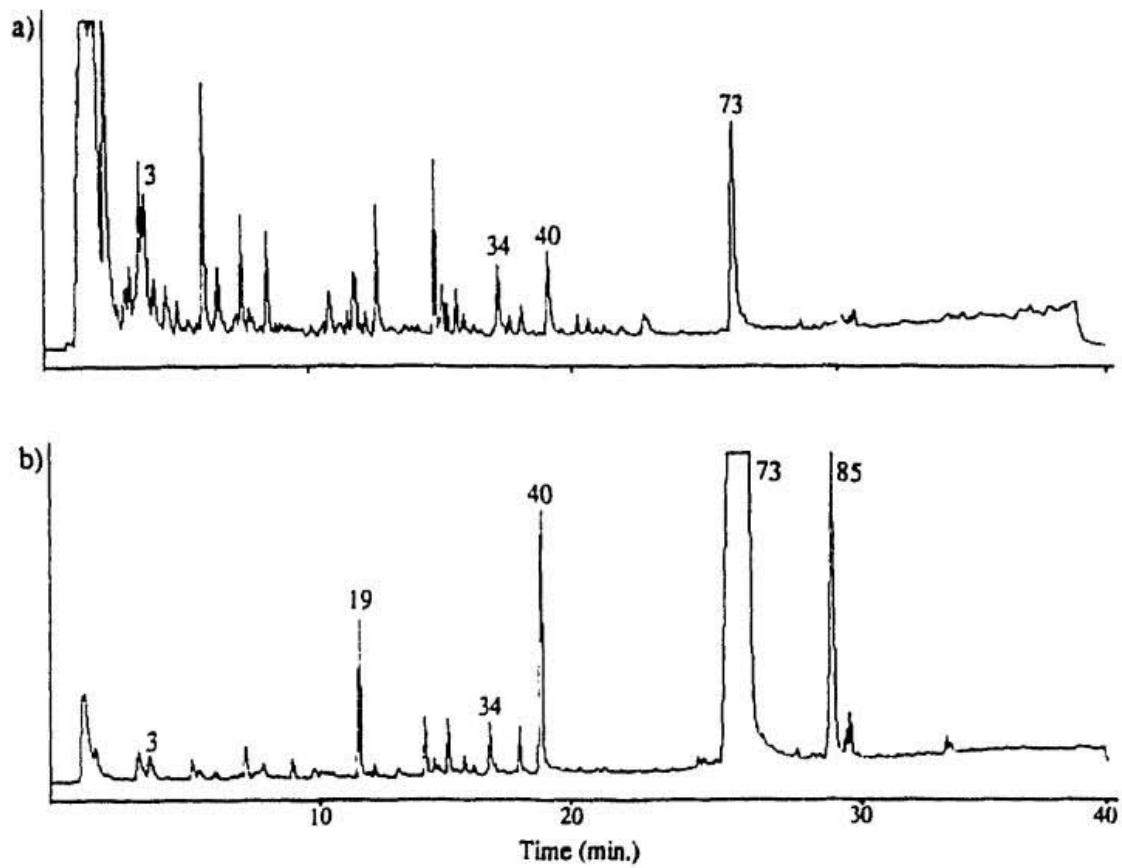


Figure 3.2: Pyrograms of Amylose (a) Untreated and (b) Acid-Washed.

3.2. Heteropolysaccharides

3.2.1. Locust Bean Gum

Locust bean gum is a galactomannan found in the seed of the tree, *Ceratonia Siligua*. It consists of a linear chain of ß-1-4 linked mannopyranose units, with side groups of single galactopyranose units attached at the C_6 position of every third or fourth mannose (87). It has a DP_n of approximately 1500.

Pyrolysis of the untreated gum gave the pyrogram illustrated in Figure 3.3a. Yields of all chromatographable pyrolyzates were low. However, proton-exchange of a solution of the polymer (section 2.4.1) reduced the ash content from 0.99% to 0.19%. This greatly improved the anhydrosugar yields from both mannose and galactose (Fig. 3.3b). Either method of isolation (i.e., freeze-drying or non-solvent precipitation) were equally effective. The optimum pyrolysis temperature for anhydrosugar production was found to be 450^{0}C, which is considerably lower than that for cellulose. This is not surprising as it has been demonstrated that the hemicellulosic and pectic substances in wood, some of which closely resemble locust bean gum (i.e., the galactoglucomannans), begin to thermally decompose at low pyrolysis temperatures (11).

The ratio of the peak areas of 1,6-anhydromannopyranose, **65**, and the sum of the areas of the galactose anhydrosugars, **60**,**70** and **84**, was approximately 4:1. This anhydrosugar ratio was in reasonably good agreement with saccharide composition obtained using acid hydrolysis - derivatization - GC analysis (i.e., 3.5:1). Glucose and arabinose residues are present in trace quantities in the gum and their anhydrosugars were also observed in the pyrogram.

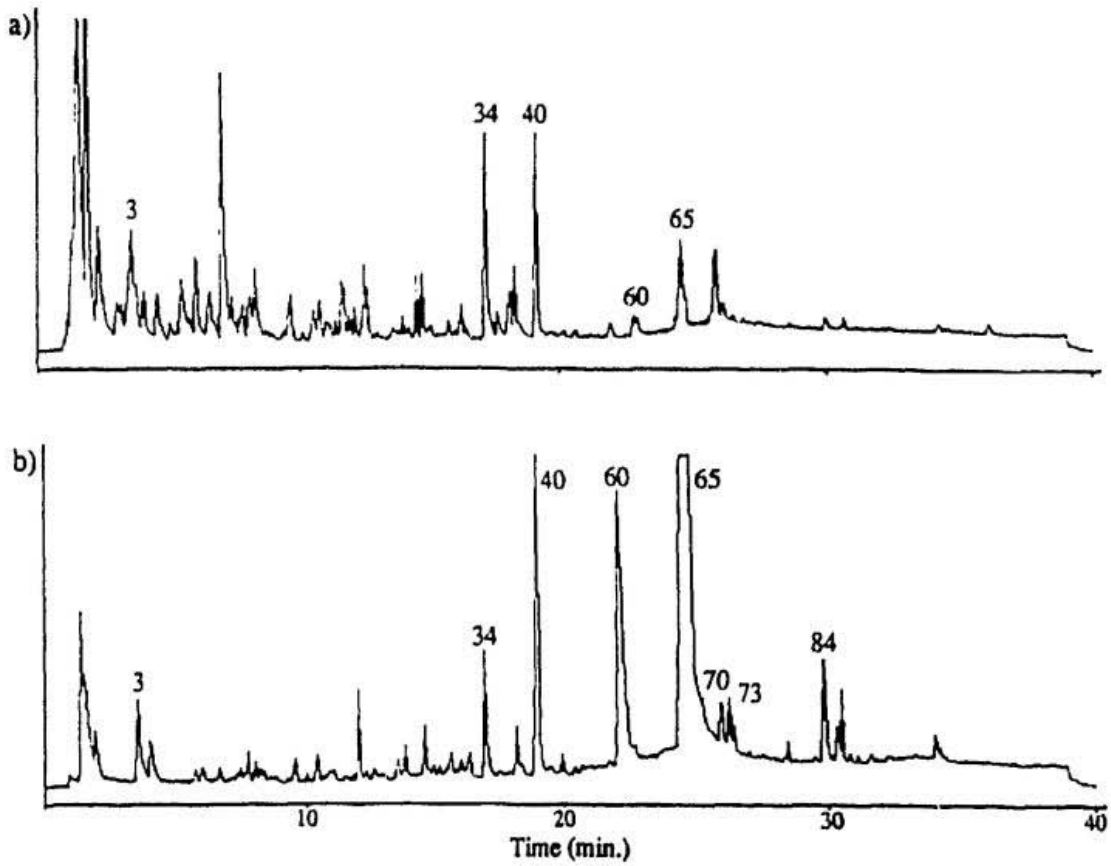


Figure 3.3: Pyrograms of Locust Bean Gum (a) Untreated and (b) Proton-Exchanged.

However, even under optimum conditions, the percent conversion rate into anhydrosugars was not as large as for the ashless glucans (36.1% for mannose and 34.8% for galactose). The fact that the polysaccharide is not entirely ash free may be responsible for this. However, it was observed that the polymer melted during pyrolysis. Anhydrosugars formed within the melt would be unable to volatilise quickly out of the pyrolysis zone thus allowing secondary reactions to occur. Melting was not observed with the glucans.

3.2.2. Arabinogalactan

The arabinogalactan used here is isolated from larchwood. It consists of a linear chain of ß-1-3 linked D-galactopyranose units with sidegroups, composed of L-arabinofuranose and D-galactopyranose, attached at the C_6 position (90). From acid hydrolysis it has been determined that the galactose to arabinose ratio is approximately 5:1. Upon pyrolysis, the untreated polysaccharide charred extensively and gave a pyrogram with only low molecular weight peaks. However, chromatographic peaks corresponding to the anhydrosugars of arabinose and galactose were clearly present in the pyrogram of an arabinogalactan sample which had been previously allowed to undergo proton exchange using the same procedure as described for locust bean gum (Figure 3.4).

Despite the fact that the proton-exchanged sample contains very little ash, the conversion rate of the saccharide units into anhydrosugars is again low, especially for arabinose (9.1% for arabinose and 20.5% for galactose). This is in contrast with the results obtained by Essig et al. (91). They reported a 79% conversion rate for arabinose in acid-washed corn bran using low temperature, vacuum pyrolysis (260-300°C). In this present study, however, it was found that the best conversion rate was achieved at a pyrolysis temperature of 400°C.

These low yields may have a number of causes. It is known that arabinose decomposes very readily at low pyrolysis temperatures (92), and it is probable that the Py-GC used here failed to remove the arabinose products from the pyrolysis zone rapidly enough to prevent secondary reactions. Furthermore, it was observed that, like locust bean gum, the arabinogalactan melts readily even at low pyrolyis temperatures.

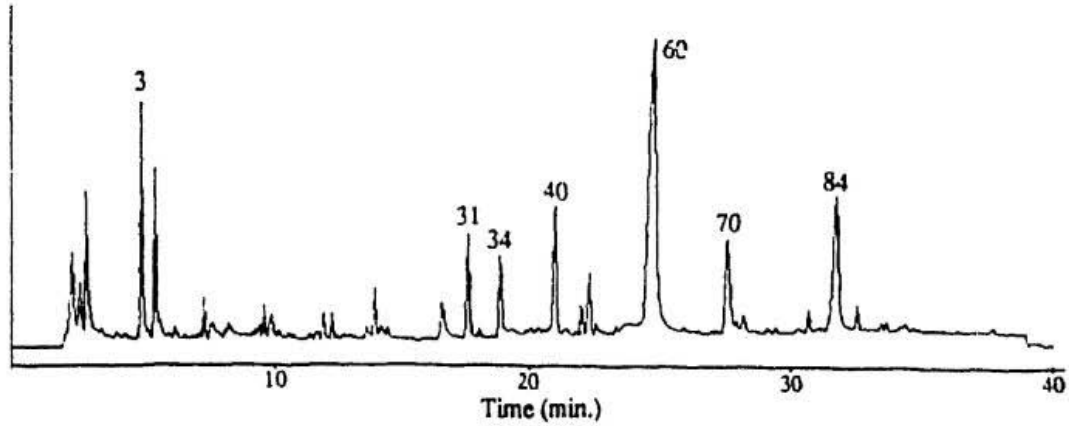


Figure 3.4: Pyrogram of Proton-Exchanged Arabinogalactan.

3.2.3. D-Xylan

D-Xylans are a group of plant polysaccharides that consist primarily of a linear chain of β-1-4 linked D-xylopyranose units. This particular sample, xylan from oat spelts, has side groups of arabinose, glucose, and glucuronic acid (most likely as the 4-O-methyl ester) attached at the C_2 and C_3 positions (90). The ratio of neutral saccharides was determined by acid hydrolysis to be approximately 1.00 : 0.10 : 0.12 for xylose, arabinose, and glucose respectively. Xylose makes up almost 80% of the treated polymer.

The untreated sample has a very high ash content (4.6%) and therefore it is not surprising that only trace quantities of anhydrosugars were observed (Fig. 3.5a). Treatment with H^+ exchange resin reduced the ash to 0.09% and there was a corresponding increase in the production of the anhydrosugars for all three neutral saccharides (Fig. 3.5b). The optimum pyrolysis temperature was

proposed that this pyrolyzate is formed via the intermediate formation of glucuronolactone. It is possible that 4-O-methyl-glucuronic acid, like galactouronic acid, is unable to form a lactone and therefore this pathway is unavailable to it. In fact galactouronic acid, both in monosaccharide and polymeric form, did not produce any unique chromatographable pyrolyzate.

The percentage rate of conversion of the saccharides components of xylan into anhydrosugars via Py-GC was low and varied for each neutral saccharide (10.5%, 19.0%, and 26.5% for xylose, arabinose, and glucose respectively). Here again, as with the other heteropolysaccharides, the xylan sample was observed to melt and char during pyrolysis. The yield of 1,4-anhydroxylopyranose, **42**, is especially low and may be partly due to the fact that some units are attached to uronic acids.

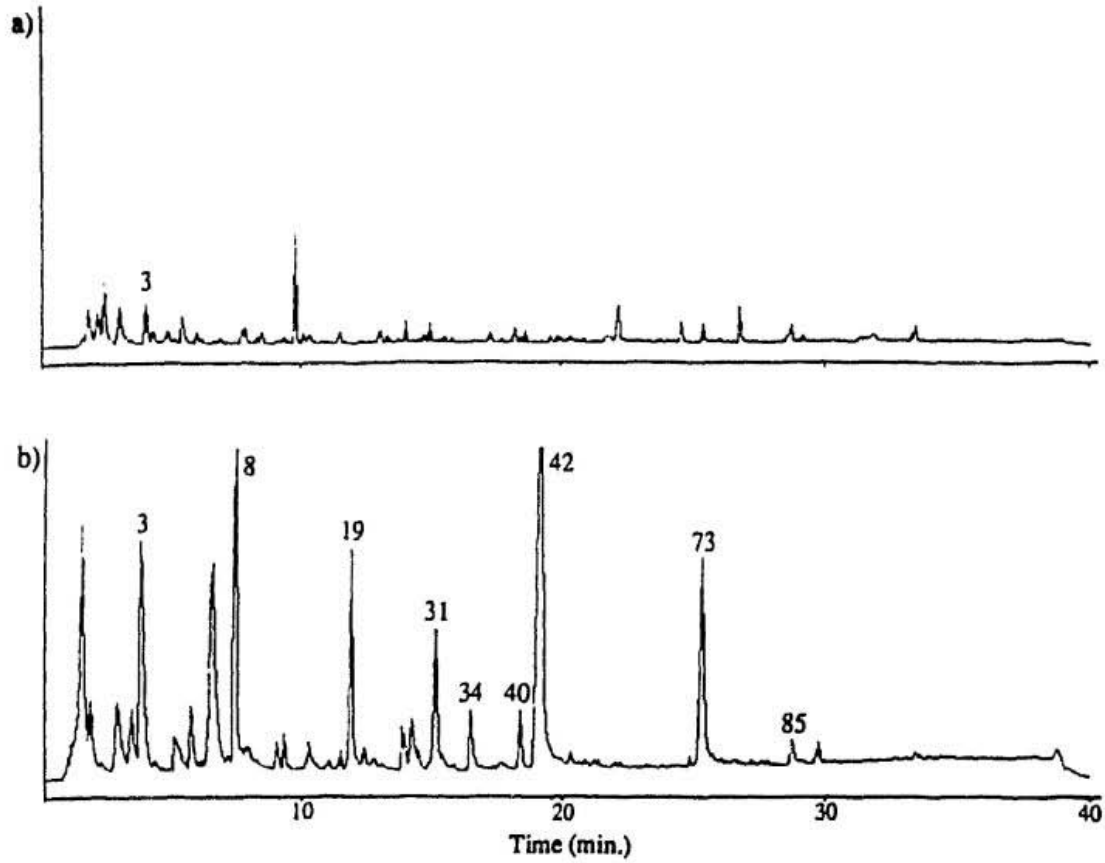


Figure 3.5: Pyrograms of Xylan from Oat Spelts (a) Untreated and (b) Proton-Exchanged.

found to be 400 - 450°C. 4-Hydroxy-5,6-dihydro-2H-pyran-2-one, **8**, was identified in the pyrogram by it's EI mass spectrum. It is known to be unique to the pyrolysis of polymeric xylopyranose units (93).

The uronic acid content of a number of oat xylans has been determined to be approximately 3% (94,95). However, no chromatographable pyrolyzate was observed that could be attributed exclusively to 4-O-methyl-glucuronic acid. This contrasts with the fact that glucuronic acid forms a unique pyrolyzate, tentatively identified as 1-deoxy-glucofuranosyl-urono-6,3-lactone (43). It was

CHAPTER 4: PYROLYSIS OF WOOD PULPS

4.1. Introduction

It has been recognised that analytical pyrolysis can provide a fast and inexpensive alternative for the chemical characterization of wood and wood products (45,96,97). In particular, analytical pyrolysis has proven to be an excellent method for the characterization of lignins (44,98,99,100). However, in most cases, the analytes are pyrolyzed with little or no pretreatment. It is clear from the pyrolysis of the isolated polysaccharides that even trace quantities of some inorganic ions can seriously affect the nature and yield of the pyrolysis products. In the following sections the influence of sample pretreatment on the pyrolysis of wood pulps will be investigated, with emphasis on anhydrosugar formation from the constituent polysaccharides.

All pulps were received as well separated fibres or particles of less than 1mm thickness. Therefore no further size reduction was considered necessary. The samples, original and treated, were oven-dried at 50°C for 8 hours prior to pyrolysis. The following pyrograms were obtained by pyrolyzing the pulps (150 ± 10 µg) with the Pyroprobe as described in section 2.5.2. The fibres were teased apart prior to weighing in order to minimize the creation of temperature gradients during pyrolysis and to prevent the retention of volatile pyrolyzates within the sample. The identity of the major carbohydrate pyrolyzates observed in the pyrograms of the wood pulps are listed in Table 3.1 in the previous chapter.

4.2. Influence of Deashing

A study was conducted to determine the optimum method of lowering the ash in black spruce

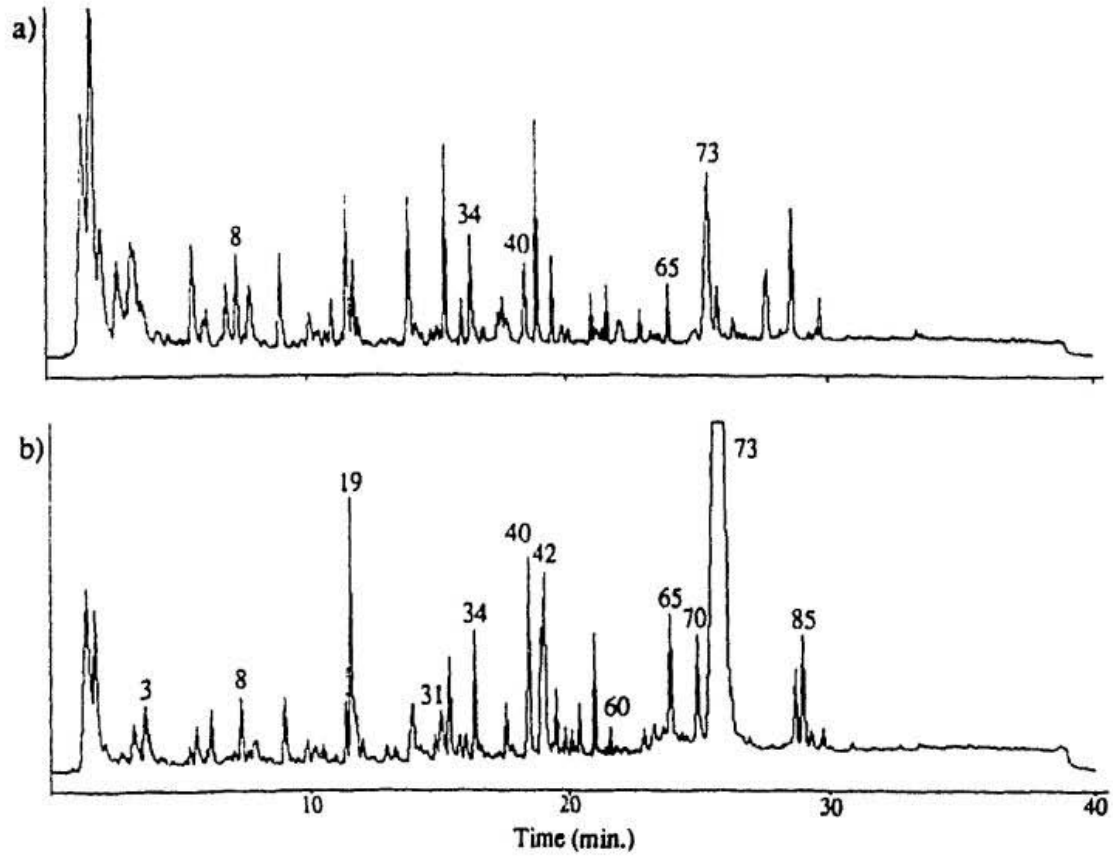


Figure 4.1: Pyrograms of Black Spruce Mechanical Pulp (a) Untreated and (b) Acid-Washed.

mechanical pulp. Washing the pulps with deionised water or 0.05 N HCl failed to remove much of the ash. Proton exchange of an aqueous suspension of the pulp with a strong H^+ exchange resin proved a little more effective. However, washing with 0.1 N or 0.5 N HCl proved to be the most effective. It was found that there was little or no difference in the pyrogram profiles of pulps acid-washed with either concentration. Therefore it was decided to use 0.1N HCl in the following studies. The procedure followed is as described in section 2.4.2.

4.2.1. Black Spruce Mechanical Pulp

This is a high yield, stone ground pulp. Much of the constituents of the original wood are retained. As might be expected, pyrolysis of untreated pulp gave a complicated pyrogram (Fig. 4.1a). It is dominated by the low molecular weight pyrolyzates and only the glucose anhydrosugars are readily identifiable. Washing the pulp with 0.1N HCl, as described in section 2.4.2, reduced the ash content of the pulp from 0.45 to 0.05 % and also changed the pyrogram profile quite significantly

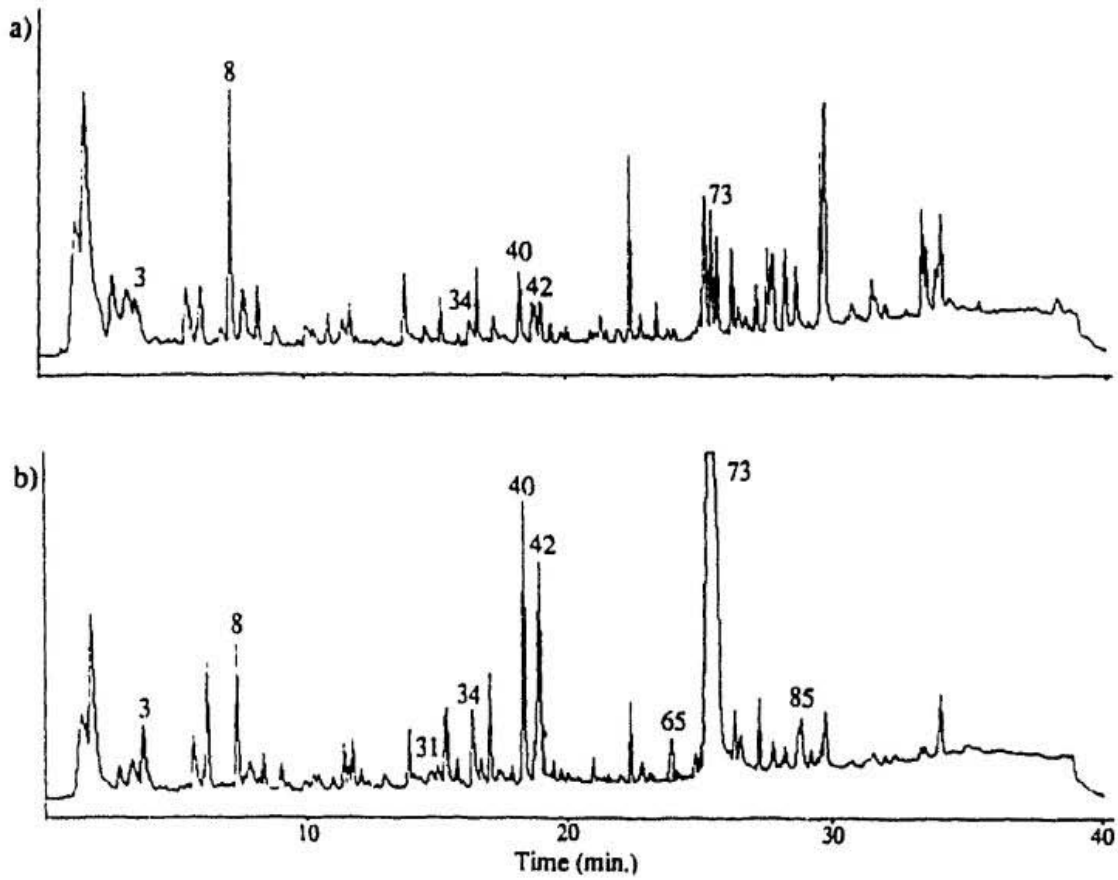


Figure 4.2: Pyrograms of Birch Mechanical Pulp (a) Untreated and (b) Acid-Washed.

(Fig. 4.1b). Levoglucosan, **73**,is now the dominant pyrolyzate and the volatile frontal peaks are greatly reduced in size. In addition, the anhydrosugars of xylose, **42**, arabinose, **31**, mannose, **62**, and galactose, **60** and **70**, are observed. Black spruce is a softwood and its hemicellulose is composed of xylans and galactoglucomannans. This is clearly reflected in the pyrogram.

4.2.2. Birch Mechanical Pulp

This pulp was manufactured in the same manner as the black spruce pulp. The pyrograms before and after acid treatment are illustrated in Figure 4.2. Here again there is a dramatic increase in anhydrosugar production after washing. There is also a corresponding decrease in the ash content of the pulp (0.29 to 0.04 %). Being a hardwood, birch hemicellulose is composed mainly of xylan and contains only small quantities of glucomannans. This is confirmed by the size of the respective anhydrosugar peaks, **42** and **65** in the pyrogram..

4.2.3. Black Spruce Kraft Pulp

The kraft process has been discussed in section 1.2.3. Kraft pulps are usually extensively delignified and much of the hemicellulose content is also removed (low yield pulp). Once again acid washing greatly enhances anhydrosugar formation and decreases the size of the volatile frontal peaks (Figure 4.3). The cellulose content of this pulp is almost 90% and the large size of the anhydroglucose peaks, **73** and **85**, reflect this. Lignin and hemicellulose make up the remaining 10%. Though galactoglucomannans are preferentially lost during processing, the presence of 1,6-anhydromannopyranose, **65**, in the pyrogram indicates that removal was not complete. However, there are no galactose anhydrosugars which suggests loss of pendant groups. These results were

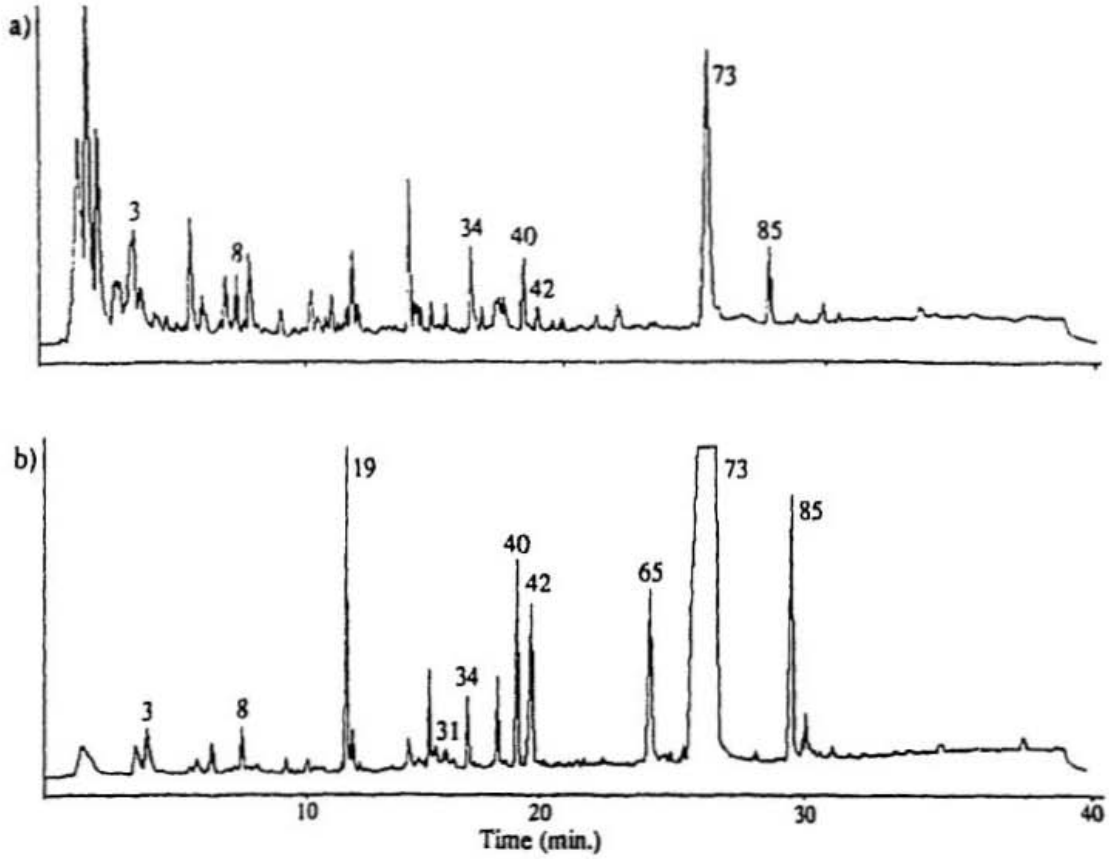


Figure 4.3: Pyrograms of Black Spruce Kraft Pulp (a) Untreated and (b) Acid-Washed.

confirmed by acid hydrolysis - derivatization / GC. The presence of 1,4-anhydroarabinopyranose, 31, has been confirmed by mass spectrometry (both EI and CI), but only in trace quantities.

In contrast to the previous two pulps, the ash content remains relatively high (0.33% in the acid washed pulp). This may be due to the inability of the acid solution to access all regions within the fibres or to the presence of inert material (e.g., silica). In either case it does not seem to affect the pyrolysis process.

4.2.4. Alcell® Birch Organosolv Pulp

The Alcell® process has been discussed previously (section 1.2.3). This pulp is also extensively delignified. Acid washing significantly improved the yield of the glucose, **73** and **85**, and xylose, **42**, anhydrosugars (Fig. 4.4). Traces of anhydroarabinose, **31**, and anhydromannose, **65**, were also observed. The similarity of the pyrograms of the Alcell and kraft pulps is remarkable considering that two different wood species and two different pulping processes were used.

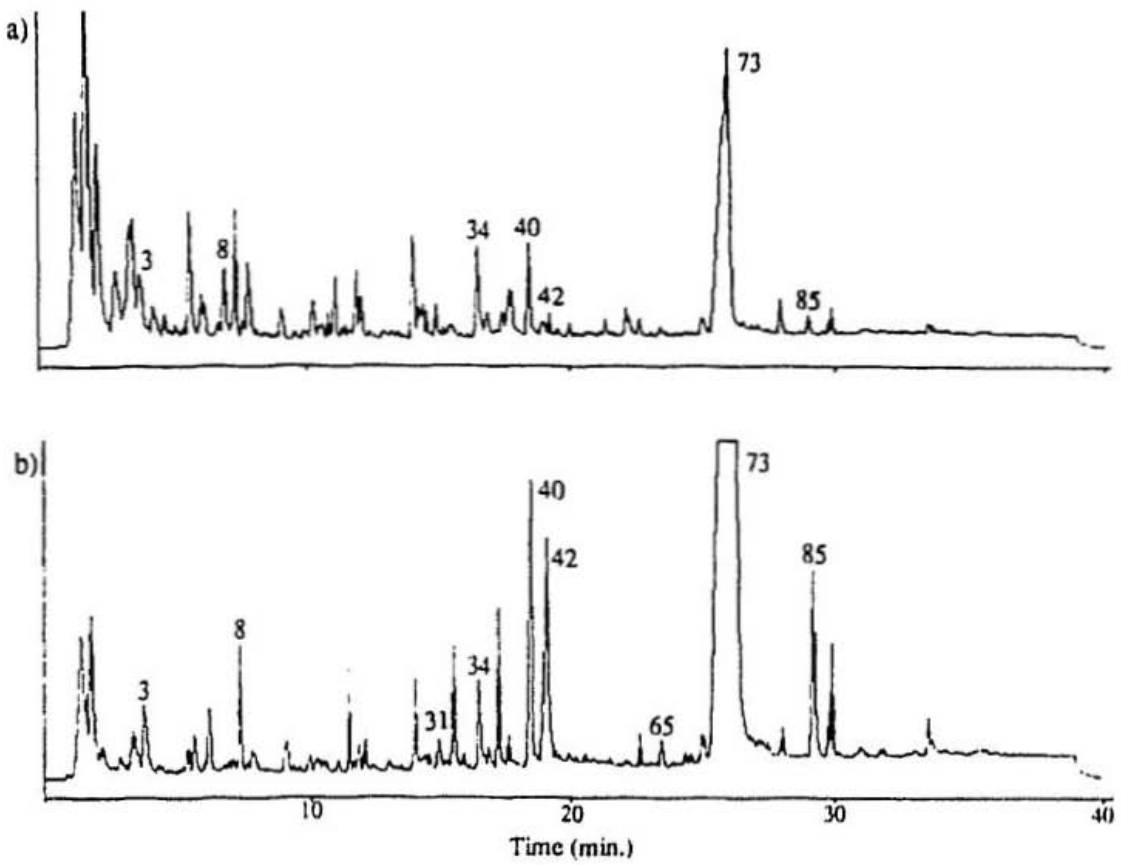


Figure 4.4: Pyrograms of Birch Alcell® Pulp (a) Untreated and (b) Acid-Washed.

4.3. Reintroducing Metal Cations - The Effect on Pyrolysis

Acid washed black spruce kraft pulp was resuspended in dilute solutions of potassium, calcium, or iron (II), as described in section 2.4.4. The pyrolysis profile of the potassium-exchanged pulp is completely different from that of the acid washed (Fig. 4.5a). Those pyrolyzates eluting after approximately 11 minutes in the acid-washed pyrogram are almost entirely eliminated in this case. It was also observed that there was a significant increase in the amount of char remaining in the pyrolysis tube. Such a result is in keeping with the findings of other researchers, that alkali metals promote the char forming reactions at the expense of those leading to anhydrosugar formation (73,78).

In contrast, the pyrogram profiles of the calcium- and iron-exchanged pulps (Fig. 4.5b,c) are not radically different from that of the acid-washed pulp. Differences, however, do exist in the yields of anhydrosugars with the best results being obtained for acid-washed pulp. Essig et al. observed a similar trend for levoglucosan, **73**, in the pyrolysis of cellulose containing the chloride salts of sodium and magnesium (73). Recently, Richards et al. carried out a more comprehensive study on the pyrolyzates obtained from the pyrolysis of wood exchanged with a variety of metal ions (101). Here again they found that the poorest yields for the anhydroglucoses were obtained with alkali metals, followed by alkali earth, and transition metals, with Fe (II)-exchanged pulps giving the best overall yield.

4.4. The Influence of Suspension pH on the Pyrolysis of Wood Pulps

Acid-washed black spruce kraft and mechanical pulps were resuspended in solutions of

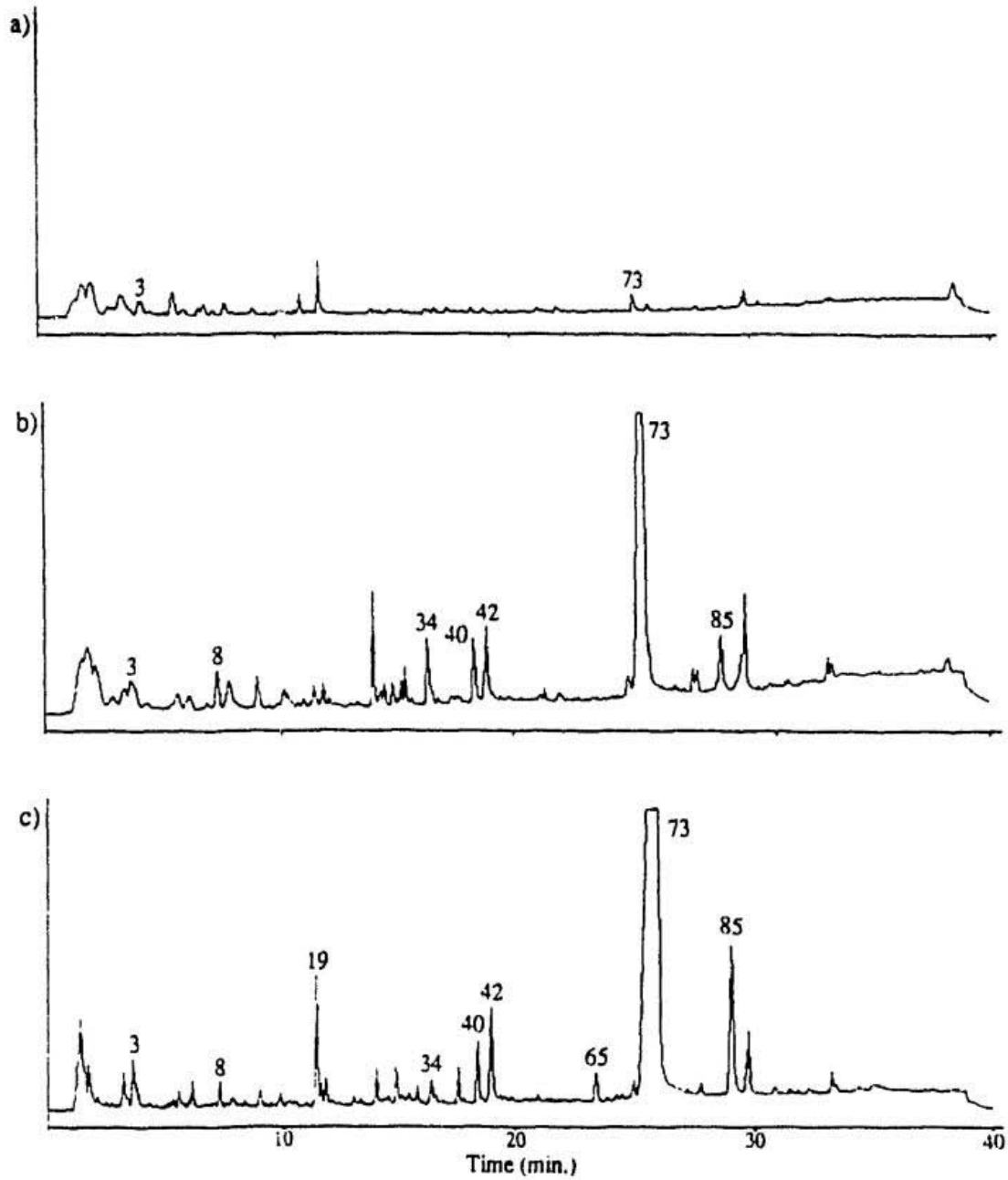


Figure 4.5: Pyrograms of Acid-Washed Black Spruce Kraft Pulp Treated with (a) 0.1 N KCl, (b) 0.1 N $CaCl_2$ and (c) 0.1 N $FeCl_2$.

differing pH and then isolated as described in section 2.4.3. These pulps were pyrolyzed to examine the influence of the suspension pH of the production of anhydrosugars. The suspension pH was plotted against the peak area for the combined anhydroglucoses, **73** and **85**, and for anhydroxylose, **42**, per microgram of pulp pyrolyzed (Fig. 4.6a and Fig.4.6b). Anhydrosugar production was maximized when the suspension pH was approximately 1. As the pH was increased, the anhydrosugar yield dropped quickly and levelled off at approximately 10% the maximum attainable value. Over a number of days, those pulps treated with very acidic solutions (pH's 0.1 and 0.45) began to degrade and the anhydrosugar yield upon pyrolysis dropped to zero. However, pulps suspended in solutions at pH 1 or above remained stable over the same period.

It has been proposed that the production of levoglucosan from cellulose occurs via an acid catalyzed pathway such as that described in Figure 1.8 (73). Such a hypothesis would explain the phenomenon observed here. To test this theory, Avicel MC and acid-washed ∝-cellulose were also suspended in solutions of differing pH. In this instance, however, anhydroglucose production was relatively constant for both samples over the pH range examined (Fig. 4.7a). ∝-Cellulose also contains a small amount of xylan and the yield of 1,4-anhydroxylopyranose is plotted in Figure 4.7b. Here again, there is very little variation over the pH range examined.

While these results do not discount the possibility of an acid catalyzed pathway for anhydrosugar production, they do suggest that other factors are responsible for the phenomenon observed here. Lignin is present to a greater or lesser extent in all the pulps under investigation and it may be that this polymer can interfere in the pyrolysis of polysaccharides. Such a theory may be quite plausible if free radicals are formed during lignin pyrolysis as was tentatively proposed by Evans et al. for the formation of coniferyl alcohol (99). The possibility of lignin interference was

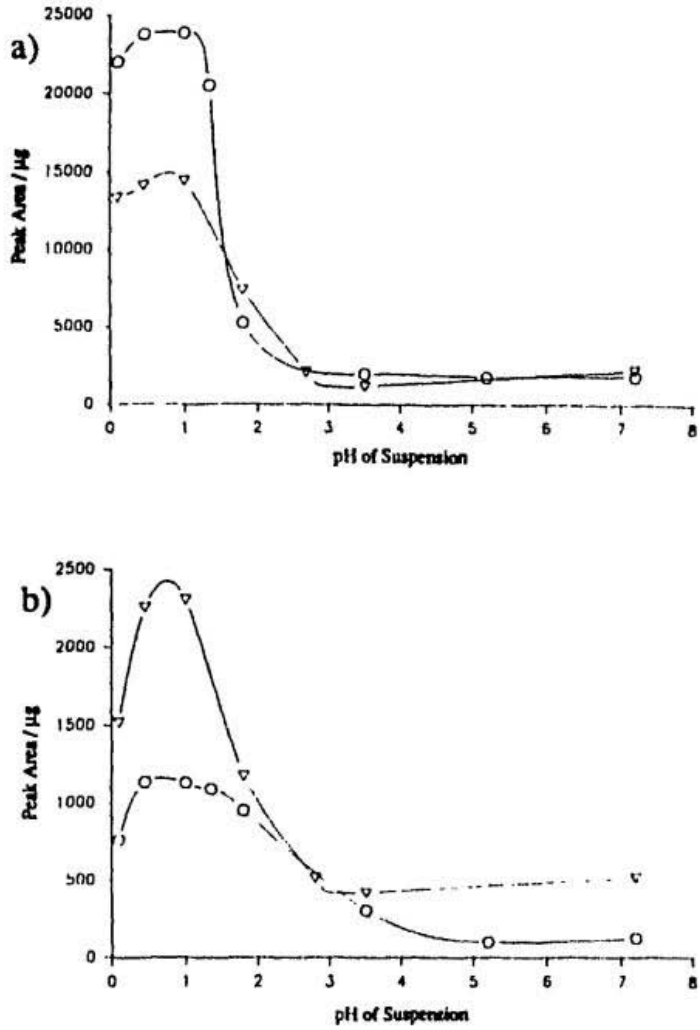


Figure 4.6: The Influence of Suspension pH on the Anhydrosugar Yields for Black Spruce Mechanical (▽) and Kraft (○) Pulps. (a) Anhydroglucoses and (b) 1,4-Anhydroxylopyranose.

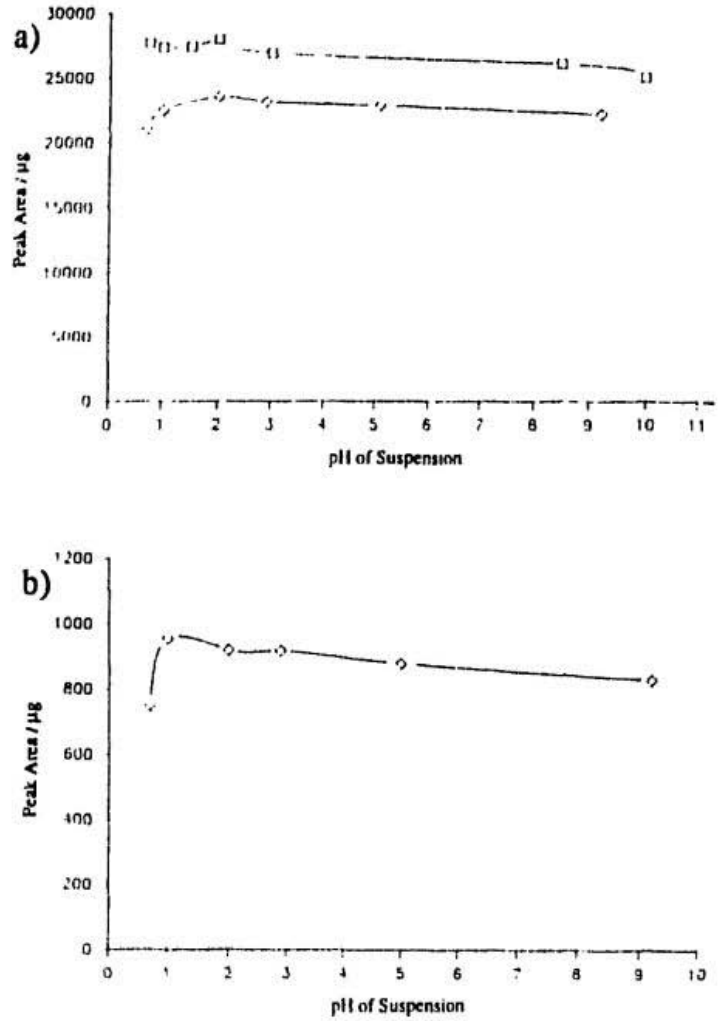


Figure 4.6: The Influence of Suspension pH on the Anhydrosugar Yields for Avicel Microcrystalline Cellulose (□) and ∝-Cellulose(◇), (a) Anhydroglucoses and (b) 1,4-Anhydroxylopyranose.

proposed previously by Shafizadeh et al. to explain the relatively low yield of levoglucosan from acid-washed cottonwood (102). This theory was discounted when cottonwood lignocellulose gave excellent yields of levoglucosan after extensive washing with deionised water. However, it was not taken into account that the lignocellulose was isolated by digestion in 1% sulfuric acid at high temperature and pressure. These aggressive conditions could easily have changed the chemical composition of the remaining lignin and effect the way in which the polymer pyrolyzes.

Milled wood lignin is isolated from wood without the use of acids or bases (103) and is considered to be reasonably similar in structure and composition to native lignin. A sample obtained from sprucewood was pyrolyzed before and after being treated with 0.1N HCl for two hours (Fig. 4.8a,b). While the pyrolysis profile was not altered radically, the pyrolyzate yields were significantly reduced by acid washing. This does not prove that lignin interferes with the pyrolysis of polysaccharides, but it does show that lignin pyrolysis is affected by acid washing, possibly due to the presence of trace quantities of acid in the sample.

Finally, it was reported in section 4.2 that the pyrogram profile of Fe^{2+}-exchanged kraft pulp is very similar to that of the acid-washed pulp, and that Richards et al. found that Fe^{2+}-exchanged wood gave good yields of the anhydroglucoses (101). It is their hypothesis that the metal ions prevent lignin from interfering in the production of levoglucosan from cellulose. It is possible, therefore, that acids also block lignin interference.

Because our present understanding of polysaccharide and lignin pyrolytic mechanisms is poor, it is not possible to explain the exact cause of the pH dependency. However, our results may help to begin to explain the low yields of levoglucosan obtained from acid-washed wood that has

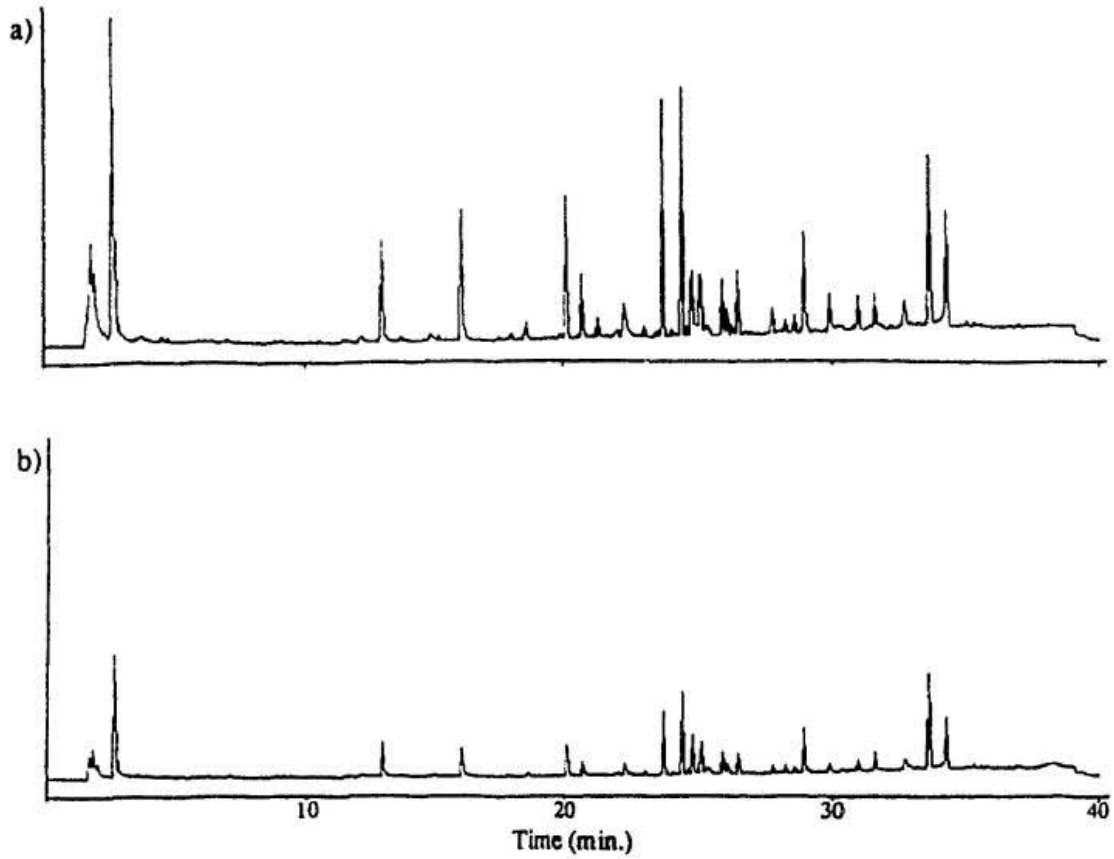


Figure 4.8: Pyrograms of Spruce Milled Wood Lignin (a) Untreated and (b) Acid-Washed.

subsequently been washed to neutrality with deionised water (101,102).

4.5. Identification of the Pyrolyzates of Acid-Washed Black Spruce and Birch Mechanical Pulps by Py-GC-MS.

The pyrograms of the mechanical pulps are complex and, while capillary GC can separate the pyrolyzates quite well, it is frequently difficult to make a definite peak assignment when using

a non-specific detector such as an FID. Mass spectrometry, on the other hand, is ideal for product identification and is easily interfaced with modern capillary GC columns. The use of on-line pyrolysis with GC-MS (Py-GC-MS) makes it possible to separate and identify the volatile components of complex pyrolyzate mixtures.

Though all the pulps were investigated using Py-GC-MS, only the acid-washed mechanical pulps of black spruce and birch are discussed here. These pulps gave the most complex pyrogram profiles and were the most challenging to characterize. In addition, it was found that pyrolysis of the kraft and organosolv pulps failed to produce any pyrolyzates not already observed in the pyrograms of the mechanical pulps. In order to acquire as much information as possible on each product, both electron impact (EI) and chemical ionization (CI) mass spectrometric techniques were utilized.

4.5.1. Electron Impact Mass Spectrometry

EI mass spectrometry (EIMS) of polysaccharide pyrolyzates suffers from a number of problems, as it does for carbohydrates in general. The strong ionization conditions (i.e, 70 eV) cause extensive fragmentation and rearrangement, resulting in spectra that are dominated by low molecular weight ions. The molecular ion is usually absent from the spectrum or is present at very low intensities. Moreover, it is impossible to distinguish between compounds that differ only in their stereoconfiguration. For example, the 1,6-anhydropyranose sugars of glucose, mannose and galactose give the same EI spectrum, as do the 1,4-anhydropyranose sugars of xylose and arabinose. Figure 4.9 illustrates the representative EI spectra for the anhydrosugars observed during this investigation.

The total ion chromatograms (TIC's) for black spruce and birch mechanical pulps are illustrated in Figures 4.10 and 4.11. Peak assignments (Table 4.1) were made by comparing their mass spectra with those previously reported in the literature (45,75,96,104-106) or, where possible, with spectra obtained for known compounds. The anhydrosugars were identified both by their EI mass spectra and their retention times. The pyrolysis products obtained from lignin have been well documented (44,99-100,104-106). All lignin pyrolyzates observed here are monomeric, phenolic compounds, though there is evidence from other researchers that dimeric pyrolyzate species are also produced by pyrolysis (99). The mass spectra of lignin-derived pyrolyzates are usually quite distinctive and always contain the molecular ion. Pyrolyzates with similar molecular weights and EI spectra (i.e., eugenol, **33**, and cis- and trans-isoeugenol, **38** and **44**) can be differentiated by their relative retention times as reported by Faix et al. (104).

Problems were encountered when the identification of the lower molecular weight carbohydrate pyrolyzates was attempted. This was due to the lack of characteristic ions in their mass spectra. In some cases the molecular weights obtained from CIMS and/or their relative retention times aided in their identification. Finally, because the initial oven temperature was 100°C, many of the early eluting pyrolyzates are not resolved and no attempt was made to identify them.

The origin of the pulps can be readily determined by examining their TIC's. For example, the pyrogram for birch mechanical pulp contains quite a few syringol derivatives which is indicative of a hardwood. As expected, the pyrogram for black spruce pulp does not contain these pyrolyzates. In addition, it can be seen that the mannose and galactose anhydrosugars are quite abundant in the softwood pyrogram whereas only small quantities are present in the hardwood pyrogram. Initially in the study, a wide bore DB-1701 column (0.329 mm i.d.) was used to resolve the pyrolyzates.

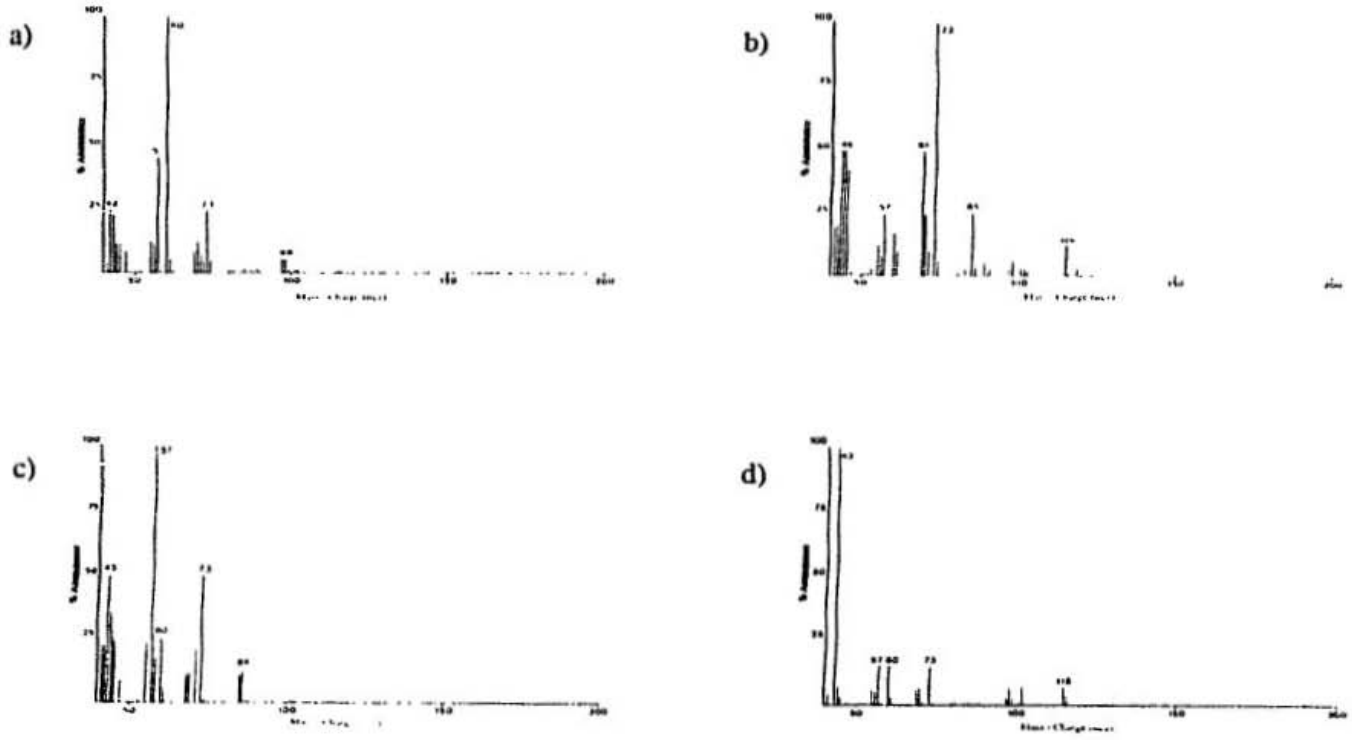


Figure 4.9: Representative Electron Impact (EI) Spectra of the Anhydrosugars, (a) 1,6-Anhydrohexopyranose, (b) 1,6-Anhydrohexofuranose, (c) 1,4-Anhydropentopyranose, and, (d) the EI Spectrum of 1,4-Anhydrogalactopyranose.

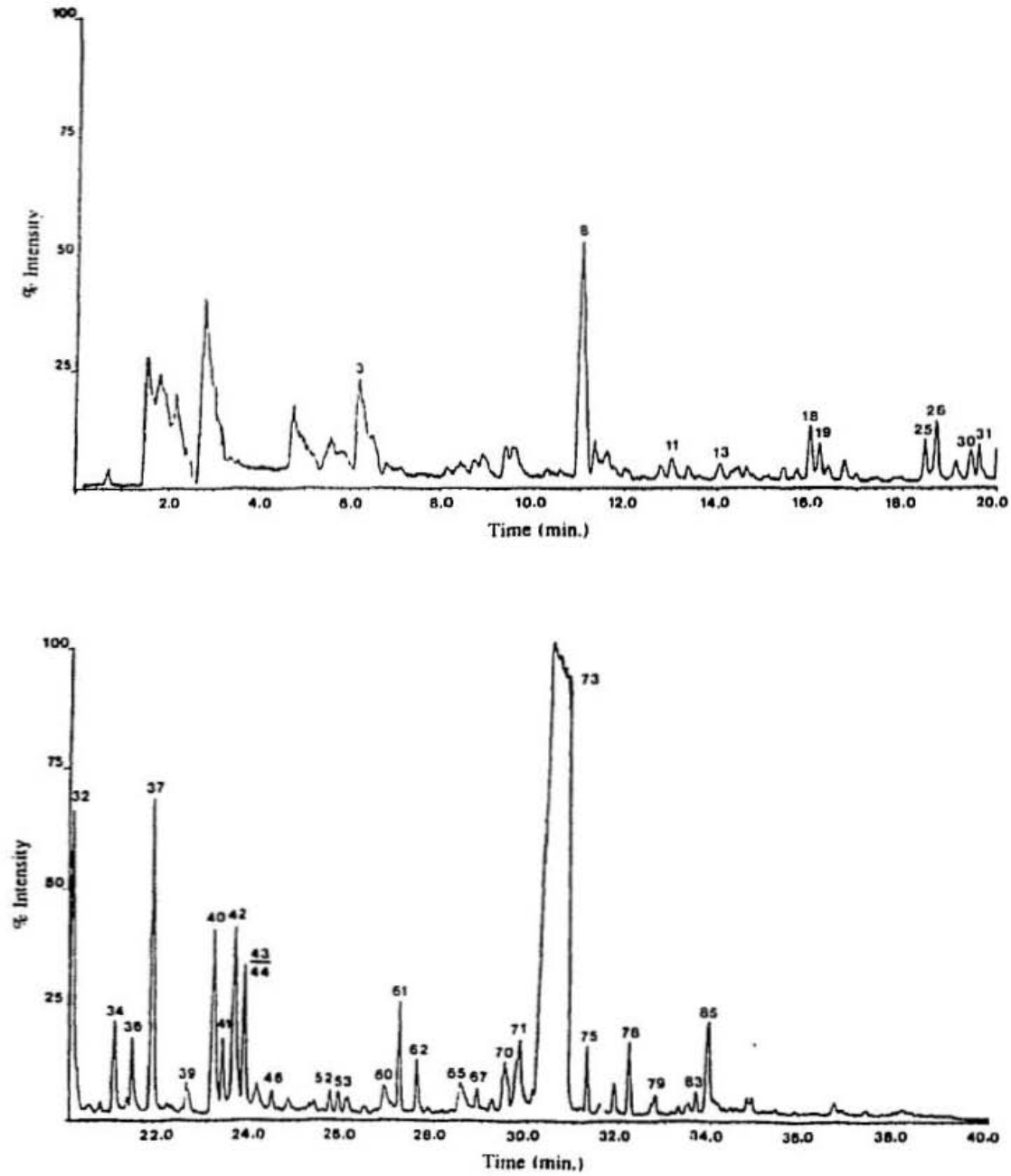


Figure 4.10: Total Ion Chromatogram (TIC) for Acid-Washed Black Spruce Mechanical Pulp Obtained by Py-GC-MS(EI).

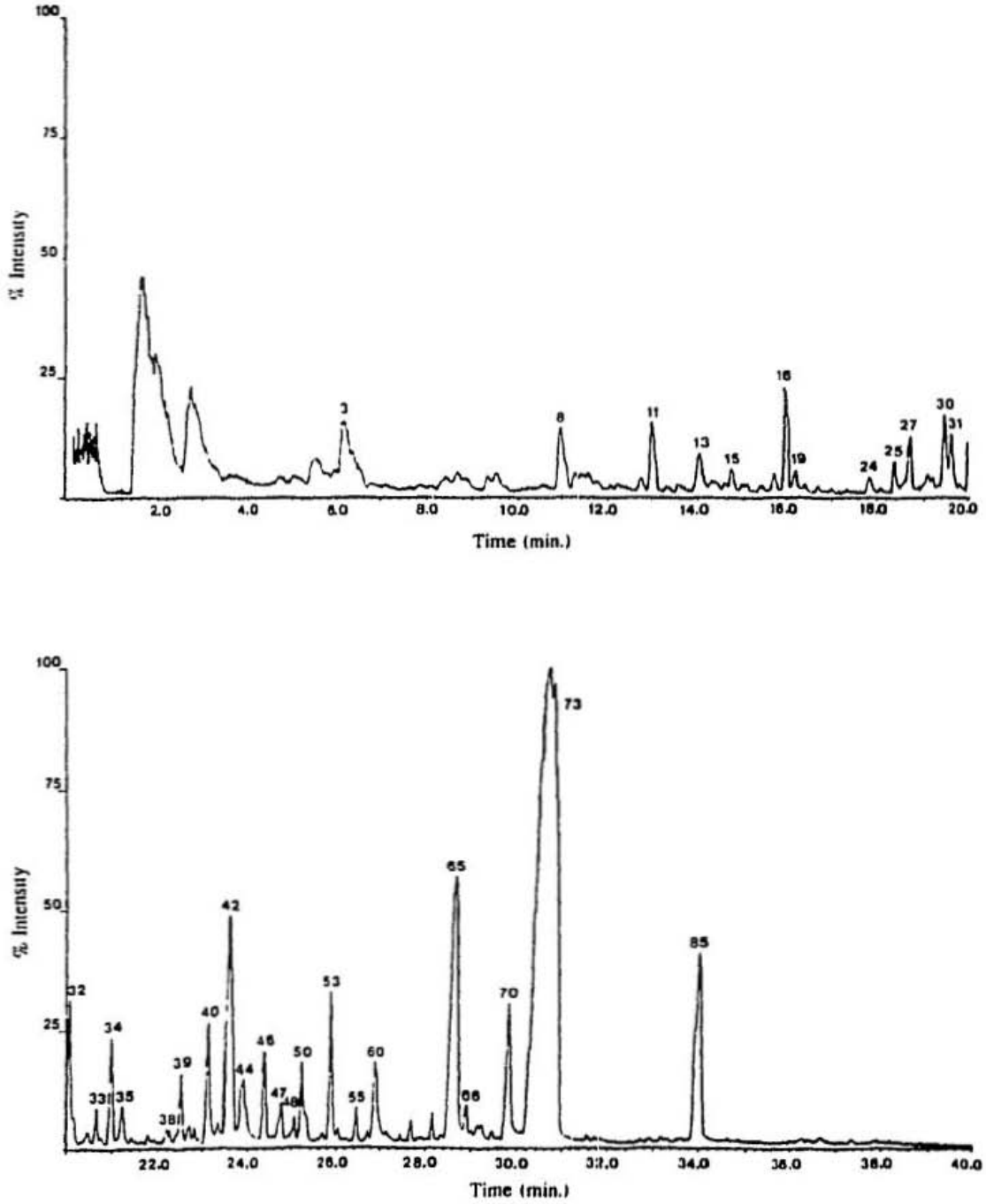


Figure 4.11: Total Ion Chromatogram (TIC) for Acid-Washed Birch Mechanical Pulp Obtained by Py-GC-MS(EI).

Table 4.1: Identification of the Peaks in the PY-GC-MS (EI and CI) Total Ion Chromatograms for Black Spruce (Acid-Washed) and Birch (Acid-Washed).

Peak No.	Identification	Mass	R_t*	$(MH^+)^\S$	$(MNH_4^+)^\S$	Origin
2	3-Furaldehyde	96	N.O.	---	114	P
3	2-Furaldehyde	96	6.22	---	114	P
7	3-Methyl-2-cyclopentenone	96	N.O.	---	114	P
8	4-Hydroxy-5,6-dihydro-(2H)-pyran-2-one	114	11.06	---	132	P
10	3-Methyltetrahydrofuran-2,4-dione	96	N.O.	---	132	P
11	Guaiacol	124	13.04	125	---	L
13	2-Acetylfuran	110	14.04	N.O.	N.O.	P
15	m-/p-Cresol	108	14.78	N.O.	N.O.	L
18	Guaiacol, 4-methyl	138	16.22	125	---	L
19	Levoglucosenone	126	16.01	---	134	P
24	Unknown	?	17.82	N.O.	N.O.	P
25	Guaiacol, 4-ethyl	152	18.39	153	---	L
26	1,4:3,6-dianhydro-α-D-glucopyranose	144	N.O.	---	162	P
27	Unknown	?	18.71	N.O.	N.O.	P
30	Unknown	156	19.46	174	---	P
31	1,4-Anhydroarabinopyranose	132	19.64	---	152	P
32	Guaiacol, 4-vinyl	150	20.10	151	---	L
33	Eugenol	164	20.65	165	---	L

Table 4.1 (continued):

Peak No.	Identification	Mass	R_t*	$(MH^+)^1$	$(MNH_4^+)^1$	Origin
34	5-Hydroxymethyl-2-furaldehyde	126	21.01	---	144	P
35	Cathecol	110	21.23	N.O.	N.O.	L
36	Syringol	154	21.39	155	172	L
37	Unknown	174	21.81	175	192	P
38	Isoeugenol (cis)	164	22.23	N.O.	N.O.	L
39	Unknown	156	22.56	---	174	P
40	1,4-Dideoxy-D-glycero-hex-1-eno-pyranos-3-ulose	144	23.15	145	162	P
41	Unknown	?	23.35	N.O.	N.O.	P
42	1,4-Anhydroxylopyranose	132	23.62	---	150	P
43	Syringol, 4-methyl	168	23.83	169	186	L
44	Isoeugenol (trans)	164	23.83	165	---	L
46	Vanillin	152	24.39	153	170	L
47	1-(4-Hydroxy-3-methoxyphenyl)propyne	162	24.78	163	---	L
48	1-(4-Hydroxy-3-methoxyphenyl)allene	162	25.06	163	---	L
50	Unknown	186	25.22	187	---	L
52	Syringol, 4-ethyl	182	25.70	183	200	L
53	Homovanillin	166	25.88	167	184	L
55	Acetoguaiacone	166	26.41	167	184	L

Table 4.1 (continued):

Peak No.	Identification	Mass	R_t*	$(MH^+)^\$$	$(MNH_4^+)^\$$	Origin
60	1,6-Anhydrogalactopyranose	162	26.88	---	180	P
61	Syringol, 4-vinyl	180	27.22	181	198	L
62	Syringol, 4-allyl	194	27.61	195	212	L
65	1,6-Anhydromannopyranose	162	28.58	---	180	P
66	Coniferyl Alcohol	180	28.58	181	198	L
67	Syringol, 4-propenyl (cis)	194	28.90	195	212	L
70	1,4-Anhydrogalactopyranose	162	29.50	---	180	P
71	Unknown	?	29.78	N.O.	N.O.	P
73	1,6-Anhydroglucopyranose	162	30.50	---	180	P
75	Syringaldehyde	182	31.28	183	200	L
78	Homosyringaldehyde	196	32.22	197	214	L
79	Acetosyringone	196	32.78	197	214	L
80	Unknown	204	N.O.	205	---	L
83	Coniferaldehyde	178	33.65	179	196	L
85	1,6-Anhydroglucofuranose	162	33.95	---	180	P

*: Retention time from EI total ion chromatograms (TIC's).

$: Protonated molecular ion and ammoniated adducts in ammonia CI spectra.

N.O.: Compound not observed in specified ion chromatogram.

L: Lignin Origin.

P: Polysaccharide Origin.

However, it was found that 1,4-anhydroxylopyranose, **42**, co-eluted with 4-methyl syringol, **43**, and was only partially separated from trans-isoeugenol, **44**. This problem was solved by using a narrower bore column (0.255 mm i.d.).

4.5.2. Chemical Ionization Mass Spectrometry

Carbohydrates and their derivatives are labile molecules and, as is observed in Figure 4.9, extensively fragment under the strong ionisation conditions of EIMS. Extensive fragmentation is also observed in CIMS when the major ionization mode is proton transfer (107). For example, when methane is used as the CI reagent gas, the mass spectrum for levoglucosan shows extensive, though simple, fragmentation with a relatively small MH$^+$ adduct ion (Fig. 4.12a). The major reagent ions, CH_5^+ and $C_2H_5^+$, have relatively low proton affinities (130.5 and 163.5 kcal.mol^{-1} respectively) and will exothermically lose a proton to most organic molecules (107). Isobutane CI also protonates levoglucosan, though the molecular ion intensity is higher and less fragmentation occurs (Fig. 4.12b). Because of the higher proton affinity (PA) isobutane's reagent ion (196.9 kcal.mol^{-1} for $C_4H_9^+$) proton transfer is less exothermic. In contrast, when ammonia is used as the reagent gas, an intense $[M+NH_4]^+$ cluster ion is formed with little or no fragmentation (Fig. 4.12c). NH_4^+ has a high PA (205.0 kcal.mol^{-1}) and will only protonate molecules of similar or higher affinities. Furthermore, carbohydrates are also polar and polyhydroxyl in nature which facilitates the formation of stable cluster ions with NH_4^+ through hydrogen bonding.

Ammonia was selected as the reagent gas in the CIMS studies because of the simplicity of the resulting carbohydrate mass spectra. The TIC's obtained for black spruce and birch mechanical pulps using Py-GC-CIMS are given in figures 4.13 and 4.14. Many of the peak assignments made

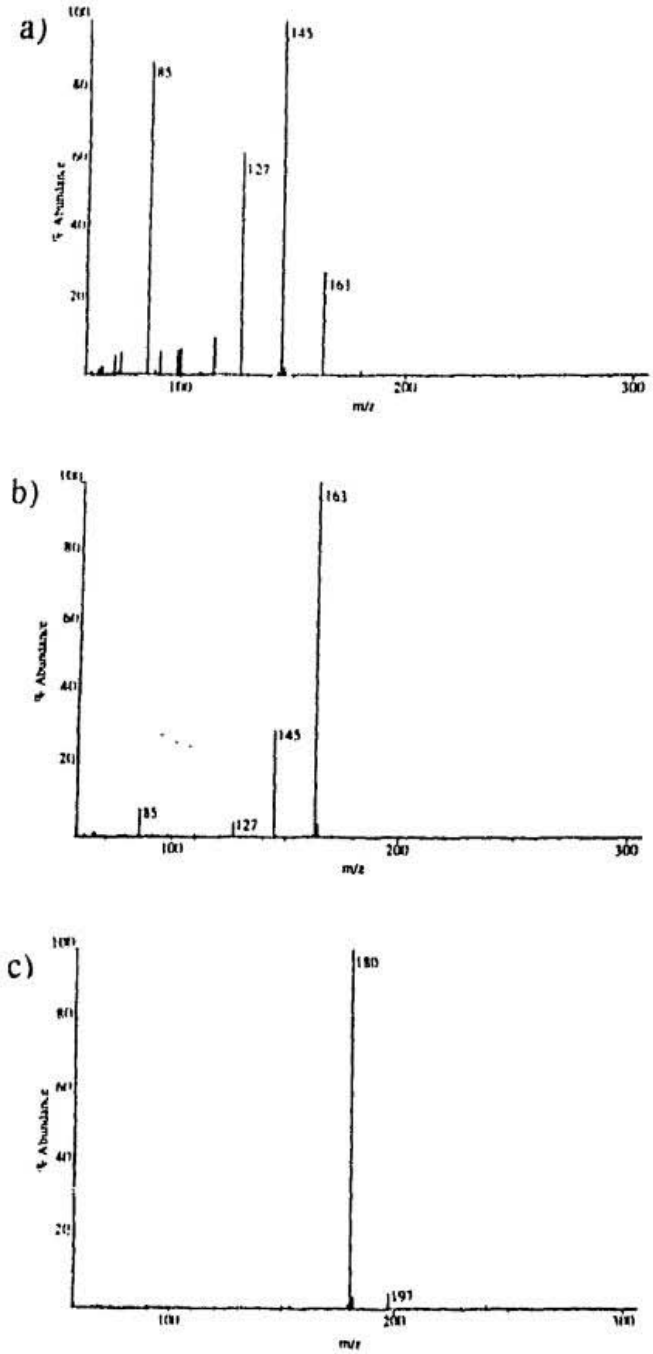


Figure 4.12: Chemical Ionization (CI) Mass Spectra of Levoglucosan using different reagent gases. (a) Methane, (b) n-Isobutane and (c) Ammonia.

in Table 4.1 were confirmed on the basis of their molecular weights obtained by CIMS. Despite a higher background signal in the Py-GC-CIMS TIC's, the majority of the pyrolyzates identified by Py-GC-EIMS are observed here also.

Table 4.1 also lists the molecular mass of each pyrolyzate and the observed adduct formed with the reagent ions (i.e., $[M+H]^+$ or $[M+NH_4]^+$). By and large the carbohydrate pyrolyzates exclusively formed the $[M+NH_4]^+$ adduct, exceptions being **37** and **40** which formed both. On the other hand, the mass spectra of all the lignin pyrolyzates contained the protonated molecular ion (Fig. 4.15a). However, many, including all the pyrolyzates derived from syringol moieties, also formed the ammoniated adduct (Fig. 4.15b). The fact that the lignin pyrolyzates preferentially form the protonated molecular ion is not surprising. It has previously been shown that the addition of polar functional groups such as hydroxyls, aldehydes and methoxyls increase the PA of benzene to values very close to that for the ammonium reagent ion (108). Under these conditions proton transfer would be the preferred pathway. However, the more highly substituted lignin pyrolyzates are also more polar and are probably better able to form a stable cluster ion.

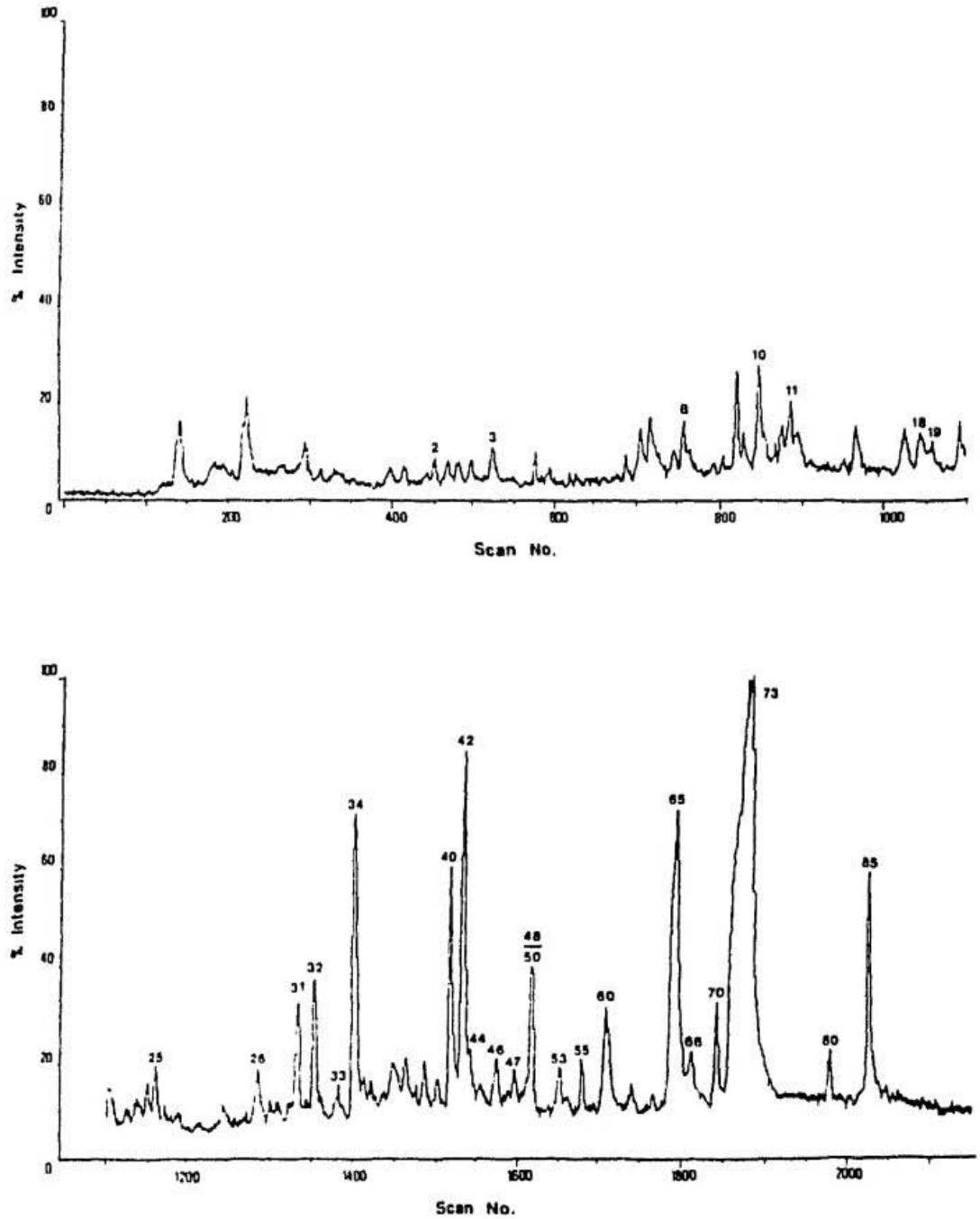


Figure 4.13: Total Ion Chromatogram (TIC) for Acid-Washed Black Spruce Mechanical Pulp obtained by Py-GC-MS(NH_3-Cl).

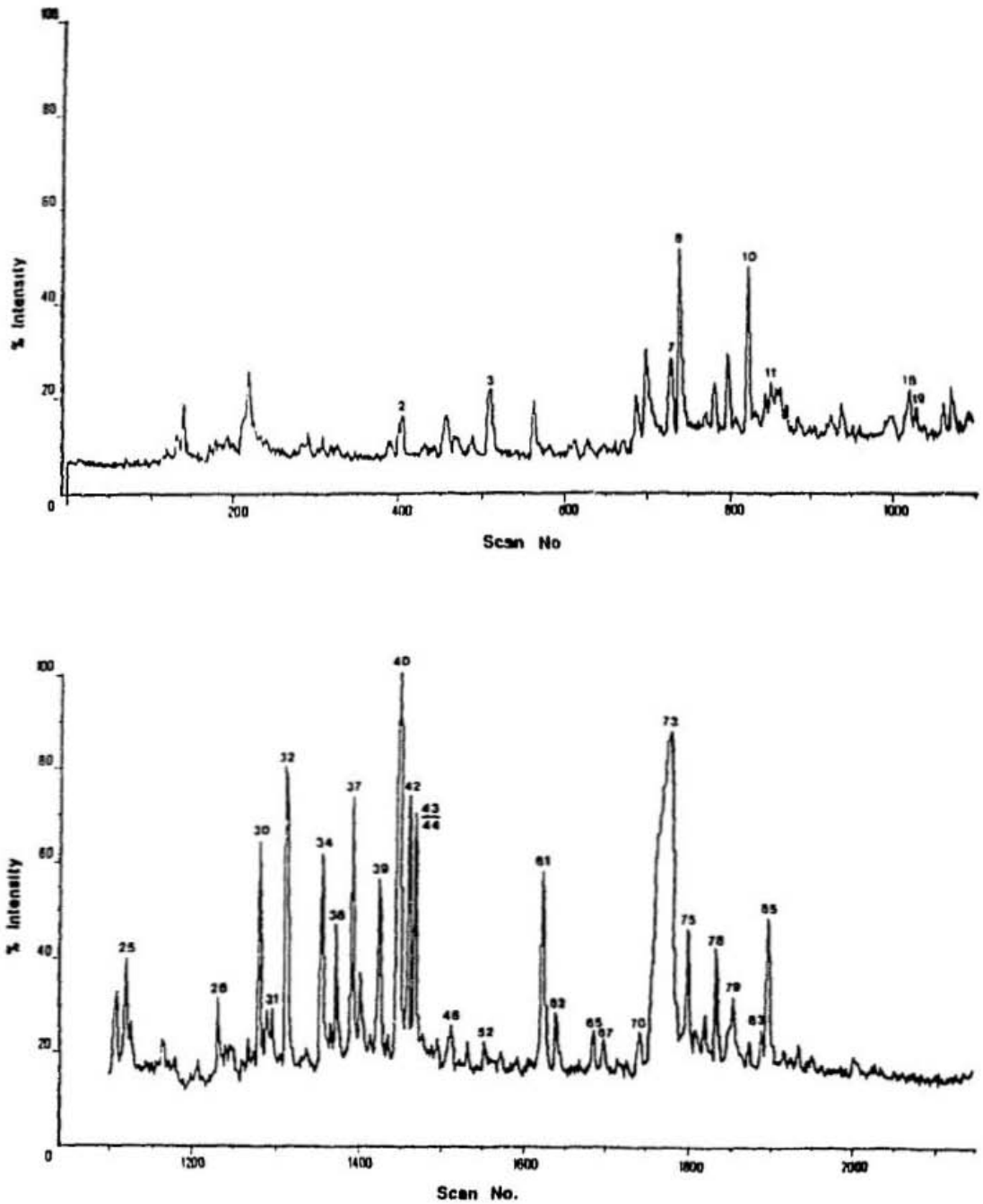


Figure 4.14: Total Ion Chromatogram (TIC) for Acid-Washed Birch Mechanical Pulp obtained by Py-GC-MS(NH_3-CI).

CHAPTER 5: PYROLYSIS OF SULFITE-TREATED PULPS

5.1: Introduction

In the previous chapter it has been demonstrated that acid-washing greatly improves the yields of anhydrosugars from pulps and that the pyrograms clearly reflect their saccharide composition. However, the pyrogram profile of acid-washed steam-exploded aspen pulp was quite different from those of the other pulps investigated (Fig. 5.1b). The dominant peak was determined by mass spectrometry (section 5.3.2) to be levoglucosenone, **19**, and, as will be discussed in section 6.1.2, the yields of glucose, **73** and **85**, and xylose, **42**, anhydrosugars were significantly lower than expected.

This phenomenon could not be attributed to residual ash in the washed pulp, as acid-washing was effective at removing almost all of it (from 1.28% to 0.10%). The production of large quantities of levoglucosenone is significant. It has been reported that acid catalysts enhance the formation of levoglucosenone from cellulose (77,109). These finding were confirmed when microcrystalline cellulose samples, sorbed with a variety of dilute acid solutions (sulfuric, HCl and acetic acids), were pyrolyzed (Fig. 5.2). It was found that levoglucosenone was formed at the expense of the anhydroglucoses, and that sulfuric acid was by far the most effective catalyst. It has also been shown that levoglucosenone is the dominant peak in the pyrograms of sulfated algal polysaccharides (110). However, this is unlikely to be due to residual HCl remaining in the pulp after acid-washing, as all pulps were treated in an identical manner and no other pulp exhibited this phenomenon.

Steam-exploded aspen differs from the other pulps in that it was treated with 8% Na_2SO_3 during processing. As illustrated in Figure 1.5, sulfonic acid groups are incorporated into lignin during sulfite pulping. Because steam-eploded aspen is a high yield pulp, much of its lignin content

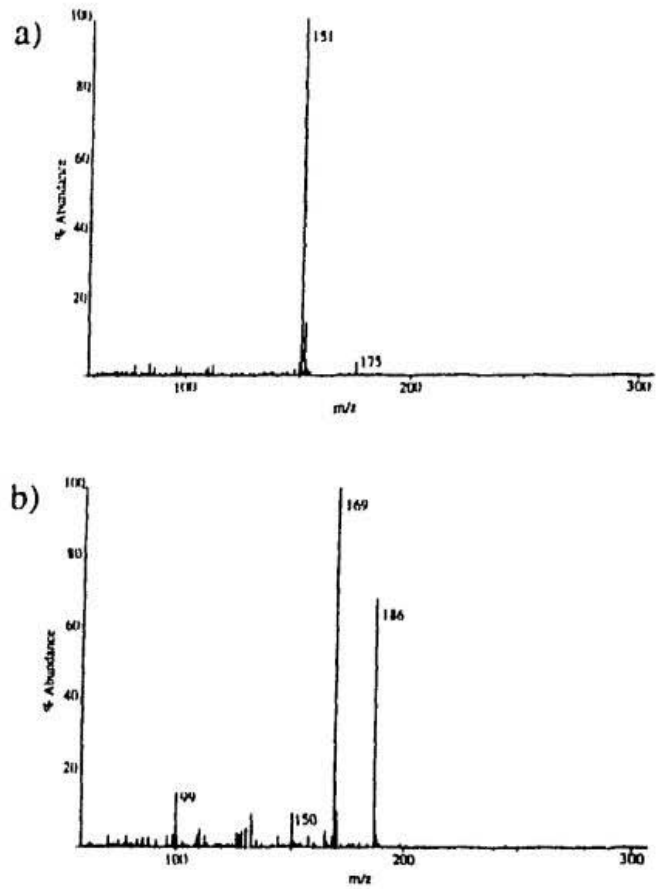


Figure 4.15: Ammonia Chemical Ionization Mass Spectra of (a) 4-Vinyl Guaiacol, **32**, and (b) 4-Methyl Syringol, **43**.

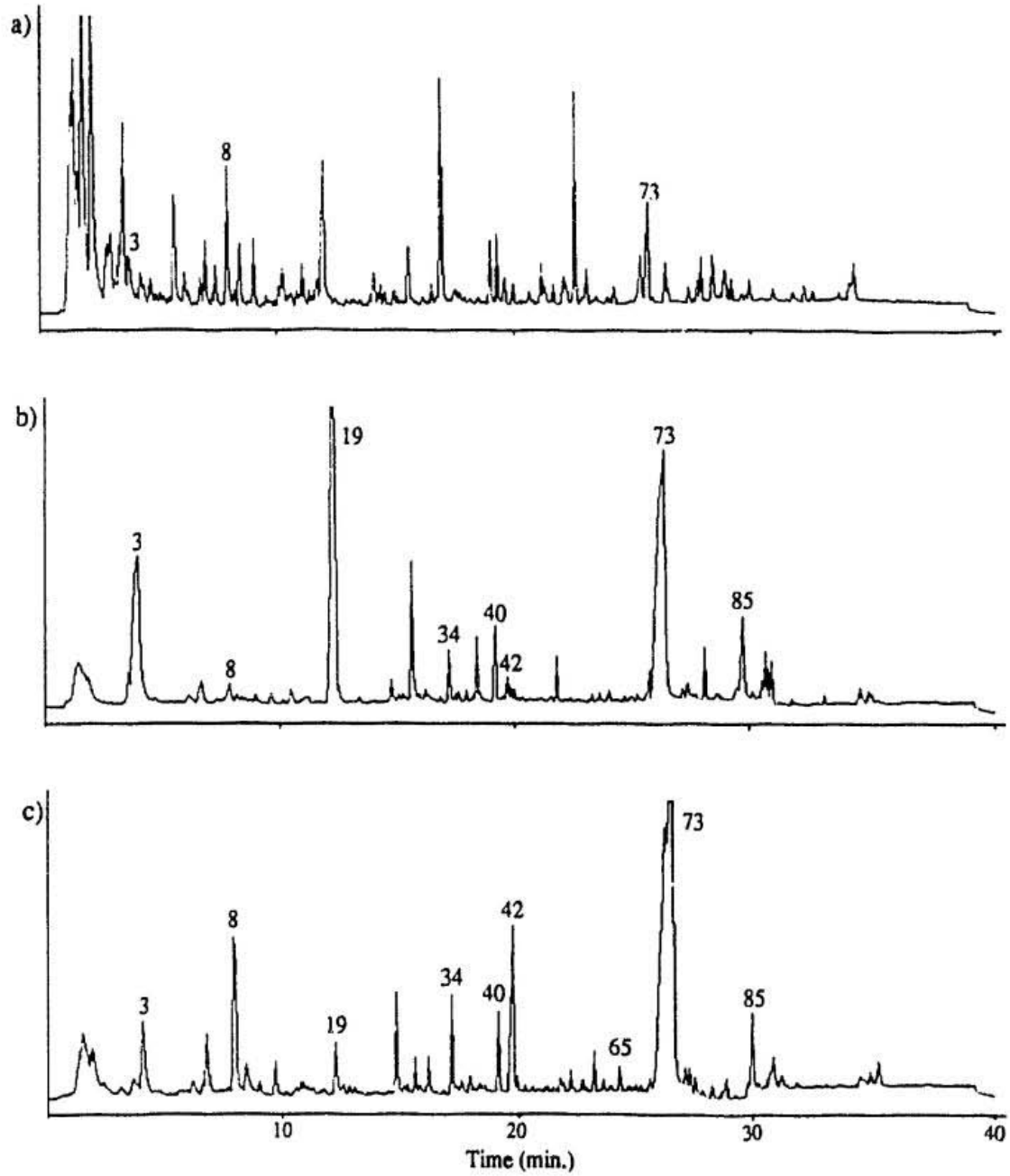


Figure 5.1: Pyrograms of Aspen Steam Explosion Pulp (a) Untreated, (b) Acid-Washed and (c) Fe^{2+}-Exchanged.

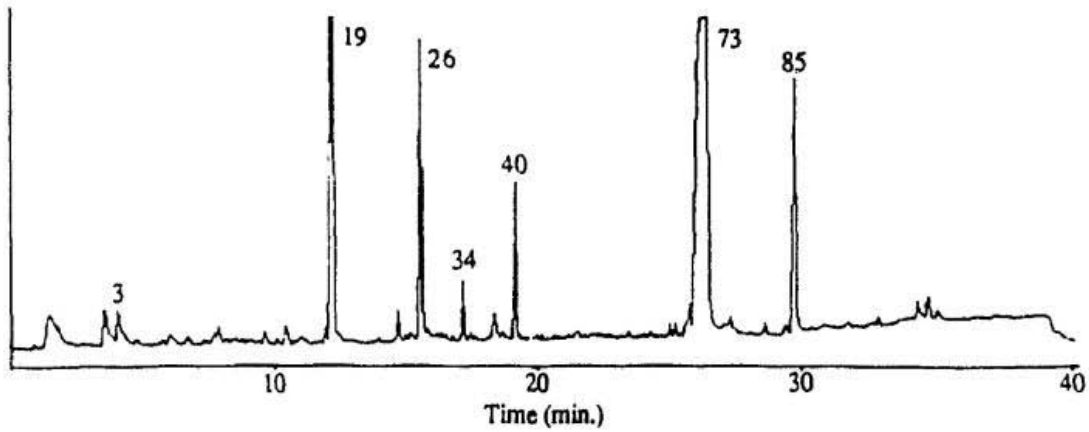


Figure 5.2: Pyrogram of Avicel Microcrystalline Cellulose sorbed with dilute H_2SO_4.

is retained. It is possible, therefore, that the presence of sulfite or lignin sulfonic acid groups in the pulp itself greatly affects the pyrolysis of the pulp carbohydrates. Interestingly, pyrolysis of the untreated pulp did not produce levoglucosenone (Fig. 5.1a). This is probably due, at least in part, to the fact that the sulfonate groups are "blocked" with metal cations. Acid-washing, however, protonates the sulfonic groups and thus promotes the in situ production of a strong acid during pyrolysis.

5.2. The Effect of Ion-Exchange with Fe^{2+}

The incorporation of Fe^{2+} ions into the acid-washed pulp of steam-exploded aspen, as described in section 2.4.4, significantly improved the yield of anhydrosugars, and the production of levoglucosenone was drastically lowered (Fig.5.1 c). The pulp was exchanged with iron (II) ions because it was demonstrated that this metal species does not radically alter the pyrogram profile of

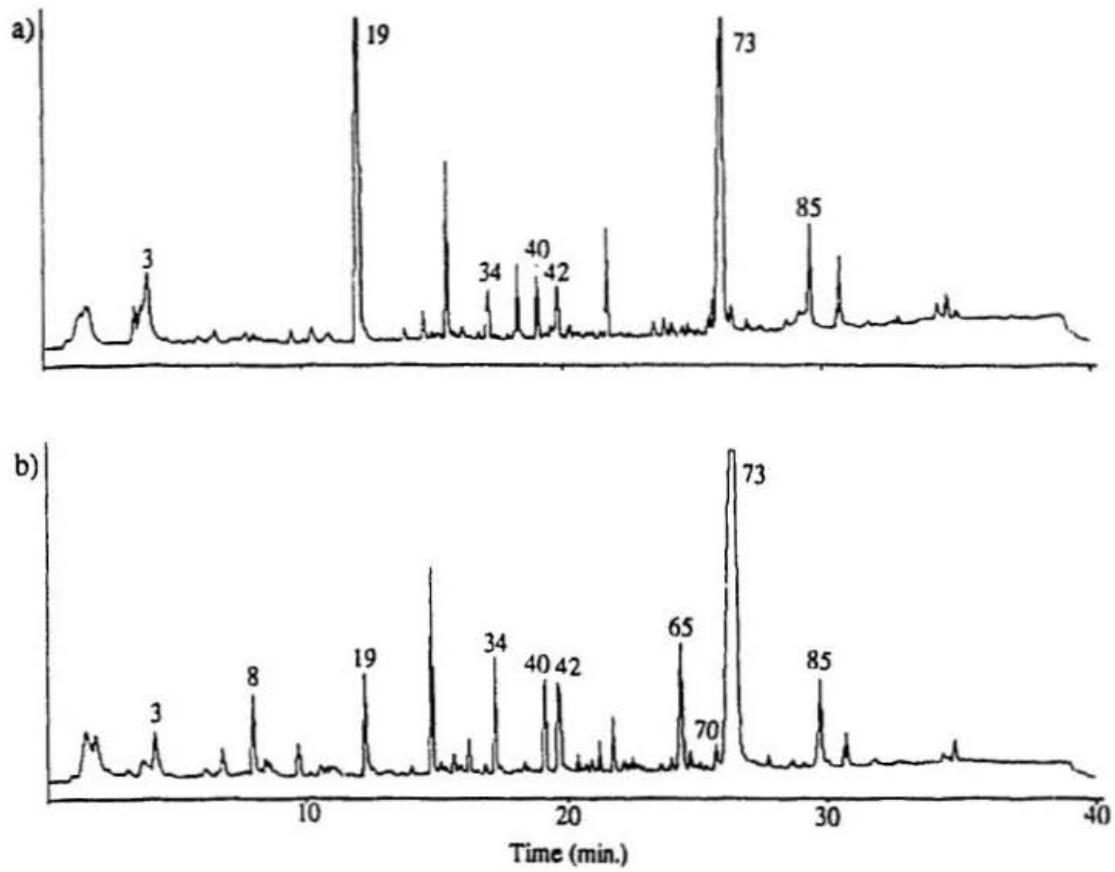


Figure 5.3: Pyrograms of Noranda Sulfite Pulp (N.S.P. #231090) (a) Acid-Washed and (b) Fe^{2+}-Exchanged.

black spruce kraft pulp (section 4.2). In most respects this Fe^{2+}-exchanged pulp pyrogram resembles those of the mechanical, high yield pulps discussed in the previous chapter (Fig 4.1 and Fig. 4.2). Aspen is a hardwood species and this is reflected in the pyrogram of the Fe^{2+}-exchanged pulp. The peak intensity for 1,4-anhydroxylopyranose, **42**, is relatively large whereas that for 1,6-anhydromannopyranose, *65*, is quite small.

By way of comparison, a high yield spruce sulfite pulp was treated and pyrolyzed in the same manner. Here also, pyrolysis of the acid-washed pulp produces large quantities of levoglucosenone and the yields of the anhydrosugars are significantly lowered (Fig. 5.3a). However, this trend is reversed when Fe^{2+} ions are exchanged into the acid-washed pulp (Fig. 5.3b). The softwood nature of the pulp is apparent from the size of the 1,6-anhydromannopyranose peak, **65**, in this pyrogram.

It would seem, therefore, that it is possible to prevent the unwanted catalytic properties of the sulfonic acid groups during pyrolysis by blocking them with a "benign" metal ion. However, this hypothesis would seem, at first glance, to clash with the observations reported in section 4.3 where it was suggested then that the presence of acid is beneficial to the production of anhydrosugars from wood pulps. It may be that the nature of the acid anion is important. The pyrogram in Figure 5.2 illustrates the effect of sorbing microcrystalline cellulose with dilute sulfuric acid. Avicel MC was also sorbed with equivalent amounts of HCl and acetic acid solutions, however, these samples produced far less levoglucosenone and the yield of the anhydroglucoses was almost as good as for the original polymer. H_2SO_4 is a very involatile acid and may, if formed during pyrolysis, persist long enough in the sample to influence the pyrolysis of the carbohydrates. On the other hand, HCl and CH_3COOH are both volatile and would be removed almost immediately from the sample.

5.3. Comparative Yields of Anhydrosugars Obtained from a Sulfite Pulp Exchanged with a Variety of Cations.

A number of metal ions, as well as NH_4^+, were exchanged into a spruce sulfite pulp, as described in section 2.4.4, in order to determine which cation gives the maximum yields of anhydrosugars upon pyrolysis. The yields of the anhydrosugars of glucose, xylose and mannose from

the cation-exchanged pulps are presented in Table 5.1. The results are expressed relative to the yields obtained for the Fe^{2+}-exchanged sample. It was observed that the best overall yields were obtained for the Fe^{2+}- and Fe^{3+}-exchanged pulps. The potassium-exchanged pulp gave by far the lowest yields for all anhydrosugars.

Although fewer cationic species were investigated in this instance, it would seem that the trend observed in Table 5.1 follows that reported by Richards et al. (101) for levoglucosan production from ion-exchanged wood. In the present study, however, the extent to which anhydrosugar production is affected varies for each saccharide species present. For example, calcium ions are more detrimental to the production of anhydroxylose than to that of the other anhydrosugars, and Hg^{2+} ions have a greater effect on the yield of 1,6-anhydromannopyranose. It was decided that Fe^{2+} would be

TABLE 5.1: Comparative Yields of Anhydrosugars from a Cation-Exchanged Noranda Sulfite Pulp

Cation	% Anhydroglucose[1] (peaks 73 + 85)	% Anhydroxylose[1] (peak 42)	% Anhydromannose[1] (peak 65)
Fe^{2+}	100%	100%	100%
Fe^{3+}	97%	101%	120%
Ni^{2+}	84%	65%	49%
Hg^{2+}	22%	20%	9%
Ca^{2+}	22%	3%	41%
K^{+}	0%	0%	11%
NH_4^{+}	105%	74%	74%

[1] : Calculated as the peak area per microgram of the pyrolyzed pulp sample relative to the Fe^{2+}-exchanged pulp. Average of duplicate analysis.

used for all future qualitative and quantitative investigations involving ion-exchanged pulps.

5.4. Py-GC-MS (EI) of Softwood Sulfite Pulps

Because the pyrolysis product profile of the sulfite-treated pulps changed quite considerably with the type of pretreatment, it was decided to investigate the identity of the pyrolyzates using Py-GC-MS (EI). Only electron impact mass spectrometry was used in this instance. The pyrolyzates were identified in the same manner as described in the previous chapter. The experimental conditions were as described in section 2.7.1.

5.4.1. Py-GC-MS (EI) of Untreated Spruce Sulfite Pulp.

The TIC for an untreated spruce sulfite pulp is given in Figure 5.4 and the identities of the pyrolyzates are listed in Table 5.2. The dominant peaks in the pyrogram are derived from lignin, **11,18,32,44** and **46**. The majority of the lignin pyrolyzates were also observed in the pyrograms of the mechanical pulps discussed in the previous chapter. The production of the anhydrosugars, **42,65,73** and **85**, is very low and the anhydrosugars of galactose are totally absent even though results obtained by acid hydrolysis - derivatization / GC indicate a residual presence (approx. 2%) in the pulp. Furthermore, this TIC is notable for the absence of levoglucosenone. The large peak at 19.45 minutes, of similar retention time to levoglucosenone, has been positively identified as 4-methyl guaiacol,**18**.

The lignin pyrolyzates were identified using reference EI spectra from the literature (45,75,96,104-106). Most of the lignin pyrolyzates were also observed in the acid-washed black

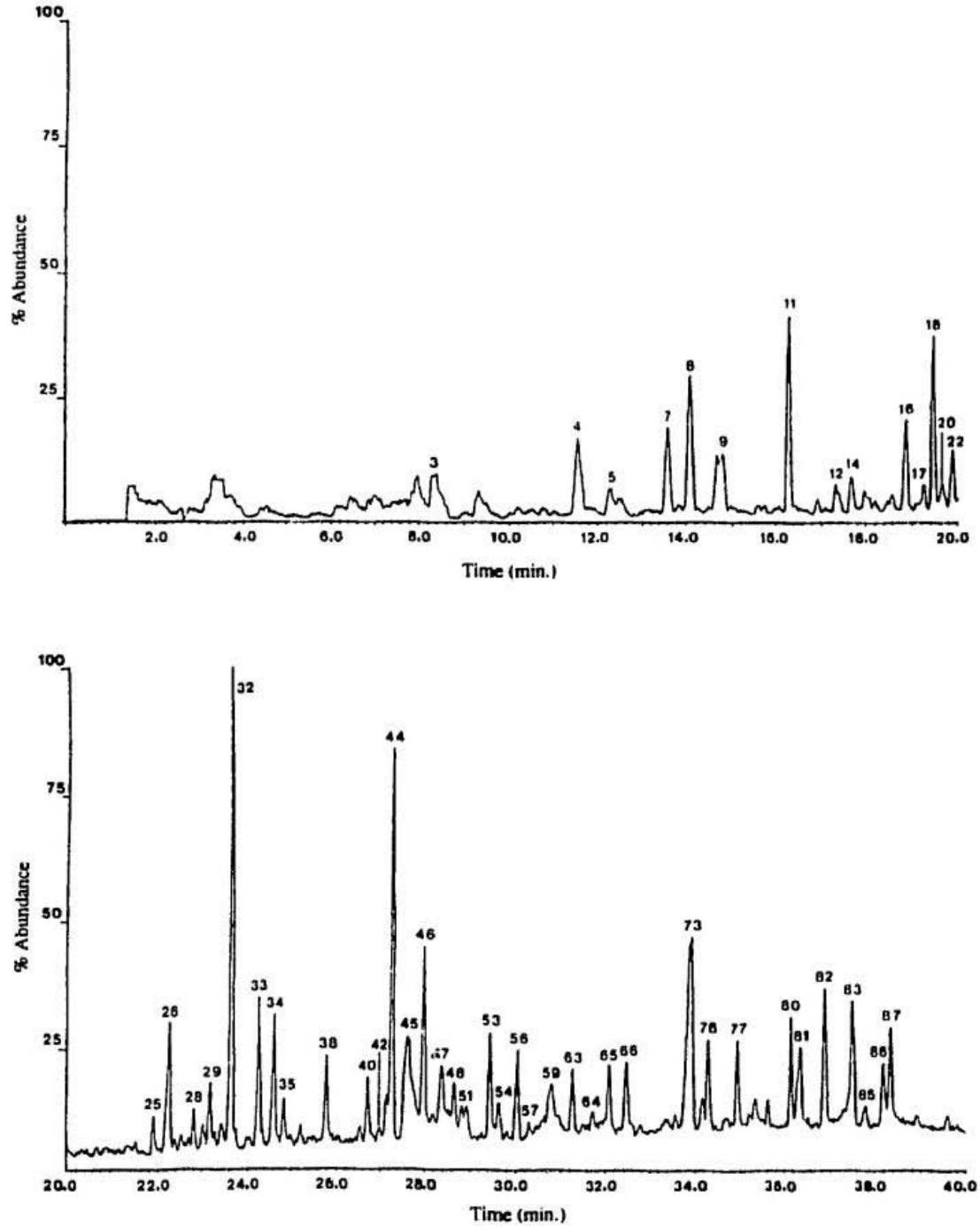


Figure 5.4: Total Ion Chromatogram (TIC) of Untreated Noranda Spruce Sulfite Pulp Obtained by Py-GC-MS(EI).

spruce mechanical pulp (section 4.4.1).

5.4.2. Py-GC-MS (EI) of Acid-Washed Spruce Sulfite Pulp

Acid washing significantly alters the TIC of the sulfite-treated pulps (Fig. 5.5). The pyrogram is now dominated by levoglucosenone, **19**, and levoglucosan, **73**. The anhydrosugar of xylose, **42**, is also greatly enhanced. On the other hand, most of the early eluting pyrolyzates observed in the TIC of the untreated pulp, **4-26**, are either absent or greatly reduced in size. Two carbohydrates pyrolyzates, **23** and **49**, are unique to the TIC of the acid-washed pulp and may possibly be pyranones.

Furthermore, the lignin pyrolyzate profile is completely changed. Most of the lignin pyrolyzates observed in Figure 5.4, **11,18,25,33,35,38,44,56,57,76** and **80** are absent from this trace. The remaining identifiable lignin pyrolyzates of significance are either aldehydes, such as vanillin, **46**, and homovanillin, **53**, or ketones, such as guaiacyl acetone, **63**.

Three new and unusual lignin pyrolyzates, **58**, **72** and **88**, were observed in this TIC. Their molecular weights are considered to be 192 a.m.u. and their mass spectra are very similar (Fig. 5.6). Faix et al. (105) observed two peaks of the same molecular weight in the pyrogram of milled wood lignin from a hardwood. However, neither their spectra nor their retention times, relative to vanillin on a similar column, correspond with those of the pyrolyzates observed in this sulfite pulp. Due to the similarity of their spectra, it is probable that these pyrolyzates are isomeric.

Considering the structures of the other lignin pyrolyzates, the most likely empirical formula

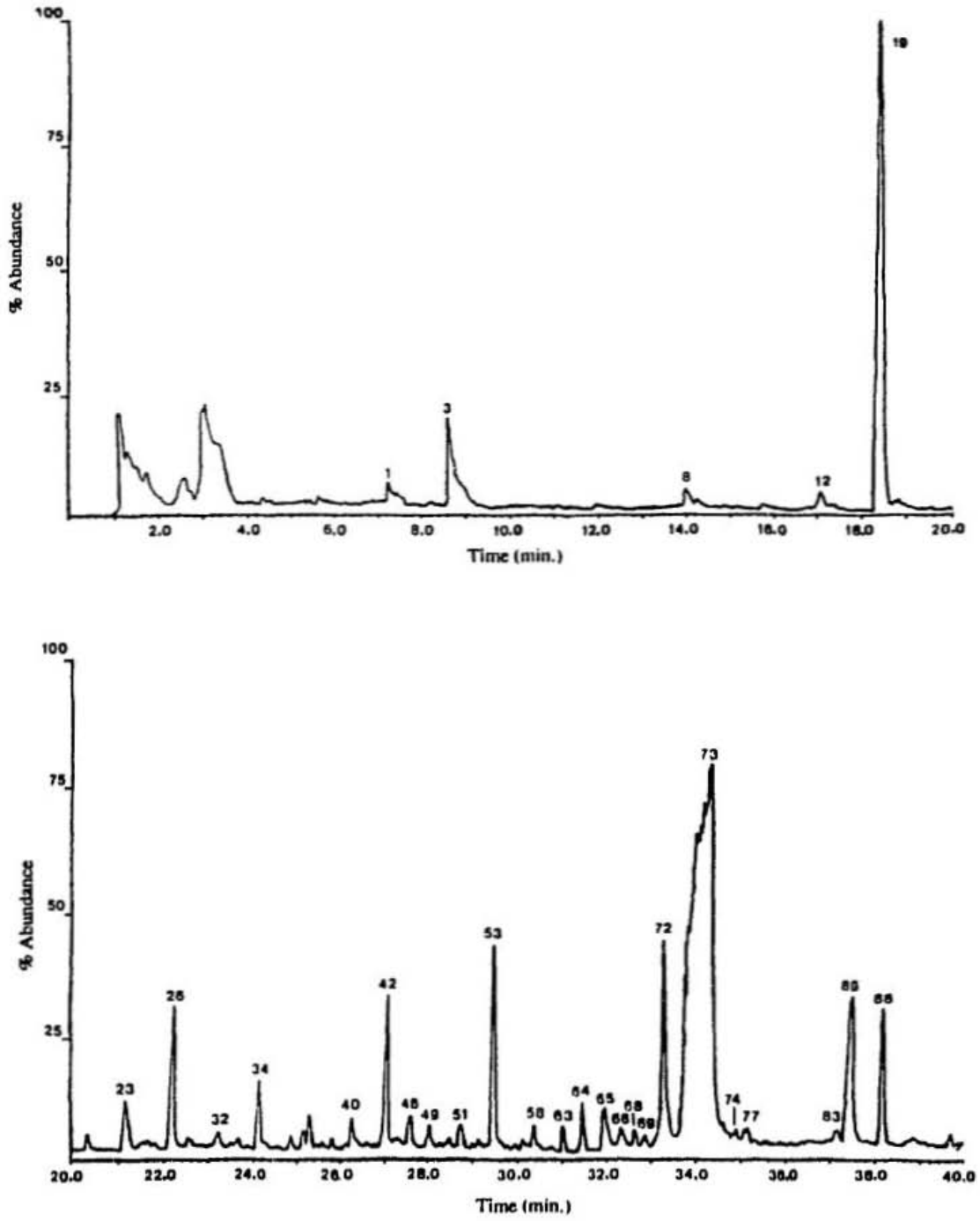


Figure 5.5: Total Ion Chromatogram (TIC) of Acid-Washed Noranda Spruce Sulfite Pulp Obtained by Py-GC-MS(EI).

Table 5.2: Identification of the Pyrolyzates in the Sulfite Pulp TIC's.

Peak No.	Identification	Mass	Origin
1	(2H)-Furan-3-one	84	P
3	2-Furaldehyde	96	P
4	2,3-Dihydro-5-methylfuran-2-one	98	P
5	5-Methyl-2-furaldehyde (+ unknown)	110	P
7	3-Hydroxy-2-methyl-2-cyclopenten-1-one	112	P
8	4-Hydroxy-5,6-dihydro-(2H)-pyran-2-one	114	P
9	2-Hydroxy-3-methyl-2-cyclopenten-1-one + Unknown	112	P
11	Guaiacol	124	L
12	2-Fucoic acid methyl ester	126	P
14	3-Hydroxy-2-methyl-(4H)-pyran-4-one	126	P
16	5-Hydroxymethyl-2-tetrahydrofuraldehyde (t)	144	P
17	2-(propan-2-one) tetrahydrofuran	128	P
18	Guaiacol, 4-methyl	138	L
19	Levoglucosenone	126	P
20	Methylformyl-(4H)-pyran-4-one	138	P
21	3,5-Dihydroxy-2-methyl-(4H)-pyran-4-one	142	P
22	Unknown	?	P
23	Unknown (possible pyranone)	126	P
25	Guaiacol, 4-ethyl	152	L
26	1,4-3,6-Dianhydro-∝-D-glucopyranose	144	P
28	Unknown	174	L
29	Unknown	174	L
30	Unknown	?	P
32	Guaiacol, 4-vinyl	150	L
33	Eugenol	164	L

Table 5.2 (continued):

Peak No.	Identification	Mass	Origin
34	5-Hydroxymethyl-2-furaldehyde	126	P
35	Cathecol	110	L
38	Isoeugenol	164	L
40	1,4-Dideoxy-D-glycero-1-enepyranos-3-ulose	144	P
42	1,4-anhydroxylopyranose	132	P
44	Isoeugenol (trans)	164	L
45	Unknown	?	P
46	Vanillin	152	L
47	1-(4-Hydroxy-3-methoxyphenyl)propyne	162	L
48	1-(4-Hydroxy-3-methoxyphenyl)allene	162	L
49	Unknown (possible pyranone)	126	P
51	Unknown	?	P
53	Homovanillin	166	L
54	Unknown anhydropentose	132	P
55	Acetovallinone	166	L
56	Acetoguaiacone	166	L
57	Propiovallinone	180	L
58	$C_{11}H_{12}O_3$	192	L
59	Structural Isomer of Coniferaldehyde	178	L
60	1,6-Anhydrogalactopyranose	162	P
63	Guaiacyl Acetone	180	L
64	Structural Isomer of Coniferaldehyde	178	L
65	1,6-Anhydromannopyranose	162	P
66	Structural Isomer of Coniferyl Alcohol	180	L
68	Unknown	?	P
69	Guaiacol, vinyl ketone	178	L

Table 5.2 (continued):

Peak No.	Identification	Mass	Origin
70	1,4-Anhydrogalactopyranose	162	P
72	$C_{11}H_{12}O_3$	192	L
73	Levoglucosan	162	P
74	$C_{11}H_{12}O_3$	192	L
76	Dihydroconiferyl alcohol	182	L
77	Coniferyl alcohol (cis)	180	L
80	Unknown	230	?
81	Unknown	?	P
82	Coniferyl alcohol	180	L
83	Coniferaldehyde	178	L
85	1,6-Anhydroglucofuranose	162	P
86	Unknown	?	P
87	Unknown	?	?
88	$C_{11}H_{12}O_3$	192	L

P: Polysaccharide Origin.

L: Lignin Origin.

for these unknowns is $C_{11}H_{12}O_3$. The number of rings plus double bonds is 6 for this formula (111). The most informative fragment ions are m/z 163, 109 and 68. The m/z 163 ion is probably formed by loss of 29 a.m.u. from the molecular ion. This could be due to the loss of $CHO^{\cdot}$ or $C_2H_5^{\cdot}$. Given the unsaturation indicated by the molecular formula, the former radical is the likelier candidate, suggesting the presence of an aldehyde group. The prominence of the m/z 109 fragment ion is also significant, as this is also the base peak in the spectrum of guaiacol. A second fragment ion, m/z 81, is also present in all spectra, including that of guaiacol. This strongly suggests that the pyrolyzates

(58) 69[100], 109[92], 192[84], 44[50], 97[49], 96[46], 55[42], 43[42], 81[42], 163[30].

(72) 109[100], 68[100], 192[71], 81[50], 55[50], 53[42], 52[42], 43[30], 163[25], 110[25].

(88) 109[100], 68[59], 192[51], 55[50], 81[42], 163[30], 110[30], 53[30], 78[27], 96[25].

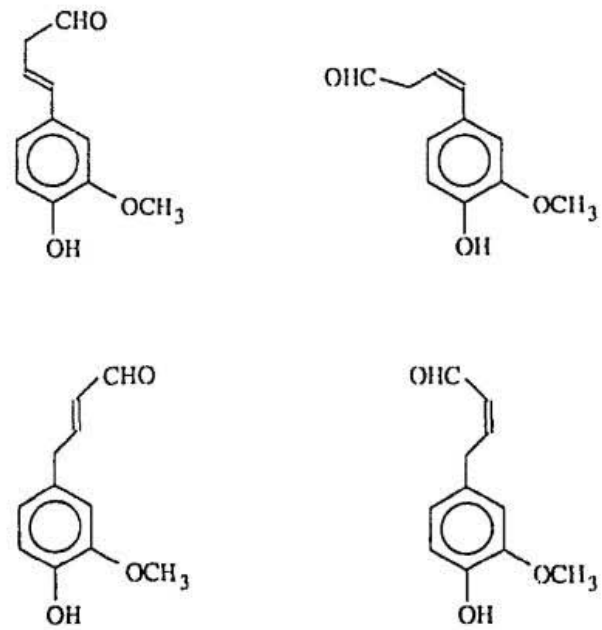


Figure 5.6: The EI Mass Spectral Data and Proposed Structures for the Unknown Lignin Pyrolyzates 58, 72 and 88.

resemble guaiacol in structure. Finally, the prominent ion at m/z 68 can be explained if all the atoms not included in the guaiacol structure are contained in a single sidechain. This ion could be formed by fission of the sigma bond to the aryl group plus a proton migration. This may also explain the relatively large ion at m/z 110 found in the spectra of compounds **72** and **88**.

Based on this information a number of tentative structures are proposed (Fig. 5.6). If these structures are correct then there should be another pyrolyzate with a mass spectrum similar to that

of **58**. By extracting the m/z 192 mass from the TIC a fourth peak, **74**, was observed at 34.87 minutes. Though it's mass spectrum was heavily contaminated with that of levoglucosan, it is similar to that of **58**. This peak was originally overlooked due to its small size and its position at the end of the downslope of the very large levoglucosan peak.

The formation of these proposed pyrolyzates is difficult to explain due to the length of the 4-carbon alkyl chain. Lignin is mostly composed of phenyl propanoid groups and it is difficult to see how these proposed pyrolyzates could be formed from them, especially using conventional lignin pyrolytic mechanisms. However, as these structures are only tentative suggestions at this stage, any proposals as to their formation would be purely speculative. On the other hand, their mass spectra show little evidence for alternative structures. For example, there is no evidence to suggest the presence of a bicyclic structure in any of the mass spectra, nor is it likely that there is a fourth side-chain attached to the phenyl ring.

5.4.3. Py-GC-MS (EI) of Fe^{2+}-Exchanged Spruce Sulfite Pulp

The TIC of the Fe^{2+}-exchanged pulp (Fig. 5.7) is quite similar in most respects to that of the acid-washed spruce mechanical pulp discussed in section 4.3.1. Levoglucosan, **73**, is the dominant peak and the other anhydrosugars, including those of galactose, **60** and **70**, are quite prominent. 1,4-Anhydroarabinopyranose was not observed and its absence in the pulp was confirmed by acid hydrolysis - derivatization / GC. Finally, levoglucosenone, although present in this pyrogram, is greatly reduced in size.

The lignin pyrolyzates are the same as those observed in the pyrogram of the untreated pulp.

However, in most cases their abundances have been lowered quite considerably. The same phenomenon was observed during the study discussed in section 5.2 for those pulps exchanged with other transition metal ions. In contrast, the samples exchanged with potassium and calcium ions yielded greater quantities of lignin prolyzates. This particular sulfite pulp (Noranda 231090) was manufactured using a sodium bisulfite solution and therefore the counter cation in the untreated pulp is almost exclusively sodium. The pyrogram of the untreated pulp would be expected to be very similar to that of the potassium-exchanged pulp, and in fact it is.

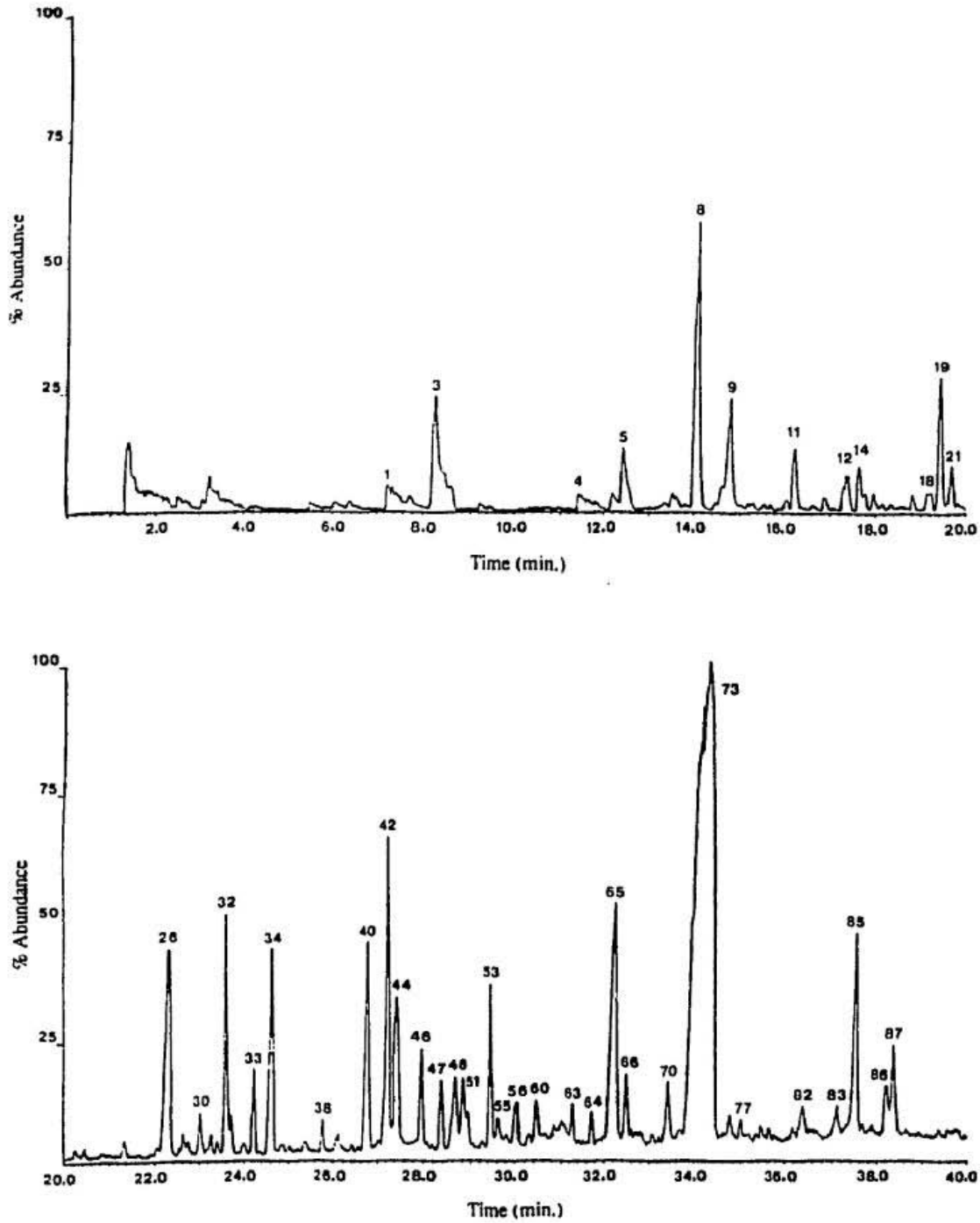


Figure 5.7: Total Ion Chromatogram (TIC) for Fe^{2+}-Exchanged, Noranda Spruce Sulfite Pulp Obtained by Py-GC-MS(EI).

CHAPTER 6: QUANTITATIVE ANALYSIS OF SACCHARIDES IN WOOD PULPS BY PY-GC

6.1. Introduction

Traditional methods for the quantitative analysis of neutral saccharides in complex biomaterials such as wood pulps are difficult, time-consuming, and expensive. There is a clear need for a faster and cheaper alternative. The results discussed so far have indicated that Py-GC could potentially be used as a quantitative method. It is fast, reproducible and relatively simple. Only one attempt has been made to quantify the saccharide composition of wood pulps by Py-GC-MS(EI) (96). However, while it was stated that good correlation was found between the relative peak intensities of the anhydrosugars and the results obtained by acid hydrolysis, in many instances the anhydrosugar peaks were barely above background and were only partially resolved from neighbouring peaks (96). Moreover, no direct comparison could be made between the anhydrosugar yields of different pulp species. This was due partly to their differing chemical compositions and partly to the variation in their inorganic ion content.

A series of studies were performed in order to assess the potential of Py-GC for use as a quantitative tool for wood pulp saccharide analysis. In each case the optimum pretreatment procedures were used. Samples were deashed as described in section 2.4.2 and the acidity of the suspensions were adjusted to pH 1 as described in section 2.4.3. This was done in order to ensure the maximum possible yields of anhydrosugars and so that all samples could be directly compared with one another. Flame ionisation and mass spectrometry are equally sensitive for the detection of anhydrosugars. However, a flame ionisation detector is many times less expensive than a mass spectrometer, and would therefore be more widely used. For this reason it was decided to use an FID

detector in the following investigations.

Two different methods were used to quantify the saccharide content of the pulps. In the initial study, the glucose and xylose contents of a number of pulps were determined by comparing their anhydrosugar yields with those of isolated polysaccharides (external standard method). In the second and third investigations, one of the pulps was used as a comparative standard. The Py-GC results were then compared with the saccharide analysis obtained by normal acid hydrolysis / derivatization - GC as described in sections 2.2 and 2.3.

In all cases the Pyroprobe®, modified as discussed in section 2.5.2, was used. However, by way of comparison, the second study was repeated using the Pyrojector®.

6.2. Quantification of Glucose and Xylose in a Number of Wood Pulps by an External Standard Method

6.2.1. Optimization of the Pyrolysis Conditions

In the previous chapters much emphasis was placed on the importance of sample pretreatment. However, it is also important to fully control the pyrolysis process itself, in order to ensure the maximum possible reproducibility between runs. It has been reported that wood polysaccharides begin to pyrolyze at different temperatures (92). This same phenomenon was observed during the investigation of the isolated polysaccharides discussed in chapter 3.

Acid-washed black spruce mechanical pulp was used as the standard to optimize the pyrolysis conditions. The pulp was pyrolyzed at various temperatures using the Pyroprobe® in order to find

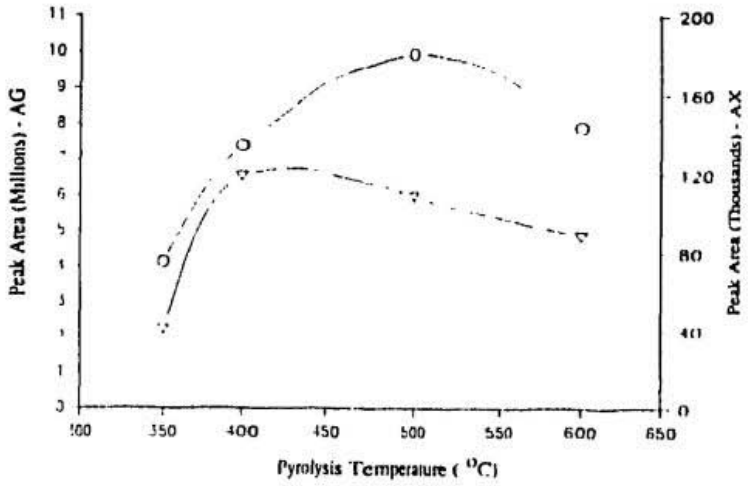


Figure 6.1: The Effect of Pyrolysis Temperature on the Anhydrosugar Yields for Glucose (o) and Xylose (v) from Acid-Washed Black Spruce Mechanical Pulp.

the optimum pyrolysis temperature. Figure 6.1 plots the peak area per microgram for anhydroxylose (AX) and the anhydroglucoses (AG) obtained at each temperature setting used. The optimum temperature for maximum anhydroglucose production is approximately 500°C and that for 1,4-anhydroxylopyranose lies closer to 450°C. It was decided to use 500°C as the pyrolysis temperature for future quantitative analysis because anhydroglucose production drops off quickly at lower temperatures and 1,4-anhydroxylopyranose yield is only slightly less than at the maximum.

Normally, each set of runs was performed using the same pyrolysis tube in order to guarantee the maximum possible reproducibility. As Table 6.1 illustrates (samples 1,2 and

Table 6.1: Optimization of Pyrolysis Parameters.

Sample No.	Tube O.D. (mm)	Wall Thickness (mm)	Sample Site	Temp. Ramp	% Int. Glucose[1] (73+85)	% Int. Xylose[1] (42)	% Int. Mannose[1] (65)
1	2.50	0.46	Centre	None	100	100	100
2	2.39	0.54	Centre	None	84.6	86.1	84.4
3	2.48	0.56	Centre	None	85.2	85.2	89.5
4	2.50	0.46	Centre	5^0C/ms	91.9	72.2	88.2
5	2.50	0.46	Centre	$.1^0$C/ms	50.4	71.8	82.7
6	2.50	0.46	End	None	85.9	108.1	106.8

[1]: Peak area per µg of sample relative to sample 1. Average of triplicate analysis.

3), the effect of using quartz tubes with differing diameters and wall thickness on pyrolysis is pronounced. The thicker walled tubes clearly have a detrimental effect on the production of all anhydrosugar products. This may well be due to the higher heat capacity of the thicker walls. The more heat the walls absorb, the longer the temperature rise time within the sample, and the greater the possibility of altemate pyrolysis pathways been taken. It is also probable that the outer diameter of the quartz tube is important. The tube used in sample 1 was the widest that would fit within the platinum coil and it is probable, in this case, that heat transfer during pyrolysis is less hampered by gas flow between the coil and the tube's outer surface. Unfortunately, it was found that no two tubes taken from the same batch had the same wall thickness and outer diameter. This is due to the method of manufacture. Larger quartz tubes are "drawn" to the required diameter by weighting one end and heating the tube until soft. A large variaton in wall thickness and outer diameter would therefore be expected along the length of the drawn tube. For this reason the same tube was used for each set of samples in the following studies.

Table 6.1 also includes the results of ramping the pyrolysis temperature (samples 4 and 5). In both cases all anhydrosugar yields are lower than for sample 1 (ramp off) which has a temperature rise of approximately 50°C/ms to 500°C (maximum ramp speed of this instrument). Interestingly, the anhydroglucoses exhibit the greatest sensitivity to temperature ramp rate. It is believed that the slower increase in sample temperature would permit the occurence of the low temperature charring reactions discussed by Shafizadeh (71). The hemicelluloses are less affected because they begin to pyrolyze at lower temperatures. In the following quantitative studies, samples were pyrolyzed in the "ramp off" mode.

Sample 6 in Table 6.1 illustrates the effect of analyte positioning within the pyrolysis tube. By moving the pulp sample away from the centre of the tube (which is also the centre of the platinum coil) the anhydrosugars for xylose and mannose increase in intensity by approximately similar magnitudes, suggesting that the lower pyrolysis temperatures experienced at the end of the tube are more suitable for the pyrolysis of the hemicelluloses. However, the yield for the anhydroglucoses is lowered. In addition, the coefficient of variation (C.V.) in peak area increases to approximately 15% for the anhydroglucoses, whereas the C.V. for all anhydrosugars in samples 1 to 3 and for 1,4-anhydroxylopyranose and 1,6-anhydromannopyranose in sample 6 is typically around 2%. This phenomenon most probably occurs for the same reasons discussed in the previous paragraph. Because of this large increase in variation, care was taken to position the analyte in the centre of the tube.

Finally, the linear dynamic range was measured for 1,4-anhydroxylopyranose and for the anhydroglucoses from acid-washed black spruce mechanical pulp (Fig. 6.2a,b). Good linearity was obtained for both from 36 to 123 µg of pulp sample. Anhydrosugar yields began to drop off at

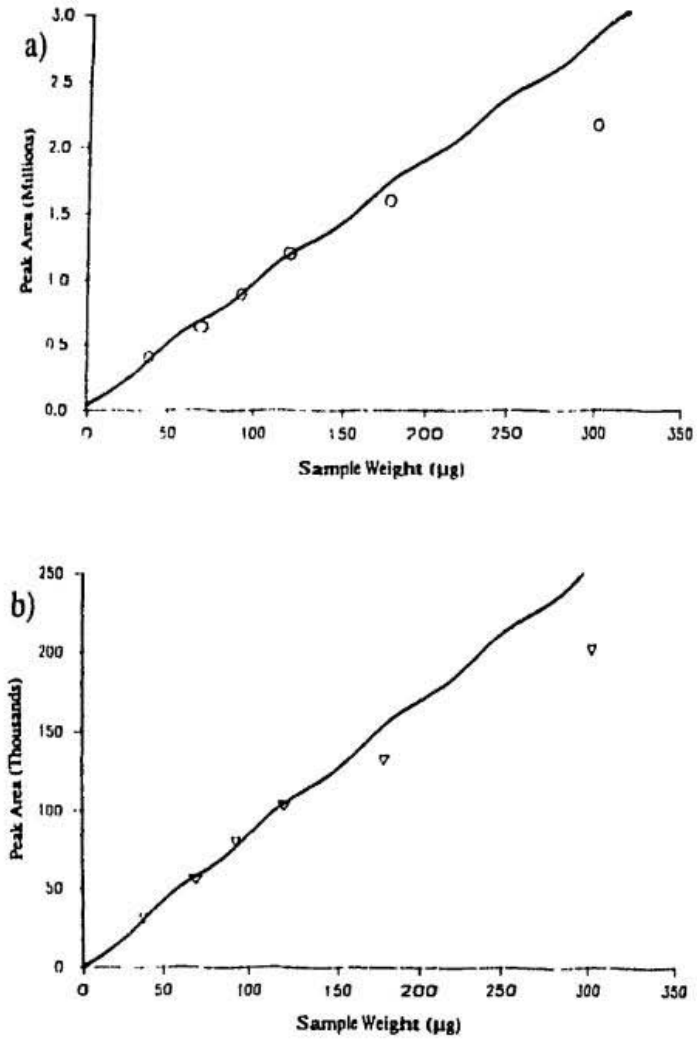


Figure 6.2: Linear Dynamic Range Curves for Anhydrosugar Production for Acid-Washed Black Spruce Mechanical Pulp. (a) Anhydroglucoses and (b) 1,4-Anhydroxylopyranose.

around 182 µg. At the largest sample weight pyrolyzed (302 µg) the pyrolysis tube was well packed with sample which could have partially restricted the flow of carrier gas through the tube. It would have also created large temperature gradients within the bulk of the sample during pyrolysis. This may be the case with the 182 µg sample also. Very small samples (< 30 µg) were also a problem because it proved difficult to properly introduce such samples into the pyrolysis tube and then weigh them accurately using the microbalance. For these reasons it was decided to keep the sample weight between 100 and 150 µg for future quantitative analysis.

6.2.2. Quantification of Glucose and Xylose

In the initial quantitative investigation it was decided to use Avicel microcrystalline cellulose (MC) and xylan from oat spelts as external standards for the following reasons. Both are relatively simple and pure polysaccharides whose structure and saccharide composition have been well characterised. In addition, their similarity to the corresponding polysaccharides found in the pulps under investigation makes them effective substitutes. Moreover, there was no pure 1,4-anhydroxylopyranose available for use as a standard. Finally, because no solvent is involved in the method, consecutive runs are very reproducible.

Standard curves were prepared for the anhydroglucoses from ashless Avicel MC and for 1,4-anhydroxylopyranose from the deionised xylan (Fig. 6.3a,b). In both cases the X-axis scale is calibrated to display the weight of the saccharide in question as measured by acid hydrolysis-derivatization/GC and not the total weight of the sample (i.e., 98% glucose in MC and 79.8% xylose in xylan). The trace for the anhydroglucoses was linear over the range tested whereas the trace for 1,4-anhydrpxylopyranose began to plateau after 133 µg. This was possibly due to the fact that the

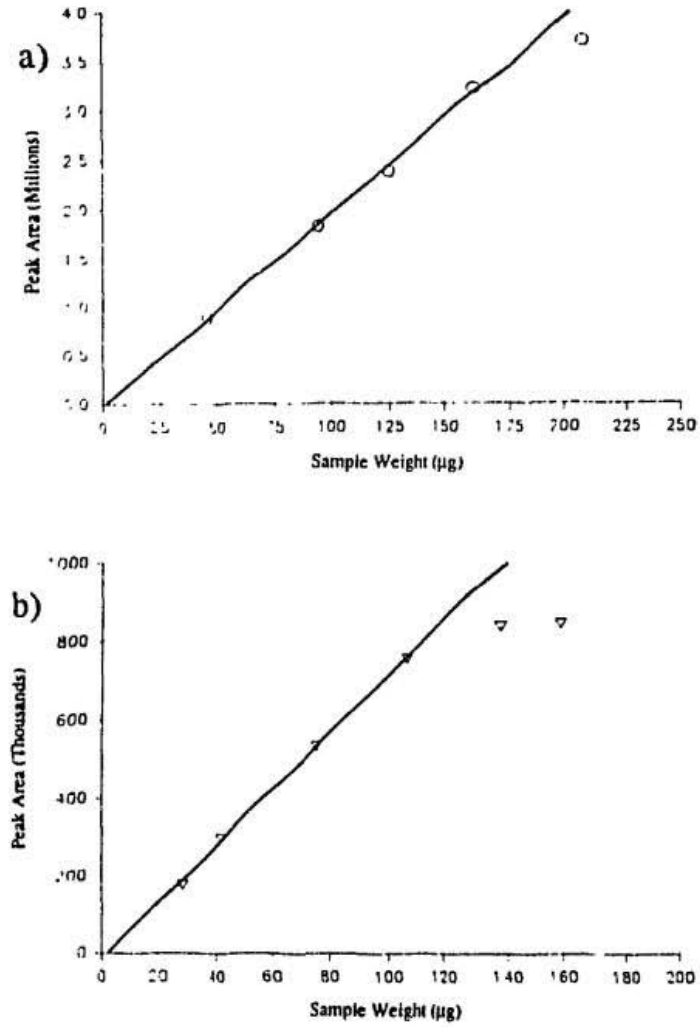


Figure 6.3: Standard Plots of Anhydrosugar Yield versus Sample Weight for (a) the Anhydroglucoses (73 + 85) from Avicel Microcrystalline Cellulose and (b) 1,4-Anhydroxylopyranose (42) from Deionised Xylan from Oat Spelts.

xylan tends to melt during pyrolysis.

For quantitative comparison, pulp samples (100-150 μg) were pyrolyzed in triplicate under identical conditions. Their glucose and xylose contents were calculated using the equation of the line of best fit for the linear portion of each standard curve and are listed in Table 6.2, together with the results obtained by the acid hydrolysis - derivatization / GC (A.H.) procedure described in sections 2.2 and 2.3.

It can be seen that there is reasonably good correlation between the A.H. and Py-GC results in most cases. Aspen steam-explosion pulp which contained sulfite in its pulping process is the exception. The glucose and xylose content as measured by Py-GC are significantly lower than the results obtained by acid hydrolysis. An explanation for this observation has already been offered in section 5.0. Both birch mechanical and Alcell® pulps exhibit higher than expected xylose compositions when measured by Py-GC. The reason for this was not obvious at first. However, subsequent Py-GC-MS analysis indicated that 1,4-anhydroxylopyranose co-elutes with 4-O-methyl syringol, a lignin pyrolyzate not formed by softwoods such as black spruce. It was found that the two peaks could be resolved by using a normal bore DB-1701 capillary column. This column was used in the third, and more extensive, investigation reported in this chapter.

Finally, an important conclusion can be drawn from this study. The constituents of wood pulp are intimately associated with one another forming a complex analyte. However, given the proper sample pretreatment, the yields of the anhydrosugars investigated here are identical to those obtained from matrix-free, isolated polysaccharides. This strongly suggests that the polymers are pyrolyzing independently of one another. This is quite remarkable considering the complexity of the

Table 6.2: Percentage Glucose and Xylose Composition of Acid-Washed Pulps as Determined by Acid Hydrolysis (A.H.) and Pyrolysis - Gas Chromatography (Py).

Sample	% Glc. (A.H.)	% Glc. (Py)[1]	C.V.[2]	% Xyl. (A.H.)	% Xyl (Py)[3]	C.V.[2]	% Ash
Microcryst. Cellulose	98.0	98.0	1.4	---	---	---	0.0
Xylan from Oat Spelts	14.8	---	---	79.8	79.8	1.9	0.09
Black Spruce - Mechanical	50.5	51.4	2.2	11.3	12.0	1.8	0.05
Black Spruce - Kraft	89.2	89.8	1.5	4.3	6.0	3.2	0.33
Birch - Mechanical	46.3	45.6	3.1	15.8	21.7	1.7	0.04
Birch - Alcell	84.1	85.2	1.1	12.3	17.8	0.9	0.16
Aspen - Steam Explosion	57.8	29.3	9.7	9.61	2.1	4.4	0.15

[1]: Calculated as the peak areas of the anhydroglucoses per µg of sample relative to that for Avicel microcrystalline cellulose.

[2]: Coefficient of variation of triplicate Py-GC analyses.

[3]: Calculated as the peak area of 1,4-Anhydroxylopyranose per µg of sample relative to that for deionised xylan from oat spelts.

samples and the highly energetic conditions they experience when pyrolyzed.

6.3. Quantitative Analysis of the Major Saccharides in Fe^{3+}-Exchanged, Sulfite-Treated Pulps using a Standardized Sulfite Pulp

The problems encountered when acid-washed sulfite pulps were pyrolyzed have already been discussed (section 5.0). The initial quantitative study clearly indicated that an alternative method of

sample preparation and quantification of sulfite-treated pulps was required. By incorporating Fe^{2+} ions into the pulp the anhydrosugar yields were increased and levoglucosenone was greatly reduced (section 5.1). Moreover, Py-GC-MS (EI) analysis revealed that all the major pyrolyzates were the same as those produced by the mechanical pulps. Therefore it was decided to attempt to quantify the saccharide content of Fe^{2+}-exchanged pulps. In addition to the steam-exploded pulp, a series of high yield sulfite pulps were obtained from Noranda. Only glucose, xylose and mannose were quantified. In almost every case, acid hydrolysis revealed that galactose and arabinose were absent from the pulps under investigation.

Initially, saccharide quantification was attempted by using isolated polysaccharides as external standards in the same manner as described in the previous section. However, this was found to be unsuitable as the calculated percentages for the saccharides were still low when compared with the acid hydrolysis results. Further work with non-sulfite pulps demonstrated that anhydrosugar yields were lowered by exchanging the pulp with a metal of any kind.

An alternative approach is to use a well characterised, Fe^{2+}-exchanged sulfite pulp as a standard. The saccharide composition of all pulp samples under investigation was determined using a classical acid hydrolysis - derivatization method, and therefore reliable data were available for comparison. Furthermore, because a pulp of similar chemical composition and matrix was used as standard, any problems or characteristics unique to the pyrolysis of these pulps would be normalised.

6.3.1. Quantification using the Pyroprobe®

The Py-GC results tabulated in Table 6.3 were obtained by using the first Noranda sulfite

pulp (N.S.P. 231090) as the comparative standard. The data obtained in this manner correlated reasonably well with those obtained by acid hydrolysis. All samples were analyzed in triplicate and it can be seen that the reproducibilty is quite good in most cases. The Py-GC value for the glucose content for aspen steam-explosion pulp is slightly higher than expected with respect to the acid hydrolysis results. This could not be attributable to error in pyrolysis as the variation between the triplicate runs is low (C.V. is 2.3%). The error may actually be in the acid hydrolysis results. Of interest here also is the fact that in one case, aspen steam-explosion pulp, mannose was not detected by acid hydrolysis while Py-GC measured it at 1.3%.

Table 6.3 also includes the results of the inorganic analyses carried out on the pulps. It can be seen that the elemental sulfur content, as determined by XRF (section 2.8.3), varies between the pulps. The iron content, as determined by atomic absorbtion spectroscopy (section 2.8.2), follows this trend also, suggesting a direct relationship between the sulfonic acid content and the quantity of iron exchanged into the pulp. More importantly, the results obtained by Py-GC do not seem to be affected by the variation in the sulfur and iron contents.

By way of comparison, a non-sulfite pulp (Noranda kraft pulp) was prepared and pyrolyzed in the same manner as the sulfite pulps. The Py-GC results (Table 6.3) for glucose and mannose do not correlate well with those obtained by acid hydrolysis. In contrast, the results obtained for xylose by both methods are comparable. This may be due to the fact that wood xylans contain exchange sites (i.e., uronic acids). Other non-sulfite pulps, both high and low yield, behave in this manner also. It is probable that the differences in matrix composition (i.e, the lack of sulfonic acid groups in the lignin) can account for this difference.

TABLE 6.3 : Percentage Saccharide Composition of Fe^{2+}-Exchanged Pulps as Determined by Acid Hydrolysis/Derivatization-GC (A.H.) and Pyrolysis-Gas Chromatography (Py).

Pulp Sample	Glucose			Xylose			Mannose			% S	% Ash	% Fe
	A.H. (%)	Py[1] (%)	C.V.[2] (%)	A.H. (%)	Py[1] (%)	C.V.[2] (%)	A.H. (%)	Py[1] (%)	C.V.[2] (%)			
N. S. P. (# 231090)	59.0	59.0	0.4	3.5	3.5	1.9	12.2	12.2	0.7	1.8	1.0	0.7
N. S. P. (# 25907)	55.9	52.3	7.5	3.7	3.6	6.9	10.9	9.4	2.7	1.2	0.9	0.6
N. S. P. (# 25910)	53.7	51.9	0.1	3.3	2.5	12.3	12.4	9.2	2.8	3.6	1.4	1.4
N. S. P. (# 25912)	58.1	55.7	1.8	2.6	1.8	3.6	6.1	6.2	0.7	4.1	1.6	1.5
N. S. P. (# 25936)	53.0	50.2	3.4	3.6	3.4	1.5	9.3	9.6	4.0	1.9	1.1	0.9
Aspen - Steam Exploded Pulp	56.9	61.6	2.3	8.1	7.1	1.5	0.0	1.3	3.3	1.5	1.1	0.9
Noranda Kraft Pulp	85.4	96.0	2.4	14.8	13.2	5.7	1.4	0.6	15.6	0.3	0.8	0.2

[1]: % Glucose, xylose and mannose as determined by Py-GC, are calculated using the areas of peaks 73 + 85 (anhydroglucoses), 42 (anhydroxylose) and 65 (anhydromannose); relative to sample N.S.P. # 231090.

[2]: Coefficient of variation of triplicate Py-GC analyses.

6.3.2. Quantification using the Pyrojector®

For comparative purposes, the same experiment was repeated using the Pyrojector®. The conditions used in the previous investigation were repeated as closely as possible here. The pulp samples were introduced into the microfurnace using the wide-bore syringe as described in section 2.5.2. The results obtained are given in Table 6.4.

The correlation between the acid hydrolysis and Py-GC results for the three saccharides is poor in many cases. In addition, the larger coefficients of variation for the triplicate analyses indicate that pyrolysis with this pyrolyzer is not as reproducible as with the Pyroprobe®. Great care was taken to ensure that sample introduction was repeated exactly the same way each time. Furthermore, tests with microcrystalline cellulose prove that it is possible to carry out a reproducible pyrolysis with this instrument (Fig. 6.4).

When a sample is introduced into the microfurnace it falls through the heated zone and comes to rest on the quartz wool plug (Fig. 2.2). During its descent it is exposed to a temperature gradient as the carrier gas is gradually heated by the furnace oven. Wood pulps contain hemicellulose and lignin that may begin to pyrolyze during the descent. If this is the case, then it is probably impossible to guarantee reproducible pyrolysis conditions. On the other hand, microcrystalline cellulose (MC) begins to pyrolyze at a higher temperature than most other polysaccharides and probably resists degradation during descent. Furthermore, the pulp samples are light and bulky by comparison with MC and their passage down the narrow quartz tube may be slowed by contact with the walls, especially if the sample becomes tacky from the melting of the hemicellulose polymers. This would affect the temperature at which pyrolysis occurs and the temperature rise time

Table 6.4: Percentage Saccharide Composition of Fe^{2+}-Exchanged Pulps Obtained using the Pyrojector®.

Pulp	Glucose			Xylose			Mannose		
	A.H. %	Py[1] %	C.V.[2]	A.H. %	Py[1] %	C.V.[2]	A.H. %	Py[1] %	C.V.[2]
N.S.P. (23/10/90)	59.0	59.0	9.8	3.5	3.5	28.4	12.2	12.2	33.0
N.S.P. (25936)	53.0	51.4	1.7	3.6	5.5	8.4	9.3	9.4	6.1
N.S.P. (25912)	58.1	38.9	30.2	2.6	8.2	36.5	6.1	8.2	16.8
N.S.P. (25910)	53.7	52.9	4.4	3.3	3.0	27.0	12.4	9.6	9.6
N.S.P. (25907)	55.9	42.8	13.6	3.7	3.2	0.5	10.9	8.7	4.9
Aspen-Steam Exploded	56.9	65.1	5.3	8.1	3.5	10.4	ND	1.6	8.2
Noranda Kraft Pulp	85.4	77.1	11.1	14.8	10.3	13.9	1.4	0.5	28.1

[1]: % glucose, xylose and mannose as determined by Py-GC, are calculated using the areas of peaks **73 + 85** (anhydroglucoses), **42** (anhydroxylose) and **65** (anhydromannose); relative to sample N.S.P. # 231090.

[2]: coefficient of variation of triplicate Py-GC analyses.

within the sample itself.

Unfortunately, it appears that the microfurnace-type pyrolyzer is unable to meet the demands put on it by this kind of analysis. The Pyrojector® was designed as a simple and easy to use pyrolyzer and qualitatively it matches the Pyroprobe®. However, the latter

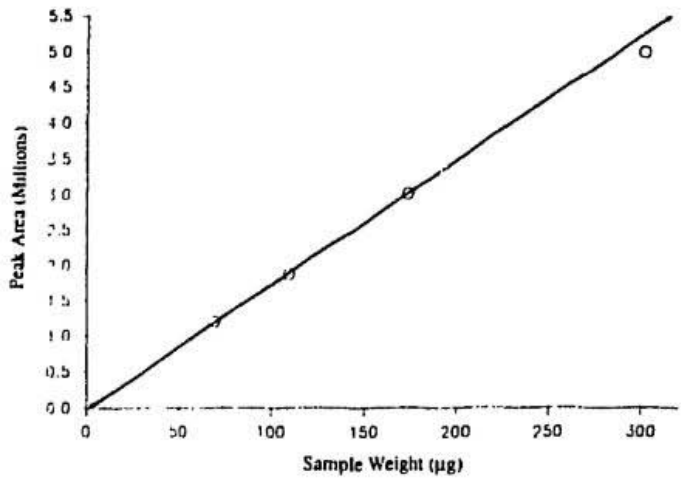


Figure 6.4: Sample Weight versus Anhydroglucose Peak Area (73 + 85) for Avicel Microcrystalline Cellulose using the Pyrojector®.

instrument is more suitable for quantitative analyses because of the greater degree of control it offers over the pyrolysis process.

6.4. Quantitative Analysis of All Neutral Saccharides in the Acid-Washed, Non-Sulfite Pulps using a Standardized Pulp.

In the first quantitative study, the glucose and xylose contents of a number of acid-washed pulps were determined using isolated polysaccharides as external standards. Though the results were encouraging, the procedure was very slow. Each pyrolysis run took

approximately 40 minutes and the preparation of the standard curves alone was a full day's work. However, in the investigation of the Fe^{2+}-exchanged sulfite pulps it was demonstrated that one of the pulps could be successfully used as a comparative standard. In addition to the advantages discussed at that time, this approach accelerated the speed of analysis because it eliminated the need for standard calibration curves. Moreover, all the major saccharides present in the pulps were quantified simultaneously.

It was decided to use this approach in this study also. Black spruce mechanical pulp was chosen as the comparative standard because it contained reasonable quantities of all the saccharides to be assayed. Samples were prepared and pyrolyzed in the same manner as described in section 6.1.1. However, in this instance a normal bore DB-1701 column was used in order to resolve 1,4-anhydroxylopyranose and 4-O-methyl syringol in the birch mechanical pulp pyrogram. The results are tabulated in Table 6.5. Galactose was determined solely as 1,6-anhydrogalactopyranose because the other anhydrogalactoses were often undetectable.

By and large the Py-GC results compare well with those obtained by acid hydrolysis. The one exception is the result for xylose in birch mechanical pulp. It is significantly lower than than the acid hydrolysis result, which is considered to be a reasonable value for a hardwood mechanical pulp such as this. Furthermore, the discrepancy cannot be attributed to irreproducible pyrolysis runs as the C.V. is only 1.8%. The problem may lie with the polysaccharide itself. Hardwood xylans can be acetylated at the C2 or C3 positions on the

Table 6.5: The Percentage Composition for all the Saccharides in the Acid-Washed Pulps using Acid-Washed Black Spruce Mechanical Pulp As Standard.

Pulp	Glucose			Xylose			Mannose			Galactose			Arabinose		
	A.H. %	Py[1] %	C.V.[2]	A.H. %	Py[1] %	C.V.[2]	A.H. %	Py[1] %	C.V.[2]	A.H. %	Py[1] %	C.V.[2]	A.H. %	Py[1] %	C.V.[2]
Black Spruce - Mechanical	50.5	50.5	2.7	11.3	11.3	0.6	9.5	9.5	1.6	1.6	1.6	8.6	1.0	1.0	1.3
Black Spruce - Kraft	89.2	87.2	3.8	4.3	6.3	1.5	1.8	2.3	3.3	ND	ND	---	ND	ND	---
Birch - Mechanical	46.3	48.4	6.1	15.8	11.8	1.8	1.4	1.8	7.2	ND	0.27	23.1	0.5	0.3	8.1
Birch - Alcell	84.1	85.6	1.4	12.3	10.2	0.2	ND	1.0	2.6	ND	ND	---	ND	<0.1	22.4
Noranda Kraft Pulp	85.4	86.1	1.9	14.8	14.6	3.7	1.0	1.4	19.5	ND	ND	---	ND	ND	---

[1]: % Glucose, xylose and mannose as determined by Py-GC, are calculated using the areas of peaks 73 + 85 (anhydroglucoses), 42 (anhydroxylose), 65 (anhydromannose), 60 (anhydrogalactose) and 31 (anhydroarabinose); relative to acid-washed black spruce mechanical pulp.

[2]: Coefficient of variation of triplicate Py-GC analyses.

N.D.: Not Detected.

Table 6.6: The Percentage Xylose in the Acid-Washed Pulps determined by the method of Kleen et al. (96).

Pulp	% Xylose A.H.	% Xylose PY[1]	C.V.[2]
Black Spruce - Mechanical	11.3	11.3	0.4
Black Spruce - Kraft	4.3	6.1	1.5
Birch - Mechanical	15.8	14.9	2.5
Birch - Alcell	12.3	14.3	1.3
Noranda Kraft Pulp	15.1	14.4	3.3

[1]: Determined after summing the areas of peaks **8** + **42**; relative to acid-washed black spruce mechanical pulp.

[2]: Coefficient of variation of triplicate Py-GC analyses.

xylopyranose units whereas softwood xylans are not. If the acetyl groups prevent the xylose units to which they are attached from forming the anhydrosugar then the yield of 1,4-anhydroxylopyranose would be lower than expected. This restriction is not present when samples are analyzed by acid hydrolysis. This phenomenon is not as pronounced with the Alcell® birch pulp as most of the pendant acetyl groups are removed during the Alcell® pulping process (112).

In their investigation, Kleen et al. (96) determined the relative intensity of xylose in wood pulps after summing the peak intensities of 1,4-anhydroxylopyranose,**42**, and 4-hydroxy-5,6-dihydro-(2H)-pyran-2-one, **8**, a known xylose marker. The intensities for the latter pyrolyzate was always significantly higher for hardwood pulps than for softwoods. This same phenomenon was observed here also. Table 6.6 lists the % xylose results determined after summing the peak areas of both

pyrolyzates. There is a marked improvement in the correlation of the Py-GC and acid hydrolysis results for the two hardwood pulps. The xylose content of the softwood pulps are not greatly altered from Table 6.5. Therefore, it would appear that this is a more suitable approach for xylose determination.

Galactose and arabinose are either absent from the pulps or are present only in small amounts and so it is not surprising that the reproducibility of their pyrolysis results is poorer than for the other saccharides. However, at such low concentrations the accuracy of the acid hydrolysis results can also be challenged, especially considering the sensitivity of arabinose to the aggressive acidic conditions required to hydrolyze the wood polysaccharides. Furthermore, small amounts of galactose and arabinose were detected by Py-GC in two of the pulps even though they were not observed by acid hydrolysis.

CHAPTER 7: CONCLUSION

The objective of this investigation was to assess the ability of on-line pyrolysis - gas chromatography (mass spectrometry), Py-GC (MS), to characterize and to quantify the neutral saccharide composition of wood pulps by the analysis of distinct anhydrosugar products. The initial studies concentrated on optimizing the production of anhydrosugars from isolated polysaccharides. For the glucans investigated, microcrystalline cellulose and amylose, ash removal and the temperature of pyrolysis had the greatest effect on the yields of the anhydroglucoses. Furthermore, after proton exchange the anhydrosugar yield was almost identical for all glucans, suggesting that neither the degree of polymerization nor the anomeric configuration of the glycosidic linkages had any effect on anhydrosugar formation. A number of heteropolysaccharides were also investigated and, in each case, proton exchange was required for optimum anhydrosugar production. Moreover, the best temperature for pyrolysis was significantly lower than that for the glucans. However, the anhydrosugar conversion rates of the heteropolysaccharides were substantially lower than those of the glucans. This may be due in part to the higher thermal instability of some of the anhydrosugars, particularly the pentoses, and in part to melting of the heteropolysaccharides.

Attention was then turned to the wood pulps. Here again the yields of anhydrosugars from all the neutral saccharides in the pulps were greatly increased by lowering the ash content. This was best achieved by suspending the pulp in 0.1 N HCl solution. In addition, a relationship was observed between anhydrosugar production and the pH of the suspending solution, with maximum yields being obtained at approximately pH 1. It was postulated that the presence of trace acid in the pulp prevents lignin from interfering in the pyrolysis of the wood polysaccharides. Furthermore, from a qualitative point of view, deashing allows direct comparisons to be made between the pulp pyrograms irrespective of origin, composition and method of pulping.

However, sulfite-treated pulps behaved differently from the other pulps. The pyrograms of the acid-washed sulfite pulps were dominated by levoglucosenone. It was proposed that acid-washing protonates sulfonate groups present in the pulp and, during pyrolysis, promotes the in situ formation of acid. This would enhance the formation of LGO from cellulose at the expense of the anhydroglucoses. This effect can be counteracted by exchanging the pulps' acidic groups with cations, effectively preventing acidic catalysis. The best anhydrosugar yields were obtained for pulps exchanged with Fe^{2+} or Fe^{3+} ions.

The pyrolyzate profiles of a number of pulps were investigated by Py-GC/MS. Both EI and NH_3 CI mass spectrometry were used to investigate the acid-washed mechanical pulps of black spruce and birch. The major carbohydrate and lignin pyrolyzates were successfully identified . However, some difficulty was encountered when attempting to identify the smaller pyrolyzates, particularly those derived from carbohydrates. Nevertheless, most of the key pyrolyzates were posivitely identified.

The pyrolyzate profiles of a reference sulfite pulp at various stages of pretreatment were also investigated by Py-GC/MS(EI). The type of lignin and carbohydrate pyrolyzates produced by the untreated and Fe^{2+}-exchanged pulps were basically the same, though the yields of many differed quite considerably. In contrast, the lignin profile of the acid-washed pulp was quite different. Many of the lignin pyrolyzates typical of the other pyrograms were absent and those that were identified were either aldehydes or ketones. In addition, a number of new, isomeric lignin pyrolyzates were observed and these too are believed to be aldehydes.

The ultimate objective of this research was to quantify the saccharide composition of the

pulps by analytical pyrolysis. Initially, the glucose and xylose content of a number of acid-washed pulps was determined by Py-GC (-FID) using isolated polysaccharides as external standards. By and large, the results were encouraging and correlated reasonably well with those obtained by acid hydrolysis / derivatization - GC. However, this approach did not suit the analysis of acid-washed, sulfite-treated pulps as their anhydrosugar yields were low and irreproducible. This problem was overcome by analyzing pulps exchanged with Fe^{2+} ions and, for quantitative analysis, using one of them as a comparative standard.

A final and more extensive study was carried out on the acid-washed, non sulfite-treated pulps. A narrower bore capillary column was used to resolve 1,4-anhydroxylopyranose from 4-methyl syringol in the pyrogram of birch mechanical pulp. Also, black spruce mechanical pulp was used as a comparative standard for quantitative analysis (as opposed to the use of external standards). Here again the saccharide composition results obtained by Py-GC and by acid hydrolysis / derivatization - GC correlated quite well except in the case of xylose for birch mechanical and, to a lesser extent, birch Alcell® pulps. It is believed that the low xylose values for these pulps obtained by Py-GC are due to the fact that hardwood xylans are heavily acetylated. However, better correlation was obtained if the peak areas of 1,4-anhydroxylopyranose, **42**, and 4-hydroxy-5,6-dihydro-(2H)-pyran-2-one, **8**, a known xylose marker, were combined for quantitation.

The above quantitative studies were carried out using the Pyroprobe® fitted with a platinum coil. For the major saccharides, the reproducibility in measurement was very good (C.V. was usually 5% or better). However, when the second quantitative study was repeated using the Pyrojector®, the results did not compare well with those obtained by acid hydrolysis. Furthermore, the reproducibility was quite poor in many cases. This was attributed to the design of the Pyrojector® pyrolyzer which

allowed more variation in the pyrolysis conditions.

However, the Pyroprobe® had a number of flaws. For example, it was found that the dimensions of the pyrolysis tube influenced the anhydrosugar yields and, because no two tubes had the same diameter and wall thickness, it was necessary to use the same one for a set of analyses. Moreover, it was necessary to place the analyte in the same position (the centre of the tube) each time in order to ensure good reproducibility. These problems may prove to be major obstacles to the development of a routine or automated quantitative method based on Py-GC. However, it may be possible to avoid these problems by using an alternative pyrolyzer design. For example, the Pyrola® pyrolyzer fitted with detachable platinum boats would eliminate the need for quartz tubes and would allow for easy weighing and manipulation of the samples. In addition, because the heat no longer has to pass through a quartz barrier before reaching the sample, a faster and more reproducible temperature rise would result.

The quantitative studies were carried out using a flame ionization detector (FID). By and large this proved to be a good choice as it has excellent sensitivity for the type of compounds encountered here and because of the long term stability of its signal. However, due to the complexity of the pyrograms, problems were often encountered when attempting to identify small peaks, especially 1,4-anhydro-arabinopyranose, **31**, and the anhydrogalactoses, **73** and **85**. In addition, some anhydrosugars were not fully resolved from neighbouring pyrolyzates as in the case of 1,4-anhydro-xylopyranose, **42**, in the hardwood pulp pyrograms, thus making quantitation difficult.

These problems can be overcome by using a mass spectrometer as a detector. Modern EI quadrupole mass spectrometers are easily interfaced with capillary GC, and their ion signal is usually

stable over relatively long periods of time. Furthermore, the sensitivity of EIMS is at least as good as FID for these type of compounds, especially in ion selective mode. Another advantage of using selected ion monitoring is its ability to discriminate between compounds. By choosing to monitor only those ions unique to, or at least most prominent in, the mass spectra of the desired analyte, the chromatogram can be greatly simplified. For example, Figure 7.1 contains the extracted ion chromatogram (EIC) for the ion m/z 57 for acid-washed black spruce mechanical pulp. When compared to the TIC (Fig. 4.10), this EIC is simpler and clearer, and this despite the m/z of the extracted ion. The anhydrosugars are the only prominent peaks and even 1,4-anhydroarabinopyranose, **31**, is clearly identifiable.

This study has shown that, under the right conditions, analytical pyrolysis can be used as an alternative method for the qualitative and quantitative analysis of the neutral saccharides in wood pulps. Typically, it takes two to three days to carry out the acid hydrolysis / derivatization - GC

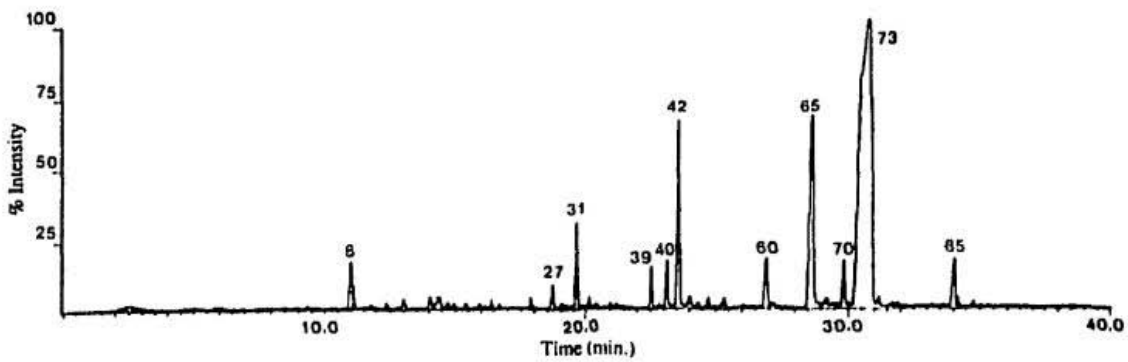


Figure 7.1: Extracted Ion Chromatogram (m/z 57) for Acid-Washed Black Spruce Mechanical Pulp.

method described in sections 2.2.2 and 2.3. Furthermore, the method is laborious and difficult. By comparison, a pulp can be pretreated (i.e., deashed, pH adjusted and oven-dried) and pyrolyzed in a single working day. In addition, each step in the pretreatment is simple to carry out. Finally, if an FID detector is used, the only additional capital costs would be the pyrolyzer itself.

The advantages of using analytical pyrolysis as a fast and simple screening method for a wide range of biomaterials are now appreciated. It is hoped that in the near future analytical pyrolysis will reach its full potential both as a qualitative and quantitative tool for the analysis of polysaccharides, lignins and other biopolymers.

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