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Title: Carbohydrates in a Thermomechanical Pulp, a Sulfite Pulp, and a Solvent Sulfite Pulp from Western Hemlock

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A thermomechanical pulp, a high yield sulfite pulp, and a high yield solvent sulfite pulp from western hemlock [Tsuga heterophylla (Raf.) Sarg.] wood were investigated to determine the amount of carbohydrate material present and to elucidate the structures of the carbohydrates. The effects of the three pulping processes, which were quite different, on the carbohydrates are compared.

Attempts to isolate hemicelluloses from the pulps

were only moderately successful. The high lignin content interfered with hemicellulose extraction. However, a xylose-rich hemicellulose and a glucomannan hemicellulose was isolated from each pulp. Most of the pulp materials remained insoluble in the alkaline media ordinarily used for hemicellulose extractions.

The structures of the carbohydrate polysaccharides were clarified by the use of the quite new technique of cross polarization/magic angle spinning nuclear magnetic resonance of solid samples. This physico-chemical spectroscopic method is a non-destructive method in which the solid samples are recovered unchanged. The method requires no preparation of derivatives and hence reduces the chance of polysaccharide degradation. It replaces, in part, the classical methylation procedure ordinarily used to determine the positions of linkages in polysaccharides. The xylan, the glucomannans, and the celluloses of the samples reported in this work were shown by carbon-13 cross polarization/magic angle spinning nuclear magnetic resonance of their solid forms to be linked by 1→4 glycosidic linkages. In addition, peaks at 90 ppm and 85 ppm in the spectra of cellulose samples from the high yield solvent sulfite pulp indicate at least two crystalline polymorphs of cellulose.

Carbohydrates in a Thermomechanical Pulp, a Sulfite Pulp, and a Solvent Sulfite Pulp from Western Hemlock

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CARBOHYDRATES IN A THERMOMECHANICAL PULP, A HIGH YIELD SULFITE PULP, AND A HIGH YIELD SOLVENT SULFITE PULP FROM WESTERN HEMLOCK

I. INTRODUCTION

Carbohydrates have always been a good material resource and energy resource. Green plants transform solar power into materials and energy in the form of synthesized carbohydrate compounds. As a consequence, forests have always been regarded as a renewable source of material and energy which we humans need. We are not only concerned with materials and energy these days from the carbohydrates, but bio-related information has also become increasingly important and is under very detailed research. Considerable genetic and identification information involves the sequence and types of carbohydrates. We in this laboratory have investigated methods of both qualitative and quantitative analyses of carbohydrates in previous efforts. These methods are often employed when carbohydrate analyses are needed.

Pulp and paper are major products of the forest industry. Research into the chemistry of pulp and paper includes basic and fundamental investigations of the

quality of the products. In this laboratory, we have carried out this type of research to have a better understanding of the characteristics of samples prepared by three different pulping processes.

A variety of physico-chemical analytical techniques have been employed for carbohydrate samples, such as proton nuclear magnetic resonance, carbon-13 nuclear magnetic resonance, ultraviolet-visible and infrared absorption spectroscopy and mass spectroscopy. These are widely used. Until recently the nuclear magnetic resonance methods have not been too useful for pulp and paper studies because they have required dissolution of the samples in a suitable solvent. The most desirable form for the investigation of the carbohydrates in pulp and paper is the intact solid form. Recently cross polarization/magic angle spinning (CP/MAS) carbon-13 nuclear magnetic resonance (NMR) technology has been developed enough to have spectra of good quality that can be meaningfully interpreted. This technique has been employed in this thesis for the analyses of intact pulp samples. By using standard chemical tests, spectral data from authentic carbohydrates, and carbon-13 CP/MAS NMR spectra of isolated pulp polysaccharides, the chemical properites of the pulps investigated are better understood.

The specific objectives of this work were: 1) to analyze and compare the carbohydrates in the three different pulps; 2) to isolate and characterize the polysaccharides in the pulps to ascertain the effects of the pulping processes on the polymers; 3) to elucidate the structures of the polysaccharides utilizing the new non-destructive technique of carbon-13 CP/MAS NMR of solid materials.

II. HISTORICAL REVIEW

To go from a raw material such as wood chips to a final product of fiberous material such as paper, the pulping process has always been a decisive factor. In order to obtain maximum yield and best quality, pulping processes have been under investigation for almost as long as human history.

Recently, to adapt to economic situations, a very high yield thermomechanical pulping process has been developed to supply the daily demand for newsprint. The thermomechanical process involves pressurized presteaming of the wood chips and usually pressurized refining in rotating disk refiners. There is ordinarily no chemical treatment of the wood, but there is some loss of solids through dissolution in the steam and resulting water removal. Collicutt, Frazier, Holmes, Joyce, Machie and Torza (12) comment that thermomechanical pulping technology is not yet mature and that the growth rate is still about 25% per year. Thermomechanical pulps (TMP) are thus important materials and an understanding of the carbohydrates in TMP is considered important.

The commercial production of pulp by the sulfite

process, on the other hand, is over 100 years old. It is one of the major commercial processes by which lignin is chemically removed from wood. The product remaining is generally called sulfite pulp. Typical conditions of sulfite pulping involves bubbling sulfur dioxide gas through a suspension of calcium carbonate in water or through a tower packed with wet calcium carbonate. The resulting liquor in essence is a solution of sulfurous acid with the pH regulated by the amount of base. In the above description the base is calcium although magnesium, sodium, and ammonium bases are also used. Thus, in sulfite pulping the wood chips are typically treated with a solution of sulfurous acid at a pH below 4.0 in a pressurized vessel. The temperature is increased to as high as 170° and the cooking time can be as long as 3-7 hours. The sulfite process is quite a harsh chemical treatment and considerable of the carbohydrates are dissolved along with the lignin so that pulp yields can be as low as 50%.

Solvent pulping, in which all or part of the aqueous medium is replaced by organic solvents, is presently under investigation in many laboratories. Some of these solvent pulping processes result in higher yields and better quality pulps than do the present commercial methods. The carbohydrates in these solvent pulps have not been

extensively investigated.

Of the three pulps investigated in this work, only the carbohydrates of a sulfite pulp have been extensively studied. Hamilton, Kircher and Thompson (31) isolated two distinct types of hemicellulose materials by an alkaline extraction of wood pulp produced from western hemlock by the sulfite process. The principal constituent was a glucomannan in which the ratio of mannose residues to glucose residues was 4 to 1. The hemicellulose was a linear polysaccharide in which the mannose and glucose residues were β-linked and joined predominantly by 1→4 glycosidic bonds. The second hemicellulose isolated from the sulfite pulp from western hemlock was a xylan polyuronide (32). The backbone of the polysaccharide was a chain of D-xylopyranose residues joined predominantly by 1→4-β-glycosidic bonds. At frequent intervals a 4-O-methyl-D-glucuronic acid residue was attached to position 2 of a xylose moiety of the main chain. These uronic acid groups occurred on an average of one to every 4 to 8 xylose residues.

The isolation of the hemicelluloses of wood and wood pulps is difficult. Beélik, Conca, Hamilton and Partlow (9) developed what has become essentially a standard procedure for the isolation and separation of hemicelluloses from softwoods. Holocellulose was first

prepared from wood chips by the acidified sodium chlorite delignification procedure. The hemicelluloses were then selectively extracted from the holocellulose. The key feature of this isolation sequence was the selective blocking of the dissolution of mannose-containing polysaccharides by impregnation with barium hydroxide. This allowed the extraction of hemicellulose-A, a xylan polysaccharide, with 10% potassium hydroxide. After removal of the barium hydroxide, hemicellulose-B, a galactoglucomannan, was extracted from the residue with 1% sodium hydroxide. Hemicellulose-C, a glucomannan, was finally extracted from the residue with 15% sodium hydroxide. The insoluble residue which did not dissolve in any of the extraction media was called residue C and was a glucose-rich material, undoubtedly cellulose. This selective extraction of hemicelluloses was applied in the present work in an attempt to isolate hemicelluloses from the pulps under investigation.

Nuclear magnetic resonance spectroscopy (NMR) has long been known and has been used very effectively for the characterization of the protons in various compounds. However, not until about 15 years ago were some carbon-13 NMR spectra actually obtained for the purpose of carbon identification. For example, Perlin and Casu (49) reported some carbon-13 NMR and some proton nuclear

magnetic resonance (PMR) data of glucose in which all the carbons were approximately uniformly enriched isotopically. Several pentose and hexose aldopyranoses were examined by Dorman and Roberts spectroscopically (18). The samples they used were in solution and the carbon-13 chemical shifts were found to be heavily dependent on the proximity of the substituents on the pyranose ring which can be explained in terms of steric hinderance or proximity effects. The spectrum assignments basically agreed with Perlin and Casu's (49). Dorman, Angyal and Roberts (17) proved later that the very important sources of chemical shift differences is from steric or proximity effects in their investigation of carbon-13 NMR spectra of some inositols and their O-methylated derivatives. Dorman and Roberts (17) also assigned the spectra of some common oligosaccharides in 1971 but these were later shown to be incorrect by Walker , London, Whaley, Barker, and Matwiyoff (69). They did some pH dependence studies and worked on saccharide derivatives also.

Gorin (27) rationalized the carbon-13 NMR spectra of a yeast mannan and structurally related oligosaccharides by using this technique. Several types of linkages were identified and both α- or β- linked mannopyranosyl units were found attached to C-2 or C-3 of the sugar unit in

the (1→6)-linked main chain. Chemical shifts and relative peak heights of several carbons in certain mannans were interpreted (27). Gorin and Mazurek (28) later assigned the spectra of aldoses and derived methyl glycosides by employing deuterium substitition techniques Usui, Yamaoka, Matura and Tusimura (66) reported the spectra of glucose oligomers and polymers. They not only determined the anomeric configurations of glucobioses but also the conformation of some glucose oligomers.

Colson, Jennings and Smith (13) assigned several cyclodextrins and linear glucans completely and they determined the composition, structure and major sequence of a number of glucans. They found that methylation of a hydroxyl group of a glucose unit always produced a downfield shift of the bearing carbon. Also they explained the relationship between pD change and intramolecular hydrogen bonding, especially those involved in linkages other than anomeric or exocyclic. In their later work, Colson and King (14) found that changing the nature of the reducing end had no important influence on the chemical shifts of the carbons in the non-reducing ring. This was also true for the converse.

Some malto- and isomalto-oligosaccharides, amylose and dextrans were analyzed by Friebolin, Frank, Kerlich and Siefert (23). They found the resonances of the central glucose units are independent of the chain length with the exception of C-1 and C-4 of amylose. Walker, London, Whaley, Barker and Matwiyoff (69) used 1-C-13 labeled monosaccharides to reevaluate the spectra of monosaccharides run previously by others (19) and found several very interesting results, such as C-2 and C-3 exhibit a coupling to C-1 only in the β anomer while C-5 is coupled to C-1 only in the α anomer.

Lichenine, containing three glucose units, has had its spectrum interpreted by comparison with laminarine and cellulose as references (24). The carbon-13 NMR technique has also been widely employed in the bio-related disciplines. It has been used to prove the structures of nucleoside disaccharide antibiotics (68). Balza, Cyr, Hamer, Perlin, Koch and Stuart (7) applied the catalytic, hydrogen-deuterium exchange technique in C-13 NMR spectroscopic analyses of sugar derivatives. They assigned the spectra with less ambiguity.

The spectra of unmodified, 1→4 linked β-D-gluco-oligosaccharides obtained by acetolysis of cellulose were fully interpreted. It was found that chain length has no influence on the chemical shift of

the resonances of the central residues (35). The carbon-13 NMR studies of mono-, oligo-, and polysaccharides, especially the procedures for structural determination of polysaccharides, were reviewed and described in detail by Sugiyama (65). The combined techniques of selective proton decoupling, carbon-13 selective spin labeling, and isotopic chemical shift induced by deuterium allowed a complete and unambiguous assignment of cellobiose and maltose. Also the chemical shifts variation of the carbon-13 signals with the degree of polymerization of each α or $\beta(1\rightarrow4)$ series has been discussed (33). Mono- and disaccharide spectra were further reevaluated in the light of the differential isotope shift (DIS) technique. The uniqueness of this method is the use of a dual coaxial NMR cell to measure simultaneously the differential shift positions in the magnetic field (50).

Equilibrium compositions of maltulose and isomaltulose solutions in D_2O along with the resonance assignments of some natural saccharides have been done by Jarrell, Conway, Moyna and Smith (36). They also found that resonances of the non-reducing group is sensitive to the ring and anomeric forms of the adjacent residue. Higher oligosaccharides and those of the corresponding disaccharides were compared spectroscopically and found

to closely resemble each other. There is a very important difference between the xylo- and cello-oligosaccharides in the chemical shifts of the internal C-4 atoms of each residue. This was interpreted as due to the absence of C-6 in xylo-oligosaccharides (25,26). Carbon-13 NMR spectra of some partially and fully acetylated L-rhamnose derivatives were assigned by Pozsgay and Neszmeleyi (54). A series of xylo-oligosaccharides was further analyzed and their carbon-13 NMR spectra were shown to be very useful in naturally occurring xylo-rich polysaccharide analyses (41). Six disaccharides composed of D-xylopyranose units with hexa-O-acetyl substituents were analyzed and compared with one another and also the effects on chemical shifts due to the presence or absence of acetyl groups was discussed (67).

A two-dimensional C-13 NMR spectra was developed for the analyses of oligosaccharides and ^{1}H-^{13}C coupling measurements were found that can facilitate interpretation (29). This two-dimensional NMR technique was further elaborated into double Fourier-transform (2D) NMR which measures simultaneously the proton and C-13 chemical shifts of each directly bonded carbon-proton pair in a molecule. It also facilitated the assignment of conventional proton and C-13 NMR spectra (45).

C-13 NMR was also used to observe the unusual upfield shifts of various forms of 1,1'-linked glycopyranosyl-glycopyranosides (40). Quantitative data on tautomeric equilibria of sugars in solution were obtained by using the improved technique of partially relaxed carbon-13 pulse Fourier-transform NMR spectroscopy (34). Another improved one carbon-13 NMR deuterium-induced differential isotope-shift (DIS) technique was introduced and three fructose-containing disaccharide spectra were assigned successfully which could not have been done previously. Pfeffer and Hicks (51) also in their work determined the percentages of each tautomeric form in solution.

Steric interaction is well known to influence NMR chemical shifts in many ways. It is believed that the most stable isomers show the largest carbon-13 chemical shifts and smaller ^{1}H-chemical shifts. Dais and Perlin (16) used disaccharides of D-glucose to examine the effect of the glycosidic bond on conformational stablity and chemical shifts. An advanced technique for a definitive analysis of carbon-13 NMR spectra is believed to be the two-dimensional version of the INADEQUATE experiment (48). Patt,Sauriol and Perlin (48) applied this technique to β-cellobiose to demonstrate its potential for locating the positions of glycosidic

linkages in higher saccharides. An entire sequence of carbon-carbon bonds in a molecule can be determined in a totaly unambiguous manner by employing this method which produces a pattern of spin-spin coupling known as "carbon-carbon connectivity plot (CCCP)". A combination of ^{31}P- and ^{13}C-NMR spectra have been used to characterize the end group and repeating unit components of oligosaccharides derived from natural sources (73).

The traditional carbon-13 NMR spectra analyses are performed in solution. The samples have to be dissolved in a solvent of some kind in order for their spectra to be obtained. Thus it creates a limitation for those polymers having higher degrees of polymerization. Substances with higher molecular weights are difficult to dissolve. With materials such as wood and paper, there is simply no way to obtain their spectra without going through some chemical treatment first. Consequently, the characteristics of these substance are usually altered after having been treated chemically. A better method which can be applied directly to examine intact samples is undoubtly needed.

The initial theory came out in 1973 of possible spectra of solid materials (53). The dilute nuclear spins in solids that initiate the NMR signals can be

enhanced by transferring polarization repeatedly from a more abundant species to which they are coupled. This cross polarization (CP) of carbons is usually accomplished with the more abundant protons. After the CP transfer the protons are dipolar decoupled leaving the carbon-13 resonances clear. Although dipolar broadening can be removed by strong decoupling during data acquisition, chemical shift anistropy may still severely complicate the carbon-13 spectrum. In many systems of interest to the chemist, overlapping anistropies are not interpretable, and the carbon-13 spectrum of a solid becomes a poorly resolved and disappointing wide-line spectrum (60). However, improvement in resolution can often be achieved by high-speed mechanical sample rotation at the magic angle (3,21,42). By fashioning the solid into a rotor (59) (or placing the solid inside a hollow rotor) and aligning the axis of rotation of the rotor at $54.7 \pm 1^{\circ}$ relative to $H_{\circ}$, chemical shift anistropic dispersions are averaged to their isotopic values (59,60). The combination of the two techniques of cross polarization and magic-angle spinning (CP/MAS) has made the resolution of carbon-13 NMR spectra of solids a practical reality.

Natural abundance, dipolar-decoupled carbon-13 NMR spectra have been obtained of some solid, glassy polymers

at room temperature. The line broadening due to ^{13}C-^{1}H dipolar interaction was removed by strong proton decoupling (dipolar decoupling) and made feasible good determinations of the spectra (59). The same authors compared the cross polarization (CP) carbon-13 NMR spectra of a dense wood under nonspinning and spinning conditions and to a standard Fourier transform (FT) spectrum of a solution of glucose. They did not perform detailed analyses except to note that the resolution of the spectrum was adequate to identify lines associated with each of the major components of wood (60). Later Schaefer, Stejskal and Buchdahl (61) acquired dipolar-decoupled, natural abundance Fourier transform and cross-polarization carbon-13 NMR spectra of glassy polymers at 22.6 MHz, both with and without 3-KHz mechanical spinning at the magic angle (54.7°). The 100 Hz resolution of spectra of the solids achieved by the spinning was almost as detailed as the standard spectra of the same polymers in solution.

The CP/MAS NMR technique has been used in conformational analyses of various (1→3)-β-D-glucans. It was found that high molecular weight glucans adopt mainly helix forms while those with lower molecular weights adopt considerable additional proportions of random coil forms in the solid state (57). This method has also

been employed by the same authors to investigate the conformational changes from solid state to solution state. They found that linear and cyclic amyloses perform a rapid conformational isomerism in aqueous solution with life times shorter than 10^{-3} seconds with reference to those obtained in the solid state (58).

Hall and Yalpani (30) determined the primary structure of some polysaccharides, mainly xanthan gum I and their derivatives by CP/MAS carbon-13 NMR spectroscopy. A bullet-shaped rotor and an Andrew-type stator have been introduced to the CP/MAS technique. The authors claimed that simple, but effective and major advantages, could be achieved and the remaining disadvantages had been minimized (8).

High resolution carbon-13 NMR spectra of two crystralline polymorphs of cellulose and an amorphous sample were obtained by a group of chemists using the cross polarization/magic angle spinning (CP/MAS) technique (5). They demonstrated the nonequivalence of adjacent anhydroglucose units which are consistent with conformational differences between the polymorphs. Based on the spectra, they proposed two types of glycosidic linkages between the anhydroglucose units and suggested that dimeric anhydrocellobiose must be viewed as the basic repeating unit in the crystalline structure.

Similar work was done by Earl and VanderHart (20). They also showed the unequivalent environments in dry cellulose I which appeared to show two types of glucose monomers. It was not the glycosidic linkages this time, but one in which the proton on C-6 seems to have more freedom and is easier to access (21).

Cellulose in the solid state has been extensively investigated by CP/MAS. The peak assignments listed below are from a publication by Maciel, Kolodziejski, Bertran and Dale (44) and are firmly established on the basis of comparison with oligosaccharides and model compounds of research referenced in (44). The C-1 (anomeric) carbons of the cellulose chain show a peak at 105 ppm. A sharp resonance at 89 ppm and a broader resonance at 84 ppm are assigned to C-4. There are several resonance features between 70-80 ppm which are assigned to C-2, C-3, and C-5 collectively because at present there is no basis for individual assignment. A narrower peak at 66 ppm and a broader peak at 63 ppm are due to C-6 resonances.

The use of CP/MAS in studying the crystal structure and polymorphy of cellulose has been reviewed by Atalla (6). The samples studied included a bacterial cellulose from Acetobacter xylinum, an algal cellulose from Valona

ventricosa, and two fibrous celluloses (cotton linters and ramie). The solid-state spectra of these native celluloses yielded fine structure that demonstrated chemical nonequivalencies within the resonance bands of the carbons involved in the glycosidic linkage (C-1 and C-4) and provided insight into the questions of crystal structure and morphology in relationship to sharp and broad components in the C-4 and C-6 resonance regions. The sharper components of the C-4 (89 ppm peak) and C-6 (66 ppm peak) resonance, as well as the C-1 (105 ppm peak) resonance, possessed a multiplicity (multiple peaks) that suggested that they arose from magnetically nonequivalent sites within crystalline domains. The most important feature of the spectra was the pattern of variation of the multiplets. These narrow lines, which arise from molecular chains within crystalline domains, differed among the samples of cellulose. Atalla concluded that the native celluloses are composites of two or more crystalline modifications dientified as $I\alpha$ and $I\beta$, which coexist in all native forms but in different proportions.

Maciel, Kolodziejski, Bertran and Dale (44) approached the question of crystallinity and polymorphism in cellulose from a different perspective.

They ground samples of cotton linters in a ball mill for intervals ranging from 0 to 80 hours. In this way they altered the crystallinity of the cellulose: the longer the the ball milling the less the crystallinity. The authors reported CP/MAS spectra of the samples. As the apparent crystallinity decreased, the relative intensities of the broader peaks at 85 ppm (C-4) and 63 ppm (C-6) increased, while those of the narrower peaks at 89 ppm (C-4) and 66 ppm (C-6) decreased. Also, with decreasing crystallinity, the relative intensity within the 70-80 ppm band (C-2, C-3, C-5) shifted from higher shielding to lower shielding and this band became increasingly featureless. Overall the resonance lines appeared broader in the samples with lower apparent crystallinities. In fact, the CP/MAS spectrum of the cellulose considered to be amorphous (80 hours ball milling) showed only a broad peak at 105 ppm (C-1), a shoulder at 98 ppm (C-1 carbons of end monomer units), a broad peak at 84-85 ppm (C-4), a broad peak at 75-76 ppm (C-2, C-3, C-5), and a broad peak at 64 ppm (C-6). All multiplicities for individual carbons had disappeared.

The observed trends clearly identified the narrower peaks at 89 ppm and 66 ppm with cellulose monomers in a more highly ordered environment and the broader peaks at 84 ppm and 63 ppm with less ordered environments. These

less ordered enviromnents could occur in amorphous regions in the interior of fibrils or at the surfaces of highly crystalline fibrils, the extent of the latter being largely dependent on sample morphology. The authors assumed that morphology was more likely than internal fibril crystallinity to be affected dramatically by ball milling. Hence, they attributed the C-13 NMR spectral changes of cellulose samples ranging from highly crystalline to amorphous to changes in the degree of monomer order, or regularity, associated with changes in morphology. It is clear that the hydrogen bonding within cellulose will be the next to be better elucidated through the use of CP/MAS.

The spectra of lignins in the solid state and solution state were compared by Maciel, O'Donnell, Ackerman, Hawkins and Bartuska (43). The solution-state spectra had a longer number of sharper and more distinct peaks, while each corresponding solid-state spectrum consisted of a collection of peak envelopes of various ranges of linewidth. However, most of the information presented by the solution-state spectra could also be ascertained from the solid state CP/MAS spectra. The solid state CP/MAS spectrum of a Norway spruce lignin showed two intense peaks, one at 56 ppm and one at 147 ppm, which are characteristic of lignin. The peak at 56

ppm was assigned to the ArO-CH_3 (methoxyl) groups on the aromatic rings. The peak at 147 ppm was attributed primarily to the ring carbons of the guaiacyl grouping which has a phenolic-OH adjacent to a methoxyl group on the aromatic ring. Additional signals at 131 ppm to 135, 120 to 124, and about 115 ppm were attributed primarily to the other carbon atoms in the aromatic ring. Smaller resonances between the sharp, strong signal at 56 ppm and the more intense group of resonances beginning at 112 ppm were attributed to the three-carbon side chains of lignin with differing types of substitution.

The solution-state spectrum of a lignin from red alder, a hardwood, showed a distinct peak in the 105-108 ppm region (with an apparent shoulder at about 105 ppm) which was assigned to the unsubstituted ring carbons when a syringyl substitution of the ring was present. In the solid-state spectrum of red alder this was manifested as a more pronounced extension of a broad band into this region. The peaks at 56 ppm for methoxyl groups and at 147 ppm for ring carbons substituted as for guaiacyl groups were intense in the spectra of this lignin as they were in the spectra of lignin from Norway spruce. Thus the lignin from a hardwood can be distinguished from the lignin of a softwood by the

presence of the resonances in the 105-108 ppm region because of the presence of syringyl units in hardwoods which are not present, or at least only in small numbers, in softwoods.

The CP/MAS techniques have also been used in the characterization of the catabolic transformation of lignin in culture by using fungus (62). Maltulose and lactulose have also been examined by CP/MAS and their spectra reported by Pfeffer and their tautomeric compostion defined and chemical shifts assigned (52).

Solid state CP/MAS NMR allows for physico-chemical spectra of materials which are not soluble. Wood is a natural choice for investigation although it is a complex substance. It is interesting that one of the first substances investigated by solid state CP/MAS NMR was wood. Schaefer and Stejskal (60) in 1976 published a carbon-13 NMR CP/MAS spectrum of a dense wood (ebong). The authors commented that wood is a complicated blend of celluloses, hemicelluloses, and lignin and they attempted no detailed analysis of the spectrum except to note that the resolution of the CP/MAS spectrum was adequate to identify lines associated with each of the three major components. In truth the spectrum showed amazing resolution, and peaks that were later assigned to cellulose and lignin were clearly evident.

Kolodziejski, Frye and Maciel (39) in 1982 published an article pertaining directly to CP/MAS carbon-13 NMR spectrometry of solid wood and components isolated from the same wood. Carbon-13 CP/MAS was applied to whole and processed lodgepole pine wood to investigate the relative amounts of and interactions between the major constituents. The authors obtained spectra on both a solid plug of lodgepole pine wood and a ground sample of the same wood and found no difference in the spectra. However, ball milling did produce signigicant spectral changes which were interpreted as changing crystalline cellulose to the amorphous form.

The spectrum of ground wood (same as solid wood) showed broad peaks at 149 and 133 ppm which were attributed to aromatic signals from lignin. A sharp peak at 56.2 ppm was assigned to the methoxyl groups of lignin and possibly of hemicelluloses. There were several peaks between 60 and 110 ppm which were assigned to the carbohydrates, both cellulose and the hemicelluloses. Side-chain groups of lignin provided a minor contribution to this region. A distinct, sharp peak at 105.5 ppm was assigned to C-1 of cellulose. This peak had a shoulder at 103 ppm which was attributed to C-1 of the hemicelluloses. Peaks at 89.4 and 83.9

ppm were C-4 signals from cellulose with a background from C-4 resonances of the hemicelluloses. Two peaks at 65.6 and 62.6 ppm were assigned to C-6 of cellulose; between these two peaks at 64 ppm appeared a C-6 resonance peak from the hexose units of the hemicelluloses. There were two peaks at 75.3 and 72.6 ppm due to C-2, C-3, and C-5 of cellulose superimposed on the broad signal due to the corresponding carbons of the hemicelluloses. A weak peak was evident at 174 ppm which was assigned to the carbonyls of lignin and of the acetate and carboxyl groups of the hemicelluloses. A weak peak at 21.5 ppm was attributed to the methyls of the acetate groups known to be on the hemicelluloses. The amount of information obtained from this CP/MAS spectrum of ground wood (same as solid wood) was indeed remarkable.

Kolodziejski, Frye and Maciel (39) isolated five lignins (Klason lignin, dioxane lignin, periodate lignin, enzymatic lignin, Brauns native lignin) from the lodgepole pine wood. A large peak at 149 ppm of the CP/MAS spectra was assigned to the aromatic ring carbons substituted with methoxyl and hydroxyl groups (the guaiacyl grouping). The other aromatic ring carbons resonated in the region of 100-140 ppm. All except Klason lignin showed an aromatic region similar to that

of whole wood suggesting that the structure of Klason lignin was signigicantly changed from or at least was not typical of native lignin. A sharp peak at 56.2 ppm was assigned to aryl methoxyl groups. Peaks between 60 and 100 ppm were attributed to side chain signals.

The authors (39) attempt to make a case for a lignin-cellulose complex by subtracting the spectrum of a chlorine gas holocellulose from the spectrum of extractives-free wood. The carbohydrate signals could not be removed which they claim means that lignin is removed with cellulose during holocellulose preparation. However, it is well known that carbohydrates are solubilized as well as lignin during holocellulose preparation so whether or not this really indicates a lignin-carbohydrate complex is unclear.

Kolodziejski, Frye and Maciel (39) reported CP/MAS spectra for hemicelluloses A and B which they isolated from lodgepole pine wood. They reported no difference in the spectra of the two hemicelluloses. Both spectra showed residual lignin peaks at 110 and 160 ppm. An intense peak at 180 ppm was attributed to carbonyl groups which came from carboxylic groups and acetate groups. A relatively strong peak at 22 ppm was attributed to the methyl groups of the acetate moieties. Signals from 50 to 110 ppm were assigned by analogy to

cellulose although it is doubtful that hemicellulose A contained any cellulose. The C-1 peak appeared at 103 ppm whcih is slightly upfield from the 105.5 ppm peak usually assigned to C-1 of cellulose. The authors do not speculate as to whether a CP/MAS spectrum can differentiate between a α-(1→4) linkage of a hemicellulose and the β-(1→4) linkage in cellulose. However, they do attribute a shoulder at 103 ppm on the 105.5 ppm peak of cellulose in the whole wood spectrum to the C-1 of hemicelluloses. In the hemicellulose spectra a central envelope included C-2, C-3, C-4 and C-5 resonances. It is interesting that the C-4 peaks were not resolved as they were in cellulose although hemicellulose A was said to be an arabino-(4-O-methylglucurono)xylan, and hemicellulose B was said to be a glucomannan. Both of these polysaccharides are mainly joined by β-(1→4) glycosidic bonds just as is cellulose. However, the C-4 peaks did not show at 89 and 84 ppm in the spectra of the isolated hemicelluloses. This may be due to in part to the non-crystallinity of the hemicelluloses. A peak at 64 ppm in both spectra was attributed to C-6 of the hemicellulose hexoses. Actually the carbohydrate peaks of the hemicelluloses were quite broad and the spectra were reminiscent of the spectrum of amorphous cellulose

(44). This reinforced the knowledge that hemicelluloses tend to be amorphous and non-crystallne materials.

The work reported by Kolodziejski, Frye and Maciel (39) is somewhat less than it should be. They extracted hemicellulose A from a holocelllose with 5 % aqueous potassium hydroxide. Although they claimed that hemicellulose A was mainly an arabino-(4-O-methylglucurono)xylan they presented no analytical evidence to support this. The 5% aqueous potassium hydroxide undoubtedly did not preferentially extract a xylan away from the other hemicelluloses because there was no initial treatment with barium hydroxide to complex with the mannose containing polysaccharides. Therefore, the hemicellulose A isolated by these authors (39) was likely a mixture of a xylan, a galactoglucomannan, and a glucomannan. The spectrum for their hemicellulose A clearly shows the presence of some hexoses because of the 64 ppm peak for C-6 . A true hemicellulose A is mainly composed of xylose with some arabinose and 4-O-methylglucuronic acid side chains and should not show the usual resonances for hexose C-6 carbons. Hemicellulose B was extracted with 24% aqueous potassium hydroxide, which is again too strong a base to separate a glucomannan from some short-chain celluloses. Thus the spectra of these

authors (39) were identical because the separation of the hemicelluloses was not complete.

Kolodziejski, Frye and Maciel (39) showed some spectra of holocellulose isolated from lodgepole pine wood and some spectra of α-celluloses prepared by extraction of the holocellulose with 24% aqueous potassium hydroxide. The holocellulose spectrum showed peaks which could be attributed to both cellulose and the hemicelluloses. A peak at 105 ppm was assigned to C-1 of cellulose and a shoulder on this peak at 103 ppm was assigned to C-1 of the hemicelluloses. Peaks at 89 and 84 ppm were assigned to C-4's and at 65 and 62 ppm were assigned to C-6's. An envelope of peaks between 69 and 80 ppm was attributed to C-2, C-3, and C-5 carbons. The spectrum for α-cellulose showed broad peaks in these general regions and was very similar to the spectrum of amorphous cellulose (44). This substantiates what is known about the cellulose residues remaining after treatment of cellulose with alkali. It is known that alkali can penetrate the crystalline regions of cellulose, break down the hydrogen bonding, and make the hydroxyl groups more accessible for chemical reactions. In fact the treatment of cellulose with alkali to prepare alkali cellulose is an early step in the preparation of such common cellulose derivatives as

rayon and cellulose acetate. The CP/MAS spectra clearly show that crystalline cellulose is changed to amorphous cellulose upon treatment with alkali. The work published by Kolodziejski, Frye and Maciel (39) is a great contribution to the chemistry of wood and demonstrates that CP/MAS of solid materials is indeed a powerful tool.

III. EXPERIMENTAL

A. High Performance Liquid Chromatography

Separation and quantitative analyses of monosaccharides was accomplished by high performance liquid chromatography (HPLC) using a borate buffer eluant. The procedure was established by Ni (46) and is a modification of the method of Sinner, Simatupang and Dietrichs (63) and Sinner and Puls (64). A schematic diagram of the system is shown in figure 1.

The eluant solvent borate buffer was prepared by dissolving potassium hydroxide (51.05 g) in distilled deionized water (3 liters) followed by the dissolution of boric acid (112.48 g). Additional distilled deionized water (500 ml) was added to yield a solution 0.52 M in boric acid (H_3BO_3) or 0.13 M in potassium borate ($K_2B_4O_7$) in accordance with the balanced chemical equation:

$$2\ KOH + 4\ H_3BO_3 \longrightarrow K_2B_4O_7 + 7\ H_2O\ .$$

The pH of the buffer was 8.9. This solution was vacuum filtered through a 47 mm diameter, 0.45 μm pore size filter. The eluant was pumped through the column by a Waters Associates M-45 solvent delivery system (pump A

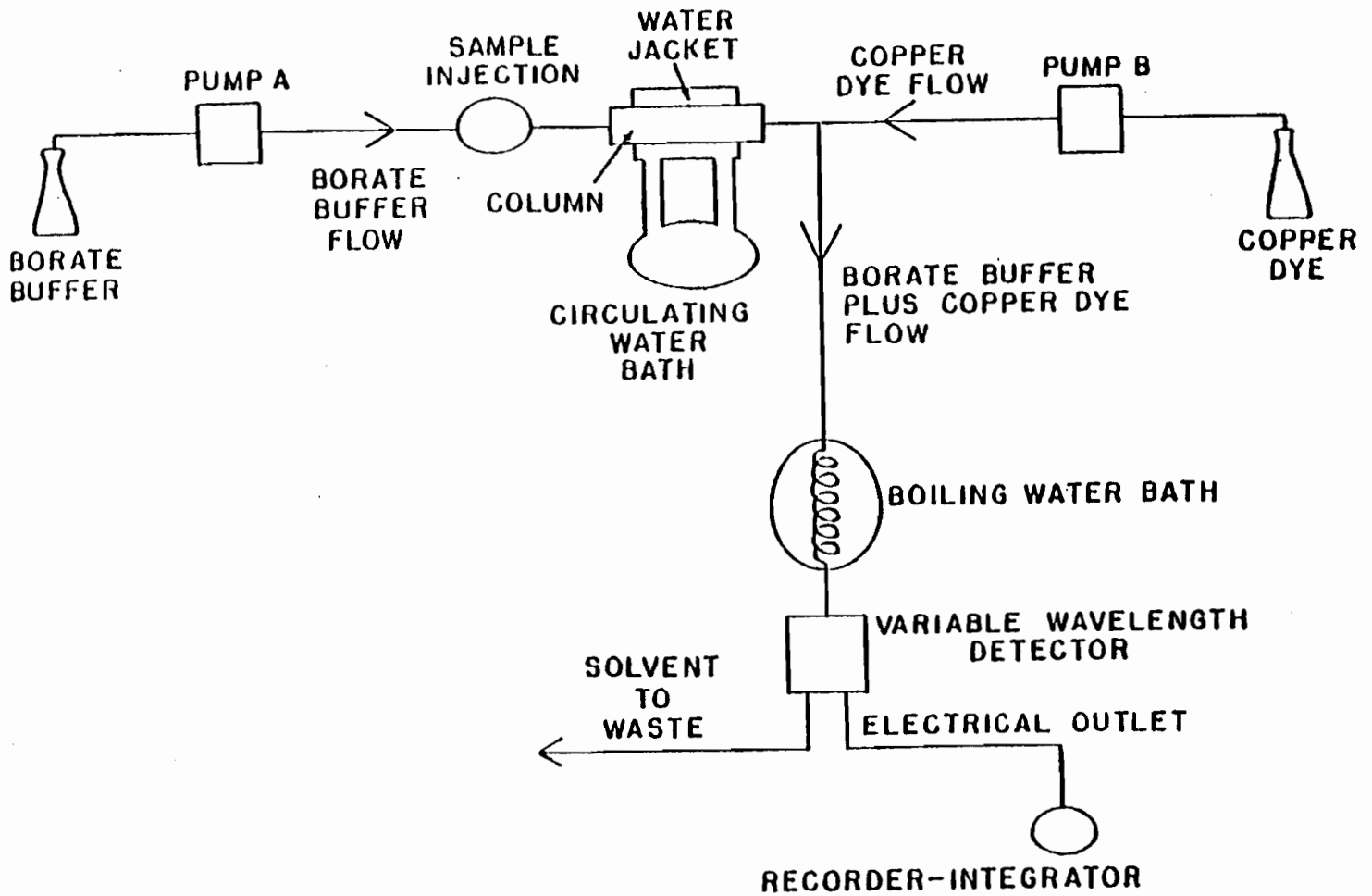

Figure 1. Schematic diagram of the apparatus used for carbohydrate analyses.

in figure 1) at a flow rate of 0.2 ml/min. The temperature of the column was 57.5°which was maintained by pumping water of this temperature through a water jacket.

A magnetic stirrer was used to keep the stock solution aggitated while in use.

The monosaccharides were detected after elution from the column by reaction with a copper dye reagent. The copper dye reagent was initially formulated in two parts, "solution A" and "solution B". "Solution A" was prepared as follows: anhydrous sodium carbonate (215.0 g) was added to 3 liters of distilled water. After complete dissolution, dipotassium 2,2'-bicinchoninate (6.2 g) [$(C_9H_5NCOOK)_2$, F.W. 420.9, assay 82.4%, from Hach Chemical Co., Ames, Iowa] and 450 ml of distilled deionized water was added with stirring. "Solution B" was prepared as follows: L-aspartic acid (3.7 g), anhydrous sodium carbonate (5.0 g) and cupric sulfate 5-hydrate (1.0 g) was added to 150 ml of distilled deionized water.

"Solution A" and "solution B" were mixed to yield about 3600 ml of final reagent in a volume ratio of 23 parts of "solution A" to one part of "solution B". The reagent was pumped into the eluant from the column with a small pump (pump B in figure 1, Model A-30-5 from Eldex

Laboratories Inc., Menlo Park, CA) at a flow rate of 0.5 ml/min. The column, 250 mm long with a 3.2 mm internal diameter and 0.25 inches outside diameter, was packed with a Durrum anion-exchange resin described as " Chromex DA-X4-11" (Dionex Corporation, Sunnyvale, CA). A T joint combined the borate buffer eluant from the anion exchange column and the copper dye reagent. The mixed solvent streams then passed through 15 meters of teflon tubing (0.8 mm internal diameter) which was immersed in a boiling water bath. The solvent stream was then passed through an ISCO absorbance monitor. The absorbances were measured at 546 nm, and recorded on a Hewlett Packard Model 3380A integrator which provided a spectrum of the peaks and also integrated the areas under the peaks.

B. Ash Determinations

The standard procedure of the Technical Association of the Pulp and Paper Industry, TAPPI standard T-211 m-58 (4), was followed to determine the ash content of samples. Samples were weighed into weighed silica crucibles and heated in a Lindberg Heavy-Duty muffle furnace at 575° for 3 hours. The weights of the ash were determined by difference and the ash contents were calculated as percentages of the oven-dried samples. Ash contents are reported as the average of triplicate determinations.

C. Klason Lignin and Acid-Soluble Lignin Determinations

The Klason lignin content of samples was determined by a modification of the TAPPI standard prodedure T-222 os-74 (2). Each sample was treated with 77% sulfuric acid for 1 hour at room temperature and then water was added to 3% sulfuric acid. The resulting samples were refluxed for 5.5 hours and filtered through sintered glass crucibles. The residues in the crucibles were dried for 10 hours in an oven at 105° and weighed. The weights of the acid-insoluble residues were reported as Klason lignin as percentages of the oven-dried samples.

The acid-soluble lignin was determined by a method described by Browning and Bublitz (1,10). The analysis is done by the characteristic lignin absorptions at 280 nm and 215 nm. The instrument used was a Beckman ACTA TM III-UV-Visible spectrophotometer. The sample and reference cells (1.0 cm cell width) were filled with 3.0% sulfuric acid which were then used to standardized the instrument. The sample was scanned over the ultraviolet spectral range from 400 nm to 180 nm with the filtrate from the Klason lignin determination in the sample cell. Absorptions were recorded at 280 nm and at 215 nm for each determination.

D. Viscosity Determinations

Viscosities of polysaccharide materials were determined by dissolution in cupriethylenediamine by the falling ball method described in TAPPI standard T230su-66 (15). Samples weights of 0.2 g - 0.3 g were first wetted with 15 cc of 0.167 N cupriethylenediamine followed by the addition of 10 cc of 1 N cupriethylenediamine and shaken until complete dissolution. The time for the ball to fall between two lines on the viscometer was recorded as was the temperature.

E. Hydrolyses of Polysaccharide Samples

1. Dissolution and Primary Hydrolysis

Pulp samples (500 mg each) were dissolved in 5 ml of 77% sulfuric acid at room temperature. Occasional application of vacuum to the reaction flask helped the dissolution of the samples. However, it was found that the most effective way was to keep stirring with a glass rod. "Squelching" the unsoaked sample often made the dissolution go faster. The dissolution was usually completed within 45-60 minutes. Complete dissolution of

samples could be checked by examination of the solutions to see if the solutions were viscous or not. A non-viscous solution meant the dissolution was complete. The solutions always appeared dark brown or black.

2. Total Hydrolysis

The pulp solutions were transferred to 250 ml round bottom flasks with 50 ml of distilled deionized water. After mixing well, another 130 ml of water was added to make a 3.0% concentration of sulfuric acid. The solutions were then refluxed for 5.5 hr. Proper amounts of D-lyxose were added as internal monosaccharide standards and adequate volumes (usually 20 μl) of the hydrolyzates were injected into the HPLC for monosaccharide analyses.

F. Preparation of Pulp Samples

1. Thermomechnical Pulp (TMP)

The sample of a commercial thermomechanical pulp (TMP) was obtained from the Weyerhaeuser Company on March

19, 1980. It was prepared at the Weyerhaeuser Company's Longview, Washington facility from essentially 100% western hemlock [Tsuga heterophylla (Raf.) Sarg.] wood. The sample had the appearance of brownish fibers and was frozen until ready for analyses.

2. High Yield Sulfite Pulp

A laboratory sample of "high yield sulfite pulp" was prepared from western hemlock chips on August 13, 1981 at the Forest Research Laboratory, Oregon State University, by Dr. Walter Bublitz and Mr. Jerry Hull, Department of Forest Products, Oregon State University, Corvallis, Oregon 97331. The pulp was prepared by a sulfite process of 20% SO_2, 7/1: liquor/wood ratio, 45 minutes cook at 165°C. The yield was 61.6% based on oven-dry wood. The Kappa number was 121.0. The material still retained much of the shape of the original chips.

3. High Yield Solvent Sulfite Pulp

The high yield solvent sulfite pulp was prepared on August 14, 1981 under exactly the same conditions as described previously for the high yield sulfite pulp,

except that the pulping liquor contained 30% by weight of methanol. The yield of solvent sulfite pulp was 57.1% based on oven-dry wood. The Kappa number was 81.4. The material retained much of the shape of the original chips.

4. Preparation of Samples for Analyses

Each of the prepared pulps was air-dried in the hood for at least four days. Triplicate samples (20-30 g) of each pulp were taken and heated in an oven at 105° overnight to determine the non-volatile solids content (47).

An aliquot (80.0 g) of the TMP pulp (91.2% non-volatile solids) was freeze-dried and used for further analyses (see chart 1).

Since the high yield sulfite pulp still retained much of the physical shape of the original wood chips, it was defiberized in a Waring blender (see chart 2). An aliquot (60.0 g non-volatile solids 90.0%) of the air-dried pulp with 900 ml of distilled-deionized water was placed in the blender and blended for 3 min at high speed. The solids were recovered on a coarse, fritted-glass filter and washed three times with distilled deionized water. This

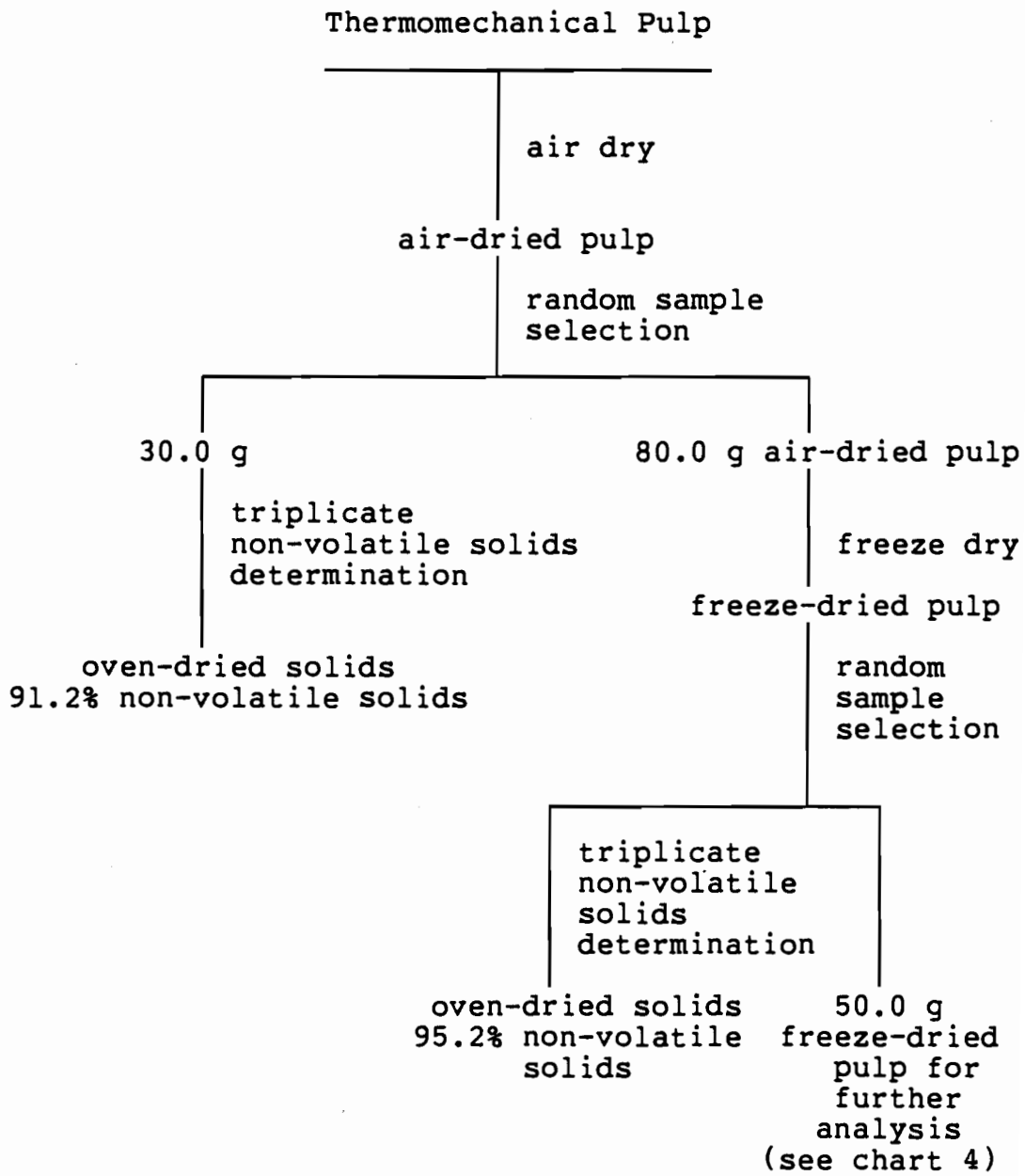

Chart 1. Sample preparation from thermomechanical pulp.

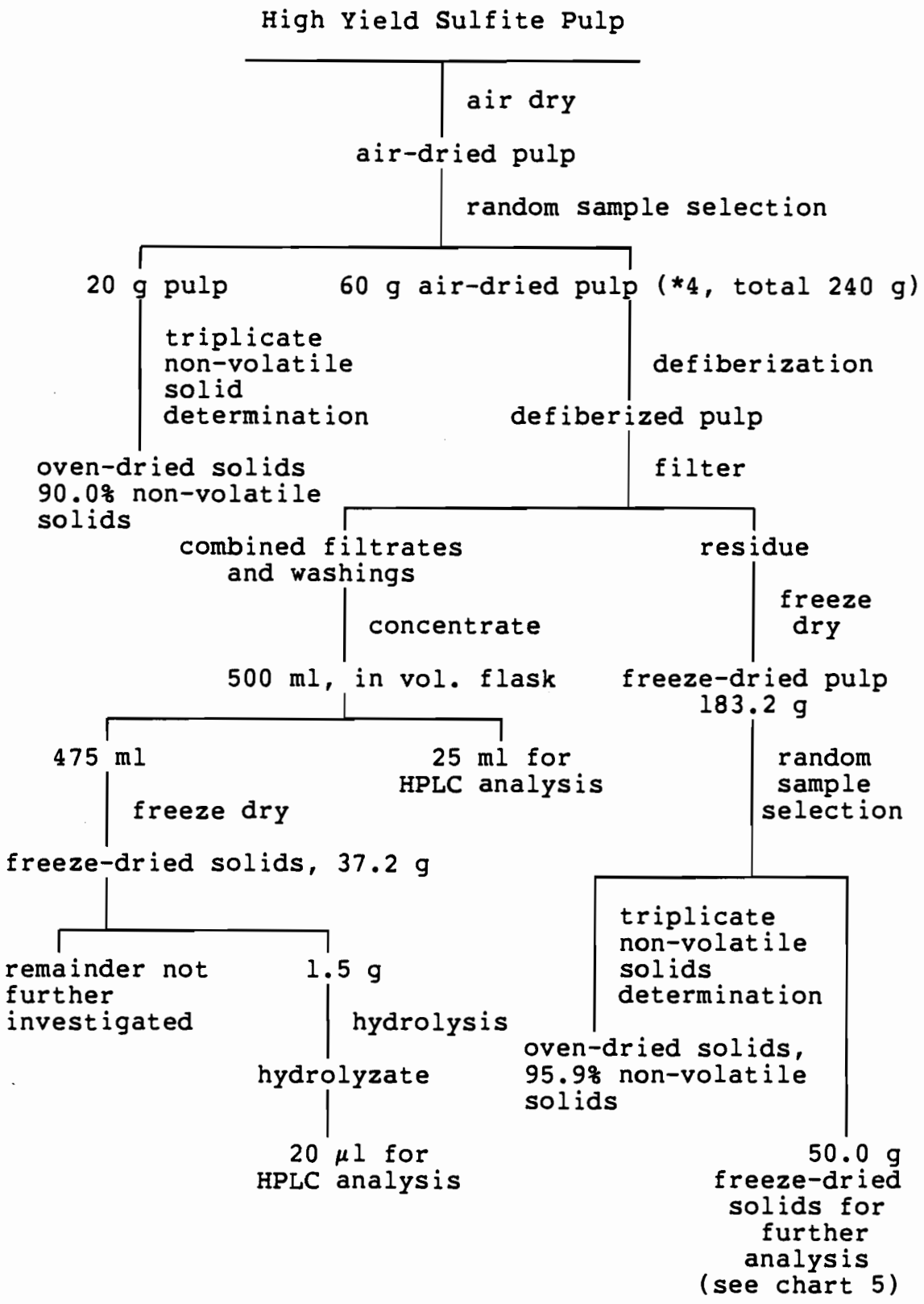

Chart 2. Sample preparation from high yield sulfite pulp.

procedure was repeated on three additional aliquots (60.0 g each) of the air-dried pulp. The defiberized solids were combined and freeze-dried, yield 183.2 g, non-volatile solids 95.9%, oven-dried weight 175.7 g.

The filtrates plus washings were combined, concentrated on a rotary evaporator, and finally diluted to 500 ml in a volumetric flask. An aliquot (25.0 ml) was taken and D-lyxose (10.2 mg) was added as an internal standard. A portion (20 μl) was injected into the HPLC for monosaccharide analysis. The result showed the presence of glucose, mannose, galactose, xylose and arabinose but the spectrum was not well enough resolved to allow quantitative determinations.

The remaining solution (475.0 ml) was freeze-dried to yield a powder: weight 37.2 g. A sample (1.5 g) of the powder was dissolved in 3% sulfuric acid (150.0 ml) and refluxed for 5.5 hours. D-lyxose (15.0 mg) was added as an internal standard. A 20 μl aliquot was injected into the HPLC for monosaccharide analysis. The results showed the presence of glucose, mannose, galactose, xylose and arabinose but the spectrum was not well enough resolved to allow quantitative detrminations.

The high yield solvent sulfite pulp also retained much of the physical shape of the original wood chips.

Therefore, it was also defiberized in a Waring blender (see chart 3). An aliquot (120.0 g, 88.9% non-volatile solids content) of the air-dried pulp with 900.0 ml of distilled deionized water was placed in the blender and blended for 3 min at high speed. The solids were recovered on a coarse, fritted-glass filter and washed three times with distilled deionized water. This procedure was repeated on an additional aliquot (120.0 g) of the air-dried pulp to make a total sample of 240.0 g defiberized. The defiberized solids were combined and freeze-dried, yield 168.8 g, non-volatile solids 93.3%.

The filtrate plus washings were combined, concentrated on a rotary evaporator, and finally diluted to 500 ml in a volumetric flask. An aliquot of 25.0 ml was taken and D-lyxose (10.2 mg) was added as an internal standard. A portion (20 μl) was injected into the HPLC for monosaccharide analysis. The results showed glucose, mannose, galactose, xylose and arabinose but the spectrum was not well enough resolved to allow quantitative determinations.

The remaining 475.0 ml were freeze-dried, yield 37.8 g. A sample (1.5 g) was dissolved in 3% sulfuric acid and refluxed for 5.5 hr. D-lyxose (10.4 mg) was added as an internal standard. A 20 μl aliquot was injected into

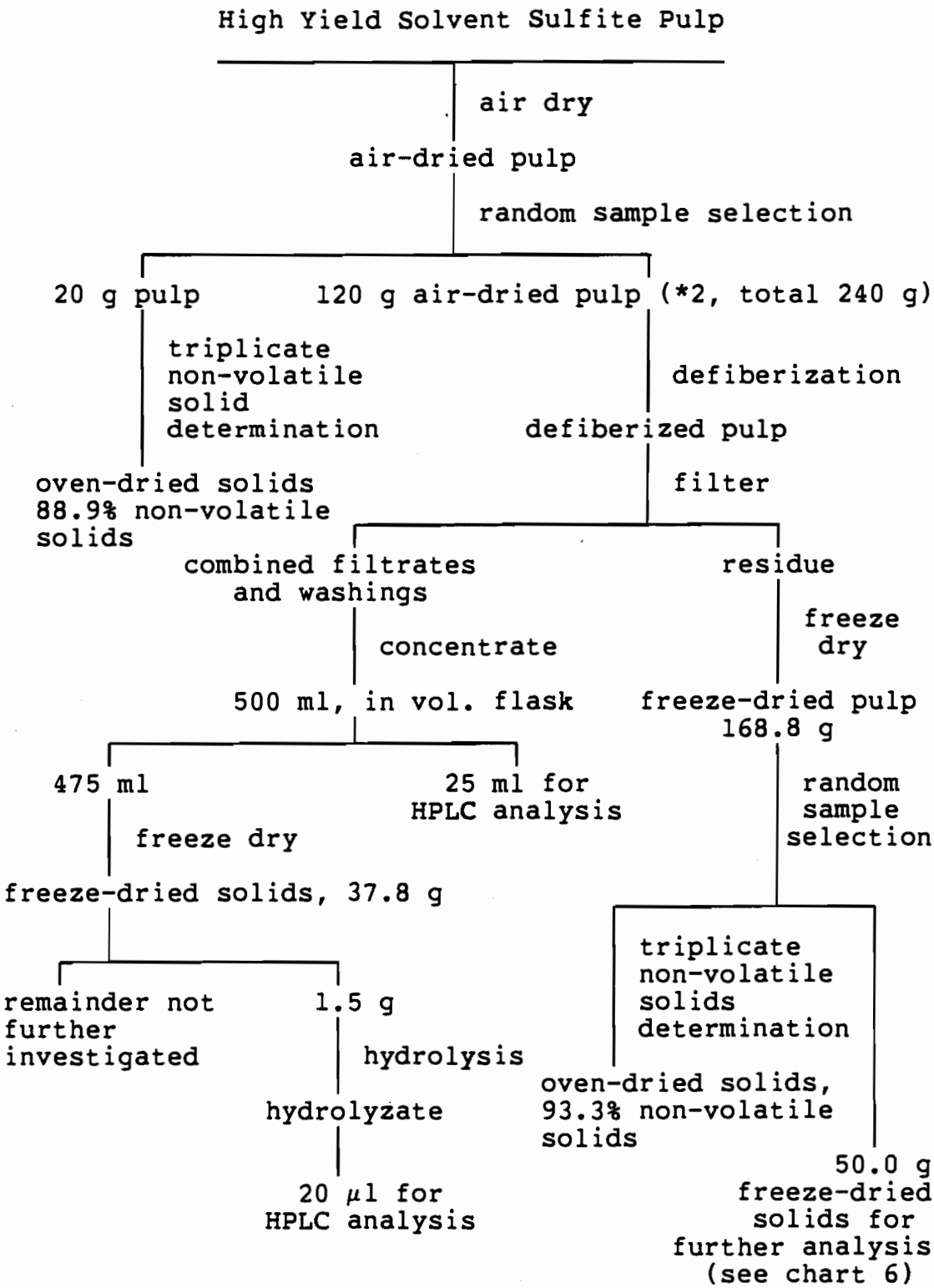

Chart 3. Sample preparation from high yield solvent sulfite pulp.

the HPLC for monosaccharide analysis. The results showed glucose, mannose, galactose, xylose and arabinose but the spectrum was not well enough resolved to allow quantitative determinations.

G. Fractionation of The Pulp Samples Into Their Component Polysaccharides

The fractionation of the pulp samples into their component polysaccharides was accomplished by a modification of the method of Beélik , Conca, Hamilton and Partlow (9) (see charts 4, 5 and 6).

1. Isolation of Hemicellulose A

Freeze-dried pulp (50.0 g) was slurried into 800 ml (817.2 g) of saturated barium hydroxide solution. After stirring for 30 min, 900 ml (1027.2 g) of 18.5% potassium hydroxide solution was added and stirred for 40 min. The solids were recovered by filtration with a sintered glass filter with two Whatman No.1 papers on the top. The pulp was washed three times with 300 ml portions of a combination of saturated barium hydroxide and potassium hydroxide solutions similar to those previously used. The

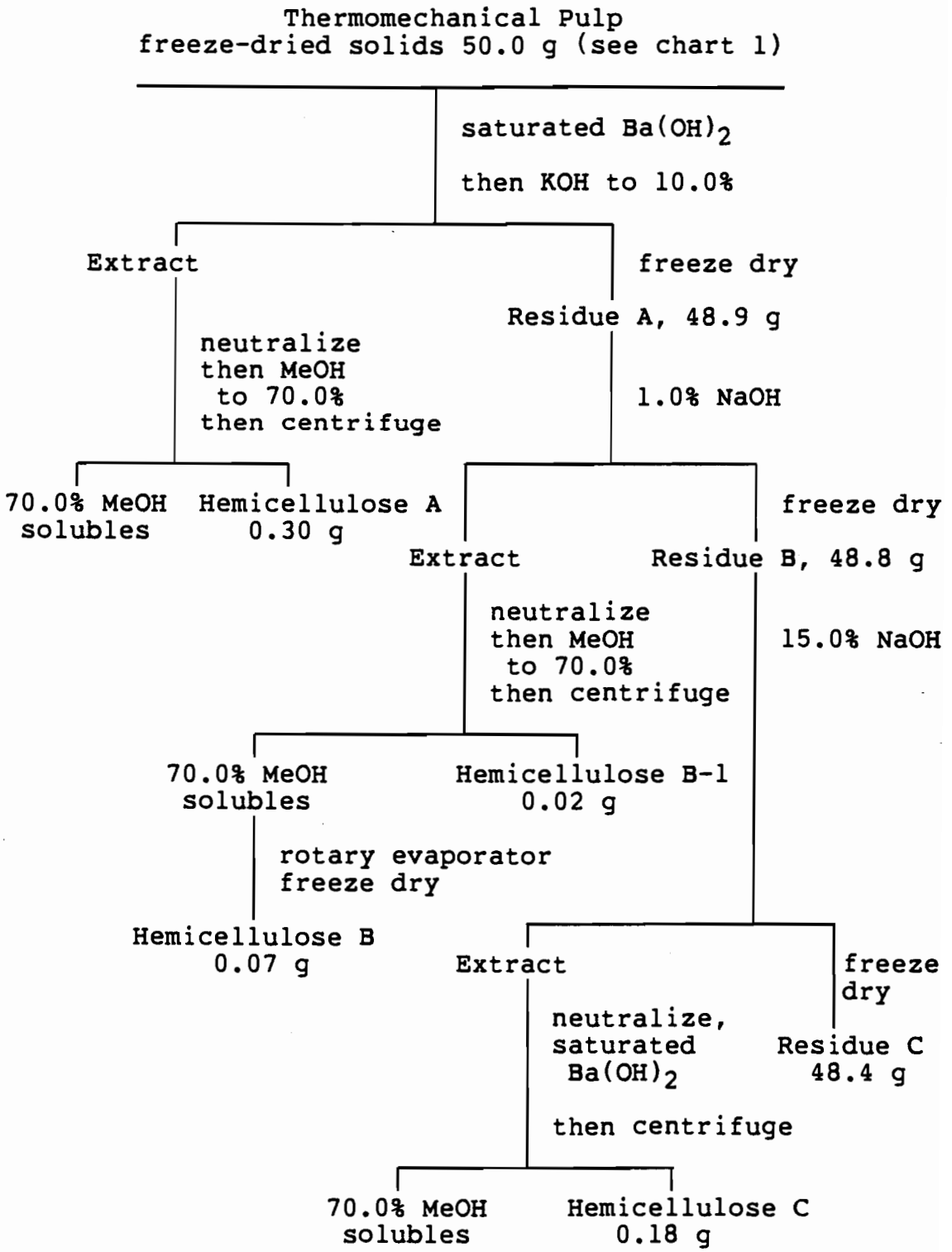

Chart 4. Fractionation of the thermomechanical pulp into its component polysaccharides.

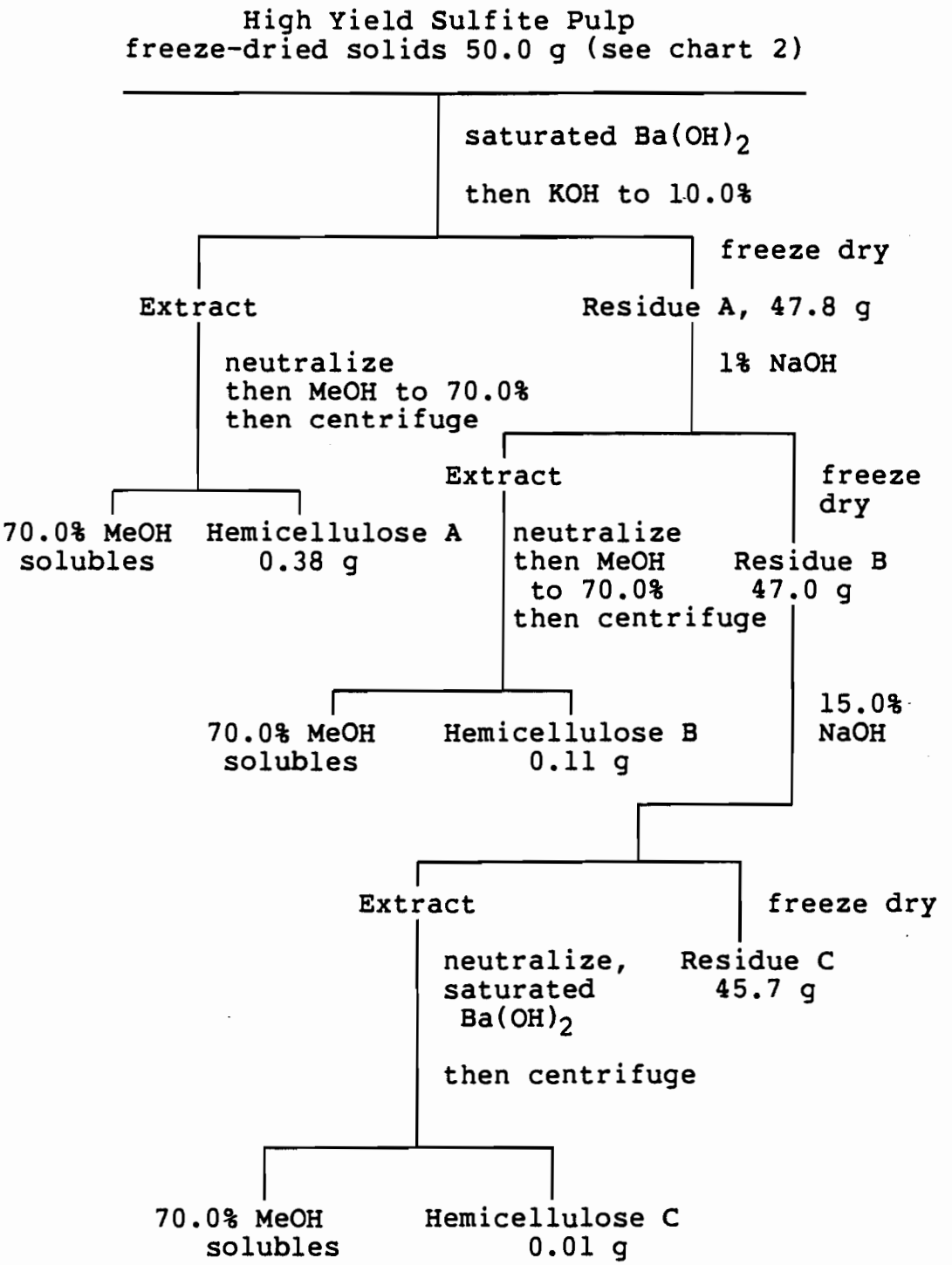

Chart 5. Fractionation of the high yield sulfite pulp into its component polysaccharides.

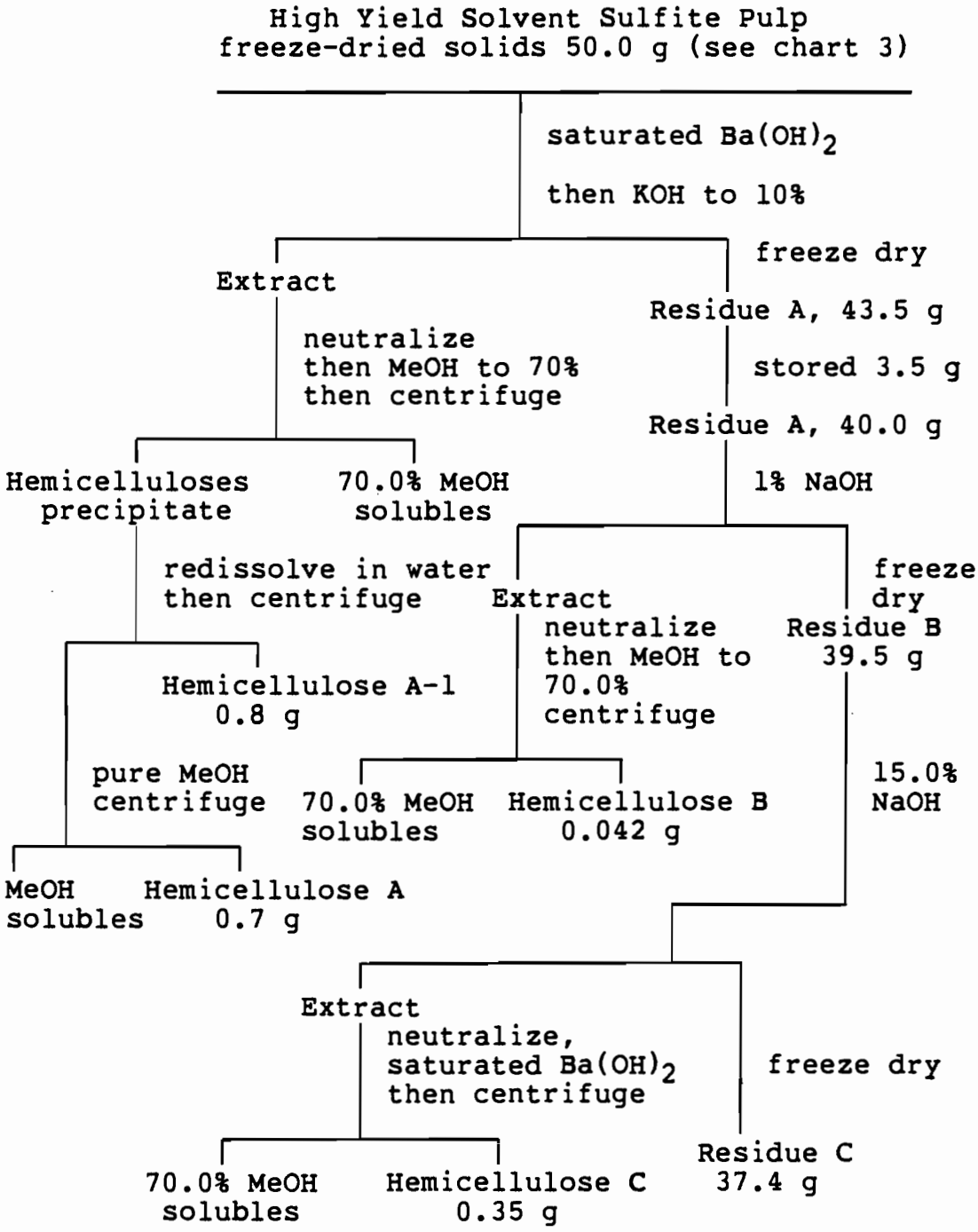

Chart 6. Fractionation of the high yield solvent sulfite pulp into its component polysaccharides.

pulp was then washed thoroughly with distilled deionized water.

The pulp solids were dispersed in 3.0% acetic acid solution for 6 hours. The solids were recovered by filtration after been washed thoroughly with distilled deionized water. They were poured into a dialyzing tube and dialyzed for 5-11 days. The residue was then freeze-dried and labeled "Residue A".

The filtrate plus washings were acidified with 50.0% acetic acid to a pH of 5.5. The solution was concentrated to 1.0 liter on a rotatory evaporator and 3.0 liters of absolute methanol was added. A white, fluffy precipitate formed. After standing overnight, the precipitate was recovered by centrifugation. To obtain a purer product, the precipitate was redissolved and reprecipitated. After centrifugation, the bottom layer (precipitate) was recovered. Methanol was removed by using a rotatory evaporator and the material was then freeze dried. The residue was labeled "Hemicellulose A". However, for the solvent sulfite pulp, it was thought that some hemicelluloses remained in the upper light-yellow 70.0% methanol layer after precipitation and centrifugation. Therefore, additional excess absolute methanol was added. A new precipitate formed. After the removal of methanol

and freeze-drying, it was named "Hemicellulose A-1". The purpose of separation and comparison of these two hemicelluloses was to see if there were any differences between them which accounts for this solubility difference (see chart 6). All the procedures of redissolution and reprecipitation were conducted 2-3 times to ensure purity.

Each of the solid hemicellulose A samples was hydrolyzed with sulfuric acid as described in section III-E of this thesis and the hydrolyzates were analyzed by HPLC as described in section III-A of this thesis.

2. Isolation of Hemicellulose B

All of residue A from the TMP pulp (see chart 4) and from the high yield sulfite pulp (see chart 5) and 40.0 g of residue A from the solvent sulfite pulp (see chart 6) were intermittently stirred into 1050 g of 1.0% sodium hydroxide solution for 30 minutes. The solids were recovered by filtration and washed with another 250 ml of 1.0% sodium hydroxide solution and thoroughly with distilled deionized water. The residue solids were dialyzed for at least three days. The dialyzed solids were then freez-dried and labeled "Residue B". The extracts and

washings were combined and condensed to about 500 ml on a rotary evaporator. Three volumes of methanol (1500 ml) were added and the mixture was let stand in the refrigerator overnight. The precipitate which formed was recovered by centrifugation. It was redissolved, and reprecipitated and freeze dried. The product obtained was labeled "Hemicellulose B". A slightly opaque solution resulted after precipitation of the hemicellulose B from the TMP extraction. The whole solution was then evaporated to near dryness and freeze dried. It was labeled "Hemicellulose B-1" (see chart 4).

Each of the solid hemicellulose B samples was hydrolyzed with sulfuric acid as described in section III-E of this thesis and the hydrolyzates were analyzed by HPLC as described in section III-A of this thesis.

3. Isolation of Hemicellulose C and Residue C

All of "Residue B" was dispersed in 900 ml (996.0 g) of 15.0% sodium hydroxide solution, and stirred for 30 minutes. The solids were recovered on a sintered glass filter and washed with another 250 ml of 15.0% sodium hydroxide solution followed by 2 liters of distilled deionized water. The residue was then dialyzed for 6

days and freeze dried. It was labeled "Residue C".

The filtrate plus washings were added to 750 ml of saturated barium hydroxide solution and allowed to stand for 1 day. Some precipitate formed. After recovery of the precipitate by centrifugation, it was redissolved in 400 ml of 2 N acetic acid solution. Three volumes of absolute methanol were added. The resulting precipitate was recovered by centrifugation. Redissolution and reprecipitation were conducted several times to purify the product. This product is called " Hemicellulose C" and freeze dried.

Each of the solid hemicellulose C samples was hydrolyzed with sulfuric acid as described in section III-E of this thesis and the hydrolyzates were analyzed by HPLC as described in section III-A of this thesis.

H. Isolation of Holocellulose Fractions

Air-dried pulps were slurried with water to give samples of 1% consistency. These were blended by an Osterizer blender at low-liquefy speed for 10 minutes and then at high-liquefy speed for 10 minutes. The slurrey was evaporated to a 3.33% consistency and blended at

high-liquefy speed for 5 minutes. The blending completely broke up the wood bundles or shives. The pulps were filtered, washed with water and oven-dried on a filter paper to oven dryness.

Each pulp was delignified by the acidified sodium chlorite method of Wise, Murphy and D'Addieco (72) and Whistler, Bachrach and Bowman (70). A sample (1.0 g) of each pulp was placed in a 500 ml Erlenmeyer flask and stirred with water (100 ml). The slurrey was heated in a water bath at 80±5° and glacial acetic acid (1.0 ml) was added followed by sodium chlorite (1.5 g). The slurrey was stirred intermittently and every 15 minutes an additional aliquot of glacial acetic acid (1.0 ml) and sodium chlorite (1.5 g) was added until a total of four additions had been made. The white, insoluble holocellulose material was recovered on a coarse fritted glass funnel and washed thoroughly with water and finally with methanol. The solids were dried in an oven at 105 for one hour and weighed. The weight of holocellulose from the TMP pulps was 0.81 g. A second delignification was done to ensure complete removal of the lignin and the holocellulose recovered weighed 0.76 g. The weight of holocellulose from the high yield sulfite pulp was 0.72 g and from the solvent sulfite pulp was 0.83 g. Viscosity determinations were made on these holocellulose samples according to section III-D of this thesis.

I. Carbon-13 Nuclear Magnetic Resonance of Solid Samples of Carbohydrate Materials

Selected samples of carbohydrate materials were sent to the Colorado State University Regional NMR Center, Fort Collins, Colorado. Carbon-13 NMR spectra were obtained on solid samples using a Nicolet NT-200 spectrometer equipped with cross-polarization and magic angle spinning (CP/MAS) capability. Chemical shifts are given relative to external tetramethylsilane (Me_4Si).

IV. RESULTS AND DISCUSSION

A. High Performance Liquid Chromatography

This routine and fairly easily maintained method was improved and modified from the method developed by Sinners and his co-workers (63,64). In this laboratory, this method is still used as our main tool of monosaccharide analyses. The advantages of this system are: (1) the acid hydrolyzate does not have to be neutralized, it can be diluted and directly injected into the instrument to be analyzed, (2) the hydrolyzate does not have to be concentrated as a consequence of neutralization, (3) most importantly the monosaccharides released on hydrolysis of polysaccharide materials can be well separated and thus each sugar can be quantitatively analyzed, (4) the monosaccharides can be analyzed without derivatization.

There is on the market a carbohydrate column manufactured by Bio-Rad Company, Richmond, CA (Aminex carbohydrate HPX-87C). It uses only water as eluant and the retention time for all common wood sugars are within 30 minutes. It seemed that this column should be the most ideal column for monosaccharide analyses. The detector

recommended for use with the column was a refractive index (RI) detector. However, the sensitivity of the RI detector connected with the column was found not to be sensitive enough. The resolution of the monosaccharides was also not well enough to allow accurate quantitative determinations. We tried to mix the eluant water with acetonitrile in different proportions, but the analyses still did not work.

Meanwhile, we also looked into the possibilities of obtaining a good spectrum by employing different combinations of temperature, flow rate, acetonitrile-water ratios on a prepacked carbohydrate column made by Waters Associates Co. [μ carbohydrate (P/N 84038)]. We also investigated the results obtained from linking together the two columns described above with different combinations of ratios of acetonitrile and water. Again we found that the major failing factors were the poor resolution as a direct consequence of inadequate columns used and the low sensitivity of the RI detector employed. Finally, in order to obtain more accurate and confident results, we readopted the borate buffer and Chromex DA-X4-11 anion exchange column system described by Ni (46).

Internal standards were added into the solution

after the refluxing was completed. Comparisons were made to see if there were any differences derived if we added the internal standards before refluxing, because possibly the internal standards may degrad during refluxing and the amount of sugars then would be calculated incorrectly. The results showed no significant difference and we concluded that the degradation of the internal standard (D-lyxose) was not serious.

Instrumental K factors were calibrated in a different situation and conditions of this particular instrument has its own factors. We found that the present set up has about 10-25% differece from the one we previously obtained.

HPLC chromatograms of a known sugar mixture, hydrolyzates of the high yield solvent sulfite pulp, its hemicellulose C and its residue C are shown in figures 2, 3, 4, 5 respectively.

B. Ash Determination

The ash content is generally 0.1% - 0.5% in wood and usually includes calcium, potassium, magnesium, carbonates, phosphates, silicates and sulfates (38). As a

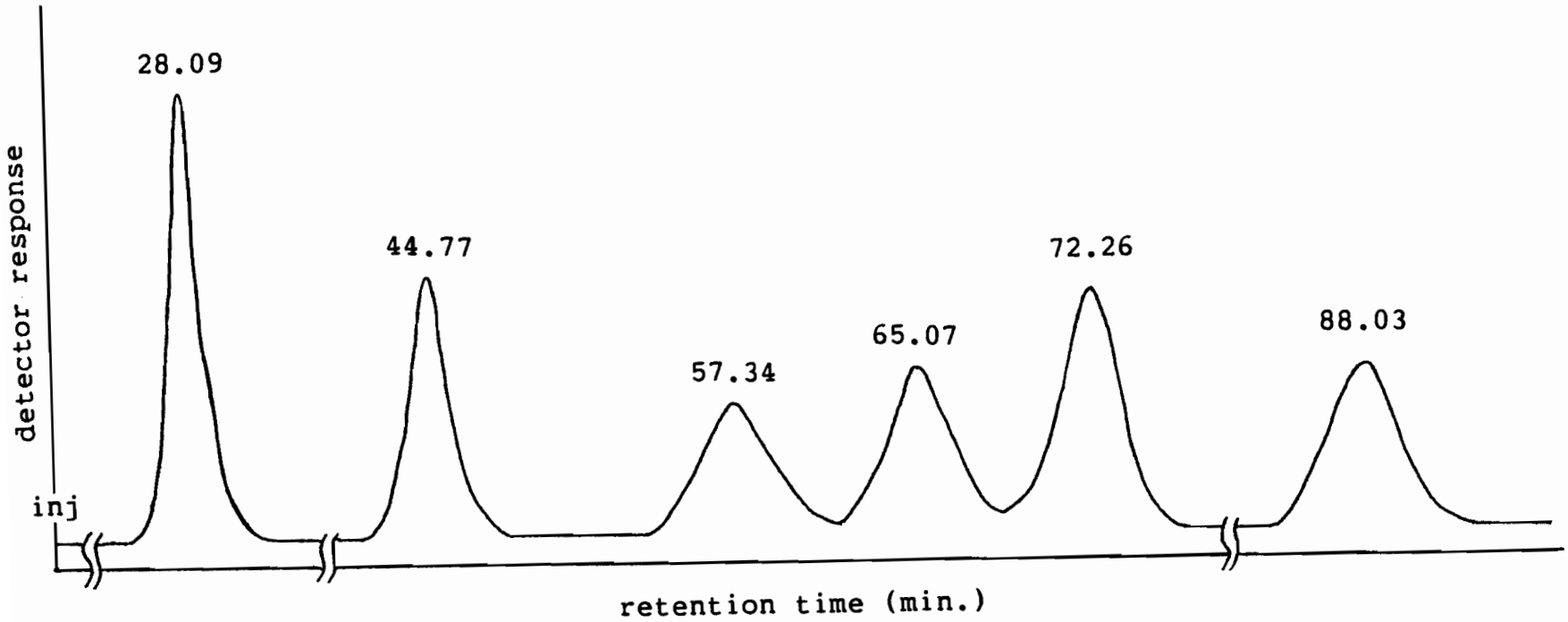

Figure 2. HPLC chromatogram of known sugars on a Dionex DA-X4-11 ion exchange resin using a borate buffer. The peaks from left to right are: rhamnose, mannose, arabinose, galactose, xylose and glucose.

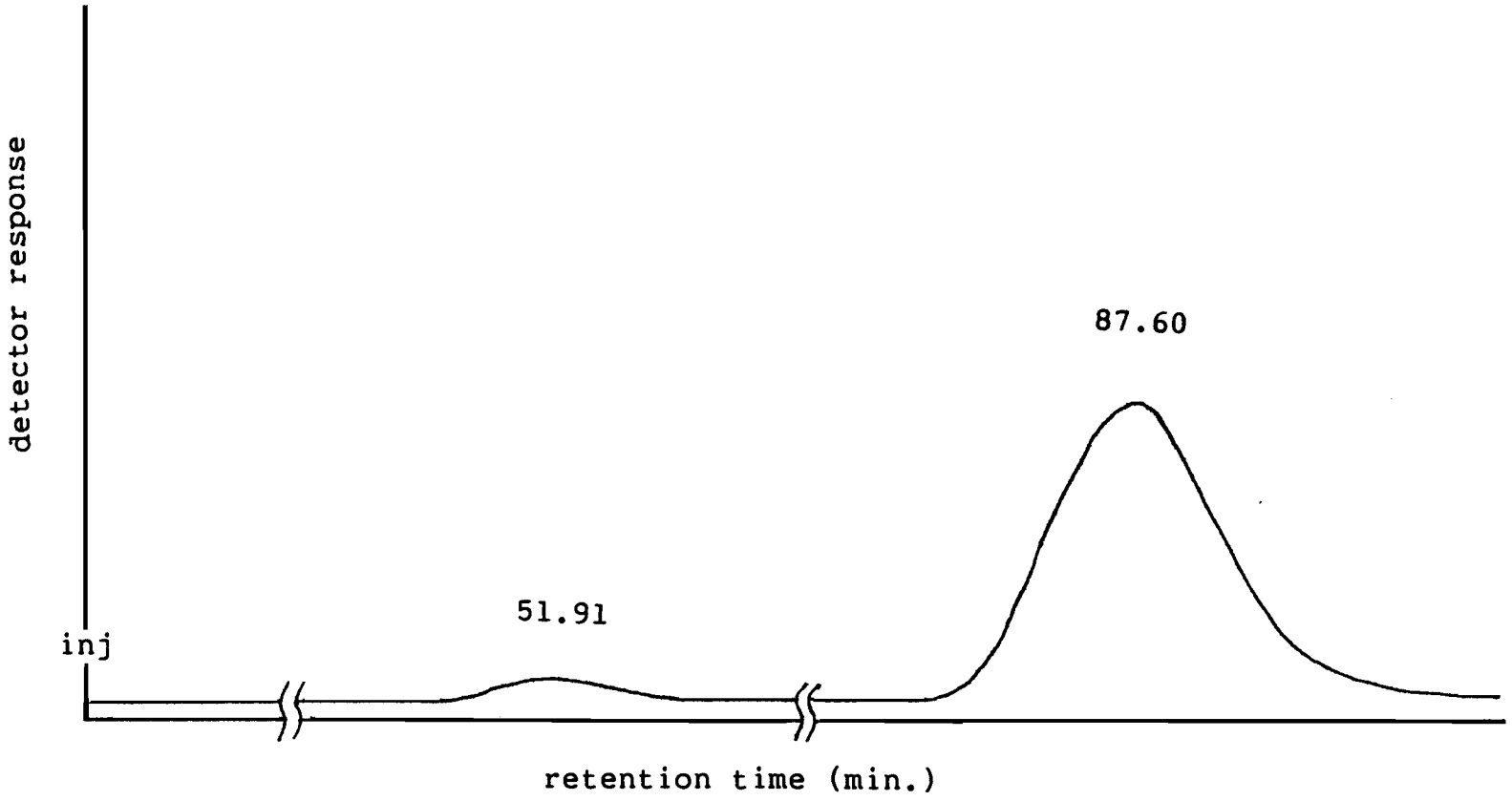

Figure 3. HPLC chromatogram of the hydrolyzate of freeze-dried solvent sulfite pulp.

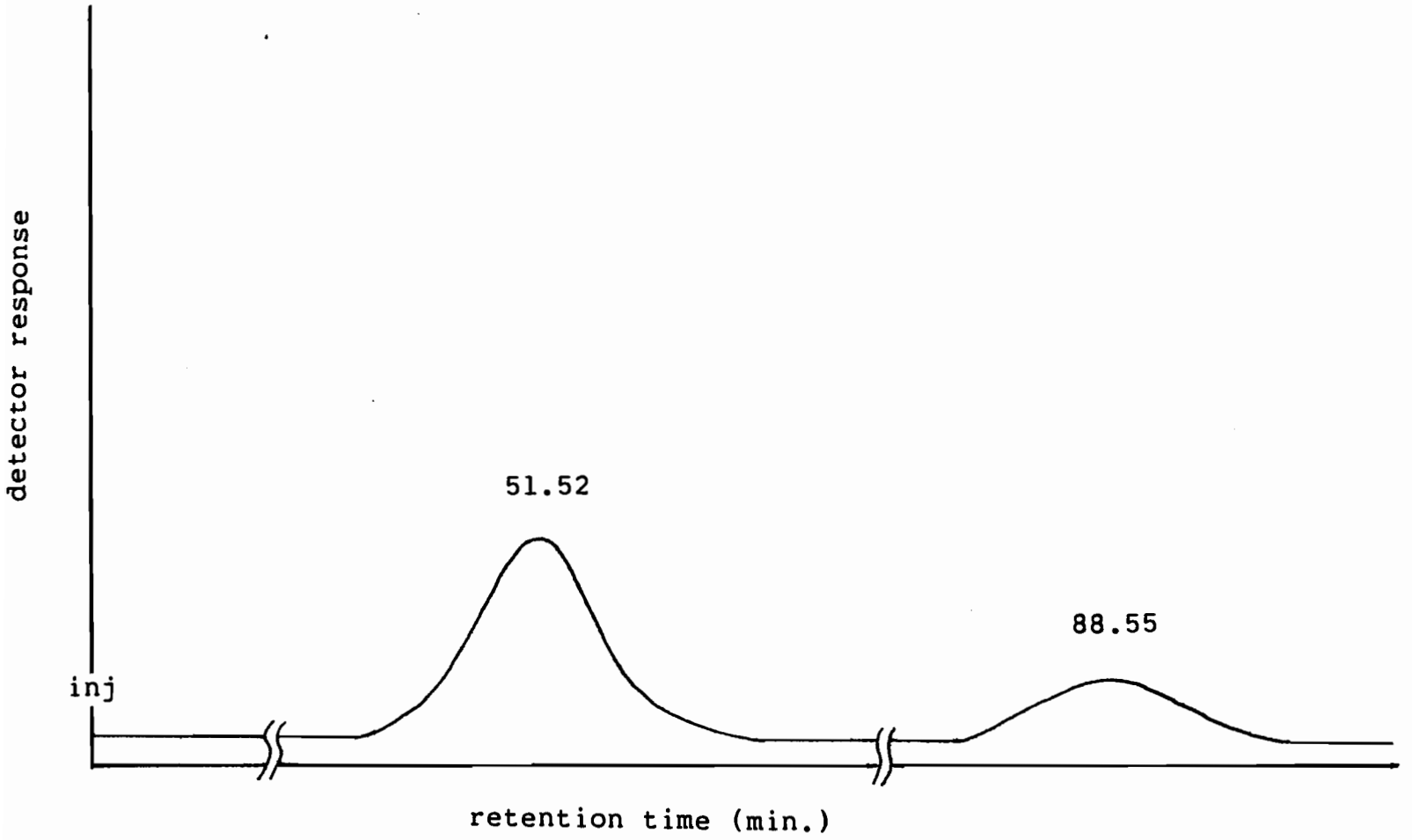

Figure 4. HPLC chromatogram of the hydrolyzate of Hemicellulose C from solvent sulfite pulp.

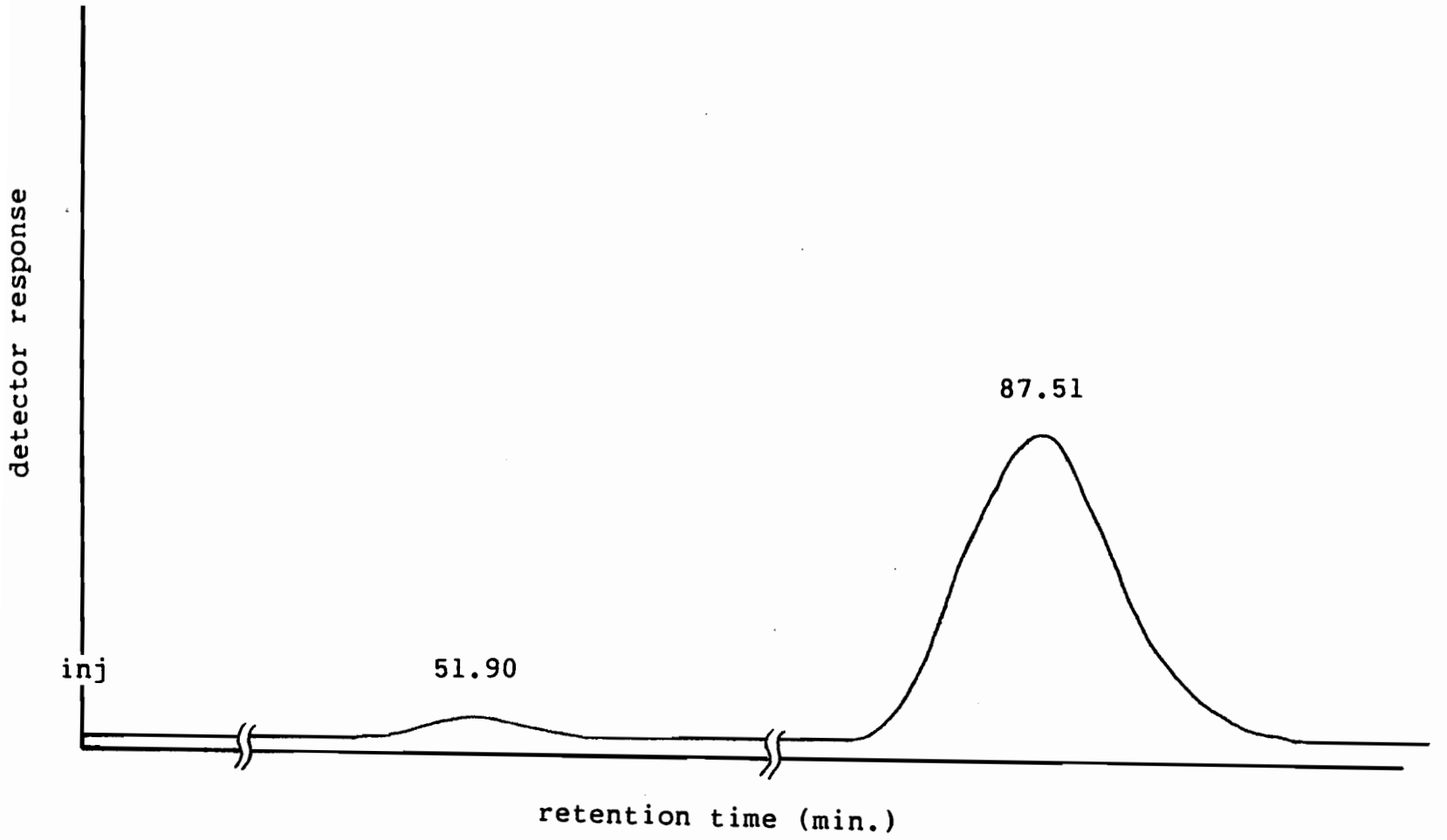

Figure 5. HPLC chromatogram of the hydrolyzate of Residue C from solvent sulfite pulp.

consequence, pulps inherit the ash from wood, plus the chemicals retained in the pulping process if the pulp was treated chemically. Thus we usually consider that pulps have higher ash contents than the raw wood material. However, this is not exactly the case we found in the data we obtained (see table 1). The defiberized, washed, and freeze-dried sulfite pulps have approximately the same ash content (1.0%-1.2%) as the TMP pulp which was not treated with chemicals. We interpret this to indicate that any excess inorganic elements introduced by the pulping processes were thoroughly washed away during the defiberization and washing stages. However, after the fractionation of the pulps into their component polysaccharides was completed, the ash content of the residue C fractions increased because of the use of barium hydroxide, potassium hydroxide, and sodium hydroxide in the separation processes (see section G). The ash contents of all three residue C's are in the range of 2.3%-3.6% (see table 1).

C. Klason Lignin and Acid-Soluble Lignin Determination

Pulping processes attempt to separate the lignin and retain as much as possible of the cellulosic fiber. Therefore, the lignin content has become a good indicator

Material	%Glucose	%Mannose	%xylose	%Klason Lignin	%Acid-soluble Lignin	%Ash	Total%
TMP	50.1	15.0	3.9	28.9	0.2	1.0	99.1
Hemicell. A[a]	5.4	0	23.7	N.A.[d]	N.A.	N.A.	29.1
Hemicell. B[a]	0	4.5	2.4	N.A.	N.A.	N.A.	6.9
Hemicell. B-1[a]	0	0	0	N.A.	N.A.	N.A.	0
Hemicell. C[a]	16.8	50.9	0	N.A.	N.A.	N.A.	67.7
Residue C[a]	49.4	13.9	4.2	29.4	0.2	2.3	99.4
High Yield Sulfite Pulp	54.8(33.8)[e]	1.2(0.7)	0.9(0.6)	29.2(18.0)	0.1(0.1)	1.0(0.6)	87.2
Hemicell. A[b]	0	0	44.4	N.A.	N.A.	N.A.	44.4
Hemicell. B[b]	17.1	11.9	3.2	N.A.	N.A.	N.A.	32.2
Hemicell. C[b]	2.1	0.1	0	N.A.	N.A.	N.A.	2.2
Residue C[b]	60.5	1.1	0	38.6	0.8	3.6	104.6
High Yield Solvent Sulfite Pulp	74.0(42.3)	3.2(1.8)	trace	17.6(10.0)	2.0(1.1)	1.2(0.7)	98.0
Hemicell. A[c]	0	0	50.5	N.A.	N.A.	N.A.	50.5
Hemicell. A-1[c]	0	0	35.7	N.A.	N.A.	N.A.	35.7
Hemicell. B[c]	4.8	0	0	N.A.	N.A.	N.A.	4.8
Hemicell. C[c]	19.0	59.2	0	N.A.	N.A.	N.A.	78.2
Residue C[c]	79.5	4.1	0	14.9	0.7	3.2	102.4

a. Isolated from TMP (see Chart 4). b. Isolated from high yield sulfite pulp (see chart 5). c. Isolated from high yield solvent sulfite pulp (see chart 6). d. N.A. indicates the sample was not analyzed for this component, usually because the weight of sample available was too small. e. numbers in the parentheses are percentages based on the original oven-dried wood.

Table 1. Chemical analyses of the pulp samples and their fractions.

to show how successful and effective the pulping process is.

The Klason lignin test is commonly regarded as a good way to determine the lignin content of wood and wood products. We found that the results obtained correlate well with the holocellulose content (see table 1). For example, the high yield solvent sulfite pulp had a Klason lignin content of 18% and a holocellulose content of 83%, for a sum of 101%. The high yield sulfite pulp had 29% Klason lignin and 72% holocellulose for a sum of 101%. The Klason lignin content of the TMP was 29%. After a first delignification the holocellulose content was 81.0% and after a second delignification was 77.0%. The sum of the Klason lignin content and the holocellulose content exceeded 100%, which probably indicates that the holocellulose still contained some lignin. TMP materials are not chemically treated during manufacture and so the wood lignin is not degraded. Therefore, the lignin would be difficult to remove by a mild delignification treatment such as holocellulose preparation.

In the Klason lignin determination, we found that the particle size of the lignin from the high yield solvent sulfite pulp was much finer than the other two. The size is estimated to be the same as the pore size of

a medium glass crucible because it was extremely difficult to filter through it. Thermomechanical pulp had the most even Klason lignin particle while the high yield sulfite pulp had relatively coarse and random size lignin particles.

The procedure for Klason lignin determination by the TAPPI standard is to dissolve the sample in 72% sulfuric acid, dilute to 3% and reflux for 4 hours. We used two TMP samples, one dissolved in 72% and the other in 77% sulfuric acid, both diluted to 3% and refluxed for 4 hours. We found no significant difference (0.04%). Later we dissolved a set of all three pulp samples in 77% sulfuric acid, diluted to 3% and refluxed for 5.5 hours. The amounts of lignin also gave no significant difference compared with the lignin obtained from another set refluxed for 4 hours except for the high yield solvent sulfite pulp with a difference of 2.4% (the one refluxed for 5.5 hours had the lower lignin content). This again indicated the solvent sulfite pulping process did alter the nature of the lignin which allowed it to dissolve away as the refluxing proceeded.

Besides the Klason lignin which is generally regarded as "lignin", there is also acid-soluble lignin released in the process of Klason lignin determination. We found low acid-soluble lignin contents in all pulps

and in the residue C's except for 2.0% in the freeze-dried high yield solvent sulfite pulp. The solvent sulfite method results in a high yield of pulp. The methanol solvent undoubtly did not remove the same type of lignin fractions as did the TMP and sulfite methods. Therefore, more of the lignin fractions dissolved in the 72% sulfuric acid digestion of the Klason lignin determinations. Thus the analyses for acid-soluble lignin was higher in the case of the solvent sulfite pulp. The content was as high as 2.0% vs. 0.1% in the high yield sulfite pulp and 0.2% in TMP (see table 1).

The Klason lignin content was much lower (17.6%) for the solvent sulfite pulp than for the other two pulps (28.9% for TMP and 29.2% for the high yield sulfite pulp) indicating a different mechanism for delignification. The low lignin content of the solvent sulfite pulp demonstrated a promising method of delignification for such a mild, fast process.

D. Viscosity Determination

Viscosity is a measure of the average degree of polymerization (DP) of the pulp sample, mainly that of

cellulose. The changes in paper strength properties can be related to the viscosity changes. The viscosity test makes it possible to check the extent of degradation caused by cooking and pulping processes and is a measure of oxidative degradation which is probably fairly parallel to the attack on whatever the strength-bearing elements may be of the fibers. The changes in the chain length of cellulose is likely to mean little on paper strength, but nevertheless a fair correlation exists between paper strength properties and pulp viscosity (56).

After the first delignification, that is holocellulose preparation, the viscosity of the TMP was 111 centipoise if it were considered as an unbleached pulp (if it was considered as bleached pulp, the viscosity would be 108 centipoise). However, after a second delignification treatment, the viscosity lowered to only 56 centipoise. Apparently severe degradation occurred in the process of delignifying the pulp.

The high yield solvent sulfite pulp had a significantly higher viscosity of 195 centipoise, almost three times as high as the 68 centipoise of the high yield sulfite pulp if both were treated as bleached pulp. From the results of the viscosity determinations, we concluded that the high yield solvent sulfite pulp was

significantly superior to the high yield sulfite pulp in terms of DP. The sulfite pulping process apparently degraded the average DP of the pulp appreciably while the solvent sulfite pulping process did not.

E. Hydrolysis of Polysaccharide Samples

The procedure followed is the one modified and discussed by Ni (46). Attempts were made to dissolve the hemicelluloses in 3% sulfuric acid without subjecting them to the harsh 77% sulfuric acid but without success. Standard procedures were followed step by step.

F. Preparation of Pulp Samples

1. Thermomechanical Pulp

Thermomechanical pulp is produced most efficiently and the yield can approach 100%. Essentially it is very finely ground wood. Wood chips are passed through a chamber of high pressure steam and then defiberized mechanically by a refiner (12). Compared to refiner mechanical pulp (RMP) produced by only sending wood chips

through a refiner, TMP undergoes an extra treatment of high pressure steam. This significantly improves the quality of the pulp. Theoretically TMP should not be greatly different in chemical composition from the wood itself except probably for some loosely attached sugars that are dissolved away in the process. This is the reason we chose TMP as one of our test materials as a comparable starting material for both the sulfite pulps. To avoid bio-degradation, the pulp was frozen until ready for analysis.

No Kappa number was determined for the TMP sample because Kappa numbers are accurate only for pulps of less than 70% yield (37).

2. High Yield Sulfite Pulp

The high yield sulfite pulp was considered as a comparison for the solvent sulfite pulp. The pulp was produced by an acid-sulfite pulping process (total 20% SO_2 based on wood chip weight). It was cooked for 45 minutes and the yield was 61.6%. The Kappa number is the number of cc's of 0.1 N potassium permanganate solution consumed by one gram of moisture-free pulp under the conditions specified in the TAPPI standard (37) and generally is considered a good indicator of lignin

content. The Kappa number of the high yield sulfite pulp was high at 121 indicating a high residual lignin content. The short cooking time of 45 minutes clearly did not remove as much lignin as a regular acid-sulfite cook which is usually cooked for 3-7 hours.

Results of the Kappa number test performed by Jerry Hull are as follows:

	high yield sulfite pulp	high yield solvent sulfite pulp
freeze-dried pulp	121	81
residue C	135	78

Only the high yield sulfite pulp showed an increase in lignin content of residue C above that of the freeze-dried pulp. This matches the data we obtained from the Klason lignin determination (29.2% in freeze-dried pulp and 38.6% in residue C) which again demonstrates the ineffectiveness of the sulfite pulping process comparing with the solvent sulfite process in the capability of altering and dissolving away lignin.

3. High Yield Solvent Sulfite Pulp

Solvent pulping is the subject of considerable research. It has several unique advantages: easier to

bleach, high yield, less smell and possibly shorter cooking times. Dr. Bublitz and Mr. Jerry Hull kindly provided us with the pulp which was investigated. They found that the optimum percentage of methanol in the pulping liquor was 30%. This solvent sulfite pulp was manufactured under exactly the same conditions as the high yield sulfite pulp described above except for the addition of 30% methanol in the pulping liquor. The yield was 57.1% and the Kappa number was 81.4 which was significantly lower than the sulfite pulp. Therefore, the Kappa number indicated a lower lignin content. This was substantiated by the Klason lignin tests which showed 29.2% Klason lignin for the high yield sulfite pulp and 17.6% Klason lignin for the solvent sulfite pulp (see table 1).

We also examined the data obtained from Mr. Jerry Hull concerning the paper properties of handsheets made from these two sulfite pulps. The solvent sulfite pulp had much better paper properties than the sulfite pulp. All the test results showed the superiority of the solvent sulfite pulp except for the tear test. It seems that all the advantages described above for solvent pulping are significant and thus the chemical composition becomes very interesting to see if we can explain the reasons for these advantages.

4. Preparation of Samples for Analyses

Each of the three pulp samples was air-dried in the hood so that they could be easily handled for future investigations. The non-volatile solids contents of these three pulps after air-drying were: TMP 91.2%, high yield sulfite pulp 90.0%, and high yield solvent sulfite pulp 88.9%. The high yield sulfite pulp and the high yield solvent sulfite pulp still retained much of the configuration of the wood chips from which they were made. The pulps had been taken directly from the digester with no effort to disintegrate the wood into fibers. Therefore, these two pulps were defiberized in a Waring blender (see charts 2 and 3). A careful monitoring of the materials solubilized during the defiberization was established.

The oven-dried weight of the starting material for the high yield sulfite pulp was 216.0 g and the oven-dried weight of the solids recovered after defiberization was 175.7 g or 81.3% recovery. The monosaccharides that were solubilized in the water during the defiberization were analyzed by HPLC and were shown to be glucose, mannose, galactose, xylose and arabinose. However, the resolution was not good enough for accurate quantitative analyses. The solubilized solids were acid

hydrolyzed to hydrolyze the soluble polysaccharides to their simple sugars. The monosaccharides were detected by HPLC as glucose, mannose, galactose, xylose and arabinose but again resolution was not good enough for quantitative analyses. However, the HPLC results demonstrated that both monosaccharides and polysaccharides were solubilized by the defiberization of the pulp.

The oven-dried weight of the starting material for the high yield solvent sulfite pulp was 213.4 g and the oven-dried weight of the solids recovered was 157.5 g or a recovery of 73.8%. Therefore, considerably more of the high yield solvent sulfite pulp was solubilized during defiberization than was the high yield sulfite pulp. The solubilized material was analyzed by HPLC and was shown to contain both monosaccharides and polysaccharides of the sugars glucose, mannose, galactose, xylose and arabinose.

Portions of the TMP pulp, the defiberized high yield sulfite pulp, and the defiberized high yield solvent sulfite pulp were freeze-dried for further investigations (see charts 1, 2, and 3).

G. Fractionation of the Pulp Samples into Their Component Polysaccharides

The quantitative analyses of the monosaccharides which comprised the three pulp samples and their fractions are shown in table 1. The TMP sample contained no detectable amounts of arabinose or galactose although it is expected that the wood from which it was made must certainly have contained some of these monosaccharides. Apparently these sugars were lost in the liquor which resulted from steaming and grinding the wood to produce TMP. It is well known that both galactose and arabinose moieties are readily cleaved from polysaccharides (71).

The extracts from the blending of the high yield sulfite pulp and the high yield solvent sulfite pulp contained both galactose and arabinose (see charts 2 & 3). However, neither of the residues from the blending contained galactose and arabinose (table 1). This again indicates the ease with which galactose and arabinose are removed from the hemlock polysaccharides. The loss of galactose and arabinose moieties are consistent with the results of Hamilton, Kircher and Thompson (31) and Hamilton and Thompson (32) who investigated the hemicelluloses of a commercial sulfite pulp manufactured from western hemlock wood. They extracted the pulp with

18.5% sodium hydroxide solution and from the solubilized material isolated two hemicelluloses, a glucomannan and a xylan polyuronide. Neither of the hemicelluloses contained either galactose or arabinose. Apparently the galactose and arabinose moieties were lost during the sulfite processing.

The TMP sample (table 1) contained a high proportion of glucose (50.1%) and of mannose (15.0%). The Klason lignin content was also high (28.9%) which indicated that very little lignin was lost in pulp manufacturing. This was to be expected because the wood was never treated with chemicals but was simply steamed and ground. The over-all total recovery of 99.1% of the chemical entities shows that the analysis was essentially complete. The amounts of hemicelluloses isolated (see chart 4) were very small: hemicellulose A 0.60%, hemicellulose B 0.14%, hemicellulose B-1 0.04%, hemicellulose C 0.36%. Apparently the high content of lignin prevented the solubilization of the hemicelluloses. It is well known that essentially all lignin must be removed from wood substances prior to hemicellulose extraction.

In the process of extracting hemicellulose A, the pulps were slurried with saturated barium hydroxide and 18.5% potassium hydroxide solution. We found it was extremely difficult to filter the slurry through a coarse

sintered glass filter. The problem was later solved by placing two extra Whatman #1 filter papers on top of the filter. The rationale was that when the pulp was soaked with these solutions, the pulp was swollen and softened and was sucked into the pores of the filter which essentially blocked and plugged all the pores.

The hemicellulose A isolated from the TMP (see chart 4) consisted of xylose residues (23.7%) and glucose residues (5.4%). Thus hemicellulose A is primarily a xylan which coincides very well with the results from the isolation procedure used and explained by Beélik, Conca, Hamilton and Partlow (9). These researchers showed that by first complexing the mannan containing polysaccharides with barium and then extracting with 10.0% potassium hydroxide, a xylan-rich hemicellulose would be extracted. The fact that only 29.1% of hemicellulose A was accounted for is due to all of the lignin that was present in the sample. Hamilton and Thompson (32) showed that western hemlock sulfite pulp contained a xylan polyuronide. They demonstrated that the structure consisted of a backbone of D-xylopyranose residues linked predominantly with 1→4-β-glycosidic bonds and at frequent intervals a 4-O-methyl-D-glucuronic acid residue was attached to position 2 of a xylose moiety of the main chain. These uronic acid residues occurred on an average of one to

every 4 to 8 xylose residues. Hemicellulose A of the present work is undoubtedly this xylan polysaccharide but there was no indication of the presence of any 4-O-methyl-D-glucuronic acid moieties although no special effort was made to detect them.

Hemicellulose B, according to the researchers mentioned above, should be a galactoglucomannan but since the starting TMP sample contained no galactose, hemicellulose B seemed to be a mixture of a mannan-rich polysaccharide contaminated with a xylan. Only a small amount, 0.14% of the starting TMP, was isolated.

Hemicellulose B from TMP was the residue obtained from the rotary dried opaque methanol-water solution after hemicellulose B-1 was precipitated. The results of monosaccharide analyses showed no detectable sugars in hemicellulose B-1 and only a very small amount of mannose (4.5%) and xylose (2.5%). We conclude that there is no hemicelulose B as we first expected and what we obtained here were essentially lignin and some very soluble monosaccharides which dissolved during the process.

Hemicellulose C contained mannose residues and glucose residues in the ratio of 3 to 1. This indicated a glucomannan which is consistent with hemicelluloses

isolated by the method of Beélik, Conca, Hamilton and Partlow (9) and with the glucomannan isolated from western hemlock sulfite pulp by Hamilton, Kircher and Thompson (31). However, only a small amount, 0.36% of the starting TMP, was isolated. Residue C from the TMP (see chart 4) analyzed essentially the same as the starting sample of TMP (table 1). This was because the high amount of lignin in the starting material interfered with the dissolution of the hemicelluloses so that nothing was removed. The higher amount of ash in the residue probably resulted from the addition of barium hydroxide, potassium hydroxide and sodium hydroxide during the extractions of the hemicelluloses.

According to the theses of Chen (11) and Fernandez (22), hemicelluloses are purified by redissolution in water and reprecipitation with methanol and then centrifuged. Here I propose that hemicelluloses should be redispersed in methanol and then centrifuged for the following reasons. Salts are used in the process of extraction (barium hydroxide, potassium hydroxide and sodium hydroxide), as a consequence, they always exist in the solution with the hemicellulose precipitate. If we use methanol to redisperse the precipitate instead of water and methanol, the ions in the precipitate will have a much greater chance to get out with the residual

water in the hemicellulose and mix with the methanol which will allow the methanol insoluble hemicelluloses to be free of contamination of ions and water solubles after centrifugation.

The analyses of the high yield sulfite pulp showed a low amount of mannose (see table 1) compared to that in the TMP pulp. The high yield sulfite pulp was treated with sulfite pulping chemicals which apparently dissolved out most of the mannose containing polysaccharides. Hamilton, Kircher and Thompson (31) also reported quite low amounts of mannose in a western hemlock pulp prepared by sulfite pulping. Although these workers isolated some glucomannan hemicelluloses, the amounts were low (4.2%) and these hemicelluloses contained considerable percentages of glucose, xylose and glucuronic acid residues in addition to mannose residues. Thus it seems that sulfite pulps prepared from western hemlock wood are low in mannose. The TMP pulp sample contained 15.0% mannose (table 1), but the TMP material was never treated with chemicals.

The glucose content of the high yield sulfite pulp was quite high at 54.8% (table 1). Since there was little mannose in the sample this must be cellulosic material. The Klason lignin content was high (29.2%) but this is

undoubtedly due to the short cooking time (45 minutes) of the wood in the sulfite pulp liquor. The high lignin content explains why the yield of the pulp was high (61.6%). The total analytical recovery of 87.2% of the starting sample was lower than it should be but all of the analyses were repeated and the values could not be improved.

As with the TMP material, the isolation of hemicelluloses from the high yield sulfite pulp was unsuccessful. This was again due to the high lignin content. Hemicellulose A (see chart 5) proved to be rich in xylose residues but there was very little dissolved (0.67%) (table 1). Hemicelluloses B and C again proved to be glucomannans but there was not much material recovered (0.22% and 0.02% respectively). Residue C was exceptionally high in glucose residues (60.5%) and Klason lignin (38.6%) (table 1). Some of this can be accounted for by the extraction of the hemicelluloses, leaving the percentages of cellulose and lignin higher but some of the high values must be due to the limits of the analytical methods as demonstrated by the total recovery value 104.6%. The increase in ash content in residue C (3.6%) is because of the addition of barium hydroxide, potassium hydroxide, and sodium hydroxide during the isolation of the hemicelluloses.

The analyses of the solvent sulfite pulp (table 1) clearly demonstrated that this material was different from the other two pulps. The pulp contained an exceptionally high amount of glucose (74.0%) which indicated that its cellulose content was high. The Klason lignin centent of 17.6% was considerably lower than either of the other two materials. Thus the analyses demonstrated that the addition of methanol to the standard sulfite pulping chemicals assisted in removing lignin and left a high cellulose content pulp. The cooking time (45 minutes) was also much less than usually used for a sulfite cook (3-7 hours) (56). However, the amount of Klason lignin in the pulp (17.6%) was still very high. The hemicelluloses extracted were the typical xylan (hemicelluloses A and A-1) and a glucomannan (hemicellulose C). The ratio of mannose to glucose in hemicellulose C was 3.1 to 1.0, very close to the ratio of mannose to glucose in the hemicellulose C from TMP and to that reported by Hamilton, Kircher and Thompson (31). Residue C was primarily glucose units which indicates that this particular pulping media caused little or no degradation of the cellulose units.

H. Isolation of Holocellulose Fractions

The holocellulose of wood represents the carbohydrate portion of the wood. It is, in effect, the sum of the cellulose and the hemicellulose fractions. The method used to isolate the holocellulose was a mild pulping reaction designed to solubilize the lignin and leave the carbohydrates insoluble. Although this goal is never precisely achieved, the holocellulose content of a material is quite a good indication of its total carbohydrate content.

The holocellulose fraction of TMP represented 81.0% after one delignification. However, the holocellulose was not quite white so the delignification was repeated. The yield of holocellulose this time was 76.0%. This fitted very well with the 28.9% Klason lignin in TMP for the sum of the two equaled 104.9%. This analysis again demonstrated that TMP still retained most of its lignin.

The high yield sulfite pulp yielded 72.0% holocellulose which was somewhat lower than the yield of 76.0% from TMP. This indicated that the treatment of wood with sulfite pulping chemicals, although only for a short period of time, somewhat altered the carbohydrates since more of them dissolved in the delignification reaction. The solvent sulfite pulp yielded 83.0%

holocellulose which showed that the solvent sulfite pulping process was mild to the carbohydrates. This again substantiated the data which showed the solvent sulfite to be a promising process. Viscosities were obtained on each of these holocellulose fractions with the results that TMP holocellulose showed a viscosity of 56 centipoise, the high yield sulfite pulp showed a viscosity of 68 centipoise, and the high yield solvent sulfite holocellulose showed a viscosity of 195 centipoise. The viscosities again demonstrated that the high yield solvent sulfite process did the least damage to the carbohydrates.

I. Carbon-13 Nuclear Magnetic Resonance of Solid Samples of Carbohydrate Materials

Three carbohydrates were used as standards for the purpose of peak assignments. They were cellobiose, maltose and gentiobiose. The spectra of these three standards are shown in figures 6, 7 and 8 respectively as well as their peak assignments.

For ease and convenience in the following discussion we use the superscript prime referring to the nonreducing

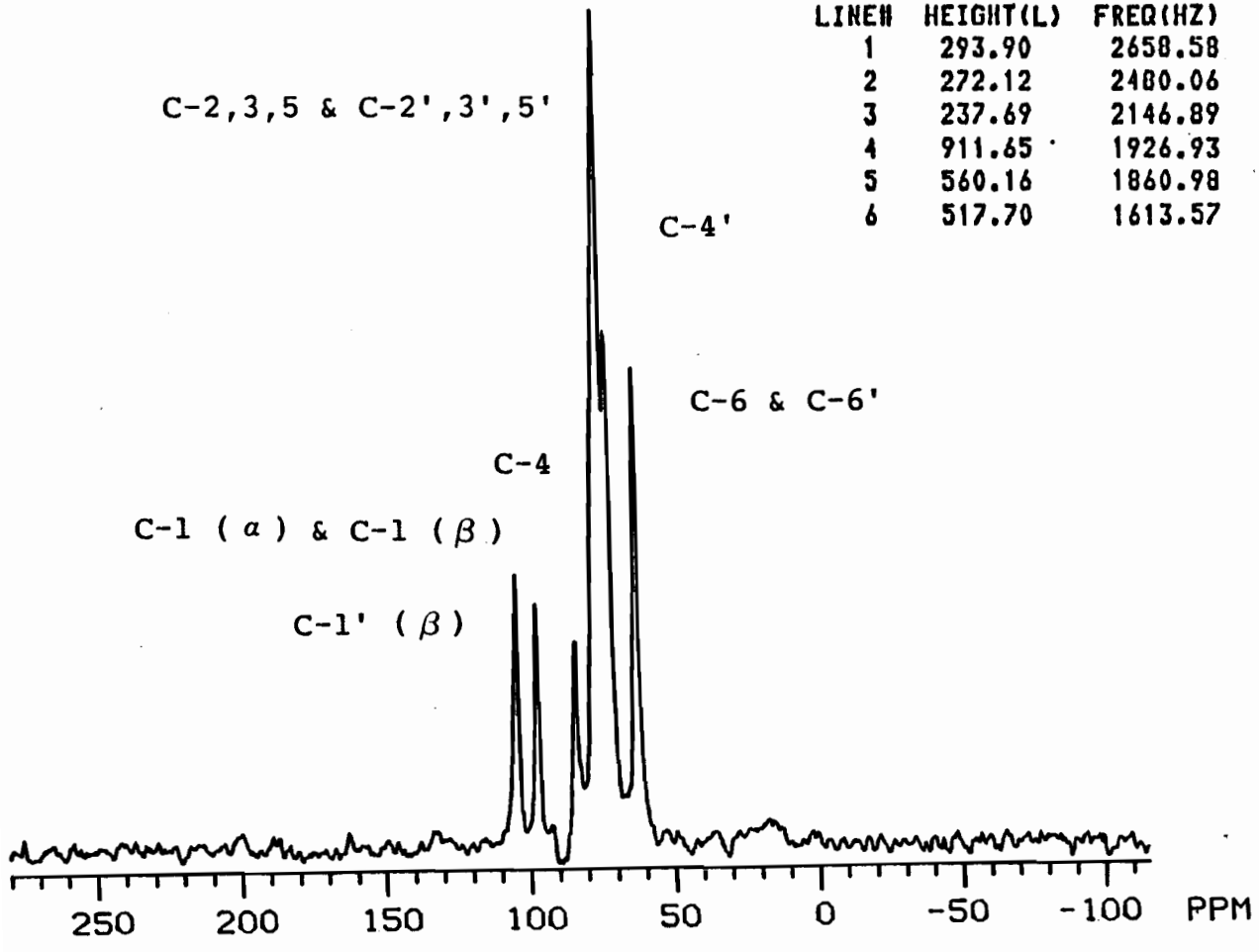

Figure 6. CP/MAS Carbon-13 NMR spectrum of cellobiose.

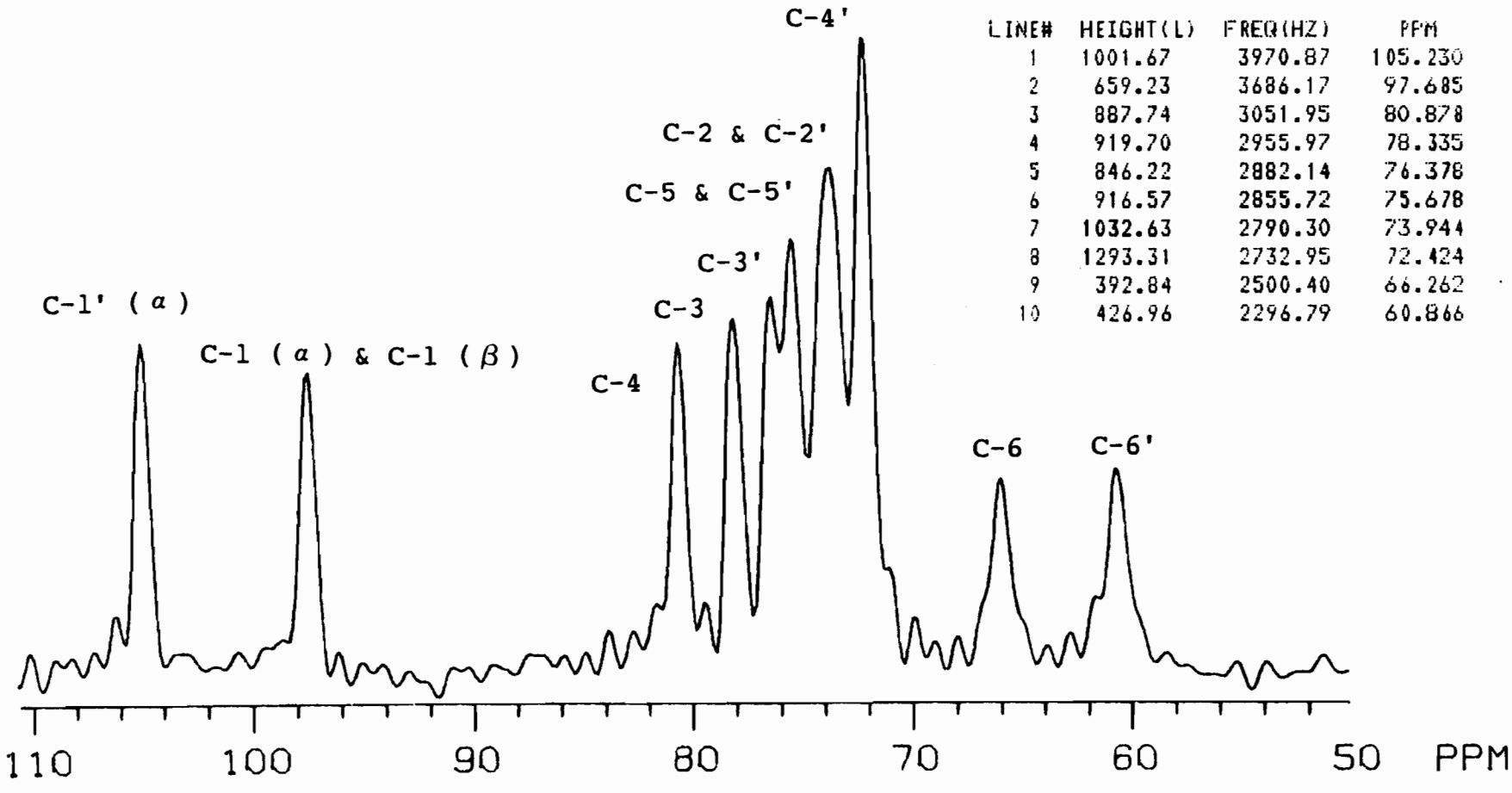

Figure 7. CP/MAS Carbon-13 NMR spectrum of maltose.

LINE#	HEIGHT(L)	FREQ(HZ)	PPM
1	491.21	3807.21	100.893
2	448.25	3678.05	97.470
3	968.37	2886.25	76.487
4	935.30	2840.22	75.267
5	1007.94	2796.65	74.113
6	772.08	2615.76	69.319
7	309.40	2549.89	67.573
8	278.89	2291.01	60.713

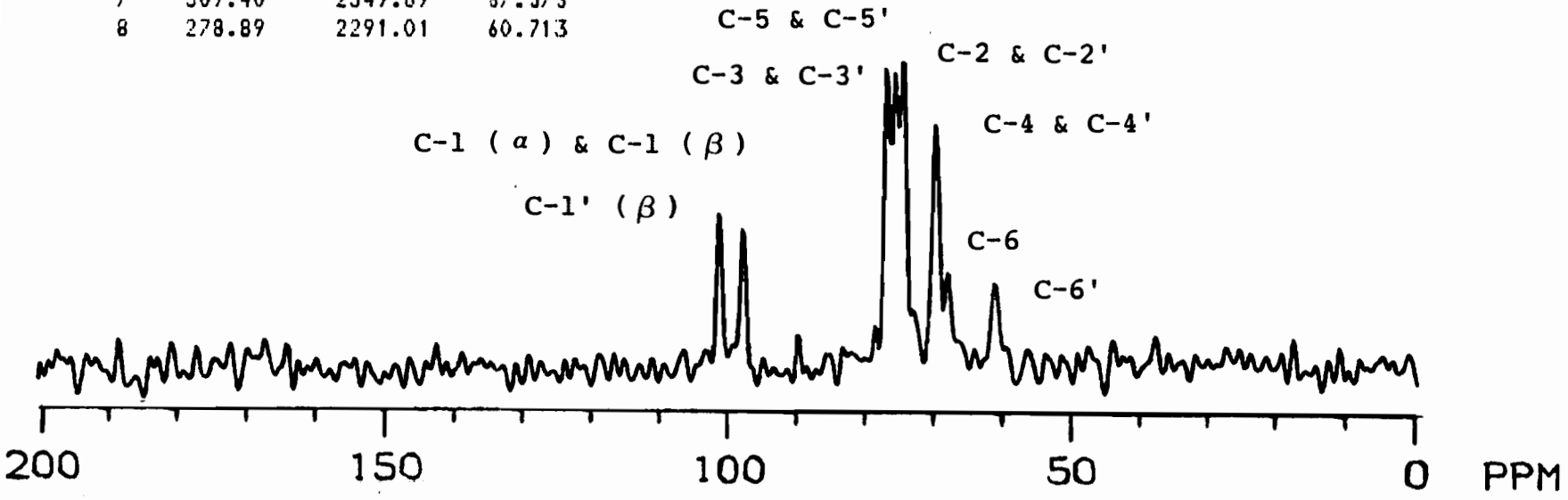

Figure 8. CP/MAS Carbon-13 NMR spectrum of gentiobiose.

unit and C represents carbon atoms.

In the spectrum of cellobiose (figure 6), the peak at 105.2 ppm was assigned to the C-1' which is the linkage carbon on the non-reducing unit. The peak at 98.1 ppm is assigned to both C-1α and C-1β on the reducing end. Several previous researchers showed well separated peaks for C-1α, C-1β and C-1' for cellobiose and similar compounds in the solution state (16,26,33,35,50,65). Ideally and theoretically it should also be this way for solid-state CP/MAS NMR analysis according to suggestions made by Saito and Tabeta (58) stating that there is no substantial differences in the chemical shifts observed between solution and solid state NMR. However, Pfeffer and Hicks (52) later pointed out that strict correspondence of chemical shifts between solution and solid state NMR cannot be assumed. Due to the limitation of the instrument, we see only two very well separated peaks instead of the three we first expected. Since it is well known that the anomeric carbon on the non-reducing unit (C-1') which exists in the 1→4 linkage type of disaccharides always shows its peak above 100 ppm both in solution and solid state NMR, spectra, C-1' is assigned easily to the 105.2 ppm peak (5,14,16,20,26, 27,33,35,36,41,50,55,58,65,66). The assignment of both

C-1α and C-1β on the reducing end to the peak at 98.1 ppm instead of assigning them separately is because: 1) the peaks from these carbons are never seen above 100 ppm in solution and 2) in the solid state the more stable form (β in this case) usually dominates and appears as seemingly the only form which is unlike the situation in the solution state (52).

The peaks at 85.0 ppm and 73.6 ppm are assigned to C-4 and C-4' respectively. For monosaccharides and their derivatives, peaks from C-4 always appear solely in the higher field (70-75 ppm) whereas in disaccharides linked 1→4 and oligosaccharides (linked 1→4) two major peaks show for C-4 (14,19,33,35,66). The one at lower field is from the linkage carbon on the reducing unit and the other one is from the C-4' of the non-reducing unit. It is interpreted as the carbon more tightly bonded or with less mobility, the corresponding peak usually shifts downfield (16). Since the C-4 on the reducing unit is in the linkage it is apparently more rigidly tied than the C-4' in the non-reducing end.

The main peak at 76.3 ppm is assigned collectively to all the carbons at the 2, 3 and 5 positions on both units. Also the peak at 63.8 ppm is assigned to both C-6 and C-6'. This pattern of assignments agrees very well with the one suggested by Gast, Atalla and McKelvey (26).

The maltose spectrum (figure 7) is composed of a total of 10 peaks. The 105.2 ppm peak is from C-1' which is the α-linkage carbon on the non-reducing unit. We regretfully report here that there is no significant difference between the chemical shifts of the C-1'α and the C-1'β (cellobiose, see figure 6) which was previously aimed as one major breakthrough in our research. However, there is possibly a small peak on the downfield shoulder which possibly presents an indication of some saccharide contamination with a β-linkage. If this proposal can be proved later, the assignments of C-1'α and C-1'β can then be easily done. The following peak at 97.7 ppm is also assigned to both C-1α and C-1β.

For maltose, the peaks at 80.9 ppm and 72.4 ppm are assigned to C-4 and C-4' respectively. The four peaks between are assigned following the pattern suggested by Heyraud, Rinaudo and Vignon (33). The peak at 78.3 ppm is from C-3 and the one at 76.4 ppm is assigned to C-3'. The argument behind this is that it is well known the C-3 is hydrogen bonded with the oxygen in the ring of the non-reducing unit and becomes more rigidly bonded than is C-3' on the non-reducing unit which results in a shift to downfield (16,17,18). The peak at 75.7 ppm is

assigned to both C-5 and C-5' as well as the one at 73.9 ppm to both C-2 and C-2'.

There are two well separated peaks for C-6 and C-6'. The one downfield (66.3 ppm) is assigned to C-6 which is also considered to be hydrogen bonded and is more restricted and shifted downfield.

The 100.9 ppm peak in the spectrum of gentiobiose (figure 8) is assigned to C-1' which is β-linked with C-6 on the reducing unit. The interpretation of the higher field than is seen in cellobiose and maltose is due to the 1→6 linkage which is believed to allow more mobility and less restriction in space than in the 1→4 linkages and results in shifting to higher field. As described above, the peak at 97.5 ppm is assigned to both C-1 α and C-1β on the reducing end.

In light of the work of Usui, Yamaoka, Matura and Tusimura (66), three peaks at 76.5 ppm, 75.3 ppm and 74.1 ppm are assigned to carbons 3, 5 and 2 on both units. The following peak (69.3 ppm) is assigned to C-4 and C-4' since neither of them are in any kind of linkage. This is justified as in the monosaccharides (glucose) being at least two carbons away from the linkage. The final two peaks at 67.6 and 60.7 ppm are assigned to C-6 and C-6' respectively. C-6 is in the

linkage but apparently the mobility is not reduced enough to move it further downfield because it appears at the same resonance as in maltose (66.2 ppm) where it is not in the linkage.

The spectra of the high yield solvent sulfite pulp and of its residue C were derived following successive extractions of hemicelluloses. These spectra plus those of the hemicelluloses are shown in figures 9-14.

The collective peaks shown around 60 ppm and 150 ppm are characteristic of lignin (39,43,62). This lignin assignment applies to all the spectra of hemicelluloses discussed later.

The peak at 106.3 ppm in the spectrum of the high yield solvent sulfite pulp (figure 9) and the one at 106.4 ppm in the spectrum of residue C (figure 14) are from carbon 1 of every unit (the reducing unit becomes insignificantly important as the DP goes up). It is assumed to be a β-linkage but without any persuasive evidence from within the spectrum itself. Both the peaks at 90.0 ppm and 85.0 ppm are from C-4. It is now well established that there are two major cellulose crystalline polymorphs and an amorphous cellulose which exist in nature (5,20,21,44). We did not observe two peaks for each C-1 and C-4 as shown in the previous

LINE#	HEIGHT(L)	FREQ(HZ)	PPM
1	117.00	5639.37	149.446
2	116.26	4487.23	118.914
3	121.99	4324.94	114.613
4	169.71	4203.94	111.407
5	1034.10	4009.93	106.265
6	673.08	3395.78	89.990
7	501.08	3207.39	84.997
8	2065.90	2855.69	75.677
9	2159.56	2766.40	73.311
10	537.61	2498.60	66.214
11	197.61	2194.60	58.158

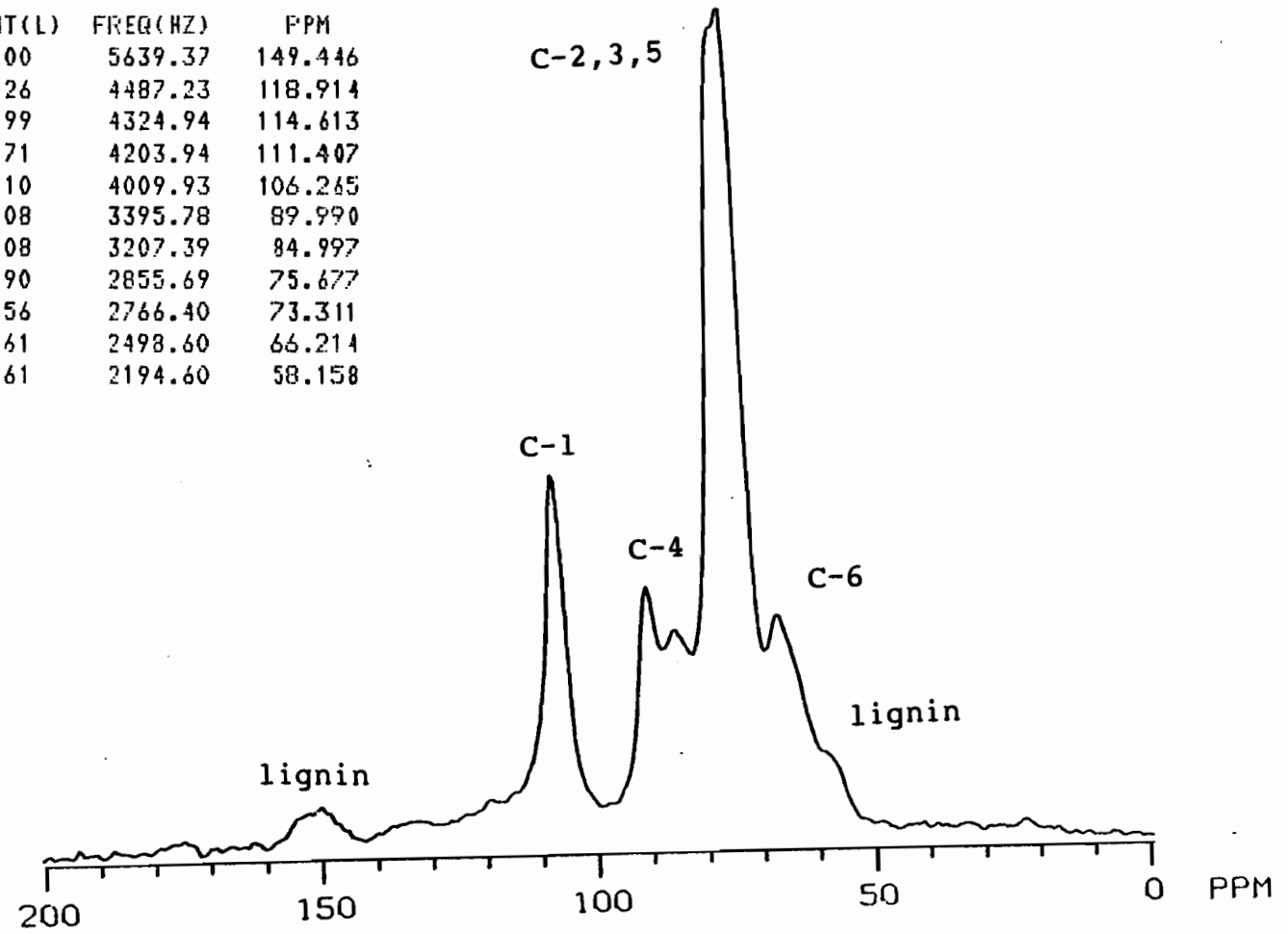

Figure 9. CP/MAS Carbon-13 NMR spectrum of the high yield solvent sulfite pulp.

LINE#	HEIGHT(L)	FREQ(HZ)	PPM		
1	165.95	6845.61	181.412		
2	185.37	6735.11	178.484		
3	128.49	5645.25	149.602		
4	106.96	5507.43	145.950		
5	108.62	5248.64	139.092		
6	144.59	5098.28	135.107		
7	123.55	4959.07	131.418		
8	159.45	4781.06	126.700		
9	160.55	4711.55	124.858		
10	162.47	4644.22	13	889.25	891.38
13	889.25	891.38	3924.56	104.003	
14	2717.00	2745.64	2852.61	75.596	
15	901.19	910.34	2462.42	65.255	
	266.65		2175.55	57.653	
18	281.54	281.59	1002.45	26.565	
19	233.75	233.78	885.21	23.458	

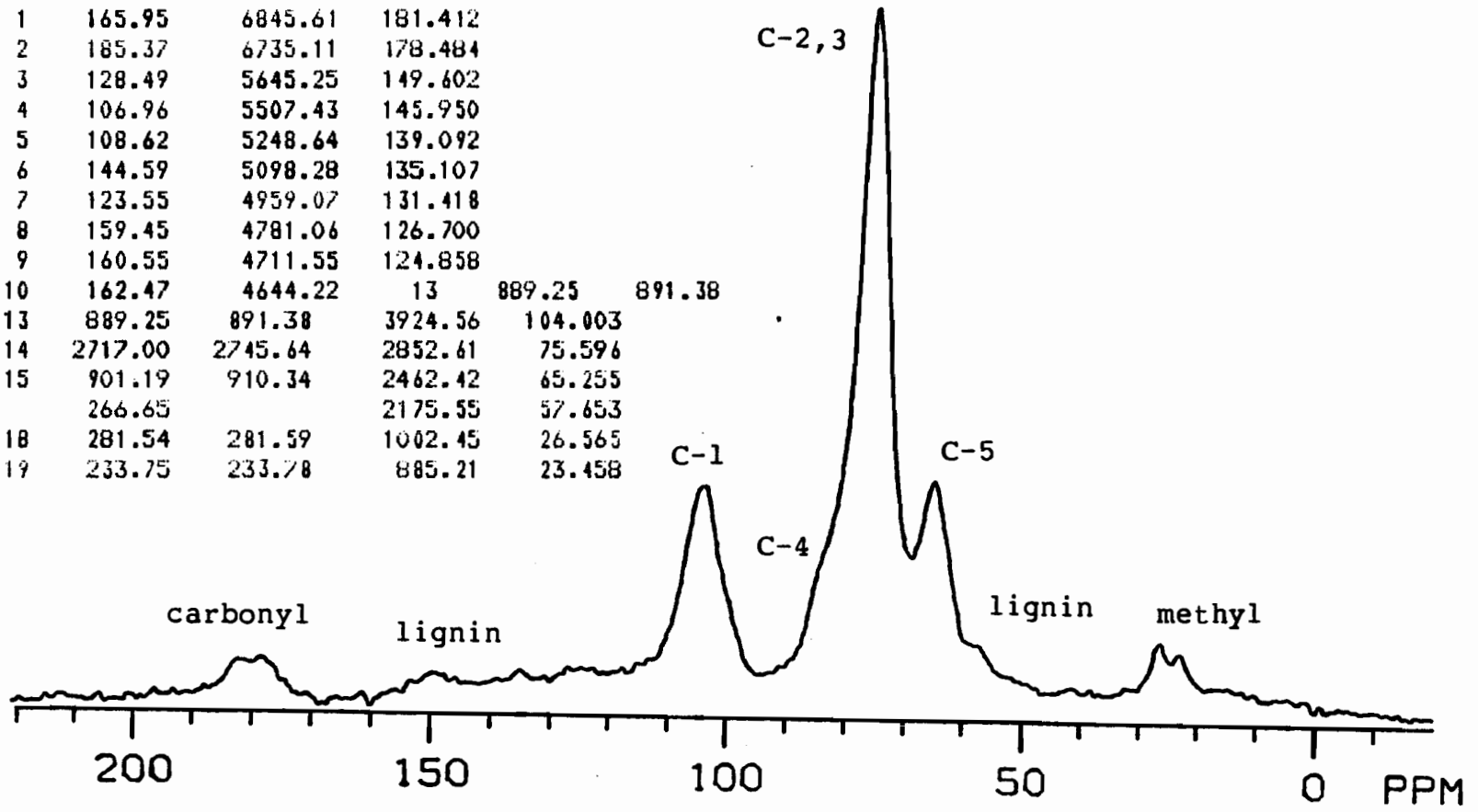

Figure 10. CP/MAS Carbon-13 NMR spectrum of hemicellulose A from the high yield solvent sulfite pulp.

LINE#	HEIGHT	HEIGHT(L)	FREQ(HZ)	PPM
1	125.05	125.06	5719.48	151.569
	59.21		6853.29	181.616
	97.32		6687.27	177.216
	52.39		6550.55	173.593
3	138.39	138.39	5671.26	150.292
4	152.58	152.87	5606.79	148.583
5	110.23	110.86	5481.03	145.250
6	108.73	109.51	5032.34	133.360
17	354.96	355.00	3883.28	102.909
18	294.57	294.87	3139.10	83.188
19	1162.10	1163.31	2816.11	74.628
20	422.67	422.77	2427.18	64.321
	398.73		2380.63	63.088
22	293.62	293.62	2136.65	56.622
25	122.65	122.73	966.63	25.616
26	117.41	117.58	840.72	22.279

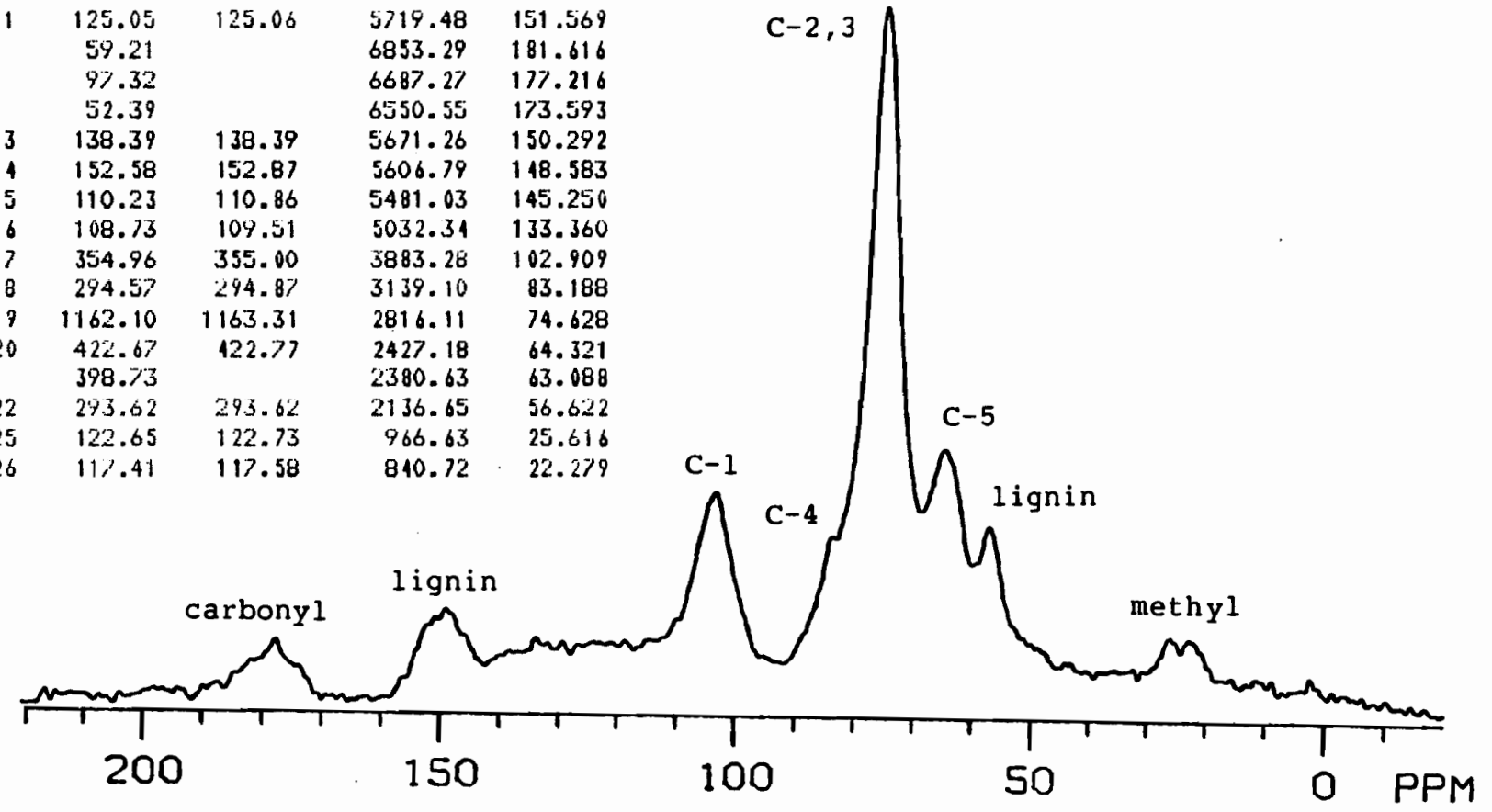

Figure 11. CP/MAS Carbon-13 NMR spectrum of hemicellulose A-1 from the high yield solvent sulfite pulp.

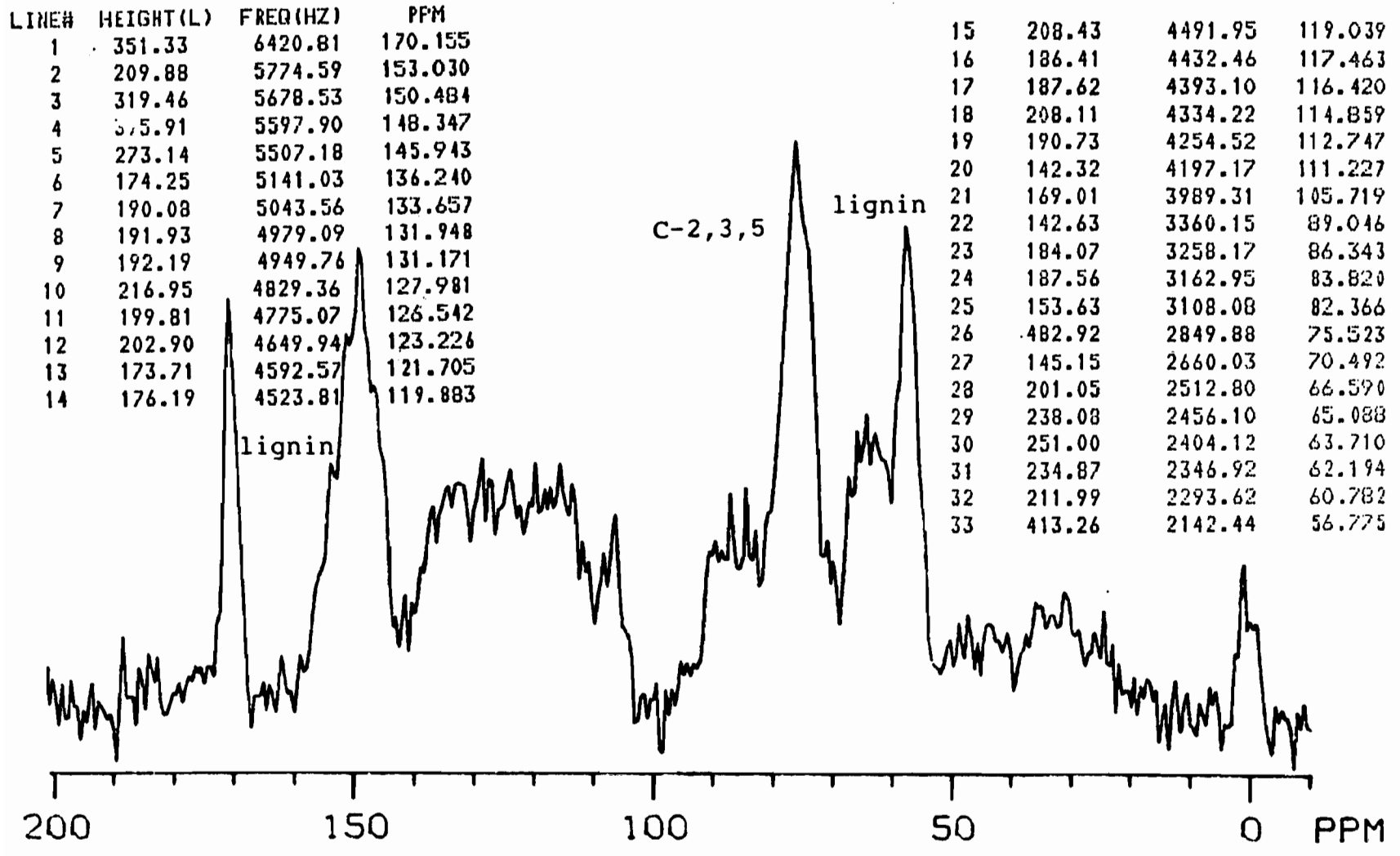

Figure 12. CP/MAS Carbon-13 NMR spectrum of hemicellulose B from the high yield solvent sulfite pulp.

LINE#	HEIGHT	HEIGHT(L)	FREQ(HZ)	PPM
1	480.00	480.01	3875.13	102.693
	65.32		5603.29	148.490
3	138.61	138.80	3294.76	87.313
	339.86		3152.11	83.533
5	459.05	459.06	3054.99	80.959
6	1194.20	1194.36	2773.15	73.490
7	403.82	403.85	2362.25	62.601
	345.84		2302.50	61.017

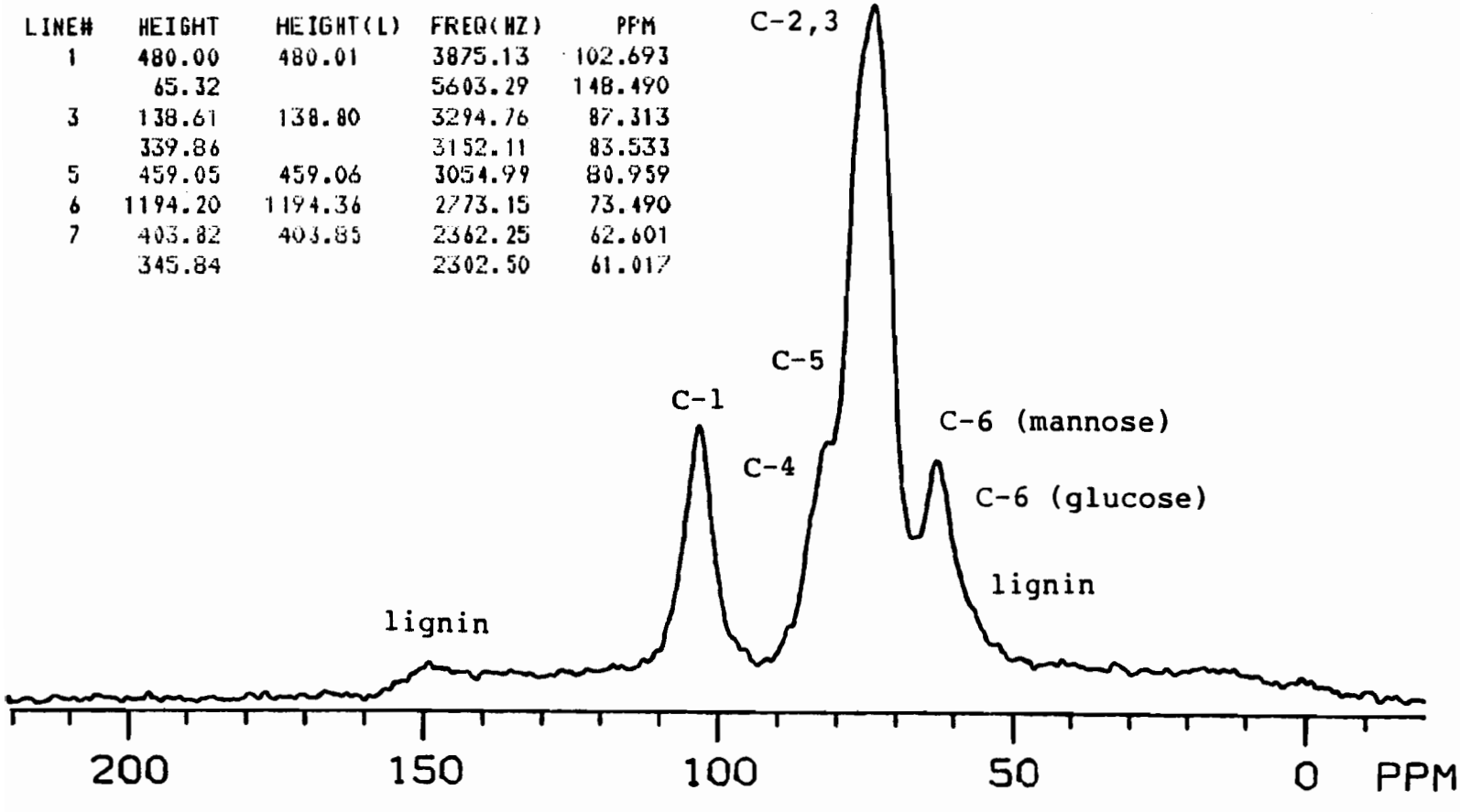

Figure 13. CP/MAS Carbon-13 NMR spectrum of hemicellulose C from the high yield solvent sulfite pulp.

LINE#	HEIGHT(L)	FREQ(HZ)	PPM
1	106.50	4233.54	112.191
2	118.11	4142.67	109.783
3	349.25	4014.41	106.384
4	118.82	3886.73	103.000
5	240.15	3357.10	88.965
6	200.29	3301.84	87.500
7	230.71	3197.98	84.748
8	200.73	3136.29	83.113
9	217.01	3078.87	81.591
10	913.82	2857.59	75.727
11	166.93	2606.94	69.085
12	175.12	2511.30	66.551
13	213.18	2448.99	64.899
14	232.64	2379.15	63.049
15	153.08	2319.44	61.466
16	112.39	2155.31	57.117
17	105.98	2105.83	55.805

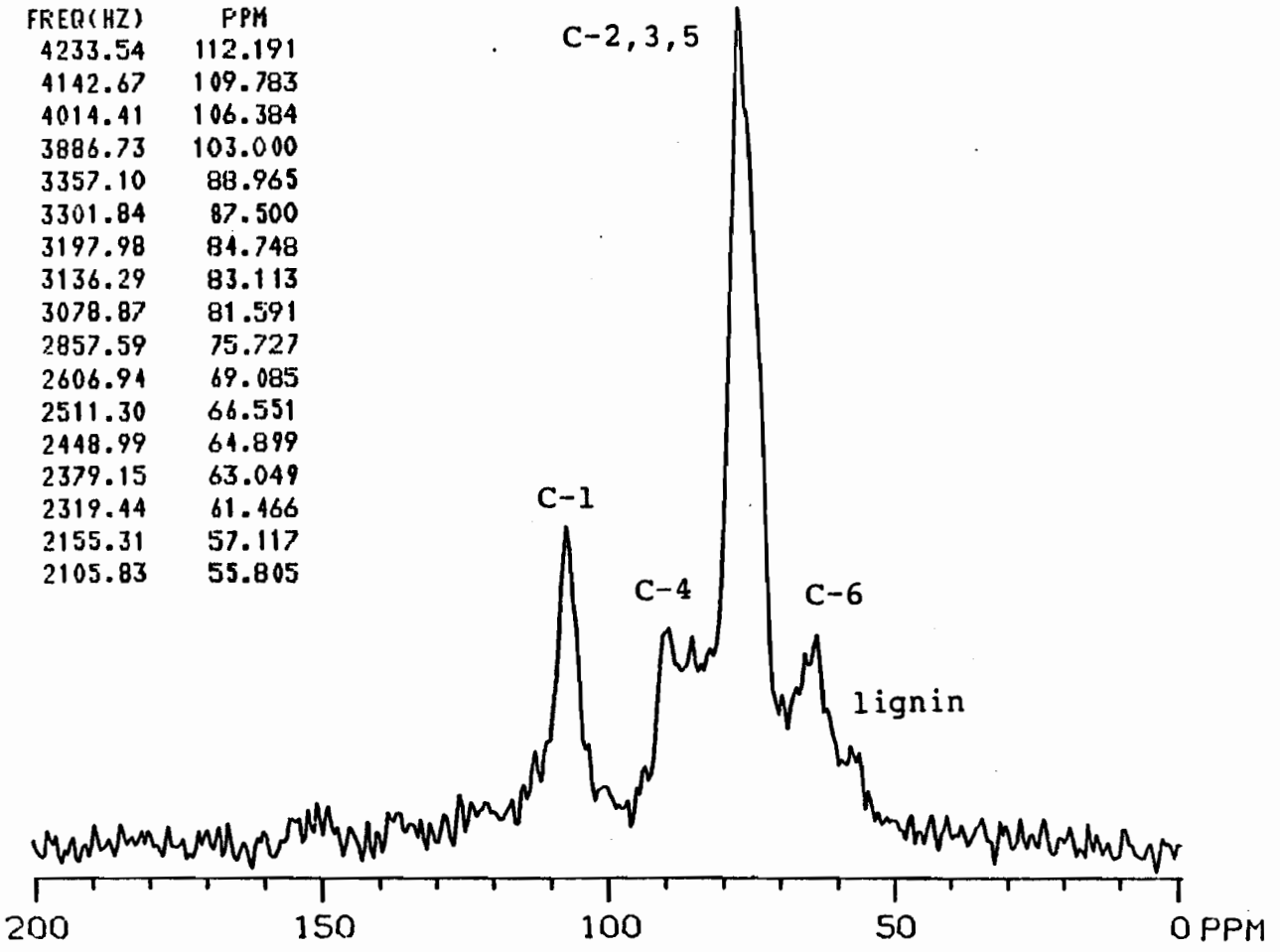

Figure 14. CP/MAS Carbon-13 NMR spectrum of residue C from the high yield solvent sulfite pulp.

research. Instead we see a single peak for C-1 and two for C-4. It is nevertheless clear enough to demonstrate the presence of at least two types of glycosidic linkages which means two types of polymorphs exist in the pulp sample.

The peak at 75.7 ppm and 73.3 ppm (also the one at 75.7 ppm in the spectrum of residue C) are assigned collectively to carbons 2, 3 and 5. The following peak at (66.2 ppm and also at 63.0 ppm in the case of residue C) is assigned to C-6.

From all the information given above, we can conclude that regardless of the type of monosaccharide they are linked in 1→4 as in cellulose and it is not as yet possible to tell unambiguously whether the linkage is α or β.

The spectra of hemicellulose A and A-1 (figures 10 and 11) are similiarly assigned. The difference between hemicellulose A and A-1 from the solvent sulfite pulp is the solubility in water. After the original hemicellulose A was extracted, it was redissolved in water and after centrifugation a layer of precipitation and a layer of light yellow solution appeared. Hemicellulose A was obtained by reprecipitating the light yellow solution with pure methanol while hemicellulose A-1 was the

material recovered from the precipitation. Hemicellulose A is definitely more soluble in water than is hemicellulose A-1. From the Carbon-13 NMR spectra of these two hemicelluloses, we observe that they are relatively the same except hemicellulose A-1 has a better separated C-4 peak according to our assignment. We conclude that hemicellulose A has a lower degree of polymerization which is more soluble in water and the C-4 peak from the reducing end and from the internal groups are so small compared to the nearby C-2,3 peak and is thus merged. Hemicellulose A-1 is less soluble in water and having a higher degree of polymerization the C-4 peak is starting to becoming significant and can be seen separately from the nearby C-2,3 peak. According to the results shown in previous sections in this thesis (table 1), only xylose exists in these two hemicelluloses. According to previous research, the peak at 65.3 ppm in hemicellulose A and the one at 64.3 ppm in hemicellulose A-1 are assigned to C-5 (26,28,41).

Although Hamilton and Thompson (32) showed that western hemlock sulfite pulp contained a xylan polyuronide, no evidence of the 4-O-methyl-D-glucuronic moiety was found by the chemical methods used in the present work. The CP/MAS spectra for hemicellulose A and A-1 (figures 10 & 11), however, showed a relatively

intense envelope of peaks at 181.4 and 178.5 ppm which Kolodziejski, Frye, and Maciel (39) assigned to carbonyl groups in their spectrum of a similar hemicellulose A isolated brom lodgepole pine wood. They commented that the carbonyl resonances came from carboxyl groupings of 4-O-methyl-D-glucuronic acid and from acetate groups. There were also peaks at 25.6 and 22.3 ppm (figures 10 and 11). Kolodziejski, Frye and Maciel (39) assigned similar resonances in their work to methyl groups of acetate moieties. Acetyl groups are common in wood substituents and the CP/MAS technique shows them. Although hemicelluloses A and A-1 in the present work were isolated by neutralization of the extraction medium with acetic acid (see chart 6), it is unlikely that the solid hemicelluloses contained any residual acetic acid which would account for the carbonyl peaks at 181.4 and 178.5 ppm and the methyl peaks at 60.9 and 66.3 ppm. The hemicelluloses were redissolved and reprecipitated a number of times and were considered quite pure. The resonance peaks from hemicelluloses A and A-1 undoubtedly came from 4-O-methyl-D-glucuronic acid moieties and acetate moieties in the xylan polysaccharides. Thus the CP/MAS technique is useful for indicating the presence of certain groupings in polysaccharides which are often very difficult to detect by chemical means. It is also to be noted that there

are no peaks around 63 or 66 ppm for a C-6. Thus solid state CP/MAS can differentiate between hexosan and pentosan polysaccharides.

Only three lignin peaks and a small peak at 75.5 ppm are seen in the spectrum of hemicellulose B (figure 12) which indicates the presence of lignin and probably a very small amount of saccharides. Efforts for further investigations were not made.

Hemicellulose C from the high yield solvent sulfite pulp is a glucomannan as shown in table 1. Hamilton, Kircher and Thompson (31) also isolated a glucomannan from western hemlock pulp by a similar procedure. The CP/MAS spectrum of hemicellulose C from the high yield solvent sulfite pulp (figure 13) can be interpreted very well on the basis of a glucomannan. The peaks at 148.5 and 61.0 ppm are characteristic of lignin. The peak at 102.7 ppm is assigned to C-1 in the linkage. Although the linkage is undoubtedly β-(1→4) as in cellulose (31), the resonance is downfield from the 105 ppm peak in cellulose. However, the 102.7 ppm peak matches the 103 ppm peak for C-1 of a glucomannan reported by Kolodziejski, Frye and Maciel (39) isolated from lodgepole pine wood. Overlapping peaks at 87.3 and 83.5 ppm are assigned to C-4 in the linkage. The glucomannan

is not crystalline enough to show the usual two peaks for C-4 in crystalline cellulose. A shoulder at 81.0 ppm is assigned to C-5 and a broad envelope from 73.5 to 80.0 ppm is assigned to the C-2 and C-3 carbons. A peak at 62.6 ppm is assigned to C-6 of glucose (28,69). Thus when the resolution is good enough mannose monomers can be differentiated from glucose monomers so that CP/MAS spectra do give some indication of which monosaccharides are present in polysaccharides. The spectrum of hemicellulose C (figure 13) shows no peaks around 180 ppm for carbonyl groups or at 22 ppm for methyl groups. Although glucomannan hemicelluloses from softwoods are known to contain acetate groups, apparently the solvent sulfite pulping conditions hydrolyzed them away. Kolodziejski, Frye and Maciel (39) reported peaks for carbonyl and methyl groups in their glucomannan (hemicellulose B) from lodgepole pine wood but their hemicellulose B was isolated by direct alkali extraction of a holocellulose. Their materials were never subjected to the acid conditions of sulfite pulping so the acetate groups apparently survived.

We can conclude that the hemicelluloses, according to the available information, are linked in the form of 1→4 regardless of the type, and none of them possessed significant degrees of crystalline polymorphs.

Although it is not with absolute certainty that all of the assignments are correct, we have reasons to believe they are generally true. More standards need to be analyzed before precise assignments can be made. Based on the limited source, we are pleased to get all the possible information out of it in understanding the pulps and their fractions.

V. SUMMARY AND CONCLUSIONS

1. A thermomechanical pulp (TMP), a high yield sulfite pulp and a high yield solvent sulfite pulp prepared from western hemlock were investigated in detail. They were found to be diverse both in chemical composition and in physical properties, and the high yield solvent sulfite pulp appeared superior to the other two in most of the comparisons.

2. The pulps were acid hydrolyzed and analyzed for monosaccharids residues by HPLC. The TMP was composed of glucose 50.1%, mannose 15.0%, and xylose 3.9%. The high yield sulfite pulp was composed of glucose 54.8%, mannose 1.2%, and xylose 0.9%. The high yield solvent sulfite pulp was composed of glucose 74.0%, mannose 3.2%, and a trace of xylose. Therefore, it was concluded that the high yield solvent sulfite pulp was a higher content cellulose pulp than the other two.

3. The TMP analyzed for 28.9% Klason lignin, the high yield sulfite pulp analyzed for 29.2% Klason lignin, and the high yield solvent sulfite pulp analyzed for

17.6% Klason lignin. Therefore, it was concluded that the high yield solvent sulfite pulping conditions were superior for removing lignin from wood.

4. A hemicellulose rich in xylose residues was isolated from each of the pulps. A second hemicellulose rich in mannose and glucose was also isolated from each of the pulps. These hemicelluloses were not isolated in high yields because of interference from the lignin still remaining in the pulps. However, sufficient quantities were obtained to characterize them as a xylan and a glucomannan.

5. Solid state CP/MAS carbon-13 NMR spectra were obtained on three known disaccharides , cellobiose, maltose, and gentiobiose. These spectra showed good resolution and all of the peaks were assigned.

6. The solid state CP/MAS carbon-13 NMR spectra of the known disaccharides showed good separation of the C-1' anomeric carbons involved in the linkage, and the C-1 anomeric carbons of the reducing end. Unfortunately the α and β forms of these anomeric carbons were not well separated. Therefore, it appears unlikely that whether the linkages are α

or β in polysaccharides can be determined by the solid state CP/MAS technique. However, the positions of attachment of the glycosidic linkages (1→4 or 1→6) can be readily ascertained.

7. Since the anomeric C-1' carbon in the β-(1→4) linkage of cellobiose resonated at 105.5 ppm, and the anomeric C-1' carbon in the β-(1→6) linkage of gentiobiose resonated at 100.9 ppm, it was concluded that the more restricted the space and mobility of the anomeric carbon atoms the more downfield they resonated. Thus the anomeric carbons in the glycosidic linkages almost always appeared downfield from any of the other carbons in the carbohydrates.

8. Solid state CP/MAS carbon-13 NMR spectra were obtained on the high yield solvent sulfite pulp and on hemicelluloses A and A-1 (xylans), hemicellulose B (mostly a lignin fraction), hemicellulose C (a glucomannan), and on residue C from the high yield solvent sulfite pulp.

9. The solid state CP/MAS carbon-13 NMR spectra of the high yield solvent sulfite pulp and residue C from the pulp showed that these materials contained cellulose, and that the cellulose was composed, in

part, of at least two crystalline polymorphs.

10. The solid state CP/MAS carbon-13 NMR spectra of hemicellulose A and A-1 showed that the polysaccharides were amorphous and not crystalline. The spectra also showed that the hemicelluloses were composed primarily of pentoses linked 1→4 with no resonance peak for C-6 of the hexoses. There were also peaks for carbonyl groups and methyl groups and it was concluded that these peaks indicated the presence of 4-O-methyl-D-glucuronic acid side chains and acetate groups because these moieties are known to be a part of similar hemicelluloses isolated from wood.

11. The solid state CP/MAS carbon-13 NMR spectrum of hemicellulose C showed that the polysaccharide was amorphous and not crystalline. The spectrum also showed 1→4 linkages of hexoses. It was concluded that the hexoses were mannose and glucose residues.

12. It was concluded that the solid state CP/MAS carbon-13 NMR technique established the positions of the glycosidic linkages, whether the polysaccharides were pentosans or hexosans, and if they contained any 4-O-methyl-D-glucuronic acid or acetate substituents.

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