ESTIMATION OF HARDWOOD LIGNIN CONCENTRATIONS BY UV SPECTROSCOPY AND CHLORINE DEMETHYLATION

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Major projects are underway in our laboratory focusing on mildly acidic (pH>3) and alkaline (pH<10) pretreatments of hardwood chips prior to incineration for electric power or prior to pulping for paper manufacture. Production of lignocellulosic ethanol from the hemicelluloses in the hydrolyzates will be attempted. It is of great interest to quantify the concentrations of lignin in these hydrolyzates, since lignin fragments are suspected as fermentation inhibitors. UV spectroscopy is normally used to estimate the concentration of aqueous soluble lignin. However, the 203 nm absorbance gave unreliable results for these hydrolyzates, and on some occasions the 278 nm absorbance was unduly influenced by high absorbance in the 260-265 nm range. A credible method that uses chlorination to generate methanol from the methoxyl groups in lignin will be described. Model compound experiments showed that syringyl lignin units, with two methoxyl groups, gave a methanol yield of ~1.0 mmole/ mmole of aromatic rings.

Keywords: Chlorination, Lignin, Guaiacyl units, Syringyl units, Methanol, Methoxyl groups,

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INTRODUCTION

In the first of two projects presently underway in our laboratory, mildly acidic solutions (acidolysis or A-stage) are been used to extract some of the hemicelluloses from hardwood chips prior to combustion or pulping. Approximately 70% of the hemicelluloses dissolve in the cooking liquor during kraft or soda/anthraquinone pulping and consume a significant fraction of the alkali in the process of being degraded to low molecular weight compounds. It would be technically difficult to remove these low molecular weight compounds from alkaline pulping effluents and purify their product streams to a degree adequate enough for use as commodity chemicals. Native hemicelluloses in wood or their degradation products in alkaline pulping effluents have high oxygen to carbon ratio and relatively low calorific values during combustion. A superior route would be to pre-extract the hemicelluloses and further hydrolyze them to monomers. Fermentation can then be used to convert the monomers to ethanol. Since lignin sub-structures are known fermentation inhibitors (Parajo et al. 1996; Jonsson et al. 1998; Cantarella et al. 2004), it would be helpful if the A-stage could be optimized to minimize the amount of lignin in the hydrolyzate (A-stage effluent).

The second project involves a pre-cooking stage with Na_2CO_3 before kraft (NaOH and Na_2S) or soda/anthraquinone (SAQ) pulping. Hooper (1932) reported on the ability of this pretreatment (C-stage) to subsequently decrease the alkali demand in straight soda (NaOH only) and kraft pulping. After a C-stage we are observing improvements in the SAQ process that are much more significant than for the kraft process. The C-stage significantly improves selectivity (pulp yield at a given residual lignin content) for the SAQ but not the kraft process. It is of great interest to discover if lignin or carbohydrate reactions in the C-stage are primarily responsible for these improvements. The ability to estimate the quantity of lignin solubilized in the C-stage is obviously critical. It should also be noted that mildly alkaline solutions can be used to extract hemicelluloses (Carpita 1984; Matulewicz and Cerezo 1987) instead of the acidic process above.

The quantification of acid insoluble or Klason lignin is a well-established and widely used procedure in the wood chemistry community (TAPPI Method T222 om-88, 1988). However, quantification of soluble lignin has been much more unreliable and controversial. Lignin determination methods have been reviewed fairly recently (Hatfield and Fukushima 2005; Dence 1992), with much of the discussion focusing on the weakness or inconvenience associated with almost all of them. The more common techniques can be divided into four categories; 1) UV spectroscopy, 2) chemical modifications combined with UV/visible spectroscopy, 3) NMR spectroscopy, and 4) chemical consumption during exhaustive oxidation.

In the present investigation unreliable results were being obtained from the most widely used of the UV spectroscopic methods, i.e. lignin dissolved in 3 wt. % sulfuric acid (Tappi Method UM 250, 1985). A wide range of the other UV methods (some including solvent/water mixtures), NMR spectroscopy and oxidant consumption methods were preliminarily investigated and deemed to be unreliable or overly complicated.

This report considers the use of chlorination to estimate the lignin content of hydrolyzates by quantifying the amount of methanol generated from the methoxyl groups by the proposed mechanism in Fig. 1 (Gierer and Sundholm 1971). Time and resources were invested in this technique because it gave credible results for softwood lignin (Ni et al. 1990) and it was known that the lignin in A-stage effluents (pH>3.0) from softwoods (Casebier et al. 1969) and hardwoods (Casebier et al. 1973) were rich in methoxyl groups.

MATERIALS AND METHODS

Lignin Model Compounds (LMCs)

The chemical structures of the LMCs used are shown in Figure 2. Compounds **I, II, IV,** and **VI** were obtained from Aldrich Chemical Company while **III** and **V** were obtained from Fluka Chemical Corp.

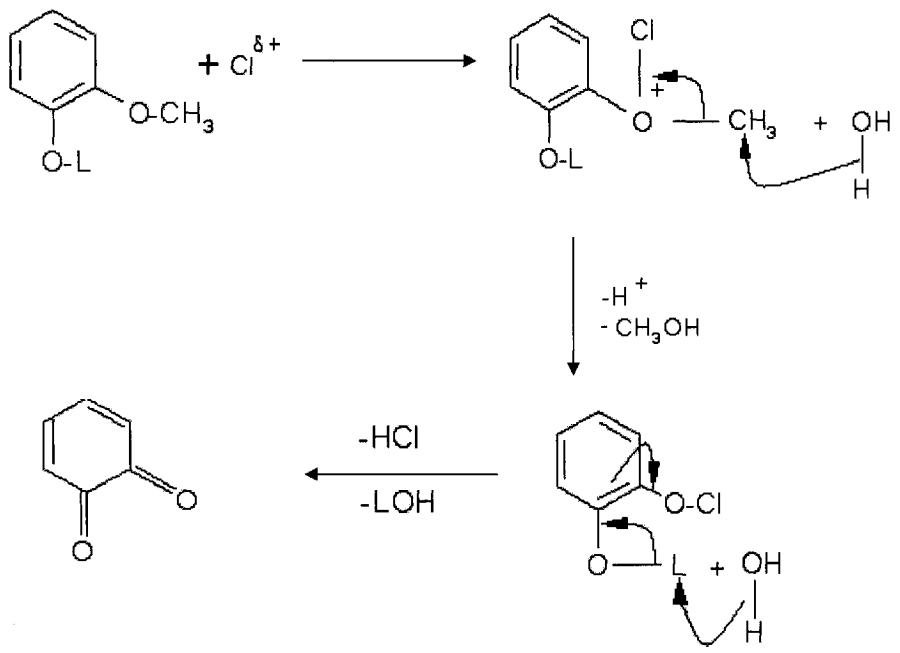
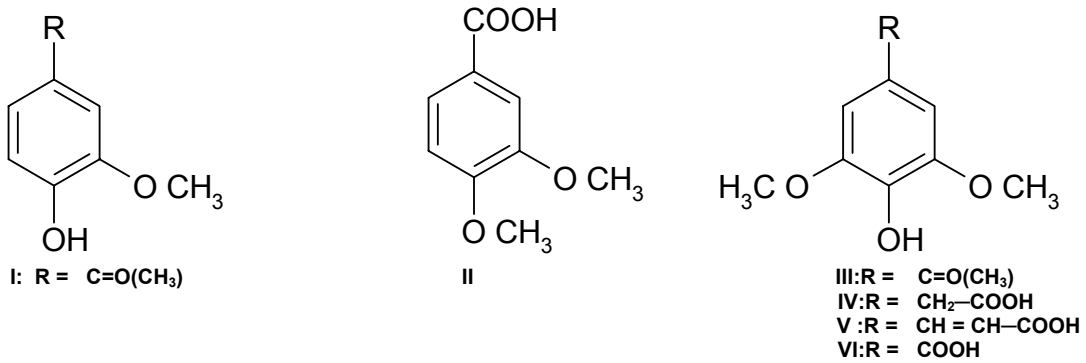


Fig. 1. Proposed mechanism for methanol generation during lignin chlorination.



I: R = C=O(CH$_3$) **II** **III:R = C=O(CH$_3$)** **IV:R = CH$_2$—COOH** **V :R = CH = CH—COOH** **VI:R = COOH**

Fig. 2. Lignin model compounds (LMCs) used in this investigation

Chlorination of Lignin Model Compounds

One mmole of each LMC was dissolved in 2ml of dioxane then diluted to 25ml with warm water. One-tenth of this solution was added to 4ml of 4M H_2SO_4 in a polyethylene bottle, and then 5 ml of household bleach (6% NaOCl) was added. The bottle was sealed, mixed, and placed in a 60°C water-bath for at least 2h. At the end of the treatment, 1ml of a solution containing 150 g/l of both $NaHCO_3$ and Na_2SO_3 was added, as well as 1ml of a solution containing the internal standard (1.6 g/l ethanol in H_2O).

Chlorination of Lignin-Containing Solutions

Hydrolyzates were adjusted to pH~9 and filtered through a 1.2µm Versapor porous disk (Pall Life Sciences) with the aid of a slight pressure difference. A volume of the filtrate (2.0 – 5.0 ml) was then taken and added to 4ml of 4M H_2SO_4. The chlorination treatment was identical to that used for the LMCs. This procedure is a slight modification to that of Ni et al. (1990) who used an aqueous solution of chlorine directly.

Acid and Carbonate Pre-extraction Stages

Sugar maple *(Acer saccharum)* chips were treated at a 4:1 liquor to wood (L:W) ratio with water or dilute acetic acid solutions at 120°C, 150°C, and 165°C. The time to temperature was 30 minutes for 120°C and 150°C, and 60 minutes for 165°C. The acetic acid dose was 3.0%, 1.5%, and 0% respectively for the 3 temperatures. The pH of all of the A-stage hydrolyzates that were collected was in the range of 3 to 4. A-stages were also applied to 20-mesh woodmeal from 13 poplars after they were extracted with ethanol-toluene in accordance with TAPPI method T204 om-88 (1988). Woodmeals were placed in 100ml autoclaves at a L:W≥20. Several autoclaves were placed simultaneously in an M&K digester with recirculating hot water. The first condition was 1h to 175°C and 45 min at that temperature. The extracting solution was comprised of 20% ethanol (v/v) and 0.01M H_2SO_4. The second condition was 30 minutes to 150°C and 1h at that temperature with only water as the extracting fluid.

A chip mixture comprised of ~60% of an eastern cottonwood (*Populus deltoides*) clone, ~20% white birch (*Betula papyrifera*), and ~20% sugar maple was used for the carbonate pretreatment. The Na_2CO_3 dose was 7.7% on chips, the L:W ratio was 4:1, and 60 minutes to 165°C was used. Once165°C was attained hydrolyzates were collected after 15, 30, and 60 minutes.

UV and Gas Chromatographic Analyses

The methanol in the reaction products was analyzed on a HP5890 gas chromatograph with flame ionization detection. A 1.83m long Tenax TA 80/100 packed column was used with a column head pressure of ~120kPa. A Perkin Elmer Lambda 650 UV/Vis spectrometer was used to obtain UV spectra of lignin-containing solutions. The filtered solutions above (pH~9) were diluted by a factor of at least 100 and adjusted to 3 wt. % H_2SO_4 before analysis.

RESULTS AND DISCUSSION

Thirteen poplars were acidolyzed at 150°C and 175°C to see if there was any correlation between syringyl to guaiacyl (S:G) ratio and acid soluble lignin. The S:G ratios of the 13 poplar woodmeals were known beforehand. The ratio was determined by a previously described method that involved both nitrobenzene and potassium permanganate oxidations (Francis et al. 2005). UV spectra with well defined peaks at 203 nm and 278 nm were obtained for 10 of the 13 A-stage effluents. However, three of the effluents did not show a peak at 278nm due to higher than normal absorbance in the 260-265 nm range. A possible explanation is that those samples had higher than normal

generation rates of furfural and/or hydroxymethylfurfural (Dence 1992). However, there may be other explanations. The spectra for 150°C and 175°C effluents (different dilution factors) for one of the three poplars are shown in Figure 3. If the 260-265 nm absorbance were primarily due to furfurals and the 278 nm to lignin, then it would be highly coincidental that the 260 nm to 278 nm absorbance ratio was nearly identical for treatments at 150°C (without ethanol) and 175°C (with ethanol).

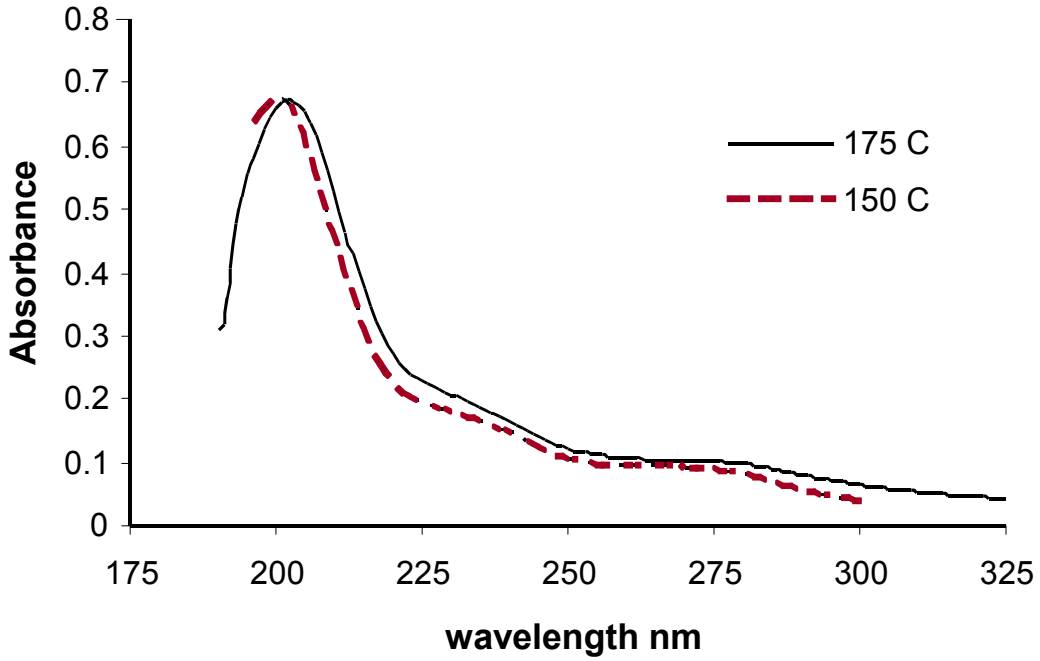


Fig. 3. UV spectra without a well defined 278 nm peak for acid hydrolyzates (150°C and 175°C) from a specific hybrid poplar.

The 203 nm absorbance readings were very erratic for these effluents, particularly when the acidolysis temperature was 175°C. Several diluted effluent samples were further diluted 1:1 with 3% H_2SO_4. Typically the 278 nm absorbance decreased by 50 ± 3%. However, the 203 nm absorbance would decrease anywhere from 10-80%. An excellent example of this is shown in Fig. 4 for acidolysis of sugar maple chips at 165°C. Effluents were collected at 15, 30 and 60 min and diluted in exactly the same manner to a final dilution factor of 800. The 278 nm absorbance increased in a logical manner from 0.106 to 0.160 to 0.217. However, the corresponding 203 nm absorbance were 1.04, 1.34, and 1.07.

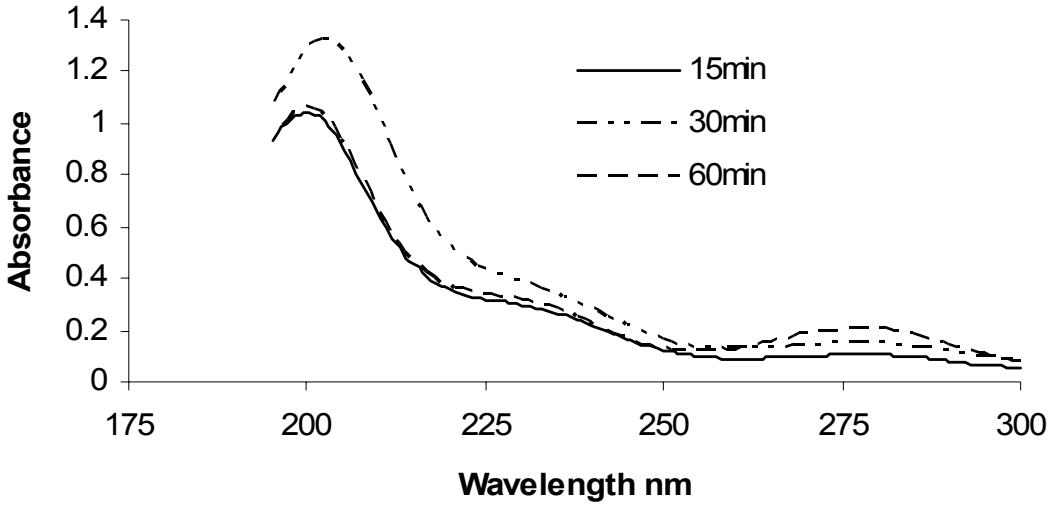


Fig. 4. UV spectra of acid hydrolyzates from sugar maple collected after 15, 30 and 60 minutes at 165°C. The dilution factor was 800 for all three samples.

It appears as if acidolysis temperature is a significant variable in the erratic nature of the 203 nm absorbance. In a simultaneous investigation, the leaching of sugar maple lignin into 0.1M NaOH at room temperature has been investigated. Close to classical UV lignin spectra were obtained despite the alkaline conditions. It can be seen in Figure 5 that the ratio of 203 nm to 278 nm absorbance was fairly constant at ~6.5. Similarly, acid soluble lignin from the Klason method (TAPPI Method T 222 om-88, 1988; TAPPI Method UM 250, 1985) was analyzed for 4 of the poplars. The 203 nm absorbance is also plotted against the 278 nm absorbance in Fig. 5 after dilution by factors of 15 and 30.

It can be seen that the ratio was very similar to that of the alkali-leached lignin; a slope of 6.2 was obtained with an R^2 value of 0.992. In the Klason test the slurry is refluxed in 3% H_2SO_4 at a low temperature of ~100°C. The data for most of the 150°C poplar effluents are also shown in Fig. 5 at various levels of dilution. It can be seen that the ratio of 203 nm to 278 nm absorbance was approaching that of the earlier two cases. However, for a 278 nm absorbance of 0.048, the 203 nm absorbance varied from 0.30 to ~0.55. Dilution experiments with selected samples indicated that most of the error was in the 203 nm absorbance.

UV spectroscopy has some clear drawbacks when used to estimate the concentration of some aqueous soluble lignin and other analytical techniques were investigated.

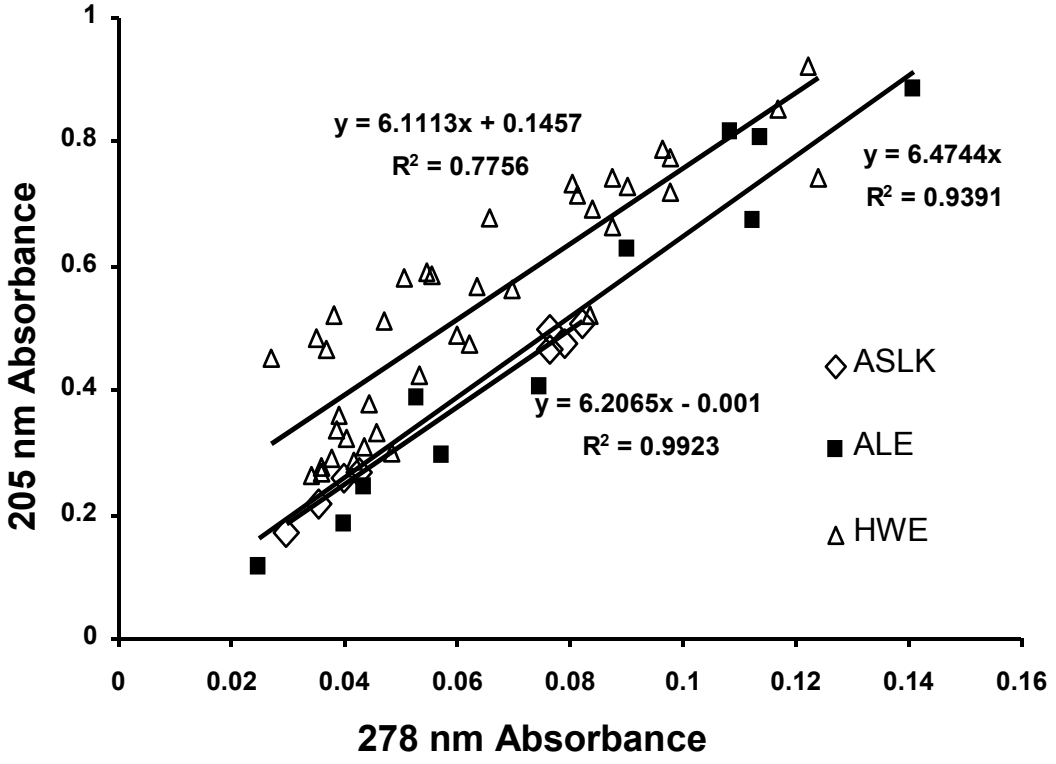


Fig. 5. Ratio of 203 nm to 278 nm absorbance for acid-soluble lignin from Klason test (ASLK), alkaline leachate at room temperature (ALE), and hot water extraction of poplar woodmeals at 150°C (HWE).

Preliminary Investigation of Other Analytical Techniques

Some of the more credible methods discussed by Dence (1992) were attempted. The first alternative UV method to be investigated was a solvent comprised of phosphoric acid and water (8:2 v/v) (Bethge et al. 1952; Francis and Reeve 1987). This solvent appeared to give higher absorbance in the 260-265 nm region of the spectrum. In another approach, A-stage effluents were further acidified then extracted with dichloromethane. The dried extracts were dissolved in 2-methoxyethanol/water (8:2 v/v) and 95% ethanol and analyzed by UV spectroscopy (Lin 1992). The two major problems observed with 3% H_2SO_4 were still apparent, i.e. absence of a sharp 278nm peak in some samples and erratic readings for the 203 nm peak.

Proton NMR is used in our laboratory for quantification of carbohydrates in hydrolyzates (Kiemle et al. 2004; Francis et al. 2006). Signature peaks for lignin were observed in both A- and C-stage effluents. However, the clearly identifiable lignin peaks were different for A- and C-stage effluents. Also, under both acid and alkaline conditions the quality of the spectra (peak resolution and baseline) varied significantly with treatment time. The methoxyl protons (~3.8 ppm) were observed in all spectra. This method maybe effective if the lignin is extracted from the hydrolyzates and acetylated

prior to NMR analysis. Sample preparation for such an approach can be found in the literature (Lundquist 1992).

The kappa number was the first and last of the oxidant consumption methods to be investigated. The only change to the method (Tappi T 236 cm-85, 1985) was replacing fibers with effluent. In one of early trials 25ml of a particular effluent consumed 25.1ml of 0.1N $KMnO_4$, but when the effluent volume was decreased to 20ml it consumed 24.3 ml of the permanganate.

At this stage it was decided that a method with an established and specific reaction would be the next one investigated, and chlorine demethylation was selected.

Methanol Yields from Syringyl Units

An excellent review on dealkylation of lignin by chlorine is in the literature (Dence, 1996). That publication referred to the high methanol yields from guaiacyl (G) LMCs (Gierer and Sundholm 1971) and the residual lignin in softwood kraft pulps (Ni et al. 1990). However, the Dence review made no mention of quantitative data for substrates such as hardwood lignin that contain significant amounts of syringyl (S) units, and we have been unable to find any such data published since 1996.

Methanol yield was quantified for the LMCs in Fig. 2, and the results are documented in Table 1. It can be seen that the methanol yield for the 4 syringyl monomers averaged ~1.0 mmole/mmole of aromatic rings. Repeat experiments were performed for three of the LMCs at a later date and those results are in parentheses in Table 1. The method appears to be decently reproducible. From the present data for two guaiacyl monomers, coupled with data from the literature (Gierer and Sundholm 1971; Ni et al. 1990), we estimated a methanol yield of ~0.8 mmole/mmole of phenylpropane (C_9) units in the guaiacyl fraction of lignin. The present yield of 0.7 for **II** is almost equal to the 0.67 obtained Gierer and Sundholm (1971) for 1, 2-dimethoxybenzene (veratrole). A decent estimate of methanol yield from chlorination of hardwoods would be 0.9-1.0 mmole/mmole of aromatic rings, since most hardwoods appear to contain more S than G units. Ten of the thirteen poplars involved in this investigation had S:G ratios ≥ 1.2.

Table 1. Methanol Yield from LMCs

Compound	Methanol Yield[1]
I	0.8
II	0.7
III	0.9 (0.8)[2]
IV	1.1
V	1.2 (1.1)
VI	0.8 (1.0)

[1] mmoles/mmole of aromatic rings [2] Experiments performed at a later date

The effect of substituents on methanol yield is beyond the scope of the present investigation. Our hope was that the methanol yield for S units would be close to that obtained for G units (~1.0 mmole/mmole) and this was indeed observed. If that were not the case, then the S:G ratio would have to be involved in the calculations correlating methanol yield to lignin content.

An average methanol yield of 1.0 mmole/mmole of aromatic rings along with an average molecular weight (MW) of 210 for the C_9 units in hardwood lignin will be used

in our calculations. A wide range of literature data was examined, and it appears as if the structural formula for various native hardwood lignins is fairly constant, except for the number of methoxyl groups. The average formula is close to $C_9H_{8.7}O_{2.9}(OCH_3)_x$ with x varying from 1.30 to 1.57 (Gellerstedt et al. 1988, Fengel and Wegener 1989; Dence and Lin 1992). If x = 1.50 is assumed, then a MW of 210 is obtained. It should also be noted that varying x from 1.30 to 1.57 only changes the C_9 MW by 8.4 units.

Lignin Content of Hydrolyzates

The number of mmoles of methanol in a given volume of hydrolyzate was obtained by GC. This value was multiplied by 210 to give mg of lignin. All of the A- and C-stages conducted with chips used 4 liters of liquor/kg of wood (oven dried or OD basis). Typically 5ml of hydrolyzate was chlorinated and that corresponded to 1,250mg of chips at the beginning of the process.

A 1-cm quartz cuvette was used for UV analysis on all occasions. Therefore, lignin concentration in g/l can be obtained by simply dividing the absorbance by the absorptivity in accordance with equation [1] (Dence 1992). The value obtained is then multiplied by the dilution factor to arrive at the lignin concentration in the hydrolyzate. One liter of solution would have treated 250g of chips.

$$A = \epsilon \ell C \qquad [1]$$

where A = absorbance; ϵ = absorptivity in $lg^{-1}cm^{-1}$; ℓ = light path in cm; C = lignin concentration in g/l

Many prior investigations have concluded that the absorptivity of native softwood and hardwood lignin is ~20 $lg^{-1}cm^{-1}$ at 278 nm (Johnson et al. 1961; Sjöström and Enström 1966; Marton 1967; Hardell et al. 1980; Iiyana and Wallis 1988). This value was used in our calculations, and an assumed value of 124 $lg^{-1}cm^{-1}$ (20 x 6.2, Fig. 5) was used for 203 nm. The chlorination method is compared to the UV method (203 nm & 278 nm) for several hydrolyzates in Table 2.

It can be seen that the chlorination method compared favorably with the 278 nm absorbance results. Therefore, for samples where the 278 nm absorbance appears to be unduly influenced by chromophores absorbing in the 260-265 nm or 300-310 nm range, chlorination can be used. The 203 nm absorbance should be used with great care on all occasions. Once again decent reproducibility was obtained.

Table 2. Comparison of Lignin Content of Hydrolyzates by Chlorination and UV Spectroscopy

Hydrolyzate	Chlorination	278nm	205nm
A-stage (Maple chips)	1.4[1] 2.3 (2.6)[2] 3.1	1.7 2.6 3.5	2.7 3.5 2.8
A-stage (Poplar,150°C)	1.4 (1.2)	1.4	1.7
C-stage (MH chips[3])	0.4 (0.4) 2.2	0.4 1.8	0.4 1.5
[1]% on chips or wood meal [2] Repeat experiment at a later date			
[3]MH = mixed hardwoods			

Wood chips were subjected to A- and C-stage treatments, and the chlorination method was used to estimate lignin content of the hydrolyzates (Fig. 6). The value was 0.3% lignin on chips after 60 minutes at 120°C; 0.7% on chips after 20 minutes at 150°C; and 1.4% on chips after 15 minutes at 165°C. These results appear to be internally consistent, based on the temperature effect normally observed in wood depolymerization. The results for the C-stage hydrolyzate were somewhat unusual. After a one-hour heat up time and 30 minutes at 165°C, the lignin content in the hydrolyzate was only 0.4%. This chlorination analysis was repeated, and a nearly identical result was obtained. Also, the UV estimate (278 nm) was in the range of 0.4% on chips for several dilutions. However, the lignin content then increased to 2.2% on chips with 30 minutes of additional retention. A credible explanation is that atmospheric O_2 in the digester at the start of the treatment oxidized and degraded lignin during the early stages. On no occasion did we purge the digester at the start of a C-stage. The pH of the hydrolyzate was 9.6 when 165°C was attained (0 minutes at temperature) and 8.4 after 30 minutes. In that pH range one would expect oxidation by O_2, catalyzed by the carbonate radical (HCO_3•), as reported by Mih and Thompson (1983). The explanation for the significant increase in lignin content after 30 minutes would be the complete consumption of the O_2 in lignin and other oxidation reactions.

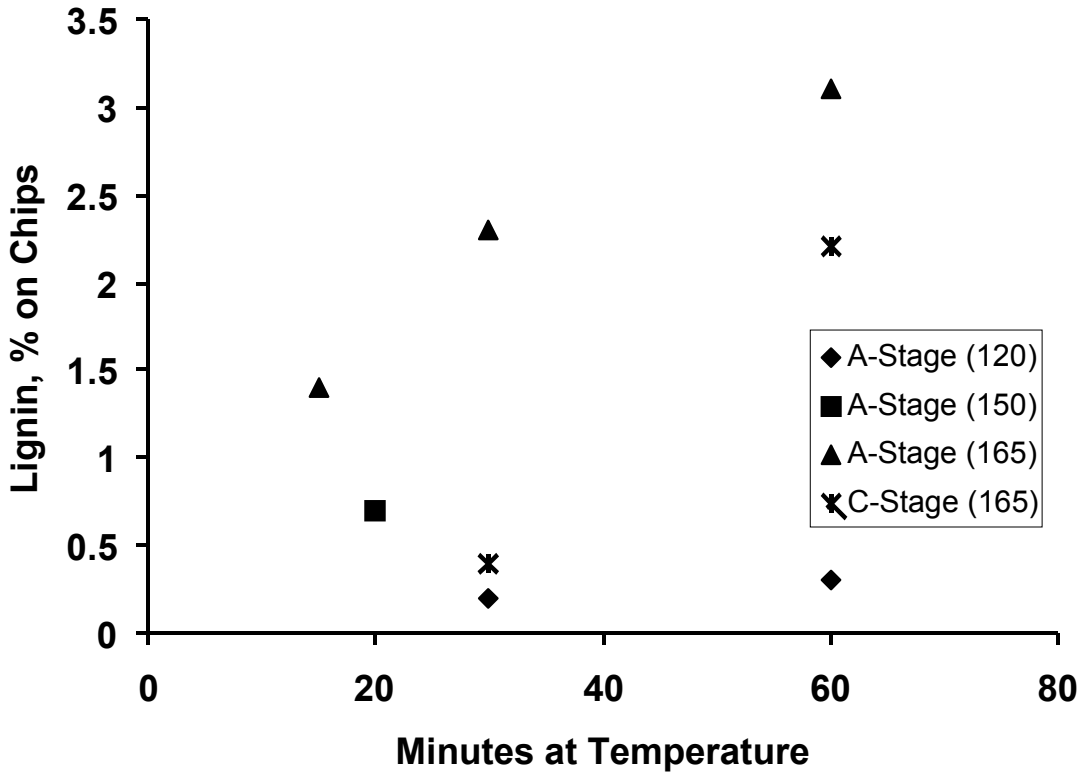


Fig. 6. Lignin content of A-stage and C-stage hydrolyzates determined by chlorination

CONCLUSIONS

In the process of investigating mild acid and alkaline pretreatments of hardwoods we found the UV method of quantifying lignin in the hydrolyzates to be unreliable. In some cases a well-defined 278 nm peak was not obtained, and in many cases the 203nm peak did not afford a credible result. Preliminary investigations were conducted with different solvents for UV spectroscopy, NMR spectroscopy, and quantification of oxidant consumption per ml of effluent (kappa number). These methods were either inconvenient or unreliable. A method based on methanol formation from methoxyl groups during lignin chlorination was investigated for hardwoods. Syringyl model compounds were investigated and they averaged ~1.0 mmole of methanol/mmole of aromatic rings. When the chlorination method was used to analyze both acid and alkaline hydrolyzates, the results obtained all fell in the expected ranges and were always close to the estimate based on the 278 nm UV absorbance, i.e. when the 278nm peak was not unduly influenced by chromophores absorbing in either the 260-265nm or 300-310nm range. Based on several repeat experiments, an error of plus or minus 10% (maximum) is estimated for the chlorination method. However, enough data were not collected for a detailed statistical evaluation. It should be noted that a 10% error is quite reasonable for these samples. Even larger errors were reported by Hatfield and Fukushima (2005) for determinations using some of the more conventional methods and involving larger masses of lignin.

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