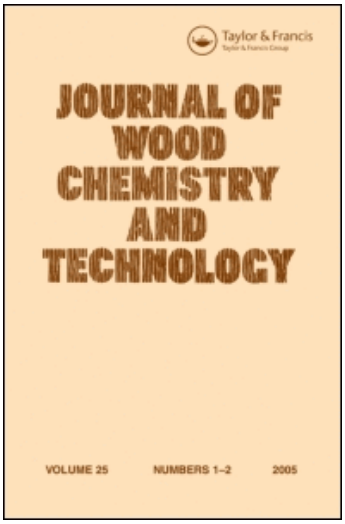

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

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To cite this Article Kaar, William E. and Brink, David L.(1991) 'Simplified Analysis of Acid Soluble Lignin', Journal of Wood Chemistry and Technology, 11: 4, 465 — 477
To link to this Article: DOI: 10.1080/02773819108051087
URL: http://dx.doi.org/10.1080/02773819108051087

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY, 11(4), 465-477 (1991)

SIMPLIFIED ANALYSIS OF ACID SOLUBLE LIGNIN

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ABSTRACT

The paper describes a novel method for the determination of ultraviolet (UV) light absorptivities for acid soluble lignin (ASL). It was found that the rehydrolysis, or reKlasonation, of Klason lignin continues to release a quantifiable amount of ASL. Thus, previously prepared Klason lignin was repeatedly subjected to reKlasonation and the mass loss after each stage of hydrolysis was quantified. The resulting hydrolysates were satisfactory for determining the absorptivity of ASL at 205 nm. The reKlasonation hydrolysates were found to be spectroscopically identical to hydrolysates prepared from the parent wood species.

INTRODUCTION

An acid hydrolyzed wood mixture typically consists of an aqueous solution and a flocculent residual solid. The residual solid has historically been called "acid insoluble lignin", as is evidenced by the title of TAPPI Standard T 222 om-83, "Acid-insoluble Lignin in Wood and Pulp"[1]. This residue has also been named "Klason" lignin after the Swedish chemist, Peter Klason, by whom it was first isolated using a similar procedure. When using a Klason-type procedure to determine the lignin content of a lignocellulosic material, the Klason lignin is frequently reported as representing the total lignin content of the sample.[1] However, a

portion of the lignin is solubilized in the Klason procedure. Since many of the frequently used techniques in the chemical analysis of wood begin with some variation of the Klason procedure, the concept of "acid soluble lignin" (ASL) has been discussed extensively in the literature.[2-12]

The most widely used method to analyze for ASL is by ultraviolet (UV) spectroscopy. The UV determination of ASL relies on an adherence to Beer's law, **A = a * c** (assuming a 1 cm path length), where:

A is the absorbance at a given wavelength
a is the absorptivity in L/g-cm
c is the concentration in g/L

The absorptivity, **a**, must be empirically determined. Researchers have determined that ASL obeys Beer's law fairly well over narrow concentration ranges.[2,3]

Earlier protocols for ASL determinations specified UV measurements at 280 nm.[4,5] However, interference from 2-furaldehyde (2-F) and 5-(hydroxymethyl)-2-furaldehyde (HMF), common acidic degradation products of carbohydrates which have absorbance maximums near 280 nm, led to a recommended change to 205 nm. Some researchers have also used measurement at 240 nm.[6] Nevertheless, a UV scan from 200 nm to 300 nm of ASL (Figure 1) reveals that measurement at 205 nm is the superior choice.

Regardless of the method used to analyze for ASL, some type of standard ASL solution must be prepared. Unfortunately, the procedure for preparing such a solution has been the irresolvable problem involved in ASL analysis. Basically, there has been no model compound which can be used to serve as ASL for this purpose. Thus, it has not been possible to determine a reliable absorptivity for ASL.

The literature reports that preparative liquid chromatography[3,5] and chemically isolated lignin preparations, such as Braun's native lignin[7] and milled wood lignin[2], have been used to obtain "pseudo" ASL. However, in the case of the ASL obtained by liquid chromatography, only a portion of the ASL is recovered and the material that is isolated is not soluble in acids, alkali, or most

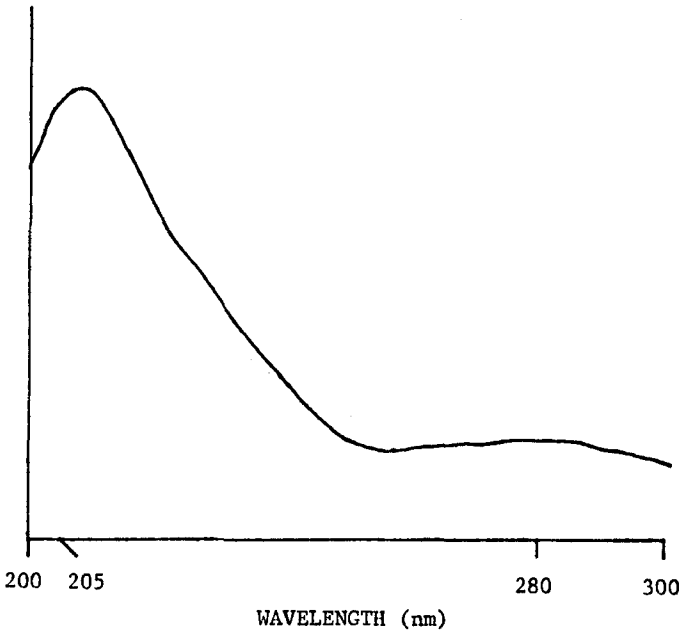

Figure 1. UV spectrum of ASL.

organic solvents; the UV spectra of the isolated "acid soluble lignins" are determined by redissolving the material in the eluting solvent.[3,5] Since the "acid soluble lignin" is no longer acid soluble, some chemical changes must have occurred during the recovery or, indeed, the material recovered is not ASL. Similarly, the use of "native" lignins as lignin model compounds for the determination of the absorptivity of ASL is also subject to serious questions. Considering that the lignins are isolated and purified by repeated dissolution in various solvents and solutions, the structural and, indeed, spectral resemblances of the preparation, compared to those of proto-lignin, must be considered dubious. This problem is exacerbated by the fact that only 35% of the lignin, or less, based on the Klason lignin, is typically isolated using these procedures.[8,13]

Clearly, the resemblance of the fraction isolated in any of the above mentioned procedures to ASL that is obtained in the Klason acid hydrolysis procedure is doubtful. This is especially true of the absorptivity that would be calculated using these fractions. Furthermore, the procedures used to obtain the "pseudo" ASLs are time-consuming and tedious. This complicates any process in which a large number of different samples needs to be analyzed.

Since the model compounds used by various investigators to establish the absorptivities for ASLs have varied, it would be expected and has been found that the absorptivities have varied as well. For example, for a given species of eucalyptus, the 205-nm absorptivity was calculated to be in the range of 88 L/g-cm and 103 L/g-cm, depending on the model compound used.[2] However, a review of the literature indicates that the 205-nm absorptivities for all of the woods that have been studied are in the relatively narrow range of 88 L/g-cm to 113 L/g-cm.[2,3,7]

Although the general methods reported in the literature for the determination of ASL are, except for the model compounds used, consistent, there is, unfortunately, very little agreement between investigators relative to the amount of ASL present in the wood. Though it has been generally agreed that hardwoods yield a greater percentage of ASL than do softwoods, determinations of ASL made by different investigators on the same species of wood have been quite variable. For example, in the case of ASL in beechwood (*Fagus sylvatica*), values of 1.3%[7] and 7.4%[5] have been reported. Similar discrepancies have been reported for other woods as well.[7] This wide range of values clearly cannot be attributed to differences in the absorptivity, **a**, as the reported values for **a** at 205 nm are all within 25%. If it is assumed in the determination of ASL that the sample materials and analytical techniques used were essentially the same, then an explanation for the disparity of ASL values reported in the literature must lie in the nature of ASL itself. Specifically, ASL does not occur in wood as a separate, isolable entity, such as anhydroglucose, but instead is formed as a lignin degradation product during acid hydrolysis. Applying this concept, one would expect the ASL content of a hydrolysate to increase as the

hydrolytic conditions became more severe; similar to the increase seen in 2-F and HMF production. Furthermore, if ASL production is governed by kinetic behavior, it should be possible to hydrolyze Klason lignin repeatedly and obtain some ASL from each hydrolysis to the point of extinction of the precursor.

To test this hypothesis, a series of experiments was conducted using Klason lignin from American beech (*Fagus grandifolia*) in which the same sample of Klason lignin was repeatedly "reKlasonized". The resulting mass loss observed during these experiments was quantitatively determined and the "solutions" were analyzed using UV spectroscopy and HPLC. It should be noted that when referring to an aqueous phase of ASL the use of the term "solution" may not be strictly correct. ASL may be a colloidal suspension in an acid phase, rather than a dissolved solid. The term "system" might be better applied to ASL present in an aqueous phase. However, in the absence of a demonstration of the state of the matter involved, the phrase "ASL solution" will be used throughout this work to refer to aqueous systems that contain ASL.

EXPERIMENTAL

Correction of UV absorbance for 2-F and HMF

Although determining the UV absorbance of wood hydrolysate solutions at 205 nm reduces the influence of 2-F and HMF, the UV scans of 2-F and HMF (Figure 2) demonstrate that the absorbances of these compounds are not zero at 205 nm. Thus, a correction must be applied to account for the absorbance due to 2-F and HMF. To quantify the portion of the absorbance at 205 nm that is due to 2-F and HMF, the following procedure was devised in this study. The 205-nm absorbance, and thus the absorptivity, of a 2-F and HMF solution of known concentrations was determined. Since 2-F and HMF do not have the same absorbance spectra, as can be seen in Figure 2, the concentrations of 2-F and HMF in the mixture were chosen to approximate the concentrations of these components in an actual sample hydrolysate to minimize the error in the absorptivity calculation. Alternatively, the 205-nm absorptivities of 2-F and

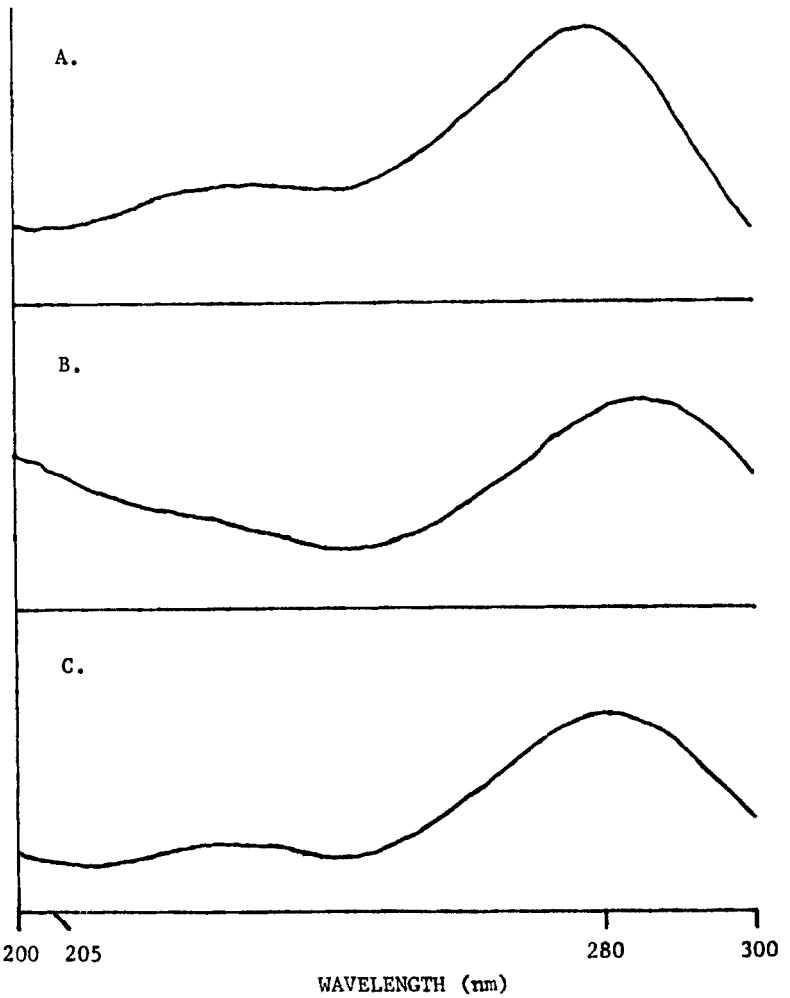

Figure 2. UV spectra of 2-F (A), HMF (B), and a mixture of equal parts of 2-F and HMF (C).

HMF could have been determined independently, although the results clearly would not differ very much and would not reflect the possible interaction between 2-F and HMF when present in the same solution.

Once the 2-F/HMF absorptivity was obtained, it was a simple matter to determine the concentrations of 2-F and HMF in the hydrolysate sample using High Performance Liquid Chromatography (HPLC), as has been described elsewhere[14], and apply the corresponding correction factor to the 205-nm absorbance. The equation below allows calculation of the percent ASL in a wood hydrolysate with known 2-F/HMF concentrations.

$$\%ASL = \frac{A * V - 19.14 * 250 * M_f}{a_L * S_w} * 100$$

where A = absorbance at 205 nm (assumes 1 cm pathlength)
V = theoretical total volume of UV solution (dilution factor times hydrolysate volume, in ml)
19.14 = 205-nm absorptivity of an equal mixture of 2-F and HMF (ml/mg-cm)
250 = volume of hydrolysate (ml)
M_f = concentration (mg/ml) of 2-F and HMF in hydrolysate (by HPLC)
a_L = absorptivity of ASL (ml/mg-cm)
S_w = sample weight of wood, in milligrams

Preparation of Klason Lignin Sample

The reKlasonation experiment was performed using Klason lignin prepared from American beech. Approximately 10 g of Klason lignin was prepared using a scaled-up procedure of TAPPI Standard T 249-cm-85.[1] After oven drying at 105 °C, the Klason lignin was pulverized using a mortar and pestle and stored in a brown glass bottle.

Hydrolytic Conditions

The Klason lignin was subjected to a series of modified secondary hydrolysis steps used in the Klason procedure. Each step in sequence used the same experimental technique although the mass of lignin used progressively diminished. However, each secondary

hydrolysis step in a given sequence utilized only lignin that had been hydrolyzed in the previous step of the sequence. For example, all of the material that was hydrolyzed in the fourth reKlasonation procedure had been run through a similar procedure three times previously. In steps 1, 2 and 5 parallel samples, denoted by the letters a and b, were run concurrently; the parallel samples were recombined before sampling for the next step in the sequence. The method used for these hydrolyses involved the use of hydrolysis bombs, which have been described in an earlier work.[14] The use of the bombs ensured that no material or degradation products were lost during the procedure. In all cases the acid concentration was 4%; no concentrated acid step was performed in the reKlasonation procedure. The hydrolysis time used for each stage was 2 h. The hydrolysis temperature, 120 °C (103 kPa), was obtained in an autoclave.

Quantification of Residual Polysaccharide

For each reKlasonation trial, the residual polysaccharide moieties that were hydrolyzed were analyzed by HPLC, using the sample preparation procedures that have been reported elsewhere.[14] Each of these HPLC preparations (excepting the 2-F analysis), utilized 50 ml of hydrolysate instead of the 20 ml that was typical of wood HPLC analyses. It should be noted that only the hydrolysate from the first Klason procedure had a significant amount of 2-F; no HMF was observed in the reKlasonation hydrolysates.

As the chemical analyses for each Klason procedure were obtained, the mass of ASL for the procedure was determined by subtracting the following quantities from the starting dry mass of Klason lignin solids: The post-hydrolysis mass of dry solids, the mass of 2-F (calculated as xylan), the mass of uronic acids (anhydro), the mass of monosaccharides (anhydro), and any other degradation products that appeared in the various HPLC chromatograms (calculated as the respective precursors).

TABLE 1
Data from ReKlasonation Trials Using American Beech Lignin

run #	Kla Lig wt (grams)	solubles (mg)	gluc (mg)	xyln (mg)	galac (mg)	2-F (mg)	GUA-X (mg)	GUA (mg)	other (mg)	ASL (mg)	a (L/g-cm)
1a	5.8462	DNA ---------> (sample mishandled)									
1b	5.2697	93	2.27	8.76	1.03	0.15	0.93	0.72	0.75	79	37
2a	5.15360	88	0.77	2.90	--	--	1.51	0.80	--	82	42
2b	4.37387	72	0.64	2.04	--	--	1.20	0.65	--	67.5	42
3	9.31197	DNA ---------> (sample mishandled)									
4	9.22474	173.4	1.16	5.09	1.28	--	2.45	--	1.50	162	40.0
5a	0.97550	15.8	DNA ---------->							est. 14.5	60.2
5b	0.96482	15.5	DNA ---------->							est. 14.3	60.5

All of the above trials, except trial 5a, were performed using 174 ml of 4% sulfuric acid; trial 5a utilized 87 ml. Furthermore, the same Klason lignin was used in each of the successive trials. Thus, the lignin used in trials 5a and 5b had been reKlasonized four times previously.
Key: gluc = glucan, xyln = xylan, galac = galactan, GUA-X = 4-O-methyl-glucurono-xylose, GUA = 4-O-methyl-glucuronic acid (anhydro), other = formic acid & HMF, DNA = did not analyze, est. = estimated, -- = absent or too low to detect

HPLC Analysis of ASL

For comparison purposes, HPLC analyses were made of wood Klason and reKlasonation hydrolysates, and of a hot water Soxhlet extract of Klason lignin. The column used was a Brownlee Labs 3-cm PPH-GU guard cartridge, using 0.01 M H_2SO_4 as the eluant. For detection, a variable-wavelength UV detector, operated at 205 nm, was used. The column was used at ambient temperature, and all of the hydrolysates were analyzed neat.

RESULTS AND DISCUSSION

After correcting for the mass of polysaccharides that was hydrolyzed in the reKlasonation procedure, the apparent concentration of ASL and an analysis of the ASL solution by UV spectroscopy at 205 nm were used to calculate the ASL absorptivity using Beer's Law. (See Figure 1 for an example of a UV spectrum). The data from each of the reKlasonation procedures, including the calculated mass of ASL and the resulting absorptivities for ASL at 205 nm, are listed in Table 1.

The phenomenon of repeatedly hydrolyzing Klason lignin in reKlasonation is clearly demonstrated in Table 1, above. In fact, the reKlasonation of Klason lignin can seemingly be accomplished to the extinction of precursor moieties giving rise to ASL. This result indicates that ASL comprises relatively low molecular weight

Klason lignin fragments. The observation that, in each procedure, an amount of ASL was produced that was consistent with the amount of initial Klason lignin, irrespective of the amount of acidic solution, indicates that a kinetic relation exists for ASL production from Klason lignin. Clearly, the production of ASL from Klason lignin is governed by the kinetic relation for acidic degradation of Klason lignin, not the solubility of Klason lignin in sulfuric acid. The utility of this concept immediately becomes apparent in the application to the analysis of ASL. Thus Klason lignin, itself, can be used to prepare solutions containing ASL that can then be used to obtain ASL absorptivities. For this set of experiments, the calculated absorptivities were essentially the same for runs in which the amount of initial Klason lignin was relatively constant. However, the variability of the absorptivities between runs 1,2 & 4 and run 5 suggests that in order to use reKlasonation of Klason lignin as an accurate method for the determination of ASL absorptivities, the reKlasonation hydrolysates must contain approximately the same ASL concentration and acid concentration as the corresponding wood hydrolysate.

The analysis of ASL by HPLC could actually prove to be a valuable technique if a column could be found that would provide a reproducible analysis. The Brownlee column was promising except for the inability to achieve reproducible peaks areas with repeated injections of the same sample. This phenomenon was undoubtedly due to irreversible adsorption of the ASL onto the column media. A similar difficulty was encountered with several silica gel GPC columns. Thus, the HPLC chromatograms obtained are useful solely for qualitative comparisons. The chromatograms obtained using the Brownlee column of a beech wood hydrolysate, a reKlasonation hydrolysate, and a hot water Soxhlet extract of Klason lignin are presented in Figure 3. These chromatograms are significant because they indicate that a reKlasonation hydrolysate is almost exactly the same as a wood hydrolysate, whereas a water extract of the same Klason lignin material is quite different.

The values for the absorptivities in Table 1 are of concern, though they are believed to be correct. It should be recalled that

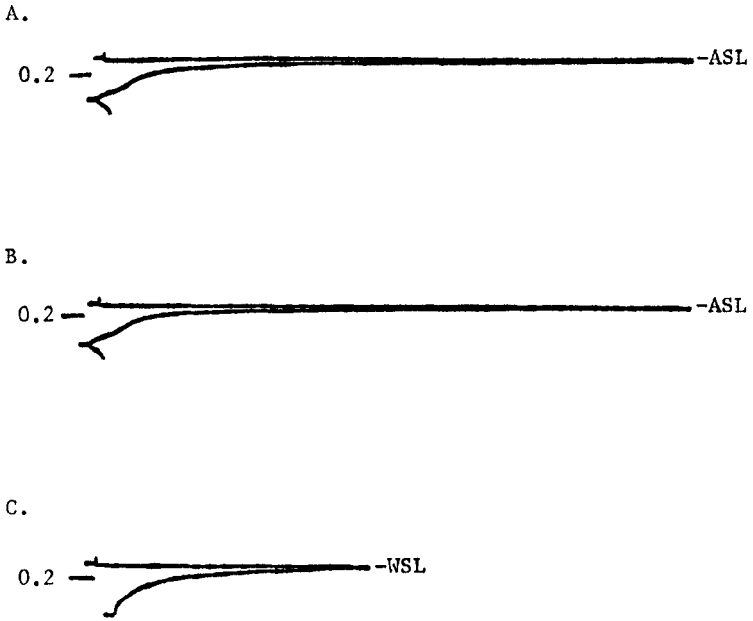

Figure 3. HPLC analyses of a beechwood hydrolysate (A), a beechwood reKlasonation hydrolysate (B), and a beechwood Klason lignin water extract (C). The detection was by UV at 205 nm.

a number of reported hardwood ASL 205-nm absorptivities were in the range of 110 L/g-cm, approximately twice that of the range found in this study. However, the determinations of ASL in this study involved a readily measured weight loss. Thus, experimental error was of less significance. A possible explanation for the difference between the absorptivities that were determined in this study and those that have been reported in the literature may be that the method used in this study resulted in the formation of true ASL. Furthermore, the ASL solutions were produced by the same procedure that was used to produce the ASL in the original hydrolysis of the wood. Therefore, the ASLs used to determine the absorptivities were essentially the same as those present in a normal wood hydrolysate. Clearly, the process of acid hydrolysis must alter the lignin in

such a way as to change its spectral properties, but the mechanism of this change is unknown.

The appearance of monosaccharides and uronic acids in the hydrolysates from the reKlasonation sequence suggests that a strong lignin-carbohydrate complex (LCC) is present in beechwood. Since the uronic acids and xylose are present, the LCC probably involves xylan fragments that are bonded to the lignin by ester linkages through the uronic acid moiety. The nature of the LCC will be a topic of a future work.

CONCLUSIONS

A method for the determination of ASL was the goal of this study. Although methods for ASL analysis have been reported in the literature, the lignin model compounds and/or analytical procedures that were used in the reported studies were inappropriate for the determination of ASL.

In the literature, Klason lignin is known as "acid-insoluble lignin". However, in this study, it was conclusively demonstrated, using Klason lignin from American beech wood, that Klason lignin can be repeatedly acid hydrolyzed and a predictable amount of material removed. Furthermore, Klason lignin was shown to be an ideal model compound to use for calibration of ASL analytical methods. The rehydrolysis, or "reKlasonation", of Klason lignin resulted in a solution whose UV spectral properties were identical to those of a wood hydrolysate. This similarity was confirmed using HPLC.

The ASL analysis method that was developed utilized UV spectroscopy as the analytical tool. The UV measurements were made at 205 nm and the ASL contributions were determined using Beer's Law. ReKlasonation solutions were found to be reliable and reproducible for use in determining the ASL absorptivities used in the ASL analyses.

ACKNOWLEDGMENTS

Portions of this work were used by WEK as partial fulfillment of the requirements for the Ph.D. degree at the University of

California, Berkeley. The current address of WEK is: VPI & SU, Dept. of Wood Science and Forest Products, 210 Cheatham Hall, Blacksburg, VA 24061-0323.

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