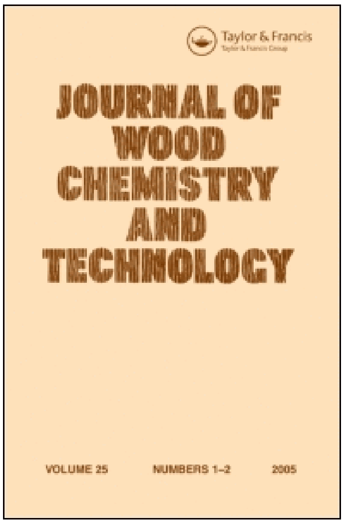

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Anna-Stiina Jääskeläinen[a]; Anna-Maija Saariaho[a]; Tapani Vuorinen[a]
[a] Laboratory of Forest Products Chemistry, Helsinki University of Technology, Espoo, Finland

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# Quantification of Lignin and Hexenuronic Acid in Bleached Hardwood Kraft Pulps: A New Calibration Method for UVRR Spectroscopy and Evaluation of the Conventional Methods

**Anna-Stiina Jääskeläinen, Anna-Maija Saariaho, and Tapani Vuorinen**
Laboratory of Forest Products Chemistry, Helsinki University
of Technology, Espoo, Finland

**Abstract:** Lignin content determination is an important task when pulp bleaching is studied. However, none of the conventional methods were developed for bleached pulps and therefore they are accurate mainly for unbleached pulps. This article describes a new, rapid method to determine lignin and hexenuronic acid contents of bleached hardwood kraft pulps based on UV resonance Raman (UVRR) spectroscopy. The lignin contents of pulp samples were determined from the aromatic band heights of the UVRR spectra. Therefore the measurements gave the content of aromatic lignin in pulp, and did not include extensively oxidized lignin structures. The method was applicable for hardwood kraft pulps with lignin content less than 1%. The measured lignin content correlated linearly with the kappa number. The lignin content (% on pulp) equaled $0.15\kappa + 0.16$. The constant 0.16 was presumably caused by the incomplete oxidation of the lignin in the kappa number determination. Klason lignin or total lignin determinations were not accurate for these kinds of pulps. Hexenuronic acid content was simultaneously determined from the UVRR band height of unsaturated C═O and C═C structures. The linear correlation of this band with hexenuronic acid indicated that the content of other unsaturated structures was constant in all the pulps, was proportional to the hexenuronic acid content of the pulps, or was insignificantly low. When compared to conventional methods, the UVRR spectroscopic method is fast, requires little sample and pretreatment, and the procedure has good

Address correspondence to Anna-Maija Saariaho, M-real Oyj, Technology Centre Äänekoski, P.O. Box 600, 44101, Äänekoski, Finland. E-mail: anna-maija.saariaho@m-real.com

repeatability. In addition, the accuracy of this technique increases with decreasing lignin content (<1%) making it a very attractive method for bleaching studies.

**Keywords:** Bleached pulp, hardwood kraft pulp, hexenuronic acid, kappa number, Klason lignin, lignin content determination, UV resonance Raman spectroscopy

## INTRODUCTION

Lignin content determination of bleached pulps is a challenging task. Kappa number determination and total lignin (i.e., Klason lignin + acid soluble lignin) content analysis are the most commonly applied methods to determine lignin in pulps.[1] However, the repeatability and reproducibility of these methods are poor for bleached pulps with less than 2–5% lignin content.

The kappa number determination is related to the number of oxidizable structures in the pulp by treating the pulp with acidic potassium permanganate under the specified conditions.[2] It has been demonstrated[3,4] that most aromatic units in pulp consume a constant amount of permanganate, whereas the native carbohydrate components do not react to a significant extent. However, in alkaline pulping the 4-O-methylglucuronic acid groups of wood xylans are converted to unsaturated hexenuronic acids (HexA),[5] structures that consume permanganate in the kappa number determination. Therefore, in determining the lignin content of alkaline pulps, the amount of hexenuronic acid groups is often determined separately, and their influence on the kappa number is subtracted to obtain a corrected lignin concentration. This makes the method time consuming and laborious.

When studying bleached kraft pulps, the kappa number determination as a measure for lignin content becomes even more questionable. The residual lignin in bleached pulps is oxidized and consumes less permanganate than the original lignin structures.[6] These limitations imply that kappa number measurement is a debatable method to determine the lignin content in bleached pulps.

Despite its limitations, the total lignin content measurement (Klason lignin + acid-soluble lignin) is reportedly the best procedure to determine the lignin content in wood and pulp samples.[7] However, this method is recommended mainly for unbleached pulps, as its accuracy decreases remarkably when the lignin content of the sample decreases below 5%.[1]

We recently developed a method to simultaneously determine the lignin and hexenuronic acid contents in softwood pulps by UV resonance Raman (UVRR) spectroscopy combined with PLS calibration.[8] The calibration of this method was based on the lignin kappa number of the pulps, and therefore the method gives results that are comparable to kappa number. This approach was selected because the UVRR spectra of unbleached pulp showed no bands arising from carbohydrates that could be applied as a reference, like in FT Raman spectroscopy.[9] Moreover, the multivariate calibration minimizes directly the error caused by the overlapping Raman bands.

However, in kraft pulps with a fairly low lignin content (~4% or less) the band arising from cellulose, clearly resolved from the baseline of the UVRR spectrum, could be assigned as a reference band.

This article describes a new and rapid calibration method for UVRR spectroscopy to determine lignin content in bleached hardwood kraft pulps. The method is based on the height ratio of the aromatic and cellulose bands in UVRR spectra. Two conventionally applied lignin quantification methods, kappa number and Klason lignin determinations, were evaluated based on the UVRR spectroscopic results. In addition to lignin content determination, UVRR spectroscopy was applied also to determine the hexenuronic acid content in pulps. The band height ratio of unsaturated structures (C=O and C=C) and cellulose was applied as a measure of hexenuronic acid groups in the pulp.

## MATERIALS AND METHODS

### Calibration Samples

Seven calibration samples with known lignin contents were prepared in order to calibrate the UV resonance Raman method. Hardwood kraft lignin was precipitated from the black liquor of conventional kraft pulping of birch. The pH of the black liquor was adjusted to 2 with 1 M HCl. Thereafter the precipitated lignin was centrifuged, washed three times with ion exchanged water and dried in air. Pulp samples (100 mg) with a low lignin content (PULP-7, Table 1) were weighed into a series of glass vials, and 0.125–2.5 mL of hardwood kraft lignin solution (2 g/L in dioxane:water, 85:15) was added to each vial. This was followed by filling the vials to 1.5 mL with the dioxane:water solution. The samples were mixed with a magnetic stirrer and the solvent was evaporated at room temperature. The dried samples

***Table 1.*** Bleaching sequences of the hardwood kraft pulps used in this study

| Sample | Bleaching sequence |
|---|---|
| PULP-1 | O |
| PULP-2 | O-A |
| PULP-3 | O-(PO)-(EOP) |
| PULP-4 | O-A-D-(EOP) |
| PULP-5 | O-D-(EOP)-D-D |
| PULP-6 | O-D-(EOP)-D-P |
| PULP-7 | O-D-(EOP)-D-Paa |
| PULP-8 | O-D-E |
| PULP-9 | O-A-D-(EOP)-D-P |
| PULP-10 | O-P (=PULP1 + P) |

were carefully collected, placed in a ball mill, and mixed for a few minutes. Finally, the samples were pressed (10 kN, 30 s) to form pellets. Reference pellets without lignin addition were prepared in a similar manner. PULP-7 was selected for the preparation of the calibration samples because its hexenuronic acid content was the lowest of all the pulps. This was done to ensure minimal band overlapping contribution to lignin calibration from hexenuronic acid band (see the later discussion).

## Hardwood Kraft Pulp Samples

The hardwood kraft pulps were obtained from Finnish pulp mills. The pulps were bleached using different bleaching sequences as shown in Table 1. The pulps, except PULP-10, were from three different pulp mills and none of them from consequent bleaching stages of the same mill. The detailed information on cooking and bleaching conditions was not available to us. Furthermore, this kind of information is not relevant in this context.

## UV Resonance Raman Spectra

The UV resonance Raman spectra were collected with a Renishaw (Cloucestershire, UK) 1000 UV Raman spectrometer equipped with a Leica (Wetzlar, Germany) DMLM microscope and an Innova 300C FreD frequency-doubled Ar+ ion laser (Coherent Inc., Santa Clara, CA, USA). The excitation wavelengths applied were 244 nm or 257 nm for the calibration samples and 244 nm for the pulp samples.

The output power of the laser was adjusted to 20 mW and the laser beam was attenuated with a neutral density filter of 10% transmittance. The beam was directed through a 15× objective (OFR Inc., Caldwell, NJ, USA) on the sample. Due to some losses in the light path, the final power at the sample level was 1.3 mW. The backscattered Raman light was directed through the microscope and focused on a 50 μm slit. The Raman light reflected via a prism mirror to a diffraction grating of 3600 grooves/mm and finally to the detector (CCD camera). The spectral range was 400–2300 $\mathrm{cm}^{-1}$ and the resolution was about 7 $\mathrm{cm}^{-1}$.

The calibration samples were measured without any further pretreatments whereas the pulp sheets were pressed between two metal plates (10 kN, 30 s) to smooth the surface prior to measurement and cut to ~1 cm × 1 cm pieces. During the spectral collection the samples were spun (6 rpm) in order to avoid any chemical changes in the sample due to the intense UV light. Another advantage of the spinning was that average spectra of the samples were obtained, which was important due to their nonhomogeneity. The spectral collection time was 2–4 min per sample, and 3–6 parallel spectra were collected from each sample.

## Spectral Processing

The band heights of the spectra were calculated using Bio-Rad Win-IR Pro (Bio-Rad Laboratories, Cambridge, MA) software. The baseline was corrected between 1900 and 750 $\mathrm{cm}^{-1}$. The cellulose, lignin, and hexenuronic acid band heights were measured precisely at 1093.5, 1604.5, and 1655.0 $\mathrm{cm}^{-1}$, respectively. In each spectrum these wavenumbers corresponded to the band height maximum within $\pm 2\,\mathrm{cm}^{-1}$.

## Conventional Methods

The kappa number measurement was performed using the standard analytical procedure SCAN-C 1 : 00.[2] After the kappa number determination, the pulp was washed thoroughly with distilled water prior to determining the UVRR spectrum. Hexenuronic acid was determined by the acid hydrolysis method combined with UV spectroscopy as described elsewhere.[10] After the acid hydrolysis, the pulp was washed with distilled water prior to determining the spectrum of the acid-treated pulp.

The "lignin kappa number" was calculated by subtracting the kappa number caused by hexenuronic acids from the total kappa number. The calculations were performed by utilizing the experimental observation[11] that 10 meq HexA/kg pulp equals 0.86 kappa units.

The total lignin content was measured by Klason lignin and acid soluble lignin determinations using the standard analytical procedure.[12] The results presented are averages of 2–4 replicates.

# RESULTS AND DISCUSSION

## Lignin Content Determination—Description of the Method

The UVRR spectra of the six calibration samples are shown in Figure 1. It can be observed that in the sample with no lignin addition, the cellulose band at 1094 $\mathrm{cm}^{-1}$ is easily discerned in the spectrum. Despite the very low lignin content of the sample, the intensity of the band arising from aromatic and other unsaturated structures at 1700–1500 $\mathrm{cm}^{-1}$ is as high as the cellulose band. This clearly illustrates that the resonance enhancement increases the sensitivity of this technique to lignin, as has been shown also in earlier studies.[13]

Addition of lignin in the pulp increased the band at $\sim$1600 $\mathrm{cm}^{-1}$, which arises from the aromatic ring stretching (Figure 1). The aromatic band height (1605 $\mathrm{cm}^{-1}$) relative to cellulose band height (1094 $\mathrm{cm}^{-1}$) correlated with the lignin addition of the sample, as is shown in Figure 2. It should be noted that in most of the samples, the variation between the measurements originated

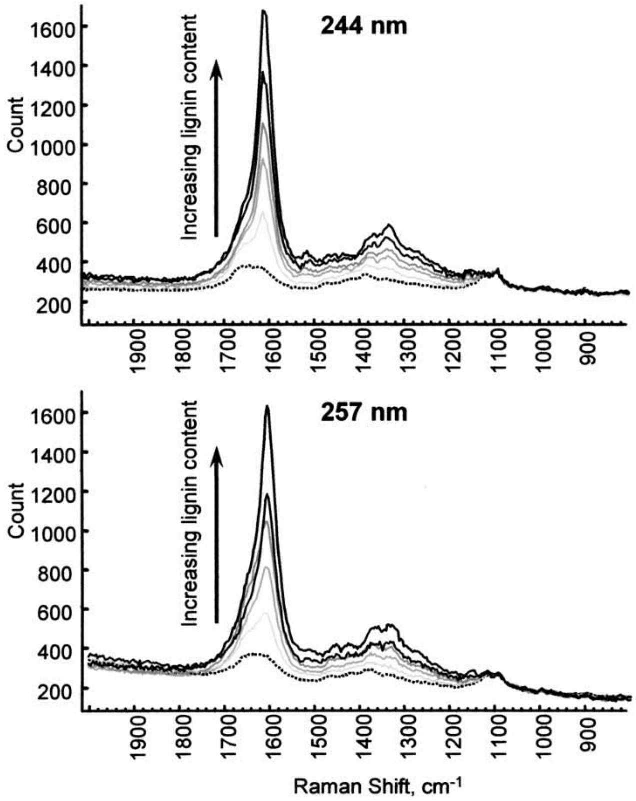

***Figure 1.*** UVRR spectra (244 nm and 257 nm) of calibration samples with lignin addition of 0, 0.5, 1.0, 2.0, 3.0, and 5.0% on pulp. The spectra were normalized to the cellulose band at 1093.5 $cm^{-1}$.

mainly from the nonhomogeneity of the samples rather than from the repeatability of the UVRR measurement. The repeatability of the UVRR measurement improved with decreasing lignin content both in absolute and relative terms. This can be explained by the fact that for the pulp with the highest lignin addition (5%) the noise in the spectrum was comparable to the magnitude of cellulose band intensity, as can be seen in Figure 1. This results in the conclusion that the upper quantification limit of this method is close to 5%, which corresponds to the lignin content of unbleached kraft pulp. On the other hand, even extremely low lignin contents can be detected and quantified with high precision, indicating the suitability of the method for bleached pulps with low lignin content.

In hardwood pulps, depending on the applied bleaching stages, the content of hexenuronic acid can be rather high. Hexenuronic acid also gives rise to the resonantly enhanced Raman band at about 1655 $cm^{-1}$.[13] The

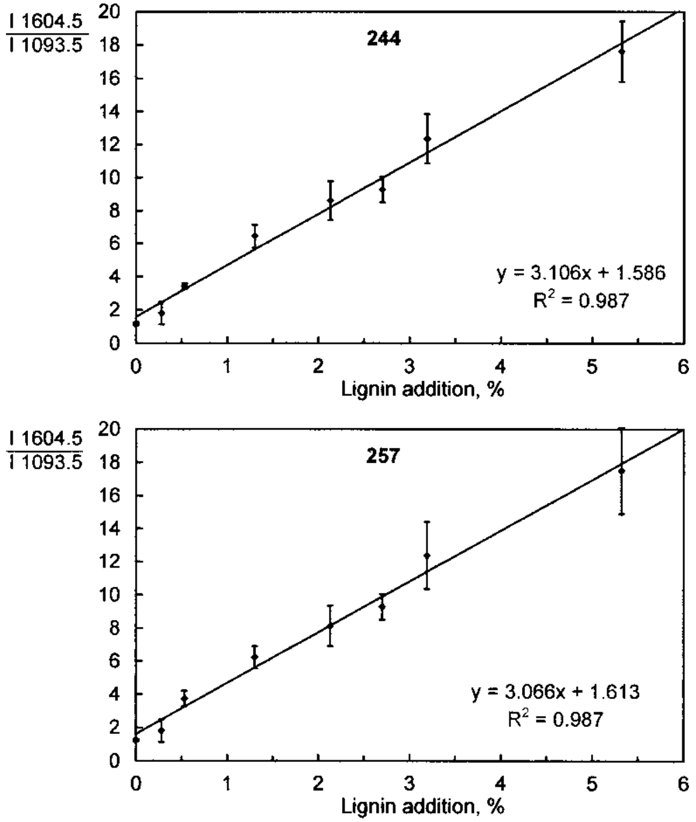

***Figure 2.*** Correlation of the band height ratio to the amount of lignin addition in PULP-7. UVRR spectra were recorded using 244 or 257 nm excitation wavelengths. The vertical bars illustrate the variance of 4–5 parallel measurements.

hexenuronic acid and lignin bands overlap with each other (Figure 3). In order to obtain very accurate results from the UVRR spectroscopy, this phenomenon has to be taken into account by multivariate calibration[8] or by band fitting. The former method has been demonstrated with softwood pulps.[8] The latter method is laborious and time-consuming and needs to be done for each spectrum separately.

Because the purpose of this work was to develop a rapid method for quantification of lignin and hexenuronic acid, the band fitting procedure was omitted. This simplification may cause some error to the UVRR results depending on relative contents of these two components. If the content of lignin is low and the content of hexenuronic acid is high, the error in the analyzed lignin content would be bigger than in a case where a higher lignin content is determined from a pulp with a low hexenuronic acid content.

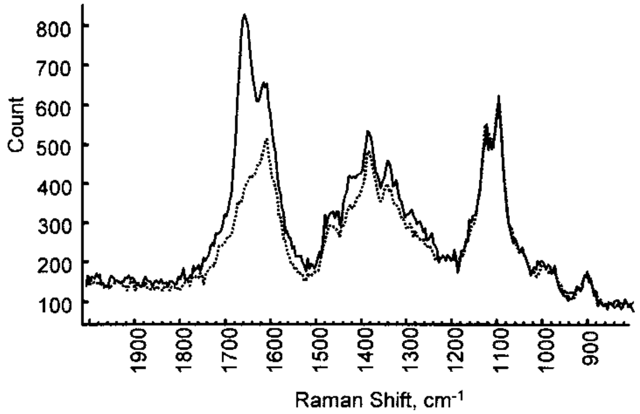

***Figure 3.*** UVRR spectrum of bleached hardwood kraft pulp 9 before (solid line) and after (dotted line) kappa number measurement.

However, when the results were compared to those obtained with the conventional methods (as discussed and demonstrated later), it was noted that they correlated significantly and that the accuracy was still quite high.

Remarkably, more fluctuation was observed in the baseline of the spectra obtained at 257 nm than at 244 nm (Figure 1), which increased the variation in the band height determination at the 257 nm excitation. These results indicated that the excitation at 244 nm was better for lignin content determination than the excitation at 257 nm.

To calculate the lignin content of the pulp based on the relative band height of aromatic vibration, the regression line (Figure 2, 244 nm) was written in the form:

$$Lignin(\%) = 0.322 \cdot \frac{I(1605cm^{-1})}{I(1094cm^{-1})} - 0.511 \qquad (1)$$

As expected, the regression line did not cross the origin because the initial pulp contained some residual aromatic structures. The y-intercept of the regression line (−0.511) implies that the lignin content of the original calibration pulp (PULP-7) was approximately 0.5% on pulp, because it is reasonable to assume that the band height at 1605 $cm^{-1}$ should equal zero when there are no aromatic lignin structures in the pulp. However, some minor contribution from hexenuronic acid band may give rise to the intensity at 1605 $cm^{-1}$ meaning that the intensity at 1605 $cm^{-1}$ might be more than zero even though there was no aromatic lignin left in the pulp. This contribution should be rather small because the hexenuronic acid content of the original PULP-7 was very low. According to the slope of the regression line, the lignin content of an unknown pulp sample can be calculated from the UVRR band height ratio using Equation (2):

$$Lignin(\%) = 0.32 \cdot \frac{I(1605cm^{-1})}{I(1094cm^{-1})} \tag{2}$$

## Comparison of UVRR Method to Conventional Methods

Ten semi- and fully bleached hardwood kraft pulps (Table 1) were subjected to lignin content measurements by UVRR spectroscopy (Equation 2) and the results were compared to traditional kappa number titration. As shown in Figure 4, there was a linear correlation between the kappa number and the UVRR lignin content of the pulps. This high

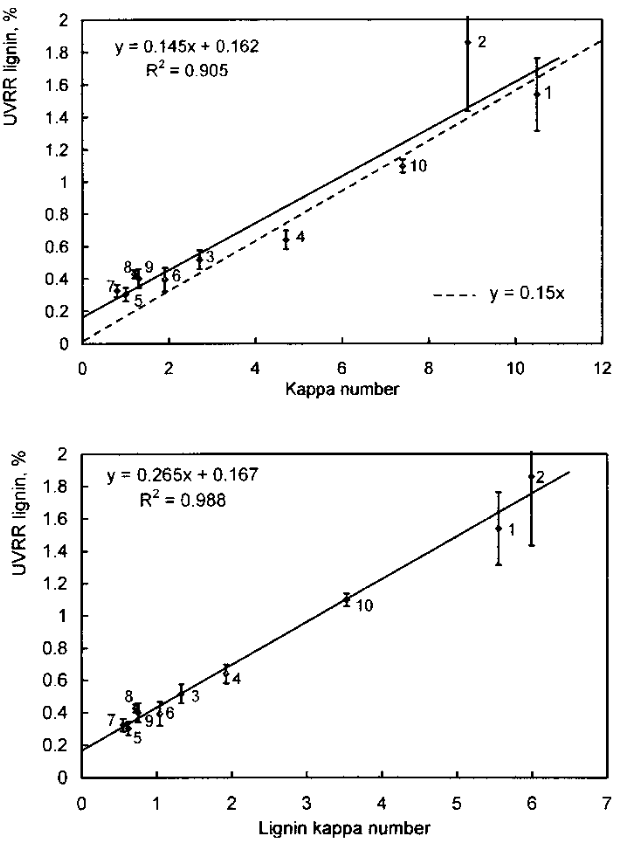

*Figure 4.* Correlation of UVRR lignin content with kappa number and lignin kappa number of the bleached hardwood kraft pulps. The numbers next to the data points refer to the pulp sample (Table 1).

correlation was rather surprising, because these two methods measure different chemical characteristics: UVRR lignin content is a measure of aromatic structures present in pulp and kappa number is a measure of oxidizable structures in pulp. The higher correlation to lignin kappa number than to kappa number itself (see Figure 4) was evident because the hardwood pulps contain rather high amount of hexenuronic acid that contributes to the kappa number determination. It is reasonable to believe that in bleached pulps there are also oxidized lignin structures that are not included in the results, which indicates that the values obtained with these two methods are lower than the actual lignin content of the pulp. The UVRR result reflects the number of unreacted aromatic structures that remained in the pulp. On the other hand, the kappa number determination indicates the number of structures that are oxidized with potassium permanganate regardless of their origin.

The dashed line in Figure 4 illustrates the traditional assumption of the linear correlation between the kappa number and lignin content.[1] The correlation factor of 0.13 for kraft pulps has been published in the standard method,[14] whereas factors between 0.15–0.20 are more commonly applied.[1] Indeed, the results in Figure 3 indicate that the factor of 0.15 is fairly applicable for the semi-bleached hardwood kraft pulps, but for fully bleached pulps it gives values that are lower than those obtained by the UVRR method. According to the regression line, a more accurate equation to convert kappa number to total lignin content would be:

$$Lignin(\%) = 0.15 \cdot \kappa + 0.16 \tag{3}$$

The regression line in Figure 4 does not cross the origin, but intersects the y-axis at 0.16. This indicates that in a hypothetical pulp with a kappa number zero, there are still aromatic structures present giving rise to the band at $1605\,\mathrm{cm}^{-1}$. The intersect can also be partly due to the HexA band that slightly overlaps the lignin band at $1605\,\mathrm{cm}^{-1}$.

The incomplete oxidation of aromatic structures by potassium permanganate was confirmed by UVRR spectroscopy. Figure 3 shows the UVRR spectrum of a bleached kraft pulp before and after the kappa number measurement. During the permanganate oxidation, some of the aromatic and unsaturated structures react, whereas a certain amount of those structures remain intact. The presence of aromatic structures in pulp after permanganate oxidation has also been observed with pyrolysis combined with gas chromatography.[15] This result clearly indicates a source of error in the kappa number determination when it is applied as a measure of lignin content in bleached pulps.

An alternative conventional method to determine lignin content in pulps is to measure the sum of Klason and acid-soluble lignin contents. This procedure has been suggested to be the most reliable method to quantify lignin in unbleached pulps.[7] It is generally known[1,12] that in hardwood kraft pulps the lignin is partly acid-soluble, and therefore more accurate results could be obtained by determining the total lignin content than using Klason lignin alone. For these pulps the correlation between the Klason lignin content and UVRR lignin content was poor, as shown in Figure 5. The correlation between total lignin and UVRR lignin content was even less significant. The high variance between replicate measurements illustrates that the repeatability of the Klason lignin method is not high enough to determine the lignin in bleached hardwood kraft pulps.

## Hexenuronic Acid Group Determination by UVRR

The band at 1655 cm$^{-1}$ in the UVRR spectra of pulps arises from the stretching vibration of C=C and C=O double bonds. It has been demonstrated[13,16] that the main origin of these structures in pulps is the hexenuronic acids, whereas other structures are responsible for a minor contribution. In wood samples, extractives also give rise to this band.[17] However, in these pulp samples the extractives contents were very low (acetone extract <0.3%), and extraction of these samples did not affect the spectra (Figure 6) indicating that in these pulps extractives did not contribute to the band at 1655 cm$^{-1}$ to any remarkable extent.

A linear correlation between the hexenuronic acid content and the relative band height at 1655 cm$^{-1}$ was observed (Figure 7). A regression line was fitted to the data, resulting in Equation (4). This equation can be applied when the hexenuronic acid group content of a hardwood kraft pulp is determined by UVRR spectroscopy.

$$HexA(mmol/kg) = 4.39 \cdot \frac{I(1655cm^{-1})}{I(1094cm^{-1})} - 1.14 \tag{4}$$

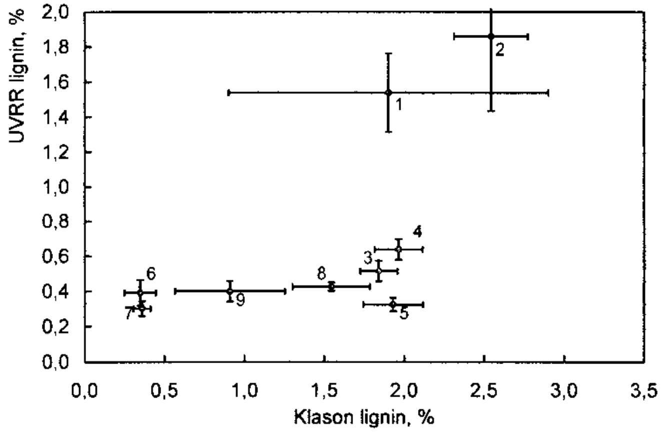

*Figure 5.* Correlation of UVRR lignin content with the Klason lignin content of the pulp samples. Horizontal bars show the variance of 2–4 replicate measurements.

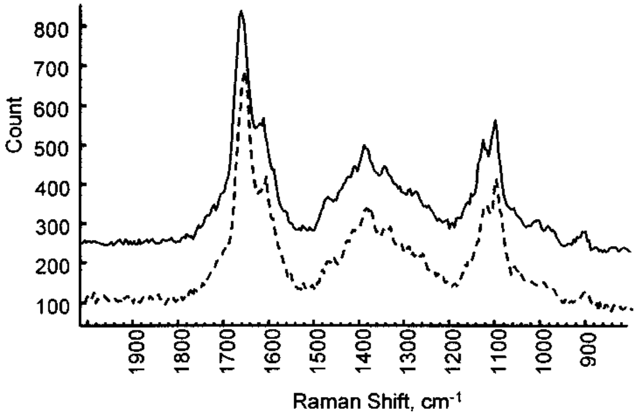

*Figure 6.* UVRR spectra of the initial (solid line) and acetone-extracted (dashed line) pulp. The spectra were offset for clarification.

The high value of the regression coefficient ($r^2 = 0.97$) indicates that this equation is a good fit of the data. The regression line intercepts the y-axis at $-1.14$, indicating that pulps without hexenuronic acid groups contain some other C=C or C=O structures arising in a band at 1655 cm$^{-1}$ or perhaps that a slightly overlapping lignin band at 1605 cm$^{-1}$ is at least partly responsible for the intercept. According to our earlier results,[18] other C=C or C=O structures may possibly originate from quinones as well as from some other unidentified structures.

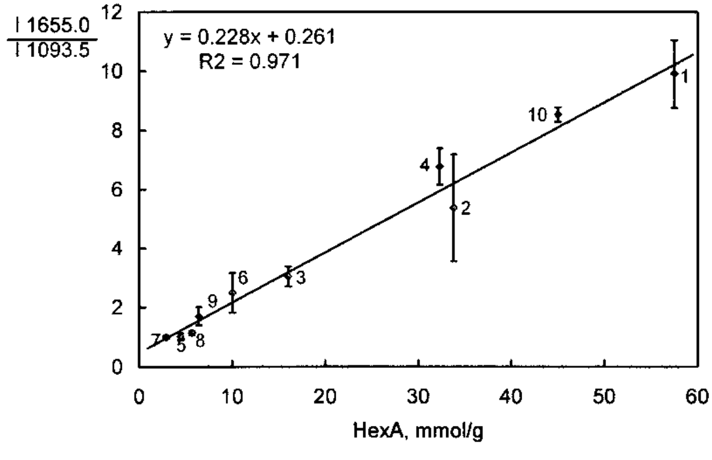

*Figure 7.* Correlation between the relative band height at 1655 cm$^{-1}$ and the hexenuronic acid group content of the pulps.

It is presumable that in different types of pulps the content of quinones and other unsaturated structures (e.g. extractives and oxidized lignin) may differ and thus would lead to false hexenuronic acid contents. However, despite the different origins and bleaching sequences of these pulps (Table 1) the correlation between hexenuronic acid and the relative band height at 1655 cm$^{-1}$ was very high. This indicates that the content of other structures remained constant in each pulp, their concentrations were proportional to hexenuronic acid contents, or their concentration was insignificantly low giving only minor errors. Ultimately, it was concluded that this technique is very applicable to the determination of the hexenuronic acid groups in bleached hardwood kraft pulps. When compared to alternative techniques to determine hexenuronic acids in pulps,[19] this method is fast and requires only a small sample size and practically no sample pretreatment.

## CONCLUSIONS

The lignin quantification method based on the band height ratio of UVRR spectra is a rapid method. One spectrum is collected in a few minutes and minimal sample pretreatment is required. When compared to chemical methods, no chemicals are needed and the operational costs are small. However, it is obvious that the method requires an expensive spectrometer.

Even though the overlapping Raman bands may give some minor error to the results obtained by UVRR method, the results had a good correlation with those obtained by conventional methods. This source of error, presumably insignificantly, can be taken into account by a band fitting procedure. This, however, makes the analysis more time-consuming and laborious. Therefore, the scientist should always judge whether time of analysis or somewhat greater accuracy is more important.

In this UVRR method, the content of aromatic lignin is determined. The oxidized lignin residues, such as quinones or muconic acids, are not included in the result. This may be regarded as a strength of the method because in this case we know the structures that are included in the result. For comparison, kappa number determination gives a figure of all oxidizable structures regardless whether they originate in lignin or in other wood components.

A good correlation between UVRR lignin content and lignin kappa number reveals that despite the speculations in the literature, the lignin kappa number determination is a surprisingly good method for lignin quantification in bleached pulps. However, the lignin content values calculated from the kappa number using the correlation factor 0.15 give systematically too low lignin contents for these kinds of pulps.

The hexenuronic acid content of pulp can be determined simultaneously with lignin content because the intensity of the band 1655 cm$^{-1}$ was found to correlate linearly with hexenuronic acid content. The method is fast and does not require much sample, which can be considered as an advantage in comparison to alternative time-consuming and laborious wet-chemical methods.

This UVRR method is applicable for semi- and fully bleached pulps with lignin content less than 3%. The accuracy decreases with increasing lignin content, because the cellulose band becomes very weak and therefore its height determination is affected by the noise in the spectrum. If the lignin content is above 5%, UVRR spectroscopy can be applied to determine the lignin content, but then a multivariate calibration would be required.

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