4286

Rapid secondary analysis of lignocellulose: comparison of near infrared (NIR) and fourier transform infrared (FTIR)

T. P. Schultz
Associate professor, Mississippi Forest Products Lab, Mississippi State University, P. O. Drawer FP, Mississippi State, Miss. 39762

D. A. Burns*
President, NIR Resources, 54 Canopus Hollow Road, Putman Valley, N.Y. 10579

ABSTRACT *The primary (wet-lab) analysis of wood is a lengthy process. We compared near infrared (NIR) and Fourier transform infrared (FTIR) spectrometry as secondary (indirect) analytical methods. A number of pine and sweetgum samples were delignified to varying degrees and were analyzed using traditional methods. These samples were divided into two sets. The first set was used to calibrate each instrument for the lignin, hemicellulose, and cellulose composition, and the second set used to verify or confirm the calibration equation. NIR had a faster and simpler sample preparation and faster scan time. It generally gave a calibration equation with a lower error value than FTIR. FTIR generally gave a smaller increase in the error going from calibration to confirmation. Both NIR and FTIR performed best in analyzing the lignin component of the hardwood and softwood samples. The calibration equations required no more than four spectra points for either FTIR or NIR. The use of fiber optics interfaced with NIR may make possible an on-line monitoring system for lignocellulosic products.*

KEYWORDS
Analysis
Hemicellulose
Near infrared spectroscopy
Lignin
Statistics
Wood

Wood is a complex material composed mainly of three polymers with relatively small amounts of other organic and inorganic materials. As with many polymeric composites, the primary (wet-lab) quantitative analysis of wood is a difficult and lengthy procedure.

Recent work with near-infrared (NIR) and Fourier transform infrared (FTIR) spectrometry has shown that these instruments are capable of the rapid secondary (indirect) analysis of various solid and liquid products (*1*, *2*). Briefly, secondary analysis consists of obtaining a set of samples, analyzing this set using traditional direct methods, and then dividing this set into two subsets where one subset is used to calibrate the NIR or FTIR and the second subset is used to confirm or verify the calibration equation. Similar samples can then be indirectly analyzed using the calibrated and verified technique. Benefits of indirect analysis include minimal sample preparation, the use of nonskilled labor, and rapid sample analysis. Also, the technique may be applicable for remote, on-line analysis via NIR optical fibers (*2*). Fiber optics may be available in the future for FTIR (*3*).

An assumption made is that all samples analyzed by this secondary technique belong to the same universe defined by the training set (*2*). A problem that often occurs is that the operator does not identify or recognize samples whose composition, processing history, or materials lie outside the training set universe. Anyone using indirect analytical techniques should

---

*Burns is currently staff scientist, Los Alamos National Laboratory, Mail Stop E-535, Los Alamos, N.M. 87545.

frequently check the calibration, use reproducible conditions, have a healthy amount of suspicion, and remember to think (*4*).

I. Primary analytical error and range for all samples

	Pine		Sweetgum[a]	
Component	*Range, %*	*Pooled std. dev.*	*Range, %*	*Pooled std. dev.*
Lignin	8-31	0.85	10-30	0.69
Hemicellulose	20-26	1.25	12-20	0.74
Cellulose	39-53	1.28	41-50	1.01

[a]The control sweetgum sample had unusually high lignin and low hemicellulose values.

Another consideration is that the total error of the secondary technique will be the direct (wet-lab) error plus the additional error of the secondary instrument and procedure. Thus, the error of the secondary method will always be greater than that of the direct procedure used to calibrate the indirect method. In addition, any consistent error in the direct analytical technique will carry over into the indirect method.

NIR has been found to be an effective method for quantitative analysis and is now widely used in the agricultural field for analyzing grains and grasses or forages (*5-7*). The components analyzed have included lignin present in the 2-6% range. A comprehensive monograph has been recently published on the use of NIR for quality control measurements in agriculture (*8*).

Only recently has FTIR been used to quantify wood and the components of wood (*9-15*). Diffuse reflectance (DRIFT), transmission and photoacoustic techniques have been used. One of the problems with analyzing wood by FTIR is that the peaks are rather broad and overlapping. While derivative and deconvolution procedures are available to enhance the spectral resolution (*16-18*), these techniques tend to increase the noise and may give meaningless peaks.

Experimental procedure

Fresh sweetgum (*Liquidambar styraciflua* L.) and loblolly pine (*Pinus taeda* L.) logs were chipped, air-dried, ground in a Wiley mill, and extracted using ethanol:benzene (1:1). Samples of each wood were delignified to various levels using sodium chlorite at a reduced temperature (55°C). About 30 delignified samples of each wood were obtained. These samples were analyzed in duplicate for total lignin (Klason plus soluble lignin). They were analyzed for sugars by gas chromatography, with the glucose content verified by a glucose analyzer using procedures described earlier (*19*). The hemicellulose and cellulose contents were estimated from the sugar analysis, with the glucose (cellulose) content of the pine samples corrected for the mannose content.

The control sweetgum sample had an unusually high lignin and low hemicellulose composition. This may have been due to juvenile wood since the sweetgum wood was taken from a tree of relatively small diameter (4 in.). Also, the lignin content of the samples treated for the longest time (4.5 h) was not as low as desired, as a result of the reduced temperature used for the chlorite procedure (55°C vs. the 70-80°C normally used). The estimated error of the wet-lab analysis was obtained using the duplicate analysis values and is reported as the pooled standard deviation. The averaged analysis of each sample was used for calibration and confirmation of the NIR and FTIR; thus, the wet-lab error should not be directly compared to the indirect error.

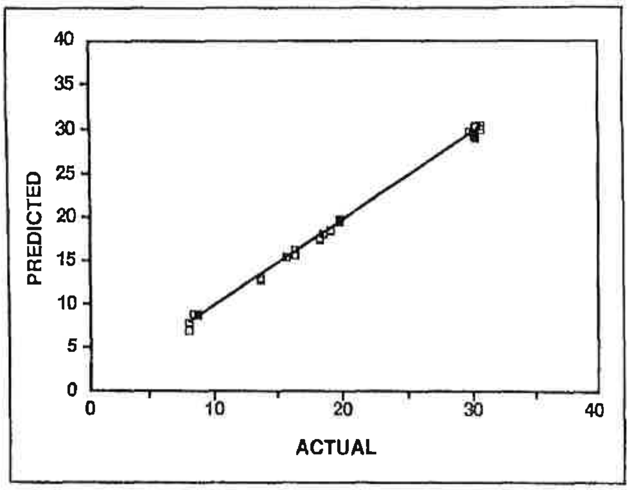
FTIR spectra were obtained using the KBr pellet method as described earlier (*18*), with 300 scans per sample collected. The first derivative spectra were then generated and smoothed. The derivative spectra were used to avoid problems caused by a sloping baseline and to accentuate small spectral differences. The absolute value of the first derivative peak was determined in the 1600-900 cm^{-1} region, giving a total of 19 points for the sweetgum and 16 for the pine samples. An allowance of ±10 cm^{-1} was used to allow for slight frequency shifts often observed with polar samples. Once the peak maximum or minimum was located, the absorbances of that point and the two adjacent points were averaged together in order to minimize noise.

Quantitative interpretation of the spectra was based on each of the 19 (sweetgum) or 16 (pine) absorbance values being a possible "internal" standard. Thus, a total of 18 or 15 different normalized data sets were generated. The total set of about 30 samples per wood species was then divided into 25 calibration and 5 confirmation samples. Each calibration data set was separately regressed against the averaged lignin, hemicellulose, and cellulose content using Minitab stepwise regression. To ensure that only those peaks which were significant were used, the Minitab procedure removed any nonsignificant absorbance values. Each calibration curve was verified using the 5 remaining confirmation samples.

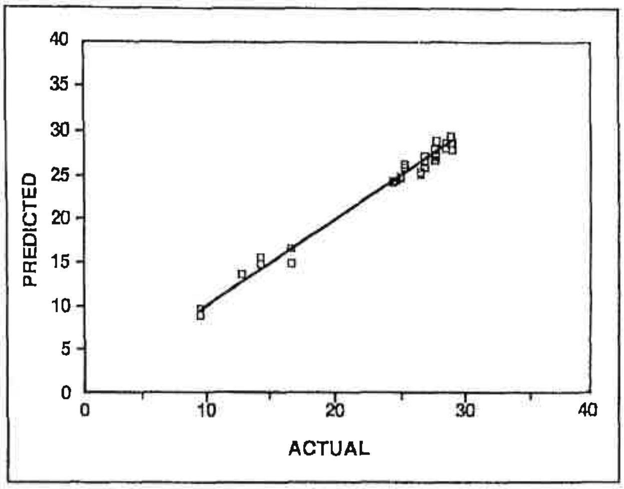
The samples were also analyzed using a Technicon NIR with the air-dried, ground samples directly analyzed using no sample preparation other than being placed in a sample cup. The total samples were equally divided into the calibration and confirmation subsets, and each sample was run twice. Thus, the total number of NIR samples run was essentially twice that of the FTIR samples. Samples were scanned in the 1100-2500 nm (4000-9000 cm^{-1}) region, and the files were saved in 4-nm increments. One pine sample that gave a highly reflective spectra was eliminated.

The standard error of calibration (SEC) was calculated using the difference between the averaged wet-lab analysis and the value generated by

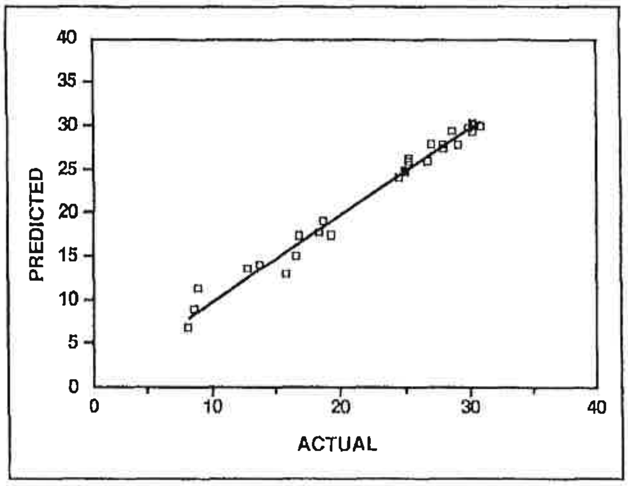
1. NIR calibration of pine lignin



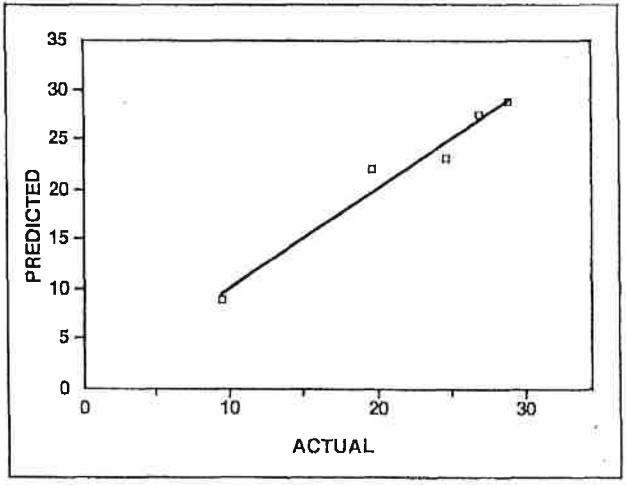
2. Confirmation of pine lignin analysis by NIR



3. FTIR calibration of pine lignin



4. Confirmation of pine lignin analysis by FTIR



the best fit of the NIR or FTIR data. The standard error of prediction (SEP) was calculated using the difference between the confirmation and wet-lab values. Both SEC and SEP are essentially the square root of the mean square error. Direct comparison of the SEC and SEP should be done carefully, since different samples were used for calibration and confirmation of the FTIR and NIR methods and because each NIR sample was run twice.

Results and discussion

The primary (wet-lab) analyses are shown in **Table I**. As mentioned, each sample was analyzed in duplicate, but the averaged analysis was used for the indirect calibration and confirmation analyses. Thus, the pooled standard deviation of the wet-lab analysis cannot be directly compared to the indirect SEC and SEP errors. Although the major change in the sodium chlorite-treated samples was a lowering of the lignin content, smaller changes were also observed in the hemicellulose and cellulose contents. In general, an indirect analytical method may be possible provided that the change in the composition is at least five times greater than the direct sampling error. Based on this rule-of-thumb, the change in the cellulose and hemicellulose composition was considered large enough so that these components were also examined.

The individual NIR and FTIR calibration and confirmation plots are shown only for the pine lignin analysis (**Figs. 1-4**). As mentioned earlier, the NIR samples were each run twice, and thus the NIR confirmation plot has more points (30) than the FTIR confirmation plot (5). The duplicate NIR runs also provide a visual means of determining the NIR reproducibility. The NIR indirect pine lignin method gave an excellent calibration fit ($R^2 = 0.99$, Fig. 1), which was slightly better than the FTIR calibration ($R^2 = 0.98$, Fig. 3). The NIR and FTIR pine lignin confirmation plots also gave good fits, although the confirmation plots, as expected, had slightly more scatter.

The statistical data and the number of wavelengths used for all calibration and confirmation equations are given in **Table II**. In general, NIR gave a smaller SEC in the calibration equations than FTIR. The exception was that FTIR gave better results for the sweetgum cellulose and hemicellulose

II. Secondary NIR and FTIR analysis results for pine and sweetgum samples

	FTIR				NIR			
	Calibration			Conf.[a]	Calibration			Conf.[a]
Component	No. of spectral points	R^2	SEC[b]	SEP[c]	No. of spectral points	R^2	SEC	SEP
				Pine				
Lignin	2	0.98	1.17	1.43	4	0.99	0.41	0.64
Cellulose	3	0.83	2.33	2.20	3	0.92	1.10	2.24
Hemicellulose	NSR[d]	NSR	NSR	. . .	4	0.79	0.91	1.03
				Sweetgum				
Lignin	4	0.97	1.54	1.23	3	0.99	0.89	1.29
Cellulose	3	0.91	0.88	2.02	4	0.85	1.01	2.76
Hemicellulose	4	0.90	0.82	1.00	4	0.88	0.93	3.94

[a] Conf. = Confirmation. [b] SEC = standard error of calibration. [c] SEP = standard error of performance.
[d] NSR = no significant relationship.

III. Comparison of pine lignin analysis by FTIR and NIR

	FTIR	NIR
Sample		
Prep. time, min	10	1
Scan time, min	5	1
Calibration		
R^2	0.98	0.99
F Test	1181	2764
SEC	1.17	0.41
Confirmation		
SEP	1.43[a]	0.64[b]
SEP/SEC	1.22	1.56

[a] 5 samples. [b] 30 samples.

calibration. FTIR did not find a significant relationship for pine hemicellulose, and NIR gave only a relatively poor fit ($R^2 = 0.79$). The reason for the difficulty with the pine hemicellulose component may be that the pine hemicelluloses are mainly composed of hexoses, as is the cellulose. Thus, the FTIR and NIR data may have had no unique spectral points to differentiate between the hemicelluloses and cellulose. The lignin component of both pine and sweetgum gave the best results for both instruments. This may be because the aromatic lignin gives a spectra with several unique peaks.

All calibration equations performed adequately, as shown by the confirmation samples. As expected, however, the SEP was greater than the SEC. Historically, an increase of approximately 50% is observed for NIR in going from SEC to SEP. FTIR showed an average increase of about 40%, while NIR showed a much larger increase of over 100%. In general, the FTIR SEP was less than the NIR SEP for every component except the pine lignin.

A direct comparison of the two procedures using the results from the pine lignin is given in **Table III.** NIR had a much faster and simpler sample preparation and shorter scan time, and it generally gave a better calibration fit. The error increase in going from calibration to confirmation (SEP/SEC) was less for FTIR. Both NIR and FTIR gave the best results for the lignin component, and the calibration equation needed only 2–4 spectral points for any component. Both indirect techniques had difficulty in quantifying the pine hemicelluloses.☐

Literature cited

1. Computerized quantitative infrared analysis (McClure, G. L., Ed.), ASTM, Philadelphia, 1987.
2. McDonald, R. S., Anal. Chem. 58: 1906(1986).
3. Anon., 1989. Chem. Eng. News (Jan. 9): 30(1989).
4. Compton, D. A. C., Young, J. R., Kollar, R. G., Mooney, J. R., and Grasselli, J. G., Computerized quantitative infrared analysis, ASTM, Philadelphia, 1987, p. 36.
5. Barton, F. E., II, and Burdick, D., J. Agric. Food Chem. 27: 1248(1979).
6. Burdick, D., Barton, F. E., II, and Nelson, B. N., Agronomy J. 73: 399(1981).
7. Shenk, J. S., Landa, I., Hoover, M. R., and Westerhaus, M. O., Crop Sci. 21: 355(1981).
8. Williams, P. and Norris, K., Near-infrared technology in the agricultural and food industries, Am. Assoc. of Cereal Chemists, St. Paul, Minn., 1987.
9. Faix, O., Holzforschung 40: 273(1986).
10. Kuo, M-l., McClelland, J. F., Luo, S., Chien, P.-L., Walker, R. D., and Hse, C.-Y., Wood Fiber Sci. 20: 132(1988).
11. Grandmaison, J. L., Thibault, J., Kaliaguine, S., and Chantel, P. D., Anal. Chem. 59: 2153(1987).
12. Berben, S. A., Rademacher, J. P., Sell, L. O., and Easty, D. B., Tappi J. 70(11): 129(1987).
13. Schultz, T. P., Templeton, M. C., and McGinnis, G. D., Anal. Chem. 57: 2867(1985).
14. Schultz, T. P., McGinnis, G. D., and Bertran, M. S., J. Wood Chem. Tech. 5: 543(1985).
15. Schultz, T. P. and Glasser, W. G., Holzforschung 40(Suppl.): 37(1986).
16. Michell, A. J., Carb. Res. 173: 185(1988).
17. Faix, O. and Beinhoff, O., J. Wood Chem. Tech. 8: 505(1988).
18. Schultz, T. P. and Nicholas, D. D., Internat. Analyst 1(9) :35(1987).
19. Schultz, T. P., Biermann, C. J., and McGinnis, G. D., Ind. Eng. Chem. Prod. Res. Dev. 22: 345(1983).

Funds were provided by the State of Mississippi.

Received for review Sept. 6, 1989.
Accepted Nov. 13, 1989.