Combining near and mid-infrared reflectance spectra: Any spectral advantage?

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Introduction

The near infrared (NIR) spectral region roughly defined as the region from 1100 to 2500 nm (9091–4000 cm^{-1}), has over the last twenty years or so been investigated and used extensively for the determination of material composition. It has been applied to the compositional determination of everything from animal feedstuffs[1] and foods[2] to medical diagnostics[3] and industrial process monitoring.[4]

Until recently, the mid-infrared (4000–400 cm^{-1}) has been limited to aiding in spectral interpretation[5] when it comes to many applications for which NIR spectroscopy has proved so useful. This is due to a general belief that mid-infrared spectroscopy required samples to be diluted with KBr and even then would not work for determining parameters such as fiber in dried forages (Personal observations). Recent efforts have shown, however, that mid-infrared spectroscopy in the reflectance mode can determine the composition of dried forages and by-products as well as or even better that NIR spectroscopy.[6–8]

Finally, if at least some information in both regions is unique then calibrations based on both spectral regions might outperform those based on a single region. The objective of this study was to investigate the potential advantage of combining spectral regions for use in calibration development.

Materials and methods

Sample generation and chemical analysis

One hundred and seventy-four samples[9] consisting of six hays (alfalfa, tall fescue, orchardgrass, red clover, timothy and a grasslegume mix of mainly orchardgrass and clover, with some timothy), two straws (barley and wheat), corn cobs, four stovers (two corn and two soybean) and three hulls (peanut, rice and soybean) treated at one of 11 different levels of sodium chlorite were assayed chemically and spectroscopically. Samples were assayed in triplicate for neutral and acid detergent fiber (NDF and ADF respectively), permanganate lignin, total crude protein (CP), cell wall and dry matter digestibility (CWDG and DMDG respectively) and seven nitrobenzene oxidation products (NBOPS) of lignin: *p*-hydroxybenzaldehyde (pHB), vanillin (VAN), a mixed

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product of acetovanillone and 4-allyl, 2-methoxyphenol (MIX), vanillic acid (VA), syringaldehyde (SYAL), an unknown (UNK) and syringic acid (SA). Due to the wide range of feedstuffs available and the effects of the chlorite treatment, a wide range of compositions were present in the data set.[9]

Spectra

Samples, ground using a cyclone grinder (20 mesh), were scanned by diffuse reflectance using a DigiLab FTS-65 Fourier transform spectrometer equipped with a mid-IR (KBr beamsplitter, ceramic source, TGS detector and dry air purge) and an NIR bench (quartz beamsplitter, halogen source, PbSe detector and dry air purge). Samples were scanned neat and diluted to 5% in KBr. For background spectra, sulfur for the NIR (sublimed, 100 mesh) and KBr for the IR region were used. NIR spectra were taken from 10,000 cm^{-1} (1000 nm) to 4,000 cm^{-1} (2500 nm) at a resolution of 4 cm^{-1}, corresponding to 0.4 nm at 1000 nm, 1.2 nm at 1750 nm and 2.5 nm at 2500 nm and at a resolution of 16 cm^{-1}, corresponding to 1.6 nm at 1000 nm, 5 nm at 1750 nm and 10 nm at 2500 nm. Mid-infrared spectra were taken from 4000 cm^{-1} to 400 cm^{-1} at resolutions of 4 and 16 cm^{-1}. In addition, samples had previously been scanned[7] on a grating scanning monochromator (GNIRM) in the NIR region using a Pacific Scientific Model 6250 spectrometer.

Statistical analysis

All spectroscopic data were analyzed using PLS. Because of previous data analysis efforts, the data were analyzed using a series of 36 pretreatment/data point combinations previously found useful.[6] Briefly, spectra were pretreated by either "Mean and Variance Scaling" or "Multiplicative Scatter Correction" followed by either a first or second derivative treatment. In addition, spectral data points were sometimes averaged. A complete list of all pretreatments used maybe found in Reference 6. Finally, the data sets were randomized and the same random order was used for all variables (NDF, ADF, etc.) and all calibration efforts. The best results [highest R^2, lowest relative square difference or RMSD) between actual and predicted values] are reported for each calibration discussed.

To produce a single spectrum from the NIR to the mid-infrared (10,000–400 cm^{-1}, the data points in the region of 4000 cm^{-1}, common to both spectral ranges, were used to match the spectra. Briefly, the average of the three overlapping data points was taken and the ratio of the mid-infrared to the NIR average used to scale all the remaining points in the NIR spectrum. The mid-infrared spectrum and the NIR spectra (minus the three common spectral points) were then simply added to produce one continuous spectrum at the resolution in question (4 or 16 cm^{-1}).

Results and discussion

Chemical analysis

Table 1 shows a summary of the chemical values for the chlorite treated materials.[9]

PLS results for fiber-based assays

In Table 2, the calibration results are presented, using various spectroscopic scans and spectra, for NDF, ADF, lignin, CWDG, DMDG and CP. Comparing the results obtained showed the following: First, the results obtained using the combined spectra at 4 cm^{-1} resolution with KBr dilution of the Fourier mid-infrared scanned samples (COM4KBr) out-performed (higher R^2, lower RMSD) the best results obtained under similar instrument configurations using only NIR (BFT-NIR) or mid-infrared (BFT-IR) spectra on all assays. The results were also better than those obtained on the GNIRM for lignin, CWDG, and DMDG,. Second, the results obtained with other

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Table 1. Summary of chemical values (% DM or % of total nitrobenzene products, *N* = 174).[9]

Assay[a]	MIN[b]	MAX[c]	Mean	Std
NDF	33.4	91.6	68.3	14.3
ADF	26.6	80.3	47.3	10.6
Lignin	2.47	26.2	9.35	5.39
CWDG	1.52	100.2	65.9	27.2
DMDG	11.5	100.2	75.0	22.0
CP	1.55	16.8	7.87	5.21
pHB	.0	26.5	6.36	6.44
VAN	10.2	82.8	36.1	13.5
MIX	.0	76.6	11.4	15.0
VA	.0	37.9	10.3	7.59
SYAL	.0	33.8	13.5	11.3
UNK	.0	47.9	17.8	12.2
SA	.0	24.6	4.53	4.04

[a]NDF = Neutral detergent fiber, ADF = Acid detergent fiber, Lignin = permanganate lignin, CWDG = cell wall digestibility, DMDG = dry matter digestibility, CP = total crude protein, pHB = *p*-hydroxybenzaldehyde, VAN = vanillin, MIX = mixed product of acetovanillone and 4-allyl, 2-methoxyphenol, VA = vanillic acid, SYAL = syringaldehyde, UNK = unknown, SA = syringic acid.
[b]Minimum.
[c]Maximum.

spectral combinations (4 cm^{-1} resolution without KBr dilution and 16 cm^{-1} resolution with or without KBr dilution) showed improvement over the GNIRM, BFT-NIR and BFT-IR results in only a few instances. For example, the results obtained for DMDG were better for the combined spectra at 16 cm^{-1} without KBr dilution (COMB16). Possible reasons for the lack of success with the other combined spectra include sample heating by the mid-infrared beam for the non-KBr diluted samples and lack of spectral resolution for those spectra obtained at a resolution of 16 cm^{-1}.

PLS results for lignin oxidation products

In Table 3, the calibration results are presented for various spectroscopic scans and spectra variations for seven NBOPS of lignin. Unlike the previous results for fiber based assays, the best results for combined spectra for three of the assays (pHB, UNK and SA) were found using

Table 2. Partial least squares analysis results for near infrared analysis for fibers, permanganate lignin, digestibility and crude protein.

Spectra[a]	NDF[b]		ADF		Lignin		CWDG		DMDG		CP	
	R^2	RMSD[c]	R^2	RMSD	R^2	RMSD	R^2	RMSD	R^2	RMSD	R^2	RMSD
GNIRM	0.974	2.29	0.980	1.51	0.946	1.26	0.945	6.39	0.962	4.28	0.994	0.39
COMB4	0.960	2.85	0.967	1.94	0.926	1.47	0.942	6.64	0.959	4.53	0.986	0.62
COM4KBr	0.968	2.56	0.978	1.56	0.957	1.12	0.960	5.53	0.970	3.88	0.989	0.55
COMB16	0.964	2.71	0.963	2.06	0.930	1.44	0.942	6.63	0.965	4.19	0.989	0.55
COMB16KBr	0.959	2.92	0.969	1.88	0.932	1.41	0.923	7.55	0.938	5.50	0.986	0.62
BFT-NIR	0.952[d]	3.12	0.964e	2.01	0.923[f]	1.50	0.936e	6.94	0.949[e]	4.99	0.980[f]	0.73
BFT-IR	0.965[d]	2.70	0.969e	1.88	0.941[f]	1.32	0.956[f]	5.84	0.965[f]	4.22	0.988[d]	0.57

[a]GNIRM = scanning (grating) near infrared monochromator, 64 scans; COMB 4, 16 = combined Fourier transform near and mid-infrared spectra at 4 or 16 cm^{-1} resolution 64 scans' COMB 4, 16 KBr = with KBr diluted samples; BFT-IR, BFT-NIR = best mid-infrared or near infrared results achievied without combining spectra using 64 scans.[7]

[b]NDF = neutral detergent fiber, ADF = acid detergent fiber, CWDG = cell wall digestibility, DMDG = dry matter digestibility, CP = total crude protein.

[c]RMSD = relative means square difference.

[d]16 cm^{-1} resolution no KBr.

[e]4 cm^{-1} resolution KBr diluted.

[f]16 cm^{-1} resolution KBr diluted.

Table 3. Partial least squares analysis results for lignin nitrobenzene oxidation products.[a]

Spectra[b]	pHB		VAN		MIX		VA		SYAL		UNK		SA	
	R^2	RMSD[c]	R^2	RMSD	R^2	RMSD	R^2	RMSD	R^2	RMSD	R^2	RMSD	R^2	RMSD
GNIRM	0.946	1.49	0.932	3.56	0.933	3.99	0.868	2.82	0.911	3.37	0.841	5.17	0.687	2.28
COMB4	0.917	1.92	0.928	3.76	0.950	3.42	0.872	2.75	0.967	2.06	0.899	3.96	0.746	2.11
COMB4 KBr	0.869	2.51	0.948	3.13	0.951	3.36	0.884	2.62	0.971	1.91	0.862	4.57	0.717	2.17
COMB16	0.902	2.03	0.925	3.91	0.927	4.19	0.852	2.94	0.947	2.62	0.885	4.16	0.609	2.52
COMB 16KBr	0.897	2.12	0.932	3.70	0.900	4.77	0.838	3.20	0.921	3.19	0.845	4.83	0.582	2.61
BFT-NIR	0.885[d]	2.19	0.931[e]	3.62	0.888[f]	5.18	0.781e	3.54	0.886[g]	3.89	0.768[e]	5.98	0.560[g]	2.71
BFT-IR	0.937[d]	1.63	0.946[e]	3.20	0.952[d]	3.37	0.903e	2.37	0.970[e]	1.95	0.903[d]	3.92	0.748[d]	2.11

[a]pHB = *p*-hydroxybenzaldehyde, VAN = vanillin, MIX = mixed product of acetovanillone and 4-allyl, 2-methoxyphenol, VA = vanillic acid, SYAL = syringaldehyde, UNK = unknown, SA = syringic acid.
[b]GNIRM = scanning (grating) near infrared monochromator, 64 scans; COMB4, 16 = combined Fourier transform near and mid-infrared spectra at 4 or 16 cm^{-1} resolution, 64 scans; COMB4, 16 KBr = with KBr diluted samples; BFT-IR, BFT-NIR = best mid-infrared or near infrared results achieved without combining spectra using 64 scans.[7]
[c]RMSD = relative means square difference.
[d]4 cm^{-1} resolution no KBr.
[e]16 cm^{-1} resolution KBr diluted.
[f]4 cm^{-1} resolution KBr diluted.
[g]16 cm^{-1} resolution no KBr.

non-KBr diluted samples at 4 cm^{-1} resolution, with the other results (VAN, MIX, VA SYAL) best using KBr diluted spectra at 4 cm^{-1}, as found for the fiber based measures. While the results using the combined spectra were an improvement over those obtained using NIR spectra alone (better than all but pHB for the GNIRM spectra and all the BFT-NIR results), the results were only a slight improvement over those obtained in the mid-infrared (BFT-IR) with results worse for pHB and VA and about the same for VAN, MIX, SYAL, UNK and SA. It would thus appear that combining NIR and mid-infrared spectra offered little or no benefit over mid-infrared spectra alone for analysis of lignin NBOPS.

Conclusions

Results have shown that for some assays and under some spectral conditions, the combining of mid-infrared and NIR spectra can result in improvements in PLS calibration results. Finally, results indicated that the combining of mid-infrared and NIR spectra would be of most advantage when spectra of low quality are being used for developing calibrations.

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