Mature instrument, immature technology: is near infrared spectroscopic analysis of high-moisture materials a serious proposition?

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Introduction

Increasingly, near infrared (NIR) spectroscopic analyses are used for high-moisture materials. Zero or minimal sample preparation and immediacy of analytical results are real incentives for their development. Their availability facilitates at-line and on-line applications. However, development of calibrations, whether global or local, represents a considerable investment. Calibration viability largely relies upon instrument stability so that contemporarily collected spectra fall within the domain of the calibration population. Instrument changes that jeopardise this sustainability negate the investment inherent in calibration data. Stable reference cells that spectrally mimic the high-moisture material being analysed only can provide insurance against this. Such reference cells are rare. This paper discusses an example where two maintenance events radically altered an NIR instrument and rendered a large, global calibration database for fresh sugarcane useless.

Materials and methods

This paper focuses on an NIRSystems (Silver Spring, MD, USA) Model 6500 scanning monochromator (#3422-9405) acquired in August 1995. Applications were developed for Brix, commercial cane sugar (CCS), fibre, moisture and polarisation reading of disintegrated sugarcane stalk tissue.[1] Samples, drawn from clonal evaluation trials conducted on the northeast coast of Queensland (16° 15´ to 18° 15´ S Lat.), were scanned in a large cassette module (LCM).[1,2] An NIRSystems remote reflectance probe, with an 1800 mm fibre-optic bundle, provided the sample to instrument interface. Earlier research established the feasibility of analysing sugarcane stalk tissue, a high-moisture material, and demonstrated the impracticality of using NR7080 cells.[3,4] The instrument has been used primarily in this configuration but also has been used extensively when configured with either sample-transport or indexing-cup modules for analysis of dried materials.

A second NIRSystems Model 6500 scanning monochromator (# 6189-9812), fitted with auto-gain detectors and a "fast" motherboard, was acquired in September, 1999. The instrument was interfaced to a second LCM with an NIRSystems remote reflectance probe fitted with a 600 mm fibre-optic bundle.

Standardisation to "slave" the 1999 instrument to the 1995 instrument was undertaken in September 1999. A black-anodised cell, 100 mm (H) × 160 mm (L) × 125 mm (W), fitted with one quartz face, was used. Clean mature stalks from ten random clones were passed through a Dedini (Piracicaba, SP) disintegrator and mixed for 90 s. The cell was packed full with a sub-sample of this material so no voids were present on the window. The cell's contents were scanned via the remote reflectance probe

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to yield a mean spectrum. Each sample was scanned three times on each instrument, with sequential scans taken on alternate instruments. The first scan for sequential samples commenced on alternate instruments. A mean spectrum was generated for each sample's spectra from each instrument. A "SCORE" (WinISI, ISI, PA, USA) was generated for each instrument's spectra, and the sample (clone) with mean spectra nearest the population mean in each population identified.

A "10% check" population captured each year (1996–2000) allowed retrospective monitoring of the 1995 calibrations. Each population contained a random 10% of scanned samples plus significant *H* and *t* outliers. Supplementary populations were captured in 1996 and 1997.[5] Population structure and the relationship to the calibration domain were monitored using the "SCORE" function.

Seven spectral populations (1995–1998) were subjected to "evolutionary" calibration development.[5] A random 50% of each population was spectrally selected using the SCORE function and combined for calibration. The residual of each was combined for a "prediction" population.

In March 1999, the detectors in the remote reflectance probe were "adjusted" during a routine service. In June 2000, the PbS detectors in the remote reflectance probe were replaced.

Results and discussion

Internally, the pre-maintenance populations (1996–98), showed variation for median *H* value (0.32–0.93) and the number of components required for the SCORE (13–32; Table 1). The proportion of samples with a significant global *H* value (> 3.0) varied from 1 / 80 (L6, 1996) to 40 / 711 (10%–CHK, 1997). Relative to the 1995 base population, only the 10%–CHK (1998) population had an average $H > 3.0$. A relatively large number of samples (156/770 ≈ 20%) had an *H* value > 3.0. Unusual crop conditions cannot explain this.

The SCORE details for the post-maintenance populations (1999 and 2000) themselves are benign, with median *H* values less than the 1995 base population, showing each populations was quite cohesive. The number of components for the SCORE file equalled that for the 1995 base population (Table 2). Relative to the 1995 base population, the populations were in marked contrast to the pre-maintenance situation (Table 1). The two populations fell well outside the bounds of the 1995 base population, as indicated by *H* values of 12.4 and 20.4, respectively. All samples fell outside the bound set by the global $H = 3.0$. Cyclonic disturbances experienced in the pre-harvest period influenced crop conditions in 1999 and 2000, but these are not considered to have impacted the relationships of the check

Table 1. Details of pre-maintenance spectral populations used for calibration development and monitoring of NIR applications for analysis of quality components of mature sugarcane stalk tissue, with results of internal global distance (*H*) analyses for each population and results for *H* analyses for each population relative to the base calibration population.

Population			Internal			Relative to base population (1995)		
Year	Detail	*N*	Median *H*	# *H* > 3.0	# components for *H*	Average *H*	Median *H*	# *H* > 3.0
1995	Base	1,764	0.686	42	32	1.000	0.709	43
1996	D1X	252	0.687	10	28	1.055	0.890	3
	L5	471	0.655	18	22	0.603	0.530	0
	L6	80	0.927	1	17	1.023	0.803	2
	10%–CHK	332	0.518	17	19	1.434	0.861	22
1997	10%–CHK	711	0.366	40	32	1.573	0.881	58
	NIR	372	0.773	9	13	0.916	0.828	2
1998	10%–CHK	770	0.316	44	32	3.362	1.536	156

Table 2. Details of post-maintenance spectral populations used for monitoring of NIR applications for analysis of quality components of mature sugarcane stalk tissue, with results of internal global distance (*H*) analyses for each population, and results for *H* analyses for each population relative to the base calibration population.

Population			Internal			Relative to base population (1995)		
Year	Detail	*N*	Median *H*	# *H* > 3.0	# components for *H*	Average *H*	Median *H*	# *H* > 3.0
1995	Base	1,764	0.686	42	32	1.000	0.709	43
1999	10%–CHK	293	0.336	21	32	12.400	7.957	293
2000	10%–CHK	749	0.403	38	32	20.422	15.207	749

populations relative to the 1995 base population. Crop conditions in 1997 were influenced also by a cyclonic disturbance, yet spectral populations in that year fell within the domain of the 1995 base population (Table 1). The maintenance performed on the instrument in 1999 and 2000 is the most likely explanation for the marked shift in relationships relative to the 1995 base population.

Analysis of disintegrated, mature-stalk tissue of sugarcane using NIS has been successful, with marginal benefit being gained from evolutionary calibration development using supplementary data gathered from 1996 to 1998.[5] Population statistics for the amalgamated calibration population (Table 3) show that there was ample variation (for example, *CV* % ($=\sigma / \bar{x}$) values from 3.5 for moisture, to 12.6 for fibre) for all components. Calibration statistics generally were excellent, with R^2 values ranging from 0.94 to 0.99. Standard errors of calibration (*SEC*) were small, gauged either as a *CV* % value or as a ratio of the population standard deviation. Comparison of these calibration statistics with those for the base 1995 population[1] indicates only a marginal deterioration in key statistics. These results make two clear statements. The care taken in structuring the initial calibration population allowed de-

Table 3. Population statistics for five quality components of disintergrated, mature-stalk samples for a spectrally-selected portion of combined populations analysed from 1995–1998, together with a summary of calibration statistics.

Measure[a]	Brix	CCS	Fibre	Moisture	Pol. reading
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(°Z)
No. samples	2258	2266	2275	2134	2263
Mean	213.9	152.2	130.1	688.4	82.2
Minimum	103.9	63.4	85.7	617.7	38.6
Maximum	273.9	193.9	200.9	824.8	105.0
SD	19.45	17.31	16.34	24.24	9.39
No. terms	13	14	15	15	15
SEC	2.14	3.33	3.91	2.51	1.13
R^2	0.99	0.96	0.94	0.99	0.99
SECV	2.20	3.44	4.06	2.59	1.16

[a]*SD* = standard deviation; *SEC* = standard error of calibration; R^2 = multiple coefficient of determination; *SECV* = standard error of cross-validation

Table 4. Prediction statistics for five quality components of disintegrated mature-stalk sugarcane samples, resulting from application of calibrations developed on a spectrally-selected portion of the combined 1995–1998 populations, to the residual portion of the combined population.

Measure[a]	Brix	CCS	Fibre	Moisture	Pol. reading
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(°Z)
No. samples	2374	2368	2374	2210	2372
$\bar{x}$	213.76	151.90	129.16	689.79	82.06
Slope (b)	1.008	0.967	0.991	0.991	0.981
r^2	0.976	0.897	0.890	0.979	0.954
SEP	3.10	6.05	4.85	3.43	2.09
Bias	0.061	0.008	–0.170	0.299	–0.026
SEP(*C*)	3.10[b]	6.05[b]	4.85	3.42[b]	2.09[b]

[a] r^2 = simple coefficient of determination; *SEP* = standard error of prediction; *SEP*(*C*) = standard error of prediction, corrected for bias
[b] *SEP* and *SEP*(*C*) values fall above threshold of significance designated by WINISI

velopment of robust calibrations that really performed well in three seasons after 1995. There was little benefit in performing evolutionary calibration development because of this robustness. The predictive value of these calibrations on the residual 1995–1998 population shows their general acceptability (Table 4), with r^2 values of ≈ 0.89 for CCS and fibre and > 0.95 for the other components. Slope (b) values were near 1.0 for all components except CCS (0.967) and all bias values were small.

The standardisation exercise to slave the 1999 instrument to the 1995 instrument was unsuccessful. The only recourse was to incorporate the spectra used as a rep. file for use with the transferred calibrations, which would have been unsatisfactory. The reason for this failure is obvious. Comparable spectra from the master and slave populations suggest that the two instruments are radically different. The spectrum for the "master" is markedly compressed in the "*Y*" axis (Figure 1). This is after only the first of the two maintenance events. Obviously, this standardisation was attempted too late. A SCORE test revealed the standardisation populations fell well outside the bounds of the 1995 base population, as defined by a global $H = 3.0$. Alone, this could be considered a possible sampling artifact, but is unlikely as the calibrations displayed robustness prior to conduct of any instrument maintenance. The hypothesis that maintenance altered the instrument, so that post-maintenance spectral populations statistically fell outside the domain of the 1995 base population, appears increasingly real.

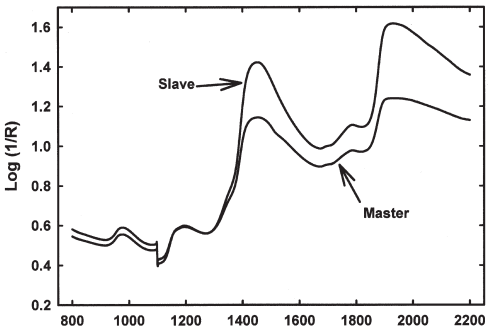

Figure 1. Comparative mean spectra for a sample of disintegrated sugarcane culm from the master (1995) and slave (1999) Model 6500 scanning monochromators.

Calibrations developed from the 1995–98 data (Table 3) performed poorly on the spectral data collected in 1999 (data not shown) and 2000 (Table 5). Few of the critical statistics were acceptable. This is not surprising given the relation-

Table 5. Prediction statistics for five quality components of disintegrated mature-stalk sugarcane samples, resulting from application of calibrations developed on a spectrally-selected portion of the combined 1995–1998 populations, to the 10% check population, 2000.

Measure[a]	Brix	CCS	Fibre	Moisture	Pol. reading
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(°Z)
No. samples	749	749	749	742	749
$\bar{x}$	211.80	154.13	127.34	692.68	82.31
Slope (b)	1.012	1.037	0.993	1.148	1.033
r^2	0.847	0.856	0.829	0.741	0.897
SEP	8.05	11.68	10.30	13.58	3.67
Bias	–5.11[b]	9.62[b]	7.51[b]	–5.71[b]	2.08[b]
SEP(*C*)	6.23[b]	6.63[b]	7.06[b]	12.33[b]	2.65[b]

[a]r^2 = simple coefficient of determination; *SEP* = standard error of prediction; *SEP*(*C*) = standard error of prediction, corrected for bias
[b]*SEP* and *SEP*(*C*) values fall above threshold of significance designated by WINISI

ship of these spectral populations to the 1995 base population (Table 2). Calibration redevelopment for the five components using the 1995–2000 data yielded statistics (Table 6) that were comparable to those resulting for calibration re-development using the 1995–1998 data (Table 3). A rigorous independent test of these calibrations was not possible. Use of the 1995–2000 calibrations on the residual

Table 6. Population statistics for five quality components of disintegrated, mature-stalk tissue for a spectrally-selected portion of combined populations analysed from 1995–1998 plus the 1999 and 2000 10% check populations, together with a summary of calibration statistics.

Measure[a]	Brix	CCS	Fibre	Moisture	Pol. reading
	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(°Z)
No. samples	3235	3194	3243	2996	3199
Mean	212.70	152.85	129.11	689.56	82.15
Minimum	103.90	37.06	81.20	617.70	25.53
Maximum	273.90	193.90	200.90	824.80	104.98
SD	18.91	17.08	16.36	23.63	9.10
No. terms	13	14	15	13	14
SEC	2.36	3.40	4.11	2.89	1.18
R^2	0.984	0.960	0.937	0.985	0.983
SECV	2.41	3.62	4.34	3.20	1.26

[a]*SD* = standard deviation; *SEC* = standard error of calibration; R^2 = multiple coefficient of determination; *SECV* = standard error of cross-validation

1995–1998 population, not surprisingly, gave statistics comparable (data not shown) to those produced by use of the 1995–1998 calibrations on the same population. Application of the 1995–2000 calibrations to the 1999 or 2000 populations (data not shown) produced statistics that were not as bad as obtained from use of the 1995–1998 calibrations (Table 5). Obviously, they were less desirable than could be achieved for a population falling within the domain of the calibration population.

The above scenario demonstrates the vulnerability of calibrations for high-moisture materials, particularly when based on single instruments. There is a real need for the development of stable reference cells that spectrally mimic the high-moisture material of interest. Their existence would allow frequent checks of instrument functionality. Differences resulting from maintenance may be resolvable with the existence of such standards. In multi-instrument sites, or networks, the need for standard cells is not as critical, but still would be highly desirable.

In the absence of standard cells, instrument manufacturers, particularly for single-instrument or isolated installations, should exercise a greater duty of care. Such instruments should be slaved frequently (for example, six-monthly) to a protected "master" maintained by the manufacturer. This should provide insurance against an instrument being changed by maintenance. Within limits, standardisation can negate maintenance-related changes and protect investment in the development of high-moisture material calibrations. In the case reported here, "maintenance" altered an instrument. The most basic duty of care obligation should dictate that a manufacturer repeat maintenance until an acceptable instrument, i.e. one that can be standardised, and so remain true to existing calibrations, results.

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