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Calibration Transfer from Dispersive Instruments to Handheld Spectrometers

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The Foss NIRSystem 6500 is one of the most commonly used laboratory instruments in agriculture and in particular in feed. New technological developments include micro-electro mechanical system (MEMS) technology, used in miniature handheld instruments such as the Polychromix Phazir spectrometer that are increasingly required for on-site analysis. The objective of this study was to assess the potential of a calibration transfer from the Foss NIRSystem 6500 to the Polychromix Phazir. The results show that good calibration models were obtained for various feed properties (fat, fiber, protein, and starch) developed on a Foss NIRSystem 6500, based on a spectral database of 9164 samples transferred to a Polychromix Phazir handheld spectrometer.

Index Headings: **Near-infrared spectroscopy; NIR calibration; Transfer: Micro-electro mechanical systems; MEMS.**

INTRODUCTION

As explained by Dardenne et al.,[1] many research centers and industries focusing on forage and feed have developed important near-infrared (NIR) data sets associated with particular reference methods. In some cases, these datasets contain more than 2000 samples per commodity. When new instruments arrive on the market, clearly the same work cannot be reproduced for each instrument (i.e., each instrument cannot be individually calibrated). There is, therefore, great interest in being able to use already-available information by transferring the NIR datasets from one instrument to another. Calibration transfer is regarded as a mathematical procedure whereby a model or data are transformed so that they are compatible with multiple analytical instruments,[2] in other words the aim is to make spectra from one instrument look like they were taken on another instrument. These calibration transfer techniques are used to format data for compilation from multiple instruments to extend their applicability. In this way, we avoid the need not only for new measurement developments, but also for costly and time-consuming reference method analyses. Shenk et al.[3] did some work on transferring NIR calibrations between seven NIR scanning monochromators, with one of them selected as the master instrument. The equations developed with this master instrument were transferred to the other instruments, with a satisfactory transfer of all the properties. They concluded that the computerized transfer of equations could be a satisfactory alternative to individual instrument calibration. A large number of techniques, such as direct standardization (DS), piecewise direct standardization (PDS), orthogonal signal correction (OSC), direct orthogonalization (DO), finite impulse transform (FIR), and wavelet transform (WT), have been described extensively in the literature.[4–16] In addition, interesting reviews by Fearn[17] and Feundale et al.[18] have featured some of these techniques for calibration transfer in NIR.

New technological developments in NIR spectroscopy include the use of micro-electro mechanical system (MEMS) technology.[19] MEMS technology is the integration of mechanical elements, sensors, actuators, and electronics on a common silicon substrate through microfabrication technology. This technology brings together silicon-based microelectronics and micromachining technology, allowing the development of smart products, augmenting the computational ability of microelectronics with the perception and control capabilities of microsensors and microactuators, and expanding the prospect of possible designs and applications. Many new MEMS applications are emerging; these include spectroscopy in order to meet the increasing demand for on-line analysis. Miniature handheld instruments are increasingly required in the forage and feed industry.

The objective of this study was to assess the potential of a calibration transfer from dispersive instruments (Foss NIRSystem 6500) to a handheld spectrometer, the Polychromix Phazir, based on MEMS. A similar study was performed by Barreiro et al.[20] when trying to transfer a calibration model from a Foss 6500 to a portable prototype. The best results they obtained were obtained using piecewise direct standardization (PDS). In this paper, a simple transfer algorithm, which uses paired spectra to assess a transfer function between the signals of both instruments, is applied and compared to PDS. For this, a set of 25 samples was analyzed using both instruments, with the handheld spectrometer Polychromix Phazir considered the master and the Foss NIRSystem 6500 the slave. Linear partial least squares (PLS) regression was used to adjust for gain and bias differences between the two spectrometers.

MATERIALS AND METHODS

Samples. To carry out this study, two feed sample sets were used:

(1) The first was a set of 9164 feed samples comprising various ingredients collected by CRA-W over a few years from several projects and analyzed using a Foss NIRSystem 6500. This feed database (or calibration set) was used to build different calibration models using reference values for fat, fiber, protein, and starch content.
(2) The second set consisted of 25 well-known feed samples. This second set was split into two subsets: a standardization set (14 samples) and a test set (11 samples). Both subsets were analyzed with the Polychromix Phazir and Foss NIRSystem 6500 instruments, both including the reference values for fat, fiber, protein, and starch content. The standardization set and the test set were used for the

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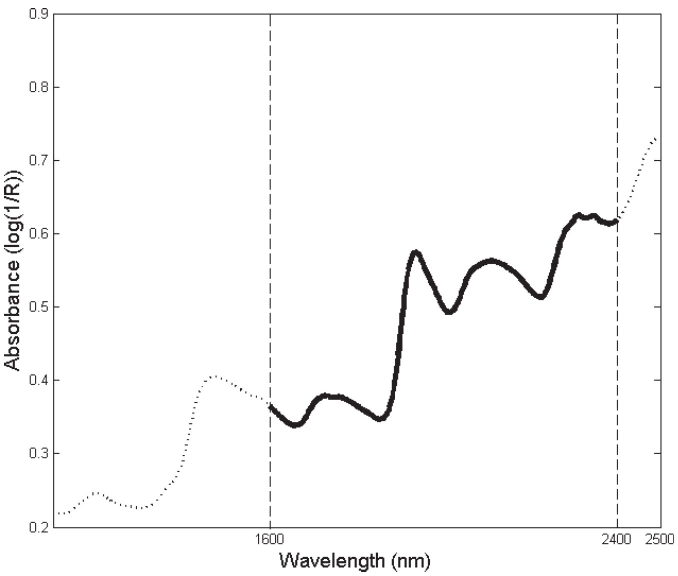

FIG. 1. Spectrum of the Foss NIRSystem 6500 (1100–2500 nm) (dotted line) and of the range of the Polychromix Phazir (1596–2394 nm) (continuous line).

standardization of the instruments and the validation respectively.

Instruments. ***The Foss NIRSystem 6500 Instrument.*** The Foss NIRSystem 6500 operates in reflection mode in the 1100 to 2500 nm range, with a step size of 2 nm. Each spectrum acquired over 1 min is the average of 32 scans performed on the sample analyzed in a quarter cup. Within the framework of this study, each feed sample was ground and spread in two quarter cups. Two spectra were acquired for each feed sample.

The Polychromix Phazir Instrument. The Polychromix Phazir is an integrated handheld NIR spectrometer using MEMS technology. It combines a digital transform spectrometer (DTS) engine, a reflectance probe, rechargeable batteries, an integrated computer, color LCD display, and software into one unit that can be used remotely, such as in field applications. It requires minimal training, is easy to use, gives an instant answer, and produces a nondestructive measurement. The spectra are acquired within a wavelength range of 1600 to 2400 nm by a non-constant step of about 8 nm. Each spectrum acquired over 2 s is the average of five scans performed on the sample analyzed. Within the framework of this study, five measurements were taken at different positions on each quarter cup prepared for the analysis on the Foss NIRSystem 6500. Ten spectra were acquired for each feed sample.

Methodology. In order to be able to transfer the calibration dataset of 9164 samples measured with the Foss NIRSystem 6500 (slave) to the handheld Polychromix Phazir spectrometer (master), the 14 samples of the standardization set and the 11 samples of the test set were analyzed using both instruments and used for the standardization of the instruments and the validation, respectively. Although it might be possible to transfer spectra without samples being measured in both instruments (using a covariance matrix), it is an advantage to have all the samples measured in both the Foss NIRSystem 6500 and the Polychromix Phazir.[14]

Because the Polychromix Phazir spectrometer works in the range of 1600 to 2400 nm with a non-constant step, the most important modification is the interpolation of the 700 wavelengths (1100–2500 nm with a constant step of 2 nm) of the Foss NIRSystem 6500 to the 100 wavelengths of the Polychromix Phazir. The interpolation used is a shape-preserving piecewise cubic interpolation, which preserves convexity to scattered convex data.[21] Figure 1 shows a spectrum of the Foss NIRSystem 6500 (dotted line) and the range of the Polychromix Phazir (continuous line).

After interpolation, a simple correction based on the spectral difference at each wavelength was performed. For this, the difference between the value of the mean spectrum of the standardization set measured with the Polychromix Phazir and the mean spectrum of the same samples measured with the Foss NIRSystem 6500 was determined at each wavelength. This difference was then simply added to each of the spectra constituting the large calibration dataset. After this correction was done, PLS models could be constructed to verify the performance of the transfer.

TABLE I. Global models using the Foss NIRSystem 6500 full reduced range (1600–2400 nm).

Property (in % MS)	N	Min	Max	Factors	RMSECV	R^2
Fat	3994	0.66	33.87	13	0.79	0.98
Fiber	2946	1.01	29.96	14	1.1	0.93
Protein	7665	8.04	63.64	12	1.3	0.97
Starch	1315	2.11	74.01	12	1.78	0.99

RESULTS

Based on the spectral database of 9164 feed samples analyzed using a Foss NIRSystem 6500, different calibration models were constructed using reference values for fat, fiber, protein, and starch content. Because the spectral transfer is dedicated to calibration purposes, the optimization of the calibration must be carried out for one specific calibration model at a time. These models were constructed using PLS as the chemometric method and based on the same range of wavelengths as the Polychromix Phazir (1600–2400 nm). The results are summarized in Table I. Because not all the 9164 calibration samples have reference values for all the properties, for each property, N represents the number of samples used to build the model, "Min" and "Max" represent the minimum and the maximum value, respectively, for that property, the third column indicates the number of PLS factors used, and the last two columns show the root mean squared error when using leave-one-out cross-validation (RMSECV) and R^2, respectively.

As noted earlier, in order to transfer the database from the Foss NIRSystem 6500 to the Polychromix Phazir, the standardization set of 14 feed samples analyzed with both instruments was used. In order to perform this transfer, the first modification was the interpolation, for all the spectra, of the 700 wavelengths of the Foss NIRSystem 6500 to the 100 wavelengths of the Polychromix Phazir. Figure 2 shows the standardization set measured by both instruments after the interpolation of the spectra coming from the Foss NIRSystem 6500.

A second modification consisting of a simple correction based on the spectral difference at each wavelength was necessary. This correction was performed using only the 14 samples included in the standardization set and applied to both

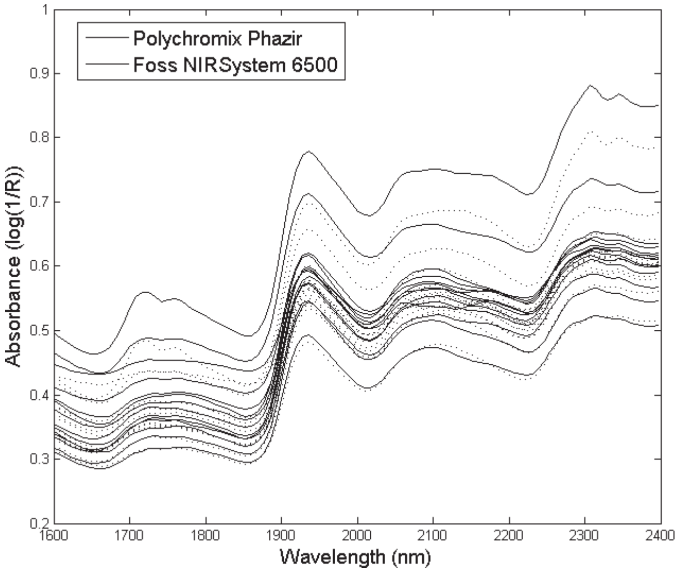

FIG. 2. Interpolation of the spectra of the standardization set (14 samples) from the Foss NIRSystem 6500 range (1100–2500 nm, with a constant step of 2 nm) to the Polychromix Phazir range (1600–2400 nm, with a non-constant step of about 8 nm).

the test set and the calibration set. At each wavelength, the difference between the mean spectrum of the standardization set measured with the Polychromix Phazir and the mean spectrum of the same samples measured with the Foss NIRSystem 6500 after interpolation was determined and added to each of the spectra constituting the large calibration dataset.

In order to check the performance of this simple transfer method, a well known technique, the piecewise direct standardization method, was also applied.[16] PDS involves local multivariate models and it is based on the hypothesis that the spectral information given at a certain wavelength on the Polychromix Phazir spectrometer is contained in a small spectral region of a few neighboring wavelengths on the Foss NIRSystem 6500 instrument. Both transfer methods, the one based on the spectral difference at each wavelength and PDS, have been applied using the standardization set and applied to the test set (11 samples). Figure 3 shows the mean square error (reconstruction error) before and after the transfer methods for the test set. The continuous line corresponds to the reconstruction error between the spectra measured with both instruments after interpolation but before standardization, whereas the other lines show the results after each standardization technique.

As can be observed from the figure, both transfer methods allow the reconstruction error to be reduced for the test set in a similar way, indicating the correct performance of the proposed methodology.

After the large calibration set has been transferred using the method based on the spectral difference at each wavelength, PLS models for each property have been constructed. To build the models, the 14 samples from the standardization set were added to the transferred calibration dataset. The results are shown in Table II. As for Table I, for each property, *N* represents the number of samples used to build the model, but in this case including the 14 samples of the standardization set measured with the Polychromix Phazir.

When looking at Table II, the results obtained reveal that the models developed with the transferred data have the same performances as those shown in Table I when using models constructed on the Foss NIRSystem 6500 in the same range as

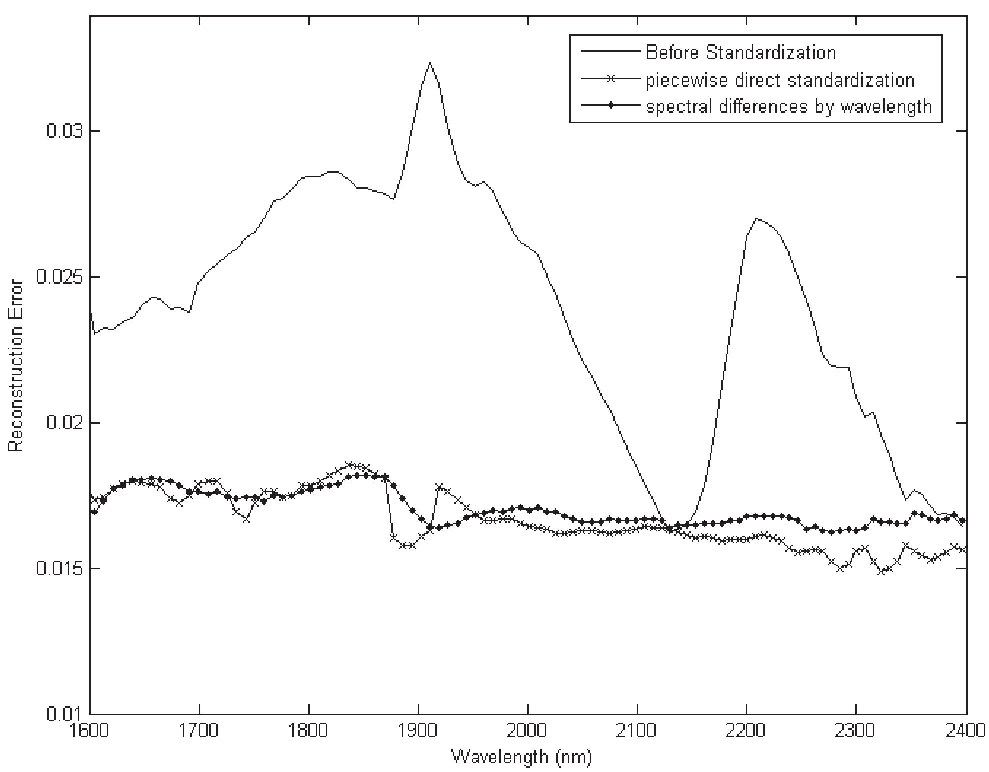

FIG. 3. Reconstruction error before (continuous line) and after the application of the transfer methods (PDS and spectral differences by wavelengths) for the test set (11 samples).

TABLE II. Model transferred to the Polychromix Phazir (1600–2400 nm).

Property (in % MS)	N^a	Min	Max	Factors	RMSECV	R^2
Fat	4008	0.66	33.87	13	0.81	0.98
Fiber	2960	1.01	29.96	14	1.1	0.93
Protein	7679	8.04	63.64	12	1.31	0.97
Starch	1329	2.11	74.01	12	1.8	0.99

[a] The 14 samples of the standardization set (from the Polychromix Phazir) have been added to the database.

TABLE IV. Prediction results for the test set (11 samples) using, at the left side, the model developed with the Foss NIRsystem 6500 before transfer (Table I) and, at the right side, the model developed in the Polychromix Phazir instrument after the transfer of the data (Table II).

Property (in % MS)	Foss reduced model		Transferred model	
	RMSEP	R^2	RMSEP	R^2
Fat	1.32	0.83	1.13	0.91
Fiber	1.65	0.98	2.18	0.87
Protein	1.14	0.87	1.34	0.82
Starch	2.75	0.94	2.66	0.95

the Polychromix Phazir (1600–2400 nm). For example, for fat content and for the same number of factors, a RMSECV of 0.79 and 0.81 were obtained for the Foss NIRsystem 6500 model and the model developed with the data transferred to the Polychromix Phazir, respectively, with the same R^2.

After the models were built, they could be used for predicting the samples included in both the standardization set (14 samples) and the test set (11 samples), this test set being the real validation for the models. Tables III and IV show the prediction results for the standardization set and the test set, respectively, using, at the left side, the model developed with the Foss NIRsystem 6500 before transfer (Table I) and, at the right side, the model developed in the Polychromix Phazir instrument after the transfer of the data as previously explained (Table II). The model parameters used for evaluation are RMSEP (root mean square error in prediction) and R^2.

For fat content, the RMSEP obtained with the transferred data in both cases was lower than that obtained when using the Foss NIRsystem 6500. For fiber content, an increase in RMSEP for both the standardization and the test set was observed. A similar result, but with a lower extension, was obtained for protein content. For starch content, when applying the model to the standardization set there was also an increase in RMSEP but a decrease in the test set in RMSEP (from 2.75 to 2.66), with almost the same R^2. Even if the models could be improved, the prediction results indicate that correct values for RMSEP and R^2 are obtained when the applied equation has been constructed using the transferred data.

CONCLUSION

From the study described here, it can be concluded that the huge dataset on feed built over several years with a Foss NIRSystem 6500 can be easily transferred using new technological developments in NIR spectroscopy, such as MEMS. The methodology proposed here was based on a simple calculation of the spectral differences at each wavelength and proved to give performance similar to that of the well-known PDS method. The PLS models developed with this transferred data to the handheld Polychromix Phazir spectrometer for different feed properties, including fat, fiber, protein, and starch, performed in a way similar to the models developed using the original instrument for measuring the samples (i.e., the Foss NIRSystem 6500). This study has shown that MEMS technology can be easily and rapidly introduced into laboratory and routine analyses by the computerized transfer of the data obtained using classical NIR instruments. This data set transfer should require fewer individual instrument measurements, avoid costly and time-consuming reference method analyses, reduce time when generating models using MEMS, and produce robust models. In order to increase the robustness and longevity of the constructed models, however, we would recommend continually adding new samples and recalculating the models.

TABLE III. Prediction results for the standardization set (14 samples) using, at the left side, the model developed with the Foss NIRsystem 6500 before transfer (Table I) and, at the right side, the model developed in the Polychromix Phazir instrument after the transfer of the data (Table II).

Property (in % MS)	Foss reduced model		Transferred model	
	RMSEP	R^2	RMSEP	R^2
Fat	1.41	0.98	1.26	0.97
Fiber	1.3	0.96	1.74	0.87
Protein	1.19	0.97	1.23	0.97
Starch	1.92	0.98	2.3	0.97

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