PHAZIR™

Determination of Moisture in Carbohydrates

Authors Dr. Suzanne Schreyer and Dr. Frederick Haibach

Polychromix offers self-contained spectrometers that can be rapidly deployed for at-line analysis of powders and granulations. While the procedures described below have been developed for the PHAZIR^{TM}, the principles apply to any NIR spectrometer offered by Polychromix. The goals of this paper are to demonstrate, the PHAZIRs capability of quantitatively measuring moisture in a carbohydrate mixture, a viable standards preparation procedure for full calibration and an abbreviated 2- and 3-level calibration for rapid assessment of drying endpoint.

Implementing the procedures assumes that representative raw materials, a blender, balance and a drying oven are available to reproduce the procedure. Because NIR is used as a secondary method, spectra are often correlated to reference measurements. Use of a reference instrument, like loss-on-drying (LOD) balance or Karl Fischer titrator, for moisture determination is useful for validation of the obtained calibrations.

Introduction

Moisture content determination is important for the processing of many powders. Powders have a large surface area and can absorb a large fraction of water by weight. The absorbed water changes the interactions between individual particles changing the way the powder processes. Water changes the flow, compaction and response to heat of the material. Hygroscopic materials, like sugar, starch and, to a lesser degree, cellulose can absorb relatively large quantities of water.

Wet powders can also affect the finished product. In food and pharmaceutical manufacturing, moisture can reduce the shelf-life of the product. In food, moisture can result in off-flavors as the water enables enzymatic activity and bacterial or mold growth. In pharmaceuticals, the moisture can result in degradation of the active ingredient or crumbling of tablets. Rapid assessment of moisture in powders, in meal, flour, sugars, starch and microcrystalline cellulose is needed to control quality.

Near-infrared (NIR) spectroscopy is sensitive to moisture, and has been used to assess moisture in ground agricultural materials for over 25 years. Water has strong, specific optical absorptions near 925, 1425, and 1925 nm. These arise from NIR photons exciting the vibrational modes of water. Other absorptions in the NIR correspond to other “functional groups” in the molecules, like CH_3, CH_2, CH, OH, NH_2. There are a few challenges to building first principles calibration models for water in the NIR. First, carbohydrates have OH functional groups absorb at similar wavelengths to water. Second, the adsorbed water has a different spectrum from pure water because the water-host interactions change the energy of the water vibrational modes. Finally, the presence of water has competing effects on the transparency of the host material. It fills microscopic voids increasing transparency, and absorbs light at other wavelengths decreasing transparency. By conducting a series of experiments, it is possible to discover mathematical transformations that minimize these effects and result in accurate water calibrations that can be built in about 30 minutes using only a couple of samples.

Partial Least Squares (PLS) is a method commonly used in the NIR community to correlate values, like water content, with spectral changes in the spectrum. Because it is a correlation method, the resulting accuracy depends upon low-noise spectra, representative samples and on the accuracy of the reference method.

Experimental

The process of building the calibration requires: creation of the wet material, mixing and homogenization of wet and dry materials, verification of the moisture content and collecting the NIR data. Much of the equipment required is typical for a formulation development laboratory. The method for creating wet material is adapted from Vojnovic *et. al*.[1] Vials containing mixed wet and dry material having a moisture content spanning 3-20 % w/w were created. The moisture content is validated using a LOD balance. NIR spectra are taken through the bottom of the 20 mL glass vials.

---

[1] Vojnovic, D., Selenati, P., Rubessa, F., Moneghini, M., Zanchetta, A. “Wet Granulation in a Small Scale High Shear Mixer.” *Drug Development and Industrial Pharmacy*, 1992, **18**, 961-972.

Wet lactose/starch blend

Adjustments to the procedure were made for the Patterson- Kelly blender and an 8 quart shell. The use of the intensifier bar allowed for water addition and rapid homogenization of the blend.
A 4 kg batch of lactose (2.7 kg) and corn starch (1.3 kg) was added to the Patterson-Kelley blender with intensifier bar in place. The materials were blended for 5 minutes using both the rotation blending and the intensifier bar. After 5 minutes the mixture was tested for homogeneity by taking out a sample and smearing it on paper to determine if the material was homogenous. No segregation of materials was observed. Two 500 mL amber bottles were used to collect samples of the dry materials.

Next the blending was re-started (rotation and intensifier bar), and 800 mL of water was added through the liquids inlet. After addition of the water, mixing continued for 10 minutes, for a total time of 15 minutes, as recommended by the Patterson-Kelley Blend Master manual. Once mixing was complete, samples were taken of the wet samples and stored in amber bottles for further experimentation.
To estimate the moisture in the wet and dry materials, the volatiles content was determined on the LOD balance using the manufacturer's recommended settings for determining the moisture content of cornstarch. A 3 g sample was dried at 90 °C for a total time of 11 minutes. Weight before and after drying was recorded, and the difference was used to determine the percent moisture of the sample. Mean and standard deviations were determined from triplicates.

Sample preparation

Samples were made up of varying ratios of the wet and dry blend. A total of 11 samples were made for the training data set, by adding from 0 to 100% wet blend to the dry blend, in 10% increments. An additional 6 samples were made for the independent test set, which also spanned the experimental range. The pure wet and dry materials were added directly to the 40 mL borosilicate glass vials, rapidly capped and weighed on an analytical balance. Weights were recorded to the nearest 0.1 mg. The calibration values are shown in Table 1. The contents were briefly mixed using a steel spatula before final capping. The vials were allowed to rest in a 60°C oven for 5 hours to speed equilibration and allowed to cool overnight. The contents of each 40 mL vial were divided into two 20 mL vials, one for NIR measurement and the other for LOD measurement.

NIR measurement

Spectra were taken using a stock PHAZIR^{TM} 1624 using the "Collect M1624" application. The spectrometer was stabilized for 30 minutes and wavelength calibrated before spectra were collected. The reference spectrum was a 10-scan average of a sintered Teflon puck. Each sample spectrum was the result of 5 scans through the bottom of the 20 mL vial. The sample spectra are taken of 3 independent locations at the bottom of the vial.

Materials:

- 2.7 kg lactose monohydrate (SuperTab Spray Dried - Lactose, New Zealand, Batch # BN290120)

- 1.3 kg corn starch (Argo Pure Corn Starch)

- 800 mL tap water

- 500 mL amber jars

- 40 mL pre-cleaned EPA vials with Teflon septa

Equipment:

- Laboratory blender (Patterson-Kelley Master Lab Blender, Model B with intensifier bar)

- Laboratory oven (Precision Scientific 25EM with Watlow 942 temperature controller)

- LOD balance (Mettler Toledo HG63)

- PHAZIR^{TM} 1624, firmware v. 2.26

- PHAZIR Method Generator^{TM} v. 1.5.6.1

Results and Discussion

Preparation of uniform samples is important for NIR. The sampling volumes of the LOD and the PHAZIR^{TM} are very different. The sampling volume for the LOD experiment is about 5 cm^3, whereas the NIR volume is about 5 mm^3. This results in a greater sensitivity of the NIR measurement to inhomogeneities on the scale of 0.5 mm^3 to 50 mm^3. The procedure was developed to provide rapid results without creating moisture inhomogeneity. Adding partially wet material, mixing and then equilibration at an elevated temperature allows moisture to rapidly distribute itself between the wet and dry material.

The uniformity of the material can be demonstrated. The moisture content of fully wetted material and as-received material was determined. The as-received material had a volatiles fraction of 3.17 ± 0.03 % w/w, while the fully wetted material had a moisture content of 19.4 ± 0.8 % w/w. The 1 standard deviation error bars were determined using triplicate measurements. These values are similar to those obtained for the entire calibration set, as shown in Table 1.

Set	Sample	design	actual
Calibration	1	3.17	3.12
	2	4.72	4.49
	3	6.49	6.34
	4	7.92	7.97
	5	9.52	9.42
	6	11.50	11.62
	7	13.10	13.38
	8	14.93	15.32
	9	16.83	17.36
	10	17.79	18.3
	11	19.40	20.15
Test	2	4.29	3.99
	4	7.56	7.37
	5	9.49	9.41
	7	13.12	13.28
	8	15.43	15.77
	10	17.15	17.6
average		11.32	11.46
error (standard dev.)		0.34	

TABLE 1: The differences between the design and the measured moisture values for the calibration and test sets.

The process described by Vojnovic *et. al.*[1] provides wet material sooner than constant-humidity chambers. Partial calibrations using 2 or 3 standards, as discussed below can be completed within 30 minutes. Full calibrations can generally be completed within a working shift, if confirmatory analyses are not needed. Running confirmatory analyses can consume another 2-3 working shifts. Constant humidity experiments generally require 20-40 days of equilibration. At the end, uniformity of moisture in the equilibration jars still need to be determined. Under these conditions, crystal, mold and bacterial growth are possible.

Borosilicate glass vials are a common, and excellent container for NIR spectroscopy. The glass is very transparent and has relatively flat surfaces that allow for uniform presentation of the sample to the spectrometer. The primary source of presentation variation is in material packing and particle size.

A subset of the raw spectra generated from PHAZIR™ is shown in Figure 1. The absorption band, centered on 1940 nm, is assigned to water. The strength of the band increases with moisture content. The spectra are offset from each other. This is likely to be due to changes in the material transparency with moisture and packing.

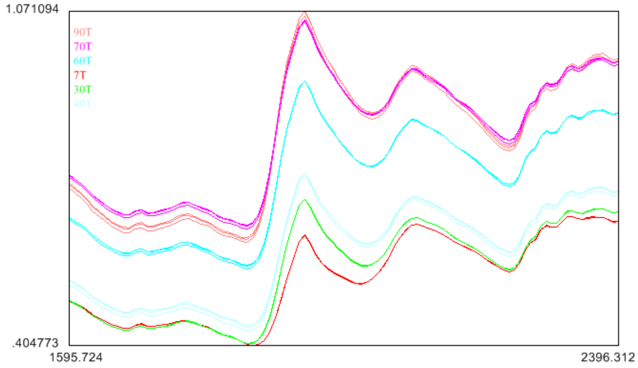

FIGURE 1. Raw spectra of samples with varying % moisture.

Frequently, spectra are manipulated to remove variations that are not related to the property of interest. Baseline correction is one common technique. Because bands are wide and overlapping in the NIR, additional methods are used. Preprocessing conditions were optimized to separate the different moisture levels, and to minimize PLS prediction errors. The best preprocessing was found to be Savitsky-Golay 1st derivative (3 point window, 2nd order polynomial), followed by range normalization. The preprocessed data is shown in Figure 3. PLS is chosen as the calibration method because the response to water in powders is inherently non-linear in diffuse reflectance measurements due to changes in particle refractive index, absorption and particle size. For the PLS model the wavelength range was truncated, and only the region between 1665 – 2232 nm was used. This eliminated regions where absorbance values were too high to obtain a good signal.

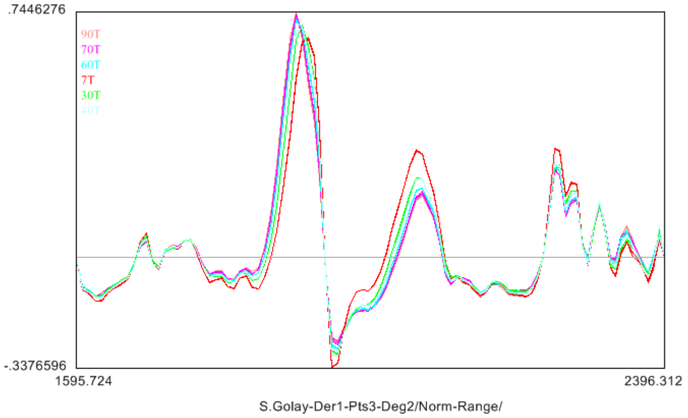

FIGURE 2. Preprocessed spectra of samples with varying % moisture.

A 3-factor PLS model was generated. Leave-five-out cross-validation using PHAZIR MG resulted in root-mean square error (RMSE) for the training data set of 0.17 % moisture, and a correlation (R^2) of 0.999. Model validation on the independent test set gave a RMSE of prediction of 0.28% and an R^2 of 0.997, as shown in Figure 3. These errors are indistinguishable from the reference method.

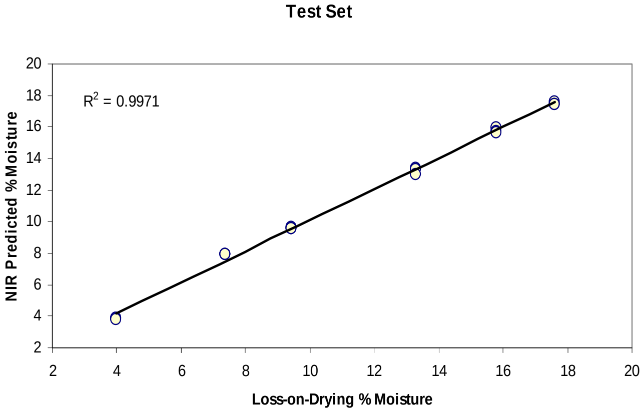

FIGURE 3. Predicted versus LOD values of the test set. RMSE of prediction = 0.27% w/w

A similar study was done using a FOSS in line NIR analyzer for the monitoring of the mixture of lactose, micro-crystalline cellulose and water.[2] The moisture was determined by LOD over a range of 5 to 10%. A 2-factor PLS model was generated, and resulted in a RMSE of calibration of 0.22%, with an R^2 of 0.99. The RMSE of prediction for a test set was higher, at 0.42%. Both of the results are higher than those recorded for the PHAZIR™. The increase in the RMSE of prediction is likely to be a result of the challenges of implementing on-line optical measurement with a laboratory instrument.

Fast calibrations

Using the knowledge gleaned from building a full calibration, a testable and general route to rapid calibrations on carbohydrate powders can be developed for drying end-point determination or qualification testing. The goal is to use a single sample to re-build a working prediction model.
We already know from building the calibration, that the response to moisture is non-linear. The general response to this would be to build a 3-point calibration to incorporate some of the curvature. Using additional samples does allow averaging to occur, and a general improvement of the predictive ability of the calibration. However, additional samples cost the time to create and measure those samples.

A two-point calibration can be created from a single sample and a LOD balance. The sample can be measured using the PHAZIR™ in its wet state, the sample allowed to dry in a LOD balance to obtain a moisture value, and, when cool, the sample can be measured again on the PHAZIR™ in its dry state. If a set of preprocessing conditions can be found that minimize the non-linear response in the NIR spectra, then a 2-point calibration model can be built in the space of 30 minutes or less.

We have simulated this procedure by using the spectra from the driest and wettest materials that were used to build the full calibration. The candidate preprocessing conditions and modeling parameters were tested using leave-five-out cross-validation. Calibration models were tested using the test-set and can be directly compared to the values arrived at above.

The most successful preprocessing conditions are different from the full calibration. Standard Normal Variate (SNV), Savitzky-Golay 2nd derivative (5-point window, 2nd order polynomial) and maximum normalization were used for the 1759 – 2114 nm region. SNV corrects for effective pathlength changes and removes a multiplicative effect. Two factors were used in the PLS model to account for moisture change and other variations. The level of the error, expressed as RMSE of prediction is 0.61 % w/w. A large fraction of this error is the residual non-linearity in response and not a reduction in precision. This change in preprocessing conditions and factor of two error increase is expected since the goals of the calibration are different. Figure 4 summarizes the prediction results.

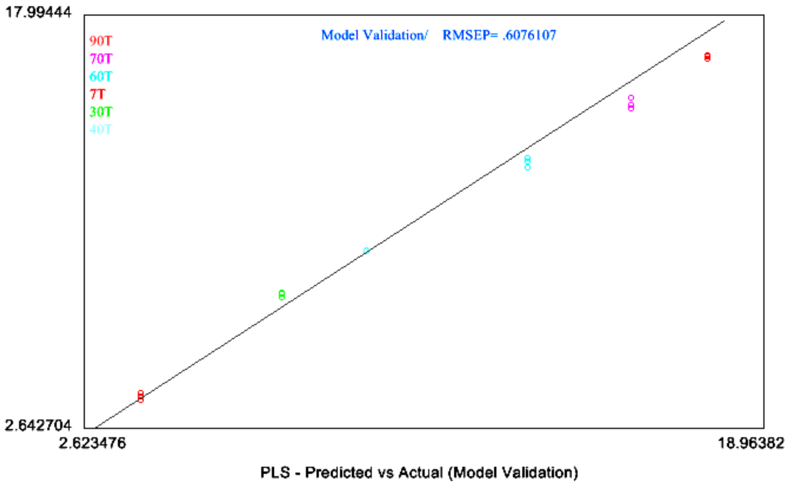

FIGURE 4. Prediction results for the 2-point moisture calibration.

---

[2] Mattes, R.A., Root, E., and Birkmore, A.P. *Spectroscopy Supplement: The Role of Spectroscopy in Process Analytical Technologies*, January 2005, 14-17.

For comparison, a 3-point calibration was created using the same methodology. The additional sample was mid-point containing 11.5 % w/w water. The optimal preprocessing was SNV, Savitzky-Golay 1st derivative (3-point window, 2nd order polynomial) and normalize unit vector over 1759 – 2114 nm. A 2 factor PLS calibration was used. The error of prediction is 0.37 % w/w, which is a minor improvement over the 2-point calibration. The non-linear response is modeled better leading to a reduction in the RMSE of prediction. Precision is slightly improved as shown in Figure 5. The results for the three methods are compared in Table 2. The differences in normalization emphasize different features in the spectra, but have a very similar effect.

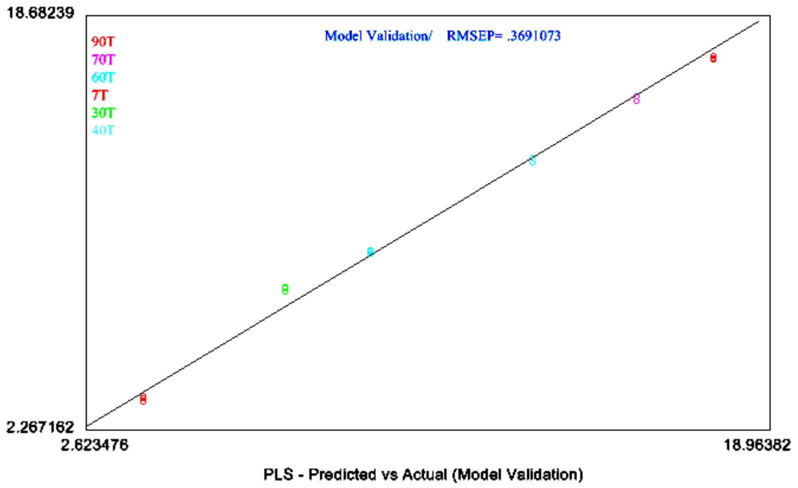

FIGURE 5. Prediction results for the 3-point moisture calibration.

# calibration samples	preprocessing steps	PLS factors	RMSE, prediction (% w/w)
11	SG 321, RN	3	0.28
3	SG 321, VN	2	0.37
2	SNV, SG 522, MN	2	0.61

TABLE 1. Comparison of full calibration, 3-point and 2-point calibrations. SG=Savitzky-Golay, RN=range normalization, VN=vector normalization, MN=maximum normalization.

Conclusions

Accurate and precise calibrations for moisture in carbohydrates can be created on the PHAZIR™ in as little as 30 minutes using a single sample. The RMSE of prediction for 11, 3, and 2 point calibrations are similar and produce robust PLS calibrations for moisture ranges between 3 and 20% w/w, which is adequate to determine drying end points, moisture addition and granulation of carbohydrate-based materials. The calibration can be completed in slightly more time than the reference method when applied to a single sample. Accuracy is comparable to the reference method, and has the advantage of rapid assessment in prediction.

These calibrations are most likely to be used for initial assessment of moisture from multiple containers or drying end-point determination, where the processes are long or multiple samples need to be taken. NIR has the advantage of precision and short analysis time.

Like any secondary method, these calibration procedures do not take into account batch-to-batch variation. Rebuilding the calibrations for multiple lots and archiving the spectra for later analysis will build up the calibrations to the level where they are robust and can be used without the need for 2-point recalibrations.