THE APPLICATION OF IN-LINE SPECTROSCOPIC TECHNOLOGY FOR TWO PHARMACEUTICAL UNIT OPERATIONS

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Real-time, in-line spectroscopic technology for process monitoring and end point determination of pharmaceutical unit operations is of great interest. Recent success has been achieved in our laboratories specific to determination of particle size of drug compounds using nano-crystal technology milling unit operation and for moisture determination in a fluid bed drying unit operation. The in-line process analytical techniques both utilize Near-IR spectroscopy to provide real-time data for process monitoring and control.

In-Line Particle Sizing for Nano Milling Unit Operations
A common strategy for increasing the bioavailability of low solubility drugs is reducing the drug particle size to the low micron range, and in some cases, down to the 100's of nanometer range. During the process, the change in particle size is often monitored through sampling at time points across the milling unit operation run, and taking the samples for analysis at a laboratory instrument. Laboratory analytical particle size measurements typically require significant dilution (often several orders of magnitude) and may impact the integrity of the sample. Shown below (**Figure 1**) is particle size data generated by laser light scattering (Coulter LS-230) for an eight hour nano-milling operation illustrating both the desired size reduction and increased homogeneity of the particle size distribution.

Figure 1. Coulter LS-230 particle size distribution results at indicated milling time points.
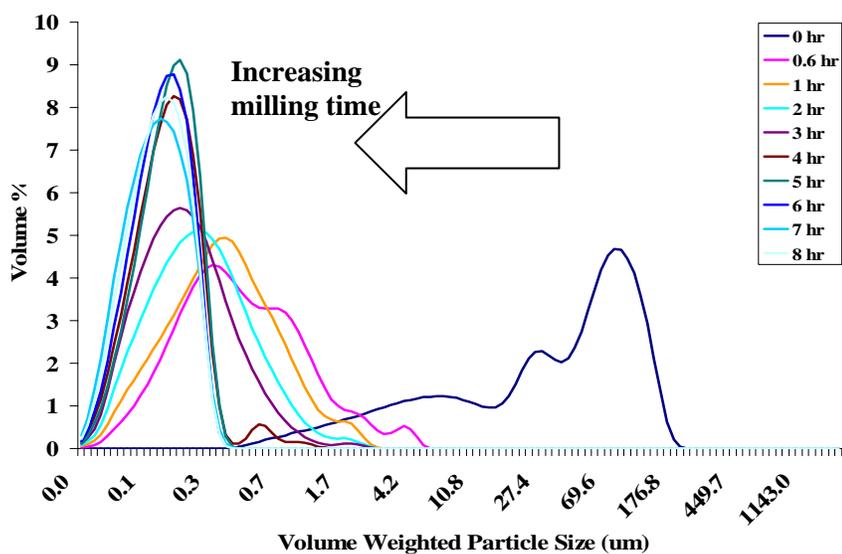
Concern with taking a representative sample increases for broad particle distributions, and the time involved with making the measurement in another laboratory (particularly for end point determination), both serve as drivers to consider in-line technologies.

There are several critical requirements of an in-process tool to determine endpoint for this specific nano-milling unit operation. Included are the ability to monitor particle size ranges from 100's of microns down to 100 nm, the ability to perform these measurements in approximately 30 % wt/wt solid dispersions, and the ability to achieve precision on the order of nanometers. In this light, several technologies were investigated, as summarized in **Table 1**, along with the outcome of each evaluation.

Table 1. Evaluation of Techniques for On-line Particle Sizing of a Media Milling operation.

Technique	*Limitation*
Reflectometry	Lack of robustness
FBRM	Not applicable to particle sizes < 1 um
Acoustic Attenuation Spectroscopy	Difference in response is small, may work with further development
Electroacoustic Spectroscopy	Technology was not successful in our hands
Acoustic Backscattering	Theory requires more development
NIR Spectroscopy	**In our hands, the tool of choice**

NIR spectroscopy was found to be uniquely qualified to achieve the desired measurement specifications. Furthermore, the in-line NIR method overcomes the limitations that are encountered using laboratory-based instrumentation for nanometer particle size determinations (see above) in addition to providing the opportunity for real time control of a critical quality attribute of the complex formulation. Spectra taken from 400 – 2500 nm using an in-line Foss reflectance probe across a milling run (Figure 2) illustrate

Figure 2. NIR spectra from 400-2500 nm collected across a milling run on a Foss
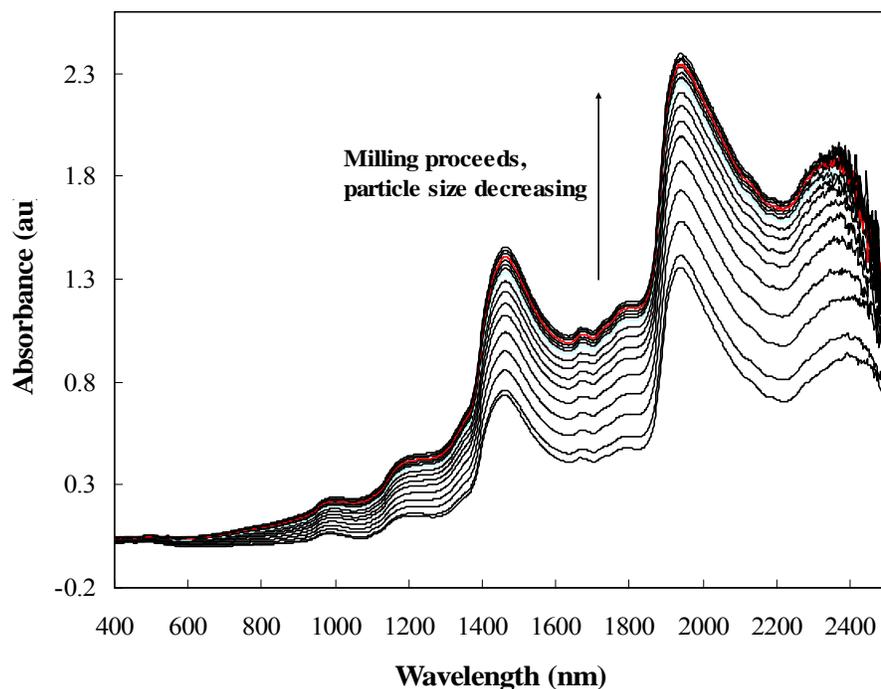
a significant and consistent trend across the milling operation. The spectral changes can be attributed to two phenomena; 1) the shifting base line representative of loss of light reflection back into the probe and 2) the increased of absorbance of the water overtone (**Figure 3**) resultant of increased effective path lengths for the

Figure 3. Second derivative NIR spectra from 1225 – 1525 nm collected across a milling run on a Foss
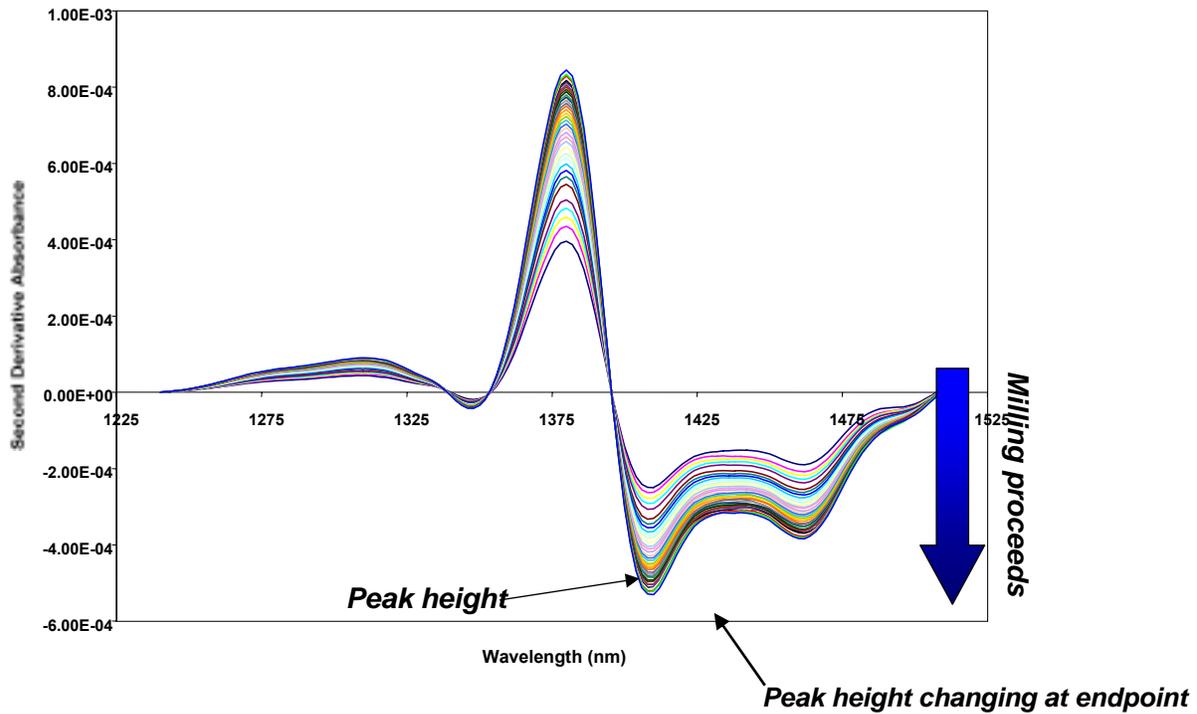
light that remains reflected back into the probe. The spectral changes in **Figure 3** can be correlated to particle size (**Figure 4**) using samples taken at identical time points and analyzing by the laboratory Coulter LS-230 method.

Figure 4. **NIR Predicted Particle Size as a Function of Milling Time for Runs 6-7 (Validation set)**
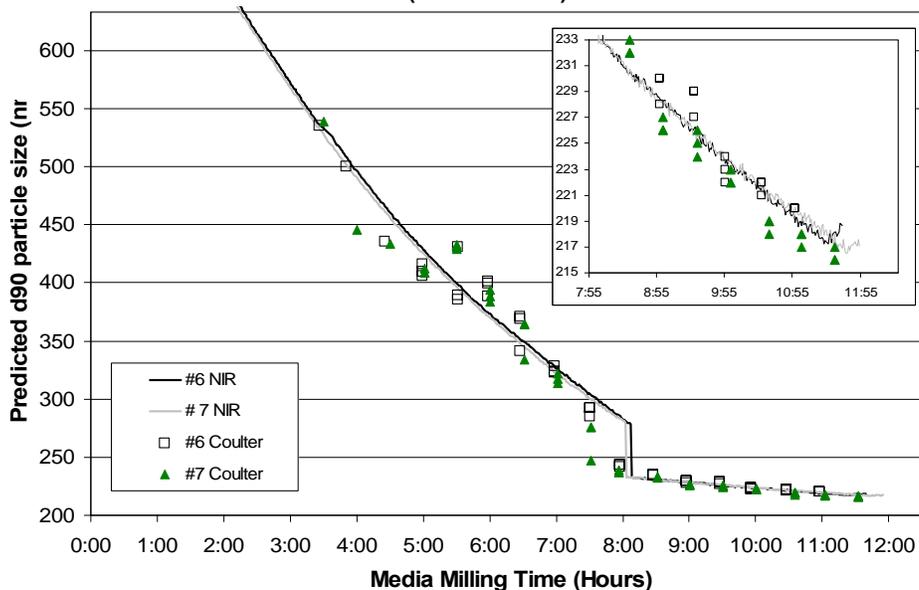
In our hands, in-line Near-IR spectroscopy is capable of providing particle size data across the range of 100's of um to 100 nm. Furthermore, the technology achieves an unprecedented accuracy of 1-5 nm (**Table 2**) near the endpoint of nanomilling where the D90 particle size is in the range of 200-220 nm.

Table 2. NIR method Calibration and Validation data comparison at the Media Milling operation end point, developing a calibration set with data from five milling runs and predicting on the subsequent four runs.

Run		Media Milling Time	On-line NIR (D90)	Coulter LS-230 (D90)	Residual (pred-measured)
1		12:35	218.3	219	-0.7
2	Calibration set	10:01	220.0	220	0.0
3		12:28	209.4	211	-1.6
4		11:24	212.2	211	1.2
5		13.26	208.9	208	0.9
6		10:58	219.3	220	-0.7
7	Validation set	11:33	217.1	218	-0.9
8		11:29	211.8	217	-5.2
9		11:45	212.4	216	-3.6

The sensitivity of the measurement was assessed for a number of process and product parameters, including particle size, temperature, fiber optic probe, etc. The effect observed was clearly most dependent on the particle size, with the second most significant contribution to the signal being the fiber optic probe. A minor dependence on the process temperature as also noted. (**Figure 5**).

Figure 5.   **Analysis of Effects of NIR absorbance of MK-0869 media milled samples (Brimrose 2-probe data for Biobatch/MCSS)**
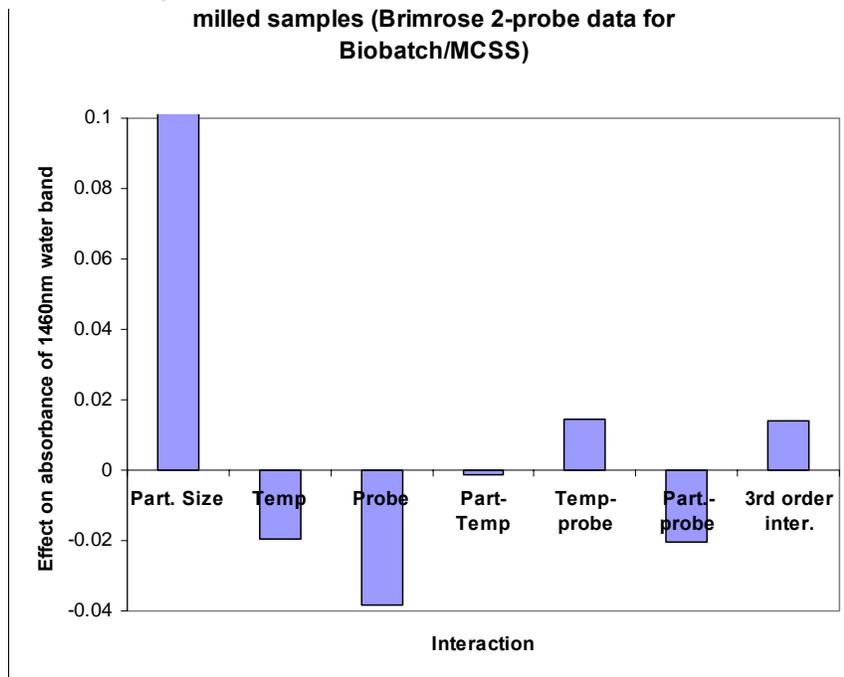
Future research will focus on assessing the ability to fit the data obtained in the reflectance Near-IR spectrum to a model particle size distribution, eliminating the need for laser light scattering as a primary measurement technique.

In-Line Moisture Determination of a Fluid Bed Drying Unit Operations

A common approach to the manufacture of a oral dosage form (tablet or capsule) involves incorporation of the drug particles into a granule, often by addition of a fluid (water is most common) to bind the drug to other non-active components of the product. After the granules have been created, a drying unit operation is generally employed. A cartoon schematic of a fluid bed dryer is shown in **Figure 6**.
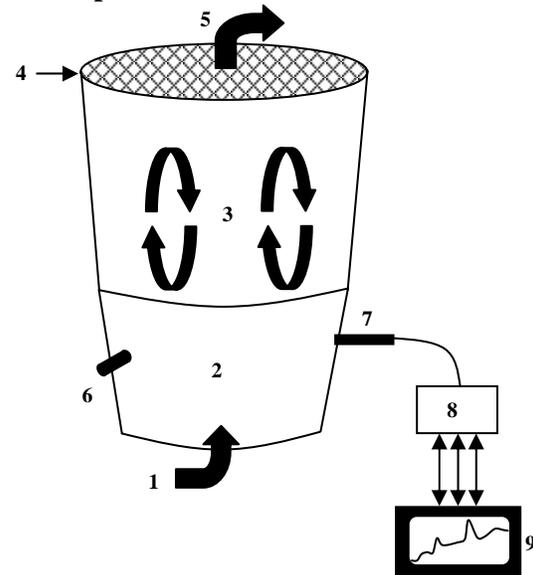
Figure 6. Side view schematic representation of the drying equipment and sampling configuration used in these studies. 1) inlet air, 2) product bowl, 3) drying column, 4) filters, 5) effluent air, 6) sample thief, 7) Near-IR probe, 8) spectrometer, and 9) computer control and display

The ideal in-line application for moisture determination also utilizes Near-IR spectroscopy since the water overtones represent the strongest signals in the NIR spectral region.

Sample presentation to the probe has been found to be one of the most difficult challenges when monitoring a process of dispersed solids in a fluidized system. This is illustrated in the top of **Figure 7**, where a probe has been inserted into a 300 L fluid bed dryer as indicated in the schematic in **Figure 6** and spectra taken as a function of the drying time. Clearly the spectra are very erratic, which can be attributed to the highly varied content of air versus sample for each spectrum taken. In light of this issue, the probe was modified to allow the capture of a static volume while immersed within the fluid bed by adding a cup to the end of the probe. The resulting spectra are shown in the bottom of **Figure 7**, again taken as a function of the drying time, and clearly illustrate the improved quality of the data which then allows one to monitor the moisture content in the fluid bed. The overall apparent accuracy of the method was found to be 0.92% over the 4-10% moisture range.
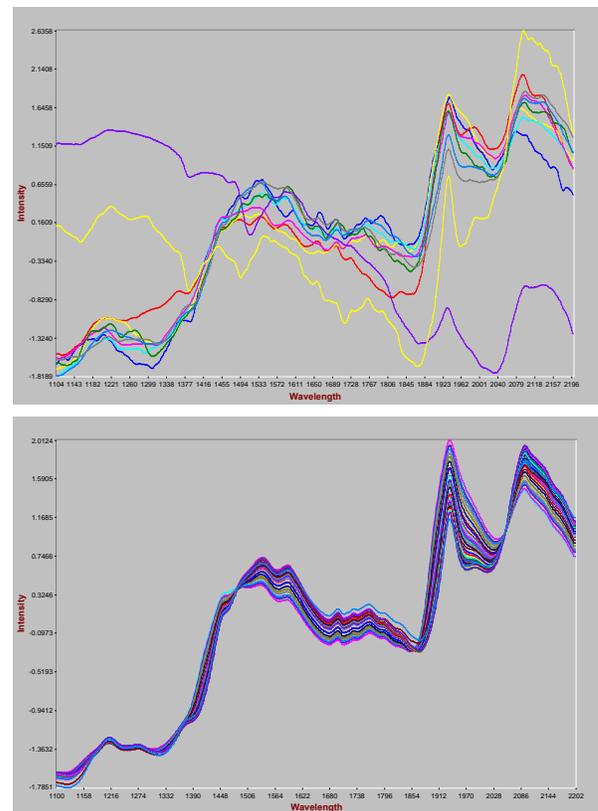
Figure 7. Consecutive NIR data acquired without the sample cup (Top) and with the sample cup (Bottom).

After issues related to probe sample presentation were addressed, the next challenge was how to calibrate data collected in the probe to actual moisture content of the granules through comparison of NIR spectra to samples taken from the dryer and delivered to the laboratory for analysis by either LOD or Karl Fisher. As can be seen in the schematic in **Figure 6**, the NIR fiber optic probe (location 7) samples a different volume of the bed than that of the sample thief (location 6). Hence, the moisture content of the two samples may not be the same, and thus limits the ability to accurately calibrate the probe. This issue was overcome by making one last modification to the sample probe, which was introduce thieving capability to the sample cup (**Figure 8**), such that the NIR spectra and the reference moisture determination were made from the same sample volume.

Figure 8. Representation similar to final probe sheath used in this work. 1) probe fibers leading back to instrument, 2) air line used to purge sample and clean probe window, 3) bushing interfacing probe sheath to product bowl, 4) sample cup, 5) stopper to allow thieving
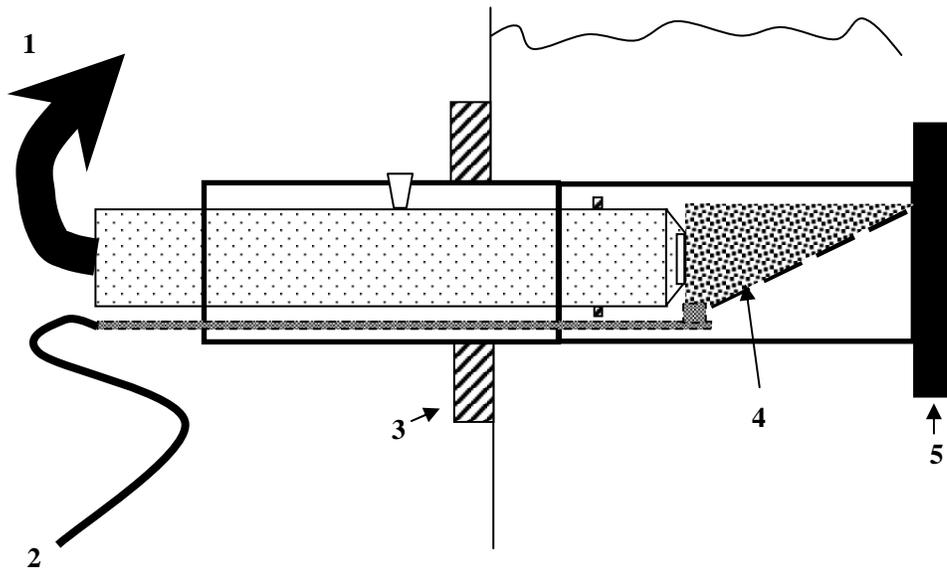
The application of this probe to the process showed a dramatic increase in the accuracy of the method, reducing the RMSEC from 0.92 to 0.39 over a 4 – 10 % moisture range.

The outcome of the combined improvements in the probe design and the calibration of the probe have allowed the real time monitoring of the fluid bed drying process in a 300 L drier (**Figure 9**). The traditional approach to monitoring the moisture in the bed is taking one sample and sending to the lab for analysis, which results (X's in Figure 9) a moisture curve with a smooth decline during drying. In contrast, the in-line NIR data (Δ's in Figure 9) shows an oscillatory drying process, which can be attributed to a volume of

Figure 9. Representative in-line Near-IR process profiles (-Δ-), Karl Fischer values (**X**), and calibration limits (--) with a 70 second acquisition interval
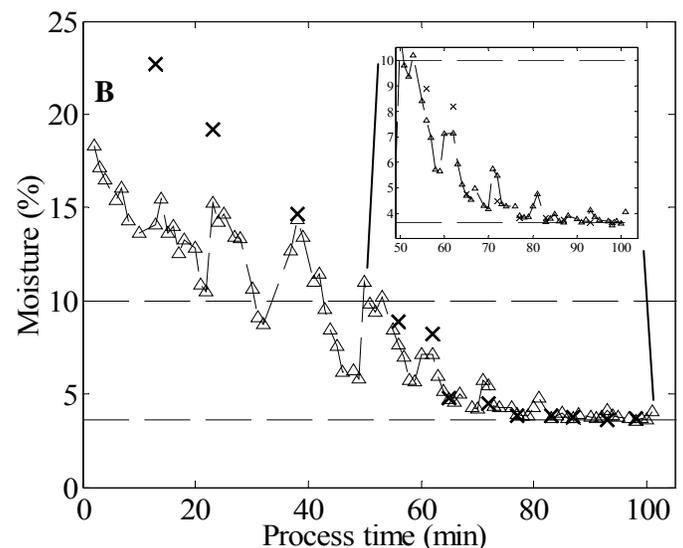
the bed material being trapped on a filter in the drying unit (location 4 in **Figure 6**), which is periodically tapped to allow the material to fall back into the fluidized bed. The material on the filter has a higher moisture content, and when added to the bed, shows an overall increase in the moisture level, which then begins to dry again, hence the oscillatory pattern in the drying cycle. The in-line NIR probe has also been applied to the 600L scale fluid bed drier, which does not show the oscillations but a smooth drying curve, resultant of the filter to batch size ratio difference and the frequency of filter cleaning at the 600 L scale. Overall, the technology provides rapid consecutive moisture determinations throughout each batch, thereby providing a more accurate picture of the drying process than typical thieving based analyses that are performed for a small number of time points and sample volumes.

Finally, it is worthwhile to consider the best application of the modified probe (**Figure 8**). Even though the utility to provide the best calibration has been demonstrated above, the sample cup technology may not provide the best measure of the overall batch. For example, if one examines the data in Figure 9; the KF data represents 12 data points for 12 sample volumes, the NIR data for a sample cup represents approximately 100 data points for 100 sample volumes, while an unmodified or open probe would represent 100 data points for up to 3200 sample volumes (a function of the material transfer rates at the probe and the integration time of the NIR analyzer). If one considers that the end of drying is represented by the last 20 data points for the NIR probe in Figure 9, this represents up to 640 sample volumes, versus the two sample volumes examined by the laboratory KF method over the same end of drying cycle time period. Clearly, the unmodified probe gives the best overall assessment of the moisture content in the entire batch, and should be the preferred choice for an in-line probe, if the process permits the generation of good data. At the 600 L scale, the open probe did not exhibit the spectral variability seen in the top of **Figure 7** for the 300 L scale process, and thus the open probe was utilized as the instrument of choice.

Specification Setting for Process Analytical Technology
The onset of the application of in-line, on-line process analytical technologies requires consideration of approaches to setting acceptance criteria. For any given process, if a large number of data points are collected, the data can often be represented as a distribution of values (an example of a gaussian distribution is shown in **Figure 10**). The distribution of the points can be then considered in two extremes. The first extreme is where the variability of the measurement is small relative to the sample variability, and the distribution then largely reflects the homogeneity of the samples in the process. The second extreme is where the variability of the measurement is large relative to the variability of the samples in the process, and the distribution of data points is reflective of the measurement precision. Traditional approaches to process characterization then often involve taking a "representative" sample from the batch and sending it to the laboratory for analysis, generating a relatively small number (<10) data points. This may then be repeated for several runs of the same process. The data is then often combined and statistically evaluated to determine the mean and standard deviation (σ), to which an acceptance criteria is then proposed at the mean plus three standard deviations, which places the acceptance criteria at the edge of the distribution curve. In this scenario, the end point of the process will rely on a combination of the sample variability and measurement variability to determine the end point, which may or may not reflect the same end point of the previous batch.

Figure 10. Representation of a Gaussian Distribution of Data Points, with the mean, 1, 2 and 3 standard deviations indicated on the positive side.
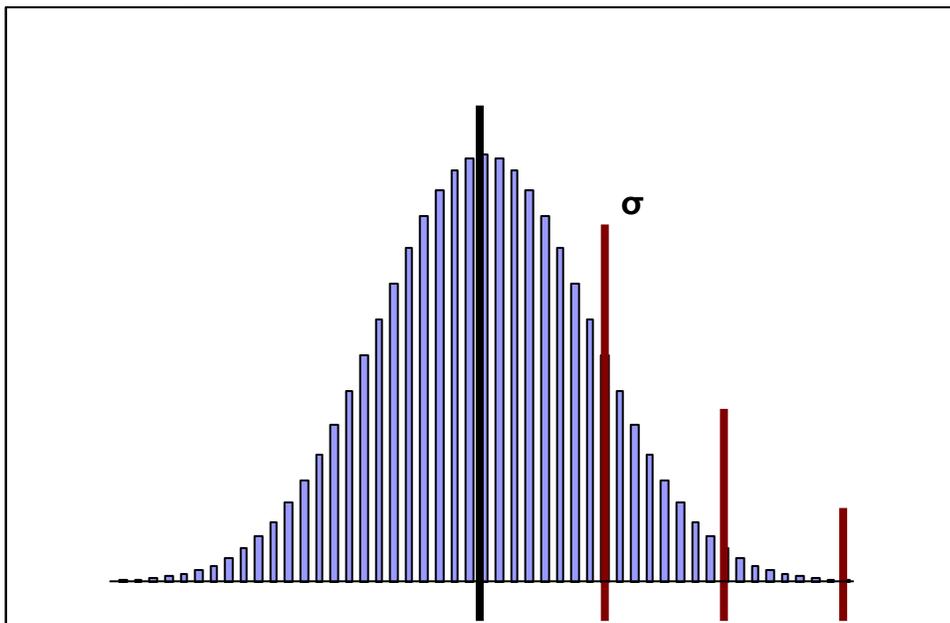
In contrast, for an in/on-line measurement, where spectra are continuously taken (for our NIR applications above, 32 scans per time point and multiple time points across the end of a run) on a continually changing volume, 640 volumes (or samples) are determined over the last 20 time points. Under these conditions, one is determining the mean value of the distribution (not the mean + 3σ) which encompasses both the variability of the sample and the measurement. If the acceptance criterion is then established at the mean value, then one rests assured that the next, or previous, process will have a common end point, if one can assume that the sample variability and measurement variability are comparable from run to run. There are two key outcomes of this assessment; 1) the in/on-line method provides the best definition of end point determination for a process, and 2) if a PAT and traditional method are considered for a process end point determination, they are likely to have different acceptance criteria, one aimed at capturing the mean and the other aimed at capturing the mean plus three standard deviations.