Differentiating Analytical Error from Sampling Errors in PAT Methods through Variographic Analysis

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In variographic analysis the nugget effect provides an estimate of the total process measurement system error. The value of variographic analysis can be further increased by differentiating the analytical error from the sampling errors. The ratio of the minimum practical error (MPE) to the analytical error provides an estimate of the suitability and performance of a sampling system since the analytical error would still remain even if sampling errors could be completely eliminated. The sampling and analytical errors for four systems used to obtain 1-D lots of pharmaceutical powder blends is presented. The first studies were conducted with blends moving over a conveyor belt and with the feed-frame of a tablet press. The researchers then developed and patented a new stream sampler and chute for sampling and analysis of pharmaceutical powder blends. Near Infrared or Raman spectra were obtained as the powder blends flowed or moved and used to determine the drug concentration in the blends. Even though these methods do not require sample extraction and sample preparation in a laboratory, they are still subject to sampling errors which were estimated through the variographic analysis. The MPE was compared to the analytical error for these four systems. The results obtained show that it is often possible to reduce sampling errors to less than ten times the analytical error. These studies represent the first efforts to estimate and reduce sampling errors in the analysis of the powder blends used to manufacture the tablets that many patients take daily.

Introduction

In 2004 the Food and Drug Administration published its Process Analytical Technology (PAT) Guidance to encourage innovative pharmaceutical development, manufacturing, and quality assurance.[1] The agency defined PAT as a "system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality". This guidance recognizes that the raw materials used in a process may show some variation in their physical properties and chemical composition. However, the manufacturing process may be controlled using the information provided by the real time analyses.

The real time analysis is performed through fast non-destructive analytical techniques such as near infrared and Raman spectroscopy. The PAT system differs significantly from many pharmaceutical processes that depend on performing an operation for a certain time, without any monitoring of the process, relying on testing after the production process has been completed. However, PAT systems are also affected by sampling errors.[2, 3] This research group is focused on studying the analytical and sampling errors in PAT for pharmaceutical manufacturing. In all the systems under study a 3-D system is transformed to a 1-D system making variographic analysis possible. The group first performed this transformation which permitted analyses in conveyor belts and the feed-frame of a tablet press.[4-8] The group then developed and patented a new stream sampler and chute for sampling and analysis of pharmaceutical powder blends.[9-12]

Variographic analysis permits the estimation of the sum of analytical and sampling errors through the nugget effect as indicated in a number of previous studies. [13-15] However, there is also a need to differentiate the sampling errors from the analytical errors. The analytical error may be estimated through a repeatability or short-term precision study. The repeatability study consists of six or more consecutive spectra on the same sample (the sample does not move or

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flow while the spectra are obtained). The group has performed repeatability studies in every project where variographic analysis was performed.

Several previous studies from the TOS community have indicated that the sampling error is often 2 – 3 orders of magnitude larger than the analytical error.[16-18] However, if sampling systems were to be fully optimized, the sampling errors would approach the magnitude of the analytical error. Analytical methods will always be subject to an unavoidable random error.[19] This study describes a quantitative effort to investigate the ratio of sampling and analytical errors in PAT methods with 1-D sampling and variographic analysis. The repeatability study is used as a measure of the Total Analytical Error (TAE) and compared to the minimum practical error (MPE) obtained through variographic analysis.[16] The MPE should be of higher magnitude since it provides an estimate of both the sampling and analytical errors. The MPE should approach the variance of the repeatability study as the sampling system is improved. As the sampling system is improved the ratio of the MPE and the repeatability study should approach a value of 1. This hypothesis is evaluated by comparison of the results of four sampling systems including: a conveyor belt[4], freed frame[7, 8, 20-22] stream sampler [10-12] and chute[23], which have used for sampling and analysis of pharmaceutical blends.

Experimental

1-D Sampling Systems:

A total of four 1-D sampling systems have been developed for analysis of powder blends. In these systems the powder blend is not removed for analysis at a laboratory. Near infrared or Raman spectroscopy are used to obtain spectra of the powders, providing real-time non-destructive analysis. When NIR spectroscopy is used, 32 scans are averaged into a single spectrum. A composite sample is obtained since the material is flowing or moving as the scans are obtained. The active pharmaceutical ingredient (API) concentration was predicted through a partial least squares (PLS) regression calibration model for each single spectrum.[3] The lag distance between pairs of drug concentration values was used in the calculation of variograms.

The first 1-D sampling system used was a conveyor belt. [4-6] The pharmaceutical powder mixture was deposited on a 3 m long rig with 10 cm width that moved at a linear velocity of 10 mm/s over the conveyor belt. The NIR spectra were obtained while in motion, at a point further along the conveyor belt. The fiber optic probe is fixed over the conveyor belt as shown in Figure 1.[4]

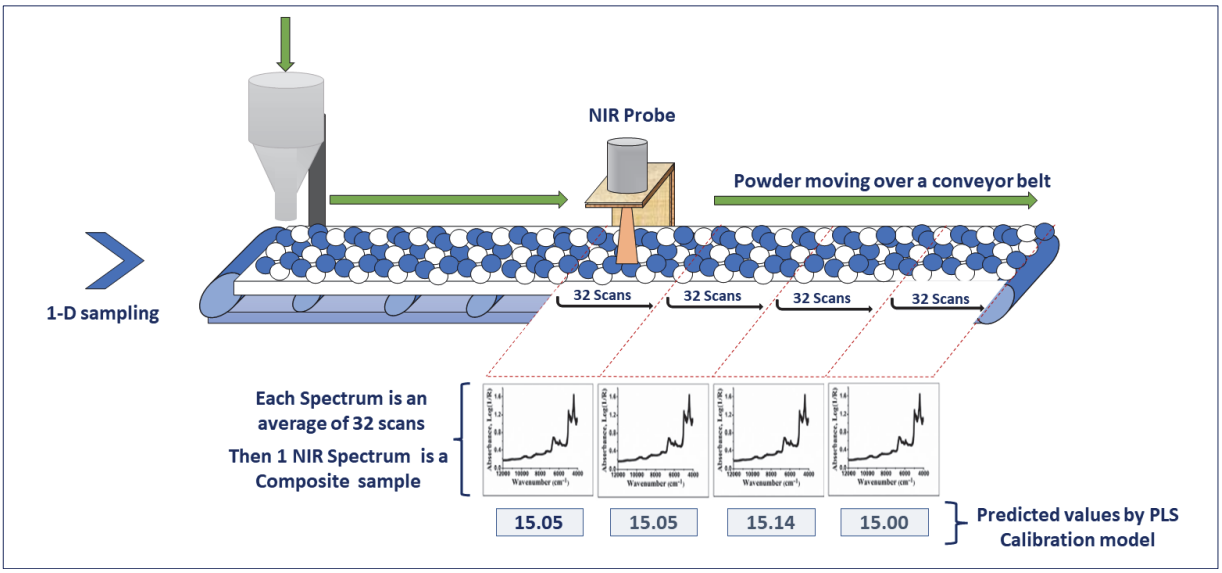

Figure 1. NIR spectral acquisition in a1-D optical sampling system for a conveyor belt

The second experimental set up consisted of a hopper, a feed frame from a Fette 3090 tablet press (Fill-O-Matic, Fette Compacting, Schwarzenbek, Germany) adapted on a table and high-density polyethylene disc. The thickness of the disc is 12.5 mm and has 36 holes of 10 mm diameter each one. This set up is used to simulate the tablet press turret.[20, 24] Figure 2 shows the experimental set-up for the feed frame sampling system.

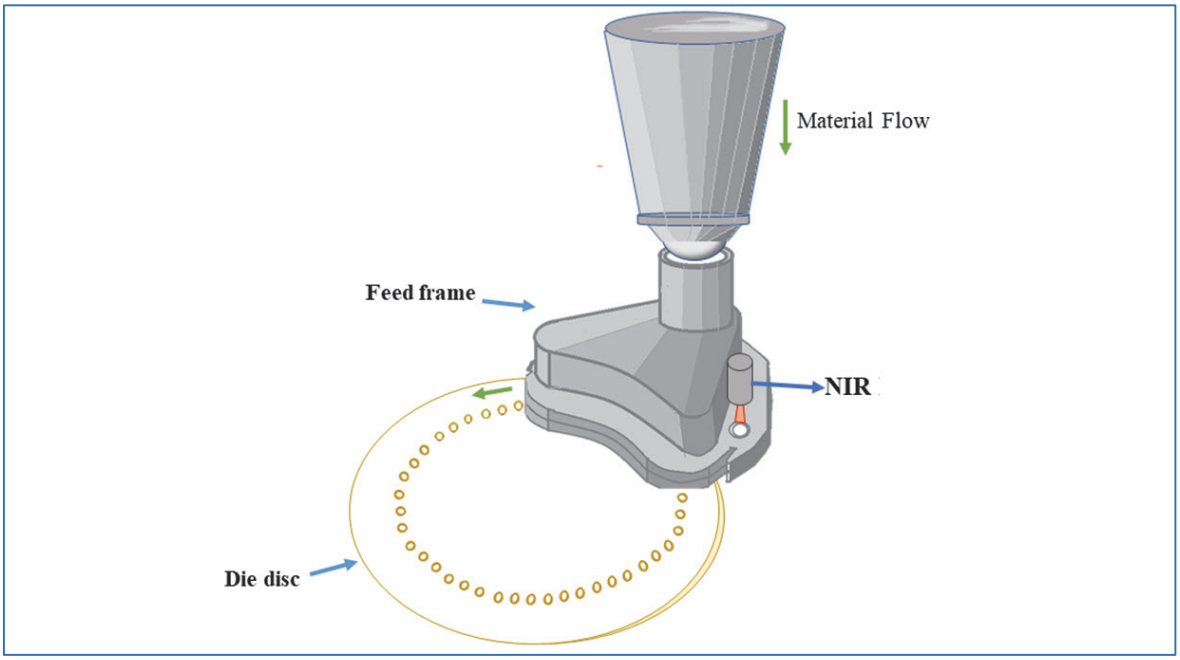

Figure 2. Example experimental set-up for a feed frame sampling system

The feed frame consists of three chambers, it has two levels and two inspection windows. The distributing chamber receives the powder from the hopper and transports it, using a paddle wheel, to fill and to set the dose to the chambers through two orifices in the partition plate [20, 24]. The paddle wheel speed and die disc speed were varied in a number of experiments. The procedure is to add powder blend to the hopper; while the paddle wheel and die disc stand still. The feed frame is turned on and it transports the powders to the chambers and inspection windows, where the NIR probe is installed over the left sapphire window to acquire spectra.

Figure 3 shows: 1) the transition chute and NIR (2) and stream sampler where spectra can be obtained.[23] The chute and stream sampler were designed to comply with the Fundamental Sampling Principle (FSP) which stipulates that all parts of the lot must have the same opportunity to be selected, and samples should be collected without affecting the material composition [3, 14]. The transition chute has a length of 41 cm a 15 cm width, with a 1 cm thickness and 2.54 cm diameter. The NIR spectra collected at the chute are obtained through a sapphire window placed 30 cm after the entry of the flowing powder. The chute is placed at an angle of 75 degrees as shown in Figure 3. The force of gravity and stream sampler revolutions allows the flowing powder blend to pass through the chute, generating more stability and continuity in the powder flow.[23]

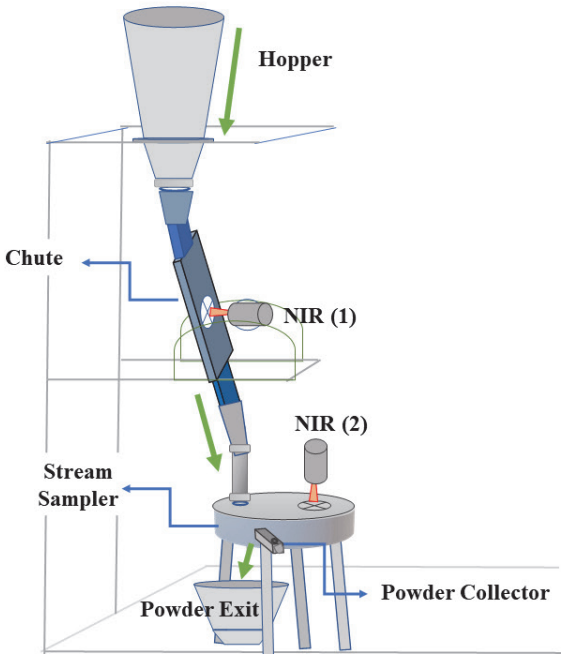

Figure 3. Schematic of the experimental sampling system set-up for the chute and the stream sampler.

The stream sampler has a chamber of approximately 150 mm diameter. The powder flows into the chamber by a wheel of rectangular paddles with a 5 mm thickness, which rotate counterclockwise at controllable speeds. The center of the paddle wheel has a diameter of approximately 86 mm, more than fifty percent of capacity of stream sampler.[9, 12] The powder leaves the system at 270° after its entry, avoiding powder re-circulation and changes in the drug concentration. This design reduces the probability of attrition and rupture of particles, which could affect the heterogeneity in the material. At the top of stream sampler, there is a sapphire window in the cross section of the flow powder. The stream sampler has an off-line powder collector, this collector is slid into the sampler to acquire the powder sample from a full cross-section of flow area. The sampler uses a 1.56 cm^3 volume, stainless-steel cup. The sample collector is used to select and analyze samples with a reference method [9, 10] However, all the results described in this study were obtained as the powder flowed through the stream sampler. This study does not report work with the powder collector.

Multivariate data analysis

The calibration models were developed and evaluated using multivariate data analysis software. The algorithm used is based on nonlinear iterative partial least squares (NIPALS) algorithm.[25] In the development of the calibration models, the spectra can be transformed by mathematical pretreatments such as standard normal variate (SNV), first and second derivative (Savitzky-Golay algorithm), etc. The PLS model was evaluated with the Root Mean Square Error of Prediction (RMSEP), the Relative Standard Error of Prediction (RSEP), and bias as shown in equations 1 - 3.

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{m}(\hat{y}_i - y_i)^2}{m}} \quad (1)$$

$$\%\mathrm{RSEP} = 100 * \sqrt{\frac{\sum_{i=1}^{m}(\hat{y}_i - y_i)^2}{\sum_{i=1}^{m}(y_i)^2}} \quad (2)$$

$$\mathrm{Bias} = \frac{\sum_{i=1}^{m}(\hat{y}i - y_i)}{m} \quad (3)$$

Variographic analysis

Variographic analysis was performed to estimate the sampling and analytical errors in the in-line PAT measurements. Figure 4 shows an example used to illustrate the information provided by the variogram. The variograms were

generated through the use of a Matlab code (Matlab version R2013b, The Math Woks, Natick, MA). The variograms were calculated according to Equation 4.

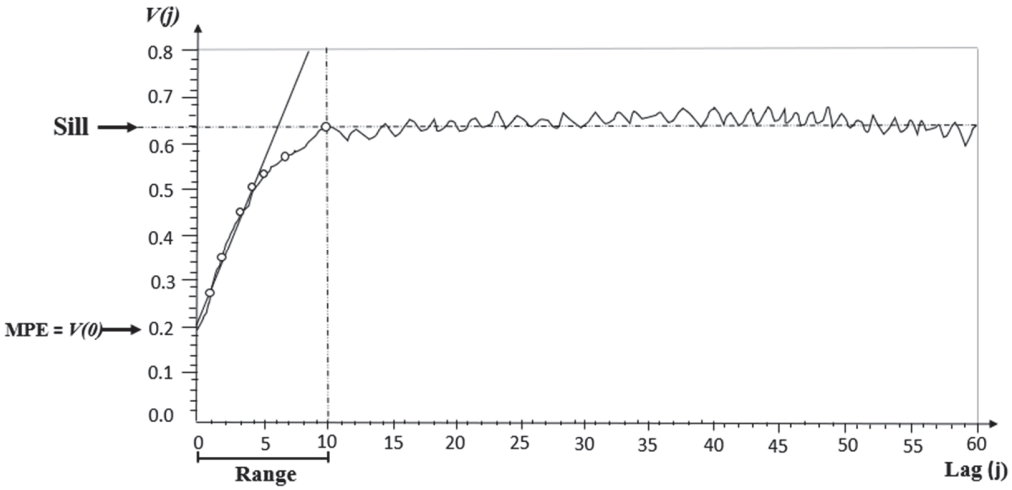

Figure 4. Example of variogram and variogram elements; Sill, MPE, Range, Lag (j) distance.

$$V(j) = \frac{1}{2(Q_{total} - j)} \sum_{q=1}^{Q_{total-j}} (h_{(q+j)} - h_q)^2 \quad (4)$$

The variogram function **V(j),** represents the variation between the predicted drug concentration for the composite samples. The Q_{total} represents the total number of composite samples, $h_{(q+j)}$ and h_q is the concentration value in each sampling point (expressed as the heterogeneity contribution). The ***j*** is the lag distance defined as the inverse of the sampling frequency in 1-D sampling. The lag is the distance between increments (or distance between samples) and can be expressed by time when the material is sampled [4, 14-16].

Variographic analysis makes it possible to calculate the contribution errors in the measurements. The total error variance (S_0^2) contribution to the analytical measurement uncertainty is: the sum of the sampling variance (S_s^2) and analytical method variance (S_m^2). Eq.5.

$$S_0^2 = S_s^2 + S_m^2 \quad (5)$$

The minimum practical error (MPE) – or nugget effect - is the minimum variance expected for any measurement situation, determined by back-extrapolating the first 3-5 points of V(j). In Equation 6 it is possible to calculate the total sampling error (TSE), and the total analytical error (TAE) as a variance calculated by from the analytical repeatability study.

$$MPE = TSE(S_s^2) + TAE(S_m^2) \quad (6)$$

The sill is the mean variation of a variogram (the flat ceiling of the variogram). The 'corrected-sill' is calculated by subtracting the MPE from sill (relative to the dynamic of process and residual heterogeneity). The range is the lag at which the variogram becomes effectively constant [15, 16].

Procedure to performing the repeatability studies

The analytical repeatability study was performed by obtaining six consecutive spectra of the same sample (without moving the fiber optic probe). The drug concentration associated with these spectra was determined through a PLS calibration model. The variance of the drug concentration obtained through the six consecutive spectra was then

calculated. The repeatability study was performed 3 to 10 times; then, the pooled variance was calculated as an estimation of total analytical error (TAE).[4]

Data

The data shown in Tables 1 and 2 were obtained from previous publications by the present research group during the last five years.

Materials

A number of active pharmaceutical ingredients (API) and excipients were used in the blends, The API used were metformin milled granules with a purity of 98 [%w/w] [4], acetaminophen (APAP) [7, 8], ibuprofen powder[11, 22], anhydrous caffeine [10, 12, 21, 23]. Microcrystalline cellulose, lactose monohydrate as excipients non-co-processed excipients, magnesium stearate as lubricant and colloidal silicon dioxide as glidant.

Results

The four sampling systems were evaluated through variographic analysis. The repeatability study was used to estimate the TAE. The sampling error and total analytical error were differentiated through the ratio of the MPE and TAE. Table 1 shows the (MPE/TAE) ratio results of studies employing the conveyor belt or feed frame and spectrometer (NIR and/or Raman). The studies were performed with concentrations of the active pharmaceutical ingredients which varied from 3 to 100% (w/w), and with several common excipients.

Table 1. Summary of studies performed with the conveyor belt and feed frame.

Reference (#)	Sampling System	API	Conc. %(w/w)	MPE $(\%w/w)^2$	TAE $(\%w/w)^2$	MPE/TAE
4	Conveyor (NIRS) Model 1	Metformin	95.0	0.0513	0.0002	257
4	Conveyor (NIRS) Model 1	Metformin	97.0	0.0414	0.0002	207
4	Conveyor (NIRS) Model 1	Metformin	100.0	0.0013	0.0007	2
4	Conveyor (NIRS) Model 2	Metformin	95.0	0.0683	0.0002	342
4	Conveyor (NIRS) Model 2	Metformin	97.0	0.0559	0.0003	186
4	Conveyor (NIRS) Model 2	Metformin	100.0	0.0009	0.0010	1
4	Conveyor (NIRS) Model 3	Metformin	95.0	0.0750	0.0010	75
4	Conveyor (NIRS) Model 3	Metformin	97.0	0.0500	0.0010	50
4	Conveyor (NIRS) Model 3	Metformin	100.0	0.0005	0.0020	0.3
7	Feed Frame (NIRS)	acetaminophen	3.00	0.0023	0.00061	3.8
7	Feed Frame (NIRS)	acetaminophen	3.00	0.0020	0.00061	3.3

7	Feed Frame (NIRS)	acetaminophen	3.00	0.0034	0.00061	5.6
8	Feed Frame (NIRS)	acetaminophen	10.0	0.019	0.00012	158
22	Feed Frame (NIRS)	Ibuprofen (Co-processed excipients)	42.5	0.136	0.0365	3.73
22	Feed Frame (NIRS)	Ibuprofen (Co-processed excipients)	50.0	0.166	0.0160	10.4
22	Feed Frame (NIRS)	Ibuprofen (Co-processed excipients)	57.5	0.138	0.0788	1.75
22	Feed Frame (NIRS)	Ibuprofen (non-Co-processed excipients)	42.5	1.74	0.127	13.7
22	Feed Frame (NIRS)	Ibuprofen (non-Co-processed excipients)	50.0	1.88	0.215	8.74
22	Feed Frame (NIRS)	Ibuprofen (non-Co-processed excipients)	57.5	1.76	0.148	11.9
21	Feed Frame (NIRS)	Caffeine	4.00	0.0047	0.0016	2.9
21	Feed Frame (NIRS)	Caffeine	5.00	0.0061	0.00096	6.4
21	Feed Frame (NIRS)	Caffeine	6.00	0.0068	0.00078	8.7
21	Feed Frame (Raman)	Caffeine	4.00	0.046	0.00048	96
21	Feed Frame (Raman)	Caffeine	5.00	0.0389	0.00084	46
21	Feed Frame (Raman)	Caffeine	6.00	0.045	0.0012	38

Ratio of MPE to the TAE in the conveyor belt studies

The ratio of the MPE to the TAE on the conveyor belt varied from 0.3 to 342 as shown in Table 1. This very large variation is related to the heterogeneity of the blends. Table 1 shows three experiments with the conveyor belt, where the drug concentration was predicted with three calibration models.[4] Each calibration model had a different range of concentrations: 90-100 % (Model 1), 85-100% (Model 2) and 65-100 % w/w metformin (Model 3). Three test set blends with concentrations of: 95.0, 97.0 and 100.0 %w/w of metformin were predicted by the calibration models. The experiment was purposely designed with high concentrations to obtain blends with low heterogeneity. The use of the single component (100% w/w metformin granules) provided an opportunity to reduce the heterogeneity to a maximum level, and thereby quantify the analytical error. The MPE/TAE for the test set blends varied from 257 to 2 for Model 1, and from 342 to 1 for Model 2 and from 75 to 1 in Model 3. The lowest MPE/TAE were obtained, as expected, for the 100% (w/w) metformin granules.

Table 1 also shows three examples with the conveyor belt where the metformin concentration was predicted by NIR spectroscopy when only one material (metformin granules) was present in the conveyor belt. The use of a single component was done to minimize the heterogeneity and reduce the sampling errors. In this case the MPE/TAE values obtained were: 2, 1, and 0.3. These results show that the sampling error is minimized, and it approaches the TAE. The differences obtained were related to the calibration model used to predict the drug concentration. The MPE/TAE of 2 was obtained with a calibration model which included blends that varied from 90 to 100% w/w. The MPE/TAE of 1 was obtained with a different calibration model which included blends that varied from 85 to 100% w/w. The MPE/TAE of 0.3 was obtained with a different calibration model which included blends that varied from 65 to 100% w/w. These experiments confirm that the MPE was reduced by using a material with a low heterogeneity.

Figure 5A shows the variograms of a test set blend with 95% w/w metformin analyzed in the conveyor belt. Figure 5B shows the variogram for the 100% w/w metformin. The MPE is 150 times lower in the 100% (w/w) metformin granules. The greater heterogeneity of the 95% w/w metformin resulted in the larger MPE.

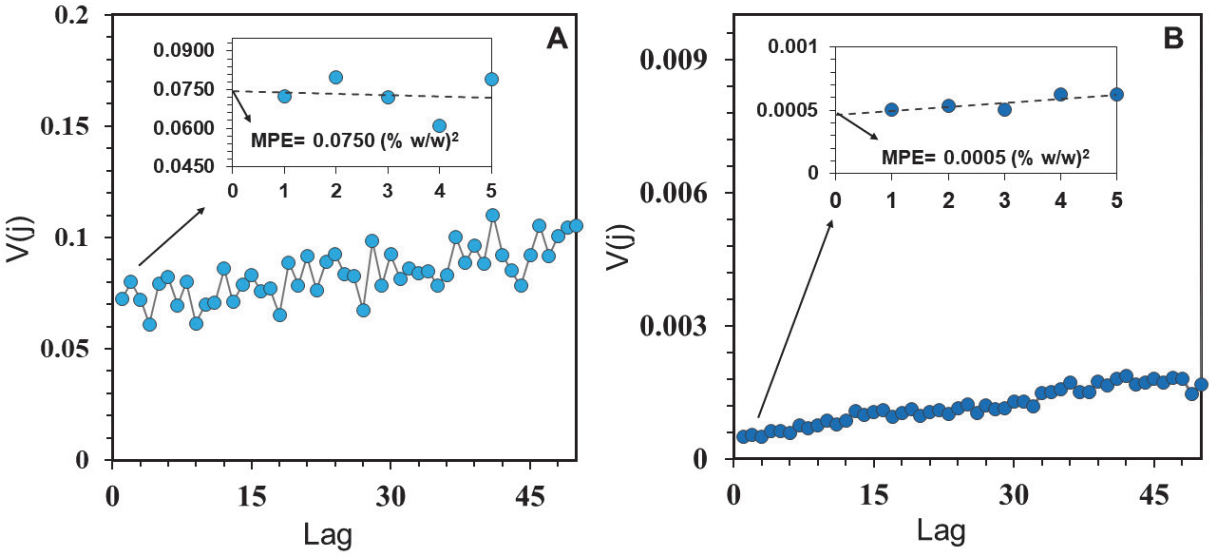

Figure 5. Comparison of variograms of test set blend 95 % w/w (A) and 100 % w/w (B) of API obtained over conveyor belt with NIR spectrometer.

The ratio of the MPE to the TAE in the feed frame

The ratio of the MPE to the TAE in the feed frame varied from 2.9 to 158. The feed frame was used with materials with a wide range of physical properties. The ratio of 158 was obtained at the continuous manufacturing facility at Rutgers, and it includes the effects of various components of the manufacturing line.[8] The experimental set-up is more complex than in other studies where the feed-frame was used. These results were also with acetaminophen, a cohesive material that affects flow properties.

Table 1 shows another case, the comparison of spectrometers in the simultaneous analysis of drug concentration, the NIR and Raman spectra was obtained over the feed frame of the tablet press.[21] Figure 6 shows the variograms for the test blend of 5 % w/w caffeine, the MPE is 6 times less when the NIR is used. The TAE is similar when using each spectrometer, but the ratio (MPE/TAE) is 6.4 for NIR and 46 with Raman spectroscopy. The difference of ratio obtained suggests a larger sampling error when using a Raman spectrometer. More studies of this difference will be illuminating.

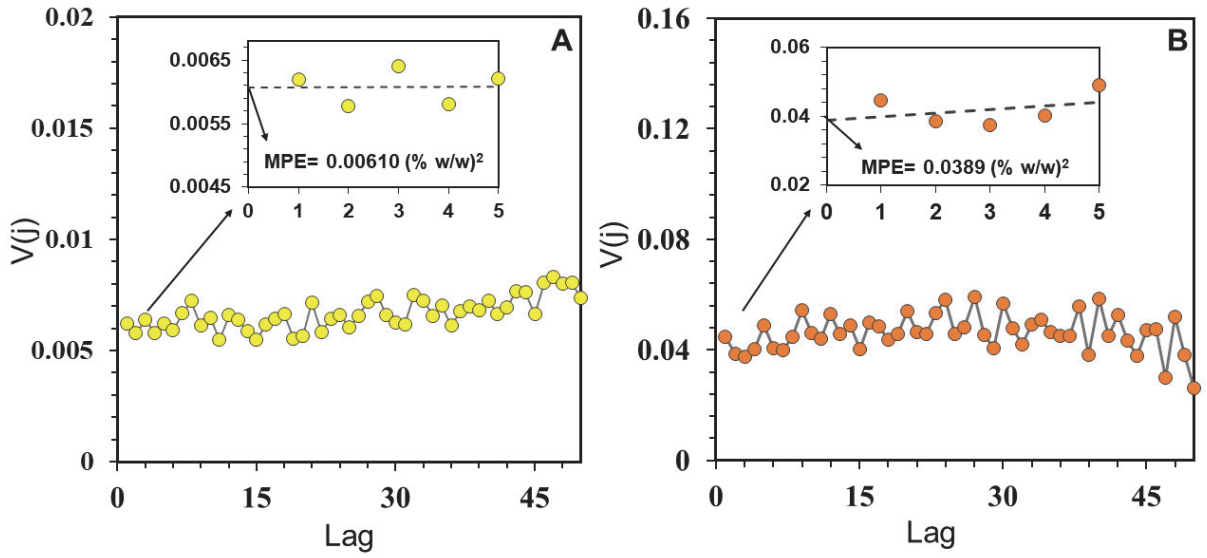

Figure 6. Comparison of variograms of test set blend of 5% by NIR(A) and Raman (B) in simultaneous analysis.

Ratio of MPE to the TAE in the stream sampler and chute

Table 2 shows the (MPE/TAE) ratio results of studies employing the stream sampler and the chute respectively. The results obtained with the stream sampler varied from 1.3 to 34. The first study with the stream sampler involved the prediction of test set blends with 12.00, 15.00, and 18.00% w/w caffeine.[10] The MPE/TAE ranged from 2.3 – 3.4 for these test set blends. The second study with the stream sampler involved test set blends with only 2.00, 3.00 and 4.00 % w/w) ibuprofen in cohesive powder blends.[11] The MPE/TAE for these blends varied from 7.6 – 24. The greater ratio may be due to the cohesiveness of these powder blends, which influence the powder flowability. The stream sampler was also used with low drug concentration powder blends where the concentration of caffeine in the test set blends ranged from 0.76 – 4.02 % w/w.[12] The MPE/TAE for these blends varied from 2.9 to 34.

The results obtained with the patented chute, used in the experimental setup illustrated in Figure 2, are also shown in Table 2.[23] The MPE/TAE varied from 29 to 153 for these blends. These are preliminary results with the chute, and additional studies are planned.

Table 2. Studies performed with stream sampler and chute.

Reference (#)	Sampling System	API	Conc. %(w/w)	MPE(%w/w)²	TAE(%w/w)²	MPE/TAE
10	Stream sampler (NIRS)	Caffeine	12.00	0.192	0.083	2.3
10	Stream sampler (NIRS)	Caffeine	15.00	0.217	0.083	2.6
10	Stream sampler (NIRS)	Caffeine	18.00	0.279	0.083	3.4

11	Stream sampler (NIRS)	Ibuprofen	2.00	0.0044	$5.8 *10^{-4}$	7.6
11	Stream sampler (NIRS)	Ibuprofen	3.00	0.0076	$9.3 *10^{-4}$	8.2
11	Stream sampler (NIRS)	Ibuprofen	4.00	0.0104	$4.4 *10^{-4}$	24
12	Stream sampler (NIRS)	Caffeine	2.02	0.0019	$5.6 *10^{-5}$	34
12	Stream sampler (NIRS)	Caffeine	3.09	0.0042	$3.6 *10^{-4}$	12
12	Stream sampler (NIRS)	Caffeine	4.02	0.0034	$2.3 *10^{-4}$	15
12	Stream sampler (NIRS)	Caffeine	0.76	0.0020	$6.8 *10^{-4}$	2.9
12	Stream sampler (NIRS)	Caffeine	1.51	0.0018	$3.6 *10^{-4}$	5.0
12	Stream sampler (NIRS)	Caffeine	2.26	0.0028	$2.2 *10^{-4}$	13
23	Chute (NIRS)	Caffeine	2.02	0.0041	$9.3 *10^{-5}$	44
23	Chute (NIRS)	Caffeine	3.09	0.0094	$3.2 *10^{-4}$	29
23	Chute (NIRS)	Caffeine	4.02	0.011	$7.2 *10^{-5}$	153

Conclusion

The hypothesis in this work was that improvements in a sampling system could lead to an MPE/TAE value as low as of 1 in the best of all possible cases. This assessment may be used to improve the performance of powder samplers. The MPE and TAE were compared in four different sampling systems. The results obtained indicate that this ratio is a valuable source of information with which to compare sampling performance of PAT systems. This is the first comparison of MPE and TAE in pharmaceutical PAT systems.

The use of the single component (100% w/w metformin granules) reduced the heterogeneity to a minimum level and indeed provided MPE/TAE values close to 1. Metformin blends of 95% and 97% (w/w) blends were also analyzed, but the lowest MPE/TAE values were obtained, as expected, for the 100% (w/w) metformin granules.

This kind of performance comparison is only possible in PAT systems where 1-D sampling and variographic analysis are performed. The value of TOS for PAT applications is evident.

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