On-Line Content Uniformity Determination of Tablets Using Low-Resolution Raman Spectroscopy

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Analytical techniques for rapid and nondestructive content uniformity determination of pharmaceutical solid dosage forms have been studied for several years in an effort to replace the traditional wet chemistry procedures, which are labor intensive and time consuming. Both Raman spectroscopy and near-infrared spectroscopy have been used for this purpose, and predictability errors are approaching those of the traditional techniques. In this study, a low-resolution Raman spectrometer was utilized to demonstrate the feasibility of both rapid at-line and on-line determination of tablet content uniformity. Additionally, sampling statistics were reviewed in an effort to determine how many tablets should be assayed for specific batch sizes. A good correlation was observed between assay values determined by high-performance liquid chromatography and Raman analysis. Due to rapid acquisition times for the Raman data, it was possible to analyze far more samples than with wet chemistry methods, leading to a better statistical description of variation within the batch. For at-line experiments, the sampling volume was increased by rotating the laser beam during the acquisition period. For the on-line experiments, the sampling volume was increased by sampling from a stream of tablets moving underneath the Raman probe on a conveyor system. Finally, an approach is proposed for monitoring content uniformity immediately following the compaction process. In conclusion, Raman spectroscopy has potential as a rapid, nondestructive technique for at- or on-line determination of tablet content uniformity.

Index Headings: **Process analytical technology; Raman spectroscopy; Content uniformity; Sampling; Central limit theorem.**

INTRODUCTION

There has been an increasing interest in process analytical solutions for the control of pharmaceutical processes, which has escalated since the release of the process analytical technologies (PAT) initiative by the U.S. Food and Drug Administration (FDA).[1] This interest has led to the integration of many spectroscopic techniques with the majority of the unit operations used in the pharmaceutical manufacturing process in order to assess different critical product attributes.[2–11] Raman spectroscopy has been shown to be a useful tool for determination of the physical form of pharmaceutical solids,[12] as well as for assaying the content of active pharmaceutical ingredient (API) in drug products.[13,14] Recent instrumentation developments have enhanced the feasibility of using Raman spectroscopy for in-/on-line analysis in place of traditional off-line analysis.[15] One potential opportunity is content uniformity determination of tablets immediately after the compaction stage. Traditionally, high-performance liquid chromatography (HPLC) is used to evaluate the uniformity of the dosage units off-line in the quality control (QC) lab. Due to the heavy workloads usually imposed on QC departments, it is not uncommon for results to take up to two weeks before they are fully analyzed, reviewed, and approved. For a drug product in high demand, this may mean that additional batches have subsequently been manufactured. Thus, control over the manufacturing process cycle is limited and feedback about the success/failure of the unit operation is slow. By incorporating process analytical technology into the compaction stage, the process can be controlled and immediately corrected for any unexpected deviation.[1] It was recently demonstrated that spectroscopic analysis of a fraction of a tablet is a valid substitute for analyzing the whole tablets, as with traditional wet chemistry methodologies.[16] However, the inter-tablet variation will be larger due to incorporation of intra-tablet variation into the results, i.e., sub-sampling. Thus far, both near-infrared (NIR) and Raman spectroscopy have been used to quantitate API concentration in pharmaceutical solid dosage forms, thereby establishing the feasibility of these approaches.[13,14,17–23] However, there appears to be a deficit of publications demonstrating successful application of monitoring techniques in an in-/on-line manner. The first publication on this subject appeared recently,[24] where the feasibility of on-line NIR spectroscopy for content uniformity determination was demonstrated.

With the availability of rapid monitoring techniques, a common approach has been to attempt to analyze as many samples as possible. However, from a statistical perspective, this may not be necessary. Hence, the goal of this study was not only to evaluate the applicability of Raman spectroscopy for in-/on-line monitoring of tablet content uniformity, but also to establish the number of tablets that need to be analyzed for representative statistical sampling using the central limit theorem. Firstly, it was necessary to evaluate the effects of various parameters on spectral quality and to establish a reasonable sampling frequency. The spectrometer chosen to perform the analysis was a low-resolution Raman spectrometer with the capability of obtaining several spectra per second. The effects of ambient fluorescent lighting, sampling distance, and exposure time were investigated, in addition to the influence of sampling volume. A calibration set was analyzed and used for the calibration model and a prediction set was used to validate the accuracy of the calibration model. Lastly, at-/on-line content uniformity analysis of a batch of tablets was performed using optimized acquisition parameters to demonstrate the potential of the technique.

Received 3 February 2006; accepted 28 March 2006.
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0003-7028/06/6006-0672$2.00/0

EXPERIMENTAL

Materials. Acetaminophen (APAP) was obtained from Ruger Chemical Co., Inc. (Linden, NJ). Avicel-PH-101 microcrystalline cellulose (MCC) was obtained from FMC Corporation (Newark, DE). Colloidal SiO_2 was obtained from Cabot Corporation (Billerica, MA). Magnesium stearate was obtained from (Mallinckrodt Chemical, St. Louis, MO). Methocel E5 hydroxypropyl methylcellulose (HPMC) was obtained from Dow Chemicals (Midland, MI). Purified water was retrieved from a PURELAB Ultra Analytic water purification system (ELGA LabWater, High Wycombe, UK). LiChrosolv methanol gradient grade (MeOH) was used for chromatographic analysis of tablets (Merck KGaA, Darmstadt, Germany). Commercial prednisone tablets were obtained from the Purdue pharmacy (West Lafayette, IN). Felodipine was acquired from AstraZeneca Bulk Drug Production (Södertälje, Sweden). Theophylline anhydrous (AT) was obtained from Rhodia (Cranbury, NJ) and compacts of different thickness were obtained by compressing different amounts of AT using an IR press (International Crystal Laboratories, Inc., Garfield, NJ) with a 13 mm die and an applied force of 2000 psi with a dwell time of 5 s. The compact thickness was determined using a micrometer (Mitutoyo America Corp., Aurora, IL).

Raman Spectroscopy. Raman spectra were acquired using an adjustable probe, which is connected by a five-meter fiber-optic umbilical to a mobile console (RP-1 Identification System, SpectraCode Inc., West Lafayette, IN). The Raman spectral excitation source was a 787 nm diode laser with a power of 1.2 W. Raman scattered light was collected through a 100 μm slit and dispersed by a 627 grooves/mm grating in the SpectraPro monochromator/spectrograph (Acton Research Corporation, Acton, MA). The dispersed light was then collected on a 1024 × 256 pixel charge-coupled device (CCD) (model 150 RTE/CCD-256-H, Roper Scientific, Inc., Trenton, NJ). The laser beam is focused to a 500 μm spot in close proximity to the probe window and the instrument could be set to rotate the laser beam at a frequency of 5 Hz.

Static Raman Sampling (At-Line Analysis). Static Raman sampling was carried out in two different ways, either by sampling from a single spot on the tablet, or by using a rotating laser beam and sampling a circular region with a diameter of 5 mm on the tablet. The integration time (exposure time) was set to 200 ms, which in the case of the rotating laser beam experiment corresponded to the analysis of a full circle on the tablet (15.7 mm circumference) with the laser beam rotating at a speed of 5 Hz. For comparison, tablets were also rotated in a sample holder while using a stationary laser beam. The rotational speed of the tablets was approximately 5 Hz with an integration time of 200 ms.

Dynamic Raman Sampling (On-Line Analysis). The tablets were passed directly underneath the stationary laser beam using a conveyor belt in order to mimic tablets passing by the Raman probe following ejection from a tablet press. The experiments were carried out using a custom-made Siemens conveyor belt (Siemens Dematic, Indianapolis, IN) with the integration time (exposure time) being increased to 300 ms in order to maintain single tablet analysis with the speed of that conveyor and the instrument read-out time. Figure 1 depicts the experimental setup.

High-Performance Liquid Chromatography (Off-Line Analysis). Individual tablets were analyzed by HPLC using the appropriate USP analytical procedure.[25] A 3.9 × 300 mm column packed with 10 μm C18 silica particles (Waters μBondapak, Waters Chromatography Ireland Ltd., Dublin, Ireland) was used for the separation of APAP from its organic impurities using a 25:75 MeOH/H_2O (v/v) mobile phase with a 1.5 mL flow rate. The chromatographic system also included a Waters 600 quaternary pump, a Waters 717+ auto-injector, and a Waters 486 variable single wavelength detector (all Waters Corporation, Milford, MA). Sample solutions had a concentration of about 10 μg/mL and 20 μL sample solution was injected onto the column. The only deviation from the USP procedure was that the sample preparation was performed by disintegrating intact tablets in pure water on a mechanical shaker instead of ultrasonicating crushed tablets in mobile phase. The alternate sample preparation methodology was deemed sufficient because of the rapid disintegration of the tablets along with the high solubility of APAP in water. The maximum concentration of APAP in any solution used was below 1/15th of the solubility at room temperature, which has been reported to be about 14 mg/mL. A short validation was successfully performed, which established the equivalence of

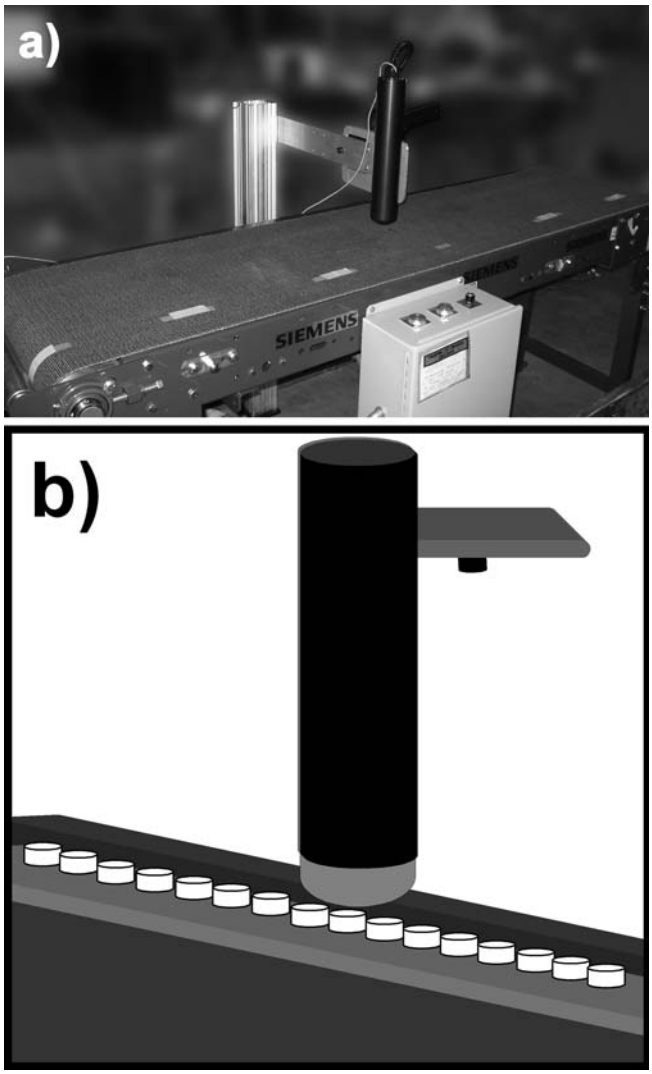

FIG. 1. Experimental setup for the dynamic analysis system. (**a**) Picture of the Raman probe and the conveyor belt (digitally manipulated to reduce background clutter); (**b**) illustration shows how tablets are lined up on a sample holder that is placed on the conveyor belt, which moves the stream of tablets underneath the Raman probe enabling sampling of individual tablets.

TABLE I. Tablet formulations used with each ingredient in percent of total weight. The target weight was 330 mg for calibration tablets and 485 mg for production tablets.[a]

Ingredient	Batch 75	Batch 85	Batch 90	Batch 95	Batch 100
APAP	18.65% (w/w)	21.12% (w/w)	22.37% (w/w)	23.62% (w/w)	24.89% (w/w)
SiO_2	0.02% (w/w)	0.02% (w/w)	0.02% (w/w)	0.02% (w/w)	0.02% (w/w)
MCC	75.47% (w/w)	73.17% (w/w)	72.00% (w/w)	70.83% (w/w)	69.64% (w/w)
HPMC	5.36% (w/w)	5.20% (w/w)	5.11% (w/w)	5.03% (w/w)	4.95% (w/w)
Mg stearate	0.50% (w/w)	0.50% (w/w)	0.50% (w/w)	0.50% (w/w)	0.50% (w/w)
Level	74.9%	84.9%	89.9%	94.9%	100.0%
Ingredient	Batch 105	Batch 110	Batch 115	Batch 125	Production
APAP	26.23% (w/w)	27.38% (w/w)	28.68% (w/w)	31.09% (w/w)	24.89% (w/w)
SiO_2	0.03% (w/w)	0.03% (w/w)	0.03% (w/w)	0.03% (w/w)	0.02% (w/w)
MCC	68.39% (w/w)	67.32% (w/w)	66.10% (w/w)	63.85% (w/w)	69.64% (w/w)
HPMC	4.86% (w/w)	4.78% (w/w)	4.69% (w/w)	4.53% (w/w)	4.95% (w/w)
Mg stearate	0.50% (w/w)	0.50% (w/w)	0.50% (w/w)	0.50% (w/w)	0.50% (w/w)
Level	105.4%	110.0%	115.2%	124.9%	100.0%

[a] APAP: acetaminophen (paracetamol); SiO_2: collodial silicon dioxide; MCC: microcrystalline cellulose; HPMC: hydroxypropyl methylcellulose; and Mg Stearate: magnesium stearate.

this alternative sample preparation methodology to that listed in the USP monograph.

Calibration Tablet Manufacturing. Calibration tablets were manufactured by direct compression using a DIAF TM20 single station tablet press (A/S Maskinfabriken DIAF, Copenhagen, Denmark). APAP was first mixed with the SiO_2 before addition of MCC and HPMC. Following blending, magnesium stearate was added and the formulation was mixed for a short period prior to compaction. Tablets weighing 330 mg were then made with flat 10 mm tooling.

The calibration tablets spanned API concentrations from 75% API to 125% of the intended concentration, as shown in Table I. A total of 28 tablets from each level were weighed and the average weight and weight variation for each batch are shown in Table II. The weight variation between tablets confirmed that the die filling was quite repeatable, although it should be noted that there might be some concentration variations within each tablet and between the target and actual concentration.

Production Tablet Manufacturing. A 1 kg batch of uncoated, biconvex tablets was manufactured using 13/32-inch tooling. The formulation was identical to that of the calibration Batch 100 as shown in Table I, but the excipients were wet granulated in order to improve flowability and homogeneity.

Production tablets were manufactured using a Stokes B2 16-station rotary tablet press (FJ Stokes Machine Company, Philadelphia, PA) with a production rate of about 10 000 tablets per hour. All the ingredients, except magnesium stearate, were weighed and placed in a Diosna P 1/6 high shear mixer-granulator (Dierks & Söhne GmbH, Osnabrück, Germany) equipped with a 2 L stainless steel bowl. The granulation liquid was sprayed onto the mix using a Masterflex Quick Load Model 7021–24 pump (Cole-Palmer Instrument Co., IL), and the amount of binder solution added was determined gravimetrically using a Mettler PC 8000 balance (Mettler Toledo, Inc., Hightstown, NJ). About 25% water was added during the wet granulation step and was removed by storing the granules at low humidity (~15% relative humidity (RH)) for 48 h at room temperature. Magnesium stearate was added to the dried granules and the mixture was blended in a bin blender (Tote Systems International, Burleson, TX) for 5 min before being added to the tablet press hopper. The average tablet weight was 486 mg with an average crushing strength of about 50 N. Crushing strength and average tablet weight were

TABLE II. Characterization of tablet batches. Weight variation and API concentration as determined by HPLC.[a]

	Batch 75		Batch 85		Batch 90		Batch 95		Batch 100	
	Weight	% API	Weight	% API	Weight	% API	Weight	% API	Weight	% API
Theoretical API conc.		18.65		21.12		22.37		23.62		24.89
Average	327.14	18.47	317.48	20.92	323.36	22.04	328.34	23.46	319.35	24.68
Max	333.70	18.72	326.00	23.45	328.70	22.49	332.50	26.76	323.40	26.42
Min	321.40	18.19	313.90	20.24	316.10	21.69	322.80	22.84	314.10	24.13
Std. dev.	4.06	0.13	2.53	0.62	3.21	0.19	2.57	0.94	2.83	0.50
% RSD	1.24	0.73	0.80	2.95	0.99	0.84	0.78	4.01	0.88	2.04
	Batch 105		Batch 110		Batch 115		Batch 125		Test set	
	Weight	% API	Weight	% API	Weight	% API	Weight	% API	Weight	% API
Theoretical API conc.		26.23		27.38		28.68		31.09		24.89
Average	323.06	26.05	332.96	26.97	324.61	28.38	322.35	30.80	483.15	24.63
Max	326.90	26.86	340.20	27.72	334.10	30.28	329.90	33.44	502.10	25.22
Min	318.30	25.62	327.80	26.50	315.40	27.97	313.80	30.38	461.10	23.57
Std. dev.	2.34	0.30	3.46	0.23	4.46	0.49	4.46	0.62	9.12	0.35
% RSD	0.72	1.15	1.04	0.86	1.37	1.73	1.38	2.02	1.89	1.43

[a] $n = 28$ tablets for calibration batches and $n = 30$ tablets for production batch.

monitored throughout the process so that any deviation could be corrected.

Software. The RP-1 software, which was built using LabVIEW version 5.1 (National Instruments, Inc., Austin, TX), was provided with the Raman instrument. This software was used to control the Raman spectrometer. In order to achieve a faster data acquisition, some modifications were made to the software. These modifications included the ability to store spectra in individual files in order to control the read-out time between samples and enhancement of the procedure for storing multiple spectra to one file. Millennium32 chromatography data system (version 4.0, Waters Corporation, Milford, MA) was used to control the HPLC analysis, as well as for analyzing the HPLC results. SIMCA-P+ (version 10.5, Umetrics AB, Umeå, Sweden) was used for principal components analysis (PCA) and partial least squares (PLS) regression analysis, as well as discriminant analysis. All PLS models were constructed using standard normal variate (SNV) transformed Raman spectra[26] unless otherwise noted. Additionally, data matrices were always mean centered before PLS regression was carried out. For the APAP calibrations, three different models were calculated. First, the average Raman spectrum at each level was calculated and used as the X values. Second, the average spectrum for each individual tablet was determined and used as the X values. Finally, each individual Raman spectrum was used as the X values. This latter approach introduced slightly more noise into the calibration and the first approach did not include enough variation to reliably predict unknown tablet; thus, the second methodology was used for all PLS calibrations unless otherwise noted. Excel 2000 (build 9.0.2720, Microsoft Corporation, Seattle, WA) was used for calibration calculations. Sigma Plot (version 8.02, SSPS Inc., Chicago, IL) was used for curve fitting and graph plotting. PhotoShop CS (version 8.0, Adobe Systems Inc., San Jose, CA) was used for figure manipulations.

RESULTS AND DISCUSSION

System Performance. Prior to performing an analysis of tablet content, the system performance of the Raman instrument was evaluated with respect to the effects of ambient fluorescent lighting, probe-to-tablet sampling distance, integration time, and the sampling volume in order to establish the system suitability and optimize analysis conditions. In an effort to minimize tablet-to-tablet drug content variability as a contributing factor, commercial prednisone tablets were used for the system performance tests unless otherwise noted.

Effect of Light. Fluorescent lighting produces characteristic peaks in the near-infrared spectrum, which can interfere with the Raman scattered light produced when using a 785 nm excitation laser. Hence, the effect of fluorescent lighting on the spectral quality was investigated by comparing the spectra of 30 tablets analyzed either with ambient fluorescent room lighting or in the dark. No significant effect of light on the spectral signature could be visually observed. The Raman spectra were further analyzed by partial least squares discriminate analysis (PLS-DA) following pretreatment by SNV transformation. The PLS-DA model attempts to position the principal components so that they describe the maximum difference between the two types of measurements.[27,28] One-third of the measurements were used as the prediction set and the rest were used to construct the discriminant model. Only six out of 20 tablets from the prediction set could be successfully predicted as having been collected in dark conditions or under fluorescent lighting. This lack of predictability indicates that the effect of light on sampling is negligible. One reason for this may be that the effective probe-to-tablet sampling distance is only a few millimeters, with the probe being significantly wider than a tablet. This feature would minimize the stray light from overhead lighting that can enter the analyzer and interfere with the Raman spectrum of the tablets.

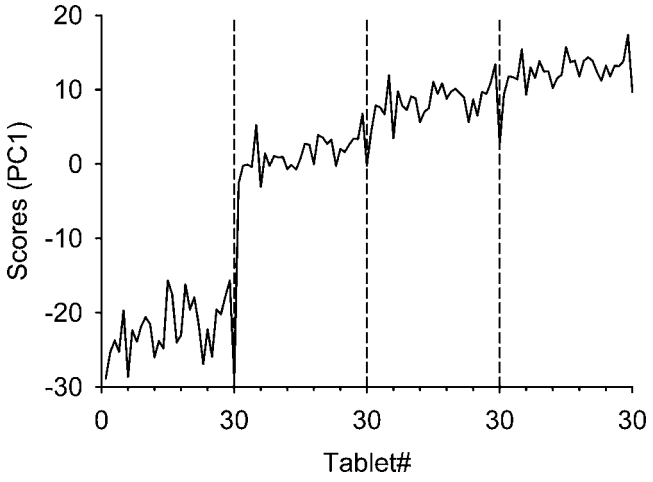

FIG. 2. The effect of integration time as represented by score values of the first principal component. This principal component was interpreted as spectral quality following analysis of the loadings.

Effect of Integration Time. An investigation into the effect of integration time on spectral quality was performed using the rotating laser beam configuration. Spectra were collected from 30 tablets using four different integration times, namely, 100, 200, 400, and 800 ms, corresponding to 0.5, 1, 2, and 4 laser beam rotations. The results were analyzed using PCA of SNV transformed data. The first principal component (PC), which described 59% of the variation in the data, was interpreted as describing the change in signal-to-noise ratio (SNR) of the measurements. In Fig. 2, score values of the first principal component for each tablet and integration time have been plotted. The most significant improvement in SNR occurs as the integration time is increased from 100 ms to 200 ms, the latter being equivalent to one revolution of the laser beam. Only marginal improvements are seen by further increasing the integration time, presumably because the sampling area is not increased any further. The optimum SNR with minimal integration time was determined to be 200 ms. Hence, this integration time was used for all experiments unless otherwise noted.

Effect of Sampling Distance. The effect of probe-to-tablet sampling distance on spectral quality was evaluated by assessing the SNR for an APAP calibration tablet at different sampling distances. The SNR was determined by comparing the peak height at 1683 cm^{-1} to the peak-to-peak noise between 1500 cm^{-1} and 1540 cm^{-1}, as well as between 1850 cm^{-1} and 1900 cm^{-1}, which marked the baseline. As seen in Fig. 3a the SNR decreases exponentially as the sampling distance increases and reaches a value of 10 at a sampling distance of approximately 5.3 mm. The International Conference of Harmonization (ICH) guidelines suggest that an SNR of 10 is the minimum necessary for quantitation;[29] thus, for these experiments, the sampling distance must be maintained between 0 and 5.3 mm throughout the analysis. The sample holder used in these investigations was tested at five different

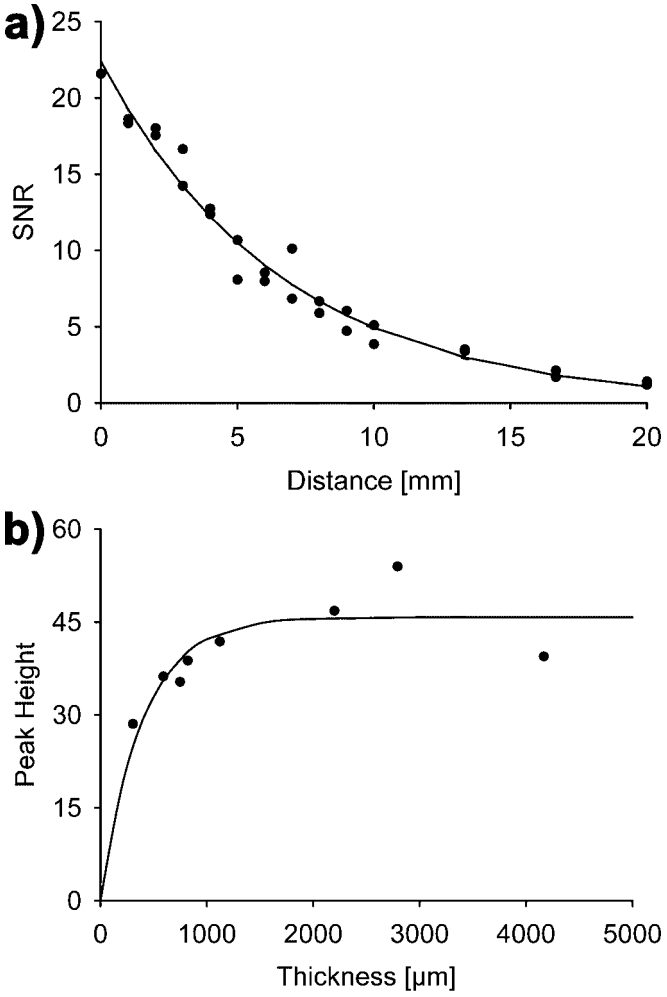

FIG. 3. Evaluation of Raman instrument performance. (**a**) Effect of sampling distance on signal-to-noise ratio; (**b**) determination of penetration depth.

positions and at no time was the sampling distance larger than 3.7 mm.

Effect of Sampling Volume. The effect of sampling volume on data variability was assessed by comparing the scattering of the score values for different sampling methodologies in the 2D score plots (Fig. 4). Three different sampling methodologies for tablet analysis were compared, namely, a stationary laser beam sampling from a single spot, a rotating laser beam sampling from a circular pattern, and a stationary laser beam with a rotating sample holder, which basically also samples in a circular pattern. Each tablet was analyzed at three different points when using the stationary laser beam, and these results were averaged to provide another sampling area measurement. Following PCA of SNV transformed data, the scores from the first two PCs were plotted in principal component space and ellipses were drawn to encompass the score values for each sampling technique (see Fig. 4). A larger ellipse indicates greater spectral variation. It can be clearly seen from Fig. 4 that the rotating laser beam yields the smallest ellipse, i.e., the tablets give similar spectral signatures, as expected, since they are all "identical". The area of this ellipse is only 6% of the ellipse obtained from the single-point measurements. Furthermore, the latter area is nearly six times larger than the ellipse from the averaged data. The larger ellipse obtained from tablet rotation as compared to the rotating laser beam is believed to arise because

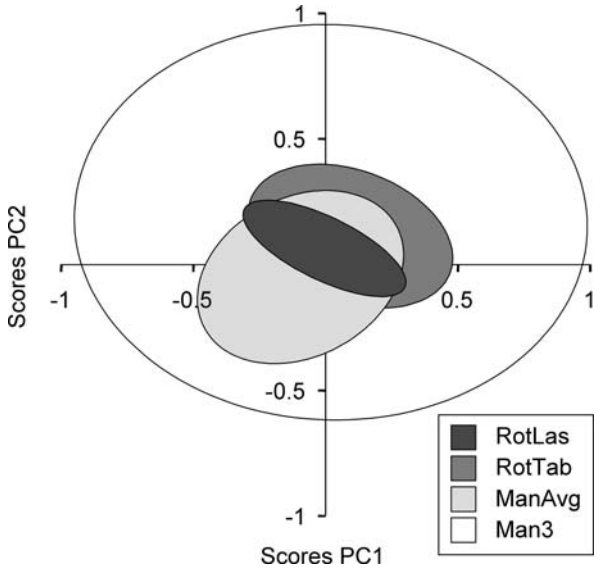

FIG. 4. Score plot illustrating the effect of sampling methodology on subsampling of commercial prednisone tablets. The white area is individual spot determination (Man3); the light gray area is the average of three spots (ManAvg); the medium gray area is from a rotating tablet (RotTab); the dark gray area is when using a rotating laser spot (RotLas).

of some vertical motion when rotating the tablet. These data illustrate how inhomogeneous the tablet surface is at the resolution of the laser beam diameter and highlight one of the limitations of Raman spectroscopy for bulk analysis, namely, the small scale of scrutiny relative to the scale of the sample.

Sampling volume was further evaluated by analyzing the depth of penetration using a previously described methodology.[30] The maximum penetration depth was estimated to be approximately 2 mm from the data presented in Fig. 3b. Laser beam diameter estimates were provided by the instrument manufacturer and were reported to be 500 µm. The apparent sampling volume for the single-spot measurement was then estimated by multiplying the maximum penetration depth by the area of illumination, assuming a cylindrical sample volume. For the rotating laser beam, the sampling volume was estimated by multiplying the circumference of the circle analyzed with the laser beam diameter and the penetration depth. For dynamic sampling, i.e., when the tablet is moving through the laser beam, the length of analysis (integration time times the conveyor speed) was multiplied by the laser beam diameter and the penetration depth. The sampling volume was found to be 0.38 µL, 15 µL, and 7.5 µL for single spot, rotating laser beam, and moving tablet, respectively. Thus, the difference in sampling volume is not that large between the rotating laser beam and a moving tablet, but vastly different compared to the single-spot analysis. The estimations of sampling volumes can be used to explain the results shown in Fig. 4. It can be concluded from these experiments that sampling over an area larger than a single spot will be necessary to accurately predict API concentrations of individual tablets and that an integration time of at least 200 ms must be used. Ideally, a rotating pattern should be used to maximize the sampling volume, but a single line across the tablet may also be sufficient to provide representative data, suggesting that it might be possible to analyze a moving stream of tablets.

Conveyor Belt Calibration. For the on-line experiments it was imperative to establish that the Raman spectrometer could

be used to collect data corresponding to a single tablet with no overlap from neighboring tablets. Tablets were placed on a conveyer belt and passed underneath the Raman probe at a speed of 25.7 mm/s, which theoretically enabled sampling of individual tablets at a rate of about 2.5 tablets per second. In order to verify that only one tablet at a time was actually sampled, a set of alternating placebo and active tablets (in-house prepared tablets containing felodipine as the active ingredient), both with a 10 mm diameter, were placed on the conveyor belt. The peak height of the most intense drug peak was then used to verify the presence or absence of the drug. The peak height values of 28 spectra obtained from a moving stream of 28 tablets are plotted in Fig. 5. It can clearly be seen that it is possible to discriminate between active and placebo tablets since the average peak height of the placebo tablets was 0.10 units compared to the average peak height of 64 units for the active tablets, verifying that only individual tablets contribute to the spectral information. This calibration was re-run at the start of each day to ensure that the conveyor belt was operating correctly.

The Process Analytical Approach to Content Uniformity. The currently accepted procedure[31] to establish content uniformity for a batch of pharmaceutical solid dosage forms, regardless of the batch size, is to randomly select at least 30 tablets from the entire batch. Of these selected tablets, 10 tablets are individually assayed using the specified analytical procedure in the monograph. An acceptance value is then calculated based on the results of the individual tablets analyzed. If this acceptance value is greater than the specified limit, an additional 20 tablets are assayed individually using the same procedure. If the calculated acceptance value, based on the results of the 30 tablets assayed, is lower than or equal to the specified limit, the batch passes the content uniformity requirement. A major advantage of at-/on-line monitoring of content uniformity using a rapid analytical method would be a better estimation of the actual variation of API concentration across a batch of tablets. This can only be achieved by analyzing many more tablets than the currently recommended maximum of 30 tablets per batch in pharmaceutical guidelines. A recent publication established sampling guidelines for content uniformity based on the central limit theorem.[32] The authors showed that the minimum number of tablets that need to be analyzed in order to estimate the average API concentration in tablets could be calculated using Eq. 1:

$$N = \frac{z^2 \cdot p \cdot (1-p)}{E^2} \quad (1)$$

where z is the standard normal random variable for a given confidence level, p is the sample proportion, and E is the margin of error.

By setting the sample proportion to the worst case of 0.5, the minimum number of tablets needed to establish the API concentration for an infinitely large batch of tablets at the 99.9% confidence level with a ±5% margin of error would be 1083 tablets. The ±5% margin of error was chosen to match typical content requirements in regulatory guidelines and the 99.9% confidence limit was used to minimize the risk of approving substandard batches.

Furthermore, it has been established that the minimum number of tablets that need to be analyzed for a finite batch size can be determined using Eq. 2.[32]

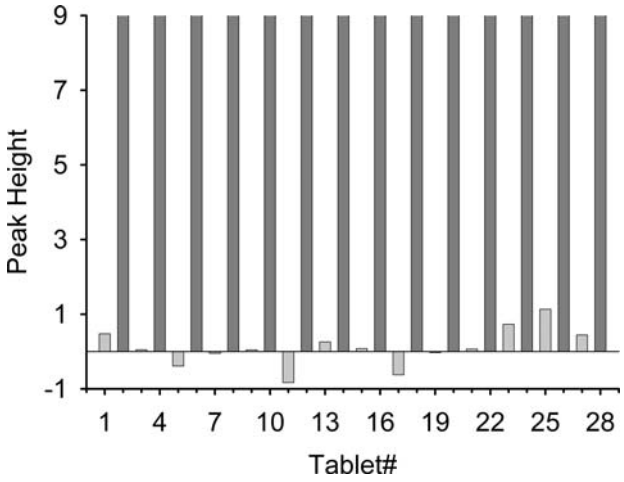

FIG. 5. Confirmation that individual tablets are analyzed using the dynamic setup. The average peak height of placebo tablets (light gray columns) is 0.1, whereas the average peak height of active tablets (dark gray columns) is 64 (note the Y axis has been truncated to illustrate the randomness of the placebo peak). Thus, as there is no trace of active tablet being detected when analyzing the placebo tablets, individual tablets are being measured.

$$n = \frac{N \cdot B}{B + N - 1} \quad (2)$$

where B is the tablet batch size. For a batch size of 2000 tablets prepared for this study, the minimum number of tablets that need to be analyzed according to Eq. 2 is 703 tablets with a 99.9% confidence limit. For an ideal batch, analysis of an appropriate number of tablets should give a normal distribution of API concentrations around the mean concentration. The variability of the batch could then be retrieved from the standard deviation of the Gaussian curve describing the results. If there were problems during the manufacturing processes leading up to the compaction stage, the resulting distribution will either broaden, representing a batch with too large variability for release, or have a skewed distribution whereby even a binomial distribution could be envisioned if there were serious segregation issues during the transfer of granules from the hopper to the compaction area.

Based on the central limit theory calculations, it was decided to analyze 703 tablets out of the production tablets with both at-line and on-line Raman, followed with analysis by HPLC of a subset of 30 tablets out of the 703, the latter corresponding to the conventional number of tablets typically tested.

Raman Spectroscopy. ***Static Raman Sampling (At-Line Analysis).*** A static calibration was obtained by analyzing 28 tablets of each API concentration level, with each tablet being analyzed three times using the rotating laser beam sampling methodology. A Raman spectrum of a calibration tablet at the 100% level is shown in Fig. 6a. The Raman spectra were SNV transformed and the data matrix mean centered before performing the PLS regression analysis, where the Raman spectra were correlated to APAP concentrations obtained from the HPLC analysis. The HPLC results of the calibration tablets are summarized in Table II. One principal component was needed to fit the Raman data to the assay results from the HPLC. The loading weights of this principal component could be interpreted as the increase of APAP in the tablet formulation (Fig. 6b). The R^2Y and Q^2 for the static Raman calibration model were 0.905 and 0.905, respectively. The RMSEP when predicting 45 calibration tablets (five at each level, not used in

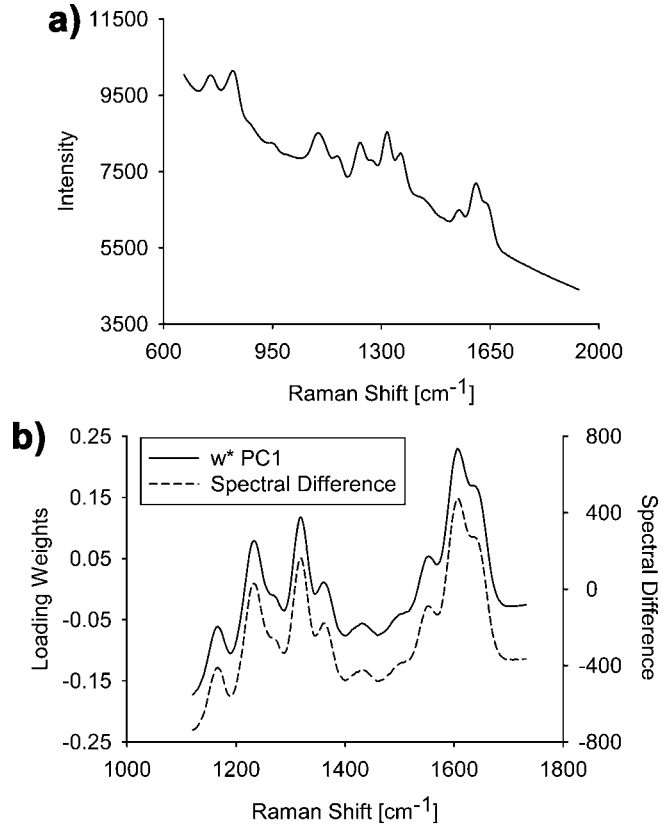

FIG. 6. Spectral data. (**a**) Raman spectrum of a calibration tablet captured using the rotating laser beam setup; (**b**) loading weights of the first PC compared to the spectral difference of high and low APAP content tablets.

the calibration model) was 4.0% APAP, with respect to the intended APAP concentration, and the mean value was 0.6% higher than for the HPLC analysis. In addition, 30 randomly selected production tablets were selected and analyzed using the static Raman setup. The average concentrations predicted by the model were about 1.0% lower than those reported from the HPLC analysis, with an RMSEP of 3.5% APAP, with respect to the intended APAP concentration. A summary of these results is shown in Table III. The variability of the spectroscopic predictions was almost three times that of the HPLC. The reason for the increased variability is that the errors in both the Raman measurement and in the HPLC results are combined, and a secondary calibration can never improve on the quality of the reference method.[16]

Once the static Raman calibration model above had been established, 703 randomly selected tablets from the production batch were analyzed using the rotating laser beam setup. The average APAP concentration predicted from the 703 tablets analyzed by the rotating laser beam setup was 100.5% with an RSD of 3.8%. The highest concentration found was 113.9% of the intended concentration and the lowest concentration found was 87.5%. Thus, no tablets were outside the ±15% limit. A histogram of the API concentration of the 703 tablets as predicted by the static calibration is shown in Fig. 7c. For comparison, histograms of the HPLC results for 10 and 30 tablets are shown in Figs. 7a and 7b, respectively. Clearly, the conventional testing regimen does not provide any information about the quality of the batch beyond confirming that the few selected tablets meet specifications. For a hypothetical poorly controlled production batch with the API concentration of the

TABLE III. Comparison between HPLC results of 30 randomly selected tablets and the results of the same tablets analyzed by Raman spectroscopy using the dynamic and static setup, respectively.[a]

	HPLC assay	Dynamic prediction		Static prediction	
	Conc. APAP	Predicted	% HPLC	Predicted	% HPLC
Average	24.6	23.9	97.2	24.4	99.0
Max	25.2	26.6	105.4	25.8	102.1
Min	23.6	20.9	88.5	22.0	93.3
Std. Dev.	0.4	1.1		0.9	
% RSD	1.4	4.8		3.8	

[a] $n = 30$ tablets.

tablets still normally distributed around the theoretical mean, but with 12% of the tablets being outside the ±15% specification limit, there would still be a 30% chance that a random selection of 10 tablets would fail to show a single tablet outside this limit. Additionally, using Eq. 1, the confidence level that the true mean is within 5% of the estimated mean is only 25% when using only 10 tablets for the estimation. Hence, the selection of 30 tablets and testing of 10 is statistically more likely to result in a passing batch, whereas the analysis of a larger number of tablets will highlight problems with a certain population of the batch.

Dynamic Raman Sampling (On-Line Analysis). Initially the calibration model described above was used to predict 30 randomly selected production tablets, which were analyzed individually as a moving stream with a stationary laser beam mounted over a conveyor belt. For the moving samples, the APAP concentration was consistently predicted about 6% lower than the analysis using the rotating laser beam setup and stationary tablets. This prediction error illustrates the importance of using the same sampling procedure for both calibration and test samples.

The calibration tablets were then analyzed individually while in motion using the stationary laser beam mounted over a conveyor belt. The tablets were placed on a sample carrier and passed underneath the Raman probe at a speed of 25.7 mm/s, which allowed sampling of individual tablets at a rate of about 2.5 tablets per second, or 150 tablets per minute. The R^2Y and Q^2 for the one PC dynamic Raman calibration model were 0.934 and 0.933, respectively. This PC was, as expected, very similar to that of the static calibration. The RMSEP when predicting 45 calibration tablets (5 at each level and not used in the calibration model) was 3.7% APAP with the average 1% below that of the HPLC analysis. Next, 30 randomly selected production tablets were selected and analyzed using the dynamic Raman setup. This dynamic calibration followed the same trend as the static calibration with the prediction of the production tablets by the Raman method underestimating the APAP concentration relative to the HPLC results. A comparison between the HPLC results and the Raman calibrations can be seen in Table III.

Finally, 703 randomly selected production tablets were quantified using the same setup as described earlier. Since the production tablets were slightly larger than the calibration tablets, an adjustment was required to facilitate individual tablet sampling through either increasing the integration time by 10 ms or increasing the conveyor speed by 0.8 mm/s. The former was chosen, since reliably adjusting the conveyor speed for such a small change was deemed impossible. The average

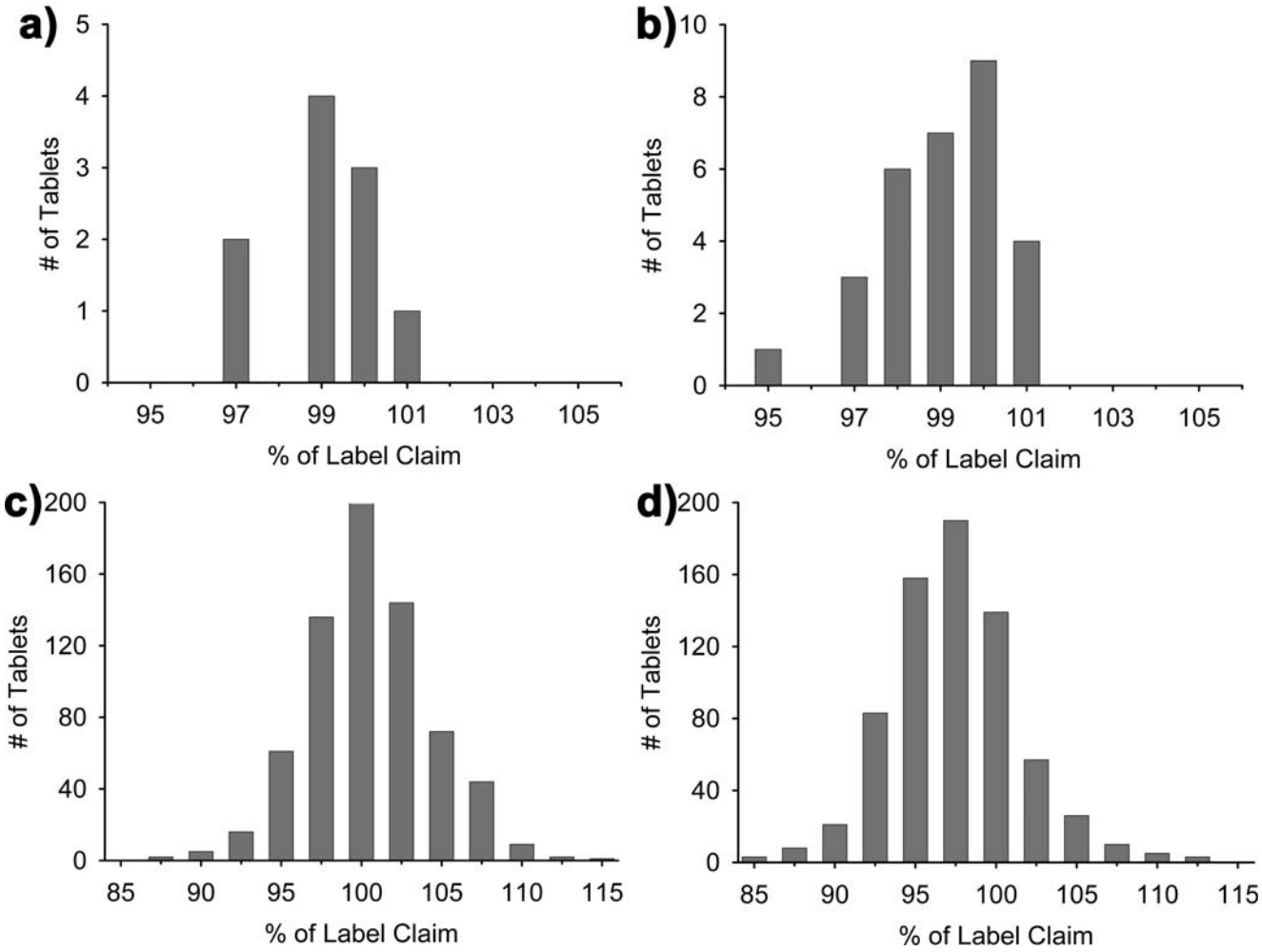

FIG. 7. Histograms of API concentration of individual tablets as assayed by HPLC and Raman spectroscopy. (**a**) HPLC result on 10 random tablets; (**b**) HPLC result on 30 random tablets; (**c**) Raman spectroscopy result on 703 random tablets analyzed using the static setup with the rotating laser beam; and (**d**) Raman spectroscopy result on 703 random tablets analyzed using the dynamic setup.

APAP concentration of the 703 tablets was found to be 97.4% with an RSD of 4.2%. The highest concentration found was 113.6% of the intended concentration and the lowest 83.3%, which was the only tablet outside the ±15% level. According to regulatory guidelines,[31] two tablets can be outside the ±15% level, but no tablet is allowed to be outside the ±25% level. Thus, this would be a passing batch; however, the guideline refers to testing only 30 tablets. This raises the question of a new approach for the regulatory framework in order to encompass these new process analytical approaches to testing. As the histogram of the analysis results shows in Fig. 7d, this batch of tablets has a normal distribution of API content, as would be expected for a manufacturing process that is in control. This result also demonstrates the validity of the central limit theorem for determining an appropriate number of tablets to be analyzed and suggests that a new guideline could offer an alternative for passing batches by emphasizing the coefficients of a Gaussian curve, i.e., mean and standard deviation, instead of content values of individual tablets. By fitting a Gaussian peak to the dynamic results, the peak maximum was described at 97.1% with a standard deviation of 3.6%. Such values, in combination with weight variation, should be adequate for release of a tablet batch. Furthermore, if problems arose during tablet manufacturing, the moments about the mean can play an important role in understanding the reason for any deviation from specification. As can be seen from the histogram of the dynamic results in Fig. 7d, the results are comparable to the initial results obtained using the static calibration in Fig. 7c; however, the average value has dropped 3%. This decrease in the estimated API concentration might arise for several reasons. However, the most probable explanation in this case is that the calibration samples are not identical to the manufactured tablet batch. This is another example of the phenomenon discussed earlier and highlights how critical it is that calibration samples are identical to, and analyzed in the same manner as, future unknown samples. In this case, the calibration samples were flat-faced tablets, whereas the production samples were biconvex tablets. When the tablets were analyzed using the static Raman setup using the rotating excitation laser beam, the tablet height variation would not affect the analysis since the circle analyzed by the laser is at a constant distance from the probe, just as it is for a flat-faced tablet. During the dynamic sampling, the tablets move under the laser, which, being biconvex, results in a height variation of approximately 1 mm. In contrast, the flat calibration samples will present a constant distance to the laser. Such variation was not built into the model and is the most likely explanation for the observed discrepancy in the

estimated content by Raman spectroscopy between stationary and moving samples.

In-Line Monitoring for Control of the Tabletting Process. The PAT initiative published by the FDA states that if a process can be shown to be monitored and controlled throughout a manufacturing stage, the need for an end-point test can be omitted, provided that significant understanding of the process has been reached.[1] One approach to control a tabletting process could be by interfacing a fast Raman instrument, like the RP-1, with a tablet press. Sampling every individual tablet in-line is not feasible with the current Raman instrumentation since commercial tablet presses can have production rates in excess of 30 tablets per second.[32] However, one approach to analyze this number of tablets could be by collecting the Raman spectra from several tablets at a time, i.e., integrating as several tablets pass under the laser. Out-of-specification events could still be detected, and the process could be automatically or semi-automatically adjusted to get back within the specification limits. To illustrate this methodology, 122 tablets segmented into various concentrations were lined up and analyzed repeatedly using an integration time of 1.9 s with the conveyor speed set such that 10 tablets were integrated per scan. The theoretical pattern based on the individual tablet concentrations is compared with the predicted patterns as shown in Fig. 8. The patterns from the predicted values have similar features to those constructed using the theoretical concentrations, indicating that this could be a feasible approach to monitor and control the compaction stage of a pharmaceutical product manufacturing process.

Comparisons with Other Techniques. To date, only one other study has been published demonstrating on-line determination of content uniformity,[24] even though several analytical techniques have been used to assay pharmaceutical tablets at-line or off-line. Colón Soto et al. established that they could assay up to 110 ibuprofen tablets per minute using on-line NIR spectroscopy. They reported prediction errors of about 4.7% of the intended ibuprofen concentration. In this study, tablets were assayed with a prediction error of 4.9% of the intended APAP concentration, using Raman spectroscopy at a sampling frequency of 150 tablets per minute. Comparing the results of these studies, it would appear that the two spectroscopic techniques yield comparable results in terms of prediction errors and sampling speed and that either technique is potentially useful for content uniformity determination. It is likely that the technique of choice will ultimately depend on the specific formulation, with NIR spectroscopy being better in some cases while for other products, Raman spectroscopy will offer an advantage. However, regardless of the analytical technique employed, taking sampling statistics into consideration will lead to a better description of the drug product batch and avoid unnecessary data generation and handling.

CONCLUSION

The feasibility of using Raman spectroscopy as a process analytical tool for determining content uniformity of pharmaceutical solid dosage forms has been demonstrated. A complete study on the effect of sampling properties such as integration time and sampling distance, as well as influence of fluorescence lighting, has been performed in order to optimize sampling. Furthermore, at-line and on-line methodologies were investigated and compared. While the choice of methodology did not play a major part in the final result, it was found to be crucial that the calibration was carried out in exactly the same manner as for the unknown samples. Additionally, a new approach for assessing the quality of the batch in terms of content uniformity was presented based on central limit theorem sampling statistics. Using such an approach at a 99.9% confidence level and ±5% margin of error, no more than 1083 randomly selected tablets need to be analyzed to fully characterize a batch, which is a realistic number for on-line analysis, and perhaps even for some at-line methods. Analyzing this number of tablets can give greater understanding of the quality of the batch compared with traditional analysis of no more than 30 tablets per batch, since the moments of the mean can be extensively interpreted. Finally, a promising approach for in-line monitoring and control of a production-scale tablet press was presented.

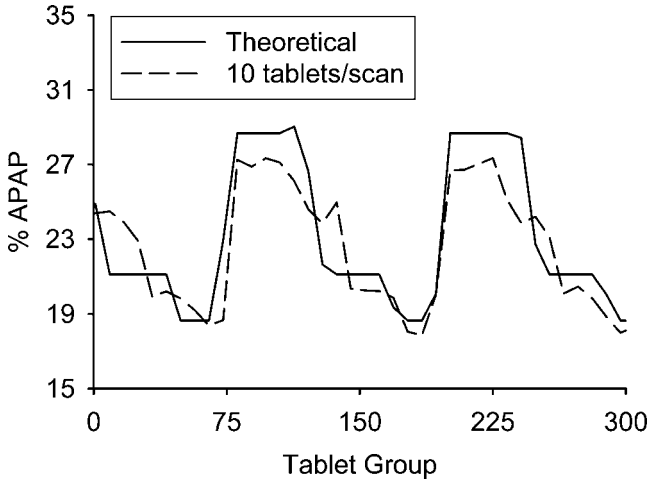

FIG. 8. Control chart from in-line Raman experiment. The theoretical pattern, based on the individual tablet concentrations, is compared with the predicted patterns when analyzing 10 tablets per scan.

ACKNOWLEDGMENTS

Winston Bonawi-Tan (Tranzact Technologies, Elmhurst, IL) is acknowledged for his assistance in analytical work. Paul Hunckler of Siemens Dematic (Material Handling Automation Division, Indianapolis, IN) is thanked for providing the vital conveyor system. The Center for Advance Manufacturing and the E-Enterprise Center at Purdue University are thanked for financial support. AstraZeneca R&D Mölndal is also gratefully acknowledged for providing analytical support and funding for H.W.

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