I, Debra Sue Hausman-Manning, hereby submit this work as part of the requirements for the degree of:

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Dedication

This dissertation is dedicated to my advisor Dr. Adel Sakr for his vision, to my parents for their constant encouragement, friendship, and wisdom, and to my husband, Philip, of whom I could not ask for more.
Abstract

Process Analytical Technology (PAT) was utilized for on-line evaluation of drug hydration state and its effect on final product quality, as well as the effect of blending parameters on low dose blend and tablet uniformity.

Experiments were conducted to elucidate the relationship between risedronate sodium (RS) hydration state and the physical stability of tablets containing RS. The RS crystal lattice contains channels occupied by water which is removed by drying processes at temperatures below the boiling point of water, causing a reversible contraction of the crystal lattice. In this study, RS was wet granulated followed by fluid bed drying and compression into tablets. During drying, RS solid-state form was continuously monitored using on-line Raman spectroscopy. It was determined that final granulation moisture had a significant effect on change in RS hydration state measured by Raman and on change in tablet thickness over time. In addition, change in RS hydration state during fluid bed drying, measured by on-line Raman, was correlated to the increase in tablet thickness and subsequent loss of tablet integrity. Evaluation of RS
solid-state during drying with Raman enabled establishment of relationships between fundamental hydration dynamics associated with RS and final product performance attributes.

On-line Raman was also used to evaluate the effect of blending parameters on uniformity of a low dose, 1%, blend of azimilide dihydrochloride. Parameters investigated were blend time, blender speed, azimilide placement in the blender, filler particle size and density, multiple tablet components, and sampling. At the 8qt scale used, there was no effect of azimilide placement in the blender on time to reach uniformity. However, there was an effect of filler particle size/density and blender speed on time to reach uniformity. On-line Raman analysis of blend uniformity provided more information about the blending process as compared to traditional thief sampling and off-line analysis, due to the feasibility of large numbers of samples across the run (sampling every 20 seconds) and the availability of spectral information on all blend components. Raman measurements of blend uniformity from univariate and multivariate analyses were significantly correlated to HPLC blend thief sample and tablet sample results.
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1. Introduction

The use of Process Analytical Technology (PAT) in the pharmaceutical industry is being endorsed by regulatory agencies, specifically the FDA, and investigated by drug companies. The goal is to produce even higher quality pharmaceuticals and gain a more fundamental understanding of the critical process parameters. This work presents the results of research using spectroscopic techniques on-line, specifically Raman, to control the hydrate state of a model drug during fluid bed drying and to ensure blend and tablet content uniformity at low dose.

1.1 Background

Every pharmaceutical process has one or more mechanisms to control product quality. Historically, these mechanisms have been associated with the process. In order to relate them to product quality, inferences are made based on developed cause/effect relationships and/or physical chemical relationships. One example is control of wet granulation particle size by setting mixing speed, mixing time, granulating fluid flow rate, and total amount of granulating fluid. Another example is
controlling blend uniformity by setting mixing speed, mixing time, and batch size (fill level). While these approaches can provide a repeatable process and high likelihood of a consistent product, they can become problematic if the process and formulation are not robust. For example, the process and formulation may not compensate for changes in raw material properties. Aggressive project timing often requires development of a pharmaceutical process with only a handful of different batches of materials. Therefore, minimal knowledge is acquired on the effect of batch to batch variation prior to marketing a drug product.

When the product controls are process-related, a significant amount of time and money can be spent troubleshooting a process when the impact of variation is experienced over time. In addition, final product quality is traditionally determined at the end of the process. There is a delay of days to weeks between manufacture of the product and release testing. By the time a problem is discovered, multiple batches can be affected.

Methodology which directly measures an aspect of interest and quickly provides feedback on the quality of product, or could control the quality of product, can save time and money and ensure the final product
The analytical tools that are being developed have more informing power than the process controls that are widely used, such as temperature, flow rate, and time. These tools have been termed Process Analytical Technologies (PAT). Process Analytical Technologies are used in a variety of industries and encompass a broad range of systems. One definition for PAT is the “systems for continuous analysis and control of manufacturing processes based on real-time measurements, or rapid measurements during processing, of quality and performance attributes of raw and in-process materials and processes to assure acceptable end product quality at the completion of the process” (Hussain, 2002). This requires a synergy of multiple systems including process analytical chemistry tools, information management tools, feedback process control strategies, and strategies for product/process design and optimization.

The PAT initiative in pharmaceuticals began in the 1990’s. In 1993, an AOAC International Symposium was held entitled “Pharmaceutical Process Control and Quality Assessment by Non-Traditional Means.” Then in 2001, an Advisory Committee for Pharmaceutical Science (ACPS) meeting was held and discussed PAT. This meeting prompted
FDA Science Board endorsement of the formation of a PAT subcommittee within ACPS (Hussain, 2001). The PAT subcommittee is comprised of four working groups: 1) Benefits, technology, definitions/terminology, 2) Process and analytical validation, 3) Chemometrics, and 4) Product/process development. These working groups are composed of people from the FDA, industry, and academia. In addition, a new journal has been published to address the expanding role of PAT in the pharmaceutical industry, The Journal of Process Analytical Technology. The first issue was September/October 2004.

It is apparent that PAT will become an important part of the pharmaceutical industry as it has in other industries. The reason for this is that PAT can improve efficiency and capability of pharmaceutical processes while verifying and improving product quality. Results that the pharmaceutical industry anticipates from optimal application of PAT include reduced risk of scrap and recalls, improved process understanding with quality “by design”, improved capacity utilization and reduced cycle times, and eventual reduction in product development time. The current methods to verify product quality, final product testing at the end of manufacturing and some process operation ranges, cause
low manufacturing efficiency and require a very high level of regulatory scrutiny (Hussain, 2002). For example, if a batch fails final product release testing for product quality control, these results are generally not known until weeks after the batch was manufactured. In that time period, multiple batches could have been manufactured with the same problems and would have to be rejected. In contrast, PAT allows for rapid identification of out of the ordinary situations when the critical product properties are defined up front. Therefore, the time to respond to problems is greatly reduced and much time and money can be saved. PAT also allows for statistical process control analysis (trending) of critical in-process product attributes.

1.2 FDA Perspective

The FDA is a great proponent of PAT due to the reasons discussed earlier. In fact, companies can submit PAT based applications or submissions any time they are ready to do so. The FDA encourages companies to contact CDER to discuss the proposed applications or submissions. Currently, the agency is working with two firms that are preparing applications containing PAT. The approach will be a “team” review and inspection process. The team will consist of four reviewers
and four inspectors for PAT applications. The team will be trained and certified in PAT (PDA, 2002). The development of the pharmacy, chemistry, and engineering components of the PAT training program is being accomplished by three National Science Foundation “process centers.” These centers are located at Purdue University, the University of Washington, and the University of Tennessee (The Gold Sheet, 2002).

The FDA recognizes industry hesitation to implement PAT. To eliminate this regulatory uncertainty, the FDA will facilitate PAT introduction in a number of ways. These include accepting PAT applications based on good science (develop standards for PAT), recognizing that the current system is “adequate for intended use.” The use of PAT will not be a requirement. FDA will also define situations in which PAT may replace current end product release testing, develop a process for addressing existing “invisible” problems in marketed products, adjust review and inspection processes, and strive for international harmonization. Part of the implementation of these ideas is a written general guidance on PAT, as well as invitations to companies to propose PAT-based submissions. This includes involving the FDA earlier in concurrent product
development review. This guidance is available as PAT – A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance, September 2004. The PAT guidance currently has five sections: Introduction, Guidance Development Process and Scope, Background, PAT Framework (Process Understanding, Principles and Tools, Strategy for Implementation), and PAT Regulatory Approach. The Principles and Tools section has four subsections: PAT Tools, Risk-Based Approach, Integrated Systems Approach, and Real Time Release. The goal of the guidance is to encourage voluntary development of innovative manufacturing and quality assurance approaches. The FDA wants to make “safe harbor” standards that reassure companies that if the use of PAT uncovers previously unknown information on a process, the company will not be penalized.

The FDA is committed to moving PAT forward in the pharmaceutical industry. They believe that PAT can be a “win-win” opportunity for the FDA and industry. The optimal application of modern analytical techniques in–process can reduce the rate of scrap/recalls, increase the efficiency of manufacturing and quality control, and provide a better scientific and engineering foundation for current FDA-industry debates.
1.3 Industry Perspective

In general, industry recognizes the advantages that PATs allow. However, there is hesitation to introduce them to processes due to regulatory uncertainty and risk that often lends itself to a “don’t tell” or “don’t use” approach. For example, new technology introduces new questions. Industry is often struggling to meet the current regulatory expectations while maintaining a quick time to launch. There is hesitation to introduce technologies that will cause more questions and increase the time to launch. Another concern is related to marketed products. There is a concern that adding PAT to older validated processes will uncover issues that are not visible with current quality control methods. In other cases, there is simply a resistance to change. Industry development and regulatory infrastructures already have systems in place that make it difficult to allow for new technology.

In addition to these concerns, PAT requires more effort up front to develop methods specific for the drug product being considered. There is also the additional complexity of the interpretation of data collected by
PAT methods. While some methods are very straightforward, others collect a large amount of data and require sophisticated data analysis methodology to relate the output to an actual physical/chemical phenomenon. A “fingerprint” of the process is not very useful unless there is a good understanding of what the fingerprint is indicating. When there is a change in the fingerprint, it is necessary to know the significance of the change as it relates to product attributes and quality.

In spite of these considerations, PAT is already being implemented to various degrees in the pharmaceutical industry, mainly by what are considered “big pharma” companies, because of the advantages of PAT and the apparent interest of the FDA. For example, Pfizer is utilizing PAT in a number of capacities in its manufacturing processes. These applications include: testing packaging components, raw materials testing in the warehouse rather than the lab, at-line or on-line blend uniformity testing, tracking drying processes, at-line or on-line tablet potency and content uniformity, measuring tablet coating thickness, on-line monitoring of clean-in-place efficiency (rather than cleaning and testing in the lab and then cleaning again if results are insufficient), and surface monitoring of equipment to verify cleaning. All of these uses
allow more rapid response when issues arise and save time and money (Hussain, 2001). In addition to using PAT in the manufacturing process, PAT is also being used during development. PAT can shorten product development time, help develop understanding of critical in-process properties, aid in scale-up and technology transfers, and aid in troubleshooting.

1.4 Examples of Process Analytical Technologies in Pharmaceuticals

A number of different technologies have been utilized to develop relationships between the physical chemical properties of drug product actives, excipients, or intermediates and final product performance. The most widely used on-line and at-line tool is near infrared reflectance (NIR) spectroscopy. Table 1 is a summary of the different technologies with examples of their uses. In addition, PAT was addressed in a recent supplement to the Pharmaceutical Technology September 2004 issue with articles on monitoring granulation drying on-line with near-infrared, monitoring and control of fluid bed drying with thermal effusivity, and near-infrared PAT method development and validation.
Table 1. Summary of PATs and Pharmaceutical Applications

<table>
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<th>Raman</th>
<th>Fluorescence</th>
<th>Miscellaneous</th>
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Near-infrared (NIR)

NIR methods are becoming more common in evaluation of pharmaceutical preparations. Overtones and combinations of the fundamental mid-IR bending and stretching modes are detected in the near infrared region. Absorbances observed by NIR result mainly from the C-H, O-H, and N-H functional groups. NIR has several characteristics which enable its use for diverse applications. NIR has a fast response time, requires no sample preparation, and is non-destructive. The instrumentation is well suited for use in production facilities for on-line measurements. One of the more difficult aspects of NIR methods is the calibration needed. Most compounds absorb in the near infrared and thus cause overlapping bands and interference. Therefore, a chemometric calibration must be used (complicated mathematical technique). Because NIR instruments are accurate and precise, once the calibration is complete, accurate comparisons can be accomplished (Mark et al., 2002). NIR is intrinsically well-suited to measuring the amount of water in a sample, because the greatest extinction coefficient in NIR is provided by water (Burns and Ciurczak,
2001). However, NIR sensitivity to water absorbance can also be a disadvantage if the O-H band interferes with other bands of interest.

NIR has been used to evaluate real-time moisture during the drying process in a fluid bed. Specifically, a “fast-drying” method was developed with the use of NIR to dry granulation at temperatures above the melting point of the active ingredient. This method uses the effect of evaporative cooling during the fluid bed drying process. The active ingredient does not see the inlet air high temperature due to the cooling effect of water evaporating off of the granule surface. There are two stages of drying in a fluid bed: evaporative and diffusion. The NIR curves show this as a linear evaporative region and an exponential diffusion region. The point at which diffusion becomes rate-limiting can be determined from the NIR curve. Until this point, a temperature higher than the melting point of the active can be used because the active is protected by evaporative cooling. An example curve for acetaminophen is shown in Figure 1. The fast drying process gave physically equivalent product and can cut the drying time in half (Morris et al., 2000).
Figure 1. Fluid bed drying curves for acetaminophen granules at 60°C

(Morris et al., 2000)
The fast-drying method was further developed by Wildfong et al. (2002). Wildfong et al. developed a combined expression for the drying profile (evaporative and diffusion) for an ibuprofen granulation. They then wrote a macro that can determine if a process will benefit from fast drying by identifying phenomenological trends. It can predict the time for drying at a certain set of formulation and dryer parameters. This prediction method should be further verified with actual data.

Steck et al. (2001) compared and contrasted two ways of gathering NIR spectra to monitor fluid bed drying. One way is to have a probe in the powder bed. The other way is to have the probe in the effluent stream. They found that both methods can technically determine the end point of drying with equal accuracy. However, there are some practical constraints to both methods. The effluent sampling is more practical, because it does not need access to the interior of the dryer; therefore it is more flexible and can be used with any dryer or material type. However, the probe may need to be heated to prevent condensation of solvent vapor from the dryer onto the probe. The effluent signal is also less noisy. The probe in the powder bed poses the challenge of keeping the probe clean during the drying process. Also, readjustment of the
probe may be necessary if the material drops below the level of the probe. However, if it is necessary to take the product to a specific solvent content, then this method may be better than the effluent method.

Thus far, only drying process evaluation with NIR has been discussed. Fluid bed granulation processes can also be monitored by NIR methods. However, this is more complicated. The complicating factors are the temperature of the sample, the particle density, and the particle size. Also, the detected signal is affected by the state of water in the matrix. This is due to solid-water interactions that affect hydrogen bonding by changing the median interaction energies of the hydroxyl bond. During fluid bed granulation, all of these complicating factors are occurring which lead to non-linearities in the data. Rantanen et al. (2001) were able to extract the factors that explain the non-linear calibration using a four-wavelength NIR set-up and a multivariate calibration method (partial least squares and artificial neural networks). The active in the test granulations was theophylline. They found that the most predictive model used the artificial neural network with a back-propagation algorithm.
The film-coating process has also been monitored using NIR methods. The methods are based on the changes in spectra obtained when the thickness of the coating varies. They make use of chemometrics to relate the thickness or amount of coating to spectral absorbance. This method is attractive compared to other methods that are destructive, such as chemical tests, and that are very time consuming, such as microscopy. It also does not require knowledge of the core tablet thickness, as is needed with tablet thickness testing. Andersson et al. (1999) were able to monitor coating thickness on a tablet consisting of two halves, each with a different active ingredient. The coater used was a 24” Accela Cota. The chemometric calibration was done by first pre-treating the data with multiplicative signal correction followed by a partial least squares analysis. It was based on two variables related to coating thickness: the total amount of coating solution used in the process and the time the tablets were being coated. The recommended calibration offered a correlation of 0.94 and a prediction error of 0.12. The two variables used in calibration correlated well with the coating thickness as determined by microscopy. However, further work was recommended to
base the calibration directly on the coating thickness determined by microscopy.

Kirsch and Drennen (1996) evaluated a similar NIR method used for coating thickness, except they were coating tablets in a Wurster column. Both an ethylcellulose and hydroxypropylmethylcellulose based coating were evaluated. The models had r-squared values of 0.993 or better and standard errors of prediction of 1.03% or less. The method was performed at-line. Future work was planned to develop an on-line method using a fiber optic probe inside the column.

Andersson et al. (2000a) used in-line NIR spectrometry to monitor the coating of pellets in a fluid bed. They used a fiber optic probe that was placed inside the fluid bed. They also used a partial least squares calibration. They then compared the calibration to the coating thickness as determined by image analysis with a CCD camera and a fluorescence microscope. Also, the NIR results were compared to multiple core tablet and coated tablet thickness measurements. They spanned the calibration by analyzing pellets made at different process conditions. They varied water concentration, atomizer pressure, coating
solution flow rate, and inlet temperature. This improved their correlation coefficient. Figure 2 shows good correlation between coating thickness from image analysis and the coating thickness from NIR. The solid line in the middle is the theoretical growth model they developed. Since real time analysis of coating thickness can be performed with NIR, the end point can be determined with more precision. Also, an estimate of variation can be obtained that further characterizes the process.
Figure 2. Theoretical coating growth model (line) and coating thickness from image analysis (dots) (Andersson et al., 2000a)
NIR spectroscopy was also used in the development of a tablet with an active in the coating. The coating was applied in a fluid bed with a Wurster column. Buchanan et al. (1996) used NIR to scale-up the process from pilot plant to production scale. It allowed rapid uniformity and coating precision results. It also helped to identify dead zones in the Wurster, so that the experimental plan could quickly be adjusted. Both multiple linear regression and partial least squares calibrations were evaluated and gave similar and acceptable results.

The use of NIR to analyze blend homogeneity on-line has been investigated. This work was done in part due to the FDA’s draft guidance on blend uniformity which stated that blend uniformity should be done as a regular in-process check. This guidance, however, was rescinded. There is still technical merit to using on-line NIR for analysis of blend homogeneity. The traditional thief sampling method is well known to disturb the powder bed and compromise the validity of the samples. NIR was initially used to analyze thief samples. In 1996, Wargo and Drennen used NIR to evaluate the homogeneity of a powder blend containing hydrochlorothiazide, lactose, croscarmellose sodium, and magnesium stearate. In this study, thief samples were taken from
the 8 qt blender and then measured with the NIR methods. They concluded that NIR had good potential for blend uniformity measurements, but at that time the results were qualitative.

NIR has also been expanded to in-line use. El-Hagrasy et al. (2001) investigated the in-line use of NIR to monitor blending of salicylic acid and lactose through optical ports installed at six positions in an 8 qt V-blender. They found that using multiple sampling points with NIR provided information on the mechanism of blending which was consistent with other studies involving the V-blender. They also found value in monitoring each blend. Differences in end point were observed between situations where the two components were blended together immediately versus when the components were held in a container together for one week before blending.

Duff et al. (1994) used NIR on-line to monitor the uniformity of pellets as they were added to capsules. The set-up used a probe that was mounted onto the encapsulator feed chute. The probe was positioned such that its window was flush with the inner surface of the chute. Some of the issues that were encountered were the flow consistency of the
pellets in the chute and the need to have the probe window covered with pellets in order to reduce the interference of stray light. The calibration was performed by comparing laboratory HPLC data to second derivative NIR data. Both multiple linear regression and partial least squares analysis were used. The partial least squares calibration gave a correlation coefficient of 0.97 and a standard error of 0.5%. The NIR on-line measurement of uniformity was successfully applied in this case.

NIR methods have also been shown to quantitate levels of different polymorphs in mixtures. Polymorphs have different IR spectra, because molecular vibrational patterns of polymorphs vary due to differences in the crystal lattice. There are other methods already being used to evaluate polymorph levels (x-ray crystallography and NMR), however they require more sample preparation and longer analysis time (Blanco and Villar, 2000). Patel et al. (2001) used NIR to quantify the sulfathiazole forms I and III in physical binary mixtures. The calibration was done by plotting the weight percent of form I against the ratio of second derivative of log (1/relative reflectance) to wavelength. The correlation coefficient was greater than 0.998. It was found that the method could quantitate polymorph levels as low as 0.3% in binary
physical mixtures. The most variability introduced was method error related to the mixing of the two components together.

NIR was also used to evaluate the pseudopolymorphic changes of theophylline during wet granulation (Rasanen et al., 2001). Anhydrous theophylline was the only component in the wet granulation, which was performed in a planetary mixer. When a small amount of water was used, NIR bands were detected that had maxima identical to theophylline monohydrate. X-ray diffraction was used to confirm the conversion from anhydrate to monohydrate during wet granulation. When more water was used, NIR spectra were observed that had bands due to OH vibrations of “free” water. Figure 3 shows the transformation that occurs during granulation.
Figure 3. Transformation of theophylline during wet granulation using NIR (Rasanen et al., 2001)
It was also noted that only the outside of the granules were evaluated with this NIR method. If this method were moved to a process on-line, this would need to be taken into account.

There are still other applications for NIR in pharmaceuticals. These include prediction of dissolution rate (Zannikos et al., 1991). Carbamezepine tablets were used to develop a correlation between dissolution time and NIR spectra. When exposed to humidity, these tablets degrade. The presence of moisture also results in a decrease in dissolution. A NIR method was developed that predicted the dissolution rate with a correlation coefficient of 0.985, standard error of estimate of 4.5%, and a standard error of prediction of 6.8%. Another application of NIR is to predict tablet hardness without destroying the tablet (Kirsch and Drennen, 1999). Cimetidine tablets were used for the study at a potency range of 1-20% and hardness range of 1-7 kp. Finally, NIR has been used to determine the median particle size of lactose monohydrate (Frake et al., 1998). The NIR method was calibrated versus laser diffractometry as the reference method. The correlation coefficients were about 0.98 for all the chemometric NIR data analysis techniques, with a root mean square error of prediction of about 5 microns.
Raman and UV/VIS

Raman spectroscopy offers similar advantages to NIR, which enables it to provide on-line physical chemical information linked to final product quality. Raman and NIR probe the vibrational transitions of molecules, providing complimentary information due to different selection rules. Raman spectroscopy utilizes light scattering where an incident photon beam of a specific wavelength is inelastically scattered by molecules. Most incident radiation is either absorbed or elastically scattered (Rayleigh Scatter). A small amount of radiation is modified due to coupling between the photon and the electron cloud of the molecule. Energy can be lost or gained in this process. A “Stokes” shift to longer wavelengths (lower energy) or “Anti-Stokes” shift to shorter wavelengths (higher energy) is detected experimentally. Raman intensity is weak when produced by polar chemical bonds with localized electron clouds. In contrast, non-polar chemical bonds with delocalized electron clouds produce strong Raman intensity. Significant increases in Raman intensity are associated with aromatic ring systems in highly conjugated molecules. In addition, Raman intensity is generally stronger for crystalline materials as compared to non-crystalline (Pelletier, 1999).
Some advantages of Raman for certain applications are that water does not have a strong Raman spectrum, some functional groups can only be observed with Raman, and dipolar broadening that can interfere with IR spectra are not active in Raman (Adar et al., 1997; Vankeirsbilck et al., 2002).

One key attribute that enables implementation of quantitative measurements by Raman is the relationship between signal intensity and the concentration of material present in the sampled region (equation 1, Pelletier, 1999). This quantitative relationship is a critical measurement attribute for successful implementation as a process/product control technique.

\[
I_R = (I_L a K) PC
\]

Where

- \( I_R \) = measured Raman intensity (photons per second)
- \( I_L \) = laser intensity (photons per second)
- \( a \) = absolute Raman cross-section (cm\(^2\) per molecule)
- \( K \) = measurement parameters
- \( P \) = sample pathlength (cm)
- \( C \) = concentration (molecules per cm\(^3\))
A pharmaceutical application that is well-suited to the advantages of Raman is evaluation of drug hydrate state. Raman has been coupled with thermogravimetric analysis (TGA) and simultaneous thermogravimetry and differential thermal analysis (TG/DTA) to monitor desolvation and recrystallization of materials to characterize hydration states of active pharmaceutical ingredients (Bigelow-Kern et al., 2005; Chang, H. and Huang, P., 2001; Ghule et al., 2003; Jager and Prinsloo, 2001). Raman measures the effect of water loss on the crystal lattice by the associated change in vibrational states of the molecule. The principle of this application can be extended for monitoring changes in hydration state during fluid bed drying. Fluid bed drying is often used in pharmaceutical manufacturing to remove bulk water from materials after wet granulation. The drying process can produce unstable granulation containing a drug in a non-equilibrium state of hydration. To avoid formation of unstable granulation, processes that do not require drying are utilized, such as direct compression into tablets, encapsulation, or dry granulation. However, in many cases wet granulation yields a more robust process. If wet granulation is being pursued for a formulation where drug hydration state is critical, careful control of the drying
process endpoint is necessary to maintain the optimum drug hydration state necessary for proper final product performance. In pharmaceutical drying processes, the endpoint is often determined using a bulk moisture measurement, such as loss on drying. With this method, drug hydration state is inferred from the bulk measurement. In contrast, Raman provides a direct measurement of drug hydration state, which enables a drying endpoint determination that ensures tablet quality.

Clarke et al. (2001) developed a method that used both NIR and Raman microscopy to evaluate pharmaceutical formulations. They called the procedure CIF for chemical image fusion. Two formulations were used, a four component and a five component formulation. The method acquires data from the exact same part of the sample for both NIR and Raman analysis. The combined spectral information produce images that allow visualization of the entire formulation and were used to determine the cause of sticking of a formulation during tableting.

Raman has been used to determine the active content in a drug pellet producing results in good agreement with the reference HPLC method (Dao and Jouan, 1993). The coefficient of variation had a mean value of
2% and the correlation coefficient ranged from 0.994 to 0.999. The limit of detection was 0.8 mg for a 123 mg tablet containing 10 mg of active. A similar application of Raman spectroscopy for rapid quantitative characterization of illegal narcotics has been reported. These studies involved quantitative measurements of cocaine in solid mixtures comprised of cocaine, caffeine, and glucose (Ryder et al., 2000). The level of cocaine varied from 9.8 to 80.6wt%. Multivariate analysis was able to account for 98% of the variation with a root mean standard error of prediction of 4.1% for cocaine, 5.2% for caffeine, and 6.6% for glucose. The experiments demonstrated the feasibility of using Raman for rapid quantitative characterization of illegal narcotics.

**Fluorescence**

Fluorescence imaging has been used to analyze the coating thickness and surface area of controlled release pellets (Andersson et al., 2000b). The pellets were coated in a fluidized bed process. Samples were then taken from the batch and cut in half. After being cut, the pellet was photographed with a camera attached to an incident light fluorescence microscope. The pictures were then converted to bitmap files that could be used in a processing program. They were then able to determine the
coating thickness and the surface area of the pellet and predict the
effect of these parameters on release rate. It was also suggested that
this method could be used as a reference method for calibration of on-
line NIR methods for assessing coating thickness.

Another similar method is frequency-domain photon migration (FDPM).
This technique can measure either the absorbance of a sample or
fluorescence. It is based upon the time-dependent diffusive scattering of
light. This allows for minimization of analysis and measurement
variation. This method was used by Shinde et al. (1999) to analyze the
concentration of riboflavin in lactose-riboflavin mixtures. They were able
to determine a linear relationship between the riboflavin absorption
coefficient and its concentration in the mixture. It has the potential for
analysis of very low doses due to its ability to detect the active at
wavelengths where its absorption is small.

Banerjee et al. (1999) investigated the use of FDPM for analysis of the
structure of a colloid-polymer suspension. As the concentration of
colloid increases, the distance between particles leads to an interaction
which causes an ordered structure. This interparticle interference
becomes significant to the spectroscopy. The FDPM method is robust enough to analyze high concentration dispersions without causing disruption of the ordered structure. Acoustic techniques have also been used to analyze high concentration dispersions, however they negatively impact the structure because of mechanical disruption and energy deposition. In contrast, optical measurement techniques have been limited to relatively dilute dispersions. Overall, the use of fluorescence for Pharmaceutical PAT applications is very promising. In the future, the possibilities should be further explored.

**Miscellaneous**

Solid state NMR and IR have been used to evaluate the polymorphs of fosinopril sodium (Brittain et al., 1993). NMR can be used to evaluate polymorphs, because the nuclei resonance frequency will be different when variations in molecular packing changes their spatial relationship. IR can be used because as the spatial relationship of functional groups changes, the vibrational frequencies also change. The use of these techniques allowed determination of enantiotropic polymorphs. Also, the results suggested that the two forms may differ conformationally due to cis-trans isomerization along the peptide bond.
Dielectric analysis (DEA) was used to assess the film curing of Eudragit RS30D plasticized with 20% acetyltributyl citrate (Guma et al., 1997). The change in dielectric response was correlated with dissolution rate from film-coated beads. The response had distinct regions that were identified as under-cured, optimally-cured, over-cured, and super-cured. The response decreased to an optimally-cured minimum, then increased due to over-curing, and then decreased in the super-cured region. The beads were coated in a fluid bed with Wurster and then cured at 60°C. The beads were tested at 0, 3, 10, and 30 hours. During the initial curing (under-cured), the DEA result decreases as the mobility of ions is decreased due to the loss of water. When the beads were optimally cured, a minimum in the DEA response was reached and stayed constant for a time. There was a continuous loss of water and plasticizer molecules. This was the state of least mobility. The slowest and most consistent dissolution rate was observed. If curing continues past this stage, then there is non-uniform loss of plasticizer and residual water which can result in micro-voids and cracks. These cracks and voids cause an increase in the DEA response. In the super-cured phase, the DEA decreases due to more loss in plasticizer that causes
the collapse of micro-voids. Polymer movement results, as there is a relative increase in film density due to the collapse. Figure 4 shows the relationship between curing time and dissolution. The DEA results followed the same trend as dissolution rate. This is shown in Figure 5.
Figure 4. Dissolution rate of film-coated chlorpheniramine beads versus curing time at 60°C (Guam et al., 1997)

Figure 5. DEA response (permittivity) of film-coated chlorpheniramine beads versus curing time at 60°C (Guam et al., 1997)
DEA provides a non-invasive measurement of curing that correlates with dissolution rate. This technique would be useful when evaluating a new coating polymer dispersion.

2. Objectives, hypotheses, and specific aims

2.1 Objectives

The Background discussed a variety of process analytical techniques that could be used to monitor pharmaceutical processes to ensure the quality of the final dosage form. For this research, there were two overall objectives.

1. To study the effect of drug hydration state on tablet physical stability by applying PAT during fluid bed drying.

2. To evaluate and optimize blending in a tumbler mixer by applying PAT.
2.2 Hypotheses

There were two hypotheses for this work. One hypothesis was that using on-line Raman spectroscopy to monitor drying in a fluid bed will identify conditions where drug hydration state is maintained and ensure final product integrity over time. The second hypothesis was that using on-line Raman spectroscopy will be better than traditional thief sampling at measuring blend content uniformity and predicting tablet uniformity.
2.3 Specific Aims

There were three main specific aims of this research.

- Evaluate the effect of drug hydration state on final tablet physical stability
- Evaluate the effect of the following factors on blend homogeneity
  - Drug/filler ratio
  - Drug placement
  - Drug particle size distribution (PSD) to filler PSD
  - Drug and filler density
  - Multiple tablet components
  - Sampling
- Correlate blend uniformity to final tablet uniformity
3. Part I: Evaluation of Drug Hydration State

One purpose of this work was to study the effect of moisture content on drug hydration state, measured by Raman, and its impact on the physical stability of tablets. Three drugs were evaluated for their potential use in this study: etidronate disodium, risedronate sodium, and azimilide dihydrochloride. Risedronate sodium (RS) was chosen due to its solid-state properties. The solid-state form of risedronate sodium is hemi-pentahydrate (Physician’s Desk Reference, 2003). RS is a mixed hydrate containing both lattice water and channel water. The theoretical moisture content is 12.9% which corresponds to one mole of lattice water and one and a half moles of channel water. Lattice water is an integral part of the crystal lattice and cannot be removed below the boiling point of water. Removal of lattice water destroys the crystal lattice. In contrast, channel water is not an integral part of the lattice, rather it occupies channels formed by the lattice. It can be removed below the boiling point of water and this process is reversible. The hydrate will equilibrate to a stoichiometric amount of water above 20% relative humidity (Redman-Furey, 2005). These properties are not unique to RS. Many materials exhibit similar solid-state hydration properties. For RS channel hydrate, contraction of the crystal lattice
occurs when water is removed and expansion occurs when water is gained. If the RS is compressed into tablets while in a partially dehydrated solid-state form, over time changes in tablet properties will be observed as the RS crystal lattice expands and returns to the equilibrium solid-state form.

In these experiments, RS was wet granulated followed by fluid bed drying to produce final granulation moisture contents between 1 and 7%. This moisture content range was chosen to induce variability in RS hydration state. Final granulation moisture at the end of drying can affect the amount of RS solid-state change measured by Raman. In addition, the final granulation moisture, and thus the RS hydration state, can affect the change in tablet properties over time. The granulation was compressed into tablets and the tablets were placed in environments of 22-25°C with 60% and 75% relative humidity. During drying, the RS solid-state form was continuously monitored by Raman spectroscopy. Raman was the enabling technology used to understand the effect of drug hydration state on final product physical stability.
3.1 Experimental

3.1.1 Materials

The materials used for these experiments are shown in Table 2. Sodium dichromate dihydrate and sodium chloride were used to create constant relative humidity chambers. Five excipients were used in the benchtop experiments. Lactose monohydrate and microcrystalline cellulose are common tablet fillers. Povidone is a common binder and crospovidone is a common disintegrant. Magnesium stearate is a common lubricant. Three drugs were chosen for evaluation in benchtop experiments due to their solid state properties: etidronate disodium, risedronate sodium, and azimilide dihydrochloride. Raman spectra of the drugs and excipients are provided in Appendix A. Risedronate sodium, microcrystalline cellulose, povidone, crospovidone, and magnesium stearate were chosen for the pilot scale experimental formulation. This decision is discussed further in Section 3.2 Results and Discussion.
Etidronate Disodium

Etidronate disodium is the disodium salt of (1-hydroxyethylidene) diphosphonic acid. It is a white powder, freely soluble in water, with a molecular weight of 250. The structural formula is shown in Figure 6 (Physician’s Desk Reference, 2003). It is a mixed hydrate containing water bound as part of the crystal lattice.

Figure 6. Chemical Structure of etidronate disodium
Risedronate Sodium (RS)

The solid-state form of risedronate sodium is the hemi-pentahydrate with small amounts of monohydrate. The theoretical moisture content is 12.9% which corresponds to one mole of lattice water and one and a half moles of channel water. The hydrate will equilibrate to a stoichiometric amount of water above 20% relative humidity (Redman-Furey, 2005). The empirical formula for risedronate sodium hemi-pentahydrate is $\text{C}_7\text{H}_{10}\text{NO}_7\text{P}_2\text{Na}\cdot2.5\text{H}_2\text{O}$. The chemical name of risedronate sodium is [1-hydroxy-2-(3-pyridinyl)ethylidene]bis[phosphonic acid] monosodium salt. The molecular weight of risedronate sodium anhydrous is 305.10 and the hemi-pentahydrate is 350.13. Risedronate sodium is a fine, white to off-white, odorless, crystalline powder. It is soluble in water and in aqueous solutions, and essentially insoluble in common organic solvents (Physician’s Desk Reference, 2003).
The chemical structure of risedronate sodium hemi-pentahydrate is the following:

Figure 7. Chemical Structure of risedronate sodium

![Chemical Structure of Risedronate Sodium](image_url)
Azimilide Dihydrochloride

The solid-state form of azimilide dihydrochloride is a dihydrochloride hemi-hydrate. The chemical name is (e)-1-[5-(4-Chlorophenyl)-2-furanyl]methylene]amino]-3-[4-(4-methyl-1-piperazinyl)butyl]-2,4-imidazolidinedione dihydrochloride. The molecular weight is 530.4 and the chemical structure is the following:

Figure 8. Chemical Structure of azimilide dihydrochloride
Table 2: Materials for evaluation of drug hydration state

<table>
<thead>
<tr>
<th>Material</th>
<th>Trade Name</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etidronate disodium</td>
<td>N/A</td>
<td>Procter &amp; Gamble, Norwich, New York, USA</td>
</tr>
<tr>
<td>Risedronate sodium</td>
<td>N/A</td>
<td>Procter &amp; Gamble, Norwich, New York, USA</td>
</tr>
<tr>
<td>Azimilide dihydrochloride</td>
<td>N/A</td>
<td>Procter &amp; Gamble, Norwich, New York, USA</td>
</tr>
<tr>
<td>Lactose monohydrate</td>
<td>N/A</td>
<td>Hollandse Melksuikerfabriek, Uitgeest, Holland, The Netherlands</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>Avicel PH102</td>
<td>FMC, Philadelphia, Pennsylvania, USA</td>
</tr>
<tr>
<td>Povidone</td>
<td>Plasdone K29-32</td>
<td>International Specialty Products, Wayne, New Jersey, USA</td>
</tr>
<tr>
<td>Crospovidone</td>
<td>Polyplasdone XL</td>
<td>International Specialty Products, Wayne, New Jersey, USA</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>N/A</td>
<td>Peter Greven, Bad Münstereifel, Germany</td>
</tr>
<tr>
<td>Sodium dichromate dihydrate</td>
<td>N/A</td>
<td>VWR, West Chester, Pennsylvania, USA</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>N/A</td>
<td>VWR, West Chester, Pennsylvania, USA</td>
</tr>
</tbody>
</table>
3.1.2 Equipment

Instrumentation used for benchtop experiments was a FP90 Central Processor (Mettler, Columbus, OH, USA), FP82HT hot stage (Mettler), Raman Holoprobe with an excitation wavelength of 785nm and an intensity at sample of approximately 50mW (Kaiser Optical Systems, Ann Arbor, MI, USA), probe head model HFPH-785 (Kaiser Optical Systems), optic NCO-1.3-NIR (Kaiser Optical Systems), DigiSense DualLogR thermocouple thermometer model 91100-50 (Cole-Parmer, Vernon Hills, IL, USA), type K wire thermocouple, AT261 DeltaRange balance (Mettler).

The pilot scale manufacturing equipment used was a N-50 Hobart mixer, a Masterflex peristaltic pump (Cole-Parmer), a #6 handscreen, a GPCG-1 fluid bed (Glatt Air Techniques, Ramsey, NJ, USA), a 10-station Piccola instrumented tablet press (Specialty Measurements Inc., Lebanon, NJ, USA), and modified oval tooling (Natoli, St. Charles, MO, USA). Instrumentation utilized in these experiments was a moisture balance (Denver Instruments, Denver, CO, USA), a Raman Holoprobe with an excitation wavelength of 785 nm and an
intensity at sample of approximately 50mW (Kaiser Optical Systems), a ball probe (Center for Process Analytical Chemistry, University of Washington), a PE360 balance (Mettler), a Schleuniger hardness tester (Copley Scientific, Nottingham, UK), and digital calipers (Mitutoyo America Corp., Aurora, IL, USA).

A moisture balance was utilized for testing in-process granulation moisture. Because lattice water associated with the model drug, risedronate sodium (RS), is not removed under fluid bed drying conditions, moisture balance parameters were optimized to remove bulk and RS channel water. Moisture balance results are directly related to changes in RS solid-state form produced during the drying process. In contrast, Karl Fischer analysis includes RS lattice water producing a bias not related to solid-state changes associated with the removal of channel water.

Tablet storage relative humidity (RH) was controlled using saturated salt solutions in sealed chambers. Tablets were stored at 22-25°C under 60% and 75% relative humidity. The 60%RH chamber was made using a saturated solution of sodium dichromate dihydrate.
(VWR, West Chester, Pennsylvania, USA). The 75%RH chamber was made using a saturated solution of sodium chloride (VWR).

3.1.3 Software

Fluid bed drying experiments were designed and data were statistically evaluated using Design-Expert version 6.0.4 (Stat-Ease Inc., Minneapolis, MN, USA). Raman spectra were collected using HoloGRAMS version 4.0 (Kaiser Optical Systems, Ann Arbor, MI, USA) and plotted using Grams/AI version 7.02 (Thermo Galactic Industries Corp., Salem, NH, USA). Raman peak areas were calculated using Holomap 2.1 rev. 1 (Kaiser Optical Systems) in MatLab R12 (The MathWorks, Inc., Natick, MA, USA). Microsoft Excel 2002 was used to create tables, graphs, and for basic calculations.
3.1.4 Formulations

Table 3 shows the formulation used in experiments to evaluate the effect of granulation moisture content on risedronate sodium (RS) hydration state. Components in Table 3 above the dashed line were used in the granulation. Components below the dashed line were used in the final blend and lubrication blend. Purified water was used as granulating fluid at a level of 20% based on the initial blend weight. The amount of purified water used as granulating fluid was 204.4 mL. The formulation in this study is representative of formulations often used in commercial manufacturing, utilizing drug, filler, binder, disintegrant, and lubricant.
Table 3. Wet granulation formulation

<table>
<thead>
<tr>
<th>Component</th>
<th>Unit (mg)</th>
<th>Batch (g)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>risedronate sodium</td>
<td>118.5</td>
<td>500.0</td>
<td>47.42</td>
</tr>
<tr>
<td>povidone</td>
<td>5.2</td>
<td>22.0</td>
<td>2.09</td>
</tr>
<tr>
<td>microcrystalline cellulose</td>
<td>118.5</td>
<td>500.0</td>
<td>47.42</td>
</tr>
<tr>
<td>crospovidone</td>
<td>7.6</td>
<td>32.0</td>
<td>3.03</td>
</tr>
<tr>
<td>magnesium stearate</td>
<td>0.1</td>
<td>0.5</td>
<td>0.05</td>
</tr>
<tr>
<td>total</td>
<td>250.0</td>
<td>1054.5</td>
<td>100.0</td>
</tr>
</tbody>
</table>

20% purified water as granulating fluid
A RS level of 47.4% was chosen because it was the minimum amount required to produce suitable signal-to-noise for quantitation of on-line Raman peaks in the C-H stretching region and the spectral region containing the 3-picoline ring and PO$_2^-$ stretches. Microcrystalline cellulose is a common tablet filler that aids in compression, and its Raman spectrum displays less interference with RS than lactose, another common filler. Lactose produced Raman bands that interfere with the RS Raman bands used to monitor the solid-state form of RS. Povidone is the binder and crospovidone is the disintegrant, and they are routinely used in tablet formulation (Rowe et al., 2003). For this formulation, 2% povidone and 3% crospovidone were below the limit of detection of Raman spectroscopy. A minimal amount of magnesium stearate was required for lubrication, due to additional lubrication provided by microcrystalline cellulose (Rowe et al., 2003). Due to the hydrophobic nature of magnesium stearate, the amount in the formulation was minimized to reduce potential interference with the rehydration of RS.
3.1.5 Manufacturing Process

Risedronate sodium, microcrystalline cellulose, and povidone were combined in the mixer, then purified water was sprayed onto the mixture at a rate of 100 mL/minute. The wet granulation was hand screened through a #6 screen into the fluid bed. The wet granulation was dried in the fluid bed to moisture contents between 1 and 7%, as determined by a moisture balance. Throughout the drying process, the granulation was monitored by Raman spectroscopy. Raman allowed detection of changes in drug hydration state during the drying process. The fiber optic probe head was inserted into the fluid bed through a solution addition port (Figure 9). This single point probe placement allows for representative sampling during the fluid bed drying process due to the small size of the fluid bed bowl, combined with the high probability that the wet granulation contains homogeneous distribution of RS. The Glatt GPCG-1 is a small fluid bed (bowl height 22in) with a high number of turnovers of the material and no significant temperature gradient across the bowl. During the drying process, Raman spectra were collected every two minutes, with a five second delay between acquisitions to allow for shaking of the filter bags. The two minute sampling interval allowed for
averaging of about 60 bed turnovers which is a representative sampling of the entire mass. The Raman sample size was estimated using an estimated particle velocity based on the size of the fluid bed bowl and the rate of turnover, an average granulation density and granule particle size, and the Raman acquisition time. The estimated sample size is 18mg per acquisition. A typical thief sample for loss on drying analysis is 1-3g. In addition, Raman spectra were acquired from material near the location in the fluid bed bowl that was sampled by the thief. The Raman sampling is representative of the total mass when compared to loss on drying. The dried granulation was blended with crospovidone and then magnesium stearate in the mixer. The final blend was then compressed into tablets. The number of tablets compressed for each batch was 4,220. The manufacturing process is illustrated in Figure 10.
Figure 9. Fluid bed and Raman set-up

[Image of a fluid bed and Raman set-up]

Raman Probe
Figure 10. Wet granulation manufacturing flowchart
3.1.6 Testing

Tablets from each experiment were stored at 22-25°C, 60%RH and 75%RH, and tested at 0, 1 hr, 2 hrs, 3 hrs, 15 hrs, 24 hrs, 1 month, 2 months, 3 months, 6 months, and 9 months. The tablet properties monitored as a function of time were expected to change if the RS rehydrated during storage. These tablet properties were appearance, weight, thickness, hardness, and moisture content. These properties can impact routine release testing and downstream processing. Tablet appearance is a routine release test in which the severity of the problem, as well as the size of the batch, determines the number of tablets tested and the number of failures allowable. Tablet weight is used in weight variation analysis to demonstrate uniformity of dosage units (US Pharmacopeia XXVII, 2004). If tablet weight increases due to hydration over time, tablet weight variation could exceed the limit of 85.0 – 115.0%. Tablet thickness is important for further processing such as coating, printing, and packing. Tablet moisture content is important since it affects the tablet mass and thus weight variation results. In addition, it can have an effect on the
chemical stability of the tablet, however the effect on chemical stability was not addressed in this work.

3.1.7 Experimental Design and Methodology

Bench experiments were performed prior to pilot scale experiments to study three model drugs, etidronate disodium, risedronate sodium, and azimilide dihydrochloride, while they were heated and cooled to 1) obtain reference spectra of the model drugs and 2) determine if transformations occur at temperatures that can be safely reached in the fluid bed dryer. The fluid bed inlet air temperature cannot reach temperatures higher than 75°C. Therefore, to be observed, drug transformations need to occur at temperatures lower than 75°C. Bench experiments were also performed to study the excipients and binary mixtures of drug and excipient. These experiments were to determine if the excipients chosen caused spectral interference in the spectral region of drug transformations. The excipient that showed the least interference was chosen to proceed to pilot scale experiments. All bench experiments included collecting data while the material was exposed to a temperature ramp from 25 to 200°C followed by a ramp back down to 25°C. The mixtures of drug and
excipient were prepared by equal parts of each shaken in a vial, poured out and cut into four sections, then added back to the vial and shaken again. The samples were then placed on a device that spun the sample under the laser and an average spectrum was collected. When the sample was then placed on the hot stage, a sample site was chosen that closely matched the average spectrum from the spinning sample. Ten bench experiments were completed. They are outlined in Table 4.
Table 4. Outline of Raman benchtop experiments

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>Materials for Benchtop Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Risedronate only</td>
</tr>
<tr>
<td>2</td>
<td>Etidronate Disodium only</td>
</tr>
<tr>
<td>3</td>
<td>Lactose only</td>
</tr>
<tr>
<td>4</td>
<td>Microcrystalline cellulose (MCC) only</td>
</tr>
<tr>
<td>5</td>
<td>Povidone only</td>
</tr>
<tr>
<td>6</td>
<td>Risedronate/lactose 50/50 mixture</td>
</tr>
<tr>
<td>7</td>
<td>Risedronate/MCC 50/50 mixture</td>
</tr>
<tr>
<td>8</td>
<td>Etidronate Disodium/lactose 50/50 mixture</td>
</tr>
<tr>
<td>9</td>
<td>Etidronate Disodium/MCC 50/50 mixture</td>
</tr>
<tr>
<td>10</td>
<td>Azimilide only</td>
</tr>
</tbody>
</table>
During pilot scale experiments, the drug hydration state was varied by performing a design of experiments (DOX) on the fluid bed drying process. Fluid bed drying processes are controlled by inlet air humidity, flow rate, and temperature. In this experiment, the inlet air temperature and final granulation moisture content were varied to produce a range of drug hydration states. Although there was no humidity control on the GPCG-1, the inlet air humidity during the experiments was consistent from lot to lot. The inlet air flow was controlled from lot to lot to maintain a consistent state of fluidization in the bed and prevent entrainment of particles into the filter bags. The DOX was a two-factor full factorial with one centerpoint. The DOX is outlined in Table 5.
Table 5. Design of experiments for evaluation of drug hydration state during fluid bed drying

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>Inlet Air Temp (°C)</th>
<th>Final Moisture Content (%LOD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>3.5</td>
</tr>
</tbody>
</table>
During experimentation, the condition of 30°C inlet air temperature and 1% final granulation moisture content could not be reached within the time scale of the process due to the thermodynamics of the system. The design of experiments was then modified to the conditions illustrated in Table 6. Two additional conditions were added to replace the 30°C inlet air temperature and 1% final granulation moisture content condition. One condition was 30°C inlet air temperature and 4.6% final granulation moisture content. The second condition was 45°C inlet air temperature and 1.5% final granulation moisture content. This modification allowed for an adequate number of experiments for statistical analysis. The original and modified design of experiments are illustrated in Figure 11. In addition, a placebo run was performed to produce granulation with a moisture content of 1.4%. Controlling the final moisture content by thief sampling and testing with the moisture balance produced slight differences between actual and target final granulation moisture values. Differences between target and actual moisture content are due to a 5 minute delay between taking a sample and receiving the
moisture balance result, during which the product is exposed to the drying conditions of the process.
Table 6. Revised design of experiments for evaluation of drug hydration state during fluid bed drying (* placebo run)

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>Inlet Air Temp (°C)</th>
<th>Final Moisture Content (%LOD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>5.8</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>1.1</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>6.6</td>
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<td>5</td>
<td>45</td>
<td>3.7</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>1.5</td>
</tr>
<tr>
<td>7*</td>
<td>60-75</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Figure 11. Design of experiments for evaluation of drug hydration state during fluid bed drying
In these experiments, the effect of fluid bed inlet air temperature and final granulation moisture content on the change in RS Raman peak areas from the start to end of the drying process was evaluated using the statistical software package Design-Expert version 6.0.4, Stat-Ease, Inc. A running median approach (n=5) was used to preprocess the peak areas. In addition, the effect of fluid bed inlet air temperature and final granulation moisture content on the change in tablet properties was analyzed for statistical significance. This analysis utilized the difference between initial results and results after 15 hours at 60%RH. The condition of 15 hours at 60%RH was used for analysis rather than 24 hours at 60%RH, because at 24 hours the thickness of tablets manufactured with low moisture granulation (<2% moisture) could not reliably be measured due to substantial tablet swelling. Similarly, data from tablets stored at 75%RH were not used for statistical analysis, because tablets made from low moisture granulation (<2% moisture) swelled to the point of breaking after only 3 hours.
3.2 Results and Discussion

3.2.1 Effect of heating/cooling on solid-state form using Raman during benchtop experiments

To obtain reference spectra and observe spectral changes of active ingredients and excipients during heating and dehydration, the materials were analyzed with Raman spectroscopy as they were heated from 25°C to 200°C and then cooled back to 25°C on a hot stage. The active ingredients analyzed were risedronate sodium, etidronate disodium, and azimilide dihydrochloride. The excipients analyzed were povidone, lactose, and microcrystalline cellulose. In addition, 50/50 mixtures were analyzed of risedronate/lactose, risedronate/microcrystalline cellulose, etidronate disodium/lactose, and etidronate disodium/microcrystalline cellulose.

Risedronate sodium had a good Raman signal, approximately 40,000 counts, with a 30 second acquisition time. Therefore, the signal with a 1 second acquisition time would be approximately 1,333 counts. It had about one quarter the scattering cross-section as that of naphthalene, which is commonly used for Raman calibration. As risedronate was heated, a number of peak changes were observed
consistent with the loss of channel water. This was determined by comparison to the spectra of risedronate monohydrate. Noticeable changes were observed even in the C-H stretching region (approximately 2900 to 3100 cm\(^{-1}\)) indicating significant changes in the crystalline lattice structure. These changes began at temperatures below 60°C, making this compound a candidate for fluid bed drying experiments with Raman spectroscopy, in which the temperature cannot exceed 75°C.

The Raman intensity of etidronate disodium, even at 1 minute acquisition time, was poor (approximately 9,000 counts). Therefore, the signal with a 1 second acquisition time would be approximately 150 counts. Prior to heating, the etidronate disodium was in the tetrahydrate form. The Raman spectra showed no significant change in hydration state until 95°C. This high temperature was expected because the water in etidronate is bound as lattice water. Etidronate disodium was not a good choice for fluid bed experiments, because the fluid bed temperature cannot exceed 75°C.
The Raman intensity of azimilide dihydrochloride was excellent. With a 1 second acquisition time, the Raman intensity was about 10,000 counts. The highly conjugated chemical structure produced the large Raman scattering cross-section. The Raman scattering cross-section of azimilide was double that of naphthalene, which is commonly used for Raman calibration. No significant spectral changes were observed upon heating until the onset of degradation. Azimilide is a good model drug for low dose blending studies due to its large Raman scattering cross-section and its stability.

Povidone had a poor Raman intensity. With a 1 minute acquisition time, the intensity was only 13,000 counts. Therefore, the signal with a 1 second acquisition time would be approximately 217 counts. When added to blends, povidone will not interfere with the Raman spectra of the other components. Lactose and microcrystalline cellulose both had Raman intensity at a level which could potentially interfere with the Raman spectra of other components. Raman intensity of lactose was 40,000 counts with a 1 minute acquisition time. Therefore, the signal with a 1 second acquisition time would be approximately 667 counts. No significant changes in lactose spectra
were observed until the onset of degradation. Raman intensity of microcrystalline cellulose was 30,000 counts with a 40 second acquisition time. Therefore, the signal with a 1 second acquisition time would be approximately 750 counts. There were some spectral changes as microcrystalline cellulose was heated, however the magnitude of the changes was minimal.

When 50/50 mixtures of risedronate and lactose were analyzed, there was considerable overlap between the Raman peaks of both compounds, making it difficult to monitor risedronate changes. However, minimal overlap was observed between risedronate and microcrystalline cellulose. Therefore, microcrystalline cellulose is the excipient of choice to be used with risedronate. The same observations held true for 50/50 mixtures of etidronate disodium with lactose and microcrystalline cellulose.

From these experiments, it was determined that risedronate sodium (RS) with microcrystalline cellulose would be used for pilot scale fluid bed drying experiments to study the effect of moisture content on RS solid-state form using on-line Raman spectroscopy. In addition, it
was determined that azimilide dihydrochloride would be used to study low dose blend uniformity on-line with Raman spectroscopy and its correlation to tablet uniformity.

3.2.2 Effect of moisture content on risedronate sodium (RS) hydration state

3.2.2.1 Effect of moisture content on RS hydration state measured by on-line Raman

Benchtop experiments and previous studies showed that Raman spectroscopy was capable of monitoring RS solid-state changes due to dehydration (Bigalow-Kern et al., 2005). Results from those experiments demonstrated feasibility of Raman for monitoring RS solid-state changes during fluid bed drying. In these experiments, Raman was used to relate risedronate solid-state changes during fluid bed drying to the physical stability of tablets. The applicability of Raman to these measurements is limited to materials which display a detectable change in their Raman spectrum due to crystal lattice changes upon dehydration. Raman spectra from the start of fluid bed drying and the end of drying to 1.1% moisture are shown in Figure 12.
Figure 12. On-line Raman granulation spectrum (a, start of drying; b, end of drying to 1% moisture)
These spectra illustrate the changes that occur during risedronate granulation dehydration in a fluid bed. Figure 13 shows specific spectral changes in the C-H stretching region and the region containing the 3-picoline ring and \( \text{PO}_2^- \) stretches corresponding to changes in RS hydration state observed under static laboratory conditions. The largest spectral changes were observed in the C-H stretching region. As peak area decreased at a Raman shift of 2963 cm\(^{-1}\), a new peak formed at 2936 cm\(^{-1}\). Significant changes also occurred in the region including the 3-picoline ring and \( \text{PO}_2^- \) stretches, as peak area decreased at a Raman shift of 1000 cm\(^{-1}\). Definitive assignment of this band is difficult due to the overlap of bands associated with the 3-picoline ring deformation and \( \text{PO}_2^- \) stretches. Additionally, assignment of this band is difficult because it is a minor band in both IR and Raman and the fluorescence of microcrystalline cellulose interferes with these spectral features. Changes in this region during dehydration were expected based on hydrogen bonding with channel water. However, changes in the C-H stretching region were not expected since typically C-H functional groups are not associated with hydrogen bonding. For change to occur in the C-H stretching
region, the loss of channel water must be significantly disturbing the crystal lattice.

The Raman spectral changes that occurred during fluid bed drying at Raman shifts of 2963 cm$^{-1}$, 2936 cm$^{-1}$, and 1000 cm$^{-1}$ are shown in Figure 14. These spectral changes were consistent with the laboratory feasibility experiments shown in Figure 13. Raman spectra collected on-line during fluid bed drying displayed poorer signal-to-noise as compared to spectra collected under static laboratory conditions. On-line measurements produced spectra at the threshold of measurement capability, displaying a signal-to-noise ratio of approximately 3 in these stretching regions. A number of factors affect Raman signal including probe design, analyte concentration (i.e. number of particles per unit volume), and Raman scattering cross-section of the analyte. The movement of material in the fluid bed caused slight differences in local RS concentration. This variation in particle concentration is reflected in Raman signal intensity. In addition, RS has a modest Raman scattering cross-section which is approximately an order of magnitude less than naphthalene. To overcome limitations
caused by modest scattering cross-section of RS, the amount of
RS in the formulation was set at slightly above that needed to
achieve measurement capability. The resulting percent RS in the
formulation is within the limits of routine formulation development.
Figure 13. Effect of dehydration on Raman spectral bands using benchtop hot stage

Figure 14. Effect of dehydration on Raman spectral bands during fluid bed drying
The effect of inlet air temperature and final granulation moisture on the solid-state form of RS was analyzed using the change in peak area at Raman shifts of 2963 cm$^{-1}$, 2936 cm$^{-1}$, and 1000 cm$^{-1}$. These peaks were chosen for analysis because their change in peak area correlated best with the change in tablet properties. Peaks at 2963 cm$^{-1}$ and 1000 cm$^{-1}$ are associated with the equilibrium RS hemi-pentahydrate and 2936 cm$^{-1}$ is associated with dehydrated RS. The results showed that final granulation moisture had a significant effect on the solid-state form. Inlet air temperature had a moderate effect on the change at 2963 cm$^{-1}$ and no effect on changes at 2936 cm$^{-1}$ and 1000 cm$^{-1}$. The significance of inlet air temperature and final granulation moisture on the change in Raman peak areas is shown in Table 7. The effect of final granulation moisture content on the change in RS hydration state measured by the change in Raman peak areas at 2963 cm$^{-1}$ and 1000 cm$^{-1}$ is shown in Figures 15 and 16, respectively. The correlation coefficients from regression of the change in Raman peak areas at 2963 cm$^{-1}$ and 1000 cm$^{-1}$ and the final granulation moisture after drying were 0.79 and 0.94, respectively. The root mean square error was 364 for 2963 cm$^{-1}$
and 2822 for 1000 cm⁻¹. These figures show a strong correlation between the final moisture content of the dried granulation and the hydration state of RS. The dehydration profiles of RS measured by Raman at 2963 cm⁻¹ and 1000 cm⁻¹ during fluid bed drying are shown in Figures 17 and 18, respectively. The results were normalized for these profiles, to better illustrate the effect of drug hydration state on the change in peak area.
Table 7. Significance of inlet air temperature and final granulation moisture on the change in Raman peak areas

<table>
<thead>
<tr>
<th>Source</th>
<th>Change in Peak Area</th>
<th>2963 cm-1</th>
<th>2936 cm-1</th>
<th>1000 cm-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inlet Air Temperature</td>
<td>Prob &gt; F</td>
<td>0.0907</td>
<td>0.3308</td>
<td>0.4952</td>
</tr>
<tr>
<td>Final Granulation Moisture</td>
<td>Prob &gt; F</td>
<td>0.0080</td>
<td>0.0490</td>
<td>0.0050</td>
</tr>
</tbody>
</table>

- **Statistically significant**
- **Moderately significant**
- **Not significant**
Figure 15. Effect of final granulation moisture on the change in RS hydration state (Raman shift of 2963 cm$^{-1}$)
Figure 16. Effect of final granulation moisture on the change in RS hydration state (Raman shift of 1000 cm⁻¹)
Figure 17. Effect of RS hydration state on Raman peak area at 2963 cm$^{-1}$
Figure 18. Effect of RS hydration state on Raman peak area at 1000 cm\(^{-1}\).
Substantial changes in RS Raman spectral bands were observed when the final granulation was dried to < 5.8% moisture, indicative of substantial changes in the RS solid-state structure. The theoretical moisture content of RS at equilibrium is 12.9%. RS contains one mole of lattice water, which accounts for 5.14% moisture, and one and a half moles of channel water, which accounts for 7.71% moisture. Only channel water is lost during the fluid bed drying process. In addition, the microcrystalline cellulose can lose water during fluid bed drying. The moisture content of the microcrystalline cellulose was 4.35%. Assuming RS and microcrystalline cellulose dehydrate at similar rates, blends dried to less than 6% moisture should produce detectable changes in RS hydration state for this formulation. This is consistent with experimental results that showed final granulation dried to <5.8% moisture produced substantial changes in the RS Raman spectral bands.
3.2.2.2 Effect of RS hydration state on tablet properties

Directly after the RS granulation was dried, it was final blended and compressed into tablets. Tablet appearance, weight, thickness, and hardness were measured over time for tablets in 60% relative humidity (RH) and 75%RH chambers at 22-25°C. The condition of 22-25°C and 60%RH was used because 25°C/60%RH is the long-term stability condition in the ICH stability guideline entitled Stability Testing of New Drug Substances and Products Q1A(R2) (ICH Steering Committee, 2003). An accelerated condition of 22-25°C and 75%RH was also used. The initial properties of the tablets from each batch are shown in Table 8. Tablets made with granulation at less than 5.8% moisture fractured in a 24 hour period at 60%RH and 75%RH, due to RS rehydration. Placebo tablets made with granulation at 1.4% moisture remained integral under the same conditions, confirming that the RS rehydration was causing the tablets to lose integrity.
Table 8. Initial tablet properties (* placebo run)

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>Inlet Air Temp (°C)</th>
<th>Final Moisture Content (%LOD)</th>
<th>Average Weight (g)</th>
<th>Average Thickness (mm)</th>
<th>Average Hardness (kp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>5.8</td>
<td>0.2487</td>
<td>3.87</td>
<td>11.8</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>1.1</td>
<td>0.2510</td>
<td>4.02</td>
<td>12.3</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>6.6</td>
<td>0.2459</td>
<td>3.79</td>
<td>12.9</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>4.6</td>
<td>0.2527</td>
<td>3.99</td>
<td>12.2</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>3.7</td>
<td>0.2501</td>
<td>3.98</td>
<td>11.5</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>1.5</td>
<td>0.2484</td>
<td>3.96</td>
<td>10.6</td>
</tr>
<tr>
<td>7*</td>
<td>60-75</td>
<td>1.4</td>
<td>0.2516</td>
<td>4.43</td>
<td>30.4</td>
</tr>
</tbody>
</table>
Final granulation moisture has a significant effect on RS hydration state. Therefore, to understand the effect of RS hydration state on the change in tablet properties, a statistical analysis to determine the effect of final granulation moisture on the change in tablet properties was performed. Also, the effect of inlet air temperature on the change in tablet properties was determined, but was expected to have little to no effect since it had little effect on RS hydration state. The change in tablet properties used for statistical analysis was the difference between the initial tablet property and the tablet property after 15 hours at 60%RH. The statistical analysis showed that the final granulation moisture, thus the RS hydration state, had a significant effect on the increase in tablet thickness and increase in tablet moisture due to RS rehydration. Final granulation moisture had a moderate effect on the decrease in tablet hardness. The increase in tablet weight was significantly affected by the interaction of inlet air temperature and final granulation moisture. The significance of inlet air temperature and final granulation moisture on the change in tablet properties is shown in Table 9. As the RS rehydrated, the moisture in the tablet increased, which caused an increase in tablet weight. Also,
tablet thickness increased as the RS rehydrated and caused expansion of the tablet. This increase in tablet thickness decreased the tablet hardness.
Table 9. Significance of inlet air temperature and final granulation moisture on the change in tablet properties

<table>
<thead>
<tr>
<th>Source</th>
<th>Weight Prob &gt; F</th>
<th>Thickness Prob &gt; F</th>
<th>Moisture Prob &gt; F</th>
<th>Hardness Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inlet Air Temperature</td>
<td>0.0502</td>
<td>0.9541</td>
<td>0.1601</td>
<td>0.6264</td>
</tr>
<tr>
<td>Final Granulation Moisture</td>
<td>0.1105</td>
<td>0.0015</td>
<td>0.0287</td>
<td>0.0990</td>
</tr>
<tr>
<td>Inlet Air Temp*Final Gran. Moisture</td>
<td>0.0033</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- statistically significant
- moderately significant
- not significant
Tablet thickness was most indicative of changes in the RS solid-state form. Tablets made with granulation at a moisture of 5.8% or greater showed little increase in thickness over 24 hours at 60% RH. In contrast, tablets made with granulation at moistures less than 5.8% showed significant increases in tablet thickness. After 15 hours at 60% RH, tablets made with granulation at moistures less than 1.5% swelled due to expansion of the RS crystal lattice as it rehydrated. These tablets lost structural integrity and their thickness could not be measured. The results are shown in Figure 19. A similar trend was observed for tablets at 75% RH. However, tablets made with granulation at moistures less than 1.5% lost structural integrity after only 3 hours of exposure to this condition. These results are shown in Figure 20. Placebo granulation was dried to 1.4% moisture, and tablets made with this granulation did not lose structural integrity. This confirms that the increase in tablet thickness and loss of structural integrity is due to RS rehydration.
Figure 19. Effect of RS hydration state on tablet thickness at 22°C, 60%RH (24 hours)

Tablet Thickness (mm)

Time (hours)

- Inlet air 60C, final granulation, moisture 6.62%
- Inlet air 45C, final granulation, moisture 3.69%
- Inlet air 30C, final granulation, moisture 5.78%
- Inlet air 45C, final granulation, moisture 1.46%
- Inlet air 30C, final granulation, moisture 4.63%
- Inlet air 60C, final granulation, moisture 1.13%

After 15hrs, tablets cracked too much to measure
Figure 20. Effect of RS hydration state on tablet thickness at 22°C, 75%RH (24 hours)

At 1.13 and 1.46% granulation moisture, tablets broke up after 3 hours.

Tablet Thickness (mm)

Inlet air 60°C, final granulation moisture 6.62%
Inlet air 45°C, final granulation moisture 3.69%
Inlet air 30°C, final granulation moisture 5.78%
Inlet air 45°C, final granulation moisture 1.46%
Inlet air 30°C, final granulation moisture 4.63%
Inlet air 60°C, final granulation moisture 1.13%
Tablets from each lot were held for nine months at 22-25°C, 60%RH and 75%RH. Tablets were tested at 1, 2, 3, 6, and 9 months. By one month, tablet moisture equilibrated across the lots to a level of 6-7.5% at 60%RH and 7.5-9% at 75%RH. The rate of equilibration due to water absorption depends on the intrinsic properties of the raw materials and the water permeability of tablets. Tablets made with granulation at moisture levels of 5.8% and above maintained a high degree of structural integrity for up to nine months at 60%RH. However, the structural integrity of tablets made with granulation at moisture levels less than 5.8% was severely compromised within one month. At the one month time point and beyond, thickness could not be measured for tablets made with granulation at a moisture level of 1.46%, because the tablets crumbled when handled. Thickness measurements could only be collected for a few tablets made with granulation dried to 1.13%, because most of the tablets crumbled when handled at one month and beyond. Placebo tablet thickness increased in the first month, but the tablets maintained structural integrity for up to nine months. Figure 21 shows the effect of final
granulation moisture, and thus RS hydration state, on tablet thickness at 60%RH over a nine month period.
Figure 21. Effect of RS hydration state on tablet thickness at 22°C, 60%RH (9 months)
3.2.2.3 **Comparison of Raman results to tablet thickness**

Raman spectroscopy is a technique that can probe the structural functionality of a sample. Raman probes the impact of water on the crystal lattice structure of a molecule, without measuring water directly. Removal and addition of water affects hydrogen bonding within the crystal structure and impacts the fundamental vibrational frequencies. For RS, loss of channel water during drying resulted in compression of its crystal lattice. This change was observed by Raman spectroscopy as a peak shift in the C-H stretching region and loss of a peak in the region containing 3-picoline ring deformation and PO$_2^-$ stretches. As RS rehydrated after compression into tablets, expansion of the material occurred as channel water was regained, producing Raman spectra consistent with equilibrated RS hemi-pentahydrate. Rehydration also caused an increase in tablet thickness over time.

These experiments have shown that final granulation moisture has a significant effect on specific Raman spectral changes associated with dehydration of RS. Final granulation moisture also has a significant effect on the change in tablet properties, such as
thickness, due to rehydration of RS. A regression analysis was performed to determine if the Raman changes were correlated with the observed tablet thickness changes. The results showed that changes in Raman peak areas in the C-H stretching region (shifts of 2963 cm\(^{-1}\) and 2936 cm\(^{-1}\)) and the region containing 3-picoline ring deformation and PO\(_2^+\) stretches (1000 cm\(^{-1}\)) correlated with the change in tablet thickness at 60% RH after 15 hours. These results are shown in Table 10.
Table 10. Correlations between change in tablet thickness and change in specific RS Raman peak areas during fluid bed drying

<table>
<thead>
<tr>
<th>Raman Shift (cm$^{-1}$)</th>
<th>Adjusted R-squared</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2963</td>
<td>0.79</td>
<td>0.011</td>
</tr>
<tr>
<td>2936</td>
<td>0.61</td>
<td>0.041</td>
</tr>
<tr>
<td>1000</td>
<td>0.93</td>
<td>0.001</td>
</tr>
</tbody>
</table>
3.3 Conclusions

This study demonstrated feasibility for using Raman spectroscopy as an on-line method to monitor drug hydration state during fluid bed drying. On-line Raman spectra collected in these experiments provided critical information that enabled understanding the relationship between risedronate hydration state and the physical stability of tablets. This study also identified a critical process variable, final granulation moisture, for fluid bed drying of risedronate sodium. This variable was critical due to its affect on the solid-state form of risedronate, as measured by Raman spectroscopy, which, in turn, impacted the physical stability of the drug product over time.

Experimental variables not characterized in these experiments include: alternate drug substances with similar hydration properties, drug and excipients with different Raman scattering cross-sections, the drug concentration, the sensitivity and signal-to-noise characteristics of the Raman instrument, and the batch size. Batch size will affect the particle concentration the Raman laser probes during fluidization, and thus the signal intensity detected. Under certain conditions, these variables may
impact the ability of on-line Raman spectroscopy to monitor solid-state changes during fluid bed drying.

### 3.4 Future Work

To develop this technology as a process control tool, extended characterization of process variables in the context of analytical informing power would need to be completed. More rigorous analysis of the spectral features associated with the change in drug hydration state, and a thorough understanding of critical measurement parameters and their edges of failure is needed. With further study, a model could be developed to control the endpoint of fluid bed drying using on-line Raman spectroscopy to produce a product that would have acceptable physical stability. This model could also be used to aid in efficient scale-up of a fluid bed drying process for a drug where the hydration state was critical to product stability.
4. Part 2: Evaluation of Blend and Tablet Uniformity

Blending is also a critical process in the manufacture of pharmaceutical dosage forms. Homogeneous distribution of the active pharmaceutical ingredient is necessary to meet expectations of dosage unit uniformity (US Pharmacopeia, 2004). In addition, the homogeneity of functional excipients is necessary for consistent product performance. While the blending or mixing of liquid preparations is well-understood and predictable utilizing tools such as finite element analysis, evaluating the blending of dry powders continues to be a considerable challenge. The importance of a uniform powder blend cannot be minimized, as it affects the final product uniformity and thus the quality, safety, and efficacy of the product. In a formulation optimized for blending, all ingredients would be added in equal amounts. Each ingredient would be at least 10% of the total amount, and all ingredients would have similar physical properties such as density, particle size, and particle morphology. However, the majority of formulations do not meet all of these criteria. In many cases, one or more of the ingredients is added at less than 5% of the total. Often the active pharmaceutical ingredient is potent and the
amount per dosage unit is 1% or less. Therefore, optimization of blending processes requires considerable research and development.

The most widely used method for assessing drug content uniformity of a blend involves sampling of the blend followed by off-line analysis, typically using an HPLC method. It has been well documented that the act of sampling, traditionally using a thief while the powder bed is stationary, disturbs the powder bed and can cause sampling that is not representative of blend uniformity (Muzzio et al., 1997; Hwang and Wu, 2004). To address the possibility of sampling methods introducing error into blend uniformity results, the Product Quality Research Institute Blend Uniformity Working group recommended stratified sampling. If blend sample %RSD is greater than 5% and/or the individual results are outside the mean +/- 10%, and a mixing problem is not identified, then dosage units (i.e. tablets) should be tested (PQRI, 2002). Error is decreased when samples are taken after the powder is compressed into discrete units due to the lack of powder mobility (Chang et al., 2004). However, understanding uniformity in the blender itself is still expected (FDA, 2003) and sampling for this purpose is performed most accurately when the powder bed is in motion. To avoid error involved in sampling a
static powder bed, on-line analytical methods are being evaluated. These methods involve instrumenting a blender with an analytical system, such as near-infrared (NIR), thermal effusivity, or Raman spectroscopy, to allow real-time blend uniformity analysis.

The spectra produced from analytical methods, such as Raman and NIR, can be analyzed with univariate and multivariate methodologies. An example of a univariate method is to measure area or height for a peak that is known to be produced by the compound of interest. However, more sophisticated chemometric data analysis techniques allow additional information to be extracted from the spectra. There are a number of multivariate techniques that take into account the change of the entire spectrum. Some of the techniques used are partial least squares (PLS), multiple linear regression (MLR) (Blanco et al., 1994), linear discriminant analysis (LDA), quadratic discriminant analysis (QDA), regularized discriminant analysis (RDA) (Wu et al., 1996a), artificial neural networks (Wu et al., 1996b), Conformity Index (CI) (Ritchie et al., 2003), and Mahalanobis Distance (MD) (Ritchie et al., 2003; Whitfield et al., 1987). Mahalanobis Distance (MD) compares the distance of an observed point to the points that comprise the model.
space. The model space is created during calibration with a training set. The training set is comprised of spectra produced from material exhibiting the desired property. For example, when the goal is to measure blend uniformity, the training set is comprised of spectra produced by a uniform blend. MD has been described as a multivariate equivalent of a confidence interval (Ritchie et al., 2003).

This work utilized on-line Raman spectroscopy with univariate and multivariate methodologies to characterize low dose (1%) blend uniformity, as compared to blend and tablet uniformity based on traditional thief sampling and HPLC analysis. Typical direct compression powder blends were used consisting of azimilide dihydrochloride, spray-dried lactose, crospovidone, and magnesium stearate.
4.1 Experimental

4.1.1 Materials

The materials used for these experiments are shown in Table 11. The drug used for these experiments was azimilide dihydrochloride. Three common fillers were evaluated due to differences in their physical properties: spray-dried lactose, microcrystalline cellulose, and dibasic calcium phosphate. A description and the density of each of these materials can be found in Table 12. Figures 22-25 show microscopy images of azimilide, microcrystalline cellulose, spray-dried lactose, and dibasic calcium phosphate, respectively. Crospovidone is a common disintegrant and magnesium stearate is a common lubricant. Raman spectra of the drug and excipients are provided in Appendix A. The additional materials in Table 11 were used during HPLC testing.
Table 11. Materials for evaluation of blend and tablet uniformity

<table>
<thead>
<tr>
<th>Material</th>
<th>Trade Name</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azimilide dihydrochloride</td>
<td>N/A</td>
<td>Procter &amp; Gamble, Norwich, New York, USA</td>
</tr>
<tr>
<td>Spray-dried lactose</td>
<td>Pharmatose DCL-11</td>
<td>DMV, Veghel, The Netherlands</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>Avicel PH102</td>
<td>FMC, Philadelphia, Pennsylvania, USA</td>
</tr>
<tr>
<td>Crospovidone</td>
<td>Polyplasdone XL</td>
<td>International Specialty Products, Wayne, New Jersey, USA</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>N/A</td>
<td>Peter Greven, Bad Münstereifel, Germany</td>
</tr>
<tr>
<td>Dibasic calcium phosphate</td>
<td>Emcompress</td>
<td>JRS Pharma LP, Patterson, New York, USA</td>
</tr>
<tr>
<td>Ammonium acetate ACS grade</td>
<td>N/A</td>
<td>JT Baker, Phillipsburg, NJ, USA</td>
</tr>
<tr>
<td>Acetonitrile HPLC grade</td>
<td>N/A</td>
<td>JT Baker, Phillipsburg, NJ, USA</td>
</tr>
<tr>
<td>Tetrahydrofuran HPLC grade</td>
<td>N/A</td>
<td>EMD Chemicals, Inc., Gibbstown, New Jersey, USA</td>
</tr>
</tbody>
</table>
Table 12. Azimilide and excipient characteristics

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
<th>Density (g/mL)</th>
<th>Mean Particle Size (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azimilide dihydrochloride</td>
<td>Crystalline, flat plates</td>
<td>0.36</td>
<td>70</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>Irregular, fiber-looking</td>
<td>0.34</td>
<td>100</td>
</tr>
<tr>
<td>Spray-dried lactose</td>
<td>Spherical particles</td>
<td>0.63</td>
<td>110</td>
</tr>
<tr>
<td>Dibasic calcium phosphate</td>
<td>Crystalline, aggregates</td>
<td>1.14</td>
<td>150</td>
</tr>
</tbody>
</table>
Figure 22. Azimilide microscopy

Figure 23. Microcrystalline cellulose microscopy
Figure 24. Spray-dried lactose microscopy

Figure 25. Dibasic calcium phosphate microscopy
4.1.2 Equipment

Feasibility experiments utilized a turbula mixer (Glen Mills, Clifton, NJ, USA). Pilot scale manufacturing equipment was a variable speed 8qt V-blender (Patterson-Kelley, East Stroudsburg, PA, USA), a pocket thief sampler with two 1.0mL dies, a 10-station Piccola instrumented tablet press (Specialty Measurements Inc., Lebanon, NJ, USA), and modified oval tooling (Natoli, St. Charles, MO, USA).

Instrumentation utilized in these experiments was a Raman Holoprobe with an excitation wavelength of 785nm and an intensity at sample of approximately 50mW (Kaiser Optical Systems, Ann Arbor, MI, USA), a custom made immersion probe (Center for Process Analytical Chemistry, University of Washington), probe head model HFPH-785 (Kaiser Optical Systems), optic NCO-1.3-NIR (Kaiser Optical Systems), homemade linear motion device and spinner for Raman tablet testing, Caliper LS Tablet Processing Workstation II v.2.2 (Caliper Life Sciences, Hopkinton, MA, USA), High-Performance Liquid Chromatography pump, UV detector, column: ODS 2 Inertsil (Metachem Tech. Inc., 50 cm x 3.0 mm, Part No.
0296-050x030) (Varian Inc., Palo Alto, CA, USA), column heater, various balances (PE11, AM50, PE360) (Mettler, Columbus, OH, USA), Schleuniger hardness tester (Copley Scientific, Nottingham, UK), and digital calipers (Mitutoyo America Corp., Aurora, IL, USA).

4.1.3 Software

Raman spectra were collected using HoloGRAMS version 4.0 (Kaiser Optical Systems, Ann Arbor, MI, USA). Raman peak areas were calculated using Holomap 2.1 rev. 1 (Kaiser Optical Systems) in MatLab R12 (The MathWorks, Inc., Natick, MA, USA). Multivariate calibration and analysis of Raman data was performed using Grams/AI version 7.02 (Thermo Galactic Industries Corp., Salem, NH, USA). HPLC analysis used Turbochrom v.6.1.1 (Perkin-Elmer, Wellesley, MA, USA). Microsoft Excel 2002 was used to create tables, graphs, and for basic calculations.
4.1.4 Formulations

For low dose blending feasibility experiments, 1% azimilide was mixed with three different excipients: microcrystalline cellulose, spray-dried lactose, or dibasic calcium phosphate. The formulation used is shown in Table 13. To evaluate the effect of drug placement in the blender on uniformity, two formulations were used. These formulations are shown in Tables 14 and 15.
Table 13. Formulation for evaluating feasibility of monitoring blending with Raman

<table>
<thead>
<tr>
<th>Component</th>
<th>Unit (g)</th>
<th>Batch (g)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azimilide dihydrochloride</td>
<td>0.0026</td>
<td>0.52</td>
<td>1.0</td>
</tr>
<tr>
<td>Microcrystalline cellulose or spray-dried lactose or dibasic calcium</td>
<td>0.2474</td>
<td>49.48</td>
<td>99.0</td>
</tr>
<tr>
<td>phosphate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.2500</td>
<td>50.00</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Table 14. Formulation for drug placement experiments with microcrystalline cellulose

<table>
<thead>
<tr>
<th>Component</th>
<th>Unit (g)</th>
<th>Batch (kg)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azimilide dihydrochloride</td>
<td>0.0019</td>
<td>0.0239</td>
<td>0.8</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>0.2394</td>
<td>3.0449</td>
<td>99.2</td>
</tr>
<tr>
<td>Total</td>
<td>0.2413</td>
<td>3.0688</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 15. Formulation for drug placement experiments with spray-dried lactose

<table>
<thead>
<tr>
<th>Component</th>
<th>Unit (g)</th>
<th>Batch (kg)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azimilide dihydrochloride</td>
<td>0.0025</td>
<td>0.0575</td>
<td>1.0</td>
</tr>
<tr>
<td>Spray-dried lactose</td>
<td>0.2388</td>
<td>5.4913</td>
<td>99.0</td>
</tr>
<tr>
<td>Total</td>
<td>0.2413</td>
<td>5.5488</td>
<td>100.0</td>
</tr>
</tbody>
</table>
The formulation in Table 16 was used for pilot scale experiments to evaluate low dose azimilide blend and tablet uniformity with Raman spectroscopy and HPLC. The formulation in this study is representative of direct compression formulations often used in commercial pharmaceutical manufacturing, utilizing drug, filler, disintegrant, and lubricant. An azimilide level of 1% enabled study of low dose blend uniformity, using on-line Raman spectroscopy, due to suitable signal-to-noise for quantitation of Raman peaks in the azimilide ring breathing bands. Spray-dried lactose is a common tablet filler that aids in flow and compression, and its Raman bands did not interfere with the azimilide Raman bands used for quantitation. Crospovidone is the disintegrant and magnesium stearate is the lubricant, and they are routinely used in tablet formulation (Rowe et al., 2003). For this formulation, the amounts of crospovidone and magnesium stearate were below the Raman limit of detection.
Table 16. Direct compression formulation for pilot scale uniformity experiments

<table>
<thead>
<tr>
<th>Component</th>
<th>Unit (g)</th>
<th>Batch (kg)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azimilide dihydrochloride</td>
<td>0.0025</td>
<td>0.058</td>
<td>1.0</td>
</tr>
<tr>
<td>Spray-dried lactose</td>
<td>0.2388</td>
<td>5.491</td>
<td>95.5</td>
</tr>
<tr>
<td>Crospovidone</td>
<td>0.0075</td>
<td>0.173</td>
<td>3.0</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>0.0013</td>
<td>0.029</td>
<td>0.5</td>
</tr>
<tr>
<td>Total</td>
<td>0.2501</td>
<td>5.750</td>
<td>100.0</td>
</tr>
</tbody>
</table>
4.1.5 Manufacturing Process

For low dose blending feasibility experiments, lactose, microcrystalline cellulose, or dibasic calcium phosphate and azimilide were added to a 250mL bottle and mixed in a turbula mixer. At set time intervals, the mixer was stopped and the jar was removed. The Raman probe was inserted approximately one inch deep into the center of the blend and a spectrum was collected with an acquisition time of 20 seconds.

For pilot scale experiments, azimilide, microcrystalline cellulose or lactose, crospovidone and magnesium stearate were added to an 8qt V-blender. The components were mixed until the endpoint for the experiment was reached. Throughout the blending process, the blend was monitored by Raman spectroscopy. The fiber optic probe head was inserted into the blender through the I-bar port (Figure 26). This single point probe placement allowed for representative sampling during the blending process, due to the small size of the blender combined with frequent Raman acquisitions. During the blending process, Raman spectra were collected every 10, 20, or 40 seconds depending on the experiment. At a blender speed of
6.75rpm, the 20 second sampling interval allowed for averaging of about two rotations, which is a representative sampling of the entire mass. The estimated sample size with a 20 second sampling interval is about 1mg per acquisition. The Raman sample size was estimated using particle velocity, an average blend density and particle size, and the Raman acquisition time. Particle velocity was estimated using the size and speed of the blender. When blending was completed, five thief samples of about 500mg were taken, two down each side of the blender and one in the middle under the Raman probe. The blend was then compressed into tablets, and samples were collected every 4 minutes throughout the 70 minute compression operation. The manufacturing process is illustrated in Figure 27.
Figure 26. Blender and Raman set-up

[Image of Blender and Raman set-up]

Raman Probe
Figure 27. Direct compression manufacturing flowchart

- **Azimilide**
- **Spray-dried lactose**
- **Crospovidone**
- **Magnesium Stearate**

Blend for various times
8qt V-blender

Compression at 15kN
Piccola 10-station

Continuous on-line Raman monitoring
4.1.6 Testing

In pilot scale experiments to assess azimilide blend and tablet uniformity, blend thief samples and tablet samples were tested for azimilide content utilizing isocratic reversed phase high-performance liquid chromatography on a C\textsubscript{18} column with pH 6.0 acetate buffer:acetonitrile:tetrahydrofuran (1000:385:154) as the mobile phase using a column temperature of 40°C and UV detection at 345 nm. Further details on the HPLC method are provided in Appendix B. For each blend, Raman spectra were collected every 20 seconds throughout the blending process. Raman spectra were also collected off-line on tablets from five batches with an acquisition time of 10 seconds.

4.1.7 Experimental Design and Methodology

Experiments were performed using the turbula mixer to determine the feasibility of detecting azimilide at 1% with Raman spectroscopy during blending with different excipients. The excipients used were microcrystalline cellulose, spray-dried lactose, and dibasic calcium phosphate. At the following times, blending was stopped and the Raman probe inserted into the powder bed to obtain a spectrum
using a 20 second acquisition time: 30 sec, 1 min, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20 minutes. For each blend, azimilide peak area at 1600 and 1620 cm⁻¹ was calculated and plotted versus time to create blend profiles. Also, the %RSD of peak area was plotted versus time by calculating a running %RSD with n=3. From these experiments, excipients were chosen to proceed into pilot scale blending studies.

At pilot scale, the effect of drug placement in the blender on uniformity using two different excipients was evaluated. The excipients were microcrystalline cellulose and spray-dried lactose. Azimilide at 1% was initially placed in one of four locations: bottom of the blender (lactose only), middle of the blender in between excipient under the Raman probe, on top of the excipient in the ear of the blender in front of the probe, on top of the excipient in the ear of the blender behind the probe. These locations are illustrated in Figure 28. The Raman acquisition time was 20 seconds with microcrystalline cellulose and 10 seconds with lactose. Two different Raman blending approaches were taken. For the microcrystalline cellulose blend, one batch was analyzed with on-line Raman continuously as it blended at 25rpm; the azimilide was placed in the
middle of the blender to start. An additional three batches were made with the azimilide initially placed in the middle, top in front of probe, and top behind probe. For these three batches, the blender was rotated one revolution and then stopped to collect a Raman spectrum. This was repeated until 100 revolutions of Raman spectra were collected, then the blend was mixed at 25rpm and monitored with Raman spectroscopy. For the lactose blend, four batches were made with azimilide initially in each of the four locations in the blender. For these four batches, the blender speed was reduced to 6.75rpm and the blend was continuously monitored with on-line Raman spectroscopy. For all blends, a running %RSD of azimilide peak area at 1600 and 1620 cm⁻¹ was calculated and plotted versus time and number of revolutions to create blend profiles. Mixing at different speeds in the V-blender can be compared through the number of blender revolutions, because it has been shown that the mixing mechanism which occurs in tumbler blenders, consistent with this size and range of speed, are essentially unchanged (Brone et al., 1998; Brone and Muzzio, 2000).
Figure 28. Drug placement locations
Using the formulation in Table 16, pilot scale experiments to assess azimilide blend and tablet uniformity with Raman spectroscopy and HPLC were performed by blending to a range of endpoints: 3 min, 4, 5, 6, 7, 8.5, 10, 15, 20, 30, 60, 90, and 120 min. Blend thief samples and tablet samples were tested for azimilide content using HPLC. Blend profiles were created by plotting the mean percent label and percent relative standard deviation versus blend time. A Raman spectrum was collected on-line every 20 seconds while the blending process was occurring. These spectra were analyzed by both univariate and multivariate methodology. The univariate methodology involved pre-processing the spectra using standard normal variant (SNV), followed by calculation of the peak area of two azimilide ring breathing bands at 1600 and 1620 cm\(^{-1}\) (Figure 29). Blend profiles were created by plotting these peak areas versus blend time and number of revolutions. Also, the %RSD of peak area was plotted versus time and number of revolutions by calculating a running %RSD with n=3.
Figure 29. Raman spectrum of 1% azimilide blend with spray-dried lactose, crospovidone, and magnesium stearate.
The multivariate analysis utilized the Mahalanobis Distance (MD), which is a conformity index. The MD is similar to the Euclidean distance (ED), except that it additionally takes into account correlation in the data (De Maesschalck et al., 2000; Blanco and Villar, 2003; Shen et al., 2003; Lachenbruch, 1997; Furutani et al., 1984). The MD for each object, \( x_i \), is computed by the following equation.

\[
MD_i = \sqrt{(x_i - \bar{x})(C_x^{-1})(x_i - \bar{x})^T}
\]

where \( \bar{x} \) is the mean, \( C_x^{-1} \) is the inverse of the variance-covariance matrix, and \( T \) indicates the transpose of the matrix.

A calibration set was created from spectra collected in the last 40 minutes of the 120 minute blend. These spectra were confirmed to be from a uniform blend using HPLC blend thief sample results. The calibration was created using discriminate analysis with standard normal variant pre-processing of the calibration set. Using this calibration, the MD was calculated for each Raman spectrum from each blend. Blend profiles were created by plotting the MD values versus blend time. The upper threshold of MD was determined empirically from the maximum MD of the calibration set. Spectra with
MD below this threshold were considered uniform, because they conformed to the population created by the calibration set.

To determine if a correlation existed between HPLC blend and tablet uniformity results and Raman blend uniformity results, regression was performed on the standard deviation of HPLC azimilide blend content and Raman blend SNV azimilide peak area (using the last seven spectra of each blend). Regression was also performed for the standard deviation of HPLC azimilide tablet content and Raman blend SNV azimilide peak area.

In addition, Raman spectra were collected off-line on tablets from batches with uniform and non-uniform distribution of azimilide using an acquisition time of 10 seconds. Batches with blend times of 3, 5, 10, 20, and 120 minutes were analyzed. HPLC analysis of tablets from batches with 3, 5, and 10 minute blend times showed that azimilide distribution was non-uniform. HPLC analysis of tablets from batches with 20 and 120 minute blend times showed that azimilide distribution was uniform. To collect Raman spectra of the tablets, they were moved linearly under the laser so that it impinged on the
length of the dome. The linear motion device moved the tablets at a speed of 30 cycles per minute. Movement of the tablet under the laser allowed for sampling of a larger portion of the tablet, as compared to collecting a spectrum from a stationary tablet. In addition to collecting average tablet spectra with a linear motion device, a spinning device at a speed of 100 rpm was also used. The spinning device was used to evaluate tablets manufactured with a 3 minute blend time and 120 minute blend time. Two locations on the tablet were tested with the spinning device, 1) the dome, as was tested with the linear motion device, and 2) the inside of the tablet after breaking it in half. To evaluate azimilide uniformity using the Raman spectra collected, the azimilide peak area at 1620 and 1600 cm\(^{-1}\) was calculated. The total peak area of each spectrum was also calculated and used to normalize the azimilide peak area. Normalization was accomplished by dividing the azimilide peak area by the total peak area and multiplying by 100. Normalized peak area was plotted versus the tablet sample number. Higher within batch variability of the azimilide normalized peak area indicates non-uniform tablets, whereas lower within batch variability indicates uniform tablets.
4.2 Results and Discussion

4.2.1 Feasibility of detecting 1% azimilide in blend using Raman

Three small blends of 50g were made in the turbula mixer and tested by Raman spectroscopy to determine if azimilide at a level of 1% could be detected in the presence of microcrystalline cellulose, spray-dried lactose, or dibasic calcium phosphate. A spectrum was collected at 30 sec, 1 minute, 2, 3, 6, 8, 10, 12, 14, 16, 18, and 20 minutes using a 20 second exposure time. Azimilide peaks were detected in all three blends, therefore blending could be monitored with Raman spectroscopy. Two blend profiles were created by plotting peak area of azimilide peaks at 1620 and 1600 cm\(^{-1}\) versus time and %RSD of peak area versus time. These profiles are shown in Figures 30 and 31, respectively.
Figure 30. Effect of excipient type and blend time on azimilide blend uniformity (Raman peak area)
Figure 31. Effect of excipient type and blend time on azimilide blend uniformity (%RSD Raman peak area)
Even though the three excipients used have different physical properties which would indicate a difference in the time to reach uniformity, at small scale in the turbula mixer they produced similar blend profiles. All excipients allowed for adequate azimilide signal, between 60,000 and 220,000 counts. The largest azimilide signal was observed with dibasic calcium phosphate, followed by lactose, and then microcrystalline cellulose. Lactose and microcrystalline cellulose were chosen to move forward to drug placement studies in the blender, because they would be more challenging for on-line Raman monitoring.
4.2.2 Effect of drug placement in the blender on uniformity at pilot scale measured by on-line Raman

An 8qt V-blender was used to evaluate blending of 1% azimilide in microcrystalline cellulose and 1% azimilide in lactose. Azimilide was initially placed in one of four locations: bottom of the blender (lactose only), middle of the blender in between excipient under the Raman probe, on top of excipient in the ear of the blender in front of the probe, on top of excipient in the ear of the blender behind the probe. As mentioned in the Experimental Design and Methodology section, the microcrystalline cellulose blends were monitored two different ways, with the blender running continuously and with the blender stopped. One batch was monitored continuously as it blended at 25rpm for 120 minutes with the azimilide initially placed in the middle of the blender. Three batches were made, with azimilide placed in the middle, top in front of the probe, and top behind the probe, where the blender was stopped after each revolution and a Raman spectrum was collected. This was repeated until 100 spectra were collected, then the blend was monitored continuously at 25rpm with Raman spectroscopy for a total of 120 minutes of blending. Blend profiles were created by plotting %RSD of azimilide peak area at
1620 and 1600 cm\(^{-1}\) versus time and number of revolutions. Blend uniformity is achieved when the %RSD stabilizes. The profiles for microcrystalline cellulose are shown in Figure 32. The negative values for RSD observed early in the blending process are due to a high degree of variability in a small azimilide Raman peak area at the initial stages of blending.
Figure 32. Effect of azimilide placement on blend uniformity with microcrystalline cellulose using on-line Raman
Azimilide blend uniformity using microcrystalline cellulose as the main filler was achieved at approximately 20 revolutions or 0.8 minutes. Figure 32 shows that the time needed to achieve uniformity was not impacted by the two different approaches of monitoring blending with Raman spectroscopy. However, the two different approaches produced very different measurement variability. The %RSD range when the blender was stopped during Raman acquisition was 5 times larger than when the blender was in motion. When the blender was in motion, the Raman sampled a larger amount of material as a spectrum was collected, as compared to when the blender was stopped. In addition, at this scale there was no effect of azimilide placement in the blender on the time to reach uniformity.

The effect of azimilide placement in the blender on the time to reach uniformity with lactose as the main filler was also studied using Raman spectroscopy. The four locations in the blender (Figure 28) were tested with a blender speed of 6.75rpm. The speed of the blender was reduced from the previous experiments with microcrystalline cellulose to aid in observation of the blending process. Blend profiles were created by plotting %RSD of azimilide...
peak area at 1620 and 1600 cm\(^{-1}\) versus time and number of revolutions. The profiles for lactose are shown in Figure 33.
Figure 33. Effect of azimilide placement on blend uniformity with lactose using on-line Raman
As with the microcrystalline cellulose blend, there was no significant effect of azimilide placement in the blender on time to reach uniformity. Uniformity was reached in approximately 30 revolutions (5 minutes). The lactose blends took six times as long to reach uniformity when compared to the microcrystalline cellulose blends. This was a combination of two factors, 1) reducing the blender speed to approximately one quarter of the original speed and 2) the greater difference in physical properties between azimilide and lactose as compared to azimilide and microcrystalline cellulose.
4.2.3 Effect of blend time on azimilide uniformity in blends and tablets measured by Raman and HPLC

To determine the effect of blend time on azimilide uniformity in blends and tablets, the experimental design utilized on-line Raman spectroscopy while blending 1% azimilide at 6.75rpm to a range of endpoints: 3 min, 4, 5, 6, 7, 8.5, 10, 15, 20, 30, 60, 90, and 120 min. The blends were then compressed into tablets. Blend thief samples collected at the end of blending and tablet samples were analyzed with HPLC. Tablets from the 3, 5, 10, 20, and 120 minute blends were also analyzed off-line with Raman spectroscopy.

4.2.3.1 Determination of Raman acquisition time and excipient

Prior to beginning this experiment, the main excipient to be used and Raman acquisition time had to be determined. The optimal excipient would allow the greatest azimilide signal and produce low measurement variability when uniformity was reached. The optimal acquisition time would maximize the azimilide signal while still being frequent enough to observe the blending process. To determine the excipient and acquisition time, four blends were
monitored with on-line Raman spectroscopy. Two of the four blends were made using microcrystalline cellulose as the excipient, one with a 5 second acquisition time and the other with a 10 second acquisition time. This was repeated using spray-dried lactose as the excipient. The blend time was 120 minutes. Blend profiles were created by plotting %RSD of azimilide peak area at 1620 and 1600 cm\(^{-1}\) versus time and number of revolutions. The %RSD blend profiles are shown for microcrystalline cellulose and lactose in Figures 34 and 35, respectively.
Figure 34. Effect of blend time on blend uniformity of 1% azimilide with MCC measured by azimilide Raman peak area %RSD

Figure 35. Effect of blend time on blend uniformity of 1% azimilide with lactose measured by azimilide Raman peak area %RSD
The lactose blends produced the largest azimilide signal, which is consistent with the feasibility experimental results using the turbula mixer. The azimilide peak area at 1620 and 1600 cm\(^{-1}\) using microcrystalline cellulose with Raman acquisition times of 5 and 10 seconds was 10,000 and 20,000 counts*cm\(^{-1}\), respectively. Using lactose, the peak areas with 5 and 10 second acquisitions were 34,000 and 65,000 counts*cm\(^{-1}\), respectively. An acquisition time of 10 seconds produced double the azimilide signal observed with 5 seconds and was frequent enough to observe the blending that occurred in the first 5 minutes. In addition, when uniformity was reached in the lactose blends the variability in %RSD was approximately half the variability of the microcrystalline cellulose blends.

From these results, it was determined that lactose would be used as the main excipient for the 1% azimilide blend and tablet uniformity experiments with a Raman acquisition time of 10 seconds.
4.2.3.2 Effect of blend time on 1% azimilide blend and tablet uniformity measured by Raman and HPLC

Azimilide Raman peak area at 1600 and 1620 cm\(^{-1}\) was pre-processed using standard normal variant (SNV). It was determined that azimilide Raman SNV peak area reaches equilibrium by 20 minutes of blending at 6.75 rpm (135 rotations). This is equivalent to 5.4 minutes of blending at 25 rpm. It has been shown that the mixing mechanism which occurs in tumbler blenders, consistent with this size and range of speed, are essentially unchanged (Brone et al., 1998; Brone and Muzzio, 2000). Azimilide content is homogeneous at this point, since Raman peak area is proportional to concentration of the material. These results are shown in Figure 36. The shape of the blending profile shown in Figure 36 is characteristic of the blending dynamics, placement of the monitoring probe, and initial placement of the drug (El-Hagrasy et al., 2001). The %RSD of azimilide SNV peak area is shown in Figure 37.
Figure 36. Effect of blend time on azimilide uniformity of blends measured by azimilide Raman peak area

Note: blender speed was 6.75 rpm
Figure 37. Effect of blend time on azimilide uniformity of blends measured by azimilide Raman peak area %RSD
Multivariate analysis supports blend homogeneity after 20 minutes of blending at 6.75rpm. The Mahalanobis Distances for each blend are shown in Figures 38 and 39. Mahalanobis Distances of four or less indicate conformity to the calibration set. In these experiments, the calibration set consisted of Raman spectra from a homogeneous blend. After 20 minutes of blending, the majority of Mahalanobis Distances (MD) are less than four, indicating that the blend is homogeneous.

In Figures 37 and 39, some outliers can be observed after 20 minutes of blending. Outliers were classified as values greater than three standard deviations from the mean. There were three outliers in the %RSD data set and five outliers in the MD data set. The spectra corresponding to these data points were reviewed and no gross spectral abnormalities were observed. A possible cause of the MD outliers is the level of variation contained in the calibration set. The calibration set was created from only one blended batch. Outliers are expected within the large data sets produced by on-line monitoring techniques. The topic of outliers is currently under discussion in the PAT community.
Figure 38. Effect of blend time on blend uniformity measured by MD of Raman spectra

Note: blender speed was 6.75 rpm
Figure 39. Effect of blend time on blend uniformity measured by MD of Raman spectra

Note: blender speed was 6.75 rpm
One of the advantages of on-line monitoring is the blending profile that is created. At 20 seconds of blending at 6.75 rpm (equivalent to 5.4 seconds at 25 rpm), the blend was not uniform. The %RSD of Raman peak areas ranged from 40 to 160%. The MD ranged from 100 to 550 also indicating a non-uniform blend. After 40 seconds of blending at 6.75 rpm (equivalent to 10.8 seconds at 25 rpm), the %RSD of Raman peak area was 18 to 80% and the MD was 200 to 500. These results were improved from the 20 second results indicating that blending was occurring. At 1.3 minutes of blending at 6.75 rpm (equivalent to 21.6 seconds at 25 rpm), the %RSD of Raman peak area was 5 to 70% and the MD was 190 to 470. These results were similar to the results at 40 seconds. At 1.7 minutes of blending at 6.75 rpm (equivalent to 27 seconds at 25 rpm), the Raman peak area %RSD was 15 to 75% and MD was 120 to 420. The MD continued to decrease, indicating that blending was still occurring. At 2 minutes of blending (equivalent to 32.4 seconds at 25 rpm), the Raman peak area %RSD was 3 to 60% and MD was 50 to 300. At 2.7 minutes of blending at 6.75 rpm (equivalent to 43.2 seconds at 25 rpm), the Raman peak area %RSD was 10 to 52% and MD was 2 to 210. The MD continued
to decrease, however the %RSD and MD were still very high indicating a non-uniform blend. At 3.7 minutes of blending at 6.75 rpm (equivalent to 59.4 seconds at 25 rpm), the Raman peak area %RSD was 3 to 26% and MD was 5 to 70. At 5 minutes of blending at 6.75 rpm (equivalent to 1.4 minutes at 25 rpm), the Raman peak area %RSD was 3 to 15% and MD was 3 to 36. The %RSD and MD continued to decrease. At 10 minutes of blending at 6.75 rpm (equivalent to 2.7 minutes at 25 rpm), the Raman peak area %RSD was 3 to 7% and MD was 0.6 to 10. At 15 minutes of blending at 6.75 rpm (equivalent to 4 minutes at 25 rpm), the Raman peak area %RSD was 1.6 to 6.4% and MD was 0.4 to 4. At 20 minutes of blending at 6.75 rpm (equivalent to 5.4 minutes at 25 rpm), the Raman peak area %RSD was 2 to 4% and MD was 0.5 to 2. At this time, the blend is uniform. The %RSD is less than 6% and the MD is less than 4. The results at 30 to 90 minutes of blending at 6.75 rpm (equivalent to 8.1 to 24.3 minutes at 25 rpm), also showed uniformity.

Blend thief samples and tablet samples were tested for azimilide content using HPLC. These results are shown in Figures 40 and
41. The blend %RSD was consistently less than 6% after 20 minutes of blending, indicating blend homogeneity after 20 minutes. The tablet %RSD was consistently less than 6% after 8.5 minutes of blending. The difference in time to reach uniformity between blends and tablets is likely due to additional blending that occurs in the tablet press feed frame, where the blend is subjected to mixing due to two rotating feeder paddles (Stahl and Langenbucher, 1981). The blend mean % label after 20 minutes of blending was 97%, and the tablet mean % label was 99%.
Figure 40. Effect of blend time on azimilide uniformity of blends measured by HPLC

Note: blender speed was 6.75 rpm
Figure 41. Effect of blend time on azimilide uniformity of tablets measured by HPLC

Note: blender speed was 6.75 rpm
In addition, the % label of each spectrum was estimated using the standard normal variant pre-processed peak areas. To produce the estimated % label, the mean peak area of the calibration set spectra was calculated. It was shown through HPLC analysis that the calibration set spectra were from homogenous blend with approximately 100% label. Then each peak area from the blends was calculated as a percentage of this mean. The results are shown in Figure 42.
Figure 42. Effect of blend time on estimated blend % label from Raman spectra:

Note: blender speed was 6.75 rpm
Tablets from the 3, 5, 10, 20, and 120 minute blends were tested off-line with Raman spectroscopy with the goal of differentiating uniform and non-uniform tablets formulated to contain 1% azimilide. Tablets from 3, 5, and 10 minute blends were determined to be non-uniform using HPLC. Tablets from blends at 20 and 120 minutes were uniform. A linear motion device was used to obtain average Raman spectra of the tablets. The laser impinged across the tablet dome using the linear motion device. Movement of the tablet under the laser allowed for sampling of a larger portion of the tablet, as compared to collecting a spectrum from a stationary tablet. See Figure 43 for a picture of the linear motion device set-up.
Figure 43. Set-up of Raman linear motion device for tablet testing
For each tablet tested, the azimilide peak area at 1620 and 1600 cm\(^{-1}\) was calculated and normalized to the total spectrum peak area. It was expected that tablet batches with uniform azimilide distribution would show lower within batch variability of the normalized azimilide Raman peak area. In contrast, tablet batches with non-uniform azimilide distribution would show higher within batch variability of the normalized azimilide Raman peak area. Normalized azimilide Raman peak area results for each batch using the linear motion device are shown in Figure 44. Mean and standard deviation of the normalized azimilide Raman peak areas for each batch are shown in Table 17.
Figure 44. Normalized azimilide Raman peak area of tablets using linear motion device.
Table 17. Mean and standard deviation of normalized azimilide Raman peak area for each batch of tablets tested using linear motion device

<table>
<thead>
<tr>
<th>Tablet Batch Blend Time (min)</th>
<th>3</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.74</td>
<td>0.80</td>
<td>0.63</td>
<td>0.74</td>
<td>0.78</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.27</td>
<td>0.31</td>
<td>0.25</td>
<td>0.26</td>
<td>0.31</td>
</tr>
</tbody>
</table>
The Raman methodology using the linear motion device was not able to differentiate between uniform and non-uniform tablets. The mean and standard deviation of the normalized azimilide peak areas were similar for both uniform and non-uniform batches. Possible causes of the lack of differentiation were 1) the linear method used to average the spectra, 2) an annealing phenomenon at the surface of the tablet, and/or 3) the sample size detected by the Raman. To test the first possible cause, the exterior (dome) of the tablet was tested using a spinning device to produce average Raman spectra. To test the second possible cause, the interior of the tablet was tested by breaking the tablet in half. Due to the diameter of the tablet, the interior could not be tested with the linear motion device. Tablets from only the 3 and 120 minute blends were tested using the spinning device. The normalized azimilide Raman peak area results using the spinning device for both the tablet exterior and interior are shown in Figure 45. Mean and standard deviation of the normalized azimilide Raman peak areas for each batch are shown in Table 18.
Figure 45. Normalized aziminide Raman peak area of tablets using spinning device
Table 18. Mean and standard deviation of normalized azimilide Raman peak area for each batch of tablets tested using spinning device

<table>
<thead>
<tr>
<th>Sample Location</th>
<th>Exterior</th>
<th>Exterior</th>
<th>Interior</th>
<th>Interior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet Batch</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blend Time (min)</td>
<td>3</td>
<td>120</td>
<td>3</td>
<td>120</td>
</tr>
<tr>
<td>Mean</td>
<td>1.01</td>
<td>0.70</td>
<td>1.10</td>
<td>0.99</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.59</td>
<td>0.30</td>
<td>0.42</td>
<td>0.38</td>
</tr>
</tbody>
</table>
It can be seen from Figure 45 that using the spinning device to average the Raman spectra still could not allow differentiation of the uniform and non-uniform tablets. In addition, the variability does not appear to be caused by a surface phenomenon, since the results from tablet interiors also could not differentiate between uniformity and non-uniformity (Table 18). It is still possible that the sample size is too small to detect a large enough number of azimilide particles to differentiate between a uniform and non-uniform low dose tablet.
4.2.3.3 Correlations of 1% azimilide blend and tablet uniformity results from Raman and HPLC

In section 4.2.3.2, the Raman results of 1% azimilide blends and HPLC results of 1% azimilide blends and tablets were presented and discussed. Both Raman and HPLC results agreed that uniformity was reached by 20 minutes of blending at 6.75 rpm. Regression was performed to determine if there was a correlation between uniformity, or lack of uniformity, determined by Raman and HPLC methods at each blend time. For regression, blend and tablet uniformity determined by HPLC at each blend time was quantified by the standard deviations of % label. Raman blend uniformity at each blend time was quantified by the standard deviations of MD and SNV peak area from spectra collected at the end of blending, just before blend thief samples were taken. The p-values determined from regression are shown in Table 19.
Table 19. Correlations between uniformity determined by Raman and HPLC

<table>
<thead>
<tr>
<th>P-values</th>
<th>HPLC tablet stdev</th>
<th>Raman blend SNV peak area stdev</th>
<th>Raman blend MD stdev</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC blend stdev</td>
<td>0.018</td>
<td>0.00006</td>
<td>0.012</td>
</tr>
<tr>
<td>HPLC tablet stdev</td>
<td></td>
<td>0.026</td>
<td>0.03</td>
</tr>
<tr>
<td>Raman blend SNV peak area stdev</td>
<td></td>
<td></td>
<td>0.00004</td>
</tr>
</tbody>
</table>
There was significant correlation between each of the methods of assessing uniformity. The Raman MD and SNV peak area standard deviations showed a coefficient of determination, $r^2$, of 0.80. This regression plot is shown in Figure 46. The Raman SNV peak area and HPLC blend standard deviations showed a $r^2$ of 0.78. This regression plot is shown in Figure 47.
Figure 46. Regression of Raman MD and SNV peak area standard deviations of blends

$R^2 = 0.80$
Figure 47. Regression of Raman SNV peak area and HPLC standard deviations of blends

\[ R^2 = 0.78 \]
4.3 Conclusions

On-line Raman spectroscopy was capable of assessing uniformity of blends containing 1% azimilide, spray-dried lactose or microcrystalline cellulose, crospovidone, and magnesium stearate. At the 8qt scale used, drug placement in the blender had no effect on the time to reach uniformity. Both univariate and multivariate Raman methodologies were studied. The univariate method employed the standard normal variant of azimilide peak area using two specific azimilide peaks (ring breathing bands) to evaluate blend uniformity. The multivariate method evaluated the entire spectrum using standard normal variant pre-processing followed by determination of Mahalanobis Distance of the spectrum as compared to a calibration set.

Both univariate and multivariate Raman analyses of blend uniformity agreed that uniformity was reached by 20 minutes of blending. In addition, HPLC uniformity results of blend and tablet samples confirmed uniformity was reached at 20 minutes of blending. Variability of Raman and HPLC results decreased as blend time increased; variability reached equilibrium when the blend was uniform. Significant
correlations were found between Raman and HPLC variability at each blend time.

A number of variables that were held constant during this experiment will also affect the ability of on-line Raman spectroscopy to monitor blend uniformity. These variables are the chemical structure of the drug and its subsequent Raman scattering cross-section, drug concentration, sensitivity and signal-to-noise ratio of the Raman system, spectral properties of blend excipients, and the blend density (as it effects the concentration of particles that the laser impinges on during blending and thus the signal that is measured)

4.4 Future Work

Additional work needs to be completed to use Raman spectroscopy off-line for assessment of low dose tablet uniformity. The Raman sample size could be optimized for detection of enough drug particles to make an accurate measurement of drug uniformity in low dose tablets. In addition, to produce a consistently uniform product, a model could be developed using on-line Raman spectroscopy to control the endpoint of blending and aid in scale-up.
5. References


http://www.fda.gov/ohrms/dockets/ac/02/slides/3841s1_01_hussain/sld01.htm


Parenteral Drug Association, XXXVIII (6), 1-12.


PQRI blend uniformity working group recommendation to FDA, December 2002. The use of stratified sampling of blend and dosage units to demonstrate adequacy of mix for powder blends.


6. Appendix A – Raman Spectra of Materials

Figure A.1. Risedronate sodium (RS) Raman spectrum at equilibrium

(30 second acquisition time)
Figure A.2. Azimilide dihydrochloride Raman spectrum

(1 second acquisition time)
Figure A.3. Etidronate disodium Raman spectrum

(20 second acquisition time)
Figure A.4. Lactose monohydrate Raman spectrum

(1 minute acquisition time)
Figure A.5. Microcrystalline cellulose Raman spectrum

(40 second acquisition time)
Figure A.6. Dibasic calcium phosphate Raman spectrum

(20 second acquisition time)
Figure A.7. Povidone Raman spectrum

(1 minute acquisition time)
Figure A.8. Crospovidone Raman spectrum

(20 second acquisition time)
Figure A.9. Magnesium stearate Raman spectrum

(15 second acquisition time)
7. Appendix B – HPLC Method

Azimilide was quantitated in tablets and blends using an isocratic reverse phase high-performance liquid chromatographic (HPLC) procedure with automated sample preparation. The method utilized a C-18 stationary phase with a pH 6.0 acetate:acetonitrile:tetrahydrofuran mobile phase and UV detection with a 5 minute run time. An automated workstation was used to disperse, filter and dilute the samples. Azimilide was quantitated using an external standard procedure with the active pharmaceutical ingredient as a standard. The accuracy, precision, linearity and specificity of the method were validated using ICH procedures and showed excellent analytical performance.