

**NEAR INFRARED SPECTROSCOPIC TRANSMISSION
MEASUREMENTS FOR PHARMACEUTICAL POWDER MIXTURES
AND THEORY OF SAMPLING (TOS) APPLIED IN
PHARMACEUTICAL INDUSTRY QUALITY CONTROL (QC)
PRACTICES.**

By

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ABSTRACT

This thesis presents two projects that involve the implementation of near infrared spectroscopy. NIR spectroscopy is a fast and non-destructive spectroscopy that in combination with multivariate analysis is a very useful tool for the development of pharmaceutical processes and the implementation of continuous manufacturing.

The first project is based on measurements of transmission in powder blends (chapter 2). This study shows the development of calibration models using NIR transmission in powders. The study explains the necessary instrumental settings for transmission measurements in powders. The transmission measurements for powders were obtained using the Bruker Optics, Fourier Transform Near Infrared Spectrometer (FT-NIR) where a modified sample measurement cup was used. Transmissions in powders were developed because there is an absence of commercial instruments for transmission measurements in pharmaceutical powder processes. The comparison between NIR transmission spectra for powder and tablet samples and the development of calibration models was performed at three different resolutions of 64, 32, and 16 cm^{-1} . Transmission in powder and diffuse reflectance in powder was also compared in this study

The second project is based in the implementation of the Theory of Sampling (TOS) using NIR spectroscopy (chapter 3). This study has two parts, the first part consists in the evaluation of levels of heterogeneity of several samples using NIR and the determination of sampling error (Part a). The heterogeneity of the material has a large influence on the sampling process; this statement was demonstrated in this study. The second part consists in the development of a stream sampling method to facilitate the implementation of variographic analysis and use of replication experiments in the development of pharmaceutical formulations (part b). This second study shows that variographic analysis and TOS could be very valuable in the development of pharmaceutical formulations in combination with NIR spectroscopy.

RESUMEN

Esta tesis presenta dos proyectos que implican la aplicación de la espectroscopia de infrarrojo cercano. La espectroscopia de infrarrojo cercano, es una espectroscopia rápida y no destructiva que en combinación con el análisis multivariado es una herramienta muy útil para el desarrollo de procesos farmacéuticos y la implementación de fabricación continua.

El primer proyecto se basa en el desarrollo de medidas de transmisión en mezclas de polvo (Capítulo 2). Este estudio muestra el desarrollo de modelos de calibración de polvos utilizando infrarrojo cercano en modo de transmisión. El estudio explica los ajustes instrumentales necesarios para las mediciones de transmisión en polvos. Las mediciones de transmisión en polvos fueron desarrolladas utilizando un Bruker optics, Espectrómetro de Infrarrojo Cercano de Transformada de Fourier (FT-NIR). Transmisión en polvos se desarrollaron porque hay una ausencia de instrumentos comerciales para las mediciones de transmisión en procesos de polvos farmacéuticos. La comparación entre espectros de Transmisión de mezclas de polvos y tabletas y el desarrollo de modelos de calibración con tres diferentes resoluciones 64, 32 y 16 cm^{-1} fueron realizados. La comparación entre transmisión en polvo y reflectancia difusa en polvo fue hecho.

El segundo proyecto se basa en la aplicación de la Teoría de Muestreo (TOS) mediante espectroscopia de infrarrojo cercano (NIR) (capítulo 3). Este estudio tiene dos partes, la primera parte consiste en la evaluación de los niveles de heterogeneidad de varias muestras utilizando infrarrojo cercano y la determinación del error de muestreo (parte a). La heterogeneidad del material tiene gran influencia en el proceso de muestreo, Esta declaración fue demostrada en este estudio. La segunda parte consiste en el desarrollo de un método de muestreo corriente para facilitar la aplicación del análisis variográfico y el uso de experimentos de replicación en el desarrollo de formulaciones farmacéuticas (Parte B) .Este estudio muestra que el análisis variográfico y TOS podría ser muy valioso en el desarrollo de formulaciones farmacéuticas en combinación con la espectroscopia de infrarrojo cercano.

Dedication

To GOD

My family

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List abbreviations and symbols

MCC	Microcrystalline cellulose
APAP	Acetaminophen
API	Active Pharmaceutical Ingredients
DOE	Design Of Experiment
NIR	Near-infrared
NIRS	Near-infrared spectroscopy
MIR	Mid Infrared
MPA FT- NIR	Multi-Purpose Analyzer Fourier Transform Near Infrared
InGaAs	Indium gallium arsenide
PCR	Principal Component Regression
MLR	Multi Linear Regression
PCA	Principal Component analysis
PLS	Partial least squares regression
RMSEP	Root Mean Square Error of Predictions
RSD	Relative standard deviation
RSEP	Relative standard errors of predictions
SNV	Standard Normal Variate
TOS	Theory Of Sampling

Basic notations used throughout this work are resumed as follow:

Cal	Calibration
Val	Validation
1 st - der	First Derivative
Std	Standard deviation

CHAPTER 1 THEORETICAL BACKGROUND

1.1 Near infrared spectroscopy and chemometric

Near infrared spectroscopy (NIRS) is used in agricultural, food, oil, and pharmaceutical industries for real time analysis to reduce costs through the elimination of sample preparation and reagent costs used in conventional laboratory tests. NIRS is non-destructive analytical method and provides information on both the chemical and physical properties of materials.¹

Near Infrared Spectroscopy (NIRS) is a vibrational spectroscopy that covers the region of the electromagnetic spectrum from $12821- 3959 \text{ cm}^{-1}$. The NIR region lies in the middle of the visible and mid infrared (MIR) regions. The absorption vibrational bands in this spectroscopy correspond to overtone and combinations of fundamental vibrations of functional groups such as $-\text{NH}$, $-\text{OH}$, $-\text{CH}$, and $-\text{SH}$. The overtone and combination bands can be explained using anharmonic model (Fig 1.1).

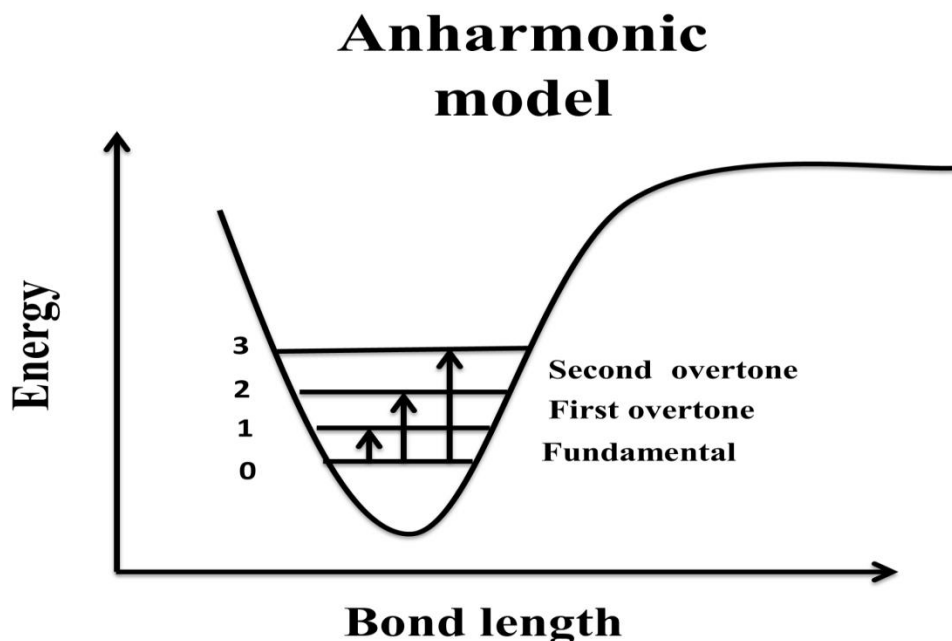


Figure 1.1 The anharmonic model

The NIR absorption bands are between 10–100 times weaker than their corresponding fundamental mid-IR absorption bands. The most common measurement modes employed by NIR spectroscopy are transmittance, transfectance, diffuse transmittance, and diffuse reflectance (Fig 1.2).

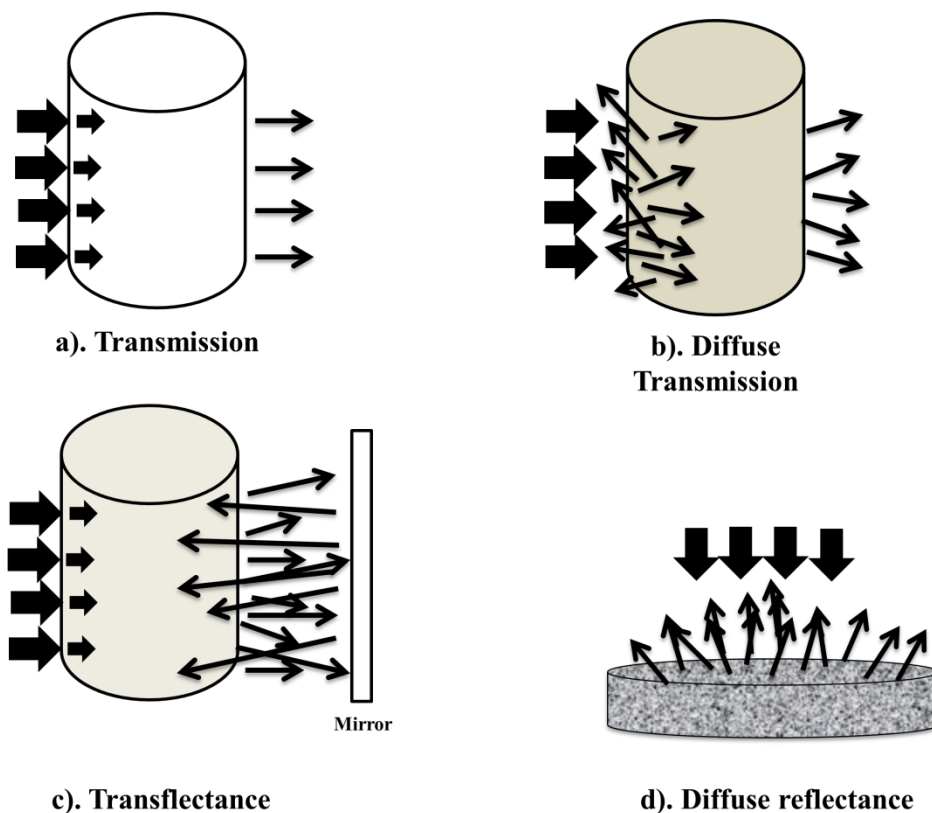


Figure 1.2 Measurement modes used in NIR

In transmission the radiation interacts with many particles and the light passes through the material. NIR transmission mode can be also used in transparent liquids. During the transmission analysis a sample liquid is often placed in a glass cuvette and analyzed in similar manner as in UV–Vis (Fig. 1.2 a). Diffuse transmission (Fig. 1.2 b) is employed in materials such as solid, semi-solid and turbid liquids samples. The light passes through many particles in the sample and finally the detector captures the diffuse radiation that emerges from the bulk of the sample. Transfectance (Fig. 1.2 c) is a combination of transmission and reflectance; in this

mode the radiation passes through the sample twice. The advantage of the measurement modes that involve transmission is that a large volume of the sample is analyzed ⁸.

Transmission measurement can be developed within the sample compartment and transmission unit accessories of the NIR spectrometer (Figure 1.3).

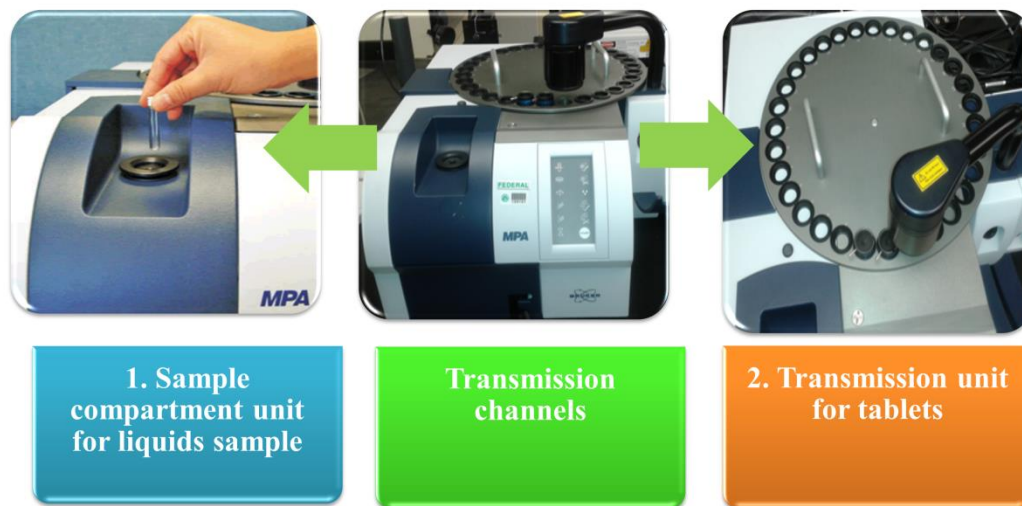


Figure 1.3 Transmission channels in a Bruker MPA FT-NIR spectrometer

In diffuse reflectance (Fig. 1.2 d) mode, near infrared radiation is irradiated on a solid surface; a portion of the radiation penetrates the sample and is absorbed⁹. Part of the radiation is refracted through each particle, a segment is scattered in all directions, and finally the incident radiation that emerges from the sample surface is called diffuse reflectance. The interaction between radiation and materials mainly depends of particles size and refractive index. The disadvantage in this analysis arises by the limited penetration capacity of the beam in the sample¹⁰.

Diffuse reflectance and diffuse transmission can be used to study particle size¹⁰ due to the interaction between the radiation and solid particles.

Diffuse reflectance measurements can be performed with the fiber optic probe and integrating sphere accessories that are part of the FT-NIR spectrometer. Reflectance spectra which depend

of absorption and scatter characteristics of sample are obtained as instrumental response. (Figure 1.4)

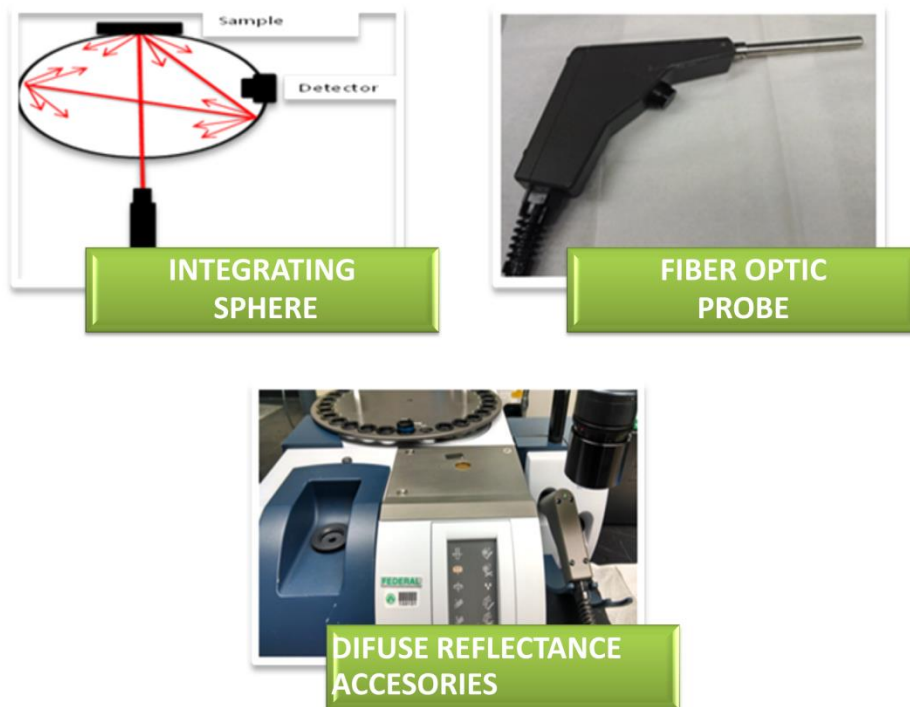


Figure 1.4 Diffuse reflectance channels in Bruker MPA FT-NIR spectrometer.

1.1.2 Type of analysis in NIR spectroscopy

Qualitative analysis is used for classification of the sample according to their NIR spectra; this type of analysis is based on the development of a library of compounds and the identification is based on a pattern recognition method.

The identification of an unknown material may be performed through the comparison of the NIR spectrum of the sample tested with the spectra collected in the library. There are two classification types, the unsupervised and the supervised classification. In the unsupervised classification, the sample is classified without a prior assignment of class; an example of an unsupervised method is the principal component analysis which is used for identifying spectral

characteristic and patterns. In the supervised classification, the spectroscopist has prior knowledge of the sample which can be assigned to a class. Partial least square discriminant analysis (PLS – DA) is an example of supervised method, PLS – DA is a method based in correlation and distance.

Quantitative analysis is used to quantify properties such as moisture, drug concentration in blends, density of materials and others. The quantification of a property of interest is done through regression methods such as multilinear regression (MLR), principal component regression (PCR), partial least square (PLS), artificial neural network, and support vector machines. The regression methods are built with a calibration set (samples with concentration known) and is validated with a validation set (sample with unknown concentration). A good calibration set would contain all the variability with respect to the properties of the samples. A good validation set would be in the range of specification of which the calibration model was developed. Quantitative analysis is performed for: real time process measurements for drug concentration in blends³, analysis of incoming raw materials, and for the determination of drug concentration in tablets.

1.1.3 Chemometrics

The NIR is sensitive to chemical changes and physical properties of materials. The development of qualitative and quantitative analysis in NIR spectroscopy requires relating spectral variables with a property of the sample. This relationship is developed with chemometric, where statistical and mathematical methods are applied to do NIR spectroscopy.

The absorption bands of the NIR spectrum are broad and overlapping. These factors do not lead an easy interpretation of the spectrum of a sample. Chemometric methods provide maximum relevant chemistry information from the data obtained.

1.1.4 Data pretreatment

The NIR spectra can be affected by particle size, humidity, scattering of the light, instrumental noise, path length of the radiation, concentration of the materials. For reducing the interference in the NIR spectra, the mathematical data pretreatments or mathematical corrections in the data were used. The reduction of the impact of these effects helps to facilitate the information extraction and the development of robust calibration models

The most commonly pretreatments used are: SNV (Standard Normal Variate), MSC (Multiplicative Scatter Correction), first and second derivative, smoothing, mean centering, and orthogonal signal correction (OSC).

1.1.5 Chemometric methods

Two commonly chemometric methods have been used in this research. The first is Principal component analysis (PCA) which is able to transform the original correlated variables to a new set of uncorrelated latent variables. PCA is a mathematical procedure that transforms the spectral data into orthogonal components whose linear combinations approximate the original data. In a PCA the loadings are linear combinations of the original variable, the principal component describe the highest variance of all linear combinations, the second component, the third and the n-component represent the remaining variation.

The second method is the Partial Least Square (PLS) regression, which is able to establish a linear relation between two matrices; the matrix corresponding to the spectral data (\mathbf{X}) and the matrix corresponding to reference values (\mathbf{Y}). This technique is modelling both (\mathbf{X}) and (\mathbf{Y}) to find out the variables in (\mathbf{X}) matrix that will best describes the (\mathbf{Y}) matrix.

1.2 Theory of sampling (TOS)

TOS is a set of proven principles and practices to decrease the influence of the heterogeneity of materials on sampling error ¹. The TOS has been used in different studies in the mining, metallurgical and environmental fields². The implementation of this theory in the manufacturing process is important because TOS improves quality control and quality assurance of sampling methods in the industry.

TOS is focused on the sample and the procedure for sample selection. There are two types of sample selection (Non – probabilistic and probabilistic)². In a Non-probabilistic sampling all part of the elements of a lot do not have the probability of being selected, while in a probabilistic sampling all parts of a lot have the same probability of being sampled. If a sampling procedure is probabilistic and the sample is not affecting in any way, a correct sampling is performed. In an incorrect sampling the procedure is non- probabilistic and the sample is affected by the sampling process². The result of an incorrect sampling is a specimen not a sample.

Heterogeneity is important in TOS , and depends on the scale of scrutiny⁴. Solids materials can be evaluated at fragment level (individual unit, unalterable and indivisible), where each unit present variability in composition. Evaluation at the fragment level is called: constitutional heterogeneity¹. The evaluation of a group of fragments (composite sample) is called: distributional heterogeneity¹.

In solid materials a correct sampling procedure of a heterogeneous sample consists in taking sample increments from different parts of the material bulk which are chosen at random ⁵. The procedure used for a correct sampling in solid materials should not affect the sample; in addition all material should have the same probability to be sampled⁵. In non-representative sampling it is common to find high values in sampling bias and sampling variance (reproducibility).

Liquids, gas solutions and pure substance are “homogenous”, because all fragments or units are identical. The homogeneity in solutions is down to the molecular level⁶. A correct sampling procedure of a homogenous sample, consists in taking only one sample increment to determine the properties of the material bulk¹.

The implementation of TOS provides representative sampling, which is necessary for quality control ⁷. Measurement uncertainty for analytical methods is related to the variance of the sampling process (S_s^2) and the method of analysis (S_m^2).

$$S_0^2 = S_s^2 + S_m^2$$

For system with low heterogeneity the sampling error is near to zero, therefore the method error dominate the measurement uncertainty¹. Sampling error can be determined using NIRS to

collect spectral data fast and without sample preparation. The data analysis can be developed with multivariate tools through the development of a chemometric model.

TOS also include repeatability and reproducibility studies. Repeatability consist in repeat the runs under identical or similar conditions, analyzing the same materials¹¹. Repeatability study evaluates short term precision. Reproducibility consists in repeat the experiments using another technician, researcher and/or in another laboratory¹¹.

An estimation of total sampling error can also be done with replication experiments¹¹, which include all contributions of the sampling procedure¹¹. The replicated analytical results show the accuracy and precision of the analysts, methodologies and instruments used. The replication experiments are needed to validate a sampling methodology.

For determination of Total Sampling Error (TSE) + Total Analytical Error (TAE), replication experiments should be realized. The replication is done first in the primary sampling, which is repeated ten times using the same sampling protocol. The next sampling stage (secondary sampling state (first mass reduction), tertiary sampling state (laboratory mass reduction), aliquot preparation, and finally the analysis of the samples) also are replicated^{11, 12}. For example to do replication experiment for aliquot preparation consists in preparing ten aliquots of the sample. The variability of a group of replications is quantified by extracting and analyzing a number of replicate primary samples¹² thereby the replication experiment determines the different errors associated with sampling and analytical errors.

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CHAPTER 2 NEAR INFRARED SPECTROSCOPIC TRANSMISSION MEASUREMENTS FOR PHARMACEUTICAL POWDER MIXTURES

2.1 SUMMARY

This study describes the development of near infrared (NIR) calibration models using transmission measurements of powder samples. The results obtained were compared those obtained with calibration models developed for tablets and diffuse reflectance in powders. Transmission near infrared spectroscopy is a method widely used for the analysis of tablets in the evaluation of drug concentration due to the larger sample volume analyzed, but it is not commonly used for the analysis of powder samples. Diffuse reflectance near infrared spectroscopy is used in both powder and tablets for the evaluation of quality attributes. In this study NIR transmission measurements were obtained using Bruker Optics, Fourier Transform Near Infrared Spectrometer (FT-NIR). Spectra were obtained with three different resolutions for the analysis of powder and tablet samples of 7.50 to 22.50 % (w/w) acetaminophen. The Partial Least Squares (PLS) calibration models developed include pretreatments such as Standard Normal Variate (SNV) and the first derivative in the region from 9500 - 7500 cm^{-1} . Transmission in powder method presented low Root Mean Square Error of Prediction (RMSEP) values that varied from 0.23- 1.15 % (w/w) APAP with resolution of 64 and 16 cm^{-1} . The lowest RMSEP values (0.23-0.39 % (w/w) APAP) were obtained using resolution of 64 cm^{-1} . The RMSEP values for powder transmission measurements were 2.35 to 5.61 times lower than diffuse reflectance measurements of the powder mixtures.

2.2 MATERIALS AND METHODS

2.2.1 Materials

Two pharmaceutical excipients and one active pharmaceutical ingredient (API) were used in this study. The excipients were: lactose monohydrate (Granulac, Meggle Pharma), that passed through a U.S. Standard Sieve 60 (250 μm opening) before mixing, and microcrystalline cellulose (MCC) (Vivapur 102 provided by JRS Pharma). The API used was semi-fine acetaminophen (APAP) received from Mallinckrodt Inc. (Raleigh, NC).

2.2.2 Preparation of powder blends.

Blends of three components (APAP, MCC and lactose) were prepared in a concentration range of 7.50 % - 22.50 % (w/w) APAP as shown in Table 2.1. Eight calibration blends were used for development of the calibration models, each with a weight of approximately 100 g. The materials

were placed in a vibration blender for 15 min to break up agglomerates and then 30 minutes in a tumble blender. In addition a set of five validation blends (8.99, 13.00, 15.00, 17.01, 21.01 % (w/w) APAP) were used to evaluate the calibration models. The validation blends were prepared with the same mixing procedure used for the calibration blends as shown in Table 2.1.

	% (w/w) APAP	% (w/w) MCC	% (w/w) LACTOSE
Cal 1	7.50	30.00	62.50
Cal 2	7.50	90.00	2.50
Cal 3	7.50	60.00	32.50
Cal 4	14.00	63.50	22.50
Cal 5	15.00	30.00	55.00
Cal 6	16.25	83.75	0.00
Cal 7	22.50	77.50	0.00
Cal 8	22.50	30.00	47.50
Val 1	8.99	44.02	73.97
Val 2	13.00	31.00	69.81
Val 3	15.00	66.67	18.33
Val 4	17.01	39.74	59.55
Val 5	21.01	37.99	53.85

Table 2.1 Composition of calibration and validation blends used in study

The compositions shown in Table 2.1 for the calibration blends were chosen based on a D-optimal, linear model (MODDE 8.0.0.0 software from Umetrics (Umeå, Sweden)). The DOE for the calibration blends has a formulation of three components with 7.50 to 22.50 % (w/w) APAP; 0.00 to 62.50 % (w/w) lactose and 30.00 to 90.00 % (w/w) MCC.

The correlation coefficients between components are shown in Table 2.2. MCC and lactose shows a high negative correlation indicating that if the value of a component increases, the value of the other component decreases. APAP shows a weak relationship (values less than 0.5) with the other components in the blends. The low correlation coefficients between APAP and the excipients should contribute to the development of a robust calibration model.

	APAP	LACTOSE	MCC
APAP	1	-0.104	-0.096
LACTOSE	-0.104	1	-0.980
MCC	-0.096	-0.980	1

Table 2.2 Correlation coefficients between APAP, MCC, and lactose in the blends

2.2.3 Tablet preparation

Tablets were prepared using a model C Carver laboratory press, manually operated. The compaction pressure was of 68.95 Mpa and a total of four tablets per concentration were obtained. The blends used for tablet preparation are described in section 2.2.2. The tablets prepared have an average weight of approximately 0.50 g. Tablet thickness was determined using an electronic digital micrometer MARATHON CO030025 that operates from $0-25 \pm 0.001$ mm. The average thickness measured was of 2.830 ± 0.024 mm.

2.2.4 Spectral acquisition for transmission in powder

Spectra were acquired with a Bruker Optics (Billerica, MA) Multi-Purpose Analyzer (MPA), Fourier Transform Near Infrared Spectrometer (FT-NIR), equipped with an RT-InGaAs detector. The OPUS® software package (version 7.2) Build: 7, 2, Bruker Optics, Germany) was used to control the spectrometer. A glass cell was placed at the bottom of the tablet holder as shown in Figure 2.1 for the transmission measurements of powder. The mass of powder placed in the holder was approximately of 0.30 g. Each spectrum was the average of 128 scans over the range of $12500-5800 \text{ cm}^{-1}$, with a resolution of 64, 32 or 16 cm^{-1} . NIR reference and background single beam spectra were obtained with an NG9 filter. This is an internal filter within the Bruker system and is selected with the Opus software. Ten aliquots of each blend were evaluated and six spectra obtained for each aliquot.

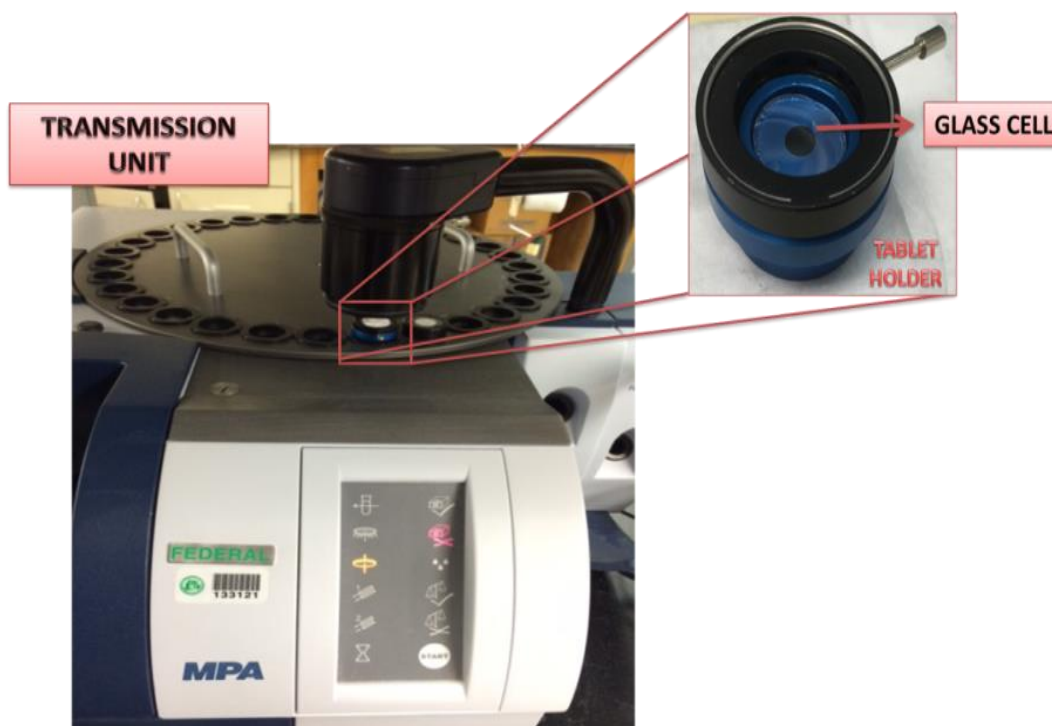


Figure 2.1 Setting for transmission measurements in sample powder

Deposition methods of powder sample to tablet holder

Figure 2.2 shows the two deposition methods. The first method consisted of using a stainless steel laboratory spatula to fill the tablet holder with the powder to complete a weight between approximately 0.30g. All calibration blends and validation blends were sampled with this method except the validation blend of 15 % (w/w) APAP. The second deposition method (in-house developed feeder) was used in a validation blend of 15 % (w/w) APAP. The powder sample flowed through the feeder according to the movement of a screw until the last sample was collected.

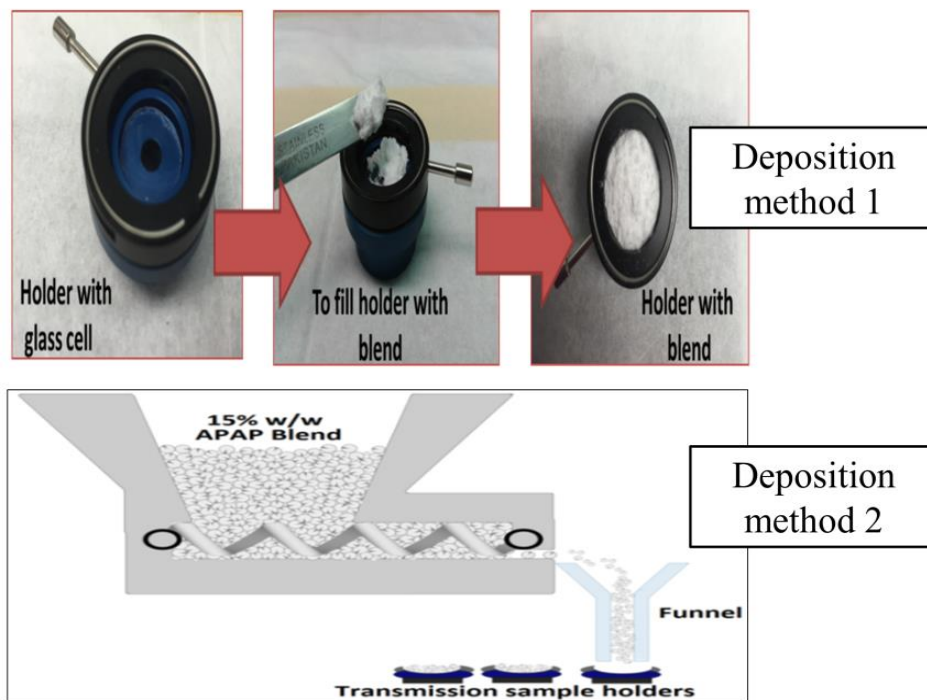


Figure 2.2 Deposition methods to place the powder within the tablet holder

2.2.5 Spectral acquisition for transmission in tablets

Spectra were acquired with the same instrument used for transmission in powder (Section 2.2.4.1). The tablet was placed in a tablet holder (Bruker Optics, IN103-I1) and three spectra were obtained without moving the tablet. Each spectrum was the average of 128 scans over the range of $12500\text{--}5800\text{ cm}^{-1}$, with a resolution of 16 cm^{-1} . The internal NG9 filter was used during the data collection.

2.2.6 Development of calibration models

Partial least squares (PLS) calibration models were developed using SIMCA v. 13.0.3. The standard normal variate (SNV) transformation was used to reduce the multiplicative and additive effects of scattering on the spectra. First derivative spectra were obtained by using the Savitzky–Golay algorithm with a 15-point segment size and a second-order polynomial.

The effectiveness of the calibration was evaluated on the basis of the bias (average of residuals between NIR predictions and reference value) and the Root Mean Square Error (RMSEP) for both leave class out cross-validation and validation samples¹.

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

Where \hat{y}_i corresponds to the predicted drug concentration and y_i is the reference drug concentration in each sample, and finally n is the number of samples predicted.

The error was also estimated in terms of relative standard error of prediction:

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

Calibration models were built from the transmission spectra of pharmaceutical powder blends over a concentration range of 7.50 - 22.50 % (w/w) of the API. Calibration models were developed with spectra of different resolutions (64, 32, 16 cm^{-1}) and using three different spectral regions (11000-7500; 10500-7500 and 9500 -7500 cm^{-1}).

A PLS calibration model was also built from the transmission spectra of tablets over a concentration range from 7.50 -22.50 % (w/w) API. The calibration model was developed using training sets composed for 32 tablets. SNV followed by first derivative with 15 point segment size and the spectral region of 9500 – 7500 cm^{-1} was used for developing of the calibration model.

2.3 RESULTS AND DISCUSSION

2.3.1 Spectra analysis

Figure 2.3 shows the NIR transmission spectra for powders and tablets of 15.00 % (w/w) APAP and the spectrum of 100 % (w/w) APAP. Significant noise is observed in the region of 7000-6000 cm^{-1} because of the small amount of radiation reaching the detector; therefore this

region was not used to develop calibration models. The limited region for transmission measurements has been discussed in several previous studies²⁻³.

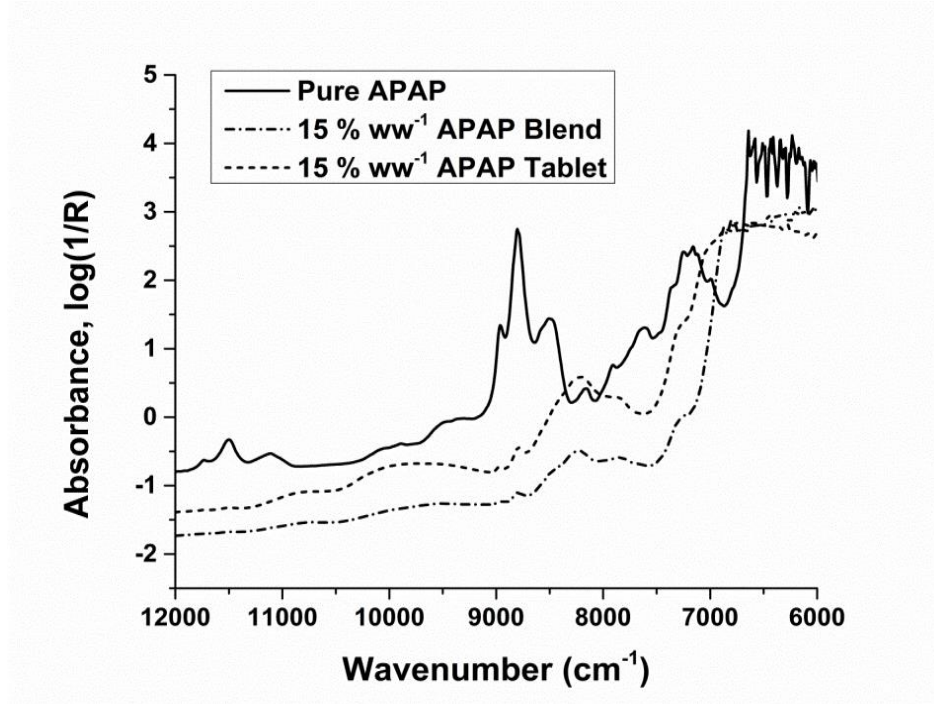


Figure 2.3 NIR transmission spectra for powder and tablet sample of 15.0 % (w/w) APAP and the spectrum of 100 % (w/w) APAP.

Figure 2.3 shows that transmission spectra in powder and tablets present a baseline that varies in absorbance between -1 and -2. This condition can be attributed to the intensity of the radiation that reaches the detector when the sample and the reference are analyzed. The baseline is negative if the intensity of radiation received by the detector from the sample is higher than the intensity of radiation received when the reference is measured. The absorbance value of - 1.7 indicates that the intensity of radiation received by the detector (I_{det}) is approximately 50 times higher than the reference (I_0) as shown below:

$$A_{detected} = -\log(R_{detected}) = -\log\left(\frac{I_{det}}{I_0}\right) = -\log\left(\frac{1}{c}\right)\left(\frac{I_{refl}}{I_0}\right) = \log c + A \quad (3)$$

Figure 2.4 shows the near infrared spectra in transmission mode of a 15.00 % (w/w) APAP blend and the tablet spectra collected. The transmission spectra in powders (Figure 2.4a) present differences in baseline for each spectrum since the particles in the blends are dispersed. The baseline difference in the transmission spectra in tablets is minimal due to the compaction of the powder (Figure 2.4b). The difference in baseline can be explained by the additive effect in scattering, where the light intensity detected by the detector of the NIR spectrometer is equivalent to the fraction of the reflected radiation⁴. The change in baseline shows that the fraction of the radiation that reaches the detector is not always the same way as indicated by equation (3).

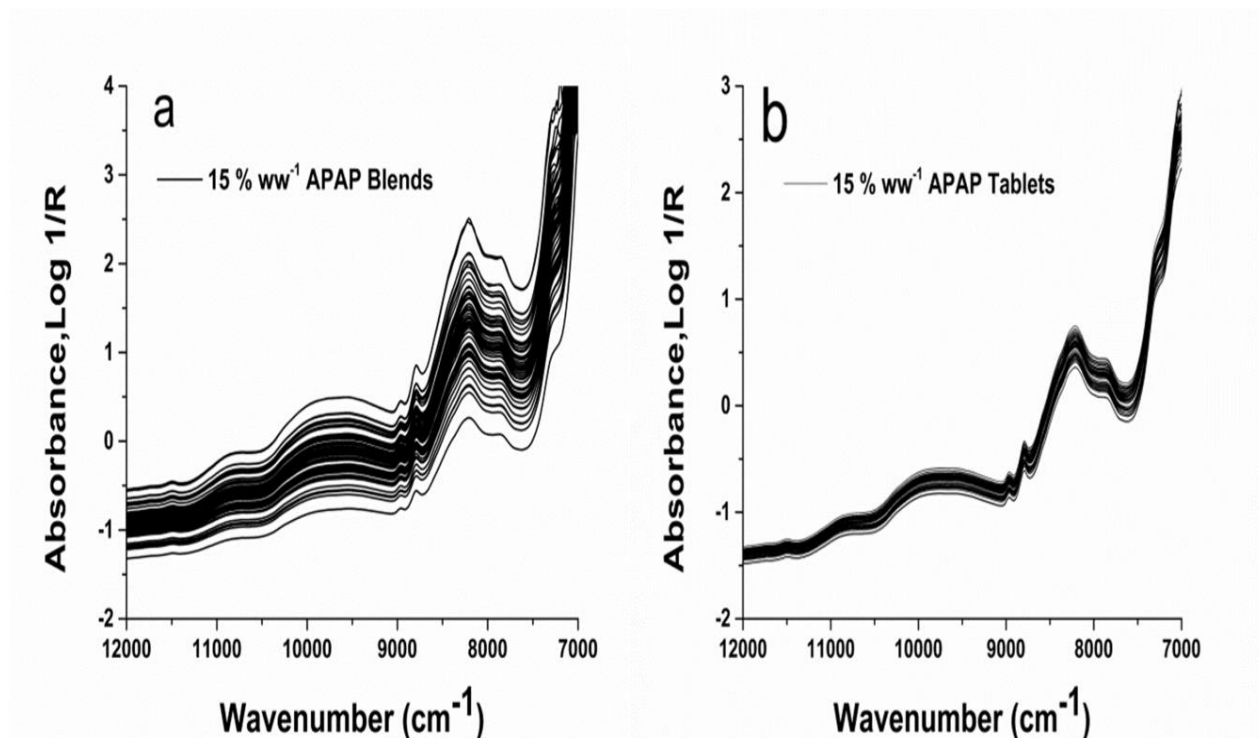


Figure 2.4 a. Transmission spectra of 15 % ww-1APAP blend to resolution 32 cm⁻¹. b. Transmission spectra of 15 % ww-1APAP tablet to resolution 16 cm-1.

APAP shows weak bands of 11876 - 10903 cm⁻¹, and stronger bands in the spectral region of 9193 - 8090 cm⁻¹, as shown in the top spectrum in Figure 2.3. The spectra of 15.00 % (w/w) APAP tablet and 15.00 % (w/w) APAP powder blend in Figure 2.3 (short dash line and dash dot

line respectively) presents one weak absorption band in the region from 9100 - 8500 cm^{-1} , which corresponds to the absorption band of APAP. The calibration models were developed to include the spectral range of API bands.

Figure 2.5 presents powder and tablet spectra with concentrations of 7.5, 15.0, and 22.5 % (w/w) APAP. The figure 2.5a and 2.5b shows the different spectra obtained. Figure 2.5b shows in the spectral region of 9100- 8500 cm^{-1} that if crease the concentration of APAP in the tablets, the absorbance increased.

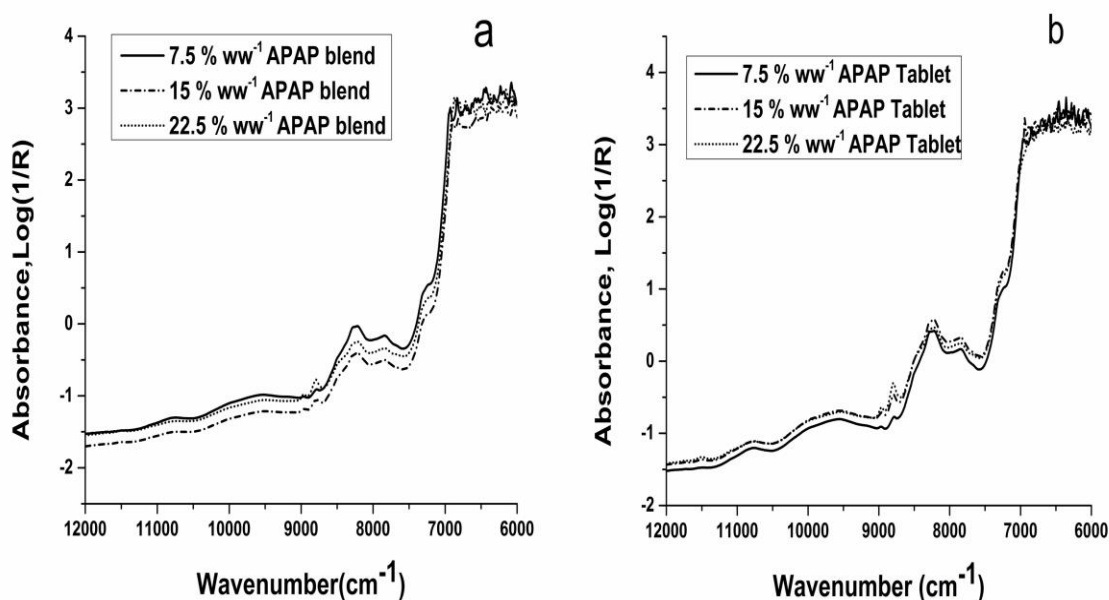


Figure 2.5 Powder (a) and tablet (b) spectra with concentrations of 7.5, 15.0, and 22.5 % (w/w) APAP

Figure 2.6 shows transmission spectra of powder blends and tablets in the spectral region with bands characteristic of the acetaminophen. Standard normal variate (SNV) and first derivative with 15 point segment size were applied.

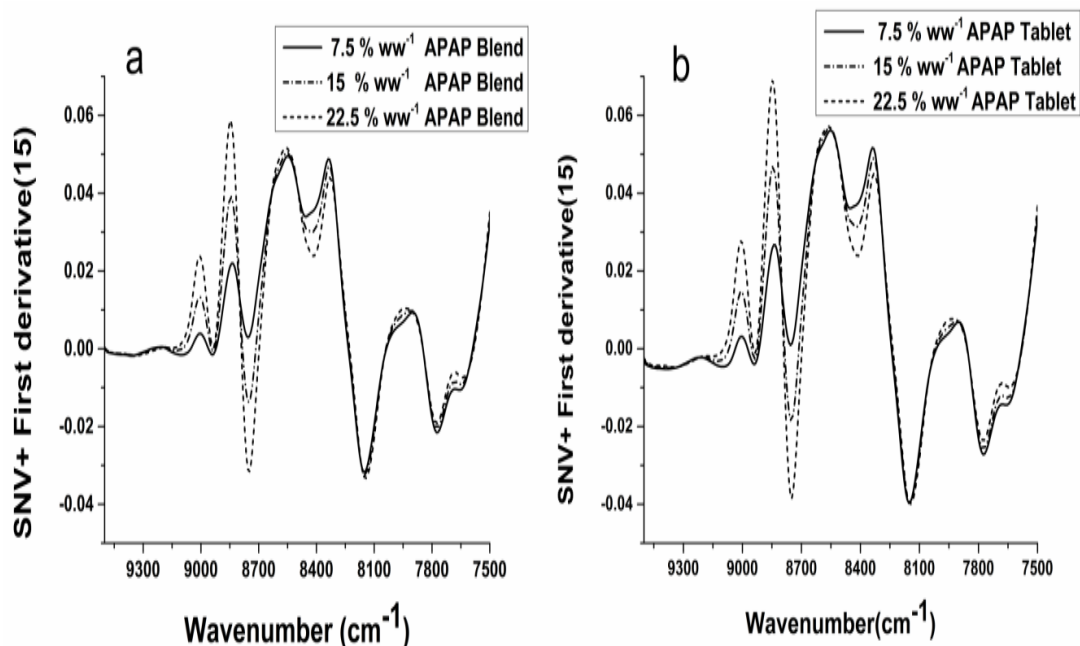


Figure 2.6 Transmission spectra of blends and tablets after SNV and first derivative with 15 point size in the spectral region of 9500 - 7500 cm⁻¹.

Figure 2.7 shows transmission spectra of powder blends with 15.00 % (w/w) APAP (Fig. 2.7a) and spectra with SNV and first derivative of powder blends with 15.00 % (w/w) APAP (Fig. 2.7b) obtained with different resolutions. Figure 2.7a shows significant differences between the three spectra with different resolutions. Figure 2.7a shows that with an increase of resolution more spectral features can be seen in the spectra; in addition more spectral noise is detected. The evaluation of different spectral resolutions was important since a future real time transmission method would likely require a low resolution to obtain spectra in less time and with an improved signal to noise ratio.

Figure 2.7b shows that the spectrum of 16 cm⁻¹ resolution presents one small absorption band associated with acetaminophen in the region of wavenumber from 9000 - 8900 cm⁻¹; This band is lost in the spectrum with 64 cm⁻¹ resolution. The SNV and first derivative spectrum with 64 cm⁻¹ resolution shown in Fig. 2.7b, presents two bands defined in the region of 9500 -7500 cm⁻¹. In the same spectral region the spectrum with 16 cm⁻¹ resolution shows a greater number of bands. The progressive increase of resolution from 64 to 16 cm⁻¹ leads to a greater number of APAP bands.

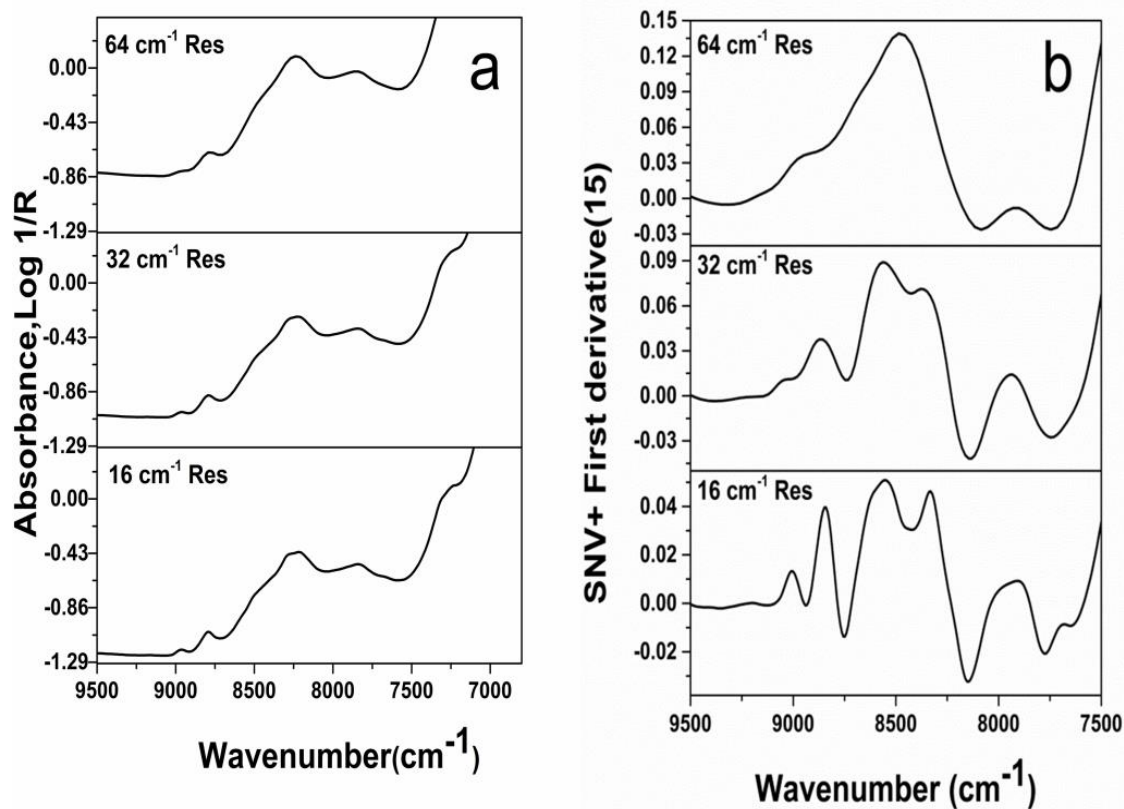


Figure 2.7 a. Transmission spectra of blends with 15 % (w/w) APAP, different resolutions were used. b. Transmission spectra of blends after SNV and first derivative with 15 point size in the spectral region of 9500 to 7500 to cm-1 with different resolution

2.3.2 Calibration models for transmission spectra of powder blends

PLS calibration models were developed to determine drug concentration in validation blends. Different spectral regions, pretreatments, resolutions and validation sets of different levels of APAP concentration were analyzed. Table 2.3 presents the predictions of four validation sets with concentration levels of 8.99, 13.00, 17.01, and 21.01 % (w/w) APAP. A total of nine calibration models were developed: three models using spectra with resolution of 64 cm⁻¹, SNV+1st-der (15), and spectral regions of 9500-7500 cm⁻¹, 10500 – 7500 cm⁻¹ and 11000 – 7500 cm⁻¹; three models using spectra with resolution of 32 cm⁻¹ and three with 16 cm⁻¹. These last six models also were developed with the same pretreatments and spectral regions mentioned previously. The three calibration models developed with spectra of

resolution 64 cm^{-1} showed the lowest root mean square error of prediction values, the next lowest RMSEP values were obtained with the three calibration models developed with spectra of resolution 16 cm^{-1} (Table 2.3). The best calibration models were obtained using the spectral region from $9500 - 7500\text{ cm}^{-1}$, which includes a number of APAP absorption bands (Table 2.3).

Prediction of APAP Validation Blends, General results						
Data Pretreatment	Spectral Range (cm^{-1})	Resolution (Res) (cm^{-1})	PLS Factors	RMSEP	RSEP (%)	Bias
SNV+1st-der (15), N=40	9500-7500	64	2	0.51	3.25	-0.09
		32	2	1.04	6.63	-0.28
		16	2	0.77	4.93	0.10
	10500 – 7500	64	3	0.40	2.54	0.10
		32	2	1.14	7.27	-0.27
		16	3	0.83	5.32	0.07
	11000 – 7500	64	2	0.63	4.05	0.06
		32	2	1.14	7.27	-0.26
		16	3	0.80	5.14	0.11

Table 2.3 Standard errors; PLS factors and bias values of the calibration models evaluated using different regions and pretreatments. A total of 40 validation blend samples were analyzed, ten samples at each of the four concentration levels.

Table 2.4 shows the results of the three calibration models developed with the best spectral region ($9500 - 7500\text{ cm}^{-1}$) evaluated. The unique difference between the three calibration models is the use of spectra with different resolutions, the pretreatments and the spectral region are the same. Table 2.4 shows the individual evaluation of four different blends, each blend with ten sub-samples. The lowest RMSEP values were obtained with the validation blends evaluated in the calibration model with the spectra of 64 cm^{-1} resolution. The relative standard errors of prediction (RSEP) values were less than five percent in all validation sets with 64 cm^{-1} resolution.

Prediction of APAP Powder Blends							
Data Pretreatment	Spectra Range	Res (cm ⁻¹)	PLS Factors	Validation sets % (w/w) APAP n=10	RMSEP	RSEP (%)	Bias
SNV+1 st der (15)	9500-7500 (cm ⁻¹)	64	2	8.99	0.23	2.57	0.07
				13.00	0.39	2.97	0.3
				17.01	0.25	1.49	0.13
				21.01	0.32	1.52	-0.23
		32	2	8.99	1.11	12.39	1.07
				13.00	0.24	1.86	0.12
				17.01	0.81	4.75	-0.77
				21.01	1.53	7.3	-1.52
		16	2	8.99	1.15	12.84	1.15
				13.00	0.30	2.29	0.25
				17.01	0.40	2.34	-0.37
				21.01	0.90	4.27	-0.64

Table 2.4 Standard errors, PLS factors, bias values of the calibration model evaluated using 64, 32 and 16 cm⁻¹ of resolution and SNV + First derivative (15) (9500 – 7500 cm⁻¹) as pretreatments.

2.3.3 Repeatability study

A repeatability study was performed taking six consecutive spectra for each sample analyzed without moving the powder holder. The spectra were used to predict APAP concentration with the calibration models developed. Repeatability was determined to evaluate the short-term precision using the same operational conditions⁵. The values presented correspond to the standard deviation of the predictions (Std) obtained using a validation set of 8.99 % (w/w) APAP and evaluating ten samples. Table 2.5 shows the repeatability results in three different resolutions evaluated (16, 32 and 64 cm⁻¹). Table 2.5 presents low standard deviations (high precision) from 0.001 to 0.013 for the three spectral resolutions evaluated.

Repeatability Study								
16 cm ⁻¹ Res n= 10			32 cm ⁻¹ Res n = 10			64 cm ⁻¹ Res n = 10		
Std Ave	Std Max	Std Min	Std Ave	Std Max	Std Min	Std Ave	Std Max	Std Min
0.003	0.007	0.001	0.004	0.007	0.002	0.006	0.013	0.002

Table 2.5 Standard deviations observed in repeatability study at the three spectral resolutions evaluated.

2.3.4 Comparison of powders and tablet transmission results

The calibration models developed with spectra of 64 cm⁻¹ resolution (low resolution), have less spectral noise and are acquired in less time. The low spectral noise in the spectra contributed to development of calibration models with a good predictive capacity. The calibration models developed with spectra of 16 cm⁻¹ resolution (high resolution) have more spectral information and are more robust models. In this study the calibration models development with spectra of resolution 16 cm⁻¹ and SNV+1st-der (15) in the spectral regions of 9500-7500 cm⁻¹ was selected to do the comparison between transmission in powder and tablets. Calibration models developed with spectra of 16 cm⁻¹ resolution present more spectral bands.

Blend concentration results were compared to the results of a PLS calibration model developed using tablets to determine drug concentration (% (w/w) APAP). Tablets were prepared with blends with the same concentration range used for the transmission powder spectra. The two models were evaluated using the four validation sets shown in Table 2.6.

Each validation set evaluated in the calibration model of tablets was composed of four tablets. Each validation set for the powder samples was composed of ten aliquots of the blend or subsample. A spatula was used to deposit the powder sample in the tablet holder for subsequent analysis (Deposition method 1). Table 2.6 shows RMSEP and RSEP values for four validation sets evaluated in transmission in powder and tablet. The largest RMSEP and RSEP values observed in transmission in powder was found for the lowest concentration (8.99 % (w/w)APAP) of the validation sets, the respective values were 1.15 and 12.84 % and the lowest RMSEP value

observed when the concentration was evaluated at 13 % (w/w) APAP of the validation sets. The RSEP values were less than 5% for all concentration except for the validation set with concentration of 8.99 % (w/w) APAP. The largest RMSEP value (1.14) observed in transmission in tablet correspond to the validation set with the highest concentration (21.01 % (w/w) APAP). The lowest RMSEP value observed was found with the concentration 13 % (w/w) APAP of the validation sets.

Validation sets.	Model 7.5-22.5 % (w/w) APAP, 16 cm ⁻¹ Res.									
	TR powder, off-line Bruker MPA					TR tablets, off-line Bruker MPA				
	SNV + 1 st der (15) (7500-9500 cm ⁻¹), 2 PLS factor					SNV + 1 st der (15) (7500-9500 cm ⁻¹), 2 PLS factor				
Ref % (w/w) APAP	N	Avg	RMSEP	RSEP (%)	Bias	N	Avg	RMSEP	RSEP (%)	Bias
8.99	10	10.14	1.15	12.84	1.15	4	9.93	0.95	10.56	0.94
13.00	10	13.25	0.30	2.29	0.25	4	13.21	0.21	1.63	0.21
17.01	10	16.64	0.40	2.34	-0.37	4	16.42	0.59	3.49	-0.59
21.01	10	20.37	0.90	4.27	-0.64	4	19.88	1.14	5.41	-1.13

Table 2.6 Comparison between transmission in powder and tablet using different validations set

2.3.5 Comparison between the calibration models of powders and tablets developed to 64, 32 and 16 cm⁻¹ of resolution.

Using one validation set of 15 % (w/w) APAP and the feeder deposition method the results of transmission in powder and tablets were compared at different resolutions. Transmissions in powder presented the lowest prediction errors for the three different resolutions (Table 2.7)

Parameters		Model 7.5-22.5 % (w/w) APAP.									
		TR powder, off-line Bruker MPA					TR tablets, off-line Bruker MPA				
		SNV + 1 st der (15) (7500-9500 cm ⁻¹)					SNV + 1 st der (15) (7500-9500 cm ⁻¹)				
Res (cm ⁻¹)	N	PLS Factors	Avg	RMSEP	RSEP (%)	Bias	PLS Factors	Avg	RMSEP	RSEP (%)	Bias
64	60	3	15.17	0.26	1.73	-0.17	2	15.06	0.53	3.55	-0.06
32	60	2	14.54	0.49	3.27	0.46	2	14.91	0.58	3.85	0.09
16	60	2	15.15	0.31	2.08	0.15	2	14.66	0.48	3.17	-0.45

Table 2.7 Comparison between transmission in powder and tablet using different resolutions and one 15 % (w/w) APAP validation set.

2.3.6 Comparison of an experimental mean with a known value

Table 2.8 shows the comparison between the experimental mean and the target value of the formulation (15.0 % (w/w) APAP). The statistical comparison was performed for a prediction set of 60 powder samples and a second prediction set of 60 tablets at three different resolutions using transmission mode.

Parameters		Transmission in powder				Transmission in tablet			
Res (cm ⁻¹)	N	Mean	Std	t _{value} calculated	Confidence interval	Mean	Std	t _{value} calculated	Confidence Interval
64	60	15.17	0.20	6.58	15.17 ± 0.05	15.06	0.53	0.88	15.06 ± 0.13
32	60	14.54	0.17	20.96	14.54 ± 0.04	14.91	0.57	1.22	14.91 ± 0.14
16	60	15.15	0.27	4.30	15.15 ± 0.07	14.66	0.29	9.08	14.66 ± 0.07

Table 2.8 Comparison between an experimental mean with a known value

The null hypothesis adopted considers that the analytical method is not subject to systematic error and considering the t critical value and the t calculated value it is possible to compare the experimental mean and a known value. For this statistical comparison there are 59 degrees of freedom, as ($P = 0.05$) and the value critical of $t_{critical}$ is 1.96. As the calculated value of t (see table 2.8) is greater than the value critical of $t_{critical}$ in transmission in powder for all resolutions, the null hypothesis is rejected indicating a systematic error. For tablet the calculated value of t (see table 2.8) is lower than the value critical of $t_{critical}$ for 64 and 32 cm⁻¹ resolution, the null

hypothesis is accepted, at 16 cm^{-1} resolution indicating a systematic error. Table 2.8 shows that the lowest confidence interval was observed for transmission in powder at all resolutions evaluated and for transmission in tablet at only one resolution (16 cm^{-1}).

2.3.7 Comparison between transmission and diffuse reflectance in powder

NIR transmission and diffuse reflectance measurements for powders were compared. The diffuse reflectance powder measurements were from a previous study with calibration models developed for blends from 7.50 to 22.50 % (w/w) APAP^{6,7}. The diffuse reflectance spectra were collected using a Bruker Optics (Billerica, MA) Matrix Fourier Transform (FT)-NIR spectrometer as the powder moved to velocity linearity of 10 mm/s, the work resolution was 8 cm^{-1} . Different validation sets were evaluated in the calibration model and RMSEP determined^{6,7}. Table 2.9 shows the comparison between transmission and diffuse reflectance measurements. This study shows that the RMSEP values for diffuse reflectance measurements are between 2.35 to 5.61 times higher than transmission measurement. Transmission present better predictions than diffuse reflectance because of the larger sample mass analyzed.

COMPARISON BETWEEN TRANSMISSION IN POWDERS AND DIFUSSE REFLECTANCE IN POWDER								
	Description	Model 7.5-22.5 % (w/w) APAP	Test set Spectra #	Avg	Bias	RMSEP	RSEP (%)	Std
TR powder	off-line Bruker MPA	SNV + 1 st der (15) (7500-9500 cm^{-1}), 2 PLS factor	TR ; n=60	15.15	0.15	0.31	2.08	0.27
DR Powder	In-line Bruker Matrix	SNV + 1 st der (17) (5000-9100 cm^{-1}), 2 PLS factor	T _S 1;n=68	15.58	0.58	0.73	4.89	0.46
			T _S 2;n=390	14.93	-0.07	0.78	5.19	0.78
			T _S 3;n=390	16.39	1.39	2.10	14.02	1.58
			T _S 4;n=258	15.17	0.17	1.74	11.63	1.74
			All test sets ;n=1106	15.54	0.54	1.59	10.58	1.49

Table 2.9 Comparison between transmissions in powder and diffuse reflectance in powder

2.4 CONCLUSION

The transmission spectra of powders and tablets are very similar. The most remarkable difference is the change in baseline due to the scattering of radiation by the particles in the blend and the minimum baseline differences in tablet spectra.

The calibration models developed for transmission in powder present a good predictive capacity, especially the calibration models developed with spectra of resolution 64 and 16 cm^{-1} . The promising results obtained in this study indicate that a real time method could be developed for powders in the future. However, sample deposition to the transmission compartment would have to be improved or a more suitable design developed.

2.5 REFERENCE

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CHAPTER 3 THEORY OF SAMPLING (TOS) AND PHARMACEUTICAL INDUSTRY QUALITY CONTROL (QC) PRACTICES

Overview

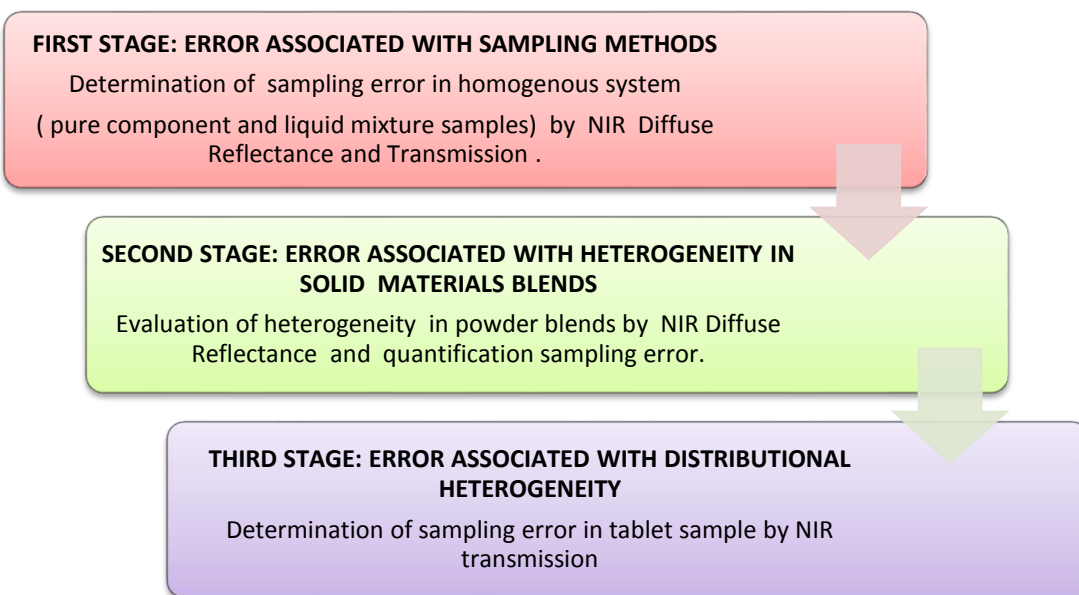
Sampling is a determinant stage in every analytical process. The objective of sampling is obtaining a representative sample of a lot or population. The Theory of Sampling (TOS) specifies that the complexity of sampling depends on the heterogeneity of the sample¹⁵. The most important factor in the sampling procedure is reducing the risk of introducing a systematic error in the sampling procedure. This research is focused in the implementation of NIR spectroscopy in the development of sampling theory. Two approaches were established in this research. The first approach is to determine the sampling error using Near Infrared Spectroscopy (NIRS) and evaluate the influence of material heterogeneity on NIR results (part a). The second approach is development a stream sampling method to facilitate implementation of variographic analysis and use of replication experiments in the development of pharmaceutical formulations (part b).

a) A STUDY OF SAMPLING ERROR IN NIR SPECTROSCOPY

3.1 SUMMARY

This research involves the development of an effective method capable of measuring sampling error in NIR spectroscopy and demonstrates the difference in the sampling error between materials of different heterogeneity levels. The basis for adequate sampling is a proper understanding of the phenomenon of material heterogeneity. Heterogeneous materials present differences in the observable fragments with respect to composition, size, density, shape. The methods developed to determine the sampling error using NIR Spectroscopy constitute an opportunity to thoroughly understand the uncertainty of analytical methods. In this study near infrared spectra were obtained in transmission and diffuse reflectance mode. Partial least square calibration models were developed for pure component, liquid mixtures (cyclohexane, decane

and heptane mixture), powder blends and tablets that ranged from 0-100 % (w/w) of Acetaminophen (APAP), microcrystalline cellulose (MCC) and lactose. Sampling error is contributes to deviations in the accuracy and precision of the methods. Three experimental stages were designed for determine and evaluate the sampling error (Scheme 3.1).



Scheme 3.1: Stages for evaluation of sampling error in NIR spectroscopy

3.2 MATERIALS AND METHODS

3.2.1 Materials

This work was performed with liquid and solid materials. The solid materials are: acetaminophen (APAP) used as API and received from Mallinckrodt Inc. (Raleigh, NC), lactose monohydrate (Granulac, Meggle Pharma), and microcrystalline cellulose (Vivapur 102 provided by JRS Pharma) used as excipients in the blends prepared. The liquids materials are: cyclohexane, heptane and decane .

3.2.2 Preparations of powder and liquids blends.

3.2.3 Powder Blends

Blends of three components (APAP, MCC and lactose) in concentration range of 0 - 100 % (w/w) APAP were prepared as shown in Table 3.1. The 15 calibration blends, each with a weight

of approximately 100 g were placed in a tumble blender for 30 min. The validation blends in total four (Table 3.1) were prepared with the same mixing procedure used for the calibration blends.

3.2.4 Liquids mixture

Blends of three components (cyclohexane, decane and heptane) in concentration range of 0 - 100 % (w/w) cyclohexane were prepared as shown in Table 3.1. The blends weights were of approximately 3 g, each blend was placed in a vial, followed of agitation for homogenization. The validation blends in total four (Table 3.1) were prepared with the same mixing procedure used for the calibration blends.

	% (w/w) APAP/ cyclohexane	% (w/w) MCC/ heptane	% (w/w) lactose/ decane	TOTAL
Mix 1	100	0	0	100
Mix 2	75	25	0	100
Mix 3	75	0	25	100
Mix 4	50	50	0	100
Mix 5	50	25	25	100
Mix 6	50	0	50	100
Mix 7	25	75	0	100
Mix 8	25	50	25	100
Mix 9	25	25	50	100
Mix 10	25	0	75	100
Mix 11	0	100	0	100
Mix 12	0	75	25	100
Mix 13	0	50	50	100
Mix 14	0	25	50	100
Mix 15	0	0	100	100
Val 1	66.25	16.87	16.88	100
Val 2	33.19	33.26	33.55	100
Val 3	16.78	16.72	66.49	100
Val 4	16.69	66.41	16.90	100

Table 3.1 Calibration sample, Five concentration levels are being evaluated (0, 25, 50, 75,100 % (w/w) respect to each components) and validation sample.

3.2.5 Tablet preparation

Tablets were prepared using a Carver laboratory presses model C, manually operated; 0- 165.47 Mpa Analog pressure gauge. The blends used for tablet preparation are described in section 5.2. The compaction pressure was of 68.95 Mpa and a total of 20 tablets per concentration were obtained. The tablets prepared have an average weight of 0.5g approximately. Thickness was determined using an electronic digital micrometer MARATHON that operates from 0-25 ± 0.001 mm, the average thickness measured was of 3.128 mm.

3.2.6 Spectral acquisition

3.2.6.1 NIR diffuse reflectance measurements in powder sample

Spectra were acquired with a Bruker Optics Multi-Purpose Analyzer (MPA), Fourier Transform Near Infrared Spectrometer (FT-NIR), equipped with an RT-InGaAs detector. Each spectrum was the average of 64 scans over the range of 12500-5800 cm^{-1} , with a resolution of 8 cm^{-1} . Diffuse reflectance spectra were collected by fiber optic probe at ten different points, six spectra for each point in the mixture were taking.

3.2.6.2 NIR transmission measurements in liquid sample

Spectra were acquired with a Bruker Optics Multi-Purpose Analyzer (MPA), Fourier Transform Near Infrared Spectrometer (FT-NIR), equipped with an RT-InGaAs detector. NIR transmission spectra were collected within the sample compartment unit of the instrument (Fig 1.3). Three spectra for each sample were obtained with 8 cm^{-1} of resolution, 64 sample scan time and 128 background scans; three spectra for each liquid sample were taking.

3.2.6.3 NIR transmission measurements transmission in tablets

Spectra were acquired with a Bruker Optics Multi-Purpose Analyzer (MPA). The tablet was placed in a fitting tablet holder and three consecutive spectra were obtained without moving the tablet. Each spectrum was the average of 128 scans over the range of 12500-5800 cm^{-1} , with a resolution of 16 cm^{-1} . A NG9 filter was used for both reference and background single beam spectra.

3.3 RESULTS AND DISCUSSION

3.3.1 First stage (Scheme 3.1): Determination of sampling error in “homogenous system” (liquid mixture and pure components) and evaluation of the uncertainty of measurements.

Development of this approach involves the use Near Infrared technology in transmission and diffuse reflectance mode. Partial least square (PLS) calibration model was constructed to obtain the concentration of mixture components. PLS calibrations are developed based by developing a mathematical relationship between the variations of the NIR spectra and acetaminophen (APAP) and cyclohexane content in the sample.

3.3.1.1 Liquid sample analysis by NIR transmission

The first evaluation consisted in the analysis of liquid samples by transmission sampling method. Liquid mixtures are considered homogeneous because they present only one phase. The sampling process in homogeneous materials does not contribute in the error propagation in the chemical analysis.

Mixtures with known composition of cyclohexane, decane and heptane were studied. Five concentration levels were evaluated (0, 25, 50, 75, 100 % (w/w) respect to each component). FT-NIR spectra of fifteen calibration mixtures were obtained and four unknown samples (Table 3.1). Figure 3.1 show transmission spectra for the liquid sample. The spectra do not present significant change in the baseline. In the $9000\text{-}8000\text{ cm}^{-1}$ and $5500\text{-}4500\text{ cm}^{-1}$ regions there is a relation proportional between concentration of cyclohexane and absorbance.

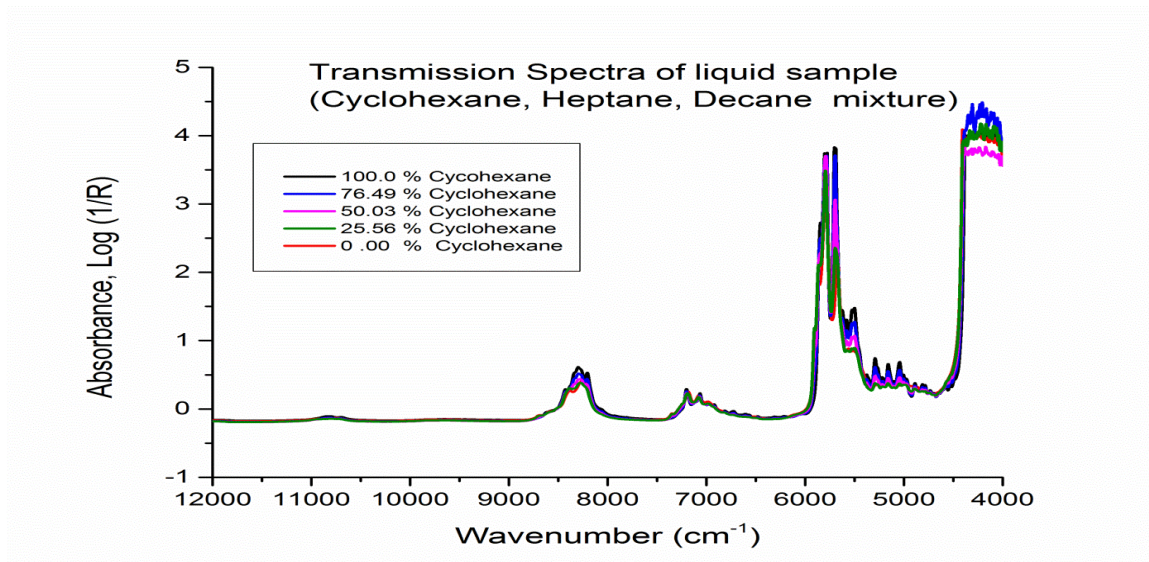


Figure 3.1 Transmission spectra obtained of the alkanes mixture at five concentration levels of cyclohexane.

Data preprocessing consisted of using mean centering in the 5403-4493 cm^{-1} region. Principal component analysis was performed with the pretreated spectra (Figure 3.2). PCA is a multivariate approach that allows the understanding of the variation in the spectra of the samples¹⁰. Figure 3.2 shows that the main source of variation in the data is due to different cyclohexane concentration levels in each sample.

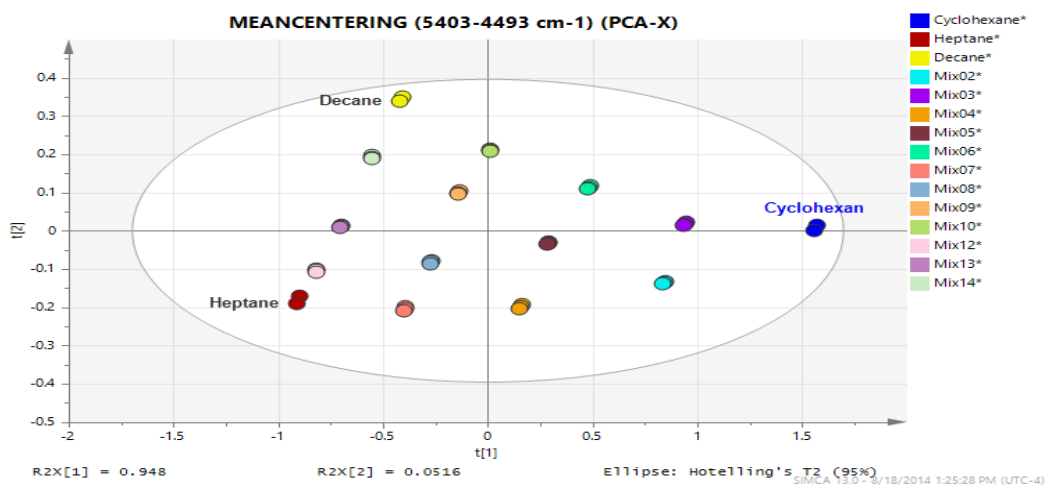


Figure 3.2 PCA Score plot show the distribution of the liquid samples within pure components

Partial least square calibration model was done with fifteen calibration samples (Table3.2). Predictions of independents samples (validation set) were done. The Root Mean Square Error of Prediction (RMSEP) was calculated (Table 3.2). The RMSEP was low in all validation sets evaluated due to the homogeneity of the sample.

PLS Calibration model for cyclohexane in liquid sample 0-100 % (w/w) cyclohexane				
NIR Transmission sampling method,				
Validation sets.	Mean centering (5403-4493 cm⁻¹), 4 PLS Factors			
Ref. % (w/w) Cyclohexane	Avg	RMSEP	Bias	Std
63.50	63.31	0.19	-0.19	0.05
34.08	33.65	0.44	-0.43	0.11
17.62	17.46	0.17	-0.16	0.08
16.77	17.02	0.27	0.25	0.14

Table 3.2 Results for prediction of Cyclohexane content in independent samples.

3.3.1.2 Pure component and blends with high concentration level of APAP analysis by NIR diffuse reflectance

Pure components were analyzed by NIR diffuse reflectance sampling method as examples of homogeneous samples. Pure components are homogenous materials since they present units strictly identical to each other; the sampling process in this type of material is easy. The drug distribution in binary blends of acetaminophen and lactose with high concentration level of API (89-100 % (w/w) Acetaminophen) will also be studied. Six blends calibration containing 89.70, 91.56, 93.58, 95.75, 97.73 and 100.00% (w/w) APAP were prepared (Table 3.3). The method was also challenged with six validation blends.

Experiment design matrix		
Calibration sample	Components (%w/w)	
	APAP	LACTOSE
Mix1	89.697	10.303
Mix2	91.564	8.436
Mix3	93.577	6.423
Mix4	95.749	4.251
Mix5	97.734	2.266
Mix6	100	0

Table 3.3 Experimental design matrix for binary blends with high concentration level of API.

Diffuse reflectance spectra were collected with a fiber optics probe. Fifty spectra of each sample were obtained in line using a conveyor belt set to 6.7 mm/s. The operating conditions of the instrument were 16 cm⁻¹ resolution, 16 scans were averaged and 64 background scans. Data preprocessing was made in the spectral data using mean centering in the region of wavelength 6800.2 - 6098.2 cm⁻¹.

Partial least square (PLS) calibration models were developed and used to predict the concentration of validation samples. The sampling error was determined in six validation sample. RMSEP values obtained were low in all concentration level evaluate. The sample with 100 % (w/w) APAP has the lowest sampling error. (Table 3.4)

PLS Calibration model for APAP in blends with high concentration of API				
NIR Diffuse reflectance sampling method				
Validation Set	Mean centering (6800.2-6098.2 cm⁻¹), 3 PLS Factors			
Ref. % (w/w) APAP	Avg	Bias	RMSEP	Std
89.7	89.98	-0.28	0.92	0.88
91.56	91.7	-0.14	0.4	0.38
93.58	93.58	0	0.32	0.33
95.75	95.23	0.52	0.42	0.37
97.73	97.67	0.06	0.32	0.32
100	100.17	-0.17	0.18	0.06

Table 3.4 Prediction results for APAP content in binary model

Preliminary results indicate that the sampling errors arise directly by heterogeneity, thereby materials with less heterogeneity are analyzed with less bias and variance.

3.3.2 Second stage (Scheme 3.1): error associated with heterogeneity in solid material blends. Evaluation of heterogeneity in powder blends by NIR diffuse reflectance and quantification sampling error.

PLS calibration model were developed to estimate the sampling error in powder blends. Powder blends present a high level in heterogeneity due to variability in particle size and distribution of components. The heterogeneity in the sample determines the sampling process. NIR diffuse reflectance was used as sampling approach in powder samples.

Blends prepared in a matrix composed by acetaminophen (APAP), microcrystalline cellulose (MCC), and lactose where studied. Concentration levels in the sample vary of 0-100 % (w/w) APAP (table 3.1). Diffuse reflectance spectra were obtained with the fiber optic probe. Ten different sampling points in each blend were evaluated and six spectra for each point were collected. The spectral data show high variability between each spectrum and differences in baseline. The variation in the spectra is due to concentration constituent and physical properties of the powder. (Figure 3.3)

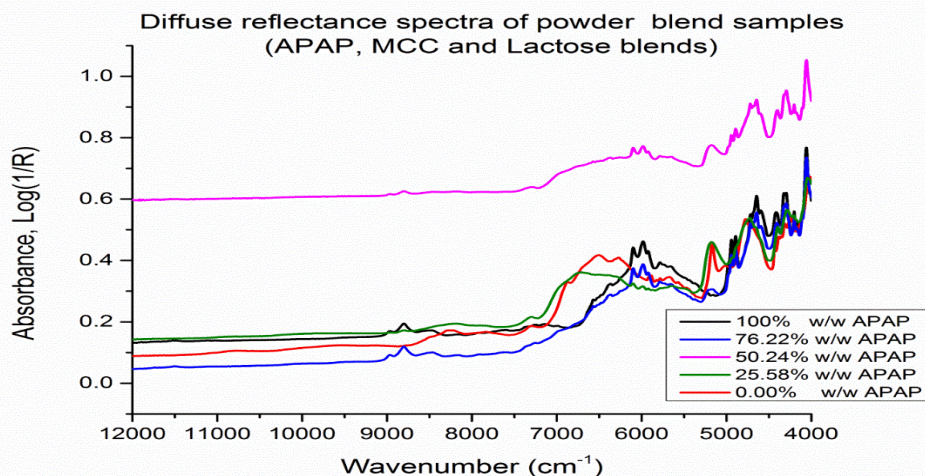


Figure 3.3 Diffuse reflectance spectra obtained of powder blends sample showing variability in baseline, effect of heterogeneity associate to particle size.

Calibration spectral data in the wavelength range of 9100 - 5000 cm^{-1} was pretreated with Standard Normal Variate (SNV) to reduce multiplicative and additive interference of scatter of radiation and particles size. The difference in baseline between the spectra was reduced using first derivative with 25 point segment size. A PLS calibration model relating to APAP content was developed with the pretreated data. Validation set sample were evaluated in the model and APAP content determined in each sample. The RMSEP was calculated (Table 3.5).

PLS Calibration model for APAP in powder blend				
NIR Diffuse reflectance sampling method				
Validation Set	SNV + First derivative (25) (9100-5000 cm^{-1}), 2 PLS Factors			
Ref. % (w/w) APAP	Avg	Bias	RMSEP	Std
66.25	68.02	-1.77	3.61	3.18
33.19	30.79	2.4	2.85	1.54
16.78	16.18	0.6	1.23	1.08
16.69	17.88	-1.19	1.93	1.53

Table 3.5 Results for prediction of APAP content in independent powder blends samples.

Table 3.5 shows that the RMSEP values were high in all concentration level evaluated in comparison to RMSEP values for liquid sample. This condition is related to heterogeneity in these blends and high bias and standard deviations were obtained.

3.3.3 Third stage: error associate to composite sample. determinations of sampling error in tablets by NIR transmission

A tablet is a composite sample. In the TOS; by definition a composite sample is made up of several, independently taken increments (An increment is a partial sample unit). The analyses of composite sample were done using NIR transmission and a calibration model developed.

Figure 3.4 shows the PCA corresponding to transmission in tablet, the principal component explains the 94.4 % of the variation corresponding to concentration of APAP while the second

component explains the remaining 2.88 % of the spectral variation. In the score plots, 15 groups are distributed along of principal component and can be distinguished (concentration of 0-100 % (w/w) APAP).

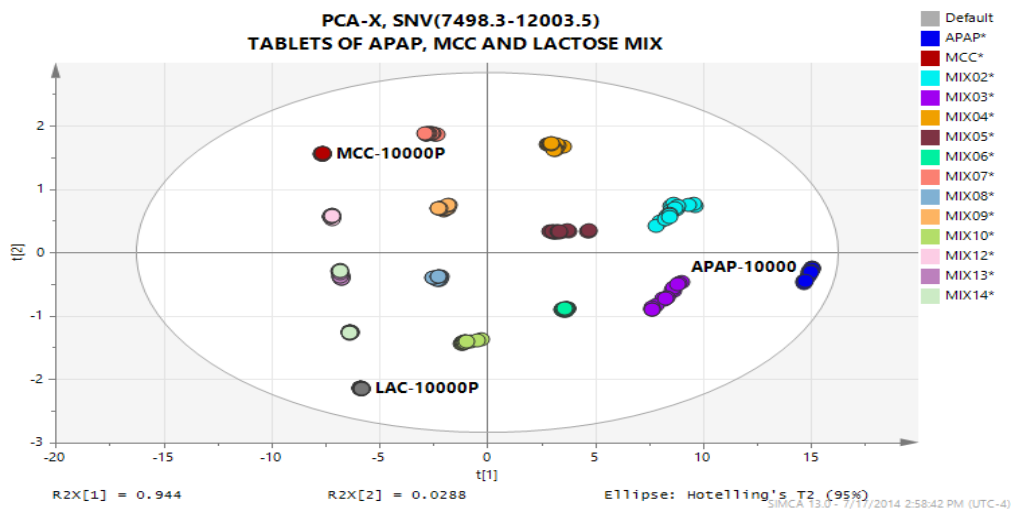


Figure 3.4 Principal Component Analysis of calibration set corresponding to transmission in tablets.

A PLS calibration model was developed using tablets obtained from same powder blends (acetaminophen, lactose and microcrystalline cellulose mixture). For PLS regression as calibration set were used 300 tablets of different APAP concentrations (0-100 % (w/w) APAP) and as validation set were used eighty tablets of four different concentrations as show in table 3.6. The RMSEP values is between 0.90- 1.81 and low standard deviation values were obtained to except for the validation set of 66.25 % (w/w) APAP (Table 3.6).

PLS Calibration model for APAP in tablets				
NIR transmission sampling method				
Validation Set	SNV(12003.5-7498.3-cm⁻¹), four PLS factors			
Reference value	Predictions average	Bias	RMSEP	Std
66.25	67.3	-1.05	1.81	1.52
33.19	33.39	-0.20	0.70	0.68
16.78	17.62	-0.84	0.94	0.46
16.69	16.01	0.68	0.90	0.61

Table 3.6 Results for prediction of APAP content of independent tablet sample.

3.4 CONCLUSION

The heterogeneity has an effect on the sampling procedure. In materials less heterogeneous the sampling process is easier and the sampling error is near zero (solutions or transparent liquid samples and pure component). In materials with high level of heterogeneity the sampling process is more difficult and probabilistic sampling is needed for powder blends.

b) When “homogeneity” is expected – Theory of Sampling in Pharmaceutical Manufacturing

3.5 SUMMARY

A stream sampling method has been developed to facilitate implementation of variographic analysis and use of replication experiments in the development of pharmaceutical formulations. These methods are thoroughly developed in the Theory of Sampling but are not currently used in pharma. Pharmaceutical formulations have very strict requirements as drug products are expected to deliver specific drug content to patients and are required to avoid possible consequences of over-dosing or under-dosing. Formulation developers currently rely on grab sampling, the use of a sample thief (spear) to extract material from areas suspected of having incomplete mixing (“dead spots”). This study applies an alternative stream sampling approach based on the Theory of Sampling in connection with testing two alternative mixing processes.

The mixing process based on vibration and tumbling can be shown to provide lower end-point heterogeneity and is therefore preferable. The results obtained show the usefulness of the variographic approach in combination with replication experiments; both are effective in identifying areas of unacceptable heterogeneity in pharmaceutical blends, and point to the need to continue improving the mixing processes described in this study.

3.6 MATERIALS AND METHOD

3.6.1 Materials

The blends were prepared from lactose monohydrate Granulac (Meggles Pharma), microcrystalline cellulose Vivapur 102 (JRS Pharma) and semi-fine acetaminophen, APAP (Mallinckrodt Inc. Raleigh, NC). The lactose monohydrate was passed through a U.S. Standard Sieve 60 (250 μm opening) before mixing.

An experimental design was followed to minimize correlation between components and obtain a robust FT-NIR calibration model. Three components blends were prepared, (correlation between majority components is unavoidable, and this process reduces the other two) amounts of each calibration blend were developed by using the experimental design software MODDE 8.0.0.0

Umetrics (Umeå, Sweden). Settings were 14 runs, objective: screening, in a D-optimal design linear model. The concentration range was 50% above and below the 15.00 % (w/w) APAP (target concentration), resulting in a calibration set spanning 7.50 - 22.50 % (w/w) APAP. The experimental design is thoroughly described in the table 3.7.

	% (w/w) APAP	% (w/w) MCC	% (w/w) LACTOSE
Cal 1	7.50	30.00	62.50
Cal 2	7.50	90.00	2.50
Cal 3	7.50	60.00	32.50
Cal 4	14.00	63.50	22.50
Cal 5	15.00	30.00	55.00
Cal 6	16.25	83.75	0.00
Cal 7	22.50	77.50	0.00
Cal 8	22.50	30.00	47.50
Val 1	15.00	66.67	18.33

Table 3.7 Experiment design

3.6.2 Preparation of powder blends

For the validation of sampling method three blends were prepared, two of 1.5kg and one of 400 g. The blends consisted of 15.00 % (w/w) acetaminophen (APAP), 66.67 % (w/w) microcrystalline cellulose (MCC), and 18.33 % (w/w) lactose (LAC). Two mixing procedures were evaluated: 1. mixing in tumble blender in one hour – this was called the T process; 2. 30 minutes of vibration and 90 minutes of tumble blending- called the VT process. A test set blend (400 g) to challenge the calibration model was prepared with a mixing time of 30 min in each blender.

3.6.3 Spectral acquisition

A Bruker Optics (Billerica, MA) Matrix FT-NIR spectrometer was used to obtain spectra. Calibration and test set spectra were obtained at a spectral resolution of 8 cm⁻¹ and a total of 32 scans were averaged. Each spectrum (average of 32 scans) requires about 4.4 seconds. All spectra were obtained as the powder moved at a linear velocity of 10 mm/s, except for the static

repeatability test (see below). Under these conditions, each spectrum can be estimated to represent approximately 180 mg of powder mixture, based on a depth of penetration of 1.2 mm measured for this spectroscopic system.^{1,2} Calibration models were developed in SIMCA 13.0 Umetrics (Umeå, Sweden), partial least squares algorithm (PLS). NIR spectra were pre-treated with a standard normal variate transformation and a first derivative based on 17 points. The chemometric model was performed on the 9100 – 5000 cm⁻¹ NIR spectral range. The performance of the calibration model was evaluated with independent test blends, aka test set validation.⁴⁻⁶ Table 3.8 shows the results obtained in the prediction of an independent test set.

A sampling system was designed to deposit blends over the conveyor belt for simulating a 1-dim industrial blender outflow sampling/analysis system: Each powder mixture (both calibration – and validation blends) was deposited in a 3 m long, 4 cm wide and 3 cm deep rig by the use of an in-house developed screw feeder.² The feeder was operated so as to provide a thick powder bed over the rig. FT-NIR spectra obtained along the entire 3 m length rig corresponded to approximately 250 g of the 1.5 kg lot powder mixture. The powder surface was left uneven and no attempt was made to obtain a flat surface of powder in the recipient, aiming to produce a highly realistic industrial situation.

Validation Blend prepared with tumble mixer + vibration mixer, mixed by one hour (T+V)					
Deposition	Avg (% (w/w) APAP)	Std	RSD (%)	RMSEP	RSEP (%)
n=1	15.58	0.46	2.93	0.54	4.71
Spectra (#)	68				

Table 3.8 Results of predictions of test set blend (tumble + vibration blender) by the FT-NIR calibration model.

3.7 RESULTS AND DISCUSSION

Real-time analysis of drug concentration was performed with near infrared spectroscopy, as a non-destructive analytical method applied to blender output streams.^{3, 7} Figure 3.5 shows the stream sampling system used to obtain the NIR spectra. The drug concentration associated with

each spectrum was predicted with the validated PLS calibration model and are shown in Figure 2 for three different blends.⁸ The blend marked VT involved both vibration and tumbling mixing as described in the Experimental section, and the blends marked T1 and T2 only included tumble mixing.

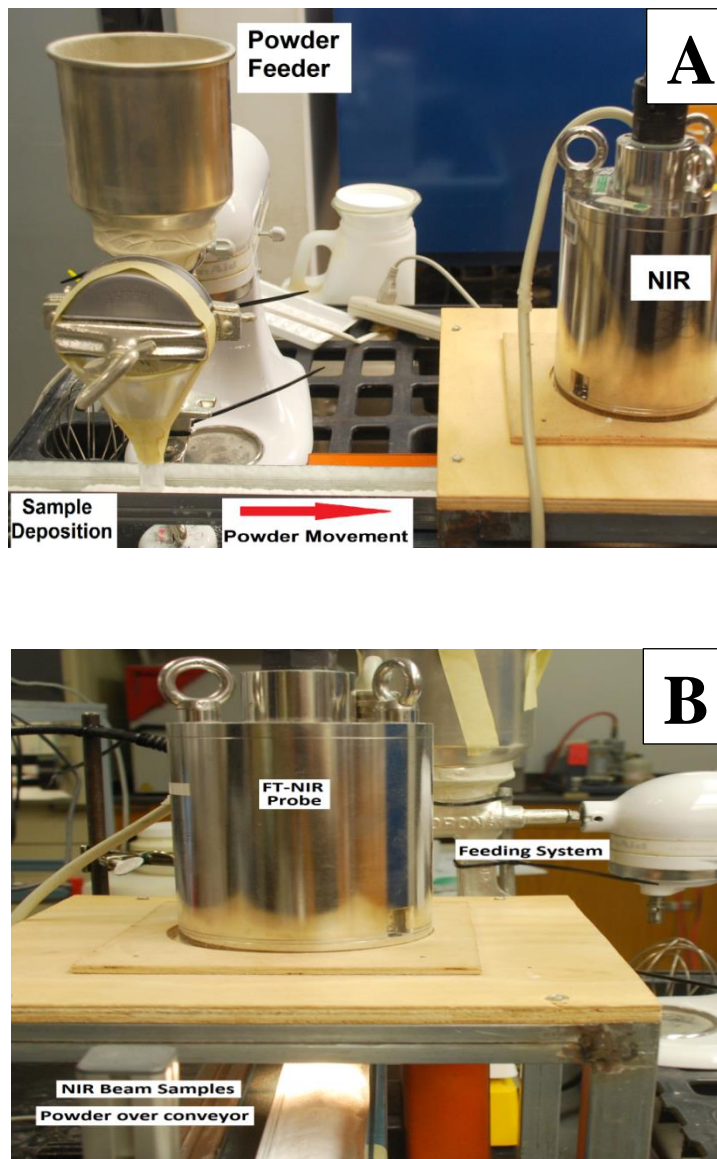


Figure 3.5 Powder depositions into the 3 meter rig used for moving the powder at 10 mm/sec towards the FT-NIR spectrometer.

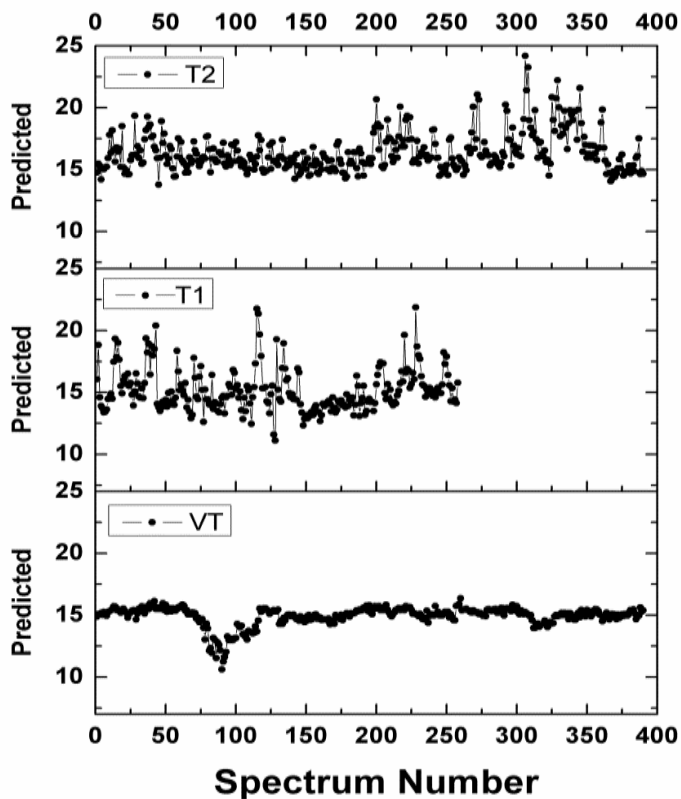


Figure 3.6 Prediction of drug concentration in three different blends using NIR spectroscopy and the rig shown in Figure 3.5.

The stream sampling approach also facilitates the use of variographic analysis and the replication experiment^{4-6, 19}, which are virtually new in pharmaceutical blending.^{2,3} The replication experiment was performed with the three blends (six successive rig depositions, 10 times to-and-fro over just one deposition), and the results are shown in Table 3.9. Figure 3.6 shows drug concentration results from a replication experiment where six depositions of 250 g are made onto the 3 m rig shown in Figure 3.5.

Figure 3.6 clearly shows that the VT process was superior in mixing to obtain concentrations near the 15.0 % (w/w) APAP target level. The breaking up of particles due to vibration also improved the flow properties of the powder mixture. The central graph (T1) shows less drug concentration results due to difficulties in powder flow and deposition onto the 3 m rig. The VT process showed the lowest standard deviation (0.78 % (w/w) APAP) as shown in Table 3.9, at

least half of those obtained with the T process. The VT process should still be improved due to a drop in concentration observed from spectra #78 – 116.²

	Deposition n=6 % (w/w) APAP			Replicate of single deposition n=10 % (w/w) APAP				Repeatability study (n =6, at 10 points) % (w/w) APAP	
	VT	T1	T2	VT	T1	T2	VT (n=6)	T1 (n =10)	T2 (n=10)
Avg	14.93	15.17	16.39	15.21	14.63	16.24	15.78	15.82	15.54
Std	0.78	1.74	1.58	0.34	1.06	2.02	0.14	0.14	0.17
RSD (%)	5.20	11.46	9.62	2.23	7.25	12.46	1.3	0.88	1.12
Spectra (#)	390	258	390	647	570	650	36	60	60

Table 3.9 Comparison of the two mixing Methods

Table 3.9 also shows that the T process has a much higher standard deviation in the replication experiment (n = 10) for a single deposition. The standard deviation of the VT process is 0.34% (w/w) APAP, while the T processes show standard deviations of 1.06 and 2.02. This replication experiment shows the significant differences in heterogeneity observed. Table 3.9 also shows similar repeatability study for all blends, since this study is a measurement of instrument (measurement) performance. The repeatability study was conducted by obtaining six consecutive spectra of the same static powder.

The results shown in Figure 3 are important because of the novelty of stream sampling in pharmaceutical blending¹⁵ since most processes have been developed with sample thief (“spear”) extracts.¹⁰ Thief sampling has been used to find “dead spots” – areas of incomplete mixing within the blender. The stream sampling approach is effective in showing areas of heterogeneity as shown in this study. The use NIR spectroscopy to develop pharmaceutical processes is also increasing but most NIR spectroscopic methods are based on a NIR spectrometer installed at a single point (interface) to a blender.^{11, 12}

Figure 3.7 shows the variograms obtained for the three processes^{13, 14}. The three variograms clearly show the differences between the blending processes. The T process shows a significantly

higher sill and nugget effect, demonstrating higher heterogeneity. Comparisons of the three approaches also indicate that the VT process provides a superior distribution of the drug in the blend. However, none of these processes would meet pharmaceutical regulatory expectations. A recently withdrawn draft guidance required: 1) a relative standard deviation $\leq 5\%$, and 2) all individual results within 10.0 percent (relative) of the mean drug concentration.¹ Thus, the stream sampling is clearly effective in finding areas of heterogeneity in the powder blend and does not hide their presence.

The VT process shows a nugget effect - minimum practical error (MPE) of only 0.04% as shown in Figure 3.7. Thus, the sampling system is capable of providing a low MPE- sum of correct and incorrect sampling errors and total analytical error (TAE). However, this MPE also depends on the heterogeneity of the blend. The MPE is greater for the more heterogeneous blends. The correct sampling errors, Fundamental Sampling Error (FSE) and Grouping and Segregation Error (GSE), depend on the heterogeneity of the blend.

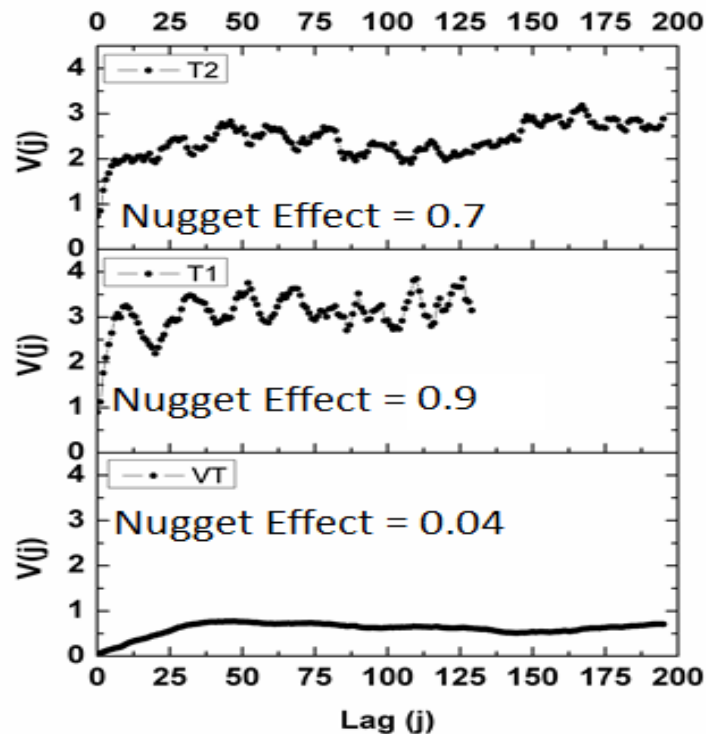


Figure 3.7 Variograms for the three blending procedures

3.8 CONCLUSIONS

The stream sampling method was effective in identifying areas of significant heterogeneity in the powder blends and the need to continue improving the process.

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