Evaluation of blend homogeneity with large differences in particle sizes using Near Infrared Spectroscopy (NIRS)

Evaluation of Blend Homogeneity with Large Differences in Particle Sizes using Near Infrared Spectroscopy (NIRS)

by

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ABSTRACT

Solid dosage forms are the most commonly prescribed pharmaceutical formulations. They consist of a homogeneous mixture of ingredients that provide desired characteristics. The process of blending during manufacturing processes is important to assure the quality of the product and it may be assessed by blend uniformity analysis. The sampling process is, also, very important and has been widely studied since it has been demonstrated that the sample thieves, the most common sampling method used in the pharmaceutical industry, introduce errors in the process. Some of these sampling errors are segregation, weight variability and product adulteration. Sample thieves do not follow Allen "golden rules" of sampling that state that a powder should always be sampled when in motion. Near Infrared Spectroscopy (NIRS) and stream sampling are alternative sampling techniques to evaluate blend homogeneity.

In this work, lactose granules were prepared to obtain large differences in particle size, creating a blend difficult to sample and evaluate. Three sampling techniques: a pocket thief, Bellamy's thief and stream sampling were used with a 20% (m/m) ibuprofen – granules blend. Sample weight variation and Relative Standard Deviation (RSD), for ibuprofen content, between 10 and 13 % (m/m) were observed. Also, the distribution of ibuprofen by particle size of granules was determined and its concentration was found to vary with particle sizes: as the particle size increases the ibuprofen concentration decreases.

Also, a NIR Blend Monitor with wireless connection was evaluated as a tool for the in-line determination of blend homogeneity. Granules of different particle sizes were sieved and placed in a V-blender to observe the blend homogeneity from a physical point of view. The spectra showed differences in baseline and absorption due to the differences in particle sizes when multiplicative scattering correction (MSC) was applied to original data. Small particles showed less absorption and baseline than larger particles. Different approaches such as mean square of differences, moving block standard deviation and absorbance at 1542 nm were evaluated to analyze changes in spectra.

RESUMEN

Las formas de dosificación sólidas son las formulaciones farmacéuticas mayormente prescritas. Estas consisten en una mezcla homogénea de ingredientes que proveen unas características deseadas. El proceso de mezclado durante los procesos de manufactura es importante para asegurar la calidad de los productos y es evaluado mediante los análisis de uniformidad de mezclado. El proceso de muestreo también es muy importante y ha sido ampliamente estudiado ya que se ha demostrado que los pillos, el método de muestreo más común utilizado en la industria farmacéutica, introducen errores en el proceso. Algunos de estos errores durante el muestreo son segregación, la variación en el peso y la adulteración del producto. Los pillos no siguen las "reglas de oro" de Allen sobre muestreo donde se establece que un polvo siempre debe ser muestreado cuando está en movimiento. La espectroscopía de infrarrojo cercano (NIRS) y "stream sampling" son técnicas alternas de muestreo para evaluar la homogeneidad de mezclado.

En este trabajo, gránulos de lactosa fueron preparados para obtener grandes diferencias en tamaños de partículas creando así mezclas difíciles de muestrear y evaluar. Tres técnicas de muestreo: pillo de bolsillo ("pocket thief"), pillo creado por Bellamy ("Bellamy's thief") y "stream sampling" fueron utilizadas con una mezcla 20% (m/m) de ibuprofen – gránulos. Variación en el peso de las muestras y una desviación estándar relativa (RSD) para el contenido de ibuprofen entre 10 y 13 % (m/m) fueron observados. También se determinó la distribución de ibuprofen por tamaños de partículas de los gránulos y se

encontró que su concentración variaba con el tamaño de partícula; entre mayor el tamaño de partículas menor la concentración de ibuprofen.

Además un monitor de mezcla de NIR sin conexión se evaluó como herramienta para la determinación en línea de la homogeneidad de mezclado. Gránulos de diferente tamaños de partículas fueron tamizados y puestos en el mezclador para observar la homogeneidad de mezclado desde un punto de vista físico. Los espectros muestran diferencias en línea base y absorción debido a las diferencias en tamaño de partículas cuando la corrección multiplicativa de la dispersión (MSC) es aplicado. Partículas pequeñas muestran menor absorción y línea base que las partículas grandes. Diferentes métodos como el cuadrado promedio de las diferencias, desviación estándar “moving block” y la absorbancia a 1542 nm fueron evaluados para observar cambios en los espectros.

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To my daughter, ***Adriana***,
for being my inspiration and my strength …

To my husband, ***Alex***,
for being with me and loving me …

To my parents, ***Nancy and Eduardo***,
for their love and support during my entire life …

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1 INTRODUCTION

Solid dosage forms are the most commonly prescribed and manufactured formulations in the pharmaceutical industries. Solid dosage forms include tablets and capsules, and are often manufactured in batches of millions of units. This manufacturing process in the pharmaceutical industry involves many steps, all of them critical and very important as the final product is obtained. One of these fundamental and critical steps is the blending of solid powders. During the blending process, the maximum homogeneity of the solid system is produced or is intended to be produced prior to tabletting or encapsulation of the final product (1). It is only when a homogeneous blend is obtained; that the mixture can be subdivided into individual doses to provide the correct amount of the active compound (2).

The current Good Manufacturing Practices (cGMPs) as described in 21CFR §211.110 require in-process controls and tests to be conducted on appropriate samples of in-process materials to assure batch uniformity and integrity of each batch of drug products. The required in-process control procedures include testing to evaluate the adequacy of mixing to ensure uniformity and homogeneity. This process is known as blend uniformity and during the last decades has received significant attention from industry, academia and the Food and Drug Administration (FDA). In 1993 the trial of United States of America (USA) vs Barr Laboratories was conducted and it opened many questions about the way that blend uniformity testing had been performed. Barr Laboratories was a producer and manufacturer of about 150 drug products by the time the trial took place. Barr Laboratories was accused of

adulteration of its products, validation failures and several cGMP's irregularities. In response to the trial, the "Barr Decision" was established for all the pharmaceutical industries. The "Barr Decision" stated that the sampling techniques should be representative for the entire batch, the blending process should not generate weak spots in the blend and the sample size must not exceed 3 times the weight of the dosage unit.

Many questions remained unanswered with the Barr decision, so FDA established the Guidance for Industry: ANDA's Blend Uniformity Analysis in 1999, but in 2002 this guidance was withdrawn and a Product Quality Research Institute (PQRI) - Blend Uniformity Working Group was established to provide a neutral forum for industry, academia and FDA. They proposed "The Use of Stratified Sampling of Blend and Dosage Units to Demonstrate Adequacy of Mix for Powder Blends" (3) where they established the criteria for blend uniformity testing in accordance with cGMP's and United States Pharmacopeias (USP) Guidelines.

Blend Uniformity testing is usually performed only at the validation for a manufacturing process in a pharmaceutical industry. Samples are taken following the mixing process to be analyzed and to assure that the process met the requirements. Those requirements are established in the 2003 Guidance for Industry: Powder Blends and Finished Dosage Units- Stratified In-process Dosage Unit Sampling and Assessment. This guidance established that for exhibit/ validation of batch powder mix homogeneity at least 10 sampling locations had to be identified in the blender that represents potential areas of poor blending.

For each location, collect at least three replicate samples. These samples should meet the following criteria: assay one sample per location ($n \geq 10$), the RSD of all individual results ≤ 5.0 percent and all individual results are within 10.0 percent (absolute), of the mean of the results.

The sampling process is, also, a critical step during the manufacturing process particularly during the blend uniformity testing. The way the sampling is conducted will influence the final result of the analysis. The sampling process has variables that should be controlled such as the variability of the bulk material being sampled, the size of each individual sample, and the number of samples taken (4).

The blending and sampling processes during the manufacturing of any pharmaceutical product have been widely studied during the last decades (5,6). The sampling of powder solids has been studied since it has shown many problems when analysis of drug content is performed. These problems came as consequence of the use of thief sampling. Samples thieves are the most common technique used for gathering samples to perform blend uniformity testing in the pharmaceutical industry as shown in Figure 1.1. The first one (left) is a side sampling thief widely used in the pharmaceutical industry, also known as pocket thief and the second (right) is an in house sampling thief referred to as Bellamy's thief (7).

Figure 1-1 - Two examples of sample thieves

Sampling thieves have shown several problems such as segregation, due to differences in particle sizes and perturbation of powder blend, weight variability due to differences in flow properties of the powders and product adulteration. These problems have been associated with the use of thief sampling when the blend is static, because it doesn't follow the "Two Golden Rules" established by Allen (8). These "Golden Rules" state that the powder should be sampled when in motion and samples should be collected throughout the entire powder stream. Alternatives to the sample thieves are stream sampling and on-line measurements with Near Infrared Spectroscopy (NIRS).

NIRS is a versatile technique with wide applications in the pharmaceutical industrial processes. In this research, NIRS was used as a method to monitor the blend uniformity process. In the first part of the research, sampling techniques such as sample thieves and

stream sampling were evaluated using a 20% (m/m) ibuprofen - lactose granules blend where differences in particles sizes existed. In the second part, the blend homogeneity for blends with great differences in particle size was monitored using a wireless in-line NIR.

1.1 Literature Review

The blending of solids is a critical step in the production of many pharmaceutical products. The intent of any blending process is to achieve a uniform mixture of all ingredients including active drug and excipients. To have an uniform mixture, particle size has to be taken in consideration because the differences in particle size could lead to segregation. The particle-size distribution of a powdered solid is usually determining through analytical sieving (9).

Brittain indicated that all the methods for the determination of particle size distribution have no value unless the composition of analyzed samples is representative of the bulk from which they were removed. Besides obtaining representative samples, the sampling plan should avoid any bias in the selection of these samples (4). The accuracy of the sampling plan will depend on the variability of the bulk material being sampled, the size of each individual sample, the number of samples taken, and the method in which these samples are prepared for analysis.

Different sampling methodologies are available to ensure that the sampling scheme is developed in such a manner that it will yield samples that are representative of the bulk. Some of these methods are samples thieves, stream sampling and the spinning riffler or rotatory sampling device (4). However, the evaluation of blending in pharmaceutical companies is usually done with a sampling probe called a thief. These thieves are inserted into the blender and extract sample from its interior. Errors may result from the distortion of the powder bed when a sample thief probe is inserted into the mixture, or the powder can adhere preferentially to the probe and be displaced as the thief passes through the bed. On the other hand, sampling thieves analyze areas where blending takes longer such as along the axis, where blending is based on diffusion instead of convection (10). It is generally recognized that limitations in current sampling technology and subsequent handling (powder segregation) limit the effectiveness of using blend sample analysis to ensure adequacy of blending (11).

Different sample thieves are available and their design may prove more suitable for a specific blend than another so their effectiveness could be compared. Bellamy et al. designed a novel sample thief that reduces the segregation caused by the insertion of the thief in the blend. Their design minimizes the disruption of the powder bed as it is inserted and collects the sample without having powder flow into the sample (7).

Many problems have been reported with the use of thief sampling for determination of blend uniformity. Tanino et al. reported segregation using samples thieves with granulations. They found that the Active Pharmaceutical Ingredient (API) was more distributed on coarse particles than in fine particles due to the hydrophilicity / hydrophobicity of API, the granulation technique or the properties of additives. Tanino also observed variation in drug content due to particle sizes distribution (12).

Berman et al. reported several problems when using a sample thief with granulations such as product adulteration, particle attrition, segregation and sampling bias. Variability of samples weights (80 -360%) was found and the causes identified were variation in physical properties of granulations, poor thief design and erratic sampling technique (6).

Garcia et al. compared the use of two samples thieves: plug and grain thief using a 2 %(w/w) drug substance blend. Their comparison was based on content uniformity and sample weights. For grain thief, the results obtained for content uniformity approached or exceeded ± 15% of label claim and for plug thief all the values are within ± 10%. The RSD values for plug thief were approximately half of the ones obtained for a grain thief. For sample weights, the results for plug thief varied between them and were difficult to obtain close values to the desire sample weight. For a grain thief, the results were more consistent based on their RSD. Grain thieves depend on the ability of the blend to flow into their chamber to collect sample. Garcia et al. found that the plug thief is better for poor flow

characteristics of the blend. Segregation and variability in content uniformity results were obtained (13).

Despite the size and design of the sample thieves, they do not follow the “golden rules” described by Allen. Stream sampling is an alternative method for sampling. It reduces the bias of sample thieves because it does not disturb the sample blend inside the mixer; the samples are taken with the blend flowing from the mixer and the sample is in motion at the time that it is obtained. As presented in the work of Popo, stream sampling provides an adequate sampling system for blends (14).

Another method for sampling is the on-line measurements using NIRS. Sekulic et al. used NIRS as an on-line technique for monitoring the homogeneity of a pharmaceutical mixture during the blending process. A tumble blender was re-engineered, using a modified 8 qt Patterson-Kelley twin shell dry blender equipped with a diffuse reflectance fiber optic probe at the axis of rotation. With the probe in contact with the component being blended, spectral information about the formulation was collected in real time.

Sekulic used multivariate statistical analysis (chemometrics) to determine the point at which the blend is considered to be homogeneous. The authors used a moving block standard deviation on the collected spectra. The calculated standard deviations show the expected trend, large standard deviations at the beginning of the blending process with a decrease over

time. This work demonstrated that after 10 minutes of mixing the standard deviation does not change, showing a good state of homogeneity, and the powder homogeneity can be monitored using an in line fiber optic accessory to obtain near infrared spectra (15).

Blanco et al. used NIRS for monitoring powder blending in pharmaceutical process. In that study, they reported a new, faster method for assessing mixture uniformity that requires no prior knowledge of the spectrum for a homogeneous sample and relies on the difference between two spectra recorded at two consecutive times. To this end, a method for calculating the Mean Square of Differences between two consecutive spectra was developed with a view to expeditiously identifying the time mixture homogeneity was attained (16).

1.2 Summary of Following Chapters

We first develop the necessary background theory in Chapter 1. Chapter 2 deals with background theory of Near Infrared Spectroscopy (NIR), mixing theory, chemometrics and granulation theory. Chapter 3 described the material and methods used for the experiments. The results and the discussion of them are presented on Chapter 4. Conclusions are described on Chapter 5.

2 THEORETICAL BACKGROUND

2.1 Near Infrared Spectroscopy

Near Infrared Spectroscopy (NIRS) is a versatile analytical technique when analyzing solids powders (14, 15, 16). It is based on dipole moment changes and molecular vibrational transitions of molecules. NIR covers the range from 12,500 – 4000 cm^{-1} (800 – 2500 nm). The absorptions in this region are due to overtones and combination bands of the fundamentals bands of the mid-IR molecular vibrational bands.

The majority of overtone peaks observed in a NIR spectrum arise from the X-H stretching modes. They are between 10 and 1000 times weaker than the fundamental bands. The radiation in the NIR can interact with matter in three different ways as shown in Fig. 2.1.

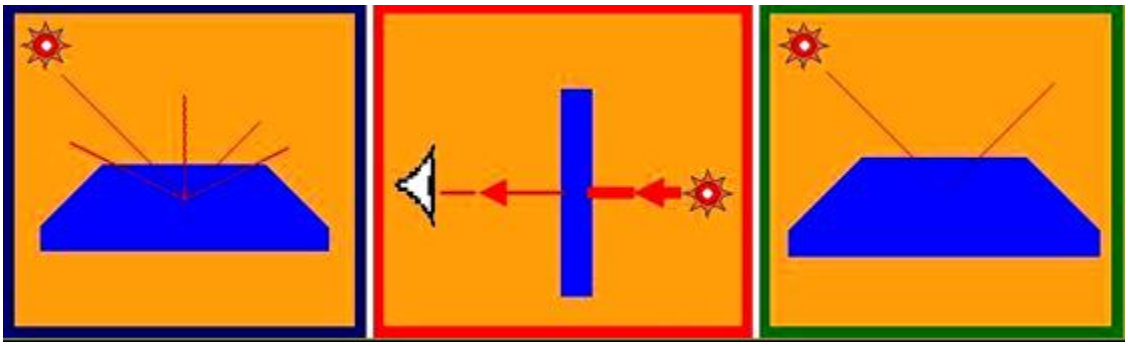

Figure 2-1 - Sample irradiation in NIR

The three different ways that the radiation can interact with the matter are diffuse reflectance, transmission and specular reflectance (17,18). In diffuse reflectance, radiation penetrates the sample and is reflected in all directions. In transmission, radiation penetrates the sample and is detected after it passes through it. Specular reflectance is the reflection of radiation at an angle equal to its radiation of incidence.

2.2 Mixing Theory

The process of powder mixing is one of the most important unit operations during production of pharmaceutical solid dosage forms (1). The blending process is a combination of three processes:

- Diffusion - the redistribution of particles by random movement of individual particles relative to one another.

- Convection - the movement of groups of adjacent particles from one place to another.

- Shear - the change in configuration of ingredient particle locations through the formation of slip places in the mixture.

Theories of powder mixing include random mixing, non random mixing, ordered and total mixing (1). Random mixing is the operation in which motion is transmitted to particles to cause them to rearrange in such a manner that any particle can be found at any given point in the mix at any one time. Non random mixing theory accepts that the probability of finding many constituents particles in a mix is not equal. Another type of mixing theory is the order mixing. It involve different methods as mechanical ordering, adhesional or coating forces or methods to prepare ordered units in the mix such that the ordered unit will be the smallest possible sample of the mix and will be nearly identical in its structure to all ordered units in the mix. The last type of mixing theory is total mixing theory. This theory applies when the powder mixes are not totally randomly distributed nor completely ordered.

2.3 Chemometrics

Chemometrics is the application of statistical and mathematical methods for the design and optimization of chemical experiments and for the efficient extraction of information from chemical data (19, 20).

- Multiplicative Scattering Correction (MSC) - is an approach to compensate for scattering by solids. In this pretreatment each spectrum is regressed linearly against an ideal spectrum to yield a slope and intercept. It removes the variation between the spectra related to physical properties.

- Subtract - is a background removal method. This approach subtracts a variable from the spectra to observe the changes in absorption through the spectra.

- Standard Normal Variate (SNV) – is another approach to compensating for scattering by solids. The mean and the standard deviation of a sample are first computed based on included variables; the value for each included variable is corrected by first subtracted the mean, then dividing by the standard deviation (21).

- Second Derivative – removes baseline features by taking derivatives with respect to variable number.

2.4 Granulation Theory:

Granulation technology is a widely used process in the industry to prepare materials for tablets or capsules. Granulation process is a size-enlargement process during which small particles are formed into larger, physically strong agglomerates in which the original particles can still be identified (22). Granulations can be prepared using two different methods: wet process and dry process. For this research, the wet granulation process was used

Wet granulation processes involve the use of a granulating agent to facilitate the agglomeration process and different steps such as wet massing, drying and screening. Table 3.1 shows the advantages and disadvantages of wet granulation process.

Table 2.1 - *The Advantages and Disadvantages of Wet Granulation

Advantages	Disadvantages
Improved Flow Properties	Multiple processing steps add complexity and make validation and control difficult
Densification	Time, space, and equipment required are costly
Improved compression characteristics	Stability may be a concern for moisture-sensitive or thermolabile drugs
Better distribution of color and soluble drugs if added a binder solution	Loss of material during various stages of processing
Reduction in dusting	
Prevention of segregation of powder mix	
Makes hydrophobic surfaces more hydrophilic	

*Adapted from Handbook of Pharmaceutical Granulation Technology

2.5 Raman Spectrsocopy

Raman spectroscopy is a technique based on the interaction of the oscillating induced polarization or the dipole moment of the medium with the inelastic scattering of radiation by a non-absorbing medium. In Raman spectroscopy a monochromatic laser beam is passed

through the sample and the radiation scattered from the sample is monitored. The phenomenon of Raman scattering results from the same type of quantized vibrational changes that are associated with infrared spectroscopy (IR). Differences in functional groups active in these techniques (Raman and IR) make them complementary techniques for analysis.

2.6 ATR-IR Spectroscopy

Infrared spectroscopy (IR) is a technique widely used as a tool for investigating chemical processes and structure. It is based on the dipole moment changes and molecular vibrational transitions of molecules. Attenuated Total Reflectance (ATR) is a specific IR reflectance technique that combines the infrared spectroscopy and the reflection theory. ATR accessory allow to take infrared spectra directly to solid powders without sample preparation. The phenomenon of ATR is based on the principle that when the beam of radiation passes from a denser to a less dense medium, reflection occurs. The fraction of the incident beam that is reflected increases as the angle of incidence become larger. If the less dense medium absorbs the evanescent radiation (penetrating radiation), attenuation of the beam occurs at the wavelengths of absorption bands.

3 MATERIALS AND METHODS

3.1 Materials

The materials used for the blend preparation were Lactose Monohydrate VIT Holland USP-NF/EP/JP Impalpable and Starch 1500 LM Low Moisture Pregelatinized Starch NF, used for granules preparation. Ibuprofen 70 grade USP/BP/EP, Albemarle, Baton Rouge, LA was used as Active Pharmaceutical Ingredient (API). The structures for these components are shown in Figure 3.1, 3.2 and 3.3. Methanol – HPLC grade Fisher Scientific was used as solvent for blend uniformity analysis.

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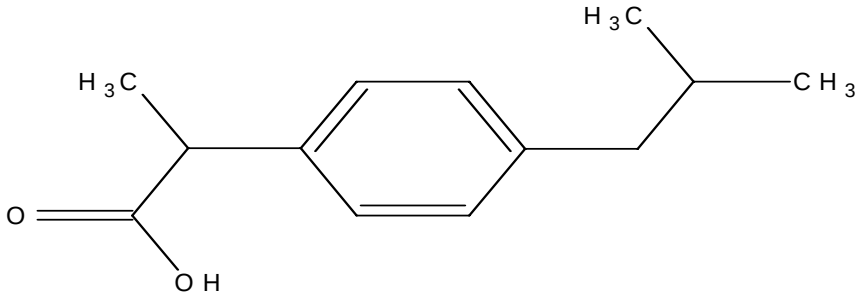


Figure 3-1 - Ibuprofen structure

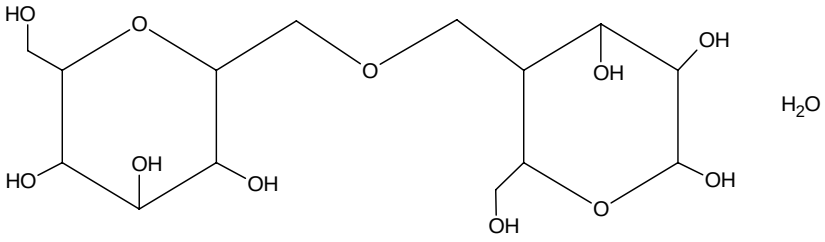


Figure 3-2 - Lactose structure

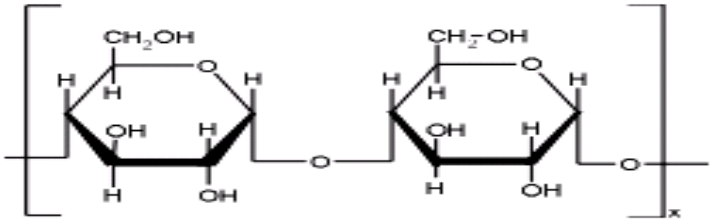


Figure 3-3 - Corn starch structure

3.2 Equipment

- Blend Uniformity Monitor, Control Development, Inc. (CDI)
  - NIR spectrometer: 256 element InGaAs, T.E. cooled
  - Wireless connection
  - Lamp source: Dual Tungsten Lamp, 5.4 watts each
  - Wavelength range: 900 nm to 1700 nm
  - CDI Spec 32 Software version 1.5.3.3

- Bruker Vector 33 FT-NIR System:
    - Fiber optic probe
- Beckman DU 650 UV-Vis Spectrophotometer
    - UV quartz cell: 10 mm and 2 mm
- Blender “Model V” Blend Master, with 4 quart acrylic cross flow shell and 16 quart stainless steel cross flow shell, both Lab Blender Model “V” from Patterson-Kelly Co. East Stroudsburg, PA.
- Isotemp Oven from Fisher Scientific
- Ro-tap Model RX-2P. W.S. Tyler. Mentor, OH
- USA standard test sieves from Fisher Scientific – different mesh sizes (4.75 mm, 2 mm, 710 µm, 500 µm, 300 µm, 250 µm, 125 µm and 63 µm)
- Ohaus Analytical Plus Balance (max. cap: 200 g)
- Ohaus Adventurer Top Loading Balance (max. cap: 4100 g)
- Kitchen Aid stand mixer
- Sample thieves: pocket and Bellamy’s thief
- Cups for stream sampling samples
- Lab Liner Shaker
- Sonicator – Aquasonic VWR Scientific product model 250HT
- Centrifuge – Fisher Scientific
- Volumetric flasks – 100.0 mL, 200.0 mL and 250.0 mL
- Vials
- Pirouette 3.10 and 3.11 Multivariate Data Analysis for Windows

- OPUS software version 5.1
- Grams AI version 7.01

3.3 Preparation of Excipient Granules

For one kilogram of granules, 450 g of Lactose monohydrate and 50 g of corn starch 1500 pregelatinized were sieved through a 125μm mesh sieve and mixed for five minutes into an acrylic V-Blender of four quarts. The blend was mixed in a Kitchen Aid® and distilled water, approximately 125 mL, was added until it formed a damp mass. This process, also, was performed in large scale using a High Shear Granulator located in the Chemical Engineering Department. The mass obtained was, then, sieved through a 4.75 mm and 2 mm meshes. The granules sieved were placed in a Fisher Scientific Isotemp Oven for 12 hours at 40°C. The dry granules were then sieved through different mesh sizes sieves (>710 μm, >500 μm, >300 μm, >125 μm) to obtain granules of different sizes as shown in Figure 3.4.

Figure 3-4 - Granules of different particle sizes

3.4 Blend preparation for different sampling techniques

The blend preparation for one kilogram of blend consisted in weighing 800 g of granules (excipient) and 200 g of ibuprofen (API) previously sieved through a 125 µm sieve mesh to make a 20% (m/m) ibuprofen – granules blend. The granules and ibuprofen were filled, into 4-quarts acrylic shell V-Blender, in layers (300 g of granules, 100 g of ibuprofen, 300 g of granules, 100 g of ibuprofen and 200 g of granules), see Figure 3.5. The blend was mixed for five minutes.

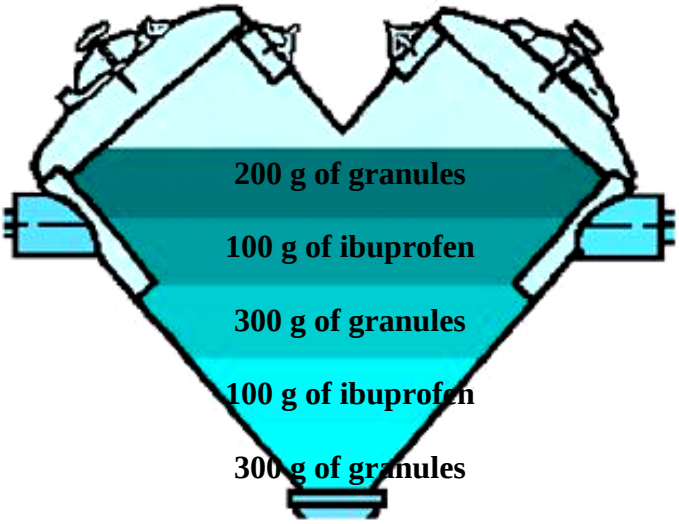


Figure 3-5 - Schematic view of filling process of the V-Blender with layers of granules and ibuprofen

3.5 Sampling Process

After the blend was mixed, four samples were taken as stream sampling, where the blend was allowed to flow from the V-blender to the sampling cups as shown in the Figure 3.6. Samples were then obtained from the axis of rotation of the V-Blender. Two sample thieves were used for this part: a pocket thief and an in-house developed thief, called Bellamy's thief, in honor of its developer (7). With each sample thief, four samples from the blender were taken as shown in Figure 3.7.

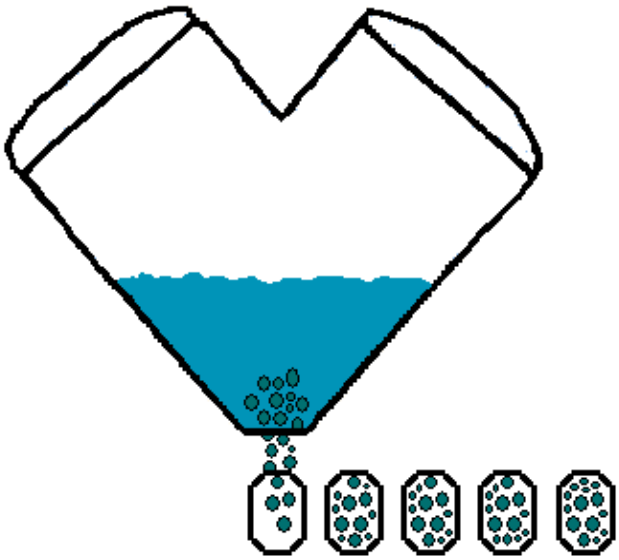


Figure 3-6 - Schematic view of the stream sampling process

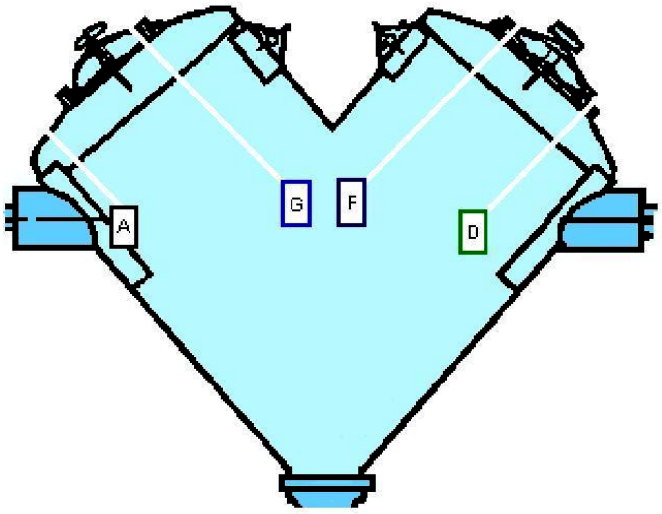


Figure 3-7 - Schematic view of sample points taken with sample thieves

3.6 Ibuprofen distribution by particle sizes

After the 20% (m/m) ibuprofen – granules blend was sampled; the rest of the blend was placed on a Ro-tap with different mesh sizes sieves (500 µm, 300 µm, 250 µm, 125µm and 63 µm). The Ro-tap was turned on for 20 minutes to allow the blend to distribute along the different sieves. Two samples were taken from each sieve for determination of ibuprofen content.

3.7 NIR spectral acquisition for samples of ibuprofen distribution by particle sizes

Spectra for the different samples were acquired using a NIR Vector 33 Spectrometer from Bruker Optics. For the spectral acquisition, the fiber optic accessory was used to take sample spectra. OPUS version 5.1 software was used to manage the data obtained. The spectral acquisition parameters used were a resolution of 4 cm^{-1} with 64 scans.

3.8 UV-Vis Spectrophotometric analysis for Ibuprofen content

The analysis for ibuprofen content for the samples taken was done using UV-VIS spectrophotometric analysis. The analysis was performed at a wavelength of 245 nm and using a 2 mm cell. The preparation of the different sample solutions are described below.

- Standard Solutions Preparation:

    - Two stock solutions were prepared weighing about 100 mg and 200 mg of ibuprofen and transferred quantitatively to two 100.0 mL volumetric flasks. The content was diluted and completed to volume with methanol 75% (v/v).
    - From stock solution 1 (100 mg of Ibuprofen), 5.0 mL, 10.0 mL, 20.0 mL and 30.0 mL aliquots were transferred into four 100.0 mL volumetric flasks, respectively. The content was completed to volume with methanol 75% (v/v).
    - From stock solution 2 (200 mg of ibuprofen), 25.0 mL and 30.0 mL aliquots were transferred into two volumetric flasks, respectively. The content was completed to volume with methanol 75% (v/v).

- Solutions for Accuracy Study:

    - Three solutions were prepared for accuracy study weighing ibuprofen and excipients in different ratios as shown in Table 3.1.
    - Both quantities were transferred to a 100.0 mL volumetric flask and diluted with methanol 75% (v/v).
    - The solutions were agitated for 20 minutes in a shaker and sonicated for three minutes. The solutions were let to stand for 10 minutes and completed to volume with methanol 75% (v/v)

- Approximately 10 mL were transferred to a vial and centrifuged for 10 minutes at 6 rpm to separate the suspended particles and obtained a transparent solution with the analyte in solution.

Table 3.1 - Weighing ratios of API (ibuprofen) and excipients (lactose and corn starch) for accuracy study solutions

Accuracy Study Solutions	API (mg)	Excipients (mg)
Solution 1 (R1)	300	1200
Solution 2 (R2)	400	1600
Solution 3 (R3)	500	2000

- Sample Solutions Preparation:

  - The samples taken with side sampling thief and stream sampling were transferred to a 100.0 mL volumetric flask, and dissolved in methanol 75% (v/v).
  - The solutions were agitated for 20 minutes in a shaker and sonicated for three minutes. The solutions were let to stand for 10 minutes and completed to volume with methanol 75% (v/v).
  - Approximately, 10 mL was transferred to a vial and centrifuged for 10 minutes at 6 rpm to separate the suspended particles and obtained a transparent solution with the analyte in solution.

- For samples taken with Bellamy's thief, the samples were transferred to a 100.0 mL volumetric flask and dissolved in methanol 75% (v/v) at room temperature.
- The solutions were agitated for 20 minutes and sonicated for three minutes. They were let to stand for 10 minutes and completed to volume with methanol 75% (v/v).
- From that solution, 20.0 mL aliquot was transferred to a 100.0 mL volumetric flask and completed to volume with methanol 75 % (v/v).
- Approximate, 10 mL aliquot was transferred to a vial and centrifuged for 10 minutes at 6 rpm to separate the suspended particles and obtained a transparent solution with the analyte in solution.

3.9 Blend preparation for granules blend uniformity

The granules were sieved to obtain fractions of particle size greater than 710 μm, 500 μm, 300 μm, 125 μm, and less than 125 μm. These fractions of the particles had a range size value instead of an absolute value that includes, for example, particles greater than 500 μm but less than 300 μm. The granules with the different particle sizes fractions were placed in layers inside a stainless steel V-Blender of 16 quarts as shown in Figure 3.8.

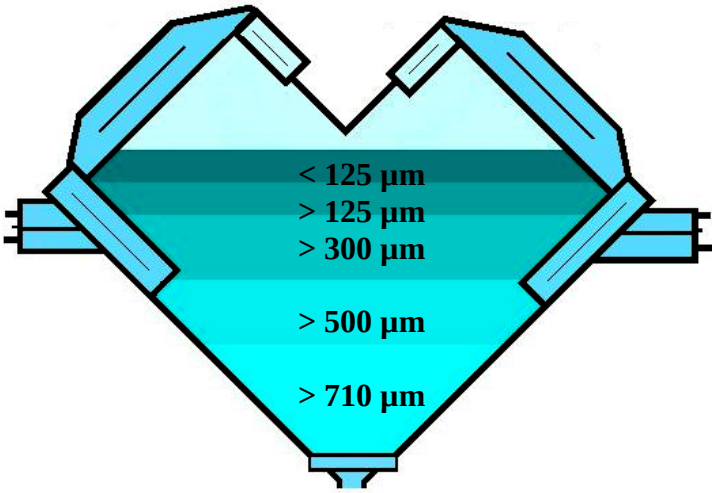


Figure 3-8 - Schematic view of the filling process of the V-Blender with layers of granules of different particle sizes

The greatest layer of all is the layer of particles sizes greater than 710 µm that covered, approximately 40% of the blender. Particles sizes greater than 500 µm and 250 µm covered another 40% and the last fractions were the one with particles sizes of greater than 125 µm and less than 125 µm. Although the weights of the fractions changed throughout the experiments, the layer proportion of the granules volume remained roughly constant.

3.10 Blend Homogeneity and NIRS

After the blender was filled with the granules, the blend process was monitored using a diode array near infrared spectrometer. This equipment has a wireless connection, with a 256

element InGaAs detector and with a dual Tungsten lamp. This equipment is attached to a V-Blender, as shown in Figure 3.9.

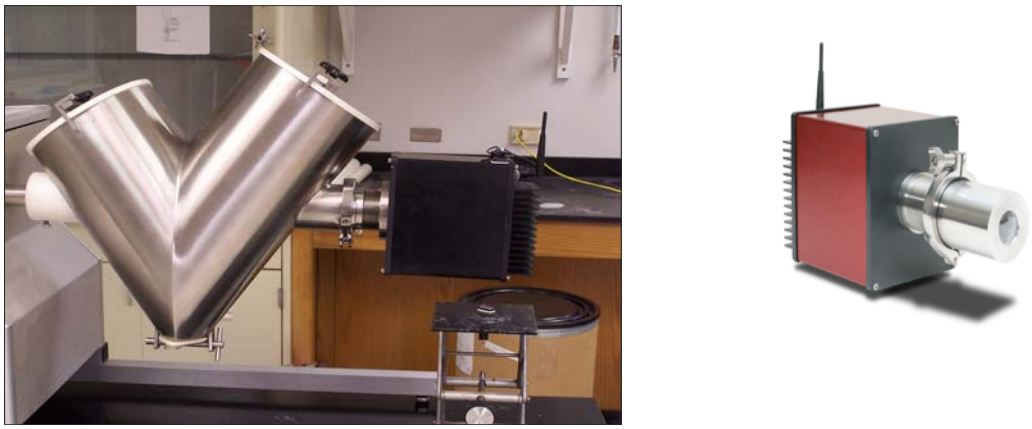


Figure 3-9 - V-Blender with a wireless NIR spectrometer used for spectral acquisition

Spectra were acquired using the CDI Spec 32 software during 32 minutes in the first experiment and about an hour in the last three experiments. An optical trigger was used to obtain a spectrum during each revolution (approximately every 5 seconds), as shown in Figure 3.10. The optical trigger has a photocell that synchronizes the recording of spectra. The spectra were acquired as showed average fill buffer mode where the spectra displayed are the average of 32 spectra taken during the integration time. The integration time used for the spectra acquisition varied from 0.0015 to 0.0017 seconds in each experiment. For each experiment, an internal auto calibration procedure was performed and the integration time was set to it.

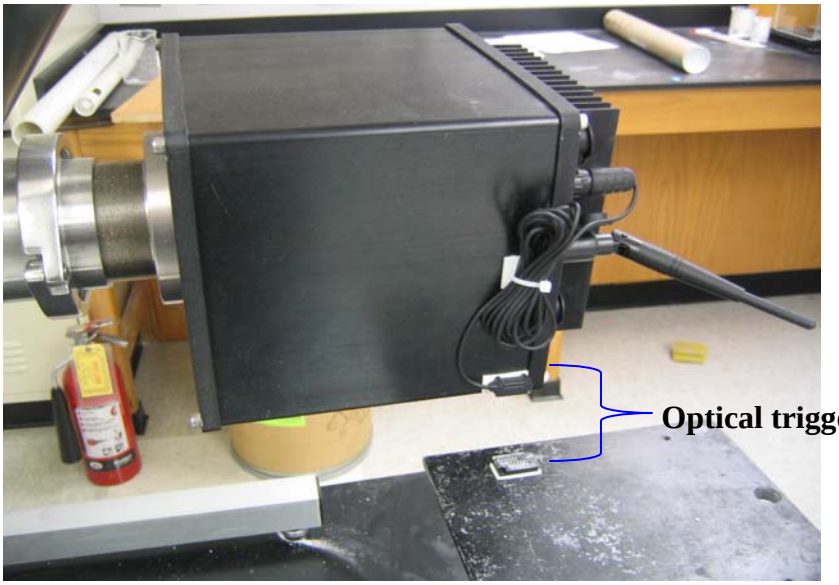


Figure 3-10 - NIR spectrometer with optical trigger

3.11 Raman Spectroscopy Spectra Acquisition

Raman spectra for the different size fractions (> 710 µm, > 500 µm, > 300 µm and > 125 µm) were acquired using a RXN-1/RA-785 System from Kaiser Optical System. For spectra acquisition, an immersion probe was used with an exposure time of 10 s and accumulation of 5.

3.12 ATR IR-Spectroscopy Spectra Acquisition

Spectra for the different sizes fractions (> 710 µm, > 500 µm, > 300 µm and > 125 µm) were acquired using a Nicolet Nexus 470 FT-IR. For spectra acquisition, an Attenuated Total Reflectance (ATR) accessory was used with a resolution of 16 cm^{-1} using OMNIC E.S.P. software version 5.1.

4 RESULTS AND DISCUSSION

This research work has been divided in two parts. The first part was focused on the evaluation of different sampling techniques, such as stream sampling and sample thieves, to evaluate the ibuprofen drug content when large differences in particle size were present in a blend. This large difference in size was obtained by using excipient granules that consisted of lactose, corn starch, and water. The second part of this research involved the use of an in-line NIR system to monitor the blend uniformity from a physical point of view, and also, when large differences in particle size were present.

4.1 Calibration curve used for the evaluation of sampling techniques

A calibration curve was obtained analyzing the standard solutions by UV-VIS spectroscopy and plotting their absorbance against their concentration. The equation line obtained was $y = 0.2013x + 0.001$ with a regression coefficient of 0.9996. Figure 4.1 shows the calibration curve used to calculate the ibuprofen content in the analyzed samples.

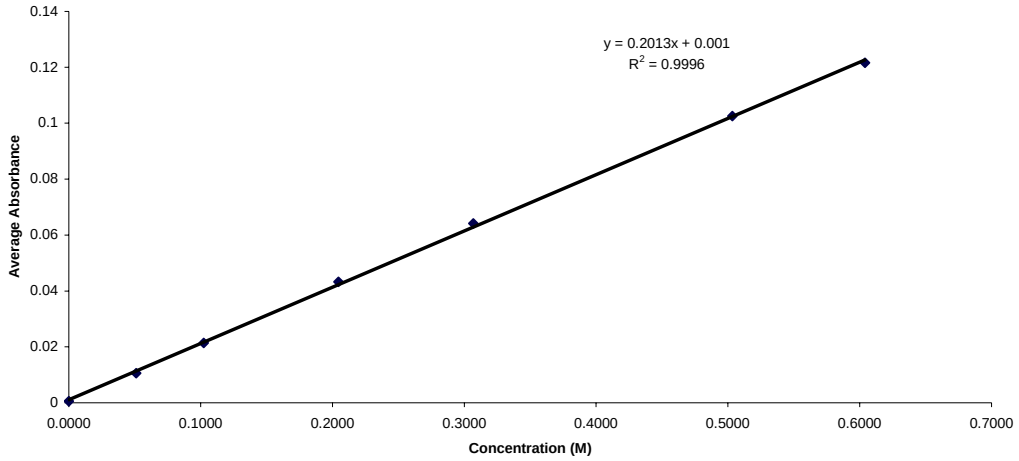


Figure 4-1 - Calibration curve for sampling techniques

4.2 Samples withdrawal using stream sampling, pocket thief and Bellamy's thief

4.2.1 Stream Sampling

The samples taken using stream sampling were analyzed by UV-VIS Spectroscopy to determine their ibuprofen content. The samples weight varied between 80.0 g and 115.9 g. These variations were a consequence of variation in powder flow from the V-Blender to the cups and differences in particle sizes. Four samples were analyzed with an average concentration of 240.9 mg/g of ibuprofen (24% (m/m)) for a target concentration of 200.0 mg/g (20% (m/m)). The corresponding standard deviation was ± 30.7 mg/g and a relative standard deviation (%RSD) 12.7%. The complete results are shown in Table 4.1.

Table 4.1 - Stream sampling results

Samples	Sample Weight (mg)	Concentration (%m/m)	Concentration (mg/g)
1	115.9	20.03	204.8
2	80.0	22.39	230.7
3	94.6	24.55	251.0
4	108.9	27.25	277.1
	Average	24	240.9
	Standard deviation	3	30.7
	%RSD	13%	12.7%

4.2.2 Pocket Thief

The samples obtained using the pocket thief were analyzed by UV-VIS spectroscopy to determine their ibuprofen content. The samples weight varied between 121.7 g and 156.2 g. These variations corresponded to differences in powder flow from the V-Blender to the sample thief compartment. These variations, although are high values, are expected since the differences in particle sizes were present Four samples were analyzed with an average concentration of 221.5 mg/g of ibuprofen (22% (m/m)) for a target concentration of 200.0 mg/g (20% (m/m)). The corresponding standard deviation was ± 21.9 mg/g and a relative standard deviation (%RSD) 9.9%. The complete results are shown in Table 4.2.

Table 4.2 - Pocket thief results

Samples	Sample Weight (mg)	Concentration (%m/m)	Concentration (mg/g)
1	121.7	22.47	200.5
2	143.5	20.86	156.3
3	156.2	19.38	190.8
4	155.1	24.49	193.3
	Average	22	221.5
	Standard deviation	2	21.9
	%RSD	10%	9.9%

4.2.3 Bellamy's Thief

The samples obtained using Bellamy's thief were analyzed by UV-VIS spectroscopy to determine their ibuprofen content. The samples weight varied between 209.6 g and 513.6 g. This thief has a greater capacity for sample collection than the stream sampling cups and pocket thief used in this work. Also, this thief does not depend on the ability of the powder to flow; instead, the thief gathered the sample inside the compartment. Four samples were analyzed with an average concentration of 185.2 mg/g of ibuprofen (19% (m/m)) for a target concentration of 200.0 mg/g (20% (m/m)). The corresponding standard deviation was ± 19.7 mg/g and a relative standard deviation (%RSD) 10.7%. The complete results are shown in Table 4.3.

Table 4.3 - Bellamy's thief results

Samples	Sample Weight (mg)	Concentration (%m/m)	Concentration (mg/g)
1	433.0	20.05	200.5
2	209.6	15.63	156.3
3	366.2	19.08	190.8
4	513.6	19.33	193.3
	Average	19	185.2
	Standard deviation	2	19.7
	%RSD	11%	10.7%

Similar standard deviation and relative standard deviation (RSD) were obtained for the stream sampling and the sample thieves. For the three methods, the standard deviation and RSD results were higher than expected. The RSD result for stream sampling was 12.7% higher than the one reported by Popo et al. for a 20% (m/m) ibuprofen – lactose blend with a mixing time of 5 minutes (13). For that blend, Popo et al. reported a RSD of 3.47%. The differences in these results are due to greater differences in particle sizes present in the blend with granules. For pocket thief (grain thief or side-sampling thief), the results were comparable when compared with Garcia et al. (12) who obtained a 6 to 10% RSD for a 2% (w/w) drug substance blend. For Bellamy's thief, a blend of KCl and dextrose was used. The RSD of KCl content was less than 6.0% for a mixing time of 30s and 60s, the differences in the RSD are due to differences in particle sizes obtained in the blend using granules while a KCl and dextrose blend only demonstrate a moderate tendency to segregate because the particle sizes did not vary greatly (7).

4.3 Ibuprofen distribution by particle sizes

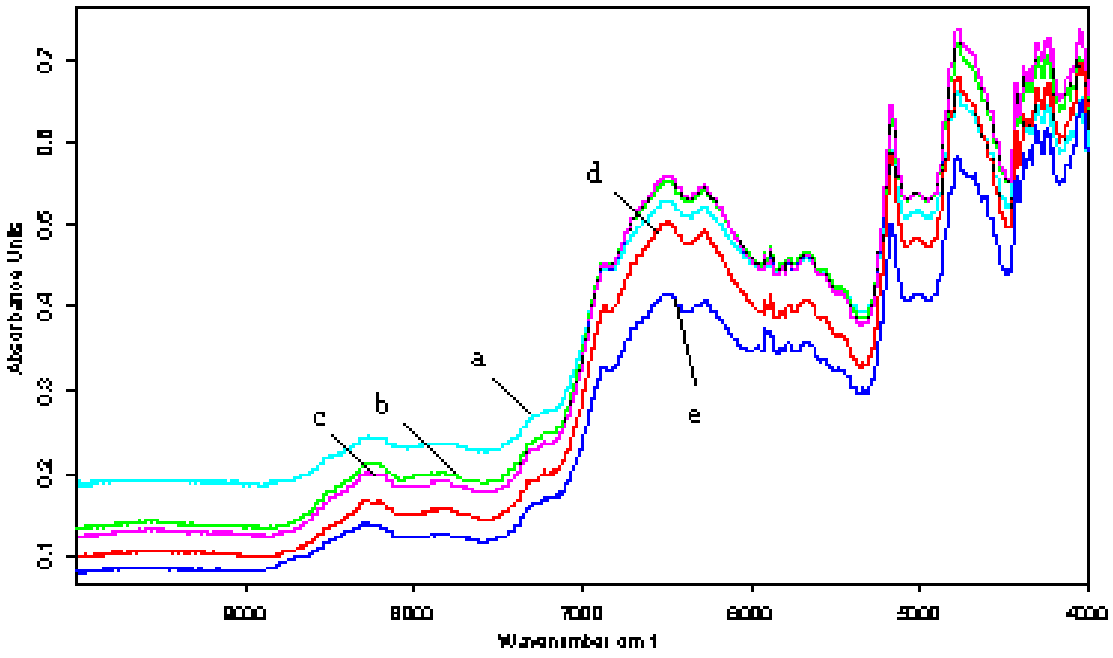
Samples were taken from different fractions of particle sizes to determine the ibuprofen distribution in these fractions in order, to see if the ibuprofen adheres to the granules surfaces. The samples were analyzed using UV-Vis spectroscopy to determine the ibuprofen content. The results obtained are shown in Table 4.4 and as the particle size of granules decreased, the ibuprofen content increased. The results for ibuprofen concentration varied from 0.8 mg/m (0.08% (m/m)) to 710.9 mg/m (71% (m/m)) from a target concentration of 200 mg/g (20% (m/m)) with a concentration average of 202.8 mg/g (20% (m/m)). The average concentration was of 20.26 %m/m. Another way to obtain an average concentration was correcting the concentration by the total sample weight obtained. This average was of 17.6 %m/m. This average reflects better the effect of sample weight variability obtained during the experiment. This tendency was expected since ibuprofen had a smaller particle size than the greater part of the granules, and the ibuprofen was added after the granules were formed. These results indicated that the ibuprofen did not adhere to the surface of the granules since the greatest part of the blend was separate into its components: granules and ibuprofen, even though, the ibuprofen is a cohesive powder.

Table 4.4 - Ibuprofen particle sizes distribution results

Samples	Sample Weight (mg)	Concentration (%m/m)	Concentration (mg/g)
Particles >700µm-1	137.8	0.46	4.6
Particles >700µm-2	104.9	0.08	0.8
Particles >500µm-1	235.1	1.08	10.8
Particles >500µm-2	163.4	0.51	5.1
Particles >300µm-1	117.1	3.02	30.2
Particles >300µm-2	106.5	1.37	13.7
Particles >250µm-1	123.7	2.18	21.8
Particles >250µm-2	136.1	2.24	22.4
Particles >125µm-1	103.1	7.96	79.6
Particles >125µm-2	121.1	8.67	86.7
Particles > 63µm-1	104.0	58.19	581.9
Particles > 63µm-2	102.5	56.21	562.1
Particles < 63µm-1	125.2	70.54	705.4
Particles >63µm-2	104.3	71.09	710.9
	Average	20.26	202.6

4.4 Spectral acquisition for granules with different particles sizes

Spectra for the different fractions were acquired using Vector 33 NIR spectrometer. The spectra showed differences in baseline and the absorption band due to changes in particle sizes. The spectra baseline increases as the particle sizes increases due to differences in light scatter. Small particles exhibit greater total reflection than larger particles, and as a result show less absorbance (20). The spectra for particles sizes greater than 710 µm to greater than 125 µm are shown in Figure 4.2.



Legend:

a – particles >710µm b - particles >500µm c - particles >300µm
d - particles >250µm e - particles >125µm

Figure 4-2 - FT-NIR spectra of different granules particle sizes at a resolution of 4 cm^{-1}

4.5 NIR inline monitoring of blend uniformity

Spectra for the different excipients (lactose, granules and corn starch) were attained using a NIR blend monitor spectrometer in static form. The spectra for the excipients are shown in Figure 4.3.

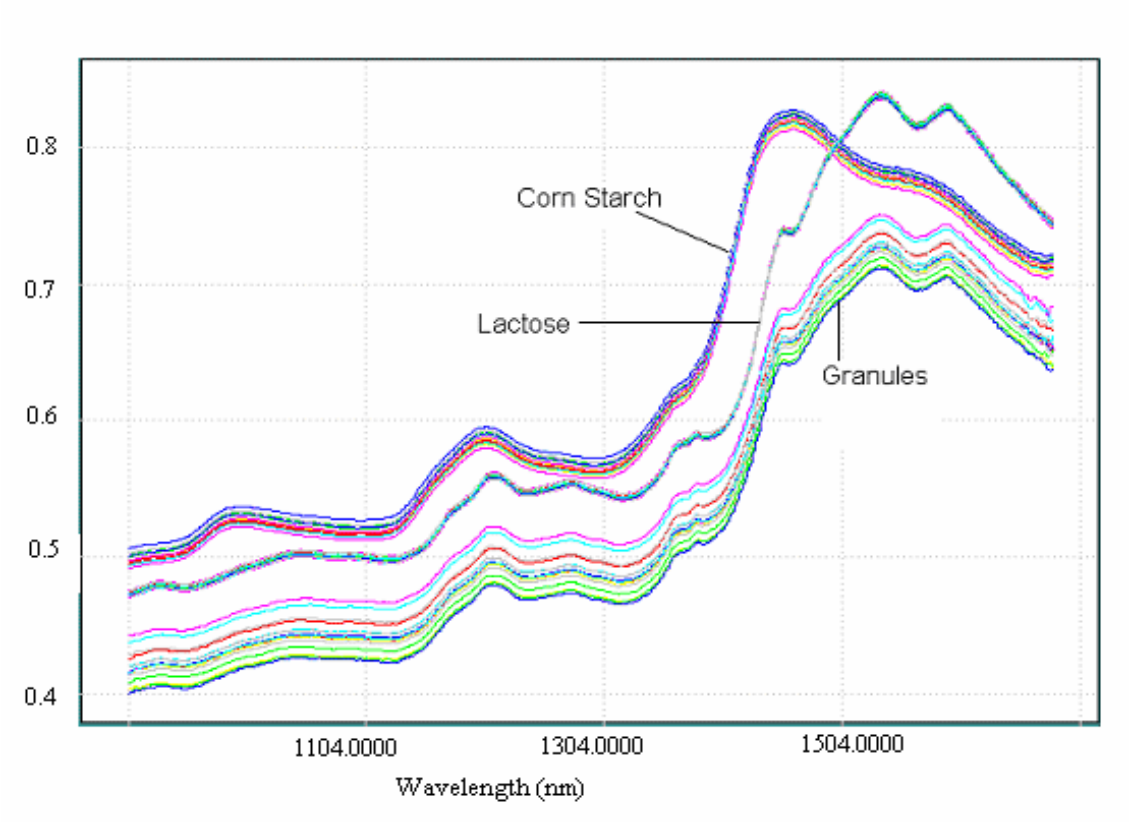
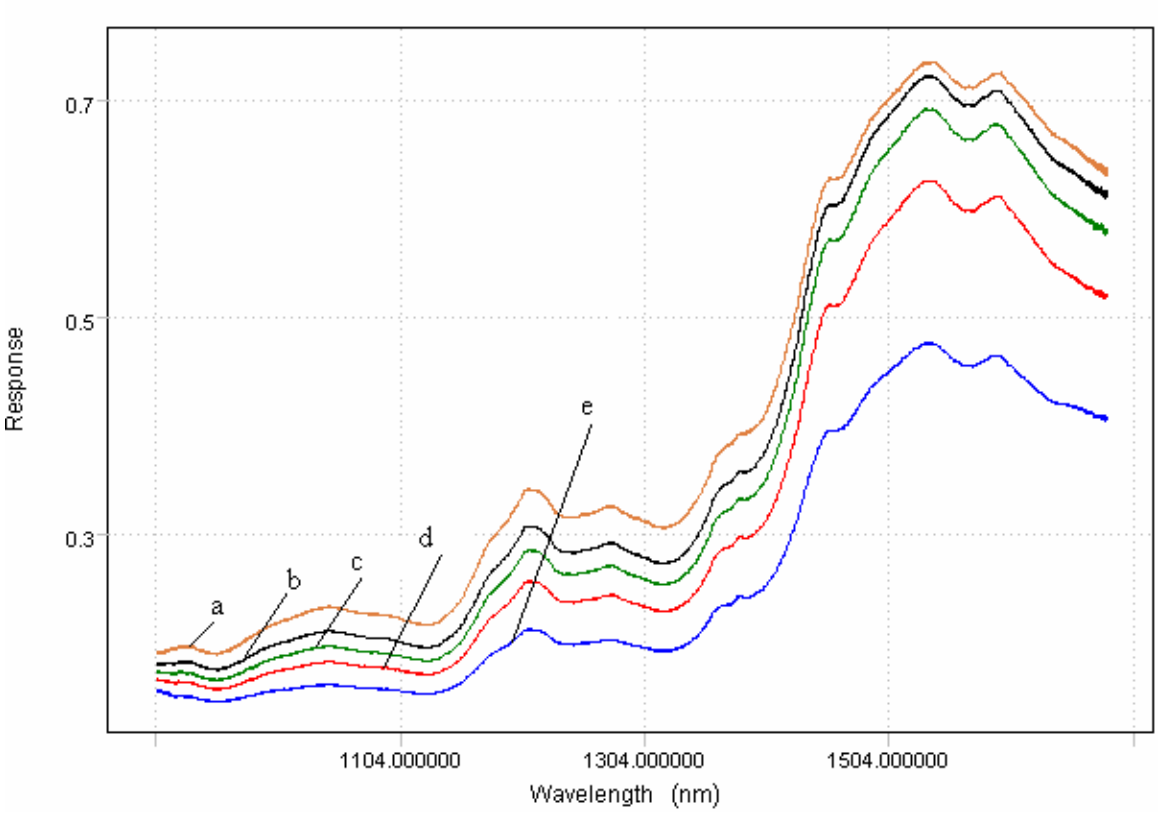


Figure 4-3 - Static form spectra for different excipients

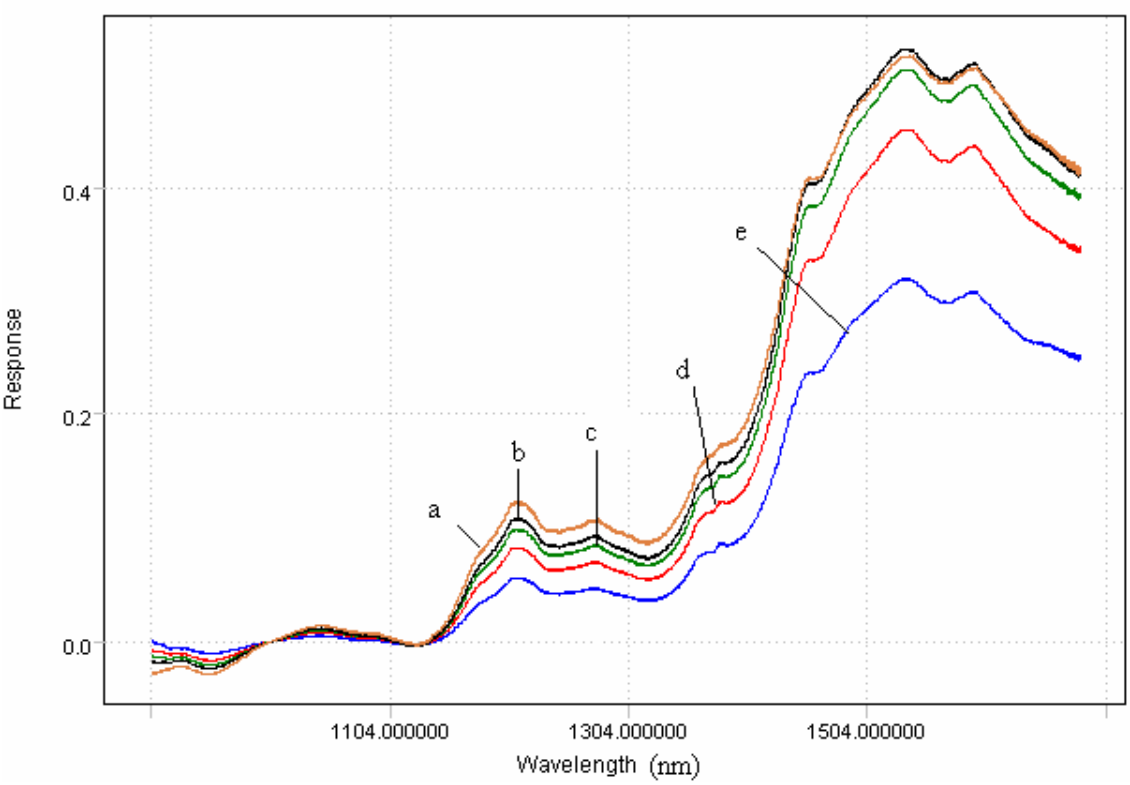
Granules of different particle sizes were prepared and their spectra were taken off-line using the NIR Blend Monitor. The multiplicative scatter correction (MSC) transform was performed to the spectra obtained to observe more clearly the differences between them. These spectra showed differences in baseline and absorption band with changes in particle sizes. As particle sizes increases, the baseline and absorption band, also, increases. Figure 4.4 shows the spectra of different granule fractions obtained off-line with MSC transform.



Legend: a - granules > 710µm b - granules > 500µm c- granules > 300µm
d - granules > 125µm e - granules < 125µm

Figure 4-4 - Off-line MSC spectra of different granules fraction

The spectra obtained off-line were transformed using subtract where in variable100 (1004 nm), it was set to zero; this data treatment will emphasize the changes in absorption. The treated spectra are shown in Figure 4.5.



Legend: a - granules > 710µm b - granules > 500µm c - granules > 300µm
d - granules > 125µm e - granules < 125µm

Figure 4-5 - Subtract spectra of different granules fraction off-line

4.6 Blend uniformity using an inline NIR spectrometer

4.6.1 First Experiment

The blend homogeneity of the blend with large differences in particle sizes was monitored for 32 minutes using the in-line NIR spectrometer. Before the mixing process started, the NIR probe sees only the granules with particle size of less than 125 µm. After the mixing process ended, the blend inside the V-Blender was observed. The blend was very different compared to the initial blend. The granules with the largest particle sizes dominated the blend and the near infrared spectra reflected the changes in the blend.

The complete spectra taken during the mixing process are shown below in Figure 4.6. One spectrum was obtained for every revolution of the blender. Approximately 383 spectra were collected during the 32 minutes as shown in Figure 4.6. As the mixing process was taking place, the spectral baseline and absorption band changed. The change in baseline and absorption varied with time of mixing and corresponds to changes in particle sizes. As shown in the Figure 4.6, the spectra with small baseline and absorption are the first spectra taken by the probe, which correspond to the smaller particle size granules.

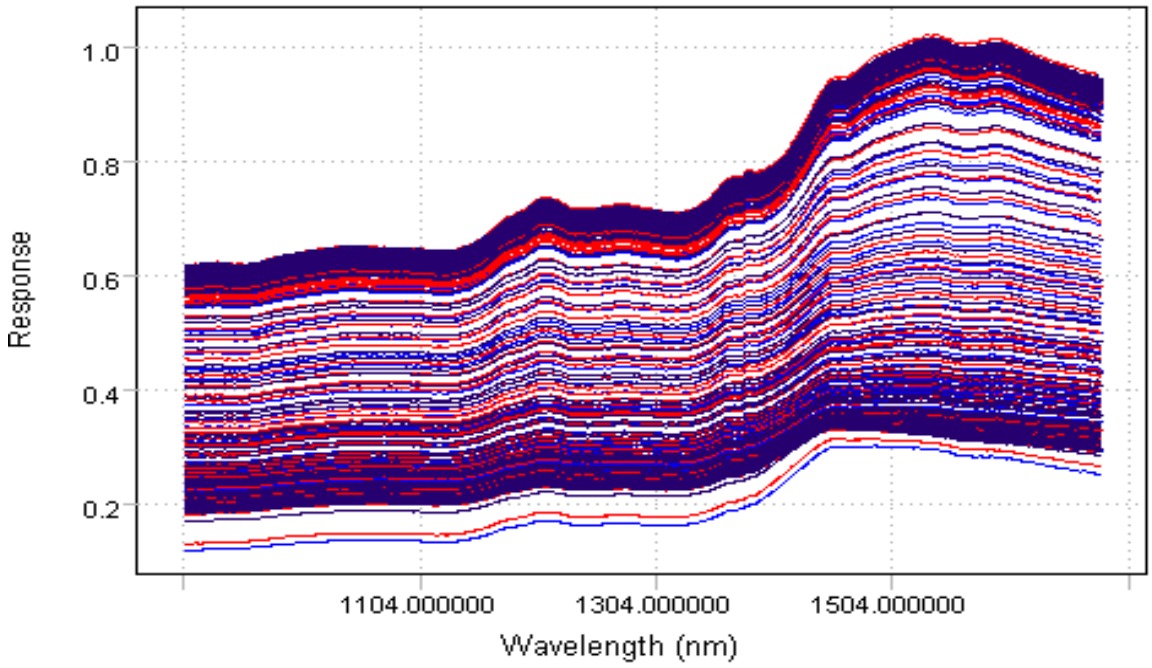
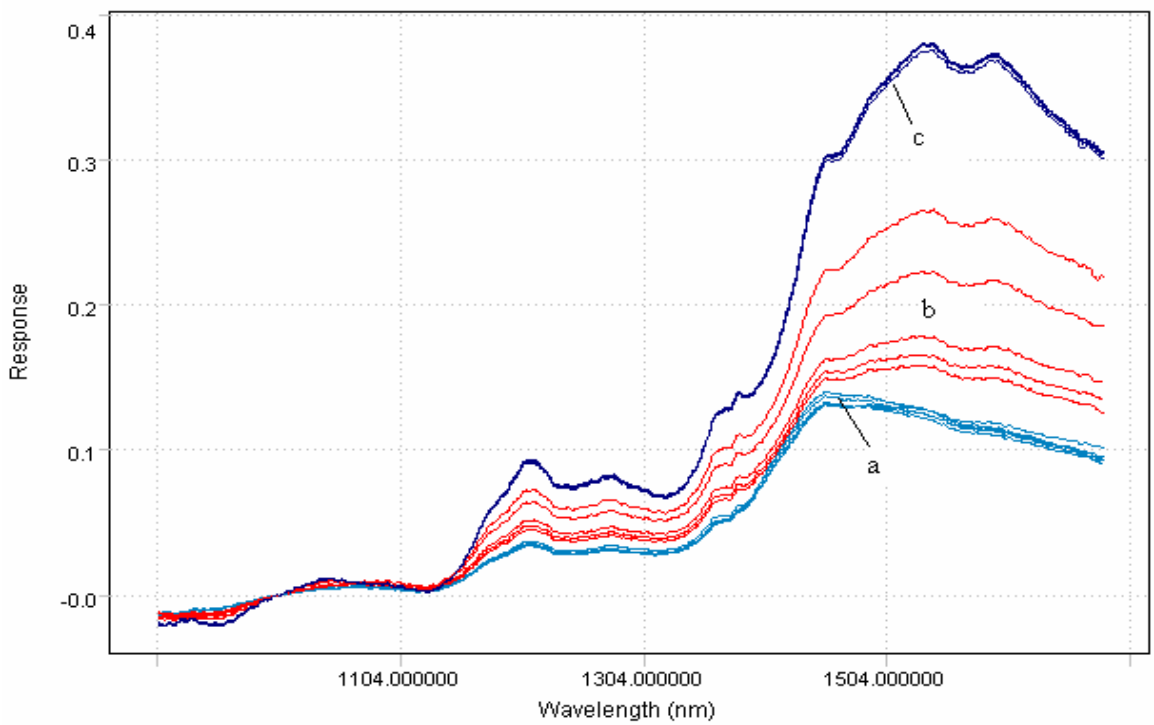


Figure 4-6 - Spectra of different sizes granules blend during 32 minutes

During the first five minutes the spectra showed little variation, then the variations increase and during the last minutes it decreases. The decrease in variation was due to homogeneity of the blend during the last 12 minutes as shown in Figure 4.7. Differences in spectra appearance are shown in the first minutes of the mixing process. These changes were vanishing as the blending process was taken place and the largest particles are the greatest part of the blend. These differences were due to light scattering effects when smaller particles are in top of the blend and the NIR radiation penetrates the particles. These effects were not longer seen when the blend homogeneity was getting accomplished.



Legend of stages: a - first 5 minutes b - from 6 to 20 minutes c - from 21 to 32 minutes

Figure 4-7 - Spectra for 32 minutes showing the three stages of the blending process

The spectra obtained during the mixing process were transformed using subtract, where variable100 (1004 nm) was set to zero. It allowed to emphasize the changes in absorption. The spectra with the subtract transformation are shown in Figure 4.8.

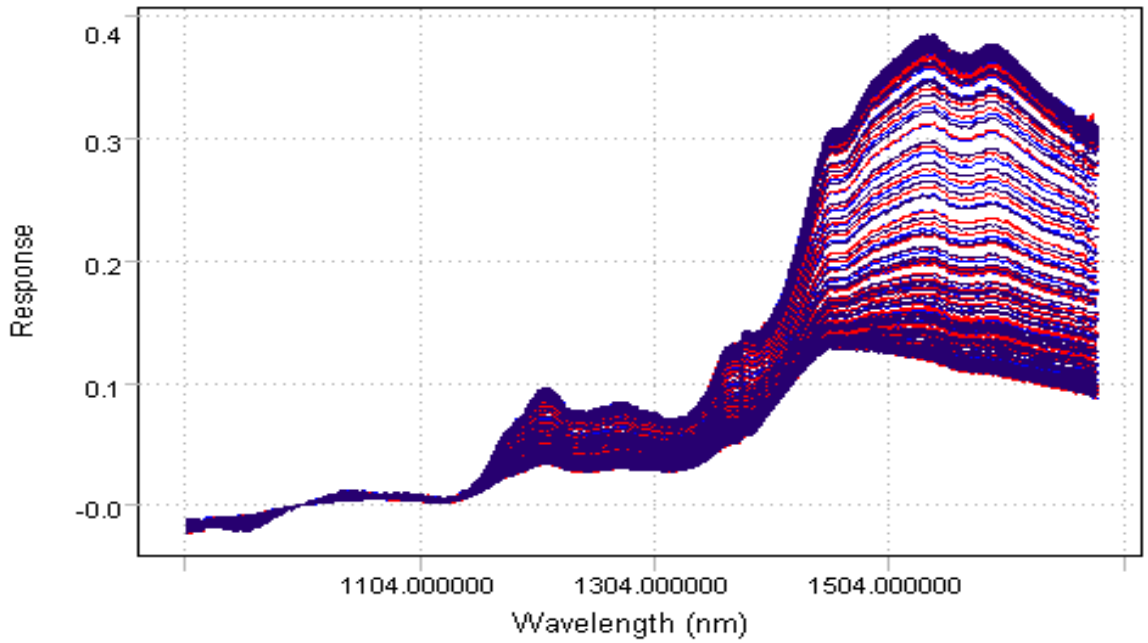
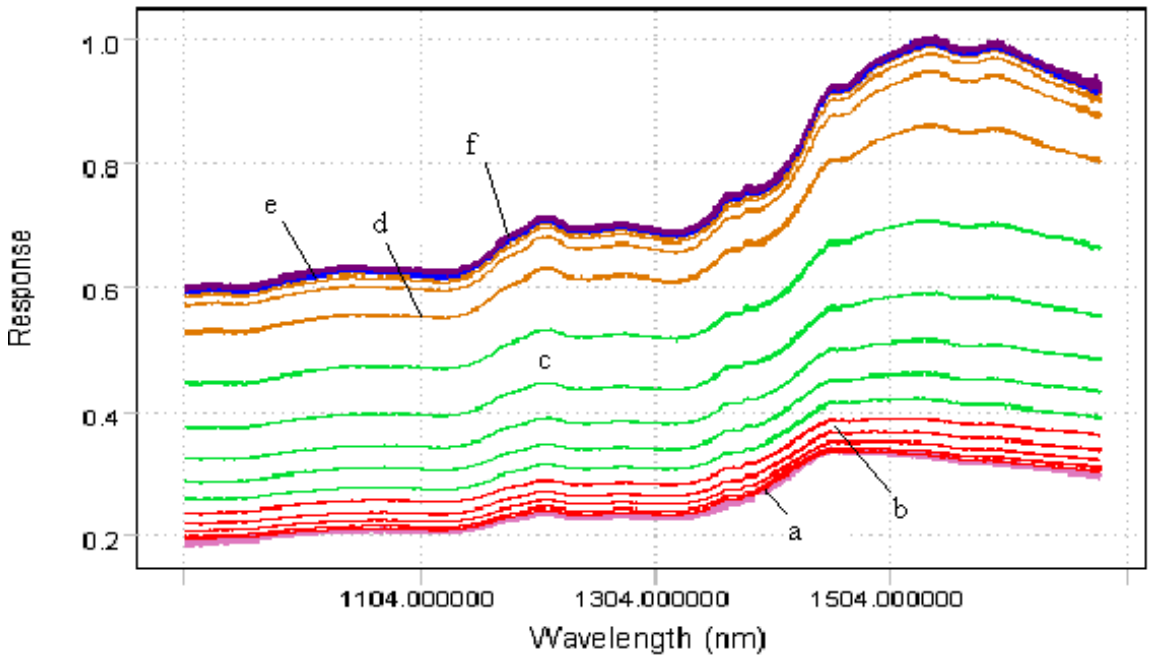


Figure 4-8 - Spectra with subtract transformation for the 32 minutes blending experiment

Approximately, 12 spectra were obtained per minute. For each minute, the spectra were transformed with MSC to remove the variation between the spectra. The MSC spectra for each minute were plotted together to evaluate the variation in the spectra with time. The spectra obtained are shown in Figure 4.9. These spectra showed differences in baseline and absorption bands as time varies. The changes in absorption are due to differences in particle sizes since the radiation penetrates the particles and the greatest ones have less area to be in contact with the radiation and the reflection decreases so the absorption increases (20). The greatest variations in the spectra were observed after 11 to 15 minutes of mixing. The spectra did not show significant changes after 21 minutes of mixing.



Blending time fractions for 32 minutes blending experiment:

a -1-5 min b - 6-10 min c - 11-15 min d -16-20 min e - 21-25 min f - 26-32 min

Figure 4-9 - MSC spectra for the mixing process of the 32 minutes blending experiment

4.6.2 Second Experiment

The blend homogeneity for a blend with large differences in particles sizes was monitored using NIR during approximately 49 minutes. As described in the first experiment, the NIR probe sees only the smallest particles at the beginning of the experiment. After the mixing process ended, the blend inside the V-Blender was visually analyzed. The blend observed was very different compared to the blend that started. The granules with the greatest particle sizes dominated the blend and these were near the probe window.

The spectra taken during the entire mixing process are shown below in Figure 4.10. Each spectrum corresponds to a revolution of the V-Blender, and hence a total of 585 spectra were collected. As shown, the baseline and the absorption were changing during time of mixing. These changes correspond to changes in particle size. As particle sizes increase, the baseline and absorption band also increases.

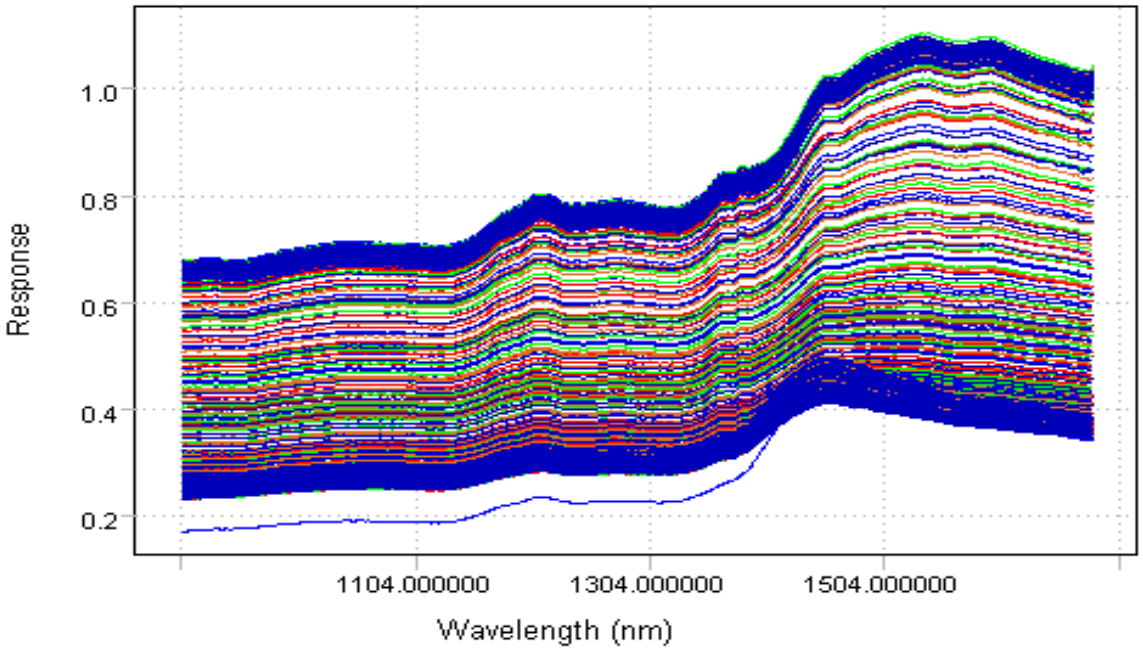


Figure 4-10 - Spectra of different particle sizes granules blend during 49minutes

The spectra showed during the first 15 minutes little variation in the baseline and absorption band of the spectra, as time goes on, the variation increases, but in the last minutes the variation decreases again.

As before, data obtained during the mixing process were treated using subtract where variable100 (1004 nm) was set to zero. Figure 4.11 shows the spectra after mentioned treatment data.

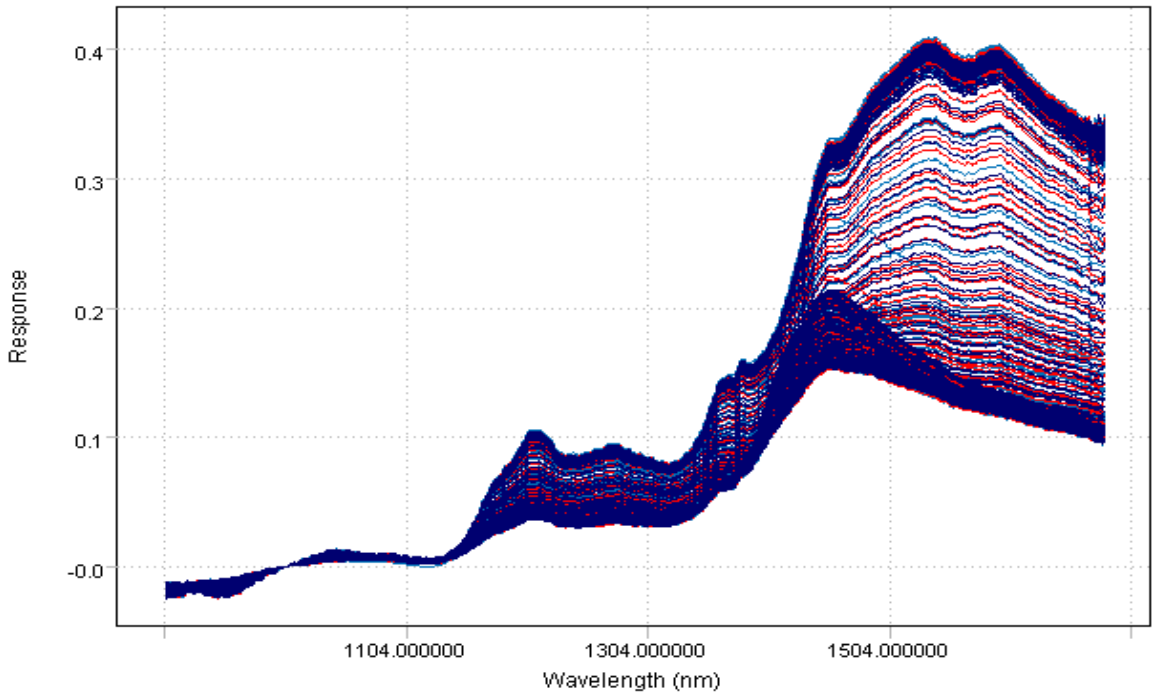
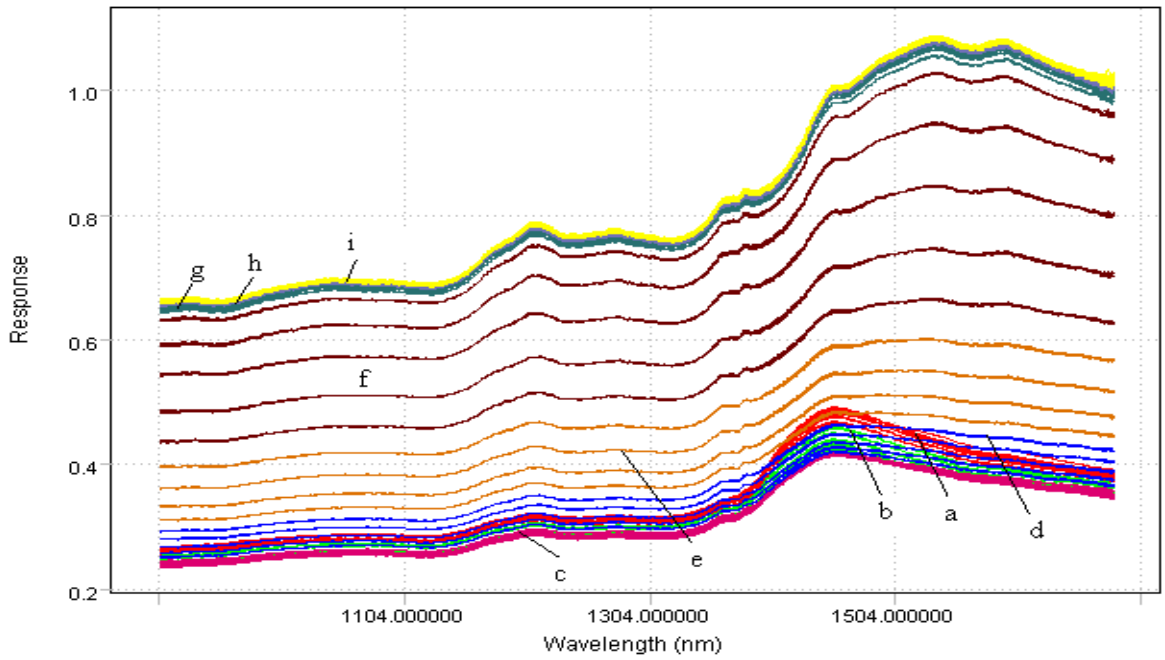


Figure 4-11 - Spectra with subtract transformation for the 49 minutes blending experiment

Approximately, 12 spectra were obtained per minute. For each minute, the spectra were transformed with MSC to remove the variation between the spectra. The MSC spectra for each minute were plotted to evaluate the variation in the spectra with time. The spectra obtained are shown in Figure 4.12. These spectra showed differences in baseline and absorption bands as time varies as for the first experiment. Differences in particle sizes led to differences in absorption and baseline. A greater particle size reflects less radiation so the

absorption increases (20). The greatest variations in the spectra were observed after 15 to 30 minutes of mixing. A reduction in spectral change was observed after 31 minutes of mixing.



Blending time fractions for 49 minutes blending experiment:

a - 1-5 min b - 6-10 min c - 11-15 min d - 16-20 min e - 21-25 min f - 26-30 min g - 31-35 min h - 36-40 min i - 41-49 min

Figure 4-12 - MSC spectra for the mixing process of the 49 minutes blending experiment

In comparison with the first experiment, the variation in these spectra decreases later in time. To observe this variation as a function of time, the absorbance at 1542 nm, a wavelength were the absorption is nearly a maximum was plotted versus the number of blender revolutions after performing subtract as shown in Figure 4.13.

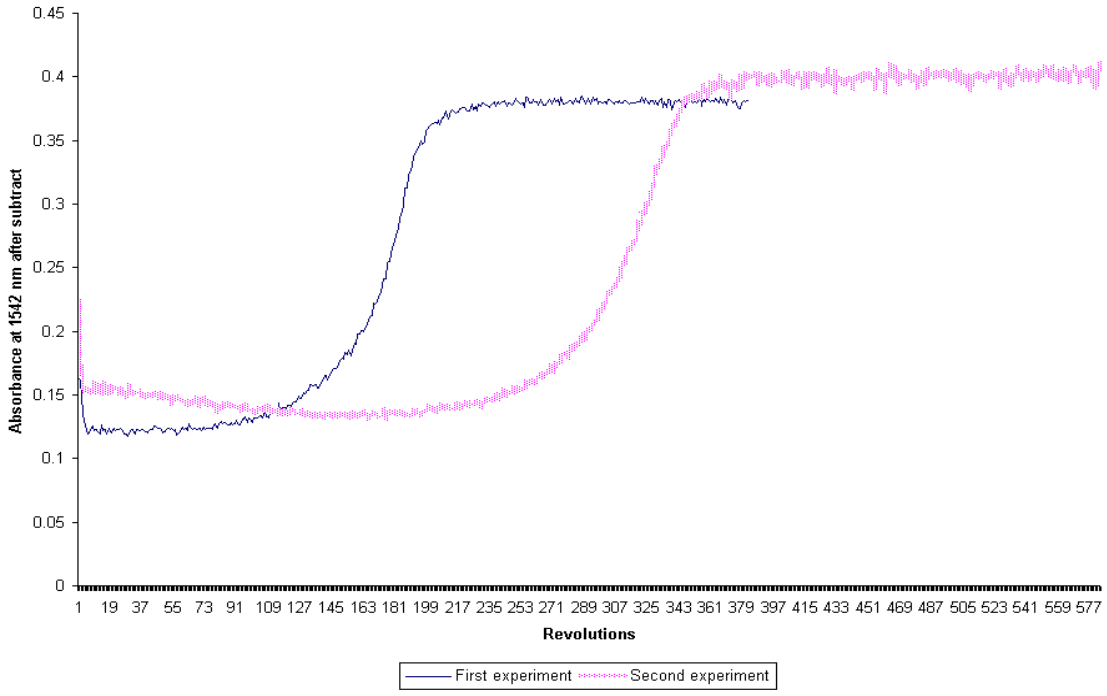


Figure 4-13 - Graph of absorbance at 1542 nm after subtract of each spectra versus revolutions (number of spectra) at 6 rpm

The graphs for the two experiments are shown in Figure 4.13. These graphs give a blending profile of the blending process performed on these two experiments. Both blending profiles have similar behavior, the absorbance during the first minutes remained almost constant, then the absorbance increases, but at the end the absorbance at 1542 nm remained constant although it is higher than at the beginning. This behavior can be because at the beginning the particles had smaller sizes so the absorbance remained almost constant and small because smaller particles show less absorption and greater reflection than larger particles (20). As the experiment progressed, the absorbance increased since greater changes in absorbance are observed due to the rearrangements of the particles until it remained, again,

almost constant, but this time their absorbance was higher. Even though, the behavior is similar, in the first experiment the absorbance at 1542 nm for small particles increases faster than in the second experiment. For the second experiment, more time is needed to obtain a constant absorbance.

Typical blending profiles were shown by Sekulic (15) and Popo (14) where the standard deviations are higher at the beginning of the blending process and then decrease as the blend became homogeneous. These blending profiles corresponded to blend uniformity from a chemical point of view while the one showed in Figure 4.13 described the blend homogeneity from a physical point of view.

Another method to analyze changes in spectra is calculating the mean square of differences (S^2) as described by M. Blanco (15), where the differences between absorbance of two consecutive spectra along all the wavelengths were calculated and graphed as a function of spectral revolutions. The graph obtained for the first experiment is shown in Figure 4.14 and for the second experiment in Figure 4.15. In both cases, minor differences were observed at the beginning of the experiment were S^2 had the greatest value. For the first experiment, differences in the graph were observed between 161 and 201 revolutions where S^2 values increased. This behavior can be compared to the one obtained with the absorbance at 1542 nm graph, see Figure 4.13. For the second experiment, the biggest differences in the graph were observed between 365 and 475 revolutions were S^2 values

increased. This behavior, also, can be compared to the one obtained with the graph shown in Figure 4.13.

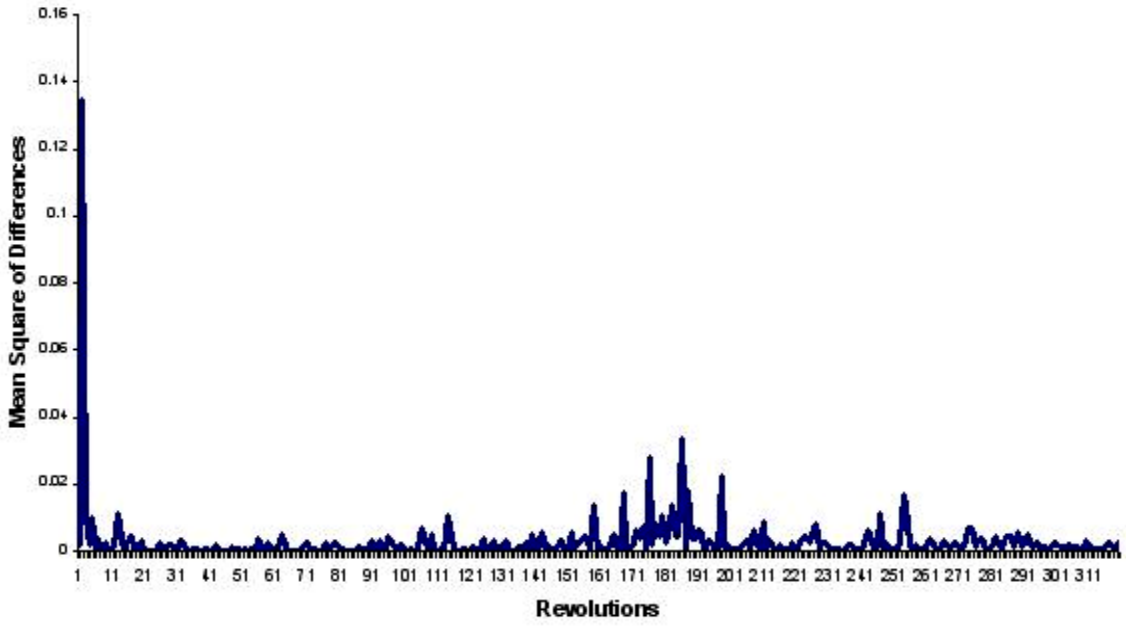


Figure 4-14 - Mean Square of Differences graph for the first experiment

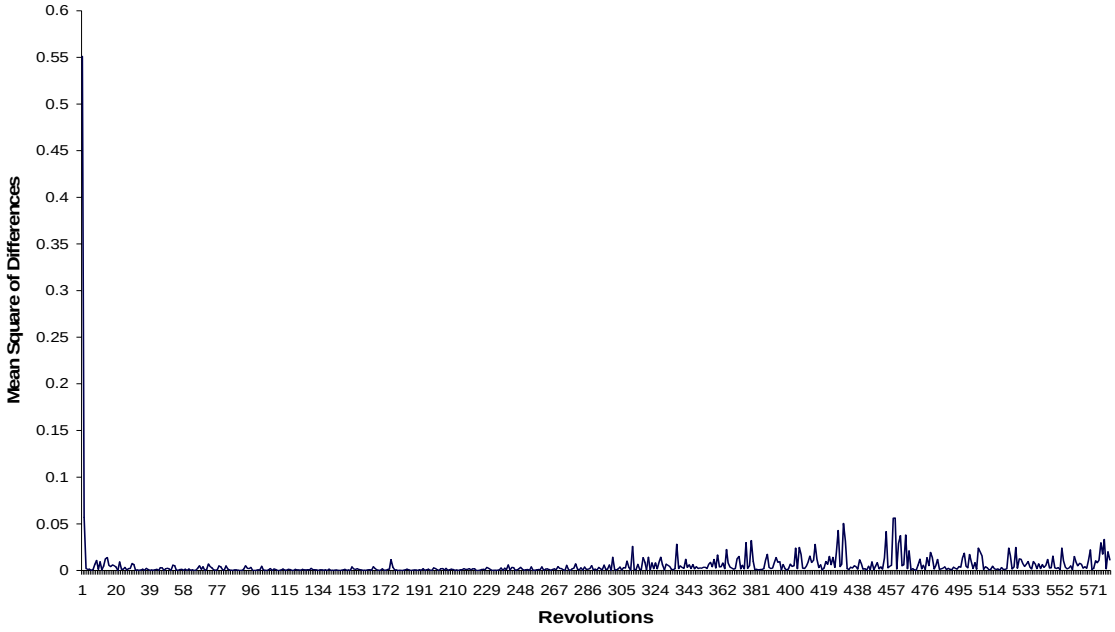


Figure 4-15 - Mean Square of Differences graph for the second experiment

An additional method to analyze changes in spectra was obtained calculating the standard deviation between the absorption intensity in three consecutives spectra (moving block) along all the wavelengths, as described by Sekulic (15). The standard deviations obtained were graphed versus spectral revolutions for the first two experiments as shown in Figure 4.16. This graph had a similar behavior as the one obtained with the mean square of differences method shown in Figure 4.14 and Figure 4.15.

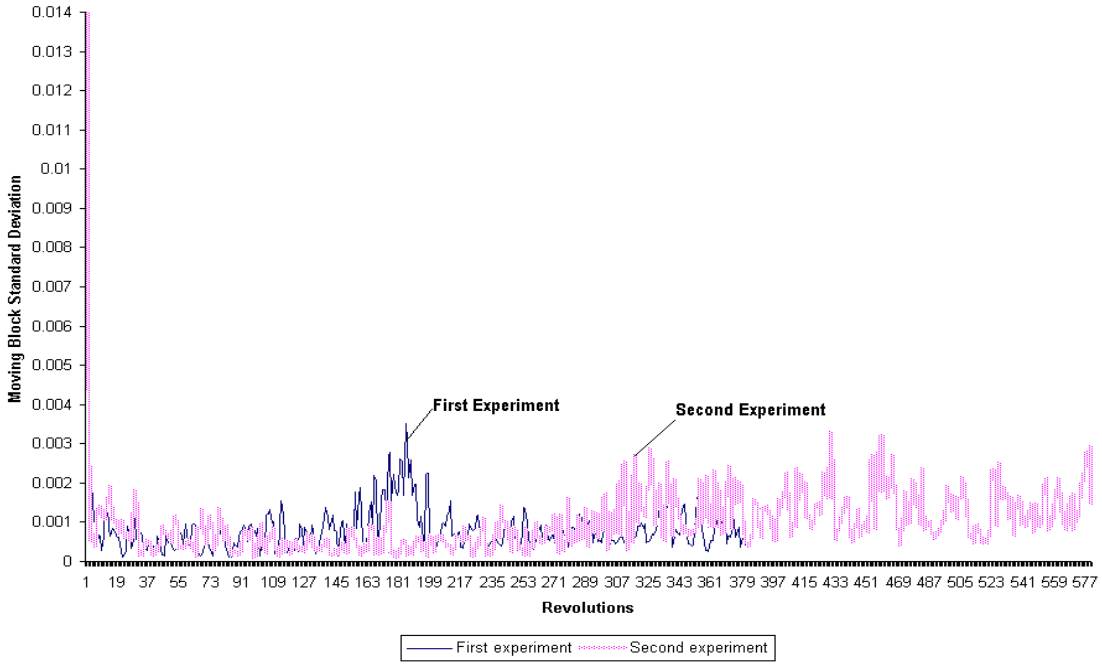


Figure 4-16 - Graph of the moving block standard deviation for the first two experiments

The two approaches, mean square of differences (S^2) and moving block standard deviations allowed observing changes in the spectra along the number of revolutions. Both approaches are used to determine the point where the mixture is homogeneous. In the case, where the homogeneity is a physical one and not chemical, as described for these experiment, the information obtained is only about the performance of the mixture with time. Greater values of standard deviations and of S^2 indicated changes in the behavior of the mixture where the particles were rearranging into a physical homogeneous blend. .

After the mixing process was completed, the granule mixture left in the V-Blender was emptied by fractions. Each fraction of approximately 500g was sieved through different mesh sizes (>710µm, >500µm, >250µm, >125µm and <125µm) and each sub fraction was

weighed to observe the distribution of particles sizes in each fraction. Table 4.5 showed the mass distribution for each granule fraction before the blending process and the sub fraction mass obtained after the distribution of the granules by particle size. The mass percent of each sub fraction was calculated to determine the fraction distribution along the V-Blender and a graph is shown in Figure 4.17.

Table 4.5 - Mass fractions for the granules before the blending process and after the distribution by particle sizes for 45 minutes blending experiment

Particle Sizes (μm)	Mass before blending process (g)	Mass after distribution of particle sizes (g)
> 710	3065.6	2996.5
> 500	1768.7	1096.2
> 250	1257.5	1168.1
> 125	N/A	582.8
< 125	N/A	454.0
Total	6320.0	6297.6

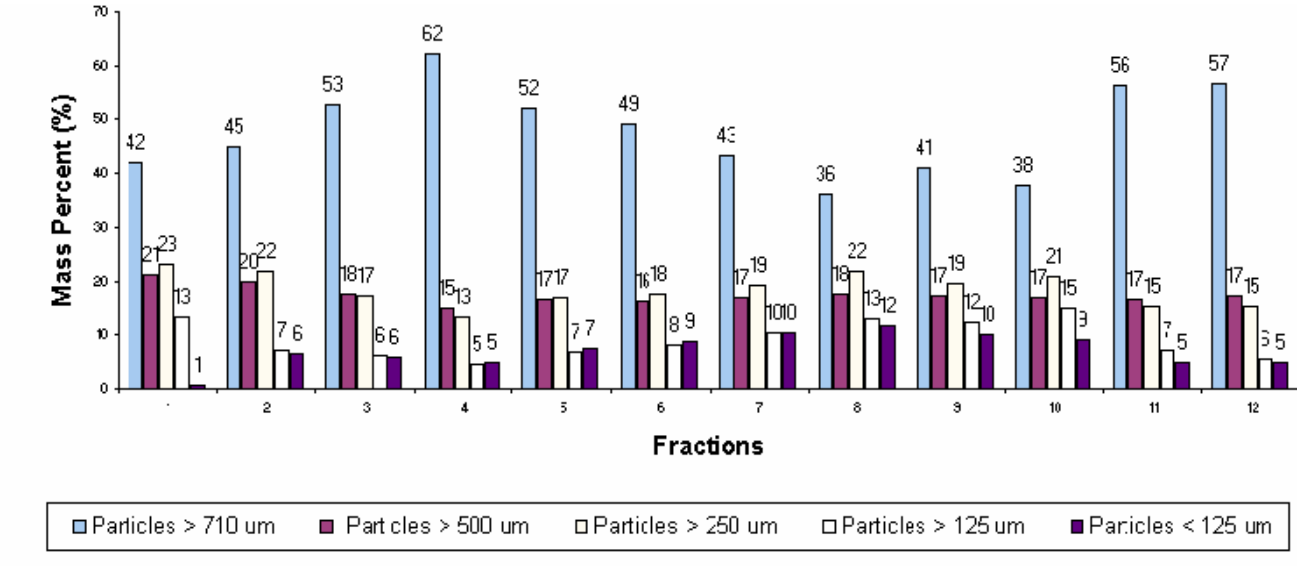


Figure 4-17 - Fraction distribution of the granules left on the V-Blender

As shown in Figure 4.17, the fractions along the V-Blender were very similar in terms of their particle size distribution. The sub fraction of particles greater than 710µm dominated all the fractions as expected because this particle size is the largest fraction inside the blender. Particles with sizes greater than 500µm and 250µm followed in order of mass percent, with percent that do not vary much between the fractions. The last two fractions (>125µm and < 125µm) were the least ones. Each fraction had similar proportions of each particle sizes according with the fractions that compose the V-Blender at the beginning of the experiment. Although these fractions gave an idea of the distribution of the granules, they are not representative of the layers of the blend when the blender is being emptied since the first granules to be collected are the ones in the middle of the blender.

4.6.3 Third Experiment

The blend homogeneity for a blend with large differences in particles sizes was again monitored using NIR. As described in the first and second experiment, the NIR probe sees only the smallest particles at the beginning of the experiment. This time the blending time was approximately one hour and a total of 932 spectra were collected. The complete spectra are shown in Figure 4.18.

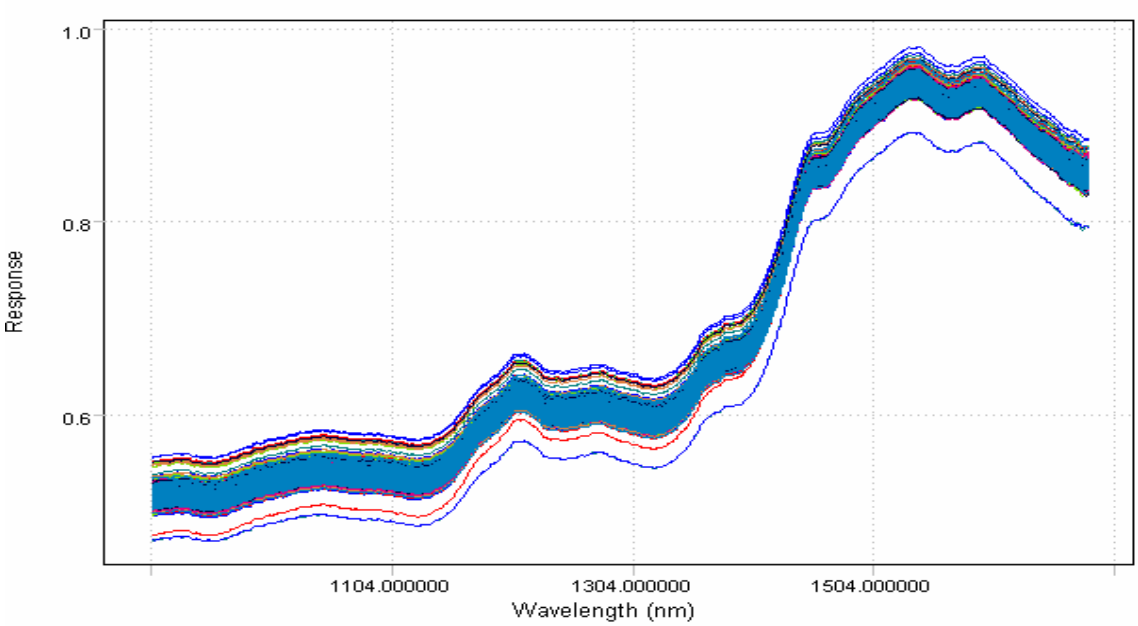


Figure 4-18 - Spectra for different particle sizes granules blend during an hour

These spectra showed little variation throughout time. Differences in baseline and absorption band are not significant in contrast with the first two experiments. The differences are notable only in the beginning and at the end of the blending process. This behavior could

be a consequence of the variation in the ratio of small particles (> 125µm and < 125µm) in the blend. The fractions for small particles in this experiment were less than for the first two. The spectra could discriminate at the beginning the layers of small particles from the rest of the blend, but these layers weren't enough to remain too long. The baseline and absorption band for these spectra covered a small range, approximate, between 0.4 and 0.6 for the baseline, and between 0.9 and 0.10 the absorption band. When these results are compared with the first two experiments where the baseline are approximate between 0.2 and 0.6 and the absorption band between 0.3 and 1.1, the difference in these ratios is obvious. For the first two experiments, the time to observe the larger particle sizes and, eventually, the homogeneity for the blend is higher than for this experiment. The blend rapidly became homogeneous and the variations in the spectra were minimal.

The spectra obtained during the mixing process were transformed using subtract where variable100 (1004 nm) was set to zero, letting the changes in absorption to be more significant. The spectra with the transformation are shown in Figure 4.19.

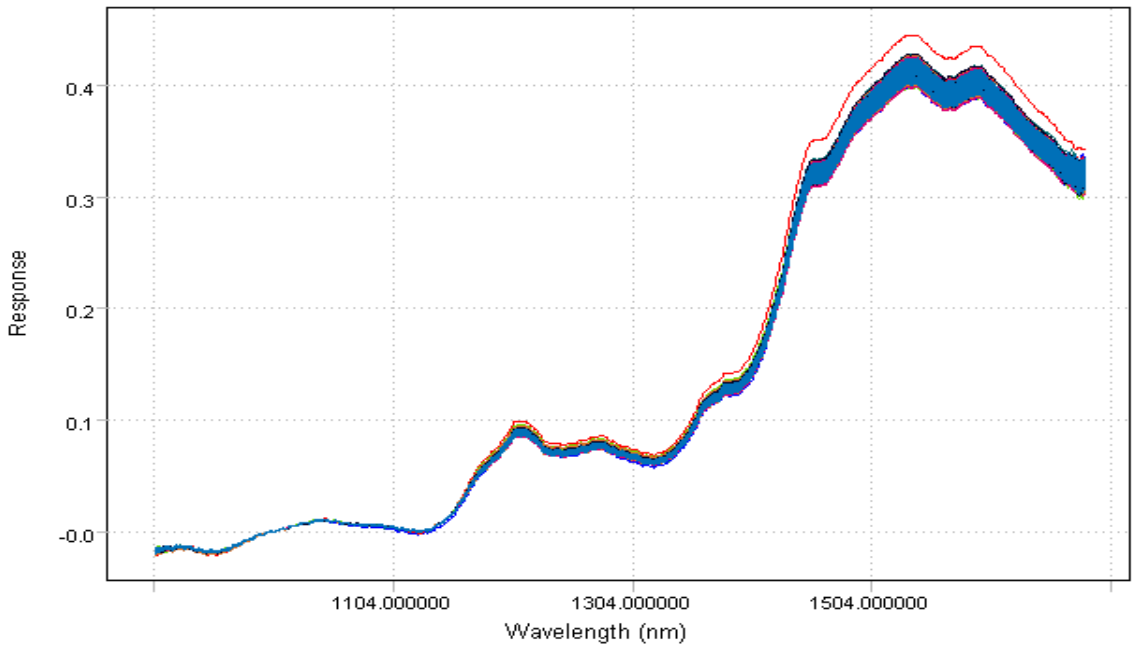


Figure 4-19 - Spectra with subtract transformation for an hour blending experiment

The differences in absorbance after subtraction were not significant as shown in Figure 4.19.

The moving block standard deviation was calculated for the spectra shown in Figure 4.19 and a graph as a function of time was obtained as shown in Figure 4.20. The standard deviations for these spectra were similar between them; the values fluctuate between 0.0002 and 0.008. The differences in standard deviation are not significant at any point of the graph, only at the beginning. The behavior for this graph is similar from the graph for the first two experiments as showed in Figure 4.16.

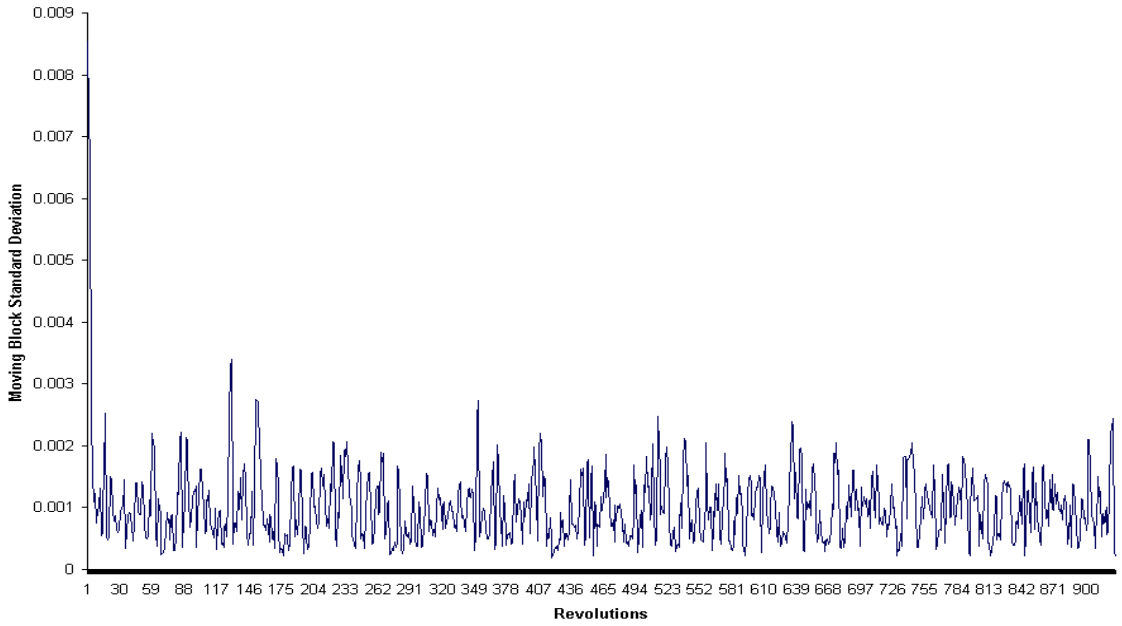


Figure 4-20 - Graph of the moving block standard deviation versus revolutions (number of spectra) for an hour blending experiment

An approach to observe if changes were present in the spectra along the revolutions was graphing the absorbance at 1542 nm of all the spectra versus revolutions as shown in Figure 4.21. In this case, no significant differences were notable in any spectrum and the blending profile obtained for the first two experiments are not longer observed.

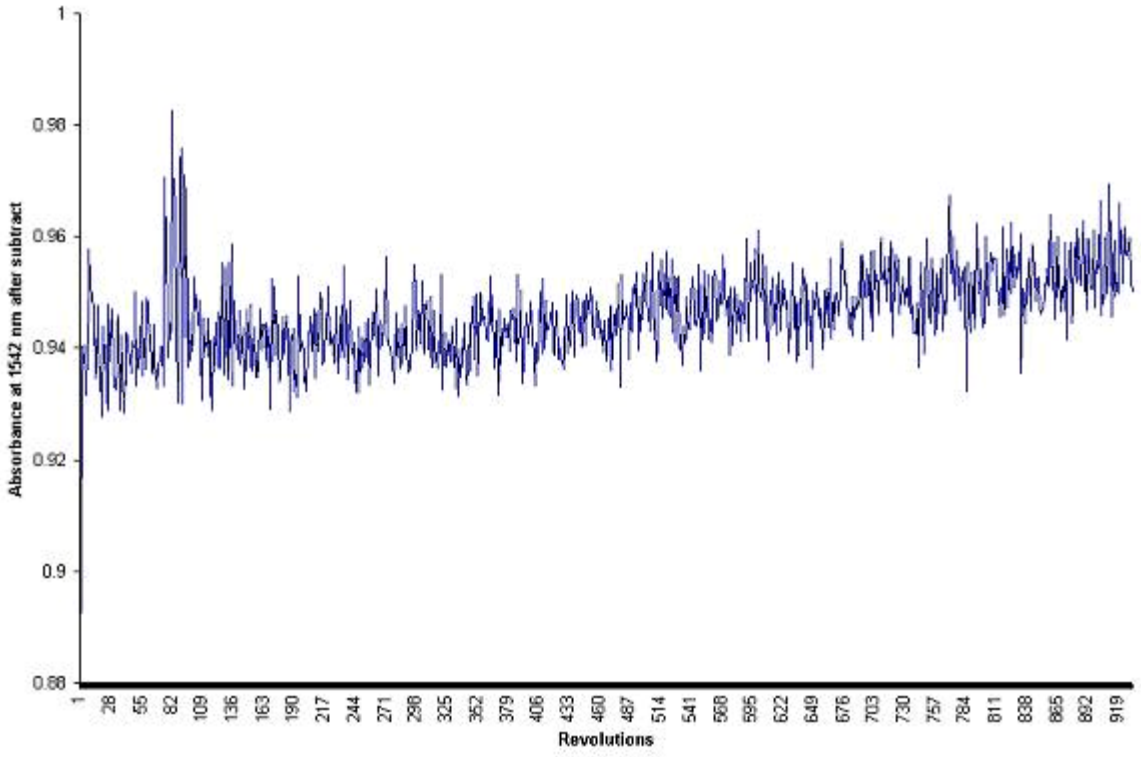


Figure 4-21 - Graph of absorbance at 1542 nm after subtract of each spectra versus revolutions (number of spectra)

The granules left into the V-Blender were emptied in fractions and sieved through different mesh sizes (>710µm, >500µm, >250µm, >125µm and <125µm) and each sub fraction was weighed to observe the distribution of particles sizes in each fraction. Table 4.6 showed the mass distribution for each granule fraction before the blending process and the sub fraction mass obtained after the distribution of the granules by particle sizes. As seen in Table 4.6, the mass distribution for the smaller sub fractions (>125µm and <125µm) increased due to the attrition of the particles during the blending process. The mass percent of each sub fraction was calculated to determine the fraction distribution along the V-Blender and a graph is shown in Figure 4.22.

Table 4.6 - Mass fractions for the granules before the blending process and after the distribution by particle sizes for an hour blending experiment

Particle Sizes (μm)	Mass before blending process (g)	Mass after distribution of particle sizes (g)
> 710	3228.7	3094.9
> 500	1384.0	1066.2
> 250	1553.6	1198.2
> 125	150.0	751.6
< 125	125.7	270.3
Total	6442.0	6381.2

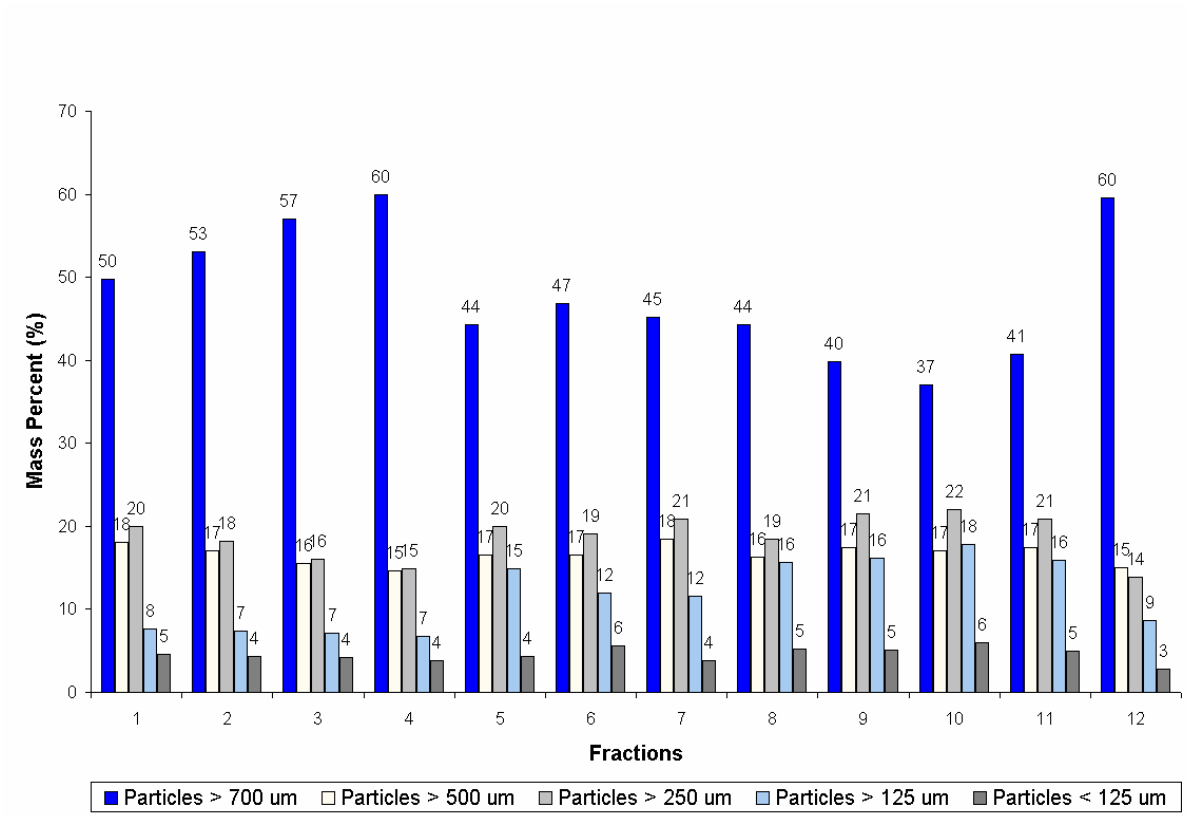
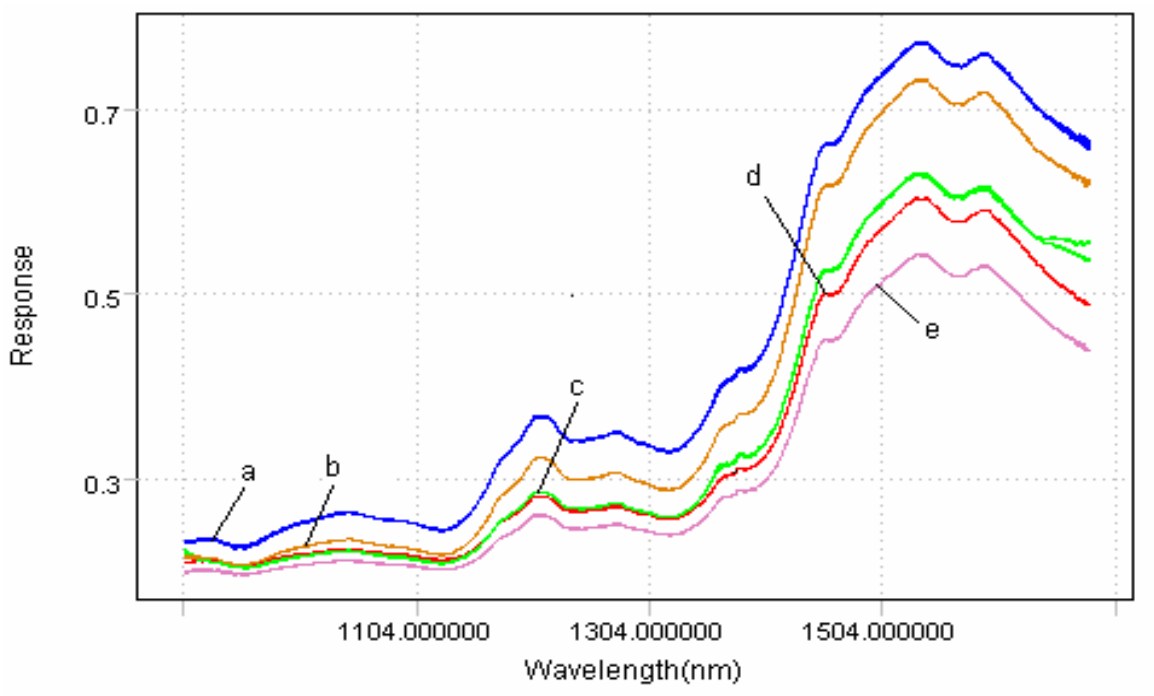


Figure 4-22 - Fraction distribution of the granules left on the V-Blender

As shown in Figure 4.22, the fractions are similar along the V-Blender. Particles sizes greater than 710 µm dominated all the fractions as expected because they present in greater quantity. The mass percent for particle sizes greater than 500 µm and 250 µm were very similar in all the fractions. The last two sub fractions varied from fractions, but were the least ones. As can be observed, each fraction had similar proportions of each particle sizes according with the fractions that compose the V-Blender at the beginning of the experiment.

4.6.4 Fourth Experiment

The experiment to monitor blend homogeneity with NIR was again performed keeping the entire procedure constant, but this time spectra of granules of different particle sizes were taken in static form. To observe the differences in baseline and absorption band, MSC and subtract transforms were applied to the spectra. Figure 4.23 showed the spectra after the MSC transform.

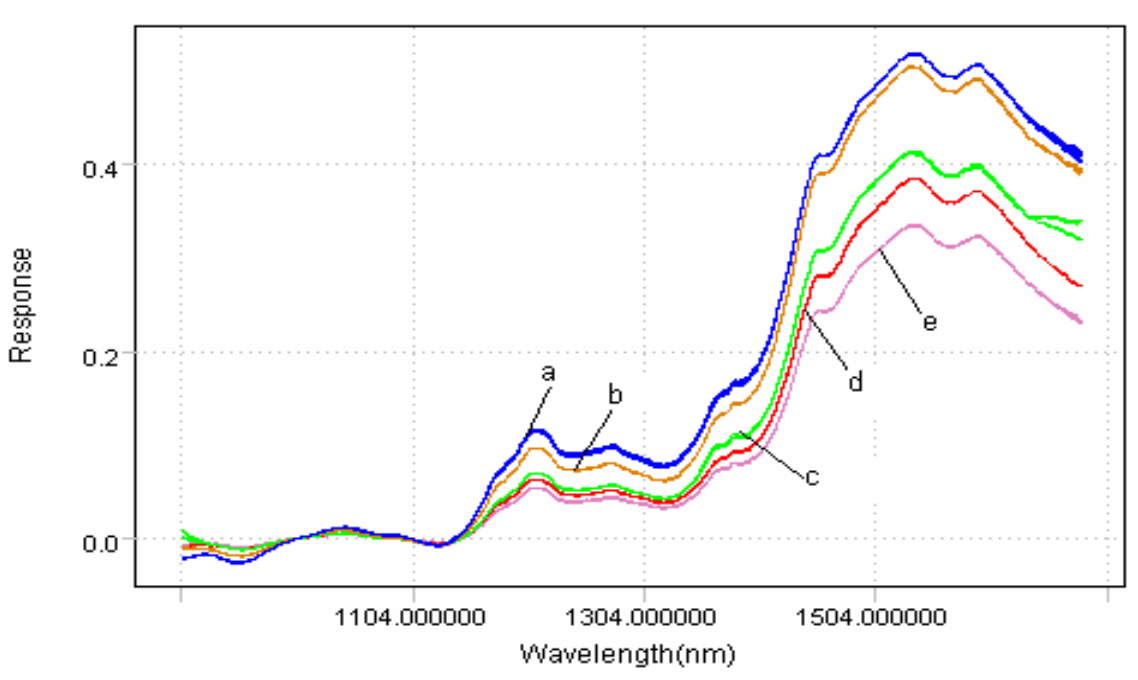


Particle Sizes:

a > 710µm b > 500µm c > 250µm d > 25 µm e < 125 µm

Figure 4-23 - MSC spectra for granules with different particle sizes - static form

In Figure 4.23, each spectrum shown corresponds to one particle sizes. As particle sizes increases the baseline and absorption band also, increases as expected. Figures 4.24 showed the spectra in static form with MSC and subtract transformations to observe better the changes in absorption.



Particle Sizes:

a > 710µm b > 500µm c > 250µm d >125 µm e < 125 µm

Figure 4-24 - MSC and subtract spectra for granules with differences in particle sizes - static form

After corroborating that the differences in particle sizes are large enough to be discriminated by NIR the blend homogeneity was monitored for approximately an hour. A total of 785 spectra were taken. All the spectra obtained are shown in Figure 4.25. These spectra showed little variation in baseline and absorption bands throughout time. The behavior of these spectra was similar to the third experiment where the differences in particles sizes are not observed during the blending process. The differences can be observed only at the beginning of the process.

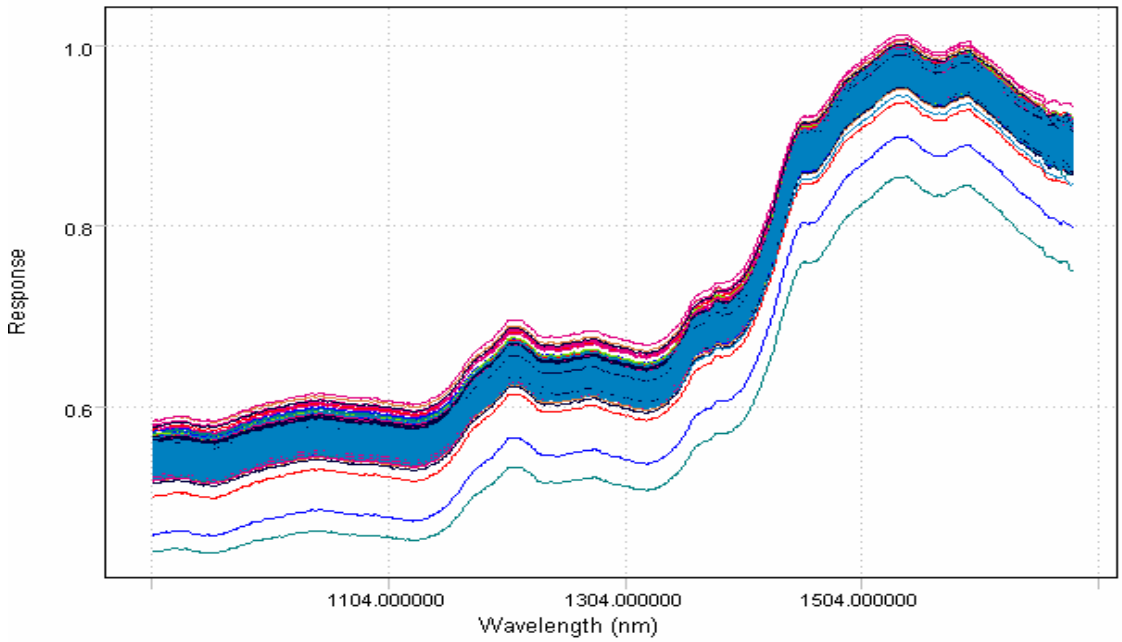


Figure 4-25 - Spectra for different particle sizes granules blend during an hour

The spectra obtained during the mixing process were transformed using subtract where variable100 (1004 nm) was set to zero, letting the changes in absorption to be more significant. The spectra with the transformation are shown in Figure 4.26.

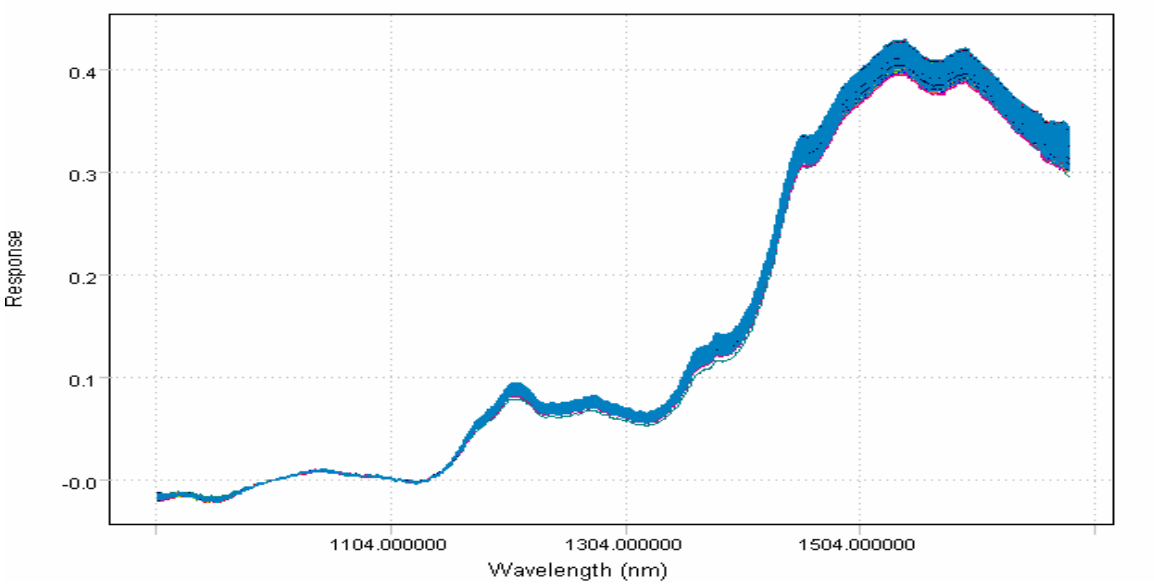


Figure 4-26 - Spectra with subtract transformation for an hour blending experiment

As shown in the spectra in Figure 4.26, the variation of the absorbance band is not significant during any time during the blending process. Like the third experiment, this one had a lower fraction of small particles sizes (> 125 µm and <125 µm) than the first two experiments. The baseline and the absorption band had a small range. For the first two experiments, the time to observe the larger particle sizes and, eventually, the homogeneity for the blend is higher than for this experiment. The blend rapidly became homogeneous and the variations in the spectra were minimal as observed in the third experiment.

The moving block standard deviation was calculated for these spectra and a graph as a function of time was obtained as shown in Figure 4.27. The standard deviations for these spectra were similar between them; the values fluctuate between 0.0005 and 0.005. The

behavior for this graph is very different for the first two experiments, but similar to the third experiment.

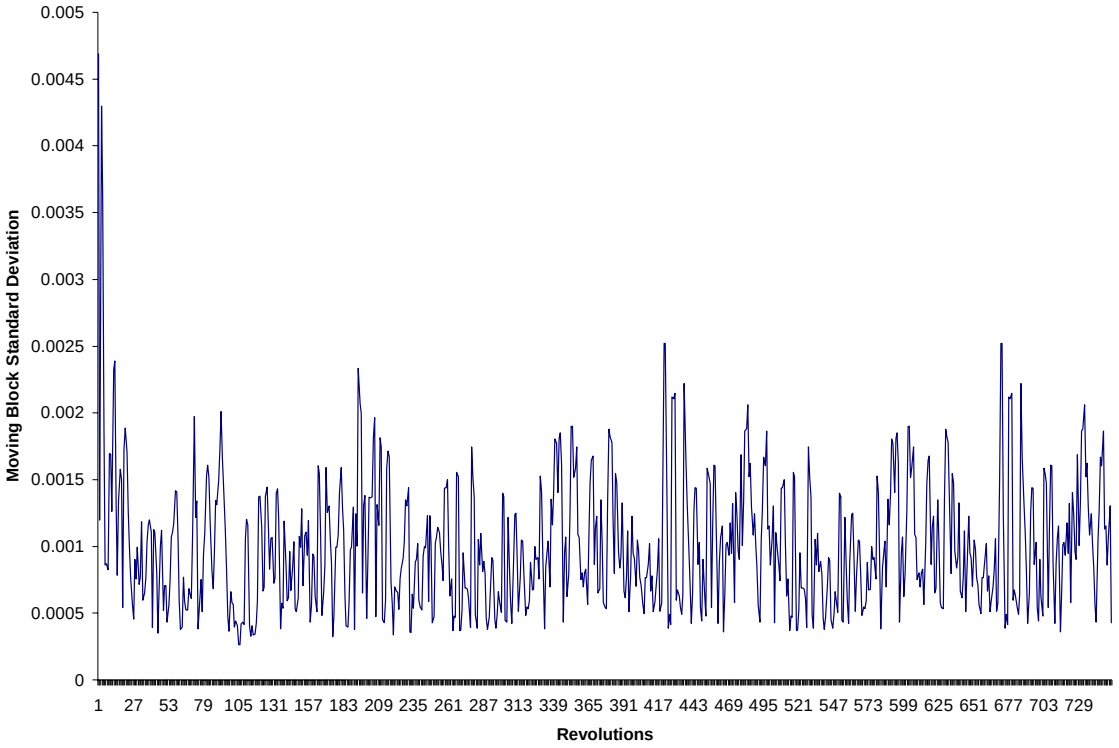


Figure 4-27 - Graph of the moving block standard deviation versus revolutions (number of spectra)

The absorbance at 1542 nm for all the spectra was graphed versus revolutions to observe the differences between spectra. Difference between them was not significant and blending profile wasn't obtained as shown in Figure 4.28.

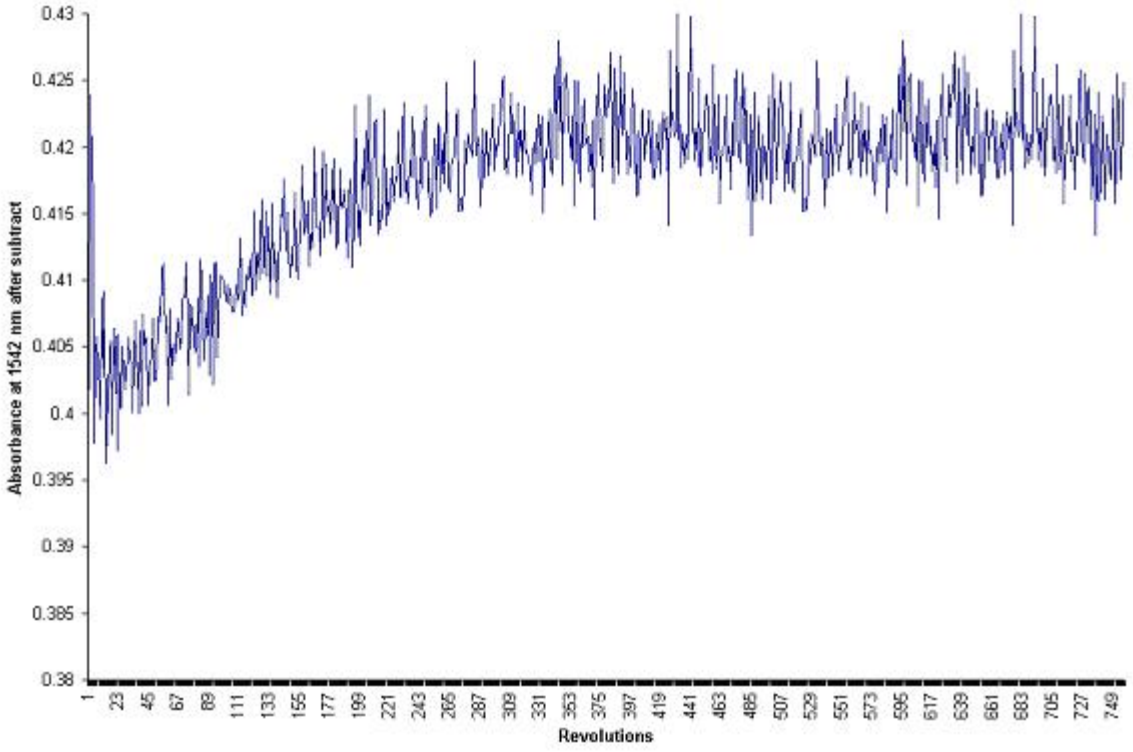


Figure 4-28 - Graph of absorbance at 1542 nm after subtract of each spectra versus revolutions (number of spectra)

4.6.5 Lactose and Granules Experiment

A lactose and granules blend was prepared to monitor the blend homogeneity of this blend by NIR for approximate an hour and a half. This time, the blend consisted only in granules with particle sizes greater than 710 µm and ground lactose monohydrate (obtained with a mortar and pestle) to get two layers into the V-blender with notable differences in particle sizes. At the beginning of the experiment, the smallest particles (lactose) were near the NIR probe window. After the mixing process ended, the blend inside the V-Blender was observed. The blend observed was dominated by lactose.

The spectra taken during the entire mixing process are shown below in Figure 4.29. Each spectrum corresponds to a revolution of the V-Blender, a total of 1038 spectra were collected. As shown in the spectra, the baseline and the absorption of them were changing during the first two minutes of the blending process. The blend rapidly became physically homogeneous where the probe only sees a mixture of lactose and granules with small changes in baseline and absorption band.

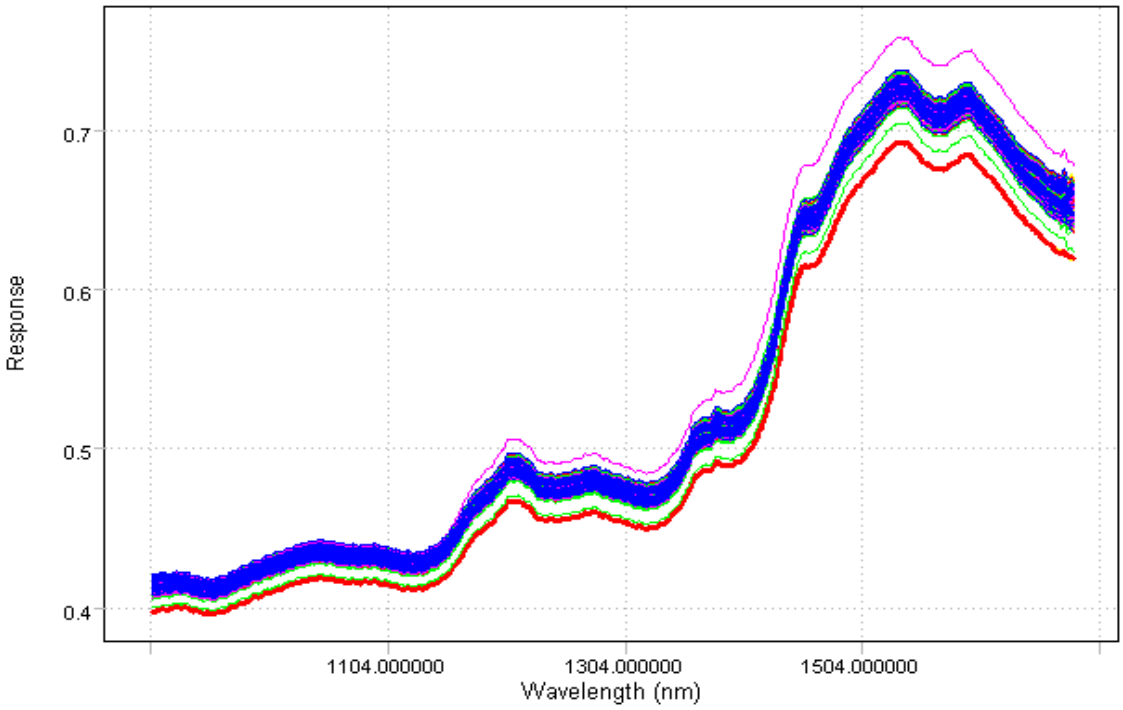


Figure 4-29 - Spectra of lactose and granules blend

The spectra obtained during the mixing process were transform using subtract where variable100 (1004 nm) was set to zero, emphasizing the changes in absorption. The spectra with the transformation are shown in Figure 4.30.

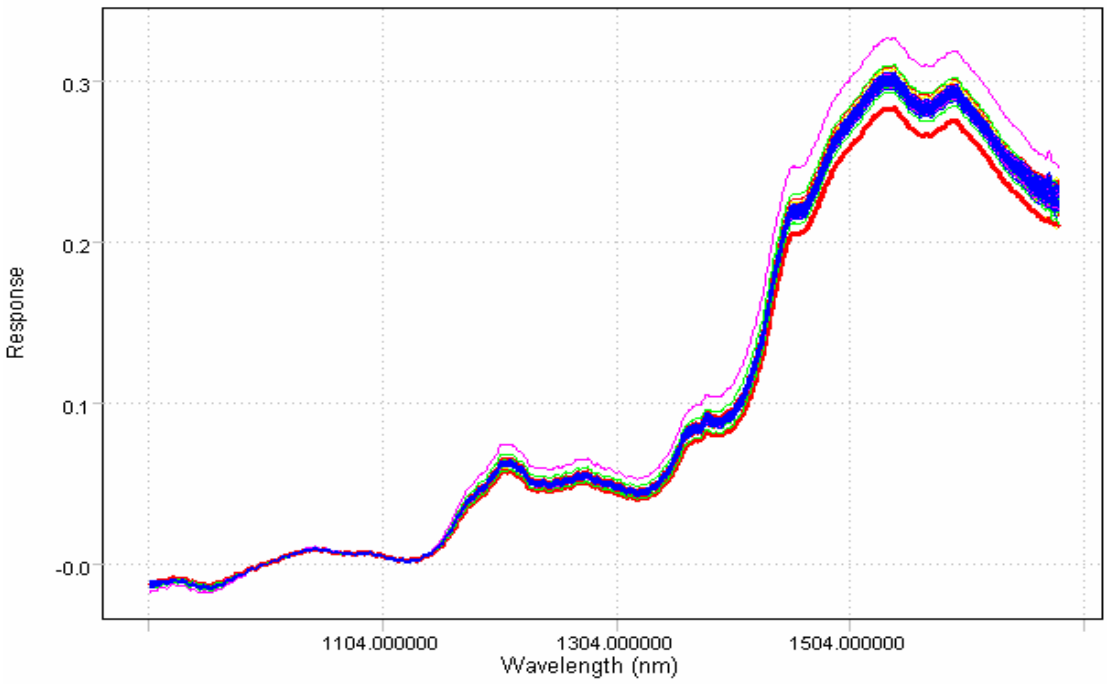


Figure 4-30 - Spectra with subtract transformation for the lactose and granules blend

As shown in the spectra in Figure 4.30, the variation of the absorbance band is not significant during any time during the blending process.

The moving block standard deviation in was calculated for these spectra and a graph as a function of time was obtained as shown in Figure 4.31. The standard deviations for these spectra were similar between them, no significant changes were observed through the entire spectra.

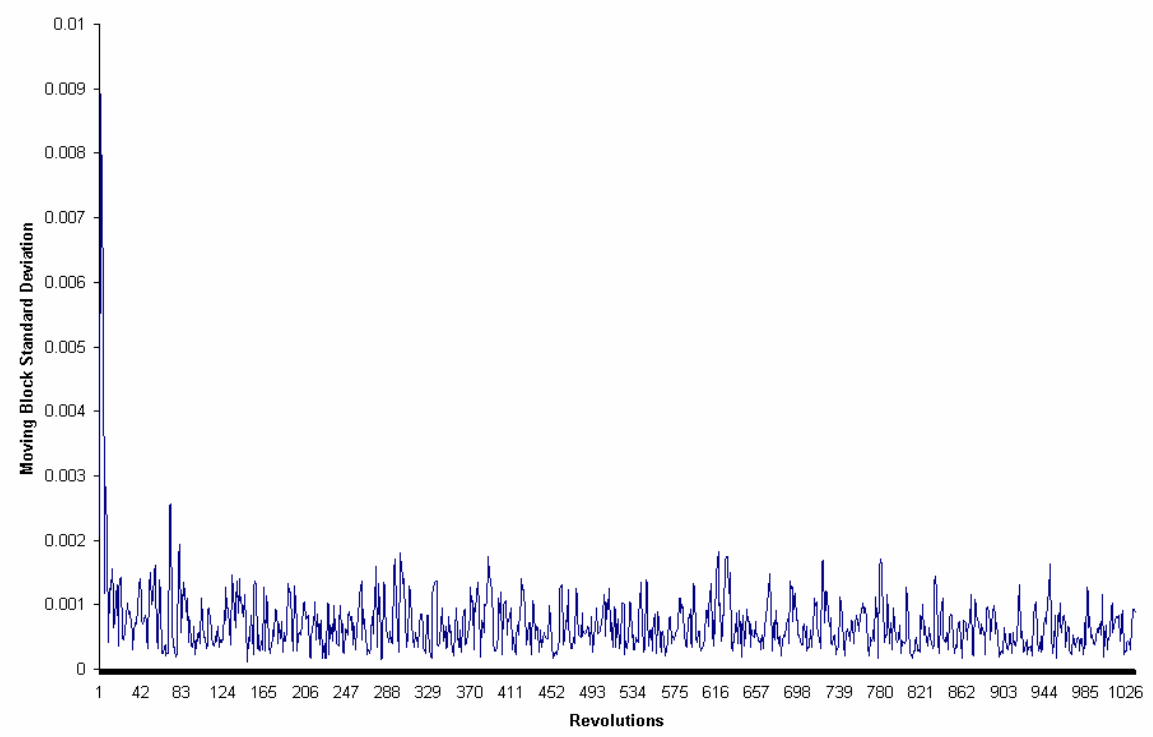


Figure 4-31 - Graph of the moving block standard deviation versus revolutions (number of spectra)

The absorbance at 1542 nm for all the spectra was graphed versus revolutions to observe the differences between spectra. Difference between them was not significant and blending profile was not obtained as shown in Figure 4.32.

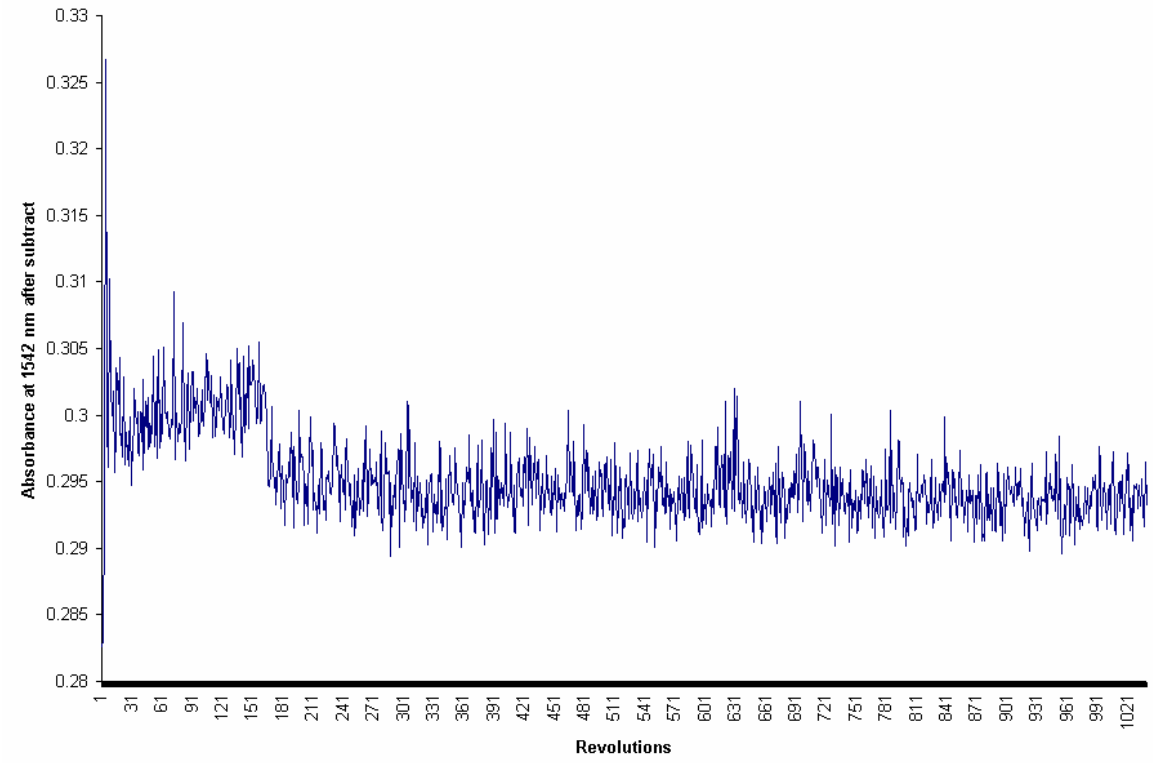


Figure 4-32 - Graph of absorbance at 1542 nm after subtract of each spectra versus revolutions (number of spectra)

4.6.6 Graphs of absorbance at 1542 nm using different transformations

Different transformations were used to observe if the physical information were retained in the spectra for the first experiment (32 minutes blending experiment). The transformations used were first derivative (25 points), second derivative (25 points), multiplicative scattering correction (MSC) and standard normal variate (SNV). Graphs were plotted using the absorbance at 1542 nm after the transform versus the number of revolutions.

The first transformation used was the first derivative to remove the slope from the spectra and the graph is shown in Figure 4.33. The absorbance at 1542 nm after first derivative did not show significant changes in absorption. The range of absorbance was between -0.00035 to -0.0002.

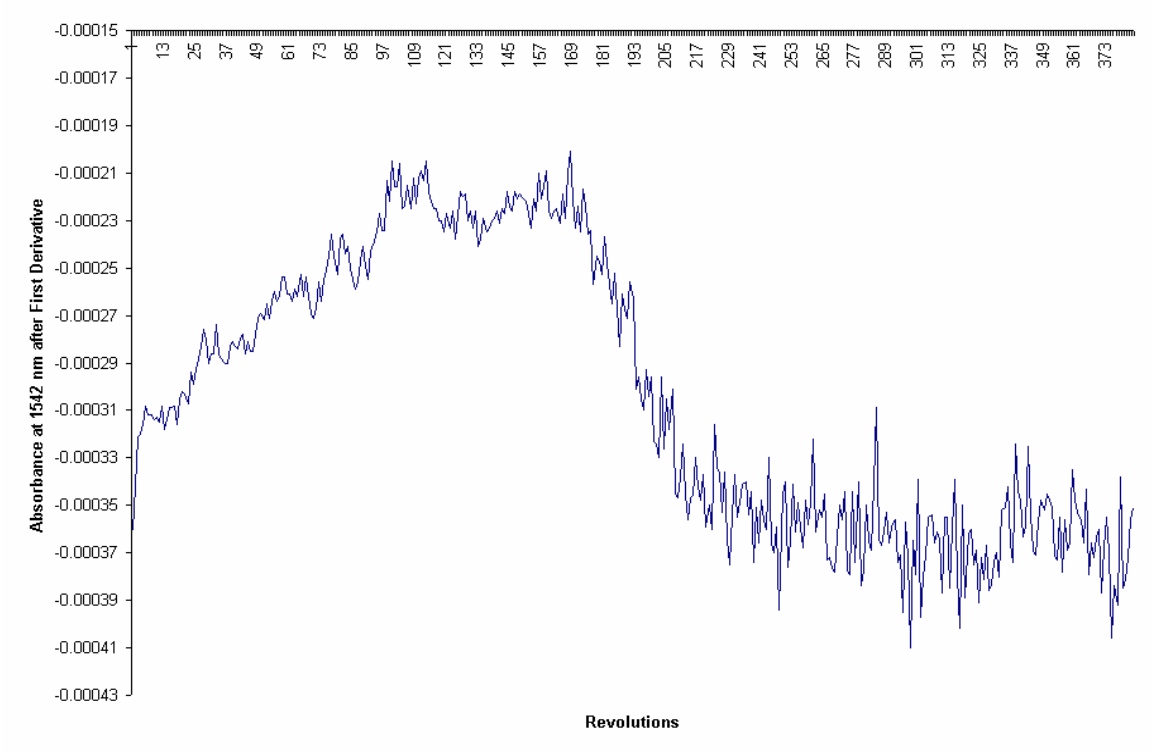


Figure 4-33 - Graph of absorbance at 1542 nm after First Derivative versus revolutions for the 32 minutes blending experiment

Other transformed used was second derivative and the graph is shown in Figure 4.34. The absorbance at 1542 nm after second derivative did not show significant changes in absorption. The range of absorbance was between -0.00001 to -0.00008.

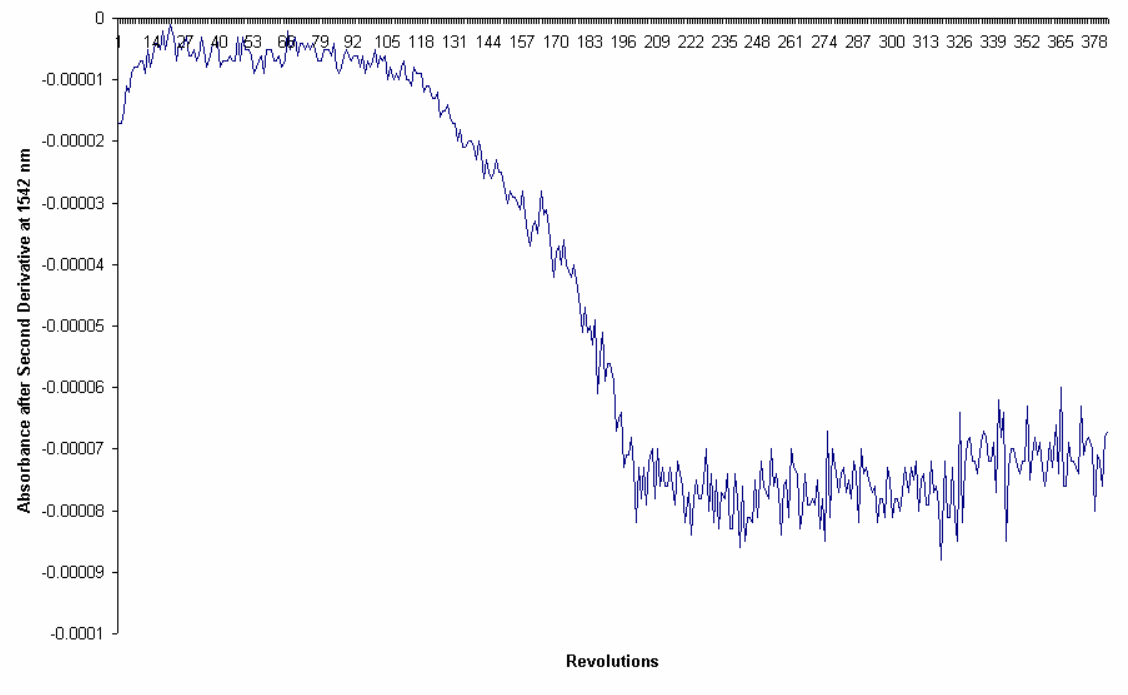


Figure 4-34 - Graph of absorbance at 1542 nm after second derivative versus revolutions for the 32 minutes blending experiment

Another transform used was MSC. The graph of the absorbance at 1542 nm after MSC was plotted against revolutions as shown in Figure 4.35. The graph has the same tendency as the one obtained for subtract (Figure 4.13), but the range of absorbance is a narrow one. The absorbance range obtained with this transform was, approximately, between 0.693 and 0.72.

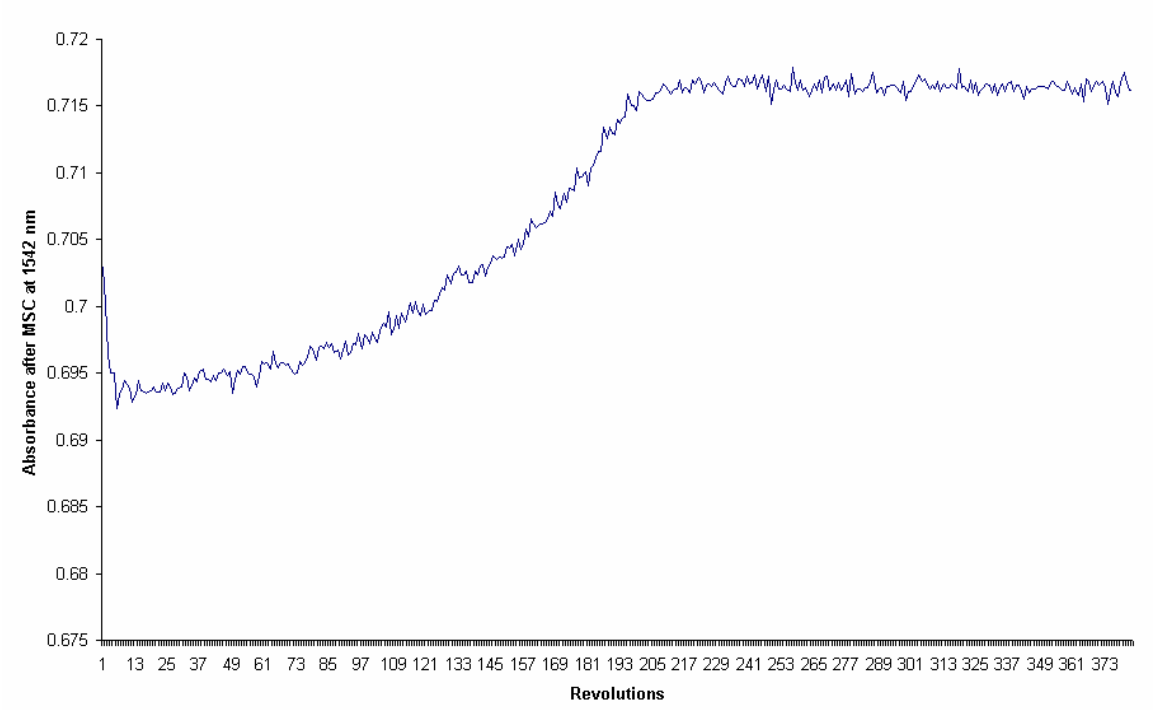


Figure 4-35 - Graph of absorbance at 1542 nm after MSC versus revolutions for the 32 minutes blending experiment

An additional transform used was SNV. This transform removes the multiplicative interferences of scatter and particle sizes. SNV removes by mathematical calculation the slope variation on an individual sample basis (21). This transform had a similar effect on the spectra as MSC. The graph of the absorbance at 1542 nm after SNV was plotted against revolutions as shown in Figure 4.36. The graph has the same tendency as the one obtained for subtract (Figure 4.13) and the graph for MSC (Figure 4.35). The absorbance range obtained was obtained 1.37 to 1.64.

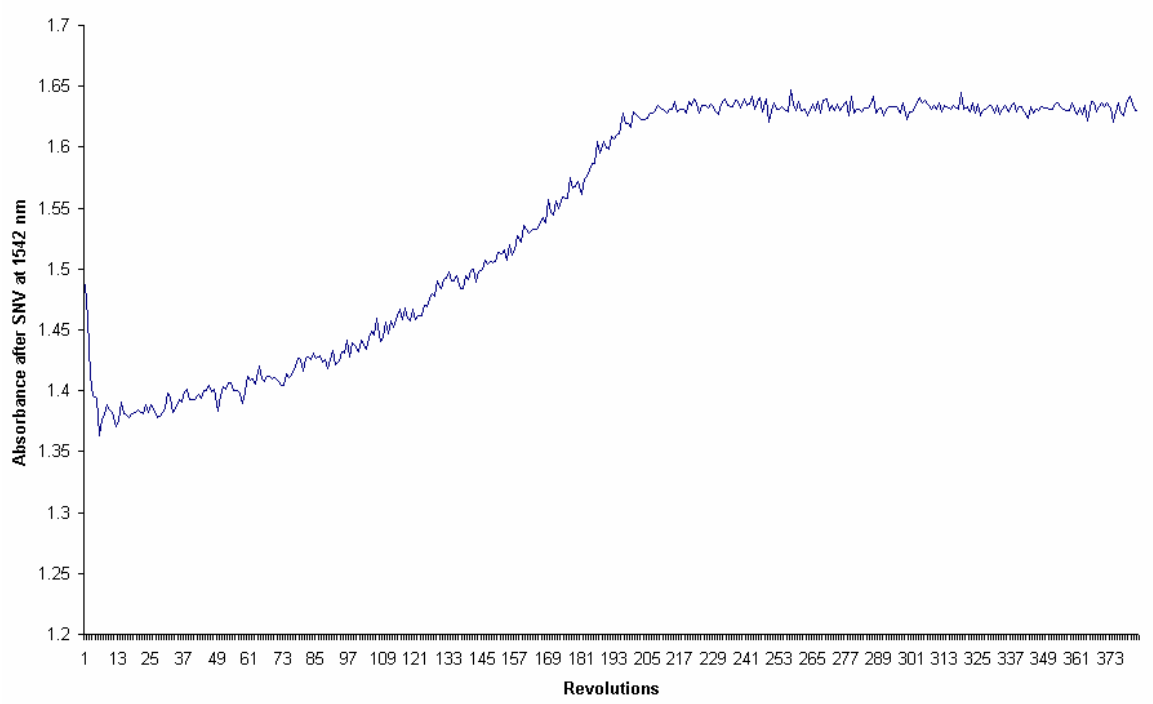


Figure 4-36 - Graph of absorbance at 1542 nm after SNV versus revolutions for the 32 minutes blending experiment

To observe the blending profiles obtained with subtract, SNV and MSC transform in the same scale, the three transform were plotted versus number of revolutions as shown in Figure 4.37. As shown in Figure 4.37, the blending profiles obtained with SNV and subtract have similar magnitude when they are compared. With MSC, the blending profile is like a straight line because the range is a narrower than for SNV and subtract. The blending profiles after SNV, MSC and subtract transform are still observed although with MSC, the physical information is almost completely eliminated.

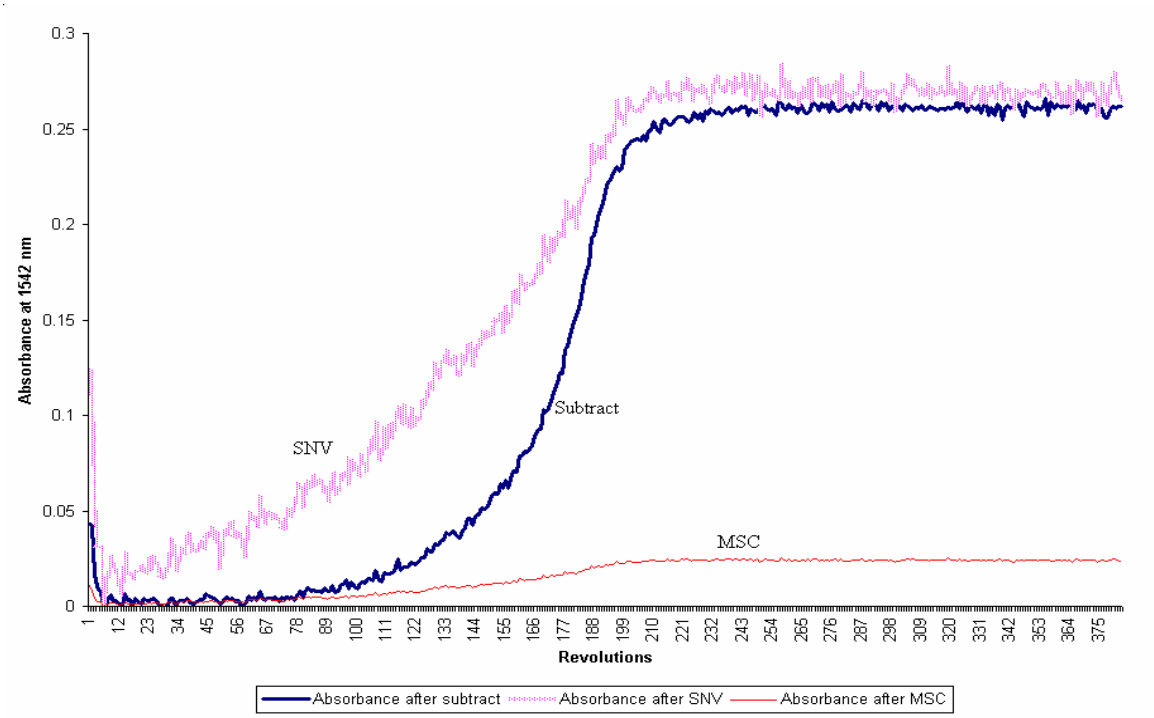


Figure 4-37 - Blending profile of absorbance at 1542 nm after SNV, MSC and Subtract versus revolutions

4.6.7 Granules spectra using Raman Spectroscopy and ATR IR-Spectroscopy

4.6.7.1 Raman Spectroscopy

Spectra for granules of different particle sizes (> 710 µm, > 500 µm, > 300 µm and > 125 µm) were acquired using Raman Spectroscopy to confirm that these granules had the same spectrum. A spectrum for the granules is shown in Figure 4.38. The spectra did not show any difference when they are compared between them. The spectra confirmed that all

the granules are chemically the same. An area of the spectra was zoomed in between 1600 and 400 cm-1 to observe clearly each spectrum as shown in Figure 4.39.

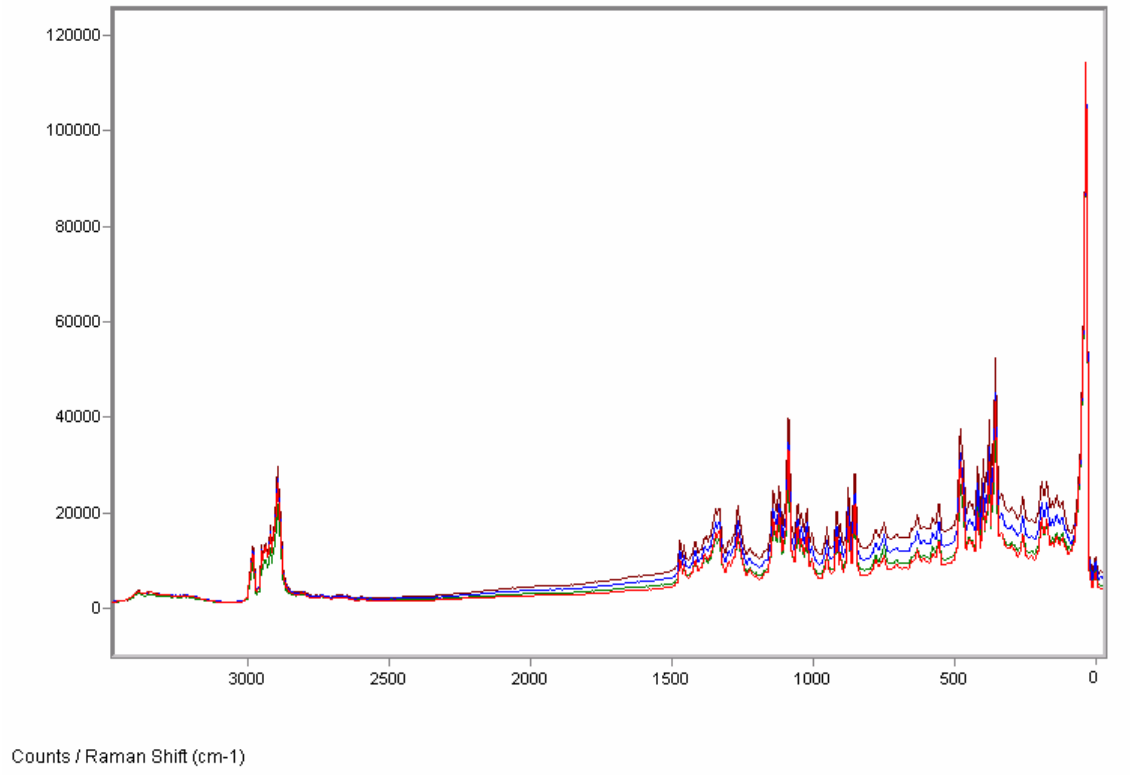


Figure 4-38 - Raman spectra for granules of different particle sizes

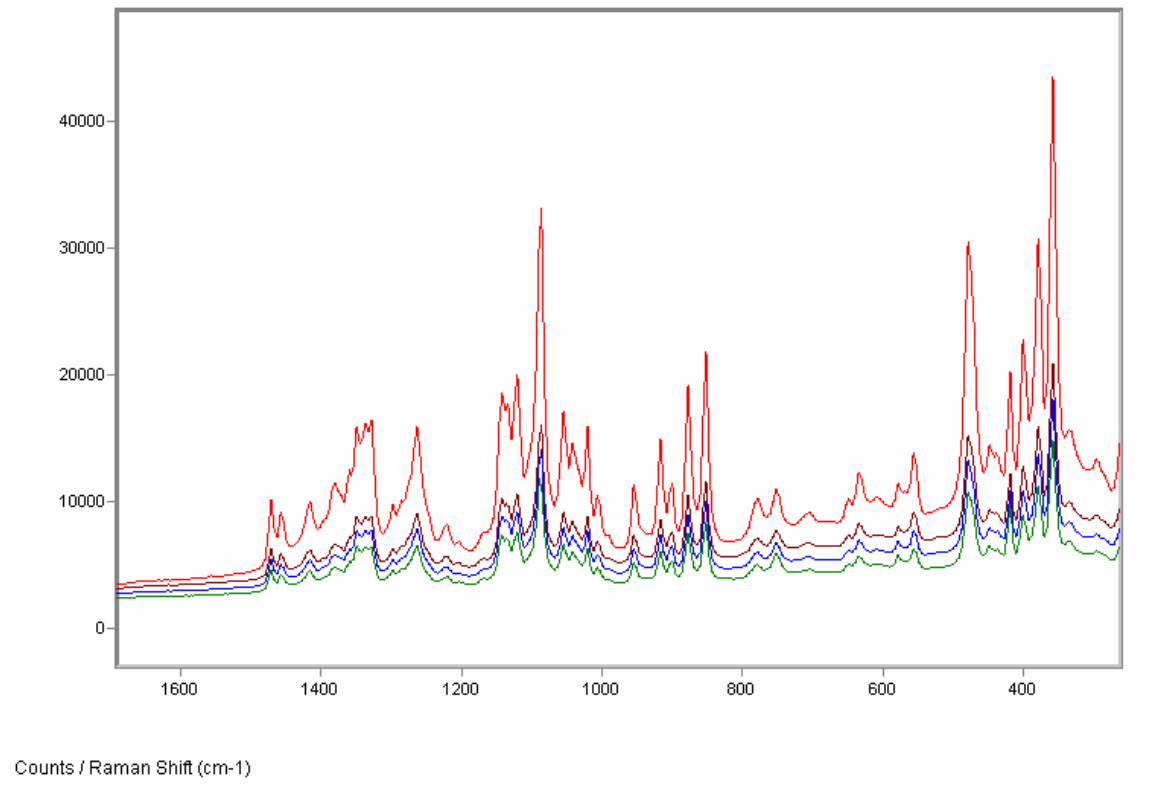


Figure 4-39 - Raman spectra for granules of different particle sizes with a zoom view from 1600 to 400 cm^{-1}

4.6.7.2 ATR-IR Spectroscopy

Spectra for granules of different particle sizes (> 710 µm, > 500 µm, > 300 µm and > 125 µm) were acquired using ATR-IR Spectroscopy to confirm that these granules had the same spectrum. A complete spectrum for the granules at a resolution of 16 cm^{-1} is shown in Figure 4.40. Also, a spectrum from the fingerprint region at the same resolution is shown in Figure 4.41. The spectra did not show any difference when they are compared between them in the fingerprint area. The spectra confirmed that all the granules are chemically the same.

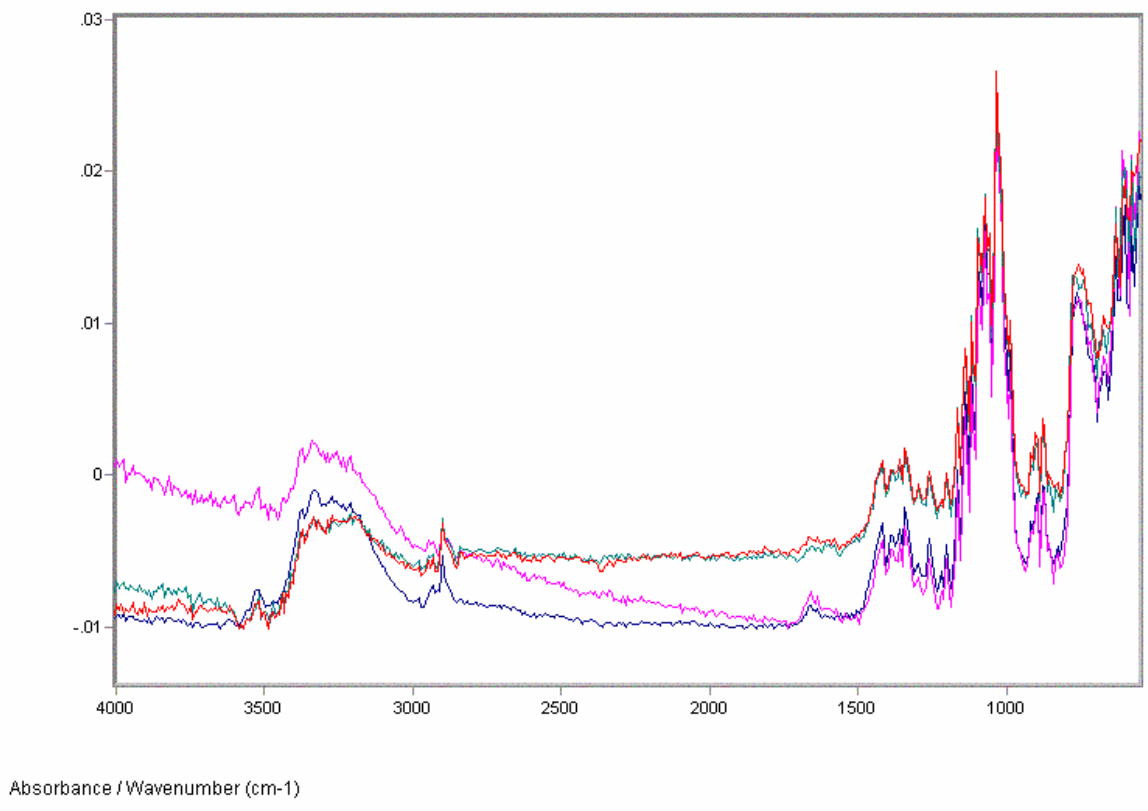


Figure 4-40 - ATR spectra for granules at a resolution of 16 cm^{-1}

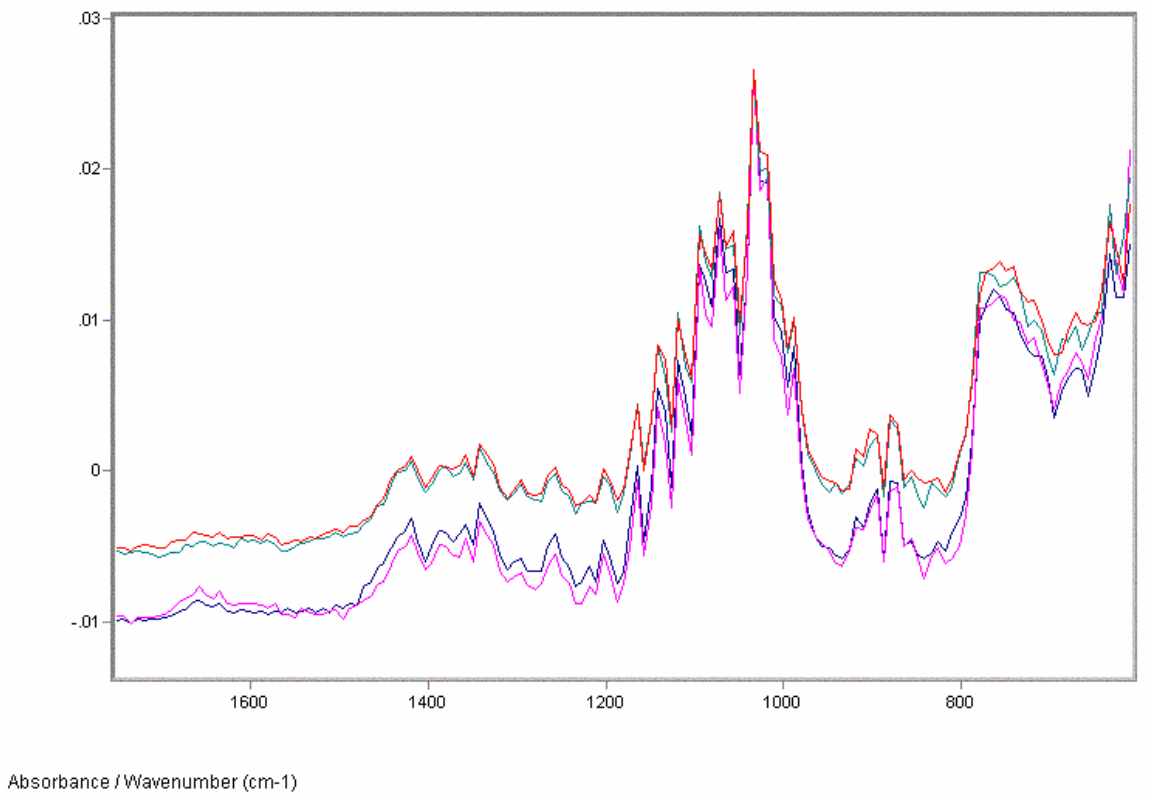


Figure 4-41 - ATR spectra for granules of different particle sizes from the fingerprint region at a resolution of 16cm^{-1}

5 CONCLUSIONS

Three sampling methods: pocket thief, Bellamy's thief and stream sampling were evaluated during the first part of this work. The results for the three methods were comparable when using a 20% (m/m) ibuprofen- granules blend. Sample weight variation and RSD between 10 to 13 % for ibuprofen content were obtained. After performing the distribution of ibuprofen by particle sizes of granules was observed that as the particle sizes increases, the ibuprofen content decrease.

The on-line NIR spectrometer allowed monitoring the blend uniformity from a physical point of view. Differences in baseline and absorption band were observed through the blending process. These differences were due to differences in particle sizes of the granules. Smaller particles had less absorption than the greater ones because they reflected more. Subtract transform allowed these changes in absorption to be more significant. Also, MSC, another transform used, was a versatile one to observe changes in baseline and absorption. Blending profiles were obtained using SNV, MSC and subtract transform and plotting the absorbance at 1542 nm versus revolutions. These blending profiles gave an idea of the blending process. The differences between layers of smaller particles and the greater ones inside the V-blender influence in the time that takes the blend to be homogeneous.

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