



Ana Sofia Mendonça Cortes

Bachelor in Chemical and Biochemical Engineering

**Comparative study of particle size distribution
analysis by laser diffraction between dry and liquid
dispersion methods**

Dissertation submitted in fulfilment of requirements for degree of Master in
Chemical and Biochemical Engineering

Advisor: Dr. Sérgio Silva, Scientist – Particle Engineering, Hovione
Farmaciencia, SA

Co-advisor: Dr. Mário Eusébio, Assistant Professor, Faculdade de
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Jury:

President: Dr. Susana Filipe Barreiros

Arguer: Dr. Ana Isabel Nobre Martins Aguiar de Oliveira Ricardo

Member: Dr. Sérgio Manuel Campos da Silva



FACULDADE DE
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Particle size is an important variable, especially in the pharmaceutical industry, since this is one of the parameters that can influence medicines performance.

Drugs can be administered by different routes of administration and in different dosage forms. The active products analyzed in this study are characterized as inhalation products to be administered as dry powder inhalers.

This type of active product is characterized by particles between 1 μm and 5 μm . Particles in this diameter range can travel through the lungs to the bronchi and alveoli area, where they settle by sedimentation. The performance of these dosage forms is compromised if the API does not meet the specifications regarding particle size.

Two techniques based on laser diffraction were used to determine the particle size distribution of several active pharmaceutical ingredients. Both work under the same principles, however, over the last 20 - 30 years, they have evolved differently. They present two major differences: the mathematical algorithm and the means of dispersion of the particles.

Two types of equipment were used for this type of analysis: Malvern Mastersizer Hydro 2000 S and Sympatec (consisting of Helos, Rodos / M and Aspiros units) for the liquid and dry dispersion, respectively. To verify the accuracy of the results an electronic microscope, the Phenom ProX Generation 5, was used.

After all the analyses and the comparison of the results it was observed that it is possible to obtain similar results of particle size distribution with different techniques. However, this is only verified if the analysis method of both equipments allow a complete particle deagglomeration.

In this study, due to the inherent characteristics of the products and the limits of the equipment, difficulties appeared and made the particle deagglomeration process difficult.

Keywords: particle size, laser diffraction, liquid dispersion, dry dispersion

O tamanho das partículas é uma variável importante, especialmente na indústria farmacêutica, sendo que este é um dos parâmetros que pode influenciar a eficácia dos medicamentos.

Os medicamentos podem ser administrados através de diferentes vias e em diferentes formas farmacêuticas. Os produtos ativos analisados neste estudo são caracterizados como produtos de inalação para serem administrados através de inaladores de pós secos.

Este tipo de produto ativo é caracterizado por partículas entre os 1 μm e 5 μm . Partículas nesta gama de diâmetro conseguem percorrer os pulmões até à zona dos brônquios e alvéolos, nos quais se depositam por sedimentação. Se o produto ativo não estiver de acordo com as especificações, um dos parâmetros afetados será a sua eficácia que diminuirá.

Para averiguar a distribuição do tamanho das partículas foram usadas duas técnicas que têm por base a difração a laser. Ambas têm o mesmo fundamento, no entanto, ao longo dos últimos 20 - 30 anos, evoluíram de forma distinta. Elas apresentam duas grandes diferenças: o algoritmo matemático e o meio de dispersão das partículas.

Foram usados dois equipamentos para este tipo de análises: Malvern Mastersizer Hydro 2000 S e o Sympatec (constituído pelas unidades Helos, Rodos/M e Aspiros) para a dispersão líquida e seca, respetivamente. Para verificar a veracidade dos resultados foi usado um microscópio eletrónico, o Phenom ProX Generation 5.

Após as análises e a comparação dos resultados foi possível verificar que através de diferentes equipamentos é possível obter resultados semelhantes no que diz respeito à distribuição de tamanhos das partículas. No entanto, isto só é verificado se os métodos de análise de ambos equipamentos possibilitarem uma desagregação total das partículas.

Neste estudo, devido às características inerentes dos produtos e limites dos equipamentos, dificuldades surgiram que dificultaram o processo de desagregação das partículas.

Palavras-chave: tamanho de partículas, difração a laser, dispersão líquida, dispersão seca

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Glossary

Excipients = also known as inactive ingredients, they are agents that combine with active ingredients to facilitate drug transport in the body [1].

Potency = is a measure of drug activity expressed in terms of the amount required to produce an effect of given intensity [2].

Potent APIs = a pharmacologically active ingredient or intermediate with biological activity at approximately 15 $\mu\text{m/kg}$ of body weight or below in humans. Characterized with high selectivity and/or potential to cause cancer, mutations development effects or reproductive toxicity at low doses [3].

Acronyms

API = Active Pharmaceutical Ingredient

EDS = Energy Dispersive Spectroscopy

MALVERN = MALVERN Mastersizer HYDRO 2000S

nm = nanometers

PS = Particle Size

PSD = Particle Size Distribution

RN = Record Number

SEM = Scanning Electron Microscope (Phenom ProX)

SYMPATEC = HELOS + RODOS/M + ASPIROS

US = ultrasounds

WP = Wet Polishing

Chapter 1

Introduction

1.1 Analysis of Particle Size

Particle size influences many properties of particulate materials and is a valuable indicator of quality and performance. Particle size can also influence a large variety of important physical properties, manufacturing processability and quality attributes. Some examples are dose uniformity, aerosolization behavior and performance [6].

Considering particulate materials, the particle size distribution (PSD) is an important parameter to analyze. PSD requirements of materials depend on the industry and on the materials' final use. For instance, in paint and pigment industries, it is important that the PSD is within specified limits or else it may influence the gloss appearance. The shape and size of glass beads may affect the reflectivity of the highway paints, if PSD is not within the specifications. Another example can be found in the food industry where the PSD of chocolate powders impacts its flavor and color [5].

The PSD is undoubtedly important in diverse industries [5].

In the pharmaceutical field, drug substances and dosage forms can be highly affected by the particle size, therefore PSD is a critical process parameter in pharmaceutical production [6]. For example, the particle size and shape may affect the dissolution rate and bioavailability of active pharmaceutical ingredients, as well as the drug release rate for sustained and controlled release formulations [6].

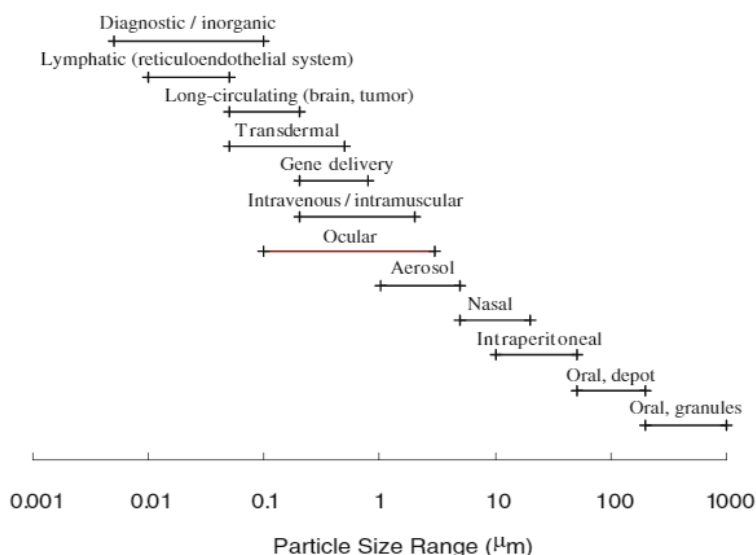


Figure 1-1 Particle size range general requirements for different dosage forms and routes of administration [6]

In Figure 1-1, it is presented the variety of ideal particle size (PS) ranges for different dosage forms, depending on the routes of administration [6]. Different routes of administration will have different particle size limitations to obtain an ideal performance.

The particle size distribution and shape of the delivered dose is more critical for inhalation than most of the other conventional drug products because these factors greatly influence the deposition profile in the lungs of the patient [7].

This study is focused on APIs (active pharmaceutical ingredients), designed for dry powder inhalers, which means that they must be designed to have an aerodynamic diameter less than 5 μm , to be suitable for respiratory delivery [8]. The optimum aerodynamic particle size distribution for most inhalation aerosols has generally been recognized as being in the range of 1 – 5 μm [5-6]. If the particles are within this range, they can deposit themselves in the bronchial and alveolar regions predominantly by sedimentation, and have the best pulmonary penetration [6].

The particle size will not only greatly vary with the dosage form or route of administration, but also with the place where it must deposit to achieve an optimum response.

APIs are formulated with excipients, so that they can be dispersed into their primary respiratory size upon inhalation through dry powders inhalers [8]. Normally, this type of inhalers contain an active ingredient and one or more excipient to aid powder dispersion and flow [7].

In pulmonary delivery of pharmaceuticals, the particle size distribution is the most important parameter affecting the formulation performance [6].

In the scope of this study and the measurement range of the particles (nanometers), a particle can be described as a 3-dimensional object, which unless a perfect sphere, it cannot be fully described by a single dimension such as diameter [4]. To describe the size of a particle it is used the spherical form as a reference. In other words, it is determined the spherical equivalent diameter and that way it is possible to evaluate every particle equally [3-4].

Since the spherical shape is the only shape that can be described as one measurement, using the spherical equivalent diameter makes comparing results easier between analyses.

As mentioned, if the particle is spherical, the diameter is used to describe the particle. However, when the particle has an irregular shape, depending on the direction it is measured, vertically or horizontally, for instance, the diameter is going to have different dimensions (Figure 1-2) [5].

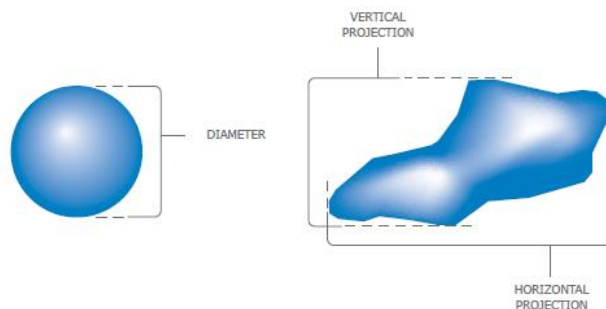


Figure 1-2 Example of particle shape: spherical particle (left) and irregular particle (right) [5]

To describe an irregular particle, it is necessary to have multiple lengths and widths to provide a measurement with a good accuracy. To simplify, many PSD techniques make the assumption that every particle is spherical, being the reported value the equivalent diameter [4]. It is conveyed that this assumption has no impact in most products, however, that may change with particles with large aspect ratio such as needle shaped particles.

The results can be presented in diverse ways or types of distributions, for example: weighted, number weighted, volume weighted or intensity weighted [4]. Usually, they are represented by a frequency or a cumulative distribution curve [9].

Although there are different types of distributions that can be used to display PS results, equipments that use techniques such as laser diffraction, will present the results as a volume weighted distribution [3-4]. This is often useful from a commercial perspective as the distribution represents the composition of the sample as volume per mass, and therefore its potential monetary value [4].

The volume weighted distribution is more used than the others mentioned. However, it is important to know that if samples are being compared or re-analyzed, they must be done with the same technique, because if not, they cannot be compared, since the results will be too different due to the different calculation mechanism [4].

However, if needed, it is possible to convert the results for the number weighted distribution, for instance, but that will increase greatly the number of errors induced [5].

When using the volume weighted distribution, it is common to have the results displayed based on the maximum particle size for a given percentage of the sample, in which the most common percentiles reported are Dv10, Dv50 and Dv90 [4].

Table 1-1 Definition of the reported percentiles

D	Diameter
v	Volume weighted distribution
10, 50, 90	Percentage of sample below this particle size (it can be written in decimal fraction)

In this study Dv10, Dv50 and Dv90 will be the percentiles reported, however, they will be written as d(0.1), d(0.5) and d(0.9). The v can be dropped because if omitted, the assumed distribution shall be the volume weighted since it is the most commonly used.

In Figure 1-3 it is possible to observe the percentiles d(0.1) and d(0.9) on a distribution, as well as the median which is represented by d(0.5) [5].

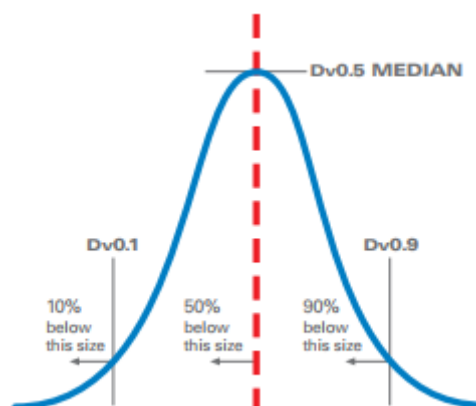


Figure 1-3 Percentiles reported: d(0.1), d(0.5) and d(0.9) [5]

Using the percentiles $d(0.1)$, $d(0.5)$ and $d(0.9)$ it is possible to calculate another PSD parameter: span. The span is calculated as shown in the equation from Figure 1-4.

$$Span = \frac{d(0.9) - d(0.1)}{d(0.5)}$$

Figure 1-4 Equation for span parameter

Span parameter is important to understand if the particle size range is wide or narrow [5]. If the span value is high (for example, higher than 2) it is probable that the distribution has two distinct populations regarding the PS. Typically a PSD with span higher than 2 displays two distinct populations, one of fine particles and other of larger particles. Usually, a normal (gaussian) distribution has a span between 1 and 2.

1.2 Methods to Measure Particle Size

When analyzing a new product, it is necessary to assess all possible parameters such as particle size and shape, surface and mechanical properties, so that we get all the information needed for later analyses and/or for interpreting the results obtained [4]. The particle size can be measured by different methods in different conditions, usually, dry or wet.

For the techniques per se, in this study, they are focused solely on two: image analysis for SEM and laser diffraction using both MALVERN (Malvern Mastersizer HYDRO 2000S) and SYMPATEC (Symptec HELOS, RODOS/M and ASPIROS). The main difference between the two last mentioned, is the particle size calculation algorithm and the mechanism of the equipment. However, the original concept behind is similar.

The SEM works almost like an optical microscope but with a greater range. However, by itself, it is not enough to determine the PSD of a sample. Errors can be induced, especially if the sampling is not well executed [4], because it will mean that what is being observed is not the reality of the bulk and this will lead to incorrect results. However, this is a common problem to all techniques.

It is imperative not to use the SEM alone, but with other techniques as executed in this study, not only to get a closer and an actual PSD, but to ensure that the PS method used by other techniques is correct and accurate since errors can be induced through different sources.

Each of the equipments used in this study have an analytical method to follow. MALVERN's method is stricter than SYMPATEC's in the way that is always the same for the same product, since it is valid for a limited range of PS. However, with SYMPATEC, even with the same product, if the PS of the batch is different, probably, new conditions must be studied to achieve a good dispersion for the analyses.

For MALVERN and SYMPATEC, PS is determined by laser diffraction. This technology has been used in the last 20 – 30 years and it has been optimized throughout the years resulting in a well-established technique that it is the standard technology and widely used in the pharmaceutical industry [6, 10].

With laser diffraction methods, it is possible to have fast analyses (usually less than a minute), wide measurement range, high precision, reproducibility, instant feedback and low maintenance [6, 10]. Another strong point is that the sample can be analyzed in both states, liquid or solid [10].

The determination of PS using laser diffraction technique is based on the angular variation in the intensity of the light scattered as a laser beam passes through a dispersed particulate sample, as can be observed in Figure 1-5 [4, 11].

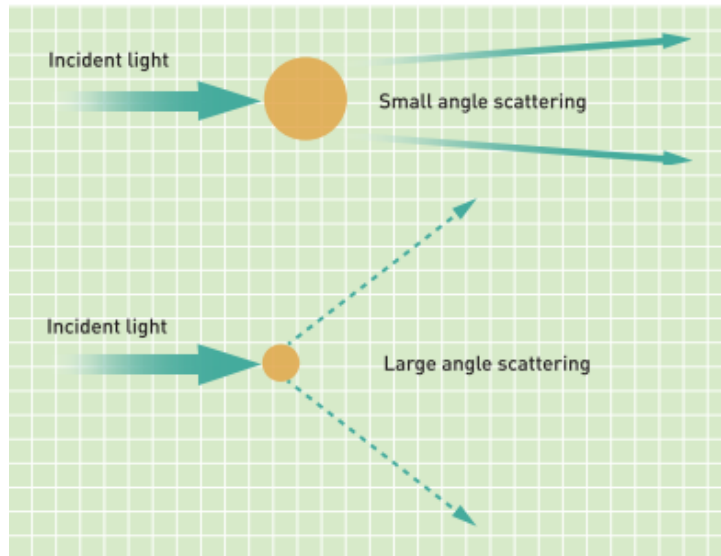


Figure 1-5 Scattering angle of large particles (above) and small particles (below) [12]

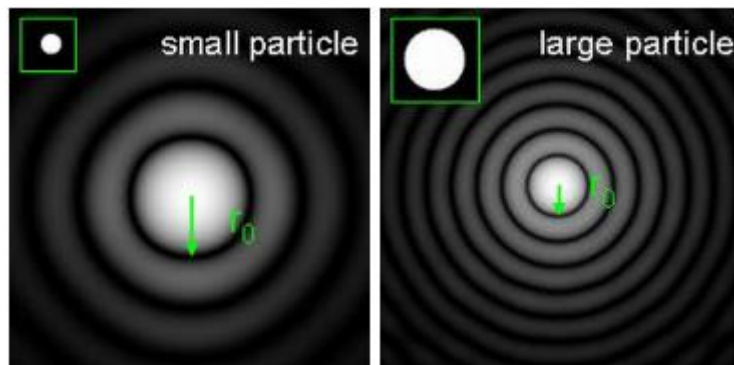


Figure 1-6 Diffraction pattern of a small particle (left) and a large particle (right) [13]

The effect of the laser beam is different depending on the size of the particles in the sample. Smaller particles scatter the light at larger angles while large particles scatter the light at smaller angles [12]. This can be demonstrated through both Figure 1-5 and Figure 1-6.

After analyzing the angular intensity data, it is possible to calculate the PS of the particles responsible for the scattering patterns applying the Mie or the Fraunhofer diffraction theories [4].

Both consist of mathematical expressions that use the input information to originate the link between both variables, angular intensity and particle size. These theories are used to calculate what kind of light intensity distribution patterns are produced by particles of various sizes, and this data is stored beforehand on a computer as a parameter table (numerical table) containing a vast amount of information [14].

A considerable amount of time is required for calculating this parameter table. However, in actual measurement of PSD, the measurement time is not affected since there are parameters tables already calculated and stored in computer memory [14].

Both theories are based on the same principle. However, since the Fraunhofer is a simplified version of the Mie theory, it cannot be used without restrictions, that will lead to errors. Because of that, normally, two conditions must be satisfied [14]:

1. The particle size must be relatively large (at least 10 times bigger than the laser wavelength)
2. Small scattering angle (30° or less) must be observed.

Because of the above-mentioned requirements, this theory is not applicable for measurements in the sub-micron region. This theory was especially used when the computers could not be fast enough to decode the complex programming that the Mie theory requires. Nowadays, due to the technological evolution, the latter is mostly applied. The only exception occurs when the PS is extremely large. In those cases Fraunhofer diffraction theory is used to avoid calculation error building up that can occur with the Mie theory [14].

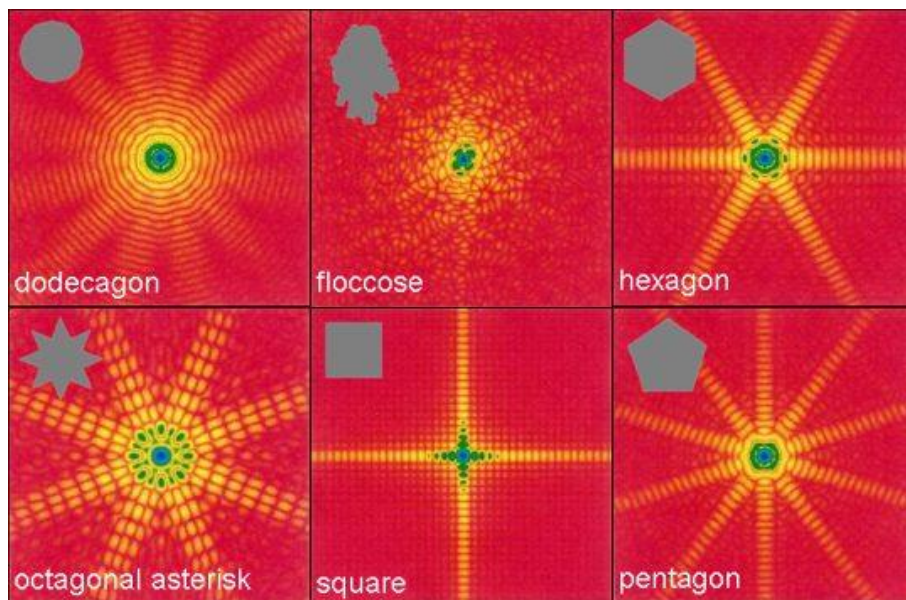


Figure 1-7 Diffraction patterns of non-spherical particles [13]

However, if the sample particles are not spherical, but they are instead more irregularly shaped, as seen in the Figure 1-7, the pattern acquired is different. The further away the particles are from the spherical shape, more differences the pattern will have.

The presence of irregular shapes is one of the major difficulties this technique faces that may induce to major errors, but it is not the only one. Aggregation is another problem that can happen and may difficult the analyses.

Companies research different methods and try diverse algorithms to have exact analyses' results, resulting in a wide-range of analytical instruments.

1.2.1 Scanning Electron Microscopy

Desktop scanning electron microscopes (SEM) are considered a high-performance SEM for imaging and analysis [15].

The SEMs provide excellent imaging due to the high brightness and long-life electron source, as well as fast SEM imaging with high resolution. There are several models to fit the diverse application needs. They are user friendly equipments with low maintenance required [16].

One of the most significant differences between a modern light microscope and a SEM is the magnification range. In a light microscope, the maximum magnification a microscope can reach is limited not only by the number and quality of the lenses, but especially by the wavelength of the light used as illumination source. These microscopes usually have a maximum magnification of approximately 1000x due to the limitations that the white light wavelength has (400 nm to 700 nm) [17].

To have higher resolution on higher magnifications, electrons started to be used, since they have shorter wavelengths.

To be able to scan the sample, first, the electrons are produced at the top of the column, which then will travel down through diverse lenses and openings to produce a beam of electrons. The beam will hit the sample mounted on the chamber area which should be under vacuum. Due to the presence of scan coils above the lens, it is possible to scan different areas of the sample. In Figure 1-8, it is shown a diagram of a SEM mechanism [18].

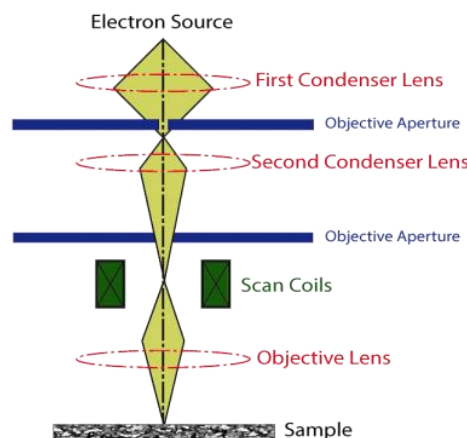


Figure 1-8 Schematic of a scanning electron microscope [18]

Due to the interaction between the electrons and the sample, secondary electrons and backscattered electrons are emitted along with characteristic X-rays. These secondary electrons after being collected by detectors, form images that are then displayed on the computer screen with help of the software [18-19]

Generally, SEM is considered a nondestructive technique, even though in the case of some more sensitive products, sample degradation may occur.

The SEM technique has a wide range of applications and is used by different industries in the pharmaceutical and medical fields providing imaging analysis and EDS (Energy-dispersive X-ray spectroscopy) [21].

1.2.2 Laser Diffraction with Wet Dispersion

The analysis of particles size using laser diffraction method is often performed using MALVERN equipments. With this equipment it is possible to analyze the particle size dry or wet, but the process to do it is not as straightforward as on the SEM since different factors and parameters must be considered. On a first-time analysis, the analysis conditions are unknown and several tests must be carried out to determine optimal analysis conditions.

In this equipment, the parameters on Table 1-2 should be evaluated.

Table 1-2 Parameters to be evaluated [MALVERN] [22]

Dispersant	Must wet the sample, add energy to improve the dispersion, as well as provide stabilization
Sampling	The sample quantity must be representative of the bulk
Obscuration range	Sample concentration read by the laser
Measurement duration	Must be long enough to allow a representative sample of the particles to circulate through the measurement cell
Stir speed	Must ensure homogeneity and that the sample passing through the measurement cell is representative

All the variables mentioned above may lead to PS determination with errors if not evaluated properly. One of the problems that may affect sample measurement that can originate from the obscuration parameter is the multiple scattering effect. This can happen if the obscuration range being used is not the recommended (although even the same types of particles may react differently) [22].

On Table 1-3 it is possible to see the recommended obscuration ranges for different particle sizes.

Table 1-3 Recommended obscuration ranges dependent on particle size [22]

Particle Size	Obscuration range
Fine particles	~5% to 10% (less than 5% may be required for <1 μ m)
Coarse particles	5% to 15%
Polydisperse samples	15% to 20%

It is important to mention that fine particles are more easily affected by multiple scattering effects, while coarser particles are more likely to be affected by sampling [22].

As for the laser diffraction technique used to calculate the PSD, the MALVERN uses the Mie theory, which means it requires knowledge of optical properties (refractive index and imaginary component) of the sample and of the dispersant. Typically, when the optical properties are unknown, an estimated value is used, since it is possible to fit the modeled data with the actual collected data with help of the software [23].

The other option available when the optical properties are not known is using the Fraunhofer theory, however, MALVERN does not recommend this approximation if the sample has particles below 50 μ m or if they are relatively transparent.

1.2.3 Laser Diffraction with Dry Dispersion

The analysis of particles size using laser diffraction method can also be performed using SYMPATEC equipments. This equipment is often composed of independent modules according

to the customer needs. The HELOS (Helium-Neon Laser Optical System) is the main part of the equipment, since it contains the laser. Its measuring range is dependent on which one of the 4 lenses is being used. The measuring range varies from 0.18 μm to 875 μm [24]. The RODOS/M is then attached to the main part as the dry dispersion unit. It can be used for almost all kinds of dry powders with a PS range of <0.1 μm to 3500 μm . This component is completely automatic, which means all the parameters are selected from the database in the software. To disperse the particles, the unit uses compressed air that will generate a dry aerosol that will then pass in front of the laser beam. After the analyses, the powders are collected by suction into a vacuum [25]. To have an easy and homogeneous dosing, especially for small quantities or potent products it is recommended to use the ASPIROS accessory. Potent products must be prepared inside a glove box. Vials are filled with a few milligrams of the product and capped. Since the vial is placed inside the ASPIROS accessory, the risk is minimized [26].

The laser diffraction technique used in SYMPATEC equipment is considered a parameter free and a model independent mathematical algorithm, since it does not need the parameters refractive index and imaginary component, like on MALVERN. This was possible with the Philips-Twomey algorithm [27].

The HELOS unit laser can evaluate the PSD with [24]:

- FREE (Fraunhofer Enhanced Evaluation) applying the Fraunhofer theory down to 0.1 μm . This theory is possible to be applied without knowledge of optical parameters.
- MIEE (Mie Extended Evaluation) through the Mie theory which is appropriated for spherical, isotropic, homogeneous particles with known complex refractive index and more.

It is important to mention that the FREE is different from the Fraunhofer theory, since it is the “upgraded” version with the Philips-Twomey algorithm, which is meant to counter the problems associated with the theory.

To summarize, in both equipments (MALVERN and SYMPATEC) a good dispersion must be achieved to obtain correct values of the particle size. Bad dispersions originate misleading PSD values as a result of the formation of the particle aggregates that will shift PSD to higher values.

In this work, whenever it was possible, the samples were analyzed with both the wet method (MALVERN) and the dry method (SYMPATEC). Additionally, product samples were also analyzed by SEM.

All the products analyzed were considered potent by Hovione’s internal procedures. They were categorized with a category 3a, which means that all products were handled by following the requirements and strict procedures [28].

Chapter 2

Materials & Methods

All the equipment used were property of Hovione's analytical R&D Products laboratory.

2.1 Desktop Scanning Electron Microscope Phenom ProX Generation 5



Figure 2-1 Desktop scanning electron microscope (SEM) Phenom ProX Generation 5 [15]

The Phenom ProX Generation 5 (Figure 2-1) was the SEM used.

The SEM has a simple preparation method and it can only be used to analyze solids. The preparation of samples must be done in a contained environment such as a glove box if it is a potent product, to ensure the protection of the operator.

For the sample preparation, the powder sample is placed on a carbon double sided adhesive tape previously mounted on a clean stub pin. Subsequently the pin is gently sprayed using compressed air to remove the excess powder that did not adhered to the carbon tape.

Afterwards, the stub pin is placed on the phenom sample holder (Figure 2-2). The stub pin must be rotated to lower the sample until 2 mm below the top surface (4 vertical marks) and then placed on the equipment.



Figure 2-2 Phenom sample holder (left); stub pins (right)

Next, captures of the sample at different magnifications are done and measurements of particle size taken manually.

The equipment specifications are presented in Appendix I.

2.2 Malvern Mastersizer Hydro 2000 S



Figure 2-3 Malvern Mastersizer (back) Hydro 2000 S (front)

One of the equipment used for the PSD analyses was a MALVERN Mastersizer Hydro 2000 S (Figure 2-3) which is composed of two separated units: the laser unit (Mastersizer) and the dispersion unit (Hydro 2000 S), which can be replaced by other units depending on the type of analyses to be done.

By connecting the two units together, the laser beam will pass through the dispersed particulate sample on the measurement cell of the dispersion unit, enabling the analyses to happen.

The MALVERN equipment can be used with product in form of suspensions or powders.

Before analyzing a new product, a new method must be created following the directions described in Hovione's Corporate Operating Procedure (COP037) which can be summarized in the steps below. It is advisable to perform a SEM analysis before initiating the method development to determine the measuring range of the PSD.

The first step consists of testing dispersion solutions and surfactants and select the combination that produces a good suspension. The surfactants are chosen based on the polarity of the solvent used during the production of the product and the dispersants available (Table 2-1)

Table 2-1 Dispersant and surfactant list [MALVERN]

Dispersant	Surfactant
Water	Tween 80/20, SDS, IGEPAL
Methanol	
Isopropanol	
N-hexane	Span 85, Lecithin
Heptane	
Isoparaffin	

Subsequently, it is necessary to select the sample amount. It is advisable to use 30 to 50 mg, being the minimum limit 20 mg. To be considered representative, at least half of the suspension must be used.

Afterwards, an ultrasounds (US) titration curve for different values of US is done (must be done with independent samples). On this step, the default conditions on the software are

employed. It is considered satisfactory only if the obscuration does not decrease during the analysis.

If the obscuration decreases during the analysis, changes in the default conditions of parameters like stirrer speed, obscuration and/or optical model (refractive index and absorption) must be made or eventually, a new dispersant/surfactant must be tested.

To proceed to the last step a plateau must be reached and the obscuration must remain unchanged. To finalize the method, tests of stability, repeatability and robustness are done.

In the case of an existing validated method the sample preparation and analysis should be performed according to that method. For the analyses to be considered valid, the residual values must be inferior to 2.

The equipment specifications are presented in Appendix I.

2.3 Sympatec Helos, Rodos/M plus Aspiros



Figure 2-4 Sympatec equipment :Helos (right), Rodos/M (middle) and Aspiros (left)

The equipment used was made of three different components: HELOS, RODOS/M plus ASPIROS (Figure 2-4) previously described. The equipment specifications are presented in Appendix I.

With dry samples on SYMPATEC, the method is more linear and simple since only two variables are considered critical.

The variables are pressure, feed rate velocity and density, in which the pressure is usually the most critical parameter. In this work, the only variables studied are the two first mentioned, since the density of the products is unknown, it was used a standard value of 1 g/cm^3 for all the products analyzed.

The preparation of the sample is done by filling a glass vial with some milligrams of the powder sample. The quantity required may vary with the product, however, it must be enough to obtain a minimum of 5% of optical concentration.

When analyzing a new product and having previously obtained a SEM image, the next step is to choose the appropriate lens so the analysis is carried out within the range of the lens (Table 2-2).

Table 2-2 Lens range [SYMPATEC]

Lens	Range
R1	0.18 μm – 35 μm
R2	0.45 μm – 87.5 μm
R4	1.80 μm – 350 μm
R5	4.50 μm – 875 μm

In case of the analysis of a new product, after selecting the appropriate lens, it should be carried out several tests to determine the optimal velocity values regarding the feeding rate and pressure that provides dispersion without damaging the sample particles.

If the product was previously analyzed and studied, after the vial preparation, it is only needed to place the sample vial inside the sample holder inside the equipment, set the conditions on the software and run the program to obtain the results.

In the Figure 2-5 it is possible to see the differences between the vials of the last two equipments mentioned, MALVERN and SYMPATEC.



Figure 2-5 Sample vials for MALVERN (left) and SYMPATEC (right)

Chapter 3

Results and Discussion

The particle size of the different APIs was studied using the techniques mentioned in chapter 1: SEM, liquid and dry dispersion techniques, Malvern and Sympatec, respectively.

The APIs used in these studies were produced using Hovione's particle engineering technology wet polishing (WP). Using this technology it is possible to produce stable crystalline APIs with a desired PS in a wet environment, that is isolated by spray drying [29].

Hovione's internal code for the APIs can be explained with the example on Figure 3-1.

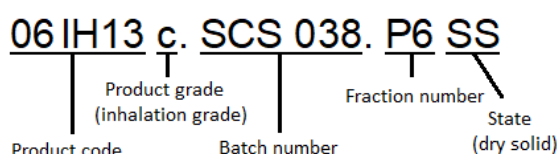


Figure 3-1 APIs nomenclature (example)

In this chapter are presented and discussed the results obtained. Throughout this chapter only results of dry fractions are presented. Meaning that the omitted fractions were analyzed while in suspension (before being feed to the spray dryer) to provide a better understanding of the PSD and in an attempt to maximize the quantity of API to be retrieved.

3.1 IH13c

IH13 is the Hovione's internal code of specific API intended to be used in a dry powder inhaler. IH13, an inhalation API, was micronized to produce samples with two different and distinct particle size distributions:

- i. d(0.5) approximately 3 μm and d(0.9) below 7 μm
- ii. d(0.5) approximately 4 μm and d(0.9) below 9 μm

3.1.1 SEM

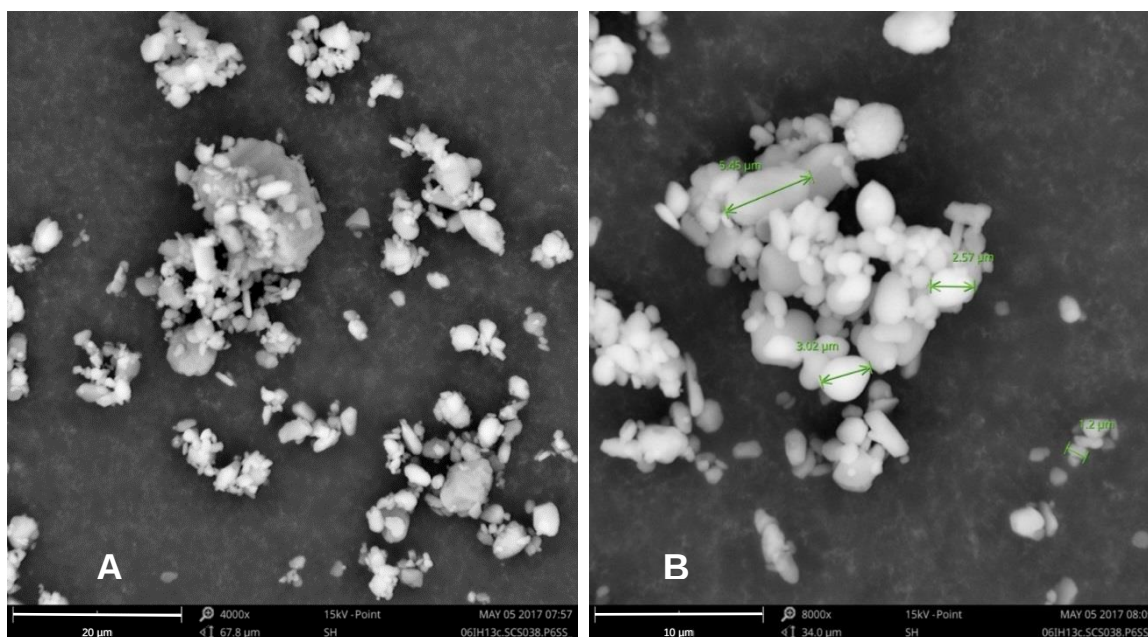


Figure 3-2 Spray dried product after micronization with magnification 4000x and 8000x with 10 kV - Point [06IH13c.SCS038.P6SS]

Figure 3-2 obtained by SEM shows oval to circularly shaped particles with variable particle size. Smaller particles are approximately 1 µm in size and the larger ones 5 µm, approximately.

3.1.2 MALVERN vs SYMPATEC

Since IH13 was a new product there was not an existing method for PS determination on MALVERN. The development of the PS method was performed according to the procedure described previously on sub chapter 2.2. It is shown in detailed in Appendix II.

After determining the optimum analysis parameters for dry powders on liquid dispersion as described in Table 3-1, it was possible to proceed with the analysis. The dispersant selected was 0.1 % (w/v) Lecithin in n-heptane.

Table 3-1 Analytical conditions on liquid dispersion for solid powders for product [IH13c]

SOLID POWDERS	
Accessory	
Sample handling unit	Hydro 2000S
Stirrer speed (rpm)	2100 rpm
Ultrasonic (%)	50 %
Time of ultrasounds	150 seconds
Measurement options	
Material	
Refractive index	1.65
Absorption	0.1
Dispersant name	N-heptane
Refractive index of the dispersant agent	1.39
Result calculation	
Model	General Purpose
Calculation sensitivity	Normal sensitivity
Particle shape	Irregular
Measurement	
Sample measurement time	10 seconds (10000 snaps)
Background measurement time	10 seconds (10000 snaps)
Number of measurements	3
Delay	10 seconds
Average	Create average result
Obscuration limits	5 % - 10 %

After the development of the analytical method with the last part of the batch and analyzed, it was achieved the results described in Figure 3-3 and Figure 3-4.

Record n...	Sample name	d (0.1)	d (0.5)	d (0.9)	Obscuration	Residual	Residual - weig...	Span
92	06IH13c.SCS038.P6SS Rep_1	1.235	2.697	4.976	7.02	1.045	1.712	1.387
93	06IH13c.SCS038.P6SS Rep_1	1.234	2.697	4.976	7.02	1.059	1.719	1.387
94	06IH13c.SCS038.P6SS Rep_1	1.234	2.698	4.981	7.03	1.071	1.728	1.388
95	06IH13c.SCS038.P6SS Rep_1 - Average	1.234	2.697	4.977	7.02	1.059	1.720	1.388

Figure 3-3 Table results (3 analyses plus average) on liquid dispersion [06IH13c.SCS038.P6SS]

The analyses were considered valid because the obscuration values were within the limit proposed and the residual values were inferior to 2.

When comparing the d(0.1), d(0.5) and d(0.9) average values obtained on MALVERN (1.234 / 2.697 / 4.977) with the SEM images, it can be observed that the results are in agreement.

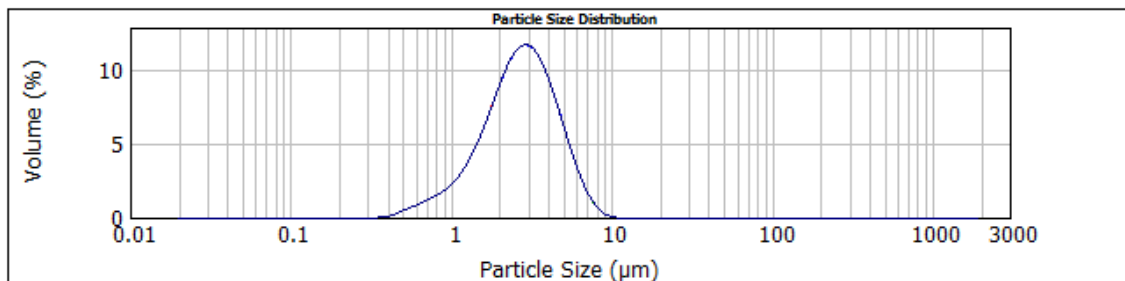


Figure 3-4 Normal distribution obtained through liquid dispersion [06IH13c.SCS038.P6SS]

The Figure 3-4 shows the presence of a fraction of fine powder (particles with PS lower than 1 µm). No turbulence effects were observed during the analysis.

In the case of SYMPATEC analyses (dry dispersion), a method was also developed by studying the pressure and feed rate velocity titration curves of the micronized product (shown in Appendix III). After analyzing and comparing the curves and with the help of SEM images it is possible to establish the ideal conditions for the analysis (1 bar at 36 mm/s), using the lens, R1.

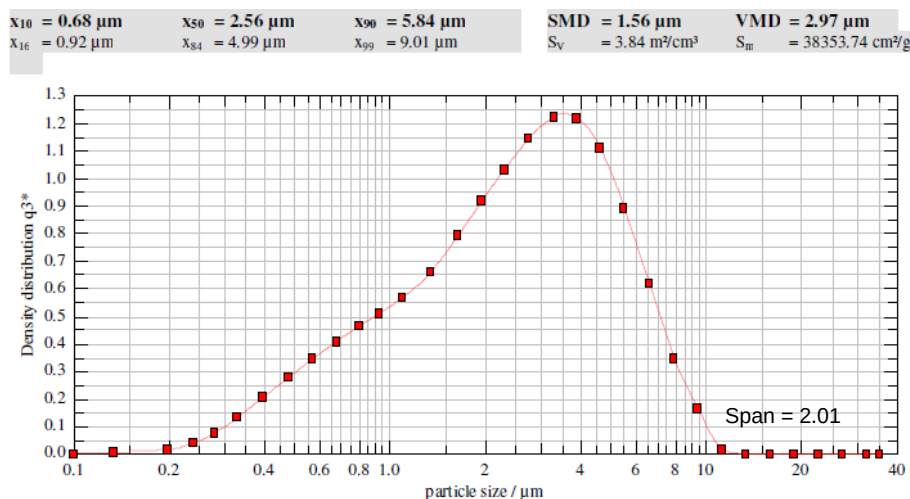


Figure 3-5 Analysis results on dry dispersion [06IH13c.SCS048.P6SS]

The histogram shown in Figure 3-5 is in good agreement with the histogram obtained through liquid dispersion (Figure 3-4). The most obvious difference found was a bigger ratio of fine powders/big particles than in MALVERN analysis, resulting in a higher span and a broader curve.

Table 3-2 PSD results obtained from liquid dispersion vs dry dispersion analyses for [06IH3c]

	D (0.1)	D (0.5)	D (0.9)
MALVERN	1.234	2.697	4.977
SYMPATEC	0.68	2.56	5.84
DIFFERENCE	0.554	0.137	0.863

When comparing the PSD, it can be observed that the SYMPATEC's sample shows a lower value of d(0.1) and a higher d(0.9), however d(0.5) values were similar in both methods. For this product specifications the d(0.1) is not a critical parameter so the difference between both results is not significant. On the other hand, d(0.9) is critical but the difference is less than 1 μm and both values are still within the limits required by the client (specification i).

On SEM images it is possible to observe particles with a size bigger than 5 μm so we can assume that the probability of d(0.9) from SYMPATEC being closer to the real seem higher than the MALVERN's d(0.9) values.

3.2 ST71c

ST71 it is the Hovione's internal code for a steroid API. This product that was micronized to originate batches with two different PS targets, specifically for d(0.5) and d(0.9):

- i. d(0.5) approximately 3.5 μm and d(0.9) below 8 μm
- ii. d(0.5) approximately 4.5 μm and d(0.9) below 10 μm

With the wet polishing method there were 3 different and independent batches obtained (06ST71c.SCS042.P5SS, 06ST71c.SCS043.P1SS and 06ST71c.SCS044.P2SS). The samples from the 3 batches were analyzed by SEM, MALVERN and SYMPATEC.

3.2.1 SEM

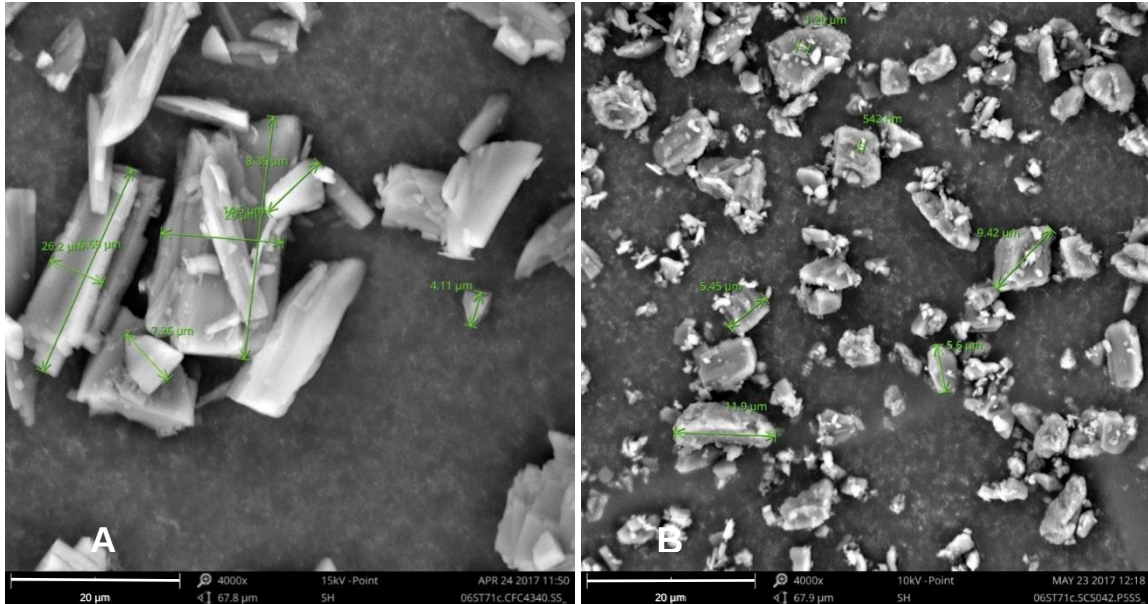


Figure 3-6 **A.** Powder sample of unmicronized material at magnification of 4000x - 15kV - Point [06ST71.CFC4340.SS]

B. Image of ST71c at magnification 4000x - 10 kV - Point [06ST71c.SCS042.P5SS]

Throughout the analysis of the Figure 3-6 A it is possible to see that the unmicronized material does not have a spherical shape but instead an irregular/rectangular shape. Since the unmicronized material does not have a spherical shape we can only say roughly that the measurements have an extended range from 2 µm to 55 µm although the majority appears to be within 15 µm to 20 µm range (more SEM images on Appendix IV).

For the batch 06ST71c.SCS042 (Figure 3-6 B) it is possible to see a clear change in the particle size and shape. After the WP micronization, the particles are smaller but still with irregular outlines. There is a lot of small particles but the majority seems to be between 3 µm to 6 µm.

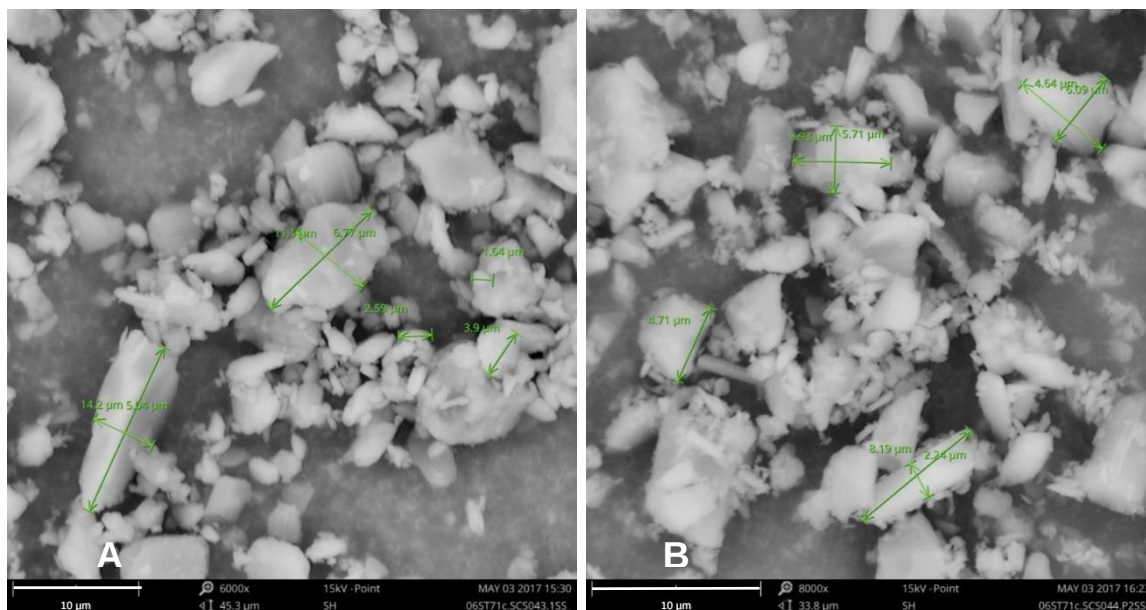


Figure 3-7 **A.** Image of ST71c at magnification 6000x - 15 kV - Point [06ST71c.SCS043.P1SS]

B. Image of ST71c at magnification 8000x - 15 kV - Point [06ST71c.SCS044.P2SS]

The figure above represents the two batches obtained (06ST71c.SCS043.P1SS and 06ST71c.SCS044.P2SS) that complied with the client's desired specifications regarding the particle size.

The pictures shown in Figure 3-7 seem to be similar between them in appearance with subtle differences since the batches variations are small and the particles irregular.

3.2.2 MALVERN vs SYMPATEC

There was an analytical method validated by Hovione available for PS analyses using MALVERN which was the one used in this study. The optimal analytical conditions used are described in Table 3-3.

The dispersant used was water plus 0.1 mL of surfactant 10% (w/v) of SLS aq. solution.

Table 3-3 Analytical conditions on liquid dispersion for solid powders for product [ST71c]

SOLID POWDERS	
Accessory	
Sample handling unit	Hydro 2000S
Stirrer speed (rpm)	2100 rpm
Ultrasonic (%)	50 %
Time of ultrasounds	210 seconds
Measurement options	
Material	
Refractive index	1.52
Absorption	0.1
Dispersant name	Water
Refractive index of the dispersant agent	1.33
Result calculation	
Model	General Purpose
Calculation sensitivity	Normal sensitivity
Particle shape	Irregular
Measurement	
Sample measurement time	10 seconds (10000 snaps)
Background measurement time	10 seconds (10000 snaps)
Number of measurements	3
Delay	10 seconds
Average	Create average result
Obscuration limits	5 % - 9 %

After preparing the slurry with the samples and with all the information provided, the unmicronized material and three batches were analyzed. The results acquired included several parameters which are shown in Figure 3-8 below.

Record n...	Sample name	d (0.1)	d (0.5)	d (0.9)	Obscuration	Residual	Residual - weig...	Span
4	06ST71c.CFC4340.SS - Average	3.543	14.632	35.849	7.58	0.965	1.232	2.208
32	06ST71c.SCS042.P5SS - Average	0.910	2.710	6.164	5.73	1.817	2.902	1.939
56	06ST71c.SCS043.P1SS - Average	0.978	2.896	6.030	5.86	1.774	2.888	1.744
60	06ST71c.SCS044.P2SS - Average	1.048	3.723	8.533	6.08	1.472	2.500	2.010

Figure 3-8 Table results of the average value of liquid dispersion for unmicronized material [06ST71.CFC4340/SS] and three batches [06ST71c.SCS042.P5SS] [06ST71c.SCS043.P1SS] [06ST71c.SCS044.P2SS]

The analyses were valid since the obscuration is within the range specified and the residual value is lower than 2.

For the unmicronized material, when comparing the results from the liquid dispersion (Figure 3-8 RN4) with the SEM images (Figure 3-6 A) it is possible to observe that the results seem to be in agreement. For the three micronized batches (Figure 3-8 RN32, RN56 and RN60), the PSD shows to be close to each other which is supported by the similar SEM images obtained.

On the Figure 3-9 it is possible to compare the PSD evolution from the unmicronized material [06ST71.CFC4340.SS] to the three micronized batches (06ST71c.SCS042.P5SS, 06ST71c.SCS043.P1SS and 06ST71c.SCS044.P2SS).

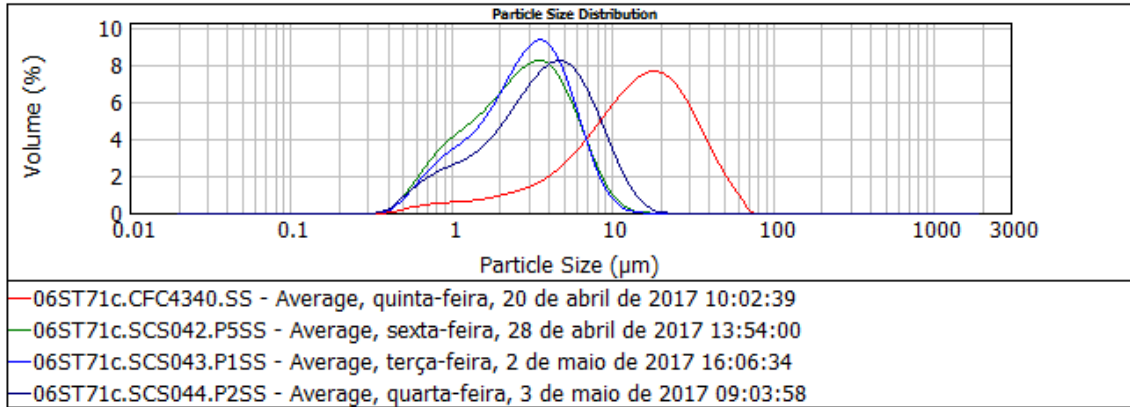


Figure 3-9 Comparison of normal distribution obtained through liquid dispersion between the unmiconized material [06ST71.CFC4340.SS] and the three distinct batches [06ST71c.SCS042.P5SS] [06ST71c.SCS043.P1SS] [06ST71c.SCS044.P2SS].

In Figure 3-9, it is possible to see that the unmiconized material distribution shifted more to the left side after micronization. The three micronized batches have identical PS distributions as expected, however, since the two batches with smaller PSD (06ST71c.SCS042.P5SS and 06ST71c.SCS043.P1SS) have a bigger population of fine particles, their histograms seem leaner and more uniform than the last batch (06ST71c.SCS044.P2SS).

ST71c had not been analyzed on dry dispersion before, therefore a new method was developed. A pressure and a feed rate velocity titration curve was done for both the unmiconized material and the micronized products (Appendix V).

By comparing the titration curves with SEM images it was determined the optimum analysis conditions (lent R2, 50 mm/s and 2 bar and lens R1, 50 mm/s and 3 bar for unmiconized material and for micronized product, respectively).

Param. 2	x10 μm	x50 μm	x90 μm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
06ST71c.SCS044.P2SS_7	0.89	4.30	9.23	1.94	7.30	3.02	50.00	R1: 0.1/0.18...35μm
06ST71c.SCS043.P1SS_4	0.74	3.43	7.62	2.00	6.14	3.01	50.00	R1: 0.1/0.18...35μm
06ST71c.SCS042.P5SS_2	0.82	3.98	8.90	2.03	8.67	3.01	50.00	R1: 0.1/0.18...35μm
06ST71c.CFC4043.SS_3	4.82	16.58	33.15	1.71	6.11	2.04	50.00	R2: 0.25/0.45...87.5μm

Figure 3-10 Table results on dry dispersion for unmiconized material [06ST71.CFC4340/SS] and three micronized batches [06St71c.SCS042.P5SS] [06ST71c.SCS043.P1SS] [06ST71c.SCS044.P2SS]

From the Figure 3-10 it is possible to distinguish the unmiconized material from the micronized product. By looking at the values of d(0.1), d(0.5) and d(0.9), the discrepancies on the results of the three micronized batches are not as pronounced as in the results on MALVERN with liquid dispersion. In this equipment, the batch 06ST71c.SCS042.P5SS is more similar to the 06St71c.SCS.P2SS than to the 06ST71c.SCS043.P1SS as seen before.

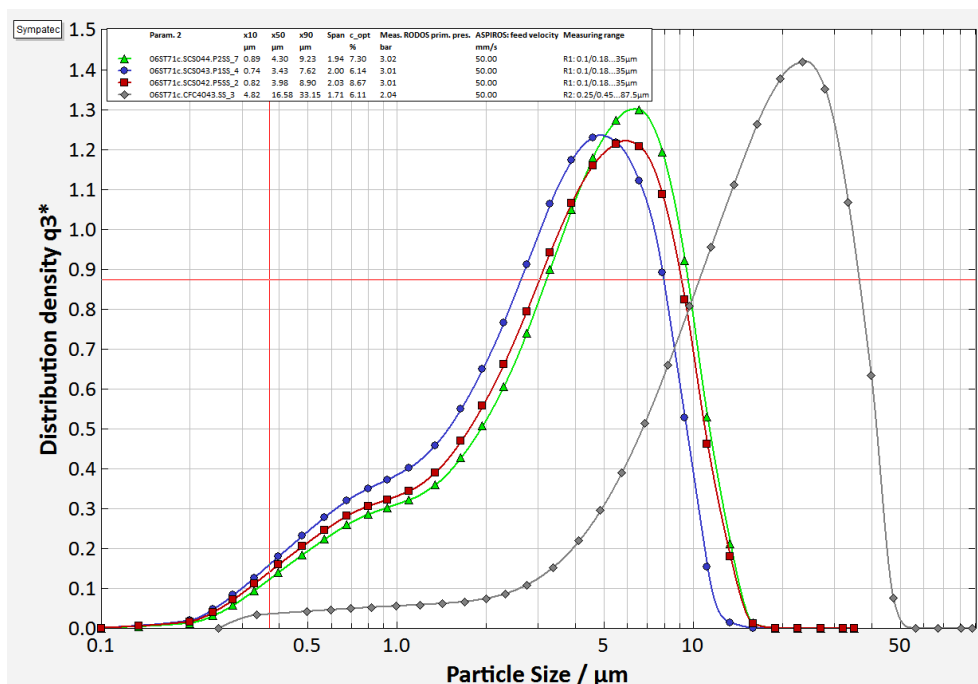


Figure 3-11 Comparison of normal distribution obtained through dry dispersion between the unm micronized material [06ST71c.CFC4340.SS] and the three different batches [06ST71c.SCS042.P5SS] [06ST71c.SCS043.P1SS] [06ST71c.SCS044.P2SS].

As seen in Figure 3-11 and as mentioned before, the unm micronized material PSD histogram is shifted to the left side as the micronization process proceeds. It is possible to see that three micronized batches histograms are quite similar unlike on liquid dispersion.

On SYMPATEC's histogram it is possible to see small variations between the batches, however, the changes seem more obvious on the histogram obtained by MALVERN's liquid dispersion.

Table 3-4 PSD results obtained from liquid dispersion vs dry dispersion analyses for [06ST71c]

	D (0.1)	D (0.5)	D (0.9)
06ST71.CFC4340.SS			
MALVERN	3.543	14.632	35.849
SYMPATEC	4.82	16.58	33.15
DIFFERENCE	1.28	1.95	2.70
06ST71C.SCS042.P5SS			
MALVERN	0.91	2.71	6.164
SYMPATEC	0.82	3.98	8.9
DIFFERENCE	0.09	1.27	2.74
06ST71C.SCS043.P1SS			
MALVERN	0.978	2.896	6.03
SYMPATEC	0.74	3.43	7.62
DIFFERENCE	0.24	0.53	1.59
06ST71C.SCS044.P2SS			
MALVERN	1.048	3.723	8.533
SYMPATEC	0.89	4.3	9.23
DIFFERENCE	0.16	0.58	0.70

When comparing both methods (Table 3-4) it is possible to see that the biggest discrepancies occur with the unm micronized material and the first batch (06ST71c.SCS042.P5SS). Since the unm micronized material has bigger particles and the MALVERN's method is optimized for the micronized product, it is normal to see a discrepancy between the values.

On the other hand, it is not expected to observe a big difference on the micronized product results. Although the differences observed are not exorbitant, on smaller PSD it makes a difference. The last batch (06ST71c.SCS044.P2SS) is the only one that does not display big fluctuations.

Analyzing the results from both types of equipment and comparing them with the SEM images makes it is easier to understand which one is closer to what appears to be the reality.

For the 06ST71c.SCS042.P5SS batch, it is possible to see that there is a significant quantity of particles with a size bigger than the d(0.9) from MALVERN, so it is safe to assume that the result from SYMPATEC it is closer to the real.

Like the previous batch, 06ST71c.SCS043.P1SS has a small difference between both results, more than 1 μm . SEM images show the same problem as the previous batch, presence of larger particles that can influence the results of d(0.9) in particular.

The last batch, 06ST71c.SCS044.P2SS shows to be the one with more concordant results between the three types of equipment since the variations are not as high as on the other three samples.

It is safe to affirm that there is a higher fluctuation with this product since the particles have an irregular shape and it does not present a uniform normal distribution. Due to the higher difficulty of the analyses, more errors can be reflected in the final results.

3.3 ST75c

ST75 it is a steroid product as well, that was micronized to have specific targets, regarding d(0.9) and the span. It was required three different batches, as specified below.

- i. d(0.9) between 2 μm and 2.5 μm
- ii. d(0.9) between 2.5 μm and 3 μm
- iii. d(0.5) approximately 2 and span between 2 – 2.2

Since during the micronization process by WP, the product produces foam and since the particles are hard to break, it took several trials to have the PSD within the specifications imposed. Four different trials were done, the first with a different unm micronized material than the following ones.

3.3.1 Batch 06ST75c.SCS048

The first trial was an attempt for the iii specification.

3.3.1.1 SEM

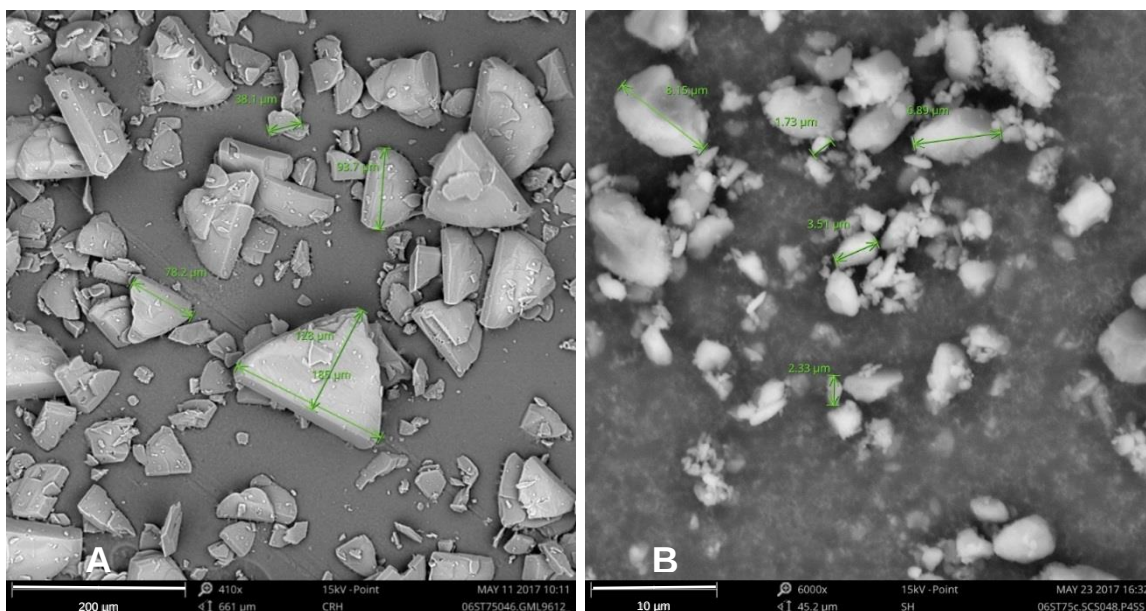


Figure 3-12 A. Powder sample of unm micronized material at magnification of 410x - 15kV - Point [06ST75046.GML9612]

B. Image of ST75c at magnification of 6000x - 15 kV - Point [06ST75c.SCS048.P4SS]

The unm micronized material shown to be a mixture of small and large particles with an irregular shape (Figure 3-12 A). The biggest particle found was more than 100 μm in diameter but the majority appears to have a diameter between 50 and 90 μm .

After being processed by WP, the particles appear to be smaller and rounder than before. The smaller particles show to be approximately less than 2 μm and the larger particles have around the 8 μm of diameter (Figure 3-12 B).

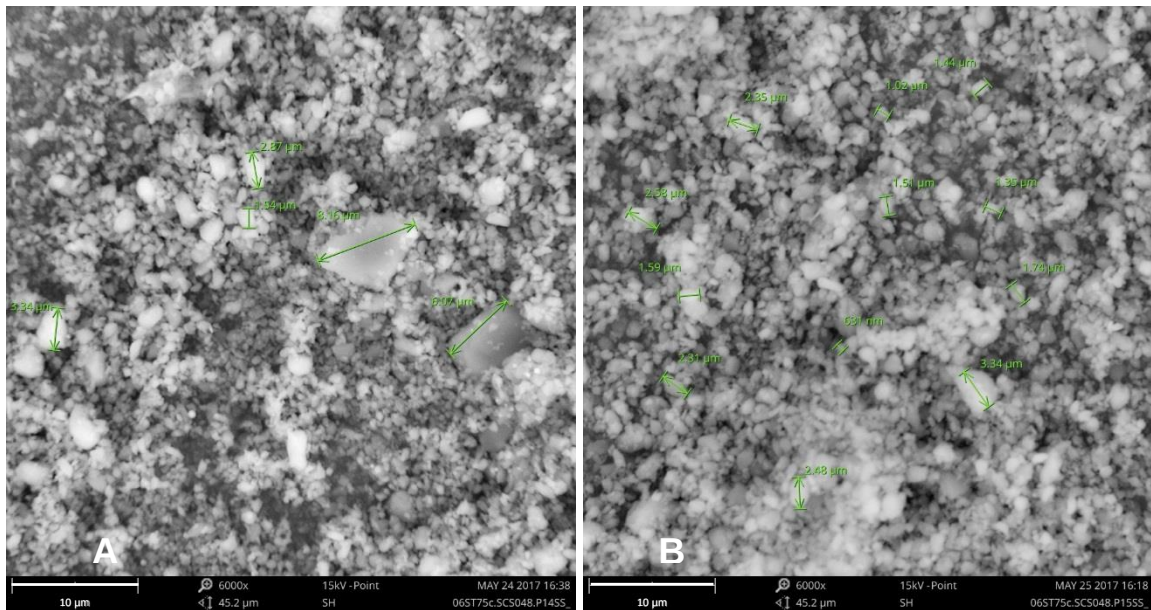


Figure 3-13 A. Image of ST75c at magnification of 6000x - 15 kV - Point [06ST75c.SCS048.P14SS]
 B. Image of ST75c at magnification of 6000x - 15 kV - Point [06ST75c.SCS048.P15SS]

The last two fractions micronized were obtained in a low quantity which means it may not be representative.

In Figure 3-13 A it is possible to see large particles (6 μm – 8 μm in diameter) that certainly will increase the PSD. On the other hand, in Figure 3-13 B, it is possible to see a more homogeneous population. Not many differences are seen between the two.

3.3.1.2 MALVERN vs SYMPATEC

The PS analyses by MALVERN were done following a validated existing method which used a water-based dispersant with Tween 80 as surfactant (Table 3-5).

Table 3-5 Analytical conditions on liquid dispersion for solid powders for product [ST75c]

SOLID POWDERS	
Accessory	
Sample handling unit	Hydro 2000S
Stirrer speed (rpm)	2100 rpm
Ultrasonic (%)	100 %
Time of ultrasounds	270 seconds
Measurement options	
Material	
Refractive index	1.52
Absorption	0.01
Dispersant name	Water
Refractive index of the dispersant agent	1.33
Result calculation	
Model	General Purpose
Calculation sensitivity	Normal sensitivity
Particle shape	Irregular
Measurement	
Sample measurement time	15 seconds (15000 snaps)
Background measurement time	10 seconds (10000 snaps)
Number of measurements	3
Delay	10 seconds
Average	Create average result
Obscuration limits	10 % - 15 %

On this first batch, it was analyzed the unmiconized material as well as three different fractions of the same batch by following the method described in Figure 3-14.

Record n...	Sample name	d (0.1)	d (0.5)	d (0.9)	Obscuration	Residual	Residual - weig...	Span
8	06ST75046.GML9612 - Average	7.473	30.051	59.690	12.56	0.477	0.646	1.738
38	06ST75c.SCS048.P4SS - Average	1.653	3.625	7.709	9.33	0.594	0.865	1.671
174	06ST75c.SCS048.P14SS_rep1 - Average	0.616	1.681	5.573	13.60	0.308	0.565	2.949
186	06ST75c.SCS048.P15SS_rep1 - Average	0.563	1.287	2.834	11.85	0.827	0.658	1.765

Figure 3-14 Table results of the average value on liquid dispersion for unmiconized material [06ST75.GML9612] and three different micronized fractions of SCS048 batch [06ST75c.SCS048.P4SS] [06ST75c.SCS048.P14SS] [06ST75c.SCS048.P15SS]

Since the method is validated for the micronizing PSD range, to analyze the unmiconized material with the same method while having the obscuration within the limits and residual values below 2, four times more than the quantity of product stated was used (approximately, 100 mg).

The equipment reads the number of particles and since the unmiconized material has a PSD larger than the micronized product, it is necessary more quantity to have the same number of particles.

In Figure 3-14 it is possible to see that all analyses were considered valid since the residual value is below 2. However, when comparing the result of the unmiconized material (Figure 3-14 RN8) analysis to the SEM image (Figure 3-12 A) it is possible to see a great discrepancy since the d(0.9) value obtained is 59.690 µm and it was seen particles with diameters above 100 µm.

Even though the unmicronized material analysis (RN8) has the obscuration and residual values within the limits, it is reasonable to say that to analyze it, it is necessary a different method because changing the product quantity is not enough.

It is possible to see that the last fraction (06ST71c.SCS048.P15SS) is within the value projected (requirement ii).

The SEM does not provide a PSD, it is only possible to estimate the PS by analyzing the SEM images. For this reason, SEM is an important technique used to validate PS methods.

The three micronized fractions show the evolution of the micronization process. This evolution is possible to see graphically through the Figure 3-15.

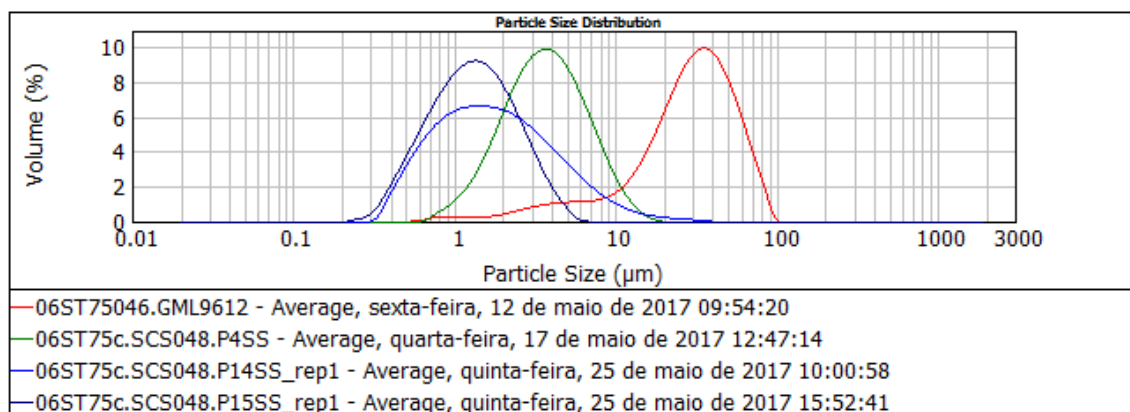


Figure 3-15 Comparison of normal distribution obtained through liquid dispersion between the unmicronized material [06ST75046.GML9612] and the fractions of batch SCS048 [06ST75c.SCS048.P4SS] [06ST75c.SCS048.P14SS] [06ST75c.SCS048.P15SS].

On the Figure 3-15 it is possible to see that the unmicronized material has a histogram with two distinct populations. A big population of large particles ($d(0.9)=59.690 \mu\text{m}$) with a small population of smaller particles ($d(0.1)=7.473 \mu\text{m}$).

On the micronized fractions it is possible to see that the first (06ST75c.SCS048.P4SS) and the last fractions (06ST75c.SCS048.P15SS) show a normal distribution. The fraction 06ST75c.SCS048.P14SS shows the presence of bigger particles, which were shown by the SEM image (Figure 3-13 A).

A SYMPATEC method was developed for ST75c by doing the titration curves (pressure and feed rate velocity) for the unmicronized material and for the micronized product. The conditions changed based on the PS of the material since the smaller the particles, the more pressure is needed to deagglomerate them.

In Figure 3-17 it is possible to see that since the last micronized fraction (06ST75c.SCS048.P15SS) has a smaller PSD compared to the other two micronized fractions, more pressure was needed to deagglomerate the particles.

The titration curves for both unmicronized and micronized product are in Appendix VI.

	Param. 2	x10 μm	x50 μm	x90 μm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
▲	06ST75c.SCS048.P15SS	0.49	1.28	2.99	1.95	3.80	5.04	5.00	R1: 0.1/0.18...35μm
●	06ST75c.SCS048.P14SS	0.71	3.41	8.24	2.21	9.13	3.02	50.00	R1: 0.1/0.18...35μm
■	06ST75c.SCS048.P4SS	0.82	3.74	8.03	1.93	12.25	3.01	50.00	R1: 0.1/0.18...35μm
◆	06ST75046.GML9612_10	29.44	71.96	118.44	1.24	6.74	0.16	50.00	R4: 0.5/1.8...350μm

Figure 3-16 Table results of the average value on dry dispersion for unm micronized material [06ST75.GML9612] and three different micronized fractions of SCS048 batch [06ST75c.SCS048.P4SS] [06ST75c.SCS048.P14SS] [06ST75c.SCS048.P15SS]

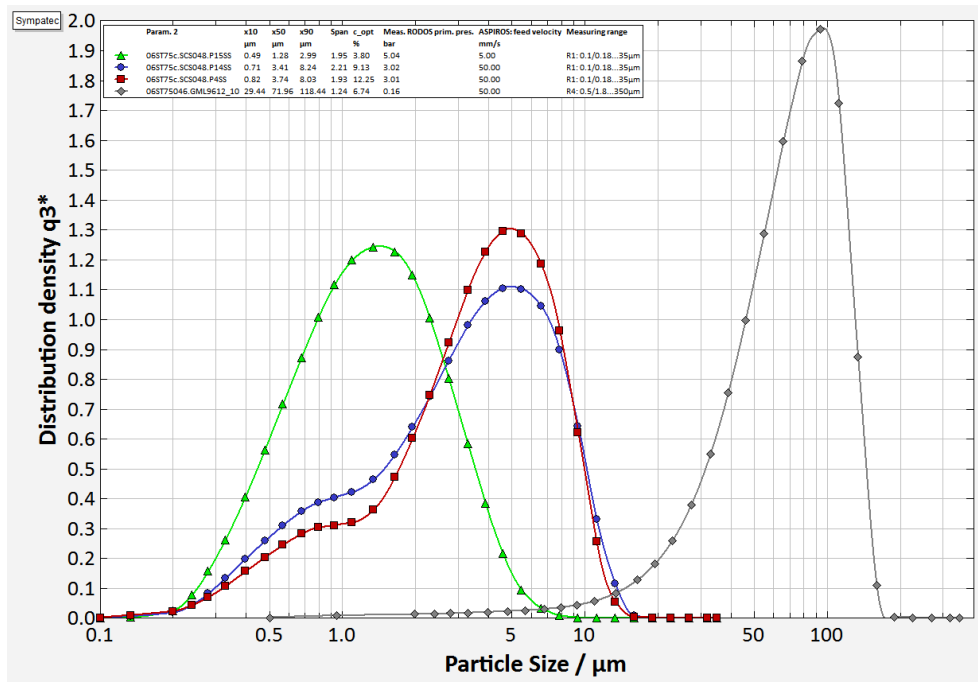


Figure 3-17 Comparison of normal distribution obtained through dry dispersion between the unm micronized material [06ST775046.GML9612] and the three different fractions from batch SCS048 [06ST75c.SCS048.P4SS] [06ST75c.SCS048.P14SS] [06ST75c.SCS048P15SS].

The SEM analysis (Figure 3-12 A) shows large particles, more than 100 μm in diameter, which appear on SYMPATEC analysis ($d(0.9)=118.44\text{ }\mu\text{m}$) but not on MALVERN ($d(0.9)=59.690\text{ }\mu\text{m}$).

The fractions 06ST75c.SCS048.P4SS and 06ST75c.SCS048.P14SS have similar percentiles values and histograms (Figure 3-16). The last micronized fraction (06ST75c.SCS048.P15SS) has an inferior PSD and a normal distribution, unlike the previously fractions mentioned.

Table 3-6 PSD results obtained from liquid dispersion vs dry dispersion analyses for [06ST75c.SCS048]

	D (0.1)	D (0.5)	D (0.9)
06ST75046.GML9612			
MALVERN	7.473	30.051	59.690
SYMPATEC	29.44	71.96	118.44
DIFFERENCE	16.97	41.91	58.75
06ST75C.SCS048.P4SS			
MALVERN	1.653	3.625	7.709
SYMPATEC	0.82	3.74	8.03
DIFFERENCE	0.83	0.12	0.32
06ST75C.SCS048.P14SS			
MALVERN	0.616	1.681	5.573
SYMPATEC	0.71	3.41	8.24
DIFFERENCE	0.09	1.73	2.67
06ST75C.SCS048.P15SS			
MALVERN	0.563	1.287	2.834
SYMPATEC	0.49	1.28	2.99
DIFFERENCE	0.07	0.01	0.16

In Table 3-6 it is possible to see a huge difference in the values of the percentiles reported. As mentioned before, the MALVERN's percentiles values obtained for the unmiconized material are not correct. To analyze the unmiconized material on MALVERN a new method is necessary since the one used is valid only for micronized particles.

By further analysis on Table 3-6 it is possible to see that the fractions 06ST75c.SCS048.P4SS and 06ST75c.SCS048.P15SS have a small difference in the percentiles values obtained by the two equipments.

On the other hand, the fraction 06ST75c.SCS048.P14SS shows differences regarding PSD between both analyses. When comparing the image from SEM (Figure 3-13 A) with results obtained by SYMPATEC, it is observed particles with size of 6-8 μm but as they were dispersed, it was not expected an increase in PSD on d(0.90).

The titration curves were done with fractions 06ST75c.SCS048.P4SS and 06ST75c.SCS048.P15SS only. Since there was not enough product from fraction 06ST75c.SCS048.P14SS, the conditions for the analysis were the same as the first micronized fraction. Due to the results it can be affirmed that there was not enough pressure to deagglomerate.

The results indicate that for the unmiconized material the SYMPATEC method is preferable, unless a new method is developed for that specific range on MALVERN.

On the other hand, for the micronized particles, even though the same MALVERN method is viable, the same does not happen with SYMPATEC, since the same pressure is not enough for the different levels of micronization. Small sample quantity makes it difficult to do titration curves.

3.3.2 Batch 06ST75c.SCS050

The second trial was an attempt for the ii specification (as obtained in the first trial) but this time the objective was producing more quantity of spray dried product.

3.3.2.1 SEM

On this batch, the unm micronized material was from a different batch.

On this batch the product suspensions were analyzed during the micronization process. However, the whole suspension was spray dried after the desired PS value was reached. The resulting micronized powder was 06ST75c.SCS050.P6SS.

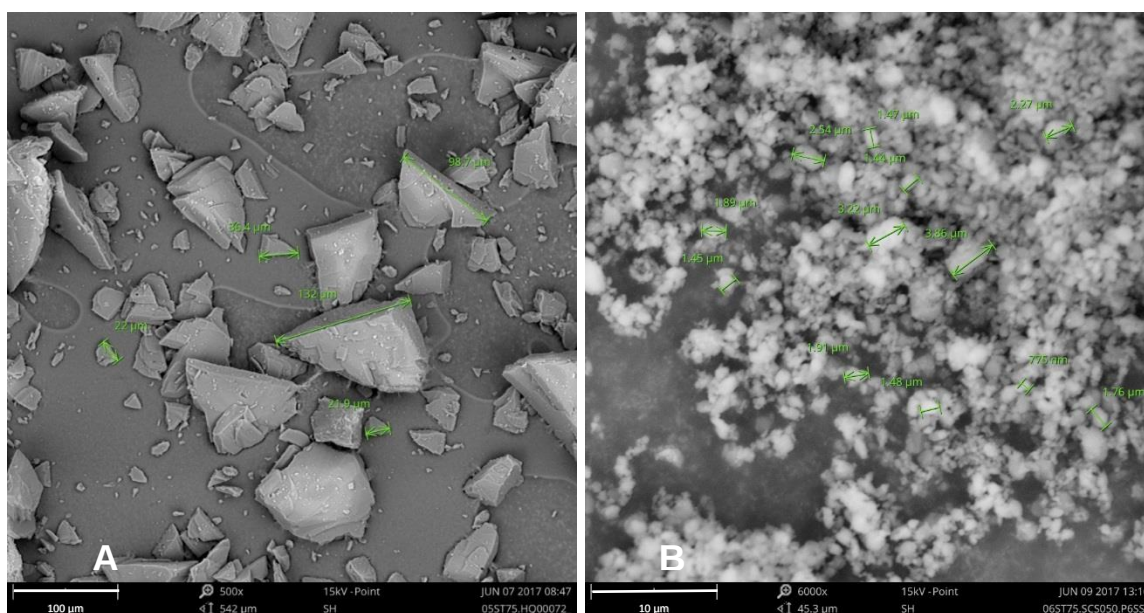


Figure 3-18 **A.** Powder sample of unm micronized material at magnification of 500x - 15kV - Point [05ST75.HQ00072]

B. Image of ST75c at magnification of 12500x - 15 kV - Point [06ST75c.SCS050.P6SS]

In Figure 3-18 A it is possible to see great similarities with the previous unm micronized material batch (06ST75046.GML9612) since its appearance is the same (Figure 3-12 A). The most noticeable difference is the presence of bigger particles compared to the previous one.

For this micronized fraction 06ST75c.SCS050.P6SS, although it was supposed to have the ii specification, a $d(0.9)$ between 2.5-3 µm, the SEM figure analysis shows that this batch has some large particles (PS of 3-4 µm) that are above the customer requirements mentioned beforehand. If in smaller quantity, they are not going to influence the parameters result, however, that does not appear to be the case.

3.3.2.2 MALVERN vs SYMPATEC

For this batch, since all the product was spray dried at once, the micronization evolution consists only of unmicronized material and final micronized product.

Record n...	Sample name	d (0.1)	d (0.5)	d (0.9)	Obscuration	Residual	Residual - weig...	Span
190	05ST75.HQ00072 - Average	4.846	27.630	58.387	14.35	0.422	0.630	1.938
242	06ST75c.SCS050.P6SS - Average	0.937	1.992	3.900	12.17	0.415	0.447	1.487

Figure 3-19 Table results of the average value on liquid dispersion for unmicronized material [05ST75.HQ00072] and micronized fraction of SCS050 batch [06ST75c.SCS050.P6SS]

When analyzing the new unmicronized material with the MALVERN method, the same problem occurred, the PSD is not in agreement with the SEM analysis (Figure 3-18 A). The values obtained are lower than expected. As mentioned before, this can be explained by the fact that the MALVERN method used is optimized for micronized product range (1 μm – 5 μm), and therefore cannot be used for both micronized and unmicronized products (range higher than 70 μm).

In the case of the micronized sample (06ST75c.SCS050.P6SS) analyzed, it is possible to observe that a few particles above the limit of the specifications have a great impact on the PSD results, especially the d(0.9). As seen in Figure 3-19, with this method, the product obtained does not meet the particle size requirement (d(0.9) between 2.5 μm and 3 μm).

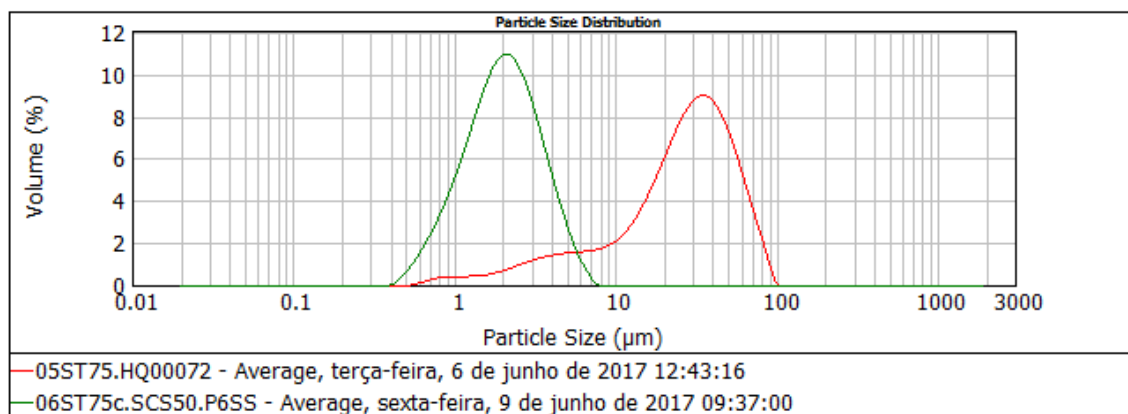


Figure 3-20 Comparison of normal distribution obtained through liquid dispersion between the unmicronized material [05ST75.HQ00072] and the fraction from batch SCS050 [06ST75c.SCS050.P6SS]

On the Figure 3-20, it is possible to observe that the unmicronized material distribution shows the presence of large particles (up to 100 μm) and a small amount of particles with size between 1 μm and 10 μm . As previously mentioned the PSD values do not convey the results from the SEM analysis.

On the other hand, the micronized product PSD shows a normal distribution, which is normally the type of distribution that the client wants since it means a uniform PSD.

On SYMPATEC, the method development involved titration curves for the micronized product, since the analysis conditions will change due to the different PSD. Pressure and feed rate velocity values were optimized. The analysis conditions for the unmicronized material were the same as previous batch, 0.1 bar and 50 mm/s, pressure and feed rate velocity, respectively.

Param. 2	x10	x50	x90	Span	c_opt	Meas. RODOS prim. pres.	ASPIROS: feed velocity	Measuring range
	μm	μm	μm		%	bar	mm/s	
06ST75c.SCS050.P6SS_F2_8	0.57	2.11	5.38	2.28	6.41	6.22	18.00	R1: 0.1/0.18...35 μm
05ST75.HQ00072_7	28.23	78.17	139.99	1.43	9.47	0.12	50.00	R4: 0.5/1.8...350 μm

Figure 3-21 Table results of the average value on dry dispersion for unmiconized material [05ST75.HQ00072] and micronized fraction of SCS050 batch [06ST75c.SCS050.P6SS]

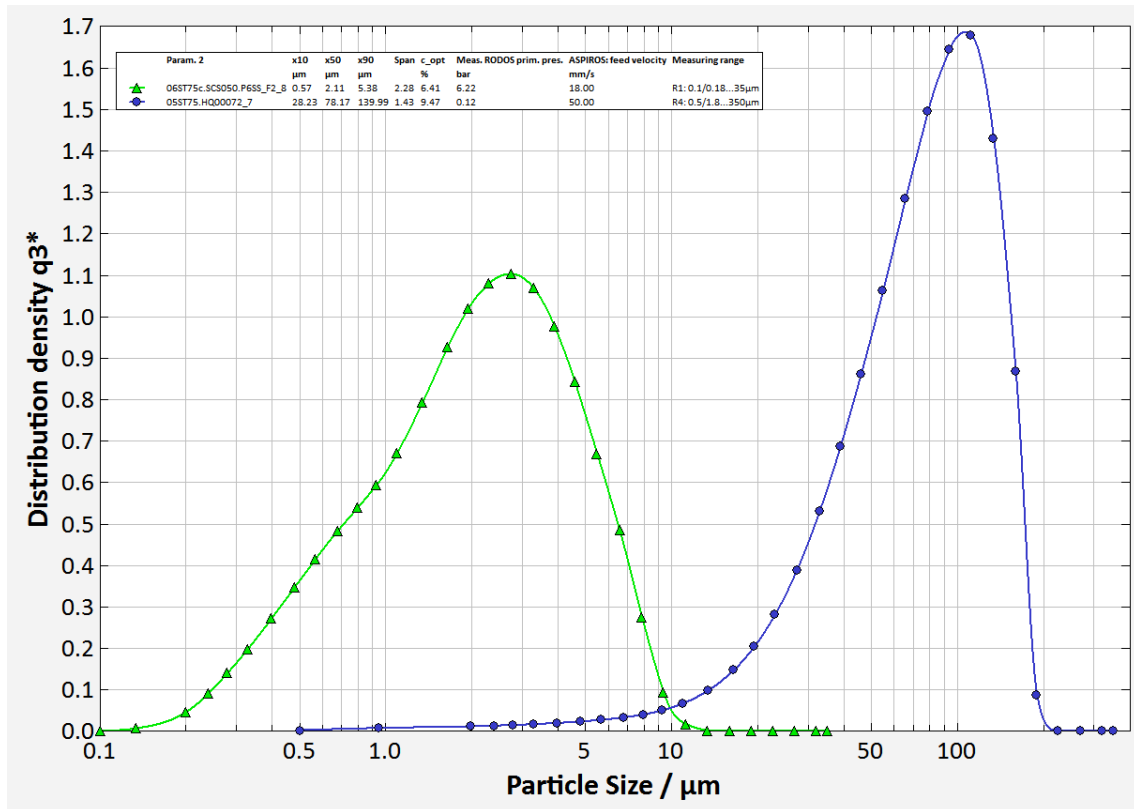


Figure 3-22 Comparison of normal distribution obtained through dry dispersion between the unmiconized material [05ST75.HQ00072] and the fraction attained [P6SS].

In Figure 3-22, it is possible to see that the unmiconized material, despite being from a different batch it has a PSD similar to previous unmiconized material (06ST75046.GML9612). The PSD and the values obtained with SYMPATEC are supported by SEM analysis (Figure 3-18 A).

With the micronized sample (06ST75c.SCS050.P6SS), it is possible to see some differences. Although a titration curve (Appendix VII) was done for both velocity and pressure parameters, the PSD obtained is not a "perfect normal distribution" as it was obtained with the MALVERN's method.

In this case, the results show a higher quantity of fine and bigger particles than what was previously obtained. However, when looking at the SEM image it is possible to see that there are not many particles with PS above 3 μm and none with a size presented in d(0.9), 5.38 μm (Figure 3-21).

Table 3-7 PSD results obtained from liquid dispersion vs dry dispersion analyses for [ST75c.SCS050]

	D (0.1)	D (0.5)	D (0.9)
05ST75.HQ00072			
MALVERN	4.846	27.630	58.387
SYMPATEC	28.23	78.17	139.99
DIFFERENCE	23.38	50.54	81.60
06ST75c.SCS050.P6SS			
MALVERN	0.937	1.992	3.900
SYMPATEC	0.57	2.11	5.38
DIFFERENCE	0.367	0.118	1.480

When comparing the results for the unm micronized material with the different methods, again we see that the MALVERN's method is not acceptable because it is not optimized for the particle size range of the unm micronized material. So, again, the SYMPATEC shows that, if the right pressure and velocity is selected, it is possible to get a PSD that agrees with the SEM analysis.

For the micronized sample the opposite is observed. The MALVERN method proves to be better at deagglomerating the coarse aggregates, since a smaller d(0.9) was obtained when comparing the value with the SYMPATEC result. On SYMPATEC, although the titration curves were performed, probably a higher pressure was needed to deagglomerate the aggregates into discrete particles.

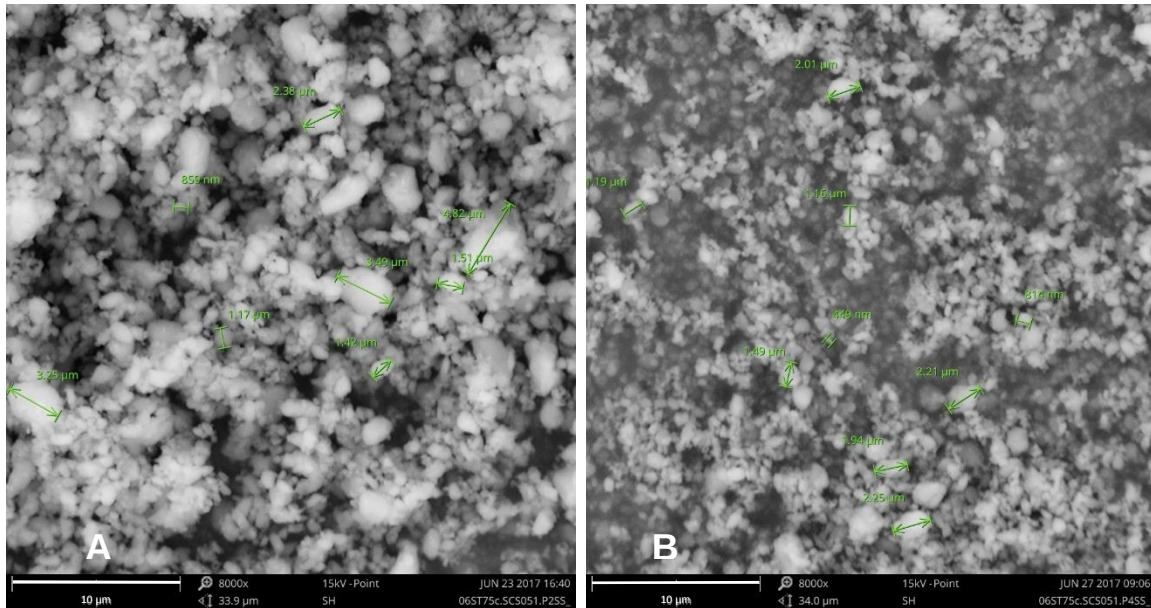
However, when looking at Table 3-7 at d(0.1) values and the SEM image of the final product (Figure 3-18 B), it is possible to observe that the smaller particles may not deagglomerate so well on MALVERN when comparing with SYMPATEC.

Even though the micronization process was basically the same as in the previous batch (06.ST75c.SCS048), it was not possible to obtain the same results or even a product within the specifications required, since the d(0.9) attained by both methods are above the client requirements. However, MALVERN showed to be more accurate when analyzing the micronized samples.

3.3.3 Batch 06ST75c.SCS051

A new batch was produced using the same unm micronized material as the one used in the second trial (batch 06ST75c.SCS050). Since the previous batch proved that the API particle size can change after being spray dried, more fractions were produced.

3.3.3.1 SEM



On the Figure 3-23 A it is possible to observe the first spray dried fraction and, it is noticeable the presence of big particles (3.5 μm - 5 μm) that will shift the PSD to higher values above the client's requirements.

On the other hand, when comparing both fractions with the same magnification, in Figure 3-23 B it is possible to notice smaller particles corresponding to a micronized product. Sub-micron sized particles and a few large particles that are close to the client's requirements ($d(0.9)$ between 2.5 μm and 3 μm).

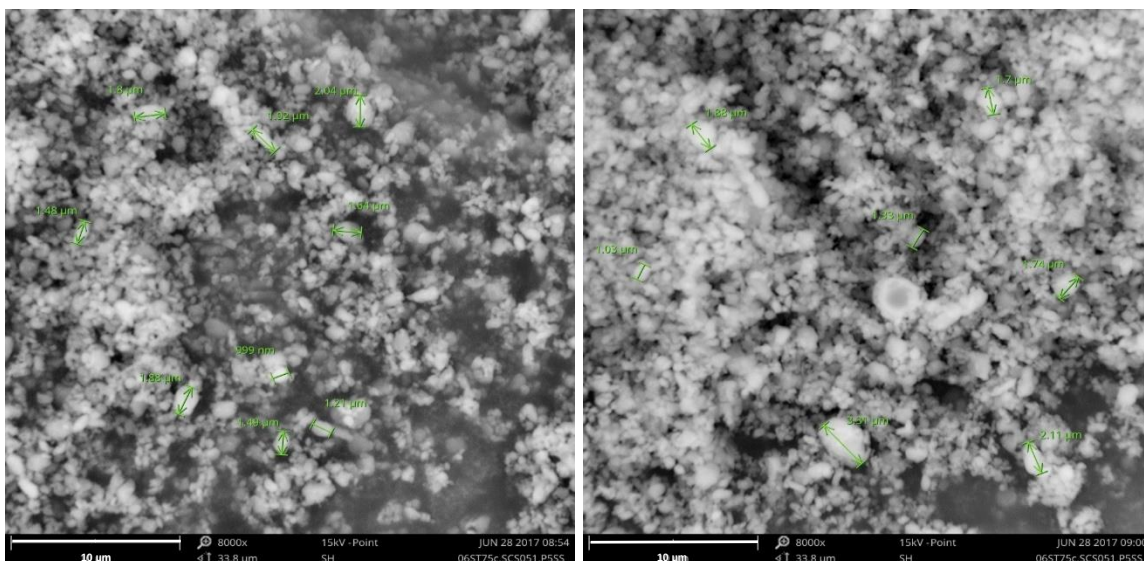


Figure 3-24 Images of ST75c at magnification of 8000x - 15 kV - Point – Different sections
[06ST75c.SCS051.P5SS]

In Figure 3-24 two different sections of the fraction 06ST75c.SCS051.P5SS are shown. A very similar view to the previous fraction (06ST75c.SCS051.P4SS) is seen, a lot of sub-micron particles and particles with size not bigger than 3.5 µm. This is to be expected since the only difference between both fractions is the quantity spray dried.

In the previous batch (06ST75c.SCS050) the PSD shifted greatly from the suspension analysis to the dried product analysis. In this batch, to guarantee that the product was within specification values, approximately 20 ml were spray dried first (06ST75c.SCS051.P4SS), and afterwards all the remaining product was spray dried resulting on fraction 06ST75c.SCS051.P5SS.

3.3.3.2 MALVERN vs SYMPATEC

The unmiconized material used was the same as the on the previous batch so the same conclusions apply: the MALVERN's method is not optimized for that PS range, so the SYMPATEC method is more accurate to analyze the unmiconized material.

Record n...	Sample name	d (0.1)	d (0.5)	d (0.9)	Obscuration	Residual	Residual - weig...	Span
190	05ST75.HQ00072 - Average	4.846	27.630	58.387	14.35	0.422	0.630	1.938
274	06ST75c.SCS051.P2SS - Average	0.971	2.090	4.121	11.60	0.389	0.486	1.507
298	06ST75c.SCS051.P4SS - Average	0.136	0.572	1.513	10.86	2.344	2.379	2.405
310	06ST75c.SCS051.P5SS - Average	0.122	0.604	1.902	13.94	0.710	2.081	2.945

Figure 3-25 Table results of the average value on liquid dispersion for unmiconized material [05ST75.HQ00072] and three different micronized fractions of batch SCS051 [06ST75c.SCS051.P2SS] [06ST75c.SCS051.P4SS] [06ST75c.SCS051.P5SS]

By the Figure 3-25 above together with the SEM images previously analyzed, it is possible to take some conclusions. For the first fraction spray dried, 06ST75c.SCS051.P2SS, where it was still possible to see big particles (3.5 µm – 5 µm), the MALVERN's method shows a good agreement (RN274). The d(0.9) value is too high according to the specifications.

Additionally, the fractions 06ST75c.SCS051.P4SS and 06ST75c.SCS051.P5SS are similar to each other regarding its PSD (RN298 and RN310, respectively). It is expected the slight increase in d(0.9) value since different quantities of product were spray dried.

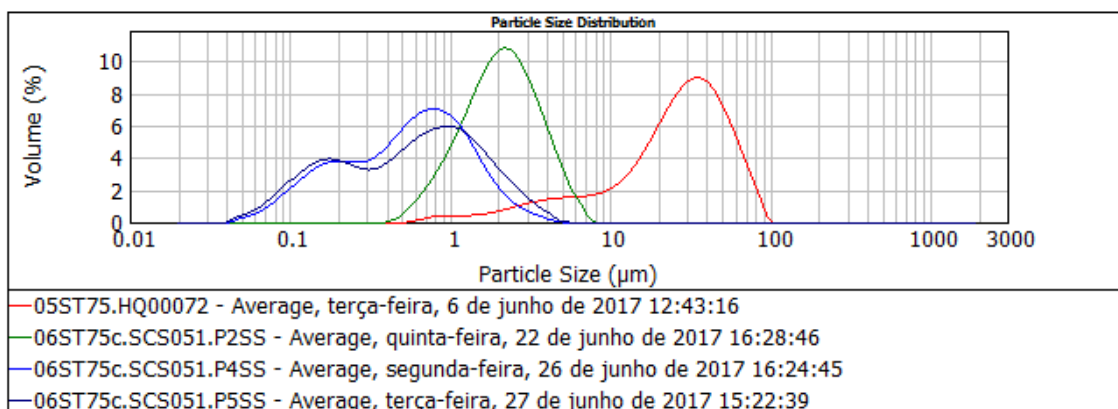


Figure 3-26 Comparison of normal distribution obtained through liquid dispersion between the unmicronized material [05ST75.HQ00072] and three fractions from batch SCS051 [06ST75c.SCS051.P2SS] [06ST75c.SCS051.P4SS] [06ST75c.SCS051.P5SS].

In Figure 3-26 a clear difference is seen between the first (06ST75c.SCS051.P2SS) and the final fraction (06ST75c.SCS051.P5SS) regarding the PSD, as well as the similarities between P4SS and P5SS fractions.

The first micronized fraction, P2SS, shows a normal distribution where the PS range is still relatively high due to the presence of big particles, resulting in a broader distribution. On the other hand, the more micronized fractions, P4SS and P5SS, have two distinct populations of particles with different PS, the sub-micron and micron sized particles.

Param. 2	x10	x50	x90	Span	c_opt	Meas. RODOS prim. pres.	ASPIROS: feed velocity	Measuring range
	μm	μm	μm		%	bar	mm/s	
▲ 06ST75c.SCS051.P4SS_1	0.52	1.56	4.03	2.25	7.67	6.24	18.00	R1: 0.1/0.18...35μm
● 06ST75c.SCS051.P2SS_6	0.58	1.98	4.79	2.13	7.69	6.21	18.00	R1: 0.1/0.18...35μm
■ 05ST75.HQ00072_7	28.23	78.17	139.99	1.43	9.47	0.12	50.00	R4: 0.5/1.8...350μm

Figure 3-27 Table results of the average value on dry dispersion for unmicronized material [05ST75.HQ00072] and two different micronized fractions of batch SCS051 [06ST75c.SCS051.P2SS] [06ST75c.SCS051.P4SS]

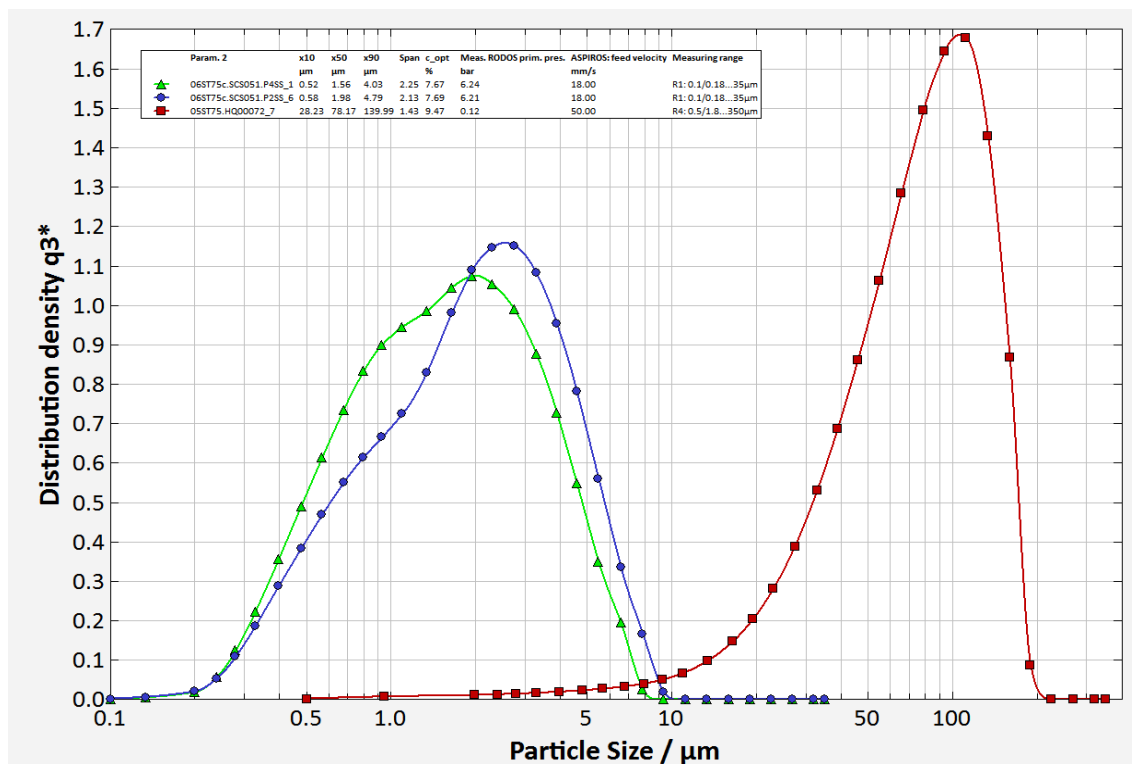


Figure 3-28 Comparison of normal distribution obtained through dry dispersion between the unmicronized material [05ST75.HQ00072] and two fractions from batch SCS051 [06ST75c.SCS051.P2SS] [06ST75c.SCS051.P4SS].

With SYMPATEC, as seen on the Figure 3-28, the fraction P5SS was not analyzed.

For the fractions 06ST75c.SCS051.P2SS and 06ST75c.SCS051.P4SS, the optimal parameters defined by the titration curves were a low feed rate velocity and the pressure limit of the equipment, 18mm/s and 6.2 bar, respectively. Titration curves in Appendix VIII.

Due to the low feed rate velocity, sometimes, 100 mg of product is needed to achieve an optical concentration superior to 5% (minimum value to be considered valid).

The results for the last fraction (06ST75c.SCS051.P5SS) are not expected to be much different from 06ST75c.SCS051.P4SS fraction as concluded with the previous analyses (Figure 3-27). Due to the low quantity obtained, it was not possible to perform titration curves with this fraction (P5SS).

When looking at the curves obtained from fractions P2SS and P4SS, it is not possible to see a significant difference between the two, which can be seen by both previous methods, SEM and MALVERN. Especially for fraction 06ST75c.SCS051.P4SS where a population of nanoparticles was shown in Figure 3-23 B.

On SEM images, fraction P2SS does not show a significant population of nanoparticles such as showed in fraction P5SS, so SYMPATEC method was able to analyze the fraction. However, as seen in Figure 3-28, a less uniform distribution was obtained when comparing it with the MALVERN result.

Table 3-8 PSD results obtained from liquid dispersion vs dry dispersion analyses for [ST75c.SCS051]

	D (0.1)	D (0.5)	D (0.9)
05ST75.HQ00072			
MALVERN	4.846	27.630	58.387
SYMPATEC	28.23	78.17	139.99
DIFFERENCE	23.38	50.54	81.60
06ST75C.SCS051.P2SS			
MALVERN	0.971	2.090	4.121
SYMPATEC	0.58	1.98	4.79
DIFFERENCE	0.391	0.110	0.669
06ST75C.SCS051.P4SS			
MALVERN	0.136	0.572	1.513
SYMPATEC	0.52	1.56	4.03
DIFFERENCE	0.384	0.988	2.517
06ST75C.SCS051.P5SS			
MALVERN	0.122	0.604	1.902
SYMPATEC	-	-	-
DIFFERENCE	-	-	-

When comparing both results for each fraction (Table 3-8), it is possible to see that the presence of nanoparticles, affected the results obtained by SYMPATEC. Nanoparticles are harder to deagglomerate due to the bonding forces between the particles.

Even though the fraction 06ST75c.SCS051.P2SS does not exhibit a lot quantity of small particles, the difference between both methods may be due to aggregates that did not fully deagglomerate. On this case, with the SEM images it was clear that there was a significant number of particles above the specs, however, the difference between both results is not considered significant.

In conclusion, apart from the unm micronized material analysis on MALVERN, the remaining showed to be in better agreement with the SEM than the SYMPATEC analyses. On the last mentioned, a complete deagglomeration could not be achieved.

On this trial, the MALVERN method provided a better particle deagglomeration. It also showed that only a small quantity of product is required to perform a valid analysis. This was an advantage because the dried product available to do the analyses was minimal.

3.3.4 Batch 06ST75c.SCS052

In this last trial done for API ST75, it was used the same unm micronized material as the last two previous batches. Just like the 06ST75c.SCS050 batch, this was an attempt to produce more product within the specification ii.

3.3.4.1 SEM

On this subchapter, only the two last fractions were analyzed by SEM analysis, P5SS and P6SS, due to the low quantity obtained on the other previously spray dried fractions (06ST75c.SCS052.P2SS and 06ST75c.SCS052.P3SS).

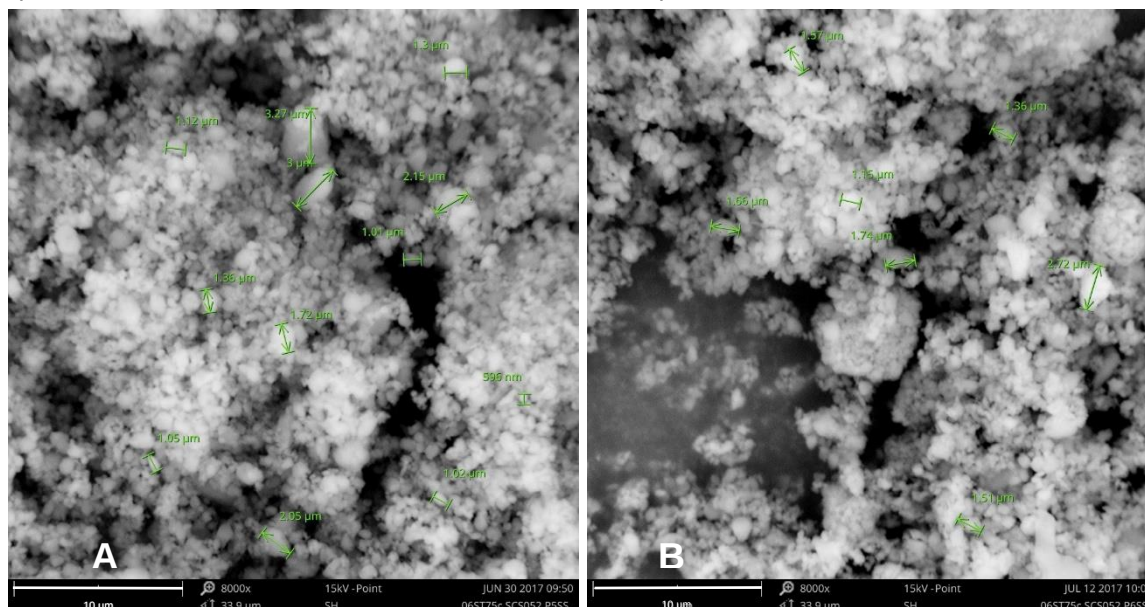


Figure 3-29 **A.** Images of ST75c at magnification of 8000x - 15 kV - Point [06ST75c.SCS052.P5SS]

B. Images of ST75c at magnification of 8000x - 15 kV - Point [06ST75c.SCS052.P6SS]

The last two fractions analyzed are somewhat different from the previous batch, because the fraction 06ST75c.SCS052.P5SS is the final micronized product and 06ST75c.SCS052.P6SS fraction was isolated foam from the suspension spray dried.

On both images in Figure 3-29 above it is possible to see an important and critical difference when comparing it with the other batch SEM image analysis: clear agglomerations of particles.

By comparing Figure 3-29 A and B, it is possible to see that there are more scattered big particles on the fraction P6SS than on P5SS, which is expected since the last dried fraction is a result of residual suspension and trapped powder from the spray dryer.

These batch also showed to be more electrostatic than the previous ones.

3.3.4.2 MALVERN vs SYMPATEC

As mentioned before, the starting material is the same as the one used on 06ST75c.SCS050 and 06ST75c.SCS051 batches. As previously mentioned, the SYMPATEC method was the only one that proved to be capable to analyze the unmiconized material.

Record n...	Sample name	d (0.1)	d (0.5)	d (0.9)	Obscuration	Residual	Residual - weig...	Span
190	05ST75.HQ00072 - Average	4.846	27.630	58.387	14.35	0.422	0.630	1.938
370	06ST75c.SCS052.P2SS - Average	0.161	0.706	2.076	10.80	2.940	1.521	2.712
386	06ST75c.SCS052.P3SS - Average	0.670	1.790	3.726	15.21	0.944	0.485	1.707
410	06ST75c.SCS052.P5SS_rep2 - Average	0.723	1.592	3.139	9.64	0.436	0.572	1.518
426	06ST75c.SCS052.P6SS_F2 - Average	0.655	1.892	5.813	11.19	0.497	0.450	2.726

Figure 3-30 Table results of the average value on liquid dispersion for unmicronized material [05ST75.HQ00072] and four fractions from batch SCS052 [06ST75c.SCS052.P2SS] [06ST75c.SCS052.P3SS] [06ST75c.SCS052.P5SS] [06ST75c.SCS052.P6SS]

As seen in Figure 3-30, there were two more fractions apart from the ones analyzed by SEM, 06ST75c.SCS052.P2SS and 06ST75c.SCS052.P3SS. These fractions were only analyzed with MALVERN.

It is possible to see that, from P2SS to P3SS fraction, the PS increased, which immediately may not make sense since P3SS is more micronized than P2SS. However, this can be explained by the quantity of suspension spray dried (approximately 20 ml), which might not be representative. Because the product recovered from the spray dryer corresponding to P2SS was less than 1 gram, the sample is not considered representative.

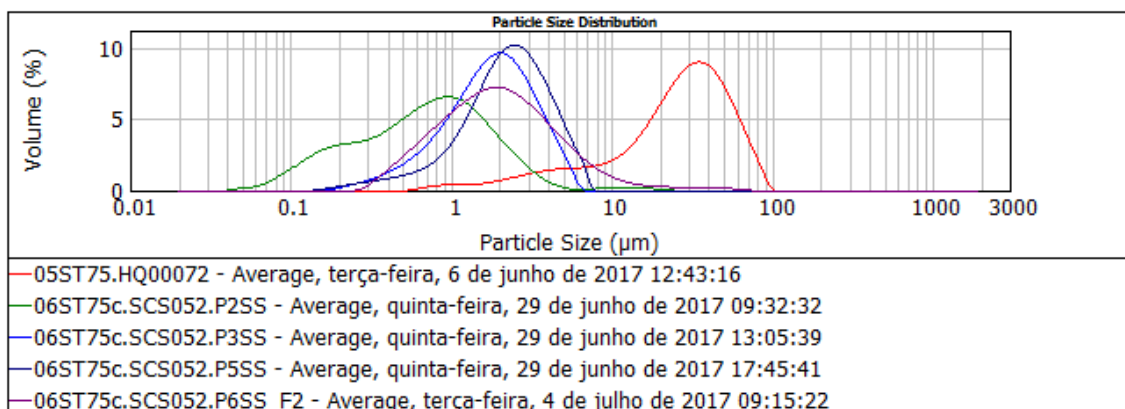


Figure 3-31 Comparison of normal distribution obtained through liquid dispersion between the unmicronized material [05ST75.HQ00072] and four fractions from batch SCS052 [06ST75c.SCS052.P2SS] [06ST75c.SCS052.P3SS] [06ST75c.SCS052.P5SS] [06ST75c.SCS052.P6SS]

In Figure 3-31, the three last fractions do not display a fine particle distribution unlike the one obtained in the previous batch (fractions 06ST75c.SCS051.P4SS and 06ST75c.SCS051.P5SS).

Because the last collected fraction, 06ST75c.SCS052.P6SS, is a mixture of the powder resulting from the suspension foam, which provides bigger particles, and residual powder from the previous fractions, the histogram obtained shows a wide span.

Due to the high electrostatic energy of the isolated powder inside the spray dryer, a lot of the product was clinging to the walls of the nozzle resulting in mass losses from the previous batch. Especially since a lot of fine particles were probably part of the ones trapped on the glass cyclone of the spray dryer, creating a fraction with a PSD above to the expected.

Another point to consider, is that because the sample has such high concentration of nanoparticles, the PSD may be outside of the method's range. As seen in the introduction chapter (Table 1-3), the obscuration range varies with the PS and it is a critical analytical parameter that influences the quality of the results obtained.

Param. 2	x10 μm	x50 μm	x90 μm	Span	c_opt %	Meas. RODOS prim. pres. bar	ASPIROS: feed velocity mm/s	Measuring range
06ST75c.SCS052.P6SS_F2_5	0.55	1.77	4.67	2.33	6.87	6.21	18.00	R1: 0.1/0.18...35μm
05ST75.HQ00072_7	28.23	78.17	139.99	1.43	9.47	0.12	50.00	R4: 0.5/1.8...350μm

Figure 3-32 Table results of the average value on dry dispersion for unm micronized material [05ST75.HQ00072] and the last fraction from batch SCS052 [06ST75c.SCS052.P6SS]

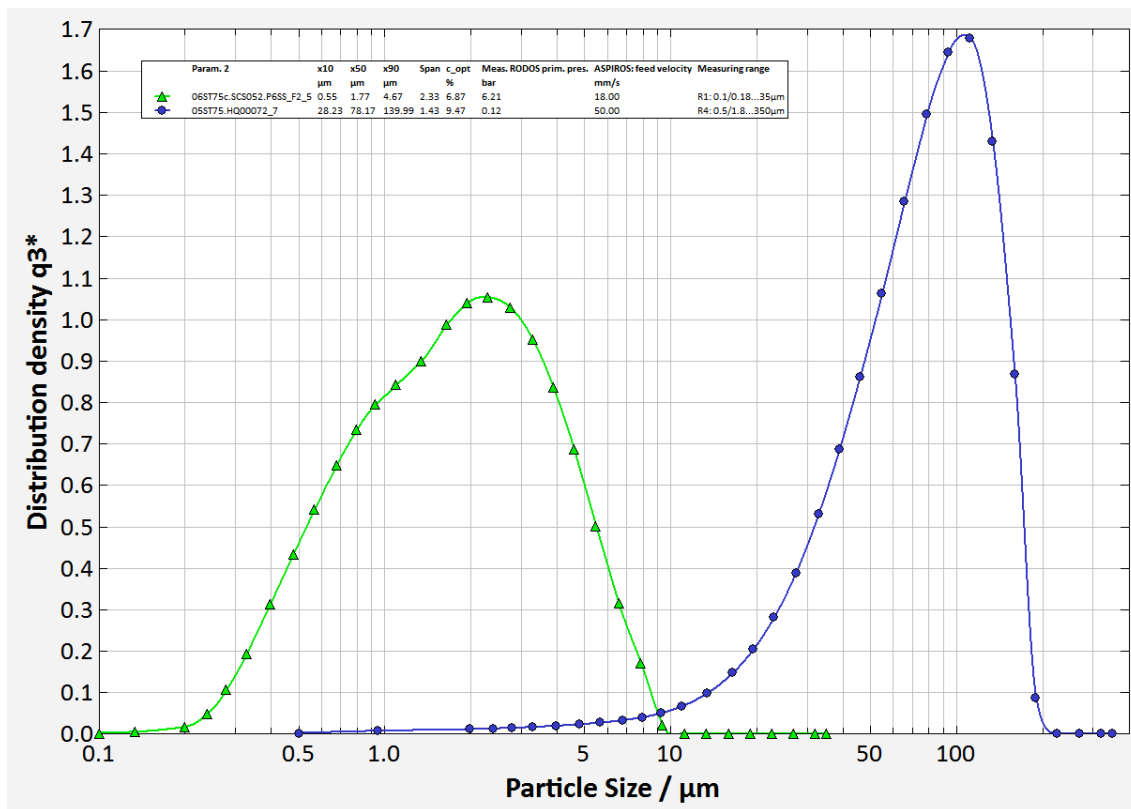


Figure 3-33 Comparison of normal distribution obtained through dry dispersion between the unm micronized material [05ST75.HQ00072] and the fraction from batch SCS052 [06ST75c.SCS052.P6SS].

On SYMPATEC, as seen in Figure 3-33, it was only possible to analyze the last fraction, because low amounts of the previous fractions were produced and it was not enough to do titration curves and to provide to the client.

For the purpose of this study, the last fraction 06ST75c.SCS052.P6SS was used, and once again, just like in the MALVERN analysis, there is not a second distribution on the histogram. However, in the SEM it is possible to observe fine particles much smaller than what obtained in d(0.1), 0.55 μm (Figure 3-32).

Table 3-9 PSD results obtained from liquid dispersion vs dry dispersion analyses for [ST75c.SCS052]

	D (0.1)	D (0.5)	D (0.9)
05ST75.HQ00072			
MALVERN	4.846	27.630	58.387
SYMPATEC	28.23	78.17	139.99
DIFFERENCE	23.38	50.54	81.60
06ST75C.SCS052.P2SS			
MALVERN	0.161	0.706	2.076
SYMPATEC	-	-	-
DIFFERENCE	-	-	-
06ST75C.SCS052.P3SS			
MALVERN	0.670	1.790	3.726
SYMPATEC	-	-	-
DIFFERENCE	-	-	-
06ST75C.SCS052.P5SS			
MALVERN	0.723	1.592	3.139
SYMPATEC	-	-	-
DIFFERENCE	-	-	-
06ST75C.SCS052.P6SS			
MALVERN	0.655	1.892	5.813
SYMPATEC	0.55	1.77	4.67
DIFFERENCE	0.105	0.122	1.143

Once again, this product proved to be quite difficult to be analyzed by SYMPATEC due to the high electrostatic energy originated from the high concentration of fine particles. It was observed that during the analyses the product clung to the glass walls of the vial.

Conditions considered optimal include low velocity and high pressure, 18 mm/s and 6.2 bar, respectively, which means more product was needed to reach at least 5% of optical concentration. Titration curves in Appendix IX.

Although when looking at the PSD results on Table 3-9 from MALVERN and SYMPATEC for 06ST75c.SCS052.P6SS, the latter has a slightly lower PSD which is closer to what is seen on SEM. This difference may be explained by the obscuration at which the analysis carried out on MALVERN, because it may influence the results obtained.

Even though the analyses were quite challenging, the fraction P5SS has the values close to the specification ii (d(0.9) between 2.5 - 3 μm), although its validity needs to be further investigated.

In conclusion, this product showed that the combination of high electrostatic energy and high concentration of fines test the limits of both types of equipment and its respective methods.

Chapter 4

Conclusion

The studies carried out during this thesis allowed to take several conclusions regarding PS analysis and the limitations of each equipment.

With SEM it is possible to observe the particles and measure them individually (one by one). This type of equipment does not report the PSD of the sample, it only allows to observe the shape and size of the particles visually.

The amount of powder used in the stub pin for SEM is less than the used in the MALVERN and SYMPATEC vials, so the sampling must be performed properly to be representative of the bulk.

Since the SEM analysis is the first step in a particle size analysis, if the data collected from imaging analysis is not correct, this may jeopardize the validity of the PS methods used for the analyses. To minimize errors induced by the analyst, several sections of the stub pin must be analyzed to cover a larger area. Thus, it becomes easier to perceive the PS, especially of the larger particles. This data is an important reference for the $d(0.9)$ reported by the PSD analysis.

Although in most SEM analyses described only one SEM image was shown per sample, during the analysis, several sections were analyzed.

The liquid and dry dispersion particle size methods are very different in their procedure.

MALVERN has several variables which need to be specified in the software before an analysis (for example, dispersant, refractive index, sample measurement time, ultrasounds and others¹). However, with SYMPATEC software only two parameters are considered: feed rate velocity and pressure.

The SYMPATEC method is more dependent on SEM than MALVERN, because small variations in the particle size of the samples easily influence the conditions of the analyses. Nevertheless, all analyses should consider the SEM results.

Small variations in the particle size can influence the interaction force between the particles (the force increases as the particle size decreases) and complete deagglomeration can be harder to achieve.

In liquid dispersion, despite the variation of the interaction forces, due to the presence of a surfactant, the deagglomeration is not usually affected. However, since in dry dispersion the medium is air, the deagglomeration must be forced by increasing the pressure.

The MALVERN method may appear to be more inflexible, however it can overcome several problems that may arise due to its robustness. The SYMPATEC method being simple is more susceptible to changes. This may not be a major problem if there is no limited quantity of product available for the analyses.

¹ All these parameters were described in tables throughout the previous chapter.

For the APIs IH13 and ST71, the MALVERN and SYMPATEC performance was similar since both provided complete deagglomeration of the samples, however that was not verified with ST75.

The presence of fine particles (nanometers range) in ST75 and high electrostatic force made it difficult to have complete deagglomeration during the analyses. This issue was specifically problematic on SYMPATEC.

Because of the presence of surfactant on the liquid dispersion, the deagglomeration was easily achieved. Only when the fine particles decreased from micrometers to nanometers the method seemed to not perform so well, since the PSD of those samples were starting to be outside the method's range.

For SYMPATEC, the pressure available was up to 6 – 6.2 bar², which showed to not be enough to provide a complete deagglomeration with high velocity feed rate. To try counter the problem, low velocities for feed rate were used, however two problems developed: still not enough pressure (although the PSD values were decreasing) and more quantity of product needed at low velocities.

At low velocities was needed 100 mg of product, approximately, for each analysis to be able to reach an optic concentration of 5%. When having to do titration curves for both pressure and feed rate velocity, a considerable amount of sample is needed, which is a critical problem with new developed products. Sometimes at high feed rate velocities, with other products, less than 20 mg can be enough for the analysis to reach 5% of optical concentration.

Other limitation that may arise during PSD analyses is the potential breakage of particles as a result of excess ultrasounds for MALVERN, or excessive pressure for SYMPATEC.

Both equipments have their limitations and advantages, for the products analyzed in this study, MALVERN showed to be the better choice overall. This does not mean that it is better than SYMPATEC, it only shows that different inherent product properties influence the analyses results and so, the method chosen must have that in consideration.

² These values were due to the equipment installation, the software enables pressures up to 20 bar.

Chapter 5

Future Work

Due to the limitations associated with lack of time it was not possible to perform further studies and analysis with the active pharmaceutical ingredients. More tests would help clarify some doubts and questions that arouse throughout this work.

Thus, it is suggested as future work:

- On Sympatec: more titration curves should have been done to better understand the impact of velocity and pressure in the deagglomeration process of the particles
- On Malvern: more analyses at different absorptions range would provide more useful information on the deagglomeration process since this is one of the principal variables when analysing small particles.
- Turbidity measurement on Malvern method development to further cement the results obtained.

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EQUIPMENT'S SPECIFICATIONS

I. Scanning Electron Microscope (Phenom ProX)

Table A-1 Equipment specification: Phenom ProX

Light optical magnification range	20 – 135 x
Electron optical magnification range	80 – 130000 x
Resolution	≤ 10 nm
Digital zoom	Max 12 x
Light optical navigation camera	Color
Acceleration voltages	Default: 5 kV, 10 kV and 15kV Advanced: adjustable range between 4.8kV and 15 kV imaging and analysis mode
Vacuum modes	Standard mode and charge reduction mode
Detector	BSD and EDS
Sample size	Up to 32 mm
Sample height	Up to 100 mm

II. MALVERN HYDRO 2000S

Table A-2 Equipment specification: Malvern Hydro 2000 S

Dispersion type	Wet
Capacity	50 – 150 ml
Typical applications	Solvent-based suspensions, Pharmaceuticals
Dispersion mechanisms	Continuously variable combined pump/stirrer Continuously variable ultrasonics
Modes of operation	Automatic via SOPs Manual via computer operating dialogs
Weight	16.8 kg
Dimensions	Width: 348 mm Height: 333 mm Depth: 365 mm
Power	100 – 120 V / 200/240 V AC, 50/60 Hz, 240 VA
Maximum size of particles	Up to 600 microns, depending on particle shape and density

III. SYMPATEC (laser diffraction sensor HELOS)

Table A-3 Equipment specification: Sympatec Helos

Sensor:	HELOS	BR: 0.1 μm - 875 μm KR: 0.1 μm - 8750 μm VARIO: 0.1 μm - 8750 μm
Principle:	Laser diffraction	$\lambda = 632,8 \text{ nm}$
Dispersion:	Adaptable modules	Aerodispersion, sprays, suspensions, emulsions
Measurement:	Multi-element detector frequency	31 semi-circular elements 2000/s, permanent autofocus
Evaluation:	Fraunhofer Enhanced Evaluation (FREE)	Mie Extended Evaluation (MIEE) as an option
Ranges:	Optical modules	R1 to R8
Performance:	Accuracy, repeatability, comparability, X_{10} , X_{50} , X_{90}	$s < 2\%$ mean rel. SD to absolute value $s < 0.04\%$ typical (repeated sample) $s < 0.3\%$ typical (riffled sample) $s < 1\%$ mean rel. SD $ Dx < 2.5\%$ rel max. deviation $< 5\%$ rel. deviation in the submicron range
Operation:	WINDOX software	WINDOWS 7 / Vista Prof. / XP Prof.
Applications:	laser power protection class/type beam diameter ($1/e^2$)	5 mW 3R/IP40 R1 & R2 : 2.2 mm R3-R5: 13 mm R6 & R7: 26 mm R8: 35mm
QA-system	Certification reference material validation	Standard test procedure SiC - F1200 ($x_{50} = 4.5 \mu\text{m}$) SiC - P600 ($x_{50} = 27 \mu\text{m}$) SiC - P80 ($x_{50} = 260 \mu\text{m}$) SiC - P50 ($x_{50} = 430 \mu\text{m}$) FDA approved

IV. SYMPATEC (dispersion unit RODOS/M)

Table A-4 Equipment specification: Sympatec Rodos/M

Principle	Aerosol jet	Particle – Particle collisions Particle – Wall collisions Velocity gradients
Dispersion	Pressure Injector airflow Extraction	0.1 – 6 bar 300 l/min Streamlined
Feeding	VIBRI funnel dosing ASPIROS tube feed	Vibratory feeder: mg – kg Micro feeder: µg – mg
QA-system	Guarantee	50000 shots: 4 g cement PZ35
Operation	Software-controlled	Standard operating procedures (SOPs)
Application	HELOS laser diffraction measuring ranges	0.1 µm – 3500 µm R1 – R7

V. SYMPATEC (Accessory ASPIROS)

Table A-5 Equipment specification: Sympatec Aspiros

Applications	Controlled dosing of dry powders as accessory to RODOS, RODOS/m and OASIS
Sample quantity	< 1 mg to about 1g
Measuring range	0.1 µm to 875 µm
Dosing	Speed controlled
Sample preparation	Glass tubes
Capped tube	For safe handling and processing
Bar-code	Adhesive bar-code label for identification and parameter set-up prior to measurement supports all standard codes, i.e. CODE 39, Interleaved 2 of 5, CODABAR, Code 128
Operation	Manual feeding of the sample glass tube containing the sample into the transport sled, operation starts only if cover is closed and locked; Sample glass detection; Automatic un-capping
Simplified cleaning	All parts in contact with the sample are easily removable
Control	By software via RS485 Interface or manually via panel with alphanumeric display; Supported by WINDOX 5 software

06IH13c.SCS038.P6SS method development [MALVERN]

DISPERSANT AND SURFACTANT

The first step of the PS method development is discovering the perfect combination of dispersant and surfactant by knowing the polarity of the solvent used during the micronization. The best dispersant was expected to be heptane, hexane or isoparaffin with surfactants being Span 85 or lecithin (as described by Table 2-1 in subchapter 2.2). The testing results are detailed on Table A-6.

Table A-6 Dispersant/surfactant test [IH13c method development]

Dispersants	Results
0,2% (w/v) Span 85 in n-hexane	Dissolves
0,1% (w/v) Lecithin in n-heptane	Good suspension
0,1% (w/v) Span 85 in isoparaffin	Product accumulation

The dispersant/surfactant combination chosen was 0.1% (w/v) lecithin in n-heptane.

ULTRASOUND TITRATION CURVES AND FIT

The first step to the method development is performing ultrasound curves to understand the product behavior and to see if the default conditions are appropriate for the analysis. The default conditions are shown in Table A-7.

Table A-7 Default conditions for ultrasound curves [IH13c method development]

Accessory	
Sample handling unit	Hydro 2000S
Stirrer speed (rpm)	2100 rpm
Ultrasonic (%)	First variable
Time of ultrasounds	First variable
Measurement options	
Material	
Refractive index	1.52
Absorption	0.1
Dispersant name	N-heptane
Refractive index of the dispersant agent	1.39
Result calculation	
Model	General Purpose
Calculation sensitivity	Normal sensitivity
Particle shape	Irregular
Measurement	
Sample measurement time	10 seconds (10000 snaps)
Background measurement time	10 seconds (10000 snaps)
Number of measurements	3
Delay	10 seconds
Average	Create average result
Obscuration limits	5 % - 10 %

The ultrasound curves were done at 30%, 50% and 80% out of 100% maximum percentage. The product was analyzed every 30 seconds until 300 seconds total.

In the end it was possible to create a trend curve for $d(0.1)$, $d(0.5)$ and $d(0.9)$. Since the curves related to $d(0.1)$ and $d(0.9)$ did not appear to have a steady decline behavior, to make the decision it was used the trend curve from $d(0.5)$ for the three different ultrasound percentages. From the US curves shown in Figure A-1 below, it was possible to determine the optimal US value condition for the analysis.

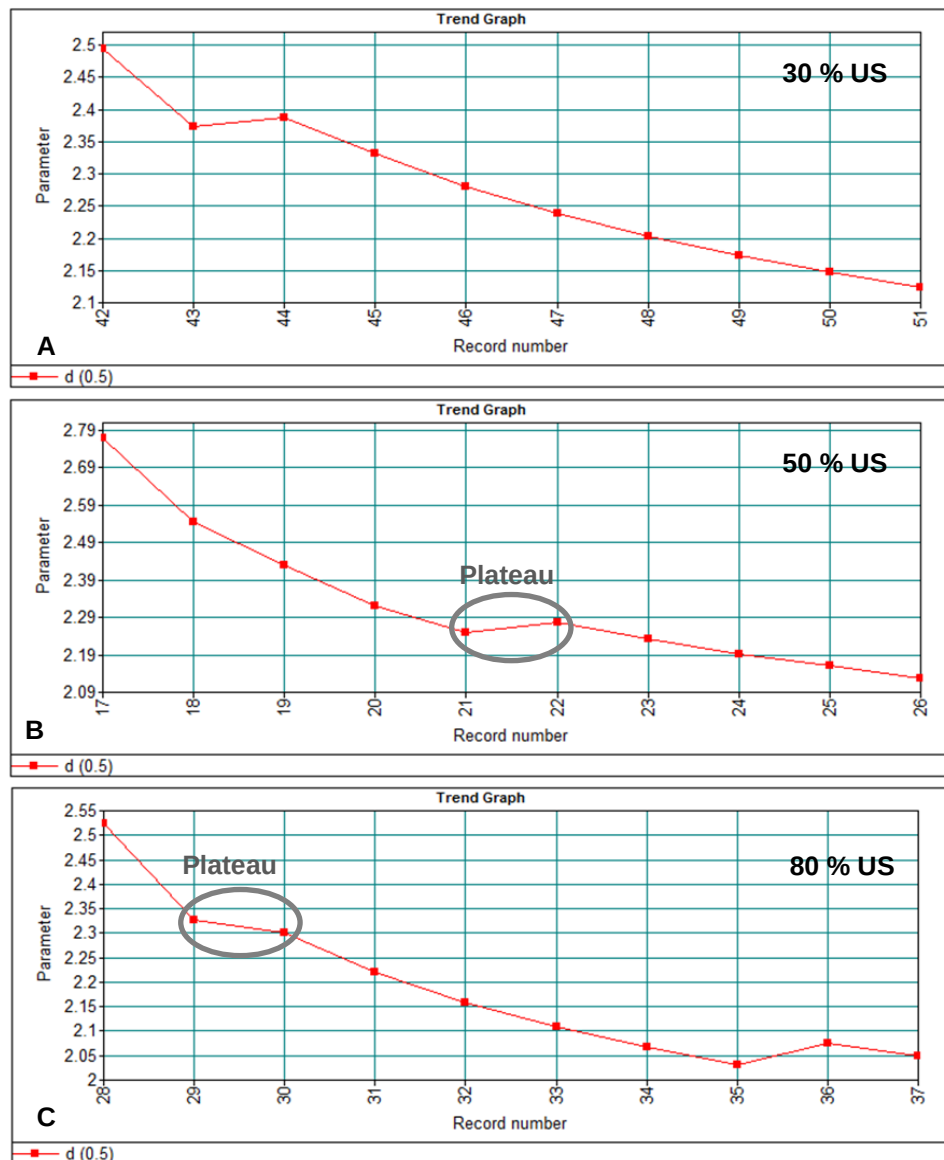


Figure A-1 Trend graphs for $d(0.5)$ at different US percentage: **A** 30% ultrasounds; **B** 50% ultrasounds; **C** 80% ultrasounds [IH13c method development]

On the curve A from the Figure A-1 it is possible to see a continuous decrease which means 30% of ultrasounds are not enough to deagglomerate all the particles. On B and C curves, it is possible to see a plateau, an interval between two analyses with little variations, which means that all particles are deagglomerated and more quantity of US may break the particles.

When comparing the curves B and C, it is possible to see that the plateau is reached faster when more US are applied. However, applying a high US percentage within a small period of time causes more turbulence. It is preferable to have a lower US percentage for an extended period of time, so that the stabilization may be easier and faster.

It was concluded that 50% US for 120 seconds was the best condition, but since the residual values were higher than 2, it was adjusted using a tool from the software, fit, as seen in Figure A-2. The adjustments were done by changing the refractive index and the absorption values.

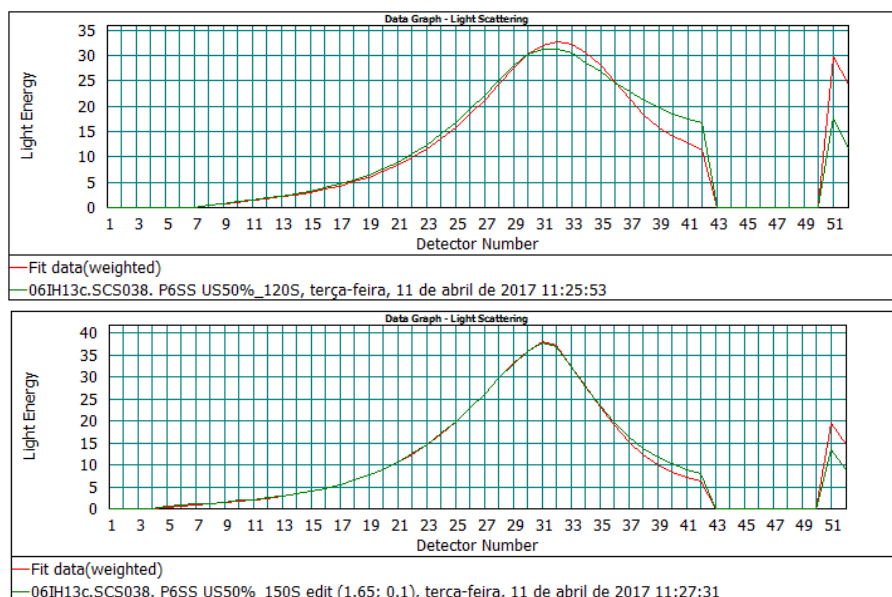


Figure A-2 Adjustment of parameters: refractive index and absorption. Before (above) and after (below) [IH13c method development]

The tool fit showed that the absorption value was not needed to change. An adjustment on the refractive index resulted in a smaller residual value. The values were adjusted from (1.52; 0.1) to (1.65; 0.1) for the refractive index and absorption, respectively.

Further repeatability and a robustness tests were carried out to verify the optimal analysis conditions (involving the speed of the stirrer, obscuration limits and sample time analysis).

The final analysis conditions are presented in the sub chapter 3.1.2. in Table 3-1.

Appendix III

IH13 TITRATION CURVES: PRESSURE AND FEED RATE VELOCITY

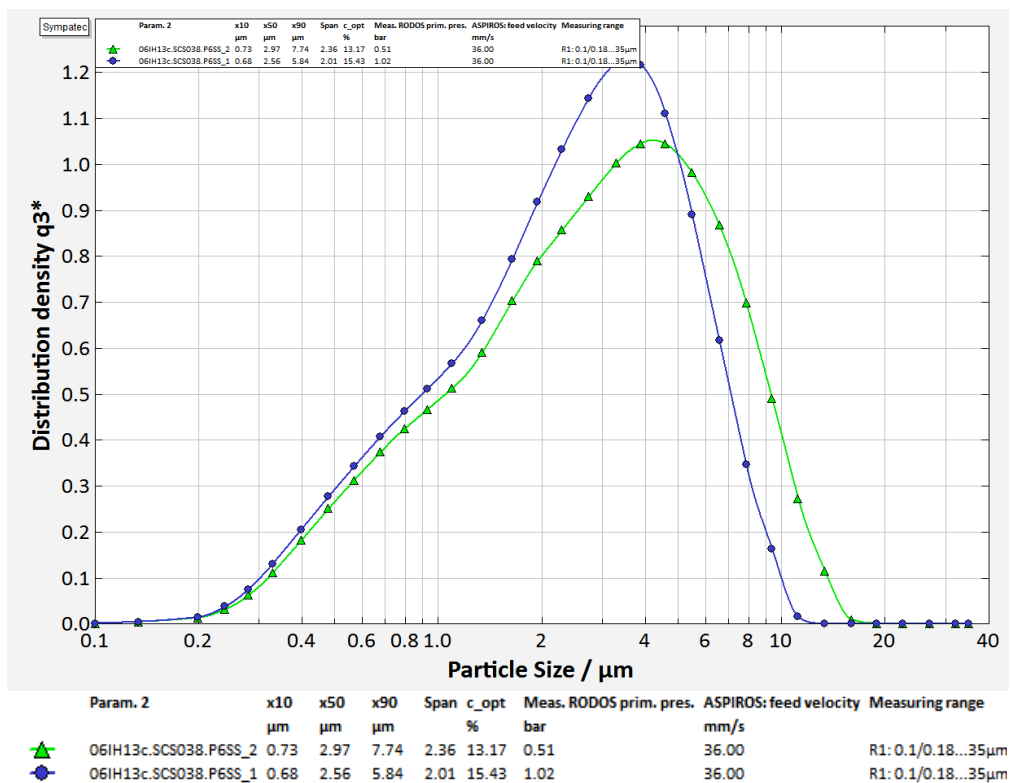


Figure A-3 Titration curve: pressure [IH13c method development]

By comparing both curves from Figure A-3, it was concluded that for complete deagglomeration the optimal pressure was 1 bar.

The feed rate velocity was selected from analyses of previous fractions.

The optimal conditions chosen were: 1 bar, 36 mm/s and lens R1.

UNMICRONIZED MATERIAL OF ST71C

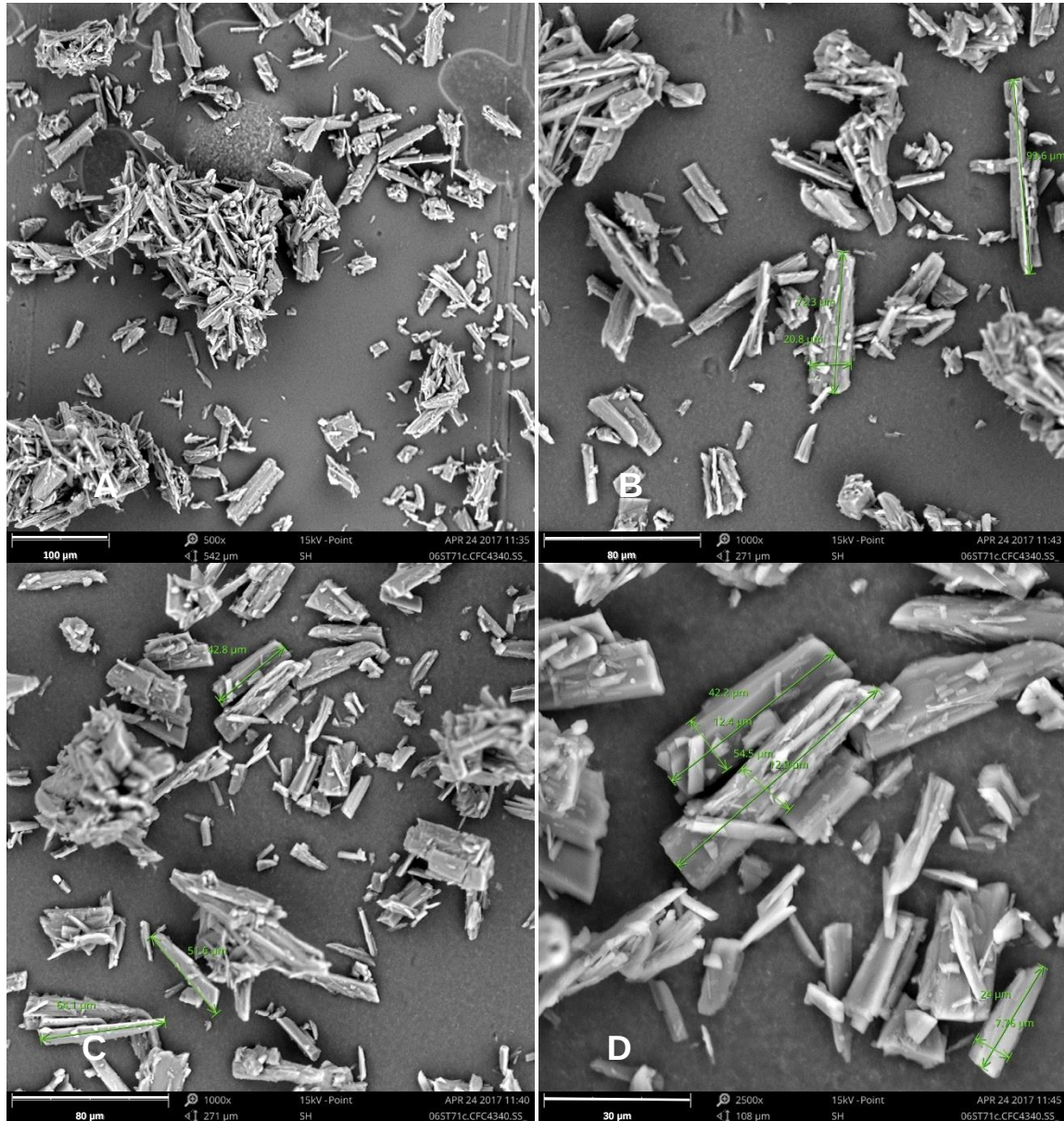


Figure A-4 Powder sample of unmicronized material [06ST71.CFC4340.SS] at 15kV – Point and magnification of: 500x (A), 1000x (B and C – Different sections) and 2500x (D)

Figure A-4 shows different sections of the stub pin at different magnifications where it is possible to observe agglomerates and a wide range of PS. Due to its rectangular like shape, it is harder to perceive its spherical equivalent diameter.

TITRATION CURVES FOR 06ST71C: PRESSURE AND FEED RATE VELOCITY

I. Unmicronized material [06ST71.CFC4340.SS]

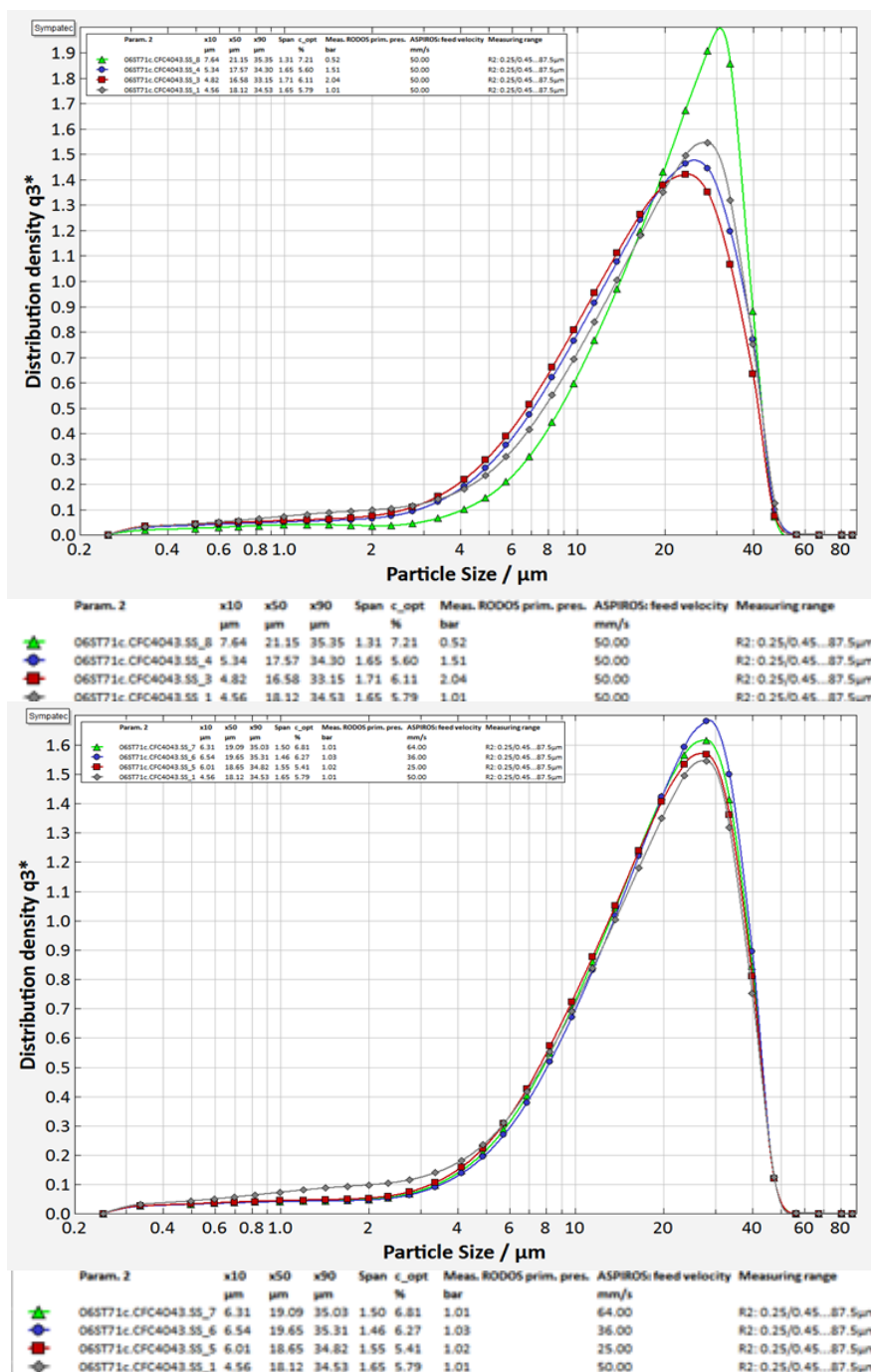


Figure A-5 Titration curves for unmicronized material [06ST71.CFC4340.SS] : pressure (above) and feed rate velocity (below) [ST71c method development]

From the Figure A-5 it is possible to see that at different pressures and feed rate velocities the PSD result does not show great discrepancies between the analyses. This may be due to its rectangular shape.

The optimal conditions chosen were: 2 bar, 50 mm/s and lens R2.

II. Micronized material

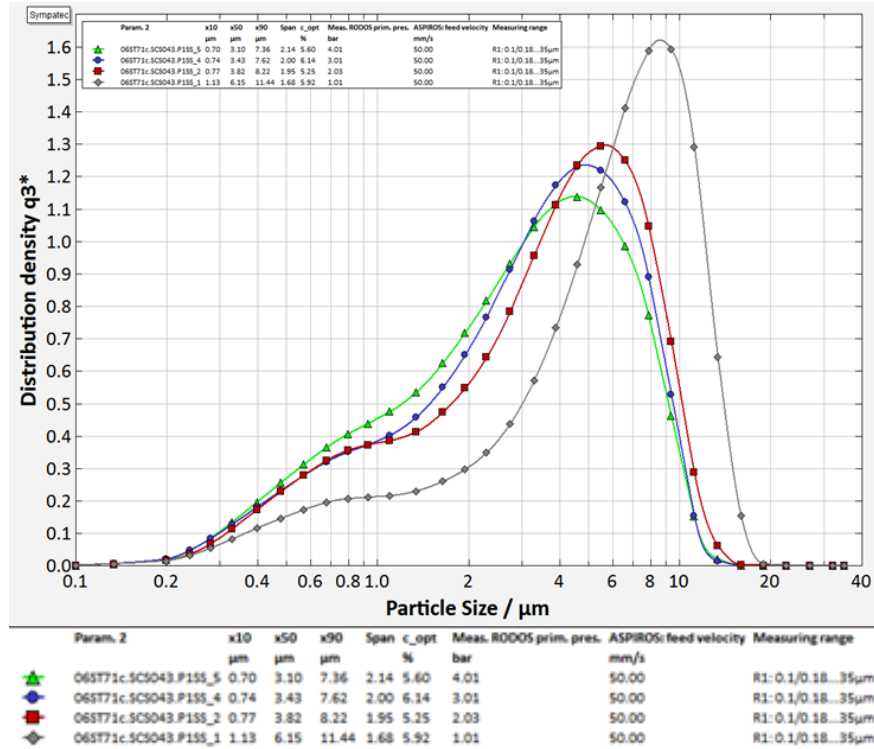


Figure A-6 Titration curves for micronized material : pressure [ST71c method development]

The titration curve for pressure (Figure A 4 6) was done with fraction 06ST71c.SCS043.P1SS at high velocity feed rate. By comparing the histograms and PSD results with the SEM images, 3 bar was the pressure chosen.

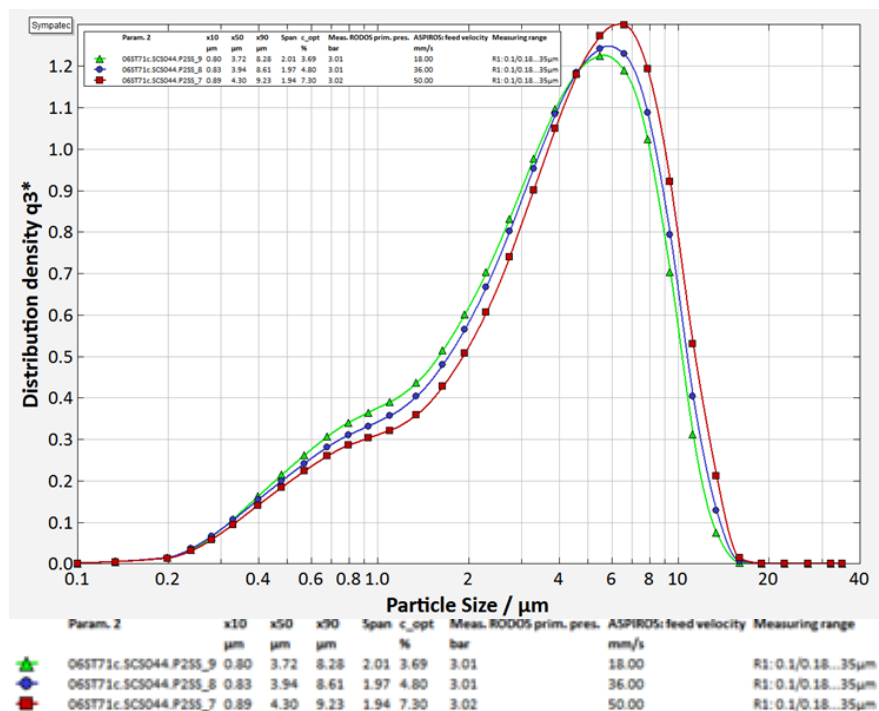


Figure A-7 Titration curves for micronized material: feed rate velocity (below) [ST71c method development]

With the fraction 06ST71c.SCS044.P2SS it was confirmed that the velocity feed rate did not influence the PSD results.

The optimal conditions chosen were: 3 bar, 50 mm/s and lens R1.

Appendix VI

TITRATION CURVES FOR ST75.SCS048: PRESSURE AND FEED RATE VELOCITY

I. Unmicronized material [06ST75046.GML9612]

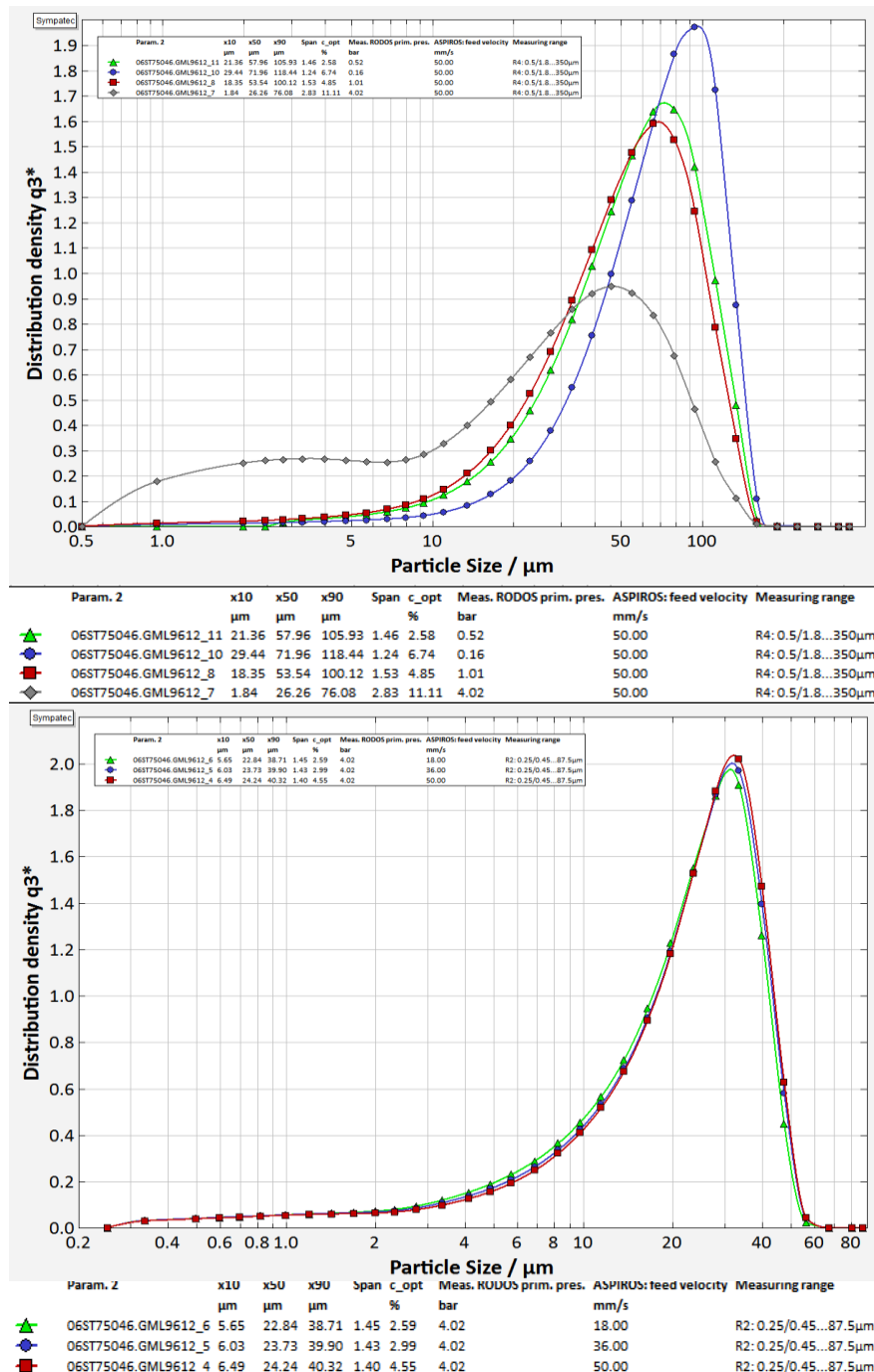


Figure A-8 Titration curves for unmicronized material [06ST75046.GML9612] : pressure (above) and feed rate velocity (below) [ST75c method development]

In Figure A-8 it is possible to see that the feed rate velocity does not influence the deagglomeration and so, the pressure titration curves were done with high velocity, 50 mm/s.

By comparing the results from the pressure titration curves with the SEM images it was possible to observe that the lowest pressure was enough to deagglomerate.

The optimal conditions chosen were: 0.1 bar, 50 mm/s and lens R4. These were the same conditions used with the unm micronized material 05ST75c.HQ00072, from batches 06ST75c.SCS50, 06ST75c.SCS051 and 06ST75c.SCS052.

II. Micronized material [06ST75c.SCS048.P4SS]

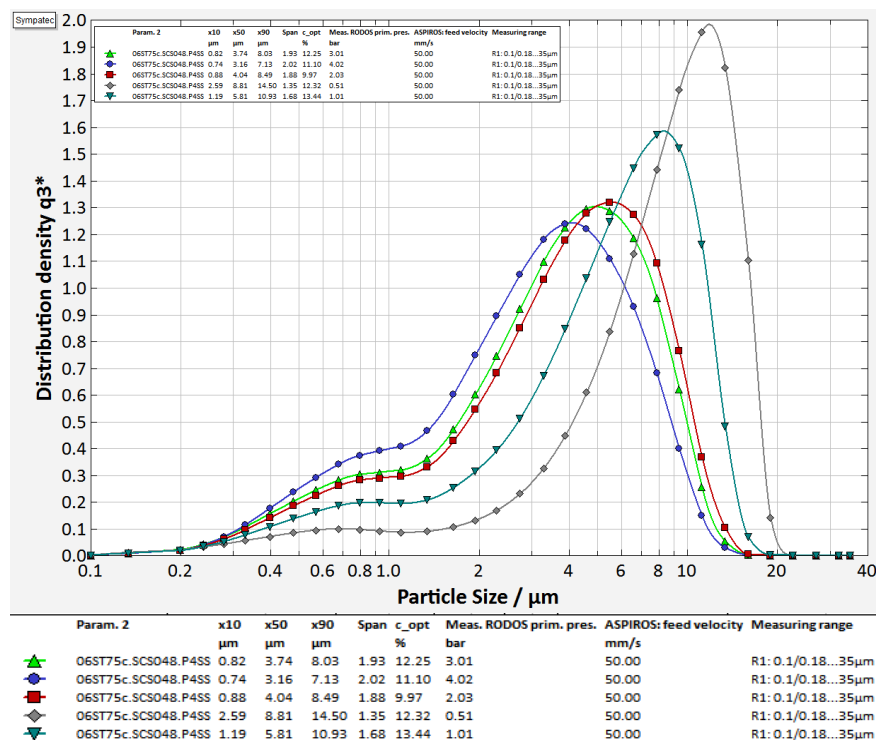


Figure A-9 Titration curve for micronized material [06ST75c.SCS048.P4SS]: pressure [ST75c method development]

For the micronized fraction 06ST75c.SCS048.P4SS, only the pressure was measured since the high velocity, 50 mm/s, proved to help deagglomeration.

With the histograms from Figure A-9 and SEM images it was concluded that medium pressure was enough to deagglomerate. Since pressures 2, 3 and 4 bar were similar it was chosen 3 bar as the ideal.

The optimal conditions chosen were: 3 bar, 50 mm/s and lens R1.

III. Micronized material [06ST75c.SCS048.P15SS]

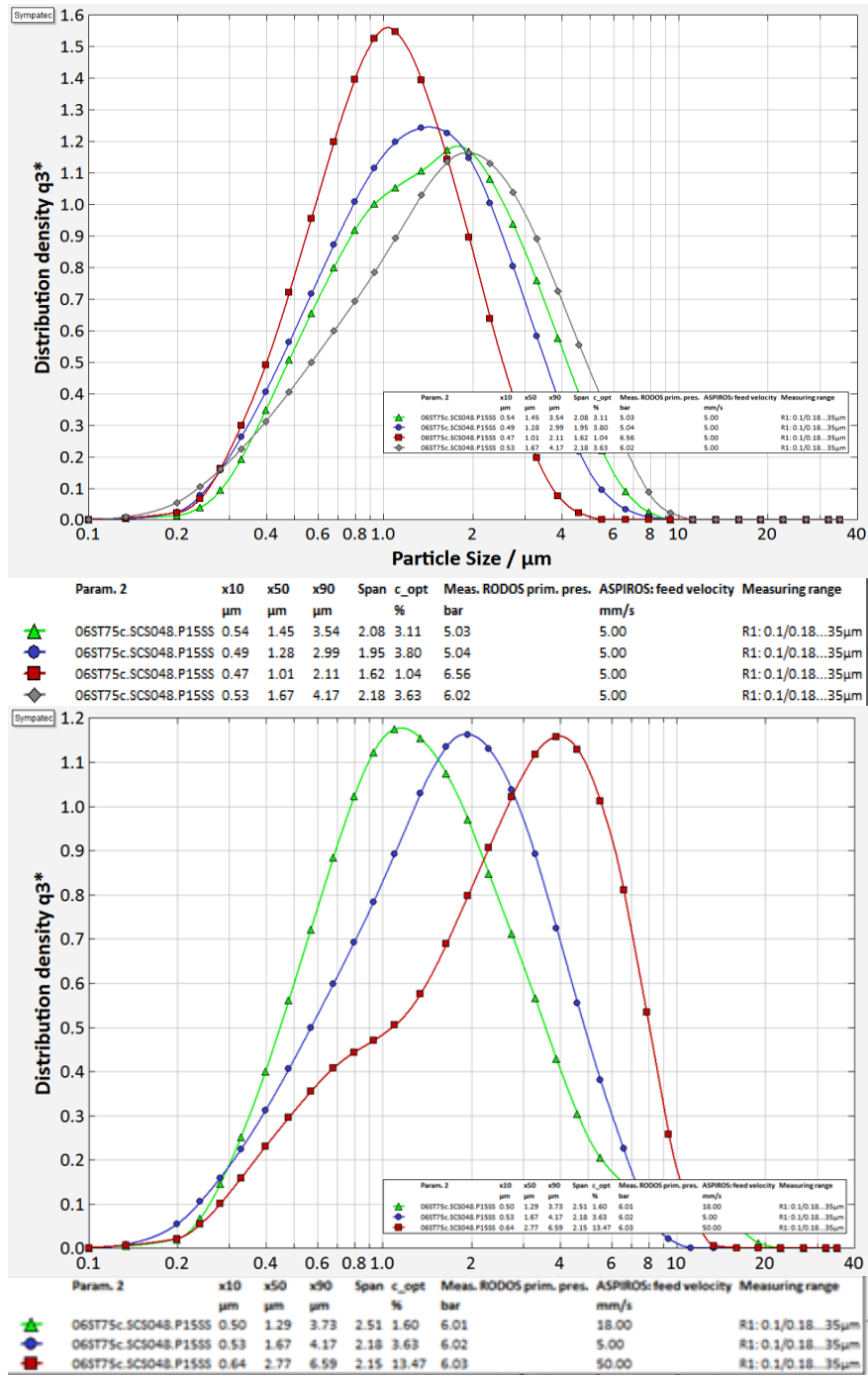


Figure A-10 Titration curve for micronized material [06ST75c.SCS048.P15SS]: pressure (above) and feed rate velocity (below) [ST75c method development]

Due to the more micronized particles, new conditions were chosen to provide a complete deagglomeration.

In Figure A-10 image below, it is possible to observe that with low feed rate velocity, a better deagglomeration is attained. For the pressure parameter, good results were obtained at 5 bar.

The optimal conditions chosen were: 5 bar, 5 mm/s and lens R1.

Appendix VII

TITRATION CURVES FOR MICRONIZED FRACTION ST75.SCS050.P6SS: PRESSURE AND FEED RATE VELOCITY

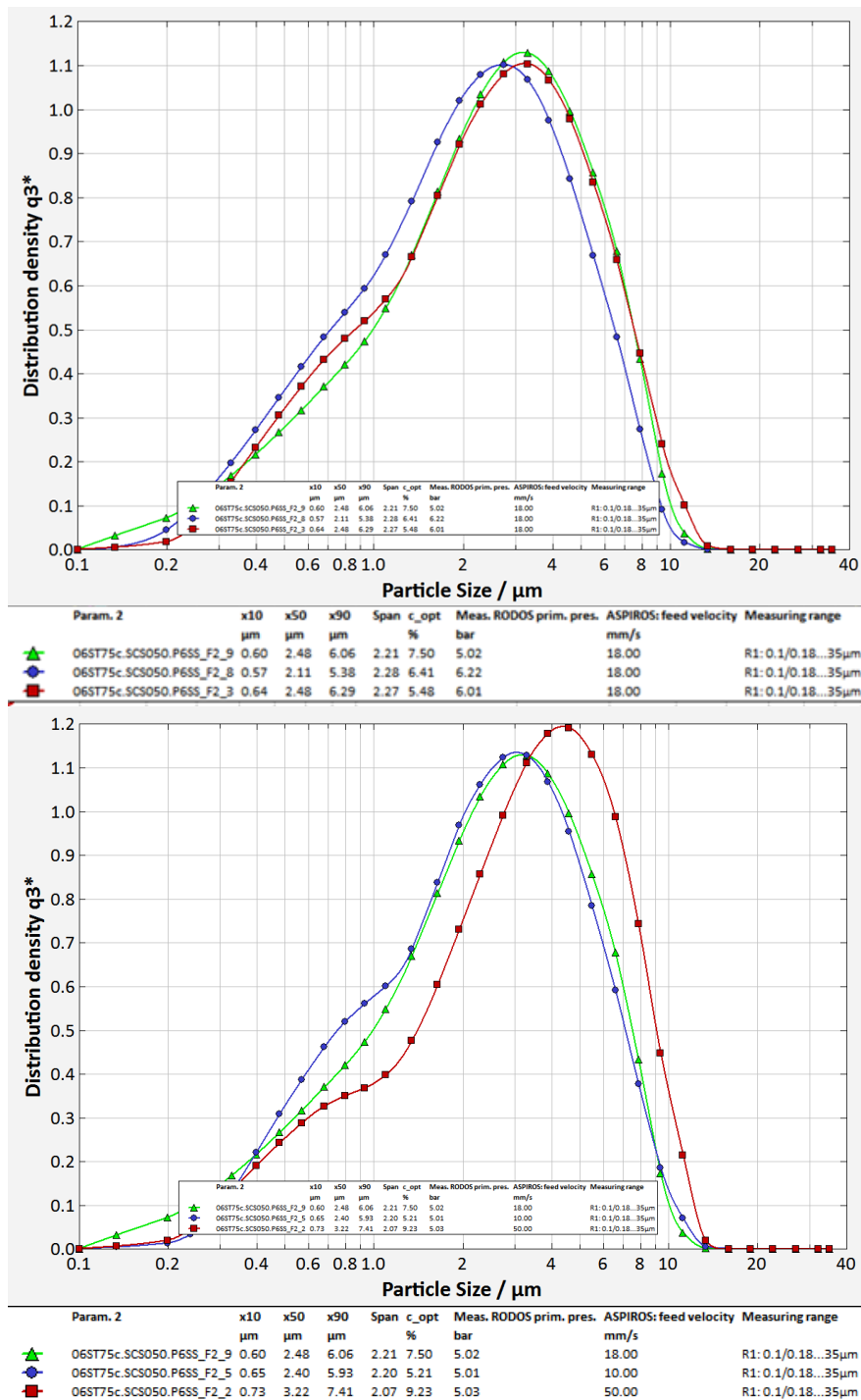


Figure A-11 Titration curves for micronized fraction 06ST75.SCS050.P6SS : pressure (above) and feed rate velocity (below) [ST75c method development]

For this fraction (06ST75c.SCS050.P6SS), low feed rate velocities were also proven to ensure a good deagglomeration. Because velocities 10 mm/s and 18 mm/s did not appear to be much different, the higher was chosen.

In Figure A-11 first image, it is possible to observe that all results are similar with high pressure. The higher pressure was chosen to guarantee complete deagglomeration.

The optimal conditions chosen were: 6.2 bar, 18 mm/s and lens R1.

Appendix VIII

TITRATION CURVES FOR MICRONIZED FRACTIONS FROM ST75.SCS051 BATCH: PRESSURE AND FEED RATE VELOCITY

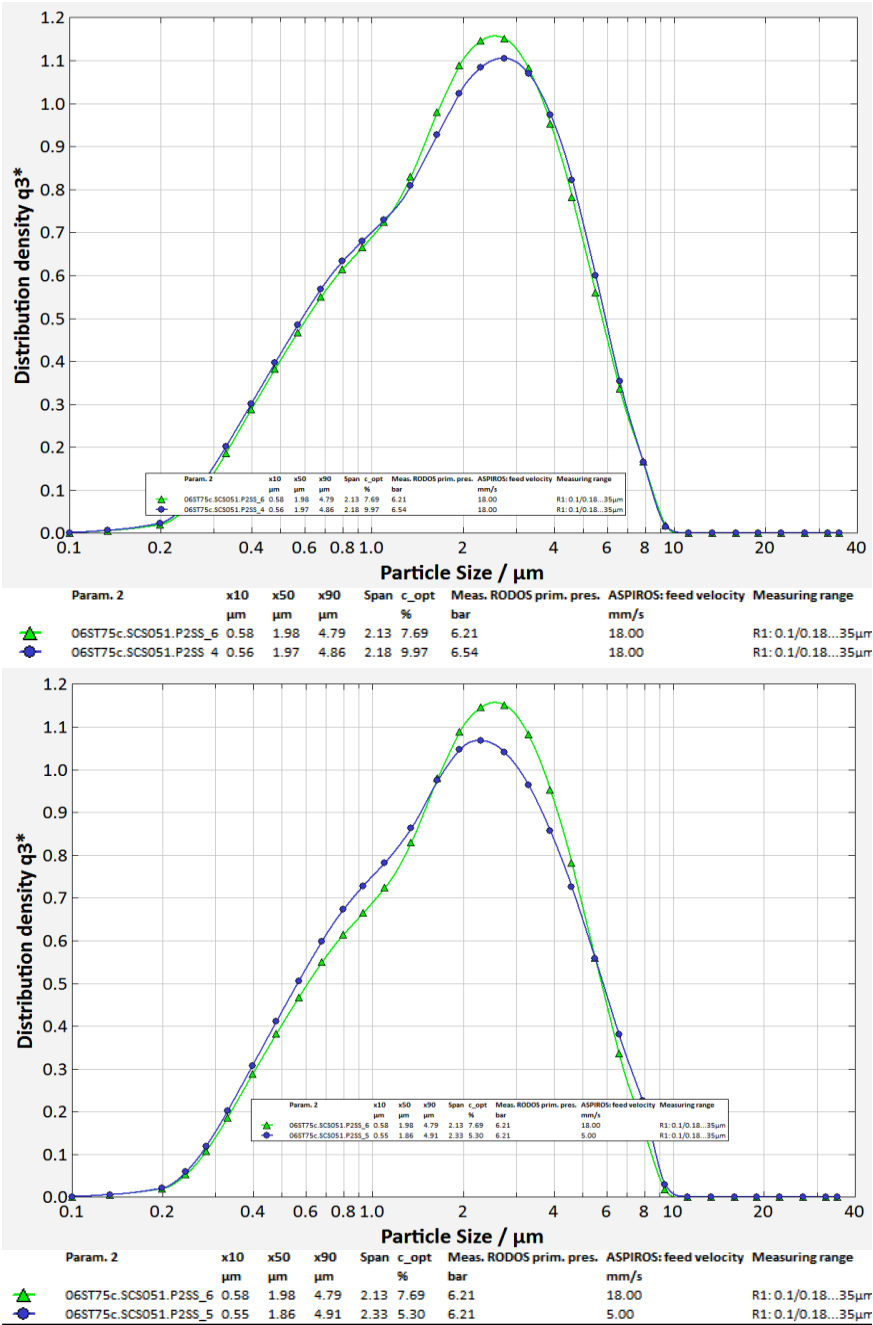


Figure A-12 Titration curves for micronized fractions from 06ST75.SCS051 batch : pressure (above) and feed rate velocity (below) [ST75c method development]

For the micronized fractions from batch 06ST75.SCS051 the same conditions from batch 06ST75c.SCS050 were used.

The Figure A-12 shows that by lowering the feed rate velocity to the minimum and pressure to the maximum, does not affect the PSD results.

The optimal conditions chosen were: 6.2 bar, 18 mm/s and lens R1.

Appendix IX

TITRATION CURVES FOR MICRONIZED FRACTION ST75.SCS052.P6SS: PRESSURE AND FEED RATE VELOCITY

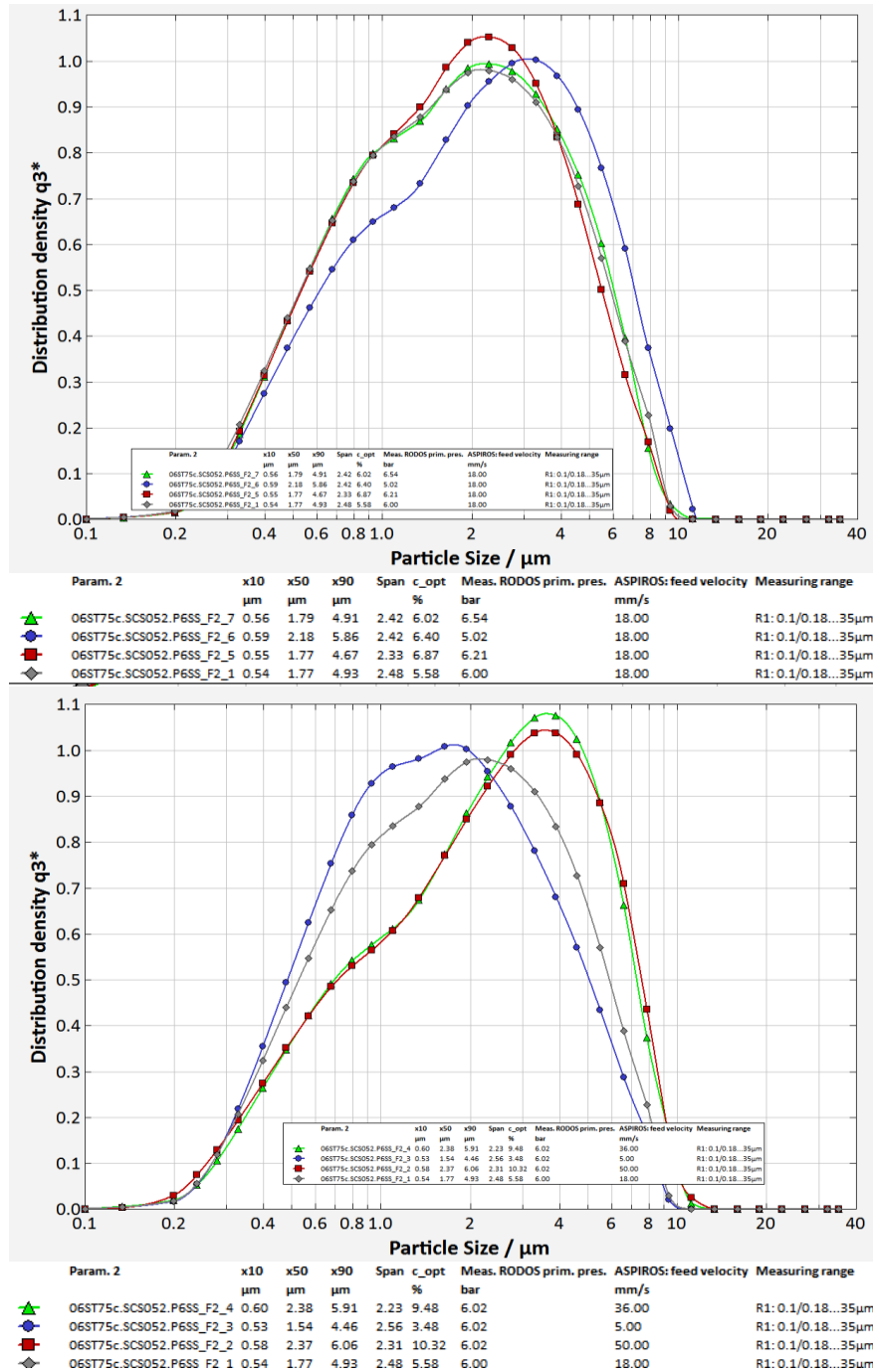


Figure A-13 Titration curves for micronized fraction 06ST75.SCS052.P6SS: pressure (above) and feed rate velocity (below) [ST75c method development]

For the micronized fraction 06ST75c.SCS052.P6SS, once again low feed rates velocities and high pressure were proven to be the ideal conditions to provide complete deagglomeration (Figure A-13).

The optimal conditions chosen were: 6.2 bar, 18 mm/s and lens R1.