



Method Development for Droplet and Particle Size Distribution Measurements using Laser Diffraction

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Abstract

This degree project aims to develop standard methods for determination of the size distribution in different samples using laser diffraction, as well as to create methodologies for development of new methods for unknown samples.

The size distribution is crucial to understand for pharmaceutical products since it affects properties such as the stability and bioavailability of the product. A way to measure the size distribution is to use laser diffraction. Laser diffraction is a technique in which laser beams are directed through a sample flow. When the laser beam hits a particle in the sample flow, the light scatters at different angles and intensities depending on the size and shape of the particle. The scattered light is then detected and translated into a size distribution, in this degree project by using the Mie theory.

Different parameters suspected to affect the size distribution when using laser diffraction were analysed and evaluated for both emulsion- and powder samples. Due to the small droplet- and particle sizes, the sample preparation technique was believed to have a great impact on the size distribution. Other than this, the parameters pump speed, sonication intensity, obscuration and delay time were evaluated.

The pump speed as well as the sonication time and intensity were found to affect the size distribution the most, while the delay time had a slight effect, and the obscuration barely affected the size distribution. It was further found that the powder samples were less sensitive than the emulsions and hence could withstand more energy in terms of higher pump speeds and sonication intensities. This also contributed to different optimal dispersing techniques during sample preparation; the powders were dispersed by sonication while the emulsions were dispersed by stirring on a magnetic stirrer plate. Other than this, the temperature was found to greatly impact the size distribution of the emulsions. In short, it was concluded that the pump speed and sonication intensity affected the size distribution the most, and that the emulsions should be treated with less energy than powders to maintain a representative sample.

Metodutveckling för Mätning av Dropp- och Partikelstorleksfördelning med hjälp av Laserdiffraktion

Partiklar finns i princip överallt runtomkring oss, och har en stor betydelse inom många industrier. Partikelstorleken i målarfärg avgör dess kvalitet, partiklar i miljön påverkar vår hälsa, och partikelstorleken i läkemedel påverkar dess effektivitet och stabilitet, för att nämna några exempel. Fokus för detta examensarbete har varit att bestämma partikelstorleken i olika läkemedel, såsom emulsioner och torra pulver.

Två olika typer av emulsioner analyserades; hudkräm och hudlotion. I dessa emulsioner har en oljefas blandats i en vattenfas, likt beredningen av majonnäs. Oljefasen bildar då små oljedroppar i vattenfasen, eftersom olja inte löser sig i vatten. Droppstorleken av oljedropparna är viktig att känna till då den påverkar formuleringens egenskaper, såsom hur den känns på huden och hur snabbt den absorberas. Utöver emulsionerna analyserades två pulver; laktos och mometasonfuroat. Laktos kan användas i inhalationsprodukter medan mometasonfuroat kan användas som antiinflammatorisk komponent i exempelvis hudkrämer. Det är viktigt att veta partikelstorleken för inhalationsprodukter eftersom de måste vara tillräckligt små för att ta sig ner i lungorna i stället för att exempelvis andas ut, medan partikelstorleken för det antiinflammatoriska pulvret påverkar huruvida det kan tränga igenom huden.

Vid mätningar av partikelstorlek är det väsentligt att förstå att partikelstorleken i en formulering inte har ett specifikt värde, utan snarare är en distribution av olika partikelstorlekar. Exempelvis kan det påstås att partikelstorleken är 5 μm , men i verkligheten kan partiklarnas storlek variera från 3 till 7 μm . För att bestämma distributionen är laserdiffraktion en passande teknik. Principen bakom laserdiffraktion är att en laserstråle riktas mot ett prov bestående av tusentals partiklar, varpå laserstrålen kommer att ändra riktning när den träffar en partikel. Små partiklar är mer krökta och ändrar därför riktningen på lasern mer än vad stora partiklar gör. Ändringen av laserns riktning detekteras sedan, utifrån vilken distributionen beräknas baserat på alla tusentals partiklar.

Inställningarna som används under mätningarna behöver optimeras, eftersom olika sorters prov är olika tåliga och därmed kan utsättas för olika mängder energi utan att förstöras. Exempelvis är emulsioner normalt sett mer känsliga än pulver, vilket gör att proven behöver behandlas olika. Dessutom är det viktigt att partiklarna som analyseras inte sitter ihop i klumpar, i så kallade aggregat, eftersom dessa då kommer att uppfattas som en enda stor partikel. För att motverka detta behövs olika mycket energi appliceras på de olika proven, exempelvis i form av ultraljud och pumphastighet. Under examensarbetet har det därför undersökts hur mycket energi, och vilken energikälla, som behövs för att minska antalet aggregat och därmed få ett mer tillförlitligt resultat. Även andra inställningar som kan påverka resultatet, såsom fördröjning innan mätningen startas, provkoncentration, och hur provet bereds, har undersökts. Sammanfattningsvis resulterade undersökningarna i att både pumphastighet och ultraljud hade stor påverkan på både emulsioner och pulver, medan fördröjningstid och koncentration inte hade lika stor påverkan. Dessutom resulterade undersökningarna i att emulsioner behöver beredas på ett annorlunda sätt jämfört med pulvren, då emulsioner är mer känsliga och därför inte kan behandlas med lika mycket energi under beredningen.

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Lastly, we appreciate the hospitality from Malmö University when letting us use their refractometer, Sympatec for enabling measurements of the droplet size distributions using dynamic light scattering and Malvern Panalytical for enabling additional measurements using multi-angle dynamic light scattering.

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Appendix I - Lotion

Appendix II – Cream

Appendix III – Lactose

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1. List of Abbreviations

DLS – Dynamic Light Scattering

DSD – Droplet Size Distribution

IPA – 2-propanol

(O/W) Emulsion – Oil in Water Emulsion

PCA – Principal Component Analysis

PC – Principal Component

PSD – Particle Size Distribution

RI – Refractive Index

RSD – Residual

SEM – Scanning Electron Microscopy

SOP – Standard Operating Procedure

(W/O) Emulsion – Water in Oil Emulsion

2. Introduction

In this part of the report, the background to why this degree project is of interest and its aim are explained. Furthermore, the theories behind laser diffraction and size distribution measurements as well as the properties of the analysed samples are presented.

2.1 Background

According to M. Petersson, Principal Scientist Analytical Development at Galenica, traditional microscopy is the main method used by Galenica for measuring particle and droplet size today. However, traditional microscopy only allows measurements of a limited number of particles since the maximum magnification is 1000 times and does not enable analysis of the distribution of particles (Personal communication, 8th of January 2024). Consequently, laser diffraction is a superior technique for measuring the size distribution, but the method must be optimised for the specific analyte. In this degree project, different sample preparation parameters suspected to affect the size distribution, such as temperature, sonication, and dispersion technique, was firstly investigated to ensure that representative samples were obtained. The dispersion technique is especially important for finer particles since they easily agglomerate while the sampling technique is more important for coarser particles since they quickly settle heterogeneously (Sympatec, 2023/24). Subsequently, parameters controlled from the software, such as the obscuration range, internal sonication intensity within the instrument and pump speed were evaluated to examine which influenced the size distribution and which did not. Based on this, methodologies for development of new methods for unknown samples using laser diffraction were created. The samples analysed were two oil in water (O/W) emulsions, a water-soluble powder (both micronised and non-micronised) as well as a micronised water-insoluble powder.

2.2 Aim

In short, the aim of this degree project was to investigate how the sample preparation as well as parameters controlled from the software affected the obtained droplet size distribution (DSD) and particle size distribution (PSD) using laser diffraction as analytical method. Analytical standard

methods for the previously mentioned samples were constructed and additionally, based on the findings from the development of these methods, methodologies for developing new analytical methods for unknown samples using laser diffraction was written.

2.3 Droplet Size Distribution and Particle Size Distribution

It is of great importance to understand and control the size distribution of pharmaceuticals to obtain the desired product. The size distribution does not only affect the stability of the product, but also the drug release, uniformity between batches and rheological properties to state some examples (Kulkarni & Shaw, 2016).

The DSD and PSD are used to analyse the distribution of droplet- and particle sizes respectively. The distribution is commonly presented as a centre value, i.e. D50, together with the values D10 and D90. These percentiles describe which size 10 %, 50 % and 90 % of the particles are below, thereby indicating the size distribution within the sample (Horiba Scientific, n.d.). For a schematic representation of a PSD, see Figure 1. The data obtained from the laser diffraction measurement can either be derived from a histogram or a cumulative curve (the black line in Figure 1). The cumulative curve is generated from adding the size classes from a histogram. The obtained size distribution is volume-based, meaning that the diameter of a hard sphere having the same volume as the detected particle is presented. This diameter is also referred to as D [4,3] (Kulkarni & Shaw, 2016).

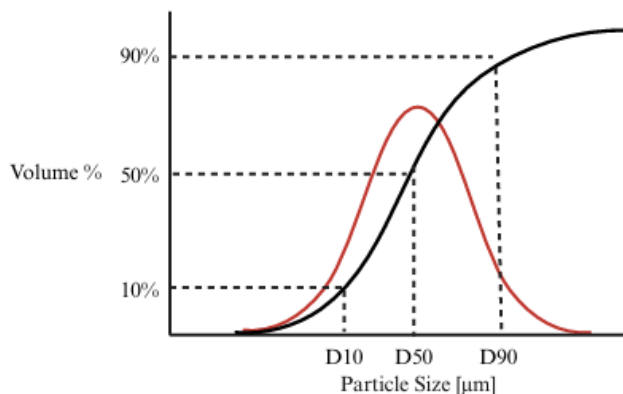


Figure 1: Visual representation of a monomodal PSD presenting the values of D10, D50 and D90 based on the cumulative curve. The figure was created using biorender.com.

The size distribution can be characterised either as monomodal or multimodal. A monomodal size distribution is obtained from samples only consisting of similarly sized particles and hence, only one peak is obtained as shown in Figure 1. A multimodal size distribution is on the other hand obtained from samples consisting of particles of more varied sizes and hence, more than one peak is obtained (Anton Paar, n.d.).

For a method to be considered robust, the residual (RSD) between six measurements for D50 should be below or equal to 10 %, while it for D10 and D90 should be below or equal to 15 %. However, for particles smaller than 10 μm, all RSD limits are doubled (USP 429, 2008). The RSDs for D10, D50 and D90 respectively were calculated according to Equation 1 below:

$$RSD Dx = 100 \cdot \frac{\sigma Dx}{\overline{Dx}} \quad (1)$$

where σDx is the standard deviation of the D-value between the replicates and $\overline{Dx}$ is the mean D-value of the replicates.

Other than this, the weighted residual and mode value are used for data analysis. The weighted residual implies the fit between the measured data and the calculated model data, and should be below 1 % (Malvern Instruments Ltd., 2013). The mode value refers to the particle size with the highest volume %. For a normally distributed monomodal size distribution, the mode value is the same as D50, while these values differ for non-normally distributed monomodal- as well as multimodal size distributions.

2.4 Laser Diffraction

Laser diffraction is a technique based on directed laser beams hitting particles within a sample, which causes the light to scatter and form different patterns depending on the size and shape of the particle. A red laser, sometimes in combination with a blue laser, is commonly used. The blue laser increases the sensitivity for measurements of small particles due to its shorter wavelength (Horiba Scientific, 2016). The instrument used during this degree project used a red laser with a wavelength of 633 nm and a blue laser with a wavelength of 466 nm (Malvern Instruments Ltd., 2007a). An integrated pump within the instrument creates a sample flow through the sample cell in which the particles collide with the laser beams, which causes the light to scatter. The scattered light pattern is then captured by detectors set up at different angles from the originated laser beam. Based on which detectors detected the light and the intensity detected by each detector, the captured light is translated to a size distribution by software calculations. For a schematic representation of a traditional laser diffraction instrument, see Figure 2.

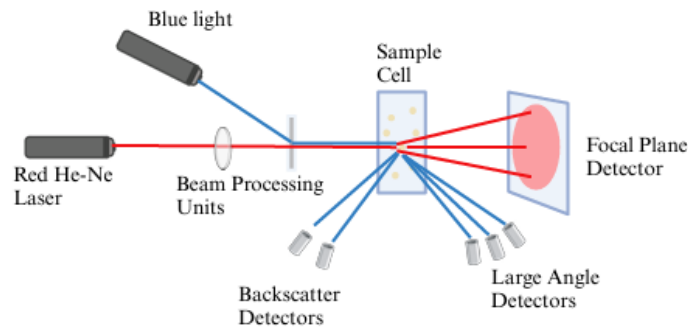


Figure 2: Schematic representation of a general laser diffraction instrument. The figure was created using biorender.com.

As mentioned before, a hard sphere model is implemented during laser diffraction measurements, meaning that the assumption is made that all particles are spherical and non-porous. Even though this is not always the case, the assumption must be made to model the size distribution. However, this might result in a broader PSD for irregularly shaped particles than for perfectly spherical particles (Microtrac, n.d.). As can be seen in Figure 3, the angle of the scattered light differs

depending on where the laser beam hits the irregular particle and hence, the same particle will be perceived as different sizes, generating a broader PSD.

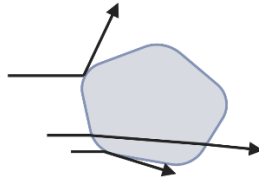


Figure 3: Schematic representation of how the light scatters differently for an irregularly shaped particle depending on where the laser beams hit the particle. The figure was created using biorender.com.

There are four main types of interactions between the laser beam and the particle; diffraction, refraction, reflection, and absorption, see Figure 4. The diffracted light is the portion of light bent in a specific angle when hitting the particle, thereby changing direction. As mentioned before, the diffraction angle depends on the size and shape of the particle. Larger particles cause the light to scatter at narrower angles, while the opposite is true for smaller particles due to their higher curvature (Horiba Scientific, 2013). This is the main light measured by laser diffraction. The refracted light is the portion of light that passes through the particle in which it changes angle and intensity. This light is especially important to consider for smaller particles where the particle size tends to be in the range of the wavelength of the laser. The refractive index (RI) is an important parameter in optical methods, describing the ratio of the speed of light in vacuum compared to the speed of light passing through a medium (Gallegos & Stokkermans, 2023). The RI describes how the light is bent in a medium or particle, used to understand the scattered light pattern taking the refracted light into account. The reflected light is the portion of light that is reflected when hitting the particle. The absorbed light is the portion of light that is absorbed within the particle, resulting in less light reaching the detectors and hence affecting the amount of detected light. In some cases, the absorbed light is reradiated as infrared heat (Horiba Scientific, 2013).

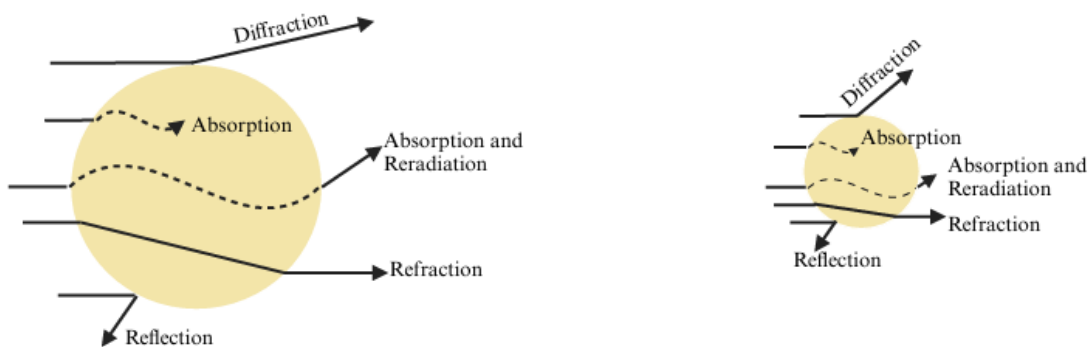


Figure 4: Schematic representation of diffraction, absorption, refraction, and reflection of the light after interaction with a droplet or a particle. The angle of the diffracted light depends on the size of the droplet or particle. The figure was created using biorender.com.

Two different calculation theories can be used to calculate the size distribution from the detected light: the Mie theory and the Fraunhofer theory (Horiba Scientific, 2013). Both theories describe how the light scatters when hitting the particle, but the Fraunhofer theory is slightly simpler since

it only accounts for the diffracted light (USP 429, 2008). The Mie theory on the other hand takes the diffracted, refracted, and absorbed light into account, which makes the calculations more accurate but more complex. To do the calculations using the Mie theory, the complex RI for the analyte is required, which might not always be known. The complex RI consists of two parts: the real RI and the imaginary RI. The real RI defines how the light is refracted through the particle and is extra important for smaller particles when the contribution from refraction becomes significant (Horiba Scientific, 2013). The imaginary RI, also known as de absorption, describes the light intensity loss due to absorption into the particle, presented as absorption in Figure 4 (Malvern Panalytical, 2010). The imaginary RI was 0.001 for emulsions and 0.01 for particles such as lactose and mometasone furoate (Malvern Panalytical, 2010). Furthermore, the Mie theory is recommended for particles smaller than 50 μm (SIS, 2000) and was therefore used in this degree project.

2.4.1 Parameters Affecting the Size Distribution Using Laser Diffraction

It is of greatest importance to obtain a representative sample and optimal software settings to ensure correct measurements. Some of the parameters suspected to affect the resulting size distribution are presented below.

Firstly, the concentration of the sample must be high enough to obtain a sufficient signal to noise ratio, yet low enough to avoid multiple particles being hit by the laser beam, as will be further discussed in Section 2.4.1.1 below. For emulsions, the temperature is an important factor as well. The temperature must be below the melting point of the oil phase to avoid it melting, which otherwise would affect the properties and stability of the emulsion.

Secondly, certain parameters controlled from the software, such as the obscuration and calculation theory, do not affect the properties of the sample itself but are believed to affect the measurement or translation into the size distribution, thereby affecting the result. However, the laser diffraction instrument used during the degree project does not allow for measurements using the Fraunhofer theory and hence, only measurements using the Mie theory were performed.

Other parameters such as the sonication intensity, pump speed during the measurement and the dispersant unit used, have a direct effect on the properties of the sample and are therefore believed to affect the result as well. These mentioned parameters will be further discussed in the following sections.

2.4.1.1 Obscuration

The portion of light that is unable to reach the detectors due to blockage from the particles is known as obscuration. The obscuration is set as an interval between two percentages and determines the amount of sample required for the analysis, thereby indirectly determining the concentration of the analysed sample (Ma, 2021).

A phenomenon occurring if the obscuration is too high is multiple scattering. Multiple scattering happens when the laser beam hits more than one particle before reaching the detector, causing the light to diffract more than once and thereby to change angles multiple times. This results in the laser light reaching the detectors at a wider angle than if only hitting a single particle. Consequently, the particle is interpreted as a smaller particle than its actual size, since smaller

particles scatter light at wider angles (Kokhanovsky & Weichert, 2001). On the other hand, if the obscuration is too low, a very small amount of sample is analysed, which can make it hard to distinguish the sample from the noise and a representative sample might not be obtained. By optimising the obscuration, multiple scattering is avoided while enough sample is analysed to ensure that the signal from the sample is distinguishable from the noise (Ma, 2021).

2.4.1.2 Sonication

Sonication is an energy force generated by sound waves applied to the sample to break aggregates to obtain single droplets or particles during the measurement. During sonication, sound waves of high frequency, i.e. ultrasound, are directed through the sample, causing the aggregates to break due to cavitation (Bader Ul Ain et al., 2023). Furthermore, sonication can be used as dispersant technique to homogenously disperse the analyte (Kaur et al., 2017). However, it is important not to add too much energy through sonication since this might disrupt the droplets and particles, affecting the size distribution. Micronised powders are difficult to de-agglomerate due to their high cohesiveness (Adi et al., 2007), leading to the hypothesis that micronised powders need more sonication than non-micronised powders.

2.4.1.3 Pump Speed During the Measurement

A higher pump speed during the measurement faster disperses the sample in the dispersant unit, making the measurement more efficient since less delay time is needed for the sample to homogenously disperse. Other than this, a high pump speed aids in avoiding re-agglomeration of the particles inside the dispersant unit. On the other hand, as for sonication, it is important not to add too much energy to the sample in order not to break the particles and thereby affect the sample itself. Due to this, the pump speed is a parameter that needs to be optimised for the specific analyte as well.

2.4.1.4 Dispersant Unit

Two main types of dispersant units can be used for analysis using laser diffraction: a wet dispersant unit and a dry dispersant unit. However, only wet dispersant units were available during this degree project and hence, the dry samples were analysed wet as well. To do this, the dry samples had to be dispersed in a liquid, which might cause swelling of the particles. This could result in the particles being perceived as larger than their actual size, generating a slightly incorrect PSD.

2.5 Samples

Below, the samples used in this degree project are described.

2.5.1 Emulsions

An emulsion is a mixture of two non-miscible liquids that has become homogenous at a macroscopic level due to treatment during the formation process. A common treatment to generate an emulsion is homogenisation. During homogenisation, the heterogenous mixture is forced through a small opening under high pressure, forcing the two phases to create smaller droplets and form a uniform distribution (Brittanica, 2021). However, on a microscopic level, the mixture is still heterogeneous. Depending on the homogenization speed, different DSDs will be formed.

There are different types of emulsions where the distinction often is made between oil in water (O/W) emulsions and water in oil (W/O) emulsions. The difference is whether the oil is dispersed in water, forming spherical oil droplets in the continuous water phase, or if the water is dispersed in oil, forming spherical water droplets in the continuous oil phase (Akhondi & David, 2023). In this degree project, only (O/W) emulsions were analysed.

Since emulsions are thermodynamically unstable, several mechanisms can cause separation between the two phases, thereby affecting the properties of the emulsion. The four main separation mechanisms are flocculation, coalescence, Ostwald ripening and creaming, see Figure 5. Flocculation is a mechanism in which two or more oil droplets stick together, forming a cluster of droplets, also known as aggregates. Coalescence is another mechanism during which two or more oil droplets merge into one larger droplet (Ravera et al., 2021). Ostwald ripening is a mechanism causing smaller, less stable, oil droplets to dissolve in the water phase to some extent. The dissolved oil droplets subsequently diffuse and merge into larger oil droplets, causing the droplet size to increase (Taylor, 1998). The resulting droplets formed by flocculation, coalescence as well as Ostwald ripening will be perceived as larger than the actual droplet size, resulting in an incorrectly presented DSD. The thermodynamic instability of emulsions is furthermore caused by the difference in density between the oil- and water phase. This difference causes the water phase to, over time, descend to the bottom by the gravitational force. Hence, the oil droplets will eventually settle at the top, creating an oil layer. This separation mechanism is known as creaming.

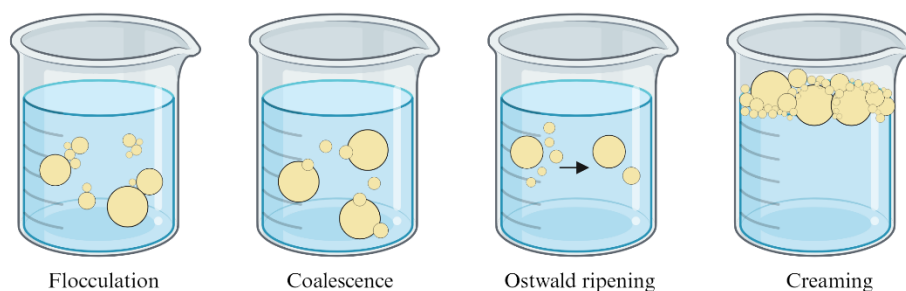


Figure 5: Schematic representation of the separation mechanisms flocculation, coalescence, Ostwald ripening and creaming. The figure was created using biorender.com.

Two different emulsions, a cream and a lotion, were analysed in this degree project. Both are (O/W) emulsions but the lotion has a higher water content than the cream, therefore having a lower viscosity and a more sheer consistency (Cetaphil, n.d.).

2.5.2 Powders

Powders are generally not spherical but consist of particles of more irregular shapes. As discussed previously, this might generate a broader PSD since the scattered light angle from the same particle differs depending on where the laser hits. The same particle will therefore be perceived as differently sized particles.

Coarser particles can be micronised, meaning that the particle size is reduced, most commonly done by milling. The purpose of micronisation is to increase the surface area to increase the solubility or dissolution of the particles and thereby increase the bioavailability (Mercuri, 2022).

Other than this, if the particles are to be inhaled, the particle size must be between 1 and 5 μm for the particles to be able to travel all the way to the lower respiratory tract where they are absorbed (Shetty et al., 2019). A problem with such small particles is that they generally are cohesive and easily form agglomerates that are too large to reach the lower respiratory tract. To avoid agglomeration, a carrier particle, i.e. a larger inert particle that the micronised particles adhere to, can be used (Hebbink et al., 2022). The size of the carrier particle should be large enough for it to be swallowed instead of reaching the lower respiratory tract, in order not to affect the health effect of the micronised particle. Lactose particles are a common example of carrier particles since they are inert, non-toxic and water-soluble (Dominici et al., 2022). Both micronised and non-micronised lactose particles were analysed during this degree project with respect to size specifications required for their application.

Furthermore, micronised mometasone furoate was analysed, which possesses similar properties to the micronised lactose powder. However, in contrast to lactose, mometasone furoate is a highly water-insoluble corticosteroid used for anti-inflammatory purposes (Spada et al., 2018).

3. Materials and Methods

The materials and methods used in this degree project are presented in the following sections. All materials and software programs were provided by Galenica, except for the refractometer that was provided by Malmö University.

3.1 Samples

The analysed samples were three different lotions, produced with a homogenisation speed of 5000, 8500 and 12000 RPM respectively, one cream, micronised and non-micronised lactose as well as micronised mometasone furoate. All samples were provided by Galenica. For batch numbers, see Table 1.

Table 1: Batch numbers for the analysed samples.

Sample	Batch number
Lotion – homogenisation 5000 RPM	529-2307-12
Lotion – homogenisation 8500 RPM	529-2307-08
Lotion – homogenisation 12000 RPM	529-2307-03
Cream	N801A
Micronised lactose	10CM079
Non-micronised lactose	1092TS4
Micronised mometasone furoate	C0264/I 10020

3.2 Chemicals

The ethanol (95 %) used for cleaning the instrument was purchased from KiiltoClean (Malmö, Sweden). The 2-propanol (99.9 %) used for dispersing the lactose was purchased from Sigma-Aldrich (France). The Tween® 80 used to when dissolving the mometasone furoate in Milli-Q water was purchased from VWR (France).

3.3 Equipment

The equipment used for the laser diffraction measurements was a Malvern Mastersizer 2000, in which particles of 0.02 μm to 2000 μm could be detected (Malvern Panalytical, n.d.). The dispersant unit used was Hydro 2000 μP with a built-in ultrasonic probe (48 kHz, 0-20 W). The scale used was a Mettler Toledo XS105DU, which had a dual range with two weighing intervals, reaching from 0 g-120 g in total. The heating plate used was an IKA® RET basic. The sonication bath used was a Bandelin Sonorex Digitec wieh (35 kHz, 480 W). The refractometer used was an Abbe Refractometer NAR-1T Atago®. The Milli-Q water used was obtained by using Milli-Q Advantage A10 Q-Pod. The syringes used to fill the dispersant unit with liquid were 20 mL HENKE-JECT® single use syringes. The microscope used was a Leica DMLB with a digital camera Leica DMC 2900, a heating unit Linkam MC60 and the software Leica Application Suite X.

3.4 Measurement of the RI of the Lotion

Since the lotion consisted of many different substances, a representative RI could not be found in literature. The RI was therefore measured for the oil- and water phase separately at Malmö University. The two phases were separately mixed according to a recipe provided by Galenica since the analyte only was the oil droplets, while the water phase was a part of the dispersant. The water phase was diluted to three concentrations representative for its final concentrations used during the measurements since it was diluted in Milli-Q water within the dispersant unit. The oil phase was not diluted since its concentration was not affected during the measurements. The RI of the water phases were measured at room temperature as well as heated to examine whether the temperature affected the RI, while the oil phase only was measured heated since it had to be melted to measure the RI.

3.5 Lotions and Cream

Since the oil droplets are insoluble in water, Milli-Q water was used as dispersant. Furthermore, water is already a part of the formulation, thereby affecting the sample properties as little as possible when dispersing in water. Since no literature was found on similar experiments containing cream or lotion, the initial settings were based on laser diffraction measurement of milk, an emulsion with fat globules similar to a dispersed lotion or cream. In an article regarding the DSD of milk, it was stated that the RI and absorption index of milk was 1.46 and 0.001 respectively, and the optimised obscuration was 1.5-3.5 % (Ransmark et al., 2019). These values were used during the early stages of the degree project, before the refractometer was available, after which the RI was changed.

3.5.1 Setup of the Screening Test

To evaluate the effect of the sample temperature, sonication intensity and stirrer speed during dispersion on the lotion homogenised at 5000 RPM, a 3³ factor trial was performed. The values of the analysed points are presented in Table 2 below. Triplicate measurements were performed for all 27 combinations.

Table 2: The analysed points of temperature, sonication intensity and stirrer speed during the screening test of lotion.

Point	Sample Temperature [°C]	Sonication Intensity [%]	Stirrer Speed [RPM]
Min	30	0	500
Centre	45	20	750
Max	60	50	1000

The evaluation of the factor trial was performed using the software MODDE®, with a D-optimal design. This design is often used for screening tests as well as optimisation, in which it selects points from the factor trial to be tested (Eriksson, 2008). However, in the screening test, all combinations were tested and evaluated in MODDE®. The results analysed from the screening test were the values of D10, D50 and D90.

The samples analysed in the screening test were prepared by weighing up approximately 1.25 g of lotion, transferring it to a glass bottle and adding a magnetic flea. 25 mL Milli-Q water was then added by using a volumetric pipette. Subsequently, the glass bottle was placed on a combined magnetic stirrer and heating plate for 20 minutes at the desired stirrer speed and temperature. For experiments where the lotion was dispersed at 500 or 750 RPM, a 50 mL glass bottle was used, while a 100 mL glass bottle was required for stability during dispersion at 1000 RPM.

3.5.2 Setup of the Robustness Test

To evaluate the robustness of the developed method for cream, a reduced factor trial was set up in MODDE® using the model fractional factor trial with resolution III (Frac Fac Res III). A 2^{6-3} fractional factor trial was chosen since all corners of the geometric box in space were examined, symbolising the extremes of the parameters. For a schematic figure, see Figure 6. This enabled analysis of the effect caused by the parameters. However, the model can be inadequate to estimate how factors co-vary with respect to each other (Eriksson et al., 2008).

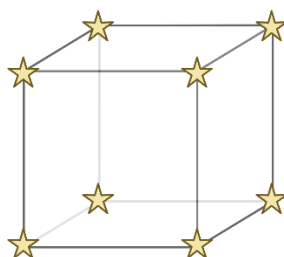


Figure 6: Schematic representation of the measurement points for a 2^3 factor trial. The figure was created using biorender.com.

Based on previous experiments, the pump speed, delay time after internal sonication, internal sonication intensity, obscuration, and time of day, i.e. morning or afternoon, were identified to affect the size distribution and were therefore the independent variables evaluated in the robustness test. The time of day was examined since it previously had been noted that the instrument got heated during the day, whereby the results were believed to be affected. Other than this, two analysts performed the measurements to examine whether the analyst influenced the result.

In addition to the centre points, which were the experiments with all parameter values according to the developed method described in Section 4.1.5, a low and a high value were entered in MODDE®. The experimental set up is presented in Table 3 below. The run order was randomised by MODDE® to minimise systematic errors. However, the run order was altered from the generated order to enable performing all morning- and afternoon measurements respectively at the same time but the order within the two time periods was kept in the same order as MODDE® suggested. The presented run order in Table 3 is the actual order the experiments were performed in. The centre points were run three times (experiments 9-11) to ensure that the results from these points were consistent. All experiments were performed in triplicates.

Table 3: The experimental set up of the reduced factor trial for the robustness test of cream. The time of day “A” represents the morning experiments while the time of day “B” represents the afternoon experiments.

Experiment No.	Run Order	Analyst	Time of Day	Pump Speed [RPM]	Equilibrium Time [s]	Sonication Intensity [%]	Obscuration [%]
1	3	A	A	650	90	22	5
2	6	B	A	650	30	18	5
3	10	A	B	650	30	22	2
4	11	B	B	650	90	18	2
5	5	A	A	850	90	18	2
6	2	B	A	850	30	22	2
7	8	A	B	850	30	18	5
8	9	B	B	850	90	22	5
9	1	A	A	750	60	20	3.5
10	4	A	A	750	60	20	3.5
11	7	A	A	750	60	20	3.5

During the robustness test, $0.75 \text{ g} \pm 0.02 \text{ g}$ of cream was weighed and transferred into a 50 mL glass bottle. 40 mL Milli-Q water was added to the glass bottle by using a volumetric pipette as well as an approximately 2 cm long magnetic flea. The glass bottle was then placed on a combined magnetic stirrer and heating plate for 20 minutes at 600 RPM without heating, after which it was set aside without stirring for at least 5 minutes before measuring.

The results noted from the experiments were D10, D50, D90, mode value and weighted residual. From the triplicates, the RSDs for the D-values were calculated according to Equation 1, where the standard deviations were calculated by the function *STDEV.P* in Excel.

3.6 Powders

As for the emulsions, the powder samples should be affected as little as possible during sample preparation to ensure analysis of a representative sample. For the lactose powders, 2-propanol (IPA) was used as dispersant since it was found the most suitable in literature (Adi et al., 2007). In the same article, it was stated that the optimal obscuration was 10-30 % when dispersing 500 mg lactose powder in 5 mL IPA.

For the micronised and non-micronised lactose, 0.1 g powder was weighed on a weighing boat, transferred to a 50 mL glass bottle, and dispersed in 10 mL IPA by external sonication. The concentration was decreased from the one found in literature since the dispersant unit used in this

degree project had a capacity of 20 mL instead of 150 mL. Both samples were sonicated during varying times in an external ultrasonication bath before measurements at different pump speeds, depending on the experiment.

The method for analysing the micronised mometasone furoate was based on the developed method for micronised lactose, but Milli-Q water was used as dispersant instead of IPA. However, due to the high hydrophobicity of mometasone furoate, 5 droplets of Tween® 80 were added to the 10 mL Milli-Q water before the 0.1 g mometasone furoate was added.

3.6.1 Setup of the Robustness Test for Mometasone Furoate

The same reduced factor trial as described in Section 3.5.2, i.e. a 2^{6-3} factor trial with resolution III, was set up in MODDE® for the method developed for micronised mometasone furoate as well. The developed method is presented in Section 4.3.1.

Based on previous experiments, the pump speed, internal sonication intensity, delay time and obscuration were identified to affect the results and hence, these factors were investigated in the robustness test. In addition, two analysts performed the experiments and the amount of Tween® 80 was varied since the method stated that 5 droplets should be added, and the actual volume therefore might differ between preparations. From the centre point, a high and low value were investigated in similar manner as for the cream. The experimental set up is presented in Table 4 below. The centre points were analysed three times (experiments 9-11) to ensure consistency of the method. All experiments were performed in triplicates.

Table 4: Experimental set up of the reduced factor trial for robustness test of micronised mometasone furoate.

Experiment No.	Run Order	Pump Speed [RPM]	Sonication Intensity [%]	Delay Time [s]	Analyst	Obscuration [%]	Droplets of Tween®80 [nr]
1	9	900	8	60	B	17	6
2	5	1100	8	60	A	13	6
3	7	900	12	60	A	17	4
4	4	1100	12	60	B	13	4
5	11	900	8	120	B	13	4
6	6	1100	8	120	A	17	4
7	2	900	12	120	A	13	6
8	8	1100	12	120	B	17	6
9	10	1000	10	90	A	15	5
10	3	1000	10	90	A	15	5
11	1	1000	10	90	A	15	5

During the robustness test, 10 mL Milli-Q was added to a 50 mL glass bottle by using a volumetric pipette after which 5 droplets of Tween® 80 were added and dissolved by shaking the bottle. 0.1 g $\pm$ 0.01 g of micronised mometasone furoate was weighed on a weighing boat and transferred to the glass bottle, followed by sonication for 2 minutes in an external sonication bath. The measurements were performed with a pump speed of 1000 RPM. The values noted from the experiments were D10, D50, D90, mode value and weighted residual. From the D-values, the RSDs were calculated according to Equation 1, where the standard deviations were calculated by the function *STDEV.P* in Excel.

3.7 Performing the Laser Diffraction Measurements

A Malvern Mastersizer 2000 was used to perform the laser diffraction measurements with the 2000 μ P dispersant unit. The dispersant unit had a capacity of 20 mL and the possibility to sonicate the sample within the unit. Firstly, the dispersant inside the dispersant unit was drained with the help of the computer software. Fresh dispersant was introduced by using a 20 mL HENKE-JECT® single use syringe after which the function “anaerobic fill” was used to reach 500 RPM above the desired pump speed, since this was recommended by Malvern to avoid interference from air bubbles (Malvern Instruments Ltd., 2001). During the screening test for lotion, the dispersant was sonicated at 50 % intensity for 30 seconds before starting the background measurement to remove air bubbles. However, during the robustness test, the background measurements were started directly after anaerobic fill since no air was detected in the system when skipping the pre-sonication.

A 3 mL Pasteur pipette was used to transfer the sample from the glass bottle into the sample well of the dispersant unit. When sample was added, a SOP with the software settings already entered was started via the software. The SOP was varied between experiments when any software parameter was changed. After each measurement, the sample unit was cleaned by flushing dispersant through the system three times before filling the dispersant unit with new dispersant.

4. Results and Discussion

In this section, the results obtained from the experiments and developed standard methods are presented and discussed.

4.1 Emulsions

The results from the RI measurements and the screening test for lotion are presented below. Furthermore, the experiments and the robustness test of the standard method developed for cream will be presented. Lastly, the created methodology for development of new methods for unknown samples are presented.

4.1.1 RI of Lotion

The RIs for the water- and oil phase were found to be 1.33 and 1.45 respectively. For all measured values, see Appendix Table A1. The RI of the water phase was the same as for pure water, which is reasonable since the main component in the water phase was water. In addition, the water phase was diluted during sample preparation as well as within the dispersant unit during the analyses, which further contributed to a water phase similar to pure water. The RI for the oil phase was similar to the one used for the analysis of milk, i.e. 1.46 as described in Section 3.5. Due to this, the results obtained when using an RI of 1.46 were still considered representative.

It should further be noted that the measured RI might not be completely accurate. The measurements were performed with a refractometer without the ability to heat the sample, causing the oil phase to solidify during the measurement. This created difficulties when trying to observe the light boundary for estimating the RI. In addition, the RI of the melted oil phase might not be the same as the solid oil phase, as it is in the lotion.

4.1.2 DSD of the Lotion

Measurements were initially performed to determine whether there was a difference between the DSDs of the lotions homogenised at different speeds, see Figure 7.

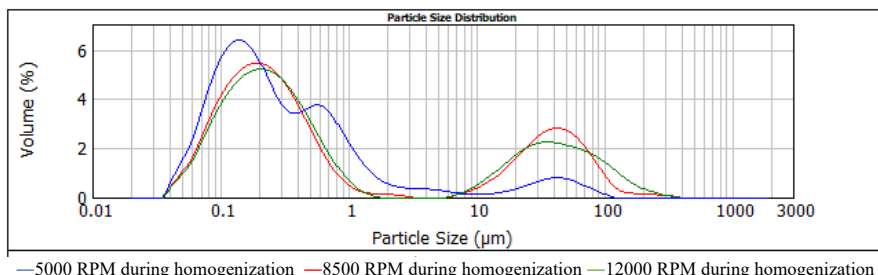


Figure 7: DSD for three different lotions. The measurements were performed at a constant pump speed of 750 RPM. The intensity of the internal sonication applied prior to the measurement was 25 % for the lotions homogenised at 5000 RPM and 8500 RPM while it was 35 % for the lotion homogenised at 12000 RPM. The obscuration was 1.5-3.5 % for all measurements. The DSDs presented are an average of triplicate measurements within the instrument of each lotion.

Looking at Figure 7, the peaks that appeared at approximately 10-110 μm were suspected to be aggregates. Moreover, the DSD of the lotion homogenised at 5000 RPM differed from the others, while the DSDs of the lotions homogenised at 8500 RPM and 12000 RPM were similar. Furthermore, for the lotion homogenised at 5000 RPM, three peaks instead of two were obtained, and the two separate peaks at the lower droplet sizes were narrower than the single peaks obtained for the other lotions. In addition, the lotion homogenised at 5000 RPM had a mode value of around 0.6 μm , while the lotions homogenised at 8500 and 12000 RPM both had a mode value of around 0.2 μm . It is reasonable that the lotions homogenised at higher speeds had smaller droplet sizes, since they were exposed to higher forces during manufacturing, generating smaller droplets. However, it was expected to see a difference in DSD between all lotions. The undetected difference between the lotions homogenised at 8500 and 12000 RPM could be due to too poor resolution of laser diffraction to distinguish such small particles, or the lotions having the same distribution.

To further examine whether there was a difference between the DSDs, the lotions were analysed in a light microscope. Unfortunately, the oil droplets were too small to be seen in the light microscope. Therefore, dynamic light scattering (DLS) was used as an alternative technique to measure DSD, since this technique had better resolution and was better suited for small droplets within the nano-range. DLS is based on Brownian motion of the droplets, where larger droplets diffuse slower than smaller droplets and vice versa, which is detected and transformed into the DSD. From these measurements, it was seen that all lotions had different DSDs when prepared at room temperature and dispersed at 750 RPM for 20 minutes. The lotions homogenised at 5000, 8500 and 12000 RPM were found to have a mode value of around 900 nm, 530 nm and 350 nm respectively, this data was however not included in the report. As can be noted, these values differed a bit from the ones obtained using laser diffraction, which was expected since different measurement principles were used. However, based on the small size of the droplets, the results using DLS were considered more reliable. From these analyses, it was concluded that the sample

preparation technique generated representative samples and hence, it was decided to prepare the samples at these conditions from here on.

It was noted that the peak at approximately 0.13 μm detected for the lotion homogenised at 5000 RPM in Figure 7 mostly appeared when the volume % of aggregates was low. This led to a suspicion that the compound generating this peak was present during all measurements but did not appear in the graphs when many aggregates were present, since the aggregates then overshadowed the smaller fractions in terms of volume %. It was further believed that the compound generating the peak at 0.13 μm might cause the oil droplet size peak of the lotions homogenised at 8500 and 12000 RPM to fuse with this peak due to the poor resolution of small droplets, thereby generating a single and broader peak. This theory was further supported from additional multi-angle DLS measurements where it could be seen that some broad peaks consisted of two narrower peaks. However, this data was not included in the report.

According to AK. Morén (personal communication, 28th of February 2024), the fine droplets at 0.13 μm could be micelles formed by macrogol stearate, encapsulating the oil within the formulation when the lotion was dispersed in water. To test this hypothesis, analyses were performed on water phase with macrogol stearate only, water phase with oil only, and water phase with both macrogol stearate and oil. Neither the oil nor the macrogol stearate in the water phase gave rise to the structures at 0.13 μm themselves. However, when both oil and macrogol stearate were mixed, a structure with a droplet size of approximately 0.20 μm appeared, which could be due to micelle formation from macrogol stearate and oil. It is reasonable that the detected size differed slightly from the one in Figure 7, since not all ingredients of the lotion were added to the formulation, and since the lotion had not been homogenised. Pure water phase was also analysed but did not generate any peak of interest. On the other hand, when comparing the measurements performed using DLS with laser diffraction, the fraction of fine particles in volume % was much lower for DLS. Another explanation could therefore be that the peak originated from something in the technique used or the calculation theory for laser diffraction, rather than fine particles.

4.1.3 Screening Test for Lotion

During the screening test, a pump speed of 2000 RPM and an obscuration of 1.5-3.5 % was used throughout all experiments.

To evaluate which parameters (i.e. temperature, stirrer speed during dispersion and internal sonication intensity) had a significant effect on the DSD of the lotion, the obtained values of D10, D50 and D90 were analysed in coefficient plots, see Figure 8 below. For a significant model, the uncertainty level should not span across $y=0$ and the area of the box should be large (Sartorius, 2017).

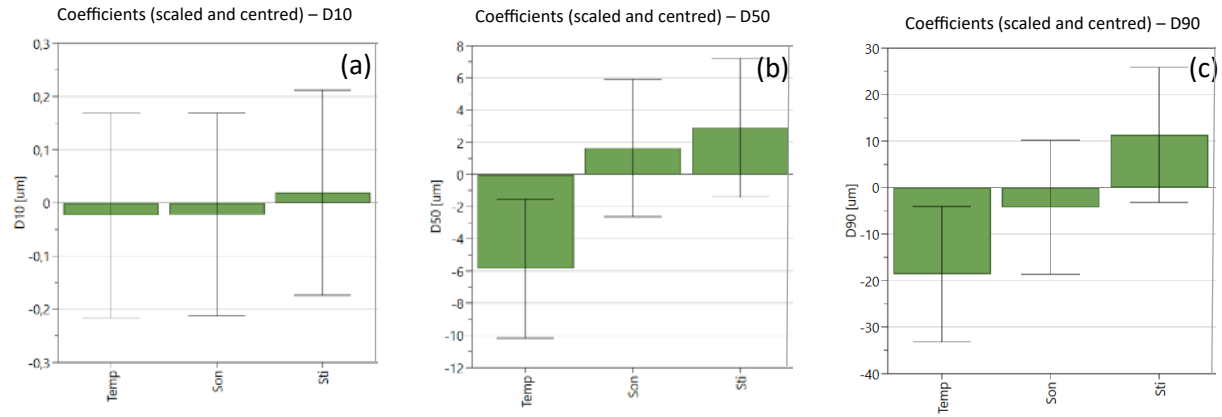


Figure 8: Coefficient plots for (a) D10, (b) D50 and (c) D90. The plots depict the influence of temperature, internal sonication intensity and stirrer speed during sample preparation on the percentiles.

In Figure 8a, it can be seen that the D10 values were not affected by either temperature, stirrer speed or internal sonication intensity while in both Figure 8b and 8c, it can be seen that the temperature had a significant effect on both D50 and D90. In both cases, the temperature was negatively associated with the D-values, meaning that an increased temperature decreased the D-values. This is reasonable since the oil droplets and aggregates most probably had disrupted at high temperatures, resulting in smaller droplets. It was therefore concluded that the samples should not be heated during sample preparation. Nevertheless, it should be noted that the standard deviations for the triplicates occasionally were very high, see Appendix Table A2, and hence, the results might not be completely reliable. This could be due to the multimodal shape of the DSD, which caused the D-values to differ a lot between some replicates.

In addition to the temperature affecting the D-values, it seemed as the stirrer speed during sample dilution could have some impact on the size distribution as well, even if it was not significant. To further examine how the temperature and stirrer speed correlate, contour plots were created where the sonication intensity was set constant at 20 %, while the temperature and stirrer speed were plotted on the y- and x-axis respectively, see Figure 9.

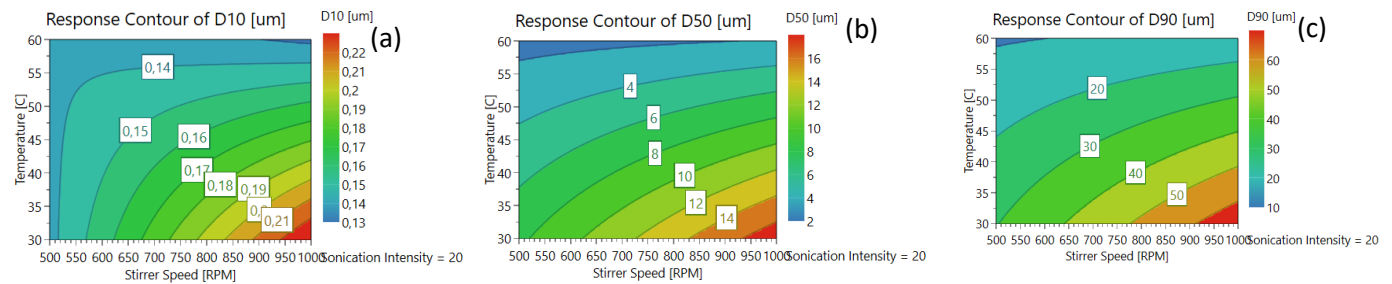


Figure 9: Response contour graphs for (a) D10 (b) D50 and (c) D90. In all figures, the x-axis presents the stirrer speed and the y-axis presents the temperature during sample dispersion while the sonication intensity was kept constant at 20 %.

From Figure 9a, it was found that at lower stirrer speeds, the stirrer speed affected D10 the most while at higher stirrer speeds, both the stirrer speed and temperature affected D10. Furthermore, it

was seen that at higher temperatures, D10 was only affected by the temperature. As mentioned for the coefficient plots, the values of D50 and D90 were significantly affected by the temperature. This tendency was also found in Figures 9b and 9c, in which both D50 and D90 decreased with increased temperatures. Additionally, at higher temperatures, these values were found to almost solely be affected by the temperature. Again, it should be noted that the results from the screening test are somewhat unreliable since the standard deviation between some triplicates were high, which can be seen in Appendix Table A2. However, the indication that high temperature disrupted the oil droplets remained and it was therefore concluded that the samples should not be heated during sample preparation. In addition, it was concluded that dispersing the sample at 600 RPM for 20 minutes was optimal, since the sample then became fully homogenous while affecting the sample properties as little as possible by not using a too high stirrer speed.

The pump speed in the instrument was also investigated for lotion, but since it was hard to distinguish the oil droplet peak from the peak at 0.13 μm , these experiments were performed more thoroughly for the cream, presented in the following sections. Other than this, measurements using different concentrations of lotion were performed to see if multiple scattering occurred. For this, masses of 0.5 g, 1 g and 2 g lotion were dispersed in 10 mL Milli-Q water. It was concluded that the concentration did not affect the size distribution, and 1.25 g lotion in 25 mL Milli-Q water was decided to be optimal for lotion. The optimal concentration was also based on the amount of sample required to obtain the wanted obscuration, which in turn results in a more representative sample.

4.1.4 Software Parameter Tests for Cream

As for the lotion, the cream gave rise to a multimodal size distribution, see Figure 10. The peak appearing at approximately 0.13 μm was still suspected to arise from micelles since the cream essentially is the same formulation as the lotion, only with a lower water content. The peak at 3 μm was thought to be the actual oil droplet size, which was confirmed when analysing the cream in a light microscope, see Appendix Figure A4. In the microscope, it could also be seen that the detected fraction slightly below 30 μm were aggregates of several droplets.

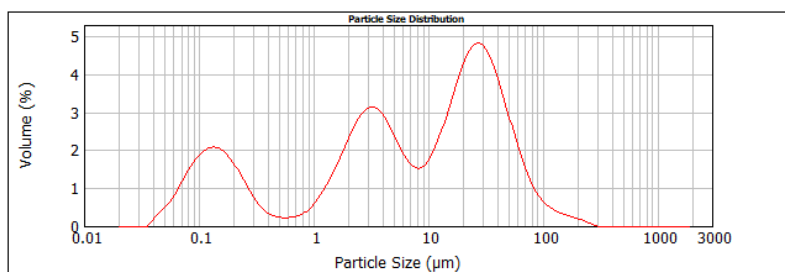


Figure 10: DSD for the cream when analysing at 750 RPM and 20 % internal sonication 30s prior to the measurement. The DSD presented is an average of triplicate measurements within the instrument.

Firstly, the pump speed in the instrument was examined for the cream. As it was found that approximately 750-850 RPM was suitable for the lotion, nearby pump speeds, i.e. 500, 750 and 1000 RPM, were tested for the cream. 1000 RPM was tested since the hypothesis was that cream could withstand more energy than the lotion, since it appeared more stable due to its lower water

content. The D-values are presented in Table 5 below and the obtained DSD graph can be found in Appendix Figure A1.

Table 5: The percentiles and weighted residuals obtained from different pump speeds during the measurement of cream. The obscuration was set to 1.5-3.5% and no sonication was used. The values presented are an average of triplicate measurements within the instrument.

Pump Speed [RPM]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
500	4.7 ± 0.52	31 ± 0.89	74 ± 2.4	0.33 ± 0.010
750	2.4 ± 0.26	24 ± 1.8	61 ± 8.0	$0.37 \pm 7.8 \cdot 10^{-3}$
1000	1.9 ± 0.20	19 ± 1.7	50 ± 4.6	0.38 ± 0.032

It was seen that higher pump speeds resulted in more disaggregation and an increased volume % of finer droplets, leading to lower D-values. As suggested above, it seemed like the cream was less sensitive to the mechanical force applied through the pump speed than the lotion, and a pump speed of 1000 RPM seemed the most appropriate for the cream. This was considered appropriate since the peak of 3 μm was distinguished from the other peaks and its volume % was high.

Internal sonication intensities of 0 %, 15 % and 25 % respectively were examined for the cream, see Table 6 below. The cream was sonicated for 30 s within the instrument prior to the measurement start, whereby the pump speed had to be reduced to 750 RPM not to disrupt the oil droplets. The graph of the DSD is presented in Appendix Figure A2.

Table 6: The percentiles and weighted residuals obtained from different sonication intensities during 30 s within the instrument prior to the measurement of cream. The pump speed was set to 750 RPM and 1.5-3.5% obscuration was used. The values presented are an average of triplicate measurements within the instrument.

Sonication Intensity [%]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
0	2.4 ± 0.26	24 ± 1.8	61 ± 8.0	$0.37 \pm 7.8 \cdot 10^{-3}$
15	$0.12 \pm 3.7 \cdot 10^{-3}$	5.7 ± 1.9	63 ± 11	0.83 ± 0.15
25	$0.089 \pm 2.2 \cdot 10^{-3}$	1.4 ± 0.40	40 ± 30	1.1 ± 0.11

It was noted that higher sonication intensities resulted in more disaggregation. As can be seen in Appendix Figure A2, sonication at 15 % and 25 % intensity generated very similar volume % for the peak at approximately 3 μm , while the volume % of the aggregates and suspected micelles differed. For the non-sonicated sample, a lot of aggregates and a low volume % of the oil droplet peak of around 3 μm were detected, which was reasonable. Based on these results, it was decided that 20 % internal sonication was optimal for the cream, when having a pump speed of 750 RPM. This was considered optimal since 15 % internal sonication generated peaks where the oil droplets and aggregates were not separated satisfactory, while 25 % internal sonication generated peaks where the oil droplets and finer fraction were not separated satisfactory. Due to this, a sonication intensity between these, i.e. 20 %, was considered most favourable. It should also be noted that the optimal pump speed was changed, from 1000 RPM to 750 RPM, since it was seen that the pump speed and sonication intensity affected the results in the same way. This was considered reasonable, since both parameters increased the energy that the sample is exposed to during the

measurement. Because of this, when sonication was added, the pump speed had to be lowered not to disrupt the oil droplets.

A source of error regarding the sonication during the measurements was that it sounded inconsistent between measurements. It sounded intensely during some measurements while it was quiet during others even though the same sonication intensity was used. It was therefore suspected that the intensity might have differed between measurements when the samples were supposed to be treated with the same sonication intensity, which would affect the reliability of the results.

After the samples were sonicated, there was an opportunity to set a delay time during which the sample was allowed to circulate in the sample cell before starting the measurement. The effect of the delay time was investigated by immediately starting the measurement, and by a delay time of 30 s, 60 s and 90 s, see Table 7.

Table 7: The percentiles and weighted residuals obtained from different delay times before the measurement of cream. Obscuration was set to 2.5-4.5%, the pump speed was set to 750 RPM and the sample was internally sonicated at 20% intensity 30 s prior to the measurement. The values presented are an average of triplicate measurements within the instrument.

Delay Time [s]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
0	0.12 ± 0.013	7.4 ± 8.0	130 ± 570	0.91 ± 0.35
30	$0.13 \pm 1.2 \cdot 10^{-3}$	6.6 ± 0.42	49 ± 4.4	0.74 ± 0.067
60	$0.12 \pm 8.2 \cdot 10^{-4}$	6.6 ± 0.52	52 ± 2.8	$0.61 \pm 7.9 \cdot 10^{-3}$
90	$0.12 \pm 8.2 \cdot 10^{-4}$	4.9 ± 0.20	49 ± 2.2	0.76 ± 0.020

The distribution did not seem to be greatly affected by the delay time. However, peaks at around 1000 μm appeared when having no delay time, mainly affecting the D90. The conclusion was drawn that an equilibrium time of 60 s was sufficient. It was not clear why the peak at around 1000 μm appeared, but since the distribution was measured in volume %, it is enough that a single aggregate or contamination that big was detected to appear in the DSD graph. This theory was further supported by the high standard deviation, indicating that the large peak was caused by interference of a few aggregates or contaminations, and not droplets representative for the whole sample. Furthermore, since the peak did not appear in the other experiments, it most likely was an outlier.

The effect from the obscuration was further investigated by performing analyses at four different obscuration intervals, see Table 8.

Table 8: The percentiles and weighted residuals obtained from different obscuration intervals during the measurement of cream. The pump speed was set to 750 RPM and the sample was internally sonicated at 15% intensity 30 s prior to the measurement. The values presented are an average of triplicate measurements within the instrument.

Obscuration [%]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
1.5-3.5	$0.15 \pm 5.0 \cdot 10^{-3}$	17 ± 2.3	80 ± 10	0.52 ± 0.12
10-13	$0.15 \pm 4.7 \cdot 10^{-4}$	22 ± 0.80	90 ± 7.0	$0.41 \pm 5.7 \cdot 10^{-3}$
17-20	$0.15 \pm 8.2 \cdot 10^{-4}$	21 ± 0.46	82 ± 6.1	$0.43 \pm 3.3 \cdot 10^{-3}$
23-25	$0.14 \pm 1.4 \cdot 10^{-3}$	22 ± 0.34	88 ± 6.8	$0.43 \pm 6.2 \cdot 10^{-3}$

As can be seen, the obscuration did not seem to have great impact on the DSD, but it was noted that the standard deviation for the low obscuration range was higher than for the others. However, it was decided to continue analyses at a rather low obscuration, i.e. 2.5-4.5 %, since the amount of sample needed thereby was reduced and the risk of multiple scattering was decreased. The high standard deviation was reduced when optimising other parameters.

The maximum time the sample could be used for analysis was set to 60 minutes based on a stability test performed for 3.5 hours, see Table 9 below. The DSD graphs are presented in Appendix Figure A3.

Table 9: The percentiles and weighted residuals obtained from the stability test. The obscuration was set to 2.5-4.5% and the pump speed to 750 RPM. The sample was internally sonicated at 20% intensity 30 s prior to the measurement. The values presented are an average of triplicate measurements within the instrument.

Time [h]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
0	$0.12 \pm 4.7 \cdot 10^{-4}$	4.8 ± 0.059	34 ± 0.41	0.91 ± 0.030
0.5	0.11 ± 0.0	3.3 ± 0.042	33 ± 0.16	1.6 ± 0.21
1.5	$0.10 \pm 4.7 \cdot 10^{-4}$	2.8 ± 0.056	30 ± 0.57	$0.90 \pm 7.3 \cdot 10^{-3}$
2.5	0.090 ± 0.0	1.6 ± 0.013	26 ± 0.089	2.7 ± 0.086
3.5	0.098 ± 0.0	1.9 ± 0.028	25 ± 0.45	1.5 ± 0.66

The DSD changed a lot between 0 and 0.5 hours, was stable between 0.5 and 1.5 hours and thereafter changed a lot again before it stabilised. Generally, the peak at 3 μm was consistent in all measurements except for the one analysed directly, which can be seen in Appendix Figure A3. The increase in volume % of the peak at 0.13 μm could be due to the micelle formation when the sample was left for a longer time. As seen before, the volume % of the aggregate peak decreased with approximately the same volume % as the 0.13 μm peak increased. Due to this, the sample was considered stable between 0.5 and 1.5 hours and the maximal time to leave the sample was therefore set to 1 hour, to ensure that a representative sample was analysed.

4.1.5 Developed Standard Operating Procedure for Cream

Based on the results presented in the sections above, a standard method was developed for measuring the DSD of the cream. The standard method includes how to prepare the sample to make it representative, the software settings of the standard operating procedure (SOP) as well as how to perform the measurement and which outputs to note. The full instructions were written in a separate document and sent to the company since they are product specific.

In short, to prepare the sample, $0.75 \text{ g} \pm 0.02 \text{ g}$ cream was weighed in a 50 mL glass bottle and 40 mL Milli-Q water was added with a volumetric pipette. The sample was then dispersed on a magnetic stirrer place at 600 RPM for 20 minutes and set aside to rest for at least 5 minutes, but maximum 1 hour. In the software, an RI of 1.45 and absorption of 0.001 were used. The pump speed was set to 750 RPM and 20 % internal sonication was applied for 30 s before starting the measurement. A delay time of 1 minute was used before starting the measurement. The optimal obscuration was 2.5-4.5 %. Using these conditions, the DSD graph presented in Figure 11 should be obtained with its corresponding D-values as presented in Table 10.

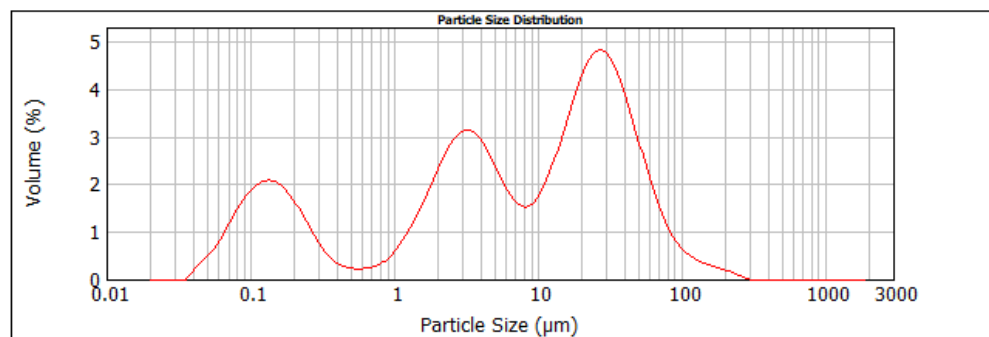


Figure 11: The resulting DSD for the cream when analysing according to the SOP. The DSD presented is an average of triplicate measurements within the instrument.

Table 10: The percentiles for the cream when analysing according to the SOP. The values presented are an average of triplicate measurements within the instrument.

D10 [μm]	D50 [μm]	D90 [μm]
0.14 ± 0.0	8.3 ± 0.90	48 ± 3.2

4.1.6 Robustness Test of the Method Developed for Cream

During the robustness test, the independent variables obscuration, pump speed, delay time, internal sonication intensity, time of day (morning or afternoon) and analyst were evaluated. The dependent responses of interest were the mode value, the weighted residual as well as the RSDs for D10, D50 and D90 respectively. For numerical values, see Appendix Table A3.

A Principal Component Analysis (PCA) plot from the robustness test is presented in Figure 12 below, giving an overview of how the parameters possibly correlate with each other. The yellow dots represent the independent variables while the purple dots represent the dependent variables. If the dots are in a right angle in relation to each other from the point at which the axes meet, it means that the variables do not correlate. However, if the points are opposite diagonal to each

other, the parameters are negatively associated with each other, and close dots means that the variables are positively correlated with each other.

Since a PCA plot transforms and simplifies data to be able to interpret all data in two dimensions, some information is lost. This leads to no actual numerical values on the axes. Instead, the x- and y-axis are presented as principal components (PCs). The two PCs of highest variance are designated as the presented axes. As can be seen in Figure 12, only 49 % of the total variation is presented, indicating missing information of some correlations. However, in terms of a robustness test, a low total variation presented in the PCA plot is desired since it means that there is no strong correlation between specific parameters. It should be kept in mind that the PCA plot cannot be used as a definite result, but should be used as an overview to give an idea of the general relationships between the parameters.

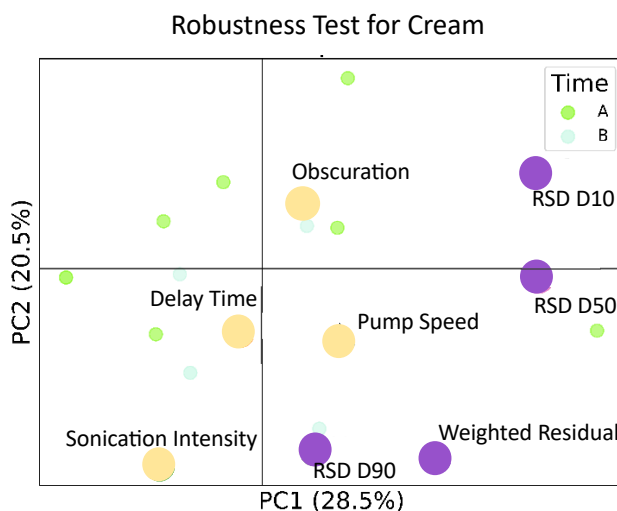


Figure 12: PCA plot from the robustness test of cream. The time of day, i.e. in the morning (A) or afternoon (B), obscuration, equilibrium time, pump speed and internal sonication intensity were plotted together with RSD D10, RSD D50, RSD D90 and weighted residual.

As can be seen in Figure 12, no strong positive correlations were overall found between the variables, indicating a robust method. Furthermore, the plotted observations (i.e. the time in green and blue dots) did not show any strong groupings, indicating that the time did not strongly correlate with any of the other variables. However, the sonication intensity appeared to be negatively associated with the RSD D10, given their positions in the PCA. Nevertheless, the PCA plot included limited variation from the dataset, and more thorough statistical analyses should be performed to be able to draw conclusions.

To further evaluate the robustness of the method, the effect caused by the independent variables were analysed separately in coefficient plots, see Figures 13 and 14 below. For the method to be considered robust, all model parameters should be close to $y=0$ and the uncertainty levels should span across $y=0$ (Sartorius, 2017).

The coefficient plots for the droplet size at the mode value and the weighted residual are presented in Figure 13 below.

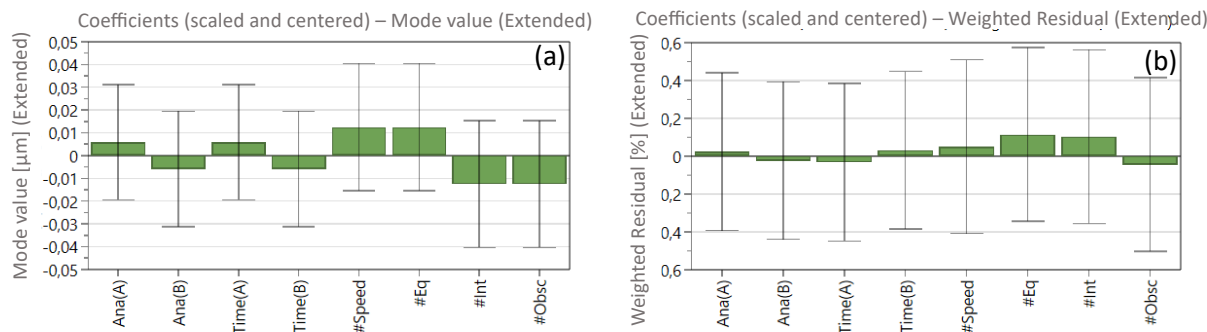


Figure 13: Coefficient plots for (a) the mode value and (b) the weighted residual, with regards to analyst, time of day, pump speed, equilibrium time, internal sonication intensity and obscuration.

As can be seen, all uncertainty levels spanned across $y=0$, meaning that all model parameters were non-significant, indicating a robust model with respect to the mode value and weighted residual. For the mode value, it can be seen that the analyst and time of day were the parameters that seemed to affect the results the least, while the pump speed, delay time, internal sonication intensity and obscuration affected the size distribution slightly but not enough to become significant. For the weighted residual, all parameters were very close to $y=0$, indicating that none of the parameters affected the result.

Throughout the robustness test, the droplet size at the mode value was very consistent at $3.1 \mu\text{m}$ with the only exception in experiment 5, where the mode value was calculated to $3.2 \mu\text{m}$, see Appendix Table A3. Furthermore, the weighted residual was under 1 % only for four of the experiments, i.e. experiments 2, 7, 10 and 11. The generally high weighted residual could be due to that the DSD was multimodal, making it harder to get a good fit to the calculation model. It should however be noted that the peak of interest was very consistent throughout all experiments while the peaks arising from the fine fraction and aggregates tended to vary, which generated the high weighted residuals.

The coefficient plots for the RSDs for D10, D50 and D90 respectively are presented in Figure 14 below.

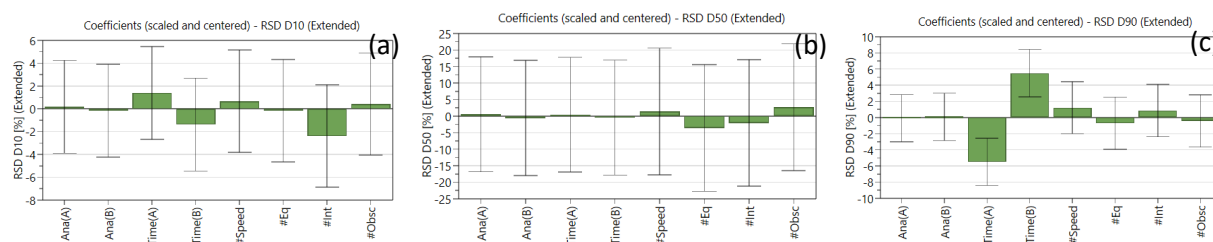


Figure 14: Coefficient plots for (a) the RSD D10, (b) the RSD D50 and (c) the RSD D90, with regards to analyst, time of day, pump speed, equilibrium time, internal sonication intensity and obscuration.

As can be seen, all uncertainty levels but the ones for time of day for RSD D90 spanned across $y=0$, indicating a non-significant model for all other model parameters. It can further be noted that the sonication intensity seemed to have the greatest effect on RSD D10, but not great enough to become significant. This is reasonable since the energy added to the sample during sonication may cause disaggregation and therefore a higher fraction of smaller, individual droplets. As previously

mentioned, the parameter “time of day” was added since it was noted that the temperature within the instrument increased throughout the day, and since emulsions are heat sensitive, this temperature increase was suspected to affect the properties of the sample. It is reasonable that the increased temperature mainly affected aggregates and thereby D90 since less energy is required to disrupt aggregates than single droplets. The correlation between the disaggregation caused by the higher temperature within the instrument and the value of D90 did not seem to be linear since the obtained size distributions were very irregular at higher temperatures. This behaviour caused the standard deviations of the D90 values to differ between the measurements performed in the afternoon, which, in combination with the inconsistent mean values, generated inconsistent RSD D90s according to Equation 1. Because of this, a note was added in the developed standard method to check that the dispersant temperature is below 28 °C before starting the measurement as the irregular results appeared when the dispersant temperature was about 30 °C or higher.

According to USP <429>, the RSDs for D10, D50 and D90 should be equal to or below 10 %, 15 % and 10 % respectively. As can be seen in Appendix Table A3, the RSD D10 was acceptable for all experiments but experiment 7, the RSD D50 was only acceptable for four experiments and the RSD D90 was acceptable for seven experiments. As stated in the background, for particles under 10 µm, which would have been the case if complete disaggregation was achieved, the limits for RSD of D10, D50 and D90 are doubled according to USP <429>. This resulted in all RSD D10 and RSD D90 values acceptable and only two RSD D50 values above the limit. A plausible explanation for the generally high RSD D50s is the multimodal shape of the DSD and the varied fractions of fine particles and aggregates. However, it should again be noted that the peak of interest was very consistent, and as the main purpose was to verify the actual droplet size distribution of the cream, and that this peak did not change in mode value or volume % between measurements, the results were still considered acceptable.

4.1.7 Methodology for Method Development of DSD

Based on the results from the experiments of the cream, a standard methodology for development of analytical methods for unknown (O/W) emulsions was created.

Firstly, the sample preparation was concluded to be of great importance when analysing (O/W) emulsions. Finding a dispersant in which the oil droplets are insoluble is crucial, where water is suggested as a good starting point. The obscuration should be around 2.5-4.5 %. Different concentrations of the sample should be tested to make sure that multiple scattering is avoided, which otherwise can be seen by graphs shifting towards the smaller particle sizes with higher concentrations.

The sample was preferably dispersed on a magnetic stirrer plate. The stirrer speed should however be investigated since this can cause the droplets to break, resulting in smaller droplets than their actual size. However, the optimal stirrer speed during sample dispersion was 600 RPM according to our findings.

The pump speed in the instrument should also be investigated since a too high pump speed might cause the droplets to break. Nevertheless, our findings suggest that a pump speed of 750 RPM was appropriate for the cream and that it should be slightly lower for lotions. In addition, the sonication

intensity during the measurement should be investigated since it is desired to have sufficient disaggregation and avoid reaggregation, but at the same time not to disrupt the oil droplets. Based on the results, a sonication intensity of 20 % was considered optimal. It should also be noted that the pump speed and sonication intensity correlated and can have varied effects depending on the other variable. In addition, a delay time should be used to allow the sample to stabilise in the instrument before starting the measurement. For this, one minute was found to be sufficient.

4.2 Lactose

The results obtained from measurements involving both micronised and non-micronised lactose are presented in the following sections. When evaluating the results, the specification by the manufacturer was used as a reference. The specifications for both the micronised and non-micronised lactose powders are presented in Table 11 below.

Table 11: Specification limits from the manufacturer of the micronised and non-micronised lactose.

Sample	D10 [μm]	D50 [μm]	D90 [μm]
Micronised lactose	-	≤ 5	≤ 10
Non-micronised lactose	35-65	95-125	160-190

Both the micronised and non-micronised lactose were dispersed in 99.9 % IPA since it was found that the water content in 70 % IPA was too high, causing the particles to dissolve and thereby changing the properties of the sample. This was detected as the obscuration quickly decreased to 0 % during the measurements when 70 % IPA was used, indicating that the particles dissolved in the water. In addition, the sample container with the dry powder had to be turned upside down about three times before sampling to ensure that a representative sample was obtained. This was detected since the PSD was slightly lower when not turning the sample container, indicating that mostly the smaller fraction was analysed. Furthermore, the RI for the lactose was found to be 1.533 in literature (Adi et al., 2007). However, according to Malvern Instruments Ltd, the RI was 1.54 (Malvern Instruments Ltd, 2007b). In addition, the RI of IPA was 1.39.

4.2.1 Micronised Lactose

In contrast to the emulsions, the micronised lactose generated monomodal PSD graphs. Furthermore, as for the emulsions, the concentration was reduced from the referenced method in Section 3.6 to enable analysis of more particles to make the measured sample more representative as well as to reduce product waste. Tests were again performed to see that the changed concentration did not affect the results before moving on with the lower concentration.

Due to the differences in RIs for lactose previously described, experiments were performed to see whether the small difference in RI affected the result, see Table 12.

Table 12: The values of the percentiles for the measurement using an RI of 1.533 and 1.54 for the micronised lactose powder. The pump speed was set to 2000 RPM, the obscuration was 2-5% and no internal sonication was used. The sample was externally sonicated for 3 minutes. The values presented are an average of triplicate measurements within the instrument.

RI	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
1.533	2.6 ± 0.025	4.9 ± 0.018	9.3 ± 0.019	1.4 ± 0.11
1.54	2.6 ± 0.016	$4.9 \pm 8.3 \cdot 10^{-3}$	9.2 ± 0.044	1.2 ± 0.11

As can be seen, the percentiles for the measurements using both RIs were very similar, and both were within the specification limits presented in Table 11. Because of this, it was decided to use an RI of 1.54, since this was the RI stated in the manual of the instrument.

The dispersant technique was evaluated next. Comparisons were made between dispersing the sample by using a magnetic stirrer plate at 300 and 800 RPM respectively for 10 minutes followed by 5 minutes external sonication, as well as only sonicating the sample for 5 minutes, see Table 13.

Table 13: The values of the percentiles when dispersing the micronised lactose powder by 5 minutes sonication only or by a combination of stirring and 5 minutes sonication. The obscuration for the sample prepared at 800 RPM was 2% and the other two samples had an obscuration of 10%. The pump speed was 2000 RPM for all measurements. The values presented are an average of triplicate measurements within the instrument.

Stirrer Speed [RPM]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
No stirring	3.1 ± 0.035	5.5 ± 0.075	10 ± 4.4	0.89 ± 4.8 · 10 ⁻³
300	3.3 ± 0.013	5.7 ± 0.031	9.9 ± 0.062	1.2 ± 4.9 · 10 ⁻³
800	3.2 ± 0.051	5.6 ± 0.025	9.9 ± 0.24	1.5 ± 0.036

As can be seen, the PSD did not differ a lot between using a magnetic stirrer plate or only dispersing the sample by external sonication. However, stirring the sample at 300 RPM generated a slightly higher volume % of the peak than stirring the sample at 800 RPM, which could be due to disruption of some of the particles when using a higher stirrer speed. The lower response for the peak generated from only sonicating the sample could be explained by the same theory, that the particles were only exposed to additional energy by external sonication, generating less disruption. However, the graph for PSD is not included in the report.

As can be seen in Table 13, all D50-values were a bit too high with respect to the specification value presented in Table 11, and all D90 values were very close to the limit. The generally high D-values could be explained by the fact that the powder was dispersed in IPA during the measurements since no dry dispersant unit was available. Because of this, the dry powders had to be analysed wet, which might have caused the particles to swell and thereby be detected as larger particles than the dry particle size. Furthermore, these measurements were performed before optimising the other parameters, which could have resulted in slightly misleading D-values. However, since the PSD was not affected much when removing the stirring procedure, it was decided to only disperse the sample by sonication. This was decided since it would result in more efficient sample preparation and reduce the risk of introducing errors.

Next, the external sonication time was examined. As can be seen in Table 14, external sonication had a large impact on the PSD. This could clearly be seen in the graphs of the PSDs in Appendix Figure A5 as well.

Table 14: The values of the percentiles for measurements after 0-, 1- and 5 minutes external sonication for the micronised lactose powder. The pump speed was 2000 RPM, and the obscuration was 2-5% for the sample treated with 1 minute external sonication, while it was 10% for the other two samples. The values presented are an average of triplicate measurements within the instrument.

Sonication Time [min]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
0	2.5 ± 0.13	6.1 ± 0.60	74 ± 14	0.63 ± 0.020
1	2.7 ± 0.044	5.1 ± 0.12	10 ± 0.83	1.1 ± 0.27
5	3.3 ± 0.013	5.7 ± 0.031	9.9 ± 0.062	$1.2 \pm 4.9 \cdot 10^{-3}$

As can be seen, none of the external sonication times resulted in D-values within the specification limits presented in Table 11. However, it was decided to sonicate the sample for 5 minutes since the aggregate peak at around 85 μm did not appear after this time, see Appendix Figure A5, indicating that the sample was the most representative after this time.

Pump speeds of 500, 1000, 2000 and 3000 RPM respectively were investigated next, see Table 15. For the graphs of the PSD, see Appendix Figure A6.

Table 15: The values of the percentiles for measurements of 500-, 1000-, 2000-, 3000- and 4000 RPM for the micronised lactose powder. The obscuration was 10% and the samples were treated with 5 minutes external sonication. The values presented are an average of triplicate measurements within the instrument.

Pump Speed [RPM]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
500	4.3 ± 0.060	10 ± 0.23	22 ± 0.42	$0.70 \pm 7.3 \cdot 10^{-3}$
1000	2.7 ± 0.022	5.2 ± 0.024	10 ± 0.015	0.77 ± 0.012
2000	3.1 ± 0.035	5.5 ± 0.075	10 ± 0.11	$0.89 \pm 4.8 \cdot 10^{-3}$
3000	$2.8 \pm 2.4 \cdot 10^{-3}$	$5.1 \pm 5.0 \cdot 10^{-3}$	9.4 ± 0.016	$0.73 \pm 6.9 \cdot 10^{-3}$
4000	$2.9 \pm 5.3 \cdot 10^{-3}$	$5.1 \pm 9.4 \cdot 10^{-3}$	9.1 ± 0.024	1.2 ± 0.011

As can be seen, measurements performed with a pump speed of 500 RPM generated higher D-values than the others. This could be due to agglomeration of the cohesive micronised particles as a consequence of the low force the particles encounter. The PSD obtained from a pump speed of 1000 RPM tended to shift more towards the larger particle sizes, which also could be explained by possible agglomerates forming within the instrument. The D-values obtained after analysis at 3000 RPM seemed to be the closest to the specification. Hence, this pump speed was considered most optimal.

To see if the obscuration affected the results, obscurations of 10 %, 15 %, 20 % and 30% were tested, since the article the method was based on recommended an obscuration of 10-30 % (Adi et al., 2007), see Table 16.

Table 16: The values of the percentiles for measurements of an obscuration of 10-, 15-, 20- and 30 % for the micronised lactose powder. The pump speed was 3000 RPM, and the samples were treated with 5 minutes external sonication. The values presented are an average of triplicate measurements within the instrument.

Obscuration [%]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
10	$2.6 \pm 4.9 \cdot 10^{-3}$	$4.94 \pm 6.1 \cdot 10^{-3}$	$9.35 \pm 7.6 \cdot 10^{-3}$	0.98 ± 0.097
15	$2.4 \pm 6.5 \cdot 10^{-3}$	$4.81 \pm 5.7 \cdot 10^{-3}$	$9.64 \pm 7.3 \cdot 10^{-3}$	$0.65 \pm 1.9 \cdot 10^{-3}$
20	$2.5 \pm 5.2 \cdot 10^{-3}$	$4.93 \pm 2.6 \cdot 10^{-3}$	$9.75 \pm 2.9 \cdot 10^{-3}$	$0.60 \pm 1.7 \cdot 10^{-3}$
30	2.7 ± 0.015	5.26 ± 0.032	10.3 ± 0.060	$0.58 \pm 1.9 \cdot 10^{-3}$

When analysing the D-values obtained from the different obscurations, only an obscuration of 30 % generated a PSD outside the specification limits presented in Table 11. Since an obscuration of 10 %, 15 % and 20 % all resulted in percentiles within the specification limits, 15 % was considered the most suitable. This was because it was quite hard to reach an exact obscuration, and it was therefore desirable to have some margins inside which the PSD was not affected.

A stability test was performed for 2 hours to see for how long the sample was stable, see Table 17. The graphs of the PSDs are presented in Appendix Figure A7.

Table 17: The values of the percentiles for measurements after 0-, 0.5-, 1- and 2 hours for the micronised lactose powder. The pump speed was 3000 RPM, and the obscuration was 15%. The samples were externally sonicated for 5 minutes. The values presented are an average of triplicate measurements within the instrument.

Time [h]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
0	$2.4 \pm 6.5 \cdot 10^{-3}$	$4.81 \pm 5.7 \cdot 10^{-3}$	$9.64 \pm 7.3 \cdot 10^{-3}$	$0.65 \pm 1.9 \cdot 10^{-3}$
0.5	2.8 ± 0.022	5.18 ± 0.042	9.61 ± 0.068	0.69 ± 0.017
1	3.1 ± 0.062	6.00 ± 0.25	11.7 ± 0.65	1.1 ± 0.035
2	3.6 ± 0.13	6.40 ± 0.44	11.4 ± 1.1	1.2 ± 0.026

As can be seen in Table 17, the distribution was quite similar between 0 and 0.5 hours but started to change more after 1 and 2 hours. Since only the immediately analysed sample was within all specification limits and the sample analysed after 0.5 hours only exceeded the limits slightly, it was concluded that the sample could be analysed for up to 0.5 hours after sample preparation. The short stability time could potentially be extended by sonicating the sample during the measurements, but since IPA was used as dispersant that has a low ignition point, it was chosen not to sonicate the sample within the instrument due to safety reasons.

4.2.2 Non-micronised Lactose

For the same reason as discussed for the micronised lactose, the particle size of the non-micronised lactose powder might have been affected due to that it was dispersed in IPA, which might have resulted in slightly higher PSDs.

When using the same concentration as for the micronised lactose powder (i.e. 0.1 g powder in 10 mL IPA), a large sample volume was required to reach the desired obscuration since the non-

micronised lactose sample was more translucent. Due to this, 0.5 g non-micronised lactose was dispersed in 10 mL IPA instead, see Table 18 for percentiles from the measurements.

Table 18: The values of the percentiles for measurements after dissolving 0.1 and 0.5 g of non-micronised lactose powder in 10 mL IPA respectively. The pump speed was 2000 RPM, and the obscuration was 5%. The samples were externally sonicated for 3 minutes. The values presented are an average of triplicate measurements within the instrument.

Mass of Powder [g]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
0.1	34 ± 1.7	92.2 ± 0.89	176 ± 1.4	$0.62 \pm 5.7 \cdot 10^{-3}$
0.5	33 ± 1.6	92.0 ± 0.79	176 ± 0.56	$0.63 \pm 9.8 \cdot 10^{-3}$

When comparing the obtained D-values to the specification presented in Table 11, the values of D10 and D50 were slightly lower for both measurements, while D90 was within the specification limits. This could be explained by other parameters not yet being optimised when performing the concentration test. Nevertheless, the increased concentration did not seem to affect the results and the higher concentration was therefore used.

The external sonication time was evaluated for the non-micronised lactose powder as well, see Table 19. The PSD graphs are presented in Appendix Figure A8.

Table 19: The values of the percentiles for measurements after 0-, 1-, 3-, 5- and 10 minutes external sonication for the non-micronised lactose powder. The pump speed was 2000 RPM, and the obscuration was 10%. The values presented are an average of triplicate measurements within the instrument.

Sonication Time [min]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
1	61 ± 1.2	103 ± 1.5	171 ± 4.5	0.66 ± 0.13
3	30 ± 2.9	92.0 ± 1.2	176 ± 1.7	$0.58 \pm 2.4 \cdot 10^{-3}$
5	15 ± 0.54	86.1 ± 0.92	169 ± 1.2	$0.55 \pm 6.2 \cdot 10^{-3}$

The non-micronised powder was not analysed without external sonication since it was suspected that fine particles were stuck to the coarse lactose particles, that detached during sonication (Adi et al., 2007). This hypothesis was explored further by looking at non-sonicated as well as sonicated non-micronised lactose dissolved in IPA in a light microscope, but the fine particles were too small to be visualised using this method (the pictures taken through the microscope are presented in Appendix Figure A11). However, a difference between the non-sonicated and sonicated samples was detected, supporting that sonication was needed. As seen in Table 19 and the PSD graphs in Appendix Figure A8, the PSD after external sonication for 1 minute differed quite a lot from the other two measurements. All percentiles after 1 minute external sonication were within the specification limits presented in Table 11, while both the D10 and D50 for the samples sonicated for 3 and 5 minutes respectively were too low. This probably was due to the particles breaking when the sample was exposed to too much energy, which also could be seen by the increasing peak at around 10 μm in Appendix Figure A8. Due to this, it was decided to disperse the sample by external sonication for 1 minute.

The delay time after sample addition before starting the measurement was evaluated to ensure that the sample was stable during the entire measurement, see Table 20.

Table 20: The values of the percentiles for measurements after a stirring time of 0-, 1- and 2 minutes respectively for the non-micronised lactose powder. The pump speed was 2000 RPM, and the obscuration was 2-5%. The samples were externally sonicated for 1 minute. The values presented are an average of triplicate measurements within the instrument.

Delay Time [min]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
0	64 ± 2.3	113 ± 1.6	193 ± 3.0	0.61 ± 0.11
1	62 ± 1.2	110 ± 1.2	187 ± 1.7	$0.56 \pm 6.6 \cdot 10^{-3}$
2	59 ± 1.4	108 ± 1.1	186 ± 1.4	0.56 ± 0.020

As can be seen, the D90 for the analysis without delay time was outside the specification limits presented in Table 11, while all other D-values were within the limits. This could be due to that the particles had not been circulated long enough within the instrument for complete deagglomeration to occur. Furthermore, the higher standard deviations should be noted for the measurement without delay time as this indicated that the PSD changed over time, meaning that the sample was not stable. A delay time of 1 minute was considered the most optimal to generate an efficient analysis while still ensuring a stable sample.

Next, the pump speed during the measurement was analysed, see Table 21 below. For the PSD graphs, see Appendix Figure A9.

Table 21: The values of the percentiles for measurements at 500-, 1000-, 2000- and 3000 RPM respectively for the non-micronised lactose powder. The obscuration was 10% and the samples were externally sonicated for 1 minute. The values presented are an average of triplicate measurements within the instrument.

Pump Speed [RPM]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
750	55 ± 2.1	118 ± 0.95	220 ± 0.77	$0.62 \pm 4.9 \cdot 10^{-3}$
1000	57 ± 2.6	114 ± 1.1	207 ± 3.1	0.60 ± 0.060
2000	61 ± 1.3	103 ± 1.5	171 ± 4.5	0.66 ± 0.13
3000	37 ± 2.3	93.0 ± 1.3	175 ± 1.6	$0.75 \pm 6.4 \cdot 10^{-3}$

As can be seen, only the D-values obtained from analysis with a pump speed of 2000 RPM were inside all specification limits presented in Table 11, which indicated this as optimal pump speed. This was further supported when analysing the PSD graphs since a pump speed of 2000 RPM generated the highest response in volume % and narrowest peak, see Appendix Figure A9.

Subsequently, the obscuration was evaluated to see whether the non-micronised lactose powder had the same optimal obscuration as the micronised lactose powder, see Table 22 below.

Table 22: The values of the percentiles for measurements at 10-, 15-, 20- and 30 % obscuration respectively for the non-micronised lactose powder. The pump speed was 2000 RPM, and the samples were treated with 1 minute external sonication. The values presented are an average of triplicate measurements within the instrument.

Obscuration [%]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
10	38 ± 1.2	94.7 ± 0.71	178 ± 0.97	$0.67 \pm 2.9 \cdot 10^{-3}$
15	42 ± 1.6	97.8 ± 1.1	183 ± 1.1	$0.66 \pm 1.7 \cdot 10^{-3}$
20	24 ± 1.7	89.8 ± 0.81	172 ± 1.1	$0.63 \pm 4.7 \cdot 10^{-4}$
30	9.8 ± 0.058	76.6 ± 0.39	155 ± 0.78	$0.55 \pm 4.9 \cdot 10^{-3}$

As can be seen, an obscuration of 10 % and 15 % generated very similar PSDs while an obscuration of 20 % and 30 % generated lower PSDs. This could be a sign that multiple scattering occurred from approximately 20 % obscuration and hence, the obscuration should be below 20 %. When further analysing the D-values, it was seen that D10 and D50 were slightly below the specification limits presented in Table 11 for measurements with 10 % obscuration, while all values were within limits for the measurements with 15 % obscuration. Due to this, 15 % obscuration was considered optimal for the non-micronised lactose powder as well.

It was detected that the obtained PSD changed a lot when leaving the samples even for a short time since the particles settled very fast, causing the sample to become heterogenous. Due to this, it was stated that the sample should be analysed immediately after preparation to avoid analysing a non-representative sample.

4.2.3 Developed Standard Operating Procedures for Lactose

Based on the results presented in the sections above, two different standard methods were developed for measuring the PSD of the micronised and non-micronised lactose respectively. The methods include how to prepare the sample, the software settings as well as how to perform the measurements and which outputs to note. The full instructions were written in a separate document and sent to the company since they are product specific.

4.2.3.1 Micronised Lactose

In short, the RI should be set to 1.54, the pump speed should be set to 3000 RPM and the delay time to 1 minute. The optimal obscuration was found to be 15 % when dispersing $0.1 \text{ g} \pm 0.01 \text{ g}$ micronised lactose powder in 10 mL IPA by 5 minutes external sonication. Using these conditions, the PSD graph presented in Figure 15 should be obtained with its corresponding D-values as presented in Table 23.

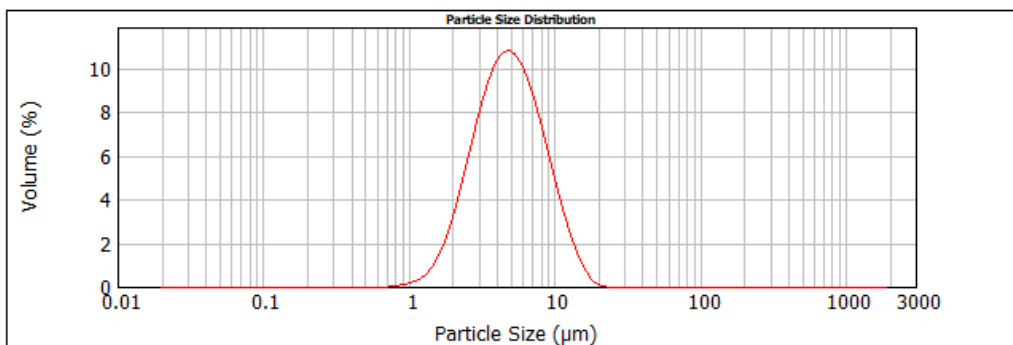


Figure 15: The resulting PSD for micronised lactose powder when analysing according to the SOP. The PSD presented is an average of triplicate measurements within the instrument.

Table 23: The percentiles for the micronised lactose powder when analysing according to the SOP. The values presented are an average of triplicate measurements within the instrument.

D10 [μm]	D50 [μm]	D90 [μm]
$2.38 \pm 6.48 \cdot 10^{-3}$	$4.81 \pm 5.71 \cdot 10^{-3}$	$9.64 \pm 1.89 \cdot 10^{-3}$

4.2.3.2 Non-micronised lactose

In short, the RI should be set to 1.54, the pump speed should be set to 2000 RPM and the delay time to 1 minute. The optimal obscuration was found to be 15 % when dispersing $0.5 \text{ g} \pm 0.01 \text{ g}$ non-micronised lactose powder in 10 mL IPA by external sonication for 1 minute. Using these conditions, the PSD graph presented in Figure 16 should be obtained with its corresponding D-values as presented in Table 24.

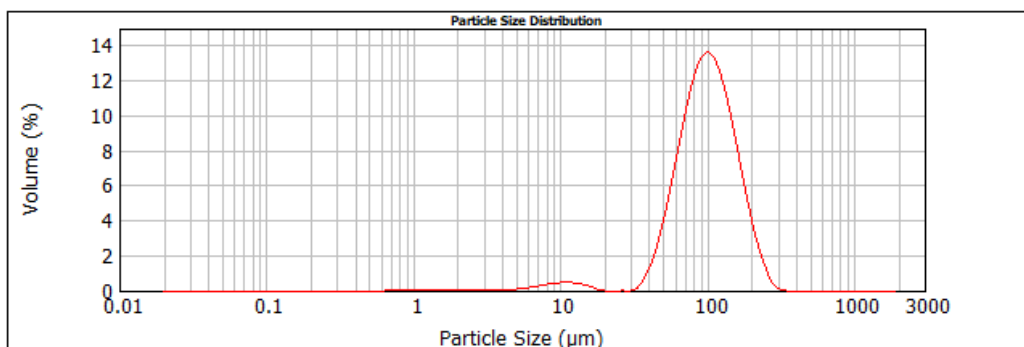


Figure 16: The resulting PSD for non-micronised lactose powder when analysing according to the SOP. The PSD presented is an average of triplicate measurements within the instrument.

Table 24: The percentiles for the non-micronised lactose powder when analysing according to the SOP. The values presented are an average of triplicate measurements within the instrument.

D10 [μm]	D50 [μm]	D90 [μm]
54.8 ± 1.56	99.0 ± 1.18	171 ± 1.50

4.3 Mometasone Furoate

Since mometasone furoate is water-insoluble, water was chosen as dispersant. However, due to its high hydrophobicity, a few droplets of Tween® 80 was required to be able to properly disperse the

powder. Other than this, the micronised mometasone furoate was suspected to have similar properties as the micronised lactose, leading to a starting point of using the same method developed for micronised lactose. Nevertheless, it should be mentioned that the mometasone furoate powder used in all experiments had expired, generating a source of error. A specification of percentiles from the manufacturer was used to evaluate whether the results were satisfactory or not. These values are presented in Table 25. The RI for mometasone furoate, i.e. 1.603, was already entered in the software and was used during the measurements. In addition, the RI of water was 1.33.

Table 25: Specification limits from the manufacturer of the micronised mometasone furoate.

Sample	D10 [μm]	D50 [μm]	D90 [μm]
Mometasone furoate	≤ 3	≤ 5	≤ 10

When analysing according to the method developed for micronised lactose, it was found that the mometasone furoate formed agglomerates when no internal sonication was applied. Therefore, the same method was run with the addition of 20 % continuous internal sonication during the measurement, see Table 26 below.

Table 26: The values of the percentiles for measurements without and with 20 % continuous internal sonication for mometasone furoate. The pump speed was set to 2500 RPM, and the obscuration was set to 15 % during both measurements. The samples were externally sonicated for 2 minutes. The values presented are an average of triplicate measurements within the instrument.

Sonication [%]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
0	2.20 ± 0.0359	5.19 ± 0.158	99.8 ± 2.29	0.542 ± 0.0205
20	1.49 ± 0.0862	3.36 ± 0.140	7.28 ± 0.190	1.49 ± 0.0212

As can be seen, internal sonication during the measurement was required to obtain percentiles within the specification limits presented in Table 25. In Appendix Figure A12, it was further seen that sonication was required since the agglomerate peak of around 80 μm disappeared when continuous internal sonication was applied. Furthermore, a monomodal distribution was thought to be more correct for the micronised mometasone furoate powder. This hypothesis was further supported by analysing the sample in a light microscope where no particles of about 80 μm were found, see Appendix Figure A13.

The same obscurations were tested for mometasone furoate as for the lactose powders to ensure that the most suitable obscuration was used, see Table 27.

Table 27: The values of the percentiles for measurements at different concentrations of mometasone furoate. The pump speed was set to 1000 RPM, and a continuous internal sonication of 10 % was used. The values presented are an average of triplicate measurements within the instrument.

Obscuration [%]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
10	1.63 ± 0.0479	3.57 ± 0.102	7.46 ± 0.117	0.744 ± 0.0103
15	1.66 ± 0.0426	3.63 ± 0.0781	7.58 ± 0.130	0.669 ± 0.0156
20	1.61 ± 0.0426	3.56 ± 0.0767	7.52 ± 0.125	0.678 ± 0.0121
30	1.44 ± 0.0968	3.39 ± 0.0916	7.52 ± 0.0784	$0.599 \pm 6.80 \cdot 10^{-3}$

As can be seen, an obscuration of 10 %, 15 % and 20 % generated very similar results while an obscuration of 30 % generated lower PSD values. This could be due to that multiple scattering occurred, but more tests are required to state that this was the case. For the same reasons as for the micronised lactose powder, i.e. that the D-values were within the specification limits and to generate a margin, an obscuration of 15 % was considered optimal.

Delay times of 60 s as well as 90 s were examined next, see Table 28.

Table 28: The values of the percentiles for measurements of different delay times for mometasone furoate. The pump speed was set to 2500 RPM, the continuous internal sonication intensity to 20 % and the obscuration was 15 %. The samples were externally sonicated for 2 minutes. The values presented are an average of triplicate measurements within the instrument.

Delay Time [s]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
60	1.49 ± 0.0862	3.36 ± 0.140	7.28 ± 0.190	1.49 ± 0.0212
90	1.39 ± 0.0602	3.21 ± 0.0852	7.01 ± 0.0776	$0.443 \pm 8.50 \cdot 10^{-3}$

A delay time of 90 s instead of 60 s generated lower standard deviations and hence, it was concluded that the delay time had an impact on the stability of the sample. Furthermore, all D-values were within the specification limits presented in Table 25 for both measurements. A delay time of 90 s was therefore considered to be optimal.

The optimal concentration of mometasone furoate was investigated next, based on the concentration of micronised lactose, which was 0.1 g dispersed in 10 mL IPA. To examine whether this concentration was appropriate for mometasone furoate as well, samples containing 0.1 g as well as 0.03 g, 0.05 g and 0.15 g dispersed in 10 mL Milli-Q water and 5 droplets of Tween® 80 were analysed, see Table 29.

Table 29: The values of the percentiles for measurements using different concentrations of mometasone furoate. The pump speed was set to 2500 RPM, the continuous internal sonication intensity to 20 % and the obscuration was 15 %. The samples were externally sonicated for 2 minutes. The values presented are an average of triplicate measurements within the instrument.

Mass Mometasone Furoate [g]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
0.03	1.55 ± 0.0684	3.34 ± 0.0932	7.01 ± 0.169	0.443 ± 0.0188
0.05	1.60 ± 0.0792	3.33 ± 0.127	6.80 ± 0.168	2.37 ± 0.0119
0.1	1.49 ± 0.0862	3.36 ± 0.140	7.28 ± 0.190	1.49 ± 0.0212
0.15	1.25 ± 0.0310	2.71 ± 0.0356	$6.12 \pm 6.55 \cdot 10^{-3}$	$2.63 \pm 7.36 \cdot 10^{-3}$

The measurement using a sample mass of 0.15 g shifted to the lower particle sizes compared to the other concentrations, which could be an indication of multiple scattering. No difference was found between the other concentrations whereby 0.1 g was chosen since it had been proven optimal for micronised lactose previously as well. In addition, the amount of sample needed to obtain the desired obscuration was considered when optimising the concentration.

Since the dispersed sample was externally sonicated in a sonication bath before the measurement, different sonication times were investigated, see Table 30 below.

Table 30: The values of the percentiles for measurements of different external sonication times in a sonication bath. The pump speed was set to 2500 RPM, the continuous internal sonication intensity to 20 % and the obscuration was 15 %. The values presented are an average of triplicate measurements within the instrument.

Sonication Time [min]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
1	1.64 ± 0.0492	3.58 ± 0.0912	7.51 ± 0.150	0.589 ± 0.0274
2	1.66 ± 0.0426	3.63 ± 0.0781	7.58 ± 0.130	0.669 ± 0.0156
5	1.30 ± 0.0143	2.94 ± 0.107	6.76 ± 0.0385	0.526 ± 0.0108
10	1.62 ± 0.0557	3.59 ± 0.0956	7.58 ± 0.147	0.521 ± 7.59·10 ⁻³

The samples sonicated for 1, 2 and 10 minutes respectively generated very similar PSDs while the sample sonicated for 5 minutes differed from the others. Due to the irregular pattern, new samples were prepared and sonicated for 5 and 10 minutes, which generated identical PSDs as before. It is not known why the PSD obtained after 5 minutes external sonication differed from the others. The D-values for all samples presented in Table 30 implied that the sonication time before the measurement did not have a great impact on the distribution. However, the PSD for the sample sonicated for 1 minute tended to change a bit over time while the samples sonicated for 2, 5 and 10 minutes were more stable. Since it is preferable to affect the sample as little as possible while still ensuring stability, 2 minutes external sonication was considered optimal.

As previously discussed, the pump speed should be considered due to its mechanical force affecting the sample. For this reason, different pump speeds were investigated for mometasone furoate, see Table 31, based on the pump speeds used during analysis of the micronised lactose powder.

Table 31: The values of the percentiles for measurements using different pump speeds in the instrument. The continuous internal sonication intensity was set to 20 % and the obscuration was 15 %. The samples were externally sonicated for 2 minutes. The values presented are an average of triplicate measurements within the instrument.

Pump Speed [RPM]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
1000	1.31 ± 0.0200	2.90 ± 0.0262	6.44 ± 0.0177	0.505 ± 0.0203
1500	1.29 ± 0.0311	2.94 ± 0.0405	6.66 ± 0.0334	0.452 ± 0.0168
2000	1.29 ± 0.0257	2.88 ± 0.0307	6.45 ± 7.79·10 ⁻³	0.477 ± 0.0181
2500	1.33 ± 0.0698	3.1 ± 0.0977	6.98 ± 0.0450	0.467 ± 0.0266

As can be seen, all D-values were within the specification limits presented in Table 25. The PSD did not change remarkably when the pump speed was changed. However, it was noted that the consistency differed between the triplicates for the different pump speeds, indicated by the increased standard deviation with increased pump speeds. For this reason, 1000 RPM was considered optimal. As can be noted, the optimal pump speed differed from the 3000 RPM used during analysis of micronised lactose. As previously mentioned, the pump speed and sonication

intensity both increased the amount of energy that the sample was exposed to and hence, when sonication was added, the pump speed had to be lowered.

The internal sonication intensity during the measurements was further examined during the experiments. Since no internal sonication during the measurements was applied on the lactose particles, no previously optimised intensity could be used as starting point. Therefore, an intensity of 20 % was applied as a first step, which seemed to generate a desired PSD. However, since it was not known whether 20 % was optimal, intensities of 0 %, 10 % and 50 % respectively were tested as well, see Table 32.

Table 32: The values of the percentiles for measurements using different internal sonication intensities within the instrument. The pump speed was set to 1000 RPM, and the obscuration was set to 15 %. The samples were externally sonicated for 2 minutes. The values presented are an average of triplicate measurements within the instrument.

Sonication Intensity [%]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
0	$1.41 \pm 4.71 \cdot 10^{-4}$	$3.11 \pm 1.70 \cdot 10^{-3}$	6.90 ± 0.0149	$0.482 \pm 4.78 \cdot 10^{-3}$
10	1.33 ± 0.0180	2.94 ± 0.0241	6.55 ± 0.0194	$0.572 \pm 9.09 \cdot 10^{-3}$
20	1.31 ± 0.0200	2.90 ± 0.0262	6.44 ± 0.0177	0.505 ± 0.0203
50	1.04 ± 0.0440	2.61 ± 0.0432	6.54 ± 0.0396	0.753 ± 0.0285

As can be seen, 50 % internal sonication intensity seemed to disrupt the particles, resulting in a lower D10 and D50, while the other intensities as well as no internal sonication seemed to generate similar distributions. When comparing internal sonication intensities of 10 % and 20 %, the PSD seemed to decrease with increased sonication intensity, which was reasonable since the force applied to the particles increased. However, the finding that analysis without internal sonication resulted in approximately the same distribution as when applying sonication was not expected since it was concluded in the beginning of this section that internal sonication was required. This finding was further investigated to find an explanation.

During the initial analyses, a pump speed of 2500 RPM was used, while a pump speed of 1000 RPM was used when examining the sonication intensity, since the pump speed was changed after its optimisation. These parameters had previously been suspected to correlate and therefore, experiments at both 1000 and 2500 RPM were conducted without internal sonication during the measurement. As can be seen in Figure 17, agglomerates were detected for the measurement with a pump speed of 2500 RPM, but not for the measurement with a pump speed of 1000 RPM. This finding was contradictory to the previous results regarding pump speeds, see Table 31, which most probably was because sonication was applied during the pump speed experiments but not in the experiment presented below. A possible explanation to this could be that the higher pump speed caused the particles to collide more frequently, and since the particles are very cohesive, they formed agglomerates as seen in Figure 17. However, the sonication applied during the pump speed experiments presented in Table 31 probably disrupted the formed agglomerates and hence, the behaviour between the PSDs differed.

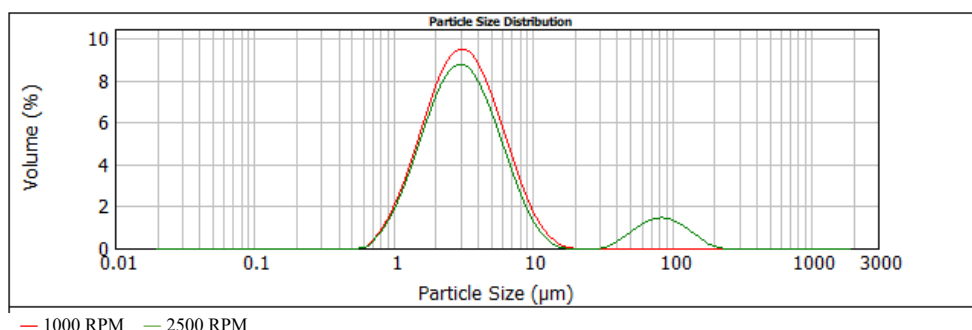


Figure 17: Graphs presenting the obtained PSDs from using different pump speeds without internal sonication during the measurements. The obscuration was set to 15 % and the samples were treated with 2 minutes external sonication. The PSDs presented are an average of triplicate measurements within the instrument.

Furthermore, it was found that the sample needed to be continuously sonicated during the measurement to avoid reagglomeration. Due to this, 10 % internal sonication intensity in combination with a pump speed of 1000 RPM was considered an optimal combination, since this affected the sample as little as possible while still obtaining a stable sample.

The stability of the dispersed sample was lastly evaluated to see for how long the sample could be used for analysis, see Table 33.

Table 33: The values of the percentiles for measurements over a 3-hour period for mometasone furoate. The pump speed was set to 2500 RPM, and the continuous internal sonication intensity to 20 %. The obscuration was set to 15 % and the samples were externally sonicated for 2 minutes. The values presented are an average of triplicate measurements within the instrument.

Time [h]	D10 [μm]	D50 [μm]	D90 [μm]	Weighted Residual [%]
0	1.49 ± 0.0862	3.36 ± 0.140	7.28 ± 0.190	1.49 ± 0.0212
0.5	1.55 ± 0.0955	3.41 ± 0.155	7.22 ± 0.202	2.09 ± 6.13·10 ⁻³
3	1.44 ± 0.0803	3.26 ± 0.115	7.00 ± 0.103	1.78 ± 5.44·10 ⁻³

The study was conducted for three hours and as can be seen, the D-values did not differ much over time. Hence, the conclusion was drawn that the same sample could be used for analysis for up to three hours. If the analysis time is to be prolonged, further stability tests should be conducted to ensure that the sample properties does not change. It was however noted that the sample separated and became heterogeneous quite fast, so before performing the analysis, the bottle should be shaken to ensure that the sample is homogenous.

4.3.1 Developed Standard Operating Procedure for Mometasone Furoate

Based on the results presented above, a standard method was developed for measuring the PSD of the micronised mometasone furoate. The method includes how to prepare the sample, the software settings as well as how to perform the measurement and which outputs to note. The full instructions were written in a separate document and sent to the company since they are product specific.

To prepare the sample, 10 mL Milli-Q water was added to a 50 mL glass bottle. 5 droplets of Tween® 80 was the added to the Milli-Q water whereby the bottle was stirred by hand to dissolve

the droplets. $0.1 \text{ g} \pm 0.02 \text{ g}$ mometasone furoate was then added to the glass bottle and dispersed by sonication for 2 minutes in an external sonication bath.

In the software, an RI of 1.60 and absorption of 0.01 was used. The pump speed was set to 1000 RPM and 10 % internal sonication was applied continuously during the measurement. A delay time of 1.5 minutes was set before starting the measurement. The obscuration used was 15 %. Using these conditions, the PSD graph presented in Figure 18 should be obtained with its corresponding D-values as presented in Table 34.

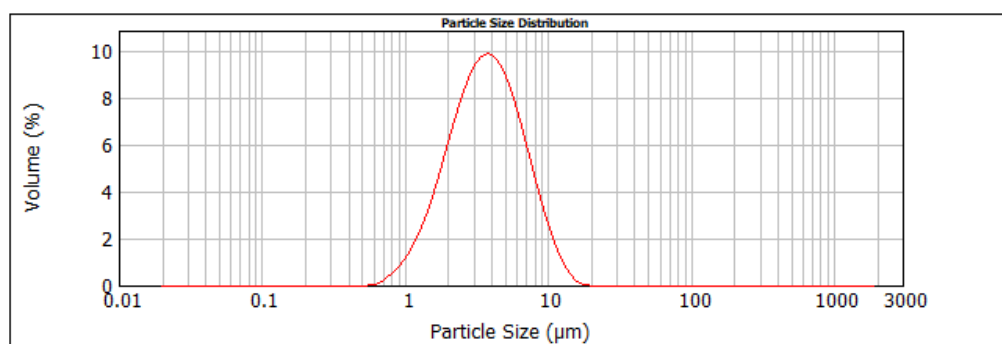


Figure 18: The resulting PSD for mometasone furoate when analysing according to the SOP. The PSD presented is an average of triplicate measurements within the instrument.

Table 34: The percentiles for mometasone furoate when analysing according to the SOP. The values presented are an average of triplicate measurements within the instrument.

D10 [μm]	D50 [μm]	D90 [μm]
1.66 ± 0.0537	3.67 ± 0.0936	7.72 ± 0.146

4.3.2 Robustness Test of the Method Developed for Mometasone Furoate

During the robustness test, the independent parameters pump speed, internal sonication intensity, delay time, analyst, obscuration, and the number of droplets of Tween® 80 were examined. The dependent responses of interest were the mode value, the weighted residual as well as the RSDs for D10, D50 and D90 respectively. For numerical values, see Appendix Table A4. As can be seen, the weighed residual was below 1 % for all experiments and the RSDs for D10, D50 and D90 were far below 10 %, 15 % and 10 % respectively for all experiments, indicating that the method was robust according to USP <429>.

In Section 3.6.1, it was stated that the centre value for the sonication intensity was 10 % and that a low sonication intensity of 8 % therefore should be used. However, it was found that the minimum sonication intensity that could be used in the instrument was 10 % and therefore, the low value was changed to 10 % and the centre value was changed to 11 %. This means that experiments 1, 2, 5 and 6 in Table 4 were performed using 10 % sonication intensity, experiments 3, 4, 7 and 8 were performed using 12 % sonication intensity and experiments 9-11 were performed using 11 % sonication intensity. This change was however corrected for in the software before statistical analysis, so the statistics presented below are based on the intensities used during the measurements.

An overview of how the parameters possibly correlated with each other is presented in the PCA plot in Figure 19. As can be seen, 55.1 % of the total variation is presented, which is relatively low, indicating that the system is complex. Due to this, a conclusion about which independent variables affect the dependent variables cannot be drawn from the PCA plot, but an overview of the correlations can be seen. However, since the PCA plot did not imply any strong correlations, and the variation was low, it was indicated that method was robust.

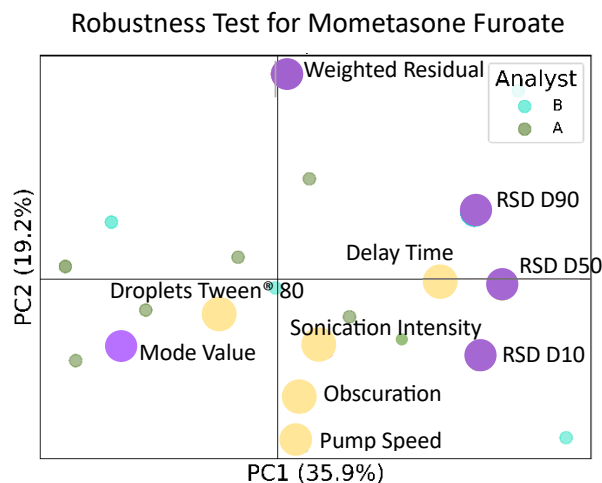


Figure 19: PCA plot from the robustness test of mometasone furoate. The pump speed, internal sonication intensity, delay time, analyst (A) and (B), obscuration and the number of droplets of Tween® 80 were plotted together with RSD D10, RSD D50, RSD D90 and weighted residual.

From Figure 19, it was implied that the number of Tween® 80 droplets and the mode value positively correlated. All other independent parameters were in an approximately right angle to the mode value, indicating that they did not have any impact. It further seemed like the sonication intensity, obscuration and pump speed negatively affected the weighted residual. This could be due to that an increased pump speed and increased sonication intensity generated a higher fraction of single particles rather than aggregates, which would make the calculations fit better to the theoretical model (for which the weighted residual decreases). The higher obscuration increased the signal from the analyte since more analyte was added during these experiments, which could result in a better fit between the theoretical and measured distribution. It was furthermore implied that the delay time had an effect on the RSD D50. This could be due to that the sample was pumped around for a longer time before the measurement, which possibly led to more disaggregation within the dispersant unit, causing detection of differently sized particles. This would affect the obtained distribution between the triplicates, which would increase the RSD. The sonication, obscuration and pump speed were suspected to positively correlate with RSD D10. This could be explained since the particles were exposed to more energy, which was suspected to cause more irregular behaviour between triplicates, as well as more particles were exposed when having a higher obscuration. Lastly, it was implied that the number of Tween® 80 droplets negatively correlated with RSD D90, possibly due to a more stable sample. However, as mentioned before, the total variance presented in the PCA plot is very low, so the implications are not fully reliable since the variance lays in more parameters than the ones presented. Because of this, further analyses were required for statistical evaluation of the model.

Since no statistical conclusions could be drawn from the PCA plot, the implications found were further evaluated in coefficient plots to examine if any parameter had a statistically significant effect, see Figures 20 and 21 below. As previously mentioned, the model parameters should be close to $y=0$ in combination with an uncertainty level that spans across $y=0$ for the method to be considered robust.

The coefficient plots for the droplet size at the mode value and the weighted residual are presented in Figure 20 below.

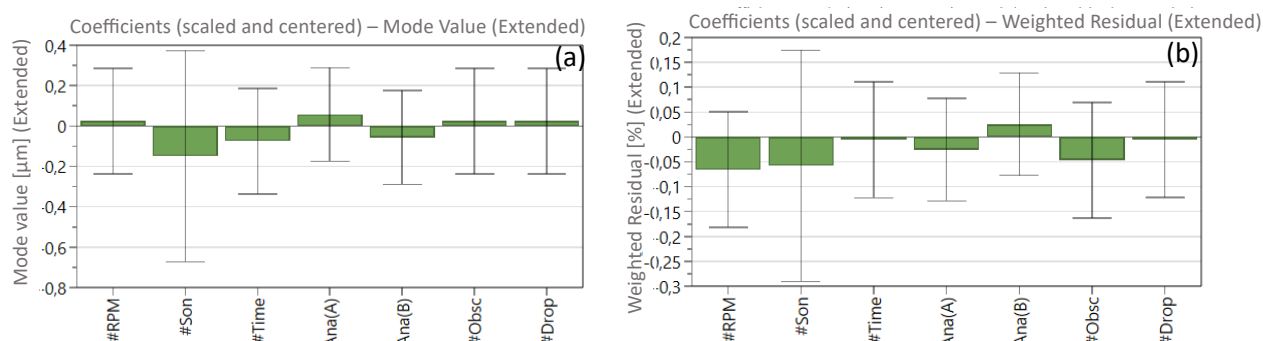


Figure 20: Coefficient plots for (a) the mode value and (b) the weighted residual, with regards to pump speed, internal sonication intensity, analyst, obscuration and droplets of Tween® 80.

As can be seen, all model parameters were close to $y=0$ and all uncertainty levels spanned across $y=0$. This means that all model parameters were non-significant, indicating a robust model with respect to the mode value and weighted residual. The coefficient plot for the mode value indicated that the sonication intensity affected the mode value the most but not enough to become significant, and that an increased sonication intensity generated a lower mode value. This is reasonable since more deagglomeration occurred with higher sonication intensity, which would result in smaller particles being detected. Furthermore, the sonication intensity seemed to be negatively correlated with the weighed residual. In addition, as can be seen in Figure 20b, the pump speed and obscuration seemed to have some negative correlation with the weighted residual as well. This could be explained by the same theory discussed above for the PCA plot. However, the effect from neither of these parameters was great enough to become significant.

The coefficient plots for the RSDs for D10, D50 and D90 respectively are presented in Figure 21 below.

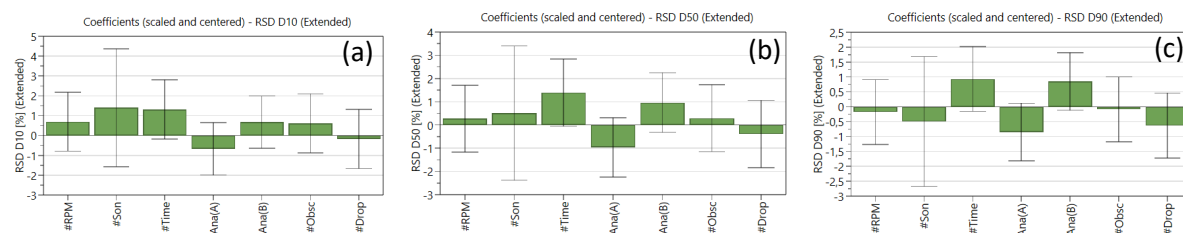


Figure 21: Coefficient plots for (a) the RSD D10, (b) the RSD D50 and (c) the RSD D90, with regards to pump speed, internal sonication intensity, analyst, obscuration and droplets of Tween® 80.

As can be seen, all model parameters were close to $y=0$ and all uncertainty levels spanned across $y=0$, indicating a robust model in terms of the RSDs. As can be seen in Figure 21a, most model

parameters seemed to positively correlate with RSD D10 but none of the effects were great enough to become significant. The sonication intensity and delay time seemed to affect the RSD D10 the most, which could be due to that more disaggregation possibly occurred before the measurement when the delay time was longer. This, in combination with increased sonication intensity, which also could cause the D10 to change during the measurement, could contribute to an increased RSD. For the RSD D50 and RSD D90, presented in Figure 21b and c, it can be seen that the uncertainty level for the delay time only just spanned across $y=0$, indicating that this parameter almost had a significant effect, explained by the same theory as for RSD D10. Furthermore, the analyst had a slight effect on the RSD D50 and RSD D90, but not great enough to become significant. This finding was harder to explain but could be due to that the particles settled to the bottom of the glass bottle quite quickly, making it necessary to shake the bottle prior to the measurement to obtain a proper dispersion before sampling. The shaking technique could have differed between analysts, which might have caused more inconsistently analysed distributions for one of the analysts, which in turn would affect the RSD.

Since all weighted residuals were below 1 % and all independent parameters were non-significant for all dependent parameters, the developed method was considered robust. However, according to USP <429>, the robustness test should be conducted by analysing six replicates. Since only three replicates were analysed, the test should be complemented with additional analyses to ensure that it is robust.

4.4 Methodology for Method Development of PSD

A standard protocol for development of analytical methods for unknown powder substances was created based on the discussed sample properties.

To start with, the sample preparation is crucial to obtain a correct PSD. Generally, the powder should be dispersed in a dispersant in which the powder is insoluble, to affect the sample properties as little as possible. The obscuration should be between 10 % and 20 %, which can be achieved by dispersing the powder at different concentrations, which in turn affects how much light passes through the sample. To ensure that multiple scattering is avoided, different concentrations should be tested to examine that the graphs are consistent and does not drift towards the smaller particle sizes with higher concentrations.

The sample could be dispersed either by stirring on a magnetic stirrer plate or by sonicating the sample, or a combination of both. However, our findings suggested that only external sonication was the most suitable. Since all powders examined during the degree project resulted in different optimal external sonication times, a range between 1 and 10 minutes should be tested. This should be optimised since too long sonication time can cause disruption of the particles, whereas too short sonication time can result in incomplete deagglomeration.

The pump speed within the instrument should further be investigated and optimised. A range between 750 RPM and 3500 RPM should be tested since different powders had different optimal pump speeds depending on the stability of the sample. The optimal delay time was about 1-2 minutes but should generally be slightly longer than it takes for the obscuration to stabilise and should therefore also be examined to ensure repeatability.

5. Conclusions and Future Work

To conclude, the findings from this degree project suggest that the parameters mainly affecting the DSD and PSD are the pump speed used during the measurements as well as the sonication intensity and time. Both these parameters contribute to the disaggregation of the samples to obtain single droplets or particles as well as to avoid reaggregation during the measurement, which was found to otherwise frequently occur. In general, it was found that the powder samples could withstand more external energy without disrupting and hence, the pump speed and sonication intensity used were higher for the powder samples than for the emulsions. Furthermore, the optimal dispersing technique differed between the samples. For the emulsions, the most suitable dispersing technique was stirring using a magnetic flea and a magnetic stirrer plate, while for the powders, it was found that the samples were properly dispersed by using external sonication. However, the optimal sonication time differed depending on whether the powder was micronised or non-micronised.

Other than this, it was found that a delay time during which the sample was allowed to properly disperse within the dispersant unit was required to obtain a sample stable throughout the entire measurement time. It was furthermore found that the obscuration did not affect the size distribution as long as it was not high enough for multiple scattering to occur. The limit for multiple scattering was found to differ between different samples and should therefore be examined for each new sample but in general, the optimal obscuration was lower for emulsions than for powders.

An interesting finding was that the time of day had a significant impact on the DSD for the cream. This was believed to be due to the increased temperature within the instrument that gradually occurred throughout the day when the pump was on for a long time. Since emulsions are heat sensitive, the increased instrument temperature affected the properties of the sample and thereby affected the obtained DSD.

To further evaluate these findings and to assess that the obtained size distributions are correct, the same samples should be analysed using another technique known to generate reliable results, such as scanning electron microscopy (SEM). This technique could also be used to examine whether the peak frequently appearing at approximately 0.13 μm for the emulsions arises due to actual structures present in the diluted sample or not. Different dispersants could also be tested, e.g. ethanol instead of IPA for lactose, to see how it affects the PSD. It would also be interesting to measure the PSDs of the powder samples using a dry dispersant unit to see how the size distribution is affected by the dispersion. Other than this, it is required to analyse more replicates in the robustness tests to fulfil the requirements in USP <429>, and to improve the statistical accuracy.

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Appendix I - Lotion

The raw data obtained during measurements of the lotion is presented below.

RI measurements

All measurement results for the RI measurements of the water- and oil phase separately of the lotion are presented in Table A1 below.

Table A1: Raw data from the RI measurements. The water phase was measured in three different concentrations relevant to the measurements performed. The oil phase was only analysed heated since it was solid at room temperature. The RI of the oil phase was measured three times.

Sample	RI at Room Temperature	RI at ~ 60°C
Water phase 1	1.331	1.331
Water phase 2	1.331	1.330
Water phase 3	1.331	1.330
Water phase mean	1.331	1.330
Oil phase	-	1.452
	-	1.455
	-	1.450
Oil phase mean	-	1.452

Screening Test

The numerical results from the screening test of the lotion are presented in Table A2.

Table A2: The numerical values from the screening test of the lotion, including the parameters. The parameters tested were temperature and stirrer speed during sample preparation, as well as internal sonication intensity during the measurement.

Experiment No.	Conditions	D10 [μm]	D50 [μm]	D90 [μm]
1	30 °C 0 % sonication 500 RPM	0.0660 ± 0.00	$0.129 \pm 4.71 \cdot 10^{-4}$	$0.266 \pm 4.71 \cdot 10^{-4}$
2	60 °C 0 % sonication 500 RPM	$0.0677 \pm 4.71 \cdot 10^{-4}$	$0.138 \pm 2.16 \cdot 10^{-3}$	$0.305 \pm 2.49 \cdot 10^{-3}$
3	45 °C 0 % sonication 500 RPM	0.114 ± 0.0328	21.5 ± 30.0	109 ± 11.9
4	30 °C 50 % sonication 1000 RPM	0.149 ± 0.0950	22.7 ± 31.8	75.4 ± 58.0
5	60 °C 50 % sonication 1000 RPM	0.0823 ± 0.0210	12.2 ± 17.1	36.4 ± 51.1
6	45 °C 50 % sonication 1000 RPM	$0.0677 \pm 1.25 \cdot 10^{-3}$	$0.134 \pm 2.62 \cdot 10^{-3}$	0.286 ± 0.0185

7	30 °C 20 % sonication 750 RPM	0.111 ± 0.0618	21.0 ± 29.5	45.4 ± 63.8
8	60 °C 20 % sonication 750 RPM	$0.0677 \pm 4.71 \cdot 10^{-4}$	$0.137 \pm 1.70 \cdot 10^{-3}$	$0.309 \pm 4.55 \cdot 10^{-3}$
9	45 °C 20 % sonication 750 RPM	1.87 ± 2.55	25.1 ± 35.3	54.0 ± 75.9
10	30 °C 50 % sonication 500 RPM	0.0843 ± 0.0273	18.2 ± 25.5	41.4 ± 58.2
11	30 °C 20 % sonication 500 RPM	$0.0663 \pm 4.71 \cdot 10^{-4}$	$0.131 \pm 2.36 \cdot 10^{-3}$	0.253 ± 0.0275
12	60 °C 0 % sonication 1000 RPM	$0.0723 \pm 5.44 \cdot 10^{-3}$	0.165 ± 0.0349	28.4 ± 39.7
13	60 °C 20 % sonication 1000 RPM	$0.0693 \pm 4.71 \cdot 10^{-4}$	$0.146 \pm 2.87 \cdot 10^{-3}$	0.386 ± 0.0348
14	45 °C 0 % sonication 750 RPM	0.0817 ± 0.0207	0.316 ± 0.258	37.5 ± 52.6
15	45 °C 50 % sonication 750 RPM	0.0760 ± 0.0134	0.210 ± 0.112	34.8 ± 48.8
16	30 °C 0 % sonication 1000 RPM	0.291 ± 0.262	15.3 ± 15.6	72.1 ± 42.6
17	30 °C 0 % sonication 750 RPM	0.0913 ± 0.0168	0.376 ± 0.185	66.9 ± 47.1
18	60 °C 50 % sonication 500 RPM	0.0670 ± 0.00	$0.134 \pm 8.16 \cdot 10^{-4}$	$0.279 \pm 3.77 \cdot 10^{-3}$
19	60 °C 50 % sonication 750 RPM	0.0670 ± 0.00	$0.134 \pm 4.71 \cdot 10^{-4}$	$0.279 \pm 1.63 \cdot 10^{-3}$
20	45 °C 20 % sonication 500 RPM	$0.0667 \pm 9.43 \cdot 10^{-4}$	$0.133 \pm 2.83 \cdot 10^{-3}$	0.280 ± 0.0109
21	45 °C 20 % sonication 1000 RPM	0.109 ± 0.0310	20.8 ± 23.6	79.8 ± 56.4
22	30 °C 20 % sonication 1000 RPM	0.113 ± 0.0354	19.4 ± 27.1	56.4 ± 52.0
23	30 °C 50 % sonication	0.0903 ± 0.0358	18.6 ± 26.2	43.0 ± 60.5

	750 RPM			
24	45 °C 0 % sonication 1000 RPM	$0.0677 \pm 4.71 \cdot 10^{-4}$	$0.138 \pm 2.83 \cdot 10^{-3}$	0.402 ± 0.117
25	45 °C 50 % sonication 500 RPM	$0.0653 \pm 9.43 \cdot 10^{-4}$	$0.126 \pm 4.71 \cdot 10^{-4}$	$0.248 \pm 8.01 \cdot 10^{-3}$
26	60 °C 0 % sonication 750 RPM	$0.0683 \pm 4.71 \cdot 10^{-4}$	$0.141 \pm 1.41 \cdot 10^{-3}$	0.327 ± 0.0130
27	60 °C 20 % sonication 500 RPM	0.0680 ± 0.00	$0.139 \pm 8.16 \cdot 10^{-4}$	$0.306 \pm 4.25 \cdot 10^{-3}$

Appendix II – Cream

The raw data obtained during measurements of the cream is presented below.

DSD Graphs from Software Parameters Test

Figure A1 presents how the DSD of the cream is affected by the pump speed.

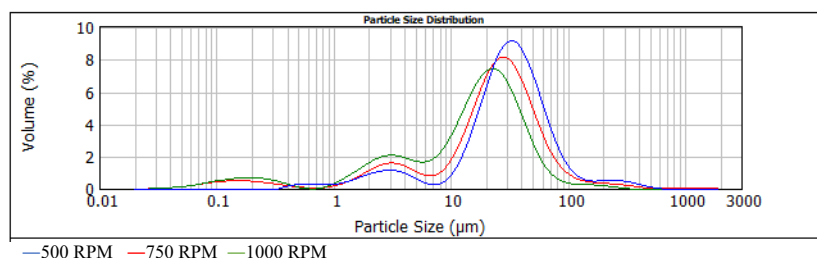


Figure A1: DSD of the cream using three different pump speeds: 500 RPM, 1000 RPM and 2000 RPM. The obscuration was set to 1.5-3.5 % and no sonication was used. The DSDs presented are an average of triplicate measurements within the instrument.

Figure A2 presents how the DSD of the cream is affected by the internal sonication intensity.

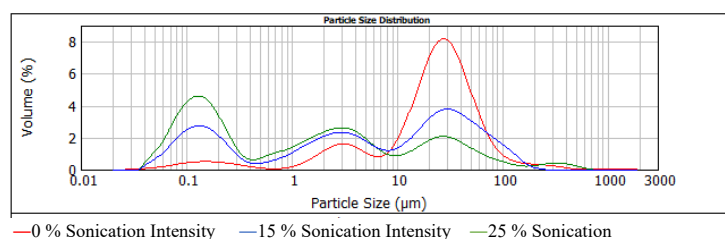


Figure A2: DSD of the cream using three different internal sonication intensities: 0 %, 15 % and 25 %. The pump speed was set to 750 RPM and 1.5-3.5 % obscuration was used. The DSDs presented are an average of triplicate measurements within the instrument.

Figure A3 presents how the DSD of the cream changes over time, indicating for how long the prepared sample is stable.

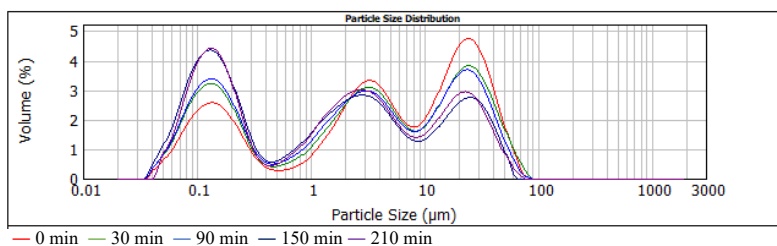


Figure A3: Stability test of the cream for 3.5 hours. The obscuration was set to 2.5-4.5 %, and the pump speed to 750 RPM. The sample was internally sonicated at 20 % intensity 30 s prior to the measurement. The DSDs presented are an average of triplicate measurements within the instrument.

Robustness Test

Table A3 presents the numerical results and standard deviation from the robustness test of cream.

Table A3: Results from the robustness test for the mode value, weighted residual as well as RSDs for D10, D50 and D90 for the cream. The values presented are an average of triplicate measurements of three separate sample preparations.

Experiment No.	Mode Value [μm]	Weighted Residual [%]	RSD D10 [%]	RSD D50 [%]	RSD D90 [%]
1	3.1 ± 0.0	1.1 ± 0.23	0.43	3.2	2.5
2	3.1 ± 0.0	0.66 ± 0.056	9.2	34	4.0
3	3.1 ± 0.0	1.1 ± 0.73	3.1	25	17
4	3.1 ± 0.0	1.2 ± 0.25	2.9	8.0	12
5	3.2 ± 0.090	1.0 ± 0.043	8.9	21	6.2
6	3.1 ± 0.0	1.1 ± 0.049	1.8	11	7.0
7	3.1 ± 0.0	0.88 ± 0.20	4.9	21	15
8	3.1 ± 0.0	1.4 ± 0.53	3.5	29	17
9	3.1 ± 0.0	1.9 ± 0.45	13	49	8.5
10	3.1 ± 0.0	0.78 ± 0.072	5.4	21	0.64
11	3.1 ± 0.0	0.92 ± 0.039	3.1	11	2.7

Microscopy Results

The pictures taken in the microscope of the cream sample are presented in Figure A4. A few oil droplets were measured in the picture using the software LASX to approximate their size. The figure presents the cream diluted in water without external sonication.

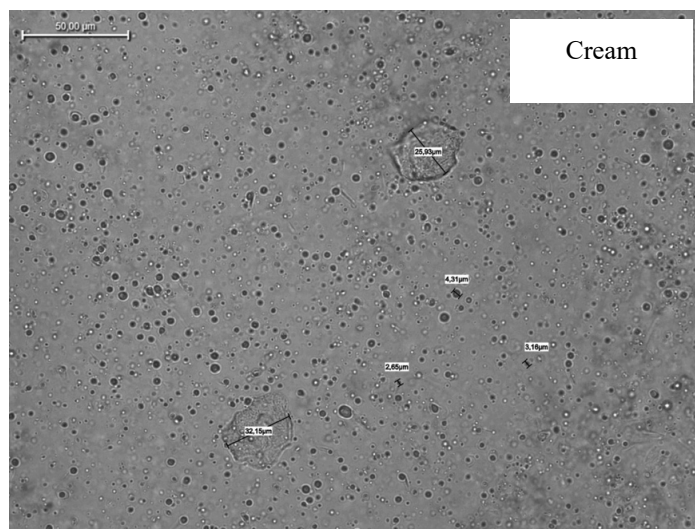


Figure A4: Picture taken through the microscope using LASX of the cream ~54x diluted in water. The sizes of three droplets and two aggregates were measured and are presented in the picture.

Appendix III – Lactose

The raw data obtained during measurements of the cream is presented below.

PSD Graphs from Parameter Trials for Micronised Lactose

Figure A5 displays the different graphs obtained for micronised lactose by sonicating the samples externally or by not sonicating it before entering the laser diffraction instrument.

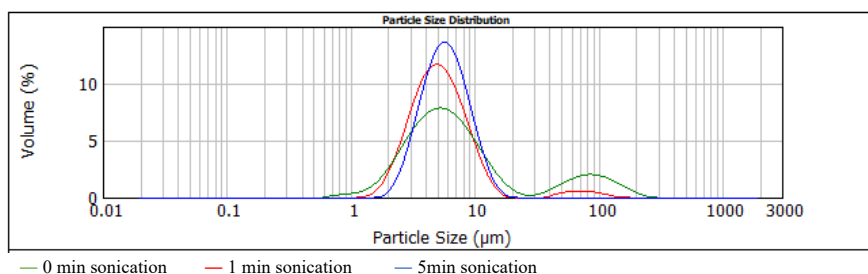


Figure A5: Graphs for using different external sonication times of the micronised lactose. The pump speed was 2000 RPM, and the obscuration was 2-5 % for the sample treated with 1 minute external sonication, while it was 10 % for the other two samples. The PSDs presented are an average of triplicate measurements within the instrument.

Figure A6 displays the different graphs obtained from micronised lactose by varying the pump speed in the laser diffraction instrument.

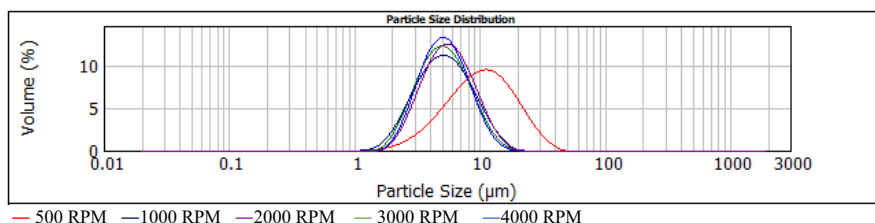


Figure A6: Graphs for using different pump speed for the micronised lactose. The obscuration was 10 %, and the samples were treated with 5 minutes external sonication. The PSDs presented are an average of triplicate measurements within the instrument.

Figure A7 shows how the graphs are changing based on how long the micronised lactose sample has been dispersed.

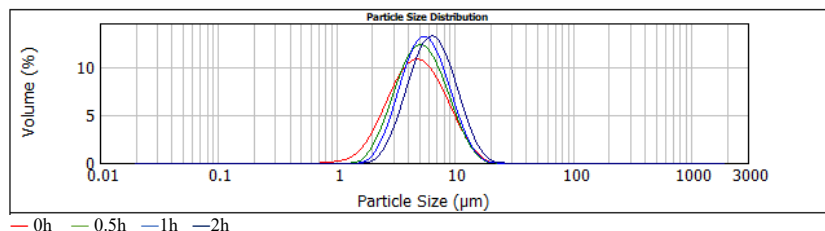


Figure A7: Graphs from the stability test of the micronised lactose. The pump speed was 3000 RPM, and the obscuration was 15 %. The samples were externally sonicated for 5 minutes. The PSDs presented are an average of triplicate measurements within the instrument.

PSD Graphs from Parameter Trials for Non-Micronised Lactose

Figure A8 shows the graphs obtained by varying the external sonication time in a water bath for non-micronised lactose.

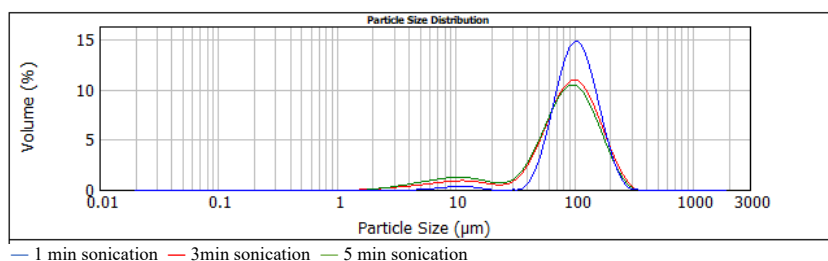


Figure A8: Graphs for using different external sonication times of the non-micronised lactose. The pump speed was 2000 RPM, and the obscuration was 10 %. The PSDs presented are an average of triplicate measurements within the instrument.

Figure A9 displays the difference in graphs obtained when changing the pump speed of the non-micronised lactose.

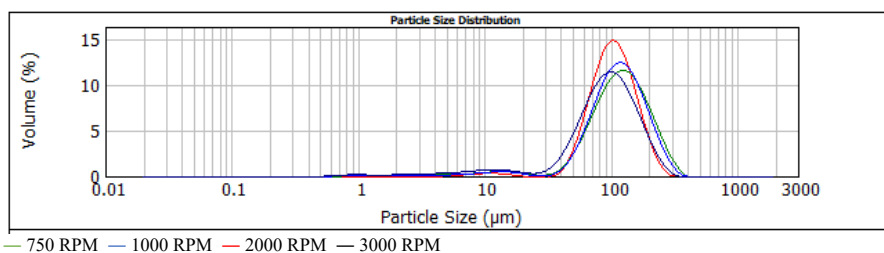


Figure A9: Graphs for different pump speeds of the non-micronised lactose powder. The obscuration was 10 %, and the samples were externally sonicated for 1 minute. The PSDs presented are an average of triplicate measurements within the instrument.

Microscopy Results

Below, pictures of the micronised lactose with the LASX software connected to the microscope are shown. Figure A10a presents the micronised lactose without external sonication while Figure A10b presents the micronised lactose after 3 minutes external sonication. In both figures, the lactose was dispersed in IPA.

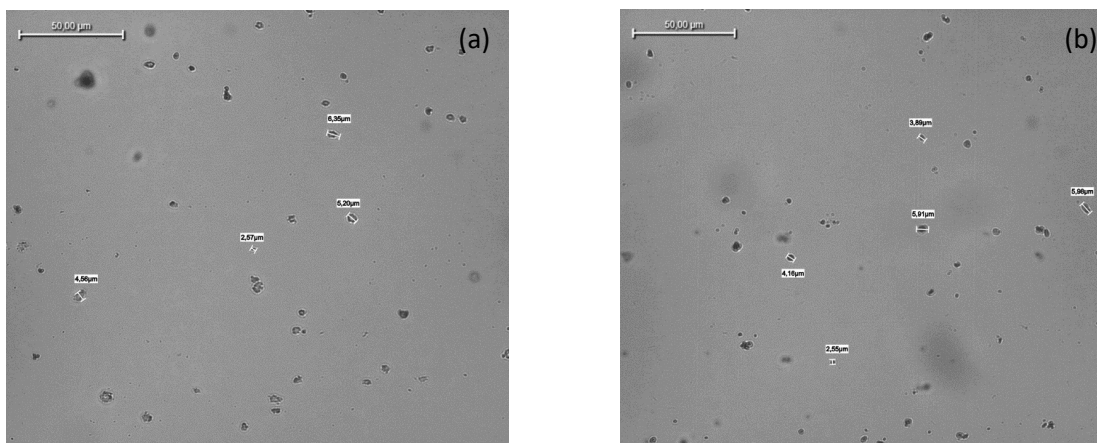


Figure A10: Picture of the micronised lactose powder taken with bright field and 40x magnification. In (a) the sample was not externally sonicated while in (b) the sample was externally sonicated for 3 minutes.

Below, pictures of the non-micronised lactose with the LASX software connected to the microscope are shown. Figure A11a presents the non-micronised lactose without external sonication while Figure A11b presents the non-micronised lactose after 3 minutes external sonication. In both figures, the lactose was dispersed in IPA.

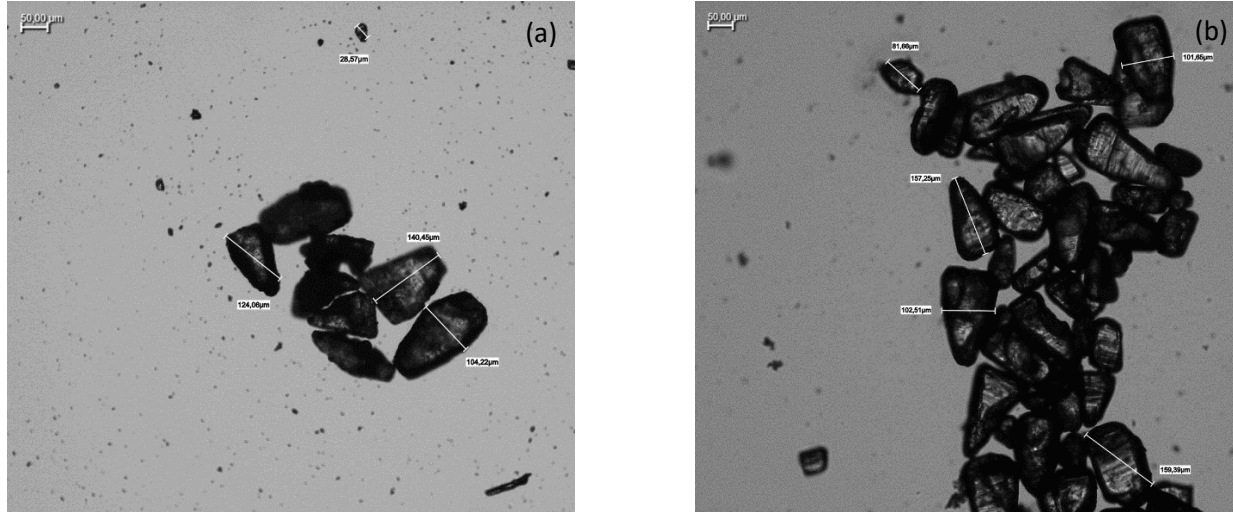


Figure A11: Picture of the non-micronised lactose powder taken with bright field and 10x magnification. In (a) the sample was not externally sonicated while in (b) the sample was externally sonicated for 3 minutes.

Appendix IV – Mometasone furoate

The raw data obtained during measurements of mometasone furoate is presented below.

PSD Graph from Parameter Trial

Figure A12 presents how the PSD of mometasone furoate is affected by the addition of internal sonication.

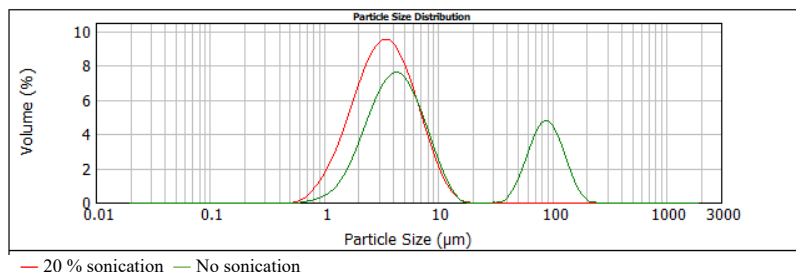


Figure A12: Graphs showing a measurement with and without internal sonication during the measurement. The pump speed was set to 2500 RPM, and the obscuration was set to 15 % during both measurements. The samples were externally sonicated for 2 minutes. The PSDs presented are an average of triplicate measurements within the instrument.

Robustness Test

Table A4 presents the numerical results and standard deviation from the robustness test of mometasone furoate.

Table A4: Results from the robustness test for the mode value, weighted residual as well as RSD for D10, D50 and D90 for mometasone furoate. The values presented are an average of triplicate measurements of three separate sample preparations.

Experiment No.	Mode Value [μm]	Weighted Residual [%]	RSD D10 [%]	RSD D50 [%]	RSD D90 [%]
1	3.7 ± 0.28	0.68 ± 0.31	1.6	1.7	1.6
2	3.7 ± 0.28	0.65 ± 0.060	1.7	1.1	0.51
3	3.5 ± 0.0	0.64 ± 0.088	3.2	2.4	1.5
4	3.5 ± 0.0	0.60 ± 0.035	3.5	2.9	2.2
5	3.4 ± 0.19	0.75 ± 0.13	4.1	5.7	5.8
6	3.6 ± 0.14	0.58 ± 0.055	5.1	4.2	2.6
7	3.4 ± 0.19	0.73 ± 0.14	3.5	2.8	1.4
8	3.7 ± 0.19	0.46 ± 0.028	7.8	6.6	3.5
9	3.7 ± 0.28	0.59 ± 0.075	0.68	0.91	1.0
10	3.7 ± 0.28	0.64 ± 0.19	1.4	1.6	1.7
11	3.7 ± 0.28	0.60 ± 0.10	2.0	1.7	1.1

Microscopy Results

A picture of the micronised mometasone furoate dispersed in 50mL Milli-Q water and Tween® 80 taken with the LASX software connected to the microscope are presented in Figure A13.

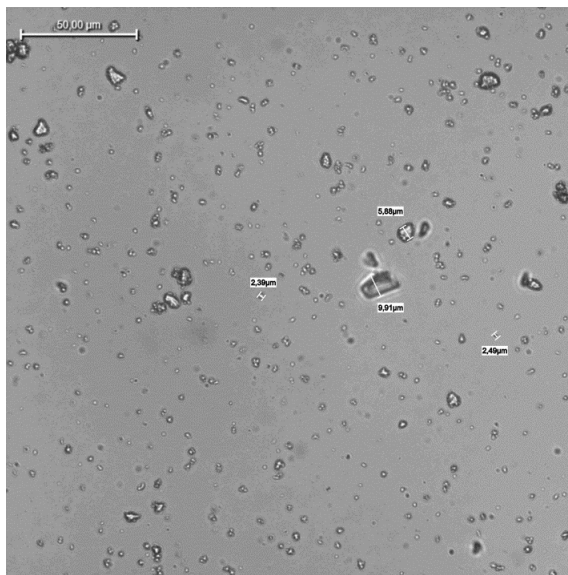


Figure A13: Mometasone furoate dispersed in 60 mL Milli-Q water and 5 droplets Tween® 80. The picture was taken using bright field and 40x magnification.