Formulation and characterization of liposomal spray dried powders intended for inhalation

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Abstract

This master thesis has evaluated the possible use of a spray dryer to produce powders suitable for use as inhalants and whether the addition of liposomes to the solution to be spray dried would improve the properties of the powder. The powders created consisted of Lysozyme as model drug, Lactose as filler and stabilizer, and different types of phospholipids to create liposomes using an ethanol injection method.

It was shown that powders suitable for use as inhalants were possible to create. By adjusting the parameters of both the spray dryer and by changing the proportions of the ingredients it was possible to achieve various powders, some of which displayed strong signs of having the proper parameters for use for inhalation.

The quality parameter deemed most important for the powders was the fine particle fraction, which corresponds to the fraction of powder that reaches the target area of the lungs. The fine particle fraction was tested using a cascade impactor and it was discovered that this fraction was dependent on the concentration of both lysozyme and liposomes as well as the inlet temperature and feed flow rate of the spray dryer.

The detected fine particle fraction varied between just a few percent for some of the powders without liposomes up to more than 50 % for one of the powders created using liposomes.

Other investigated parameters were the water activity (important for the stability of the powder), the liposome size distribution (to see how this affected the particles), the yield and the outlet temperature from the spray dryer.

Sammanfattning

Under detta examensarbete har det studerats hur man kan använda spraytorkning för att producera pulver som passar för att användas som inhalationsläkemedel samt huruvida man kan få förbättrade egenskaper på pulvret genom att lägga till liposomer. Pulvret bestod av lysozym som användes som modelläkemedel, laktos som användes som utfyllnads- och stabiliseringsmedel samt olika sorters fosfolipider i form av liposomer, skapade med hjälp av en ”ethanol injection method”.

Det upptäcktes att det var möjligt, och genom att variera inställningarna både på spraytorken och på ingrediensernas proportioner så kunde man påverka pulvrets egenskaper så att vissa av pulvren visade klara tecken på att vara lämpliga som inhalationsläkemedel.

Den parameter som bedömdes viktigast för pulvrets egenskaper var finpartikelfraktionen, vilket motsvarar den fraktion av pulvret vars partiklar är små nog att nå målområdet i lungan. Det visade sig att denna fraktion påverkades av koncentrationen av både lysozym och liposomer samt av torktemperaturen och matarflödet i spraytorken.

Den uppmätta finpartikelfraktionen varierade mellan allt ifrån några få procent för vissa pulver utan liposomer upp till mer än 50 % för ett av pulvren som innehöll liposomer.

Under arbetets gång mättes även vattenaktiviteten (den kan ha en stor påverkan på pulvrets hållbarhet), liposomstorleksfördelningen (för att se hur den påverkade partiklarna) samt utbytet och ut-temperaturen från spraytorken.

Popular Summary – Creating a fat powder for inhalation

By using tiny fat bubbles, is it possible to improve a protein powder intended for inhalation? This question was the basis of this thesis, and the answer seems to be in the positive.

So how do you get a protein medicine, such as insulin or a vaccine, into the body? The usual answer is to inject it, as it doesn't survive the stomach, but a recent trend is to try to breath it in instead. This would be an improvement for many reasons, as first of all no one likes needles, let alone having to use one on yourself several times a day. But for it to be breathed in, there are several problems. How does one make the protein become absorbed by the lungs and enter the blood? One answer is to make sure the medicine is in particles small enough to be breathed in to the very bottom of the lungs, where it can easily pass through the thin boundary between the air and the blood. These small particles are often called fine particles, and obviously it is better for more of the medicine to consist of these fine particles, but in reality many medicines only have half of the medicine in this size range.

To create such small particles special equipment is needed. One such device is the spray dryer, which is a device for turning a liquid into a fine powder. By tweaking the settings, it is possible to create a powder with particles small enough to reach the bottom of the lungs.

The powder we produced consisted of lysozyme, a protein, lactose, used mainly as something to take up space and make the powder cheaper, and different types of fat in the form of liposomes. The powers were created both with and without these liposomes to test what effect their presence had. It was tested for not only its size, but also for its water activity and the yield of the spray dryer that created the powder. Water activity is a term for the humidity of the powder, which is important as it affects how long the powder can be stored before it is destroyed.

An effect of most protein medicines is that when the protein has reached the blood, it quickly has its effect and is then quickly removed. This is sometimes great when you need a quick effect, but sometimes you want something with a lasting effect instead. For these occasions, one tactic is to have the protein kept inside of a tiny fat bubbles called a liposome. By having the protein inside of the liposome it takes a longer time for it to be released into the blood and in consequence makes it last longer. This effect was not studied in this project, but instead it was studied if the liposome had any other effect on the powder.

It was discovered that when you added liposomes to the powder, a lot of things happened. The powder became stickier and the yield became lower. On the other hand, the amount of fine particles became larger; in fact, it more than tripled for one of the powders with liposomes when compared with a powder created the same way but without the liposomes. All powders created with liposomes had more fine particles than any created without them, leading to the conclusion that the addition of liposomes were indeed a step forward.

This discovery, that there is not only a potential increase in effective time of the medicine in the body, but also a significant effect on the amount of fine particles in the powder could be used to improve the powders created for inhalation purposes after further studies.

Content

Introduction

There are several ways to deliver drugs to the body. One is the pulmonary delivery path, i.e. through the lungs. For many drugs, the lungs provide a safe and easy route and the administration can be easily managed by the patient. The most common use of pulmonary drugs is for local administration where the drug targets are situated in the lungs, such as most drugs used for asthma. Currently there is a growing interest in using the lungs for systemic drug delivery, i.e. delivery to drug targets outside of the lungs. Inhalation is becoming an increasingly researched delivery method for drugs, such as biologics (protein, or other natural products such as RNA, based drugs) that traditionally only could be delivered using i.v. or s.c. injections. (Kjems, et al., 2012) However, for systemic delivery the drug particle must be small enough for it to reach the parts of the lungs where they can be transferred to the blood stream. There are several manufacturing methods available to manufacture such particles, one of these being the spray dryer.

Spray drying is a method for transforming a liquid to a dry powder. The method is frequently used for production of everything from food and pharmaceutical productions to general chemicals and electronics. Within the pharmaceutical industry, it is for example used for biologics, as it is a mild technique that does not harm the heat-sensitive proteins as much as many other techniques. (Parikh, 2005)

One of the effects of using the pulmonary pathway is that it provides a fast onset action. While fast onset of action is an advantage in some cases, it is sometimes preferable to have a delayed release profile for less frequent drug administration. There are several ways to create such a delayed release inhalant, one of which is the use of liposomes. (Shahiwala, et al., 2005)

Liposomes are spherical lipid bilayers which can be used to encapsulate the active pharmaceutical ingredient (API) inside of them. An advantage of using liposomes is that they can incorporate both hydrophilic and hydrophobic particles. By encapsulating the API inside of the liposome, it takes an additional time for the API to enter the circulation of the patient, thereby creating the desired delayed release. Liposomes can also have an interesting effect on spray dried powder, since surface active chemicals such as lipids have a tendency of modifying the properties of the created particles. (Shahiwala, et al., 2005)

The idea of using these techniques in conjunction is not new. In a preliminary pharmacokinetic study, the protein Leuprolide was placed inside of liposomes and spray dried, the powder to be used for inhalation. This is similar to what was performed in this project, though the type of liposomes and protein is different, as are the manufacturing method of the liposomes and the excipients used. (Shahiwala, et al., 2005)

Aim

The aim of this project was to verify the feasibility of using a spray dryer to formulate a powder suitable for inhalation and to investigate possible positive effects of adding liposomes to the formulation. Another aim was to investigate how the variation of input parameters affected the performance of the created powder.

The properties investigated were the water activity, the aerodynamic size of the particles, as well as the yield and the outlet temperature of the spray drying process. The parameters varied were the spray dryer settings, concentration of lysozyme, the model drug, and lipids, and type of lipids used. The project was split into four phases named A, B, C and D.

A. The pre-testing phase when the settings to be used during the actual testing phases were determined. The only thing tested was the feasibility of using these techniques at later phases.
B. In phase B the settings for the spray dryer, when producing powder without lipids, were determined. The settings varied were the inlet temperature and feed flow rate of the spray dryer as well as the concentration of lysozyme. These powders were tested for water activity, particle size, yield and outlet temperature of the spray dryer.
C. In phase C the focus was on how to add liposomes to the process in order to establish how this affected the system. Here the settings varied were the type, concentration and manipulated liposome size of the lipids. These powders were tested for water activity, particle size and yield and outlet temperature of spray dryer as well as for liposome size.
D. During this phase some of the powders from phase C were reproduced for further analysis. The powders were first subjected to the same tests as in phase C and then to a more thorough particle size distribution testing.

Theory

Inhalation

Inhalation as a drug delivery method has a long history. The use of aromatic and medicinal vapors has been in use for hundreds, if not thousands, of years to treat lung conditions. The oldest known example of this medicinal use is the ancient Egyptians who treated asthma using incense created from frankincense (common as both a modern and traditional ingredient in incense and perfume) and grapes. Such use has been described in documents from as early as 3000 BC (Sheldon G. Cohen, 1992).

In modern times, the use of inhalation has become a commonly used drug delivery method to treat lung diseases such as asthma and Chronic Obstructive Pulmonary Disease (COPD). In more recent years the use of inhalations has also been developed for systemic treatments, as the large surface area and the avoidance of the metabolic pathways of the gastric tract are attractive for oral medications. Pulmonary administration is also a way of avoiding the risks and discomforts of s.c. or i.v. administrations. (Aulton, 2013)

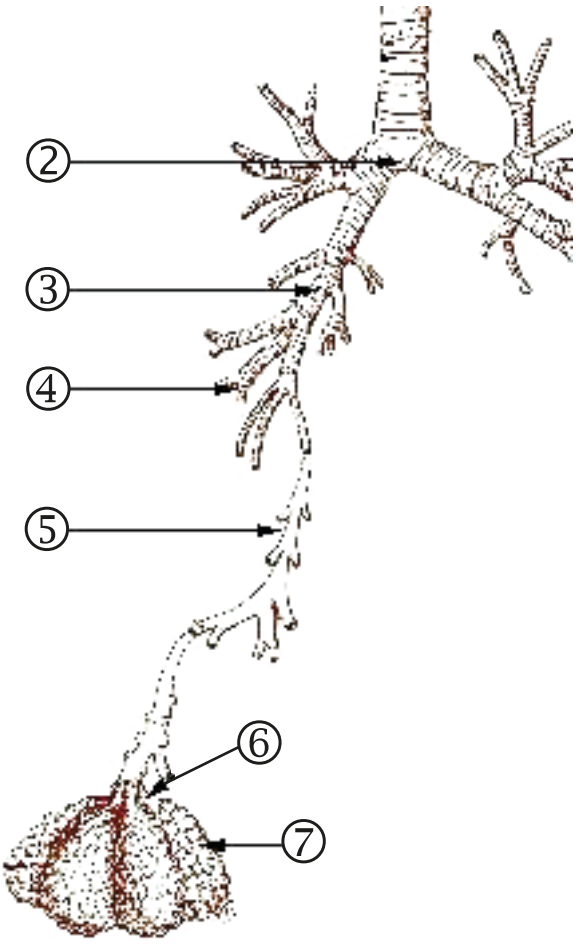

Figure 1. In this figure the general structure of the lung is demonstrated. The numbers 2 through 6 shows different parts of the lung where the air flow splits, causing smaller and smaller particles contained in the air to impact with the walls of the bronchi the further down in the lungs the split is. Number 7 points to an alveolar sack, where the air-blood exchange occurs. (Wikipedia Commons, 2006)

To understand the delivery of inhaled drugs, one must first understand how the target areas, the lungs, work. The lungs are organs which are primarily used to oxygenate the blood and rid the body of excess carbon dioxide. To achieve this, the part of the lungs that does the exchange between the blood and the air, the alveoli, have a very thin barrier to allow for the transport of the gases. This means that the alveoli are also very sensitive to damage, meaning the body has to have a way of protecting itself. The lungs solve this by having a branch-like structure of pipes called bronchi, where the

bronchi are divided further and further into smaller and smaller pathways. This is set up to allow the particles in the air to sediment in the bronchi and be transported by the cilia of the epithelial cells lining the conducting airways. The epithelial cells trap the particles in mucus and transport them upwards to the throat, where they are swallowed. After 23 of these junctions, the branches lead to the alveolar sacks, containing approximately 30 billion alveoli that, in total, have a surface area of up to 100 m^2 in adults. (Martini, et al., 2013)

To deliver a drug to the lungs, it must be administered as an aerosol. An aerosol is a suspension of either solid or liquid particles dispersed in a gas, usually air. To be stable, the particles have to be small enough not to fall out of the gas directly. The size of the particles is one of the most important parameters of an inhaled drug, as only very small particles can reach the alveoli without getting stuck on the way. To describe the size of the aerosol one often talks about the aerodynamic size of the particle, which is a way of standardizing the particle size by calculating the size of an ideal spherical particle, d_a, that has the same velocity as the particle in question. (Aulton, 2013) This can be done by using Equation 1, which assumes mostly spherical particles:

$$d_a = d_p \sqrt{(\frac{\rho}{\rho_0})} \tag{1}$$

In this equation, d_p is the physical size of the particles, ρ is the particle density and ρ_0 is the unit density 1 g/cm^3. To reach the deep lung region, the particles should have an aerodynamic size between 5 and 0.5 µm. As aerosols used in pharmaceutics are polydisperse and often assumed to have a log-normal distribution, one can insert the mass median diameter (MMD) into the equation to acquire the mass median aerodynamic diameter (MMAD), a parameter commonly used to compare sizes between pharmaceutical aerosols. (Aulton, 2013)

For local treatments of many diseases, such as asthma and COPD, the use of the strict size distribution described above might be unnecessary. The most common class of drugs against this type of disease, β_2 adrenergic antagonists, act on receptors found on the walls of bronchi and bronchiole (“small bronchi”, found lower down in the lung) as well as in the alveoli. This means that whether the drug is deposited at the very lowest part of the lungs or not is not of as much importance as the question of whether the drug deposits in the lung at all or not. (Barnes, 2004)

There are several advantages with using pulmonary drug delivery, some of which are:

- No first pass effect, as the drugs do not pass though the gastric tract. This allows for inhalation of drugs that would normally only be able to be administered by parental formulations.
- Fast onset of the drug if the target is the lungs.
- Use of smaller doses locally compared to systemic treatments, leads to lower drug costs and possibly also to a lower frequency of adverse drug reactions.
- Can be used for drugs with poor oral absorption.

- Large surface area, abundance of capillaries and thin air-blood barrier make it an excellent target for systemic treatments.

There are however several disadvantages with this administration route, such as:

- The delivered dose often depends on the breathing pattern of the patient, leading to non-uniform doses.
- Narrow particle size needed for efficient drug delivery, especially for systemic delivery.
- More complex delivery method than either oral or parental.

There are several types of devices used for inhalation, but the one used for these studies is called a Dry Powder Inhaler or DPI. In this device, the drug is formulated as a powder that is inhaled as an aerosol of fine particles using the patient's own lung capacity to transport the drug to the lungs. This device can either contain only a single dose or multi-dose and doses can be either preloaded or inserted manually. When inserted manually, the drug is often contained in a hard capsule. (Aulton, 2013)

Figure 2. In this figure, the Breezehaler device used in the experiment is shown. The yellow parts seen at the sides of the device can be depressed to cause two "needles" to be inserted to the cavity of the device, causing an inserted capsule to be pierced and the powder inside to accessible.

The inhalation device used in the current studies was a Breezhaler® device from Novartis (figure 2). In this device, a single hard gelatin capsule is inserted into the cavity seen in the opened device. The sides off the device are then depressed to pierce the capsule. After these preparations the device is inserted into the mouth and the patient inhales. When inhaling, the air enters through the openings seen in the right side of figure 2 and the spiral air flow caused by the geometry of the device causes the capsule to vibrate and release the powder into the inhaled air. This vibration can be heard clearly when the capsule has been properly pierced, with a sound similar to a rattlesnake. (Pascual, et al., 2015)

The performance of the formulation depends on more than the size of primary particles in a formulation. Another important parameter is the rheology of the powder. The rheology describes how well the powder flows, as well as how much it tends to aggregate (particles spontaneously stick together, requiring a force to de-aggregate). The deposition can also depend on the type of device used, as certain devices make it easier for some types of powder to reach further down in the lungs. The breathing pattern and characteristics also create a large variation between how many particles reach the target area. Even individual differences in airway geometry and inhalation technique between patients can cause differences in particle deposition, making it hard to predict the lung deposition using only measurements on the particle size distribution. (Aulton, 2013)

Spray Drying

The general mechanics of spray drying is that the solution is first atomized into droplets, then each droplet is evaporated and finally the dried powder is separated from the gas stream, as seen in figure 3.

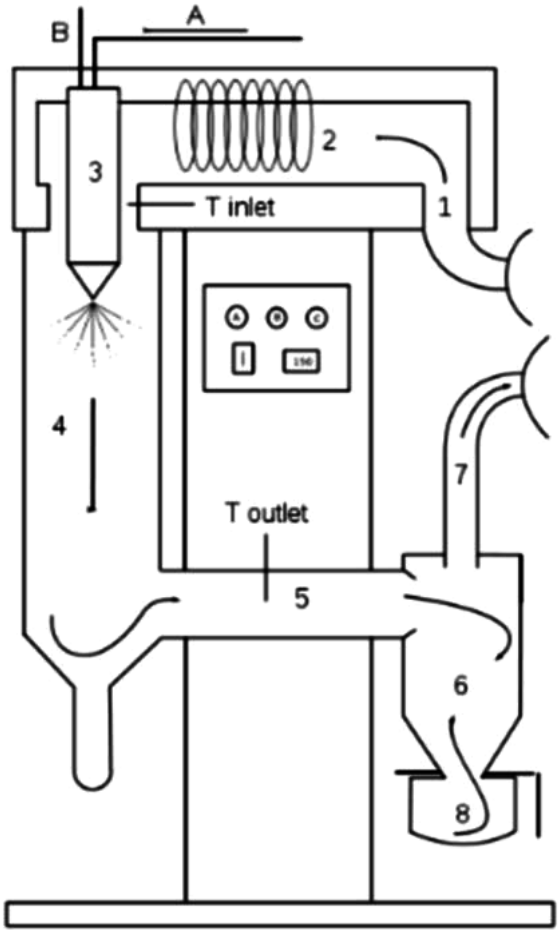

Figure 3. In this schematic figure the general principle of a spray dryer of the same type as the one used during this thesis can be seen. The numbered parts are: A: The inlet for the solution to be dried, B=The inlet for the atomization gas, 1: Inlet for the drying gas, 2: Heating of the drying gas, 3: The atomizer, 4: Drying chamber, 5: The connection between the drying chamber and cyclone, 6: The cyclone, 7: Outlet of drying gas, 8=Collection vessel of product. The arrows show the air flow and mean that this is a co-current spray dryer. (Pelkonen, 2009)

The atomizer

In the atomization stage of the spray drying process, the purpose is to separate the liquid into fine droplets. For optimal evaporation, the spray must have a large surface to volume ratio, allowing for a faster drying time. Optimally, all of the droplets should be the same size, but in reality a narrow size distribution is seen as a success. There are three main types of atomizers; Centrifugal Atomizers, 2-Fluid Nozzles and Pressure Nozzles. (Parikh, 2005)

Centrifugal Atomizer:

These are often in the form of a wheel that spins to disintegrate the liquid stream into droplets. By altering the atomizer speed, as well as the size of the equipped atomizer, spray characteristics can be altered. The droplet size distribution is narrow and the mean droplet size can be anything from 15 µm to 250 µm (larger mean sizes requires a larger drying chamber diameter. (Parikh, 2005)

Centrifugal atomizers typically have a speed of 5000-25000 rpm with a wheel diameter between 5 and 50 cm. The mean size of the droplet produced is inversely proportional to wheel speed and directly proportional to feed rate and viscosity of the feed solution. The porous structure of the produced powder makes it more soluble and the relatively low density and friability makes it easier to compress. The reproducibility and transferability between machines is high. (Parikh, 2005)

2-Fluid Nozzles:

This atomization technique is the most commonly used in the pharmaceutical industry as it is effective when using small spray dryers. The atomization is accomplished by the interaction of the feed solution and a second fluid, often compressed air. The liquid feed breaks up into a spray of fine droplets due to the high pressure of the second fluid, typically between 200-350 kPa. By varying the ratio of the compressed airflow to that of the liquid,

particle size can be controlled. As the liquid has a relatively low velocity exiting nozzle the drying chamber can be smaller than for other atomizers, caused by the shorter flight path of each droplet. This simple design allows for easy cleaning, a sterile operation and minimal contamination. As such, it can be designed to meet the strictest requirements for sterile or aseptic applications. (Parikh, 2005)

Pressure Nozzles:

The second most common form of atomization for pharmaceutical applications, the pressure nozzle, works by utilizing hydraulic pressure. The feed solution is pressurized by a pump and forced out of the nozzle orifice as a high-speed film. Because of the pressure, this film then disintegrates into fine droplets. As the feed rotates within the nozzle the film acquires a cone shaped pattern as it leaves the opening. Compared to 2-fluid nozzles, the solution exits the nozzle with a higher velocity, leading to the requirement of a far larger drying chamber. The drying chamber often has to be at least 2.5 m in diameter and 3.0 m in cylinder height to allow the droplets to dry. (Parikh, 2005)

Figure 4. This figure is a photograph of the spray dryer used dung the production of powders in this thesis, a Büchi Mini Spray Dryer 290. This type of spray dryer has a 2-fluid nozzle for atomization.

The droplet size, and thereby the particle size, is determined by the difference in pressure across the nozzle. The particle size is therefore usually more narrow compared to the other two other types of atomizers. The average size of the particles is directly proportional to feed rate and inversely proportional to pressure. Pressure nozzles are generally used to form coarse spray-dried particles of a few hundred microns (120 - 300 µm) with good flow properties, used e.g. in the production of antibiotics. (Parikh, 2005)

Spray-air contact and evaporation

The purpose of the drying chamber is to bring together the droplets with the heated drying gas, causing the liquid in the droplets to evaporate simultaneously over their entire droplet surface. By introducing the drying gas via an air dispenser, the gas is spread out to all parts of the chamber. Air entering the disperser, the device which introduces air into the drying chamber, must be well mixed as a temperature gradient across the duct leading to it, will cause the drying to become uneven. (Parikh, 2005)

The air flow can be either co-current, counter-current or mixed flow. A co-current flow means the spray and the drying air have same direction, which is ideal for heat-sensitive products. This is because the evaporation is fast, minimizing the risk of heat degradation. A

counter-current flow means the spray and the drying air have opposite directions. The advantage of this is that it allows for optimal heat utilization, being the most energy efficient of the air flows. This causes the driest particles to interact with the hottest air and thereby increasing the dryness of the powder. Logically, a mixed flow incorporates both above mentioned flows. This flow acts as a mix of both flows, and is most often used for coarse powders. The air flow design is selected depending on the desired particle size and the heat sensitivity of the dried particles. (Parikh, 2005)

The selection of drying chamber is based primarily on the type of atomizer used, as different atomizers require different volumes and shapes to allow the droplets to dry and not have enough momentum to impact with the drying chamber walls, but instead follow the air flow to the next part of the machine. (Parikh, 2005)

In many applications of spray drying, especially pharmaceutics, water is the most common solvent. Other common solvents are classical organic solvents such as ethanol and methanol. The reasons these solvents are used is partly that they are able to dissolve some substances that cannot be dissolved in water. They also have the additional advantage of having a short residence time, making evaporation much faster but creating the risk of explosions. This is why the common practice when using these solvents is to use an inert gas, such as nitrogen, as the drying gas. This also requires a closed system, where the solvent is recovered and the amount of gas used is limited. (Parikh, 2005)

Droplet Drying Mechanism

A drying curve for a droplet, as seen in figure 5, describes the change in drying rate as a function of time. This is affected by several factors, such as the temperature, humidity and the transport properties of the solvent in the droplet as well as in the drying gas surrounding it. The drying curve generally has three arbitrary parts: the initial, the constant drying rate and falling rate parts. The initial part starts directly when the droplet comes into contact with the drying gas and the drying rate increases as the droplets surface temperature increases. When equilibrium across the droplet-air interface has been reached, the drying becomes constant and one enters the second part of the drying curve, the constant drying rate. This is the highest rate of evaporation achieved for this droplet, and during this part the liquid inside of the droplet migrates to the surface, as the surface liquid is evaporated. When the migration starts to slow down, the drying enters the third part. In this part, the drying slows down until the now formed particle has achieved the same moisture as the surrounding drying gas, unless the particle is removed from the gas before this. This drying process is fast, and the entire process can be achieved in under 1.5 seconds. It is important to note that the drying process described here is only a theoretical representation, and many factors can influence this curve to change its appearance radically. (Parikh, 2005)

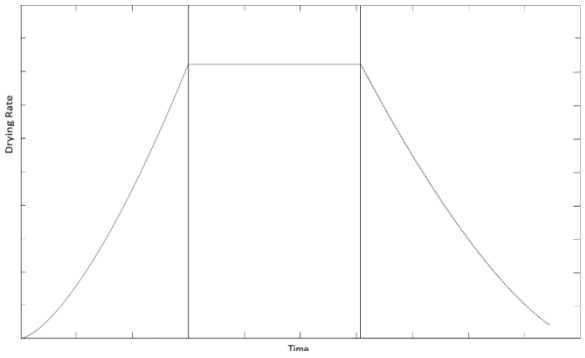

Figure 5. In this figure, the general shape of a drying curve is shown. The curve has been split into three parts corresponding to the three different drying parts: initial, constant drying rate and the falling drying rate part.

Separation of Particles

After the drying stage comes the final stage, where the powder is separated from the drying gas. In principal, there are two systems used to achieve this; primary separation and separation equipment. The primary separation takes place at the base of the drying equipment, which is often cone-shaped to facilitate this process. For industrial sized spray dryers, this is the main separation system, if not the only. The other separation equipment is placed after the drying chamber, and is most commonly a cyclone. The addition of a cyclone is most common in smaller spray dryers, where this is often the only used separation system. A cyclone separator, as can be seen in figure 6, rotates the airflow in a downward helical pattern until it reaches the bottom, where it then travels upwards towards the gas outlet tube. The large and dense particles have too much inertia to follow the air flow and impacts with the walls of the cyclone, whereby they fall to the bottom of the cyclone toward the collection jar. As the rotating air comes to the conical section, the turns become sharper and smaller and smaller particles are separated. The geometry of the cyclone, as well as the air speed, determines the size of the particles that are removed from the air stream. (Huard, 2010)

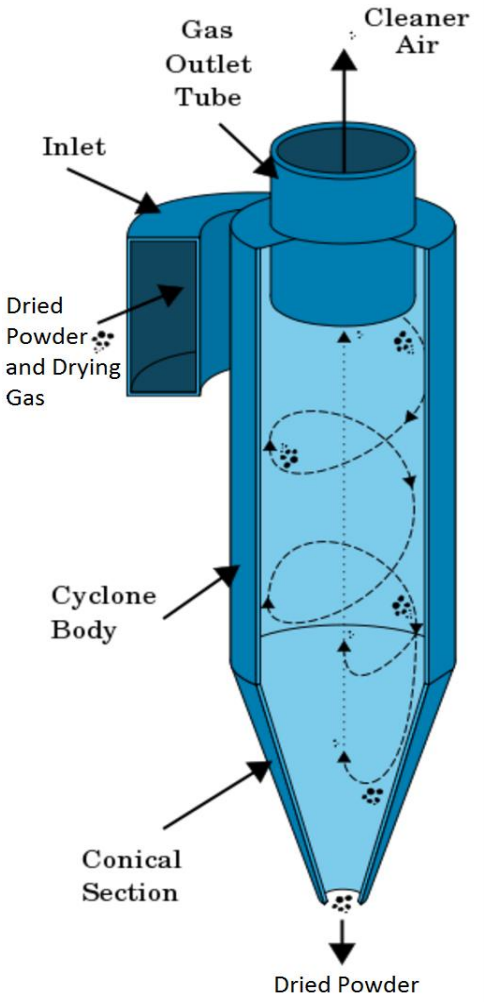

Figure 6. In this figure the general principle of a cyclone used in many spray dryers of laboratory scales. The different parts of the spray dryers have been labeled with their names. (CBurnett, 2007)

Main advantages and disadvantages of Spray Drying

As a manufacturing technique spray drying has several advantages, the main being that it is a continuous process. As long as supplies are fed, the spray dried powder continues to be manufactured, a process that can be continued for months without interruptions. Another commonly stated advantage is that it is easy to scale up from a small lab-scale production to pilot-plant scale production and further to a production plant production without the need to adjust many parameters, as opposed to many other micronization techniques where this is a common issue. Another advantage is the degree of control that can be achieved when it comes to determination of the physical properties of the particles produced, as long as much attention and the proper equipment is used. Because of the fast drying time, spray drying is ideal for heat sensitive products, and the absence of many moving parts makes it possible to eliminate the contact between the device and the produced powder. Even though a spray dryer is expensive to buy, it supplies a cost-efficient process that can be fully automated. (Parikh, 2005)

The main issue with the spray dryer is the narrow range of particle size that can be achieved (2-200 µm). In addition, utilization of the whole range is only possible if changes of the

atomizer are made, which may be impossible due to the pre-existing size of the drying chamber. Another issue is the poor thermal efficiency at low inlet temperatures. (Parikh, 2005)

Spray drying applications in pharmaceutics

Granulation

Granulation is a process that gathers small particles into larger permanent masses in which the original particles still can be identified. The use of granulates is common for tablets, where the rapid breakdown of granules is important to increase the available surface area and aid in solution of the active drug. (Aulton, 2013)

Granulates are often produced using spray drying, as the method is consistent in terms of density, particle size and compaction behavior. This means that spray drying is suitable for production of powdered lactose, cellulose and mannitol, as such excipients are usually directly compressed to produce the granules. In fact, spray dried lactose is the most common excipient to be prepared using a spray dryer. The granules produced using spray drying have several differences when compared with more traditional granulation techniques, causing some to reject that the term granulation should apply. (Parikh, 2005)

One additional advantage of using spray drying when granulating is that the powder is never in contact with any moving parts, as opposed to most other granulation methods. This means that the spray dryer is far easier to clean, making the entire process more user friendly. (Parikh, 2005)

Inhalation Dosage Forms

Inhalation requires the drug to be deposited in the lower airways for them to be clinically effective. Because of this, spray drying is a good manufacturing method for inhalants, as it can produce particles with a narrow size distribution at the right size range. This can be done at a single step instead of the several steps needed for other production methods. Another advantage of this manufacturing method is its “mildness” i.e. how little it tends to degrade the final products. The reason for this is that the particles are dried during a fast process where the core temperature of each particle only increases very little, and the entire drying process takes place in a matter of seconds. There is also little or no mechanical degradation of the ingredients during this process. (You, et al., 2007)

Liposomes

A liposome, as can be seen in figure 7, is a spherical vesicle with at least one lipid bilayer. A lipid bilayer is a two-layered configuration of lipids with the hydrophobic parts facing each other and the hydrophilic parts facing the outside or, in the case of a liposome, one side facing the outside and one side facing the inside of the vesicle. The bilayers are created by hydrophobic interactions which cause the hydrophobic tails of the lipids to be pushed away from the water, and the hydrophilic heads to be turned towards the aqueous solution and thereby creating bilayers.

Liposomes are created by forces acting on the naturally occurring lipid bilayers. These bilayers can be formed into liposomes with the addition of energy; sufficient concentrations of lipids are needed for the formation of liposomes. There are many techniques that can be used to create liposomes under more or less controlled circumstances, and each of these techniques has their pros and cons. (Aulton, 2013)

There are several types of liposomes, and they can be classified either by their size, their creation method or the number of bilayers in the vesicle. This classification may be confusing, but it is the standards used. When classifying liposomes based on number of bilayers, they are often split into single layered unilamellar vesicles (ULV) and multilayered multilamellar vesicles (MLV). When classifying them based on size, usually only the unilamellar vesicles are classified. The vesicles are classified as either small, medium or large unilamellar vesicles (SUV, MUV and LUV) with size ranges of 20-40 nm, 40-100 nm and 100-1000 nm respectively. When classifying them based on manufacturing method, they are called “manufacturing method” vesicle, e.g. ethanol injection vesicle (EIV) for vesicles created using the ethanol injection method. (Vemuri, et al., 1995)

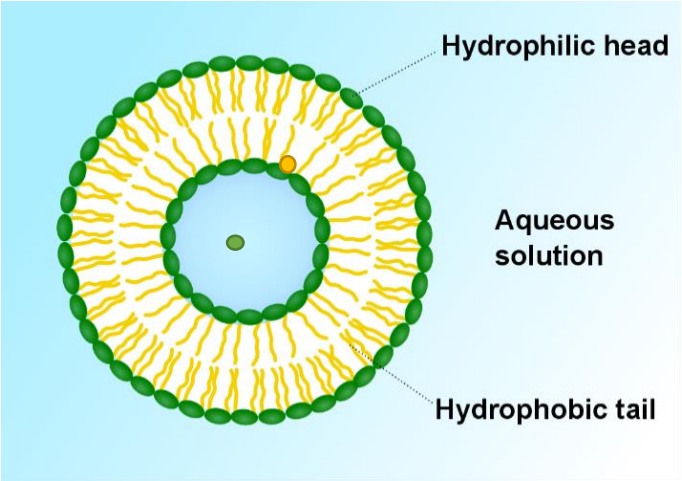

Figure 7. In this figure, the basic principle of a liposome is demonstrated. The two main parts of a lipid, the hydrophilic head and the hydrophobic tail, are clearly visible and the resulting configuration is the typical, if small, lipid bilayer configuration.
The two spheres seen in the figure, one orange and one green, are symbolic of how an API can be encapsulated in a liposome depending on if the lipid is hydrophobic (orange) or hydrophilic (green). (SuperManu, 2007)

The main use for liposomes in pharmaceutical formulations is to encapsulate APIs. The API can be encapsulated inside the liposome in two different ways, depending on the type of API to be encapsulated. If the API is hydrophilic, then it will be contained in the water on the inside of the liposome. If the API instead is hydrophobic it will be contained inside of the lipid bilayer, surrounded by the hydrophobic tails of the lipids, as seen in figure 7. (Aulton, 2013)

Liposome encapsulation can have several advantages, e.g.:

- It can protect the API from hostile environments such as the gastrointestinal tract.
- It can create a delayed release profile, as the API has to pass through the liposomes before reaching the target.
- By attaching targeting molecules such as antibodies to the surface of the liposome, it can become a targeted medication that only releases the API at the target location instead of systemically.
- It can be used to increase the permittivity of the API, e.g. when the liposome is absorbed into another cell, the API is released into the cell instead of outside it. (Aulton, 2013)

The degree of encapsulation varies depending on several characteristics, such as the manufacturing method and the type of API to be capsuled. (Aulton, 2013)

To manufacture the liposomes used for the studies the ethanol injection method was used. In this method, the lipid is first mixed with pure ethanol. This mix is then put into a syringe and an aqueous solvent is prepared. The ethanol solution is then injected into the solution, where the lipids are dispersed evenly and form liposomes. (Justo, et al., 2011)

One of the main advantages of this technique is how easy it is to use without extra equipment, the only thing needed is a syringe. Another advantage is that the process has no degenerative effects on the phospholipids, and the size distribution is reasonable. The major shortcoming is that it is limited by the amount of lipids soluble in ethanol and the amount of ethanol that is stable in the aqueous solution, leading to a rather dilute liposome suspension. This in turn means that the encapsulation rate is low if the API is solved in the aqueous solution. (Justo, et al., 2011)

The size, type and stability of the liposomes created by this method depend on several parameters. These are the temperatures of the solutions, the amount of API in the aqueous solution, the amount of liposomes in the ethanol solution, the amount of ethanol solution added to the aqueous solution, the mixing speed of the aqueous solution, the speed of injection, the type of lipid and the pH of the solutions. (Justo, et al., 2011)

There is some confusion of what happens to liposomes when spray dried. According to some studies (Goldbach, et al., 1993)the liposomes remain intact and the resulting powder is composed of dried liposomes. Other says that the liposomes are, at least in part, destroyed by the forces of the atomizer and the surface active lipids which form the liposomes can migrate to the edges of the particles. (Heurtault, et al., 2003) As this was not examined during this study, the use of the term liposome will be used to describe the detected liposomes in the solutions and the term lipids or phospholipids will be used to describe the possible liposomes in powder form.

Analytical Methods Used

Impactor Theory

An impactor is a common way to measure the aerodynamic size distribution of a pharmaceutical powder. This is a device which functions by using similar mechanisms as the lungs, where air streams are split up into ever finer jets with sharp turns where particles impact and later can be removed. In the case of an impactor, there are specific parts in the apparatus that can be removed and the amount of powder collected there analyzed, allowing for a chance to measure a size distribution or a size cut-off. At each stage where particles are collected, there is a cut-off diameter that can be calculated or drawn from a table found in the European or American Pharmacopeia. All particles smaller than this size pass through this stage, while particles bigger than the cut-off impact there. (Kristensen, 2004)

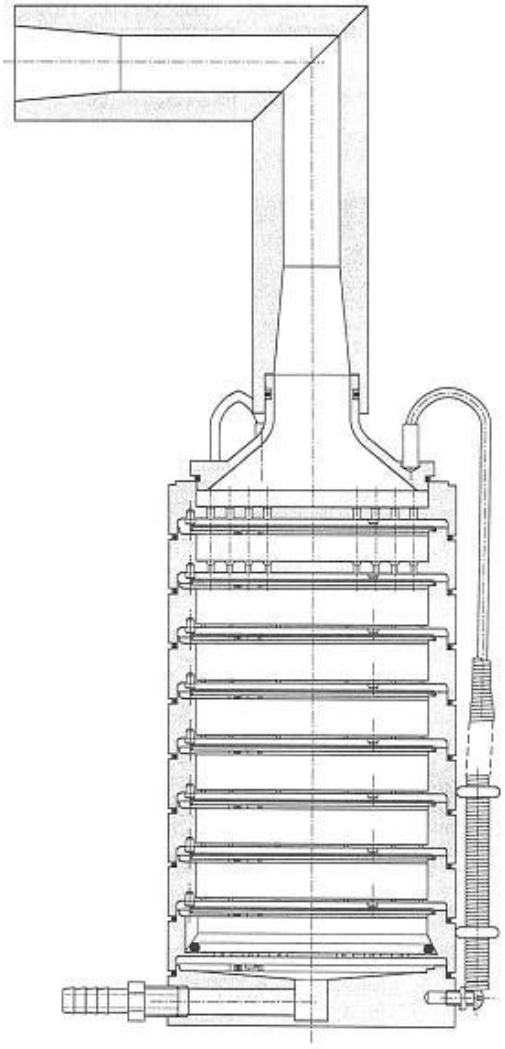

Figure 8. This figure shows the general schematic of an Andersen impactor. The inhaler device is inserted into the top of the impactor, and the extruding part at the bottom is where a vacuum source is attached. (Kristensen, 2004)

To ensure reproducibility, the aerosol is drawn through the device at a known flow rate that can be set to different standards. The most common flow rate for DPIs is a flow rate that generates a pressure drop of 4 kPa over the inhaler, but others such as 2 and 6 kPa are also used in product characterization, as specified by the Pharmacopeia. (Kristensen, 2004)

For the Breezehaler® used in this thesis, a different approach was used. As the low air resistance in the device limits the maximum pressure drop to around 3 kPa, which corresponds to ~90 liter/minute, the maximum inhalation speed of patients using this type of device. As the usual inhaled air speed for patients is approximately 40-90 liter/minute, these air speeds were chosen.

To create this air flow, a vacuum source is needed, typically vacuum pump or another vacuum source. Between the vacuum source and the impactor, a flow regulating valve plus an on/off value is positioned, as specified in the Pharmacopeia. (Kristensen, 2004)

The inhalation device is inserted into the induction port or “throat” of the device at the top, and a vacuum source is connected to the bottom to act as the simulated breath. The stages

in the device are perforated and the holes in the stages are smaller the further down in the device they are to be placed. This means larger particles are deposited higher up in the device, while smaller travel further down before their momentum is too high for them to avoid impacting on one of the collection plates. To ensure that no particles that impact on the collection plates bounce off, the collection plates are coated with a sticky coating. In the final stage a filter is placed, to remove the last of the fine particles so that the vacuum source is not contaminated. (Kristensen, 2004) Two types of impactors were used in this project; the Andersen impactor and the Next Generation Impactor.

In the Andersen impactor, seen in figure 8, there are 8 stages where the air stream is split up and flows through a sharp turn around a collection plate. These collection plates can be removed after the experiment and the powder stuck on them can be measured to create a size distribution. (Kristensen, 2004)

Another type of impactor is the Next Generation Impactor (NGI), which can be seen in figure 9. This impactor functions by the same principles as the Andersen impactor, but there are a few structural differences. The geometry is changed so all stages are placed at the same height and are accessible at the same time to make repeated experiments easier. The collection plates are replaced with collection cups that can be removed all at the same time and cleaned easier. It is not uncommon to add a pre-separator to the NGI, which functions as an additional first stage where the largest particles are removed. This pre-separator has the same principles as the rest of the impactor, but the cut-off of the particles is higher than stage 1 and also the collection capacity is far larger. (Kristensen, 2004)

Figure 9. In this figure one can see two next generation impactors. In the right hand part if the figure, the opened NGI demonstrates the collection cups placed in a tray that can easily be extracted from the machine. In the left hand part of the figure a closed NGI is seen, including a pre-separator placed in between the mouthpiece of the impactor and the first collection cup. In both parts of the figure, the small extruding part situated at the bottom left part of the device is where the vacuum source is attached. (Svensson, 2013)

Water Activity Meter

Water is abundant in most biological compounds and play a large role both for the stability and the adhesive properties of powders. A high amount of moisture can cause structural changes to the powder, degradation of protein and increase the microbial growth in the powder. Because of this, it is necessary to determine the amount of water in a sample.

However, not all water in a sample is equal. In most biological systems, such as foods or protein-based pharmaceutics, the water in the system can be said to exist with either hindered or unhindered mobility, also called bound or unbound water, respectively. The bound water is hard to define, but it is often thought of as being much harder bound to the substrate than the unbound water, which acts as normal bulk-water. The changed characteristics of the bound water are e.g. higher vapor pressure, higher binding energy, reduced mobility and unavailability as a solvent. This means that when discussing the stability of the product, the most important type of water is the unbound water, which has a far higher effect on the powder than the bound water. (Al-Muthaseb, et al., 2002)

One way of measuring only the unbound water is to measure the water activity, which is a type of partial water pressure of the substance to be analyzed compared to water:

$$a_w = {}^{p}/_{p_0} = \frac{relative\ humidity}{100} \tag{2}$$

In this equation p is the partial pressure of the substance, p_0 is the pressure of pure water and a_w is the water activity at the same temperature. The value given is a number between 1 and 0, where 0 means there is no free water in the analyzed substance and 1 means the substance is pure water, i.e. only free water. (Al-Muthaseb, et al., 2002)

There are different techniques used to measure the water activity of samples, but the one used by the equipment used during this project is the chilled-mirror dewpoint technique. In this technique the measurement is based on a mirror placed above the sample in the sealed interior of the machine and the means of detecting condensation on the mirror. The mirror is cooled gradually until the dew point is reached, which is the temperature where condensation first starts to form on the mirror. At this temperature the relative humidity of the air in the sealed interior is the same as the water activity. (Decagon Devices, Inc, 2007)

Ultraviolet and Visual Spectroscopy

UV spectroscopy works by having a light source pass through a sample while measuring the light intensity before and after. The decrease at a specific wavelength is recorded as the absorbance at that wavelength. Different substances absorb light at different wavelengths and one use of this technique is to use it to identify a substance using the particular signature absorbance spectrum of this substance. Another use of this technique is the determination of concentration of a substance in a solution. To do this, one typically determines a wavelength for which the substance has a peaked and distinct absorbance and uses only this wavelength to determine the concentration. One such wavelength is 280 nm, which is often used when analyzing the concentration of proteins. (Pavia, et al., 2001)

To determine the concentration of a compound using UV spectroscopy, the first step is to establish how the absorbance varies depending on concentration of the sample to be analyzed. This is typically done by creating a calibration curve by way of a dilution series. This means creating a link between the measured absorbance and a known concentration of the sample to be analyzed. This link is called a conversion factor. After this the absorbance

of your unknown sample and multiply the absorbance with the conversion factor to acquire the concentration. (Pavia, et al., 2001)

One challenge with spectroscopy is that factors other than the sample can affect the recorded absorbance. Air bubbles in the sample can e.g. increase the absorbance at all wavelengths at the same time. Another challenge is that addition of particles, such as liposomes, in the solution can absorb light based not only on particle volume, but also on the size of each particle. (Pavia, et al., 2001)

One way to partly counteract the effects of air bubbles is to make an additional measurement at a different wavelength of light that does not interact with the sample to be measured. If the sample has an increased signal at this wavelength, then it must interact with something other than the sample. If one assumes this is caused by air bubbles then the increase is the same at all wavelengths and by subtracting it from the measured signal from the sample this effect can be removed. The easiest way to counteract the disturbance from liposomes is to destroy these liposomes. (Pavia, et al., 2001)

Laser Diffraction Particle Size Determination

The use of laser diffraction as a tool to determine the size of particles is common in several fields, but it is especially common in the pharmaceutical industry. The method works by having the particles pass through the light of a single or several lasers while measuring the angle of reflection. The particles can be suspended in either air or a solution. Large particles have a low reflection angle, while smaller particles have a higher angle. (Sperazza, et al., 2004)

The reflection angles can be used to calculate the size of particles using two different types of calculations, Frauhofer and Mie. The Frauhofer technique was the first to be used with this type of equipment, and it requires no additional input data. A disadvantage with this technique is that is becomes more inaccurate the smaller the analyzed particles are. (Sperazza, et al., 2004)

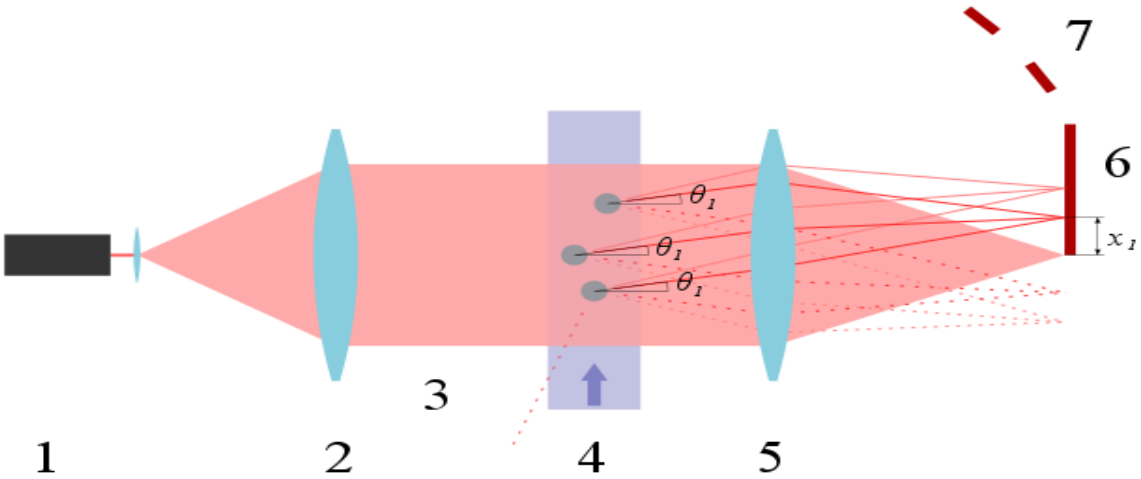

Figure 10. In this figure, the general principle of a laser diffraction particle size determination device is shown. 1: The laser, 2 and 5: Optical lenses, 3: The spread laser light which impacts with the particles, 4: The medium transporting particles, 6: The photo detector; Θ: The angle at which the laser light is diffracted by the particles, symbolized by small green blobs. (Sunspeanzler, 2014)

The other technique is called Mie. This technique usually gives more reliable results, but they require the additional input of the particles refractive index (RI) and absorption (Abs). These parameters can be acquired either from the literature, experimentally or they can be estimated using the term “weighted residual” supplied by the analyzer. This term represents how well the recorded data fits the calculated size distribution. When this term is large (more than 1) the RI and Abs are usually wrong. By changing these terms, it is possible to create new calculated size distributions from the same experimental data, and this changes the weighted residual. By repeatedly changing these terms, the weighted residual can be minimized and a more reliable size distribution can be acquired. (Sperazza, et al., 2004)

Another possible explanation for a high residual is that the received signal was too low and the influence of random noise was too large. This cannot be compensated for after the measurements are made. The only alternative is to either accept the results or make a new measurement using more of the sample. (Sperazza, et al., 2004)

The advantage of using laser diffraction as a tool to determine the size of particles is that it can make accurate particle size distributions over a large size range. Disadvantages with the method include that it requires the knowledge of some of the particle parameters to make an accurate calculation and that the equipment only measures the size indirectly, meaning artifacts from the calculations can become a problem. (Sperazza, et al., 2004)

Methods and Materials

Materials

The chemicals used during the experimental phase of this master thesis were:

- Lysozyme, 85 % (CAS no 9001-63-2)
- α-Lactose monohydrate (CAS no 5989-81-1)
- SPRINK spray grease
- Sodium Dodecyl Sulphate (SDS, CAS no 151-21-3)
- Ethanol, 99 % (CAS no. 64-17-5)
- Brij 35 (CAS no 9002-92-0)
- Glycerol, 85 % (CAS no 56-81-5)
- Mix of phospholipids from a natural source
- Two single types of phospholipid, synthetic
- Distilled water

The chemicals given at the start of the project to work with was the lysozyme, water and the two single types of phospholipids. The other chemicals were selected over the course of the experimentation as they were deemed necessary.

The first chemical to be added was lactose, which is a common filler and carrier material for inhalation powders. Lactose was added since the amount of dry matter in the solution to be spray dried was too small and the resulting powder would be too cost-inefficient to produce.

The Sprink was added as a simple and efficient coating to use for the collection plates for the Andersen impactor. The reason this was acceptable was the fact that the collection plates were not to be used for the analysis of the FPF, but instead the filter paper was to be used. This meant that the only criterion of the coating was that it had to be sticky enough to prevent the bouncing off of particles that impacted with the collection plates.

The third chemical to be added was the mix of phospholipids, which was added as a cheap alternative to the single types of phospholipids. This mix was added as it had a similar melting point and properties to one of the single types of phospholipids.

The SDS was added as a way to dissolve the liposomes when the lysozyme content were to be analyzed using a spectrometer. The SDS cause the liposomes to break apart by first forming a monolayer around the liposomes. It then starts to be incorporated into the liposomes in a matter of minutes, where it increases the permeability and cause the liposomes to break apart into much smaller micelles, which no longer give any signal in a spectrometer. (Deo, et al., 2003)

When it was decided that the liposome production was to be done using the Ethanol Injection method, the use of ethanol was self-explanatory.

The Brij 35 and the glycerol was a mix used as a standard coating for the collection plates when using the Next Generation Impactor. The mix consisted of 3 % Brij 35, 81 % glycerol and 16 % ethanol.

Methods

Naming conventions for the produced powders

The powders produced during the experiments have an id consisting of two letters and one number, such as A-X3. The rules for how the powders were named and the reasons for these rules are found below. Figure 11 shows the principle for how the powders were named. When only a part of the name of a powder is used in this thesis, it is meant as a group. This means that if, e.g. powders A-X was mentioned then that means all the powders A-X1, A-X2, A-X3 and A-X4.

- The first letter of the name is the same as the phase during which the powders were produced.
- The second letter is based on which of the parameters that were changed.
    - For powders produced in phase A, -P means that they were early prototypes only used to verify if the proposed manufacturing technique was suitable. For the powders found in table 2, the change in the second letter corresponds to a change in the lysozyme concentration, with -X, -Y and -Z corresponding to 0.5 %, 1 % and 0.75 % respectively.
    - For powders produced in phase B (table 3), the same conventions as those in table 2 apply.
    - For powders produced in phase C (table 4), the second letter corresponds to whether the sample had any after-treatment or not. -X did not have this treatment while -Y had.
    - For powders produced in phase D (table 5) only one letter, -X, is used.
    - The reason for using the second letter this way is to make it easier to see similarities and differences in what was at the start believed to be the largest factor to determine these powders behaviors. Two powders with identical first letter and number are the same in every respect except the parameter mentioned above.
- The number 1 through 4 is used to differentiate between the powders. The principle is that number 1 and 3 are paired (share one input parameter), as well as the numbers 1 and 2, 2 and 4 as well as 3 and 4. For example, in series B, 1 and 2 have the same inlet temperature, while 1 and 3 have the same feed flow rate. The exception to this rule are the powders A-Z and B-Z, which all are copies of each other created as a means of controlling reproducibility.

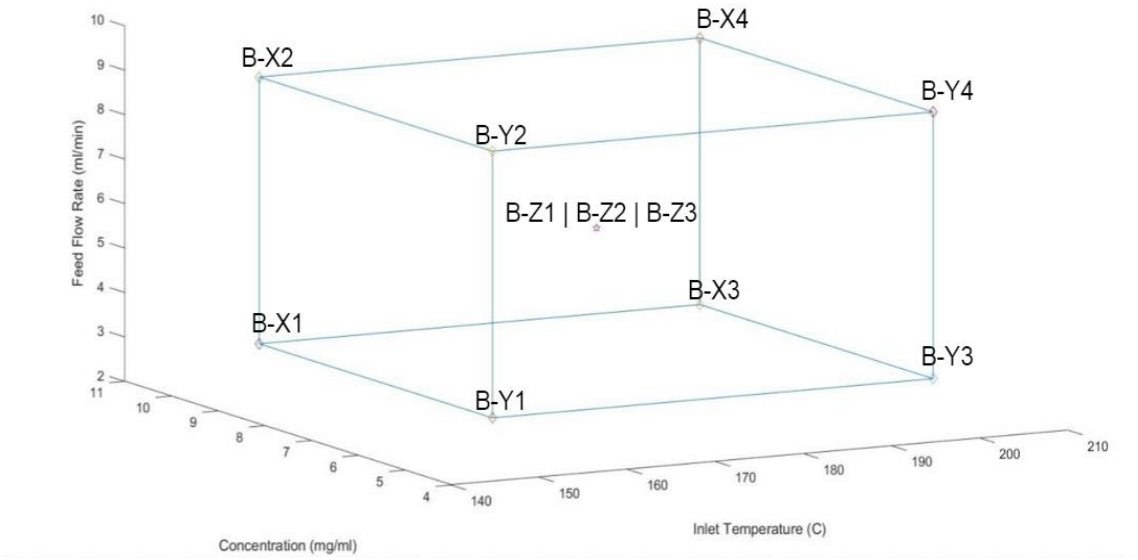

Figure 11. Powders produced in phase B are plotted in a 3D plot based on their input parameters. Here it is possible to see the pattern described above, where each powder occupies a corner in a cube, with the exception of the three center points.

Manufacturing methods

Spray drying

The spray drying was done using a Büchi Mini Spray Dryer 290 with a 2-fluid nozzle and a cyclone as a separator. The following procedure was used:

1. The solution to be spray dried was prepared. This was done by weighing the chemicals according to tables 1-5 found below and adding them to a magnetically stirred container. For production of powder containing lipids, see Ethanol Injection Method below.
2. A second container containing distilled water was prepared and placed by the spray dryer.
3. The collection bin was weighed and then inserted into the spray dryer.
4. The spray dryer was turned on, and the inlet temperature was set according to table 1 through 5 below. The aspirator rate was set to 100 %. The feed tube was blocked so no water could pass through the system prematurely.
5. When the inlet temperature was reached, the desirable feed flow was turned on according to table 1, 2 or 3, using only the distilled water and the airflow was set to 30 mm.
6. When the system had stabilized and the outlet temperature no longer fluctuated, the feed was switched to using the sample. This was continued until all of the sample solution was used up. The outlet temperature was noted.
7. After switching to the pure solvent for a short while, the aspirator, as well as the inlet temperature and feed flow controls were turned off.
8. The collection bin was removed when it had cooled off sufficiently to be handled comfortably.
9. Finally, the filled collection bin and a sealable container were weighed. The powder was transferred from the collection bin to the sealable container using a paint brush

and a spoon. The sealable container was then weighed and marked with its sample name.

The following settings were used for the different experiments.

Table 1. In this table the first spray dried powders are displayed. For each process, 100 g of each solution was produced and spray dried.
**The reason for the temperature interval is that it was first started at 100 degrees, but the water that was used to stabilize the system did not dry enough and moisture appeared in the collection bin. This continued until when the temperature was increased to 120 degrees, but stopped when further increased to 150 degrees and when the outlet temperature had stabilized the powder was produced.*

Sample Name	Feed Flow Rate (ml/min)	Inlet Temperature (C)	Concentration Lysozyme (% w/w)	Concentration Lactose (% w/w)
A-P1	9	200	1	0
A-P2	6	150	1	4
A-P3	6	100-150*	0.75	4
A-P4	3	175	0.75	4
A-P5	6	175	0.75	4

Table 2. In this table are the settings for the first full factorial production of powders, found in phase A. For each powder, 100 g of each solution was produced and spray dried. In this production, the amount of lactose was kept constant at 4 % (w/w).

Sample Name	Feed Flow Rate (ml/min)	Inlet Temperature (C)	Concentration Lysozyme (% w/w)
A-X1	3	150	0.5
A-X2	9	150	0.5
A-X3	3	200	0.5
A-X4	9	200	0.5
A-Y1	3	150	1
A-Y2	9	150	1
A-Y3	3	200	1
A-Y4	9	200	1
A-Z1	6	175	0.75
A-Z2	6	175	0.75
A-Z3	6	175	0.75

Table 3. In this table are the settings for the powders produced in phase B. For each powder, 100 g of each solution was produced and spray dried. As in table 1, the concentration of lactose was 4 % (w/w).

Sample Name	Feed Flow Rate (ml/min)	Inlet Temperature (C)	Concentration Lysozyme (% w/w)
B-X1	3	150	0.5
B-X2	9	150	0.5
B-X3	3	200	0.5
B-X4	9	200	0.5
B-Y1	3	150	1
B-Y2	9	150	1
B-Y3	3	200	1
B-Y4	9	200	1
B-Z1	6	175	0.75
B-Z2	6	175	0.75
B-Z3	6	175	0.75

Table 4. In this table are the settings for the production of powders in phase C. For each powder, 120 g of each solution was produced and 100 g was spray dried.
In this production the settings are based on the powder B-X3. This means the inlet temperature is 200, the feed flow rate is 3 and the concentration of lysozyme is 1 %. The amount of lactose was varied to keep the amount of dry matter in the solution constant.

Sample Name	Type of Lipid	Concentration of Lipids (% w/w)	Concentration of Ethanol (% w/w)	After-treatment	Concentration of Lactose (% w/w)
C-X1	Mix	0.2	2.8	Yes	3.8
C-X2	Single	0.2	2.8	Yes	3.8
C-X3	Mix	0.4	2.6	Yes	3.6
C-X4	Single	0.4	2.6	Yes	3.6
C-Y1	Mix	0.2	2.8	No	3.8
C-Y2	Single	0.2	2.8	No	3.8
C-Y3	Mix	0.4	2.6	No	3.6
C-Y4	Single	0.4	2.6	No	3.6

Table 5. In this table are the settings for the production of powders in phase D. For each powder, 200 g of each solution was produced and 180 g was spray dried.
The powders D-X3 and D-X4 are direct copies of the powders C-X3 and C-X4. The powder D-X2 is a copy of the powder C-X2, but the amount of lysozyme was halved. No other changes were made.

Sample Name	Type of Lipid	Concentration of Lipids (% w/w)	Concentration of Ethanol (% w/w)	Concentration Lysozyme (% w/w)
D-X2	Single	0.2	2.8	0.5
D-X3	Mix	0.4	2.6	1
D-X4	Single - New	0.4	2.6	1

Preparation of liposomes

In this project, the liposomes were produced using the ethanol injection method, and the following procedure was followed:

1. The lipids and ethanol were weighed and put into a small sealable container. This container was mixed using a vortex mixer until no lipids could be seen, which took approx. 60 seconds.
2. The water and the lysozyme was weighed and mixed together using a magnetic stirrer set at 400 rpm until the no powder was visible.
3. The ethanol solution was placed inside of a disposable plastic syringe. It was then injected into the water solution.
4. The lactose was weighed and added to the solution.
5. For the solutions that had after-treatments (C-Y1 though C-Y4), the solution was then kept on the magnetic stirrer for 10 minutes with a magnetic stirring speed of 1000 rpm.
6. 20 g of the solution was removed for particle size determination; the remaining solution (100 or 180 g) was transferred to the spray dryer.

During pre-testing, the parameters described above were chosen based both on trial and error as well as based in literature. (Pons, et al., 1993) (Justo, et al., 2011). The additional after-treatment was a product of trial and error based on one of the pre-tests, where the

two solutions were produced with the only difference being that one of the solutions had been after-treated. Another discovery done during the pre-testing of this method was the fact that when using the two single type phospholipids, one of them was unable to dissolve after step 3 above. The problem remained despite decreasing the amount of lipid; therefore, this type of lipid was not further used. All pre-tests were performed as the list above describes, but only 20 g of the solution was produced, since it was not to be spray dried.

Analytical methods

Impaction methods

When doing impaction tests using the Andersen impactor, the following was done:

1. Between 20 and 30 mg of powder was inserted into hard gel capsules. For the pre-tests, one capsule was filled for each powder, while for the following tests five capsules were filled for each powder. The capsules were weighed both empty and full.
2. The impactor was assembled and a filter paper was inserted after the third stage to create a cut-off at 4.66 µm. The collection plates placed above this were coated with Sprink.
3. The pump was turned on and the air flow was tested using a manometer and was adjusted until it was 60 l/min.
4. A capsule was inserted and then pierced by the Breezhaler inhalation device.
5. The Breezhaler was inserted into the throat of the device and the trigbox was set to produce two puffs of 5 seconds each. The capsule is removed and for the tests after the pre-test, the next capsule is inserted into the Breezhaler and the process is repeated until all capsules containing the same type of powder have been used. Because of reasons explained during the pre-test section, the impaction test during phase A was achieved using a single capsule per sample while later experiments were performed using five capsules and an average was used to eliminate statistical fluctuations.
6. The filter paper was removed and placed in a beaker containing 30.0 g of either a 2 % SDS-solution for powders containing lipids or distilled water for powders without lipids.
7. The used capsules were weighed to examine how much powder remained.
8. The filter papers were allowed to rest for at least 1 or 2 hours (1 for the powder without lipids and 2 for the powder with), after which they were analyzed using the UV-Vis spectrophotometer.

For the Next Generation Impactor, the procedure was as follows:

1. The collection cups were assembled and coated with 1.5 ml of the Brij 35 coating using a Kleenex paper towel.
2. Between 20 and 30 mg of powder was inserted into each hard gel capsule, five capsules for each type of powder. The capsules were weighed both empty and full.

3. The NGI was closed and the mouth piece was inserted. A manometer was used to confirm that the correct air flow was achieved.
4. A capsule was inserted and pierced by the Breezhaler, and the trigbox was activated to provide two five seconds long puffs needed to empty the capsules. This procedure was repeated until all capsules using this powder were used.
5. After this the NGI was opened and 15 ml of a 2 % SDS solution was put into each collection cup, as well as in the mouthpiece. The tray with all of the collection cups was removed and the device was ready for the next powder.
6. The used capsules were weighed to examine how much powder remained.
7. The collection cups were allowed to rest for at least 20 minutes before they were analyzed using a UV-Vis spectrophotometer.

To calculate the FPF, the following equation was used for both impactors:

$$Amount = \frac{Absorbance * K * V * C_{Lysozyme}}{Load} \quad (3)$$

Where K is the calculated conversion factor, V is the volume of the solution that the collected powder has been dissolved in (30 ml for the Andersen impactor and 15 ml for the NGI), $C_{Lysozyme}$ is the concentration of lysozyme in the powder and $Load$ is the amount of powder loaded into the capsule(s). For the Andersen impactor, this $Amount$ was the same as the FPF, as the entire FPF was collected on the filter paper. For the NGI, the same equation was used for each collection cup and the FPF was then calculated using a program supplied by Emmace Consulting AB. This program calculated the FPF based on the collected amount of each cup with a cut-off smaller than the determined size limit of the particles (4.46 µm).

Water Activity Testing

When testing the water activity of the powders, the powder was put into a cup and inserted into the water activity meter (Aqualab series 3TE). After 15 to 30 minutes the results were displayed by the water activity meter. The powder was removed from the cup and put back into its container. This measurement was performed during all phases for all powders, with the exception of powders A-P.

UV-Vis Spectrophotometry

After the impaction runs, the samples were analyzed using a spectrometer either a Varian Cary 50, or a Hewlett Packard 8453, and the Varian was used using the following procedure:

A cuvette was filled with distilled water and measured at 280 nm to zero the equipment. Each sample was mixed in the container to ensure the powder had dissolved. The cuvette was filled with the solution to be analyzed, and four measurements were made. The highest and the lowest absorbance were saved, and the cuvette was emptied, cleaned and filled again with the same solution. Four more measurements were made from this sample, with the highest and lowest values extracted again. After this the cuvette was cleaned and ready for a new sample.

For the Hewlett Packard 8453, which was stationed at Emmace Consulting, the procedure was slightly different:
The cuvette was filled with water and zeroed for both 280 and 500 nm. After this the cuvette was emptied and filled with the solution to be analyzed. The absorbance was measured at both 280 nm and 500 nm and it was measured only once. The cuvette was then cleaned and ready for the next solution.

The calibration curve needed to calculate the conversion factor was acquired by creating a solution using the same recipe as one of the solutions to be spray dried. The amount of absorbance of this solution was measured. The solution was thereafter diluted and for every new concentration the measurement was repeated. The dilution process was done by removing half of the solution to be diluted and transferring it to a new container, where the same amount of solvent as solution was added, creating a solution with half the concentration of protein as the previous solution in the dilution series. For these dilution series, 30 g of the original solution was created, and 15 g was then removed to create the next stage of the dilution series.

The concentrations of protein used during the creation of the calibration curve were (in % w/w): 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024.
The solution was diluted either with distilled water (for phase A and B) or an aqueous solution containing 2 % SDS (for phase C and D).

When creating the calibration curve using the spectrometer at Emmace, instead of using a combination of ingredients with the same recipe as one of the solutions to be spray dried, the powder D-X4 was used and diluted to create the calibration curve. After measuring the absorbance of this dilution series, a small amount of Brij 35 solution was added to some of the solutions, to verify if this solution created any increased absorbance values.

When using the conversion factor to calculate the amount of protein in a sample, the conversion factor used was an average of all previously calculated conversion factors using this spectrometer, as different spectrometers give slightly different absorbance values.

Laser Diffraction Particle Size Analyzer
The particle size was measured as follows:

1. The dispenser unit was filled with distilled water and the stirrer was set to 1200 rpm.
2. After this the program used was started and the machine measured the water to remove background noise.
3. Thereafter the sample was added to the dispenser unit until either the obscuration reached approx. 7 % or the sample was used up. Type of substance was selected and the measurement was started.
4. After a minute the Particle Size Analyzer displayed the particle size distribution.
5. If this was the first test on this type of liposome, the residual is controlled, and if it is too large the absorbance and refractive index is modified to decrease the residual.

Results and Discussion of the Different Phases

Pre-Testing – Phase A

Determination of usable Spray Drying Temperatures

As the spray dryer did not sufficiently dry the pure solvent at temperatures lower than 150 °C, as seen when producing powder A-P3 (table 1), this temperature was decided to be the lowest spray drying temperature. The highest temperature used, 200 °C, was decided on as this had been demonstrated to work earlier in A-P1, as well as in literature. (Maltesen, et al., 2008) (Porras-Saavedra, et al., 2015) (BÜCHI Labortechnik AG, 2002)

Testing of Ethanol Injection Method

During the experiments with the ethanol injection method, the after-treatment (extra high speed stirring for 10 minutes) was tested and decided upon. The basis for this decision was the results found in figure 12, showing the size distribution of two identical samples where only one of the samples had received the after-treatment. The resulting change of size distribution is clearly visible, and this was deemed sufficient to make this the standard techniques to reduce the size of the liposomes in half of the samples in phase C.

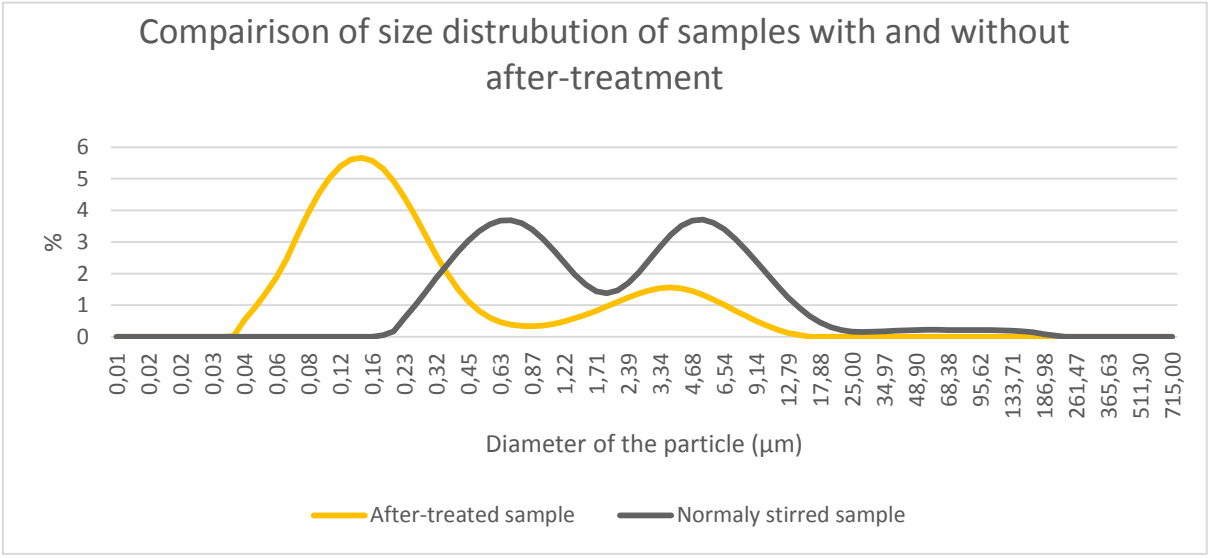

Figure 12. In this figure, the size distribution of two samples with the same experimental parameters except that one of the samples had been after-treated, i.e. had additional stirring. The two samples contained: 1 % lysozyme, 3.7 % lactose, 0.3 % mixed phospholipids, 2.7 % ethanol and 92.3 % water (w/w for all percentages).

Testing of Dissolution of powder containing liposomes with SDS

During testing of ethanol injection, a part of the experiment was to see if the same analysis method, i.e. the UV-Vis spectrometer, that was used to measure the amount of protein in the sample of samples without liposomes worked to analyze samples containing liposomes. Early tests made it clear that this was not possible without some modifications, as the

results acquired were more than a factor two higher than expected. This led to the idea that the liposomes had to be dissolved in order to use the spectrometer. When the SDS was added to the same solution, the results acquired were as expected. This was further verified when a new calibration curve was produced based on a solution created using the ethanol injection method and adding SDS as the diluting solution.

Determination of time needed to dissolve the powder.

After all of the impactor tests, the collected powder had to be dissolved. The time this required can be split into three parts; the powder without lipids, the powder containing lipids tested in the Andersen impactor and the powder with lipids tested in the NGI. For the first category, the time one hour was chosen arbitrarily, but as the measured concentration was not effected when the same sample was analyzed an hour later, it was determined that the powder was fully dissolved.

For the powder containing lipids, it was clear that it was slower to dissolve as the measured concentration increased after an hour of dissolution. As this no longer occurred after two hours, it was determined that at least this much time was needed.

For the NGI tests, the absorbance was measured every 5 minutes for the first sample, and it increased until after 15 minutes where it stabilized. The reason this was so much faster is likely that it is much faster to dissolve a powder laying at the bottom of a metal cup than to remove it from a filter paper.

Calibration curves for the UV-Vis.

To measure the concentration of protein in the samples inserted into the spectrometer, a conversion factor used to transform the absorbance into a concentration was established, as described in the methods section. These calibration curves were created repeatedly to acquire a reliable conversion factor, and to verify if the use of SDS and liposomes changed this conversion factor, which it did not. The conversion factor was calculated by plotting the absorbance and the known concentrations and then calculating the trend line using the Matlab command polyfit. The conversion factor was the slope of the curve, and the conversion factor used to calculate the results was an average of all earlier used calibration curves, see figure 13.

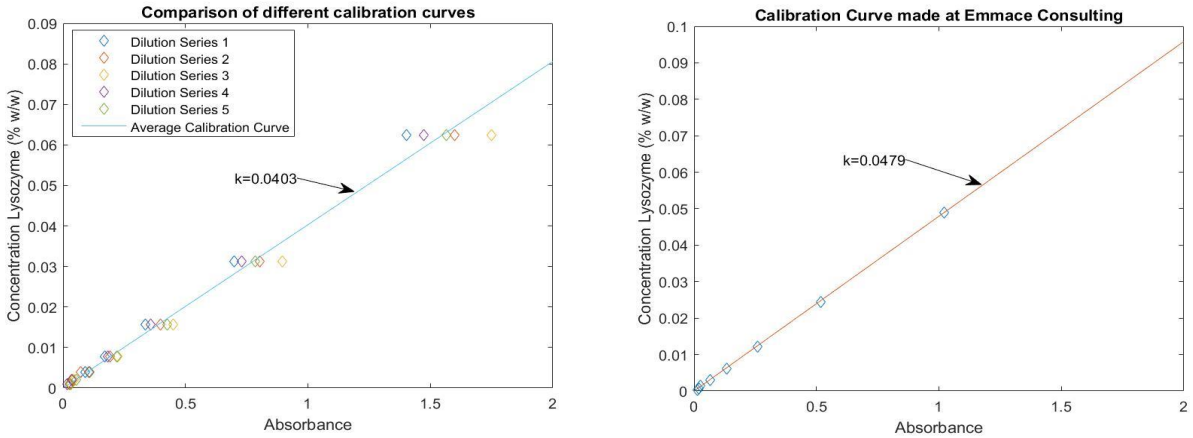

Figure 13. In this figure, the two calibration curves used during the calculations of results from the impactor experiments are shown.

When switching from one spectrometer to another, it is always prudent to create a new calibration curve, as there are often differences between the different measuring equipment's given absorbance values. The new calibration curve was used to create a new conversion factor, and it was indeed different from the earlier conversion factor. The two conversion factors used during the analysis were 0.0403 and 0.0479 [Concentration Lysozyme (% w/w)/Absorbance]. This difference can come from several different avenues, such as the difference between the equipment, the cuvettes or something else entirely.

Testing if the Brij-35 effected the absorbance values

During the measurements of the calibration curve at Emmace, the effects of the Brij solution was tested. This was done by adding 1.5 ml of the Brij solution to various samples in the dilution series. These samples were then retested to see if any measurable differences could be seen. No change in absorbance values was measurable when Brij solution was added. This led to the assumption that the Brij solution had a negligible effect on the absorbance values of the solution.

First Testing of Impactor

When the A-X, A-Y and A-Z powders had been produced, they were tested using the Andersen impactor. During these tests, a single capsule was used for each powder. The resulting fine particle fraction (FPF) can be seen in table 6 below. In table a large variation between the FPF determined during different experiments can be seen. This discrepancy is now believed to be caused by a combination of uneven loading of the capsules and contaminations that entered the system. As there is only a small amount of sample in each experiment, the addition of even a small amount of contamination can have a staggering effect on the resulting FPF. Due to the large variation between experiments, the laboratory practices for the experiments were changed to using 5 capsules per powder instead of using only one, and an increased attention was paid to contaminants. Because a limited amount of powder was left, it was decided that the manufacturing process of creating A-X, A-Y and A-Z was to be repeated.

Table 6. In this table, the impactor results from phase A are shown. Here the large variations between consecutive experiments can be seen clearly.

Sample name	First Experiment (%)	Second Experiment (%)	Third Experiment (%)	RSD (%)
A-X1	22.87	23.09	11.64	34.10
A-X2	15.85	35.47	8.68	69.35
A-X3	18.97	29.12	9.65	50.59
A-X4	16.88	10.94	7.04	42.64
A-Y1	23.18	30.86	37.83	23.93
A-Y2	27.03	62.22	35.41	44.24
A-Y3	24.27	20.76	11.05	36.62
A-Y4	25.19	47.54	10.84	66.39
A-Z1	9.82	31.89	12.24	67.32
A-Z2	6.33	19.77	12.80	51.84
A-Z3	14.45	19.35	11.51	26.22

Water Activity Testing, Outlet Temperature and Yield of Spray Dryer

The reason for the powder deficiency that caused the need for recreating the powders was that due to an error; the powder that had been used for testing the water activity, a non-destructive experiment, had been discarded instead of being reclaimed. The resulting water activity can be seen in table 7 where the outlet temperature and yield from the spray drying is also shown. In this table, one can see that all water activity was between 0.20 and 0.32.

The reason for the abnormally low yield of sample A-Y2 was that the powder was stuck to the bottom of the collection jar, which made the removal of the powder to the storage container difficult.

Table 7. In this table the results from the water activity and spray drying parameters of samples A-X, A-Y and A-Z can be seen.

Sample name	Outlet Temperature (°C)	Yield (%)	Water Activity
A-X1	103	51.02	0.205
A-X2	85	50.81	0.282
A-X3	137	27.91	0.234
A-X4	121	53.88	0.249
A-Y1	103	44.78	0.233
A-Y2	78	16.76	0.312
A-Y3	127	41.85	0.231
A-Y4	124	52.45	0.279
A-Z1	115	44.35	0.248
A-Z2	109	39.72	0.274
A-Z3	103	46.53	0.253

Lysozyme Based Powder – Phase B

Aim

The aim of this phase was to reproduce the powders A-X, A-Y and A-Z and repeat the impactor test using five capsules instead of one as well as pay stricter attention to avoiding contamination during the experiments. The powders were also tested for water activity and were photographed in a microscope for additional information.

Results

The results from the impaction experiments can be seen in tables 8 and 9, where the results from the individual impaction experiments are displayed in table 8. Table 9 contains the water activity and the FPF, as well as the outlet temperature and yield from the spray dryer.

Table 8. In this table the resulting FPF found during the impaction experiments performed during phase B are found. The three results in red are measurements where there is suspected contamination, as they are significantly more varied than the other results. For these three samples (B-X2, B-Y1 and B-Z2) two averages and RSDs were calculated; the first value includes all three measurements, and the other value excludes the likely contaminated measurement.

Sample name	Experiment 1 (% FPF)	Experiment 2 (% FPF)	Experiment 3 (% FPF)	Average FPF (%)	RSD (%)
B-X1	6.55	5.74	6.25	6.18	6.58
B-X2	3.90	3.50	6.56	4.66 (3.70)	35.74 (7.70)
B-X3	13.20	15.26	13.23	13.90	8.51
B-X4	5.15	4.45	4.47	4.69	8.48
B-Y1	8.27	6.01	5.70	6.66 (5.86)	21.04 (3.80)
B-Y2	5.04	6.87	5.75	5.88	15.65
B-Y3	9.06	7.82	8.18	8.35	7.68
B-Y4	6.85	5.40	5.22	5.82	15.31
B-Z1	4.97	4.21	3.92	4.37	12.35
B-Z2	15.14	4.99	6.59	8.91 (5.79)	61.30 (19.50)
B-Z3	5.18	4.54	5.37	5.03	8.69

Table 9. In this table the results from the water activity and spray dryer measurements in phase B are found.

Sample name	Outlet temperature (C)	Yield (%)	Water activity
B-X1	107	42.55	0.181
B-X2	79	54.72	0.195
B-X3	135	23.37	0.135
B-X4	121	52.80	0.163
B-Y1	102	40.70	0.124
B-Y2	88	40.07	0.164
B-Y3	138	41.75	0.214
B-Y4	104	47.72	0.178
B-Z1	102	51.60	0.137
B-Z2	111	54.87	0.133
B-Z3	118	57.57	0.153

Discussion

As can be seen, the increase of capsules used as well as a more stringent attention to decontamination had a large effect on the FPF, both decreasing the differences between each sample as well as decreasing the measured values significantly. As can also be seen in table 8, there were still some outliers seen during the impactor testing (samples B-X2, B-Y1 and B-Z2), in which contamination issues were suspected. Other than this the overall trend was toward more consistent results.

The water activity was lower for all powders during this phase than they were in phase A. The cause for this was believed to be the lower air humidity of the spray drying in phase B than in phase A. No numbers were acquired for this air humidity, but as it was a wet December (raining most of the time) when phase A was produced and a dry January

(temperature below freezing) when phase B results were produced the likelihood of there being a difference of air humidity is high.

Liposome and Lysozyme Based Powder – Phase C

Aim

The aim of this phase was to look at how the addition of liposomes affected the characteristics of the produced powder. It was decided that three parameters were to be varied; the type of phospholipid, the amount of phospholipids and if the sample had received any after-treatment. As the sample B-X3 had the highest FPF in phase B, it was decided that all samples in phase C were to be based on this sample, which meant the use of:
200 C inlet temperature, 3 ml/min feed flow rate and 1 % (w/w) lysozyme.

Results

The results from the impaction experiments can be seen in tables 10 and 11, where the results from the individual impaction experiments are displayed in table 10. Table 11 contains the water activity as well as the outlet temperature and yield from the spray dryer and the particle size, obscuration and weighted residual from the particle sizer. Figure 14 contains 4 graphs that show the size distribution of the liposome size of the samples.

Table 10. In this table, resulting FPF calculated from the impaction experiments can be seen. Experiment 2 on C-Y2 is marked red since it is suspected that this sample was contaminated. As in table 8, there are two calculated averages and RSD; one value including and one excluding the suspected value.

Sample name	Experiment 1 FPF (%)	Experiment 2 FPF (%)	Experiment 3 FPF (%)	Average FPF (%)	RSD (%)
C-X1	28.35	31.16	27.46	28.99	6.65
C-X2	29.22	30.93	31.81	30.65	4.30
C-X3	35.26	35.72	35.74	35.57	0.76
C-X4	26.87	26.00	30.19	27.68	7.99
C-Y1	28.25	29.96	29.38	29.19	2.98
C-Y2	29.92	47.61	27.78	35.10 (28.85)	31.00 (5.24)
C-Y3	34.44	36.89	33.82	35.05	4.63
C-Y4	26.67	30.24	30.80	29.24	7.66

Table 11. In this table, the results from the particle sizer as well as the water activity and spray dryer measurements can be seen.

Sample Name	Surface weighted mean size (µm)	Obscuration (%)	Weighted residual (%)	Water activity	Yield (%)	Outlet temperature (C)
C-X1	0.13	5.44	1.55	0.250	55.45	128
C-X2	0.12	2.28	1.74	0.165	63.64	135
C-X3	1.11	7.78	0.43	0.161	47.46	133
C-X4	12.72	7.83	0.61	0.179	48.77	133
C-Y1	0.14	4.32	1.17	0.187	62.82	134
C-Y2	0.33	2.77	0.87	0.189	61.56	135
C-Y3	1.21	6.47	0.51	0.211	50.2	134
C-Y4	11.69	8.01	0.88	0.180	52.4	138

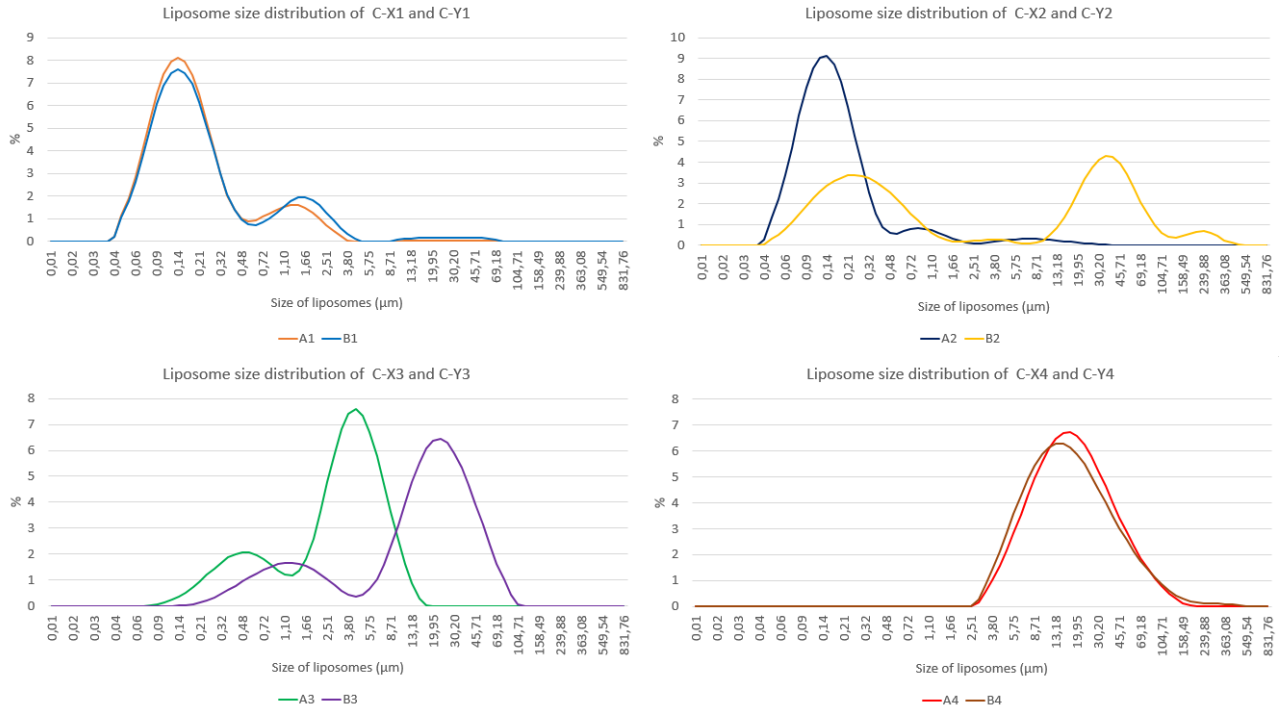

Figure 14. In this figure the size distributions of particles in the liposome-solutions can be seen. As seen in these graphs, the effect of the after-treatment was not the one intended, though it sometimes had an effect. The unexpected part of this is that the effect was different for different samples, but for two of the samples pairs (C-X4/C-Y4 and C-X1/C-Y1) the effect was negligible. For C-X3/C-Y3 the effect was that the size distribution was moved to the right, increasing the size with a factor of 10. For C-X2/C-Y2 the effect was a complete shift of size distribution, from an untreated single peak with its highest point at around 0.15 µm to the treated double peak with its highest points at 0.25 µm and 40 µm.

Discussion

The FPF of the samples were effected positively by the addition of lipids to the powder, as all powders displayed a higher FPF than any produced during phase B. Despite this, the variation of parameters had a surprisingly small effect on the FPF of the samples, with the exception of samples C-X/Y-3 which had an average of 6 percentage points higher FPF than the other powders produced during this phase ($p < 0.0001$).

The effect on liposome size of the after-treatment was sporadic and unreliable, causing a size change to only half of the samples. In the samples that were effected, one them (C-X/Y3) had size shift of the entire distribution, while the other (C-X/Y2) had another type of distribution entirely. But even though this after treatment had such a chaotic effect on the liposome sizes the impact on powder properties were negligible. This means this is a very stable system, and raises the question of what happens to the liposomes after they have been spray dried, which will be discussed further in the general discussion.

The low obscuration and high residual of C-X2 occurred when the entire sample removed from the spray dryer for this use (~20 g) was added to the particle sizer. Despite this, the obscuration was still only at 2.28, so this low obscuration was used.

Final Powders – Phase D

Aim

For this phase, the aim was to select a small number of powders and do a more thorough examination of their aerodynamic particle size distribution using an NGI. The obvious selection was the powder which was the most distinct, and best, of the powders produced in phase C in terms of FPF, C-X3/C-Y3. The second powder selected to copy was C-X4/C-Y4, as it was a solution that had both a different type of lipid as well as the highest liposome size, making it an interesting powder to take a closer look at. The third powder to be selected was based on the parallel research done by the former collaborator Hung Nguyen, who had seen an increased FPF when the amount of lysozyme in the samples containing lipids was decreased. Because of this, the third powder to be produced was a modified variant of sample C-X2 with a halved amount of lysozyme (0.5 % instead of 1 %). These powders were to be created and first brought through the tests previously performed during phase C, as well as a further examination performed using the NGI. To simplify comparisons between the results from the NGI and the Andersen impactor, the resulting FPF from the NGI will be based on the same cut-off size as the Andersen impactor (less than 4.46 μm).

Results

The results from the NGI can be seen in table 12. It contains the FPF based on both the amount of powder loaded into the capsules (called load or L) and the amount of powder that was detected in the NGI (called delivered dose or DD). This table also contains the relative standard deviation between the duplicates performed, which was used as a basis to select if further retesting was needed. The calculated averages of the FPF as well as the calculated MMAD can be seen in figure 15. Table 13 contains the FPF from the Andersen

impactor as well as water activity, outlet temperature and yield from the spray dryer and the particle size, obscuration and weighted residual from the particle sizer. Figure 16 shows the particle size distribution before spray drying. Figure 17 shows the normalized particle size distributions acquired from the NGI. Figure 18 is a photograph of the three powders.

Table 12. In this table the calculated results from the NGI experiments (n=n2) can be seen. In three of the tests (the second experiments for D-X4 at 44 l/min, D-X3 at 44 l/min and D-X2 at 90 l/min) the results from test 1 and 2 were inconsistent and when a third experiment was performed, the results from the second experiments (shown in red above) were dismissed and a new relative standard deviation was calculated.

Sample name and flow rate (l/min)	FPF/DD Experiment 1 (%)	FPF/DD Experiment 2 (%)	FPF/DD Experiment 3 (%)	RSD (%)	FPF/L Experiment 1 (%)	FPF/L Experiment 2 (%)	FPF/L Experiment 2 (%)	RSD (%)
D-X4 at 44	17,58	21,72	16,80	10,99 (2,22)	15,05	19,50	13,60	15,65 (5,07)
D-X4 at 60	18,14	18,09		0,13	15,95	16,00		0,15
D-X4 at 90	22,80	21,88		2,07	21,07	20,93		0,33
D-X3 at 44	52,34	52,16	52,76	0,48 (0,40)	32,98	24,55	35,22	14,86 (3,28)
D-X3 at 60	48,00	51,88		3,88	37,53	42,57		6,29
D-X3 at 90	38,18	41,64		4,34	35,05	30,53		6,89
D-X2 at 44	14,38	14,73		1,23	13,43	14,45		3,65
D-X2 at 60	17,26	17,89		1,80	17,68	17,72		0,12
D-X2 at 90	18,80	22,22	17,71	9,37 (2,91)	17,23	25,70	17,90	18,97 (1,92)

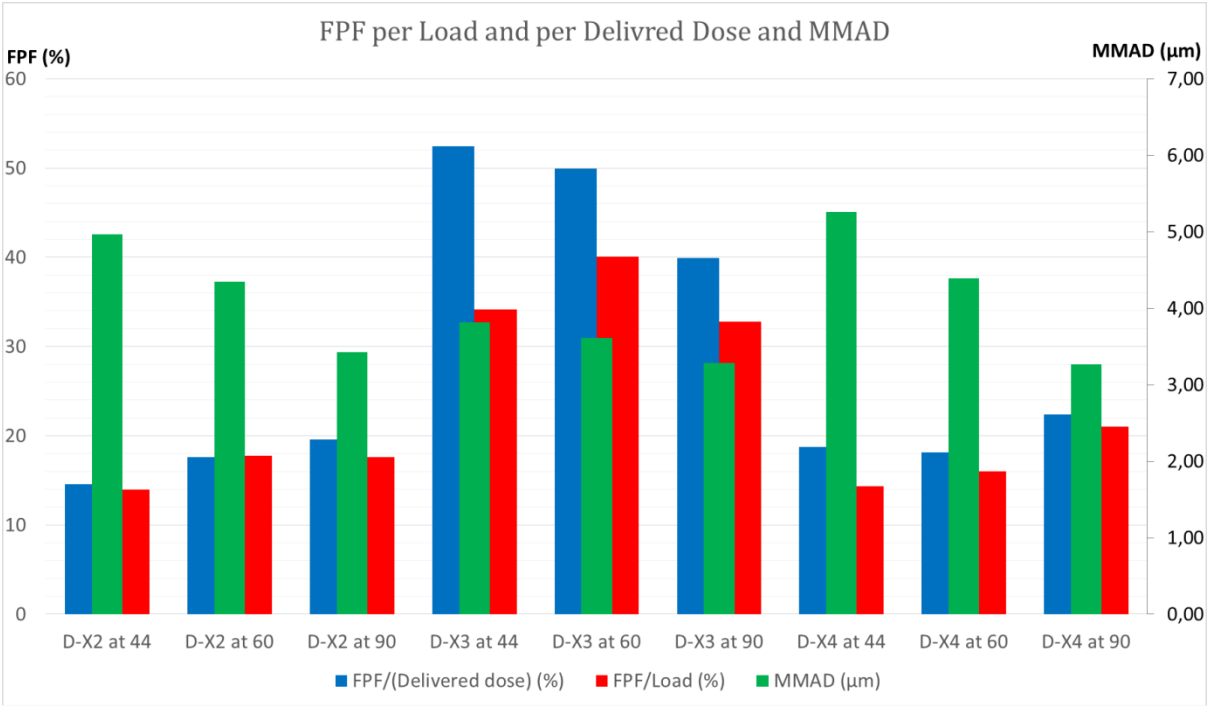

Figure 155. In this figure FPF per Load and per Delivered Dose as well as the MMAD of the powders from phase D are shown. The y-axis to the left shows the FPF (blue and red columns) and the y-axis to the right shows the MMAD (green column).

Table 13. In this table the FPF acquired using the Andersen impactor, the liposome size, water activity and the output parameters from the spray dryer, are shown. As expected, the FPF of D-X3 and D-X4 are highly similar to the results from C-X3 and C-X4. The sample D-X2 showed more than 50 % increase in FPF when compared to the closest previously produced powder (C-X2 had an FPF of 30.65). As one can see for D-X2, the obscuration is lower than usual, even though the full amount of sample put aside for this particle sizing experiment was used. This may well have caused the introduction of random noise into the measurement. One can also see that the yield of both X2 and X3 are lower than any the powders they are based on. It was found that during the spray drying of D-X2 the powder stuck to the bottom of the collection bin.

Experiments:	**D-X2**	**D-X3**	**D-X4**
Average FPF from the Andersen Impactor (%)	46.80	29.40	23.94
Surface weighted mean size (µm)	0.19	2.09	32.33
Obscuration (%)	1.13	7.26	7.9
Weighted residual (%)	1.302	0.708	0.385
Water activity	0.403	0.253	0.324
Yield (%)	30.86	29.95	54.34
Outlet temperature (C)	105	125	114

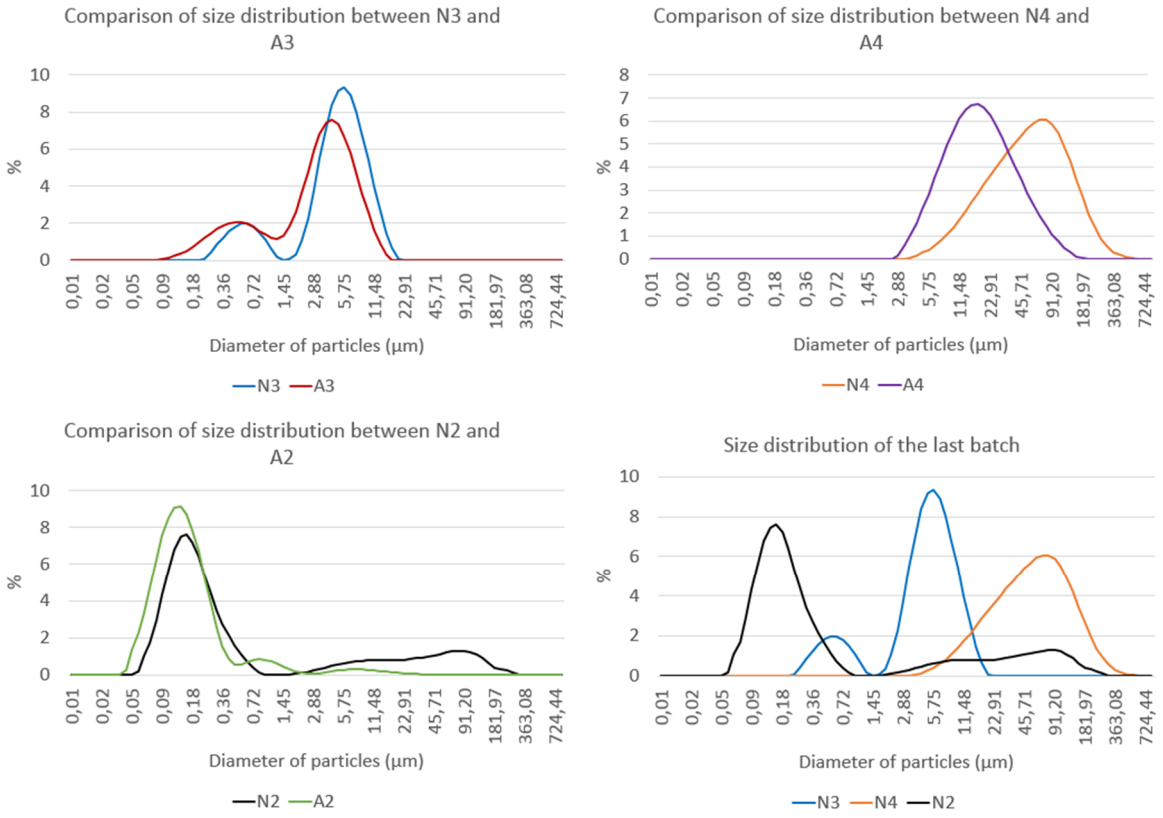

Figure 16. In this figure the resulting particle size distribution of the samples before they were spray dried are shown. As one can see, they all share the general shape with their predecessors, but there are differences. In D-X2 there is an extra population of particles that have a size range at 2-200 µm, though the validity of this measured size is in question when one considers the low obscuration and high weighted residual. In D-X3 the shape is generally the same but the populations have been narrowed and the average shape has been increased.
In D-X4 the size distribution is the same type as in C-X3, but the distribution has been widened and the average size has been increased.

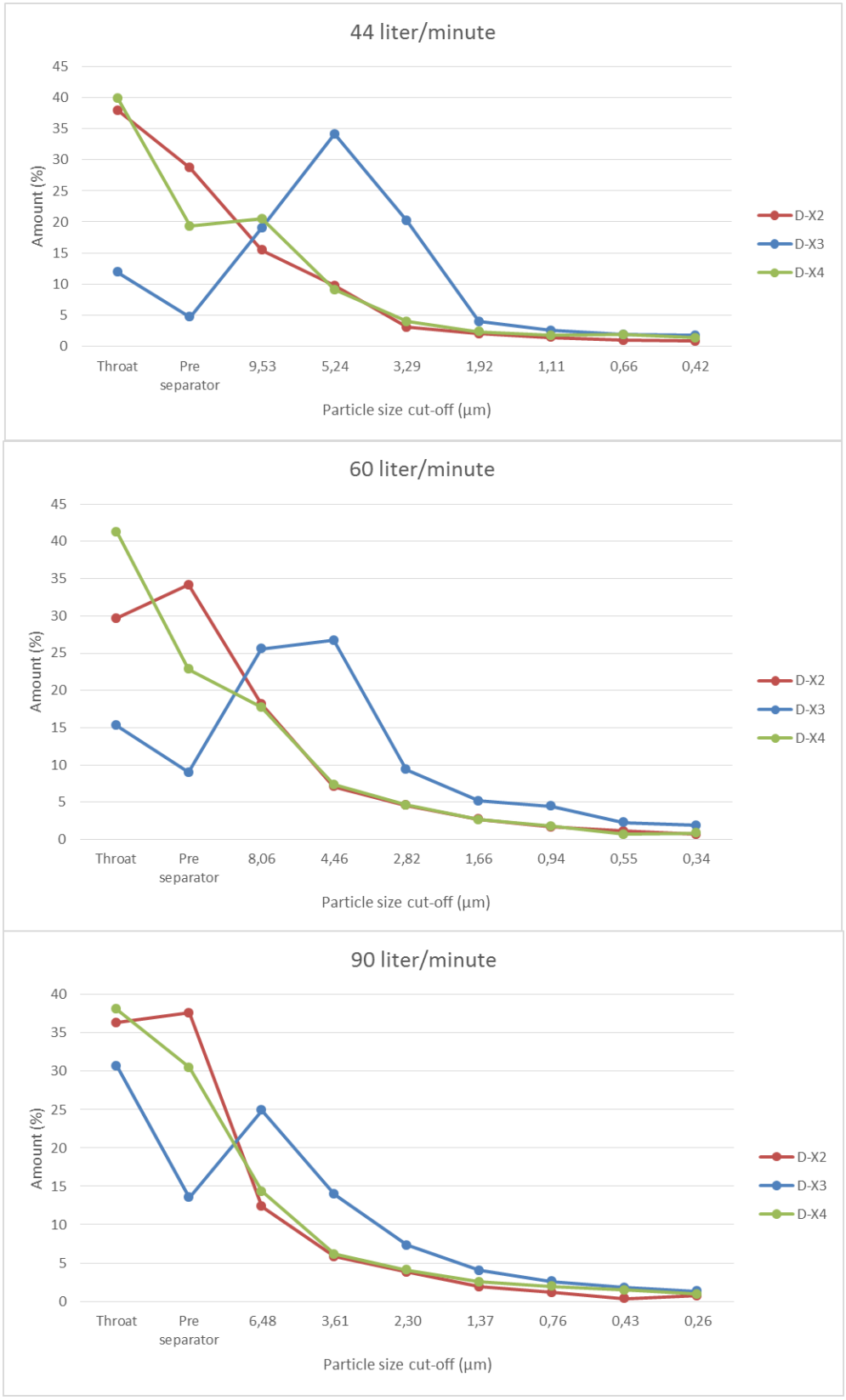

Figure 17. In this figure the size distribution acquired from the NGI is seen. The distributions have been normalized to make the proportions easier to see where 100 % is the entire delivered dose. The particle size cut-offs seen in the graphs show the maximum size of particles found on this stage. This means that, e.g. for the third measuring point on the bottom graph, the collected particles had a size between 6.48 and 3.61 µm, while the particles collected at the same measuring point of the top graph had a size between 9.54 and 5.24 µm.

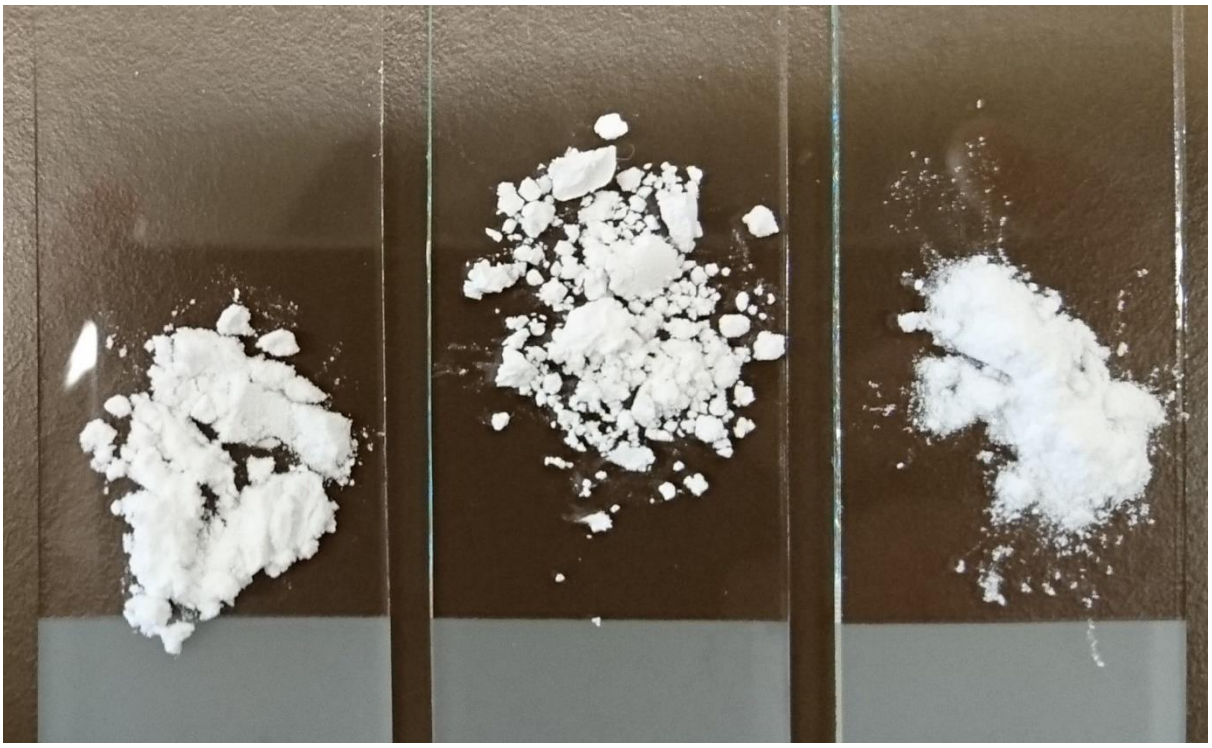

Figure 18. This figure is a photograph of the three powders D-X2, D-X3 and D-X4, in that order. One can see clear differences between the three powders. D-X3, the middle powder in the figure, is the most divergent of the three, as it is composed of mm-sized aggregates that break at the smallest touch. D-X2 is the stickiest of the three powders, and when touched one can feel sharp particles inside of it that are absent from the other powders. D-X4 has a more traditional fine powder appearance, having the feel of fine powdered sugar. Most previously produced powders had this texture and appearance.

Discussion

There was a shift between the results from the NGI and the Andersen impactor, especially for the powder D-X2. During the Andersen impactor tests, which were performed the same and the following two days as the powder was created, the FPF was found to be at an average of 46.8 %. When retested using the NGI a week later the corresponding measurement (FPF/Load at 60 l/min) was found to be only 17.7 %. A likely explanation for this is that the unusually high water activity of this powder (0.404) caused the powder to become unstable and change during the short time period the powder was stored between the measurements. This change was possibility caused by the water in the sample making the powder to “cake up” causing an increase in aggregates and thereby lowering the FPF. It is known that lactose, the main component of all produced powders, starts to crystalize at room temperature and water activities above 0.37. This causes an increased stickiness of the powder as well as the possible release of excess water in the powder (Roos, 2002). This possibility is further supported by the fact that when touched the powder seems to have sharp particles inside of it, something none of the other powders have. Another possibility is that the powder

During the spray drying the yield for samples D-X2 and D-X3 was less than two thirds as high as the powders they were a reproduction of. The reason for this is believed to be that the spray dryer was slightly damp at the start of the spray drying process. This would cause the

powder to get stuck to the cyclone in a higher frequency than previously and lower the yield.

The cause for the unusually high water activity of D-X2 is thought to have been caused by a combination of factors. One of these is the low dry matter of the spray dried sample, which causes each droplet to contain more liquid and therefore causes an increase in water activity (BÜCHI Labortechnik AG, 2002). This explanation would have been more convincing if this trend had been visible during the rest of the experiments, but it was not. Another explanation is the abovementioned possible reason for the lower yield for D-X2 and D-X3; the moisture suspected inside of the spray dryer during production. Another effect of additional water inside of the spray dryer would be the increase of moisture content of the powder. This is also supported by the water activity of sample D-X3, as it is higher during this experiment compared to C-X3.

There were also some inconsistencies between the results from the liposome size distributions of the solutions before they were spray dried. When they are compared with the samples they are reproductions of, the samples from phase D all have larger particles. When discussing the two reproductions D-X3 and D-X4, this is likely caused by the scale up process, as the scale up caused the average energy added to each liposome to decrease, thereby changing the results. For sample D-X2, the low obscuration and high weighted residual casts doubts to the validity of the measurement, especially the extra “tail” of the distribution with particles with a size of 2-200 μm which cannot be seen in C-X2.

When looking at the results from the NGI, it is clear that all three of the samples had special characteristics. The two powders that were created using the single type of lipid, D-X2 and D-X4, both had a similar response to the increase in air flow, increased air flow led to an increased FPF/DD and FPF/L, which is the normal response for DPIs. There were some differences between their results as well, such as an increased FPF/DD for D-X4 of an average of 2.5 percentages. Another interesting aspect is that while their size distribution has large similarities to their general shape, sample D-X4 had a higher proportion at the pre separator for all air speeds, especially for the two higher air speeds, where this was where the largest portion of the powder was detected. As the largest particles get stuck in the throat and only the particles that pass the throat get stuck in the pre separator, this means that D-X2 in particular has a large amount of large agglomerates that cannot leave the throat of the device unrelated to changes in the air flow, while these same type of large agglomerates in D-X4 can be separated by increased air speed.

One property that is shared by all samples was that MMAD increased with increased air flow, which is expected for DPI products. One possible and likely explanation for this is that the increased air flow corresponds to an increase in the mechanical force that drags the powder out of the capsule and into the device. An increase in this force should also increase the stress on the agglomerates, causing a larger amount of separation and thereby lowering the MMAD. This is also deemed the most likely explanation for the positive correlation between the FPF and the air flow for samples D-X2 and D-X4.

For sample D-X3 there seems to be a negative correlation between the FPF/DD and air flow. This might seem inexplicable until one compares this number with the size distribution found in figure 18. There one can see that the major difference between the distributions for the different air flow is the amount of powder that gets stuck in the throat of the device. As seen in table 15, the amount of powder that reaches the NGI is significantly higher at 60 l/min than at 44l/min. This could mean that most of the extra powder that reached the NGI at 60 l/min got stuck in the throat of the device, thereby lowering the FPF/DD.

The delivered dose per load (DD/L) should, according to the explanation used repeatedly above, have a positive correlation with the air flow, and this is partly the case but not as often as expected. In fact, it is only strictly correct for D-X4, as both D-X3 and D-X2 have their highest DD/L at 60 and not at 90 l/min, though the DD/L is always higher at 44 than it is at 90 l/min. The reason for these discrepancies might be that at increased air flows another effect competes with the earlier mentioned tendencies. One such effect might be that at higher air flows the powder has a higher likelihood of escaping the device and instead enter the atmosphere.

General Discussion

Effects of changing parameters

In this section all parameters that were varied during the creation of the powders are scrutinized to see if they are correlated with any of the measured parameters. When the effects of a parameter are discussed, only this parameter is changed to eliminate the effects of other parameters. A correlation is only suggested when at least 75 % of the experimental data supports this correlation.

To study the likelihood of these suggested correlations, the data has been analyzed using the statistical analysis software JMP using a Least Square Fit function to retrieve the p-values of said correlation. Based on these p-values, the correlations will be classified as likely ($p < 0.05$), marginal ($0.05 < p < 0.1$), unlikely ($0.1 < p < 0.25$) and highly unlikely ($p > 0.25$)

Concentration of API

This parameter was primarily tested during phase A and B, though it was also changed for sample D-X2 in phase D. In phase A and B, the concentration was 0.5, 0.75 or 1 % (w/w).

- There seems to be a negative correlation between the API concentration and the FPF, as seen in 75 % of measured cases ($p = 0.6760$, highly unlikely). Unless this is caused only by random fluctuations, it may be caused by the fact that there is less dry matter in the solution, causing each created droplet in the spray dryer to contain fewer particles and thereby decreasing the size of the resulting dried particle. (BÜCHI Labortechnik AG, 2002)
- The concentration of API seemed to have little to no clear effect on the yield, outlet temperature or water activity based on the detected data.

Inlet temperature

This parameter was only varied during phase A and B, where it was 150, 175 or 200 C.

- There is a clear and predictable positive correlation between the inlet and outlet temperature seen in all cases ($p < 0.0001$, likely). This is likely caused by the fact that not all energy (heat) added to the system is used, and when increasing the input energy, an increased amount of energy is left. (BÜCHI Labortechnik AG, 2002)
- There seems to be a positive correlation between FPF and inlet temperature seen in 75 % of cases ($p = 0.1956$, unlikely). If this is not random chance, it is thought to be caused by the fact that the use of increased temperatures increases the drying temperatures, making the particles more porous (less dense) and thereby lowering the aerodynamic size (and thereby the FPF), as porous particles are faster than solid particle of the same size. (Vanbever, et al., 1999)
- The inlet temperature seemed to have little to no clear effect on the yield, outlet temperature or water activity based on the detected data.

Feed flow rate

The feed flow rate was varied in phase A and B, where it was 3, 6 or 9 ml/minute.

- There was a clear negative correlation between the feed flow rate and the outlet temperature seen in all experiments ($p < 0.0001$, likely). This is likely caused by the fact that there is more solvent to evaporate, requiring more energy from the system and thereby lowering the outlet temperature. (BÜCHI Labortechnik AG, 2002)
- There seems to be a positive correlation between the water activity in the sample and the feed flow rate seen in 75 % ($p = 0.2989$, highly unlikely). If this effect is more than random noise then it is likely caused by the fact that when there is more solution added to the system and the drying is therefore not as complete when the particles leave the drying chamber as it would be at a lower feed flow rates. (BÜCHI Labortechnik AG, 2002)
- There seems to be a negative correlation between the FPF and feed flow rate seen in 75 % of cases ($p = 0.0809$, marginal). This is probably caused by the fact that there is not as much energy per volume of feed to be atomized when the feed flow rate is increased. This makes each droplet, and in consequence particle, larger and thereby decreases the FPF. (Maltesen, et al., 2008)
- There seems to be no connection between the feed flow rate and the yield of the powder.

Effect of lipid type

There were two types of lipids used during phase C and D. One of them was a single type of phospholipid and the other was a natural mix of several types of phospholipids.

- There seems to be a correlation between the choice of lipid and the liposome size, where the liposomes are larger for the single lipids in 80 % of cases ($p = 0.0380$, likely). This is likely a consequence of the type of lipids used.

- There seemed to be a correlation between type of lipid used and yield from the spray dryer, where the use of the single type results in a higher yield 80 % of the time (p = 0.4969, highly unlikely).
- No simple or clear correlation between type of lipid and FPF, water activity or outlet temperature.

Concentration of lipids

The concentration of lipids used was varied during phase C and D, and it was either 0.2 or 0.4 % (w/w).

- There is a clear and significant positive correlation between the liposome size and the concentration of lipids (p = 0.0325, likely). This is likely caused by the fact an increased concentration of lipids in the solution increases the chance for more lipids to be in the same area as each other and be able to form structures together. The increased detected size might also be caused by an agglomeration of many liposomes, and if there is a larger amount of liposomes then these agglomerations can become larger. (Pons, et al., 1993)
- There seems to be a negative correlation between the weighted residual and the concentration of lipids, seen in 75 % of cases (p = 0.1466, unlikely). Unless this is caused by random chance, this may be connected with the decreased detected liposome size and caused by the increased interference of random noise for smaller particles seen in this type of particle sizers.
- There seems to be a positive correlation between FPF and concentration of lipids seen in 75 % of cases (p = 0.2498, unlikely). Unless this is pure chance, it might be caused by the fact that lipids are surface active, meaning that they are drawn to the surface of the particles. There they would decrease the surface tension of the droplets, causing the amount of energy needed to separate droplets decrease and thereby decreasing the droplet size and in consequence the FPF. (Vanbever, et al., 1999)
- There seems to be a negative correlation between yield and concentration of lipids seen in all tested samples (p = 0.1449, unlikely). If this correlation is not purely generated by random noise, it is believed to be caused by the increased “stickiness” of the particles containing more lipids which might cause the particles to stick to the inner surfaces of the spray dryer and thereby decreasing the yield.
- There seems to be no simple or clear connection between concentration of lipids and outlet temperature as well as water activity.

As seen when comparing the results of phase B and C, the addition of any lipids at all had a significant effect on the system, especially the FPF. This might mean that only a minimum amount of lipids are needed to generate this change, such as the amount of lipids needed to coat the surface of the droplets dried in the spray dryer.

After-treatment of solutions containing lipids

The after-treatment of samples was performed during phase C, and the reason these were not repeated during phase D is that they caused no changes larger than the random noise

seen during all experiments after the solution had been spray dried. This lead to the assumption that the after-treatment had no controllable effect and that the effect was mostly neutralized by the spray drying, after which the differences between the after-treated and non-after-treated were caused mainly by random noise.

Conclusions

In the aim of this thesis there were two main questions that were asked. The first question was whether it was possible to create a sufficiently usable powder suitable for inhalation using a spray dryer and whether the addition of liposomes would improve the properties. The second question was how the variation of input parameters affected the performance of the created powders.

As all powders containing lipids had a higher FPF than even the best powder created without lipids, the addition of lipids can be seen as a large improvement of the properties of the powder. As for the first part of this question, it could be concluded that at certain parameters one could create a powder such as D-X3 which had a FPF of over 50 % at certain ways of testing, which qualifies as a suitable powder, though further optimization is required.

As for the second question, table 14 is a summary of the correlations found between input and output parameters during this thesis. As one can see in this table, there are 4 correlations that are deemed as likely, 1 deemed as marginal, 4 deemed as unlikely and 3 deemed as highly unlikely. The reason for the small amount of likely correlations can be seen as an indication of how chaotic this experimental system is and the need for further experimentation.

Table 14. In this table the detected correlation between the manufacturing parameters and the measured output parameters are displayed. To illustrate the statistical significance of the correlations, all likely correlations are colored green, all marginal are colored blue, all unlikely are colored yellow and all highly unlikely are colored red.

	Increase of API Concentration	**Increase of Inlet Temperature**	**Increase of Feed Flow Rate**	**Lipid Type**	**Increase of Lipid Concentration**
Outlet Temperature	None/Unknown	Positive	Negative	None/Unknown	None/Unknown
Yield of Spray Dryer	None/Unknown	None/Unknown	None/Unknown	Single Increases	Negative
Water Activity	None/Unknown	None/Unknown	Positive	None/Unknown	None/Unknown
Fine Particle Fraction	Negative	Positive	Negative	None/Unknown	Positive
Liposome size	-	-	-	Single Increases	Positive
Weighted Residual from liposome sizing	-	-	-	None/Unknown	Negative

Future work

Even though much was achieved during this project, there are still several areas where further work is needed. One of the most obvious areas where further work would improve results is further optimization and testing, where one could try to use all of the knowledge found in table 14 to create a powder with optimal performance. Said table could also be vastly improved with the addition of further data, which might be able to confirm or dismiss the various correlations proposed there.

Another area that would be interesting would be a further study of the powder particles composition. During the thesis, it was supposed that the particles were a homogeneous mix of all ingredients, but a further study of the composition of the particles using e.g. RAMAN spectroscopy would have revealed where in the spray dried particles each different component of the particles were situated. It would also have been interesting to study the particles using an electron microscope to study their structure, which would have revealed the actual physical shape and additional such information. There was also suspected effects of the air humidity during the spray drying, and if one could control the air humidity one could test how this parameter affected the properties of the created powder.

As one of the main purpose of introducing liposomes in pharmaceutical formulations is to affect the release profile of the drug, a study of the dissolution would have been informative. An interesting experiment would have been to use two powders, one of which contained liposomes and one of which did not and study how the release profile was affected by this.
Another of the main uses of a liposome is to encapsulate the API, causing the API to be protected and affecting the properties of the drug, e.g. long term stability. Further study could reveal how much of the API was encapsulated and perhaps improve this fraction.

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Appendix - Additional Data

Table 15. In this table the calculated Delivered Dose per Load is shown. In the second column the residual powder in the capsule is not included in the calculations.

Sample name and flow rate (l/min)	Average Delivered Dose/ Load (%)	Average Delivered Dose / (Load-remaining in capsule) (%)
D-X2 at 44	95.86	99.89
D-X3 at 44	62.99	67.28
D-X4 at 44	83.40	86.67
D-X2 at 60	100.72	104.53
D-X3 at 60	80.13	83.60
D-X4 at 60	88.19	91.70
D-X2 at 90	96.18	98.34
D-X3 at 90	78.70	80.84
D-X4 at 90	97.94	99.76

Table 16. In this table the three tested FPF for the Andersen impactor as well as their relative standard deviation is displayed.

Sample name	Experiment 1 FPF (%)	Experiment 2 FPF (%)	Experiment 3 FPF (%)	RSD (%)
D-X2	49.20	43.90	47.29	5.73
D-X3	29.08	30.46	28.67	3.18
D-X4	24.98	22.14	24.71	6.55

Table 17. In this table the averaged results derived from the NGI testing are shown. These averages do not take into account the three tests that were dismissed.

Sample name and flow rate (l/min)	Average FPF/ (Delivered dose) (%)	Average FPF/ Load (%)	Average MMAD (μm)
D-X2 at 44	14.55	13.94	4.97
D-X3 at 44	52.55	34.10	3.82
D-X4 at 44	17.19	14.33	5.26
D-X2 at 60	17.58	17.70	4.35
D-X3 at 60	49.94	40.05	3.61
D-X4 at 60	18.12	15.98	4.39
D-X2 at 90	18.26	17.56	3.43
D-X3 at 90	39.91	32.79	3.28
D-X4 at 90	22.34	21.00	3.27

Table 18. In this table the amount of residual powder in the capsules after the impaction run is shown in percentage of original load.

Sample name	Remaining in capsule (%)
A-X1	1.05
A-X2	0.66
A-X3	1.74
A-X4	2.81
A-Y1	2.45
A-Y2	1.91
A-Y3	1.99
A-Y4	2.56
A-Z1	1.19
A-Z2	1.83
A-Z3	1.61
B-X1	1.75
B-X2	5.22
B-X3	8.18
B-X4	4.19
B-Y1	9.80
B-Y2	4.27
B-Y3	1.12
B-Y4	3.06
B-Z1	4.41
B-Z2	3.33
B-Z3	3.56
C-X1	2.64
C-X2	1.40
C-X3	2.33
C-X4	3.04
C-Y1	1.89
C-Y2	0.06
C-Y3	0.37
C-Y4	1.33
D-X1	5.15
D-X2	3.81
D-X3	4.33