FACULTEIT FARMACEUTISCHE WETENSCHAPPEN

NEAR INFRARED SPECTROSCOPY AS IN-LINE PAT TOOL FOR A ROBUST MOISTURE CONTENT DETERMINATION OF SPIN FREEZE-DRIED SAMPLES

Laurine Vannieuwenhuyze

A Master dissertation for the study program Master in Pharmaceutical Industry

Academic year: 2020 - 2021

UNIVERSITEIT GENT

FACULTEIT FARMACEUTISCHE WETENSCHAPPEN

NEAR INFRARED SPECTROSCOPY AS IN-LINE PAT TOOL FOR A ROBUST MOISTURE CONTENT DETERMINATION OF SPIN FREEZE-DRIED SAMPLES

Laurine Vannieuwenhuyze

A Master dissertation for the study program Master in Pharmaceutical Industry

Academic year: 2020 - 2021

UNIVERSITEIT GENT

PREAMBLE

"This master's thesis was executed in a period where corona measures have influenced research and education activities in various ways. These unusual circumstances may have had an impact on this thesis to a greater or lesser extent, despite all the efforts of the student, daily supervisor(s) and promoters. This generic preamble aims to frame this and was approved by the faculty."

SUMMARY

Freeze-drying is a widely used technique to improve stability and increase shelf-life of heat sensitive and water labile medicines. At the moment the pharmaceutical industry is shifting from the standard batch manufacturing to a continuous freeze-drying way. Continuous freeze drying has already proven to increase the stability of the product compared to these produced in batch mode.

In this project a PLS model for spin freeze-dried formulations containing mannitol and sucrose was developed to determine the residual moisture content of samples based on NIR spectra. 51 spin freeze-dried samples were measured with NIR spectroscopy. These samples differ in weight ratio mannitol/sucrose, composition and relative moisture. Wherefore a multivariate analysis was carried out. The moisture content was taken in the range of 0.34% to 6.89%.

It was shown that quantification of moisture content in spin freeze-dried samples of mannitol-sucrose mixtures via multivariate analysis of NIR spectra, correlated with the moisture content measured by Karl-Fisher. Therefore all NIR spectra were SNV corrected and Savitzky Golay filtered in a range of 1100 to 2200 nm. Also taking a first derivative led to better results. Multivariate analysis yielded a Root Mean Squared Error of Estimation of 0.28%. The obtained model was also validated according to the ICH guidelines. Due to lack of time, only a limited number of samples were used to set up the model. If the reference set were more extensive, the model would get better and better.

Additionally, it was shown that the model could be applied to samples with changing composition. A Root Mean Squared Error of Prediction of 0.74% and 0.31% was attained when using the model to predict the moisture content of samples with a different filling volume of 6:4 weight ratio of mannitol and sucrose or containing proteins in a concentration. The PLS model based on NIR spectra obtained in this project was not that robust, but this was mainly because the model was not sufficiently trained and the range was taken too large. Based on a better model, robustness is likely to increase.

In general, it can be concluded that if the dataset were more extensive, a better trained model would be obtained. As a result, the residual moisture content of samples with a different composition would also be better predicted.

SAMENVATTING

Vriesdrogen is een veelgebruikte techniek om de stabiliteit en de houdbaarheid van water labiele en warmte gevoelige geneesmiddelen te vergroten. De farmaceutische industrie schakelt momenteel over van de standard batchproductie naar een continue vriesdroogproductie. De continue vriesdroogtechniek heeft aangetoond dat de stabiliteit gelijk of beter is dan die van batch vriesdrogen.

In dit project werd een PLS-model ontwikkeld voor spin gevriesdroogde formuleringen met mannitol en sucrose om het vochtgehalte van monsters te bepalen op basis van NIR-spectra. 51 spin gevriesdroogde vials werden gemeten met NIR spectroscopie. De stalen verschilden in gewichtsverhouding mannitol/sucrose, samenstelling en relatieve vochtigheid. Daarom werd een multivariate analyse uitgevoerd op deze data. Het vochtgehalte lag in een bereik van 0,34% tot 6,89%.

Er werd aangetoond dat kwantificering van het vochtgehalte in spin gevriesdroogde vials van mannitol-sucrose mengsels via multivariate analyse van NIR spectra, correleerde met het vochtgehalte gemeten met Karl-Fisher. Voor dit doel werden alle NIR spectra SNV gecorrigeerd en SG gefilterd in een bereik van 1100 tot 2200 nm. Ook het nemen van een eerste afgeleide leidde tot betere resultaten. Multivariate analyse leverde een RMSEE van 0,28% op. De ontwikkelde NIR-methode voor de kwantificering van vocht werd ook gevalideerd volgens de ICH-richtlijnen. Wegens tijdgebrek werd slechts een beperkt aantal monsters gebruikt om het model op te zetten. Als de referentieset uitgebreider zou zijn, zou het model steeds beter worden.

Bovendien werd aangetoond dat het model kon worden toegepast op stalen met wisselende samenstelling. Een RMSEP van 0,74% en 0,31% werd bereikt bij gebruik van het model om het watergehalte te voorspellen van stalen met een verschillende vulvolume bij een 6:4 gewichtsverhouding van mannitol en sucrose of met eiwitten in de formulatie. Hieruit blijkt dat NIR-modellen kunnen worden opgesteld die het vochtgehalte van stalen met verschillende samenstellingen kunnen voorspellen. Het PLS-model op basis van de in dit project verkregen NIR-spectra was niet zo robuust, maar dat kwam vooral doordat het model niet voldoende was getraind en de range te groot genomen was. Op basis van een beter model zal de robuustheid waarschijnlijk toenemen.

In het algemeen kan worden geconcludeerd dat als de dataset uitgebreider zou zijn, er een beter getraind model zou worden verkregen. Hierdoor zou ook het vochtgehalte van stalen met een andere samenstelling beter kunnen worden voorspeld.

ACKNOWLEDGEMENTS

Finishing this POL project, I would like to give my appreciation to everyone involved in its realization.

First of all, I would like to thank my academical promotor ***Prof. dr. Thomas De Beer*** *for giving me the opportunity to participate in this research project in the Laboratory of Pharmaceutical Process Analytical Technology at* ***Ghent University****.*

A special appreciation for my supervisor ***Laurens Leys*** *for the pleasant collaboration. Thank you for the good guidance, advice and follow-up. I wish you the best with your PhD.*

Aurélie Verniers *thank you for the pleasant company and cooperation during this past weeks.*

Most importantly, I would like to thank my ***parents, sister, family & friends*** *for their support, interest and the many enjoyable walks during this strange COVID period.*

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

EMA	European Medicines Agency
FDA	Food and Drug Administration
GMP	Good Manufacturing Practice
ICH	International Conference on Harmonization
IR	Infrared
LN_2	Liquid nitrogen
NIR(S)	Near Infrared (Spectroscopy)
PAT	Process Analytical Technology
PC	Principal Component
PCA	Principal Component Analysis
Ph Eur	European Pharmacopeia
PLS	Partial Least Squares
QbD	Quality by Design
RMSEC	Root Mean Square Error of Calibration
RMSEE	Root Mean Squared Error of Estimation
RMSEP	Root Mean Square of Prediction
Rpm	Rounds per minute
SG	Savitzky-Golay
SNV	Standard Normal Variate

LIST OF SYMBOLS

R_p Dried product resistance to water vapor transfer

T_f Freezing temperature

T_g Glass transition temperature of the dried product

$T_{g'}$ Glass transition temperature of the maximally cryoconcentrated material

1 INTRODUCTION

1.1 FREEZE DRYING

Freeze-drying, also known as lyophilization, is a low temperature drying technique to convert unstable drug solutions into stable solids with a low residual moisture content. Freeze-drying is applied in varied sectors, most frequently in the pharmaceutical and food industry. (4) In the pharmaceutical and bioindustry it is used in the manufacturing of heat sensitive and water labile drugs, such as biopharmaceuticals. The freeze-drying process is a complex and expensive method, but has many advantages such as an increasing shelf life and the higher stability of products. (1, 4-6)

1.1.1 The freeze-drying process

The freeze-drying process is a complex procedure that consists of three fundamental stages: 1) freezing, 2) primary drying and 3) secondary drying. During this process the shelf temperature and chamber pressure are the most important process settings (Figure 1.1). (1, 4, 7, 8)

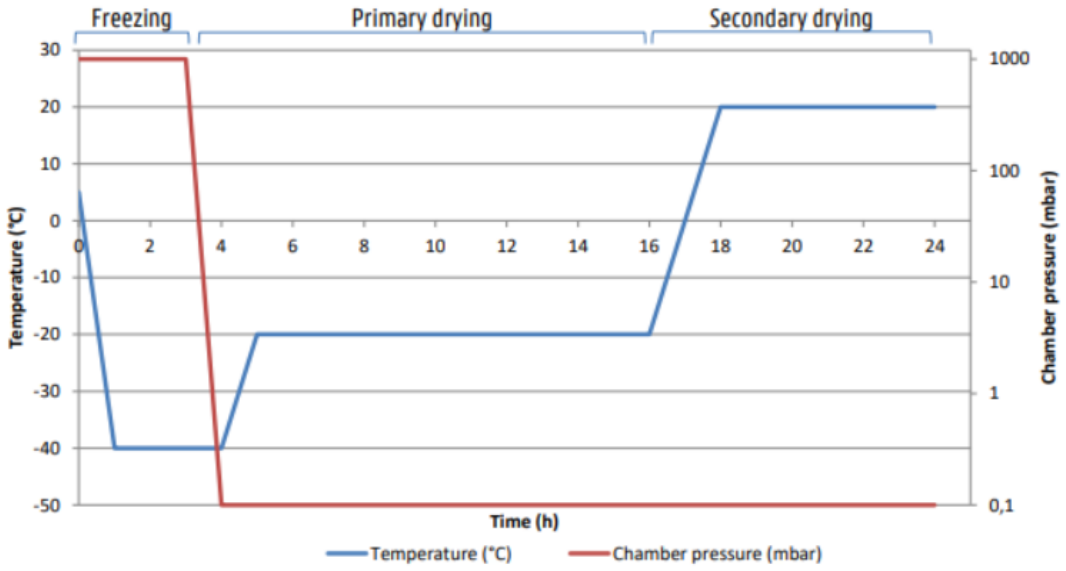

Figure 1.1. Shelf temperature and chamber pressure during the three stages of a typical freeze drying cycle. During freezing the shelf temperature is lowered at atmospheric chamber pressure. In the primary drying step the chamber pressure is reduced to vacuum and the shelf temperature is raised, so sublimation can occur. At last, during secondary drying the chamber pressure is lowered to a deeper vacuum and the shelf temperature is increased even more. (1)

During freezing the temperature drops and most water is converted into ice. In the second stage a vacuum is introduced and unbound water is removed by sublimation. After primary drying the product still contains some bound or unfrozen water. These moisture content is usually too high and can result in an unstable product with a short shelf life. Therefore a second drying step is required. Finally, during secondary drying, bound water is removed by desorption. (1, 7, 8) Figure 1.2 shows the phase diagram of water which shows an overview of the three states. (1, 8)

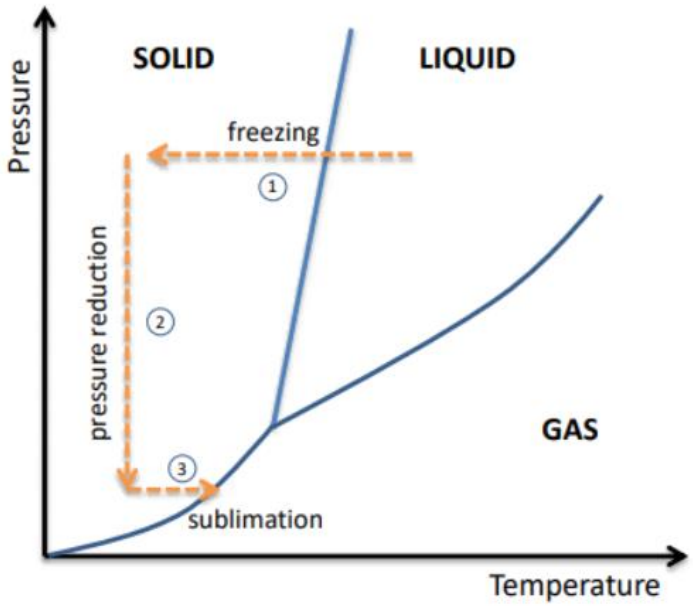

Figure 1.2: The stippled line shows the trajectory of the freeze-drying process. During freezing the temperature drops and water changes from a liquid into a solid state. Primary drying occur by a pressure reduction and an increase in temperature, leading to the sublimation of water. (1)

1.1.1.1 Freezing

Freezing is the first step and an important desiccation step of the freeze drying process. During this step the purpose is to convert most water into ice and separate the solvent from the solutes. (1, 7)

Different actions take place during freezing. At first at a temperature 10 to 20 degrees below the equilibrium freezing point the solution supercools. Depending on the solution properties supercooling occurs at different degrees. During supercooling the solution stays the whole time liquid. Once crystallizations occurs, which is an exothermic process, the temperature rises rapidly. The temperature will rise until balance is reached, after that the temperature will fall back until the shelf temperature is reached. By decreasing the temperature and the occurrence of

crystallization there is an increasing concentration of solutes in progressively less and less water. When the freezing point of that solution continually goes down because the concentration goes up, this is termed the freeze concentration of the solute. Freeze concentration continues on freezing until a supersaturated solution is reached. By the end of the freezing step the formulation is in a solid state and contains a residual moisture content around 20%. (1, 7)

The freezing step affects the following steps of the freeze-drying process. So plays the freezing rate an important role on the further freeze-drying steps. Fast cooling provides a large degree of supercooling, a minimal solute concentration, small ice crystal size and a slow rate of drying. On the opposite, slow cooling provides a small degree of supercooling, a maximal solute concentration, large ice crystal size and a fast rate of drying. The effect of the freezing rate on the ice crystal size is important in the further progress of the process. The microstructures of ice crystals formed affect the primary drying rates. Large ice crystals will result in large pores and a low mass transfer resistance (Rp) which leads to a short primary drying time. The total surface of the cake is smaller, this has consequences for the secondary drying step. (1, 7) Figure 1.3 shows two examples. The top row has a low degree of supercooling and forms large ice crystals. The bottom one has as result smaller ice crystals due to a higher degree of supercooling.

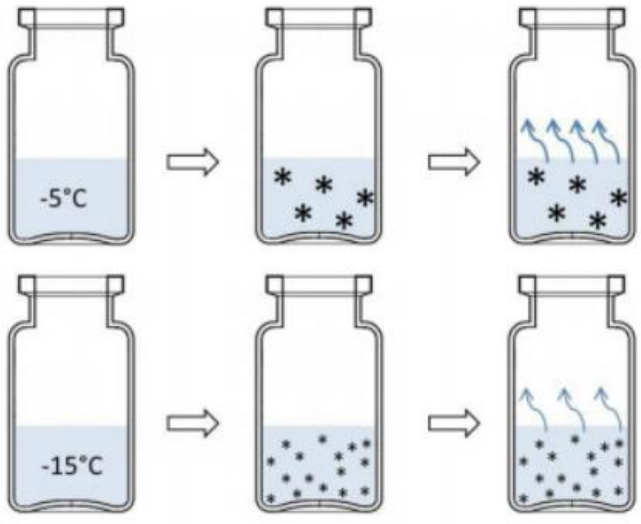

Figure 1.3: Impact of the degree of supercooling. Large ice crystals are formed by a low degree of supercooling, smaller ice crystals by a higher degree of supercooling. (1)

1.1.1.2 Annealing during freezing

Freezing is considered the most important step in the freeze-drying process because it determines the size of the ice crystals and thus affects the further process time. During freezing an additional annealing step can be introduced. Hereby the product temperature is kept at a determinate subfreezing temperature above the glass transition temperature of the freeze concentrate, Tg', for a certain time. In this period the ice crystals size distribution is influenced. Their size distribution is ruled by Oswald ripening what gives rise to growth of the smaller crystals to larger ones. (9, 10)

Introducing this step improves the homogeneity within the batch. A second reason for introducing annealing is to complete crystallization of a partially crystallized bulking agent. Overall, annealing leads to a cake of good quality in a shorter amount of time. (1, 9, 10)

1.1.1.3 Primary drying

When the liquid formulation in the vial has completely converted to a solid form, primary drying is induced. This step is the longest stage of the freeze-drying process and is responsible for the removal of surface water by sublimation of ice crystals. To accomplish this the shelf temperature is raised and a vacuum is set into the freeze-dryer chamber. An increase of the shelf life is necessary because sublimation is an endothermic process. When the process progresses the sublimation front is moving slowly downwards. When this occurs a pore is formed at the place where an ice crystal is removed, creating a porous network of dried product. The formed water steam is eventually deposited on the cold condenser plates. To ensure a solid and rigid cake, the temperature at the sublimation front must be kept below the collapse temperature, T_c. When all the ice crystals are removed primary drying is completed. (1, 5, 7)

1.1.1.4 Secondary drying

After primary drying, the dried product still contains some residual water that is bound to the components in the cake. In the final drying phase, the residual moisture is reduced as much as possible by removing most of the bound or unfrozen water by desorption. This process takes place under a deep vacuum and at a higher shelf temperature. To avoid collapse of the cake the product temperature should be kept under the glass transition temperature of the dried product (Tg). (1, 5)

At the end of the primary drying stage the product still contains 5 to 10 % of residual moisture. Water performs as plasticizer which could decrease the glass transition temperature and thereby reducing the storage stability. At the end of the secondary drying step and the thus the end of the whole process, the goal is to become a product with a moisture content usually amounts to 1 to 3%. This lead to a product with an optimal level for stability. (1, 7)

1.1.2 Freeze-drying equipment

A typical batch freeze-dryer regulatory consists of 4 essential components: a drying chamber, a condenser, a vacuum pump and a refrigeration plant. (1, 4) These components are illustrated in figure 1.4.

Temperature and pressure are the two most important variables during the process. To foresee the requisite temperature in all steps of the process the drying chamber contains an external heating system and temperature controlled shelves. The condenser is associated with the drying chamber and provides place for deposition of water steam generated during primary and secondary drying. The necessary pressure during the process is controlled by the vacuum pump, these evacuate the air from the drying chamber. (1, 4)

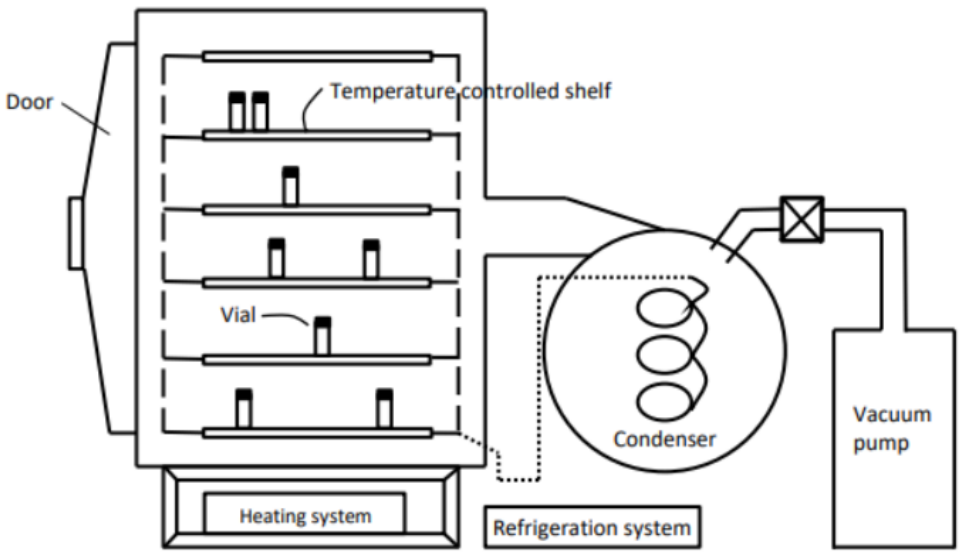

Figure 1.4. Schematic representation of a typical batch freeze-dryer. A drying chamber with temperature controlled shelves, a condenser, a vacuum pump and a refrigeration plant. (1)

1.1.3 Formulation and stabilty

To provide a stable product with an acceptable lyophilized cake structure and critical quality characteristics such as residual moisture content and reconstitution time, using the correct excipients is necessary. Supplementing the formulations with suitable excipients not only improves the stability of the biopharmaceutical, but also affect the parameters of the lyophilization process. Most lyophilized products are intended for parenteral administration hereby prescriptions for excipients are very strict and the number of additional excipients is limited. Different categories of freeze-drying excipients and their roles are short described. (11-13)

During freezing and drying different stresses can occur which could affect the stability of the product. To maintain stability stabilizers are added, which are the main group of excipients. Stabilizers can be divided into two large groups: the cryoprotectants and the lyoprotectants. Cryoprotectants ensure hydration of the molecule and preventing changes during freezing. Lyoprotectants prevent changes during drying by acting as a water substitute. Several product can act as stabilizers: PVP, PEG, dextran, sugars (trehalose, sucrose, glucose, maltodextrins, lactose), amino acids (glycine, arginine), albumin, etc. At present threalose and sucrose are the most used stabilizers, but new stabilizers are very promising. (11-13)

To have sufficient dry matter to provide the appropriate structure to the lyophilized cake a bulk agents such as mannitol, sucrose and lactose are added. They also contribute to the morphology of the cake, with the formation of pore structures. Larger pores provide a faster sublimation which can lead to shorter drying times. Most product now on the market contain mannitol as bulking agent because of its high crystallization tendency. (11-13)

Also surfactants are often added for wetting the cake during reconstitution, minimize the surface-adhesion, reduce the risk of aggregation. In addition, buffers are often added as well as salt, to provide stability of the drug and the tonicity of the injectables. These can influence the freezing concentration, attention must be paid to this. (11, 12)

1.2 PHARMACEUTICAL FREEZE DRYING

Pharmaceutical freeze-drying is traditional performed as a batch wise process. But a new trend in the pharmaceutical industry as an alternative manufacturing technique is continuous processing. Continuous processing is a radically different system. In the pharmaceutical industry this is a relatively new way of manufacturing, about 5% of all processes take now place in a continuous manner. (14) In batch-wise manufacturing, a specific quantity of

materials follows a sequence of steps in a specific order. Quality control of the finished product occurs in an off-line way. During each operation a limited number of samples are examined. A batch-wise process does not provide real-time information and is associated with a retardation of the overall production process time. Furthermore, if the quality of the samples is not satisfactory, the whole batch is rejected. Because of these inefficiencies in batch-wise production, a new concept for freeze-drying is presented, namely continuous freeze-drying. Continuous production works according to the principle of "one in, one out". Due to in-line quality control there is an increase of the process efficiency and real-time release has been made possible. Continuous production has many advantages compared to batch wise manufacturing, such as a lower cycle time and faster release of manufactured goods. (1, 2, 14, 15)

1.2.1 Disadvantages of batch freeze-drying

Despite the fact that batch freeze drying is the standard process to freeze drying in pharmaceutical industries, it has several drawbacks. First of all there is an uneven heat transfer in the freeze-drying chamber. Because of that vials at different places on the shelves have a various energy input. For example outer vials will receive more radiation energy and will sublimate more quickly than vials in the center of the shelves which are shielded by the cold vials next to them. See figure 1.5. This lead to vial to vial variability which can lead to variability in the final product. Quality control is done on only a small number of vials. It is therefore important to place probes in the vials on the center of the plates so that certainly every vial is frozen during freezing for example. (1, 2, 14)

Further the freezing step during batch freeze-drying is uncontrolled, which has an impact on the following steps. This can lead to different ice crystal sizes between the vials, which affects the individual vial sublimation rate during primary drying. These drawbacks result in various freeze-drying process conditions in each vial, this leads to uncontrolled variability from vial to vial and batch-to-batch final product. (1, 2, 15)

Freeze-drying is also a slow, time-consuming and expensive proces, the process is faster in a continuous manner than when the process is carried out batch-wise. These disadvantages prompt the pharmaceutical industry to evaluate a new way of freeze drying, namely continuous freeze drying. (1)

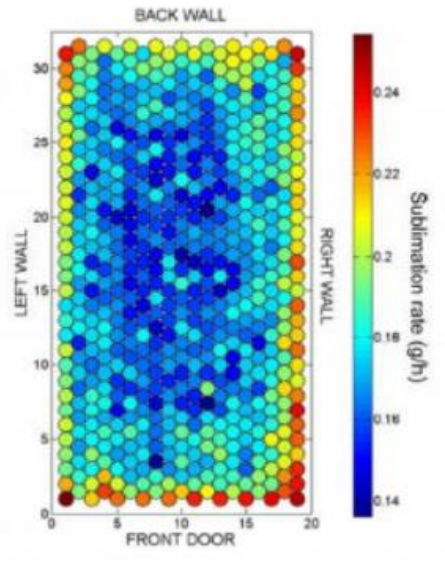

Figure 1.5 Effect of sample position on sublimation rate. Heat transfer occurs by various means but is not uniform throughout the batch. Outer vials receive more radiation energy and will sublimate more quickly. Center vials have a greater shielding form side wall radiation. It is therefore important to insert the probe into the samples which will take longer to sublimate, so the vials in the center of the batch. (1)

1.3 A CONTINUOUS APPROACH OF FREEZE-DRYING

Continuous processing is an alternative method than the current way of manufacturing. During batch-wise freeze-drying the process starts with a fixed amount of starting materials. These undergo a sequence of segmented unit operations. During these operations off-line quality control is carried out. There is a delay on these results and when results of an intermediate are not met, the entire batch can be rejected or need to be reprocessed. Continuous freeze-drying on the other hand integrates all unit procedures into a single production unit. During this process there is a continuous feed of start material and a continuous outcome of the final product. This way of manufacturing has a lot of advantages. (1, 14)

During continuous freeze drying vials are filled with a fluid formulation and are rotated rapidly, so the liquid spreads over the entire vial wall. This happens in a freeze-unit using a flow of an inert, sterile and cold gas, as result a solid product is formed over the entire vial wall. This is called spin freeze drying and is illustrated in figure 1.6 (1, 2)

Additional solidification and ice crystallization of the solutes takes places in a temperature-controlled chamber to obtain the desired morphological structure.

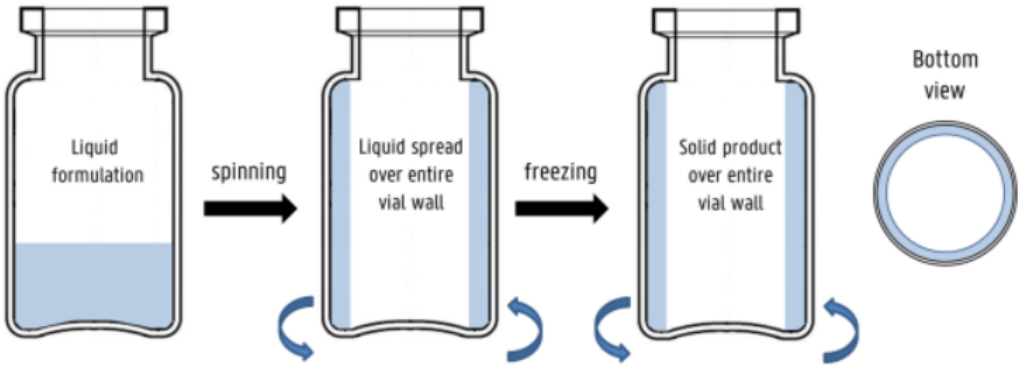

Figure 1.6 Illustration of spin freezing. (1)

When freezing is completed, the vials are transported by a continuous load-lock system to the drying unit. There are two drying chambers, one for primary drying and one for secondary drying. Spin freeze drying creates a thinner vial thickness and so a large surface area, which will shorten the drying steps. To become an efficient and homogeneous drying the heat energy must be uniformly and adequately to the thin product layer. Infrared (IR) radiation can provide the energy needed for sublimation and desorption. This is illustrated in figure 1.7. When freeze drying is completed, the load-lock system removes the vials from the vacuum and transport them to a final unit where there are stopped and capped. (1, 2)

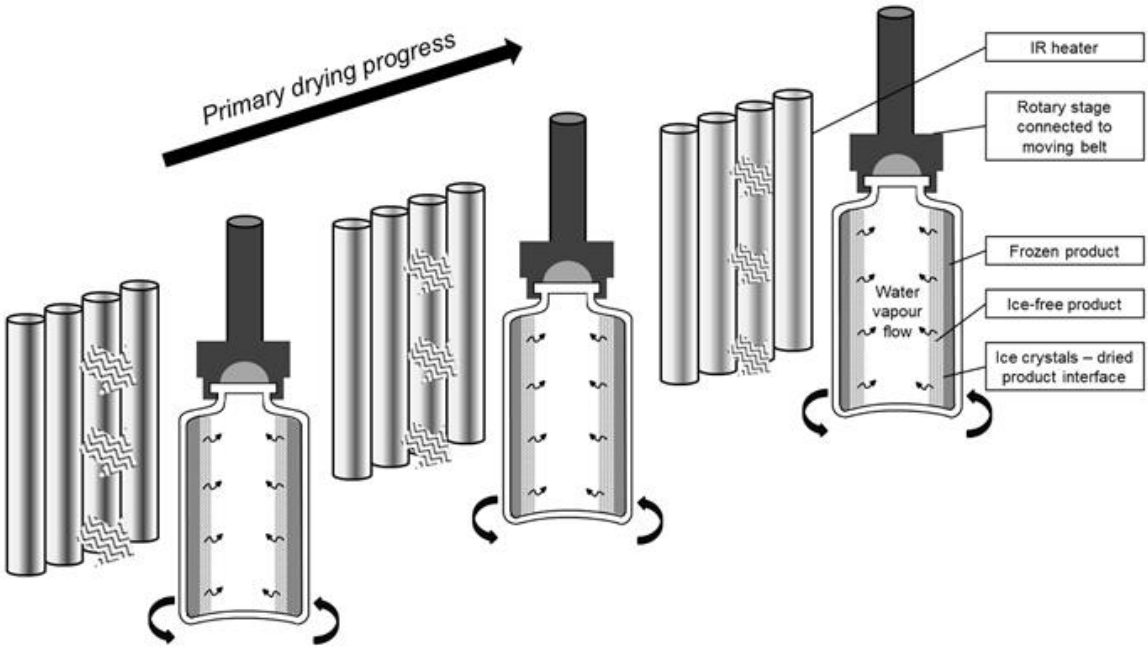

Figure 1.6 Illustration of the Infrared assisted continuous primary drying of spin frozen vials. (2)

1.3.1 Advantages of continuous freeze-drying

Continuous processing has a major impact on many aspects of the freeze-drying aspect. First, systems used for freeze drying on a continuous way are smaller because there are less quality control areas. Furthermore there is no real-time information during batch freeze-drying so quality control is done off-line by sampling during each unit operation. If the quality is not met the batch need to be rejected. Continuous freeze drying uses in-line quality control so there is a reduction of waste because less batches are rejected. (1)

By spin-freezing the vials, the product is spread as a thin layer instead of a cake. Therefore the primary and secondary drying process will occur more efficient. (1) Also less personnel is required during the continuous process. Taking all this into account, the cost is also much lower than batch wise and there is a faster release of manufactured goods.

1.4 QUALITY BY DESIGN IN FREEZE DRYING

Conventional pharmaceutical manufacturing, like batch unit operations, have an off-line quality control in quality control labs. The quality control is done away and independently from the central processing. This current way of manufacturing has a lot of drawbacks, as already mentioned in 1.2.1. (1, 16)

Two major evolutions are driving the pharmaceutical industry to assume new production models: the cost-cutting and the regulatory pressure. The Food and Drug Administration (FDA) published in 2004 its process analytical technology (PAT) guideline to tackle the quality problem in the manufacturing process. (17) PAT is defined as "a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality." (16) The aim of PAT is also to understand and control the manufacturing process which must lead to quality by design. (16)

One of the many advantages is that measurements of samples can be done in-line with PAT. Hereby the sample is not removed from the process stream. Near infrared (NIR), Raman spectroscopy and particle size analyzers have been more and more used for real time measurements of critical process and product attributes during pharmaceutical processing. In this master dissertation NIR will be used. (16)

1.4.1 Near infrared spectroscopy

NIR energy was discovered in the 19th century by William Herschel and was first used in an industrial application in 1950. (18) Nowadays NIR is a widely used analytical technique in the pharmaceutical industry, because of its non-destructive, non-invasive and fast measurements. Also in-line implementation is a big advantage. (19, 20)

Water has strong signals in NIR spectra with an overtones band at 1420nm and a combination band at 1920nm. Therefore NIR is often used as a non-destructive technique for residual moisture content analysis in samples or as moisture monitoring during processing. During freeze-drying NIR can also be a powerful tool to monitor the whole freeze-drying process such as determinate the end of primary and secondary drying. (19, 20)

Beside this NIR spectra are tough to understand and contain quantitative and qualitative chemical and physical information. It is characterized by overlapping and broad absorption peaks and thousands of wavelengths. Therefore chemometrics is used to extract useful information. (19, 21)

1.4.1.1 Instrumentation

A NIR Spectrometer consist of 4 main components: a sample holder, a monochromator, a light source and a detector. The light source is usually a tungsten halogen lamp which generates a beam that can irradiate the samples. Within the wavelength selection systems the monochromators are the most commonly used, classified under the dispersive instruments. Also the non-dispersive instruments are widely used with a broad variety of selection systems. The main detector can differ, silicon, lead sulfide (PbS) and indium gallium arsenide (InGaS) can be used and all have different properties such as different speed or sensitivity in different regions. (22)

1.4.2 Multivariate data analysis

Spectroscopic data are often multivariate. A multivariate analysis should be performed to make it possible to make an illation from the data. Multivariate calibration by principle component analysis (PCA) and partial least squares analysis (PLS) are frequently used in quantitative NIR analysis. Before performing PCA and PLS data are often pretreated and usually mean centered. (22, 23)

1.4.2.1 Reduction of variables by analysis of principal component

NIR spectral data contains an immense number of correlated variables. The most used and best known method for variable reduction is the principal component analysis or PCA. PCA makes it possible to extract the required information, while eliminating overlapping information. It is a mathematical procedure that extracts information from data tables by transforming them into plots. This leads to new variables, called principal components (PC's). Most of the time the first two PC's represent the maximum variance, but sometimes information is present in the third of even higher PC. The obtained information about the variables and the role they play is found in the so called loading plot. (22-24)

1.4.2.2 Multivariate calibration for quantitative analysis by partial least squares

Partial least-squares (PLS) regression is one of the frequently used multivariate regression methods used in quantitative NIR analysis. PLS is useful when a large set of independent variables has to be predicted. The ultimate goals of PLS is to predict the regression coefficients in a linear model with a large numbers of x variables. PLS combines the spectroscopic data matrix X (a set of independent variables) and the concentration matrix Y (a set of

dependent variables). The PLS algorithm tries then to find the direction of the X space, which explains the maximum multidirectional variance direction in the Y space. (22, 23)

2 OBJECTIVES

Freeze-drying is a widely used technique to improve stability and increase shelf-life of heat sensitive and water labile medicines. Ordinary pharmaceutical freeze-drying is a batch-wise process. The traditional batch approach to freeze-drying is associated with many inefficiencies such as inhomogeneous heat transfer and long drying times. Partly because of this, the pharmaceutical industry is presently shifting to continuous manufacturing. During continuous freeze drying the vials are spin freeze-dried which results in a formed solid product over the entire vial wall. This continuous freeze-drying technology has shown better stability compared to batch freeze-drying.

The goal of this POL project was to obtain a predictive and robust PLS model for the residual moisture content of spin freeze-dried vials filled with a mannitol-sucrose solution using NIR as in-line PAT tool.

To develop the model solutions with a determine distribution were prepared. The solutions consisted of mannitol/sucrose mixtures with different weight ratios (1:1, 7:3 and 9:3). Mannitol and sucrose were chosen since they are frequently used as excipients in freeze-dried products. The vials were filled and spin frozen. After spin freezing, drying was performed in an batch freeze-dryer. The dry samples with a remaining certain moisture content were measured in-line with NIR in a single vial freeze-drying prototype and Karl Fisher titration was used as a reference method to define the residual moisture content. Multivariate data analysis (PCA/PLS) was performed to understand the results. The NIR method for the quantification of moisture content was validated in accordance with the ICH guidelines by check linearity, range, limit of quantification, limit of detection, robustness and accuracy.

Furthermore, small, but deliberate variations in method parameters were made in two different test sets to measure of the model has the capacity to remain unaffected and reliable during normal usage. A protein, BSA, was added to one of the test sets, the filling volume of the other test set was adjusted as well as the weight ratio of mannitol and sucrose. From this, the robustness of the obtained model can be determined.

Lastly, of each weight ratio one sample was characterized after freeze-drying by off-line Raman Spectroscopy, to become additional information concerning the solid state of mannitol.

3 MATERIALS AND METHODS

3.1 SAMPLE PREPERATION

Samples for lyophilization were prepared by filling 3.9 ml solution into 10 ml glass freeze-drying vials (Schott, Mullheim, Germany). The solutions of 50 mg/ml consist out of mannitol (Abc Chemicals, Nazareth, Belgium) and sucrose (Merck, Darmstadt, Germany) in different weight ratios. The following weight ratios were used 1:1, 7:3 and 9:1 (binary mixtures). This resulted in 26 samples and were used ass reference set. Mannitol and sucrose are common excipients in a freeze-dried product. Mannitol acts as bulking agent which led to good cake structures. Sucrose is added as a lyoprotectant.

Besides this, two test set were prepared for testing the robustness of the model. One test set consist of mannitol and sucrose in a different weight ratio, the 6:4 ratio. These samples were prepared by filling 3 ml of the solution into a glass vial. Another test set was prepared by adding Bovine Serum Albumin (BSA) to a 50 mg/ml mannitol-sucrose solution (7:3) (tertiary mixture). Both test sets resulted in 25 samples in total. An overview of the sample distribution is given in table 3.1.

Table 3.1 Schematic overview of the sample composition.

Mixture	Mannitol-sucrose ratio	Content of mannitol and sucrose mixture (mg ml^{-1})	Filling volume vial (ml)	BSA (mg/ml)
		Reference set		
Binary	1:1, 7:3 and 9:1	50	3.9	/
		Test set		
Binary	6:4	50	3.0	/
Tertiary	7:3	50	3.9	5

Samples with different residual moisture were obtained by using different temperatures during secondary drying .

3.2 FREEZE-DRYING

Vials filled with the mixture were attached to a vial holder which vertically rotated the vial at about 4.000 - 5.000 rounds per minute (rpm) (Wiggens High Speed Overhead Stirrer WB6000-D, Straubenhardt, Germany) to become a homogeneous product layer across the whole vial wall. Subsequently, a compressed air which is cooled by liquid nitrogen (LN_2) was blown onto the rotating vial until the vial was completely solidified. The freezing rate during spinning was adjusted by controlling the gas flow, a freezing rate of approximately 30°C/min was used. A self-programmed program in LabVIEW, was used for this. The spin frozen vials were partially stoppered and quickly transferred to a metal cylindrical vial holder which was placed inside a pre-cooled batch freeze-dryer (GEA Group, Köln, Germany) of -40°C.

After spin freezing, drying was performed in an Amsco FINNAQUA GT4 freeze-dryer. This is a single chamber freeze-dryer with three shelves. Vials were placed in an aluminum vial holder. After transferring the vials into the freeze dryer the pressure inside the chamber was dropped to 100 µbar. The pressure inside the drying chamber was regulated by addition of inert gas. Next the temperature of the shelves was increased to -28 degrees, to provide the energy needed for sublimation. This temperature and pressure were held constant for 12 h to perform primary drying. Based on comparative pressure measurement the termination of primary drying was detected. Secondary drying was achieved using variable shelf temperatures, from 28 °C to 30 °C, in order to acquire samples with different residual moisture contents. The chamber pressure stays at 100 µbar during secondary drying. When the freeze-drying cycle was completed vials were sealed with a silicon Wester RS stopper (West Pharmaceutical Services, Exton, Pennsylvania, USA).

The dried vials with a remaining certain moisture content are transferred to a single vial freeze-drying prototype (Rhea Vita, Belgium) where there NIR spectra were measured while rotating.

3.3 NEAR INFRARED SPECTROSCOPY MEASUREMENT

In the single vial freeze-drying prototype there is a possibility to record in-line the NIR spectra of the rotating vials through the implementation of a fiber optic probe in the vacuum chamber. A SentroPAT FO NIR spectrometer

(Sentronic, Dresden, Germany) was used. Samples were measured through the side of the rotating vial. Every ten seconds a spectrum was collected in the 1100 to 2200 nm region with a wavelength accuracy of 1 nm. About three spectra were measured per specific sample conditions.

Furthermore, a FLIR A655sc IR thermal camera (FLIR Systems Inc, Wilsonville, United States) was implemented at the single freeze-dryer prototype which continuously measured the temperature of the vial. The camera was hanged outside the freeze-drying chamber in front of an IR transparent window. The emissivity of the glass vial was 0.93. Also an IR heater with a Voltcraft PPS-11360 power supply (Conrad Electronic, Hirschau, Germany) was added to allow the vial to be heated to a stable temperature of around 38-40 degrees. The heater was located in the freeze-dryer at an angle of 90° to the thermal camera.

Spectra were measured for each individual sample in three ways. First, the spectra was measured from the vials at room temperature. Then the heater was switched on, around 13 V. When a stable temperature was reached around 38-40 degrees the spectra was measured. The heater was then switched off and the spectra recorded immediately afterwards. Up to three spectra were recorded for each state.

3.4 KARL FISHER TITRATION

The reference analysis used to measure the residual moisture content for the samples was performed using Karl Fisher titration. After recording the spectra of the samples, the real moisture content was determined using this method. The cake was dissolved with approximately 3 ml of a 1:1 solution of dry methanol (Honeywell, Germany) and dry formamide (Honeywell, Germany). The entire volume of the vial was injected into the system, a Mettler Toledo V30 volumetric Karl Fisher titrator (Schwerzenbach, Switzerland) and titrated with Hydranal titration solvent (Honeywell, Germany).

3.5 MULTIVARIATE DATA ANALYSIS

The obtained NIR spectra were analyzed by using multivariate data analysis. A Principle Component Analysis (PCA) and a Partial Least Square (PLS) are performed using the multivariate data analysis software SIMCA® 16 (Umetrics, Umea, Sweden).

Spectra were imported and y variables were indicated. Subsequently data preprocessing methods were done to improve the results of qualitative and quantitative multivariate applications. Various wavenumber ranges and data

preprocessing techniques were compared to obtain the best possible model. All NIR spectra were mean-centered, Standard Normal Variate (SNV) and Savitzky-Golay (SG) corrected before analysis. SNV correction is used to reduce the physical influence on the spectrum. A Savitzky-Golay filter has the purpose of smoothing the data. Further the data were processed by PCA and PLS. PCA and PLS are projection methods. These methods reduce the dimension in a multivariate dataset.

3.6 RAMAN SPECTROSCOPY

Extra information regarding the solid state of mannitol can be acquired by using Raman spectroscopy. Of each weight ratio one sample was characterized after freeze-drying by off-line Raman Spectroscopy. A Kaiser Raman Rxn2 Spectrometer (Kaiser Optical Systems Inc, Ann Arbour, Michigan, USA) was used, equipped with a non-contact fibre-optic pharmaceutical area testing (P^hAT) HEAD Raman probe (laser wavelength 785 nm). The exposure time for data collection was set at 30 s. All samples were analyzed over the range 175 to 1890 cm^{-1} and with 3 accumulations per sample. iC Raman™ V4.1 software (Mettler-Toledo, Columbia, Maryland, USA) was used for data acquisition. Data analysis was performed using the software SIMCA® 16 (Umetrics, Umea, Sweden). The spectra were pre-processed by Standard Normal Variate (SNV).

4 RESULTS AND DISCUSSION

4.1 PRINCIPAL COMPONENT ANALYSIS

All 51 spin freeze-dried samples were measured with NIR spectroscopy. The samples differ in weight ratio mannitol-sucrose, composition and relative moisture. Therefore a multivariate analysis was performed on the data. Twenty-six of the 51 samples were included in the development of the PLS model. The moisture content was in the range of 0.34% to 6.89%. The appearance of the spin freeze-dried samples was in general uniform. To acquire a dataset suitable for analysis scaling of the data is essential. Wherefore, all spectra were mean-centered.

4.1.1 Principal Component Analysis of untreated data

For every single vial three spectra were in-line collected in three different ways. The NIR spectra were analyzed in the range from 1100 to 2200 nm. First the untreated data were analyzed. In figure 4.1 the raw NIR spectra are shown. Three groups can be distinguished on the spectra of the raw data. Furthermore the raw spectra are hard to interpret. Preprocessing steps are applied to focus on the information of interest and to reduce the impact of disturbing variations.

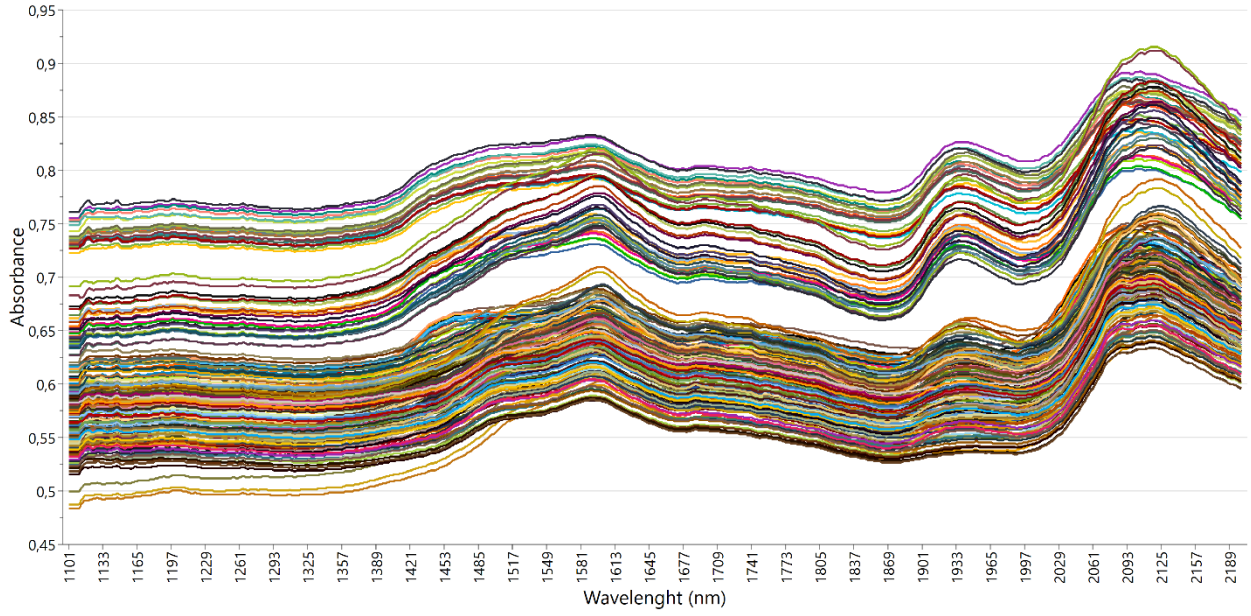

Figure 4.1 Raw NIR spectra.

Analysis of the untreated data can be helpful to remark outliers. Figure 4.2 shows the principal component analysis score plot of the untreated data of PC1 and PC2. The points in the PCA score plot were colored by mannitol-sucrose ratio, clusters of samples having the same weight ratio. Most of the samples are clustered around the centrum of the plot. One group of samples is located on the edge of the score plot. These are samples of the 1:1 weight ratio mannitol-sucrose. These samples were more unstable at a higher moisture content, so fewer samples of the 1:1 ratio were measured in this moisture content range. The reason for their instability could be explained by the fact that the mannitol is amorphous instead of crystalline when a higher content of sucrose is present in the sample. If more samples were measured in this higher residual moisture range, the score plot would probably look different and these samples would not be considered outliers. Therefore these samples were not excluded from the model.

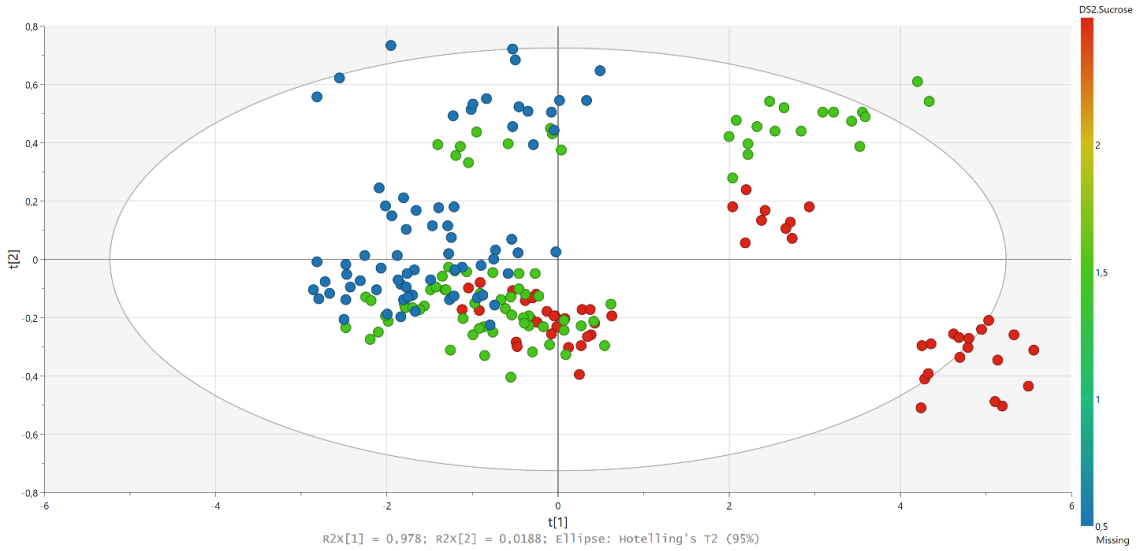

Figure 4.2 Principal component analysis of untreated spectra. Score plot of PC1 against PC2., samples coloured according to sucrose weight ratio.

A trend can be seen, when looking diagonally from the 4th to the 2nd quadrant a decrease in sucrose content in noticed. Conversely, an increase in mannitol content can be observed. This distribution might explain a difference in the presence of different forms of mannitol. Figure 4.3 shows the loading plots op PC1 and PC2 in the range from 1100 to 2200 nm.

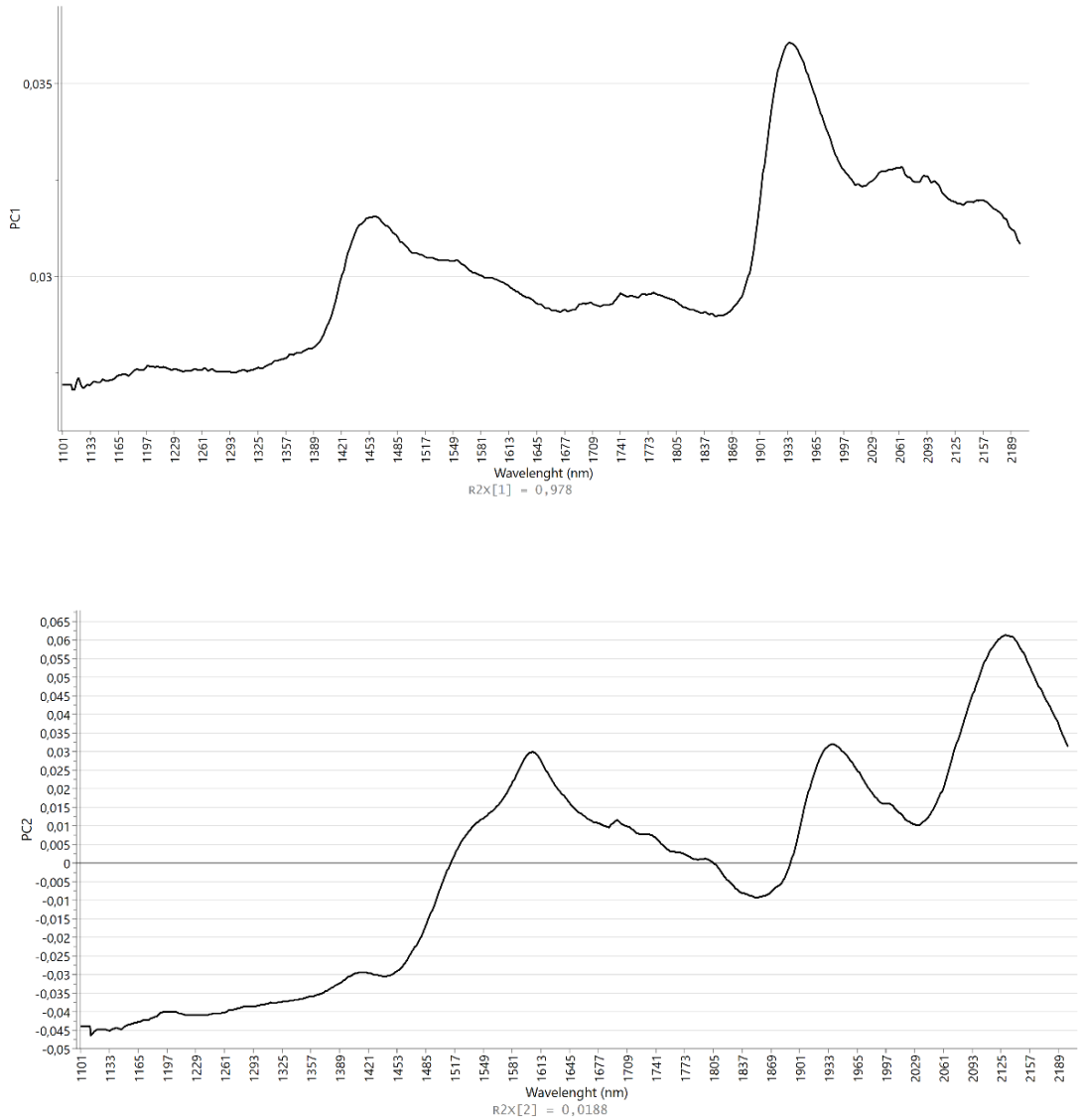

Figure 4.3 Loading plot of the first (top figure) and 2nd (bottom figure) principal component of the untreated spectra.

When coloring the samples according to relative moisture no trend is observed on the PCA score plot of PC 1 and PC2. However, on the PCA score plot of PC1 and PC3 a trend can be seen. An increase of residual moisture of the samples is noticed when moving from the 3th to the 1st quadrant (Figure 4.4). The loading plots (Figure 4.5) of these components show a peak at 1920 nm, which corresponds to the OH combination band of water.

PCA analysis of the untreated data allowed to detect difference in mannitol-sucrose weight ratio and residual moisture content between the different samples. Also no strong outliers were observed.

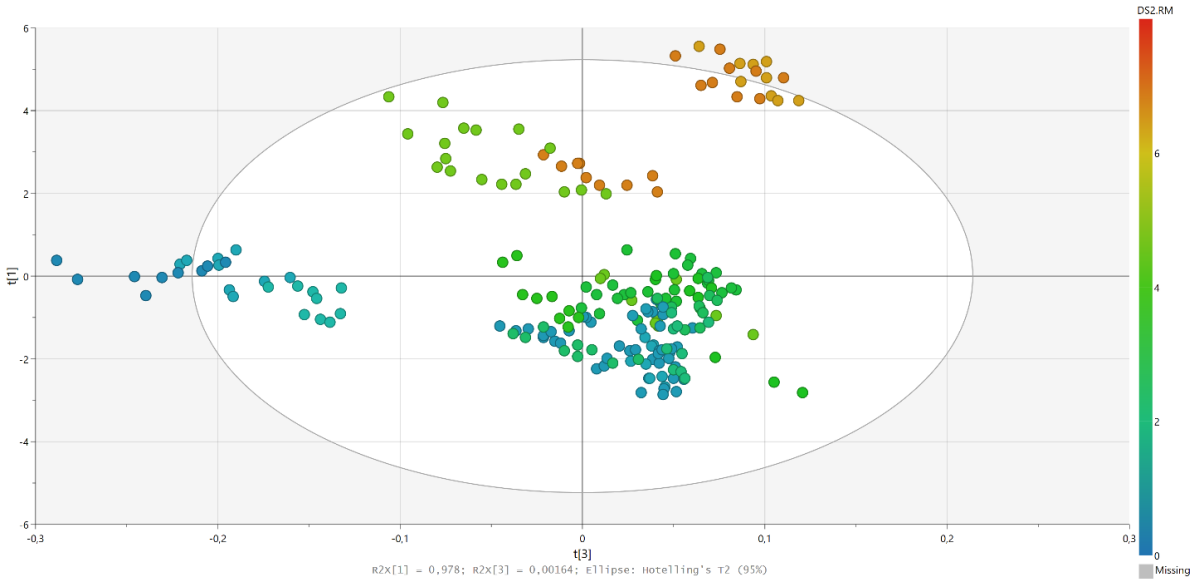

Figure 4.4 Principal component analysis score plot of PC 1 and PC3. Samples coloured according to residual moisture content.

Figure 4.5 Loading plot of PC3.

4.1.2 Principal Component Analysis of the pretreated data

Before performing the PCA analysis preprocessing steps were performed. It was shown in earlier studies (12) that mean-centering and SNV (Standard Normal Variate) transformation of the spectra lead to good results to achieve a reliable model for the determination of moisture in freeze-dried mannitol-sucrose samples. Therefore all spectra were mean-centered and SNV corrected. By mean-centering and scaling each individual spectrum SNV corrects for scattering and particle size effects. A Savitzky-Golay (SG) filter was also added next to the SNV correction to smooth the spectra. This is a moving window averaging method. Hereby a window is selected where the data are fitted by a polynomial of a certain degree. The central point in the window is replaced by the value of the polynomial. (25) This preprocessing step was also always applied in the lab because it was found to give better results. The visual inspection of the SNV corrected spectra with additional SG smoothing are shown in figure 4.6. This did not give refined data.

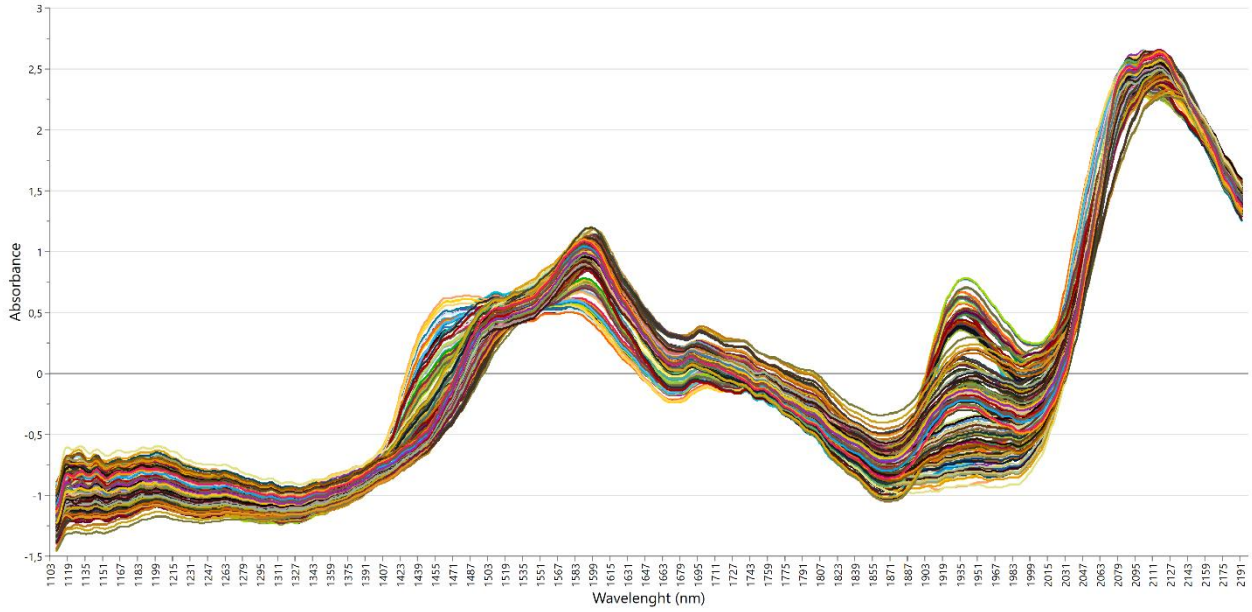

Figure 4.6 Visual inspection of the SNV corrected spectra with additional SG smoothing.

Furthermore several other preprocessing techniques were tested. A first derivative transformation, a quadratic smoothing with 15 points in each sub-model, was added. Small differences between NIR spectra are sometimes not obvious to observe. Deriving spectra decreases S/N ratio and ensures also smoothing of the spectra because noise can

have a large derivative value. Figure 4.7 shows the NIR spectra of the pretreated data with the extra first derivative transformation. A more interpretable spectrum is obtained which will lead to a simpler and reliable model.

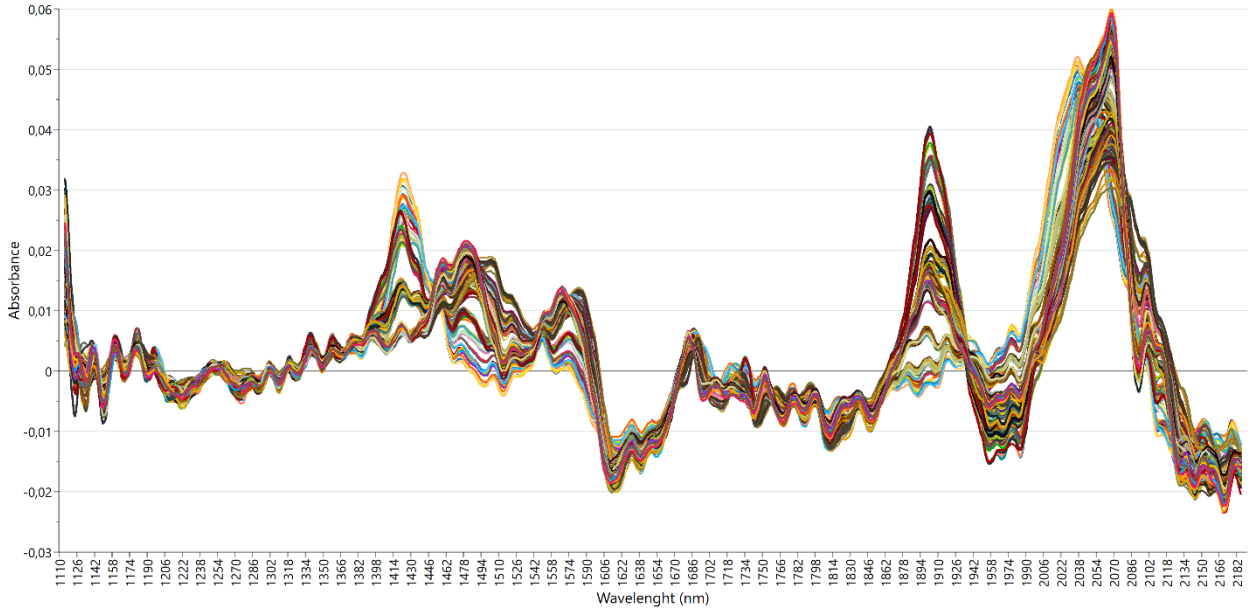

Figure 4.7 Visual inspection of the SNV corrected spectra with additional SG smoothing and a first derivative taken.

The spectra in figure 4.6 some enthralling peaks can be identified. The peak at a wavelength of approximately 1935 nm corresponds to the combination band of O-H stretching and bending of water molecules while the region of 1420 to 1740 nm can be related to the ratio of sucrose and mannitol in the formulation. The combination band of water should also be found in this region, however the interpretability of this information is difficult since mannitol and sucrose also absorb in this region. In the spectra with the additional first derivative (Fig.4.7), the moisture region can be found in the range of 1860 – 2020 nm. The peaks between 1380 and 1620 nm can be used to model to the sucrose and mannitol content. Figure 4.8 and 4.9 shows the SNV corrected NIR Spectra with additional SG smoothing and a first derivative taken colored by mannitol content and by residual moisture content to get a better view.

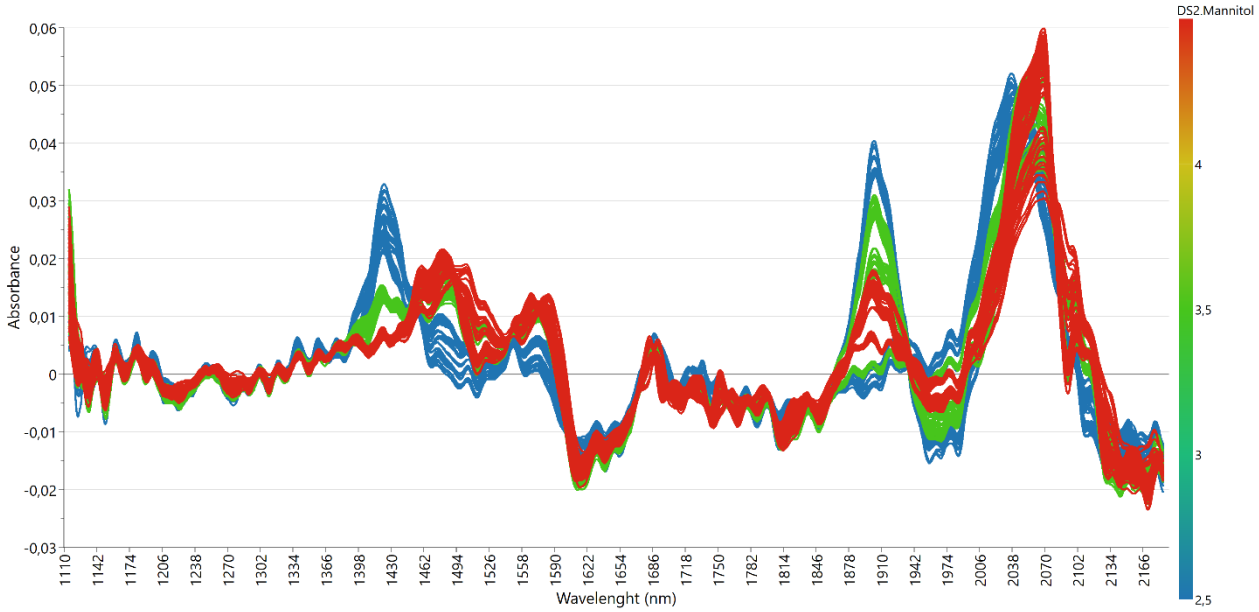

Figure 4.8 SNV corrected NIR Spectra with additional SG smoothing and a first derivative taken colored by mannitol content. The difference in mannitol content is best discerned around 1420 nm.

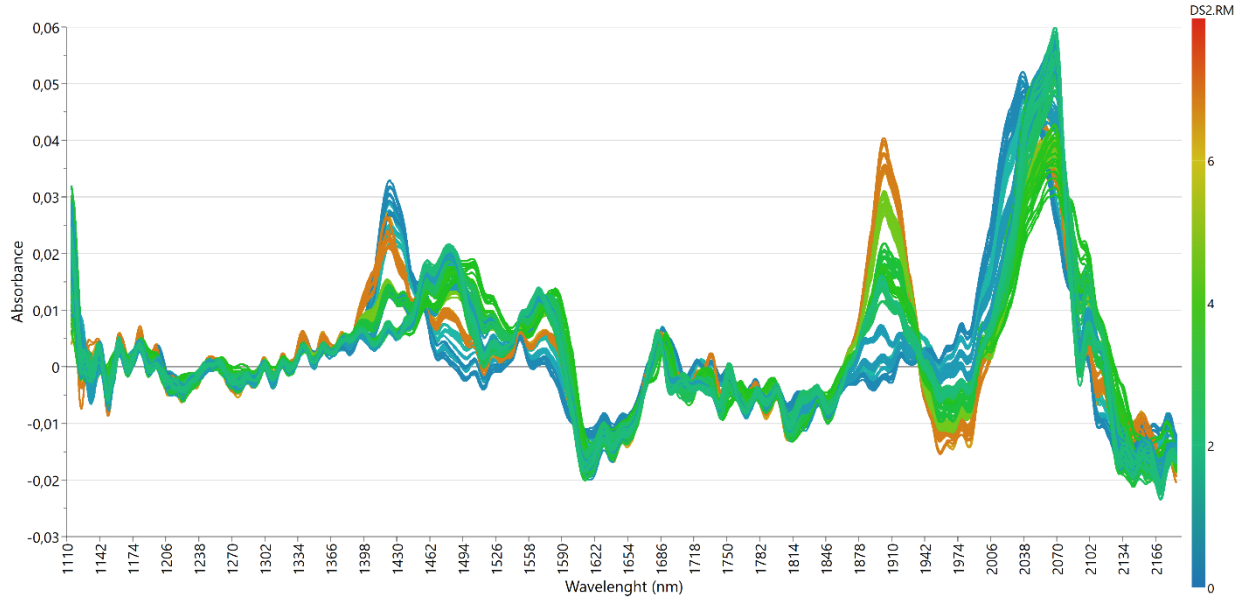

Figure 4.9 the SNV corrected NIR Spectra with additional SG smoothing and a first derivative taken colored by residual moisture content. The different moisture content are best distinguished around 1910 nm.

4.1.2.1 Comparison of the first and the second principal component

Figure 4.10 shows the principal component analysis score plot of PC1 and PC2. Coloring the samples according to residual moisture content, a trend of samples along the first principal component is seen. Samples with a higher residual moisture content are seen most on the right of the first principal component, when samples with a low residual moisture content lie to the left of the first PC.

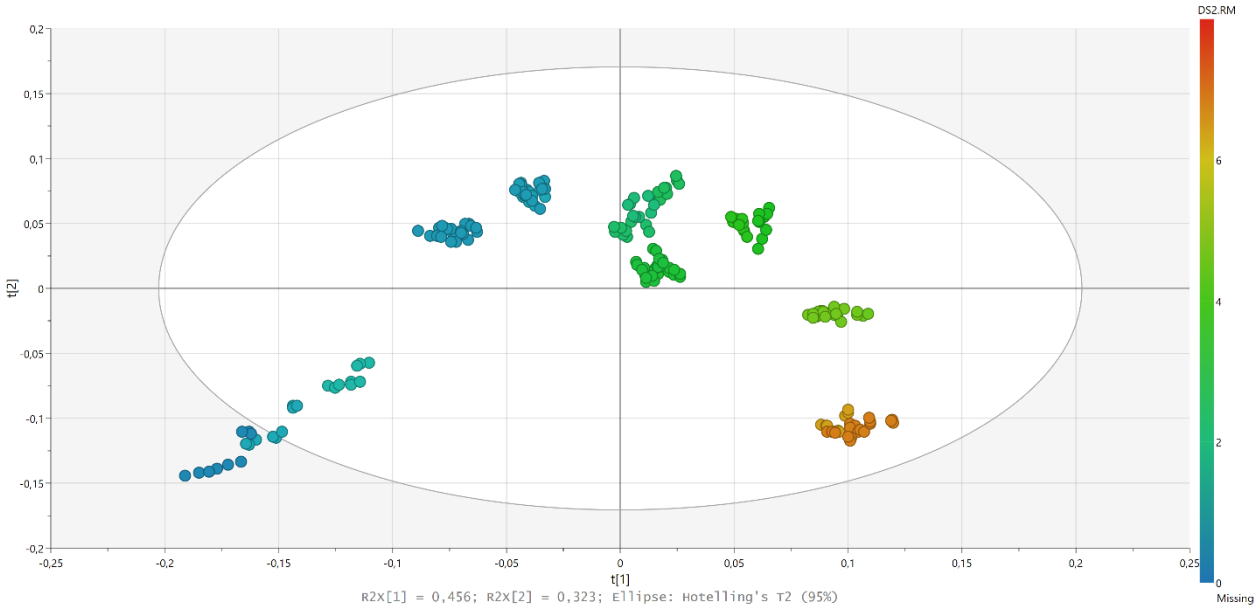

Figure 4.10 PCA score plot of PC1 and PC2. Points coloured according to residual moisture.

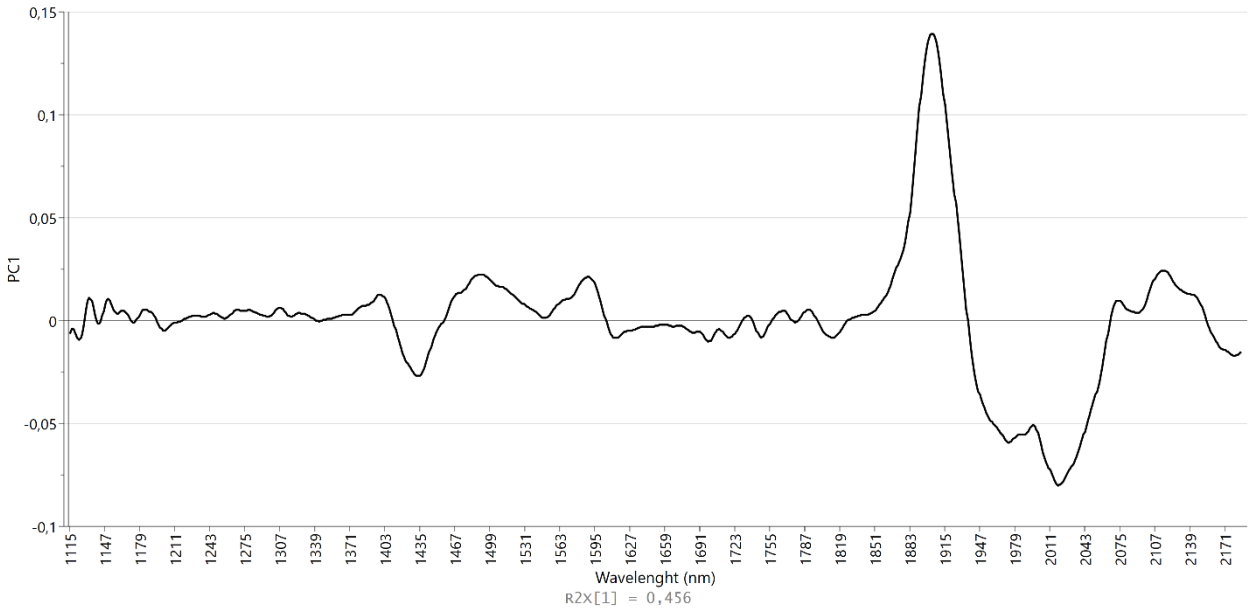

Figure 4.11 Loading plot of PC1.

The trend can be explained when looking at the loading plot of PC1 in Figure 4.11. The plot shows a peak around 1900 nm, corresponding to a strong band in the spectra of water, the combination band of O-H stretching and bending of water molecules.

Figure 4.12 shows the scattered score plot of PC1 and PC2, now the samples are colored by sucrose content. A random distribution of the samples is seen along the first principal component. But a tendency can be seen along the second principal component. Samples containing a high amount of sucrose have a low value for PC2 when samples containing a higher amount of sucrose have a higher value for PC2.

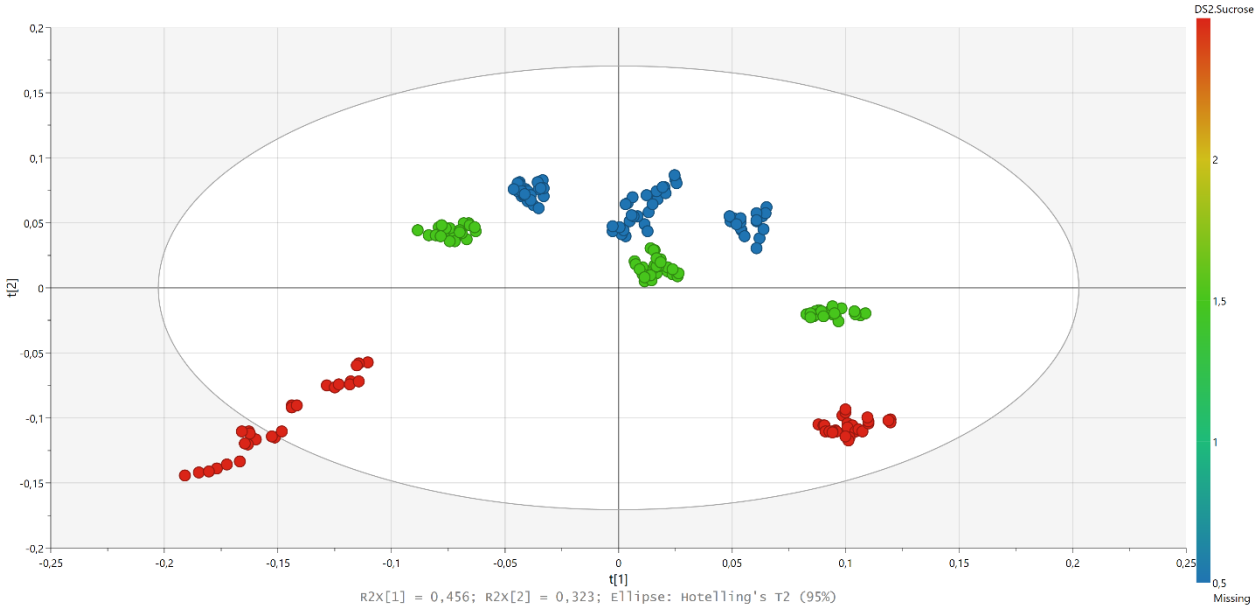

Figure 4.12 PCA score plot of PC1 and PC2. Points colored according to sucrose weight ratio.

Looking at the loading plot of PC2 shown in figure 4.13, the plot shows a strong maximum around 1480 and 2100 nm, similar to strong bands in the spectra of mannitol and sucrose.

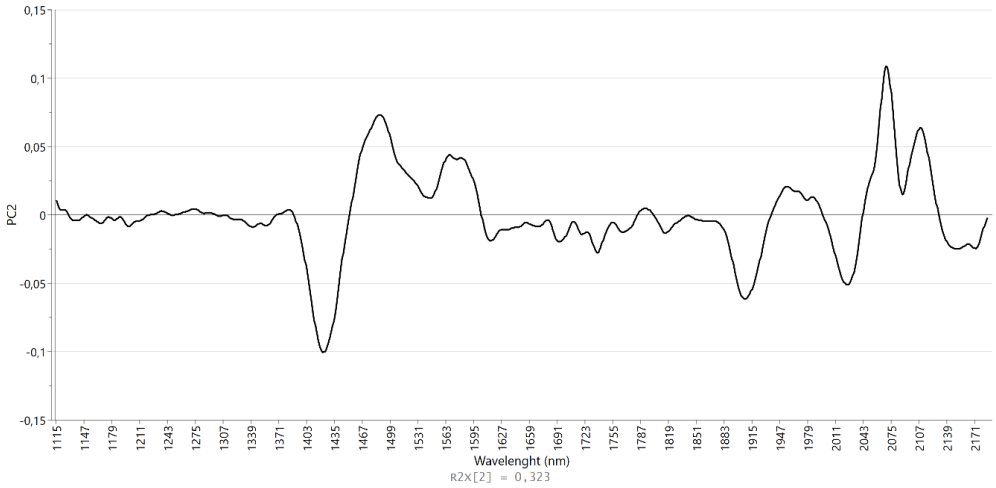

Figure 4.13 Loading plot of PC2.

4.1.2.2 Comparison of the other principal components

Residual moisture content and the mannitol-sucrose content can be seen in respectively PC1 and PC2. A third variable, the heater, could not be seen in the first 2 components. When looking at the PCA score plot of PC 3 and PC4 (Figure 4.14) no clear conclusion can be drawn. The red points (heater on) lie more to the right of the score plot, but no clear separation can be made between the samples measured with heater on or off.

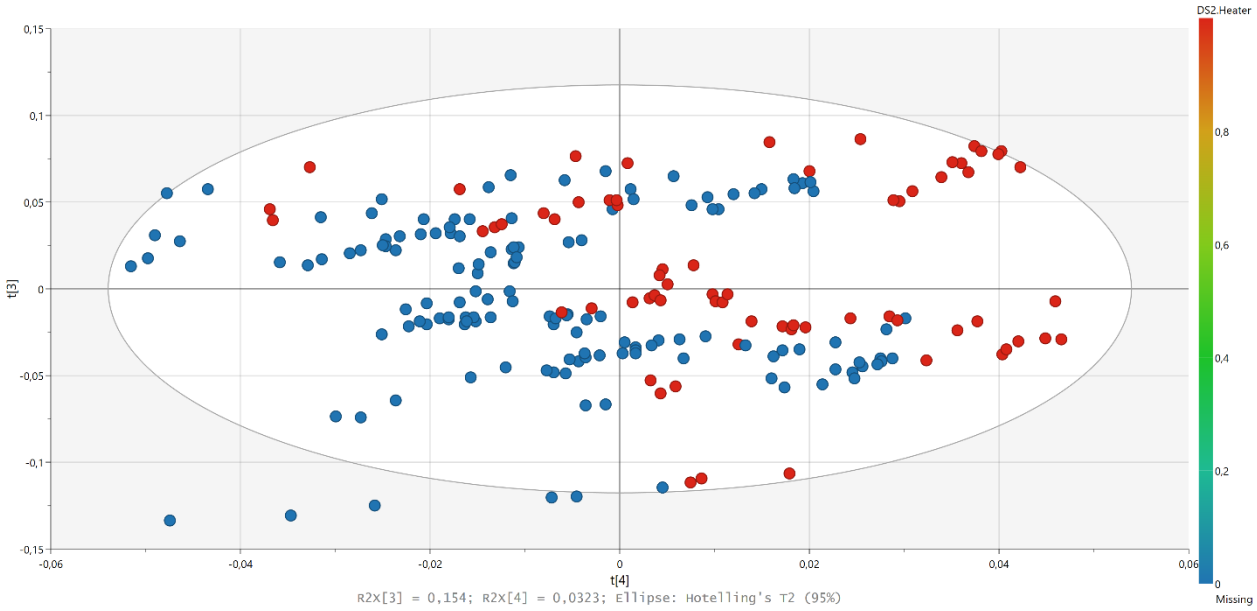

Figure 4.14 PCA score plot of PC3 and PC4. Point colored according to heater on or off.

Out of this it can be concluded that the greatest variability between the samples can be explained by the difference in residual moisture content and mannitol-sucrose weight ratio because these variables lie in the first two components which explain the greatest variability. The heater is more difficult to see in the components and will therefore only determine non to a small part of the variability. This, for example, due to the reflection of the light on the glass.

4.2 ESTABLISMENT OF THE NIR MODEL

The results concerning the construction of a NIR model for the quantification of residual moisture content are mentioned in this section. Ad first the correlation was examined among the observed and the predicted moisture content. Further the development and the validation of the obtained model for residual moisture quantification is reviewed. Last the robustness of the model is discussed when varying the sample composition.

4.2.1 First look at the Observed versus Predicted plot

A PLS model including 4 PLS components was built based on the centered and SNV corrected NIR spectra of all samples included in the development of the model in a range from 1100 to 2200 nm. Focusing on smaller wavelength ranges only had minor effects. Also a Savitzky-Golay filter was added to the spectra and a first derivative was taken to smooth the spectra. As already described above, this 1st derivative was taken because it led to better results. Figure 4.15 shows a good correlation between the residual moisture observed with Karl Fisher titration and the predicted residual moisture based on NIR spectra. A RMSEE (Root Mean Square Error of Estimation) of 0.283363 % was obtained.

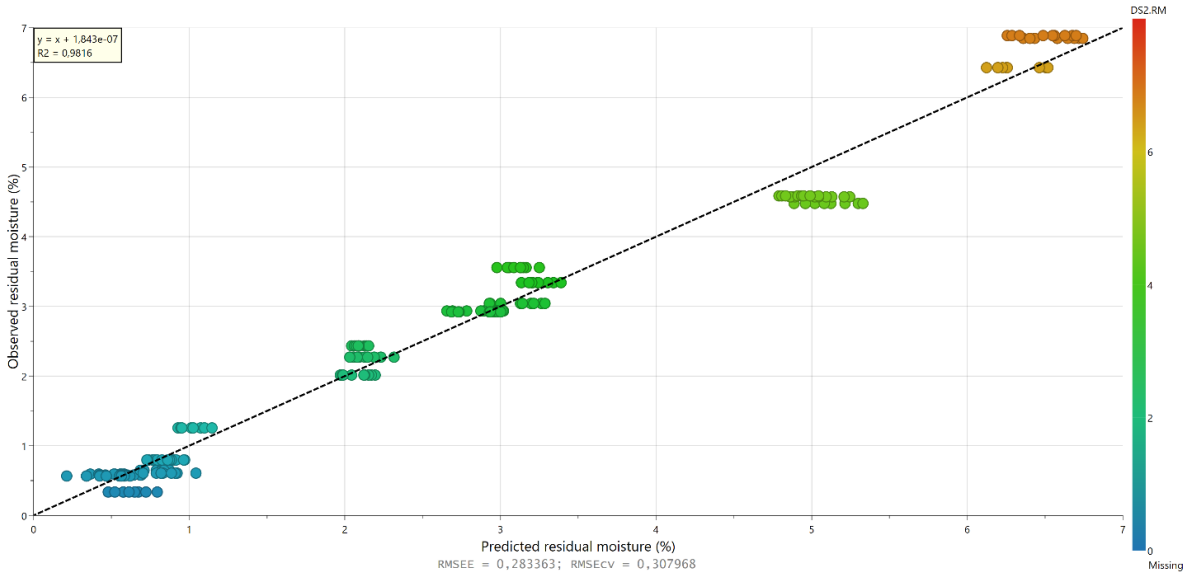

Figure 4.15 Plot of residual moisture observed with Karl Fisher titration versus the predicted residual moisture based on the NIR spectra (RMSEE = 0.283363 %).

A slope value of 1 and a y-intercept value close to 0 were found which indicates there is no relative and absolute bias of the model predictions. The R2 was 0.9816.

The first two PLS components explain about 77% of the correlation between the NIR spectra and the Karl Fisher titration results. On the loading plot of PC1 of the PLS model, figure 4.16, the highest peaks at 1424 and 1910 nm corresponding to the first overtone of OH and the OH combination band of water.

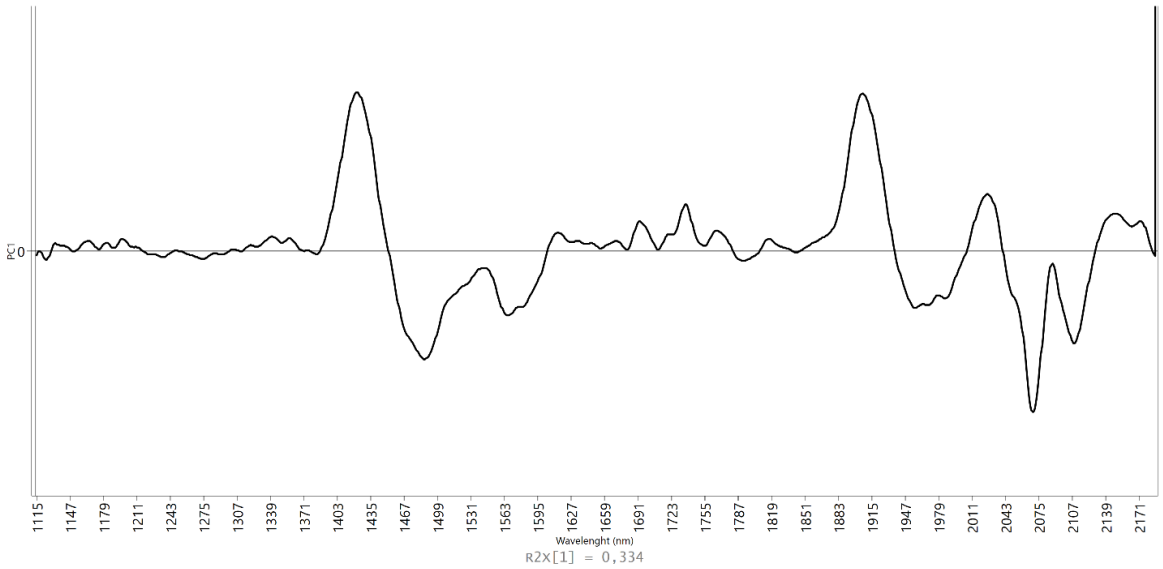

Figure 4.16 Loading Plot of PC1.

4.2.2 Model for the quantification of water

4.2.2.1 Development of the model

To obtain the best possible model, three different PLS models were developed. The same preprocessing steps were added in all models and all data were centered. Three spectral ranges were evaluated: 1100 – 2200 nm (complete range in which the NIR spectra had been taken); 1400 – 2200 nm including the OH combination band and the first overtone of OH vibrations in water and 1800 – 2200 nm including only the OH combination band of water. Based on the predictive power of the model as shown in the RMSEE, the best model was chosen. An overview of the PLS models is given in Table 4.1.

Table 4.1 Results of PLS model in different spectral ranges.

Wavelength (nm)	Number of PLS components	RMSEE (%)
1100-2200	4	0.283
1400-2200	4	0.271
1800-2200	4	0.277

No meaningful differences were observed in the RMSEE: all values are between 0.27 and 0.28%. The model ranging from 1100 to 2200 nm corresponding to the complete range in which the NIR spectra were taken was chosen for further validation.

Further the optimal number of components for the model was determined based on the RMSECV of the model in order to avoid over fitting. On figure 4.17 the number of components in function of the RMSECV is plotted. A model based on 4 PLS components was chosen.

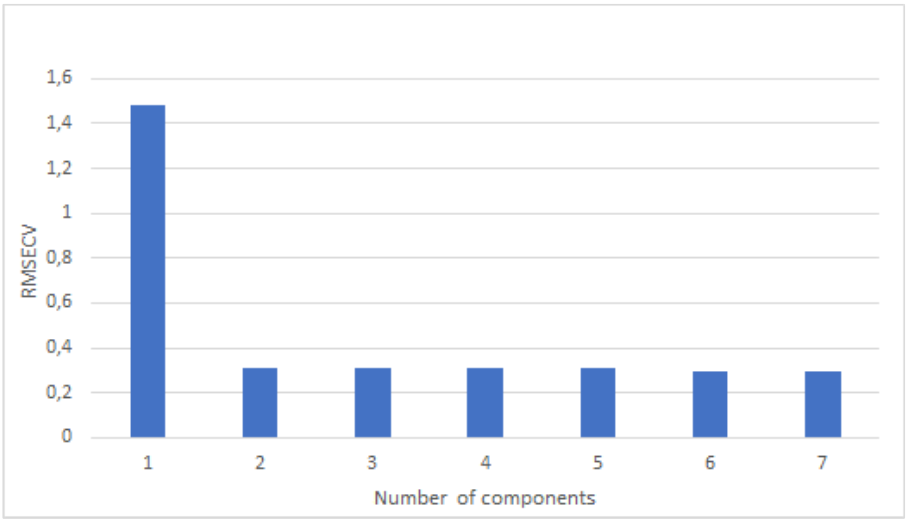

Figure 4.17 Number of components in function of RMSEP.

4.2.2.2 Validation of the model

In this project the validation of the method for the quantification of moisture was processed according to the ICH (International Conference on Harmonization) guideline. This guideline is written for chromatographic techniques, such as HPLC and designed for the validation of methods involving univariate calibration. For data evaluation of NIR spectra, multivariate data analysis is mandatory but specific guidelines for validation of methods involving multivariate data have not been developed yet. The following validation parameters as described in the ICH guidelines were evaluated: linearity, range of application, limit of detection (LoD) and limit of quantification (LoQ), accuaracy, precision and robustness.

Linearity. The linearity is the capacity within the range to become test results which are directly proportional to the concentration of analyte in the sample. (26) To examine the linearity of the method, a regression line was developed between the moisture content observed with Karl Fisher titration and the predicted values based on the NIR measurements. The regression line is showed in figure 4.15 The method is linear when the regression line has a zero intercept and a unity slope. This indicates that the NIR measurements provide equal results as the results obtained with the Karl Fisher titration. The regression equation $y=1x + 1.84*10^{-7}$ was obtained with y being the observed value and x the predicted value. A slope value of 1 and a y-intercept value close to 0 ($1.84*10^{-7}$ exactly) and a regression coefficient (R^2) of 0.9816 were found. The method is qualified as linear.

Range. The range is the interval between the upper and lower limit of analyte concentration that can be determined with an acceptable precision, accuracy and linearity when applying to the described method. (26) In the range from 0.34% to 6.89% moisture content the method was confirmed to be linear, precise and accurate.

Limit of detection (LoD) and Limit of quantification (LoQ). The Limit of detection is the lowest amount of a substance that can be detected but not necessarily quantitated as an exact value. The limit of quantification is the lowest amount of a substance that can be determined with suitable precision and accuracy. (26) The LoD and the LoQ were calculated by measuring a low humidity sample three times and calculating the standard deviation the measurement prediction. The calculated standard deviation was then processed according to the ICH guidelines, as shown in Eqs.. This resulted in a limit of detection of 0.047% and a limit of quantification of around 0.145%.

LoD = 3.3 σ/S

LoQ = 10 σ/S

Where: σ: standard deviation of the response

S: Slope of the regression line

Accuracy. The accuracy expresses the closeness of agreement between the value which is accepted either as a conventional accepted value and the value found. (26) The accuracy profile was generated using the prediction values of PLS models. For the validation of accuracy of the PLS model the profile below was obtained and show the relative error in function of the residual moisture content. Also the acceptance limits of ±20% are indicated on the figure 4.18. The accuracy of the method is roughly accurate below 1.5%.

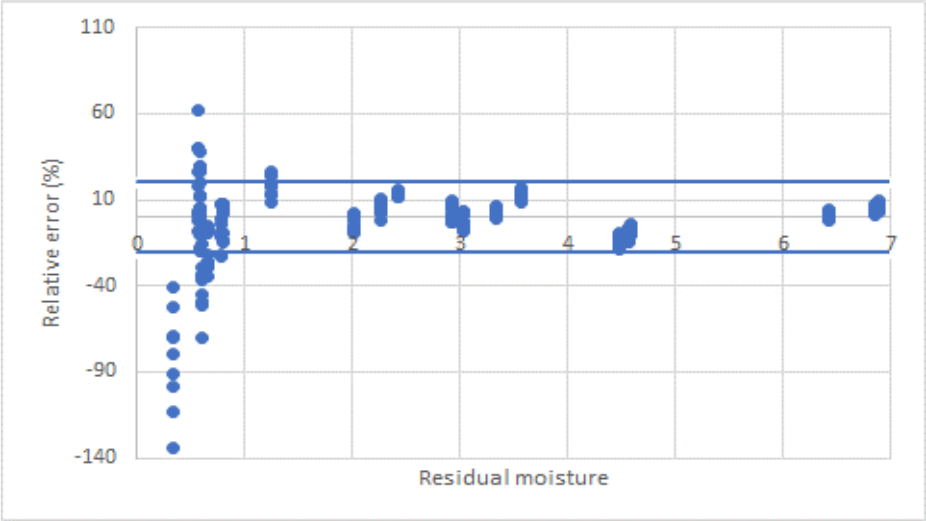

Figure 4.18 The accuracy profile obtained for the validation of the PLS model. The full black lines are the acceptance limits (±20%).

Precision. The precision expresses the closeness of similarity between a series of measurements obtained from multiple sampling of the same sample under the prescribed conditions. In accordance to the ICH guidelines the repeatability was evaluated. The repeatability was determined by measuring three samples, covering the range, three times by the same person, at the same time and with the same instrument. (26) The results are shown in Table 4.2.

Table 4.2 Results of the repeatability precision study.

Sample	Mean moisture content (%)	Absolute spread moisture content (%)	Percent relative standard deviation (RSD) %
1	0.48	0.42 - 0.56	11.6
2	0.73	0.67 - 0.79	6.8
3	2.03	1.98 - 2.12	3.0

The mean moisture content was calculated as well as the absolute and percent relative standard deviation of the moisture content. A percent relative standard deviation from 3 to 12% was found for the moisture content in the repeatability precision study. The highest percent relative standard deviation in moisture content was found in the sample with the lowest moisture content.

Robustness. The robustness is the measure of its capacity to stay unaffected by small variations in method parameters. This provides an indication of its reliability during normal usage. (26) The robustness is discussed in the section below.

4.3 PREDICTION OF SAMPLES OF VARYING FORMULATION COMPOSITION

The next phase was to examine whether the model based on 50 mg/ml mannitol-sucrose mixtures could be applied to predict the residual moisture content of samples with a varying composition, here with samples containing the protein BSA or samples having a different mannitol-sucrose weight ratio with a filling volume of the vial of 3 ml instead of 3.9 ml. This allows a decision to be made about the robustness of the model. Robustness is, according to the ICH guidelines, investigated for small, but deliberate variations.

The estimation of the robustness of the model was done by specifying two prediction sets. The model, discussed above, based on the samples of a mannitol and sucrose solution in different weight ratio's was used to predict the residual moisture content of the test sets. The PLS model was SNV corrected and SG filtered in the range from 1100 to 2200 nm as described above. Also a first derivative was taken.

4.3.1 Prediction set 1

The first prediction set consist out of vials containing mannitol and sucrose in a 6:4 weight ratio. Instead of filling the vials with 3.9 ml of the solution only 3 ml was added. The slope in the observed versus predicted plot had a value of 1.17, a y-intercept of 0.07516 and a R^2 value of 0.9443. A RMSEP (Root Mean Square Error of Estimation) of 0.73309 % was achieved, which was considered too high. Figure 4.19 Shows the plot of the observed residual moisture content versus the residual moisture content forecast based on the NIR spectra for prediction set 1.

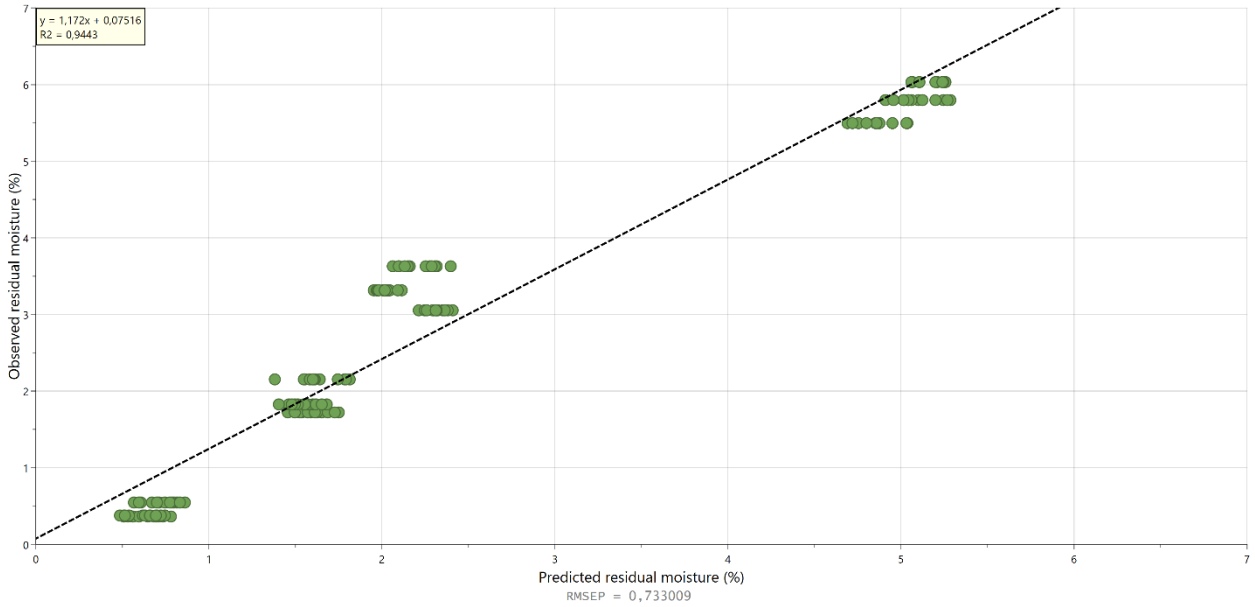

Figure 4.19 Plot of the water content observed with Karl Fisher titration versus the predicted residual moisture content based on the NIR spectra of the test set with weight ratio mannitol/sucrose 6:4 and a 3ml filling volume of the vial.

The mostly underprediction of the 6:4 mannitol-sucrose weight ratio samples may be because of to the presence of amorphous mannitol. A different degree of crystallinity has an effect on the distribution of moisture. The binding of moisture is affected by an increasing amount of amorphous mannitol. If multiple amorphous samples were included in the model, amorphous samples would probably be better predicted.

One sample of each different reference and test set was measured with Raman spectroscopy, this to get an idea of the different physical stated of mannitol. This is described in 4.4.

4.3.2 Prediction set 2

The second prediction set consist out of vials containing mannitol and sucrose in a 7:4 weight ratio. To this solution Bovine Serum Albumin (BSA) was added. The observed versus predicted plot slope had a value of 1.17, a y-intercept of 0.4898 and a R^2 value of 0.9712. A RMSEP (Root Mean Square Error of Estimation) of 0.327769 % was obtained. This is a fair outcome since the difference in with the RMSE of the used model is small. Figure 4.20 shows the plot of the observed water content versus the moisture content predicted based on the NIR spectra.

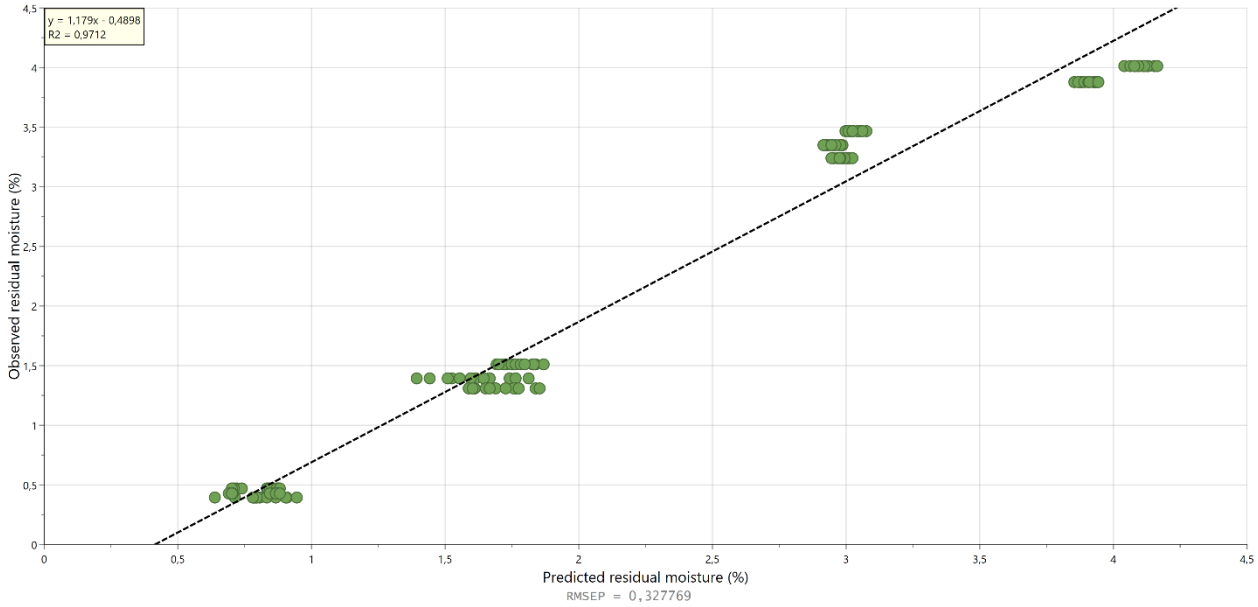

Figure 4.20 Plot of the water content observed with Karl Fisher titration versus the predicted residual moisture content based on the NIR spectra of the test set with weight ratio mannitol/sucrose 7:3 and BSA.

Unlike the 6:4 mannitol-sucrose weight ratio sample, these samples containing the protein BSA in a 7:3 mannitol-sucrose ratio are mostly overestimated by the NIR method. This can be seen from the negative y-intercept of Fig 4.20 ,which is relatively large. A reason for this variation in moisture content might be found in the influence on the crystallization behavior of mannitol.

The obtained PLS model based on NIR spectra is currently not that robust, but this was mainly because the model was not sufficiently trained and the range was taken too large. Based on a better and more comprehensive model, robustness is likely to increase. In spite the fact that the accuracy of the prediction was low for samples which were not included in the calibration set, the model still provides a solid estimation (low or high) of the moisture

content. In addition, the total amount of samples included in the calibration set was still low, which leads to the conclusion that this model can likely be improved by adding more samples.

4.4 RAMAN MEASURMENTS

Mannitol has different polymorphs. Raman spectroscopy is a method to investigated these different polymorphs. One sample of each different composition was analyzed with the Raman microscope. A sample consisting of 100% sucrose was also analyzed. In a range from 270 to 1900 cm^{-1} a PCA analysis was performed. The data were all mean-centered and SNV corrected.

On the score plot (Figure 4.21) of PC 1 versus PC2 a distribution can be seen. The sucrose sample has a low value for PC1, the more mannitol the sample contains the higher the value for PC1 becomes. The sample containing the protein BSA does not differ greatly from the sample in the same weight ratio (7:3) without the BSA.

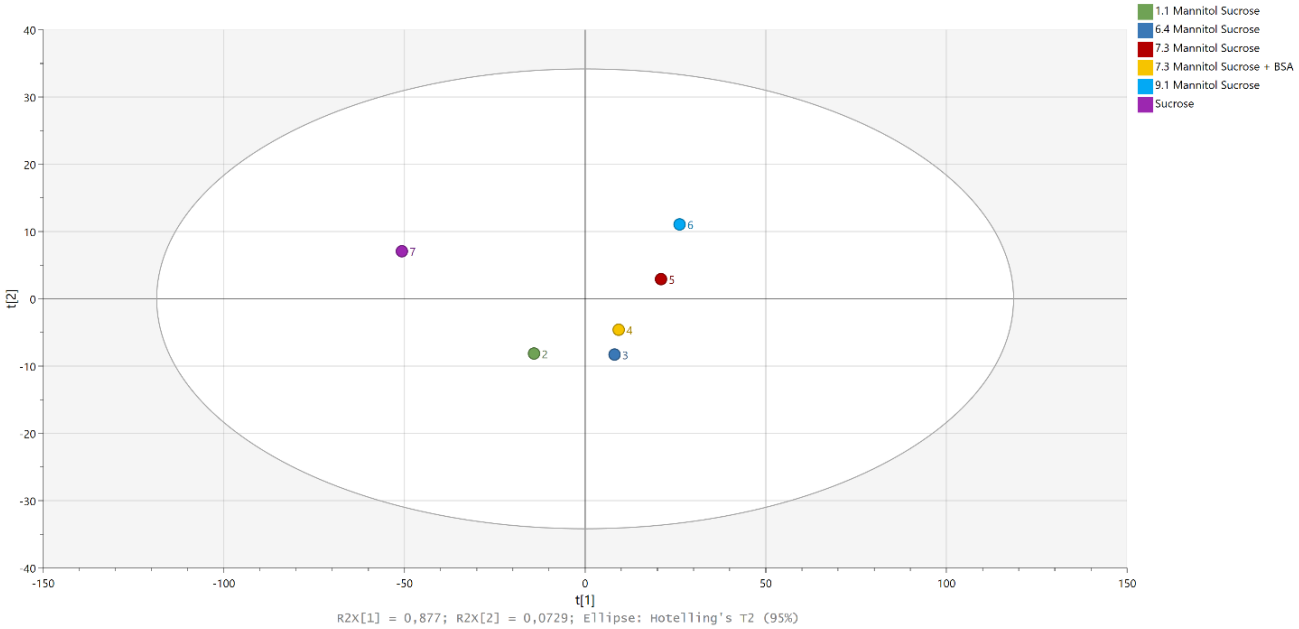

Figure 4.21 Score plot of PC1 and PC2. Samples colored by composition.

The Raman spectra were compared with reference spectra found that are described in the literature. Mannitol has different polymorphs: α-mannitol, β-mannitol, δ-mannitol and a hemihydrate form. For the different polymorphs of mannitol, a reference spectra is used which was published by De Beer et al. (3). The reference spectra of mannitol are shown in figure 4.22. They describe that the different polymorphs forms can be clearly distinguished in the 1000–1170 cm^{-1} region. The β -polymorphic form of mannitol has a characteristic peak at 1037 and at 1135 cm^{-1}. The δ -mannitol form has characteristic peaks at 1054 and 1147 cm^{-1}. The hemihydraat from has a characteristic peak at 1140 cm^{-1}. (3)

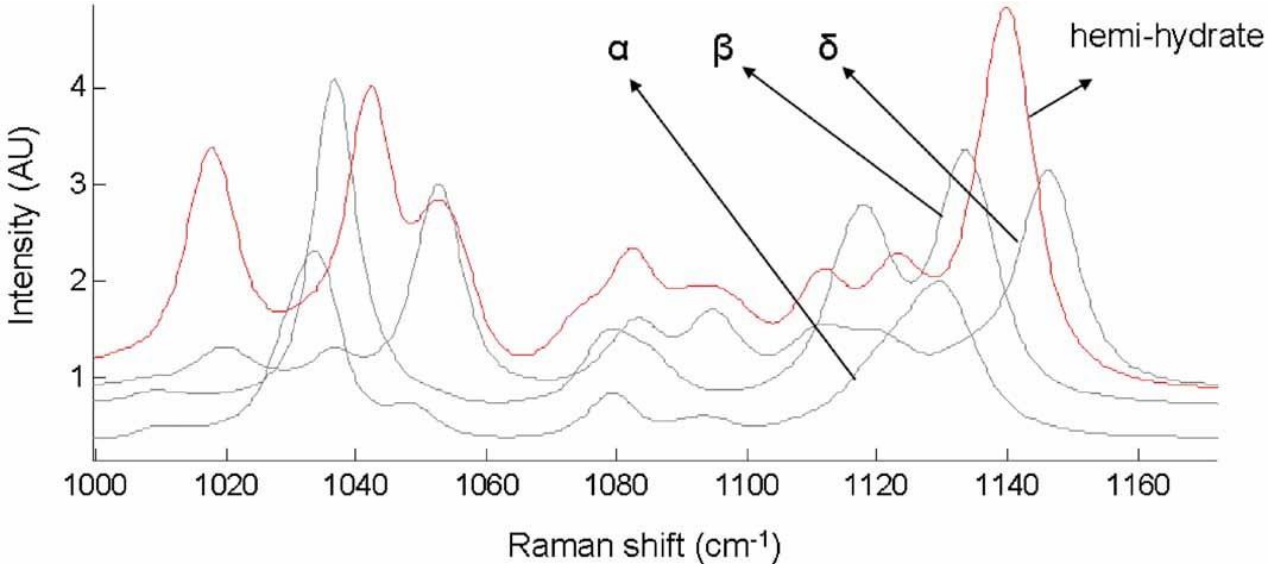

Figure 4.22 Raman spectra of α-mannitol, β-mannitol, δ-mannitol and a hemihydrate form. (3)

A Sucrose Raman spectrum is given by Kerem et al. (2015) (27). The spectra of sucrose has no characteristic peaks in the same position as the characteristic peaks of the different polymorphic forms of mannitol. Figure 4.23 shows the obtained Raman spectra in the full range from 270 to 1900 cm^{-1}. Figure 4.24 shows the Raman spectra in a smaller range from 1000 to 1200 cm^{-1} because as mentioned before the different polymorphs forms can be clearly distinguished in the 1000–1170 cm region. No peak characteristic of mannitol hemihydrate was observed in any of the samples. Samples with a high amount of mannitol, the 9:1 weight ratio and 7:3 weight ratio without the BSA, contained β-mannitol, indicated by peaks at 1037 and 1135 cm^{-1}. These samples also contained the δ polymorphic form which can be seen from the peak at 1054 cm^{-1}. Samples with a decreasing amount of mannitol such as the 6:4 and 1:1 weight ratio do not show any characteristic peaks for a polymorphic form of mannitol. In these samples, mannitol is probably amorphous.

The obtained model can reasonably distinguish between different forms of mannitol and this is therefore not an issue when making a PLS model. But if multiple amorphous samples were included in the model, amorphous samples would probably be better predicted.

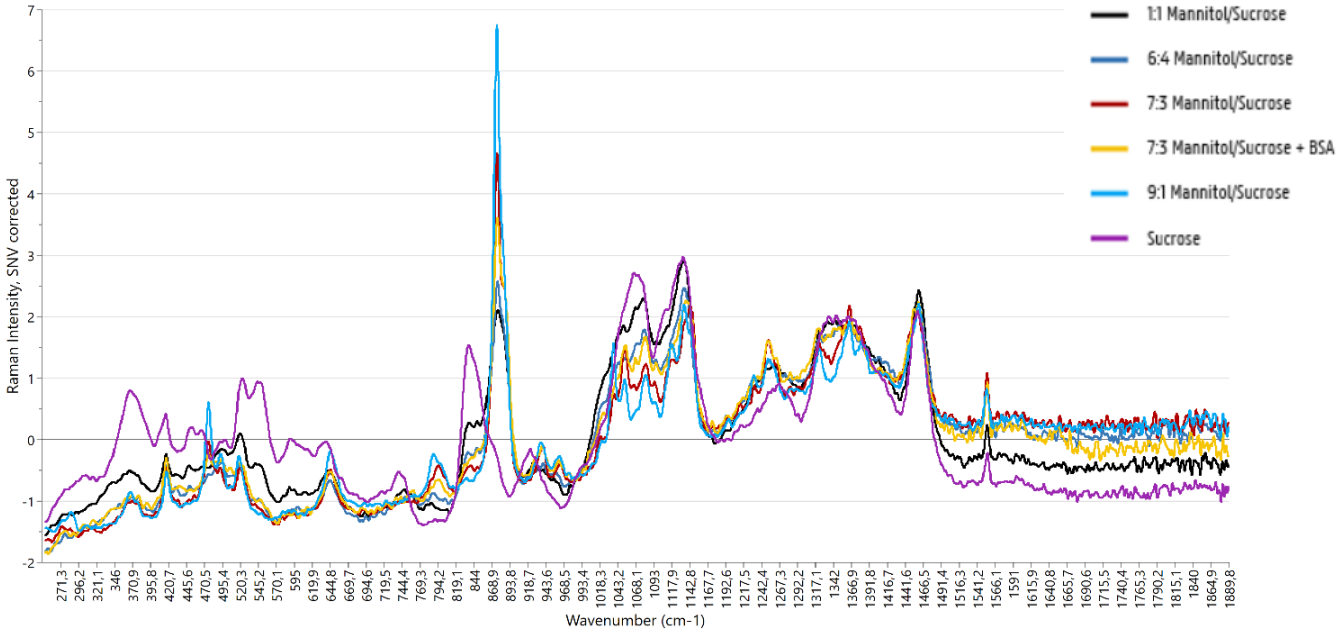

Figure 4.23 Raman Spectra over the total range of 270 - 1900 cm-1.

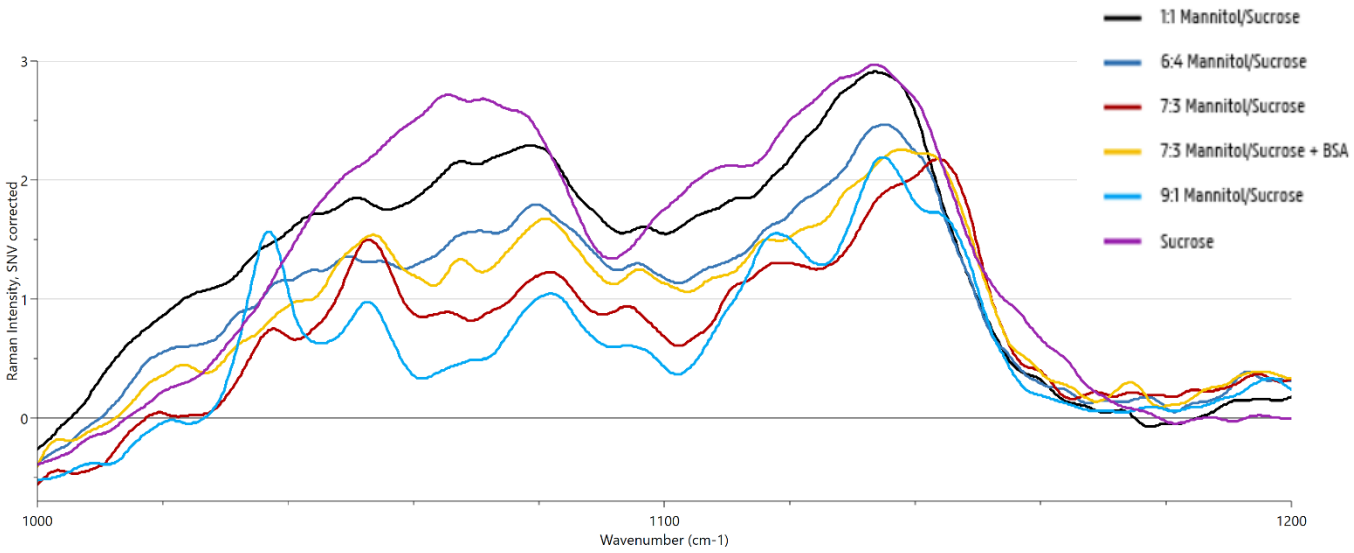

Figure 4.24 Raman spectra in the range of 1000 to 1200 cm-1.

5 CONCLUSION

A PLS model for spin freeze-dried formulations containing mannitol and sucrose was developed to determine the residual moisture content of the samples based on the NIR spectra. It was shown that quantification of moisture content in spin freeze-dried samples of mannitol-sucrose mixtures via multivariate analysis of NIR spectra, correlated with the moisture content measured by Karl-Fisher. For this purpose all NIR spectra were SNV corrected and SG filtered in a range of 1100 to 2200 nm. Also taking a first derivative led to better results. Multivariate analysis yielded a RMSEE of 0.28%. The developed NIR method for the quantification of moisture was also validated according to the ICH guidelines. The model was found to be linear in a range from 0.34 to 0.64%. A LoD of 0.047% and a LoQ of 0.145% was determined. Due to lack of time, only a limited number of samples were used to set up the model. If the reference set were more extensive, the model would get better and better.

Furthermore, it was shown that the model could be applied to samples with varying composition. A RMSEP of 0.74% and 0.31% was achieved when using the model to predict the residual moisture content of samples with a different filling volume of 6:4 weight ratio of mannitol and sucrose or containing proteins in a concentration. The obtained values are not yet optimal. But it does shows that NIR models can be established which can predict unknown samples with varying compositions. The PLS model based on NIR spectra was not that robust, but this was mainly because the model was not sufficiently trained and the range was taken too large. Based on a better model, robustness is likely to increase.

From the research of the solid state properties of the spin freeze-dried samples with Raman spectroscopy it could be concluded that samples containing lower mannitol may be amorphous. A varying degree of crystallinity will influence the distribution of water. An increasing amount of amorphous mannitol can affect the binding of moisture. This also explains why the cakes looked less good from the higher concentrations of mannitol as amorphous mannitol has a low Tg. The obtained model can reasonably distinguish between different forms of mannitol and this is therefore not an issue when making a PLS model. But if multiple amorphous samples were included in the model, amorphous samples would probably be better predicted.

6 FUTURE PERSPECTIVES

The goal of this POL project was to become a model based on a mannitol-sucrose solution and being capable of using this model to predict the residual moisture content of a vial by using NIR as in-line PAT tool. More experiments are required. So gives the obtained model an indication that this goal can be achieved if the model is expanded and refined.

Furthermore more thorough validation of the obtained model is vital and may be done via way of means of evaluating prediction values with experimental observations from samples with an equal composition as samples used to construct the model. Eventually, it must be viable to estimate the residual moisture content of the vial more accurate.

Also more investigation of the solid state properties of the spin freeze-dried samples with Raman spectroscopy as well as with XRPD and NIR spectroscopy would be significant.

7 REFERENCES

1. L. DM. Realisation and evaluation of an innovative continous freeze-drying technology for (bio-) pharmaceutical cylindrical unit doses. Ghent University2018.

2. Jos Corver P-JVB, Thomas De Beer. A continuous and controlled pharmaceutical freeze-drying technology for unit doses. European Pharmaceutical Review. 2018.

3. De Beer TRM, Vercruysse P, Burggraeve A, Quinten T, Ouyang J, Zhang X, et al. In-line and real-time process monitoring of a freeze drying process using Raman and NIR spectroscopy as complementary process analytical technology (PAT) tools. Journal of Pharmaceutical Sciences. 2009;98(9):3430-46.

4. Bermúdez-Aguirre LEG-AJW-CFTV-BD. Freeze-drying: The Basic Process. Encyclopedia of Food and Health. 2016:104-9.

5. Adams GDJ, Cook I, Ward KR. The Principles of Freeze-Drying. In: Wolkers WF, Oldenhof H, editors. Cryopreservation and Freeze-Drying Protocols. New York, NY: Springer New York; 2015. p. 121-43.

6. Nail S. GL. Freeze-drying: principles and practice. Pharmaceutical Dosage forms - parental medications. 2010;2:353-81.

7. Pikal XTaMJ. Design of Freeze-Drying Processes for Pharmaceuticals: Practical Advice. 2004;21.

8. Meyer LD. Realisation and evaluation of an innovative continous freeze-drying technology for (bio-) pharmaceutical cylindrical unit doses. Ghent University2018.

9. Bingquan (Stuart) Wang MJP. Stabilization of Lyophilized Pharmaceuticals by Process Optimization: Challenges and Opportunities. American Pharmaceutical Review. 2012.

10. P. Fonte PRL, V. Seabra, A. J.Almeida, S. Reisa, B. Sarmento. Annealing as a tool for the optimization of lyophilization and ensuring of the stability of protein-loaded PLGA nanoparticles. International Journal of Pharmaceutics. 2016;503(1-2):163-73.

11. Maja Bjelošević AZP, Odon Planinšek, Pegi Ahlin Grabnar. Excipients in freeze-dried biopharmaceuticals: Contributions toward formulation stability and lyophilisation cycle optimisation. International Journal of Pharmaceutics. 2020;576.

12. D. G. Solid state properties of freeze-dried protein formulation Universiteit Gent; 2009.

13. Challener CA. For Lyophilization, Excipients Really Do Matter. BioPharm International. 2017;30(1):32-5.

14. Luigi C. Capozzi BLT, and Roberto Pisano. From Batch to Continuous: Freeze-Drying of Suspended Vials for Pharmaceuticals in Unit-Doses. Industrial & Engineering Chemistry Research. 2019;58:1635-49.

15. Bockstal P-JV. The development of a continuous and controlled freeze-drying concept for biopharmaceutical unit doses.: Universiteit Gent 2017.

16. George L. Reid HWWI, Andrew S. Palm, Koji Muteki, Ph.D. Process Analytical Technology (PAT) in Pharmaceutical Development. American Pharmaceutical Review 2012.

17. Yongjiang Wu YJ, Yerui Li, Di Sun, Xuesong Liu, Yong Chen. NIR spectroscopy as a process analytical technology (PAT) tool for on-line and real-time monitoring of an extraction process. Vibrational Spectroscopy 2012;58:109 - 18.

18. Davies AMC. An introduction to near infrared (NIR) spectroscopy. Norwich Near Infrared Consultancy.

19. M. Wiggenhorn GW. The current state of PAT in freeze drying. 2005.

20. A. Funke RG, S. Tosch, A. Tulke. PAT Series: Recent achievements in NIR-based on-line monitoring of lyophilisation processes. European Pharmaceutical Review 2016;3.

21. C. Schaefer CL, D. Clicq, A. Merschaert, E. Norrant, F. Fotiadu. On-line near infrared spectroscopy as a Process Analytical Technology (PAT) tool to control an industrial seeded API crystallization. Journal of Pharmaceutical and Biomedical Analysis. 2013;83:194 - 201.

22. Espitia VC. Use of NIR spectroscopy and multivariate process spectra calibration methodology for pharmaceutical solid samples analysis. In: Universitat Autònoma de Barcelona S, editor. 2012.

23. Heyden JVaYV. Introduction to herbal fingerprinting by chromatography. Chemometrics in Chromatography. 2018.

24. D.L. Massart and Y. Vander Heyden. From Tables to Visuals: Principal Component Analysis, Part 1. LC•GC Europe 2004;19:586-91.

25. Teukolsky WHPaSA. Savitzky-Golay Smoothing Filters. Computers in Physics 1990;4:669-72.

26. Agecy EM. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology. 1995.

27. Kerem Ilaslan IHB, Ali Topcu. Rapid analysis of glucose, fructose and sucrose contents of commercial soft drinks using Raman spectroscopy. Food Control. 2015;48:56-61.

Master dissertation submitted to the faculty of Pharmaceuticals Sciences, performed in collaboration with the Laboratory of Pharmaceutical Technology.

Academic Promotor: Prof. Dr. Thomas De Beer

Industrial Promotor: Apr. Laurens Leys

Commissionairs: Prof. Thomas De Beer (UGent, voorzitter)

Prof. Guy Van den Mooter (KULeuven)

Prof. Yvan Vander Heyden (VUB)

Prof. Filip Kiekens (UAntwerp)

The information, conclusions and point of view in this master dissertation are those of the author and do not necessarily represent the opinion of the promotor or his research group.