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DÉVELOPPEMENT D'OUTILS NIR ET DE METHODES POUR MONITORER DES PRODUITS DE LYOPHILISATION

DEVELOPMENT OF NIR-BASED TOOLS AND METHODS FOR MONITORING FREEZE-DRYING PRODUCTS

Mémoire de maîtrise
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To,

My Daughter, My Wife and My Parents!

ABSTRACT

The demand to achieve improved drug product quality has been accelerated with the advent of quality by design (QbD) guidance launched by regulatory agencies around the world. This extends to freeze-drying processes, where bio-pharmaceutical products are dried under an extremely controlled environment. Freeze-drying, or lyophilization, is a low-temperature dehydration process that involves multistep transformations making use of the principles of heat & mass transfer. This often renders the process complicated and time-consuming, resulting in large operating costs.

Multiple process analytical technology (PAT) tools have been introduced to monitor product quality attributes in batch dried vials, as these tools help in keeping an eye on the product/process to achieve acceptable product quality attributes. Despite significant advances, many topics remain to be addressed. One of them being the impact of spatial variations in the product attributes, thus rendering the accuracy of in-process results obtained from a single point on the vial surface questionable. Another being the aesthetic appearance of the product, specifically collapse inside the products, which is usually assessed by visual inspection. However, relying completely on human input can be fallible and unrealistic in the production environment as thousands of product vials roll out from the freeze-dryers. Failure to detect an aesthetic defect in the finished freeze-dried product cake may put a patient's life at risk as any defect might be a result of product collapse or meltback affecting the drug safety and efficacy.

This project consisted of two main areas of work. 1) Using NIR Chemical Imaging (NIR-CI) and NIR spectroscopy (NIRS) to investigate the spatial variability of moisture on the surface of the vials undergoing drying. Furthermore, it demonstrates the necessity of using multiple measurement points on the vial surface to quantify moisture inside the freeze-drying products. 2) Using NIRS to identify the physical properties of the product, such as normal or collapsed product. This is performed by leveraging the ability of NIRS to exhibit unique spectra relative to the physical characteristics of the product. Two intensities of collapse were induced in the freeze-drying products, and the potential of NIRS in identifying the collapse during the process and in the finished freeze-dried products was demonstrated.

Results show the promising nature of the NIR-CI and NIRS in combination with the multivariate data analysis (MVDA) methods to monitor product quality attributes and better understand their variability. Overall, this thesis work presents a detailed investigation about the moisture distribution and collapse inside the freeze-dried products.

Keywords: QbD; PAT; NIRS; NIR CI; Freeze-drying, Moisture distribution; Collapse; MVDA

RÉSUMÉ

La demande d'amélioration de la qualité des produits pharmaceutiques a été accélérée avec l'avènement des directives de qualité par la conception (QbD) lancées par les agences de réglementation du monde entier. Cela s'étend aux procédés de lyophilisation, où les produits biopharmaceutiques sont séchés dans un environnement extrêmement contrôlé. La lyophilisation est un de déshydratation à basse température qui implique des transformations en plusieurs étapes utilisant les principe de transfert de chaleur et de masse. Cela rend souvent le procédé compliqué et long, ce qui entraîne des coûts d'exploitation importants.

Plusieurs outils de technologie d'analyse de processus (PAT) ont été introduits pour surveiller les attributs de qualité du produit dans des flacons séchés par lots, car ces outils aident à garder un œil sur le produit / procédé pour obtenir des attributs de qualité de produit acceptables. Malgré des avancées significatives, de nombreux sujets restent à traiter. L'un d'eux est l'impact des variations spatiales dans les attributs du produit, rendant ainsi la précision des résultats en cours de procédé obtenus à partir d'un seul point sur la surface du flacon discutable. Un autre est l'aspect esthétique du produit, qui est généralement évalué par une inspection visuelle. Cependant, se fier entièrement à l'apport humain peut être problématique et irréaliste dans l'environnement de production, car des milliers de flacons de produit sortent des lyophilisateurs. Le fait de ne pas détecter un défaut esthétique dans le gâteau de produit lyophilisé fini peut mettre la vie d'un patient en danger, car tout défaut peut être le résultat de l'effondrement du produit (*meltback*) affectant l'innocuité et l'efficacité du médicament.

Ce projet comprenait deux thèmes principaux. 1) Utilisation de l'imagerie chimique NIR (NIR-CI) et de la spectroscopie NIR (NIRS) pour étudier la variabilité spatiale de l'humidité à la surface des flacons en cours de séchage. 2) Utilisation de NIRS pour identifier les propriétés physiques du produit, en tirant parti de la capacité du NIRS à présenter des spectres uniques par rapport aux caractéristiques physiques du produit. Deux intensités d'affaissement ont été induites dans les produits de lyophilisation, et le potentiel du NIRS dans l'identification de l'effondrement pendant le procédé et dans les produits lyophilisés finis a été démontré.

Les résultats montrent la nature prometteuse du NIR-CI et du NIRS en combinaison avec les méthodes d'analyse de données multivariées (MVDA) pour surveiller les attributs de qualité

des produits et mieux comprendre leur variabilité. Dans l'ensemble, ce travail de thèse présente une étude détaillée de la répartition de l'humidité et de l'effondrement à l'intérieur des produits lyophilisés.

Mots clés: QbD; PAT; NIRS; NIR CI; Lyophilisation, distribution de l'humidité du produit; Effondrement du produit; MVDA

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LIST OF ACRONYMS

Acronym	Definition
US FDA	United States Food and Drugs Administration
EMA	European Medicines Agency
WHO	World Health Organization
USP	United States Pharmacopeia
ICH	International Conference on Harmonization
GMP	Good Manufacturing Practices
QbD	Quality by Design
PAT	Process Analytical Technology
CQA	Critical Quality Attribute
CPP	Critical Process Parameter
RMC	Residual Moisture Concentration
ASTM	American Society of Testing and Materials
PARD	Processes, Administration, Research and Development
NIRS	Near-infrared spectroscopy
NIR-CI	Near-infrared chemical imaging
SWIR	Short Wave Infra-Red
TDLAS	Tunable Diode Laser Absorption Spectroscopy
MVDA	Multivariate Data Analysis
SNV	Standard Normal Variate
SG	Savitzky-Golay
PCA	Principal Component Analysis
MCR	Multivariate Curve Resolution
CLS	Classical Least Squares
AWA	Apparent Water Absorbance
PLS	Partial Least Squares
PC	Principal Component
PLS-DA	Partial Least Squares-Discriminant Analysis
LDA	Linear Discriminant Analysis
LD	Linear Discriminant
RMSEC	Root Mean Square Error in Calibration
RMSECV	Root Mean Square Error in Cross-Validation
RMSEP	Root Mean Square Error in Prediction
RMSE	Root Mean Square Error
NIPALS	Nonlinear Iterative Partial Least Squares
VIP	Variable Influence in Projections

1. INTRODUCTION

1.1 Context and the research problem

The global biopharmaceuticals market is a multibillion industry and is expected to grow approximately 10% to $446 billion by the middle of the next decade[1]. Roughly 50% of biopharmaceutical products approved by FDA and EMA are freeze-dried[2]. Biotech formulations containing aqueous solutions and which are sensitive to heat are dried in a highly controlled low-temperature environment. This helps in achieving enhanced product stability for distribution and storage. But freeze-drying is a complex process and may result in in-process and in-storage instabilities if the drying cycle is not properly designed and monitored. To address such issues encountered in different pharmaceutical unit operations, regulatory guidelines such as ICH Q8/Q9/Q10 focused on quality by design (QbD) approach were introduced. Following these guidelines, it is expected that the quality of the product is built into the process rather testing only a few samples or batches at the end of the process. These guidelines answers the questions related the current procedures of the ICH Quality Implementation. Process analytical technology (PAT) tools are a vital part of QbD in achieving the product quality. US-FDA states that the PAT is "a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e. inline/online) of critical quality and performance attributes of raw and in-process materials and processes with the goal of ensuring final product quality"[3]. The goal to achieve QbD can be achieved using a combination of PAT tools such as chemometric tools, process analyzers, endpoint monitoring tools, and knowledge management tools[4]. Since the launch of guidance on the use of PAT, researchers have put immense efforts in the development and implementation of PAT for monitoring the freeze-drying processes.

PAT tools used in the monitoring of pharmaceutical freeze-drying operations are broadly classified into single vial and batch process monitoring technologies. Single vial focused techniques give information related to a given vial located at a specific position inside the freeze dryer, whereas the batch monitoring techniques yields the information of an overall batch undergoing the drying process. However, it was recently understood that vials exhibit variable

temporal drying profiles based on their spatial position inside the freeze-drying chamber, as there is a variability in pressure and temperature effects inside the freeze-dryer[5,6,7,8]. Neither single vial focussed, nor the batch monitoring approaches, can account for the spatial variations in the quality attributes of the product. These temporal differences result in variability in quality attributes of the products within a batch, which can be minor variations in the quality of the product or a major product defect. For instance, if the vials in the center of the chamber still contain traces of ice compared to vials on the sides of the product chamber, and if the shelf temperature is increased it will selectively cause collapse or product defects in the vials located at the center of the chamber. Despite significant advances, there is still no in-line monitoring method to monitor defects during the freeze-drying process.

In light of this problem, this thesis centers on the evaluation of non-invasive NIR tools to implement them in-line to monitor freeze-drying processes. NIR chemical imaging and spectroscopy were used for the qualitative and quantitative examination of vials in order to clarify the spatial moisture variations on the surface of the vials during the freeze-drying process. Subsequently, NIR spectroscopy was evaluated to identify the physical characteristics of the product cake during the freeze-drying process. The ultimate objective of this thesis was to explore the potential of NIR tools and deepen our understanding of the freeze-drying products and the processes.

1.2 Definition and objectives of the current research project

Freeze-drying is a dehydration process that plays an important role in stabilizing the pharmaceutical formulations that are unstable in aqueous solutions. Residual moisture content (RMC) can have a significant impact on the quality of the product. It can alter the physical state of the in-process products resulting in cake defects, whereas in the finished products it may affect product stability via microbial growth. Considering the consequential nature of residual moisture, it is deemed as one of the most significant critical quality attributes (CQAs). Various studies indicate that RMC influences other quality attributes in the finished products[9,10,11,12]. RMC inside the product has been a topic of much work and NIR tools were developed and implemented for monitoring moisture inside the products during the process[4,7,13,14,15] and inside the finished freeze-dried cakes[16,17,18]. Despite significant work in this field, many topics remain

to be addressed. One of them being the impact of spatial variations in product attributes, thus the accuracy of in-process results obtained from a single point on the vial surface remain questionable. Also, the aesthetic appearance of the product is subject to acceptance by visual inspection. However, relying completely on human input can be fallible in the production environment as thousands of product vials roll out from the freeze-dryers. Failure to detect an aesthetic defect in the finished freeze-dried product cake may put a patient's life at risk as any defect might be a result of product collapse or meltback affecting drug safety and efficacy.

The main goal of this project is to develop tools and methods to monitor quality attributes of the freeze-dried products.

The core objectives of this project are:

1. To determine the spatial variability of moisture in the in-process freeze-dried products. A combination of NIR tools-chemical imaging and NIR spectroscopy were used to investigate spatial moisture variability. This was achieved by studying the intra-vial spatial variability using NIR-CI images acquired at different measurement positions, and the comparative PLS figures of merit obtained using the NIR spectra obtained at multiple measurement positions.
2. To identify the product state of in-process products and identify the characteristics of the finished freeze-dried products using NIR spectroscopy. This was achieved by inducing different degrees of melt back/collapse in products during freeze-drying and studying the product state/characteristics in the in-process and finished freeze-dried products.

1.3 Original contributions

This research work proposes a method to assess spatial variability of moisture within products during the freeze-drying process. This is needed to select an appropriate number of measurement spots on the product vial while working with point focused measurement PAT tools, more specifically while using NIR spectroscopic tools. This work also imparts understanding about the use of the NIR spectroscopy to identify the aesthetic appearance of the product both during the process and in the finished freeze-dried products. Contributions of this thesis work are presented in the form of research articles:

Paper 1: Peer-reviewed research article

An investigation into the spatial distribution of Moisture in Freeze-dried products using NIR spectroscopy and chemical imaging.

Paper 2: Research article

Identifying collapse in freeze-dried products via NIR spectroscopy.

1.4 Document structure

This thesis is organized into five main chapters, following is the outline of each chapter

Chapter 1 presents introduction and the context, motivation and the defined objectives of this research work.

Chapter 2 consists of a review of the lyophilization process with emphasis on the critical quality attributes (CQA) and a special focus on the state of art tools/methods used throughout this thesis.

Chapter 3 presents the research work detailing an Investigation into the spatial variability of moisture inside in-process freeze-dried samples using near infrared (NIR) chemical imaging (CI) and spectroscopy

Chapter 4 presents the feasibility of using NIR spectroscopy to monitor the aesthetic appearance of in-process and the finished freeze-dried products.

Chapter 5 presents the summary of preliminary evaluation trials using different NIR spectrometers for monitoring the product moisture in in-process freeze-dried products.

Chapter 6 presents the overall conclusions and potential future work for this project.

2. STATE OF THE ART

The main focus of this thesis work involves developing NIR based methods to investigate spatial variations in moisture distribution & monitor the aesthetic appearance of the freeze-dried products. This work encompasses the use of NIR tools in tandem with chemometric methods to analyze properties of freeze-dried products, as data generated by the NIR tools alone is not enough to draw conclusions on the targeted properties. The first part of this chapter presents the freeze-drying operation and criticalities involved in monitoring the freeze-drying product/process, this is presented to enable one to understand the background of this research work. Then the basics of NIR based PAT tools, NIR spectroscopy and NIR chemical imaging, and their recent applications in the freeze-drying industry have been presented along with the chemometrics data analysis methods.

2.1 Freeze-drying operation and the criticality of monitoring moisture inside the freeze-dried products

Freeze drying is an important unit operation in the manufacturing of pharmaceutical injectable-products, as it enhances product lyophilic nature and stability of the formulations[19]. The product is subjected to highly controlled process conditions inside the freeze-dryer in order to perform drying[20]. First, the liquid formulation is filled inside glass vials and the vials are half stoppered such that vapors can evacuate from the product. The vials are then placed on the trays and loaded on the shelves of the freeze-dryer. The shelves are cooled in a stepwise manner (typically -30 to -60°C) to freeze the product, which results in the formation of ice nuclei called nucleation phase and the solute is maximally freeze-concentrated.

The product is subjected to drying in steps after freezing, ensuring the temperature of the product is below its critical temperature representing the maximum temperature that the product can withstand during drying without melting or collapsing the product. These temperature limits are product specific and are determined beforehand using methods such as freeze-drying microscopy, differential scanning calorimetry and dielectric resistance analysis. For details of these methods following reference could be consulted[21]. In general, frozen products are categorized as either crystalline or amorphous glass in structure. Crystalline products have a

defined “eutectic” freezing/melting point that is also its collapse temperature. Amorphous products have a corresponding “glass transition” temperature and their collapse temperature is typically a few degrees celsius warmer than the glass transition temperature. For freeze-drying, the frozen product is exposed to high vacuum to sublimate the ice where the frozen liquid transforms to a gaseous state directly without going through a liquid phase which is referred to as initial drying phase, called as primary drying[22]. The shelf temperature is elevated in the range of -30°C to -10°C which is done to provide suitable conditions for sublimation. The duration of the primary drying varies from hours to days depending upon the volume and nature of the product. Once the ice sublimation is finished the residual moisture bounded inside the product interstitial spaces is removed by desorption, also called secondary drying. In this step temperature of the shelf is elevated while maintaining the product temperature below glass transition temperature, typically between 0 to +40°C. Secondary drying usually takes a few hours and is relatively short compared to the whole length of the process. The product chamber is back-filled usually with nitrogen gas, and then vials are fully stoppered by pressing the shelves together hydraulically once the product is dried. Vials are unloaded and further sealed, labeled and packed after stoppering, and then stored under appropriate temperature conditions.

Freeze drying equipment includes a temperature control system, vacuum System, product chamber, and condenser.

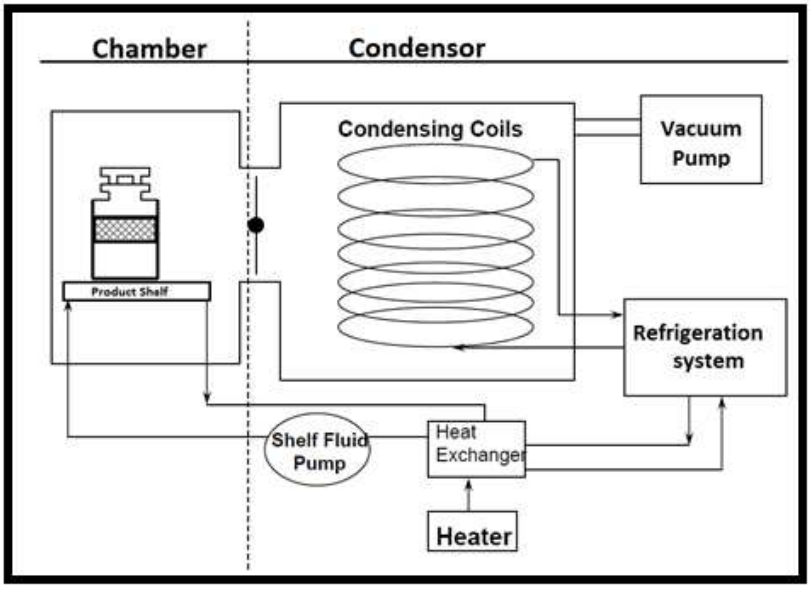


Figure 2-1 Schematic view of a freeze-drying equipment (Adapted from Nathaniel Milton, Eli Lilly and Co[23])

Figure 2-1 presents different components of the freeze-drying equipment. The temperature is controlled by refrigeration and heating systems. The refrigeration system cools the condenser coils and shelves in the product chamber for adsorbing sublimed vapors on the condenser coils and freezing product inside the vials. The heating system with the aid of heat exchanger applies small amount of heat to the shelves during the drying stages. Advanced shelf freeze dryers consisting of microprocessor-based controllers are capable of controlling the shelf temperature within ±1°C of the set point within the control range of -55°C to 65°C.

The vacuum system consists of a separate vacuum pump connected to both an airtight condenser and a product chamber. Typically, vacuum levels for freeze drying are between 50mTorr and 300mTorr with 100mTorr to 200mTorr being the most common range. The required pressure inside the product chamber is maintained through a controlled flow of nitrogen, called nitrogen breathing.

The purpose of the condenser is to capture the vapors being sublimed off of the product. The condenser is maintained at a lower temperature relative to the product in the chamber, since vapors evacuating from the product chamber flows into the condenser chamber and gets deposited on the condenser coils in the form of an ice. The temperature in the condenser is usually between -55°C and -85°C.

The conditions under which the drying takes place determines the quality attributes in the finished freeze-dried products. Critical quality attributes, such as API state (e.g., protein conformation and stability), residual moisture content, freeze-dried product cake appearance, and reconstitution time are evaluated on randomly selected samples after the completion of the drying[2]. Studies have shown that moisture content is the most significant attribute that directly or indirectly affects other mentioned product quality attributes[9,10,11,12]. A product containing residual ice when subjected to secondary drying results in product collapse, and this affects the aesthetic appearance of the product[10]. The stability of the finished freeze-dried formulations is also affected by their excess residual moisture. The following reference[24] presents a review of different studies highlighting the criticality of residual moisture in the freeze-dried products.

2.2 NIR tools used as Process Analytical Technologies (PATs) for monitoring Freeze-dried products

Considering the regulatory requirements for the aseptic manufacturing of biopharmaceutical products, non-invasive and non-destructive testing tools are considered a boon in monitoring the freeze-dried products. Over the last decades, near-infrared (NIR) spectroscopy in combination with the light-fiber optics, new in and on-line probe accessories, and chemometrics evaluation procedures has emerged as a powerful PAT tool in various pharmaceutical applications. The widespread use has been further enhanced with the advancement in technology such as miniaturized optics, small footprints, and wireless functionality, making it a widely used non-invasive and non-destructive tool in in-line monitoring applications[25]. Additionally, chemical imaging helps in studying the spatial variations on the surface of the product. Both techniques are complementary as NIR-CI provides spatial information related to moisture on the surface of the product, whereas spectroscopy allows penetrating the surface layers of the product.

2.2.1 NIR Spectroscopy (NIRS)

NIR spectroscopy is one of the most widely used PAT tools in manufacturing several different pharmaceutical formulations, it is embedded as an essential tool in monitoring various stages of pharmaceutical unit operations. The American Society of Testing and Materials (ASTM) defines the NIR region of the electromagnetic radiation as a narrow spectral region ranging in the wavelength range of 780-2520 nm. Many of the NIR spectral bands originate as a result of overtones and a combination of fundamental vibrations of C-H, O-H, N-H and S-H. For details about the principles and theoretical aspects of NIR measurement following reference may be consulted[26].

Guidelines specific to the use of NIRS have been published by regulatory agencies like European Medicines Agency (EMA) and FDA considering the increasing use of NIR as PAT in monitoring various pharmaceutical operations [27,28]. NIR spectroscopy is used in-line for monitoring several different attributes such as in-line product moisture[15,7], the progress of the drying operation[4,29] and polymorphic forms of products which is caused by the change in the

process conditions[30] during the freeze drying process. NIRS spectra sensitivity to various forms of water such as ice, liquid or vapour adds to its uniqueness in identifying normal (ice) or deviated (liquid) state of the product during the freeze-drying[31]. Figure 2-2 shows different energy levels of absorbance overtone bands of water exhibited with the change in its state[31]. The water absorbance band shifts from higher energy levels to lower energy levels when there is a change in the product's physical state from liquid to ice.

Absorption Peaks of Water, Ice, and Vapor

Ice (nm)	Ice (cm^{-1})	Liquid Near Freezing Point (nm)	Liquid Near Freezing Point (cm^{-1})	Liquid Near Boiling Point (nm)	Liquid Near Boiling Point (cm^{-1})	Vapor (nm)	Vapor (cm^{-1})
800	12,500	770	13,000	740	13,500	723	13,831
909	11,000	847	11,800	840	11,900	823	12,151
1025	9760	979	10,210	967	10,340	942	10,613
1250	7990	1200	8310	1160	8640	1135	8807
1492	6700	1453	6880	1425	7020	1380	7252
1780	5620	1780	5620	1786	5600	—	—
1988	5030	1938	5160	1916	5220	1875	5332

Figure 2-2 Absorbance overtone bands of water specific to its physical state in the NIR region (Reprinted with permission from[31].Copyright ©2007,Taylor &Francis Group LLC)

Besides, NIRS has sensitivities to the physical characteristics of products, and also to additional characteristics such as pore structure, which can helps in determining the normal or collapsed nature of the finished freeze-dried products. Despite the challenges in monitoring the freeze-drying products/process such as an isolated space, and limited access to the product undergoing drying inside the freeze-dryer, it is possible to monitor product inside the freeze-dryer chamber with the present generation of the miniaturized spectrometers and carry out real time qualitative and quantitative studies.

2.2.2 NIR Chemical Imaging (NIR-CI)

NIR-CI (NIR Chemical Imaging) facilitates visualizing surface of the sample to map the distribution of chemical components at wavelengths between 700-2500nm. A NIR imaging system essentially consists of a light source, optics, a spectrograph, and an array detector[26].

NIR-CI is a new technique for the monitoring of freeze-dried products, and only a few studies have been reported for freeze-drying monitoring applications. However, a recent study[32]

specific to spin freeze-dried products, assessed the potential of NIR-CI in monitoring distribution of Mannitol solid form and moisture inside the freeze-dried products. Whereas, in the other pharmaceutical areas, researchers have successfully demonstrated the feasibility of using NIR-CI for different applications, a few of them include pharmaceutical powder blend concentration monitoring[33], tablet uniformity[34], and identifying the product polymorphic forms[35]. The advantage of chemical imaging lies in the ability to demonstrate qualitative surface heterogeneities with the quantitative information of the sample. However, the selectivity of the measurement method depends on the nature and distribution properties of samples.

2.3 Chemometrics and Multivariate data analysis (MVDA) tools

The biggest challenge in implementing analytical tools for monitoring any chemical processes is studying the vast number of variables to find the right variables that help to efficiently monitor and control processes. This is possible using Chemometrics, which is the science of extracting information from the acquired data with the use of mathematical and statistical procedures. Chemometrics is indeed an interdisciplinary branch that utilizes the knowledge from other sectors like multivariate statistics, applied mathematics, and computer sciences. Because of its multidisciplinary nature, chemometrics has emerged as an important subject in extracting information from the process data helping in enhanced process understanding thus enabling efficient process control.

Chemometrics include[36]: 1) data preprocessing, 2) classification, 3) calibration, and 4) prediction and validation of the data. Statistical multivariate projection methods, also called multivariate data analysis (MVDA) methods are often used for the data exploration and calibration. These methods are being increasingly used in the PARD (Processes, administration, research and development).[36] Several MVDA methods have been developed in the recent years, review of these methods and their applications in various pharmaceutical processes has been presented by Rajalahti et.al. [37]

Vibrational spectroscopic methods such as NIR and Raman spectroscopy in combination with multivariate data analysis methods such as PCA and PLS are most commonly used to monitor the freeze-drying processes.[7,38] In two separate studies published by Beer et.al[4,29] simultaneous use of Raman and NIR spectroscopy was demonstrated in order to monitor quality attributes of

products during the freeze-drying process. In freeze-drying applications, PCA is generally used for data visualisation, and PLS is used for data interpretation.[37] Other methods such as, partial least squares discriminant analysis (PLS-DA), linear discriminant analysis (LDA), multivariate curve resolution (MCR) and classical least squares (CLS) have also been reported for studying the attributes of freeze dried products and processes.[4,38,39]

A few of the MVDA methods used throughout this project are discussed in the following sections.

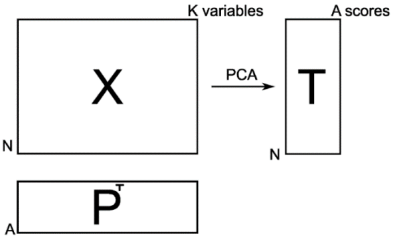
2.3.1 Principal component analysis (PCA)

Principal component analysis (PCA) is one of the most widely used multivariate statistical method that forms the basis for multivariate data analysis.[40] It transforms the large number of original variables into a set of new orthogonal variables called principal components such that most of the information is contained in the first few components explaining maximum variance in the data.[41] The first principal component (PC1) explains the largest variation in the data set, PC2 explains the second largest variation and so forth.

In PCA analysis, a data matrix is modelled as[42]

$$\mathbf{X} = \mathbf{TP^T} + \mathbf{E} \tag{2.1}$$

Where, **T** *(N×A)* is Scores, **P** *(K×A)* is loadings and **E** *(N×A)* is an error.



The product of **T** and $\mathbf{P^T}$ is equal to the sum of the considered PCs and **E** is an error matrix calculated after subtracting original matrix by the matrix originated after PCs were considered. **E** matrix has the same size as original matrix or **X**. The equation is given by

$$\mathbf{X} = \mathbf{t_1 p_1^T} + \mathbf{t_2 p_2^T} \ldots + \mathbf{t_n p_n^T} + \mathbf{E} \tag{2.2}$$

The matrix product $\mathbf{TP^T}$ models the structure and the residual matrix **E** contains the noise. The principal component scores of the first, second, third components ($\mathbf{t_1,t_2,t_3}$…) are columns of the score matrix **T**. The scores are coordinates of the observations in the model hyper plane. Alternatively, these scores may be seen as new variables which summarises the old ones and they are sorted in order of descending importance. The meaning to the scores is given by loadings. Loadings of the first, second, third components ($\mathbf{p_1,p_2,p_3}$…) build up the loading matrix $\mathbf{P^T}$. Loadings define the orientation of PC plane with respect to the original **X**-variables, and informs the magnitude of correlation (large or small) and the manner in which the measured variables contribute to the scores.

Prior to carrying out PCA analysis, data is typically pre-processed by centering and scaling to unit variance. Plots of the PCA show the relationship among observations' including the outliers.

2.3.2 Partial least squares (PLS)

PLS is the regression extension of PCA, PLS stands for projection to latent structures by means of partial least squares. It is used to connect the information in two blocks of variables **X** and **Y** to each other by linear multivariate model[43]. PLS is mainly useful in dealing with the noisy, co-linear and incomplete variables in both **X** and **Y**[36]. PLS models the relations between the **X** and **Y** blocks by means of score vectors, and it decomposes the (N×K) matrix of variables **X** and the (N×M) matrix of variables **Y** into the form represented as[43]

$$\mathbf{X} = \mathbf{TP^T} + \mathbf{E} \tag{2.3}$$

$$\mathbf{Y} = \mathbf{UC^T} + \mathbf{F} \tag{2.4}$$

Where the **T**, **U** are (N×A) matrices of the p extracted score vectors respectively, the columns of **P** and **C** represents the matrices of loadings. **E** and **F** are just model residuals. In PLS, summary of the importance of X-variables for both **Y** and **X** is given by variable influence on projection (VIP) plot. The VIPs are a weighted sum of squares of PLS weights that are calculated by explained **Y**-variance in each dimension[36]. The attraction of VIP lies in its mode of providing information in the simplest form with only vector summarizing all the components and Y-variables.

Several methods have been developed to calculate PLS model such as the NIPALS algorithm, SIMPLS and Kernel algorithm. However, the NIPALS algorithm developed by Wold et.al is

known to be simplest. Using this iterative approach, scores (**T**), loadings (**P**) and additional weights (**W**) which has the same dimensionality as loadings are calculated. The addition of weights in PLS is required to maintain the orthogonal scores. For the details about the NIPALS algorithms Wold et.al[43] work could be consulted.

To validate the model fit and predictive ability of the developed calibration models following root mean square errors must be evaluated[44]:

Root Mean Square Error of Calibration (RMSEC) – It gives a measure of the average difference between predicted and measured response values at the calibration stage; a model with a perfect fit will yield a RMSEC of zero.

$$RMSEC = \sqrt{\sum_{i=1}^{N} \frac{(\hat{y}_i - y_i)^2}{(N - A - 1)}} \tag{2.5}$$

where, $\hat{y}_i$ is the model-estimated Y-values whereas y_i is the known Y-values; A = number of PLS factors; N is the number of samples in the test set

Root Mean Square Error of Cross Validation (RMSECV) – It gives a measure of the average difference between predicted and measured response values of samples from the calibration subset that were placed aside; this provides more realistic estimates of the model's prediction and optimal complexity performance of the model.

$$RMSECV = \sqrt{\sum_{i=1}^{N} \frac{\left(\hat{y}_{CV,i} - y_i\right)^2}{N}} \tag{2.6}$$

where, $\hat{y}_{CV,i}$ = model-estimated Y-values without the calibration subset i.

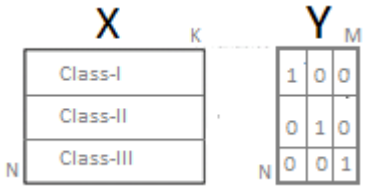
Root Mean Square Error of Prediction (RMSEP) – It gives a measure of the average difference between predicted and measured response values at the validation stage; it can be used to provide a reasonable assessment of the model's prediction performance on future samples.

$$RMSEP = \sqrt{\sum_{i=1}^{N} \frac{(\hat{y}_i - y_i)^2}{N}} \quad (2.7)$$

Here, *N* is the number of samples in the test set.

2.3.3. Partial least squares discriminant analysis (PLS-DA)

PLS-DA is a supervised classification method developed with an objective to separate different classes of observations on the basis of their X-variables. Similar to the PLS, in this method **Y**-matrix encodes observations into different categories by means of dummy variables. Each class of observations in the dummy **Y**-matrix is denoted with ones and other classes as zeros, number of vectors in **Y**-matrix increases with the increase in the number of classes of observations. As presented in the example below, an **X**-matrix with three different classes of observations are attributed to the three variables of the Y-matrix.



The PLS model is fitted between the (N×K) matrix of **X** and the dummy (N×M) matrix of **Y**, this way a discriminant plane is found in which observations are separated according to their respective classes[45]. More specifically, PLS-DA finds a linear regression model by projecting the predicted dummy variables and the observed variables into a new space[46]. Through the obtained weights and loadings insights related to discrimination are achieved. PLS-DA holds well for the classification of samples based on their properties such as origin, activity and other physical characteristics[36].

2.3.4. Linear discriminant analysis (LDA)

LDA, synonymously called Fischer's LDA is an established supervised classification method for classification and discrimination of observations belonging to different classes. The main objective of LDA is to find a new dimension that best segregates different groups of the samples

projected onto it, maximizing the ratio between the sum of squares between and within the group[46].

Formally the projection matrix ($\mathbf{P}_{\mathrm{LDA}}$) is calculated as following

$$\mathbf{P}_{LDA} = \underset{\mathbf{P}}{argmax}\left(\frac{\det(\mathbf{P^T S_b P})}{\det(\mathbf{P^T S_w P})}\right) \tag{2.8}$$

Where, **P** is the eigenvector**,** $\mathbf{S_b}$ is between class variance and $\mathbf{S_w}$ is within class covariance.

LDA works well if all the observations are homogenous and the number of variables is much smaller than number of samples in the training set [47].

The following table (Table 2-1) summarizes the recent studies that have reported the use of chemometric methods specific to the pharmaceutical freeze-drying processes and products monitoring.

Table 2-1 Application of NIR-based Chemometric methods in freeze-drying

Application	Chemometric method	Analysis Type	Year & Reference number
Design of Experiments based Near-Infrared Spectroscopy models to monitor moisture content in the freeze-dried products.	PCA, PLS	Qualitative and Quantitative	2020 (48)
Demonstration of NIR based multivariate model to obtain information on liposome structure and integrity, and determination of endpoints of primary and secondary drying.	PCA	Qualitative	2018 (49)
Determination of endpoint of primary drying using in-line Near-Infrared Spectroscopy and mechanistic model produced using temperature as an input during the continuous freeze-drying.	PCA	Qualitative	2018 (50)
Monitoring moisture content and distribution inside the spin freeze-dried vials using Near-Infrared Chemical Imaging	PCA, PLS	Qualitative Quantitative	2018 (32)
Demonstration of reliability of multi-vial Near-Infrared approach in	PLS	Quantitative	2014 (7)

monitoring product moisture during the freeze-drying process			
Monitoring of changes induced in the multicomponent formulation, identification of solid state form of mannitol and sublimation of solvents during the drying process	PCA	Qualitative	2014 (30)
Using NIR based models to distinguish formulations with varying virus pretreatments and virus volume.	PCA, PLS-DA	Qualitative	2013 (51)
Using Near-Infrared probes to monitor moisture content inside multiple vials during the freeze-drying process.	PCA, PLS	Quantitative	2013 (15)
Monitoring interactions between protein and lyoprotectant, and detection of protein unfolding during the freeze-drying process.	AWA	Qualitative	2012 (15)
Using DOE approach and combination of multiple PAT tools for freeze-drying process and formulation optimization: NIR for investigation of drying phase and selection of optimal process step temperatures	PCA	Qualitative	2011 (52)
Study on complementary properties of Raman and NIR: NIR for monitoring drying steps and solid state characterization of mannitol	PCA	Qualitative	2009 (4)
Study on complementary properties of multiple PAT tools: NIR for detection removal of hydrate water, determination of drying end point and solid-state characterization of mannitol	PCA	Qualitative	2009 (29)

3. RESEARCH PAPER: An investigation into the spatial distribution of moisture in freeze-dried products using NIR spectroscopy and chemical imaging.

Title in French

Une étude de la distribution spatiale de l'humidité dans les produits lyophilisés par spectroscopie NIR et imagerie chimique

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Date of submission: 20th October 2020

Status: In review

Submitted to Journal: PDA Journal of Pharmaceutical Science and Technology.
Contributions to the thesis: This paper allowed to determine the distribution of moisture on the surface of the freeze-dried products using near-infrared chemical imaging, and spatial variabilities in the distribution of moisture on the surface of the vials have been confirmed. Using NIR measurements from multiple spots on the surface of the vial it was confirmed that to obtain robust quantitative results measurements from multiple spots on the surface of vial must be acquired.

ABSTRACT

Near-infrared spectroscopy (NIRS) is a widely accepted method of measuring moisture in pharmaceutical freeze-dried products, both during the process and in the finished products. Multiple NIR measurement approaches have been introduced to monitor product moisture in freeze-dried vials. However, the spatial moisture gradients within a vial have not been investigated in depth. Like any other point-focused process analytical technology (PAT) tool, a spectrum produced by NIRS represents an average over a given area of the product vial. Implementing a point-focused NIR on any random position without proper understanding of spatial moisture variations within the vial may severely impact the reliability of the results.

The present work focuses on understanding the moisture distribution within freeze-dried vials. We performed an investigation using NIR tools, NIR chemical imaging (NIR-CI), and NIRS to understand the spatial variations in moisture on the outer surface (i.e., periphery) of the freeze-dried vials. To achieve this, the moisture distribution within individual vials was mapped using NIR images. Then, NIRS was used to determine the necessity of using multiple measurement points to produce robust models quantifying moisture inside freeze-dried products. Overall, the results confirm the non-homogenous distribution of moisture, as well as the non-uniform drying front, in the products undergoing freeze-drying. The findings from the NIRS-based partial least squares (PLS) models indicate that, to achieve reliable product/process information, measurements must be drawn from multiple measurement points on the surface of the freeze-dried products.

Keywords: Freeze-drying, NIR, NIR Imaging, PAT, Chemometrics

RÉSUMÉ FRANÇAIS

La spectroscopie proche infrarouge (NIRS) est une méthode largement acceptée pour mesurer l'humidité dans les produits pharmaceutiques lyophilisés, à la fois pendant le procédé et dans les produits finis. Plusieurs approches de mesure NIR ont été introduites pour mesurer l'humidité du produit dans les flacons lyophilisés. Cependant, les gradients d'humidité spatiaux dans un flacon n'ont pas été étudiés. Comme tout autre outil de technologie d'analyse de procédé (PAT), un spectre produit par NIRS représente une moyenne sur une zone donnée du flacon de produit. La mise en œuvre d'un NIR ponctuel sur n'importe quelle position aléatoire sans une bonne compréhension des variations spatiales d'humidité dans le flacon peut gravement affecter la fiabilité des résultats.

Le présent travail se concentre sur la compréhension de la distribution de l'humidité dans les flacons lyophilisés. Nous avons effectué une enquête à l'aide d'outils NIR, d'imagerie chimique NIR (NIR-CI) et de NIRS pour comprendre les variations spatiales de l'humidité sur la surface externe (c'est-à-dire la périphérie) des flacons lyophilisés. Pour ce faire, la distribution de l'humidité dans les flacons individuels a été cartographiée à l'aide d'images NIR. Ensuite, le NIRS a été utilisé pour déterminer la nécessité d'utiliser plusieurs points de mesure pour produire des modèles robustes quantifiant l'humidité à l'intérieur des produits lyophilisés. Globalement, les résultats confirment la répartition non-homogène de l'humidité, ainsi que le front de séchage non-homogène, dans les produits en lyophilisation. Les résultats des modèles des moindres carrés partiels (PLS) basés sur le NIRS indiquent que, pour obtenir des informations fiables sur les produits / procédés, les mesures doivent être effectuées à partir de plusieurs positions sur la surface des produits lyophilisés.

Mots clés : Lyophilisation, NIR, Imagerie NIR, PAT, Chimiométrie)

3.1 INTRODUCTION

Freeze-drying, or lyophilization, is a low-temperature dehydration process that involves freezing a product, placing it in a relative vacuum, and then removing the resulting ice by sublimation[53]. It is often used in the pharmaceutical industry to dry thermally sensitive products, such as biopharmaceutical formulations[54]. During the freeze-drying process, liquid formulations are subjected to controlled multistep transformations, making use of the principles of heat and mass transfer. This is done to achieve a uniform, amorphous dry cake, which often renders the process complicated and time-consuming, resulting in high operating costs[55,2]. It is therefore crucial to monitor the product during the process to ensure that the desired critical quality attributes (CQAs) of the product are achieved[56].

In general, CQAs are related to the physical, chemical, biological, or microbiological properties of the drug product that define the final product quality[57]. The most common CQAs associated with freeze-dried products are potency and purity, reconstitution time, aesthetic appearance and residual moisture concentration (RMC) in the finished drug product[58]. Among these, RMC within the product has been the topic of much work, both in the products undergoing drying and also in the finished freeze-dried products. Various studies indicate that RMC influences the other quality attributes in the finished products[9,10,11,12]. Considering the importance of RMC in freeze-dried products, several process analytical technology (PAT) tools have been developed to directly or indirectly monitor product moisture during the freeze-drying process.

Monitoring RMC is done on two levels: the individual vials (vial-focused monitoring) or the overall freeze-drying unit (batch monitoring). A review of both types of applications can be found elsewhere[59]. In contrast to vial-focused monitoring tools, batch monitoring tools have so far been preferred, as they comply with good manufacturing practices (GMP) requirements and provide information on the complete batch of vials[19]. Among the different batch monitoring tools, tunable diode laser absorption spectroscopy (TDLAS) shows promising results for the quantitative moisture characterization of a batch of vials[10,60,61,62]. However, research has shown that vials exhibit variable temporal drying profiles based on their spatial position inside the freeze-drying chamber, as there is variability in the pressure and temperature effects inside the freeze dryer[5,6,7,8]. Although TDLAS promises to obtain information related to the overall process, it cannot account for spatial variations or provide vial-specific information. Unlike

batch monitoring tools, point-focused tools such as near-infrared spectroscopy (NIRS) have successfully been demonstrated to monitor the moisture of specific vials during freeze-drying[13,4]. In these works, the NIRS probe was implemented only on a single vial among the whole batch, and cannot account for variations between vials undergoing drying. Going a step further, Kauppinen et al.[7,15] introduced an in-line multi-vial NIRS approach to study the variability in product moisture in several vials located in different positions throughout the freeze dryer shelf. Such an approach occupies a middle ground between single-vial and whole-batch monitoring and enables increased understanding of product variability specific to the spatial locations inside the freeze dryer over the entire lyophilization run.

Considering the potential of IR imaging to monitor spatial variations in the physical and chemical properties of the sample, several tools have been developed and implemented as PATs. IR thermal imaging has been used to monitor temperature gradients on the surface of individual vials as well as on the bulk product spread over the entire tray of a freeze-drying unit[63]. In both cases, spatial temperature variations were observed between the vials and within the bulk product. This further strengthens the rationale behind using the multi-vial NIR approach for monitoring moisture during the freeze-drying process, as the temperature is closely related to the residual moisture inside the vial. In another work, thermal imaging was used to show variability in the axial and radial temperature profiles of the vial throughout the drying process[64]. With the recent use of NIR chemical imaging (NIR-CI) in continuous freeze-drying, the presence of an axial moisture gradient within the vials was observed as the product was deposited on the vial wall as a film[32].

The present study aims at investigating spatial moisture variations in freeze-dried vials and at determining the suitability of acquiring measurements on a single measurement spot using point-focused probes. This is because the product presentation in freeze-dried vials is significantly different from that in spin-dried vials, resulting in markedly different drying profiles. Here, we used NIR-CI and NIRS to accomplish these objectives. These techniques are complementary, as NIR-CI provides spatial information related to moisture on the surface of the product, whereas spectroscopy enables penetration into the surface layers of the product. The use of NIRS in combination with a partial least squares (PLS) regression is an established approach for quantifying moisture in freeze-dried products[15,32]. Images acquired using NIR-CI

were used to examine the spatial variability in the distribution of moisture on the surface of the product. NIRS data through quantitative moisture prediction was used to confirm the spatial moisture variabilities in the freeze-dried products. The images and spectra were acquired on the surface of the sample on six different sides (every 60° angle) by rotating the vial on a rotation stage. This allows inspection of the spatial variability in moisture around the whole circumference of the vial. Here, we show the spatial variability of moisture within the vials during the shelf freeze-drying process. Furthermore, we determine the robustness of single-point NIR measurement while the product is undergoing batch freeze-drying. In the end, this work seeks to provide tools, and methods, to better understand spatial variations within freeze-dried products.

3.2 MATERIALS AND METHODS

3.2.1 Materials

Glycine (≥98.5%), a common pharmaceutical excipient, was purchased from Fischer Scientific Canada and was used without further treatment prior to the experiments. Deionized water was used as a solvent to prepare 15% (w/v) glycine solution. The solution was filled in 50-ml capacity glass bottles (DWK Life Sciences, Millville, NJ, USA), where 120 samples were prepared by pouring 20 ml glycine solution into the glass vials, resulting in a 1-inch product fill height in liquid form.

3.2.2 Freeze-drying and sample collection procedure

Prior to drying, the samples were first frozen in lab freezer (16.6 ft^3 upright freezer, Frigidaire, Charlotte, NC, USA) at -18°C for 4 hours, then transferred to a -86°C freezer (MDF-U700VXC-PA, Panasonic, Kadoma, Osaka, Japan) and frozen for another 4 hours. A stepwise freezing procedure was carried out to avoid vial breakage due to thermal shock and to allow complete crystallization of the product formulation[65].

Drying was performed using a ModulyoD laboratory-scale freeze dryer (Thermo Electron Corporation, Waltham, MA, USA). A total of 120 vials were used for the experiments. The samples were collected at different time points to achieve samples with moisture in the range of 85% to <1% (w/w). To produce samples with differing moisture levels while minimizing interruptions to the process, the vials were collected at 4-hour intervals to achieve samples with

a moisture spacing of ≈5% between the 85% and 20% (w/w) moisture range. In the latter stages of drying, the samples were collected more frequently, reducing the time to 2-hour intervals to achieve moisture spacing of ≈2% (w/w).

Two vials were damaged during the drying process, and the remaining 118 vials were used in this study. The moisture content from the collected samples was determined using gravimetry, where the difference in the initial and final product weight was calculated[66,67]. All collected samples were stored in an -18°C freezer for further analysis.

3.3.3 NIR-CI

A NIR greyscale InGaAs area scan camera (Bobcat 320, Xenics Infrared Solutions, Leuven, Belgium) with a 25-mm infrared lens (Navitar, Rochester, NY, USA) was used as an imaging tool. The resolution of the camera was 320 × 256 pixels, and the operating wavelength range was 900–1700 nm. The shortwave IR (SWIR) camera was not equipped with a spectroscope, but a 1460 ± 11 nm selective wavelength bandpass filter (Spectrogon Inc., Mountain Lakes, NJ, USA) was mounted in front of the camera lens. This was done to selectively capture the images in the water combination overtone band region (1450–1500 nm) exhibited by water in near freezing and ice states[31]. These overtone bands are a result of stretching vibrations in the water molecule. The output from the NIR camera was a 2D greyscale image with varying pixel intensities relative to the moisture content on the surface of the vial. Thus, the image pixels representing moisture would have lower pixel intensities and appear darker than the pixels representing the dry product inside the vial. The sample presentation to the camera was controlled by optimizing parameters such as lighting, relative distances, and angles between the camera and the vial, as well as the acquisition parameters. To ensure image-to-image comparability, all parameters were kept constant throughout the measurements.

3.3.3 NIR Spectroscopy

A diffuse reflectance NIR spectrometer (MicroNIR 1700, Viavi Solutions, Inc., Milpitas, CA, USA) covering the wavelength range of 900–1700 nm was used. Each spectrum acquired by the probe consisted of an average of 128 spectra obtained with an integration time of 12 ms.

Before acquiring the spectra, the NIR spectrometer was calibrated for 0% and 100% reflectance. For 0% reflectance (**D**), a spectrum was acquired in the absence of IR light; for 100%

reflectance (**B**), the NIR spectrum was recorded by shining the light on a white reflectance standard. Every raw spectrum (**R**) acquired from the sample during the measurements was internally corrected using the 0% and 100% reflectance spectra according to the following equation:

$$\mathbf{C} = \frac{\mathbf{R} - \mathbf{D}}{\mathbf{B} - \mathbf{D}} \tag{3.1}$$

Where, **C** is the corrected spectra.

3.3.4 Procedure to acquire images and spectra on the surface of the product vial

To better understand spatial variability, NIR-CI and NIRS acquisitions were carried out at different positions on the vials. Measurements were carried out at 60° intervals around the cylindrical vials (Figure 3-1). Using both the NIRS and NIR-CI systems separately, spectra and images were acquired at different positions of the vial by rotating them on a fixed stage. Whereas the NIRS probe was positioned in direct contact with the vial, as the probe head size was equivalent to the product height inside the vial, the NIR-CI camera was located approximately 40 cm away to acquire a full picture of the vial. For illumination with the NIR camera, a 75-W halogen bulb was used. At each position on the vial, three replicate measurements were acquired and averaged prior to the analysis.

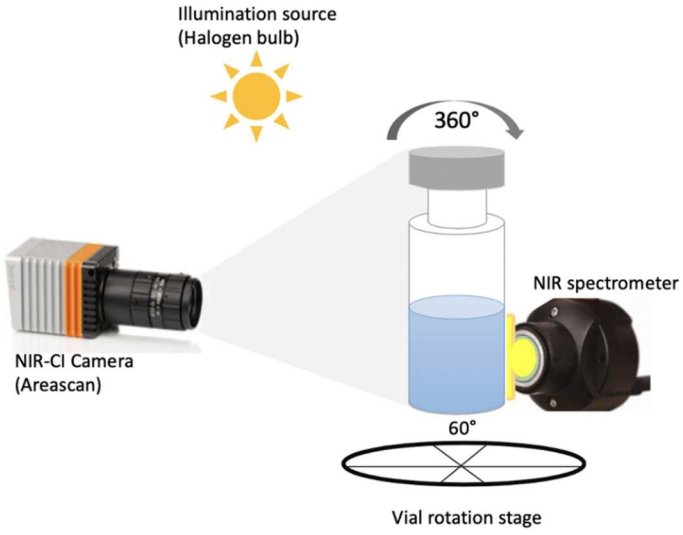

Figure 3-1. Schematic representation of the vial showing the six angular positions and the field of view with the NIRS and NIR-CI tools.

3.3.5 Treatment of the NIR-CI images

To correct for minor variations in the lighting conditions, the raw images were standardized based on the intensity of the image background with the following equation:

$$\mathbf{C} = 100\frac{\mathbf{X}}{\boldsymbol{b}} \qquad \text{(eq2)},$$

Where, $\mathbf{C}$ is the corrected image, $\mathbf{X}$ is a raw image, and b is the mean pixel intensity of the given portion (100 × 50) of the image background in the top corner (Figure 3-2).

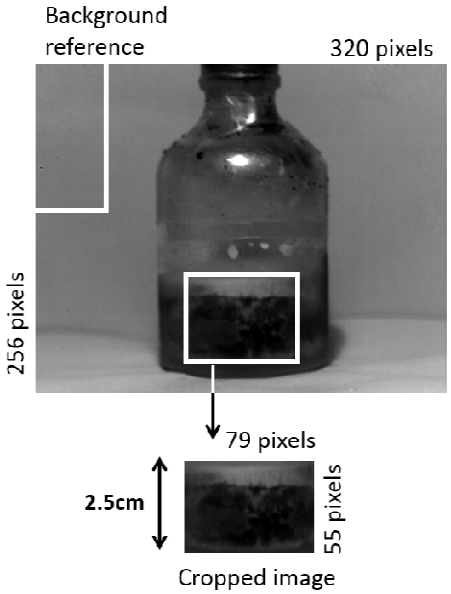

Figure 3-2. Typical vial illustrating a typical cake as well as the background reference area used for standardizing the images.

After standardizing the images, the portion of the image representing the cake was cropped and used for the analysis. This area is based on the fill height (approximately 1 inch) and limited to a 60° window of the vial (Figure 3-2). Each cropped image consists of 55 × 79 pixels. As mentioned, NIR spectra and images were acquired at six positions (at 60° intervals) to capture the overall moisture of the vials. These six images could be averaged into a single image representing the drying front of a cylindrical vial (Figure 3-3). Otherwise, the six images could be concatenated into a larger (55 × 474) pixel image representing the full periphery of the vial (Figure 3-3). The NIR images were colored using a gradient coloring scheme, where the color bar increases with the dryness of the product; dark blue represents wet products whereas pale blue and yellow represent the dry product.

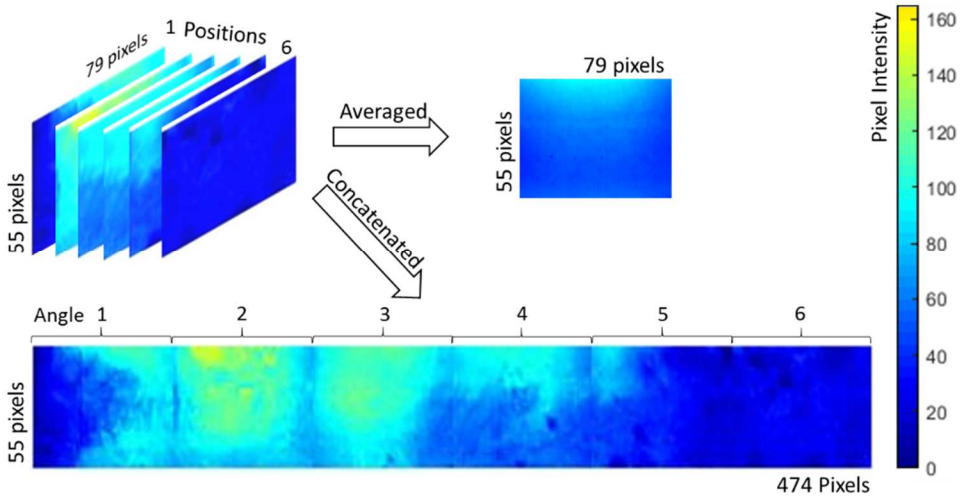

Figure 3-3. Representation of the averaged and concatenated images using a typical freeze-dried sample (a single vial). The horizontal dimension of the image represents the diameter of the vial; the vertical dimension represents the product height inside the vial.

3.3.6 Representation of the NIR spectra

Spectra were obtained at six different angles on the surface of the samples; the combined dataset can be represented as a 3D matrix (Figure 3-4), where the x-axis represents the wavelength (nm), and the y- and z-axis represent sample spectra respective to the position/angle on the product vial.

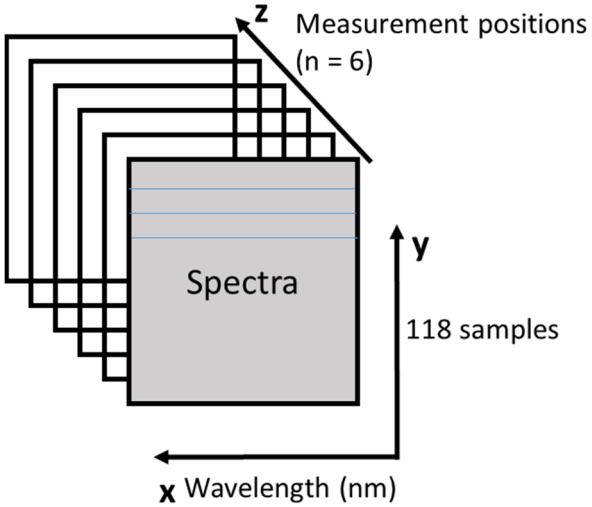

Figure 3-4. NIR spectra acquired on the vial surface at six different measurement angles represented in a 3D matrix.

3.4 RESULTS AND DISCUSSION

3.4.1 Qualitative analysis of the NIR images

Figure 3-5 represents average images of 10 categories of freeze-dried samples containing moisture in the range of 85% to <1% (w/w). These were obtained by averaging the images of the vials in each moisture category. As the products dried, a decrease in pixel intensity could be observed (from dark blue to pale blue), and the lighter pixels gradually moved from the top to the bottom of the product. The symmetric color gradients observed on the sides of the images may be linked to the curvature of the vials. This observation is in line with the mathematical models of top–down drying in vial freeze-drying[68,69]. In Figure 3-6, the histogram shows a linear relationship between product moisture, gravimetric measurement, and the average pixel intensity of the NIR-CI images, a surface measurement. This relation serves to strengthen the hypothesis that, on average, non-invasive measurement of the product at the vial periphery can be representative of the bulk of the powder within the vial. However, as illustrated in Figure 3, the moisture gradients imply that no single position at the periphery of a given vial can be a reliable indicator of the overall moisture content of that vial.

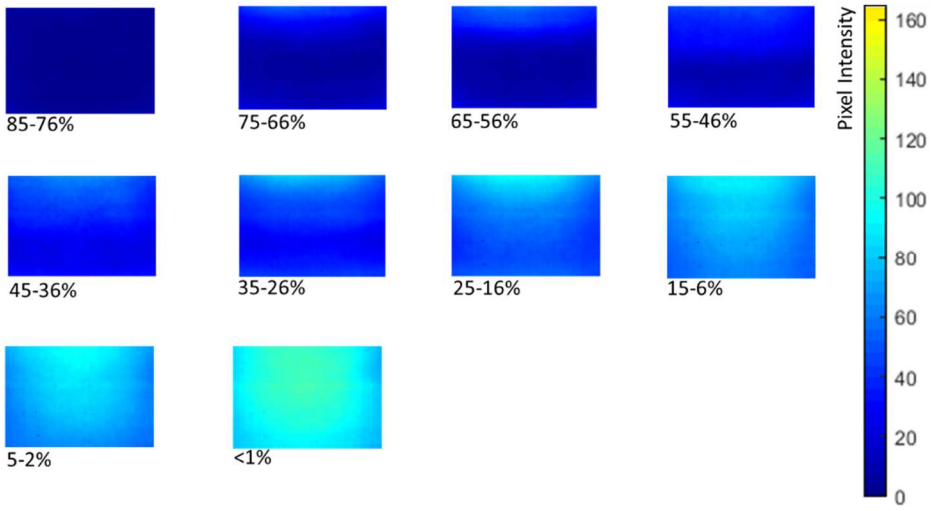

Figure 3-5. Average images of freeze-dried samples containing moisture in the range of 85% to <1% (w/w). Averages represent all 118 samples divided into 10 different categories.

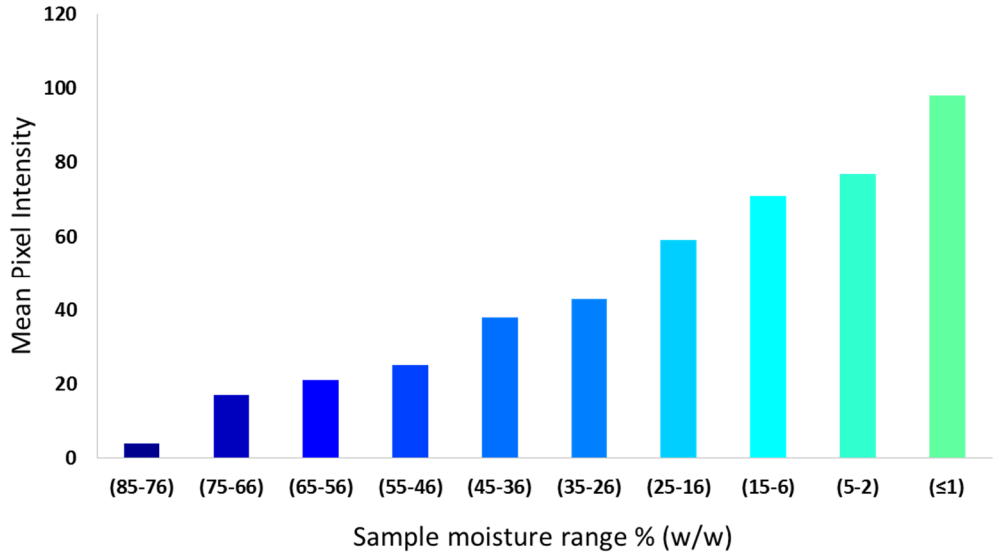

Figure 3-6. Histogram of the mean sample moisture vs. mean pixel intensity of NIR-CI images in the range of 85% to <1% (w/w) moisture.

The individual NIR-CI images of the individual samples were compared to investigate the drying front and moisture distribution within the freeze-dried products. The images of the freeze-dried samples containing different moisture levels illustrate a variable drying profile on the surface of the batch-dried vials (Figure 3-7). On one hand, high-moisture samples, i.e., >45% (w/w), showed a relatively uniform drying front at the top of the images and exhibited homogenous moisture distribution. On the other hand, lower-moisture samples, i.e., ≤45% (w/w), exhibited a non-uniform drying front and heterogeneous moisture distribution on different sections of a given vial. For example, the 5% (w/w) moisture sample presented in Figure 3-7 clearly shows a high-moisture area (2.5 images shown in dark blue) surrounded by a larger low-moisture area (3.5 images shown in pale blue).

This indicates a discrepancy between the drying profile and the amount of moisture present inside the samples. It also presents a clear problem with the notion of a horizontal drying front of the vials. Figures 3-7, 3-8, and 3-9 clearly show seemingly random drying fronts in which horizontal, vertical, and diagonal patterns are present, respectively.

A possible reason for the non-homogenous moisture distribution on the product surface could be attributed to the orientation of the vial on the drying shelf. Rambhatla et al. have demonstrated inter-vial differences (edge effect on the freeze dryer shelf) in the sublimation

rates specific to spatial position on the freeze dryer shelf. This was attributed to the atypical heat transfer on the freeze dryer shelf; therefore, vials on the edges of the shelf may dry faster compared to the vials on the center of the shelf. A similar phenomenon may occur within a vial, causing the side of the vial exposed toward the outer edge of the dryer shelf to dry faster compare to that exposed towards the center of the drying chamber.

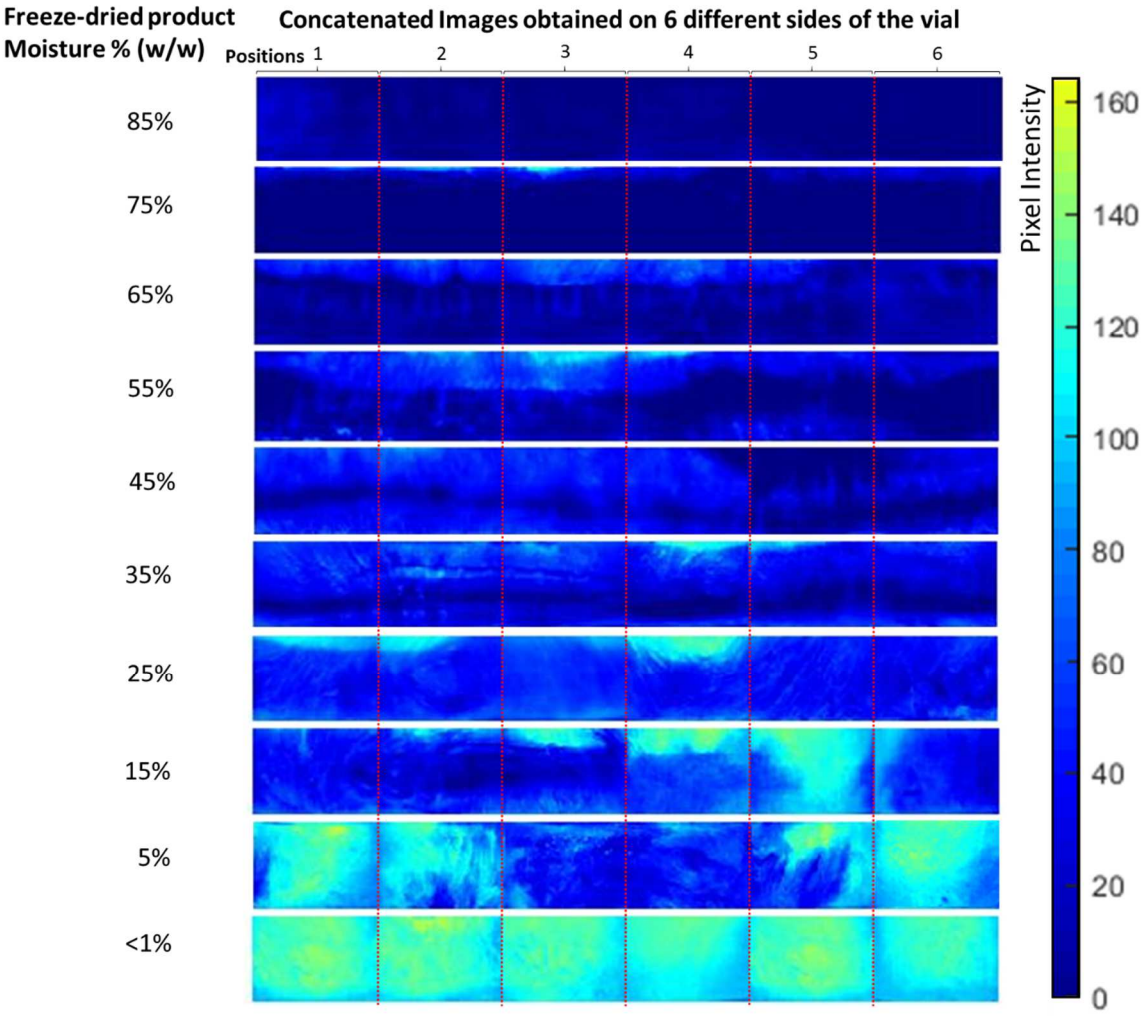

Figure 3-7. Representative images of the freeze-dried samples containing moisture in the range of 85% to <1% (w/w).

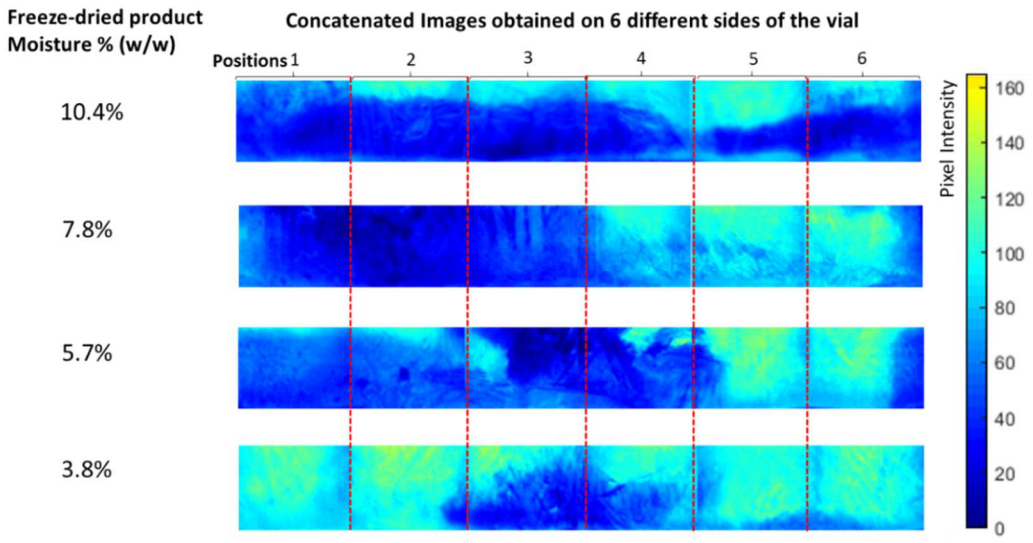

Figure 3-8. Images of vials in the 3–11% (w/w) moisture range. This range presents the largest spatial variability. These vials are representative of the population.

Furthermore, the moisture patterns of some of the batch freeze-dried vials containing low moisture levels, i.e., 5 ± 1% (w/w), show a different drying profile, although they have similar theoretical moisture levels, as illustrated in Figure 3-9. Thus, none of the vials presented a horizontal drying front, which leads to the conclusion that measurements must be acquired on multiple points around the periphery of each sample to ensure appropriate estimation of the drying level at a selected height of the vial.

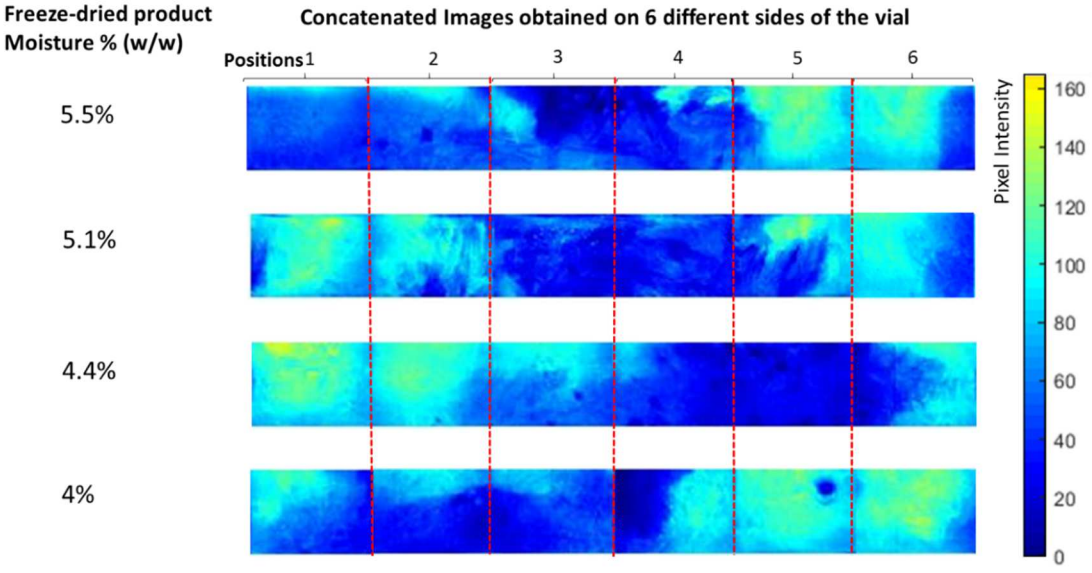

Figure 3-9. Images of a few representative vials containing similar moisture levels, i.e., 5 ± 1% (w/w).

This section presents a qualitative assessment of the use of NIR-CI to identify, map, and characterize the moisture distribution on the surface of freeze-dried vials. However, we stopped short of using this single-filter NIR-CI technology to produce a quantitative model predicting sample moisture, as there was huge variability in the moisture layout in the images acquired from the surface of the vials, rendering it impossible to determine an accurate moisture level for each image. Furthermore, imaging a cylindrical vial presents further challenges when building robust PLS models due to a relatively complex measurement setup for ensuring adequate lighting without overheating the frozen samples.

For this reason, moisture in the batch–freeze-dried products was quantified using a NIR spectrometer. An important factor in considering the NIR spectrometer was its relatively higher sample penetration depth in comparison with the NIR-CI system. Using the same equipment setup as our experiments, Dalvi et al.[33] performed a comparative penetration depth analysis to monitor the concentration of pharmaceutical powders. They showed that NIR-CI was sensitive to the first 0.75 mm of powder, whereas NIRS was sensitive to 1.5 mm of powder. Based on this, we assumed that the NIRS probe may also provide greater penetration depth in freeze-dried samples.

3.4.2 Quantitative analysis using NIRS

The objective was to determine if NIRS can be used to predict the moisture of heterogeneous batch–freeze-dried samples. Having established the spatial variability through NIR-CI, multiple NIR spectra were used for each sample. We acquired six spectra (one at every 60°) around each vial. While it is possible to simply average the six spectra for each sample, it is time-consuming and labor-intensive. Furthermore, we sought to determine a middle ground to limit the number of acquisition points to monitor vials in situ. However, the impact of reducing the number of NIR spectra per vial needs to be understood.

To answer this question, PLS regression models were used to study the quantitative relationship between NIR spectra and moisture in the freeze-dried samples. The original 3D data **X** matrix (111 wavelengths × 118 samples × 6 angles) was split into calibration and validation sets, both covering the full range of moisture levels. Spectra were pretreated using the Savitzky-Golay (SG) first derivative (15 points, second-order quadratic), and mean centered to minimize the baseline shifts.

To include spectra from a variable number of measurement angles, multiple PLS models were built taking into account an average of 1–6 measurement angles. Furthermore, the measurement points were randomly selected to account for the rotational ambiguity of the cylindrical vials. To illustrate, consider the case where a single angle was used per vial. This angle was randomly chosen for each of the 118 vials and was used to compute a PLS model predicting the gravimetric results (**Y**). This step was repeated 500 times to determine the variability of the results. Such a methodology was repeated using two, three, four, five, and six angles per vial. Using cross-validation[70], we determined that a minimum of two principal components (PC) were required to build the PLS models. From the different combinations of PLS models obtained with variable angles, on average, the first component explains the majority of the variance (71%), and the second component explains 22% of the variance.

To determine which wavelengths are most important to the model, we computed the PLS variable influence in projections (VIP) values. VIP variables with values > 1 are typically considered more important[36]. Figure 3-10 illustrates the case where all six angles are used. Large VIP values in the wavelength range of 1400–1600 nm are a result of the SG first derivative water overtone band exhibited by the ice in the sample. Furthermore, wavelengths < 1200 nm exhibit VIP values > 1 resulting from weaker absorbance bands of water and other alkyl groups present inside the glycine formulation[31].

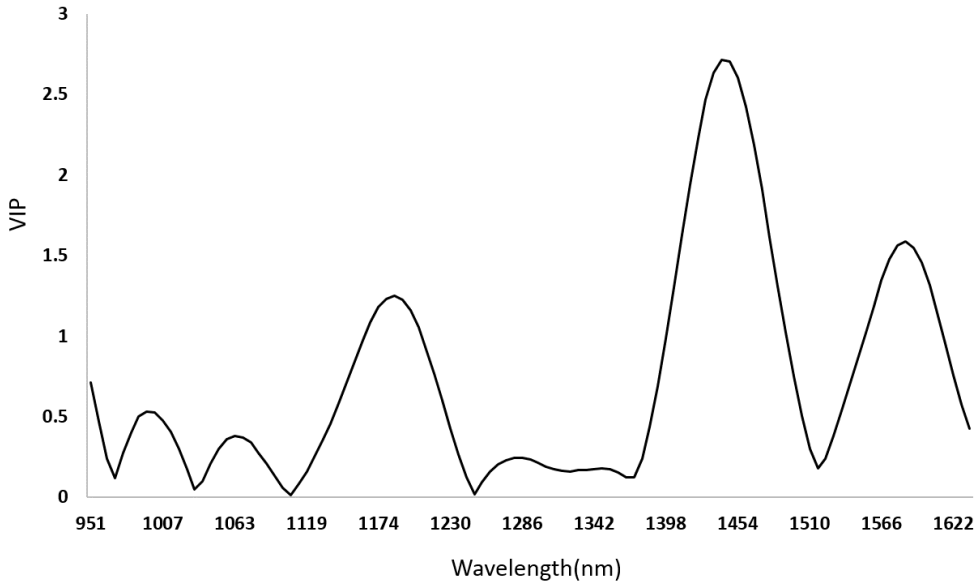

Figure 3-10. PLS VIP. Wavelengths with values >1 are considered important for predicting moisture.

The root mean square error of calibration (RMSEC) and prediction (RMSEP) of the PLS models were compared using a box and whisker plot (Figure 3-11). Three observations were drawn from this figure. The first was that the RMSE values decreased as the number of measurement angles increased. This is fully consistent with the fact that heterogeneous samples require more measurement points to be fully characterized. The second observation was that the RMSE values (i.e., height of the whisker box) decreased as the number of measurement angles increased. Again, while a single NIR spectrum may be biased, the odds that ≥2 measurements are all biased decrease rapidly, thus limiting the variability of the PLS results. The third observation was that the RMSEP results were similar, but slightly superior, to the RMSEC results. On average, RMSEP was 0.4% higher than RMSEC, indicating that the model appeared to be performing well in validation.

The RMSE values decreased between one and four measurement angles, then plateaued when five or six angles were used. The error values indicate that constant figures of merit in the models were achieved only when the measurements acquired from different spatial positions were considered by the model. In this situation, it can be inferred from the error values that spectra from a minimum of four measurement positions is an appropriate bid for PLS quantification models.

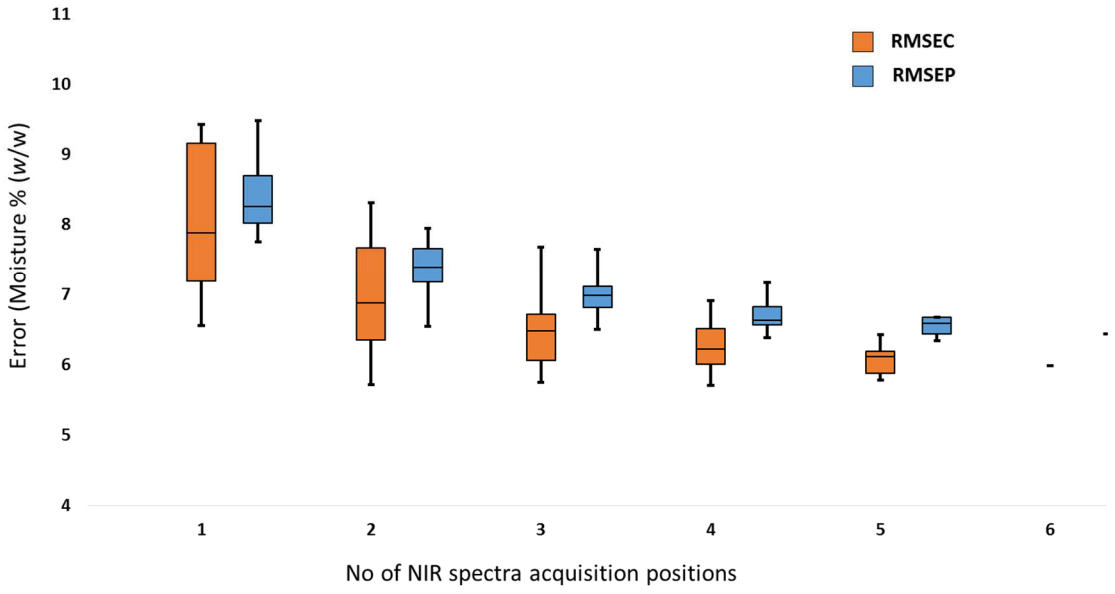

Figure 3-11. Box and whisker plot of the moisture predictions comparing the RMSEC and REMSEP values.

Based on these results, the spatial moisture variability within the batch–freeze-dried vials, while working with a point-focused surface measurement tool, should be considered. It is important to acquire measurements from multiple positions on the vials. As the specifics thereof depend on the vial geometry, processing parameters, and product properties, moisture mapping via NIR-CI appears to be prudent. Achieving useful models may be challenging, and careful consideration should be given to the product volume–vial size ratio, vial arrangement, and invasion of the probe inside the freeze-drying chamber.

3.5 CONCLUSIONS

Using a combination of NIR tools (spectroscopy and chemical imaging), this study sought to aid understanding the spatial moisture variability present in batch–freeze-dried vials. It also sought to determine the effects of spatial variability on the results obtained using single-point-focused measurement tools, which is demonstrated through the PLS analytical figures of merits.

To do so, 15% (w/v) glycine solution was used as a model formulation, and the vials were freeze-dried using a batch process. The vials were collected at different time points to achieve variable moisture levels. NIR images and spectra were acquired at six different angles around the vial surface to consider the spatial variabilities around the vial circumference. The NIR images were used for investigating the moisture distribution on the peripheral surface of the product, whereas the NIR spectra were used to confirm the spatial variations in moisture through the figures of merit of the PLS models.

Qualitative analysis of the NIR images demonstrated the heterogeneous distribution of moisture on the surface of the batch freeze-dried vials. We observed that the drying front on the product surface was not horizontal as often hypothesized in mathematical models. Further, the PLS figures of merit obtained using NIRS quantification models indicated that measurements must be collected from multiple peripheral points, in this case four points, on the vial surface to achieve constant error values. This approach could be beneficial in the early implementation of point-focused PAT tools, as knowing the spatial variations between the vials and within the vial could allow appropriate positioning of PAT tools.

Further work will be needed to investigate the peripheral distribution of moisture between the vials relative to their spatial position inside the freeze dryer so that single-point-focused PAT tools can be appropriately implemented therein. Further investigation is also required to determine intra-vial spatial moisture variability in batch–freeze-dried products.

ACKNOWLEDGEMENTS

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4. Identifying collapse in freeze-dried products via NIR spectroscopy

Title in French

Identification d'effondrement des produits lyophilisés par spectroscopie NIR

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Submitted to Journal: American Pharmaceutical Review Journal.

Contributions to the thesis: This paper explores the potential of NIR spectroscopy in identifying the collapse in the in-process and finished freeze-dried products. NIRS data analyzed by MVDA methods shows the categorization of the freeze-dried products into different groups, which opens the doors to monitor freeze-dried products in real-time and in the finished freeze-dried product.

ABSTRACT

Aesthetic appearance is a critical quality attribute (CQA) of a freeze-dried drug product and plays a key role in its release to the consumer. A collapsed cake may be a result of poorly designed process conditions or a change in physical parameters that may have gone unnoticed during the drying process. This work presents the application of near infrared spectroscopy (NIRS) and multivariate data analysis (MVDA) methods as potential process analytical technology (PAT) tools for testing collapse in freeze-dried products, both for in-process and finished freeze-dried products. The sensitivity of NIRS to the cake microstructure and physical state of water was leveraged for detecting and classifying collapse in products undergoing drying and in the finished freeze-dried products. Our results show the suitability of the NIRS for identifying in-process collapse/melt-back during freeze-drying and for classifying the collapse in the finished freeze-dried products. Comparative analysis of the NIR data using different MVDA methods showed the strong potential of linear discriminant analysis (LDA) for identifying the appearance of the in-process and finished freeze-dried products.

Keywords: Freeze-drying, Cake collapse, NIRS, PAT, Chemometrics

RÉSUMÉ FRANÇAIS

L'apparence esthétique est un attribut de qualité critique (CQA) d'un produit médicamenteux lyophilisé et joue un rôle clé dans sa diffusion chez le consommateur. Un gâteau effondré peut être le résultat de conditions de procédé mal conçues ou d'un changement de paramètres physiques qui peuvent être passés inaperçus pendant le processus de séchage. Ce travail présente l'application de la spectroscopie proche infrarouge (NIRS) et des méthodes d'analyse de données multivariées (MVDA) en tant qu'outils potentiels de technologie analytique de processus (PAT) pour tester l'effondrement des produits lyophilisés, à la fois pour les produits lyophilisés en cours de fabrication et finis. La sensibilité du NIRS à la microstructure du gâteau et à l'état physique de l'eau a été mise à profit pour détecter et classer l'effondrement dans les produits en cours de séchage et dans les produits lyophilisés finis. Nos résultats montrent la capacité du NIRS à identifier l'effondrement / refonte en cours de processus pendant la lyophilisation et à classer l'effondrement dans les produits lyophilisés finis. L'analyse comparative des données NIR utilisant différentes méthodes MVDA a montré le fort potentiel de l'analyse discriminante linéaire (LDA) pour identifier l'apparence des produits lyophilisés en cours de fabrication et ainsi que des produits finis.

Mots clés: Lyophilisation, Effondrement du gâteau, NIRS, PAT, Chimiométrie

4.1 INTRODUCTION

Following Quality by Design (QbD) guidelines,[57] biopharmaceutical manufacturers put enormous effort in developing and implementing process analytical technology (PAT) tools for monitoring freeze-drying processes. These tools help monitor both processes and products to achieve the desired quality in the product.[3] Despite significant technological advances, it is still not possible to ensure that 100% of freeze-dried cakes reach the critical quality attributes (CQA). International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use [ICH Q8 (R2)] defines a CQA as a property (i.e., physical, chemical, biological, or microbiological) or characteristic that should be within the appropriate range for ensuring the desired quality of the product.[57] One CQA is the aesthetic appearance of the cake, described as a uniform and elegant cake structure of the dried product.[71] Aesthetic appearance is determined while the product is undergoing the drying process. A possible factor affecting cake appearance is product collapse, defined as loss to cake microstructure that is established during freezing.[72] An increased product temperature caused by early ramping into secondary drying results in product melt-back during the drying process, and may have a significant impact on the appearance of the cake.[58]

In accordance with good pharmaceutical practice, the United States Pharmacopeia (USP) recommends inspecting all vials in a batch, also termed 100% batch inspection.[73] Product acceptance with regards to its aesthetic appearance is subject to acceptance by visual inspection in the production environment. However, sole reliance on human intervention to inspect cake appearance can be fallible on the production line, as thousands of vials are produced. Failure to detect a cake defect in the finished cake, such as collapse or melt-back, can have a deleterious effect on the patient, as this defect might be the result of excess product moisture that may affect the drug's safety and efficacy.[73] In some instances, cake collapse may cause poor solubility and increased reconstitution time.[74] Recently, Patel et al.[71] summarized the challenges related to the visual inspection of lyophilized drug products and provided insights on acceptable cake appearance from an industrial perspective.

Several offline tools have been developed for characterizing the appearance of freeze-dried cakes, but as these techniques are invasive and require longer testing times, they are only commonly used in the development stages.[75] Here, we present the potential of near infrared spectroscopy (NIRS) in combination with multivariate data analysis (MVDA) methods for

identifying collapse in products during the process and in the finished freeze-dried cake. The proposed approach could provide assurance and accelerate the practice of visual inspection of freeze-dried cakes.

4.2 MATERIALS AND METHODS

4.2.1 Sample preparation and NIRS measurement procedure

A 15% (w/v) glycine solution was used as a model formulation for the freeze-dried products. The samples were dried using a ModulyoD laboratory-scale freeze dryer (Thermo Electron Corporation, Waltham, MA, USA). Three categories of samples were produced: 1) partially collapsed cakes, 2) fully collapsed cakes, and 3) normal freeze-dried cakes; each category comprised 25–35 samples. Cake collapse was produced by manipulating the vials during drying. We observed that the degree of collapse was closely related to the amount of moisture in the freeze-dried samples; therefore, two different moisture levels were selected for inducing partial and full collapse (Figure 4-1).

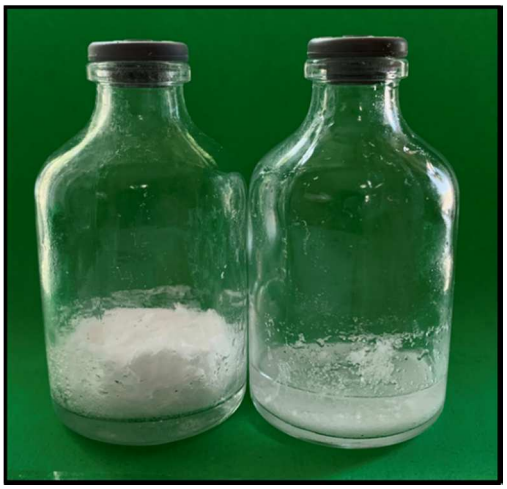

Figure 4-1 *Representation of collapse in typical freeze-drying samples. (Left) Partially collapsed and (right) fully collapsed/melt-back samples.*

To induce partial collapse, samples containing 5 ± 2% (w/w) moisture were removed from the freeze dryer and left at room temperature (20°C) until cake shrinkage was observed. To induce full collapse, samples containing 20 ± 5% (w/w) moisture were removed from the freeze dryer and left at room temperature until cake melt-back was observed. The moisture content was determined by gravimetry, where the difference between the initial and final product weight was calculated[66,67].

The NIR spectra were acquired using a diffuse reflectance NIR spectrometer (MicroNIR 1700, VIAVI Solutions, Inc., Milpitas, CA, USA) covering the wavelength range of 900–1700 nm. The measurements acquired consisted of an average of 128 spectra, with an integration time of 12 ms. The vials assigned to partial and full collapse were scanned before and after collapse was induced. After the NIR spectra had been obtained, the vials were replaced in the freeze dryer to complete the drying cycle. Once dry, all three categories of the samples (Figure 4-2) were scanned to obtain the spectra for the different categories of finished freeze-dried products. NIR spectra were acquired in triplicate at 60° intervals around the cylindrical vials; later, the spectra obtained on each vial were averaged to obtain one spectrum per vial.

Figure 4-2 Representation of typical finished freeze-dried samples. (Left) Normal cake, (center) partially collapsed, and (right) fully collapsed/melt-back samples.

4.2.2 Data structure and Chemometric methods

The dataset of the finished samples consisted of 92 spectra × 125 wavelengths, one for each of the 92 samples in the three categories: partially collapsed, completely collapsed, and normal products. The dataset of the in-process samples was composed of 114 spectra × 125 wavelengths. The 114 spectra were: 32 completely collapsed samples and 25 partially collapsed samples, all of which were scanned twice (before and after product collapse).

The raw and preprocessed spectra were examined to obtain information related to the collapse and melt-back in the freeze-dried cakes. This was carried out via chemometrics methods such as principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and linear discriminant analysis (LDA), applied on the finished and in-process sample dataset

for classifying the samples into the different categories. PCA is a projection method for maximizing the variance of the X matrix (spectra), and does not take the Y matrix (CQA) into account. PLS-DA is a projection method for maximizing the covariance between X and Y, where Y is a diagonal matrix representing the class of each sample. LDA is a projection method for maximizing the separation of each X group based on the Y matrix, that is, a known class of each sample; however, this does not seek to maximize variance. All data analysis, spectral pre-processing, and chemometric model building steps were performed using Python 3.7.

4.3 RESULTS AND DISCUSSION

4.3.1 Identifying and classifying collapsed vs normal finished freeze-dried products

In the finished freeze-dried products, the spectral baseline information was used to classify complete collapse, partial collapse, and normal freeze-dried products. The spectra were simply mean centered prior to model building. Figure 4-3 (a, b, c) shows the comparative score plots of the different methods (PCA, PLS-DA, LDA); the samples are colored according to category (partial and full collapse, and normal freeze-dried). In all cases, two latent variables were sufficient for discriminating the spectra belonging to different categories. In PCA and PLS-DA, spectra separation could be seen on the first component axis (PC1), but the samples tended to overlap, as PCA and PLS-DA tend to maximize the variance and covariance between samples. A clear separation and clustering of spectra respective to the categories could be seen in the LDA score plot, as LDA tends to maximize the separation of the group of samples based on the known sample category. The LDA loadings (Figure 4-3 d) were mostly flat, which means that the entire baseline is used to separate the samples into different categories. And there is limited to no impact of moisture on different categories of the samples. The LD1 loadings separate the samples based on the product's aesthetic appearance contributed by the baseline changes in the samples' spectra. The LD2 loadings separate the samples based on the product concentration changes. The partially and completely collapsed samples had lower scores on the LD2 axis, as the product in some cases had shrunk and was not homogeneously distributed inside the glass vial. However, the higher LD2 scores of the normal freeze-dried products could be correlated with the higher product concentration, as the cake in this condition was intact and homogenously distributed in the glass vial.

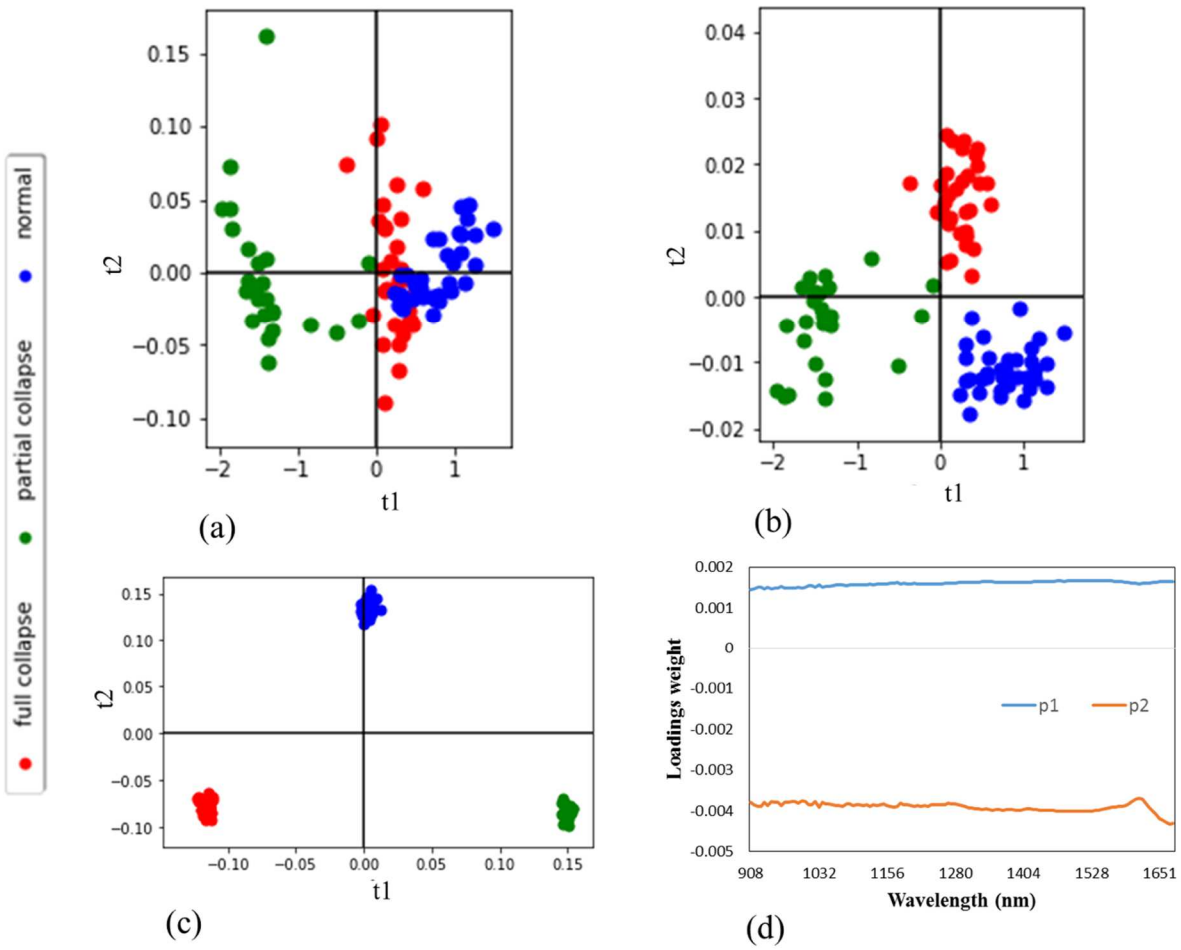

Figure 4-3. Scores and loadings plot of finished freeze-dried products. (a) PCA score plot. (b) PLS-DA score plot. (c) LDA score plot. (d) LDA loadings plot.

4.3.2 Identifying in-process collapse (meltback) in the products during the freeze-drying process

In the in-process products, partial and complete collapse were identified using the spectral signature. The spectra of both categories of samples, obtained before and after collapse as induced, were pretreated using the standard normal variate (SNV). The spectra showed a distinct water overtone band when there was a change in the product's physical state (from ice to liquid). Figure 4-4 shows comparative score plots of the different methods (PCA, PLS-DA, LDA); the samples are colored according to category (partial and full collapse, and normal freeze-dried). In all methods, two latent variables were sufficient for discriminating between the samples.

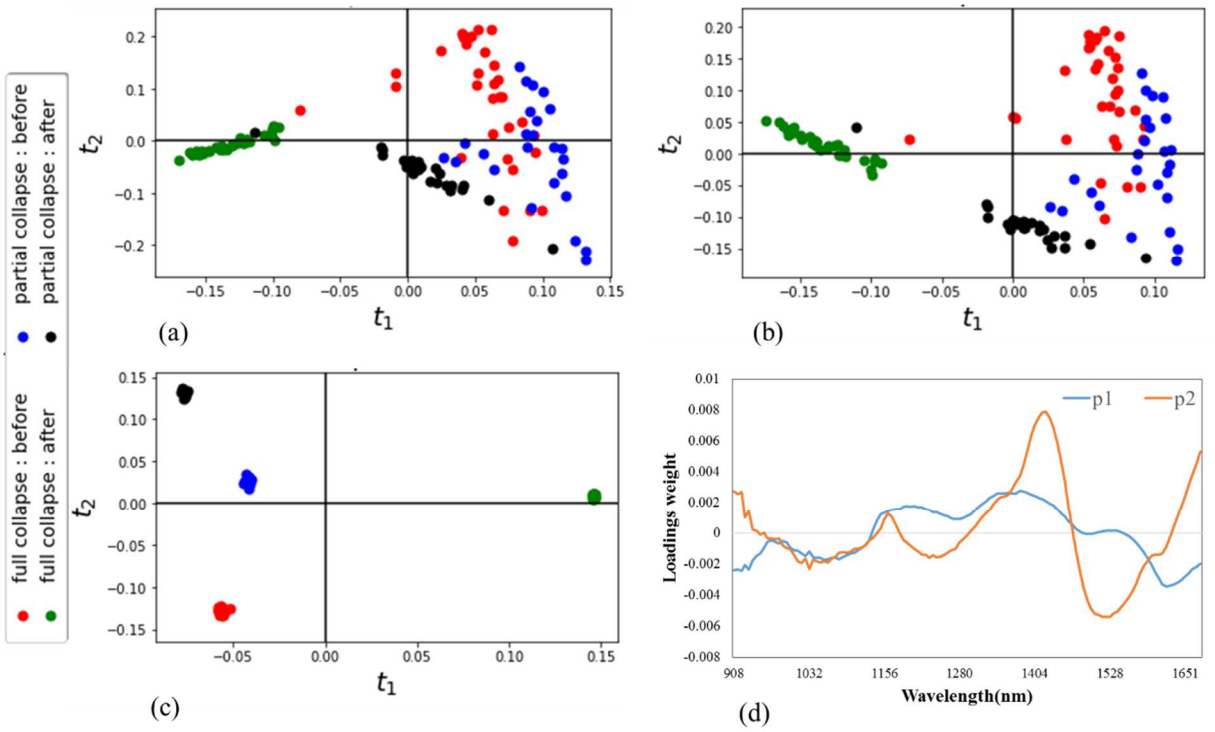

Figure 4-4 Scores and loadings plots of freeze-drying (in-process) products. (a) PCA score plot. (b) PLS-DA score plot. (c) LDA score plot. (d) LDA loadings plot

In the PCA and PLS-DA score plots, the separation of the samples into different categories could be seen only on the PC-1 axis; however, the LDA score plot showed a clear separation of the samples on both the LD1 and LD2 axes. On the LD1 axis, the samples were separated into two broad categories. The LD1 loadings had higher weights at 1366 nm, indicating that the product concentration changes (the alkyl group, in this case) contributed to the separation seen on the LD1 axis. Samples existing in a full collapse state showed sedimentation of the product at the bottom of the vial; therefore, they had comparatively higher scores. The LD2 loadings indicate that the free moisture present in the samples' interstitial spaces contributed to the grouping of spectra on the LD2 scores axis. The partially collapsed samples had higher LD2 scores, as they contained larger amounts of free moisture, whereas samples prior to full collapse had lower scores, as they contained less free moisture. This is in accordance with the literature that presents NIRS ability in identifying the state and nature of water present inside the samples[31]

4.4 CONCLUSION:

This study evaluates the potential of NIRS for identifying the cake defects encountered in the manufacturing of freeze-dried drug products. Defects in the products, such as partial and complete collapse, were produced by mimicking abnormal process conditions that might arise during the routine manufacturing process. The samples were tested during the process (in-process) and after the process had been completed. The in-process samples were tested before and after collapse had been induced. The NIRS data were analyzed using PCA, PLS-DA, and LDA methods, and the comparative results from these three methods have been presented.

The results show the potential of NIRS for identifying the different cake defect intensities in the finished freeze-dried products, and also for identifying product collapse during freeze-drying. Moreover, the comparative results showed that choosing the appropriate method is important for characterizing the samples into different categories based on their nature. Among the methods used, LDA was most appropriate for discriminating samples.

5. Preliminary experiments to assess the suitability of NIR spectrometers and test formulation for monitoring moisture inside the freeze-dried products

Title in French

Expériences préliminaires pour évaluer l'aptitude de spectromètres NIR et tester la formulation pour monitorer l'humidité à l'intérieur de produits lyophilisés

Contributions to the thesis: Work in this chapter was intended to make a preliminary evaluation of several low-cost NIR spectrometers using a model formulation in order to identify a suitable NIR spectrometer for monitoring moisture in freeze-dried products. To carry out this work, mimicked freeze-dried samples with different moisture levels were used. Findings of this experiment helped in identifying an NIR spectrometer that can potentially be used for monitoring moisture in the freeze-dried products. In addition, this chapter provides a methodology that allows a one-to-one comparison between three different NIR spectrometers.

CHAPTER OVERVIEW

NIR Spectroscopy (NIRS) is an established method to monitor moisture in in-process and in the finished freeze-dried products. In the past, several studies have reported the application of NIRS for monitoring moisture inside the freeze-dried products using pharmaceutical products. The selectivity of NIRS to monitor moisture inside the products depends on factors such as the NIR wavelength range, choice of formulation ingredients and spectral properties of NIR in implementing in the real-time process settings.

The present work evaluates several low cost NIR spectrometers such as Viavi MicroNIR-1700, Spectral engines (N-1.7 & N-2.2), and SCIO-NIR to identify a suitable one for monitoring moisture inside the glycine model formulation. PLS based predictive models were built using data from different NIR spectrometers and their performance was determined by comparing the obtained PLS figures of merit. Comparison of NIR spectrometers' PLS figures of merit was done in order to identify a NIR spectrometer suitable for monitoring moisture inside the freeze-dried products. Results demonstrate the spectral selectivity and suitability of a NIR to monitor the moisture in freeze-dried products containing variable moisture levels.

RÉSUMÉ DU CHAPITRE EN FRANÇAIS

La spectroscopie NIR (NIRS) est une méthode établie pour mesurer l'humidité dans les produits lyophilisés en cours de séchage et dans les produits finis. Dans le passé, plusieurs études ont présenté l'application du NIRS pour surveiller l'humidité à l'intérieur des produits lyophilisés à l'aide de produits pharmaceutiques. La sélectivité du NIRS pour surveiller l'humidité à l'intérieur des produits dépend de facteurs tels que la plage de longueurs d'onde NIR, le choix des ingrédients de formulation et les propriétés spectrales du NIR lors de la mise en œuvre dans les paramètres de processus en temps réel.

Le présent travail évalue plusieurs spectromètres NIR à faible coût tels que le Viavi MicroNIR-1700, les moteurs spectraux (N-1.7 et N-2.2) et SCIO-NIR pour identifier un spectromètre approprié pour la surveillance de l'humidité à l'intérieur de la formulation du modèle de glycine. Des modèles prédictifs basés sur des modèles PLS ont été construits à l'aide de données provenant de différents spectromètres NIR et leurs performances ont été déterminées en comparant les chiffres de mérite PLS obtenus. Une comparaison des valeurs de mérite des spectromètres NIR PLS a été effectuée afin d'identifier le NIR pour surveiller l'humidité à l'intérieur des produits lyophilisés. Les résultats démontrent la sélectivité spectrale et l'aptitude d'un NIR à surveiller l'humidité dans les produits lyophilisés contenant des niveaux d'humidité variables.

5.1 INTRODUCTION

IR based Process analytical technologies (PAT) tools are increasingly popular to monitor the critical quality attributes (CQA) of the product during the freeze-drying, making it possible to monitor the progression of the process. NIR spectroscopy (NIRS) is one of the preferred methods to monitor the moisture in the freeze-dried products because of its non-product-invasive and non-destructive nature. Early work identified the potential of NIRS to determine the moisture inside the freeze-dried products with the precision equivalent to the Karl-Fischer (KF) titration method[14]. Most recently, Kauppinen et al[15] demonstrated the implementing of multiple NIR probes on vials specific to spatial position inside the drying chamber, this was done to monitor moisture variability in vials during the freeze-drying process. Despite the existence of numerous studies on the use of the NIR probes, there are still challenges in implementing the probes in freeze-drying equipment:

- The introduction of a probe inside the freeze-drying chamber presents challenges because of the presence of an isolated product chamber space and it requires major retrofitting to the drying chamber.
- Maintaining the position of the NIR probe on the sample surface throughout the drying process.
- Difficulties in transferring the technology from the lab scale to production scale.

This study was focussed on exploring the low-cost NIRS that addresses all the challenges and allows easy adaptability inside the freeze-dryer. Furthermore, it promises to reduce the cost and enhance the technical feasibility of implementation. The selection of the NIRS specific to the formulation is crucial as there can be an interference of absorbance bands coming from formulation ingredients with the water overtone bands.

Therefore, the objectives of this study are, 1) evaluate the suitability of model formulation for moisture quantification studies, 2) compare NIR spectrometers' performance to identify a suitable NIRS for monitoring moisture inside the mimicked freeze-dried products.

5.2 MATERIALS AND METHODS

Glycine (≥98.5%), a pharmaceutical excipient, was purchased from Fischer Scientific Canada and was used as a model formulation for producing samples. Deionized water was used as a

solvent to prepare different moisture levels inside the samples. For this study, 50mL capacity glass vials were used (DWK Life Sciences, Millville, NJ, USA). Sample freezing was done in -18°C freezer (RCA, NY, United States). In order to achieve homogenous distribution of moisture in the damp samples a household coffee blender was used (Cuisinart-DCG-12BCEC, Woodbridge, ON, Canada).Three different NIR spectrometers were used for testing the experiments and the following are their specifications:

Table 5-1 Working wavelength range of NIR spectrometers and their focused moisture band

NIR	**Working wavelength range**	**Focused moisture band**
MicroNIR (Viavi Solutions Inc.)	950-1650nm	1440-1470nm
N-2.2 Sensor (Spectral Engines GmbH)	1750-2150nm	1900-1950nm
ScioNIR (Consumer Physics Inc.)	760-1052nm	950-980nm

5.2.1 Sample preparation:

Both the calibration and validation samples were prepared in % (w/w) concentrations for a total sample weight of 50 grams (water + glycine). Calibration samples were prepared in triplicates for each moisture level, whereas, one sample per moisture level was prepared in the validation group. Proportions of glycine and water used to produce samples in the moisture range of 0-30% (w/w) are presented in Table 5-2 & 3.

Table 5-2 Proportions of water and glycine required for calibration samples

Sample S.no	**Water weight (g)**	**Glycine weight (g)**	**Moisture (%) w/w**
1	0	50	0
2	1	49	2
3	2	48	4
4	3	47	6
5	4	46	8
6	5	45	10
7	6	44	12
8	7	43	14
9	8	42	16
10	9	41	18
11	10	40	20
12	11	39	22
13	12	38	24
14	13	37	26
15	14	36	28
16	15	35	30

Table 5-3 Proportions of water and glycine required for validation samples

Sample Number	Water weight (g)	Glycine Weight (g)	Moisture (%) w/w
1	1.5	48.5	3
2	3.5	46.5	7
3	5.5	44.5	11
4	7.5	42.5	15
5	9.5	40.5	19
6	11.5	38.5	23
7	13.5	36.5	27

In order to achieve homogenous distribution of water inside the glycine, the weighed sample mixture was mixed in a coffee blender for 15 seconds, this was done by flattening the blades of the blender with a duct tape. Samples were filled in glass vials and labelled appropriately. Samples with >20% (w/w) moisture were prepared directly in a glass vial as it was relatively easy to mix the sample slurry with a spatula to achieve homogenous spread of moisture. Samples containing >20% (w/w) moisture concentration were frozen inside -18°C freezer for 2 hours, whereas samples ≤20% (w/w) moisture were maintained at the room temperature; this was done to mimic the product form that exists during the freeze-drying process. To acquire the NIR measurements, initially samples with ≤20% moisture were scanned using an individual NIRS, later the frozen samples were taken out of the freezer one by one and the measurements were made ensuring the product was in the frozen state, each vial was kept in an ambient temperature environment for not more than one minute. NIR measurements were acquired around three different sides on each sample vial at an angle of approximately 120° and at each measurement angle 3 replicate spectra were acquired, the height of the NIR sensor was adjusted in a way to keep the sensor head always in the middle of the sample fill volume.

5.2.2 Data analysis

The following steps were used to analyse the data:

Step 1. Pre-processing of Raw Spectra

NIRS raw spectra were inspected to observe the spectral trend relative to the moisture concentration present inside the samples. Spectra obtained from each NIR spectrometer were pre-processed using several methods such as SNV, derivatives, and a combination thereof. After preprocessing, spectra were inspected to observe the trend in the water absorbance band

with the change in product-moisture concentration. Pre-processed spectra that exhibited an ascending trend in water absorbance maxima with the increase in water concentration was later used for PLS model building. All the spectral pre-processing and model building was done using SIMCA 14.1 (MKS Umetrics, Sweden).

Step 2. Chemometric and statistical analysis

Several PLS calibration models were built using data obtained from different pre-processing methods such as SNV, derivatives, their combinations, the best among the developed models is presented in the following section. To include possible variations in the model, all the spectra were included, although a few of the replicate scans seemed to deviate in terms of water band absorbance intensity.

The reference moisture content for the samples was taken based on gravimetrically acquired moisture values presented in Tables 5-2 & 3.

To choose the appropriate number of components in a PLS-model, an optimum balance between model fit and predictive ability was considered, reference for the goodness of model fit was taken by the parameters, explained variation (R^2) and goodness of prediction by parameter predicted variation (Q^2), the point where there was a good agreement between the R^2 and Q^2 values was used as a basis for selecting the appropriate number of components. Cross validation was done to avoid overfitting the model. Additionally, residual component plots were inspected to ensure there was no significant pattern in the error matrix.

Step 3. Validation of the developed PLS model:

The accuracy of the model is demonstrated by the RMSECV and RMSEP. To obtain the RMSECV, cross-validation was done using a random grouping method in which the training set was divided into 7 subgroups such that each group contained observations with random moisture levels. The RMSECV was obtained by a repeated protocol in which a model is trained with 6 subgroups to predict the values of the kept out subgroup. To compute the RMSEP, external validation was done using the spectra obtained from the validation sample group containing water concentration in the range of 3-19% (w/w), mentioned in Table 5-3.

5.3 RESULTS AND DISCUSSION

In the MicroNIR spectra, water show a prominent absorbance band in the wavelength range of 1400-1500 nm (Figure 5-1 a) another small peak is observed at 1170nm and it is related to a weak water absorbance band.

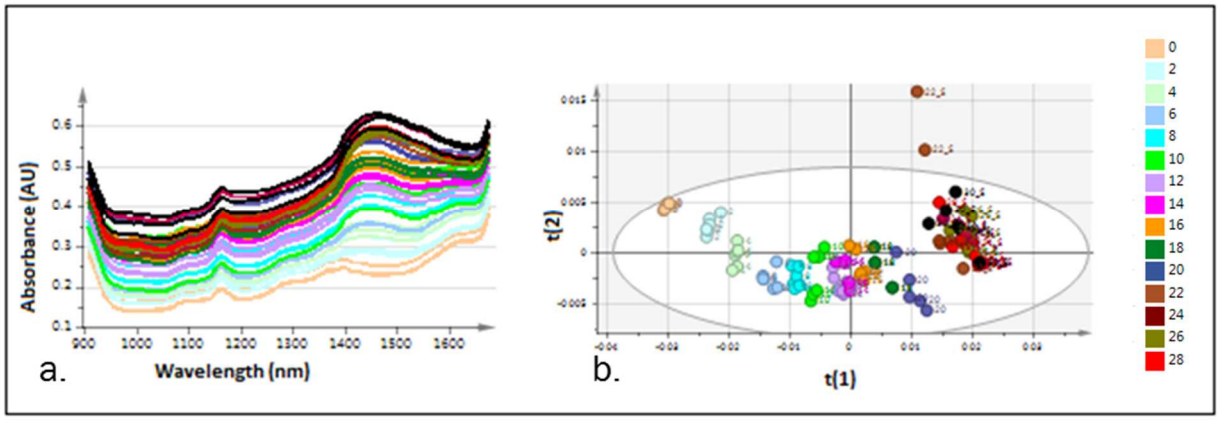

Figure 5-1 (a) MicroNIR raw spectra of freeze-dried samples with 0-30% (w/w) Moisture. (b) PCA score plot of MicroNIR spectra; spectra and data points in PCA plot are labelled and colored according to theoretical moisture%.

Spectra of the frozen samples >20% (w/w) moisture exhibit no clear separation of spectra and there was an overlap in spectral response, this was due to sedimentation of product at the bottom of the vial. A PCA model was built using the MicroNIR spectra in order to evaluate the effect of product sedimentation in frozen samples on their spectral response. In the PCA score plot (Figure 5-1 b) PC1 and PC2 explained a total of 98% variance. The score plots shows that the samples with water concentration in the range of 0-20% (w/w) are well separated on a PC1 axis based on the amount of water present in them, whereas all the frozen samples with water concentration in the range of 22-30% (w/w) seem to closely cluster with no clear trend. This confirms the heterogeneous distribution of moisture inside the frozen samples.

However, a few of the replicate spectra of the samples <20 % (w/w) water seem to show overlapping spectral response with the other sample concentrations, despite these deviations all the spectra were retained for the model building.

In the SE N-2.2 NIR and ScioNIR spectra (Figure 5-2 a & b) water shows an absorbance band at approximately 1950 nm and 960nm. Similar to MicroNIR, spectra of SE N-2.2 NIR and ScioNIR (Figure 5-2 a) also show no clear segregation in spectra of samples >20% (w/w)

moisture. Therefore, for all NIRS, spectra of samples with moisture concentration in the range of 0-20% (w/w) were used for building PLS models.

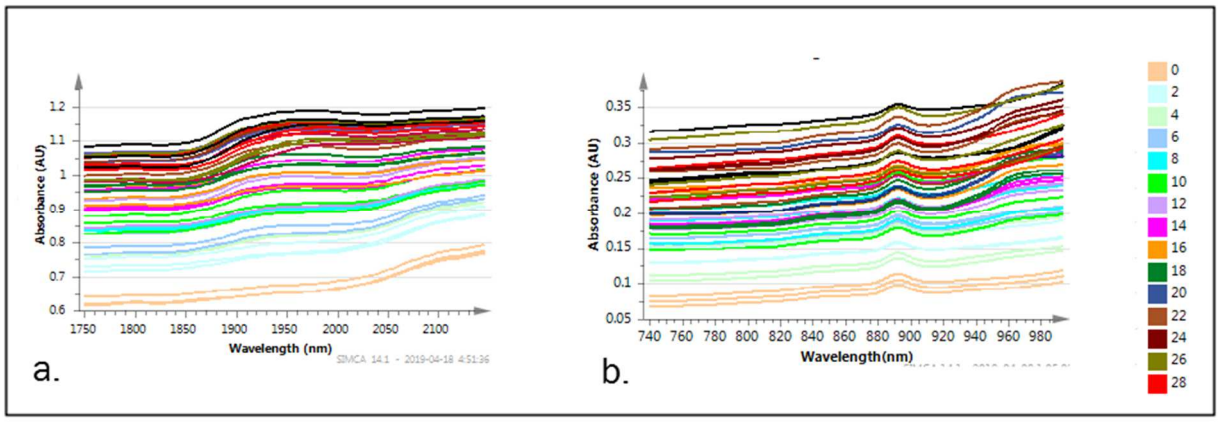

Figure 5-2 (a) SE N-2.2 NIR *raw spectra of freeze-dried samples with 0-30% (w/w) moisture. (b) ScioNIR raw spectra of freeze-dried samples with 0-30% (w/w) moisture; spectra are labelled and colored according to theoretical moisture%.*

Calibration models for MicroNIR and SE N-2.2 NIR spectrometer were built using first derivative spectra, and for the ScioNIR standard normal variate pre-processed spectra were used.

PLS calibration models were built separately for each NIR spectrometer. In the MicroNIR spectrometer, wavelengths <1100nm were excluded from building PLS models as there was no clear trend in the spectra. In the SE N-2.2 spectrometer, all the wavelengths were taken into account. Whereas, in ScioNIR spectrometer wavelengths lower than 950nm were excluded as there was a more prominent absorbance band contributed by the alkyl group present inside the product. In all the cases PLS based calibration models with two latent variables showed R^2adj values ranging between 0.91 to 0.96. And the maximum RMSEC was ≤1.82% (w/w) moisture. And the calibration models used for predicting the test dataset showed RMSEP of ≤2% (w/w) moisture. Detailed PLS figures of merit are presented in Table 5-4.

Table 5-4 Summary PLS figures of merit of different NIRS

NIR	**Pre-treatment method**	**No of PC**	**R^2**	**Q^2**	**RMSEC**	**RMSECV**	**RMSEP**
MicroNIR	1st Derivative	2	0.99	0.98	0.69	0.69	1.01
Spectral Engines (SE) N-2.2	1st Derivative	2	0.92	0.91	1.85	1.84	2.02
ScioNIR	SNV	2	0.96	0.96	1.24	1.23	1.32

Among the three evaluated NIRS, PLS figures of merit were comparatively better for MicroNIR spectrometers, and it was observed that there was no interference of glycine functional groups on the water absorbance band. Also, a clear separation in spectra respective to the amount of moisture present inside the samples was observed.

5.4 CONCLUSIONS

Conventional NIRS tools require major retrofitting to the freeze-drying equipment to monitor product-moisture during the process, however, by using small size tools, retrofitting to the equipment can be avoided. This enhances the feasibility of implementing NIRS for real-time monitoring of the freeze-drying products and processes. The study sought at assessing the feasibility of using mini NIR spectrometers to predict moisture inside the simulated freeze-drying samples via PLS calibration models. Results of this experiment with three different low-cost NIR spectrometers demonstrate their abilities in measuring the amount of water present inside the samples in the concentration range of 0-20% (w/w). Among the evaluated NIR spectrometers, MicroNIR showed better separation in spectra respective to the amount of moisture present in the samples. And comparatively better PLS figures of merit were seen, which indicates the possible potential of MicroNIR in real-time monitoring of moisture in the freeze-drying products.

Further work will be needed with In-process freeze drying samples in order to assess the capabilities of these NIR sensors for freeze-drying monitoring, however, these initial trials with the simulated samples open the doors for considering the low-cost NIR probes for freeze-drying monitoring.

6. CONCLUSION

PAT tools are increasingly being explored and developed to monitor pharmaceutical manufacturing operations in order to achieve high quality products. The intent behind continuous PAT development is not just to comply with current regulatory requirements but also to enhance the process understanding so that the products and processes can be efficiently monitored and controlled. Knowing the criticality of moisture on product stability and its impact on several different quality attributes in products, numerous PAT tools have been developed focussed on monitoring and quantifying moisture inside the freeze-dried products. In order to obtain robust results using point focussed measurement tools, further exploration is needed to determine the spatial intra-vial distribution of moisture in the freeze-dried products. In addition, there is a need to explore tools that can potentially be used for monitoring collapse in products during the freeze-drying and in the finished freeze-dried products; such that the batch failure can be avoided and the practice of visual inspection of 100% of vials in the batch can be accelerated.

Part of this thesis focuses on the use of a combination of NIR tools (Chemical imaging and NIR spectroscopy) to investigate the spatial distribution of moisture inside the freeze-dried vials. Qualitative and quantitative studies were carried out to gain in depth understanding about the intra-vial moisture distribution in the freeze-dried vials. These studies showed the usefulness of NIR CI in studying the spatial moisture on the surface of the vials. The results confirm the non-homogenous distribution of moisture, as well as the non-uniform drying front, in the products undergoing freeze-drying. Findings from the NIRS-based partial least squares (PLS) models indicate that, to achieve reliable product/process information, measurements must be drawn from multiple measurement points on the surface of the freeze-dried products.

Later work was aimed at evaluating NIRS for monitoring the collapse inside the freeze-dried products. Results show the potential of NIRS in identifying different intensities of cake defects in the finished freeze-dried products, and also in identifying the product collapse during the freeze-drying process. Besides, from the comparative results obtained using different MVDA methods, it was understood that in order to classify samples based on their nature into different categories it is crucial to choose an appropriate method. Owing to the LDA's nature of

maximizing separation between sample groups it was found to be the most appropriate method to discriminate normal and collapsed categories of freeze-dried samples.

Overall, this thesis work presents interesting findings of the freeze-dried products using NIR tools (Chemical imaging and spectroscopy). During freeze-drying cycle development stage, presented approach of investigating the spatial distribution of moisture inside the freeze-dried products enables appropriate positioning of point focussed PAT tools and targeting the right sample inside the freeze-drier. Besides, the implementation of NIRS in monitoring the product collapse during freeze-drying helps in mitigating product defects. Also, this helps in reassuring existing practice of 100% visual inspection of finished product vials in the production environment.

Conclusion en Français

Pour obtenir des produits de haute qualité, les outils PAT sont de plus en plus explorés et développés pour surveiller les opérations de fabrication pharmaceutique. L'intention derrière le développement continu de PAT n'est pas seulement de se conformer aux exigences réglementaires actuelles, mais d'améliorer la compréhension des procédés afin que les produits et les processus puissent être efficacement monitorés et contrôlés. Connaissant l'importance de l'humidité sur la stabilité et son impact sur plusieurs attributs de qualité différents dans les produits, de nombreux outils PAT axés sur la surveillance et la quantification de l'humidité à l'intérieur des produits lyophilisés ont été développés. Afin d'obtenir des résultats robustes en utilisant des outils de mesure focalisés sur un point, une exploration plus approfondie est nécessaire en ce qui concerne la distribution spatiale intra-flacon de l'humidité dans les produits lyophilisés. En outre, il est nécessaire d'explorer des outils qui peuvent être utilisés pour surveiller l'effondrement des produits pendant la lyophilisation et dans les produits lyophilisés finis; de sorte que la défaillance du lot puisse être évitée et que la pratique de l'inspection visuelle de 100% des flacons du lot puisse être accélérée.

Une partie de cette thèse se concentre sur l'utilisation de la combinaison d'outils NIR (imagerie chimique et spectroscopie NIR) pour étudier la distribution spatiale de l'humidité à l'intérieur des flacons lyophilisés. Des études qualitatives et quantitatives ont été menées pour acquérir une compréhension approfondie de la distribution de l'humidité intra-flacon dans les flacons

lyophilisés. Ces études ont montré l'utilité du NIR CI pour étudier l'humidité spatiale à la surface des flacons. Les résultats confirment la répartition non homogène de l'humidité, ainsi que le front de séchage non homogène, dans les produits en lyophilisation. Les résultats des modèles des moindres carrés partiels (PLS) basés sur le NIRS indiquent que, pour obtenir des informations fiables sur les produits / procédés, les mesures doivent être effectuées à partir de plusieurs points de mesure sur la surface des produits lyophilisés.

Des travaux ultérieurs visaient à évaluer le NIRS pour surveiller l'effondrement à l'intérieur des produits lyophilisés. Les résultats montrent le potentiel du NIRS dans l'identification de différentes intensités de défauts de gâteau dans les produits lyophilisés finis, et également dans l'identification de l'effondrement du produit pendant le processus de lyophilisation. En outre, à partir des résultats comparatifs obtenus à l'aide de différentes méthodes MVDA, il a été compris que pour classer les échantillons en fonction de leur nature dans différentes catégories, il est important de choisir une méthode appropriée. En raison de la nature de la LDA de maximiser la séparation du groupe d'échantillons, il a été jugé plus approprié de distinguer les catégories normales et regroupées d'échantillons lyophilisés.

Dans l'ensemble, ce travail de thèse présente des découvertes intéressantes sur les produits lyophilisés à l'aide d'outils NIR (imagerie chimique et spectroscopie). Au cours de la phase de développement du cycle de lyophilisation, cette approche d'étude de la distribution spatiale de l'humidité à l'intérieur des produits lyophilisés permet le positionnement approprié des outils PAT focalisés et ciblant le bon échantillon à l'intérieur du lyophilisateur. En outre, la mise en œuvre du NIRS aide à surveiller l'effondrement des produits lors de la lyophilisation et dans les produits finis. En outre, cela contribue à rassurer la pratique existante d'inspection visuelle à 100% des flacons dans l'environnement de production.

7. FUTURE WORK

This thesis provides tools and methods for investigating the spatial distribution of moisture on the surface of the freeze-dried vials and identifying collapse inside the freeze-dried products. The following research can be potentially carried out in the future:

i. Considering NIR CI findings related to the spatial distribution of moisture on the surface of vials, PAT scientists can use this work as a proof of concept during the development stages to identify the most appropriate vial and most appropriate position on the vial for implementing point focussed PAT tools. This can be done by imaging vials located at different representative positions (i.e. corner, middle, and on the center of the freeze-dryer shelf) and identifying the best suitable vial and measurement spot on the vial surface for monitoring moisture from the start until the end of the freeze-drying process.

ii. Further evaluation of NIRS for identifying collapse inside the freeze-dried products can be focussed on inducing different levels of collapse inside the freeze-dried products and exploring the potential of NIRS in the real-time process settings.

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