EVALUATION OF DRYING TECHNOLOGIES FOR STORAGE AND SHIPMENT OF RECOMBINANT PROTEIN DRUG SUBSTANCE

By

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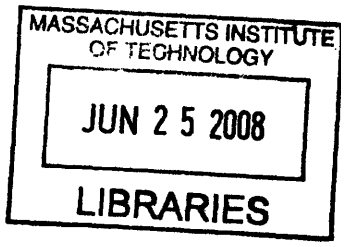
Submitted to the MIT Sloan School of Management and the Engineering Systems Division in partial fulfillment of the requirements for the degrees of



Master of Business Administration
and
Master of Science in Engineering Systems

In conjunction with the Leaders for Manufacturing Program at the

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June 2008

ARCHIVES

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ABSTRACT

With growing markets and increasing pipelines, biotechnology companies face a supply chain challenge to manufacture and distribute products using economically feasible methods that protect protein integrity. Adequate storage and shipment of drug substance is an important operation and product quality issues are dependent upon success at this stage of the manufacturing process. While cryopreservation technologies are widely in use today, they may become prohibitively expensive in the future due to increasing product volumes and high operational costs.

This thesis presents an evaluation of drying technologies as an alternative to cryopreservation for recombinant protein drug substance storage and shipment. After presenting an assessment of current cryopreservation technologies, the potential of drying technologies to protect protein integrity is examined through process optimization and product characterization at laboratory scale. The economic impact of such technologies and the implications of their implementation in the manufacturing environment are discussed. Recommendations on storage technologies for drug substance are proposed based on results of the analysis. Finally, the thesis builds on this particular study to research the specifics of process development in the biopharmaceutical industry and to discuss implications for future process innovation.

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1. INTRODUCTION

This thesis presents the results of a six month project completed at Amgen Inc. headquarters in Thousand Oaks, California, between June and December of 2007. The project was sponsored by the Amgen Process Development organization and most specifically by the Drug Product and Device Development (DP&DD) group within Amgen Operations. The objective of the study was to evaluate the potential of drying technologies (freeze drying and spray drying) as an alternative to cryopreservation for the bulk storage of recombinant protein drug substance, prior to fill-and-finish operations. The evaluation consisted of a series of lab scale technical studies, a thorough financial analysis, as well as the understanding of the business implications of a potential introduction into Amgen operations. This project is one of the Leaders for Manufacturing (LFM) projects undertaken as part of the Amgen and LFM program partnership.

In order to protect proprietary Amgen information, the data presented throughout this thesis has been altered and does not represent the actual values used by Amgen, Inc. The dollar values have been disguised in order to protect competitive information where necessary.

1.1. Statement of the problem

Currently, large biotechnology firms face a growing supply chain challenge to manufacture and distribute products at reasonable costs while protecting protein integrity. With growing markets and increasing pipelines, the current methods used for storage and shipment of drug substance (DS) may become too costly to use in the future. Drug substance is currently held in inventory using cryopreservation methods. This technology has been safely used in the biotechnology environment for decades, but limits the flexibility at the manufacturing sites. It requires large freezer facilities, and complicates product handling because containers must be moved to different areas of the facilities after the freeze, storage and thaw process steps, in addition to sometimes long freeze and thaw cycle times. Moreover, a cold environment must be maintained along the supply chain, complicating shipments to fill-and- finish manufacturing

facilities. Finally, the freeze-thaw cycles can have an impact on the protein integrity and therefore the final product quality.

Drying technologies are a strategic alternative to cryopreservation, since their use could improve the manufacturing and supply chain operations and financial figures. Notably, freeze drying and spray drying have been widely used in the biopharmaceutical industry for years, especially to increase shelf-life stability of the final drug product. The use of drying technologies for drug substance storage could help overcome some of the current challenges encountered with cryopreservation techniques, by effectively removing the majority of the water contained in the DS and generating a powder. First, it is well recognized that the product stability in storage is increased in the powder form. Second, removing the majority of the water will help reduce the bulkiness of the drug substance held in inventory. Finally, this would defeat the cold chain requirements currently necessary during the shipment of drug substance to fill-and-finish operation sites.

Using drying technologies as a storage method for drug substance may be proven very beneficial, but it would require extensive development and validations prior to be implemented in the manufacturing environment. In the pharmaceutical and biotech industry, government or other regulatory agencies are an important stakeholder in the process. In particular, validations would have to demonstrate product safety with the use of these new technologies, and prove that drug substance prior to the drying step and after the reconstitution process are equivalent. Further, the product and facility would be allowed to implement this new process as long as the FDA is satisfied that the product is being made under cGMP (current Good Manufacturing Practices). The study presented in this thesis therefore only highlights the initial steps towards a future validation of drying technologies to store a powder form of the drug substance.

1.2. Summary of approach

Freeze drying and spray drying equipment at manufacturing scale are not only massive (up to thirty square meters footprint for a large freeze dryer) but also very expensive. It is therefore

realistic to first conduct a scaled down evaluation study to assess if drying technologies have the potential to become an alternative platform for drug storage and shipment. In addition, process results obtained at lab scale are (especially in the case of freeze drying) a good representation of process cycle time and outputs that can be obtained at larger scale.

This thesis therefore describes a series of experiments conducted with lab scale freeze drying and spray drying equipment which were run by a small team comprised of the LFM intern and several scientists from the Drug Product and Device Development group. This experimental approach was intended to demonstrate a preliminary feasibility to dry biological drug substance (by meeting specific measures of success) and to produce results which could ultimately be scaled up into process conditions for a commercial manufacturing facility. In addition to experimental results, financial expenses related to technology validations, introduction into manufacturing and day to day operations are modeled. Non quantifiable impacts of the introduction of drying technologies are approached, and recommendations on storage technologies for drug substance are proposed. Finally, specifics of process development in the biotechnology industry are discussed and implications for future process innovation are discussed.

1.3. Organization of this thesis

Chapter One describes the problem statement and summarizes the approach used in this thesis.

Chapter Two provides some background information on the biopharmaceutical industry and briefly describes Amgen's massive growth since the company's creation. Amgen's current position in the industry is presented in broad terms. Finally, the Drug Product & Device Development group is introduced and its function within the Process Development organization is detailed.

In the context of the drying technologies evaluation, Chapter Three describes the different steps of the biotechnology manufacturing process and the importance of the drug substance storage step. The different cryopreservation technologies currently in use or in development are described and advantages / problems associated with each are discussed. The two drying technologies evaluated in this thesis are then presented in more detail.

Chapter Four presents the strategy used to conduct the technical evaluation of freeze drying at lab scale. Research methodology is described, as are the different experiments and the major results. The results implications are then discussed in the context of the technical evaluation.

Chapter Five describes the similar approach used to evaluate spray-drying at lab scale and presents the data obtained through experimentation, before drawing conclusions on the results obtained.

In Chapter Six, the strategy used to conduct the financial analysis of the different storage technologies is described and the major model assumptions presented. Results obtained through Net Present Value analysis are then presented and major conclusions are drawn from the data.

Chapter Seven discusses non quantifiable impacts of the introduction of drying technologies in manufacturing, regarding equipment utilization and process flexibility. Finally, the different aspects of the evaluation presented throughout the previous chapters are summarized and recommendations are presented.

Chapter Eight first presents a literature review on innovation in various industries, and then elaborates on the specifics of process innovation in the pharmaceutical and biotechnology industries. Finally, implications for future process innovation in biotechnology firms are discussed.

Chapter Nine concludes the thesis by wrapping up the results and ideas discussed in the previous eight chapters.

2. COMPANY BACKGROUND

2.1. The biopharmaceutical industry

Two major events have contributed to the strong development of the biotechnology industry. The first was the discovery of the DNA structure by Watson and Crick in 1953, and the second the discovery of recombinant DNA cloning technology (also known as genetic engineering) in 1973 by Cohen and Boyer. The new biotechnology industry grew rapidly from the mid seventies, recombinant human insulin (Genentech and Eli Lilly and Co) becoming the first biotech therapy to earn FDA approval in 1982. By 1988, only fourteen biotech drugs and vaccines had been approved by the FDA, but this number multiplied during the nineties before slowing down in these last few years, partially because of increasing regulation requirements (Figure 1).

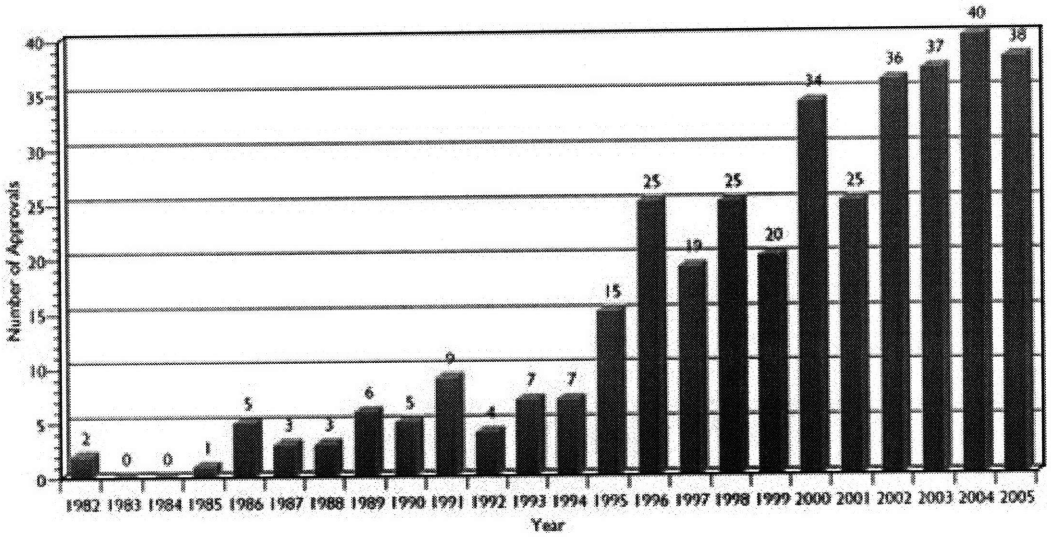

Figure 1[1]: New Biotech Drug and Vaccine Approvals/ New Indication Approvals by Year

The biopharmaceutical industry has grown steadily over time, with revenues increasing from 8 billion in 1992 to 50.7 billion in 2005. As of Dec. 31, 2005, there were 1,415 biotechnology companies in the United States, of which 329 were publicly held (with a market capitalization of $410 billion) [2]. Biotechnology is one of the most research-intensive industries in the world. The U.S. biotech industry spent $19.8 billion on research and development in 2005, with the top five biotech companies invested an average of $130,000 per employee in R&D in 2005[2].

Despite billions of dollars invested in capital and stunning growth in revenues for the industry as a whole, it is paradoxical to see that most biotechnology firms earn no profit. The revenues of public biotech companies have grown dramatically but their profits have oscillated near zero. Without the biggest biotech firm Amgen, the industry has consistently been in the red.

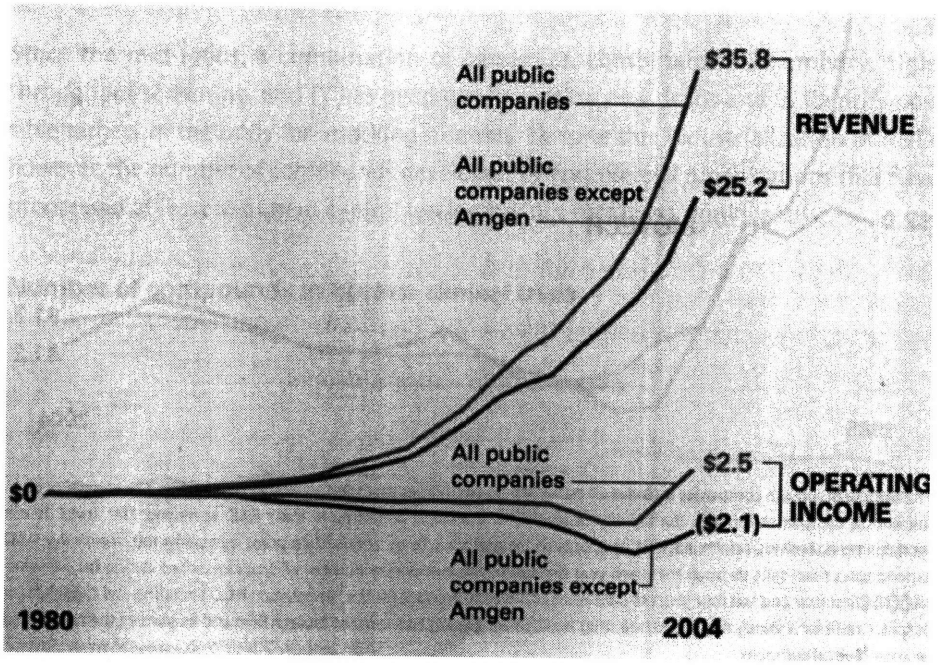

Figure 2[3]: Revenue and operating income before depreciation ($ billions 2004)

In his book "Science Business"[3], Gary Pisano mentions that the challenges seen in biotechnology come directly from the way the industry is structured. Similarly to high tech and nanotechnology industries, the biotech model consists of interrelated elements:

- Technology transfers from universities through the private sector through firms creation
- Venture capital and public equity market providing funding at critical stages
- A market in which young companies provide their intellectual property to large companies in exchange for funding.

However, Pisano argues that this model is flawed because biotech drug R&D differs radically: the R&D process involves high uncertainty and long term risks, there are strong interdependencies between technical activities making problem solving very complex, and it is extremely difficult to interpret data from experiments and learn from them, making IP protection more challenging. Very few companies are offered the time to learn through experience because

relationships with investors are often centered on reaching specific, short term milestones. As a result, it becomes very difficult for new companies to succeed in this environment. While Pisano calls for a more integrated organization focused on long-term relationships, it is currently difficult to envision how the industry as a whole will evolve.

2.2. Amgen's early successes and growth

Amgen was established in 1980 as Applied Molecular Genetics Inc. by venture capitalists, four years after the first biotech firm, Genentech, was founded. At first, Amgen tried using genetic engineering to create organisms that would extract oil from shale, proteins that would make chickens grow faster and new specialty chemicals for the textile industry. None of these initial studies proved fruitful. In 1983, a molecular biologist at Amgen, Fu-Kuen Lin, cloned the gene for erythropoietin, the kidney hormone that controls levels of red blood cells. During that same year, the company changed its name to Amgen Inc., and raised $40 million through the initial public offering.

This discovery led to the FDA approval of anemia drug EPOGEN® in 1989. This first success was quickly followed by the approval and launch of NEUPOGEN® in 1991, a protein that increases the level of white blood cells. These two drugs became the biotechnology industry's first blockbusters, and both were named "Product of the year" by Fortune Magazine the same years they were launched[4]. Amgen enjoyed through the nineties a phenomenal growth thanks to these two products, with a stock compounded annual return of 52% over that period, and sales reaching $3.2 billion in 2000[5]. In 2001, Amgen completed the biggest deal in the biotech industry with the $11 billion acquisition of Immunex and its breakthrough drug for rheumatoid arthritis ENBREL®. Over the following years, Amgen launched a set of five new drugs and acquired two other biotech companies.

2.3. The company today

With total revenues reaching $14.3 billion and R&D investments $3.2 billion in 2006[6], Amgen is today the biggest biotechnology firm in the world. Headquartered in Thousand Oaks,

California, the company employs just fewer than 20,000 people worldwide. The company owns seven manufacturing sites located in the United States and Puerto Rico and distribution centers worldwide.

However, recent safety warnings raised by the FDA concerning the risks of overuse of anemia drugs as well as changes in coverage rules have resulted in a sales decline of the company's anemia drugs throughout 2007, causing a significant loss in revenues for the year. This triggered a corporate restructuring plan to reduce costs, including an extensive re-evaluation of global manufacturing capacity. In addition, the company has recently launched a new corporate initiative to introduce lean manufacturing methods in its manufacturing organization and become more operationally efficient.

2.4. Process Development in the Drug Product and Device Development Group

Biotechnology manufacturing is substantially different from traditional pharmaceutical manufacturing, because of the complexity and inherent uncertainty of using living organisms as production systems to manufacture complex proteins. Changes in the manufacturing process can impact the activity of the protein by inducing subtle changes in its structure or conformation. Therefore, controlling the manufacturing process using well-defined operating parameters and raw materials is critical to assuring the safety, potency and consistency of the product. Process development organizations in biotechnology have therefore an essential role to select the appropriate technologies and process parameters to ensure product safety and operation reliability, short development time and ease of process validation.

At Amgen, the Drug Product & Device Development (DP&DD) group is part of the Process Development organization, in the Operations division. Most of the staff is located at the corporate headquarters in Thousand Oaks, with some presence at the manufacturing sites. The group's major responsibilities include:

- Pre-commercial and commercial drug product process development and manufacturing support
- Drug delivery and device engineering
- Packaging and container engineering
- Small molecule drug product process development support

As a subset of the multiple deliverables captured in these broad responsibilities, DP&DD is accountable for the process development, characterization and validation of protein drug substance freeze-thaw cycles in all primary bulk containers used at Amgen. The group is also responsible for the development and characterization of the drug product freeze drying processes used at fill-and-finish manufacturing facilities.

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3. DRUG SUBSTANCE STORAGE TECHNOLOGIES

3.1. Overview of biologics manufacturing

Biotechnology's unique approach to making medicines is to use living cells (microbial cells like *E. coli*, or mammalian cells, primarily Chinese Hamster Ovary, or CHO cells) to produce the appropriate proteins. Once the protein is fully formed, it is separated from the cells to create the final product that is delivered in proper dosage form into patients. The first step in manufacturing a protein or antibody is to genetically engineer a cell so that it produces the desired protein. This requires introducing the genetic information — DNA — that provides the cell with the instructions it needs to produce the protein or antibody. Once a cell has been engineered to express the product of interest, it is used to establish a cell line, i.e. thousands of copies of this original cell. This cell line is then frozen and stored for use in the manufacturing process.

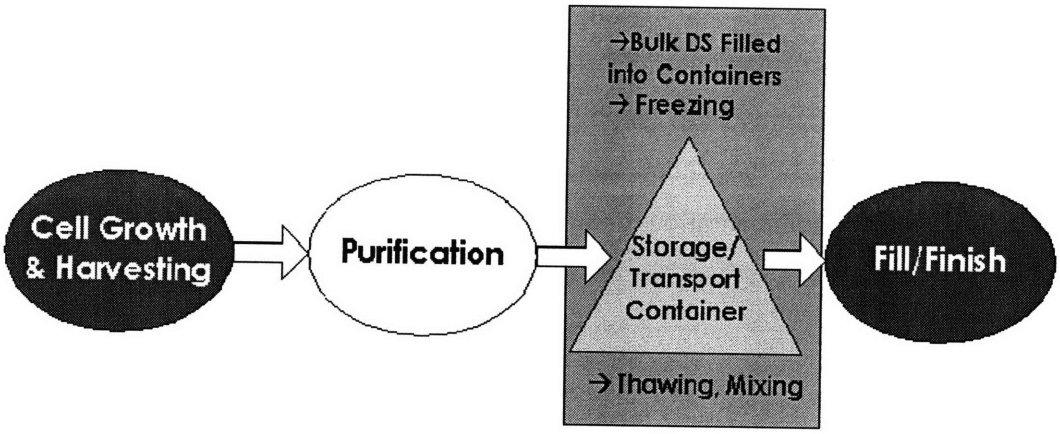

Figure 3: Simplified overview of biologics manufacturing

To begin the production cycle of the drug substance, a small vial of these genetically engineered cells is thawed and allowed to grow in culture for several days. Once the cells have undergone several rounds of replication, they are transferred to a larger container with specific growth parameters to favor cell multiplication. When the cells grow to sufficient numbers, they are once again transferred, this time to large-scale production tanks (thousands of liters), where

they are induced to produce the specific protein. At this point in the process, the protein or antibody can be harvested (Figure 3).

After harvesting, the purification process first consists of separating the cells debris from the growth media containing the protein product of interest. The protein is then subjected to several additional purification steps (including more refined separation chromatography, filtration, buffer changes, concentration and dilution processes). When the final concentration and formulation is obtained, the drug substance (purified protein prior to final formulation and fill into the final drug product vials) is filled into the appropriate storage container, frozen to stabilize it and transported to cold storage area until being shipped to fill/packaging facility.

The final drug product (drug in its final form for use in the patient population) fill-and-finish manufacturing is usually completed at a different facility than drug substance cell culture and purification manufacturing processes. At the fill-and-finish plant, the drug substance is first thawed, and the product goes through additional formulation steps as needed (i.e. surfactant addition, dilution, etc) and sterile filtration to obtain the correct dosage. The drug product is then filled into individual vials (or syringes). Certain drug products, containing proteins not stable over time in a liquid solution, must be lyophilized (freeze dried) to ensure the protein stability over the required shelf life of the product (this implies that the solution must be reconstituted with sterile water before administration to the patient). After inspection, labeling and packaging of the vials, the drug product is ready for shipment to physicians, hospitals and pharmacies around the globe.

3.2. Importance of the drug substance storage step

One of the biggest challenges of biotechnology firms is to maintain protein stability throughout manufacturing and during product shelf life. In their article "Protein Drug Stability[7]", Frokjaer & Otzen mention that "the therapeutic activity of proteins is highly dependent on their conformational structure. However, the protein structure is flexible and sensitive to external

conditions, which means that production, formulation and handling of proteins needs special attention in optimizing efficacy and safety, including minimized immune responses." Formulated drug substance is usually produced at a bulk manufacturing facility in large campaigns, due to the complexity of customized processes, the time required to retrofit a line for new product manufacturing, as well as the size and scale of production requirements. In consequence, large quantities (thousands of liters) of bulk drug substance must be stored for months before being transferred to fill/finish facilities. Given the protein sensitivity to pH or temperature changes, surface interactions or contamination, and the high cost of the product at this stage of manufacturing, it is important to use technologies that will maintain the safety and efficacy of the product throughout the storage period.

3.3. Review of cryopreservation technologies as a storage option

There are several common conditions for protein storage and tradeoffs associated with each method. For example, proteins stored in solution at 4°C can be dispensed conveniently as needed but require more diligence to prevent microbial contamination. In addition, such proteins may not be stable for more than a few days or weeks. The long storage period required explains why cryopreservation has become the overwhelmingly dominant technology in use for drug substance storage. By storing drug substance in a frozen state, the rates of the most common reactions leading to physical and chemical degradations are retarded. Minimization of microbial growth and elimination of agitation and foaming during transport are other advantages. Frozen storage offers several advantages over liquid storage, but it is not free from risks since freeze-thaw cycles may induce protein aggregation (proteins bonding together to form aggregates, hampering filtration processes and increasing final defect rates).

In light of these points, the following sections will present different containers currently used or in development in the biopharmaceutical industry. References to these containers will be used later on in this document for a quantitative and qualitative comparison of technical, financial and operational outcomes with respect to drying technologies.

3.3.1. Plastic Carboys

Plastic carboys are the most common containers used for frozen storage of drug substance. Polycarbonate and Teflon carboys of various capacities (up to tens of liters) are filled with drug substance batches and placed on pallet spaces to complete the freezing process (Figure 3). Plastic carboy use is widely spread because of the disposable nature of the container and its low cost. In addition, the relatively low volume of these containers permits an increased flexibility for variations in batch volumes produced within the same product line, or in the case of a multi-product facility.

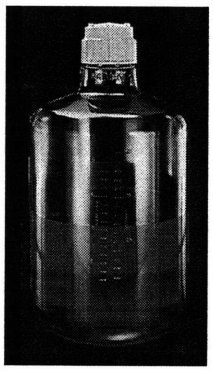

Figure 4: Polycarbonate carboy used for storage of drug substance

While it is a proven technology, it is still complex to design and validate freeze-thaw cycles with plastic carboys. The cooling and freezing process can take several days, and the thawing process up to two to three weeks (in a 2 to 8°C room temperature environment). There are no good solutions to reduce these cycle times, because high temperature gradients within the carboy would lead to several sources of protein denaturation. For example, as the liquid water converts to ice, the protein and formulation excipients are progressively concentrated in the regions between the ice crystals. This augments the probability of molecular collisions between the protein molecules and can potentially lead to denaturation through aggregation. These long cycle times imply complicated logistics to handle and ship these carboys. Carboys need a certain distance between each other on the pallet to obtain an optimal freezing process. In the final storage area, carboys positions are usually reconfigured on pallets to optimize space, requiring new manual labor. Given the large number of carboys per drug substance batch (carboy containers' volumes are limited to tens of liters to avoid high temperature gradients during the

cycles), freezer storage space required becomes extremely important, as well as cold room space during the thawing process at the fill/finish facilities. Finally, shipping logistics are also impacted by these large volume requirements.

3.3.2. CryoVessels®

A more advanced technology is sometimes used in protein manufacturing processes. The CryoVessel (Figure 5) is "a portable jacketed, stainless steel freeze-thaw vessel for cryopreservation of biopharmaceuticals, vaccines, blood products, and gene therapy products" [8]. These vessels are available in volumes ranging from 20L to 500L. The main advantage of this type of container is the presence of extended heat transfer surfaces that increase heat flux and help control the freeze-thaw process. The cycle time to thaw drug substance at the fill/finish sites is reduced to hours instead of days for carboys. Most importantly, this heat control technology provides reproducible and recordable temperatures profiles, limiting variations between cycles and gradients that could cause product denaturation. Finally, the system is designed for sterile filling operations.

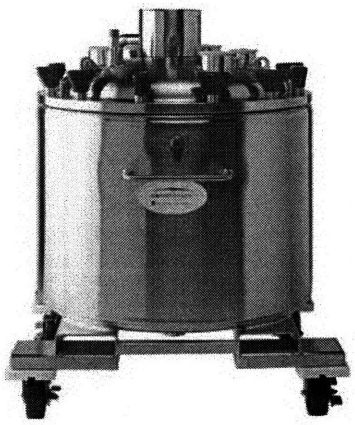

Figure 5: CryoVessel container (Sartorius Stedim Biotech Group)

As explained above, CryoVessels present many advantages. They however require a large initial capital investment, many expensive containers being necessary during the long storage period. The major benefits of CryoVessels come from the controlled rate freeze/thaw cycle,

deriving a large price premium. However, the containers spend the majority of the time stored in freezers for months; the initial investment is therefore questionable because it is usually difficult to quantify the dollar value of the quality benefits of a controlled rate freeze/thaw process (i.e. how much denaturation is potentially avoided?). In addition, the reuse costs of these containers are high (around 40% of operating expenses), including shipment back to the bulk manufacturing facility, preventive maintenance and refurbishing as well as sterilization. Finally, the use of large vessels limits process flexibility, as you may have to pay for use and shipment of a container filled only with 10%, because of volume variations in production.

3.3.3. Celsius-Paks®

Celsius-Pak (Figure 6) is the new container technology proposed by Stedim Biotech Group. The disposable container bag is filled while mounted on a structural frame for a protected handling of the bag and product. It combines the benefits of a disposable container with the advantages of a controlled freeze/thaw technology, reducing thawing time to hours versus days in the uncontrolled carboys. Because of the relative smaller bag volume, the Celsius technology provides improved flexibility in manufacturing and reduces logistics issues compared to the CryoVessel technology (bags are single use, and only frames are shipped back, eliminating the costs of cleaning and maintenance associated with the vessels).

Figure 6: Celsius Paks containers (Sartorius Stedim Biotech Group)

3.4. The potential of drying technologies as a storage option

Freeze drying and spray drying are the two most popular methods of drying protein solutions in the biopharmaceutical industry. Usually, drying is employed if the storage stability of the active ingredient (physical and/or chemical) is unsatisfactory. For example, "spray-dried powders are commonly prepared when the intended route of administration is via inhalation, since the desired control over particle size can be obtained, but freeze drying is most commonly employed for injectable products since sterility and particle-free quality attributes are more easily obtained in freeze drying"[9]. These two technologies are currently employed to improve the stability and therefore the shelf life of the final drug product. But they have yet to be implemented as an alternative method to cryopreservation to maintain the stability of drug substance in storage. At this time, numerous products in biotechnology firms' pipelines, (notably monoclonal antibodies) are expected to necessitate high delivery volumes for patients (the individual dose volume) and higher target concentration than current products to achieve therapeutic dose levels. From the high dose levels and the large patient populations, it is anticipated that these compounds need to be manufactured in high volumes, complicating the current technical and logistical issues associated with drug substance storage. In light of the issues outlined above and due to the potential need to increase the protein concentration in future products, it is becoming imperative to explore a new technology that could enable the manufacture of high volume protein products at a lower cost. The reasoning behind the choice of drying technologies stands in the hypothesis that cost savings realized during a product manufacturing lifetime (i.e. no cold chain requirements, improved logistics...) will offset the initial capital investment costs, while ensuring superior product stability during the storage period.

3.4.1. Freeze drying

Freeze drying, also known as lyophilization, is the most common drying method for producing powders for parenteral administration. Figure 7 represents a typical freeze drying cycle, with

time in hours on the X axis and temperature in Celsius on the Y axis. The different numbers represents the different phases of the cycle

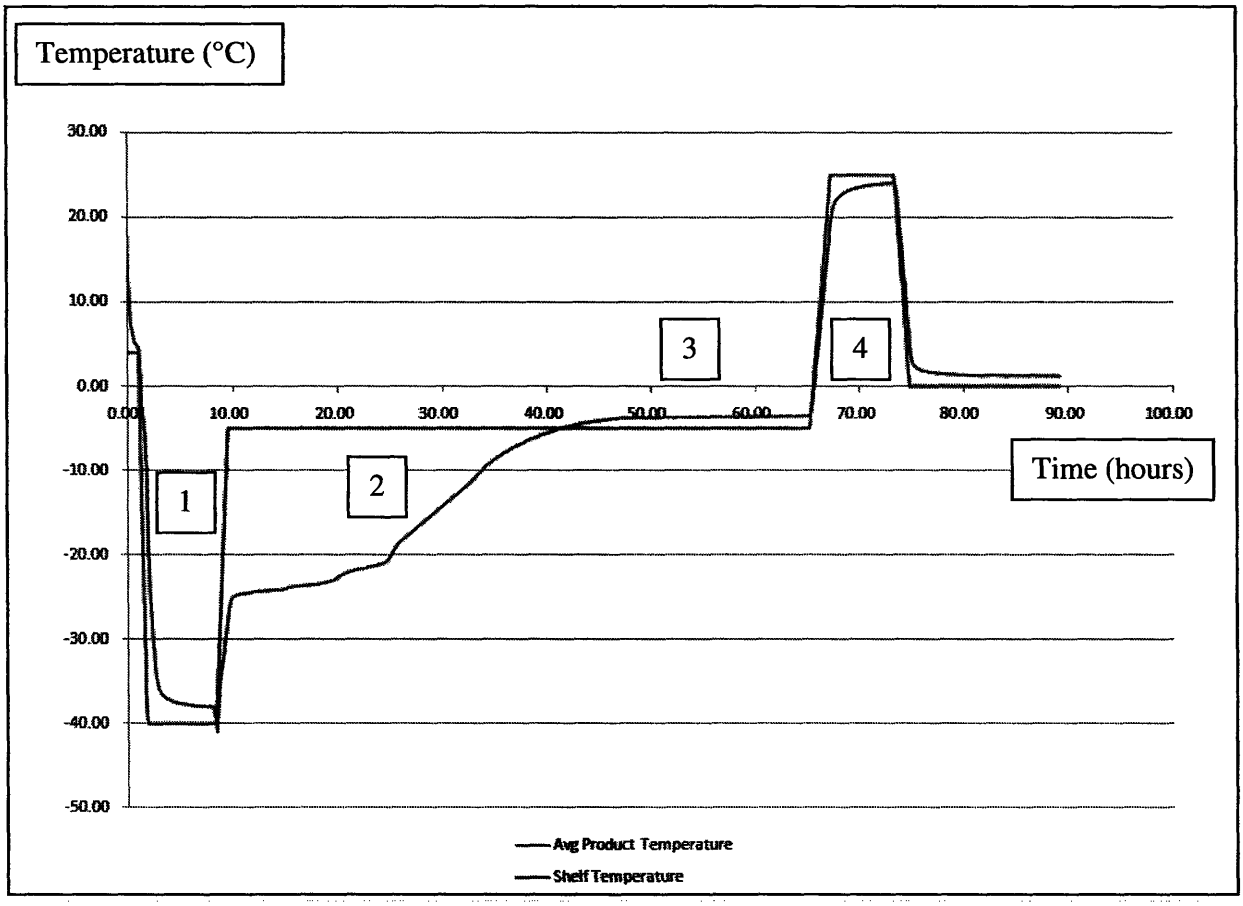

Figure 7: Typical freeze drying cycle

In phase 1, the shelf temperature is cooled down to a very low temperature and maintained there until the frozen solution temperature in the tray reaches an equivalent value and stabilizes. In phase 2, vacuum starts in the drying chamber and shelf temperature is raised to the defined primary drying temperature. Water is slowly sublimated and the product temperature rises slowly as product is dried. In phase 3, the primary drying is completed and the dry cake temperature is equivalent to the shelf temperature. In order to decrease the moisture content level in the cake, the shelf temperature is raised again to a set temperature. This is the secondary drying phase (phase 4), designed to eliminate additional water contained in the solid form by desorption.

Manufacturing scale freeze drying requires very large equipment (Figure 8), with up to 40 square meters of shelf surface necessary for large batch volumes. In addition, an automated loading line is usually linked to the freeze dryer, whose role is to fill trays containing the product and load them on the freeze dryer shelves. After the cycle, trays can be stored in the appropriate location at room temperature if a low moisture environment can be maintained over time (moisture content is a critical factor in storage stability of dried protein).

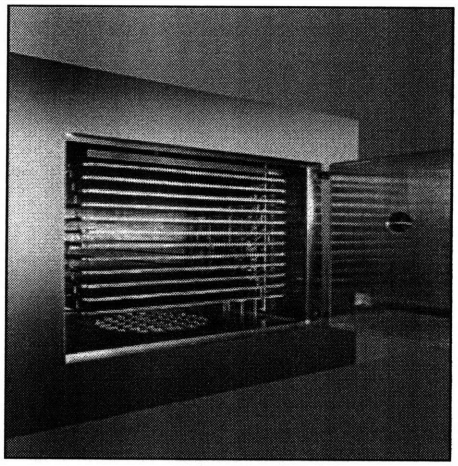

Figure 8: Manufacturing scale freeze dryer[10]

As mentioned previously in this thesis, storing drug substance at the dry stage will ensure a better stability over time of the active content, i.e. the protein. It will also ensure easier logistics of transportation, since the product can be shipped at room temperature with reduced weight (volume reduction is not significant since the solid content is still contained in trays with enough volume to fill the defined amount of solution). On the other hand, downsides of using freeze drying in manufacturing include the loss of some flexibility (massive fixed volume capacity whether batch size is low or high, long lead time involved if additional capacity needs to be installed) and the potential difficulty to move protein manufacturing from site to site (FDA requires identical processes and cycles at the different sites, meaning that a freeze dryer equipment must be available at all sites as well).

3.4.2. Spray drying

Spray drying (drying by evaporation) is usually a more economical drying method widely used to produce powders for pulmonary delivery. The drying method in a spray dryer (Figure 9) involves feeding the solution through an atomizer nozzle (placed inside a drying chamber) at a controlled rate. The liquid stream emerges from the nozzle as very fine droplets in the drying chamber by the aid of an atomizing gas (inert gas such as nitrogen). The solvent contained in the fine droplets evaporates in the drying chamber due to high temperatures, and the solid content is collected in the final container through cyclone recovery.

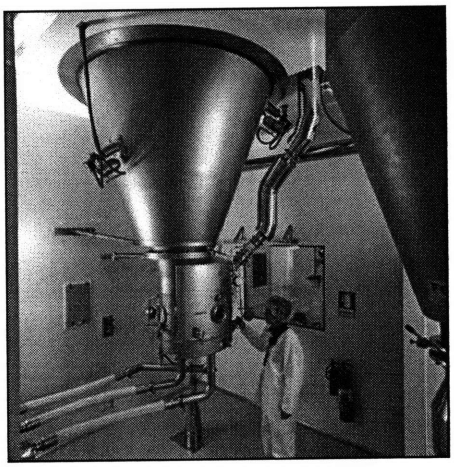

Figure 9: Manufacturing scale spray dryer[11]

In addition to all the benefits of drying drug substance for storage purpose, spray drying presents the advantage to process identical volumes of drug substance in shorter times than freeze drying, to reduce both volume and weight (the final container requires only volume for the solid content of the solution). The spray drying process induces more stress on the protein structure (heat and atomization) and must therefore be carefully understood. Finally, process yields are usually lower at manufacturing scale than for other equipments like freeze dryers (solid particles containing proteins can get stuck in the spray drying chamber and not collected).

3.5. Attributes of importance

Evaluating the different drug substance storage technologies requires defining the attributes on which to compare their performance. The following list presents these attributes of importance in more detail:

- Product integrity: Safety is the most important concern in biotechnology manufacturing. A validated process must not alter or damage the manufactured protein. The initial assessment of drying technologies presented in the next chapters will measure key characteristics of the solution (protein concentration and pH) and the dry cake (moisture content, powder glass transition) and compare them against defined tolerances to establish the safety of these process technologies for the protein integrity.

- Cost of implementation and operations: A financial model based on Net Present Value analysis will compare the long term cost of the different technologies implementation in the manufacturing environment.

- Handling logistics: The supply chain logistics vary greatly depending on the technology used (container size, disposable container versus reusable equipment, etc) and on the storage conditions. A subjective analysis will be conducted to compare the supply chain implications of the different technologies.

- Manufacturing flexibility: A similar analysis evaluating the impact of the different technologies on manufacturing flexibility will be conducted. Process cycle times and equipment capacity limitations will be listed to compare the performance of the different technologies on this attribute.

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4. FREEZE DRYING: A TECHNICAL EVALUATION

This chapter presents the strategy and methods used and the results obtained during the evaluation of bulk drug substance freeze drying at lab scale (Appendix 1 presents a picture of trays on the shelves of the lab scale freeze drying equipment).

4.1. Lab scale evaluation strategy

The goal of this evaluation is to assess if drying bulk drug substance using freeze drying technology is feasible. This implies that:

- the process cycle time obtained is equivalent or shorter than current cycle times experienced in manufacturing for typical commercial products,
- the dry product after freeze drying has the characteristics necessary to withstand storage during an extended period of time (preferably at controlled room temperature),
- the reconstituted drug substance solution (completed by adding back the water eliminated through the drying process) has similar parameters to the initial drug substance solution.

It is also necessary to demonstrate before any introduction into manufacturing that this technology can be used for different formulations (final drug product in a liquid state (liquid formulation) or in a lyophilized state (lyophilization formulation)) and protein concentrations. In addition, the effect of the tray fill depth on the overall freeze drying process cycle time will be evaluated. Table 1 presents the different parameters varying in this study.

Protein type	One mimic protein (B.S.A.: Bovine Serum Albumin)
Protein concentration	30 mg/ml and 100mg/ml)
Drug substance base formulation	Liquid formulation& Lyo formulation
Tray Fill volume	1L and 1.8L

Table 1: Varying parameters for the evaluation

All experiments are conducted using the same four-step methodology, as presented in Figure 10. First, protein concentration and pH of the initial solution are measured. In addition, the characteristics of the frozen solution are determined (collapse temperature, Tc, and glass transition temperature of the maximum freeze concentrate, Tg'). Second, the freeze drying cycle optimization is completed, by determining the adequate shelf temperature for primary and secondary drying leading to the overall cycle time optimization.

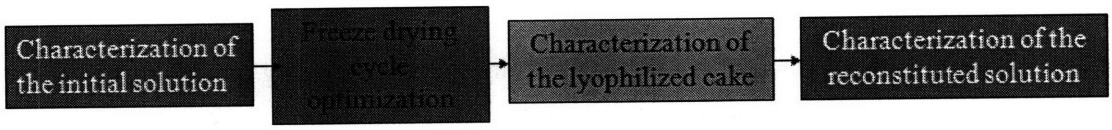

Figure 10: Freeze drying cycle evaluation methodology

Third, the lyophilized "cake" contained in the tray is analyzed and the two most critical parameters to ensure good storage stability are measured: moisture content and glass transition temperature of the cake. Finally, after recording the time necessary to reconstitute the liquid drug substance (by adding the volume of water that was eliminated during the drying process), pH and protein concentration are measured immediately, and after a period of time on stability, and compared to the initial data prior to the drying process.

4.2. Frozen solution characterization

4.2.1. Tc: Collapse Temperature

At the end of the freezing cycle, the temperature of the frozen solution in the tray is stabilized well below 0° C, at a temperature equivalent to the shelf temperature. At this point of the cycle, the primary drying can start. The shelf temperature is raised to a higher temperature and the pressure in the drying chamber is reduced to a minimum: solvent is then removed by sublimation of ice. During this process, the temperatures of both the frozen solution and newly formed dried product temperatures slowly increase. Two physical parameters have a critical importance during primary drying to ensure a high quality product and adequate shelf life. The first parameter is the

collapse temperature (Tc) of the lyophilized cake which represents the eutectic point, or melting temperature of the cake. Primary drying should always be performed 2 to 5° C below the collapse temperature of the cake. It is therefore critical to determine this parameter to establish the right shelf temperature during primary drying.

Collapse temperature is determined using a technique called freeze drying microscopy. This technique permits reproducing the primary drying phase using a small amount of drug substance solution, and observe *in situ* the drying process using a microscope. As the product temperature is raised, the dried product collapses and the Tc value can be determined. The images below in Figure 11 present an illustration of freeze drying microscopy pictures obtained.

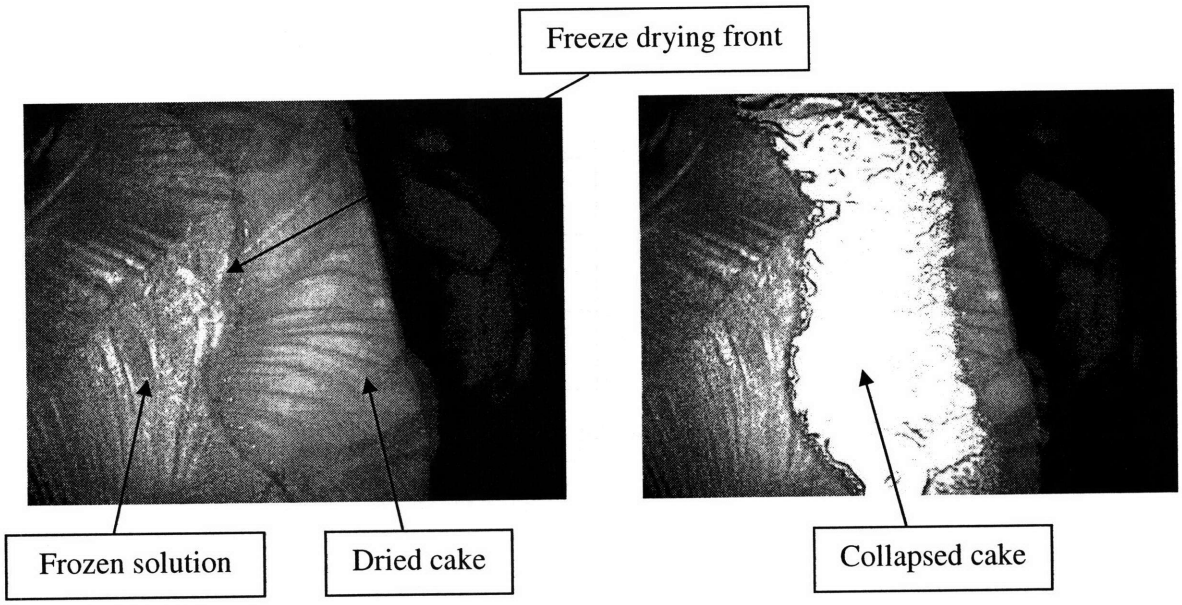

Figure 11: Freeze drying microscopy images

In the left picture, lyophilization of the frozen solution is in progress, with the freeze drying front progressing from right to left. As the temperature of the cake is progressively raised, it reaches the eutectic point and the cake collapses as shown in the right picture. The collapse temperature is recorded when the melting process starts.

For the lyophilization formulation experiments, the collapse temperature can be greatly increased through an annealing process, in order to ensure complete crystallization of some of the formulation excipients (critical to process performance and quality attributes of the dry

product). In this testing, the frozen solution was heated up for 30 minutes then cooled again prior to collapse temperature measurement.

Table 2 presents all results obtained through freeze drying microscopy for the different formulations and concentrations used.

Sample Type	# of replicates	Annealing Time at -12° C (min)	Collapse Temperature (Tc, °C)
BSA 30 mg/ml Liquid formulation	3	N/A	-28.0 ± 0.8
BSA 70 mg/ml Liquid formulation	3	N/A	-22.5 ± 0.7
BSA 100 mg/ml Liquid formulation	3	N/A	-18.0 ± 0.4
BSA 30 mg/ml Lyo formulation	3	0	-33.5 ± 0.9
BSA 100 mg/ml Lyo formulation	3	0	-31.0 ± 0.5
BSA 30 mg/ml Lyo formulation	3	30	-21.0 ± 0.7
BSA 100 mg/ml Lyo formulation	3	30	-19.0 ± 0.6

Table 2: Observed collapse temperature for different formulations and concentrations

In light of these results, two comments can be made. First, for both formulations, the collapse temperature increases with protein concentration. The product temperature can therefore be raised at a faster rate (thanks to a higher shelf temperature setting) during primary drying, and that the overall cycle time of the drying process can be reduced. Second, the implementation of the annealing step led to an increase of the collapse temperature of the cake by around 10°C for the lyophilization formulation samples. This confirms the importance of this step in the cycle time optimization for this formulation.

4.2.2. Tg': Glass transition temperature of the maximum freeze concentrate

The glass transition temperature of the maximum freeze concentrate Tg' is the second parameter critical to a successful primary drying phase. Below Tg', the frozen solution effectively behaves as a solid in the amorphous state. At a temperature reaching Tg', the frozen solution starts to become viscous with water diffusion in the freeze concentrate. This chemical property must be avoided because it also impacts the primary drying process, protein stability and product shelf life. The primary drying process should therefore always be performed a few degrees below the Tg' value. Usually, the Tg' value for a specific protein formulation is found slightly lower than the collapse temperature Tc[9].

Tg' is determined using MDSC (Modulated Differential Scanning Calorimetry) equipment. MDSC is a thermal analysis technique which is used to measure the temperatures and heat flows associated with transitions in materials as a function of time and temperature (such as the glass transition of the maximum freeze concentrate). Samples of each protein formulation are frozen to very low temperatures, and then progressively heated until the glass transition temperature Tg' is reached. This provokes a thermal flow change and can be recorded on a graph such as Figure 12 below.

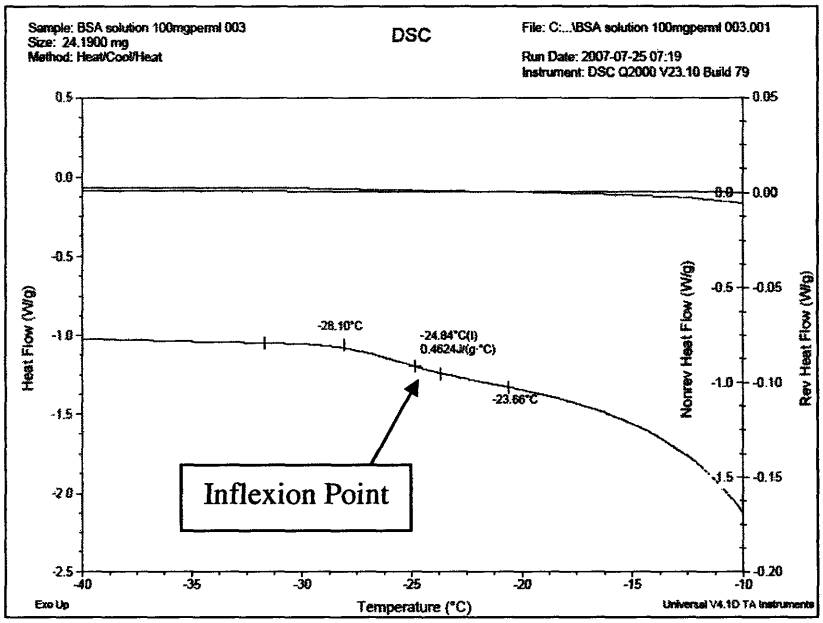

Figure 12: MDSC Thermal graph

The inflexion point found in the curve corresponds to the glass transition temperature Tg'. This process was repeated for all the different protein solutions and concentrations. For the lyophilization formulation samples, Tg' was determined with and without a preliminary annealing step, similarly than for Tc determination. Table 3 below presents all results obtained during the testing.

Sample Type	# of replicates	Annealing Time at -12° C (min)	Glass Transition Temperature (Tg', °C)
BSA 30 mg/ml Liquid formulation	3	N/A	-29.1 ± 0.4
BSA 70 mg/ml Liquid formulation	3	N/A	-27.3 ± 0.3
BSA 100 mg/ml Liquid formulation	3	N/A	-24.8 ± 0.7
BSA 30 mg/ml Lyo formulation	3	0	-35.4 ± 0.6
BSA 100 mg/ml Lyo formulation	3	0	-28.4 ± 0.1
BSA 30 mg/ml Lyo formulation	3	30	-23.3 ± 0.5
BSA 100 mg/ml Lyo formulation	3	30	-22.2 ± 0.2

Table 3: Tg' values for different protein formulations and concentrations

Comments made for the Tc values can be repeated when looking at these results. As expected, Tg' temperatures increase with protein concentration. In addition, the annealing process contributed to an increase in Tg' values for the lyophilization formulation by roughly 5 to 10°C. Finally, this data confirms that Tg' is a few degrees lower than the collapse temperature Tc for a given protein formulation, with one exception. For all but one samples, this was verified in our experiments, as shown in Appendix 2.

4.3. Success criteria used for the evaluation

The goal of this study was to demonstrate feasibility of drug substance freeze drying, and the following measures of success were established (Table 4).

Process	
Cycle time	In line w/ manufacturing run rates. Best if below 48h.
Dry Cake	
Glass Transition Tg	Above 40°C
Moisture Content	Less than 3%
Reconstituted Solution	
Protein Concentration	Within +/- 10% of labeled conc.
pH	Within +/- 0.3 of initial pH
Reconstitution Time	Record for info

Table 4: Measures of success for the freeze drying lab scale study

The glass transition temperature and moisture content of the dry cake are critical for long term storage stability of the dry product. Protein concentration and pH of the reconstituted solution must be found within tolerances of the values measured on the initial solution to confirm that the reconstituted drug substance has similar properties after drying. Meeting successfully all criteria for each solution tested in this initial lab scale assessment of drug substance freeze drying would therefore indicate a major probability of success of drying technologies at pilot and manufacturing scale. In addition, optimized cycle times at lab scale should be similar to or lower than manufacturing run rates to make sure that the drying process does not become a bottleneck in the manufacturing environment.

4.4. Optimization of the freeze drying cycle

During the primary drying steps of the freeze drying cycle, one critical parameter is the product temperature Tp. Tp influences both the stability and the sublimation rate (e. g. cycle time). The two major process parameters that determine the product temperature are the shelf temperature and chamber pressure. Several combinations of shelf temperature and chamber

pressure will result in the desired Tp. The goal is to find a combination that yields a high sublimation rate while maintaining the product temperature below Tc and Tg'.During the secondary drying step of the cycle, a critical parameter is the glass transition temperature of the dry cake (Tg). Tg influences the stability of the dry cake. If Tg is exceeded, the cake collapses. The parameters that influence Tg are shelf temperature, and secondary drying time.

In order to demonstrate feasibility for different formulations and protein concentrations, all process parameters could not be varied in depth with a full factorial design and therefore the following parameters were fixed, based on empirical experience within the DPDD department:

- Freezing step at -40°C for six hours
- Drying chamber pressure of 150 mTorr during primary drying
- Annealing step at -15°C for six hours for the lyophilization formulation (value comprised between the Tg' value of the frozen solution and the eutectic melt temperature of the excipient to cristallize)
- Secondary drying for six hours

For the base formulations used in this study, it is recommended to use a shelf temperature of 5°C or less during primary drying. The first round of experiments was therefore conducted to evaluate if a shelf temperature of 5°C could be withstood by the product during primary drying.

The optimization process consisted of defining adequate shelf temperature during primary and secondary drying to produce an elegant cake (without collapse) and minimize process cycle time. The optimization started with the liquid formulation, 30 mg/ml BSA concentration. This decision was made because this solution presented the lowest Tc and Tg', indicating that the drying cycle time would probably be the longest for this formulation. The first two runs were respectively conducted with a shelf temperature of 5°C and 0°C during primary drying, and a shelf temperature of 20°C during secondary drying. For both runs, we discovered some collapsed cake in the trays, indicating that the product temperature increase during the sublimation phase was too rapid. A third run with a shelf temperature of -5°C during primary drying and 25°C during secondary drying finally resulted in optimized conditions and a pharmaceutically elegant cake without collapse.

Solution	Primary Drying Shelf Temp.	Secondary Drying Shelf Temp.	Cake Collapse
BSA 30 mg/ml Liquid Formulation	5°C	20°C	Yes
BSA 30 mg/ml Liquid Formulation	0°C	20°C	Yes
BSA 30 mg/ml Liquid Formulation	-5°C	25°C	No
BSA 70 mg/ml Liquid Formulation	5°C	25°C	No
BSA 100 mg/ml Liquid Formulation	5°C	25°C	No
BSA 30 mg/ml Lyo Formulation	5°C	25°C	No
BSA 100 mg/ml Lyo Formulation	5°C	25°C	No

Table 5: List of freeze drying runs completed

While no more collapsed cake was found after the third run, there was still an opportunity to reduce the overall process cycle time. During the initial testing, the primary drying duration was unknown and it was necessary to monitor the product temperature in the trays and run the process manually. When the controls indicated that the cake temperature had reached the shelf temperature (and therefore that the primary drying is completed), the secondary drying phase was launched. The brackets on the product temperature graph in Appendix 3 show the potential time savings that can be obtained in a final run with automatic shelf temperature changes.

Similar testing was conducted with the other solutions. Shelf temperature of 5°C during primary drying and 25°C during secondary drying gave adequate results. New runs for all solutions were finally repeated with defined shelf temperatures conditions and an optimized primary drying time. Measured freeze drying cycle times can be found in Table 6 and temperature profiles are shown in Appendix 4. Process cycle times are calculated from start of the freezing process to end of the secondary drying phase (as shown on the product temperature profiles).

Solution	Primary Drying Shelf Temp.	Secondary Drying Shelf Temp.	Process cycle time
BSA 30 mg/ml Liquid Formulation	-5°C	25°C	57h
BSA 70 mg/ml Liquid Formulation	5°C	25°C	48h
BSA 100 mg/ml Liquid Formulation	5°C	25°C	45h
BSA 30 mg/ml Lyo Formulation	5°C	25°C	51h
BSA 100 mg/ml Lyo Formulation	5°C	25°C	51h

Table 6: Optimized freeze-drying process cycle times

Table 6 cycle times were measured based on the assumption that the processes were considered optimized after analyzing both the dry cake and the reconstituted solution, and demonstrating that the defined criteria for success were met (this data is presented in the next section). While the majority of the optimized cycle times obtained are slightly above the 48h criteria, they are in line with run rate usually seen in manufacturing. Slight modifications to predefined process parameters could help reaching the 48 hour by:

- Slightly increasing the vacuum in the drying chamber, or the cooling and heating ramp rates used between phases
- Decreasing the fixed temperature plateau durations in the cycle

This fine tuning exercise was not completed for this thesis because of the time constraints.

4.5. Assessment of dry product and reconstituted solution

The list of criteria for success (Table 4) includes glass transition temperature and residual moisture content of the dried product, as well as reconstitution time, protein concentration and pH of the reconstituted solution. Before presenting the results obtained, this section will introduce the method and equipment used to measure these different parameters.

Glass Transition of the cake (Tg): This parameter is determined with Modulated Differential Scanning Calorimetry methodology, similarly to the Tg' determination. Cake samples are heated until their glass transition temperature is reached, inducing melting and change in heat flow.
Cake residual moisture content: The determination is completed using the Karl Fischer method. The water in the sample is vaporized and carried by dry oxygen free nitrogen into a reaction vessel with methanol. The methanol traps the water which is titrated to an end point with a Karl Fischer reagent to determine the amount present. This method is more accurate than simple weight loss because in the weight loss method, volatiles other than water can be lost which is translated into artificially high water content.
Reconstitution Time: The tray is weighed empty, full of solution prior to freeze drying, and after the cycle. The weight loss corresponds to the weight of water that was removed through sublimation and desorption during the cycle. The same amount of water is poured back into the tray, and the time to dissolve the entire dry product into solution is recorded.
Protein concentration and pH: Protein concentration in mg/ml is determined using an A280 spectrophotometer and pH is measured using a pH meter.

Even though all physical characteristics of the cake and reconstituted solution were measured during the optimization phase, Table 7 below only presents the results obtained for the final runs from Table 6.

	Dry cake		**Reconstituted solution**		
Solution	Glass Transition Tg	Moisture Content	Protein Concentration Δ with initial value	pH Δ	Reconstitution time
BSA 30 mg/ml Liquid Formulation	48.6 ± 0.6	3.0%	W/in specifications -1.8%	0.09	4 min
BSA 70 mg/ml Liquid Formulation	53.1 ± 0.9	1.6%	W/in specifications -1.7%	0.02	23 min
BSA 100 mg/ml Liquid Formulation	76.3 ± 0.7	1.5%	W/in specifications -1.7%	-0.06	70 min
BSA 30 mg/ml Lyo Formulation	41.7 ± 0.4	1.6%	W/in specifications -1.2%	-0.09	5 min
BSA 100 mg/ml Lyo Formulation	46.6 ± 0.4	1.8%	W/in specifications 3%	-0.06	45 min

Table 7: Dry cake and reconstituted solution characteristics

For the different formulations and concentrations tested, we were able to meet the defined success criteria. This initial assessment confirms that freeze drying could be an adequate technology for drug substance storage. Looking at "dry cake" data, we can see that the glass transition Tg increases with protein concentration for both formulations, with significant differences in values obtained for the liquid formulation samples. Residual moisture content levels are mostly linked to shelf temperature. The desorption rate is higher when using a higher shelf temperature, and explains why moisture content is nearly half for solutions lyophilized at 5°C, compared to the solution dried at -5°C (this also affects the duration of the primary drying, as seen in the cycle optimization section).

Protein concentration and pH values obtained with the reconstituted solutions were found similar to the values obtained on the same solutions prior to freeze drying, and well within the success criteria defined prior to the experiments. This proves that the freeze drying process did not significantly modify the solution (protein denaturation, modification of base formulation), and that the method used to reconstitute was appropriately measuring the water loss during the lyophilization process. Finally, it is interesting to note that reconstitution time is greatly linked to protein concentration. Dry cakes from low protein concentration solution were dissolved in minutes, while it took up nearly an hour or more for high concentration solutions. This must be taken into account when considering dry product reconstitution at manufacturing scale. While the times recorded in this evaluation are highly dependent on the quantities used (1L volume fill), the same time variations can be expected with higher volumes of product in manufacturing.

4.6. Effect of fill depth in tray

As mentioned in section 4.1, it is important to evaluate the effect of tray fill depth, because it is one of the factors that needs to be considered when choosing a freeze dryer capacity. Indeed, the number of trays required for an entire production batch is reduced greatly if the tray fill depth can be increased (and therefore the volume of drug substance in each tray). In consequence, a freeze dryer with smaller total shelf surface could be used (given the condenser has enough

capacity to retain all the water extracted by sublimation). The disposable trays used in this evaluation were filled with one liter of solution, but the tray has a capacity to hold up to 1.8L of solution.

The objective of this experiment was to test the effect of fill depth on the process cycle time. We used the BSA 30 mg/ml solution, liquid formulation, because it led to the longest cycle time in our previous experiments and therefore is a worst case scenario for overall cycle time. The same optimized process parameters were set (primary drying shelf temperature: -5°C; secondary drying shelf temperature: 25°C).

Figure 13 below shows the basic process of sublimation as it happens in the tray during the primary drying process. The lyophilization process starts from the upper surface of the frozen solution, and the depth of the dry cake layer increases overtime in the bottom direction, until all water is removed and only dry cake remains in the tray.

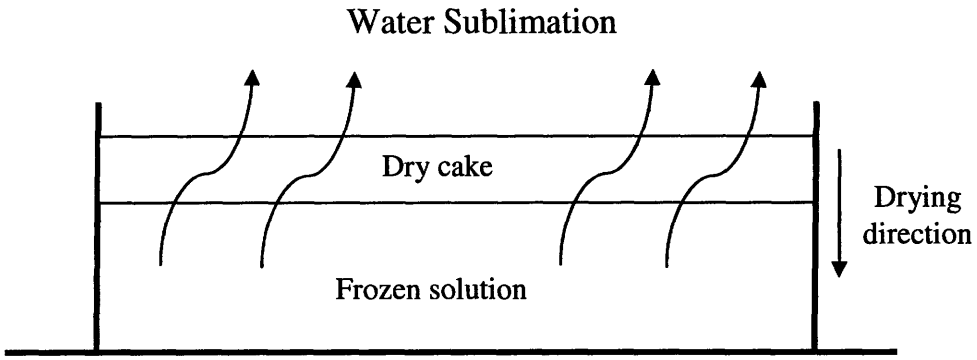

Figure 13: Water sublimation process in tray

Filling the tray with 1.8L of solution increased by almost 2.5 times the primary drying time, from 42 hours for 1L fill to 103 hours for 1.8L fill (see Appendix 5 for product temperature profiles during primary drying). The residual moisture content in the cake was measured at 3.7% (compared to 3% measure for the 1L fill experiment). Other parameters were found equivalent. Different reasons can explain this increase in drying time. For example, the increased volume can modify the solution freezing pattern at the beginning of the cycle. This could lead to different size of ice crystals, and consequently modify the sublimation rate seen previously. But more importantly, this result shows that fill depth can become a limiting factor if a freeze drying

process is to be implemented at a drug substance manufacturing site. Filling trays above a certain height could lead to very long cycle times and this step could potentially become a process bottleneck. On the other hand, lower volume in trays will increase the number of trays necessary for a batch production. In consequence, the process would require a freeze dryer with higher shelf surface capacity.

4.7. Technical summary

In this chapter, we presented the overall strategy and methods used to design and optimize a freeze drying process for a given formulation. We applied this methodology to different drug substances formulations and protein concentrations. Physical characteristics and cycle times obtained from solution to solution varied. However, we were successful in meeting a set of baseline requirements indicating that it is possible to dry drug substance using tray freeze drying, and later reconstitute the initial solution to specifications. Optimized cycle times are in line with current manufacturing run rates in drug substance manufacturing facilities, and measured properties of the dry cake demonstrate the possibility to store and ship the product at controlled room temperature. From a technical and qualitative standpoint, lab scale results therefore indicate that freeze drying is a feasible alternative to cryopreservation for the storage and shipment of drug substance.

5. SPRAY DRYING: A TECHNICAL EVALUATION

This chapter presents the methodology and results obtained during the evaluation of bulk drug substance spray drying at lab scale, similarly to the content presented in chapter 4 (See Appendix 6 to see a picture of the lab scale spray dryer equipment used).

5.1. Lab scale evaluation strategy

Demonstrating feasibility to use spray drying technology to dry drug substance has the same implications than for freeze drying in terms of cycle time, dry product and reconstituted solution characteristics. Success criteria used in chapter 4 are therefore valid for the spray drying evaluation. The methodology is however very different, since the drug substance is not frozen but sprayed in a liquid state into a drying chamber (Figure 14). First, it is important to conduct a mass flow analysis on the spray dryer used. This step is essential to understand the relationship between the different inputs settings (liquid feed rate, atomizing gas flow, inlet temperature) and the output temperature obtained (the major drying parameter). When this analysis is completed, different inputs combinations can be selected. The test solution is then sprayed using these different settings and dry product collected and analyzed. The solution can then be reconstituted and its physical characteristics measured. The optimal spray drying for one formulation is the cycle that will yield a low cycle time, good dry product characteristics (high Tg, low residual moisture content) and meet the specifications of the reconstituted solution in terms of pH and protein concentration.

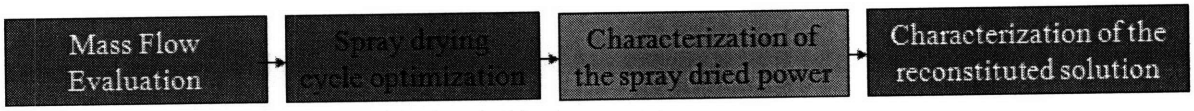

Figure 14: Spray drying cycle evaluation methodology

5.2. Mass flow analysis

Figure 15 describes the functioning principle of a lab scale spray dryer. An inert gas under pressure (usually nitrogen) enters the system through the air inlet 1 and flows through an electric heater 2 that raises its temperature to the set level. Liquid solution is sprayed through a nozzle 3 and mixed with hot air right at the nozzle tip. The fine liquid droplets formed by atomization fall down the drying chamber 4. Water contained in the droplets rapidly evaporates during the residence time in the drying chamber and solid particles left are separated from gas in the cyclone 5 and are collected in the vessel 6. Hot gas flows through the outlet filter 7 due to the aspirator 8 whose role is to pump air through the system.

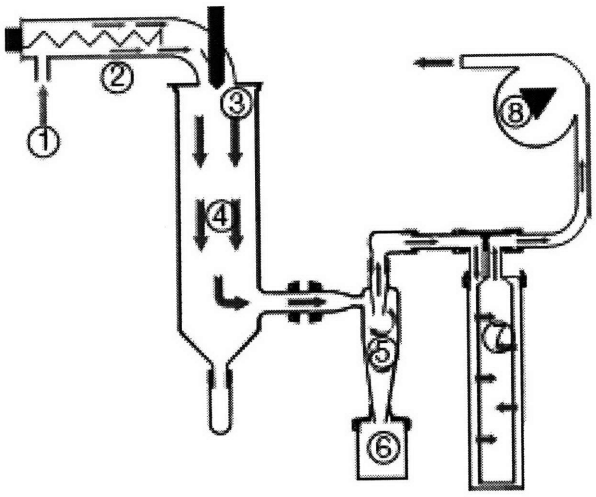

Figure 15: Functional principle of the drying air (lab scale equipment)[12]

Inlet temperature and liquid feed rate are the two parameters that most influence outlet temperature. Specifically, the outlet temperature increases with increasing inlet temperature and decreases with increasing liquid rate[13]. Outlet temperature is the determining factor in spray drying, because it will affect the drying quality. If outlet temperature is too low, the product will either not dry, or the residual moisture content in the powder will be too high (which would impact long term stability). If outlet temperature is above the glass transition temperature of the powder, the dry product will melt, inducing quality issues. In order to understand the relationship between liquid feed rate, inlet and outlet temperature, and therefore to choose the right set of

spray drying parameters (leading to an adequate outlet temperature) a mass flow analysis was run on the lab scale spray dryer.

The objective of the study was to test different inlet temperatures and liquid feed rates using water, and record outlet temperatures obtained. Based on expected Tg (glass transition of the dry powder) values for each solution, we were able to determinate a set of potential optimal conditions for the different formulations and concentrations. The test results are presented in Appendix 7. As expected, the variations between inlet temperature and outlet temperature at a given liquid feed rate were linear. The Tg values measured for the different powders after freeze drying were comprised between 40 and 76°C. Tg values of dry products obtained with spray drying are therefore likely to vary within the same interval. We decided to test for each solution several input conditions that lead to an outlet temperature lower or equal to the expected Tg values of each solution, and maximize liquid feed rate, as presented in Table 8 below.

	Inlet temperature (°C)	Liquid feed rate (ml/min)	Expected outlet temperature (°C)
BSA 30 mg/ml, Liquid Formulation	40	1	32
	40	2	29
	50	1	39
	50	2	36
BSA 70 mg/ml, Liquid formulation	50	2	36
	60	2	44
	60	5	34
	70	2	51
	70	5	42
BSA 100 mg/ml, Liquid formulation	50	2	36
	70	2	51
	90	2	67
	90	5	58
	90	10	41
BSA 30 mg/ml, Lyophilization formulation	40	2	29
	50	2	36
	60	5	44
BSA 100 mg/ml, Lyophilization formulation	50	2	36
	70	2	51
	90	2	67

Table 8: Experiment conditions for each solution

5.3. Optimization of the spray drying cycle

A defined volume of solution was spray dried using the lab scale equipment to yield enough dry product for powder and reconstituted solution testing. The volume level chosen is directly related to protein concentration: at constant formulation and volume, a 30 mg/ml protein concentration solution contains less solid content than a 100 mg/ml solution. All experiments were conducted using the same experimental method. Cycle inputs were set and actual spray drying was not started until inlet temperature was stabilized. Pressure levels in the system were kept constant in each experiment to avoid variations. Table 9 below presents the results obtained for each cycle. Residual moisture content was evaluated for each powder produced as an optimization criteria, as it affects protein stability over time in storage.

	Inlet temperature (°C)	Liquid feed rate (ml/min)	Drying cycle success (Y/N)	Moisture Content
BSA 30 mg/ml, Liquid Formulation	40	1	Y	1.704%
	40	2	Y	3.712%
	50	1	Y	1.356%
	50	2	Y	2.461%
BSA 70 mg/ml, Liquid formulation	50	2	Y	2.579%
	60	2	Y	2.626%
	60	5	No: drying not effective	XXXX
	70	2	Y	2.714%
	70	5	Y	5.142%
BSA 100 mg/ml, Liquid formulation	50	2	Y	1.901%
	70	2	Y	1.732%
	90	2	Y	1.905%
	90	5	Y	3.836%
	90	10	Y	5.491%
BSA 30 mg/ml, Lyophilization formulation	40	2	Y	4.01%
	50	2	Y	2.25%
	60	5	No: drying not effective	XXX
BSA 100 mg/ml, Lyophilization formulation	50	2	Y	1.70%
	70	2	Y	2.02%
	90	2	No: Inlet temperature too high	XXX

Table 9: Cycle optimization – results

Red cells in the table represent cycle conditions that led to an incomplete drying process. For two sets of conditions (liquid feed rate 5 ml/min), water contained in the atomized droplets did not fully evaporate during the residence time in the drying chamber. Residual wet solution accumulated on the drying chamber walls, sign of an inadequate drying process. For the third set of conditions, inlet temperature ended up being too high for the sprayed solution: "burned" product accumulated at the nozzle and the experiment was stopped. Other combinations seemed to be adequate and produced actual powder as expected. But residual moisture content measured after the cycle was found above the 3% success criterion that was established (yellow cells in the table). These combinations were therefore also rejected.

All the remaining cycles met the residual moisture content criterion. The optimized sets of conditions for each solution were then determined using the following steps. First, liquid feed rate for the cycles were compared. If liquid feed rate was found equal, the optimized cycle corresponded to the set of conditions that gave the lowest residual moisture content in the dry powder. This short analysis gave us a single optimized set of conditions for four of our five tested solutions (optimized cycles are the green colored rows).

For the BSA 30 mg/ml, liquid formulation solution, we had to choose between a low liquid feed rate and very low moisture content in the product, or a faster spray drying process producing a dry powder with a higher moisture content (but still meeting the success criterion). It is assumed that a lab scale feed rate leads to a feed rate hundred times higher at manufacturing scale. Our study assumes a theoretical batch volume of 300L in production. This means it would take 50 hours at the lower rate to spray dry a production batch and 25 hours at the faster rate. Only the 2 ml/min feed rate process is therefore rapid enough to meet a production process cycle time requirement of 48 hours or less.

5.4. Assessment of dry product and reconstituted solution

Assessment of the dry products and reconstituted solutions was conducted with the same test equipment and techniques presented in Chapter 4. The methodology for solution reconstitution was however different. Lab scale spray drying yield does not usually reach more than 60%. Indeed, lots of powder particles stick to the drying chamber walls or are lost because of air tightness issues (small leaks in the system) and are not collected in the vessel container. It is therefore difficult to reconstitute a solution based on the initial volume to spray dry. The most consistent technique is to measure a certain weight of dry powder in a beaker, and complete the reconstitution process by adding the corresponding theoretical volume of water (based on theoretical solid content in the solution). For example, add 6.25 ml of water to 1 gram of dry powder if the theoretical solid content in the original solution (protein + excipients) is 16 grams of solid for 100 ml of solution.

Table 10 presents measured physical characteristics of all dry powders and reconstituted solution for the process cycles chosen:

	Cycle conditions		**Dry Powder**		**Reconstituted solution**			
Solution	Inlet Temp.	Liquid feed rate	Glass Transition Tg	Moisture Content	Protein Concentration Δ w/ initial solution	pH Δ	Reconst. time	Expected prod. process cycle time
BSA 30 mg/ml, Liquid solution	50	2	48.7 ± 0.6	2.50%	W/in specs -3.5%	0.07	10 min	≈ 25 hours
BSA 70 mg/ml, Liquid solution	50	2	48.7 ± 0.4	2.60%	W/in specs -2.4%	-0.01	11 min	≈ 25 hours
BSA 100 mg/ml, Liquid solution	70	2	59.0 ± 0.7	1.70%	W/in specs -6.7%	-0.04	16 min	≈ 25 hours
BSA 30 mg/ml, Lyo solution	50	2	40.3 ± 0.8	2.25%	W/in specs -4.5%	-0.13	10 min	≈ 25 hours
BSA 100 mg/ml, Lyo solution	50	2	57.2 ± 0.3	1.70%	W/in specs -5.8%	-0.12	13 min	≈ 25 hours

Table 10: Dry powder and reconstituted solution characteristics

Similarly to the freeze drying evaluation, all acceptance criteria were met with the optimized spray drying cycles, including the expected process cycle time for a manufacturing scale spray drying process (given a 300L batch hypothesis). Glass transition temperatures Tg increase with protein concentration increase and moisture content decrease. We can also note that protein concentration variations obtained are higher than those for the freeze drying evaluation. This is likely due to the less precise method used for solution reconstitution volume, based on theoretical solid content rather than actual measurements of water loss like for freeze drying. Finally we can observe that reconstitution times seem shorter on average (especially for high concentration solution). This can be explained by the fact that only a few grams of dry powder were reconstituted for each solution (rather than a full tray of dry product for freeze drying), hastening the overall process.

5.5. Technical summary

Throughout the chapter, we discussed an optimization method for spray drying cycles at lab scale. When applying this methodology to a set of solutions with different protein concentrations and base formulations, we were able to design cycles that meet all of our predefined success criteria. These lab scale results indicate a major probability of success for spray drying technology at pilot and manufacturing scale to process drug substance into a dry powder that could be stored and later reconstituted without major changes to the solution characteristics. From a technical standpoint, spray drying is therefore a credible alternative to cryopreservation to maintain drug substance stability under safe storage conditions.

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6. FINANCIAL ANALYSIS

We demonstrated in previous chapters that it was technically feasible to produce a dry powder that had physical characteristics adequate for long term storage of drug substance. Freeze drying and spray drying process cycle times obtained were also found in line with manufacturing rates currently seen in biological production facilities. In this chapter, we explore the methodology used to develop a cost model comparing the financial impact of the introduction of drying technologies in the manufacturing process of a drug protein with the costs associated with the use of cryopreservation technologies in the same process.

6.1. Strategy and methodology

Data obtained through cost modeling is not an exact representation of actual costs incurred in manufacturing. Modeling requires assumptions needed for simplification purposes. In addition, it is sometimes very complicated to allocate adequately indirect and overhead costs to a single product. Finally, it is difficult to accurately measure future capital investments or expected process yields for a manufacturing process when the model represents costs incurred over a long period of time.

The goal of the financial model developed here is to provide some general cost information on different storage technologies over the lifetime of a new drug protein. The scope of the analysis is therefore limited to all processes related to storage and shipment of the drug substance at the bulk manufacturing and the fill/finish facilities. This includes:

- Costs associated with freezing or drying of drug substance (capital investment for buildings, equipment, disposable materials, labor costs, etc)
- Costs associated with storage of the product (mostly facility investment in storage space)
- Costs associated with shipment of the product and equipment (return shipment cost for Cryovessels for example)
- Costs associated with drug substance thawing or reconstitution

The financial model is based on a scenario where a high volume pipeline product is transferred to manufacturing for validation and commercial production. The model aggregates all costs incurred yearly at the bulk manufacturing and the fill/finish sites for storage and shipment of drug substance when using five different types of equipment (cryopreservation: plastic carboys, Cryovessels, Celsius Paks; drying technologies: freeze drying and spray drying). The net present value of all costs for each technology is then compared over a 12-year period (2 year on site process validation and 10 year commercial period).

Two major assumptions can be used to build this model. The first one consists of considering that the bulk manufacturing and fill/finish facilities are both greenfield facilities that have to be built. In this option, all costs for facility construction and validation at both sites need to be included (cost per square foot necessary to host equipment and processes associated with cryopreservation or drying methods, as well as storage capacity). Even though this seems to be a fair method to compare the cost of implementing different technologies in manufacturing, it does not take into account that most biotechnology firms and Amgen in particular have already an extensive network of facilities with actual freezer storage capacity available for new product introduction. We therefore decided that only building modifications and extensions related to the new product introduction should be considered in the model, and that costs incurred to build existing freeze capacity were sunk costs not relevant to the analysis.

6.2. Data Collection

Having defined a costing approach and developed a strong understanding of the processes involved in drug substance storage, the next step was to collect the required data. Production volumes were based on an actual Amgen pipeline product commercial forecast (see Appendix 8 for representation of storage volume over time). We decided to choose a high volume product because this is a scenario relevant to this study. The higher the production volume is, the higher the quantity of stored drug substance will be (increasing the potential benefits of using drying technologies over cryopreservation methods). Facility modifications and expansions due to this

product introduction are based on actual Amgen facilities capacities. These facilities are representative of facility size found in biotechnology operations.

- Material and direct labor

The direct labor cost is the number of workers operating the equipment times the average yearly labor rate and was obtained from plant management. This rate represents the average pay across the various pieces of equipment, different shifts and for employees with different levels of experience. Another yearly labor rate in the model was used for employees involved in validations activities during new product introduction to manufacturing. Material costs are highly dependent on the technology used (Celsius-Paks versus disposable plastic trays for freeze drying for example) and the production batch size (300L) used for this study.

- Capital Equipment

Capital equipment investment was estimated over the 12-year period based on utilization levels over time. For example, for the Cryovessels technology, we evaluated the number of containers necessary to manage year to year operations given the production volume requirements and safety stock in storage. New facility building (for example to hold a freeze dryer) or expansion costs (freezer storage capacity required over time) were based on required capacity. Equipment prices were either available through quotes from the manufacturer, or were estimated by plant management and cost data from past projects.

- Shipment costs

Shipments costs were directly quoted by the shipper company, based on pallet space requirement and weight per shipment, as well as transportation mode.

- Vessels maintenance and Re-use costs

Average cost for refurbishment and cleaning of non disposable containers were included in the model and were calculated based on past production data obtained from other manufacturing processes for which these containers are used.

6.3. Major model assumptions

Some major assumptions were made to keep the model relatively simple in order to calculate the net present value of costs incurred over the period 2008 – 2020.

First, the model assumes that the production batch volume for this new protein manufacturing process was kept constant at 300L over the whole time period. In addition, we considered that the bulk manufacturing site chosen has enough capacity to manufacture this protein until 2020. This implies that only this single manufacturing site will produce the new protein over the 12-year period considered. Even though biotechnology firms are usually constrained to move protein production from one site to another in order to balance capacity, it would considerably increase the complexity if we had to integrate a process transfer from one site to another into the model.

Second, it was assumed that some initial freezer capacity was available at the bulk site when commercial production starts, delaying by a few years capital investment need for freezer expansion. The requirement of months of inventory at hand in storage was kept constant during the 12-year time period. We also considered for the freeze drying and spray drying scenarios that process validation on site would be completed throughout the period 2008-2010.

Third, it was considered that process yield for all three cryopreservation technologies and for freeze drying were equivalent. Manufacturing scale spray drying process yield is known to be slightly lower than yield obtained with other technologies. We therefore implemented a 2% yield loss for spray drying, based on actual yield estimates from the equipment manufacturer. The costs of additional production necessary to meet volume production was not included in the model (for complexity issues once again).

Finally, the scenario assumes that the fill/finish process will be completed at a contract manufacturer site. This contract manufacturer usually charges an identical price whether drug substance needs to be thawed or reconstituted prior to process it in the fill/finish operation. We therefore used an equivalent cost value for thawing or reconstitution process in the model.

6.4. Net Present Value analysis and results

We built the financial model on assumptions stated above and populated it with expenses incurred per year for each storage technology scenario. We used a 3% inflation rate, a 38% tax rate and a constant year to year wage merit increase for headcounts. Cash outflows were discounted using the standard rate at Amgen for capital investment projects. Finally, any equipment value in the model is depreciated over a 10 years period, using a straight line depreciation method.

We consider that most of the cost data for the carboys, Cryovessels and Celsius-Pak scenarios is pretty accurate because numbers are either inferred from similar manufacturing processes used at Amgen (for carboys and Cryovessels) or are taken from a current evaluation (Celsius-Paks as a potential strategic technology for future projects). Unfortunately, we cannot have the same degree of confidence for some cost estimates obtained for freeze drying and spray drying scenarios. Two line items are especially problematic: the first one concerns the cost estimate of a building addition at the bulk manufacturing site for the freeze or spray drying equipment, from construction start to validation process. Determining an accurate cost estimate for a project of this scope would require a deep analysis of all activities involved that the author could unfortunately not complete in the timeframe of the project. We therefore had to rely on the experience of the plant management to come up with a number. The cost of the disposable containers for the dry powder is also difficult to evaluate in the long run: we used during the study containers for lab scale studies, and obtain an estimate for a manufacturing scale size container was purely based on the willingness of the supplier to come up with a reasonable number (we considered that the container prices would decrease much more than the estimate we were given due to the massive economies of scale realized with the production volumes we anticipated). We therefore decided to complete the Net Present Value analysis with two different cases:

- A base case using the reference cost values we obtained,
- An optimistic case where a low point estimate was used for capital costs necessary for the building addition, and a 40% reduction in disposable container costs for the drying process.

- A pessimistic case where a high point estimate was used for capital costs necessary for the building addition.

Table 11 presents the dollar net present value obtained for each storage technology for all cases.

Storage Technology scenario	**Base Case** Net Present Value ($ millions)	**Optimistic Case** Net Present Value ($ millions)	**Pessimistic Case** Net Present Value ($ millions)
Carboys	-3.53	-3.53	-3.53
Celsius Paks	-3.75	-3.75	-3.75
Cryovessels	-8.96	-8.96	-8.96
Freeze Dryer	-8.7	-5.61	-11.86
Spray Dryer	-11.29	-9.4	-13.58

Table 11: Net Present Value of costs incurred – All scenarios

(Note: Numbers have been altered to protect sensitive data. However, the basic significance has been preserved)

In all cases, the analysis demonstrated that carboys and Celsius-Paks are the two most economical methods to store and ship drug substance over the period of time considered. The present value of costs incurred when using Cryovessels or freeze drying technology is significantly more expensive. Finally, spray drying is in all cases the most expensive technology.

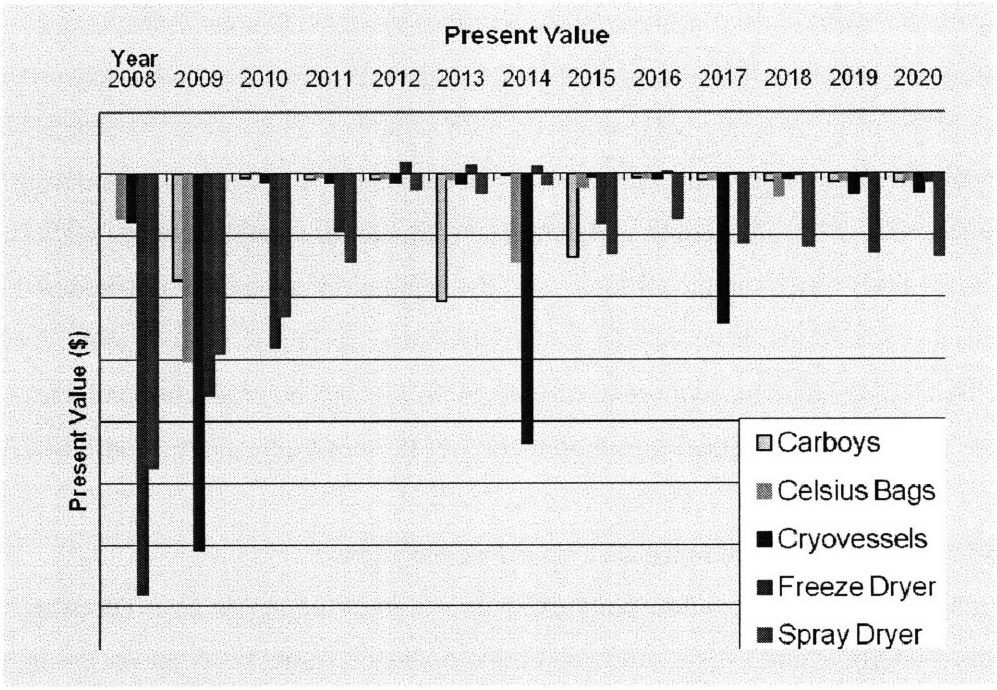

Figure 16: year to year present value of costs for each technology (base case scenario)

Figure 16 presents the present value of costs per year and is very useful to understand at what moment major expenses are realized for each technology. In addition, Appendix 9 presents the pallets space requirement in the freezer over the period considered.

Carboys: Major expenses are realized in three specific years (2009, 2013 and 2015). Appendix 9 shows that these expenses correspond to investments for freezer capacity increase, to store increasing levels of frozen drug substance. Shipment cost increases over time are not dominant because they happen later in the time period considered and are therefore heavily discounted.

Cryovessels: Major investments are linked to freezer capacity increase (years 2009 and 2014, refer to Appendix 9) as well as purchase of expensive vessels (in years 2009, 2014 and 2017) to support the increasing production volumes over time.

Celsius-Paks: in our scenario, the Celsius-Pak technology is not yet validated and used in production for drug substance storage in 2008. The major costs seen in the early years are related to equipment purchases as well as validation activities. The large expense in 2014 is related to freezer capacity expansion and purchase of additional equipment to support the increasing production levels.

Freeze drying: Major expenses occur all in the early years in the period considered: the cost of the building, equipment and validation activities is spread over three years, and a first freezer capacity expansion occurs in the fourth year.

Spray drying: Identically to the freeze dryer scenario, major expenses occur over the first four years to build and validate the spray drying facility. Towards the end of the study period, the 2% yield loss becomes very expensive as the volumes of drug substance produced increase rapidly.

6.5. Discussion

The financial analysis clearly shows that carboys and Celsius-Paks are the two most economical container technologies to store and ship drug substance. When using carboys, several investments in additional freezer capacity are necessary to support the production of a high volume protein. But these investments remain reasonable since the facility infrastructure does not require large modification for these expansions. For Celsius-Paks, the initial purchase of equipment is offset by the fact that less frozen storage space is necessary with this technology.

Drying technologies are not cost competitive with carboys and Celsius-Paks for several reasons. First, drying equipments are very expensive by nature, and their implementation in the current manufacturing environment requires large additional expenses (building addition, supporting equipment and line validations). These costs need to occur early on and are therefore not heavily discounted in a net present value model, compared to costs incurred for other technologies (the significance of the results is not sensitive to discount rate changes). Since the overwhelming majority of the production facilities of biotechnology companies are currently using cryopreservation as a storage method, the costs of switching to drying technologies become therefore non-economical at this point.

Next, we can see in Appendix 9 that the bulk manufacturing site would still require some freezer expansion if using freeze drying or spray drying technologies. This seems counter-intuitive since dry powders could be stored in controlled room temperature. But there is a specific reason for this: in current biological manufacturing processes, process intermediate products are stored as a buffer step between the upstream harvesting process and the downstream purification steps. With very high volume production, there is not enough freezer space to store the increasing volumes of process intermediate products, and adding freezer capacity becomes therefore inevitable at some point.

Additional specific reasons to each drying technology can also explain the costs gap. Disposable containers for freeze drying have a limited volume capacity. As discussed in Chapter 4, fill depth is critical to obtain an adequate process cycle time. It therefore impacts greatly the number of trays needed per batch, and in consequence the shelf surface required to process a full

batch with only one freeze dryer. Buying a freeze dryer with higher capacity and a large number of trays for each batch make the overall project costs increase therefore consequently. In the case of spray drying, the lower yield has a huge impact on the overall process costs. Drug substance is very expensive to produce and 2% yield loss has a massive impact on the overall process costs when production levels reach a very high volume and the manufacturing lines are used at full capacity.

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7. DRUG SUBSTANCE STORAGE: MANUFACTURING PROCESS STRATEGY

While the initial technical assessment demonstrated that it was feasible to produce at lab scale a dry powder with adequate physical characteristics for long term storage, the financial analysis showed that drying technologies were significantly more expensive to implement than some of the cryopreservation technologies presented in the thesis. This chapter builds on the previous results and discusses different aspects of the drug substance storage process step in relation to the evaluation of drying technologies. Based on all findings obtained throughout this study, recommendations are made regarding the strategic potential of drying technologies as a storage technology for drug substance.

7.1. Storage technologies cycle times

Drug substance manufacturing campaigns (leading to the storage of product for months) allow some scheduling flexibility between drug substance and drug product manufacturing, but this process has an obvious lack of connection with lean "pull" processes. Womack & Jones define Lean Thinking[14] as the removal of "muda" (Japanese word for waste), i.e. any activity that absorbs resources and create no value. Value stream mapping methodologies help determine value added activities (any action that transforms information / material into a capability for the customer at the right time and the right quality) and design a lean flow. Following this definition, the drug substance storage step is a non value added activity, but it cannot be eliminated at this point based on potential product quality issues, technologies currently available and manufacturing processes used nowadays.

Cycle times to freeze drug substance using cryopreservation can vary from a few hours for a controlled freezing process to up to several days for an uncontrolled freezing process with carboys. For drying technologies, it has been established in the initial evaluation that it could vary from one day for spray drying and up to over two days for freeze drying. For all technologies, it would be difficult to drastically improve these cycle times due to their dependence on defined characteristics that are not easily modifiable (for example the liquid flow

rate of an optimized spray drying process). However, over the years, manufacturing run rates in other areas of the bulk manufacturing process are constantly being driven down through process improvements and implementation of lean practices. This does not have a significant impact for a process using cryopreservation technologies since only the filling process occurs in the manufacturing line: the freezing process itself is completed in a different storage area. This would however not hold true for drying technologies. Drying equipment is connected to the last tank of the bulk manufacturing line. With continuous improvements on manufacturing run rates over the lifetime production of a specific protein, there is a potential risk that the drying process becomes the process bottleneck. This aspect must therefore be taken into consideration when evaluating the implementation of drying technologies in a protein bulk manufacturing process.

7.2. Manufacturing flexibility

Agile manufacturing is seen as the winning strategy to be adopted by manufacturers looking for dramatic improvements and continuous performance in increasingly competitive markets. Led by the success of Japanese auto maker Toyota, it has transformed the old mass production system into a lean model, where the business strategy is focused on flexibility and adaptability and the operations is designed for synchronized flow and pull systems[15]. Big biotechnology firms like Amgen have in the past vertically integrated by heavily investing in large sized plants of considerable complexity because of scale economics and costs of capital. Today, numerous efforts are made to modify processes and make these manufacturing sites much more responsive to demand size changes, multi product manufacturing lines, capacity balance and reduced lead times.

When evaluating the different technologies proposed in this thesis for drug substance storage, one must also keep in mind flexibility as an important decision factor. At the manufacturing site level, drying technologies will improve storage space and logistics required for the transportation and storage of drug substance. However, spray drying and freeze drying equipments are not at all flexible: because of their defined capacity, it can become very inefficient in case of demand changes during the lifetime of the product. Similarly, the decision to buy and implement this

equipment must be completed several years before a new protein commercial production start. With great uncertainties on pipeline products volumes, it is possible to implement equipment with a capacity that will not match the actual demand in the long run. These issues are much less impactful when using cryopreservation technologies, since volume changes will mainly impact the number of containers used. In addition, biotechnology companies tend to balance production capacity by shifting manufacturing locations for certain products, within the company network of facilities or through contract manufacturers' services. New manufacturing sites will most probably not have the massive drying equipment available in their facilities, requiring building constructions and validation exercises and increasing significantly the effective lead times necessary for a manufacturing location change.

7.3. Drug substance inventory levels

The major operational benefit of using drying technologies for drug substance storage is to reduce freezer space requirements and complicated logistics for drug substance shipment from the bulk production facility to the fill / finish manufacturing site. But one of the fundamental principles of lean manufacturing is to remove waste from the system, and inventory is one of the seven categories of waste. Drying technologies can certainly become a valid answer to reduce the burden of increased inventory levels necessary to meet a growing demand. But one should not keep out of sight that it is a work around the problem of inventory control. Steven Spears explains in one of his articles how applying techniques from the Toyota Production System was able to significantly improve operations by eliminating ambiguities in the systems and stopping working around problems[16]. The same philosophy can be applied to the drug substance storage problem: improving inventory control cannot be solved by local solutions like implementing a new technology to reduce storage space, but must be seen as a system issue. Major improvements can only be made in the long run at the system level, by transforming the entire supply chain organization into an agile system able to adjust more quickly to changes in demand.

7.4. Drug substance storage technologies: a path forward

Based on the analyses conducted in this thesis, Table 12 provides some performance comparisons between the different technologies discussed (flexibility and cost rating scale of 1-5 = best to worst).

	Plastic Carboys	Cryovessels	Celsius Paks	Freeze drying	Spray Drying
Proven Technology	Yes	Yes	In process	Not for use w/ drug substance	Not for use w/ drug substance
Product Integrity	Medium Risk	Low Risk	Low Risk	Low Risk	Low to medium Risk
Handling logistics	Difficult (Long freeze/thaw cycle, high number of carboys required)	Difficult (Return shipment, Refurbishment)	Simple (Disposable container Short freeze/thaw cycle)	Simple (Disposable trays, no cold chain requirement)	Simple (Disposable bag , no cold chain requirement)
Flexibility	2	3	1	5	4
Cost	1	3	2	4	5

Table 12: Performance comparison between technologies

It is not surprising to observe that no technology best performs in all categories and that tradeoffs need to be made when analyzing the overall potential to meet the varied sets of requirements for drug substance storage. Drying technologies could improve greatly in handling and shipment logistics while improving product stability during the storage period. However, we demonstrated that operating these massive equipments would offer a very poor manufacturing flexibility and would be very costly to implement in a manufacturing network. With the current cost pressures and the momentum to implement some lean manufacturing methodologies in the biotechnology industry, drying technologies are probably not attractive enough at this point to justify the process development at larger scale and an implementation in the near future in a manufacturing environment for drug substance storage.

The main challenges faced when using cryopreservation technologies reside in the fact there is currently no container that can best protect the protein integrity during the freeze / thaw cycles (i.e. with a temperature controlled cycle) while offering operational advantages (simple logistics, flexibility to changes) at a low cost. Celsius Pak technology, currently under test, seems to have the features to respond to this demand, but it is still uncertain if this equipment will meet all

container requirements and be finally be implemented for drug substance storage. If it does not happen, plastic carboys may likely stay the container of choice for drug substance storage in the industry for the next years.

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8. PROCESS INNOVATION IN THE BIOPHARMACEUTICAL INDUSTRY

Building on the evaluation of drying technologies for storage of drug substance presented in this thesis, this chapter evaluates the role of process innovation in the biopharmaceutical industry. After a literature review on innovation in various industries, as well as a presentation of the benefits of manufacturing innovation, the specifics of process development and innovation in the pharmaceutical and biotechnological industry are presented. Finally, implications for future process innovation in biotechnology firms are discussed.

8.1. Product and process innovation

The importance of process development dates back to the work of Abernathy[17] and Utterback[18] on the rate of product and process innovation throughout the product's life cycle (see Figure 17). The model shows that during the early years of a product's life, the rate of product innovation would exceed the rate of process innovation. Later, a dominant design would emerge and radical product innovation would begin to decline.

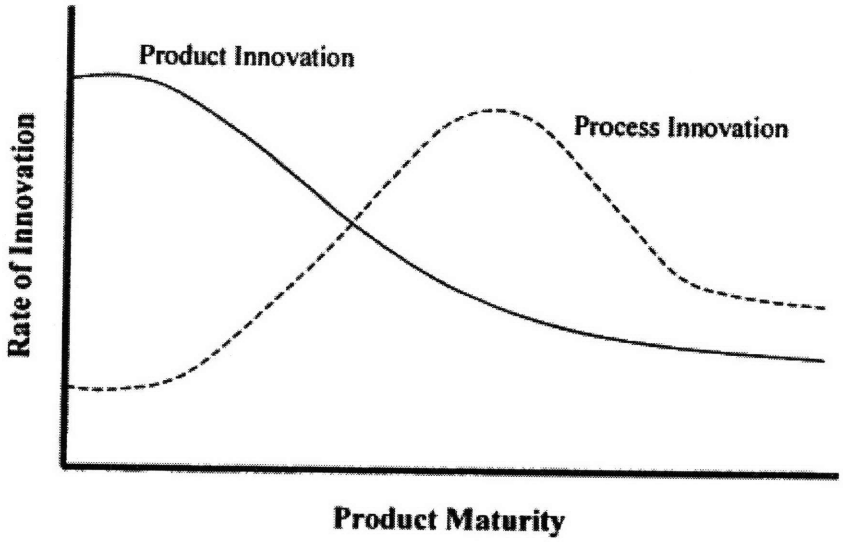

Figure 17: Innovation through product maturity

At that point, competitors would shift to producing similar designs at lower costs and firms would start focusing on process innovation. Thus according to this model, process innovation

becomes important only later in the life of an industry. Yet, the assumptions of the model are not always applicable. It mostly focuses on cost reduction as the primary goal of process innovation, implying that firms have an incentive to develop new processes only in the intermediate phases of an industry's life, after opportunities for product innovations have been depleted and production volumes are sufficiently high to justify standardized processes.

However, there are many firms and industries where strong product and process development capabilities need to be enabled jointly. In advanced materials, biotechnology, pharmaceutical and semi-conductor industries, new products can often not be commercialized without breakthroughs in process technology. "Intel Corporation, as an example, is able to continually introduce ever higher-performing microprocessors because it has built strong capabilities to develop and scale-up the complex manufacturing processes required to produce these sophisticated devices" [19]. Pisano describes the relationship between product and process innovation and highlights the role of manufacturing process innovation in different types of industries (see Figure 18).

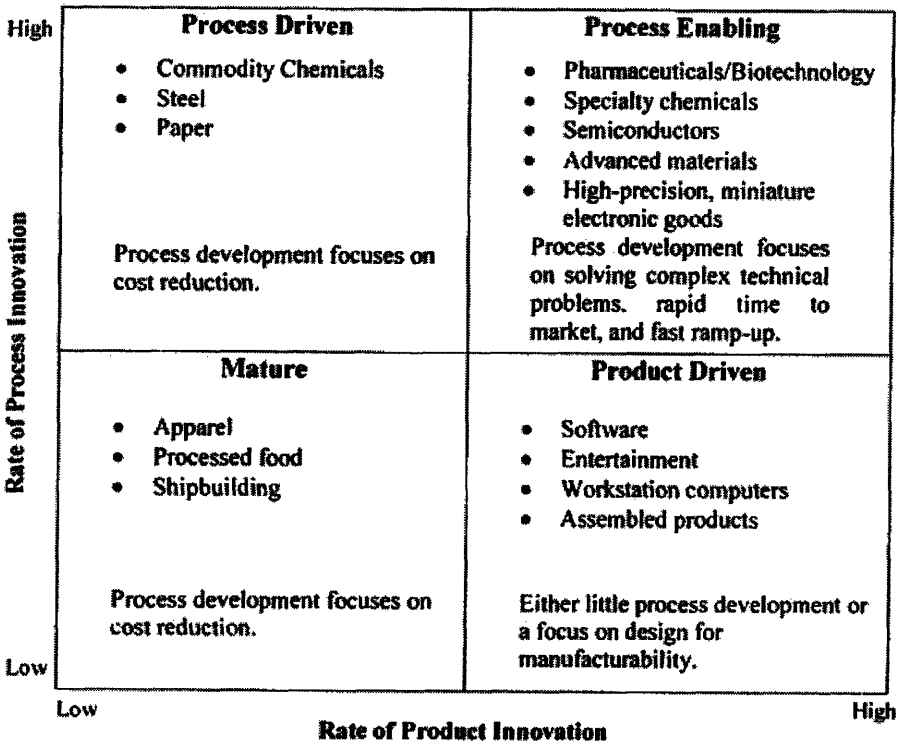

Figure 18[20]: Relationship between product and process innovation

The two left quadrants of the matrix represent the intermediate and mature phase of the product life cycle model, where there is a low rate of product innovation. In both quadrants, the role of manufacturing is to improve productivity and to adopt more efficient process technologies. The lower right quadrant (product driven) represents the initial phase, where product innovation is very strong but process technologies are quite stable. It is the upper right quadrant (Process Enabling) that is largely ignored by the product life cycle model. In this quadrant, the capability for fast, efficient and quality process development has direct impact on the commercial success of new products. As new products are designed, they are continually in need of new manufacturing capabilities and require a high degree of process development and innovation to be able to produce the system.

8.2. Purpose of Manufacturing Innovation

In this section, the importance of developing new manufacturing capabilities is presented in order to justify investment in manufacturing innovation. "Real leverage comes from an aggressive pursuit of process technology changes rather than a simple focus on operating existing technology better to increase volume and boost capacity utilization"[21]. Many industries are exposed to a multitude of factors that support and drive the adoption of new models of development. There is an increasing complexity of product technology, minimal sustainable competitive advantage and shorter product life cycles. By investing in manufacturing innovation early in the product development life cycle, a company can generate a number of competitive benefits:

- Difficulty to copy manufacturing technologies (more sustainable competitive advantage)
- Develop sophisticated technical problem solving capabilities
- Decrease time to market
- Faster manufacturing ramp-up
- Reduce risk and complexity of development

Pisano presents a model towards which companies must be headed in to develop new manufacturing capabilities (see Table 13).

	Conventional Model	New Model
Primary Goals	Reduce manufacturing costs of existing products	Proactive support of timely, efficient, and high-quality launches of new products
Technical focus	• Incremental process improvement • New capacity/ equipment/automation • Troubleshooting • Product modifications for enhanced manufacturability	• Exploration/development of new process architectures needed for new product designs
Product Development Role/Influence	Peripheral	• Central • Process developers as core members of product development teams
Customer	Plant	• Plant • R&D
Key Capabilities	• Process Engineering • In-depth knowledge of current manufacturing environment • Minimize product disruptions	• Process science • Ability to anticipate future manufacturing requirements • Responsiveness to project level uncertainty
Learning	Maximize learning curve *within* product/process generations	Capture learning *across* product/process generations
Metrics of Performance	Improvements in yield, cost, quality, and capital over the life of a product	• Improvements in *initial* yield, cost, quality, and capital across products • Lead time, efficiency, quality

Table 13: Two models of process development[20]

For companies following the new model (such as pharmaceutical and biotechnology companies), the process development organization becomes central to the development team, and requires early involvement in the product development stages. Its role becomes much more strategic, with the development of capabilities necessary to understand future manufacturing requirements and technologies associated with. Superior process development therefore reduces manufacturing costs, but it most importantly helps companies achieve faster time to market and a stronger proprietary position. As Hayes and Wheelwright mention, "proprietary processes are just as formidable competitive weapons as proprietary products, and more enduring competitive barriers are created when a firm couples product innovation with process innovation" [22].

8.3. Process innovation in the pharmaceutical and biotechnology industry

Pharmaceutical and biotechnology are process enabling industries that both face similar risks (rising costs of developments, increasing regulatory requirements and pressures on pricing). But the innovation process differs quite significantly on the process development side.

Traditional pharmaceutical use chemically synthesized organic molecules to create new compounds. In recent years, the pharmaceutical industry has turned into rational drug design, working backwards from a detailed knowledge of a disease's biochemistry to determine what chemical compounds can inhibit the chemical reaction involved. Process technologies development associated with drug discovery rest on chemistry and chemical engineering based activities. These fields have now well-established, highly articulated and formalized knowledge base. As a consequence, more and more commercial manufacturing processes can be developed through computer simulations and verified in small scale experiments to gather physical data needed for chemical studies of the process. Even though all potential issues are not solved in research laboratories, Gary Pisano showed that process development organizations could actually anticipate and respond to manufacturing issues without doing all the work in the plant. He characterized that the development time for a process technology was not correlated to how early or late it was transferred to a manufacturing environment (see Figure 19), and called this development concept as "learning before doing" [23].

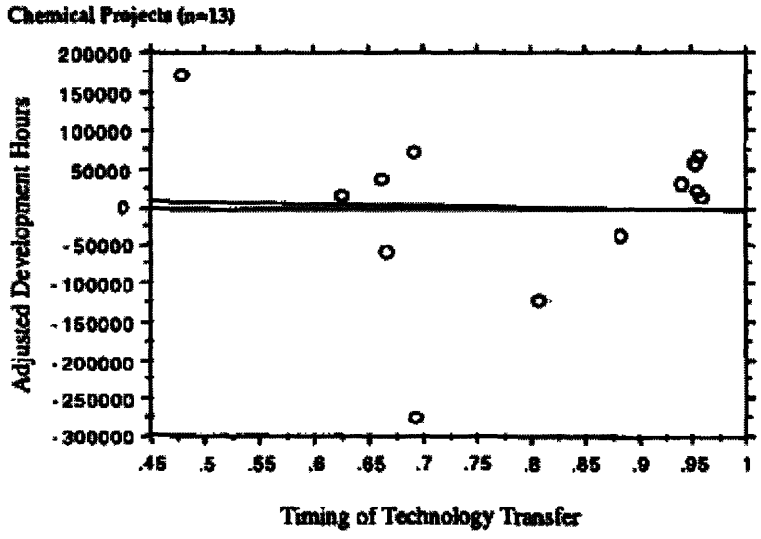

Figure 19: Relationship between development hours and technology transfer for chemical projects[23]

The biotechnology industry uses synthesized protein molecules to create new treatments. Diseases are studied at the cell level using molecular biology to identify potential treatments. Genetic engineering then allows scientists to manipulate the genetic structure of cells to produce certain proteins, thereby manufacturing the critical treatment. Contrary to chemistry and chemical engineering, the field of biotechnology process development and innovation is fairly new. Factors affecting biotechnological processes even at small scale are not well understood. In consequence, it is in general very difficult to predict commercial process performance from laboratory data. The best strategy in biotechnology for process technology development is therefore the "learning-by-doing" approach as demonstrated by Pisano[23] in his study. The earlier the technology transfer happens to a more scaled up environment (pilot and manufacturing scale equipment), the lower the development hours required. The regression in Figure 20 clearly shows the need for early technology transfer and "learning-by doing" methodologies.

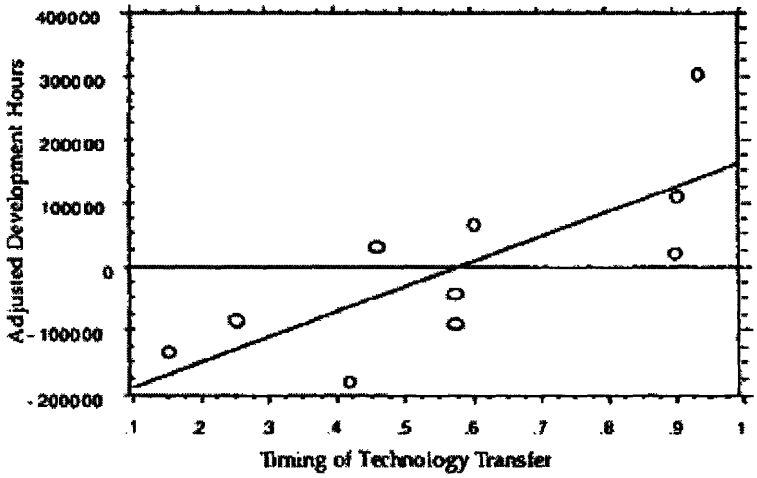

Figure 20: Relationship between development hours and technology transfer for biotech projects (n=10)[23]

8.4. Implications for biotechnology firms

The specifics of process innovation in biotechnology highlight some important implications for both process development and manufacturing strategy of firms in this industry.

8.4.1. Better understanding of scaling processes

As demonstrated by Gary Pisano, process innovation in biotechnology is characterized by the "learning-by-doing" methodology. This implies that physical plant and equipment must be installed long before a new product is approved, in order to support the process development activities. Extensive site specific characterization studies are conducted to support the technology transfer, often requiring several full scale manufacturing runs worth millions of dollars. With the current financial pressures on the big biotechnology firms, it becomes necessary for process development organizations in the industry to focus on better understanding and anticipating scaling problems. Laboratory models that could address process scaling issues would be beneficial in reducing human and material resources requirements, development times and full scale runs at the final manufacturing sites.

8.4.2. Platform technologies

With the exponential growth seen in the biotechnology industry in the last 15 years, the most important role of Operations organizations during that period was to build enough capacity and safely produce enough products to meet the increasing demand requirements for blockbuster protein drugs. This evidently resulted in differences between facilities in existing equipments (design, scale and specifications), facility design and procedures. With the current shift in the industry towards lean manufacturing practices, the development and implementation of standardized technologies and practices across the manufacturing network of a biotech firm becomes critical to ensure smooth technology transfer and rapid production ramp-up for new products, as well as facilitating the transfer of commercial production drugs from one site to another.

8.4.3. Flexible manufacturing plants

Process development is an essential competitive advantage for a biotechnology firm in terms of strategic expertise and potential for stronger proprietary position on the market. It implies

early and risky investments in additional commercial manufacturing capacity to support process development in the factory. In recent years, it led some companies to more and more utilize outside partners or contractors for manufacturing. It could however be a risky strategy in an industry where feedback from production to R&D is critical to improve the scientific knowledge about the technology used. The development of in-house flexible and multi-purpose manufacturing plants, as currently seen in the industry, is a strategic answer to the capital risks required for early technology transfer and the future risks of loosing process expertise to biotechnology contract manufacturers.

9. CONCLUSION

Recombinant protein manufacturing is very complex, with product and process designs highly interdependent, and where changes in process technology can have a significant impact on product characteristics. Process development capabilities are strategic to succeed in this highly uncertain industry. Developing new technologies require a deep analysis before envisioning any implementation in the manufacturing environment. In this thesis, we evaluated the possibility of using drying technologies to store drug substance in a powder form rather than the current frozen state obtained using cryopreservation methods. Through the development and optimization of process cycles and analyses of the drug substance in the dry and liquid form, we were able to demonstrate at lab scale that we could meet a set of predefined physical and chemical criteria indicating that drying technologies could become a valid technology to use for drug substance storage. However, further analyses based on financial estimates and on manufacturing aspects showed that drying technologies were not competitive at this point compared to existing cryopreservation technologies.

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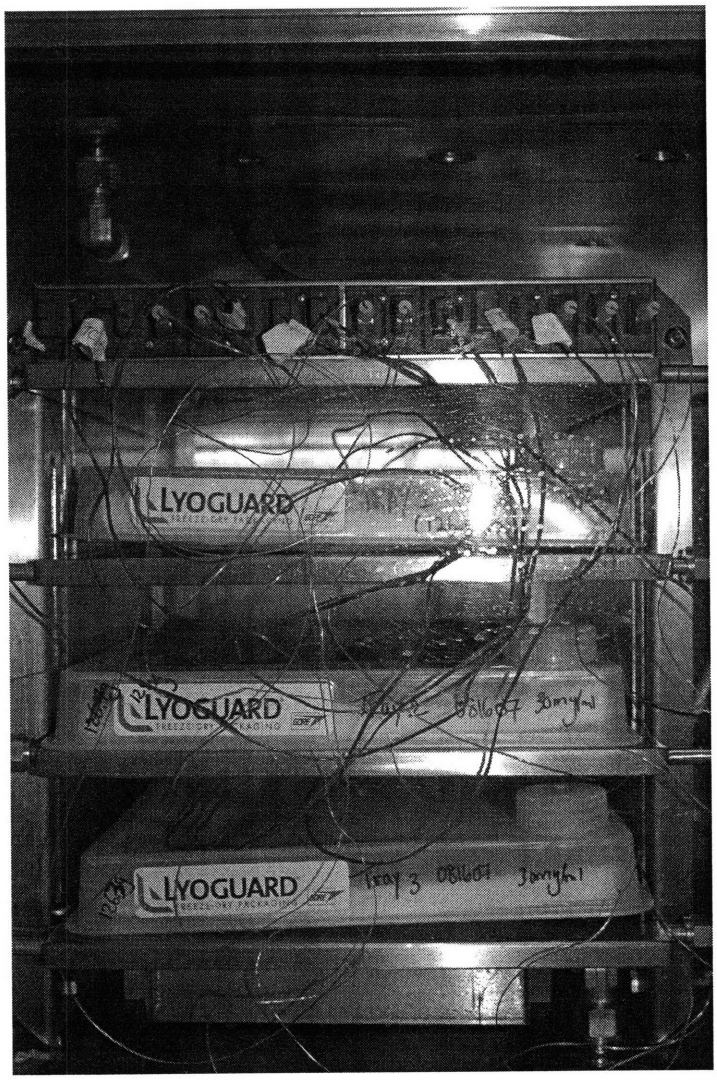
11. APPENDICES

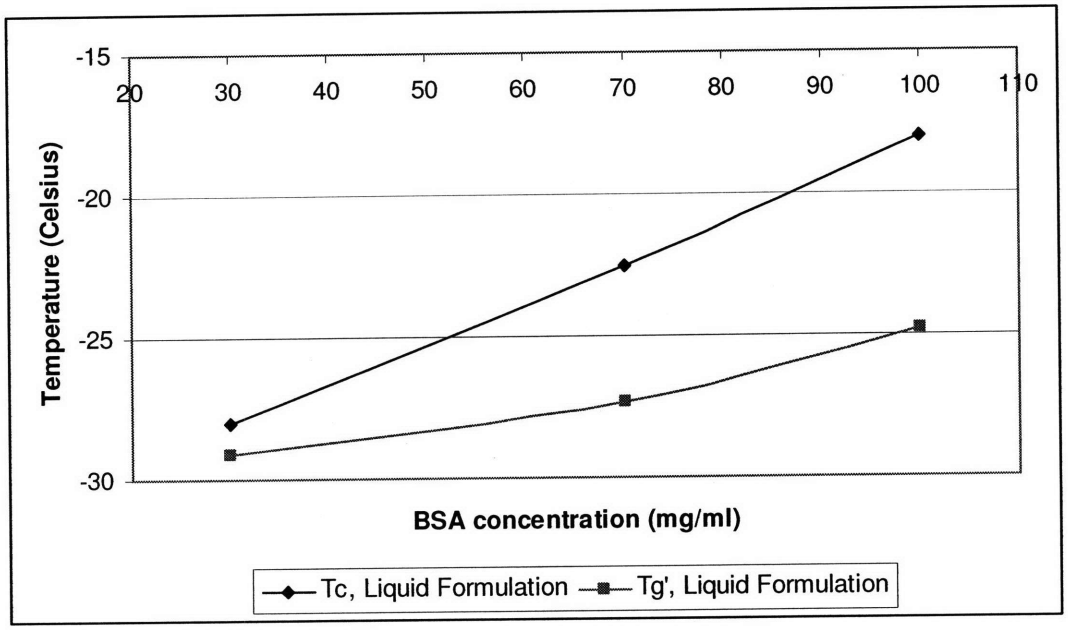
Appendix 1



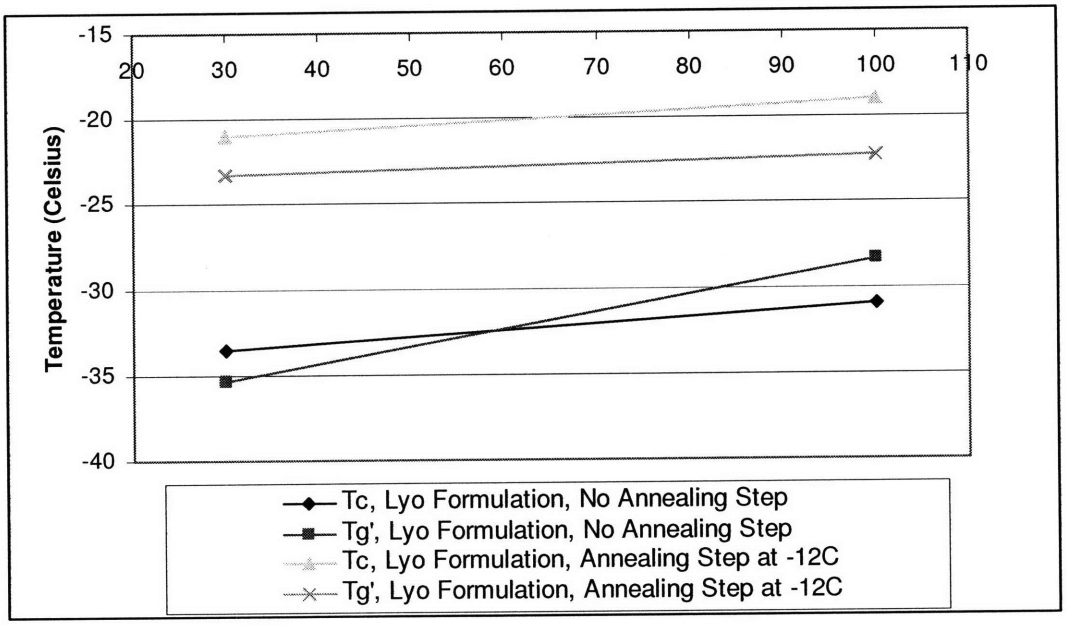
Disposable trays in the lab scale drying chamber at the end of the freeze drying cycle

Equipment characteristics: 316L drying chamber with a capacity of 3 shelves

Appendix 2



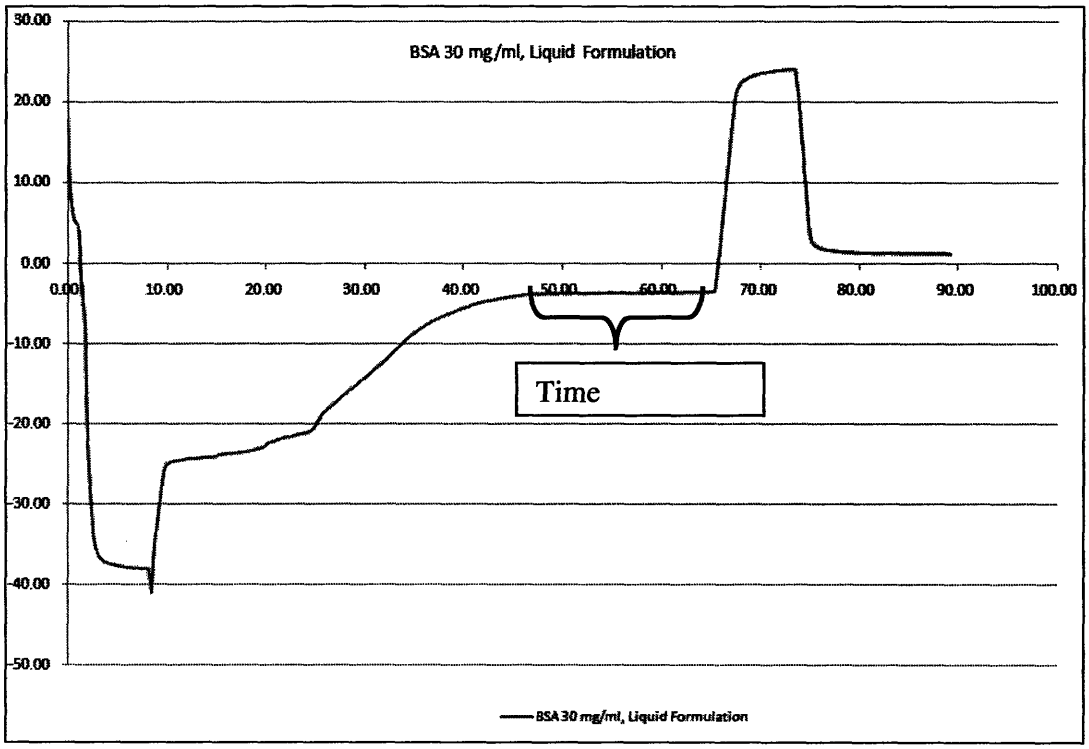
Tc and Tg' values for a liquid formulation containing BSA at different concentrations



Tc and Tg' values for a lyophilization formulation containing BSA at different concentrations

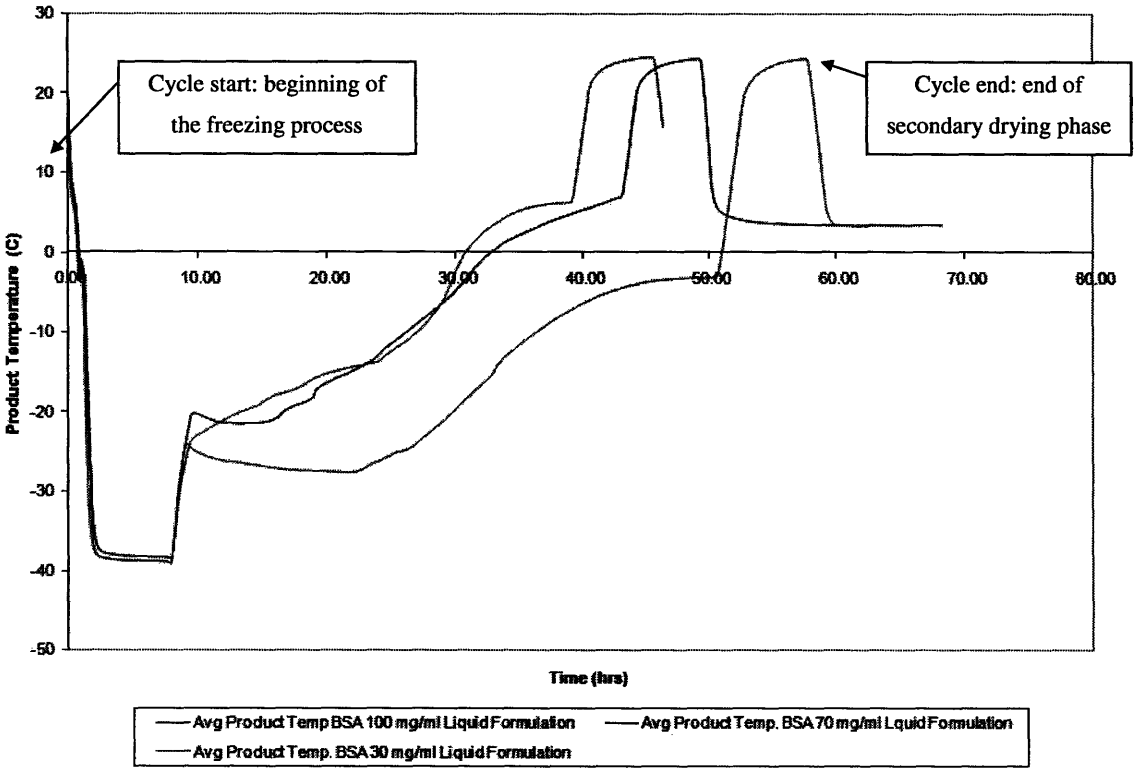
Appendix 3

Initial Freeze drying cycles parameters

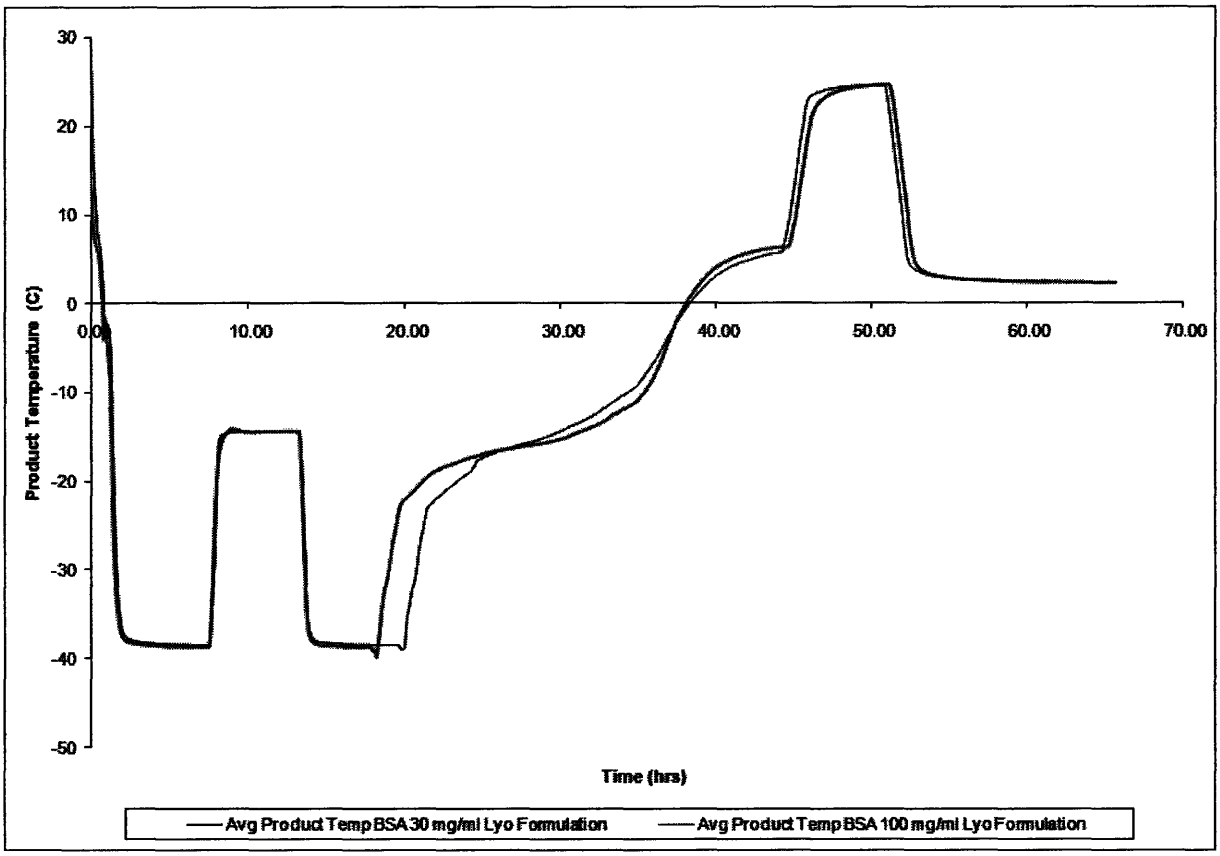


Freeze drying cycle, BSA 30 mg/ml, Liquid formulation, Run 3

Appendix 4

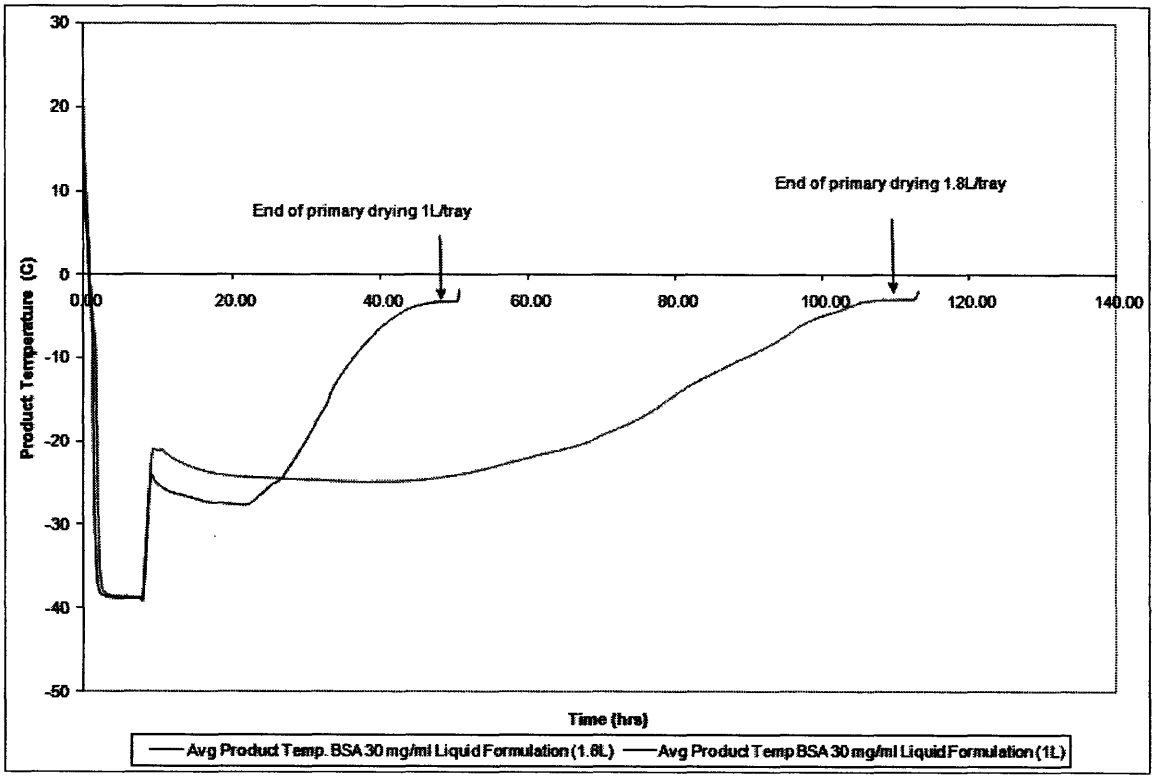


Optimized freeze drying cycles, liquid formulations



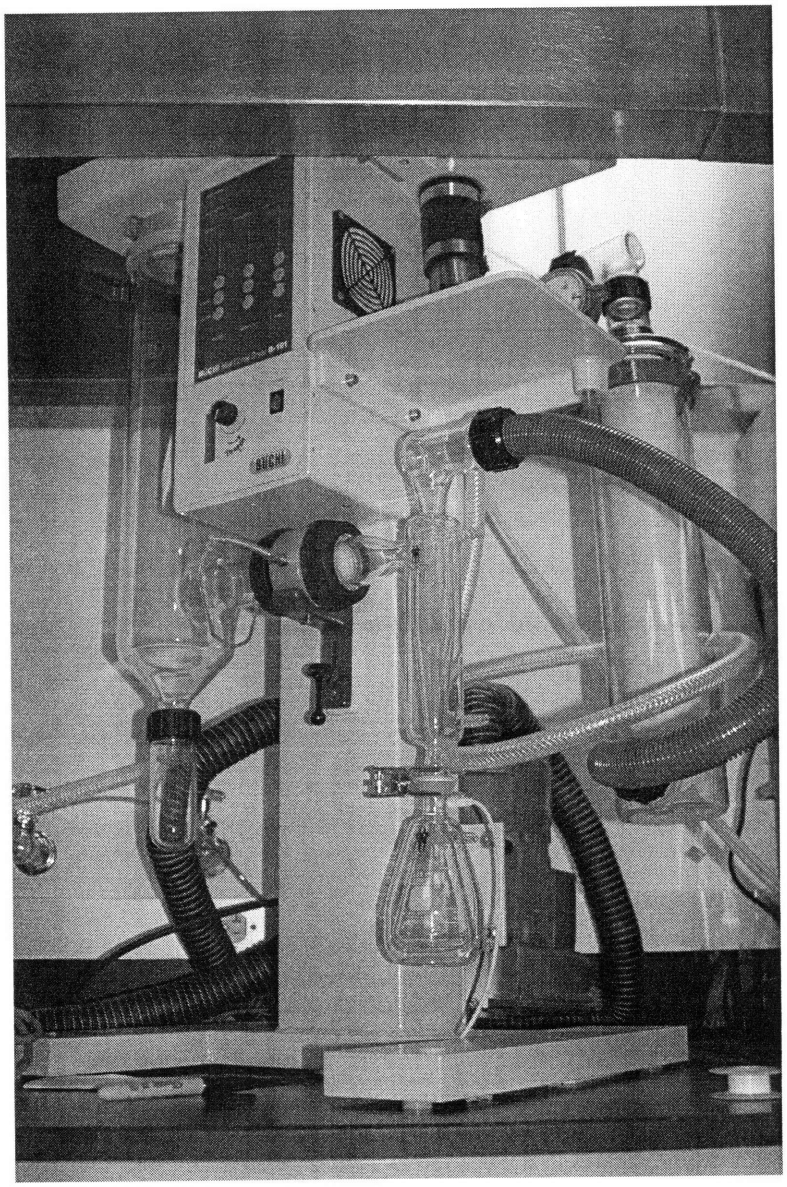
Optimized freeze drying cycles, lyophilization formulation

Appendix 5



Effect of tray volume fill on primary drying duration

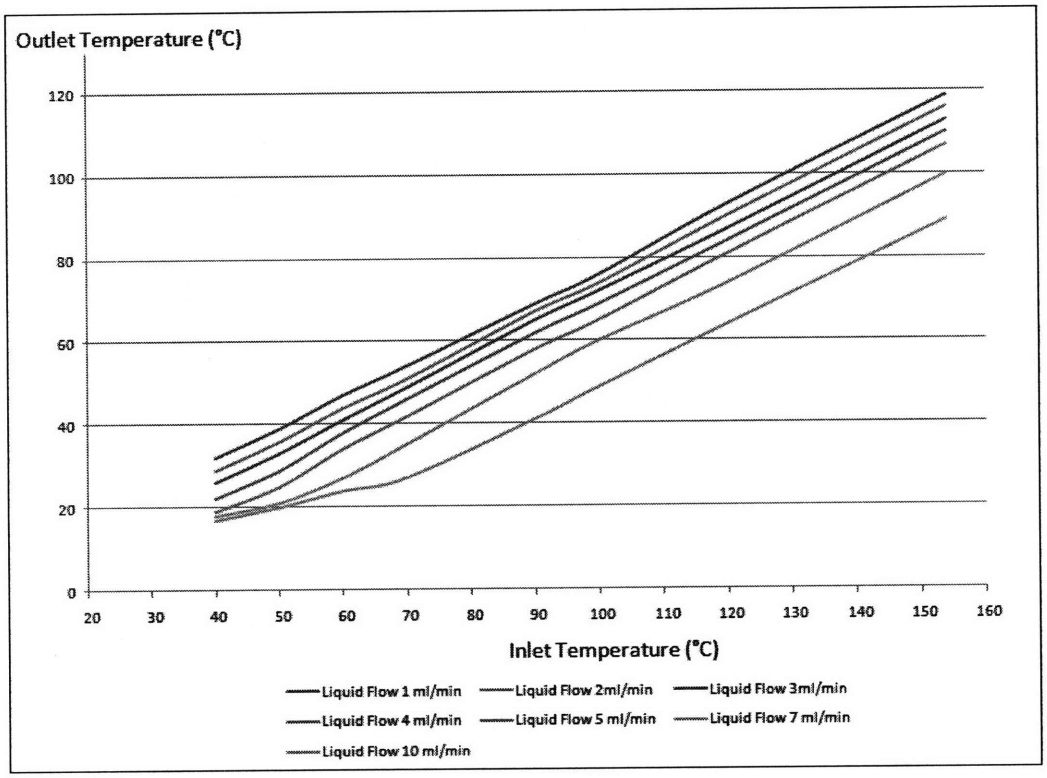
Appendix 6



Lab scale spray dryer used for the evaluation

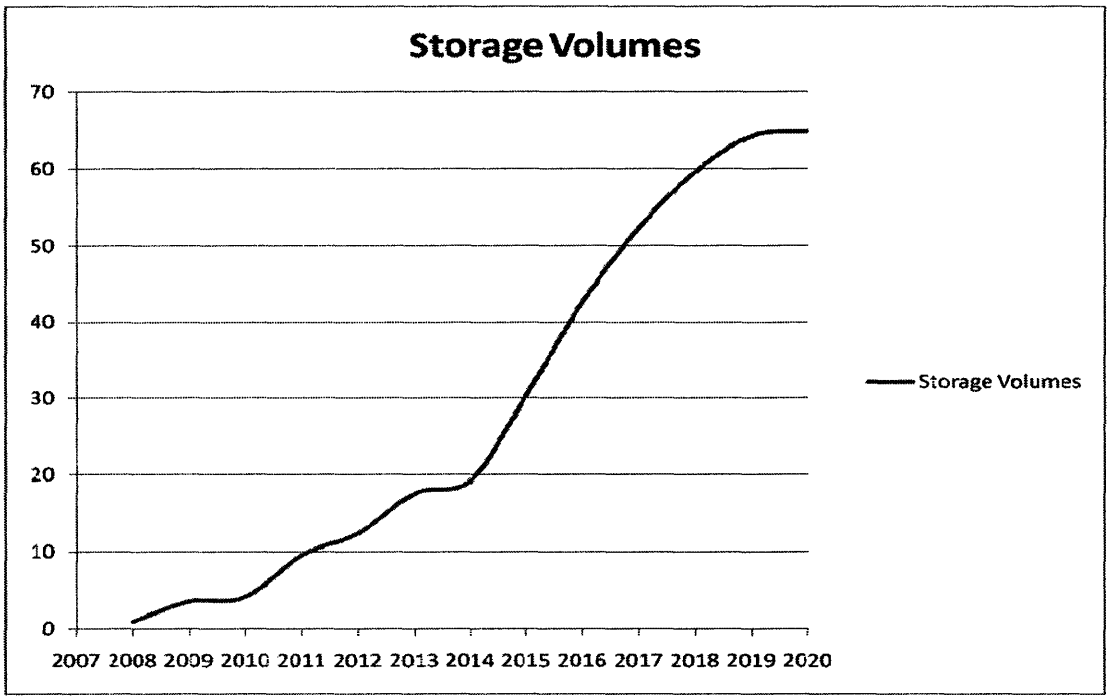
(maximum air flow of 35 m^3/hour and maximum evaporation of 1 liter/hour)

Appendix 7



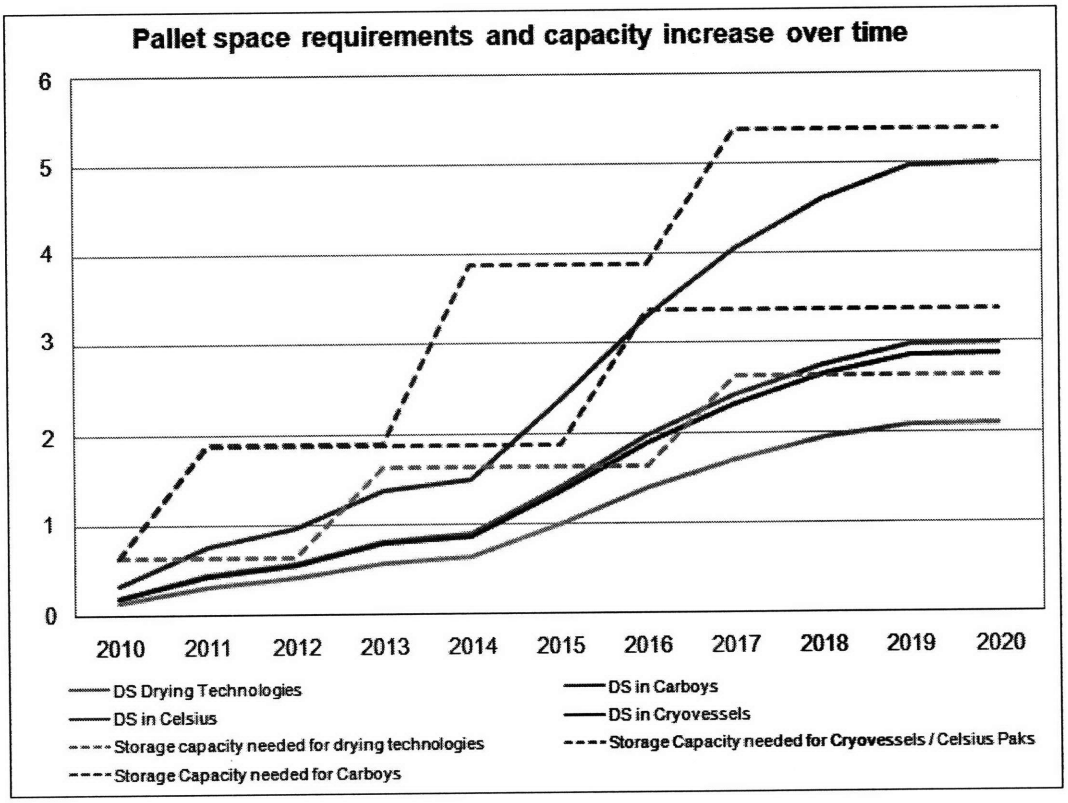
Mass Flow analysis – Lab scale spray dryer

Appendix 8



Drug substance storage volume over time
(Numbers have been indexed to the 2008 volumes to protect sensitive information)

Appendix 9



(Numbers have been indexed to 2010 capacity to protect sensitive information)