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Review

Innovative Drying Technologies for Biopharmaceuticals

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ABSTRACT

In the past two decades, biopharmaceuticals have been a breakthrough in improving the quality of lives of patients with various cancers, autoimmune, genetic disorders etc. With the growing demand of biopharmaceuticals, the need for reducing manufacturing costs is essential without compromising on the safety, quality, and efficacy of products. Batch Freeze-drying is the primary commercial means of manufacturing solid biopharmaceuticals. However, Freeze-drying is an economically unfriendly means of production with long production cycles, high energy consumption and heavy capital investment, resulting in high overall costs. This review compiles some potential, innovative drying technologies that have not gained popularity for manufacturing parenteral biopharmaceuticals. Some of these technologies such as Spin-freeze-drying, Spray-drying, Lynfinity® Technology etc. offer a paradigm shift towards continuous manufacturing, whereas PRINT® Technology and Microglassification™ allow controlled dry particle characteristics. Also, some of these drying technologies can be easily scaled-up with reduced requirement for different validation processes. The inclusion of Process Analytical Technology (PAT) and offline characterization techniques in tandem can provide additional information on the Critical Process Parameters (CPPs) and Critical Quality Attributes (CQAs) during biopharmaceutical processing. These processing technologies can be envisaged to increase the manufacturing capacity for biopharmaceutical products at reduced costs.

1. Introduction

Biopharmaceuticals, composed of either peptides, proteins, glycoproteins and nucleic acids or composite combinations of biomolecules, include antibodies, antibody-drug conjugates, recombinant proteins, nanobodies, enzymes, hormones, vaccines and gene therapy products etc. (Gervasi et al., 2018; Walsh, 2010). The stability of protein-based biopharmaceuticals can be negatively affected by temperature, moisture, prolonged storage, denaturants, organic solvents, shear, oxygen, changes in pH etc. (Declerck, 2012; Li et al., 2015). Protein aggregation is one of the major challenges that impacts the CQAs of biopharmaceutical products (Wang and Roberts, 2018). This can be encountered at any stage of the manufacturing process to the administration of drugs.

Parenteral biopharmaceuticals are marketed as either liquid or solid products manufactured by various fill finish technologies (Martagan et al., 2020). With about 34 % of biopharmaceuticals marketed as Freeze-dried products in the European market (Gervasi et al., 2018), dehydration via Freeze-drying is the gold standard for manufacturing solid biopharmaceuticals. Several advantages of Freeze-drying have been identified. Freeze-drying allows improved shelf-life of heat-

Abbreviations: ADH, Alcohol dehydrogenase; API, Active Pharmaceutical Ingredient; BSA, Bovine Serum Albumin; cGMP, current Good Manufacturing Practices; CPP, Critical Process Parameters; CQA, Critical Quality Attributes; DNase, Deoxyribonuclease; DPPC, Dipalmitoyl phosphatidylcholine; DP, Drug Product; DSPC, Distearoyl phosphatidylcholine; DS, Drug Substance; DLS, Dynamic Light Scattering; ELP, Elastin-like Polypeptide; FDA, Food and Drug Administration; FDKP, Fumaryl diketopiperazine; FTIR, Fourier Transform Infrared Spectroscopy; IgE, Immunoglobulin E; IgG, Immunoglobulin G; IR, Infrared; IVIG, Intravenous Immunoglobulin; LDH, Lactate dehydrogenase; mAb, Monoclonal Antibody; MS, Mass Spectrometry; PAT, Process Analytical Technology; PFPE, Perfluoropolyether; PCA, Polycyano acrylate, PVA, Polyvinyl alcohol; QbD, Quality by Design; QC, Quality Control; rhGH, recombinant Growth Hormone; RMC, Residual Moisture Content; RTD, Resistance Temperature Detector; SEC, Size Exclusion Chromatography; ssHDX-MS, solid-state Hydrogen Deuterium Exchange – Mass Spectrometry; siRNA, small interfering Ribonucleic Acid; SSA, Specific Surface Area; ssPL, solid-state Photolytic Labelling; SRCD, Synchrotron Radiation Circular Dichroism Spectroscopy; TOSAP, Temperature-controlled organic assisted precipitation.

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sensitive and labile biopharmaceuticals along with a reduced requirement for cold chain (Carpenter and Chang, 1996; Tang and Pikal, 2004). Chemical decomposition is reduced at very low temperatures during the process, with low residual moisture content (RMC) post-processing, keeping the product stable (Matejtschuk, 2007). However, several drawbacks associated with conventional Freeze-drying have been reported. Firstly, processing times could vary from days to weeks (Nail and Gatin, 1993; Tang and Pikal, 2004) with high capital costs (Stratta et al., 2020) and high energy consumption leading to reduced efficiency in the overall process (Bando et al., 2017; Liapis and Bruttini, 2008; Liu et al., 2008). Secondly, being a static process, it increases the risk of rejection of the entire batch in case of process failure. A batch process is limited to processing small volumes of unit doses which increases the requirement for a large number of vials. Human intervention during the process can increase the risk of contamination despite current Good Manufacturing Practices (cGMP) (FDA, 2014a). Thirdly, concerns related to heat and mass transfer and scaling up the process have been discussed which depend on the dryer design (Barresi et al., 2010), container closure, load condition and product formulation etc. (FDA, 2014a; Patel and Pikal, 2011). Vials situated on the edge of the shelves dry faster than vials present in the center due to radiation from the chamber walls which causes issues of vial to vial heterogeneity (Rambhatla and Pikal, 2003). Furthermore, certain high concentration Freeze-dried protein cakes can take very long to reconstitute compared to free-flowing powders produced by alternative drying techniques (Cao et al., 2013; Dani et al., 2007).

Though having not gained widespread acceptance in the biopharmaceutical industry, some potential, continuous drying technologies open avenues for research to combat challenges associated with conventional Freeze-drying technology. Readers are referred to the cited reviews herein for detailed information on other continuous Freeze-drying technologies (Adali et al., 2020; Ishwarya et al., 2015; Pisano, 2020; Pisano et al., 2019). This review encompasses some innovative drying technologies, namely, Spin-freeze-drying, continuous Freeze-drying of suspended vials, Active-freeze-drying, Spray-freezing and Dynamic Freeze-drying, Lynfinity® Technology, Spray-drying, PRINT® Technology and Microglassification™ for biopharmaceuticals along with some considerations for the selection of a drying process. A variety of biopharmaceutical characterization techniques (section 3) and PAT (section 5) in tandem with drying processes can provide an in-depth assessment of product CQAs and process CPPs, ensuring safety, quality and efficacy of products before being delivered to patients. Moreover, formulation components play an important role in conferring biopharmaceutical stability and the rationale of choosing excipients specific to the drying process has been discussed in section 4. In addition to the above considerations, some of the alternative drying technologies can reduce complexities associated with validation processes and also allow successful scale-up with a Quality by Design (QbD) approach (section 6). Some alternative drying technologies have been summarized in Table 1. While some of the technologies are categorized as bulk drying technologies, these technologies can also be employed to produce single unit doses via an integrated powder/product filling system (Dalton Pharma, 2021; Nova Laboratories, 2015).

2. Biopharmaceutical Drying Technologies

Some of the single dose and bulk drying technologies have been discussed in sub-section 2.1 and sub-section 2.2, respectively.

2.1. Single Dose Drying Technologies

2.1.1. Conventional Batch Freeze-drying

A batch Freeze-drying process involves the removal of solvent, typically water, from a solution based on the principle of sublimation. A typical batch Freeze-dryer consists of a drying chamber with multiple shelves, a condenser and a vacuum pump. The process of Freeze-drying consists of 3 major steps: freezing, primary drying and secondary drying. Fig. 1 depicts a laboratory-scale Freeze-drying cycle. Vials containing the desired volume of liquid product are partially stoppered and loaded into the drying chamber. Freezing of the product is carried out at very low temperatures ranging between – 40 to – 60 °C to ensure solidification below the eutectic point (T_m) of crystalline components or below the glass transition temperature of the frozen product (T_g') of amorphous materials (Pisano, 2019). The average product temperature, in a non-cGMP Freeze-drying cycle, can be monitored using thermocouples and other wireless temperature probes (Wang and McCoy, 2015). Annealing is an optional, additional step performed to crystallize bulking agents and to improve product homogeneity (Al-Hussein and Gieseler, 2012; Pisano, 2019). Post freezing, primary drying involves the transformation of frozen water into water vapor below its triple point. Partially stoppered vials allow the migration of water vapor from the vials to the condenser. Increased shelf temperature at a lower chamber pressure is ideal for sublimation, however, the product temperature is maintained below its T_g' or T_m (Tang and Pikal, 2004). The final step, secondary drying, is also known as the desorption phase. This phase is carried out to reach the optimum residual moisture level (Wang et al., 2015). While the bound water molecules are removed at much higher temperatures, the product temperature during the secondary drying phase is maintained below its solid-state glass transition temperature (T_g). Post secondary drying, all vials are typically backfilled with sterile nitrogen gas and stoppered and capped with silicone stoppers and aluminium cover seals. All vials undergo inspection before they are finally released and shipped.

2.1.2. Spin-freeze-drying of Unit doses

The method of Spin-freezing was first patented by Becker in 1957, patent no. DE967120 (Becker, 1957). This method was then employed for Freeze-drying and patented by Broadwin in 1965, patent no. US3203108A and Oughton et al. in 1999, patent no. US5964043 (Broadwin, 1965; Oughton et al., 1999). With modifications to the patents and to the conventional Freeze-drying process, authors invented a novel continuous Freeze-drying process for unit doses (Corver, 2012; De Meyer et al., 2015). A major characteristic of this continuous Freeze-drying technology is the rotation of vials containing the liquid product of interest along their longitudinal axis, therefore, this is known as Spin-freeze-drying (Fig. 2).

The process begins with the continuous Spin-freezing step wherein vials containing the liquid product are spun rapidly, typically at 2500 – 3000 rpm, along their longitudinal axis for a period of time. The axial rotating motion results in the formation of a dispersion layer on the inner walls of the vial with a relatively uniform thickness of 1 mm (Corver, 2012). Subsequently, the rotating vials are exposed to a flow of sterile cryogenic gas, such as nitrogen or carbon dioxide, which is temperature controlled. As the frozen product is spread all over the inner walls of the vial, this results in a large surface area, thereby, allowing fast and homogenous freezing/heating of the dispersion layer (De Meyer et al., 2015). The process of solidification takes about 1 – 2 min and the product is typically subjected to a temperature between – 40 °C and – 60 °C for another 10 – 20 min (Corver, 2012). To achieve crystallization and the desired morphology of the excipients, further modifications are made to the cooling process conditions in a temperature-controlled chamber. Following the cooling step, the vials are transferred to the primary drying chamber through a conveyor belt system. Each vial is held in a heat conducting jacket or a pocket in the chamber with the desired pressure and temperature conditions. The jacket surrounds the outer surface of the vials to facilitate homogenous distribution of heat through conduction or radiation. Subsequently, the vials are transferred to the secondary drying chamber for the desorption of residual water. Fig. 3 depicts a schematic for the continuous drying system. The drying step lasts for about 30 min to 2 h (Corver, 2012). It was reported that the total processing time is reduced by 10 – 40 times depending on the vial dimensions and the product formulation (De Meyer et al., 2015).

Table 1
Potential drying technologies for biopharmaceutical application.

Drying Technology	Key Features	Molecule / Biomolecule Processed	Batch / Continuous processing	Product Yield	Achievable RMC (w/w)	Dry Product Characteristics	Potential Process-induced Stress	Aseptic Equipment Manufacturer	References
Single Dose Drying Technologies									
Batch Freeze-drying	Gentle drying, improved shelf-life, reduced cold chain requirement.	A wide range of enzymes, antibodies, hormones, vaccines, gene therapy products etc.	Batch.	$\geq$ 98 %	$\leq$ 1 %	• Intact, porous cake. • Cake surface area ~ 300 – 400 mm^2.	• Cold denaturation. • Ice-liquid interfacial denaturation.	SP Scientific, IMA Life, GEA Lyophil, Optima Pharma, MillRock Technology.	(De Beer et al., 2009; Ganguly et al., 2018; Gervasi et al., 2018; Harguindeguy and Fissore, 2021; Nail et al., 2017; Tang and Pikal, 2004).
Spin-freeze-drying	Spinning on longitudinal axis, large surface area and heat/mass transfer, no shear stress.	Alcohol dehydrogenase, IVIG.	Continuous.	Not available.	$\leq$ 1 %	• Intact dry layer along the inner surface of vials. • Dry layer surface area ~ 2500 mm^2.	•Cold denaturation. • Ice-liquid interfacial denaturation.	Prototype available by RheaVita.	(Corver, 2012; De Meyer et al., 2015; Lammens et al., 2018; Vanbillemont et al., 2020a).
Continuous Freeze-drying of suspended vials	Controlled nucleation via VISF and homogeneous heat transfer, continuous flow of vials.	Aqueous solutions of sucrose and mannitol.	Continuous.	Not available.	~ 1 %	• Intact, porous cake.	• Cold denaturation. • Ice-liquid interfacial denaturation.	Not available.	(Capozzi et al., 2019).
Foam drying in vials	Rapid evaporation/ boiling at low vapour pressure and ambient temperature, no freezing required, lower energy consumption.	Vaccines, rhumAb, bacteria.	Batch.	Not available.	> 1 – 3 %	• Dry foam structure. • Lower specific surface and lower water desorption rate.	• Stress due to surface tension and cavitation.	Not available.	(Abdul-Fattah et al., 2007; Ohtake et al., 2011a, 2011b)
Microwave vacuum drying	Rapid dehydration, reduction in freeze-drying cycle time by $\geq$ 80 %, comparable product appearance and enzyme activity.	Haemoglobin, catalase, live virus vaccine.	Semi-continuous.	Not available.	~ 1.8 %	• Dried cake with an average SSA of 1.52 m^2/g comparable to the SSA of freeze-dried (1.61 m^2/g) counterpart.	• Cold denaturation during flash freezing. • Thermal denaturation due to plasma discharge at high electromagnetic field intensity. • Heterogenous heating and arcing may cause burning of product.	EnWave.	(Bhambhani et al., 2021; Durance et al., 2020; EnWave, 2021).
Bulk Drying Technologies									
Active-freeze-drying	Bulk processing of product, free flowing powder, small particle size, improved properties with continuous stirring for certain products.	Ketoconazole.	Batch.	85 – 94 %	< 0.5 %	• Free-flowing powder. • Particle size: 1 – 100 μm.	• Stress due to continuous stirring by impeller. • Ice-liquid interfacial denaturation. • Cold denaturation.	Hosokawa Micron B.V.	(Hosokawa Micron, 2019; Touzet et al., 2018; Van Der Wel, 2012).
Spray-drying	Continuous and rapid drying, particle engineering, free-flowing powder, low energy, and equipment cost.	Raplixa®, Exubera®, Lysozyme, BSA, mAbs, siRNA etc. and some other biologics listed in Table 2.	Continuous.	> 50 – 95 %	$\gtrsim$ 1 – 2 %	• Free-flowing powder. • Particle size: 300 nm – 100 μm.	• Shear due to atomization. • Air-liquid interfacial denaturation. • Thermal denaturation due to residence time at high outlet temperatures.	SPX Flow Technologies - Anhydro, GEA Niro, Fluid Air, Ohkawara Kakohi Co. Ltd.	(Bowen et al., 2013; FDA, 2015a; Silva et al., 2013; Uddin et al., 2021; Vehring, 2008; Vehring et al., 2020; Walters et al., 2014; Wang et al., 2018; White et al.,

(continued on next page)

Table 1 (*continued*)

Drying Technology	Key Features	Molecule / Biomolecule Processed	Batch / Continuous processing	Product Yield	Achievable RMC (w/w)	Dry Product Characteristics	Potential Process-induced Stress	Aseptic Equipment Manufacturer	References
									2005; Wu et al., 2019; Ziaee et al., 2020).
Spray-freezing and Dynamic Freeze-drying	Uses frequency driven prilling nozzle. Primary, secondary drying occurs in rotary freeze drying chamber.	mAb.	Continuous.	> 97 %	< 1 %	• Free-flowing powder. • Particle size: < 300 μm – 1000 μm.	• Shear due to frequency nozzle and atomization. • Cold denaturation.	Meridion Technologies.	(Lowe et al., 2018; Luy et al., 2018; Struschka et al., 2016).
Lynfinity® Technology	Uses piezoelectric spray nozzle to produce dried spheres. Primary, secondary drying occurs on cascading vibratory shelves.	Not available.	Continuous.	Not available.	≤ 1 %	• Free-flowing powder. • Particle size: ~ 600 μm.	• Shear due to piezoelectric nozzle and atomization. • Cold denaturation.	IMA Life.	(IMA Life, 2019; DeMarco and Renzi, 2015).
PRINT®	Tunable shape, size, and morphology of nano and microparticles, enhanced surface properties and API bioavailability, large-scale production is possible.	Lysozyme, BSA, DNase, IgG, siRNA, ribavirin, vaccines.	Continuous.	Not available.	Not available.	• Free-flowing powder. • Particle size: 2 – 200 μm. • Customizable particle shape and morphology.	• Cold denaturation. • Roller pressure may induce shear stress.	Liquidia Corporation.	(DeSimone, 2016; Galloway et al., 2013; Garcia et al., 2012; Kelly and DeSimone, 2008; Wilson et al., 2018; Xu et al., 2013).
Microglassification™	Controlled particle size, morphology, release and dissolution, no excipients used.	BSA, lysozyme, α-chymotrypsin, catalase, horseradish peroxidase, ELP.	Batch.	Not available.	Assumed comparable to Freeze-drying by the authors.	• Particle beads. • Particle size: 1 - > 10 μm.	Not available.	Not available.	(Aniket et al., 2015a, 2015b, 2014).
Electrospinning	Gentle, rapid drying (~0.1 s) and reconstitution due to high surface area. Ultra-fine particles by electrical atomization. Requires viscous solutions. Comparable product stability.	Infliximab, zein, siRNA, inulin, β-galactosidase, lysozyme.	Continuous.	~ 80 %	~ 6.5 %	• Dry particles size: < 10 μm.	• Shear due to electrical atomization.	Bioinicia.	(Abraham et al., 2019b; Domján et al., 2020; Jain et al., 2014; Karthikeyan et al., 2015; Wagner et al., 2015).
Supercritical Fluid drying	Rapid drying, drying can occur via Spray-drying in the presence of supercritical fluid (e.g., CO_2) at low temperatures (>32° C) and pressure (~103 Bar) or via supercritical antisolvent precipitation. Particle engineering possible, no freezing required.	Albumin, alkaline phosphatase, catalase, chymotrypsin, insulin, lactase, rhDNase, trypsin, urease, lysozyme, myoglobin, IgG, LDH.	Continuous.	Not available.	≳ 1 %	• Free-flowing powder. • Micron-sized particles (≥200 nm – 50 μm).	• Shear due to atomization. • Fluid-fluid interfacial denaturation. • High pressure denaturation.	Extratex, Natex, Separex.	(Bouchard et al., 2007; Jovanović et al., 2008a, 2008b, 2004; Long et al., 2019; Sellers et al., 2001).

API, Active Pharmaceutical Ingredient; BSA, Bovine Serum Albumin; DNase, Deoxyribonuclease; ELP, Elastin-like Polypeptide; IgG, Immunoglobulin G; LDH, Lactate dehydrogenase; mAb, monoclonal antibody; rhDNase, recombinant human Deoxyribonuclease; siRNA small interfering Ribonucleic Acid; SSA, Specific Surface Area.

De Meyer et al. showed that the freezing method significantly contributed to the sublimation kinetics of the Spin-frozen vials compared to static batch freezing (De Meyer et al., 2015). This was due to the fact that the presence of a thinner product layer and large surface area in the inner walls of the vials contributed to much higher sublimation rates. Amongst the other factors studied, increasing the shelf temperature and the chamber pressure increased the sublimation rates, however, the freezing rate and the fill volume did not have a significant impact on the sublimation rates post 2 h of Spin-freeze-drying and batch Freeze-drying (De Meyer et al., 2015). Furthermore, it was shown that the shelf temperature and chamber pressure were observed to have similar effects on the sublimation rates of Spin-frozen vials post 2 h of

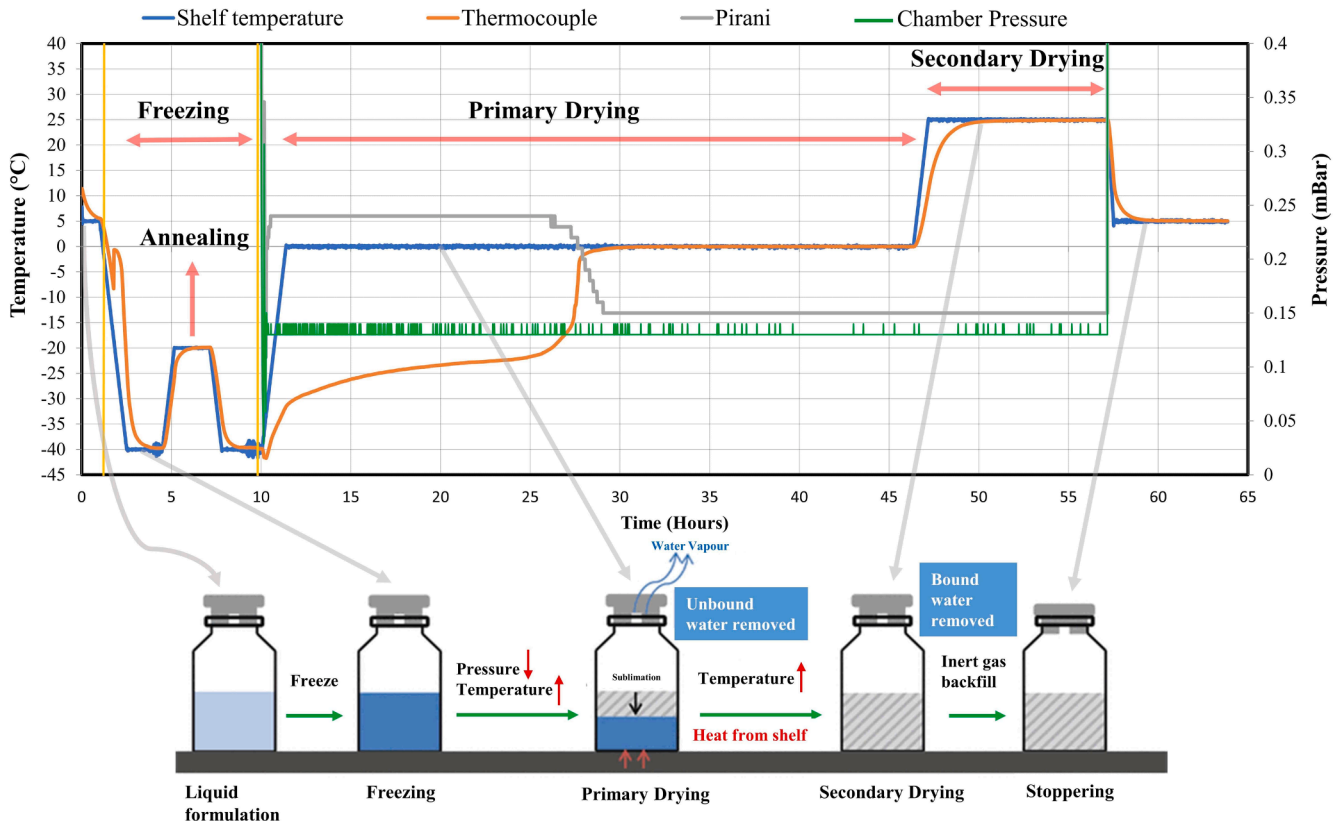

Fig. 1. Schematic evolution of a Freeze-drying cycle. Adapted from (LyophilizationWorld, 2020).

primary drying but shelf temperature was reported to be the most significant factor on the rate of sublimation of Spin-frozen vials when there was an adequate contact between the vial and the shelf. The effect of chamber pressure, compared to the shelf temperature, was much lower for the batch frozen vials. This was again due to the fact that that the product surface area on the inner walls of the vial was much greater for Spin-frozen vials (2533 mm^2) than the traditionally frozen vials (373 mm^2) (De Meyer et al., 2015).

Through another study, the effects of shear, sedimentation and diffusion velocity were studied on the stability of Alcohol dehydrogenase (ADH) during Spin-freeze-drying (Lammens et al., 2018). The calculated shear rates at 2900 rpm, 800 rpm and 400 rpm for 2 – 4 min were 2145 s^{-1}, 591 s^{-1} and 295 s^{-1}, respectively. These values were comparatively lower than the shear rates (4000 s^{-1} – 20,000 s^{-1}) generated during some processes such as cross-flow filtration and lobe pumping (Bee et al., 2009; Gomme et al., 2006; GE, 2014), indicating that Spin-freezing would not negatively impact ADH. It is worthwhile understanding that the liquid in the vial experiences maximum shear when the vial is accelerated from rest. As the vial attains the maximum desired rotational velocity, the relative rotational velocity of the vial with respect to the liquid reduces, thereby, reducing the shear rate. This is analogous to a person sitting in a moving aircraft experiences negligible force with respect to the aircraft. However, further product-specific evaluation is required to study the effect of Spin-freezing on labile biopharmaceuticals. Moreover, no significant loss in the activity of ADH and the absence of permanent aggregates in the Dynamic Light Scattering (DLS) results confirmed that the shear rate experienced during Spin-freezing did not affect the stability of ADH. Furthermore, inhomogeneity associated with sedimentation velocity (6.59×10^{-9} m s^{-1}) and stress experienced due to diffusion velocity (diffusion coefficient = 6.10×10^{-11} m^2 s^{-1}) during Spin-freezing were shown to have negligible effects on proteins within 10 min of Spin-freezing (Lammens et al., 2018). It was found that the sedimentation velocity of viruses was 5730 times higher and that of bacteria was up to 20,000 times higher compared to the sedimentation velocity of proteins. This meant that viruses and bacteria are more prone to inhomogeneity in the frozen product layer due to sedimentation. More recently, the impact of Spin-freeze-drying was studied on the stability of a commercial polyclonal antibody, human intravenous immunoglobulin (IVIG), manufactured by Baxter Healthcare Corporation (Vanbillemont et al., 2020a). The authors concluded that the stability of the Spin-freeze-dried protein was comparable to its conventionally Freeze-dried counterpart. Since low shear rates and no major air–liquid interfaces were generated, Spin-freezing did not impact the stability of the protein. These results were consistent with results shown previously (Lammens et al., 2018).

In terms of aseptic manufacturing, a GMP-like engineering prototype for Spin-freeze-drying has been developed by RheaVita and Ghent University (Corver et al., 2018). The authors propound that this technology can be scaled-up by adding 5 parallel lines within an area of 25 m^2 to produce 10,000 Spin freeze-dried vials per day. In comparison to the throughput delivered by the Spin-freeze-drying prototype, a commercial batch Freeze-dryer, within an area of 30 m^2, can deliver 100,000 vials of a capacity of 2 mL over a 3-day cycle. In conclusion, Spin-freeze-drying technology has shown to be a potential competitor to batch Freeze-dying in terms of process associated stresses and PAT, though the feasibility of implementing>5 parallel lines to generate a higher throughput along with the associated costs in a cGMP environment would be an interesting area of study.

2.1.3. *Continuous Freeze-drying of suspended vials*

More recently, a new concept of continuous Freeze-drying known as continuous Freeze-drying of suspended vials has been developed based on patent PCT no. WO2018204484 (Trout et al., 2018). As shown in Fig. 4, the Freeze-drying setup consists of a sequence of modules for different unit operations connected together to ensure a continuous flow of vials. The vials are suspended over a track with multiple rows that

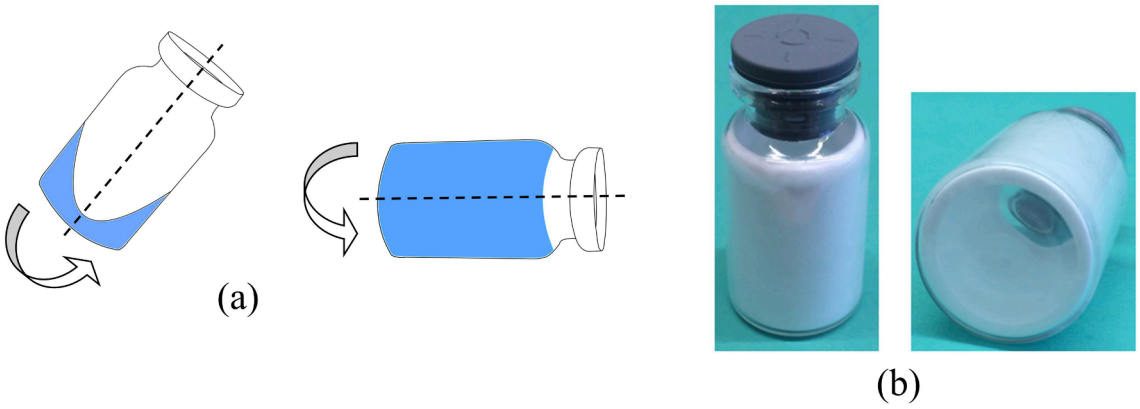

Fig. 2. (a) Spin-freezing of a vial along its longitudinal axis and (b) Spin-freeze-dried vials. Reprinted from (De Meyer et al., 2017) with permission from Elsevier.

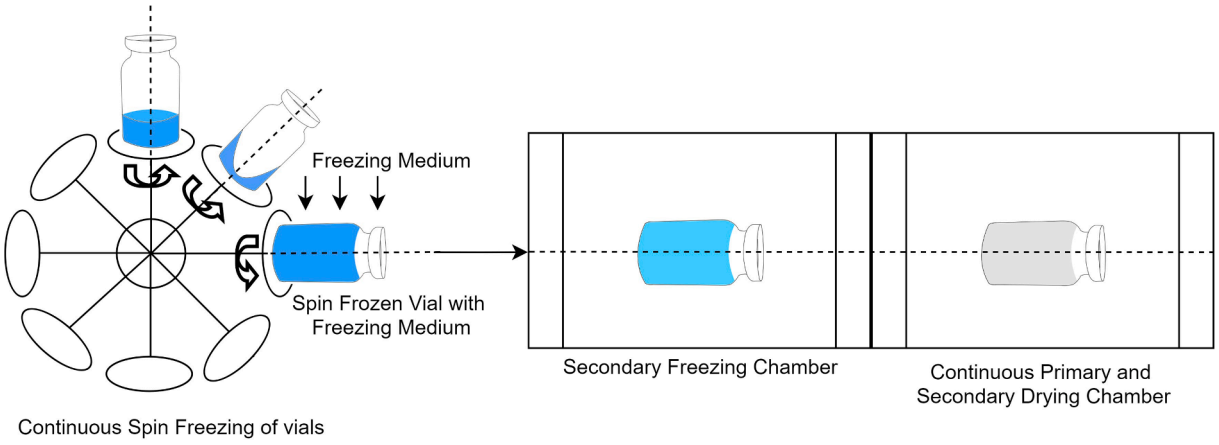

Fig. 3. A schematic of the continuous Spin-freeze-drying System.

allow the transfer of vials through chambers with different temperature and pressure conditions. Each chamber is separated by a load-lock system that facilitates the transfer of vials between different modules. As the vials are continuously being loaded into the Freeze-dryer, they are filled and partially stoppered. After filling, the vials enter the freezing zone where freezing occurs either through spontaneous or controlled nucleation using Vacuum Induced Surface Freezing technique (VISF). Heat transfer during freezing is achieved by cooling gas through forced air convection or radiation. A significant reduction in temperature gradients within the product results in the formation of larger and uniform pores which is achieved by the suspended-vial configuration. This further results in a faster sublimation rate, thereby, reducing the total drying time compared to conventional Freeze-drying. In addition, controlled nucleation reduces vial-to-vial ice crystal inhomogeneity which results in a homogenous porous structure amongst vials. Following the process of freezing, the vials subsequently move into the primary and secondary drying chamber. The desired pressure is applied to initiate sublimation. Controlled heat transfer is achieved through a circulating heat transfer fluid in the radiating surfaces. This feature helps to overcome the drawback of heterogeneous heat and mass transfer during conventional Freeze-drying. The authors asserted that continuous Freeze-drying would require a cycle time of only about 6 h compared to a 51 h hour batch Freeze-drying cycle (Pisano et al., 2019). Moreover, the size of this continuous Freeze-drier would be 6 – 8 times smaller compared to a conventional batch Freeze-dryer (Capozzi et al., 2019).

In conclusion, this concept of continuous Freeze-drying offers a greater advantage in terms of reduced drying times, continuous throughput and PAT for all individual vials, however, the feasibility of setting up this continuous process in a cGMP environment with associated scale-up and validation processes need to be addressed.

2.2. *Bulk Drying Technologies*

2.2.1. *Active-freeze-drying*

In contrast to tray-based bulk Freeze-drying, Hosowaka Micron B.V. developed stirred bulk Freeze-drying known as “Active-freeze-drying” based on patent no. EP1601919A2 (Van Der Wel, 2012). The Active-freeze-drying process allows drying of heat-sensitive bulk materials ranging from solutions, suspensions and pastes to wet solids with minimal handling (Hosokawa Micron, 2019). The final dried product is obtained as free-flowing powder, unlike Freeze-dried cakes. As an additional feature, the characteristics of certain products can be improved by stirring. Moreover, a higher rate of heat transfer and reduced drying times can be achieved. The process flow includes a jacketed conical vacuum dryer, an impeller, a collection filter, a product collector, a condenser and a vacuum pump as shown in Fig. 5.

The working principle involves dynamic freezing of the product in a conical stirred drying chamber with the help of a freezing medium. The chamber is surrounded with a controlled heating/cooling jacket. Frozen granules of different sizes and shapes are obtained as a result of VISF and stirring motion. Subsequently, sublimation takes place at a suitable pressure and heat is distributed through the jacket along with the stirring motion. Mixing provides a large surface area for sublimation leading to a higher rate of heat transfer, thereby, reducing the drying time (Touzet et al., 2018). Sublimation starts at the outer layers eventually moving towards the inner layers of the frozen granules. Due to the stirring motion, the dried layer is continuously disintegrated into

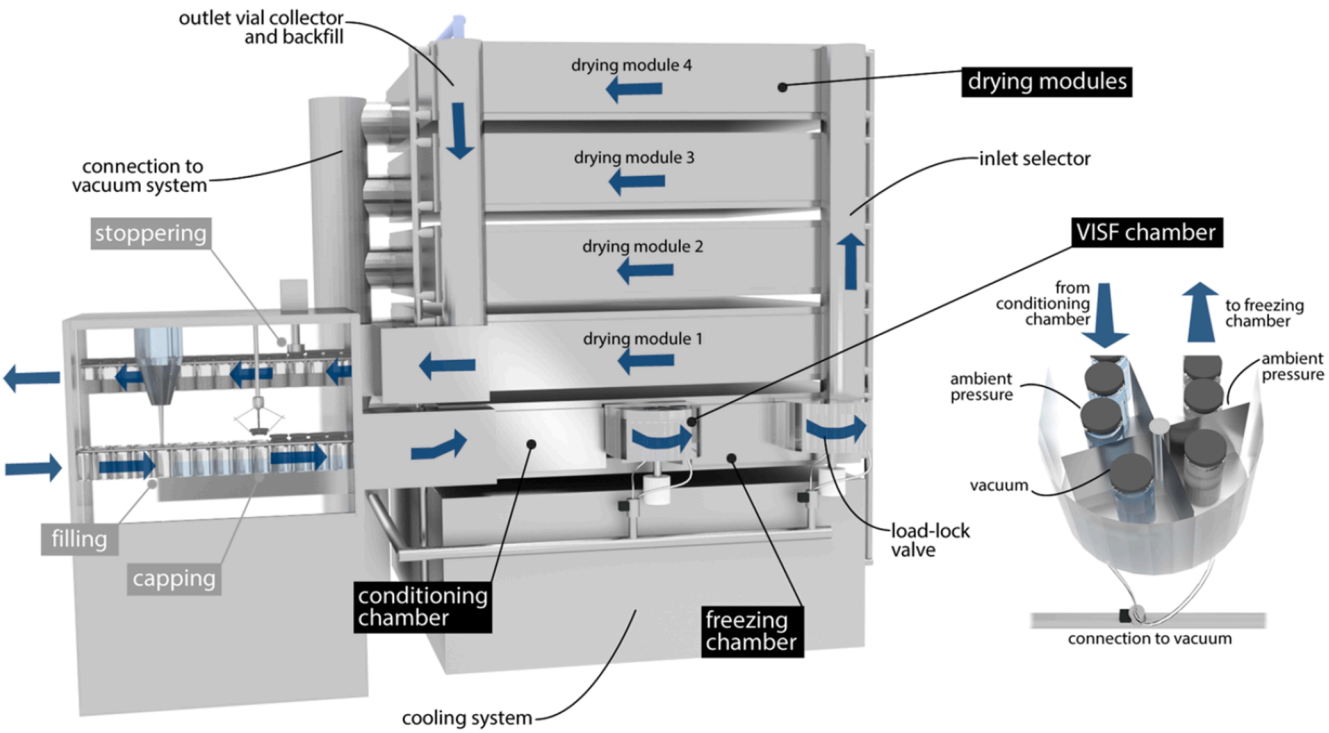

Fig. 4. Continuous Freeze-drying of Suspended Vials. Reprinted from (Capozzi et al., 2019) with permission from ACS.

fragments which reduces resistance to vapour flow. These fragments are driven into the collector by vacuum. The drying process is finished when the product temperature is in equilibrium with the chamber wall temperature.

A pilot-scale study conducted on Active-freeze-drying of nanocrystal-based ketoconazole drug showed that the technique efficiently produced reconstitutable nanocrystal powder (Touzet et al., 2018). Out of the process parameters evaluated, namely, freezing method, nanocrystal concentration, jacket temperature and screw rotation, the jacket temperature significantly contributed to the rate of sublimation and the yield. An increase in the jacket temperature approximately doubled the rate of sublimation and resulted in the collection of significantly larger fragments due to the corresponding increase in the vapour flow rate and allowing the transfer of large fragments from the chamber into the collector, thereby, leading to a higher yield. Large fragments, as shown by Scanning Electron Microscopy (SEM) (Fig. 6), were also obtained by

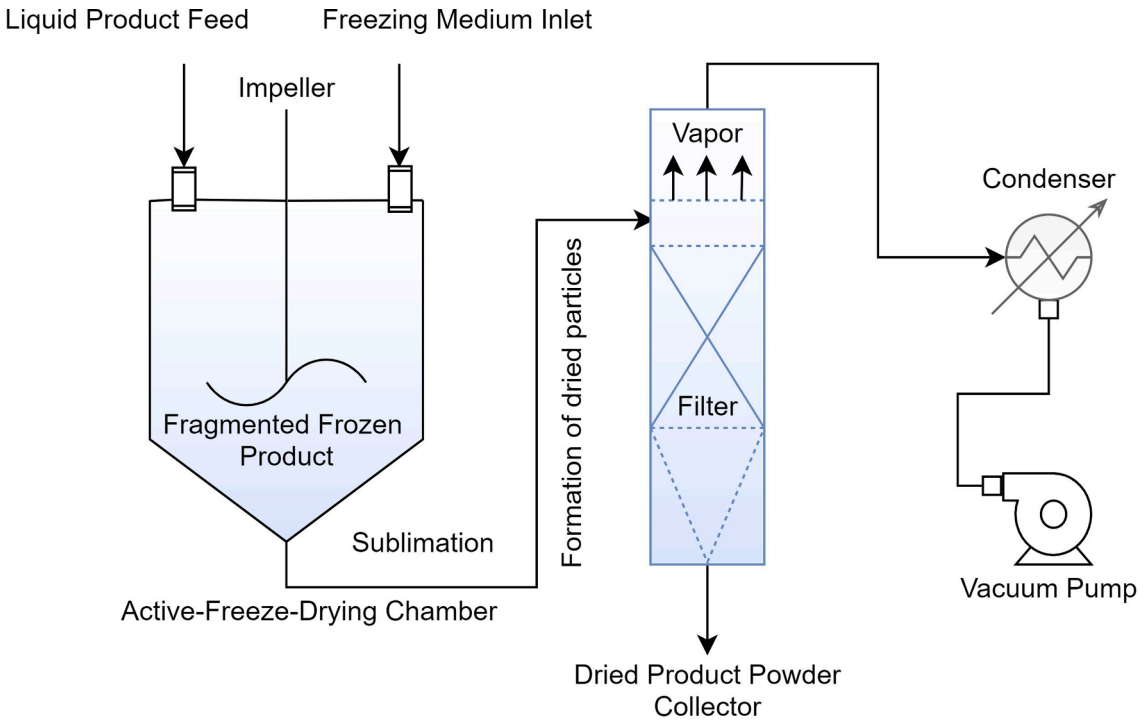

Fig. 5. Pictorial representation of the Active-freeze-drying Process.

increasing the nanocrystal concentration as a result of a stronger nanocrystal network structure. Furthermore, particle size analyses of the reconstituted Active-freeze-dried nanocrystal suspensions showed the presence of nanocrystal aggregates between 1 and 100 μm along with reduced presence of the original population by volume. The presence of aggregates induced by Active-freeze-drying, reduced the surface area available for rehydration by 45 – 50 %. A significant reduction in the fraction of aggregates in the formulation was observed by increasing the concentration of D-α-tocopherol polyethylene glycol 100 succinate (TPGS) by three-fold. This resulted in an increased availability of the surface for re-dispersion, however, this did not reduce the reconstitution time of the dried nanocrystals. Lastly, they also demonstrated that lowering the freezing temperature from – 19 °C to – 33 °C led to reduced aggregation. It was stated that no surface loss was observed over the period of 2 weeks of storage post Active-freeze-drying and the nanocrystal suspensions were physically stable (Touzet et al., 2018).

While this technology employs the principle of Freeze-drying to produce bulk powder, it is still a batch process. Continuous operation and automated recovery of the dried product is yet to be addressed. Moreover, the continuous stirring motion throughout the freezing and drying process can be detrimental for the stability of proteins. Continuous stirring can lead to increased foaming in the liquid product, thereby, exposing the protein to interfacial denaturation and aggregation (Duerkop et al., 2018). During the freezing step, breaking up of ice crystals by continuous stirring can interfere with ice nucleation and the cooling rate which may lead to instability at the ice-liquid interface (Authelin et al., 2020). Furthermore, continuous stirring in an insulted vessel can lead to a relative increase in temperature and so, it is crucial to accurately monitor the product temperature during sublimation. Sublimation above the T_g' may lead to product collapse (Meister et al., 2009; Meister and Gieseler, 2009; Ohori and Yamashita, 2017). Active-freeze-drying technology may be more suited for small molecules and stable biopharmaceuticals, though the feasibility of this technique needs investigation for commercially manufactured parenteral biopharmaceuticals.

2.2.2. *Spray-freezing and Dynamic Freeze-drying Technology by Meridion Technologies*

Meridion Technologies developed the SprayCon Lab® Spray-freeze-dryer based on two patents by Sanofi Pasteur SA, patent no. US10006706B2 (Luy et al., 2018) and US9347707B2 (Struschka et al., 2016). A schematic of the production-scale Spray-freeze-drying process and Spray-freeze-dried microspheres generated by Meridion Technologies are shown in Fig. 7. The transfer liquid vessel and the Spray-freezing chamber are positioned over the rotary Freeze-dryer connected through a cooled tube and a flap that tightly separates both process areas. The dried product is transferred from the drying chamber through a transfer tube into filling vessels.

This process is divided into two broad steps, namely, Spray-freezing (SprayCon® Technology) and dynamic bulk Freeze-drying (LyoMotion® Technology). The spraying process employs a frequency-driven prilling nozzle. The liquid feed disintegrates into round droplets at the resonance frequency and are guided by gravity into the freezing chamber. The flow rate, nozzle frequency, viscosity and orifice diameter influence the droplet size. It has been reported that approximately 1000 – 5000 droplets/s can be generated (Luy and Stamato, 2020). The freezing chamber is a double-walled, cylindrical vessel into which the droplets are frozen using a cryogenic medium (liquid N_2 and gas N_2). The droplets are not directly in contact with N_2 (l). Sterile N_2 (g), at an operating temperature in the range of – 80 °C to – 150 °C is filled inside the chamber where heat exchange occurs by convention. Additionally, deflector jets are installed along the trajectory of the falling droplets that facilitate droplet dispersion and prevent agglomeration (Sebastião et al., 2019a). Several parameters such as gas temperature, droplet size, glass transition temperature, total solid content and the height of the freezing chamber impact the rate of freezing of the droplets. Typically, uniform frozen spheres of a size of 300 – 1000 μm can be generated in 1 – 3 s of travelling 1.5 m – 3.5 m (Luy and Stamato, 2020).

Post Spray-freezing, the dynamic Freeze-drying process involves a rotary Freeze-dryer made of a cylindrical drum located inside a double-walled vacuum drum. For sterile operation, the freezing chamber and rotary Freeze-dryer are connected through an isolation valve. The rate of sublimation is increased as a result of the constant rotary motion of the drum along its longitudinal axis and increase in temperature. Conductive heat transfer to the product is achieved by silicon oil that flows through the double-walled surface and by infrared (IR) radiators installed inside the drum and the pressure is set to 100 μbar or lower. Moreover, the cross-sectional area for the flow of water vapour is significantly increased through the opening located at either ends of the drum. By reverse rotation of the drum, the dried bulk product spheres are taken up by discharge scoops that are directed downwards through a funnel for final collection or filling (Luy and Stamato, 2020).

Sebastião et al. described mathematical models, including a derived set of equations, for droplet kinetics (Sebastião et al., 2021, 2019a, 2019b). These models could predict the temperature of a single sprayed droplet at different positions in the freezing column in terms of mass, velocity, sublimation rate etc. They found that the predicted droplet temperatures were consistent with their experimental data within an error of 10 %. Readers are referred to the cited paper herein for details on the model parameters and their relationships (Sebastião et al., 2019a). In a complimentary parametric study, the authors described the impact of spraying conditions on the freezability of droplets in the freezing column (Sebastião et al., 2019b). They elucidated that the droplet diameter and the cryogenic gas temperature significantly influenced the freezing distance of droplets compared to the nozzle flow rate, initial droplet temperature, solute concentration, and the supercooling degree. The initial droplet temperature and the volumetric flow

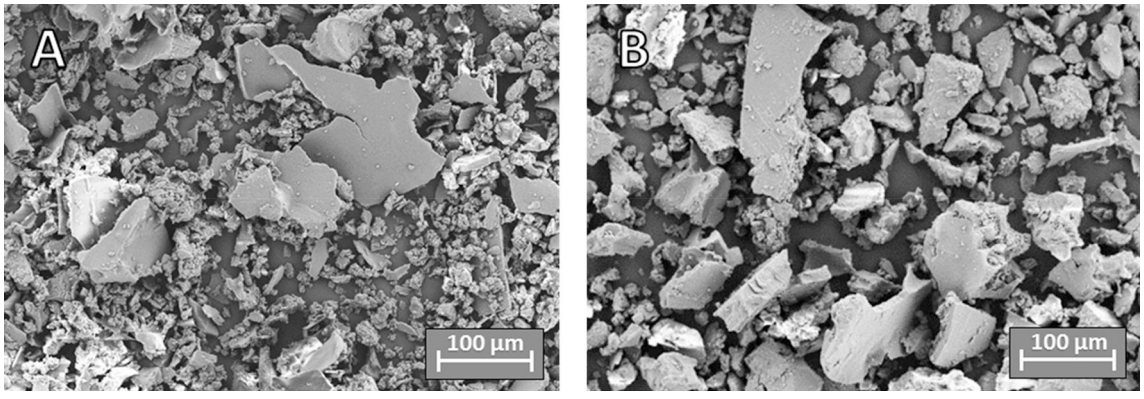

Fig. 6. (A) SEM image of 10 % w/w ketoconazole drug crystals (B) SEM image of 20 % w/w ketoconazole drug crystals. Reprinted from (Touzet et al., 2018) with permission from Elsevier.

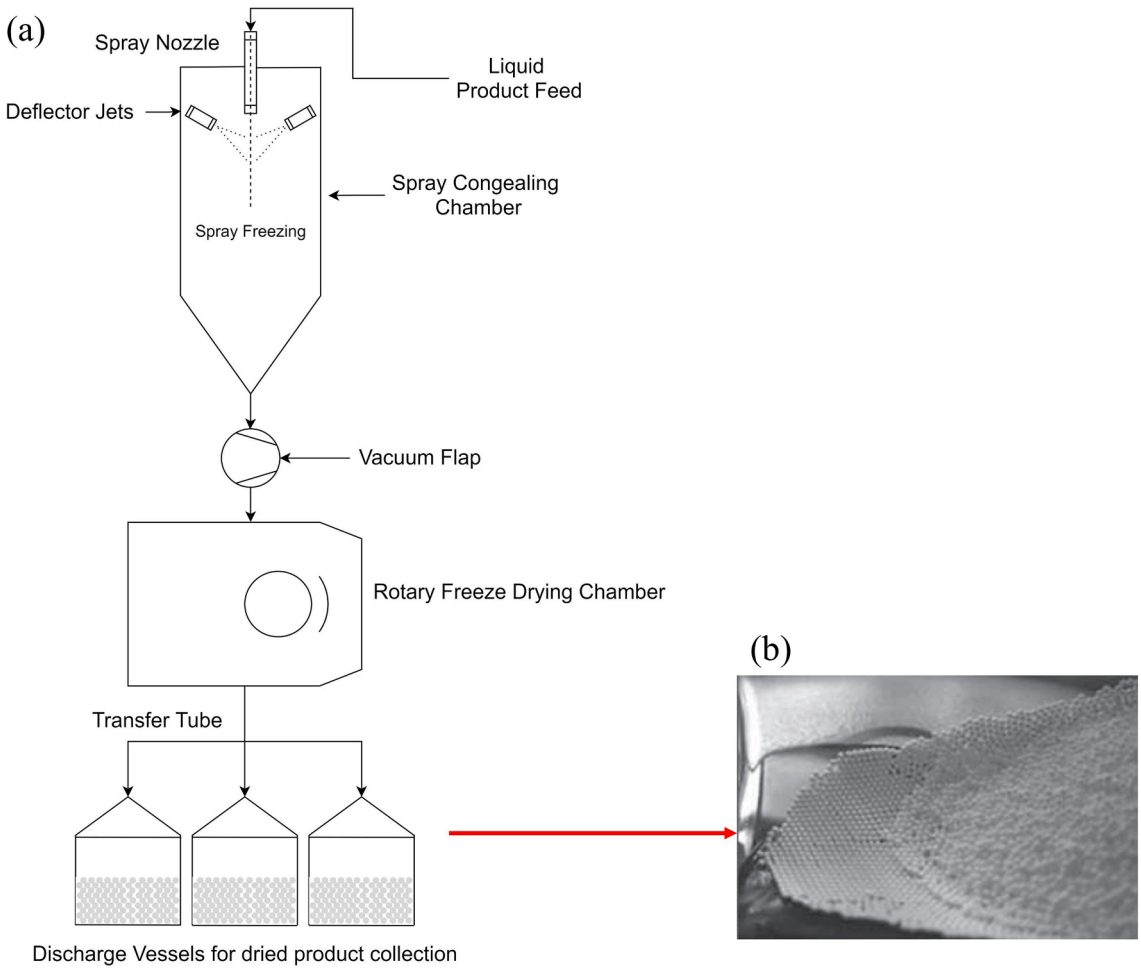

Fig. 7. (a) A schematic diagram of the Spray-freeze-drying process by Meridion Technologies and (b) Spray-freeze-dried microspheres. Reprinted (adapted) from (Luy and Stamato, 2020) with permission from John Wiley and Sons.

rate from the spray nozzle did not have a major impact on the freezing distance. This meant that viscous solutions could be sprayed at higher temperatures, thereby, preserving pumping efficiency and performance. Moreover, reduced freezing distances could be achieved by increasing the initial solute concentration at higher droplet temperatures though the impact of solute concentration was not significant at lower temperatures. Understanding the effect of these parameters is essential to ensure proper freezing of droplets before entering the drying phase. Though these results have not been generalised, they are crucial in developing and optimizing a Spray-freeze-drying equipment and process.

Reported benefits of this technology are described here. The cryogenic gas, the rotating drum surface as well as the IR radiators and the large surface area of the frozen microspheres confer increased heat and mass transfer, thereby, reducing the total drying time. Reduction in the water vapour diffusion length is achieved in a Spray-frozen microsphere with a diameter of 1 mm and a maximum diffusion length of 500 μm compared to a 10 mm thick Freeze-dried cake with a maximum diffusion length of 10000 μm (Luy and Stamato, 2020). While a recommended total solid content of 5 – 10 % w/w in the product feed allows strong spherical structures, drying of a monoclonal antibody (mAb) formulations with mAb concentrations in the range of 50 – 200 mg/mL and a total solid content of 10 – 36 % w/w were achieved in about 24 – 30 h (Lowe et al., 2018). Moreover, free-flowing powder, rapid reconstitution and minimal increase in protein aggregation was achieved at 25 °C, over a 9-month storage period for the formulation containing a ratio of 1:1 (mAb : sugar) (Lowe et al., 2018). Even though particle size of < 300 μm is achievable during Spray-freezing, the presence of high solid content reduces the risk of loss of particles due to a high rate of sublimation. The risk of particle loss for product feed with low solid content can be reduced by generating larger particle sizes i.e., 2 – 3 mm. It has been reported that the time required for Spray-freezing a 100 L bulk with 20 % solid content is $\sim$ 10 to 20 h and the time required for dynamic Freeze-drying is $\sim$ 24 h with $>$ 97 % yield (Luy and Stamato, 2020).

2.2.3. *Continuous Aseptic Spray-freeze-drying Technology by IMA Life*

IMA Life America INC. invented and patented a bulk Freeze-drying process design using a combination of Spray-freezing and Stirred Drying, patent no. US9052138B2 (DeMarco and Renzi, 2015). The process flow begins with the freezing step involving spraying of the bulk product along with an aseptic freezing medium into an aseptic freezing vessel. This is followed by introduction of a vacuum to the frozen powder to initiate sublimation. The frozen material is stirred using a spiral blade agitator at a low speed. Subsequently, the frozen powder is heated to increase the rate of sublimation. Lastly, the vacuum is released to obtain the final Freeze-dried product.

With modifications to the patent, IMA Life developed the Lynfinity® Spray-freeze-dryer (IMA Life, 2019). A schematic diagram of the Lynfinity® process and microparticles generated by the process are depicted in Fig. 8. The spraying process begins with the generation of uniform droplets under the influence of frequency vibrations as the product feed is made to flow through a temperature-controlled droplet zone. The disintegrated product feed passes through the nozzle into the freezing column where the freezing process begins. The stainless-steel freezing

column is lined with a double walled jacket that utilizes liquid nitrogen and silicone oil for controlling the temperature in the chamber. The cooling gas is maintained at temperatures below – 130 °C. As the droplets are sprayed into the column, they are instantaneously (in<5 feet from the point of ejection with an average volumetric diameter of 500 μm) frozen allowing them to maintain their shape (IMA Life, 2019). The frozen spheres are collected at the base of the freezing chamber. Before entering the drying module, an intermediate chamber allows the product to be transferred from the freezing column at atmospheric pressure to the drying chamber under vacuum conditions without disrupting the continuous process. The movement of frozen spheres inside the drying chamber is done at a controlled rate on cascading shelf stacks using gentle vibratory agitation. Agitation through vibration along with heat through heat transfer fluid initiate rapid sublimation and prevent agglomeration of the spheres inside the drying chamber. Post drying, the dried spheres are collected as bulk in the collection chamber. Unlike a conventional Freeze-dryer, the Lynfinity® contains dual ice condensers allowing continuous operation.

The Lynfinity® production-scale Spray-freeze-dryer is show in Fig. 9. Anticipated benefits of this technique over conventional Freeze-drying include bulk processing with minimal handling of trays without the need for post-processing operations such as granulation and milling (Siow et al., 2018) along with higher productivity and lower downtime (IMA Life, 2019). Secondly, it confers increased efficiency of heat and mass transfer between the product, trays and shelves. Thirdly, it may establish a continuous process in a sterile environment with greater throughput flexibility (DeMarco and Renzi, 2015). The specific surface area of the dried spheres was measured using Brunauer–Emmett–Teller (BET) theory and was found to be 7.03 m^2/g, compared to that of Freeze-dried cakes (0.47 m^2/g), allowing greater interaction with solvent during rehydration (IMA Life, 2019). Moreover, the dried spherical product can be distributed into syringes, vials, inhalation systems etc. which is not possible with Freeze-dried cakes. Despite the benefits, a slight increase in the turbidity of a rehydrated Spray-freeze-dried product, compared to its Freeze-dried counterpart, was attributed to particle agglomeration. They observed that increasing the concentration of surfactant reduced turbidity due to aggregation.

In conclusion, continuous bulk processing can allow the use of more PATs for real-time process and product monitoring, though further consideration is required in terms of product yield, stability of Spray-freeze-dried parenteral biopharmaceuticals, footprint associated with the commercial-scale equipment and suitable powder filling options in a cGMP environment.

2.2.4. *Spray-drying*

Spray-drying is a technique that has potential applications in the biopharmaceutical industry. It is one of the few techniques used to produce dried powder formulation from liquid, slurry or low-viscosity paste (Celik and Wendell, 2010). Several advantages of Spray-drying have been reported. Firstly, it eliminates the need for a large number of unit operations which makes it cost-effective and improves production efficiency. The manufacturing cost associated with Spray-drying is

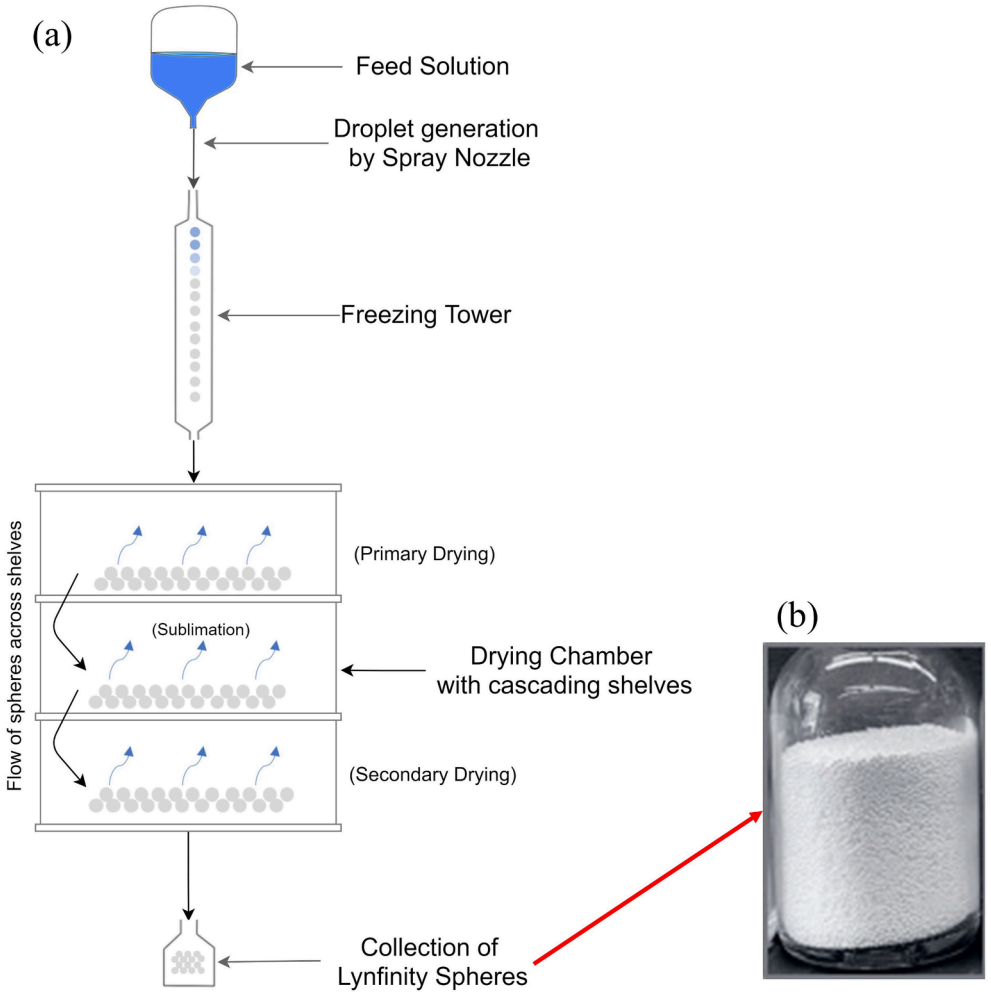

Fig. 8. (a) A schematic diagram of the LYnfinity® Spray-freeze-drying Process and (b) Spray-freeze-dried microparticles generated by LYnfinity® Technology (IMA Life, 2019).

only 20 % of the manufacturing cost associated with Freeze-drying (Roser, 1991; Santivarangkna et al., 2007). Secondly, it is a rapid one step process, allows continuous production of flowable powders and can be implemented across a range of biopharmaceuticals (Walters et al., 2014). Properties such as particle size and shape can be controlled and engineered by Spray-drying (Vehring, 2008). Furthermore, this technique can take thermolabile products into consideration. The evaporation process takes about milliseconds to a few seconds and the process is very instantaneous, thereby, minimizing exposure to high inlet air temperatures (Celik and Wendell, 2010). The production of millions of small droplets provides a large surface area for heat and mass transfer allowing rapid evaporation. SPX Flow Inc. has had aseptic Spray-dryers that have been inspected by the FDA and produced clinical supplies for phase 3 pivotal studies. The authors have demonstrated the use of Anhydro MS-35 Spray-dryer to successfully produce dry powder-based mAbs (Bowen et al., 2013; Gikanga et al., 2015). However, some of the factors that may induce instability in proteins have been depicted in Fig. 10.

Fig. 10 represents a schematic diagram of the Spray-drying process using a cyclone-based separator. The liquid feed is drawn towards the spray nozzle at a flow rate using a peristaltic pump. With the help of an atomizing gas flow and desired size of spray nozzle orifice, the liquid is atomized and sprayed into the upper drying chamber. The temperature of the sprayed droplets in the upper chamber is lower or equal to the wet bulb temperature. The droplets experience evaporative cooling and are pulled towards the lower chamber under the influence of gravity and drying gas. The outlet gas temperature is measured at the bottom of the lower drying chamber. With the help of a cyclone gas flow rate, the particles are guided towards the cyclone where particles are separated based on their densities. The dry product is collected at the bottom as dry powder and the low density fine particles can be recovered using a bag filter (GEA, 2020). Possible sites for protein denaturation in the upper drying chamber include shear at the nozzle, air–liquid interfacial denaturation and dehydration due to evaporative cooling. As the particles exit the lower drying chamber, they experience temperatures greater than the wet bulb temperature but lower or equal to the outlet gas temperature. Outlet gas temperatures greater than the T_g of the product and the residence time of the particles in the cyclone collector may affect the stability of proteins.

In comparison to Spray-drying, drug administration methods such as nebulization and nasal spray expose proteins to air–liquid interfaces, shear and temperature, which may cause deterioration in the CQAs of biopharmaceuticals (Albasarah et al., 2010; Bodier-Montagutelli et al., 2020; Fröhlich and Salar-Behzadi, 2021; Hertel et al., 2015; Niven et al., 1996, 1995). Examples of some commercial and clinical dry powder-based biopharmaceuticals administered via the parenteral route, inhalation or nebulization are collated in Table 2. The stability and composition of such products can provide more insight while studying the impact of the atomization process during Spray-drying or Spray-freeze-drying.

More recently, authors have demonstrated approaches to identify CPPs and formulation components for Spray-drying proteins (Batens et al., 2018; Grasmeijer et al., 2019; Ziaee et al., 2019). The outlet temperature was found to be the most critical factor that affected the enzymatic activity of lysozyme. Along with high outlet temperatures, ultrasonic vibrations and mechanical stress produced from ultrasonic nozzles had a negative impact on the activity of lysozyme (Ziaee et al., 2020).

While the impact of Spray-drying on proteins has been studied in terms of temperature, the effect of shear and interfacial denaturation during atomization and spraying is also crucial as some proteins are susceptible to such stresses (Broadhead et al., 1993; Grasmeijer et al., 2019; Koshari et al., 2017; Maa et al., 1998; Maa and Hsu, 1997; Mumenthaler et al., 1994; Wilson et al., 2019; Ziaee et al., 2020). Understanding the impact of spraying conditions prior to dehydration, can provide more insight while developing and choosing excipients for Spray-drying proteins. Typically, the liquid feed is drawn into a two-fluid nozzle at velocity, ν_{liq} and exits the nozzle tip with a diameter, d_i

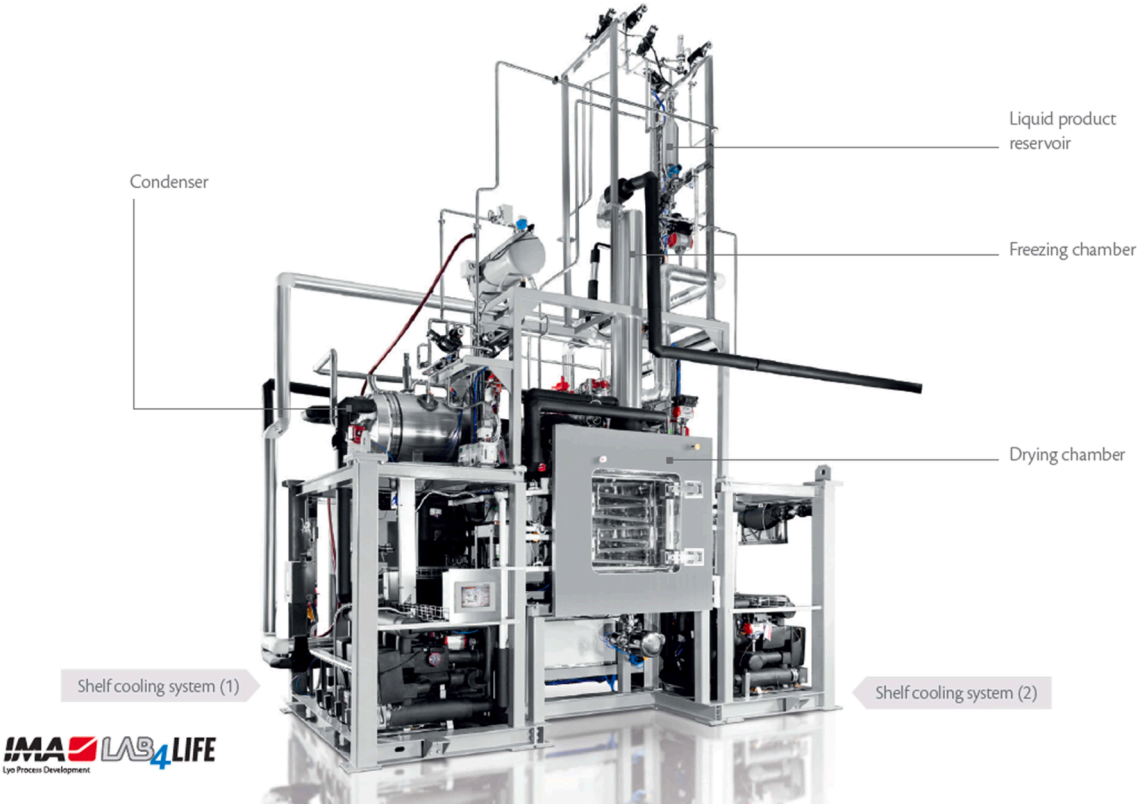

Fig. 9. LYnfinity® Production-Scale Spray-freeze-dryer (IMA Life, 2019).

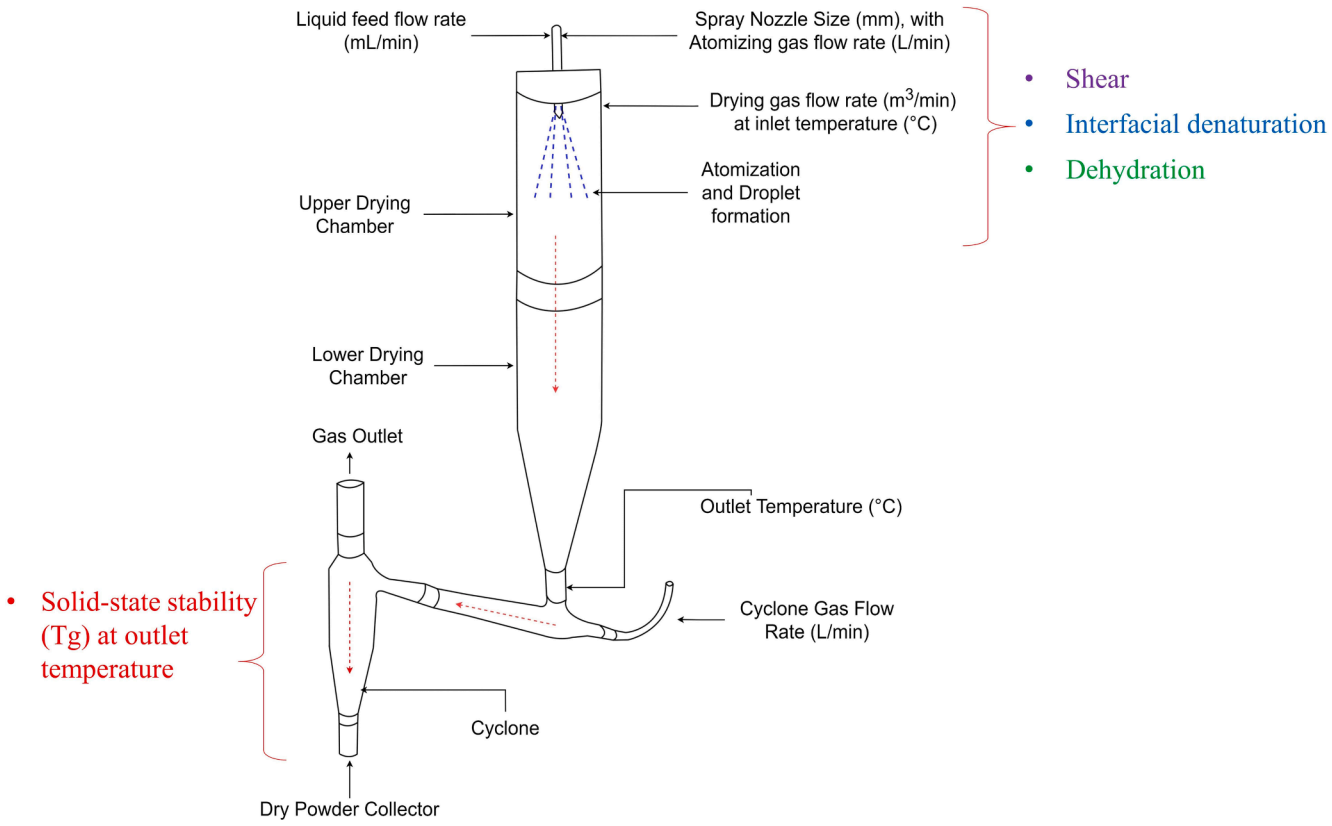

Fig. 10. A schematic diagram of the Spray-drying process with a cyclone separator and possible sites for protein denaturation. Blue dotted lines represent the sprayed droplets. The red dotted lines represent the trajectory of the particles.

(Fig. 11). A resultant velocity, ν_{av} is generated at the mixing zone with the help of an atomizing gas flow rate, ν_{gas}. The shear rate generated from a two-fluid spray nozzle has been estimated using Equation (1) (Ghandi et al., 2012; Hede et al., 2008).

$$\gamma = \frac{\left[2\left(\nu_{av} - \nu_{liq}\right)\right]}{d_i} \quad (1)$$

Where γ is the shear rate in s^{-1}, ν_{av} is the average velocity at mixing point in m/s, ν_{liq} is the velocity of liquid in m/s and d_i is the inner diameter of the nozzle tip in mm. ν_{av} is a function of the mass flow rates (kg/s) of the gas and liquid and must not be confused with the arithmetic mean of their velocities.

Using Equation (1), authors have shown that the shear rates generated from a two-fluid nozzle at different atomizing flow rates and liquid densities can range from 97,000 s^{-1} to 992,000 s^{-1} (Ghandi et al., 2012; Morgan et al., 2020). Shear-induced inactivation for some proteins can occur at $< 2000\ s^{-1}$ for ≥ 20 min (Ashton et al., 2009; Charm and Wong, 1981), while some proteins may remain stable up to a shear rate of $> 250{,}000\ s^{-1}$ for > 30 min (Bee et al., 2009; Duerkop et al., 2018). Bekard et al. elucidated that the α-helical content in poly-L-lysine was inversely proportional to the square root of shear strain and the extent of unfolding decreased with increasing molecular weight due to greater cohesive forces (Bekard et al., 2011). Such shear levels can be experienced during similar drying methods such as Spray-freeze-drying and so, it is crucial to study the stability of biopharmaceuticals as a function of shear and atomization.

In conclusion, Spray-drying is one of the most popular industrial drying technology and has been studied over a wide range of products over the past few decades. The challenges associated with the CPPs of Spray-drying of labile parenteral biopharmaceuticals, choice of formulation excipients and their molecular mechanism of interactions with biopharmaceuticals during Spray-drying require further product-specific study.

2.2.5. PRINT® Technology

Particle Replication in Non-Wetting Templates, also known as PRINT® technology, originated from lithographic techniques applied in the microelectronics and semiconductor industry. PRINT® is a micro-moulding based particle design / engineering technology employed to generate monodisperse, uniquely shaped (i.e. filaments, rods, spheres, discs, toroids) micro and nano-particles of hydrogels, polymers, APIs etc. with tunable size and morphology (Galloway et al., 2013; Garcia et al., 2012; Kelly and DeSimone, 2008). The fabrication of PRINT® particles was first demonstrated by Rolland et al. (Rolland et al., 2005) and the pharmacokinetic characteristics of these particles as delivery vectors were first studied by Euliss et al. and Gratton et al. (Euliss et al., 2006; Gratton et al., 2007). Kelly and DeSimone demonstrated the generation of protein particles, namely, insulin and albumin using PRINT® technology (Kelly and DeSimone, 2008). With the incorporation of cGMP practices, this technique has been scaled-up with continuous roll-to-roll system which allows continuous particle production for pre-clinical and clinical study of pharmaceutical inhalation powders by Liquidia Corporation (DeSimone, 2016; Liquidia Corporation, 2021).

The process flow for generating PRINT® particles reported by authors is discussed here (Garcia et al., 2012; Gratton et al., 2007; Kelly and DeSimone, 2008). Perfluoropolyether (PFPE) was poured onto a prepared silicon master template containing the desired etching patterns of 2 μm, 5 μm and 200 nm sized shapes to produce a mould containing the same sized cavities (Fig. 12 (a)). Following the preparation of the PFPE mould, aqueous protein samples containing insulin, albumin and albumin mixtures with siRNA or paclitaxel were sandwiched between the cavities present in the mould and a high surface energy polyethylene film (Fig. 12 (b)). A pressure of 50 psi was applied through a roller to prevent the formation of layers between the filled cavities and to

Table 2
Some commercial/clinical dry powder-based biopharmaceuticals and some biopharmaceuticals administered via spraying or nebulization.

Biopharmaceutical and Manufacturer	Biomolecule API	Formulation Excipients	Manufacturing Process (Administration Method)	Reference
Exubera® by Pfizer / Nektar (Discontinued).	Insulin (Hormone)	Sodium citrate dihydrate, mannitol, glycine, sodium hydroxide.	Spray-dried powder. (Inhaled for diabetes)	(FDA, 2006; White et al., 2005)
Raplixa® by Profibrix, The Medicines Company (Commercial).	Fibrin and Thrombin.	Trehalose, calcium chloride, human albumin, sodium chloride, sodium citrate, L-arginine hydrochloride.	Spray-dried powder. (Powder applied on surface of bleeding tissue for uncontrolled bleeding)	(FDA, 2015a; Manufacturing Chemist, 2015)
Afrezza® / Technosphere insulin (TI) by Mannkind (Commercial).	Recombinant Human Insulin.	FDKP, Polysorbate 80.	Technospheres® by precipitation, adsorption and Freeze-drying. (Inhaled for diabetes)	(McElroy et al., 2013; Sarala et al., 2012; Tsai-Turton, 2014)
Inbrija® by Acorda Therapeutics (Commercial).	Levodopa (aromatic amino acid).	DPPC and sodium chloride.	Arcus® Technology – Spray-dried powder. (Inhaled for off episodes in patients with Parkinson's disease).	(Acorda Therapeutics, 2021; FDA, 2018)
Somatuline® LA by Ipsen (Commercial).	Lanreotide acetate (octapeptide analogue of somatostatin hormone).	PLGA, mannitol, carmellose sodium, polysorbate 80.	Phase separation and spray-dried microspheres. (Powder and solvent for prolonged-release suspension for injection against multiple conditions)	(EMA, 2013; HPRA, 2019; Pinto et al., 2021)
Trelstar® LA by Verity Pharmaceuticals (Commercial).	Triptorelin pamoate (synthetic decapeptide analogue of GnRH hormone).	PLGA, mannitol, carboxymethycellulose sodium, polysorbate 80.	Phase separation and spray-dried microspheres. (Powder and solvent for prolonged-release suspension for injection for the treatment of prostate cancer)	(Pinto et al., 2021; Vhora et al., 2019)
Sandostatin® by Novartis (Commercial).	Octreotide acetate (cyclic octapeptide).	Mannitol, D,L-lactic and glycolic acids copolymer, carboxymethylcellulose sodium.	Dry powder prepared by Phase separation and Spray-drying. (Injectable suspension for the treatment of acromegaly etc.)	FDA, 2008; Hou et al., 2018; Vhora et al., 2019)
TOBI® Podhaler™ by Novartis (Commercial).	Tobramycin (Antibacterial aminoglycoside).	DSPC, calcium chloride, and sulfuric acid.	PulmoSphere™ by Spray-drying. (Orally inhaled for cystic fibrosis against *Pseudomonas aeruginosa*).	(FDA, 2015b; Weers and Tarara, 2014)
Fludase® by Ansun Biopharma (NexBio) (Clinical Trial Phase 2).	DAS181 sialidase (recombinant neuraminidase).	Histidine, trehalose, citric acid, magnesium sulphate, acetate buffer.	TOSAP. (Dry powder for oral inhalation against influenza like illness)	(Bodier-Montagutelli et al., 2018; ECRI, 2011; Mack et al., 2012; Moss and Li, 2015)
CSJ117 by Novartis (Clinical Trial Phase 2)	Anti-TSLP antibody fragment.	Leucine, trileucine, mannitol and trehalose.	PulmoSol™ engineered powder. (Dry powder inhaled for asthma).	(Fröhlich and Salar-Behzadi, 2021; Liang et al., 2020; NCT04410523, 2021)
Aerovant® by Aerovance / Bayer (Clinical Trial Phase 2).	Cytokine – Pitrakinra.	Different formulations including sucrose, mannitol or trehalose, leucine or poly (amino acid) and citrate, acetate or lactate buffer were evaluated.	Spray-dried powder. (Inhaled for asthma)	(Bodier-Montagutelli et al., 2018; Liang et al., 2020; Otulana, 2011; Vehring et al., 2020; Wenzel et al., 2007)
Abrezekimab (VR942) by UCB Pharma, Vectura. (Clinical Trial Phase 2).	CDP7766 (IL-13 mAb fragment).	Trehalose dihydrate, L-leucine and phosphate buffer.	Spray-dried powder. (Inhaled for asthma)	(Burgess et al., 2018; Giles Morgan et al., 2017; Liang et al., 2020; Vectura, 2015; Vehring et al., 2020)
Pulmozyme® by Genentech / Roche (Commercial).	Deoxyribonuclease I.	Calcium chloride dihydrate, sodium chloride.	Liquid formulation. (Inhaled through jet nebulizer against cystic fibrosis to improve pulmonary function).	(Bodier-Montagutelli et al., 2018; FDA, 2014b)
Miacalcin® by Novartis / Mylan (Commercial).	Polypeptide hormone (Calcitonin).	Sodium chloride, benzalkonium chloride, hydrochloric acid.	Liquid formulation. (Nasal spray for the treatment of postmenopausal osteoporosis).	(FDA, 2017; Ozsoy et al., 2009)

DPPC, Dipalmitoyl phosphatidylcholine; DSPC, Distearoyl phosphatidylcholine; FDKP, Fumaryl diketopiperazine; GnRH, Gonadotropin releasing hormone; IL-13, Interleukine-13, PLGA, Poly(lactic-co-glycolic acid); TOSAP, Temperature-controlled organic assisted precipitation.

laminate the samples present between the mould and the film (Fig. 12 (c)). Subsequently, the polyethylene film was removed and the mould containing the samples was Freeze-dried (Fig. 12 (d)). The dehydration process can also occur through either photocuring, vitrification or evaporation (Xu et al., 2013). A liquid harvesting layer, made of either polycyano acrylate (PCA) or polyvinyl pyrrolidinone (PVP), was casted onto a glass slide (Fig. 12 (e)). Post Freeze-drying, the PFPE mould was placed over the adhesive harvesting film (Fig. 12 (f)). Once the harvesting layer was dried, the PFPE mould was removed yielding dried protein particles onto the adhesive film (Fig. 12 (g)). Finally, free-flowing protein powder was recovered by dissolving the adhesive film (Fig. 12 (h and i)). SEM images of the uniquely shape powders are shown in Fig. 13.

It was shown that no aggregation was observed in albumin and insulin particles generated via PRINT® technology. Additionally, Pulmozyme (DNase) and siRNA therapeutic molecules were processed using PRINT® technology (Garcia et al., 2012). Minimal aggregation was observed in the size exclusion chromatography (SEC) profile of DNase PRINT® microparticles with comparable enzyme activity to native DNase and the chemical structure of PRINT® generated siRNA particles

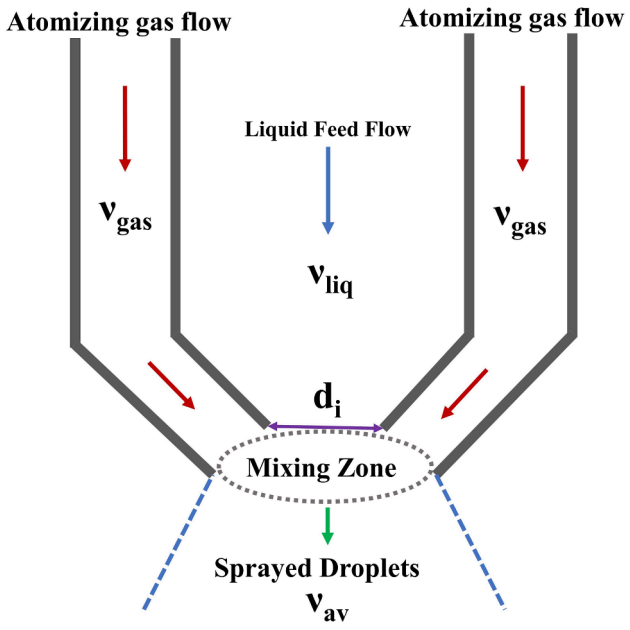

Fig. 11. A schematic representation of external mixing in a two-fluid nozzle.

was preserved without any denaturation.

Furthermore, PRINT® technology was combined with a scalable Spray-Assisted layer-by-layer (LbL) technique to enhance the characteristics of the fabricated nanoparticles (Morton et al., 2013). The combination of these techniques offer improved stability, sustained drug release and improved physicochemical properties of nanoparticles (Morton et al., 2013; Poon et al., 2011b, 2011a). In this case, the polyvinyl alcohol (PVA) harvesting layer was crosslinked using 50 % glutaraldehyde and 10 % hydrochloric acid to reduce its solubility in water. This was done to prevent any loss of particles during the spraying process. An aqueous solution of cationic polyelectrolyte was sprayed at a concentration of 1 mg/mL onto the nanoparticles for 3 s. To remove excess cationic polyelectrolyte, a wash step with water was included for another 3 s. This was followed by another spray of anionic polyelectrolyte at a concentration of 1 mg/mL for 3 s and a final wash with water. The sprayed-LbL particles were finally recovered by sonification, 0.45 μm filtration and ultracentrifugation. DLS analysis showed consistent monodisperse particles with the polydispersity ranging between 0.01 and 0.1 and hydrodynamic diameter between 190 nm and 246 nm for uncoated and coated nanoparticles (Morton et al., 2013). A decrease in the hydrodynamic diameter of coated particles was observed due to contraction forces on the PVA layer. The shape and integrity of the recovered particles was confirmed by electron microscopy images wherein particles were entirely coated by polyelectrolytes. Moreover, the biological functionality of these particles was retained and could be fine-tuned by altering the film thickness as per its application.

Furthermore, researchers developed cylindrical nanoparticles of commercially available vaccines, such as Fluzone® (Sanofi-Pasteur), Fluvirin® and AgriFlu® (Novartis) and Afluria® (Merck), using this technology (Galloway et al., 2013). PRINT® fabricated vaccine particles showed 200-fold improved antigen binding along with enhanced immune response. It was elucidated that the shape, size along with other surface properties play an important role in the interaction of these nanoparticles with other biomolecules in their surroundings (Galloway et al., 2013). Xu et al. demonstrated the fabrication of transiently insoluble BSA particles after being cross-linked by a disulphide-based cross-linker using PRINT® with new opportunities for drug and gene delivery (Xu et al., 2012). More recently, results of a phase 1 clinical trial study reported for a PRINT® fabricated dry-powder ribavirin

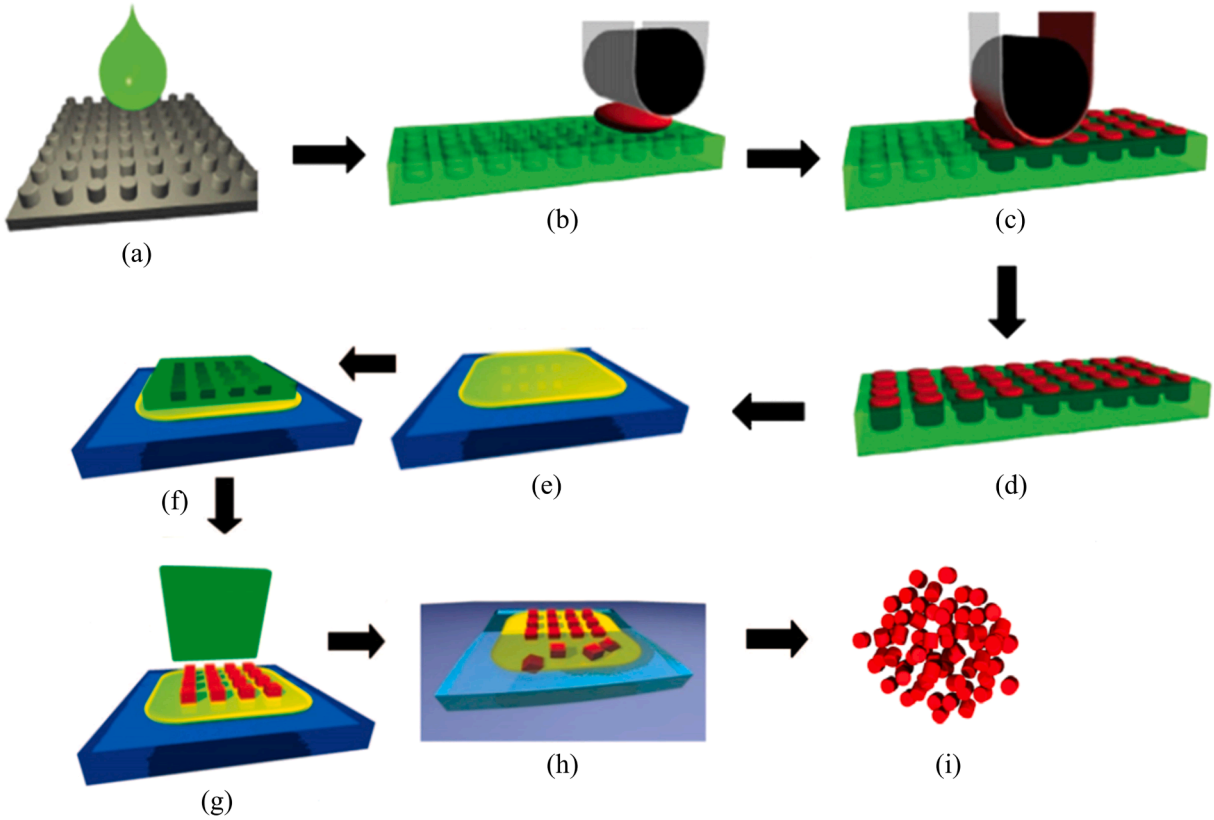

Fig. 12. The process flow diagram of PRINT® Technology. Reprinted (adapted) from (Garcia et al., 2012; Hofmann et al., 2019; Kelly and DeSimone, 2008) with permission from ACS and Hindawi Publishing Corporation.

formulation by GSK showed improved physicochemical properties, efficient and convenient delivery of API to the lungs (Dumont et al., 2020).

Overall, this technology has shown promising results for some inhaled proteins, gene therapy products and vaccines with the ability for continuous production in a large-scale cGMP facility, though the stability of large parenteral mAbs and enzymes via PRINT® will be an interesting area of study.

2.2.6. *Microglassification™*

In therapeutic protein formulations water substitution by excipients, generally saccharides and polyalcohols, stabilize therapeutic proteins in their dehydrated state (Allison et al., 1999; Liao et al., 2002; Mensink et al., 2017). With modifications to previously described micropipette manipulation techniques in literature (Duncan and Needham, 2006, 2004; Rickard et al., 2010), Aniket et al. developed a new technique called Microglassification™ for the preservation of proteins by dehydrating protein microdroplets in an immiscible drying solvent (Aniket et al., 2014). The technique was performed at room temperature and produced stable, excipient-free protein microglassified beads. The technique was performed using two chambers; one containing BSA solution and the other filled with an organic solvent (Aniket et al., 2014; Su et al., 2010). A small plug of organic solvent was withdrawn into a micropipette. The micropipette was then positioned into the chamber containing BSA solution and the desired amount of protein was pulled into it. The micropipette was then positioned back into the organic chamber, releasing a single droplet of the protein solution which was held firmly at the tip of the micropipette in the organic chamber. By doing so, water from the single droplet was extracted into the organic chamber, thereby, leading to the formation of a Microglassified™ bead (Fig. 14).

The authors demonstrated this technique on BSA and showed that the protein exhibited full recovery and restoration of its secondary structure upon rehydration of the Microglassified™ beads (Aniket et al., 2014). The fluorescence spectra of native and rehydrated Microglassified™ BSA were comparable, showing that the tertiary structure of BSA was preserved. Moreover, the Microglassified™ beads were purely amorphous which was shown through X-ray diffraction spectroscopy. This was due to rapid dehydration of the microdroplet which did not give enough time for crystallization. A significantly lower percentage of aggregates was reported in the rehydrated Microglassified™ (3.3 %) sample compared to rehydrated Freeze-dried (10 %) sample. Furthermore, accelerated storage results for Microglassified™ BSA showed that by increasing the saturation factor of water in alcohol from 0.4 to 0.5, a significant decrease in the soluble monomeric content of BSA was observed just after the first 10 min of storage at 65 °C. This meant that by increasing the availability of water to the Microglassified™ bead, an increase in the formation of protein aggregates was observed for a total of 60 min of storage at 65 °C.

Through another study, this technique was performed on lysozyme, α-chymotrypsin, catalase and horseradish peroxidase (Aniket et al., 2015a). Five different organic solvents, namely, n-pentanol, n-octanol, n-decanol, triacetin and butyl lactate were used as an immiscible drying solvent. It was reported that butyl lactate showed higher water solubility compared to other solvents. Fourier transform infrared (FTIR) spectroscopic analyses for dried and rehydrated enzyme microspheres showed that even though distortions in the secondary structural conformation of Microglassified™ enzymes were observed, these changes reverted to

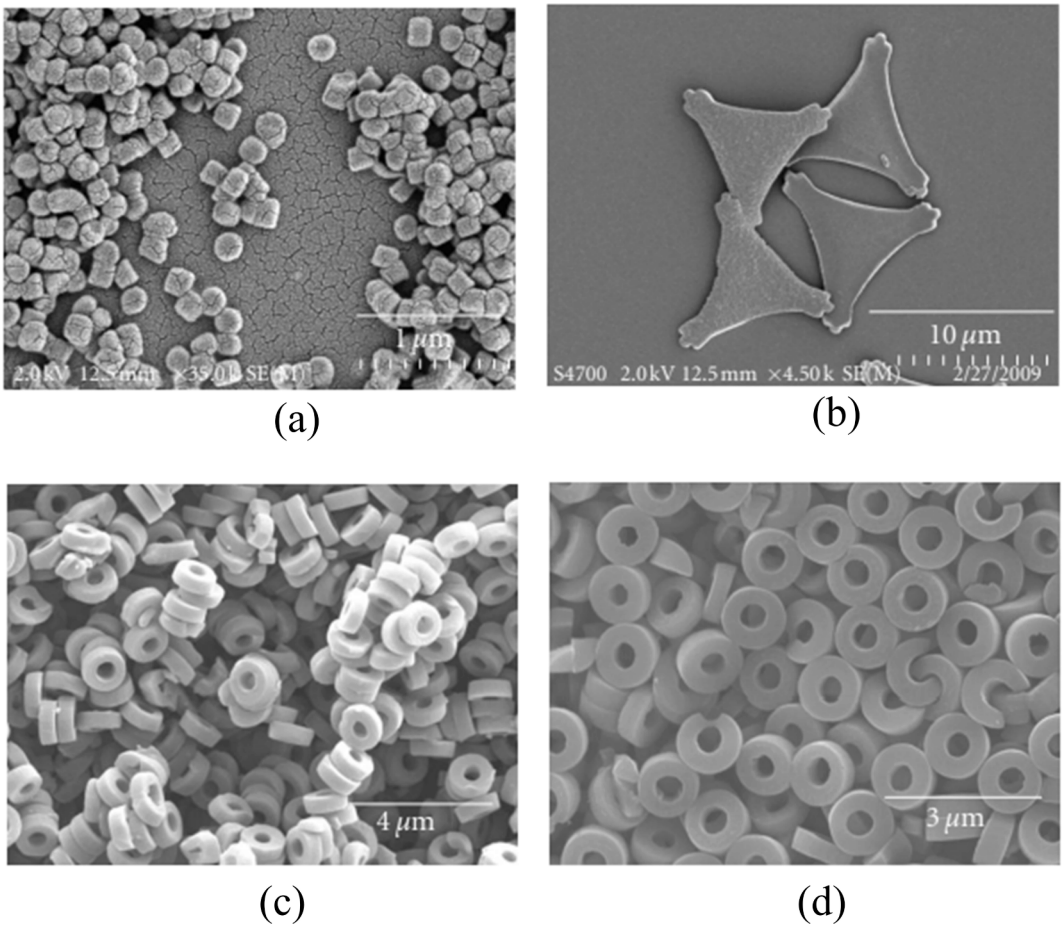

Fig. 13. SEM images of (a) 200 × 200 nm cylindrical BSA-Lactose, (b) 10 μm pollen IgG-Lactose, (c) 1.5 μm torus DNase and (d) 1.5 μm torus siRNA PRINT® fabricated particles. Reprinted (adapted) from (Garcia et al., 2012) with permission from Hindawi Publishing Corporation.

their native-like conformation upon reconstitution. Moreover, the Microglassified™ enzymes exhibited significantly higher activity in n-pentanol compared to triacetin and butyl lactate. Accelerated storage results for lysozyme showed a significant reduction in enzyme activity of the Microglassified™ and Freeze-dried samples after 1 month of storage at 40 °C. Bioactivities of both Microglassified™ and Freeze-dried enzyme were comparable. Post 1 month and up to 3 months of storage, minimal changes in the enzyme activity and secondary structure content was observed. The authors assumed similar low levels of residual moisture, as both Microglassified™ and Freeze-dried lysozyme absorbed the same amount of water as a function of water activity.

Furthermore, the potential to Microglassify™ a recombinant biopolymer – elastin-like polypeptide (ELP) with controlled size and morphology for chemotherapy has been shown (Aniket et al., 2015b). More recently, this technique has seen application in the fabrication of biolasers for biosensing and optical device implantation purposes (Nguyen et al., 2019; Nguyen and Ta, 2020). In summary, this technique has demonstrated the potential as a novel drying technique on a wide range of enzymes without the incorporation of any excipients at room temperature. Further study is required to elucidate its application to large-scale biopharmaceutical manufacturing along with the time associated with the formation of Microglassified™ beads and their reconstitution. Moreover, the evaluation of Microglassification™ on commercial enzymes, mAbs, vaccines and the development of high concentration biopharmaceutical parenteral formulations are areas that can increase the scope of this technique.

2.3. Other Drying Technologies

In addition to some of the potential alternative drying technologies described in this review, other drying techniques such as Microwave drying, Foam drying, Vacuum drying, Supercritical Fluid drying, Electrospinning, Fluidized bed drying, Hybrid drying etc. are gradually gaining popularity as alternatives to Freeze-drying of biopharmaceuticals as well but are beyond the scope of this review. Recently, authors have demonstrated Microwave vacuum drying via REV™ technology to produce efficacious and stable biopharmaceuticals, including a live virus vaccine, with an 80 % reduction in the time associated with batch Freeze-drying (Bhambhani et al., 2021). Similar studies exploiting microwaves for the drying of mAbs, have been found in literature as Microwave-assisted Freeze-drying (Gitter et al., 2019, 2018). Moreover, various other microsphere generation technologies under evaluation have been listed in Table 3. For further reading on other drying technologies, readers are referred to the cited reviews herein (Emami et al., 2018; Lovalenti and Truong-Le, 2020; Pardeshi et al., 2021; Thorat et al., 2020; Vass et al., 2019; Walters et al., 2014; Durance et al., 2020).

3. Biopharmaceutical Characterization

As described in the ICH Q5E guidelines, product comparability subject to any changes in the manufacturer's manufacturing process requires the evaluation of the impact of an alternative process on the safety, quality and efficacy of biopharmaceutical products (ICH, 2004). Any aberrations in the CQAs of biopharmaceutical products post drying can be assessed using various analytical and characterization techniques. Fig. 15 shows a comprehensive list of techniques currently employed to study some of the product CQAs in the solid and liquid-state. Some of these techniques are not employed for routine analyses but can provide additional information in understanding the impact of CPPs on product CQAs.

While some chromatographic and spectroscopic techniques are employed as QC release tests, they fail to provide intricate and high-resolution information in understanding protein stability and their interactions with excipients in the solid-state. ssHDX-MS (Kammari and Topp, 2020; Moorthy et al., 2014; Wilson et al., 2019) and ssPL-MS (Iyer et al., 2016, 2013) are novel, high resolution mass spectrometric techniques that have provided further insights in understanding and elucidating protein stability. HDX-MS has been previously demonstrated to study the conformational stability of proteins in liquid solutions (Houde et al., 2011; Tsutsui and Wintrode, 2007; Wales and Engen, 2006), in frozen solutions (Zhang et al., 2012, 2011) and protein adsorption onto solid surfaces (Buijs et al., 2003, 2000, 1999; Zhang and Smith, 1993). One of the potential applications of this technique is predictive stability. Moreover, this technique offers an insight to study protein degradation in the solid-state which is a poorly understood area. In addition to the application of NMR in studying protein-excipient interactions (Mensink et al., 2016; Tian et al., 2007; Yoshioka et al., 2011), time-domain Nuclear Magnetic Resonance Spectroscopy (TD-NMR) has been demonstrated to determine the RMC in Freeze-dried biopharmaceuticals (Abraham et al., 2019a). Also, NMR coupled with Magnetic Resonance Imaging (MRI) has been studied to determine complete reconstitution of Freeze-dried products (Partridge et al., 2019). More recently, the determination of reconstitution time of Freeze-dried BSA has been demonstrated using fluorescence spectroscopy (ElKassas et al., 2021). Furthermore, advancements in CD spectroscopy have allowed researchers to study intricate information in the lower vacuum ultraviolet (VUV) region (<190 nm) using SRCD spectroscopy (Miles et al., 2008; Miles and Wallace, 2020, 2006; Wallace, 2019, 2009; Wallace et al., 2004). SRCD has also seen application in protein photostability,

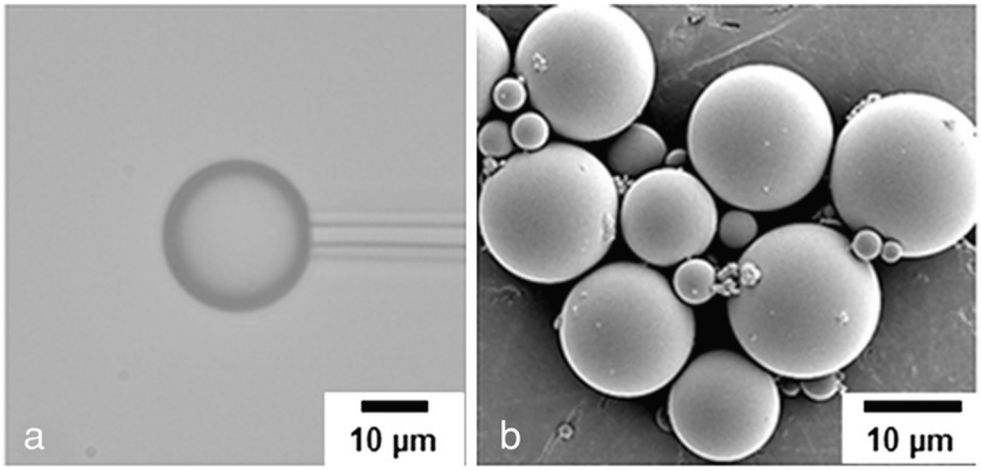

Fig. 14. (a) Optical image of Microglassified™ BSA in decanol. (b) SEM image of Microglassified™ beads. Reprinted (adapted) from (Aniket et al., 2014) with permission from Elsevier.

Table 3
Microsphere Technologies under evaluation for pharmaceuticals.

Microsphere Technology	Manufacturer	Reference
Q-Sphera™ Technology	MidaTech Pharma	(MidaTech Pharma, 2021; Seaman et al., 2019)
iSPHERE™ Technology	Pulmatrix	(Pulmatrix, 2021)
Kureha Microsphere Technology	Kureha	(Kureha, 2019; Vhora et al., 2019)
Plexis® Technology	Auritec Pharmaceuticals	(Auritec Pharmaceuticals, 2016; NCT03626714, 2019)
Stratum™ Technology	Orbis Biosciences	(Dormer and Berkland, 2016; Vhora et al., 2019)
FormEZE™ Microparticle Technology	Evonik Industries	(Evonik, 2015; Vhora et al., 2019)

photoisomerization, protein–ligand interactions and RNA characterization (Auvray et al., 2019; Hussain et al., 2018; Nasser et al., 2018; Wien et al., 2021).

Overall, the characterization techniques listed in Fig. 15 may have certain merits and demerits on their own but if employed as complimentary techniques, they can provide further insights on biopharmaceutical stability. These characterization techniques combined with different drying technologies can help the biopharmaceutical industry in choosing appropriate methods for manufacturing and testing their products.

4. Formulation aspects for Drying Technologies

Majority of the Freeze-dried biopharmaceutical formulations contain buffers, salts, amino acids, sugars, bulking agents, surfactants, tonicifiers, preservatives etc. (Bjelošević et al., 2020; Gervasi et al., 2018). Minimizing pH shifts, protein mobility in the solid-state and denaturation at air–liquid interfaces, increasing colloidal stability and providing increased solubility etc. are some of the key roles played by formulation components. Buffer salts such as sodium phosphate show a tendency to crystallize, thereby, causing pH shifts during freezing and depress critical temperatures of formulations crucial for Freeze-drying (Kolhe et al., 2009; Wu et al., 2015). These challenges may be eliminated for Spray-drying of formulations containing such buffers.

Some of the amorphous saccharides, provide improved stability for Freeze-dried and Spray-dried proteins (Carpenter et al., 1994; Chang et al., 2005a; Green and Angell, 1989; Kreilgaard et al., 1999). Trehalose, compared to sucrose, is less frequently used in Freeze-dried formulations but is the most preferred disaccharide for Spray-drying (Pinto et al., 2021). Trehalose can significantly protect biopharmaceuticals at higher temperatures during Spray-drying due to its high glass transition temperature (>100 °C) (Liao et al., 2004; Massant et al., 2020; Simperler et al., 2006). Authors have demonstrated that while sucrose preserves the protein's secondary structure during dehydration, trehalose provides protection during long-term storage of Freeze-dried and Spray-dried lysozyme (Starciuc et al., 2019). Apart from disaccharides, cyclodextrin is widely used in Spray-dried protein formulations (Pinto et al., 2021). A good stabilizing effect was observed at a 2:1 (protein : sugar) ratio, respectively and an optimized inclusion of both trehalose and sucrose could improve the overall stability of lysozyme (Liao et al., 2003, 2002). A 9:1:10 blend of mannitol, trehalose and lysozyme, respectively, exhibited higher bioactivity and stability post Spray-drying (Hulse et al., 2008). Moreover, the inclusion of ethanol as a co-solvent improved the aerosol performance of Spray-dried lysozyme by exhibiting a higher percentage of fine particle fraction (FPF) compared to the water-based lysozyme formulation (Ji et al., 2016). In spite of their benefits, it is important to note that trehalose and sucrose may crystalize in their frozen state as well as in their dried state during storage at accelerated temperature and moisture and cause damage to protein structure and stability (Singh, 2018; Singh et al., 2011). Therefore, biopharmaceutical products are processed and stored below their glass

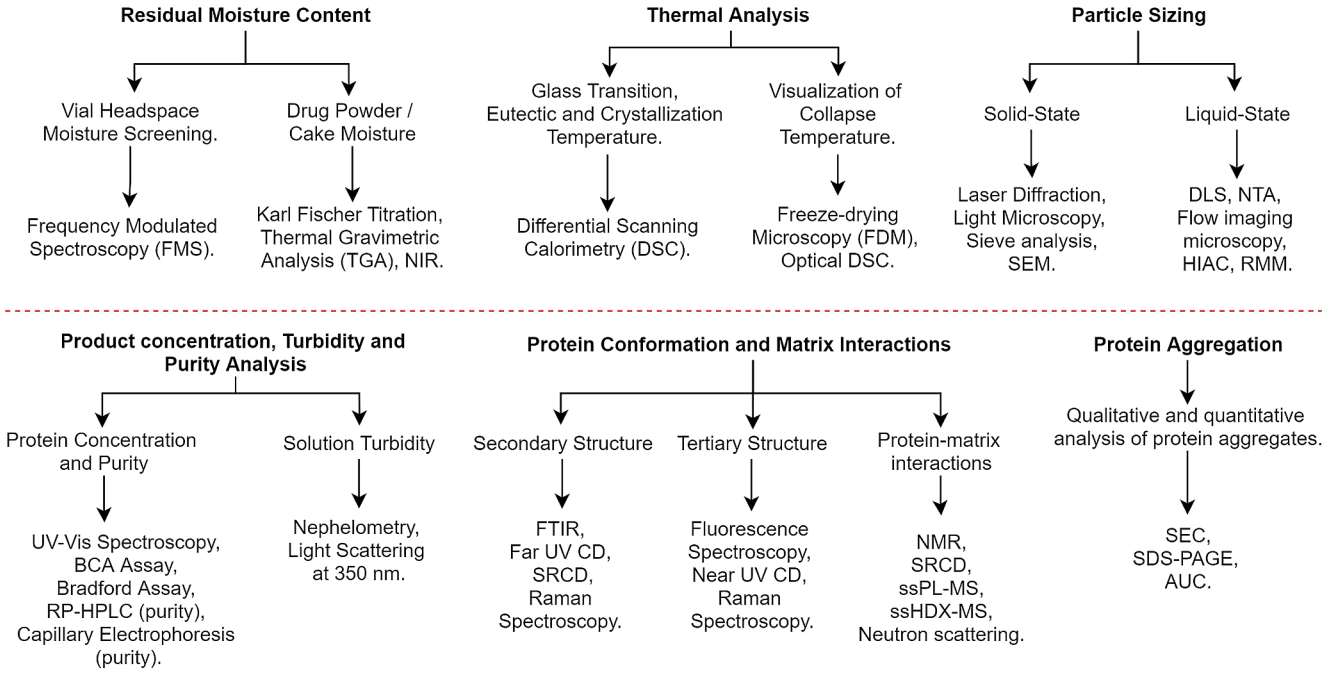

Fig. 15. Offline analytical and characterization techniques for biopharmaceutical products. AUC, Analytical Ultracentrifugation; BCA, Bicinchoninic acid Assay; CD, Circular Dichroism Spectroscopy; DLS, Dynamic Light Scattering; FTIR, Fourier Transform Infrared Spectroscopy; HIAC, High Accuracy Fluid Particle Counting; NIR, Near Infrared Spectroscopy; NMR, Nuclear Magnetic Resonance Spectroscopy; NTA, Nanoparticle Tracking Analysis; RMM, Residual Mass Measurement; RP-HPLC, Reverse Phase – High Performance Liquid Chromatography; SDS-PAGE, Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis; SEC, Size Exclusion Chromatography; SEM, Scanning Electron Microscopy; ssHDX-MS, solid-state Hydrogen Deuterium Exchange – Mass Spectrometry; ssPL, solid-state Photolytic Labelling; SRCD, Synchrotron Radiation Circular Dichroism Spectroscopy; UV–Vis, Ultraviolet–Visible Spectroscopy.

transition or eutectic temperature.

On the other hand, crystalline bulking agents such as mannitol and glycine may not necessarily confer protein stability but provide robust and elegance Freeze-dried cakes structures (Johnson et al., 2002; Peters et al., 2016; Varshney et al., 2007). Mannitol has also been reported to reduce the reconstitution times in high concentration Freeze-dried cakes (Kulkarni et al., 2018) and improve the aerosol performance of Spray-dried anti-IgE formulations and salbutamol (Costantino et al., 1998; Kaialy et al., 2010; Molina et al., 2019). Mannitol is the most popular monosaccharide in dried biopharmaceuticals (Gervasi et al., 2018; Pinto et al., 2021), although, crystallization of mannitol in the absence of amorphous stabilizers can negatively impact protein structure and stability. Crystallization of mannitol leads to phase separation that reduces the possible number of interactions between the protein and excipient, thereby, rendering the protein unstable (Wilson et al., 2019). Approximately, 20 – 40 % of sugar content is sufficient to safe guard the antibody in terms of its stability (Dani et al., 2007; Maury et al., 2005). In spite of studies carried out on the stability of antibodies, the effect of elevated temperature stress (>180 °C) on mAbs due to high inlet temperatures of large-scale Spray-dryers is still a concern. Bowen et al. conducted studies by testing different commercial mAb : trehalose formulations (2:1 and 1:2 ratio by weight, respectively) and stated that the percentage of monomers was lower in the Freeze-dried product even though the residual water content in the Freeze-dried product was quite low compared to the Spray-dried product (Bowen et al., 2013). A yield of > 95 % and improved storage stability was reported for commercial mAbs post Spray-drying (Gikanga et al., 2015). More recently, authors have shown comparable stability of sucrose-containing myoglobin and lysozyme post Freeze-drying and Spray-freeze-drying (Mutukuri et al., 2021). Along with trehalose, sucrose and mannitol, excipients such as polyethylenimine, hyaluronic acid, leucine, phenylalanine, arginine, cysteine, glycine etc. have been used for Spray-freeze-drying of some biopharmaceuticals (Adali et al., 2020; Chaurasiya and Zhao, 2020).

Amongst amino acids, L-arginine and L-arginine hydrochloride have been reported to increase protein stability and solubility and reduce the viscosity of protein solutions (Inoue et al., 2014; Shah et al., 2012; Stärtzel, 2018; Stärtzel et al., 2015). Interestingly, it has been reported that arginine along with other excipients and by itself in protein formulations is capable of acting as the main stabilizer (Baynes et al., 2005; Reslan et al., 2017; Shukla and Trout, 2011; Tsumoto et al., 2004). Moreover, the stabilizing effect of a large number of amino acids and their combinations were studied on Spray-dried catalase, lysozyme and Pandemrix influenza vaccine containing haemagglutinin (Ajmera and Scherließ, 2014). A combination of arginine, glycine, and protein in the ratio of [(1 + 1) + 1] resulted in a very good stabilizing effect post Spray-drying. Amongst the commonly used excipients for proteins, a combination of trehalose, arginine and protein in a ratio of 1:1 (excipient : protein) by weight improved the properties of Spray-dried mAbs in terms of reconstitution time and stability (Massant et al., 2020). Furthermore, leucine, isoleucine and trileucine have been reported to effectively protect the protein from sheer stress caused during atomization and improve powder flowability, dispersibility and aerosolization (Ganderton et al., 1999; Lechuga-Ballesteros et al., 2008; Schüle et al., 2008; Staniforth et al., 2001). While histidine is the most popular amino acid used in liquid and Freeze-dried protein formulation (Gervasi et al., 2018), leucine is the most preferred in Spray-dried protein formulations (Pinto et al., 2021).

In addition, surfactants play a major role in reducing aggregation due to protein exposure at air–liquid, solid–liquid and liquid–liquid interfaces (Chen et al., 2021; Chernysheva et al., 2018; Maa et al., 1998). These interfaces may be generated during freezing, atomization, reconstitution etc. Therefore, consideration to select appropriate excipients specific to the product and process right at the formulation development stage is crucial to ensure product stability. A summary of some of the commonly used excipients for Freeze-drying and Spray-drying has been described in Table 4, though further investigations are required to elucidate the mechanism of stabilization by excipients for other drying technologies.

5. Feasibility of PAT for Drying Technologies

As per the 'Pharmaceutical Development' ICH Q8(R2) guidelines, PAT is a QbD approach to design, analyse and control manufacturing (ICH, 2009). Several PATs have been explored in literature, however, some drawbacks associated with their feasibility during batch Freeze-drying have been identified. Most of the PATs provide an average result of the batch and cannot be implemented in-line, invasively or non-invasively for all individual product vials during processing. The risk of damage due to sterilization in the drying chamber makes these tools unfit for commercial cGMP cycles. On the contrary, most of these PATs can be potentially employable, in-line or at-line for unit doses or bulk product for some of the alternative drying technologies.

Typically, pressure and temperature sensors are used to monitor Freeze-drying cycles (Fissore et al., 2018; Nail et al., 2017; Nail and Johnson, 1992). This is essential for the development and optimization of Freeze-drying cycles and to account for the RMC in Freeze-dried cakes. Optical fiber sensors (OFS) (Kasper et al., 2013) and wireless data loggers such as temperature remote interrogation system (TEMPRIS) (Schneid and Gieseler, 2008) and TrackSense® (Ellab, 2020) are other available options for product temperature monitoring during Freeze-drying. Comparative measurements between the pirani gauge and the capacitance manometer, and between the temperature probes and the shelf temperature are used to determine the primary drying end-point (Fissore et al., 2018; Nail et al., 2017). In comparison, Tunable Diode Laser Absorption Spectroscopy (TDLAS) and Mass Spectrometry (MS) have been used as better alternatives (Ganguly et al., 2018; Gieseler et al., 2007; Patel et al., 2010a, 2010b). Along with estimating the primary drying end-point, vapour flow rate, average product temperature, heat transfer coefficient and mass transfer resistance, these tools can also be used to monitor the secondary drying end-point and RMC with high sensitivity and accuracy (Ganguly et al., 2018; Gieseler et al., 2007; Patel et al., 2010a; Schneid et al., 2009). Ganguly et al. showed that MS was highly sensitive to an average cake moisture of < 3 % in the late secondary drying phase (Ganguly et al., 2018). Additionally, MS can be used to detect silicon oil and helium gas leaks in a Freeze-dryer. In contrast to some of the drawbacks associated with these tools during batch Freeze-drying, TDLAS and MS can be potentially configured with most of the continuous drying technologies to estimate the RMC for all individual vials or bulk product.

More interestingly, authors explored potential applications of Near Infrared – Chemical Imaging (NIR-CI) and 4D Micro-Computed X-ray tomography and imaging for Spin-freeze-drying (Brouckaert et al., 2018; Goethals et al., 2020; Vanbillemont et al., 2020b). NIR-CI was able to capture different polymorphs of mannitol and the distribution of residual moisture in mannitol and mannitol-sucrose containing Spin-freeze-dried vials whereas 4D Micro-Computed X-ray tomography and imaging were able to detect intra-vial differences in the mass transfer resistance and primary drying end-point. Also, a NIR probe coupled to a FT-NIR analyser was connected to the vial holder to demonstrate the primary and secondary drying end-point (De Meyer et al., 2015). However, concerns relating to heat generation from NIR radiating halogen bulbs, mechanical challenges, and the feasibility of implementing this PAT in a cGMP environment need to be addressed. Moreover, Near Infrared – Frequency Modulated Spectroscopy (NIR-FMS) is a non-invasive, easy and a quick method for determining headspace oxygen and moisture levels in Freeze-dried vials (Cook and Ward, 2011a, 2011b; Lin and Hsu, 2002; Victor et al., 2017). Correlation observed between Karl Fisher analysis and NIR spectroscopy can make it easier to predict the RMC post drying (Affleck et al., 2021; Brouckaert et al., 2018; Carfagna et al., 2020). This technique can be employed in-line or at-line to analyze all dried vials or bulk product generated via some of the alternative drying technologies. Furthermore, NIR, MIR and Raman

Table 4
Key roles of some commonly used excipients for Freeze-drying and Spray-drying of biopharmaceuticals.

Excipients	Examples	Freeze-drying	Spray-drying
Amorphous saccharides	Sucrose	Preservation of protein secondary structure by glassy-state stabilization, H-bonding (Liao et al., 2003, 2002; Starciuc et al., 2020).	
	Trehalose	Protection during long-term storage by glassy-state stabilization, H-bonding and high glass transition temperature (>100 °C) (Liao et al., 2004; Massant et al., 2020; Simperler et al., 2006; Starciuc et al., 2020).	
	Raffinose	n/a	Glassy-state stabilization, high glass temperature (114 °C), increased fine particle fraction and aerosolization (Alhajj et al., 2021; Amaro et al., 2011; Zhao et al., 2018).
	Glucose		Glassy-state stabilization (Ying et al., 2012).
	Lactose	Reducing sugars not preferred due to pH shifts and Maillard reaction (Mensink et al., 2017).	Improved particle dispersibility (Horn et al., 2020; Pilcer et al., 2012; Seville et al., 2007) but high hygroscopicity (Hebbink and Dickhoff, 2019).
Polyols	Mannitol	Bulking agent and reduced reconstitution time (Kulkarni et al., 2018; Mehta et al., 2013).	Improved aerosol performance (Costantino et al., 1998; Kaialy et al., 2010; Molina et al., 2019).
	Sorbitol Glycerol	Plasticize α-motions but antiplasticize β-motions in combination with non-reducing amorphous sugars (Chang et al., 2005b; Cicerone and Soles, 2004).	
Amino Acids	Leucine, isoleucine, trileucine	n/a	Protection from atomization stress, improved powder flowability, dispersibility and aerosolization (Alhajj et al., 2021; Lechuga-Ballesteros et al., 2008; Seville et al., 2007).
	Arginine	Increased protein stability, solubility and reduced viscosity (Inoue et al., 2014; Shah et al., 2012; Stärtzel, 2018; Stärtzel et al., 2015).	Improved protein stability, reduced turbidity and reconstitution time (Ajmera and Scherließ, 2014; Massant et al., 2020).
	Glycine	Bulking agent (Varshney et al., 2007).	Improved protein activity and stability (Ajmera and Scherließ, 2014).
	Histidine	Amino-acid buffer (Al-hussein and Gieseler, 2013; Liao et al., 2013).	Improved protein activity and stability (Ajmera and Scherließ, 2014).
Surfactants	Non-ionic (Tween 80, 20)	Reduced air–liquid interfacial protein adsorption, reduced aggregation, improved protein refolding (Arsiccio and Pisano, 2018; Chernysheva et al., 2018; Maa et al., 1998).	
	Anionic (Sodium stearate, magnesium stearate)	n/a	Moisture protectant, improved pore formation and aerosolization (Parlati et al., 2009; Tewes et al., 2014; Yu et al., 2018).
	Pulmonary (DPPC, DSPC)	n/a	Improved aerosolization and surface enrichment properties (Cuvelier et al., 2015; Miller et al., 2015; Weers and Tarara, 2014).
Other polysaccharides	Cyclodextrin	Improved protein stability, elegant cake appearance (Haeuser et al., 2020).	Glassy-state stabilization, improved powder flowability, anti-hygroscopicity (Branchu et al., 1999; Serno et al., 2010; Zhao et al., 2018).
	Inulin	Improved protein stability (Hinrichs et al., 2001; Ke et al., 2020).	

DPPC, Dipalmitoyl phosphatidylcholine; DSPC, Distearoyl phosphatidylcholine; n/a, not applicable or not available.

spectroscopy have been used as potential PATs for process control and quality assurance of infant formula and dairy ingredients powders (Wang et al., 2018). NIR and Raman spectroscopy have also been employed in-line to study the protein conformational stability and aggregation (Nitika et al., 2021; Pieters et al., 2013, 2012). A NIR or a Raman probe can be positioned to analyze vials as they move across different drying chambers during continuous Freeze-drying of suspended vials. Similarly, a probe can be placed in-line at the different stages of Active-freeze-drying, Spray-drying, and Spray-freeze-drying or at-line during powder filling into unit doses. These PATs can also reduce batch release test time from fill finish to the market.

Particle size is one of the major CQAs for free-flowing powder-based products. Laser diffraction has been used both in-line and at-line to measure the particle size distributions during Spray-drying (Chan et al., 2008). Moreover, a variety of different PATs such as Spatial Filtering Velocimetry (SFV), Focused Beam Reflectance Measurements (FBRM), Photometric Stereo Imaging (PSI) and Eyecon® technology have been explored for analysing dry powder particle size (Silva et al., 2013). These PATs can be configured in-line or at-line for Active-freeze-drying, Spray-freeze-drying and PRINT® Technology to measure the particle size of dry powder. However, discrepancies observed in the particle size measurements of the PATs have been discussed by the authors (Silva et al., 2013) and so, users must consider pre-requisite knowledge on the theory, mechanism and equipment for appropriate results.

In summary, RMC, protein structural conformation and aggregation, particle size and polymorphism of excipients are some of the major CQAs for biopharmaceutical products that can be monitored using PATs. Monitoring CPPs such as product temperature, drying rate etc. are as important as product CQAs and so, some of the continuous drying technologies offer a greater advantage from PAT perspective. Table 5 summarizes the application of some PATs based on authors' assessments for only some of the drying technologies. The potential compatibility of these PATs with other drying technologies listed in Table 5 has not been found in literature and is based on opinion.

6. Scale-up, Packaging and Validation aspects for drying Technologies

Scale-up and technology transfer involve moving a pharmaceutical manufacturing process from one facility to another i.e. from a development / pilot-scale to a commercial-scale or an intra-site / inter-site fill finish line to line transfer. As per ICH Q12 guidelines, technology transfer may also be required for lifecycle changes across different commercial facilities (ICH, 2019).

Freeze-drying in vials requires the qualification of not only the drying process, but several other critical fill finish operations such as compounding, filtration and vial filling. This brings in further technical and compliance requirements such as mixing studies, filter bacterial retention, fill volume cycle development, media fill qualification and environmental monitoring (FDA, 2014a). Moreover, large loading times (4 – 12 h) for vial filling impacts process efficiency, may lead to issues such as product splashing and/or foaming and may also impact product

stability upon increased validated time out of refrigeration (Patel et al., 2017; Rathore and Rajan, 2008). Along with large loading times, large unloading times and vial inspection also impact the efficiency of commercial operations and slows the inventory turnover time in the most expensive footprint area of a commercial site i.e., controlled areas for aseptic fill finish. Such additional steps are eliminated for Active-freeze-drying, Spray-drying and Spray-freeze-drying technologies. These drying technologies can also provide the option for drug substance (DS) – drug product (DP) validation as continuous processes and eliminate the requirement for additional validation steps, thereby, minimizing the complexities associated with the regulatory filing and qualification of an end-to-end fill finish process (Pisano, 2020). Also, with the bulk product stored in the dried state as opposed to the liquid or frozen state, cold chain shipping validation of the bulk can be reduced. On the negative side, a more comprehensive cleaning validation for alternative drying technologies may be a requirement as per cGMP.

Furthermore, many of the Freeze-dried biopharmaceuticals such as Eloctate® (FDA, 2014c), Alprolix® (FDA, 2014d), Fabrazyme® (FDA, 2010) etc. are manufactured in multiple dose strengths. Batch Freeze-drying for multiple dose strengths require completely different fill finish processes with additional qualification and validation for primary packaging components supply chain, fill finish line equipment, sterilization methods, fill volume, Freeze-drying cycles, capping, inspection, container closure integrity etc. In contrast, alternative drying technologies such as Active-freeze-drying, Spray-drying, Spray-freeze-drying etc. make it easier to fill and pack free-flowing product into different container types such as vials, ampules, syringes, sachets etc. at multiple dose strengths. This also helps in simplifying infusion requirements at

Table 5
Potential / compatible PATs for drying technologies.

PAT	Application	Batch Freeze-drying	Active-freeze-drying	Spin-freeze drying	Spray-freeze-drying	Continuous Freeze-drying of suspended vials	Spray-drying	PRINT® Technology
Temperature Probes: • **Thermocouples and RTDs** (Fissore et al., 2018; Nail et al., 2017). • **Wireless Probes: TrackSense®, TEMPRIS** (Schneid and Gieseler, 2008; Ellab, 2020). • **Optical Fibers** (Kasper et al., 2013).	Average product temperature mapping.	Yes	Yes (RTDs can be installed At-line)	No	Yes (RTDs can be installed At-line)	Yes (Wireless probes can be installed in-line)	Yes (RTDs can be installed At-line)	n/a
	Product temperature mapping for all individual vials or bulk product.	No	Yes (At-line)	No	Yes (At-line)	No	Yes (At-line)	n/a
• **IR Thermography** (Harguindeguy and Fissore, 2021).	Product temperature mapping for individual vials or bulk product only within the camera's field of view.	Yes, but the center vials are calculated based on average.	Yes	Yes	Yes	Yes	Yes (At-line)	Yes (At-line)
• **NIR Spectroscopy** (De Beer et al., 2009; Mensink et al., 2015; Pieters et al., 2012; Wang et al., 2018). • **Raman Spectroscopy** (De Beer et al., 2009; Nitika et al., 2021; Pieters et al., 2013; Wang et al., 2018).	In-line protein structure analysis, protein aggregation and distribution of excipient polymorphs for all individual vials or bulk product.	No, but can provide an average measurement.	Yes	Yes	Yes (At-line)	Yes	Yes (At-line)	Yes (At-line)
• **NIR-FMS** (Carfagna et al., 2020; Cook and Ward, 2011a, 2011b; Lin and Hsu, 2002; Victor et al., 2017).	In-situ vial headspace oxygen and moisture measurements for all individual vials or bulk product.	No, but can provide an average measurement.	Yes	No	Yes (At-line)	Yes	Yes (At-line)	n/a
• **NIR-CI** (Brouckaert et al., 2018).	In-situ RMC estimation and distribution of excipient polymorphs in all individual vials or bulk product.	Yes	Yes	Yes	Yes	Yes	Yes (At-line)	Yes
• **TDLAS** (Gieseler et al., 2007; Kessler et al., 2006; Kuu et al., 2011, 2009; Schneid et al., 2011, 2009; Sharma et al., 2019). • **MS** (Fissore et al., 2018; Ganguly et al., 2018; Nail et al., 2017).	In-situ RMC, drying end point, vapor flow rate estimation for all individual vials or bulk product.	No, but can provide an average measurement.	Yes	Yes	Yes	Yes	Yes	Yes
• **MS** (Barfuss, 2014; Connelly and Welch, 1993; Ganguly et al., 2018).	In-line silicon oil or gas leak detection.	Yes	Yes	Yes	Yes	Yes	Yes	Yes
• **Laser Diffraction** • **Light Microscopy** • **SFV** • **PSI** • **FBRM** • **Eyecon®** (Chan et al., 2008; Dos Reis et al., 2021; Petrak et al., 2018; Silva et al., 2013).	In-line / at-line dry powder particle sizing.	n/a	Yes	n/a	Yes	n/a	Yes	Yes

RTDs, Resistance Temperature Detectors; n/a, not applicable or not available.

clinics.

To enable successful scale-up and technology transfer, drying processes require a QbD approach where the process boundaries are well defined and provide adequate robustness for commercial operations (Nail and Searles, 2008). Small scale process modelling for Freeze-drying is complex as each individual vial behaves as its own drying system, subject to variability, as a function of vial heat transfer coefficient, freezing temperature and location on the shelf. Active-freeze-drying, Spray-drying and Spray-freeze-drying, for example, are not as hindered by the challenges of drying in individual containers as they provide a more predictive and consistent manufacturing performance.

In terms of scalability, moving an existing commercial product from a batch Freeze-drying process to an alternative drying process is regarded as a major regulatory change and comprehensive comparability data would be required as part of implementation and approval (Pisano, 2020). The significant cost of biopharmaceutical DS, commercial line time for engineering, validation batches, long term stability data and the requirement for filing a change mean any efficiency gain obtained by alternative drying processes may be offset by such costs. Overall, the most viable route to introduce alternative drying processes may very well be on the back of the development and industrialization of new products.

7. Conclusion and Future Directions

At present, batch Freeze-drying is a well-established drying technology for the majority of biopharmaceutical products. Many of the alternative drying technologies have increasingly shown promising prospects for manufacturing solid biopharmaceuticals without compromising on the safety, quality and efficacy of biopharmaceutical products. These potential drying technologies are significant to the biopharmaceutical industry as they will not only reduce time, energy consumption and associated costs with the manufacturing of life-saving drugs but also help in mitigating any risks with the supply of drugs during pandemics such as Covid-19. While some of the alternative methods offer continuous manufacturing at reduced operational costs, the impact of CPPs such as temperature, shear, etc. on product CQAs is the fundamental requirement for the selection of drying technologies. Although drying technologies, namely, Spin-freeze-drying, Spray-freeze-drying, Spray-drying, PRINT® and Microglassification™ have shown positive results on the stability of some proteins and inhaled biopharmaceuticals, their impact on a wide range of parenteral biopharmaceuticals is yet to be studied. Through product-specific research, sufficient stability data is required to move from conventional Freeze-drying to continuous manufacturing. Along with CPPs, the choice of formulation components with respect to the drying process as well as the product is crucial to ensure product stability. Moreover, the molecular mechanism of interaction of biopharmaceuticals with specific excipients in the solid-state is poorly understood. Some of the advanced characterization techniques and PATs in tandem can offer faster and in-depth analysis in understanding and evaluating the product-process relationship. While most of the alternative drying methods can offer significant benefits with the usage of PATs, their feasibility at commercial scale requires further exploration. In terms of scale-up, packaging and validation aspects, some of the alternative drying processes offer a greater advantage in reducing the complexities associated with the validation of multiple fill finish unit operations. The commercial scale operation for some alternative drying technologies has been demonstrated with proven potential in the biopharmaceutical industry though some scale-up challenges are yet to be addressed.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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