Sphärische Lyophilisate zur pulmonalen Proteinapplikation

Dissertation

zur Erlangung des Doktorgrades (Dr. rer. nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

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Bonn, 2017

Angefertigt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn.

Erster Gutachter:	Prof. Dr. A. Lamprecht
Zweiter Gutachter:	Prof. Dr. R. Süverkrüp
Tag der Promotion:	03. September 2018
Erscheinungsjahr:	2019

ACKNOWLEDGEMENT

The work for the present thesis has been performed between February 2013 and December 2016 at the Department for Pharmaceutical Technology and Biopharmacy, Rheinische Friedrich-Wilhelms-Universität Bonn.

This thesis would not have been successful without the guidance from Dr. Alf Lamprecht. Thank you for the highly interesting topic, the constant interest and support in the progress of the project and the excellent working conditions at the department.

I would like to thank the Deutsche Forschungsgemeinschaft (DFG) for financing this project within the framework of the SPP 1423 (grant. No. LA1362/2-1).

Prof. Dr. Richard Süverkrüp for all the hours in the laboratory, the brilliant ideas and very competent advice regarding practical and theoretical work and the extremely interesting and entertaining talks, which had a very positive impact on this thesis.

Within the Department of Pharmaceutical Technology I would first of all like to thank Kai Berkenfeld for the interesting professional and private discussions we had and all that amazing time we spent or wakeboarding on the Bleibtreusee. Huge thanks to Anne Cossé, Bernadette Kettel and Fabian Simons for the great times we had in southern Germany – Rock ‘n’ Roll! Also very special thanks to the members of “MC Techno” Stefan Lorscheidt and Dr. Florian Schorr - may the Eifel will fear us again one day! I also would like to thank Dr. Sarah Barthold, Dr. Maryam Shetab Bousheri and Henusha Jhundoo for proof reading my work. Martina Gerlitz for all her administrative support around the university, scheduling appointments with our professors and everything else! Also many thanks to my colleagues Dr. Ehab Ali, Esther Bochmann, Katrin Grüneberg, Veronika Hagelstein, Ozan Hirlak, Bashar Ibraheem, Markus Jäger, Alvaro Lopez Marmol, Dr. Julia Potschadel, Simone Putzke, Markus Ries, Anna Schlüter, Dr. Thomas Schmal, Mert Serim and Kristina Steffens for the friendly atmosphere in the department.

Acknowledgment

I would like to further address sincere thanks to Franz-Josef Willems, Thomas Vidua, Jürgen Hohmann and their workshops for the amazing support concerning technical and mechanical challenges.

The work of the students of pharmacy Farieba Rahmaty, Cedric Tuch, Elli Husso, Johanna Lübbert and Ilona Janzen also is acknowledged.

I especially want to emphasize my deepest gratitude to Pia Steinlein. Thank you for being brave enough to join me at the “Kerb” in Münter-Sarmsheim. Thank your for all the after-work hangouts which made me forget all worries on stressful days. Thank you for your open ears, your precious advice (which I should have maybe followed a little bit more) and of course all the laughter. The past years were a blast and I am truly thankful for almost all those memories we now share.

Outside the University I am much obliged to the “Minschterer Bube” Eric, Isa, Johannes and Maxim for all those fun times we had in Mü-Sa and to the “Allgäuer Bube” Eder, Hubsi, Max and Schützi making Oberstdorf my second home. I especially want to thank Tim Escher for all of those endless days on the mountain. Ride in heaven my friend - we truly miss you.

Above all, I am grateful to my parents Dr. Martin and Petra Wanning for their endless support and advice. Sincere gratitude to my brothers Andreas and Markus for every single creative, funny or crazy moment we had together. If we stick together, there is nothing that will ever stop us.

For family,
for friends,
and of course: for me.

PUBLICATIONS

The following research papers, reviews and conference posters were published in context of this thesis.

CONFERENCE POSTERS:

Serim, T.M., Wanning, S., Lamprecht, A., 2014. Determination of Residual Moisture in Spray-Freeze-Dried Particles and Optimization of the Freeze Drying Time. *Presented at the AAPS Annual Meeting and Exposition;* 2014, San Diego, CA, USA.

Wanning, S., Süverkrüp, R., Lamprecht, A., 2014. Aerodynamic droplet stream expansion for the production of uniform pulmonary low density spherolyophilisates. *Presented at the AAPS Annual Meeting and Exposition;* 2014, San Diego, CA, USA.

RESEARCH PAPERS, REVIEW ARTICLES AND BOOK CHAPTERS

Wanning, S., Süverkrüp, R., Lamprecht, A., 2015. Pharmaceutical spray freeze drying. *Int. J. Pharm.* 488, 136–153.

Süverkrüp, R., Eggerstedt, S., Wanning, S., Kuschel, M., Sommerfeld, M., Lamprecht, A., 2016. Collisions and coalescence in droplet streams for the production of freeze-dried powders. *Colloids Surf B* 141, 443–449

Wanning, S., Jäger M., Eggerstedt S.N., Süverkrüp R., Lamprecht A., Dietzel M., Kuschel M., Darvan A., Sommerfeld M., 2016. Droplet-Stream Freze Drying for the Production of Protein-Formulations - From Simulation to Production -. *Process Spray, Springer International Publishing*, 1st Ed. p314-381

R. Süverkrüp, S. Wanning, A. Lamprecht:, 2016. Continuous Atmospheric Droplet-Stream Freeze-Drying Processes . *Pharmind* 78; 741-749.

Wanning, S., Süverkrüp, R., Lamprecht, A., 2016. Aerodynamic Droplet Stream Expansion for the Production of Spray-Freeze-Dried Powders. *AAPS Pharm Sci Tech.*

Wanning, S., Süverkrüp, R., Lamprecht, A., 2017. Jet-vortex spray freeze drying for the production of inhalable lyophilisate powders. *Eu J Pharm Sci* 96, 1–7.

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INTRODUCTION

NEW BIOLOGICAL ENTITIES

New biological entities (NBE, or short: biopharmaceuticals or biologicals) are proteins, peptides, antibodies, tumor necrosis factors, interferones, interleukines and vaccines, which are used to treat or prevent diseases. They are completely or almost completely identical with endogenous proteins/peptides of the human body, mostly used for substitution of such (tumor necrosis factors, monoclonal antibodies, insulin, and more). NBEs can either be isolated from human, animal or microorganismic sources and may be yielded by biotechnological methods. The development and approval of NBEs has constantly risen over the past 10 years and has had a peak in 2015 with 45 approvals by the Food and Drug Administration (FDA) in the United States. 36 % of the biologicals approved in 2015 are 'first in class' which reflects the innovation potential that lies within the treatment with NBEs (Sargent, 2016). In Germany, sales of biopharmaceuticals increased by 9.7 % and amounted to € 8.2 billion compared to 2014. They had a share of 22.9 % of the total pharmaceutical market in Germany. Of the 50 newly approved drugs in Germany 2015, 15 were biopharmaceutical products, which is a new record. This trend will probably continue, as the number of biologicals in clinical development increased from 604 to 627 with a strong increase in phase I (+11 %). (Lücke et al., 2016). These numbers show that development of NBEs and their formulation into medicinal products is on the rise and a promising future market.

ROUTES OF ADMINISTRATION FOR BIOPHARMACEUTICAL PRODUCTS

Compared to new chemical entities, biologicals have a relatively large molecular mass and are often only bioavailable after administration by intravenous injection. It is of general interest to find new ways for the delivery of macromolecular drugs, as they may facilitate the administration process and increase the patient's compliance. Intradermal needle-free injection (Schiffter et al., 2010), nasal (Garmise et al., 2007), pulmonary (Maa et al., 1999) and ophthalmic delivery (Süverkrüp et al., 2009) have been discussed as possible options for

local and systemic drug delivery. Of the alternative routes of administration, the pulmonary pathway is highly interesting for protein delivery. It offers a large surface area (> 80-120 m^2), good vascularization and a low thickness of the alveolar epithelium, which facilitates drug transport (Scheuch and Siekmeier, 2007). It has been reported that this route has a low amount of metabolic enzymes, bypasses the hepatic first past effect and provides very fast absorption rates (Patton, 2004; Patton et al., 1999).

The systemic delivery of proteins via the lung provides a higher bioavailability than any noninvasive route of administration for reasons not yet fully understood (Patton, 2004). Efforts were made by Pfizer to establish an inhalable insulin (Exubera®) on the global drug market, but due to the large dimensions and complicated handling of the inhaler, compliance and acceptance among patients was very low. Exubera® was discontinued, as the sales expectations were not met.

With Afrezza®, a promising inhalative insulin treatment based on the Pulmosphere® technique has been developed and marketed by Mannkind and Sanofi Aventis and was approved by the FDA in June 2014. Due to the highly porous and lightweight characteristics of the particles, the inhaler could be kept simple and small. In June 2016, Sanofi stopped support for Afrezza® due to profits below expectation. Mannkind still continues Afrezza®, as they strongly believe that a change in patients' habits is a slow but constant process. They are currently adjusting and improving the Pulmosphere® technology to allow the administration of other APIs (*e.g.* epinephrine).

DEVELOPMENT OF A NOVEL SPRAY-FREEZE-DRYING METHOD

Gruner and Süverkrüp first studied spray-freeze-drying (SFD) at the University of Bonn while working on lyophilisates for ophthalmic application (Gruner, 2008). They injected a monodisperse droplet-stream into a cold gaseous atmosphere which yielded frozen droplets that were then dried by sublimation. This gave the idea of developing an inhalable lyophilized powder, which consisted of highly porous and monodisperse particles that may further reduce the product related variability. A patent application for this method was filed in 2008 and published in 2010 (Süverkrüp, 2010). In the following years, Eggerstedt

characterized this method and its products. He compared spray lyophilization to conventional spray-drying (Eggerstedt and Lamprecht, 2012), investigated protein stability in droplet stream freezing and the influence of formulation composition on particle properties such as particle size and surface morphology (Eggerstedt et al., 2012), and studied droplet collisions in fast droplet streams (Baroud et al., 2012). This phenomenon has been investigated in detail later (Süverkrüp et al., 2016, 2013). Based on these findings, it was suggested to inject the droplet stream into a cross-flowing gas stream inside a swirl tube to increase the horizontal distance between the droplets and accelerate droplet freezing to prevent coalescence. First proof of concept experiments showed that the setup successfully led to an expansion of the droplet stream and smaller median particle sizes. A patent application was filed in 2015 (Süverkrüp, 2015).

AIM AND SCOPE OF THIS THESIS

This work focuses on the development of an aerodynamic droplet stream expansion method to prevent droplet collisions and yield lyophilized powders which are applicable to the lung. It consists of three main parts, each of which has two chapters. The first part elucidates the theoretical background. The second and third parts focus on process optimization and formulation characterization respectively. To conclude, a chapter on atmospheric freeze-drying gives a brief outlook on the future of the project.

The first chapter was published as a review entitled “Pharmaceutical Spray Freeze Drying” (Wanning et al., 2015) and gives an extended overview over the current state of research in the field of spray-freeze-drying. Within this section, major SFD techniques and fields of application for spray-lyophilized products are presented and reviewed in detail. A book chapter that has been published separately (Wanning et al., 2016a) describes the achievements on implementing the droplet-stream freeze drying technique at the University of Bonn. It focuses on giving a brief summary of the technical development of spray-freeze drying apparatuses throughout the thesis and the overall spray-freeze drying project. Droplet-stream freeze-drying proved to be suitable for the production of spherical lyophilisate particles containing protein based active pharmaceutical ingredients (APIs). However, the

median particle size was larger than expected and droplet collisions prior congelation has been identified by high-speed imaging as the root cause for this phenomenon.

As the aim of this thesis was the development and generation of an inhalable SFD powder, it is of the utmost importance to keep the droplet and particle size as low as possible. Therefore, it was sought to establish a method to prevent the aforementioned droplet collisions and thus reduce the particle size. The second chapter introduces and adds a swirl tube to the spray freezing apparatus, in which the droplets are to be deflected from their original trajectory by two cross-flowing tangential streams of cold process gas. It was assumed that the increase in distance between the unfrozen droplets would reduce the incidence of detrimental droplet collisions, as well as the faster freezing process prevent droplet-merging. The influence of the horizontal and vertical droplet stream generator position and the total airflow on gas velocity in the vortex and the median particle size was studied. Furthermore, efforts were made to implement a screening method for the fast evaluation of various factorial levels (Wanning et al., 2016b), as complete SFD cycles are time consuming, due to the long freeze-drying step.

The next step was to improve the spray-freeze-drying apparatus based on the findings of the previous chapter (Wanning et al., 2017) to enable the generation of small, inhalable spherolyophilisates with low inter-batch variability, so further experiments with a focus on formulation development can be carried out. In chapter four, experiments were carried out to find the ideal combination of two factors (horizontal position distance of the DSG to the gas-jet nozzle and gas flow of the vortex) with regard to four target parameters, namely the median particle size, the width of the particle size distribution, the inter-batch variability and the fine particle fraction (FPF).

It was hypothesized that besides optimizing the process parameters and SFD device, further improvements regarding the aerodynamic behavior as well as the handling characteristics and storage stability of the SFD powder could be achieved by adjusting the excipient composition in the spray solution. It was expected that the mechanical robustness might decrease with increasing porosity of the particles. As literature describes the stabilizing effect of PVP in freeze-dried cakes, various polymers were tested as stabilizer in the final composition to improve the mechanical stability, as already described for freeze-dried cakes. The fourth

chapter thus describes the screening of various formulations for spray-freeze drying which consist of a mono/dimeric- and/or a polymeric constituent in graded concentrations.

The effect of the formulation composition on the spray-freeze drying process and the lyophilized powders was assessed. The dried particles were additionally characterized regarding their morphological properties, particle size, relative mechanical strength and their aerodynamic performance in cascade impaction experiments. In the subsequent chapter, these powders were studied under variable humidity levels in dynamic-vapor-sorption analysis and cascade impaction experiments with a focus on the influence of the relative humidity on the powder's aerodynamic performance and the particle's structural integrity.

In the 6th chapter, the SFD apparatus is modified to allow atmospheric freeze-drying. First 'proof of concept' experiments are carried out and suggestions for future studies are made regarding drying conditions.

REFERENCES

Baroud, Y., Eggerstedt, S.N., Klaiber, M., Süverkrüp, R., Simon, S., Lamprecht, A., 2012. Monitoring of Collisions in Fast Droplet Streams by Real-Time Image Processing with Line Sensors. ILASS-Americas 24th Annual Conference on Liquid Atomization and Spray Systems.

Eggerstedt, S.N., Dietzel, M., Sommerfeld, M., Süverkrüp, R., Lamprecht, A., 2012. Protein spheres prepared by drop jet freeze drying. Int. J. Pharm. 438, 160–166.

Eggerstedt, S.N., Lamprecht, Alf, 2012. Spray Freeze Dried versus Spray Dried Particles for Pulmonary Delivery. Presented at the AAPS Annual Meeting and Exposition, Chicago, Illinois, USA.

Garmise, R.J., Staats, H.F., Hickey, A.J., 2007. Novel dry powder preparations of whole inactivated influenza virus for nasal vaccination. AAPS PharmSciTech 8, 2–10.

Gruner, K., 2008. Tröpfchenlyophilisate für die Augenheilkunde. Rheinische Friedrich-Wilhelms-Universität Bonn.

Lücke, J., Bädeker, M., Hildinger, M., 2016. BCG Report 2016 [WWW Document]. URL https://www.vfa-bio.de/download/bcg-report-2016.pdf (accessed 11.29.16).

Maa, Y.-F., Nguyen, P.-A., Sweeney, T., Shire, S.J., Hsu, C.C., 1999. Protein inhalation powders: spray drying vs spray freeze drying. Pharm. Res. 16, 249–254.

Patton, J.S., 2004. The Lungs as a Portal of Entry for Systemic Drug Delivery. Proc. Am. Thorac. Soc. 1, 338–344.

Patton, J.S., Bukar, J., Nagarajan, S., 1999. Inhaled insulin. Adv. Drug Deliv. Rev. 35, 235–247.

Sargent, B., 2016. 2015 FDA New Drug Approvals – 13 Biologics Receive Approval [WWW Document]. Cell Cult. Dish. URL http://cellculturedish.com/2016/02/2015-fda-new-drug-approvals-13-biologics-receive-approval-2/ (accessed 21.10.16)

Scheuch, G., Siekmeier, R., 2007. Novel approaches to enhance pulmonary delivery of proteins and peptides. J. Physiol. Pharmacol. 58, 615–625.

Schiffter, H., Condliffe, J., Vonhoff, S., 2010. Spray-freeze-drying of nanosuspensions: the manufacture of insulin particles for needle-free ballistic powder delivery. J. R. Soc. Interface 7, S483–S500.

Süverkrüp, R., 2015. Vorrichtung und Verfahren zur Erzeugung monodisperser gefrorener Tropfen. DE 102015105375 A1.

Süverkrüp, R., 2010. Vorrichtung und Verfahren zur Herstellung eines pulverförmigen, lyophilisierten Materials, bestehend aus annähernd gleich großen Teilchen. 10 2008 017 461.

Süverkrüp, R., Eggerstedt, S., Wanning, S., Kuschel, M., Sommerfeld, M., Lamprecht, A., 2016. Collisions and coalescence in droplet streams for the production of freeze-dried powders. Colloids Surf B 141, 443–449.

Süverkrüp, R., Eggerstedt, S.N., Gruner, K., Kuschel, M., Sommerfeld, M., Lamprecht, A.,

2013. Collisions in fast droplet streams for the production of spherolyophilisates. Eur. J. Pharm. Sci. 49, 535–541.

Süverkrüp, R., Gruner, K., Diestelhorst, M., 2009. Spherolyophilizates as Ophthalmic Drug Delivery Systems. Invest. Ophthalmol. Vis. Sci. 50, 5557–5557.

Wanning, S., Jäger, M., Eggerstedt, S.N., Süverkrüp, R., Lamprecht, A., Dietzel, M., Kuschel, M., Darvan, A., Sommerfeld, M., 2016a. Droplet-Stream Freeze-Drying for the Production of Protein Formulations: From Simulation to Production, in: Fritsching, U. (Ed.), Process-Spray. Springer International Publishing, pp. 341–381.

Wanning, S., Süverkrüp, R., Lamprecht, A., 2017. Jet-vortex spray freeze drying for the production of inhalable lyophilisate powders. Eu J Pharm Sci 96, 1–7.

Wanning, S., Süverkrüp, R., Lamprecht, A., 2016b. Aerodynamic Droplet Stream Expansion for the Production of Spray-Freeze-Dried Powders. AAPS Pharm Sci Tech.

Wanning, S., Süverkrüp, R., Lamprecht, A., 2015. Pharmaceutical spray freeze drying. Int. J. Pharm. 488, 136–153.

PHARMACEUTICAL SPRAY-FREEZE-DRYING

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This chapter was published as follows:

'Pharmaceutical Spray-Freeze-Drying'

Wanning S., Süverkrüp R., Lamprecht A., *International Journal of Pharmaceutics*, 488, 1-2, p136-153

1.1 ABSTRACT

Pharmaceutical spray-freeze-drying (SFD) includes a heterogeneous set of technologies with primary applications in apparent solubility enhancement, pulmonary drug delivery, intradermal ballistic administration and delivery of vaccines to the nasal mucosa. The methods comprise three steps: droplet generation, freezing and sublimation drying, which can be matched to the requirements given by the dosage form and route of administration. The objectives, various methods and physicochemical and pharmacological outcomes have been reviewed with a scope including related fields of science and technology.

Keywords: Spray freeze drying, Protein formulations, lyophilisation, porous particles, pulmonary application, vaccination

1.2 WHY TO SPRAY-FREEZE-DRY MEDICINAL PRODUCTS

Since its introduction by Werly in 1964, 'spray-freeze-drying'(SFD) has attracted much interest in various areas of research, though for the fulfillment of different objectives. The process has been widely used in pharmaceutical research, as well as food science & technology (Ishwarya et al. 2015).

In this review, we aim to provide an overview about the potentials of SFD for the development of pharmaceutical products. We will discuss the main steps involved within the production process (i.e. spraying, freezing and drying) and introduce, describe, and evaluate different available technical approaches related to each step. The findings of the reviewed papers are then discussed in terms of physical and therapeutic characteristics and are subsequently evaluated with regard to the intended pharmaceutical application.

Different approaches have been developed to enable the delivery of biologicals to the body. In addition to the well-known routes of administrations, less conventional pathways such as the pulmonary and nasal routes and delivery to the epidermis by needle-free injection have been investigated. The prime goal of exploring such administration pathways is to develop alternatives to parenteral injection (Schiffter et al. 2010, Klingler et al. 2009, Bi et al. 2008) and to enhance the drug targeting potential (Roa et al. 2011, Gao et al. 2011).

As an approach facilitating the development of dosage forms for alternative delivery pathways, SFD is preferred over classical spray-drying (SD) or freeze-drying (FD) for various reasons. Firstly, using SFD methods can enhance the apparent solubility of poorly water-soluble drugs which is a common problem with newly developed active pharmaceutical ingredients (API) (Vu et al. 2013). Additionally, due to an ultra-fast freezing process, the drug is embedded amorphously in the excipient thereby minimising the possibility of phase separation between drug and excipients and therefore leading to a molecular distribution of the drug in the excipient material.

Within the context of delivering biologicals as sustained release injectables, some research groups have used SFD for preprocessing the protein/peptide ingredient prior to encapsulation in poly(lactic-co-glycolic acid) (PLGA) microspheres. Others have used the process to enable pulmonary, nasal, and needle-free epidermal drug delivery (*e.g.* non-invasive vaccination). Biologicals are usually freeze-dried in vials to enhance storage stability, but when aiming for

the latter applications, development of a flowable powder is mandatory. SFD offers the possibility to produce such powders with controlled particle-size distributions, and is hence more favourable than classic FD. On the other hand, SFD is preferable over SD due to the possibility of processing thermo-sensitive ingredients (Cheow et al. 2011) and the improvement of the reconstitution characteristics of polymeric nanoparticles (Ali and Lamprecht 2014). Furthermore, SFD is economically preferable over the conventional FD in vials. Lyophilisation of spray-frozen products is more favourable than that of parenteral formulations in vials both in terms of time and energy consumption (Bosshammer 2014, Claussen et al. 2007). Moreover, SFD allows the production of a flowable bulk ware. This leads to an enormous increase in flexibility of a production site, as the dosage in vials can be adjusted very easily. Table 1-1 presents an overview of different applications of SFD techniques.

Table 1-1 Usage of spray-freeze-drying to improve drug formulation processes

Route of Delivery/Purpose	Active or model ingredients	Researcher
Apparent solubility enhancement	Danazol	(Hu et al., 2004a; Rogers et al., 2003b, 2003c, 2002b)
	Carbamazepine	(Rogers et al., 2002a)
	Phenytoin	(Niwa et al., 2009)
	Ciclosporine	(Niwa et al., 2012, 2010)
	Tolbutamide	(Kondo et al., 2011, 2009a)
	Oleanolic acid	(Tong et al., 2011)
	Baicalein	(He et al., 2011; Schiffter et al., 2010)
Preprocessing for sustained release injectables	recombinant human growth hormone (rhGH)	(Costantino et al., 2004; Johnson et al., 1997; Kennedy et al., 2008)
	recombinant human insulin like growth factor-I (rhIGF-I)	(Lam et al., 2000)
	recombinant human vascular endothelian growth factor (rhVEGF)	(Cleland et al., 2001; Daugherty et al., 2011)
	recombinant human nerve grow factor (rhNEGF)	(Lam et al., 2001)
	bovine serum albumin (BSA)	(Carrasquillo et al., 2001; Leach et al., 2005)
Pulmonary (local treatment)	rhDNAse	(Maa et al., 1999)
	bovine DNAse	(Zijlstra et al., 2009)
	Anti-IgE monoclonal antibodies	(Maa et al., 1999)
	Ciclosporine	(Zijlstra et al., 2007)
	Ciprofloxacin	(Sweeney et al., 2005)
	Rifampicin	(Ohashi et al., 2009)
	Rapamycin	(Carvalho et al., 2014)
	Kanamycin	(Her et al., 2010)
	D^9-Tetrahydrocannabinol	(van Drooge et al., 2006)
	Doxorubicin nanoparticles	(Roa et al., 2011)
	Salbutamol sulphate	(Mueannoom et al., 2012)
	Terbutalin sulphate	(Sharma et al., 2013)

Table 1-1 (continued): Usage of spray-freeze-drying to improve drug formulation processes

Route of Delivery/Purpose	Active or model ingredients	Researcher
	Voriconazole	(Beinborn, 2012)
	Itraconazole	(Vaughn et al., 2007)
	Tacrolimus	(Watts et al., 2013)
Pulmonary	Cetrorelix	(Zijlstra et al., 2004)
(systemic delivery)	liposomal insulin	(Bi et al., 2008)
	pCMV-Luc plasmid DNA	(Mohri et al., 2010)
	Influenza vaccine	(Amorij et al., 2007; Saluja et al., 2010)
Nasal	Anthrax vaccine (antigens)	(Jiang et al., 2006; Mikszta et al., 2005; S. H. Wang et al., 2012)
	Influenza vaccine	(Garmise et al., 2007, 2006)
	Plague vaccine	(Huang et al., 2009)
Epidermal	Influenza vaccine	(Amorij et al., 2008; Dean and Chen, 2004; Maa et al., 2004)
(needle-free injection)		
	Diphteria toxoid	(Maa et al., 2003)
	Tetanus toxoid	(Maa et al., 2003)
	Hepatitis B vaccine	(Maa et al., 2003)
	Insulin	(Schiffter et al., 2010)
Colonic	Lipid-polymer composite microspheres	(Gao et al., 2011)
Ophthalmic	Sodium-fluorescein	(Süverkrüp et al., 2009)

1.3 HOW TO SPRAY-FREEZE-DRY MEDICINAL PRODUCTS

The term ‘spray-freeze-drying’ (SFD) refers to processes with the following three steps in common:

- dispersion of bulk liquid solutions into droplets,
- droplet freezing, and
- sublimation drying of the frozen material which may comprise particles or a film that can be subsequently pulverised.

Obviously, some aspects of SFD are closely related to SD and lyophilisation operations which are widely employed in both pharmaceutical and food industries. However the intricate interactions between rheological and surface phenomena with the transfer of matter and energy and fast transitions from the liquid to the solid and from the solid to the gaseous phase generate opportunities for new products with unique features but also new challenges, particularly with respect to current good manufacturing practices (GMP) and process analytical technologies. Similar combinations of spray-congealing and drying operations have been in use for some time in the production of uniform spherical particles for fertilizers, detergents and explosives, where the process is known as ‘prilling’.

In contrast to SD, where the size and shape of particles emerge upon drying, the size and essential features of the internal structure of lyophilised spherules originate from the freezing step, and with qualifications this holds also for the surface morphology. When droplets are frozen in flight, the particle size is nearly equal to that of the droplet. The surface of dried particles is spherical, usually covered with a smooth shell, which may be partially or completely missing, so that the irregular honeycomb of the internal structure becomes visible. Due to the high specific surface area, the maximal diffusion path length of solvent molecules is short and in combination with the absence of container walls, which impede the transport of matter and energy, the drying step is much faster than for the lyophilisation of comparable quantities in vials. The compressibility, friability and density of the dry spherical particles depend upon the types and the concentrations of solids in the starting solution. Considering their minute size and low density, lyophilized microdroplets have excellent flow properties, but the electrostatic charge may cause problems.

1.3.1 GENERATION OF SPRAYS AND DROPLET STREAMS

Different product specifications require different processing conditions. The dispersion of bulk liquid and the formation of droplets is the first step in SFD. A wide range of methods is available for this purpose, which differ in technical sophistication, mean size and uniformity of the droplet population, throughput, scale-up potential and adaptability to pharmaceutical GMP requirements. They can be broadly categorized into hydraulic and pneumatic sprays in the strict sense, where random effects cause the droplet size distribution to be non-uniform, while dispensers or generators produce essentially mono-disperse droplets.

1.3.1.1 Hydraulic nozzles

Research in the formation of spray cones or spray clouds by gasless liquid nozzles with a single orifice has been focused on the injection of hydrocarbon fuels in internal combustion or jet engines (Dan et al., 1997; Dumouchel, 2008; Schneider, 2003) (Figure 1-1), but the method is also important for the airless coating of surfaces.

Cylindrical jets and flat or conical sheets of liquid disintegrate into small fragments by surface instabilities, friction with the boundary layer of the surrounding gas and induced vortices or eddies.

A quantitative mathematical model for the breakup of drops with sizes equal to the nozzle exit diameter into spray plumes has been developed by Reitz and Diwakar (1987). The mean droplet size decreases with increasing pressure, but due to incomplete control of fluid dynamics the spray is never uniform. The transfer of momentum from the fast moving liquid to the boundary layer is complex and causes the formation of eddies and vortexes.

Since droplets are decelerated by atmospheric friction and collide, both the mean volume and the spread of the volume distribution of the disperse phase increase by coalescence.

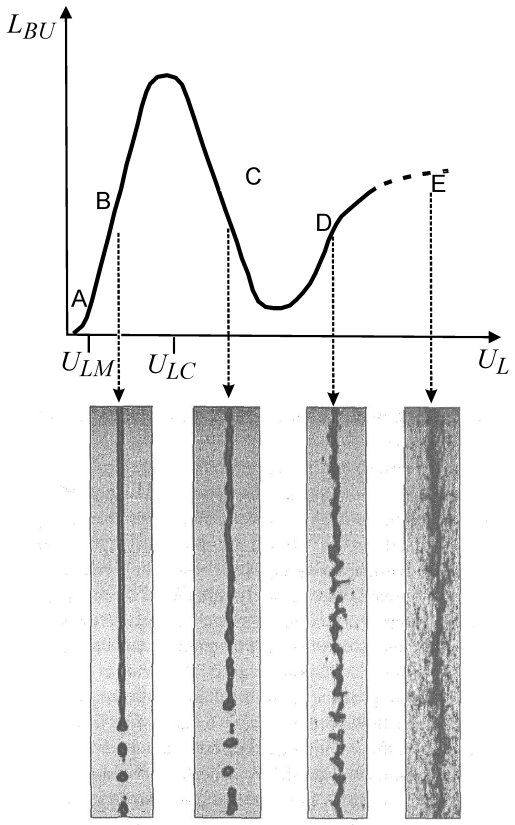

Figure 1-1: Stability of hydraulic jets.
Liquid jet stability curve **A:** Dripping regime, **B:** Rayleigh regime (Re_L = 790; We_G = 0,06), **C:** first wind induced regime (Re_L = 5,500; We_G = 2,7) , **D:** second wind induced regime (Re_L = 16,500; We_G = 24), atomization regime (Re_L = 28,000; We_G = 70).
L_{BU}: breakup length (mm), **U_L:** Liquid jet velocity (m/s), **U_{LM}:** minimum liquid jet velocity (m/s), **U_{LC}**: critical liquid jet velocity (m/s).

1.3.1.1.1 Hydraulic droplet aerosol generators

Hydraulic spray nozzles are preferred for liquids with low viscosity. The simplest devices of this type are hand-operated like the BD Accuspray (Becton-Dickinson, USA-Franklin Lakes, NJ), which produces a spray cloud for intranasal droplet delivery (Alchas, 2007). Huang et al. (2009) thus generated a spray containing recombinant F1-V fusion protein of Y. pestis, which was then frozen in liquid nitrogen and administered to the nasal mucosa of mice following lyophilisation.

For the delivery to the alveolar region of the airways, dry powder inhalants with smaller particles are required. Tsukamoto et al. (2012) and Audouy et al. (2011) used a Micro SprayerTM Aerosolizer (Penn-Century, Inc., USA-Wyndmoor, PA) developed for the intratracheal generation of droplet sprays in small animals (Century, 2001) to generate a fine mist which was frozen in liquid nitrogen and lyophilised. The authors produced dry powder inhalants containing luciferase-tagged CMV plasmid DNA for pulmonary delivery. Operated manually with a cannula of only 0.032" ID at pressures up to 700 psi, droplets with mean mass diameters between 25 and 30 μm are obtained, and a helical insert near the tip of the blunt capillary imparts a swirl to the liquid (Figure. 1-2a). The combination of high constant pressure and centrifugal forces disrupts the coherent liquid exiting from the orifice, so that a spray plume is formed in the narrow space available in the airways. An empirical equation used in the dairy industry to describe the relationship between process variables and the mean size of droplets for a hydraulic nozzle is given by Westergaardt, 2010.

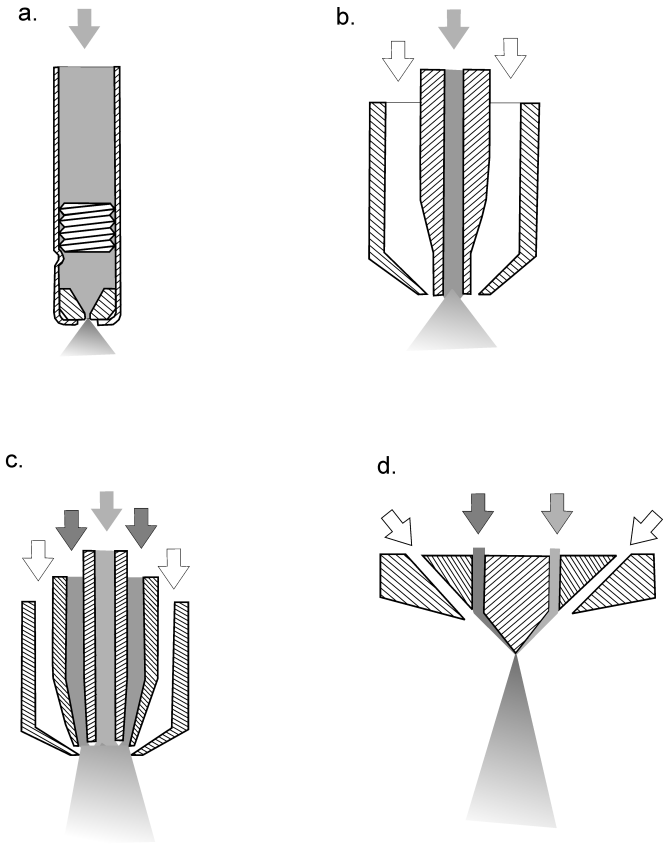

Figure 1-2: Spray nozzles
(a) pneumatic intrapulmonary cannula atomizer, **(b)** two-fluid external mixing, **(c)** three-fluid internal mixing, **(d)** Venturi- and Coanda-effect four fluid nozzle.

1.3.1.1.2 Spray freezing into liquid (SFL)

Liquid sprays in gases are used for many purposes, while submerged liquid jets are rare except in marine propulsion and underwater cleaning. SFL comprises both the disintegration of a fast coherent liquid thread into droplets and the freezing step. The latter will be addressed in section **1.3.2.5**. The technique was developed and used for the production of protein/peptide microparticles containing zinc insulin (Yu et al., 2004) and bovine serum albumin (BSA), and to improve the solubility of poorly water soluble active ingredients like danazol and carbamazepine (Hu et al., 2004a, 2004b, 2003, 2002; Rogers et al., 2003a, 2003b, 2003c, 2002a, 2002b) by embedding them in small particles of soluble excipients.
At temperatures close to the boiling point of the receiving liquid, extensive formation of gas bubbles by evaporation and cavitation (Wright et al., 2013) is to be expected. This may limit the rate of transfer of thermal energy from the droplets to the coolant. Scanning electron microscopic (SEM) images indicate, that under certain conditions, the time available is sufficient for the formation of globular particles, but frequently the high shear rate, turbulent fluid dynamics and complex freezing conditions yield irregularly shaped fragments. Cryogenic liquids in contact with a small nozzle bear the risk of clogging by ice formation which can be temporarily overcome by using a polymer with low thermal conduction such as polyether-ether-ketone (PEEK) as the capillary material and by high feed pressures (5000-6000 psi) leading to jet velocities between 58 and 157 m/s. Considering the size and shape of the particles (Rogers et al., 2002b; Yu et al., 2004), the atomisation conditions appear to correspond to Dumouchel's Regime E **(**Figure 1-1**)**. The formation of droplets from a jet submerged in a cryogenic liquid is only the first aspect of this process step, the second one – freezing is addressed below.

1.3.1.2 Pneumatic atomisation

For coating, painting, food processing and granulation with viscous or non-Newtonian liquids concentric two-fluid nozzles (2N) are more efficient than hydraulic pressure nozzles. The coherent liquid is dispersed by a co-directional expanding and turbulent gas flow, and the atomisation may occur within or outside of the nozzle cavity **(**Figure 1-2b**).**

This nozzle type is widely used in the pharmaceutical industry for coating and granulation. Leuenberger and co-workers developed an early atmospheric SFD technique for solubility enhancement based on standard fluid bed granulation equipment (Leuenberger, 1986; Mumenthaler and Leuenberger, 1991). They used solid carbon dioxide as an auxiliary freezing agent and dried the particles in a stream of cold dry air. Westergaardt (2010) gives a second empirical equation relating the mean droplet size for a specific type of pneumatic nozzle to the operating conditions. Frequently, the droplets are simply collected and frozen by sedimentation into liquid nitrogen (LN_2) (spray freezing into vapour, SFV) but in general it is not clear to what extent supercooling and freezing take place in the vapour layer or in contact with the cryogenic liquid. Costantino et al. (2000) studied the effect of atomisation conditions in 2N. The receiving gas phase in which the droplets were frozen was cooled by injection of liquid nitrogen jets.

Some SD systems have been modified for SFD by cooling the process gas and isolating or cooling the walls of the drying chamber while maintaining the temperature of the spray gas and the two-fluid pneumatic nozzle above the freezing point in order to prevent clogging. Laboratory-scale apparatuses of this category, e. g. Büchi type B 19, B290 (Büchi AG, CH-Flawil) and Tokyo Rikakikei SD 1000 (Tokyo Rikakikai Co. Ltd., J-Tokyo) have been used (*e.g.* by Cheow et al., 2011; Mohri et al., 2010; S. H. Wang et al., 2012; Y. Wang et al., 2012).

1.3.1.2.1 Three- and four-fluid nozzles

Layered droplets for the production of lyophilised microcapsules with a dissolution-rate limiting coat can be produced by concentric three-fluid nozzles (3N) (Figure 1-2c**),** where the solution of the active ingredient is sprayed from the central orifice, the coating solution from the inner and the atomisation gas from the outer ring nozzle. Pabari et al. (2012) produced layered microparticles by this technique which can be combined with ultrasonic fluid excitation to obtain a narrower droplet size distribution as studied by Whelehan and Marison (2011). How such layered droplets freeze and how they can be dried are still open questions.

A four fluid-nozzle (4N, Figure 1-2d) (MDL-050B, Fujisaki Electric, Co., Ltd., J-Tokushima) has been used to produce two jets or fast-moving liquid sheets with solvents of different

polarity. Initially, the liquids are accelerated and dispersed by pressure and pneumatically by the Venturi effect of gas flowing across the orifices. The Coandă effect keeps the biphasic flows close to the solid surfaces of the nozzle until they collide, mix and form a cloud of fine droplets with diameters in the 2 to 10 μm range which yield open, sponge-like spherical particles upon lyophilisation. Drug substances with low solubility in water are dissolved in water-miscible organic solvents, *e.g*. acetonitrile or t-butanol, and dispersed by collision with aqueous solutions of suitable matrix-forming excipients. The technique has been used to enhance the apparent solubility of BCS class IV drugs, *e.g*. tolbutamide (Chen et al., 2004), phenytoin (Niwa et al., 2009) and ciclosporin (Niwa et al., 2010) by embedding them in small porous freeze dried particles with soluble excipients like mannitol or hydroxypropyl methyl cellulose. It is also applicable to the formulation of freeze-dried controlled-release microparticles with polyacrylates (Niwa et al., 2010) and for the preparation of rifampicin powder aerosols (Ohashi et al., 2009).

1.3.1.3 Ultrasonic spray nozzles

Slow moving clouds of droplets are shed from liquid-covered surfaces oscillating at high frequencies. In ‘soft mist’ generators marketed by Sono-Tek Inc (USA-Milton, NY), the liquid feed channel runs down a cylindrical stem of high-strength and chemically inert titanium alloy, in which standing waves are induced by a ceramic piezoelectric transducer. The lengths of the stems in various types are matched to their operating frequencies, so that the amplitudes of the shedding surfaces are maximised by their location in the antinodes. For solutions with different viscosities and surface tensions, the magnitude of the oscillations can be adjusted via the power of the piezoelectric input signal. The mean size of droplets is inversely related to the operating frequency which is determined by the dimensions of the generator. This type of spray nozzle has been used to produce lyophilised particles with high density for needle-free injection (Rochelle and Lee, 2007; Schiffter et al., 2010) (Figure 1-3).

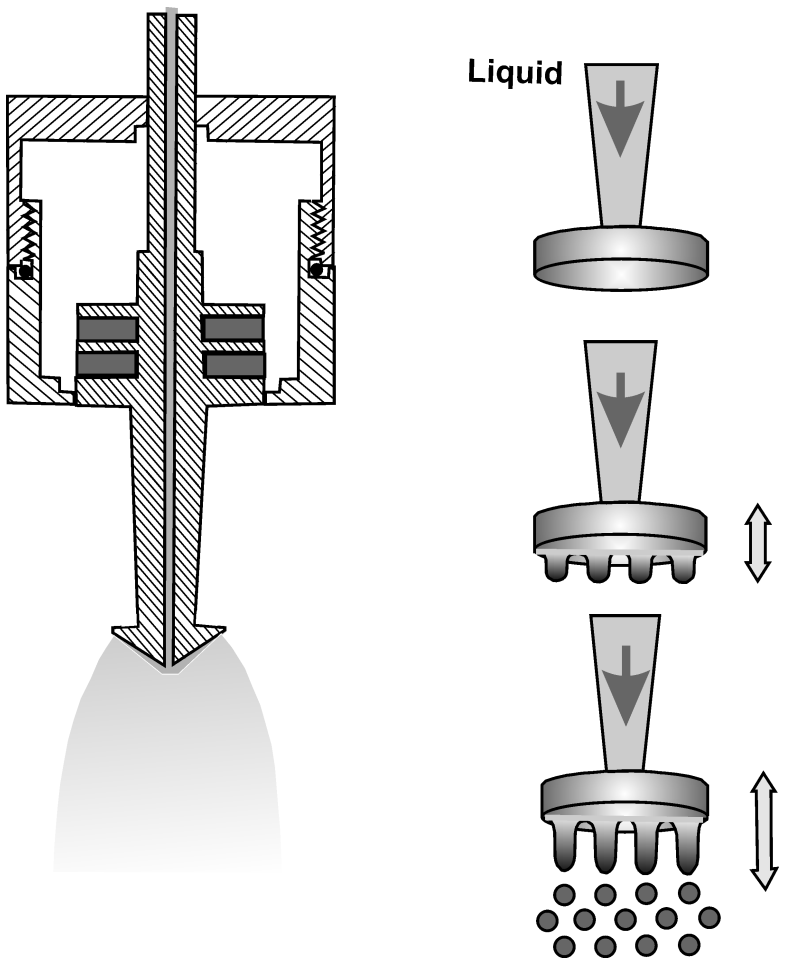

Figure 1-3: Piezoelectric soft mist generator

The droplet size distribution is narrow and the slow sedimentation rate facilitates freezing in a cold process gas. D'Addio et al. (2010) prepared solid dispersions of β-carotene nanoparticles stabilized by polyethylene glycol using the hydrogen bonding coacervate precipitation (HBCP) process, which were subsequently frozen in LN_2 and lyophilised.

1.3.1.4 Piezoelectric droplet stream generation

The generation of droplet streams has reached a high degree of perfection in ink-jet printing and automated analytical reagent dispensing systems (Wijshoff, 2008). This technology has the potential for the production of nearly monodisperse low-density lyophilisate powders with small spherical particles if the initial droplet size can be maintained during the freezing step.

Thus, the formulation-dependent variability component of pulmonary deposition and bioavailability can be minimised.

Lord Rayleigh (Strutt, 1878) demonstrated that coherent jets disintegrate into equally-sized droplets due to the growth of initially small disturbances and the spontaneous minimisation of the fluid surface. In capillary dispensers without piezoelectric excitation, the breakup length *LBU* in the Rayleigh regime depends upon the fluid dynamic conditions (Figure. 1-1), while the mean diameter of the droplets *d* is proportional to the diameter of the orifice *D*: $d = 1.89\ D$.

For aqueous solutions exiting from small circular orifices at low pressure, the formation of small droplets with diameters down to a few micrometres is limited by the surface tension and the adhesion of the liquid to the nozzle wall. By piezoelectric excitation, the breakup length of the liquid jet can be shortened and the signal type (*e.g.* sinusoidal, rectangular), frequency and amplitude affect both the mean size and the uniformity of the droplets.

According to Brenn et al. (1997), mono-disperse droplet streams are obtained if the dimensionless wavelength k of the piezoelectric excitation lies within the range $0.3 \leq k \leq 1.0$, where k is given by

$$k = \frac{\pi \cdot D \cdot f_G}{u_i} \qquad \text{Equation 1-1}$$

with

D:	diameter of the orifice (m)
f_G:	excitation frequency (1/s)
u_i:	initial velocity of the jet (m/s)

and the diameter of the droplets is

$$d = \left(\frac{3 \cdot u_i \cdot D^2}{2 \cdot f_G}\right)^{1/3} \qquad \text{Equation 1-2}$$

A system of standing waves generated by a ring-shaped actuator in a barrel ejects one droplet per cycle in pinhole-type piezoelectric droplet generators and with a sinusoidal or rectangular signal, the droplet diameter is roughly equal to the diameter of the orifice.

Capillary (Figure 1-4a) and pin-hole type droplet generators (Figure 1-4b) differ with respect to maintenance requirements and scale-up potential. Capillaries must be cleaned in place, which is difficult, while clogged pin-hole diaphragms are easy to remove and clean. For an increase in throughput, the addition of more capillaries with actuators is costly, while pin-hole diaphragms with single or multiple orifices can simply be replaced by equivalents with more pores. For production-scale operations, droplet generators with many orifices have been developed and tested (Brenn et al., 1996).

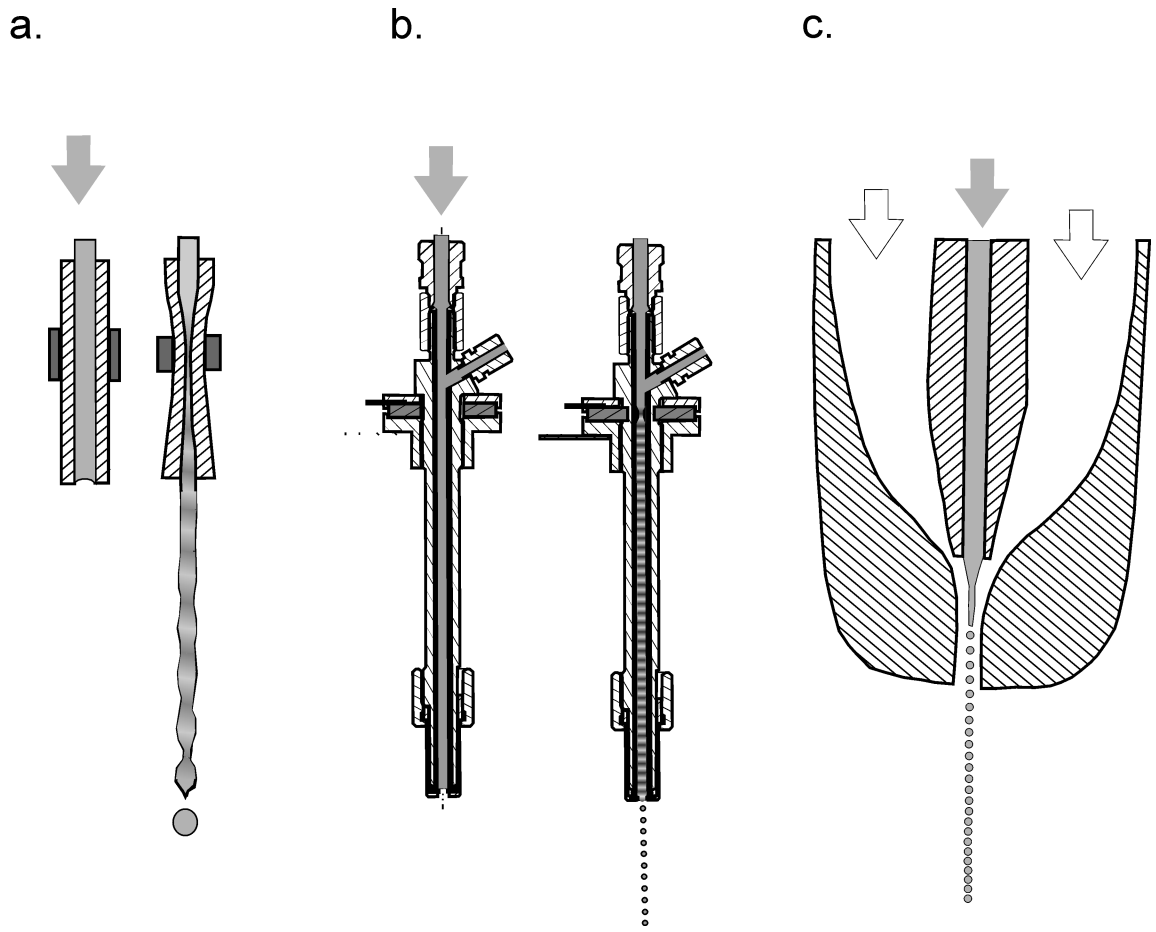

Figure 1-4: Monodisperse droplet generators
(a) piezoelectric capillary type, **(b)** piezoelectric pinhole-type, **(c)** gas dynamic extension nozzle

Streams or monodisperse droplets with diameters smaller than the orifices from which the liquid fluid is ejected can also be produced under the conditions of the Rayleigh regime if the coherent jet is compressed and accelerated by a surrounding gas flow (Figure 1-4c) as

described by Gañán-Calvo (1998), Walzel et al. (2001) in general, and by Webb et al. (2002) for spray lyophilisation, where pressure waves in either fluid can be generated by acoustic excitation, but early collisions are difficult to control due to the high volume and velocity of the gas. Nevertheless, Webb et al. (2002) applied this method to the production of spray-freeze dried particles containing recombinant human interferon-γ.

1.3.1.5 Thermal droplet stream generation

In most low-cost ink-jet printers thermal print heads are used. A small quantity of the ink is evaporated by a heating element and drives a droplet out of the nozzle. Harker et al. (2008), Mueannoom et al. (2012) and Sharma et al. (2013) have used this technology to produce droplet lyophilisates for pulmonary delivery with a high content of relatively heat-resistant APIs. The droplets are uniformly sized and easily spaced. The throughput per nozzle is minimal, which can be favourable for the production of very small amounts of product, but their number can easily be multiplied. The technique may be less suitable for solutions of thermolabile compounds, but apparently the risk has not yet been assessed experimentally.

1.3.1.6 Electrohydrodynamic droplet (EHD) generation

Lastow et al. (2007) produced inhalable budesonide particles on a laboratory scale by generating monodisperse positively charged droplets of ethanolic solutions in a low-voltage nozzle and drying them at ambient temperature.

1.3.1.7 Droplet stabilisation

The technology of droplet generation is highly developed for many purposes and one option for the next step, atmospheric freezing, is apparently simple, because the frozen particles can easily be separated from the refrigerant. Unexpectedly, freezing the droplets in a cold gas without degrading essentially mono-disperse initial size distributions is difficult because fast streams of small droplets are subject to aerodynamic braking (Süverkrüp et al., 2013). Three approaches to solve this problem have been discussed in the context of spray lyophilisation and related technologies.

1.3.1.7.1 Electrostatic droplet size stabilisation

Droplets generated by EHD dispersion are held at a distance by their positive charge. They have not been freeze-dried, but the method is of general interest for the production of small uniform inhalable droplets and particles and because the electrostatic charge prevents droplet collisions in flight and the resulting deterioration of the particle size distribution. On the other hand, charged powders have to be discharged before processing, *e.g*. by corona needles, which increase the complexity of the system. An electrostatic droplet separation and collection system developed by Brandenberger et al. (1999) was used by Leuenberger et al. (2006) and a similar setup is featured in the Büchi B 90 Nano laboratory spray dryer (Büchi AG, CH-Flawil).

1.3.1.7.2 Aerodynamic droplet size stabilisation

Fast droplet streams injected into a stagnant gas are decelerated by atmospheric friction which also reduces the width of inter-droplet gaps. Upon contact, droplets merge and non-central collisions lead to lateral deflection. Thus, initially monodisperse and unidirectional droplet streams turn into polydisperse spray plumes (Süverkrüp et al., 2013). The initial monodispersity of droplet and particle size is lost as the mean diameter and the spread of the distribution increase with the distance from the generator. For pulmonary drug delivery, both the mean aerodynamic diameter and the uniformity of lyophilisate particles are important quality characteristics, and the stabilisation of droplet sizes before freezing is an essential step of the manufacturing process. Both the freezing rate of droplets and their horizontal distances are increased when the stream is injected into a cold gas vortex (Süverkrüp, 2014).

1.3.1.7.3 Acoustic droplet deflection

When straight droplet streams are decelerated by aerodynamic friction, spaces in the direction of flight decrease and droplets coalesce upon contact. This can be prevented by acoustic impulses, which accelerate them laterally and alter their trajectories. The method was patented for ink jet printing, but can also maintain the integrity of droplets in the freezing step (Pechtl, 2009).

1.3.2 DROPLET FREEZING

Droplets are either frozen by transfer of thermal energy from the liquid to a cold gas, another immiscible liquid or a solid in contact with the droplet surface or by the diffusion of energy-rich volatiles into the surroundings at low vapour pressure.

The mechanisms, kinetics and thermodynamics of droplet freezing were first studied in a meteorological context. The methods developed and results obtained give a theoretical background to the product-oriented approach favoured in pharmaceutics.

As early as 1911 Wegener (Wegener, 1911) considered the initiation of droplet freezing by heterogeneous and homogeneous nucleation. Fifty years later Hoffer studied the effects of solutes and suspended particles on nucleation and freezing under controlled laboratory conditions (Hoffer, 1961). He found that 'soluble salts, commonly found in the atmosphere, caused the freezing temperatures of the droplets to become colder than would be anticipated by bulk freezing point lowering calculations in all cases. Insoluble nuclei increased the freezing temperature of the droplets. The addition of soluble salts to water containing insoluble nuclei caused a marked depression of the freezing point below that originally observed. The magnitude of the depression was found to be a function of the solute concentration'.

The freezing of droplets proceeds in five steps (Hindmarsh et al., 2003, quote):

(i) Liquid cooling and supercooling: during which the liquid droplet is cooled from its initial state to a temperature below the equilibrium freezing point.

(ii) Nucleation: where there is sufficient supercooling for spontaneous crystal nucleation to occur.

(iii) Recalescence: during which supercooling drives rapid kinetic crystal growth from the nuclei. There is an abrupt temperature rise as this growth liberates latent heat of fusion. This stage is terminated when the supercooling is exhausted and the droplet has reached an equilibrium freezing temperature.

(iv) Freezing: where further growth of the solid phase is governed by the rate of heat transfer to the environment from the droplet. This process continues until the droplet is frozen throughout. During this stage, progressively greater

freezing point depression can arise due to an increased concentration of solutes in the unfrozen liquid phase.

(v) Solid cooling or tempering: where the temperature of the frozen droplets reduces to a steady-state value near that of the ambient air temperature.

With small droplets in a sufficiently cold environment, nucleation and freezing may be complete within less than a millisecond (Al Hakim K. et al., 2006). The structure of lyophilised particles indicates the separation into a solute-depleted and a solute-enriched phase. By sublimation drying, the depleted phase is converted into voids while lamellar or filamentous solid structures originate from the enriched phase.

During super-cooling thermal energy is transferred to an external heat sink through the droplet surface. Due to their small size, low mass and high specific surface, the thermal equilibrium between droplets and their environment is quickly established and internal temperature differences are probably small although gradients may be steep. Al Hakim et al. (2006) studied the size and velocity distributions of droplets generated by either pneumatic or hydraulic nozzles and obtained estimates of both their nucleation and solidifications times by phase Doppler anemometry.

In flight, the droplet/gas convective heat transfer coefficient and hence both nucleation and solidification times depend upon the slip velocity of the droplets. Upper bounds of nucleation and freezing times are therefore related to initial velocities of droplets generated by pneumatic (u_i = 90 m/s) and hydraulic nozzles (u_i = 2 m/s). Nucleation and freezing times were computed for representative slip velocities in mid-flight for fast and slow-moving droplets.

The analysis of Al-Hakim et al. (2006) was based upon research on atmospheric phenomena by Hindmarsh et al. (2003), who studied the nucleation and freezing of water drops suspended from the junction of a thermocouple. They observed nucleation temperatures between 260 and 256 K and found that a heat balance model described the relationship between nucleation and freezing times at gas temperatures between 258 and 248 K fairly well. The nucleation temperature of the solution is a critical process parameter in spray freezing because no ice nuclei are formed and the droplets will not freeze at gas temperatures above this level. Observations of Tagami et al. (1999) indicate that nuclei originate at the surface of droplets and that freezing proceeds inwards from a solidified shell. This applies particularly if droplets are cooled rapidly and a steep temperature gradient is temporarily formed between the surface

and the core. The lamellar structure of lyophilisates, where solid sheets separated by lamellar voids radiate from focal pores at the surface, appears to indicate where the process of solidification begins and how it spreads (Figure 1-5a).

Krämer et al. (1999) determined homogenous nucleation rates for water droplets of 60 μm diameter levitated inside an electro-dynamic Paul trap by analyzing the polarisation of laser light scattered by the freezing droplets. They found that the time constant of thermal equilibration depends upon the droplet size but that the nucleation rate is equal for drops of different size at a given temperature. They observed that the number of nuclei formed per second in a cm^3 of liquid increases rapidly as the temperature decreases. If the temperature is reduced by just one degree, the nucleation rate increases by a factor of almost forty.

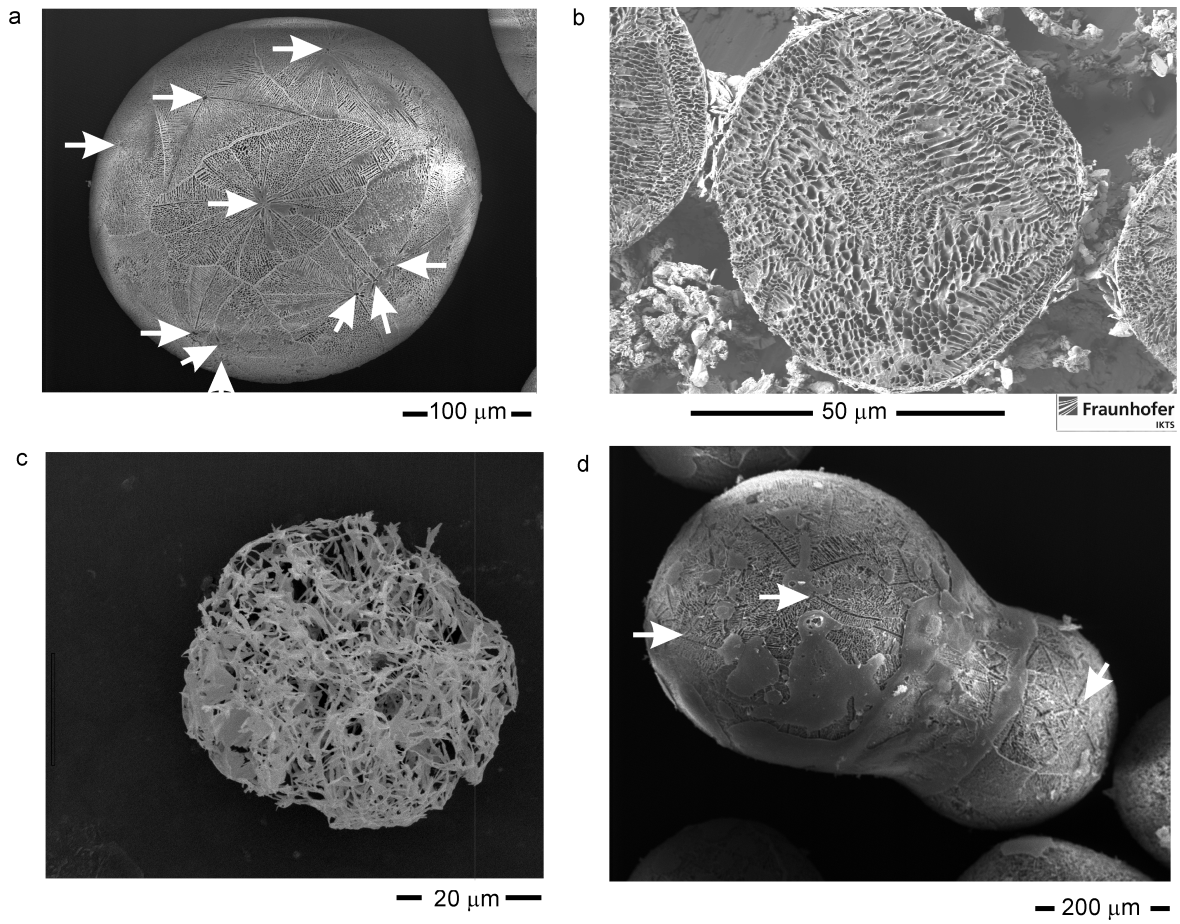

Figure 1-5: SEM images of lyophilized droplets
(a) Surface nucleation pattern (maltodextrin 10 %, mannitol 5 %, lyozym 1 %), **(b)** cross section (ion beam cut, bovine serum album 5 %), **(c)** highly porous particle (mannitol 2 %, polyvinylpyrrolidone 1 % (Kollidon™ 25)), **(d)** twin particle with nucleation traces (mannitol 5 %, lysozyme 1 %). All concentrations in % (w/w) in solution.

1.3.2.1 Overview of spray freezing techniques

In an early review of spray freezing techniques for the formation of microparticles (Rogers et al., 2001) the authors distinguish between the following categories: atmospheric spray-freeze-drying (Mumenthaler and Leuenberger, 1991; Oyler, 1993), spray-freezing into halocarbon refrigerant vapour (Briggs and Maxwell, 1975, 1973, 1976; Thomas H Adams et al., 1982, 1978), spray-freezing into a halocarbon refrigerant (Buxton and Peach, 1984; Dunn et al., 1972; Sauer, 1969), and spray-freezing onto liquid nitrogen (Gombotz et al., 1991; Herbert and Healy, 1999; Lilakos, 1990). Recent additions to the spray-freeze-drying methodology include the spray freezing into liquid nitrogen (SFL) technique (Hu et al., 2002; Rogers et al., 2003a, 2002a, 2002b; Yu et al., 2004) and the thin film freezing (TTF) procedure (Carvalho et al., 2014; O'Donnell et al., 2013; Overhoff et al., 2007).

1.3.2.2 Atmospheric freezing

In atmospheric freezing, the heat sink is gaseous, at ambient pressure with a nearly uniform temperature sufficiently low to induce the formation of ice nuclei in the solution. The frictional stress is generally low and the size and the approximately spherical shape of the droplets are not altered as they solidify. Under these conditions, the cooling rate is limited by the rate of energy transfer across the droplet surface, which depends upon the slip velocity. Slow free falling droplets may not freeze quickly. If the nozzle loses more thermal energy to the gaseous coolant than it receives from the liquid feed, it has to be heated in order to prevent clogging by ice formation.

Leuenberger and co-workers (Leuenberger, 2001, 2001, 1986; Mumenthaler and Leuenberger, 1991) developed atmospheric SFD processes with the intention of improving the dissolution behaviour of poorly water-soluble APIs by embedding nano-sized particles in hydrophilic matrices. Aqueous or organic solutions of the drug substances and ingredients like polyvinyl pyrrolidone of polyethylene glycol were injected by a heated pneumatic nozzle against the flow of a cold gas into a thermally isolated fluidised bed dryer onto a blend of carrier particles and dry ice. The drying gas was recirculated and reconditioned in a closed system, where organic solvents were recovered. Using a prilling nozzle they obtained nearly monodisperse spherical particles with diameters of about 250 μm (Leuenberger et al., 2006).

If the spray plume touches the walls of the cylindrical and conical chamber before droplets are completely frozen, they may adhere and build up a sheet of ice. Wang and Finlay (2006) solved this problem by introducing the coolant in either gaseous form or as a cryogenic liquid spray through the porous walls of the freezing chamber so that a centripetal flow of coolant fluid prevents the droplets or particles from touching the chamber walls **(**Figure. 1-6**)**.
Eggerstedt et al. (Eggerstedt and Lamprecht, 2012; Eggerstedt et al., 2012) and Süverkrüp et al. (2013) injected droplet streams at ambient gas pressure into vertical gas-filled freezing tubes with LN_2-filled coolant jackets, in which the solutions were frozen rapidly but without precise temperature control. Costantino et al. (2000) injected jets of liquid nitrogen as a volatile refrigerant into spray cones of BSA solutions.

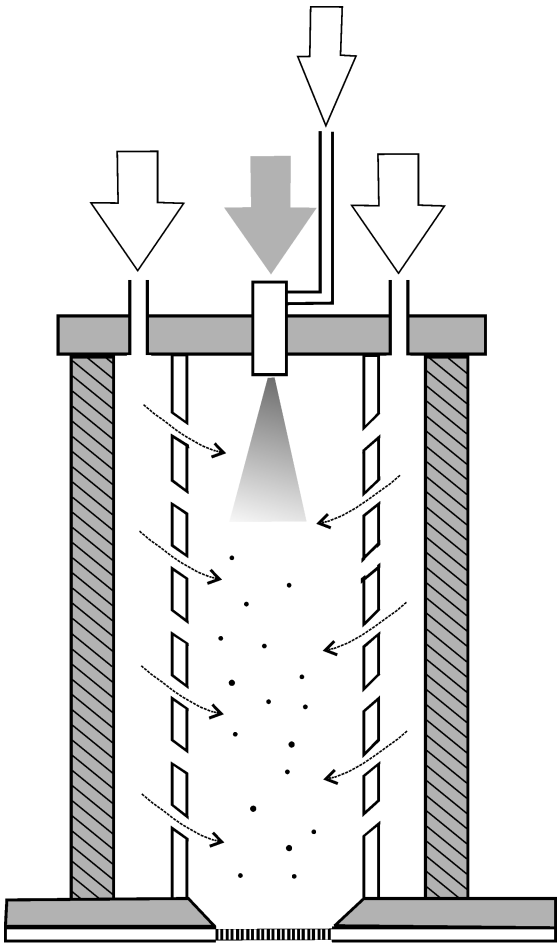

Figure 1-6: Convergent flow atmospheric freeze drying, Source: (Wang et al., 2006) redrawn

1.3.2.3 Spray-freezing with compressed carbon dioxide

The temperature of aqueous sprays can also be reduced below their freezing point by Joule-Thompson cooling of co-expanding carbon dioxide. Henczka et al. (2006) studied this process and developed a model for the prediction of particle size, temperature profile and freezing time.

1.3.2.4 Freezing by spraying into vapour over a cryogenic liquid (SFV)

When droplets are sprayed into a gaseous environment above the freezing point of the solution and sediment through the vapour layer onto the surface of a cryogenic liquid, supercooling and freezing may occur in the supernatant gas and vapour or upon contact with the condensed refrigerant. Since the velocity of small droplets decreases rapidly due to atmospheric deceleration, frictional stresses remain low and the freezing conditions are similar to those upon atmospheric freezing. The nozzle needs not be heated, but the frozen droplets have to be separated from the coolant for further processing. They can be collected by screening, but the refrigerant may also simply be evaporated. Davis and DeVack (1989) obtained a patent for freezing droplet streams with diameters between about 0.6 and 5 mm.
Murphy et al. (1974) proposed to form fine frozen particles of by spraying a solution onto a layer of liquid coolant flowing down a sloping plate and to collect and dry the frozen spherules as they drain.
A publication by Werly and Bauman (1964) is of historical interest because it appears to be the first work on SFD. The authors produced solid aerosols of a variety of materials (*e.g.* sodium sulfate, ferritin, hemoglobin, egg albumen, casein) by dispersing 30 mg of the dried powders with a 60 psi rupture disk dispenser into a 1 m^3 dust chamber and sprayed liquid aqueous dispersions containing 1-10% solids onto a liquid film of dichloro-difluoromethane (Freon 12) in an externally cooled rotating flask.
Kennedy et al. (2008) used chilled 1,2-dichlormethane and *iso*-pentane to develop heat transfer models for the freezing step of droplets of aqueous polymer solution solutions generated by an ultrasonic 25 kHz spray generators (Sonotek Inc., Milton, NY, USA) in the ACES (atomisation into cooled extraction solvents) process.

1.3.2.5 Spray-freezing into liquid (SFL)

High freezing rates can be achieved by injecting the solution directly at high flow rates through a tube of thermally insulating material into a cryogenic liquid. Under these conditions, frictional stresses are high and the fluid dynamic conditions are not well defined. The particles formed are frequently small fragments, but under low-shear conditions and with suitable excipients spherules have been obtained. It is known from submerged liquid jets used for marine propulsion and for removal of growth from underwater surfaces that cavitation, i.e. the formation of gas bubbles, is a dominant phenomenon which may limit the cooling rate in this application (Wright et al., 2013).

Alternatively, the solution may be dripped or sprayed at a lower rate from a heated nozzle into the liquid coolant. If the density of the solution is lower than that of the cryogenic fluid, it can also be injected from the bottom of the freezing vessel and the frozen particles are skimmed off the surface. Dunn et al. (1972) obtained a patent for a process by which droplets of uniform size are frozen in immiscible refrigerants under less turbulent conditions by injecting the solution through the bottom of a vessel containing a layer of dense liquid at a temperature slightly above its freezing point and a supernatant layer of colder refrigerant. Due to their low density, the droplets rise, pass through the boundary, freeze in the upper layer and are collected from its surface. A SFD method for aqueous solutions of biological materials using boiling dichlorodifluoromethane or other fluorocarbons was patented (Briggs and Maxwell, 1975, 1973, 1976) for the preparation of free-flowing lyophilised powders of biological materials.

1.3.2.6 Spray-freezing onto solid surfaces (thin film freezing, TFF)

High cooling rates and uniform particulate materials can be also produced by spraying or dripping liquids on a cold solid surface (Craig, 2002). Thus, the freezing rate is accelerated compared to volatile cryogenic liquids because the Leidenfrost effect is avoided, in which a vapour layer limits the transfer of thermal energy to the heat sink. Overhoff et al. (2007) produced danazol/polyvinyl pyrrolidone powders by dripping solutions containing various concentrations of drug and excipient in either acetonitrile or tert-butanol on a solid substrate in the temperature range between 193 and 243 K and lyophilised the frozen splashes. The

solvents differed markedly with respect to freezing and spreading behaviour, but both produced high surface area powders with low crystallinity.

Instead of freezing individual droplets, Watts et al. (2013) generated low-density microparticles by spray-coating the cryogenic surface, freeze-drying the film and comminuting the thin brittle matrix into irregularly shaped fragments. By this thin film freezing technique (TFF) they obtained respirable powders with mean geometric particle diameters ranging from 25 to 50 µm and aerodynamic fine particle fractions up to 69%. Carvalho et al. (2014) applied this technique and compared the pharmacokinetics of rapamycin powder obtained by freezing mixtures of rapamycin and lactose in acteonitrile on a stainless steel surface at -80 °C, milled the brittle product and compared its pulmonary bioavailability in rats with that of similarly ground mixtures of the same drug and excipient. They found both a significantly enhanced extent of absorption and an increased pre-systemic elimination of the TFF product.

Stabenau and Winter (2007) deposited micro-droplets of recombinant erythropoietin and of recombinant granulocyte stimulating factor on solid surfaces which were subsequently frozen and dried in a chamber-type vacuum lyophilizer.

1.3.3 SUBLIMATION DRYING

The physical principles of the final steps of the FD process in vials under vacuum have been studied and modelled in detail (Pikal et al., 1983; Waananen et al., 1993) and summarised in books, *e.g.* by Costantino and Pikal (2004), Jennings (1999) and Rey and May (2011). The diffusion of solvent molecules through the complex layer of interconnected pores which grows on top of a frozen solution during primary drying is difficult to model because the structure of the lyophilisate depends upon minute temporal and spatial fluctuations of the process variables temperature and pressure and upon the interaction of surface forces and flow of semisolids during congelation.

The drying kinetics of icy droplets differ significantly from that of frozen solutions in vials because the specific surface area of frozen droplets exceeds the ratio of the surface area available for the escape of solvent molecules and the bulk volume of ice in impermeable containers by several orders of magnitude. In spherical particles the maximal value of the

geometric shortest diffusion pathway is equal to the radius, while in vials it corresponds to the filling level of the containers.

Mobile solvent molecules separate from the surface of the frozen solid when they have acquired the energy necessary to break free from their neighbours' attraction. The sublimation energy can be transferred by thermal conduction in the condensed phase, from impinging gas molecules or by electromagnetic radiation. In the primary drying phase, frozen solvent is evaporated at temperature levels low enough to prevent flow of the residual glassy solid, which still contains a fraction of solvent bound or adsorbed to the sponge-like residue. The migration of solvent molecules from the evaporation front to the surface of the porous solid is a diffusion/effusion process hampered by collisions with the walls of the emerging porous lyophilisate. Two extreme conditions can be distinguished, depending upon the pressure of non-condensable gases present. The critical parameter is the Knudsen number *Kn*, the ratio of the free path of vaporised molecules λ and the structural dimensions of the solid phase i.e. the mean diameter of the capillaries or connected pores, *d*: $Kn = \lambda/d$.

In the pharmaceutical and food industries solutions or humid materials are frozen at ambient pressure before drying. Microdroplets injected into a vacuum are snap-frozen when part of the solvent evaporates and depresses the temperature of the remainder (Chapman, 2009; DePonte et al., 2008), but this is mainly of interest for research, *e.g.* the X-ray analysis of protein structures, not for production purpose.

The bulk of solvent is taken away in the primary drying phase either under vacuum conditions or by a stream of cold process gas at temperatures below the collapse threshold of the frozen material. Subsequently, the remaining solvent is removed at elevated temperatures in the secondary drying phase.

1.3.3.1 Atmospheric freeze-drying

Historically, atmospheric freeze-drying antedates its scientific and technical applications. It was used for preserving food, *e.g.* potatoes, by Andean people, and for drying laundry on sunny winter days in northern climates. Cold and dry air or gas passes over the frozen material or solution and removes solvent from its surface.

At ambient pressure, $Kn << 1$, and in the viscosity-dominated continuum regime a solvent molecule, which is not initially located at the surface, strikes many molecules of non-condensable gases between contacts with the walls of the porous solid and in the immediate vicinity of the particle before escaping from the region dominated by gas-surface interactions. Meryman (1963, 1959) proposed low-temperature atmospheric drying as a method for desiccating tissue specimens under mild conditions by recycling the process gas though an absorbent bed, which takes up the solvent. The theory was elaborated by Heldman (1974) and the common feature of several procedural variants is a flow of dry gas over the surface of the material to be dried. The temperature of the process gas remains low enough to prevent meltback and the efficiency of the drying operation depends upon the pore structure of the material, its specific external surface area and the flow rate of the process gas.

With particulate drying materials, the process gas can either rise through a bed of granulate or powder or, if the particles rest on a permeable support, pass through it in a descending flow. At sufficiently fast upstream flow rates, fluidised or spouting beds are formed, depending upon the inertia of the particles, the geometry of the chamber and the gas dynamics (Di Matteo et al., 2003; Haas, 2008; Leuenberger et al., 2006; Menshutina et al., 2004; Niksiar et al., 2013). Under these conditions a fraction of unspecified fine particles can be formed by attrition and some may be lost in the exhaust filters. In downstream drying, the gas percolates mainly through the voids between particles (Prat, 1993; Wang et al., 2006). Frequently, the electrostatic charge of particles makes them difficult to handle (O'Donnell et al., 2013).

For simple conditions, Seaver (1984) has developed closed model equations for the drying kinetics of droplets. The problem is treated more extensively by Gusarov and Smurov (2002) and an overview of general models for the transfer of mass and momentum by slip flows with emphasis on applications in microelectromechanical systems is given by Zhang et al. (2012).

According to Claussen et al. (2007) atmospheric FD offers a significant energy saving potential. Typical specific moisture extraction rates for atmospheric freeze drying of particulate goods with heat pumps are in the range of 4.6 to 1.5 kg water per kWh, while in conventional vacuum freeze drying 1 kWh dries only 0.4 kg of water,

1.3.3.2 Vacuum lyophilisation

In the typical vacuum freeze situation, the free path λ of vaporised solvent molecules is one or two orders of magnitude smaller than the mean diameter of interconnected pores, i.e. $10^{-2} < Kn < 10^{-1}$ and the long-range flow rate is limited by collisions of solvent molecules with the wall (Knudsen, 1909). In the terminology of contemporary microfluidics this is referred to as the slip flow regime (Zhang et al., 2012), where non-equilibrium effects dominate near the walls. This condition prevails when the contents of vials are lyophilised in evacuated chambers, when extracts or matter are desiccated at low temperatures in vacuum tunnels or when frozen particles are vacuum-dried in rotating drums.

1.3.3.2.1 Vacuum chamber lyophilisation

When frozen particles are dried in layers, the sublimation rate is determined by a bimodal pore size distribution, where the short-range diffusion of free solvent molecules is determined by the internal pores and their connectivity. The longer range movement through interparticulate spaces and collisions with the surface of particles dominate the drying rate only in bulk powders. Liapis and Bruttini (2009) developed a mathematical model for this situation, where a packed bed of frozen particles is formed and the interparticulate space renders the frozen region unsaturated. The sublimation front moves through the porous bulk phase by convection and diffusion. A more detailed model for the vacuum lyophilisation of pellets was developed and experimentally tested by Trelea et al. (2009).
With few exceptions, currently marketed lyophilised pharmaceutical products are parenterals, which are freeze-dried in containers and reconstituted before use under aseptic precautions. Current good manufacturing practices assure sterility and a low load of pyrogens. Regulatory agencies have established strict rules of inspection for processes and equipment, *e.g.* the U.S. FDA Guide to Inspections of Lyophilisation of Parenterals 7/93 (2014), which refers exclusively to vacuum-chamber type lyophilisation equipment.

1.3.3.2.2 Vacuum tunnel lyophilisation

An obvious way to reduce the drying time and to increase the energy efficiency of lyophilisation is to reduce the thickness of the layer of frozen material and to supply the

sublimation energy by infrared or microwave radiation. For freeze drying food on a large scale, the material is frozen on trays which are passed through entry locks into a vacuum tunnel and unloaded through exit locks in a quasi-continuous process. This type of FD facilities which was first used on an industrial scale for the production of instant coffee, has been developed to a high degree of sophistication and throughput *e.g.* the Conrad unit (GEA Niro, DK- Soeborg) or vacuum FD plants with capacities of up to 60 tons of drying goods per day, (ALD Vacuum Technology, D-Hanau). On this scale, aseptic processing, which it essential for parenterals, is neither technically feasible nor are such capacities required for medicinal products.
Tunnel-type lyophilisers for the production of components for immediate or modified release solid oral dosage forms need not meet these extreme microbiological standards (Raycon, GEA-Niro, DK-Soeborg).

1.3.3.2.3 Rotary drum vacuum lyophilisation

A closed SFD system has been developed recently by Meridion Technologies (D-Müllheim) for the production of nearly monodisperse lyophilised spherules in the 200 to 800 μm diameter range. It combines a droplet freezing tower with a prilling nozzle and a rotary drum lyophilisation system (Bosshammer, 2014). The LyoMotion dynamic bulk FD system appears to be the first industrial scale unit which has been validated for the production of sterile products. Major claimed advantages are significantly reduced drying times, flexibility and cost savings, product uniformity and robustness of the manufacturing process.

1.3.4 SOLVENTS AND EXCIPIENTS

In the present context solvents are understood as liquid components of formulations which are removed in the drying step to near-trace levels. If emulsions are spray-freeze dried, non-volatile liquids are persistent excipients like the surfactants which stabilize disperse systems.

1.3.4.1 Solvents

The capacity of water to solubilise and to form solvates with polar compounds, its high freezing point, vapour pressure profile and safety make it the solvent of choice for many

active ingredients and routes of administration. On the other hand, spray-freeze-drying techniques have also been used to ameliorate the dissolution behaviour of compounds with low aqueous solubility by embedding them in highly disperse form in readily soluble matrices and by increasing the surface area available for hydration by body fluids. Mixtures of water with organic solvents like t-butanol (van Drooge et al., 2006), tetrahydrofuran (Rogers et al., 2002a) and acetonitrile (Zijlstra et al., 2007) have been used successfully to enhance the apparent solubility of Δ^9-THC, danazol, ciclosporine A and other BCS Class II and IV compounds. The mixing of water with solvents of different polarity by high-speed heterogenous droplet collisions in a 4-fluid nozzle (Niwa et al., 2009) may be viewed as a special case. In any case, formulators and process operators have to make sure that the solvent content is reduced in the drying step below acceptable thresholds.

1.3.4.2 Excipients

For many SFD products monomeric (mannitol), dimeric (trehalose, lactose), oligomeric (inulin) or polymeric (dextran, HPMC) carbohydrates or other polymers (PVP, polyacrylates, chitosan) have been used as matrix forming ingredients and lyoprotectants. In several formulations amphiphilic micro-and nano-structure forming compounds or molecular compositions and O/W emulsions containing lipids and surfactants have been spray-freeze-dried.

The diversity of approaches and the large number of tested and potential materials does not yet reveal general patterns or trends, but specific examples are available. The inactivation of proteins at the liquid-gas boundary can been reduced by surfactants and amino acids (Adler et al., 2000), and cationic non-viral vectoring agents have been used for the transfer of genetic material: chitosan by Mohri et al. (2010), 1,2-dioleoyl-3-trimethyammoniumpropane by Tsukamoto et al. (2012) and polyethyleneimine by D'Addio et al. (2013).

1.4 FINDINGS

SFD is a manifold production method applied for a variety of purposes. Therefore, each research group has specifically focused on analyzing different characteristics of their generated SFD products on the basis of the particularly intended application. In this review,

the findings of the research papers are debated in two main categories: physical and therapeutic characteristics. Physical characteristics primarily cover particle size distributions, particle densities and aerodynamic behaviour, and have been categorized based on the intended administration pathway (pulmonary delivery, nasal delivery, epidermal delivery). Furthermore, solubility characteristics, dissolution rates, controlled release of encapsulated spray-freeze-dried API and stability studies of SFD powders and API are covered. The therapeutic characteristics of SFD products are categorised as protein/peptides and small molecule APIs.

1.4.1 MORPHOLOGY OF SFD POWDERS

The general morphology of SFD powders depends upon the spraying, freezing and drying technique. The excipients and the solid content also have an impact on the surface morphology of SFD particles. The majority of SFD powders consist of perfectly shaped spherical particles (Figure 1-5a). Cross-sections of SFD particles reveal the high porous interior of SFD particles elucidating the extremely low densities of spray-freeze-dried materials (Figure 1-5b) (Eggerstedt et al., 2012). It is observed that the porosity of SFD particles increases with the decrease of the solid content of the spray solution (Figure 1-5c). Should too much reduction of the solid concentration be carried out, the mechanical stability of the particles is compromised and particles tend to break into non-spherical fragments (Mueannoom et al., 2012).

The freezing rate also has a notable effect on the droplet morphology. Nucleation is a random process, and the probability and rate of formation of pre-crystalline clusters of solvent molecules is highly temperature dependent. In case of a pronounced temperature gradient within the droplet due to rapid cooling, nuclei emerge first at the droplet surface. Should the temperature distribution be flat, homogenous nucleation may also occur in the interior of the droplets. In lyophilisates, surface nucleation leads to characteristic patterns in the pore structure: trenches devoid of solid matter and lamellae radiate in all directions from focal points, and the gaps between them are filled with either isodiametric or co-directional pores.

Formation of many nuclei is indicative that supercooling was extensive and the nucleation rate was high. In small, highly supercooled droplets, few nuclei suffice to induce complete

freezing (Figure 1-5a) within a few microseconds. In some cases, droplet collisions deliver the activation energy for the freezing process to begin and colliding droplets freeze instantaneously (Figure 1-5d).

The freeze concentration process manifests itself in the morphology of the pores and the solids of the lyophilised particle. If the freezing rate is relatively slow and the concentrated solution passes through a viscous state before solidification, surface forces may convert initially lamellar foam-like condensed phases into filamentous structures with a lower surface-to-volume ratio. Conversely, morphologically diverse particles can be obtained from the same solution in one production run in case the temperature of the cold gas is incompletely controlled. Engstrom et al. (2008) studied the morphology of lysozyme/trehalose particles obtained by SFL using both LN_2 and isopentane. They found both lamellar and filamentous and intermediate structures depending upon both the concentration of the starting solutions and the cryogenic liquid.

The conditions under which a skin is formed on the surface are not yet understood, although the variables such as the chemical composition of the starting solution (Ali and Lamprecht, 2014) and the concentrations of its constituents, the surface tension and size of the droplet, its velocity and the viscosity, density and temperature of the cooling gas have been already identified.

1.4.2 PARTICLE CHARACTERISTICS AND SIZE DISTRIBUTION

SFD powders have been prepared for different purposes and various forms of application. Therefore, the obtained particle size distributions as well as particle properties such as the density, specific surface area and mass median aerodynamic diameter (MMAD) are dependent on the intended usage (Table 1-2). In the following, we will discuss the particle properties based on the application of the developed powder.

Table 1-2: SFD particle characteristics overview

Route of Administration	$d50_{Geo}$ [µm]	Solid Content (w/w) [per cent]	Bulk Density [mg/cm³]	FPF [per cent]
Pulmonary	7 – 42	3.6 – 5.0	20 - 230	22.9 - 70
Nasal	25 - 70	10	60 - 170	-
Epidermal	34 - 50	20 - 47	630 – 650	-

1.4.2.1 Powders for pulmonary application

For an effective pulmonary deposition, particles should have an aerodynamic diameter of 1–5 µm, as well as a narrow particle size distribution to minimise incorrect deposition. The aerodynamic diameter of a particle is defined as the diameter of a perfect sphere with the density of 1 g/cm³, which has the same settling velocity as the analysed particle (Equation 1–3).

$$d_{ae} = d_p \sqrt{\frac{\rho_p}{\rho_0 \chi}} \qquad \text{(Equation 1-3)}$$

with:

d_{ae}: aerodynamic diameter,

d_p: geometric particle diameter

ρ_p: particle density

ρ_0: unit density (1 g/cm^3),

χ: shape factor

As indicated in Equation 1-3, the aerodynamic diameter depends upon the geometrical particle size, particle density and shape factor (1 for spherical particles as they are obtained in most SFD methods) (Ziegler 2006). Therefore, it is preferred to produce sprays, which can form powders with a $d_{50.geo}$ around 5 µm. The pulmonary depositable fine particle fraction (FPF) of a powder can be identified by the use of analytical methods suitable for measuring aerodynamic particle size distributions such as the Anderson-Cascade-Impactor, Multi-Stage Liquid Impinger or the Next-Generation-Impactor (NGI).

Two methods have been primarily used for the production of SFD powders for pulmonary application. The majority of research groups involved in SFD have used the 2N SFV process, while one research group investigated the thermal ink jet spray method (Mueannoom et al., 2012; Sharma et al., 2013). As SD is a well-established process, Maa et al. (1999) and Zijlstra et al. (2009) used it to compare the basic applicability of SFD powders for inhalation. SD resulted in small particles between 3.4 µm (Maa et al., 1999) and 7.5 µm (Zijlstra et al., 2009) and FPF between 46 and 41.8 per cent respectively. When the same solutions were prepared by SFD, the geometrical diameter of the particles approximately doubled in size (from 3.4 µm (SD) to 7.0 µm (SFD) and 7.5 µm to 18.67 µm respectively). Yet, there was still a comparable amount of FPF (39 per cent) for the SFD powder. It was demonstrated that powders and particles prepared by SFD have lower densities than SD material and show a high porosity in SEM imaging. In SD, the removal of water from the droplets leads to the particles shrinkage during the drying process and thus the loss of the initial geometric diameter. In SFD, however, the droplets slightly grow in diameter (van Drooge et al., 2006) when frozen. After FD, the particles still have up to 84 per cent of their initial size and keep their original spherical shape. Therefore, based on Equation 1-3, it can be well justified that when starting with the same droplet size and solid content, a SD particle will have a larger aerodynamic diameter than a SFD particle given the shrinkage phenomenon during the drying process. In their study, Maa et al., 1999 succeeded in increasing the FPF of the SFD powder up to 70 per cent (compared to 46 per cent for SD) which they suspected to be due to smaller aerodynamic diameters.

In another project, thermal-inkjet SFD was used as a method to create small amounts of excipient-free salbutamol (Mueannoom et al., 2012) and terbutalin (Sharma et al., 2013) formulations suitable for inhalation. The average geometrical size of the particles created with

the print-head was around 35 µm and 41 µm respectively. However, due to the low particle density (caused by low concentrations of the spray solution between 2 and 10 per cent), a considerable amount of particles were reported to possess sufficient small aerodynamic diameters for pulmonary application. Hence, the FPF of both salbutamol and terbutalin-sulfate formulations approached those of the available commercial products. The aerodynamic diameter depends on the solid content of the droplets and consequently the solid content of the spray solution. This can impact the MMAD as shown clearly by Mueannoom et al., where the MMAD was found to grow with increasing solid content of the spray solution, while the geometric diameter remained constant. The formation of powders with MMAD values smaller or equal to 6 µm with large geometric particle sizes of 35 to 41 µm is indeed remarkable. Within this context, large porous particles offer several advantages over small and dense particles (Edwards, 1997). For instance, the smaller surface-to-volume ratio leads to lower cohesion force between the particles and therefore facilitates the dispersibility in air. The same effect leads to lower adhesion forces within the inhaler and leads to very high emitted fractions. In fact, *in-vitro* deposition patterns of cascade impaction or liquid impingement show that SFD products leave almost no residue in the inhaler. These can lead to a higher bioavailability of large porous particles. Nonetheless, there are some limitations regarding particle size and especially particle density, *i.e.* although the reduction of particle density can compensate for large geometric particle sizes, low density particles tend to be less stable against physical stress and can easily undergo breakage (Mueannoom et al., 2012).

1.4.2.2 Powders for nasal application

SFD powders for nasal application have been prepared with geometric diameters of approximately 25 µm (Garmise et al., 2007; Y. Wang et al., 2012) and 70 µm (Jiang et al., 2006; Mikszta et al., 2005). Particles for nasal application should be small enough to be applicable without generating a foreign body sensation in the nasal cavity, but also large enough to reduce the entry into the deep lungs. Within this frame, Garmise et al. (2007) also focused on flow properties, as they have an effect on further production steps such as mixing and on the performance of the final product. It was determined that SFD trehalose had a larger angle of repose (36.1°) than bulk sieved trehalose (21.6°) and therefore slightly poorer flow

performance (Garmise et al. 2007). This contradicts the assumption that spherical SFD particles have superior flow characteristics over bulk material. A reason for this could be the lower density of the SFD material (bulk density of SFD trehalose 0.17 g/cm³ vs. unprocessed trehalose 0.46 g/cm³). Additionally, the SEM image of SFD trehalose shows a large fraction of non-spherical particles, which negatively impact on the flowability.

1.4.2.3 Powders for needle-free ballistic intradermal application

The objective of SFD processes optimised for the production of nasal and pulmonary deposition is to obtain light particles with good dispersibility in air. For an epidermal application, the particles are accelerated in an application device by compressed gas and penetrate into the epidermal layers of the skin. The skin penetration is dependent on the particle velocity, particle density and the particle diameter (Kendall et al., 2004). As a consequence, the highly porous low-density particles used for nasal and pulmonary application do not suit epidermal application given their high fragility. To overcome the low mechanical stability, spray solutions with a high sugar and polymer content (35 per cent w/w or more) are required (Maa et al., 2004) which result in the formation of powders with bulk densities around 0.5 g/cm³. SFD particles with a diameter of 20-70 µm (Dean and Chen, 2004; Maa et al., 2004; Schiffter et al., 2010) and narrow particle size distributions have been prepared by a ultrasonic soft-mist generator over liquid process.

1.4.3 SOLUBILITY AND DISSOLUTION RATES OF POORLY SOLUBLE PRODUCTS

SFD has been intensively investigated for the purpose of enhancing dissolution rates of poorly water-soluble drugs such as danazol (Hu et al., 2004a; Rogers et al., 2003b, 2002a, 2002b), carbamazepine (Hu et al., 2004a), phenytoin (Niwa et al., 2009), tolbutamide (Kondo et al., 2009b) and ciclosporine (Niwa et al., 2012, 2010). The two mainly investigated methods include SFL and SFV using a 4N. Rogers, 2003 and Hu et al., 2004a explored the dissolution times of 10 mg API in 900 ml SLS/Tris solution and compared SFL to slowly frozen controls and bulk API. Danazol prepared by SFL dissolved completely within 10 minutes, while bulk danazol required 60 minutes for complete dissolution. For SFL carbamazepine, the results

indicated a thorough dissolution after 10 minutes, whereas carbamazepine controls possessed significantly slower dissolution rates with the slowly frozen carbamazepine having achieved 85% dissolution after 20 minutes, and the bulk carbamazepine merely 25 % after 60 minutes (Hu et al., 2004a). X-ray powder diffraction measurements of carbamazepine and danazol formulations revealed that the frozen formulations contained danazol in amorphous form, while the bulk material formulations comprised high amounts of crystalline danazol. Further studies demonstrated that the amorphous danazol within the SFL micronised powder is stable and shows no signs of recrystallisation when stored over 6 months at 20 °C/60 % relative humidity (rhm) and 40 °C/75 % rhm (Rogers et al., 2003b).

In conventional SFL and SFV methods using single and 2N, the API and carrier excipient have to be dissolved in a common solvent, which sometimes leads to a limited application of the technique (Niwa et al., 2009). With the 4N, separating the spray solutions allows the dissolution of the poorly water-soluble API in an organic solvent, and the excipients in water, thereby resolving the abovementioned problem. In one study, it was shown that the preparation of tolbutamide-HPMC particles by SD and SFD which enabled the incorporation of amorphous tolbutamide within the particle structure could improve the API's dissolution time in pH 1.2 and pH 6.8 medium compared to bulk tolbutamide. The composite particles produced by SFD showed a faster drug release compared to particles produced by SD. This finding can be attributed to the higher specific surface area of the highly porous SFD particles (SFD: 28.32 m^2/g vs. SD: 0.35 m^2/g) (Kondo et al., 2009a). The 4N-SFV methods were also able to significantly improve the release profiles of ciclosporine. In this study, ciclosporine was sprayed with mannitol in different concentrations, which indicated the improvement of the drug release with the increasing content of mannitol in the particles. This observation is suggestive that mannitol accelerates the penetration of dissolution medium into the particles and that the effective surface area of ciclosporine is increased. In addition to solubility enhancement, the 4N-SFV process could be used to produce phenytoin- Eudragit-L particles with sustained release properties in acid medium (Niwa et al., 2010).

1.4.4 PREPROCESSING FOR CONTROLLED RELEASE MICROSPHERES

Encapsulation of spray-freeze dried solid rhGH (recombinant human growth hormone) into PLGA microspheres was tested *in-vivo* in juvenile rhesus monkeys and rats. A zinc acetate:rhGH, molar ratio 6:1, dispersion was spray-freeze dried and mixed with zinc carbonate, added to a solution of the polymer in dichloromethane and sonicated. The rhGH-levels in the serum showed an initial drug-release wherein 20 per cent of the protein was released in 48 hours followed by constant rhGH levels for 20 days (Johnson et al., 1997). Costantino et al., 2004 determined that the initial burst was dependent on the size of rhGH particles and was therefore controllable by prior spray-freezing modifications while maintaining the sustained release characteristics. In addition to rhGH, researchers explored spray-freeze-dried recombinant human vascular endothelial growth factor (rhVEGF) PLGA microspheres. Compared to rhGH encapsulated in the same formulation, rhVEGF microspheres were associated with a lower initial burst (10 instead of 20 per cent). Henceforth, a continuous release of bioactive protein for 21 days was achieved (Cleland et al., 2001). Later, rhVEGF-incorporated PLGA:NMP (N-methyl-pyrrolidone) gel showed slower *in-vitro* release profiles compared to its PLGA microspheric counterpart. The gel also had a slower release profile *in-vivo*, while the PLGA microspheres show signs of initial burst release. Further studies considering recombinant human insulin like growth factor I (rh-IGF-I) as a microencapsulated protein for the treatment of diabetes showed that the spray-freeze dried protein loading of the PLGA microcapsules had an impact on surface area and morphology of the capsules and therefore influenced the initial burst release (Lam et al., 2000). Leach et al., 2005 used sonication of spray-freeze-dried BSA to produce sub-micron protein particles with low aggregation and denaturation. The protein was encapsulated in PLGA and PLA microspheres. Release profiles showed that compared to conventional BSA particles the burst release of BSA from spray-freeze-died microspheres could be reduced five to tenfold.

1.4.5 STABILITY OF NEW BIOLOGICAL ENTITIES (NBE) IN SFD POWDERS

The stability of protein/peptide active pharmaceutical ingredients is of major interest when formulating new drug delivery systems. Researchers have investigated the impact of the SFD

process on different protein/peptides and could show, that SFD did not have negative effects on the structural and functional stability of NBEs. Schiffter et al. (2010) studied insulin stability in a nanoparticle SFD process using an ultrasonic nozzle. Samples were taken at four steps during the process: preparation of the nanoparticles, atomisation, fast freezing in liquid nitrogen and freeze-drying. Proteins embedded in the nanoparticles remained largely intact, but spraying at ambient temperature caused a higher extent of aggregation than spray-freeze-drying. This has been explained by the longer exposure of the protein to a water-air interface during atomisation without freezing. An overview of the results is given in Table 1-3.

Table 1-3: Stability of proteins/peptides in SFD processes

Drug	Stability confirmed by	Researcher
Insulin	reverse-phase HPLC of insulin and A-21 desamido insulin degradant size-exclusion chromatography	(Rogers et al., 2002a)
Anthrax vaccine	circular dichroism FTIR spectroscopy SDS-PAGE	(S. H. Wang et al., 2012)
Influenza vaccine	SDS-PAGE	(Maa et al., 2004)
rhGH	size-exclusion chromatography reverse-phase chromatography	(Johnson et al., 1997)
rhVEGF	size-exclusion chromatography, heparin affinity chromatography, mitogenic receptor (KDR)-IgG binding affinity	(Cleland et al., 2001)
Dry Plasmid DNA	gel electrophoresis	(Mohri et al., 2010)

FTIR: Fourier-transform-infrared-spectroscopy,
SDS-PAGE: Sodium-dodecyl sulfate polyacrylamide gel electrophoresis

One of the common purposes of lyophilisation is to improve the storage stability of protein/peptides. Within this context, S. H. Wang et al. (2012) showed, that SFD powders generally had superior storage stability compared to a liquid control formulation when stored in dry conditions.

1.4.6 THERAPEUTIC EFFICIENCY OF SFD PRODUCTS

1.4.6.1 Vaccines

Several vaccines such as influenza and anthrax have been in the focus for the creation of SFD formulations for pulmonary, nasal and epidermal applications.

Monovalent influenza subunit-vaccine has been prepared by SFD for pulmonary application. SFD powder showed a higher systemic immune response, which was determined by higher haemagglutinin (HA), IgG and IgA titers than a pulmonary applied liquid control solution and an intramuscular injection. The SFD particle formulation induced stronger mucosal immune response in the nasal cavity and also in the lung. Saluja et al. (2010) compared the immunogenicity of whole inactivated influenza vaccine (WIIV) prepared by SFD and SD. The SD and SFD formulations also observed higher IgG titers compared to the liquid control as well as the intramuscular control. These findings have been attributed to the presence of high amount of inulin used to stabilise the dry powder formulations, which might have led to an increase of local viscosity and therefore reduction of the mucociliary clearance and increment of antigen uptake. Another administration route for WIIV is via the nasal cavity, where serum IgG antibody titers showed comparable results and mucosal IgA antibody titers were significantly higher compared to intramuscular control solutions (Garmise et al., 2007). Furthermore, the epidermal influenza vaccination has been investigated, based on the concept that the skin itself is not only a barrier for pathogens, but also an active immune organ. By using SFD vaccine with an epidermal powder injection method, strong antibody responses were observed, while the protective HA titers were comparable between the intramuscular and epidermal powder injections (Dean and Chen, 2004).

Efforts have been made to create a minimally invasive prophylactic vaccination for anthrax. The primary immunogenic endotoxin of anthrax (PA – protective antigen) was recombinantly produced (rPA) for a second-generation anthrax vaccine, which was administered by intramuscular injection (Jiang et al., 2006; Mikszta et al., 2005). Mikszta et al. (2005) showed that both intranasal and intradermal delivery were effective routes for vaccination. The intradermal delivery in rabbits showed similar toxin-neutralising antibody (TNA) titers ($>10^4$) and survival rates (83%-100%) to those of intramuscular injection. Intranasal application of

SFD and FD also showed high survival rates (83 to 100 per cent), though with lower serum TNA titers ($<10^4$). It is suspected that the intranasal delivery may provoke stronger local mucosal immunogenicity, which is beneficial in aerosol challenges (Mikszta et al., 2005). S. H. Wang et al. (2012) conducted further studies regarding intranasal application, and concluded that the nasal application is an effective route for the delivery of rPA.

Similar to Mikszta et al. (2005), studies have been conducted for the delivery of Plague-F1V vaccine. In rabbit survival studies, intradermal routes led to high survival rates of 70 to 90 per cent (intramuscular 80 to 100 per cent) (Huang et al., 2009). The nasal administrations of SFD plaque-F1V vaccine led to 80 per cent protection. The serum antibody responses after intranasal application were lower than those after intramuscular or intradermal injections. This can be elucidated based on the fact that intranasal applied vaccine has to be absorbed through the mucosal barrier.

Maa et al. (2003) investigated alum-adsorbed Diphtheria, Tetanus and Hepatitis B vaccines for epidermal powder injection or (reconstituted) liquid intramuscular injection. Albeit both FD and SFD are commonly utilised for the stabilisation of the biological products, SFD offers the advantage of being applicable for the products containing aluminum salts which are often used to improve the efficacy of vaccines but are sensitive to slow freezing. This sensitivity originates from the growth of ice crystals leading to alum gel coagulation due to freeze concentration. Hence, the rapid freezing step such as that involved in SFD can account for higher rates of nucleation, thereby reducing the growth of large ice crystals.

1.4.6.2 Insulin

Alternative routes for the application of insulin have been widely explored. Bi et al. concentrated on a pulmonary application using spray-freeze dried insulin-loaded nanoparticles (ILNP) (Bi et al., 2008). In an *in-vivo* study, diabetic rats were treated with an intratracheal instillation of ILNP. The instilled ILNP showed comparable hypoglycemic effects to a subcutaneous injection of insulin. The threshold of optimal hypoglycemic effect (70 per cent of initial glucose level (Park et al., 2007) is being used to determine the long-acting properties of the formulations. ILNP administered by both intratracheal instillation and subcutaneous

injection of control solution decreased the glucose level for 9.5 h and 6 h below 70 per cent respectively (Bi et al., 2008).

1.4.6.3 Recombinant human vascular-endothelial-growth-factor (rHVEGF)

PLGA microspheres containing rhVEGF microspheres were administered intravitreally and subretinally in rats. Intravitreal injection increased retinal vessel dilation and appearance of tortous new vessels, while subretinal route additionally led to neovascularisation at the injection site (Cleland et al., 2001). In later studies, treatment of peripheral vascular disease was targeted.

1.4.6.4 Dry plasmid DNA (pDNA)

Gene expression after the pulmonary application of SFD pDNA in mice has been analysed. It was shown that both the application and gene transfection had been successful (Mohri et al., 2010).

1.4.6.5 Doxorubicin

For the treatment of lung cancer, doxorubicin loaded nanoparticles (DLN) have been spray-freeze dried into inhalable carrier particles. A survival study with cancer bearing mice showed an increased survival time for mice treated with DLN compared to those which received an intravenous injection. Furthermore, administration of doxorubicin as inhalable nanoparticles could significantly reduce the cardiotoxic side effects, which is attributed to the lower concentration of free drug in the system (Roa et al., 2011).

1.5 GENERAL DISCUSSION

SFD covers a large variety of production methods and therefore offers the opportunity to choose the best fitting technique to serve different purposes. All SFD methods demonstrated higher potential to fulfil their specified mission and were often superior to the classical SD and FD methods. Compared to FD, SFD allows for the production of flowable powders with different particle sizes and various densities suitable for nasal, pulmonary (low density) and needle-free epidermal applications (high density). The FPF of the SFD powders was superior

compared to that of SD powders, thus rendering it more appropriate for pulmonary application. Nevertheless, there still remains much room for further improvement of the SFD process within the context of drug delivery through the lung. The ultra-fast freezing step minimises the effect of freeze concentration, thereby clearing several stability issues in terms of coagulation and agglomeration of the proteins and peptides during the process (Maa et al., 2004). In addition, the ultra-fast freezing rapidly immobilises proteins and peptides, preventing their access to the liquid/air surface. Moreover, the high speed of freezing results in the development of particles containing amorphous API molecules with improved dissolution characteristics. In general, the biggest advantage of SFD over SD is the ability to process extremely heat sensitive products.

Due to the diversity and complexity of SFD processes, specialised knowledge is required to produce lyophilised powders, which meet particular requirements with respect to solubility, stability, flowability and site-specific delivery. This is why most experts have focused merely on one or a few techniques and optimised them for specific objectives. Therefore, there are almost no comparable sets of data that allow drawing conclusions regarding the qualification and superiority of one specific technique for a particular objective. As a high level of know-how is required to efficiently operate an SFD set-up, joint-forces programs between research groups should be considered to evaluate the qualification of different SFD methods for specific purposes.

Almost all SFD methods are still highly experimental and only scaled for laboratory purposes. Industrial and regulatory aspects such as qualification, process validation, good manufacturing practice, scale-up and scale-down potential and expenses for purchase, operation and energy have rarely been addressed. Until now, one SFD method (Meridion Technologies) has reached this level which indicates that the technology is on the brink of commercialisation.

1.6 ACKNOWLEDGEMENTS

The authors acknowledge support by the Deutsche Forschungsgemeinschaft (DFG) in the framework of SPP1423 (grant No. LA1362/2) and thank Sören N. Eggerstedt for providing SEM images.

1.7 REFERENCES

Adler, M., Unger, M., Lee, G., 2000. Surface composition of spray-dried particles of bovine serum albumin/trehalose/surfactant. Pharm. Res. 17, 863–870.

Alchas, P.G., 2007. Nasal delivery device including spray nozzle. US 7296566.

Al Hakim K., Wigley, G., Stapely, 2006. Phase Doppler Anemotry Studies of Spray Freezing. Chemical Engineering Research and Design 84, 1142–1151.

Ali, M.E., Lamprecht, A., 2014. Spray freeze drying for dry powder inhalation of nanoparticles. Eur. J. Pharm. Biopharm. 87, 510–517.

Amorij, J.-P., Huckriede, A., Wilschut, J., Frijlink, H.W., Hinrichs, W.L.J., 2008. Development of Stable Influenza Vaccine Powder Formulations: Challenges and Possibilities. Pharm. Res. 25, 1256–1273.

Amorij, J.-P., Saluja, V., Petersen, A.H., Hinrichs, W.L.J., Huckriede, A., Frijlink, H.W., 2007. Pulmonary delivery of an inulin-stabilized influenza subunit vaccine prepared by spray-freeze drying induces systemic, mucosal humoral as well as cell-mediated immune responses in BALB/c mice. Vaccine 25, 8707–8717.

Audouy, S.A.L., van der Schaaf, G., Hinrichs, W.L.J., Frijlink, H.W., Wilschut, J., Huckriede, A., 2011. Development of a dried influenza whole inactivated virus vaccine for pulmonary immunization. Vaccine 29, 4345–4352.

Beinborn, N., 2012. Dry powder insufflation of crystalline amorphous voriconazole formulations produced by thin film freezing to mice. Eur J Pharm Biopharm 81, 620–628.

Bi, R., Shao, W., Wang, Q., Zhang, N., 2008. Spray-freeze-dried dry powder inhalation of insulin-loaded liposomes for enhanced pulmonary delivery. J. Drug Target. 16, 639–648.

Bosshammer, F., 2014. LyoMotion - Etablierte Technologie vs. Innovation in der Gefriertrocknung. Techno Pharm 3, 156–161.

Brandenberger, H., Nüssli, D., Piëch, V., Widmer, F., 1999. Monodisperse particle production: A method to prevent drop coalescence using electrostatic forces. J. Electrost. 45, 227–238.

Brenn, G., Durst, F., Tropea, C., 1996. Monodisperse sprays for various purposes—their production and characteristics. Part. Part. Syst. Charact. 13, 179–185.

Brenn, G., Helpiö, Durst, F., 1997. A new apparatus for the production of monodisperse sprays at high flow rates. Chemical Engineering Science 52, 237–244.

Briggs, A., Maxwell, T., 1975. Lyophilized biological products. US 3928566.

Briggs, A., Maxwell, Th.J., 1973. Process for preparing powder blends. US 37217256.

Briggs, A., Maxwell, T.J., 1976. Method for preparation of lyophilized biological products. US 3932943.

Buxton, I.R., Peach, J.M., 1984. Process and apparatus for freezin a liquid medium. US 4470202.

Carrasquillo, K.G., Stanley, A.M., Aponte-Carro, J.C., De Jésus, P., Costantino, H.R., Bosques, C.J., Griebenow, K., 2001. Non-aqueous encapsulation of excipient-stabilized spray-freeze dried BSA into poly (lactide-co-glycolide) microspheres results in release of native protein. J. Controlled Release 76, 199–208.

Carvalho, S.R., Watts, A.B., Peters, J.I., Liu, S., Hengsawas, S., Escotet-Espinoza, M.S., Williams III, R.O., 2014. Characterization and pharmacokinetic analysis of crystalline versus amorphous rapamycin dry powder via pulmonary administration in rats. Eur. J. Pharm. Biopharm. 88, 136–147.

Century, T.J., 2001. Intrapulmonary aerosolizer. US 6016800.

Chapman, H.N., 2009. X-ray imaging beyond the limits. Nat. Mater. 8, 299–301. doi:10.1038/nmat2402

Chen, R., Tagawa, M., Hoshi, N., Ogura, T., Okamoto, H., Danjo, K., 2004. Improved dissolution of an insoluble drug using a 4-fluid nozzle spray-drying technique. Chem. Pharm. Bull. (Tokyo) 52, 1066–1070.

Cheow, W.S., Ng, M.L.L., Kho, K., Hadinoto, K., 2011. Spray-freeze-drying production of thermally sensitive polymeric nanoparticle aggregates for inhaled drug delivery: Effect of freeze-drying adjuvants. Int. J. Pharm. 404, 289–300.

Claussen, I.C., Ustad, T.S., Strømmen, I., Walde, P.M., 2007. Atmospheric Freeze Drying—A Review. Dry. Technol. 25, 947–957.

Cleland, J.L., Duenas, E.T., Park, A., Daugherty, A., Kahn, J., Kowalski, J., Cuthbertson, A., 2001. Development of poly-(D, L-lactide–coglycolide) microsphere formulations containing recombinant human vascular endothelial growth factor to promote local angiogenesis. J. Controlled Release 72, 13–24.

Costantino, H.R., Firouzabadian, L., Hogeland, K., Wu, C., Beganski, C., Carrasquillo, K.G., Córdova, M., Griebenow, K., Zale, S.E., Tracy, M.A., 2000. Protein spray-freeze drying. Effect of atomization conditions on particle size and stability. Pharm. Res. 17, 1374–1382.

Costantino, H.R., Johnson, O.L., Zale, S.E., 2004. Relationship between encapsulated drug particle size and initial release of recombinant human growth hormone from biodegradable microspheres. J. Pharm. Sci. 93, 2624–2634.

Costantino, H.R., Pikal, M.J., 2004. Lyophilization of Biopharmaceuticals. Springer Science & Business Media.

Craig, R., 2002. Cryogenic freezing of liquids. US 6381067.

D'Addio, S.M., Chan, J.G.Y., Kwok, P.C.L., Benson, B.R., Prud'homme, R.K., Chan, H.-K., 2013. Aerosol Delivery of Nanoparticles in Uniform Mannitol Carriers Formulated by Ultrasonic Spray Freeze Drying. Pharm. Res. 30, 2891–2901.

D'Addio, S.M., Kafka, C., Akbulut, M., Beattie, P., Saad, W., Herrera, M., Kennedy, M.T., Prud'homme, R.K., 2010. Novel Method for Concentrating and Drying Polymeric Nanoparticles: Hydrogen Bonding Coacervate Precipitation. Mol. Pharm. 7, 557–564.

Dan, T., Yamamoto, T., Senda, J., Fujimoto, H., 1997. Effect of nozzle configurations of Non-Reacting Diesel Fuel Spray. SAE Transactions 106, 581–596.

Daugherty, A.L., Rangell, L.K., Eckert, R., Zavala-Solorio, J., Peale, F., Mrsny, R.J., 2011. Sustained release formulations of rhVEGF165 produce a durable response in a murine model of peripheral angiogenesis. Eur. J. Pharm. Biopharm. 78, 289–297.

Davis, R.B., DeVack, D.L., 1989. Droplet freezing method and apparatus. US 4848094.

Dean, H.J., Chen, D., 2004. Epidermal powder immunization against influenza. Vaccine 23, 681–686.

DePonte, D.P., Weierstall, U., Schmidt, K., Warner, J., Starodub, D., Spence, J.C.H., Doak, R.B., 2008. Gas dynamic virtual nozzle for generation of microscopic droplet streams. J. Phys. Appl. Phys. 41, 195505.

Di Matteo, P., Donsì, G., Ferrari, G., 2003. The role of heat and mass transfer phenomena in atmospheric freeze-drying of foods in a fluidised bed. J. Food Eng. 59, 267–275.

Dumouchel, C., 2008. On the experimental investigation on primary atomization of liquid streams. Ex Fluids 45, 371–422.

Dunn, E.B., Masavage, G.J., Sauer, H.A., 1972. Method of freezing solution droplets and the like using immiscible refrigerants of differing densities. US 3653222.

Edwards, D.A., 1997. Large Porous Particles for Pulmonary Drug Delivery. Science 276, 1868–1872.

Eggerstedt, S., Lamprecht, A., 2012. Sprühgefriertrocknung zur Herstellung von stabilen sphärischen Protein-Lyophilisaten. Presented at the Spray Conference Berlin.

Eggerstedt, S.N., Dietzel, M., Sommerfeld, M., Süverkrüp, R., Lamprecht, A., 2012. Protein spheres prepared by drop jet freeze drying. Int. J. Pharm. 438, 160–166.

Engstrom, J.D., Tam, J.M., Miller, M.A., Williams, R.O., Johnston, K.P., 2008. Templated Open Flocs of Nanorods for Enhanced Pulmonary Delivery with Pressurized Metered Dose Inhalers. Pharm. Res. 26, 101–117.

Gañán-Calvo, A.M., 1998. Generation of steady liquid microthreads and micron-sized monodisperse sprays in gas streams. Phys. Rev. Lett. 80, 285.

Gao, Y., Zhu, C.-L., Zhang, X.-X., Gan, L., Gan, Y., 2011. Lipid–polymer composite microspheres for colon-specific drug delivery prepared using an ultrasonic spray freeze-drying technique. J. Microencapsul. 28, 549–556.

Garmise, R.J., Mar, K., Crowder, T.M., Hwang, C.R., Ferriter, M., Huang, J., Mikszta, J.A., Sullivan, V.J., Hickey, A.J., 2006. Formulation of a dry powder influenza vaccine for nasal delivery. AAPS PharmSciTech 7, E131–E137.

Garmise, R.J., Staats, H.F., Hickey, A.J., 2007. Novel dry powder preparations of whole inactivated influenza virus for nasal vaccination. AAPS PharmSciTech 8, 2–10.

Gombotz, W.R., Healy, M.S., Brown, L.R., 1991. Very low temperature casting of controlled release microspheres. US 5019400.

Gusarov, A.V., Smurov, I., 2002. Gas-dynamic boundary conditions of evaporation and condensation: Numerical analysis of the Knudsen layer. Phys. Fluids 1994-Present 14, 4242–4255.

Haas, H., 2008. Spray-freeze-drying process for the preparation of pellets comprising percolation drying. EP 1915987A1.

Harker, J., Li, C.-T., Gaisford, S., Jones, M., 2008. Novel Use of a Modified Ink-Jet Print Head to Produce Spray-Freeze Dried Particles for Inhalation, in: DDL Conference.

Heldman, D.R., 1974. An analysis of atmospheric freeze drying. J Food Sci 39, 147–155.

Henczka, M., Bałdyga, J., Shekunov, B.Y., 2006. Modelling of spray-freezing with compressed carbon dioxide. Chem. Eng. Sci. 61, 2880–2887.

Herbert, P.F., Healy, M.S., 1999. Production scale method of forming microparticles. US 5922253.

Her, J.-Y., Song, C.-S., Lee, S.J., Lee, K.-G., 2010. Preparation of kanamycin powder by an optimized spray freeze-drying method. Powder Technol. 199, 159–164.

He, X., Pei, L., Tong, H.H.Y., Zheng, Y., 2011. Comparison of Spray Freeze Drying and the Solvent Evaporation Method for Preparing Solid Dispersions of Baicalein with Pluronic F68 to Improve Dissolution and Oral Bioavailability. AAPS PharmSciTech 12, 104–113.

Hindmarsh, J.P., Russell, A.B., Chen, X.D., 2003. Experimental and numerical analysis of the temperature transition of a suspended freezing water droplet. Int. J. Heat Mass Transfer 46, 1199–1213.

Hoffer, T.E., 1961. A laboratory investigation of droplet freezing. J Meteorol 18, 766–778.

Huang, J., D'Souza, A.J., Alarcon, J.B., Mikszta, J.A., Ford, B.M., Ferriter, M.S., Evans, M., Stewart, T., Amemiya, K., Ulrich, R.G., Sullivan, V.J., 2009. Protective Immunity in Mice Achieved with Dry Powder Formulation and Alternative Delivery of Plague F1-V Vaccine. Clin. Vaccine Immunol. 16, 719–725.

Hu, J., Johnston, K.P., Williams, R.O., 2004a. Rapid dissolving high potency danazol powders produced by spray freezing into liquid process. Int. J. Pharm. 271, 145–154.

Hu, J., Johnston, K.P., Williams, R.O., 2004b. Nanoparticle Engineering Processes for Enhancing the Dissolution Rates of Poorly Water Soluble Drugs. Drug Dev. Ind. Pharm. 30, 233–245.

Hu, J., Johnston, K.P., Williams, R.O., 2003. Spray freezing into liquid (SFL) particle engineering technology to enhance dissolution of poorly water soluble drugs: organic solvent versus organic/aqueous co-solvent systems. Eur. J. Pharm. Sci. 20, 295–303.

Hu, J., Rogers, T.L., Brown, J., Young, T., Johnston, K.P., Williams Iii, R.O., 2002. Improvement of dissolution rates of poorly water soluble APIs using novel spray freezing into liquid technology. Pharm. Res. 19, 1278–1284.

Jennings, T.A., 1999. Lyophilization: Introduction and Basic Principles. Taylor & Francis.

Jiang, G., Joshi, S.B., Peek, L.J., Brandau, D.T., Huang, J., Ferriter, M.S., Woodley, W.D., Ford, B.M., Mar, K.D., Mikszta, J.A., Hwang, C.R., Ulrich, R., Harvey, N.G.,

Middaugh, C.R., Sullivan, V.J., 2006. Anthrax vaccine powder formulations for nasal mucosal delivery. J. Pharm. Sci. 95, 80–96.

Johnson, O.L., Jaworowicz, W., Cleland, J.L., Bailey, L., Charnis, M., Duenas, E., Wu, C., Shepard, D., Magil, S., Last, T., Jones, A.J.S., Putney, S.D., 1997. The Stabilization and Encapsulation of Human Growth Hormone into Biodegradable Microspheres. Pharm. Res. 14, 730–735.

Kendall, M., Mitchell, T., Wrighton-Smith, P., 2004. Intradermal ballistic delivery of micro-particles into excised human skin for pharmaceutical applications. J. Biomech. 37, 1733–1741.

Kennedy, M.T., Ali-Reynolds, A., Farrier, C., Burke, P.A., 2008. Atomizing into a Chilled Extraction Solvent Eliminates Liquid Gas from a Spray-Freeze Drying Microencapsulation Process. J. Pharm. Sci. 97, 4459–4472.

Knudsen, M., 1909. Die Gesetze der Molekularströmung und der inneren Reibungsströmung der Gase durch Röhren. Ann. Phys. 333, 75–130.

Kondo, M., Niwa, T., Danjo, K., 2011. Dissolution characteristics of composite particles using a spray freeze drying. Yakugaku Zasshi 131, 711–719.

Kondo, M., Niwa, T., Okamoto, H., Danjo, K., 2009a. Particle characterization of poorly water-soluble drugs using a spray freeze drying technique. Chem. Pharm. Bull. (Tokyo) 57, 657–662.

Kondo, M., Niwa, T., Okamoto, H., Danjo, K., 2009b. Particle characterization of poorly water-soluble drugs using a spray freeze drying technique. Chem. Pharm. Bull. (Tokyo) 57, 657–662.

Krämer, B., Hübner, O., Vortisch, H., Wöste, L., Leisner, T., Schwell, M., Rühl, E., Baumgärtel, H., 1999. Homogeneous nucleation rates of supercooled water measured in single levitated microdroplets. J. Chem. Phys. 111, 6521–6527.

Lam, X.M., Duenas, E.T., Cleland, J.L., 2001. Encapsulation and stabilization of nerve growth factor into poly (lactic-co-glycolic) acid microspheres. J. Pharm. Sci. 90, 1356–1365.

Lam, X.M., Duenas, E.T., Daugherty, A.L., Levin, N., Cleland, J.L., 2000. Sustained release of recombinant human insulin-like growth factor-I for treatment of diabetes. J. Controlled Release 67, 281–292.

Lastow, O., Andersson, J., Nilsson, A., Balachandran, W., 2007. Low-Voltage Electrohydrodynamic (EHD) Spray Drying of Respirable Particles. Pharm. Dev. Technol. 12, 175–181.

Leach, W.T., Simpson, D.T., Val, T.N., Anuta, E.C., Yu, Z., Williams, R.O., Johnston, K.P., 2005. Uniform Encapsulation of Stable Protein Nanoparticles Produced by Spray Freezing for the Reduction of Burst Release. J. Pharm. Sci. 94, 56–69.

Leuenberger, H. (Ed.), 2001. Spray Freeze-drying – The Process of Choice for Low Water Soluble Drugs? J Nanopart Res 4, 111–119.

Leuenberger, H., 1986. Process of drying a particulate material and apparatus for implementing the process. US 4608764.

Leuenberger, H., Plitzko, M., Puchkov, M., 2006. Spray Freeze Drying in a Fluidized Bed at Normal and Low Pressure. Dry. Technol. 24, 711–719.

Liapis, A.I., Bruttini, R., 2009. A mathematical model for the spray freeze drying process: The drying of frozen particles in trays and in vials on trays. Int. J. Heat Mass Transf. 52, 100–111.

Lilakos, L., 1990. Method and apparatus for cryogenic crystallization of fats. US 4952224.

Maa, Y.-F., Ameri, M., Shu, C., Payne, L.G., Chen, D., 2004. Influenza vaccine powder formulation development: spray-freeze-drying and stability evaluation. J. Pharm. Sci. 93, 1912–1923.

Maa, Y.-F., Nguyen, P.-A., Sweeney, T., Shire, S.J., Hsu, C.C., 1999. Protein inhalation powders: spray drying vs spray freeze drying. Pharm. Res. 16, 249–254.

Maa, Y.-F., Zhao, L., Payne, L.G., Chen, D., 2003. Stabilization of alum-adjuvanted vaccine dry powder formulations: Mechanism and application. J. Pharm. Sci. 92, 319–332.

Menshutina, N., Korneeva, A.E., Goncharova, S., Leuenberger, H., 2004. Modeling of freeze drying in fluidized bed, in: Proceedings of the 14th International Drying Symposium, Silva, MA et Al., Eds., Sao Paulo, Brazil. pp. 680–686.

Meryman, H., 1963. Apparatus for freeze-drying at atmospheric pressure. US 3096161.

Meryman, H., 1959. Sublimation Freeze Drying without Vacuum. Science 628–629.

Mikszta, J.A., Sullivan, V.J., Dean, C., Waterston, A.M., Alarcon, J.B., Dekker, J.P., Brittingham, J.M., Huang, J., Hwang, C.R., Ferriter, M., others, 2005. Protective immunization against inhalational anthrax: a comparison of minimally invasive delivery platforms. J. Infect. Dis. 191, 278–288.

Mohri, K., Okuda, T., Mori, A., Danjo, K., Okamoto, H., 2010. Optimized pulmonary gene transfection in mice by spray–freeze dried powder inhalation. J. Controlled Release 144, 221–226.

Mueannoom, W., Srisongphan, A., Taylor, K.M.G., Hauschild, S., Gaisford, S., 2012. Thermal ink-jet spray freeze-drying for preparation of excipient-free salbutamol sulphate for inhalation. Eur. J. Pharm. Biopharm. 80, 149–155.

Mumenthaler, M., Leuenberger, H., 1991. Atmospheric spray-freeze drying: a suitable alternative in freeze-drying technology. Int Pharm J 72, 97–110.

Murphy, J.L., Grace, G.K., Rogers, R.E., 1974. Spray-Freezing apparatus and method. 378095.

Niksiar, A., Sohrabi, M., Rahimi, A., 2013. A Model for the Dynamics of Spouted Bed Dryers. Dry. Technol. 31, 295–307.

Niwa, T., Mizutani, D., Danjo, K., 2012. Spray freeze-dried porous microparticles of a poorly water-soluble drug for respiratory delivery. Chem. Pharm. Bull. (Tokyo) 60, 870–876.

Niwa, T., Shimabara, H., Danjo, K., 2010. Novel spray freeze-drying technique using four-fluid nozzle-development of organic solvent system to expand its application to poorly water soluble drugs. Chem. Pharm. Bull. (Tokyo) 58, 195–200.

Niwa, T., Shimabara, H., Kondo, M., Danjo, K., 2009. Design of porous microparticles with single-micron size by novel spray freeze-drying technique using four-fluid nozzle. Int. J. Pharm. 382, 88–97.

O'Donnell, K.P., Cai, Z., Schmerler, P., III, R.O.W., 2013. Atmospheric freeze drying for the reduction of powder electrostatics of amorphous, low density, high surface area pharmaceutical powders. Drug Dev. Ind. Pharm. 39, 205–217.

Ohashi, K., Kabasawa, T., Ozeki, T., Okada, H., 2009. One-step preparation of rifampicin/poly(lactic-co-glycolic acid) nanoparticle-containing mannitol microspheres using a four-fluid nozzle spray drier for inhalation therapy of tuberculosis. J. Controlled Release 135, 19–24.

Overhoff, K.A., Engstrom, J.D., Chen, B., Scherzer, B.D., Milner, T.E., Johnston, K.P., Williams, R.O., 2007. Novel ultra-rapid freezing particle engineering process for enhancement of dissolution rates of poorly water-soluble drugs. Eur. J. Pharm. Biopharm. 65, 57–67.

Oyler, J.R.J., 1993. Liquid substances freeze-drying systems and methods. US 5208998.

Pabari, R.M., Sunderland, T., Ramtoola, Z., 2012. Investigation of a novel 3-fluid nozzle spray drying technology for the engineering of multifunctional layered microparticles. Expert Opin. Drug Deliv. 9, 1463–1474.

Park, S.-H., Kwon, J.-H., Lim, S.-H., Park, H.W., Kim, C.-W., 2007. Characterization of human insulin microcrystals and their absorption enhancement by protease inhibitors in rat lungs. Int. J. Pharm. 339, 205–212.

Pechtl, K., 2009. Erzeugung und Ablenkung von Tintentropfen in einem kontinuierlich arbeitenden Tintenstrahldrucker. EP 08012210NWA1.

Pikal, M.J., Shah, S., Senior, D., Lang, J.E., 1983. Physical Chemistry of Freeze-drying: Measurement of Sublimation Rates for Frozen Aqueous Solutions by a Microbalance Technique. J. Pharm. Sci. 72, 635–650.

Prat, M., 1993. Percolation model of drying under isothermal conditions in porous media. Int. J. Multiph. Flow 19, 691–704.

Reitz, R., Diwakar, R., 1987. Structure of High-Pressure Fuel Sprays. SAE Technical Paper.

Rey, L., May, J.C. (Eds.), 2011. Freeze drying/lyophilization of pharmaceutical and biological products, 3rd ed. ed, Drugs and the pharmaceutical sciences. Informa Healthcare, New York ; London.

Roa, W.H., Azarmi, S., Al-Hallak, M.H.D.K., Finlay, W.H., Magliocco, A.M., Löbenberg, R., 2011. Inhalable nanoparticles, a non-invasive approach to treat lung cancer in a mouse model. J. Controlled Release 150, 49–55.

Rochelle, C., Lee, G., 2007. Dextran or Hydroxyethyl Starch in Sprayfreeze-Dried Trehalose/Mannitol Microparticles Intended as Ballistic Particulate Carriers for Proteins. J. Pharm. Sci. 96, 2296–2309.

Rogers, T., 2003. Micronized powders of a poorly water soluble drug produced by a spray-freezing into liquid-emulsion process. Eur. J. Pharm. Biopharm. 55, 161–172.

Rogers, T.L., Hu, J., Yu, Z., Johnston, K.P., Williams, R.O., 2002a. A novel particle engineering technology: spray-freezing into liquid. Int. J. Pharm. 242, 93–100.

Rogers, T.L., Johnston, K.P., III, R.O.W., 2001. Solution-Based Particle Formation of Pharmaceutical Powders by Supercritical or Compressed Fluid Co2 and Cryogenic Spray-Freezing Technologies. Drug Dev. Ind. Pharm. 27, 1003–1015.

Rogers, T.L., Johnston, K.P., Williams III, R.O., 2003a. Physical Stability of Micronized Powders Produced by Spray-Freezing into Liquid (SFL) to Enhance the Dissolution of an Insoluble Drug. Pharm. Dev. Technol. 8, 187–197.

Rogers, T.L., Nelsen, A.C., Hu, J., Brown, J.N., Sarkari, M., Young, T.J., Johnston, K.P., Williams, R.O., 2002b. A novel particle engineering technology to enhance dissolution of poorly water soluble drugs: spray-freezing into liquid. Eur. J. Pharm. Biopharm. 54, 271–280.

Rogers, T.L., Nelsen, A.C., Sarkari, M., Young, T.J., Johnston, K.P., Williams III, R.O., 2003b. Enhanced Aqueous Dissolution of a Poorly Water Soluble Drug by Novel Particle Engineering Technology: Spray-Freezing into Liquid with Atmospheric Freeze-Drying 20, 485–493.

Rogers, T.L., Overhoff, K.A., Shah, P., Santiago, P., Yacaman, M.J., Johnston, K.P., Williams III, R.O., 2003c. Micronized powders of a poorly water soluble drug produced by a spray-freezing into liquid-emulsion process. Eur. J. Pharm. Biopharm. 55, 161–172.

Saluja, V., Amorij, J.-P., Kapteyn, J.C., de Boer, A.H., Frijlink, H.W., Hinrichs, W.L.J., 2010. A comparison between spray drying and spray freeze drying to produce an influenza subunit vaccine powder for inhalation. J. Controlled Release 144, 127–133.

Sauer, H., 1969. Method and Apparatus for Freeze-Freeze Drying. US 3484946.

Schiffter, H., Condliffe, J., Vonhoff, S., 2010. Spray-freeze-drying of nanosuspensions: the manufacture of insulin particles for needle-free ballistic powder delivery. J. R. Soc. Interface 7, S483–S500.

Schneider, B.M., 2003. Strukturelle Untersuchungen zur Spraystruktur. Eidgenössische Techische Hochschule Zürich.

Seaver, A.E., 1984. Closed Form Equations for the Evaporation Rate and Droplet Size of Knudsen Droplets. Aerosol Sci. Technol. 3, 177–185.

Sharma, G., Mueannoom, W., Buanz, A.B.M., Taylor, K.M.G., Gaisford, S., 2013. In vitro characterisation of terbutaline sulphate particles prepared by thermal ink-jet spray freeze drying. Int. J. Pharm. 447, 165–170.

Stabenau, A., Winter, G., 2007. Application and Drying of Protein Drug Microdroplets on Solid Surfaces. Pharm. Dev. Technol. 12, 61–70.

Strutt, J.W., 1878. On the instability of jets. Proc London Math Soc 10, 4–13.

Süverkrüp, R., 2014. Verfahren und Anlage zur kontinuierlichen Produktion von pharmazeutischen Lyophilisaten (German Application). 10 2011 053 7732.

Süverkrüp, R., Eggerstedt, S.N., Gruner, K., Kuschel, M., Sommerfeld, M., Lamprecht, A., 2013. Collisions in fast droplet streams for the production of spherolyophilisates. Eur. J. Pharm. Sci. 49, 535–541.

Süverkrüp, R., Gruner, K., Diestelhorst, M., 2009. Spherolyophilizates as Ophthalmic Drug Delivery Systems. Invest. Ophthalmol. Vis. Sci. 50, 5557–5557.

Sweeney, L.G., Wang, Z., Loebenberg, R., Wong, J.P., Lange, C.F., Finlay, W.H., 2005. Spray-freeze-dried liposomal ciprofloxacin powder for inhaled aerosol drug delivery. Int. J. Pharm. 305, 180–185.

Tagami, M., Hamai, M., Mogi, I., Watanabe, K., Motokawa, M., 1999. Solidification of levitating water in a gradient strong magnetic field. J. Cryst. Growth 203, 594–598.

Thomas H Adams, James P Beck, Robert C Menson, 1982. Novel Particulate Compositions. US 4332478.

Thomas H Adams, James P Beck, Robert C Menson, 1978. Method and Apparatus for making novel particulate compositions. US4122015.

Tong, H.H.Y., Du, Z., Wang, G.N., Chan, H.M., Chang, Q., Lai, L.C.M., Chow, A.H.L., Zheng, Y., 2011. Spray freeze drying with polyvinylpyrrolidone and sodium caprate for improved dissolution and oral bioavailability of oleanolic acid, a BCS Class IV compound. Int. J. Pharm. 404, 148–158.

Trelea, J., Passot, S., Marin, M., Foseca, F., 2009. Model for Heat and Mass Transfer in Freeze-Drying of Pellets. J Biomech Engin 131, 4p.

Tsukamoto, M., Okuda, T., Okamoto, H., Higuchi, Y., Kawakami, S., Yamashita, F., Hashida, M., 2012. Bovine serum albumin as a lyoprotectant for preparation of DNA dry powder formulations using the spray-freeze drying method. Biol. Pharm. Bull. 35, 1178–1181.

van Drooge, D.J., Hinrichs, W.L.J., Visser, M.R., Frijlink, H.W., 2006. Characterization of the molecular distribution of drugs in glassy solid dispersions at the nano-meter scale, using differential scanning calorimetry and gravimetric water vapour sorption techniques. Int. J. Pharm. 310, 220–229.

Vaughn, J.M., Wiederhold, N.P., McConville, J.T., Coalson, J.J., Talbert, R.L., Burgess, D.S., Johnston, K.P., Williams, R.O., Peters, J.I., 2007. Murine airway histology and intracellular uptake of inhaled amorphous itraconazole. Int. J. Pharm. 338, 219–224. doi:10.1016/j.ijpharm.2007.02.014

Waananen, K.M., Litchfield, J.B., Okos, M.R., 1993. Classification of Drying Models for Porous Solids. Dry. Technol. 11, 1–40.

Walzel, P., Schmelz, F., Schneider, S., 2001. Herstellen monodisperser Tropfen mit pneumatischen Ziehdüsen. Chem. Ing. Tech. 73, 1599–1602.

Wang, S.H., Kirwan, S.M., Abraham, S.N., Staats, H.F., Hickey, A.J., 2012. Stable Dry Powder Formulation for Nasal Delivery of Anthrax Vaccine. J. Pharm. Sci. 101, 31–47.

Wang, Y., Kho, K., Cheow, W.S., Hadinoto, K., 2012. A comparison between spray drying and spray freeze drying for dry powder inhaler formulation of drug-loaded lipid–polymer hybrid nanoparticles. Int. J. Pharm. 424, 98–106.

Wang, Z.L., Finlay, W.H., Peppler, M.S., Sweeney, L.G., 2006. Powder formation by atmospheric spray-freeze-drying. Powder Technol. 170, 45–52.

Watts, A.B., Wang, Y.-B., Johnston, K.P., Williams, R.O., 2013. Respirable Low-Density Microparticles Formed In Situ from Aerosolized Brittle Matrices. Pharm. Res. 30, 813–825.

Webb, S.D., Golledge, S.L., Cleland, J.L., Carpenter, J.F., Randolph, T.W., 2002. Surface Adsorption of Recombinant Human Interferon-g in Lyophilized and Spray-Lyophilized Formulations. J Pharm Sci 91.

Wegener, A., 1911. Kerne der Kristallbildung. Thermodynamik der Atmosphäre. J.A. Barth, Leipzig 94–98.

Werly, E.P., Bauman, E.K., 1964. Production of Submicronic Powder by Spray-Freezing. Arch. Environ. Health 9, 567–571.

Westergaardt, V., 2010. Milk Powder Technology –Evaporation and Spray Drying. 5th Ed GEA Niro 93.

Whelehan, M., Marison, I.W., 2011. Microencapsulation using vibrating technology. J. Microencapsul. 28, 669–688.

Wijshoff, H., 2008. Structure- and fluid-dynamics in piezo inkjet printheads. Universiteit Twente.

Wright, M.E.B., Dropkin, A., Truscott, T., 2013. Cavitation in submerged jets 54, 1541ff.

Yu, Z., Garcia, A.S., Johnston, K.P., Williams III, R.O., 2004. Spray freezing into liquid nitrogen for highly stable protein nanostructured microparticles. Eur. J. Pharm. Biopharm. 58, 529–537.

Zhang, W.-M., Meng, G., Wei, X., 2012. A review on slip models for gas microflows. Microfluid. Nanofluidics 13, 845–882.

Zijlstra, G.S., Hinrichs, W.L.J., Boer, A.H. de, Frijlink, H.W., 2004. The role of particle engineering in relation to formulation and de-agglomeration principle in the development of a dry powder formulation for inhalation of cetrorelix. Eur. J. Pharm. Sci. 23, 139–149.

Zijlstra, G.S., J. Ponsioen, B., A. Hummel, S., Sanders, N., Hinrichs, W.L.J., de Boer, A.H., Frijlink, H.W., 2009. Formulation and process development of (recombinant human) deoxyribonuclease I as a powder for inhalation. Pharm. Dev. Technol. 14, 358–368.

Zijlstra, G.S., Rijkeboer, M., Drooge, D.J. van, Sutter, M., Jiskoot, W., Weert, M. van de, Hinrichs, W.L.J., Frijlink, H.W., 2007. Characterization of a cyclosporine solid dispersion for inhalation. AAPS J. 9, E190–E199.

AERODYNAMIC DROPLET STREAM EXPANSION FOR THE PRODUCTION OF SPRAY FREEZE DRIED POWDERS

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This chapter was published as follows:

'Aerodynamic Droplet Stream Expansion for the Production of Spray Freeze Dried Powders'

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2.1 ABSTRACT

In Spray-Freeze-Drying (SFD) a solution is sprayed into a refrigerant medium, frozen and subsequently sublimation dried which allows the production of flowable lyophilized powders. SFD allows commonly freeze-dried active pharmaceutical ingredients (e.g. proteins and peptides) to be delivered using new applications such as needle-free injection and nasal or pulmonary drug delivery. In this study a droplet stream was injected into a vortex of cold gas in order to reduce the risk of droplet collisions and therefore droplet growth before congelation which adversely affects the particle size distribution. Droplets with initial diameters of about 40-50 µm were frozen quickly in a swirl tube at temperatures around -75 °C and volumetric gas flow rates between 17 and 34 L/min. Preliminary studies that were focused on the evaluation of spray-cone footprints were performed prior to SFD. A 2^3 factorial design with a model solution of mannitol (1.5 % m/V) and maltodextrin (1.5 % m/V) was used to create flowable, low density (0.01 – 0.03 g/cm^3) spherical lyophilisate powders. Mean particle diameters sizes of the highly porous particles ranged between 49.8 ± .6 µm and 88.3 ± 5.5 µm. Under optimal conditions, the mean particle size was reduced from 160 µm to 50 µm (decrease of volume by 96 %) compared to non-expanded streams, whereas the SPAN value did not change significantly. This method is suitable for the production of lyophilized powders with small particle sizes and narrow particle size distributions which is highly interesting for needle-free injection or nasal delivery of proteins and peptides.

Keywords: Spray freeze drying, protein formulations, lyophilisation, porous particles, flowable lyophilized powders

2.2 INTRODUCTION

The process of spray-freeze drying (SFD) has been widely studied in pharmaceutical research and food sciences. Various approaches and versions have been developed which can be broken down into three basic steps: droplet generation, freezing, and sublimation drying (Wanning et al., 2015).

SFD offers the possibility to create stable and flowable peptide, protein and nucleic acid derivate formulations. The technique is suitable for the freeze-drying of bulk powders prior to dispensing them into containers or processing as it allows more flexibility than freeze drying in vials. Spherolyophilisates with diameters between 10 and 80 µm have been used for needle-free injection (Schiffter et al., 2010), delivery of vaccines to the nasal mucosa (Wang et al., 2012) and pulmonary drug delivery (Bi et al., 2008) and have shown promising results (Wanning et al., 2015). Furthermore, spherolyophilisates can be produced by droplet stream lyophilization of active ingredients, matrix forming and cryo-stabilizing excipients (Eggerstedt et al., 2012).

Droplets can be generated by different atomization techniques such as hydraulic single-fluid (Hu et al., 2003), pneumatic two-fluid (Ali and Lamprecht, 2014; Eggerstedt, 2014; Maa et al., 1999) and four-fluid nozzles (Niwa et al., 2012), thermal ink-jet printheads (Mueannoom et al., 2012; Sharma et al., 2013), ultrasonic atomizers (Maa et al., 2004; Rochelle, 2005; Schiffter et al., 2010) or piezoelectric droplet-stream generators (Eggerstedt et al., 2012). Each technique has its individual advantages and downsides. Pneumatic nozzles produce small particles but have wide particle size distributions. Thermal ink-jet printheads offer the possibility to produce very small amounts, but may inflict damage to thermo-sensitive constituents. Ultrasonic atomizers can produce small particles with narrow size distributions, but atomization is affected by the viscosity of the spray solution. Further advantages and shortcomings of the individual techniques have been discussed earlier (Wanning et al., 2015). The most common technique used for the freezing of droplets is to spray them over a vessel filled with a cryogenic liquid (mostly liquid nitrogen) where they freeze before or upon contact with the liquid phase (Maa et al., 2004, 1999; Mueannoom et al., 2012; Niwa et al., 2012; Rochelle, 2005; Schiffter et al., 2010; Sharma et al., 2013). An alternative technique

consists of atmospheric freezing where the droplets are sprayed into a cold gas (Ali and Lamprecht, 2014; Eggerstedt et al., 2012). This allows an easy collection of the product and facilitates the transport to the freeze dryer as the particles do not have to be separated from the cryogenic liquid.

The size, shape, internal structure and surface features of the particles are mostly determined in the freezing step and not during solvent evaporation as in spray drying. Since the droplet size hardly changes after congelation, the particle density and therefore the aerodynamic diameter can be controlled by adjusting the solid content of the spray solution.

The aerodynamic diameter of spherical particles is related to the geometrical diameter and the particle density and may be described by the following equation:

$$d_{ae} = d_p \sqrt{\frac{\rho_p}{\rho_0 \chi}} \qquad \text{(Equation 2-1)}$$

with:
d_{ae}: aerodynamic diameter,
d_p: geometric particle diameter
ρ_p: particle density
ρ_0: unit density (1 g/cm^3),
χ: shape factor

Droplet-stream generators (DSG) produce mono-disperse droplets and are potentially suited for the production of lyophilized powders with extremely narrow particle size distributions. In order to obtain nearly mono-disperse, small particles, the first steps of the production process, namely droplet generation and freezing, must be closely controlled to reduce the risk of droplet collisions before congelation (Süverkrüp et al., 2016, 2013). Eggerstedt et al. reported that a droplet stream generator with nozzle diameters of 20, 50 and 100 µm was used to produce lyophilized powders with mean particle sizes of 160 - 800 µm (Eggerstedt, 2014; Eggerstedt et al., 2012). For some dosage forms (e.g. needle-free injection or pulmonary application), smaller particles with narrow particle size distributions are required. Therefore,

aerodynamic droplet stream expansion, which circumvents droplet collisions, may be an alternative technique for the production of particles fitted for these requirements. The droplet stream was injected into a vortex of cold gas inside a swirl tube as depicted in Figure 2-1b which simultaneously accelerates the droplets in the horizontal direction and reduces their temperature below the freezing point. The risk of droplet collisions before congelation and therefore the mean particle size of the lyophilized particles is reduced.

The aim of the study was to assess the impact of the factors on the efficiency of the droplet stream expansion and therefore the prevention of droplet collisions, size reduction and effect on the width of the particle size distribution. Furthermore, attempts were made to establish a fast screening method, which consisted of spraying a dyed solution on ink-jet paper for at a large number of factorial levels, to predict the outcome of SFD trials. The influence of the droplet generator position and gas flow rate on the size distributions of droplet traces and lyophilized particles were studied in a spray cone screening study and in a 2^3 factorial design freeze drying experiment.

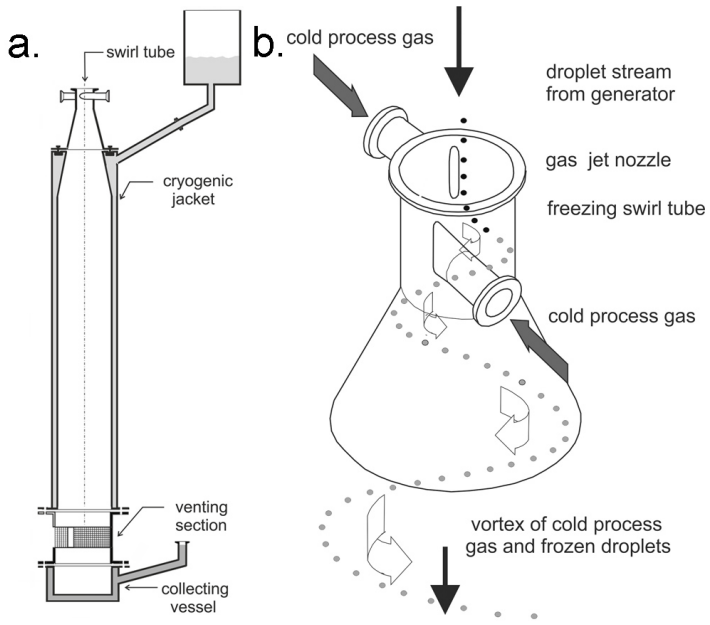

Figure 2-1: Droplet Stream Freezer, longitudinal section (a) and swirl tube (b)

2.3 MATERIALS AND METHODS

2.3.1 MATERIALS

d(−)-Mannitol [Ph. Eur.] was purchased from VWR International, NL-Amsterdam. Maltodextrin (DEX) [Roquette LAB 2509, dextrose equivalent of DE = 19] was a gift from Roquette Frères, F-Lestrem Cédex. Ultrapure water was produced with a MilliQ, Millipore Corp., USA- Billerica, Massachusetts. Liquid nitrogen was purchased from Linde, D-Pullach.

2.3.2 SPRAY FREEZE DRYING

A FMP Type MTG – 01.G1 droplet stream generator (DSG) (FMP Technologies GmbH, D-Erlangen) with a 20 µm pinhole diaphragm was used for droplet generation. The droplet generator was fed with a solution of mannitol (1.5 % m/V), maltodextrin (1.5 % m/V) and fluorescein sodium (0.05 % m/m) with a pressure of 250 kPa. The operating frequency was set to approximately 48 kHz. Correct droplet generation was checked before spraying by stroboscopic microscopy and the operating frequency was fine tuned to ensure optimal droplet generation.

The original experimental set-up from the spray-cone footprints was reduced to a 2^3 factorial design in order to minimize the time consuming process of SFD and optimize particle characterization.

The effects of the factors within the factorial design were calculated as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 \qquad \text{(Equation 2-2)}$$

with:

X_1: Volumetric gas flow

X_2: Vertical position

X_3: Horizontal position

b_0: Arithmetic mean of all observations

b_1: Effect of volumetric gas flow

b_2: Effect of vertical position of DSG

b_3: Effect of horizontal position of DSG
b_{12}: Interaction between gas flow and vertical position
b_{13}: Interaction between gas flow and horizontal position
b_{23}: Interaction between vertical and horizontal position
b_{123}: Triple interaction

A swirl tube as depicted in Figure 2-1b was used for the deflection of the droplet stream, where a gas vortex was created by injecting cooled process gas (nitrogen) through two tangentially positioned jet nozzles into a cylindrical chamber. The process gas was split in two partial flows prior to injection: one of the partial flows was cooled below -160 °C by passing through a liquid nitrogen filled heat exchanger while the second partial flow was maintained at ambient temperature and admixed in order to adjust the temperature of the gas vortex. In the SFD experiments, the DSG was positioned in the swirl chamber on top of the freezing tube in the epicyclical positioning device and was heated to prevent the nozzle from freezing. The horizontal distances of the DSG from the gas inlet were 17.05 mm and 25.07 mm. The vertical positions (V-Pos) were 0 mm and 10 mm and the gas flow for the swirl-tube was set to 17 L/min and 34 L/min. The parameters used for the 2^3 design of experiments are shown in Table 2-1 (see SFD-Trial #). The nitrogen for the gas flow in the swirl tube was cooled down to -75 °C. The droplets were frozen in a cold atmosphere inside a double walled stainless steel tube at temperatures below -80 °C (Figure 2-1a). The steel tube was cooled down by filling liquid nitrogen into the surrounding coolant jacket. Temperatures were recorded and monitored using a Testo T176 T4 temperature logger in the mixing chamber, swirl tube and freezing tubes respectively. The frozen particles were collected in a refrigerated glass vessel at the bottom of the freezing tube below the gas-permeable venting section and transferred into a STERIS Lyovac GT2 freeze dryer (STERIS, D-Hürth) and were dried for at least 48 h at 0.05 bar. The plate temperature was ramped up from -27 °C to 10 °C within the first 5 hours.

Table 2-1: 2^3 Factorial design of experiments

Spray Trial #	SFD-Trial #	V-Pos[1]) (mm)	GasFlow (L/min)	H-Pos[2]) #	α[3])	β[4])	X_D[5]) (mm)	Y_D[6]) (mm)	D[7]) (mm)
1		10	17	1	345	30	6.32	15.10	29.35
2	2	10	17	2	345	0	2.07	16.23	25.07
3		10	17	3	0	0	0.00	16.50	23.00
4		10	17	4	15	0	-2.07	16.23	20.93
5		10	17	5	30	0	-4.00	15.43	19.03
6	6	10	17	6	45	0	-5.66	14.16	17.50
7		10	34	1	345	30	6.32	15.10	29.35
8	1	10	34	2	345	0	2.07	16.23	25.07
9		10	34	3	0	0	0.00	16.50	23.00
10		10	34	4	15	0	-2.07	16.23	20.93
11		10	34	5	30	0	-4.00	15.43	19.03
12	5	10	34	6	45	0	-5.66	14.16	17.50
13		0	17	1	345	30	6.32	15.10	29.35
14	4	0	17	2	345	0	2.07	16.23	25.07
15		0	17	3	0	0	0.00	16.50	23.00
16		0	17	4	15	0	-2.07	16.23	20.93
17		0	17	5	30	0	-4.00	15.43	19.03
18	8	0	17	6	45	0	-5.66	14.16	17.50
19		0	34	1	345	30	6.32	15.10	29.35
20	3	0	34	2	345	0	2.07	16.23	25.07
21		0	34	3	0	0	0.00	16.50	23.00
22		0	34	4	15	0	-2.07	16.23	20.93
23		0	34	5	30	0	-4.00	15.43	19.03
24	7	0	34	6	45	0	-5.66	14.16	17.50

1) Vertical position of droplet generator
2) Horizontal position of droplet generator
3) Deferent angle
4) Epicyclic angle
5) Abscissa coordinate of droplet stream
6) Ordinate coordinate of droplet stream
7) Distance of droplet stream from slot nozzle

2.3.3 SPRAY CONE FOOTPRINTS

The influence of the DSG position and the volumetric gas flow rate on droplet impact patterns of a 0.5 per cent methylene blue solution dispensed from a 20 µm pinhole on an ink jet paper target at a distance of 30 cm were studied at ambient temperatures in order to retrieve some preliminary data without resorting to the time consuming lyophilization procedure required for powder production. The droplet generator was positioned at 6 horizontal (H-Pos) (Figure 2-2a) and 2 vertical positions (V-Pos) (Figure 2-2b) using an epicyclical positioning device (see supplementary Figure 2-S1a and 2-S1b). The gas flow was set to 17 L/min and 34 L/min. An overview of the experimental conditions is shown in Table 2-1. A large format shutter (see supplementary Figure 2-S2) was used to allow an exact exposure time (1 second) of the ink-jet paper to the droplet stream. The number of replicates for each setting was 5. Targets were scanned at a resolution of 600 dots per inch (dpi) and the image files were analyzed using ImageJ 1.46 r (National Institutes of Health, 2012). The distribution of droplet trace areas and their dispersion on the target surface were assessed in order to monitor operating conditions and ensure sufficient separation between droplets and maintain the losses due to the impact with the walls of the swirl tube to an acceptable level.

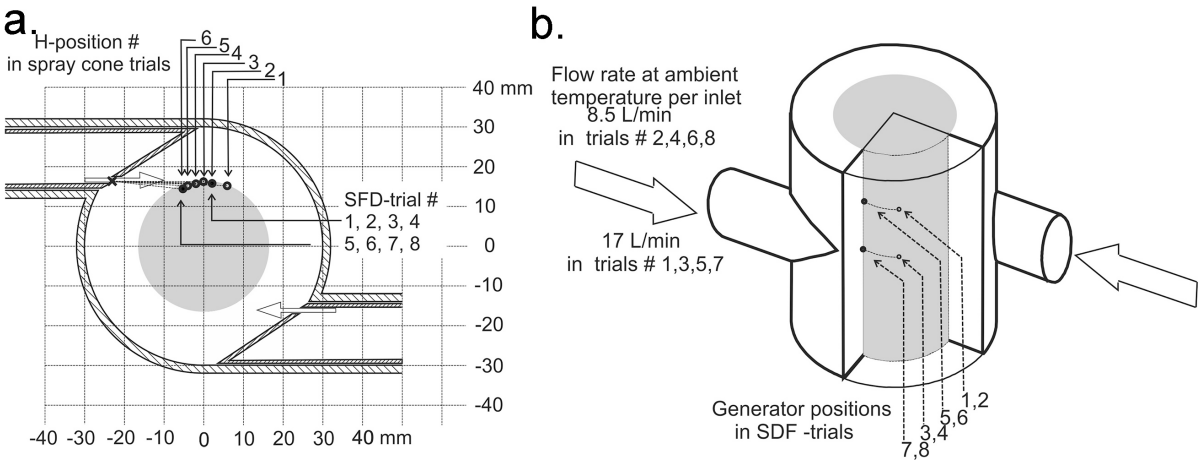

Figure 2-2: Droplet-stream generator positioning (shaded: accessible range of present positioner). Horizontal positioning in spray-cone experiments at ambient temperature **(a)** and horizontal and vertical positioning in SFD experiments **(b)**.

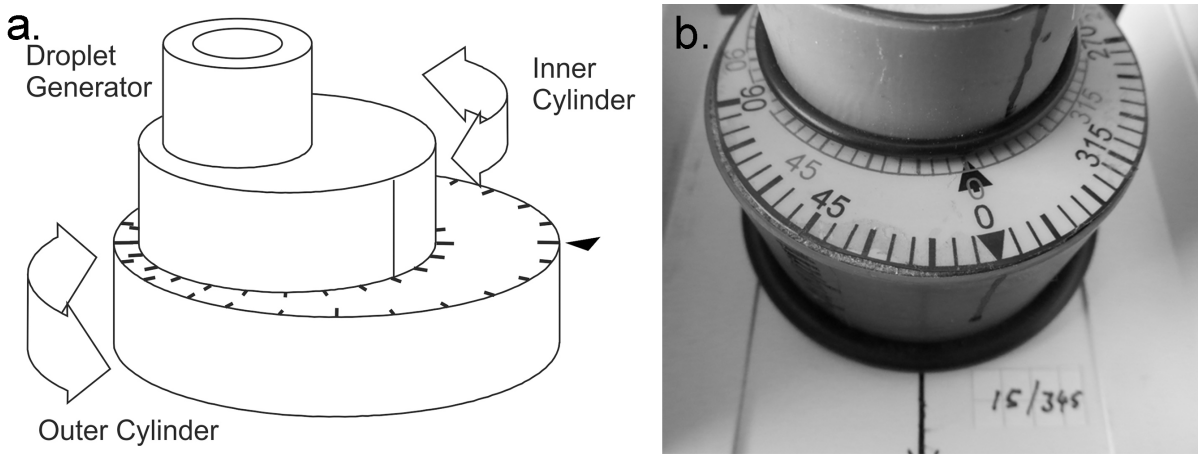

Figure 2-S1: Epicyclical positioner: Scheme **(a)** and view **(b)**

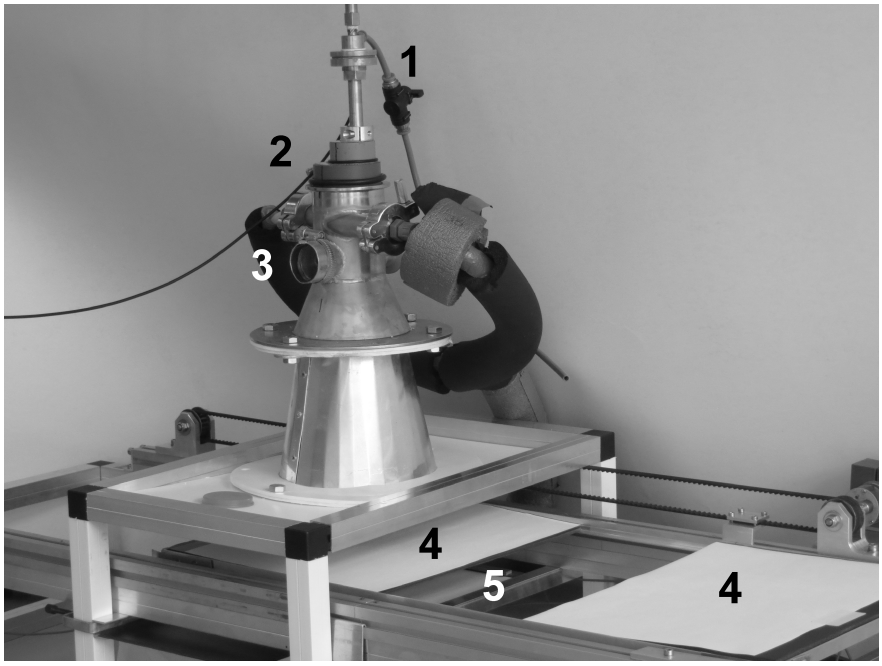

Figure 2-2: Spray cone expansion test setup. Droplet generator **(1)**, epicyclical positioner **(2)**, swirl tube **(3)**, shutter surfaces **(4)**, target surface **(5)**.

2.3.4 GAS VELOCITIES IN THE SWIRL TUBE

The gas velocities of vortex in the swirl tube were measured at the droplet-stream-generator positions summarized in Table 2-1 and volumetric gas flow rates of 17 L/min and 34 L/min, using a Testo 403 hot-wire anemometer (Testo AG, D-Lenzkirch) and a KFR-414700 rotameter (Kobold Messring, D-Hofheim). Measurements were performed at ambient temperature.

2.3.5 PARTICLE CHARACTERIZATION

The bulk and tapped densities were measured as follows: a 5 mL graduated cylinder was filled with 4 ml of powder and weighed to obtain the bulk density. The powder was subjected to 1250 taps using a Erweka SVM 22 tap densitometer for the assessment of the tapped density. The flowability was determined by calculating the Carr's compression index (CI) defined by the following equation:

$$CI = 100\ [(\ \rho_{tapped} - \rho_{bulk} / \rho_{tapped}\ ] \qquad \text{(Equation 2-3)}$$

A CI <15 is considered as good, between 15 and 25 as medium and >25 as poor flowability.

A thermo-gravimetric scale (TGA 7 with TAC7/DX Controller, Perkin Elmer, US-Waltham) was used to measure the loss on drying. Approximately 1 mg powder was dried to constant weight at 70 °C. The difference between the initial and the final mass was divided by the initial mass and multiplied by 100 to obtain the loss on drying in per cent.

For scanning electron microscopic (SEM) imaging, freeze dried particles were coated for 8 min with gold in a Polaron SC7640 sputter Coater (Quorum Technologies Ltd., UK-Newhaven) and were imaged with a Hitachi S-2460N (Hitachi High Tech. Corp., JP-Tokyo) scanning electron microscope.

The geometric size distribution was measured with a Sympatec Helos LF laser diffraction particle analyzer using a Sympatec Rodos SR dispersing module (Sympatec GmbH, D-Clausthal- Zellerfeld) with 0.5 bar of dispersion pressure. Particle size distributions were computed using the Fraunhofer option of the Windox 3.4 software. The volumetric mean particle diameters (x_{50}) are summarized in Supplementary Table 2-S1. The SPAN value was calculated as:

$$SPAN = (x_{90} - x_{10}) /(x_{50}) \qquad \text{(Equation 3-4)}$$

The x_{90} and the x_{10} represent the diameters where 90% and 10% of the particle distribution consists of particles with a smaller size.

2.4 RESULTS

2.4.1 GAS VELOCITY IN SWIRL TUBE

The gas velocities of the vortex ranged between 0.24 m/s and 4.25 m/s depending on the DSG position and the volumetric inlet gas flow in the swirl tube. In addition, the gas velocities at positions with V-Pos = 10 mm and 17 L/min increased from 0.34 m/s to 0.62 m/s with a decreasing horizontal distance from the gas inlet up to a distance of 19.03 mm. At a horizontal distance of 17.05 mm at 17 L/min, a minimum velocity of 0.24 m/s was detected. Increasing the gas flow from 17 L/min to 34 L/min increases the gas velocity 1.2 to 1.9 times. Furthermore, for the lower vertical position (00 mm) the gas velocities also increased with decreasing horizontal distance from the gas inlet from 0.87 m/s to 2.08 m/s. Doubling the gas flow also doubled the mean gas velocities as shown in Figure 2-3.

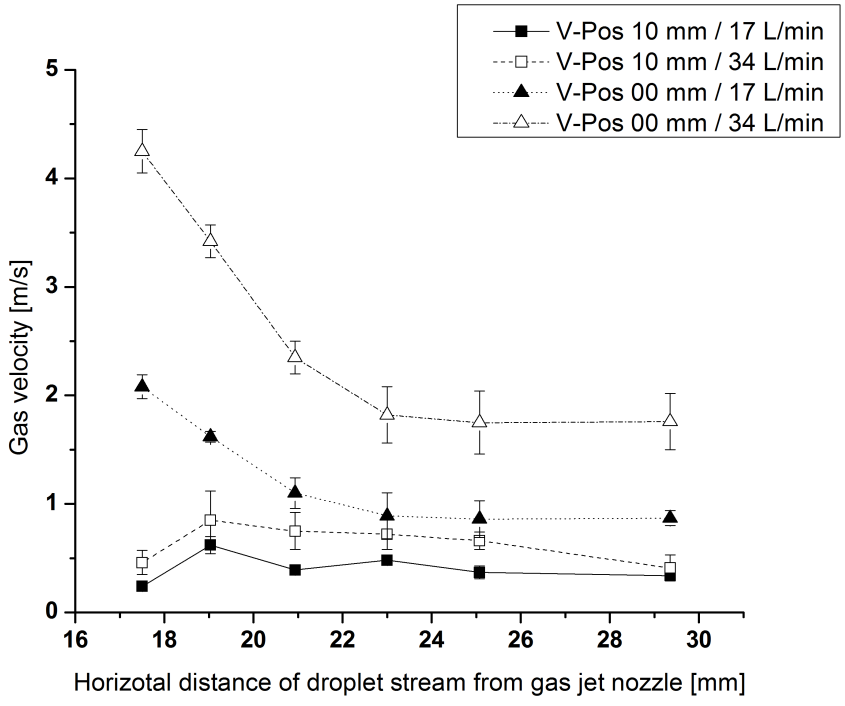

Figure 2-3: Volume flow rates and gas velocities at intersection of droplet-stream and gas jet (ambient temperature)

2.4.2 SPRAY-CONE FOOTPRINTS

An exemplary spray-cone footprint of an experiment after digitalizing and conversion into a binary black and white image is shown in Figure 2-4a. The mean droplet trace areas ranged between 5.3 ± 0.5 px and 16.6 ± 3.2 px (n=5). The absolute number of droplet traces (sum of all 5 trials) was between approx. 10 000 and 21 000 (see supplementary Table 2-S1).

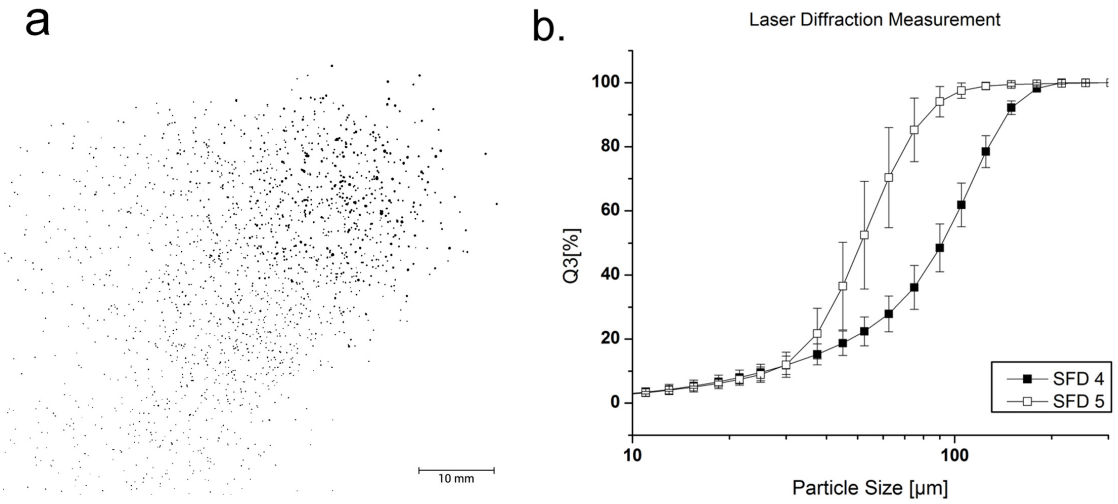

Figure 2-4: Spray cone footprints **(a)** and cumulative size distribution of SFD particles **(b)**

Table 2-S1: Results

	Conditions			**Outcomes**		
SFD – Trial #	V-pos (mm)	Gas Flow (L/min)	Distance (mm)	Droplet traces number Sum	Droplet traces Area (px) Mean±SD	Median particle diameter (mm)
1	10	34	25.07	20839	6.6 (±0.7)	85.2(±2.1)
2	10	17	25.07	19311	9.6(±1.3)	88.6(±5.4)
3	0	34	25.07	11192	15.0(±1.7)	83.3(±3.0)
4	0	17	25.07	9731	16.6(±3.2)	88.4(±11.6)
5	10	34	17.05	19129	5.3(±0.5)	49.5(±6.6)
6	10	17	17.05	16602	11.1(±1.9)	87.0(±1.3)
7	0	34	17.05	13818	11.2(±1.0)	59.8(±5.1)
8	0	17	17.05	13213	13.9(±3.5)	89.8(±3.3)

px: Pixels
V-Pos: vertical position
SD: standard deviation

The vertical position apparently had the strongest influence on the droplet trace count (Table 2-2). The count increased to 698 traces and trace areas decreased by 3.0 px when the DSG position was changed from 0 mm to -10 mm. When the gas flow was increased, the trace count increased by 153 traces and trace areas decreased by 1.6 px. In addition, an increase in the distance from the DSG to the jet nozzle resulted in the trace counts being reduced by 43 and trace areas increasing by 0.8 px.

Furthermore, as the gas flow of the vortex was increased from 17 L/min to 34 L/min, the droplet trace areas were significantly reduced whereas the droplet count did not change. This could be attributed to the loss of droplets at the inner wall of the swirl tube at higher gas. Conversely, the horizontal position did not significantly influence the droplet trace counts and mean droplet trace areas.

Table 2-2: Outcomes of factorial design experiments

Effects	**Trace Count** (n=5)		**Trace Area** (n=5)		**Gas Velocity** (n=3)		**x50** (n=3)		**SPAN** (n=3)	
		Rel. [%]	[px]	Rel. [%]	[m/s]	Rel. [%]	[µm]	Rel. [%]		Re.l [%]
Gas flow	153 ±135	0.05±4.36	-1.6 ±0.8	-0.15 ±6.7	0.4 ±0.0	0.33 ±3.0	-9.0 ±0.6	-11.5 ±0.8	-0.08 ±0.02	-5.9 ±0.8
V-Pos	698 ±124	0.23±3.99	-3.0 ±0.6	-0.27 ±5.6	-0.9 ±0.1	-0.68 ±4.7	-0.9 ±0.9	-1.1 ±1.2	-0.05 ±0.03	-4.3 ±2.1
H-Pos	42 ±181	-0.01±5.85	0.8 ±0.0	0.07 ±0.0	-0.4 ±0.0	-0.32 ±2.2	7.9 ±1.0	10.1 ±1.3	0.02 ±0.00	1.3 ±0.0
Gas flow & V-Pos	50 ±135	0.02±4.35	-0.6 ±0.3	-0.05 ±2.2	-0.3 ±0.0	-0.24 ±0.9	-1.2 ±1.1	-1.6 ±1.4	0.00 ±0.03	-0.3 ±2.6
Gas flow & H-Pos	-4 ±40	0.00 ±1.30	0.5 ±0.2	0.04 ±2.0	-0.2 ±0.0	-0.11 ±0.4	6.9 ±2.4	8.8 ±3.0	0.03 ±0.02	2.0 ±1.9
V-Pos & H-Pos	263 ±39	0.09 ±1.25	-0.8 ±0.1	-0.07 ±0.9	0.5 ±0.0	0.38 ±1.3	1.4 ±0.8	1.8 ±1.1	0.07 +0.01	5.6 ±0.7
Gas flow & V-Pos & H-Pos	-46 ±26	-0.01 ±0.83	0.2 ±0.0	0.02 ±0.2	0.2 ±0.0	0.13 ±0.9	1.7 ±0.2	2.1 ±0.3	0.02 ±0.07	1.6 ± 5.7
Mean	3095	100 %	11.1	100 %	1.3	100 %	78.4	100 %	1.27	100 %

px: pixels
Rel.: relative
V-Pos: vertical position of droplet stream generator
H-Pos: horizontal position droplet stream generator
x50: median particle size

2.4.3 POWDER AND PARTICLE PROPERTIES

SEM imaging showed that the powders consist of small, uniformly shaped spherical particles. Particles ranging from approx. 25 µm to 180 µm were obtained. Single particles as well as small agglomerates were observed as depicted in Figure 2-5a. The particle surface was partly porous and covered with a thin solid layer as can be seen in Figure 2-5b.

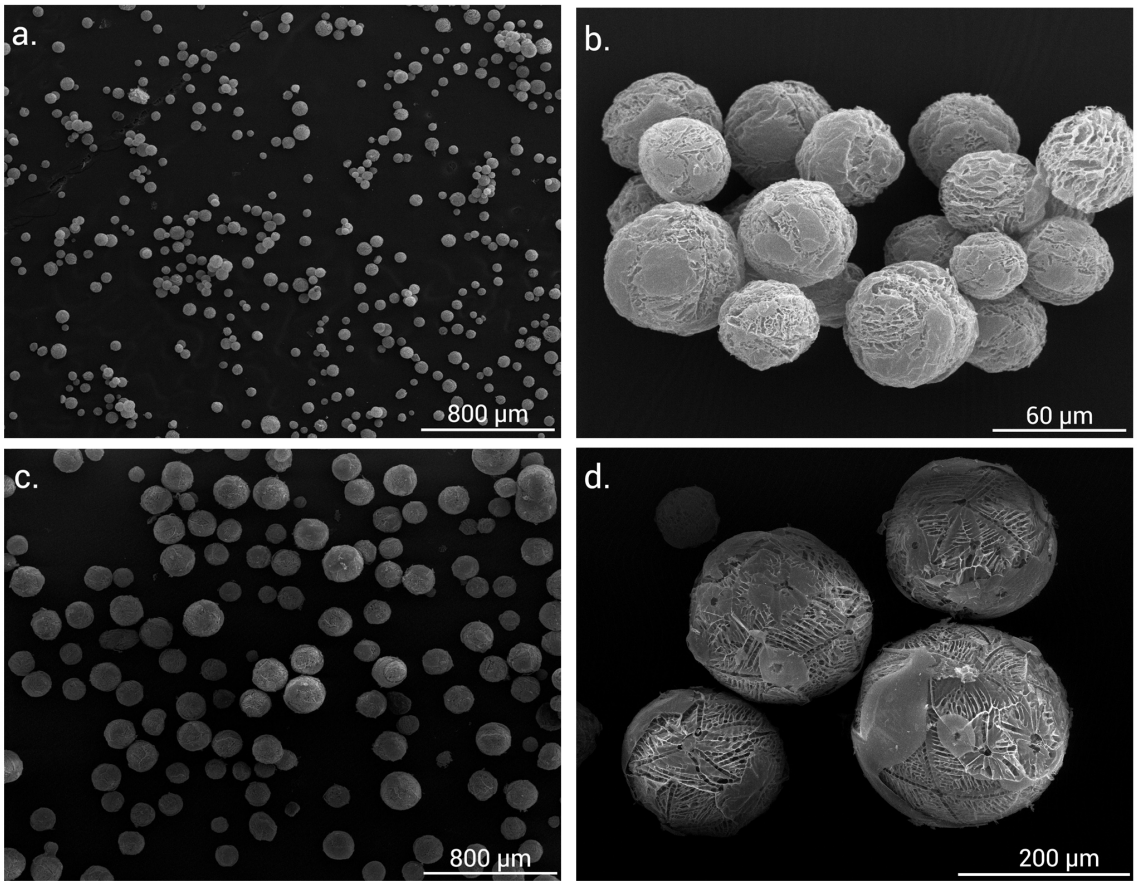

Figure 2-5: SEM images of SFD particles produced with (**a** and **b**) and without (**c** and **d**) atmospheric droplet stream expansion

The droplet-stream SFD process in this study resulted in extremely porous and light powders that are easily dispersed in air. The mean bulk density of the powders was found to be 0.012±0.006 g/mL whilst the mean tapped density was 0.016±0.007 g/mL which resulted in a CI of 27±4 and indicated medium to poor flow characteristics. The mean on drying of the powders was 3.5±0.7 %.

The factorial analysis of the laser diffraction measurements showed that increasing the gas flow from 17 L/min to 34 L/min reduced the mean particle size by 11.5 % from the mean. Elevating the V-Pos of the DSG from 0 mm to 10 mm resulted in a minor effect and decreased the x_{50} by 1.1 %. An increase in the distance of the droplet stream to the gas jet nozzle from 17.05 mm to 20.35 mm led to a 10.1 % increase in the mean particle size. The combination of increased gas flow and increased distance to the gas inlet resulted in an 8.8 % increase in particle size. Therefore, it could be inferred that the influence of the distance outweighed the influence of the gas flow (Table 2-2).

In addition, the laser diffraction measurements showed that the mean volumetric particle size (x_{50}) for the SFD trials 1,2,3,4,6 and 8 ranged between 83.3 (±3.0) µm and 88.6 (±5.5) µm and the differences in x_{50} were not statistically significant. SFD experiments 5 and 7 yielded mean particle sizes of 49.5 (±6.6) µm and 59.8 (±5.1) respectively as depicted in Figure 2-6; Supplementary Table 2-S1. All SPAN values ranged between 1.00 and 1.45. Exemplary cumulative distributions by laser diffraction measurement are shown in Figure 2-4b.

2.5 DISCUSSION

Lyophilized powders show potential for the application of peptides and proteins via the epidermal, nasal and pulmonary route (Wanning et al., 2015). It is worth noting that due to their good reconstitution characteristics, SFD particles might be considered as microparticulate carriers for nanoparticles (Ali and Lamprecht, 2014).

In this study, the porous regions on the particle surface observed in Figure 2-5b indicate a highly porous interior structure as previously reported (Eggerstedt et al., 2012).

Decreasing the horizontal distance from the DSG position to the gas jet nozzle led to an increase in the gas velocity which may be attributed to the vertical position of 0 mm being directly inside gas vortex. Subsequently, the mean gas velocities for the vertical position

10 mm were significantly lower and less affected by the H-Pos which showed that the orifice of the DSG was positioned above the gas vortex. Therefore, there is a need to understand the fluid dynamics inside the swirl tube in further studies.

Although droplet trace areas and droplet counts may be used as an indicator for the droplet stream expansion, such results have to be carefully considered. It was hypothesized that the gas velocity within the gas vortex at a distinct position of the DSG orifice would have a direct impact on droplet stream expansion, droplet trace areas and trace counts. Increasing the gas flow resulted in an increase in the gas velocity of the vortex which subsequently led to an expanded droplet stream as described earlier. However, this correlation could not be established for the vertical positioning of the DSG. When the DSG position was elevated to 10 mm, the droplet stream expansion was improved although the gas velocities were significantly lower. A possible explanation for this observation could be that by positioning the DSG orifice in the middle of the gas jet, the droplet stream was deflected by only half of the gas jet. On the other hand, when the DSG was positioned at 10 mm, the droplet stream was fully deflected over the complete profile of the gas jet which led to an improvement in the droplet stream expansion.

Furthermore, a comparison of the absolute results with the evaluated and calculated effects obtained using factorial design showed that the effects of the gas flow and horizontal position may be underestimated because all calculated effects were related to the mean value of all observations.

For droplets created by Rayleigh disintegration, the initial droplet size was approximately 1.9 times the orifice diameter (Walzel, 1990). However, Rayleigh disintegration could not be observed for orifice diameters of 20 µm and the initial droplet size was determined by evaluation of high-speed recordings of the droplet stream. The images suggested an initial droplet size of approximately 40-50 µm (Volume: 0.034 – 0.065 nL).

Particles with a size of 160 µm (2.1 nL) consist of approximately 32-64 primary droplets whilst those with a size of 50-60 µm consist of approximately 2-3 primary droplets. This observation indicates that freezing small droplets in a cold gas vortex may reduce the risk of collisions before congelation and thus enhance the size and spread of the particle size distribution.

The SPAN values of powders produced by vortex freezing are comparable to those obtained by stagnant cold gas drop-jet freezing which infers that the cold gas vortex has effect on the width of the particle size distribution. Interestingly, when compared to other SFD methods, the SPAN values of powders produced by atmospheric gas-vortex freezing were found to be relatively small. It has been reported that SFD powders prepared by spraying into or above liquid nitrogen using a single, two (Ali and Lamprecht, 2014; Eggerstedt, 2014; Garmise et al., 2007; Schiffter et al., 2010) or four-fluid nozzle (Niwa et al., 2012) exhibited wider particle size distributions (SPAN 1.5 – 23.3) compared to the powders prepared using atmospheric gas-vortex freezing in this study.

A study involving the use of a thermal ink-jet printhead for particle generation resulted in SPAN values up to 16.6 (Mueannoom et al., 2012; Sharma et al., 2013). Only the ultrasonic soft-mist atomizer used by Schiffter and Maa was able to produce SFD powders with SPAN values below 1 (Maa et al., 2004; Schiffter et al., 2010). Powders with uniformly sized particles and hence low SPAN values are expected to reduce the product-related variability component of drug delivery without affecting the individual components due to anatomical differences or breathing patterns.

It is hardly possible to make any prediction on the outcome of atmospheric spray-freezing experiments based on the data from spray-cone footprints, because these experiments were carried out at ambient temperature and not under freezing conditions. It is not possible to state that high droplet trace counts and small trace areas correlate with small mean particle sizes (see Trial 1 and 5 in Figure 2-6), although it might be possible to use the technique to reject settings, which lead to insufficient droplet stream expansion and therefore large mean particle sizes (see Trial 3,8 & 4).

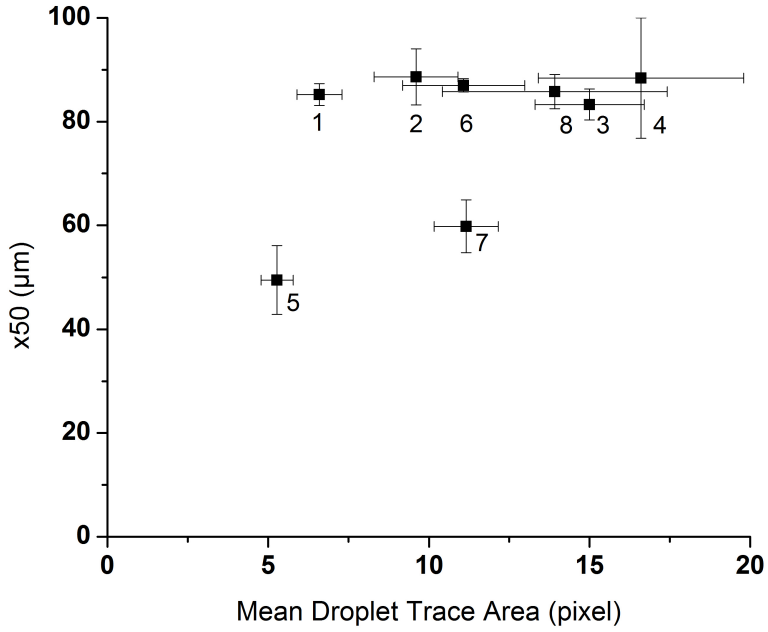

Figure 2-6: Correlation of droplet trace areas with freeze-dried particle diameters

Analysis of the effects in the factorial design also indicated that the factors have different impacts depending on the target criteria, for instance, increasing the horizontal distance of the DSG from the gas jet nozzle had no significant impact on droplet trace counts and areas but had an effect on mean particle sizes in spray-freeze drying. It is worth noting that the freezing step has a major impact on how the droplets behave in the swirl tube. Once frozen, droplet collisions are of minor importance as the frozen droplets do not merge which explains why the vertical positioning has a significant effect on the spray-cone footprints but is of minor significance in spray-freezing. Furthermore, the physical behavior of the process gas depends on its temperature that could alter the aerodynamic properties and fluid dynamics of the gas vortex as the density and the viscosity of the gas changes. Spray-cone footprint analysis is quick and allows the elimination of inefficient options although it may not be suitable for the identification of optimal freezing conditions.

The probability of droplet collisions could be further reduced by positioning the DSG closer to the jet nozzle orifice in order to obtain a better droplet-stream deflection due to higher gas velocities and faster freezing.

2.6 CONCLUSION

Atmospheric droplet-stream freezing in combination with aerodynamic droplet-stream expansion is a viable method for generating very low-density spherolyophilisate powders with small mean particle sizes and narrow particle size distributions. By reducing the horizontal distance between the droplet stream and gas jet nozzle and by increasing the volumetric gas flow rates, droplet collisions were successfully reduced and the mean particle size was reduced from 160 µm to around 50 µm, while the SPAN was not altered by the droplet stream expansion. The method could be relevant for the generation of lyophilized powders for needle-free injection or nasal application. The use of smaller nozzle orifices (e.g. 10-15 µm) could further reduce the mean particle size and offer the possibility to produce powders for pulmonary application.

2.7 CONFLICT OF INTEREST

R. Süverkrüp: German Patent Application
2015 0409 11542100 DE ‚Vorrichtung und Verfahren zur Erzeugung monodisperser gefrorener Tropfen'
(Apparatus and method for the generation of mono-disperse frozen droplets)

2.8 ACKNOWLEDGMENTS

The authors are grateful for the financial support of the 'Deutsche Forschungsgemeinschaft' (DFG) in the framework of SPP1423 (grant no. LA1362/2-1) and would also like to thank Franz-Josef Willems, Thomas Vidua and Jürgen Hohmann for technical assistance.

2.9 REFERENCES

Ali, M.E., Lamprecht, A., 2014. Spray freeze drying for dry powder inhalation of nanoparticles. Eur. J. Pharm. Biopharm. 87, 510–517.

Bi, R., Shao, W., Wang, Q., Zhang, N., 2008. Spray-freeze-dried dry powder inhalation of insulin-loaded liposomes for enhanced pulmonary delivery. J. Drug Target. 16, 639–648.

Eggerstedt, S., 2014. Sprühgefriertrocknung zur Herstellung von Protein-Inhalanda (Dissertation). Rheinische Friedrich-Wilhelms-Universität Bonn.

Eggerstedt, S.N., Dietzel, M., Sommerfeld, M., Süverkrüp, R., Lamprecht, A., 2012. Protein spheres prepared by drop jet freeze drying. Int. J. Pharm. 438, 160–166.

Garmise, R.J., Staats, H.F., Hickey, A.J., 2007. Novel dry powder preparations of whole inactivated influenza virus for nasal vaccination. AAPS PharmSciTech 8, 2–10.

Hu, J., Johnston, K.P., Williams, R.O., 2003. Spray freezing into liquid (SFL) particle engineering technology to enhance dissolution of poorly water soluble drugs: organic solvent versus organic/aqueous co-solvent systems. Eur. J. Pharm. Sci. 20, 295–303.

Maa, Y.-F., Ameri, M., Shu, C., Payne, L.G., Chen, D., 2004. Influenza vaccine powder formulation development: spray-freeze-drying and stability evaluation. J. Pharm. Sci. 93, 1912–1923.

Maa, Y.-F., Nguyen, P.-A., Sweeney, T., Shire, S.J., Hsu, C.C., 1999. Protein inhalation powders: spray drying vs spray freeze drying. Pharm. Res. 16, 249–254.

Mueannoom, W., Srisongphan, A., Taylor, K.M.G., Hauschild, S., Gaisford, S., 2012. Thermal ink-jet spray freeze-drying for preparation of excipient-free salbutamol sulphate for inhalation. Eur. J. Pharm. Biopharm. 80, 149–155.

National Institutes of Health, 2012. Image J. Bethesda MD, USA.

Niwa, T., Mizutani, D., Danjo, K., 2012. Spray freeze-dried porous microparticles of a poorly water-soluble drug for respiratory delivery. Chem. Pharm. Bull. (Tokyo) 60, 870–876.

Rochelle, C., 2005. Spray-Freeze-Dried Powders for Needle-Free Injection (Dissertation). Friedrich-Alexander-Universität Erlangen-Nürnberg.

Schiffter, H., Condliffe, J., Vonhoff, S., 2010. Spray-freeze-drying of nanosuspensions: the manufacture of insulin particles for needle-free ballistic powder delivery. J. R. Soc. Interface 7, S483–S500.

Sharma, G., Mueannoom, W., Buanz, A.B.M., Taylor, K.M.G., Gaisford, S., 2013. In vitro characterisation of terbutaline sulphate particles prepared by thermal ink-jet spray freeze drying. Int. J. Pharm. 447, 165–170.

Süverkrüp, R., Eggerstedt, S.N., Gruner, K., Kuschel, M., Sommerfeld, M., Lamprecht, A., 2013. Collisions in fast droplet streams for the production of spherolyophilisates. Eur. J. Pharm. Sci. 49, 535–541.

Süverkrüp, R., Eggerstedt, S., Wanning, S., Kuschel, M., Sommerfeld, M., Lamprecht, A., 2016. Collisions and coalescence in droplet streams for the production of freeze-dried powders. Colloids Surf B 141, 443–449.

Walzel, P., 1990. Zerstäuben von Flüssigkeiten. Chem. Ing. Tech. 62, 983–994.

Wang, S.H., Kirwan, S.M., Abraham, S.N., Staats, H.F., Hickey, A.J., 2012. Stable Dry Powder Formulation for Nasal Delivery of Anthrax Vaccine. J. Pharm. Sci. 101, 31–47.

Wanning, S., Süverkrüp, R., Lamprecht, A., 2015. Pharmaceutical spray freeze drying. Int. J. Pharm. 488, 136–153.

JET-VORTEX SPRAY-FREEZE-DRYING FOR THE PRODUCTION OF INHALABLE LYOPHILISATE POWDERS

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This chapter was published as follows:

'Jet-vortex spray freeze drying for the production of inhalable lyophilisate powders'

Wanning S., Süverkrüp R., Lamprecht A., *European Journal of Pharmaceutical Sciences*, 2017, 96, p1-7.

3.1 ABSTRACT

Spray-freeze-dried powders were suggested for nasal, epidermal (needle-free injection) or pulmonary application of proteins, peptides or nucleic acids. In spray-freeze-drying processes an aqueous solution is atomized into a refrigerant medium and subsequently dried by sublimation. Droplet-stream generators produce a fast stream of monodisperse droplets, where droplets are subject to collisions and therefore the initial monodispersity is lost and droplets increase in diameter, which reduces their suitability for pulmonary application. In jet-vortex-freezing, a droplet-stream is injected into a vortex of cold process gas to prevent droplet collisions. Both the injection position of the droplet-stream and the velocity of the cold gas vortex have an impact on the size distributions of the resulting powders. A model solution containing mannitol (1.5 % m/V) and maltodextrin (1.5 % m/V) was sprayed at 5 droplet-stream positions at distances between 1 mm and 30 mm from the gas jet nozzle and 5 gas velocities (0.8 - 6.8 m/s) at a process temperature of -100 °C. Mean geometric diameters of the highly porous particles (bulk density: 0.012±0.007 g/cm^3) ranged between 55 ± 4 and 98 ± 4 µm. Evaluation of the aerodynamic properties by Next-Generation-Impactor (NGI) analysis showed that all powders had high emitted doses (98±1 %) and fine-particle-fractions ranged between 4±1 % and 21±2 %. It was shown that jet-vortex-freezing is a suitable method for the reproducible production of lyophilized powders with excellent dispersibility in air, which has a high potential for nasal and pulmonary drug delivery.

Keywords: spray freeze drying, droplet-stream generator, protein formulations, lyophilization, porous particles, pulmonary drug delivery, droplet collision

3.2 INTRODUCTION

Spray-freeze-drying (SFD) is a process for the production of lyophilized powders with spherical particles. It combines the advantages of both freeze-drying and spray-drying as the drying process is extremely mild and most powders are flowable without further processing.

Due to their unique properties as dispersed lyophilisates, SFD powders have been widely studied for the use in nasal (Garmise et al., 2006; Jiang et al., 2006), epidermal (needle-free injection) (Schiffter et al., 2010), ophthalmic (Süverkrüp et al., 2009) and pulmonary drug delivery (Wanning et al., 2015). Moreover, they were considered for enhancing the apparent solubility of poorly water soluble drugs (Hu et al., 2003; Leuenberger, 2001; Rogers, 2003; Rogers et al., 2003, 2002a, 2002b). SFD consists of three sequential steps: droplet generation, freezing and sublimation drying. Droplets are formed by spraying mostly aqueous solutions with hydraulic (single-fluid) (Rogers et al., 2002a) or pneumatic (two-fluid (Maa et al., 1999) or four-fluid (Niwa et al., 2009)) nozzles. Ultrasonic soft mist generators were used to create clouds of slow moving droplets (Schiffter et al., 2010). Monodisperse droplet-streams were formed using thermal ink-jet print-heads (Mueannoom et al., 2012) or piezoelectric droplet-stream generators (DSG) (Eggerstedt et al., 2012). Droplets are commonly frozen by spraying over and subsequently into a cryogenic liquid (mostly liquid nitrogen) (Maa et al., 1999; Niwa et al., 2009; Schiffter et al., 2010; Zijlstra et al., 2009) or into an atmosphere of cold gas, which has several advantages such as no contact to the refrigerant liquid and easier particle recovery (Ali and Lamprecht, 2014; Eggerstedt et al., 2012). In most studies, the solvent is removed by sublimation drying under vacuum in chamber-type freeze-dryers (Eggerstedt et al., 2012; Niwa et al., 2009; Rogers et al., 2002a; Schiffter et al., 2010), but the possibility of atmospheric freeze drying also has been investigated (Leuenberger et al., 2006) (Claussen et al., 2007).

Unlike in spray drying, the size, shape and porous structure of lyophilized particles are largely determined in the freezing step and highly porous powders with spherical particles are obtained from solutions with low concentration of non-volatile solids.

For an effective and systemic pulmonary drug delivery, an aerodynamic diameter (d_{ae}) of 1-5 µm is required to access the alveolar regions in the deep lung (Lin et al., 2015).

The d_{ae} is described as follows:

$$d_{ae} = d_p \sqrt{\frac{\rho_p}{\rho_0 \chi}} \qquad \text{(Equation 3-1)}$$

with:
d_{ae}: aerodynamic diameter,
d_p: geometric particle diameter
ρ_p: particle density
ρ_0: unit density (1 g/cm^3),
χ: shape factor

The fraction of powder with aerodynamic particle sizes between 1-5 µm is also referred to as the fine particle fraction (FPF). Commercially available dry powder inhalers (DPI), which are commonly based on micronized API powders combined with larger (often spray-dried) carrier particles, have emitted doses of approximately between 50 % - 80 % and FPFs between 20-40 % (Berkenfeld et al., 2015; Son and McConville, 2008).
As an alternative formulation approach, porous particles have improved dispersion performance from inhaler devices due to their large size and low mass density (Edwards, 1997).
As a further development, SFD powders prepared by two-fluid nozzles were able to reach FPFs of 30-40 % (Ali and Lamprecht, 2014) and even up to 70 % (Maa et al., 1999) with relatively wide particle size distributions (Ali and Lamprecht, 2014; Maa et al., 1999). It is expected that narrower (or even monodisperse) particle size distributions may improve the delivery of powders to the lung by reducing the product related variability of the deposition patterns. For that reason, droplet-stream generators seem suitable, since they produce a stream of initially monodisperse droplets. Due to collisions before the droplets are frozen, the monodispersity is lost and droplet size as well as width of the size distribution increase if the droplets coalesce before freezing (Süverkrüp et al., 2016, 2013). In this study, jet-vortex freezing is introduced as an advanced developed method of prior experiments (Wanning et al., 2014) with reduced dimensions, more economical consumption of cryogenic liquid and improved control of spray parameters to reduce droplet collisions before solidification.

Droplet deflection and collision prevention depend on the temperature and velocity of the cold gas vortex as well the position of the DSG inside the cold gas vortex.
The primary aim of this study is to assess a process optimum for several target parameters, where droplet collisions can be prevented at the best possible rate so that the mean diameter and the spread of the particle size distribution and their batch-to-batch variability are minimized. Finally, the aerodynamic performance of the lyophilized powder is investigated to evaluate its suitability for pulmonary drug delivery.

3.3 MATERIALS AND METHODS

3.3.1 SPRAY SOLUTION

The spray solutions contained 1.5 g of D(−)-mannitol [Ph. Eur.] (VWR International, NL-Amsterdam), 1.5 g maltodextrin [Roquette LAB 2509, dextrose equivalent of DE = 19] (Roquette Frères, F-Lestrem Cédex) and 0.05 g sodium fluoresceine. The solid components were dissolved in 100 ml ultrapure water (MilliQ, Millipore Corp., US-Billerica, MA), filtered through a 0.22 µm aseptic filter and sonicated.

3.3.2 SPRAY FREEZE DRYING

A Type MTG – 01-G1 (FMP Technologies GmbH, D-Erlangen) droplet-stream generator (DSG) with a 20 µm pinhole diaphragm was used to create a stream of initially monodisperese droplets. The feed pressure was 250 kPa and the operating frequency was approximately 48 kHz. Droplet generation was checked visually by stroboscopic microscopy before spraying.

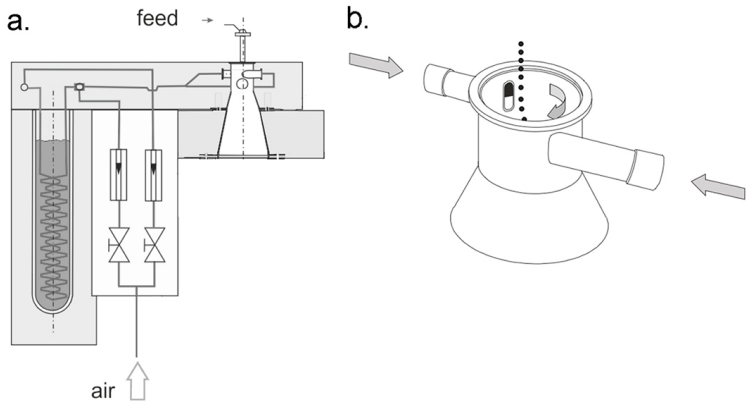

Figure 3-1: Jet vortex freezer **(a)** and swirl tube **(b)** for generation of cold gas vortex (b)

The droplet stream was injected into the jet-vortex freezing apparatus and droplets were frozen instantaneously (Figure 3-1a). Operating temperatures in the mixing chamber, the jet vortex, and the collecting sieve were monitored and recorded closely with a T390 (PCE-Instruments, D-Meschede) temperature logger. The frozen droplets were collected on a cooled sieve and sublimation dried in a lab freeze dryer (Lyovac GT2, STERIS, D-Hürth) for at least 24 h.

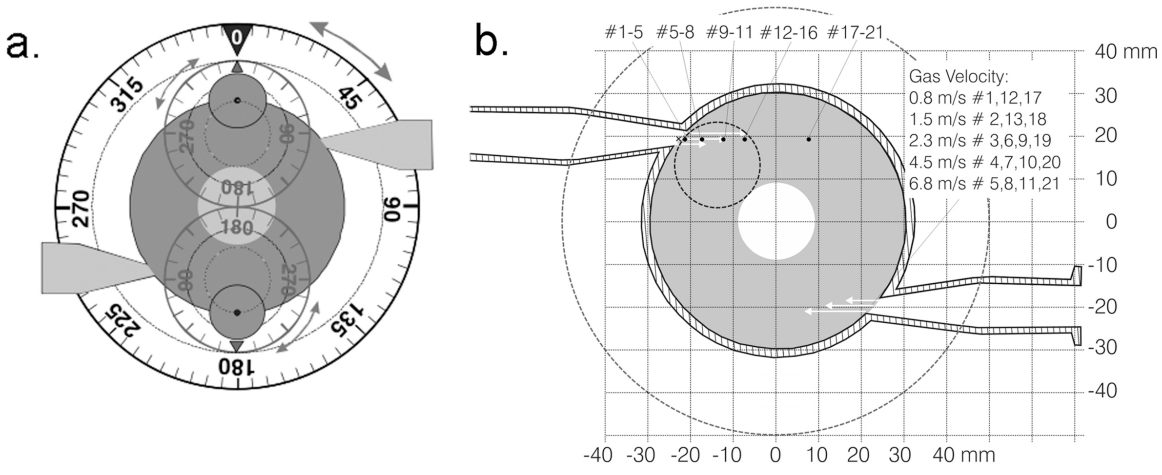

Figure 3-2: Double epicyclical droplet stream generator positioning device **(a)**. Dots in **(b)** indicate positions actually studied, grey area indicates range of accessible positions.

The vertical distance between the DSG pin-hole diaphragm and the jet-vortex nozzle was 10 mm. The horizontal distance between the droplet stream and the freezing gas nozzle was set in 3 positions ranging from 1 to 30 mm. Two additional distances were added at 5 and 10 mm (Figure 3-2b, Table 3-1) after evaluation of the first results. The gas velocity at the jet nozzle ranged between 0.8 m/s and 6.8 m/s. The freezing temperature was -100±5 °C. The complete design of experiments is shown in Table 3-1.

Table 3-1: Experimental Design and settings for process optimization trials

Trial No.	Distance from Gas Jet Nozzle	Jet Nozzle Size	Volumetric Gas Flow	Exit Velocity at Gas Jet Nozzle
#	[mm]		[L/min]	[m/s]
1	1	large	15	0.8
2	1	large	30	1.5
3	1	small	15	2.3
4	1	small	30	4.5
5	1	small	45	6.8
6	5	small	15	2.3
7	5	small	30	4.5
8	5	small	45	6.8
9	10	small	15	2.3
10	10	small	30	4.5
11	10	small	45	6.8
12	15	large	15	0.8
13	15	large	30	1.5
14	15	small	15	2.3
15	15	small	30	4.5
16	15	small	45	6.8
17	30	large	15	0.8
18	30	large	30	1.5
19	30	small	15	2.3
20	30	small	30	4.5
21	30	small	45	6.8

3.3.3 JET-VORTEX

Two tangentially positioned gas-jet nozzles create a vortex of cold gas inside a swirl tube (Figure 3-1b). Before injection into the swirl tube, the process gas (nitrogen) stream was split in two partial flows. One of the partial flows passed through a heat exchanger submerged in liquid nitrogen. The cold and the ambient gas flows were mixed before being injected into the swirl tube. The temperature of the reunited gas was controlled by the mixing ratio of the cold and the ambient gas flow. The volumetric flow rates of the partial flows were adjusted by needle valves (PFI 32031, Pfeiffer Vacuum, D-Asslar) and monitored by rotameters (KFR-414400, KOBOLD Messring GmbH, D-Sindelfingen) (Figure 3-1a). The operating temperatures in the swirl tube and above the sieve were measured with a T390 temperature logger (PCE Instruments, D-Meschede) for combinations of 3 different gas flows (15 L/min, 30 L/min, 45 L/min) and 4 different mixing ratios (25 %, 50 %, 75 %, 100 % cold gas). Temperatures below -150±4 °C can be reached for a gas flow of 45 L/min. For lower gas flows, the minimal temperatures were -140±3 °C (at 30 L/min) and -105±2 °C (at 15 L/min). Two jet nozzles with different orifice sizes (Supplementary Table 1) were used, which allowed the exhaust velocity to be set between 0.8 and 6.8 m/s for volumetric gas flows from 15 to 45 L/min at process temperatures down to -100 °C (Table 3-1).

Supplemtary Table 3-1: Calculated gas velocities at jet vortex nozzle

	Jet Nozzle				**Process Gas**		
	Height [mm]	**Width [mm]**	**Combined Area [mm^2]**	**Gas Flow [L/min]**	**Process Temp. (PT) [°C]**	**Gas Velocity at 25°C [m/s]**	**Gas Velocity at PT [m/s]**
Jet Nozzle 'large'	24	4	192	15	-100	1,3	0,8
	24	4	192	30	-100	2,6	1,5
	24	4	192	45	-100	3,9	2,3
Jet Nozzle 'small'	8	4	64	15	-100	3,9	2,3
	8	4	64	30	-100	7,8	4,5
	8	4	64	45	-100	11,7	6,8

The DSG was positioned at the top of the jet-vortex freezer with a double epicyclical positioning device (Figure 3-2a) which allowed to set the horizontal distance of the droplet stream to the gas jet nozzle within the shaded area shown in Figure 3-2b.

PARTICLE CHARACTERIZATION

Scanning electron microscopy (SEM) was used to investigate the microscopic particle characteristics and surface morphology. The spherolyophilisates were sputter-coated for 6 min with gold in a Polaron SC7640 sputter coater (Quorum Technologies Ltd., UK-Newhaven) and were imaged with a S-2460N electron microscope(Hitachi High Tech. Corp., JP-Tokyo).

The loss on drying was measured with a thermo-gravimetric scale (TGA 7 with TAC7/DX Controller, Perkin Elmer, US-Waltham, MA). 1 mg of lyophilized powder was dried to constant weight at 70 °C. The loss on drying was calculated in per cent of the initial mass.

The geometric particle size distribution was measured using a HELOS® LF laser diffraction particle analyzer in combination with a RODOS® SR dispersion module (Sympatec GmbH, D-Clausthal-Zellerfeld). The dispersion pressure was set to 0.5 bars. The volumetric median particle diameter ($x_{50,V}$) and the span (width of particle size distribution) were computed with the Windox 3.4 software (Ali and Lamprecht, 2014).

The span was calculated as:

$$span = (x_{90} - x_{10}) / (x_{50}) \qquad \text{(Equation 3-2)}$$

The x_{90} and the x_{10} represent the diameters at 90% and 10% cumulative volume.

The aerodynamic size distribution of freeze-dried powders was analyzed using a Next Generation pharmaceutical Impactor (NGI) (MSP Corporation, US-Shoreview, MN) in combination with a Copley Critical Flow Controller TPK and a Copley High Capacity Pump

HCP 5 (Copley Scientifitc Ltd., UK-Colwick Nottingham). Each capsule was filled with 0.2 mL of spherolyophilisate powder and contained a mass of 2 to 5 mg, depending on the bulk density of the powder. For application into the NGI a Handihaler® (Boehringer-Ingelheim GmbH & Co. KG, D-Ingelheim) was used. The airflow was set to 47±0.6 L/min and the timer was set to an inspirational volume of 4 L. Deposited powder in the stages was dissolved in water and assayed by fluorimetry (485 nm/535 nm) using a PlateReader 1420 Multilabel Counter VICTOR³V® with (Perkin Elmer, US-Waltham, MA). The $x_{50.M}$ (mass median diameter) values of the stages are listed in Table 3-2. The respirable fine particle fraction is defined as the fraction of particles in per cent that have an aerodynamic diameter smaller than 5 µm. The emitted dose is the sum of all powder residues from the mouthpiece on to the last stage of the impactor.

Table 3-2: NGI Cut-off diameters

Stage	Cut off at 48 L/min [µm]
1	9,05
2	5,01
3	3,15
4	1,84
5	1,06
6	0,63
7	0,39

For measuring the bulk and tapped densities, a 5 mL graduated cylinder was filled with approx. 4 mL of powder and weighed. For assessing the tapped density, the powder was subjected to 1250 taps using a tap densitometer (SVM 22, Erweka GmbH D-Heusenstamm). Carr's compression index (CI), which characterizes numerically the flow ability of powders, was computed as described earlier (Ali and Lamprecht, 2014). A CI <15 is considered as good, between 15 and 25 as medium and >25 indicates poor flowability.

DATA EVALUATION AND RESPONSE SURFACES

Response surfaces were generated using the Origin Pro 8.0 software's (OriginLab Corporation, US-Northhampton, MA), XYZ-contour plot option (increase factor 1000, smoothing factor 0.005). The dots indicate the anchor points (Figure 3-4).

To assess the suitability of different combinations of DSG position and gas velocity of the vortex, four target parameters, the median particle size ($x_{50.V}$), the SPAN, the standard deviation of the mean $x_{50.V}$ of three batches ($SD_{X50.V}$) and the FPF were evaluated.

The mean $x_{50.V}$ ($M_{X50.V}$) of three batches was evaluated to quantify the geometric particle size. The SPAN was calculated to obtain information about the width of the geometric particle size distribution and consistency of the prevention of droplet collisions during the production of one batch. The standard deviation of $M_{X50.V}$ of three batches ($SD_{X50.V}$), which were produced under the same conditions, was computed to assess the reproducibility of the DSG position and gas velocity settings.

The FPF was calculated to evaluate the overall performance of the powder in the NGI and therefore suitability of the product for pulmonary application.

3.4 RESULTS

The spray-freezing in a jet-vortex process has a parameter space outside of which particles can not be produced (hatched areas in Figure 3-3a). When the DSG position is chosen too close to the inner walls of the swirl tube, the droplet stream is deflected too much and impacts upon the swirl tube. The droplets freeze upon contact and an ice 'stalagmite' starts to grow towards the nozzle orifice. A similar effect can be observed for distances over 15 mm and gas exhaust velocities below 2 m/s, where an ice 'stalagmite' starts to grow from the sieve upwards towards the nozzle orifice because the droplet stream expansion is insufficient and unfrozen droplets impact on the sieve. These effects reduce the yield by about 50 to 75 % and have a negative impact on both process stability and reproducibility.

Inside the operating window, low-density particles which can easily be dispersed in air were produced.

The powders have a bulk density of 0.012±0.007 g/cm^3 and tapped density of 0.014±0.009 g/cm^3, the resulting CI of 22±4 indicates limited but, considering the diameters and density of the particles, above average flowability. The loss on drying of all powders is 3.1±0.4 %. SEM imaging shows that the powders consist of distinct spherical particles with a light tendency to agglomerate. The particle surface shows porous as well as smooth regions similar to spray freeze dried particles in an earlier study (Eggerstedt et al., 2012) (Figure 3-3).

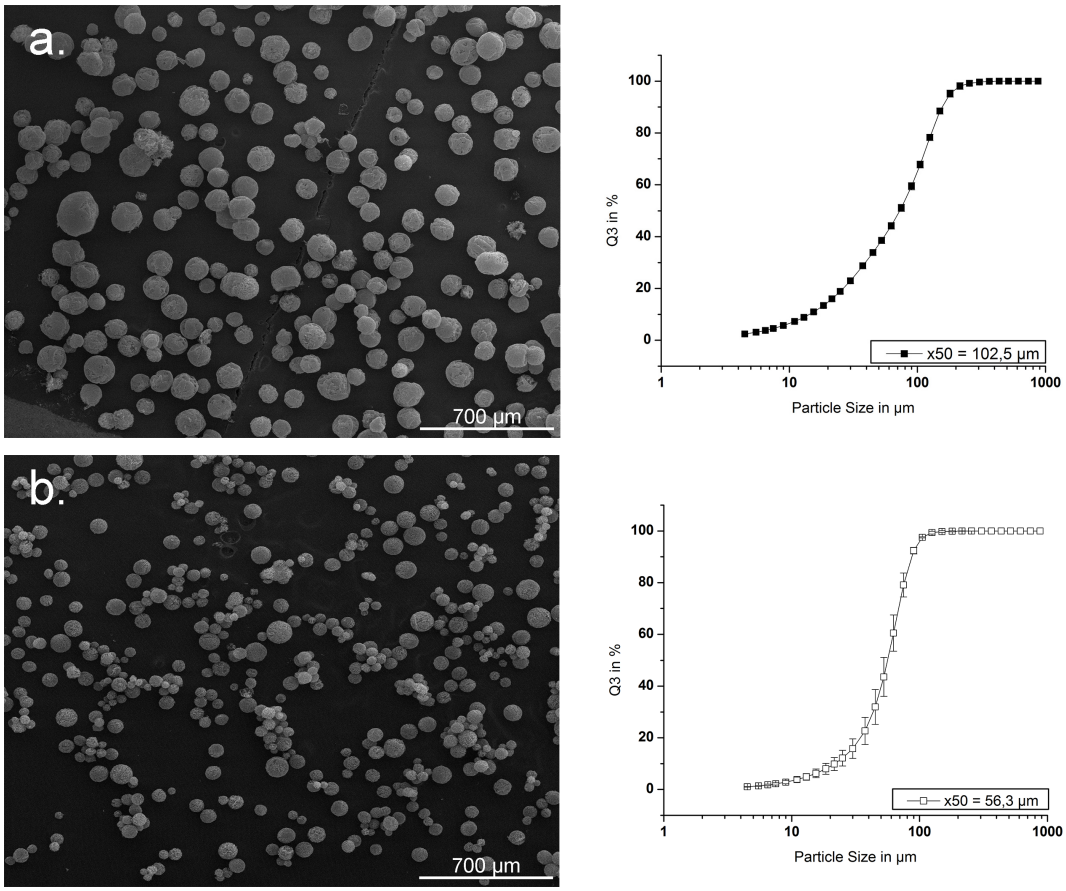

Figure 3-3: SEM overview and cumulative size distribution (n=3) for a sample with large particles (Trial #17) **(a)** and small particles (Trial #11) **(b)**.

The mean geometric diameters of the lyophilized powders range from 44.5±4.8 µm to 97.9±3.6 µm depending on the experimental settings (Table 3-1). The $M_{X50.V}$ increases with decreasing gas exit velocity as well as increasing distance from the gas jet nozzle. A particle size maximum ($M_{X50.V}$ >95 µm) was observed for gas velocities below 1.5 m/s and distances over 12.5 mm. The smallest $M_{X50.V}$ (≤57.5 µm) were generated at distances between 1-5 mm

from the gas nozzle and high gas velocities (6.8 m/s) as well at distances over 25 mm and exit velocities between 3 and 4.5 m/s, which means that the droplet stream expansion and fast freezing and consequent prevention of droplet collision was most effective at these settings. (Figure 3-4a).

Figure 3-3b shows that the $SD_{X50.V}$ is high for settings with low gas velocities or close distances of the DSG to the jet nozzle, which indicates a poor reproducibility. The area of the minimum has a width of approximately 10 mm and stretches from 10-20 mm at 3 m/s and to 1-12.5 mm at 6.8 m/s. The span ranges from 1 to 3. The smallest span value was observed at distances between 5-10 mm and exit velocities above 5.5 m/s (Figure 3-4c).

NGI deposition patterns for three exemplary samples (low FPF, medium FPF and highest FPF) are shown in Figure 3-5a. All trials have high emitted fractions of 98±1 % and small amounts of residue inside the capsule (0.9±0.5 %) indicating that the powders are readily dispersible and aerosolizable. A relatively large amount (40 to 60 %) of the lyophilized powder is deposited in the pre-separator. FPFs <8 % are found for low gas velocities and distances between 1 and 10 mm. FPF values of approx. 21 % are reached for distances < 5mm and between 7.5 and 15 mm and gas exhaust velocities above 6 m/s (Figure 3-5b). The standard deviation of the FPF has a minimum at distances between 10 and 15 m at velocities > 6m/s.

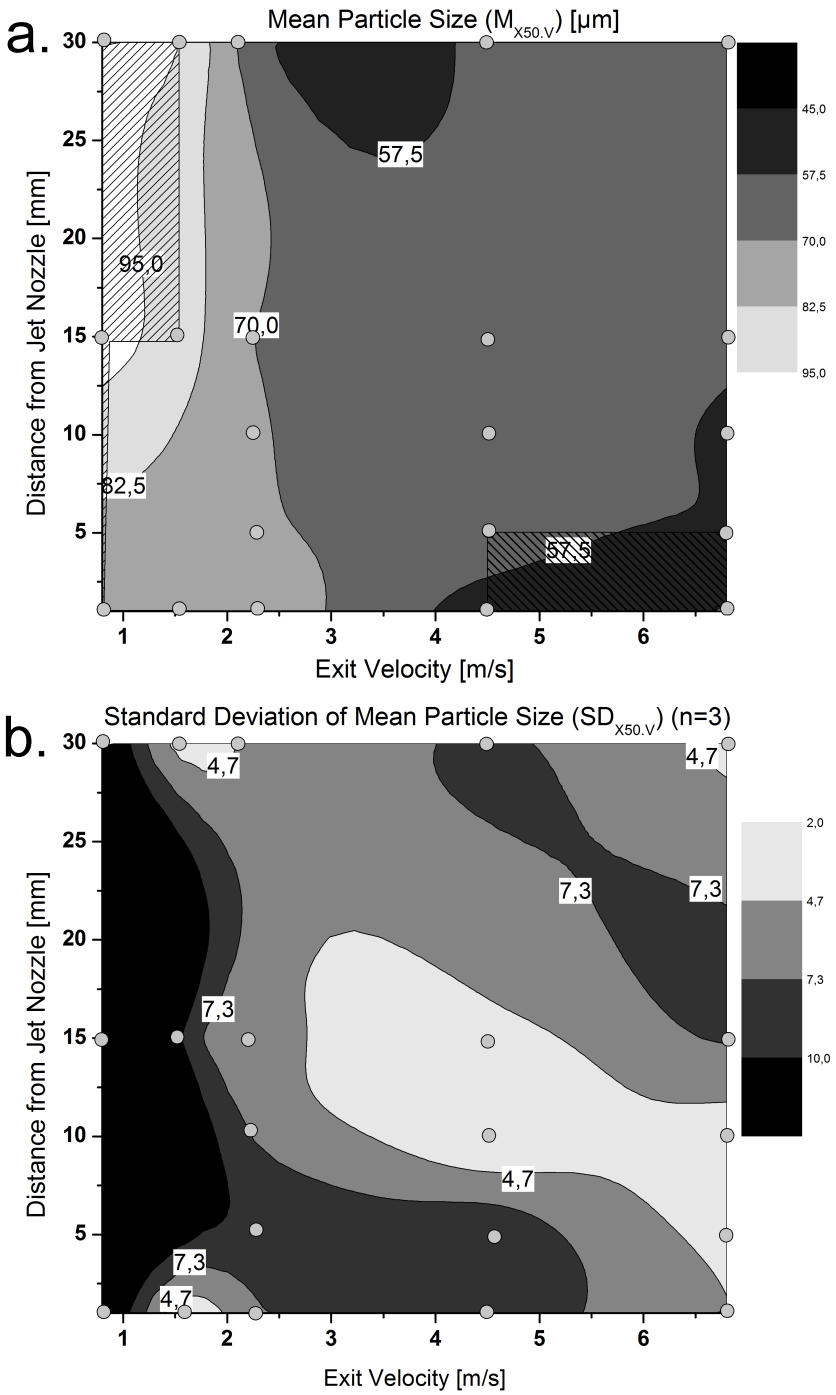

Figure 3-4: Response surfaces of the target parameters: mean particle size ($M_{X50,V}$) **(a)**, inter-batch variability ($SD_{X50,V}$)**(b)** and width of the particle size distribution (span) **(c)**.

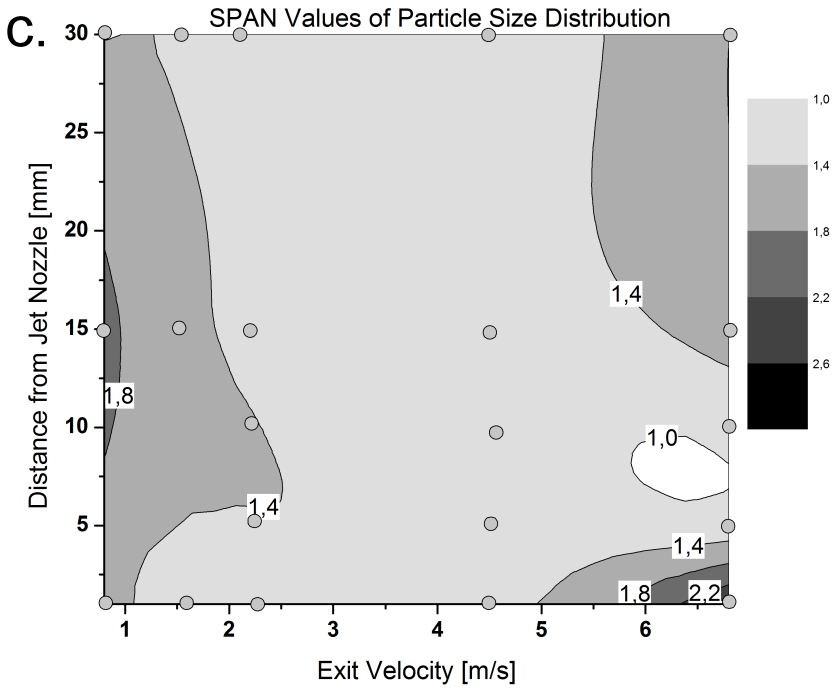

Figure 3-4 (continued): Response surfaces of the target parameters: mean particle size ($M_{X50,V}$) **(a)**, inter-batch variability ($SD_{X50,V}$)**(b)** and width of the particle size distribution (span) **(c)**.

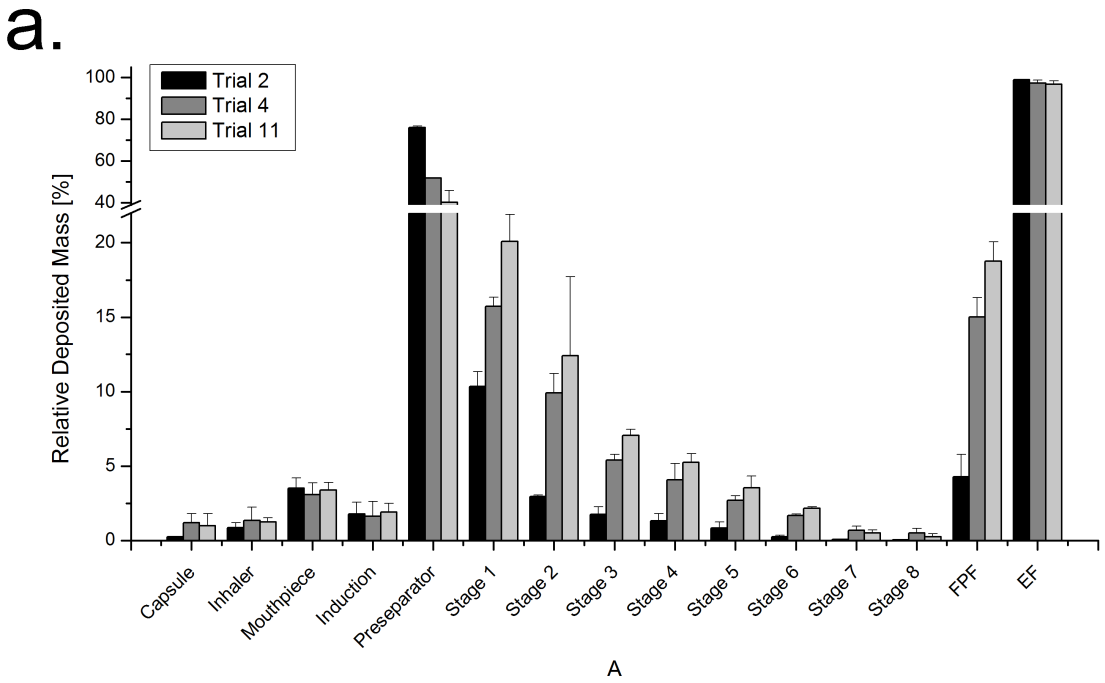

Figure 3-5: Exemplary NGI deposition patterns of a low, medium and high performing trial **(a)** and the corresponding response surfaces of the mean FPF **(b)** and the standard deviation of the FPF **(c)**.

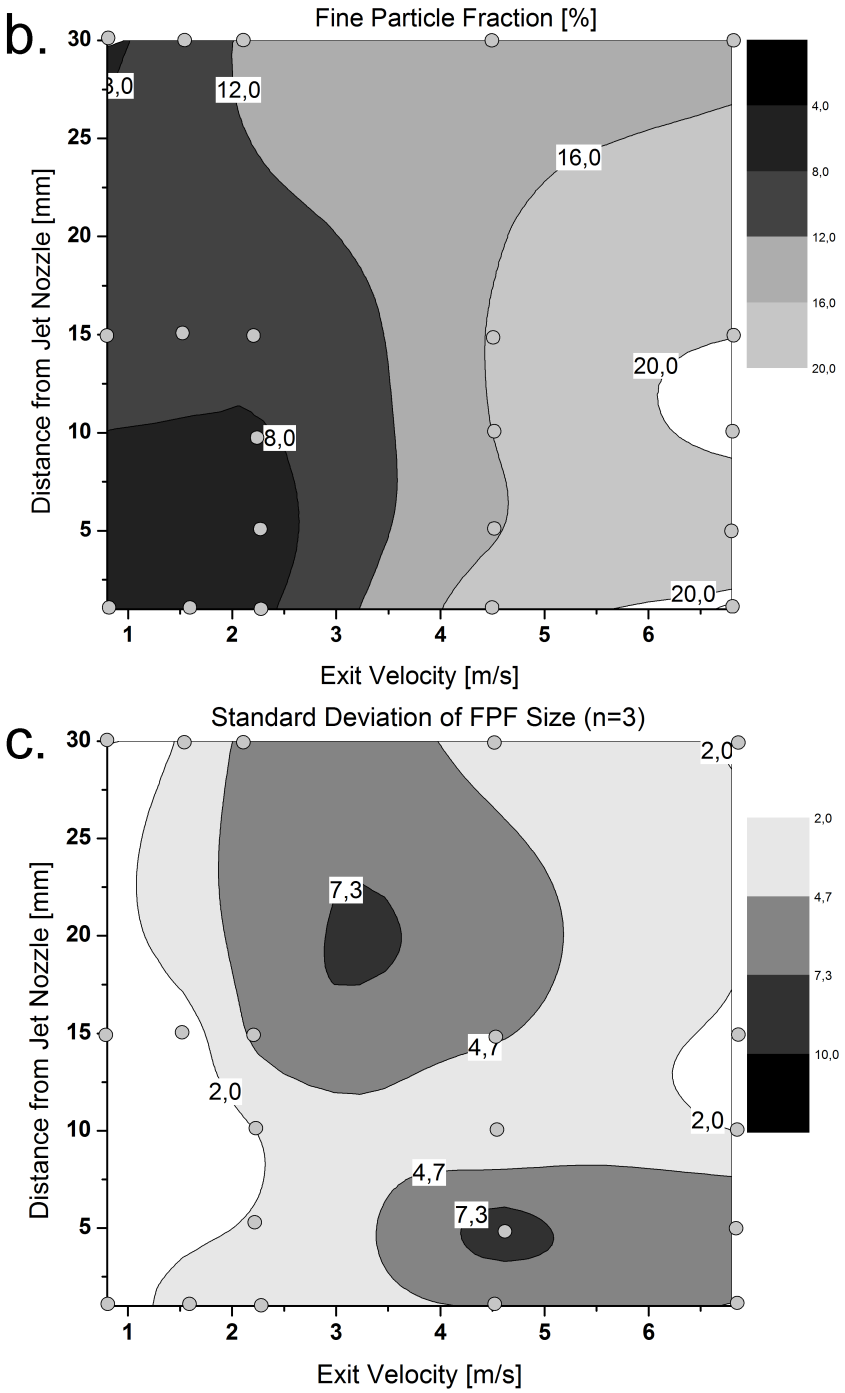

Figure 3-5 (continued): Exemplary NGI deposition patterns of a low, medium and high performing trial **(a)** and the corresponding response surfaces of the mean FPF **(b)** and the standard deviation of the FPF **(c)**.

3.5 DISCUSSION

Spray-freeze drying is a suitable method for the production of highly porous, small spherical lyophilisates with relatively good flowability considering the small particle size and low density of the powders. The response surfaces show that the positions of the DSG inside the vortex and the gas velocity have to be chosen carefully for the production of powders with small mean particles sizes and good performance in the NGI.

The high $SD_{X50.V}$ for close distances of the DSG to the gas jet nozzle could be explained by the limited accuracy of the epicyclical positioning device prototype. The closer the DSG is to the jet nozzle orifice, the more important the exact position of the DSG becomes. Interestingly the $M_{x50.V}$ shows two minima at different process settings. This might be due to the aerodynamic behavior of the gas vortex, which depends on the swirl-tube's internal geometry. For further studies regarding process optimization, the fluid dynamics of the cold gas vortex need to be investigated more closely. Up to now, the flow patterns are not yet fully understood and any explanations on why settings do or do not lead to a sufficient prevention of droplet collisions are only speculative.

Two combinations of experimental settings lead to fine particle fractions up to about 21 %, whereby one lies outside the operating window of the jet-vortex freezer.

When all optima of the four target parameters are taken into account for the determination of the process optimum, only one combination of distance (10-15 mm) and gas exit velocity (>6 m/s is suitable for the reproducible production of lyophilized powders and allows for the effective prevention of droplet collisions. The diameter of the primary droplets which are ejected from the pinhole nozzle is around 40-50 µm (Süverkrüp et al., 2016). This corresponds to a droplet volume of 0.034 – 0.065 nL. The method is able to reduce the $M_{X50.V}$ from 160 µm to approximately 50 µm compared to unexpanded atmospheric droplet-stream freezing. A droplet with a diameter of 160 µm has a volume of 2.1 nL and hence originates from the coalescence of at least 32-62 primary droplets, whereas as droplet with a diameter of 50 µm only consists of 1-2 primary droplets. Therefore, it can be assumed that droplet collisions were almost completely prevented.

SFD powders have shown their potential for pulmonary drug delivery in several studies (Amorij et al., 2007; Bi et al., 2008; Maa et al., 1999; Sharma et al., 2013).

In these studies, where two-fluid nozzles were preferred, the mean particle diameters ranged from 7 to 62 µm with FPFs of about 20 to 70 % (Wanning et al., 2015), mainly using two-fluid nozzles. The significant drawback of these earlier results caused by the use of the two-fluid nozzle is the much wider particle size distribution (span 1.9-2.6), compared to jet-vortex freezing of droplet stream (span 1.0-1.2) introduced here.

Commercially available DPIs have FPFs of approximately 10 % (Son and McConville, 2008) up to 85 % (Berkenfeld et al., 2015) and emitted doses of 50-80%. The Handihaler (which has also been the subject of this study) can achieve FPFs of 20% (Chodosh et al., 2001). The FPFs of up to 21 % in this study are comparable to commonly used DPI formulations. Most aerosol powders in commercial DPI devices are interactive mixtures, in which the micronized API is adhered to larger carrier particles. The overall performance of these powders is strongly dependent on the inhaler device and its deagglomeration potential to separate the API from the carrier particles. This might not be the case for spray-freeze dried powders. Due to their relatively large $M_{X50.V}$ and porous and low-density characteristics, the lyophilized powders in this study are flowable, readily aerosolizable and show very high emitted doses of over 90 %. These characterics might reduce the need for complex inhalers and development of such devices.

Spray-freeze drying with a jet-vortex is a suitable method to produce lyophilized powders of proteins, peptides and nucleic acids for pulmonary delivery due to their increased stability by fast freezing of the droplets. Although the emitted dose is already high, it may be discussed whether the FPF of the lyophilized powder could be further increased.

SFD particles in this study exhibit a calculated aerodynamic diameter of about 8.7 µm based on the mean geometric particle size (approx. 50 µm) and particle density of 0.03 g/cm^3, assuming only a minor droplet expansion upon freezing and no shrinkage during drying. Generally, there are two options to further reduce the aerodynamic diameter and increase the FPF. The decrease of the geometric diameter of the particles is seemingly the easier option, however smaller nozzle orifices risk increasing the production time massively as the throughput of the nozzle decreases and hence production times exponentially increase. An alternative is to reduce the aerodynamic diameter while leaving the geometric diameter unaltered, by decreasing the particle density.

3.6 CONCLUSION

Jet-vortex freezing of droplet-streams is a suitable method for producing lyophilized powders with good dispersibility in air. The incidence of droplet collisions and the mean particle sizes are reduced significantly compared to unexpanded droplet-streams. These lyophilized powders have proven to be suitable for the pulmonary delivery of drugs and still could undergo further improvements if the formulation composition was optimized.

3.7 ACKNOWLEDGMENTS

The authors are grateful for the financial support of the ‘Deutsche Forschungsgemeinschaft’ (DFG) in the framework of SPP1423 (grant no. LA1362/2-1) and would also like to thank Elli Husso for practical assistance and Franz-Josef Willems, Thomas Vidua and Jürgen Hohmann for technical assistance.

3.8 REFERENCES

Ali, M.E., Lamprecht, A., 2014. Spray freeze drying for dry powder inhalation of nanoparticles. Eur. J. Pharm. Biopharm. 87, 510–517.

Amorij, J.-P., Saluja, V., Petersen, A.H., Hinrichs, W.L.J., Huckriede, A., Frijlink, H.W., 2007. Pulmonary delivery of an inulin-stabilized influenza subunit vaccine prepared by spray-freeze drying induces systemic, mucosal humoral as well as cell-mediated immune responses in BALB/c mice. Vaccine 25, 8707–8717.

Berkenfeld, K., Lamprecht, A., McConville, J.T., 2015. Devices for Dry Powder Drug Delivery to the Lung. AAPS PharmSciTech 16, 479–490. doi:10.1208/s12249-015-0317-x

Bi, R., Shao, W., Wang, Q., Zhang, N., 2008. Spray-freeze-dried dry powder inhalation of insulin-loaded liposomes for enhanced pulmonary delivery. J. Drug Target. 16, 639–648.

Chodosh, S., Flanders, J.S., Kesten, S., Serby, C.W., Hochrainer, D., Witek Jr, T.J., 2001. Effective delivery of particles with the Handihaler® dry powder inhalation system over a range of chronic obstructive pulmonary disease severity. J. Aerosol Med. 14, 309–315.

Claussen, I.C., Ustad, T.S., Strømmen, I., Walde, P.M., 2007. Atmospheric Freeze Drying—A Review. Dry. Technol. 25, 947–957.

Edwards, D.A., 1997. Large Porous Particles for Pulmonary Drug Delivery. Science 276, 1868–1872.

Eggerstedt, S.N., Dietzel, M., Sommerfeld, M., Süverkrüp, R., Lamprecht, A., 2012. Protein spheres prepared by drop jet freeze drying. Int. J. Pharm. 438, 160–166.

Garmise, R.J., Mar, K., Crowder, T.M., Hwang, C.R., Ferriter, M., Huang, J., Mikszta, J.A., Sullivan, V.J., Hickey, A.J., 2006. Formulation of a dry powder influenza vaccine for nasal delivery. AAPS PharmSciTech 7, E131–E137.

Hu, J., Johnston, K.P., Williams, R.O., 2003. Spray freezing into liquid (SFL) particle engineering technology to enhance dissolution of poorly water soluble drugs: organic solvent versus organic/aqueous co-solvent systems. Eur. J. Pharm. Sci. 20, 295–303.

Jiang, G., Joshi, S.B., Peek, L.J., Brandau, D.T., Huang, J., Ferriter, M.S., Woodley, W.D., Ford, B.M., Mar, K.D., Mikszta, J.A., Hwang, C.R., Ulrich, R., Harvey, N.G., Middaugh, C.R., Sullivan, V.J., 2006. Anthrax vaccine powder formulations for nasal mucosal delivery. J. Pharm. Sci. 95, 80–96.

Leuenberger, H. (Ed.), 2001. Spray Freeze-drying – The Process of Choice for Low Water Soluble Drugs? J Nanopart Res 4, 111–119.

Leuenberger, H., Plitzko, M., Puchkov, M., 2006. Spray Freeze Drying in a Fluidized Bed at Normal and Low Pressure. Dry. Technol. 24, 711–719.

Lin, Y.-W., Wong, J., Qu, L., Chan, H.-K., Zhou, Q.T., 2015. Powder Production and Particle Engineering for Dry Powder Inhaler Formulations. Curr. Pharm. Des. 21, 3902–3916.

Maa, Y.-F., Nguyen, P.-A., Sweeney, T., Shire, S.J., Hsu, C.C., 1999. Protein inhalation powders: spray drying vs spray freeze drying. Pharm. Res. 16, 249–254.

Mueannoom, W., Srisongphan, A., Taylor, K.M.G., Hauschild, S., Gaisford, S., 2012. Thermal ink-jet spray freeze-drying for preparation of excipient-free salbutamol sulphate for inhalation. Eur. J. Pharm. Biopharm. 80, 149–155.

Niwa, T., Shimabara, H., Kondo, M., Danjo, K., 2009. Design of porous microparticles with single-micron size by novel spray freeze-drying technique using four-fluid nozzle. Int. J. Pharm. 382, 88–97.

Rogers, T., 2003. Micronized powders of a poorly water soluble drug produced by a spray-freezing into liquid-emulsion process. Eur. J. Pharm. Biopharm. 55, 161–172.

Rogers, T.L., Hu, J., Yu, Z., Johnston, K.P., Williams, R.O., 2002a. A novel particle engineering technology: spray-freezing into liquid. Int. J. Pharm. 242, 93–100.

Rogers, T.L., Johnston, K.P., Williams III, R.O., 2003. Physical Stability of Micronized Powders Produced by Spray-Freezing into Liquid (SFL) to Enhance the Dissolution of an Insoluble Drug. Pharm. Dev. Technol. 8, 187–197.

Rogers, T.L., Nelsen, A.C., Hu, J., Brown, J.N., Sarkari, M., Young, T.J., Johnston, K.P., Williams, R.O., 2002b. A novel particle engineering technology to enhance dissolution of poorly water soluble drugs: spray-freezing into liquid. Eur. J. Pharm. Biopharm. 54, 271–280.

Schiffter, H., Condliffe, J., Vonhoff, S., 2010. Spray-freeze-drying of nanosuspensions: the manufacture of insulin particles for needle-free ballistic powder delivery. J. R. Soc. Interface 7, S483–S500.

Sharma, G., Mueannoom, W., Buanz, A.B.M., Taylor, K.M.G., Gaisford, S., 2013. In vitro characterisation of terbutaline sulphate particles prepared by thermal ink-jet spray freeze drying. Int. J. Pharm. 447, 165–170.

Son, Y.-J., McConville, J.T., 2008. Advancements in Dry Powder Delivery to the Lung. Drug Dev. Ind. Pharm. 34, 948–959.

Süverkrüp, R., Eggerstedt, S.N., Gruner, K., Kuschel, M., Sommerfeld, M., Lamprecht, A., 2013. Collisions in fast droplet streams for the production of spherolyophilisates. Eur. J. Pharm. Sci. 49, 535–541.

Süverkrüp, R., Eggerstedt, S., Wanning, S., Kuschel, M., Sommerfeld, M., Lamprecht, A., 2016. Collisions and coalescence in droplet streams for the production of freeze-dried powders. Colloids Surf B 141, 443–449.

Süverkrüp, R., Gruner, K., Diestelhorst, M., 2009. Spherolyophilizates as Ophthalmic Drug Delivery Systems. Invest. Ophthalmol. Vis. Sci. 50, 5557–5557.

Wanning, S., Süverkrüp, R., Lamprecht, A., 2015. Pharmaceutical spray freeze drying. Int. J. Pharm. 488, 136–153.

Wanning, S., Süverkrüp, R., Lamprecht, A., 2014. Aerodynamic droplet stream expansion for the production of uniform pulmonary low density spherolyophilisates. Presented at the AAPS Annual Meeting and Exposition; 2014, San Diego, CA, USA.

Zijlstra, G.S., J. Ponsioen, B., A. Hummel, S., Sanders, N., Hinrichs, W.L.J., de Boer, A.H., Frijlink, H.W., 2009. Formulation and process development of (recombinant human) deoxyribonuclease I as a powder for inhalation. Pharm. Dev. Technol. 14, 358–368.

IMPACT OF EXCIPIENT CHOICE ON THE AERODYNAMIC PERFORMANCE OF INHALABLE SPRAY-FREEZE-DRIED POWDERS

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4.1 ABSTRACT

Spray-freeze-drying (SFD) is a process in which a solution is dispersed into a freezing medium and dried by sublimation, resulting in lyophilized powders with spherical particles. This study aims at screening and evaluating the impact of the excipient choice and spray solution characteristics in SFD on the physico-chemical characteristics of spherolyophilisates. A monodisperse droplet-stream was injected into a vortex of cold gas for the production of inhalable, uniform spherical lyophilisates with a narrow particle size distribution. Model solutions containing graded contents (0.3 %, 1.0 %, and 3.0 % m/v) of common bulk-forming excipients like mannitol, lactose, poylvinylpyrrolidone (PVP), maltodextrin or hydroxypropyl methylcellulose (HPMC) and their blends were dispersed using a single 20 µm pinhole diaphragm. Powders were analyzed regarding their geometric particle size, apparent density, mechanical stability and aerodynamic performance. The diameter of the frozen droplets partially correlated with the Ohnesorge number of the spray solutions. The spherolyophilisate powders had median geometric particle diameters ranging from 20 µm to 81 µm. Some powders showed signs of particle shrinkage during the drying step and diameters were reduced down to 30 % of their initial size. The apparent particle densities ranged from 0.009 g/cm^3 to 0.087 g/cm^3. The mechanical stability of the spheres depended on the constituents and concentration of the initial spray solution. Mannitol/maltodextrin formulations yielded large porous particles with promising performance in the Next-Generation-Impactor, emitted fractions between 92 – 98 % and fine-particle-fractions of over 55 %. The development of formulations for inhalable spray freeze-dried powders is a challenging task as the impact of excipients on the technological characteristics of the dosage form has to be considered.

Keywords: spray freeze drying, droplet-stream generator, protein formulations, lyophilization, porous particles, pulmonary drug delivery, droplet collision

4.2 INTRODUCTION

Over the last decades, research groups have focused on the development of spray-freeze drying (SFD) methods for various purposes. SFD has been studied for the generation of pulmonary (Amorij et al., 2007; Bi et al., 2008; Maa et al., 1999; Mohri et al., 2010) or nasal (Garmise et al., 2007; Jiang et al., 2006; Wang et al., 2012) particles, powders for intradermal delivery by needle-free injection (Rochelle, 2005; Schiffter et al., 2010; Ziegler, 2006) or for technological reasons, such as solubility enhancement (Hu et al., 2003; Kondo et al., 2009; Rogers, 2003; Rogers et al., 2002) or producing stable protein formulations for further processing (Cleland et al., 2001; Daugherty et al., 2011; Lam et al., 2001, 2000).

SFD is a multi-variant process for the production of lyophilized powders. Three basic and sequential steps are the basis of every spray-freeze-drying process. First, an aqueous or organic solution is atomized into small droplets by devices like pneumatic two-fluid nozzles, ultrasonic nebulizers (Schiffter et al., 2010; Sonner, 2002; Ziegler, 2006), thermal ink-jet printheads (Harker et al., 2008; Mueannoom et al., 2012; Sharma et al., 2013), four-fluid nozzles (Kondo et al., 2009; Niwa et al., 2012, 2009) or piezoelectric droplet-stream generators (Ali and Lamprecht, 2014; Eggerstedt et al., 2012). The droplets are then frozen in a cold atmosphere or upon contact with a cryogenic liquid. In the last step, the solvent is removed from the frozen droplets by sublimation drying either under vacuum or at atmospheric pressure in a dry gas flow (Wanning et al., 2015).

A large amount of newly developed biotechnological therapeutic agents are peptides or proteins, which are usually formulated in liquid solutions and administered by injection. A common technique to enhance the storage stability of such solutions is freeze-drying in vials. The dry cake is then reconstituted directly before application. It is of general interest to find non-invasive alternatives to injection and thereby improve compliance. The pulmonary epithelium provides a large surface (approximately 100 m^2) and better permeability for macromolecules than other absorption barriers which makes it a promising target for protein delivery (Patton, 2004). It has been reported that this route has a low amount of metabolic enzymes, bypasses the hepatic first-pass metabolism and provides a very fast absorption rate. (Patton, 2004; Patton et al., 1999). An efficient drug targeting of the deep lungs can be achieved by delivering drug aerosols with an aerodynamic diameter of 1-5 µm (Lin et al.,

2015). The fraction of the delivered aerosol with the given aerodynamic diameter is also referred to as fine-particle-fraction (FPF). Similar to the formulation of protein injectables, a water-free matrix is preferred for stability reasons. As several studies have shown, SFD is a suitable method for the production of particles which meet these specifications (Ali and Lamprecht, 2014; Amorij et al., 2007; Bi et al., 2008; Maa et al., 1999) and resulted in powders with FPFs from 20 % up to 70 %, which is comparable to commercially available dry powder inhalers that have FPFs of 10-85 % (Berkenfeld et al., 2015; Son and McConville, 2008).

SFD possesses an interesting option to further enhance the pulmonary performance of the designed particles by reducing the particle density as the produced particles are highly porous. Since the reduction of the nozzle diameter is accompanied by exponentially increasing production times, reducing the particle density as an alternative becomes an even more interesting aspect. The particle size, shape and interior structure in SFD are largely determined by the freezing step. If the droplets freeze fast enough, the excipient distribution is similar to the distribution in the liquid solution, as the diffusion effects are minimized. It is possible to generate highly porous, spherical particles from spray solutions with concentrations of solid constituents as low as 6 % (Eggerstedt et al., 2012) and even less (Wanning et al., 2017b). Commonly used excipients in pulmonary formulations are mannitol and lactose, both of which are also used as bulking agents in freeze-drying (Costantino and Pikal, 2004; Jennings, 1999).

Over the past decade, many studies concerning spray freeze drying and the evaluation of different techniques have been performed. Most of the studies focused on the formulation of a small number of excipients which were optimized for specific active ingredients and the individual process to allow a successful powder generation (Wanning et al., 2015). In this study, various formulations were screened to evaluate the general suitability of excipients and their combinations with respect to particle size, aerodynamics, aggregation, and shrinking of spherolyophilisates.

Mannitol and lactose solutions with variable solid contents were processed in the jet-vortex freezer (Wanning et al., 2017) to obtain particles with different apparent densities. It can be expected that mechanical stability of the particles decreases with decreasing solid content in

the spray solution and macromolecular constituents may improve the mechanical stability of freeze-dried products. Bashir et al. (Bashir and Avis, 1973) found that the mechanical stability of freeze dried cakes was significantly enhanced by the addition of polyvinylpyrrolidone.

For this reason, polymeric compounds such as maltodextrin (DEX), polyvinylpyrrolidone (PVP) and hydroxypropylmethylcellulose (HPMC) were sprayed as single constituents or in mixture with easily crystallizing excipients of low molecular weight (mannitol or lactose) and sublimation dried under vacuum in a chamber-type lyophilizer. The powders were then analyzed regarding their physico-chemical properties as well as their aerodynamic performance in the Next-Generation-Impactor (NGI).

4.3 MATERIALS AND METHODS

4.3.1 MATRIX FORMING EXCIPIENTS

D(−)-mannitol (M) and lactose-monohydrate (L) were purchased from VWR International, NL-Amsterdam. Polyvinylpyrrolidone (PVP12, Kollidon 12 PF; M=2000-3000 g/mol) and (PVP25, Kollidon 25; M=28000-34000 g/mol) were obtained from BASF, Ludwigshafen, Germany. Maltodextrin (DEX, Glucidex 19 IT, dextrose equivalent of DE = 19) was kindly gifted by Roquette Frères (F-Lestrem). Hydroxypropylmethylcellulose (HPMC, Pharmacoat, Grade 606; M=35600 g/mol) was purchased from Shin-Etsu Chemical Co., Ltd. JP-Tokyo.

4.3.2 CHARACTERIZATION OF SPRAY SOLUTIONS

Spray solutions contained graded concentrations of mono- or dimeric and polymeric components according to Table 4-1 and an additional 0.05, 0.016 or 0.005 % (m/V) sodium fluorescein respectively for assay purposes. The solid components were dissolved in 100 mL ultrapure water (MiliQ®, Milipore Corp., US-Billerica), filtered through a 0.22 µm aseptic filter and sonicated before spraying. Three batches of each solution were prepared.

The density of the solutions was measured using a borosilicate glass pyknometer (9.876 ml, Brand GmbH & Co. KG, D-Wertheim). The surface tension was measured with the drop

volume method using a FM40 Easy Drop tensiometer (Krüss GmbH, D-Hamburg). Viscosity was measured with a RheoStress® 1 rheometer by Haake GmbH (D-Karlsruhe) using a 60 mm cone-plate tool (α = 1°) at a constant shear rate of 50/s at 22.5 °C. All measurements were performed in triplicates. The properties of the solutions related to the formation of droplets were quantified by the Ohnesorge number, which represents the ratio of internal viscosity dissipation to the surface tension energy and characterizes the droplet formation in a spray process.

$$Oh = \frac{\eta}{\sqrt{(\sigma \rho d)}} \qquad \text{(Equation 4-1)}$$

Oh: Ohnesorge number
η: viscosity
σ: surface tension
ρ: density

The glass-transition temperature of solutions (Tg_s') with 3 % and 1 % m/V was measured by differential scanning calorimetry with a DSC 2 (Mettler-Toledo GmbH, D-Gießen). About 20 µL of each solution were placed into aluminium DSC pans, sealed and analyzed. The samples were cooled down to -150 °C (-10 °C/min) and then heated at a rate of 10 °C/min in a range between -150 and 10 °C using the TOPEM® (modulated DSC) method with 1 °C pulse height, 15-30 s pulse width and an underlying heating and cooling rate of 2 °C/min. DSC curves were evaluated using the STARe® Software.

Table 4-1: Spray Solutions

c(M) [%]	c(L) [%]	c(PVP12) [%]	c(PVP25) [%]	c(DEX) [%]	c(HPMC) [%]	C(Fluo) [%]	Density (ρ) [g/mL]	Surface Tension (σ) [mN/m]	Viscosity (η) [mPas]	Oh
3						0.050	1.009	72.74	1.56	0.0338
1						0.016	1.001	71.05	1.62	0.0360
0.3						0.005	0.999	73.27	1.47	0.0317
	3					0.050	1.007	69.85	1.64	0.0370
	1					0.016	1.001	65.94	1.57	0.0376
	0.3					0.005	0.998	70.10	1.53	0.0346
		3				0.050	1.002	66.77	1.65	0.0390
		1				0.016	0.999	66.71	1.55	0.0367
		0.3				0.005	0.996	68.93	1.35	0.0310
			3			0.050	1.003	65.42	1.96	0.0473
			1			0.016	0.999	66.70	1.87	0.0443
			0.3			0.005	0.997	67.60	1.39	0.0326
				3		0.050	1.008	72.07	3.94	0.0861
				1		0.016	1.000	72.11	1.63	0.0357
				0.3		0.005	0.997	72.99	1.38	0.0299
					3	0.050	1.005	46.93	5.62	0.1889
					1	0.016	0.984	47.21	4.33	0.1462
					0.3	0.005	0.998	47.49	1.75	0.0583
1.5		1.5				0.050	1.005	65.30	1.61	0.0389
0.5		0.5				0.016	1.000	67.19	1.69	0.0398
0.15		0.15				0.005	0.997	68.05	1.32	0.0307
1.5			1.5			0.050	1.005	69.06	1.36	0.0311
0.5			0.5			0.016	0.999	66.64	1.56	0.0370
0.15			0.15			0.005	0.995	68.59	1.73	0.0400
1.5				1.5		0.050	1.007	72.05	1.63	0.0356
0.5				0.5		0.016	1.000	71.68	1.64	0.0362
0.15				0.15		0.005	0.998	72.52	1.40	0.0306
1.5					1.5	0.050	1.006	47.10	5.50	0.1841
0.5					0.5	0.016	0.998	47.22	4.07	0.1364
0.15					0.15	0.005	0.996	49.02	1.74	0.0562
	1.5	1.5				0.050	1.007	64.88	1.49	0.0362
	0.5	0.5				0.016	1.002	63.82	1.57	0.0389
	0.15	0.15				0.005	0.999	68.78	1.25	0.0288
	1.5		1.5			0.050	1.007	65.11	1.75	0.0424
	0.5		0.5			0.016	1.000	66.23	1.66	0.0396
	0.15		0.15			0.005	0.998	67.72	1.42	0.0332
	1.5			1.5		0.050	1.012	71.82	2.39	0.0523
	0.5			0.5		0.016	1.000	72.72	1.51	0.0328
	0.15			0.15		0.005	0.989	74.25	1.31	0.0281
	1.5				1.5	0.050	1.006	46.35	4.86	0.1653
	0.5				0.5	0.016	1.000	47.62	4.12	0.1368
	0.15				0.15	0.005	0.997	48.35	1.52	0.0498

4.3.3 SPRAY FREEZE DRYING

A Type MTG – 01.G1 droplet stream generator (DSG) with a 20 µm single pinhole nozzle (FMP Technologies GmbH, D-Erlangen) was used for droplet generation. The feed pressure was approximately 250 kPa and the operating frequency ranged between 42 and 48 kHz. Droplet formation was checked visually by stroboscopic microscopy.

A jet-vortex freezing apparatus (JVF) with an epicyclical DSG positioning device (as described earlier in (Wanning et al., 2017) was used for droplet freezing at -105±3 °C. Both the horizontal and the vertical distance between the DSG and the jet vortex nozzle were set at the optimal values of 10 mm (Wanning et al., 2017) and the volumetric flow of the process gas was 45 L/min, which corresponds to a gas exit velocity of 6.8 m/s at the gas-jet nozzle to obtain particles of 55±4 µm. Operating temperatures in the mixing chamber, the jet vortex, the collecting sieve and room temperature were monitored and recorded with a 4 channel temperature logger (T390, PCE-Instruments, D-Meschede). The frozen droplets were collected on a cooled 700 mesh stainless steel sieve and sublimation-dried on pre-cooled plates (-30 °C) in a lab freeze dryer (Lyovac GT2, STERIS, D-Hürth) for at least 24 h at approximately 0.05 mbar. In this study, all formulations were dried under identical conditions.

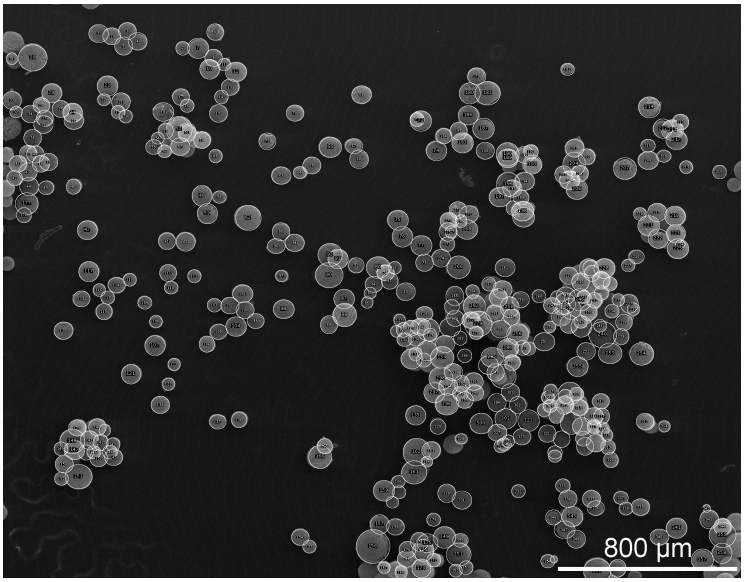
4.3.4 PARTICLE CHARACTERIZATION

4.3.4.1 Scanning Electron Microscopy

The spherolyophilisates were sputter-coated for 6 min with gold in a Polaron SC7640 sputter Coater, Quorum Technologies Ltd., UK-Newhaven and imaged with a Hitachi S-2460N (Hitachi High Tech. Corp., JP-Tokyo) scanning electron microscope (SEM) to examine the particle shape and surface morphology.

4.3.4.2 Geometric particle size determination of dry particles, ice spherules and liquid droplets

To assess the geometric diameter of dry particles, SEM images with 35-45x magnification were analyzed using the NIH open source software ImageJ 1.43 and the Multi-Measure plugin. Due to overlapping particles, an automatic determination of the parameters of the particle size distribution by image analysis was not possible. Particles were masked manually with the oval selection tool (see supplementary Figure. 4-S1) and the pixel area was translated into a geometric particle diameter in µm.



Supplementary Figure 4-S1: Visual particle size distribution analysis with Image J.

The number of analyzed particles to calculate the volumetric x_{10}, x_{50}, x_{90} and SPAN ranged between 200 and 500 per image. The expected mean particle diameter was 55±4 µm as assessed at optimal process conditions earlier (Wanning et al., 2017).

A similar procedure was used to measure the geometric particle size of the frozen droplets on the sieve, with the difference that not the SEM, but an optical microscope with a camera (MikroCam 1.3 MP, Bresser GmbH, D-Rhede) was used for imaging, as frozen particles had to be imaged quickly before melting.

4.3.4.3 Apparent particle density and shrinking

The apparent particle density was calculated from the absolute solid content (SC) in the spray solution, the estimated median droplet diameter (calculated from the median ice particle diameter from which the ice spherule originated) and the final median particle size of the dry spherolyohilisates. The expected apparent particle densities were 0.030, 0.010 and 0.003 g/cm^3 respectively.

After successful drying, the dry particle size is equivalent to the frozen matrix. A collapse of the stabilizing structure is accompanied by a decrease in diameter. To quantify the decrease in the drying process, the $x_{50.SEM}$ of the dry particles was set in relation to the x_{50} of the frozen spherules ($x_{50.FD}$) resulting in the shrinking factor (F_s). The corresponding standard deviation was computed from the root mean squares of the variance ratios. The mean deviation of all samples was 0.08.

A F_s value $< 1.0 \pm 0.08$ was considered as an indication for the reduction of particle size during the drying process.

4.3.4.4 Laser diffraction spectrometry (LD)

A Helos LF® laser diffraction particle analyzer in combination with a Rodos SR® dispersing module (Sympatec GmbH, D-Clausthal-Zellerfeld) was used to measure the geometric particle size distribution at different dispersion pressures (0.25, 0.5, 1.0 and 2.0 bars), to assess indications concerning the relative mechanical stability and aggregation tendencies of the powders. The volumetric median particle diameter ($x_{50.LD}$) and the span were computed with the Windox 3.4 software. The $x_{50.LD}$ values of the laser diffraction measurements were divided by the $x_{50.SEM}$ values determined by SEM image analysis as an indication of particle aggregation and mechanical particle breakage at different dispersion stresses and will be referred to as 'Aggregation/Breakage-Factor: F_{AB}'. The corresponding standard deviation was computed from the root mean squares of the variance ratios.

A F_{AB} value $>1.0 \pm 0.2$ was considered as an indicator for particle aggregation, whereas a value $<1.0 \pm 0.2$ indicated mechanical breaking due to shear stress in the dispersion module.

4.3.4.5 Aerodynamic particle size analysis

A Next-Generation pharmaceutical Impactor (NGI) (MSP Corporation, US-Shoreview, MA) in combination with a Copley Critical Flow Controller TPK and a Copley High Capacity Pump HCP 5 (Copley Scientific Ltd., UK-Colwick Nottingham) was used for determining the aerodynamic particle size distribution of the lyophilized powders at room atmosphere (33 ± 2 % relative humidity at 23 ± 1 °C).

Each capsule was filled with 0.2 mL of lyophilized powder weighing between 0.5 and 5 mg, depending on its bulk density. The airflow was set to 47±0.6 L/min to obtain a pressure drop of 4 kPa and the timer was adjusted to create an inspirational volume of 4 L.

A Handihaler® (Boehringer-Ingelheim, D-Ingelheim) was used to introduce the lyophilized powder into the NGI. Deposited powder in the stages was dissolved in water and evaluated by fluorimetric analysis using a PlateReader 1420 Multilabel Counter VICTOR³V® (Perkin Elmer, US-Waltham, MA). The median cutoff values of the stages at 48 L/min are 9.1 µm, 5.0 µm, 3.2 µm, 1.8µm, 1.0 µm, 0.6 µm and 0.4 µm respectively. The FPF is defined as the amount of particles in per cent that have an aerodynamic diameter smaller than 5 µm. The emitted fraction (EF) is the sum of all powder residues from the mouthpiece on to the last stage of the impactor.

4.3.4.6 Loss on drying

Loss on drying was measured with a thermo-gravimetric scale (TGA 7 with TAC7/DX Controller, $\Delta m \pm 0.1$ µg, Perkin Elmer, US-Waltham). Approximately 1 ± 0.1 mg of lyophilized powder was dried to constant weight ($\Delta m_{rel} < 0.05$ %/min) at 70 °C. The loss on drying was calculated in per cent of the initial mass.

4.4 RESULTS

4.4.1 ICE PARTICLE SIZE AND SPRAY SOLUTION CHARACTERISTICS

As the diameter of a frozen droplet is directly related to the liquid droplet size, it was plotted against the Ohnesorge number of the spray solution. As shown in Figure 4-1, the Oh of viscous spray solutions (Oh > 0.075) was positively correlated with larger frozen droplets (>70 µm) (R^2: 0.911). Formulations containing HPMC, DEX or PVP25 had median dry particle sizes larger than 55±4 µm. All other solutions were in a range between Oh 0.025 and Oh 0.06 and 50 - 65 µm and the relation between Oh and particle size cannot be correlated as the change of particle diameter and Oh is smaller than the measurement accuracy of the available methods.

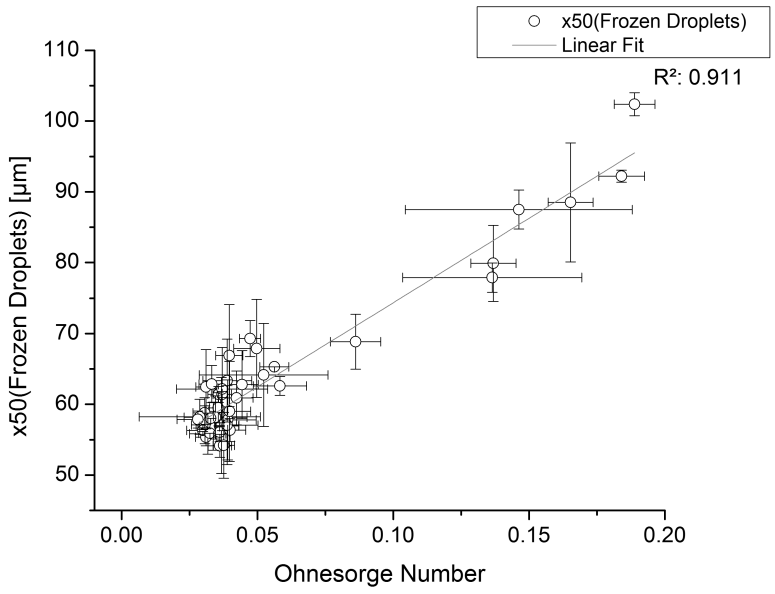


Figure 4-1: Correlation of Ohnesorge number with diameter of frozen droplets

4.4.2 GLASS TRANSITION TEMPERATURES OF FROZEN SPRAY SOLUTIONS

The crystallization and glass formation and consequently the glass transition temperature (Tg_s') of the frozen solution are affected by the freezing rate of the liquid. The Tg_s' are listed in Table 4-2 and agree well with earlier findings (Her and Nail, 1994). Upon heating, pure mannitol solution showed no glass transition but a small exotherm, which has been noted previously (Kett et al., 2003; Kim et al., 1998). No glass transitions were observed for solutions with lactose+HPMC and with low contents of solids (<1 %), which is probably due to low signal strengths.

4.4.3 PARTICLE MORPHOLOGY

The bulk appearance of the lyophilized powders differed between formulations. Most powders (*e.g.* M 3 %, M+DEX 3 %, M+PVP25 3 %, L 3 %, L+DEX 3 %) were free flowing and consisted of small particles. Other spray solutions resulted in powders with large but light aggregates *(e.g.* PVP 25 3 % and 1 %, DEX 3 % and 1%, M+DEX 1 %), which were easily disaggregated into their primary particles upon physical stress. Several lactose-based formulation powders consisted of very compact aggregates, where smaller particles were not found and which were not easily disaggregated. The particle shape and surface morphology was slightly different for every formulation. Spherical particles were found for all formulations with a solid content of 3 % (m/V). Particles with smooth and partially closed surfaces and a lamellar interior were found for all samples with a SC of 3 % (Figure 4-2). All particles originating from spray solutions with 0.3 % were less porous and mostly arranged in aggregates (Figure 4-2c, o).

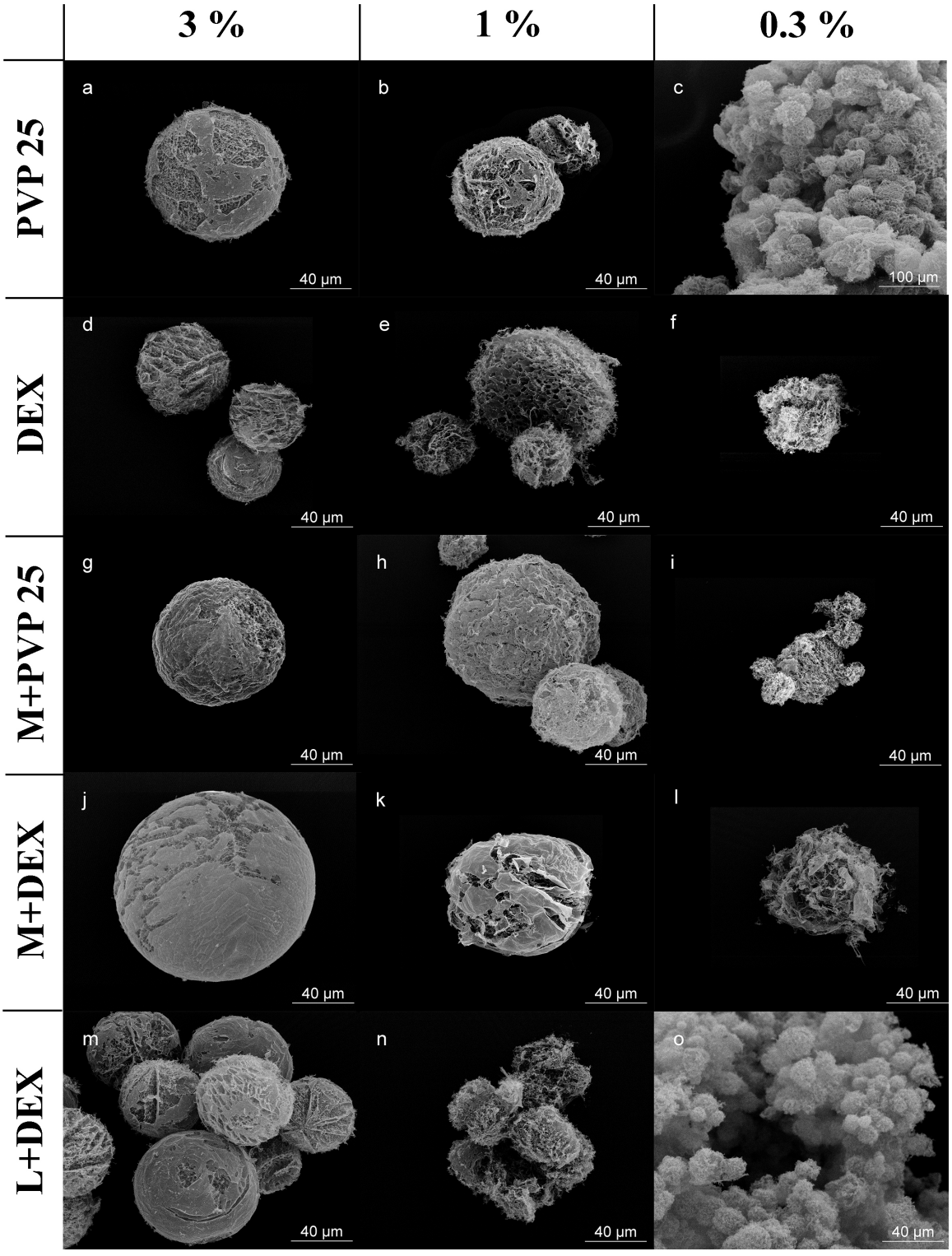


Figure 4-2: Examplary SEM Images of particles with variable excipient composition and solid contents in the spray solution

4.4.4 GEOMETRIC PARTICLE SIZE DETERMINED BY SEM IMAGE ANALYSIS

According to previous studies (Wanning et al., 2017), the expected median particle size was around 50-60 µm . Of the formulations containing single excipients, only mannitol 3 % and 1 % and lactose 3 % resulted in particles within this range. Solutions containing only PVP25, DEX, and HPMC yielded particles > 60 µm. Particles from solutions with PVP12 3 % and 1 % were smaller than 40 µm (Figure 4-3a). Combinations of mannitol and PVP12, PVP25 or DEX with a SC of 3 % had a $x_{50.SEM}$ of approximately 55 µm as originally expected. For a SC of 1% only mannitol based spray solutions (except M+PVP12) had an $x_{50.SEM}$ of 55 µm (Figure 4-3b). Particles originating from spray solutions containing 3 % of lactose and PVP25 or lactose and DEX were around 55-60 µm (Figure 4-3c). L+PVP12 3 % lead to particles smaller than 50 µm. All particles with 1 % SC and combinations of lactose and a polymer were smaller than the corresponding mannitol formulations and all those with 0.3 % SC were smaller than 40 µm.

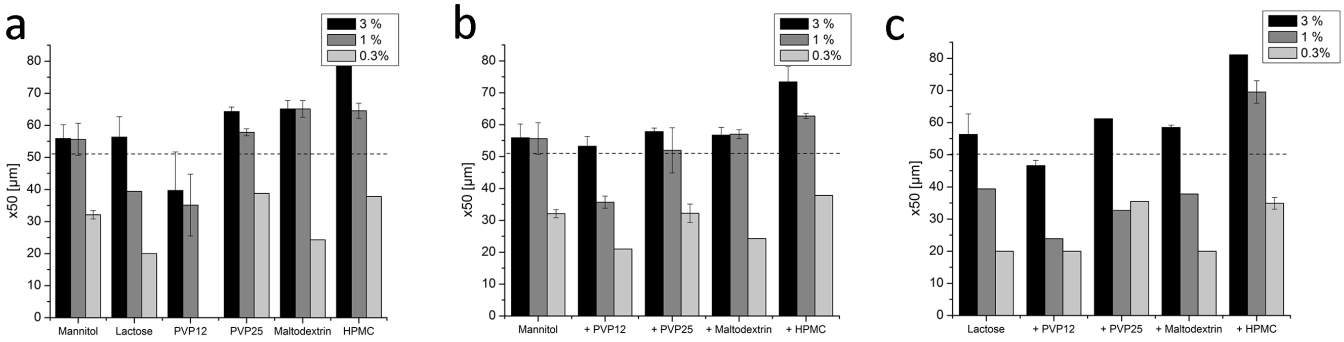


Figure 4-3: Mean particle diameters ($x_{50.SEM}$) for formulations consisting of single excipients **(a)**, a combination of mannitol and a polymer **(b)** or lactose and a polymer **(c)** (n=9, errorbars represent standard deviation)

4.4.5 COMPARISON OF FROZEN-DROPLETS AND DRIED PARTICLES

A plot of the $x_{50.SEM}$ over the F_s shows that all formulations that resulted in median dry particle diameters <40 µm also had a SF < 1 (Figure 4-4). This indicates that the particles decreased in size during the drying process. All samples with a solid content of 0.3 % in the spray solution shrank.

These results were also reflected in the apparent particle densities. Formulations which showed a size reduction had higher particle densities than expected. They ranged between 0.024 - 0.074 g/cm^3 for formulations with a SC of 3%, between 0.009 - 0.115 g/cm^3 for 1 % SC and 0.011 – 0.048 g/cm^3 for 0.3 % SC respectively (Table 4-2)

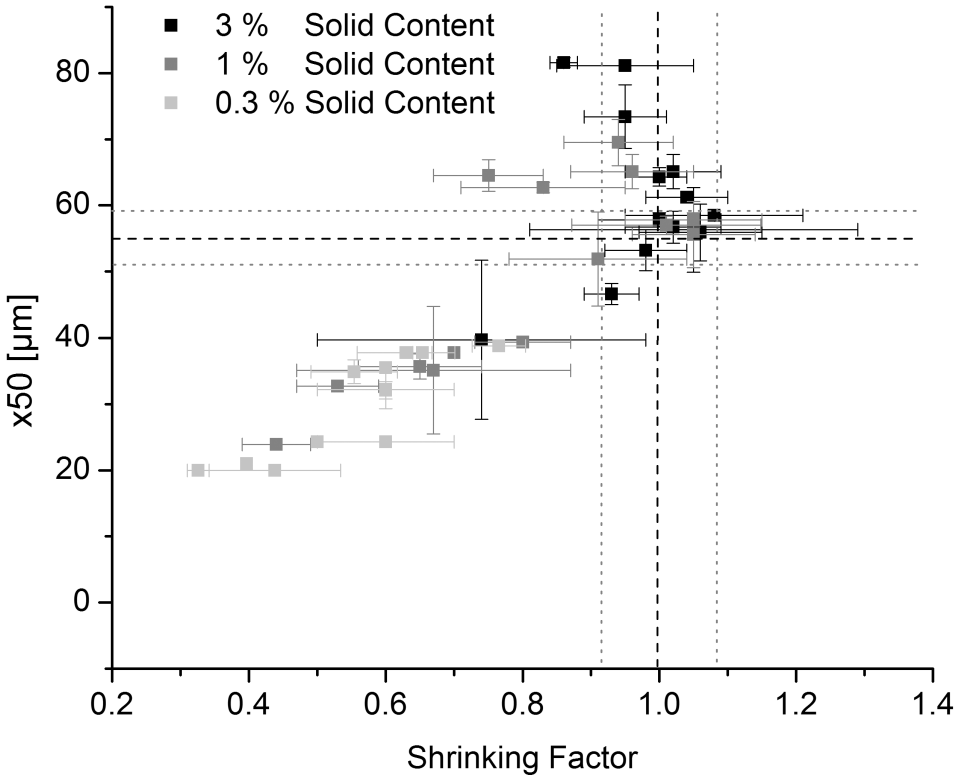


Figure 4-4: Formulations with an SF < 1 show shrinking during drying and are smaller than originally expected

Table 4-2: Results

Mono / Dimer	Polymer	$x50_{Frozen\ Droplets}$ [µm]			$x50_{SEM}$ [µm]			Apparent Particle Density [g/cm³]			Loss on Drying [%]			FPF [%]			Emitted Fraction [%]			Tg_s °C	
Solid Content (m/V)		3%	1%	0.3%	3%	1%	0.3%	3%	1%	0.3%	3%	1%	0.3%	3%	1%	0.3%	3%	1%	0.3%	3%	1%
M		56.9	57.0	55.9	55.8	55.6	32.1	0.025	0.009	0.013	0.1	0.0	0.0	15.3	34.1	65.2	94.7	97.1	98.4	-	-
L		62.2	54.2	58.2	56.3	39.4	-	0.026	0.020	-	4.7	3.8	3.8	18.5	32.3	-	98.4	99.1	-	-29.8	-
	PVP12	57.8	59	51.7	39.7	35.1	-	0.074	0.034	-	6.0	5.7	-	25.9	25.7	-	98.3	98.7	-	-27.8	-
	PVP25	69.3	62.8	54.1	64.3	57.8	38.8	0.030	0.009	0.007	7.4	6.7	5.3	1.4	2.7	-	97.6	98.5	-	-22.5	-23.1
	DEX	68.7	61.4	56.3	65.1	65.1	24.3	0.028	0.011	0.031	5.2	4.6	4.6	4.8	8.0	15.1	96.5	97.4	97.4	-14.6	-14.2
	HPMC	102.2	92.3	62.5	81.6	64.5	37.8	0.047	0.023	0.011	4.2	4.5	1.5	1.8	0.0	-	53.9	22.8	-	-15.3	
M	PVP12	58.4	57.0	55.4	53.2	35.7	21.0	0.031	0.036	0.048	2.2	2.4	2.6	16.5	21.5	-	98.3	-	-	-34.8	-36.6
	PVP25	62.4	61.4	56.4	57.8	51.9	32.2	0.030	0.013	0.013	5.5	3.2	5.3	10.0	8.7	20.5	98.4	97.6	98.4	-33.6	-36.7
	DEX	59.5	59.5	58.8	56.6	57.0	24.3	0.028	0.009	0.011	5.2	1.2	4.6	21.6	52.9	58.5	97.5	92.9	99.4	-36.8	
	HPMC	92.2	81.2	65.2	73.4	62.7	37.8	0.035	0.017	0.012	1.6	1.6	1.7	4.6	12.9	10.7	87.6	93.3	93.3	-30.2	
L	PVP12	54.2	57.1	58.3	46.6	23.9	20.0	0.037	0.115	0.036	5.0	4.0	3.2	9.3	-	-	94.7	-	-	-30.2	-29.1
	PVP25	60.8	66.9	62.9	61.2	32.7	35.5	0.027	0.066	0.013	5.2	4.6	2.8	10.6	9.7	8.2	96.5	94.4	98.3	-26.8	-26.9
	DEX	64.2	58	57.9	58.5	37.8	20.0	0.024	0.029	0.087	3.9	3.6	3.9	21.8	40.5	-	96.3	96.7	-	-23.6	-24.3
	HPMC	88.5	79.9	67.9	81.1	69.5	34.9	0.035	0.012	0.018	5.2	4.6	2.8	0.6	0.8	-	23.4	13.8	-	-	-

4.4.6 SIZE DISTRIBUTIONS OF DRIED PARTICLES BY LASER DIFFRACTION SPECTROSCOPY AT VARIOUS LEVELS OF RODOS DISPERSION PRESSURE

When the dispersion pressure is increased from 0.25 bar to 2 bar, F_{AB} of M 3 %, L 3 % and PVP12 3 % decreased, while DEX and PVP25 almost kept their original size up to 2 bar dispersion pressure. Pure HPMC could not be measured with the given setup because of plastic deformation of the bulk powder during handling (Figure 4-5a). When mannitol was added to the polymer formulations the particles or aggregates increased in size but broke up at lower pressures (Figure 4-5d). When lactose was added instead, both the particle size and the F_{AB} ratio decreased, but less than with mannitol. (Figure 4-5g). Reducing the solid content of the spray solution to 1 % led to an increased F_{AB} which indicates particle aggregation. At a dispersion pressure of 2 bar, these aggregates were then partially disaggregated (HPMC 1 %, PVP25 1 %, L+DEX 1 %) (Figure 4-5b and 5-5). PVP25 1 % and M+DEX 1 % completely disaggregated at lower dispersion pressures and then started to mechanically break apart at dispersion pressures over 1 and 0.5 bar respectively (Figure 4-5e). Due to the low yield of powder from spray solutions with a SC of 0.3 %, not all formulations could be tested with this method. Pure HPMC 0.3 % and PVP25 0.3 % as well as all formulations containing lactose showed strong aggregation (Figure 4c and i). Formulations with mannitol rather showed particle breakage upon increasing dispersion pressure (Figure 4-5f).

The higher mechanical stability and stronger agglomeration of particles produced from solutions with a SC of 0.3 % is mainly caused by particle shrinkage during the drying process. As can be seen in the SEM images (Fig. 5-2i e.g.) the particles were less porous and denser (Table 4-2), which probably leads to a higher mechanical stability. SEM images also showed solid bridges between particles which are mainly responsible for the strong agglomeration (Figure 4-2c and o).

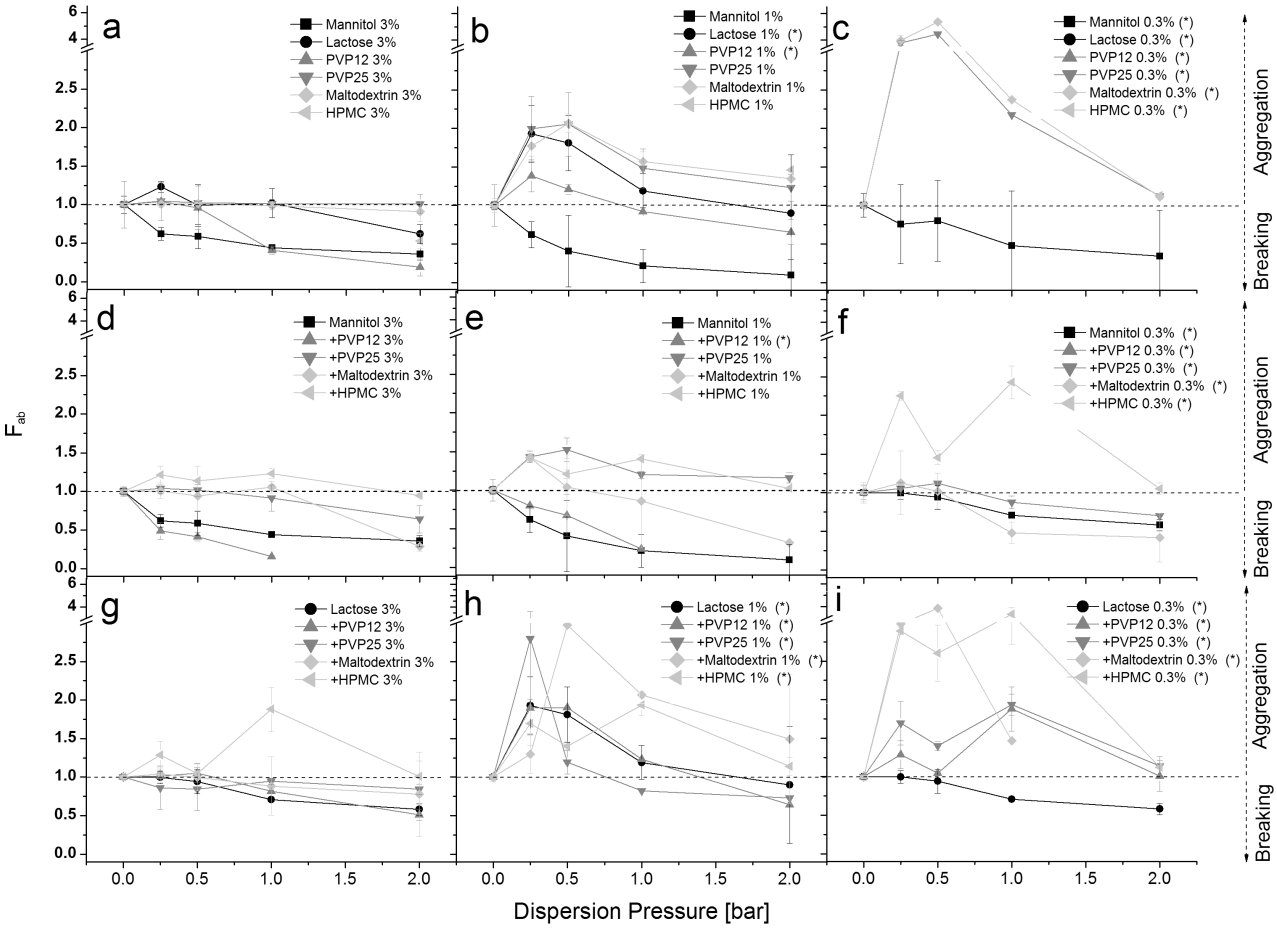


Figure 4-5: F_{AB} Ratio in dependence of dispersion pressure, 0 bar = SEM particle size (n=9, errorbars represents standard deviation)

4.4.6.1 Fine Particle Fraction

For pure mannitol, lactose, PVP25 and DEX formulations, the FPF increases with decreasing solid content of the spray solution (Figure 4-6a). Mannitol shows the highest FPF values with 15,3 % for SC 3 %, 34,1 % for SC 1% and up to 65,2 % for SC 0.3% respectively. Very low FPFs of 1.4% and 2.7% were found for PVP25 and almost no residue in the NGI was found for pure HPMC with a SC 3 % (Table 4-2). The addition of mannitol to the polymeric excipients improved their overall performance in the NGI and all formulations showed higher FPFs than the pure polymeric excipients except for M+PVP12. M+DEX 1 % and 0.3 % had

FPFs of 52.9 % and 58.8 % respectively (Table 4-2). Adding lactose to the polymeric excipients decreased the performance in the NGI for all samples (except L+DEX).

The overall emitted dose for most formulations exceeded 95 %. Only formulations containing HPMC had very low emitted doses because of plastic deformation during handling.

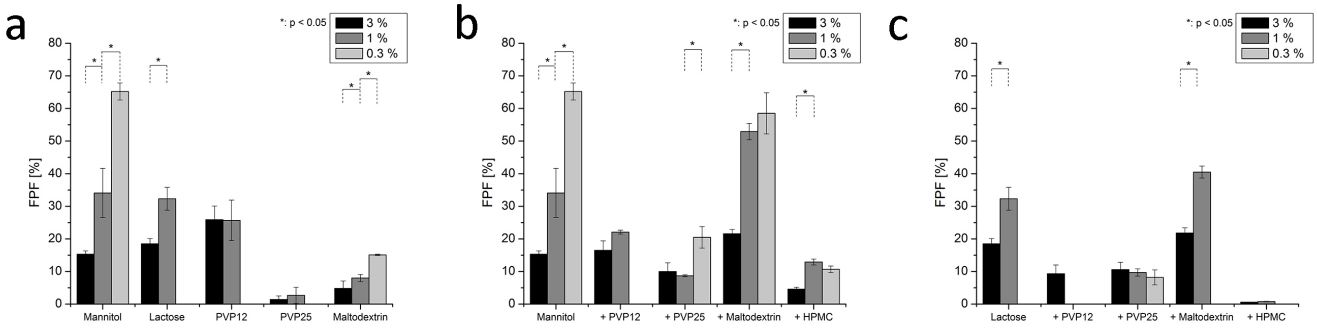


Figure 4-6: Fine particle fractions (FPF) for formulations consisting of single excipients **(a)**, a combination of mannitol and a polymer **(b)** or lactose and a polymer **(c)** (n=6, errorbars represents standard deviation)

4.4.6.2 Residual Moisture

The loss on drying (LOD) ranged between 0.1% (mannitol) and 7.4% (pure PVP25) and was not correlated with the absolute solid content of the spray solutions. Formulations containing mannitol had a significantly lower loss on drying than formulations with lactose (Table 4-2). The type of polymeric compound in the spray solution also had an influence on the LOD. It appears that more hygroscopic components such as PVP12 and PVP25 have higher LODs. In this study, all formulations were dried under identical conditions. The sorption and desorption characteristics of the formulations is discussed in further studies (Chapter 5).

4.5 DISCUSSION

The generation of spherolyophilisates for pulmonary drug delivery with the jet-vortex freezing method has many challenges. Particles should be readily dispersible in moderate gas flows, reach the distal parts of the bronchial system as well as the alveoli, and they should be flowable and sufficiently stable to withstand mechanical stresses upon handling.

The solutes have a major impact on the production process and physicochemical properties of the lyophilized powders on a micro- and macroscopic scale.

The structural and morphological appearance of the particles is primarily defined during the freezing process, where solutes are concentrated in the interstices between growing ice crystals. When the frozen solvent is removed by sublimation, the non-volatile solids form an irregular structure, which may shrink or collapse during dehydration.

The extremely rapid freezing of the droplets in jet-vortex spray freezing probably reduces the crystallization of the excipients and promotes the generation of an amorphous, glassy matrix and leads to smaller pore sizes. Furthermore, the addition of largely amorphous polymers like PVP12 and PVP25 further retards the growth of crystalline structures (Wang, 2000) and favors small pore sizes, which can be seen in Figure 4-2a.

The addition of polymeric excipients leads to mechanically more stable particles, which was indicated by laser diffraction measurements, where a reduced particle breakage for all solutions containing PVP25, DEX and HPMC was observed. PVP25 yielded the relatively most stable particles in laser diffraction experiments, which might be due to the smaller pore size compared to other formulations. Larger amounts of polymeric constituents also increase the Ohnesorge number of the solutions and led to particles larger than the originally expected 55±4 µm. The viscosity can be considered to be a limiting factor for the generation of small particles as high viscosity might prevent the creation of small droplets by forced disintegration of laminar streams. Hence, macromolecules like HPMC, which increase the viscosity, have to be used in low concentrations. With decreasing solid content the mechanical stability of mannitol particles was reduced. Particles composed of lactose and the investigated macromolecules however showed signs of agglomeration. This agglomeration might have its origin in mechanical interlocking due to rougher and more open particle surfaces as observed in DEX, mannitol and DEX or lactose. For powders with higher

amounts of residual moisture (HPMC, PVP25), adhesive capillary forces might also be a factor.

It has been hypothesized in previous studies (Eggerstedt et al., 2012; Wanning et al., 2017b, 2016) that the particles are not altered in size during sublimation drying. The current findings suggest that this statement is only valid for formulations containing mannitol, PVP25, DEX with at least 1 % or mannitol, lactose, PVP25 and DEX with at 3% of total solid content in the spray solution.

The critical temperature at which particle shrinkage occurs (often referred to as collapse temperature (T_C) during the drying step is probably related to a glass transition of the frozen spray solutions (Wang, 2000). As the freezing rate in the jet-vortex at -100 °C is considerably higher than in the DSC (-10 °C/min), the Tg_s' results have to be considered cautiously and can only be seen as ranking scale. Due to the extremely thin laminae and their sharp edges, the surface tension of the honeycomb structure is probably very high. If temperatures during drying are above the Tg_s' and T_C the frozen solution might show micro-meltbacks (McCartney, 2014) or return into a highly viscous state, allowing the polymeric components and interstitial water to become mobile and rearrange to a thermodynamically more stable and compact structure. It was reported that creating an amorphous mannitol formulation from a single-component solution was not possible, even when single droplets were dripped into liquid nitrogen. Yet, the addition of > 70 % (w/w) non-crystallizing co-solutes (sucrose, trehalose, dextran, lysozyme) apparently yielded amorphous mannitol (Kim et al., 1998). The generation of amorphous lactose proved to be easier. The solid content of the spray solution has a minor impact on the Tg_s' but clearly affects the thickness of the lamellar structure of the particle and lower concentrated formulations result in thinner lamellas (McCartney, 2014). The combination of a glass transition and extremely thin walls then may lead to a loss of the internal stabilizing structure of the particle which consequently collapses.

Although particle shrinkage increases the mechanical stability and might have no negative effect on protein stability (Wang et al., 2003), it may involve other changes such as lower rates of sublimation during the primary drying phase and lower desorption rates in the secondary drying phase due to a decrease in specific surface area and increase the required drying time. The structural collapse also influences the aerodynamic behavior of the powder

and its dissolution characteristics. When the particle diameter decreases, the particle density increases, because the absolute mass of the particle is unaltered. Based on the relationship between geometric particle diameter, particle density and aerodynamic diameter, it can easily be calculated that a smaller and denser particle has a larger aerodynamic diameter than the original, larger but more porous sphere. For example: If a spherical M+PVP12 particle with a d_{geo} of 57 µm and a density of 0.01 g/cm^3 shrinks to 35.7 µm while maintaining its original mass, the apparent density will increase to 0.036 g/cm^3. This leads to an increase of the aerodynamic diameter from 5.7 µm to 6.8 µm.

Moreover, the strong agglomeration and still more the formation of solid bridges decreases the emitted fraction and therefore the FPF.

Conventional powders for pulmonary aerosols are interactive mixtures of microparticles (<5 µm) containing the APIs+ and larger inert carriers (60-90 µm), which assure the flowability (Berkenfeld et al., 2015). Most commercially available dry powder inhalers have FPFs of around 10 to 30 % (Son and McConville, 2008) and emitted fractions between 50-80% (Berkenfeld et al., 2015). During application, the API particles have to be separated from the carrier particles to reach the deep lungs. This desagglomeration depends on the inspirational flow and the inhaler geometry. Lyophilized powders prepared by jet-vortex freezing of continuous droplets streams from earlier studies already had comparable FPFs of about 22 % and superior emitted fractions of over 95 % (Wanning et al., 2017). By optimizing the composition of the spray solution, the FPF could be enhanced to over 55%, which is remarkable if the relatively large geometric diameter (approx. 50 µm) of the particles is considered. Large, porous SFD particles have the API directly embedded in their matrix, are readily dispersible in air and do not need any carrier particles. Therefore, a formulation design with a carrier particle is not necessary anymore.

In freeze-drying it is often necessary to add supplementary excipients with cryo- and lyoprotective properties to obtain stable protein formulations. The selection of these excipients depends primarily on the active ingredient which is to be conserved (Wang, 2000). This study shows, that the selection of excipients is not only important for the stabilization of the biological API but also for the quality (*e.g.* the particle size and the aerodynamic properties) of the dosage form. In freeze-drying of parenterals, the lyophilized cake is not the

final pharmaceutical dosage form, as it hast to be reconstituted and then a solution is applied via intravenous injection. In SFD, the main goal is to create a directly applicable lyophilized powder which is the final dosage form. When the chosen route of administration places high demands on the powder, it is vital not only to investigate the chemical stability of the API, but also the pharmaceutical-technological characteristics of the drug product.
It should be, however, noted that a potential impact by the API, either by its mass contribution or by the interaction between API and excipients, cannot be neglected and may limit the selection of excipients and the composition of the SFD.

4.6 CONCLUSION

Pulmonary applicable lyophilized powders with excellent dispersion characteristics in air have been successfully produced by jet-vortex freezing of droplet-streams and subsequent sublimation drying. It has been shown that the addition of polymeric components to a spray solution may increase the mechanical stability of spherolyophilisates, but can also increase the aggregation tendency which has a significant impact on the aerodynamic performance of the powder. The aerodynamic performance in the NGI with an optimized formulation composition showed that spherolyophilisates may be superior compared to commercially available DPIs due to improved dispersion characteristics. Yet, the excipients may have an impact on the physicochemical properties of the lyophilized spheres and formulations will have to be characterized briefly for each combination of constituents to guarantee a satisfactory product quality.

4.7 ACKNOWLEDGMENTS

The authors are grateful for the financial support of the ‘Deutsche Forschungsgemeinschaft’ (DFG) in the framework of SPP1423 (grant no. LA1362/2-1) and would also like to thank Johanna Lübbert, Franz-Josef Willems, Jürgen Hohmann for technical and practical assistance.

4.8 REFERENCES

Ali, M.E., Lamprecht, A., 2014. Spray freeze drying for dry powder inhalation of nanoparticles. Eur. J. Pharm. Biopharm. 87, 510–517.

Amorij, J.-P., Saluja, V., Petersen, A.H., Hinrichs, W.L.J., Huckriede, A., Frijlink, H.W., 2007. Pulmonary delivery of an inulin-stabilized influenza subunit vaccine prepared by spray-freeze drying induces systemic, mucosal humoral as well as cell-mediated immune responses in BALB/c mice. Vaccine 25, 8707–8717.

Bashir, J.A., Avis, K.E., 1973. Evaluation of excipients in freeze-dried products for injection. Bull. Parenter. Drug Assoc. 27, 68–83.

Berkenfeld, K., Lamprecht, A., McConville, J.T., 2015. Devices for Dry Powder Drug Delivery to the Lung. AAPS PharmSciTech 16, 479–490.

Bi, R., Shao, W., Wang, Q., Zhang, N., 2008. Spray-freeze-dried dry powder inhalation of insulin-loaded liposomes for enhanced pulmonary delivery. J. Drug Target. 16, 639–648.

Cleland, J.L., Duenas, E.T., Park, A., Daugherty, A., Kahn, J., Kowalski, J., Cuthbertson, A., 2001. Development of poly-(D, L-lactide–coglycolide) microsphere formulations containing recombinant human vascular endothelial growth factor to promote local angiogenesis. J. Controlled Release 72, 13–24.

Costantino, H.R., Pikal, M.J., 2004. Lyophilization of Biopharmaceuticals. Springer Science & Business Media.

Daugherty, A.L., Rangell, L.K., Eckert, R., Zavala-Solorio, J., Peale, F., Mrsny, R.J., 2011. Sustained release formulations of rhVEGF165 produce a durable response in a murine model of peripheral angiogenesis. Eur. J. Pharm. Biopharm. 78, 289–297.

Eggerstedt, S.N., Dietzel, M., Sommerfeld, M., Süverkrüp, R., Lamprecht, A., 2012. Protein spheres prepared by drop jet freeze drying. Int. J. Pharm. 438, 160–166.

Garmise, R.J., Staats, H.F., Hickey, A.J., 2007. Novel dry powder preparations of whole inactivated influenza virus for nasal vaccination. AAPS PharmSciTech 8, 2–10.

Harker, J., Li, C.-T., Gaisford, S., Jones, M., 2008. Novel Use of a Modified Ink-Jet Print Head to Produce Spray-Freeze Dried Particles for Inhalation, in: DDL Conference.

Her, L.-M., Nail, S.L., n.d. Measurement of Glass Transition Temperatures of Freeze-Concentrated Solutes by Differential Scanning Calorimetry. Pharm. Res. 11, 54–59.

Hu, J., Johnston, K.P., Williams, R.O., 2003. Spray freezing into liquid (SFL) particle engineering technology to enhance dissolution of poorly water soluble drugs: organic solvent versus organic/aqueous co-solvent systems. Eur. J. Pharm. Sci. 20, 295–303.

Jennings, T.A., 1999. Lyophilization: Introduction and Basic Principles. Taylor & Francis.

Jiang, G., Joshi, S.B., Peek, L.J., Brandau, D.T., Huang, J., Ferriter, M.S., Woodley, W.D., Ford, B.M., Mar, K.D., Mikszta, J.A., Hwang, C.R., Ulrich, R., Harvey, N.G., Middaugh, C.R., Sullivan, V.J., 2006. Anthrax vaccine powder formulations for nasal mucosal delivery. J. Pharm. Sci. 95, 80–96.

Kett, V.L., Fitzpatrick, S., Cooper, B., Craig, D.Q., 2003. An investigation into the subambient behavior of aqueous mannitol solutions using differential scanning calorimetry, cold stage microscopy, and X-ray diffractometry. J. Pharm. Sci. 92, 1919–1929.

Kim, A.I., Akers, M.J., Nail, S.L., 1998. The physical state of mannitol after freeze-drying: Effects of mannitol concentration, freezing rate, and a noncrystallizing cosolute. J. Pharm. Sci. 87, 931–935.

Kondo, M., Niwa, T., Okamoto, H., Danjo, K., 2009. Particle characterization of poorly water-soluble drugs using a spray freeze drying technique. Chem. Pharm. Bull. (Tokyo) 57, 657–662.

Lam, X.M., Duenas, E.T., Cleland, J.L., 2001. Encapsulation and stabilization of nerve growth factor into poly (lactic-co-glycolic) acid microspheres. J. Pharm. Sci. 90, 1356–1365.

Lam, X.M., Duenas, E.T., Daugherty, A.L., Levin, N., Cleland, J.L., 2000. Sustained release of recombinant human insulin-like growth factor-I for treatment of diabetes. J. Controlled Release 67, 281–292.

Lin, Y.-W., Wong, J., Qu, L., Chan, H.-K., Zhou, Q.T., 2015. Powder Production and Particle Engineering for Dry Powder Inhaler Formulations. Curr. Pharm. Des. 21, 3902–3916.

Maa, Y.-F., Nguyen, P.-A., Sweeney, T., Shire, S.J., Hsu, C.C., 1999. Protein inhalation powders: spray drying vs spray freeze drying. Pharm. Res. 16, 249–254.

McCartney, S., 2014. Mechanical characterization of freeze-dried biopharmaceuticals. Imperial College London.

Mohri, K., Okuda, T., Mori, A., Danjo, K., Okamoto, H., 2010. Optimized pulmonary gene transfection in mice by spray–freeze dried powder inhalation. J. Controlled Release 144, 221–226.

Mueannoom, W., Srisongphan, A., Taylor, K.M.G., Hauschild, S., Gaisford, S., 2012. Thermal ink-jet spray freeze-drying for preparation of excipient-free salbutamol sulphate for inhalation. Eur. J. Pharm. Biopharm. 80, 149–155.

Niwa, T., Mizutani, D., Danjo, K., 2012. Spray freeze-dried porous microparticles of a poorly water-soluble drug for respiratory delivery. Chem. Pharm. Bull. (Tokyo) 60, 870–876.

Niwa, T., Shimabara, H., Kondo, M., Danjo, K., 2009. Design of porous microparticles with single-micron size by novel spray freeze-drying technique using four-fluid nozzle. Int. J. Pharm. 382, 88–97.

Patton, J.S., 2004. The Lungs as a Portal of Entry for Systemic Drug Delivery. Proc. Am. Thorac. Soc. 1, 338–344.

Patton, J.S., Bukar, J., Nagarajan, S., 1999. Inhaled insulin. Adv. Drug Deliv. Rev. 35, 235–247.

Rochelle, C., 2005. Spray-Freeze-Dried Powders for Needle-Free Injection (Dissertation). Friedrich-Alexander-Universität Erlangen-Nürnberg.

Rogers, T., 2003. Micronized powders of a poorly water soluble drug produced by a spray-freezing into liquid-emulsion process. Eur. J. Pharm. Biopharm. 55, 161–172.

Rogers, T.L., Nelsen, A.C., Hu, J., Brown, J.N., Sarkari, M., Young, T.J., Johnston, K.P., Williams, R.O., 2002. A novel particle engineering technology to enhance dissolution of poorly water soluble drugs: spray-freezing into liquid. Eur. J. Pharm. Biopharm. 54, 271–280.

Schiffter, H., Condliffe, J., Vonhoff, S., 2010. Spray-freeze-drying of nanosuspensions: the manufacture of insulin particles for needle-free ballistic powder delivery. J. R. Soc. Interface 7, S483–S500.

Sharma, G., Mueannoom, W., Buanz, A.B.M., Taylor, K.M.G., Gaisford, S., 2013. In vitro characterisation of terbutaline sulphate particles prepared by thermal ink-jet spray freeze drying. Int. J. Pharm. 447, 165–170.

Son, Y.-J., McConville, J.T., 2008. Advancements in Dry Powder Delivery to the Lung. Drug Dev. Ind. Pharm. 34, 948–959.

Sonner, C., 2002. Protein loaded powders by spray freeze drying. Friedrich-Alexander-Universität Erlangen-Nürnberg.

Wang, D.Q., Hey, J.M., Nail, S.L., 2003. Effect of Collapse on the Stability of Freeze-Dried Recombinant Factor VIII and α-Amlyase. J Pharm Sci 93, 1253–1263.

Wang, S.H., Kirwan, S.M., Abraham, S.N., Staats, H.F., Hickey, A.J., 2012. Stable Dry Powder Formulation for Nasal Delivery of Anthrax Vaccine. J. Pharm. Sci. 101, 31–47.

Wang, W., 2000. Lyophilization and development of solid protein pharmaceuticals. Int Pharm J 203, 1–60.

Wanning, S., Süverkrüp, R., Lamprecht, A., 2017. Jet-vortex spray freeze drying for the production of inhalable lyophilisate powders. Eu J Pharm Sci 96, 1–7.

Wanning, S., Süverkrüp, R., Lamprecht, A., 2016. Aerodynamic Droplet Stream Expansion for the Production of Spray-Freeze-Dried Powders. AAPS Pharm Sci Tech.

Wanning, S., Süverkrüp, R., Lamprecht, A., 2015. Pharmaceutical spray freeze drying. Int. J. Pharm. 488, 136–153.

Ziegler, A.S., 2006. Needle-free Injection: Attrition forces acting on spray-dried and spray-freeze-dried portein-loaded particles with needle-free injectors. Friedrich-Alexander-Universität Erlangen-Nürnberg.

HUMIDITY-RESISTANCE WITH OPTIMAL EXCIPIENT CHOICE IN INHALABLE SPRAY-FREEZE-DRIED POWDERS

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5.1. ABSTRACT

In lower segments of the respiratory tract, the humidity is high and the mass, size and adhesiveness of inhaled porous particles may change significantly with consequential alterations of the deposition pattern. In this study, the water uptake of various spray-freeze dried formulations containing graded amounts (3.0 %, 1.0 %, 0.3 % m/V) of mono/dimeric (mannitol, lactose) and polymeric excipients (maltodextrin, polyvinylpyrrolidone, hydroxypropylmethycellulose) were investigated. The collapse humidity at ambient temperature was determined and the aerodynamic performance at 33 %, 55 % and 77 % relative humidity (rH) was assessed. Formulations showed different moisture uptake profiles. Structural collapse was observed between 36 % rH and 88 % rH, depending on the formulation composition. Upon increasing humidity levels, the fine particle fraction and emitted fraction of SFD powders decreased between 5 and 35 % and 1 and 81 % respectively. It could be shown that humidity has a detrimental effect on the structural stability and aerodynamic dispersibility in air of lyophilized powders and that this effect depends on the formulation.

Keywords: Spray freeze drying, lyophilization, porous particles, pulmonary application

5.2. INTRODUCTION

In spray-freeze drying (SFD) a solution containing the active ingredient and one or more excipients is atomized into a cryogenic liquid or gaseous phase and subsequently sublimation dried to yield a flowable lyophilized powder. The three basic steps of SFD are: Droplet formation, droplet freezing and sublimation drying. There are several variations of each step, which have their individual advantages and disadvantages and lead to specific properties of the final product. SFD techniques and pharmaceutical applications of flowable lyophilized powders have been reviewed recently (Wanning et al., 2015).

Powders and particles have to fulfill geometric and aerodynamic criteria for an effective deposition in the deep lung. An aerodynamic diameter (d_{aero}) of 1-5 µm is required (Laube et al., 2011; Lin et al., 2015) to successfully deliver drug loaded particles to the deep lung. Both dense particles with small diameters and larger porous particles with low density have similar aerodynamic properties and meet these requirements. Jet-vortex freezing of fast droplet streams is a promising technique for the production of large (approx. 50 µm) spherical and porous lyophilized particles (Wanning et al., 2017). It has been shown that a variety of excipients can be processed with expanded droplet streams from piezoelectric droplet stream generators, but that the physicochemical properties (particle size, apparent particle density, mechanical stability, agglomeration) and the suitability for pulmonary application of the powders strongly depends on the chosen excipients (Chapter 4).

A commonly known characteristic of lyophilized products is their high porosity and therefore their large surface area. If functional groups, such as hydroxyl-, keto- or aldehyde groups, are available at the solid/gas interface, large amounts of water from the atmosphere can be adsorbed on the surface. This leads to strong hygroscopic characteristics. During handling of PVP-based lyophilized powders in earlier studies (Chapter 4), it was observed that powders started to shrink and collapse when they came in contact with a moderately humid atmosphere (>50 % rH). While freeze-dried cakes in vials can be protected from moisture, powders for inhalation will inevitably be in contact with different levels of humidity prior to application. Upon administration, the ambient air that passes through the inhaler disperses the powder. It then passes through the mouth, the trachea, and the bronchii until the fine particle fraction (FPF) is deposited in the alveoli. The human respiratory tract has a humid

environment (Martonen and Zhang, 1993; Son et al., 2013) and it has been reported that inhaled particles grow in humid atmospheres which has an impact on the aerodynamic behavior during inhalation (Hickey, 1996; Hickey and Martonen, 1993; Martonen et al., 1982). It was hypothethised, based on observations n earlier studies, that large and low density particles first lose their porous structure and collapse which might affect the dispersibility in air and even therefore the aerodynamic performance.

This study focuses upon the moisture uptake of these powders and its effect on the structural integrity of spherolyophilisates by evaluating various excipients, alone and in combination. Mannitol and lactose were used being standard excipients in pulmonary formulation design and bulking agents in freeze-drying. To increase the mechanical stability, polymeric excipients were added to the spray solutions. Lyophilized powders were produced by injecting a fast droplet stream into a jet-vortex of cold air and characterized with a focus on vapour sorption and emitted fraction as well as FPF at different levels of humidity at ambient temperature to determine the suitability of different SFD formulations for pulmonary application.

5.3. MATERIALS AND METHODS

5.3.1 MATRIX FORMING EXCIPIENTS AND SPRAY SOLUTIONS

The mono- and dimeric constituents were D(−)-mannitol (M) and lactose-monohydrate (L) (VWR International, NL-Amsterdam). As polymeric components, polyvinylpyrrolidone (PVP12, Kollidon 12 PF; M= 2000-3000 g/mol) and (PVP25, Kollidon 25; M=28000-34000 g/mol, BASF, D-Ludwigshafen), maltodextrin (DEX, Roquette LAB 2509, dextrose equivalent of DE = 19, Roquette Frères, F-Lestrem), hydroxypropylmethylcellulose (HPMC, Pharmacoat, Grade 606, Shin-Etsu Chemical Co., Ltd. JP-Tokyo) were used.

Spray solutions consisted of either one mono/dimeric or oligo/polymeric excipient or blends of equal amounts of both types of excipient with total solid contents (SC) of 3 %, 1 % or 0.3 % respectively. Sodium-fluoresceine was added as marker to each solution for analytical purposes.

5.3.2 SPRAY FREEZE DRYING

Droplets were generated with a MTG – 01.G1 (FMP Technologies, D-Erlangen) droplet stream generator (DSG) using a single pinhole diaphragm (ø: 20 µm), a feed pressure of 250 kPa and excitation frequencies between 42 and 48 kHz as described earlier (Wanning et al., 2017). Droplet generation was checked by stroboscopic microscopy.

A jet-vortex freezing device (JVF) (Wanning et al., 2017) was used for quickly separating and freezing droplets at temperatures around 105±3°C. An epicyclical positioner was used for mounting the DSG at the top of the JVF. The JVF was operated at optimized spray settings previously optimized settings (Wanning et al., 2017). Both the horizontal and the vertical distance of the DSG from the gas-jet nozzle were 10 mm respectively, the gas exit velocity was 6.8 m/s.

The freezing temperature in the jet vortex was monitored and recorded with a temperature logger (T390, PCE-Instruments, D-Meschede). The frozen droplets were collected on a cooled sieve and sublimation dried on pre-cooled plates (-30 °C) in a laboratory-scale freeze dryer (Lyovac GT2, STERIS, D-Hürth) for at least 24 h at approximately 0.05 mbar.

5.3.3 POWDER CHARACTERIZATION

5.3.3.1 Water sorption, desorption and loss of structural integrity

Water uptake of the powders was studied by dynamic vapour sorption (DVS, Surface Measurements Systems, UK-London) with a Cahn D-200 recording balance (Thermo Electron Corp. US-Newington, NH). Samples with initial masses of approximately 2 mg were exposed at 25 °C to gas flows with an initial relative humidity of 0 %, which was ramped up to 96 % rH at a rate of 10 % rH/h and then back down at the same rate to 0 % (see Table 5-1, Figure 5-4a).

Table 5-1: Dynamic vapour sorption measurement program (T = 24 °C)

Step:	Condition	Stop criteria
1	0 % rH	t = 60 min
2	+10 % rH/h	96 % rH
3	- 10 % rH/h	0 % rH
4	0 % rH	Δm_{rel}: ≤ 0.02 %

Both the rH in the sample chamber and the sample mass were recorded at intervals of 1 minute. The powder was also imaged with an endoscopic camera (Voltcraft BS-12, CH-Wollerau) inside the sample chamber and pictures were taken every 10 min using the ChronoLapse Software (http://www.chronolapse.com by Collin Green, US-Mointainview, CA). The relative humidity at which particles were observed to shrink will be referred to as collapse humidity..

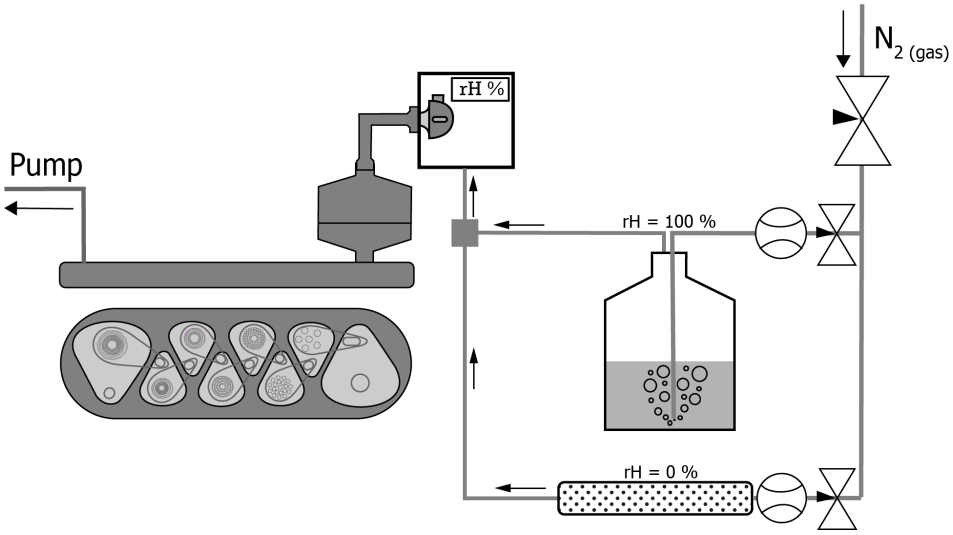

Figure 5-1 : NGI Device with climate chamber

5.3.3.2 Geometric particle size and apparent particle density

Dry powder samples were sputter-coated (Polaron SC7640, Quorum Technologies Ltd., UK-Newhaven) and imaged using a S-2460N scanning electron microscope (Hitachi High Tech. Corp., JP-Tokyo). The geometric particle size distribution was assessed by analyzing the images with the NIH open source software Image J 1.43 and the multi measure plugin as described earlier (Chapter 4). Particles were masked with the circle selection tool, the pixel area was used to calculate the diameter in pixels, which was then translated into a geometric diameter in µm. The volumetric x_{10}, x_{50}, x_{90} and the span were calculated from around 200-500 particles per sample. The apparent particle density was calculated from the absolute solid content of the droplets and the dry particle size as described earlier (Chapter 4).

5.3.3.3 Aerodynamic particle size analysis

The aerodynamic particle size distribution was assessed with a Next-Generation pharmaceutical impactor (NGI) (MSP Corporation, US-Shoreview, MA) in combination with a Critical Flow Controller TPK and a High Capacity Pump HCP 5 (Copley Scientifitc Ltd., UK-Colwick Nottingham).

To investigate the aerodynamic performance of samples at varying moisture contents and ambient temperature in nitrogen, a climate chamber was used. The chamber was supplied with dry and moisture saturated N_2 gas flow which were controlled individually to generate a specific moisture by mixture inside the chamber (Figure 5-1). The deposition pattern of powders was assessed while they were exposed to relative humidity levels of 33±2 %, 55±2 % and 77±2 % at a temperature of 23±1°C.

Capsules were filled with 0.2 mL of lyophilized powder and contained between 0.5 and 1.5 mg, depending on the powder's bulk density. The gas flow was set to 47±0.6 L/min to obtain a pressure drop of 4 kPa and the timer was set to 5.0 s.

A Handihaler® (Boehringer-Ingelheim GmbH & Co. KG, D-Ingelheim) was mounted inside the climate chamber for powder delivery into the NGI.

Powder deposited on the stages was dissolved in water and the fluoresceine was assayed using a PlateReader 1420 Multilabel Counter VICTOR³V® (Perkin Elmer, US-Waltham, MA). The $x_{50.M}$ cutoff values of the stages at 48 L/min are 9.1 µm, 5.0 µm, 3.2 µm, 1.8µm, 1.0 µm, 0.6 µm and 0.4 µm respectively. The FPF is defined as the amount of particles in per cent that have an aerodynamic diameter smaller than 5 µm. The emitted fraction (EF) is the sum of all powder minus the residue inside the capsules and the inhaler. Samples which had FPFs > 20 % at 33 % rH were used for NGI experiments at 55 % rH and 77 % rH and 23±1 °C.

5.4. RESULTS

The use of different excipients in spray freeze drying using a jet-vortex freezer led to lyophilized powders with individual physical, chemical and morphological characteristics, which are described in detail in earlier work (Chapter 4). For the sake of comparison, the median geometric particle diameters (x_{50}) and the apparent particle densities assessed in a earlier study (Chapter 4) are given in Table 5-2.

The unprocessed excipients had a clear rank order of water uptake profiles. Mannitol and lactose showed a low increase in mass. HPMC and DEX had intermediate water uptake and both types of PVP showed the highest water uptake (Figure 5-2). These findings correspond with product information supplied by the manufacturers.

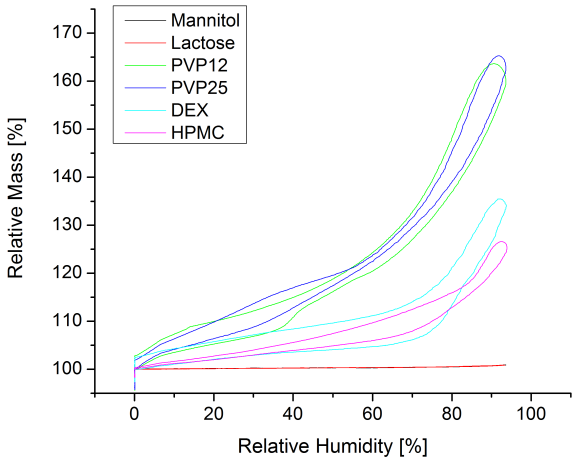

Figure 5-2: Water sorption of unprocessed excipients

Lyophilized mannitol powders showed a low water uptake of only 6 % of their initial mass during the test cycle. HPMC and DEX showed an increase in mass of approximately 25 %. The fastest and most pronounced water sorption was observed for PVP12 and PVP25, where the water uptake rises to 60-70 % of the initial mass (Figure 5-3). Mixtures of 50 % mannitol and 50 % polymeric constituents showed a decreased rate in water sorption as well as lower maximum values (Figure 3c-e) compared to the pure excipients.

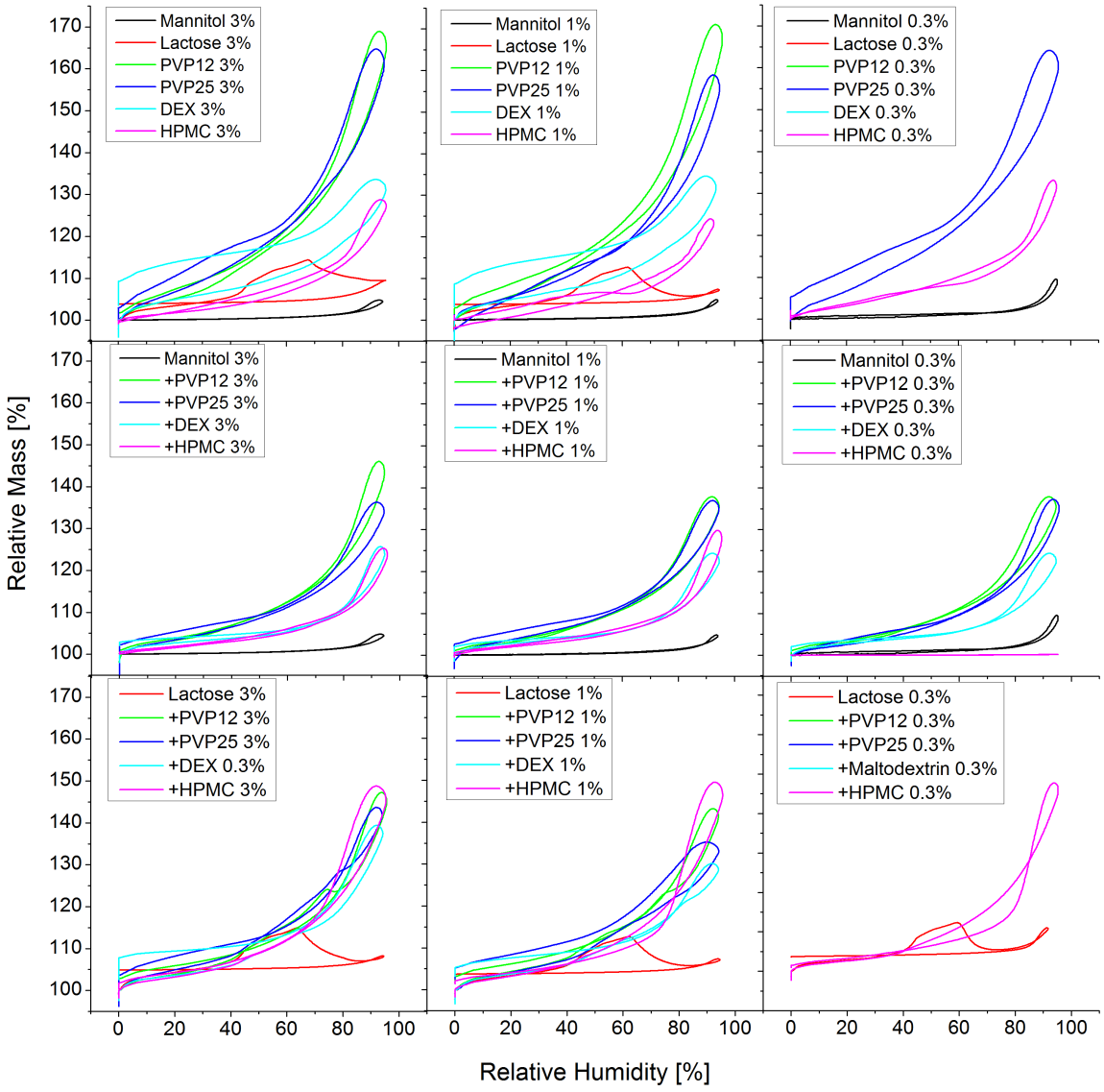

Figure 5-3: Water sorption and desorption profiles of spray-freeze dried pure excipients and 1:1 blends of mannitol or lactose with polymeric additives

For powders originating from spray solutions with lower solid contents almost no difference in the absolute water uptake or the water uptake rate was observed.

Lactose showed a peculiar water sorption profile, which has also been described by Burnett et. al (2004). After an initial increase in mass at 40 % rH, the maximum uptake was observed at around 65 % rH. The sample then decreased in mass up to 85 % rH (Figure 5-3a and 5-4b).

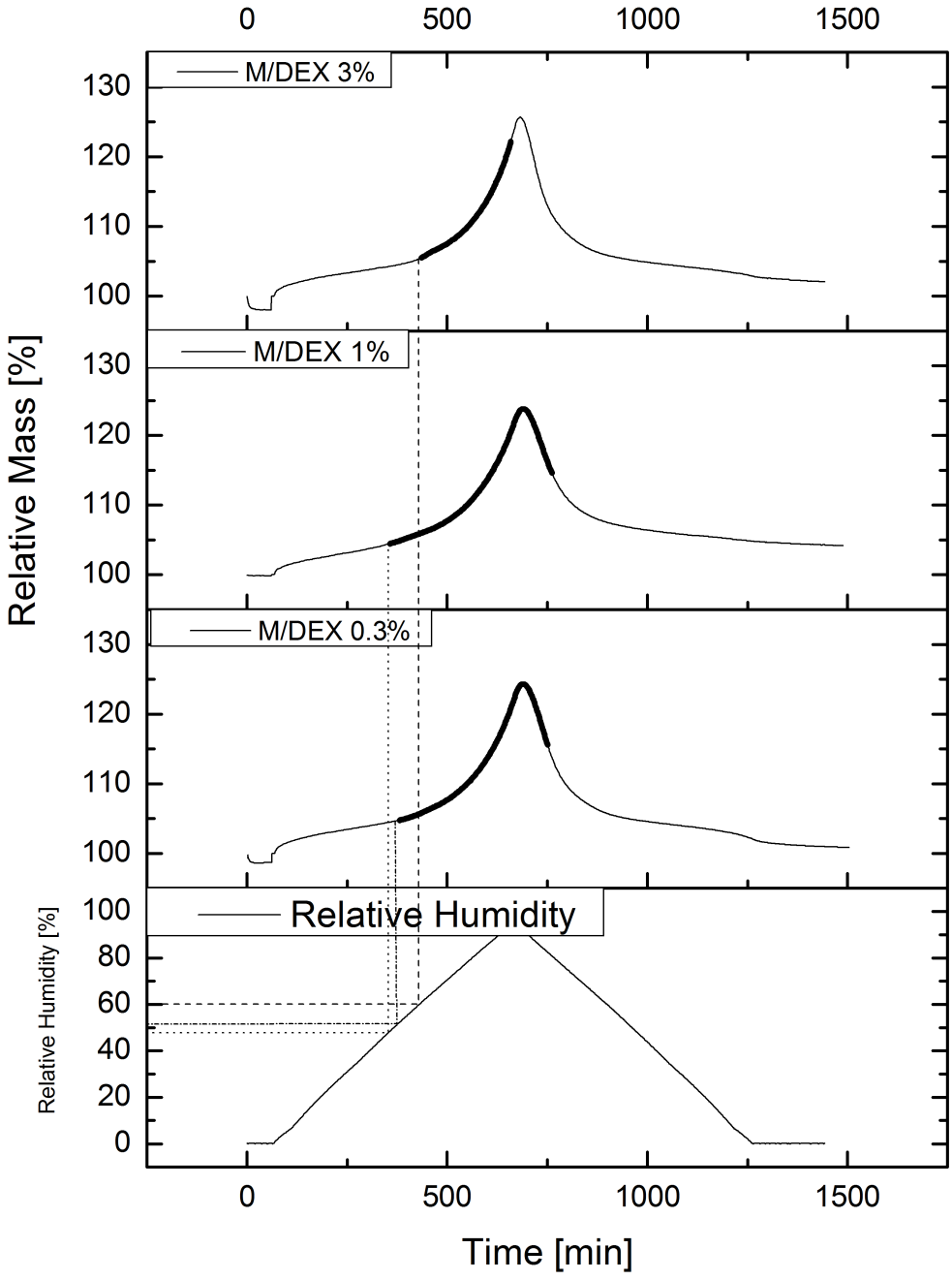

Figure 5-4a: Irreversible collapse of structure of a mannitol/maltodextrin formulation with different solid contents. The bold line indicates time-span of collapse.

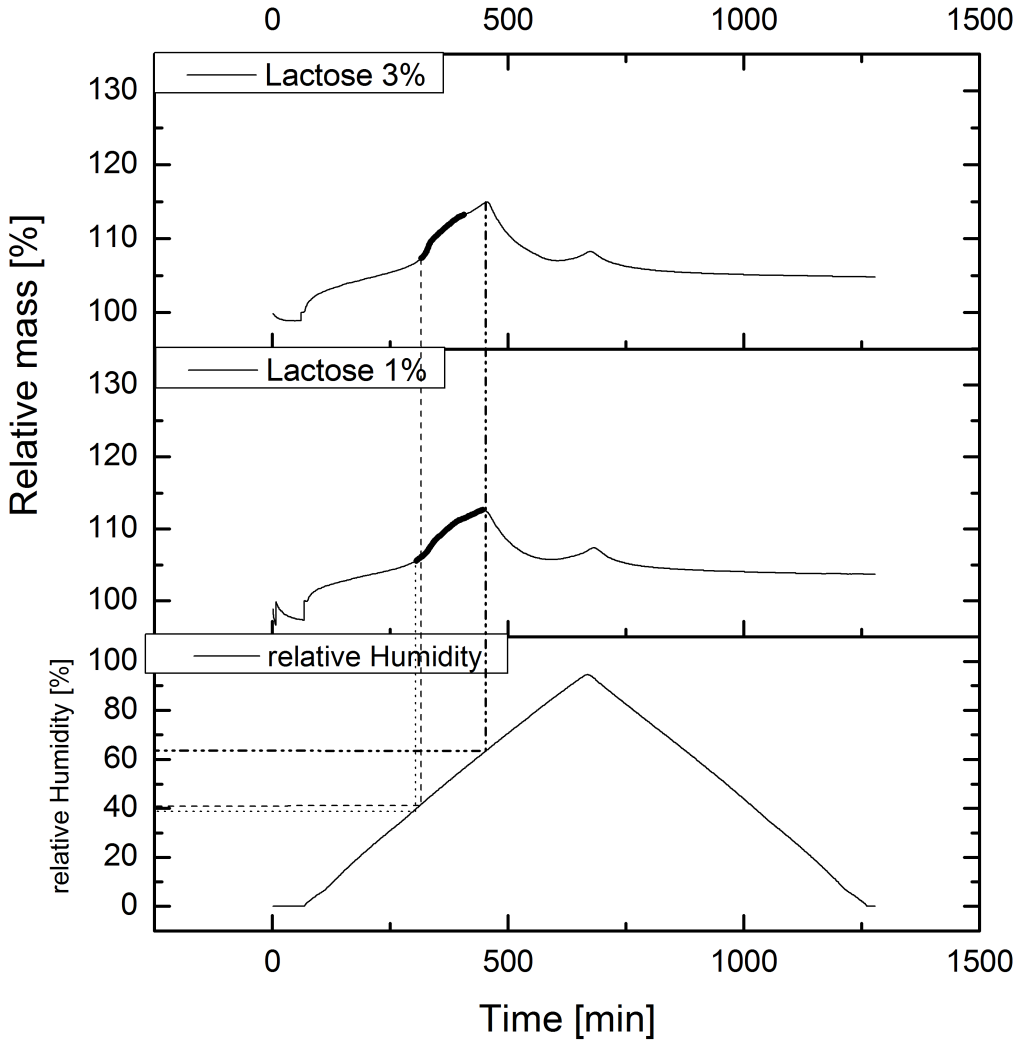

Figure 4b: Peculiar moisture uptake profile of spray lyophilized lactose powders showing the moisture induced phase transition described by Burnett et al., 2004. The bold line indicates time-span of collapse.

Reduction in bulk volume or loss in structural integrity respectively was observed for each formulation at individual relative humidity values. The collapse humidity values are listed in Table 5-2.

Table 5-2: Mean particle size, apparent particle density and collapse humidity of different formulations

Mono / Dimer	Oligo / Polymer	x50 (Dry Particles) * [µm]			Apparent Particle Density * [g/cm³]			Collapse Humidty [%]		
Solid Content (m/V)		3%	1%	0.3%	3%	1%	0.3%	3%	1%	0.3%
M		55,8	55,6	32,1	0,025	0,009	0,013	88	44	49
L		56,3	39,4	-	0,026	0,020	-	41	39	37
	PVP12	39,7	35,1	-	0,074	0,034	-	37	31	-
	PVP25	64,3	57,8	38,8	0,030	0,009	0,007	53	38	55
	DEX	65,1	65,1	24,3	0,028	0,011	0,031	57	56	-
	HPMC	81,6	64,5	37,8	0,047	0,023	0,011	53	54	53
M	PVP12	53,2	35,7	21,0	0,031	0,036	0,048	42	40	48
	PVP25	57,8	51,9	32,2	0,030	0,013	0,013	48	50	40
	DEX	56,6	57,0	24,3	0,028	0,009	0,011	60	53	51
	HPMC	73,4	62,7	37,8	0,035	0,017	0,012	52	55	53
L	PVP12	46,6	23,9	20,0	0,037	0,115	0,036	43	37	37
	PVP25	61,2	32,7	35,5	0,027	0,066	0,013	38	46	-
	DEX	58,5	37,8	20,0	0,024	0,029	0,087	47	44	-
	HPMC	81,1	69,5	34,9	0,035	0,012	0,018	46	44	43

* Values as assessed in Chapter 4

The collapse led to a drastic decrease in volume and completely changed the bulk appearance of the powder (Figure 5-5, supplementary Video V1). The collapse humidity decreased with decreasing solid content and higher porosity of the powder as shown in Figure 5-4a, but formulations with 0.3 % solid content did not collapse at lower relative humidity levels.

Powders originating from a 3 % mannitol spray solution were stable up to 88 % rH. In general, collapse humidity values for lactose-based formulations were lower than for mannitol-based formulations (Table 5-2).

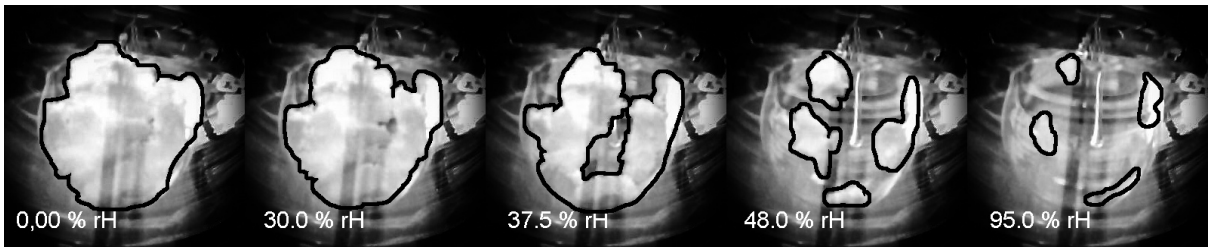

Figure 5-5: Structural collapse upon increasing relative humidty of PVP25 spherolyophilisates with 3 % solid content. data.

The aerodynamic performance was strongly influenced by the relative humidity in the climate chamber. At 33 % rH, all powders showed high emitted fractions of over 95 % and FPFs

between 15 % and 58 %. At 55 % rH, the EF of Lactose 3% and 1% dropped down to 57.7±3.8 % and 79.4±5.2 and the FPFs to 0.3±0.4 % and 0.8±0.0 % respectively. All other tested formulations had EFs >90% but showed a decrease in FPF between 3 - 35 %. For very humid atmospheres (77 % rH) the FPF of all samples was further reduced (Table 5-3). The EF of all samples containing lactose was < 65 % (Table 5-2). Figure 5-6a and b show that with increasing humidity, the powder residue is shifted to the preseparator and the amount of powder remaining in the capsules and inhaler increases. It can be seen that the emitted fraction of the lactose based formulation is decreased drastically at a relative humidity >55 %.

Table 5-3: Emitted fractions and fine particle fractions at different levels of humidity (23 °C) (Mean±SD, n=3)

	FPF			EF		
Sample	**33% rH**	**55% rH**	**77% rH**	**33% rH**	**55% rH**	**77% rH**
M 3%	15.5±0.5	10.6±1.0	10.6±1.2	98.4±0.9	94.2±2.5	96.1±1.2
M 1%	43.6±1.4	26.2±0.5	18.4±0.4	98.2±0.0	98.4±0.4	88.7±1.0
L 3%	17.8±0.1	0.3±0.4	0.8±0.2	98.4±0.0	57.7±3.8	8.9±2.4
L 1%	31.1±1.1	0.8±0.2	1.2±0.0	99.1±0.2	79.4±5.2	60.4±4.6
M/PVP12 3%	19.1±2.9	18.5±1.4	3.6±1.3	98.2±0.0	91.1±5.4	60.7±1.8
M/PVP12 1%	22.1±0.6	17.9±1.8	1.6±0.5	96.8±2.2	92.3±7.2	16.0±6.2
M/DEX 3%	21.5±0.4	14.7±1.5	0.5±0.0	97.2±0.7	98.4±0.4	92.4±3.8
M/DEX 1%	53.1±3.0	49.6±5.1	23.6±3.5	98.8±0.0	97.8±0.1	92.6±6.6
M/DEX 0.3%	58.9±5.5	38.2±5.4	27.8±1.3	99.5±0.0	98.6±0.3	98.3±0.2
L/DEX 3%	19.6±0.3	13.5±1.3	3.0±0.4	96.6±0.8	97.1±0.3	62.1±10.1
L/DEX 1%	38.9±0.7	16.1±1.2	3.9±3.1	95.9±0.3	98.1±0.3	49.2±39.2

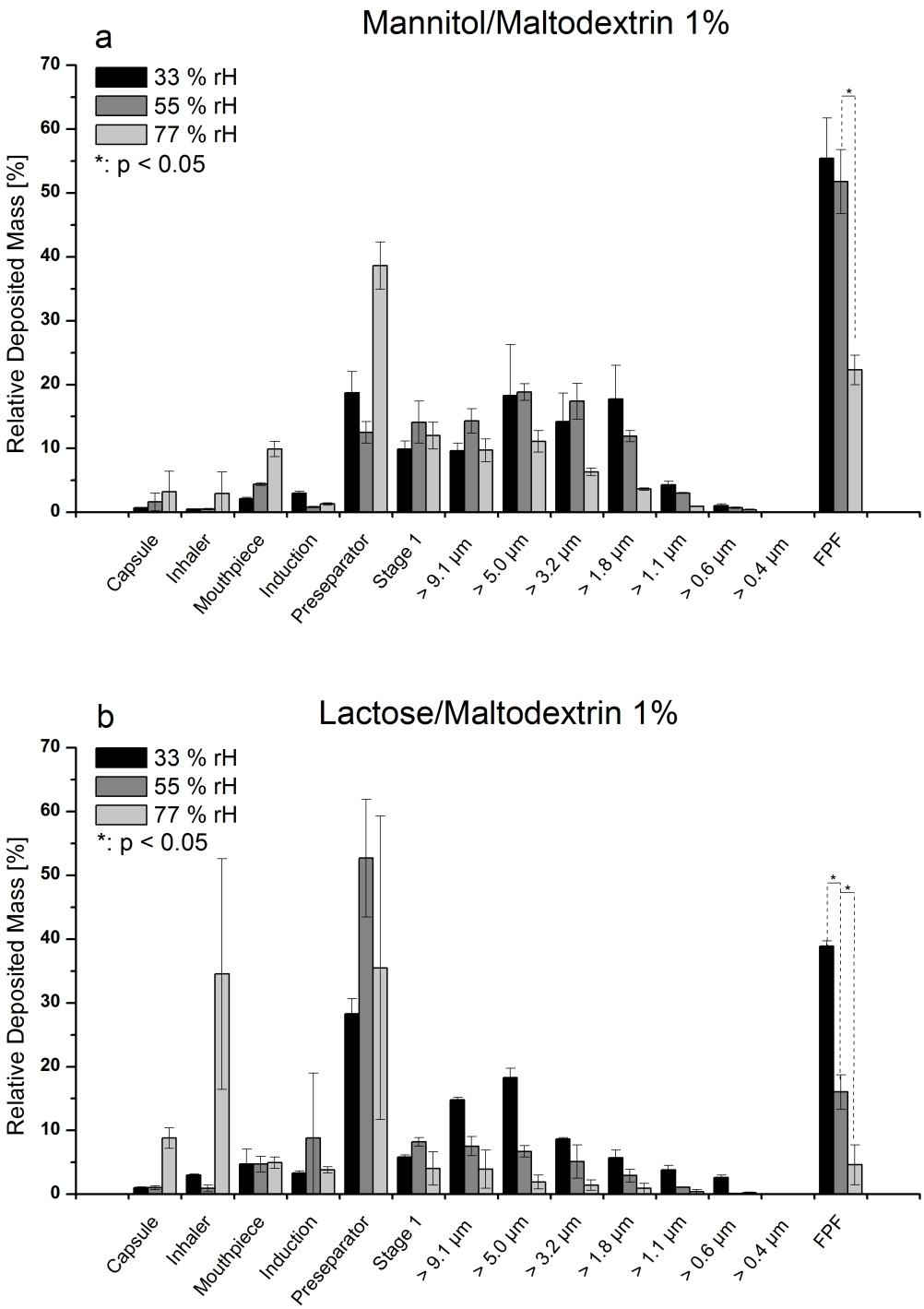

Figure 5-6: NGI deposition pattern for M+DEX 1% **(a)** and L+DEX 1% **(b)** formulations at different relative humidity levels.

5.5. DISCUSSION

Creating flowable lyophilized low density particles with relatively large particle sizes, good dispersibility in air and sufficient aerodynamic performance for inhalation is a challenging task. The production parameters have to be controlled closely (Wanning et al., 2017), the excipients have an impact on the morphological appearance, the mechanical stability and agglomeration tendency of the resulting spherolyophilisates (Chapter 4), and therefore also on the sensitivity of the powder to moisture. The microscopic droplets are frozen instantaneously and the solid constituents are immobilized quickly, which leads to a highly porous lamellar and honeycomb-like structure of the spheres after drying. It was shown recently that the solid contents of the spray solution influence the thickness of the lamellas and the pore-size (McCartney, 2014). Formulations of the same composition but with variable content of solids, different porosities and therefore apparent particle densities, showed no difference in the water uptake rate and maximum uptake. Due to the extreme porosity, differences in surface area are probably insignificantly small, which explains the similar moisture sorption and desorption profiles for samples with identical excipients but different solid contents. This suggests, besides effects by particle shrinking (Chapter 4) and given a sufficient porous structure, that the moisture uptake solely depends on the formulation composition.

The hypothesis is also supported by the collapse humidity values which show that particles with a lower apparent density collapse earlier than denser particles because the interior structure is weaker and therefore dissolves faster. Particles that already collapsed during drying (e.g. all samples with SC 0.3 % (Wanning et al., 2017b)) had higher apparent particle densities and therefore had higher collapse humidity values.

Hygroscopicity of particles for inhalation has been studied intensively (Hickey and Martonen, 1993; Martonen et al., 1982; Martonen and Zhang, 1993) in the past, as the bulk powder properties and the aerodynamic behavior of the particles may change. Most inhalable dosage forms are interactive blends, which consist of inhalable API particles (1-5 µm) adhered to larger carrier particles (60-90 µm) to guarantee sufficient flow properties (Berkenfeld et al., 2015). With increasing humidity, the particle adhesion forces are increased due to a change in in surface topology and an increase of capillary forces (Berard et al., 2002) and pulmonary

delivery of interactive blends may be affected negatively because API particles are not separated from their carrier particles. Spherolyophilisates are also affected by increased relative humidity in the atmosphere. Because of the moisture induced structural collapse, the large diameter and low-density characteristics of the particles, which are essential for the dispersibility in air, are lost. This means that the particles have to be stored and applied in a dry atmosphere.

If the dispersing gas is dry and particles are aerosolized and inhaled successfully, they are exposed to the high humidity in the bronchial tree. Small and dense API particles are then subject to particle growth by water sorption, which increases the aerodynamic diameter (Martonen et al., 1982). In contrast, the highly porous lyophilisate particles lose their supporting interior structure, collapse and therefore shrink. Thus, the particle density increases drastically and it can easily be calculated from the relationship between aerodynamic diameter, geometric diameter and particle density (Lin et al., 2015) that the aerodynamic performance would be affected negatively, if the particle collapse occurs before they reach the deep lung.

However, if the particles collapse after they have reached their target area, this effect can be beneficial, as the particle cannot be exhaled. This study primarily focused on the storage stability of inhalable lyophilized spheres and the influence of the ambient atmosphere upon powder application. Further studies concerning the water uptake in rapidly changing atmospheres, which might simulate the *in-vivo* conditions, are to be carried out.

5.6. CONCLUSION

This study showed that using a dry dispersing gas is crucial when applying pulmonary spherolyophilisates as it has a major impact on the structural integrity of the highly porous powder, its dispersibility characteristics in air and its aerodynamic performance. It was shown that the composition of formulations affects the magnitude of this effect and of the formulations tested here, mannitol or blends of mannitol and maltodextrin were preferable. As the relative humidity in the atmosphere can hardly be controlled, the use of non-hygroscopic formulations is crucial. Nonetheless, a breathing cycle is completed within a few seconds and the DVS cannot simulate such fast changes in relative moisture so these assumptions have to be treated carefully.

Spherolyophilisate powders have a high potential for the pulmonary delivery of a broad range of active pharmaceuticals. The relative humidity of the atmosphere has a significant impact on the structural integrity and the aerodynamic performance of the lyophilized powders which has to be considered when developing formulation for pulmonary application and designated inhaler devices.

5.7. ACKNOWLEDGMENTS

The authors are grateful for the financial support of the “Deutsche Forschungsgemeinschaft” (DFG) in the framework of SPP1423 (grant no. LA1362/2-1) and would also like to thank Ilona Janzen, Kai Berkenfeld, Franz-Josef Willems, and Jürgen Hohmann for practical and technical assistance.

5.8. REFERENCES

Berard, V., Lesniewska, E., Andres, C., Pertuy, D., Laroche, C., Pourcelot, Y., 2002. Dry powder inhaler: influence of humidity on topology and adhesion studied by AFM. Int. J. Pharm. 232, 213–224.

Berkenfeld, K., Lamprecht, A., McConville, J.T., 2015. Devices for Dry Powder Drug Delivery to the Lung. AAPS PharmSciTech 16, 479–490.

Burnett, D.J., Thielmann, F., Booth, J., 2004. Determining the critical relative humidity for moisture-induced phase transitions. Int. J. Pharm. 287, 123–133.

Hickey, A.J. (Ed.), 1996. Inhalation Aerosols: Physical and Biological Basis for Therapy. CRC Press, Hillsdale, N.J.

Hickey, A.J., Martonen, T.B., 1993. Behvaior of Hygroscopic Pharmaceutical Aerosols and the Influence of Hydrophobic Additives. Pharm Res 10.

Laube, B.L., Janssens, H.M., de Jongh, F.H.C., Devadason, S.G., Dhand, R., Diot, P., Everard, M.L., Horvath, I., Navalesi, P., Voshaar, T., Chrystyn, H., 2011. What the pulmonary specialist should know about the new inhalation therapies. Eur. Respir. J. 37, 1308–1417.

Lin, Y.-W., Wong, J., Qu, L., Chan, H.-K., Zhou, Q.T., 2015. Powder Production and Particle Engineering for Dry Powder Inhaler Formulations. Curr. Pharm. Des. 21, 3902–3916.

Martonen, T.B., Bell, K.A., Phalen, R.F., Wilson, A.F., Ho, A., 1982. Growth rate measurements and deposition modelling of hygroscopic aerosols in human tracheobronchial models. Ann. Occup. Hyg. 26, 93–108.

Martonen, T.B., Zhang, Z., 1993. Deposition of Sulfate Acid Aerosols in the Developing Human Lung. Inhal. Toxicol. 5, 165–187.

McCartney, S., 2014. Mechanical characterization of freeze-dried biopharmaceuticals. Imperial College London.

Son, Y.-J., Longest, P.W., Hindle, M., 2013. Aerosolization Characteristics of Dry Powder Inhaler Formulations for the Excipient Enhanced Growth (EEG) Application: Effect of Spray Drying Process Conditions on Aerosol Performance. Int. J. Pharm. 443, 137–145.

Wanning, S., Süverkrüp, R., Lamprecht, A., 2017. Jet-vortex spray freeze drying for the production of inhalable lyophilisate powders. Eu J Pharm Sci 96, 1–7.

Wanning, S., Süverkrüp, R., Lamprecht, A., 2015. Pharmaceutical spray freeze drying. Int. J. Pharm. 488, 136–153.

ATMOSPHERIC SUBLIMATION DRYING OF POWDERS PREPARED BY SPRAY-FREEZING IN A JET-VORTEX

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6.1. ABSTRACT

The majority of all spray-freeze-drying processes is based on batch-wise vacuum sublimation drying routines. In this study, a jet-vortex spray-freezer was modified to enable atmospheric sublimation drying by a percolating gas flow to generate highly porous and spherical lyophilisate particles, suitable for multiple purposes such as nasal or pulmonary drug delivery. Twelve mL of a 3 % (m/V) mannitol and maltodextrin (ratio 1:1) spray solution were spray-frozen at -100±1 °C and collected on a stainless steel sieve with a mesh width of 25 µm. The ice powders were dried atmospherically at -20, -15 and -10 °C respectively or under vacuum in a batch freeze-drier. Because the ice spherules were deposited unequally on the sieve, drying times were between 27.5 and 98.5 hours and the resulting powder showed morphological and physicochemical features which were comparable to the vacuum freeze dried control. With further improvements, atmospheric sublimation drying is a suitable technique for the generation of low density lyophilized spherules.

Keywords: Spray freeze drying, lyophilization, porous particles, pulmonary application, atmospheric freeze-drying, sublimation drying

6.2. INTRODUCTION

Spray-freeze-drying (SFD) is a family of processes for the generation of particulate lyophilized powders which are being studied for the application of small molecule or protein/peptide based drugs and vaccines via the nasal route (Garmise et al., 2006; Jiang et al., 2006), the lung (Ali and Lamprecht, 2014; Bi et al., 2008; Maa et al., 1999; Wanning et al., 2017), for needle-free intradermal injection (Rochelle and Lee, 2007; Schiffter et al., 2010) or for technological reasons (*e.g.* solubility enhancement of poorly water-soluble drugs (Niwa et al., 2012; Rogers, 2003)). A spray solution is first atomized into small droplets which are then frozen in a cold atmosphere or upon contact with a cryogenic phase. The solvent is then removed by sublimation drying under vacuum or atmospheric pressure (Wanning et al., 2015).

The lung has been discussed extensively as an alternative route of application for protein and peptide active pharmaceutical ingredients (API) and with Exubera® and Afrezza® two formulations were approved by the FDA and of which the latter is still available on the market (Bi et al., 2008; Patton, 2004; Patton et al., 1999). For delivering particles to the deep lung, an aerodynamic diameter of 1-5 µm is obligatory (Davies et al., 1976; Lin et al., 2015). Powders for inhalation are characterized by their fine particle fraction which represents the amount of powder in percent with the previously mentioned requirements

Initially monodisperse droplet-streams have previously been successfully processed in a jet-vortex freezing apparatus for the production of inhalable porous and spherical lyophilized particles (Wanning et al., 2017). It has been shown that the formulation has major effects on the physicochemical properties and the aerodynamic performance of the spherolyophilisates. Optimized compositions yielded powders with fine particle fractions (FPF) of up to 55 % (Chapter 4). Some formulations showed a structural collapse during sublimation drying which is hypothesized to be due to an insufficiently controllable temperature profile of the presently used laboratory vacuum freeze-dryer.

An alternative to vacuum sublimation drying (VSD) is the removal of water at low temperatures and atmospheric pressure which was initiated by Meryman in 1959 (Meryman, 1963, 1959). Atmospheric freeze drying has been implemented both for food (Malecki et al.,

1970) and pharmaceutical products (Leuenberger, 2001; Mumenthaler and Leuenberger, 1991) and has proven to be more economical regarding investment and operating costs.

Fluidized bed freeze drying has been successfully tested for large and denser particles (d_{geo} > 300 µm, V > 14.1 nL, porosity < 90 % (Leuenberger et al., 2006)). Lyophilized particles for inhalation prepared by jet-vortex spray freeze drying are 6 to 20 times smaller by volume (d_{geo} = 55 µm, V=0.037 nL), originate from spray solutions with solid contents < 3 %, have a very low apparent particle density (0.01 g/mL) and are therefore easily dispersed in air, become readily airborne which leads to a problematic handling in atmospheric currents. Furthermore, Leuenberger et al. found that spray solutions with solid contents <5 % could not be dried because they were crushed by the mechanical stress in the fluidized bed. An alternative atmospheric drying method was developed by Wang and Finlay (Wang Z and Finlay, 2008) who collected the particles on a filter at the bottom of a spray-freezing tube and dried them in a downstream of cold gas. Both the temperature and the flow of the percolating gas can be controlled precisely and spectroscopic PAT methods are available for measuring the residual moisture of the product (De Beer et al., 2011). Alternatively, the moisture content of the exhaust can be monitored and used to assess the progress of drying (Robinson, 2015).

Minor changes of the previously introduced jet-vortex spray-freeze-drying apparatus allowed the implementation of a percolation drying technique in the production routine for inhalable spherolyophilisates. A droplet-stream generator was fed with a model spray solution and the frozen droplets were collected on a stainless steel sieve with a mesh width of 25 µm. The drying temperature has to be chosen carefully. The vapor pressure of ice decreases drastically with decreasing temperature (Wexler, 1977). The temperature has to be chosen high enough to maximize the drying rate but low enough to prevent a particle collapse or even a melt-back.

The aim of this work was to find drying parameter settings for a sufficiently fast drying rate while not compromising the porous particle structure. The ice spherules were dried at varying temperatures between -5 and -20 °C and the water content in the exhaust gas was monitored for end-point determination. The morphology and size distribution of freeze-dried particles were assessed by scanning electron microscopic imaging.

6.3. MATERIALS AND METHODS

6.3.1 MODEL SOLUTION

1.5 g of D(−)-mannitol [Ph. Eur.] (VWR International, NL-Amsterdam) and 1.5 g maltodextrin [Roquette LAB 2509, dextrose equivalent of DE = 19] (Roquette Frères, F-Lestrem) were dissolved in 100 ml ultrapure water (MilliQ, Millipore Corp., US-Billerica, MA) and filtered through a 0.22 µm aseptic filter. The model solution was degassed by sonication shortly before spraying.

6.3.2 SPRAY FREEZING

The spray solution was fed to a Type MTG – 01.G1 droplet stream generator (DSG) with a 20 µm stainless steel diaphragm (FMP Technologies GmbH, D-Erlangen). The feed pressure was 250 kPa and the excitation frequency ranged between 42 and 48 kHz. Droplet formation was checked visually by stroboscopic microscopy before spray-freezing.

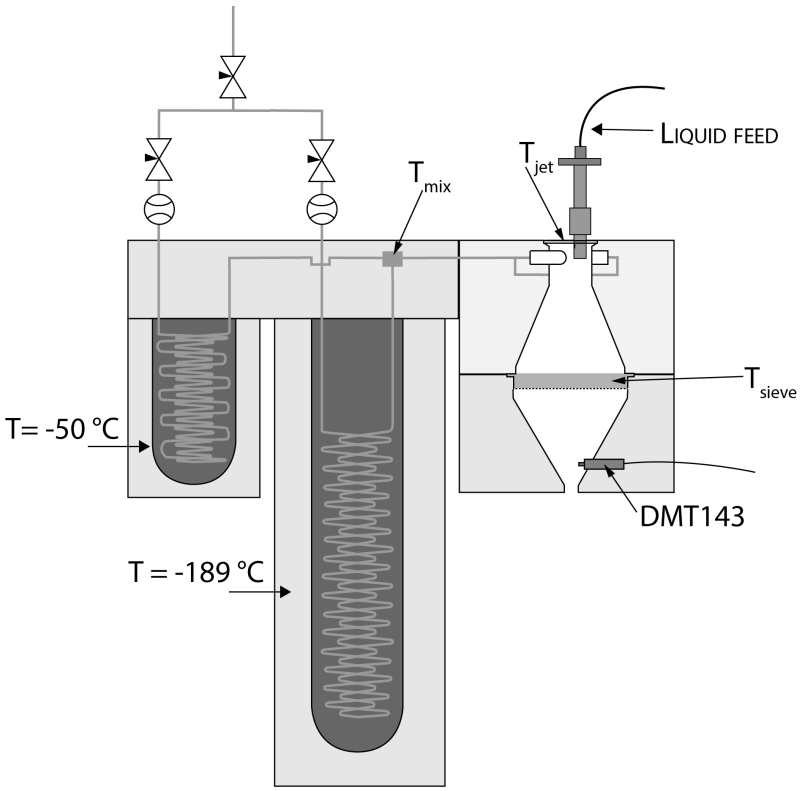

Figure 6-1: Jet-vortex spray-freezer device modified for atmospheric sublimation drying

The droplet-stream was then frozen in a cold gas vortex at -100±1 °C with optimized spray-freezing settings in a modified jet-vortex-freezer, where an exit cone was added in which the moisture of the exhaust gas was measured (Wanning et al., 2017) (Figure 6-1). The operating temperatures in the mixing chamber, the jet vortex, the collecting sieve were monitored and recorded with a 4 channel temperature logger (T390, PCE-Instruments, D-Meschede). 12 mL of spray solution was dispersed within 90 minutes and the frozen droplets were collected on a fine-meshed (mesh width: 25 µm) stainless steel sieve.

6.3.3 ATMOSPHERIC SUBLIMATION DRYING (ASD)

The spraying process was stopped and the cold gas from the vortex percolated through the product on the sieve. The gas flow through the -189 °C heat exchanger (see Figure 6-1) was stopped and the complete gas flow passed through heat exchanger submerged in the cooling bath of a cryostat (CT50W, Thermo Haake GmbH, D-Karlsruhe). The powder was dried at a flow rate of 45 L/min and at temperatures of -15 °C, -10 °C and -5 °C respectively (Figure 6-2). The water content in the exhaust air was measured and recorded with a 'DMT-143' dew point transmitter (Vaisala, FIN-Vantaa) with a measuring interval of 2 min. Drying was stopped, when the water content in the exhaust gas was < 30 ppm or after 100 h.

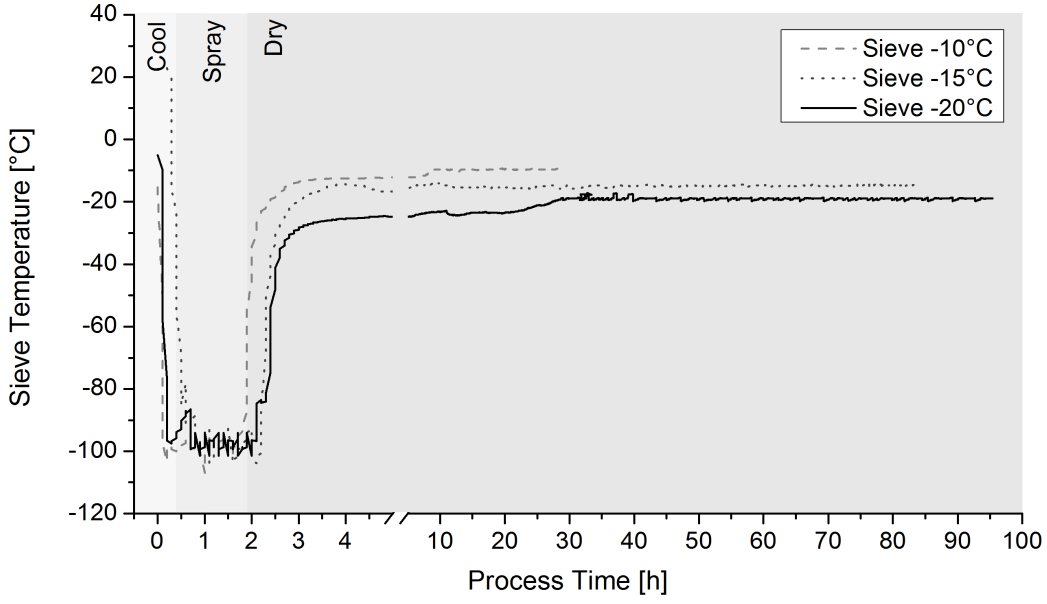

Figure 6-2: ASD temperature profiles

6.3.4 VACUUM SUBLIMATION DRYING (VSD)

For the vacuum sublimation drying, the particles were transferred into an Alpha 1-4 LSCplus batch freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, D-Osterode am Harz) and dried for 24 h at 0.01 mbar. The condenser temperature was -55 °C and the plate temperature profile is shown in Figure 6-3.

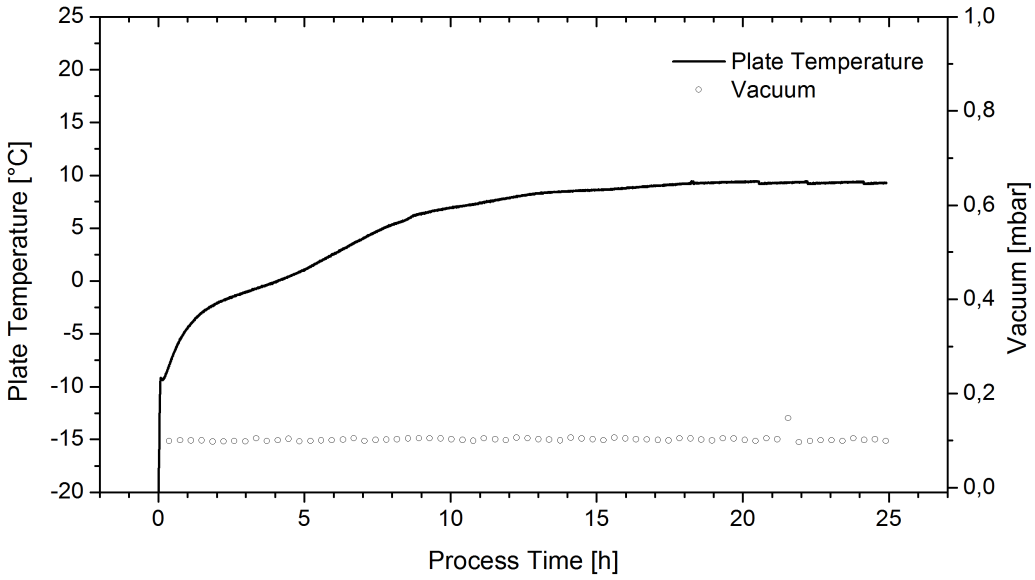

Figure 6-3: VSD plate temperature and pressure profile

6.3.5 POWDER CHARACTERIZATION

6.3.5.1 Particle Morphology and Geometric Particle Size

Samples were sputter coated (Polaron SC7640, Quorum Technologies Ltd., UK-Newhaven) and imaged using a S-2460N scanning electron microscope (Hitachi High Tech. Corp., JP-Tokyo). The geometric particle size distribution was assessed by evaluating the images with the NIH open source software Image J 1.43 and the multi measure plugin as described in detail earlier (Chapter 4). The volumetric x_{10}, x_{50}, x_{90} and the span were calculated from between 200-500 measured particles per sample.

6.3.5.2 Loss on Drying

Loss on drying was measured with a thermo-gravimetric scale (TGA 7 with TAC7/DX Controller, Perkin Elmer, US-Waltham). Approximately 1±0.1 mg of lyophilized powder was dried to constant weight ($\Delta m_{rel} < 0.05$ %/min) at 70 °C. The loss on drying was calculated in per cent of the initial mass.

6.4. RESULTS

Vacuum dried ice spherules of a mannitol and maltodextrin solution (1:1, 3 % m/V) resulted in low density, spherical particles with a porous surface and lamellar interior structure and a median particle size of 55 ± 4 µm.

Atmospheric sublimation (ASD) drying of ice spherules of the same solution at -20 °C and -15 °C yielded spherical (Figure 6-4) and median particle sizes of around 48 ± 3 µm. The surface was more closed and seemed slightly collapsed. This minimal collapse is also reflected in the median, which is slightly smaller than in vacuum freeze drying. Ice spherules which were dried at -10 °C did not result in distinct particles but in a collapsed structure with a closed surface which originated from multiple spherules (Figure 6-4).

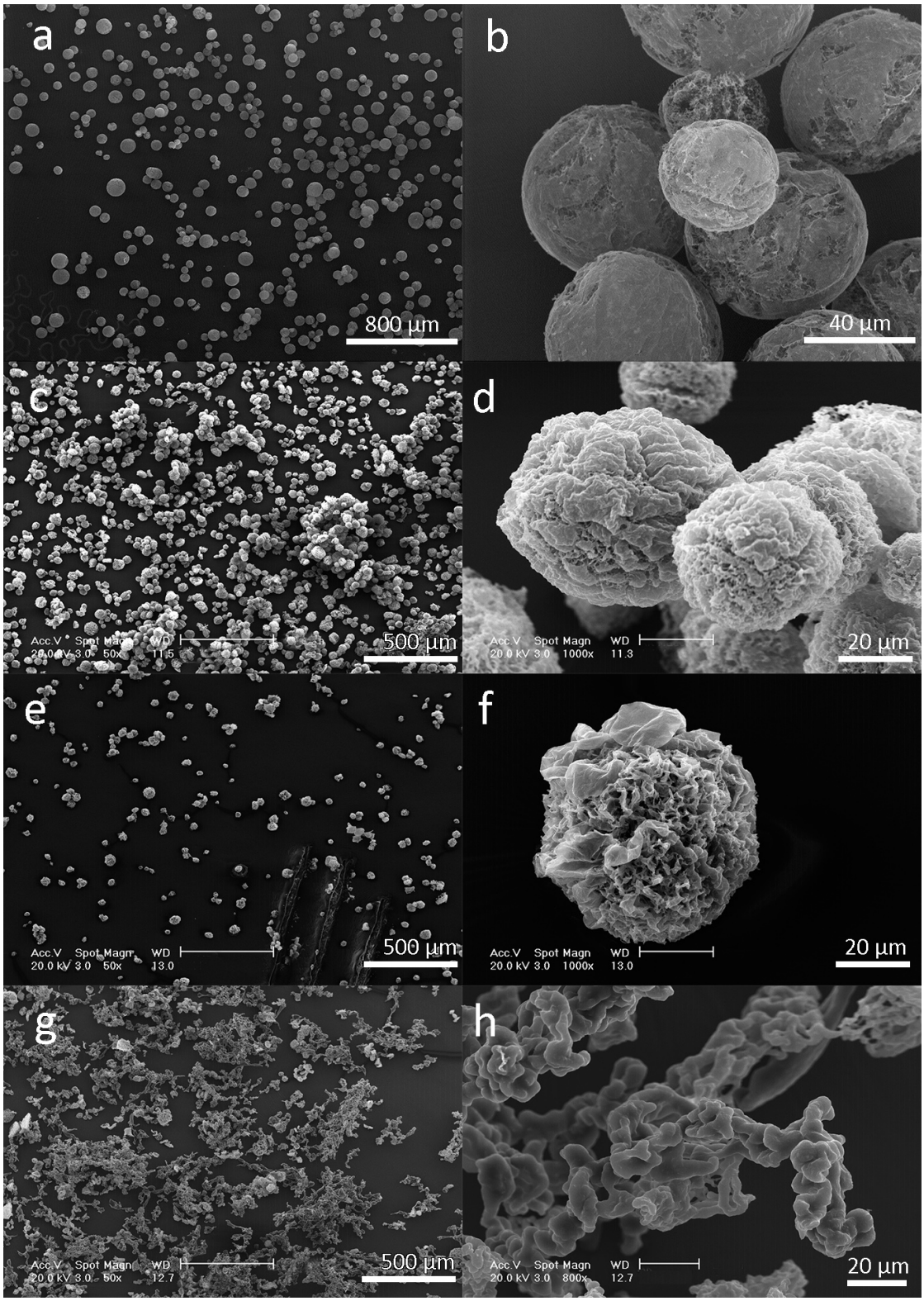

Figure 6-4: SEM images of VSD particles (a,b) in comparison to ASD particles at -20 °C (c,d), -15 °C (e,f) and – 10 °C (g,h).

Within the first 4 hours, the water content in the exhaust air increased (Figure 6-5), as the temperature in the swirl tube rose from the freezing level (-100 °C) to the drying temperature (-100 °C) to drying temperature (-20, -15, and -10 °C respectively). After the new thermal equilibrium was established, the water content in the exhaust air decreased gradually, as the sublimation surface shrunk and the diffusion resistance grew with the thickness of the porous layer. At -10 °C, the endpoint was reached after 27.5 h and at -15 °C after 82.5 h. At -20 °C drying temperature, the drying process was extremely slow and the water content was still above 200 ppm after 94.5 h (Figure 6-5). Robinson et al. showed that 50 mL of a spray solution spray-freeze dried with a two-fluid nozzle can be sublimation dried within 8 hours.

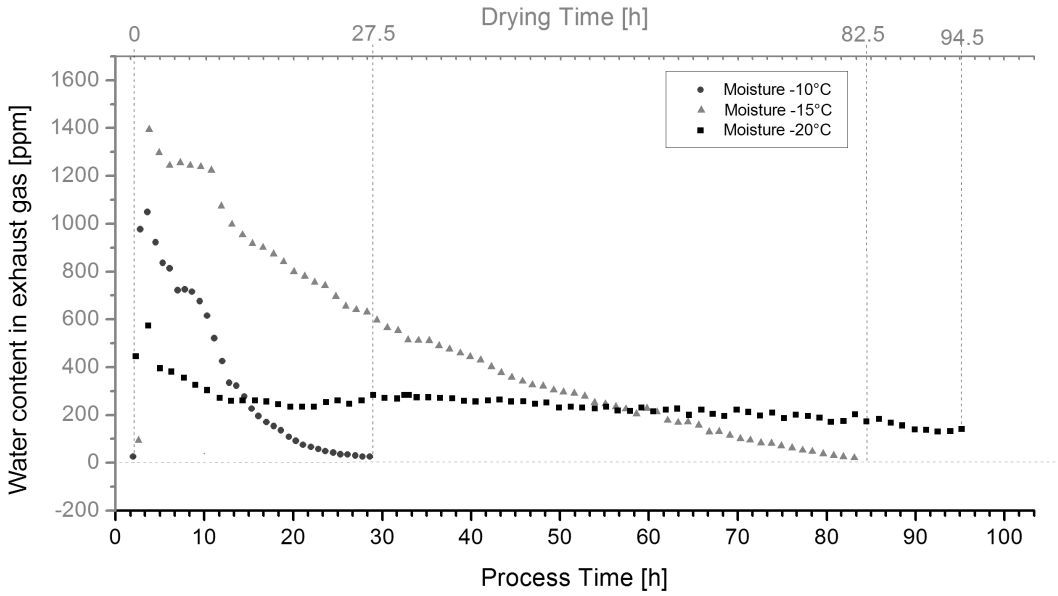

Figure 6-5: Water content in exhaust gas profile of 13 mL frozen spray solution at different drying temperatures

It was observed that after drying the powder was mostly located at the outer edge of the collecting sieve and that a ring shaped deposition pattern had developed in which the layer thickness decreased from the outside to the center of the sieve (Figure 6-6). As the drying process at -20 °C was aborted after 94.5 h, a large quantity of the particles on the outer edge were undried and still in a frozen state which drastically decreased the yield of dry particles (Table 6-1).

ASD samples dried at -15 and -20 °C had a loss on drying lower than 3.4 %, which is not significantly different from VSD samples. Particles dried at -10 °C had a significantly higher LOD of 5.8 % (Table 6-1), as the unporous and collapsed structure prevented a complete drying of the particles.

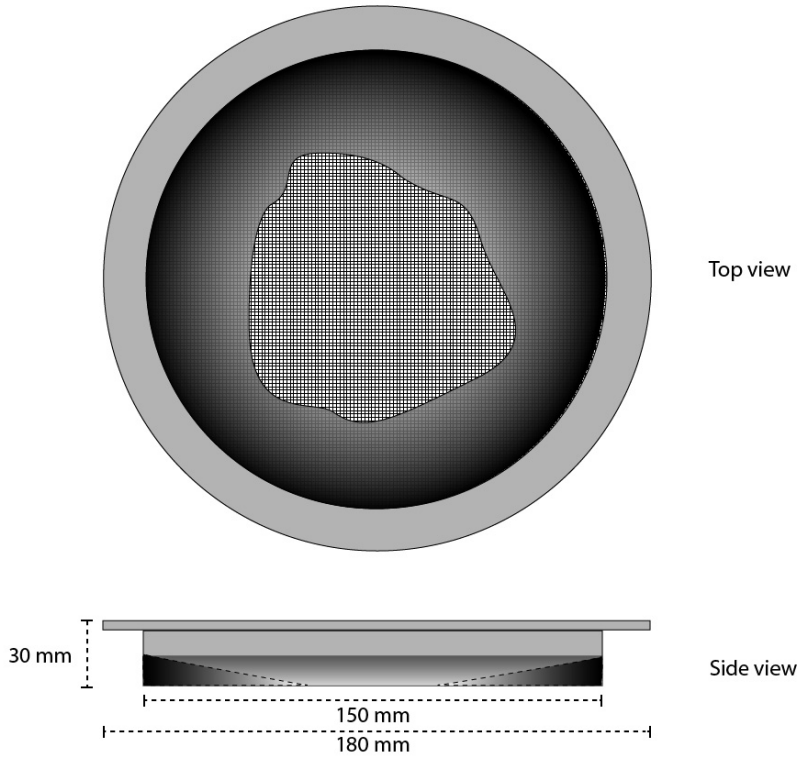

Figure 6-6: Exemplary deposition pattern of the ice spherules on the sieve. Darker areas indicate a thicker layer

Table 6-1: Results

Sample	Water Content Exhaust Endpoint [ppm]	Time of Endpoint [h]	Yield [mg]	Media particle diameter [µm]	LOD [%]
ASD - 20 °C	144	94.5	114	47 ± 2	3.1 ± 0.8
ASD -15 °C	20	82.5	339	49 ± 4	3.4 ± 0.4
ASD -10 °C	26	27.5	345	-	5.8 ± 0.7
VSD	-	24	354	55± 4	2.8 ± 0.4

6.5. DISCUSSION

Atmospheric sublimation drying of frozen spherules generated by jet-vortex freezing of monodisperse droplet-streams has the potential to be an alternative to vacuum sublimation drying after further optimization, as it yields lyophilized powders with comparable physicochemical characteristics. ASD offers various advantages over drying in a laboratory scale batch freeze-dryer. First, the drying temperature can be set at temperatures between -20 and 25 °C, which is important to prevent melt-backs during sublimation drying. Secondly, the spray-frozen product does not have to be transferred to a freeze-dryer after the freezing step, which simplifies the handling and reduces the risk of product contamination. The implementation of a continuous ASD process is easier than VSD, as described earlier by Süverkrüp et al. (2016).

The drying conditions have a direct impact on the drying time as the vapor pressure of water/ice directly correlates with the temperature and therefore has to be chosen carefully to enable an efficient sublimation while being low enough to prevent a melt back of the particles. It is suggestible to implement different conditions during the drying process as already known from vacuum freeze drying, starting with low drying temperatures for a primary drying and then increase the temperature step by step for the removal of residual water in a secondary drying-step.

Another factor that influences the drying efficiency is the layer thickness on the collecting sieve. The diffusion paths of solvent molecules are comprised of two parts: the intra-particular and the inter-particulate path. For an effective drying, the diffusion paths for the solvent molecules should be as short as possible. The highly porous surface structure facilitates the mass transfer, but ticker and multiple layers of particles inhibit the percolation, lead to longer diffusion paths through the interparticulate space and prolong the sublimation process. As the layer thickness depends on the amount of processed spray solution, the spraying time has to be adjusted to the drying time.

Further improvements have to be made, to prevent the particles from being deposited in a ring shaped pattern on the sieve, as the gas flow chooses the path of least resistance and exits through the powder-free center of the sieve, reducing the percolation and therefore the drying efficiency

The drying conditions probably depend on the formulation composition, as observed in previous studies (Chapter 4). Therefore, individual drying routines will be required for each formulation.

6.6. CONCLUSION

This proof of concept study shows that atmospheric sublimation drying is a viable method for the generation of highly porous spherical lyophilisate particles. It has several advantages over vacuum freeze drying, such as a precise temperature control and drying in a closed system.
By carefully choosing the drying conditions and implementing a step based routine, the absolute drying time can probably be reduced further.

6.7. ACKNOWLEDGMENTS

The authors are grateful for the financial support of the 'Deutsche Forschungsgemeinschaft' (DFG) in the framework of SPP1423 (grant no. LA1362/2-1) and would also like to thank Franz-Josef Willems, Thomas Vidua and Jürgen Hohmann for technical assistance.

6.8. REFERENCES

Ali, M.E., Lamprecht, A., 2014. Spray freeze drying for dry powder inhalation of nanoparticles. Eur. J. Pharm. Biopharm. 87, 510–517.

Bi, R., Shao, W., Wang, Q., Zhang, N., 2008. Spray-freeze-dried dry powder inhalation of insulin-loaded liposomes for enhanced pulmonary delivery. J. Drug Target. 16, 639–

Davies, P.J., Hanlon, G.W., Molyneux, A.J., 1976. An investigation into the deposition of inhalation aerosol particles as a function of air flow rate in a modified "Kirk Lung"†. J. Pharm. Pharmacol. 28, 908–911.

De Beer, T., Burggraeve, A., Fonteyne, M., Saerens, L., Remon, J.P., Vervaet, C., 2011. Near infrared and Raman spectroscopy for the in-process monitoring of pharmaceutical production processes. Int. J. Pharm. 417, 32–47.

Garmise, R.J., Mar, K., Crowder, T.M., Hwang, C.R., Ferriter, M., Huang, J., Mikszta, J.A., Sullivan, V.J., Hickey, A.J., 2006. Formulation of a dry powder influenza vaccine for nasal delivery. AAPS PharmSciTech 7, E131–E137.

Jiang, G., Joshi, S.B., Peek, L.J., Brandau, D.T., Huang, J., Ferriter, M.S., Woodley, W.D., Ford, B.M., Mar, K.D., Mikszta, J.A., Hwang, C.R., Ulrich, R., Harvey, N.G., Middaugh, C.R., Sullivan, V.J., 2006. Anthrax vaccine powder formulations for nasal mucosal delivery. J. Pharm. Sci. 95, 80–96.

Leuenberger, H. (Ed.), 2001. Spray Freeze-drying – The Process of Choice for Low Water Soluble Drugs? J Nanopart Res 4, 111–119.

Leuenberger, H., Plitzko, M., Puchkov, M., 2006. Spray Freeze Drying in a Fluidized Bed at Normal and Low Pressure. Dry. Technol. 24, 711–719.

Lin, Y.-W., Wong, J., Qu, L., Chan, H.-K., Zhou, Q.T., 2015. Powder Production and Particle Engineering for Dry Powder Inhaler Formulations. Curr. Pharm. Des. 21, 3902–3916.

Maa, Y.-F., Nguyen, P.-A., Sweeney, T., Shire, S.J., Hsu, C.C., 1999. Protein inhalation powders: spray drying vs spray freeze drying. Pharm. Res. 16, 249–254.

Malecki, G.J., Shinde, P., Morgan, A.I., Farkas, D.F., 1970. Atmospheric fluidized bed freeze drying. Food Technol. 24, 601–603.

Meryman, H., 1963. Apparatus for freeze-drying at atmospheric pressure. US 3096161.

Meryman, H., 1959. Sublimation Freeze Drying without Vacuum. Science 628–629.

Mumenthaler, M., Leuenberger, H., 1991. Atmospheric spray-freeze drying: a suitable alternative in freeze-drying technology. Int Pharm J 72, 97–110.

Niwa, T., Mizutani, D., Danjo, K., 2012. Spray freeze-dried porous microparticles of a poorly water-soluble drug for respiratory delivery. Chem. Pharm. Bull. (Tokyo) 60, 870–876.

Patton, J.S., 2004. The Lungs as a Portal of Entry for Systemic Drug Delivery. Proc. Am. Thorac. Soc. 1, 338–344.

Patton, J.S., Bukar, J., Nagarajan, S., 1999. Inhaled insulin. Adv. Drug Deliv. Rev. 35, 235–247.

Robinson, T., 2015. Compositions and Methods for Atmospheric Spray Freeze Drying. US 20150175716A1.

Rochelle, C., Lee, G., 2007. Dextran or Hydroxyethyl Starch in Sprayfreeze-Dried Trehalose/Mannitol Microparticles Intended as Ballistic Particulate Carriers for Proteins. J. Pharm. Sci. 96, 2296–2309.

Rogers, T., 2003. Micronized powders of a poorly water soluble drug produced by a spray-freezing into liquid-emulsion process. Eur. J. Pharm. Biopharm. 55, 161–172.

Schiffter, H., Condliffe, J., Vonhoff, S., 2010. Spray-freeze-drying of nanosuspensions: the manufacture of insulin particles for needle-free ballistic powder delivery. J. R. Soc. Interface 7, S483–S500.

Süverkrüp, R., 2015. Vorrichtung und Verfahren zur kontinuierlichen Sublimationstrocknung gefrorener Tropfen und Mikrotropfen in einem geschlossenem Gaskreislauf. 2015 0430 1507 5500 DE.

Süverkrüp, R., Wanning, S., Lamprecht, A., 2016. Continuous Atmospheric Droplet-Stream Freeze-Drying Processes. Pharm. Ind. 78, 741–749.

Wang Z, Finlay, W.H., 2008. Powder formation by atmospheric spray freeze drying. US 7363726.

Wanning, S., Süverkrüp, R., Lamprecht, A., 2017. Jet-vortex spray freeze drying for the production of inhalable lyophilisate powders. Eu J Pharm Sci 96, 1–7.

Wanning, S., Süverkrüp, R., Lamprecht, A., 2015. Pharmaceutical spray freeze drying. Int. J. Pharm. 488, 136–153.

Wexler, A., 1977. Vapor pressure formulation for ice. J. Res. Natl. Bur. Stand. Sect. Phys. Chem. 81A, 5.

SUMMARY AND CONCLUSION

SUMMARY

Literature shows various potential applications for spray-freeze-drying (SFD) techniques. Three basic operations can be combined with almost no limitations which makes SFD very versatile. SFD has been used to produce lyophilized powders for nasal, epidermal (needle-free injection), pulmonary (local and systemic), colonic and ophthalmic delivery of small molecules, proteins, peptides, and nucleic acids. Furthermore, SFD has been used to enhance the dissolution rate of poorly water soluble drugs and for preprocessing biologicals to increase their stability during microencapsulation.

In pulmonary application, SFD powders yield FPFs comparable or even better than commercially available products. It was shown that delivery of influenza, anthrax, hepatitis B, diphtheria, and tetanus vaccines via the pulmonary, nasal, or transdermal route resulted in a suitable degree of immunization. Another study reports the successful delivery of insulin via the pulmonary, as well as the transdermal route. In general, the process of SFD has only minor effects on protein/peptide structure and increases storage stability compared to liquid control formulations.

In SFD experiments a variety of different nozzle types have been utilized, all of them producing a unique droplet size distribution. It is probable that decreasing the span of the particle size distribution reduces product related variability upon administration.

Since first experiments carried out by Gruner and Süverkrüp, droplet-stream freeze-drying has been continuously refined and progress was made towards the production of inhalable lyophilized powders. Eggerstedt found that droplet-stream SFD is a suitable method for the production of lyophilized protein powders with excellent storage stability. Still, the reported median particle size was around 200-300 µm, hence not suitable for pulmonary administration. By evaluating high-speed camera recordings of the droplet stream, it was shown that primary droplets are subject to aerodynamic deacceleration and collide multiple times before congelation and thus increasing the particle size. This phenomenon, which is unique for droplet-stream generators, has been described in detail by Süverkrüp et al. in 2013 and 2015. Consequently, an aerodynamic droplet stream expansion method for the prevention

of droplet collisions was proposed, in order to maintain the initial droplet size and monodispersity.

The first experimental part of this thesis focuses on process and spray-freezing-device optimization by developing a proof of concept model of a swirl tube, intended to create a cold gas vortex. Influential factors having a major impact on droplet stream expansion were investigated in a 2^3 factorial design of experiments. Additionally, a fast screening method, which renounced the freezing and drying step was developed to evaluate a large number of additional factors. Due to the omitted freezing step, it was concluded that this screening method is suitable only to reject settings with insufficient droplet stream expansion. The DoE showed that the horizontal distance of the droplet-stream generator from the gas-jet nozzle as well as the gas flow rate have the strongest impact on droplet stream expansion. Vertical position was found to have a minor influence only. Droplet collisions were successfully prevented by aerodynamic droplet stream expansion with a cold gas jet. The median droplet size was reduced from 160 µm to 50 µm compared to unexpanded streams and the number of primary droplets forming the final droplet was reduced from 32-62 to 2-3.

Findings of jet expansion experiments were applied in the development of a novel apparatus ('jet-vortex-freezer'), showing several advances when compared to its precursor. A larger heat exchanger resulted in lower gas flow temperatures. A new double epicyclical positioning device allowed a decrease of the horizontal distance from the DSG to the gas-jet nozzle to 1 mm (previously 7 mm), resulting in a more precise droplet-stream deflection. Furthermore, jacket cooling was omitted so that the freezing apparatus notably decreased in size and LN2 consumption was reduced from 22 L/h to 10 L/h. The smaller dimensions made the device more responsive to alterations of process parameters and allowed a more dynamic production process, which was beneficial as a large number of experiments with varying conditions were conducted. A DoE was carried out to assess the optimal process settings, generating response surfaces for 4 critical target parameters (median particle size, width of particle size distribution, inter-batch variability and fine-particle-fraction). After evaluation thereof, the optimal process settings were found to be at a horizontal distance of 10 mm and a gas exit velocity at the jet nozzle of 6.8 m/s, resulting in powders with a median geometric particle size of 55 ± 4 µm and a narrow particle size distribution for a sample formulation consisting

of 1.5 % mannitol and 1.5 % maltodextrin in the aqueous spray solution. Suitability for dry powder inhalation was assessed by cascade impaction trials in which the powders achieved up to 22 % FPF, which is comparable to early commercially available inhalable drugs of the second generation.

As performance was found below expectations, further improvement of formulation and device were found essential. Based on a mathematical model describing the relation of aerodynamic diameter as product of the geometric diameter and the square root of the apparent particle density there are two approaches, *i.e.* reduction of particle diameter or density, to improve aerodynamic performance. As the droplet collisions were already minimized, further reduction of particle size by reduction of the primary particle´s diameter is achievable mainly by reducing the nozzle orifice diameter. Besides process optimization, improvement of the formulation sprayed is another approach to positively influence the product´s characteristics. Consequently, reduction of aerodynamic diameter was achievable only by reducing the apparent particle density which was achieved by adjusting the solid content (SC) in the solution sprayed. The formulation was optimized to yield high fine particle fractions whilst maintaining sufficient mechanical stability and dispersibility for powder handling and application via the DPI. Because of their established use in inhalable formulations as well as in freeze-dying, mannitol and lactose-monohydrate were chosen as structure forming excipients. Bashir and Avis (1973) reported that freeze-dried cakes of small, crystallizing carbohydrates such as mannitol had a low mechanical stability and that the addition of polymeric substances like polyvinylpyrrolidone (PVP) increased stability up to 25 times.

Therefore, Kollidon 12PF (PVP12) and Kollidon 25 (PVP25), maltodextrin (DEX) and HPMC were included as polymeric components. Solutions of individual excipients or mixtures of equal amounts of mannitol or lactose with a polymeric additive with increasing concentrations (0.3 %, 1 % or 3 %) SC were spray-freeze-dried under the previously assessed optimal process settings using the jet-vortex-freezer and characterized. Resulting powders showed different bulk properties. Scanning electron microscope imaging showed that most formulations consisted of spherical, porous particles. Other formulations showed collapsed particles and aggregates as well. To quantify size reduction, the median diameter of the dry

particles was related to the diameter of the ice spherules which showed that all formulations with a solid content of 0.3 % underwent particle shrinkage upon drying. Furthermore, solutions with 1 % solid content containing PVP12 and/or lactose also displayed a decrease in diameter. Additionally, the size analysis of the ice spherules and the rheological characterization of the spray solutions showed a positive correlation between the size of the ice spherules and the Ohnesorge number of the solutions.

The focus of this chapter is to assess the aerodynamic performance of these powders using the Next Generation Cascade Impactor. It was expected that formulations with lower solid content would show larger fine-particle-fractions, which has been confirmed for most of the powders assessed. It is unclear whether the positive correlation actually was due to a smaller aerodynamic diameter of the particles or by shear-stress induced fragmentation. Some powders (PVP12 and PVP25, HPMC) showed a lower FPF than anticipated. It was hypothesized that this observation was caused by either particle cohesion and aggregation, the larger particle size, or a combination of both effects. To quantify the relative mechanical stability of the powders and the degree of agglomeration, laser diffraction analysis with varying dispersion pressures was performed. It was found that low FPFs are mainly due to particle agglomeration. Pure mannitol and lactose displayed good performance in the NGI but showed extensive fragmentation even at low stress conditions. On the contrary, a combination of equal amounts of mannitol and DEX yielded high FPFs (52.9 % - 58.5 %) but fragmented only at high shear stress in the dispersion unit, even at solid contents as low as 1 %. Overall, it was shown that pulmonary performance of lyophilized powders can be improved by optimizing the formulation and that spherolyophilisates might be superior compared to commercially available interactive-blend based dry-powder-inhalers.

It is known that protein/peptides require an individual formulation composition for optimal cryo- and lyoprotection. As excipients used have an impact on the physicochemical properties of the lyophilized powders, formulations are to be characterized in detail regarding their flow and dispersibility characteristics, their median particle size, aerodynamic performance, and mechanical stability to guarantee a satisfactory product performance.

It was noted that on days with a high relative humidity (> 65 %) analytical procedures failed as some formulations suffered from spontaneous shrinking upon contact with ambient air.

Thus, the effect of moisture uptake on lyophilized powders and its impact on NGI experiments was investigated in depth in Chapter 5.

Dynamic vapor sorption analysis was used to determine water sorption/uptake and time-lapse images of bulk powders in the sample chamber were taken to document the loss of structural integrity and particle collapse upon increasing humidity. Additionally, samples that showed good performance in the NGI (> 20 % FPF) were re-assessed under varying humidity levels to investigate the influence of moisture on the dispersibility in air and the overall aerosol performance. It was found that spray-lyophilization increased the absolute moisture uptake of mannitol and lactose particles when compared to the unprocessed excipients. Additionally, it was shown that lactose underwent a moisture induced phase transition from the amorphous to the crystalline state, forming the monohydrate. Water uptake profiles of spray-frozen formulations from solutions with 1 % solid content were found to be comparable to ones with 3 % solid content, regarding absolute water uptake and uptake rate, but they collapse at lower RH. This indicates that formulations from 3 % SC and 1 % SC have comparable surface areas, but the walls of particles from solutions with 1 % SC are thinner and therefore soften and dissolve faster which leads to an earlier loss in structural integrity. Critical RH also depends on the formulation. PVP12 in general collapses earlier than other formulations, lactose formulation collapse earlier than mannitol formulations and particles containing DEX are relatively stable upon increasing humidity. Increasing humidity decreases FPFs and emitted fractions of lyophilized powders. The spherolyophilisates of mannitol+DEX formulations is insensitive to humidity. One must differentiate between water uptake prior to and after aerosolization as the former is assessable with the experimental setup (*i.e.* DVS and NGI/LD) used, while the latter process cannot be isolated from the overall effect. If the water uptake occurs fast and the particle mass and diameter change before it has reached its target, the effect is detrimental, but if the particle increases in aerodynamic diameter after it has entered the target area, the effect can be beneficial as less particles are subject to exhalation.

In the last chapter, first ‘proof-of-concept’ experiments of atmospheric percolation drying of ice spherules for the production of spherolyophilisate powders were carried out. The previously introduced jet-vortex-freezer was modified to allow moisture measurement of the exhaust gas for end-point determination. The ice powder was dried under atmospheric

conditions at -20 °C, -15 °C and -10 °C respectively and using a standard procedure under vacuum. It was found that atmospheric sublimation drying at -20 °C and -10 °C was suitable for the production of spherical lyophilisates, but drying times were around 72 h up to more than 98 h. SEM imaging showed that powders dried at -10 °C did not consist of spherical particles and showed signs of a melt-back during drying. The long drying times probably are due to the distribution of the particles on the sieve used. They were deposited in a peripheral ring and escaped the main flow of the drying gas which passed through the center. These experiments confirm that atmospheric freeze drying in a modified jet-vortex freezer is possible but will need further improvements to yield spherolyophilisates comparable to ones produced using a 24 h drying routine as vacuum sublimation drying does.

RATING OF THE METHOD

This method has several advantages compared to other SFD techniques. Atmospheric freezing allows controlling and varying the freezing temperature (in contrast to spraying into or over liquid nitrogen) and leads to an easy recovery of the frozen product, as no subsequent separation from the cryogenic liquid is necessary. Additionally, this allows directly implementing an atmospheric drying step. The median droplet sizes prepared by droplet-stream generators are relatively large (45-55 µm) when compared to droplets prepared by using *e.g.* two-fuid nozzles (5-15 µm). Producing smaller droplets could be possible, by further reducing the orifice diameter of the pinhole-diaphragm, but this would consequently decrease the throughput. While spraying 50 mL through a 20 µm nozzle (generating primary droplets with 40-50 mm) has an approximated process time of 4 hours, the process time of a 10 µm nozzle increases to more than 24 h.

The larger particle size could be considered as a drawback, but the width of the particle size distribution of powders prepared by SFD with droplet stream generators is narrower and handling properties (flowability and dispersibility in air) are superior. Ultrasonic nebulizers produce small particles with narrow particle size distributions, but are probably subject to nozzle freezing due to the slow movement of the atomized spray solution. However, the low throughput of droplet-stream generators require additional investigation, if scale up is

desired. Using multiple orifice nozzle-diaphragms as suggested by Eggerstedt et. al. might not be a suitable approach, as deflecting closely adjected droplet-streams could increase the occurrence of inter-stream droplet collisions.

POTENTIAL AND CHALLENGES IN FORMULATION DEVELOPMENT

Using this herein developed SFD method to generate lyophilized particles for pulmonary application opens the door for many advances in the formulation of inhalable powders. Inhalable lyophilized protein powders enable the local and systemic treatment of lung diseases with thermo-sensitive protein and peptide based APIs but also with already marketed small molecule drugs, as the latter also can benefit from the superior administration-performance of low density spherolyphilisates. The aerodynamic performance of spherolyophilisates can easily be controlled by the solid content of the spray-solution, if particles are not subject to secondary shrinking during drying. Lyophilized powders may be suitable inhalable carrier systems for new biological entities, but the excipients have to be chosen carefully. Protein/peptide based API has individual requirements of the formulation composition for the ideal cryo- and lyoprotection in freeze-drying processes. Due to the lyophilization step, excipients often change their physicochemical characteristics compared to the unprocessed excipients, which leads to an altered interparticular behavior. In contrast to freeze-drying in vials, spray-freeze dried powders not only have to protect the API from degradation but also need to meet the physical requirements given by the designated route of application, such as the geometric particle diameter, apparent density and flow characteristics. The formulation composition has a major impact on the particles' needs regarding the parameter-settings in the drying step and also its final physicochemical properties, such as flowability, mechanical stability, dispersibility in air, aerodynamic performance, water uptake profile and structural sensitivity at elevated humidity levels.

With respect to the lung as a primary target, the list of commonly used excipients for inhalational drug formulations is limited. On the one hand, SFD offers the implementation of excipients which are already well studied in freeze-drying and consequently often

administered directly into the blood stream, on the other hand almost no empirical knowledge has been assessed on the administration of polymeric components and their lung-clearance.
Also, the method is suitable only for high potency drugs, which need low doses only to become effective. As the overall solid content has a major influence on the aerodynamic performance of the particle, matrix-forming excipients will have to be replaced by API. This replacement is limited by the influence of the API on the mechanical stability of the particle.

CONCLUSION

Spray-freeze drying comprises a family of various techniques which are suitable for the production of lyophilized powders for different forms of applications, each with its individual advantages and drawbacks.
It offers the possibility to produce stable bulk-ware of lyophilized APIs which makes the filling process more flexible. By implementing the aerodynamic droplet-stream expansion using a jet-vortex the generation of inhalable powders is easily possible. Furthermore, by directly targeting the lungs, adverse effects which might occur due to systemic application, can be avoided. Nevertheless, inhalable lyophilized powders also can be used for systemic application of proteins and peptides that usually have to be administered by intravenous or subcutaneous injection.
This thesis shows that, by systematically optimizing both process and formulation, an inhalable powder consisting of low-density spherical lyophilized particles with excellent dispersibilty in air and aerodynamic performance was obtained by droplet-stream freezing. Droplet collisions, which previously were identified as the main root cause for large particles from small nozzles, were successfully prevented by aerodynamic droplet stream expansion and fast freezing. Of all formulations tested, a 1 % solution of mannitol/DEX 50/50 proved to be most suitable to generate a flowable, mechanically stable, inhalable low-density spherolyophilisate with a low sensitivity to moisture. It displayed a FPF of 55 % and an emitted fraction of > 95 %, even under elevated relative humidity levels of 77 % rH.
For future work, the influence of the formulation composition on the production process (droplet generation and drying) and on the particle characteristics (flowability, mechanical

stability) has to be assessed further. The mono/dimer to polymer ratio should be varied to further optimize the physical characteristics of the particles. Moreover, as this thesis mainly focuses on developing a carrier system, the addition of APIs to the formulation and its influence on the physicochemical properties of the particles has to be investigated. Furthermore, the optimized formulation should be tested in new inhaler devices with improved deagglomeration characteristics e.g. the Conix® or Twincer®. A first step towards an energy efficient production process has been made, but more work on scale-up measures has to be done to obtain a high throughput method.