Pharmaceutical spray freeze drying

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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 of 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.

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1. Why to spray-freeze-dry medicinal products

Since its introduction by Werly and Baumann, (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 and 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 in regard with 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. First, 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 pre-processing the protein/peptide ingredient prior to encapsulation in poly(lactic-co-glycolic acid) (PLGA) microspheres. Others have used the process to enable the pulmonary, nasal, and needle-free epidermal drug delivery (e.g., non-invasive vaccination). Biologicals are subsequently evaluated in regard with the intended pharmaceutical application.

2. 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, but 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 arise problems.

### Table 1

<table>
<thead>
<tr>
<th>Purpose/route of delivery</th>
<th>Active or model ingredients</th>
<th>Researcher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent solubility enhancement</td>
<td>Danazol</td>
<td>Rogers et al. (2002a, 2003a,b,c)</td>
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<td></td>
<td>Carbamazepine</td>
<td>Hu et al. (2004b)</td>
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<td>Phenytin</td>
<td>Rogers et al. (2002b)</td>
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<td>Ciclosporin</td>
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<td>Tolbutamide</td>
<td>Niwa et al. (2010, 2012)</td>
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<td>Oleanolic acid</td>
<td>Kondo et al. (2009, 2011)</td>
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<td>Baicalein</td>
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<td>Schiffer et al. (2010)</td>
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<td></td>
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<td>He et al. (2011)</td>
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<td>Preprocessing for sustained release injectables</td>
<td>Recombinant human growth hormone (rhGH)</td>
<td>Johnson et al. (1997)</td>
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<td>Costantino et al. (2004)</td>
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<td>Kennedy et al. (2007)</td>
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<td>Recombinant human insulin like growth factor-I (rhIGF-I)</td>
<td>Lam et al. (2000)</td>
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<td>Recombinant human vascular endothelial growth factor (rhVEGF)</td>
<td>Cleland et al. (2001)</td>
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<td>Recombinant human nerve growth factor (rhNEGF)</td>
<td>Lam et al. (2001)</td>
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<td>Bovine serum albumin (BSA)</td>
<td>Carraquillo et al. (2001)</td>
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<td>Leach et al. (2005)</td>
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<td>Pulmonary (local treatment)</td>
<td>rhDNase</td>
<td>Maa et al. (1999)</td>
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<td></td>
<td>Bovine DNase</td>
<td>Zijlstra et al. (2009)</td>
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<td>Anti-lgE monoclonal antibodies</td>
<td>Maa et al. (1999)</td>
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<td>Ciclosporin</td>
<td>Zijlstra et al. (2007)</td>
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<td>Ciprofloxacin</td>
<td>Sweeney et al. (2005)</td>
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<td>Rifampicin</td>
<td>Ohashi et al. (2009)</td>
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<td>Rapamycin</td>
<td>Carvalho et al. (2014)</td>
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<td>Kanamycin</td>
<td>Her et al. (2010)</td>
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<td>Δ9-tetrahydrocannabinol</td>
<td>VanDrooge et al. (2005)</td>
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<td>Doxorubicin nanoparticles</td>
<td>Roa et al. (2011)</td>
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<td>Salbutamol sulphate</td>
<td>Mueanoom et al. (2012)</td>
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<td>Terbutalin sulphate</td>
<td>Sharma et al. (2013)</td>
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<td>Voriconazole</td>
<td>Reinborn et al. (2012)</td>
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<td>Itraconazole</td>
<td>Vaughn et al. (2007)</td>
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<td>Tacrolimus</td>
<td>Watts et al. (2013)</td>
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<td>Pulmonary (systemic delivery)</td>
<td>Cetrorelix</td>
<td>Zijlstra et al. (2004)</td>
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<td>Liposomal insulin</td>
<td>Bi et al. (2008)</td>
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<td>pCMV-Luc plasmid DNA</td>
<td>Mohri et al. (2010)</td>
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<td>Influenza vaccine</td>
<td>Amorij et al. (2007)</td>
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<td>Saluja et al. (2010)</td>
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<td>Nasal</td>
<td>Anthrax vaccine (antigens)</td>
<td>Mikszt et al. (2005)</td>
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<td>Influenza vaccine</td>
<td>Jiang et al. (2006)</td>
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<td>Plague vaccine</td>
<td>Wang S.H. et al. (2012)</td>
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<td>Garmise et al. (2006, 2007)</td>
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<td>Huang et al. (2009)</td>
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<td>Epidermal (needle-free injection)</td>
<td>Influenza vaccine</td>
<td>Maa et al. (2004)</td>
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<td>Diptheria toxoid</td>
<td>Dean and Chen (2004)</td>
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<td></td>
<td>Tetanus toxoid</td>
<td>Amorij et al. (2008)</td>
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<td>Hepatitis B vaccine</td>
<td>Maa et al. (2003)</td>
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<td></td>
<td>Insulin</td>
<td>Maa et al. (2003)</td>
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<td>Schiffer et al. (2010)</td>
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<td>Colonic</td>
<td>Lipid–polymer composite microspheres</td>
<td>Gao et al. (2011)</td>
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<td>Ophthalmic</td>
<td>Sodium-fluorescein</td>
<td>Süverkrüp et al. (2009)</td>
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2.1. 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.

2.1.1. 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 (Dumouchel, 2008; Schneider, 2003; Dan et al., 1997, Fig. 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 vortices. 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.

2.1.1.1. 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. (2010) used a Micro Sprayer™ Aerosolizer (Penn-Century, Inc., USA-Wyndmoor, PA) developed for the intratracheal generation of droplet sprays in small animals (Century, 2000), 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 (Fig. 2a). The combination of high constant pressure and centrifugal forces disrupts the coherent liquid exiting 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 Westergaard (2010).

2.1.1.2. 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 2.2.5. The technique was developed and used for the production of protein/peptide microparticles containing zinc insulin (Yu et al., 2002) and bovine serum albumin (BSA), and to improve the solubility of poorly water soluble active ingredients like danazol and carbamazepine (Hu et al., 2002, 2003, 2004a,b; Rogers et al., 2002a,b, 2003a,b,c) 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 high feed pressures (5000–6000 psi) leading to jet velocities between 58 and 157 m/s. Considering the size and shape of the particles (Yu, 2004, Rogers, 2002a), the atomisation conditions appear to correspond to Dumouët’s Regime E (Fig. 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.

2.1.2. 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 (Fig. 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. Westergaard (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 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, Wang Y et al (2012), Cheow et al. (2011), and Mohri et al. (2010).

2.1.2.1. 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) (Fig. 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, Fig. 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 2–10 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 cyclosporine (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 (Ohashishi et al., 2009).

2.1.3. Ultrasonic spray nozzles

Slow moving clouds of droplets are shed from liquid-covered surfaces oscillating at high frequencies. In “soft mist” generators marketed by Sonotek 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; Vonhoff, 2010; D’Addio et al., 2013). (Fig. 3). 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 LN2 and lyophilised.

2.1.4. 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 \( \text{LBU} \) in the Rayleigh regime depends upon the fluid dynamic conditions (Fig. 1), while the mean diameter of the droplets \( d \) is proportional to the diameter of the orifice \( D \): \( d = 1.89 \times D \).

For aqueous solutions exiting from small circular orifices at low pressure, the formation of small droplets with diameters down to a few micrometers 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:

\[
k = \frac{\pi \times D \times f_c}{u_i}
\]

(1)
dimensionless wavelength with:
- \( D \): diameter of the orifice (m)
- \( f_c \): excitation frequency (1/s)
- \( u_i \): initial velocity of the jet (m/s)

and the diameter of the droplets is:

\[
d = \left( \frac{3 \times u_i \times D^2}{2 \times f_c} \right)^{1/3}
\]

(2)
droplet diameter

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 (Fig. 4a) and pin-hole type droplet generators (Fig. 4b) differ with respect to maintenance requirements and scale-up.
2.1.5. 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 favorable 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.

2.1.6. 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.

2.1.7. 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.

2.1.7.1. 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).

2.1.7.2. 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 atmospheric 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).

2.1.7.3. 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 (Pecht, 2009).

2.2. 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 vapor 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 favored in pharmaceutics.

As early as 1911 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.

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, 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 (2006) studied the size and velocity distributions of droplets generated by either pneumatic or hydraulic nozzles and obtained estimates of both their nucleation and solidification 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 \( u_i = 90 \, \text{m/s} \) and hydraulic nozzles \( u_i = 2 \, \text{m/s} \). Nucleation and freezing times were computed for representative slip velocities in mid-flight for fast and slow-moving droplets.

The analysis of Al-Hakim (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) and 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, appear to indicate where the process of solidification begins and how it spreads (Fig. 5a).

Krämer et al. (1999) determined homogenous nucleation rates for water droplets of 60 µm diameter levitated inside an electrodynamic Paul trap by analysing 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 cm³ 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.

2.2.2. 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, 1986, 2001, 2002; Mumenthaler and Leuenberger, 1991) developed atmospheric SFD processes with the intention to improve the dissolution behavior of poorly water-soluble APIs by embedding nano-sized particles in hydrophilic matrices. Aqueous or organic solutions of the drug substances and ingredients like polivinylpyrrolidone of

Fig. 5. Convergent flow atmospheric freeze-drying Source: Wang et al. (2006), redrawn.
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 (Fig. 5).

Eggerstedt et al. (2012a,b) and Süverkrüp et al. (2013) injected droplet streams at ambient gas pressure into vertical gas-filled freezing tubes with LN₂-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.

2.2.3. Spray-freezing with compressed carbon dioxide

The temperature of aqueous sprays can also be reduced below the 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.

2.2.4. Freezing by spraying into vapor over a cryogenic liquid (SFV)

When droplets are sprayed into a gaseous environment above the freezing point of the solution and sediment through the vapor layer onto the surface of a cryogenic liquid, supercooling and freezing may occur in the supernatant gas and vapor or upon contact with the condensed refrigerant. Since the velocity of small droplets decreases rapidly due to atmospheric braking, 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 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 Baumann (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 m³ dust chamber and sprayed liquid aequous dispersions containing 1–10% solids onto a liquid film of dichlorodifluoro-
methane (Freon 12) in an externally cooled rotating flask.

Kennedy et al. (2007) used chilled 1,2-dichlormethane and isopentane 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.

2.2.5. 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 spheres 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 vessel. 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 for Briggs and Maxwell, 1973, 1975, 1976 for the preparation of free-flowing lyophilised powders of biological materials.

2.2.6. 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 vapor layer limits the transfer of thermal energy to the heat sink. Overhoff et al. (2007) produced danazol/polyvinylpyrrolidone powders by dripping solutions containing various concentrations of drug and excipient in either acetone or tert-butanol on a solid substrate in the temperature range between 193 and 243 K and lyophilised the frozen splash. The solvents differed markedly with respect to freezing and spreading behavior, 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 25–50 μm and aerodynamic fine particle fractions up to 68%. Carvalho et al. (2014) applied this technique and compared the pharmacokinetics of rapamycin powder obtained by freezing mixtures of rapamycin and lactose in acetonitrile on a stainless steel surfaces 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 presystemic 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 lyophiliser.

2.3. Sublimation drying

The physical principles of the final steps of the FD process in vials under vacuum have been studied and modeled in detail (Pikal et al., 1983; Waananen, 1993) and summarised in books, e.g., by Jennings (1999), Costantino and Pikal (2005) and Rey et al. (1975). 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 vapourised molecules $\lambda$ 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. Micro-droplets injected into a vacuum are snap-frozen when part of the solvent evaporates and depresses the temperature of the remainder (De Ponte et al., 2008; Chapman, 2009), 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.

2.3.1. 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, $K_n \ll 1$, 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 (1959, 1963) 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 and Hohner (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; Menschüttna et al., 2004; Leuenberger et al., 2006; Haas et al., 2008; Niksir 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 4.6–1.5 kg water per kWh, while in conventional vacuum freeze drying 1 kWh dries only 0.4 kg of water.

2.3.2. Vacuum lyophilisation

In the typical vacuum freeze situation, the free path $\lambda$ 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, 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.

2.3.2.1. 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 unstemated. 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.

2.3.2.2. 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 t 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).

2.3.2.3. Rotary drum vacuum lyophilisation. A closed SFD system has been developed recently by Meridian Technologies (D-Mülheim) for the production of nearly monodisperse lyophilised spherules in the 200–800 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.

2.4. 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 stabilise disperse systems.

2.4.1. Solvents

The capacity of water to solubilise and to form solvates with polar compounds, its high freezing point, vapor 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 behavior 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., 2005), tetrahydrofuran (Rogers et al., 2002a) and acetonitrile (Zijdstra, 2007) have been used successfully.
to enhance the apparent solubility of Δ^2-THC, danazol, cyclosporine A and other BCS Class II and IV compounds. The mixing of water with solvents of different polarity by high-speed heterogeneous droplet collisions in a 4-fluid nozzle (Niwa, 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.

2.4.2. 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 be 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-trimethylammonium propane by Tsukamoto et al. (2012) and polyethyleneimine by D’Addio et al. (2013).

3. 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 reviews research papers are debated in two main categories: physical and therapeutic characteristics. Physical characteristics primarily cover particle size distributions, particle densities and aerodynamic behavior, 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.

3.1. 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 (Fig. 6a). Cross-sections of SFD particles reveal the high porous interior of SFD particles elucidating the extremely low densities of spray freeze dried materials (Fig. 6b) (Eggerstedt 2012a,b). It is observed that the porosity of SFD particles increases with the decrease of the solid content of the spray solution (Fig. 6c). 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 nonspherical fragments (Mueannoom, 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 Fig. 6a within a few microseconds. In some cases, droplet collisions deliver the activation energy for the freezing process to begin and colliding droplets freeze instantaneously (Fig. 6d).

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. (2007) studied the morphology of lysozyme/trehalose particles obtained by SFL using both LN2 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 Lamprécht, 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.

3.2. 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 2). In the following, we will discuss the particle properties based on the application of the developed powder.

3.2.1. 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 analyzed particle (Eq. (3)).

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>d50,avg (μm)</th>
<th>Solid content (w/w) (%)</th>
<th>Bulk density (mg/cm³)</th>
<th>FPF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary</td>
<td>7–42</td>
<td>3.6–5.0</td>
<td>20–230</td>
<td>22.9–70</td>
</tr>
<tr>
<td>Nasal</td>
<td>25–70</td>
<td>10</td>
<td>60–170</td>
<td>–</td>
</tr>
<tr>
<td>Epidermal</td>
<td>34–50</td>
<td>20–47</td>
<td>630–650</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 2

SFD particle characteristics overview.
d_{ae} = d_p \sqrt{\frac{\rho_p}{\rho_X}} \tag{3}

aerodynamic particle diameter with:

d_{ae}: aerodynamic particle diameter,

d_p: geometric particle diameter,

\rho_p: particle density,

\rho_X: unit density (1 g/cm^3),

X: shape factor (1 for spherical particles).

As indicated in Eq. (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 Andersen-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 SFT 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%, 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 7.5–18.67 μm, respectively). Yet there was still a comparable amount of FPF (39%) 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., 2005) when frozen. After FD, the particles still have up to 84% of their initial size and keep their original spherical shape. Therefore, based on Eq. (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 to increase the FPF of the SFD powder up to 70% (compared to 46% for SD), which they suspected to be accounted for 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 terbutaline (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%), a considerable amount of particles were reported to possess sufficient small aerodynamic diameters for pulmonary application. Hence, the FPF of both salbutamol and terbutaline 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. (2012) where the MMAD was found to grow with increasing solid content of the spray solution, while the geometric diameter remained constant. Formation of powders with MMAD values smaller or equal to 6 μm with large geometric particle sizes of 35–41 μm is indeed remarkable. Within this context, large porous particles offer several advantages over small and dense particles (Edwards et al., 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).

3.2.2. Powders for nasal application

SFD powders for nasal application have been prepared with geometric diameters of approximately 25 μm (Garmise et al., 2007; Wang S.H. et al., 2012) and 70 μm (Jiang et al., 2006; Mikzta 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, 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.

3.2.3. Powders for needle-free ballstic 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% 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 et al., 2003; 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.

3.3. 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 (Rogers et al., 2002a, 2003a,b), carbamazepine (Hu et al., 2004b), phentoin (Niwa et al., 2009), tolbutamide (Kondo, 2009) and ciclosporine (Niwa, 2010, 2012). The two mainly investigated methods include SFL and SFV using a 4N. Rogers et al. (2003a) and Hu et al. (2004b) 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 min, while bulk danazol required 60 min
for complete dissolution. For SFL carbamazepine, the results indicated a thorough dissolution after 10 min, whereas carbamazepine controls possessed significantly slower dissolution rates with the slowly frozen carbamazepine having achieved 85% dissolution after 20 min, and the bulk carbamazepine merely 25% after 60 min (Hu et al., 2004b). 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 (rh) and 40°C/75% rh (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 m²/g vs. SD: 0.35 m²/g) (Kondo et al., 2009). The 4N-SFV methods could also 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, 2010).

3.4. 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% of the protein was release in 48 h followed by constant rhGH levels for 20 days (Johnson, 1997). Costantino (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%). Henceforth, a continuous release of bioactive protein for 21 days could be 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. (2004) 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-dried microspheres could be reduced five to tenfold.

3.5. 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. Schiffer 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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Stability confirmed by</th>
<th>Researcher</th>
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<tr>
<td>Insulin</td>
<td>Reverse-phase HPLC of insulin and A-21 desamido insulin degradant</td>
<td>Rogers (2002b)</td>
</tr>
<tr>
<td>Anthrax vaccine</td>
<td>Circular dichromism</td>
<td>Wang S.H. et al. (2012)</td>
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<tr>
<td>Influenza vaccine</td>
<td>SDS-PAGE</td>
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<td>rhGH</td>
<td>Size-exclusion chromatography</td>
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<td>rhVEGF</td>
<td>Size-exclusion chromatography</td>
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<tr>
<td>Dry plasmid DNA</td>
<td>Gel electrophoresis</td>
<td>Mohri et al. (2010)</td>
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FTIR: Fourier-transform-infrared-spectroscopy.
SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Table 3
Stability of proteins/peptides in SFD processes.
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 exposition of the protein to a water-air interface during atomisation without freezing. An overview of the results is given in Table 3.

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

3.6. Therapeutic efficiency of SFD products

3.6.1. 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 IgC 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 (Mikszta et al., 2005; Jiang et al., 2006). 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 (83–100%), 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; Wang S.H. 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-F1 V vaccine. In rabbit survival studies, intradermal routes led to high survival rates of 70–90% (intramuscular 80–100%) (Huang et al., 2009). The nasal administrations of SFD plaque–F1 V vaccine led to 80% 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.

3.6.2. Insulin

Alternative routes for the application of insulin have been widely explored. Bi et al. (2008) 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% 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% respectively (Bi et al., 2008).

3.6.3. 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 tortuous 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.

3.6.4. 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).

3.6.5. 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).

4. 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 fulfill 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 epidemial 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 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 knowledge 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.

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