Application Note
No. 291/2017

Microencapsulation of Nucleic Acids by Spray Drying

1. Introduction

Nowadays, efficient treatment of several diseases involves innovative therapeutic approaches[1]. Gene therapy retains the interest of numerous researchers and was highlighted regularly as a potential approach to treat disease caused by genetic disregulations such as cystic fibrosis, hemophilia or cancer[1,2]. The use of gene therapy for vaccination is also mentioned in the literature as an alternative to conventional live/attenuated viral treatments[3,4]. Several approaches exist concerning gene therapy; for example i) DNA can be delivered in the nucleus, ii) RNA and oligonucleotides into the cytoplasm, giving the possibility to insert genes that restore/switch off a gene function or to regulate the production of active proteins for exertion of a therapeutic[1,2].

Different routes of administration (intravenous, ocular, topical or pulmonary delivery) have been investigated for nucleic acids, however, delivery system design and formulation remains challenging due to nucleic acid instability after administration[1,2,5]. To date, nucleic acid therapies are mainly based on viral and nonviral delivery system. Viral delivery systems are using the ability of viruses to enter the cells and release the nucleic acid with ease[6] whereas non viral delivery system are methods that do not involve viruses[7]. Promising results have been reported with viral delivery system concerning transfection efficiency. They remain very controversial due to the numerous safety concerns associated with them[1,8]. Non viral delivery systems are thus emerging as favorable alternatives to viral delivery systems[6].

When focusing on non-viral delivery systems, most authors of current publications are using nucleic acids in form of nanoparticles[9], packaging them with methods such as spray drying[10–14], spray freeze drying[5], freeze drying[14], liposome entrapment[6], emulsion / double emulsion[15,16] or a combination of the previous methods[1]. Amongst these techniques, spray drying, with its one-step process, scalability and control of particle properties[11,17,18], is an excellent option to process solutions, suspensions or emulsions[12,19] into dry powder formulations with engineered functional properties such as defined particle size and density[11]. Spray drying has been used extensively for a number of applications in the formulation of biopharmaceuticals intended for pulmonary delivery and inhalation and was reported to show potential to encapsulate nucleic acids[11,12,18].

The aim of this note is to present the existing possibilities to encapsulate nucleic acids using spray drying. The current options for nucleic acid delivery will be reported, the different types of delivery systems available will be specified with particular focus on non-viral delivery systems produced by spray drying. Several applications of nucleic acid therapy produced by spray drying will then be described.

2. Spray drying

Spray drying is a well-established technology to transform a liquid feedstock into a dried powder. It has been commonly used in food technology since the nineteenth century and is nowadays also used in the pharmaceutical industry for the formulation of biopharmaceutical preparations[18]. A conventional spray drying process comprise four fundamental steps[18,20,21]:

- Atomization of the feed into a spray
- Spray air contact
- Solvent evaporation
- Separation of the dried product from the drying medium

A conventional spray dryer consists of three main components, the atomization device or nozzle, the drying chamber and the collector. The atomisiation device reduces the feed into fine droplets. In the drying chamber, the solvent (water- or organic-based) is evaporated from the spray droplets and removed by the flow of heated gas (air or nitrogen). The dried particles are finally collected in a collection device, usually a cyclone[18,20,21].
Spray drying is a rapid, energy efficient, single step process. With the quick evaporation of the solvent from the droplet into the gas stream, the temperature experienced by the particles is lower than the inlet temperature of the instrument, extending the range of temperature available for

sensitive samples[18]. Thus, despite the levitated temperature of the drying gas, spray drying is a well suited method to prepare sensitive compounds such as biopharmaceuticals[18].
Several types of atomizers are available, the most common being, pressure nozzles, ultrasonic nozzles, two-fluid nozzles and rotary disk atomizers[18,20,21]. The choice of atomizer highly depends

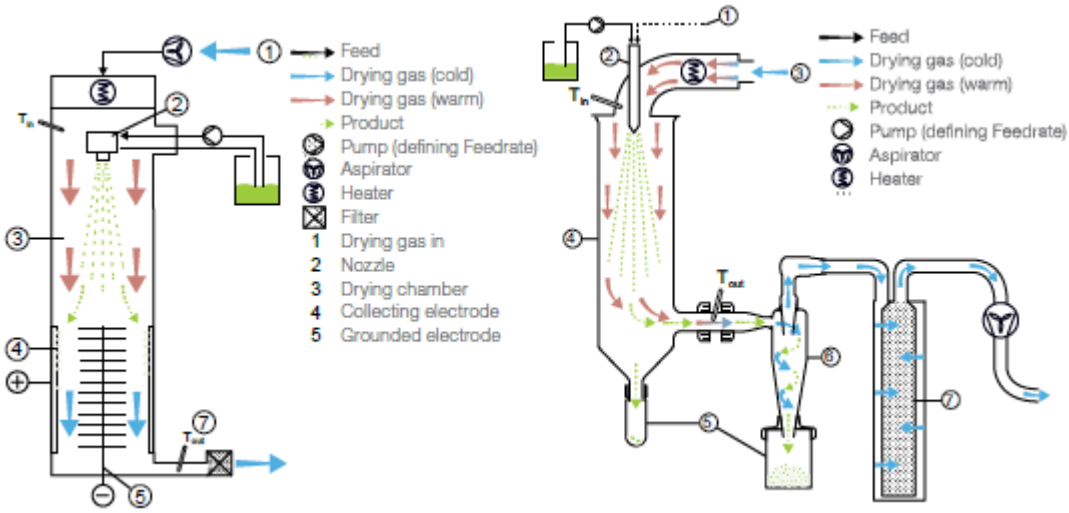

Figure 1 Functional principle of BUCHI Nano Spray Dryer (left) and conventional spray dryer - BUCHI Mini Spray Dryer B-290 (right).

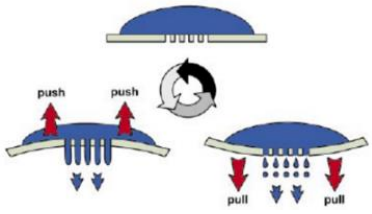

Figure 2: Droplet generation with the Nano Spray Dryer B-90 HP.

on the properties of the feed and the end product requirements[20,21]. The drying chamber main role is to ensure that the droplets are given enough time to dry before being collected. Its geometry depends mainly on the atomization type. The droplet-air contact determines several important parameters such as the evaporation rate of volatiles in the droplets, the trajectory and residence time in the drying chamber, the deposit on the chamber wall, the morphology of particles, and the product quality[18,20,21]. The direction of the air flow relative to the spray can therefore vary depending on the type of droplet-air contact system required[18]. The powder collection is mainly provided by cyclone separators which work by centrifugal forces using the inertia of the solid particles. The design of the drying chamber and collection system are dependent on the scale of production[18].

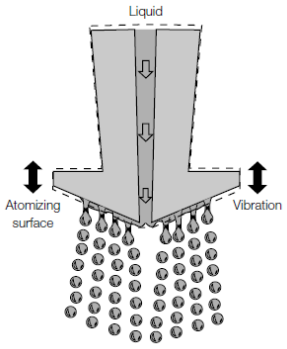

Figure 3: Ultrasonic nozzle for Mini Spray Dryer B-290.

BUCHI manufactures laboratory scale spray drying equipment (Figure 1). The Mini Spray Dryer B-290 works according to the principle of conventional spray dryer and is usually equipped with a two fluid nozzle and a cyclone collection system, while the Nano Spray Dryer B-90 and B-90 HP are equipped with piezoelectric nozzle systems (Figure 2) enabling the production of particles below the micrometer range (200 nm – 5µm) and an electrostatic particle collector in order to collect particles. Several accessories are available for these equipments, including the Inert Loop B-295 to work in an inert environment for oxygen sensitive substances or in presence of organic solvent and the Dehumidifier B-296/B-296 Nano to remove residual moisture. For the Mini Spray Dryer B-290, a three fluid nozzle is available to handle immiscible samples and an ultrasonic package (Figure 3) enables the generation of larger particles (10-60 µm) in laboratory scale spray drying, allowing an easier handling of the product and an increase of its flowability.

3. Nucleic acid therapy

3.1. How does it work?

With the discovery of nucleic acid molecules, new DNA-based therapeutics based on transgene-containing plasmids, oligonucleotides or small interfering RNA have been developed[6]. A common approach to gene delivery was for a long time to transfect cells with an encapsulated plasmid DNA (pDNA) in order to replace a defective gene in the genome. A novel therapeutic pathway consisting in using RNA to silence harmful genes has then emerged[22].
To be an efficient therapy, nucleic acids first need to be delivered to the target cells without damage. They then have to cross the cellular membrane and reach their targeted cellular location. pDNA and siRNA gene therapy differ in their mechanism of action and in the cellular location where the mechanisms are carried out[22]. DNA gene therapy requires the delivery of the pDNA in the nucleus of the host cell where it can induce the expression of the desired gene[22]. siRNA on the other hand will be used to induce degradation of mRNA molecules and prevent the synthesis of a specific targeted protein. siRNA molecules are released from the nucleus into the cytoplasm, therefore, siRNA only needs to travel to the cytoplasm to reach complementary mRNA[22]. siRNA might show some advantages over DNA and oligonucleotides molecules; they have a high degree of specificity to mRNA, are reported to be nonimmunogenic and highly resistant to ribonucleases, moreover, they do not integrate into the genome, offering a greater safety than pDNA molecules[6].

Macromolecules such as proteins and peptides have been widely investigated for pulmonary delivery therapy and the delivery of nucleic acid via the pulmonary route is told to be similar than for other macromolecules[7]. Pulmonary administration is reported to have several positive characteristics such as the avoidance of interactions with serum proteins and a comparatively low nuclease activity. Also, the large alveolar surface, the thin air-blood-barrier and the distinct vascularization make of the lungs an ideal basis for the absorption of drugs in the systemic circulation[9]. The size of the particles (expressed in mass median aerodynamic diameter MMAD) is important to determine the medicine site of deposition. Large particles with MMAD > 5 µm tend to deposit on the upper respiratory system, in the mouth and pharyngeal region; particles with MMAD between 1-5 µm sediment in the lower airways and bronchioles[23] while the movement of very small particles (<1µm) is ruled by the principles of Brownian molecular motion[2,7,9]. Recently, it was shown that for ultrafine particles below 100 nm, an effective alveolar accumulation could be observed[7,9].
Exogenous particles are mostly cleared out of the airways by the mucociliary clearance. When it comes to the alveoles, phagocytosis by macrophages and epithelial endocytosis are the main method for particle removal[2,7,9]. Alveolar macrophages can phagocyte particles with diameter from 1 to 3 µm efficiently, however it is reported that they seem to ignore particles below 260 nm[9]. Assuming that the nucleic acids particles successfully escaped the extracellular barriers and reached the cell surface, nucleic acids will need to be transported into the cell in order to exert their biological effect. Internalisation consists of endocytosis and endosomal escape[1,2,6]. Cellular uptake of naked nucleic acid molecules remains extremely inefficient due to the size, charges and stability of the molecules and will then necessitate the use of delivery systems that can be either of the viral type or non-viral type[1,2,6].

3.2. Delivery systems

A delivery system can be used to promote cellular internalization and to protect nucleic acids from degradation. The ideal system should have a high transfection efficiency and a high degree of cell specificity; it should neither be cytotoxic nor immunogenic, ideally biodegradable and able to maintain high drug concentrations over time by preventing the recognition by macrophages or the excression through the kidney. It should also be easy to formulate and to modify for customized nucleic acid release, delivery and expression[9,17]. Currently available delivery systems can be classified into two types depending on their origin: biological viral delivery systems or chemical non-viral delivery systems.[1,2,6]

3.2.1. Viral

Throughout evolution, viruses acquired the ability to enter cells and to transfer nucleic acid molecules into those cells efficiently[2,6]. For therapeutic purposes, it became interesting to exploit the viral machinery for the internalization of genetic material by using attenuated viruses as

delivery systems[2,3,6]. The nucleic acids of interest are assembled in the viral genome and the innate mechanism of infection of the virus is used to enter the cell and to release of the genetic material[2,6]. Viral delivery systems are recognized to have extremely high transfection efficiency in several human tissue[6]. They often contain plasmid DNA since they enable DNA molecules to enter the nucleus and to be integrated into the host gene pool and eventually be expressed[2,6]. Although the delivery of genetic material using viruses has many advantages, concerns regarding safety, manufacturability and efficacy of viral systems have been reported[2,6]. The first concern is the toxicity of viruses and their possibility to target immune responses[2,6]. Pre-existing antibodies against the virus would not only provoque an immune response but also neutralize the delivery system and the molecule it carries[2,6], making the therapy inefficient. Additionally, the integration of the nucleic acids into the host genome by the virus cannot be controlled and takes place randomly. Depending on the insertion site, this could have serious consequences such as mutagenesis that may inhibit expression of normal cellular genes or tumorigenesis[2,6].

3.2.2. Non-viral

Non-viral delivery systems can be used to circumvent some of the disadvantages associated with viral delivery[2,6]. With their lack of immune response and their ease of formulation and assembly, non-viral systems are emerging as a favorable alternative to viral delivery vectors[2,6]. Lipid based and polymer-based systems are the most commonly used. Their physicochemical properties can be modified to improve the biocompatibility, to increase internalization and to tailor the exact requirements for nucleic acid delivery[2,9].
For dry powder non-viral systems, several particle engineering approaches such as spray drying, spray freeze drying or freeze drying were investigated using synthetic materials[1].

4. Spray drying in gene therapy

Spray drying is commonly used in gene therapy research articles to produce delivery system for both pDNA and siRNA therapy, with most of the research focusing on inhalable dry powder for lung delivery[1].

4.1. Carrier materials

Commonly used materials for non-viral delivery system of nucleic acids includes lipids, polymers and/or peptides[7].

Lipid based delivery systems typically use cationic lipids or liposome to create complexes with the negatively charged nucleic acid through spontaneous electrostatic interactions[7]. It is crucial to optimize the lipid composition of the liposome or lipoplexe in order to reduce the toxic side effects and the inflammatory responses caused by lipid based delivery systems[7]. Cationic lipid based systems are reported to be generally more toxic than neutral or anionic systems[6,7]. The possibility to shield the surface charges of cationic lipoplexes or liposomes using polyethylene glycol (PEG) showed promising results for the reduction of the inflammatory response[7]. The use of neutral and anionic lipid based systems, although more favorable safety wise show a lack of interaction with the nucleic acid molecule and therefore have limited use as delivery vector[6,7]. Specialized lipid based systems such as lipid-like molecules or pH-sensitive lipid are also reported to show interesting results in nucleic acid encapsulation[6,7].

Polymer-based vectors have a versatile nature that allows the modification of their physicochemical characteristics to fit their purpose. For the delivery of nucleic acids, polymers such as poly(D, L-lactide-co-glycolide) (PLGA) or polycations such as polyethylenimine (PEI) or chitosan are often used[1,6,7,9]. Polycations have a high cationic charge density and can form polyplexes with the negatively charged nucleic acid through electrostatic interactions[7]. Although they show good potential for DNA entrapment, their weaker interaction with siRNA makes them less efficient for siRNA entrapment and less effective in protecting siRNA molecules from nucleases[7]. Encapsulating siRNA in particles made of chemically modifiable hydrophobic polymer such as PLGA and its derivatives molecules is yet reported to be a potential solution to protect siRNA[7].

DNA and siRNA molecule show a limited ability to enter the cells due to their negative charges. Cell penetrating peptides (CPP) and pH-responsive peptides can be used to overcome this limitation. A variety of CPPs has been synthesized and successfully employed to facilitate therapeutic transport of macromolecules into cells. This strategy was further extended to the delivery of nucleic acids[7]. CPPs molecules consist either of small amino acid sequences composed of arginine, lysine and histidine providing a positive charge that help to mediate the interaction with the cell membrane, or of structures with both lipophilic and hydrophilic sides that can mediate translocation across the membrane[1].
The use of delivery systems made with pH responsive peptides to deliver nucleic acids in cell was reported by Liang et al.[5,24] as a successful solution to deliver DNA to A549 cells. In acidic environments, these peptides can destabilize the membrane activity of the endosomes, provoque endosomal escape and thereby avoid degradation of the genetic material. The use of pH responsive peptide can therefore be considered as a solution to overcome endosomal and liposomal degradation of the nucleic acid after internalization by endocytosis[1,5,24].

In order to produce good quality powder by spray drying with the above polymers, thermoprotectants stabilizing adjuvants and excipients can be used additionally. Such molecules can include sucrose, glycine, agarose, trehalose, PEG, bovine serum albumin (BSA) and several amino acids (arginine, lysine, and histidine).

4.2. Commonly used spray drying parameters

Several studies investigating spray drying to produce gene therapy delivery systems can be found in the literature. Most of the found publications rely on BUCHI Spray Dryer and parameters are summarized in Table 1 and 2 below.
A successful drying process relies on the correlation and interdependency of the process parameters. Thus, optimization of the inlet temperature (T_{in}), outlet temperature (T_{out}), throughput/feed rate, drying air flow and atomization gas, is important to obtain the desired particles.

The **inlet temperature** is the temperature of the heated drying gas. It regulates the temperature gradient between the wet droplet surface and hot saturated gas and leads to the evaporation of the solvent. The maximum inlet temperature that can be set on the BUCHI Mini Spray Dryer B-290 is 220°C, and 120°C on the BUCHI Nano Spray Dryer B-90 HP. The more energy is put to the system, the faster the solvent is evaporated, which in turn increases the drying efficiency.

The **outlet temperature** represents the temperature of the dry powder before entering the collection vessel or at the collection vessel. It is an important parameter when working with heat sensitive samples since it is the resulting temperature of the heat and mass balance in the drying cylinder. It cannot be regulated and represents the maximum temperature to which the product is expected to be exposed. The outlet temperature is the result of the combination of many parameters, the following are the most important:

- Inlet temperature
- Aspirator flow rate (quantity of air)
- Peristaltic pump setting (feed rate)
- Concentration of the material being sprayed.

The **peristaltic pump** feeds the sample solution to the nozzle. In the Mini Spray Dryer, its speed affects the temperature difference between the inlet temperature and the outlet temperature since the pump rate directly corresponds to the inlet mass. A higher throughput will require more energy to evaporate the droplets from the particles, thus the outlet temperature will decrease. It should be noted that in the Nano Spray Dryer, an increase in pump speed will not always lead to an increase of inlet mass since those parameters are not directly related.

The **atomizing air flow** or spray flow rate is the amount of compressed air needed to disperse the sample. A gas other than compressed air can be used when it is required to work in an inert environment. The spray flow rate can be set to between 300 and 800 L/h on the B-290 device. This parameter is not relevant for the Nano Spray Dryer B-90 HP.

The **aspirator capacity** regulates the amount of air available (drying air flow) for the drying process. Because the amount of energy available for vaporization changes with the amount of drying air

available, the aspirator speed setting has a significant effect on the drying performance of the instrument.

Table 1: Parameters for nucleic acid (DNA) encapsulation via spray drying.

Nucleic acids	Spray Dryer	Main parameters	Carrier material	Microparticle size [µm]
DNA[17]	BUCHI B-191	**T_{in} [°C] :** 120-160 **Atomizing air flow [L/h] :** 500-700 **Feed rate [mL/min] :** 3-9 **Aspirator capacity: -** **T_{out} [°C] :-**	Chitosan Leucine Lactose	3-11.8
DNA[14,19]	BUCHI B-191	**T_{in} [°C] :** 150 **Atomizing air flow [L/h] :** 600 **Feed rate [mL/min] :** 7.5 **Aspirator capacity:** 35 m^3/h **T_{out} [°C] :** 80-85	Lactose DOTAP liposome Protamine sulphate (Chitosan)	1-10
DNA[25]	BUCHI B-191	**T_{in} [°C] :** 78-79 **Atomizing air flow [L/h] :** 600 **Feed rate :** 10% **Aspirator capacity:** 75% **T_{out} [°C] :** -	PLGA PVA	3-4
DNA[5]	BUCHI B-191	**T_{in} [°C] :** 50 **Atomizing air flow [L/h] :** 750 **Feed rate [mL/min] :** 2 **Aspirator capacity:** 60 (supposed %) **T_{out} [°C] :** 28	LAH or LADap peptides Mannitol	<10
DNA[4]	BUCHI B-191	**T_{in} [°C] :** 50 **Atomizing air flow [L/h] :** 800 **Feed rate [mL/min] :** 1 **Aspirator capacity:** 70% **T_{out} [°C] :** 39-40	PLGA Stearylamine	2.2-8.3

Table 2: Parameters for nucleic acid (siRNA) encapsulation via spray drying.

Nucleic acids	Spray Dryer	Main parameters	Carrier material	Microparticle size [µm]
siRNA[24]	BUCHI B-290	**T_{in} [°C] :** 50 **Atomizing air flow [L/h] :** 740 **Feed rate [mL/min] :** 2 **Aspirator capacity:** 100% **T_{out} [°C] :** 34±2	LAH and LADap peptides Mannitol	<10
siRNA[11,12]	BUCHI B-290	**T_{in} [°C] :** 45 **Atomizing air flow [L/h] :** 473 **Feed rate [mL/min] :** 0.3 **Aspirator capacity:** - **T_{out} [°C] :** 30	PLGA / DOTAP-modified PLGA Trehalose Mannitol Lactose	3.7[12]-4.99[11]
siRNA[26]	Ultrasonic nozzle Sono-Tek	**T_{in} [°C] :** 120±2 **Atomizing air flow [L/h] :** - **Feed rate [mL/min] :** 1 **Aspirator capacity:** - **T_{out} [°C] :** 65±2	Chitosan E-80 F127 HPMC Mannitol	6.2-9.8
siRNA[10]	BUCHI B-90	**T_{in} [°C] :** 30-60 **Spray power [%] :** - **Feed rate [mL/min] :** - **Aspirator capacity:** 118-121 L/min **T_{out} [°C] :** -	PLGA/PEG-PLGA BSA DOTAP	0.58-0.77

5. Recent applications in nucleic acid therapy

Nucleic acids have the potential to be used as therapy or vaccines for several diseases, although, their delivery remains challenging. Recently, promising results from a clinical trial showed that sickle cell disease could potentially be cured through an autologous hematopoietic genetically modified stem-cell transplantation[27]. The therapeutic nucleic acid was transferred *ex vivo* into the autologous heamatopoietic cells using a viral delivery vector and the cells were then implanted back into the patient for treatment[27]. Even though this procedure is rather invasive, results are promising and show the potential of nucleic therapy to cure genetic related diseases. Less invasive therapeutic products based on DNA can be found on the market nowadays[1]; they are used against cancer and some rare diseases, are administrated either as intramuscular injection or intralesional injection and are using viral gene delivery vectors[1].

Several research applications can be found using spray drying to produce non-viral nucleic acid-containing delivery systems, most of them being related to the production of inhalable dry powders for pulmonary delivery. Wu et al.[28] successfully created a powder intended for vaginal delivery by entrapping siRNA into HPMC-containing delivery vectors by spray drying. Results showed effective siRNA protection and sustained release. Moreover, the authors suggested that optimized powder formulations could have the potential to alter the steric barrier posed by mucus

and therefore hold promises for an effective siRNA vaginal delivery. Hence, this research shows the potential of spray drying nucleic acid not only for lung delivery but also for other delivery areas.

6. Conclusion

Within the past years, gene therapy evolved from experimental technology into a viable strategy for therapeutics. It holds many promises in the treatment of genetic related diseases and numerous rechearch projects are ongoing. The discovery of safe and effective carriers for the genetic material is mandatory in order to expand gene therapy in human medicine and polymer system could be part of the solution.
Over the years, spray drying has been widely investigated to produce carriers for biopharmaceuticals. While its main application is in the formulation of aerosolized therapeutics, several reviews of spray dried molecules for other applications also exist. With both the Mini Spray Dryer B-290 and the Nano Spray Dryer B-90 HP, BUCHI offers a wide range of possibility for the development of innovative new carriers. Recently, Talal et al.[29] successfully developped amphiphilic nanoparticle-in-nanoparticle drug delivery systems using BUCHI Nano Spray Dryer B-90 while Amsalem et al.[10] also effectively investigated the possibility to produce solid nano-in-nanoparticles for potential delivery of siRNA with the Nano Spray Dryer B-90.
By combining the existing knowledge in biopharmaceutical spray drying with the advantages of the equipment, innovative delivery system for nucleic acid could therefore be developed.

7. Reference

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