Spray Drying Biologics: An Alternative To Freeze Drying.

WHITE PAPER

Dr Richard Johnson
Chief Scientific Officer

Upperton
Pharma Solutions

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The need for drying technologies to stabilise drug products containing biologics.

Drug products containing active biological ingredients form one of the most important and fastest-growing sectors of the pharmaceutical industry.

The biologics market size is estimated to achieve sales of USD 429.5 billion in 2024, and is expected to reach 601.3 billion by 2029, growing at a CAGR of 6.96% between 2024-2029[1].

The term 'biologics' is used across a wide range of large, complex molecules typically 200-1,000 times bigger than small molecule drugs.

They include protein and peptide products, (such as monoclonal antibodies, enzymes and peptide hormones), through to more complex moieties including vaccines containing either proteins or active/inactivated virus particles and mRNA/RNA based drugs that are incorporated into lipid-based delivery vehicles.

Most biologics are formulated and administered to the patient in aqueous dosage forms, and because of their size they require delivery by an injection.

However, in an aqueous solution the large size, complex structure, and susceptibility of therapeutic proteins to environmental stress results in several physicochemical degradations. This includes deamidation, oxidation, hydrolysis, disulfide exchange, aggregation, precipitation, and denaturation. This results in limited shelf life, with storage requiring either frozen or refrigerated conditions.

To overcome these stability challenges, biologics can be formulated and water removed to create a dry powder. The storage stability of these complex molecules improves dramatically once water has been removed.

However, achieving the desired stability profile requires a number of processing steps, including the selection/addition of excipients and optimization of the drying conditions to protect the molecule during the drying process and improve shelf life.

02

Freeze drying: The established technology for producing dry powder biologics.

Freeze drying is the traditional process of choice for creating dry powder biologics in a range of dosage forms, including those for parenteral administration.

This technique has numerous advantages, with drying equipment available at varying scales from early research to commercial manufacture.

The process typically yields individual, sterile dosage forms in a single drying step, and regulators are comfortable with this established process. Indeed, freeze drying is widely applied and is used to produce more than half of the biopharmaceuticals commercially available in today's market[2].

Although freeze drying is a well-established technology for the production of sterile, dry powder dosage forms, it suffers from several technical challenges.

Commercial freeze drying processes can be relatively slow, often taking several days, and the development of new processes can be challenging, as difficulty controlling the physical properties of the dry powder/cake leads to variation between and within batches. Scale-up of processes can also present challenges, with significant energy and equipment costs.

The challenges posed by freeze drying have resulted in a growing interest in alternative drying technologies for creating dry powder dosage forms containing biologics, with spray drying emerging as a technology that offers several advantages over freeze drying.

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03

Spray Drying: A viable, cost-effective alternative to freeze drying.

In pursuit of a more economical and scalable technology for producing dry powder dosage forms, various strategies have been taken into consideration. Although several experimental technologies - such as spray freeze drying, thin-film freeze drying and supercritical fluid drying – have generated interesting results, these methods have not yet been taken to full commercial scale. Currently only spray drying has the existing capacity to be considered a viable option for the scale-up and manufacture of commercial-scale dry powder biologics.

As a drying process, spray drying offers many benefits over its more established counterpart. One of the most obvious advantages is improved speed and scale which in turn means lower production costs.

Some of the additional advantages are summarised below:

Table 1: The benefits of spray drying compared to freeze drying

Key feature	Freeze drying	Spray drying
Capable of formulating sensitive biologics into stable dry powder dosage forms	✓	✓
Rapid, continuous production process	✗ Slow batch process, expensive and energy-demanding	✓ Kilograms of powder are produced quickly at a lower cost
Scalable process	✓ Extensive process development needed to achieve consistency	✓ Faster to develop and scale, highly reproducible
Aseptic Processing	✓ Established technology	✓ Aseptic spray dryers are now available commercially
Avoid potentially damaging Freezing step	✗ Freezing is essential	✓
Produce a free-flowing powder that can be filled into capsules, devices	✗ Further processing is needed	✓ Dry powder can be filled into capsules, vials, devices

The first spray dried biologics to be approved are shown in **Table 2** below, with three of the four shown requiring an aseptic dosage form. Whilst this list is relatively small, there are a considerable number of spray dried biologics in clinical development that are expected to achieve approval in the coming years.

Table 2: Commercially approved spray dried products containing biologics

Product	Active pharmaceutical ingredient	Approval Date	Administration Route
Exubera® Pfizer	Insulin	Jan 2006	Inhalation
Trelstar® Verity pharmaceuti-cals	Triptorelin pamoate	Oct 2010	IM injection
Somatuline® Ipsen	Lanreotide acetate	Nov 2013	IM injection
Raplixa® Medicines Company	Thrombin/Fibrinogen	Apr 2015	Topical/wound

Spray drying is capable of producing dry powder formulations containing large, complex biologics such as monoclonal antibodies (mAbs) with similar levels of success to freeze dried formulations. Massnat et al[3] evaluated two mAbs with a range of excipients including sugars (trehalose, sucrose), and a range of amino acids as their formulation stabilizers.

Using a Design of Experiment approach (DoE), it was shown that trehalose concentration started from 30 up to 120 mM, and for amino acids, it ranged between 50 and 150 mM. Storage condition was set at 25 °C and 40 °C for 13 consecutive weeks.

Results from the study showed that most formulations preserved their amorphous state.

The trehalose concentration (at a ratio of at least 1:1) played a crucial role in improving stability and the reconstitution time.

Interestingly, stabilising effect of arginine was found to be so strong that there was no need to add a sugar stabilizer to enhance the stability as well as showing improved reconstitution times.

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CASE STUDY

The ability of spray drying to process biologics to create stable dry powders whilst maintaining tertiary structure – spray drying and freeze drying of Recombinant Human Albumin.

In a recent case study at Upperton, a large (mwt 66kDa) soluble, globular protein recombinant human albumin (rHA) was formulated and either spray dried or freeze dried to create dry powder products.

The rHA molecule has a heart-shaped tertiary structure held in place by 17 pairs of disulphide bridges with one free cysteine (Cys 34) close to the N-terminus. It is usually divided into three domains that have specific binding functions and the protein acts as a carrier for a wide range of molecules (polar and non-polar) in the blood/ serum.

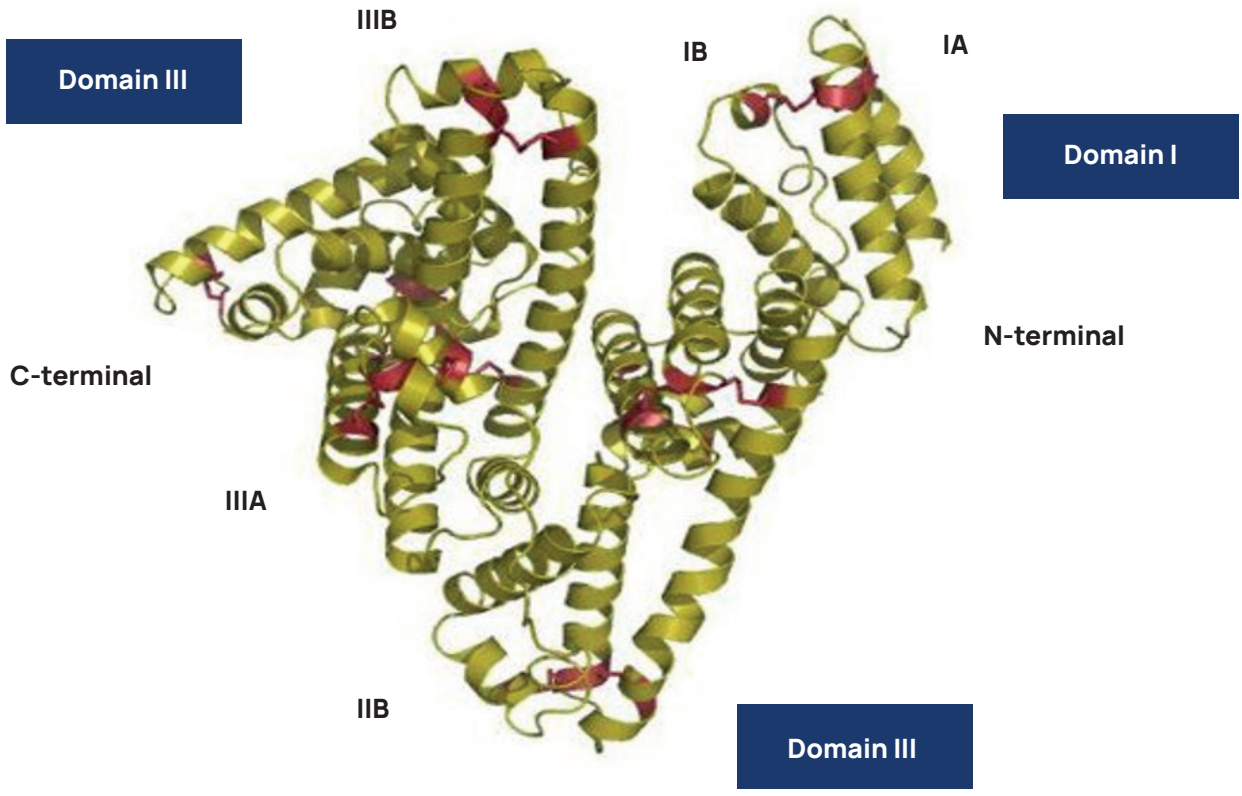
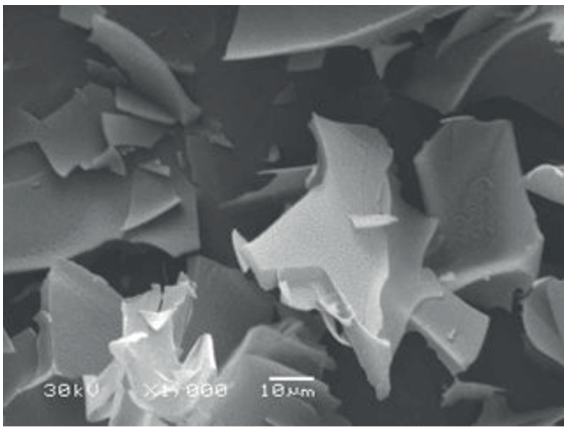


Figure 1: Representative of the 3-D structure of recombinant human albumin (rHA), showing three specific structural domains

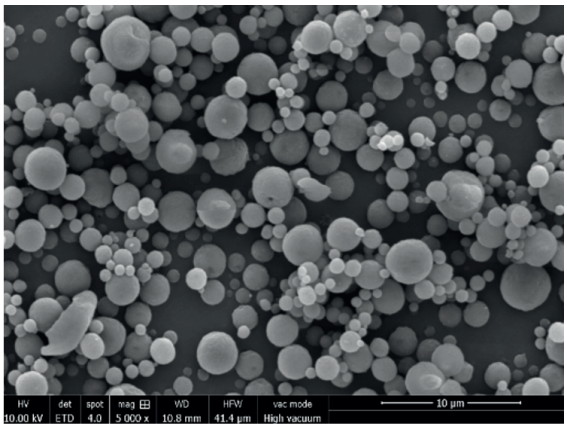
In this study, an rHA solution was formulated with trehalose (1:1 w/w) and transformed to a dry powder product using either spray drying of freeze drying. One immediate advantage of the spray drying process was that a 10g batch of stable, formulated powder could be produced on a bench top spray dryer (Buchi B-290) in less than 30 minutes. In comparison, the freeze drying process used took 36 hours to complete. The resulting spray dried powder was far easier to handle as it existed as a powder rather than the solid "cake" produced by freeze drying (see **Figure 2** for structure). This meant it could be filled into capsules or devices without further processing.

Figure 2: Electron micrographs of freeze dried (A) and spray dried (B) rHA powders

(A) Freeze dried rHA

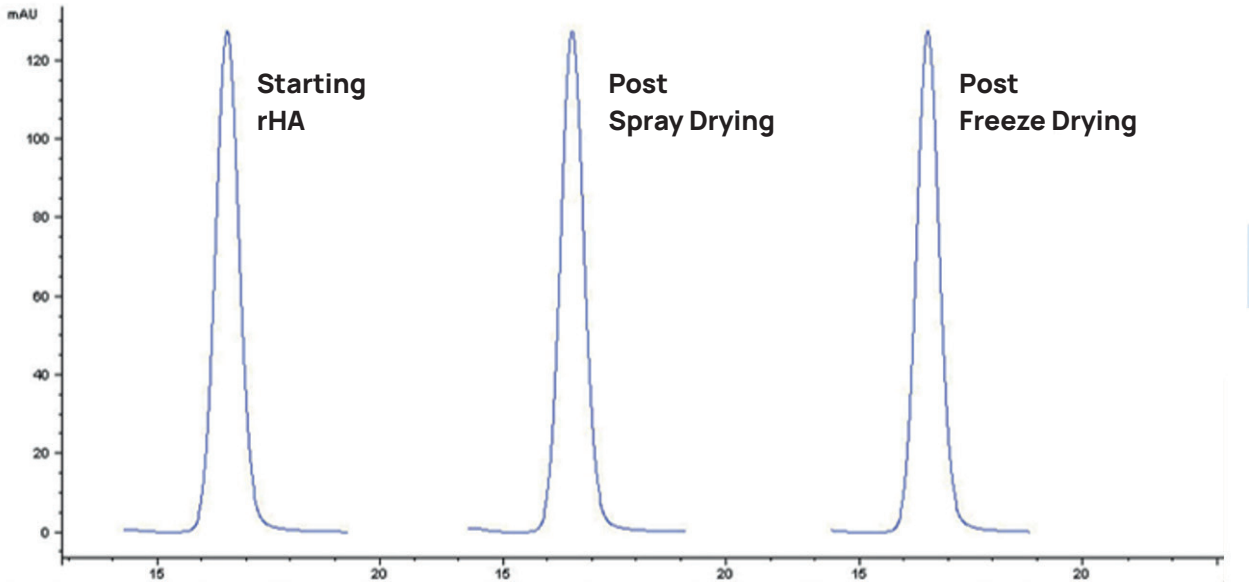


(B) Spray dried rHA



The freeze dried and the spray dried rHA powders were characterised using a number of analytical techniques to determine whether the tertiary structure changed after each drying process. rHA is prone to dimerization/polymerisation if exposed to heat or denaturing conditions, but in both cases the tertiary structure of the rHA molecule remained unchanged as can be seen in **Figure 3**, with no evidence of dimerization on the leading shoulder of the main peak using size exclusion chromatography on HPLC (SEC), which remained virtually unchanged.

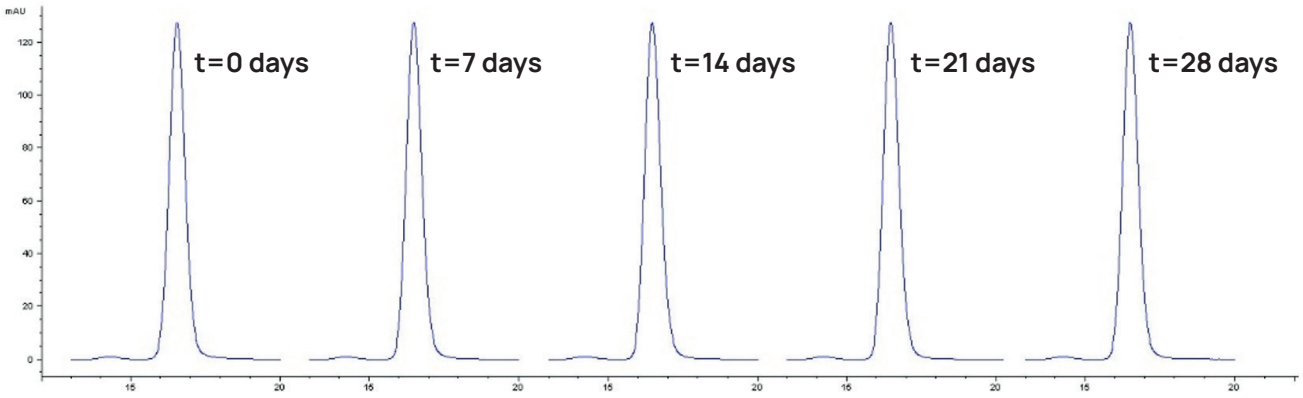
Figure 3: SEC elution profiles of rHA pre- and post-spray drying and freeze drying



Further analysis compared and contrasted the storage stability of the two dry powder formulations and again, there was no discernible difference in the HPLC profiles after 28-day storage at 40°C as shown in **Figure 4** below.

Figure 4: SEC elution profiles of spray dried and freeze dried rHA after storage at 40°C and associated peak integrations

Spray Dried rHA



Freeze Dried rHA

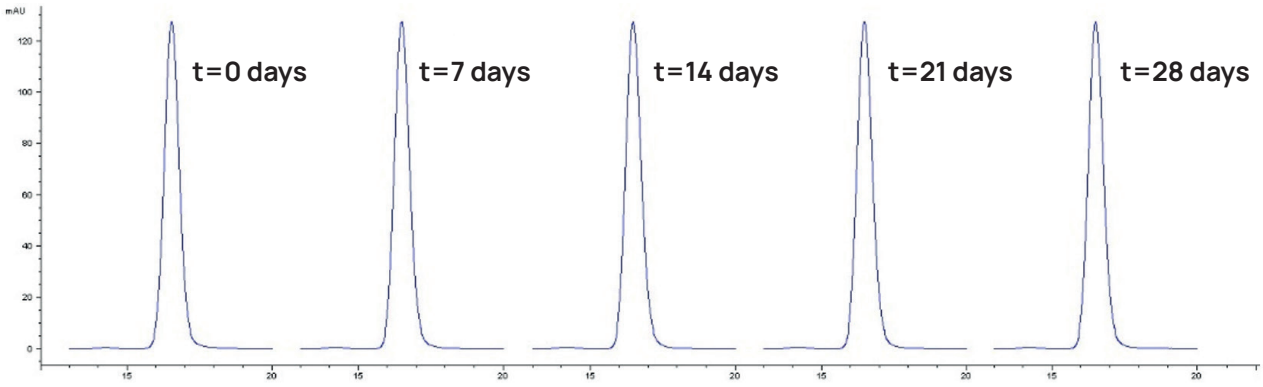


Table 3: Spray dried and reconstituted rHA SEC peak integrations (% monomer)

Sample storage time at 40°C	T=0	7d	14d	21d	28d
Spray dried rHA (% monomer by SEC)	99.3	99.1	99.1	99.2	99.3
Freeze dried rHA (% monomer by SEC)	99.6	99.3	99.1	99.5	99.4

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CASE STUDY

Spray drying complex liposomal formulations containing surface-encapsulated proteins and peptides.

Recent years have seen some new, increasingly complex biologics entering the market that use liposomes and other lipidic carriers to deliver proteins, peptides and even larger biologics such mRNA or RNA.

The pace of this development was accelerated by the recent Covid-19 pandemic with several new mRNA-based vaccines being rapidly developed and subsequently approved by regulators.

The formulation of mRNA and other biologics such as proteins and peptides in a lipid-based carrier or liposome is emerging as an important technology for creating a wide range of next-generation vaccines.

These vaccines are attracting increasing interest with the potential to deliver them via other routes such as nasal and oral delivery.

However, these lipidic carriers are notoriously unstable and shipment/storage typically requires reduced temperatures.

Extending the shelf life by removing the water is therefore an attractive proposition for not only improving stability but also enabling them to be filled into delivery devices for inhalation or formulating them into oral dosage forms such as tablets and capsules.

Delicate liposomal structures such as virosomes (vaccines based on influenza viral coat antigen extracts) and mRNA containing vaccines are irreparably damaged by processes that form ice crystals. Freeze drying is therefore not a suitable process for formulating these liposomes into a dry powder.

In a recent study[4], the team at Upperton investigated whether spray drying was capable of producing dry powder virosomal formulations since freeze drying had been shown to cause significant damage to the delicate structures.

The study involved formulating an aqueous virosomal vaccine designed to raise an immune response to the HIV virus.

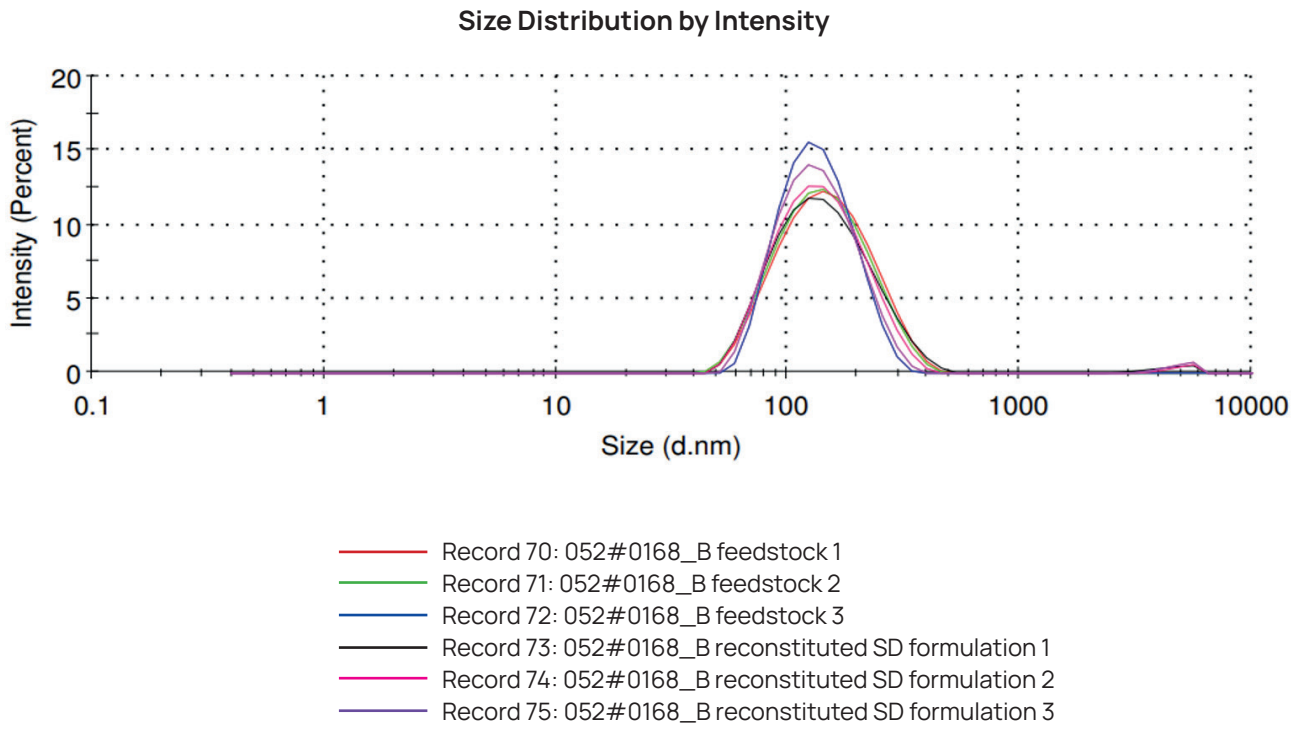
Synthetic HIV envelope glycoprotein P-1 and recombinant gp41 along with associated influenza antigens were anchored in the virosome membrane, along with the adjuvant 3M-052 (TLR7/8 agonist).

In aqueous suspension, the freshly assembled liposomes had a mean diameter generally ranging from 80 to 120 nm.

These delicate, waterfilled liposomes were spray dried at a range of drying temperatures with three different formulations, all containing trehalose as the basic matrix forming agent.

In all cases, the spray dried powders were reconstituted in water and shown to maintain the same particle size as before spray drying when analysed using a Malvern Zetasizer (see **Figure 5**).

Figure 5: Size overlay of 3 batches of formulated virosomes. Sizes are determined before spray drying and after reconstitution of the spray dried powder in water.



Closer analysis of the surface antigens using HPLC and ELISA confirmed that all of the influenza surface antigens and the critical P-1 and gp41 antigens remained unchanged after spray drying as well as the associated adjuvant.

In subsequent studies, antigenic properties of vaccinal antigens with key gp41 epitopes were maintained, preserving the original immunogenicity of the starting liquid form.

Solid forms were also exposed to a high temperature (40 °C) for up to 3 months, with minimal antigen and adjuvant content variation.

Virosomes reconstituted from the powder forms remained as free particles with similar size, and virosome uptake by antigen-presenting cells in vitro were comparable to virosomes from the liquid form.

The presence of excipients specific to each solid form did not prevent virosome transport to the draining lymph nodes of immunized mice.

Virosome integrity was also preserved during exposure to < –15 °C, mimicking accidental freezing conditions.

These 'ready to use and all-in-one' thermostable needle-free virosomal HIV-1 mucosal vaccines offer the advantage of simplified logistics with a lower dependence on the cold chain during shipments and distribution[4].

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Conclusion.

The continued growth and complexity of dosage forms containing biologics has meant that an increasing number of practitioners are assessing alternative drying technologies to traditional freeze drying technology.

Spray drying is ideally placed to become an alternative drying technology for these next-generation biologics, as sterile dosage forms or for biologics that are to be delivered via the oral, nasal or pulmonary route.

Spray drying is ideally placed to become an alternative drying technology.

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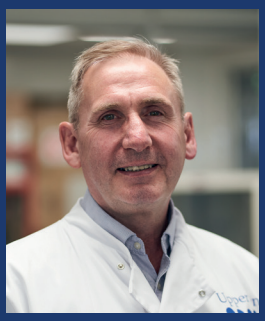
About the author



Dr Richard Johnson is the Founding Director and Chief Scientific Officer of Upperton Pharma Solutions, a UK-based Contract, Development and Manufacturing Organisation. Graduating from Warwick University with a PhD Biochemistry he was employed as a protein chemist at Delta Biotechnology. In 1994 he was part of a management buy-out team that founded Andaris Ltd, a research and development company exploiting the use of spray drying technology in the fields of diagnostic imaging and drug delivery. In 1999, Dr Johnson founded Upperton and has overseen significant growth and expansion over the past 20 years, specialising in spray drying and recently with the addition of gmp manufacturing services.

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