INHALABLE THERAPEUTIC BIOLOGICS, A PARADIGM SHIFT FOR NON-INVASIVE EFFICIENT MEDICAL TREATMENTS

In this article, Francesca Buttini, PhD, Associate Professor, University of Parma, and Susana Ecenarro Probst, Director of Scientific Business Development, Qualicaps, discuss the rising prominence of biologics in the pharmaceuticals market and how dry powder inhalers utilising hard capsules are a promising potential delivery method for these new drugs.

Therapeutic biologics led the top selling drugs in 2017[1] and are expected to experience a gradual growth in the coming years, due to a deeper understanding of some disease pathologies and further developments in biochemical engineering (Figure 1). From recombinant human insulin to interferons and monoclonal antibodies (mAbs), biologics have proved to be very effective therapeutics, improving the quality of life of patients with diabetes, infectious diseases, haemophilia and cancer.

Over 20 mAbs have been approved by the US FDA and the European Medicines Agency (EMA). Since the first approval of adalimumab in 2002, human or humanised mAbs have captured special attention and become a promising growth category within targeted therapeutic agents.[2] The FDA Center of Drug Evaluation and Research (CDER) has regulatory responsibility, including premarket review and continuing oversight, over the following categories of therapeutic biological products:

- Monoclonal antibodies for *in vivo* use.
- Plant, animal, human, or micro-organism-derived therapeutic proteins and recombinant versions of these products.
- Immunomodulators: proteins or peptides that are intended to treat or prevent disease by inhibiting or modifying a pre-existing immune response (non-vaccine and non-allergenic products).
- Monoclonal antibodies, cytokines, and growth factors intended to mobilise, stimulate, decrease or otherwise alter the production of cells *in vivo*.

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The use of the inhalation route for delivering proteins and peptides is becoming a viable proposition. Investigation into pulmonary delivery of systemic drug therapies is focused on chronic diseases and refractory ailments of both small and large molecules, including engineered macromolecules.

Although it has mainly been used to treat or alleviate local disease conditions of the lungs (asthma and chronic obstructive pulmonary disease), the advantages of pulmonary delivery over the oral route include the avoidance of subjecting the active pharmaceutical ingredient (API) to the harsh environment conditions and the enzymes in the gastrointestinal tract, as well as first-pass metabolism.[3] Additional

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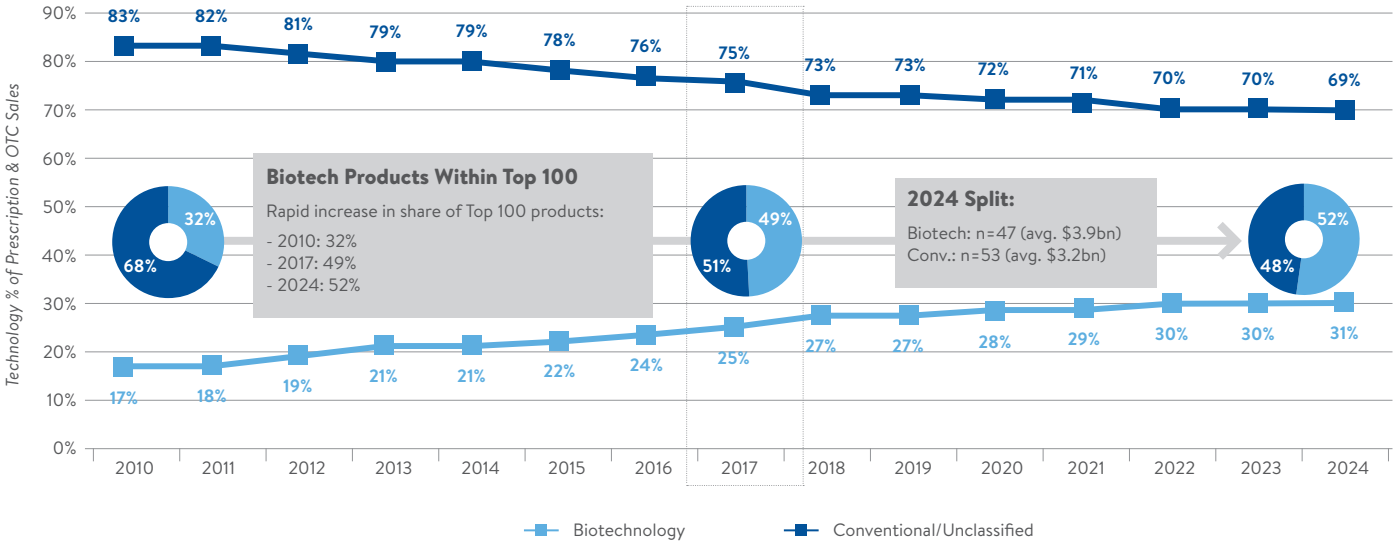

Figure 1: Worldwide prescription drug and over-the-counter (OTC) sales, biotech versus conventional technology.[1]

benefits of using pulmonary administration for therapeutic biologics include:

- Effective targeting allowing for a reduction of the total dose to be given. This reduces the adverse systemic side effects.
- Greater convenience for patients and subsequent improved compliance with the treatment compared with the invasive parenteral route.
- Dry powders for inhalation are stable, and are formulated to avoid the need for cold-chain storage or reconstitution of powders into solutions for nebulisation, which could represent an important advantage for antibiotic therapies or vaccination programmes in tropical developing countries.
- More rapid and higher extent of absorption of drugs compared with other non-invasive routes.

The biomolecules that have been studied are shown in Table 1, which includes a list of proteins/peptides and their target diseases states.

Devices for delivery via the pulmonary route have been used for a long time in their various forms: nebulisers, metered dose inhalers (MDIs) and dry powder inhalers (DPIs).[4] The first two use various solvents, both aqueous and organic, and therefore DPIs offer the best option for stabilising proteins in dry formulations. The DPI consists of unit powder doses packed in either blisters, cartridges or hard capsules. Its action is dependent on the patient's inspiratory flow rate and it is a popular device among users due to the recognised advantages, summarised in Table 2.[6]

Disease state	Therapeutic protein/peptide
Anaemia	Erythropoietin
Anticoagulant	Heparin
Cancer	LHRH analogues
Diabetes	Insulin
Diabetes insipidus	1-deaminocysteine-8-D-argenine vasopressin (dDAVP)
Growth deficiency	Human growth hormone
Multiple sclerosis	Interferon-β
Neutropenia	rhG-CSF
Osteoporosis	Calcitonin, Parathyroid hormone
Viral infections	Ribavirin, Interferon-α
Acute lung injury	Actived protein C

Table 1: Proteins and peptides proposed for delivery via inhalation. Redrawn from information in references 4 & 5.

Capsule-based DPI advantages
Breath-actuated and no need to hold breath after inhalation
Inhaled dose can be confirmed by the patient due to visibly empty transparent capsule after inhalation
Portable, designing the device with the formulation as a seperate entity minimises its dimensions
No propellant (environmentally friendly)
The powder mass in each unit is flexible, permitting use of the same device for both low and high dose volumes
Medications requiring high doses are typically delivered by single-dose reusable devices rather than multi-dose inhalers

Table 2: Advantages of capsule-based DPIs.

The manufacture of protein-based pharmaceuticals needs processes that will not damage them. This requirement has changed the formulation challenges, as these substances, because of their complex structures, are less stable than the small molecules used previously.

Important insights and challenges for inhaled proteins were thoroughly reviewed and discussed in a paper by the Université Libre de Bruxelles (Brussels, Belgium) in 2013.[7] It was highlighted that, of the three major existing inhalation delivery device types on the market, only DPIs do not use liquid formulations. The particle size and shape requirements for dry powders were related to the anatomical features of the lungs. Due to the requirement for particles with a very low aerodynamic diameter (1–5 µm), impaction in the upper respiratory tract should be considered and avoided. This could be achieved by micronisation techniques, as well as by limiting the inter-particle interactions through formulation strategies. The formulated powders must maintain the integrity of the protein and avoid physicochemical degradation, which can lead to loss of biological activity during processing and storage. In this sense, formulation strategies, such as freeze-drying, spray-drying and supercritical fluid drying, and some specific excipients to improve stability, were explained. The systemic absorption of proteins represents another potential challenge due to their high molecular weight. Absorption enhancers may overcome the crossing of the alveolar capillary membrane.

"Among proteins and peptides, insulin has, for some time, attracted the attention of several pharmaceutical companies due to the potential large sales in diabetic treatment."

A recent paper from the University of Chile (Santiago, Chile), and the University of Texas at Austin (US), summarised the challenges and prospects for delivery of biologics through inhalation.[8] For pulmonary development, product pipelines have shifted from a mix of small molecules to include biologics. The key factor for inhalation and deep penetration into the lungs is the aerodynamic particle size of the formulation. Particles with aerodynamic diameters of <3 µm will reach deep into the lungs and be absorbed into the bloodstream to treat systemic diseases. Progress requires a combination of particle engineering and device design to achieve this goal. Currently, over 30 actives are at various stages in the development process, either in preclinical testing or in Phase I and II trials.

Among proteins and peptides, insulin has, for some time, attracted the attention of several pharmaceutical companies due to the potential large sales in diabetic treatment (Table 3). Since injectable insulin was introduced into clinical practice in 1922, other routes of administration have been explored. According to the US *National Diabetes Statistics Report*, 29.1 million people have diabetes mellitus (DM) in the US, approximately 9.3% of the population, with direct and indirect costs totalling US$245 billion (£187 billion) in 2012. Zion Market Research published a report in which it stated that the global human insulin market accounted for $27 billion in 2015 and is expected to reach $43.6 billion by 2021. The market will grow at a $CAGR_{2016–2021}$ of around 8.3%.

All patients with Type 1 DM require insulin therapy. Patients with Type 2 DM may also become dependent on exogenous insulin as their disease progresses.[2] Approximately six million people in the US require insulin therapy.

The history of marketed inhaled insulin products has often been discussed, particularly the problems caused by the Exubera® insulin (Nektar, Pfizer) withdrawal from the market after a single year due to unexpectedly low sales. The failure of Exubera® may have resulted from several factors, including the high cost of the inhaler, dosing in milligrams (which may have confused patients who had been receiving conventional insulin therapy which is measured in insulin units, IU) and finally the large size of the device.

"A collaboration in 2017 between the University of Parma in Italy and Qualicaps Europe studied the chemical stability of pure spray-dried insulin produced using a patented process[11] and filled into inhalation-grade hypromellose capsules."

	Product	Generic Name	Company	Pharma Class	WW Sales (US$m) 2017	WW Sales (US$m) 2024	CAGR 2017-24	WW Market Share 2017	WW Market Share 2024	Current Status
1	Trulicity	dulaglutide	Eli Lilly	Glucagon-like peptide (GLP) 1 agonist	2,030	4,622	+12.5%	4.4%	7.8%	Marketed
2	Ozempic	semaglutide	Novo Nordisk	Glucagon-like peptide (GLP) 1 agonist	–	4,411	n/a	n/a	7.4%	Marketed
3	Jardiance	empagliflozin	Boehringer Ingelheim	Sodium glucose co-transporter (SGLT)2 inhibitor	1,139	3,510	+17.4%	2.5%	5.9%	Marketed
4	Tresiba	insulin degludec	Novo Nordisk	Insulin analogue	1,113	3,387	+17.2%	2.4%	5.7%	Marketed
5	NovoRapid	insulin aspart	Novo Nordisk	Insulin analogue	3,043	2,561	-2.4%	6.6%	4.3%	Marketed

Table 3: Predicted top five anti-diabetic products worldwide in 2024.[1]

The interest in this administration route remained high, with a second ultra-rapid-acting meal-time pulmonary insulin powder (Afrezza®, MannKind Corporation, US) having been approved by the FDA in 2015.[9] This product contains 18% insulin with fumaryl diketopiperazine used to manufacture respirable microparticles according to proprietary Technosphere® technology. Afrezza® is available in different doses (4, 6 and 8 IU), which are modulated by loading increasing amounts of powder in the device. The product has to be stored at 2-8°C and when the blister foil package is opened, it must be used within 10 days.[10]

A collaboration in 2017 between the University of Parma (Italy) and Qualicaps Europe studied the chemical stability of pure spray-dried insulin (Ins-SD) produced using a patented process[11] and filled into inhalation grade hypromellose capsules (Quali-V®-I). Capsules were semi-automatically filled with 2 mg of insulin powder using an Omnidose TT vacuum drum filler system (Harro Höfliger) and were blister packed using transparent PVC/PVDC films for storage trials. Samples were stored for six months at the ICH conditions 25°C and 60% relative humidity (climatic zone II), and at fridge conditions of 4°C.

The *in vitro* respirability test showed that Ins-SD powder had a high respirability, considering that the delivered dose was >95% and the fine particle fraction (FPF) lower than 5 µm was 91%. The percentage of the degradation products was found to be below the US Pharmacopeia limits in both storage conditions during the six months of the study. The stability outcome has demonstrated that the formulation contained in the hypromellose capsules (Quali-V®-I) together with a PVC-PVDC blister packaging material can offer a stable therapy, less dependent on cold-chain storage.

Peptides, Polypeptides (up to 50 amino acids in a chain)	Proteins (Enzymes, hormones, etc.)
SINAPULTIDE (Synthetic Peptide) Treatment: Respiratory Distress Syndrome	AFREZZA (≈6kDa) (Insulin for Diabetes)
CORUSURF (Bovine and Pig Surfactants) Treatment: Respiratory Distress Syndrome)	PULMOZYME (DNAse enzyme, 37 kDa) Treatment: Cystic Fibrosis and mucus clearance

Table 4: Marketed inhalable biologics. (Soucre: PharmaCircle)

The *in vivo* study was conducted in rats and the glycaemic plasma profile was determined after pulmonary insufflation of Ins-SD and Afrezza® powder. Male Wistar rats received a 1 g/kg glucose injection (time zero) and five minutes later 10 IU/kg of insulin were administered subcutaneously (SC) or intratracheally using a DPI device DP-4 insufflator™ (Penn-Century, Philadelphia, US). The glycaemic plasma profiles after pulmonary insufflation of Ins-SD and Afrezza® were similar, indicating that the two formulations have a similar pharmacodynamic effect. Furthermore, the glycaemic profiles after administration were similar to the plasma profile following SC insulin.

Hormones like insulin could be considered as a promising example of the paradigm shift to new, efficient, non-invasive, and therefore more convenient, medical treatments for patients. Besides the inhaled biomolecules already on the market (Table 4), there are other inhalation programmes in clinical Phase I and II.

As reviewed in this article, there are important challenges and aspects to consider in order to maintain the structural integrity and biological activity of biomolecules during the formulation process, packaging, storage, aerosolisation and in the lung environment. Capsule-based DPIs could enable more stable biologic formulations and help avoid the potential denaturation processes, monomer formation, aggregation and chemical degradation in various forms that could occur with liquid inhalation systems.

The described insulin research study has been successfully completed and provides new perspectives to develop DPI-based carrier-free formulations that could represent an encouraging future in the evaluation of high drug-load formulations with highly dispersible particles.

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ABOUT THE AUTHORS

Francesca Buttini has extensive experience in the field of development of innovative pulmonary products, in particular her main research area is the formulation and testing of DPIs. She is an expert both in particle engineering and in development of carrier-based formulations, as well as in the characterisation of the products and their dissolution. Dr Buttini is Associate Professor at the University of Parma (Italy) and in 2014 she was appointed as Visiting Lecturer at the Institute of Pharmaceutical Science at King's College London (UK). To date, she has published several original papers and patents in the field of drug delivery systems and she recently received the Drug Delivery to the Lungs (DDL) conference emerging scientist award.

Susana Ecenarro Probst is Director of Scientific Business Development at Qualicaps Europe. She supports R&D centres within the pharmaceutical industry in new drug development by providing scientific and technical expertise, as well as promoting collaborations with European universities and third parties that focus on the application of state-of-the-art capsule technologies. Prior to Qualicaps, she worked for Schering AG for 18 years, working in diverse QC positions and covering several functions, including analytical development, process validation, technology transfer, and operational excellence projects, amongst others, followed by five years of experience leading an analytical R&D unit at Bayer Healthcare. Ms Ecenarro Probst holds a MBA, and a Bachelor's degree in Pharmacy.

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