Strategies to Reduce Cold Chain Burden in Biopharmaceuticals

The availability of a wide range of therapeutic biologics has revolutionised modern medicine. Whilst the majority of pharmaceutical drug products remain conventional, small molecule medicines; the emergence of biotherapeutics has allowed for the treatment of many disease areas which were previously out of reach.[1] Despite the positive growth in biotherapeutics (Figure 1), significant challenges will need to be overcome in order to fulfil the promise of biotherapeutics across society. With a growing number of protein therapeutics and vaccines in development[2,3] one of the biggest challenges facing the product developers is the stability of these molecules.[4] Biologics including vaccines are inherently unstable and prone to degradation. Therefore these medicines typically require low temperature (cold chain) transport and storage. The result is a much higher economic burden and in some cases limited access.

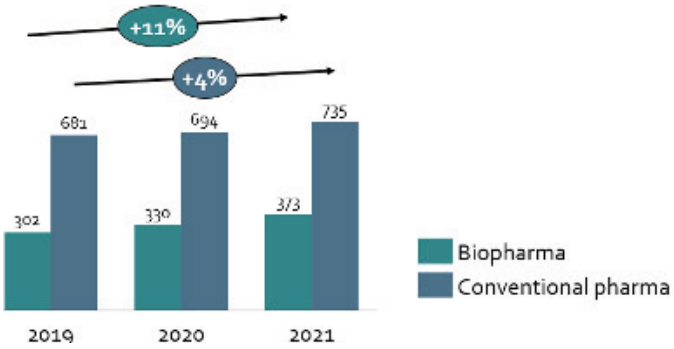


Figure 1. Source IQVIA 2022, Sales (B€) and growth (CAGR%) of Biopharmaceutical versus Conventional pharmaceutical market

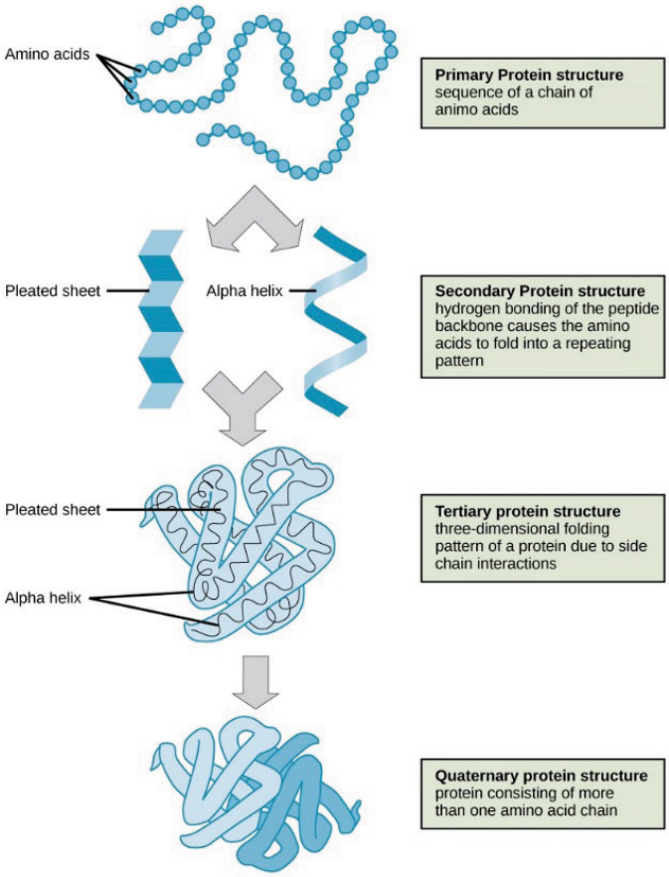


Figure 2. Multiple levels of protein structure (image: Biology by CNX OpenStax, CC BY 4.0)

Stability Challenges Slowing Future Growth

Biopharmaceuticals comprise of medium to large, complex, macromolecules that can be divided into 4 major classes; proteins, nucleic acids, lipids and carbohydrates. For the purposes of this article the focus will be proteins.

Protein Structure

Protein structure can be divided into four levels as shown in Figure 2. The 3D structure of the protein is highly complex, and inherently linked to therapeutic effectiveness. If the 3D structure is not maintained, there is a risk the therapeutic effect of the drug is reduced or lost entirely. There is also the possibility the molecule can present and immunogenicity risk to the patient. It is therefore vital to maintain the structure of the protein.

Degradation Mechanisms

The difficulty arises due to the structure of a protein being relatively weak, compared to small molecules. Numerous chemical, physical and colloidal degradation pathways can occur in biologics, a full review of these pathways is beyond the scope of this article. Henceforth, a simplified term of "stability" will be taken to mean that all degradation pathways that negatively impact activity or safety are reduced.

As a result of these numerous degradation pathways, it is typically the case that biopharmaceutics require a cold-chain to be maintained throughout the supply chain, and ultimately until the medicine is administered. If the material cannot be maintained under the required conditions then the consequence may be; reduced availability of medicines, product waste, loss of therapeutic effect or even risk of immunogenicity.

Even if a robust cold chain is in place, the financial impact of maintaining it is a significant contributor to the high price of biological medicines. Additionally, when a cold chain is available and the medicine affordable, the shelf life of biological drug product is typically short relative to conventional small molecule medicines. Below we briefly discuss the multiple strategies employed to enhance the stability of biologics and thus potentially reduce the cold chain burden.

Mitigation Strategies

There are different approaches to reducing the burden posed by

the need for cold chain storage, a non-exhaustive summary is discussed in the following sections. It is reasonable to consider that multiple strategies will ultimately be required.

Through the many steps of discovery and development of biotherapeutics there are opportunities to enhance the stability profile. Powerful *in silico* modelling tools enable screening of molecules with higher chances of developability.[5,6] During upstream processing, post translational modifications may be included to enhance therapeutic effect as well as stability. Further downstream the efficient isolation, purification and the use of an optimised buffer system offers further gains.

Downstream Processing

One of the clearest examples of a stability enhancement opportunity occurs during downstream isolation and purification. Where it is crucial to employ highly selective process steps to remove products that could lead to degradation; for example protein fragments. An often less considered area is selection of fully optimised buffer. This is a crucial step and should be made with the end use in mind. Typically the main response that is maximised is the yield. Whilst stability is also a consideration analytical methods to predict longer term stability may not be representative. As an example even trace levels of impurities introduced by lower purity ingredients, can lead to stability issues later in the products life, however rapid screening technique often cannot discern these long terms effects. Impurities may ultimately lead to aggregation and can be very challenging to debug when the aggregation is only detected in the final formulation, but actually activated during downstream processes. It is therefore prudent to take a conservative risk based approach when selecting buffer media.

Formulation

Many stability optimisation approaches are highly specific to a single molecule. They often involve expensive and time consuming studies for which the conclusions cannot be extrapolated. It would be advantageous to instead take a more generalised approach to protein stability enhancement. Degradation pathways all have a common pre-requisite, they require molecular mobility on some scale.[5] Thus if one can reduce the molecular mobility, a reduction in the rate of degradation could be expected. Removal of the continuous phase of a biological system, i.e. water, offers a great potential in the preparation of robust and stable drug product.

Several technologies are capable of removal of water from an aqueous protein solution, lyophilisation, also known also freeze drying in particular shows great promise in biopharmaceutical applications. During lyophilisation the product is initially frozen, followed by removal of water ice via sublimation. This is followed by secondary drying step resulting in a powder where a residual water content of < 1 % w/w can be achieved.[7]

There is however a complicating factor, during the freezing and drying process, the biomolecules are exposed to stresses that can lead to physical or chemical degradation of the protein. Stresses such as; pH shift, increased ionic strength, mechanical shear stress and dehydration stress may all occur[7] In order to protect the protein, a suitable combination of process parameters and excipients is required. A well-executed process optimisation and rational formulation design may result in a drug product that has good stability, in some cases a cold-chain can be entirely avoided.

Carbohydrate Systems to Maximise Stability.

The selection of excipients is critical; as the vast majority of biologics are administered parenterally[3,4] the need for high

purity excipients with an excellent safety profile is paramount, options are fairly limited. One class of molecules that show great promise are carbohydrates. Sucrose, Trehalose and Mannitol have long been known to offer cryo and lyo protection.[7] There is no general rule for best excipient selection, however trehalose has shown great promise across a wide range of difficult to stabilised molecules such as monoclonal antibodies (mAbs).

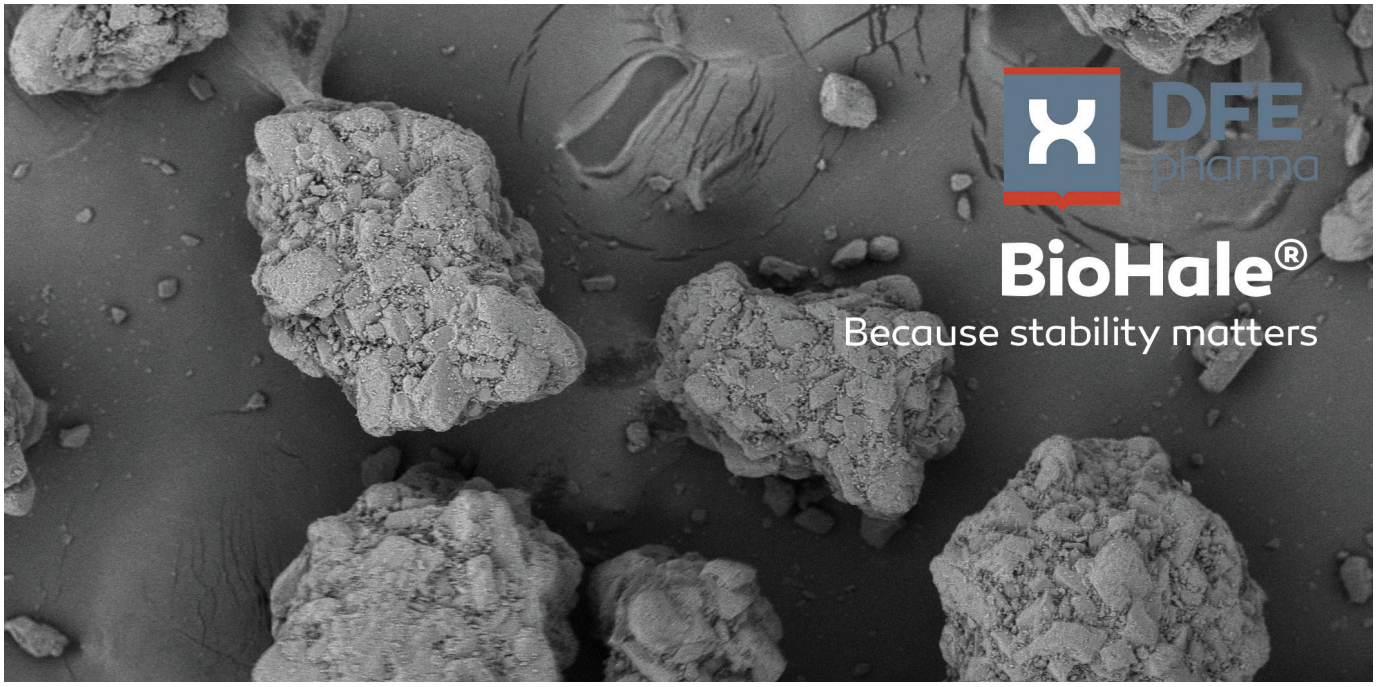
INN	Type
Durvalumab	Liquid injection
Ocrelizumab	Liquid injection
Adalimumab	Liquid injection
Blinatumomab	Lyophilised
Obinutuzumab	Liquid injection
Ranibizumab	Liquid injection
Bevacizumab	Liquid injection
Trastuzumab	Lyophilised
Brentuximab	Lyophilised
Pertuzumab	Liquid injection

Table 1. Marketed monoclonal antibody formulations containing trehalose dihydrate (non-exhaustive)[11]

The general mechanisms by which sugars stabilise proteins during lyophilisation is described by two predominant theories; water replacement theory and vitrification theory. A critical requirement in both these theories is the formation of an amorphous glass matrix, composed of the biologic, sugars and other excipients. In order to remain a glass, the product must remain below the glass transition temperature Tg of the matrix. Therefore if a glass with a very high Tg can be prepared, it is feasible that the protein may remain stable even at temperatures above ambient. Moisture also needs to be considered and it acts as a plasticiser, effectively reducing the Tg and allowing re-crystallisation at lower temperatures.

It common to utilise sugar glasses to stabilise proteins. For successful protection of the protein the sugar should have a high Tg, low hygroscopicity and a slow crystallisation rate. Additionally a high glass transition at the maximally freeze concentrated fraction (Tg') is preferable.[8] The disaccharide trehalose dihydrate exhibits all these properties and is therefore widely used. However there is compelling research indicating that incorporation of higher molecular weight carbohydrates with trehalose could enhance the stabilisation further.

In a recent study, binary mixtures of trehalose (a disaccharide) and varying levels of pullulan (a polysaccharide) were used during the preparation of lyophilised formulations using the model protein β-galactosidase.[9] It was observed that approximately 80% activity of β-galactosidase was retained for binary mixtures of specific ratios stored at 30°C / 56% RH after for 4 weeks. In environment with very low relative humidy the benefit of the binary mixture over trehalose alone was limited. However in the more realistic environment with exposure to RH > 50% the benefits were clear. The researchers noted that pullulan itself may not be the optimal polysaccharide. Despite the high molecular weight and high Tg of the material used (261°C), pullulan is a bulky molecule with a linear, rigid structure. The researchers hypothesized this could prevent tight molecular packing and limit the stabilising potential.[9]



Oligo and polysaccharides are a highly complex class of molecules and further research is ongoing.[10] However to date this formulation strategy shows great promise of a universal approach in the development of stable lyophilised protein drug product, without the necessity of an uninterrupted cold chain.

Conclusions

In order for biopharmaceuticals to reach their maximum potential across society, the industry needs to reduce the burden of cold-chain. A crucial step in achieving this ambitious goal is the availability of technologies able to stabilise proteins under more demanding environmental conditions. Lyophilisation is discussed as a critical enabling technology. Next generation sugar glass' are proposed as another critical technology. Specifically, by combining the established benefits of trehalose with higher molecular weight sugars such as inulin, pullulan or other oligo and polysaccharides, biotherapeutics that do not require cold chain storage may become reality.

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