In this article, Jan Jezek, PhD, Chief Scientific Officer, Arecor Ltd, highlights the advantages of liquid formulations of biotherapeutics compared with lyophilised powder formulations. He describes the challenges of, and numerous factors affecting, liquid formulation stability, and introduces some of the innovative techniques and technologies that Arecor has developed to improve the stability of liquid formulations of vaccines and biotherapeutics.

SMART FORMULATIONS OF LIQUID BIOThERAPEUTICS

STABILITY IS CRITICAL

The parenteral drug sector is experiencing a considerable growth, particularly due to the development of new biotherapeutics for a range of acute and chronic conditions. Although many products are still formulated as lyophilised powders requiring reconstitution prior to use, pharma companies can make considerably better use of liquid formulations, particularly in combination with convenient, user-friendly and cost-effective prefilled devices. The major benefits of the prefilled devices incorporating liquid formulations include:
• Minimisation of dosage errors due to the reduced number of steps involved
• Reduced risk of contamination
• Ease and speed of administration
• Potential for self-administration by the patient
• Improved patient compliance
• Sterility assurance
• Elimination of the need for overfill of costly biotherapeutics

Stability of a biotherapeutic, a prerequisite for the development of a successful liquid product, is a function of numerous parameters, including the formulation, interactions with the container-closure system and stress conditions. Various stress conditions must be applied during the development of a robust biotherapeutic, such as: storage (a combined function of temperature and time); shaking; freeze-thaw stress; and exposure to light.

Protein instability can be divided into two key categories: physical instability and chemical instability.1 Stability of a biotherapeutic, a prerequisite for the development of a successful liquid product, is a function of numerous parameters, including the formulation, interactions with the container-closure system and stress conditions. Various stress conditions must be applied during the development of a robust biotherapeutic, such as: storage (a combined function of temperature and time); shaking; freeze-thaw stress; and exposure to light.

Determining which stability issues are critical and which are non-critical is an important part of the product development process, together with setting specifications for impurities resulting from the degradation processes. A suitable formulation must then be developed to meet the specifications.

FORMULATION IS CRITICAL FOR REQUIRED STABILITY

Formulation is a very powerful tool for controlling biotherapeutic stability and thus achieving the target product profile. Formulation is defined by the nature and quantity of specified excipients as well as other parameters such as pH. The excipients present in the formula-
Stability improvement, particularly with osmolarity. Salts (sodium chloride, for example), are typically the dominant component in a formulation, and its level established for formulation osmolarity to maintain near-neutral pH is preferable, but generally pH 5-8 is seen as safe. However, a number of parameters should be kept within optimal limits to minimise adverse side effects. For example, there is an optimum level established for formulation osmolarity to minimise the risk of injection pain.

Typically, each component serves a specific purpose in the formulation (Figure 2). It is strongly preferable to use excipients with a history of use in approved drug products because of their established safety profile, and ideally the excipients should be used within the approved concentration limit for the intended route of administration.

Traditionally, the stability of biologics is controlled by optimisation of basic formulation parameters, such as pH, ionic strength, and the nature of the key components. Determining the optimal pH and selecting an appropriate buffer is a critical step in formulation development. Near-neutral pH is preferable, but generally pH 5-8 is seen as safe. However, a number of successful products are formulated outside of this range, for example Lantus (insulin glargine, Sanofi Aventis), which is at pH 4.0, and Fuzeon (enfuvirtide, Roche) which has a pH of 9.0. The tonicity modifier is typically the dominant component in a formulation, and its key purpose is to adjust osmolarity to a required level. Salts (sodium chloride, for example), sugars and sugar alcohols (such as trehalose and mannitol), are typically used as tonicity modifiers. Surfactants, especially non-ionic surfactants (for example, polysorbate 20 and 80), are often added to protein formulations to prevent or minimise the surface interface induced damage, particularly in the presence of shaking or agitation stress. Antioxidants (e.g. methionine) can be used to prevent chemical changes due to oxidative stress. In the case of multi-dose formulations, preservatives (e.g. m-cresol or benzyl alcohol) must be included to prevent microbial growth. Some excipients have been found to have a stabilising effect with respect to particular degradation pathways and maintaining structural integrity of the biotherapeutic, so these can be added to the formulation as stabilisers. For example, metal cations can have a stabilising effect, particularly on proteins that contain a structural metal ion. Formulations of Factor VIII contain a small amount of calcium chloride, as calcium ion binds within the structure of Factor VIII and improves its conformational stability.

Both anionic and cationic polymers have been reported to increase stability of some proteins. Cyclodextrins are another class of polymeric molecules reported and commercialised to improve stability and/or solubility of certain proteins. Amino acids, including histidine and arginine, have been described as having the ability to stabilise proteins by a variety of mechanisms, including preferential exclusion, direct protein binding and prevention of oxidation. Some proteins have also been used as excipients to improve stability, for example human serum albumin (HSA) and hydrolysed gelatin (predominately used in vaccine formulations).

In order to develop better liquid formulations of biotherapeutics it is necessary to broaden the formulation design space, including:

- Screening of new excipients
- Understanding beneficial synergistic effects between excipients
- Understanding the synergies between the effect of excipients and other conditions such as ionic strength
- Understanding the destabilising effects of specific conditions and excipients

Whilst computational tools, such as Design of Experiment can be very helpful to map out the design space, the number of possible permutations of excipients and other parameters is vast. Traditionally, the stability of biologics is controlled by optimisation of basic formulation parameters, such as pH, ionic strength, and the nature of the key components. Determining the optimal pH and selecting an appropriate buffer is a critical step in formulation development. Near-neutral pH is preferable, but generally pH 5-8 is seen as safe. However, a number of successful products are formulated outside of this range, for example Lantus (insulin glargine, Sanofi Aventis), which is at pH 4.0, and Fuzeon (enfuvirtide, Roche) which has a pH of 9.0. The tonicity modifier is typically the dominant component in a formulation, and its key purpose is to adjust osmolarity to a required level. Salts (sodium chloride, for example), sugars and sugar alcohols (such as trehalose and mannitol), are typically used as tonicity modifiers. Surfactants, especially non-ionic surfactants (for example, polysorbate 20 and 80), are often added to protein formulations to prevent or minimise the surface interface induced damage, particularly in the presence of shaking or agitation stress. Antioxidants (e.g. methionine) can be used to prevent chemical changes due to oxidative stress. In the case of multi-dose formulations, preservatives (e.g. m-cresol or benzyl alcohol) must be included to prevent microbial growth. Some excipients have been found to have a stabilising effect with respect to particular degradation pathways and maintaining structural integrity of the biotherapeutic, so these can be added to the formulation as stabilisers. For example, metal cations can have a stabilising effect, particularly on proteins that contain a structural metal ion. Formulations of Factor VIII contain a small amount of calcium chloride, as calcium ion binds within the structure of Factor VIII and improves its conformational stability.

Both anionic and cationic polymers have been reported to increase stability of some proteins. Cyclodextrins are another class of polymeric molecules reported and commercialised to improve stability and/or solubility of certain proteins. Amino acids, including histidine and arginine, have been described as having the ability to stabilise proteins by a variety of mechanisms, including preferential exclusion, direct protein binding and prevention of oxidation. Some proteins have also been used as excipients to improve stability, for example human serum albumin (HSA) and hydrolysed gelatin (predominately used in vaccine formulations).

**EXPLORING A BROADER FORMULATION DESIGN SPACE**

In order to develop better liquid formulations of biotherapeutics it is necessary to broaden the formulation design space, including:

- Screening of new excipients
- Understanding beneficial synergistic effects between excipients
- Understanding the synergies between the effect of excipients and other conditions such as ionic strength
- Understanding the destabilising effects of specific conditions and excipients

Whilst computational tools, such as Design of Experiment can be very helpful to map out the design space, the number of possible permutations of excipients and other parameters is vast.
Figure 4: Recovery of antigenic activity of hepatitis B vaccine formulations at 45°C. The formulations were: (A) original Shanvac-B formulation; (B) 40 mM phosphate, 40 mM histidine, 100 mM NaCl, pH 5.2.

The optimal formulation of hepatitis B vaccine developed at Arecor, based on 40 mM histidine and 40 mM phosphate at pH around 5.0 showed considerably improved stability at elevated temperatures compared with the currently marketed product, Shanvac-B (Shantha Biotechnics Pvt Ltd (Sanofi), Hyderabad, India) (see Figure 4). The stability of the product was also confirmed in an animal study.

The new vaccine formulation has the potential to be used outside the cold chain for part of its shelf life. This is very likely to improve the immunisation coverage, simplify the logistics for outreach immunisation, and ensure the potency of the vaccine in areas where the cold chain is insufficient.

Another example of the application of two of Arecor’s technologies is shown in Figure 5, using erythropoietin (EPO). The formulation of the existing marketed liquid product was compared with the new formulation based on Arecor’s technology, using a combination of TRIS and benzoate anion (i.e. dual buffer system with pKa values >1 unit from the pH of the composition instead of phosphate (i.e. a conventional buffer)). The formulation change resulted in a considerable improvement of stability with respect to aggregation (Figure 5).

Additional stability was achieved through using a second technology, the use of small amphiphilic species under specific salt conditions. It should be noted that the use of a small amphiphilic species (in this example, the benzoate anion) was found to be essential for achieving the required stability. It is believed that the synergistic effect of benzoate anion and salt conditions is due to non-covalent interactions with hydrophobic patches on the surface of the proteins.

CONCLUSION

Formulation is a very powerful tool to control the stability of biotherapeutics. Conventional approaches to formulation are well established and in many cases result in satisfactory stability to support suitable target product profiles. However, with increasing commercial pressures, it is essential to seek further improvements in stability to allow more convenient drug delivery options and, where possible, permit next-generation biotherapeutics. Innovation in formulation science is therefore very important.

As shown in this article, rational formulation design can lead to novel formulations with considerably improved stability. Improvement can be achieved by eliminating excipients that are conventionally used in formulations as well as exploiting specific properties of excipients to achieve the required stability. The understanding of the physical and chemical degradation
pathways of proteins has enabled this rational design approach. The design and success of this formulation approach will be continually improved to tackle new demands from the biopharmaceutical industry and regulators, based on novel insights into protein stability through the development of new analytical technologies.

REFERENCES

Advanced Formulation of Biologics

Areccor Limited was established to provide formulation solutions to pharmaceutical and biotech companies developing drugs, vaccines, medical devices and diagnostics.

Areccor has developed Arestat™, a patented set of tools for stabilization, to enable the presentation of labile biologics as stable aqueous formulations even at high concentrations or in situations where cold storage is not practical or desirable.

As a simple reformulation, Arestat™ can be readily incorporated into standard manufacturing practice, without covalent modification of the biological molecule and using excipients approved for the route of delivery.

Arestat™ can also protect proteins from degradation in the presence of ionizing radiation typically used in the sterilization of medical devices.

Areccor currently has active feasibility programmes and licenses with most of the world’s leading biopharmaceutical companies on a wide range of proteins and vaccines.

Areccor Limited 2 Cambridge Science Park, Cambridge CB4 0FE, United Kingdom
Tel: +44 (0)1223 426060  Fax: +44 (0)1223 423411 Email: info@areccor.com
www.areccor.com

Biopharmaceuticals  Diagnostics  Animal Health