

Formulation and manufacturability of biologics

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An important challenge in the pharmaceutical development of a biologic is the optimization of safety and efficacy while ensuring the ability to manufacture the drug while maintaining quality and stability. The manufacturing process consists of several operational steps referred to as 'unit operations' where the biologic is subjected to different stresses and conditions that may compromise quality and stability. Moreover, recently the requirement for the development of subcutaneous formulations for high dose drugs, such as monoclonal antibodies, at high protein concentrations has created additional challenges for many of the unit operations. These challenges can be mitigated by modification of the manufacturing process and/or development of formulations to prevent degradation. In particular, formulations have been designed to minimize protein aggregation and decrease viscosity, which has led to successful manufacture of the biologic.

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Introduction

Before the advent of recombinant DNA technology, biologics were produced from either animal tissue, for example pancreatic tissue for insulin, pituitary glands for human growth hormone or blood, for example blood coagulation proteins such as Factor VIII. However, with the commercialization of recombinant DNA technology, the shift has been to produce human versions of protein drugs from bacterial fermentation or mammalian cell culture. The pharmaceutical development of these biologics requires the selection of a formulation that is based on optimizing safety and efficacy while ensuring the ability to manufacture the drug while maintaining quality and stability. In addition the formulation needs to be appropriately designed for its intended route of administration, as well as the 'marketability' of the final drug product (DP). The latter is often tied in with the

challenges of a marketplace with a competitive environment, where products that more adequately address the needs of patients through ease of use, have an advantage. However, not to be overlooked is the industry's responsibility to address the needs of patients even in noncompetitive environments. Thus, the development of a biologic candidate into a successful DP must address all these challenges leading to a set of requirements often referred to as the 'Target Product Profile' or TPP. In this paper we will focus on the challenges and issues that need to be addressed during the design, development, and scale-up of a formulation to enable consistent and robust performance in a manufacturing environment.

The manufacturing of these biologics begins with the expression in biologic, cell-based systems of proteins that then requires recovery and purification, often by multiple chromatographic techniques. The general overall bioproduction process is executed in a series of steps often referred to as 'unit operations'. Herein lies one of the biggest differences between small molecule and biologic development. Instead of obtaining a drug candidate from chemical synthesis that leads to separate efforts to prepare formulated drug, the manufacturing step for the formulation of a biologic is often the last step in the recovery and purification process. Thus the final recovery and purification step requires a technology that can efficiently exchange the components of the chromatographic eluent into the components of the formulation for the bulk drug substance (DS). The DS is further subjected to additional unit operations to eventually obtain the final DP, each of which may subject the therapeutic protein to a variety of stresses. Thus, the success of these processing steps will be highly dependent on the appropriate design of the formulation as well as modifications of process and process equipment. The focus of this review will be on the interplay between formulation and process design, and the needs of key unit operations that occur from the initial DS manufacturing step to the DP in its final container/closure system.

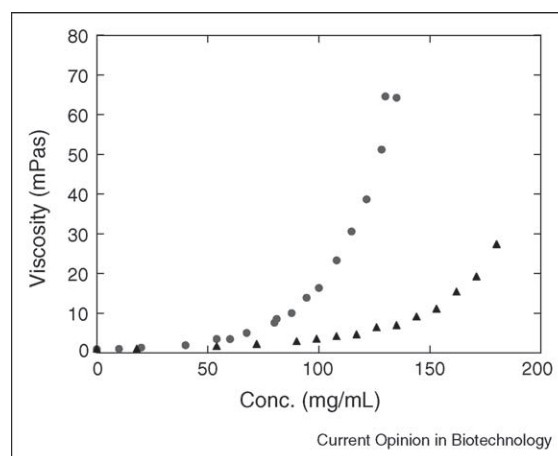
Drug substance manufacturing

Solution exchange technologies

After elution from the final chromatographic recovery/purification step a unit operation is required to exchange the components of the chromatography elution buffer with the chosen formulation components. This can be done using size exclusion chromatography or binding followed by elution from ion exchange resins. However, the use of buffer exchange or size exclusion may result in further dilution of protein and large volume handling requirements, whereas bind/elute ion exchange

chromatography may be limited by ionic strength and pH requirements of the elution buffer necessitating additional exchange steps. The predominant technology has been used in the industry for buffer exchange and concentration is a form of ultrafiltration/diafiltration called tangential-flow filtration (TFF) [1–3]. In this technology, the protein solution is recirculated under pressure, tangentially to an ultrafiltration membrane. A pressure differential is maintained across the membrane, which allows for the selective passage of water and small solutes but not macromolecule of interest. This technology works well for formulated DS at low to moderate concentrations of protein drug. However, the emergence of monoclonal antibody therapies for home-use, subcutaneous (SC) administration, has necessitated the development and manufacture of high concentration formulations >100 mg/mL [4••]. The attainment of high concentration formulations by TFF systems can be difficult because the required transmembrane flux may lead to a higher concentration gradient at the membrane boundary. Depending on a protein's propensity to interact with and unfold at the surface, this could lead to decreased flux and eventual membrane fouling. Proteins are sensitive to generated air–water interfaces, resulting in denaturation [5–7] and the continuous circulation through tubing and pulsation of the pumps may also cause cavitation [8], which results in protein unfolding and precipitation. Thus, formulations that can stabilize the native protein structure when exposed to these stresses may mitigate some of these problems. In addition to the stresses encountered by proteins during TFF, another potential limitation is the ability to concentrate highly viscous protein solutions. In particular, high viscosity may lead to high back-pressures, exceeding pump capacity during the TFF process. Moreover, as the viscosity increases during protein concentration, the consequent reduction in the diffusion coefficient can lead to a very high protein concentration at the membrane causing a further decrease in the transmembrane flux. The higher viscosity makes it increasingly difficult to remove the concentrated protein from the TFF unit, leading to low yields for the process that may be economically unacceptable. While some of these challenges can be overcome by redesign or modification of the TFF equipment, it can lead to a greater expense as well as significant delays in development timelines. Design of an appropriate formulation to decrease solution viscosity may help address some of these manufacturing process limitations. Successful implementation of a formulation design to decrease solution viscosity at high concentration is shown in the viscosity profile for an IgG1 monoclonal antibody (hereafter referred to as MAb1), where the addition of appropriate excipients lowered the viscosity dramatically (Figure 1) [9,10••]. The large viscosity in this antibody is caused by reversible protein self-association that appears to be governed by electrostatic attractive interactions at high concentration. Increase of the formulation

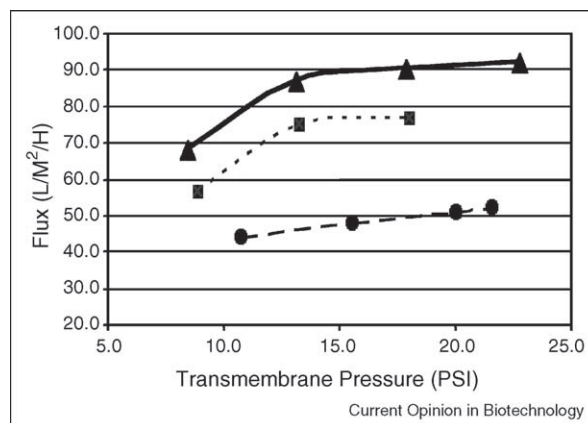
Figure 1



Viscosity versus concentration for an IgG1 monoclonal antibody (MAb1). Formulation without (●) and with (▲) added excipients that decrease viscosity.

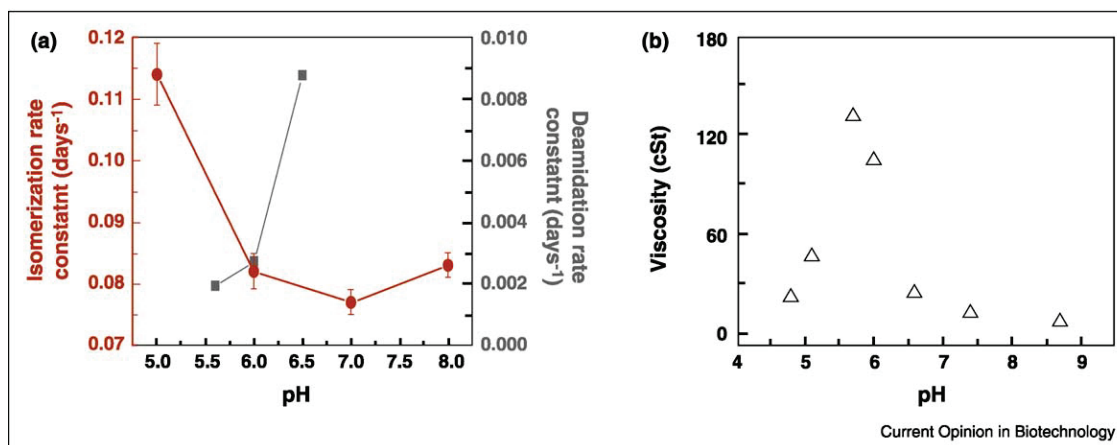
ionic strength or addition of particular compounds such as arginine can decrease these interactions and viscosity [11,12••]. In situations where greater decreases in viscosity are required, additional improvements in the TFF process can be achieved by running the process at higher temperature [13••,14]. At the higher temperatures where the viscosity is lower, greater membrane flux for MAb1 can be attained at a specific transmembrane pressure (Figure 2). However, in such cases, protein stability may be compromised by prolonged exposure to higher processing temperatures. Formulation design thus becomes critical for not only lowering the viscosity but also controlling degradation at the higher temperatures. This can be a very challenging exercise since physical degradation and chemical degradation routes may have

Figure 2



The tangential-flow filtration (TFF) flux (L/M²/H) versus transmembrane pressure (PSI) at different temperatures for MAb1, initially at 30 mg/mL and at 23 (●), 40 (■), and 46°C (▲).

Figure 3



(A) The pseudo-first order rate constants for isomerization (●) and deamidation (■) versus pH for MAb1. (B) The kinematic viscosity at 25°C versus pH for MAb1 at 130 mg/mL.

very different dependencies on solution conditions. This is exemplified by the behavior of MAb1 shown in Figures 3A and B where the isomerization and deamidation degradation kinetics are optimized at a pH where the viscosity is at a maximum value. Ultimately it may not be possible to control degradation only by formulation design, necessitating evaluation, and optimization of the impact of the processing.

Bulk DS storage

Once formulated, the DS needs to be stored in order to manage product inventory, that is the results of a manufacturing campaign may produce large quantities of DS that needs to be stored over a period of time. It is also often desirable to minimize manufacturing campaigns, and thus a robust formulation needs to be provided to ensure stability of DS that can be as long as five years. Freezing of DS bulk solutions is an obvious strategy that has been widely used in the industry [15[•]]. This requires filtration and filling of large volumes of DS into storage vessels followed by freezing. The filtration using 0.2 µm filters may be more difficult at high protein concentrations especially with high viscosity [16,17], and thus formulation to lower viscosity may be beneficial at this step. After sterile filtration the freezing step at large volumes can lead to cryoconcentration as a result of water freezing and exclusion of formulation solutes and protein [18,19]. If the solution/ice mixture is not mixed during thawing, the protein will be exposed to concentration and thermal gradients. In addition the generation of a matrix consisting of ice and protein can result in the alteration of protein conformation near the ice surface due to weakening of hydrophobic bonds and protein adsorption onto the ice surface [20,21]. The disruption of protein structure may result in the exposure of hydrophobic

portions normally found in the interior of the protein, and coupled with the increased concentration of protein during freezing, can lead to increased protein aggregation. Thus freeze-thaw vessel design is very important and several systems have been created that ensure efficient heat transfer and mixing during the thawing step [15[•],22,23]. Although the development of freeze-thaw systems can minimize the formation of gradients during thawing, the protein is still exposed to ice surfaces during the freezing process. Thus, formulation excipients such as sugars, which can function as cryoprotectants can help stabilize the protein conformation, and are often included [24[•],25]. An important consideration of such excipients is that they are not prone to crystallization during freezing [26]. Mannitol and recently sorbitol, have proven to be problematic as protein stabilizers since they readily crystallize resulting in a phase separation whereby the sugar is no longer able to interact and potentially protect the protein from damage during freezing [27–29]. Formulation components that have been shown to be effective as cryoprotectants include disaccharides such as trehalose and sucrose [24[•]], although recent evidence suggests that trehalose may be prone to crystallization during freezing making it a less attractive choice than sucrose (T Patapoff, unpublished data). When considering sugars as cryoprotectants it is important to avoid reducing sugars, which can result in glycation adducts to primary amines [30,31]. However, sucrose although not a reducing sugar, can hydrolyze at lower pH resulting in the formation of the reducing sugar glucose [32,33]. Sucrose formulations at pH 6 or greater are usually not an issue, but buffer salts that are prone to crystallization such as dibasic sodium phosphate can lead to large decreases in pH (from 7 to 4) and should be avoided [24[•]]. Another important consideration in formulation design for DS frozen bulk storage is

the glass transition temperature T_g' of the ice/water/solute frozen matrix. Storage of the frozen formulation at temperatures above the T_g' results in greater molecular mobility and can result in enhanced degradation, especially protein aggregate formation [34,35]. Typical T_g' values for carbohydrate-based formulations are at approximately -30°C [24[•]] and thus storage of large volumes at temperatures lower than -30°C is desirable, but often not practical or economical. Tonicifiers such as NaCl are typically added to formulations to create isotonic solutions that prevent cell rupture, particularly in the case of red cells. However, the use of NaCl should be carefully evaluated because at -21°C water and NaCl form a eutectic mixture where the NaCl is at 23.3% w/w, which could enhance the mobility at -20°C [36], a temperature typically used for large-scale bulk storage [15[•]]. In addition, the common stainless steel alloy 316L used in freezing/storage vessels is susceptible to corrosion induced by chloride [37]. The metal corrosion and leaching of metal ions into the protein formulation can lead to metal-induced oxidation degradation of the protein drug [38]. Thus development of formulations may require a screening of other counterions, which do not promote metal corrosion. Development of alternate materials, especially plastic disposables for freeze-thaw storage systems are attractive but will require a thorough analysis of potential leachates from the plastic materials into the protein formulation [39,40]. Another consideration as to how formulation can benefit this particular unit operation is the development of higher concentration DS formulations that can be frozen resulting in a decrease in the volumes used for storage, which then require less tank storage. Alternatively bulk freeze-drying or spray drying formulations could be developed. The literature is replete with examples on formulation development using such processes [41^{••},42–50]. Although freeze-drying for bulk storage could be done in trays, spray drying would require appropriate collection of the powder and then aseptic filling into the storage containers.

Formulation and manufacturability of drug product

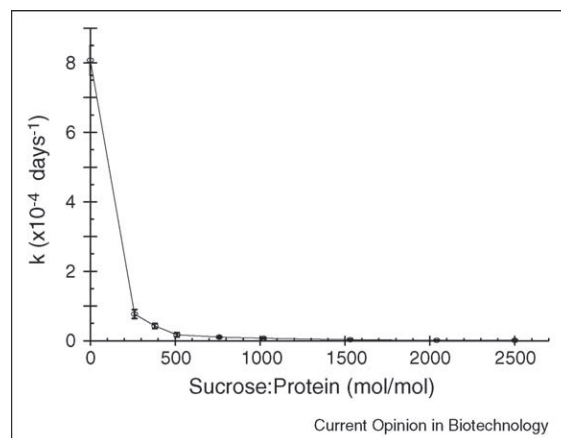
The manufacturing challenge for DP will be dependent on the final form chosen. For liquid products, especially at high concentration, addition of viscosity lowering excipients may be required since the viscosity of the product can impact sterile filtration before fill, as well as the accuracy of the filling equipment. In particular, highly viscous liquids may result in excessively long times to filter DP as in the case of DS or result in 'hanging drops' at the tip of the filling tube resulting in carry over into the next vial. Generation of air–water interfaces and pump cavitation during filling can induce protein denaturation but can be mitigated by the addition of surface-active agents such as surfactants to the DP formulation. However, some of the common surfactants that are used such as polysorbate 20 and 80 are prone to degradation, which

may impact the stability of the protein drug [51]. In particular, oxidation of amino acid residues such as methionine has been shown to occur as a result of peroxides in the surfactants [52].

The choice of the final container/closure may also dictate choices for formulation components. Prefilled syringes tend to expose the protein to higher levels of silicone oil, which can lead to increased aggregation [53,54]. Recent experience with Eprex illustrates the problems that may occur as a result of formulation changes and leachates from drug contact surfaces. In that particular example it is proposed that addition of the surfactant polysorbate 80 may have increased the amount of leachates into the formulation that enhanced an anti-erythropoietin immunogenic response leading to pure red-cell aplasia (PRCA) in patients [55]. Other potential leachates, such as tungsten salts and oxides that result from the process used to make staked needle syringes have been implicated in increasing the propensity for aggregation [56[•]]. If this problem cannot be handled by formulation then the manufacturing process may need to be altered to minimize the levels of tungsten.

In the case of solid, freeze or spray dried dosage forms excipients that can act as cryoprotectants as well as drying protectants need to be added. Excipients such as sugars and surfactants have been shown to be effective in protecting the protein from the stresses encountered during typical drying techniques such as lyophilization or spray drying [19,24[•]]. A common degradation pathway that occurs during lyophilization is protein aggregation, which can be controlled by such stabilizers (Figure 4). The addition of sugars as protective agents during drying is believed to occur via one or both of two proposed mechanisms. One is the formation of a glassy amorphous state where molecular motions are decreased relative to

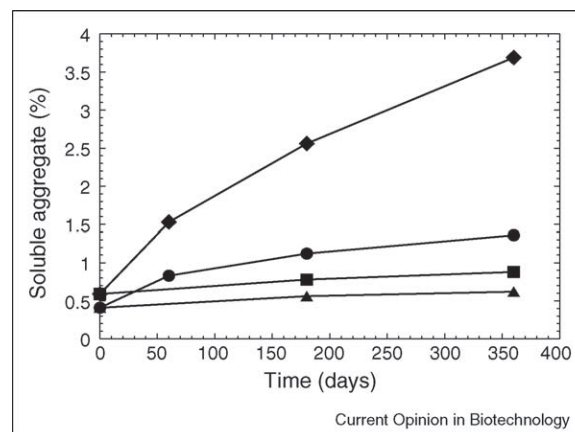
Figure 4



The pseudo-first order rate constants at 50°C for aggregation of lyophilized MAb1 versus sucrose:MAB molar ratio.

proteins in solution and the other is through the ability of sugars to replace the lost hydrogen bonding from removal of water [19,57^{*}]. Recent studies have further elucidated how sugars such as sucrose protect proteins during lyophilization [41^{••}]. Surfactants when added to solid, dried dosage forms are useful in preventing the formation of particulates during the reconstitution process. An essential property of these dosage forms is that the excipients remain in an amorphous state since crystallization results in a phase separation whereby the stabilizer no longer can interact with the protein [28]. Another aspect of solid dosage form development is the possibility of developing high dosage forms for SC administration. Although, as discussed previously, TFF for DS is the industry standard for concentrating macromolecules at large scale, there are alternatives. Attainment of high DP concentrations can be achieved by any drying process, which if done by bulk processing can substitute for the DS TFF concentration step. Essentially, any drying technique such as freeze-drying in trays [58], spray drying [45,59], fluid bed drying [60,61], or vacuum drying [23,62] with a subsequent reconstitution at lower volumes can be used. Such a manufacturing process will likely require redesign of equipment to facilitate reconstitution and removal for fill. As an example, lyophilization trays with good drainage and valve ports to assure quantitative removal of product would be required. Undoubtedly formulations would need to be designed for low viscosity to assure acceptable flow to permit good recovery. One advantage of such a process would be less of a requirement to have rapid reconstitution times since this is a bulk preparation method. Overall such technologies circumvent the problem of concentrating DS to high concentrations, and thus the traditional usage of TFF at the DS formulation stage becomes more tenable. An alternative approach of drying sterile filtered products in vials at the DP manufacturing stage essentially transfers the unit operation to concentrate DS or DP to high concentrations to the end user, that is the patient or pharmacist performs the concentration during the reconstitution step. Lyophilization is a common technology used by the pharmaceutical industry and is the logical drying method for this alternate approach to generate formulations for high dose SC administration [63^{••}]. Essentially a loading volume of protein, V_L , at loading concentration C_L is lyophilized and then reconstituted with a volume of diluent, V_R , where $V_R < V_L$. The final concentration of the DP, C_F , as well as all formulation components will be concentrated ($V_L/V_R + V_S$) fold, where V_S is the volume contributed from the solids present after lyophilization. As previously discussed, lyoprotectants such as sucrose, are added to the formulation to control protein aggregation. In the example shown in Figure 4, MAb1 aggregation at high temperature storage (50°C) is minimized at a protein:sucrose 500:1 molar ratio. Formulations were developed that resulted in ~3-fold concentration during reconstitution. Two formulations that were considered

Figure 5



Soluble aggregate kinetics for lyophilized MAb1. Lyophilized formulations for sucrose:MAB molar ratio after reconstitution of 250:1 stored at 5°C (■) and 30°C (◆) and sucrose:MAB molar ratio after reconstitution of 500:1 stored at 5°C (▲) and 30°C (●).

resulted in an isotonic solution after reconstitution with final protein:sucrose molar ratio of 250:1 or one that was hypertonic at 500:1. Although isotonicity is not necessarily required for SC administration, it may be desirable for minimizing injection pain or inflammation [64] or tissue damage [65]. Thus, in order to lower tonicity the amount of added protectants must be decreased. The challenge then is to add sufficient lyoprotectants to attain acceptable stability while assuring a final reconstituted DP solution that is isotonic. As shown in Figure 5, the formulation with a final reconstituted 500:1 sucrose:protein molar ratio for MAb1 was significantly more stable at controlled room temperature (30°C) than at the lower 250:1 molar ratio. If stability were the only concern then this would have been the appropriate choice. However, this MAB was going to be used mainly in clinics and doctors offices in the US market where refrigeration was readily available. At 5°C storage there was little difference in stability between the 250:1 and 500:1 molar ratio formulations, and thus the lower ratio was chosen to assure an isotonic formulation.

Conclusion

The manufacturing of therapeutic proteins requires several unit operations, which subject the protein to different environments and stresses. The change in conditions and stresses can lead to physical and chemical alterations of the protein drug, which may impact safety and efficacy. In addition, physical properties such as high viscosity may make it difficult to conduct the unit operation successfully to meet its targeted purpose, as is the case for attaining high protein concentration formulations for SC administration. It may be possible to mitigate these problems by redesigning processes and equipment or in

the case of final DP changing the container/closure surfaces. Alternatively, development of appropriate formulations can aid in the manufacturing step, by protecting the protein from the stresses encountered during the particular operation or overcoming the process limitations. Examples of both strategies have been discussed in this review. Overall, the development of a formulation is not required just for stability and long shelf life of the final product, but also to enable successful manufacturing of the protein drug from DS to final DP.

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