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Commentary

Development of high concentration protein biopharmaceuticals: The use of platform approaches in formulation development

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ABSTRACT

The development of highly concentrated solutions of monoclonal antibodies has become increasingly popular in biotechnology firms as doses increase, and the demand for sub-cutaneous injection formulations rises for reasons of convenience and compliance. Protein concentrations often exceed 100 mg/mL. Unfortunately, the reduced success rate of commercialization of biotechnology products result in a situation in which the majority of biologics that enter clinical trials have a very low probability of becoming commercial products. Under these circumstances, the formulation scientist must make judicious use of his or her available time and resources and must avoid over-investing in early phase 1 compounds. This circumstance has driven many laboratories to develop so-called "platform formulations" in which a suitable, high concentration, robust formulation, or dosage form is utilized broadly across a number of early stage biologics at a savings of time and effort. This highly pragmatic approach increases the efficiency of developing early clinical candidates with fewer resources, while maintaining clinical flexibility, thus saving their effort for later stage candidates for which proof of concept has been demonstrated and for which a more optimized and robust formulation and process, suitable for commercial application, must be developed.

1. Introduction

Monoclonal antibodies are being developed for a variety of indications at increasingly higher doses. They represent the largest growing segment of products from the biotechnology industry with 26 products currently approved in the United States [1]. The requirement for elevated doses (cetuximab is dosed at 250–400 mg/m^2; efalizumab at approximately 1 mg/kg), as well as a strong desire to provide a convenient sub-cutaneous dose, to increase the level of compliance and provide greater flexibility to the patient and the health care provider, results in the need for increasingly higher concentration proteins. Recently approved products have pushed the solubility limits for biopharmaceuticals and have yielded very high concentrations (omalizumab is 125 mg/mL post-reconstitution, certolizumab pegol is 200 mg/mL post-reconstitution) as well as elevated viscosities. Clearly, there is an evolving need to routinely generate highly concentrated monoclonal antibody solutions for clinical testing.

Despite the need for innovative solutions to unmet medical need, the success rate for the development of biotherapeutics is limited. An FDA report from 2004 indicated that the success rate from Initial Investigational New Drug (IND) to successful licensure is approximately 8% [2–5]. With this poor success rate, one must ask whether the extensive investment in early stage process and product development, with the goal of developing commercially acceptable types of processes and dosage forms, is warranted for an early stage biologic in which so little is known. The development of a mature dosage form requires an understanding of dose (mg/kg or fixed dose), route of administration (sub-cutaneous, intra-venous, or intra-muscular), market need (self-administered, in-home, doctors office, or clinically administered), safety, and efficacy. Often, these parameters are not well defined until late phase 2 proof-of-concept studies have been completed. Without this information, the formulation scientist should be less concerned with potential commercializability of the phase 1 product and more concerned with providing a flexible and stable dosage form for phase 1–2 clinical trials. For the clinic, the dosage form must meet certain quality requirements pertaining to safety, stability, and purity, but it must also provide the clinician with flexibility in dosing across a broad range of dose levels (typically 0.1–20 mg/kg for a monoclonal antibody, depending on target indication) as well as flexibility across routes of administration (typically sub-cutaneous and intra-venous). As a result, biotechnology firms are developing flexible, stable dosage forms that provide monoclonal antibodies at concentrations of approximately 100 mg/mL. This

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approach has reached patients in the form of commercial products such as efalizumab, palivizumab, and golimumab which are formulated at 100 mg/mL.

2. Rationale for platform approach

Standardized approaches for development and manufacturing have been utilized for many centuries. Whether mass production of automobiles or the efficient utilization of pre-existing technologies for cellular phones, firms and individuals strive to utilize existing, often proprietary approaches and experience and wish to avoid having to re-invent already existing technologies. The biotechnology industry is no different. Various laboratories have their preferred host cell lines [6], expression media, and purification processes. Recent publications describe platform purification processes for monoclonal antibodies. Tugcu et al. [7] describe the use of a protein A capture step followed by anion and/or cation exchange chromatography for a three column purification of monoclonal antibodies. Kelley et al. [8] describe a two column purification process in which protein A affinity chromatography is followed by a weak partitioning anion exchange resin. These examples illustrate that, at least for monoclonal antibodies, one can utilize routine purification processes which, although they may need to be slightly modified to accommodate the characteristics of a specific protein or host cell system, should provide an adequately reproducible and suitably pure drug substance which can then be utilized in formulation studies. One aspect to consider in the development of platform approaches, formulation or otherwise, is that they are not intended to be successful 100% of the time. One must be content to cover the majority (e.g. >80%) of monoclonal antibodies and benefit from the savings in time and effort. Early screening of expression levels, ease of purification and accelerated stability and solubility studies can help to screen the 20% of compounds that may not fit into the platform approach.

With regard to formulation design, recent articles have focused on the use of broad arrays of formulation approaches to determining the most suitable formulation for a given product. Specifically, high-throughput approaches have been developed for the thorough screening of conditions for difficult to formulate proteins [9,10]. In addition, tremendous experience has been gained in the development of lyophilized powders for proteins. Monoclonal antibodies, in contrast to cytokines and growth factors, appear to be a more homogenous set of proteins to formulate the majority of the time. One may choose, therefore, to forgo the desire to optimize a formulation for a monoclonal antibody prior to phase 1 clinical trials, especially due to the significant number of unknown elements associated with a pre-clinical project, and rather adopt the approach of a standardized or platform formulation. What is described in the following sections should be considered guidance pertaining to a platform formulation approach which utilizes a standardized or platform formulation of defined protein concentration and excipients.

3. Platform approaches to dosage form development

The first steps to designing a suitable dosage form, and formulation, is to gather information on the effect of pH with regard to the protein's behavior. The pH of the formulation must be selected to minimize a wide variety of possible degradation mechanisms including aggregation, poor solubility (partially based on the pI of the protein), and chemical degradation by oxidation, deamidation, and hydrolysis just to name a few mechanisms [11]. Many of these chemical degradation mechanisms can be managed effectively by lyophilization [12], however the formulator must also consider the effect of short-term hold times during the manufacturing process as well as just prior to administration. In order to assess a suitable pH, however, one does not necessarily have to perform large numbers of experiments with multiple buffers under a variety of stress conditions. Rather, one may wish to begin by first learning from the work of others.

Table 1 presents a list of formulations for recently approved antibodies. The information contained in this table has been taken from the publicly available sources provided by the manufacturers, in particular the appropriate package inserts. Review of Table 1 demonstrates a common theme in terms of pH as well as other excipients, often depending on antibody concentration. For example, the average reported pH for all antibodies in Table 1, when reported, is 6.3 ± 0.6 (n = 15). If we focus on the higher concentration antibodies for which information is available, the range narrows slightly to pH 6.0 ± 0.4 (n = 9). Of the 13 antibodies formulated at $\geqslant$20 mg/mL, 7 utilize L-histidine as the buffer. Further, the six most highly concentrated antibodies, from 90 to 150 mg/mL, utilize L-histidine as the buffer.

Table 1 illustrates that both liquid and lyophilized presentations are utilized for antibody based formulations. As expected, the lyophilized powders use a cryo-preservative and/or a bulking agent to aid in the lyophilization process. Choices of cryo-preservatives include sucrose and trehalose while bulking agents include glycine and mannitol. Of the five most highly concentrated antibodies, three (canakinumab, omalizumab, and efalizumab) share very similar lyophilized formulations of L-histidine as buffer, sucrose as cryo-preservative, and some level of surfactant. Despite this interest in high concentration lyophilized dosage forms, the majority of the antibody formulations in Table 1 are liquid dosage forms (14 of 21). At lower concentrations, sodium phosphate appears to be utilized broadly as a buffer (6 of 8 products at $\leqslant$10 mg/mL) in formulations often similar to phosphate-buffered saline. Sodium chloride is often utilized at lower protein concentrations, but alternate tonicity adding excipients are also utilized such as glycine, mannitol, and sorbitol. Finally, 16 of 21 antibody products utilize either polysorbate-20 or polysorbate-80 as a surfactant.

Review of Table 1 suggests that, for an antibody, the development of an early clinical dosage form may be based on one's experience, or the commercial experience of others, rather than exploring a broad formulation space *de novo*. A typical preformulation screen, for example, may explore formulation characteristics such as pH (from 4.0 to 8.0), ionic strength using NaCl, cryo-preservatives such as sucrose and the need for surfactants. Since 16 of the 21 examples presented in Table 1 contain either polysorbate-20 or polysorbate-80, there is value in asking whether it is prudent, when time and materials are limited, to experimentally assess whether one should include polysorbate in the formulation. Based on the commercial experience of the formulation scientists who developed the products described in Table 1, the simple recommendation would be to include some level of polysorbate in the formulation at a concentration suitable to that required by the protein concentration. Thus, the decision is no longer whether to include a surfactant in the formulation, but rather to ask how much to include based on its intended purpose such as protection from mechanical agitation or possible assistance during the reconstitution of a lyophilized powder [13]. Again, the level of polysorbate may be guided by the experience of others.

A similar review of the products described in Table 1 can be applied to pH. Since the majority of the high concentration antibody formulations are formulated at pH 6.0 ± 0.4, one must ask whether there is value in examining formulations outside this range. Further, given the limited number of choices for a suitable buffer in this range (succinic acid has a pK_a of 5.6, L-histidine 6.0 and sodium phosphate 7.21 all at 20 °C), we must ask why we would consider a buffer other than L-histidine.

Table 1
Formulations of several commercial antibodies.[a]

Product name	Dosage form	Concentration	pH	Formulation
ILARIS® (canakinumab)	Lyophilized	150 mg/mL	NA	92.4 mg/mL sucrose, L-histidine and L-histidine HCl, 0.6 mg/mL polysorbate-80
XOLAIR® (omalizumab)	Lyophilized	125 mg/mL	NA	125 mg/mL omalizumab, 90 mg/mL sucrose, 1.7 mg/mL L-histidine hydrochloride monohydrate, 1.1 mg/mL L-histidine, 0.3 mg/mL polysorbate-20
RAPTIVA® (efalizumab)	Lyophilized	100 mg/mL	~6.2	Approximately 82 mg/mL sucrose, 4.5 mg/mL L-histidine hydrochloride monohydrate, 2.9 mg/mL L-histidine, 2 mg/mL polysorbate-20
SYNAGIS® (palivizumab)	Lyophilized	100 mg/mL	NA	47 mM histidine, 3 mM Glycine, 5.6% mannitol
SIMPONI® (golimumab)	Liquid	100 mg/mL	~5.5	0.88 mg/mL histidine and histidine–HCl monohydrate, 41 mg/mL sorbitol, 0.16 mg/mL polysorbate-80
STELARA® (ustekinumab)	Liquid	90 mg/mL	5.7–6.3	1 mg/mL L-histidine and L-histidine–HCl, 76 mg/mL sucrose, 0.04 mg/mL polysorbate-80
HUMIRA® (adalimumumab)	Liquid	50 mg/mL	~5.2	6.2 mg/mL sodium chloride, 0.86 mg/mL sodium citrate, 1.3 mg/mL citric acid monohydrate, 12 mg/mL mannitol, 1 mg/mL polysorbate-80
CAMPATH® (alemtuzumab)	Liquid	30 mg/mL	NA	8 mg/mL sodium chloride, 1.44 mg/mL dibasic sodium phosphate, 0.2 mg potassium chloride, 0.2 mg/mL monobasic potassium phosphate, 0.1 mg/mL polysorbate-80, 0.0187 mg/mL disodium edentate dihydrate
AVASTIN® (bevacizumab)	Liquid	25 mg/mL	6.2	60 mg/mL trehalose, 5.8 mg/mL sodium phosphate monobasic monohydrate, 1.2 mg/mL sodium phosphate dibasic anhydrous, 0.4 mg/mL polysorbate-20
HERCEPTIN® (trastuzumab)	Lyophilized	21 mg/mL	~6	Approximately 20 mg/mL α,α-trehalose dehydrate, 0.5 mg/mL L-histidine HCl, 0.32 mg/mL L-histidine, 0.09 mg/mL polysorbate-20, 1.1% bacteriostatic water
VECTIBIX® (panitumumab)	Liquid	20 mg/mL	5.6–6.0	5.8 mg/mL sodium chloride, 6.8 mg/mL sodium acetate
ROACTEMRA® (tocilizumab)	Liquid	20 mg/mL	6.5	15 mM phosphate, 50 mg/mL sucrose, 0.5 mg/mL polysorbate-80
ARZERRA® (ofatumumab)	Liquid	20 mg/mL	6.5	8.55 mg/mL sodium citrate, 0.195 citric acid monohydrate, 5.85 mg/mL sodium chloride
SOLIRIS® (eculizumab)	Liquid	10 mg/mL	NA	Chloride, phosphate dibasic, phosphate monobasic, polysorbate-80
LUCENTIS® (ranibizumab)	Liquid	10 mg/mL	5.5	10 mM histidine–HCl, 10% trehalose, 0.01% polysorbate-80
REMICADE® (infliximab)	Lyophilized	10 mg/mL	~7.2	50 mg/mL sucrose, 0.05 mg/mL polysorbate-80, 0.22 mg/mL monobasic sodium phosphate monohydrate, 0.61 mg/mL dibasic sodium phosphate dihydrate
RITUXAN® (rituximab)	Liquid	10 mg/mL	~6.5	9 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate-80
ZENAPAX® (daclizumab)	Liquid	5 mg/mL	~6.9	3.6 mg/mL sodium phosphate monobasic monohydrate, 11 mg/mL sodium phosphate dibasic heptahydrate, 4.6 mg/mL sodium chloride, 0.2 mg/mL polysorbate-80
SIMULECT® (basiliximab)	Lyophilized	4 mg/mL	NA	1.4 monobasic potassium phosphate, 0.20 mg/mL disodium hydrogen phosphate (anhydrous), 0.32 mg/mL sodium chloride, 4 mg/mL sucrose, 16 mg/mL mannitol, 8 mg/mL glycine
ERBITUX® (cetuximab)	Liquid	2 mg/mL	7.0–7.4	8.48 mg/mL sodium chloride, 1.88 mg/mL sodium phosphate dibasic heptahydrate, 0.42 mg/mL sodium phosphate
REOPRO® (abciximab)	Liquid	2 mg/mL	pH 7.2	10 mM sodium phosphate, 150 mM sodium chloride, 0.001% polysorbate-80

[a] All formulations are expressed as mg/mL after reconstitution. Because of fill volume variability, some concentrations are approximate.

The rationale for the selection of an appropriate pH and the use of polysorbates in antibody products, demonstrates the concept of a "simple formulation." Protein formulations should be inherently simple and each excipient should have a clearly defined purpose. This purpose, however, may not need to be demonstrated experimentally for each compound going into phase 1 clinical trials. Recent regulatory guidance [14] suggests that the selected excipients and their respective concentrations should be justified on the basis of their impact on the product's stability, bioavailability, and manufacturability. One may wish to consider, for a phase 1 compound, whether this needs to be justified for each monoclonal antibody or can it be generalized across a class of proteins. While additional data are collected on these parameters during the late clinical stage of development, much of this information will not exist during pre-clinical development prior to the preparation of early stage clinical supplies. Limited information should encourage the formulation scientist to seek simple solutions to the design of the formulation space for the protein in question. With this in mind, the "simple formulation" approach may be broadly applicable across antibody products. If, for example, 10–20 mM L-histidine, some level of polysorbate, and pH 6.0 works for several antibodies, it is a reasonable starting point to assume that it may apply broadly. Rather than performing a broad pH screen, from pH 4 to 8, under accelerated storage conditions of 25 °C and 40 °C, the formulation scientist may simply wish to verify that the selection of 10–20 mM L-histidine at pH 6.0 provides adequate stability for the early clinical dosage form. A similar approach could be taken for identification of the level of polysorbate. For example, the products described in Table 1 encompass a range from 0.005% to 0.2% with either polysorbate-20 or polysorbate-80. A simple titration of polysorbate within the formulation, when suitably stressed, should provide a rationale for selection of an appropriate concentration. Further, this level may be fixed in conjunction with a set protein concentration across products.

In addition to a buffer, pH range and selection of surfactant, one typically requires a stabilizer to protect the protein from the rigors of ultrafiltration, possible lyophilization, and multiple freeze–thaws. While the rationale for excipient selection, for lyophilized products in particular, has been discussed elsewhere, the principles for stabilizer selection are similar to those of selecting a suitable buffer, pH, and surfactant: learn from experience and do not over-invest your time and effort for a phase 1 product unless the protein shows instability with commonly used excipients. Drug substances are often produced in multiple-liter batches that must be stored for extended periods ranging from months to years. While some drug substances, such as antibodies, may be stored under refrigerated conditions, it is often the case where drug substances must be frozen to preserve stability and provide manufacturing flexibility. A cryo-preservative is often utilized to protect the protein of interest from the rigors of repeated freeze–thaw as well as freeze-drying. The use of sucrose as a cryoprotectant is well documented as is its use as a stabilizer for lyophilized dosage forms [15]. Preformulation experiments are critical in determining whether a liquid formulation is feasible based on the analytical procedures available during the pre-clinical stage of development. The use of sucrose, at neutral pH, provides the opportunity to either lyophilize the product or develop a liquid formulation that will be stable against freeze–thaw induced damage. One aspect to consider, however, is the use of sucrose in liquid dosage forms where the pH is slightly acidic; in this case, hydrolysis

may occur even under refrigerated conditions [16]. In these circumstances, one may wish to consider the use of trehalose which has a more stable glycosidic bond. For a lyophilized dosage form, however, the use of sucrose, which is widely accepted, should be encouraged. For a liquid dosage form, alternate excipients have been utilized, but we must also consider how the drug substance is to be stored and further processed. The use of sodium chloride and mannitol as tonicity agents is well known, however these excipients tend to crystallize on storage at −20 °C which can lead to loss of protein integrity. As a result, the development of liquid based formulations must also consider cryo-preservation, when frozen storage is required, as well as processes for compounding and storage.

4. Broad applicability of a platform approach

When utilizing a platform approach, or better yet, platform formulations for antibodies at the same concentration (liquid or lyophilized), there are several advantages that arise. If one were to place two or more proteins in the same formulation (as well as the same container-closure system), they could share the same placebo. This efficiency reduces the need for product-specific placebos to be produced for each individual product that enters clinical trials. Further, the use of platform dosage forms (excipients, container-closure systems) reduces the need to carry released, qualified, expensive cGMP raw materials at a manufacturing site for a product that may be unique. Further, if one were to develop a standard formulary from which the formulation scientist can draw, it dramatically simplifies the formulation selection process for a phase 1–2 compound which, based on the discussion above, is unlikely to reach phase 3 clinical testing or commercial launch.

The pharmaceutical scientist must consider whether to utilize liquid or lyophilized dosage forms for phase 1 clinical trials. Liquid dosage forms have several advantages such as ease of manufacture, simplicity in packaging, and ease of use. Further, they may allow for the generation of a representative stability profile in phase 1 clinical trials materials which enables the establishment of suitable release and stability specifications at the latter stage of development. An obvious disadvantage is the potential for inadequate stability which may require an increased number of resupply fills, an unwanted expense. Lyophilized dosage forms have the disadvantage of additional costs associated with development, manufacture, testing, and the need for diluents for reconstitution, but they are likely to help reduce the number of resupply batches that may be required because of the superior stability of the lyophilized dosage form. The level of experience and the sense of risk with the interdisciplinary project team and the formulation scientist should guide these choices.

In addition to platform formulations or approaches to formulation development, other drug product related processes can be platformed. For example, it is not unusual for firms to utilize a specific sterile filtration membrane for all of their monoclonal antibodies. Recent work by Ho et al. [17] demonstrated the benefit of poly-ether sulfone sterile filtration membranes, relative to PVDF for example, for monoclonal antibodies of neutral or basic pI. Armed with this type of data, one could select a sterile filtration membrane, with a high probability of success, simply on the basis of the pI of the protein. In a similar manner, one could imagine crafting a platform approach, or standard procedure, for conducting freeze–thaw studies, shaking studies, and stability studies. Having a common manner in which to conduct these studies not only provides for a streamlined approach that can be utilized across a laboratory group, but it also enables one to look across compounds and analysts to compare data with the knowledge that the experiments were conducted in a comparable manner. Finally, if one were to use the same formulation, protein concentration and container-closure system, then it is reasonable to assume that these products could utilize the same lyophilization cycle, mixing process, and sterile filtration process. While these pre-defined processes should be experimentally verified, it is an advantage to have an agreed upon starting point from which to assess the applicability of a defined process.

The platform approach not only applies to formulations and drug product unit operations but also to unit operations within other biotechnology processes (cell line, bioreactor process, purification process, and analytical development). In addition, there are efficiencies when developing specifications or manufacturing documents (batch records) in which having mature, well-accepted templates further streamline the robustness, repeatability, predictability, and expectations in terms of time and expenses.

5. Phase 3/commercial process and product development

Platform approaches can extend beyond phase 1–2 clinical trial materials into phase 3/commercial dosage form development. While the specific dosage forms may vary depending on the clinical application (e.g. vialed product for intra-venous injection of an oncology medication; autoinjector presentation for self-administration of a rheumatoid arthritis medication), the general approach to process and product development should be the same.

Once the dose, route of administration, and presentation (vial, pre-filled syringe or autoinjector) have been selected, the formulation and manufacturing process must be developed, optimized, characterized for robustness, and scaled up for commercial production. Experiments to support the development activities can be standardized to ensure an efficient use of time and resources. For example, one can platform a lyophilization robustness approach to ensure that the majority of potential process deviations are addressed. Having a platform approach makes these experiments more routine and predictable. Similar approaches can be taken to robustness studies pertaining to formulation (defining acceptable excipient levels or pH range), hold-times, numbers of freeze–thaw cycles and filtration steps, compatibility with filling equipment, mixing, and so on. For phase 3/commercial processes, a platform approach is what adds value more than a prescriptive platform formulation.

6. Summary

Platform or standardized approaches have been utilized broadly in a variety of industries for hundreds if not thousands of years. Best practices, once defined and shared, have resulted in tremendous savings of time, effort, and funds. Within biotechnology, recent advances in process and formulation development have enabled the development of platform approaches for purification, cell line development and formulation development. These platform approaches will become increasingly important as the number of molecules (antibodies specifically) entering the clinic increases as well as a mature recognition that the vast majority of these compounds will not enter phase 3 clinical trials. The use of platform approaches enables the formulation scientist to efficiently develop suitable, safe, stable, reproducible phase 1–2 dosage forms with reduced effort thus creating time and effort to address additional projects or further the development of novel technologies.

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