Pharmaceutical aspects of subvisible particles in protein formulations

Weinbuch, D.

CHAPTER 2

INTRODUCTION INTO FORMULATION DEVELOPMENT OF BIOLOGICS

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Abstract

Formulation development is an essential part of every biopharmaceutical development program and important for the therapeutic and commercial success of a promising protein drug product. Assuring the quality, safety, and efficacy of a therapeutic product throughout the intended shelf life are thereby major goals. Formulation development is composed of multiple phases, interacting with other product development exercises as early as discovery research all the way until and beyond market approval. Every drug product demands a tailor-made formulation, due to the complexity of degradation pathways potentially affecting the product stability, the specific characteristics of the active pharmaceutical ingredient, the demands for patient compliance, and even marketing considerations. Formulation development can be approached using various strategies, based on a rational design, relying on scientific knowledge in low or medium throughput, or high-throughput formulation (HTF) approach screening of hundreds or even thousands of conditions employing miniaturized analytical methods. In this chapter an introduction to the field of protein formulation development is given, literature on current protein formulation development strategies is reviewed, and current challenges are summarized.

Introduction

Protein formulation development aims to render a therapeutic protein product robust for manufacturing, storage, handling and administration to patients. So, formulation development is essential for the therapeutic and commercial success of a promising protein molecule: “it is a medicine, not a molecule, that we are giving to the patient” (1). With this chapter, the reader is introduced to general concepts related to formulation development of biologics. The focus is on liquid and lyophilized protein formulations for parenteral use, as those comprise the vast majority of our current arsenal of marketed biologics. Nevertheless, most of the concepts described in this chapter also apply to other biologics, such as vaccines and DNA- and RNA-based products. Issues specific for the challenges of protein delivery systems for non-invasive administration and particles for sustained release and targeting are beyond the scope of this chapter; the interested reader is referred to the literature (2–6).

Within this chapter we discuss various elements of protein formulation development, formulation strategies during several stages of development and challenges that can be encountered. Rather than going into great detail, the intention is to present the complexity of the topic and important aspects that should be considered during formulation development (see Table 1).

Formulation Development Strategies and Approaches

Protein Formulation: Beyond Stabilization

One of the major challenges in the formulation of therapeutic proteins is to assure their stability, not only during storage but also during manufacturing, shipment, handling and administration. Nevertheless, it should be realized that the ‘optimal’ formulation is not necessarily the one that is most stable, but rather should fit the purpose depending on several factors. These include, besides sufficient stability, the stage of development, clinical requirements, regulatory requirements, packaging, and device configuration, economical issues, marketing considerations or the freedom to operate within the patent landscape (Table 1). As an example, what is ‘best’ in terms of a product’s stability is not necessarily good from a patient’s or economical perspective. For instance, suppose a certain product would be most stable in 50 mM sodium citrate, pH 4.0. If the product is meant for subcutaneous administration, this formulation probably would be not preferred, because the unfavorable combination of low pH and hypotonicity may cause pain at the injection site (7). The same formulation might, however, be acceptable if the product were intended to be diluted in an infusion liquid prior to intravenous

administration, provided that the product is stable in use and compatible with the infusion system. Another example: if a lyophilizate in a vial would be stable for five years but the same molecule could be formulated as an aqueous solution in a prefilled syringe with two years shelf life, the latter might be preferred over the more stable formulation for economical and marketing reasons and due to easier patient self-administration.

Table 1: Critical factors to be considered during formulation development.

Factor	Description / attributes / examples
Analytical methods	High- versus low-throughput, stability-indicating, QC, extended characterization
API	Type of protein, physico-chemical properties, e.g., molecular weight, pI, hydrophobicity, solubility, post-translational modifications, pegylation
Clinical factors	Patient population (e.g., age, indication, concomitant medication), therapeutic window, self-administration versus administration by professional, compatibility with infusion solution
Competitive landscape	Originator versus biosimilar product, patent situation, competitive drugs
Dosage form	Single- or multi-dose, prefilled syringe, dual chamber cartridge, pen cartridge
Drug substance	API concentration, formulation composition, available amount, purity
Excipients	Pharmaceutical quality, safety record (for intended administration route and dose), manufacturer, tested for critical impurities, stability
Manufacturing capabilities	Disposable/non-disposable technologies, dedicated equipment, filling line / pumping
Other factors	Budget, time(lines), manufacturability, company policy, marketing strategy, regulatory requirements
Phase of development	Preclinical, early clinical, late clinical, commercial
Primary packaging material	Glass, polymers, rubber, silicone oil, metals, leachables (anti-oxidants, plasticizers, etc.)
Route of administration	Subcutaneous, intravenous injection or infusion, intramuscular, intravitreal, intraarticular, intradermal
Target dose and dosing regime	Concentration, volume, indication (e.g., one-time application or chronical application)
Type of formulation	Liquid, lyophilizate, frozen liquid

Since a liquid formulation is often faster and cheaper to produce and is more user-friendly, generally it is preferred over a lyophilizate. However, it may be impossible to develop a sufficiently stable liquid formulation, either because of time constraints during (early) product development or because the molecule turns out to be insufficiently stable even after extensive formulation development exercises. The obvious alternative in such cases is a dry formulation (apart from an early-stage frozen liquid formulation), which is almost exclusively achieved by lyophilization, a process requiring dedicated formulation development.

From a formulation scientist's perspective, in an ideal world already at the earliest stage of development the final dosage form, the required stability profile as well as other needs (see Table 1) have been defined, high-throughput, stability-indicating analytical methods are in place, and material, time and resources are available in unlimited amounts. However, the real world is quite different. Consequently, the first formulation used during preclinical studies (e.g., toxicity studies) is likely going to be different from the formulation applied during later clinical phases and the final formulation used for commercialization. This may be explained, besides by the above-mentioned reasons, by changes in the dosing regime, the route of administration or the primary packaging material (e.g., switch from vials to syringes) or by instabilities occurring in a not-yet-optimized formulation as well as additional insight gained into the stability of the protein molecule and/or the excipients. Nevertheless, it is highly favored to have the final formulation composition defined as early as possible during drug product (DP) development to avoid additional studies, regulatory efforts and to align drug substance (DS) and DP composition. To this end, it is imperative that the formulation scientist acquires knowledge about the clinical needs, marketing considerations as well as regulatory requirements. Moreover, the more is known about the physical and chemical stability of a protein molecule as function of major formulation variables and external stress factors (temperature, mechanical stress, freezing and thawing etc.) early in the development, the less complex, costly and time-consuming it will be in a later stage of development to accommodate a formulation to the needs of the molecule and the product.

"There isn't just one way of doing it" holds true for formulation development of biologics and there are numerous ways and philosophies how to come to a stable and robust formulation. No matter which approach is followed for achieving a satisfactory formulation, the selection of analytical methods plays a crucial role. Already early in the process the critical routes of instability need to be identified in order to establish the important stability indicating analytical methods as well as the appropriate formulation strategy to tackle the instability issues. Formulation development usually evolves during a drug development program, and often thereafter, and can generally be divided in the following activities: preformulation, formulation development for DS, DP formulation development for preclinical phases, for early clinical phases, for late stage/commercialization, and finally formulation activities during the life cycle of a product (Figure 1). Of course, there certainly is an overlap between these phases and wherever applicable, considerations for a later stage should be reflected as early in the development process as possible. In the following sections, we describe first what typically

forms part of a protein formulation and then discuss several phases and approaches of formulation development.

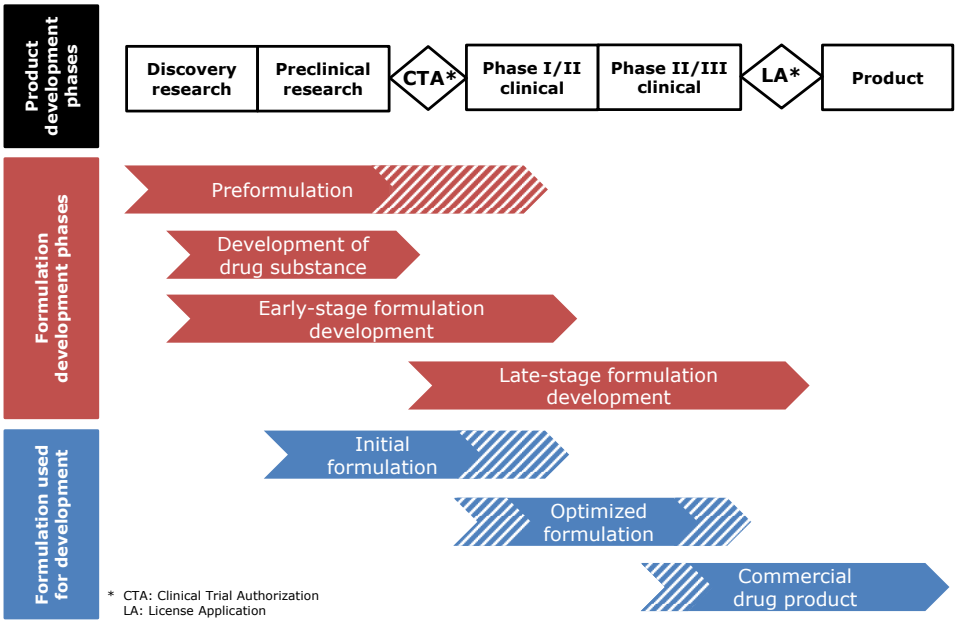

Figure 1: Diagram of a formulation development process. Modified from (8).

Components of a Protein Formulation

Active pharmaceutical ingredient and drug substance

The term active pharmaceutical ingredient (API) refers to the molecule of interest e.g., a peptide, monoclonal antibody or enzyme. In a pure state, the API would typically be a solid powder, as often found for peptides. This state however, is extremely impractical to obtain and/or presents an unstable state for most biologics. Therefore, a DS, a (sometimes frozen) liquid formulation containing the API is used for purified bulk storage. A DS typically results from a chromatographic or ultra-diafiltration step at the end of a purification process. In commercial-scale production, the formulation composition of the DS is often very similar to that of the final DP, but this can obviously not be the case when formulation development has yet to be completed. This may have consequences for DP formulation screening.

Excipients

One rule in formulation development is to avoid putting anything into the formulation that is not needed. In other words, a formulation should be kept as simple as possible and

each excipient, as well as its quantity, should be justified. Having mentioned this, it is not an easy task to combine the right excipients in the right concentration, because a stabilizing excipient potentially exhibits a destabilizing effect on a different protein instability pathway, and excipients potentially influence each other's action. For instance, polysorbates added for protection against interface related protein aggregation may contain oxidizing species, which may promote chemical instability (9). Whereas sodium chloride could help reducing a formulation's viscosity, it may negatively affect a protein's colloidal stability and also be detrimental upon freezing or lyophilization as upconcentrated in the freeze-concentrated solution (10). Finally, the most frequently used excipient, water for injection, is a natural solvent for proteins but at the same time mediates most if not all possible protein degradation reactions, reason why many products are lyophilized to reduce the water content to minimal amounts.

Table 2 gives an overview of the most commonly used excipient classes and their functions in protein formulations. Importantly, it is common practice to choose among excipients that are approved and commonly used in protein formulations (see examples in Table 2) in comparable doses and dosing frequencies for the intended route of administration. Although it would be interesting to explore novel excipients in order to expand the options for a formulation scientist, including a new excipient in a formulation is often a 'no go'. The reason is that it would greatly increase development time and cost, because – besides the need for a justification to use it instead of a more common excipient – its safety would have to be evaluated in order to get the product approved for clinical trials and registration. The same may hold true for unusually high doses of a certain excipient. Furthermore, the quality of excipients should be considered critically and their stability in the specific formulation should be assessed. For instance, sucrose might not be included in liquid formulations below pH 6, because its hydrolysis rate during storage may become significant, leading to the formation of fructose and glucose; the latter degradant can form glycation products with the protein via the Maillard reaction (11). While excipients preferably should comply with compendial standards, additional requirements may apply for specific protein formulations.

Excipients can exert several functions, e.g., glycine can act as stabilizer, buffer and tonicity modifier and may have several modes of action. The need for their inclusion in a protein formulation mainly depends on the critical instability pathways of the protein and other not protein stability related needs, such as tonicity requirements and lyophilizate appearance. Furthermore, certain excipients that may be useful in liquid formulations should be avoided in lyophilizates (e.g., volatile buffers such as acetate, or salts that lower the glass transition temperature of the maximally freeze-concentrated solution (Tg') of

amorphous formulation), whereas some excipient functions are specific for lyophilized products, e.g., bulking agent, lyoprotector.

Table 2: Common excipients encountered in protein formulations.

Excipient class	Function	Examples
Solvents	Dissolution	Water for injection
Buffers	pH control, tonicity	Acetate, citrate, glutamate, histidine, phosphate, succinate, glycine, aspartate
Salts	Tonicity, solubilization, stabilization, viscosity reduction	Sodium chloride
Sugars, polyols	Tonicity, stabilization, cryoprotection, lyoprotection*, bulking agent*	Mannitol, sorbitol, sucrose, trehalose
Surfactants	Solubilization, stabilization, adsorption prevention, reconstitution improvement*	Polysorbate 20, polysorbate 80, Poloxamer
Amino acids	Solubilization, stabilization, tonicity, viscosity reduction, pH control, bulking agent*	Arginine, glycine, glutamate, histidine, lysine, succinate
Anti-oxidants	Oxidation prevention	Methionine, sodium edetate
Preservatives	Antibacterial action (multi-dose formulations)	Benzyl alcohol, meta-cresol, phenol

* specifically for lyophilized products

Buffer species may have specific destabilizing or stabilizing effects on proteins, besides offering buffer capacity. So, buffer type and concentration should be carefully selected during formulations screening and the decision depends not only on the desired pH (typically well within about ± 1 unit from the pKa of the buffer species), but also on the protein, the route of administration, and whether it is a liquid or a lyophilized formulation. Furthermore, in high-concentration protein formulations one could consider not to include any buffer. Especially in slightly acidic, highly concentrated (>50 mg/ml) antibody formulations, the total number of His, Glu, and Asp residues in the API may provide sufficient buffer capacity to provide a stable pH value (12).

Primary Packaging Material

Since the primary packaging material may affect the quality of the DP, it is an important and integral part of the formulation development program. Obviously, the primary packaging material depends on the dosage form (see Table 1 for some examples), which in turn impacts the way a drug is administered and its user-friendliness. Implications of the primary container on formulation development, e.g., the set-up of mechanical stress

studies, are addressed in the section "*Selection of Analytical Methods and Stress Conditions*" of this chapter.

Preformulation

Preformulation studies are a prerequisite "to know your molecule", which is vital for the entire development cycle of a therapeutic protein. On the short term, preformulation studies may be used for candidate selection and will help in the optimization of upstream and downstream processes for the selected candidate molecule as well as in the development of a sufficiently stable formulation for DS, preclinical and first-in-human clinical trials. At later stages of development and after commercialization, the fundamental knowledge acquired with preformulation activities will support the rational design of (an) optimized formulation(s) and the assessment of the shelf life under appropriate storage conditions.

The term preformulation is used rather flexible and differently among research groups with respect to its transition to, or its position within, formulation development. Preformulation studies are performed in close collaboration with discovery research and should start as early as a promising drug candidate has been obtained. Preformulation studies are meant to gain insight into critical physico-chemical properties of the protein drug candidate (see Table 1), such as primary, secondary, and higher-order structure, molecular weight, extinction coefficient, isoelectric point, post-translational modifications, hydrophobicity, and biophysical properties, such as conformational and colloidal stability. Moreover, they are aimed to determine the criticality of various environmental factors, such as pH, ionic strength and buffer species, and the API's sensitivity to pharmaceutically relevant stress conditions (Table 3). The latter involves assessment of the predominant degradation pathways. The critical predominant degradation pathways, as well as the sensitivity to pH and ionic strength, may be quite different between proteins, even for relatively similar ones such as monoclonal antibodies (13–16). Preformulation should ultimately lead to the development of suitable stress conditions and a toolbox of stability-indicating analytical methods, enabling the differentiation between good and bad formulations in upcoming, more comprehensive formulation development studies. In some cases, selected excipients may already be screened to improve the stability of the molecule against critical stress factors.

Table 3. Accelerated stability and forced-degradation studies used in protein formulation screening.

Stress type	Exemplary stress conditions	Anticipated instability types
Temperature	Real-time/intended temperature, e.g., at 2-8°C Accelerated testing, e.g., at 15, 25 or 40°C	Aggregation, conformational changes, chemical changes
Mechanical, shaking	50-500 rpm, 2 h to >48 h	Aggregation, adsorption, conformational changes
Mechanical, stirring	50-500 rpm, < 1 h to 48 h	Aggregation, adsorption, conformational changes
Mechanical, freeze-thawing	1-5 cycles, e.g., between 25°C and -20°C to -80°C	Aggregation, adsorption, conformational changes
Oxidation	H_2O_2, 1-5 % for 1-2 days, oxygen purge	Chemical changes (oxidation), aggregation, conformational changes
Humidity*	0-100% RH	Aggregation, conformational changes, chemical changes, moisture content

* specifically for lyophilized products

Preformulation includes the testing of the thermal stability, e.g., by (micro-)differential scanning calorimetry (DSC) or dynamic scanning fluorimetry (DSF) as well as the testing of colloidal stability, including aggregation propensity and viscosity, e.g., by determination of the 2nd virial coefficient or the interaction parameter kd by static light scattering (SLS), dynamic light scattering (DLS) or analytical ultracentrifugation (AUC) (17,18). DSC and DSF are often applied to assess thermal events, such as unfolding, which is helpful to define relevant conditions for accelerated stability studies. However, although thermal stability studies are routinely used in formulation screening, for several reasons thermal stability may not correlate with storage stability. For example, Bam et al. (19) observed an excellent stabilization against agitation by polysorbates, although DSC experiments showed lower unfolding temperatures in presence of the surfactant. Furthermore, the ranking of melting temperatures does not always predict the order of conformational stability at storage temperature (20,21). Therefore, preformulation should include mechanical stress e.g., by shaking or stirring at temperatures, far below the Tm value (Table 3). Moreover, chemical degradation can arise from the fully native structure even without the application of thermal or mechanical stress and might in specific cases be more problematic than conformational or colloidal instability (22). Preformulation should thus test for such pathways e.g., by forced oxidation (Table 3).

Formulation Development

Formulation development strategies

Formulation development involves studying the influence of formulation variables on potential critical quality attributes upon intended storage, accelerated and forced-degradation conditions in order to identify a stable and robust formulation based on previous experience with the same API or similar molecules and the preformulation work. There are several ways and philosophies to reach a stable and robust formulation. One is a rational design methodology testing well-selected formulation conditions in low or medium throughput and a defined number of excipients based on the properties of the molecule, as established in preformulation studies. The alternative high-throughput formulation (HTF) approach involves the empirical screening of hundreds or even thousands of different formulations under accelerated conditions preferably employing miniaturized analytical methods. Finally, for some well-known molecule formats (e.g., monoclonal antibodies), platform approaches might be suitable by applying standard formulation conditions with a high chance, but no guarantee of success. For novel protein molecule designs, such a fast-track formulation approach may not be feasible, as a better understanding of the physico-chemical properties and the routes of instability is required to identify appropriate formulation conditions.

Independent of the formulation strategy followed, once a suitable formulation has been identified, its shelf life must be confirmed in real-time and accelerated stability studies and its robustness assessed under relevant stress conditions. Accelerated stability studies can never replace real-time stability assessment, because rates of the degradation routes may have different temperature dependency potentially affected by a change in protein conformation with temperature (8). Consequently, the predominant degradation pathway at elevated temperature, e.g., 25 °C/60 °C, could differ from that under refrigerated conditions (2-8 °C). Therefore, and because protein degradation processes can mutually influence each other in a complex fashion, Arrhenius kinetics often do not apply to protein formulations (23).

Early-stage formulation development

Time pressure, limited resources, the risk of a drug to drop out during the development program, or plans to sell a drug candidate after clinical phase 1, are only some arguments to define an early-stage DP for preclinical phase or clinical phase 1 without extensive formulation development. In this case, within a relatively short time frame the formulation scientist should aim to deliver such an initial formulation that can be reproducibly manufactured with a standard container closure system, while leaving enough flexibility to, e.g., alter the dosage regime and the route of administration at later

development stages. Lyophilization and reconstitution with a different volume is one approach to allow dosing flexibility and setting up different protein concentrations (24,25). The shelf life requirement of this early DP is mainly determined by the logistics of supplying the drug for clinical trials. Stability of the API in the DP until at least the end of the trial must be supported by stability data. Importantly, the more is known at this stage about the intended commercial formulation (e.g., administration route, dosage form, and primary packaging material), the better.

In preformulation and early formulation development, HTF screening can be beneficial, especially if there is no or very limited pre-existing knowledge about the sensitivity of the API to formulation and stress conditions. The high number of test formulations can be handled when working with automated pipetting systems or robots ideally combined with stress testing/stability testing in plates and plate-reader based analytics requiring low sample volumes. Typical analytical methods for this purpose are UV spectroscopy (protein content, turbidity), fluorescence spectroscopy (intrinsic or extrinsic with dyes), and DLS, all of which can be performed fully automated in multi-well plates. Moreover, intermediate-throughput methods, such as HPLC/UPLC and DSC, when performed with autosampler devices, can be conveniently used (26–28).

Late-stage formulation development

While the protein in its initial formulation is tested in clinical trials, the formulation scientist will already be working on an optimized, commercially viable formulation. This formulation should, beyond the stability required for the initial formulation, ultimately be robust against external stresses during the desired shelf life, administration (sometimes using product specific application devices), and to potential protein-specific degradation pathways. In order to test robustness, forced degradation studies at relevant stress conditions (Table 3) combined with a tailored set of stability indicating analytical methods, defined during preformulation, are employed. In this context, design of experiment (DOE) approaches can be applied to optimize experimental setups and reduce the number of required sample measurements (29). While forced degradation studies do not reflect real-life conditions, they are useful to reveal differences in stability between formulations and to give justification on why excipients are added and at which quantity. In late-stage formulation development, tasks of the preformulation phase might still be ongoing and specific molecule characterization tasks may be intensified. Since the DS is at this stage available in larger quantities (and often higher purity), the formulation scientist is not anymore tied to low-volume analytical methods used in early-stage development, but can also employ resource consuming or high-volume methods e.g. AF4, AUC, FTIR-spectroscopy, MS, and particle characterization (30) to test the stability of the protein

more in detail. Knowledge from clinical trials on application route, dosage regime, and the potential use of an application device will also influence the formulation design. The investigation of processing stability should include filter tests, tubing tests, handling test, and fill-finish tests to assure robustness towards stresses during manufacturing, if not already, at least in parts, performed during early-stage development. Finally, real-time stability studies at relevant storage conditions (e.g., 2-8 °C) using the DP in its primary container system from different production batches are to be conducted to define and justify the product's shelf-life. This is stated in the ICH guideline QC5 and for most DPs a shelf life of at least 18 - 24 months is desired.

Formulation development after commercialization

When a commercial DP has successfully entered the market, formulation development might still be needed e.g., for life cycle management to change protein concentration, packaging material, or route of administration and to support changes in the manufacturing process. In this case, knowledge from pre-, early stage, and late stage formulation activities is key to enable fast and effective formulation change and comparability studies. Since slight changes in formulation conditions potentially affect the safety and efficacy of the DP, it is necessary to perform detailed studies to assure that product quality and degradation profile have not quantitatively worsened or even qualitatively altered. If analytical characterization and non-clinical comparability studies are not sufficient for this claim, the ICH Guideline Q5E demands additional clinical comparability studies.

Challenges during Formulation Development

Amount and Quality of DS

One challenge in preformulation and early-stage formulation studies is the typically limited availability of API. The required amount depends in part on the product development stage as well as on the formulation strategy. Vice versa, if substantially limited amounts are available, this may unavoidably lead to a change in formulation strategy and/or a reduction of the number of stress testing methods applied, formulations screened, and analytical methods used (30). Obviously, analytical methods that require little sample are preferred, including well-plated based spectroscopic and light-scattering based methods as well as electrophoretic and chromatographic techniques (28).

Another challenge is the potential variation in DS quality during product development, which may be due to coinciding development and changes in production cell line, cultivation conditions, and downstream processes. In particular during early stages of

product development, the quality of the DS may not reflect that of later-stage (pilot or full-scale production) batches. In particular, aggregate and particle levels in pre-GMP technical batches do not always meet the minimum standards, such as those defined by the USP Chapter 787, which impedes proper assessment of a formulation's capability to avoid aggregation (31). Moreover, the level of impurities or contaminants may have major effects on product stability (32). For instance, variations in residual protease activity will especially affect the stability of the API in a liquid DP. Similarly, a relatively high residual lipase activity may lead to unexpectedly rapid degradation rates of polysorbates (33,34). If the root cause of such degradation processes would be identified in an early stage, one could choose to first develop a frozen liquid or lyophilized DP for early-stage (pre)clinical development, while optimizing the upstream and downstream processes in the meantime. This, however, would take additional resources and time. Ultimately, there is the risk that formulation development is focused on inhibiting a degradation process that turns out to be irrelevant as soon as higher-quality DS batches become available.

For DP formulation screening the available DS formulation will have to be exchanged with the formulations of interest e.g. by column chromatography, dialysis or ultra-/diafiltration. Such processes, which may also involve dilution or concentration of the API, pose stress upon the molecule. Consequently, it should be investigated whether the chosen method compromises the protein quality. Furthermore, in buffer exchange and concentration procedures using a semi-permeable membrane, especially at high protein concentrations, the final formulation composition may significantly differ from the intended one because of unequal partitioning of excipients. This may be due to volume exclusion, non-specific interactions and for ionic solutes, such as salts and buffer components, the Donnan effect (35). The presence of a surfactant such as polysorbates in the DS formulation e.g. introduced in the downstream process to protect the API against interfacial stress would pose a particular challenge, as it is practically impossible to remove surfactants quantitatively and they may accumulate in an unpredictable way during membrane concentration processes (36). Thus, quantification methods for each of the excipients that are part of DS and DP should be in place for guiding the proper design of formulation screening methodologies. Furthermore, once a suitable final DP formulation is chosen, the polishing step in the downstream process can be adjusted to bring the DS formulation in line with that of the DP.

Selection of Analytical Methods and Stress Conditions

The paradigm "formulation is characterization" refers to the fact that only with a proper analytical toolbox one can differentiate between good and poor formulations within the

limited time frame of a short accelerated stability and stress program. But how should one set up the analytical package and appropriate stress conditions?

Analytical methods

No matter which formulation approach is followed, the availability of low-volume, high-throughput methods is advantageous, especially in preformulation and early-stage formulation studies. Techniques used in these stages preferable provide a general indicator for stability, such as melting temperature by DSF or DSC, or colloidal stability by light scattering. Since proteins can undergo a variety of degradation reactions (22), complementary analytical methods should be used for monitoring the formation of all potential degradation products when performing stability and forced-degradation studies. Filipe et al. (30) gave an excellent overview of commonly used analytical methods outlining their measurement parameter, their sample requirement, and whether they can be operated in high-throughput. The interested reader is also referred to books by Jiskoot and Crommelin (37), and by Houde and Berkowitz (38) providing details about analytical methods beyond the scope of this chapter. Especially in later stages of formulation development, orthogonal methods should be used to verify the validity of specific methods. For instance, size-exclusion chromatography (SEC) methods only cover a limited size range of relatively small protein aggregates (up to about 100 nm) and may not detect reversible aggregates within this range (39,40). Consequently, regulatory agencies expect SEC data to be confirmed by orthogonal methods, such as AUC and AF4 (30,41). In addition, until recently the use of compendial methods such as light obscuration has been focused on the analysis of subvisible particles larger than 10 micron. However, safety concern with respect to protein aggregates and other particulates in the size range of 2 – 10 µm and more recently also the submicron size-range has facilitated the development of new particle analysis methods e.g., micro flow imaging, nanoparticle tracking analysis, and resonant mass measurement that are now increasingly being applied in formulation development (30,41–44). This has also been acknowledged by regulatory bodies and has lead to new and updated guidelines such as the USP <787> and the educational chapter USP <1787>, suggesting quantification and qualitative characterization of particles in this size range by orthogonal methods (45). With the analytical methods comes the challenge of setting specifications and their justification. For many quality attributes assessed throughout the whole manufacturing process of a DP like appearance, color, pH, sterility, osmolality, visible particles, or subvisible particles, the pharmacopoeial monographs apply. Other specifications e.g., the SEC monomer content, are not ultimately defined at early stage. A specification of more than e.g., 95 % monomer can be accepted at early stage development, may be set in accordance with platform technology experience and revised reflecting experience and stability data gathered on the way to commercialization.

Stability testing and forced-degradation studies

How to select appropriate stress conditions? The answer to this question is not straightforward, because it depends, amongst others, on the purpose, the protein, the formulation, the dosage form, and the development stage (23). For formulation screening, the stress conditions should be discriminative and allow ranking of formulations, which implies that they should be harsh enough to induce detectable changes, but at the same time not so harsh that all formulations show similar, nearly complete degradation. Preexisting knowledge from the literature and in-house experience with similar molecules may be extremely valuable to set up appropriate stress conditions. Moreover, the relevance of the stress conditions should be kept in mind. For instance, exposing a protein to a temperature above its unfolding temperature over a longer storage period would be as irrelevant as pyrolyzing a small molecule; and if a formulation is shown to be resistant to rigorous shaking for several days, rather than continuing the applied stress for another few weeks, one may conclude that the formulation is robust towards this mechanical stress factor.

Setting up appropriate stress conditions may be part of preformulation and could be done with the DS. Typical stressors include thermal, freeze-thawing, mechanical, and oxidation stress. Table 3 gives some rough indications of possible conditions that could be applied for each of these stress factors. Although extreme pH and ionic strength are sometimes mentioned as stress factors, those are in fact formulation variables that are typically studied in preformulation studies, often in combination with exposure to elevated temperatures. The outcome of such extreme pH/ionic strength exposure studies is relevant to define the design space not only in formulation development but also in downstream processing steps, such as elution conditions in chromatographic procedures, viral inactivation, hold times, and conditions between purification steps.

Light stress may be added at later-stage formulation studies and essential protection is finally provided by the secondary packaging material. One may consider using also less harsh conditions than those according to ICH, in order to assess subtle differences between formulations. If the final container is known, this may be advantageous, especially for mechanical stress studies. For instance, the influence of shaking stress (conditions) is highly dependent on not only the shaking frequency and the incubation temperature, but on container dimensions, filling volume, and solution viscosity as well.

For lyophilized formulations, storage of lyophilizates with different residual moistures levels under accelerated testing conditions needs to be considered. Moreover, the effect of freeze-thawing stress to the corresponding liquid formulation (with conditions used during lyophilization) needs to be studied. Furthermore stress stability testing after

reconstitution is highly valuable to reflect light, temperature, and mechanical stress, which the liquid could potentially be exposed to in the clinics and by the patients.

While forced degradation or accelerated stress studies are valid means to compare formulation conditions during development and are recommended by the ICH Q1 guidelines, they have limited predicting value to the stability of a protein at real-time storage conditions. Thus, one can use these data to understand degradation pathways and to define and justify formulation conditions, for instance the use of an excipient in a certain concentration, but one should not exaggerate forced degradation studies. Instead, a promising formulation should be tested by long-term studies testing at relevant storage conditions as early as possible since these studies are the basis for the determination of the product's shelf life and demonstrate the relevance of the different degradation pathways.

Manufacturability and Formulability

Formulation development has the goal to obtain a DP that serves the patient's needs and promotes stability of the protein. However, manufacturability should also play a role when defining a final formulation, because the product needs to be manufactured at large scale and commercially viable. Some steps and procedures that can be performed with ease in small scale or on a lab bench might be difficult to implement in a large-scale production facility. For example, filtration steps using very low pore size filters are easily performed in the lab, but low volume throughput and the costs of industry-sized filter systems might make implementation problematic in production scale. Also, high-concentration and viscous formulations could be difficult to handle during manufacturing and might cause problems during release testing by required compendial methods such as light obscuration. Contrary, low-concentration formulations might face the problem of protein loss through surface absorption, a factor that can become more relevant in a production facility. The same holds true for excipients in low concentrations e.g., substantial loss of polysorbate to filters at the beginning of a filling process can occur. The scale up to a commercial facility can create additional problems not observed in small scale. For example, mixing solutions in a large stainless steel tank, pumping solutions through stainless steel tubing, filtration, and filling through a high-speed filling machine can introduce unexpected stresses to the protein. In addition, the introduction of particles, e.g., by pump systems has been observed. Therefore, the relevance of such scale-up related problems should be assessed early during process development and should be considered during formulation development. Since some, if not all, of the factors mentioned above can show a certain batch-to-batch variability, regulators require stability data from multiple production batches before approval of the final DP.

Data Handling and Analysis

From the above it should be clear that protein formulation screening will involve the generation, analysis, and interpretation of huge data sets. The two goals of a formulation scientist are to make analytical data manageable as well as interpretable. For the first, a streamlined data analysis is important, which should include standardized export and analysis templates for each analytical technique (either using standard office software or dedicated data analysis programs). In addition, meaningful data folder and file structures as well as traceable sample names are crucial when handling huge data sets. For the second goal, singular-value decomposition analysis can help to condense complex data sets, e.g., spectroscopic data, by vector algorithms to a few descriptive values without loosing information. Further, visualization tools such as empirical phase diagrams and radar plots (46,47) will improve data interpretation and will allow the formulation scientist to identify the best formulation more quickly.

Conclusions

Protein formulation activities are an important part of a protein drug development process. Formulation development should start early in product development. Selecting 'the right' formulation requires extensive exercises, including analytical method development, forced-degradation studies, and accelerated and real-time stability studies. Moreover, clinical needs, company policy, and marketing strategy should be taken into consideration during formulation development. Knowledge gained during preformulation activities will help the scientist to identify potential hurdles in the subsequent formulation development program and to design a formulation to overcome those, by selecting a limited number of required excipients in appropriate amounts. Since the definition of 'the right' formulation depends in part on the development stage, early stage formulations typically differ from late-stage and commercial formulations. Despite its complexity, if formulation development is done properly, the final result is often a simple liquid or lyophilized formulation in a dosage form for parenteral administration.

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