Review

Challenges for the pharmaceutical technical development of protein coformulations

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Abstract

Objectives This review discusses challenges to stability, analytics and manufacturing of protein coformulations. Furthermore, general considerations to be taken into account for the pharmaceutical development of coformulated protein drug products are highlighted.

Key findings Coformulation of two or more active substances in one single dosage form has recently seen increasing use offering several advantages, such as increased efficacy and/or the overall reduction of adverse event incidents in patients. Most marketed coformulated drug products are composed of small molecules. As proteins are not only comparatively large but also complex molecules, the maintenance of their physicochemical integrity within a formulation throughout pharmaceutical processing, storage, transport, handling and patient administration to ensure proper pharmacokinetics and pharmacodynamics *in vivo* already represents various challenges for single-entity products. Thus, nowadays, only sparse biologics-based coformulations can be found, as additional complexity during development is given for these products.

Summary The complexity of the dosage form and the protein molecules results into additional challenges to formulation, manufacture, storage, transport, handling and patient administration, stability and analytics during the pharmaceutical development of protein coformulations. Various points have to be considered during different stages of development in order to obtain a safe and efficacious product.

Introduction

Coformulation of two or more active substances in one single dosage form has recently seen increasing use in particular with small molecule product development.[1–3] These products are generally known as fixed-dose combinations (FDCs). FDCs may synonymously be called fixed-ratio combination products as the product is composed of two or more active pharmaceutical ingredients (APIs) which are present at a fixed ratio in one single final drug product.[4] Different dosage strengths of a certain combination of two or more APIs can be developed that may furthermore contain the actives at different ratios, each of them again being itself an FDC.

Combination of several APIs into one product is performed to ideally medicate a certain disease at various different molecular targets resulting into an overall improved medical condition of the patient due to additive and/or synergistic effects as compared to the single drug(s) alone.[5] Additional to an increased efficacy, the development may be justified by the overall reduction of adverse event incidents in patients, the opportunity to reduce the dose of either one or of multiple APIs within the combination, or the treatment of two distinct diseases at the same time, as in the case of Juvisync® indicated for patients suffering from type 2 diabetes mellitus and hypercholesterolaemia.[6,7] Further advantages may lie within improved patient convenience and compliance (increased patient adherence, simplified patient guidance and education), overall reduced health care costs (manufacture and purchase of one product instead of multiple products), easier supply processes (simpler procurement and distribution of

one product than multiple ones for the end-user) and new product opportunities within life-cycle management of existing marketed products.[1,2,4] However, one size does not fit all, and the use of coformulated products may become challenging in cases where dose adjustments are required, such as weight-based dosing, paediatric dosing or load-in dosing. Thus, development of various FDCs of different dosing strengths and/or different ratios of the actives may be required to enable dosing of different patient populations. This can be easily visualized by the example of Tekturna HCT®, a coformulation of aliskiren hemifumarate and hydrochlorothiazide for the treatment of hypertension developed by Novartis Pharmaceuticals Corporation.[8] The tablets exist in four different dosage strengths (mg aliskiren/mg HCTZ): 150/12.5 (ratio 1 : 12), 150/25 (ratio 1 : 6), 300/12.5 (ratio 1 : 24), 300/25 (ratio 1 : 12).

Looking at marketed coformulated drug products reveals that most of the therapeutic actives are small molecules, containing previously approved actives targeting different therapeutic indications, such as hypertension, cardiovascular disease, HIV/AIDS, tuberculosis, glaucoma or type 2 diabetes.[1–3,6,8–29] Compared to the amount of marketed small molecule coformulations, the number of peptide- or protein-based products is very limited.[30–32] Recently, EMA approved two peptide based coformulations, namely Ryzodeg® and Xultophy®, for diabetes therapy.[33–35] Ryzodeg contains the long-acting basal insulin degludec (IDeg) and the rapid-acting prandial insulin analogue, insulin aspart (IAsp), whereas Xultophy is composed of the long-acting basal insulin degludec and a GLP-1 receptor agonist, being liraglutide.[33–35] The latter was also recommended for approval by the US FDA's Endocrinologic and Metabolic Drugs Advisory Committee under the name of IDegLira®.[36] Finally, no coformulation containing multiple protein APIs is currently marketed. Thus, one question emerges: why are there currently so little coformulated peptide- and protein-based drugs available?

As opposed to small molecules, proteins are not only comparatively large but also complex molecules composed of an amino acid chain baring various chemical groups that may underlie several degradation mechanisms (e.g. oxidation, deamidation, hydrolysis/fragmentation, isomerization).[37–39] Additionally, the amino acid chain needs a specific folding into a three-dimensional structure in order for it to be active and to ensure proper pharmacokinetics and pharmacodynamics *in vivo*. Due to the numerous reactive chemical groups as well as the fragile three-dimensional structure mainly based upon relatively weak interactions, the maintenance of physical and chemical integrity within a formulation throughout pharmaceutical processing, storage, transport, handling and patient administration already for only one protein can represent various challenges.[40–43] Thus, the pharmaceutical technical development of protein

coformulations clearly poses additional stability, analytical and manufacturing hurdles to be overcome, which will be discussed in this review. Additionally, general considerations for the development of coformulated protein drugs will be highlighted. In scope are any potential kind of protein combinations, which are meaningful from a therapeutic perspective, which can be proteins of the same type, for example two or more monoclonal antibodies, but also different protein types, such as cytokines in combination with fusion proteins and/or enzymes, as a random example. Regarding human serum albumin formulations, in which the latter functions as an excipient to competitively bind to interfaces to prevent protein adsorption, the reader may consult available literature, as those are also out of scope.[44]

General considerations, requirements and regulations

During the last decades, multiple different diseases have been shown to be effectively and safely treated by using biologics. To render treatments even more efficacious the next logical step is combination of various protein-based drugs, and thus tackling different molecular targets of a certain disease. This approach itself is also known as combination therapies.[45–52] Within a combination therapy, the different drugs can be administered consecutively one after the other, sometimes also with a certain break in between, or simultaneously. In the latter case, the two or more drugs can be either mixed immediately before patient administration (comixture) or are already combined/coformulated within a single drug product. Per definition, the latter described combination of two or more actives (i.e. independent if actives are small molecules or proteins) into one formulation is a fixed combination/FDC.[5] For a FDC, different scenarios may apply: (1) the FDC consists of two or more previously marketed APIs, (2) one or more previously marketed APIs should be combined with one or more new APIs or (3) combination of two or more new APIs. The respective scenario determines how much non-clinical and clinical data are already available that might potentially be leveraged for the FDC.

To date, no international guidelines exist for FDCs, however, several national authorities issued own guidelines, which are sometimes only applicable to very specific products or therapeutic groups.[5,53–57] Careful evaluation of differences within national regulations for coformulated products, if existent, is recommended. For example the US FDA defines per Biologics Price Competition and Innovation Act of 2009 as amendment to section 351 (1) of the PHS Act that a protein is 'any alpha amino acid polymer with a specific defined sequence that is greater than 40 amino acids in size' and differentiates those from

chemically synthesized polypeptides, which are defined as 'any alpha amino acid polymer that (1) is made entirely by chemical synthesis; and (2) is less than 100 amino acids in size'.[58] Chemically synthesized polypeptides per above definition are regulated as drugs, and thus, the combination of such a polypeptide (or any other small molecule) with a protein API will fall within the US under regulations applicable to combination products and not to FDCs.[59,60] Additionally, for most cases, numerous guidelines related to preclinical and clinical development and marketing authorization of products containing a single API/single entity may equally be applicable to FDC products and should be taken into consideration.

In general, the sponsor is required to justify the rationale including the posology and dosing frequency for the particular API combination chosen.[5,53,55] For that, it is of course of utmost importance that the biology of the disease to be treated is sufficiently understood. Data should be presented demonstrating that the combination of APIs within a FDC results into an increased benefit over the individual APIs alone and that each component contributes to the efficacy and safety of the FDC. A careful evaluation that balances the advantages over disadvantages of the FDC should be performed based upon scientific, medical and quality considerations. For example, it will be difficult to find on the one hand a therapeutic rationale for a FDC product intended to treat conditions that usually do not coexist and on the other hand have it supported with necessary non-clinical and clinical data.

Another example, for which development of FDCs may not be justifiable, are treatment regimens requiring a large flexibility of dose adjustments for the different biological drugs to be administered. In such cases, simultaneous administration of the comixture prepared immediately prior administration should be taken into consideration to enable a combination therapy. Comixture administration is also much more practical within the early stage clinical trials during the FDC development, when the exact doses and the thus resulting ratios of the biologics to be combined are yet unclear and large flexibility regarding dosing and dose ratios is required.

Furthermore, for successful FDC development and approval, special attention should be paid to the following elements:[56]

1 Potential for pharmacodynamic (PD) interactions if affinity and thus competition for same receptors or targets is given for the combined APIs.
2 Potential for pharmacokinetic (PK) interactions, such that absorption, excretion, tissue distribution and/or metabolism rate or pattern for the other API(s) might be changed. Additionally, an increase in unbound-free API and subsequent potential tissue uptake may occur when the different APIs compete for binding of serum proteins.
3 Potential for toxicological interactions in particular if a narrow safety margin is given.
4 Potential for higher toxicological signals due to synergistic interactions of the combined APIs.
5 Potential changes in the levels or activity of endogenous molecules or proteins due to the FDC.
6 Potential for impairing lifesaving therapeutics' efficacy.

Regarding analytical or pharmaceutical development activities, the principles to be applied for FDC development are similar to those of single API products. However, as two or more APIs require consideration, additional challenges and complexities originate during technical FDC product development.

This additional complexity due to combination of multiple targets can be further illustrated using the example of combination vaccines. Monovalent vaccines are designed to protect against viral or bacterial pathogens. A plethora of monovalent vaccines exists, which are made of live, attenuated or killed, inactivated viruses and/or bacteria, subunits thereof, toxoids, polysaccharides or proteins etc. To avoid multiple injections, combination vaccines have been developed, which mostly are presented as suspensions or emulsions, but also liposomal or microspherical preparations may be encountered. Individual vaccines are already complex biological systems in itself, combination of the latter inevitably results into challenges regarding formulation and manufacture. For example, the pH and ionic strength may influence the binding of different antigens to the respective adjuvant.[61] Another example is the decrease in the observed immunogenicity of inactivated poliovirus vaccines after combination with diphtheria-tetanus-whole-cell-pertussis vaccines containing the preservative thimerosal.[62] During combination vaccine manufacture, the order of addition of components may impact the observed immunogenicity.[63] As the facet of components and their pharmaceutical presentations is huge, the approaches tackling those challenges may differ tremendously depending on the kind of problem.[61–71]

Considerations for FDC pharmaceutical technical development

As pointed out previously, proteins are composed of an amino acid chain that may range from 40–50 up to 25 000 amino acids. Each protein is rendered unique not only by the specific order and total amount of amino acids but also by possible post-translational modifications resulting into a defined three-dimensional structure. As the maintenance of the three-dimensional structure is based upon weak

interactions and crucial for its activity, the environment of the protein API has a major impact on its stability. The formulation of a drug product defines the direct environment a protein encounters and should be optimized such that the proteins' stability and thus activity throughout manufacture, packaging, transport, storage and administration is given.

Before initiation of the FDC formulation development, the target product profile (TPP) should be evaluated to determine key parameters for the intended drug product and usage thereof. For example, in certain cases, it might be necessary to evaluate the FDC as well as the individual drugs in preclinical and/or clinical trials.[53,55,56] Thus, the formulation scientist has not only to consider stability of the FDC during formulation development but also of the single entities. In particular within early stage development programmes, where the scope is to quickly move into clinical trials, this may mean to choose an acceptable formulation in which the single compounds as well as the combined compounds are sufficiently stable and of acceptable quality but not choosing the most optimal formulation with regard to the FDC. For example, the combination of the different APIs at the clinical site before administration might be such a case. This means that no long-term stability data of the combined product are needed, however, sufficient stability is required to enable on-site preparation and safe administration. The TPP should also indicate whether a lyophilizate or a liquid formulation is the preferred dosage form as well as the intended administration frequency, the preferred primary packaging and the route of administration. Furthermore, particular information concerning the FDC is essential for the formulation scientist: (1) how many APIs should be combined, (2) which different kinds of API-classes should be combined (e.g. monoclonal antibodies, enzymes, growth factors or mixtures thereof), (3) which doses of each API may be required and (4) which API ratios may result out of this (one or multiple)? As the latter two points are questions usually addressed during early stage clinical trials and thus unavailable to the formulation scientist within that development stage, the combination of the different APIs at the clinical site before administration is much more practicable.

As a next step, previous information on the different APIs, if available, should be analysed. Thus, important information on the molecular weight, the amino acid sequence with information on possible degradation hotspots prone to deamidation or oxidation, the isoelectric point (pI), hydrophobicity, glycosylation, three-dimensional structure and in best case data from previous preformulation or forced degradation studies on the single entities may easily be available and may indicate potential challenges during coformulation development.[72]

Most protein drugs are administered via the parenteral route with intravenous, subcutaneous and intramuscular application being the most prominent ones. Several other parenteral administration routes exist, such as intraocular, intraperitoneal, intra-articular or intrathecal and others, but are less common. Challenges for the formulation scientist coming from the intended route of administration may be due to limited injection volumes, which is given for subcutaneous, intraocular, intrathecal or partially also for intra-articular injections.[73–75] In case of high protein doses that need to be administered, being typically the case for monoclonal antibodies, high protein concentrations are required. For single-entity products, it is well described in the literature that high protein concentrations may result into increased viscosity, protein aggregation and/or decreased solubility as the solution conditions deviate more and more from being ideal resulting into increased protein–protein interactions due to molecular crowding phenomena.[42,76–78] Elevated protein aggregation may become problematic when long-term stability issues result into insufficient product safety and/or insufficient shelf life. Additionally, increased solution viscosity can lead into manufacturing issues, for example during ultrafiltration/diafiltration, sterile filtration, filling and administration challenges (e.g. syringeability, injection time).[76–80] For a FDC, the combination of two or more protein APIs can result into an overall elevated total protein content within the final coformulated drug product. Thus, increased viscosities and/or protein aggregation may be observed in the coformulated product. If either of them is observed, the following points should be clarified: (1) what sort of aggregates are formed with regard to size and composition, (2) is only one API aggregating or are several ones involved, (3) which molecular interactions are resulting into the increased viscosity and/or aggregation, (4) are there any technical measures available, for example certain excipients or feasibility to increase the dosing volume, to reduce or even avoid inter- and/or intramolecular interactions, if those are involved. Answering these questions will help designing a high-quality FDC product. However, additional complexity from analytics renders this task significantly more challenging as compared to a single protein product (see section below).[41,43,77,78,80,81]

Excipients should always be added upon an 'as much as needed basis' into a formulation, and it needs to be ensured that safety and efficacy of the API are not negatively impacted. The choice of the particular excipients and their concentrations require sound justification within the marketing authorization application. Every single entity within the coformulation needs to be compatible with the respective excipients chosen. This may become challenging, if an excipient is needed to reduce or prevent, for example, aggregation of one API, but this in turn results into

increased degradation/instability of the other API(s) within the coformulation. Additional complexity arises, if the formulation scientist has only a very limited list of acceptable excipients to choose from for any of the following reasons: (1) an excipient commonly accepted for one parenteral administration route is not necessarily acceptable for a different parenteral route (i.e. no safety data available or toxicity was reported) and (2) due to a higher dosing frequency, the same excipient type and concentration result into a higher patient exposure as compared to another product, and no safety data concerning the 'excipient dose' are available.

A protein is uncharged, if the buffer pH is at and around the protein's pI, which in turn results into increased protein–protein interactions and consequently increased turbidity or protein aggregation may be observed. Changing the solution pH away from the pI results into changes of the protein's net charge and usually results into improved physical stability. However, different chemical degradation mechanisms may occur depending on the solution pH.[39,82–86] Thus, the formulation pH is one of the key factors determining protein drug product stability. Having multiple protein APIs within a coformulation needs careful evaluation of the different degradation reactions occurring at various pH values including their impact on protein activity. Various degradation routes may occur simultaneously in the selected formulation, and their rate and extent should be evaluated for differences within the coformulated product versus the single entities to check for potential dependencies. A risk-benefit evaluation needs to be undertaken in order to choose the pH and formulation composition at which the various degradation pathways of the coformulated APIs is minimized as far as possible, while acceptable activity is maintained. In particular, if the proteins within the FDC have different pI values and, furthermore, fast or multiple chemical degradation reactions occur at solution pH values that are needed to prevent from physical degradation, it may be difficult to get a sufficiently large formulation space in order to obtain a robust formulation.

If no sufficient stability can be obtained within a liquid formulation and the TPP allows for a freeze-dried/lyophilized product, usually achieving higher chemical and physical protein stability, a lyophilized powder product for reconstitution can be considered. The freeze-drying process typically consists of three major process steps: (1) freezing, (2) primary drying and (3) secondary drying in order to remove most of the water in the formulation (typically to below 1% residual moisture). During freezing, several phenomena may occur that can result into structural damages of the protein, as well as denaturation, destabilization and aggregation or precipitation.[42,87,88] On the one hand so called freeze- or cryoconcentration may occur and result

into increased protein aggregation due to increased effective protein concentration and facilitate intra- and/or intermolecular aggregation within the FDC. On the other hand, the elevated ionic strength obtained in the freeze-concentrate may foster chemical modifications of the different APIs with one another. It should also be evaluated, whether during freezing stepwise crystallization of the acid and base component of the buffer system used for the formulation, results into significant pH shifts. Having one or several APIs in the FDC being susceptible to pH dependent degradation, a crystallization induced pH shift during freeze-drying may have a detrimental impact on the product's long-term stability.[89,90]

During manufacture of the final FDC finished drug product, homogeneity should be in particular focus. As two or more APIs are coformulated into one product, insufficient mixing during the addition of the APIs could result into a non-homogenous solution and finally into a non-uniform finally filled product, which may not meet its specifications and represent a potential safety issue for the patient. A manufacture at large scale, such as 250 l, may take several hours for the product to be filled into vials. During this time, the previously homogenized bulk drug product solution containing multiple APIs will be sitting in the manufacturing vessel, phase separation may occur and, thus, should be studied. In such cases, stirring of the bulk containing vessels during fill and finish operations, for example, should be considered. On the other hand, excessive mixing should be prevented, as shear and interfacial stresses could result into protein degradation. If one of the APIs is particularly sensitive to mixing stresses, small-scale characterization studies should be performed to support the at scale set up of mixing parameters ensuring stability of all protein APIs and product homogeneity of the FDC at the same time. Furthermore, during manufacture, the drug product solution will be in contact with various surfaces and materials, that is tubing, filter, stainless steel and others. Adsorption of proteins to surfaces has been described and could result into removal of a great part of an active component in case of FDC products containing one API at a low concentration. A non-homogenous drug product is the consequence.[87,91,92] Additionally, potential conformational changes after protein desorption may result in long-term stability issues and may become even more challenging, if degradation reactions involving multiple APIs occur as a consequence.[43]

During storage, transport and administration, the final drug products may get exposed to light, heat and oxygen. As proteins may be sensitive towards those, the packaging material chosen should protect from these potential hazards as good as possible, while at the same time, compatibility between the formulation and the packaging materials needs to be ensured. During long-term storage of the

product, small amounts of contaminants (also known as leachables), such as metal ions, peroxides or plasticizers, may migrate into the drug product and result into deactivation and/or denaturation of proteins.[93,94] Some proteins may also be susceptible to silicon oil, which is sometimes used as lubricant for elastomeric stoppers or coating of prefilled or single-use syringes used for administration.[95,96] In case of FDC products, the different proteins might be sensitive in a different extent to any contaminant and careful evaluation of their impact on each of the APIs within an FDC should be undertaken. In case one or several, APIs are found to be sensitive to these contaminants, further evaluation is required to determine the impact on the overall FDC stability impact. Also, adsorption to the surface of the packaging materials may be observed and similar considerations regarding product homogeneity as during FDC manufacture are to be applied (see above).

Based upon in-use and compatibility testing, the formulation scientist ensures that the protein remains stable and establishes recommendations for clinical handling and administration procedures. Within this testing, factors such as adsorption to the administration materials, compatibility with different administration materials, dilution effects, storage temperature and time as well as physical and chemical protein stability are evaluated. For FDC products, all APIs need to be evaluated for these parameters to ensure accurate patient dosing. Additionally, possible physical and chemical interactions between the different APIs may occur and need careful evaluation. Sreedhara *et al.* have performed a similar compatibility study for the case of co-administration of pertuzumab with trastuzumab in which they were able to show the stability of both products within the IV bags and the absence of any interactions.[97] FDC products with one API component at low concentration and one or more API(s) at high concentration may need application of additional analytical techniques during in-use testing to ensure the low concentrated API does not get adsorbed and lost before administration. This is of particular interest for IV products, as intravenous administration usually requires further dilution of the drug product in infusion bags.[40,98]

Analytics and stability

As previously stated, a protein may experience several degradation pathways. Thus, also for single protein products, several analytical methods are required during development to detect the different degradation mechanisms that may occur. Usually, various methods with focus on protein content/concentration, aggregation, charge pattern, fragmentation, chemical modifications (e.g. oxidation, deamidation), potency/activity, visible and subvisible particles and others, such as pH, osmolality or clarity and opalescence are employed. A good analytical method performance and sensitivity are crucial to ensure high quality and safety of the final product.

A main requirement throughout overall FDC product development is to show compatibility between the different APIs in the product. Therefore, as compared to single-entity products, additional focus is to be put on the elucidation of potential drug–drug interactions. This may become challenging as certain standard methods employed during protein product development may not always be capable of differentiating one protein from another. For example, SEC read-outs, typically used for the detection and quantification of protein aggregates, may be limited if two or more proteins of comparable molecular weight resp. size are coformulated which may result into peak overlap. Thus, detection of differences in the monomer or degradation pattern between the two proteins may become difficult. Peak overlap may also occur for IEC, analysing the molecular charge profile, if the two proteins exhibit a similar or also very complex charge pattern. Therefore, it is usually crucial to compare the coformulated product with the single entities in order to check, for example for hetero-aggregate formation, chemical drug–drug interactions by comparison of the degradation patterns in absence vs. presence of each other. Additional methods for extended product characterization, such as SDS-PAGE, AUC or SEC-MALS, RP-HPLC or peptide-map/MS should be considered as different assays might be needed for differing purposes. These points should not only to be considered during formulation development, but also during evaluation of accelerated and long-term stability. Finally, once acceptance criteria are to be established, impurities in the product should be expressed with reference to the parent API and not the overall content of API, if feasible.[5] The latter may become challenging if one protein is present in only a minute amount as compared to other API(s). Furthermore, method validation may become technically more complex for FDCs as it should be performed in the presence of the other API(s).

Conclusion

Coformulation of two or more protein APIs in one final drug product offers several advantages, such as increased efficacy, the overall reduction of adverse event incidents in patients, the opportunity to reduce the dose of either one or of multiple APIs within the combination or the treatment of two distinct diseases at the same time. However, additional complexity during development is given for these so called FDCs. As protein activity is linked to its complex three-dimensional structure and various physico-chemical degradation pathways may co-exist, the maintenance of physical and chemical integrity of all protein APIs

is key for a safe and efficacious FDC product. This review has discussed various challenges that may occur throughout pharmaceutical development of coformulated protein products. Considerations for formulation, manufacture, storage, transport, handling and patient administration, stability and analytics of FDCs were presented.

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