REGIONE DEL VENETO

ULSS3 SERENISSIMA

AVVISO PUBBLICO, PER TITOLI E PROVA ORALE, PER L'ASSEGNAZIONE DI N. 1 BORSA DI STUDIO ALLA U.O.C. DI FARMACIA OSPEDALIERA DEL PRESIDIO OSPEDALIERO DI MESTRE – PER LA DURATA DI 8 MESI E PER 35 ORE SETTIMANALI.

PUBBLICAZIONE CRITERI DI VALUTAZIONE DELLA COMMISSIONE ESAMINATRICE E DELLE TRACCE DELLA PROVA ORALE

In ottemperanza a quanto disposto dall'art. 19 del D.Lgs 14 marzo 2013, n. 33 e s.m.i., vengono elencati i criteri di valutazione e le tracce della prova orale, estratti dal Verbale della Commissione esaminatrice n. 1 e n. 2 del 9 febbraio 2022:

- Omissis -

CRITERI DI VALUTAZIONE

La Commissione stabilisce, sulla base del relativo bando, che la prova orale deve riguardare argomenti relativi alla "Tecnica farmaceutica ed informatica" e che, nell'ambito del colloquio, sarà altresì accertata la conoscenza della lingua inglese attraverso la lettura e la traduzione di testi, nonché mediante una conversazione in modo tale da riscontrare il possesso di un'adeguata e completa padronanza degli strumenti linguistici.

In particolare, la Commissione stabilisce che, in relazione agli aspetti peculiari del progetto, saranno accertate la capacità e la competenza del candidato nella preparazione del farmaco, in particolare il farmaco proteico, nonché nella conversazione in lingua inglese e nella gestione dei database.

La Commissione individua, inoltre, gli ulteriori criteri di valutazione:

- comprensione delle tematiche affrontate;
- completezza e correttezza della risposta;
- chiarezza espositiva.

TRACCE DELLA PROVA ORALE:

Domande A

1. Aspetti tecnico-farmaceutici dei farmaci proteici per via sottocutanea
2. Stewardship of the researcher within a European drug logistics project
3. Read and translate BOX A

Domande B

1. Aspetti tecnico-farmaceutici dei farmaci proteici per via endovenosa
2. Setting up a data collection for a research project
3. Read and translate BOX B

Domande C

1. Principali problematiche nella formulazione dei farmaci proteici rispetto ai farmaci tradizionali
2. Databases for carrying a bibliographic search
3. Read and translate BOX C

Domande D

1. Modalità di conservazione dei farmaci proteici
2. Databases to monitor the use of drugs
3. Read and translate BOX D

- omissis –

F.TO Il Segretario della Commissione

REVIEWS

Advanced protein formulations

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BOX A

Abstract: It is well recognized that protein product development is far more challenging than that for small-molecule drugs. The major challenges include inherent sensitivity to different types of stresses during the drug product manufacturing process, high rate of physical and chemical degradation during long-term storage, and enhanced aggregation and/or viscosity at high protein concentrations. In the past decade, many novel formulation concepts and technologies have been or are being developed to address these product development challenges for proteins. These concepts and technologies include use of uncommon/combination of formulation stabilizers, conjugation or fusion with potential stabilizers, site-specific mutagenesis, and preparation of nontraditional types of dosage forms—semiaqueous solutions, nonfreeze-dried solid formulations, suspensions, and other emerging concepts. No one technology appears to be mature, ideal, and/or adequate to address all the challenges. These gaps will likely remain in the foreseeable future and need significant efforts for ultimate resolution.

Keywords: stability; viscosity; stabilizers; conjugation; suspension

BOX B

Introduction

One of the key steps in the development of a successful protein drug product is to formulate the drug candidate into a s dosage form to achieve a minimum of 18-month shelf life. Due to the high molecular sensitivity of proteins to different process stresses, temperature, and other environmental factors, formulation development for proteins can be a complex, and often challenging process.[1]

Over the past two decades, a variety of formulation approaches have been tried or being developed to stabilize a protein candidate. A commonly accepted process for formulation development is to conduct stability studies upon alteration of a variety of formulation variables, such as pH, ionic strength, type/concentration of buffering agents, and type/concentration of other stabilizing agents, such as surface-active agents, and tonicity-adjustment agents.[2] However, these traditional formulation approaches are generally not adequate to make a protein stable enough to commercialize a liquid protein product for long-term storage at room temperature.

BOX C

Many proteins are not even stable enough at 2–8 °C in solution and have to be made into a solid form for clinical evaluation or commercialization. Commercially, only few plasma products, such as Hizentra, approved in the United States in 2010, a 20% immune globulin solution for subcutaneous injection (IGSC), can be stored at room temperature (up to 25 °C) for up to 30 months (US Package Insert).

In recent years, monoclonal antibodies have become a major therapeutic class.[3,4] In association with their practical applications for subcutaneous administration, high concentrations are frequently needed to accommodate the low administration volume. Unfortunately, the physical behavior of such a product may change dramatically with increasing

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protein concentrations.[5,6] These properties may include significant enhancement of solution opalescence,[7,9] viscosity,[10–12] and protein aggregation/immunogenicity.[13–15] These altered properties challenge the drug product manufacturing processes, product administration, and marketability.

BOX D

Several nontraditional or novel concepts and technologies have been or are being developed to address the above issues. This short review summarizes these concepts and technologies as advanced formulations for stabilization of proteins (i.e., improvement in any protein stability indicators, such as T_m, aggregation tendency, etc.) and/or development of high-protein concentration products (>100 mg/mL).

Use of Complex or Uncommon Stabilizers

Proteins generally require a formulation excipient(s) as a protein stabilizer in a liquid state. Protein stabilization by a stabilizer(s) can be achieved through the traditional preferential interaction mechanism[16] and/or other proposed mechanisms such as nonspecific interaction with surface hydrophobic pockets[17] or charged amino acids,[18] specific ligand binding,[19] and enhancement of solution viscosity.[20] To enhance the stability of proteins, simultaneous use of multiple stabilizers has been tested in expectation of addressing different stability issues via different mechanisms and/or possible synergistic effect. For example, a mixture of three amino acids—L-arginine (positively charged), L-glutamic acid (negatively charged), and L-isoleucine (nonpolar) can stabilize recombinant factor VIII (FVIII) during lyophilization and storage to the same degree as achieved by using albumin as a stabilizing excipient.[21] Similarly, protein solubility, shown to correlate closely with protein aggregation tendency,[22] can also be significantly enhanced by combination of multiple excipients. Use of both L-arginine hydrochloride and L-glutamic acid together showed synergistic effect on enhancement of the solubility of proteins due to a reduction in protein–protein interactions and additional hydrogen bonding interactions between the excipients on the surface of the protein.[23] In reality, most mAbs seem to have high solubility potentials (>100 mg/mL)[24] and do not need solubilization. Most of above stabilizing excipients are charged amino acids, suggesting that excipient charges play a significant role in protein stabilization, supporting some of the above proposed stabilization mechanisms. Use of multiple charged excipients for protein stabilization/solubility enhancement is likely similar to combination of multiple buffering agents for protein stabilization.[25] On the other hand, use of multiple excipients certainly add additional burden during protein formulation characterization and stability studies.

Use of a large polymeric excipient/substance has been found to be effective in stabilization of proteins. Neutral polymers, as crowding agents, can stabilize a protein due to the excluded volume effect (steric repulsion).[26] Several neutral polymers were found to stabilize various proteins, such as PVP,[27] Ficoll-70000,[28] and hydroxyethyl (heta) starch, or PEG 4000.[29] Recently, new polymers have been synthesized and have demonstrated utility for protein stabilization. Examples include functionalized dextrans[30,31] and glycopolymers containing trehalose as the side chain units for enhanced process and storage stability.[32,33] Glycopolymers made from modified trehalose monomers were also shown to achieve stabilization for several model proteins.[34] Polyanions/polycations have long been recognized as possible stabilizers due to their delicate formation of protein–polyion interactions, such as heparin[35–39] and dextran sulfate[36,37,40] pentosan polysulfate, polyphosphoric acid, poly-L-glutamic acid, poly(acrylic acid), poly(methacrylic acid).[36,37] Interaction between polycationic chitosan and negatively charged lactate dehydrogenase (LDH) leads to significant stabilization during air-jet nebulization.[41,42] On the other hand, interaction of negatively heparin and keratinocyte growth factor 2 (KGF-2) actually facilitated protein aggregation during agitation.[43] It is apparent that strong interactions may lead to protein destabilization, as illustrated by the preferential interaction mechanism.[16]

Similarly, amphoteric polymers or proteins can be used as effective protein stabilizers. Successful examples include albumin, caseins,[44] heat shock proteins (HSPs),[45–48] valosin-containing protein (VCP),[49] α-crystallin,[50–52] and gelatin.[29,53] Among these examples, use of α-crytallin at 0.2 mg/mL greatly improved resistance of insulin to fibrillation, better than the effect of HSA at 2.5 mg/mL.[54] The chaperone-like effect is proposed due to interaction between crystallin and non-native protein species involved in the fibrillation process. A special case is the use of recombinant hyaluronidase enzyme in a protein formulation for facilitating rapid tissue distribution and thus, administration of a larger-than-normal injection volume.[55] Again, the use of polymers or proteins in a protein formulation increases the complexity of the formulation and complicates protein formulation characterization and stability studies.

The limited degree of protein stabilization achieved by commonly-used excipients prompted evaluation of uncommon excipients/substances in improving protein/peptide stability. These include resveratrol, a natural phenol,[56] hydroxybutyrate,[57] polyamines,[44] octanoic acid,[58] and quinone-tryptophan derivatives.[59] Among these, hydrophobic salts, such as salt of pentaine-1,5-dimaine and camphor-10-solfonic acid, have been shown to reduce the viscosity of mAb solutions by 10 folds.[60] However, uncommon excipients may need safety evaluations, which may include significant *in vivo* studies.