Analytical Considerations for Excipients Used in Biotechnology Products

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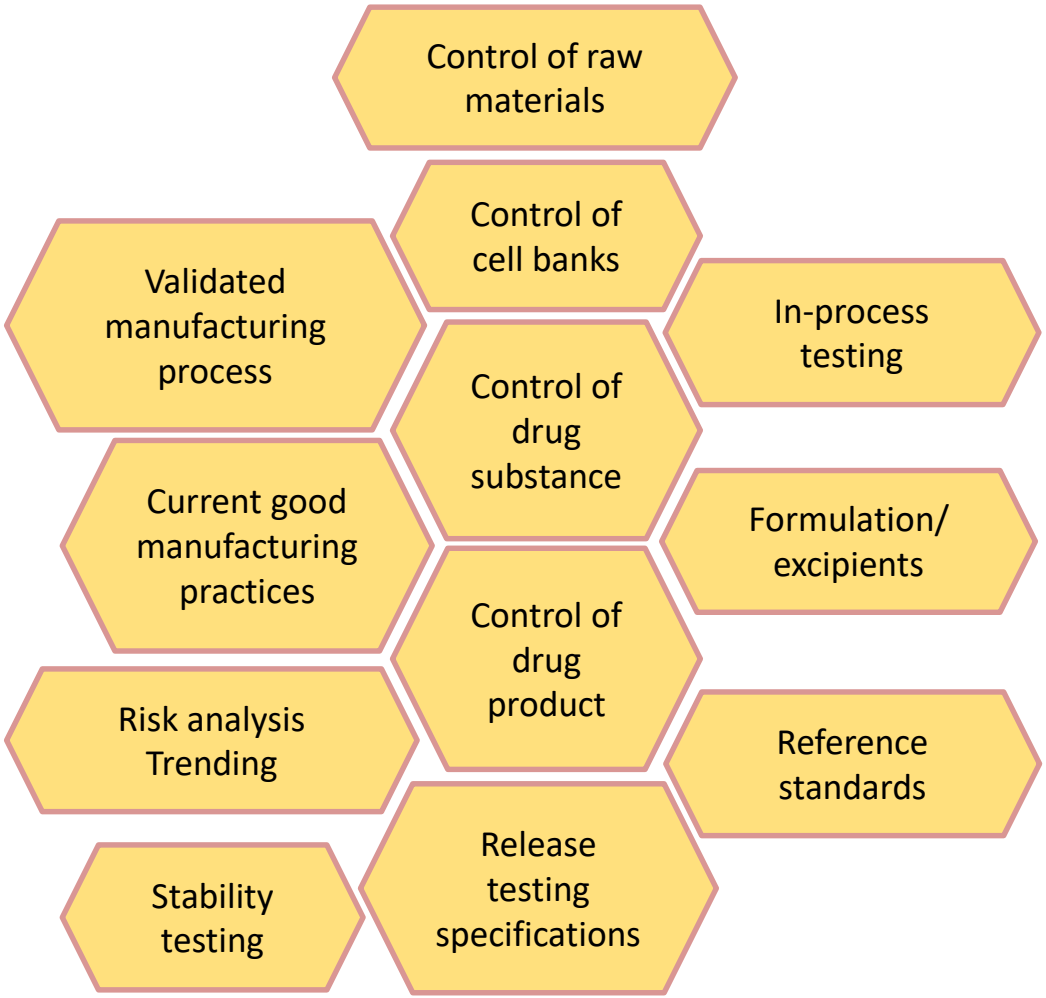
Outline of presentation

- Analytical methods and their integral role in control strategies for quality of biotechnology products
- Considerations for analytical methods with respect to excipients
  - How excipients factor into the control strategy for biotechnology products
  - Control of surfactants in biotechnology products
- Case studies on caveats and analytical challenges posed by some excipients
- The scientific principles behind regulatory expectations from analytical testing related to excipients

Elements of Control Strategies for Quality Attributes of Biotechnology Products



Regulatory basis for excipient quality and analytical considerations

21 CFR 211.84(6)(d)(2)

- “(2) Each component shall be tested for conformity with all appropriate written specifications for purity, strength, and quality. In lieu of such testing by the manufacturer, a report of analysis may be accepted from the supplier of a component, provided that at least one specific identity test is conducted on such component by the manufacturer, and provided that the manufacturer establishes the reliability of the supplier's analyses through appropriate validation of the supplier's test results at appropriate intervals.”

Life Cycle of Analytical Methods

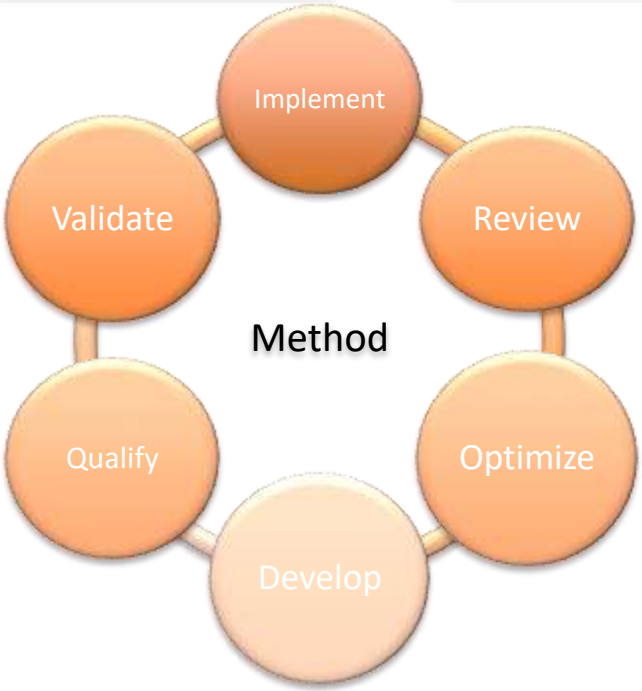
Pre-Clinical
- Selection
- Development
- Optimization
- Pre-formulation

Phase I
- Safety tests validated,
- Qualified methods
- Set tentative release/stability acceptance criteria

Phase 2
- Optimization/qualification
- Refine lot release criteria
- Set tentative validation acceptance criteria
- Delineate/initiate assay validation parameters
- Excipient control strategy

Phase 3 and BLA
- Full assay validation (strongly recommended for phase 3)

Post-Licensure
- Trend analysis
- Performance review
- Method replacement (supplement)



Excipients in OBP-regulated products

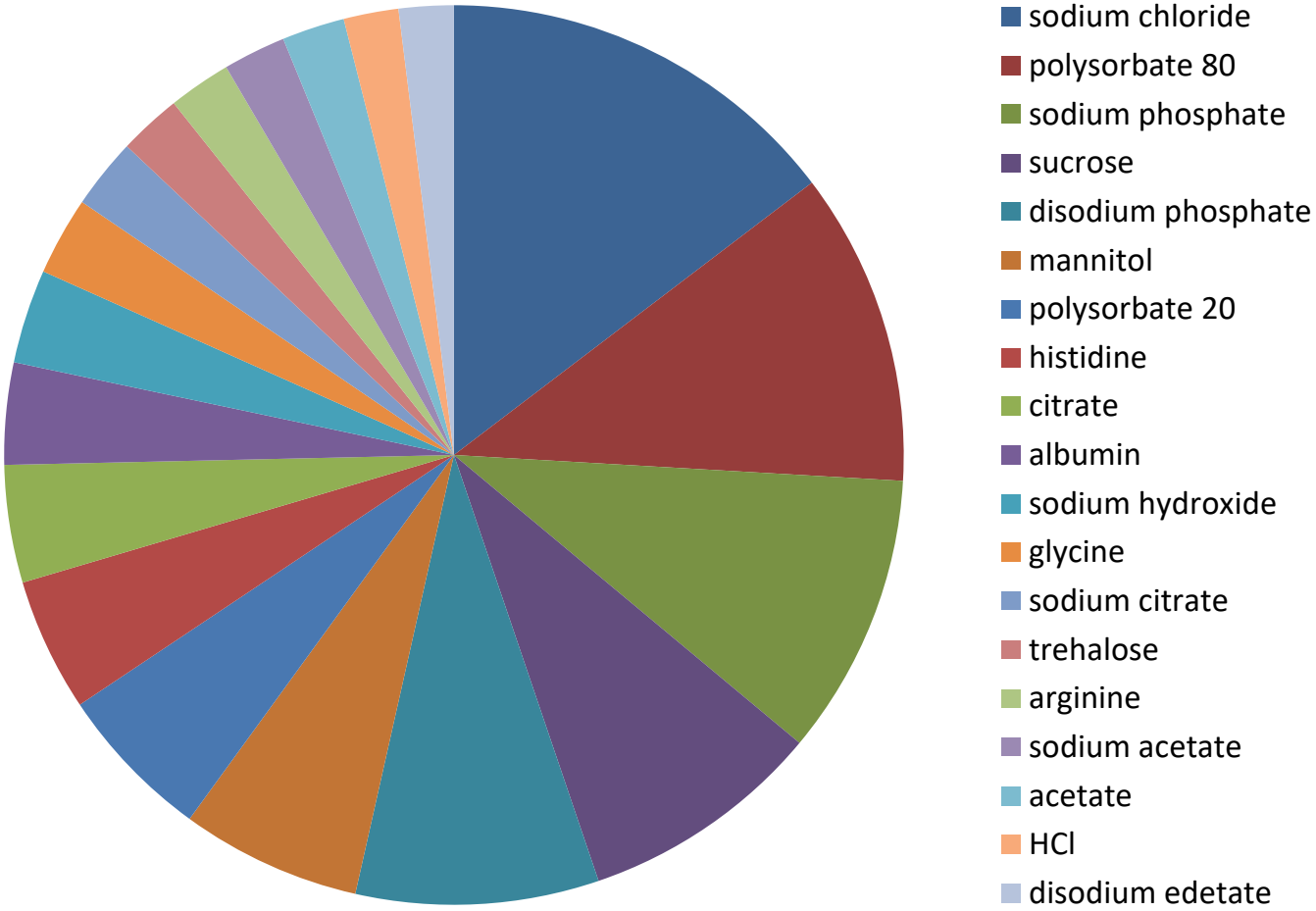
As of February 2018, the Office of Biotechnology Products had 161 licensed (under BLA) or approved (under NDA) protein therapeutic products and over 205 unique formulations or presentations of those products.

The need for excipients in biotechnology products

- pH – minimize oxidation, degradation (e.g. sodium carbonate)
- Stabilizer – prevents modification/degradation (surfactants, amino acids, antioxidants, sugars)
- Buffering agent – temperature stability, safety at larger volumes (acetate, citrate, phosphate)
- Polymers and proteins – to stabilize and increase half-life (PEG, HSA)
- Solubilizing agent – Increases protein solubility with low concentration (salts, non-ionic surfactants)
- Tonicity modifier, bulking agent – generally inert (sorbitol)
- Preservatives – to prevent microbial growth for multi-use
- Lyoprotectants – protect against freezing/unfolding (sucrose, trehalose)

Carpenter and Manning, Pharmaceutical Biotechnology, 2002

Commonly used excipients in OBP-regulated products



Commonly used excipients in OBP-regulated products

Top10 All OBP products

- Sodium chloride (52)
- Polysorbate 80 (40)
- Sodium phosphate (36)
- Sucrose (31) & disodium phosphate (31)
- Mannitol (23)
- Polysorbate 20 (20)
- Histidine (17)
- Citrate or Citric acid (15)
- Albumin (13)

Top 5 mAb Excipients

- Polysorbate 80 (24)
- Sodium chloride (20)
- Sucrose (14) and histidine (14)
- Sodium phosphate (11) and disodium phosphate (11)

Top 5 Cytokine/GF Excipients

- Sodium chloride (13)
- Sodium phosphate (11)
- Disodium phosphate (9)
- Mannitol (8) and Polysorbate 80 (8)

Caveats with biotechnology-derived drug products and their excipients

- Certain antimicrobial preservatives can induce protein aggregation
  - M-cresol>phenol>benzyl alcohol>phenoxyethanol and propensity to aggregate IFN-2α (Bis and Mallela, Int J Pharm, 2014)
  - Humanized monoclonal antibody (Gupta and Kaisheva, AAPS PharmSci, 2003)
  - Screening needed for each excipient at desired drug concentration.
- Protein oxidation by buffer components, "antioxidants" and trace metals
  - Ascorbate-and peroxide-mediated oxidation and fragmentation of enzymes and mAbs (Uehara and Rao, Pharm Res, 2015)
  - Trace metals can catalyze unintended protein oxidation and aggregation (Kryndushkin D et al, Pharm Res, 2017)

Excipients and interference with analytical methods

- Human serum albumin (HSA) – most abundant protein in human plasma and potential interference during immunogenicity testing.
- As an excipient, HSA could interfere with enzymatic activity of therapeutic proteins during potency testing. Comparability of drugs with or without HSA could be challenging.
- HSA could interfere with the higher order structure, solvent accessibility, receptor binding, metal binding, and activity of proteins. (*Peng Y et al, PLoS One, 2014; Quinlan GJ et al, Hepatology, 2005*)
- HSA (and other amino acids) can affect compositional analysis, protein concentration assays and sequencing results. (*Shintani H, Pharm Analaytica Acta, 2013*)
- Removal of excess HSA to improve sensitivity of LC-MS/MS (*Liu G et al, Anal Chem, 2014*)

Excipients and interference with analytical methods

- "The large UV/vis absorbance and broad chromatographic elution of Polysorbate 80 often makes it difficult to accurately quantitate pharmaceutically active compounds in solutions where the surfactant is present." *Wuefling WP et al, J Pharmaceutical and Biomedical Analysis (2006)*
  - Variability of the P80 between vendors and over 200-300 nm range
  - Column resin and pore size also render different retention times and chromatogram profiles
  - Polysorbate buildup on column
  - Fewer problems at 0.01-0.05% (w/w) concentration

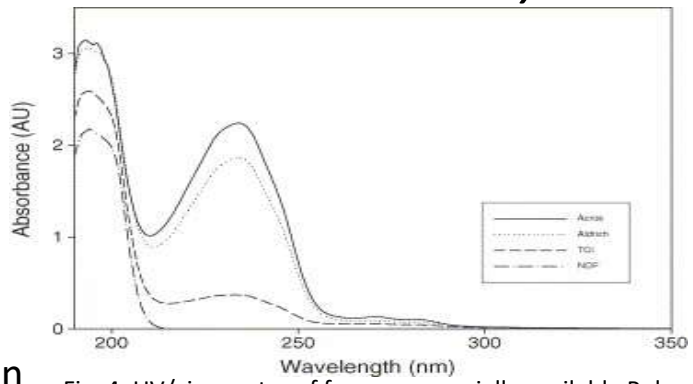


Fig. 4. UV/vis spectra of four commercially available Polysorbate 80 brands (0.1%, w/w solution). (Wuefling WP , 2006)

- "Successful dissolution chromatographic method development will include the use of a low organic mobile phase that should be followed by some high organic wash cycle throughout an analysis sequence to ensure no significant surfactant build-up. "

Excipients and interference with analytical methods

- Antibodies to polyethylene glycol (PEG) could give a false positive signal for neutralizing antibody activity in a cell-based functional assay
- Pre-existing antibodies to PEG may cross-react or interfere with other PEG-containing product during immunogenicity/bioanalytical PK/PD assays (*FDA Guidance for Industry on Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products, 2016*)
- Precipitation of IgG and IgM might give false negative results (*Sturgeon and Viljoen, Ann Clin Biochemistry, 2011*)

Case Study (polaxamer 188 used as a raw material)

A growth factor product where polaxamer 188 was used as a raw material:

- Polaxamer 188 used in cell culture production media to protect cells from shear stress from gas sparging and agitation.
- UPLC method was used to quantitate P188.
- Concentration of P188 was shown to be below LLOD after downstream purification by ion exchange chromatography.
- Data from multiple batches and worse case scenario was presented as the max theoretical amount of P188 that could be administered to the patient per dose of drug. A toxicological risk assessment was provided with daily exposure calculations from available animal and human clinical data.
- Based on the demonstrated clearance and scientific justification, no routine testing was proposed and found to be acceptable.

Case Study (polysorbate 20)

A CHO cell-derived mAb with PS20-based formulation –

- A CHO-cell specific lipase was identified to be co-purified during the manufacturing steps, beginning from initial harvest to drug substance. Spiking studies with FTIR and GC-MS showed this lipase could hydrolyze polysorbate and form free fatty acids, which over time formed particulates shown by MFI upon long-term storage.
- Multiple batches showed reduction in residual lipase content during each chromatographic purification steps. Mass spec and immune-reaction based assays were used to measure residual levels.
- Forced degradation studies such as heat and agitation showed increased levels of both LMW and HMW species by SE-UPLC and CE-SDS.
- After addition of a HIC step, the levels fell below LLOD.
- Given the efficiency of clearance process, no further testing of the lipase was proposed and found to be acceptable.
- Continued monitoring of particulates and polysorbate content at release and during stability studies using SEC and MFI.

Control of Polysorbates and other excipients before formulation

- A certificate of analysis is generally provided for the batch(es) of PS used in formulation.
- Prior to use in formulation, USP monograph-based compendial tests are generally included as specifications for PS. Other tests and acceptance criteria could be included with a scientific justification.
- For polysorbates, in addition to levels of solvents and heavy metals, acid, hydroxyl, peroxide, and saponification value, the composition of fatty acids by GC is generally included, based on compendial recommendation.
- Stability studies and process development, including clearance or spiking studies with potentially problematic impurities (e.g. HCP)
- Choice of container closure system (e.g. protect from light, N2 overlay etc)
- *Martos A et al, J Pharm Sci, 2017; Zhang L et al, Pharm Res, 2017; Li Y et al, Analytical Chem, 2014; Zhang R et al, J Chrom Sci., 2012; McShan AC et al, PDA J Pharm Sci Tech, 2016* .

During release and stability testing

- HPLC-based methods for polysorbate or other surfactants with an appropriately established reference material for relative quantitation
- MS-based methods for isosorbide and major ester derivatives, coupled with LC (e.g. Mixed mode chromatography, multidimensional UPLC, evaporative light scattering detection, electrospray ionization-MS)
- Limits for process-related impurities that impact surfactant and product stability (e.g. ELISA or LC w/reference)
- Orthogonal purity tests that can capture aggregate formation at release and during stability.
- Degradation profile during stability to establish limits for product/impurity during storage (which can be different from release testing limits)

The goal throughout a drug's developmental lifecycle

- To prevent unreasonable and significant risk of illness or injury to human subjects [21 CFR 312.42(b)(1)(i)]
- Provide sufficient information to asses risk to human subjects [21 CFR 312.42(b)(1)(iv)]

Take home messages

- A life-cycle approach to analytical methods is recommended, which includes design → develop → monitor → improve.
- Excipients are an integral and functional component of biotechnology drug products and control strategy for product quality
- Some excipients could interfere with analytical methods used for the control of the active pharmaceutical ingredients.
- Certain process-related impurities could interact with surfactants to result in product-related impurities
- Control strategies for surfactants depend on the degree of characterization of the surfactant with the drug product and supporting scientific justification
- A combination of specific characterization data, in-process testing and, if appropriate, release/stability testing is generally reported in submissions
- The analytical toolbox for excipient control includes the use of LC and MS based techniques with modified derivatization or detection techniques and specific reference materials for relative quantitation.
- Continual improvements in analytical methods provide opportunities to monitor quality and control for lot-to-lot consistency with the goal of safe and efficacious drug products

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