Excipients and biotechnology product quality attributes: a regulator's perspective on reducing the interfacial tension

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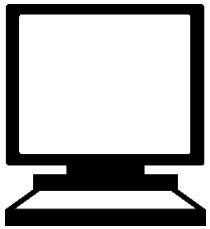
CASSS CMC Strategy Forum 2020

Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.



Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.

Drugs are no different.


FDA
Patients expect safe and effective medicine with every dose they take.

Pharmaceutical quality is

assuring *every* dose is safe and effective, free of contamination and defects.


It is what gives patients confidence in their *next* dose of medicine.

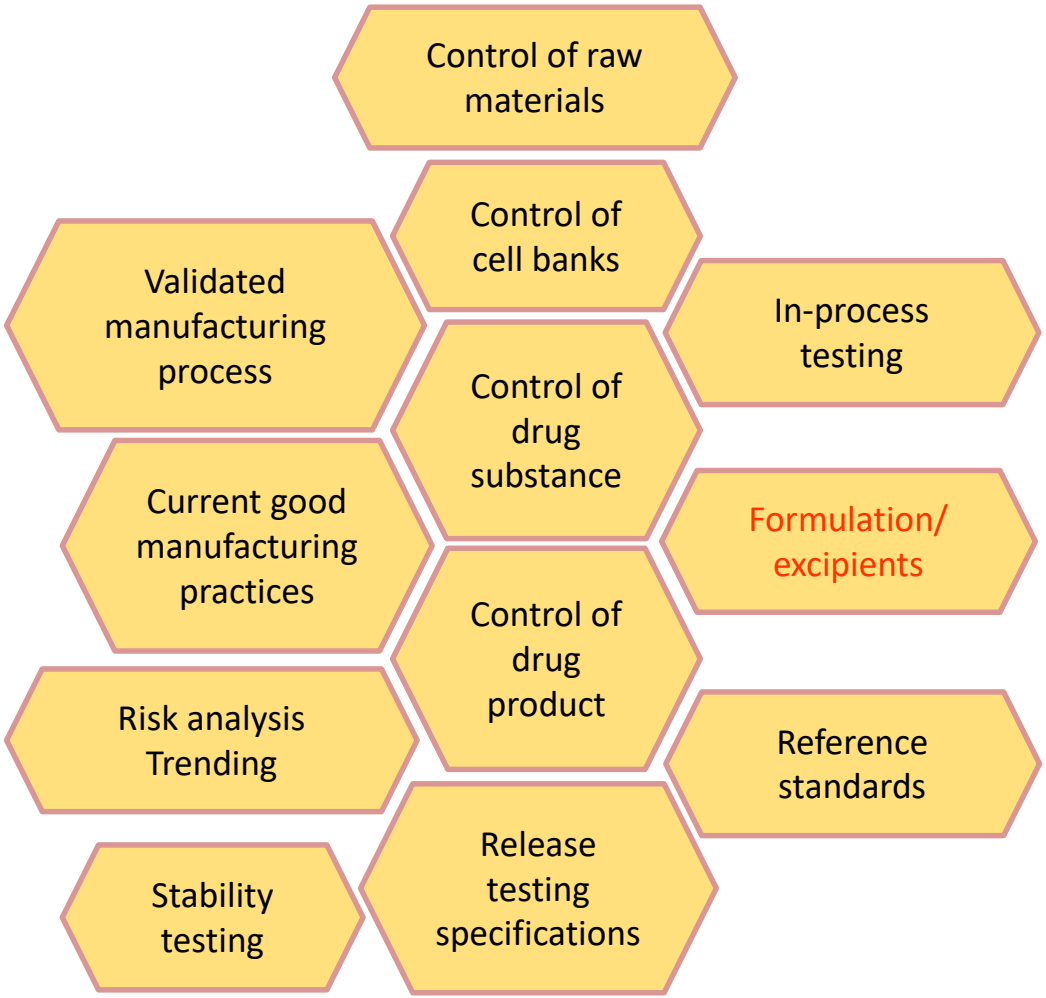
Disclaimer

The views and opinions expressed should not be used in place of regulations, published FDA guidances, or discussions with the Agency.

Outline of presentation

- Background and core regulatory expectations with respect to excipients in biotechnology products
- Product quality caveats and analytical challenges posed by some surfactants
- Recommended strategies for surfactants, aggregates and related product quality attributes
- The scientific principles behind regulatory expectations for excipients – the desired state

Elements of Control Strategies for Quality Attributes of Biotechnology Products



Regulatory basis for excipient quality 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 a Surfactant Control Strategy

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

Phase I
- Raw material qualification
- Qualified methods
- Set phase-appropriate release/stability acceptance criteria
- Excipient control strategy

Phase 2
- Dosage form optimization
- Refine lot release criteria
- Extended characterization
- Delineate/initiate assay validation parameters

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

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

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)]

Surfactants 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

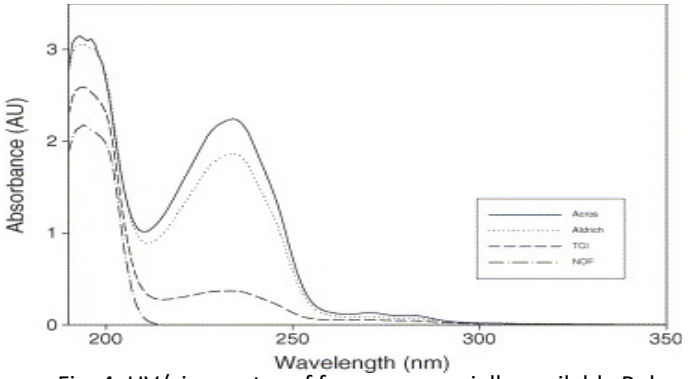
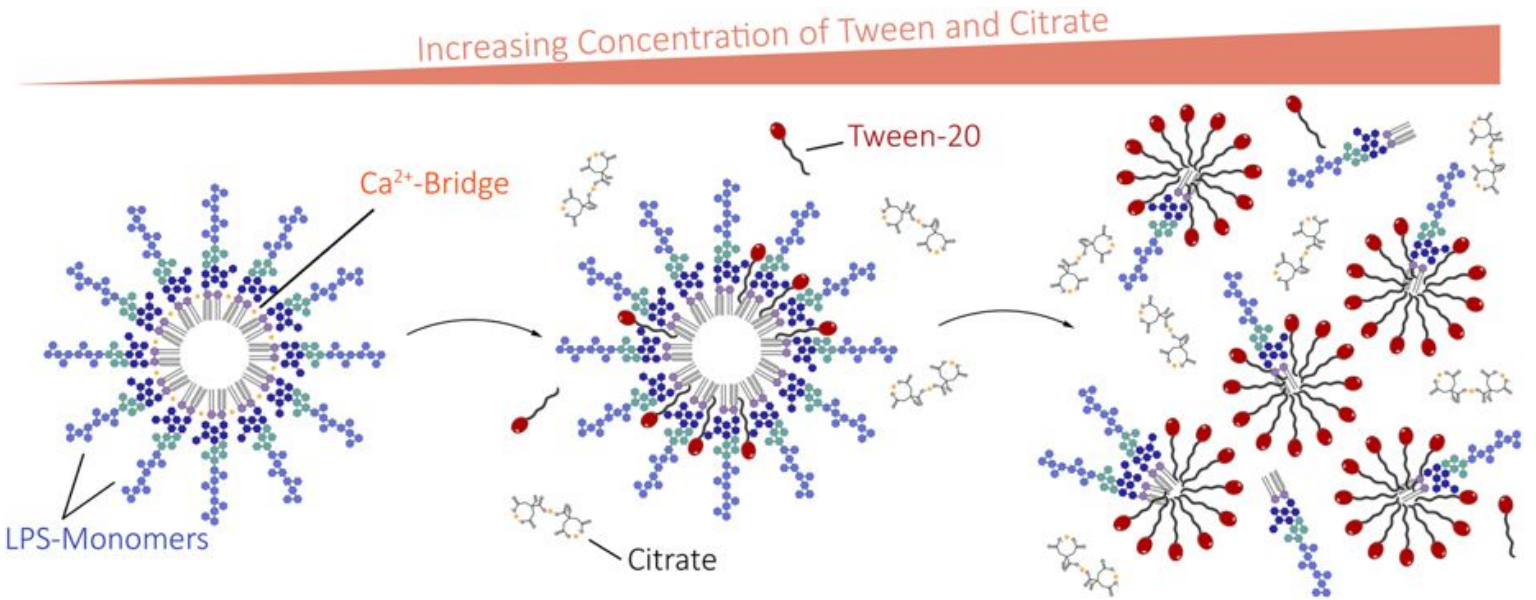


Fig. 4. UV/vis spectra of four commercially available Polysorbate 80 brands (0.1%, w/w solution). (Wuefling WP , 2006)

Surfactants and interference with analytical methods



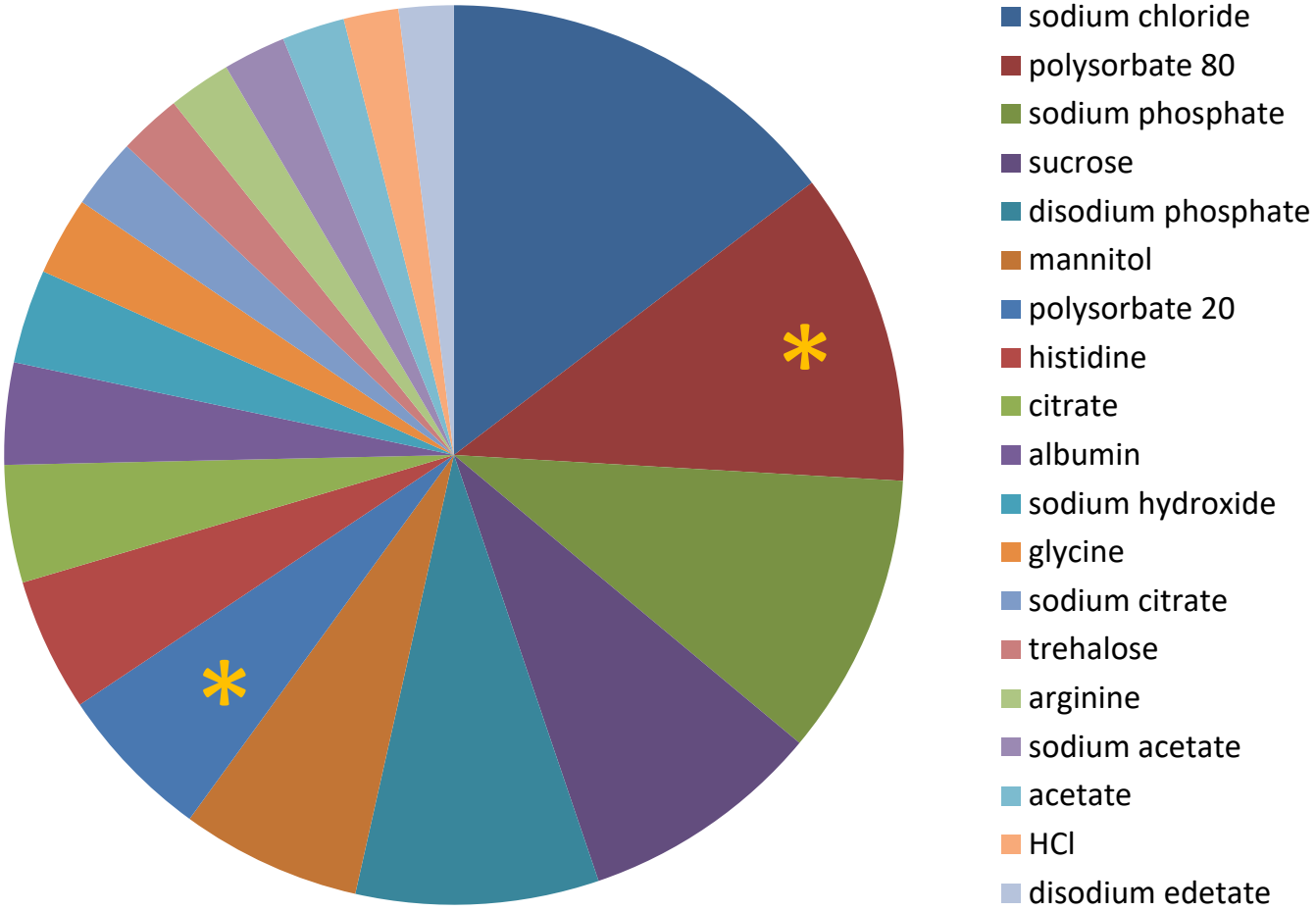
Low endotoxin recovery (LER) with limulus amebocyte lysate (LAL)-based assays to detect lipopolysaccharides (LPS) because of a masking effect caused by chelators or surfactants.

Reich J et al, Biologicals: journal of the IABS, 2016; **Schwarz H** et al, Scientific Reports, 2017; Patricia Hughes, Ph.D. (OPQ)

Excipients in OBP-regulated products

As of September 2019, the Office of Biotechnology Products had 236 licensed (under BLA) or approved (under NDA) protein therapeutic products and over 371 unique formulations or presentations of those products.

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) and 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)

Surfactants in licensed biotechnology products

- Poloxamer 188 : 2%
- Polysorbate 20 : 16%
- Polysorbate 80 : 32%
- Polysorbate (unspecified): 2%

"Polysorbates are not physiologically inert." – *Singh S et al, J Pharm Sci, 2018*

Preclinical

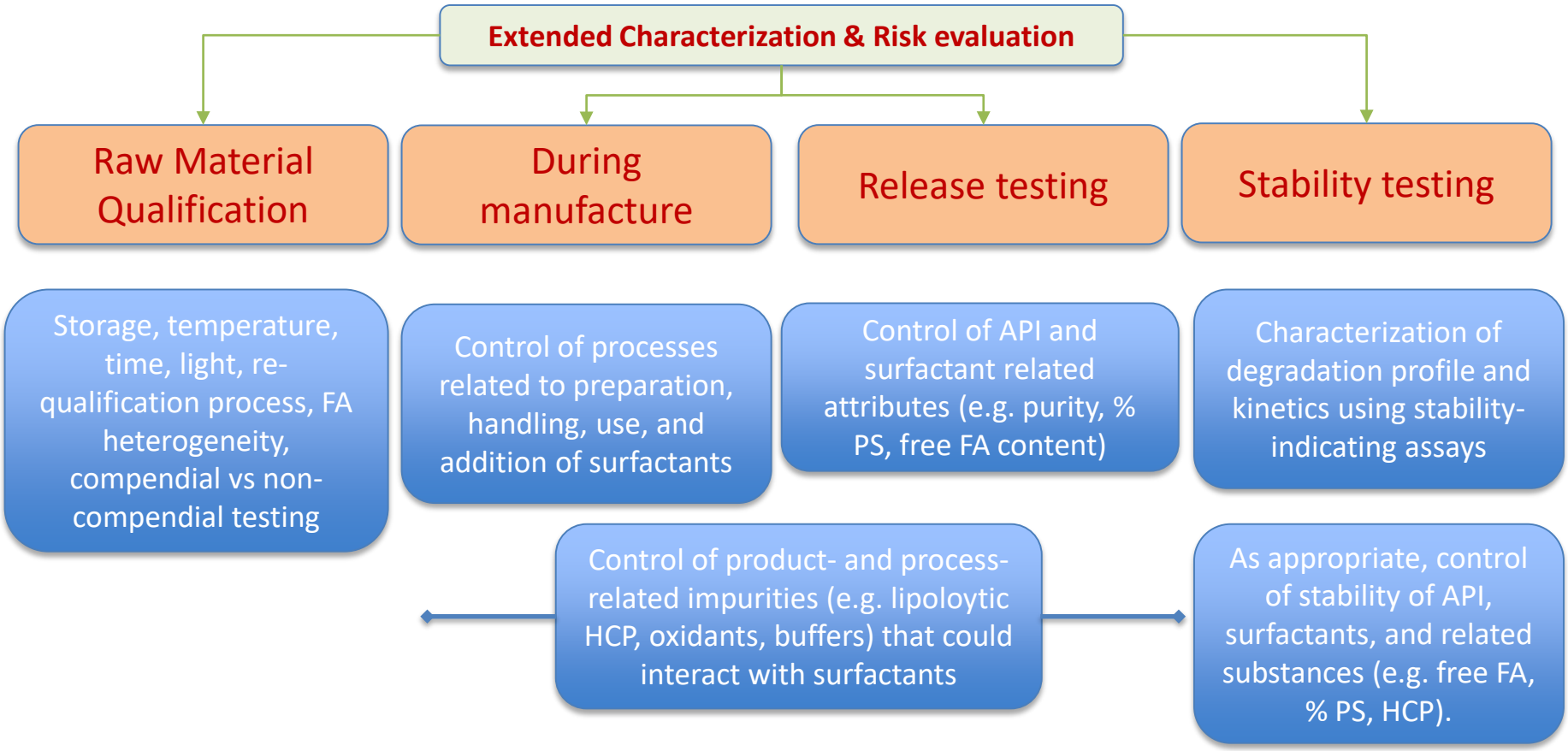
Clinical

Surfactant	Route of administration	Dose/ concentration	Event reported	References
Polysorbate 80	Systemic (dogs)	5 mg/kg	Histamine release, Hypotension, tachycardia	Masini E et al, Agents Action, 1985; Gough WB et al, J Cardiovasc Pharmacol, 1982; Munoz et al, Eur Heart, 1988.
Polysorbate 20 & Polysorbate 80	In vitro w/healthy human blood	0.5% w/v	Complement activation with production of C3a-desAr and SC5b-9 (anaphylotoxins)	Weiszhar Z et al, Eur J Pharm Sci, 2012
Polysorbate 20 & Polysorbate 80	Intravenous (Single patient reporting anaphylactoid shock rxn on infusion of multivitamin preparation during pregnancy)	0.5% v/v	Skin prick test comparing PS/non-PS products. Basophil activation but no polysorbate-specific IgE antibodies.	CoorsEA et al, Annals of Allergy, Asthma, and Immunol, 2012
Polysorbate 20 & Polysorbate 80	IV, Low-birth-weight infants	9% PS-80, 1% PS-20 (w/Vitamin-E)	Fatal vasculopathy hepatotoxicity	Bove KE, JAMA, 1985; Alade SL et al, Pediatrics, 1986; Pesce AJ and McKean DL, Ann Clin Lab Sci, 1989
Polysorbate 80	IV, Cancer patients	26 mg PS-80/mg drug (Engels)	Acute hypersensitivity reactions and cumulative fluid retention (w/docetaxel)	Engels FK et al, Anticancer drugs, 2007; Loos WJ et al, Clin Pharm Ther, 2003,; Schwartzberg LS and Navari RM. Adv Ther, 2018
Polysorbate 80	IV, Cancer patients	400 mg/m2 (w/VP-16)	Two-fold reduced AUC (for anthracylines)	Cummings J et al, Cancer Chemo Pharm, 1986
Polysorbate 80	IV, patients receiving Epo or darbepoietin	0.15 mg/mL	Pruritis, erythema and orofacial angioedema. Linked by spin prick test.	Limaye S et al, J Allergy Clin Immunol, 2002. Steele RH et al, Nephrology, 2005
Polysorbate 20	IV, psoriasis patient w/ bradolumab/infliximab/adalimumab & healthy volunteers w/bradolumab	1:10,000 dilution	Urticaria, confirmed with skin prick test	Kato M et al., J Dermatol, 2019

Current gaps in knowledge

- The **individual** and **comparative biochemical and immunological properties** of intact polysorbate (e.g. polyoxyethylene sorbitan monooleate), individual fatty acids and polyoxyethylene **byproducts** of the polysorbate synthesis such as trioleates, tetraoleates, sorbitan dioleates, isosorbide dioleates, sorbitan and isosorbide series are unknown.
- Same for polysorbate **degradation products** – peroxides, aldehydes, ketones, acids, n-alkanes, fatty acid esters
- Are there unique patterns of degradation products with **each host cell lipase or protease**?
- The relationship between hydrophilic lipophilic balance (**HLB**), the critical micelle concentration (**CMC**), protein stabilization and potential **immunogenicity** of polysorbates and related species is unknown.
- The performance and behavior of polysorbate degradation products **in the presence of each protein API** has not been generalizable.
- How is the **predictive value** of currently available preclinical and in vitro models for immunogenicity related to the impurity, drug and anticipated clinical outcome?

General strategies observed for surfactant control during manufacture



Kerwin BA, J Pharm Sci, 2008; Jones MT et al, Pharm Res, 2018; Kishore RS et al, Pharm Res, 2011; McShan et al, PDA J Pharm Sci Tech, 2016

Risk evaluation through extended characterization studies

- Extended characterization studies can provide critical information on the relationship (or lack of) between surfactant stability and drug product quality.
- Studies could include specific stress conditions, multiple simultaneous stress conditions, and end-of-shelf life studies.
- Based on the risk assessment and accompanying scientific justification, either monitoring for information purposes or specifications are generally proposed as part of a comprehensive control strategy that accounts for both the surfactant and the protein.

During manufacture - Control strategies related to host cell proteins

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Pharmaceutical Biotechnology

Residual Host Cell Protein Promotes Polysorbate 20 Degradation in a Sulfatase Drug Product Leading to Free Fatty Acid Particles

Nitin Dixit [1,*], Nazila Salamat-Miller [1], Paul A. Salinas [2], Katherine D. Taylor [1], Sujit K. Basu [1]

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Current Opinion in Biotechnology

Applications of proteomic methods for CHO host cell protein characterization in biopharmaceutical manufacturing

Kristin N Valente[1,5], Nicholas E Levy[2,5], Kelvin H Lee[3,4] and Abraham M Lenhoff[3]

Applied and Environmental Microbiology, Sept. 2011, p. 6094–6099 Vol. 77, No. 17
0099-2240/11/$12.00 doi:10.1128/AEM.05122-11

Discovery of an *Escherichia coli* Esterase with High Activity and Enantioselectivity toward 1,2-*O*-Isopropylideneglycerol Esters▽†

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ARTICLE

BIOTECHNOLOGY and BIOENGINEERING

Knockout of a Difficult-to-Remove CHO Host Cell Protein, Lipoprotein Lipase, for Improved Polysorbate Stability in Monoclonal Antibody Formulations

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Polysorbates 20 and 80 Degradation by Group XV Lysosomal Phospholipase A_2 Isomer X1 in Monoclonal Antibody Formulations

Troii Hall, Stephanie L. Sandefur, Christopher C. Frye, Tammy L. Tuley, Lihua Huang*

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ARTICLE

BIOTECHNOLOGY and BIOENGINEERING

Host Cell Protein Impurities in Chromatographic Polishing Steps for Monoclonal Antibody Purification

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Surfactants as critical raw materials

- Quality of surfactants used in formulations is important
- Raw material qualification process for both compendial and non-compendial surfactants
- Use of non-compendial material with adequate supporting data is allowed (if fit-for-purpose).
- Storage of surfactant (temperature, time, light)
- Consider usage conditions – during manufacture and as a final drug product (in-use)

Control of Polysorbates and other excipients during manufacture and 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.
- 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, N_2 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 formulation: Polysorbates are typically used at 0.001-0.2%

(Manning MC et al, Adv Protein Chem, 2018; Braun AC et al, Eur J Pharm Biopharm, 2015)

- "The hydrophobic moieties of polysorbate 80 result in the formation of micelles at concentrations above the **critical micelle concentration (CMC) of 0.01%** (weight/volume) in protein-free aqueous solution" (Schwartzberg LS and Navari RM, Adv Ther, 2018; Chuo DK et al, J Pharm Sci, 2005)
- "CMC of polysorbate 20 is in the vicinity of **0.06 mg/ml**" (Mittal KL, J Pharm Sci, 1972)
- "..concentrations **below 0.002 mg/mL** are not recommended due to the ineffectiveness in aggregation prevention" *and* "Cautions are needed when exploiting surfactants at the concentration **around or above 2 mg/mL**...due to their potential effects in protein-binding and structure-perturbation" (Wang S et al., Eur J Phar, Biopharm, 2017)

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 (ELSD), 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)

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.

Why control for aggregates?

- Protein aggregates have the potential to negatively impact clinical performance
- Current USP particulate testing is not designed to control the potential risk of large protein aggregates to impact immunogenicity
- Development of quantitative analytical methods for particle counting and characterization is important for risk assessment and control of final drug product quality, safety, and efficacy

Pharm Res (2011) 28:920–933
DOI 10.1007/s11095-010-0297-1

COMMENTARY

Strategies for the Assessment of Protein Aggregates in Pharmaceutical Biotech Product Development

John den Engelsman • Patrick Garidel • Ronald Smulders • Hans Koll • Bryan Smith • Stefan Bassarab • Andreas Seidl • Otmar Hainzl • Wim Jiskoot

Table IV Typical Use of Techniques in Industry with Respect to Aggregate Analysis

Method	Validation	Quantification	Robustness[a]	Sensitivity[a]	Sample throughput[a,b]	QC method[c]
Visual inspection	Yes	No	Medium	Medium	High	Yes
Optical microscopy	No	Possible	Medium	N/A	Low	No
Fluorescence microscopy	No	No	Low	High	Low	No
Electron microscopy	No	No	Low	N/A	Low	No
Flow imaging	No	Yes	Low	N/A	Medium	No
Atomic force microscopy	No	No	Medium	N/A	Low	No
Turbidity	Yes	No	High	Medium	Medium	Yes
DLS	No	No	Medium	High	High	No
SEC-MALLS	No	No (MALLS part)	Medium	High	High	No
Light obscuration	Yes	Yes	Medium	Medium	Medium	Yes
"Native" mass spectrometry	No	No	Low	Medium	Low	No
Macro-IMS	No	No	Low	N/A	Medium	No
AUC	No	Yes	Low	Medium	Low	No
SEC	Yes	Yes	High	Medium	High	Yes
AF4	Yes	Yes	Medium	Medium	High	No
SDS-PAGE	Yes	Possible	Medium	Medium	High	Yes
Native PAGE	Yes	Possible	Medium	Low	Medium	No
CE-SDS	Yes	Yes	Medium	Medium	High	Yes
UV-VIS spectroscopy	No	No	Medium	Medium	High	No
Infrared spectroscopy	No	No	Medium	N/A	Low	No
Raman spectroscopy	No	No	Medium	N/A	Low	No
Fluorescence spectroscopy	No	No	Medium	N/A	High	No
Circular dichroism spectroscopy	No	No	Medium	N/A	Medium	No
NMR spectroscopy	No	No	Medium	Medium	Medium	No

[a] Scoring (low, medium, or high) was based on consensus of opinion of the authors; N/A = not available; [b] Low: <10; medium; 10–25; high >25 per day and per operator; [c] QC = quality control; all listed methods can be used for extended characterization; see Table I for definitions

Regulatory expectations for soluble aggregates between 0.2-2, 2-10 or 10-25 µ

- Aggregates, SVP, and visible particles can pose a risk to patient safety and product efficacy
- Specifications should be established for SVP below 0.2 micron and above 10 and 25 micron for parenteral and inhaled products
- SVP between 2 and 10 micron should be *evaluated* using quantitative methods and an appropriate control strategy developed
- SVP between 0.2 and 2 micron should be *characterized* and an appropriate control strategy developed

Layers of risk assessment

Surfactant quality and safety

+ other drug product components

+ specific protein API

+ manufacturing conditions

+ analytical methods

+ intended patient use

Review of excipients in biotechnology-derived therapeutic protein products

- Excipients chemistry, manufacturing and controls are reviewed considering **the context of the protein ingredient** they are intended to support.
- Multidisciplinary assessment of the quality, safety, and efficacy, including its immunological and microbiological impact, is performed by a team of discipline-specific reviewers.
- Some resources used by FDA reviewers:
  - Inactive ingredients database - https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm
  - FDA Guidance for industry on non-clinical studies for the safety evaluation of pharmaceutical excipients - https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm079250.pdf
  - FDA research and testing
  - Scientific publications

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)]
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The scientific principles behind regulatory expectations for excipients – the desired state

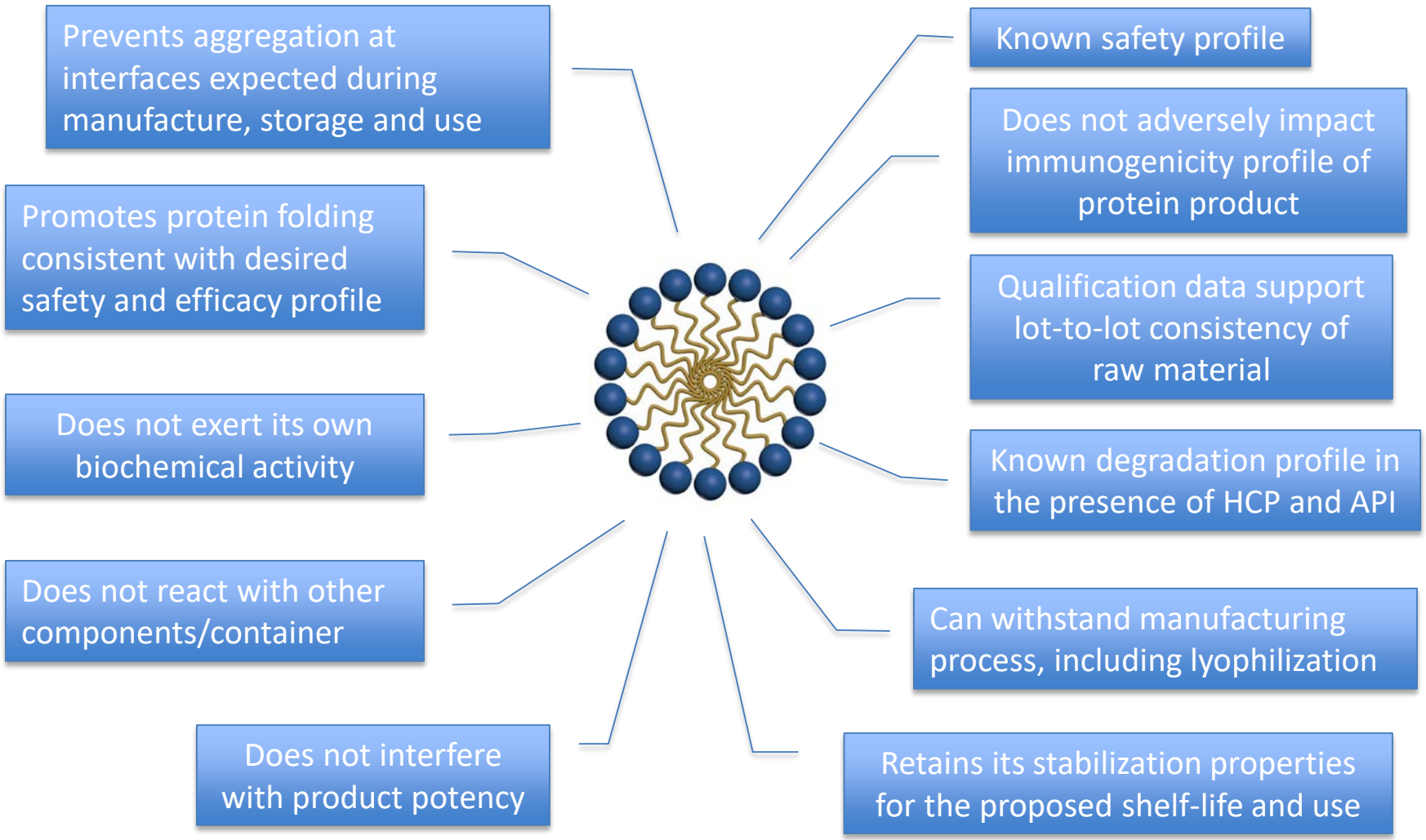

Image source: Tepavcevic V, 2017

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