Characterization of particles and practical implications

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PDA World of Pre-filled Syringes
Nov 6

Outline

- Background on Subvisible particle (SbVP) analysis for proteins
- Definitions of SbVP from <1787>
- Specifics of <787> and <1787>
- Description of Techniques
- Considerations on risks of particles
- Summary and future plans

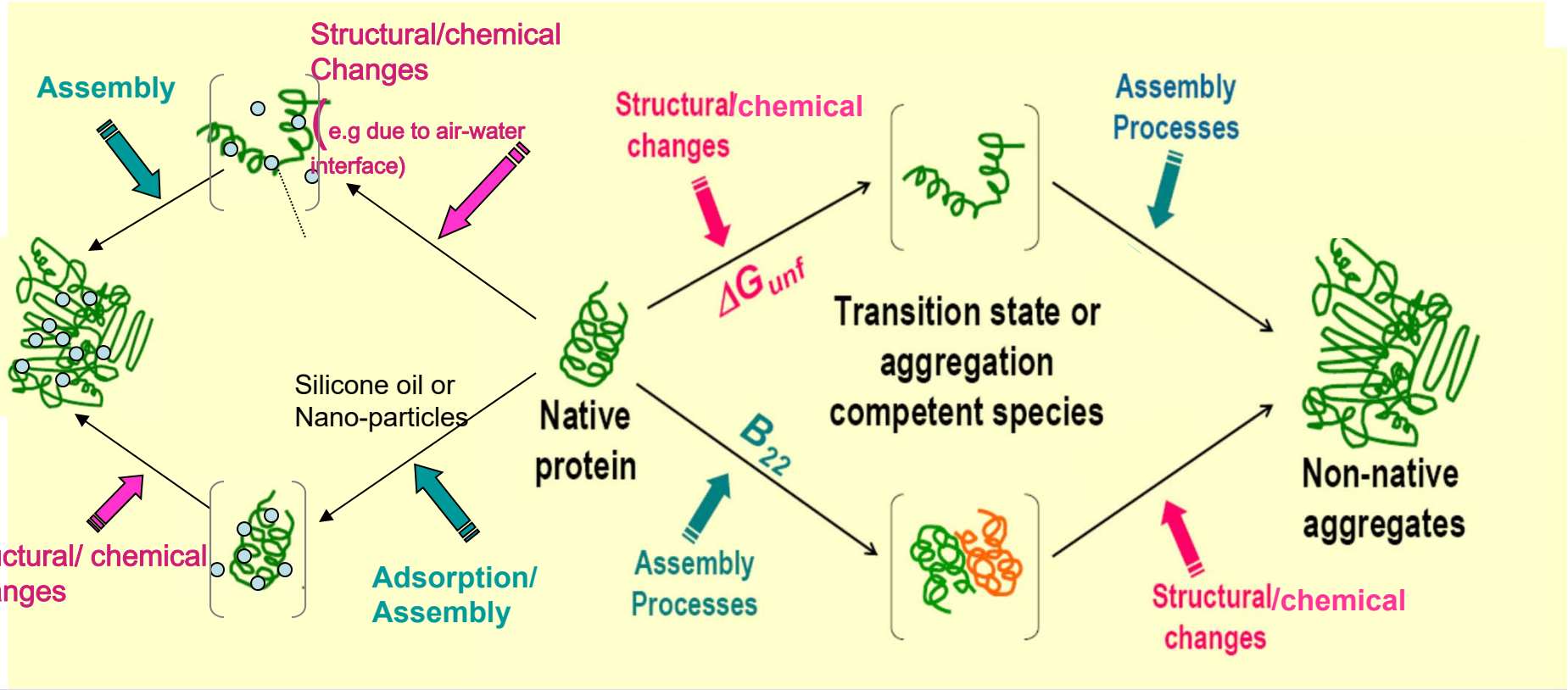
Biotherapeutics must be

- Efficacious
  - Achieve desired result at reasonable dose
  - With long enough half life (PK) to be effective
- Safe:
  - No unexpected side effects
  - No non-specific binding
  - No Toxicity
  - Minimized Immunogenicity (both neutralizing and non-neutralizing Abs)
- Manufacturable:
  - Stable shelf life for up to 2 years
  - Able to make consistently and efficiently
  - Fit existing facilities and process platforms much as possible

Proteins aggregate via different pathways

Heterogeneous nucleation or interface dependent aggregation

Conformational or colloidal stability dependant aggregation



Different mechanisms of formation can result in different types of protein aggregate particles

Synthesis of work from multiple scientists including R. Thirumangalathu, J. Bee, S Krishnan, EY Ch, H-C Mahler, M. Joubert , Q Li, S. Shire, M Cromwell, L. Narhi, et al

Aggregates are a very heterogeneous population requiring multiple descriptors*

- Size
  - <100 nm (Nanometer)
  - 100-1000 nm (Sub-μm)
  - 1-100 μm (micron, SbVP)
  - >100 μm (Visible particles have company-specific size range)
- Reversibility
  - Reversible should be restricted to aggregates for which an equilibrium constant can be measured. That is, the disassociation of proteins may be observed on the experimental time scale simply by reverting to original conditions.
  - Irreversible
  - Dissociable under physiological conditions
  - Dissociable with denaturant when conditions that disrupt structure are required to dissociate the aggregate
- Secondary/Tertiary structure
  - native
  - partially unfolded
  - unfolded
  - amyloid
  - Inherently disorded
- Covalent Modification
  - Chemical modification
  - Cross-linked
  - Reducible crosslink
  - Non-reducible crosslink
  - Intra-molecular modification
  - No modification
- Morphology
  - Aspect ratio
  - Surface roughness
  - Internal morphology
  - Homo and heteroaggregates
  - Translucent
  - Heterogeneous
- Optical properties: similar for all protein particles

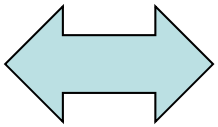
A risk based approach means we need to understand biological consequences as well

*Narhi, Linda O., Schmit, J., Bechtold-Peters, K., Sharma, D., Classification of Protein Aggregates (2012) J. Pharm. Sci. 101, 493-498.

Biopharmaceutical aggregates can be generated during all steps of the manufacturing process

Steps During the Manufacturing Process

- Fermentation
- Purification
- Formulation
- Storage
- Shipping
- Administration

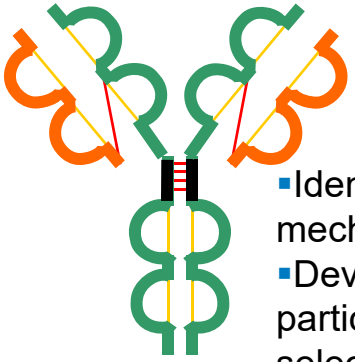


Stress Conditions

- Heat
- Freeze-thaw
- Cross-linking
- Protein concentration
- Formulation change – pH, salt
- Addition of extractables/leachables
- Chemical modification
- Mechanical Stress
- Surface effects
- Nano-particles

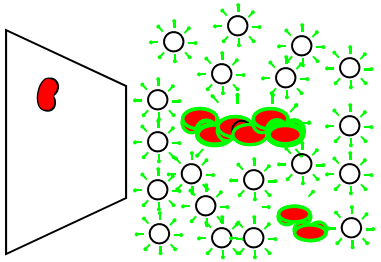
There are multiple approaches to mitigate particulation

MOLECULE



- Identify molecular mechanism
- Develop accelerated particle assay for molecule selection

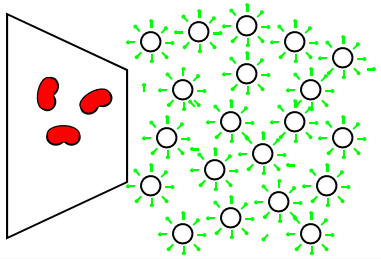
FORMULATION



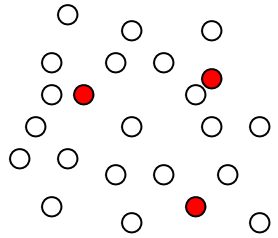
- Incorporate novel excipient screening into commercial formulation dev for particle prone molecules
- Align visual inspection analytics

CONTAINER

Determine effect of container material, vendor, and washing/depyrogenation



PROCESS

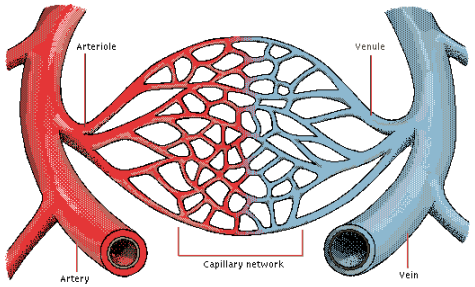


Leverage knowledge of how process differences impact particle propensity

All require reliable and sensitive particle size distribution and other analytical methods
A risk based approach means we need to understand biological consequences as well

Overview of Subvisible particles in USP/EP/JP

- Harmonized EP 2.9.19 Particulate Contamination: Sub-visible Particles and USP <788> Particulate Matter in injections both contained guidance on acceptability of ≥10 and ≥25 micron particles (6000 and 600 per container)
- Essentially created to control levels of extrinsic and intrinsic particles
- Safety concerns were around capillary occlusion by these rigid SbVP, as well as contamination, process control, etc
- Agencies were previously not concerned with specific values for biologics as long as they were under the USP limit
- No other regulatory guidance existed for subvisible particles apart from the pharmacopoeias



USP <787>, <1787>

For biologics, the focus on SbVP has changed to potential immunogenicity

COMMENTARY (by Authors from Academia and the FDA) Overlooking Subvisible Particles in Therapeutic Protein Products: Gaps That May Compromise Product Quality, John F. Carpenter, Theodore W. Randolph, Wim Jiskoot, Daan J.A. Crommelin, C. Russell Middaugh, Gerhard Winter, Ying-xin Fan, Susan Kirshner, Daniela Verthelyi, Steven Kozlowski, Kathleen A. Clouse, Patrick G. Swann, Amy Rosenberg, Barry Cherney J Pharm Sci. **2009 Apr;98(4):1201-5. doi: 10.1002/jps.21530.**

- Original USP particulate testing was not designed to measure protein particle size distribution, or to address the potential risk of large protein aggregates to impact protein immunogenicity.
- All formulated antibody drug products contain low levels of aggregates.
- The clinical immunogenic risk of aggregates is uncertain, resulting in a high risk factor being assigned to the presence of protein aggregates in biologics.
- To reduce this uncertainty, the following should be defined:
  - Aggregate attributes that cause a response
  - Amount of aggregate required to break the threshold of activation
  - Extent and nature of the response
  - Extensive studies with different proteins, stresses, and model systmes suggest the response depends on protein sequences, aggregate characteristics (including size, modification, and morphology), administration, and model systems or patient attributes. (Jiskoot et at, 2016, Ehab et al, 2016, etc)
- Analytical methods that can assess particulate characteristics (including composition, amount and reversibility of the protein aggregate) are critical for developing scientifically sound approaches for evaluating and mitigating risk to product quality caused by large protein aggregates

Definition of SbVP (sub visible particles) in <1787>

Sub visible particulate matter is defined as material between 1 and about 100 micrometers in size

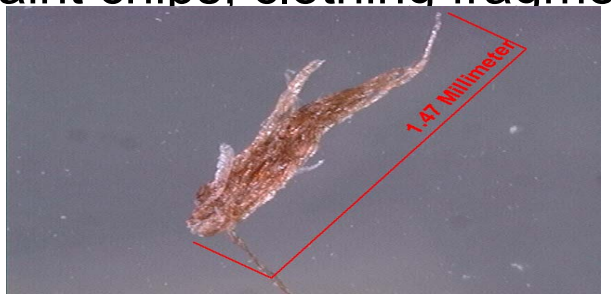
SbVP in therapeutic protein injections can arise from three general sources:

- extrinsic material (*outside, from the exterior)*,
- intrinsic (*inside, part of the whole)*, or
- inherent sources (*existing as a permanent and inseparable element*).
- Silicon oil droplets are a special type of intrinsic particle

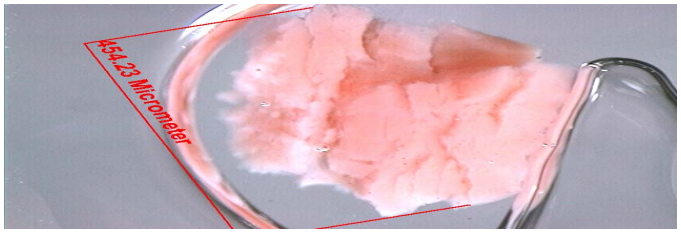
From USP <1787>

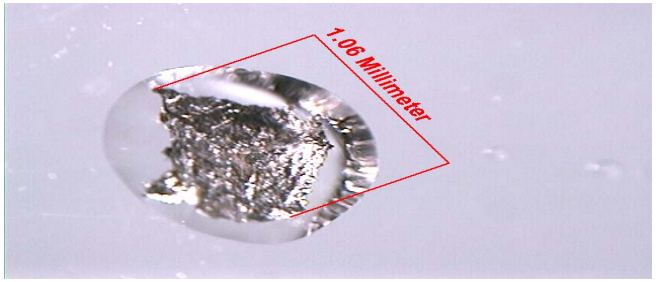
USP definitions: Visible and SbVP Particles can be assigned to one of three categories

- **Extrinsic particles** (from the outside) are materials that are not part of the drug product, package, or process, but are present due to contamination. These are truly foreign particles that are unexpected in drug product (e.g., insect parts, paint chips, clothing fragments, hair).



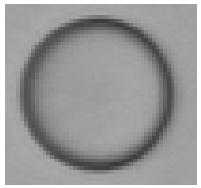
- **Intrinsic particles** (from the inside) are undesirable, non-protein material from degradation of formulation components, or related to the manufacturing and packaging processes and the device itself (e.g., glass lamellae, particles arising from packaging materials for drug product components, rubber from stoppers, silicone oil).



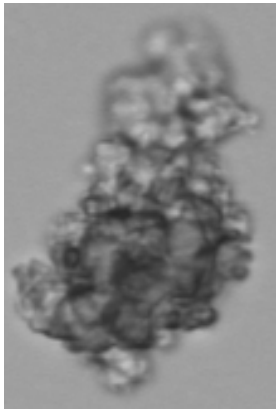


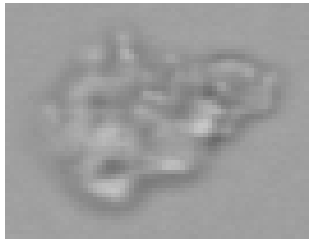
USP SbVP definitions

- **Silicone oil droplets** are important intrinsic particles resulting from the silicone oil that is a necessary lubricant in glass pre-filled syringes. They can confound the analysis of the total subvisible particle population, and also have the potential to interact with the protein depending on formulation conditions[1-4]



- **Inherent particles** are particles which originate from the drug product, either the protein therapeutic itself or formulation components. These particles can be an expected characteristic of the drug product.





<787> describes a Light Obscuration Method which addresses the needs for biologics

From Beckman Coulter
http://www.beckman.com/particle/instruments/lab-liquid-particle-counters/hiac-9703

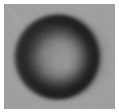
- **Benefits:**
  - Test individual units (as much as possible)
  - Reduced sample volume 5mL
  - For many biologics individual units are less than 1 ml
    - Release and stability testing: (≤) 5 mL/test
      - Characterization & investigation testing: ≤ 5 mL/test
    - Qualification and validation: < 100 ml
  - Extend to multiple (e.g. 7) size channels: ≥ 2, 5, 10, 15, 20, 25, 50 μm
  - Modify & improve sample handling procedure to reduce false negatives and positives (micro bubbles, etc)
  - Improve performance compared to <788>
- **Intended use for drug products**:
  - Release and stability testing
  - Process and product characterization
  - Investigations

Could be applied to all parenterals

Currently working on <1788> to describe best practices for dynamic flow imaging

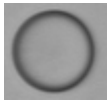
Challenges in analysis for SbVP in protein solutions

- Lack of protein particle standards (being addressed by NIST, etc)

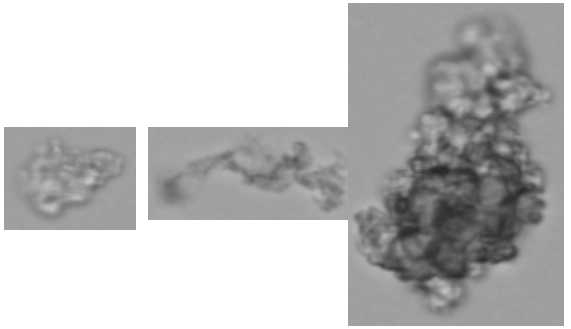


PS Latex Particle (15 μm)

Used for calibrating LO instrument



Silicone oil droplet



Protein particles

- Polystyrene, etc. counting standards have greater contrast with background, and consistent , regular shape
- Similar results obtained across techniques
- Protein particles are amorphous, and have refractive index similar to bulk solution

Causes of Sizing & Counting Errors

Reported diameter depends on particle attributes & instrument used

Method	What it measures	Source of errors	Critical parameter
Light obscuration	Scattering & absorption of light	Reduced light scattering of faint particles	Δn, over all diameters
Flow imaging	Optical image	Reduced image contrast of faint particles	Δn for small diameters
Electrical sensing zone	Displaced volume of particle	Porosity of protein aggregates	Average particle density

- Measurements of refractive index difference between particle and fluid, $\Delta \boldsymbol{n}$, are hard for protein particles—literature values just starting to be reported.
- Errors in diameter lead to misasignment of particles in size bins, and that leads to large errors in reported concentration.

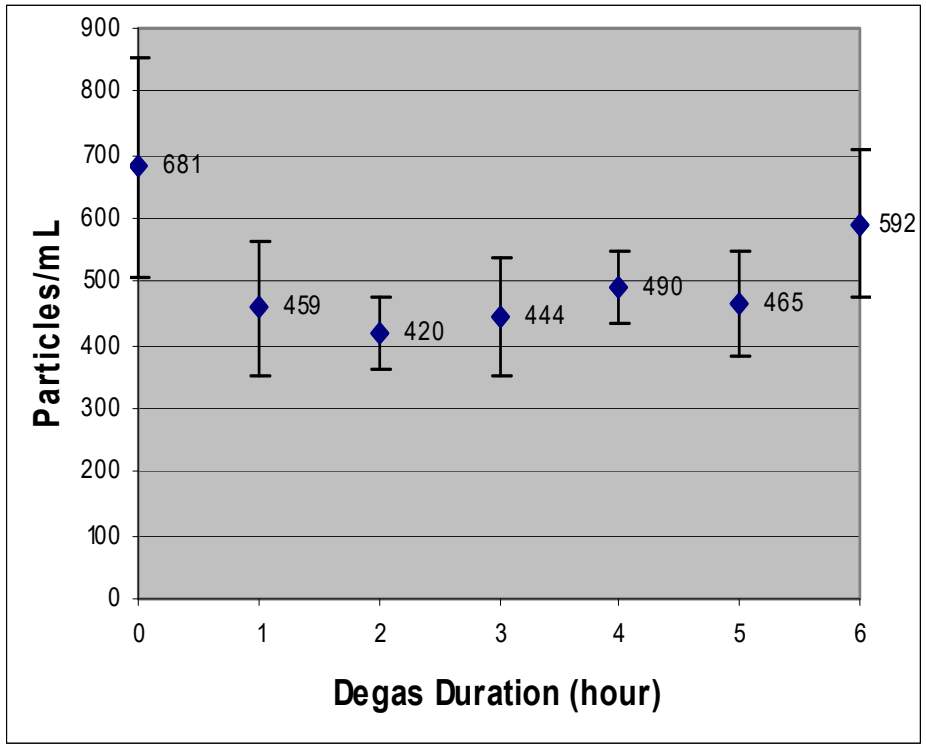
Hu Z, Ripple DC (2104) The Use of Index-Matched Beads in Optical Particle Counters. J. Res. Nation. Inst. Stand. Tech. 119:674-682. doi:10.6028/jres.119.029.

Ripple DC, Montgomery CB, Hu Z (2015) An interlaboratory comparison of sizing and counting of subvisible particles mimicking protein aggregates, J. Pharm. Sci. 104:666-677

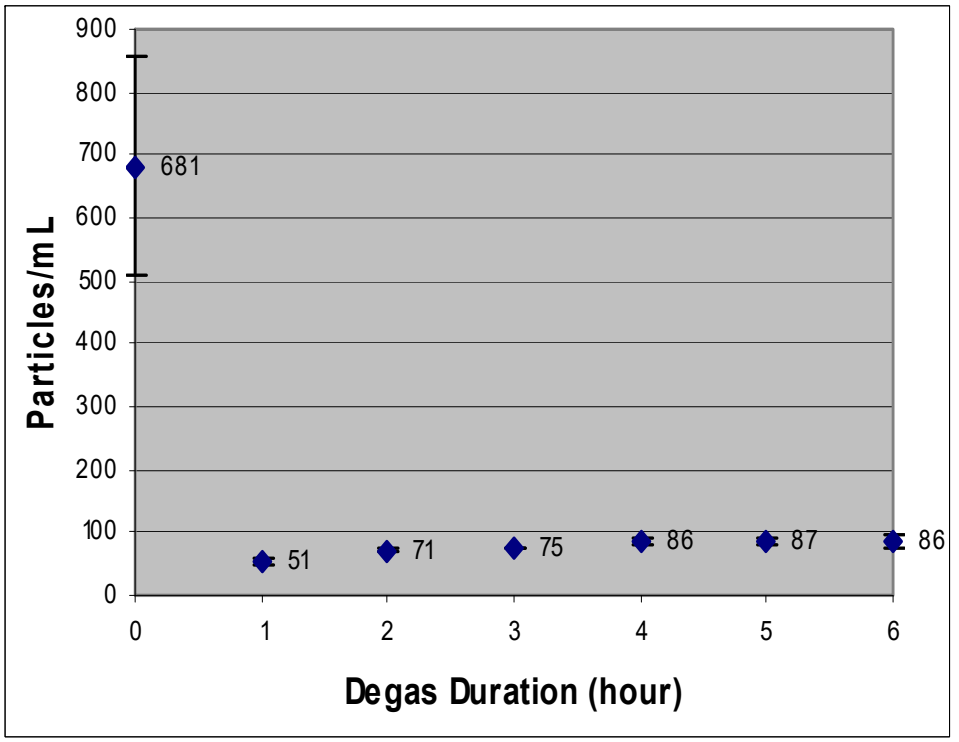
Appropriate sample handling is critical (not sonication)

≥ 10 μm particles/mL

Allow to stand



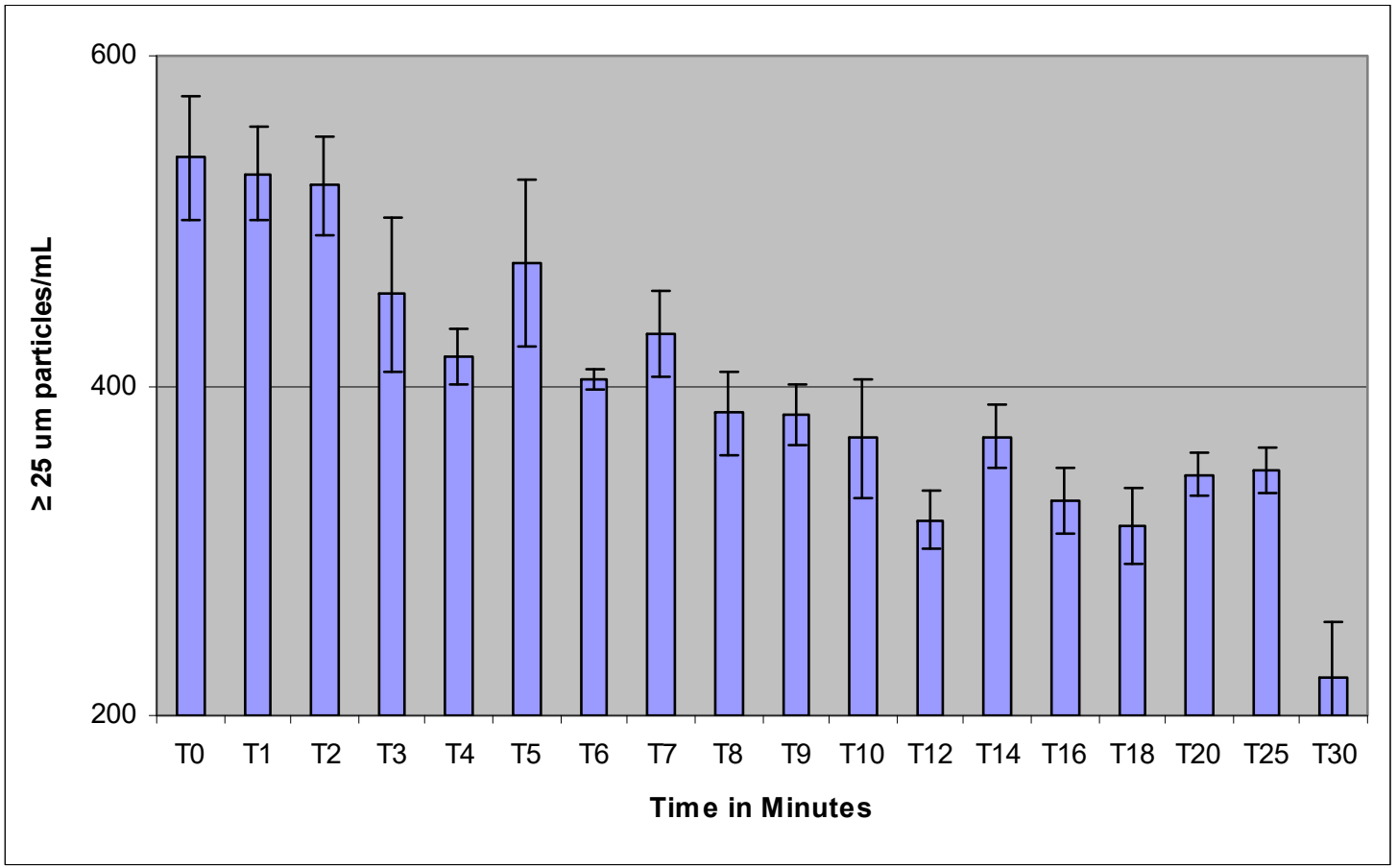
Vacuum (75 Torr) degas



Shawn Cao, Yijia Jiang, and Linda Narhi, A Light-obscuration Method Specific for Quantifying Subvisible Particles in Protein Therapeutics (2010) Pharmacopeial Forum, Vol. 36(3) 824-834

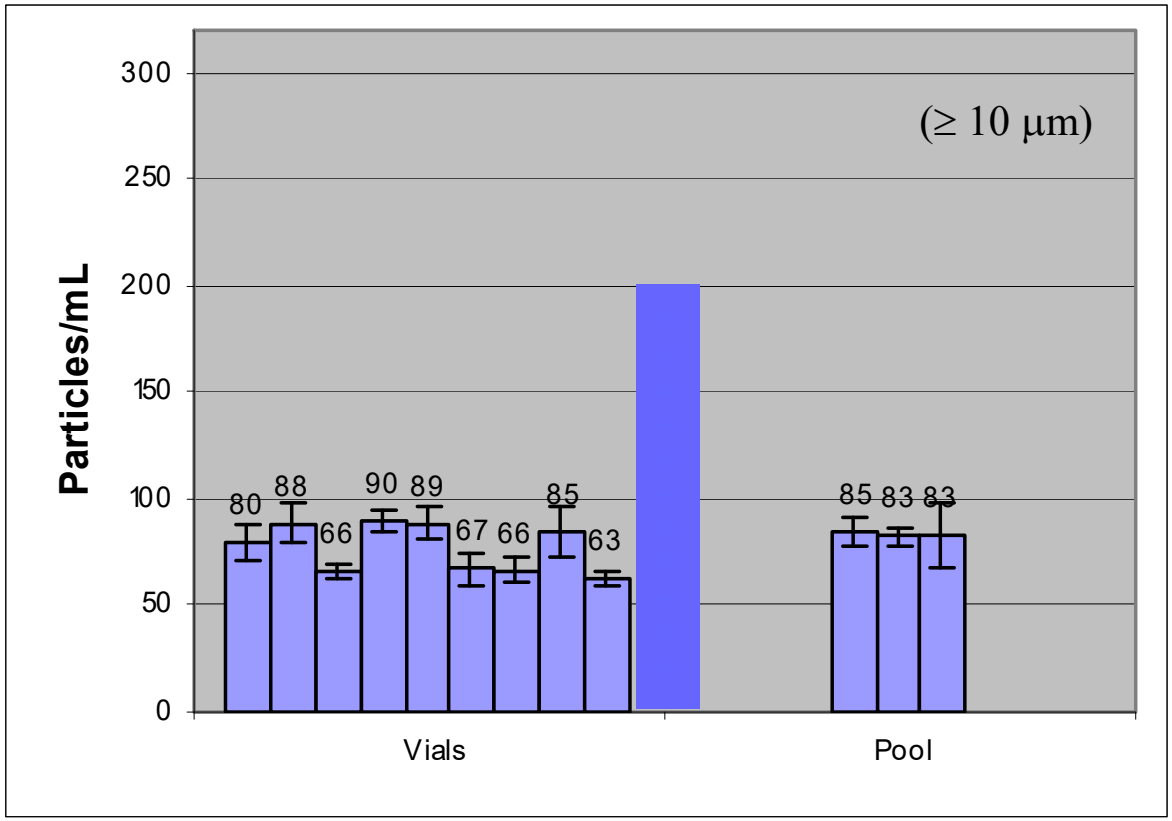
Particle Settling

30 μm Particle counting standards in H_2O



Pooling contents can lose information

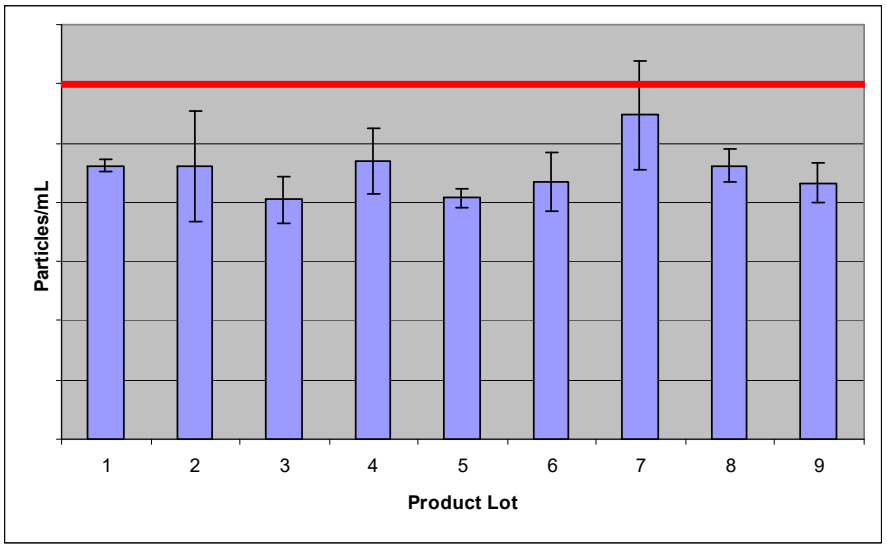
- Individual versus pooled units

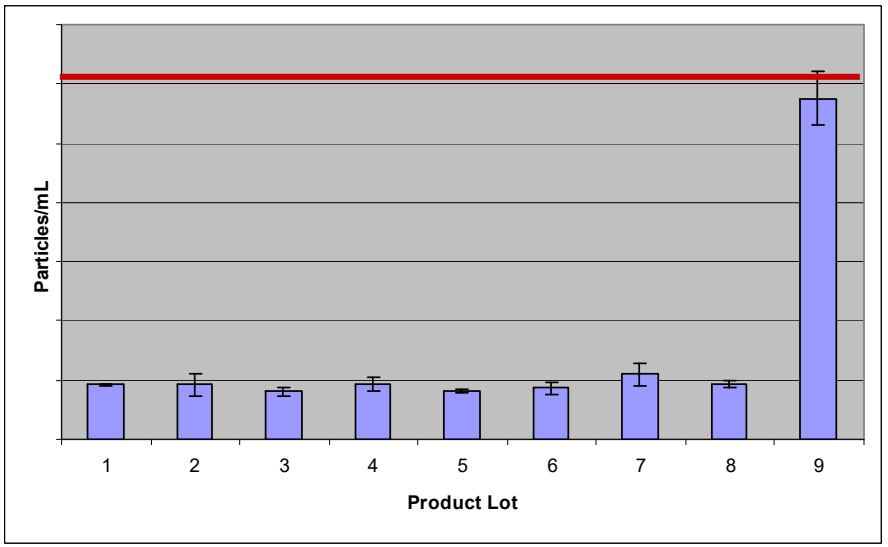


Unit to unit variability even higher with PFS and combination devices

What is the pertinence of the 6000/600 per container limits?

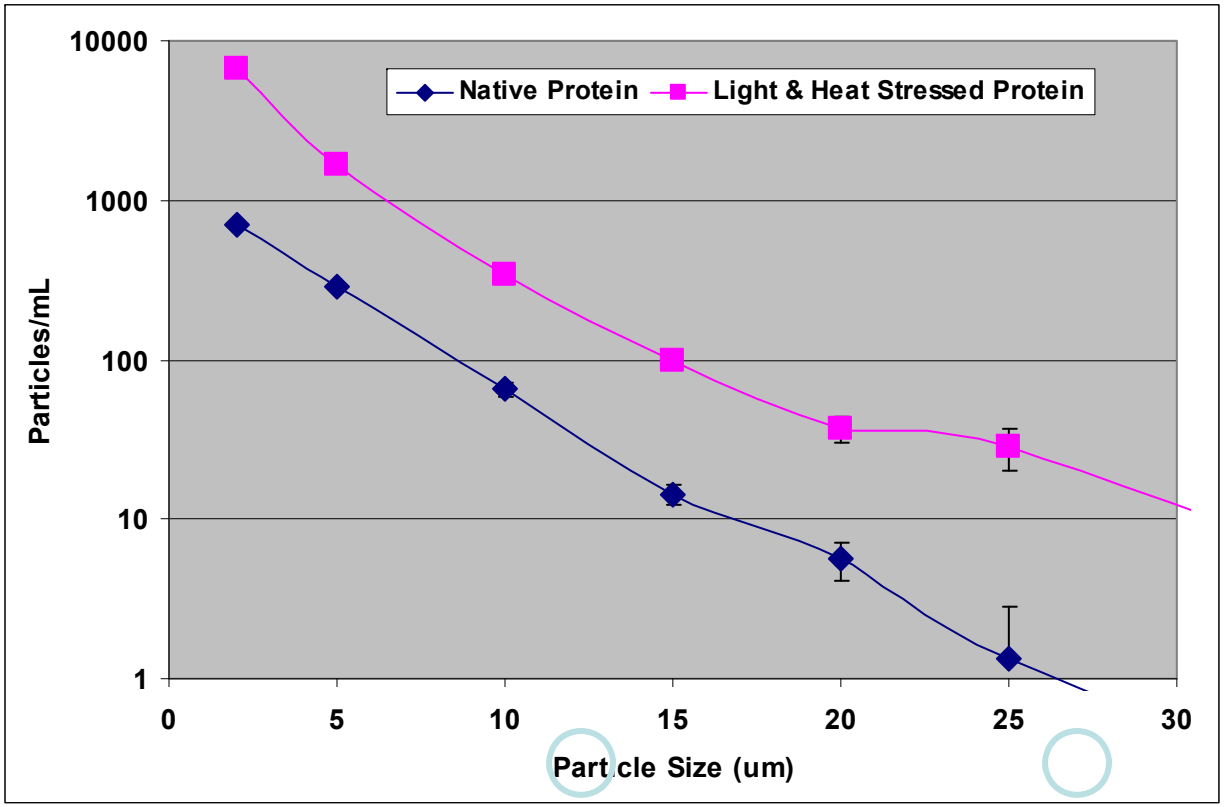
Instead of just “pass/fail”, product particle history is just as important.





Eventually have specifications based on multiple DS and DP lots

More particles are present at lower end of micron range



Population distribution for SbVP for 2 μm-50 μm size range before and after stress

Going below 10 micrometers provides valuable information and increases sensitivity of assay.
The <10 micron protein particles are more likely associated with immunogenicity

Expands information available during Process Characterization

Subvisible Particle Distribution for Processed Batches

Sample	Particles/mL at ≥ 2 µm	Particles/mL at ≥ 5 µm	Particles/mL at ≥ 10 µm	Particles/mL at ≥ 25 µm
Control (unfilt., Lot 1)	339 ± 78	29 ± 2	Below LOQ	Below LOQ
1x filtration Lot 1	29 ± 6	11 ± 5	Below LOQ	Below LOQ
2x filtration Lot 1	19 ± 9	14 ± 9	Below LOQ	Below LOQ
5x filtration Lot 1	50 ± 14	12 ± 9	Below LOQ	Below LOQ
Control (unfilt., Lot 2)	680 ± 51	36 ± 4	Below LOQ	Below LOQ
1x filtration Lot 2	20 ± 3	5 ± 2	Below LOQ	Below LOQ

- Filtration by 0.2 µm filter has removed particles at ≥ 2 µm
- 1x filtration is effective in removing the particles
- Particle concentrations are below LOQ at ≥ 10 and 25 µm

Subvisible Particle Distribution for Downstream Processing Samples

Sample	Particles/mL at ≥ 2 µm	Particles/mL at ≥ 5 µm	Particles/mL at ≥ 10 µm	Particles/mL at ≥ 25 µm
Proc. Step 1	590 ± 43	82 ± 0	Below LOQ	Below LOQ
Proc. Step 2	465 ± 69	96 ± 8	Below LOQ	Below LOQ
Proc. Step 3	17 ± 2	Below LOQ	Below LOQ	Below LOQ
Final DS	Below LOQ	Below LOQ	Below LOQ	Below LOQ

- Data on smaller particles can be used to inform process characterization, and effectiveness of step 2 versus step 3.
- Particle concentrations are below LOQ at ≥ 10 and 25 µm for all samples

Limit of quantification (LOQ): 10 particles/mL

USP <787> vs. USP <788> LO Method

	SVLO	USP <788> LO
Principle	Light Obscuration	Light Obscuration
Degassing	Vacuum degassing (75 Torr, 2 hours)	Allow sample to stand or sonicate, or as appropriate
Runs per sample	▪ **1st run results are discarded** ▪ **The results of the next 3 runs are averaged**	▪ **1st run results are discarded** ▪ **The results of the next 3 runs are averaged**
Volume per run	1 mL	5 mL
# of units needed / test	1+ (total vol. 5 mL)	10+ units (total Min. vol. 25 mL)
Particle sizes measured (μm)	≥ 2, ≥ 5, ≥ 10, ≥ 15, ≥ 20, ≥ 25, ≥ 50	≥ 10, ≥ 25

run = portion

USP<1787>

- Measurement of Subvisible Particulate Matter in Therapeutic Protein Injections
- Informational chapter (complements compendial chapter <787>) on methods and strategies for measuring and characterizing proteinaceous particles, size range 2 – 100 µm
- Objectives: provide information on orthogonal techniques for use during development, root cause analysis / nonconformance investigations, etc.

USP<1787>

- Not a recipe for when to use the methods
- Not a recipe for which methods to use
- Proposes Categorization of Aggregates in aid of Objectives (as required)
  - Size / Count
  - Composition / Identification
  - Reversibility / Dissociability
  - Structure / Conformation
  - Chemical modification
  - Morphology

Narhi et al., JPharmSci, 101, 493, 2012
Narhi et al., JPharmSci, 10.1002/jps.24437, 2015

Techniques for particle size and distribution analysis from <1787>

Technique	Principle of Operation	Range
Turbidimetry and Nephelometry	Estimation of the particle size distribution is attained by measuring the interaction of light with suspended particles, by the loss in intensity of transmitted light (turbidimetry) or light scattering (nephelometry).	0.035µm to 50µm
Light Obscuration	The size of the particle in the product fluid is determined by the amount of light that it blocks when passing between the source and the detector.	1 to 300µm
Coulter: electrical sensing zone	The size of the particle in product fluid or selected electrolyte is determined by the change in resistance as the particle passes through a micro-channel (orifice).	1 to 1600µm
Mastersizer (laser diffraction)	Intensity and angle of scattering generates a particle size distribution curve	0.01-3000 micrometers

Techniques for size and morphology analysis from <1787>

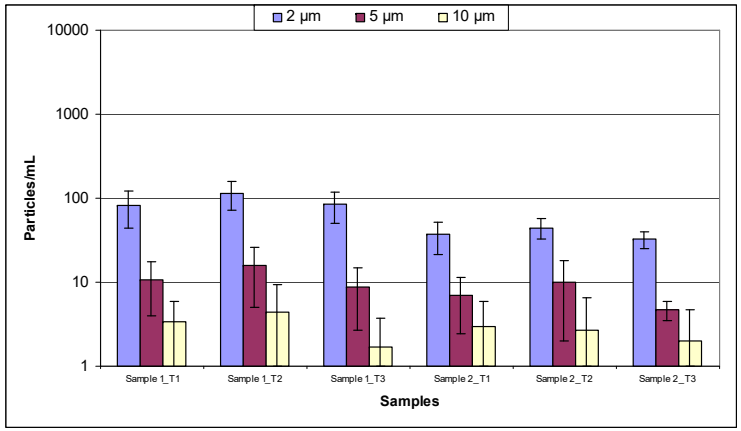
Technique	Principle of Operation	Range
Light Microscopy	Photon imaging of substances directly in product fluids or mounts or of isolated specimens on substrates.	0.3µm to mm's
Dynamic Imaging Analysis: Flow Microscopy	Digital image capture of the particles' magnified image in streaming product fluid, revealing size, shape, optical properties.	0.7 to 100µm for size distribution 4 to 100µm for morphology
Electron Microscopy (EM): Scanning EM, Scanning transmission EM and transmission EM	Electron imaging of specimen isolates on substrates. High vacuum or near-ambient pressures required.	Angstroms to mm's
Flow Cytometry: Forward scattering channel	Passage of particle across light beam increases light scattering in forward direction. Low refractive index difference, irregular morphology	1-100 micrometers

Techniques for characterization from <1787>

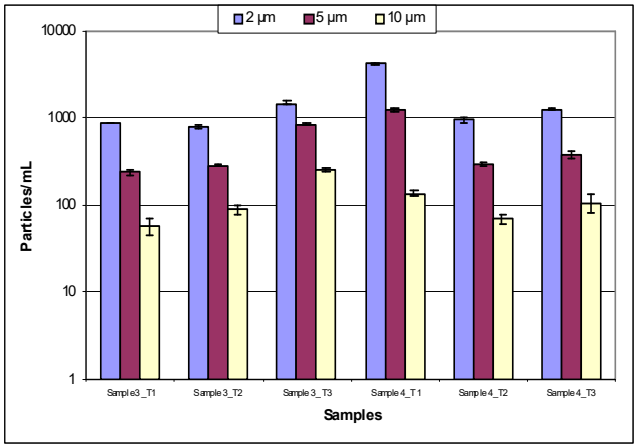
Technique	Principle of Operation	Range
FTIR Microspectroscopy	Photon imaging of isolated specimens on substrates	10μm to mm's
Dispersive-Raman Microspectroscopy	Photon imaging of isolated specimens on substrates, or in product fluids or fluid mounts	0.5μm to mm's
Electron Microscopy with Energy-Dispersive X-ray Spectrometry [EDS]	X-ray Photon emission from specimens energized by a focused electron beam	Angstroms to mm's for imaging, 1μm to mm's for elemental composition
Electron Microscopy with Electron Energy Loss Spectroscopy [EELS]	Inelastic scattering from specimens energized by a focused e-beam; e-loss is characteristic of the source element. Complementary to EDS.	Angstroms to mm's for imaging, 0.5μm to mm's for elemental composition

EXAMPLES

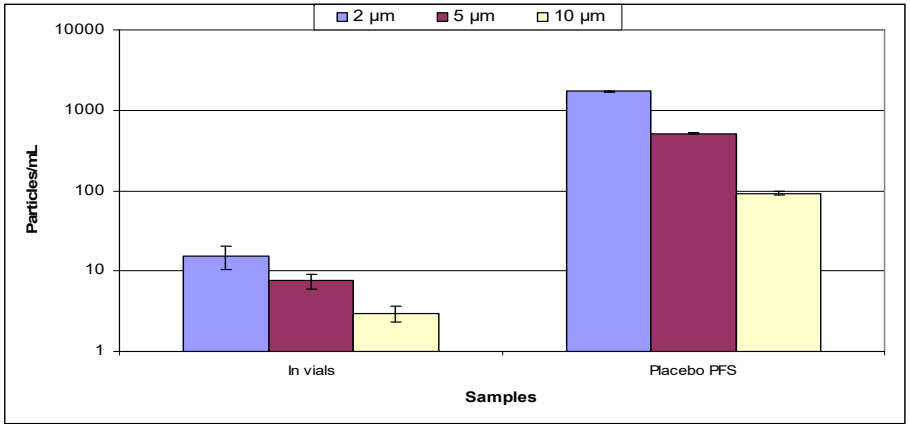
Case study: Changing from vial to pre filled syringe



vials



PFS

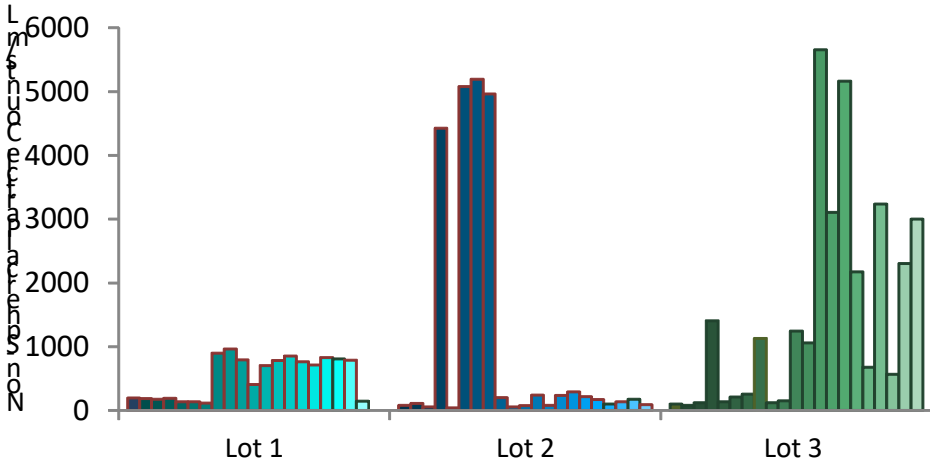


placebo

Increase in background SbVP due to Si Oil

S. Cao, N. Jiao, Y. Jiang, A. Mire-Sluis, L. O. Narhi, Sub-visible Particle Quantitation in Protein Therapeutics (2009) Pharmeuropa Bio & Scientific Notes 2009-1, 73-79.

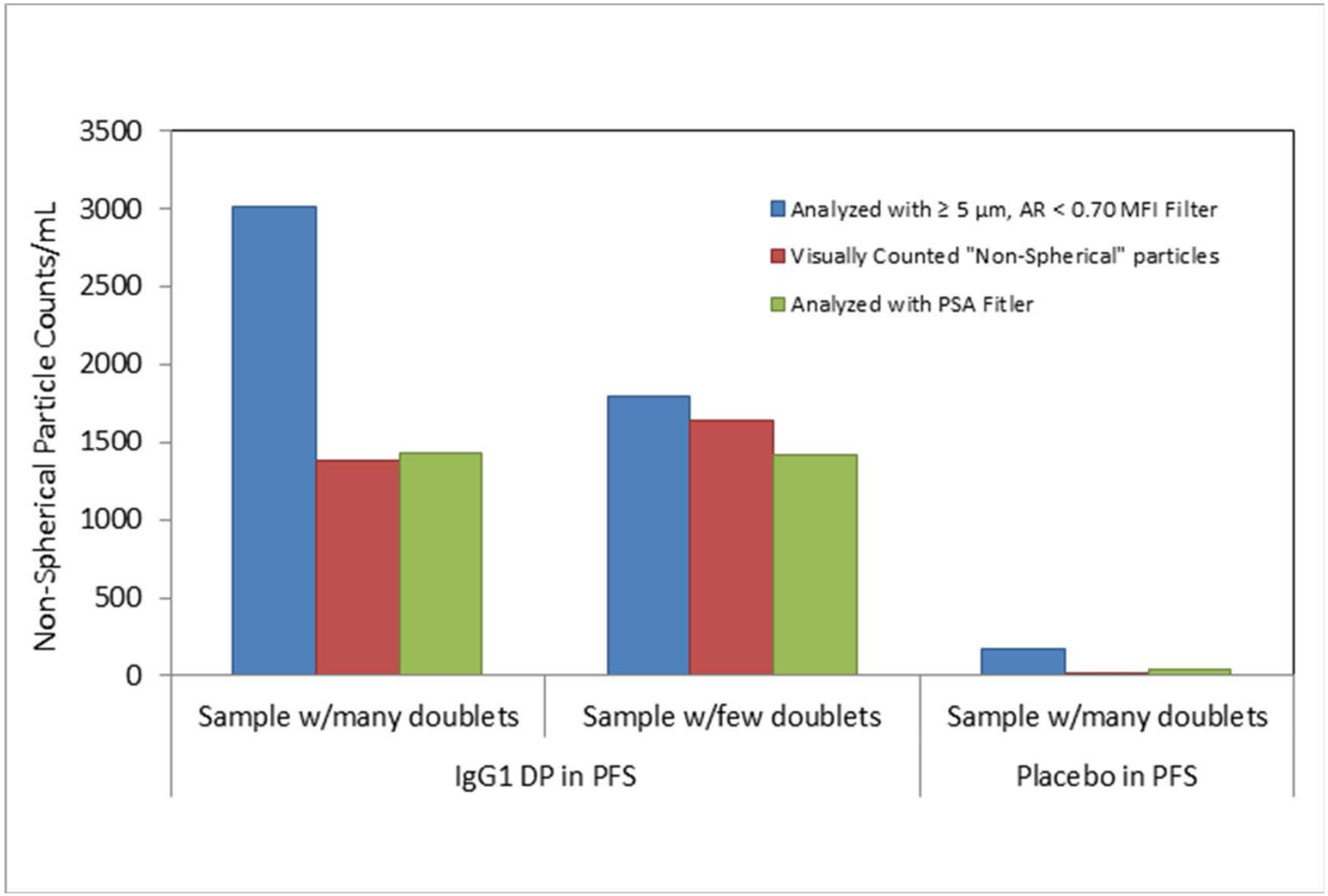
Changing from vial to PFS resulted in increase in particles labeled as protein by standard MFI algorithm



Variability in the non-spherical sub-visible particle counts ≥5 µm observed amongst drug product (DP) units derived from 3 lots

Examination of the non-spherical particle images from placebo by expert analysts demonstrated the presence of silicone oil doublets which were misclassified by the original algorithm

An improved algorithm was developed to filter the Si Oil from protein aggregates and other particles



Testing biological consequences of silicone oil

Attributes	Xeno-het mice (anti-drug antibodies)	Xeno-het mice (cytokines)	IVCIA Assay (Early phase cytokines in PBMC)	IVCIA Assay (Late phase cytokines in PBMC)	OCLA Assay (PRR Activation Signals in Cell Lines)
mAb1 SOD↑, PS80+	-	-	-	+	-
mAb1 SOD↑, PS80-	-	-	-	-	-
Buffer SOD↑, PS80+	-	-	-	+	-
Positive Ctrls (KLH-TCE-mAb1 w/ or w/o ↑[mAb1], LPS/PHA, Tri-Dap, or CL075)	++++	++++	++++	++++	++++

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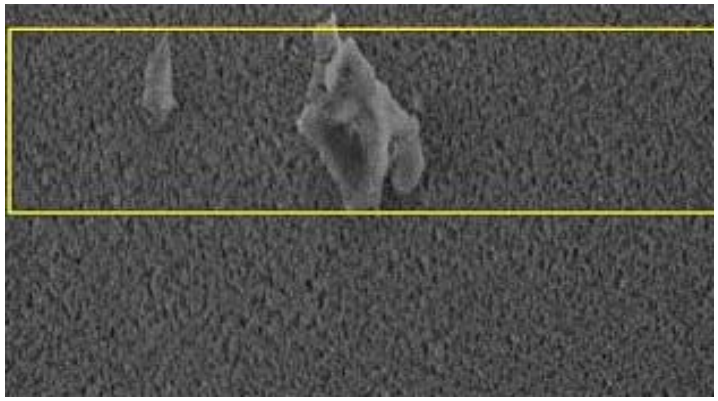
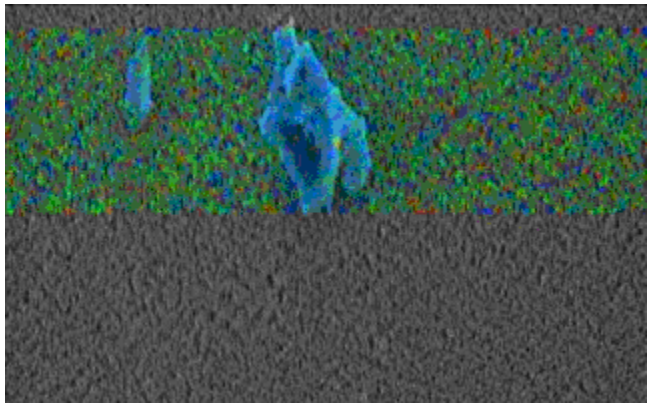
Conclusions from case study

- Changing to PFS resulted in the expected increase in SbVP due to Si oil
- Using the algorithm that came with the MFI resulted in an apparent increase in other SbVP even in placebo
- This was due to miss-assigned Si oil complexes
- Refined algorithm reflected expert assessment of images: increase due to Si oil in both placebo and DP
- Analysis requires expert insight
- Risk based approach should include assessment of impact to safety and efficacy

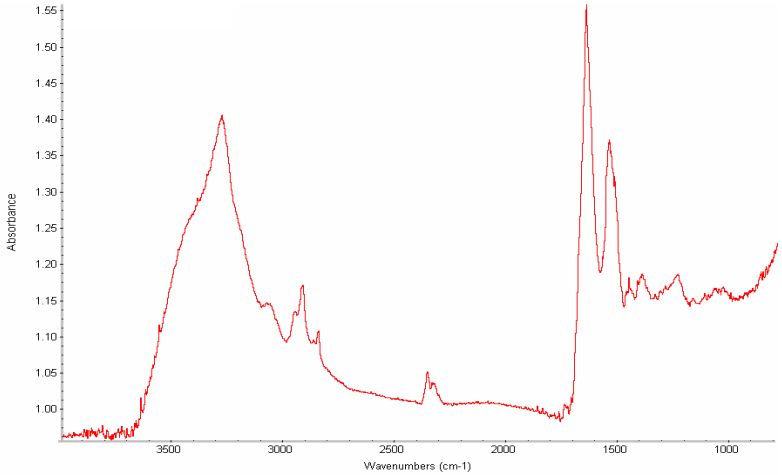
Characterization/identification of particles >10 micrometer.



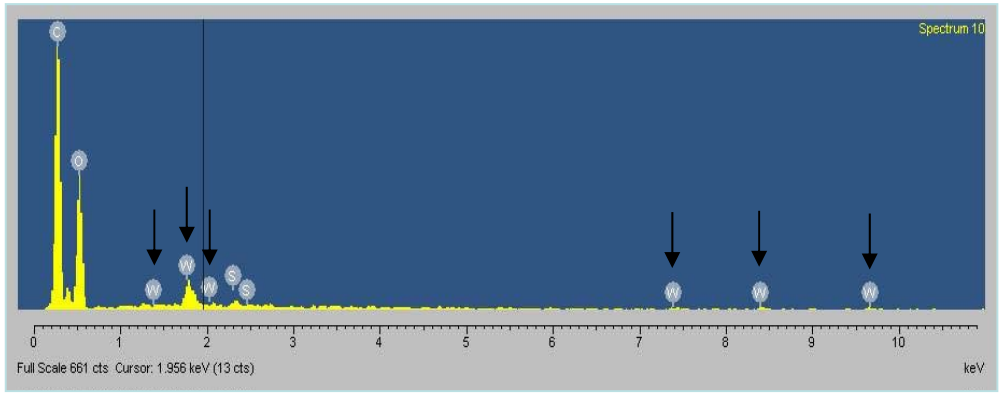
SEM micrograph



Cameo overlay of EDS and SEM image



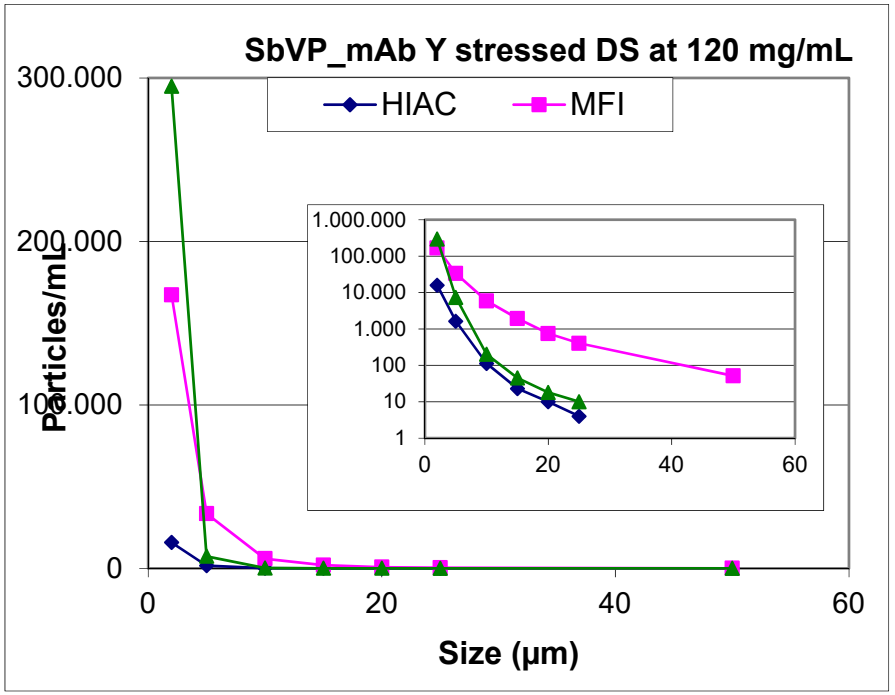
FTIR spectrum of particle



Energy Dispersive X-Ray Spectrum – Arrows Indicate Tungsten Peaks

The absolute numbers and size of micron aggregate depends on the instrument used

HIAC, MFI and Coulter Counter gave different particle concentrations and particle size distributions for protein particles in mAb Y drug substance at 120 mg/mL



- HIAC has the lowest counts of particles through all sizes and dilutions
- Coulter has highest counts of smaller particles (2-5 µm)
- MFI has the highest counts of larger particles (≥ 5 µm).
- Dean Ripple has published on underlying causes for these types of discrepancies, and ways to address them

Relative ranking of samples is usually consistent across techniques

POINTS TO CONSIDER AND FUTURE PLANS

Key points to consider

- There is increased scrutiny on SbVP in protein products due to potential risk of immunogenicity
- Characterization with orthogonal methods is important (Coulter counter, MFI and other flow microscopy techniques, etc. in addition to light obscuration/HIAC)
- Algorithms for the MFI can help differentiate between silicon oil and protein aggregates;
- For all techniques it is important to verify results with expert analysts
- Characterization during development can both minimize particles present, and also result in understanding of “what is normal”
- This should enable use of LO as the lot release method based on deep understanding of product gained during characterization
- A risk based approach should include attempts to understand safety and efficacy risks associated with particular species

Points to consider, cont'd

- The field is moving to a common nomenclature for protein aggregates,
- Sample handling is critical, including effect of dilution on particle size distribution, micro-particle removal, etc.
- Particle standards that are similar in optical properties and density to protein aggregates are being developed with NIST and others
- Several options to address count & size biases:
  - accept biases, but understand them & use new standards as controls
  - approximate corrections of bias by consensus algorithms (requires more research on particle D*n* & algorithms)
  - improved instruments that can apply bias corrections based on detected attributes of individual particles (some examples now in literature)

Summary and future plans

- Protein aggregates occur due to multiple factors, inherent molecular properties, process conditions, and interactions with formulation and device.
- Our analytical ability and understanding of the biological consequences of micron protein aggregates has improved significantly over the last few years.
  - Continued exploration of the applications of these techniques during product development
- All techniques have strengths and weaknesses. High concentration analysis is particularly difficult for all of them.
- USP expert committee finalized <787> (Biologics specific chapter), <1787>, informational chapter, and stimulus articles on submicron particles
  - is currently working on adding flow imaging (without specifications) to <788>
- **Can the adjustments in <787> be added to harmonized chapters as well?**
- Bridging studies demonstrate that products that pass <787> will pass <788> as well, so companies do not have to file with both

Ongoing activities from other groups

- AAPS focus groups on Protein aggregation and Biological Consequences is planning cross lab experiment (16 labs from industry, academia, regulatory agencies and NIST), using aggregate from same proteins (6), generated by same stresses, and characterize in the same assays
    - examine variability of characterization assays, in vitro and in vivo models, understand the variability of assays
    - Identify CQA of aggregates that have some activity in in vitro and in vivo assays
    - There will be no IP generated, and the outcome will be 3 publications, one for each phase of the study.

- Through IQ consortium a survey on submicron particles present in marketed product is underway, to begin understanding clinical exposure and baseline of these species

Some related publications:

- Rigidly organized protein arrays in the micron range may be highly immunogenic
  - VSV-G and VLV and regularly spaced acrylamide polymers (5-10 nM) are immunogenic

    Bachmann et al., Annu. Rev. Immunol, 15 (1997) 235-70.
    Denis et al., Virology, 363 (2007) 59-68.
    Dintzis et al., PNAS, 73 (1976) 3671-5.
    Chackerian et al, J Immunol, 169 (2002) 6120-6.

  - Immune response of protein coated nanobeads and preferential internalization of protein coated aluminum adjuvants by DCs

    Fifis et al., J Immunol, 173 (2004) 3148-54.
    Morefield et al., Vaccine, 23 (2005) 1588-95.

- Reports of protein aggregate immunogenicity *in vivo* give conflicting results
  - Aggregates of IFN-γ: metal-catalyzed and pH/50°C induced aggregates (but not untreated, crosslinked, hydrogen peroxide or boiled) can break tolerance in transgenic mice.

    Hermeling et al., Pharm Res, 22 (2005) 1997-2006.
    Hermeling et al., J Pharm Sci, 95 (2006) 1084-96.

  - Aggregates of FVIII: heat induced aggregates were less immunogenic than the monomeric protein.

    Purohit et al., J Pharm Sci, 95 (2006) 358-71.

  - Aggregates of GH: freeze-thaw and agitation induced aggregates were not able to break the tolerance of transgenic mice (freeze-thaw and GH absorbed onto glass or alum particles showed an enhanced response in wild-type mice).

    Fradkin et al., J Pharm Sci, 98 (2009) 3247-64.
    Fradkin et al., J Pharm Sci, (2011)

  - Only highly chemically modified aggregates (oligomers) broke tolerance in transgenic mouse model

    Bessa et al Pharm Res (2015) DOI 10.1007/s11095-015-1627-0

  - A weak transient response was obtained with aggregates in the 2-10 micron size range with some native structure and chemical oxidation in a Xeno-het modeland an aggregated murine mAb broke tolerance in wild-type mice

    Bi et al J Pharm Sci 102 (2013) 3545-5
    Freitag et al, Pharm Res 32 (2015) 430-444

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Related Publications, cont'd

- **Scott Aldrich, Shawn Cao, Andrea Hawe, Desmond Hunt, Linda Narhi, Dean Ripple, Satish K. Singh. Analytical Gaps and Challenges for Particles in the Submicrometer Size Domain. Pharmacopeial Forum 2016;42(6)**

- **Jawa V, Joubert MK, Zhang Q, Deshpande M, Hapurarachi S, Hall MP, Flynn GC "Evaluating Immunogenicity Risk Due to Host Cell Impurities in Antibody Based Biotherapeutics" 2016 AAPS J**

- **Joubert MK, Deshpande M, Yang J, Reynolds H, Bryson C, Fogg M, Baker MP, Herskovitz J, Goletz TJ, Zhou L, Moxness M, Flynn GC, Narhi LO, Jawa V. "Use of In Vitro Assays to Assess Immunogenicity Risk of Antibody-Based Biotherapeutics" 2016 PLOS One**

- **Jiskoot W, Kijanka G, Randolph TW, Carpenter JF, Koulov AAV, Mahler HC, Joubert MK, Jawa V, Narhi, LO Mouse Models for Assessing Protein Immunogenicity: Lessons and Challenges (2016) J Pharm Sci 105 1567-1575**

- **Moussa EM, Panchal JP, Balakrishnan SM, Blum JS, Joubert MK, Narhi LO, Topp EM. "Immunogenicity of therapeutic protein aggregates" J Pharm Sci 2016 (DOI: 10.1016/j.xphs.2015.11.002).**

- **S Telikepalli, HE Shinogle, PS Thapa, JH Kim, M Deshpande, V Jawa, CR Middaugh, LO Narhi, MK Joubert and DB Volkin. "Physical characterization and in vitro biological impact of highly aggregated antibodies separated into size enriched populations by FACS" *J Pharm Sci* 2015; 104 (5): 1575-91.**

- **V Bi, V Jawa, MK Joubert, A Kaliyaperumal, C Eakin, K Richmond, O Pan, J Sun, M Hokom, TJ Goletz, J Wypych, L Zhou, BA Kerwin, LO Narhi and T Arora "Development of a human antibody tolerant mouse model to assess the immunogenicity risk due to aggregated biotherapeutics" *J Pharm Sci* 2013 102 (10): 3545-55. Winner of the 2015 American Pharmacists Association Ebert Prize**

- **Narhi LO, Corvari V, Ripple DC, Afonina N, Cecchini I, Defelippis MR, Garidel P, Herre A, Koulov AV, Lubiniecki T, Mahler H-C, Mangiagalli P, Nesta D, Perez-ramirez B, Polozova A, Rossi M, Schmidt R, Simler R, Singh S,Spitznagel TM, Weiskopf A, Wuchner K Subvisible (2–100 m) Particle Analysis During Biotherapeutic Drug Product Development: Part 1, Considerations and Strategy (2015) J Pharm Sci** *DOI 10.1002/jps.24437*

- **MK Joubert, M Hokom, C Eakin, L Zhou, M Deshpande, MP Baker, TJ Goletz, BA Kerwin, N Chirmule, LO Narhi and V Jawa "Highly aggregated antibody therapeutics can enhance the in vitro innate and late-stage T-cell immune responses" *J Biol Chem*. 2012 287 (30): 25266-79.**

- **Q Luo, MK Joubert, R Stevenson, RR Ketchem, LO Narhi and J Wypych "Chemical modifications in therapeutic protein aggregates generated under different stress conditions" *J Biol Chem* 2011 286 (28): 25134-44.**

- **MK Joubert, Q Luo, Y Nashed-Samuel, J Wypych and LO Narhi "Classification and characterization of therapeutic antibody aggregates" *J Biol Chem.* 2011 286 (28): 25118-33.**

Acknowledgements

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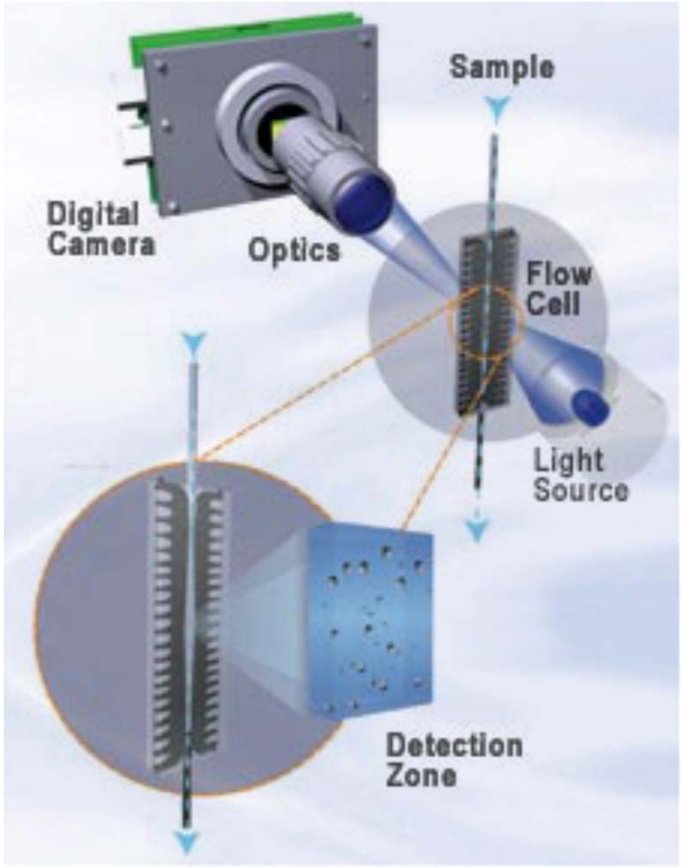
- Marisa Joubert
- Keith Kelley
- Tony Mire-Sluis

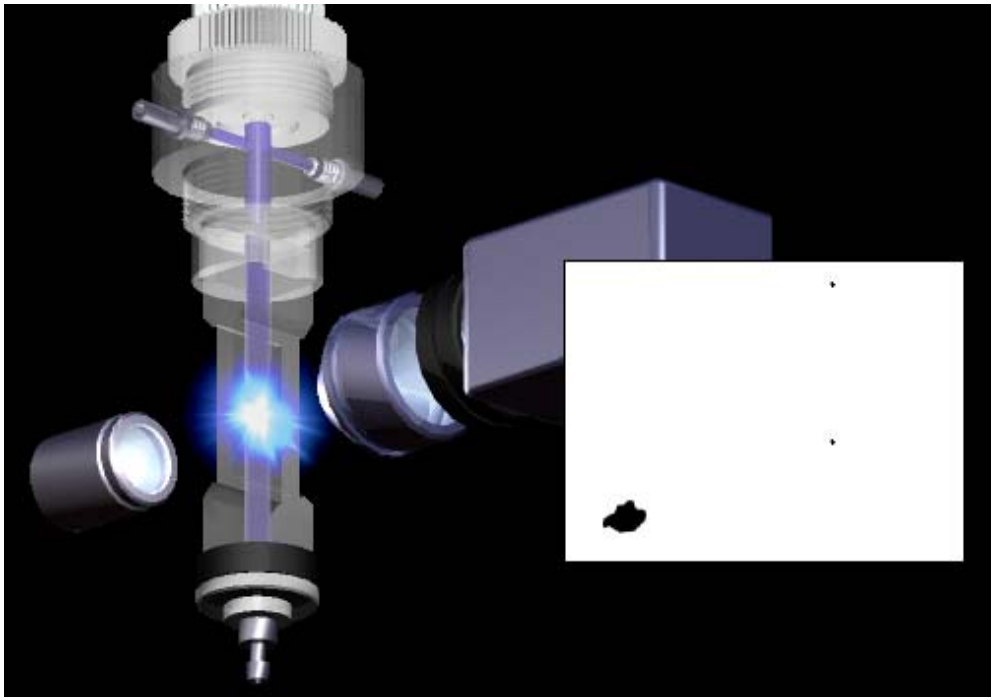
- Dean Ripple
- USP expert committed for SbVP in biologics,

Camera Based Technologies

MFI (micro-flow imaging)

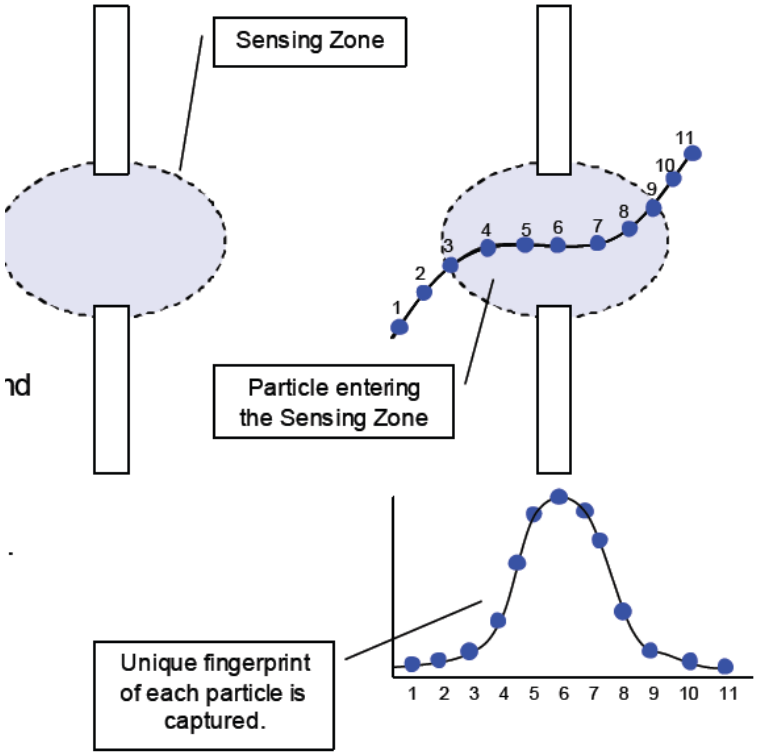


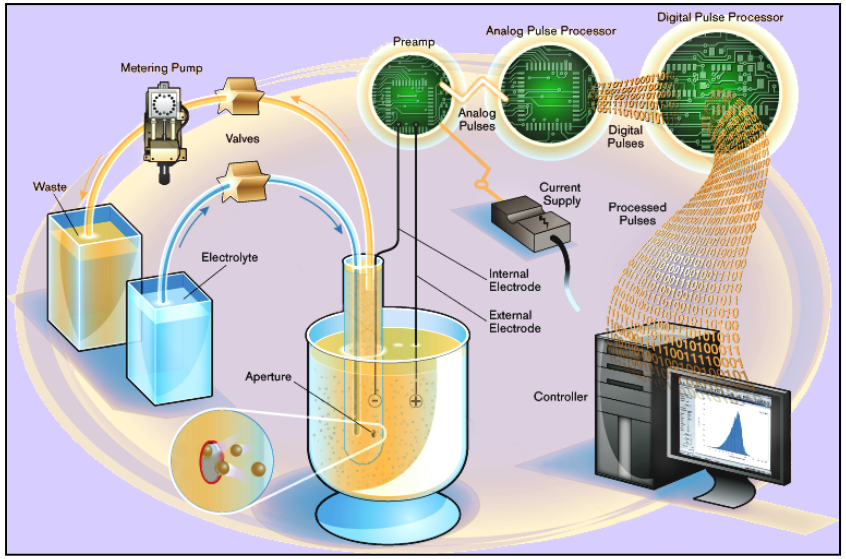
Flowcam



Taken from instrument manufactures' information

Electrical Sensing Zone (Coulter Principle)





Adapted from Coulter

Conclusions

- Xeno-het mouse is immuno-competent, shown by the TCE-KLH-mAb1 positive control inducing mAb1-specific ADA and robust cytokine responses.
- These mice are tolerant to mAb1-SOD samples (in the presence or absence of PS80, with or without increased SOD # by transport), showing little to no increase in cytokine secretion and no ADA during the entire 10 week study.
- PBMC and reporter-cell line responses to even the most potent samples comprising mAb1 in increased number of SOD with PS80 are negligible compared to known immune system activators.

Overall, *no increased risk* of immunogenicity is posed by high numbers of SO droplets from PFS for mAb1 as assessed by the different model systems here.