# Abstract

USP<787> is a new chapter that addresses the limitations of USP<788> for therapeutic proteins. This talk will cover the key aspects of USP<787> and discuss some of the orthogonal methods for characterization of these proteinaceous aggregates in the context of risk assessments during product development.

# FDA Queries -1

- *"…USP <788> testing results are critical to mitigate the risk associated with occlusion of small blood vessels and small subvisible particles may pose an immunogenicity risk. Provide USP <788> particulate testing data for in-use stability studies and an analysis of particulates between 2 and 10 microns."*

- *"…in addition to measuring particulates that are ≥10 µm in size, subvisible particulates in the 2-10 µm range should also be characterized and quantified using technique(s) that can accurately estimate the amount of subvisible protein particulates present. Sub-visible particulates in the 0.1-1 µm range should be qualitatively assessed."*



Singh, Ch 14. (Kolhe et al. Eds, Sterile Product Development ,Springer 2013)

# FDA Queries -2

- *"…additionally characterize the types and amounts of subvisible particles (2 – 10 µm) in the drug product under stress conditions, at release, and throughout the shelf-life, and also propose an appropriate control strategy based on the risk to product quality."*

*In this particular case, Specifications were also required for the under 10 micron size ranges <2010>*



Singh, Ch 14. (Kolhe et al. Eds, Sterile Product Development ,Springer 2013)

# FDA Queries - 3

- Recent information suggests that nanoparticles can be shed from a filling pump's solution contact surfaces which may then nucleate protein aggregation and/or particulate formation. More information should be submitted to the IND on the fill process, e.g. pumps used that may impact on particles. It is also recommended that in addition to USP <788> particulate testing, sub-visible particles <10 µm in size be characterized at release and at regular intervals in the drug product stability program including under accelerated and/or stressed condition. While your product should comply with compendial limits for particles greater in size than 10 micron and 25 micron during development, it is not necessary to establish acceptance criteria at this time for smaller sub-visible particles. Data from these characterization studies can be used to develop and provide support in your license application for an overall control strategy for particulate matter for the xyz manufacturing process.

# Subvisible Particulate Matter

- Original concern for Injections
  - Capillary occlusion etc from “foreign” particulates
- New concern for Therapeutic Proteins
  - Immunogenicity risk from proteinaceous (aggregates) particles
- Additionally, a measure of Product and Process consistency
  - Risk assessment and Control strategy

# Types of Particulate Matter

- Extrinsic: Material not part of formulation, package, assembly process i.e. foreign and unexpected
  - Bioburden and sterility concerns
  - Random, non-uniform, unchanging
- Intrinsic: Material arising from sources related to formulation, package, assembly process
  - Indicative of potentially systemic problems in process or product (e.g cleaning, instability)
  - Silicone oil is considered intrinsic
- Inherent: Material expected from drug substance and formulation components, a potentially accepted / expected attribute

✓ Classification is not exactly hard and fast always

✓ Classification plays a role in assessing risk

# USP<787>

- Active USP37 NF32 (1st Suppl. 01Aug2014)
- Drafts for comments in Pharmacopeial Forum 38(3) 2012; 39(2) 2013
- Rationale: To address gaps in <788> with regards to testing of Therapeutic Protein Injections for subvisible particulate matter
- Applicability: Biotechnology-derived articles <1045>, their infusion preparations, as well as natural-sourced therapeutic protein injections

# Salient Aspects of USP<787> (1)

- Use either <788> or <787>
- Recognizes “inherent” particles - particles of the protein or formulation components – as a separate species to monitor; builds upon what is in <1788>.
- Allows use of alternative analytical methods with adequately developed particulate limits
  - Light Obscuration is the current standard methodology
- Allows quantitative dilution if necessary. Requires Method verification to ensure appropriateness of sample handling, performance of method and number of units tested.
- Discounts use of Membrane Microscopy for counting and sizing proteinaceous particles – being primarily for (classification, characterization and quantitation of) extrinsic and intrinsic particles

# Salient Aspects of USP<787> (2)

- Emphasizes statistically sound sampling plans (volume, historical counts, particle size distribn, variability in counts between units)
- Calibrate with standards between 2 – 100 µm (<788> says 10 – 25 µm)
- System suitability verification added (handle USP Ref stds the same as a test sample)
- Product samples tested in a manner that most represents delivery use (e.g. expelled syringe contents)
- Test Volume flexible (0.2 – 5.0 mL) / Individual Units testing preferred.
  - No need to collect 25 mL solution
- Clarifies that for Lyo products, reconstitution with specified Diluent must be done and Diluent must also be tested. However, Diluent counts cannot be subtracted.
- Precaution about eliminating gas bubbles. Avoid Sonication.

# Salient Aspects of USP<787> (3)

- Dilution as a last resort; use smallest level of dilution and provide rationale for dilution and show suitability of dilution scheme. Bridging studies. Orthogonal technique to justify suitability of method.
- Acknowledgement that “although current historical USP limits of ≤ 6000 and ≤ 600 particles/container may be applied, the most appropriate limits for biotechnological products will depend upon the specific product and manufacturing process and taking into account the clinical safety and efficacy data as well as dose and route of administration”.
  - Setting limits is most appropriately done on a case-by-case basis during development and licensure
  - Limits derived historically from <788> should be considered provisional
- For LVIs an additional requirement: Total particle load should not exceed 6000 for ≥ 10 µm and 600 for ≥ 25 µm
- Specific statement that the limits apply to filtrates for products that require filtration just prior to administration

# More Thoughts on USP<787>

- Dilution to analyze can be tricky
  - Demonstrate no change in counts
- Where adequate volume is available, use reasonable test aliquot volume
  - Accuracy of counting increases with more counts, esp. in low count solutions
- Expect product specific limits to be required post licensure – could be lower or higher.
- Expect limits for 2 – 10 µm also
  - Characterization beyond size and count important

# USP<1787>

- Measurement of Subvisible Particulate Matter in Therapeutic Protein Injections
- Informational chapter on methods and strategies for measuring and characterizing proteinaceous particles, size range 2 – 100 µm
- Objectives: Enumeration, Orthogonal characterization, identification, root cause analysis / nonconformance investigations…

# 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
  - Reversibility
  - Structure/Conformation
  - Chemical modification
  - Morphology

Narhi et al., JPharmSci, 101, 493, 2012

# Orthogonal Methods

- Flow Microscopy Imaging
- Laser Diffraction
- Electrical Sensing Zone
- AFFF
- EMs (SEM, TEM, EM_EDXS, EELS)
- FTIR, Raman, TOF-SIMS
- Nanoparticle Tracking analysis (< 1 µm)
- Resonant Mass Measurement (< 1 µm)

# Particulates Strategy

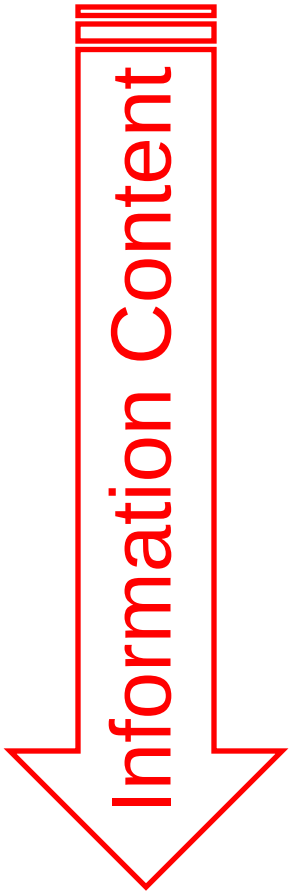
- Comprehensive strategy for particulates
  - Early: Gather typical particle profile (release and stability); characterize aggregates and particles in clinical material
  - Late: Comparability; relationship with formulation, manufacture, use; enhanced characterization; risk assessment under storage, use and stress conditions; control strategies; monitor and trend
  - Post: For management of changes during product life-cycle

Note: Archived samples are likely not very useful for particulates

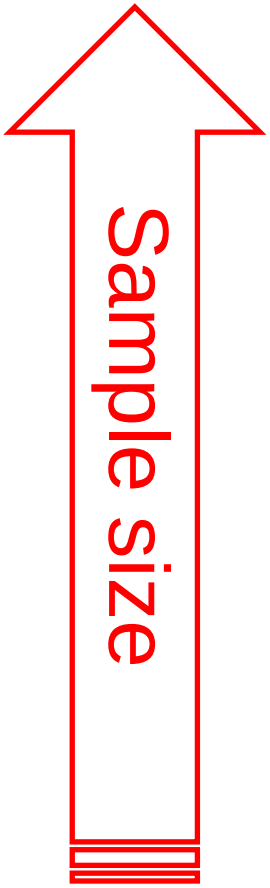
# Particulates Strategy (cont'd)

- Stress testing and Control Strategy
  - Forced Degradation (thermal, F/T, agitation, acidic, basic, oxidation, photo, in-use..)
    - Not all are relevant at DP level once formulated
  - Useful for method development / qualification
  - Use for risk assessment and control strategy
    - Materials specs, process parameters, hold-times, handling and storage restrictions, in-process controls, process limits, …

# Characterization of Particles




- Size and Count
- Categorization / Identification
- Reversibility
- Morphology
- Chemical identity
- Conformation / Structure



# Need for Orthogonal Methods

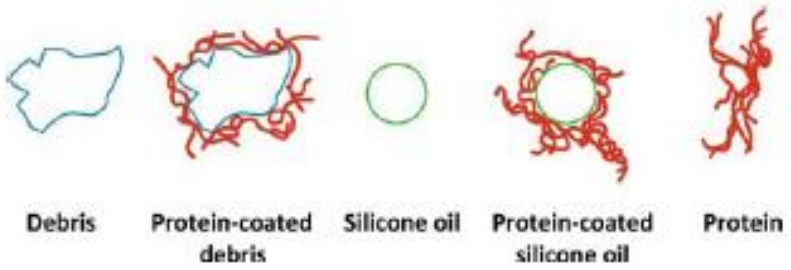
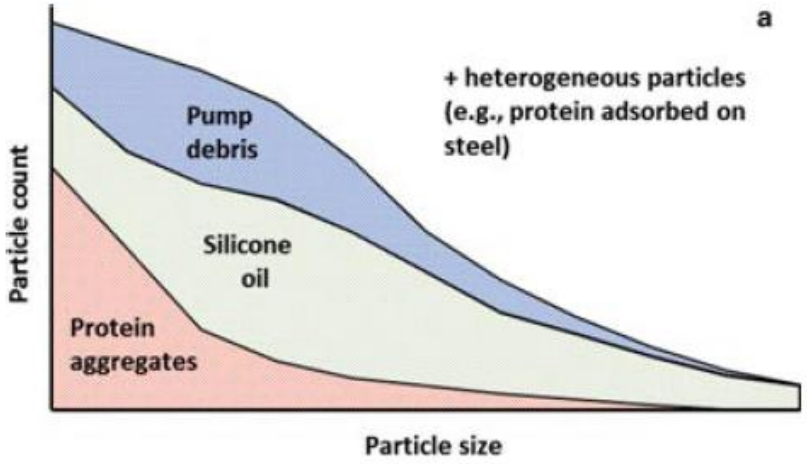
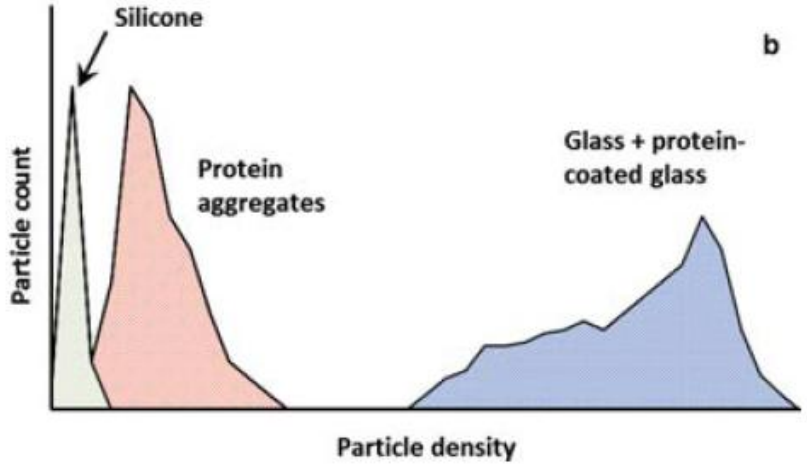



**Figure 1.** Schematic of typical particle types found in therapeutic proteins.

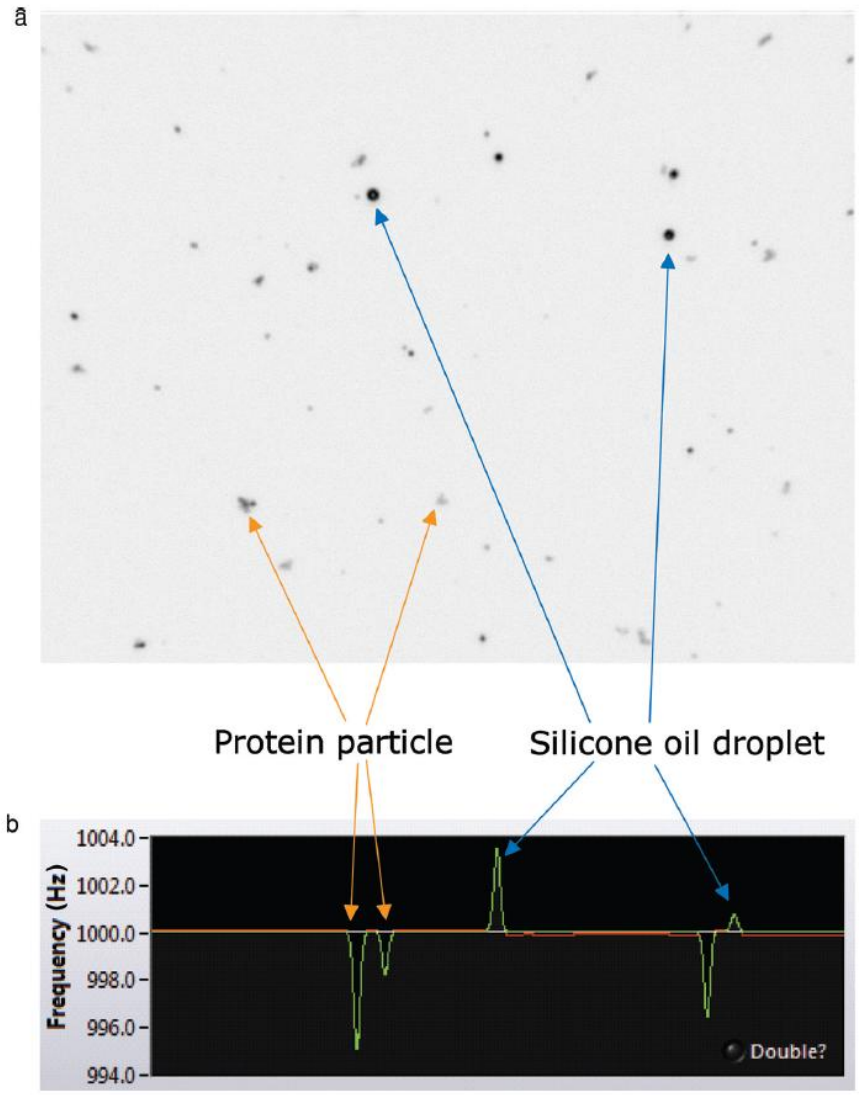


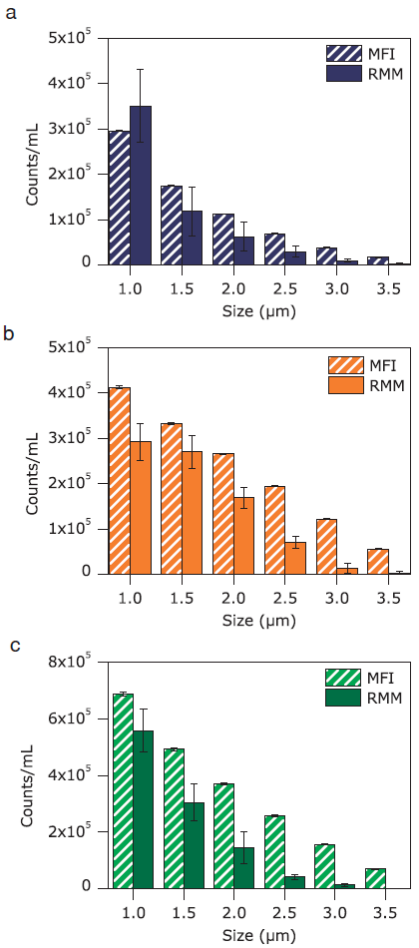






Ripple and Dimitrova, JPharmSci, 101, 3568, 2012

# Resonant Mass Measurement




Si Oil droplets

Heat-stressed mAb

40:60 mixture



Weinbuch et al., JPharmSci., 102, 2152, 2013

# Characterization

- 2 – 10 µm range
  - Size and count
  - ID, reversibility, morphology, composition, conformation (?)..
- 0.2 – 2 µm range
  - Size and count (!)
  - “classification”, reversibility,…

  Note: Number of caveats in the 0.2 – 2 µm range; limited experience;

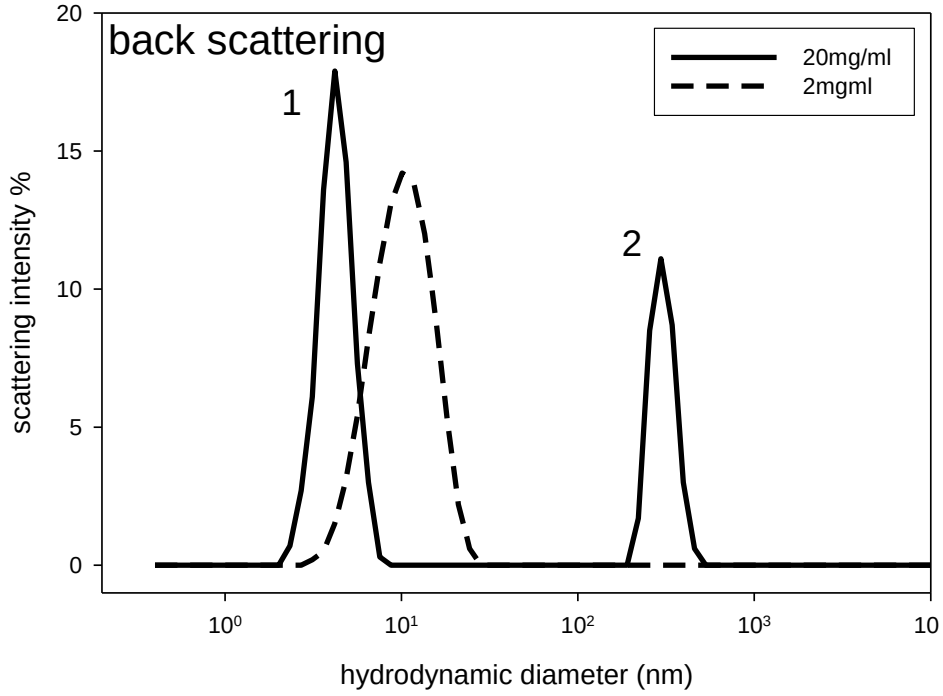
# Example 1: Product Format Switch

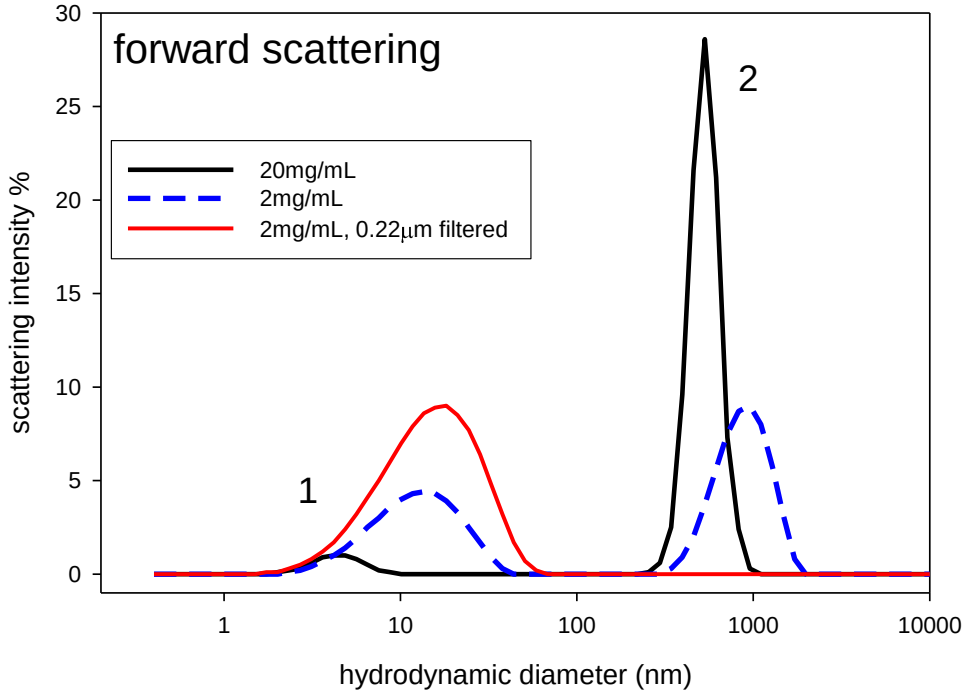
- Lyo DP in vial
  - New form developed: Liquid DP in PFS
- Much higher count in Liquid - with LO
- Fewer particles in Lyo – with MM.
- Flow Microscopy shows a large number of spherical particles
  - Characteristic typical of Silicone oil
  - Could be discounted from total counts
- Conclusion: Product change did not worsen product quality characteristic

# Example 2: Risk Assessment

- Count sizes under 10 µm, Perform Risk assessment, Propose Control Strategy
- Studied impact of stress (after storage of DS, DP release, F/T excursions, Thermal excursions, Transport, In-use infusion)
- F/T excursions led to “significant” increase
- Additional Instructions and precautions and monitoring put in place as Control Strategy

# Example 3: Reversibility of submicron aggregates by DLS




- Submicron aggregates in mAb detected by DLS in two modes
- Submicron aggregates were reversible on dilution.
- Submicron aggregates were removable through filtration.



Zou, 2009

# Example 4: Identification by FTIR and SEM/EDX

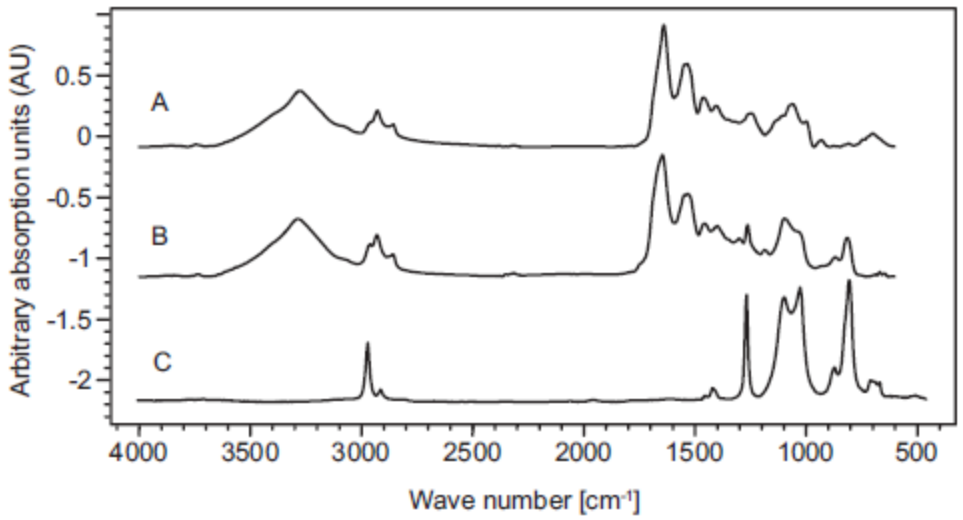



**Figure 6.** Representative ATR FT-IR spectrum of protein particle (B), overlaid with IgG1 reference spectrum (A) and polydimethylsiloxane reference spectrum (C). Protein

| FT-IR[b] | SEM/EDX[c] |
|---|---|
| Protein (6/6), silicone (6/6) | C,N,O (4/4), Si (4/4) |
| Protein (5/5), silicone (5/5) | C,N,O (3/3), Si (3/3), Al (1/3), F (1/3)[d] |
| Protein (7/7), silicone (4/7) | C,N,O (6/6), Si (4/6), Al (4/6) |
| Protein (10/10), silicone (10/10) | C,N,O (4/4), Si (3/4) |

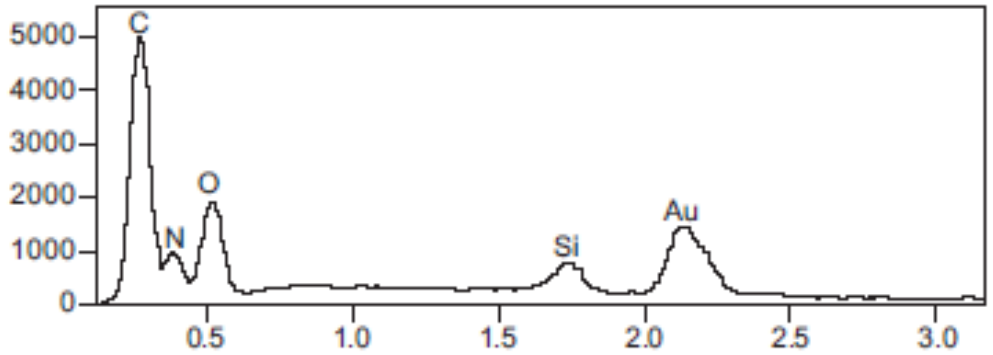
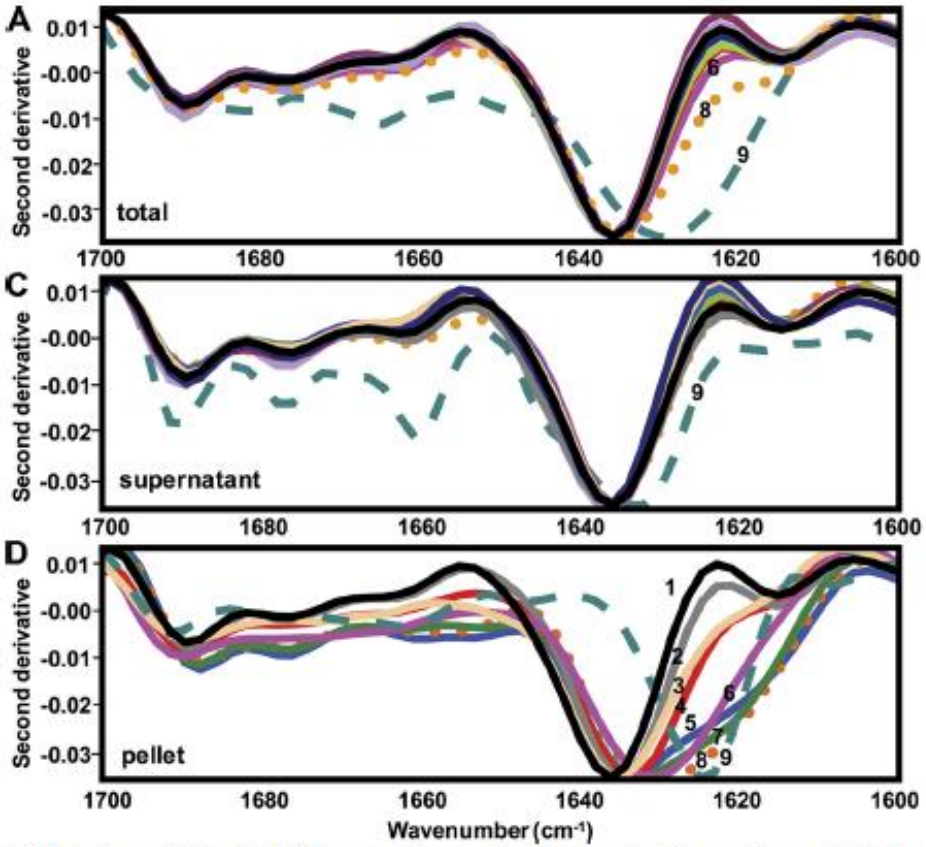



**Figure 7.** Energy dispersive X-ray spectrum of a repre-



Wuchner et al., JPharmSci, 99, 3343, 2010

# Example 5: FTIR for Secondary Structural Changes




B

| Sample | CC (%) Total | CC (%) Sup | CC (%) Pellet | ID No. |
|---|---|---|---|---|
| untreated | 100 | 100 | - | 1 |
| ft-slow | 98 | 89 | - | - |
| ft-fast | 94 | 97 | - | - |
| pH 3.5 | 98 | 98 | - | - |
| pH 11 | 99 | 92 | 71 | 2 |
| xlink | 94 | 89 | - | - |
| agitate-so⁺ | 95 | 89 | 50 | 3 |
| stir-20h | 93 | 98 | 42 | 4 |
| syringe-so⁻ | 96 | 92 | - | - |
| syringe-so⁺ | 96 | 96 | 32 | 5 |
| $H_2O_2$ | 99 | 99 | - | - |
| metal | 78 | 92 | 24 | 6 |
| 65C/pH 8.5 | 98 | 100 | 26 | 7 |
| stir-3d | 56 | 89 | 20 | 8 |
| 90C | 16 | 42 | 19 | 9 |

- not enough sample to test

FIGURE 4. **Second derivative FTIR spectra show varying degrees of mAb1 protein structural changes upon stress treatment.** *A*, overlaid spectra of the



Joubert et al., JBC, 286, 25118, 2011

# Example 6: FTIR for Secondary Structure in Particles

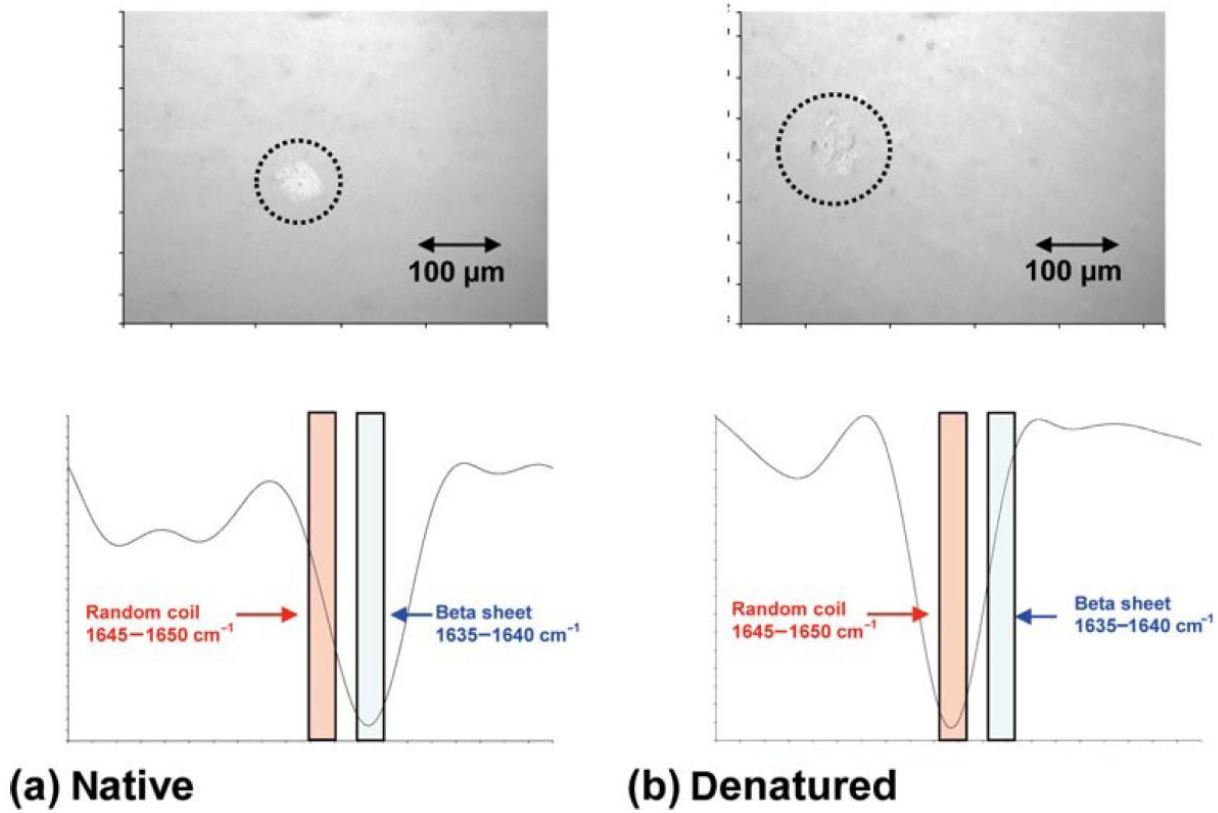



**Figure 3.** Optical images and second-derivative FTIR microscopy spectra of two particles from monoclonal antibodies. Although there is no discernible difference between the optical images, the FTIR microscopy demonstrates that the native structure is preserved in panel (a), whereas there is a substantial loss of secondary structure in panel (b).



Ripple and Dimitrova, JPharmSci, 101, 3568, 2012

# Example 7: Reversibility of Counts on Dilution

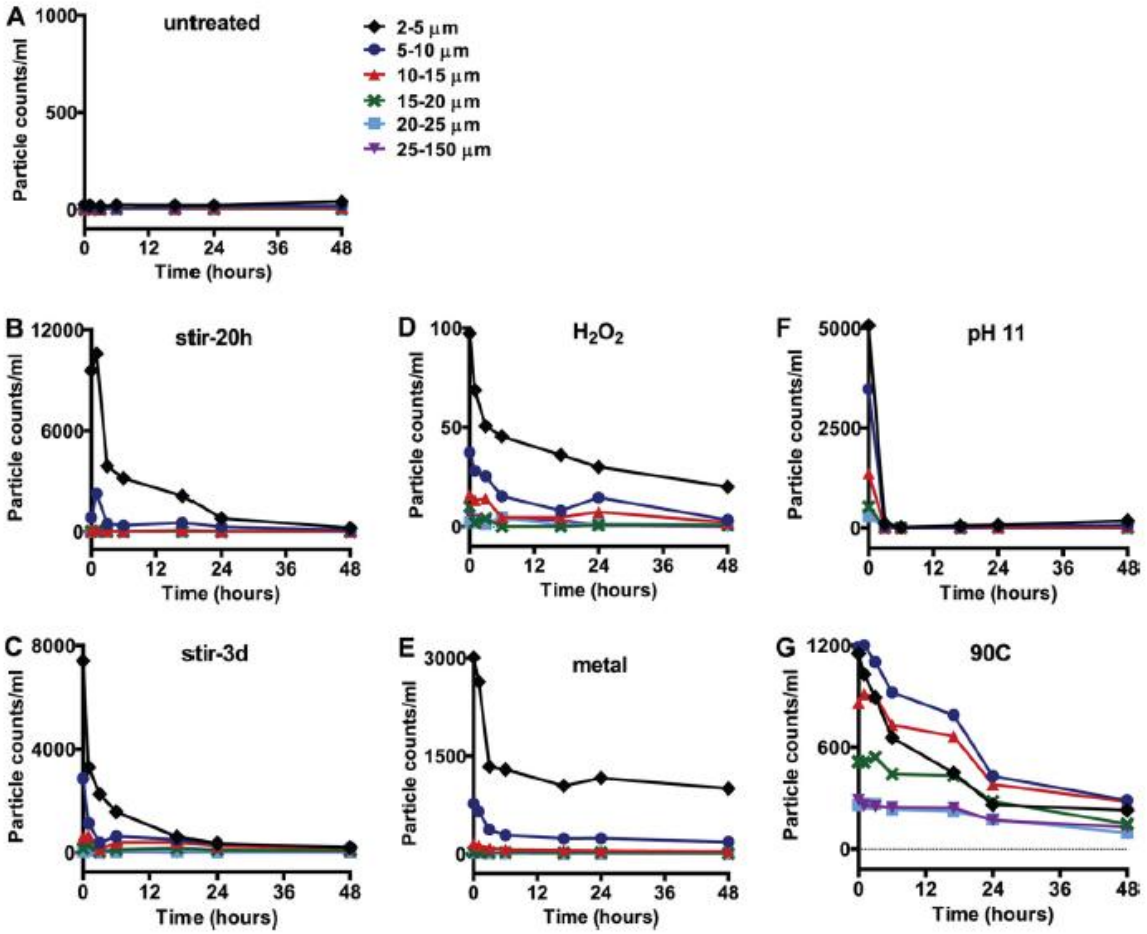
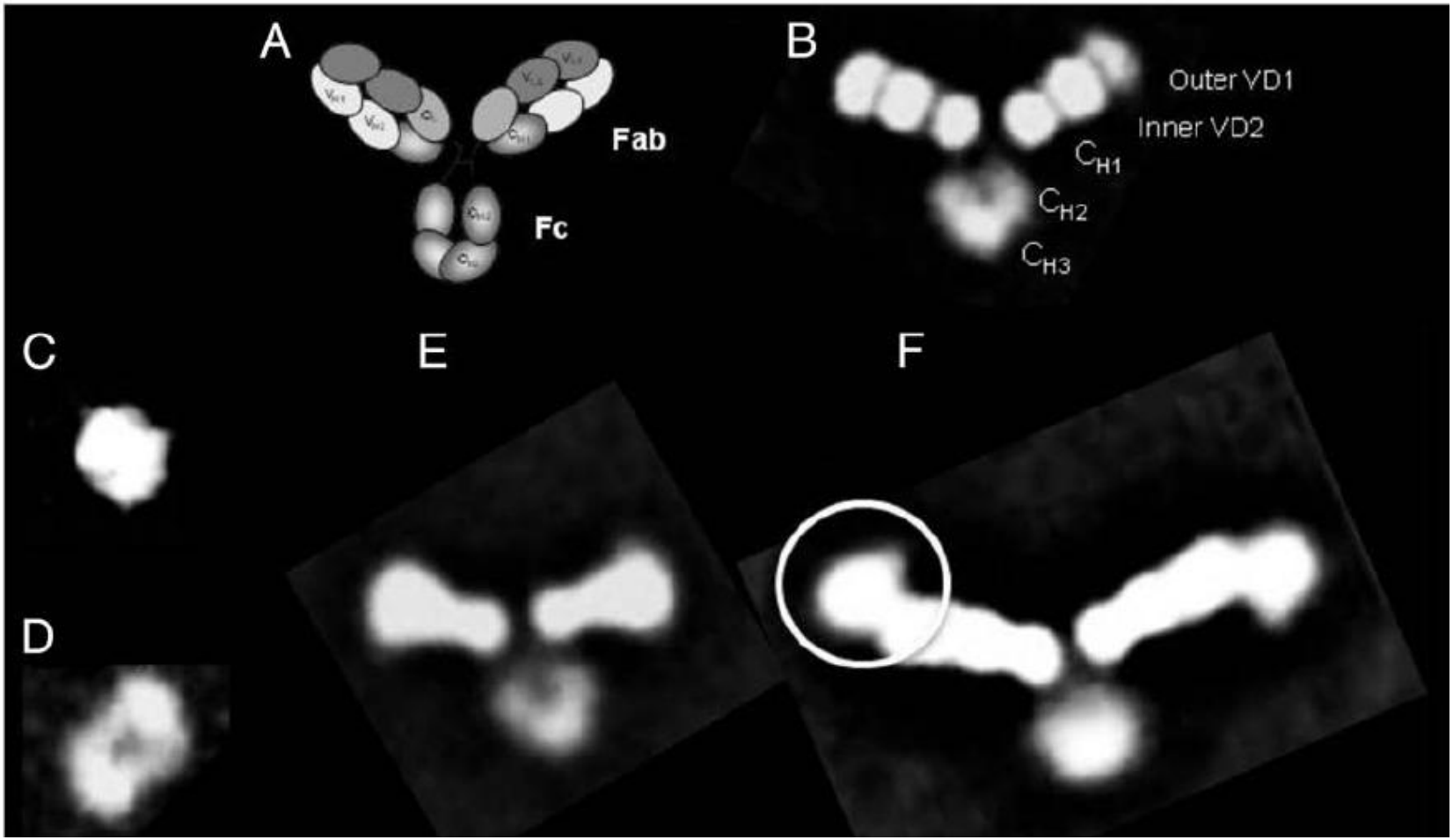



FIGURE 7. **Time course showing a decrease in particle counts upon dilution in a pH 5 buffer.**



Joubert et al., JBC, 286, 25118, 2011

# Example 8: TEM Visualization






Correia et al., mAbs, 5, 364, 2013

# Example 9: TEM Visualization

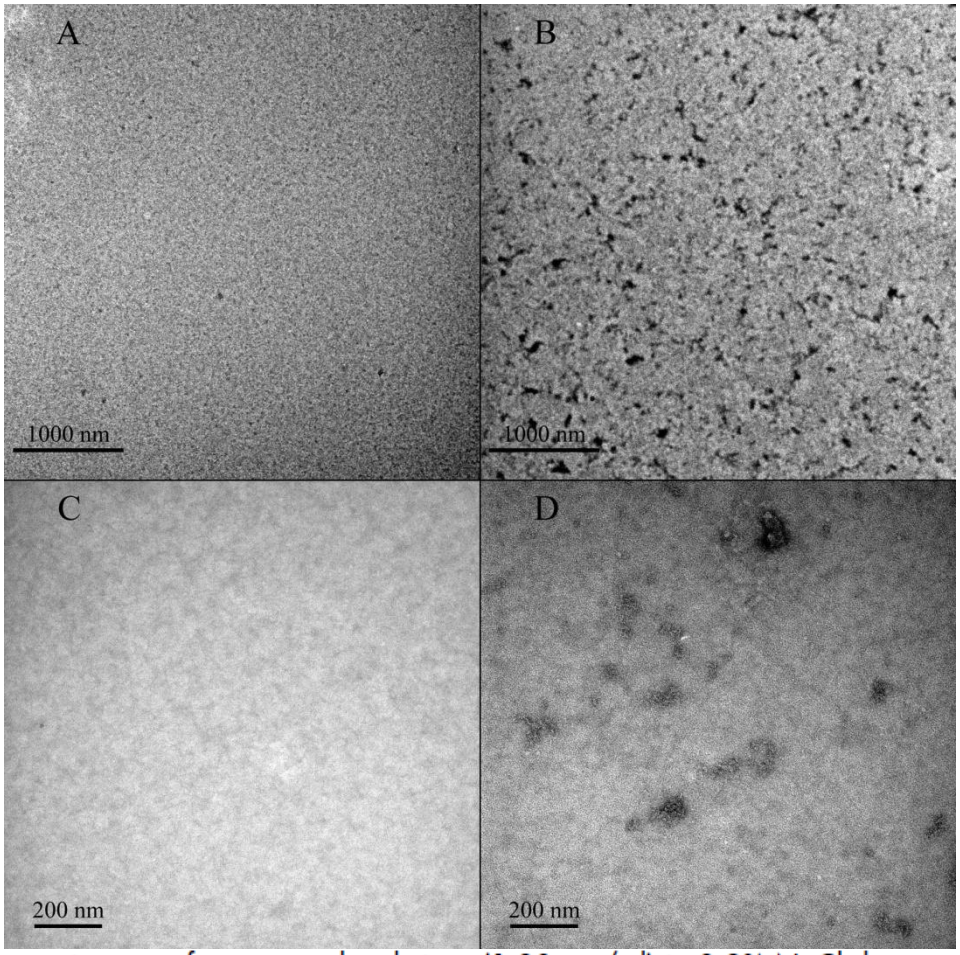
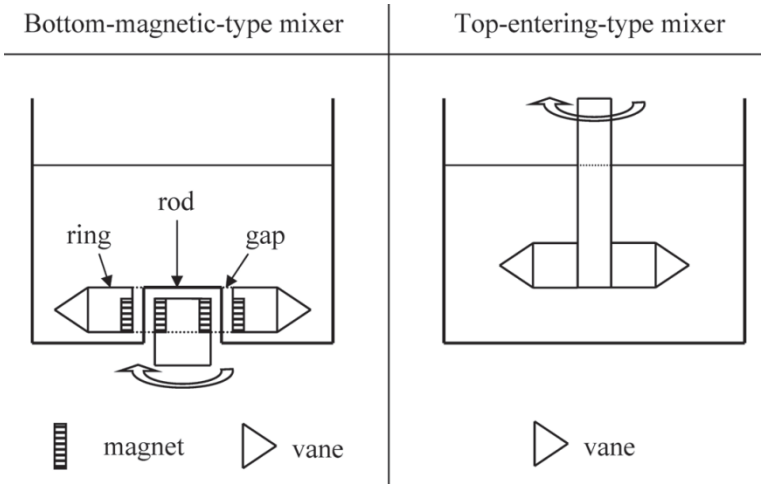
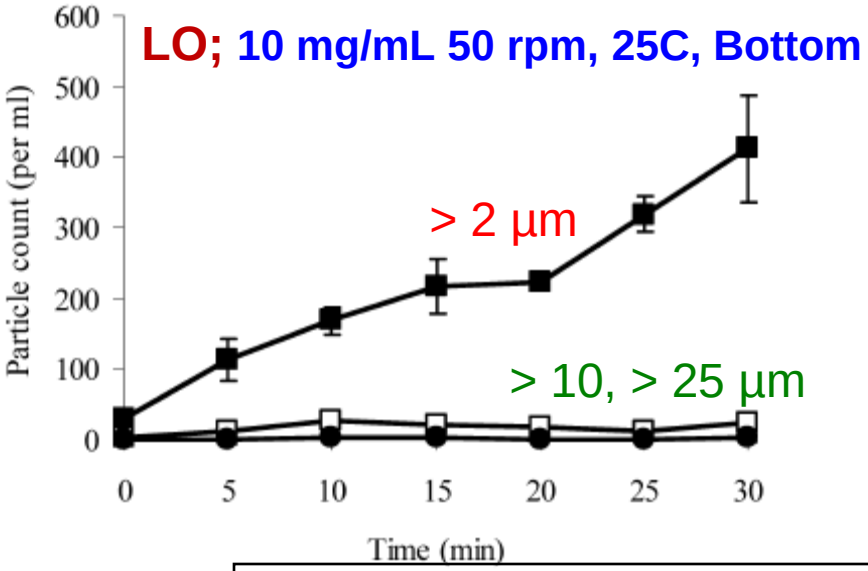


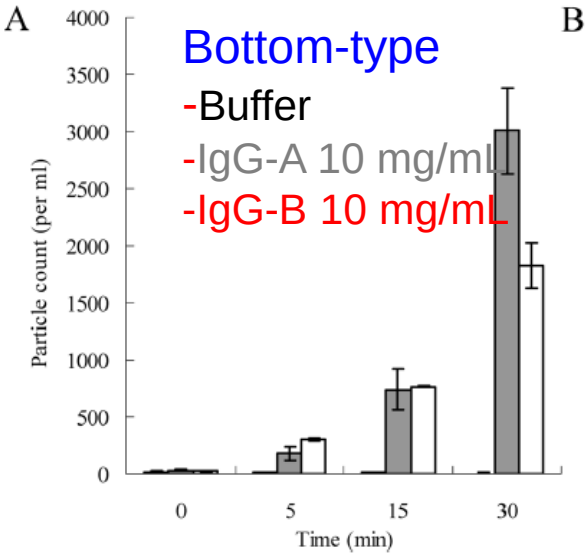

Figure 7. Transmission electron microscopy images of trastuzumab solutions (1.28 mg/ml) in 0.9% NaCl; low magnification, (A); high magnification, (C) and in 5% dextrose, low magnification, (B); high magnification, (D) Aggregates were only present in 5% dextrose and appeared as black spots (B and D).

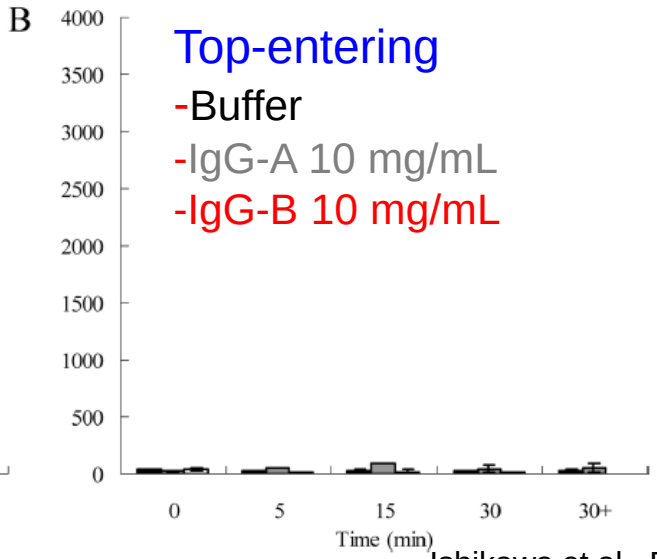
Demeule et al., mAbs. 1. 142. 2009

# Example 10: Process Equipment Selection




- Turbidity seen on agitation with bottom-type magnetic stirrer in 5 minutes
- FTIR showed only protein
- No loss of protein; No change in SEC profile
- No particles in buffer-only
- $V_{max}$ decrease seen on filter



Ishikawa et al., BiolPharmBull 33, 1043, 2010

# Summary

- USP<787> has been developed for therapeutic proteins
- USP<1787> Guidance is in development
- Orthogonal methods required to characterize particles beyond size and count
  - id, composition, reversibility, conformation, morphology
- Know your product. Monitor particulates (size > 2 µm) in development to create product (manufacturing and stability) history and understand liabilities, and to create proper control strategy.

# Thank You

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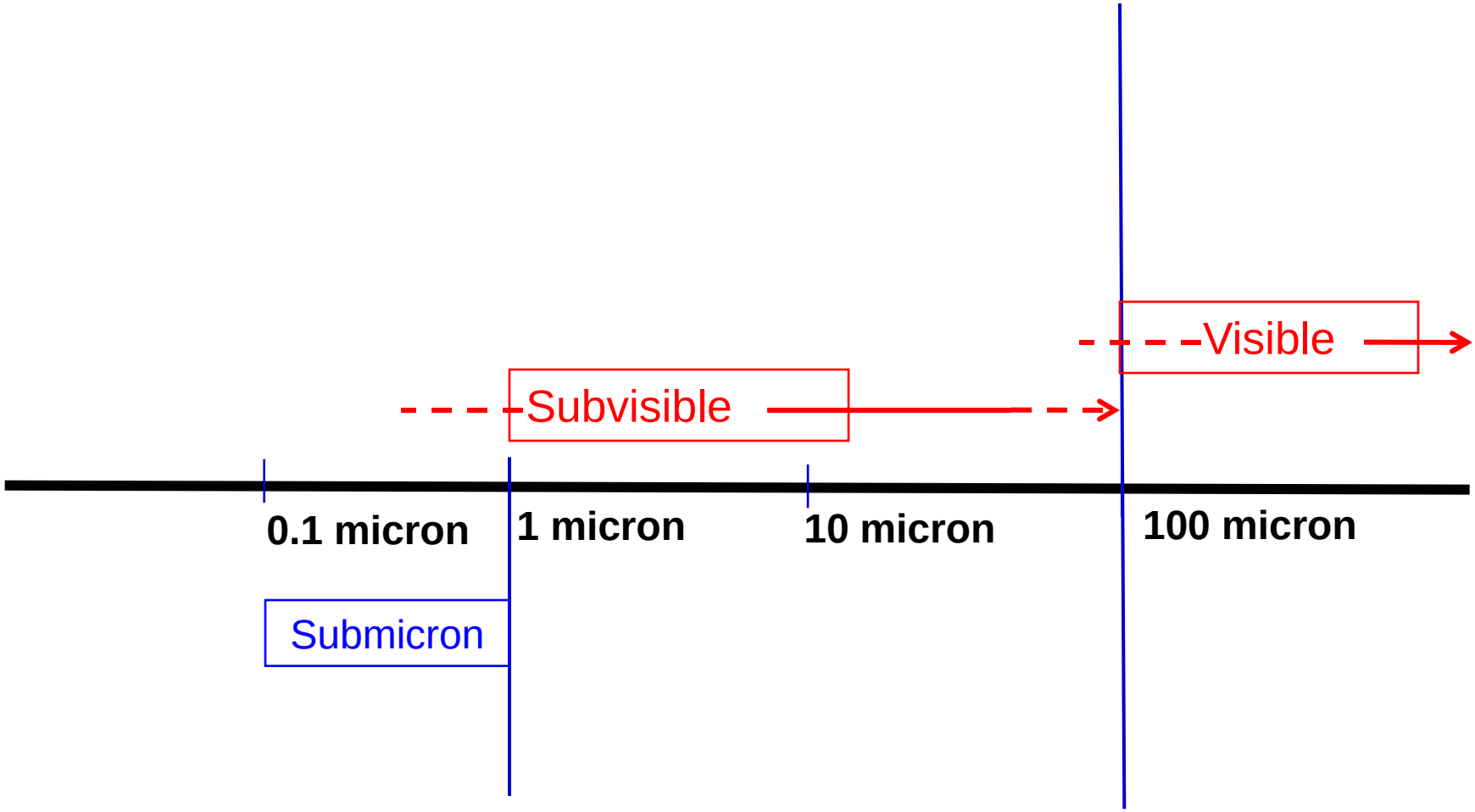
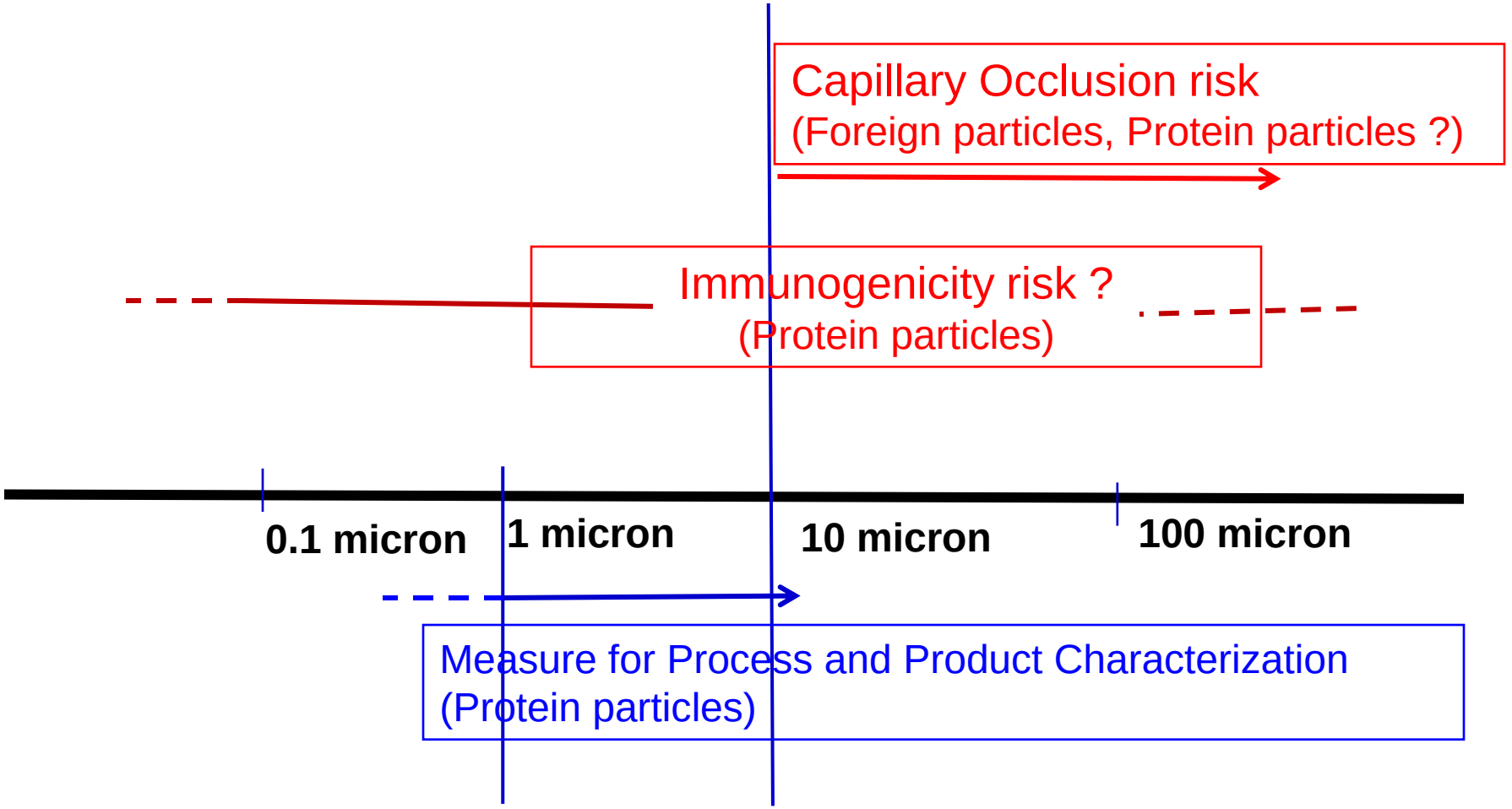
# Back-Ups

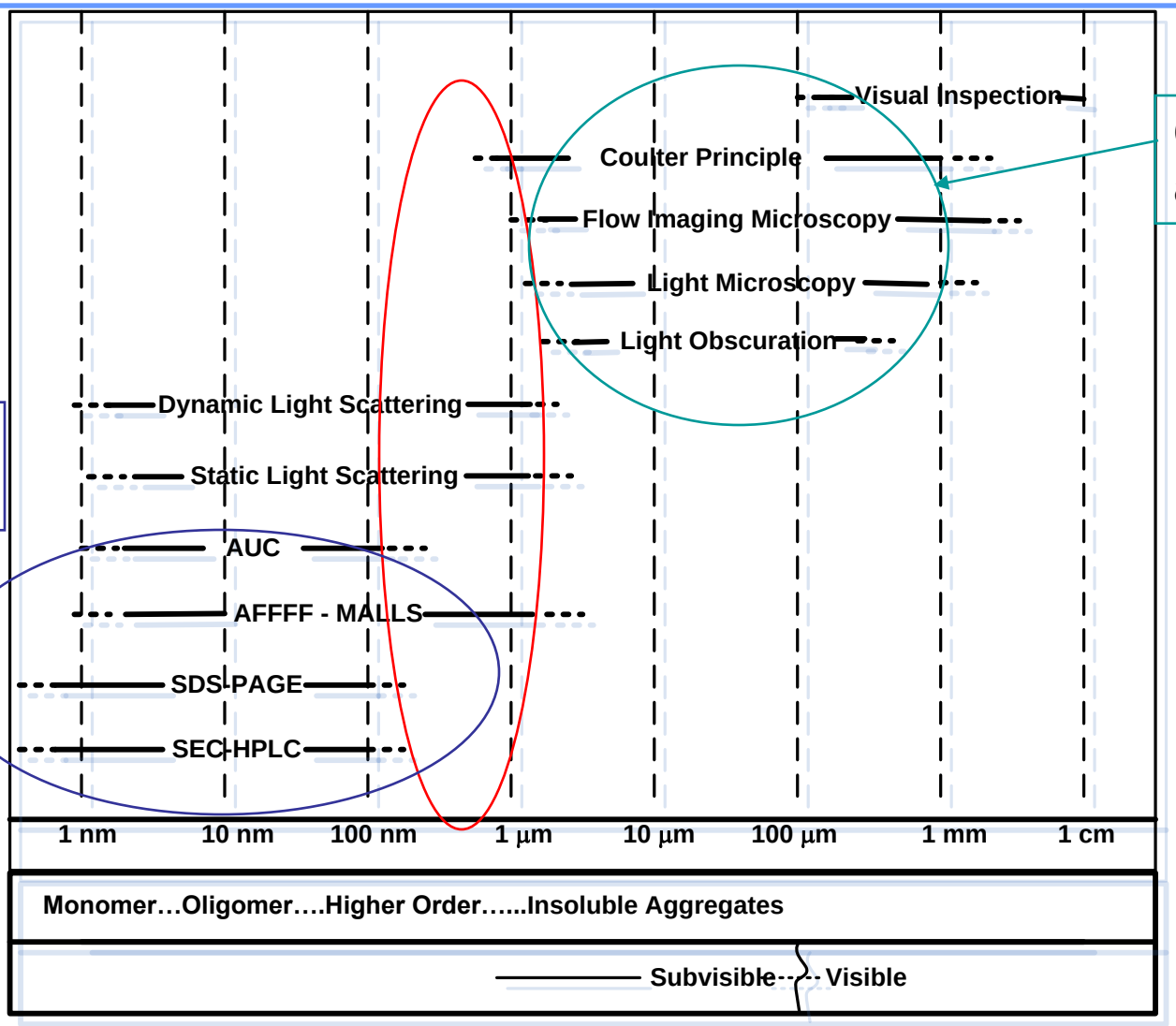
# Particles: Categories



# Particles: Risks



# Current Methods and Limitations




Quantification as Counts

Quantification as % mass



Singh et al., JPS, 99, 3302, 2010