Aggregates, Particles and Patient Immunogenicity with Biopharmaceuticals: Roles for Prefilled Syringes

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Outline

- When miracle drugs fail because of immunogenicity
- Problems and challenges with siliconized glass syringes
- Published case study: Tungsten-induced EPO aggregation and immunogenicity
- Silicone oil-induced protein aggregation and immunogenicity
- Analytical challenges with siliconized prefilled syringes

Adverse Immunogenicity: When Miracle Drugs Fail

- Therapeutic proteins (e.g., anti-TNF's, interferon-β, replacement enzymes and Factor VIII) are miracle drugs for patients.

- But in clinical practices up to 30 to 50% of patients have loss of efficacy; often because of immunogenicity.

- Consequences can be severe; including death

In products that are immunogenic, many studies show that trace amounts of particles/aggregates may play a role.

Particles as Adjuvants: Equine IgG in Humans

- Anti human lymphocyte IgG produced in horses
- Administration to organ transplant patients resulted in immune response & rapid clearance of the IgG
- Treatment of patients with equine IgG in which aggregates/particles removed by ultracentrifugation (134,500 *x g* for 1hr) resulted in no immune response and actually made the patients tolerant to foreign IgG

Wesker et al., 1970, *J. Clin. Invest.* 49:1589

Required reading

Managing Uncertainty: A Perspective on Risk Pertaining to Product Quality Attributes as They Bear on Immunogenicity of Therapeutic Proteins

AMY S. ROSENBERG, DANIELA VERTHELYI, BARRY W. CHERNEY

Division of Therapeutic Proteins, Center for Drug Evaluation and Research, US Food and Drug Administration, Bethesda, MD 20892

"Given the identification of SVPs as a CQA for which there is uncertainty regarding effects on the safety of therapeutic protein products, it is our contention that actions should be undertaken to lessen the probability of occurrence and severity of harm pertaining to such particles. Therefore, risk-reduction strategies should be undertaken that encompass a better characterization of the propensity to form aggregates, and correlative studies performed to address the probability of SVPs contributing to adverse events for individual therapeutic protein products."

JOURNAL OF PHARMACEUTICAL SCIENCES, VOL. 101, NO. 10, OCTOBER 2012

Rapid Communication

Subvisible Particle Content, Formulation, and Dose of an Erythropoietin Peptide Mimetic Product Are Associated With Severe Adverse Postmarketing Events

Joseph Kotarek [1], Christine Stuart [1], Silvia H. De Paoli [1], Jan Simak [1], Tsai-Lien Lin [2], Yamei Gao [3], Mikhail Ovanesov [1], Yideng Liang [1], Dorothy Scott [1], Janice Brown [4], Yun Bai [5], Dean D. Metcalfe [5], Ewa Marszal [1,*], Jack A. Ragheb [6,*]

From US FDA: Light obscuration misses important particles

A B S T R A C T

Peginesatide (Omontys®; Affymax, Inc., Cupertino, CA) was voluntarily withdrawn from the market less than a year after the product launch. Although clinical trials had demonstrated the drug to be safe and efficacious, 49 cases of anaphylaxis, including 7 fatalities, were reported not long after market introduction. Commercialization was initiated with a multiuse vial presentation, which differs in formulation from the single-use vial presentation used in phase 3 studies. Standard physical and chemical testing did not indicate any deviation from product specifications in either formulation. However, an analysis of subvisible particulates using nanoparticle tracking analysis and flow imaging revealed a significantly higher concentration of subvisible particles in the multiuse vial presentation linked to the hypersensitivity cases. Although it is unknown whether the elevated particulate content is causally related to these serious adverse events, this report illustrates the utility of characterizing subvisible particulates not captured by conventional light obscuration.

Responses to regulatory clinical hold due to protein product immunogenicity

- If there is a desire to renew clinical trials, sponsor must determine cause(s) and reduce risk of immunogenicity
- Typically such efforts start with improved analytical assessments and attempts to reduce relevant risk factors
- Aggregates, nano- and microparticles levels are measured, sources are identified and control strategies implemented; e.g., change filler pumps and/or container/closure
- Also risks due to host cell proteins and other impurities are reduced by improving purification and assays
- And perhaps there is a need to reduce risks due to problems with glass prefilled siliconized syringes (see below)

Protein Stability and Analytical Challenges with Siliconized Glass Prefilled Syringes

- **Residual tungsten (from formation of hole for needle) may cause protein aggregation and particle formation**
- Residual radicals from light-cured needle glue and sterilization can cause protein oxidation (and potentially aggregates and particles)
- **Silicone oil-induced protein aggregation and particles**
- Leachates from uncoated plunger stoppers, needle shields and glue used for needles may affect protein physical and/or chemical stability
- **Silicone oil droplets interfere with protein particle analysis**

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Pharm Res (2012) 29:1454–1467

Tungsten-Induced Denaturation and Aggregation of Epoetin Alfa During Primary Packaging as a Cause of Immunogenicity

Andreas Seidl, Otmar Hainzl, Marleen Richter, Robert Fischer, Stephan Böhm, Britta Deutel, Martin Hartinger, Jörg Windisch, Nicole Casadevall, Gerard Michel London & Iain Macdougall

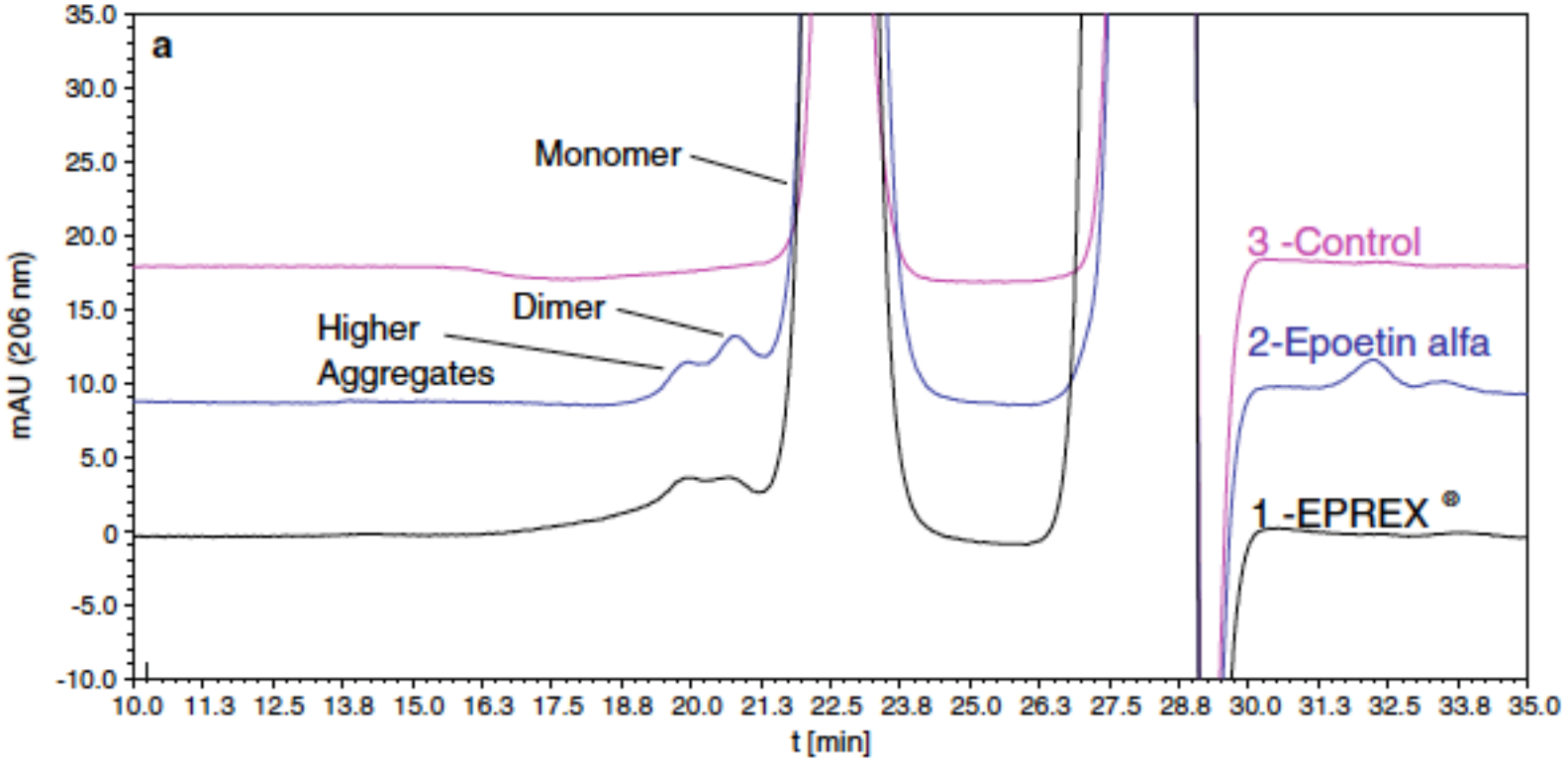
Two patients developed neutralizing antibodies, resulting in pure red cell aplasia in one patient.
Extensive root cause analysis and *in vitro* PBMC assays were performed.

blood advances™
14 FEBRUARY 2017 VOLUME 1, NUMBER 6

T-cell assays confirm immunogenicity of tungsten-induced erythropoietin aggregates associated with pure red cell aplasia

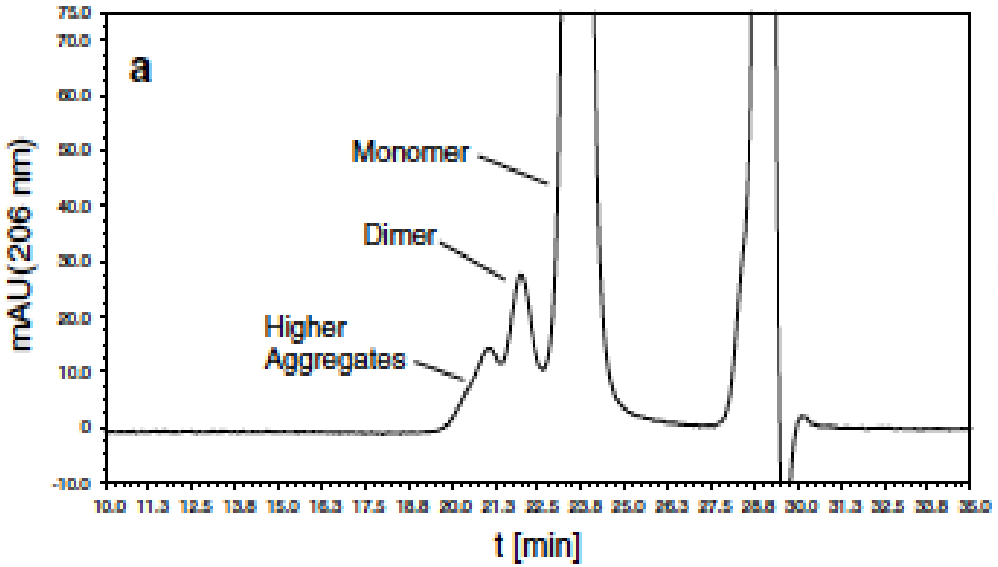
Tina Rubic-Schneider,[1] Masataka Kuwana,[2] Brigitte Christen,[1] Manuela Aßenmacher,[3] Otmar Hainzl,[3] Frank Zimmermann,[3] Robert Fischer,[3] Vera Koppenburg,[4] Salah-Dine Chibout,[1] Timothy M. Wright,[5] Andreas Seidl,[3] and Michael Kammu¨ller[1]

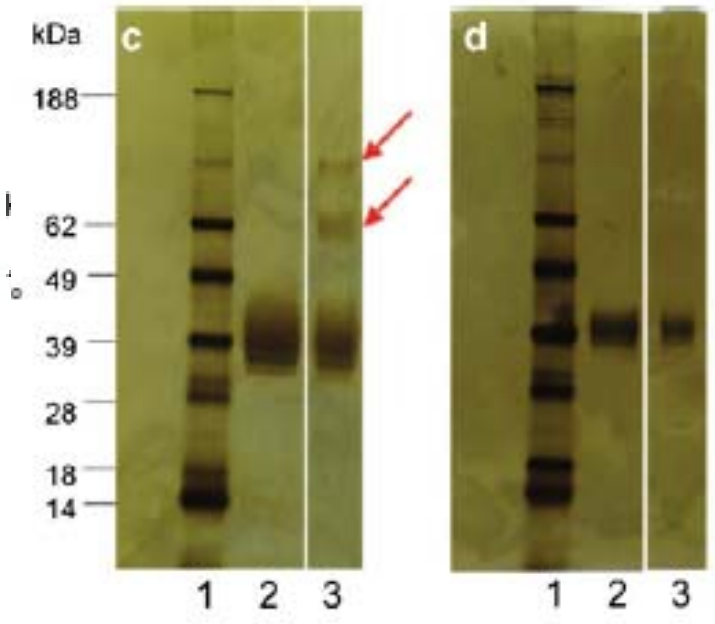
Higher aggregate levels in syringes of EPO drug product from batches given to patients who developed neutralizing antibodies



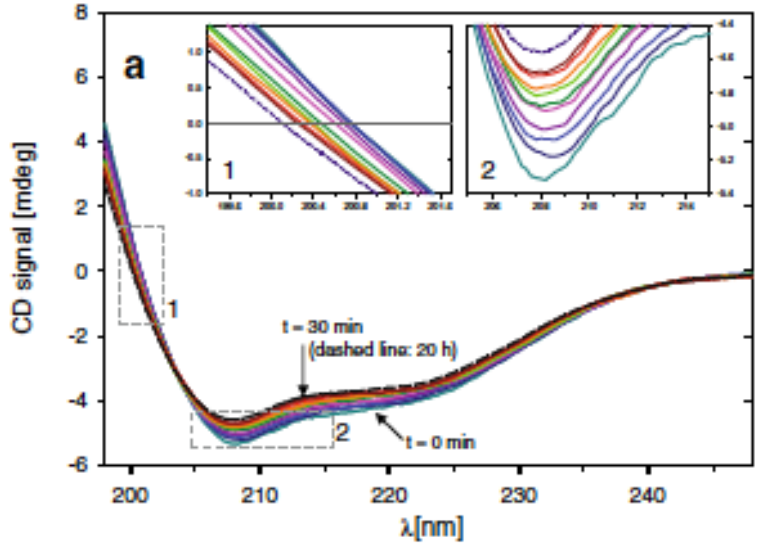
High tungsten levels also detected in these syringes.

Tungsten-induced structural perturbations and covalent aggregates of EPO



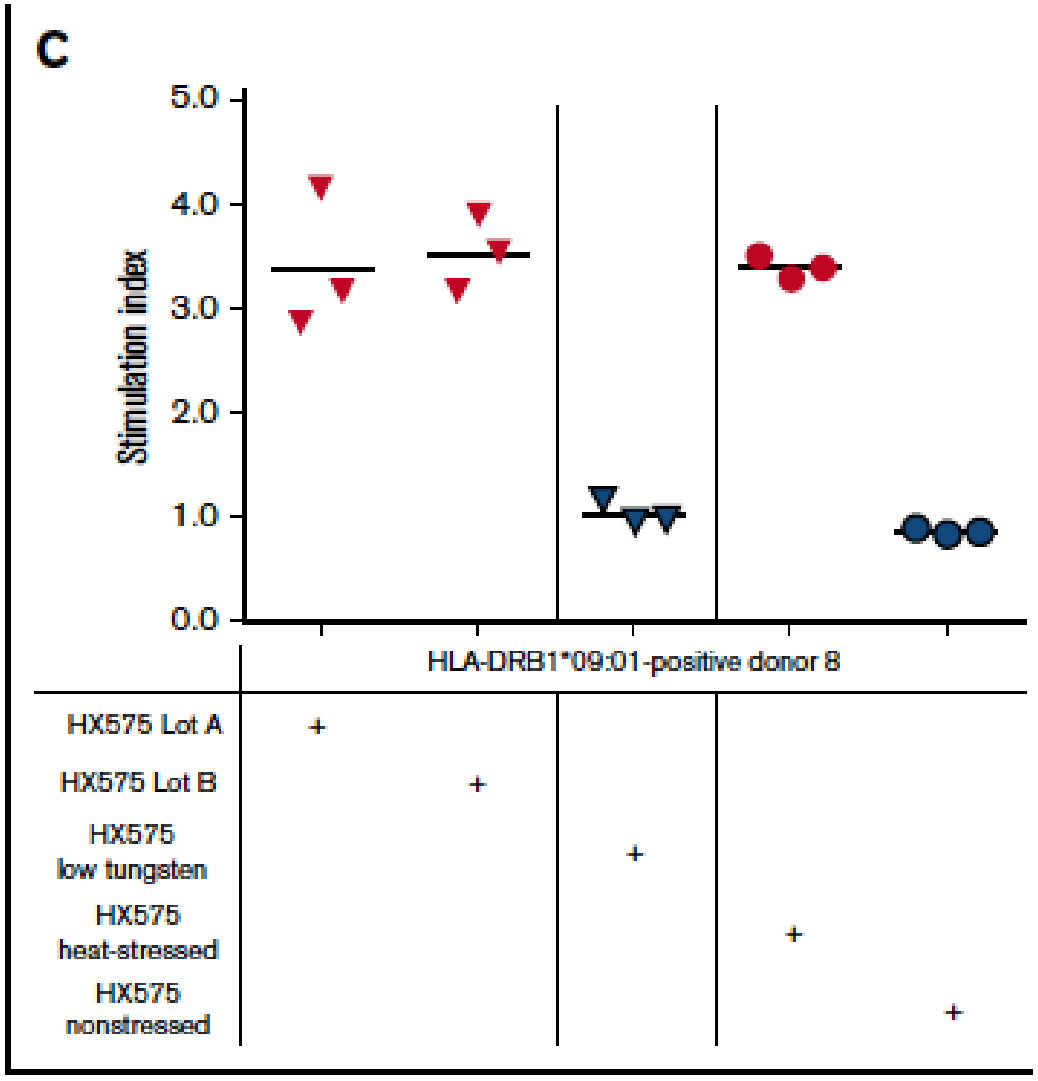


Non-reduced Reduced



Root cause analysis conclusion: Residual tungsten in prefilled syringes with EPO caused aggregates which promoted immunogenicity in patients.

PBMC's stimulated by EPO lots with tungsten-induced aggregated or heat aggregated EPO



Dimer and Aggregates (%)

Lot A: 3.2
Lot B: 2.2
Heat Stressed: 7.9
Non Stressed: ND
Low Tungsten: ND

Protein Stability and Analytical Challenges with Siliconized Glass Prefilled Syringes

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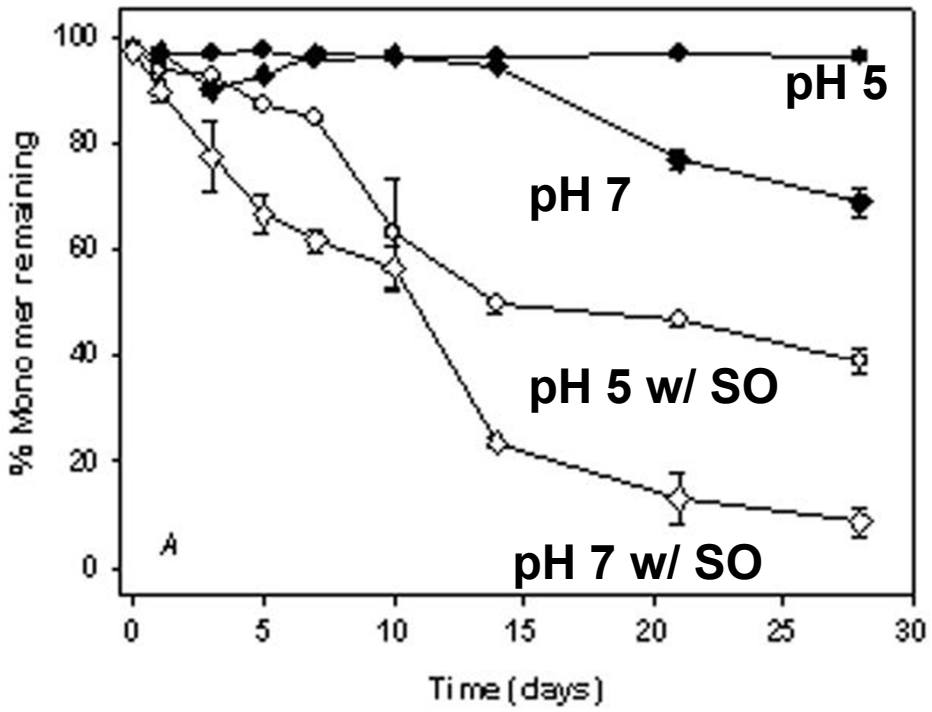
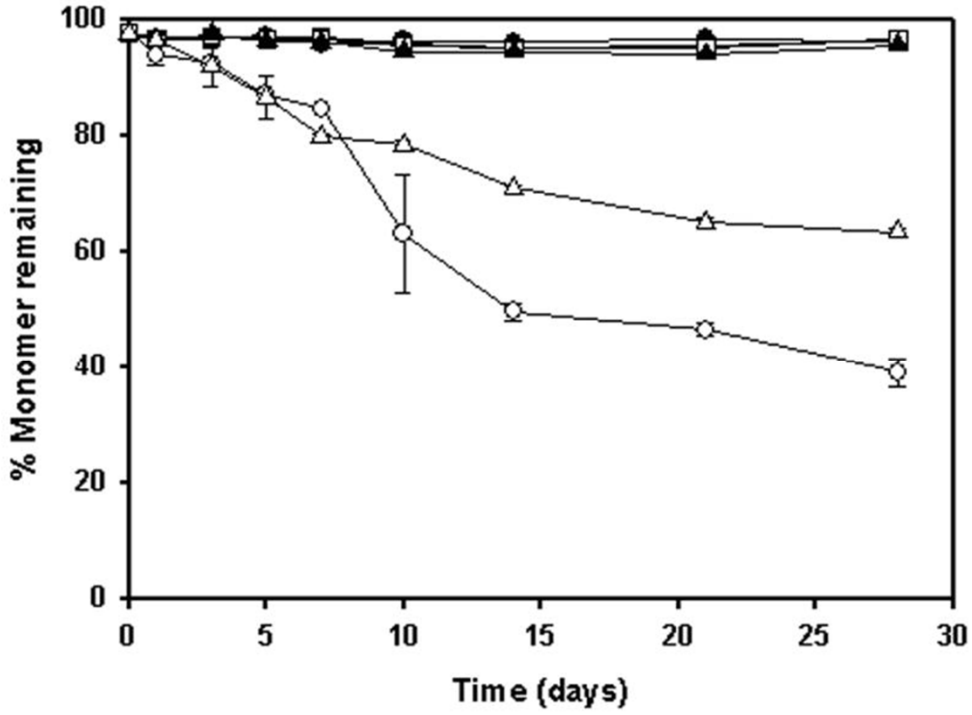
Protein Interactions with Silicone Oil: Example of Container/Closure Incompatibility with Protein

- Syringe and cartridge barrels are coated with silicone oil to facilitate smooth movement of plunger.
- Silicone oil coating can lead to droplets of silicone oil suspended in product formulation.
- Protein adsorption to droplets and inner syringe surfaces can result in particles and aggregates.
- Silicone oil-induced protein aggregates and particles greatly enhanced with agitation stress

Agitation with Silicone Oil Microdroplets Accelerates IgG Aggregation





No aggregation observed with silicone oil without agitation.

Colloidal stability reduced at pH 7.

Polysorbate 20 inhibits aggregation; due to inhibition of adsorption of protein molecules to oil-water interface

Journal of Pharmaceutical Sciences Volume 98, 3167–3181

Interfaces are Major Causes of Protein Particles

- Protein molecules adsorb to most interfaces.
- Layers of adsorbed protein form gels or films.
- Rupture or sloughing off of film can lead to particles in the bulk solution phase
  - During agitation and with bubble popping
  - Compression/dilation can disrupt film
  - Layers adsorbed (e.g., to syringe walls or to tubing used in processing) can slough off; facilitated by agitation, fluid movement or mechanical stress.
  - Also, multiple interfaces (e.g., air bubble in syringe)

Gelation of a mAb at silicone oil-water interface

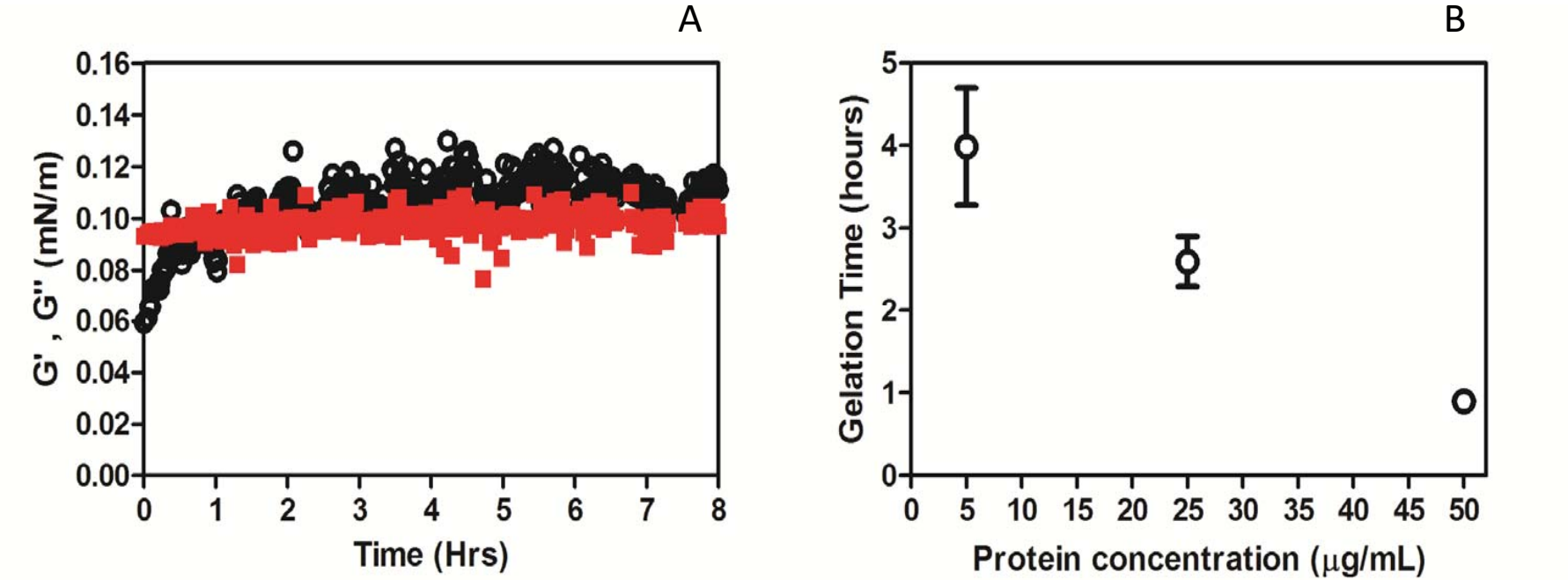
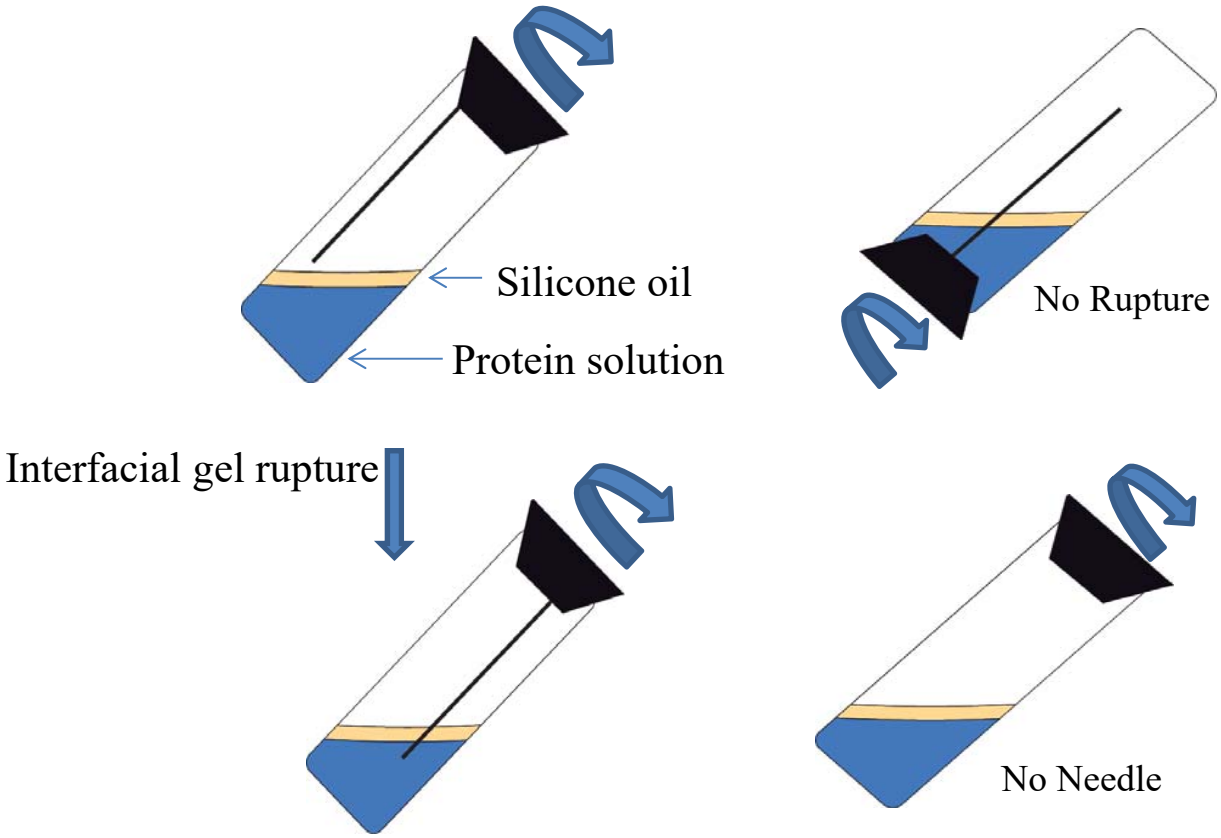


Figure : (A) Dynamic interfacial shear moduli as a function of aging time at 50 μg/mL protein concentration. Black open circles represent elastic modulus (G') and Red solid squares represent viscous modulus (G''). (B) Gelation time as a function of protein concentration. Data points represent the mean ± SD for triplicate samples. Error bars for certain data points are smaller than symbols.

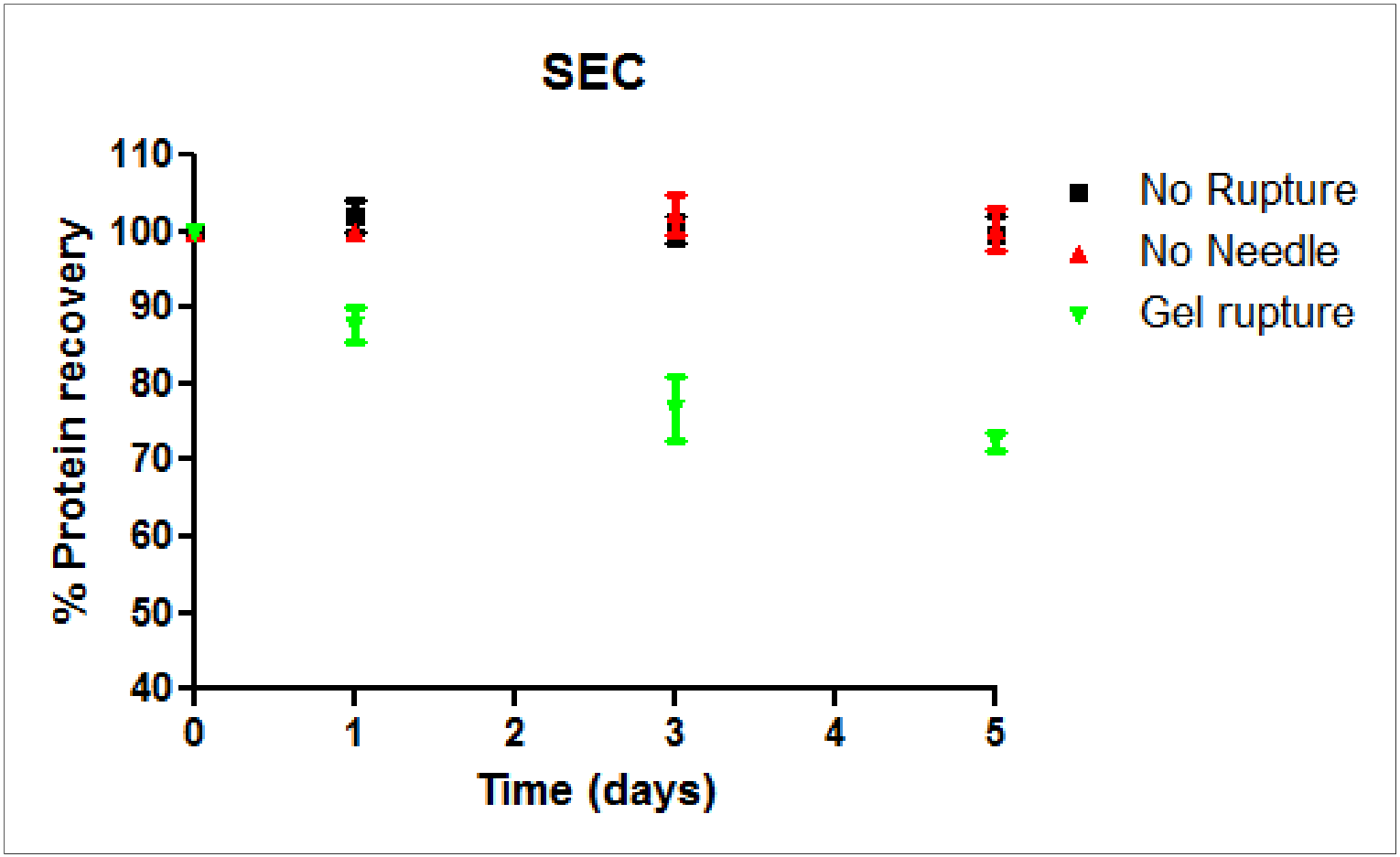
Mehta et al., 2015. *JPharmSci* 104:1282

Experimental setup for gel rupture at the silicone oil-water interface



Mehta et al., 2015. *JPharmSci* 104:1282

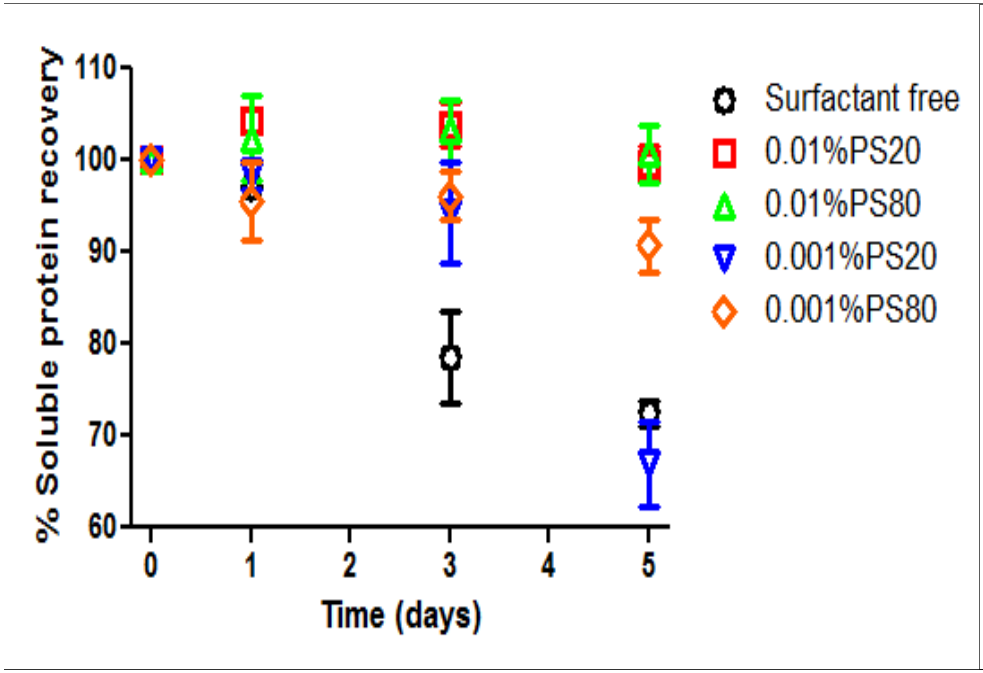
Gel rupture leads to loss of soluble protein



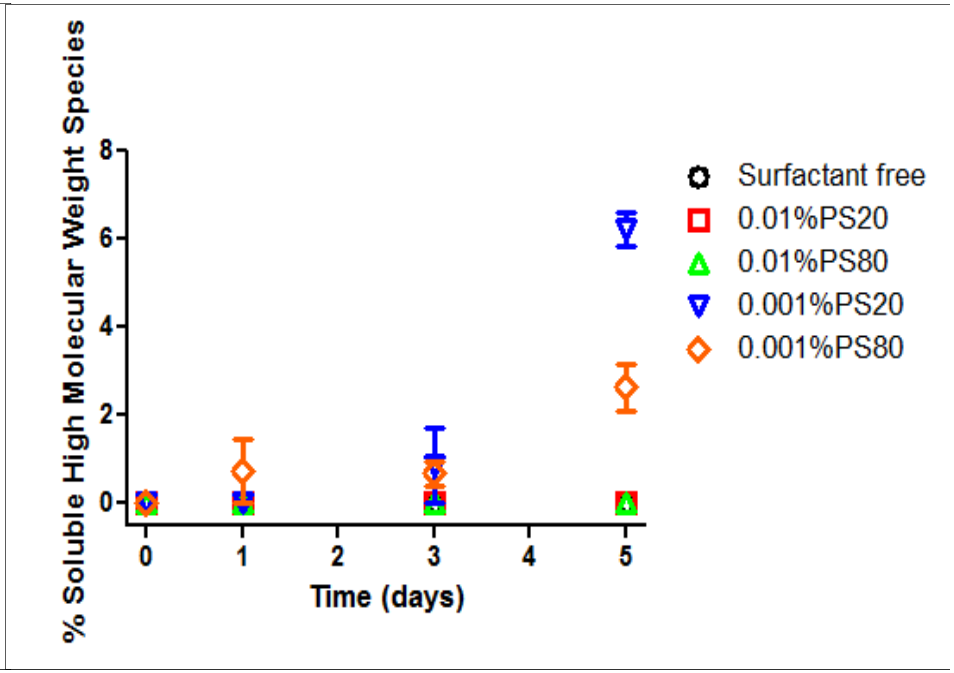
Gel rupture resulted in about 30% loss of soluble protein due to aggregation. No loss of soluble protein was detected for control samples.

Mehta et al., 2015. *JPharmSci* 104:1282

Presence of surfactants resulted in reduced aggregation



A. Soluble protein recovery

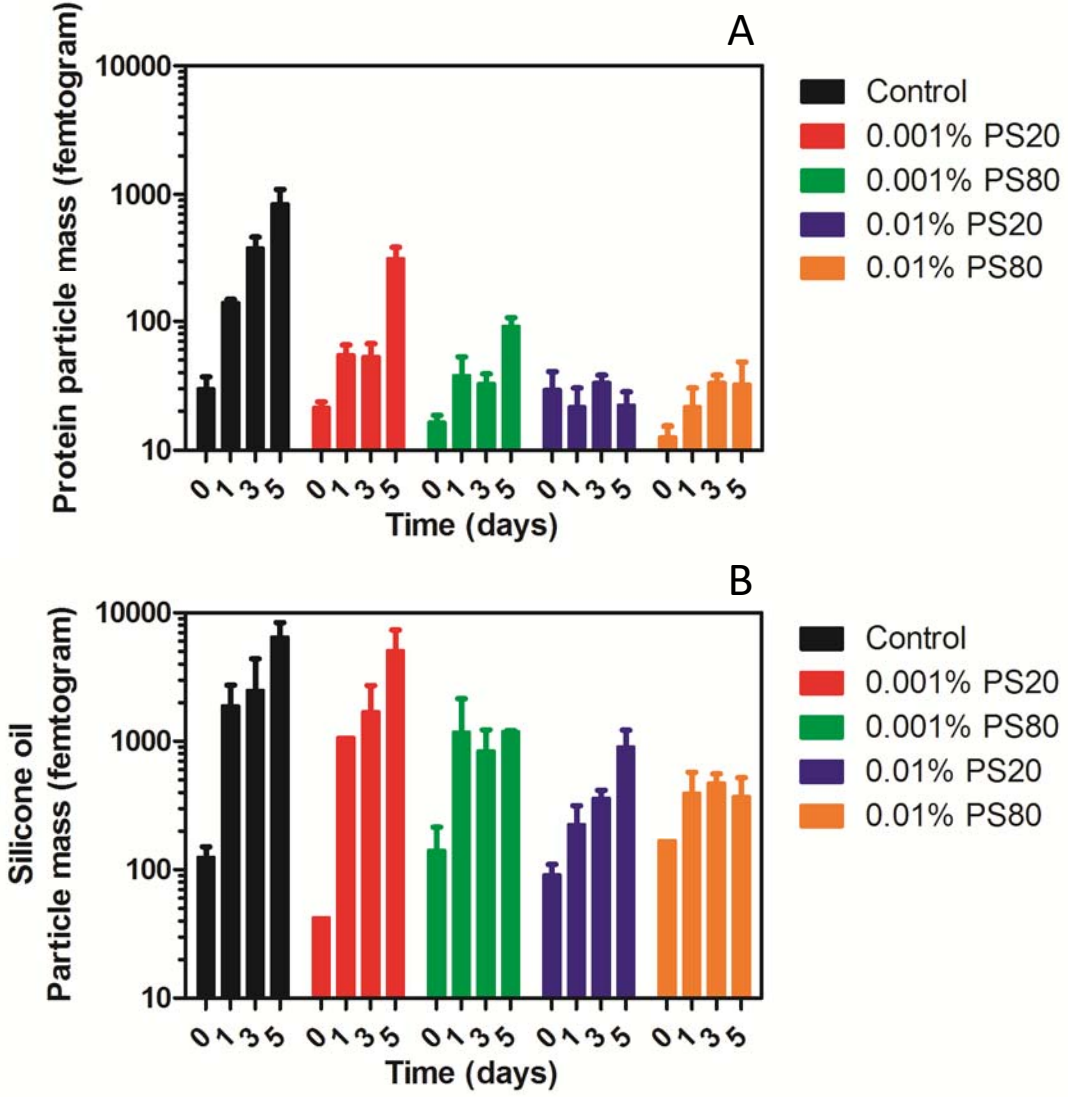


B. Soluble high molecular weight species

At above-CMC levels, the presence of surfactants provided significant protection against interface induced aggregation.

Mehta et al., 2015. *JPharmSci* 104:1282

Presence of surfactants led to a decrease in the protein particle mass detected by Archimedes



(A) Total protein particle MASS and (B) Silicone oil droplet mass as detected by RMM for gel Rupture samples in different formulations at different time points.

<u>Presence of surfactants led to a decrease in the protein particle mass detected.</u>

JPP JOURNAL OF Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY
JPP Journal of Pharmacy And Pharmacology
Research Paper
Journal of Pharmacy and Pharmacology, **69** (2017), pp. 1341–1351

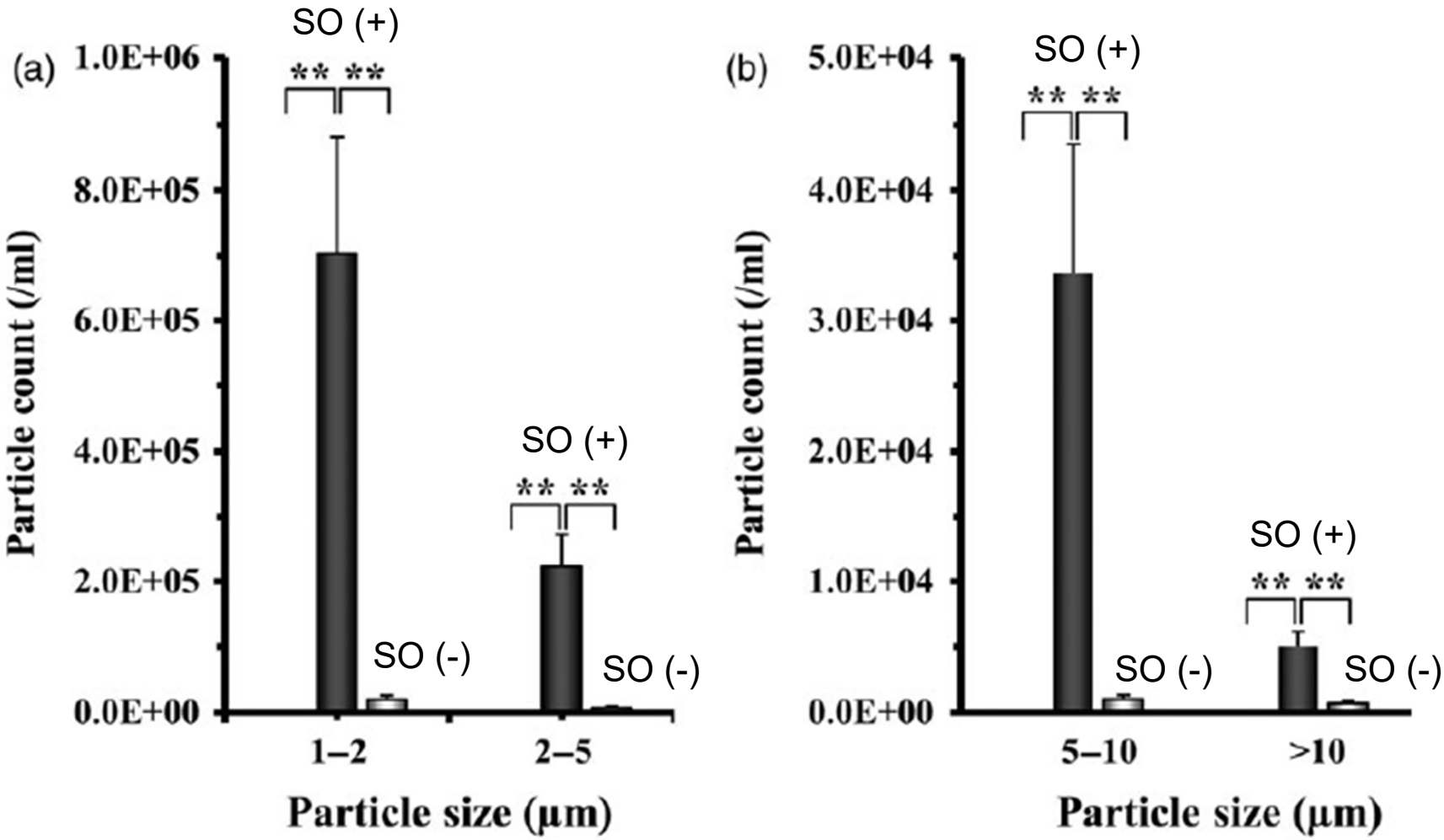
Immunogenicity of protein aggregates of a monoclonal antibody generated by forced shaking stress with siliconized and nonsiliconized syringes in BALB/c mice

Tomonobu Uchino, Yasunori Miyazaki, Takuto Yamazaki and Yoshiyuki Kagawa

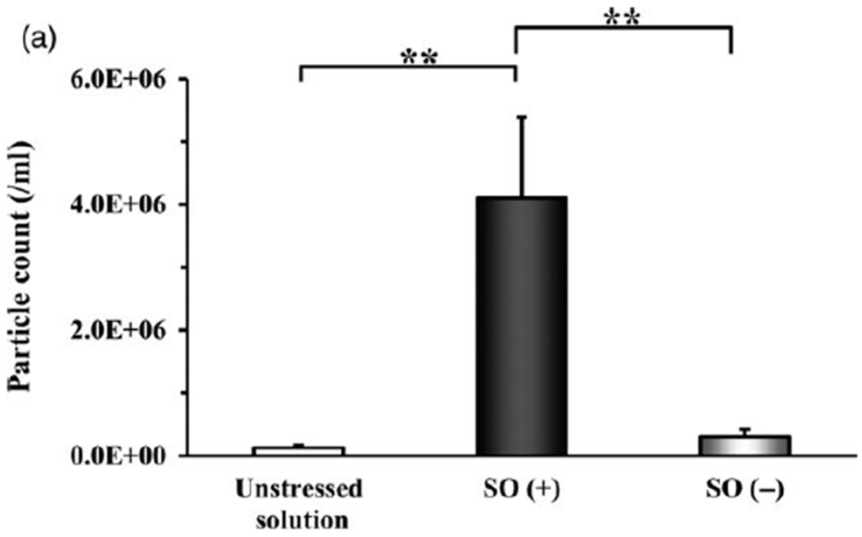
Department of Clinical Pharmaceutics, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

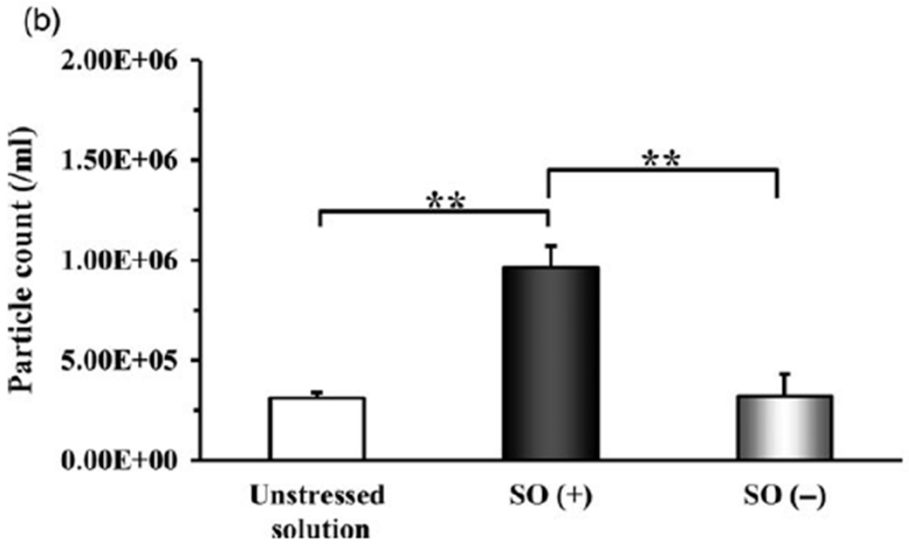
- Humira was diluted 1/50 to 1 mg/ml in PBS
- Placed in siliconized and unsiliconized Terumo COP syringes
- Syringes with headspace were agitated at 250 rpm for 5 minutes
- Particle levels and immunogenicity were tested

Effect of agitation in siliconized vs. nonsiliconized Terumo COP syringes: MFI



Effect of agitation in siliconized vs. nonsiliconized Terumo COP syringes: RMM



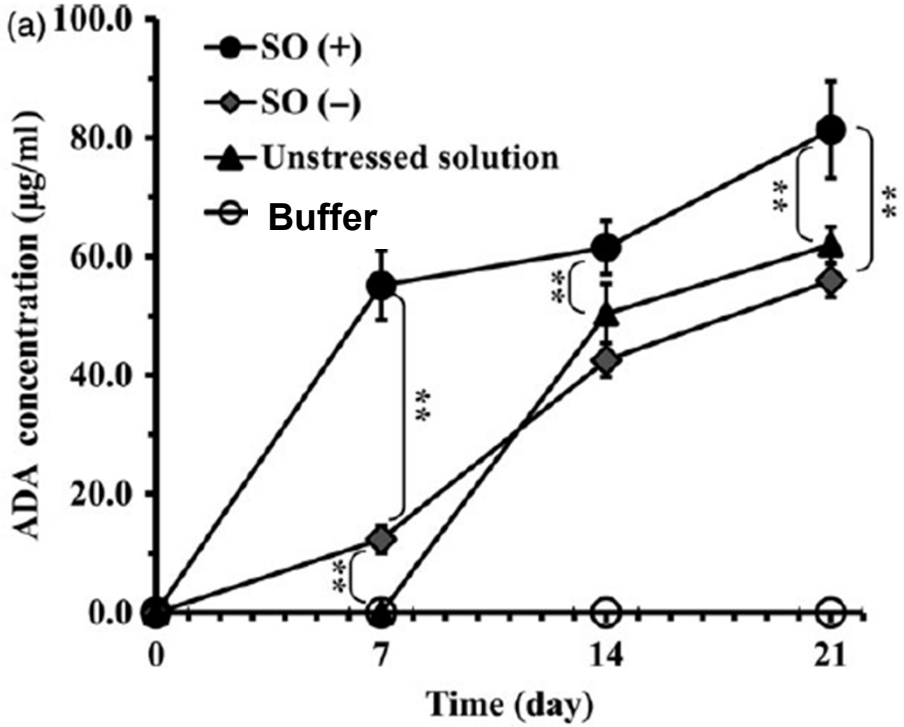


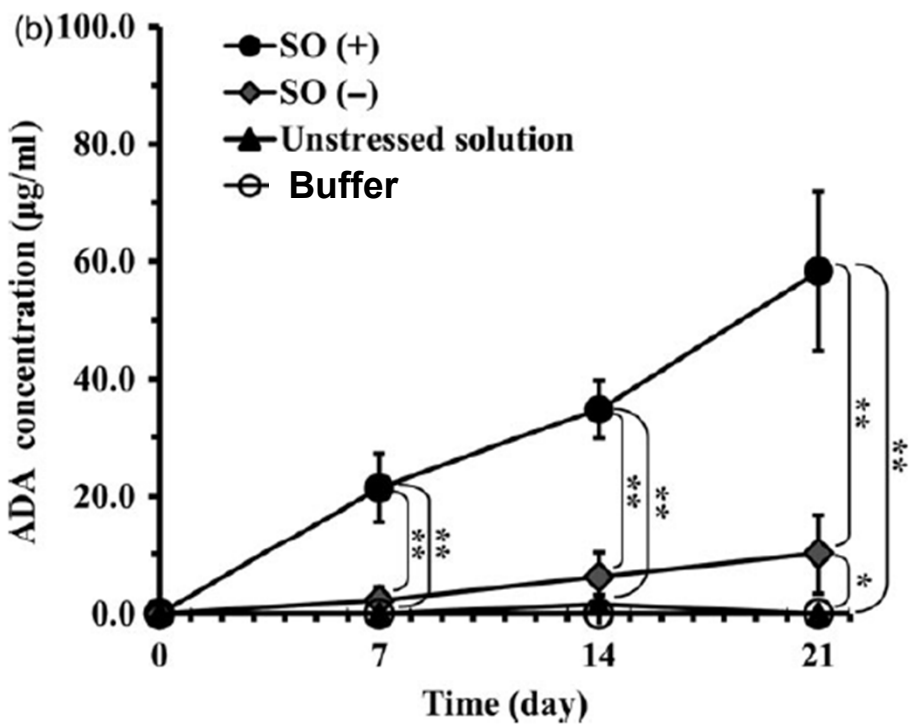
Positively buoyant particles

Negatively buoyant particles

Effect of agitation in siliconized vs. nonsiliconized COP Terumo syringes: Immunogenicity

High dose tolerance





10 μg/ml injections

100 μg/ml injections

Silicone Oil-Induced Protein Aggregation

- The effects of silicone oil on protein structure and aggregation are protein specific.
- Agitation and silicone-water interface promote aggregation and particle formation.
- Polysorbates reduce protein adsorption to silicone-water interface and protein aggregation, even with agitation.
- But the inhibition is not absolute; particles can still form.
- Also, polysorbates can promote sloughing of oil droplets from walls on containers.

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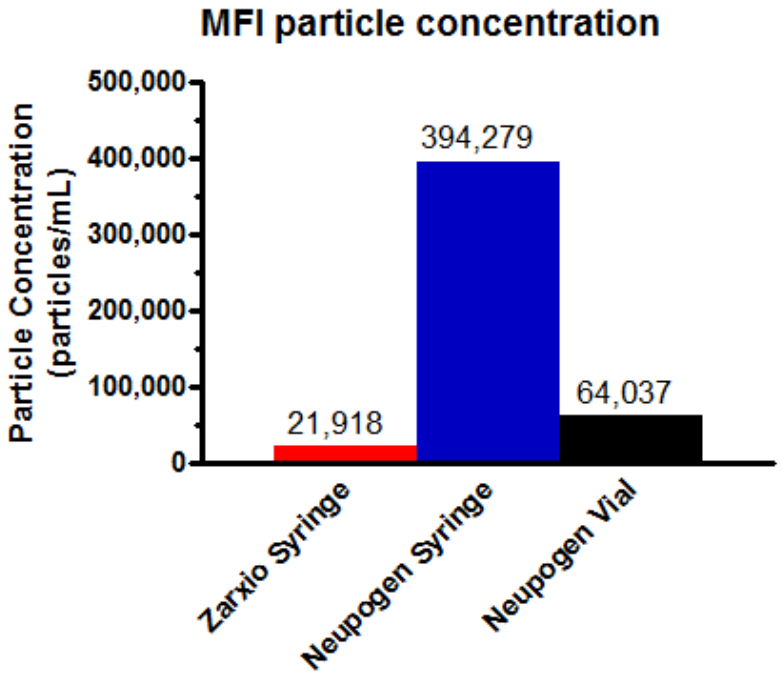
Biosimilar Zazxio vs. Innovator Neupogen

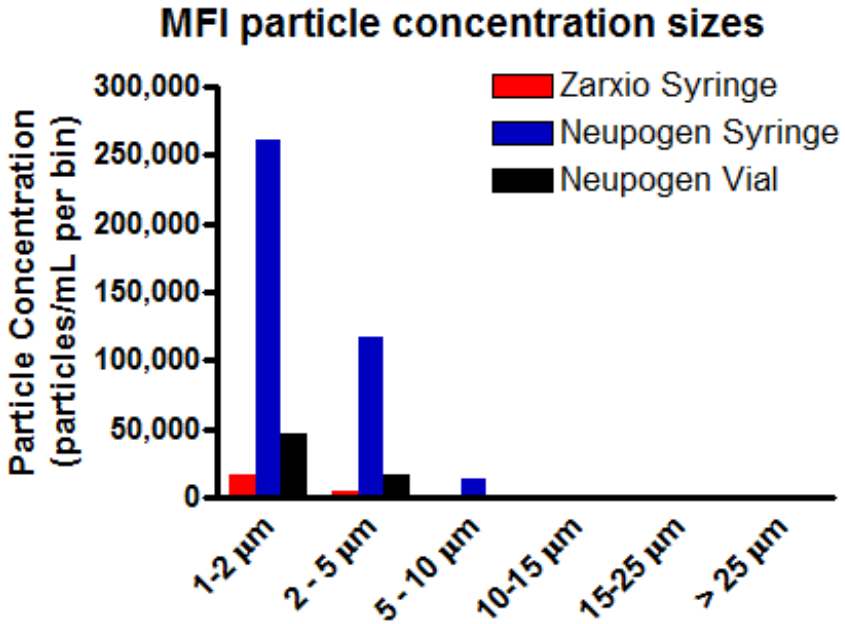
- Samples:
  - Neupogen, 300 mcg in 0.5 mL prefilled syringe
  - Zarxio, 300 mcg in 0.5 mL prefilled syringe

- Formulations:

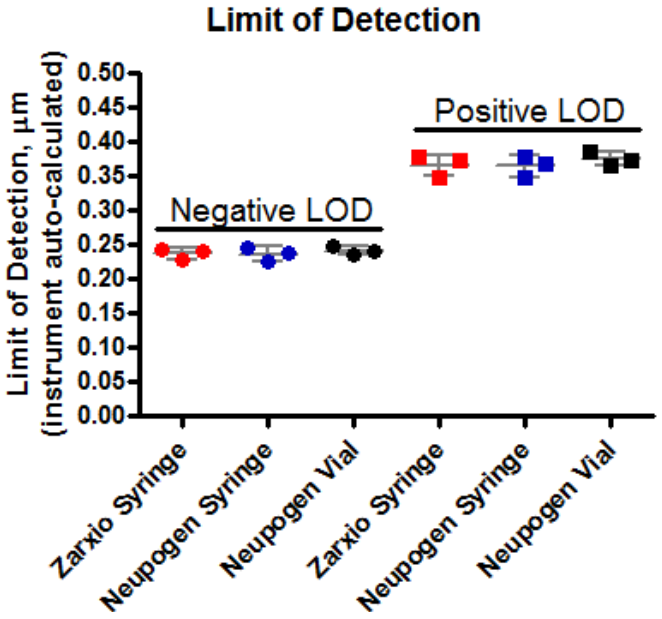
Neupogen	Zarxio
10 mM sodium acetate 5% sorbitol 0.004% PS80 pH 4.0	10 mM glutamic acid 5% sorbitol 0.004% PS80 pH 4.4

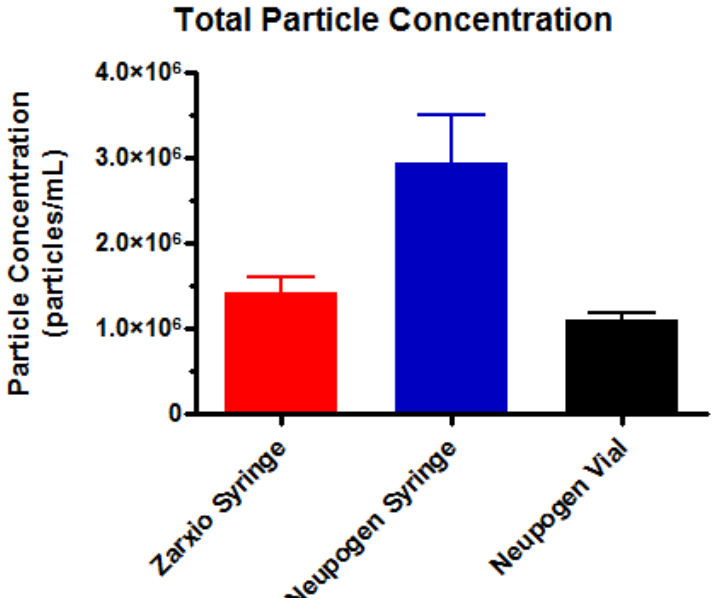
Microparticles (MFI)

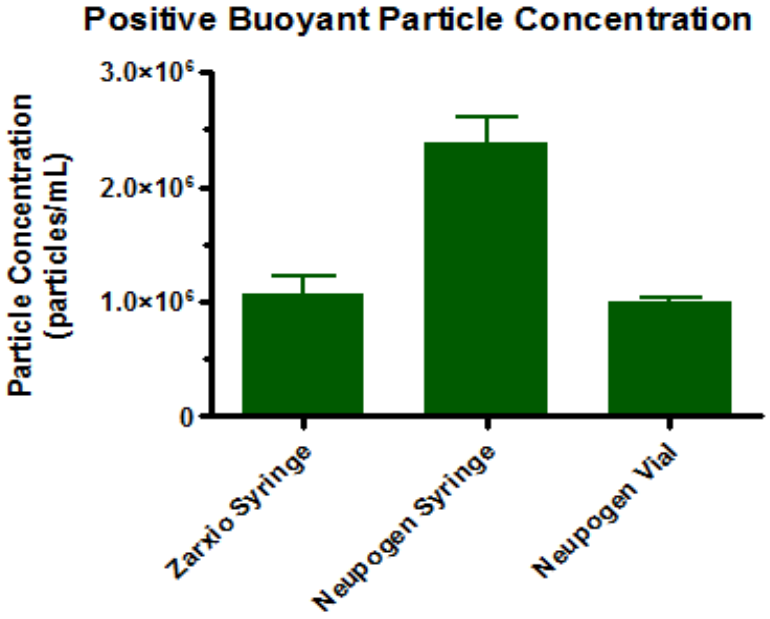


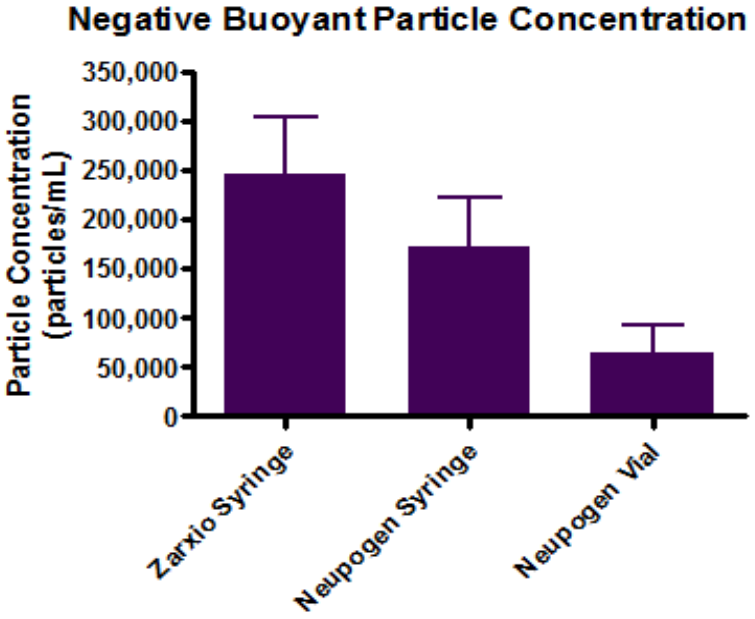


Nano- and Microparticles (Archimedes)



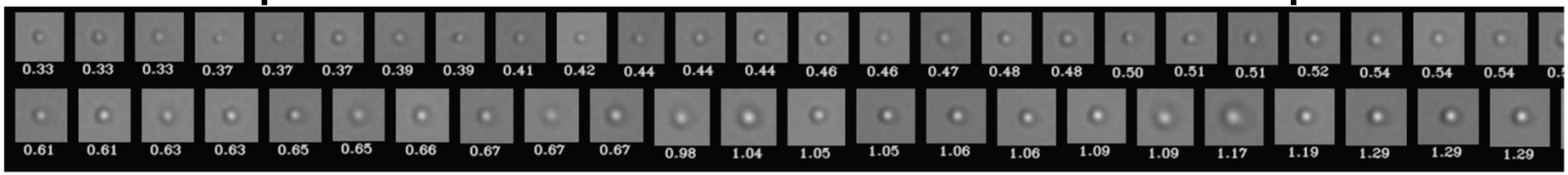


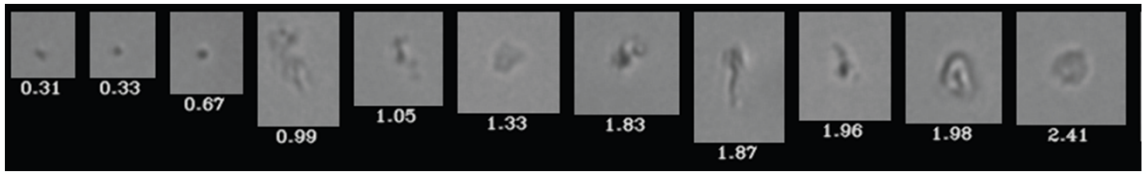




Analytical Challenges with Siliconized Prefilled Syringes

- Most protein particles and silicone oil droplets are smaller than 2-5 micron size required for typical digital filtering with flow imaging methods
- Archimedes (RMM) shows that most particles are submicron, but can distinguish protein particles from oil droplets
- Perhaps new FlowCam Nano would be helpful





Conclusions

- Several components that are present in glass prefilled syringes are stressful to proteins.
- The resulting protein oxidation, aggregation, and/or particle formation may reduce product quality and induce adverse immunogenicity.
- Also, silicone oil droplets interfere with quantitation of protein particles in products from glass prefilled syringes.
- Nonsiliconized syringes can help to mitigate these problems.