

Characterization and Quantitation of Aggregates and Particles in Inteferon- β Products

Particles as Adjuvants

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Acknowledgments

Dr. Wim Jiskoot

Jay Yang and Dr. John Philo (AUC)

Ken Babcock (Affinity Biosensor)

Duncan Griffiths (Nanosight)

Neutralizing antibody (NAb): Summary of Clinical Data for IFN- β Products

Betaseron

(summary of 6 studies)

27.8 - 47% NAb positive

Rebif

(summary of 6 studies)

5.3 - 35% NAb positive

Avonex

(summary of 6 studies)

2 - 7.5% NAb positive

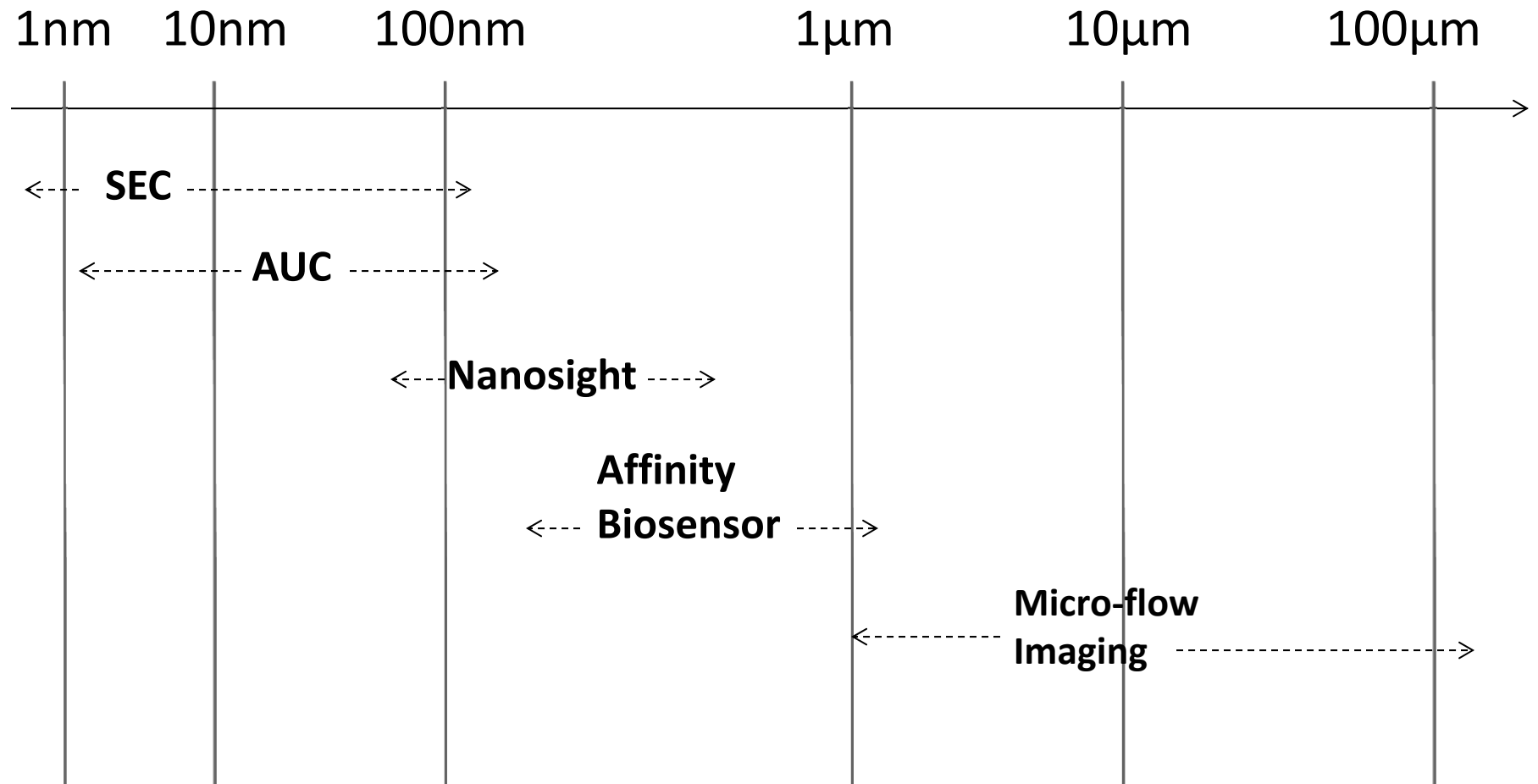
Immunogenicity of interferon beta: differences among products. Bertolotto A. J Neurol 2004

Frequency and Magnitude of Interferon β Neutralizing Antibodies in the Evaluation of Interferon β Immunogenicity in Patients with Multiple Sclerosis. Grossberg S. Journal of Interferon & Cytokine Research 2011.

Dosage Form/Formulation of Ifn- β Products Tested

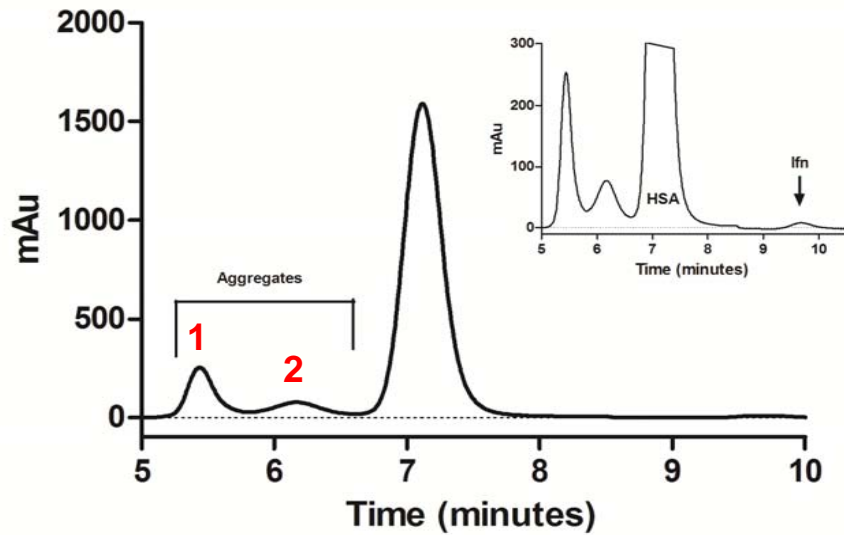
- **Betaseron(\$2,976 /14 vials) and Extavia (\$3,000/15 vials)**
 - Lyophilized powder, 15mg mannitol, 15 mg human serum albumin (HSA), 0.3 mg interferon beta 1b. Stored at room temperature.
 - Reconstituted with 1.2mL of 0.54% NaCl from a prefilled syringe. Use within 3 hours, store at 5° C post reconstitution.
 - Extavia is produced at the same manufacturing site as Betaseron.
- **Rebif (\$2,982 /12 syringes)**
 - Prefilled syringe (0.5mL), 54.6 mg/mL mannitol, 0.8mg/mL sodium acetate, 8mg/mL HSA, 44 μ g interferon beta 1a.
- **Avonex (\$2,954 /4 syringes)**
 - Prefilled syringe (0.5mL), 1.58 mg/mL sodium acetate, 0.5mg/mL acetic acid, 0.05 mg/mL polysorbate 20, 31.6 mg/mL arginine, pH 4.8, 30 μ g interferon beta 1a.

Analytical Methods for Characterizing and Quantifying Aggregates and Particles

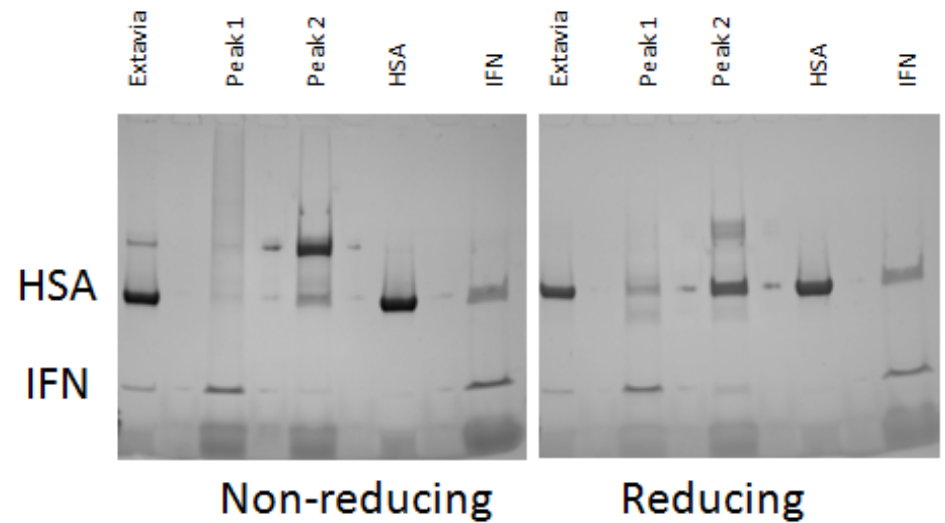
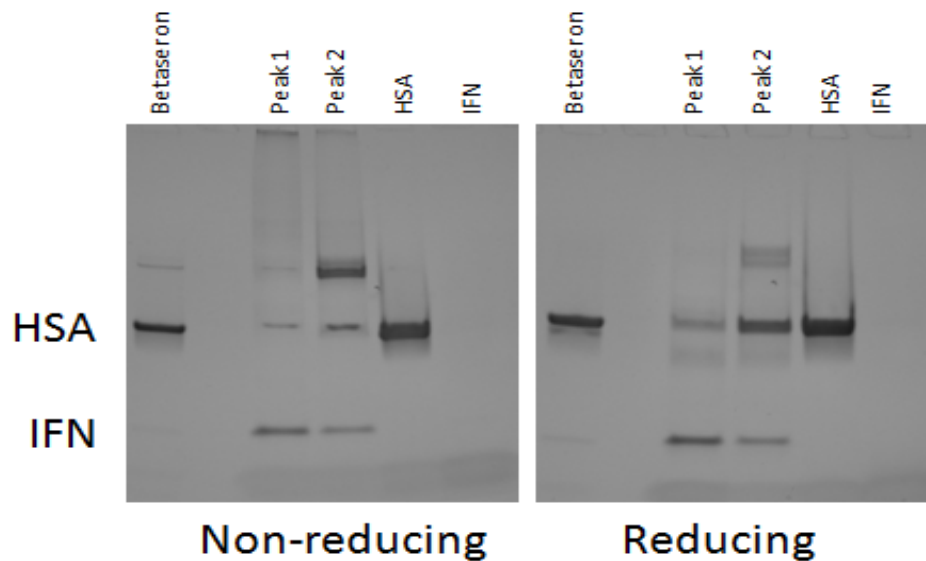
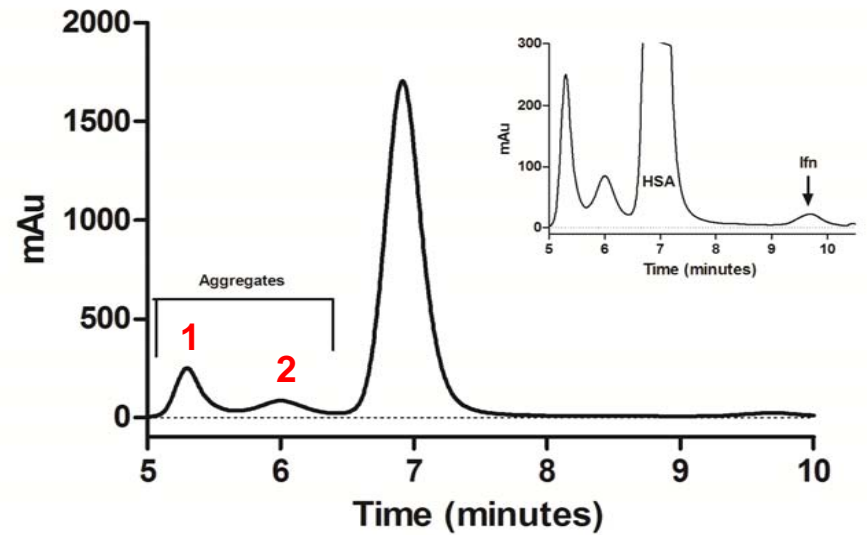


Almost no IFN monomer in Betaseron and Extavia

Betaseron

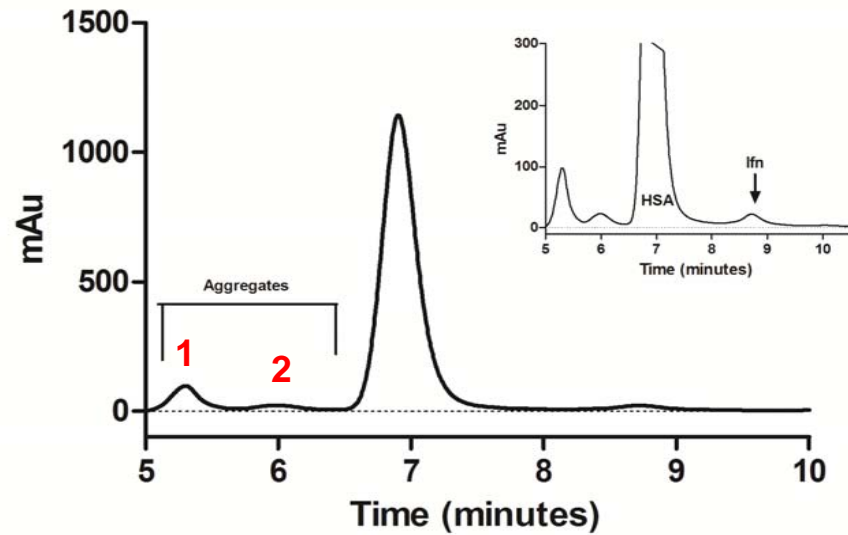


Extavia

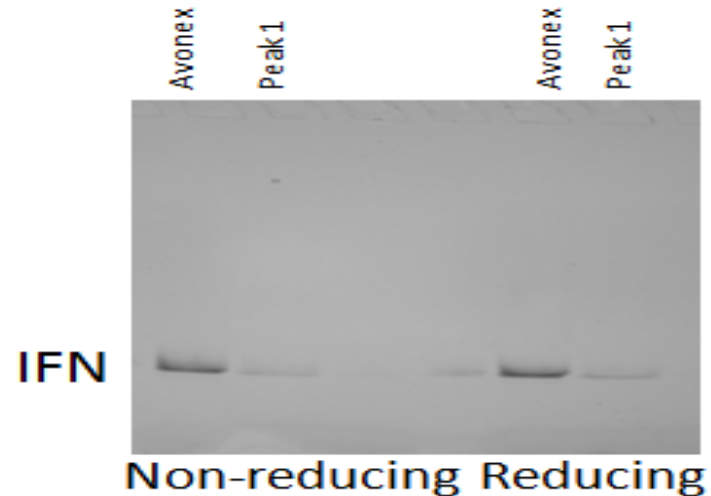
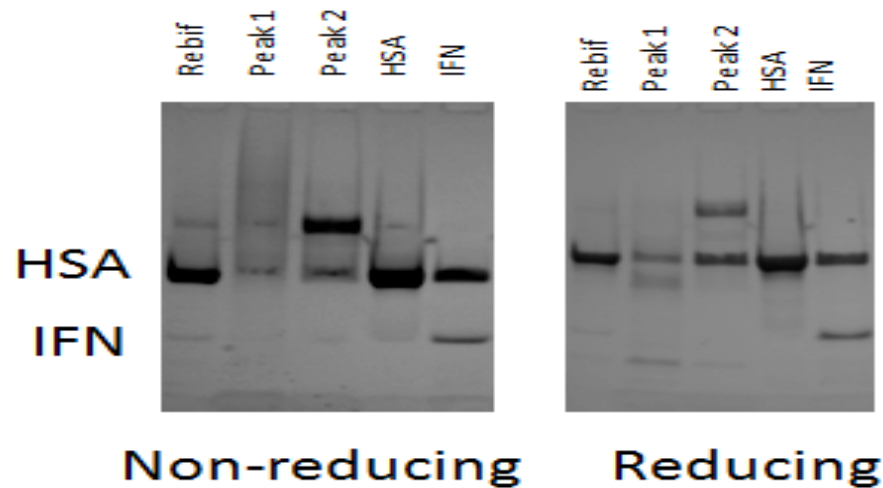
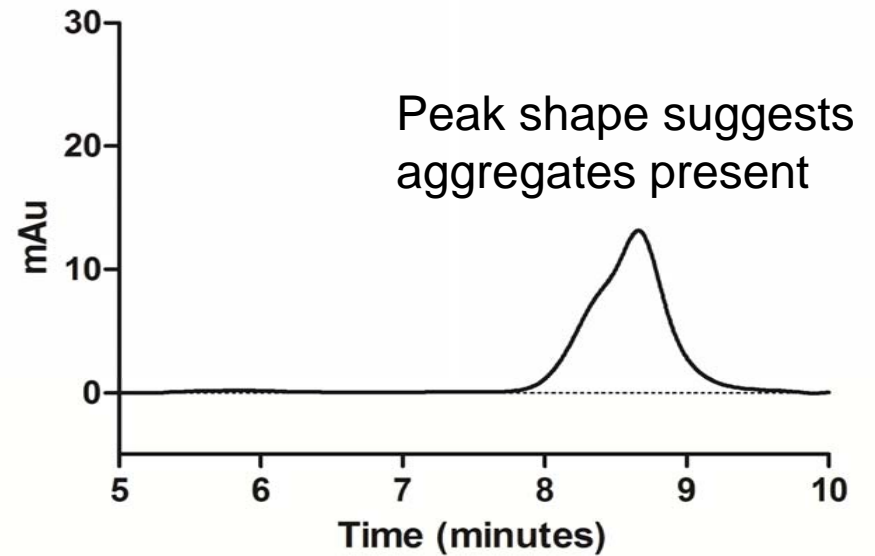


SEC and SDS-PAGE Results

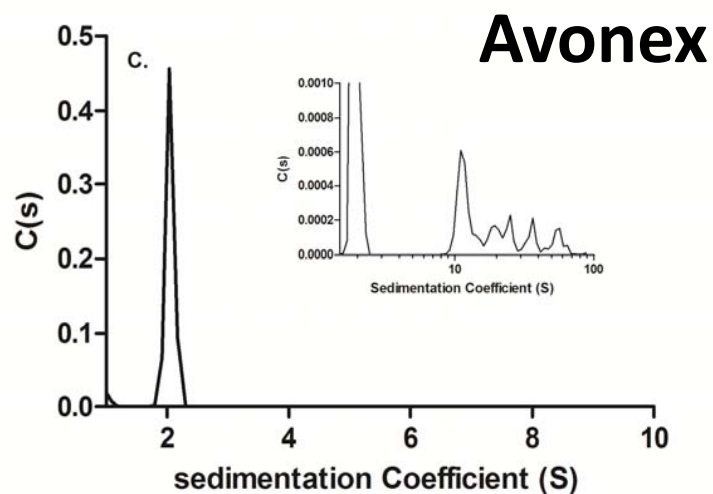
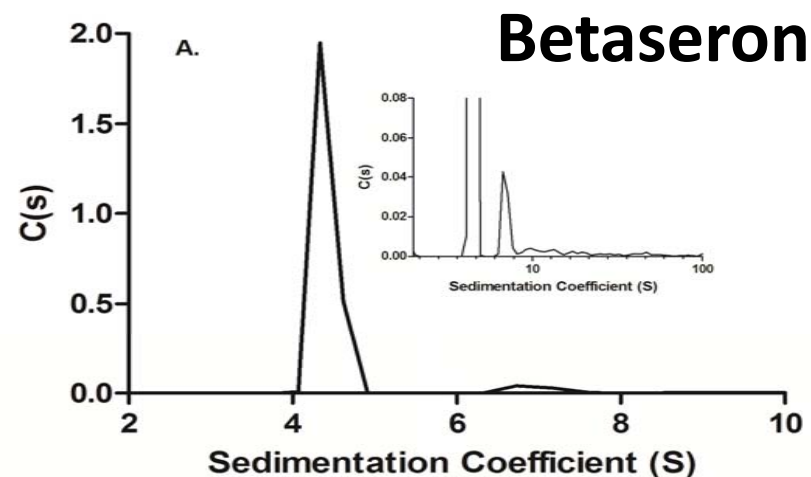
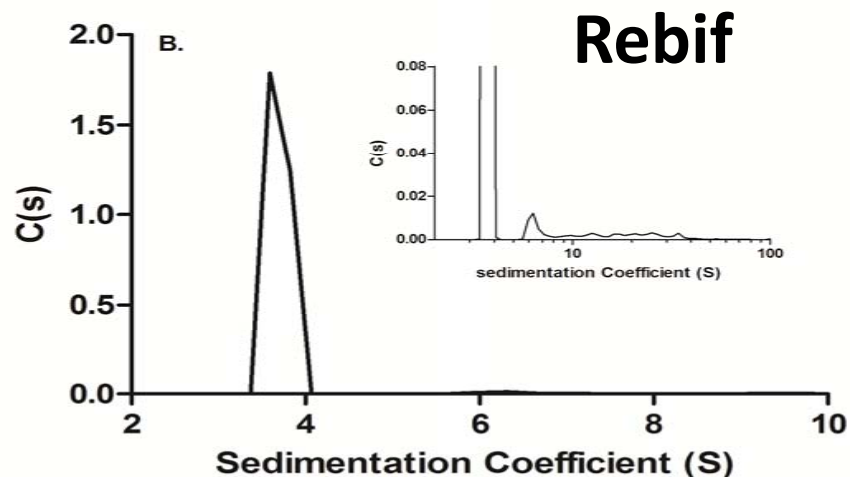
Rebif



Avonex

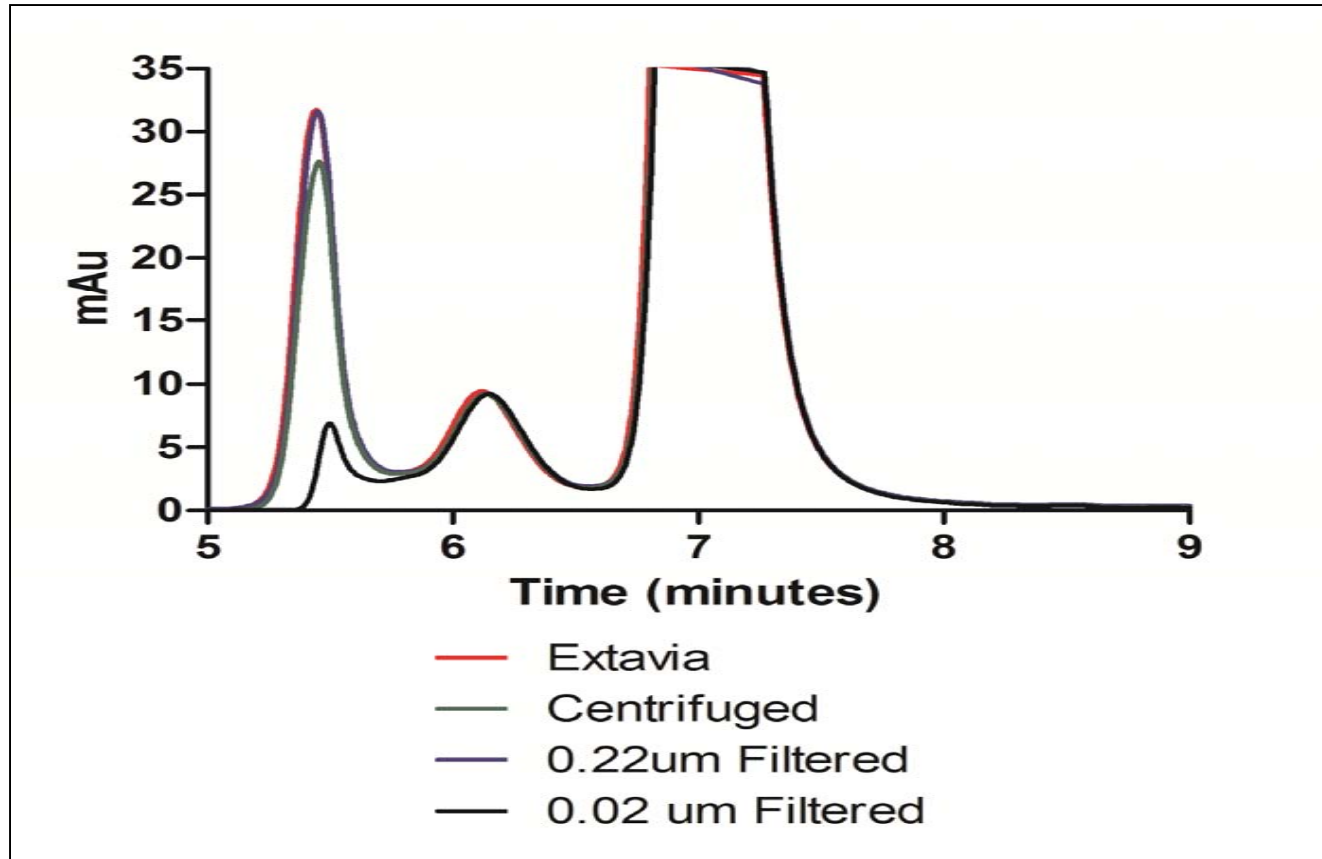


Analytical Ultracentrifugation



Product	% Aggregate SEC (pH 7 mobile phase) (n = 3, ± SD)	% Aggregate AUC (formulation)
Betaseron	14.9 ± 0.3%	14.6%
Extavia	14.5 ± 0.5%	15.5%
Rebif	8.6 ± 0.1%	10.3%
Avonex	0 ± 0%	5.5%

Filtration Prior to Performing SEC

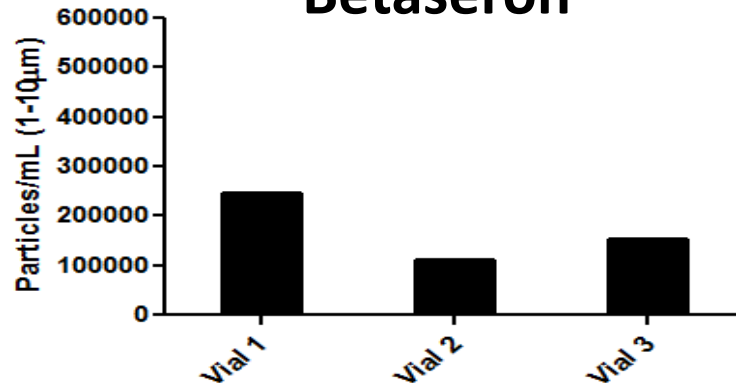


1. When Extavia is filtered with a 0.02 μ m filter prior to performing SEC, high molecular weight species eluting in the void volume are greatly reduced.
2. This result, in addition to the AUC results, indicates that these aggregates are present in the vial & not induced by SEC method.

Particle Counts by Microflow Imaging (>1 μ m)

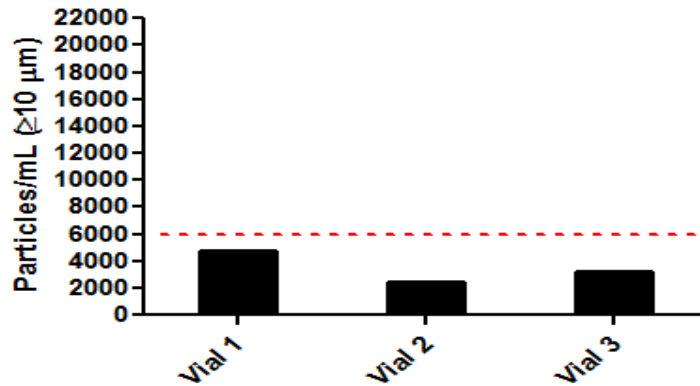
Betaseron

1-10 μ m

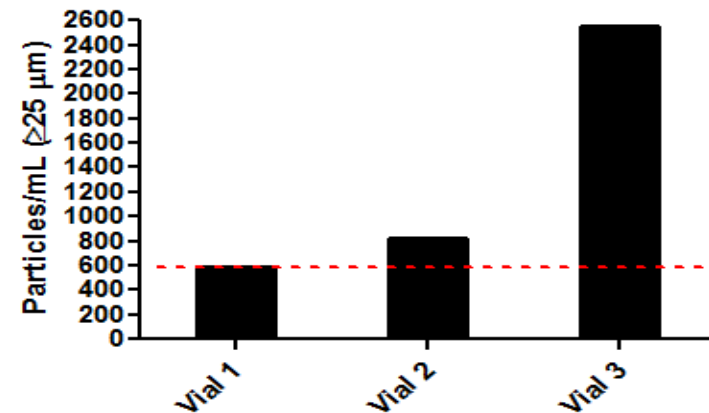
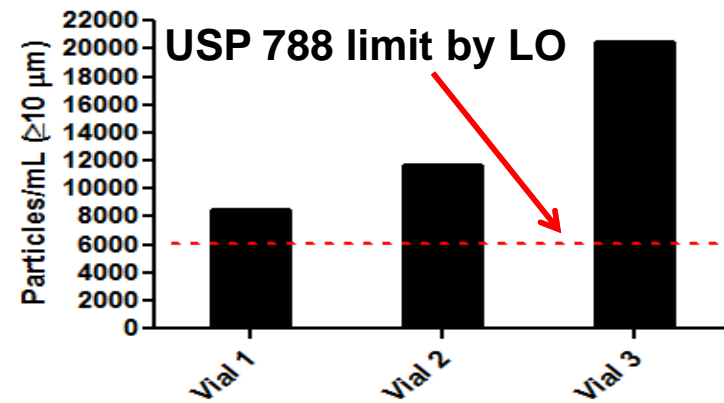
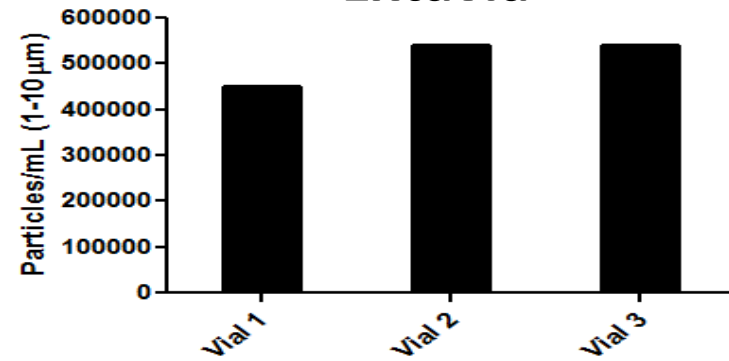
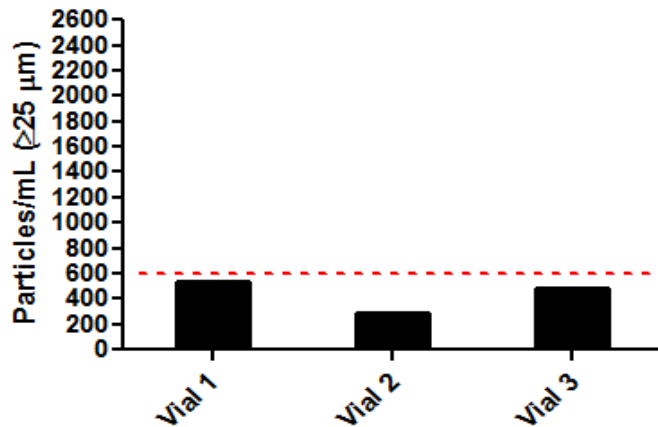


Extavia

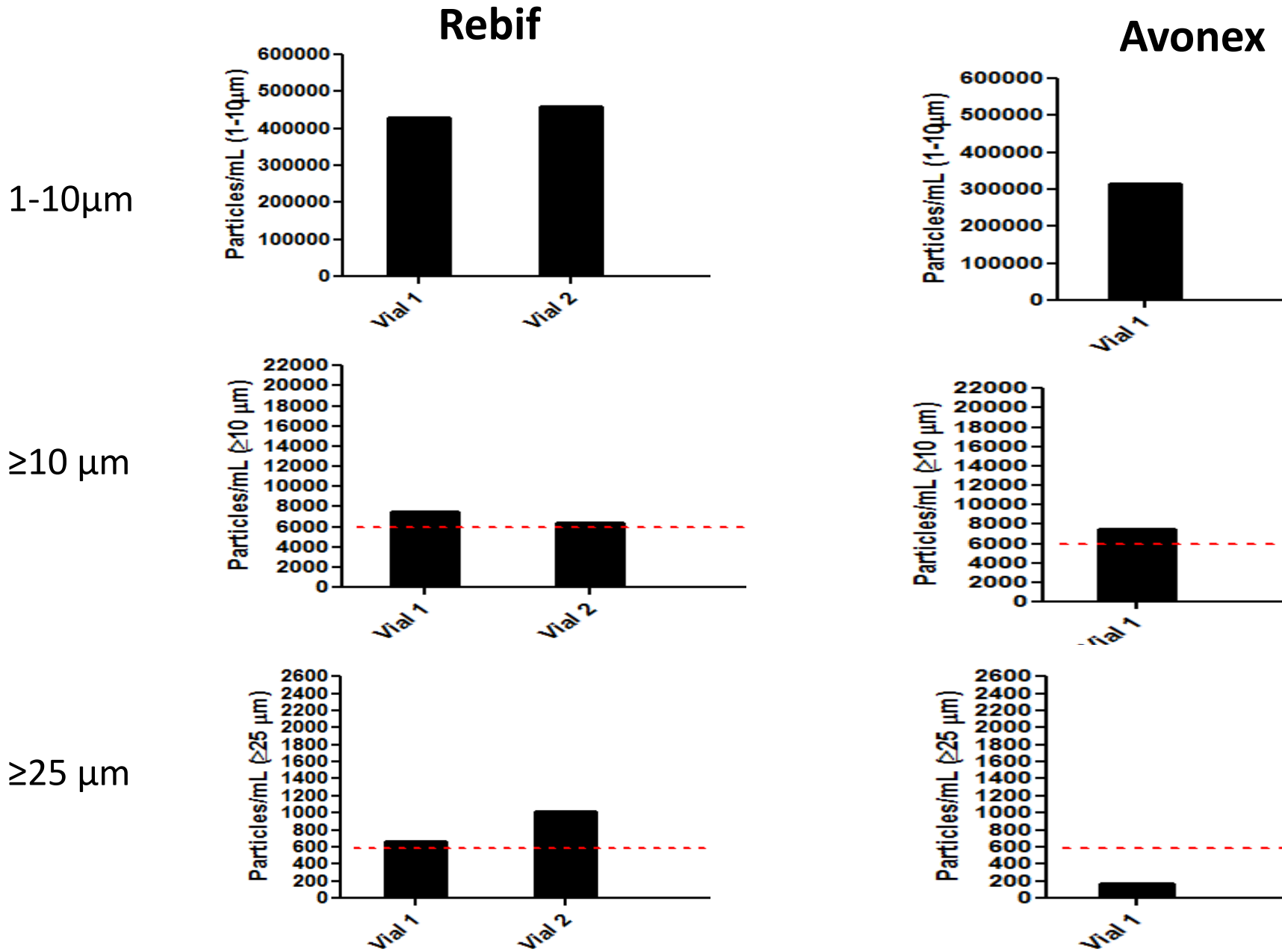
$\geq 10 \mu$ m



$\geq 25 \mu$ m



Particle Counts by Microflow Imaging (>1 μm)

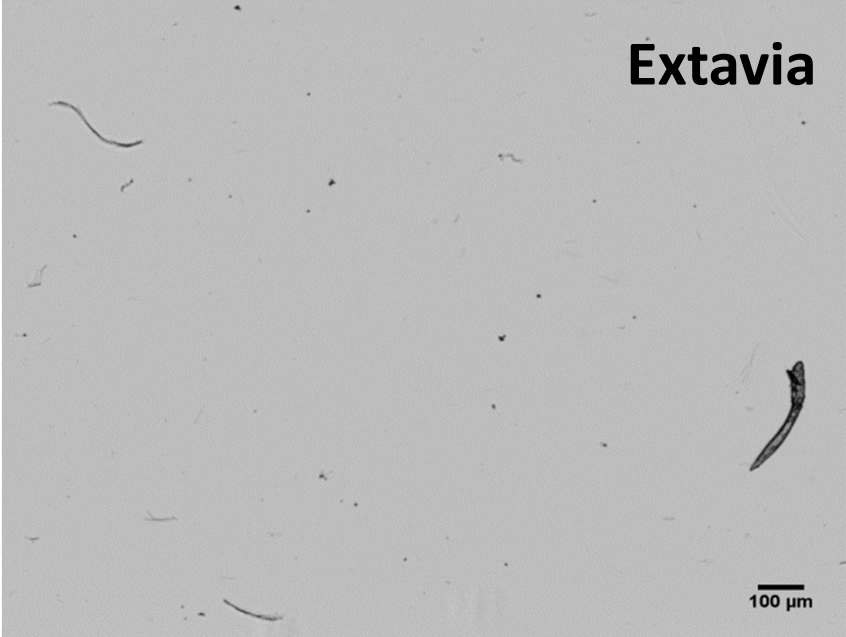


Particle Morphology

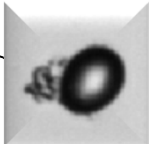
Betaseron



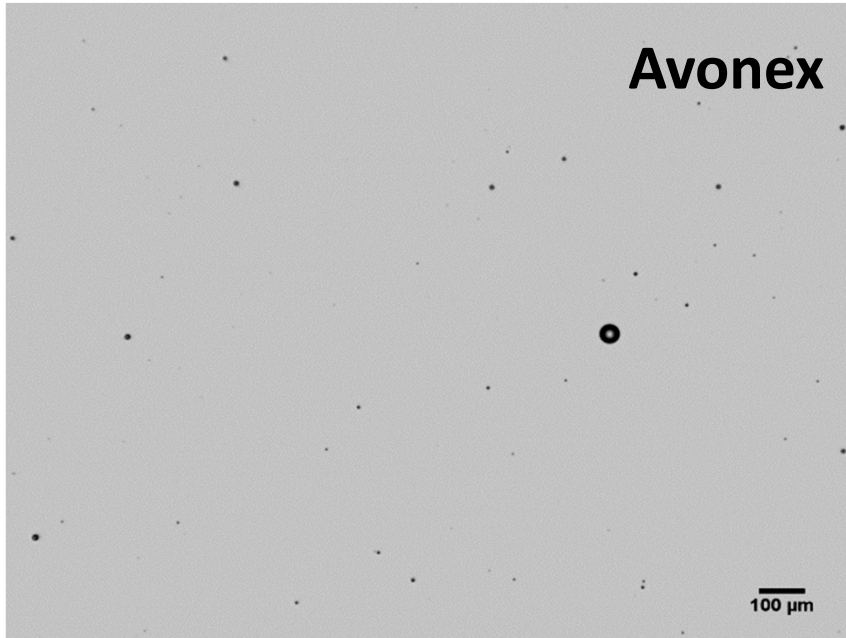
Extavia



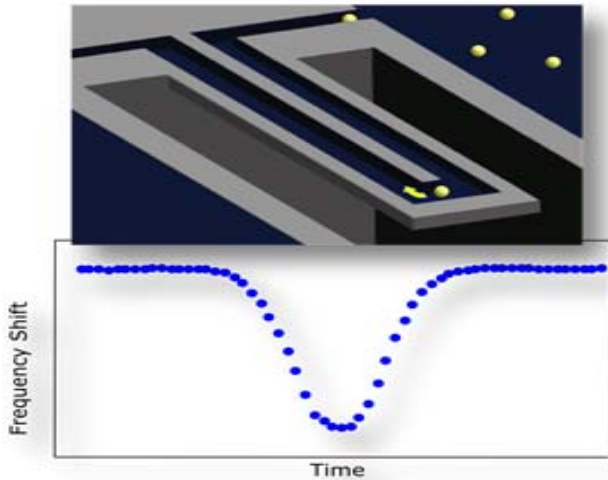
**Rebi
f**



Avonex



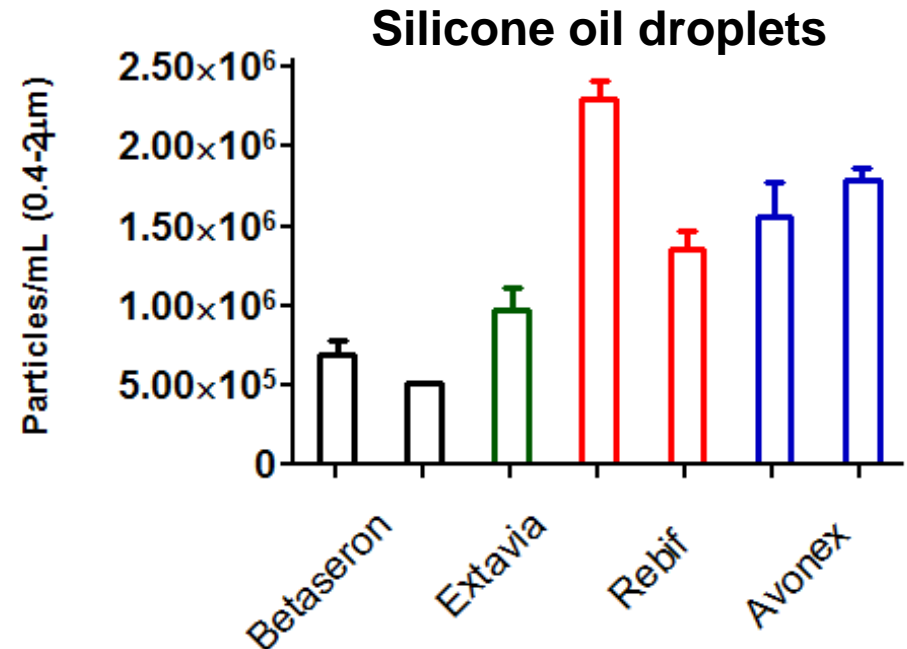
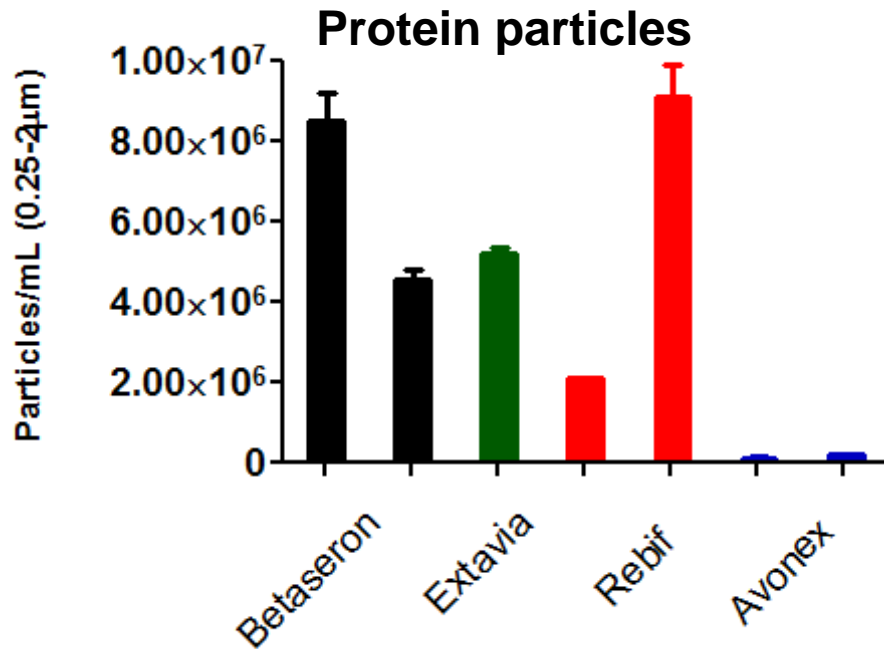
Affinity Biosensor (0.3-2 μ m) particles



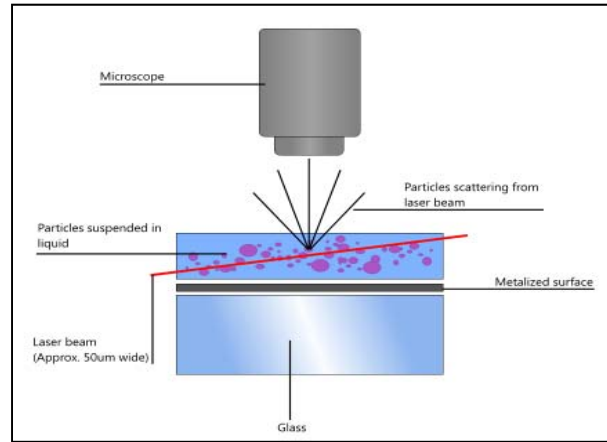
Silicone oil signal



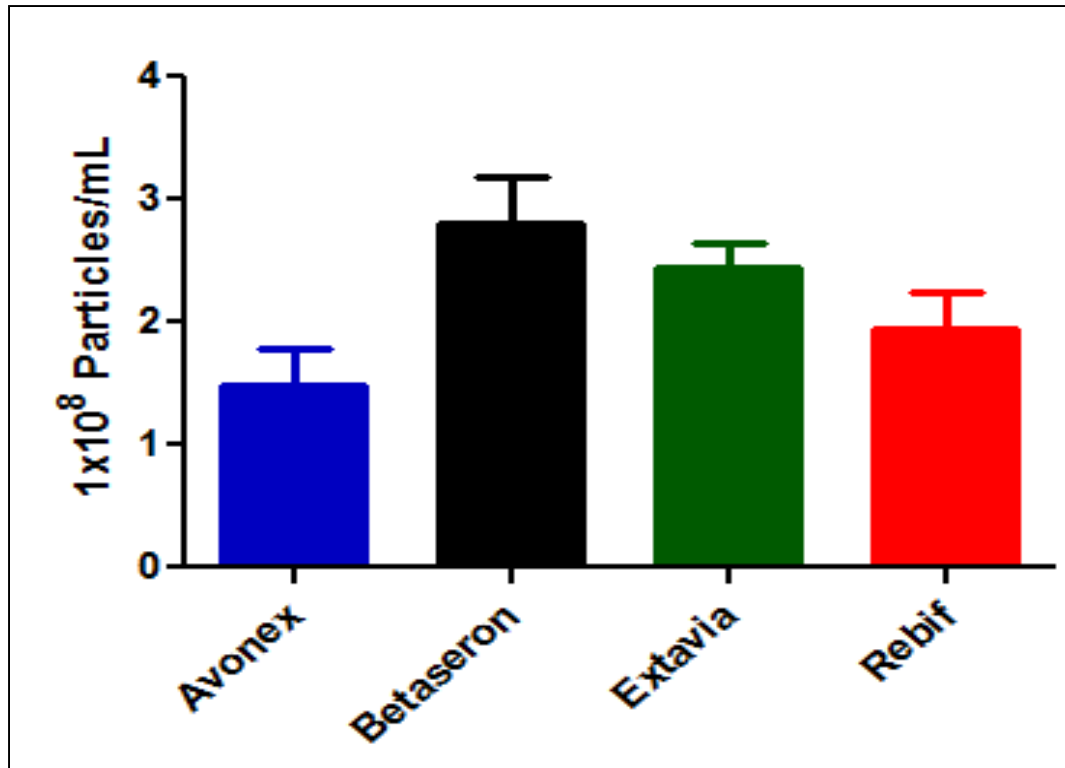
Protein signal



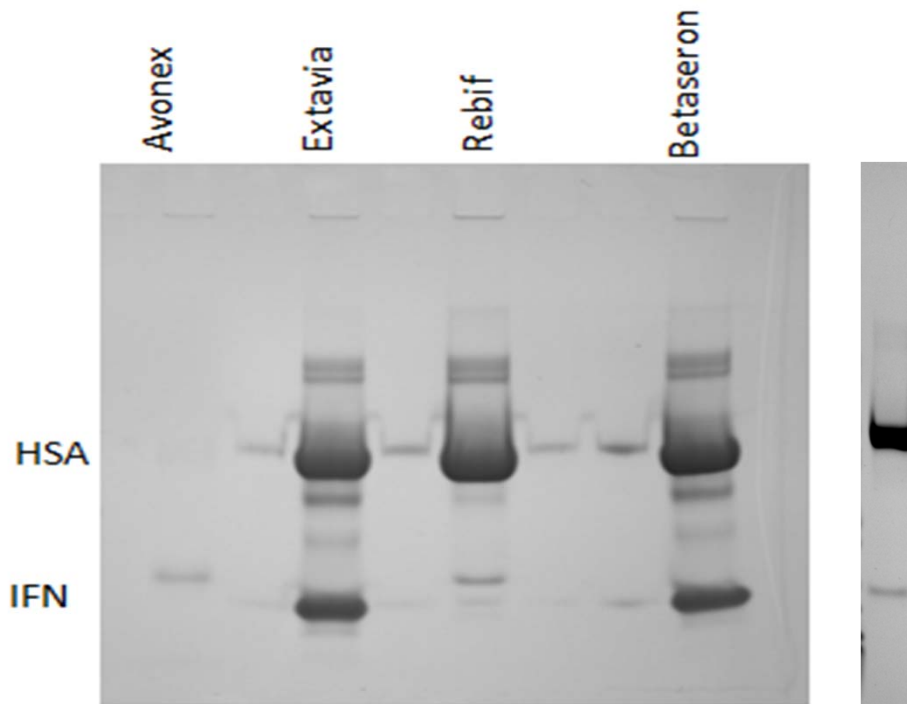
Nanosight (~70-450 nm) particles



Total Particles/mL



Centrifugation/SDS-PAGE of Pellet



1. 400 μ L of sample centrifuged @ 138,000g for 30 minutes.
2. Pellet solubilized in reducing SDS buffer.

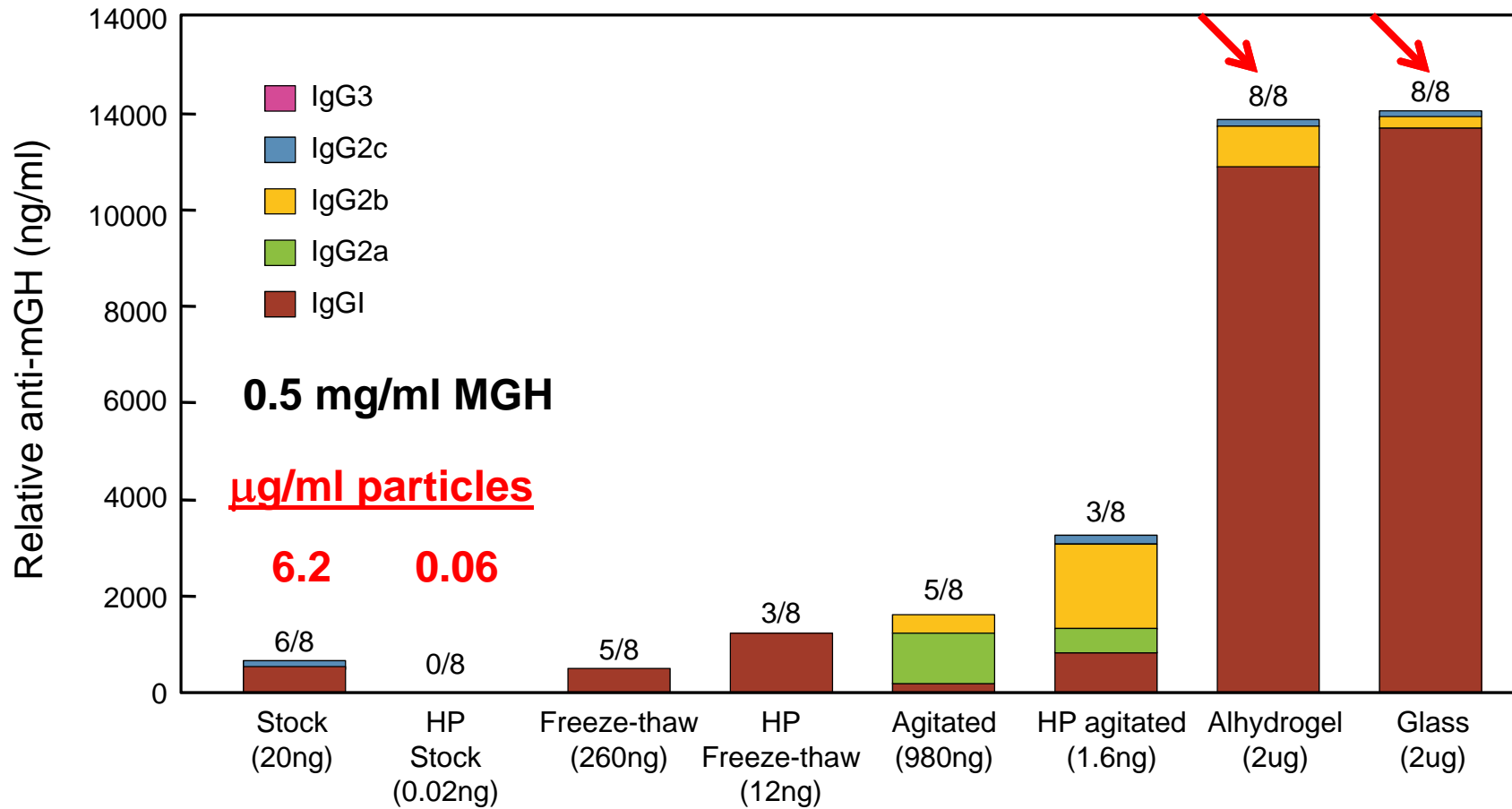
	Betaseron		Extavia		Rebif		Avonex	
Identity	Pellet	Peak 1 Fraction	Pellet	Peak 1 Fraction	Pellet	Peak 1 Fraction	Pellet	Peak 1 Fraction
%Aggregate	11.4	0	9.7	0	14.8	0	0	0
%HSA	50.3	32.9	47.9	42.3	79.6	53.0	0	0
%Fragment	7.0	13.4	7.7	14.6	1.0	47.0	0	0
%IFN	31.3	53.7	34.7	43.1	4.6	0	100	100

Conclusions from IFN- β Analyses

- 1. There is a correlation between aggregate/particle content and clinical rates of immunogenicity for the IFN- β products.**
- 2. Of course, many other factors could also play important roles in adverse immunogenicity.**
- 3. The analytical findings could have implications for follow-on biologics and for future regulatory expectations regarding particulate content.**

Should a biosimilar have the same high aggregate and particle content as the innovator product or should it meet current product quality expectations?

Particles Break Tolerance to mGH: An Adjuvant Effect



Thanks to Amy Rosenberg

Aggs (dose)

Particles as adjuvants

- Removing subvisible particles eliminates immunogenicity of “100%” monomer MGH.
- Numerous studies from 60’s and 70’s in human patients and in animals documented that removal of aggregates/particles eliminated immunogenicity and sometimes invoked tolerance to foreign proteins.

Particles as adjuvants

- Typically studies compared unprocessed sample vs. those in which aggregates/particles (often a trace amount of the product mass) were removed by ultracentrifugation or filtration.
- In other studies “cleaned up” samples were spiked with trace amounts of aggregates, which stimulated immunogenicity.

Particles as Adjuvants Equine IgG in Humans

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

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

Particles as adjuvants: Testing with current therapeutic protein products

- Purchase therapeutic protein product from pharmacy
- Characterize and quantify aggregates and particles
- As needed, develop and confirm assay protocols (e.g., SEC method)
- Develop ultracentrifugation or filtration protocol to remove aggregates and/or particles.
- In mice, compare immunogenicity of untreated product with that treated to reduce aggregates/particles
- Present and publish results
- Repeat with the next therapeutic protein product

Conclusions

- Particles serve as adjuvants and promote immunogenicity
- Protein particle formation is ubiquitous in the production, shipping, storage and delivery of therapeutic proteins.
- Subvisible particles are critical species on protein aggregation pathway and are in all therapeutic protein products.
- How do we minimize patient exposure to particles? (e.g., could filters be developed for subcutaneous injection?)

Invitation from *Journal of Pharmaceutical Sciences*

- Please consider writing Commentary or Review for the *Journal*
- For example, Commentary on timely issue for the industry
- For example, Review with critical assessment of important area
- Topics and submission dates are open.
- Please contact me:
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