Advances in the detection and characterization of subvisible and visible particles and other aggregates

Andrea Hawe

7th Open Scientific EIP Symposium on Immunogenicity of Biopharmaceuticals

*23rd to 25th February 2015 * Lisbon/Portugal*

Coriolis Pharma
Biopharmaceutical Research and Development Service

Background of SVP analysis at Coriolis Pharma

Biopharmaceutical Research and Development Service

company

privately held,
independent service
provider
established in 2008

people

interdisciplinary team of
highly qualified scientists
35 FTE, all academic,
70% with PhD

science

expert scientific board:
Prof. Dr. G. Winter
Prof. Dr. W. Friess
Prof. Dr. W. Jiskoot
Prof. Dr. J. Carpenter
Prof. Dr. C. Schöneich

techniques

innovative analytical and
technical equipment,
focus aggregate and
particle characterization

research

cutting edge research in
the field of protein
sciences with top
publications

area

biopharmaceutical
products: NBEs, protein,
mAbs, peptides, vaccines,
oligonucleotides, colloidal
systems, NCEs

Overview of Coriolis' services and key expertise

- Science driven formulation development for biopharmaceuticals
- Lyophilization cycle development and scale-up
- Forced degradation studies and stability testing
- Contract analytical services for biopharmaceuticals
- cGMP analysis of aggregates and subvisible particles

Aggregates and particles: a heterogeneous mixture

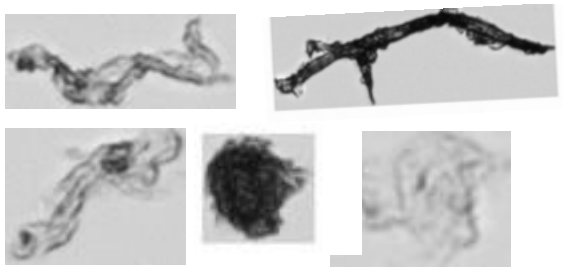
aggregate = assembly of protein molecules

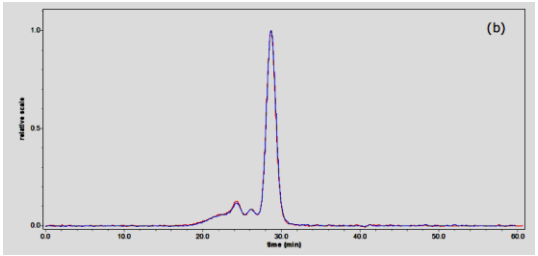
(other than the native quaternary structures)

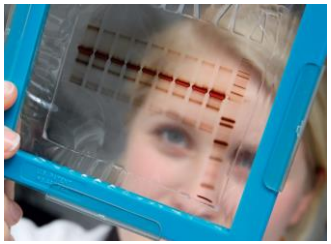
- nm-sized to µm/mm-sized
- soluble ↔ insoluble
- covalent ↔ noncovalent
- ordered ↔ disordered
- reversible ↔ irreversible

Nomenclature	Size range
Oligomers	10 to 100 nm
Submicron aggregates: nanometer aggregates	0.1 – 2 µm
Subvisible particles: micron aggregates	1 – 100 µm
Visible particles	> ~ 100 µm

Narhi et al. (2012) J Pharm Sci 101 (2): 493-498







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(Subvisible) particles --- the topic remains hot

Pharm Res
DOI 10.1007/s11095-014-1541-x

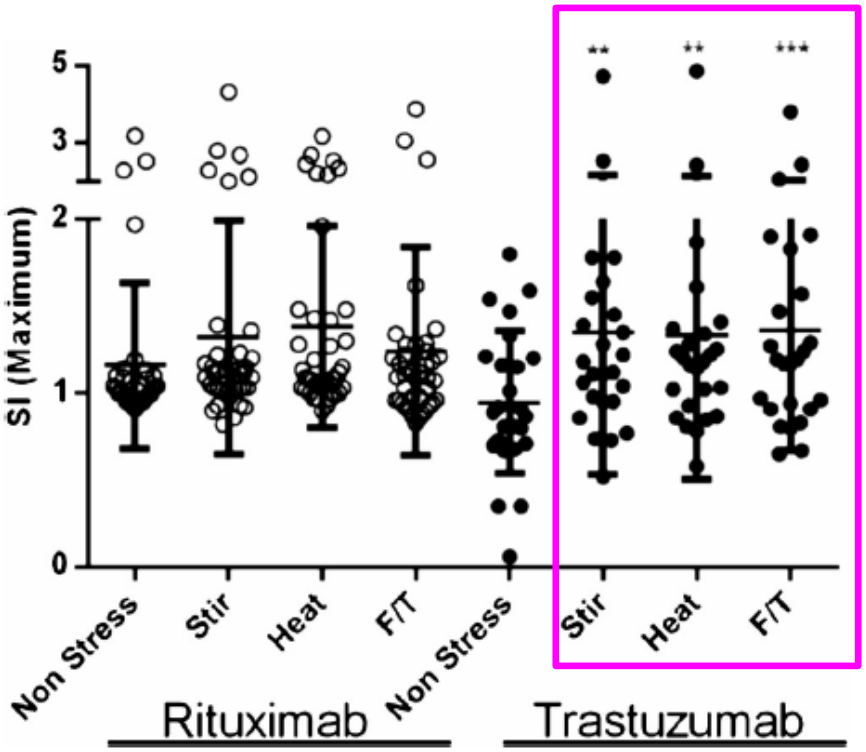
RESEARCH PAPER

Small Amounts of Sub-Visible Aggregates Enhance the Immunogenic Potential of Monoclonal Antibody Therapeutics

Maryam Ahmadi • Christine J. Bryson • Edward A. Cloake • Katie Welch • Vasco Filipe • Stefan Romeijn • Andrea Hawe • Wim Jiskoot • Matthew P. Baker • Mark H. Fogg

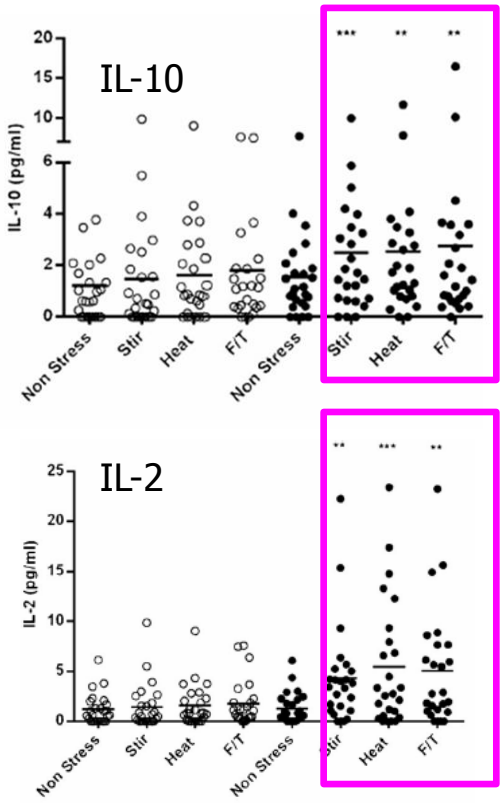
(Subvisible) particles --- the topic remains hot

Proliferation of CD4+ T cells



*** p< 0.05** p< 0.01; *** p< 0.001**

Cytokine production by CD4+ T cells



IL-1b, IL-6, IL-8 and IL-12 levels were unchanged

Ahmadi et al. (2014) Pharm Res, in press

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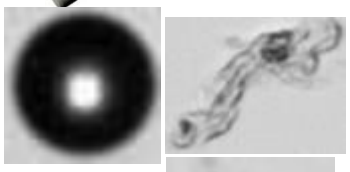
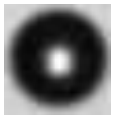
Subvisible particles --- the topic remains hot





- **Regulatory agencies** expect more than data for particles > 10 µm and > 25 µm (LO) and visual inspection
- Change of compendial approaches (e.g. **new USP <787>** allowing low volume methods for light obscuration)
- **Emerging techniques** open new possibilities and insights into products and processes (e.g. trouble shooting)
- Increasing need for particle identification for all size classes: e.g. differentiation **proteinaceous ↔ non-proteinaceous**

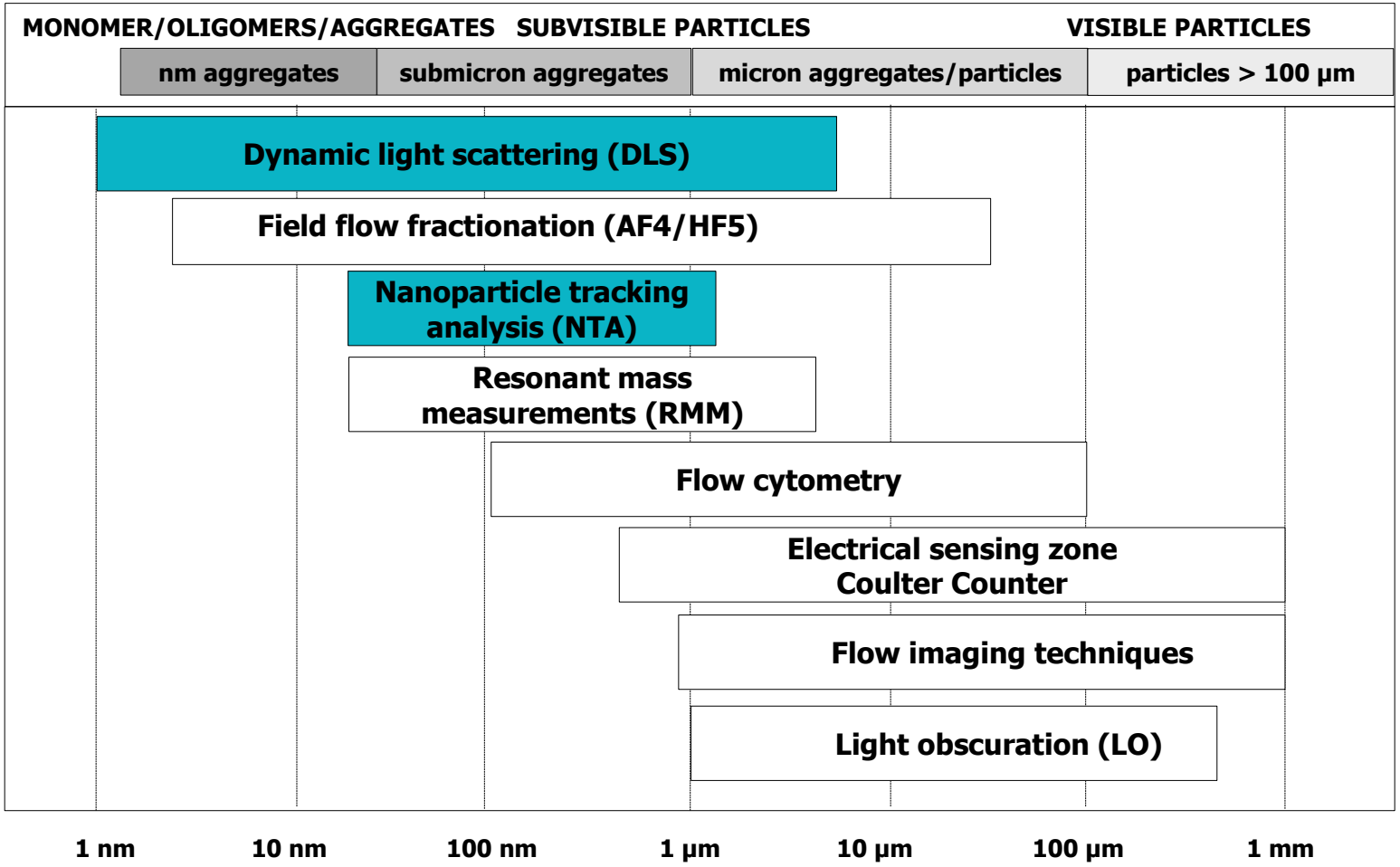
FDA expectations on aggregate and SVP analysis

- Quantification of aggregates < 0.2 µm
- Qualitative analysis of SVP from 0.2 µm to 2 µm
- Quantification of SVP from ~ 2 µm to 10 µm and characterization for shape, type (protein, silicone oil), size-distribution

- Analysis of samples from different batches, forced degradation studies, stability studies, shipment studies
- Risk assessment, including those studies, in combination with clinical data and development of a control strategy
- FDA does not have a preferred method; orthogonal methods should be used whenever possible (e.g. HP-SEC to be confirmed by AUC)

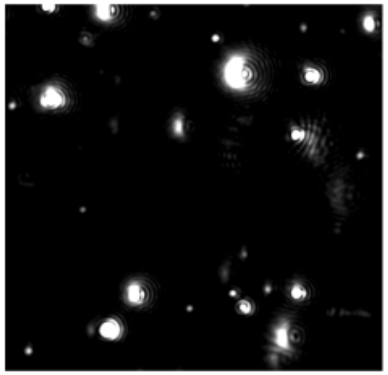
Susan Kirshner, Presentation, 2012
http://www.aaps.org/uploadedFiles/Content/Sections_and_Groups/Focus_Groups/Protein_Aggregation_and_Biological_Consequences/PABCFG_Kirshner2012.pdf

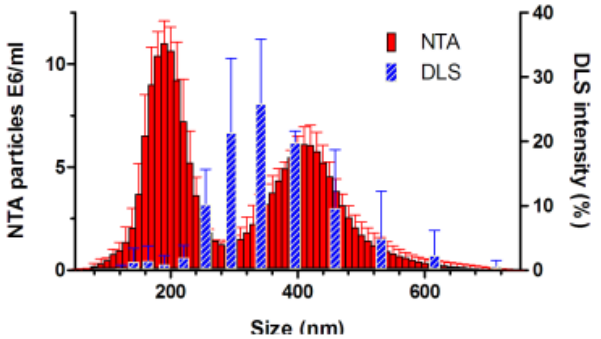
Methods for submicron particles

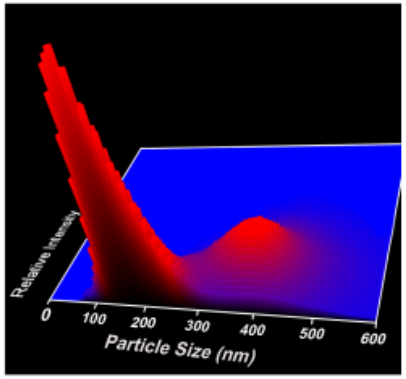


DLS vs. NTA: mixture of beads

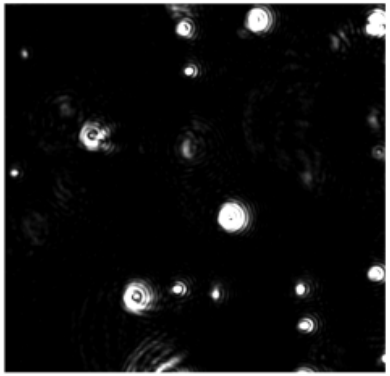
c) 200 and 400 nm beads Number ratio: 2:1

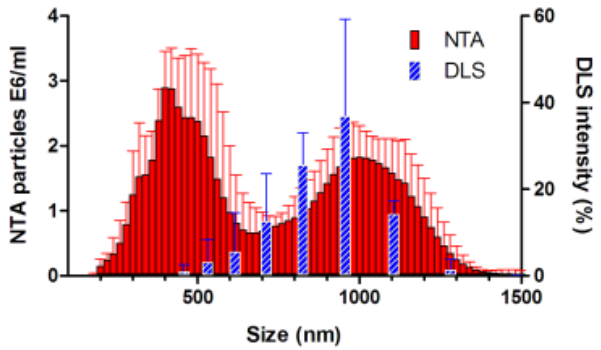


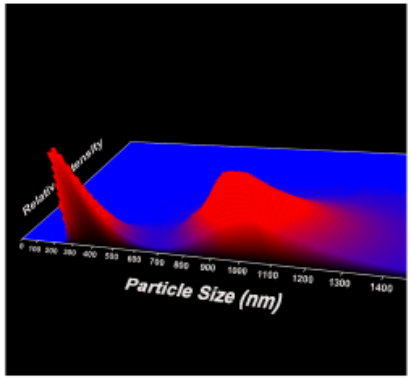




d) 400 and 1000 nm beads Number ratio: 1:1







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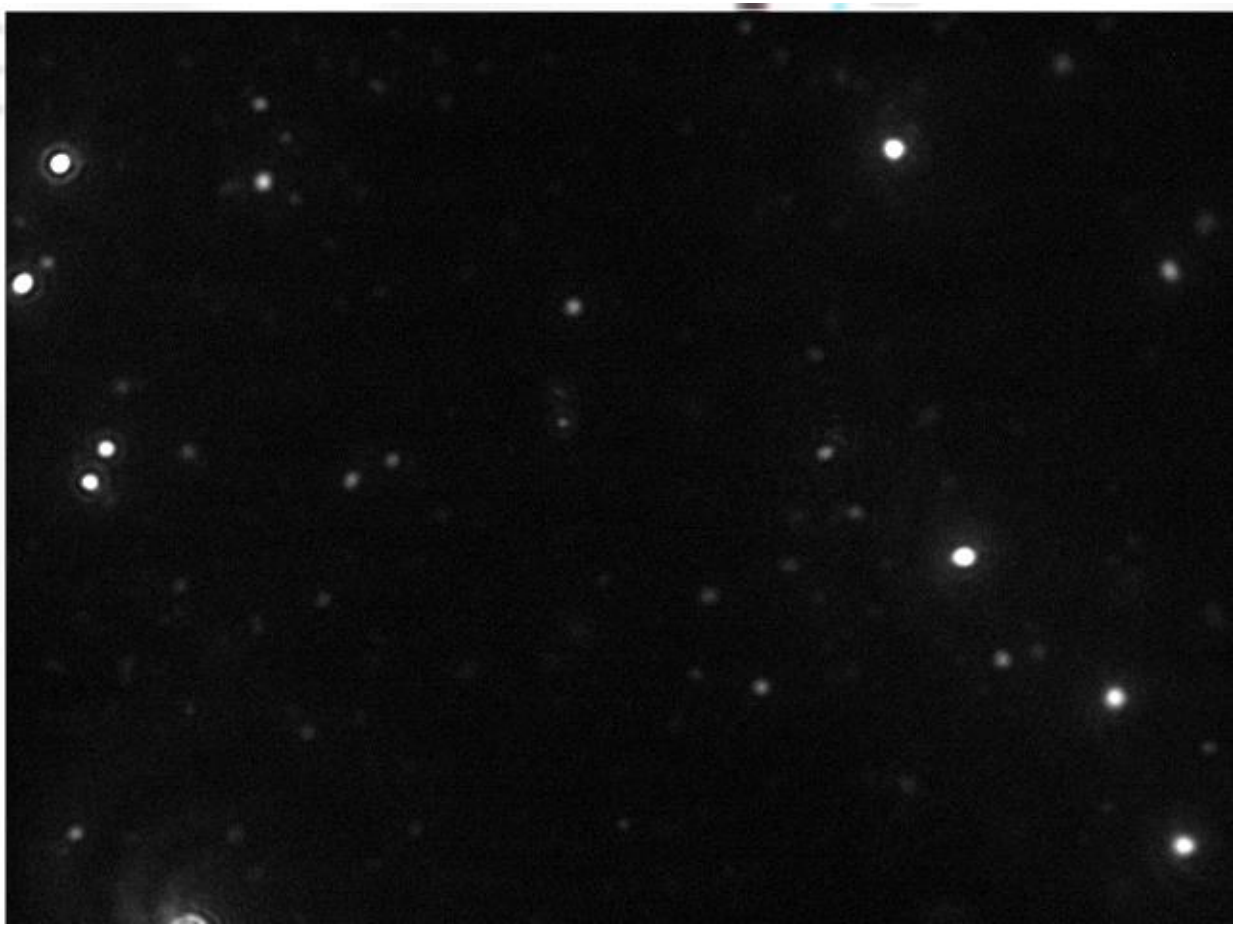
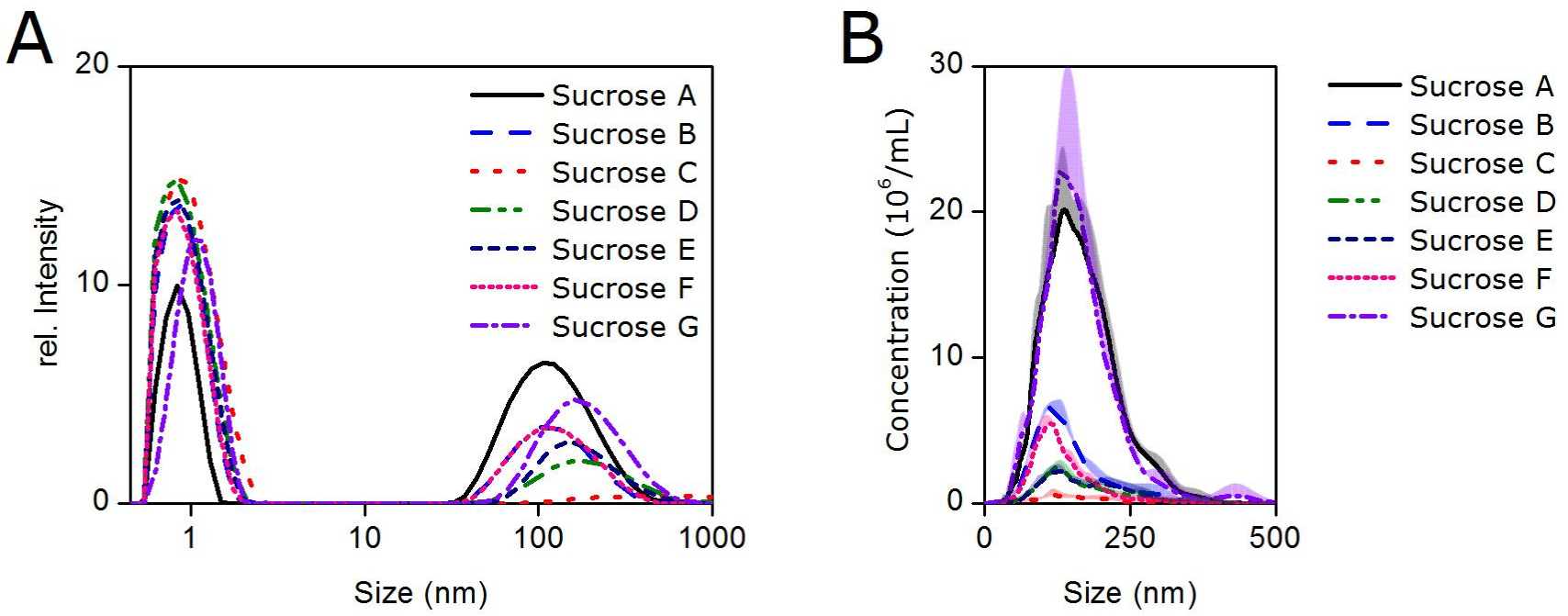
Filipe et al. (2010) Pharm Res 27:314-326.

Interference of sugar in NTA and DLS

2% (w/v) of sucrose in NTA



Sugar nanoparticles disturb DLS and NTA analysis



- Sucrose (and other sugars) show a signal at 100 - 200 nm in DLS and NTA
- Up to 10^{10} particles in 1 g sugar (Ph.Eur. grade)
- Differences between individual batches observed

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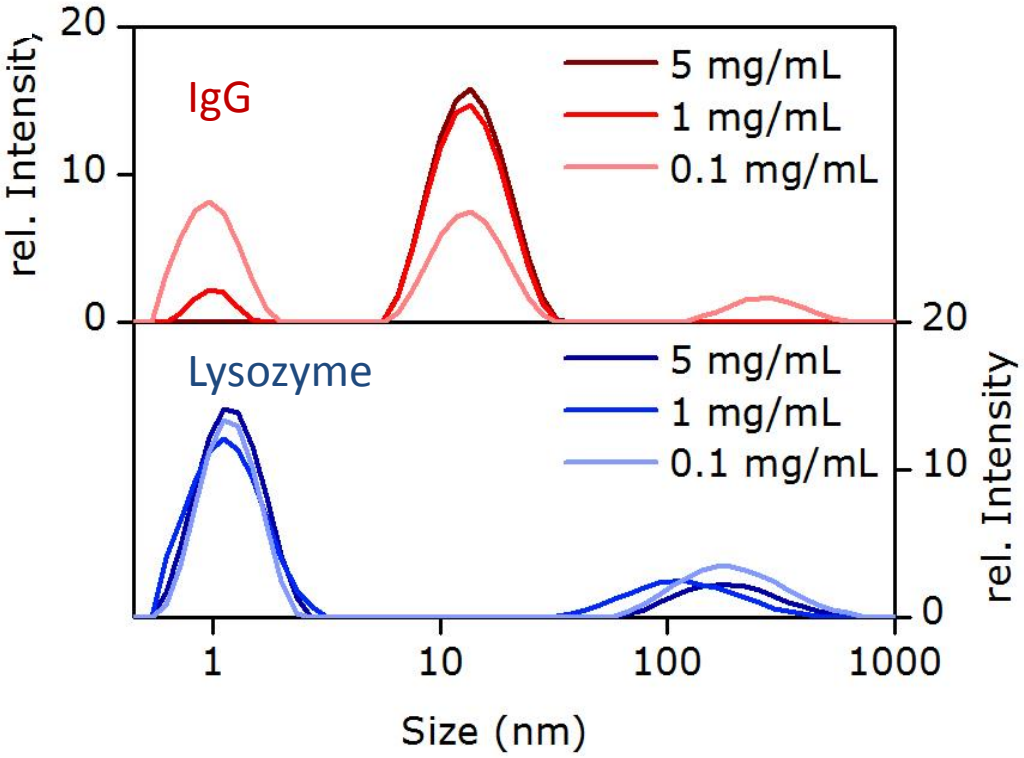
Weinbuch et al. (2015), Pharm Res, in press

Sugar nanoparticles interfere with protein analysis

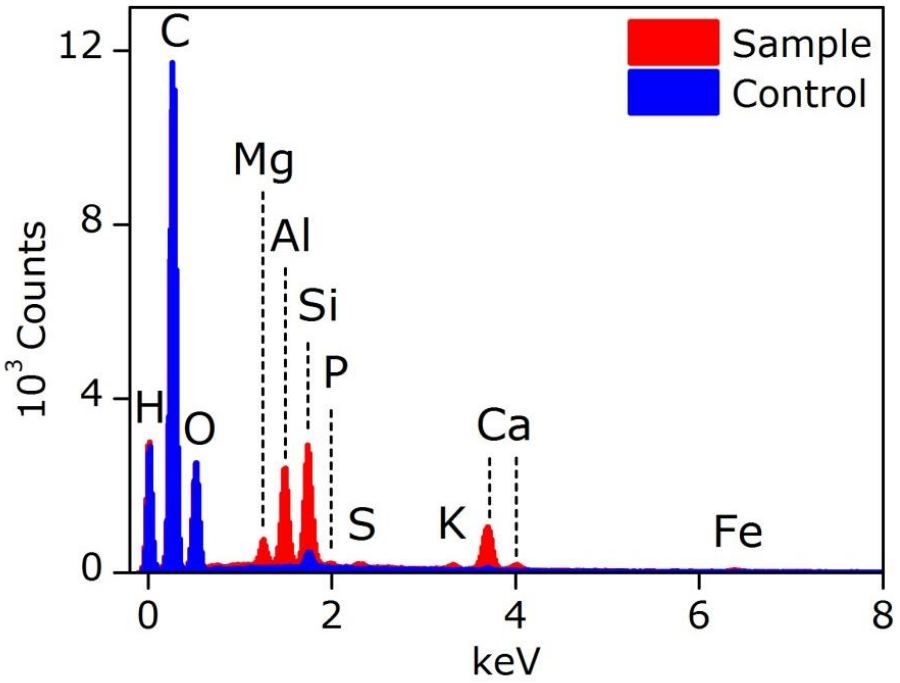


→ Sugar nanoparticles (signal at 1 nm and 100-200 nm) can falsely be interpreted as protein particles

Weinbuch et al. (2015), Pharm Res, in press

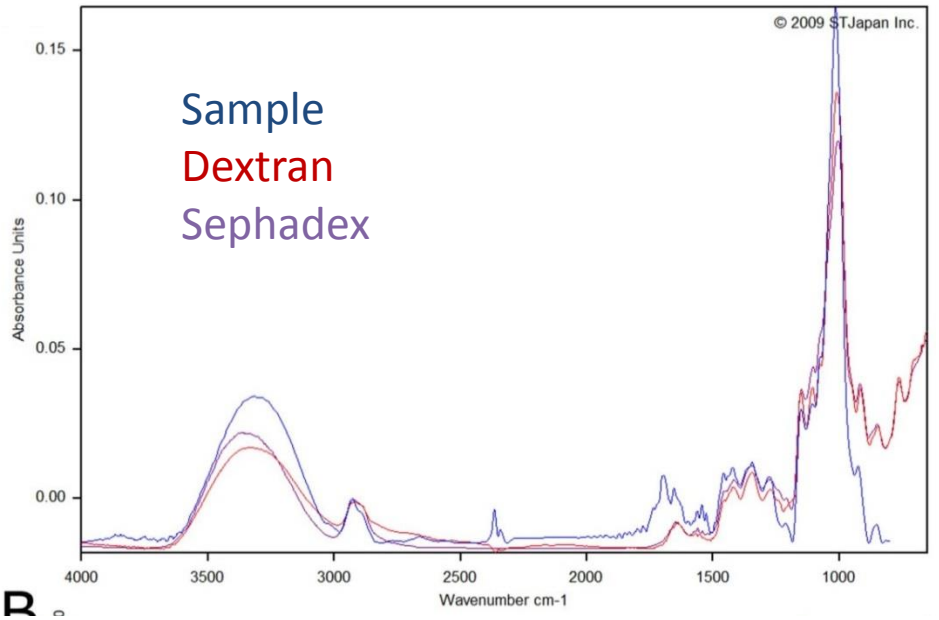
Isolation & characterization of sugar nanoparticles

SEM-EDX



→ minerals and metals

FTIR-microscopy



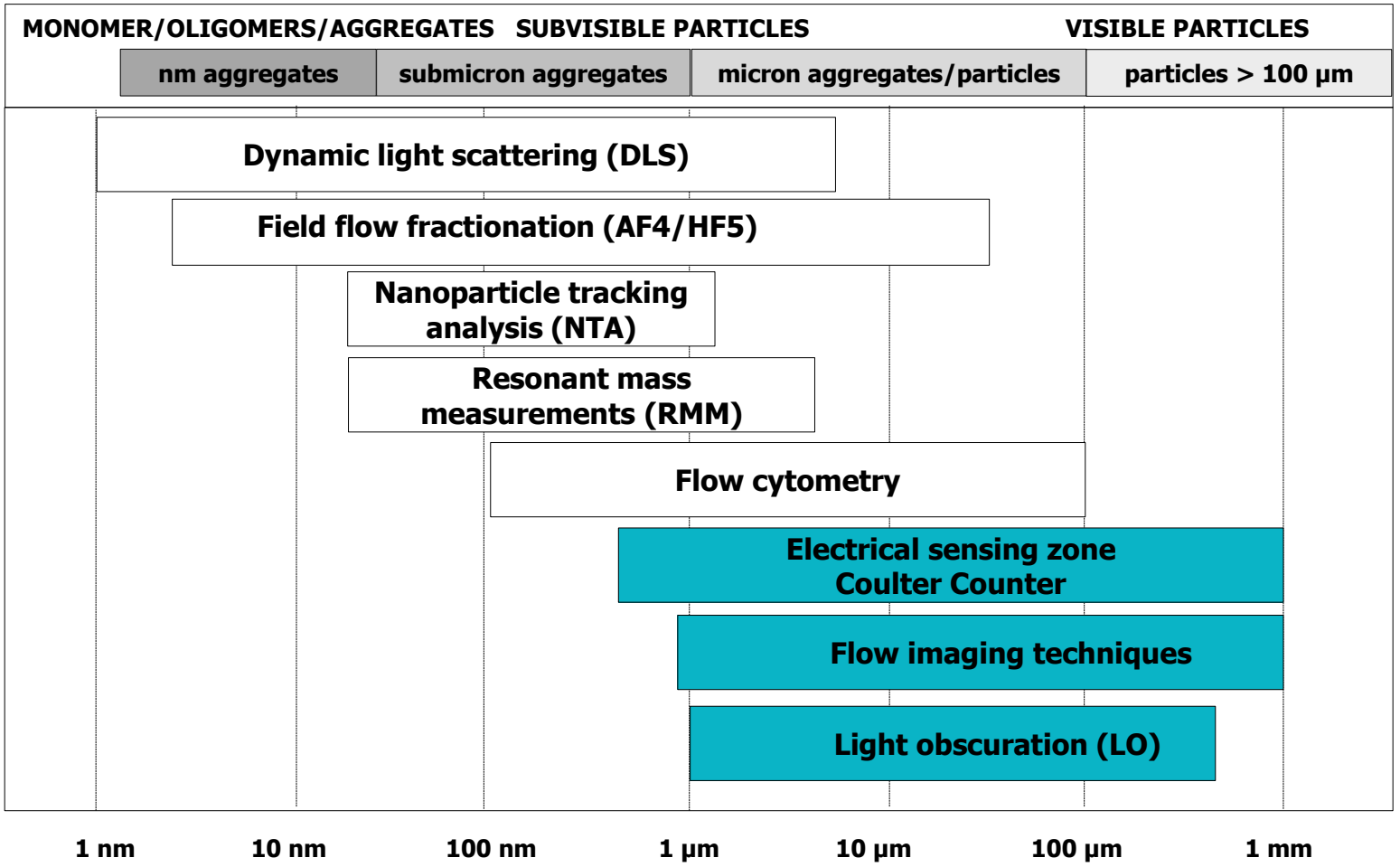
→ long chain and cross-linked dextran

Weinbuch et al. (2015), Pharm Res, in press

Summary nanoparticles in sugars

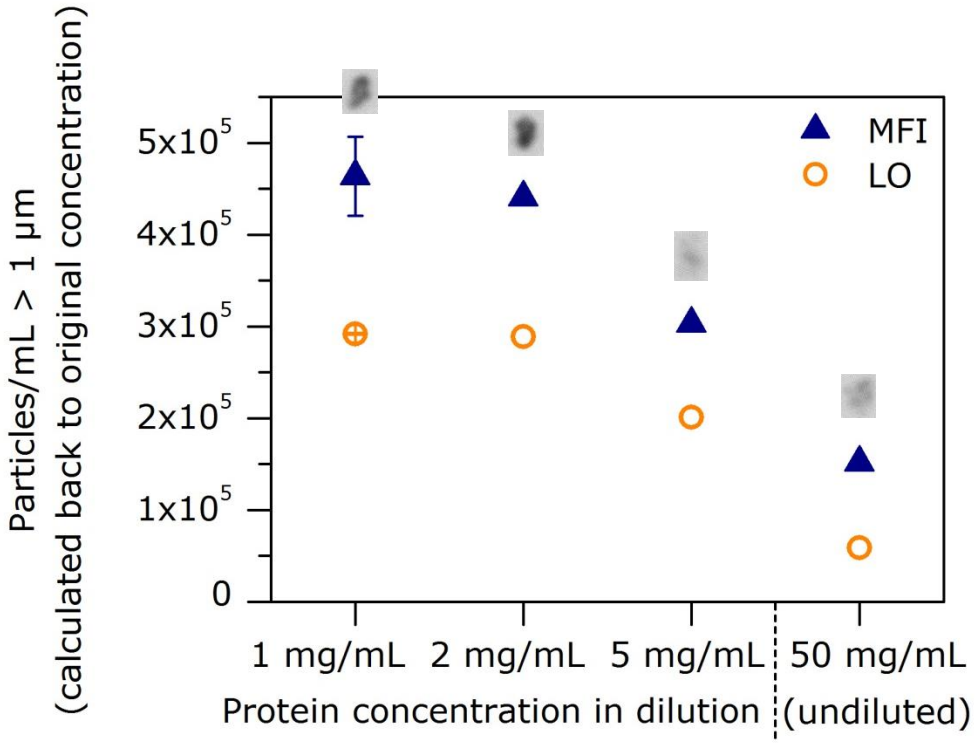
- Pharmaceutical grade sugars (e.g. sucrose, trehalose) used as excipients in protein formulations contain nanoparticles (size 100-200 nm)
- Amount can vary significantly between suppliers, as well as between production batches
- Nanoparticles found in sucrose are agglomerates of a variety of impurities (dextran like structures, ash and aromatic colorants)
- Consequences for protein formulations
  - Disturbance of analytics (DLS, NTA, MALLS)
  - **Influence on protein stability, e.g. aggregation, oxidation etc?**
    → study ongoing at Coriolis

Methods for micron particles



RI as main challenge for light based techniques

- Significant underestimation of particle concentration in pharmaceutically relevant formulations when $RI_{formulation}$ > 1.35
  e.g. 10% sugar, 100 mg/ml protein or 5% sugar + 50 mg/ml protein



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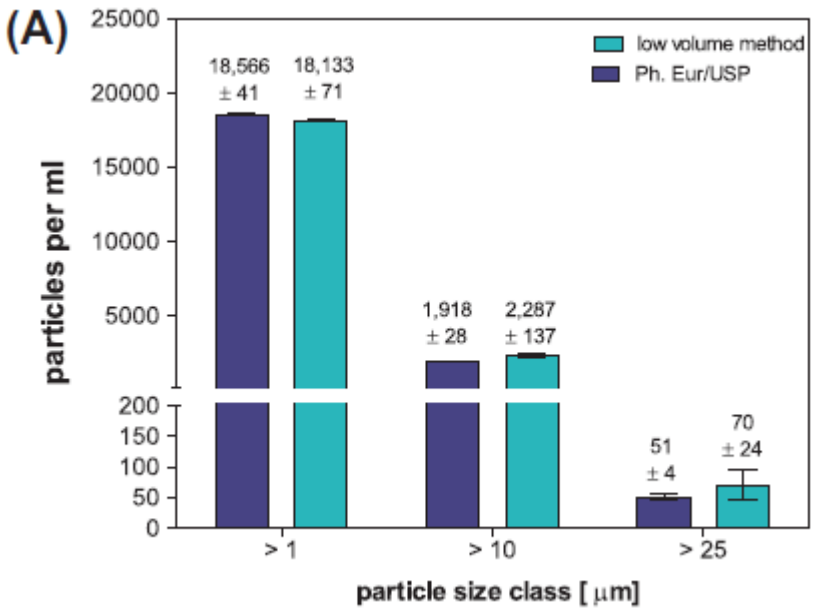
Zölls et al. (2013), J Pharm Sci, 102:1434-1446

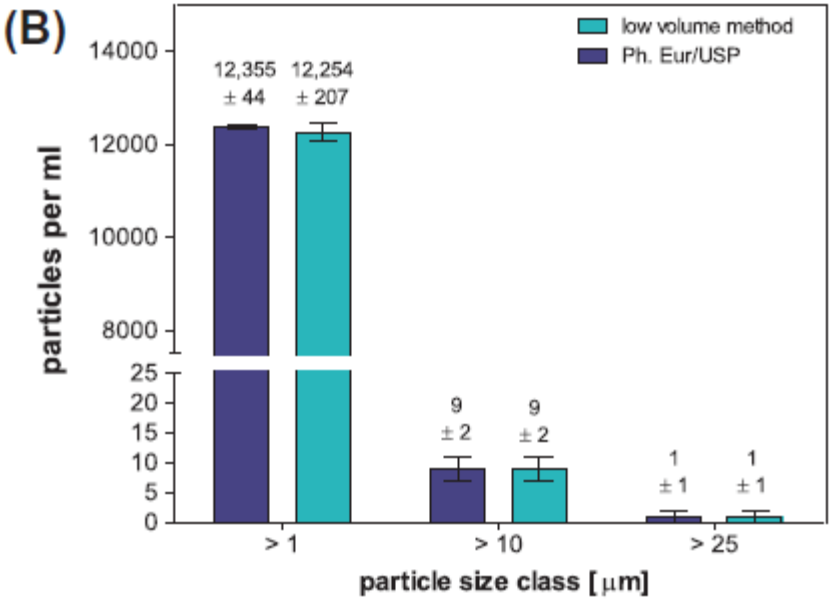
Low volume light obscuration analysis: USP <787>

- USP <787> : measurement of subvisible particulate matter in therapeutic protein injections: measurement of 4 x 0.2 ml to 5 ml allowed instead of 4x 5 ml
- Educational chapter USP <1787> will be available soon

Mixture of standard beads



IgG formulation



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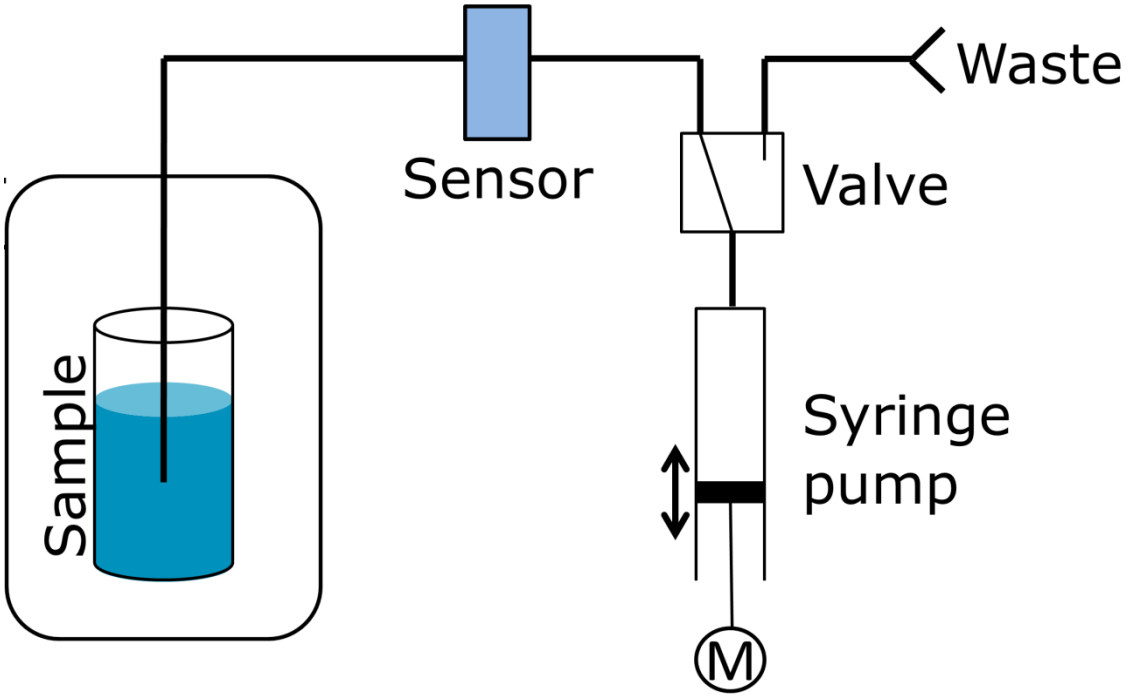
Hawe et al. (2013) EJPB 85(3 Pt B):1084-1087

Light obscuration for high viscosity samples



- Problem for LO: **introduction of air** into the system leads to a reduced analyzed sample volume
- Makes **sample dilution** necessary

Weinbuch et al. (2014) AAPS Journal 16(5):1128-31

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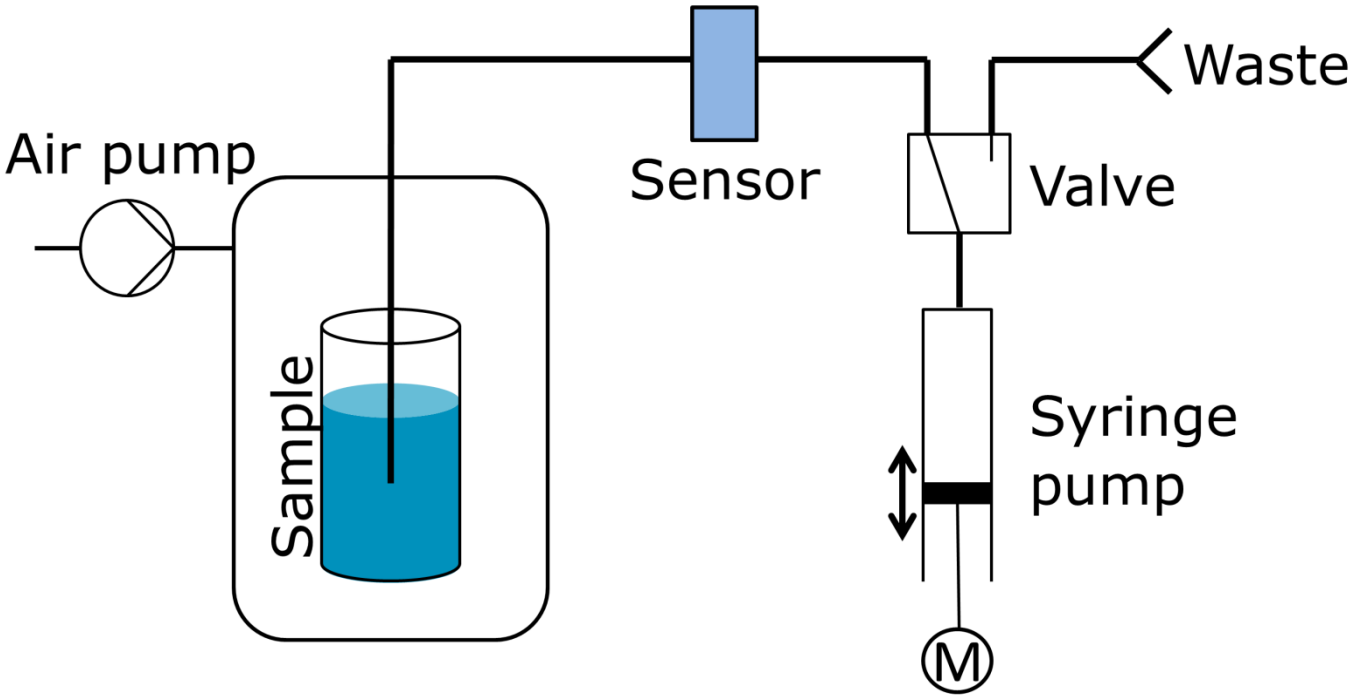
Light obscuration for high viscosity samples



- Application of pressure on the sample chamber up to 4 bar (PAMAS SBSS system)
- Supports liquid flow through the system even at high viscosities (> 50 cP)

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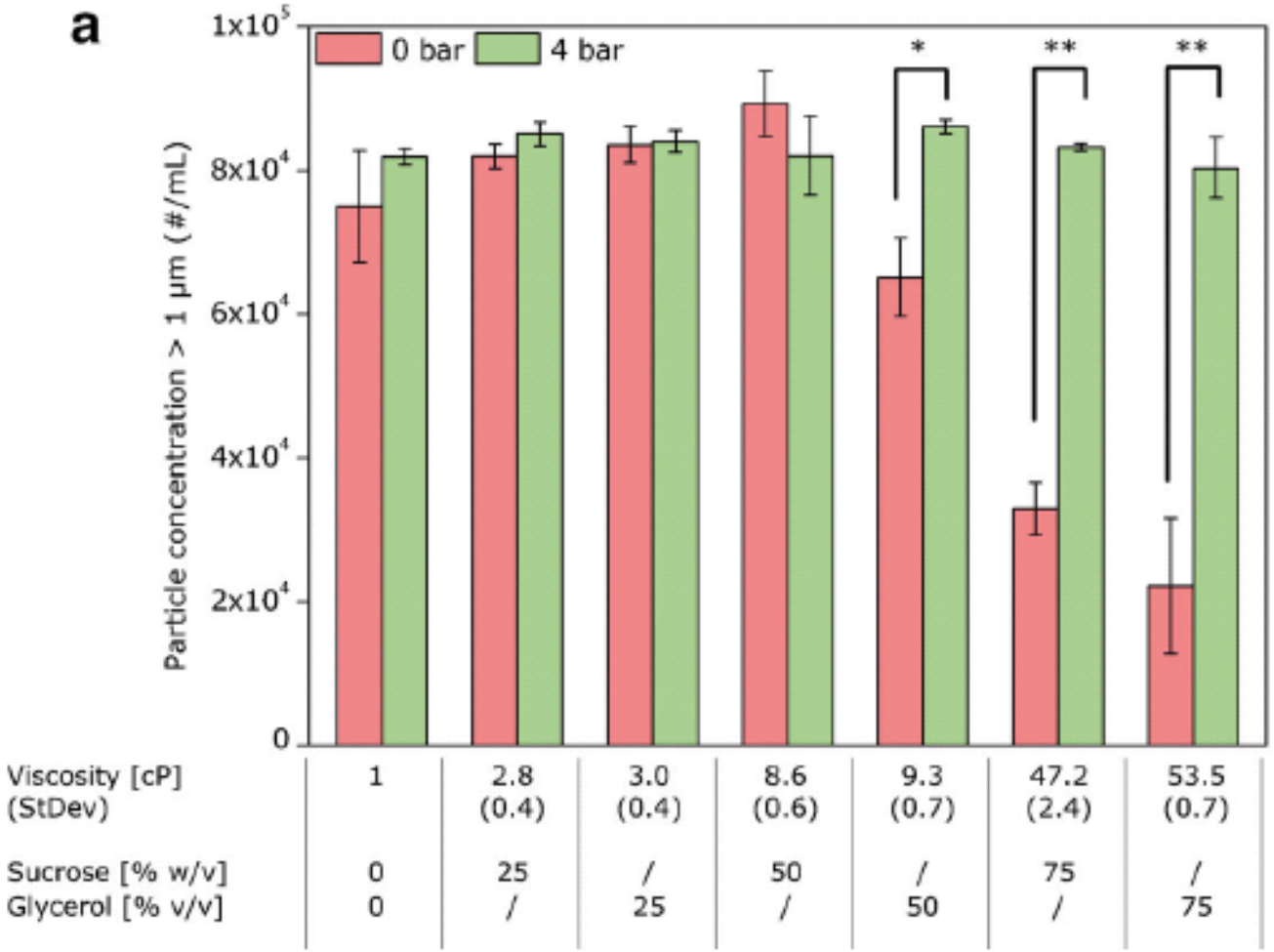
Weinbuch et al. (2014) AAPS Journal 16(5):1128-31

Light obscuration for high viscosity samples



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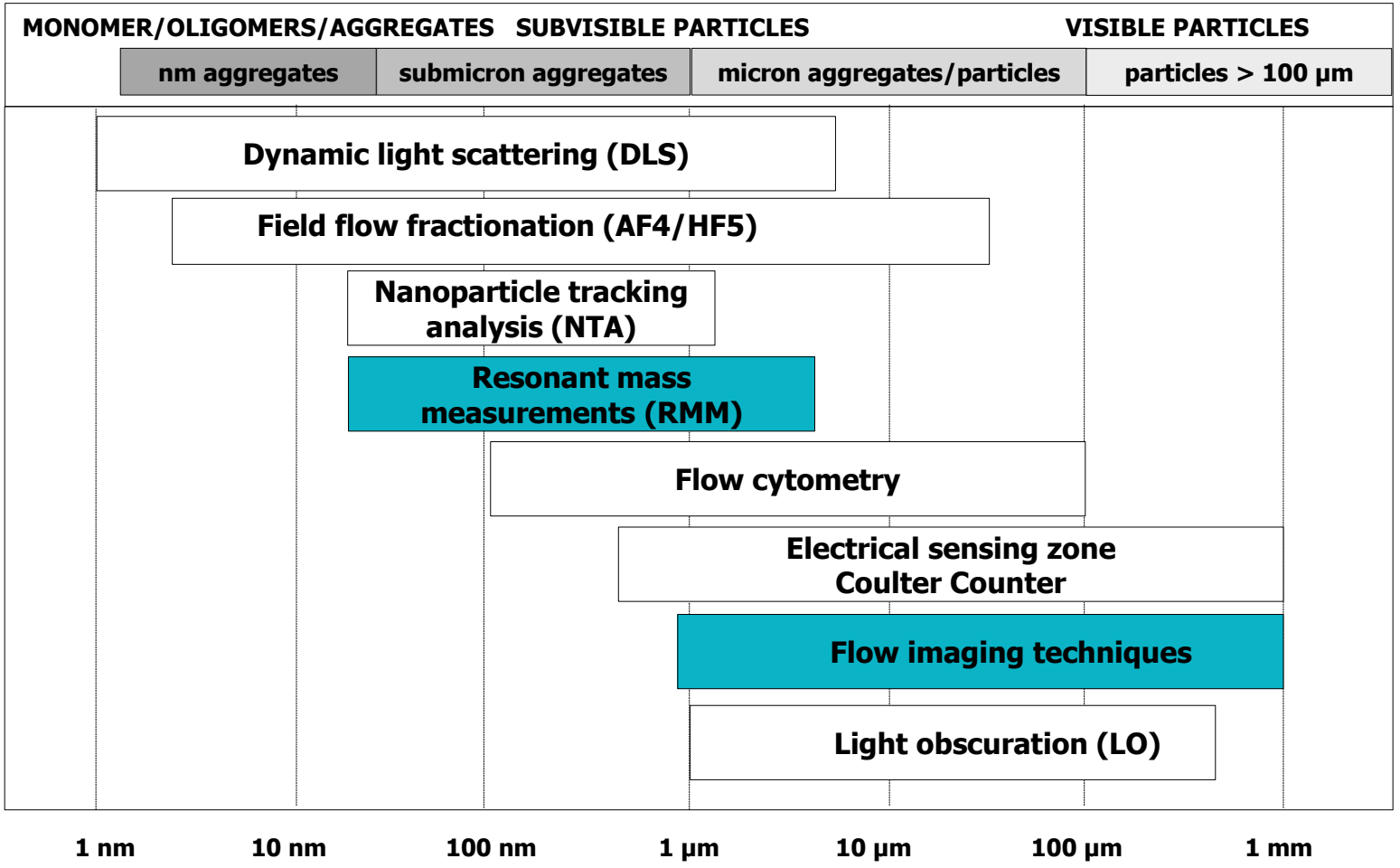
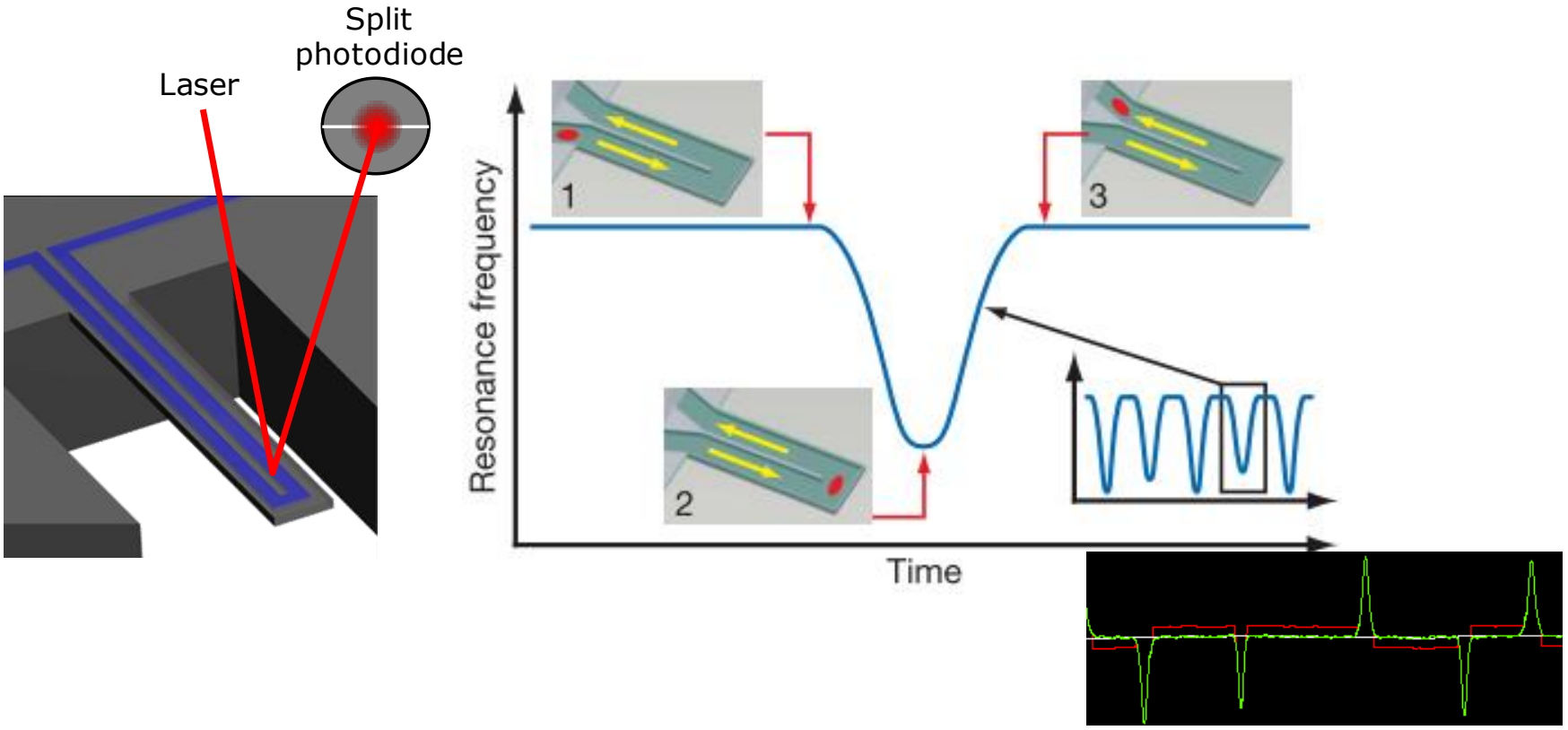
Weinbuch et al. (2014) AAPS Journal 16(5):1128-31

Differentiation protein particles from silicone oil



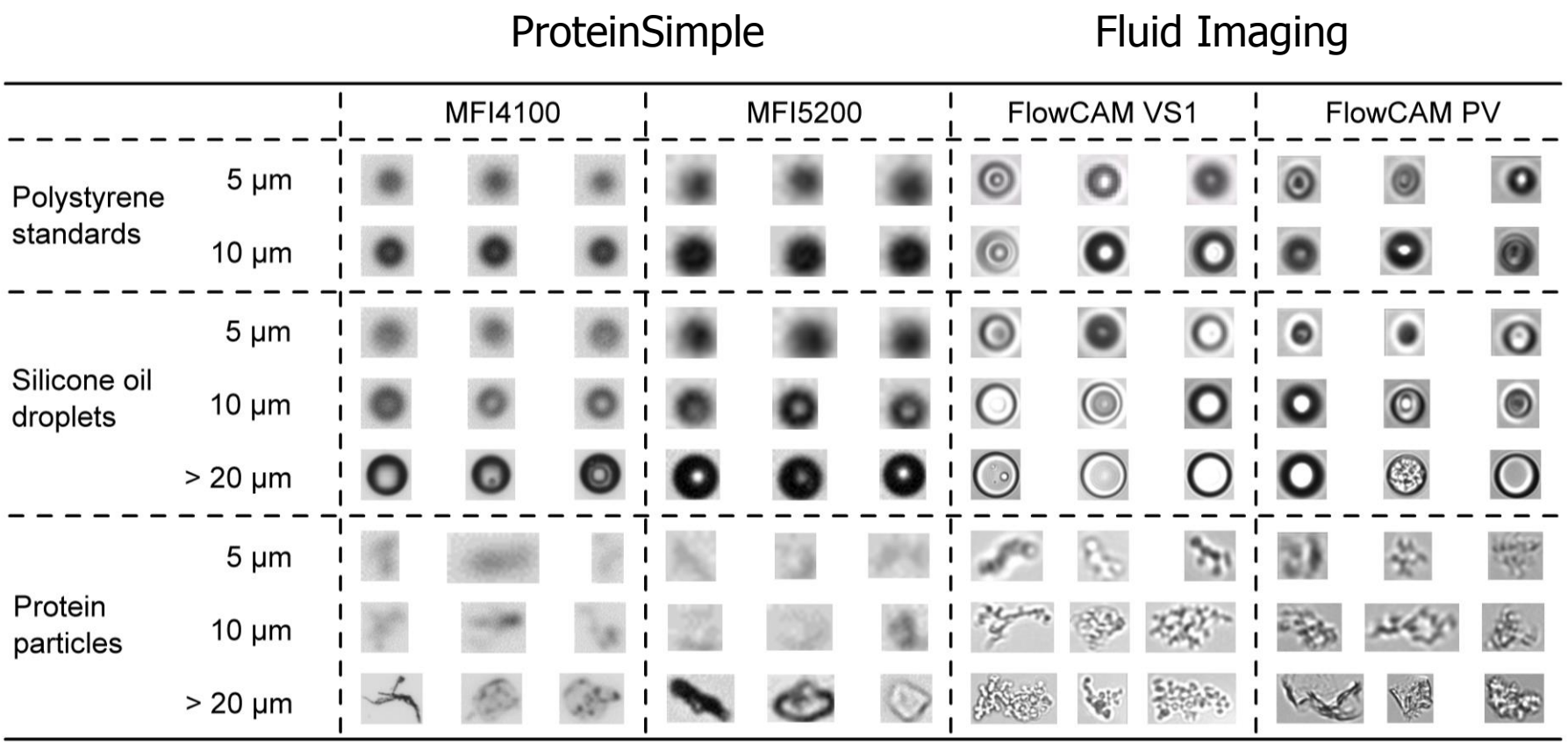
Resonant mass measurements (RMM, Archimedes)



- Individual particles are "weighed" in a mechanically resonating microfluidic channel

  → frequency changes depending on particle's buoyant mass
- Calculation of particle size based on a (known or assumed) **particle density**

Flow imaging microscopy – example images



Zölls / Weinbuch et al. (2013) AAPS Journal, 15:1200-1211

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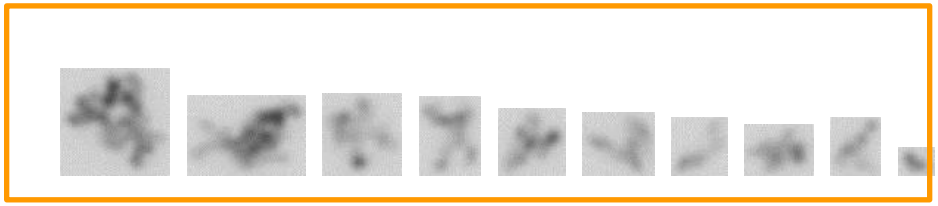
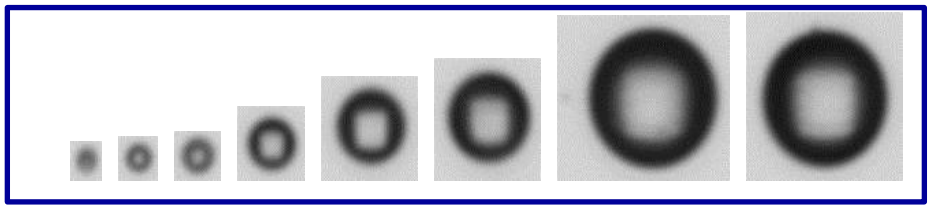
Study design for method evaluation

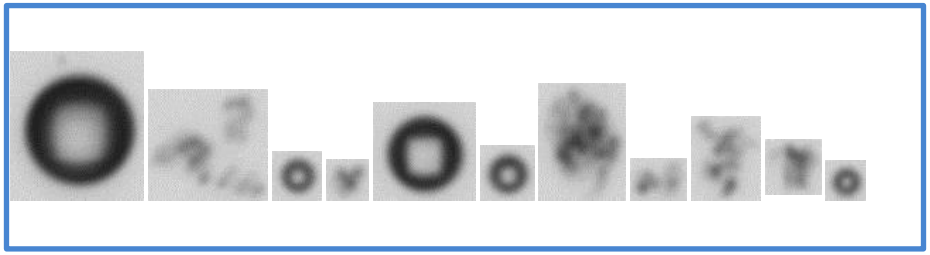
Heat stressed IgG



Silicone oil droplets



Preparation of various mixing ratios



Analysis

Flow imaging microscopy
(MFI4100, MFI5200, FlowCAM VS1, FlowCAM PV)
Resonant mass measurements

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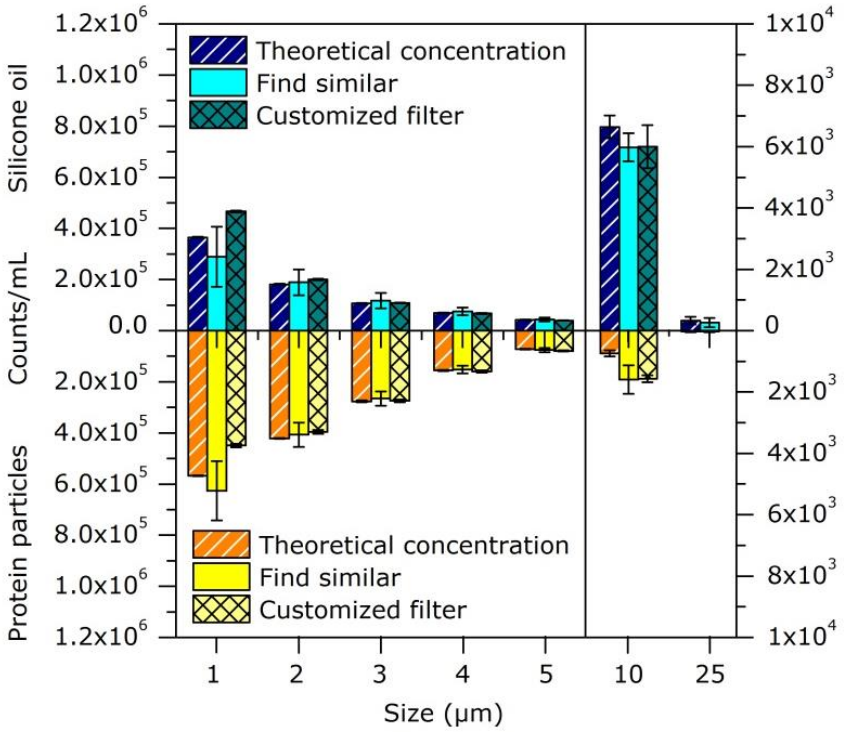
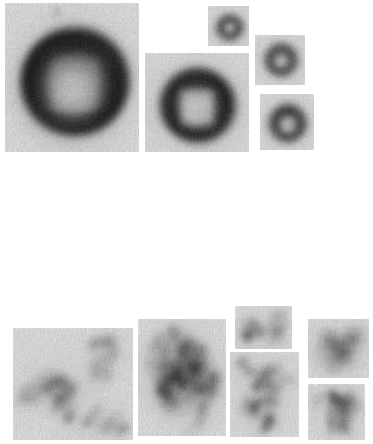
Weinbuch / Zölls et al. (2013) J Pharm Sci, 102:2152-65

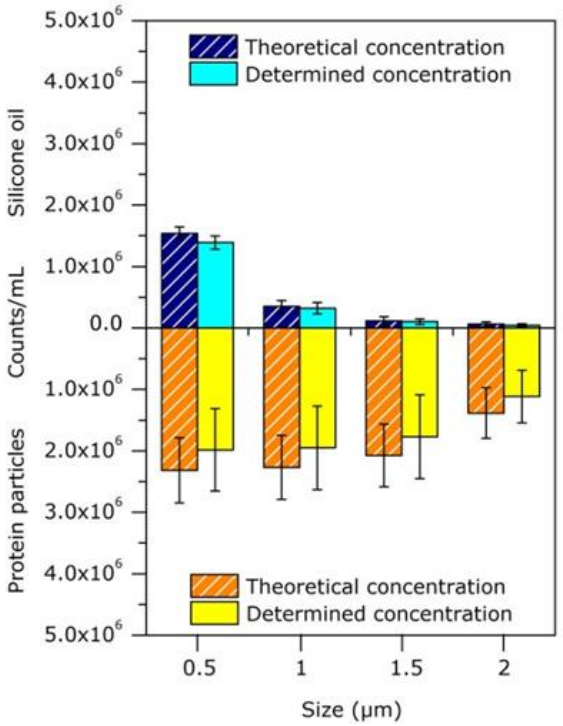
Silicone oil ↔ protein by MFI and RMM

MFI 4100





RMM



→ reliable differentiation from > 2-4 µm

→ customized filter improves repeatability

→ reliable differentiation from 500 nm to 2-3 µm

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Weinbuch/Zölls et al. (2013) J Pharm Sci, 102:2152-65

Conclusions

- Several methods (established and emerging) should be applied thoughtfully in parallel to obtain a good understanding in terms of quantity, composition, and other characteristics (e.g. size, shape, density, etc.)

  → **Which methods are most suitable for a certain molecule or formulation should be evaluated case-by-case**

- Understanding of the underlying measurement principle is crucial for selecting the right methods and for data interpretation
- New developments would be beneficial in various directions:
  - instruments that combine different measurement techniques
  - automated and high-throughput instruments
  - better software for data analysis

Acknowledgements

Coriolis Pharma
Biopharmaceutical Research and Development Service

- Sarah Zölls (Coriolis)
- Daniel Weinbuch (Coriolis)
- **Coriolis Pharma Team**

Coriolis' scientific Advisory Board

- Wolfgang Friess (LMU)
- Gerhard Winter (LMU)
- Wim Jiskoot (Leiden University)
- John Carpenter (University of Colorado)
- Christian Schöneich (Kansas University)

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