Particle analysis in biopharmaceuticals

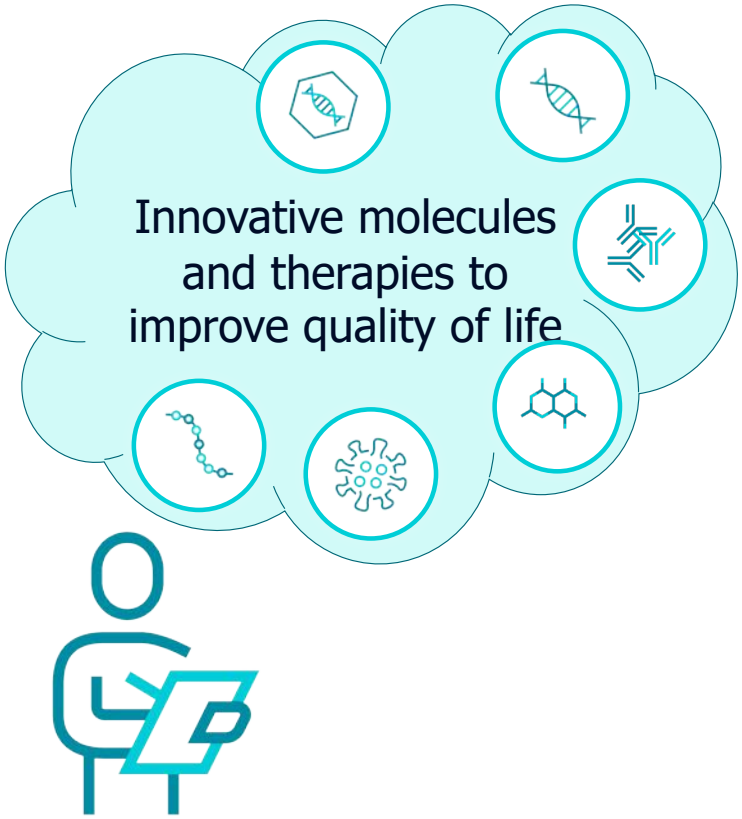
An overview form protein particles to living cells and relevance for immunogenicity

Prepared by: Dr. Andrea Hawe

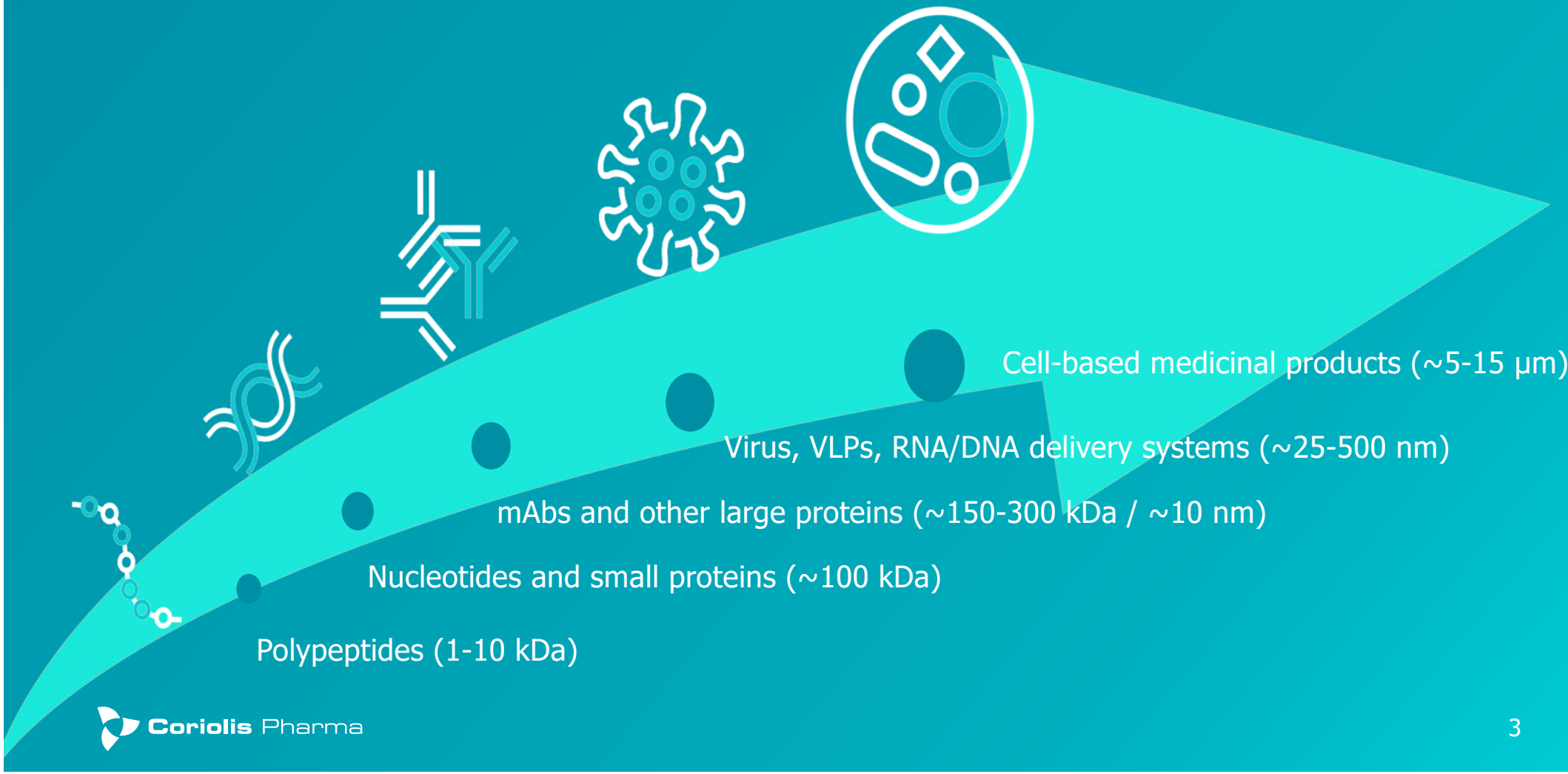
Date: 26 April 2022

Coriolis Pharma

Why are we here?



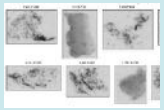
Biopharmaceuticals & ATMPs



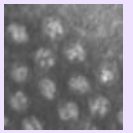
Particles in biopharmaceuticals and ATMPs

Therapeutic protein aggregates and particles

- 10-100 nm: Protein oligomers
- 0.1-1 µm: Submicron particles/ nanometer aggregates
- 1-100 µm: Subvisible particles/ micrometer aggregates
- >~ 100 µm: Visible particles



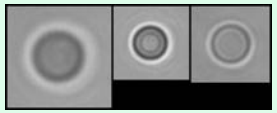
Particulate delivery systems, e.g.



- Viral vectors
- Virus-like particles (VLP)
- Lipid nanoparticles (LNP)
- Lipoplexes
- Polymer-based nano- & microparticles
- …

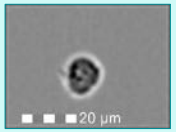
Particulate impurities, e.g.

- Cellulose, hair (foreign)
- Metal fragments, filter fibers (process-related)
- Silicone oil, glass, rubber (packaging-related)
- Fatty acid particles (excipient-related)
- Nanoparticulate impurities (NPIs) in sugars
- …



Cell-based medicinal products, e.g.

- Stem cells
- Chimeric antigen receptor T cells (CAR-T)
- …



Roesch et al. (2022) J Pharm Sci, 111:933-950

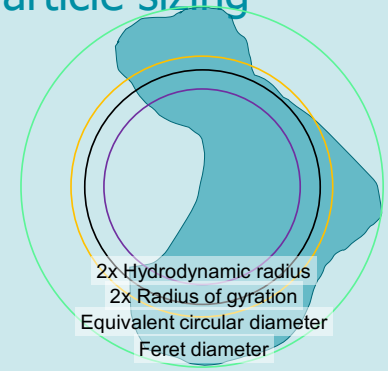
Scope of particle analysis

- Characterization of **impurities** in biopharmaceuticals and ATMPs
  - Impurities: can be from the "active" itself, excipients, primary packaging material, process-related, etc.
  - Quantification of particles
  - Classification and identification of particles

- Characterization of **the "active" itself**
  - Virus or viral vectors
  - Drug delivery systems, e.g. LNPs, polyplexes, liposomes…
  - Cells

Elements of particle analysis

Particle sizing



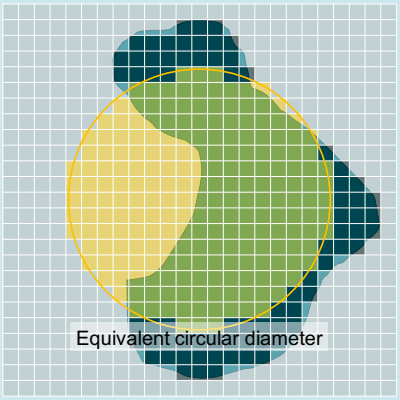
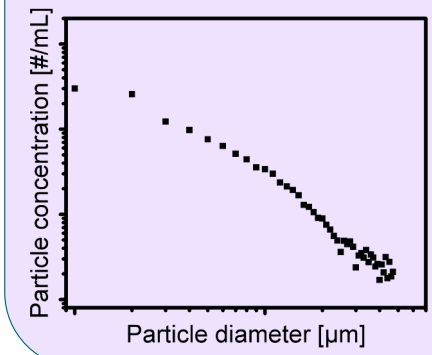
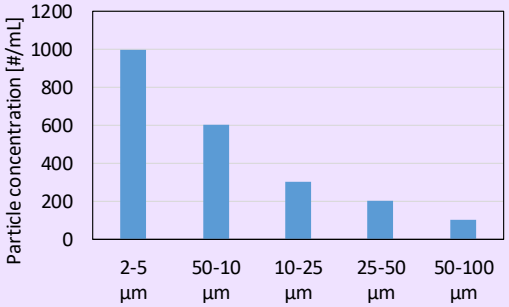


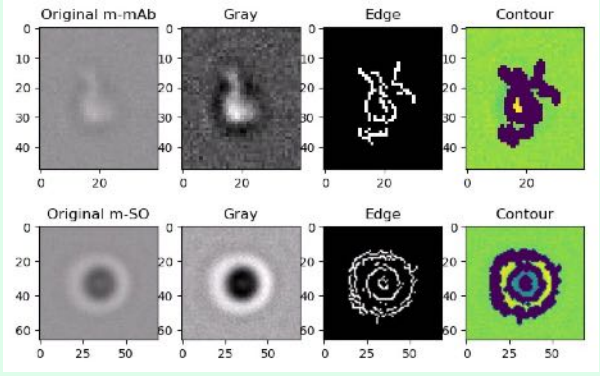
Fig. *

Particle counting

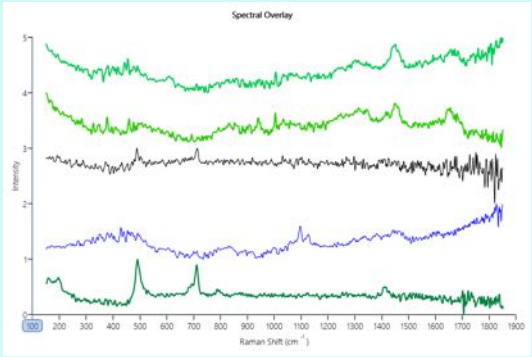


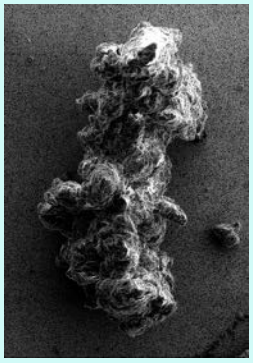


Particle differentiation



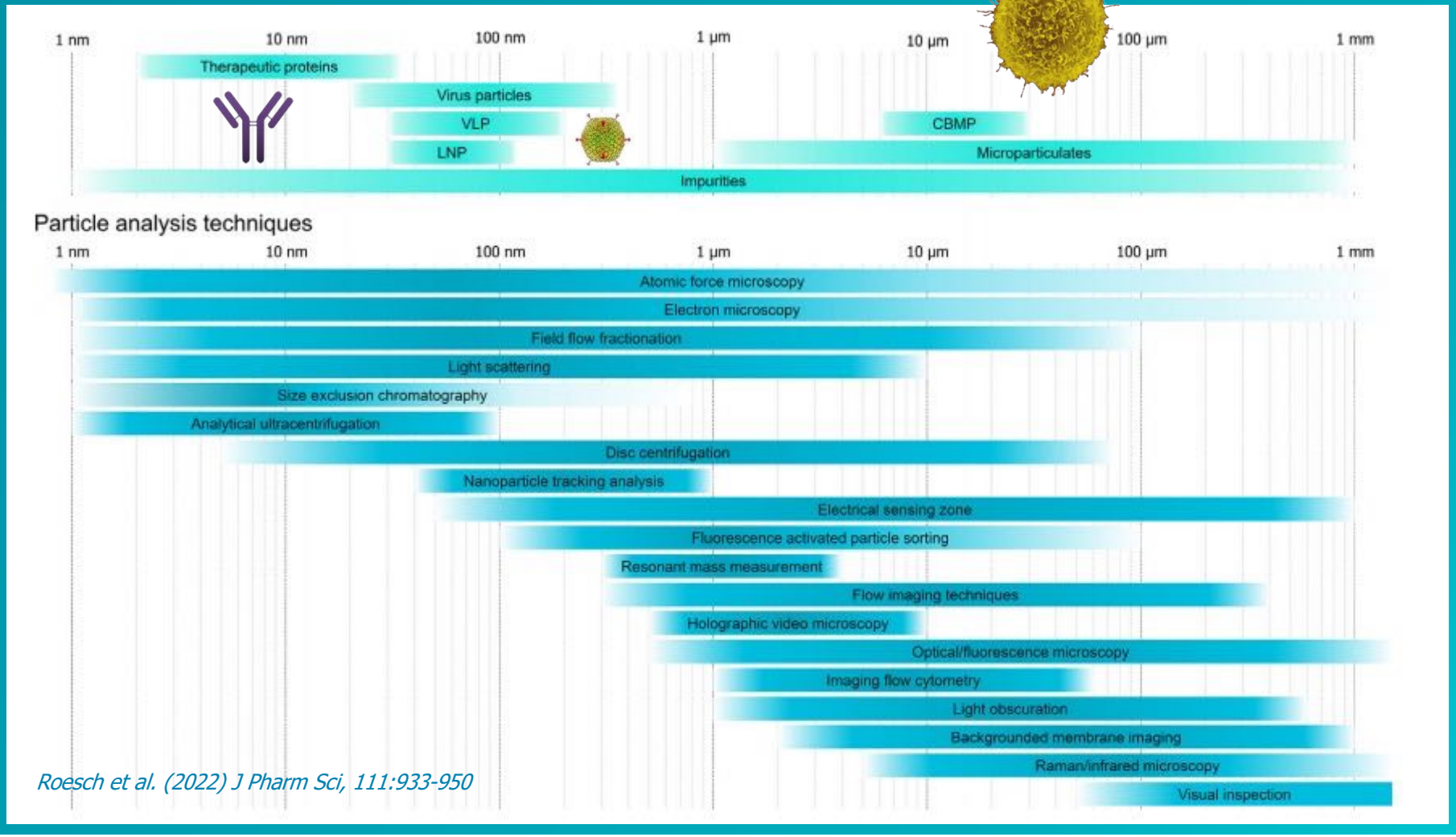
Particle identification





*Roesch et al. (2022) J Pharm Sci, 111:933-950

Methods for particle analysis in biopharmaceuticals



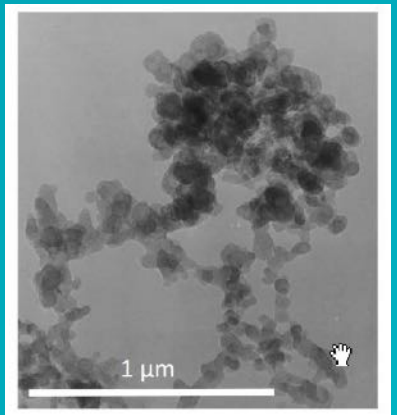
Roesch et al. (2022) J Pharm Sci, 111:933-950

Vaccines are based on particles

Vaccine category	Examples	Particle size category
mRNA – LNP vaccines	Covid-19 vaccines	Submicron: ~ 60-100 nm
Viral vector-based vaccines	Covid-19 vaccines	Submicron: ~ 70-100 nm
Virus like particles	Hepatitis B	Submicron: < 100 nm
Split and subunit vaccines	Influenza	Submicron: ~ 300 nm
Inactivated viruses	Inactivated influenza vaccine Inactivated polio vaccine	Submicron: < 100 nm Submicron: ~ 200 nm
Alum-adsorbed antigens MF59 adjuvanted antigens	Diphtheria, tetanus Influenza	Micron: low µm size range Submicron: ~ 200 nm
Live bacterial	Salmonella	Micron: ~ 1 µm
Inactivated bacteria	Whole cell pertussis	Micron: ~1 µm

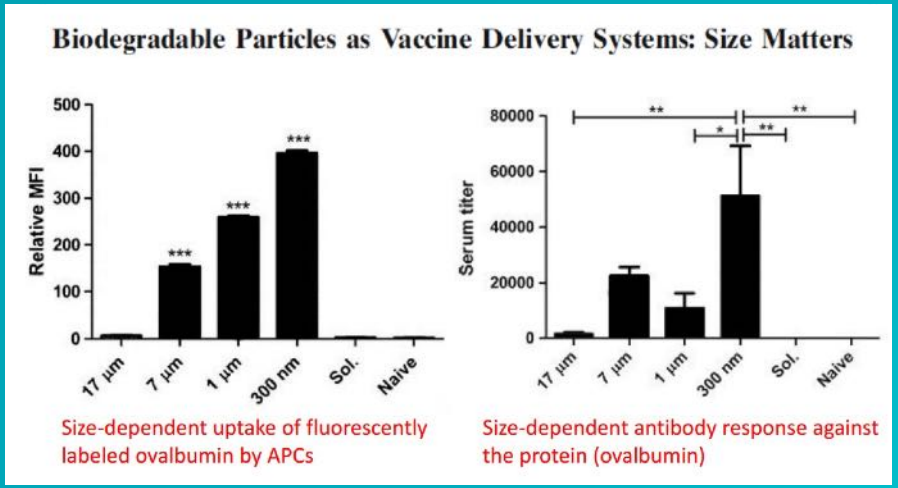
Vaccines are based on particles

- Submicron-size particles (and small micron-size particles) are more efficiently taken up by antigen-presenting cells (APCs) than soluble protein
- Uptake by APCs is a first essential step towards an immune responds
- Immune response depends on various particle properties:
  - * Composition
  - * Shape
  - * Surface characteristics
  - * Rigidity
  - * Size
  - ..



Aluminium phosphate

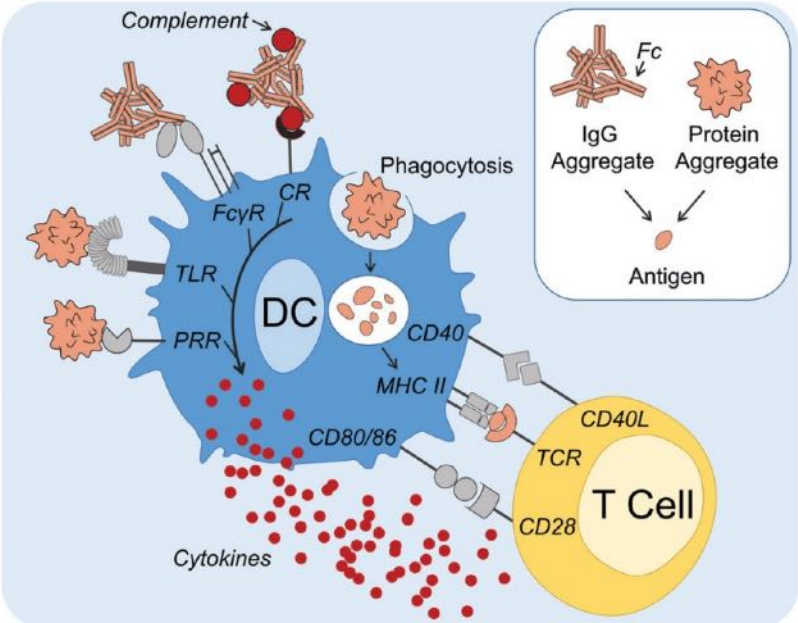
Vrieling et al. Coll Surf. (2019) 181:648-656



Joshi et al. AAPS J 15:85-94 (2013)

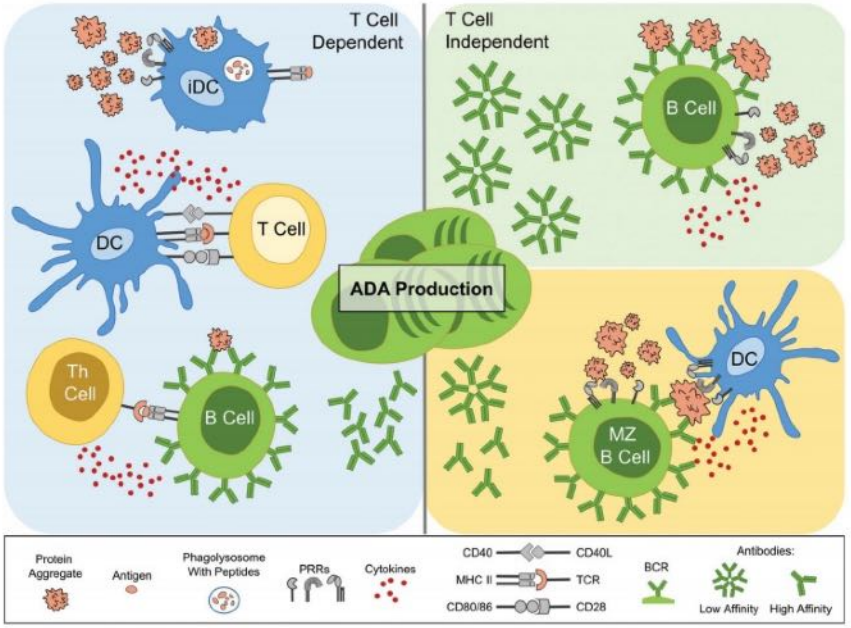
Immune activation by aggregates and particles

Activation of innate immune system



Aggregates can enhance DC maturation and antigen presentation

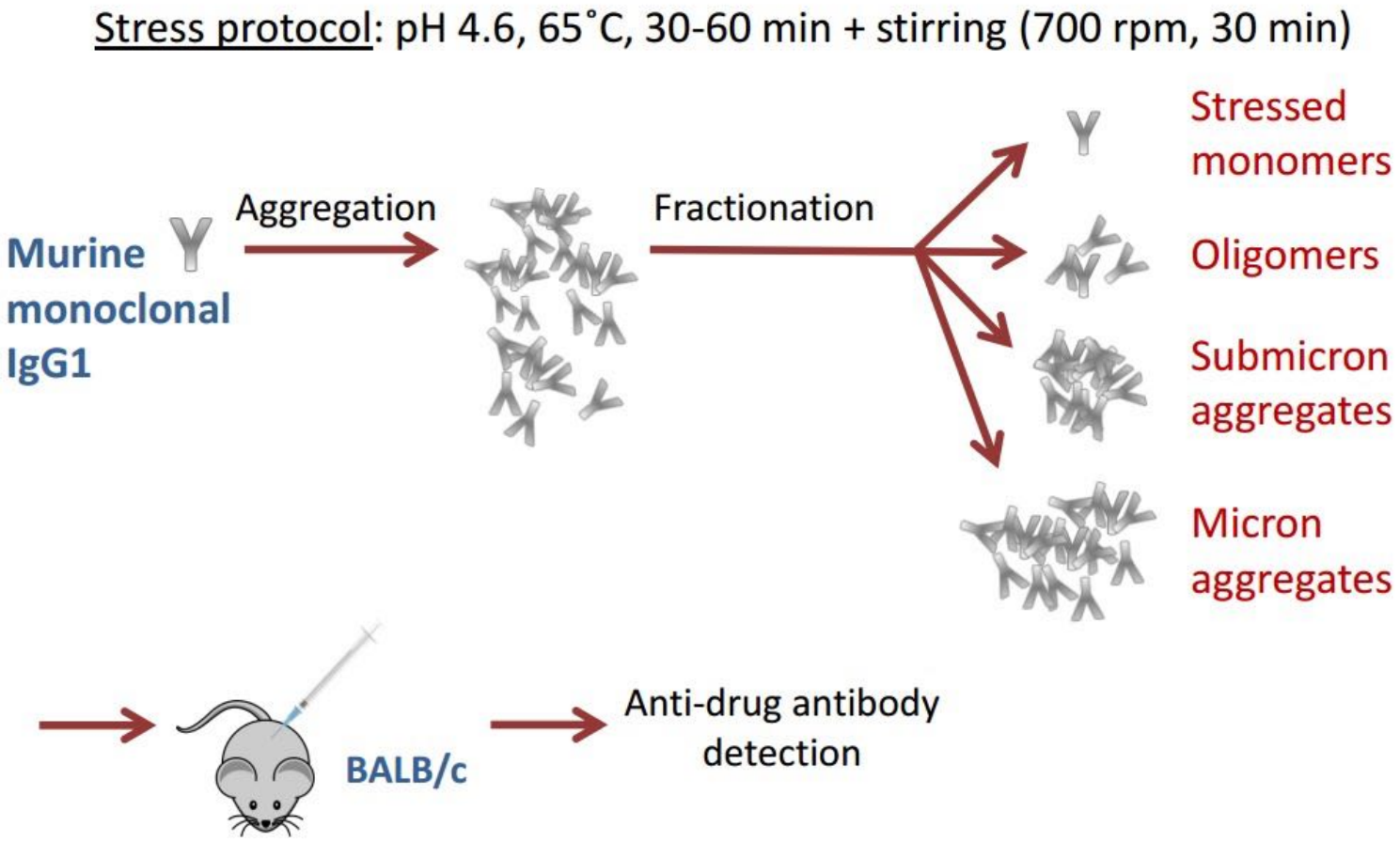
Activation of adaptive immune system



Aggregates can enhance several routes for ADA production

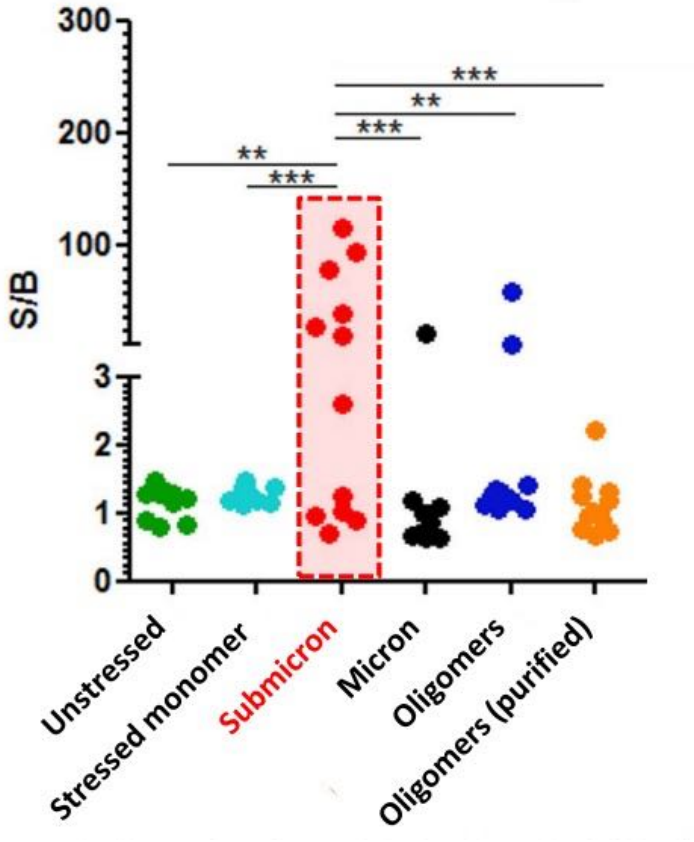
Lundahl et al. (2021), RSC Chem. Biol. 2:1004ff

mAb aggregates break tolerance in transgenic mice

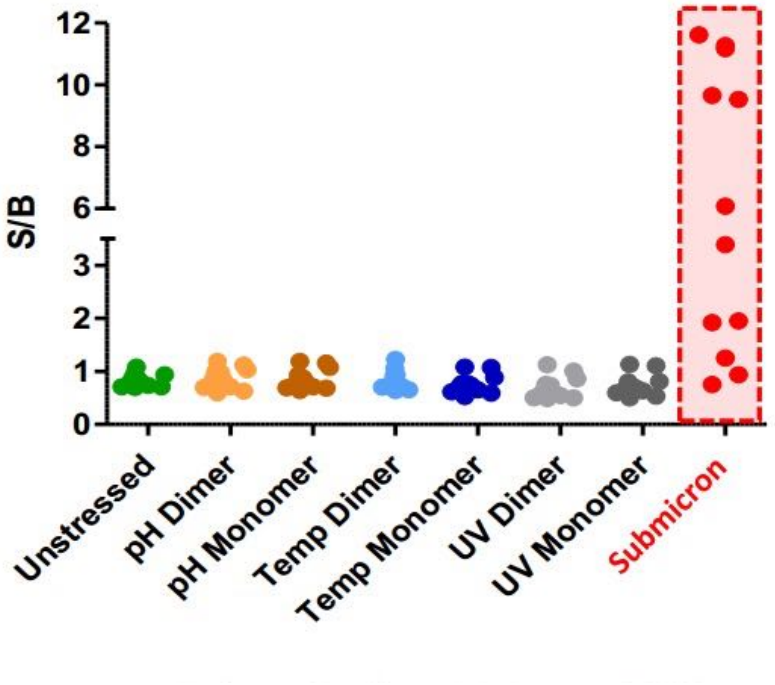


Kijanka et al. (2018) J Pharm Sci 107:2847-2859

mAb aggregates break tolerance in transgenic mice



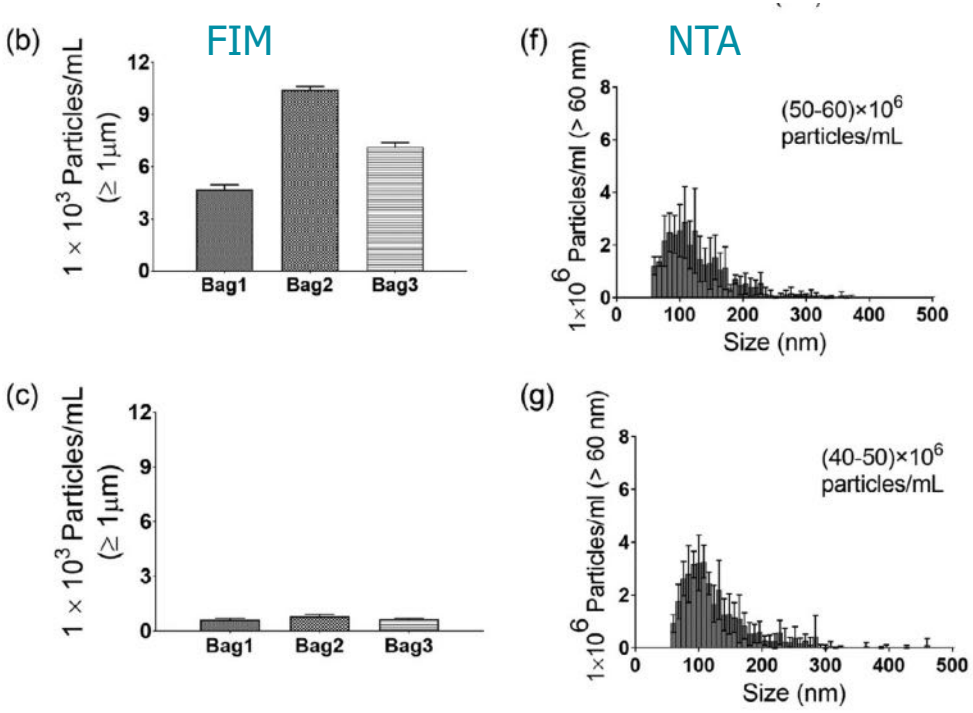
Kijanka et al. (2018) J Pharm Sci 107:2847-2859



Kijanka et al. (2020) J Pharm Sci 109:730-738

Activation of immune system by subvisible particles

- Infliximab (i.v.): > 20% adverse infusion related reactions and ~ 50% neutralizing antibodies
- What is the role of subvisible particles?



After dilution of infliximab in saline and infusion without in-line filter

In vitro effect of particles from infusion solutions:

- Induction of IL-6 secretion (whole blood samples)
- TLR stimulation
- T-cell proliferation

After dilution of infliximab in saline and infusion with in-line filter (0.2 µm)*

**similar results with 1.2 µm filter*

Coriolis Pharma

Pardeshi et al. (2021) J Pharm Sci 110:2894-2903

SVP and adverse events in Peginesatide

- **Peginesatide**: synthetic erythropoietin peptide mimetic covalently dimerized and linked to PEG
- Market approval 2012 (FDA) and withdrawn again in 2013 due to adverse events (anaphylaxis)
- FDA investigation pointed towards subvisible particles playing a role

Clinical Phase 3:
mainly single-use vial (SUV)

Commercialization:
multi-use vial (MUV)

Particles > 10 µm & > 25 µm in light obscuration (USP <788> compendial test) were comparable for SUV and MUV

Kotarek et al. (2015) J Pharm Sci 105:1023-1027

SVP and adverse events in Peginesatide

A closer look with additional particle characterization methods

Nanoparticle tracking analysis (NTA): submicron particles 50 – 1000 nm

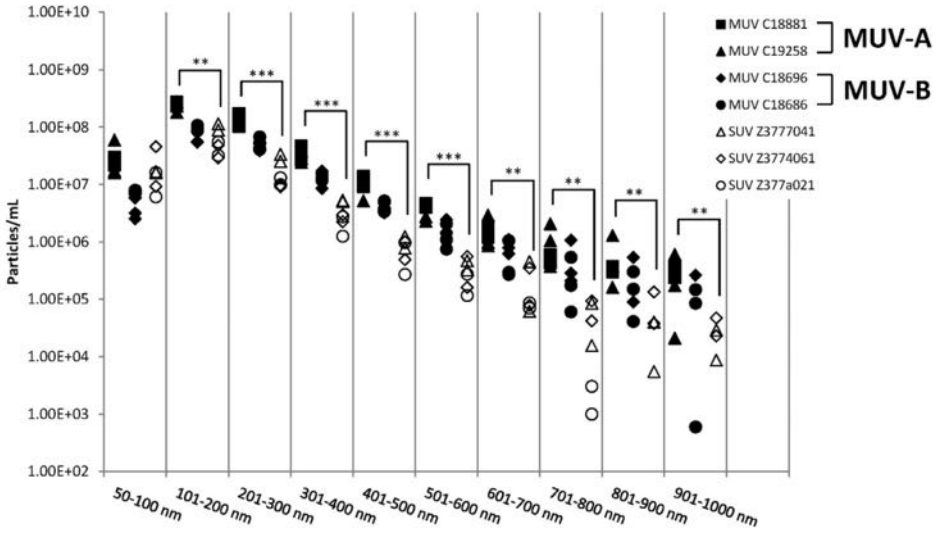


Table 1
NTA Peginesatide Median Particle Concentrations[a,b]

Hydrodynamic Diameter (nm)	SUV	MUV	p^c
50-1000	9763	29,934	0.028
50-100	1610	1197	0.673
101-200	5176	14,426	0.022
201-300	1155	8526	0.001
301-400	286	2068	0.001
401-500	88	519	0.001
501-600	30	237	0.001
601-700	8	102	0.003
701-800	3	48	0.002
801-900	2	30	0.002
901-1000	2	18	0.008

[a] Particles/mL ($\times 10^4$).
[b] SUV and MUV were independently measured 6 (each SUV lot in duplicate) and 12 (each MUV lot in triplicate) times, respectively.
[c] Mann–Whitney test.

Kotarek et al. (2015) J Pharm Sci 105:1023-1027

SVP and adverse events in Peginesatide

A closer look with additional particle characterization methods

Flow Imaging Microscopy: micron particles > 2 µm

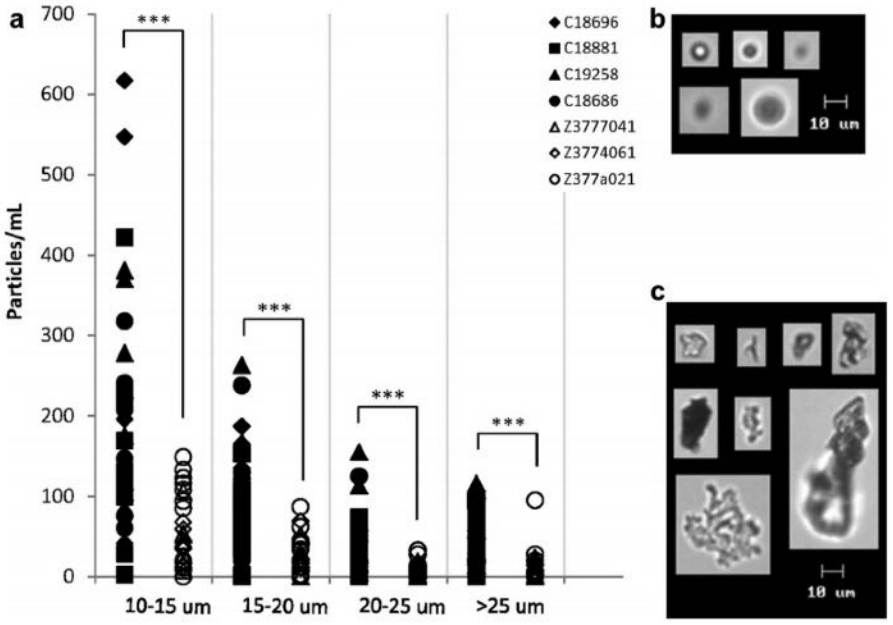


Table 2
Normalized FlowCAM Peginesatide Particle Concentrations[a]

ESD (µm)	SUV			MUV			
	Z377a021	Z3777041	Z3774061	C18686	C18696	C18881	C19258
10-15	1.00	0.40	0.54	2.24	3.28	2.28	3.23
15-20	1.00	0.76	0.48	3.13	4.29	3.11	5.49
20-25	1.00	0.49	0.43	4.05	4.73	4.54	7.28
≥25	1.00	0.82	1.92	13.60	9.02	14.26	24.69

[a] Mean particle concentrations normalized to SUV lot Z377a021 (see Materials and Methods section). Each lot was independently measured 5-10 times.

Kotarek et al. (2015) J Pharm Sci 105:1023-1027

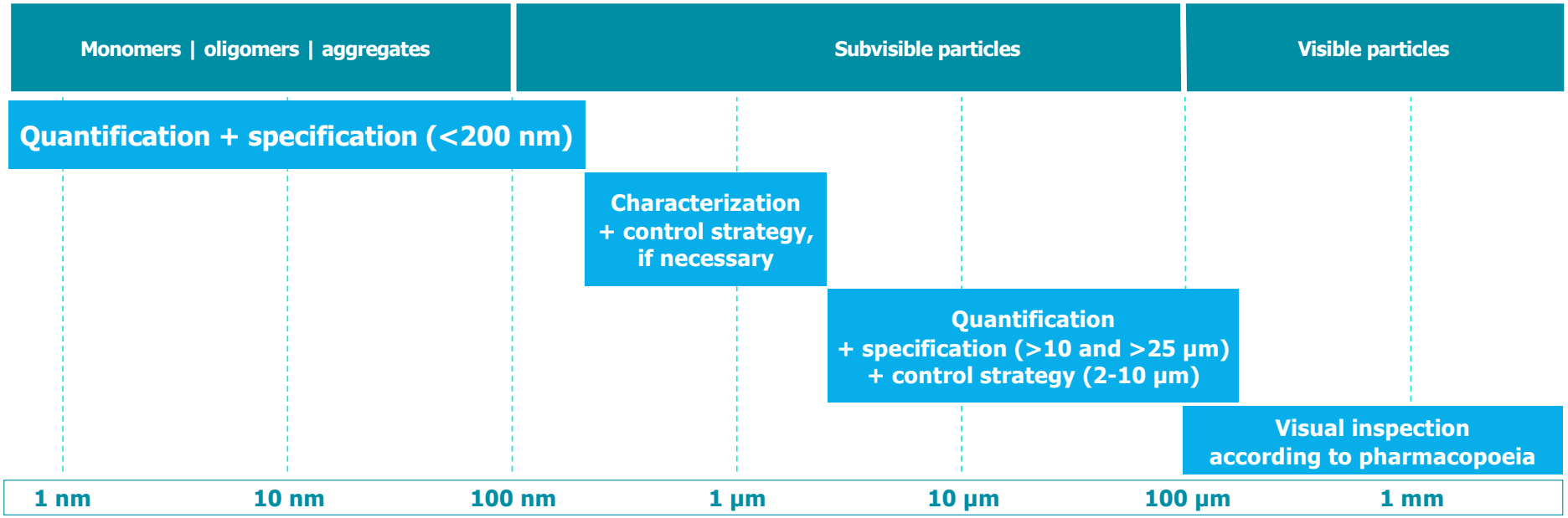
Coriolis Pharma

Particles and immunogenicity

- Particulate antigens are highly immunogenic: very low amounts (1-100 µg*) particulate protein antigen in vaccines
  **1- 100 µg of a particulate protein antigen would be ~ 0.001-0.1% of a mAb dose of 100 mg and not detectable by methods like HP-SEC*
- Protein aggregates and particles can activate innate and adaptive immune system
- Factors that play a role are size, number, morphology/surface characteristics, (native) structure, chemical modifications, … of aggregates
- Aggregates and particles in biopharmaceuticals are considered as risk factor for immunogenicity

Analytical methods for a comprehensive characterization of aggregates and particles is an important part of drug product development

Regulatory expectations particle analysis



Thorough characterization already at early development stages:

- Analysis of samples from different batches, forced degradation studies, stability studies, shipment studies, etc.
- 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

Coriolis Pharma

Susan Kirshner (2012 and 2014) Breckenridge Workshop, Breckenridge, CO
Ashutosh Rao (2021) Swiss Academy of Pharmaceutical Sciences, Online PharmaTalk 2/2021

Orthogonal methods

Orthogonal methods can compensate for limitations of an individual analytical technique

HP-SEC

Oligomers

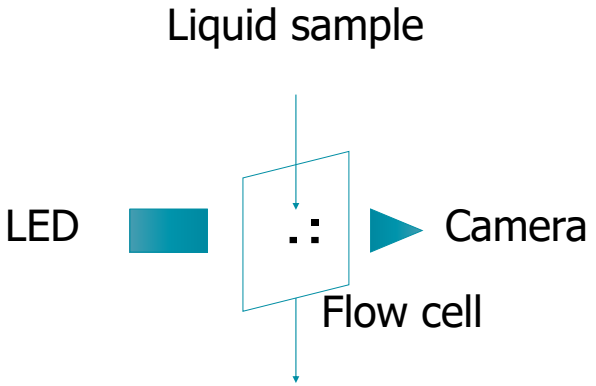
SV-AUC

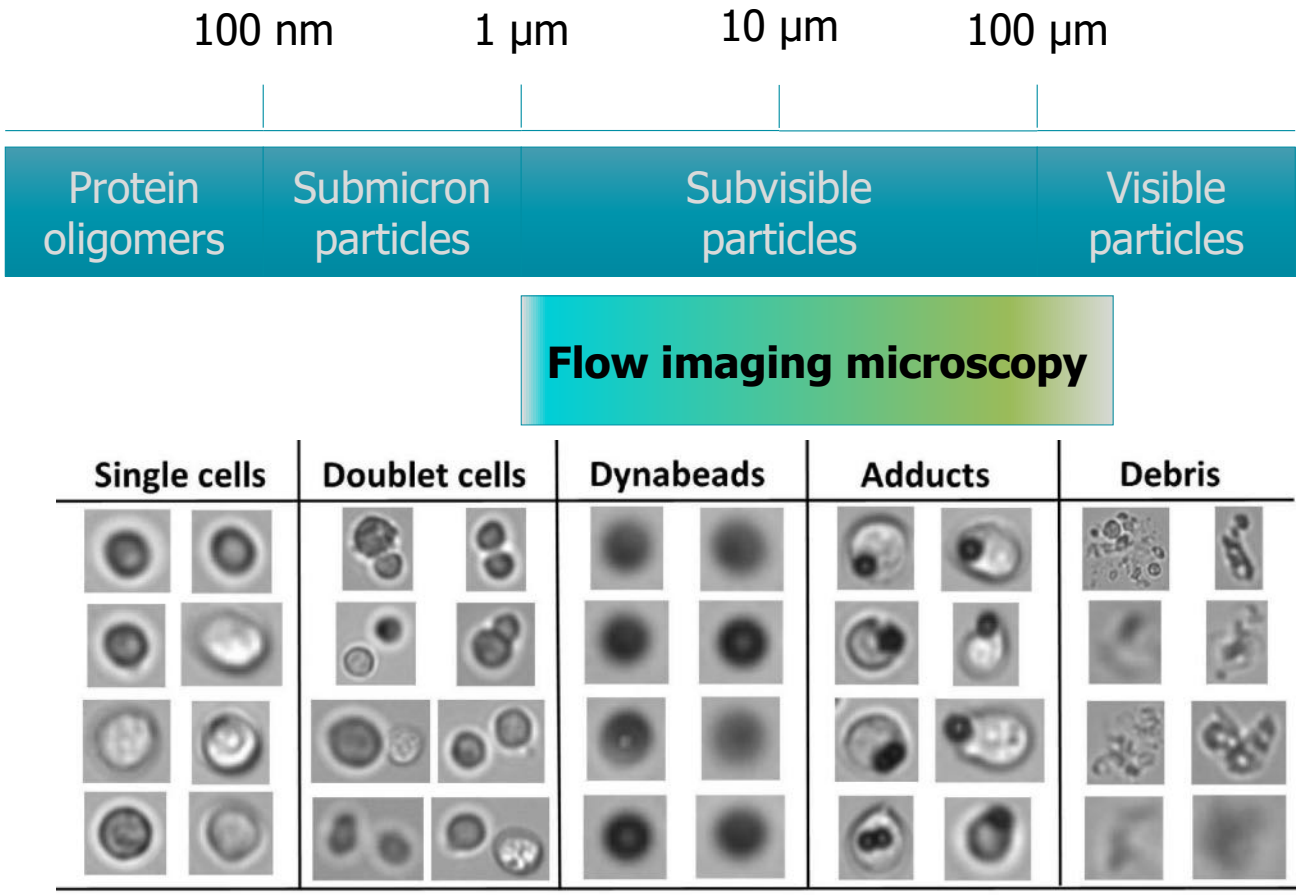
LO

Subvisible particles

FIM

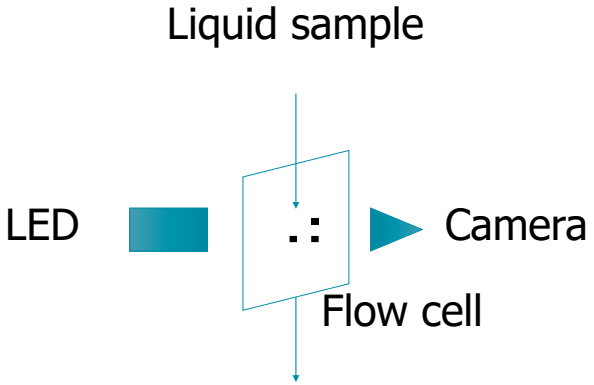
Flow imaging microscopy

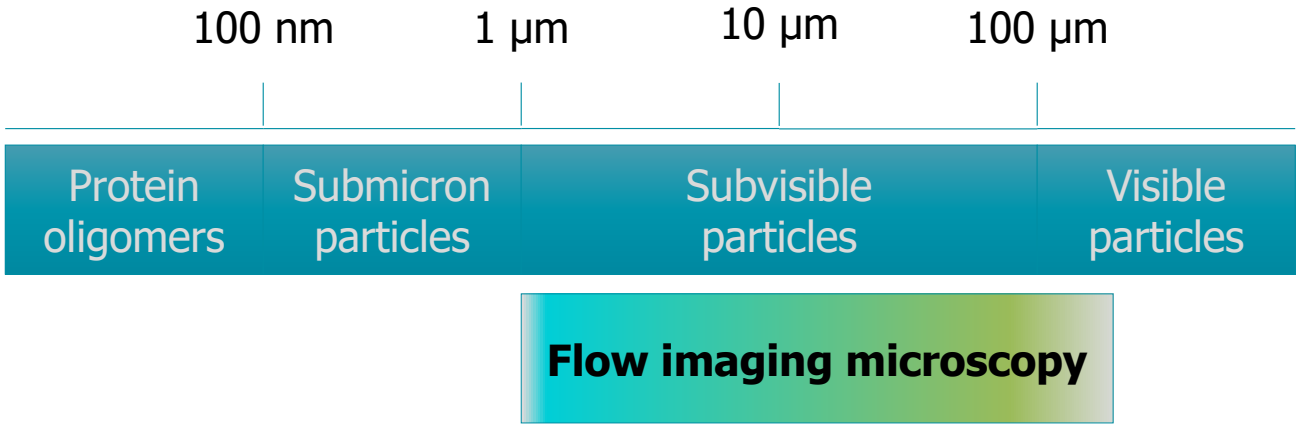




Grabarek et al. (2020) Cytotherapy, 2:S1465-3249(20)30633-2

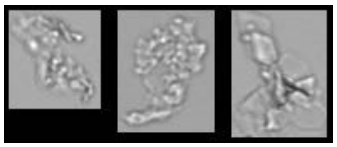
Flow imaging microscopy



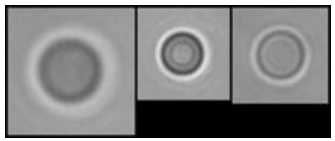


Protein (API degradation)

Fatty acid related (Polysorbate degradation)



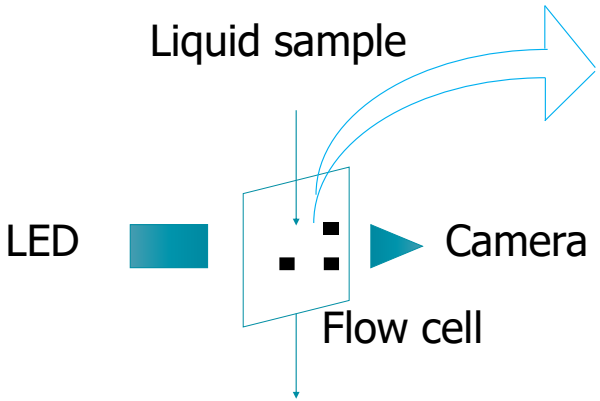
Silicone oil (Packaging material)



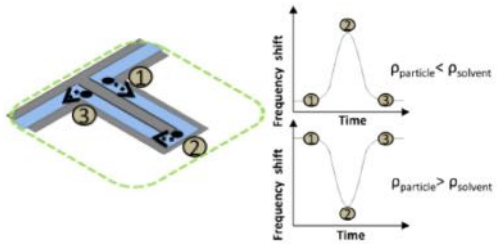
… and many more.

Differentiation protein particles and silicone oil

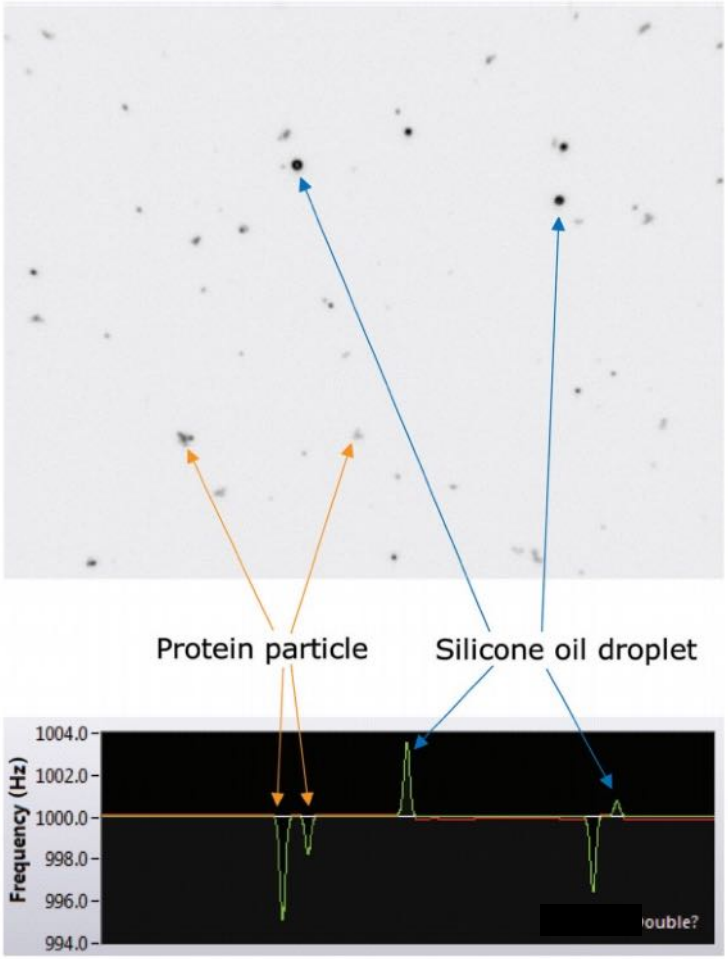
Flow imaging microscopy



Resonant mass measurement

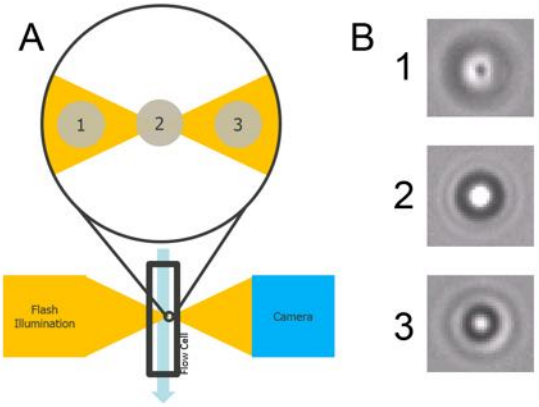


Groß-Rother et al. (2020) Pharmaceutics 12(11): 1112



Weinbuch et al. (2013) J Pharm Sci 102(7): 2152-65

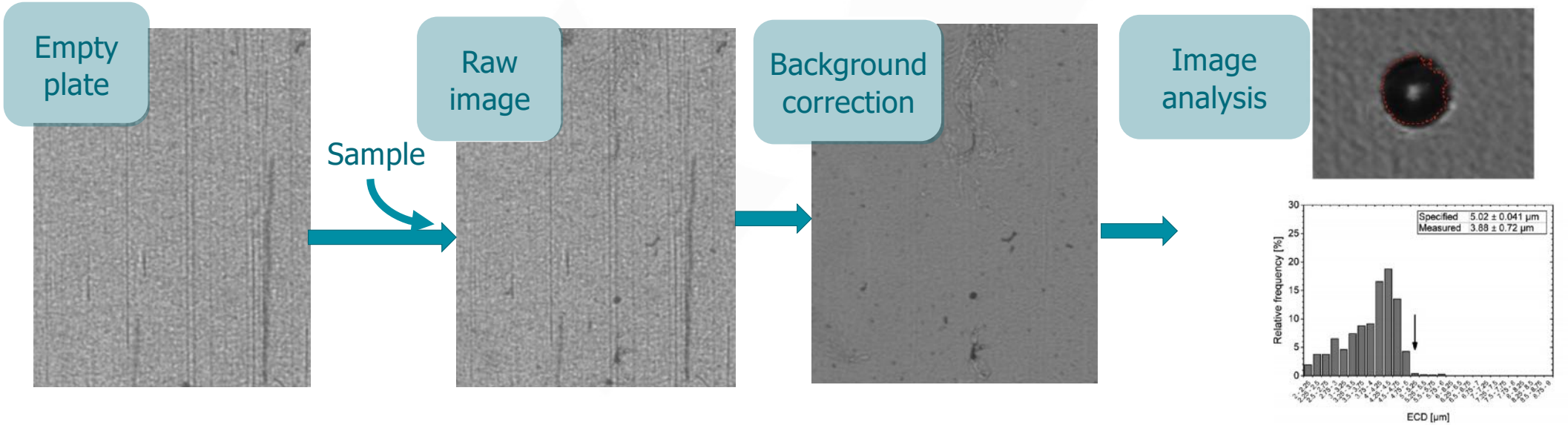
Oil immersion flow imaging microscopy



Krause et al. AAPS J, 2021 23:13

Backgrounded membrane microscopy (BMI)

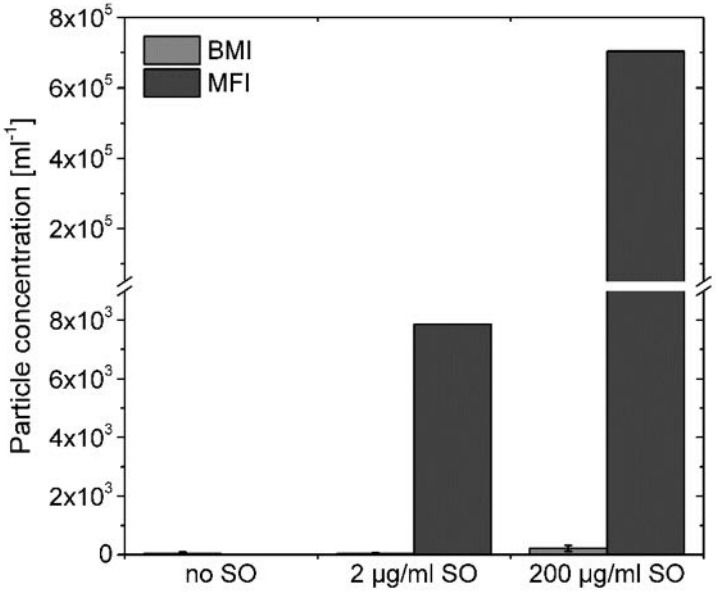
- Automated, 96-well plate-based membrane microscopy
  - Throughput: 2 to 3 hrs/plate
  - Applied volume: ~50-100 µl/well
- Analysis of particles >2 µm



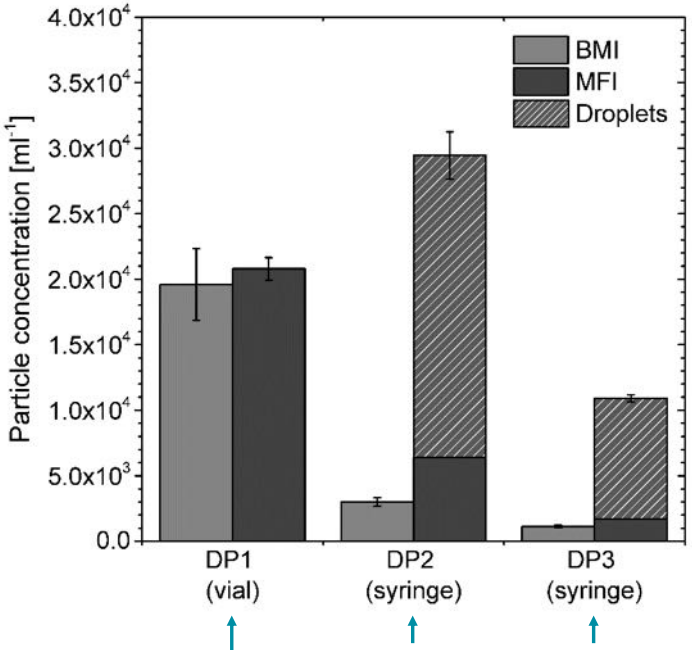
Coriolis Pharma

Helbig et al. (2019) J Pharm Sci, 109(1): 264-76

BMI: separation of protein aggregates from silicone oil droplets



Silicone oil droplets pass filter membrane;
Only ”solid“ particles >2 µm are detected



Silicone oil droplets expected

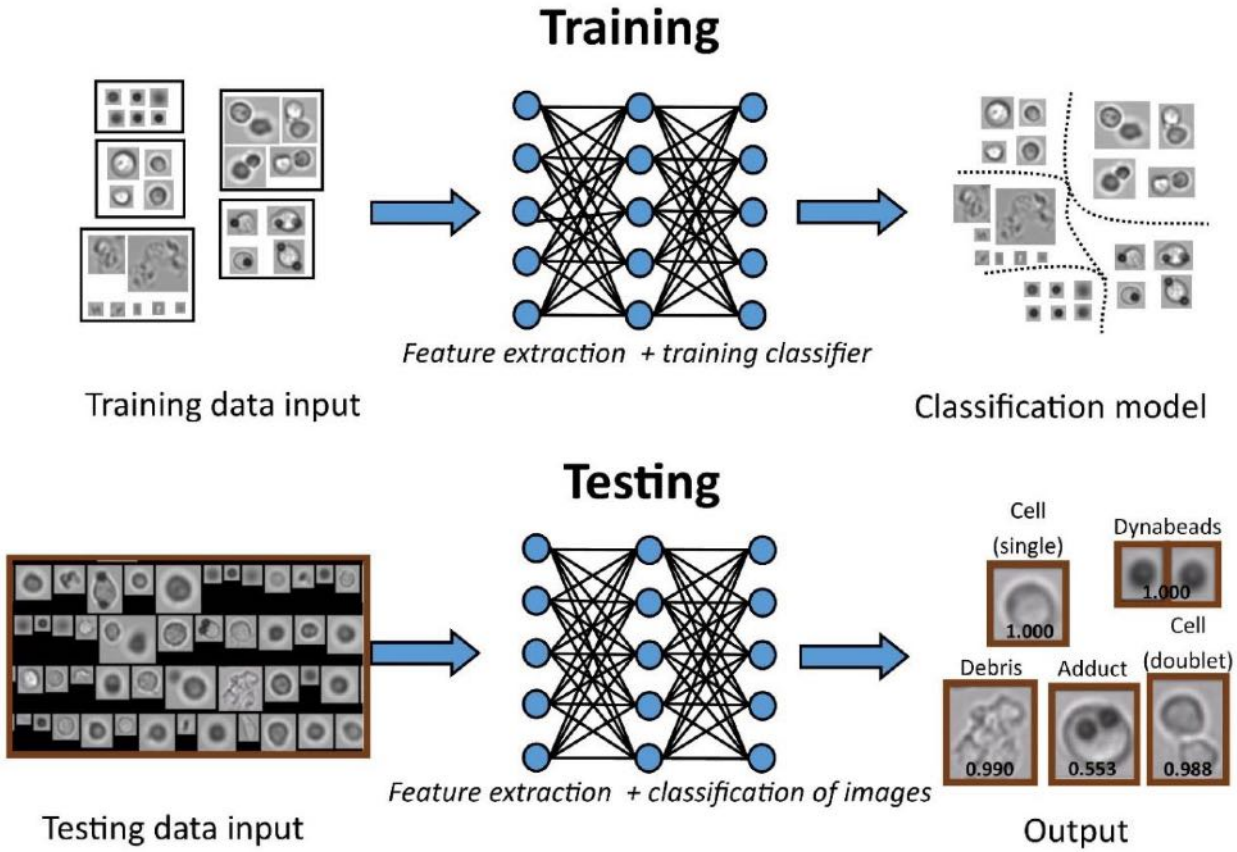
No silicone oil droplets expected

BMI can be used as orthogonal technique to flow imaging microscopy

Coriolis Pharma

Helbig et al. (2019) J Pharm Sci, 109(1): 264-76

Deep learning to improve particle classifications



Grabarek et al. (2020) Cytotherapy, 2:S1465-3249(20)30633-2

Summary and conclusions

- Particles are considered as risk factor for immunogenicity of biopharmaceuticals
- Particles in biopharmaceuticals are critical quality attributes and require close analytical characterization
- Several techniques incl. FIM, RMM, BMI (among others) can be applied to differentiate between protein aggregates and silicone oil droplets (> ca. 0.5 µm)
- The analytical tool-box is broad – depending on the scope of analysis and product a suitable method selection should be made

Acknowledgements

- Tim Menzen
- Wim Jiskoot
- Constanze Helbig
- Adam Grabarek
- Nils Krause
- All co-authors and collaborators

The AAPS Journal (2021) 23:13
DOI: 10.1208/s12248-020-00547-9

Research Article

Oil-Immersion Flow Imaging Microscopy for Quantification and Morphological Characterization of Submicron Particles in Biopharmaceuticals

Nils Krause,[1] Sebastian Kuhn,[2] Erik Frotscher,[2] Felix Nikels,[2] Andrea Hawe,[1] Patrick Garidel,[2] and Tim Menzen[1,3]

Journal of Pharmaceutical Sciences 109 (2020) 264-276

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutical Biotechnology

Background Membrane Imaging (BMI) for High-Throughput Characterization of Subvisible Particles During Biopharmaceutical Drug Product Development

Constanze Helbig [1], Gregor Ammann [1], Tim Menzen [1], Wolfgang Friess [2], Klaus Wuchner [3], Andrea Hawe [1,*]

[1] Coriolis Pharma Research, Fraunhoferstr. 18 b, 82152 Martinsried, Germany
[2] Department of Pharmacy, Ludwig-Maximilians-Universität München, Pharmaceutical Technology and Biopharmaceutics, Butenandtstr. 5-13, 81337 Munich, Germany
[3] Janssen Research and Development, DPD&S Biotherapeutics Development, Hochstr. 201, 8205 Schaffhausen, Switzerland

Journal of Pharmaceutical Sciences 111 (2022) 933–950

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Special Topic Cluster

Particles in Biopharmaceutical Formulations, Part 2: An Update on Analytical Techniques and Applications for Therapeutic Proteins, Viruses, Vaccines and Cells

Alexandra Roesch[a,c], Sarah Zölls[a], Daniela Stadler[a], Constanze Helbig[a], Klaus Wuchner[b], Gideon Kersten[a,c], Andrea Hawe[a], Wim Jiskoot[a,c,1], Tim Menzen[a,*]

[a] Coriolis Pharma, Fraunhoferstr. 18 b, 82152 Martinsried, Germany
[b] Janssen Research and Development, DPD&S Biotherapeutics Development, Hochstr. 201, 8200 Schaffhausen, Switzerland
[c] Leiden Academic Centre for Drug Research (LACDR), Leiden University, PO Box 9502, 2300, RA, Leiden, the Netherlands

Contact us

Coriolis Pharma Research GmbH
Fraunhoferstraße 18 b
82152 Martinsried | Germany
Phone: +49 (0) 89 41 77 60 – 0
Andrea.Hawe@coriolis-pharma.com

Coriolis Pharma

Inherent, intrinsic and extrinsic particles

Extrinsic particles
are not part of the formulation, package, or assembly process and may originate from

Examples:
- Biological external sources (e.g., insect parts, pollens, vegetative matter)
- Building materials (e.g., non-process-related fibers, lint, minerals, paint)
- Personnel (e.g., epithelial cells, clothing fragments, hairs)

Intrinsic particles

derive from sources within the formulation ingredients, assembly process, or primary packaging materials

Examples:

- Introduced and/or not completely removed during, cleaning and preparation (e.g., elastomers from seals and gaskets, container plastic or glass shards, stainless steel parts)
- Lubricants of primary packaging components (e.g. silicone oil in siliconized syringes)
- Changes in the drug product over time, which may be related to:

i) ionic or organic extracts (e.g., leachables from rubber stoppers)
ii) instability of the active pharmaceutical ingredient (e.g., unexpected subvisible and visible protein particles)
iii) excipient degradation (e.g., fatty acid particles from degraded polysorbate)
iv) product–package interaction (e.g., glass delamination)

Inherent, intrinsic and extrinsic particles

Extrinsic particles

are not part of the formulation, package, or assembly process and may originate from

Examples:
- Biological external sources (e.g., insect parts, pollens, vegetative matter)
- Building materials (e.g., non-process-related fibers, lint, minerals, paint)
- Personnel (e.g., epithelial cells, clothing fragments, hairs)

Intrinsic particles

derive from sources within the formulation ingredients, assembly process, or primary packaging materials

Inherent particles

are (i) intentionally present (API = particle)
or (ii) expected (product-formulation-related particles characteristic of the product if their presence is measured, characterized, and determined to be part of the clinical profile).