University of Applied Sciences and Arts Northwestern Switzerland
School of Life Sciences

Limitations of analytical ultracentrifugation in the biopharma and how to overcome them

Bölsterli David
Master thesis, Chemistry

FHWN Supervisor: Prof. Dr. Oliver Germershaus
Expert: Dr. Marius Koch, Solvias AG
Company Supervisor: Dr. Alexander Aster, Solvias AG

1. Analytical ultracentrifugation (AUC)

- Critical quality attribute (CQA) of protein drugs is the aggregate content[1,2]
- Advantages AUC to complementary techniques for aggregate content
  - \+ No additional surface
  - \+ Measurement in original formulation instead of mobile phase
- Separation based on molecular weight & shape in centrifugal field

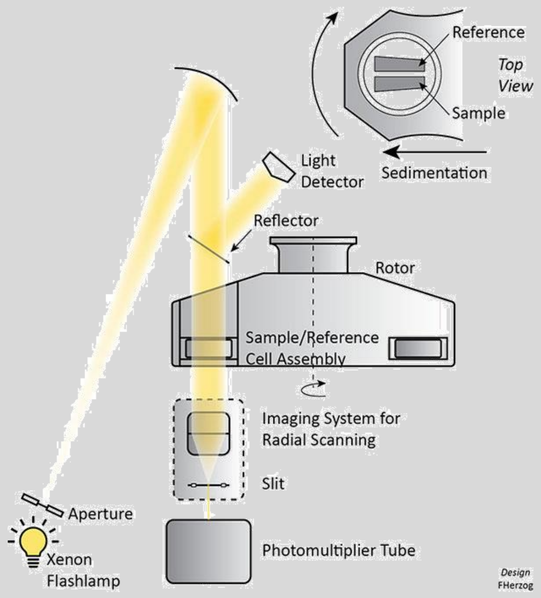


Fig 1: Schematic illustration of AUC instrument[3]

Raw data: absorbance (time, radius)

- Raw data modelled (Fig 2)

Model (see 2.):

- Extraction of underlying distribution by Lamm equation (Fig 3)

Results:

- Relative content
- Sedimentation coefficient[4,5]

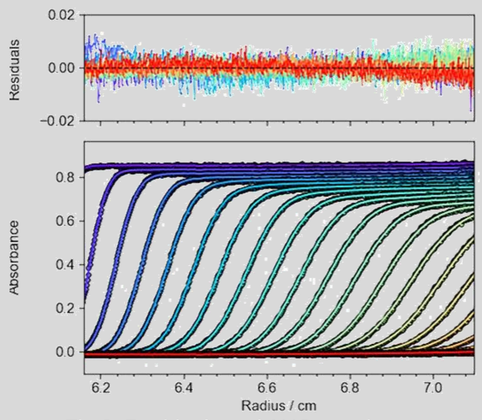


Fig 2: Residuals and AUC raw data

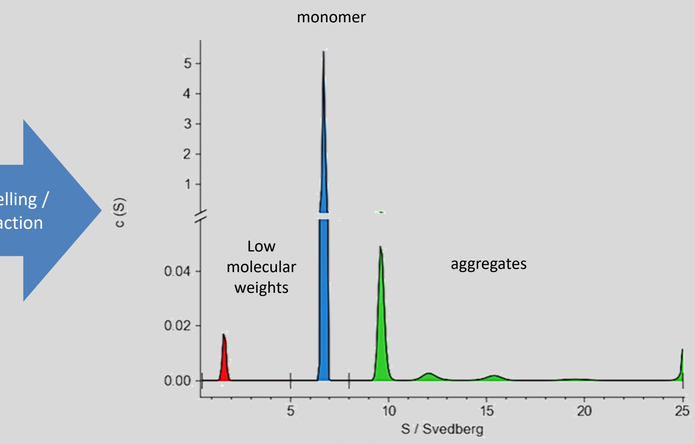


Fig 3: Species distribution

2. Model assumption

- Constant excipient concentration over time and radius

Most biological formulations:

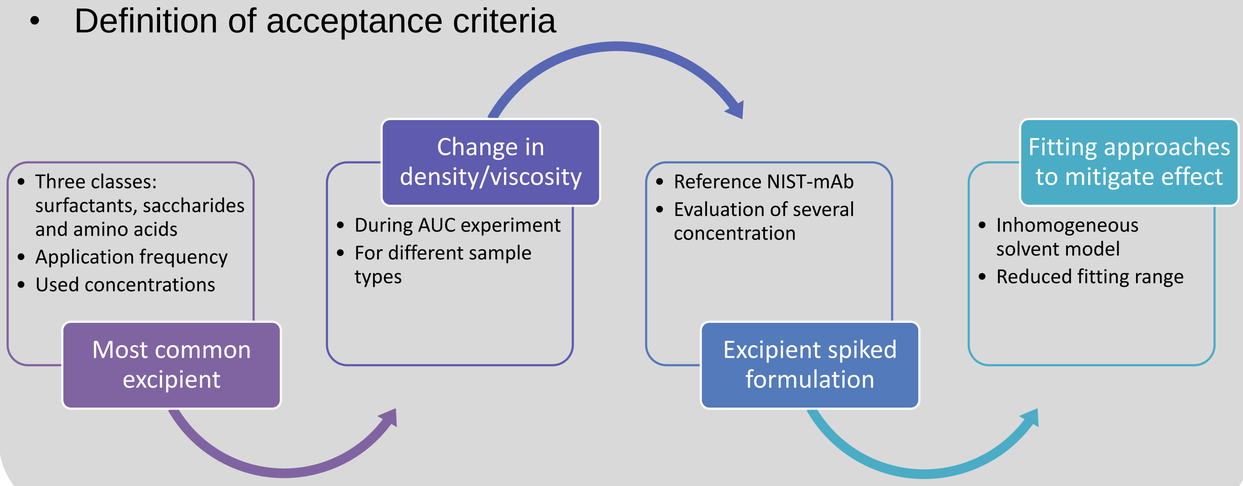
➔ high excipient concentrations ➔ can form density/viscosity gradients

➔ Inaccurate relative content determination

- Unknown which excipients can cause gradients[4-6]

3. Goals and how to get there

- Evaluation which excipients and concentration cause gradients
- Gradient manifestation in the result
- Development of mitigation strategies
- Definition of acceptance criteria



4. Mitigation strategies

Inhomogeneous solvent model
calculation of the change in density and viscosity based on the excipient's sedimentation and diffusion coefficient.

Reduced fitting range
considers only data upon the cell radius where the excipient concentration is constant[6].

5. Result and conclusion

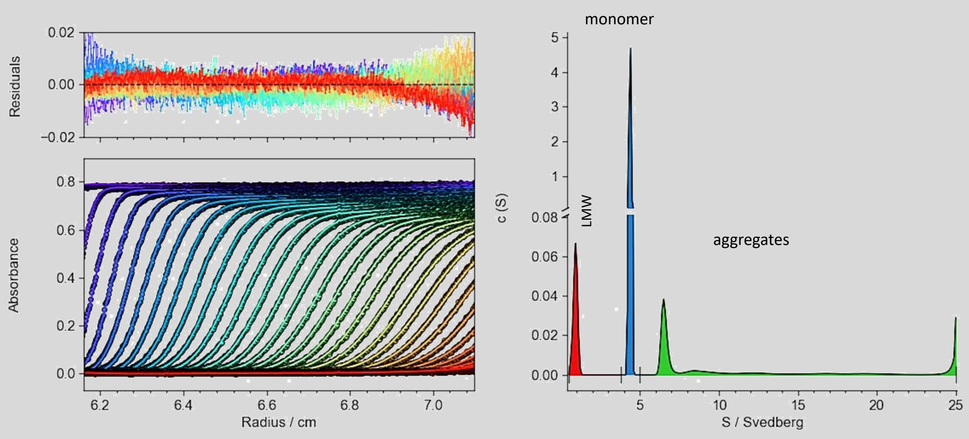


Fig 4: Example of residuals, raw data and generated distribution when a gradient is present

Gradient manifestation (Fig 4):

- Increase in residuals between model and raw data
- Appearance of LMW artifact

Gradient mitigation (Fig 5):

- Δ Density/Viscosity > 3 % ➔ significant impact in relative content determination
- Both fitting approaches ➔ corrected species content

Acceptance criteria (Fig 6):

- Density/viscosity gradient significant impact for:
  - Proteins formulated with saccharides
  - Peptides formulated with saccharides or amino acids

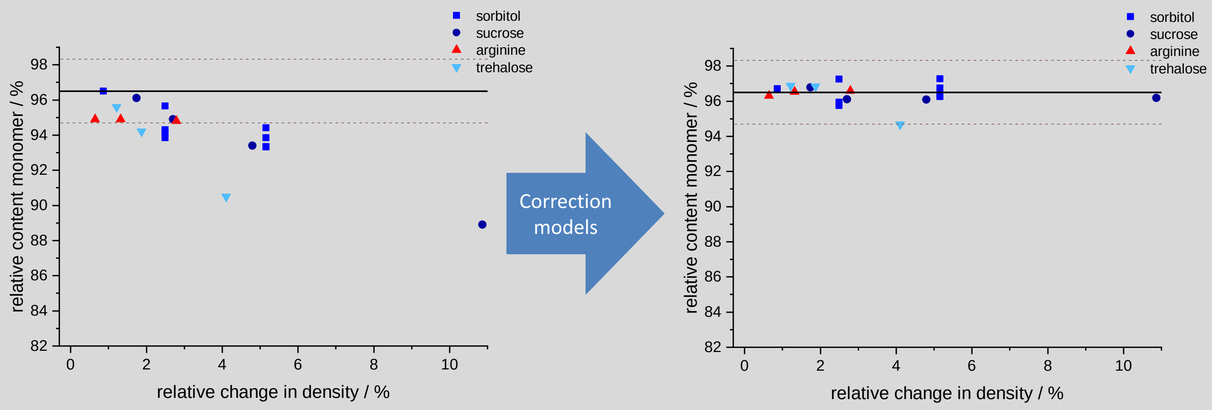


Fig 5: Relative monomer content by regular and correction model. Black line: expected content; dotted lines: expected content ± 3xSD

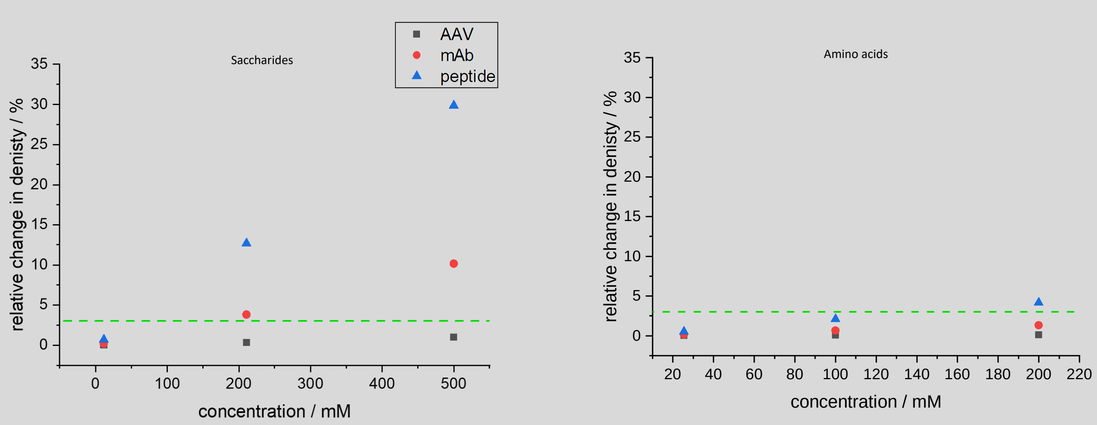


Fig 6: Δ density of excipients at min., average and max. common applied concentration

References

1. Dimitrov, D. S. Therapeutic Proteins. in *Therapeutic Proteins* (eds. Voynov, V. & Caravella, J. A.) vol. **899** 1–26 (Humana Press, 2012).
2. European Medicines Agency. Q 6 B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products. **17** (2006).
3. https://2018.igem.org/Team:Lethbridge/Experiments, 16.03.2023
4. Bou-Assaf, G. M. *et al.* Best Practices for Aggregate Quantitation of Antibody Therapeutics by Sedimentation Velocity Analytical Ultracentrifugation. *J. Pharm. Sci.* **111**, 2121–2133 (2022).
5. Brown, P. H. & Schuck, P. Macromolecular Size-and-Shape Distributions by Sedimentation Velocity Analytical Ultracentrifugation. *Biophys. J.* **90**, 4651–4661 (2006).
6. Gabrielson, J. P., Arthur, K. K., Kendrick, B. S., Randolph, T. W. & Stoner, M. R. Common Excipients Impair Detection of Protein Aggregates During Sedimentation Velocity Analytical Ultracentrifugation. *J. Pharm. Sci.* **98**, 50–62 (2009).

solvias