Structural Aspects of Immunogenicity: Aggregates

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EIP-Protein Characterization Subcommittee

Mission of the EIP-PCS:

To discuss and exchange experience with protein characterization in relation with immunogenicity, in order to increase our fundamental understanding of product related causes of immunogenicity

Current members:

Ronald Smulders (Schering-Plough/Merck)
John den Engelsman (Schering-Plough/Merck)
Partrick Garidel (Boehringer Ingelheim)
Stefan Bassarab (Boehringer Ingelheim)
Hans-Christan Mahler (Roche)
Hans Koll (Roche)
Bryan Smith (UCB)
Wim Jiskoot (Leiden University)

EIP-Protein Characterization Subcommittee

White paper:

- Definition of common strategies and methodologies for protein characterization in relation with immunogenicity
- First draft version written (to be finalized before the end of the year)

Purpose of this presentation:

- To present the topics that are currently under discussion within the EIP-PCS
- To invite you to participate in the EIP-PCS and share your experience

Outline

Physico-chemical characterization:

1. Why is it important for immunogenicity assessment
2. What types of techniques are common in the industry
3. How to apply extended characterization tests
4. When to apply extended characterization tests
5. Final conclusions

Immunogenicity assessment

Immunogenicity:

- Majority of marketed proteins products induce a human antibody response
- Clinical relevance is typically low but there have been severe incidents
- Pre-clinical models are lacking to predict the immunogenic potential of protein products

Physico-chemical characterization:

- Needed to establish product quality: what do we exactly inject into humans?
- Needed to link clinical observations to product quality
- Needed to justify “safety” specifications

Aggregates:

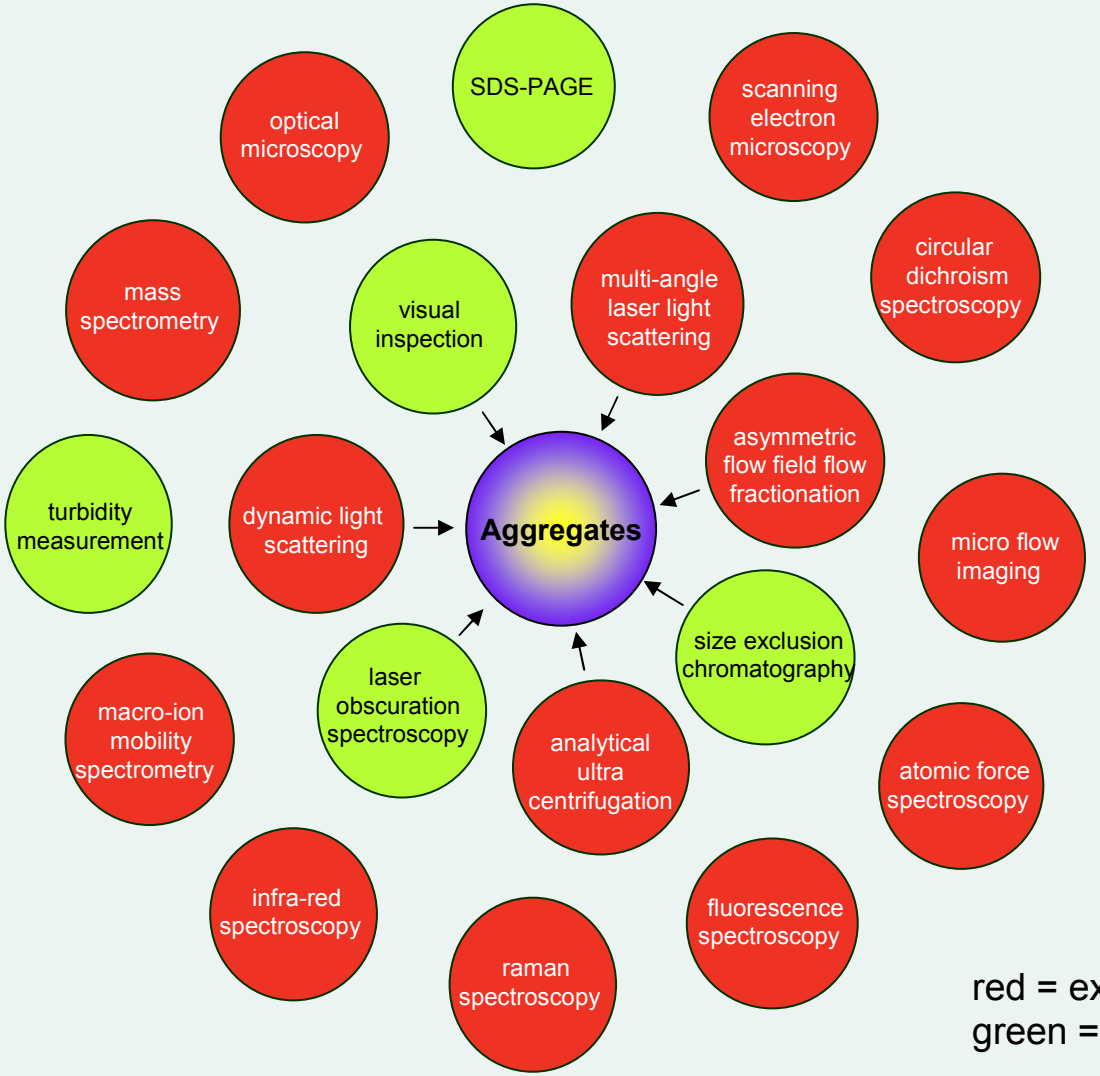
- Assumed to be a very important product-related factor causing immunogenicity
- Assemblies of protein molecules other than native quaternary structures
- Little is known about the exact structure of aggregates causing immunogenicity

Common Analytical Techniques

Physico-chemical testing for soluble and insoluble aggregates:

Type of method	Technology	ICH validation	Throughput	Data interpretation	Application
Quality control	Established	Yes	High	Operator	GMP
Extended characterization	Sophisticated	No	Low	Scientist	Development

Common analytical techniques



red = extended characterization
green = quality control

Visual inspection

Characteristics:

- Visual test: threshold for spherical particle detection is about 50 µm
- Compendial requirement: practically/essentially free from particles
- Typically performed as a 100% test by trained operators
- Test procedure described in the Ph.Eur.

Challenges:

- Probabilistic nature of the test
- No discrimination between proteinaceous particles and non-related particles
- Reject rate may depend on method/site/operators

Example: GMP batch (18000 units)

in-house VI method (2 light sources): 40% rejects
Ph. Eur. VI method (1 light source): 5% rejects

Laser obscuration testing

Characteristics:

- Determination based on particle counting: 1-100 µm
- Compendial requirements: NMT 6000 particles ≥ 10 µm, NMT 600 particles ≥ 25 µm
- Test procedure described in the Ph.Eur./USP

Current discussion industry/regulators:

- Relationship small particles (2-10 µm) and larger particles
- Variability of particle load (release vs. shelf life)
- Potential risks for safety/efficacy (particles may have adjuvant properties)

Challenges:

- Sample preparation (avoidance of contamination/artifacts)
- No discrimination between proteinaceous particles and non-related particles
- Low precision/accuracy for small particles/non-spherical particles

Size exclusion chromatography

Characteristics:

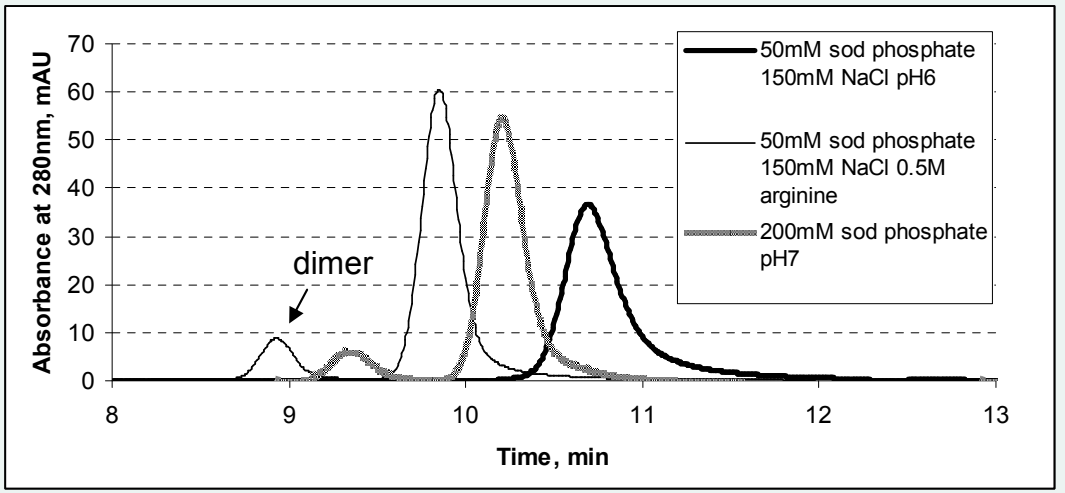
- Separation based on size and (unwanted) column interaction
- Workhorse method for development and QC applications
- Not suitable for low affinity aggregates or insoluble aggregates
- May be combined with MALLS for extended characterization (mass determination)

Challenges

- Establishment of robust separation conditions (elimination of artifacts)
- Separation of reversible aggregates
- “Creation” of relevant standards for validation studies and system suitability testing
- “Cross validation” with extended characterization tests

Size exclusion chromatography

Example: effect of chromatographic buffer on separation



	Relative peak area%		
Buffer	**Monomer**	**Dimer**	**Trimer**
50mM sod phosphate, 150mM NaCl, pH6	100	0	0
50mM sod phosphate, 150mM NaCl, 0.5M arginine, pH6	89.5	10.5	0
200mM sod phosphate, pH7	88.4	11.4	0.2
Estimate from SDS PAGE (Coomassie-stained scanned gel)	87	13	0

Asymmetric flow field flow fractionation (AF4)

Characteristics:

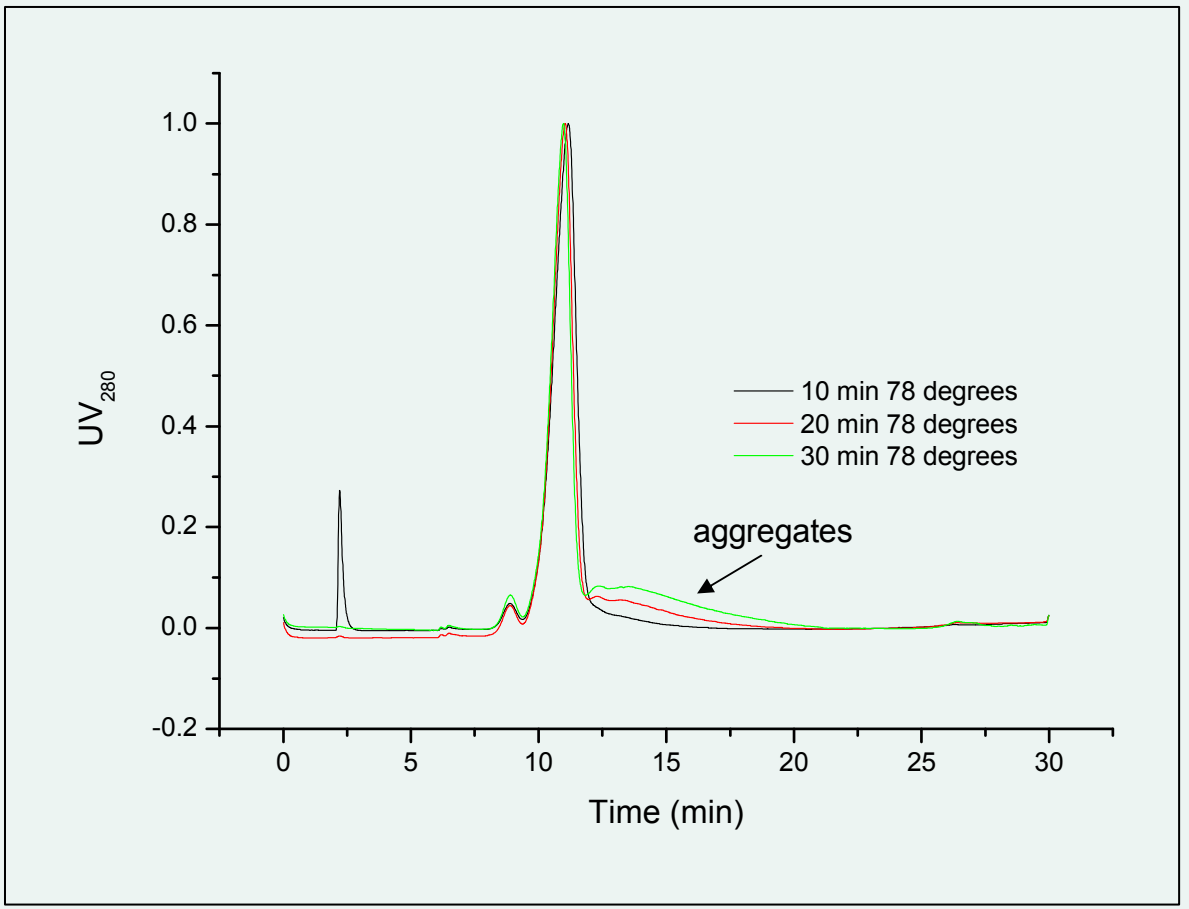
- Separation based on Brownian motion and diffusion of particles
- Chromatography like technique (separation in a flow channel + UV detection)
- Can determine particles ranging from 1 nm to 100 µm
- Can be used to determine insoluble particles (no size exclusion limit)
- Limited or no sample preparation

Challenges:

- Separation of small proteins/reversible aggregates
- Method development (success very much depends on the protein)
- Robustness (emerging technique)

Asymmetric flow field flow fractionation (AF4)

Example: effect heat stress on aggregate formation



Dynamic light scattering

Characteristics:

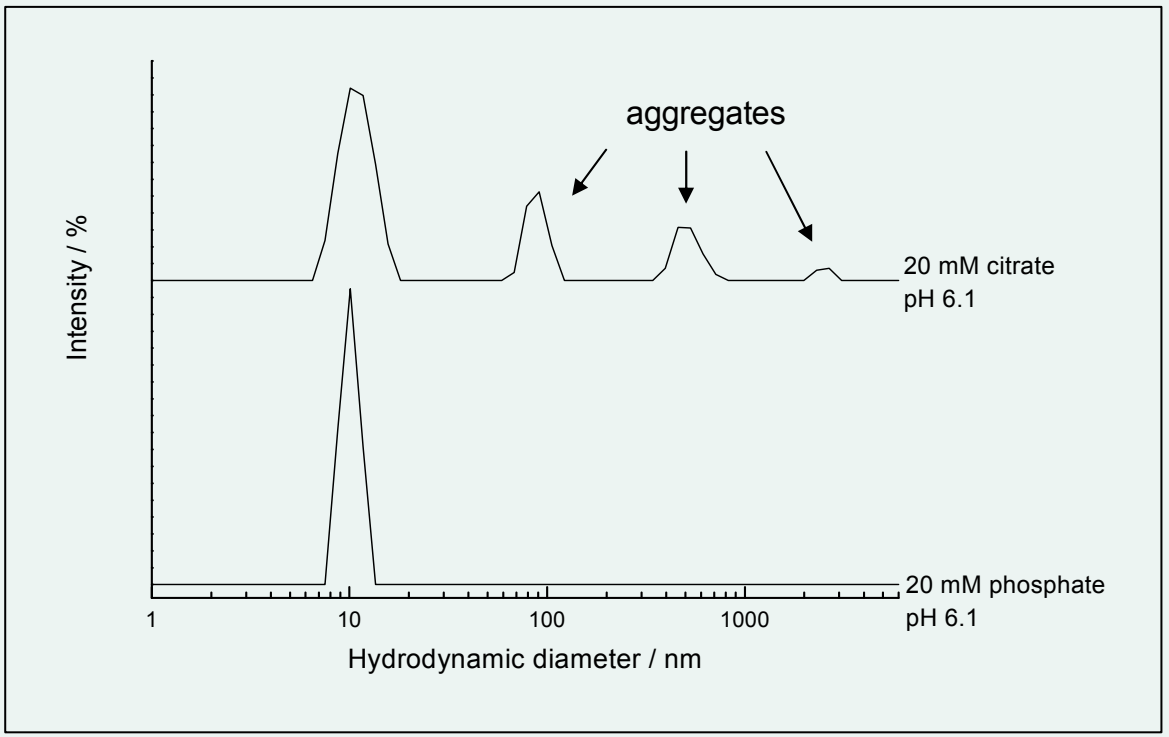
- Determination based on Brownian motion of particles
- Can be used to determine particle size distributions
- Can be used to determine hydrodynamic diameters of particles (1 nm- 5 µm)
- Very sensitive (light scattering ~ d^6) but NOT suitable for quantitation

Challenges:

- Dust contamination and excipient artifacts
- Complex data analysis
- Poor size resolution (e.g. no separation of dimers from monomers)

Dynamic light scattering

Example: effect of buffer on aggregation after agitation stress



Analytical ultracentrifugation

Characteristics:

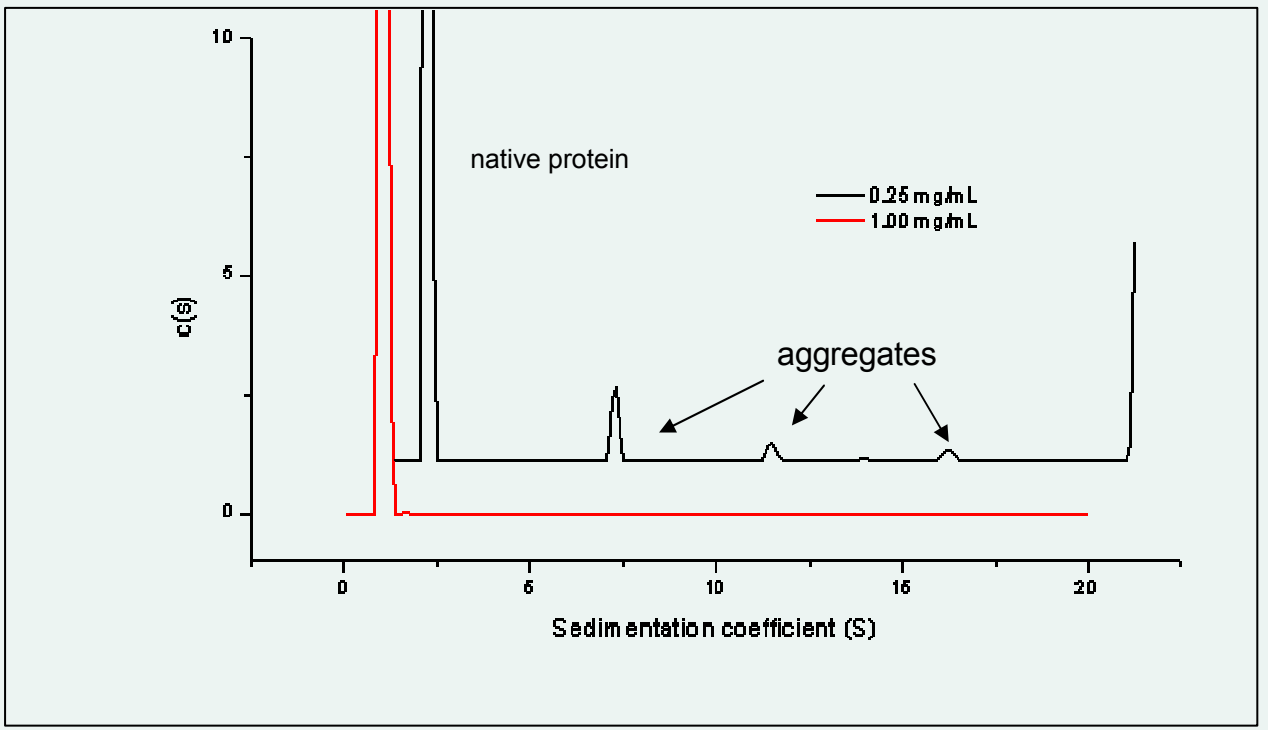
- Based on sedimentation behavior of proteins
- Seen as the “gold standard” by regulators
- Can be used for mass determination
- Can be used for quantitation
- Limited sample preparation (except for highly concentrated solutions)

Challenges:

- Complex mathematical models needed
- Relatively low sensitivity (LOQ ~ 1-2% for mAbs)
- Low throughput (few samples per run)
- Sensitive towards experimental artifacts (wear and tear of the equipment)

Analytical ultracentrifugation

Example: effect of sample dilution on aggregation



How to apply extended characterization

Product development:
- Formulation and process development
- In-use testing
- Compatibility testing

Cross validation HPSEC:
- Demonstration that HPSEC “sees” all relevant types of aggregates
- Confirmation that quantitation by HPSEC is appropriate by trending analysis

Comparability testing:
- Impact of formulation/process changes on quality
- Quality (differences) of toxicological and clinical batches
- Justification of “aggregation levels” (specification setting)

Characterization of aggregates:
- Soluble vs. insoluble, reversible vs. irreversible, covalent vs. non-covalent

When to apply extended characterization

Extended characterization tests:

- Low throughput
- Not robust (sensitive towards artifacts)
- Contradictive data are common
- Scientists rather than operators needed
- Laborious/time consuming

Example: 12 months stability study with **ONE** sample
6 time points, 3 temperatures

QC (SEC, RP, PAGE, compendial testing): 50 man-days
EC (AUC, CD, DLS, fluorescence): 30 man-days

Multiple strengths, formulations, primary packs = scientists may go mad

When to apply extended characterization

Decisions on application of extended characterization test is may be driven by:

- Complexity of the product
- Availability of resources
- Technical know how
- Priority of the development program
- CMC and clinical timelines
- Experience with regulators

Decision making should become more risk-based:

- Driven by patient safety
- The more risk the more extensive/frequent the characterization
- More in line with regulatory expectations

When to apply extended characterization

Risk based approach:

- Multidisciplinary exercise: toxicologists, clinicians and product developers
- Based on parameters that are known to impact immunogenicity such as:

Type of molecule (fully human vs. chimeric)
Route of administration (IV vs. SC)
Dose regimen (single vs. multiple dose)
Homology to endogenous counter part
Clearance rate
Patient immune status (suppressed vs. activated)

"Some bloke wants to know if we've carried out a thorough risk assessment?"

When to apply extended characterization

High risk:

- Extensive use of extended characterization tests before PoC
- Close monitoring of (pre)clinical batches with extensive characterization tests
- Very early development/cross validation HPSEC

Low risk:

- Selective use of extended characterization tests before PoC
- Sample retention strategy (low temperature storage) applied before PoC
- Retrospective characterization of relevant samples post PoC (comparability exercise)

PoC = Proof of Concept

Final conclusions

- Physico-chemical characterization is a key element of immunogenicity assessment

  absence of pre-clinical immunogenicity models
  linkage between clinical observations and quality attributes
  justification of specifications

- Extended characterization tests are laborious and require in-depth scientific expertise
- Extended characterization tests are useful throughout the whole development process
- Decisions on use of extended characterization tests should become more risk-based