Static Light Scattering and Small-Angle Neutron Scattering Study on Aggregated Recombinant Gelatin in Aqueous Solution

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Recombinant Gelatins

- Recombinant gelatins are currently being evaluated as pharmaceutical excipients.
- Behavior of recombinant gelatins in solution is not well investigated, but important for potential pharmaceutical applications.
- Suitable techniques for analyzing the solution behavior of recombinant gelatins are required.

Objectives

To evaluate the usefulness of combining static light scattering (SLS) and small-angle neutron scattering (SANS) for detecting aggregation of recombinant gelatin in aqueous solution and to obtain structural information about the aggregates.

Recombinant Gelatin: RG-15-His

- Expression in recombinant yeast cells (Pichia pastoris)
- Molecular mass = 15 kDa
- 161 amino acids
- Gelatin-typical Gly-X-Y triplets
- Amino acid sequence is given in patent WO02052342

Amino Acid	% (by number)
Ala	1.2
Asn	11.8
Glu	5.2
Gln	15.5
Gly	34.2
His	3.7
Lys	1.9
Pro	25.2
Ser	5.6

Preparation of RG-15-His Solutions and Aggregate Formation

- 2% and 0.2% (w/v) solutions in H_2O and D_2O
- Filtration through 0.2 μm filters

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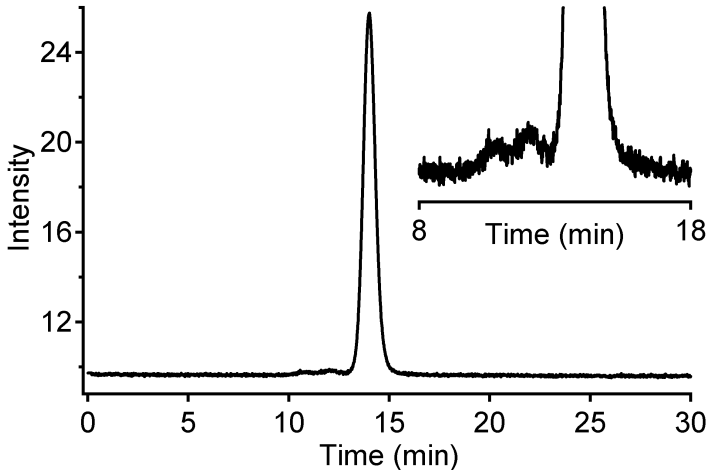
- GPC:



- Few small aggregates detected by dynamic light scattering (DLS).

Storage for 24 h at 8°C

↓

- Large aggregates detected by DLS.
- Hydrodynamic radii of several hundred nanometers.
- Highly polydisperse.

Analysis by Circular Dichroism and Fourier Transform Infrared Spectroscopy

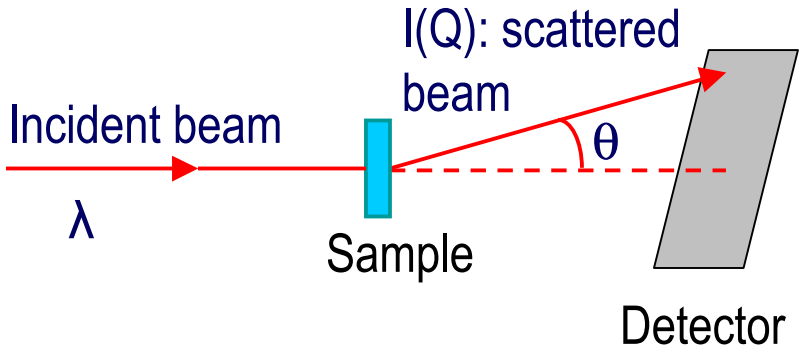
Method	RG-15-His secondary structure	RG-15-His aggregation detectable?
Circular dichroism (CD)	unordered	no
Fourier transform infrared spectroscopy (FTIR)	unordered, many turns	no

RG-15-His aggregation does not depend on the formation of particular secondary structures (e.g. helices or β-sheets).

Static Light Scattering (SLS) and Small-Angle Neutron Scattering (SANS)

SLS : Laboratory technique

SANS: Requires a neutron source (e.g. reactor)

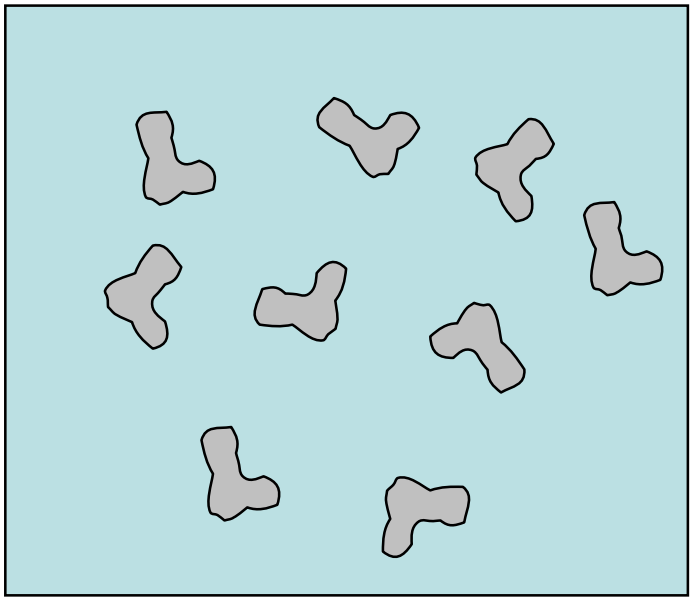


Scattering vector:

$$Q = \frac{4\pi}{\lambda}\sin\left(\frac{\theta}{2}\right)$$

Scattered intensity (I) is measured as a function of the scattering vector (Q), which makes data from SLS and SANS combinable.

Information Provided by SLS and SANS



Suspension of particles (e.g. protein aggregates) in a homogeneous medium

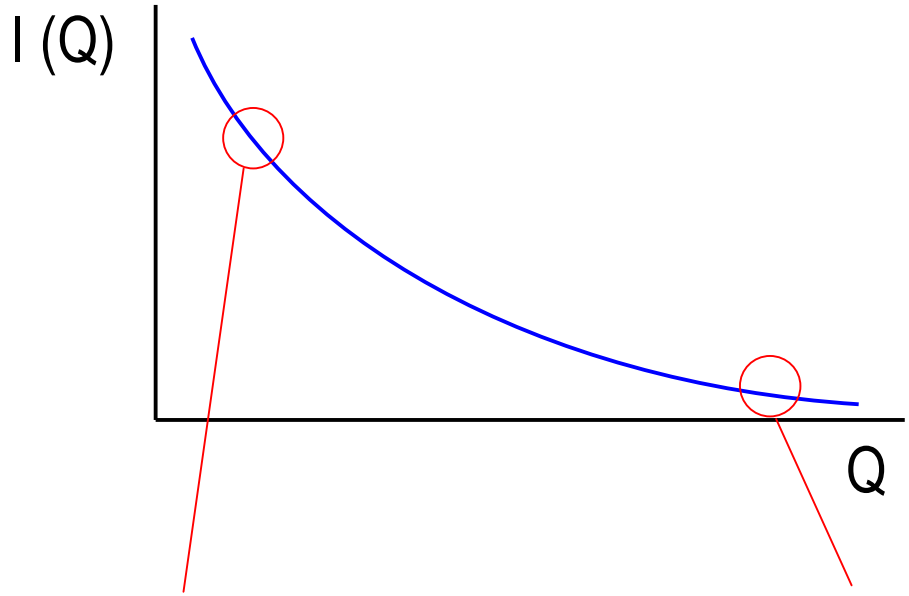
- ⇒ Particle size
- ⇒ Particle shape
- ⇒ Interactions between particles (at high concentration)
- ⇒ Molecular weight

Size Range of SLS and SANS

SANS ~ 5 – 1000 Å

SLS ~ 100 – 1500 Å

$$D = 2\pi/Q$$



Large particles (e.g. aggregates)

Small particles (e.g. individual molecules)

Scattering Function

- N = Number concentration of particles
- V = Particle volume

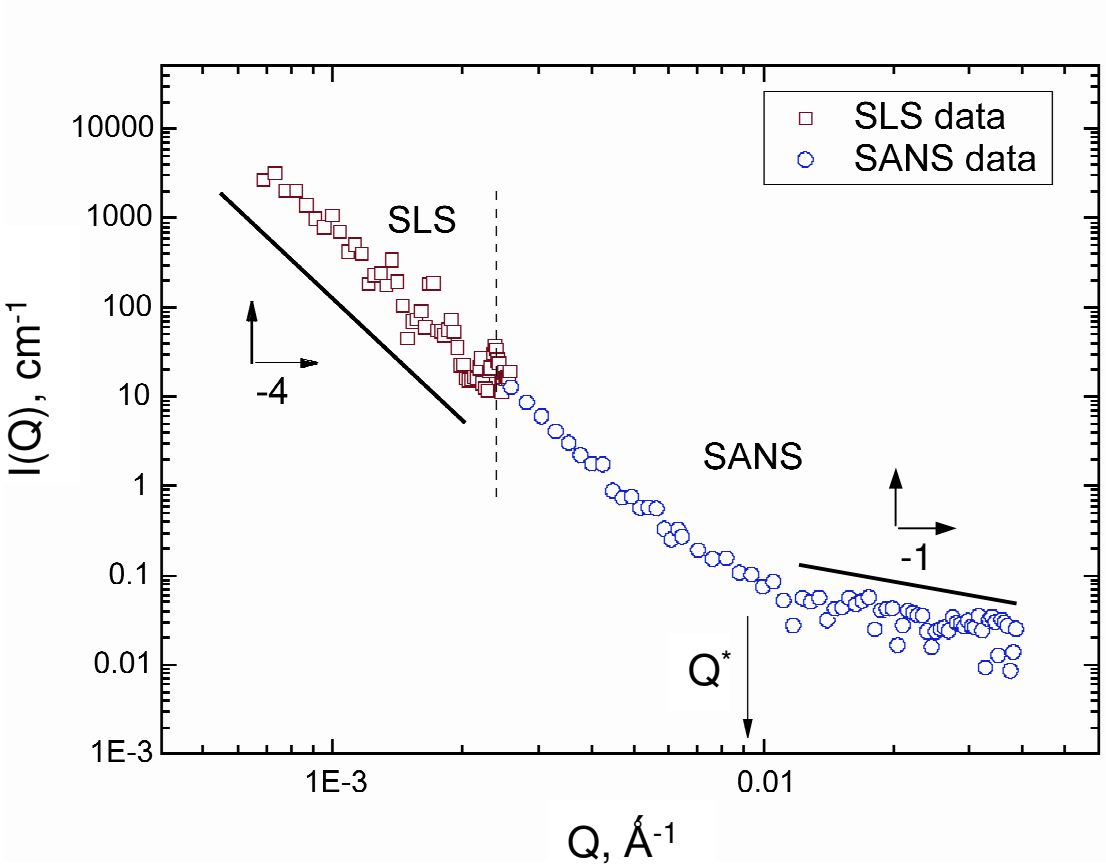
- Scattering length density difference (contrast factor)
- Can be calculated.

$$I(Q) = N \cdot V^2 \cdot (\rho_{particle} - \rho_{solvent})^2 \cdot P(Q) \cdot S(Q)$$

- Form factor
- Particle size and shape
- Form factors for various geometries are known and can be used for analyzing measured data.

- Structure factor
- Particle-particle interactions
- In dilute solutions: $S(Q) \rightarrow 1$

2% RG-15-His Solutions Upon 24 h Storage

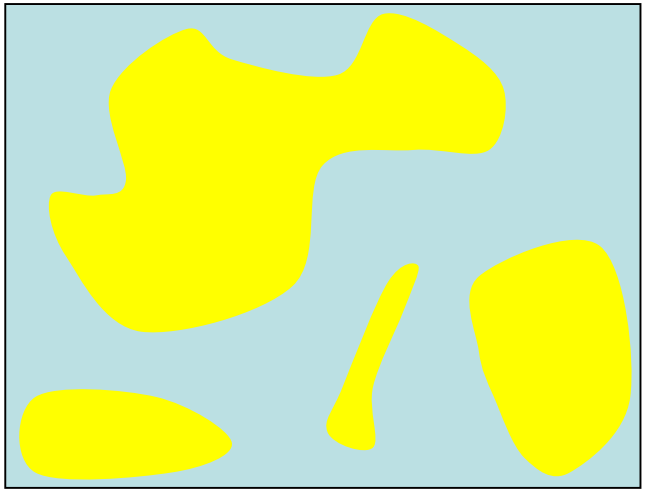


- At Q<Q*, the slope is -4, which is characteristic for particles with sharp interfaces (Porod's law).
- No plateau at low Q.
- Aggregates exceed upper size limit of instrumentation.
- At Q>Q*, the slope is -1, which is characteristic for rigid rods.

Data Analysis at Q<Q*

Real space

Two phase system (aggregates and solvent)



- Irregular aggregate shapes
- Irregular aggregate dimensions

Q-Space

Debye-Bueche model:

$$I(Q)^{-1/2} = I(0)^{-1/2} + I(0)^{-1/2} \cdot \Xi^2 \cdot Q^2$$

where

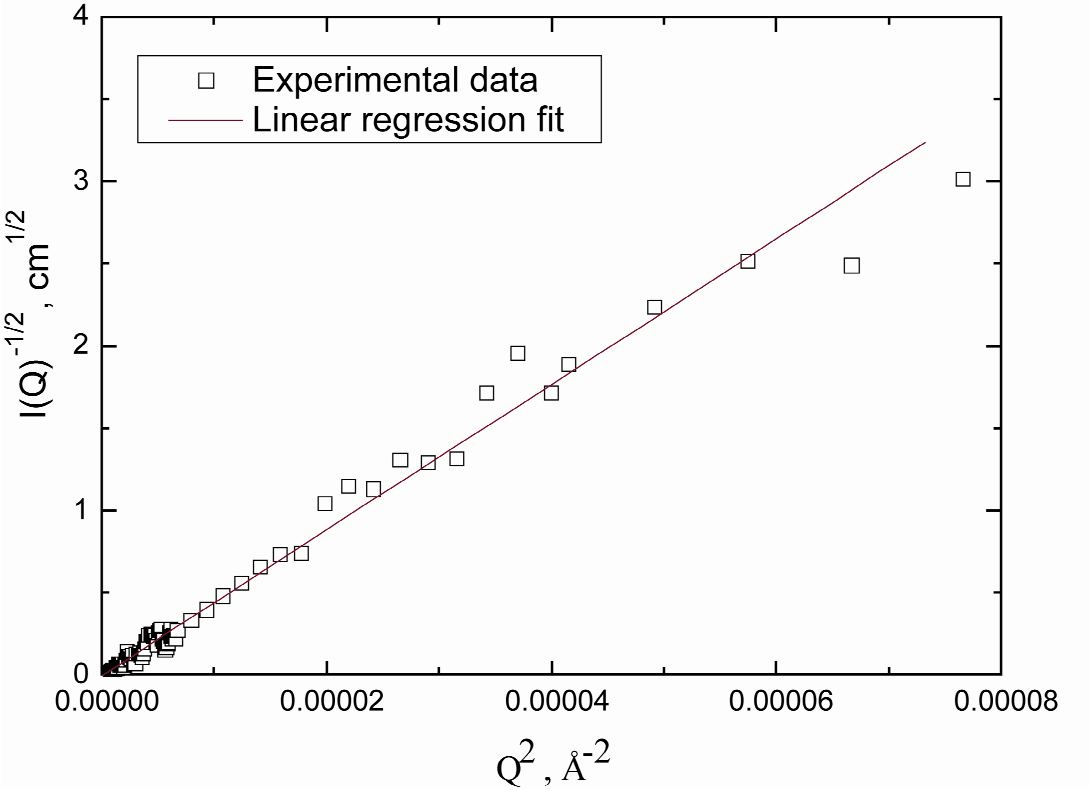
I(0) is the scattered intensity at Q = 0

and

Ξ is a characteristic distance that is a measure of aggregate dimensions.

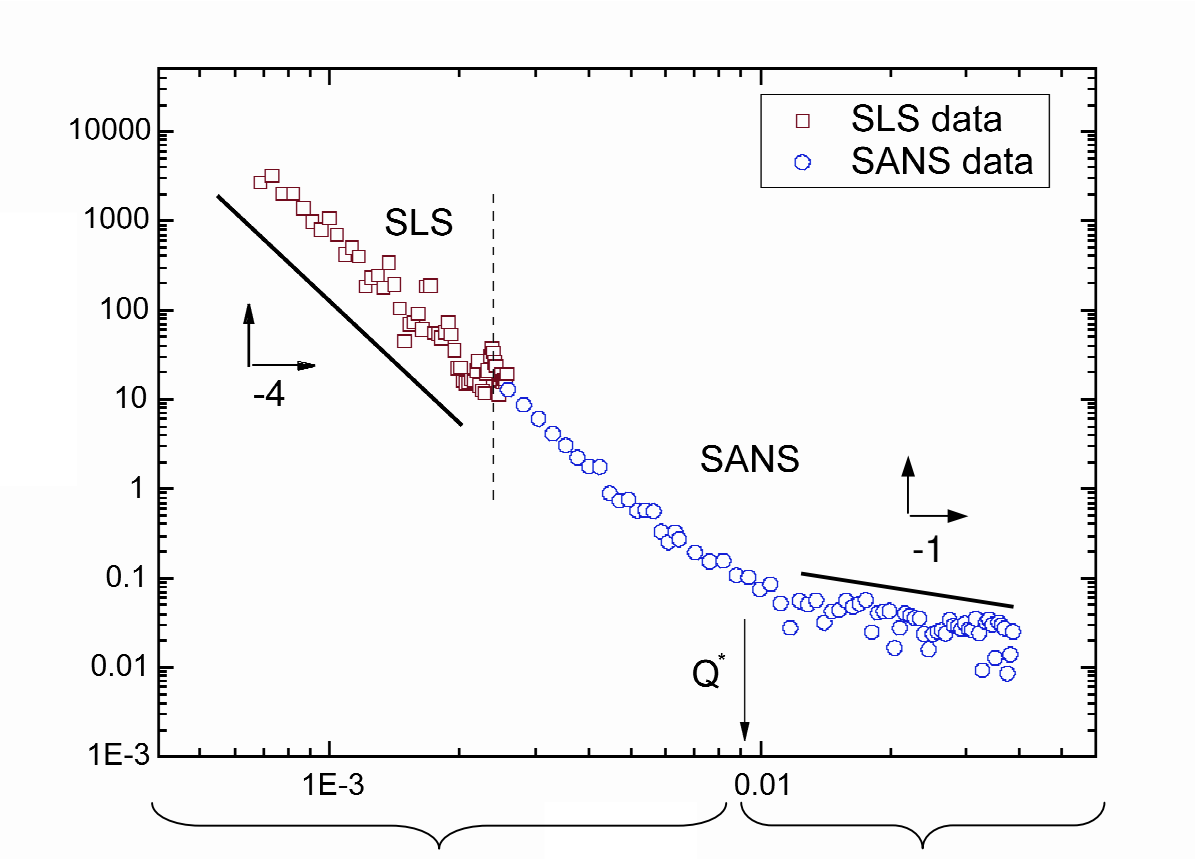
Data Analysis at Q<Q*

Analysis with Debye-Bueche model



Ξ = 332 nm

Data Analysis at Q>Q*



Scattering from large aggregates with sharp interfaces

Scattering from rigid rods

Length of the rigid rods

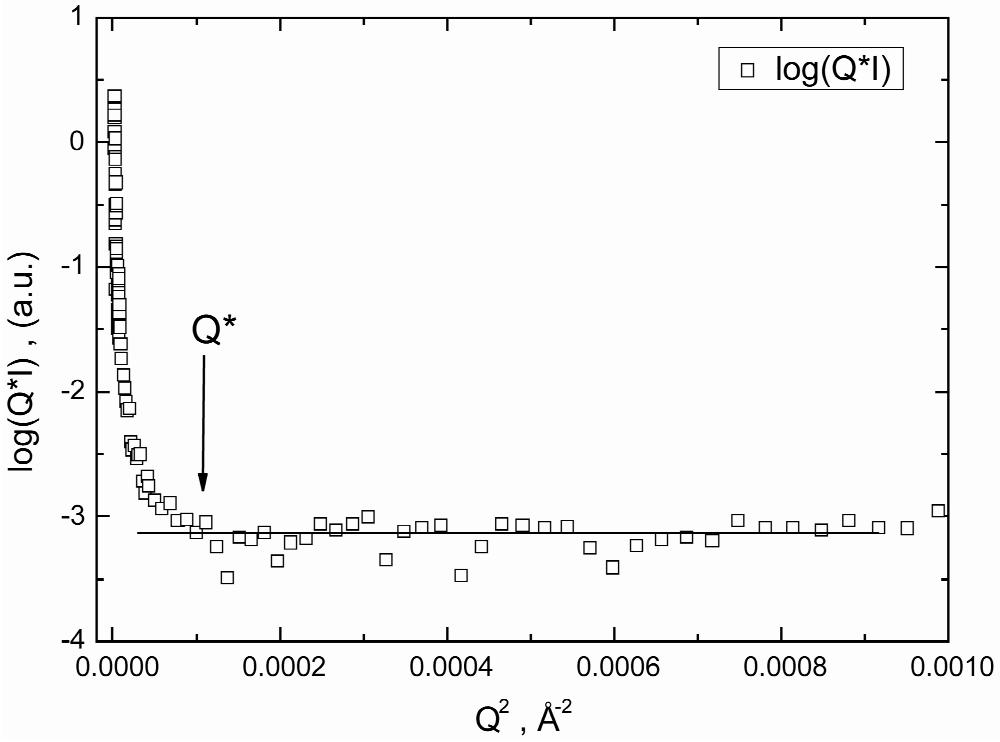
$= 12/\pi \cdot Q^*$

$= 37 \pm 5$ nm

Data Analysis at Q>Q*

For rod like structures with negligible cross-sectional radius, the logarithm of (Q·I(Q)) is independent of Q^2.

(Kratky and Porod)



Interpretation of RG-15-His Aggregation

- RG-15-His in aqueous solution forms very large aggregates with sizes $\geq$ 300 nm.
- Large aggregates grow by the association of very thin, rigid rods of about 37 nm length.
- As a consequence of their geometry, the rods are likely composed of stretched and tightly packed RG-15-His molecules.
- This aggregation behavior is not previously reported for gelatins.

Usefulness of Combining SLS and SANS

- The combination of SLS and SANS provided detailed insight into the structure of RG-15-His aggregates.
- The advantages of combining SLS and SANS are the broad size range that is experimentally accessible, the independence of signals on specific secondary structures, and the non-invasive nature of the measurement procedure.
- The combination of SLS and SANS is a potent complementary approach for studying the solution behavior and aggregation of other pharmaceutically interesting biomolecules.

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