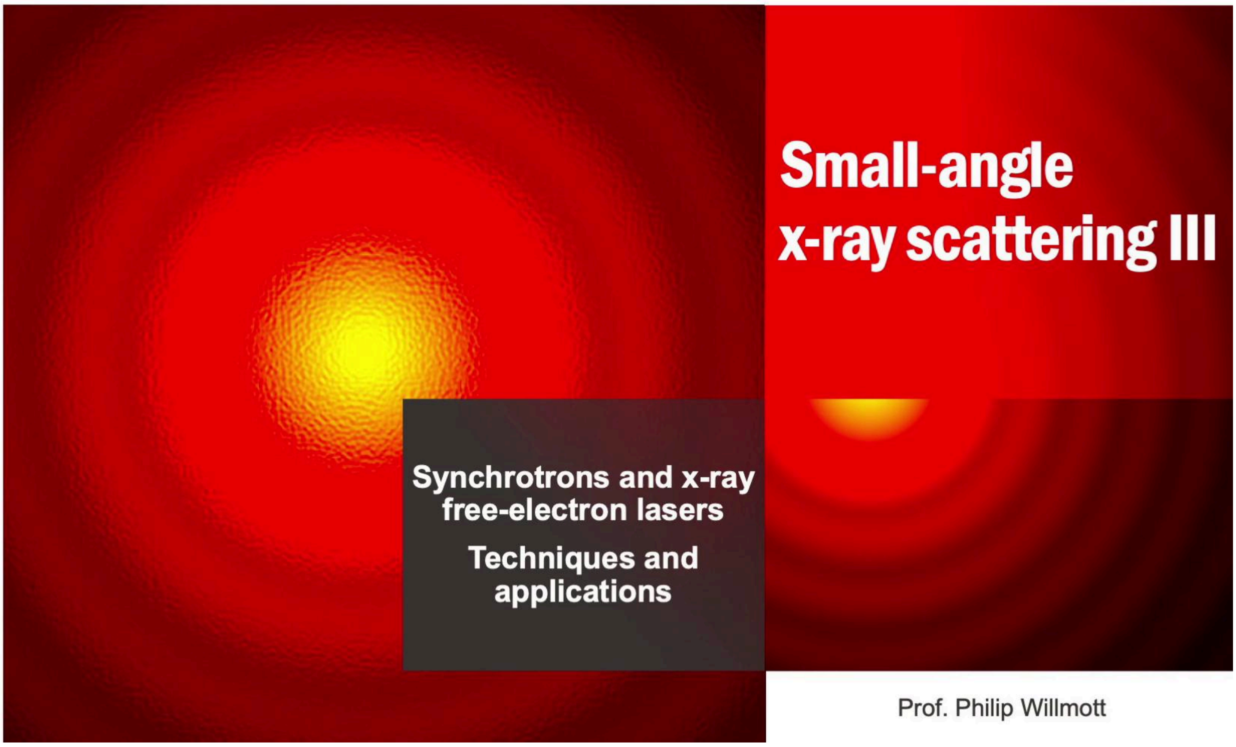
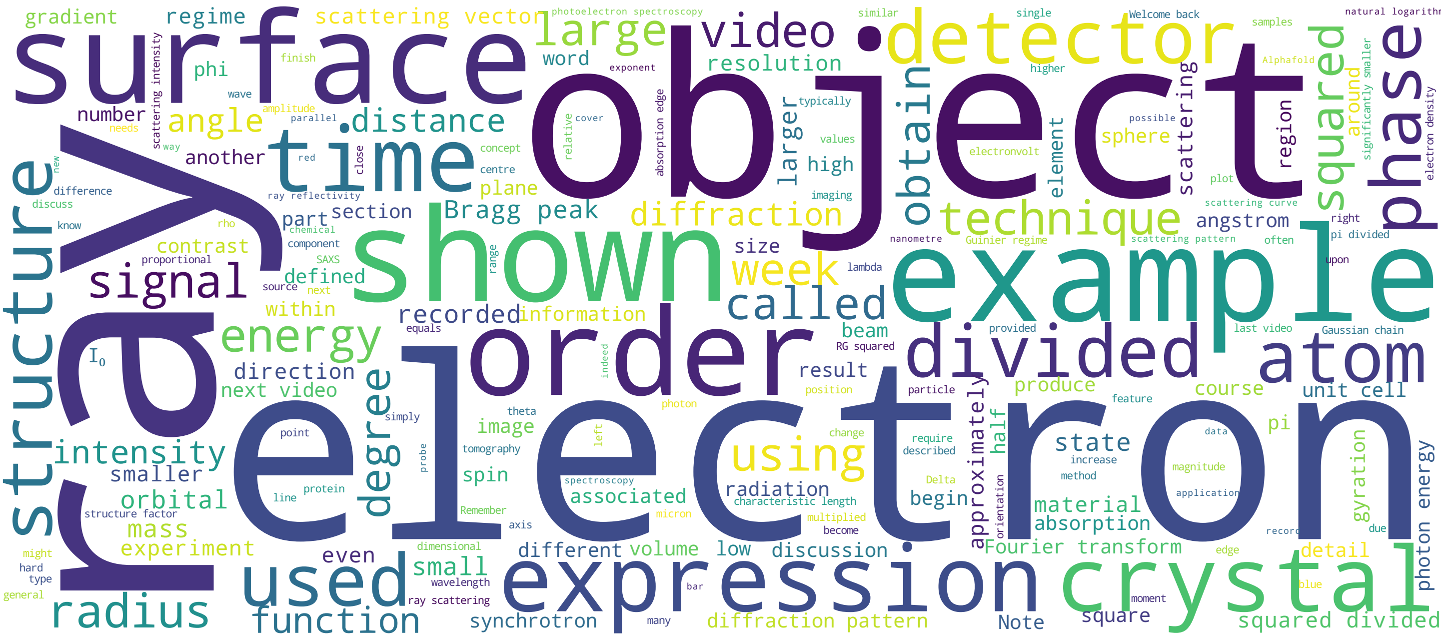
École polytechnique fédérale de Lausanne



Prof. Philip Willmott



Search MOOC

Video

Contents and objectives of this video

- Radius of gyration
- Guinier regime
- Porod regime
- Polydispersity
- Experimental details

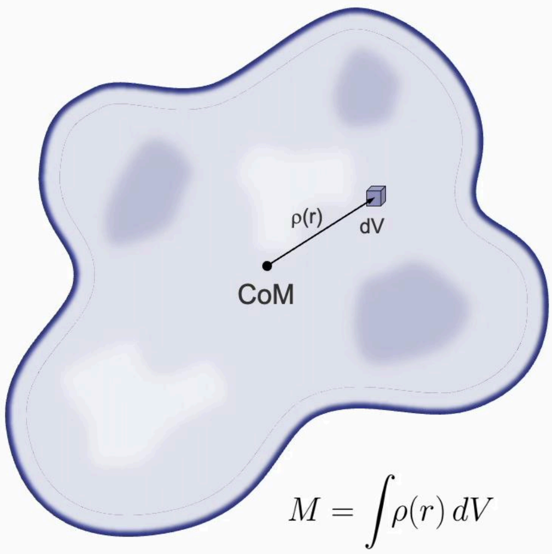
Hello and welcome back to the last video on SAXS. Well, this isn't strictly true, as the next video, the second last video of this week, is concerned with X-ray reflectivity, which is actually a form of SAXS in which the response of reflected X-rays at shallow angles is recorded. But that's for the next video. In this one, we will consider the so-called radius of gyration, which informs us about general dimensions in the low Q or Guinier regime. We will look at the other extreme of the scattering intensities at values of Q which are large compared to characteristic lengths of the sample, the effect of varying particle size, and so-called polydispersity, and finally, make one or two remarks about experimental aspects of SAXS measurements.

Notes

Summary

0m 05s

Radius of gyration



$$M = \int \rho(r)\, dV$$

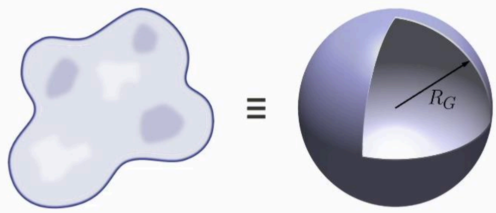
- Moment of inertia

$$I = \int \rho(r) r^2\, dV$$

- Radius of gyration

$$I = M R_G^2$$

$$\Rightarrow R_G = \left[\left(\int \rho(r) r^2\, dV \right) / \left(\int \rho(r)\, dV \right) \right]^{1/2}$$



Object, mass M

Hollow shell, mass M, radius R_G

So we begin this video with the concept of the radius of gyration, which is, in mechanical terms, equal to the root mean square distance, of the object's parts from its centre of mass. Consider some object. It will have a centre of mass at which the masses of the object's components are perfectly balanced. The total mass of the object is simply the mass-density distribution rho r integrated over the volume of the object. The moment of inertia, on the other hand, is given by the integration of the mass density in a volume element dV, multiplied by the square of the distance of that element from the centre of mass. The radius of gyration, RG, is defined, therefore, as the radius associated with the moment of inertia I and the mass M, and is therefore given by the expression for the moment of inertia divided by the mass all to the power of a half. One can therefore consider the radius of gyration of an object of mass M, to be the radius of a hollow shell with infinitesimal shell thickness with the same mass as the object, all concentrated in the shell wall.

Notes

Summary

0m 53s

Radius of gyration

Object	R_G^2
Solid sphere radius r	$\frac{3}{5}r^2$
Hollow sphere radii r_1 and $r_2 > r_1$	$\frac{3}{5}\frac{r_2^5 - r_1^5}{r_2^3 - r_1^3}$
Solid cylindrical rod radius r, height L	$\frac{r^2}{2} + \frac{L^2}{12}$
Solid rectangular beam width W, height H, length L	$\frac{W^2 + H^2 + L^2}{12}$
Hollow tube radii r_1, r_2, height L	$\frac{r_1^2 + r_2^2}{2} + \frac{L^2}{12}$
Solid ellipsoid semi-axes a, b, c	$\frac{a^2 + b^2 + c^2}{5}$
Hollow ellipsoid outer semi-axes a, b, c, inner semi-axes αa, βb, γc	$\frac{(1-\alpha^3\beta\gamma)a^2 + (1-\alpha\beta^3\gamma)b^2 + (1-\alpha\beta\gamma^3)c^2}{5(1-\alpha\beta\gamma)}$
Solid elliptical cylinder semi-axes a, b, height L	$\frac{a^2 + b^2}{4} + \frac{L^2}{12}$
Hollow elliptical cylinder outer semi-axes a, b, outer height L, inner semi-axes αa, βb, inner height γL	$\frac{3(1-\alpha^3\beta\gamma)a^2 + 3(1-\alpha\beta^3\gamma)b^2 + (1-\alpha\beta\gamma^3)L^2}{12(1-\alpha\beta\gamma)}$
Randomly folded polymer chain, n monomers of length a	$\frac{a^2 n}{6}$

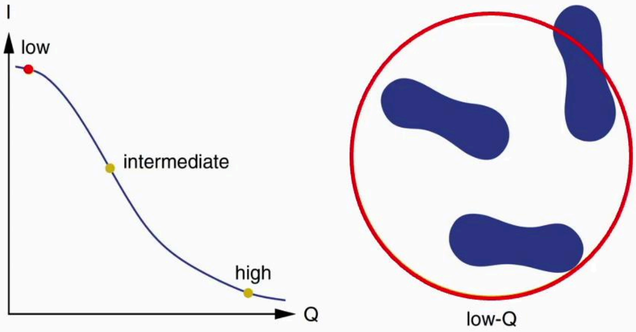
I have provided here expressions for the radius of gyration for different geometrical objects. Please look through this table at your own leisure.

Notes

Summary

2m 23s

Guinier regime



- $Q \ll 2\pi/a$
  - a = characteristic length of scattering object
  - Details of object shape excluded
  - Only information on overall size
    - i.e., radius of gyration
- Goal: simple expression for I(Q) for $Q \ll 2\pi/a$
  - Choose particle type with I(Q) that lends itself to simplification
    - Sphere!

$$I_{\text{sph}} = I_0 \left(\frac{3\left[\sin(Qa) - Qa\cos(Qa)\right]}{(Qa)^3} \right)^2$$

    - Use

$$\sin(x) \approx x - x^3/6 + x^5/120 - \cdots$$
$$\cos(x) \approx 1 - x^2/2 + x^4/24 - \cdots$$

We now consider the Guinier regime, for which the scattering vectors are significantly smaller than 2 pi divided by a, where a is the characteristic length of the object being investigated. It should come as no surprise that in such a situation, one cannot hope to obtain information about the details of the object shape, but instead, only information about the overall size, in other words, the radius of gyration. Now, our goal in the following exercise is to obtain a simple expression for the scattering intensity in this regime. Now, in order to make our lives a little simpler, we should choose a particle shape that lends itself to mathematical simplification. As you will see immediately, the obvious candidate for this is the solid sphere. We know what the expression is for scattering from a sphere, from the last video, and it is given once more here. First of all, we will substitute Qa, a dimensionless quantity, with x. Remember that we are working in the regime where Qa equals x is much smaller than unity. So in this case, expressions for sine x and cos x can be simplified by their Taylor expansions shown here and also detailed, in the supplementary materials trigonomic approximations.

Notes

Summary

2m 34s

Guinier regime

$$I_{\text{sph}}(x \equiv Qr \ll 1) \approx I_0 \left(\frac{3\left[\cancel{x} - x^3/6 + x^5/120 \cdots - \cancel{x} + x^3/2 - x^5/24 + \cdots\right]}{x^3} \right)^2$$

$$= I_0 \left(\frac{3\left[x^3/3 - x^5/30\right]}{x^3} \right)^2$$

$$= I_0 \left(1 - x^2/10\right)^2 \approx I_0 \left(1 - x^2/5\right)$$

But for a sphere of radius r,

$$R_G^2 = \frac{3}{5} r^2$$

$$\Rightarrow I_{\text{sph}}(Qr \ll 1) = I_0 \left(1 - Q^2 R_G^2/3\right)$$

$$\boxed{\approx I_0 \, \exp(-Q^2 R_G^2/3)}$$

We place these approximations, in our expression for the scattering intensity. We need to include terms up to x to the five in the numerator, as these need to be of higher order than the x-cube term in the denominator. The first thing we can do, is cancel out the two linear terms x. This then simplifies to the expression given here, which further simplifies to I_0, 1 minus x squared upon 10, all squared. But we also know that the general expression 1 plus x to the N, is approximately equal to 1 plus Nx for x much less than 1. Hence the scattering intensity is closely approximated by I_0, one minus x squared divided by 5. But x is equal to Qr, and so x squared is equal to Q squared r squared. Also, the square of the radius of gyration of a solid sphere is equal to three-fifths the square of the sphere's radius. We therefore obtain a scattering profile at low Q of I_0, 1 minus Q squared r, G squared divided by 3. We're nearly there. We now make the final approximation that e to the x is equal to 1 minus x and obtain a scattering profile equal to I_0, exponent minus Q squared RG squared divided by 3. Now, remember that we only chose the sphere as our object, as it is described by an expression that is relatively easy to manipulate.

Notes

Summary

4m 08s

Guinier regime

$$I_{\mathrm{sph}}(x \equiv Qr \ll 1) \approx I_0 \left(\frac{3\left[\cancel{x} - x^3/6 + x^5/120 \cdots - \cancel{x} + x^3/2 - x^5/24 + \cdots\right]}{x^3} \right)^2$$
$$= I_0 \left(\frac{3\left[x^3/3 - x^5/30\right]}{x^3} \right)^2$$
$$= I_0 \left(1 - x^2/10\right)^2 \approx I_0 \left(1 - x^2/5\right)$$

But for a sphere of radius r,

$$R_G^2 = \frac{3}{5} r^2$$
$$\Rightarrow I_{\mathrm{sph}}(Qr \ll 1) = I_0 \left(1 - Q^2 R_G^2/3\right)$$
$$\boxed{\approx I_0 \exp(-Q^2 R_G^2/3)}$$

- Plot ln[I(Q)] v Q^2
  - Gradient = $-R_G^2/3$
- N.B. Valid for ALL objects, not only spheres!!

We've stated that at these low-scattering vectors, we can't know anything about the detailed shape of the scattering object. It therefore follows that the highlighted equation, is valid for all objects, not only spheres. If we plot the natural logarithm of the scattering curve as a function of the square of Q, we should therefore obtain a linear gradient in the low Q regime of minus RG squared divided by 3.

Notes

Summary

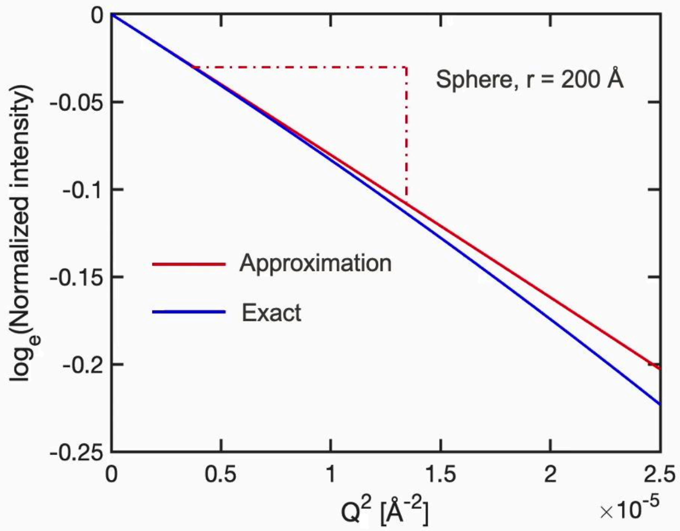
5m 54s

Guinier regime

- Plot ln[I(Q)] v Q^2
  - Valid for
    - $Q \ll 1/200$ Å^{-1}
    - $Q^2 \ll 2.5 \times 10^{-5}$ Å^{-2}
  - Gradient = $-R_G^2/3$

  - $R_G^2/3 = (0.20115/2.5 \times 10^{-5})$ Å^2
    $= 8.0459 \times 10^3$ Å^2

  $R_G^2/3 = r^2/5 \Rightarrow r =$ **200.6 Å**



If we look at the example of a sphere of radius of 200 angstroms, and take the gradient to the tangent shown in red, to the real curve shown in blue, we get a value of 8.059 times 10 to the 3 square angstroms, leading to a radius which is predicted to be 200 angstroms, almost identical to the actual value.

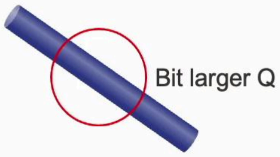
Notes

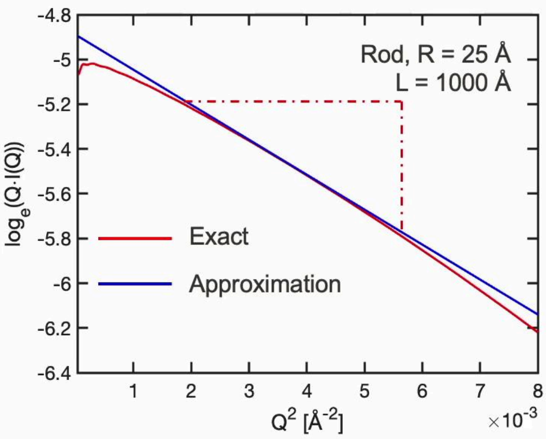
Summary

6m 22s

Extended Guinier regime for rods

- Objects with widely differing characteristic lengths
  - Thin rods L ≫ R
  - Flat platelets R ≫ H





$$I(Q) = \frac{AI_0}{Q}\exp\left(-\frac{R_G^2 Q^2}{2}\right); \quad QL > 2\pi;\ QR \ll 2\pi$$

$$R_G = R/\sqrt{2}$$

Gradient = -160
R = 25.3 Å

If objects have widely different characteristic lengths, such as flat platelets and thin rods, we can obtain information in a so-called extended Guinier regime at moderately larger Q values. Without going into the derivation, it can be shown that for thin rods, for example, for the regime where QL is larger than 2 pi and QR is significantly smaller than 2 pi, the scattering intensity is well-approximated by AI_0 divided by Q, multiplied by the exponent of minus RG squared Q squared divided by 2. A plot of the logarithm of Q multiplied by IQ against Q squared delivers a gradient of minus RG squared upon 2. But RG is equal to R divided by root 2, hence, the gradient is equal to R squared divided by 4. In the example shown here, the range of Q squared begins at 2 pi divided by 1,000, or approximately 4 times 10 to the minus 5 reciprocal square angstroms, while the upper limit should be well below 2 pi divided by 25 squared or 0.06. The grazing tangent in this regime has a gradient of approximately 160, leading to a rod radius of 25.3 angstroms, also a pretty darn good estimate of reality.

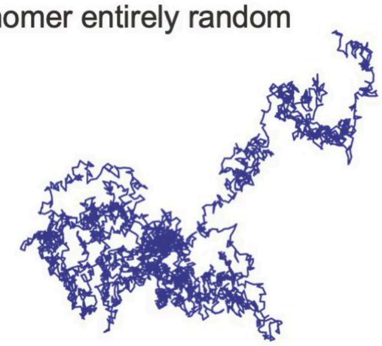
Notes

Summary

6m 49s

Gaussian chains – Guinier regime

- Polymers consisting of a single chain of monomers with the orientation of each monomer entirely random



n links, each length a, random orientation
"Gaussian chain"

$$I(Q) = \frac{2I_0}{\phi^2}\left[\exp(-\phi) + \phi - 1\right],$$

whereby $\phi = Q^2 a^2 n/6$

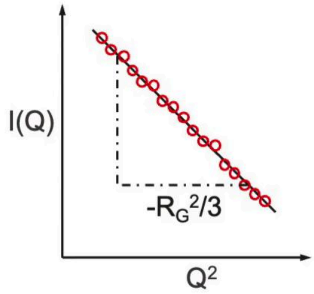
- Small Q:

$$I(Q) \approx \frac{2I_0}{\phi^2}\left(\underbrace{1 - \phi + \phi^2/2 - \phi^3/6 + \cdots}_{\approx \exp(-\phi)} + \phi - 1\right)$$

$$= I_0\,(1 - \phi/3) \approx \exp(-\phi/3) = \exp(-a^2 n Q^2/18)$$

$$\Rightarrow R_G^2 = a^2 n/6$$

- Plot I(Q) v Q^2 to determine R_G



We finish our discussion of the Guinier regime, with this discussion of Gaussian chains. For polymers that have elements or monomers whose orientation is completely random, that is, not influenced by their neighbouring monomers in the chain, this can be described by a so-called Gaussian chain. Note, by the way, that proteins in their natural state in vivo do not satisfy the condition, and only approximate it, when they are denatured, such as when bathed in a strong acid or alkaline solution. According to the expression for the scattering curve of a Gaussian chain, a Taylor expansion of the exponent term allows one to simplify the expression to be I_0 is equal to the exponent of minus RG, Q squared divided by 3, and hence a plot of IQ as a function of Q squared, will produce a gradient of minus RG divided by 3.

Notes

Summary

8m 27s

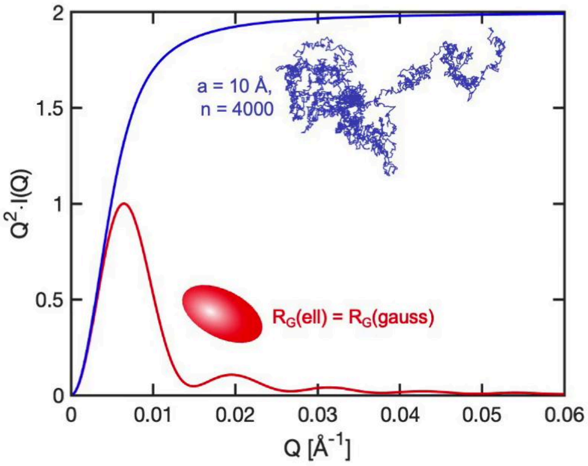
Gaussian chains – Kratky plots

- Plot of $Q^2I(Q)$ v Q
  - Provides information on compactness of polymer chains
  - Open “Gaussian” chain?
  - Closed ellipsoidal structure?
- Gaussian chain (e.g.,unfolded protein)

$$I(Q) = \frac{2I_0}{\phi^2}\left[\exp(-\phi) + \phi - 1\right]$$

$$\Rightarrow \phi I(Q) = 2I_0 \frac{\left[\exp(-\phi) + \phi - 1\right]}{\phi}$$

  - Larger Q: $\phi I(Q) \approx 2I_0$
- Compact ellipsoid (e.g., globular protein)
  - Large Q: $\phi I(Q) \approx 0$



PROVIDES IMPORTANT INFORMATION ABOUT DEGREE OF PROTEIN FOLDING!!

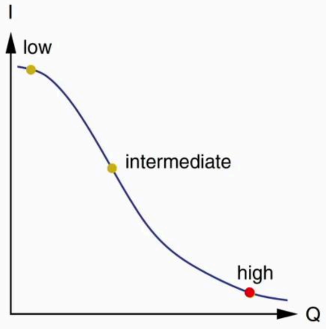
An interesting plot for polymeric chains, is the so-called Kratky plot. Remember that phi is equal to Q squared, A squared and divided by 6, so Q can still be small, but phi large. In this case, the term shown here in the square brackets, exponent minus phi plus phi minus 1, is almost equal to phi. Hence, phi IQ is asymptotically equal to 2 I_0. So a plot of phi IQ or Q squared IQ against Q will lead to a constant value for large Q for a true open Gaussian chain. A globular structure, as more commonly found in true proteins, tends to zero at large Q. Kratky plots thus provide important information about the degree of folding in protein structures.

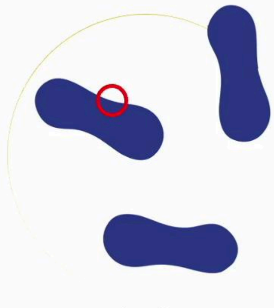
Notes

Summary

9m 26s

Porod regime, Porod's law, and the Porod plot





- Q ≫ 2π/a
  - a = characteristic length of scattering object
  - Probes local features at surface
    - i.e., interfaces with Δρ across boundary
    - If Q large enough, surface is smooth at that scale (Fresnel equations for reflectivity, see following video on x-ray reflectivity)
- Remember:

  $$I(Q) = NV^2(\Delta\rho)^2[\mathcal{F}(Q)]^2$$

  - For spheres and high Q:

$$\boxed{[\mathcal{F}(Q)]^2 = \frac{3}{2R^3}\left(\frac{S}{V}\right)\frac{1}{Q^4}}$$ Porod's law

$$\left(S = 4\pi r^2 \quad V = \frac{4}{3}\pi r^3\right)$$

$$\boxed{\Rightarrow I(Q) = 2\pi N(\Delta\rho)^2\frac{S}{Q^4}}$$

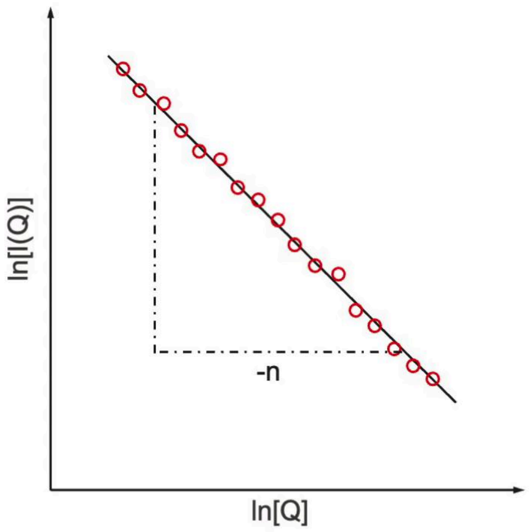
The Porod regime is that where Q is so large that it probes dimensions that are smaller than any typical characteristic length of the sample. This means it probes smooth interfaces and surfaces between regions with different electron densities. Porod's law states that for a sphere, the absolute square of the form factor is proportional to the inverse third power of the sphere's radius, the ratio of the surface to volume, and the inverse fourth power of the scattering vector. Together, this results in the intensity being equal to 2 pi N times the square of the electron density contrast, the illuminated surface divided by Q to the four.

Notes

Summary

10m 26s

Porod regime, Porod's law, and the Porod plot



- Porod plot: high Q
- Gradient -n
- n = …
  - 1: rigid thin rods (1D)
  - 2: Gaussian chain
  - 3 – 4: rough interfaces
  - 4: smooth surface (c.f. x-ray reflectivity)

A Porod plot involves a natural logarithm of the intensity against a natural logarithm of Q, for Q is much larger than 2 pi upon d. A gradient of minus 4 indicates a smooth surface from the perspective of the scattering vectors used. In other words, for resolutions much smaller than the typical length scales. Moreover, shallower gradients provide information about other sample characteristics listed here.

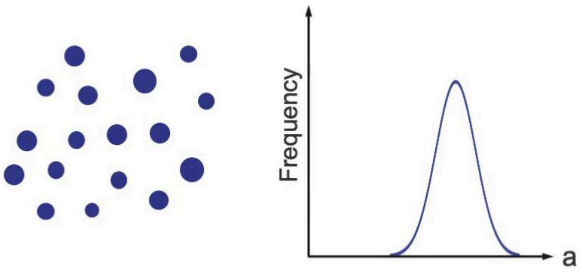
Notes

Summary

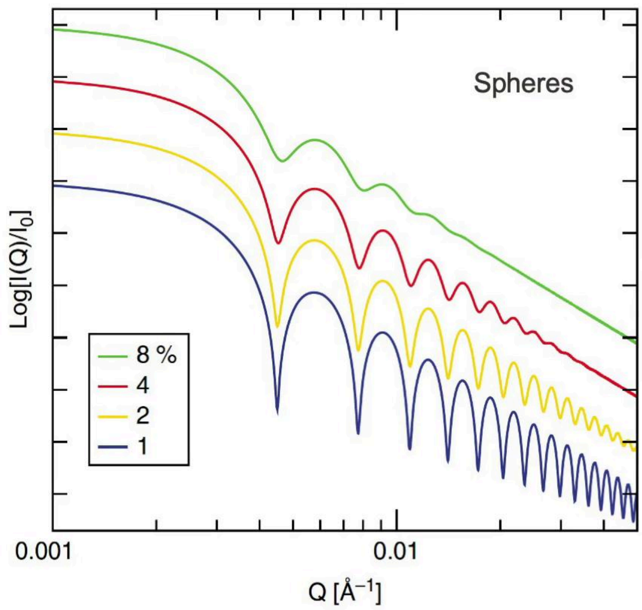
11m 13s

Polydispersity

- Ensemble of objects with same shape but range of sizes



$$\mathrm{FWHM} = (8\ln 2)^{1/2}\sigma \approx 2.355\,\sigma$$



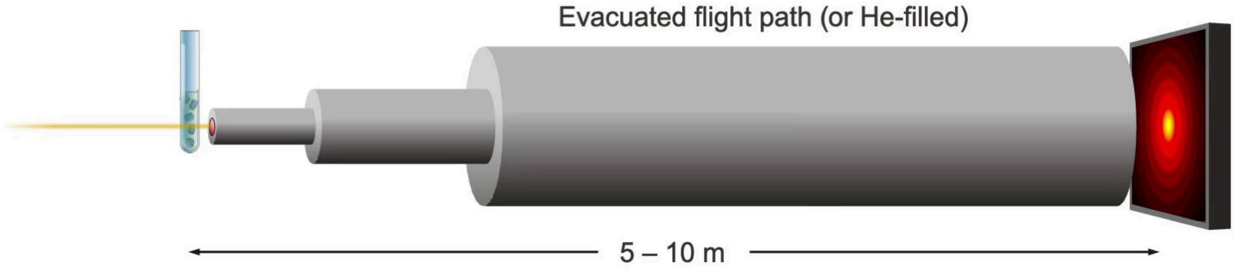
Polydispersity describes a system of particles of identical shape but different sizes, in contrast to truly identical particles, which are said to be monodisperse. The curves show, as an example, how the scattering curve for spheres, changes with polydispersity. The percentage values shown indicate sigma, the standard deviation values of a Gaussian distribution of sizes. The relationship between the full-width at half maximum to sigma is also shown here.

Notes

Summary

11m 42s

Experimental details



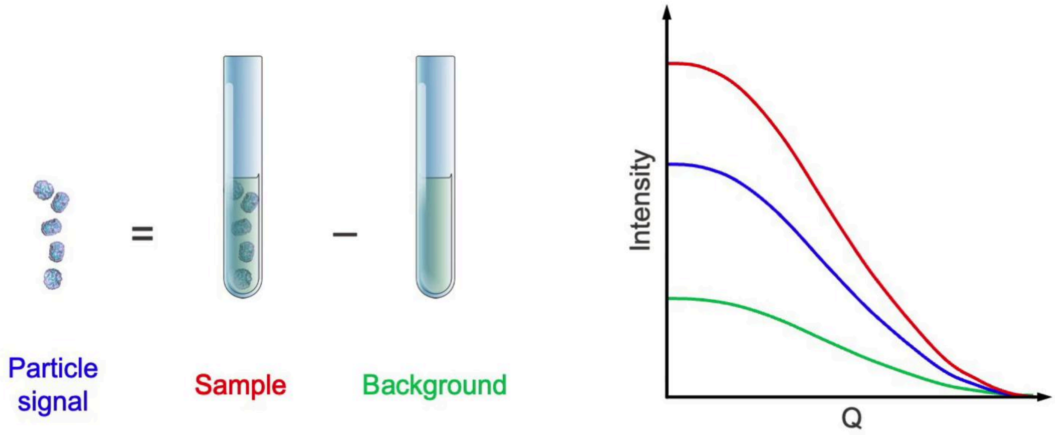
A SAXS experiment at synchrotrons normally involves the sample being separated from the detector, by an evacuated flight tube. This can also be filled with high-purity helium, which is a very weak scatterer. The distance between sample and detector is typically several metres, in order to resolve features of the scattering pattern, using the pixelated detector.

Notes

Summary

12m 20s

Background subtraction



The background signal of the sample holder, including container, and any liquid the particles might be suspended in, can be recorded by itself and subtracted from the sample signal, to obtain the true particle signal. Any vibrations of the sample should have amplitudes which are significantly smaller than the smallest feature that needs to be resolved. This can mean suppressing vibrations down to the few tens of nanometres scales or even less. Lastly, with the advent of DLSR sources, the largest Q vector that can be detected will be much larger than previously. Remember, the one upon Q to the fourth relationship between signal intensity, and scattering vector? That said, higher incident intensities are, of course, associated with more rapid degradation, due to radiation damage.

Notes

Summary

12m 45s

In the next video...

In the next and final video of this week, we will look at X-ray reflectivity, a particular variant of small-angle X-ray scattering, that measures the specular reflectivity, of X-rays from an object.

Notes

Summary

13m 38s