ANSWERS

SCATTERING [100 points total]

Please use separate pieces of paper for OM, EM, and Scattering.
Don't forget to write down your name on every piece of paper!
You can write your answers either in English or in Dutch.

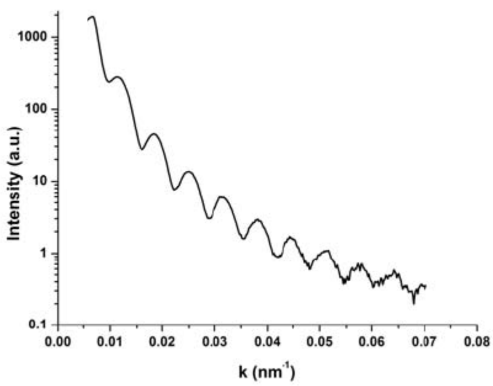
1. Explain the idea of contrast variation using the H/D substitution. Is it used in light, x-ray or neutron scattering? Why is it useful? [15 points]

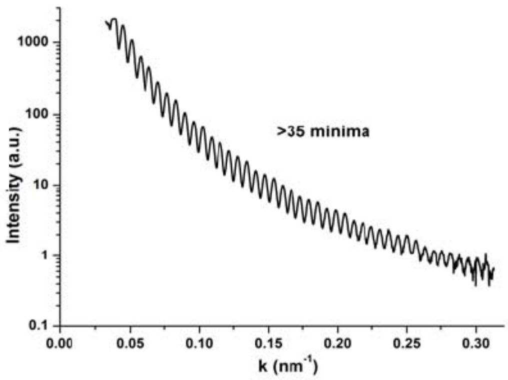
[5p] Hydrogen/deuterium substitution is used in neutron scattering.
[10p] Since both H and D have relatively large scattering length with different sign, the scattering length density ζ can be varied in a wide range. One can vary ζ of the solvent; one can also (partially) deuterate a part of your macromolecule, (nano)particles, etc. This allows one revealing fine details of the structure of the scattering objects.

2. What is the difference between the Guinier range and the Porod range? What type of information can be obtained from the measurements in these two cases? [15 points]

[5p] Guinier range is the range of small K-values, $Ka < 1$, where a is the characteristic size of the scattering objects. Porod range is in another extreme, $Ka >> 1$.
[5p] From the Guinier range the radius of gyration can be determined in dilute suspensions.
[5p] From the Porod law the total surface area can be estimated.

3. The graphs below present results of small-angle x-ray scattering measurements in a suspension of silica spheres. The left and right graphs present results for two different ranges of the scattering wavevectors: $0.006 < K < 0.07$ nm^{-1} (left) and $0.03 < K < 0.3$ nm^{-1} (right). The total K-range covered by these two measurements is therefore between $K_{min} = 0.006$ nm^{-1} and $K_{max} = 0.3$ nm^{-1}.





a). What was the minimum θ_{min} and the maximum θ_{max} scattering angle in these measurements if the x-ray wavelength was $\lambda = 0.1$ nm? [15 points]

[5p] The scattering wavevector K is related to the angle θ via $K = (4\pi / \lambda)\sin(\theta / 2)$.

[optional] For small angles it can be simplified to $K = (2\pi / \lambda)\theta$, where θ is given in radians.

[5p] By substituting numbers, one gets $\theta_{min} \approx 10^{-4}$ rad $\approx 0.006°$.

[5p] Similarly, $\theta_{min} \approx 5\ 10^{-3}$ rad $\approx 0.3°$.

b) Argue that the colloidal particles were very monodisperse. [10 points]
(The authors have estimated from these data that the variation of the diameter was less than 0.5%).

[10p] There are very many oscillations observed in the scattering pattern. This is only possible when there is little variation of the particle size, which smear out the minima, especially at large K.

c) Despite the very low polydispersity, the minima in the scattering profile are not very deep. What can be the reason? [15 points]

[15p] Instrument resolution can also limit the depth of the minima. However, this factor is the same for all minima (in contrast to polydispersity). The visibility of the oscillations in the data does not change much with K, which points out to the resolution effect.

4. Imagine that one studies the same system (as above) using light scattering.
   a) What range of K can be reached with light having the wavelength of λ = 628 nm? Assume that the average refractive index in the sample is n = 1.5 and that the scattering angle can be varied between 5 and 180 degrees. [15 points]

[5p] For light one needs to account for the refractive index *n* of the sample: $K = (4\pi / \lambda) n \sin(\theta / 2)$.

[10p] By plugging in the numbers one gets $0.0013 < K < 0.03$ nm^{-1}.

b) Can light scattering supplement the x-ray data shown above? Are there advantages/disadvantages in using light? Describe at least 3 advantages and/or disadvantages of using light in this case. [15 points]

Possible answers (list is not exhausted) are given below. You get 5 points for each correct answer with a maximum of 15 points. I might, however, subtract some points for nonsense answers.

[+] One could complement the x-ray data with light scattering, which can reach K values that are ~5 times smaller (see answer 4a). One could attempt determination of R_g.

[+] With smaller K values one could also see whether particles aggregate or not.

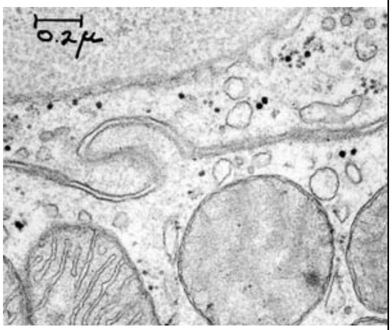
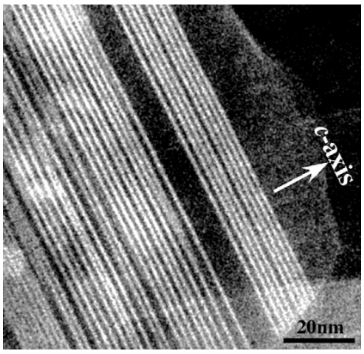
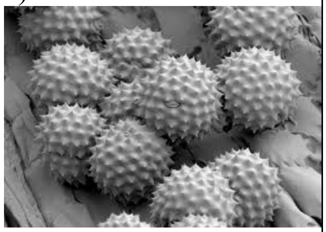
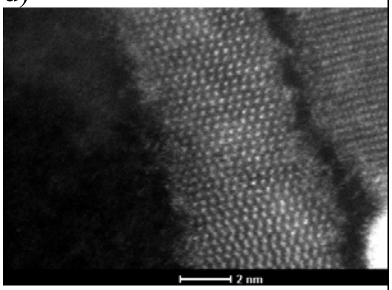
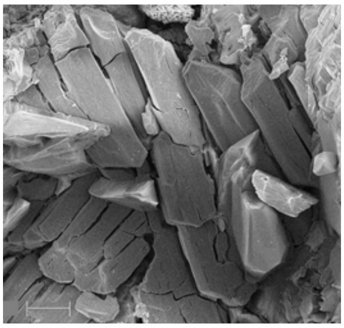
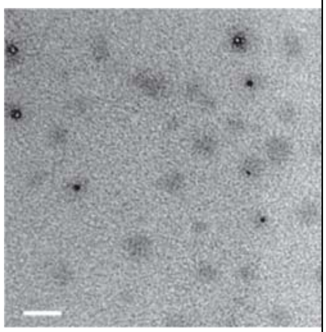
[+] Light scattering is advantageous since it is simple, fast and (relatively) cheap.

[+] With light one can measure the first couple of minima. Since the angles are much larger than for x-rays, one could improve the resolution and better measure the depth of the minima.

[-] However, one has to take care of careful index matching, which is difficult to achieve in many cases. One can, for example, easily get too much multiple scattering if the sample is too turbid.

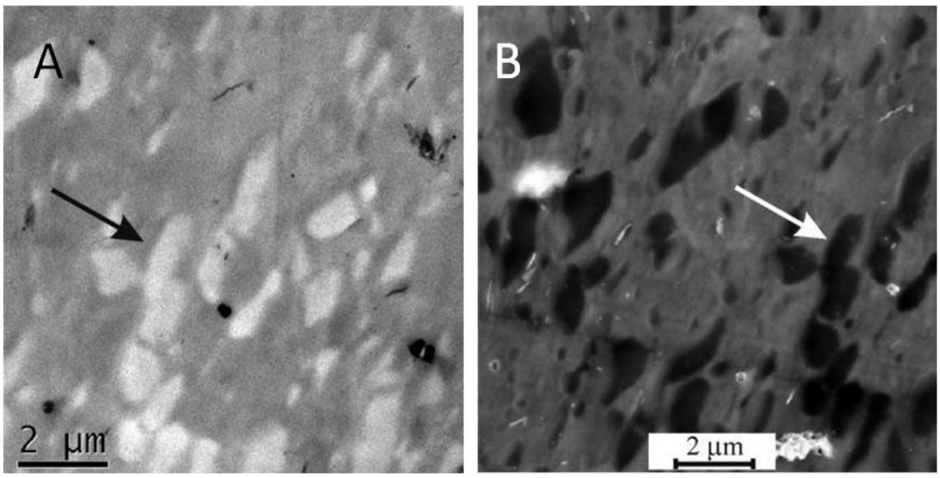
Answers Electron microscopy

Answers to each question should not exceed 2-3 sentences!

a)	b)	c)
		
d)	e)	f)
		

1. By which of those three methods have the images been acquired: Bright-field transmission electron microscopy (BF-TEM), high-angle annular dark field scanning TEM (HAADF-STEM), or scanning electron microscopy (SEM)? (3 P)

A: BF-TEM, b: HAADF-STEM, c: SEM, d: HAADF-STEM, e: SEM, f: BF-TEM



A thermoplastic vulcanized (TPV) unstained specimen imaged in different modes: (A) bright field conventional TEM image (approximately in focus); (B) high-angle annular dark field (HAADF) scanning TEM image of the same area as imaged in A (arrows indicate same specimen features).

2. Why is the contrast between A (bight field TEM in focus) and B (HAADF STEM) inverted? (3 P)

   In bright field, the image is effectively the primary beam minus the diffracted contribution, whereas the HAADF image captures only the diffracted electrons. Thus, dense areas are dark in conventional TEM, but bright in HAADF STEM.

3. The contrast in A could be increased by defocusing. (*i*) how is this defocus-based contrast called and (ii) how is this additional contrast generated? (3 P)

   (i) *phase contrast. (1 P)*

   (ii) *Defocusing causes interference of primary and scattered electron beam, which is proportional to the scattering amplitude (dark field is proportional to square!). (2 P)*

4. Why is almost exclusively bright-field defocused-based imaging used for studying biological molecules in the frozen-hydrated state ('cryo-EM')? (2 P)

   The signal per incident electron is much stronger because it is interference-based (see 2).

5. To precisely identify different types of atoms STEM imaging is often combined with Energy-dispersive spectrometry (EDS/EDX). Briefly describe how inelastic interaction of the electron beam with an imaged atom can generate characteristic X-rays. (3 P)

   An incident electron removes an electron from an inner electron shell of the respective atom. Transition of electrons from higher-energy orbitals into the electron hole causes emission of light (X-rays) with specific energies.

ANSWERS Optical Microscopy

a) $E = R_o^6 / (R_o^6 + r^6) = 4.8^6 / (4.8^6 + 5^6) = 0.44$

b) $E = 1 - \tau_{D+A}/\tau_D = 1 - \tau_{D+A}/2.8 \rightarrow \tau_{D+A} = 1.57$ ns

c) $1/\tau_D = k_{rad} + k_{nr}$, $1/\tau_{D+A} = k_{rad} + k_{nr} + k_{ET} = 1/\tau_D + k_{ET} \rightarrow k_{ET} = 0.279\ \text{ns}^{-1}$
$1/\tau_{D+nA} = k_{rad} + k_{nr} + k_{ET} = 1/\tau_D + nk_{ET}$: $n = 4 \rightarrow \tau_{D+4A} = 0.67$ ns

d) $d_{min,lat} = 0.45\lambda/NA = 0.171$ um , $d_{min,ax} = 1.4\ \lambda/(n.\sin^2\alpha) = 0.597$ um
vol = $0.171^2 \times 0.597$ um3 = $0.0175\ \text{um}^3 = 1.8 \times 10^{-17}$ litres

e) see lecture sheets

f) $d_{airy} = 1.22\ \lambda/NA = 0.697$ um, area = $\frac{1}{4}\pi d^2 = 0.38\times10^{-8}\ \text{cm}^2$
peak power = $2\times10^{-3} / (120\times10^{-15} * 50\times10^6) = 333$ W
peak intensity = $333\ \text{W}/0.38\times10^{-8}\ \text{cm}^2 = 8.8\times10^{10}\ \text{W/cm}^2$