Determination of particle size and shape using transmission electron microscopy

**Theoretical part:**

**Introduction**
The transmission electron microscope (TEM) is used, for example, to visualize colloidal systems in detail. Electron microscopes were developed due to the limitation of light microscopes which are limited by the physics of light to 500x or 1000x magnification and a resolution of 0.2 micrometers. The observation of colloidal particles with an optical microscope is limited by the resolution of the microscope. This refers to the ability to discriminate between two closely spaced points in the view of the microscope.

The resolution $d_p$ of a microscope is given by (Rayleigh resolution):

$$d_p = \frac{0.61 \lambda}{n_0 \sin \theta}$$

Here $n_0$ is the refractive index of the medium, $\theta$ is the collection semi-angle of the objective lens (limited by the lens or an aperture) and $\lambda$ is the wavelength.

The resolution can evidently be increased ($d_p$ decreased) by reducing the wavelength, or increasing $n_0$ and $\theta$. In practice in light microscopes only visible light is used ($\lambda \approx 500$ nm), but $n_0$ can be increased by filling the region between the lens and the sample with a transparent oil ($n_0 \approx 1.59$) instead of air. Wide angle lenses are also helpful (i.e. $\theta$ increased) but the angle is limited by other optical problems like spherical and chromatic aberration. In effect, the lower limit for $d$ is about 0.2 $\mu$m, so optical microscopy is limited to the upper end of the colloidal size range.

The electron microscopy depends for its operation on the wave nature of the electron and the fact that electrons are charged and can be focused by electromagnetic or electrostatic lenses. The wavelength of an electron depends on its mass $m$, its velocity $v$ and therefore on the acceleration voltage $U$. $h$ is the Planck’s constant.

The relation between $\lambda$ and $U(V)$ is given by:

$$\lambda = \frac{h}{mv}, \quad \lambda \approx \frac{1.23}{\sqrt{U}} \text{ nm}$$

1
For example: $U = 80,000$ volts (80 kV) $\rightarrow \lambda = 4.3$ pm

The higher the acceleration voltage is, the faster the electrons and the shorter the wavelength.

The limitation in most machines is in the performance of the magnetic lenses and the maintenance of stable magnetic fields. The spherical aberration is one of the principal factors limiting the resolution of the TEM. So, the theoretical resolution in consideration of the spherical aberration (spherical aberration coefficient $C_s$) and using an optimal aperture is given by $d_{th} \approx \frac{\lambda_{el}^{3/4}}{C_s^{1/4}}$. Modern TEM’s have powers of resolution of about 0.2 nm.

**Construction and function of a TEM**

The TEM contains an illumination system, comprising the electron gun and condenser lenses, and an imaging system with several magnetic lenses (objective, intermediate and projector lenses).

In our case the electron beam is produced by thermionic emission from a tungsten cathode, and is accelerated towards an aperture in the anode. The acceleration voltage is 80 kV. Typically acceleration voltages are between (80kV) 100kV and 300kV. The accelerated electrons first focused by a condenser lens and will be scattered elastic or inelastic within the specimen. The scattering angle depends on the electron density of the object. The greater the mass of the atoms, the greater is the degree of deflection. The objective lens forms a first real image of the illuminated specimen area. This image can be magnified by the intermediate lens. The projector lens system induces a further magnification of 50 to 400 times of the image as it projects the electrons on the fluorescence screen or on a CCD camera. Micrographs taken with electron microscopes are always black and white. The degree of darkness corresponds to the electron density (=differences in atom masses) and the thickness. Polymers have only weak contrasts since they consist mainly of atoms with low atomic numbers (C, H, N, O). Consequently it is necessary to treat the preparations with special contrast enhancing chemicals (heavy metals) to get at least some contrast.

Additionally the samples should not be thicker than about 100 nm depending on the atomic number and the application.
The Preparation of specimen support films

Because of the lower penetrating power of electrons in the 100 kV range, it is necessary to mount objects for examination in the electron microscope on very thin films. These films must be made from materials with a high transparency to electrons and should not be more than about 20 nm thick. The films are mounted on discs usually made of copper and containing a number of apertures. These disks are known as specimen support grids, and are available in different designs and sizes. A wide variety of grid designs is available in the standard 3 mm size. Different mesh sizes are available and the grid spacing is usually quoted in bars per inch; standard sizes are 50, 75, 100, 200, 300 and 400. In general the grids are made of copper. But they also can be supplied in stainless steel, platinum, gold etc.

Although it is possible to buy grids with support films, it is less expensive to make the films in the laboratory. Thin plastic films (20-80 nm thick), are most commonly made from Formvar (polyvinylformal) or Collodion (nitrocellulose). Collodion films are easier to make than Formvar films, but they are not that stable in the electron microscope. For high-resolution studies thinner and more stable films may be required. These can be made from evaporated carbon to produce a film thickness of 5-50 nm.
Carbon films
Plastic films are not stable under the electron beam and may cause specimen drift. A layer of carbon evaporated onto a plastic film is leading to greater stability in the beam.

A carbon film can also be used on its own and should be at least 10 nm thick. It is prepared by evaporating carbon onto a freshly-cleaved mica. Carbon is evaporated in a coating unit using two pointed hard graphite rods with their points just touching and the mica is placed 100-150 mm from the source. Afterwards, the carbon film is floated off on to a water surface by lowering the mica slowly into a dish of distilled water at a shallow angle. A filter paper disk with grids is placed before on the bottom of the dish with water. After draining off water the carbon film covers the grids.

The Preparation

The mounting of particles suspended in a liquid
This is the simplest method, but even so there are a number of pitfalls.

Mounting on coated grids:
A coated grid is held firmly in a pair of tweezers in a horizontal position. A small drop of suspension is placed on the coated grid using a fine pipette or glass rod. The suspension is allowed to dry. Aqueous suspension and suspensions in an organic solvent can be mounted on this way. The distribution of particles over the grid is often very uneven.

The Preparation of bulk material
It is necessary to prepare ultrathin sections of bulk material because of the limited penetration of the electron beam in the electron microscope by accelerating voltages up to 100 kV. For penetration and resolution the specimen should not be thicker than 100 nm.

The techniques of fixing, dehydrating and embedding specimens in preparation for thin sectioning are similar to those used in light microscopy.

The final aim of the whole procedure is to produce blocks which can be sectioned without difficulty and which contain specimens in which the fine structure is preserved.
The fixation is not necessary by preparation of polymer materials, but by preparation of biological objects.

The aim of dehydration is to remove all free water from the specimen and replace it with ethanol. Most embedding media are not soluble in water and consequently specimens are dehydrated by passing them through a sequence of solutions, the last of which is miscible with the embedding medium. Dehydration is accomplished by passing the fixed specimen through a graded series of increasing concentration of the ethanol.

In the final stage of preparing a specimen in a form suitable for thin-sectioning it is infiltrated with a liquid embedding medium which is polymerized to produce a solid block. Three main types of embedding media are in general use; the epoxy resins, the polyester resins and the methacrylates.

After embedding and trimming the specimen block the specimen can be sectioned with an ultramicrotome. The specimen holder of the ultramicrotome is moved toward the knife (diamond, glass) a given distance during each cycle. The thickness of the section is determined by the magnitude of its forward advance. A ribbon of sections float from the knife edge into the trough filled with water. The sections are picked up from above and can be investigated with the TEM.
**Practical part:**

1. Prepare two aqueous latex dispersions for the transmission electron microscopy by suspension preparation.

   Latex 1 and Latex 2: 0.001% polystyrene latex (aqueous dispersion)

   **Both** polystyrene latices were synthesized by radical emulsion polymerization using potassium peroxydisulfate (KPS) as initiator and sodium dodecylsulfate (SDS) as surfactant (compare with the manual of the experiment “dynamic light scattering”):

<table>
<thead>
<tr>
<th></th>
<th>KPS (in g)</th>
<th>SDS (in g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex 1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Latex 2</td>
<td>1.0</td>
<td>-</td>
</tr>
</tbody>
</table>

2. Determine the diameter of the polystyrene particles from the TEM micrographs (pay attention to the scale!).

   Take the measurements of 500 particles for Latex 1 as well as Latex 2.

   a. Determine the mean diameter of the particles according to the following formula:

   \[ \bar{d} = \frac{1}{n} \sum_{i=1}^{n} d_i \]

   \( n \): number of particles, \( d_i \): diameter of the particle \( i \)

   b. Determine the standard deviation of the mean diameter of the particles.

   The **standard deviation** \( \sigma \) is defined as the square root of the variance \( \sigma^2 \).

   \[ \sigma = \sqrt{\frac{\sum_{i=1}^{n} (d_i - \bar{d})^2}{n - 1}} \]

   c. Determine the root mean square (r.m.s.) error \( \sigma_m \) (standard deviation of the sample mean)!

   The r.m.s error \( \sigma_m \) is the square root of the **variance of sample mean** \( \sigma_m^2 \)

   \[ \sigma_m^2 = \frac{\sigma^2}{n} \]
\[ \sigma_m = \frac{\sigma}{\sqrt{n}} \]

d. Summarize the results in a table:

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>(d_{\text{min}}) [nm]</th>
<th>(d_{\text{max}}) [nm]</th>
<th>Mean (\bar{d}) [nm]</th>
<th>standard deviation (\sigma) [nm]</th>
<th>r.m.s. error (\sigma_m) [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex 1</td>
<td>500</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latex 2</td>
<td>500</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1

3. Plot the following histograms for each sample:
   a. absolute (class) frequency vs. diameter (classes)
   b. rel. (class) frequency vs. diameter (classes)
   c. cumulative frequency vs. diameter (classes)
   d. rel. cumulative frequency vs. diameter (classes)

determine
   - the class bounds (class intervals) of the diameter \(\Delta d\)
   - the absolute (class) frequency \(f_i\), rel. (class) frequency \(f_i/n\)
   - cumulative frequency \(\text{cum}\), rel. cumulative frequency \(\text{cum}/n\) (Table 1)

\[ f = \text{frequency} \]
\[ n = \text{number of the particles} \]
\[ d = \text{diameter of the particles} \]
\[ i = \text{class index} \]

**Example:** \(\Delta d = 5 \text{ nm}\) and \(n = 500\)

<table>
<thead>
<tr>
<th>class intervals [nm]</th>
<th>(f_i)</th>
<th>(f_i/n)</th>
<th>\text{cum}</th>
<th>\text{cum}/n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 &lt; (d \leq 5)</td>
<td>3</td>
<td>3/500</td>
<td>3</td>
<td>3/500</td>
</tr>
<tr>
<td>5 &lt; (d \leq 10)</td>
<td>0</td>
<td>0/500</td>
<td>3</td>
<td>3/500</td>
</tr>
<tr>
<td>10 &lt; (d \leq 15)</td>
<td>15</td>
<td>15/500</td>
<td>18</td>
<td>18/500</td>
</tr>
<tr>
<td>15 &lt; (d \leq 20)</td>
<td>10</td>
<td>10/500</td>
<td>28</td>
<td>28/500</td>
</tr>
<tr>
<td>\ldots</td>
<td>\ldots</td>
<td>\ldots</td>
<td>\ldots</td>
<td>\ldots</td>
</tr>
</tbody>
</table>

Table 2
Examples of histograms:

4. Discuss the particle size and particle size distribution with regard to the polymerization conditions of Latex 1 and Latex 2.

**Bibliography**

