**Lecture 8.**

**Optical techniques for studying the nanodisperse systems. Comparison of** *-* **resolving power of optical and electron microscopes. Transmission electron microscope. Scanning electron microscope. Ultramicroscopy.**

*The optical microscope- resolving power.* High dispersed particles (nanoparticles) are often too small to permit direct microscopic observation. The resolving power of an optical microscope (i.e. the smallest distance by which two objects may be separated and yet remain distinguishable from each other) is limited mainly by the wavelength λ of the light used for illumination. The limit of resolution δ is given by the expression

δ = λ / 2 n sin α (1)

 where α is the angular aperture (half the angle subtended at the object by the objective lens), *n* is the refractive index of the medium between the object and the objective lens, and *n* sin α is the numerical aperture of the objective lens for a given immersion medium.

The numerical aperture of an optical microscope is generally less than unity. With oil-immersion objectives numerical apertures up to about 1.5 are attainable, so that, for light of wavelength 600 nm, this would permit a resolution limit of about 200 nm (0.2 μm). Since the human eye can readily distinguish objects some 0.2 mm (200 μm) apart, there is little advantage in using an optical microscope, however well-constructed, which magnifies more than about 1000 times. Further magnification increases the size but not the definition of the image.

Owing to its large numerical aperture, the depth of focus of an optical microscope is relatively small (10 μm at x 100 magnification and 1 μm at x 1000 magnification).

Particle sizes as measured by optical microscopy are likely to be in serious error for diameters less than 2 μm.

In addition to the question of resolving power, the visibility of an object may be limited owing to lack of optical contrast between the object and its surrounding background.

Two techniques for overcoming the limitations of optical microscopy are of particular value in the study of high dispersed systems. They are electron microscopy, in which the limit of resolution is greatly extended, and dark-field microscopy, in which the minimum observable contrast is greatly reduced.

*The transmission electron microscope.* To increase the resolving power of a microscope so that matter of nano dimensions may be observed directly, the wavelength of the radiation used must be reduced considerably below that of visible light. Electron beams can be produced with wavelengths of the order of 0.01 nm and focused by electric or magnetic fields, which act as the equivalent of lenses. The resolution of an electron microscope is limited not so much by wavelength as by the technical difficulties of stabilizing high-tension supplies and correcting lens aberrations. Only lenses with a numerical aperture of less than 0.01 are usable at present. With computer application to smooth out 'noise' a resolution of 0.2 nm has been attained, which compares with atomic dimensions. Single atoms, however, will appear blurred irrespective of the resolution, owing to the rapid fluctuation of their location.

The useful range of the transmission electron microscope for particle size measurement is 1 nm-5 μm diameter. Owing to the complexity of calculating the degree of magnification directly, this is usually determined by calibration using characterised polystyrene latex particles or a diffraction grating.

The use of the electron microscope for studying nanodispersed systems is limited by the fact that electrons can only travel unhindered in high vacuum, so that any system having a significant vapour pressure must be thoroughly dried before it can be observed. Such pretreatment may result in a misrepresentation of the sample under consideration. Instability of the sample to electron beams could also result in

misrepresentation.

A small amount of the material under investigation is deposited on an electron-transparent plastic or carbon film (10-20 nm thick) supported on a fine copper mesh grid. The sample scatters electrons out of the field of view, and the final image can be made visible on a fluorescent screen. The amount of scattering depends on the thickness and on the atomic number of the atoms forming the specimen, so that organic materials are relatively electron-transparent and show little contrast against the background support, whereas materials containing heavy metal atoms make ideal specimens.

To enhance contrast and obtain three-dimensional effects, the technique of shadow-casting is generally employed. A heavy metal, such as gold, is evaporated in vacuum and at a known angle on to the specimen, which gives a side illumination effect. From the angle of shadowing and the length of the shadows, a threedimensional picture of the specimen can be built up. An even better picture can be obtained by lightly shadowing the sample in two directions at right angles.

A most useful technique for examining surface structure is that of replication. One method is to deposit the sample on a freshly cleaved mica surface on to which carbon (and, if desired, a heavy metal) is vacuum-evaporated. The resulting thin film, with the specimen particles still embedded, is floated off the mica on to a water surface.

The particles are dissolved out with a suitable solvent and the resulting replica is mounted on a copper grid.

*The scanning electron microscope.* In the scanning electron microscope a fine beam of medium-energy electrons scans across the sample in a series of parallel tracks. These interact with the sample to produce various signals, including secondary electron emission (SEE), back-scattered electrons (BSE), cathodoluminescence and X-rays, each of which (with their varying characteristics) can be detected, displayed on a fluorescent screen and photographed. In the SEE mode the particles appear to be diffusely illuminated, particle size can be measured and aggregation behaviour can be studied, but there is little indication of height. In the BSE mode the particles appear to be illuminated from a point source and the resulting shadows lead to a good impression of height.

The magnification achieved in a scanning electron microscope (resolution limit of 5 nm) is, in general, less than that in a transmission electron microscope, but the major advantage of the technique (which is a consequence of the low numerical aperture) is the great depth of focus which can be achieved. In colloid and surface science this large depth of focus is extremely valuable in the study of the contours of solid surfaces and in the study of particle shape and orientation.



Figure 1– Comparison of optical microscope, transmission electron microscope and scanning electron microscope

*Dark-field microscopy – ultramicroscope.* Dark-field illumination is a particularly useful technique for detecting the presence of, counting and investigating the motion of suspended highly dispersed particles. It is obtained by arranging the illumination system of an ordinary microscope so that light does not enter the objective unless scattered by the sample under investigation.

If the particles in a colloidal dispersion have a refractive index sufficiently different from that of the suspending medium, and an intense illuminating beam is used, sufficient light is deflected into the objective for the particles to be observed as bright specks against a dark background. Lyophobic particles as small as 5-10 nm can be made indirectly visible in this way. Owing to solvation, the refractive index of lyophilic particles, such as dissolved macromolecules, is little different from that of the suspending medium, and they scatter insufficient light for detection by dark-field methods.

The two principal techniques of dark-field illumination are the slit and the cardioid methods. In the slit ultramicroscope of Siedentopf and Zsigmondy (1903) the sample is illuminated from the side by an intense narrow beam of light from a carbon-arc source (Fig. 30). Dark-field methods do not help to improve the resolving power of a microscope. A small scattering particle is seen indirectly as a weak blur. Two particles must be separated by the resolution distance δto be separately visible. Dark-field microscopy is, nevertheless, an extremely useful technique for studying colloidal dispersions



Figure 2 – Principle of the slit ultramicroscope

There are another impoved types of ultramicroscopes, for example, flow ultramicroscope designed by B. Derjaguin and G.Vlasenko. In the flowing susension this device registers the number of particles that travel per unit time across the microscope field, allowing one to rapidly determine the particle concentration in sols. The use of optical electronic devices for the measurement of intensity of light scattered by indnividual particles makes it possible for one to obtain also the particle size distribution curves. Inspite of disadvantage connected with resolving power, the ultramicroscopy method plays an important role in the development of colloid science for determination the particle size of nanodisperse systems.

The surface structure and morfology can be effectively analyzed by such methods as transition electron microscopy, scanning electron microscopy, atomic force microscopy, scanning tunneling microscopy and other methods.

**Revision questions:**

1. Describe the resolving power of optical microscope.
2. Explain the principle scheme of transition microscope.
3. Explain the principle scheme of scanning microscope.
4. Compare the resolving power of optical and electron microscopes.
5. Explain the principle scheme of dark-field ultramicroscope.