**TEM grid preparation**

Carbon evaporation

Various devices are available to hold carbon rods for evaporation. Two 1/8” carbon rods are used, one flat and the other pointed or sharpened into a smaller cylinder, so that it will reach evaporation temperature.

1. Form a 1 mm diameter tip about 1 mm long on a 5 cm carbon rod. Flatten the end of another 5 cm rod with emery paper.
2. After the shadow indicator is in place and distances are made correct, place the carbon rods in the tension holders so that the specimens will be in front of the flat rod surfaces (see diagram).
3. At sufficient vacuum (0.2 um Hg) evaporate half the length of the tip. By using the formula for thickness with R=10cm, the following table can be made:

|  |  |  |
| --- | --- | --- |
| **Tip length (nm)** | **Thickness (nm)** | **Indication** |
| 0.4 | 2.5 | very light |
| 0.6 | 3.8 | light |
| 0.8 | 5.0 | medium |
| 1.0 | 6.2 | medium light |
| 2.0 | 12.5 | very dark |

Note: To carbon stabilize collodion-coated grids on the back, tilt the perforated rack of grids upside down with a toothpick at one end.

Pease, 1965, pp. 200-205; Kay,1965, pp. 66-68

Gold evaporation

Use R= 10cm and 0.008” wire

|  |  |  |  |
| --- | --- | --- | --- |
| **Wire length (cm)** | **Weight (mg)** | **t (A)** | **Indication** |
| 1 | 5 | 20 | very light |
| 2 | 10 | 40 | light (thin) |
| 4 | 20 | 80 | medium |
| 6 | 30 | 120 | medium dark |
| 8 | 40 | 160 | - |
| 12 | 60 | 240 | very dark (thick) |
| 20 | 100 | 400 | - |

Pease, 1965, pp. 200-205; Kay,1965, pp. 66-68

Support films

Electron microscope grids are analogous to the glass slides used for specimen support in light microscopy. These grids come in a variety of sizes and shapes of their perforations. Many have a regular coordinate grid pattern, e.g., 200 mesh, which have 200 “holes” per inch and about 60 -70% open space.

Grids with fewer holes have more open space and those with more holes (400 mesh) have a lesser open space for electron transmission. Other grid patterns encountered appear as long slots or ovals.

You can readily see that whenever a section or ribbon of sections is smaller than the slot, or when particulate samples are smaller than the hole, some electron transparent material (film) will be required to maintain the specimen in position on the grid.

Suitable electron transparent support films can be constructed of carbon evaporated on a freshly cleaved sheet of mica or a more easily prepared plastic film. There are three plastics which are commonly used for support films: Collodion, Parlodian and Formvar (polyvinyl formal). All can be produced by the same technique and with reasonable ease.

Colloidon is less hydrophobic and less stable in the electron beam than is Formvar. Therefore, the specimens will aggregate more on a Formvar film, but that film will sublime less in the electron beam.

While there are a number of techniques for the preparation of these plastic support films, you are asked to prepare suitable examples by the “cast on glass” method. These support films will be used in a subsequent exercise to examine bacterial specimens. Please read these directions in their entirety before commencing the exercise.

##### A few words of caution

Plastic films should be viewed within a month of preparation. The films are unstable in the electron beam and a low beam current and intensity should be used until the photography is ready to be done because there may be film and specimen drift as the energy of the electron beam is absorbed by the film.

If a higher resolution is required for your work, you will then require a carbon support film. This can be made by evaporating a carbon layer (see section on carbon evaporation) onto freshly cleaved mica surfaces. The mica is then scored with a clean razor blade (into 4 mm x 4 mm squares) and the film floated free as with Formvar films (a mixture of 5 ml butanol in 100 ml of water can reduce the surface tension effects and keep the fragile film from breaking up). The carbon film is then placed on the grids by coming up from beneath the surface with individual grids being coated by small pieces of carbon film.

Preparing Formvar grids

**Cleaning grids**

1. Put grids into a small beaker
2. Sonicate grids with 100% acetone (5 mins)
3. Sonicate with 95% ethanol and air dry on filter paper.
4. Put all clean grids into petri dish with filter paper to dry.

For used grids: sonicate in chloroform or ethylene dichloride for several minutes followed by HCl before the acetone.

**Coating grids**

1. Clean a SuperFrost microscope slide by wiping with Kimwipe and 95% ethanol.
2. Attach the clean, dry slide to a bulldog clip with a piece of string.
3. Lower the clean, dry slide into the bottle of Formvar. Tap it on the bottom to remove any bubbles.
4. Quickly raise the slide and hold in the vapor phase above the liquid for about 30 seconds. Completely withdraw the slide and allow it to drain vertically on a filter paper.
5. Score around the periphery of the slide using a razor blade to break the continuity of the film to facilitate its removal, but not to deeply scratch the glass slide.
6. Huff on the coated slide to cause condensation of water vapor immediately before immersing it into a dish of clean distilled water. Slowly lower the slide held at a slight angle into the water so that the film is detached from the slide. When all the planets are in place, the slide can be lowered very slowly vertically into the water and two films come off.
7. Pick up grids by the underside with a vacuum pump or antistatic forceps and drop them onto the film. When the film is totally covered with grids, cut a piece of parafilm to fit and lower it from one edge onto the water. Pick up the parafilm and coated grids and store them in a petri dish.
8. Score around a grid with forceps before removing it from the parafilm.