



Saint Mary's
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Electron Microscopy Centre

Title: Preparations of Support Films for TEM Grids

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NOTE: The Chemicals used for TEM grid support films are restricted to the specific EM users who have had prior training in proper use, handling and storage. **Handle solutions and cast the films in a fume hood. Wear lab coat, eye protection and latex gloves as splash protection only.**

Introduction

In general, 200-400 mesh (lines/in) grids are used in TEM experiments. Most EM grids are made of copper because it is non-ferromagnetic and thus minimally distorts the magnetic field of the objective lens. The copper mesh also rapidly removes heat away from the support film and thus prevents thermal expansion and movement of the specimen under electron irradiation. Grids may be used bare or, more commonly, filmed depending on the nature of the specimen being studied. Thin sectioned specimens, for example, are sometimes examined on bare grids, but for highest resolution work, the sections must be mounted on filmed grids or, better yet, on net films. These films should be extremely thin (20 nm or less) and made of suitable electron-transparent substance, such as plastic or evaporated carbon, without breaking when gently handled or when irradiated with the electron beam.

Support films are generally one of three types:

- Plain plastic such as Collodion, Formvar or Pioloform
- Plastic coated (stabilized) with evaporated carbon
- Plain carbon

If high resolution work is required, plain carbon films are the best choice because these films are generally tough and could withstand the bombardment of electron beam. They can also conduct electricity and heat easily from the specimen to the grid bars and hence reduce specimen drift due to both charge and thermal effects.

Preparation of TEM grids

1. Merge the TEM grids into amyl acetate solution for several hours.
2. Drain the amyl acetate solution and rinse the grids with distiller water at least 3 times.
3. Dehydrate the grids using 100% alcohol.

4. If the TEM grids are not clean after above procedures, put the grids into boiled 1% NaOH solution for 5 min., rinse off the chemical using distiller water and dehydrate with 100% alcohol.

Preparation of plastic films

1. Collodion support film

- 1.1. Prepare a concentration of 2% collodion in amyl acetate solution.
- 1.2. Place a drop of the collodion solution on the surface of distiller water and allow it to spread out and dry.
- 1.3. Use tweezers to pick up the film and discard it properly. This process will remove any dust floating on the water surface. Repeat the process twice or more if necessary.
- 1.4. In a **vibration free** environment, place a drop of the collodion solution on the surface of distiller water and allow it to spread out and dry. This leaves a thin layer of plastic on the water surface
Note: If thicker film is desired, user could add one or two more drops of collodion drops after the first drop is well spread and dried.
Note: This method tends to produce thicker and more uneven films compared to the formvar film.
- 1.5. Check if the film is even. Remove the film if the film appears to be uneven.
Note: A good film should appear an even grey to dark grey color when viewed by reflected light. If it appears yellowish or uneven, it is too thick and unsuitable.
- 1.6. Lay the EM grids shiny-side up (dull side down) onto the plastic film.
- 1.7. Remove the grid with film from the water using a strip of hard filter paper. User can also use Parafilm or a clean glass slide to pick up the grids.
- 1.8. Set the grids aside in a dust free place to dry before use.

2. Formvar support film

2.1. Prepare a concentration of 0.3% formvar in chloroform solution (user could also use ethylene dichloride to replace chloroform).

2.2. Remove dust from a glass microscope slide with Kim wipe tissue and dip the glass slide in the solution of Formvar in chloroform.

Note: The longer the slide is within the solution, the thicker the support film will be. The thickness of the film could be identified when the film is floating on the surface of the water. A good film should appear an even grey to dark grey color when viewed by reflected light. If it appears yellowish or uneven, it is too thick and unsuitable.

2.3. The slide is withdrawn and drained in the presence of chloroform vapor.

2.4. Score 2mm from the slide edge with a stout scalpel blade, breathe on the film and lower into a beaker of distilled water at an angle of 45°. The film should flow on the water.

Note: All the actions should be gentle and the hand should not be shaking when lowering the slide into the water.

2.5. Lay the EM grids shiny-side up (dull side down) onto the plastic film.

2.6. Remove the grid with film from the water using a strip of hard filter paper. User can also use Parafilm or a clean glass slide to pick up the grids.

2.7. Set the grids aside in a dust free place to dry before use.

3. Pioloform support film (Pioloform produces a stronger film than formvar)

3.1. Prepare a concentration of 1.2% in chloroform (analytical quality), adjusted with extra chloroform until films of the desired thickness are obtained.

Note: To avoid the formation of holes in the films if the conditions are humid, allow to dry in a lidded container thus retaining an atmosphere of chloroform.

3.2. Pour approx 80ml of the pioloform solution into a 100ml container with a tap. The container should be wide enough to put in a glass microscope slide.

- 3.3. Remove dust from a glass microscope slide with velin tissue, and drop into the pioloform solution. Cover the top of the container, open tap and drain solution into the stock bottle for re-use.
- 3.4. Leave the slide to dry with the cover on, thus maintaining the chloroform atmosphere within the container.
- 3.5. Score 2mm from the slide edge with a stout scalpel blade, breathe on the film and lower into a beaker of distilled water at an angle of 45°. The film should flow on the water.
- 3.6. Place acetone-cleaned grids, matt side down onto the film, separating each by 3mm.
- 3.7. Cut a piece of 'Yellow Pages' (with text printing on both sides to allow even uptake of water) and leave until the whole paper is wet. Remove the grids gently from the surface with forceps.
- 3.8. For slot grids, remove from the surface of the water when 3mm has been wetted.
- 3.9. Set the grids aside in a dust free place to dry before use.

Preparation of carbon films

Carbon-Formvar films can be easily converted into carbon-only films by dissolving away the plastic. The carbon-plastic films are placed plastic-side down onto a piece of filter paper soaked in a solvent which dissolves the plastic. After a few hours, the grids are moved to a dry piece of filter paper and allowed to air dry.

Another method for preparing pure-carbon films is to evaporate a layer of carbon directly onto the surface of a freshly-cleaved piece of mica. The carbon is then floated off onto water and transferred to EM grids in one of two ways:

- TEM grids can be brought up through the water from below the film or- the film may be carefully lowered onto grids, situated beneath the water surface on a piece of wire mesh, by slowly removing the water from the vessel.