

## Preparation of Specimen Support Films for TEM

### Materials

Microscope slides	Forceps (fine tipped)
Coplin jar	TEM grids
Glass dish	Parafilm
Razor blade	

### Procedure for Formvar support film

1. To ensure good film adhesion, grids used in this procedure should be clean. If not clean from the manufacturing process, they may be cleaned by washing four times in chloroform in the original container, followed by four washes in 95% or 100% ethanol. Dump the grids out onto a lint-free filter paper and allow to dry before attaching film to them.
2. Prepare a 0.25% Formvar solution in ethylene dichloride.
2. Fill a Coplin jar 3-4 inches deep.
3. Place a glass slide into the Formvar solution. The slide may be breathed upon to attach water vapor. Then it should be wiped clean with lint-free paper but not extensively washed or acid cleaned.
4. Remove the slide and prop up to drain on filter paper in a desiccator.
5. Allow the slide to thoroughly dry—approximately 5-10 minutes.
6. Fill a wide mouth glass container nearly full of distilled water. In order to ensure a clean surface, fill the container to overflowing and then dump off a small amount.
7. Break the edges of the film by scraping with a razor blade. Be careful to avoid scattering glass particles on the film surface.
8. Float the film off the slide by gently lowering the filmed end at a low angle into the water. The film should release at the edge and can be removed by gradually submerging the slide.
9. Place grids, shiny side down, onto the film at spacings of about one grid width apart.
10. Pick up the film/grids by placing a strip of Parafilm on top of it and lifting up off the surface of the water. Avoid bubbles between the Parafilm and the support film.
11. Allow to dry in a dust-free area.

### Butvar Support Films

Butvar (polyvinyl butyl resin) films are more difficult to prepare than formvar films but their superior qualities make the extra effort worthwhile. Butvar films are more mechanically stable, have less intrinsic structure, and

greater translucency. In addition, the hydrophilic nature of the butvar makes it an ideal substrate for negative staining and other staining procedures.

### Procedure

1. Prepare a solution of 0.25% butvar (W/V) in chloroform.
  - a. In an exhaust hood—heat a water bath (use a heatable stir plate) to 50°C.
  - b. Weigh 0.125 gms of butvar powder in a clean 100 ml beaker.
  - c. Pour 50 ml of chloroform down the sides of the beaker being careful to wash all of the crystals down.
  - d. Add a clean stir bar to this beaker.
  - e. Cover the beaker with aluminum foil, then with parafilm.
  - f. Insert thermometer through beaker coverings and set beaker in water bath.
  - g. Stir slowly until the solution has reached 50°C.
2. Cool to 40° - 42°C just before coating the slide.
3. Hereafter, the heating and cooling procedure must be followed each time the stock solution is used to coat grids.
4. Butvar films are cast on microscope slides using the same procedure as for formvar films.