

# APPENDIX 1

## Preparation of Support Films for Negative Staining

There are a variety of methods for preparing support films for attachment to electron microscope grids. Such films may be prepared from Parlodion or Formvar using techniques that are very simple—casting films on water by dropping a plastic solution onto the water surface—or complex, such as the use of very elaborate film casting chambers that precisely control draining and drying rates. The method we use is fairly simple and straightforward, and yet it will usually produce satisfactory support films. The films are generally more uniform than water-cast films and the thickness of the film can be controlled.

### Materials Required

1. Ethylene dichloride (1,2-dichloroethane), reagent grade.
2. Formvar powder.

3. Grids of suitable mesh. Fine mesh grids are needed to give good support to the plastic film, which is delicate. Rather than the usual square mesh, we use  $75 \times 300$  or  $100 \times 400$  mesh grids that have rectangular openings. When oriented properly in the electron microscope, this type of grid will allow long scanning treks uninterrupted by grid bars, yet providing excellent specimen support.

4. Glass microscope slides. These should not be pre-cleaned, as it is difficult to secure the release of Formvar films from slides that have been cleaned with a detergent. Neither should they be fire-polished nor have beveled edges, as sharp edges are required to cut the plastic film free of the slide. By the same token, these unfinished slides with their sharp edges are likely to cause cuts if they are improperly handled. Exercise caution when polishing them. Some lots of slides may work better than others for preparing films, even though they are from the same supplier and bear the same description and catalog numbers. If slides from a particular lot work especially well, then reserve an ample supply from that lot to be used solely for filmcasting. The slides that work less well can be used for preparing leaf dips and for other tasks not requiring special characteristics. Slides (most, but not all lots) that have usually worked well for us for casting films are Thomas Red Label Micro Slides, Economy Grade, Catalog No. 6685-H21, Arthur H. Thomas Company.

5. Bamboo splints (skewers).

6. Filter paper disks of assorted sizes and user-preferred grades. It is easy to pick up grids from a creped type of paper such as Fisher P8, Catalog No. 09-790-12C (for 9-cm disks).