Negative Staining of Particulate Samples

Needed Materials:

- Carbon coated grids
- Pasteur pipettes
- 1% uranyl acetate stain in dH₂O
- Self-closing grid tweezers
- dH₂O for rinsing the grid
- Pipetman and tips
- UA waste disposal container
- Grid box

Caution: All excess uranyl acetate and contaminated, filter-paper wedges, etc should go into the provided UA waste container. All lab users are responsible for properly removing materials contaminated with bio hazardous materials. Ask for assistance if you need it.
Procedures (Click on each thumbnail for a larger image. Click on the large image to close.):

1. Previously prepared uranyl acetate stain is available in the laboratory refrigerator. It was made by dissolving 1% of the granular stain (w/v) in distilled water. The resultant solution was filtered and put into Eppendorf tubes.

2. Glow discharge carbon-coated grids as described. A small drop of liquid should immediately cover the entire grid when applied if the grid is properly glow discharged. A hydrophobic grid will cause the liquid to bead up (Fig. 1).

3. Place a small drop (3-5 μl) of the sample on the grid (Fig. 2). The sample concentration will depend on a number of factors and may have to be determined by trial and error. For viruses, in the range of 20 nm to 100 nm, a protein concentration of about 1 mg/ml is sufficient. The amount of time that the sample sits on the grid is not important unless the sample concentration is very low. In that case, allow the sample to sit about 5 minutes to allow more of the sample to adhere to the carbon.

4. Rinse the grid with 10 - 20 drops of distilled water (Fig. 3). If the sample is infectious this should be done over a biohazard bag. This step is necessary because the stain does not interact well with many buffer salts. At this point the sample is well-adhered to the carbon film and will not wash off.

5. Add a drop of the UA stain to the grid and flick it off into the UA waste jar (Fig. 4). Repeat two times.

6. Take a wedge of the filter paper and wick the stain from the edge of the grid (Fig. 5). If the grid was properly glow discharged it should have a slight sheen on it from the remaining stain. Place the tip of the filter paper between the blades of the tweezer to soak up any liquid that may have gotten between them and gently guide the grid with the filter paper into a grid storage box. Failure to do this last item may result in the grid riding up along the tweezers in a drop of the remaining liquid.

7. The grid should dry in a few minutes and then will be ready to be put into the microscope.