Lead Post-Staining

Prep:

A stock solution of 1% (w/v) KMnO4 is prepared by dissolving KMnO4 in distilled water.

This is a purple-red colored solution that can be kept several months at room temperature.

KMnO4 in sulfuric acid concentrations >0.5N severely damages Lowicryl K4M ultrathin sections, and sulfuric acid concentrations <0.01 N Produce contaminants on the sections.

Prolonged incubation of > 5min. causes the loss of ultrathin sections on gold TEM grids, and <10 seconds has no effect on contrast.

Staining:

1. Just before staining, add 100μl of the KMnO4 stock solution and 100μl of 1 N H2SO4 (sulfuric acid) to 800μl of distilled water for a final concentration of 0.1% KMnO4 in 0.1 N H2SO4.

2. Dip coverslip in coplin jar filled with water

3. Remove and put into petri dish with filter paper or gauze underneath

4. Stain 1 minute in freshly made acidified permanganate staining solution.

5. Quickly remove stain and flood coverslip with large quantities of DI water

6. Put back in staining dish and add small amount of filtered 5% aqueous Uranyl Acetate

7. Stain for 30 min.

8. While coverslip is staining, make up fresh lead solution (0.01 to 0.04 g lead citrate in a 10 ml tube already weighed out). Add 1 ml of carbonate free 2N NaOH to dissolve the lead, then add 9 mls of water. Filter before using. Should be clear.

9. Remove UA staining solution and flood coverslip with large quantities of water. Store in coplin dish while getting lead stain ready. Add NaOH pellets to staining dish.

10. Add freshly made and filtered lead citrate. Stain for 1 min ONLY! Remove lead and flood coverslip with water.

11. Let dry then mount with carbon paint onto SEM mount. Let paint harden and dry thoroughly before putting in scope.

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