conditions. For TSV, *Nicotiana tabacum* and *Datura stramonium* are more suitable propagating hosts, but the same procedure as described below can be used for purification.

3.2. Extraction and Crude Purification

All purification steps should be performed in the cold with precooled buffers, equipment, and rotors. Temperatures between 0 and 4°C are sufficient.

1. Harvest the infected cucumber (*Cucumis sativus*) cotyledons and leaves 3–6 d after inoculation. Cool the plant tissues, buffers, and Waring blender to 4°C for 30 min.
2. Homogenize the tissue with a Waring blender in extraction buffer (volume equivalent to twice the weight of tissue) for 5 min at maximum speed. To prevent excessive foaming when blending, add five drops of antifoam A per 100 g leaf tissue, but this can be omitted if antifoam A is not available.
3. Centrifuge the homogenate for 20 min at 1520*g* in a Sorvall GS-3 rotor to remove cell debris. Carefully decant the supernatant into a beaker, and discard pellets.
4. Adjust the pH of the supernatant to 4.9 with glacial acetic acid under the control of a pH meter, and incubate at 4°C for 30 min. This step will precipitate most chloroplast material, host proteins, and cell debris.
5. Centrifuge for 20 min at 11,000*g*, in the same rotor as above, to remove precipitated material. Carefully rescue the clear yellow supernatant and adjust the pH of it immediately back to 7.0 with 5*N* NaOH. Discard pellets.
6. Add Triton X-100 slowly to the supernatant to a final concentration of 2% (v/v) and stir slowly at 4°C for 1 h. This step dissolves any remaining membranes and membrane vesicles, and thus prevents their sedimentation during the following ultracentrifugation.

3.3. First Concentration

Sediment the virus by centrifugation at 105,000*g* for 4 h in a Beckman (Fullerton, CA) Ti-45 rotor and let the pellets resuspend overnight at 4°C in a volume of 0.03*M* sodium phosphate, pH 8.0, equivalent to one-tenth of the original tissue weight. The pellets should be clear and glassy, with a small center of tan-colored, insoluble material. If the pellets are very difficult to resuspend, a Dounce glass homogenizer with a loose fitting piston can be applied to speed up the resuspension process.

3.4. Fine Purification

1. Centrifuge the resuspended pellet at 16,300*g* for 15 min in a Sorvall HB4 rotor and save the supernatant. This will remove most of the opaque portion of the original pellet.
2. Sediment the virus again by centrifugation for 3 h at 93,000*g* in a Beckman Ti-70 rotor. The resulting pellet should be nearly colorless and the opaque material reduced to a small dot in the center of the pellet. Most of this will be removed by the subsequent low-speed centrifugation.