3. Alternate universal buffers, which work well for all cucumoviruses tested, are: buffer A, 0.1 sodium citrate, pH 7.0, 20 m*M* EDTA, 0.1% sodium diethyldithiocarbamate (DIECA) (w/v, add just before use), 0.1% (v/v) 2-mercaptoethanol; buffer B, 10 m*M* Tris, pH 7.0, 0.5 m*M* EDTA, 2% Triton X-100; buffer C, 10 m*M* Tris, pH 7.0, 0.5 m*M* EDTA (for TAV use 20 m*M* phosphate; *see* **Note 2**); cushion I, 10 m*M* Tris, pH 7.0, 0.5 m*M* EDTA, 2% Triton X-100, 20% sucrose; cushion II, 10 m*M* Tris, pH 7.0, 0.5 m*M* EDTA, 20% sucrose. For alternate universal buffers, use 2 mL buffer A and 2 mL chloroform per gram of tissue for extraction; ultracentrifugations are done at 150,000*g* for 3.5 h.
4. One strain of CMV, M-CMV, requires a unique method for purification. Because of a modification of the CP, M-CMV is unstable in the presence of chloroform and of EDTA ***(24)***. M-CMV is extracted as follows: buffer A, 0.1*M* dibasic sodium phosphate, 0.1% thioglycolic acid (add just before use), and 0.1% DIECA (add just before use); extract in 3 mL buffer A per gram of tissue and filter the homogenate through two layers of cheesecloth and two layers of Miracloth. Centrifuge the filtrate at 15,000*g* at 4°C for 10 min. Add Triton X-100 to 2%, and stir at 4°C for 15 min. Centrifuge at 78,000*g* for 2 h. Resuspend in 0.1*M* dibasic sodium phosphate, as for CMV resuspension. Centrifuge at 5500*g* at 4°C for 10 min, and pour the supernatant into an ultracentrifuge tube. Underlay with 5 mL 0.1*M* dibasic sodium phosphate, 10% sucrose, and centrifuge, as above. Resuspend final pellet in 10 m*M* sodium borate, pH 8.0.
5. To avoid crosscontamination, especially of satellite RNAs, all plastic and glassware must be thoroughly cleaned after each use. It is preferable to bake all glass and metal components of blender jars, tubes, flasks, and stir bars overnight at 160°C. Items that cannot be baked should be soaked for 2 h in 0.1*M* sodium hydroxide, followed by thorough rinsing.
6. For less than 20 g of tissue, a polytron works best for homogenization. Alternatively, small amounts of tissue can be ground in buffer A in a mortar and pestle, squeezed through several layers of cheesecloth, and extracted with chloroform by shaking in the centrifuge bottle, before low-speed centrifugation. For 20–50 g of tissue, use a small blender jar (500-mL capacity), for 50–250 g of tissue, use a large blender jar (1000-mL capacity).
7. For very large-scale preparations, virus may be concentrated by precipitation with polyethylene glycol (PEG) prior to the first ultracentrifugation step. Replace the thioglycolic acid in buffer A with 40 m*M* 2-mercaptoethanol (S. Flasinski, personal communication). Add 10 g of PEG 8000 for every 90 mL of extract in buffer A. Stir at 4°C for 45 min. Centrifuge at 15,000*g* at 4°C for 10 min. Drain the pellets thoroughly, because residual PEG may cause the resuspended virus to precipitate. Resuspend the pellet in buffer B, using one-fourth to one-third of the original extraction volume. Stir for 45 min at 4°C. Centrifuge at 7500*g* at 4°C for 10 min. The virus will be in the supernatant, and the pellet should be very small; a large pellet indicates that the residual PEG has reprecipitated the virus. In this case, save the pellet, and resuspend it again in buffer B. Continue the purification with the second ultracentrifugation.