of 40, 30, 20, and 10% sucrose in 10 m*M* Tris-HCl, pH 7.3; let it diffuse at 4°C overnight. Centrifuge at 82,000*g* in a Beckman SW40 rotor for 2 h.

b. Remove equal volume fractions and analyze them by UV absorption and/or agarose electrophoresis (*see* **Note 2**).
c. Pool together fractions containing the virus, dilute them twofold with 10 m*M* Tris-HCl, pH 7.3, and centrifuge at 146,000*g* in a Beckman 50.2 Ti rotor for 2 h.
d. Resuspend the pellet in 10 m*M* Tris-HCl, pH 7.3. Remove unresuspended material as in **Subheading 3.1., step 7**.

6. There is an alternative method to the one described here that includes a polyethylene glycol (PEG 6000) precipitation step ***(9)***. If followed, supernatant obtained in **Subheading 3.1., step 4** is brought to 8% PEG 6000 and 200 m*M* NaCl, and stirred for 1 h at 0–4°C. Virions are then pelleted by centrifugation at 7700*g* for 20 min. Pellets are resuspended in 10 m*M* Tris-HCl, pH 7.3, and solution is finally ultracentrifuged at 146,000*g* for 2 h to pellet the virions again. We must stress, however, that in side-by-side experiments the sucrose cushion protocol described here rendered higher amounts (as much as twice) of better quality virus preparations, in a significantly shorter period of time, when compared to the PEG precipitation protocol.
7. As reported previously ***(1)***, caution must be taken with carmoviruses that have been reported to be isoelectric at acidic pHs, since virus precipitation may occur during extraction. In these cases, tissue must be extracted in neutral phosphate buffers and clarification of the homogenate achieved with organic solvents (*n*-butanol and/or chloroform) ***(1)***. We do not predict any problems with the use of centrifugation through sucrose cushions in these examples.

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