8. Wash the pellet with cold 70% ethanol and dry.
9. Resuspend the precipitated RNA in TE buffer and store at –20°C.

4. Notes

1. All the buffers used in the methods described above are stable, except for the phosphate:ascorbate buffer used for Steere's method. It is best made from the dry chemicals just before use; dissolve the ascorbic acid first, because it rapidly degrades above pH 8.0 and produces a yellow compound.
2. **Caution:** Take care with the fumes of chloroform and *n*-butanol; use these in a fume hood or well-ventilated space because chloroform is an anesthetic and *n*-butanol causes breathing difficulties.
3. The centrifugation times and *g*-values given above are only indicative and depend on the machinery available. When sedimenting the virions by centrifugation alone, be guided by the fact that long centrifugation times at large *g*-values may produce pellets that are difficult to resuspend, whereas smaller *g*-values and insufficient centrifugation times will produce unstable pellets depleted in virion-like protein shells.
4. We have found the methods described above to work well for more than a dozen tymoviruses; we always use PA buffer for extraction when the propagation host of the tymovirus produces a sap extract that oxidizes and becomes brown (e.g., *Nicotiana glutinosa* with eggplant mosaic virus). However, for other hosts, TE buffer is an alternative to PA buffer for all steps, and, for larger preparations, the first high-*g* centrifugation step can be replaced by adding 4% NaCl (w/v) and 12% w/v polyethylene glycol (mol wt 8000), stirring for 1 h, and collecting the sediment by centrifuging at 5–10,000*g* for 20 min.
5. The pI of TYMV virions is 3.75, and those of cacao, kennedya, and ononis yellow mosaic viruses are similar; those of belladonna mottle, dulcamara mottle, and eggplant mosaic viruses have pIs above 8.0 (J.-K. Mo, M. Fischer and A. Gibbs, unpublished results); however, we obtained no better preparations of the basic virions using buffers of pH 5.0.
6. An alternative method that clearly produces very pure virion preparations uses ethanol and centrifugation to prepare TYMV virions for X-ray diffraction analysis ***(13)***; 20 m*M* potassium phosphate buffer, pH 7.8, and a temperature of 4°C, is used for all stages of the procedure:
   a. Blend infected leaves in 1.5 vol/wt of buffer.
   b. Filter the fiber from the buffered extract using cheesecloth.
   c. Add 0.25 vol of 95% ethanol; stir for 30 min.
   d. Remove the sediment by centrifuging for 10 min at 5000*g*.
   e. Centrifuge the supernatant at 104,000*g* for 1.5 h to sediment and concentrate the virions.
   f. Resuspend the virions in buffer; clarify the preparation by centrifuging for 10 min at 5000*g*.
   g. Further purify the virions by another round of centrifugation; 186,000*g* for 1.5 h; 5000*g* for 10 min.