4. Notes

1. Other methods, as decribed by Van Kammen ***(6)***, and Bruening and Agrawal ***(7)***, result in lower yields of virus.
2. A pH of 6.0 has been used to prevent formation of brown-colored substances ***(8)***.
3. It may be important to carry out the initial steps in the purification in rapid succession to decrease the tendency of the virus to aggregate ***(8)***.
4. During virus purification, **steps 3**, **6**, and **7** of **Subheading 3.1.,** can be omitted. This shortens the procedure; however, the virus suspension will contain more contaminants.
5. Higher amounts of PEG have been used (up to 10%). This may be useful when the virus concentration is low.
6. To prevent aggregation and formation of brown-colored substances, it may be helpful to use a phosphate buffer containing 2 m*M* EDTA and 2 m*M* β-mercaptoethanol in this step.
7. When the virus pellet is not clear, the virus suspension can be further purified by an extra high-speed centrifugation in 0.1*M* phosphate buffer (150,000*g* for 2 h) and repetition of **Subheading 3.1., step 8**.
8. Prior to RNA extraction, virus components can be separated on sucrose density gradients ***(9)*** and/or by isopycnic centrifugation on, for example, CsCl or Nycodenz (**refs. *10*** and ***11***; *see also* Chapter 26). A 1-mg/mL suspension of purified CPMV B- and M-component has an OD_{260} of 10.0 and 6.2, respectively.
9. Other methods to extract the RNA from comovirus particles include the disruption of the particles in a detergent solution by heating ***(12)***, and treatment by proteinase K ***(13)***. After RNA extraction, the RNAs can be separated and further purified by sucrose density gradients, as described, for example, by Van Klootwijk et al. (**ref. *12***; *see also* Chapter 26).
10. Re-extraction of the phenol:chloroform layer with 0.5X extraction buffer containing 0.1% SDS can be used to improve the yield of viral RNA.

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