sequenced furoviruses: first, the 5'-proximal position of the CP cistron on a genomic RNA; second, the presence of an open reading frame (ORF) adjacent to the CP cistron, which is expressed by translational readthrough for BNYVV ***(13,14)***, SBWMV ***(6,15)***, and probably PMTV ***(10)***, and by ribosome scanning for PCV ***(7,16)***. In the case of BNYVV, this protein has been implicated in fungus transmission ***(17)***. Finally, all the characterized furoviruses have an ORF for a small cysteine-rich protein, which has been shown in the case of BNYVV to be involved in regulation of CP expression ***(18)***.

At present, a dozen viruses have been classified within the furovirus group ***(19)***, and, for each of them, a distinct purification procedure (or several such procedures) has been described. Because of this diversity, this chapter will only detail the technique described by Putz and Kuszala (*see* **ref. *20***), *which is* currently used in slightly modified form in this laboratory for large-scale purifications of BNYVV. However, significant differences with the purification methods for other well-studied furoviruses are mentioned in **Subheading 4**.

Generally speaking, furoviruses do not multiply well in their hosts ***(19)***. Another obstacle to their purification is a tendancy of the virions to self-aggregate or to adhere to large cellular components. Aggregation is reported to be particularly severe in the case of PMTV and *Nicotiana velutina* mosaic virus (NVMV) ***(21–23)***. Consequently, yields of purified virus rarely exceed 30 mg/kg of leaves. However, the method described in this chapter has given yields of BNYVV of as much as 80 mg/kg of leaves of the local lesion host *Chenopodium quinoa*.

2. Materials

1. Plant host: *C. quinoa*, aged 7–8 wk.
2. Inoculum: Freshly ground leaves of infected *C. quinoa*. Leaves that have been frozen for some weeks or lyophilized (preferable) can also be used.
3. Inoculum buffer (4X): 0.2*M* potassium phosphate, pH 7.0.
4. Abrasive powder: Celite.
5. Sterile cotton, mortar, and plastic gloves.
6. Variable speed food blender of approx 1-L vol.
7. Extraction buffer: Sterile 0.1*M* sodium tetraborate, adjusted to pH 9.0 with boric acid.
8. Clarification agent: carbon tetrachloride (CCl_4).
9. Miracloth or equivalent filter tissue.
10. Precipitation agent: NaCl (8 g/L of clarified sap), polyethylene glycol (PEG) 6000 (20 g/L of clarified sap).
11. 20% Sucrose cushion (w/v) prepared in 10 m*M* extraction buffer.
12. Low-speed centrifuge equipped with fixed-angle rotors and with tubes of capacity approx 0.5 L, 0.25 L, and 30 mL. Ultracentrifuge with fixed-angle rotor and accepting ultracentrifuge tubes of approx 25 mL.