3. Extract twice with 1 vol of diethyl ether or chloroform.
4. Add 0.1 vol of 3*M* sodium acetate, pH 5.5, and 2 vol of cold absolute ethanol. Mix thoroughly and leave at –20°C for 2 h, or at –70°C for 30 min, to allow RNA precipitation.
5. Collect the RNA by centrifugation for 15 min at top speed in an Eppendorf centrifuge at 4°C.
6. Wash the pellet with 70% ethanol.
7. Dry the pellet and resuspend it in sterile water (*see* **Note 3**).

4. Notes

1. Be aware that the pH of the homogenate does not become alkaline during extraction. Some carmoviruses tend to swell by the shift from acidic to slightly basic pHs, making the viral RNA susceptible to the degradation by nucleases. The use of low pH extraction buffers avoids this possibility, having the additional advantage that many of the host proteins precipitate at these pHs.
2. Measure the virus concentration by UV absorption (1 OD_{260} is 5 mg/mL for CarMV). We routinely follow the procedure described here in the purification of PFBV and CarMV, with yields that range from 1.6–3 mg virus/100 g tissue for PFBV, up to 2 mg/g in the case of CarMV. Purity can be checked with agarose gel-TBE electrophoresis ***(11)***; direct electrophoresis of the virion solution will render a single ethidium bromide-stained band, when free of contaminants. After ethidium bromide staining, the agarose gel is suitable for Coomassie blue staining to reveal proteins; again, a single band located at the same position should appear in reasonably pure preparations. Additionally, protein contamination can be assayed with polyacrylamide gel electrophoresis ***(12)***.
3. Measure viral RNA concentration by UV absorption; to calculate RNA extraction yield, consider that carmovirus particles have a 17–22% RNA content ***(1)***. RNA quality and contamination can be checked by agarose gel-TBE electrophoresis ***(11)***; a single sharp band should be visible; sometimes weak smaller bands corresponding to sgRNAs also appear. In order to store the viral RNA for long periods, we strongly recommend addition of 0.1 vol of 3*M* sodium acetate, pH 5.5, and 2 vol of absolute ethanol to the RNA solution; keep in aliquots as ethanol precipitate at –70°C.
4. In our experience, the virus preparation obtained by high-speed pelleting through sucrose cushions has excellent infectivity and is pure enough and suitable for several experimental approaches. For instance, we have used viral RNA extracted from virions purified in this way for in vitro translation, Northern detection, cDNA synthesis to obtain nonradioactive probes, and direct RNA sequencing by reverse transcription.
5. Should a higher purity of virions be needed, the method described above can be continued by centrifugation through a linear sucrose gradient as follows:
   a. Layer the virus fraction obtained in **Subheading 3.1., step 8** on a 10–40% sucrose in 10 m*M* Tris-HCl, pH 7.3, gradient. We recommend preparation of the gradient the day before by loading in ultracentrifuge tubes equal volumes