viruses with polyethylene glycol was first described by Hebert ***(4)***; the RNA extraction protocol is based on the procedure as described by Zimmern ***(5)***.

2. Materials

1. Stock 0.1*M* phosphate solution, pH 7.0.
2. Sucrose cushion: 40% sucrose in 0.1*M* phosphate buffer, pH 7.0.
3. Extraction buffer: 0.02*M* Tris-HCl, pH 7.6, 0.2*M* NaCl, 2m*M* EDTA, 4% SDS.
4. Phenol: Equilibrated to a pH >8.0 and containing 0.1% hydroxyquinoline.
5. Chloroform used for the RNA extraction: chloroform:isoamyl alcohol (24:1).

3. Methods

3.1. Purification of Virus

1. Homogenize infected leaves with 0.1*M* phosphate buffer, pH 7.0 (0°C, 2mL/g leaf tissue) in a blender or a mortar, and press the homogenate through two layers of Miracloth (*see* **Notes 1–3**).
2. Clarify the extract by centrifugation at 15,000*g* for 20 min.
3. Stir the supernatant for 1 min with 0.7 vol of chloroform and *n*-butanol (1:1) and centrifuge at 1000*g* for 5 min (*see* **Note 4**).
4. Remove the clear aqueous layer and add to it polyethylene glycol 6000 (PEG) to a final concentration of 4% (w/v) and NaCl to a concentration of 0.2*M*. Stir the mixture at room temperature to dissolve the PEG and NaCl, and incubate for 1 h (*see* **Note 5**).
5. Collect the precipitate by centrifugation at 20,000*g* for 15 min and resuspend the pellet in 0.01*M* phosphate buffer, pH 7.0 (0.5 mL/g leaf tissue) (*see* **Note 6**).
6. Clarify the suspension by centrifugation at 10,000*g* for 15 min at 4°C (*see* **Note 4**).
7. Layer the suspension on a sucrose cushion and sediment the virus by centrifugation at 150,000*g* for 3 h at 4°C (*see* **Note 4**).
8. Resuspend the virus pellet in sterile distilled H_2O (pH >5.2) and clarify the suspension by centrifugation at 10,000*g* for 15 min at 4°C. If the infectivity has to be preserved add phosphate buffer, pH 7.0, to a final concentration of 10 m*M* (*see* **Note 7**).
9. To determine the virus concentration, measure the OD at 260 nm. A 1 mg/mL CPMV suspension has an OD of 8.1 (*see* **Note 8**).

3.2. Extraction of Viral RNA

1. Dilute the virus suspension to a concentration of 10 mg/mL or less and add 1 vol of extraction buffer and 2 vol of phenol. Vortex for 3 min (*see* **Note 9**).
2. Add 2 vol of chloroform, vortex for 2 min and separate the phases by centrifugation at 10,000*g* for 2 min (*see* **Note 10**).
3. Repeat the extraction of the aqueous layer two times with 1 vol phenol and 1 vol chloroform added simultaneously.
4. Precipitate the RNA with 0.1 vol of 3*M* NaAc, pH 5.2, and 2.2 vol ethanol, and wash the pellet with 70% ethanol.