2. Buffer B: Buffer A containing 1% polyvinyl pyrrolidone (mol wt 40,000) and 0.5% 2-mercaptoethanol (grinding buffer).
3. Buffer C: Buffer A containing 0.5% 2-mercaptoethanol.
4. Buffer D: Buffer C containing 20% (w/v) sucrose.
5. Buffer E: Buffer A containing 10, 20, 30, and 40% sucrose (w/v), for preparation of sucrose density gradients.

2.4. Buffers for Purification of Legume-Infecting Potyviruses

1. Buffer A: 0.5*M* potassium phosphate buffer, pH 7.5.
2. Buffer B: Buffer A containing 0.02*M* sodium sulfite (grinding buffer).
3. Buffer C: 0.25*M* potassium phosphate buffer, pH 7.5 (dilute buffer A 1:1 with deionized dH_2O).
4. Buffer D: 20% (w/v) PEG in 0.02*M* Tris-HCl, pH 8.2.

2.5. Buffers for Purification of Rymoviruses

1. Buffer A: Grinding buffer: 0.01*M* K_2HPO_4. Chill buffer and tissue to 4°C before use.
2. Buffer B: 0.01*M* sodium citrate, adjusted to pH 8.0 with 1*M* HCl.

2.6. RNA Isolation

1. A 10 or 20% sodium dodecyl sulfate (SDS) stock solution made in deionized dH_2O.
2. Tris-equilibrated phenol, pH 8.0 (available from US Biochem [Cleveland, OH] and other suppliers).
3. Chloroform:isoamyl alcohol, 24:1.
4. TE buffer: 10 m*M* Tris-HCl, 1 m*M* EDTA, pH 8.0.
5. 7.5*M* Ammonium acetate.
6. Reagent grade ethanol.
7. DEPC-treated water: Add diethyl pyrocarbonate to 0.1% and stir vigorously for 1 h. Autoclave for at least 25 min (121°C). Freeze aliquots in RNase-free containers until needed. Care should be taken to avoid contamination with RNase by use of RNase-free (or disposable) containers.

3. Methods

3.1. Potyvirus Purification

This procedure is perhaps the most common starting point when attempting to purify an unknown potyvirus. It was first developed by Moghal and Francki *(3)* and is presented here. Improvements and modifications (from numerous laboratories) are incorporated into the protocol. Many of the notes for this method that follow (*see* **Subheading 4.1.**) are applicable or relevant to steps in the other protocols.

1. Grind leaves in 2 vol (400 mL) grinding buffer until thoroughly triturated. Add 0.5 vol (v/v) of chloroform and carbon tetrachloride (100 mL each). Blend again for 1 min at highest speed in the blender (*see* **Notes 1** and **2**).