plants; thus, tymoviruses are ideal for teaching virological techniques, and, unlike tobamoviruses, are not contagious, although they are readily sap transmitted.

The earliest methods used to purify the virions of TYMV exploited differences in the solubility and stability of the virions and the host sap constituents, and used only low *g*-force centrifuges. The best of these methods were the ethanol:ammonium sulfate method ***(1)*** and the pH 4.8 method ***(11)***, although we have found the latter to be unreliable.

We find that variants of Steere's method ***(12)*** work well for all the 15 or so tymoviruses that we have studied. This method uses organic solvents and differential high-speed centrifugation:

1. Blend fresh or frozen infected leaves in 1.5-4 vol/wt of PA buffer.
2. Blend further while slowly adding 0.25 vol of 50:50 chloroform:*n*-butanol to form an emulsion.
3. Centrifuge the emulsion at 5000*g*, collect, and filter the aqueous (top) layer through a small cotton wool plug.
4. Concentrate and purify the virions by one cycle, or more, of differential centrifugation (100,000*g* for 3 h, and 5000*g* for 10 min), using 50 m*M* phosphate buffer, pH 7.0–8.0, for resuspending the virions.
5. The virions can be further purified and separated from the shells by centrifuging in sucrose gradients (10–40% sucrose in TE buffer at 113,000*g* for 2 h) or in cesium chloride gradients.
6. The final virion preparation is usually dialyzed into SSC for use as a source of genomic RNA, or for use as an immunogen. If not used immediately virion preparations may be stored at 4°C, but it is best to add sodium azide to give a concentration of 1–5 m*M*.

The final suspension of virions (2 mg/mL obtained from each 10 g tissue) can be used for preparing crystals.

3.2. Genomic RNA Preparation

We prepare TYMV genomic RNA from purified virions using a proteinase K method ***(14)***.

1. Mix 1 vol of virion preparation in SSC with 4 vol of RNA extraction buffer.
2. Add proteinase K to give a concentration of 0.8 mg/mL and incubate at 56°C for 15 min.
3. Add 1*M* NaCl to restore its concentration to 0.15*M*.
4. Add 1 vol of TE-saturated phenol and mix thoroughly.
5. Add 1 vol of chloroform, mix thoroughly, then centrifuge at 5000*g* for 10 min and collect the aqueous (upper) phase carefully.
6. Repeat **steps 4** and **5** above.
7. Precipitate the RNA by adding 0.1 vol 3*M* sodium acetate, pH 5.2, and 2.5 vol ethanol, mix, place at –20°C for 15 min, and centrifuge at 15,000*g* for 10 min.