shear forces, should be avoided. Dissolution of the viral pellet may prove difficult if large quantities of virus are present. If difficulties are encountered, the volume of buffer used for solvation should be increased.

7. Ideally, the integrity of the purified virions should be confirmed by electron microscopic analysis. Samples of the virus can be stained with 2% sodium phosphotungstate, pH 7.0. The majority of particles should be 300 nm rods. Depending on the tobamovirus, rods of one or two other discrete size classes may be visible. These short rods result from the presence of encapsidated subgenomic RNAs.
8. Difficulty in extracting TMV from some hosts has been reported and extraction has been facilitated by the use of the detergent Triton X-100. Another problem encountered during the purification process is adsorption of host components to the virus. Methods involving adsorbents, such as charcoal, bentonite, and Celite, have been developed to eliminate this problem. An alternative method for the purification of TMV, which makes use of Triton X-100 and Celite, has been described by Asselin and Zaitlin ***(16)***.
9. The integrity of the purified RNA should be checked by gel analysis. A predominant band of 6.4 kb should be visible on denaturing gels. Minor bands may result from extracted subgenomic RNAs. If high levels of RNA degradation are visible, this suggests that either the particles are fragmented or that there is RNase contamination. The degree of particle fragmentation can be assessed as described above. If RNase contamination is suspected more stringent procedures should be employed to avoid this. Precautions should be taken to ensure that none of the plasticware used in the RNA extraction process is touched with bare hands and, when possible, solutions should be treated with DEPC. Solutions containing Tris cannot be treated with DEPC, because it reacts with primary amines. Therefore, it may be necessary to purchase molecular biology grade Tris, certified free of RNase, which can then be dissolved in DEPC-treated water.

References

1. *AAB descriptions of Plant Viruses*, Numbers 151 to 156, 184, 196, 330 and 351. Association of Applied Biologists, Warwick, UK.
2. Zimmern, D. (1975) The 5' end group of tobacco mosaic virus RNA is $m^7G^{5'}ppp^{5'}Gp$. *Nucleic Acids Res.* **2,** 1189–1201.
3. Gallie, D. R., Sleat, D. E., Watts, J. W., Turner, P. C., and Wilson, T. M. A. (1987) The 5'-leader sequence of tobacco mosaic virus RNA enhances the expression of foreign gene transcripts *in vitro* and *in vivo*. *Nucleic Acids Res.* **15,** 3257–3273.
4. Goelet, P., Lomonossoff, G. P., Butler, P. J. G., Akam, M. E., Gait, M. J., and Karn, J. (1982) Nucleotide sequence of tobacco mosaic virus RNA. *Proc. Natl. Acad. Sci. USA* **79,** 5818–5822.
5. Young, N., Forney, J., and Zaitlin, M. (1987) Tobacco mosaic virus replicase and replicative structures. *J. Cell Sci.* **7 Suppl.,** 277–285.
6. Pelham, H. R. B. (1978) Leaky UAG termination codon in tobacco mosaic virus RNA. *Nature (London)* **272,** 469–471.