9. Transfer the suspensions to glass or polypropylene tubes. Aliquots (~100 µL) of the resuspended polyribosomes may be pipeted into liquid nitrogen and the frozen spheres may be stored in sealed vials indefinitely. These polysomes may be used for in vitro translation, RNA extraction, or for fractionation over sucrose gradients (*see* **Subheading 3.4., Note 6**).

3.3.2. Method for Sucrose Gradient Fractionation

This procedure allows visualization of the extent of polysome polymerization and permits recovery of different size classes of polysomes. The polysomes are separated by centrifugation over sucrose gradients, as described below, to separate the polysomes according to the number of ribosomes on the mRNA molecule. **Figure 3** shows the nearly identical profiles of free and membrane-bound polysomes from uninfected and SYNV-infected tobacco leaves, and gives some indication of typical profiles recovered from this tissue. The gradients are analyzed in a density gradient fractionator that can monitor the absorbance of the eluate at 254 nm. For analysis, it is recommended that two duplicate samples are run. To prepare samples for the gradients, use 100 µL of polysomes in 1X resuspension buffer. Add 30 µL of proteinase K solution (20 mg/mL) and incubate the samples on ice for 15 min. Proteinase K treatment reduces aggregation of polyribosomes, which are sometimes formed by interactions of nascent polypeptides, to give a more reliable estimate of the polysome size distribution than would be obtained with untreated samples.

1. Sucrose gradient preparation: Prepare gradients for 18–48 h before they are needed, to permit equilibration. Protect the solutions from RNase contamination by using flamed glass pipets with a sealed tip and a small hole in the side to form the gradient layers. Normally, use a Beckman SW 41 rotor with six buckets that hold 12-mL tubes (14 × 89 mm). The sucrose layers contain 2 mL of 150 mg/mL (layer A), 4 mL of 300 mg/mL (layer B), 4 mL of 450 mg/mL (layer C), and 2 mL of 600 mg/mL of 1X gradient buffer (layer D). Load layer D on the bottom, then load the C, B, and A layers, consecutively.
2. Let the gradients equilibrate at 4°C for at least 18 h before use.
3. Load 0.5 mL samples containing 2.5–6 A_{260} units of polysomes on top of the equilibrated gradients and centrifuge at 235,000*g* for 70 min at 4°C.
4. For larger scale separations, use a SW 27 rotor with 25 × 89-mm tubes. Form a 36-mL gradient with 6 mL of 150 mg/mL, 12 mL of 300 mg/mL, 12 mL of 450 mg/mL, and 6 mL of 600 mg/mL. Layer 1-mL samples containing 10–20 A_{260} units on top of the gradients. Centrifuge at 110,000*g* for 2 h 20 min at 4°C.
5. For analytical use, and to conserve sample size, use a SW 50.1 rotor with 13 × 51-mm tubes. The 4.8-mL gradients should be composed of 0.8, 1.6, 1.6, and 0.8 mL of 150 mg/mL, 300 mg/mL, 450 mg/mL, and 600 mg/mL, respectively. The sample size should be 0.2 mL with 1–2 A_{260} units. Centrifuge at 245,000*g* for 45 min at 4°C. Alternatively, use a SW 60Ti rotor with 11 × 60-mm tubes containing