9. Centrifuge the resuspended pellets at 2000*g* at 4°C for 10 min. Remove the supernatant. Be careful not to transfer any particulate matter from the pellet and adjust the volume to 57 mL with 50 m*M* phosphate buffer, pH 6.8. Add 3 mL of 20% (v/v) Triton X-100 and stir at 4°C for 10 min.
10. Prepare six 25-mL discontinuous step gradients in 25 × 89-mm centrifuge tubes. These gradients can be prepared just prior to use. Carefully layer 7 mL 24% (w/v) sucrose, 7 mL 18% (w/v) sucrose, 7 mL 12% (w/v) sucrose, and 4 mL 6% (w/v) sucrose into the tubes. It is not essential that you keep the interfaces between the layers sharp, because the gradients are primarily being used for flotation during pelleting of the virus.
11. Layer 10 mL of the extract over each of the sucrose gradients and centrifuge at 80,000*g* in a swinging bucket rotor for 3 h at 4°C.
12. Remove the gradients and resuspend the first pellet in 5 mL of 10 m*M* potassium phosphate buffer, pH 6.8. Transfer the suspended pellet to the next tube and resuspend each pellet sequentially in the original 5 mL of buffer. Resuspend any clumps with a glass homogenizer immediately, because aggregation can occur at this point. If the pellets are green, they should be diluted to 9.5 mL with 10 m*M* potassium phosphate buffer, pH 6.8, and 0.5 mL of 20% (w/v) Triton X-100, thoroughly mixed into the solution. This is followed by repelleting through a single 6–24% (w/v) sucrose gradient, as described in **step 10**.
13. Immediately centrifuge the suspended pellets at 2000*g* for 10 min at 4°C to remove insoluble material.
14. Remove the supernatant and measure the absorbance spectrum from 220 to 320 nm. The extinction coefficient at 260 nm is 2.6 OD/mg for BSMV.
15. Normal yields for BSMV from barley leaves are 1–2 mg virus/g tissue.
16. If the preparation is to be stored, add ethylene glycol to 5% and store at –20°C (*see* **Note 2**).

3.2. Viral RNA Extraction

This protocol is based on a modification of the protocol described by Jackson and Brakke ***(15)***.

1. Dilute the virus to approx 5 mg/mL in 10 m*M* potassium phosphate buffer, pH 6.8, or H_2O to yield a volume of approx 2.5 mL.
2. Add 100 µL of 2 mg/mL proteinase K and incubate on ice for 30 min.
3. Add 2.6 mL of ammonium carbonate buffer, pH 9.0, and mix well.
4. Add 5.2 mL of phenol:chloroform and vortex well to emulsify.
5. Centrifuge at 7000*g* for 5 min to separate the aqueous and organic phases.
6. Remove the upper aqueous phase, being careful not to disturb the interface, and transfer to another tube.
7. Repeat **steps 4–6**.
8. To precipitate the RNA, measure the volume of the aqueous phase and add 1/20th volume of 3*M* sodium acetate, pH 4.8, 2 vol of ethanol, and place at –20°C for several hours.