

gation (**Fig. 2**, RNA). This procedure also provides a sensitive estimate of the contamination, since ribosomes are the major host contaminants and they contain ~65% RNA, whereas SYNIV has <2% RNA. Also, a variety of phenol extraction methods can be used, but the yields are low and are somewhat variable.

2.4. Notes on SYNIV Purification

1. About 8 d after mechanical inoculation of leaves, *C. quinoa* develops water-soaked lesions about 1 mm in diameter that turn tan-colored 2–3 d later. Virus can be transferred from single lesions during the first 1–2 d after their appearance. Upper uninoculated *C. quinoa* leaves usually develop a mild systemic mottle in 3–4 wk.
2. The infectivity of SYNIV is lost quickly unless tissue is ground in the presence of a reducing agent, such as sodium sulfite or mercaptoethanol. In the presence of 0.5% sodium sulfite, the infectivity of leaf extracts is maintained for more than 48 h at 4°C. Purified preparations of the virus stored at –80°C in maintenance buffer maintain their infectivity indefinitely.
3. The interfaces and the layers used to make gradients form more easily if a glass pipet with a sealed tip and a 2- to 3-mm hole in the side just above the tip is used.
4. The amount of tissue extracted can be increased if one has access to a large-volume zonal rotor for **step 5**. Under these conditions, a larger Buchner funnel containing a proportionately increased surface area will be needed to accommodate the larger volume recovered from the interface between the 300 and 600 mg/mL sucrose layers.
5. A particularly critical variable in the purification procedure is the thickness of the Celite pad used for filtration. Pads thicker than 7.5 mm diminish yield of virus, but pads less than 2.5 mm result in filtrates contaminated with chloroplast fragments.
6. Despite care in filtration, contamination with host components at this stage is variable, and some filtrates may contain traces of chlorophyll. These contaminants are normally difficult to remove at this stage, because repeated filtration will result in loss of virus. Moreover, a considerable quantity of the contaminating host material will sediment with the virus in subsequent stages of purification, so these preparations should be kept separate from virus preparations of higher purity. However, the partially pure preparations can be used for purposes that require less purity.

3. Polyribosome Purification from Tobacco

3.1. Introduction

Routine and reproducible procedures for isolation of polyribosomes from a variety of plant tissues was first accomplished by Jackson and Larkins (9). This procedure, which was initially developed to isolate polyribosomes from SYNIV infected tobacco leaf tissue, relied in part on the use of EGTA (ethyleneglycol *bis*-[2 amino ethyl ether] tetra-acetic acid) for chelation of metals that cause polysomes to precipitate during the first low-speed centrifugation steps. The method subsequently proved to be adaptable to most tissues of tobacco and to a wide variety of different plant, fungal, and insect species.