



Fig. 1. 1.2% formaldehyde/MOPS agarose gel of CPMV RNAs extracted from whole virus (lane 1) and the two nucleoprotein components separated by ultracentrifugation on a Nycodenz gradient (lanes 2 and 3). Each track contains approx 1 μ g of RNA. The position of RNA-1 and RNA-2 is indicated on the left. The RNAs were stained with ethidium bromide and photographed under UV light.

2. Nycodenz absorbs strongly at 260 nm. Hence, it is impossible to determine the concentration of each component by optical density after the first centrifugation. Dilute the suspension and respin as detailed in Methods. If required, separated components can be stored in the same way as whole virus (usually this means the pellet is resuspended in 10 mM phosphate buffer and stored at 4°C).
3. Phosphate ions are extremely insoluble in ethanol solutions. Avoid resuspending the separated components in phosphate buffer before RNA extraction.
4. Sugar solutions will brown or caramelize if heated at high temperatures for long periods. Ensure that solutions are autoclaved for no more than 15 min at 121°C.

Reference

1. Gugerli, P. (1984) Isopycnic centrifugation of plant viruses in Nycodenz density gradients. *J. Virol. Methods* **9**, 249–258.