Summary

A method has been developed to obtain clarified viral concentrates (CVC) from extracts of small samples. The amount of starting tissue may be as little as 1 g, and the procedure consumes minimal time and material. In a typical CVC procedure, virus-infected tissue was triturated in a buffer with a mortar and pestle and expressed through cheesecloth. The subsequent exudate was clarified with organic solvent(s) and centrifuged in a microcentrifuge. Polyethylene glycol (PEG) was added to the supernatant to precipitate the virions, and the suspension was again centrifuged. The resultant pellet was resuspended in buffer and subjected once more to microcentrifugation, and the virus-containing supernatant was retained for electron microscopy.

The CVC method was used successfully to extract, clarify, and concentrate the virions of cowpea mosaic (comovirus), cucumber mosaic (cucumovirus), nandina stem-pitting (closterovirus), peanut stripe (potyvirus), pepper mottle (potyvirus), sonchus yellow net (rhabdovirus), tobacco mosaic (tobamovirus), tobacco necrosis (tobacco necrosis virus group), and tobacco ringspot (nepovirus) viruses as well as particles associated with a disease of lettuce and escarole, which closely resemble ilarvirus virions (e.g. tobacco streak virus).

Negatively stained samples from the CVC procedure were mounted on specimen grids, and the numbers of virions were determined using an electron microscope. The number of virions found in the last step of the CVC preparations ranged from 6 to 114 times greater than those found in their comparable starting exudates. These increases of unexpected magnitude could have been only partly due to physical concentration of the sap extract (actual concentration was five to six times the concentration of the starting extract). In the course of the CVC procedure, many of those sap components that compete with the virions for available grid adsorption sites were eliminated, and this played a significant part in elevating the ratios of virion concentration.

CVC has shown potential to aid in the identification of virus hosts and to develop virus purification procedures by expediting the selection of buffers, solvent systems, reducing agents, and other additives from a spectrum of candidates. CVC can also be used in many situations when the amount of starting tissue is limited and only small amounts of virus preparation are needed. Another use for the technique is to permit the simultaneous production of a number of virus preparations. Applications for the CVC technique could also include the preparation of samples for serology, polyacrylamide gel electrophoresis, and immunosorbent electron microscopy.