Introduction

Transmission electron microscopy is frequently used in plant virus research to detect virions in crude tissue extracts (leaf dips). The discovery of virus-like particles in these extracts indicates viral infection, and may even allow the microscopist to assign them tentatively to known virus groups on the basis of particle morphology (19). Although Kitajima (15), and Hitchborn and Hills (13) have been able to detect isometric virions in leaf dips, the technique has usually been applied to the detection of elongated virions. It takes considerable skill and experience to recognize small isometric particles in the background clutter associated with crude preparations unless the virions can be found in arrays or if they are unusually numerous.

Immunosorbent electron microscopy (ISEM) (21) consists of various serological techniques that aid in the detection and identification of virus diseases. The most important of the ISEM techniques is Derrick's method of coating electron microscope grids with virus-specific antisera, and subsequently exposing these grids to extracts of known or suspected viruses (6). Derrick's technique is often used in any of several variations, most of which have been devised to increase the speed and ease of the technique (7,20), but at least one, the protein A modification (9,26), is purported to improve the sensitivity of the method.

Whenever Derrick's technique or a variation of it is used, there is seldom any problem in recognizing virions, even the isometric ones, because they are usually discovered in great concentrations. All of the ISEM techniques require the use of antisera to the viruses being studied. If these antisera are not available, or if the nature of the viruses is unknown, it is appropriate to seek other techniques for particle enhancement. The regimen described here, generically termed CVC [clarified viral concentrate(s)], comprises related procedures that are nonserological, depending rather on various clarification techniques and on the established use of polyethylene glycol (PEG) to precipitate virions. The virion precipitation characteristics of PEG were first noted by Hebert (12), and subsequently used for the purification of papaya mosaic virus by de Bokx (3). PEG has now become a primary tool for the purification of many viruses of diverse morphologies.

The CVC method is first described as the "standard" procedure, followed by descriptions of some variations. The CVC method can greatly increase the numbers of virions discovered by electron microscopy compared to the numbers found in the starting crude extracts. The use of the CVC method can produce sufficient amounts of virus suspensions to meet the requirements of electron microscopy