Virus purification method

The purpose of purification is to produce a preparation contain only infective virus particle. Purification refers to the separation of virus particles from host components in biologically active state. Purified virus is required for the production of antibodies, biochemical and molecular characterization of virus particles. Purification of virus involves various step such as propagation of virus in the host, extraction of sap, clarification, concentration and further purification. Purification methods vary with different viruses, and there are no universal methods of virus purification. Procedures that are effective for one virus may not work with the other. Stable viruses that reach high concentration in their propagation hosts are easy to purify compared to viruses that are less stable and occur in low concentration in their hosts.

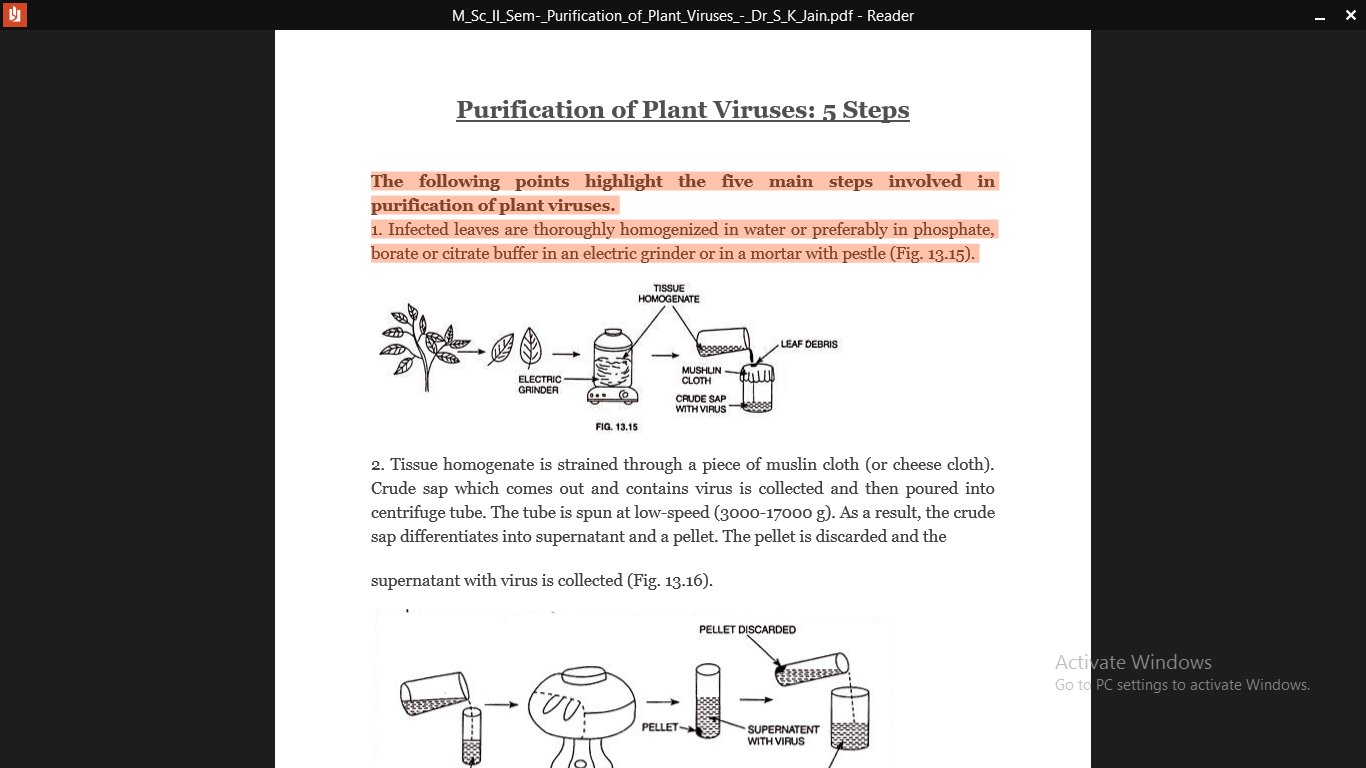
For the purification of plant virus techniques centrifuge are mainly used. Centrifugation is a technique Centrifugation is a technique that helps to separate mixtures by applying centrifugal force. A centrifuge is a device, generally driven by an electric motor, that puts an object, e.g., a rotor, in a rotational movement around a fixed axis.

A centrifuge works by using the principle of sedimentation: Under the influence of gravitational force (g-force), substances separate according to their density. Different types of separation are known, including isopycnic, ultrafiltration, density gradient, phase separation, and pelleting.

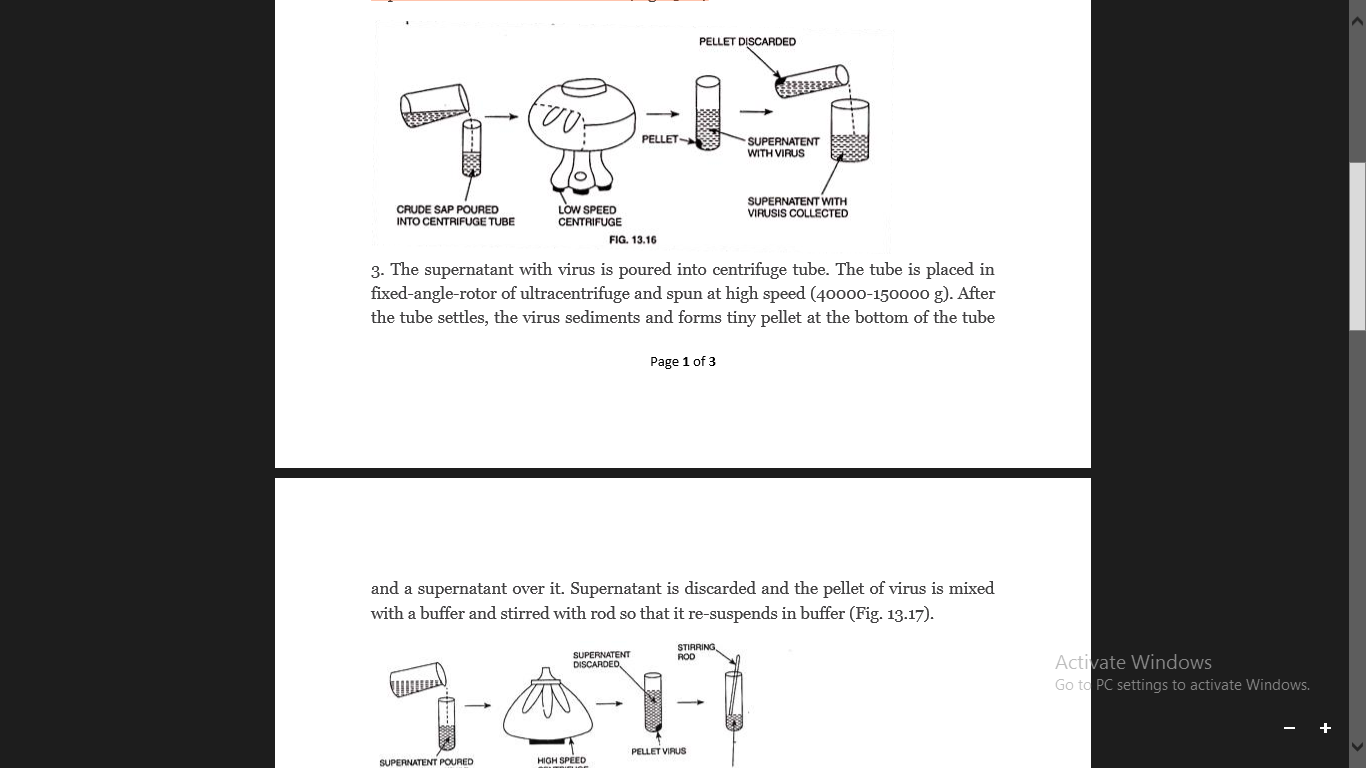
Pelleting is the most common application for centrifuges. Here, particles are concentrated as a pellet at the bottom of the centrifuge tube and separated from the remaining solution, called supernatant. During phase separation, chemicals are converted from a matrix or an aqueous medium to a solvent (for additional chemical or molecular biological analysis). In ultrafiltration, macromolecules are purified, separated, and concentrated by using a membrane. Isopycnic centrifugation is carried out using a "self-generating" density gradient established through equilibrium sedimentation. This method concentrates the analysis matches with those of the surrounding solution. Protocols for centrifugation typically specify the relative centrifugal force (rcf) and the degree of acceleration in multiples of g (g-force). Working with the rotational speed, such as revolutions per minute (rpm), is rather imprecise.

**The following points highlight the five main steps involved in purification of plant viruses.**

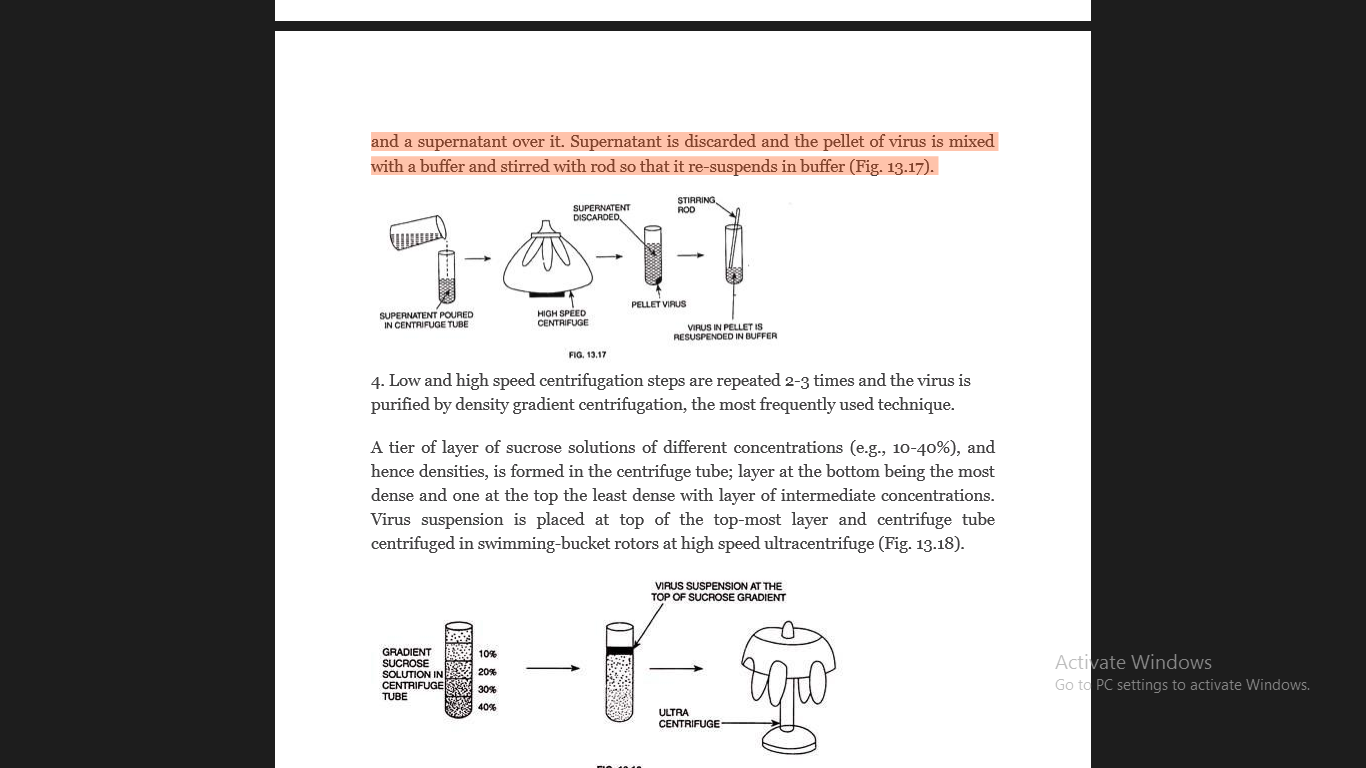
1. Infected leaves are thoroughly homogenized in water or preferably in phosphate, borate or citrate buffer in an electric grinder or in a mortar with pestle (Fig. 13.15).



1. Tissue homogenate is strained through a piece of muslin cloth (or cheese cloth). Crude sap which comes out and contains virus is collected and then poured into centrifuge tube. The tube is spun at low-speed (3000-17000 g). As a result, the crude sap differentiates into supernatant and a pellet. The pellet is discarded and the supernatant with virus is collected (Fig. 13.16)

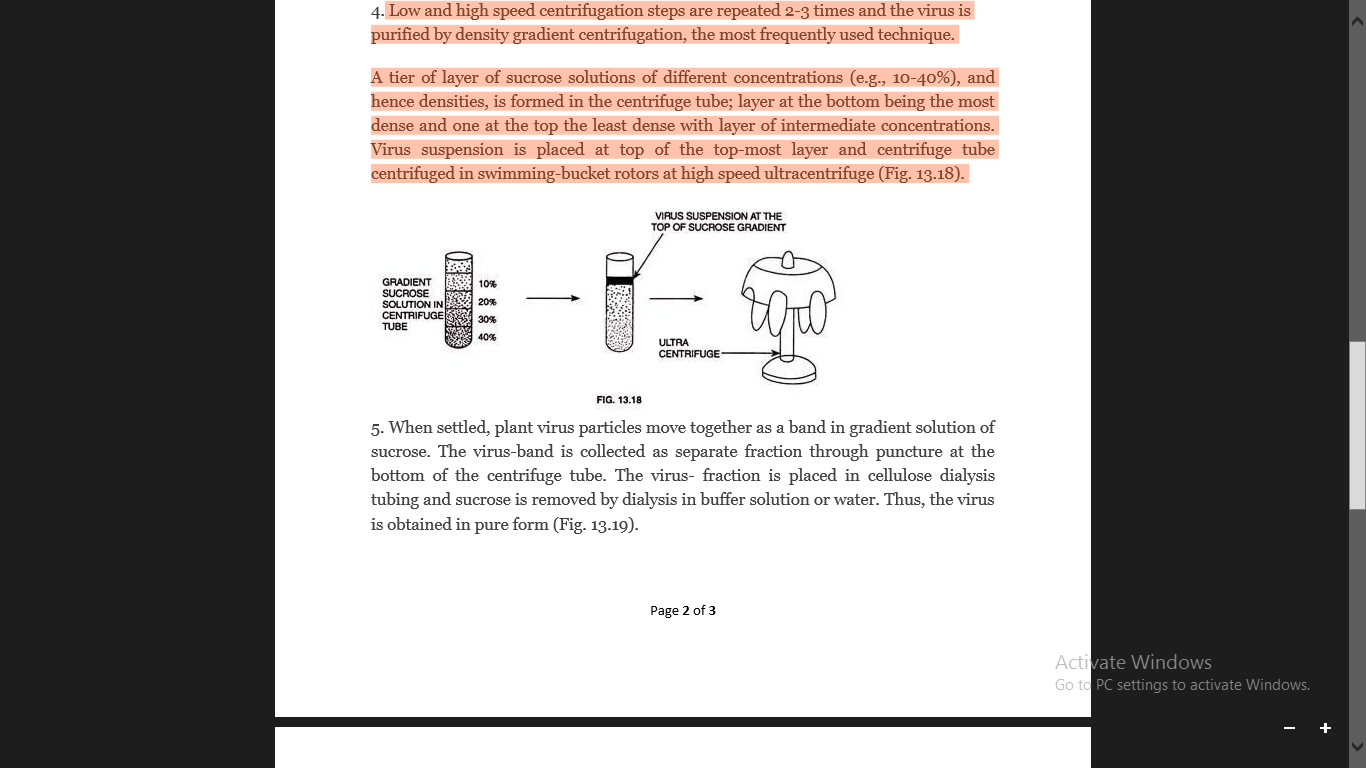


1. The supernatant with virus is poured into centrifuge tube. The tube is placed in fixed-angle-rotor of ultracentrifuge and spun at high speed (40000-150000 g). After the tube settles, the virus sediments and forms tiny pellet at the bottom of the tube and a supernatant over it. Supernatant is discarded and the pellet of virus is mixed with a buffer and stirred with rod so that it re-suspends in buffer (Fig. 13.17).

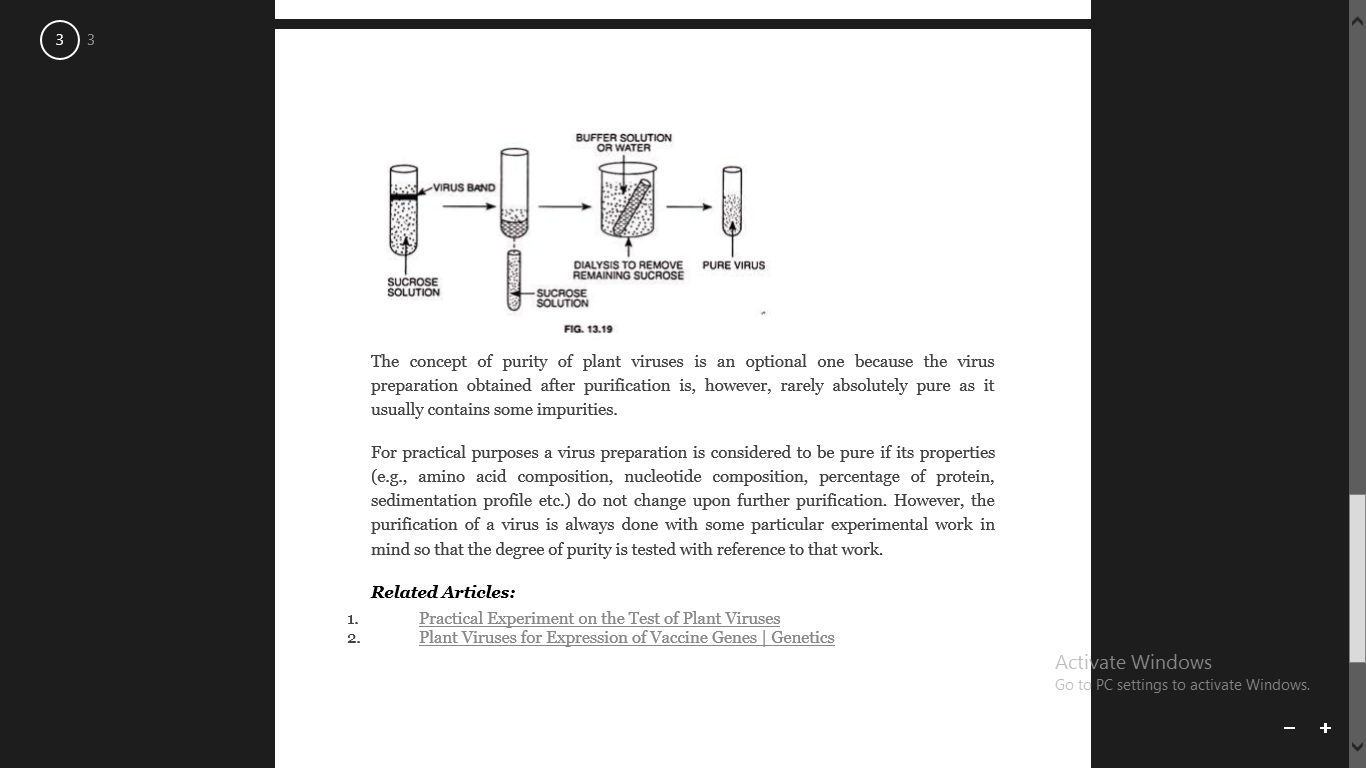


1. Low and high speed centrifugation steps are repeated 2-3 times and the virus is purified by density gradient centrifugation, the most frequently used technique.

A tier of layer of sucrose solutions of different concentrations (e.g., 10-40%), and hence densities, is formed in the centrifuge tube; layer at the bottom being the most dense and one at the top the least dense with layer of intermediate concentrations. Virus suspension is placed at top of the top-most layer and centrifuge tube centrifuged in swimming-bucket rotors at high speed ultracentrifuge (Fig. 13.18).



1. When settled, plant virus particles move together as a band in gradient solution of sucrose. The virus-band is collected as separate fraction through puncture at the bottom of the centrifuge tube. The virus- fraction is placed in cellulose dialysis tubing and sucrose is removed by dialysis in buffer solution or water. Thus, the virus is obtained in pure form (Fig. 13.19).

The concept of purity of plant viruses is an optional one because the virus preparation obtained after purification is, however, rarely absolutely pure as it usually contains some impurities.

For practical purposes a virus preparation is considered to be pure if its properties (e.g., amino acid composition, nucleotide composition, percentage of protein, sedimentation profile etc.) do not change upon further purification. However, the purification of a virus is always done with some particular experimental work in mind so that the degree of purity is tested with reference to that work