During purification some viruses lose much of the infectivity if proper care is not taken at every step. It involves a number of steps for obtaining preparations suitable for investigating the properties of the virus.

**Selection of the host**: Out of the several hosts, CYMV attained highest titre in *N. glutinosa* 10 days after sap inoculation. This host was also found to contain little amount of substances which interfere with the process of purification. So *N. glutinosa* plants were used in the purification of CYMV.

**Clarification of the sap**: All the clarification procedures removed some green host material from the diseased sap obtained from *N. glutinosa*. However, most of the treatments removed much of the infectivity (Table 11). Only butanol together with 2% triton X 100 clarified the infected sap without reducing much of the infectivity. Out of the several clarifying agents used, chloroform removed most of the infectivity.

Acidification of the diseased sap resulted in the precipitation of the plant proteins but the treatment also caused great loss of infectivity (Table 11).
Table-11. Effect of various clarifying agents, alone or in combinations and acidification on the infectivity of carrot yellow mosaic virus.

<table>
<thead>
<tr>
<th>Clarifying agent</th>
<th>Supernatant</th>
<th>Pellet</th>
<th>Infectivity (No. of local lesions/leaf*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Green</td>
<td>Green</td>
<td>85</td>
</tr>
<tr>
<td>Chloroform 10%</td>
<td>Light green</td>
<td>Yellowish brown</td>
<td>02</td>
</tr>
<tr>
<td>Chloroform + Butanol 10% (1:1)</td>
<td>Light Yellow</td>
<td>Brown</td>
<td>32</td>
</tr>
<tr>
<td>Butanol 10%</td>
<td>Light yellow</td>
<td>Light brown</td>
<td>68</td>
</tr>
<tr>
<td>Butanol + 2%</td>
<td>Light yellow</td>
<td>Light brown</td>
<td>79</td>
</tr>
<tr>
<td>Triton X 100</td>
<td>Light yellow</td>
<td>Light brown</td>
<td>39</td>
</tr>
<tr>
<td>Bentonite 2g/100 ml</td>
<td>Light yellow</td>
<td>Light brown</td>
<td>28</td>
</tr>
<tr>
<td>Carbon tetrachloride 10%</td>
<td>Light brown</td>
<td>Light brown</td>
<td>46</td>
</tr>
<tr>
<td>CCl₄ + Butanol 10% (1:1)</td>
<td>Light brown</td>
<td>Light brown</td>
<td>13</td>
</tr>
<tr>
<td>Acidification</td>
<td>Light green</td>
<td>Light green</td>
<td></td>
</tr>
</tbody>
</table>

* Average no. of local lesions on each leaf of *C. amaranticolor* based on 15 leaves.

Precipitation of the virus with Polyethylene glycol (PEG-MW 6000):

Precipitation of the carrot yellow mosaic virus was tried using 4, 6, 8 and 10% PEG (MW 6000) in the presence of salt (0.2 M). Table-12 shows that PEG 8 per cent was found to be most suitable, causing very little loss of infectivity.
It precipitated most of the virus from the sap. PEG (10\%) also precipitated the virus to some extent from the clarified sap. All the virus does not seem to be precipitated by 4 and 6 per cent PEG.

Table-12. Precipitation of the carrot yellow mosaic virus with PEG (MW 6000).

<table>
<thead>
<tr>
<th>Conc. of the PEG (NaCl 0.2 M)</th>
<th>*No. of local lesions/leaf</th>
<th>Per cent loss of infectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 per cent</td>
<td>13</td>
<td>77</td>
</tr>
<tr>
<td>6 per cent</td>
<td>20</td>
<td>65</td>
</tr>
<tr>
<td>8 per cent</td>
<td>48</td>
<td>15</td>
</tr>
<tr>
<td>10 per cent</td>
<td>30</td>
<td>48</td>
</tr>
<tr>
<td>Control (Clarified buffered extract)</td>
<td>57</td>
<td>-</td>
</tr>
</tbody>
</table>

* Average no. of local lesions/leaf developed on 3 plants having 5 leaves each.

Density gradient centrifugation:

Most of the extraneous matter from the partially purified preparation was removed by rate zonal density gradient centrifugation using a Spinco 25.1 rotor in L-3-55 Beckman preparative ultracentrifuge. Two ml of the partially purified virus was loaded on each tube and the centrifugation was continued for 3½ hours at 24,000 rpm.
Examination of tubes in a dark room after rate zonal density gradient centrifugation by projecting a narrow beam of light from the top, revealed a single light scattering zone 21-24 mm below the miniscus. Material removed from the zone when inoculated to carrot and \( N. \text{ glutinosa} \) produced typical symptoms of CYMV.

**Isolation and infectivity of RNA**

The RNA isolated by phenol-detergent method, was found to be highly infectious. UV absorption studied of the isolated RNA showed maximum absorption at 258 nm and minimum at 238 nm. Isolated RNA when inoculated to \( C. \text{ amaranticolor} \) plants produced characteristic local lesions. RNA treated with pancreatic ribonuclease when inoculated to assay host \( (C. \text{ amaranticolor}) \) failed to cause infection, suggesting that the isolated infectious nucleic acid preparation is in fact that of RNA.

**Adopted method of purification of carrot yellow mosaic virus:**

Two hundred and fifty grammes of infected \( N. \text{ glutinosa} \) leaves from plants inoculated 10 days earlier were homogenized in a Waring blender with 500 ml of 0.1 M phosphate buffer pH 7.0. The homogenate was filtered through double layers of cheese cloth. Sap thus obtained was subjected to low-speed centrifugation (10 min at 750 rpm). To this supernatant (500 ml) was added 50 ml butanol and 10 ml of Triton X-100 with continuous stirring on a magnetic stirrer for half an
hour, then incubated for one hour at 4°C. The mixture was then centrifuged at 10,000 rpm for 10 minutes. The pellet containing the plant material was discarded and the supernatant (clarified sap) was used in further purification. Thirty two grammes polyethylene glycol (PEG-MW 6000) was added to the clarified sap (400 ml) in the presence of NaCl (4.64 g for 400 ml sap) with continuous stirring on a magnetic stirrer. The stirring was continued for half an hour. The mixture was incubated for 1.5 hour and then centrifuged for 20 min at 10,000 rpm. The supernatant was discarded and the pellet containing the virus was dissolved in 100 ml buffer. Triton X-100 (2% V/V) was added to the solution with continuous stirring for 15 min. The mixture was incubated for 30 min at 4°C and then centrifuged for 10 min at 10,000 rpm. The pellet was discarded and supernatant was centrifuged for 120 min at 35,000 rpm. The pellet was dissolved in 50 ml buffer and dialysed overnight in a cellulose bag. The mixture was then centrifuged at low speed and the pellet discarded, whereas the supernatant was subjected to another cycle of high speed (2 h at 35,000 rpm) and low speed (10 min at 7550 rpm) centrifugation. After the last high speed the pellets were dissolved in 15 ml buffer. Two ml of this virus suspension was loaded at the top of each tube on a sucrose density gradient column. The tubes were centrifuged in SW 25.1 rotor for 3.5 hours at 24,000 rpm. The virus zone situated 21-24 mm below the meniscus was removed by a hypotermic needle bent twice at right angles. To the material obtained
from the virus zone was added phosphate buffer pH 7.0 and then the virus pelleted with a high speed centrifugation. The pellet was dissolved in 10 ml of buffer and subject to low speed centrifugation. The suspension thus obtained was opalescent and highly infections.
Flow diagram of adopted method for the purification of carrot yellow mosaic virus:

Macerate 250 g infected *N. glutinosa* leaves in 500 ml 0.1 M phosphate buffer pH 7.0 in a waring blender

- Squeeze through double layers of cheese cloth
- Centrifuge for 10 min at 7500 rpm

Pellet discard

- Supernatant 500 ml
- Add 50 ml butanol + 10 ml Triton X-100 with continuous stirring for 30 min
- Keep for 60 min at 4°C
- Centrifuge at 7500 rpm for 10 min

Pellet discard

- Supernatant 400 ml
- Add 8% (w/v) PEG (MW 6000)+4.64 g NaCl with continuous stirring
- Keep for 90 min at 4°C
- Centrifuge for 20 min at 10000 rpm

Supernatant discard

- Pellet
- Dissolve in 100 ml Phosphate buffer 0.1 M, pH 7.0
- Add 2%(v/v) Triton X-100 and stir for 15 min
- Keep for 30 min at 4°C
- Centrifuge for 10 min at 10000 rpm

Pellet discard

- Supernatant
- Centrifuge for 120 min at 35000 rpm

Supernatant discard

- Pellet
- Suspend in 50 ml buffer
- Dialyse for 12-15 h at 4°C
Centrifuge for 10 min at 10000 rpm

Pellet discard

Supernatant

Centrifuge for 120 min at 35000 rpm

Pellet

Supernatant discard

Suspend in 15 ml of 0.1M phosphate buffer pH 7.0

Centrifuge for 10 min at 10000 rpm

Supernatant

Pellet discard

(Virus suspension)
Fig. 11 Rate zonal density gradient centrifugation of carrot yellow mosaic virus in sucrose gradient for 3.5 h at 24000 rpm.