STUDIES ON SUGARCANE MOSAIC VIRUS

By

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C E R T I F I C A T E

This is to certify that the thesis entitled "Studies on Sugarcane Mosaic Virus" submitted to the Post Graduate School, Indian Agricultural Research Institute, New Delhi in partial fulfilment of the requirements for the award of the degree of DOCTOR OF PHILOSOPHY IN MYCOLOGY AND PLANT PATHOLOGY, is a record of bona fide research work carried out by Mr. Dilipkumar M. Joshi, under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

The assistance received during the course of these studies and source of literature cited have been duly acknowledged by him.

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_C_O_N_T_E_N_T_S_

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INTRODUCTION

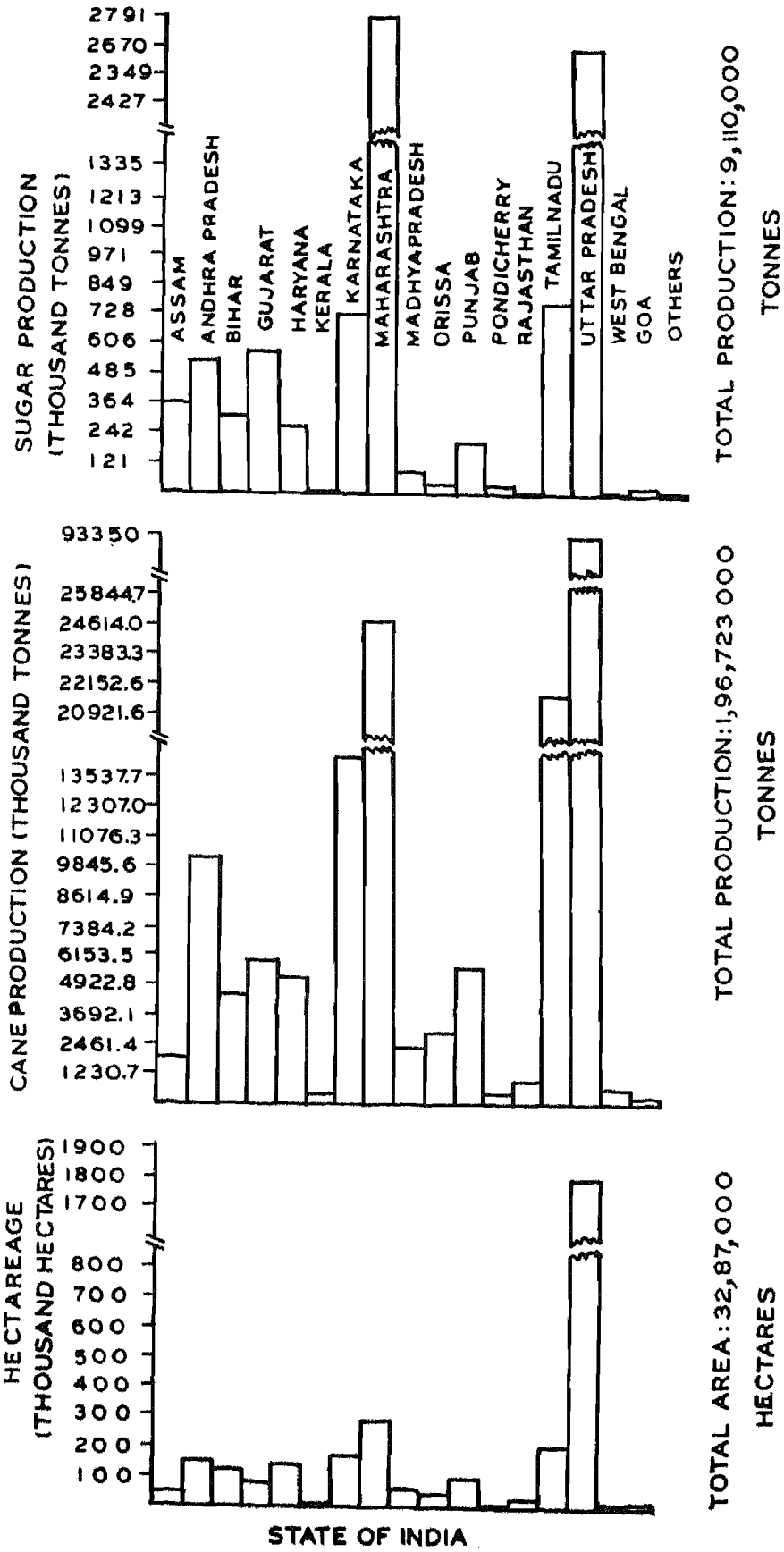
Sugarcane is the member of the genus *Saccharum* of the family Gramineae in the tribe Andropogoneae and is a perennial tropical grass. The botanical name *Saccharum* is derived from Sanskrit word 'Sharkara'. There are considerable historical evidence to show that India is a home of thin class of canes *S. barberi* Jesw. and *S. sinense* (Roxb.) Jesw. The sugarcane plant is mentioned as 'ikshu' in oldest sacred books of Hindus, the Atharva Veda (1500 B.C - 1000 B.C). The word 'ikshu' has now become 'ikh' in Hindi which is specific for *Saccharum barberi* Jesw. sugarcane.

Presently, *S. officinarum* L. which has its origin in south pacific area, is referred as thickcane. According to Budhist and Tamil literature, it is present in India from very early times. This species is now grown by and large all over the tropical and subtropical regions 30°N and 30°S of equator, and is known to tiller heavily at its base to produce a number of solid unbranched canes, 3 to 4 meter or more tall and about 5 cm thick in diameter. The juice of these canes is extracted to manufacture sugar. The development of sugar manufacture, which dates back to 300 B.C. (referred as 'gud', 'khanda', and 'sharkara') was introduced from India to Indonesia and Malaysia, through Bhuddhist influence. However, the modern and successful industry are located in all over the subtropical regions between 15° to 30° latitude as in south/central Brazil, Cuba, Mexico, South Africa, India, China, Australia, and Hawaii (Blackburn, 1984).

India was not self sufficient in respect of sugar production until 1959-60, and was the principal importer of Java sugar for a long period. By 1936-37, one hundred and forty factories were working. Nearly 12 million tons of cane were processed in that year, yielding 1,128,000 tons of white sugar, a four fold increase in four years. Imports rapidly declined from 890,000 tons in 1931 to 100,000 tons in 1936. In 1956, the five year plan had a productive target of 2,250,000 tons of white sugar. This figure was surpassed in 1959-60, one year before the target. In the subsequent year, nearly 3 million tons were produced. A considerable part of this expansion took place in tropical cane growing areas situated in states of Maharashtra, Gujarat, Andhra Pradesh, Karnataka, and Tamil Nadu, where irrigation facilities to irrigate cane crops were available. India became an exporter of sugar, with 398,000 tons being shipped in 1960-61 (Barnes, 1974). It is interesting to note that the subtropical sugar producing zone which included Gangetic Valley including Uttar Pradesh and Bihar besides east Punjab and Bengal, during these years did not contribute to this change.

During the year 1987-88, the sugarcane in India was cultivated in 3,287,000 hectares area, with sugarcane production of 1,96,723,000 tonnes, and total sugar production as 9,110,000 tonnes (Figure 1). The area of sugarcane cultivation was highest in Uttar Pradesh followed by Maharashtra, with similar trend in sugarcane production. Sugar production was, however, significantly higher in Maharashtra about 1.04 times more, as compared to Uttar Pradesh. In fact, Maharashtra occupies first place in total sugar production in spite of having 6.1 times less area and 3.7 times less sugarcane production, than Uttar Pradesh. The similar

FIG. 1. TOTAL AREA UNDER SUGARCANE, CANE PRODUCTION, AND SUGAR PRODUCTION, IN DIFFERENT STATES OF INDIA DURING THE YEAR 1987-88.



trend in sugar production was being observed for last fourteen years (Anonymous, 1989). Maharashtra stands first among the states in the matter of sugar recovery (10.90%), followed by Karnataka and Gujarat (Rao et al., 1983), probably because of the favourable climate.

In sugarcane crop, around 147 diseases have been listed from all over the world out of which 84 have been reported from India and seven of these diseases are of major economic importance, sugarcane mosaic disease is one of them prevalent all over the cane growing regions of the world. It is considered most distructive virus disease of the sugarcane. The Lousiana Sugar Industry which was threatened during 1920-27 with the mosaic disease epiphytotic affecting their breeding programme. The breeding programme is even today centered on the important objective of mosaic resistance.

In India, Barber who pioneered the sugarcane breeding programme at Coimbatore as early as 1912, recorded the mosaic disease of sugarcane infecting one of his first generation prized commercial variety Co 205 (Blackburn, 1984). Chona (1944) observed that even 100 per cent infection of sugarcane crop with mosaic caused 10 per cent loss in yield. However, he (1958) considered it to be a potential threat with the possibility of development of its more severe pathogenic strains. Singh (1971) considered the sugarcane mosaic virus disease to be of national importance.

Almost every field of Co 671 variety of sugarcane in South Gujarat surveyed currently had more than 80 per cent of plants infected with the sugarcane mosaic virus. The infected plants displayed severe chlorotic symptoms, severely affecting the photosynthetic potentials of

the infected plants and thereby reducing the recovery of the sugar.

Since sugarcane mosaic is most widely prevalent disease in all the sugarcane growing regions of the country and have the potential to cause severe losses, adversely affecting the economy of the sugarcane industry; the present studies were undertaken to properly characterize and study the extent of variation of the Indian isolates of SCMV collected from agroclimatically different sugarcane growing zones. Suitable, accurate and rapid diagnostic methods were also needed to be devised to take these steps to further strengthen the economy of sugarcane cultivation and sugar production.

2. REVIEW OF LITERATURE

Dr. Melvin Calvin, Noble Prize Winner in Chemistry, in 1961, considered the sugarcane plant as the 'champion terristrial plant in its efficiency in storing solar energy', as it is capable of producing 90 tonnes of storage tissue with barely 1 per cent of total Solar energy, available to the plant. However, almost all the parts of these plants are affected by large number of diseases. The involved pathogens are viruses, mycoplasmas, bacteria, fungi, nematodes etc. (Rao et al., 1983).

The mosaic disease of sugarcane ranks as one of the major sugarcane diseases of the world causing mosaic, stunted growth of the plants, and considerable reduction in the yield. The mottling of the leaves is accompanied by light green and yellowish patches. In India, mosaic disease of sugarcane is most widely distributed and considered as one of the major diseases of the crop.

The disease was first reported from Java in 1892 as 'Gelestrepenziekte' meaning yellow stripe disease (Musschenbroek, 1892). Subsequently thereafter the disease was observed in Hawaii in 1908 (Lyon). The disease occurred in epiphytotic forms in Puerto Rico in 1916 as reported by Earle (1918), who the named it as 'mosaic' disease of sugarcane.

A serious outbreak of a disease occurred in Lousiana completely destroying the sugarcane crop and adversely affecting industry to a chaotic conditions. This has stimulated remarkably the breeding programme for resistant varieties in U.S.A. and have led to the establishment of the Canal Point Cane Breeding Station at Florida in 1918,

and influenced the research programme of the other breeding stations in the world, especially in Java and Barbados (Barne, 1974). Edgerton (1955) gave a general description of the outbreak and the methods by which it was controlled.

Canal Point is the home of World Reference Collection (WRC) of sugarcane varieties, a duplicate collection of which is also kept at Coimbatore, India.

In India, Dastur (1923) recorded the occurrence of the sugarcane mosaic virus from Pusa (Bihar). Chona and Rafay (1950) studied the mosaic disease in detail.

2.1 HOST REACTION STUDIES OF SUGARCANE MOSAIC VIRUS

Sugarcane mosaic virus was reported to infect a large number of graminaceous hosts, in nature or on being inoculated artificially. These include cultivated crops like maize, sorghum, millet (Brandes and Klaphaak, 1923), wheat, barley, rye and rice (Abbott and Tippett, 1964), besides a number of other cultivated and wild grasses.

The SCMV was found to be the cause of common leaf mottle in maize in Venezuela (Ordosgoitty and Malaguti, 1969; Lastra and Trujillo, 1976) and in Israel (Klein et al., 1973). Rishi (1973) mechanically inoculated A and F strains of SCMV to maize cv. Deccan hybrid, and infection was reported to be maximum, when both temperature and humidity were higher. The Johnsongrass strain was found to be the major one infecting maize and sorghum in Australia and was the cause of maize ringspot mottle disease (Teakle and Grylls, 1973). The mechanical inoculation of maize roots (cv. Golden Bantam) with SCMV did not result

in systemic infection and the virus could only be isolated from 9 of 1,950 inoculated roots. Although, some SCMV was detected in root; of young leaf inoculated seedlings (Moline and Ford, 1974). In Kenya, Louie (1980) observed SCMV infecting maize in 21 districts, with infection percentage ranging from 15.2 to 19.6 per cent. Maize crop in the vicinity of sugarcane in Egypt was also recorded to be severely infected with a strain of SCMV (Allam et al., 1981). The seedborn nature of SCMV in maize cv. Kalahari Early Pearl, with inhibition of germination, and reduction of growth was demonstrated by Wechmar et al. (1984), in South Africa. Martin et al. (1984) developed cultivar Kulara of sweet corn, resistant to SCMV-Jg strain under field conditions.

Sorghum is another important crop affected by the sugarcane mosaic virus. In India, Rishi et al. (1973) reported natural infection of jowar (*Sorghum vulgare*) for the first time from India. Klein et al. (1973) identified the SCMV strain infecting sorghum and Kaffir corn (*S. caffrorum*) in Israel. Benigno and Veqgara (1977) found that a strain of SCMV caused red stripe and mosaic in grain sorghum. Different sorghum cultivars and accessions reacted either by producing mosaic or red stripe symptoms, or both. Those with mosaic reaction gave a decrease in yield of 44 to 54 per cent, while those with both symptoms died prematurely and produced no grain.

Stokes et al. (1982) observed that when sweet sorghum cultivar NM 4611 was infected with SCMV strain A and used as male or female parents in cross with cv. Planter, the presence of virus reduced the number of F_2 populations that fitted expected segregation ratios by 10 per cent. Genetic effects associated with the reductions apparently

influenced the recombination of gamate in joint segregation, indicating that the initial effect occurred prior to or during gamate formation in virus infected NM 4611. When planter was virus infected parent the changes in expected ratios varied < 2 per cent from control corsses. Virus infection may thus affect the evaluation of parental material, normal segregation ratios, and maintenance of pure line seed supply. Henzell et al. (1982) in Australia developed over 6 year research, fifteen parental grain sorghum lines (QL-6 to QL-17 and QL-20 to QL-22) homozygous for Krish source of SCMV resistance. SCMV was not detected in new growth of leaves following mechanical inoculation. QL-19 contains the Q-7539 source of resistance and confers it at high level to natural infection. QL-19 averaged 13.5 per cent infected plants in field tests. Garud and Mali (1983) identified SCMV-johnsongrass strain causing a red stripe virus disease of sorghum in India. Derrick et al. (1984) observed accumulation of dsRNA in older sorghum leaves systemically infected with SCMV.

Beside sorghum and maize, the SCMV also infected bajra (*Pennisetum typhoides*), johnsongrass (*Sorghum halepense*), ragi (*Eleusine coaracana*) and bamboo (*Arundinaria gigantea*) (Rishi et al., 1973; Klein et al., 1973; Subbayya and Raychaudhuri, 1970; Benda, 1970).

An Elephantgrass (*Pennisetum purpureum*) was reported to be a natural host of the SCMV and act as a reservoir of SCMV infection for sugarcane crops (Bhargava et al., 1969; Rishi et al., 1973). Joshi and Gupta (1976) reported *Digitaria adscendens* as a another weedhost of SCMV which was frequently and abundantly observed in the vicinity of sugarcane, maize and sorghum fields in Bhabhar belt of Nainital

district. Nikolic et al. (1973) in Yugoslavia isolated maize strain of SCMV from *Digitaria sanguinalis* Scop. and *Setaria verticillata* P.B. from nature. Teakle and Grylls (1973) identified four groups of SCMV strains designated as *Digitaria didyctyla* strain, Johnsongrass strain, sugarcane strain and *Urochloa mosambicensis* strain, in Australia. The crown foot grass (*Dactyloctenium aegyptiacum* Wild) was reported as another natural host of sugarcane mosaic virus from Gorakhpur (Singh and Bhargava, 1975), and also from Coimbatore (Singh, 1976). Ram and Chatterjee (1977) identified a strain of SCMV on the basis of host range, transmission, and physical properties, which caused mosaic of *Panicum crosgalli*.

Grasses such as *Rottboellia exaltata* in USA (Gillaspie and Koike, 1973), *Dichanthium annulatum* (Sadruddin et al., 1981) in U.P., India; and 55 grasses were found susceptible to SCMV from Mississippi (Rosenkranz, 1987).

2.2 APHID TRANSMISSION OF SUGARCANE MOSAIC VIRUS

Brandes (1920) was first to demonstrate that corn leaf aphid, *Rhopalosiphum maidis* (Fitch.) was able to transmit the SCMV from diseased to healthy sugarcane in nature. Subsequently thereafter a number of aphid species (Table 1) were rcorded as vectors of SCMV by various workers.

Tate and Vendenberg (1939) reported *R. maidis* Fitch., *Carolinaia cyperi* V.d.G., *Aphis gossypii* Glov., *Schizaphis graminum* Rond., and *Hysteroneura setariae* Ths.

Table 1. Transmission of sugarcane mosaic virus by different species of the aphids

Sl No.	Aphid species	References
1.	*Aphis craccivora* (Koch)	Teakle and Grylls (1973); Yang et al. (1984)
2.	*Aphis gossypii* (Glov.)	Bhargava et al. (1971); Teakle and Grylls (1973)
3.	*Aphis nerii* (Boy.)	Bhargava et al. (1971); Yang et al. (1984)
4.	*Aphis spiraecola* (Patch)	Yang et al. (1984)
5.	*Carolinaria cyperi*	Vedenberg (1939)
6.	*Ceratovacuna lanigera* (Zehntner)	Yang et al. (1984)
7.	*Uroleucon (Dactynotus) ambrosiae* (Thos.)	Koike (1977); Koike (1979)
8.	*Hysteroneura setariae* (Thos.)	Tate & Vendenberg (1939); Daniel et al. (1972)
9.	*Lipaphis pseudobrassicae*	Bhargava et al. (1971)
10.	*Macrosiphum euphorbiae* (Thos.)	Teakle and Grylls (1973)
11.	*Melanaphis idiosacchari*	Daniel et al. (1972)
12.	*Melanaphis sacchari* (Znht.)	Bhargava et al. (1971); David et al. (1972); Rizvi and Bhargava (1973); Beningo and Veqgara (1977)
13.	*Myzus persicae* (Sulz.)	Tosic and Sutic (1968); Daniels and Toler (1969); Shaunak and Pitre (1971); Yang et al. (1984)
14.	*Rhopalosiphum maidis* (Fitch)	Brandes (1920), Tosic and Sutic (1968); Chona and Seth (1958); Bhargava et al. (1971, 1972); Rizvi and Bhargava (1973); Teakle and Grylls (1973); Fisher and Lockhart (1974); Beningo and Veqgara (1977); Mali and Garud (1978)
15.	*Rhopalosiphum padi* (L.)	Teakle and Grylls (1973)
16.	*Rhopalosiphum rufiabdominalis* (Sasaki)	Bhargava et al. (1971)
17.	*Schizaphis graminum* (Rond.)	Daniels and Toler (1969)

The acquisition period after starving was one minute by *S. graminum* and *M. persicae*, and two-minutes by *R. maidis*. The virus persisted 6 hours in *S. graminum*, 4 hours in *R. maidis* and 2 hours in *Myzus persicae*, there were no latent periods (Tosic and Sutic,1968). Zummo and Charpentier (1965) while studying vector-virus relationship, found on the other hand &reported that *R. maidis* became viruliferous 5 minutes after being placed on infected sugarcane and transmitted the virus within 15 minutes, and lost the ability to transmit SCMV within 1 hr after removal from a diseased source. Daniels and Toler (1969) transmitted the johnsongrass strain of SCMV infecting maize by *S. graminum* with 1 to 3 minutes acquisition probes or acquisition feeding for 24 hr on infected material. The SCMV and maize dwarf mosaic virus isolates were transmitted by *Myzus persicae* from maize to maize with efficiency ranging from 3 to 19 per cent, and frequency of transmission was greater for MDMV than SCMV isolate (Shaunak and Pitre, 1970). In India, *R. maidis* and *Schizaphis graminum* had been reported as vector of SCMV (Chona and Seth, 1958; Seth and Chona, 1961). Bhargava et al. (1971) reported several aphids, colonizing sugarcane and other plants growing in the vicinity of sugarcane plantations and showed that *Aphis gossypii*, *Melanaphis (Longiunguis) sacchari*, *Lipaphis pseudobrassicae*, *Myzus persicae*, *Rhopalosiphum maidis* and *R. rufiabdominalis* could transmit strain B of SCMV from sugarcane to sugarcane, maize and sorghum; from maize to sugarcane and maize; and from sorghum to sugarcane and sorghum.

Bhargava et al. (1972) have shown that *Aphis nerii* also is capable of transmitting strain D of SCMV. David et al. (1972) have recorded *R.maidis*, *Melanaphis sachhari*, *M. idiosacchari*, *Hysteroneura*

setariae and bug *Assamia moesta* as vector of SCMV from Coimbatore. Bhargava et al. (1971) observed *Longiunguis sacchari*, *L. indosacchari* and *R. maidis* in the test plots and correlation was observed between disease incidence and the presence of these vectors. Rizvi and Bhargava (1973) reported six aphid vectors of sugarcane mosaic virus in and around sugarcane plantations throughout the year in Gorakhpur and elsewhere in Uttar Pradesh. The *M. sacchari* and *R. maidis* were considered by them as a major vector to spread SCMV.

In Australia, Teakle and Grylls (1973) grouped 4 strains of SCMV, and the aphids, *Aphis craccivora*, *A. gossypii*, *Macrosiphum euphorbiae*, *Rhopalosiphum maidis*, and *R. padi* each transmitted at least 1 of the 4 strains while *Aphis craccivora* and *Rhopalosiphum maidis* were each able to transmit all the four strains namely johnsongrass strain, sugarcane strain, *Urochloa mosambicensis* strain and *Digitaria didyctyla* strain. The johnsongrass strain is the major one infecting maize and sorghum crops.

In Morocco, Fisher and Lockhart (1974) studied SCMV and *R. maidis* which occurs naturally in Morocco, was the vector of the SCMV, but not *R. padi*. The red stripe and/or mosaic in grain sorghum is caused by a strain of SCMV, in Philippines (Benigno and Veqgara, 1977) and *R. maidis* and *Longiunguis sacchari* are the aphid vectors. While sorghum red stripe recorded from India (Mali and Garud, 1978) was caused by SCMV-Jg strain and was transmitted by maize aphid *R. maidis* to johnsongrass.

Koike (1977) observed selective transmission of SCMV strain I, or MDMV-A strain, or transmission of both strains together, by

Dactynotus ambrosiae to rio sorghum, from *Rottboellia exaltata* simultaneously inoculated with both the strains. In Lousiane, USA, Koike (1977) observed that the SCMV-H strain originally from a field grown sugarcane plant, lost its transmissibility by *Dactynotus ambrosiae* after having been maintained in the glass house on sugarcane by mechanical inoculation or vegetative propagation for 16 years, which suggested mutational change of SCMV-H to non-aphid transmissible forms, which became dominant (Koike, 1979). The other SCMV strains (A, B and D) maintained under the same glasshouse conditions as SCMV-H strain, and SCMV-I and M strain maintained for shorter period, remained aphid transmissible, as were freshly collected SCMV-H isolate. All the strains were readily transmitted mechanically.

Yang et al. (1984) in Taiwan, trapped 48 aphid vectors species during October 1977 to June 1981 in cane fields in 3 localities, the most abundant were *Aphis nerii*, *Myzus persicae*, *A. gossypii*, *Ceratovacuna lanigera*, *Capitophorus hippophaes*, *A. spiraecola* and *Aphis craccivora*. The seasonal abundance of alate and their relationship with weather conditions were also investigated.

2.3 PURIFICATION OF THE SUGARCANE MOSAIC VIRUS

Sugarcane mosaic virus is grouped under difficult to purify potyviruses which gives only low yields (Hollings and Brunt, 1981). Different problems have been encountered during purification of various potyviruses. The major problem is usually the irreversible aggregation of virus during extraction; or in the later stages, and its loss in low speed centrifugation. Although aggregation could be reversed or decreased

by adding chelating agent such as EDTA, however, EDTA did not prevent the aggregation of the sugarcane mosaic virus, in the initial extraction stage or when resuspending the pelleted virus (Damirdagh and Shepherd, 1970).

Baudin et al. (1968) partially purified sugarcane mosaic virus by gel filteration. The crude extract after clarification with chloroform (1:1) was filtered through Sephadex G-200 column and the infectious fraction, collected ahead of elution, were almost free from pigments. They gave a UV spectrum an optimum at 260 nm, and produced with immunoserum a reaction line not observed in healthy fractions. The gel sephadex technique produced an infectious extract free from many contaminating proteins and sugars and can thus constitute a preliminary purification stage prior to concentration and ultracentrifugation. The SCMV strains A, B, D and H were purified by Bond and Pirone (1971). The SCMV in the chloroform clarified crude sap was concentrated by one cycle of differential centrifugation and then floated on 10 to 40 per cent sucrose gradient and centrifuged at 90,000 g for 2 hr to give one light scattering zone. A very similar procedure, in which the sucrose for density gradients was dissolved in 0.01 M sodium citrate, was used for purification of maize dwarf mosaic virus strain A and B as well as sugarcane mosaic virus strains (Snazelle et al., 1971). The purified preparation was further purified by equilibrium centrifugation in sucrose gradients (Gillaspie, 1972). Lagenberg (1973) purified unaggregated infectious maize dwarf mosaic virus-B strain using nonionic detergent and without use of organic solvents. Yield of purified MDMV-B varied from 0.5 to 2.8 mg/100 µg of infected tissue.

Tosic et al. (1974) compared techniques for purification of maize dwarf and sugarcane mosaic viruses. Alternations to the purification procedures used for these viruses in the potyvirus groups include acidification of sap to pH 4.7 early in the clarification process and use of a smaller concentration of chloroform (3%). These viruses occur in low titer and are thus more effectively purified when the elapsed time between clarification and finally produced extraction is minimised. Moghal and Francki (1976) worked on purification of some potyviruses and found that attempts to purify viruses by methods described in literature were unsatisfactory in their hands. However, they purified sugarcane mosaic and bean yellow mosaic virus by one of the two procedure they developed. After extraction of SCMV infected tissue in borate buffer, the clarification was carried out by one half volume each of chloroform and carbon tetrachloride. After emulsion was broken at low speed the SCMV from aqueous phase was isolated by three cycles of differential centrifugation followed by sucrose density gradient centrifugation.

The SCMV purification method of Moghal and Francki (1976) proved slightly better than other purification procedures but most of the virus was found to be lost during low-speed centrifugation due to extensive aggregation of the particles (Gough and Shukla, 1981). Gough and Shukla (1981) modified procedure of Moghal and Francki (1976) and the major difference was 2 cycles of differential centrifugation instead of three, and preparation was layered on sucrose gradients and centrifuged and virus containing band was removed using syringe. The yield of the four SCMV strains purified ranged from 30-100 mg/kg of leaf material depending upon the strain. The johnsongrass strain always gave a much higher yield then the other three strains.

Rishi and Rishi (1985) purified SCMV strain A and F from India. They found that for extraction, low molarity phosphate buffer of neutral pH with 0.01M DIECA was more useful. The virus containing pellet at the end of two cycle of differential centrifugation was suspended in 0.05M borate buffer pH 8.5 supplemented with 0.01M EDTA and 0.5M urea.

2.4 SERODIAGNOSIS OF THE SUGARCANE MOSAIC VIRUS

Several antisera have been produced for the various isolate and strains of sugarcane mosaic virus (Pirone, 1972) and the titer of the antiserum produced ranged between 1/256 (Bond and Pirone, 1971) to as high as 1/2048 (Rishi and Rishi, 1985).

Sugarcane mosaic virus is a member of the potyvirus group and may be distantly related to several other member viruses of the group (Pirone, 1972). Strain A and B of maize dwarf mosaic virus, are serologically related to each other (Gordon and Williams, 1970; Snazelle et al., 1971), and to sugarcane mosaic virus strains (Shepherd, 1965; Wagner and Dale, 1966; Gordon and Williams, 1970; Bond and Pirone, 1971; Snazelle et al., 1971).

Zummo and Stokes (1973) compared various strains of SCMV reported that SCMV-K strain which is more closely related to SCMV strains A, B and D than to strains H or I. Baudin (1977) in France observed that the Ampefy strain of SCMV was closely related to seotype consisting of SCMV-A, B and D strains. Gough and Shukla (1980) studied use of protein A in immune electron microscopy and found that precoating electronmicroscope grids with protein A before coating them with specific

antiserum enhanced the number of SCMV particles at each optimum antiserum dilution by 25.7 fold, over those grids treated with specific antiserum alone. Further the protein A coated grids can be stored for up to 6 months at 4°C, but not at room temperature. Shukla and Gough (1984) studied the serological relationships among four Australian strains of sugarcane mosaic virus as determined by immune electron microscopy. Results obtained from differential trapping and decoration methods both led to the conclusion that the johnsongrass strain of SCMV is not serologically related to 3 other SCMV strains namely: Sugarcane (SC), Queensland blue couch grass (BC), and Sabi grass (SG) strains. SC strain was closely related to BC strain and SG, but is distinct from both the strains. Whereas BC and SG strains are very closely related to each other.

Devergne et al. (1982) detected SCMV-D strain using double antibody sandwich type method of Enzyme Linked Immunosorbent Assay (DAS-ELISA), in France. The purified SCMV-D was detected at 1 ng/ml concentration by DAS-ELISA. Shukla et al. (1983) used Electroblot radio immunoassay (EBRIA) to detect plant viruses and detected as low as 0.5 ng virus. The aminophenylthioether paper was found more efficient than the nitrocellulose sheet in binding the plant virus coat protein. Antisera prepared in response to intact particles and to LiCl isolated coat protein of a virus, although identical in their sensitivities in homologous situtions (0.5 ng), gave the same lower sensitivity (50 ng) when tested under heterologus conditions. The technique was found convenient in establishing relationships as demonstrated with 4 SCMV strains. EBRIA could probe a paper containing 8 virus samples using 5 µl antiserum.

Jarjees and Uyemoto (1984) determined serological relatedness of maize dwarf mosaic virus and sugarcane mosaic virus strains using microprecipitin and enzyme linked immunosorbent assays, which distinguished the same 4 serogroups among the 10 viruses tested. Strains MDMV-A and SCMV-J comprised one serogroup, MDMV-KS1 a second, MDMV-B and SCMA-A, B and D a third, and SCMV-H, I and M a fourth.

Hewish et al. (1986) used biotin conjugated antisera of 2 strains of sugarcane mosaic virus, which proved sufficiently sensitive to detect the viruses in extracts of infected plants using ELISA and electroblot immuno assays. Virus strain specificities observed for the antisera agreed with those found using immunoelectron microscopy and electroblot immuno assays. The Biotin-avidin system offers several advantage over other current method of antibody labelling, notably in speed of development and versatility.

Gillaspie and Harris (1987) determined the serological relationships amongst six strains of SCMV, namely SCMV-A, B, D, E, H and I, using ELISA, and classified the strains into four different serogroups. Their groupings are: (1) SCMV-J; (2) SCMV-A, B, D and K; (3) SCMV-E; and (4) SCMV-H, I and M. Further, they indicated that serological methods cannot be used independent of differential host tests to identify individual SCMV strains.

2.5 ELECTRONMICROSCOPY OF THE SUGARCANE MOSAIC VIRUS

Electronmicroscopic studies of Gold and Martin (1955) for the virus particles associated with sugarcane mosaic virus indicated rod shaped particles measuring 15x630 mμ in size. Paliwal and Raychaudhari

(1966) reported rod shaped virus measuring 339-964 nm x 27-35 nm as a strain of sugarcane mosaic virus causing maize mosaic in India. The SCMV-A and F strain reported from Gorakhpur, India had filamentous particles and 656 and 679 mμ length respectively (Anonymous, 1968). Tosic et al. (1978) from Italy described the sugarcane mosaic virus as typical potyvirus with 750 nm length.

From Taiwan, Chen (1978) reported after examining purified SCMV under electronmicroscope that the virus particles are long flexous rods mostly 750x13 nm in size. Liu (1979) studied negatively strained SCMV strain A and B infecting 294 variety of sugarcane in Purto-Rico, which measured 720-1446x14-15 nm and 720-1730x14-15nm, respectively. In Australia, Moghal and Francki (1981) observed 750 nm as the mean particle length of SCMV. The SCMV strain A and F purified in India by Rishi and Rishi (1985) reported the length of particles between 670-770 nm. The SCMV-H strain isolated in taxas from grain sorghum had 706 nm length (Giorda et al., 1986).

2.6 PHYSICOCHEMICAL PROPERTIES OF THE SUGARCANE MOSAIC VIRUS, SCMV COAT PROTEIN, SCMV-INCLUSIONS AND ITS PROTEINS, AND THE SCMV-RNA

The value of sedimentation coefficient of sugarcane mosaic virus particles has been differently reported by various workers such as 176±5S for the B strain by Tosic and Ford (1972) 168±6 by Shepherd (1965), 155±3 by Bancroft et al. (1966), 148±2 by Sehgal (1968) and 160, 162 by Jones and Tolin (1972). The buoyant density in CsCl was recorded as 1.3245 by Sehgal and Jean (1970) and 1.3327 by Tosie and Ford (1972).

Gough and Shukla (1981) isolated coat protein (Capsid protein) of four Australian sugarcane mosaic virus strains : Johnsongrass (JG), Sugarcane (SC), Queensland blue couch grass (BC), and Sabi grass (Sabi) and analysed on SDS-poyacrylamide gel electrophoresis. The proteins migrated as a single band in SDS-polyacrylamide gel electrophoresis with molecular weight of 33,700 (SC), 34,200 (JG), 39,100 (BC), and 40,300 (Sabi). The BC and Sabi strains have identical aminoacid compositions and tryptic peptide map but these two properties of JG strain showed very little similarity to the three other strains. On the basis of amino acid composition, relatedness index (S Δ Q values) and peptide maps, the four SCMV strains can be devided into two main groups: JG in one group and SC, BC and Sabi in another group, for which the name Johnsongrass (JG) and Sugarcane (SC) groups, respectively, were proposed.

Jensen et al. (1986) studied virus induced proteins for the 4 strains of the sugarcane mosaic virus, namely A, B, H and M strains, and identified two virus specific proteins. Whereas the capsid protein for SCMV-A, B, H and M strains were 36.9, 36.7 and 39.1 and 37.4 KDa, the virus induced protein was 66 KDa in infected plants. The virus induced protein was not found to be serologically related to the capsid protein and was probably a cytoplasmic inclusion protein. They varied from 64.2-66.5 KDa among four strains of the SCMV.

Gough et al. (1987) sequenced the capsid and nuclear inclusion protein genes from johnsongrass strain of SCMV. The results suggested that genome of SCMV encodes one or more large proteins that are processed to mature proteins. The nucleotide sequence of 3' terminal 1782 nucleotides of SCMV genome has been determined. There is an open reading frame, from the 5' end, of 1307 nucleotides upstream from a 475 nucleotide 3' non-coding region that is polyadenylated. The open reading frame encodes a polypeptide of 435 amino acids. The segment of the genome encoding the viral capsid protein (M. wt. 34,200) is adjacent to 3 non-coding regions. The predicted capsid protein was similar in sequence to the capsid protein sequence predicted for tobacco etch virus (TEV). Part of another protein encoded in the same reading frame similar to predicted nuclear inclusion protein from TEV, was identified upstream from coat protein gene.

2.7 THE DIFFERENTIATION OF SUGARCANE MOSAIC VIRUS STRAINS

Strains of SCMV were first differentiated by their reaction on sugarcane clones by Summers (1934). The sugarcane clones, generally utilized for this purpose were CP 31-294, CP 31-588, Co 281 and sweet sorghum cultivar Rio. A comprehensive account of strainal identification was discussed by Summers et al. (1948) and Abbott and Tippett (1966) and later by Tippett and Abbott (1968), Zummo and Stokes (1973), Zummo (1974), and Koike and Gillaspie (1976).

A SCMV infects maize and sorghum besides sugarcane all over the world, a satisfactory method of identifying SCMV strains

acceptable for all those crops has to be worked out. Considering that the sugarcane is a vegetatively propagated crop, a set of 10 sorghum lines were suggested to differentiate strains of SCMV, at the 1978 International Workshop on Sorghum Diseases, held at International Crop Research Institute for the Semi-arid Tropics, Hyderabad, India. These set differentiales were used by Giallaspie and Mock (1984) to differentiate the SCMV strains infecting sugarcane.

3. MATERIALS AND METHODS

3.1 COLLECTION AND MAINTENANCE OF THE SUGARCANE MOSAIC VIRUS CULTURE

Vigorously growing shoots of sugarcane cultivar Co 671 bearing profuse growth of leaves, showing typical severe mosaic symptoms of sugarcane mosaic virus (SCMV), were collected from farmers fields at Malanpur in South Gujarat. These shoots were cut into small sets of 5-6 cm each, bearing a single bud at nodal regions, and planted in 30 cm diameter earthen pots, filled with 3:1 mixture of soil and farm yard manure for fresh vegetative growth. The pots were kept in the insect proof glasshouse. Fresh leafy growth from these buds, displaying severe mosaic symptoms, characteristics of SCMV infection, were used for further experimentations.

These plantings were kept in the insect proof glasshouse for maintaining the SCMV culture.

3.2 SAP INOCULATION OF SUGARCANE MOSAIC VIRUS

The young newly emerging sugarcane leaves, showing severe mosaic symptoms (Plate 1) were collected from glasshouse potted plants, washed with tape water and blotted dried. The leaves were ground with sterilized mortar and pestle in 0.01M potassium phosphate pH 7 (3 ml/g). The crude extract thus prepared was strained through two layers of cheese cloth. A pinch of diatomaceous earth (Sigma Chemical Co., St. Louis, Missouri, U.S.A.) was added directly into the expressed sap and different test plants were inoculated with the index finger dipping in the inoculum, using 3 strokes of index finger per leaf.

Plate 1. (Top) : Severe mosaic symptoms induced by sugarcane mosaic virus on Co 671 variety of sugarcane

(Bottom) : Extensive chlorosis of the leaves due to SCMV infection on newly emerged leaves of Co 671 variety of sugarcane

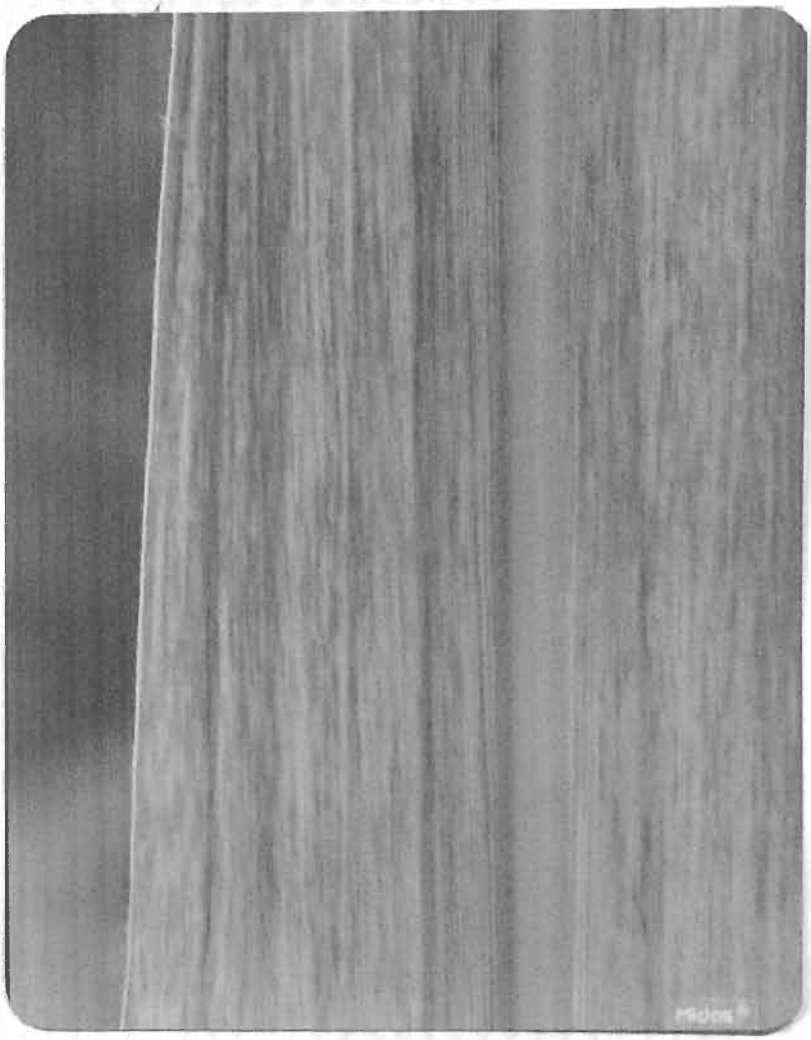


PLATE-I
(TOP)

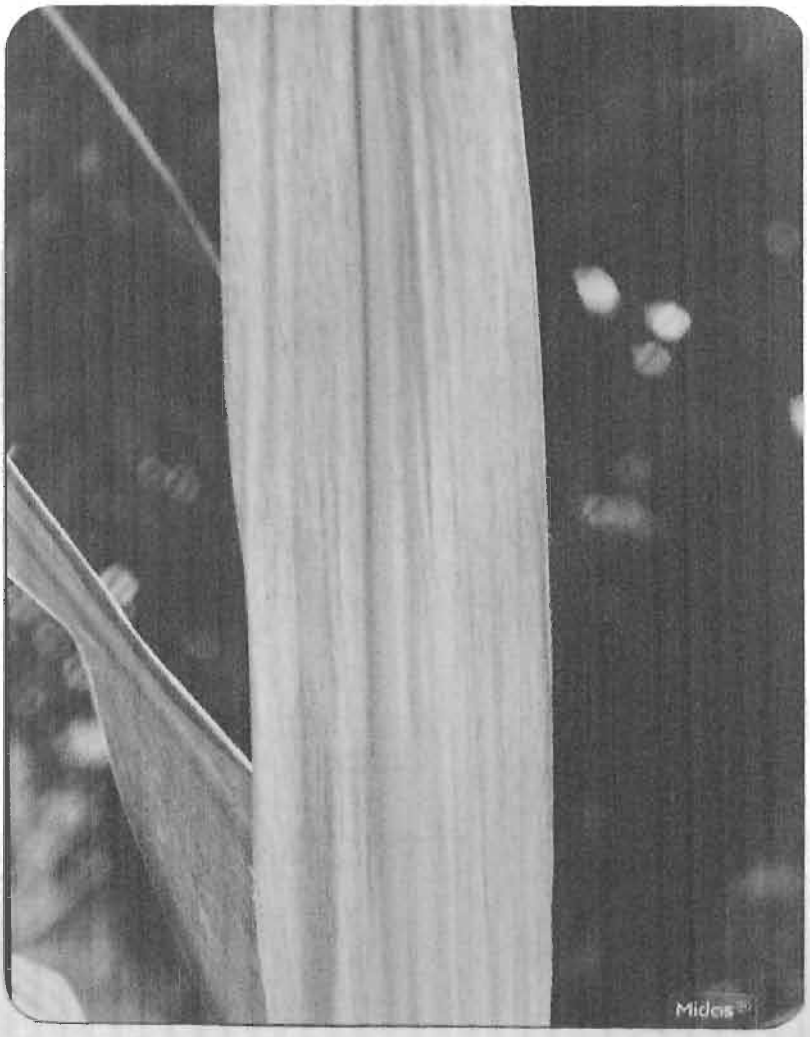


PLATE-I
(BOTTOM)

3.3 HOST REACTION STUDIES OF SUGARCANE MOSAIC VIRUS

For studying the range of host reactions, seedlings of different cultivars of sorghum (*Sorghum bicolor* (L.)Moench), maize(*Zea mays* (L.) and bajra (*Pennisetum americanum* (L.) Leeke) were directly sown in 20 cm diameter earthen pots. The rice (*Oryza sativa* L.) seedlings on the other hand, were transplanted in 25 cm diameter earthen pots in standing water. Duplicate pots for each of these test plant were also planted, one of which was kept as buffer rubbed control while the other were inoculated with the SCMV infectious sap. Ten test plants grown in each pot were inoculated with SCMV inoculum at 4 leaf growth-stage in the case of sorghum, maize and bajra and 5 leaf growth-stage in the case of rice. These test plants after inoculation were kept on the benches of the insect proof glasshouse for observations. The final observations on symptom expression were carried out at weekly intervals, up to 1 month after inoculation.

The symptomless test plants were further tested for the presence or the absence of the sugarcane mosaic virus by backassays. For these tests, CSH-9 sorghum seedlings were raised in 20 cm diameter pots and were inoculated at 4 leaf growth stage. Three grams of tissue from each of the symptomless host were ground separately in sterilized mortars with pestles in 3 ml volume of 0.01 M potassium phosphate pH 7 buffer and the extracted sap was used to inoculate ten CSH-9 sorghum seedlings after adding pinch of the diatomaceous earth in it. Some of the seedlings were left uninoculated in each pot as controls. The plants were kept in the insect proof glasshouse and observed at weekly intervals, up to one month after inoculation. The final ratings of symptoms were undertaken thereafter.

3.4 APHID TRANSMISSION EFFICIENCY OF THE SUGARCANE MOSAIC VIRUS

The nonviruliferous colonies of *Rhopalosiphum maidis* (Fitch) and *Melanaphis sachhari* (Znht.) were collected from healthy sorghum plants in October and maintained on young, healthy sorghum CSH-9 seedlings in the insect proof glasshouse. Their repeated transfers to the susceptible CSH-9 sorghum confirmed freedom of these aphid culture from SCMV infection. Healthy, nonviruliferous culture of *Aphis craccivora* (Koch) on the other hand was obtained from Division of Entomology at IARI and maintained on healthy cowpea plants in the insect proof glasshouse of the Mycology Division.

For testing the efficiency of the three aphid species, their aviruliferous colonies were disturbed carefully by breathing on them so that the aphids will withdraw their stylets and start moving on plant surface. With the moistened tip of a Camel hair brush (Camel 'O'), one hundred wingless aphids (apterous forms) were individually picked up and transferred to a petri plate with moist filter paper. The sides of the petri plates were sealed with parafilm, wrapped in a black cloth and placed in cool, shaded place for 30 minutes starvation. The SCMV infected Co 671 sugarcane leaf pieces were kept in a separate petri plate and the starved aphids were transferred on it for 5 minutes acquisition access period. The single wingless aphids (apeterous forms) were transferred individually to young and healthy CSH-9 sorghum seedlings at 3 leaf stage, for inoculation feeding for a period of 1 hour. Thus, one hundred aphids were transferred to one hundred CSH-9 sorghum seedlings. After an hour of inoculation feeding, the plants were sprayed with Malathion, @ 2 ml/l of water, to kill the aphids. The plants were

kept in glasshouse for observations and weekly sprayed with malathion. Final symptoms ratings were done after 35 days.

3.5 PURIFICATION PROCEDURES FOR SUGARCANE MOSAIC VIRUS

3.5.1 Propagation of sugarcane mosaic virus inoculum

The CSH-9 sorghum [*Sorghum bicolor* (L.) Moench] seeds were sown in 30 cm diameter pots, and kept for germination in the insect proof glasshouse. At 4 leaf growth stage, the CSH-9 sorghum seedlings were sap inoculated with SCMV inoculum prepared by macerating severely infected leaves of Co 671 sugarcane variety in 0.01 M potassium phosphate pH 7 buffer (3 ml/g of tissue) in sterilized mortar and pestle, and straining through the cheese cloth. The CSH-9 sorghum seedlings after inoculation were kept in the insect proof glasshouse for the fresh growth of leaf tissues, to be harvested after 4 weeks for extraction of SCMV for purification.

At times, the leaves of sugarcane Co 671, showing severe mosaic symptoms were also used for preparing extract for the purification of the SCMV.

3.5.2 Extraction, clarification and purification procedure

For extraction of the sugarcane mosaic virus, 100 g of leaves from inoculated sorghum or sugarcane were usually harvested at a time, washed for 3 times in tap water, blotted dried, cut into small pieces, and ground in the blendor at 4°C in a suitable extraction buffer.

For the clarification, the low speed centrifugation was done in Sorvall RC-5B superspeed centrifuge using SE-12 or SA-600 fixed angle

rotors. Sorvall OTD-65B ultracentrifuge was used for high speed centrifugation in A-841 fixed angle rotor for final pelleting of the virus particles, at 4°C, and TST 41.14 swing out rotor was used for the density gradient centrifugation at 4°C for further purification of the virus preparation. The gradients were fractionated at 1 ml/min flow rate and 30 cm/h chartspeed using ISCO density gradient fractionator Model 640 coupled with Type-6 optical unit and the UV profile of the gradients were recorded by coupling UA-5 Absorbance/Florescence Detector (ISCO, Inc., P.O. Box 5347, 4700 superior st., Lincoln, Nebraska, U.S.A. 68505). The absorbance range was adjusted to 1 to 2 for recording gradient column profiles at 254 nm.

In order to standardize a suitable method for purification 2 standard published method and their 2 modifications were attempted as described in detail, herewith.

3.5.2.1 Procedure-I (Lagenberg, 1973)
(Flow chart, Figure 2)

Fifty to 100 g of SCMV infected leaf tissue was ground in cold 0.1M TACm extraction buffer (3ml/g leaf tissue) for 3 min in a blendor at high speed. The TACm buffer consisted of 0.1M Tris (hydroxymethyl) amino methane), 0.05M citric acid, 0.8% polyvinyl pyrrolidone (PVP, average molecular weight = 40,000 daltons), and 0.2% 2-mercaptoethanol (2-ME), with pH adjusted to pH 8.2 using 1N HCl. The sap was passed through cheese cloth and stirred with a magnetic stirrer, while adding 1 ml of 2M $CaCl_2$ and 2 ml of 2M K_2HPO_4 for each 50 ml of sap. The resulting mixture was centrifuged for 10 min at 8,500 rpm (x 8,433 g). The supernatant liquid was recovered and Triton x-100

Flow Chart Figure 2. Flow chart of SCMV purification procedure-I (Lagenberg, 1973)

100 g SCMV infected leaves

Discard material on this side	*1. Homogenize in 0.1M TACm [0.1M Tris, 0.05M citric acid, 0.8% PVP (Mol. wt. 40,000), and 0.2% 2-mercaptoethanol] pH 8.2 buffer, 3 ml/g leaf tissue.
	2. Strained sap through cheese cloth
	3. Stirred, while add 1 ml 2M $CaCl_2$ and 2 ml 2M K_2HPO_4 per 50 ml of sap.
	4. Centrifuge 8,500 rpm, 10 min
Pellet	5. Supernatant, add 0.5% Triton x-100 and 6% PEG 6000.
	6. Stirred solution for 3 h at room temperature.
	7. Centrifuge at 8,500 rpm for 10 min.
Supernatant	8. Pellet suspended in 1/20th Vol. in TACm pH 8.2 buffer.
	9. 9000 rpm (x 9,459 g), 10 min.
Pellet	*10. Supernatant layered on 10 ml of 30% sucrose pad.
	11. 68,000 g (28,000 rpm) for 1 h.
Supernatant	12. Pellet dissolved in 0.01M TACm buffer pH 8.2
	13. x 6,577 g for 10 min.
Pellet	14. Supernatant layered on 10-40% sucrose gradients, centrifuge at 23,000 rpm (x 67,500g) for 2 h.
	15. Scanned gradients at 254 nm with an ISCO density gradient fractionator.
	16. Collect virus containing peaks.

*Modifications: Step 1. pH adjusted with 1N HCl instead of 1,3-propane diamine.
Step 10. Sucrose not decolorized but 'AnAlar' Grade used.

(alkylphenoxypolyethoxy ethanol) and polyethylene glycol (Molecular weight= 6000) were added to their final concentration of 0.5 and 6 per cent, respectively. The solution was stirred for 3 hr at room temperature and precipitated material was subsequently removed by a 10 min low speed centrifugation at 8,500 rpm (x 8,433 g). Pellets were suspended in cold 0.01 M TACm buffer (0.01M tris, 0.005M citric acid, 0.01M 2-mercaptoethanol, and 0.1% triton-x-100), equal to 1/20th volume of the extracted sap. The suspension was centrifuged at 9000 rpm (x 9, 454 g) for 10 min to remove insoluble material. The resultant supernatant was layered on 10 ml pad of 30 per cent sucrose dissolved in 0.01M TACm buffer in a 28 ml tube and centrifuged in fixed angle rotor at 28,000 rpm (x 68,000 g) for 1 hr. The clear, transparent, light green pellets were dissolved in 0.1M TACm buffer and given a low speed centrifugation for 10 min at 9000 rpm (x 6,577 g). The opalescent superntant solution was (1 ml) layered on 100-400 mg/ml sucrose density gradient prepared by layering 3 ml, 3 ml, 3 ml and 2 ml of 100, 200, 300 and 400 mg/ml density of sucrose, respectively. The gradients were buffered with 0.01M TACm buffer and equilibriated overnight at 4°C before use. The gradients were centrifuged at 23,000 rpm (x 67,500g) for 2 hr in swingout rotor at 4°C. The gradient column profile was recorded at 254 nm.

2.5.2.2 Procedure-II (Gough and Shukla, 1981)
(Flow chart, Figure 3)

This procedure is essentially a modification of the method of Moghal and Francki (1976). One hundred grams of SCMV infected small leaf tissue lots were stored frozen for overnight at -20°C before homogenization into one and half volume of 0.5M sodium borate buffer pH 8,

Flow Chart Figure 3. Flow chart of SCMV purification procedure-III (Gough and Shukla, 1981)

100 g SCMV infected leaf tissue

Discard material on this side	→ 1. Minced the tissue in 1.5 volume of extraction buffer (0.5M sodium borate pH 8 + 0.15% TGA and 0.01M EDTA) and 0.5 volume each of chloroform and carbontetrachloride.
	→ 2. Strain sap through cheese cloth.
	→ 3. 15,000 rpm (x 8,000 g), 10 min.
Pellet	→ 4. To supernatant, add 5% Triton X-100, Stirr 30 min at 4°C.
	→*5. Silicon antifoam, few drops to defroth.
	→ 6. 8,000 g, 10 min.
Pellet	→ 7. Supernatant, 38,500 rpm (x108,000 g) for 90 min.
Supernatant	→ 8. Pellet resuspended overnight at 4°C in extraction buffer.
	→ 9. 8,000 g, 10 min.
Pellet	→10. Supernatant, 38,500 rpm, 90 min.
Supernatant	→11. Pellet resuspended overnight at 4°C in resuspension buffer (0.05M sodium borate buffer pH 8, 0.01M EDTA)
	→12. 8,000 g, 10 min.
Pellet	→13. Supernatant layered on 10-40% sucrose gradient and centrifuged at 22,000 rpm (x 60,000 g) in TST 41.14 rotor.
	→14. Gradients fractionated at 254 nm with ISCO density gradient fractionator.
	→15. Collect virus containing peaks.
	→16. Dilute in resuspension buffer the virus peaks, centrifuge at 38,000 rpm (x108,000 g) for 90 min.
Supernatant	→17. Pellet resuspended in resuspension buffer.

*Modifications: Step 5. Silicon antifoam was used instead of 2-Octanol

containing 0.15% (V/V) thioglycollic acid and 0.01M EDTA (extraction buffer), and 0.5 volume (V/V) each of chloroform and carbontetrachloride. The extract was strained through double layers of cheese cloth and centrifuged at 10,500 rpm (x 8,000 g) for 10 min at 4°C. The supernatant was mixed with 5% (V/V) of triton x-100, while stirring for 30 min at 4°C. A few drop of silicon-antifoam (Fluka AG, Switzerland) was also added to prevent frothing. The mixture was clarified at 10,500 rpm (8,000 g) for 10 min. The supernatant was separated and thereafter centrifuged at 38,500 rpm (x 108,000 g) for 90 min at 4°C. The final pellet was resuspended overnight at 4°C in extraction buffer. The suspension was centrifuged at 10,500 rpm (x 8,000 g) for 10 min at 4°C. The virus was sedimented at 38,500 rpm (x 108,000 g), for 90 min at 4°C and the pelleted virus was resuspended overnight at 0°C in 0.05M sodium borate buffer pH 8, containing 0.01M EDTA (resuspension buffer). The final suspension was further clarified by centrifugation at 10,500rpm (x 8,000 g) for 10 min at 4°C. The final preparation was layered on 10-40 per cent sucrose gradients prepared in resuspension buffer, and centrifuged at 22,000 rpm (x 60,000 g) at 4°C, for 3 hr. The gradients column profile at 254 nm after centrifugation was recorded.

3.5.2.3 Procedure-III (Flow chart Figure 4)

The method of Gough and Shukla (1981) was used with some modifications. Different steps in the procedure were the same as detailed in procedure-II except for the clarification of the crude sap was carried out using n-butan-1-ol @ 6 per cent, instead of chloroform and carbontetrachloride. The pellets from only one cycle of differential centrifugation was suspended and layered on 10-40 per cent sucrose density gradients

Flow Chart Figure 4. Flow chart of SCMV purification procedure-III. Modified method of Gough and Shukla (1981)

100 g SCMV infected leaf tissue

Discard material on this side	→ 1. Minced the tissue in 1.5 volume of extraction buffer (0.5M sodium borate pH 8 + 0.15% TGA and 0.01M EDTA)
	→*2. Strain sap through cheese cloth and mix 6% n-butanol.
	→ 3. 15,000 rpm (x 8,000 g), 10 min.
Upper n-butanol phase	→*4. Save lower aqueous phase
	→ 5. Add triton-x-100 to 5% concentration, stirr at 4°C for 30 minutes
	→ 6. 8,000 rpm (x 5197 g), 10 min
Pellet	→ 7. Supernatant, 38,500 rpm (x100,000 g) for 3 h
Supernatant	→*8. Pellet suspended in extraction buffer overnight, 4°C.
	→ 9. 8,000 rpm (x 5197 g), 10 min
Pellet	→*10. Supernatant layered on 10-40% sucrose density gradients and centrifuged at 23,000 rpm for 3 h.
	→11. Gradients scanned at 254 nm with an ISCO density gradient fractionator
	→12. Virus containing peaks to be collected, dialysed and concentrated

*Modifications: Step 2. n-butanol used instead of chloroform and carbon tetrachloride for clarification of sap.

Step 8. Only one cycle of differential centrifugation and only overnight resuspension of pellet instead of two overnights.

Step 10. Supernatant layered on sucrose gradients only after one cycle of differential centrifugation

for final purification of SCMV. The gradient column profile at 254 nm was recorded after centrifugation.

3.5.2.4 Procedure-IV (Flow Chart, Figure 5)

The procedure of Rishi and Rishi (1985) was followed with some operational modifications particularly to adjust suitably:

1) the tissue : buffer ratio;

2) the sedimentation time (t), and

3) to eleminate earlier step of dialysis,

for achieving optimum results and avoid loss of virus titer. Eighty eight grams of leaf tissues were ground at 4°C in 240 ml volume of cold 0.067M potassium phosphate pH 7 buffer, containing 0.01M sodium diethyl dithiocarbamate. The extract was passed through double layer of cheese cloth and centrifuged at 3000 rpm (x 1,050 g) for 10 min at 4°C. To the supernatant, equal volume of chloroform was mixed gently for 20 min at 4°C on a magnetic stirrer. Four hundred eighty ml mixture of leaf extract and chloroform was centrifuged at 3000 rpm (x 1050g) for 20 min. The yellow aqueous phase was carefully pipettedout to which 0.5% (V/V) triton X-100 (alkylphenoxy polyethoxy ethanol) was added and stirred for 2 min on a magnetic stirrer. The virus was pelleted by centrifugation of aqueous phase at 25,000 rpm (x 45,500 g) for 3 hr, at 4°C. The pellets were resuspended in 28 ml volume of 0.05 M borate buffer pH 8.2 + 0.01M EDTA + 0.5M urea (resuspension buffer) and the virus suspension was clarified by centrifugation at 5000 rpm (x 2918 g) for 10 minutes. After the second cycle of high speed [at 25,000 rpm (x 45,500 g) for 3 h] the final pellet was resuspended overnight at 4°C in 2 ml of resuspension buffer. Next morning, the virus suspension was

Flow Chart Figure 5. Flow chart of SCMV purification procedure-IV. (Rishi and Rishi, 1985) modified method

88 g SCMV infected leaf tissue

Discard materials on this side	→ *1.	Minced the tissue in 240 ml volume of 0.067M potassium phosphate pH 7 buffer plus 0.01 M NaDIECA
	→ 2.	Strained extract through cheese cloth.
	→ 3.	3000 rpm (x 1,050 g), 10 min.
Pellet	→ 4.	Add, equal volume of chloroform, mixed gently for 20 min at 4°C on magnetic stirrer.
	→ 5.	3000 rpm (x 1,050 g), 20 min.
Lower chloroform phase	→ 6.	Supernatant yellow aqueous phase, add 0.5% triton X-100, stirred for 2 minutes.
	→ 7.	25,000 rpm (x 45,500 g), 3 h.
Supernatant	→ 8.	Pellet suspended in 28 ml volume of resuspension buffer (0.05 M borate buffer pH 8.2 + 0.01M EDTA+0.5 M urea)
	→ 9.	Centrifuge at 5000 rpm (x 2918 g), 10 min.
Pellet	→ *10.	25,000 rpm (x 45,500 g), 3 h.
Supernatant	→ 11.	Pellet resuspended overnight at 4°C in 2 ml resuspension buffer.
	→ 12.	5000 rpm (x 2,030 g), 10 min.
Pellet	→ 13.	Supernatant layered 10-40% sucrose gradients.
	→ 14.	25,000 rpm (x 79,700 g), 2 h, 4°C.
	→ *15.	Gradients fractionated at 254 nm in ISCO density gradient fractionator SCMV peaks collected.
	→ 16.	Dialysed 4 h, against resuspension buffer, and diluted to 30 ml vol. with it.
	→ 17.	25,000 rpm (x 79,700 g), 3 h.
Supernatant	→ 18.	Pellets suspended in 0.6 ml total volume of resuspension buffer
	→ 19.	Purified SCMV

*Modifications:
1. Tissue to buffer ratio reduced to 2.7 from 4.
2. Step-7 and 10, length of run doubled.
3. Dialysis step between step: 12 & 13 totally omitted.

centrifuged at 5000 rpm (x 2030 g) for 10 min., and the supernatant was layered on 10 to 40 per cent sucrose density gradients and centrifuged at 25,000 rpm (x 79,700 g) for 2 h at 4°C. The gradients were buffered in 0.05M borate buffer pH 8.2 containing 0.01M EDTA + 0.5M urea, and allowed to diffuse overnight before use. The gradient column profile at 254 nm was recorded.

The virus containing fractions were collected and dialysed for 4 h against resuspension buffer at 4°C. The 10 ml volume of dialysed SCMV preparation was further diluted to 30 ml with buffer, and centrifuged in swingout rotor TST 41.14 at 25,000 rpm (x 79,700 g) for 3 h to finally pellet out the highly purified virus preparation. The 3 pellets of SCMV were resuspended in 0.6 ml of resuspension buffer and the preparation was used for electronmicroscopic studies.

3.5.2.5 Procedure-V. (Flow Chart, Figure 6)

This procedure was developed during the present investigation, which involves use of cesium sulphate (Cs_2SO_4) instead of sucrose for the density gradient centrifugation. The SCMV infected leaves were harvested and frozen at -20°C for overnight duration before being used for extraction. The tissues were ground in 0.5M sodium borate pH 8 buffer containing 0.15% thioglycolic acid and 0.01 M EDTA (1.2 ml/g leaf tissue). The sap was passed through cheese cloth and emulsified with 1/3rd volume of chloroform for 2 to 3 minutes in the blendor. The resulting emulsion was centrifuged at 4000 rpm (x 1500 g) for 10 min at 4°C. The yellowish, aqueous phase was carefully pipetted out from centrifuge tubes. The polyethylene glycol (Mol. wt. 6000) and sodium chloride were added at the rate of 6 per cent and 0.3 M respectively,

Flow Chart Figure 6. Flow chart of SCMV purification Procedure-V

100 g SCMV infected tissue

Discard material on this side	→ 1.	Frozen infected tissue overnight at -20°C.
	→ 2.	Minced tissue in (1.2 ml/g) extraction buffer (0.5 M sodium borate buffer pH 8, 0.15% TGA, 0.01M EDTA)
	→ 3.	Sap strained through cheese cloth.
	→ 4.	Emulsify sap with 1/3rd vol. of chloroform in a blender for 2 to 3 minutes.
	→ 5.	4000 rpm (x 1,500 g), 10 min
Lower chloroform phase	→ 6.	Upper yellowish aqueous phase separated and add 6% PEG 6000 and 0.3M NaCl.
	→ 7.	Keep for 1 h at 4°C, stirring.
	→ 8.	15,000 rpm (x26,260 g), 20 min. 4°C.
Supernatant	→ 9.	Pellets dissolved in 10 ml 0.1M sodium borate buffer pH 8.
	→ 10.	Layered 3.5 ml virus suspension on preformed 15-30% Cs_2SO_4 gradients, with 2 ml of 10% sucrose solution on top of gradients.
	→ 11.	40,000 rpm (x204,100 g) for 2 h.
	→ 12.	Fractionated gradient at 254 nm with an ISCO density gradient fractionator and virus peaks collected.
	→ 13.	Dilute virus fructions with equal volume of 0.04M potassium phosphate pH 7 buffer.
	→ 14.	Layered on CS_2SO_4 gradients, and 40,000 rpm (x 204,100 g) for 2 h.
	→ 15.	Repeat step-12
	→ 16.	Diluted peaks with distilled water, 40,000 rpm (204,100 g), 2 h.
	→ 17.	Pellet of SCMV suspended in 0.05M borate buffer pH 8 or in 0.13M saline for immunization.

and slowly dissolved by stirring on magnetic stirrer at 4°C for 1 h. The virus containing PEG-suspension was centrifuged at 15,000 rpm (x26,260 g) for 20 min at 4°C. The pellets were dissolved in 10 ml volume of 0.1M sodium borate buffer pH 8 and the supernatant was discarded. The cesium sulphate gradients were prepared by layering 2 ml volume of 30, 22.5 and 15 per cent solutions prepared in 10 per cent sucrose solution; buffered with 0.04 M potassium phosphate buffer pH 7. On the top of Cs_2SO_4 solutions, 2 ml volume of 10 per cent sucrose prepared in 0.04 M potassium phosphate pH 7 was layered. The Cs_2SO_4 gradients (15-30% in 10% sucrose) were prepared just prior to layering of virus preparation. On the top of each gradients 3.5 ml volume of virus preparation was layered and centrifuged at 40,000 rpm (x 204, 100 g) for 2 hr in swingout TST 41.14 rotor at 4°C.

The gradient column profile at 254 nm was recorded and different fractions collected using ISCO density gradient fractionator. The fraction indicating SCMV peaks were collected and pooled. The SCMV fraction collected were diluted with equal volume of 0.04M potassium phosphate pH 7 buffer, and layered on freshly prepared Cs_2SO_4 (15-30% in 10% sucrose) and CsCl (15-30% in 15% sucrose) gradients, and centrifuged at 40,000 rpm (x204,100 g) for 2 h and 1 h respectively. The gradient columns were fractionated again and the fractions representing SCMV peaks were separately collected, diluted with distilled water. The SCMV was finally pelleted by centrifugation at 40,000 rpm (x 197,478 g) in SW 41 rotor of Beckman L8-60M ultracentrifuge at 4°C. The final virus pellet was suspended in 0.01M potassium phosphate buffer pH 7 or in saline (0.85% or 0.15 M NaCl), depending upon the use of the final purified SCMV.

The yield (mg/kg of tissue) of SCMV-nucleoprotein in the finally purified preparation was estimated spectrophotometrically by the method of Layne (1957) and compared for estimating the efficiency of each procedure.

3.5.2.6 Recovery of SCMV from supernatant of polyethylene glycol precipitation step (Step-9 of procedure-V, indicated in flow chart)

The supernatant from polyethyleneglycol precipitation step was centrifuged at 38,000 rpm (x105,000 g) for 2 h. The pellet obtained thereafter was suspended in 0.5 M sodium borate pH 8, and centrifuged at 5000 rpm (x 2918 g) in SE-12 rotor of Sorvall RC-SB superspeed centriuge for 10 minutes. The supernatant was layered on Cs_2SO_4 gradient columns and centrifuged at 40,000 rpm (x 204,100 g) for 2 h as detailed earlier in section 3.5.2.5 of this chapter. The gradient column profile was recorded by scanning at 254nm and its fraction indicating virus peaks were collected put through second cycle of Cs_2SO_4 density gradient and centrifuged again for further purification of SCMV. The fractions containing SCMV peaks were diluted with distilled water and centrifuged at 40,000 rpm for 2 h, and final purified SCMV pellet was suspended in 0.05M borate buffer, pH 8.

3.6 SERODIAGNOSIS OF THE SUGARCANE MOSAIC VIRUS

3.6.1 **Production of antiserum against sugarcane mosaic virus**

The antiserum for the isolate of the sugarcane mosaic virus from Gujarat was produced by administering, into young New Zealand white rabbit four injections of 1 ml of purified SCMV suspension prepared from second Cs_2SO_4 or CsCl gradients centrifugation. The first injection

was administered intravenously with 1 ml of purified SCMV suspension prepared in normal saline using 26 gauge sterile hypodermic needle into the marginal ear vein of the rabbit. Three intramuscular injections were administrated at weekly intervals into the hindleg using 23 gauge needle. For intramuscular injections 1 ml of purified SCMV was emulsified with equal volume of Freund's incomplete adjuvant by squirting through 18 gauge needle, repeatedly. One week after the last injection the rabbit was bled twice at a week's intervals, collecting 15 ml of blood at a time. The blood was allowed to clot at room temperature for one hour and then kept overnight in the cold room at 4°C. Next morning, the blood clots adhering to the interior wall of the centrifuge tube were removed using thin glassrod and centrifuged at 4500 rpm (x 1644g) in SE-12 rotor for 10 min at 4°C in SorvallRC-5B superspeed centrifuge. The SCMV antiserum, collected from two bleedings were pooled and mixed thoroughly with equal volume of 98 per cent purified glycerol. The antiserum was stored in autoclaved Borosil screw cap tubes at -70°C till used.

3.6.2 **Determination of titer of the sugarcane mosaic virus antiserum**

3.6.2.1 Ring interface precipitin test

The titer of the SCMV antiserum was determined using ring interface precipitin test which was carried out as detailed below:

Three grams of healthy and SCMV infected Co 671 variety of sugarcane leaf tissues, were ground separately in sterilized mortar with pestle in 12 ml volume of 0.01 M potassium phosphate buffer pH7. The healthy and diseased leaf extracts were separately pressed through double layer of cheese clothes, and centrifuged at 4,000 rpm (x 1,500g)

in SE-12 rotor at 4°C and the supernatants were separately diluted to 1:8 and 1:16 dilutions using 0.01M potassium phosphate buffer pH 7.

Two fold dilution series of SCMV antiserum from 1:4 to 1:1024 dilution were prepared in 50% glycerol prepared in normal saline (0.85% NaCl). The ring interface test was done in small serological tubes, putting 0.2 ml of antiserum dilution at the bottom of the serological tube and gently layering 0.1 ml dilution of SCMV infected plant extract. Such antiserum-antigen combinations were made in separate serological tubes with three two fold dilutions of diseased and healthy plant extracts (1:4, 1:8 and 1:16) and 9 two-fold dilutions of antiserum (1:4 to 1:1024). Thereafter the tubes were incubated at room temperature for 3 h and observations of ring formation at interface was done in dark room with a slit source of light.

3.6.2.2 Tube precipitin test

The two-fold dilutions of antiserum were made ranging from 1:16 to 1:1024 in 0.15M NaCl. One gram of SCMV infected Co 671 variety of sugarcane leaves were ground in 16 ml of 0.01M potassium phosphate pH 7 buffer, resulting in dilution of 1:16 (W/v). Similarly, 1:16 dilution (W/V) for healthy control was prepared. Both, diseased and healthy leaf extracts were centrifuged at 5000 rpm (x 2030 g) in SE-12 rotor of RC-5B centrifuge at 4°C. The supernatants were used for tube precipitin test.

To each of the serological tubes, 0.5 ml of SCMV antiserum dilutions (1:16 to 1:1024) were dispensed, beginning with highest dilution. To each of those tubes 0.5 ml of SCMV infected sugarcane leaf extract (1:16 dilution) was added. Similarly, 0.5 ml of healthy plant extract

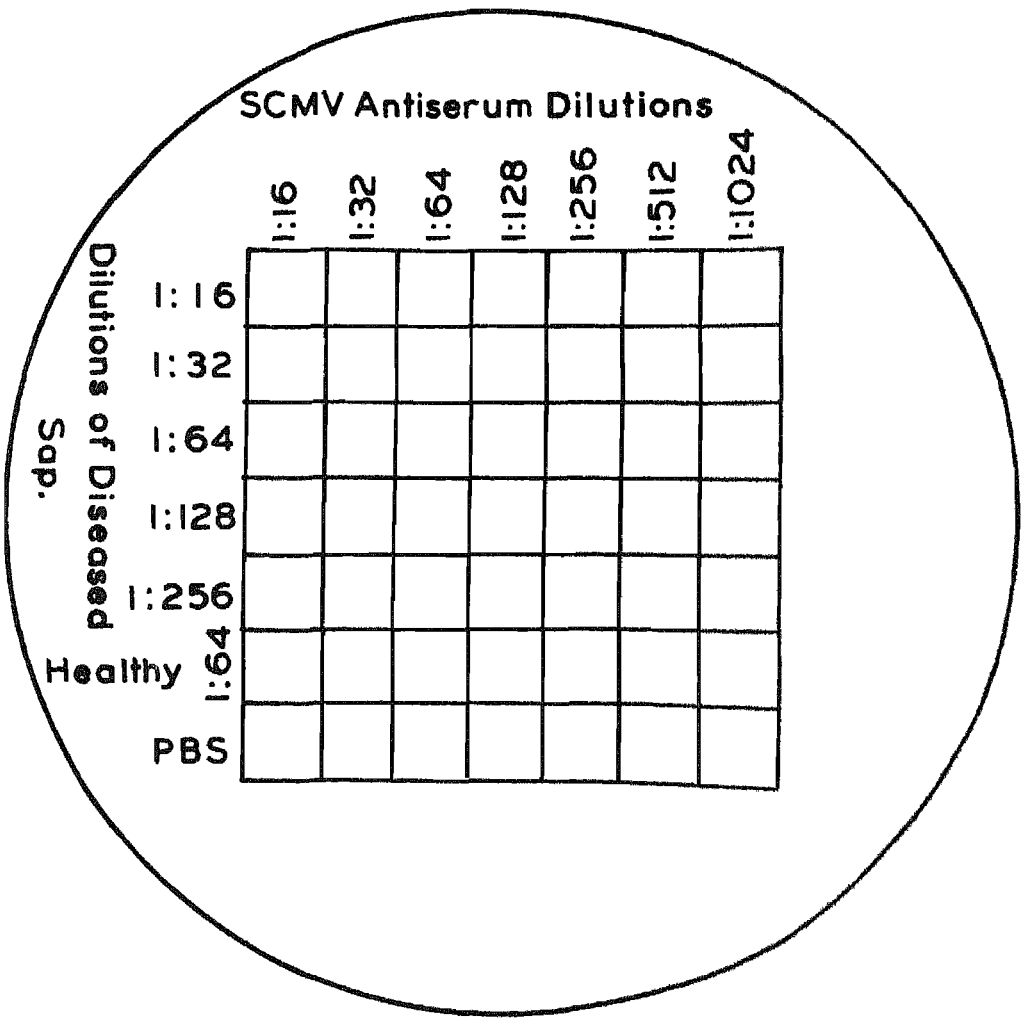
(1:16 dilution) was added to 0.5 ml of each antiserum dilution in the serological tubes. The contents were mixed and tubes were incubated at 37°C for 2 h, in serological waterbath with tubes half immersed in water. Each of the treatment were rated for the presence or absence of the precipitates.

3.6.2.3 Microprecipitin test

The two fold dilutions ranging from 1:16 to 1:1024 for sugarcane mosaic virus antiserum were prepared in 0.15M NaCl. One gram of SCMV infected Co 671 sugarcane leaf tissue was ground in 16 ml of 0.01M potassium phosphate pH 7 buffer, resulted in dilution of 1:16 (W/V). Further two-fold dilutions of SCMV infected sugarcane plant sap ranging from 1:32 to 1:256 were prepared in 0.01 M potassium phosphate pH 7 buffer. Similarly, the dilution series was prepared for healthy plant sap.

New, clean, flat bottomed glass petri plates were coated with 0.25 per cent formvar dissolved in chloroform. After pouring off the excess of fluid, the petri dishes were thoroughly dried. A grid was prepared on a sheet of paper with cross bars, spaced at equal distance to facilitate the deposition of different antigen-antiserum combinations. The checkerboard plan of the combinations of different dilutions of antigen and antiserum was prepared as shown in the figure 7. The formvar coated petri plate was placed on it. Using Gilson P-20 Pipette-man (Ranin Instruments Co., Mack road, Woburn, MA 01801) 8 µl drops of appropriate dilutions of antigen were placed in the petridish as per plan , starting from lowest dilution and proceeding towards highest

FIG. 7. THE CHECKERBOARD PLAN OF THE COMBINATIONS OF DIFFERENT DILUTIONS OF SCMV ANTIGEN AND SCMV ANTISERUM, TESTED IN MICROPRECIPITIN TEST.



dilution. Similarly, 8 µl drop of appropriate dilution of antiserum were placed on the drop of appropriate dilution of antigen. Two controls namely 1:64 healthy plant extract dilution and PBS were used and the petri dish was carefully flooded with mineral oil from one edge until all the drops were submerged in the oil without being disturbed. After closing the lids the petridishes were incubated at room temperature for 3 h and periodically observed using stereomicroscope with incident light and black background. The petri plates were transferred to a refrigerator for observation after overnight storage. The intensity of reaction was rated as very heavy (++++), heavy (+++), moderate (++), slight (+), and no reaction (-).

3.6.2.4 Immuno electronmicroscopy decoration method

Small amount of crude extract was prepared by grinding the leaves from SCMV infected Co 671 sugarcane plants in a few drops of 1% gluteraldehyde solution on a glass slide with a flattened glass-rod in the presence of a pinch of 600 mesh carborandum powder. The carbon coated grids were floated on 20 µl drops of SCMV containing 1:4 diluted leaf extract for 10 minutes with the support film facing downwards. Each of the grids were washed gently, thereafter, with 20 drops of distilled water. These grids were then floated on 20 µl drops of different dilutions of SCMV antiserum ranging from 1:2 to 1:4096, for 10 minutes. Each of these grids were then washed again gently with 20 drops of distilled water. The grids were stained with few drops of 2 per cent uranyl acetate, blotted with piece of filter paper by gently touching the edge of the grids and examined in JEOL-100 Cx-II transmission electron microscope. The electron micrographs were taken with 2 seconds

exposure time on plate or 35 mm film.

3.6.3 Enzyme-linked immunosorbent assay for detection of SCMV

3.6.3.1 Partial purification of gama globulins (antibodies) produced against SCMV

Six ml of SCMV antiserum (titer 1:512) was added with 54ml of double distilled water and mixed thoroughly. Add 60 ml of saturated ammonium sulphate solution to diluted SCMV antiserum. The mixture was stirred at 4°C for 2 h on a magnetic stirrer, centrifuged at 10,000 rpm (x11,670 g) for 10 min in SA-600 rotor of Sorvall RC-5B centrifuge at 4°C, and pellets were resuspended in 6 ml of half strength PBS (0.01M sodium phosphate + 0.075 M NaCl, pH 7). Dialysed 6 ml volume of resuspended antibodies for 48 h against half strength PBS, 500 ml/change and 4 such changes were used at interval of 12 h. The dialysed SCMV antibodies were transferred to presterilized vials (3 ml/vial) and stored frozen at -75° C till used.

3.6.3.2 Determination of concentration of partially purified SCMV antibodies

The 1:10 dilution of partially purified SCMV antibodies was prepared in half strength PBS pH 7 and absorbance was recorded at 278 nm wavelength. $E^{1\ mg/ml}_{278\ nm}$ = 1.4 relationship was used to estimate the mg/ml concentration of SCMV antibodies.

3.6.3.3 Preparation of enzyme-antibody conjugate for ELISA

Two hundred microliters of partially purified SCMV antibodies (1 mg/ml) were mixed with 36 µl (500 units) of enzyme alkaline phosphatase (Boehringer Mannheim GmbH Biochemica, P.O. Box 310120,

6800 Mannheim 31, German Federal Republic) and 2.5 µl of 25 per cent gluteraldehyde. The mixture was incubated at 22°C for 4.75 h and dialysed against three changes of PBS (0.02M $NaHPO_4$ + 0.15 M NaCl, pH 7), 500 ml PBS for 12 h/change. After dialysis, bovine serum albumin was added to give a concentration of 5 mg/ml and stored at 4°C in the cold room till used.

3.6.3.4 Enzyme-linked immunosorbent assay

The double antibody sandwich ELISA procedure of clark and Adams (1977) was employed with some modifications. The Dynatech microtiter ELISA plates were coated with 0.1 µ g/ml concentration of partially purified SCMV gama globulins in 0.5M sodium carbonate pH 9.6 buffer. Two hundred microliters of suspension of partially purified SCMV gamma globutins were dispensed in each well and incubated at room temperature for 4 h. These wells in ELISA plates were thereafter washed with PBS-Tween pH 7.4 (0.02M sodium phosphate + 0.15M NaCl, pH 7.4 + 0.05% tween-20). Total 3 washings were given for 3 minutes each. Each wells in ELISA plates were then loaded with 200 µl of SCMV antigen prepared in PBS-Tween (1 g/10 ml) alongwith their respective healthy controls. The plates after loading were incubated, overnight, at 4°C. Next morning, the wells in ELISA plates were washed with the PBS-tween pH 7.4, for 3 times for 3 minutes each. The $2x10^{-3}$ dilution of SCMV antibody-enzyme conjugate, was dispensed to each well at the rate of 200 µl per well and incubated for 4 h at room temperature. Thereafter, plates were washed with PBS-Tween, three times for 3 minutes each. Freshly prepared substrate, P-nitrophenyl phosphate disodium salt solution (0.6 mg/ml) in 10 per cent diethanolamine

pH 9.8 was dispensed in each well of the ELISA plates at the rate of 300 µl per well and incubated at room temperature for an hour. Thereafter, absorbance at 405 nm was recorded for each well using Dynatech Mini Reader-II which was attached to Gemini-100 printer.

3.6.3.4.1 Optimum coating concentration and conjugate dilution for ELISA

Three different coating concentrations of SCMV antibodies, namely 10 µg, 1 µg and 0.1 µg/ml, and four different dilutions of SCMV-antibody-enzyme conjugate, namely 1:250, 1:500, 1:1000 and 1:2000 were used for the study. Diseased Co 671 sugarcane plant sap diluted to 1:10, 1:100 and 1:1000 times were tested alongwith PBS-Tween pH 7.4 buffer control. Thus, there were 48 combinations in all (3 of coating concentration x 4 conjugate dilution x 4(3+1) host sap dilution and PBS control), distributed serially in ELISA microtiter plates.

3.6.3.4.2 Effect of blocking on the performance of ELISA for SCMV detection

Blocking treatments was carried out with 5 per cent dried skimmed milk powder(Five pints Mfd.: St. Ivel Ltd., Swindon, Wiltshire, England) Solution; prepared in PBS-Tween pH 7.4 containing 2 per cent polyvinyl pyrrolidone and 0.2 per cent albumin, 300 µl of which was dispensed in each well of the washed plates and kept for 30 min after overnight incubation with antigen. These plates were then washed 3 times, with PBS-Tween pH 7.4 before putting the conjugate.

An unidentified sugarcane plant, showing no apparent symptoms was also indexed by cross absorption of 1:2000 dilution of SCMV-conjugate, with 1:100 dilution of clarified leaf extract and loading the both unblocked and blocked wells.

3.6.3.4.3 Estimation of SCMV titer in different plant parts of infected Co 671 sugarcane and CSH 9 sorghum

To estimate the titer of SCMV in different tissues, the extract was prepared from leaf, stem and root tissues macerated in PBS-Tween (1 g/10 ml) from healthy and diseased CSH 9 sorghum, and diseased Co 671 sugarcane plants. PBS-Tween pH 7.4 buffer was taken as control. Each well in ELISA plates were loaded with 200 µl of the 10^{-1}, 10^{-2} and 10^{-3} dilutions of the SCMV infected extracts from different tissues. After treating the plates with SCMV antibody-enzyme conjugate, and incubation with substrate, the absorbance for each well at 405 nm was recorded with Dynatech Mini Reader-II attached to Gemini-100 printer.

3.7 ELECTRONMICROSCOPY TO DETERMINE THE SIZE AND MORPHOLOGY OF THE PURIFIED SUGARCANE MOSAIC VIRUS

A drop of the purified sugarcane mosaic virus was kept on the carbon coated grid of the electronmicroscope for 3 min. The grid was then washed with 20 drops of distilled water and stained with few drops of 2 per cent uranyl acetate. The grid was blotted dried by touching the edge of the grid with the piece of filter paper and examined in JEOL-100 Cx-II transmission electron microscope. Electron micrographs of well separated SCMV particles were taken at 14,000 magnification on plate film. The negatives were magnified 3 times the original magnification and the measurements of length of 100 SCMV particles in millimeters were taken from the prints. The actual length of the particles were calculated by using following formula:

$$\text{Size of SCMV particle in nm} = \frac{\text{measured size in mm}}{42{,}000} \times 1000{,}000$$

The modal length of SCMV particle was determined by taking into consideration the frequency distribution at 10 nm class intervals.

Measurements from these prints for width of the SCMV particles were also taken using "PEAK Scale Lupe-10X" mini measure. The actual width of the SCMV particles were calculated using above mentioned formula.

3.8 PHYSICOCHEMICAL PROPERTIES OF THE SUGARCANE MOSAIC VIRUS

3.8.1 Ultraviolet absorbance profile of the purified SCMV

The purified preparation of SCMV diluted 1:10 times with 0.05M borate buffer pH 8.2+0.01M EDTA+0.5M urea, was filled in a quartz cuvette for UV absorbance. Another cuvette with same amount of buffer (0.05M borate pH 8.2+0.01M EDTA+0.5M urea) was taken as a blank for zero absorbance calibration. UV absorbance from 320 to 330 nm at the interval of 5 nm, in Pye Unichem SP8-400 UV/VIS spectrophotometer were taken. The absorbance was plotted against wavelength to obtain UV profile for purified SCMV. The correction for absorption due to light scattering was made by taking absorbance from 300-400 nm and extrapolating the graph to obtain the corrected absorbance value of wavelength of interest (Noordam, 1973).

3.8.2 Determination of RNA% of SCMV

The percentage of ribonucleicacid in SCMV was determined by following two methods.

3.8.2.1 Spectrophotometric determination of percentage of SCMV-RNA from purified SCMV preparation

The dilution of 1:10 of purified SCMV was prepared in 0.05M borate buffer pH 8.2 containing 0.01M EDTA + 0.5 M urea. The net absorbance at 260 and 280 nm were recorded and corrected from the graphs for light scattering and A280/A260 ratio was estimated. The percentage of nucleic acid was estimated according to the tabulation given by Layne (1957).

3.8.2.2 Determination of percentage RNA in purified SCMV preparation using quadratic regression line relationship

Gibbs and Harrison (1976) have related ratio of A260/A280 of purified viruses to their RNA%, giving a quadratic regression curve and the equation:

$$\frac{A\ 260}{A\ 280} = 0.9320 + 0.0454\ (RNA\%) - 0.0006(RNA\%)^2$$

The absorbance values at 260 and 280 nm were taken for 1:10 dilution of purified SCMV, in Pye Unichem SP8-400 UV/VIS spectrophotometer.

3.8.3 **Determination of extinction coefficient of the SCMV**

Extinction coefficient ($E_1^{0.1\%}_{cm}$), which is the optical density of a 1 cm columns of a 1 mg/ml preparation of purified viruses; is correlated with RNA percentage. Gibbs and Harrison (1976) demonstrated a linear regression relationship between the extinction coefficient at 260 nm ($E\ 260_1^{0.1\%}_{cm}$) of purified preparations of virus particles and the RNA contents (Figure 9), represented by a formula:

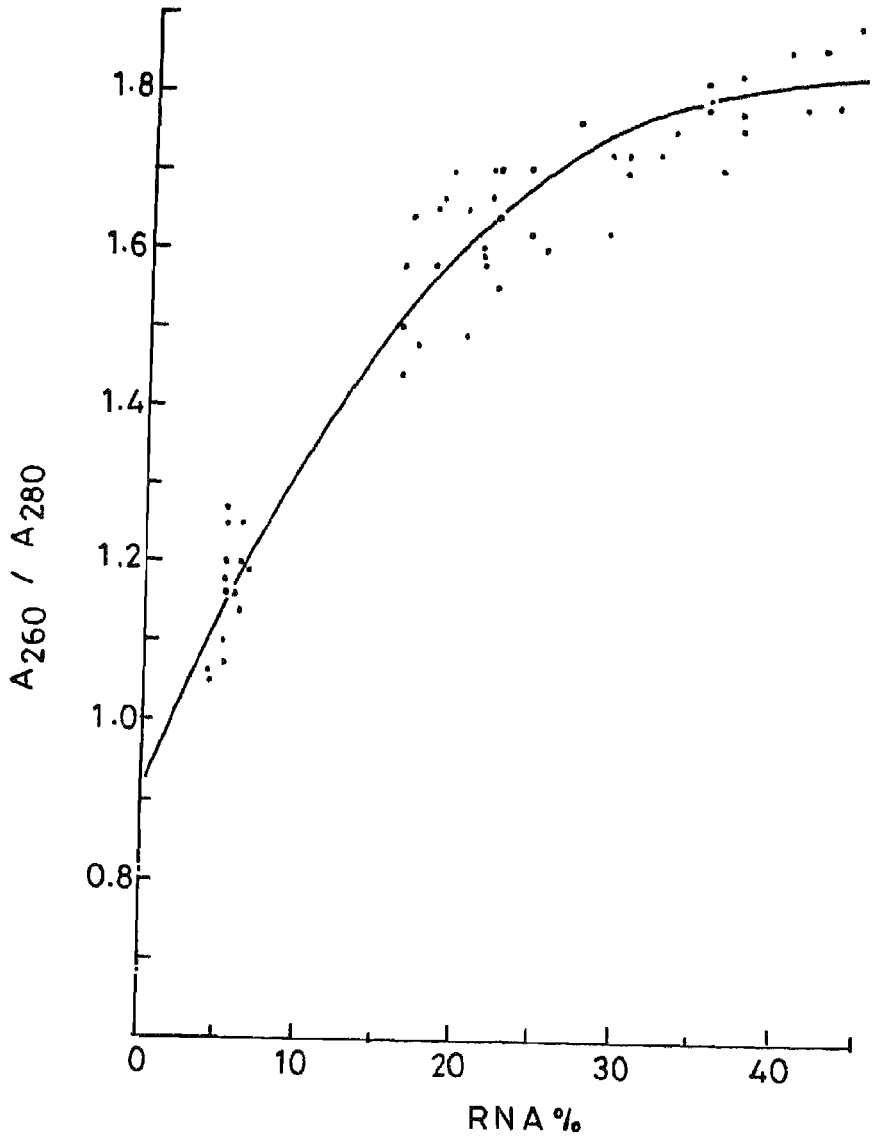


FIG. 8. GRAPH ILLUSTRATING THE RELATION BETWEEN THE A_{260} / A_{280} RATIO OF PURIFIED PREPARATIONS OF VIRUS PARTICLES AND THE RNA CONTENT OF THOSE PARTICLES. THE LINE SHOWS THE QUADRATIC REGRESSION WHICH BEST FITS THE SIXTY TWO RECORDS, SOME MULTIPLE, WITH THE RELATION.

$$A_{260}/A_{280} = 0.9320 + 0.0454\,(\text{RNA}\%) - 0.0006\,(\text{RNA}\%)^2$$

(FROM: GIBBS AND HARRISON, 1976)

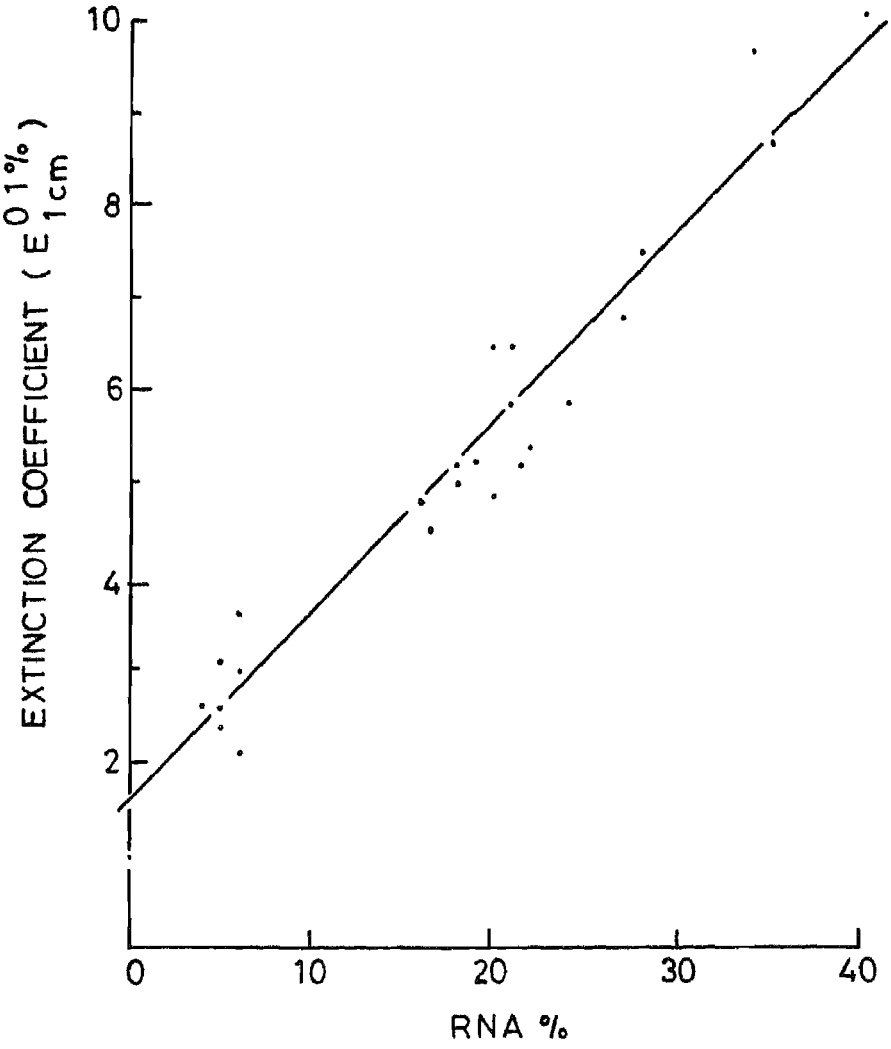


FIG. 9. GRAPH ILLUSTRATING THE RELATION BETWEEN THE EXTINCTION COEFFICIENT AT 260 nm ($E\,260^{0.1\%}_{1\,cm}$) OF PURIFIED PREPARATIONS OF VIRUS PARTICLES, AND THE RNA CONTENT OF THOSE PARTICLES. THE LINE SHOWS THE LINER REGRESSION WHICH BEST FITS THE TWENTY FIVE RECORDS AND HAS THE RELATION.

$$(E\,260^{0.1\%}_{1\,cm}) = 1.531 + 0.205\,(RNA\,\%)$$

(FROM: GIBBS AND HARRISON, 1976)

$$(E\ 260^{0.1\%}_{1\ cm}) = 1.531 + 0.205\ (RNA\%)$$

The RNA% of SCMV, determined in section 3.8.2 of this chapter was used for calculating of extinction coefficient of SCMV.

3.8.4 Determination of the sedimentation coefficient of the SCMV

The sedimentation coefficient of the SCMV was determined by band sedimentation velocity run in Beckman L8-60M ultracentrifuge hooked up with preparative UV scanner (Sussman, 1975, Beckman Bulletin No. SB-482). The purified SCMV was diluted with 0.05 M borate buffer pH 8.2 containing 0.01 M EDTA + 0.5 M urea, so as to give 0.5 absorbance at 280 nm. The analytical cell was assembled and 0.45 ml of purified SCMV preparation was filled into one sector of the cell, and the second sector was filled with the buffer used for suspending the purified SCMV. The analytical cell after loading was placed in the An-A analytical two-place rotor (No. 334238) with one counter balance, and centrifuged at 26,000 rpm up to 1 hour at 4°C in Beckman L8-60M ultracentrifuge.

The migration of nucleoprotein (SCMV) band was monitored, with the hooked up preprative UV scanner for obtaining UV absorbance tracings at 5,10,15,18,21,27,33,45 and 50 min. For each scan tracings distance between the inner edge of the cell to the mid band position was measured. Different values namely (1) Magnification factor; (2) true radial distance, and (3) lnr were calculated for each scan according to following formulas:

i) Magnification factor (F) = $\frac{d}{1.62}$ . . . . . Equation (I)

where d = the distance on chart (in cm) between inner and out signals of edges of the cell

ii) True radial distance (r) = $\frac{x}{F}$ + 5.7 . . . . . Equation (II)

where x = distance (in cm) on chart between the point of interest and the inner reference edge signals.

iii) $\ln r = 2.303 \log r$ . . . . . Equation (III)

A plot of lnr against time was plotted as straight line relationship and slope was found by the equation:

iv) Slope = $\frac{d\ln r}{dt}$ . . . . . Equation (IV)

Sedimentation coefficient was calculated with the following formula:

$$S = \frac{1}{w^2} \left(\frac{d\ln r}{dt}\right) \quad \text{. . . . . Equation (V)}$$

where w = angular velocity (sec^{-1}), $\frac{d\ln r}{dt}$ = slope

3.8.5 Determination of density of SCMV and its partial specific volume ($\bar{V}$) from the RNA% of the SCMV

Gibbs and Harrison (1976) gave a quadratic regression curve (Figure 10) to demonstrate the relationship of buoyant density and partial specific volume of nucleoprotein molecules with their nucleic acid contents. Accordingly, the buoyant density of the SCMV was calculated as per the following formula given by Gibbs and Harrison (1976).

$$\text{Density} = 1.2922 + 0.0011(\text{RNA\%}) + 0.0001\ (\text{RNA\%})^2$$

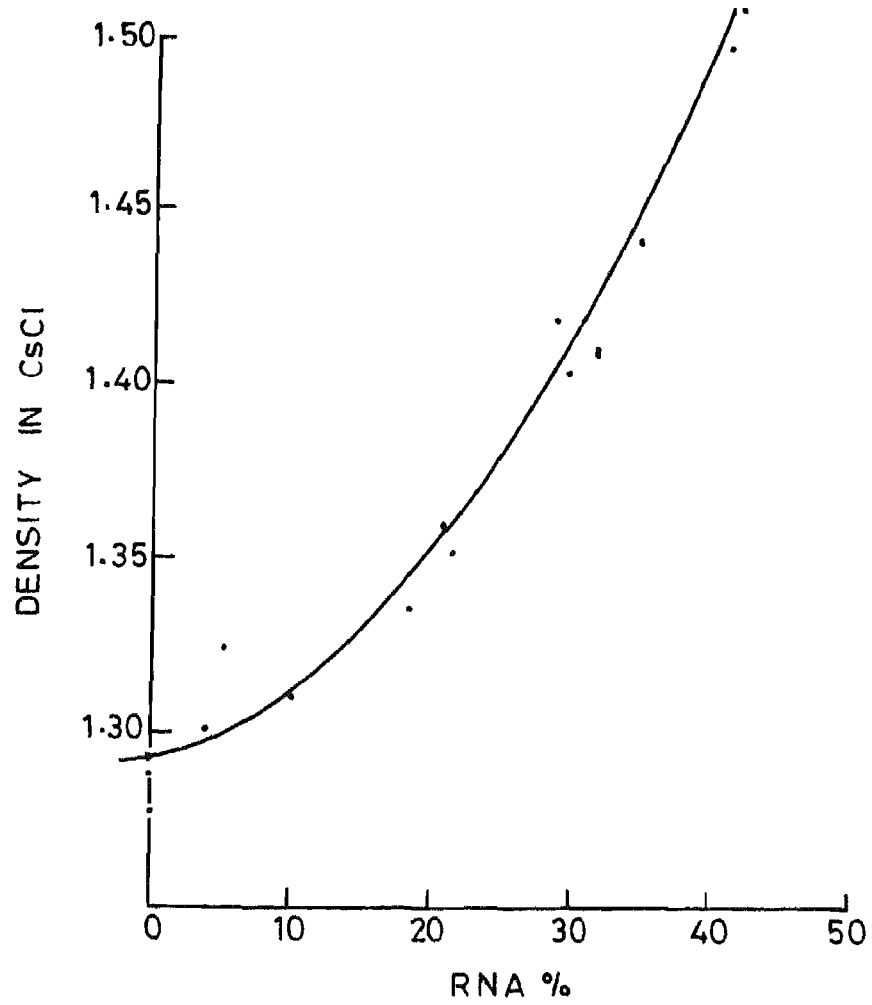


FIG. 10. GRAPH ILLUSTRATING THE RELATION BETWEEN THE DENSITY IN CsCl SOLUTION OF DIFFERENT VIRUS PARTICLES AND THEIR RNA CONTENT. THE LINE SHOWS THE QUADRATIC REGRESSION WHICH BEST FITS EIGHTEEN RECORDS (two multiple) WITH THE RELATION.

$$DENSITY = 1.2922 + 0.0011\,(RNA\,\%) + 0.0001\,(RNA\,\%)^2$$

(FROM : GIBBS AND HARRISON, 1976)

The partial specific volume ($\bar{V}$), being the reciprocal of bouyant density was also calculated from the above value (density).

3.9 PHYSICOCHEMICAL PROPERTIES OF THE SUGARCANE MOSAIC VIRUS COAT AND INCLUSION PROTEINS

3.9.1 **Viral coat protein**

3.9.1.1 Isolation of coat protein of SCMV (Flow Chart, Figure 11)

The final pellet of the purified sugarcane mosaic virus was suspended in PEN buffer (0.01M NaH_2PO_4 + 0.001M EDTA + 0.01M NaN_3, pH 7) for isolating viral coat protein. To one ml of the purified SCMV suspension, was added 1 ml of 4M LiCl, mixed throughly and kept overnight in deep freeze at -75°C. The frozen suspension was thawed, next morning, and centrifuged at 15,000 (x 18,270 g) for 20 minutes in SE-12 rotor of Sorvall RC-5B centrifuge at 4°C. The supernatant was dialysed to remove LiCl, against 0.1M sodium phosphate buffer containing 0.1M EDTA, pH 7.5, at 4°C for 8 hr, giving 3 changes of buffer and using 250 ml volume of buffer per change. The virus coat protein was precipitated by adding 5 volumes of cold acetone containing 10mM of 2-mercaptoethol and stored at -75°C for 1 h (Hames and Rickwood, 1981). Thereafter the SCMV coat protein was pelleted by centrifugation at 15,000 rpm (x 26,260 g) for 20 minutes in SA-600 rotor of the Sorvall RC-5B centrifuge at 4°C, for 1 h to remove the traces of acetone, dissolved in 225 µl of Laemmli's (1970) sample buffer and stored at 4°C till used. The Laemmli's sample buffer was composed of distilled water - 4.2 ml, 0.5M tris-HCl pH 6.8 - 1 ml, Glycerol - 8 ml, 10% sodium dodecyl sulphate - 1.6 ml, 2-mercaptoethanol - 0.4 ml. The bromophenol

Flow Chart Figure 11. The flow chart for purification procedure of the sugarcane mosaic virus coat protein

Purified SCMV suspended in 0.01M sodium phosphate + 0.001M EDTA + 0.01M NaN_3, pH 7 buffer

Discard material on this side	→ 1.	Add 1 ml of 4M LiCl solution to 1 ml purified SCMV. Mixed and stored at -75°C for overnight
	→ 2.	18,270 g, for 20 min, at 4°C
Pellet	→ 3.	Supernatant saved and dialysed at 4°C for 8 h, in 3 changes of virus suspension buffer.
	→ 4.	Add 5 volumes of cold acetone containing 10 mM-2ME, store at -75°C for 1 h.
	→ 5.	26,260 g, for 20 min, at 4°C.
Supernatant	→ 6.	Pellet air dried for 1 h to remove acetone
	→ 7.	Pellet dissolved in 225 µl Laemmli's sample buffer, stored at 4°C

blue tracking dye was added to the sample buffer at 0.05% concentration prior to processing for electrophoresis.

3.9.1.2 Electrophoresis for determination of the molecular weight

For determination of the molecular weight, the isolated SCMV coat protein was coelectrophoresed with low molecular weight protein markers (LMW) (Pharmacia Code No: 17-0446-01), in 12.5% SDS-polyacrylamide gel (Hames and Rickwood, 1981). For SDS-denaturation 100 µl of SCMV coat protein and 10 µl of Pharmacia's LMW markers were heated in boiling water bath at 100°C for 5 minutes in separate sterilized apendoff tubes and cooled immediately on ice. The opendoff tubes were centrifuged at 10,000 g for 5 min to remove any insoluble materials. The Pharmacia's protein LMW markers having molecular weight range from 14,000 to 94,000, constituted of Phosphorylase b 94,000; albumin 67,000; Ovalbumin 43,000; Carbonic anhydrase 30,000; trypsin inhibitor 20,000; and lactalbumin 14,000 daltons. The 10 µl volume of protein LMV markers and 80 µl volume of SCMV coat protein were loaded in two different tracks of 12.5% SDS-polyacrylamide verticle slab gel (2.5% cross linked). The electrophoresis was carried out at 60 V for 1 h and then at 120 V for 3 h using tris-glycine-SDS as running buffer (pH 8.3). LKB Bromma 2197 power supply was used for electrophoresis.

The gel after electrophoresis was stained over night in 0.02% Kenacid blue R (Milling blue 2BR) in water:methanol:glacial acetic acid (5:5:2 by volume). The gel was destained in methanol: double distilled water:acitic acid (3:6:1 by volume) till the protein bands were visible clearly. Usually 3 changes of destainer were given; and

the volume of destainer used was 200 ml per change. The gel was stored in 5 per cent acetic acid. The position of the Pharmacia's LMW protein markers and SCMV coat protein band were measured from the edge of the well to the middle part of the band and the relative mobility (Rf) was calculated by the following formula:

$$Rf = \frac{\text{distance migrated by a protein}}{\text{distance migrated by dye}}$$

For estimating the molecular weight of the SCMV coat protein the standard curve of molecular weight markers versus Rf and distance of migration were plotted on semilog graph paper and the molecular weight of SCMV coat protein was determined from its Rf value and also by distance of migration. The slab gel was scanned at 620 nm in Shimadzu CS-930 dual wavelength chromoscanner linked with Shimadzu DR-2 data recorder, with program Casset CCS-1 (Mfd. Shimadzu Corporation, Spectrophotometric Instrument Plant, Analytical Instrument Division, Kyoto, Japan) and the tracing was obtained.

3.9.2 Inclusion protein

3.9.2.1 Purification of SCMV induced inclusion protein (Flow Chart, Figure 12)

The inclusion purification method of Jensen et al. (1986) was further modified. Five grams of Co 671 sugarcane leaf tissue was ground in 20 ml volume of an extraction buffer consisting of 5 mM tris, 1mM KCl, 0.1mM Na EDTA, 1mM MgCl, 10% glycerol, 1% triton x-100 and 0.1% 2-mercaptoethanol. The sap was passed through cheese cloth and was centrifuged at 2000 g for 2 min in SA-600 rotor of the Sorvall RC-5B centrifuge at 4°C. The supernatant was centrifuged over 5 ml pad of 20 per cent sucrose prepared in the extraction buffer for 25 min

Flow Chart Figure 12. Flow chart for purification of SCMV inclusions

Discard materials of this side	→	1.	Ground 5 g SCMV infected Co 671 sugar-cane tissue in 20 ml extraction buffer.
	→	2.	Strain sap through cheese cloth, and 2000 g, for 2 min, 4°C.
Pellet	→	3.	Supernatant centrifuged over 5 ml pad of 20% sucrose prepared in extraction buffer at 17,000 g, 25 min, 4°C.
Supernatant	→	4.	Pellet resuspended in 1.25 ml of 175mM tris buffer pH 8.4.
	→	5.	Layered suspension on 5 ml pad of 20% sucrose, and 17,000 g, for 25 min, 4°C.
Supernatant	→	6.	Pellet resuspended in 10 ml with 175 mM tris buffer pH 8.4.
	→	7.	17,000 g, for 25 min, 4°C.
Supernatant	→	8.	Pellet of SCMV inclusions resuspended in dissociation buffer.

at 14,500 rpm (x 17,000 g) in SE-12 rotor of Sorvall RC-5B centrifuge at 4°C. The pellet was suspended in 1.25 ml volume of 175 mM tris buffer pH 8.4 and layered on 5 ml pad of 20% sucrose prepared in extraction buffer and centrifuged at 14,000 rpm (x 17,000 g) in SE-12 rotor for 25 min at 4°C. The pellet was resuspended in 10 ml volume using 175 mM tris pH 8.4 buffer. The inclusions were pelleted by centrifuging the 10 ml suspension at 14,500 rpm (x 17,000 g) for 25 min in SE-12 rotor at 4°C, and suspended in 1 ml volume of dissociation buffer pH 8.8, containing 175 mM tris, 10 mM 2-mercaptoethanol, 5% sucrose, 2% (W/V) SDS and 0.001% crystalviolet.

3.9.2.2 Electron microscopy of purified inclusions

The purified SCMV inclusions suspended in 175 mM tris pH 8.4 buffer, a drop of which was kept on carbon coated grids for 2 min, and then the grid was washed with 20 drops of double distilled water. And further stained with few drops of 2% aqueous uranyl acetate stain, blotted dried by touching filter paper at the edge of the grid, and observed in the JEOL-100 Cx-II transmission electron microscope (Mfd. Jeol Ltd., Tokyo, Japan). The pictures of inclusions were taken at x10,000 magnification and with 2 seconds exposure time.

3.9.2.3 Electrophoresis for the determination of the molecular weight

For determining the molecular weight of the inclusion protein, the isolated inclusion proteins were coelectrophoresed with LMW protein markers (Pharmacia Code No. 17-0446-01) in SDS-denaturation state. For SDS denaturation the procedure was same as detailed in section 3.9.1.2 of this chapter. The 10 µl volume of protein LMW markers and

10, 20, 40, 80 and 100 µl volume of SCMV inclusion protein were loaded in six different tracks of 12.5% SDS-polyacrylamide verticle slab gel (2.5% cross linked). The electrophoresis was carried out at 60V for 1 h and then at 120 V for 3 h, using LKB Bromma 2197 power supply.

The gel was stained with silver nitrate following the procedure of Morrissey (1981). The slab gel was given prefixation treatment in 200 ml volume of 50 per cent methanol, 10 per cent acetic acid for 30 min, followed by another treatment in 200 ml volume of 5 per cent methanol, 7 per cent acetic acid for 30 min. Thereafter the gel was fixed in 100 ml volume of 10 per cent gluteraldehyde for 30 min and washed in 1 liter volume of double distilled water for overnight duration. Next day, the gel was rinsed in double distilled water for 30 min and then soaked in 200 ml volume of dithiothreitol (DTT) at the concentration of 5 µg/ml, for 30 min. The DTT solution was poured off and without rinsing the gel was treated with 200 ml volume of 0.1 per cent silver nitrate for 30 min. The gel was once rinsed rapidly with 200 ml volume of the developer (100 µl of 37% formaldehyde in 200 ml volume of 3% sodium carbonate) until desired level of staining is attained. The staining was stopped by adding 10 ml volume of 2.3M citric acid directly into the developer and agitating for 10 min. This solution was then discarded and the gel was washed several times in distilled water over a 30 min period. The gel was soaked in 200 ml volume of 0.03% sodium carbonate and then stored in double distilled water. The position of the Pharmacia's low molecular weight markers and SCMV inclusion protein bands were measured from the edge of the respective loading wells and the relative electrophoretic mobility (Rf) was calculated. The

molecular weight of SCMV inclusion protein was determined by plotting molecular weights versus Rf or distance of migration for standards, and from Rf or distance of migration values of inclusion protein, its molecular weight ascertained.

3.10 VIRAL NUCLEIC ACID

3.10.1 Purification of the SCMV-RNA (Flow Chart, Figure 13)

The SCMV-RNA was isolated from purified SCMV preparation using the procedure of Kirby (1965) with a few modifications. The purified SCMV was suspended in 2 ml of 1xSSC (0.15M sodium chloride, 0.15M tris sodium citrate pH 7.5) in sterilized SA-600 rotor tubes. Twenty microliters of 10 per cent sodium dodecyl sulphate dissolved in 1xSSC was added to give a concentration of 1 per cent. Equal volume of water saturated phenol containing 0.1 per cent 8-hydroxyquinoline was mixed throughly in the tube for 5 minutes, before treating the mixture at 60°C in water bath for 2 min, and cooled immediately on the ice. After centrifugation at 5000 rpm (x2,918 g) for 10 min, the upper 4 ml aqueous phase was transferred to sterilized SA-600 rotor tubes. The aqueous phase was again mixed with equal volume of water saturated phenol containing 8-hydroxyquinoline and mixed for 5 min, centrifuged at 5000 rpm (x 2,918 g) for 10 min, and aqueous phase was again recovered and transferred to sterilized centrifuge tube. Two volumes of cold absolute ethanol was added to the aqueous phase, mixed thoroughly and stored frozen at -75°C for overnight. The SCMV-RNA was pelleted by centrifugation at 15,000 rpm (x 26,260 g) for 30 min in SA-600 rotor

Flow Chart Figure 13. The flow chart for purification procedure of SCMV-RNA

	1.	Purified SCMV suspended in 2 ml of 1xSSC (0.15M sodium chloride, 0.15M tris sodium citrate pH 7.5)
	2.	Add 20 µl of 10% SDS (dissolved in 1xSSC buffer) and mixed thoroughly in sterilized tube and keep on ice for 10 min.
	3.	Add equal volume of water-saturated phenol containing 0.1% 8-hydroxyquinoline, mixed throughly in tube for 5 min.
	4.	Heat at 60°C for 2 min in water bath, and cool quickly in ice bath.
	5.	5000 rpm (x 2,918 g), 10 min, 4°C.
Discard material on this side		
	6.	Upper aqueous phase recovered and mixed with equal volume of water saturated phenol.
	7.	5000 rpm (x 2,918 g), 10 min, 4°C.
Pellet and lower phenolphase		
	8.	Upper aqueous phase, add 2 volumes of cold absolute ethanol, mixed and stored frozen at -75°C, overnight.
	9.	15,000 rpm (x 26,260 g) for 30 min, 4°C.
Phenolphase		
	10.	RNA pellet dissolved in 250 µl of sterile double distilled water.
	11.	Stored in 125 µl aliquotes in sterile apendoff tubes at -75°C till used.
Supernatant		

of the Sorvall RC-5B centrifuge at 4°C. The SCMV-RNA pelleted was dissolved in 250 µl volume of sterile double distilled water, and stored at -75°C in 125 µl aliquotes in sterile apendoff tubes till used.

3.10.2 Ultraviolet absorbance profile of the purified SCMV-RNA

To obtain the UV absorbance profile, the 1:50 dilution of purified SCMV-RNA was prepared with sterile distilled water and filled into quartz cuvette. The UV absorbance was taken from 230 to 340 nm range with 5 nm intervals in the Pye-Unichem SP 8-400 UV/VIS spectrophotometer, and the graph was plotted, absorbance versus the wavelength.

3.10.3 Determination of titer of SCMV-RNA

To determine the SCMV-RNA titer 1:50 dilution of purified SCMV-RNA was prepared with sterile double distilled water in sterile apendoff centrifuge tube. The UV absorbance were taken at 260 and 280 nanometers wavelength in Pye-Unichem SP8-400 UV/VIS spectrophotometer and the titer of the SCMV-RNA was estimated using the formula:

µg RNA/ml = Absorbance at 260 nm x 40 x Dilution of RNA preparation

3.10.4 Determination of the sedimentation coefficient of the SCMV-RNA

The analytical cell of An-A two place analytical rotor was assembled and both the compartments were repeatedly flushed with sterile double distilled water. The purified 0.45 ml of SCMV-RNA sample (0.5A at 280 nm) was loaded aseptically into the one sector of the analytical cell, while 0.45 ml of sterile double distilled water was placed into the second sector. The cell was centrifuged in An-A rotor at

40,000 rpm, for 2 h, and at 4°C in the Beckman L8-60M ultracentrifuge coupled with preparative UV scanner. The UV absorbance scan tracings were recorded at 3, 5, 9, 12, 14, 22, 26 and 31 min of run. Radial distances from inner edge of the cell to the moving boundary were measured, in case of each scan. The sedimentation coefficient was calculated according to the calculation as described in section 3.8.4 of this chapter.

3.10.5 Determination of the molecular weight of the SCMV-RNA

The molecular weight of the purified SCMV-RNA was determined from its sedimentation coefficient using following formula (Sussman, 1975):

$$M = \frac{2\,RT}{(1-\bar{V}p).\ w^2} \times \frac{d\ln.r}{(dt^2)}$$

where, R = Universal gas constant 8.315×10^{-7} erg/degree . mole.

T = absolute temperature

$\bar{V}$ = partial specific volume

p = solution density, g/cm^3

$\frac{d\ln r}{dt}$ = slope of the line plotted

3.10.6 Further purification and analysis of the SCMV-RNA by electrophoresis

The SCMV-RNA was further purified and analysed in horizontal agarose and verticle polyacrylamide slab gels. The samples of the purified SCMV-RNA having different titers, with or without denaturing treatments were loaded in separate wells of different gels.

For agarose gel electrophoresis, the 0.6% agarose gel was prepared by dissolving 0.3 g of electrophoresis grade agarose in 50 ml of TAE buffer (40 mM tris base, 2mM Na_2 EDTA. $2H_2O$, 20 mM sodium acetate anhydrous, 29.6 mM glacial acetic acid, pH 7.49 and autoclaved before use) in hot water bath. For casting, the melted agarose was poured on the gel casting tray of the mini horizontal electrophoresis apparatus and the well forming comb was inserted for making wells. The agarose gel was allowed to set for 60 min. Enough electrophoresis buffer was poured in gel electrophoresis apparatus just to cover gel by approximately 2 to 3 mm.

The wells of agarose gel were loaded with samples of SCMV-RNA, adjusted to 10 and 20 µg of concentration, in a loading volume of 20 µl, containing 5 µl of 50 per cent glycerol and 0.05 per cent bromophenol blue as tracking dye. The electrophoresis was carried out at 50 volts, 60 mA for 1.5 h. The gel was stained with 5 µg/ml ethidium bromide dissolved in 0.5 M ammonium acetate for 60 min and destained by washing gel briefly in distilled water. The RNA bands were visualized by its fluroescence under ultraviolet light. The RNA bands on stained gel were photographed using incident UV light, with the gel placed on black plate, using the panchromatic film Kodacolor Gold 100 ASA, and with exposure time of 2 min at diaphragm opening (f) 5.6, in the dark room.

3.10.6.1 Denaturation of SCMV-RNA with heat, 3 M urea and 36% formamide, and their electrophoresis on 0.6% agarose gel

Treatment 1. Heat treatment (60°C).

For heat denaturation treatment the 20 µl of samples having 10 and 20 µg of SCMV-RNA were heated at 60°C for 5 min in separate sterile apendoff tube in precision water bath and then immediately cooled in ice bath prior to loading.

Treatment 2. Urea treatment (3M).

For urea denaturation treatment 20 µg concentration of SCMV-RNA was prepared in 3M urea with a loading volume 20 µl in sterile apendoff tubes. The sample was heated at 60°C for a period of 5 min in precision water bath.

Treatment 3. Formamide (36%) treatment.

For formamide denaturation treatment equal volume of 100 per cent formamide was added. SCMV-RNA preparation having a 20 µg concentration of SCMV-RNA in sterile apendoff tubes prior to loading was heated at 60°C for5min.

The electrophoresis procedure and conditions were same as detailed in section 3.10.6 of this chapter. The gel after electrophoresis was stained in 5 µg/ml concentration of ethidium bromide and the RNA bands were photographed on panchromatic film as detailed earlier in section 3.10.6 of this chapter.

3.10.6.2 Denaturation of SCMV-RNA with Glyoxal and its electrophoresis in 1% agarose

The procedure used was that of McMaster and Carmichael (1977) which underwent some modification. The glyoxal [30% (W/V) solution in H_2O] was purified by passing 1.25 ml of glyoxal through mixed bed column of strongly cationic (Searlite SRC-120) and strongly anionic (Seralite.SRC-400) resins, repeatedly for five times. The 10 µg SCMV-RNA sample was denatured by incubating it in plastic capped tubes for 1 h at 50°C in a buffer containing 1M glyoxal, 50 per cent dimethyl-sulphoxide and 10 mM sodium phosphate pH 7 buffer. The samples were cooled to room temperature, and 5 µl of 50 per cent glycerol containing 0.01 per cent bromophenol blue, was added to 15 µl volume of SCMV-RNA sample and was later loaded. The electrophoresis was carried out in 1 per cent agarose gel prepared in 10 mM sodium phosphate pH 7 buffer; at 30 volts and 44 mA, for 2 h and the reservoir buffer used was 10 mM sodium phosphate pH 7.

The gel was stained for 30 min in 200 ml of staining solution consisting of 30 µg/ml concentration of acridine orange prepared in 10 mM sodium phosphate pH 7 buffer. The gel was destained for 1 h at 22°C in 10 mM sodium phosphate pH 7 buffer and observed under UV light. Further, the gel was destained overnight in the buffer and observed in transmitted light on florescent screen and photographed.

3.10.6.3 Electrophoresis of sugarcane mosaic virus RNA in 2.6, 4 and 5 per cent polyacrylamide gels

The SCMV-RNA was also analysed in 5, 4 and 2.6 per cent polyacrylamide gels (5% cross linking), separately according to procedure outlined by Grierson (1982). For 2.6 per cent PAGE 5, 10 and 20 µg SCMV-RNA were loaded in separate tracks, while for 5 per cent PAGE 10 µg RNA, and for 4 per cent PAGE 10 µg and 20 g RNA were loaded in separate tracks. The electrophoresis conditions for 2.6 per cent PAGE were 50 volts, 38 mA, and 2.5 h; for 4 per cent PAGE they were 70V, 50 mA and 3.5 h; while for 5 per cent PAGE the 70 V, 40 mA and 2.5 h time duration was set.

After electrophoresis the gels were stained using silver nitrate staining procedure for RNA of Igloi (1983) which was slightly modified. The gels were kept for 60 min in 200 ml volume of fixative -I (50% methanol + 10% acetic acid) followed by overnight treatment to gel in 2nd change of 200 ml volume of the fixative-I. Next day, the gels were kept for 60 min in fixative-II (10% methanol + 1% acetic acid) and 2 changes of fixative (200 ml each) were given. The gels were further treated with 200 ml volume of 12 mM $AgNO_3$ solution for 60 min, followed by two brief (1 min) rinse with double distilled water, and treatment with 200 ml volume of developer solution (0.28% HCHO+ 0.75M KOH) until RNA bands and tracks became clear. The gel was rinsed for 10 min in 200 ml volume of 0.07M Na_2CO_3 solution and stored in 5 per cent acetic acid.

3.11 DIFFERENTIATION OF THE SUGARCANE MOSAIC VIRUS STRAINS

In order to investigate the distribution of SCMV strains in two different agroclimatic regions, sugarcane variety Co 671 was collected from south Gujarat and Coimbatore (Courtesy, Dr. K.C. Alexander, Sugarcane Breeding Institute, ICAR, Coimbatore-641007, Tamil Nadu) as it is one of the most popular variety. Also SCMV infected another variety Co 859 from Coimbatore was assayed to have a better appraisal of SCMV strains.

For assaying SCMV isolates, a set of 10 sorghum inbred lines were used as differentials for SCMV strains identification as suggested by Gillaspie and Mock (1984). In addition to these, sorghum lines Martin, Martin-40, Martin 5330, and QL-3-Tx and Johnsongrass (Sorghum halepense) obtained from Dr. J.M. deWet (Genetic Resource Unit, ICRISAT, Hyderabad-224016) were also used to differentiate the SCMV-strains.

These sorghum lines were grown in 10 cm diameter earthen pots filled with 3:1 mixture of soil and farm yard manure. For each inbred lines, two pots were sown at the rate of 10 seeds per pot. The pots were kept for germination of seeds in the environmental chamber 'Conviron' Model SlOH (Mfd. Control Environment Ltd., Winnipeg, Manitoba Canada). The temperature was set to 24°C during 18 h photoperiod of 19,333 lux, and 13°C during 6 h darkness, throughout the experiments. Whereas the seedlings in one of the pots were used for inoculation with SCMV, the other pots with 10 seedlings was for control.

The inoculum was prepared for each of the three SCMV infected sugarcane sample by grinding 3 g of SCMV infected leaf tissue

in 12 ml volume of 0.01 M potassium phosphate pH 7 buffer and sap was passed through double layer of cheese cloth. A pinch of the diatomaceous earth was added to the inoculum and sorghum lines were inoculated using index finger, at four leaf stage and the plants were again kept continuously in Conviron chamber under environmental conditions as specified earlier. For each sorghum lines buffer rub controls in the other pots were also kept under similar environmental conditions.

The final symptoms were rated after one month, for strainal identification on the basis of characteristic differential symptoms as indicated by Gillaspie and Mock (1984).

4. EXPERIMENTAL RESULTS

4.1 HOST REACTION STUDIES OF SUGARCANE MOSAIC VIRUS

The results clearly indicated that the sugarcane mosaic virus (SCMV) infected all the ten sorghum varieties and produced severe mosaic symptoms including CSH-9 (Plate 2) which is commercially grown in South Gujarat. None of the varieties of maize, rice and bajra tested became infected with the SCMV. The results of the host reaction studies are presented in Table 2.

The backassays of the symptomless host carried out proved that all of the six varieties of maize, three varieties of bajra and four varieties of rice were immune to SCMV strains collected from southern Gujarat (Table 3).

4.2 APHID TRANSMISSION EFFICIENCY OF THE SUGARCANE MOSAIC VIRUS

The results of studies on comparative transmission efficiency of SCMV by 3 species of aphids are represented in Table 4. Whereas the transmission of SCMV by apterous forms of *Aphis craccivora* (Koch) was 48 per cent, the transmission of SCMV by apterous forms of *Rhopalosiphum maydis* (Fitch.) and *Melanaphis sacchari* (Znht.) was 40 and 34 per cent, respectively. Although the *A. craccivora* (Koch) (Plates 3 and 4) had 8 per cent higher transmission rate of SCMV from sugarcane to CSH-9 sorghum, the colonization habit of *Rhopalosiphum maydis* (Fitch.) into the central whorl leaf of sorghum (Plates 5, 6 and 7) makes it a more important vector of SCMV. The transmission

Plate 2. The severe mosaic symptoms caused by SCMV on economically important sorghum variety CSH-9

PLATE-2

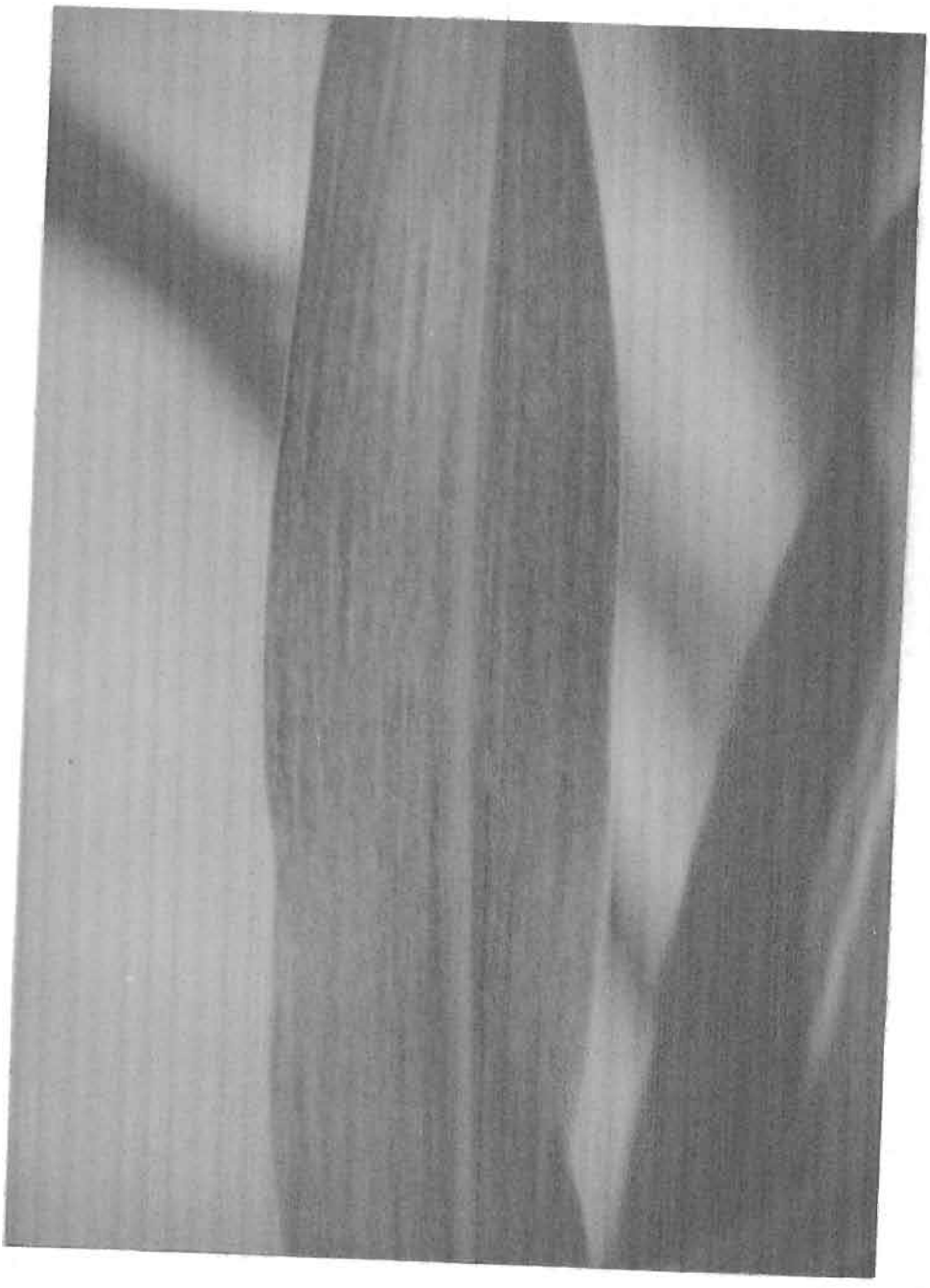


Table 2. Host reaction studies of sugarcane mosaic virus

Sl No.	Name of host	Symptoms
1.	*Zea mays* (L.) var. Ageti-76	NS*
2.	Bari local	NS
3.	Inbred line : CM-110	NS
4.	CM-600	NS
5.	var. Deccan-103	NS
6.	MDR-1	NS
7.	*Sorghum bicolor* (L.) Moench var. Surat-1	SM**
8.	M-234	SM
9.	GJ-35	SM
10.	GJ-37	SM
11.	BP-53	SM
12.	Tukadi	SM
13.	Desi white	SM
14.	Chhasathio	SM
15.	Solapuri	SM
16.	CSH-9	SM
17.	*Pennisetum americanum* (L.) Leek var. WCC-75	NS
18.	BK-560-280	NS
19.	MBH-110	NS
20.	*Oryza sativa* (L.) var. Saket-4	NS
21.	IR-8	NS
22.	TN-1	NS
23.	Jaya	NS

*NS = No symptoms; **SM = Severe mosaic

Table 3. Back assays of the symptomless hosts, which were initially inoculated with SCMV

S.No.	Back assayed host	Symptoms on CSH-9 sorghum
1.	*Zea mays* (L.) var. Ageti-76	NS*
2.	Bari local	NS
3.	Deccan-103	NS
4.	MDR-I	NS
5.	Inbred : CM-110	NS
6.	CM-600	NS
7.	*Pennisetum americanum* (L.) Leek. var. WCC-75	NS
8.	BK-560-280	NS
9.	MBH-110	NS
10.	*Oryza sativa* (L.) var. Saket-4	NS
11.	IR-8	NS
12.	TN-1	NS
13.	Jaya	NS

*NS = No symptoms

Plate 3. A colony of Aphis craccivora (Koch) on the cowpea plant

Plte 4. A wingless form of Aphis craccivora (Koch) on cowpea plant

PLATE-3

PLATE-4

Plate 5. The colony of Rhopalosiphum maidis (Fitch) in the central whorl leaf of CSH-9 sorghum

Plate 6. The closeup view of wingless form of R. maidis (Fitch.)

Plate 7. The winged adult form of R. maidis (Fitch.)

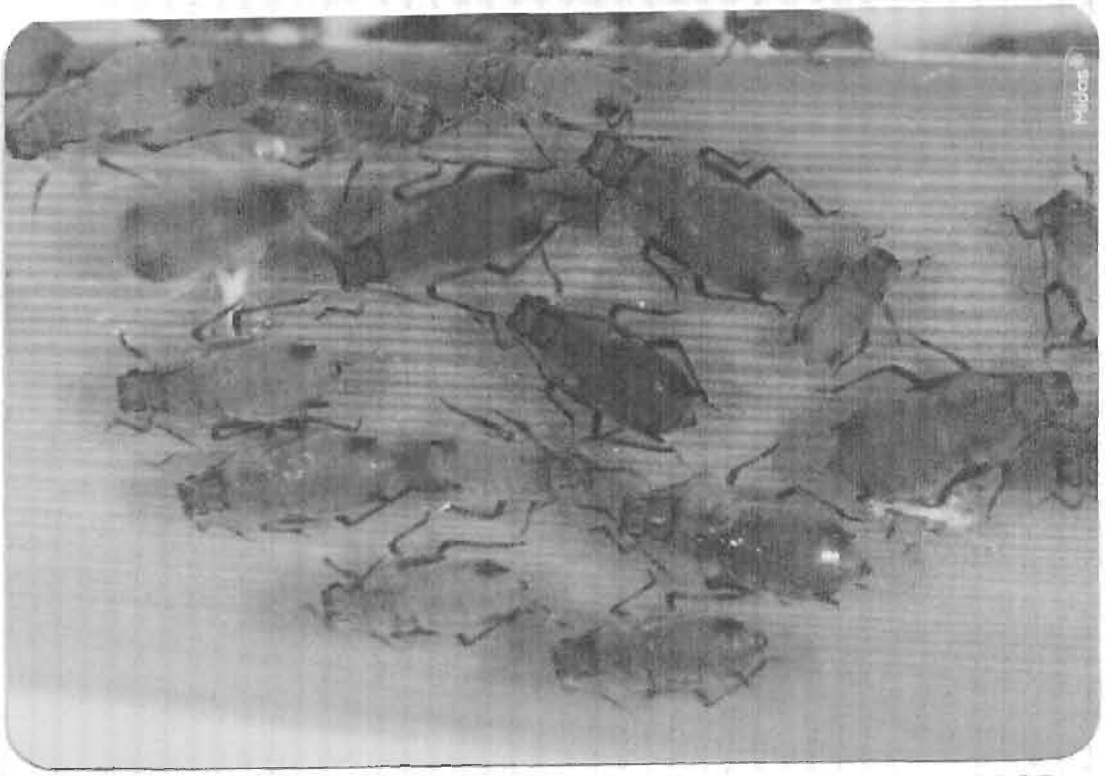


PLATE-5

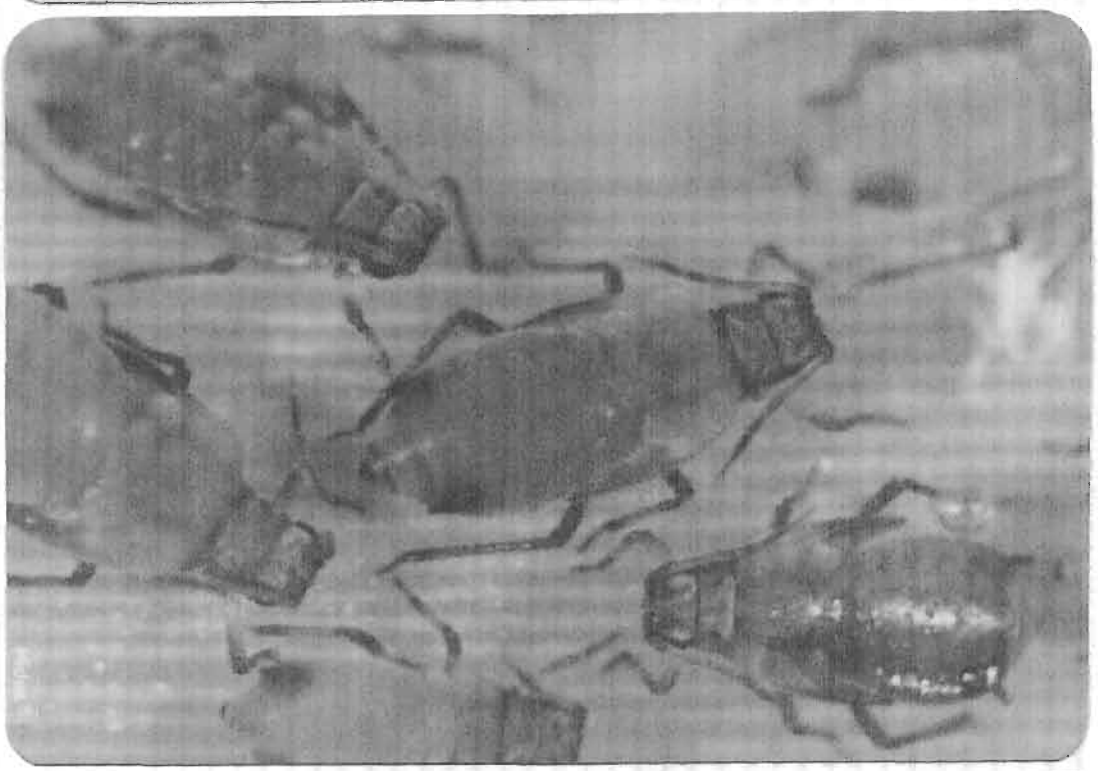


PLATE-6

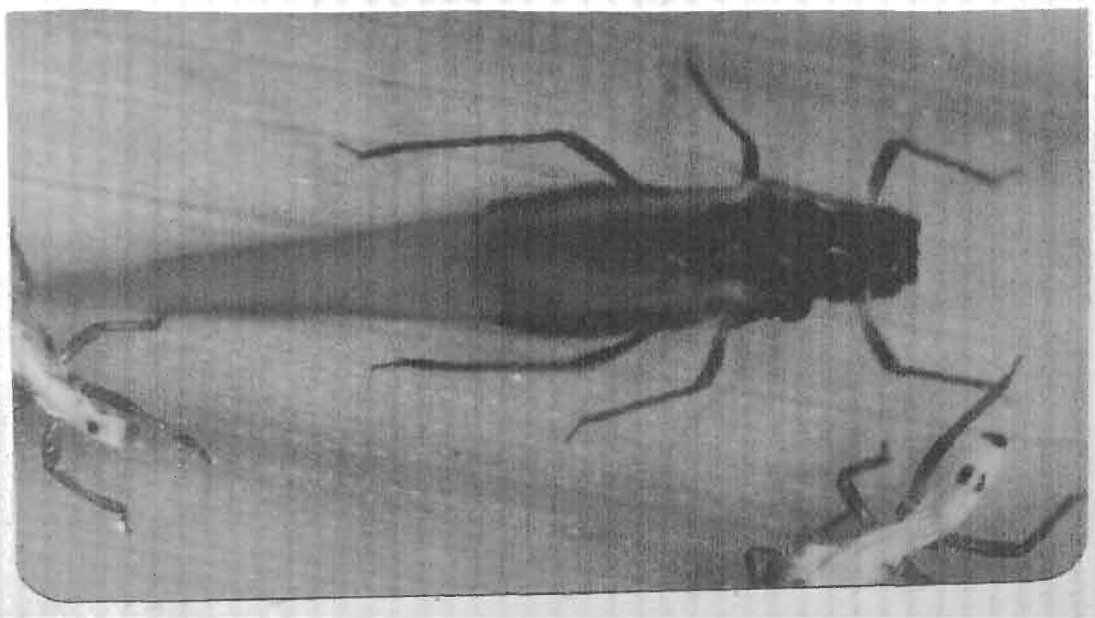


PLATE-7

Table 4. Transmission of sugarcane mosaic virus from infected Co 671 variety of sugarcane to CSH-9 sorghum by 3 different species of aphids

S.No.	Aphid species	% transmission of SCMV
1.	*Aphis craccivora* (Koch)	48
2.	*Rhopalosiphum maidis* (Fitch.)	40
3.	*Melanaphis sacchari* (Znht.)	34

rate of SCMV by the *Melanaphis sacchari* (Zhnt.) was comparatively lower than the other two aphid species tested.

4.3 PURIFICATION OF SUGARCANE MOSAIC VIRUS

As revealed by the column profiles, scanned at 254 nm, resulting from the density gradient centrifugation step in the purification procedures of Lagenberg (Procedure-I, Figure 14), Gough and Shukla (Procedure-II, Figure 15) and the modified procedure of Gough and Shukla (Procedure-III, Figure 16), no SCMV containing bands were detectable. However, the profile indicated only the prominant host component peaks in case of purification procedure-II and III. In the case of procedure-I, even the host component peak remained undetected. The modified SCMV purification procedure-IV (Rishi and Rishi, 1985) on the other hand resulted in the detection of both, host component and virus peaks as compared to the original procedure (Figures 17 and 18). However, their separation was not as distinct as in the case of scanning profile of the Cs_2SO_4 gradient column during the procedure-V (Figure 19). The second cycle of centrifugation in procedure-V when carried out on CsCl gradient column also gave a single band of SCMV (Figure 20).

The fractions showing virus peaks were collected and the virus was concentrated by high speed centrifugation. The yield of SCMV nucleoprotein in these preparation was estimated spectrophotometrically in terms of milligrams of purified virus nucleoprotein per kg of infected leaf tissues. The yield of the SCMV was 22.6 mg and 9.81 mg per kg of infected leaf tissue (Table 5) in the case of finally pelleted virus with procedures V and IV, respectively.

FIG. 14. SUCROSE GRADIENT COLUMN (10-40%), SCANNED AT 254 nm AFTER CENTRIFUGATION AT 23,000 RPM (X 67,500g) FOR 2 HOURS, USING LAGENBERG'S (1973) PROCEDURE OF SCMV PURIFICATION.

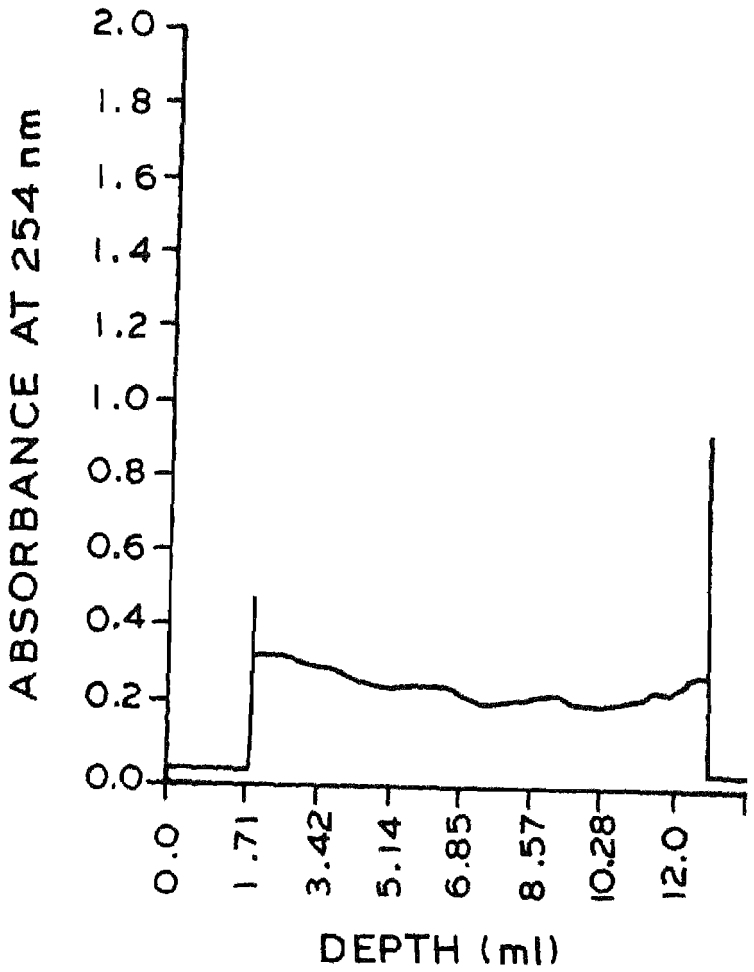


FIG.15. SUCROSE GRADIENT COLUMN (10-40%) SCANNED AT 254 nm AFTER CENTRIFUGATION AT 22,000 RPM (X60,000g) FOR 3 HOURS, USING GOUGH AND SHUKLA'S (1981) PROCEDURE OF SCMV PURIFICATION.

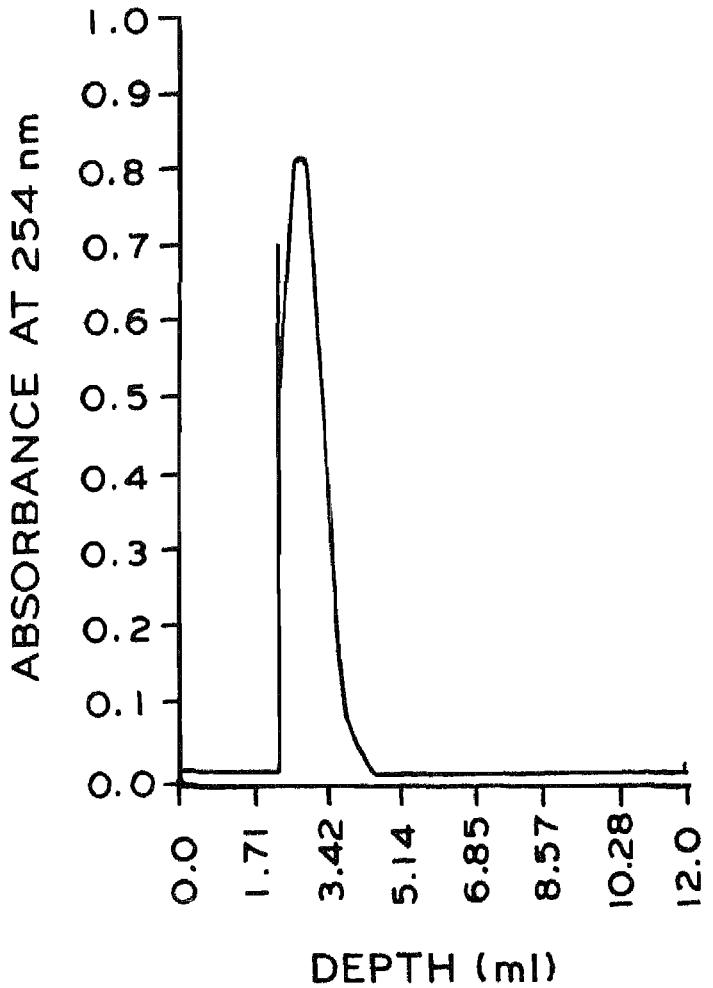


FIG. 16. SUCROSE GRADIENT COLUMN (10–40%) SCANNED AT 254 nm AFTER CENTRIFUGATION AT 22,000 RPM (X60,000g) FOR 3 HOURS, USING MODIFIED GOUGH AND SHUKLA'S (1981) PROCEDURE OF SCMV PURIFICATION.

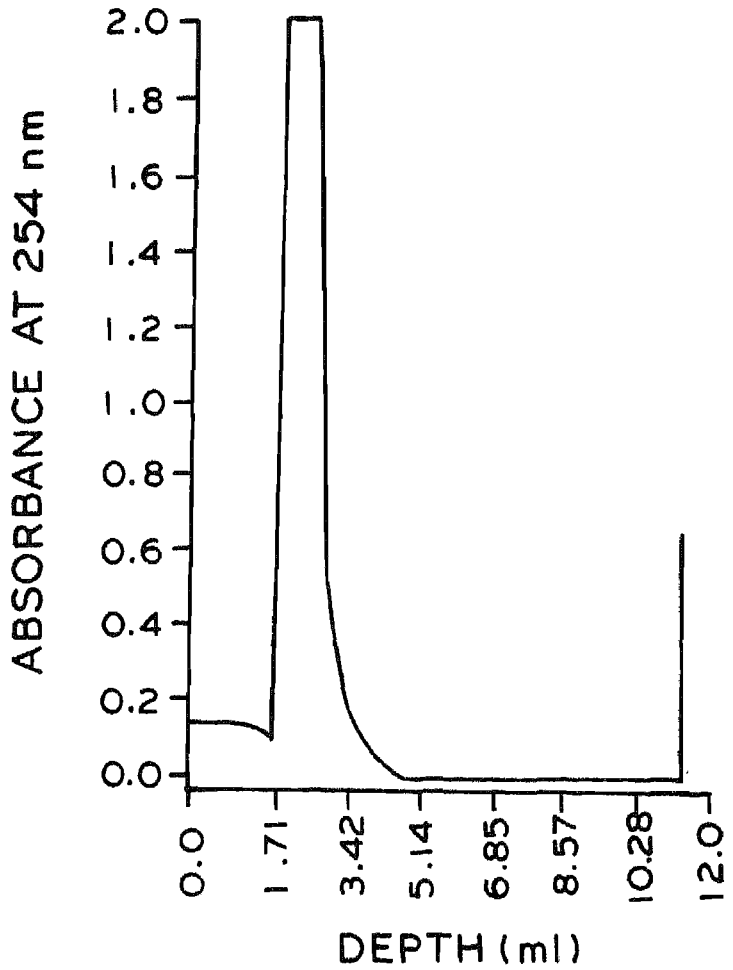


FIG.17. SUCROSE GRADIENT COLUMN (10–40 %) SCANNED AT 254 nm AFTER CENTRIFUGATION AT 25,000 RPM (X 79,700 g) FOR 2 HOURS, USING MODIFIED PROCEDURE OF RISHI AND RISHI (1985) FOR THE SCMV PURIFICATION.

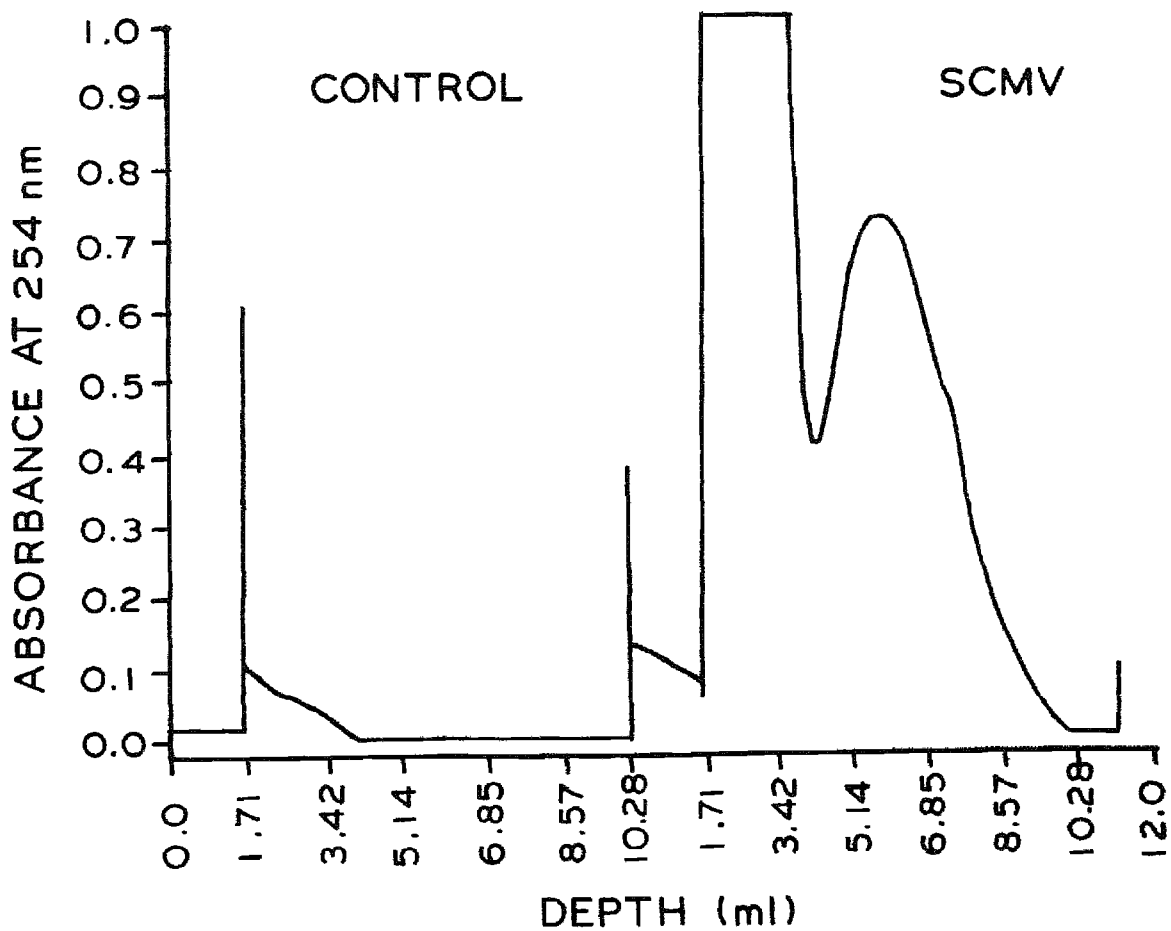


FIG.18. SUCROSE GRADIENT COLUMN (10–40%) SCANNED AT 254 nm AFTER CENTRIFUGATION AT 25,000 RPM (79,700g) FOR 2 HOURS, USING PROCEDURE OF RISHI AND RISHI (1985) FOR SCMV PURIFICATION.

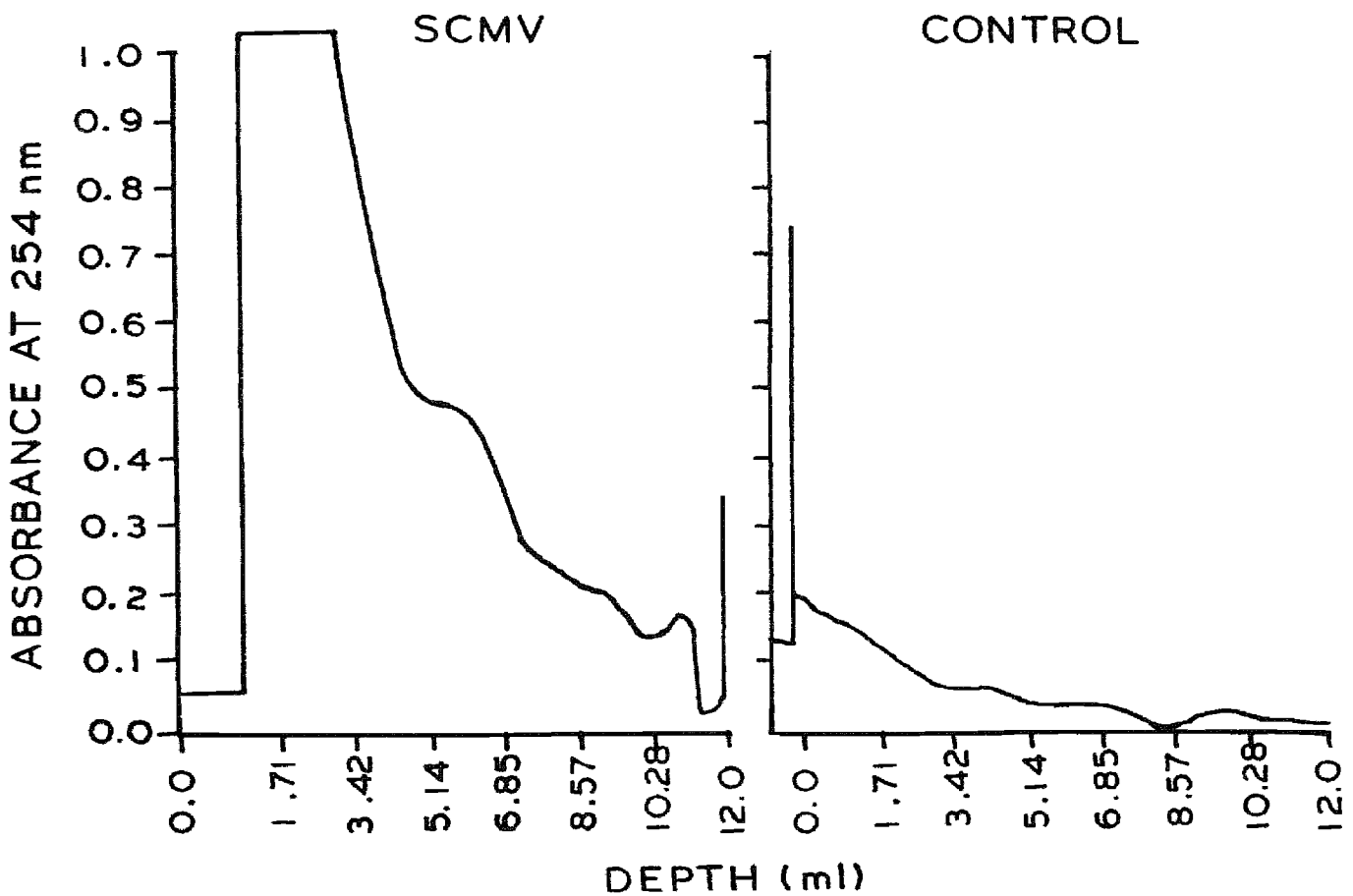


FIG.19. CESIUM SULPHATE GRADIENT (15–30 % IN 10% SUCROSE) COLUMN SCANNED AT 254 nm AFTER CENTRIFUGATION AT 40,000 RPM (X204,100g) FOR 2 HOURS.

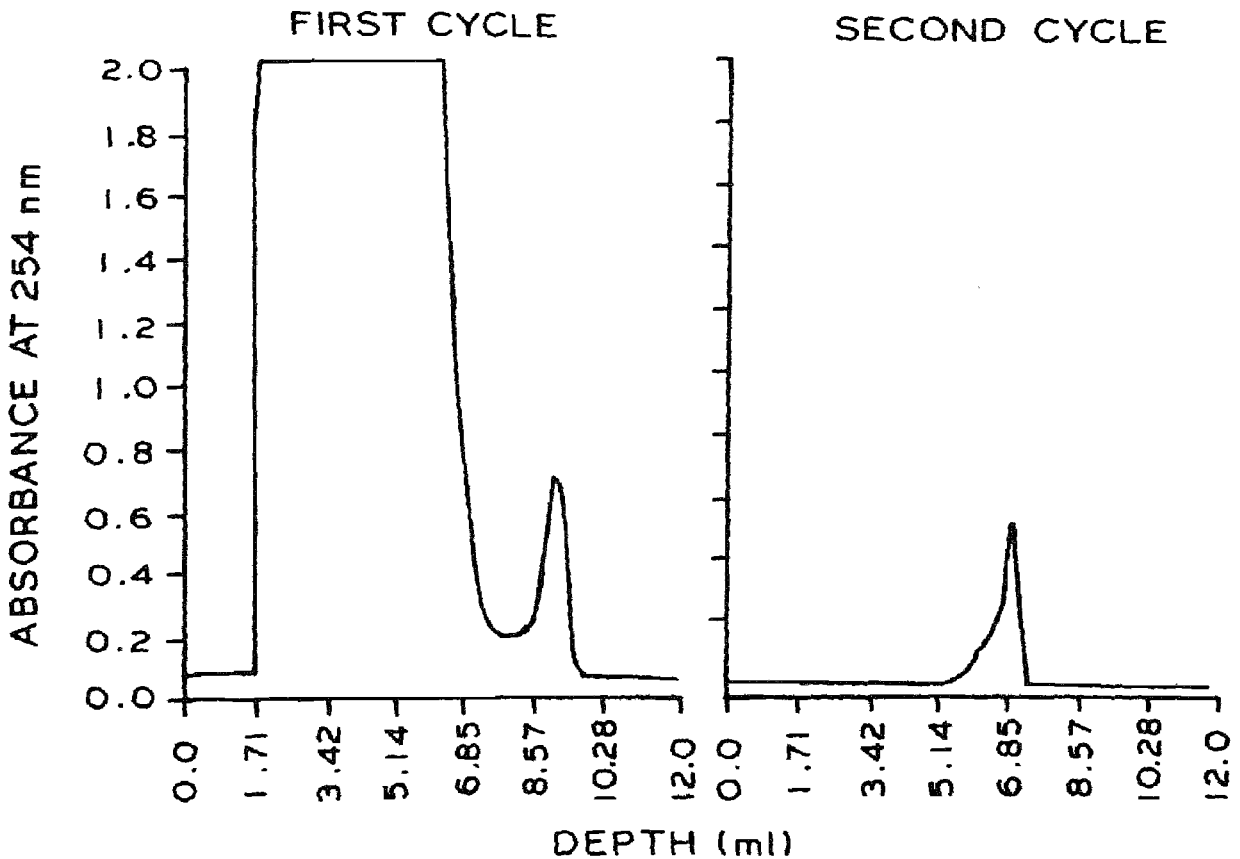


FIG. 20. I. CESIUM SULPHATE GRADIENT (15–30% IN 10% SUCROSE) COLUMN SCANNED AFTER CENTRIFUGATION AT 40,000 RPM (X204,100g) FOR 2 HOURS (1ST RUN) II. CESIUM CHLORIDE GRADIENTS (15–30% IN 15% SUCROSE) COLUMN CENTRIFUSED 2ND TIME AT 40,000 RPM (X204,100g) FOR 1 HOUR

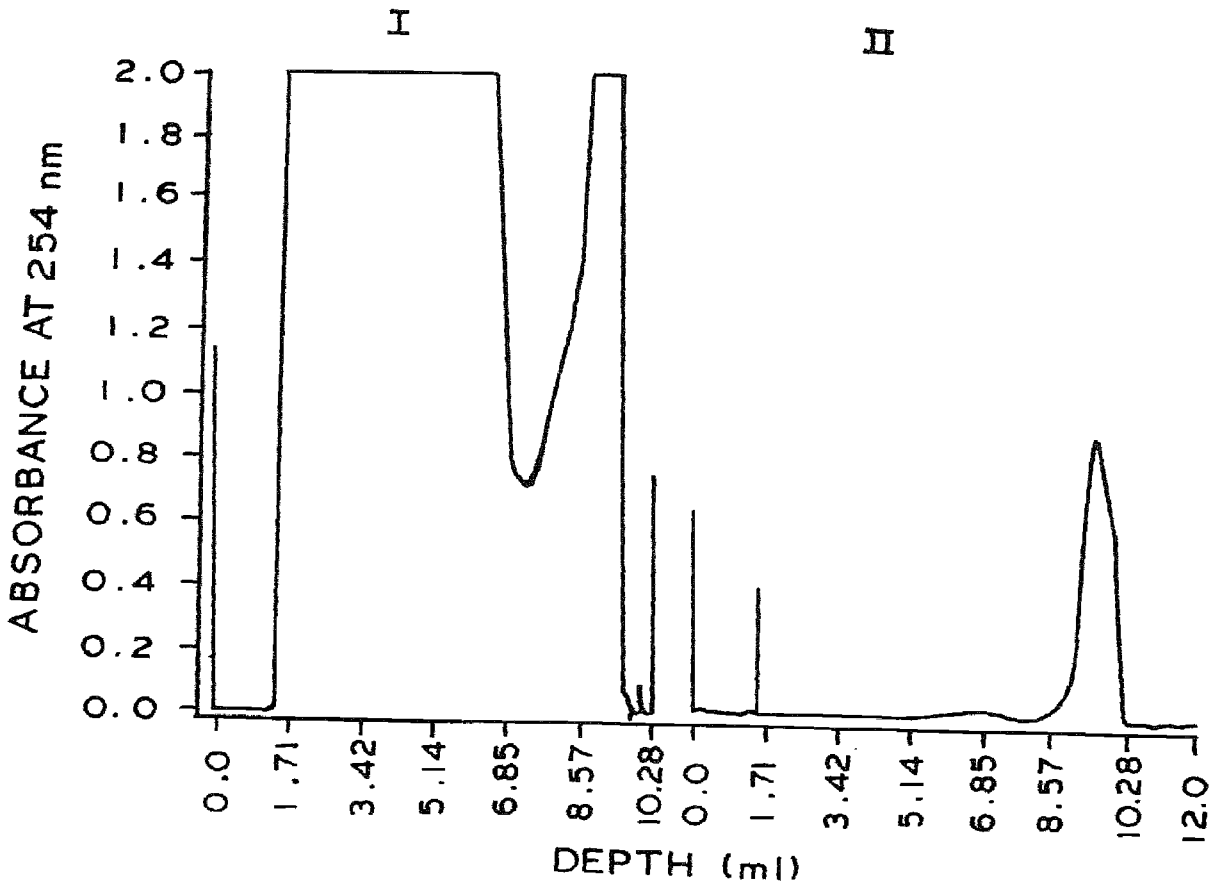


Table 5. Comparison of five different methods for the purification of SCMV

Sl No.	SCMV purification procedure	SCMV yield (mg/kg of tissue)	Column profile		Electron microscopic observations	Remarks
			Host component peak	Virus peak		
I)	Sucrose density gradient					
	1. Lagenberg (1973)	-	-	-	-	-
	2. Gough and Shukla (1981)	-	+	-	-	-
	3. Modified Gough & Shukla (1981)	-	+	-	-	-
	4. Modified Rishi & Rishi (1985)	9.81	+	+	In great numbers, the flexous rod shaped particles were observed	Sharp SCMV peak was observed at 6.8 ml depth, but was together with the host component peak
II)	Cesium sulphate density gradients					
	5. Procedure V	22.60	+	+	SCMV particles were observed in great numbers	Sharp peak of SCMV was observed at 9.0 ml depth in the gradient column

The purified preparation of SCMV when observed in JEOL-100 Cx-II transmission electron microscope, flexous rod shaped virus particles were observed (Plate 8) in great numbers.

4.4 RECOVERY OF SCMV FROM SUPERNATANT OF THE PEG PRECIPITATION STEP

The scan at 254 nm of the first cycle of Cs_2SO_4 density gradient centrifugation clearly indicated that considerable amount of SCMV from supernatant of PEG precipitation (Step-9 of procedure-V) was recovered using two cycles of Cs_2SO_4 gradient centrifugation (Figure 21). The host component associated with SCMV was also effectively separated after the second cycle of Cs_2SO_4 density gradient centrifugation.

4.5 SERODIAGNOSIS OF SUGARCANE MOSAIC VIRUS

4.5.1 **Determination of the titer of the SCMV**

4.5.1.1 Ring interface precipitin test

At 1:4 and 1:8 dilutions of SCMV infected Co 671 sugarcane leaf tissue, the SCMV antiserum reacted up to 1:512 dilution (Table 6), while no reaction was observed with 1:4 and 1:8 dilutions of healthy plant extract. At 1:16 dilution of SCMV infected Co 671 sugarcane leaf tissue the SCMV antiserum reacted from 1:4 to 1:64 dilutions. The titer of SCMV antiserum, therefore, was found to be 1:512 at 1:4 and 1:8 dilutions of the antigen, in ring interface precipitin test.

Plate 8. Electron micrograph of the purified SCMV particles stained with 2% Uranyl acetate (x28,000)

PLATE-8

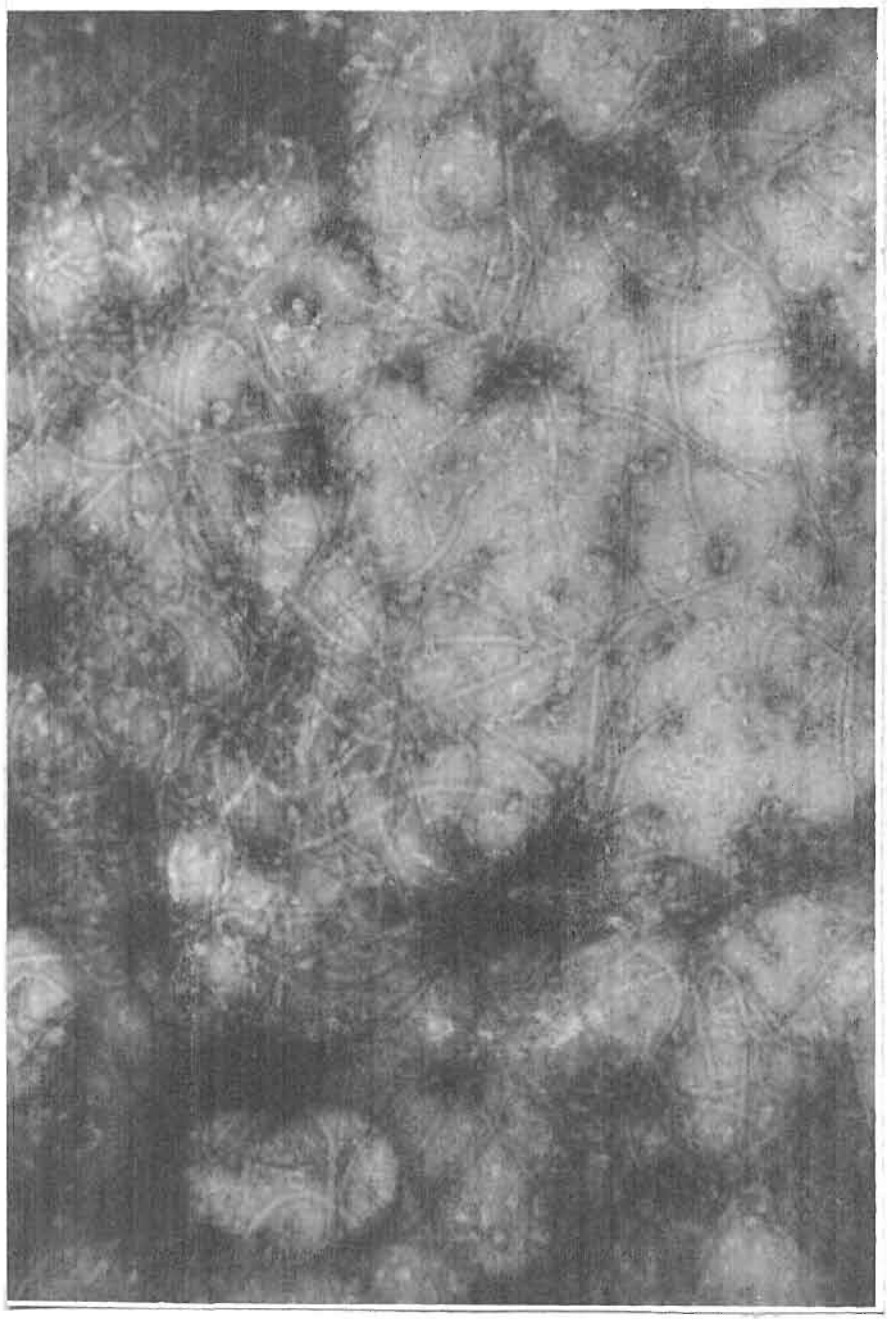


FIG.21. CESIUM SULPHATE GRADIENT (15-30% IN 10% SUCROSE) COLUMN SCANNED AT 254 nm, AFTER TWO CYCLES OF CENTRIFUGATION AT 40,000 RPM (X204,100g) FOR TWO HOURS, FOR RECOVERY OF SCMV FROM SUPERNATANT OF PEG PRECIPITATION STEP OF PROCEDURE-V.

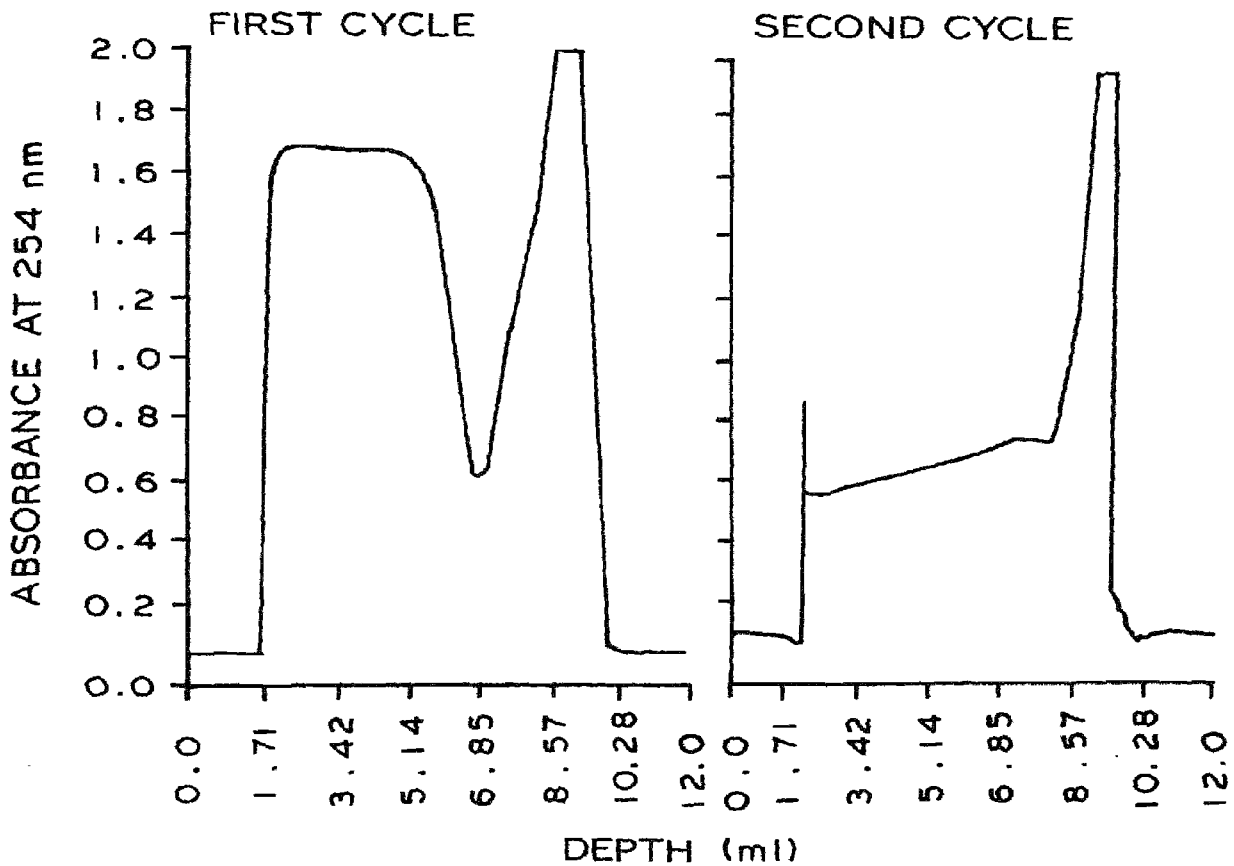


Table 6. Titer of the SCMV antiserum as determined by ring interface precipitin test

Antigens	Dilutions	SCMV antiserum dilutions								
		1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
Diseased extract	1:4	+	+	+	+	+	+	+	+	-
Healthy extract	1:4	-	-	-	-	-	-	-	-	-
Diseased extract	1:8	+	+	+	+	+	+	+	+	-
Healthy extract	1:8	-	-	-	-	-	-	-	-	-
Diseased extract	1:16	+	+	+	+	+	-	-	-	-
Healthy extract	1:16	-	-	-	-	-	-	-	-	-

+ = positive reaction;

- = no reaction

4.5.1.2 Tube precipitin test

The clarified leaf extract of SCMV infected Co 671 sugarcane variety, at the constant dilution of 1:16 gave positive precipitin reaction in the tube precipitin test from 1:16 to 1:512 dilution of the SCMV antiserum. The results are presented in Table 7. The titer of the SCMV antiserum therefore at 1:16 dilution of SCMV infected leaf extract of the Co 671 sugarcane variety was 1:512.

4.5.1.3 Microprecipitin test

The results of the microprecipitin test are presented in Table 8. At 1:16 and 1:32 dilution of SCMV infected leaf extract, the precipitation reaction was observed up to 1:1024 dilution of the antiserum. However, at 1:64, 1:128, and 1:256 dilutions of SCMV antiserum, on the other hand the positive reaction was observed only at the antigen dilution of 1:256, 1:32, and 1:16, respectively.

4.5.1.4 Immuno Electron Microscopy - Decoration method

The sugarcane mosaic virus was decorated with the SCMV antiserum dilutions of 1:2, 1:16, 1:32, 1:64, 1:128, 1:256 and 1:512 (Plates 9-15). However, antiserum dilution of 1:1024, 1:2048 and 1:4096 did not decorate the SCMV antigen particles. At 1:16 dilution of antiserum, the electron micrograph at the total magnification of x80,000 indicated a uniform decoration of SCMV particles with SCMV antibodies (Plate 10a).

Table 7. Determination of titer of SCMV antiserum using tube precipitin test

Antigens	Dilution of antigen	SCMV antiserum dilutions						
		1:16	1:32	1:64	1:128	1:256	1:512	1:1024
Diseased extract	1:16	+	+	+	+	+	+	-
Healthy extract	1:16	-	-	-	-	-	-	-

+ = reaction positive

- = no reaction

Table 8. Titer determination of SCMV antiserum by microprecipitin test

Antigens	Dilution of antigen	SCMV antiserum dilutions						
		1:16	1:32	1:64	1:128	1:256	1:512	1:1024
Diseased extract	1:16	++++	++++	+++	+++	+++	++	++
	1:32	+++	+++	++	++	+	+	+
	1:64	++	++	+	+	+	-	-
	1:128	+	+	-	-	-	-	-
	1:256	+	-	-	-	-	-	-
Healthy extract	1:64	-	-	-	-	-	-	-
PBS control	-	-	-	-	-	-	-	-

+++ = very heavy, +++ = heavy, ++ = moderate, + slight, - = no reaction

Plate 9. Immuno Electron Microscopy - decoration of SCMV-Gujarat isolate at 1:2 dilution of the SCMV antiserum (x 55,000)

Plate 10. IEM-decoration of SCMV particle with 1:16 dilution of the SCMV antiserum (x 55,000)

PLATE-9

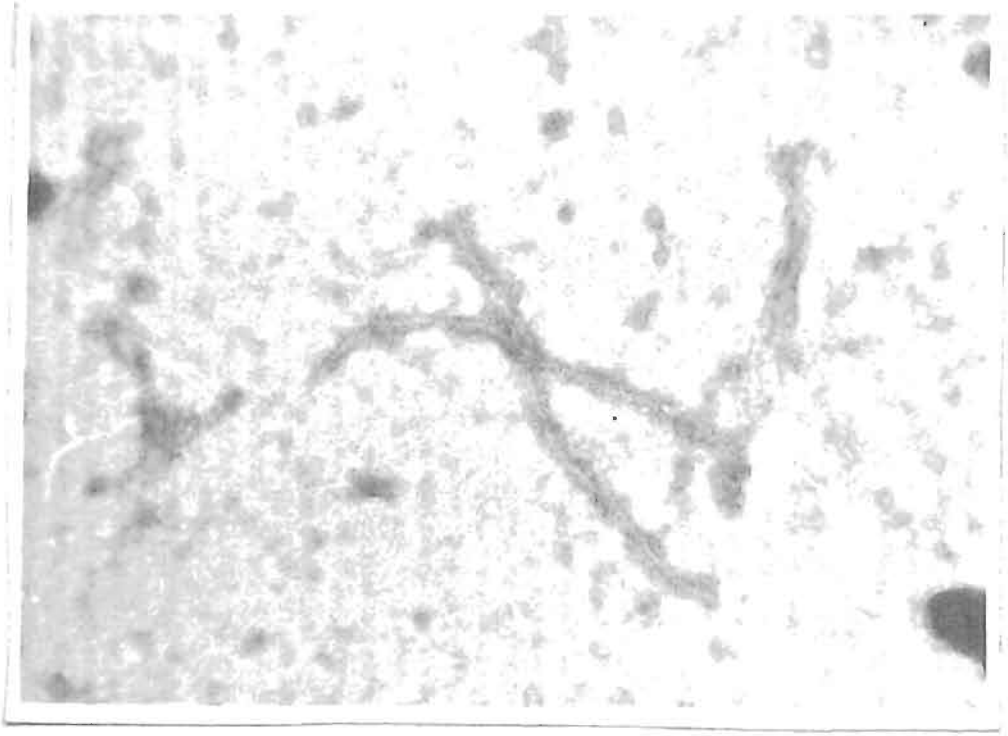


PLATE-10

Plate 10a. IEM of a SCMV particle indicating uniform decoration at 1:16 dilution of antiserum (x 80,000)

Plate 11. IEM-decoration of SCMV with 1:32 dilution of the SCMV antiserum (x 55,000)

PLATE-10a

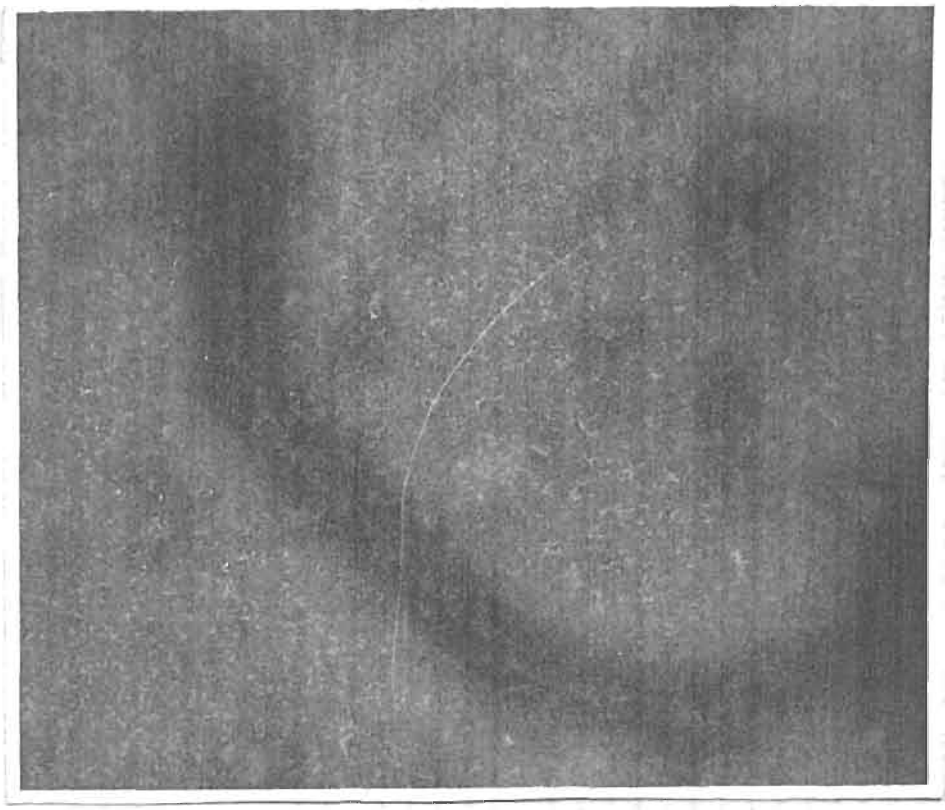


PLATE-11

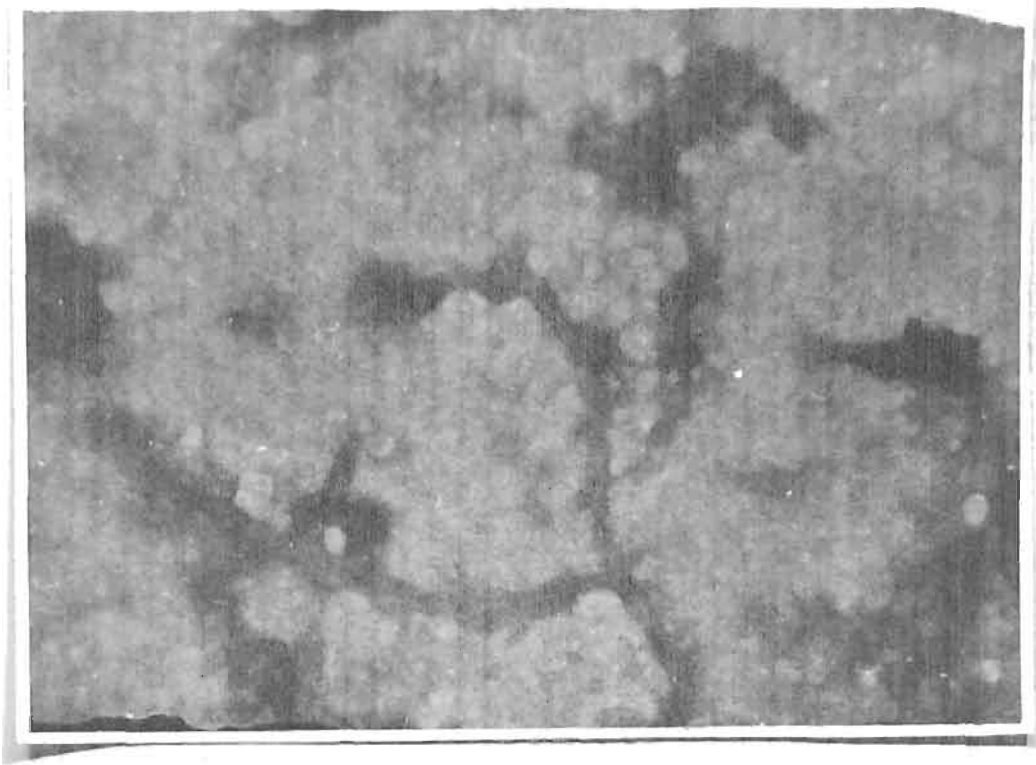


Plate 12. IEM-decoration of SCMV with 1:64 dilution of the SCMV antiserum (x 55,000)

Plate 13. IEM-decoration of SCMV with 1:128 dilution of SCMV antiserum (x 55,000)

PLATE-12

PLATE-13

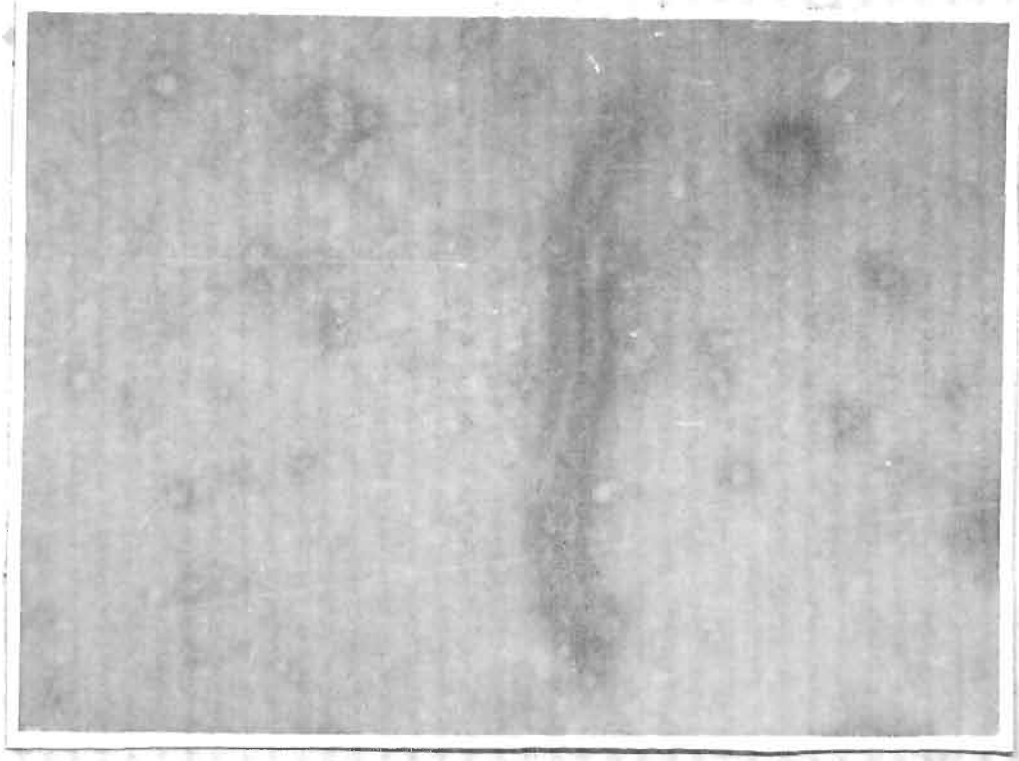


Plate 14. IEM-decoration of SCMV with 1:256 dilution of the SCMV antiserum (x 26,500)

Plate 15. IEM-decoration of SCMV with 1:512 dilution of the SCMV antiserum (x 55,000)

PLATE-14

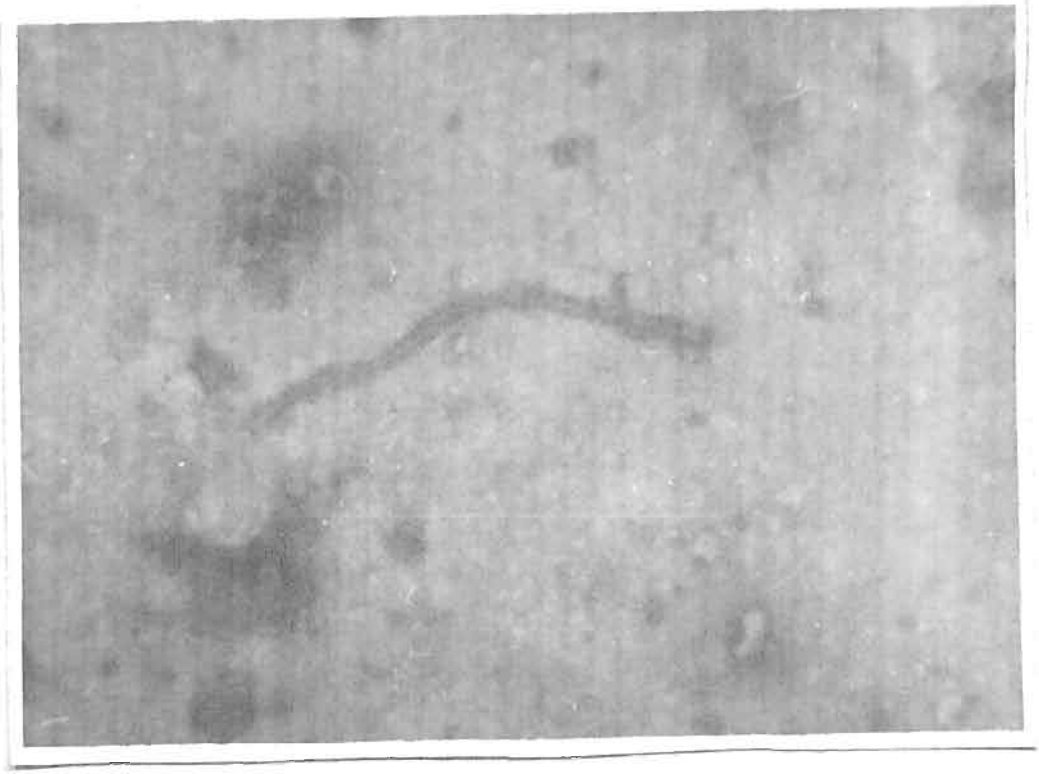


PLATE-15

4.5.2 Double antibody sandwich enzyme linked immunosorbent assay

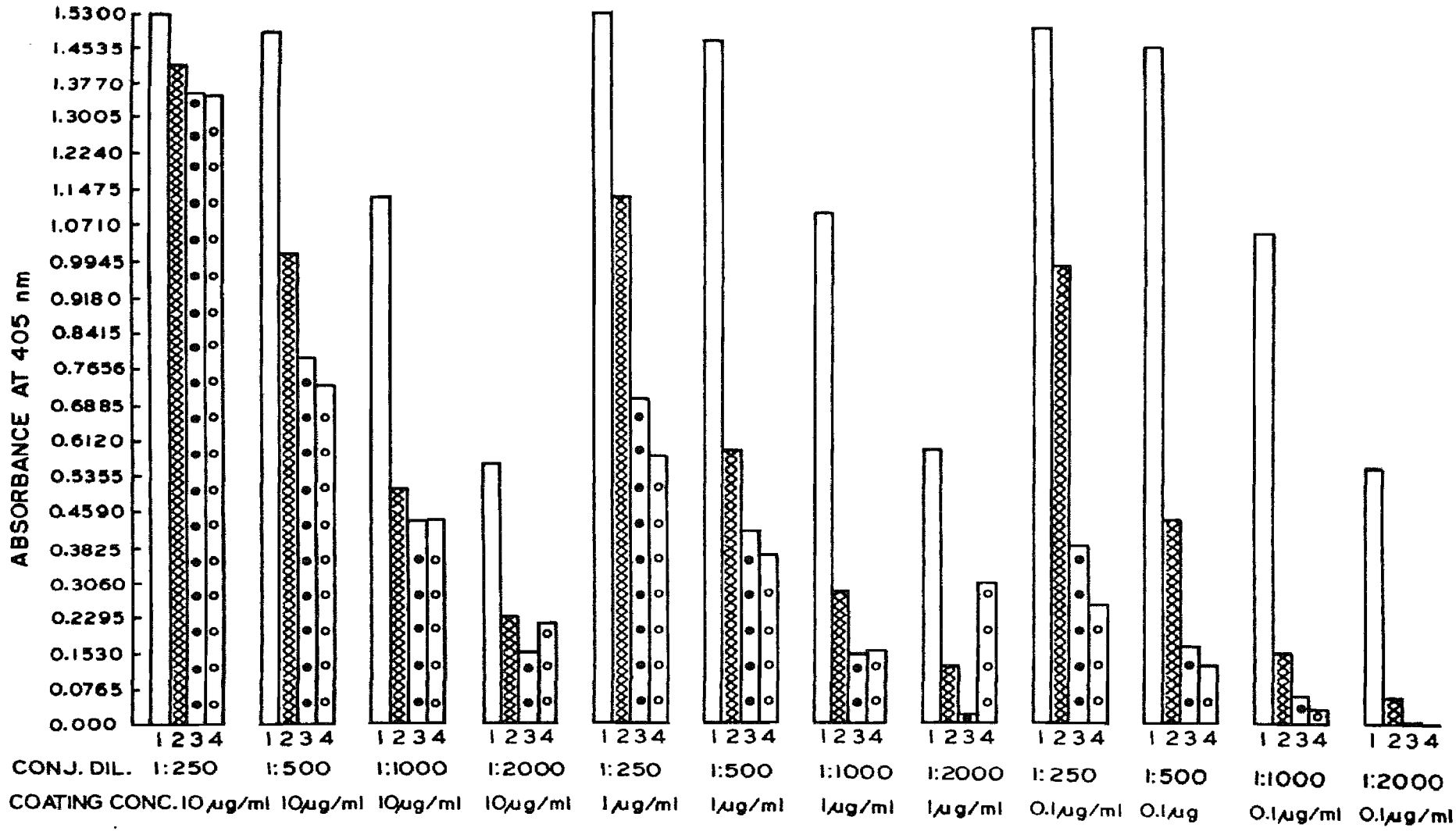
4.5.2.1 Optimum coating concentration and conjugate dilution for ELISA

At 1:250 dilution of SCMV enzyme-antibody conjugate and 10 µg/ml concentration of coating antibodies, higher absorbance values at 405 nm were observed for 1:100 and 1:1000 dilution of SCMV infected sap of Co 671 sugarcane leaf and also for PBS-Tween pH 7.4 (PBS-T) buffer controls; as compared to 1 and 0.1 µg coating concentration tried (Figure 22). Similar trend was observed with 1:500, 1:1000, and 1:2000 dilutions of conjugate, using 10, 1 and 0.1 µg/ml coating antibodies, and 1:100 and 1:1000 dilution of the Co 671 diseased sugarcane plant sap. Zero absorbance at 405 nm with PBS-Tween control was observed at 0.1 µg/ml coating combined with 1:2000 dilution of conjugate. Therefore, for further work with ELISA to detect SCMV, 0.1 µg/ml coating of SCMV antibodies and 1:2000 dilution of SCMV antibodies-enzyme conjugate was considered optimum.

4.5.2.2 Effect of blocking, on the performance of ELISA for SCMV detection

Blocking has significantly reduced the mean absorbance value (A405 nm) particularly in the samples from diseased plants, but not in the apparently healthy plants, and PBS-Tween controls. On the other hand, cross-absorption of conjugate with sap from apparently healthy sugarcane plant have considerably reduced the mean absorbance values in the treatments with diseased and apparently healthy (Table 9). The blocking and cross-absorption together has reduced the mean A405 nm

FIG. 22. OPTIMIZATION OF COATING CONCENTRATION OF SCMV-ANTIBODIES & CONJUGATE DILUTION FOR DETECTION OF SCMV, BY ELISA.



NOTE: 1= DISEASED 1:10, 2=1:100, 3=1:1000, 4= PBS-TWEEN pH 7.4

Table 9. Effect of blocking, on the performance of ELISA for SCMV detection

Sl No.	Treatment	Blocking	Cross-absorption on conjugate	Mean A405 nm
1.	Diseased (1:10)	No	No	1.035
2.	Unknown (1:10)	No	No	0.920
3.	PBS-Tween ph 7.4	No	No	0.130
4.	Diseased (1:10)	No	Yes	0.0975
5.	Unknown (1:10)	No	Yes	0.010
6.	PBS-Tween pH 7.4	No	Yes	0.000
7.	Diseased (1:10)	Yes	No	0.785
8.	Unknown (1:10)	Yes	No	0.830
9.	PBS-Tween pH 7.4	Yes	No	0.035
10.	Diseased (1:10)	Yes	Yes	0.080
11.	Unknown (1:10)	Yes	Yes	0.080
12.	PBS-Tween pH 7.4	Yes	Yes	0.000

CD at 5% = 0.132

Treatment No.	1	2	8	7	3	4	10&11	9	5	6&12
Mean A405 nm	1.035	0.920	0.830	0.785	0.130	0.097	0.080	0.035	0.01	0.000

Note: Treatments underlined with same bar are not significantly different from each other

to zero level in case of PBS-T control and significantly reduced the mean A405 nm in diseased and apparently healthy samples.

4.5.2.3 Estimation of SCMV titer in different plant parts of infected Co 671 sugarcane and CSH-9 sorghum

The absorbance values at 405 nm of the extracted sap from leaves, stem and root tissue of SCMV infected sugarcane plants as detailed in Tables 10, 11 and 12 were significantly higher at all the sap dilutions compared to sorghum tissues, indicating higher titer of SCMV. From amongst the three tissues tested for detection of SCMV titer, root tissues specifically had the highest absorbance compared to leaf and stem.

In the case of sorghum the SCMV titer in the leaves, stem and root tissues were compared using the corresponding tissue of healthy sorghum plants as controls. The absorbance values at 405 nm in the sap from leaf, stem and root tissues of healthy sorghum plants were almost at zero level as in the case of PBS-Tween control at the dilutions of 10^{-2} and 10^{-3}. In diseased sorghum plants the SCMV concentration was highest in roots, followed by leaves, and no detection in stem tissue at 10^{-2} dilution of the sap.

4.6 ELECTRON MICROSCOPY TO DETERMINE THE SIZE AND MORPHOLOGY OF THE PURIFIED SUGARCANE MOSAIC VIRUS

The SCMV particles in electron micrographs (Plate 16) appeared as long, flexous, intertwined rods. These particles measured from 600 to 880 nm long and 15.4 nm in width.

The modal length was recorded from the electron micrographs showing up distinct separate particles. From counts of 100 such particles the modal length was found to be 750 nm (Figure 23).

Plate 16. Electron micrograph of fairly well separated long flexous SCMV particles for the measurement of length, width and modal length, stained with 2% uranyl acetate. (x42,000)

PLATE-16

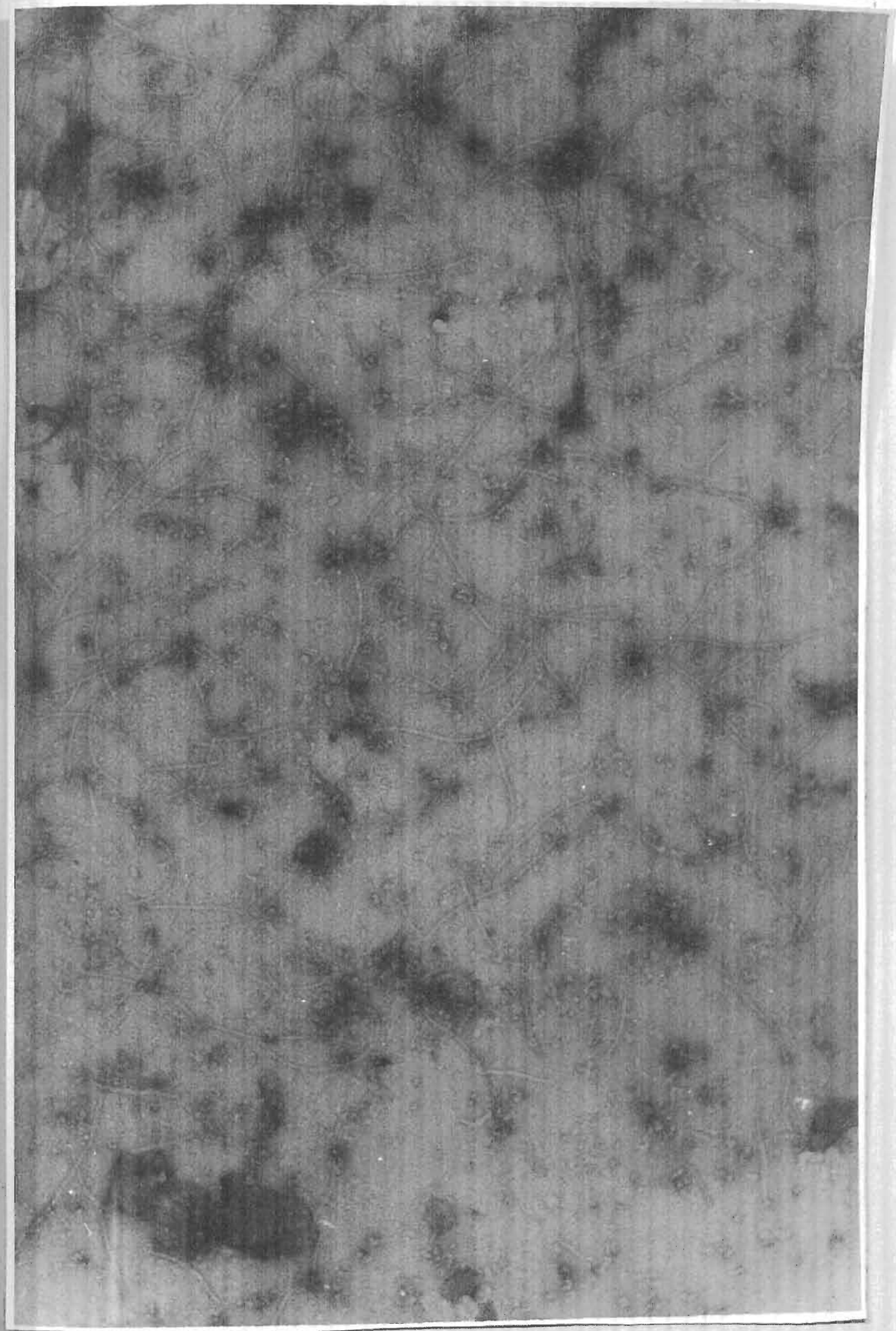


FIG. 23. HISTOGRAMS OF SCMV PARTICLE LENGTH AFTER PURIFICATION. THE INTERVALS OF GROUPS (RECTANGLES) IN 10 nm.

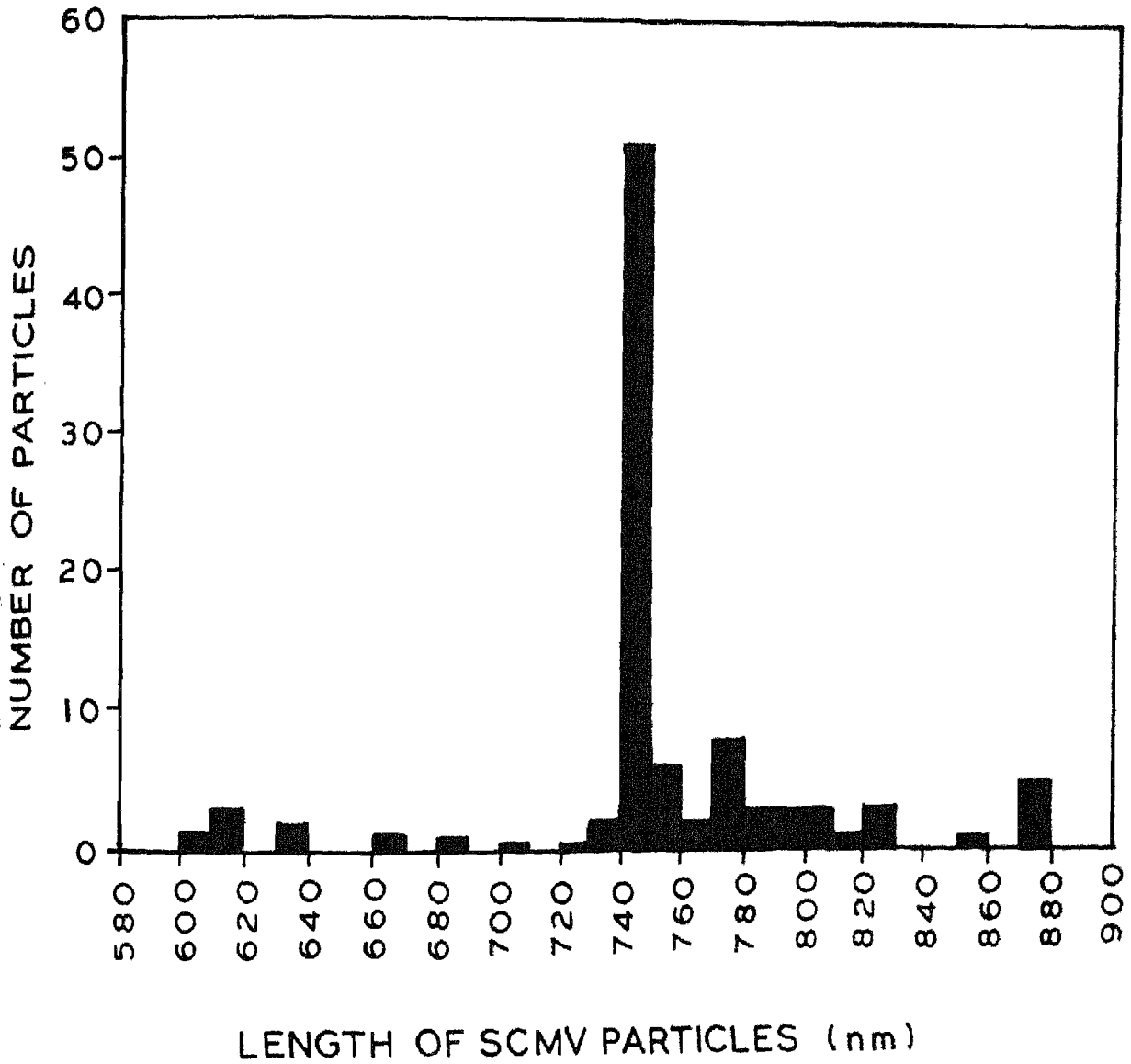


Table 10. Determination of SCMV titers in leaf, stem and root tissue of infected Co 671 sugarcane and CSH-9 sorghum plants by ELISA

Sl No.	Treatment	Dilution of sap used	Mean A405 nm
1.	SCMV infected sorghum leaves	10^{-1}	1.2000
2.	SCMV infected sorghum stems	10^{-1}	0.6725
3.	SCMV infected sorghum roots	10^{-1}	0.4650
4.	Healthy sorghum leaves	10^{-1}	0.8250
5.	Healthy sorghum stems	10^{-1}	0.2375
6.	Healthy sorghum roots	10^{-1}	0.3325
7.	SCMV infected sugarcane leaves	10^{-1}	1.1600
8.	SCMV infected sugarcane stems	10^{-1}	1.1775
9.	SCMV infected sugarcane roots	10^{-1}	1.2025
10.	PBS-Tween pH 7.4, control	-	0.0500

CD (5%) = 0.04425

Treatment No.	9	1	8	7	4	2	3	6	5	10
Mean A405 nm	1.2025	1.200	1.1775	1.16	0.825	0.6725	0.4650	0.3325	0.2375	0.050

Note: Treatments underlined with the same line are not significantly different from each other

Table 11. Determination of SCMV titers in leaf, stem and root tissue of SCMV infected sorghum and sugarcane plants by ELISA

Sl No.	Treatment	Dilution of sap used	Mean A405 nm
1.	SCMV infected sorghum leaves	10^{-2}	0.205
2.	SCMV infected sorghum stems	10^{-2}	0.095
3.	SCMV infected sorghum roots	10^{-2}	0.525
4.	Healthy sorghum leaves	10^{-2}	0.007
5.	Healthy sorghum stems	10^{-2}	0.000
6.	Healthy sorghum roots	10^{-2}	0.000
7.	SCMV infected sugarcane leaves	10^{-2}	0.383
8.	SCMV infected sugarcane stems	10^{-2}	0.400
9.	SCMV infected sugarcane roots	10^{-2}	1.040
10.	PBS-Tween pH 7.4, Control	-	0.050

CD (5%) = 0.066

Treatment No.	9	3	8	7	1	2	10	4	5&6
Mean A405 nm	1.040	0.525	0.400	0.383	0.205	0.095	0.050	0.007	0.00

Note: Treatments underlined with the same line are not significantly different from each other

Table 12. Determination of SCMV titer in leaf, stem and root tissue of infected Co 671 sugarcane and CSH-9 sorghum plants by ELISA

Sl No.	Treatment	Dilution of sap used	Mean A405 nm
1.	SCMV infected sorghum leaves	10^{-3}	0.0525
2.	SCMV infected sorghum stems	10^{-3}	0.0250
3.	SCMV infected sorghum roots	10^{-3}	0.0700
4.	Healthy sorghum leaves	10^{-3}	0.0000
5.	Healthy sorghum stems	10^{-3}	0.0000
6.	Healthy sorghum roots	10^{-3}	0.0000
7.	SCMV infected sugarcane leaves	10^{-3}	0.1675
8.	SCMV infected sugarcane stems	10^{-3}	0.2475
9.	SCMV infected sugarcane roots	10^{-3}	0.8450
10.	PBS-Tween pH 7.4, Control	-	0.0500

CD (5%) = 0.0381

Treatment No.	9	8	7	3	1	10	2	4,5&6
Mean A405 nm	0.485	0.2475	0.1675	0.07	0.0525	0.05	0.250	0.000

Note: Treatments underlined with the same line are not significantly different from each other

4.7 PHYSICOCHEMICAL PROPERTIES OF THE SUGARCANE MOSAIC VIRUS

4.7.1 Ultraviolet absorbance profile of the purified SCMV

The absorbance value of SCMV purified preparations ranging from 225 to 300 nm are given in Table 13 alongwith their respective corrected values for light scattering. The absorbance spectrum of purified SCMV preparation (Figure 24) indicated maximum absorbance at 260 nm (0.111) and minimum at 250 nm (0.103), with a little bump at 280 nm which is characteristic of nucleoprotein. The ratio of absorbance at 260/280 nanometers of purified SCMV was 1.18, however, due to light scattering these figures were not realistic and 260/280 absorbance ratio was much higher i.e. 1.329.

4.7.2 Determination of RNA per cent of SCMV

4.7.2.1 Spectrophotometric determination of percentage of SCMV-RNA from purified SCMV preparation

The corrected absorption (Noordam, 1973) of purified SCMV (1:10 dilution) at 260 and 280 nm were recorded as 0.111 and 0.094, respectively. The ratio of absorbance at 280/260 nanometers was calculated to be 0.846 and from Table 14 (Layne, 1957) the RNA% of SCMV was directly read and was found to be 5.5 per cent.

4.7.2.2 Determination of percentage RNA in purified SCMV, using quadratic regression line relationships

The percentage of nucleic acid contents of SCMV was calculated from the corrected absorbance values at 260 and 280 nm, which were 0.111 and 0.094, respectively. These values were substituted in the following equation given by Gibbs and Harrison (1976).

FIG. 24. ULTRAVIOLET PROFILE OF PURIFIED SUGARCANE MOSAIC VIRUS.

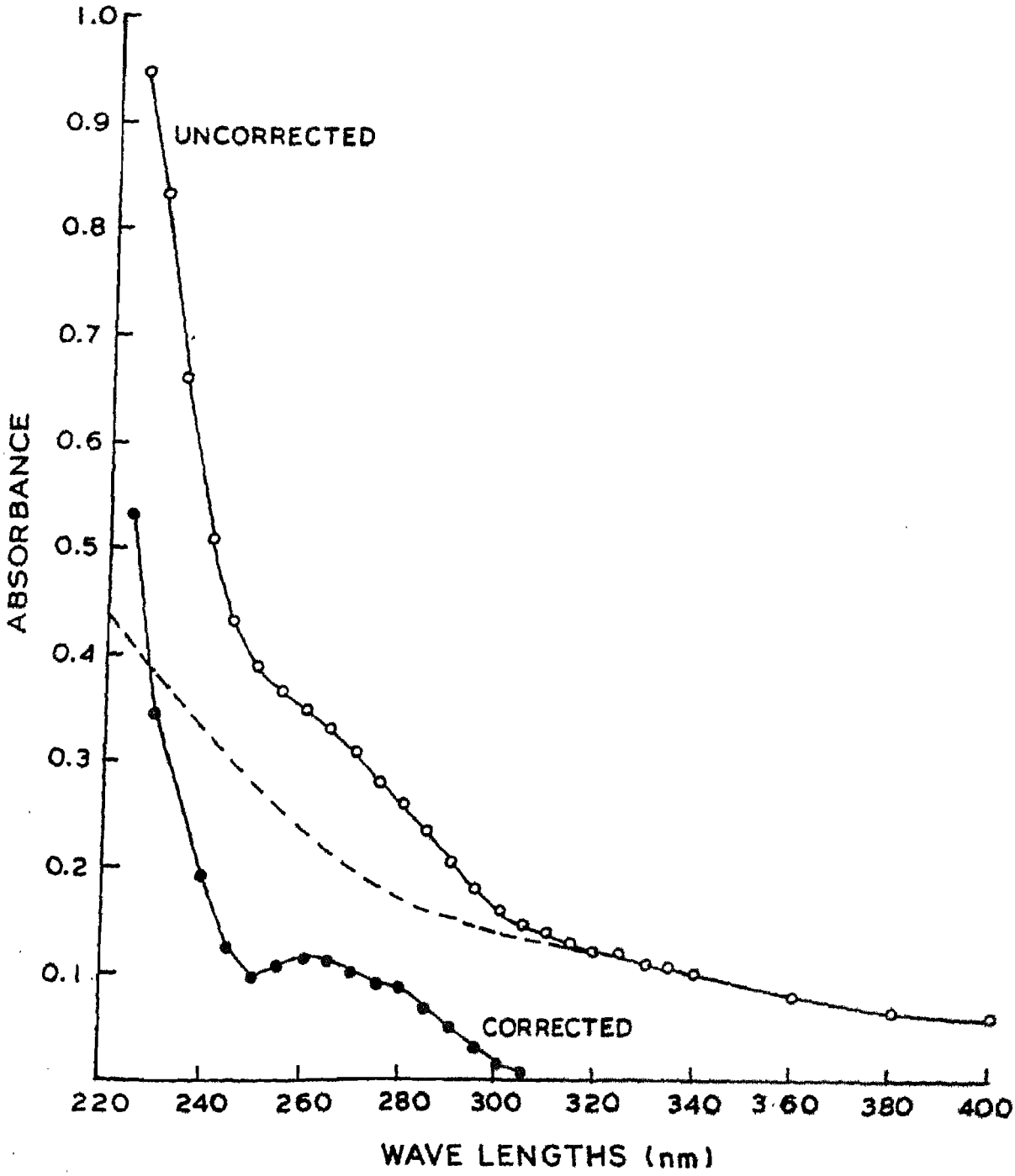


Table 13. Ultraviolet absorption spectrum of purified SCMV preparation

Wavelength (nm)	Absorbance	
	Uncorrected	Corrected
225	0.942	0.532
230	0.832	0.347
235	0.660	0.280
240	0.511	0.181
245	0.435	0.125
250	0.388	0.103
255	0.365	0.115
260	0.351	0.111
265	0.330	0.110
270	0.311	0.111
275	0.284	0.099
280	0.264	0.094
285	0.235	0.070
290	0.207	0.052
295	0.181	0.031
300	0.161	0.021

Table 14. Relationship of nucleic acid percentage with ratio of absorbance at 280/260 nm, of the nucleo protein preparation

A280/A260[a]	% Nucleic acid	F[b]
1.75	0.00	1.116
1.63	0.25	1.081
1.52	0.50	1.054
1.40	0.75	1.023
1.36	1.00	0.994
1.30	1.25	0.970
1.25	1.50	0.944
1.16	2.00	0.899
1.09	2.50	0.852
1.03	3.00	0.814
0.979	3.50	0.776
0.939	4.00	0.743
0.874	5.00	0.682
0.846	5.50	0.656
0.822	6.00	0.632
0.804	6.50	0.607
0.784	7.00	0.585
0.767	7.50	0.565
0.753	8.00	0.545
0.730	9.00	0.508
0.705	10.00	0.478
0.671	12.00	0.422
0.644	14.00	0.377
0.615	17.00	0.322
0.595	20.00	0.278

a. Ratio of optical density at 280 nm to optical density at 260 nm

b. Protein concentration (mg/ml) = $F.\frac{1}{D}$. A280

where, D is cuvette width centimeters, and

A280 is the optical density at 280 nm

From: Layne (1957)

$$\frac{A\ 260}{A\ 280} = 0.9320 + 0.0454\ (RNA\%) - 0.0006\ (RNA\%)^2$$

$$\frac{0.111}{0.094} = 0.9320 + 0.0454\ (RNA\%) - 0.0006\ (RNA\%)^2$$

$$1.1808 - 0.9320 = 0.0454\ (RNA\%) - 0.0006\ (RNA\%)^2$$

$$0.2488 = 0.0448\ (RNA\%)$$

$$\frac{0.2488}{0.0448} = RNA\%$$

5.5 = RNA% of SCMV

The SCMV has 5.5 per cent RNA according to this procedure.

4.7.3 Determination of 'Extinction Coefficient' of the sugarcane mosaic virus

The RNA% of SCMV determined as 5.5 per cent in section 4.7.2 of this chapter was taken as a basis and was used to further determine the 'Extinction Coefficient' of SCMV using the following relationship (Gibbs and Harrison, 1976).

$$(E\ 260_{1\ cm}^{0.1\%}) = 1.531 + 0.205\ (RNA\%)$$

$$= 1.531 + 0.205\ (5.5)$$

$$= 1.531 + 1.1275$$

$$= 2.658$$

Thus the extinction coefficient ($E\ 260_{1\ cm}^{0.1\%}$) of SCMV was found to be 2.658.

4.7.4 Determination of 'Sedimentation Coefficient' of the sugarcane mosaic virus

The UV scans of SCMV obtained are presented in Figures 25, 26 and 27, respectively. The details of calculation of 'F', 'r' and 'lnr' are presented in Table 15. The slope of the plot (Figure 28) was calculated to be 0.0887 $X10^{-5}$. The Sedimentation coefficient was calculated as follows:

$$S = \frac{1}{w^2} \quad \frac{d\ln r}{dt}$$

$$= \frac{0.08875 \times 10^{-5}}{0.00534 \times 10^{-7}}$$

$$= 16.610 \times 10^{-12} \text{ sec}$$

$$= 166 \times 10^{-13} \text{ sec } (\because 1 S = 10^{-13} \text{ sec})$$

= 166 Svedberg Units

Thus, the sedimentation coefficient of the SCMV was calculated to be 166 Svedberg units.

4.7.5 Determination of the density and partial specific volume ($\bar{V}$) of the sugarcane mosaic virus particles

The formula derived by Gibbs and Harrison (1967) on the basis of relationship of RNA percentage with density of viruses in CsCl (Figure 10).

Density of SCMV particle = $1.2922 + 0.0011 (RNA\%) + 0.0001 (RNA\%)^2$

$= 1.2922 + 0.0011 (5.5\%) + 0.0001 (5.5)^2$

= 1.2988 g/ml

Thus, 1.2988 g/ml density was estimated for the SCMV particles.

FIG. 25. UV SCAN PATTERNS OF SCMV OBTAINED AFTER 5, 10 AND 15 MINUTES OF CENTRIFUGATION IN AN-A ROTOR AT 40,000 RPM.

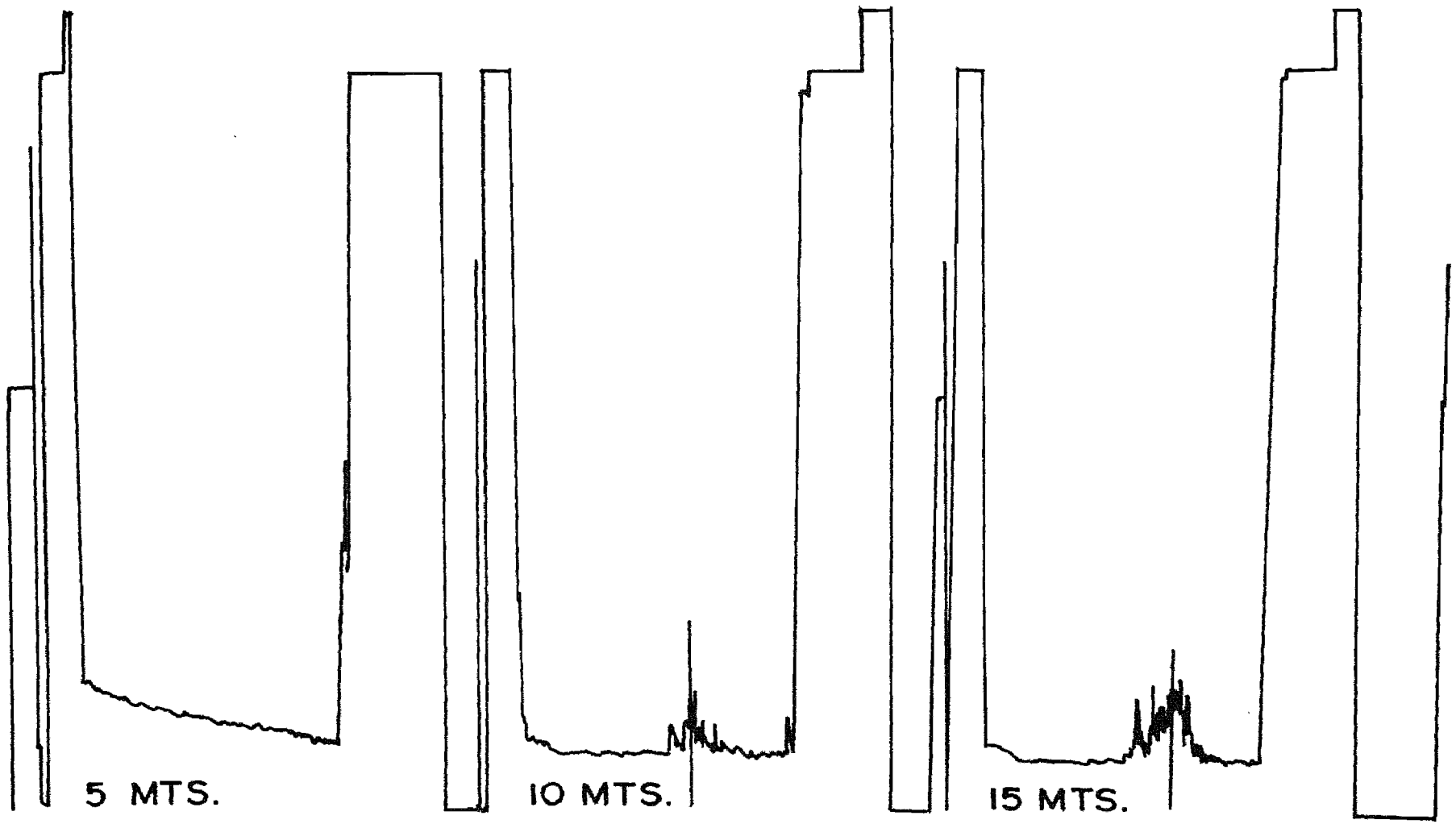


FIG. 26. UV SCAN PATTERNS OF SCMV OBTAINED AFTER 18, 21 AND 27 MINUTES OF CENTRIFUGATION IN AN-A ROTOR AT 40,000 RPM.

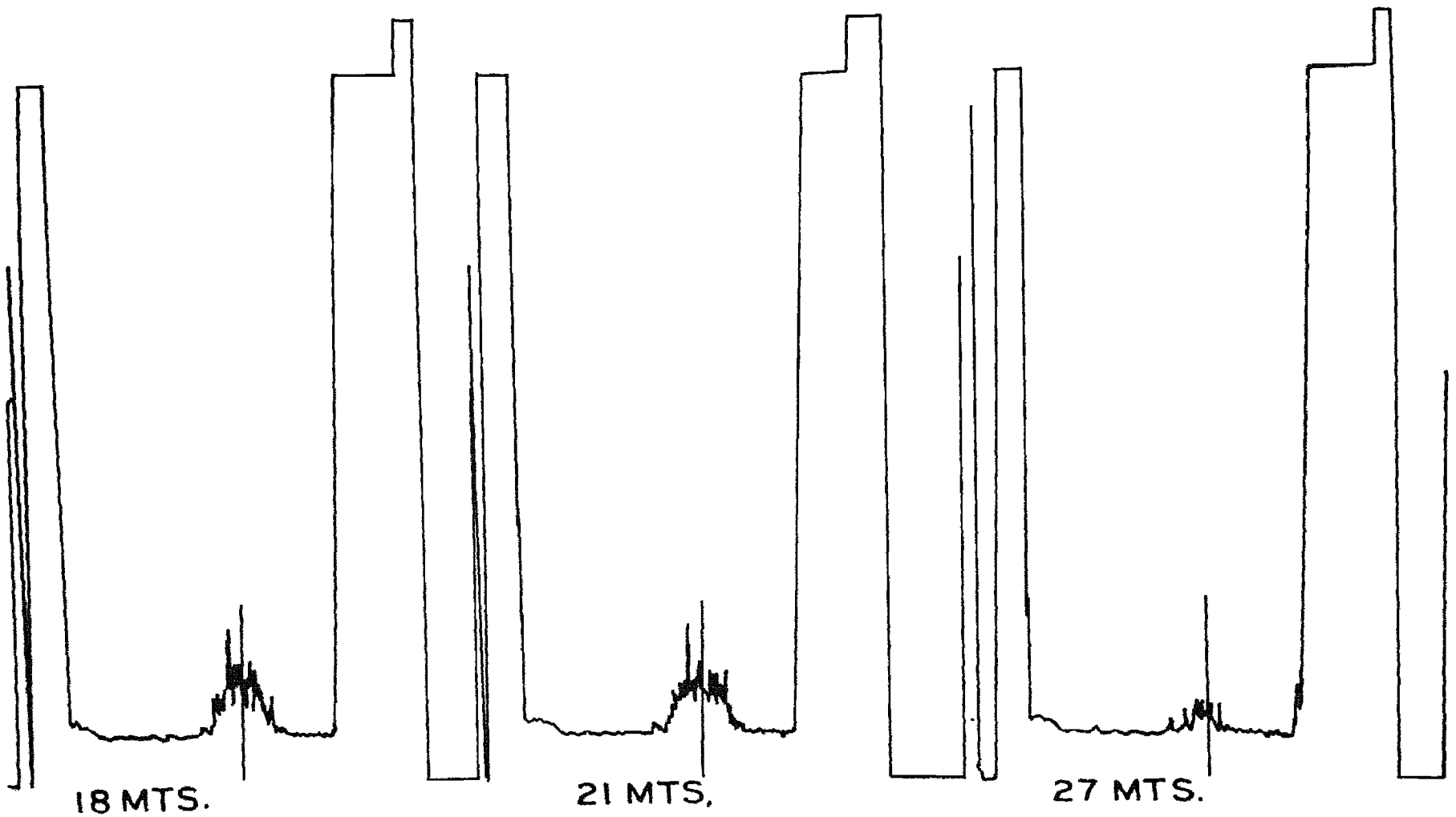


FIG. 27. UV SCAN PATTERNS OF SCMV OBTAINED AFTER 33, 45 AND 50 MINUTES OF CENTRIFUGATION IN AN-A ROTOR AT 40,000 RPM.

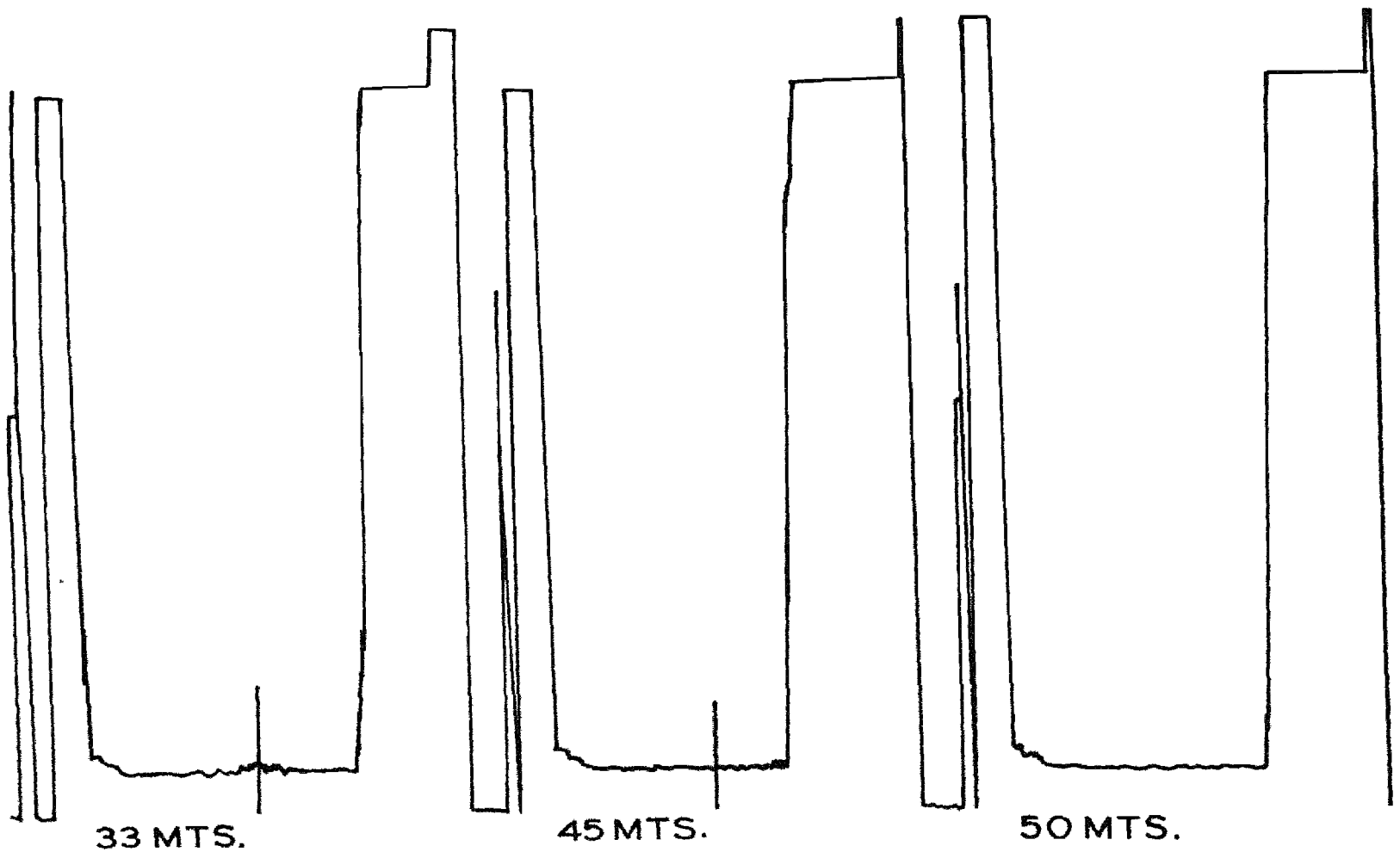
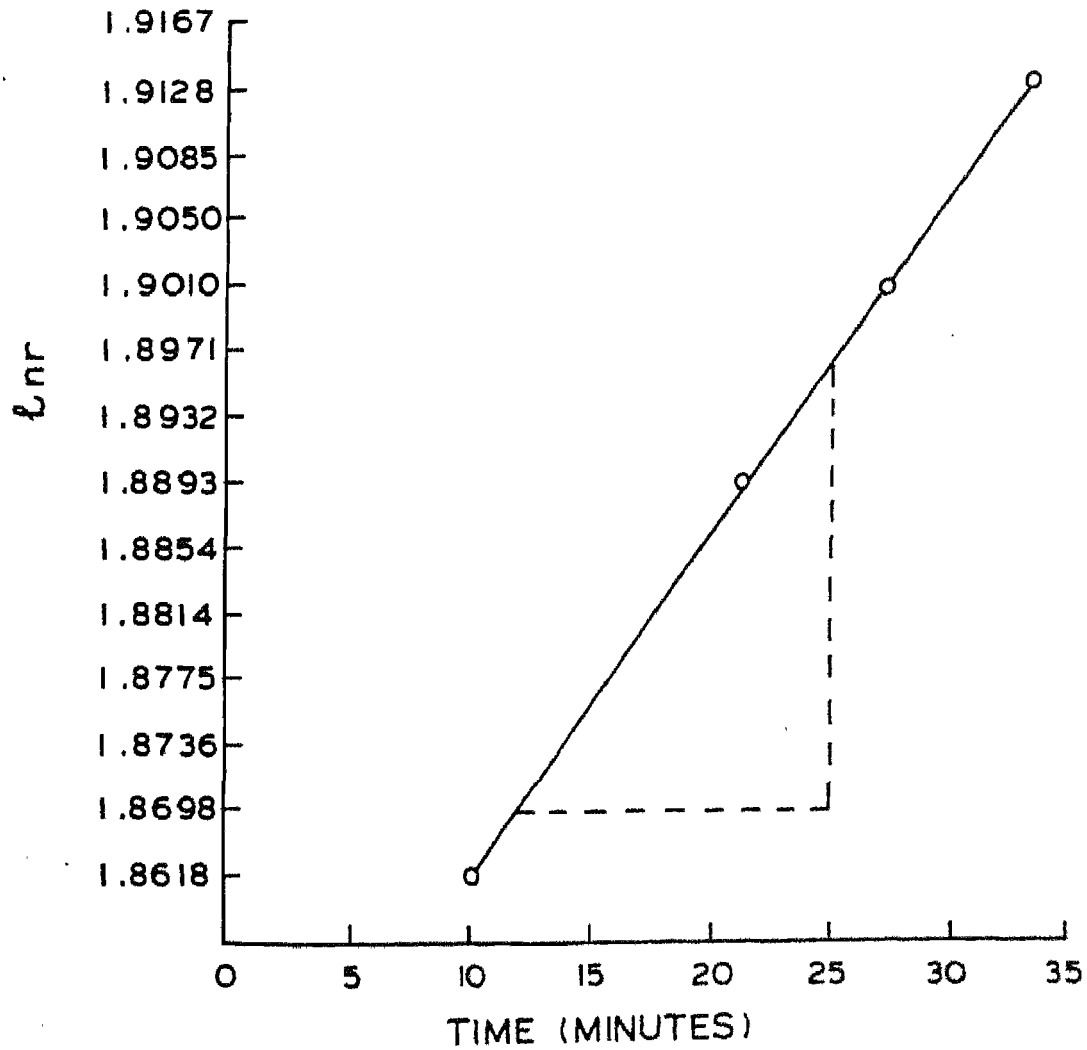


Table 15. Calculated 'x', 'r' and lnr' values from scans of the run, for measurement of sedimentation coefficient of the SCMV

Time (min)	'd' (cm)	$F = \frac{d}{1.62}$	'x' (cm)	$r = \frac{x}{F} + 5.7$	'lnr' = 2.303 log r
10	8.5	5.2469135	3.85	6.433764717	1.861895297
15	8.5	5.2469135	5.1	6.672000015	1.898261657
18	8.5	5.2469135	4.9	6.633882367	1.892531166
21	8.5	5.2469135	4.8	6.614823543	1.889653561
27	8.5	5.2469135	5.2	6.691058839	1.901114637
33	8.5	5.2469135	4.9	6.633882367	1.892531166

FIG. 28. PLOT OF ℓnr (2.303 logr) VERSUS TIME, FOR ESTIMATION OF SEDIMENTATION COEFFICIENT OF PURIFIED SUGAR-CANE MOSAIC VIRUS.



Since partial specific volume ($\bar{V}$) is the reciprocal of the density of the particle (Gibbs and Harrison, 1976), it was calculated for SCMV particle as follows:

$$\bar{V} \text{ of SCMV particles} = \frac{1}{\text{Density}}$$

$$= \frac{1}{1.2988}$$

$$= 0.76$$

Thus, the $\bar{V}$ of SCMV particle was found to be 0.76.

4.8 PHYSICOCHEMICAL PROPERTIES OF THE SUGARCANE MOSAIC VIRUS COAT AND INCLUSION PROTEINS

4.8.1 Viral coat protein

4.8.1.1 Electrophoresis for determination of the molecular weight

The isolated SCMV-coat protein was coelectrophorsed on a 12.5 per cent SDS-polyacrylamide gel with Pharmacia low molecular weight protein markers (Plate 17), having a molecular weight range from 14,000 to 94,000 daltons. The gel was stained with Kenacid blue. The viral coat protein was visualized as a single band in the track loaded with the isolated SCMV coat protein (Figure 29) in the gel. Six distinct bands were developed in the track loaded with the protein markers (Figure 30). The relative electrophoretic mobility (Rf) of SCMV coat protein band and other marker protein bands were as recorded in Table 16. Also the superimposed scan of SCMV coat protein and markers were taken (Figure 31) to know their relative position.

The molecular weight of SCMV coat protein was found to be 40,500 daltons based on two standard curve plotted, with either distance

Plate 17. Single band of SCMV-coat protein when coelectrophoresed with Pharmacias low mol. wt. markers in 12.5% SDS-Polyacrylamide gel, stained with 0.02% Kenacid blue R.

PLATE-17

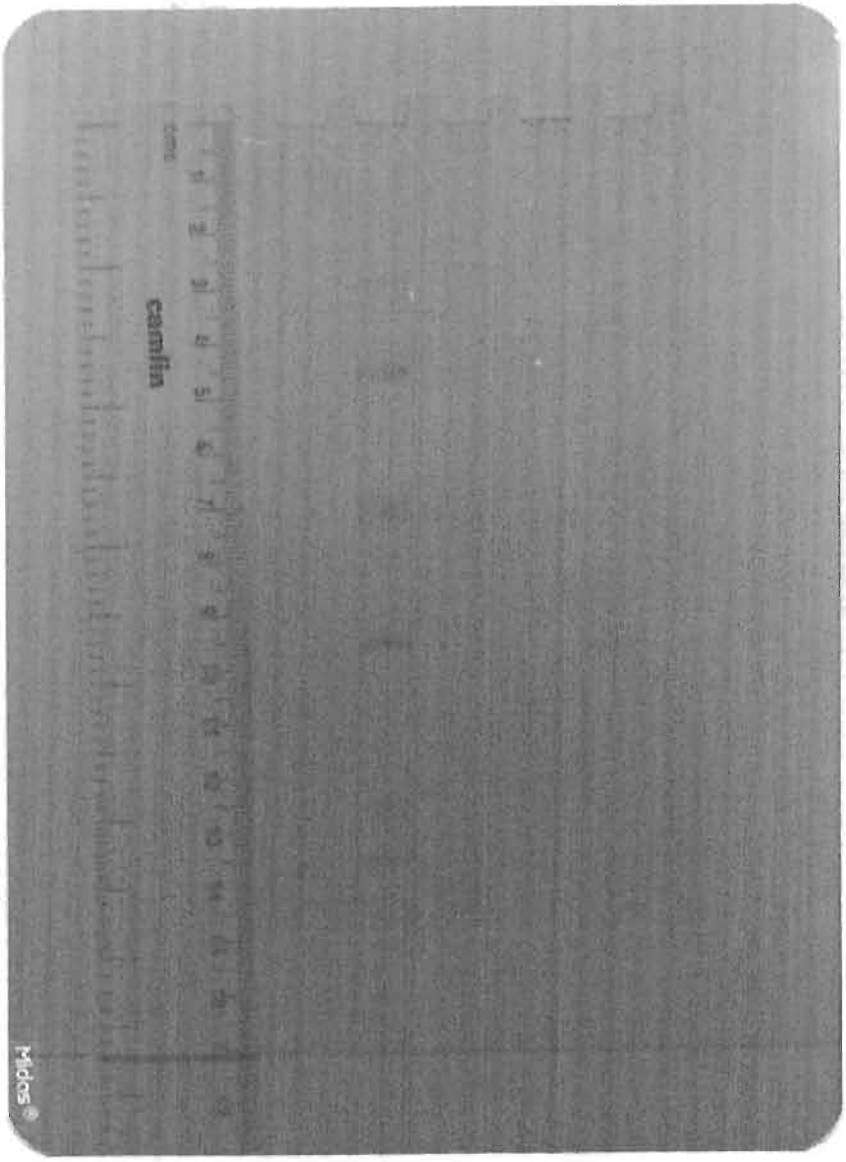


FIG.29 SCAN OF SCMV-COAT PROTEIN AFTER ELECTROPHORESIS IN 12.5% SDS POLYACRYLAMIDE GEL, STAINED WITH KENACID BLUE.

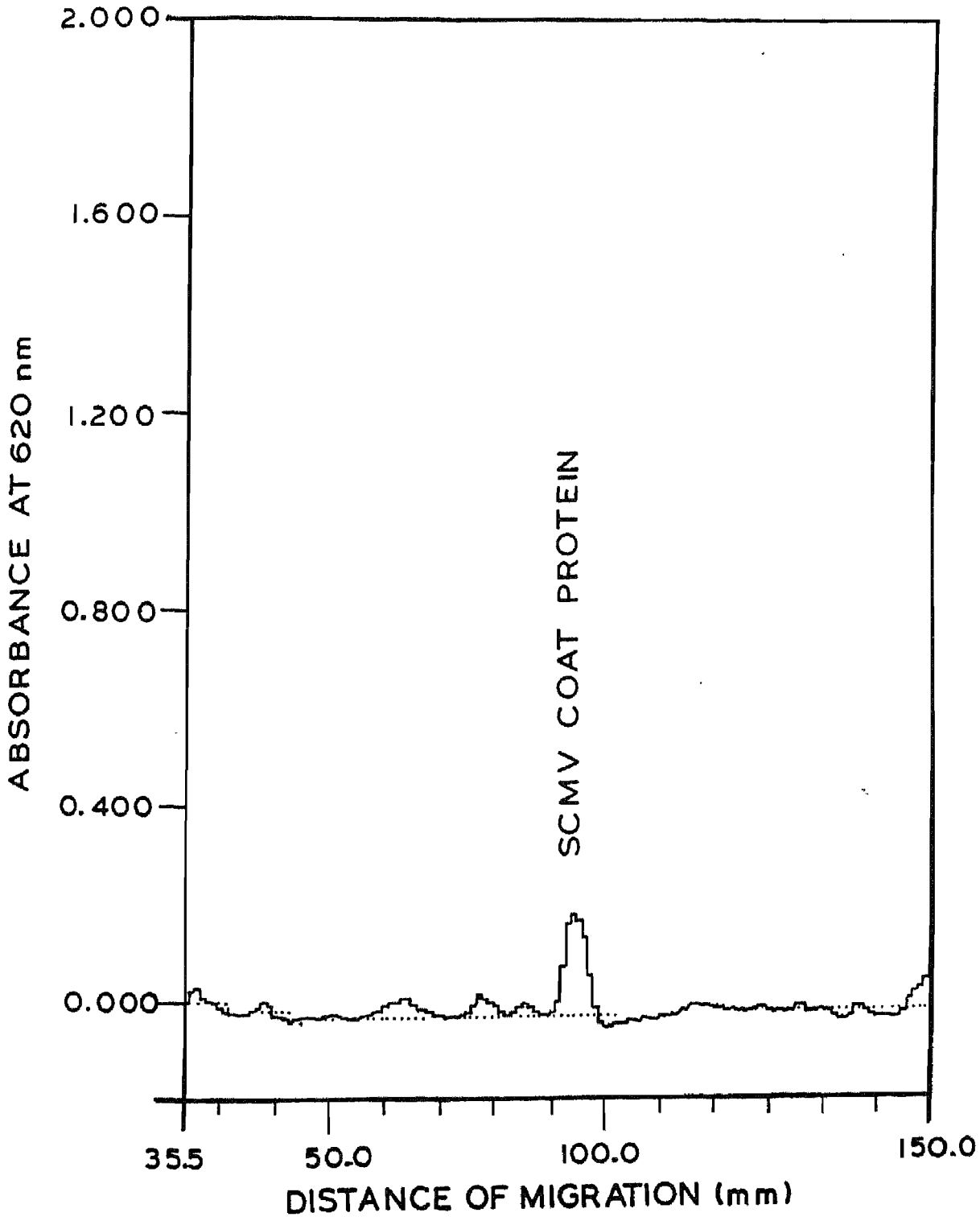


FIG.30. SCAN OF PROTEIN LOW MOLECULER WEIGHT MARKERS, CO-ELECTROPHORESED WITH SCMV-COAT PROTEIN IN 12.5% POLYACRYLAMIDE GEL CONTAINING SDS AND STAINED WITH KENACID BLUE

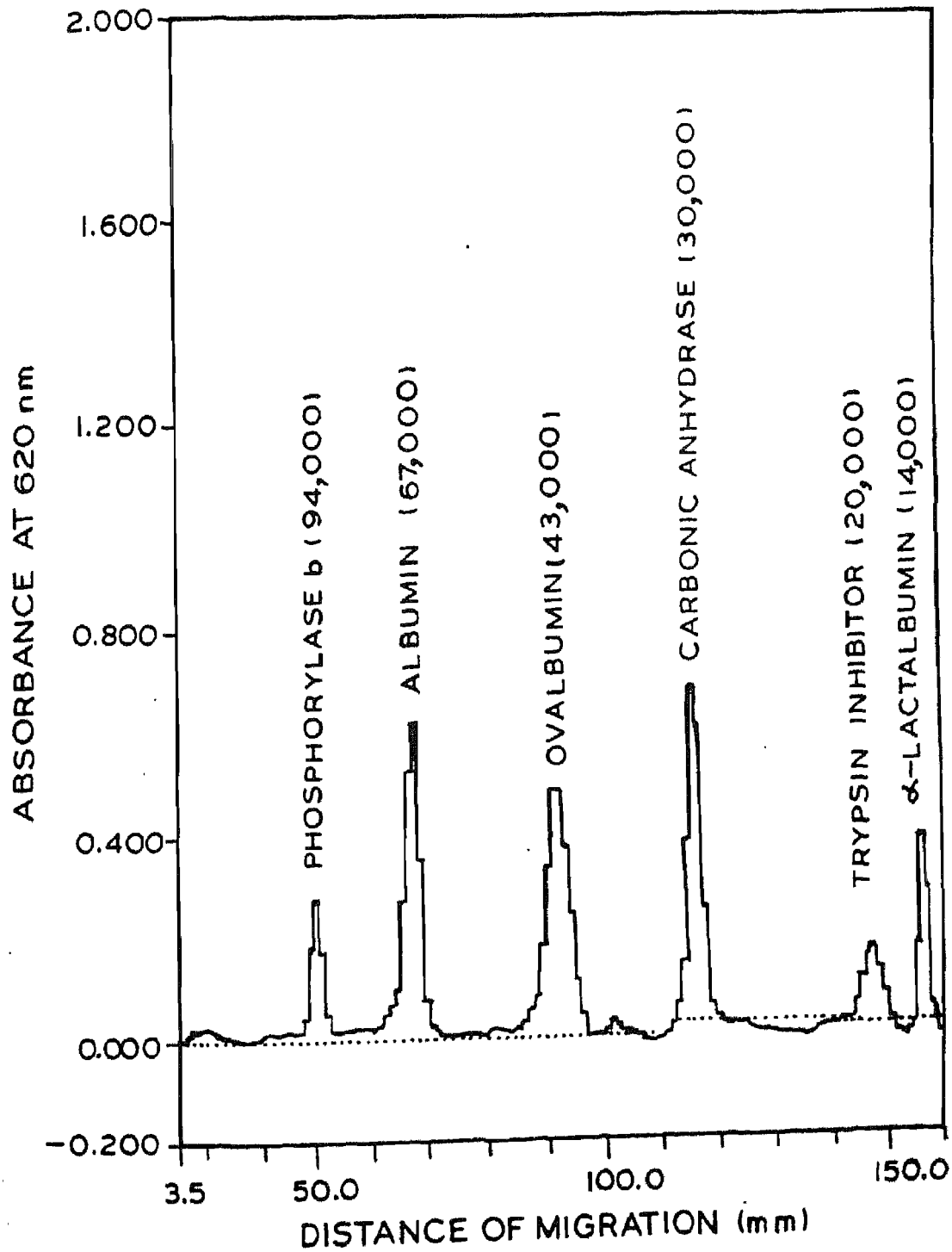


FIG. 31. SUPERIMPOSED SCAN OF SCMV COAT PROTEIN AND LOW MOLECULER WEIGHT PROTEIN MARKERS AFTER SDS PAGE

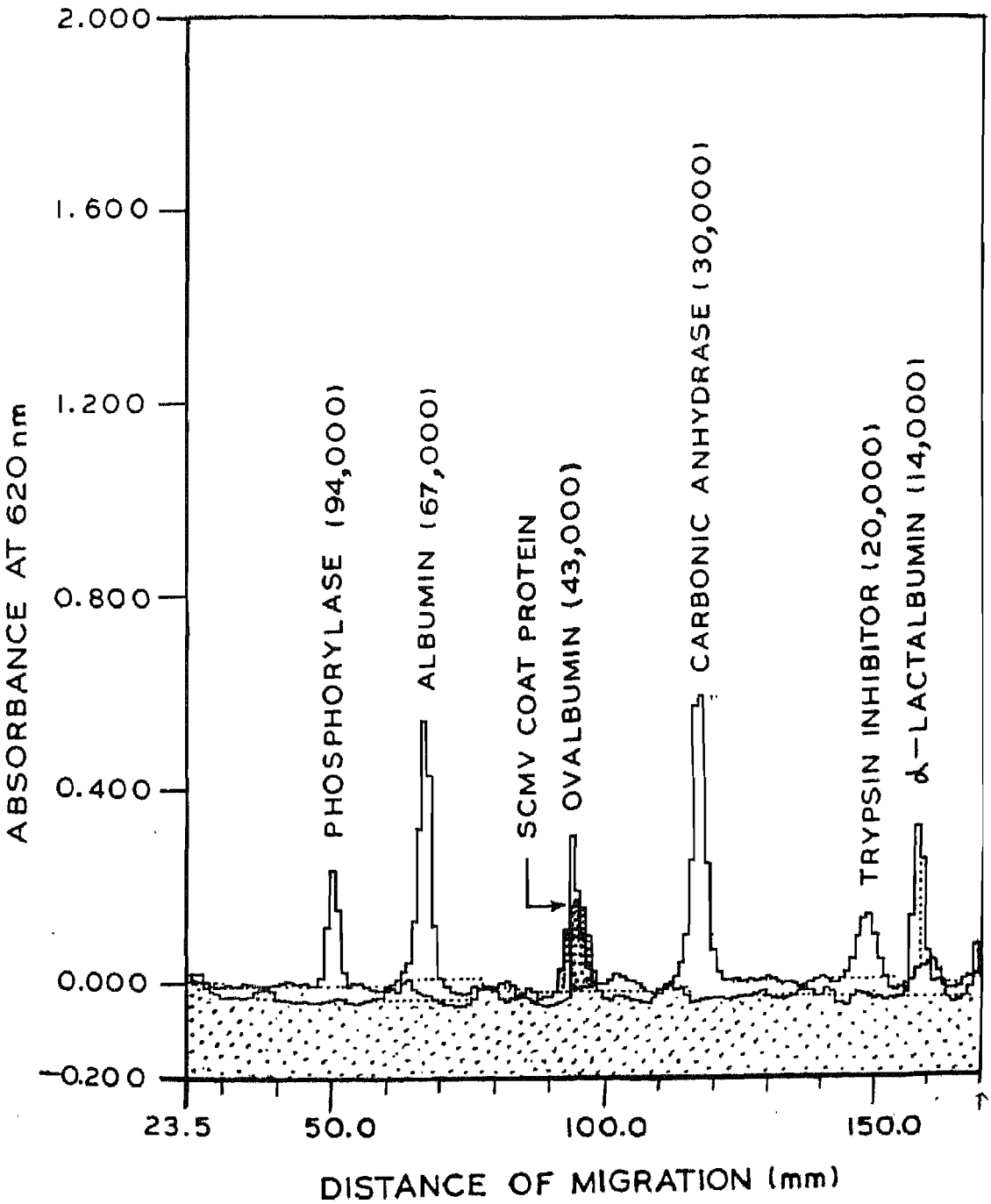


Table 16. Relative mobility (Rf) of SCMV coat protein with protein markers

Sl No.	Protein mol. wt. markers	mol. wt. (daltons)	Distance of migration (cm)	Rf
1.	Phosphorylase b	94,000	2.05	0.210
2.	Albumin	67,000	4.62	0.341
3.	Ovalbumin	43,000	7.05	0.520
4.	Carbonic anhydrase	30,000	9.62	0.710
5.	Trypsin inhibitor	20,000	12.50	0.929
6.	α-lactalbumin	14,000	13.55	1.00
7.	SCMV coat protein	-	7.4	0.546
8.	Dye edge	-	13.55	-

of migration or relative electrophoretic mobility as against the molecular weight marker proteins (Figure 32).

4.8.2 Inclusion proteins

4.8.2.1 Electron microscopy of purified inclusions

The purification procedure used for the present studies resulted in the isolation of virus induced crystalline inclusions (CI). Electron microscopic examination revealed aggregate of these crystalline inclusions as electron dense bodies when stained with 2 per cent uranylacetate. Most of these CI were rectangular or triangular in outline (Plate 18, 19) morphology and varied in size from 150 x 350 nm to 500 x 1050nm in width and length dimensions, respectively. Some of the CI were irregular pieces and appeared to be disintegrated structures. Crystalline inclusions stained with 1 per cent PTA or 1 per cent ammonium molybdate were not stable and clearly visible.

4.8.2.2 Electrophoretic determination of molecular weight

The purified crystalline inclusion proteins were dissociated by heating at 100°C for 5 minutes in Laemmli's buffer and analysed on 12.5% polyacrylamide gel by electrophoresis with marker proteins (Table 17, Figure 33). Of all the five loading volumes (10, 20, 40, 80 and 100 µl) tried, 20 µl loading volume was optimum for better visualization of all the bands (Plate 20). After electrophoresis, two bands were found to be more prominently visualized in the gel. The relative electrophoretic mobility (Rf) of these two bands were 0.42 and 0.616. The estimated molecular weight based on their Rf was 38,000 and 62,000 daltons, respectively (Figure 34). Some additional faint bands were

FIG. 32. DETERMINATION OF MOLECULAR WEIGHT OF SCMV COAT PROTEIN USING SDS-POLYACRYLAMIDE GEL ELECTRO-PHORESIS.

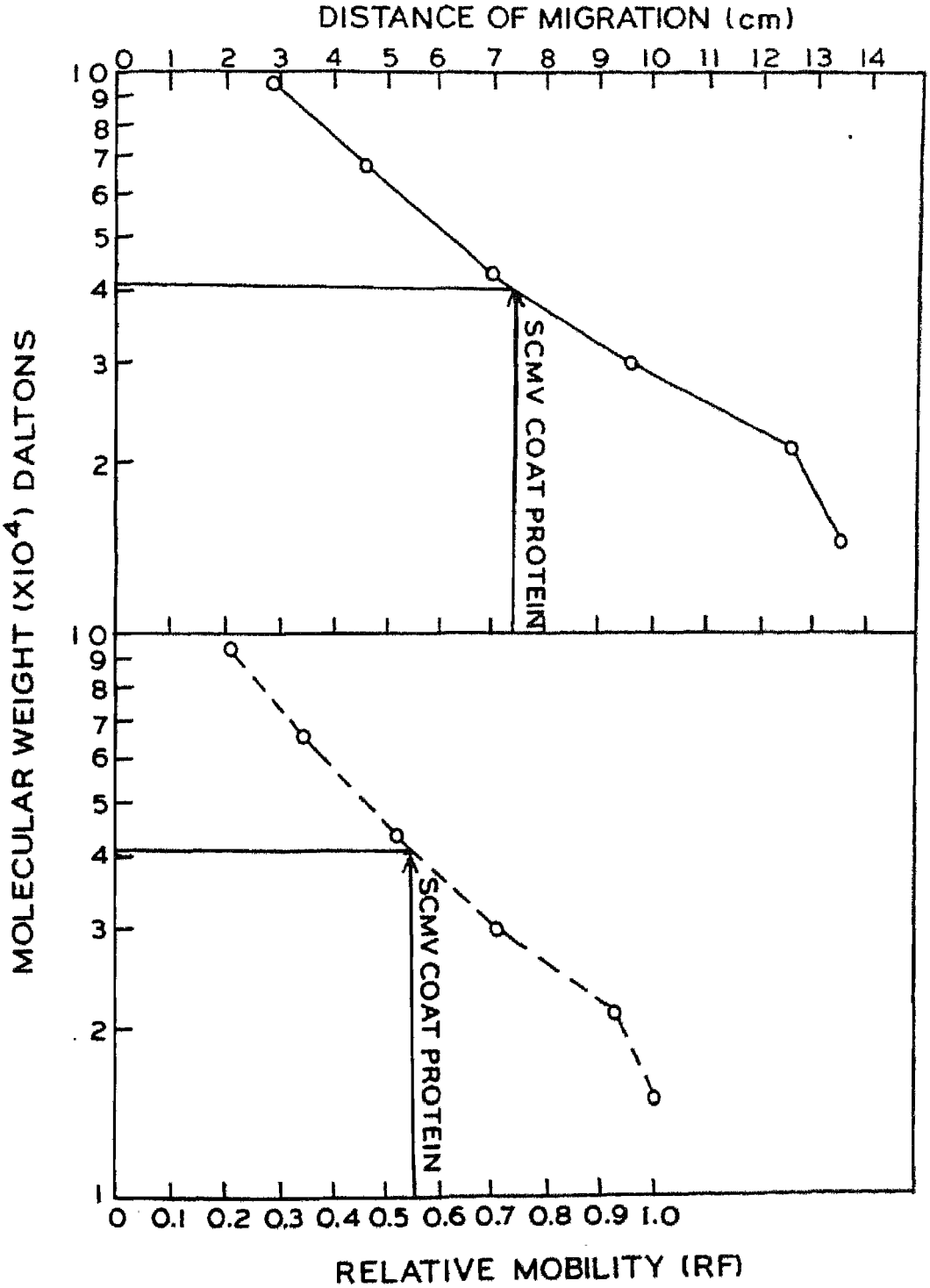


Plate 18. Electron micrograph of purified crystalline inclusions of the SCMV (x 20,000)

Plate 19. Electron micrograph of well separated crystalline inclusions of the SCMV (x 20,000).

PLATE-18

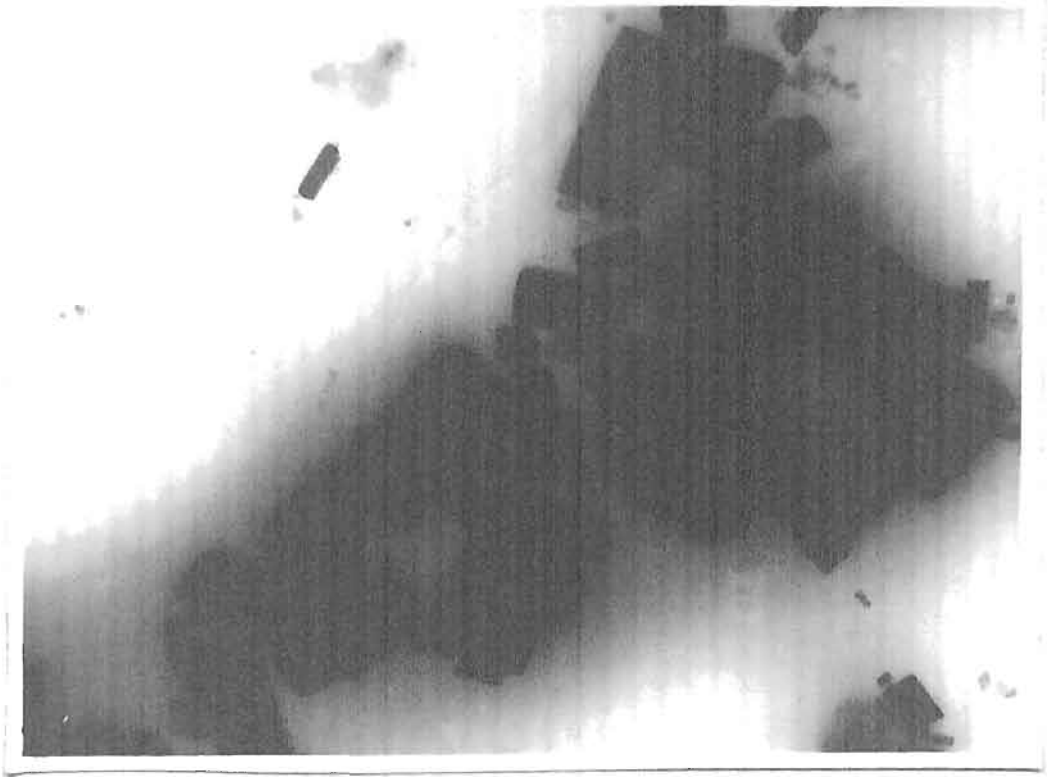


PLATE-19

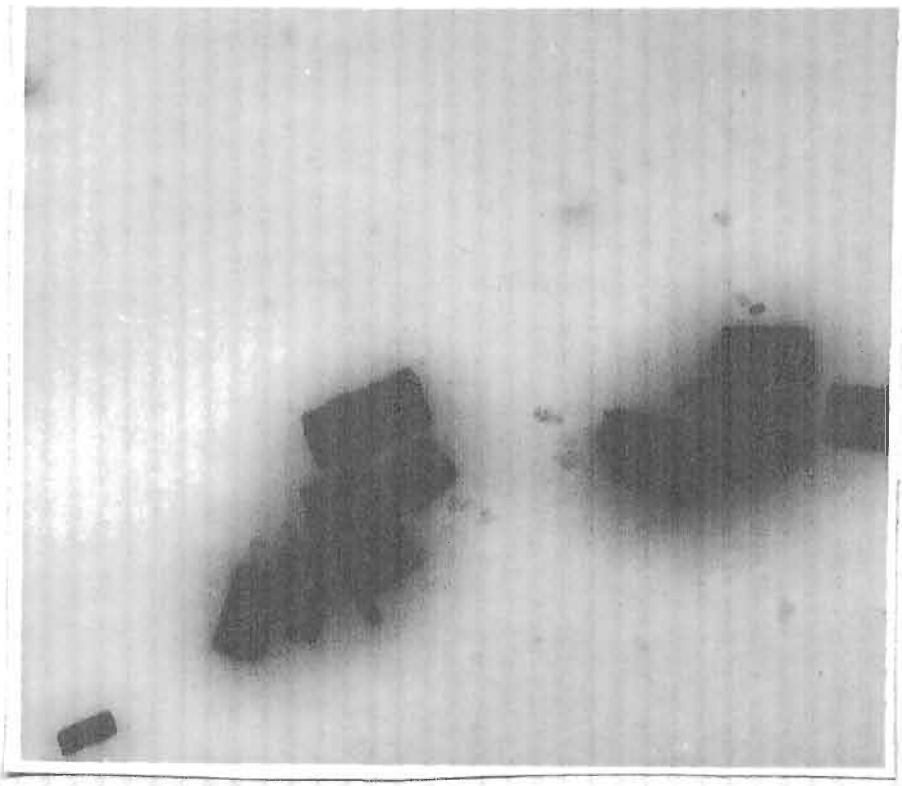


Plate 20. SCMV induced crystalline inclusion proteins, electrophoresed in 12.5% SDS-polyacrylamide gel, stained with silver-nitrate

Track 1. Pharmacia's low molecular weight markers

Track	µl	
2	10	
3	20	
4	40	
5	80	
6	100	loading of inclusion protein was done

PLATE-20

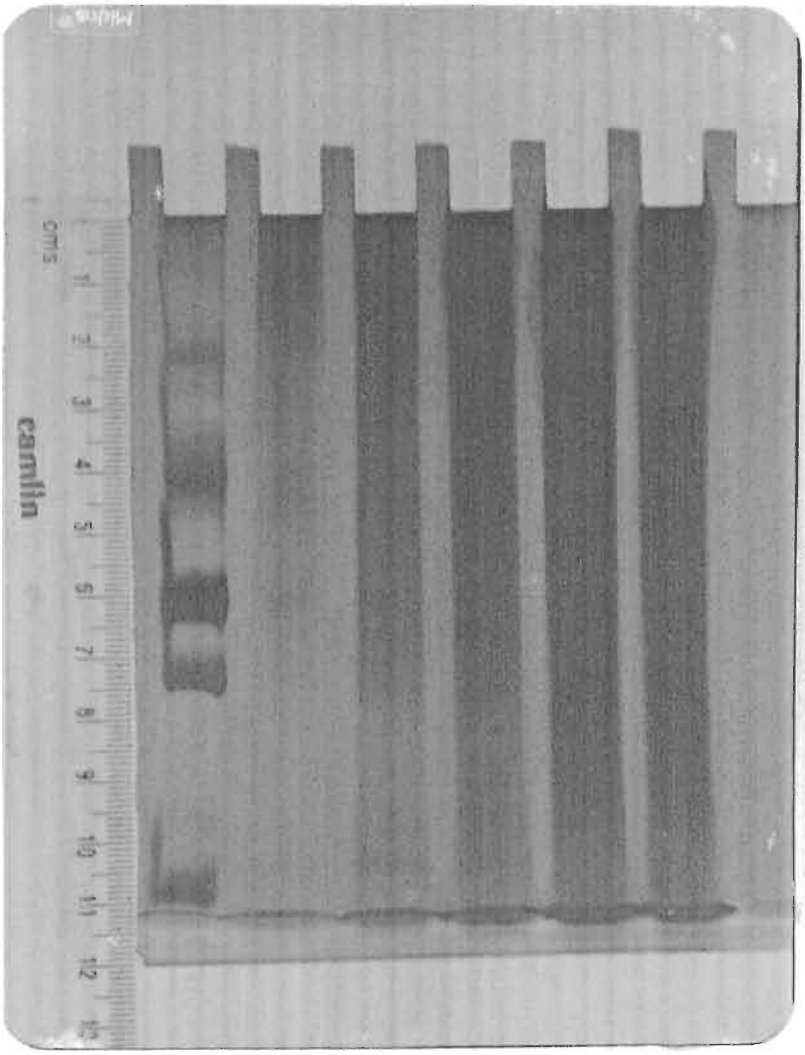


FIG. 33. SCAN OF PROTEIN LOW MOLECULAR WEIGHT MARKERS (I), COELECTROPHORESED WITH SCMV-INCLUSION PROTEIN (II), IN 12.5% POLYACRYLAMIDE GEL CONTAINING SDS AND STAINED WITH SILVERNITRATE.

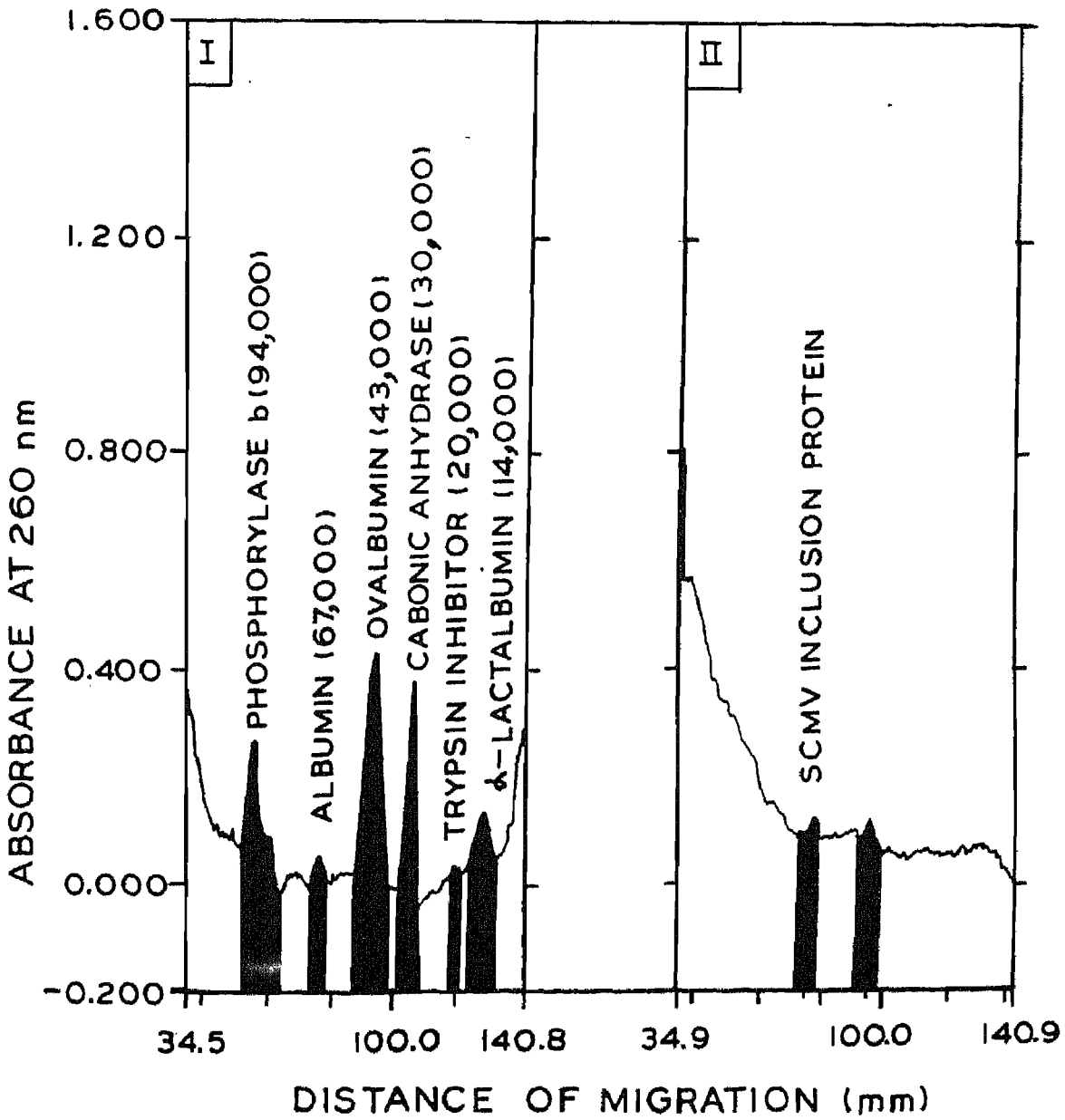


FIG. 34. DETERMINATION OF MOLECULAR WEIGHT OF SCMV-INCLUSION PROTEIN USING SDS-POLYCRYLAMIDE GEL ELECTROPHORESIS.

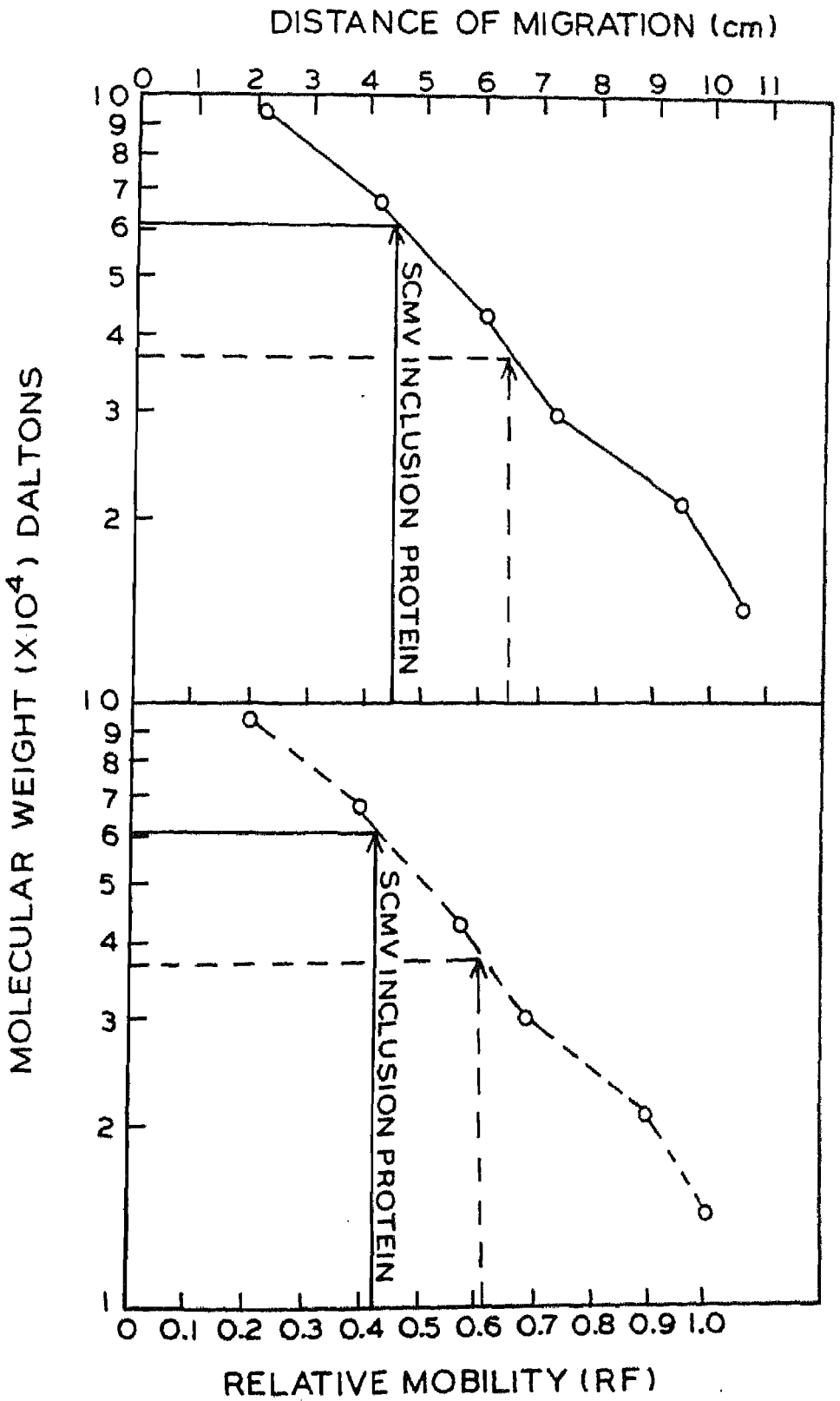


Table 17. Relative mobility (Rf) of SCMV inclusion protein with protein markers

Sl No.	Protein mol. wt. markers	Mol. wt. (daltons)	Distance of migration (cm)	Rf
1.	Phosphorylase b	94,000	2.15	0.203
2.	Albumin	67,000	4.15	0.393
3.	Ovalbumin	43,000	6.05	0.573
4.	Carbonic anhydrase	30,000	7.20	0.682
5.	Trypsin inhibitor	20,000	9.45	0.895
6.	α-lactalbumin	14,000	10.55	1.000
7.	Inclusion protein	-	4.45	0.421
8.	Dye edge	-	10.55	-

also observed, some of these may be due to host or viral coat proteins. The molecular weight of SCMV crystalline inclusion protein was found to be 62,000 daltons.

4.9 VIRAL NUCLEIC ACID

4.9.1 Isolation of SCMV-RNA from purified SCMV preparation

The SCMV-RNA was isolated from purified preparation of SCMV following a modification of Kirby's (1965) procedure. The nucleic acid was reextracted second time with water saturated phenol and use of ether was eliminated altogether. In all about 16,000 µg of RNA was isolated, as estimated on the basis of the titer of the final preparation, as calculated below:

µg RNA/ml = A260 nm x 40 x Dilution of RNA preparation

= 1.280 x 40 x 50

= 2560 µg/ml

= 2.560µg/µl

4.9.2 Ultraviolet absorption profile of SCMV-RNA

The ultraviolet absorption profile of SCMV-RNA is presented in Figure 35. The SCMV-RNA had maximum UV absorption at 265 nm and minimum at 235 nm. The absorbance value at different wavelength are presented in Table 18.

FIG. 35. ABSORBANCE SPECTRUM OF PURIFIED RNA OF SUGARCANE MOSAIC VIRUS.

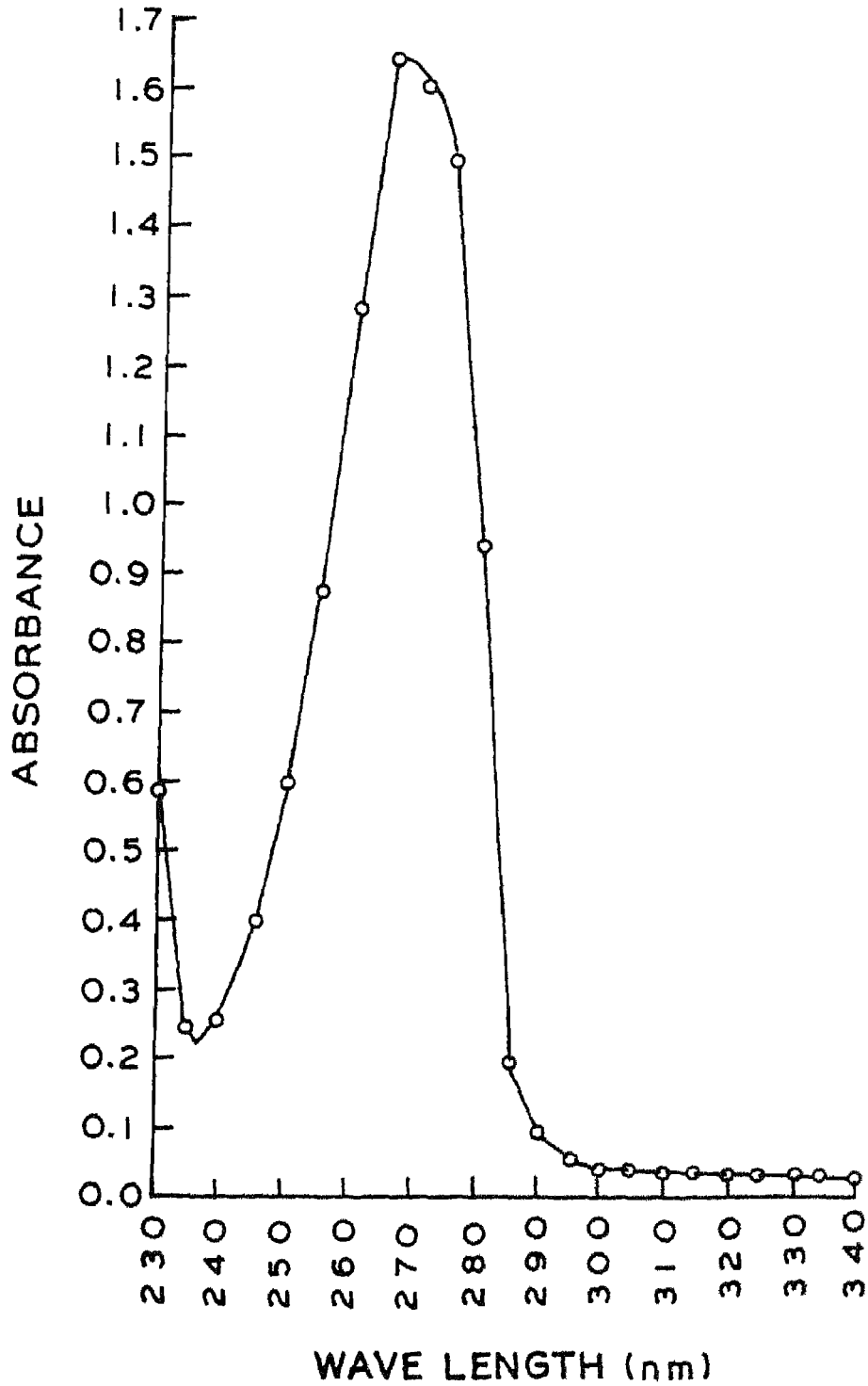


Table 18. Ultraviolet absorption spectrum of purified SCMV-RNA preparation

Wavelength (nm)	Absorbance
230	0.587
235	0.240
240	0.262
245	0.387
250	0.605
255	0.885
260	1.280
265	1.640
270	1.621
275	1.612
280	0.957
285	0.204
290	0.099
295	0.061
300	0.047
305	0.039
310	0.038
315	0.038
320	0.036
325	0.036
330	0.035
335	0.034
340	0.033

4.9.3 Determination of 'Sedimentation Coefficient' of the sugarcane mosaic virus ribonucleic acid (SCMV-RNA)

The UV scans tracings recorded at different duration are presented in Figures 36, 37 and 38, respectively. The details of the calculation of 'F', 'r', and 'lnr' are presented in Table 19. Slope of plot (Figure 39) was calculated and found to be 0.11845×10^{-5}. The Sedimentation coefficient was calculated as follows:

$$S = \frac{1}{w^2} \cdot \frac{d\ln r}{dt}$$

$$= \frac{0.1148 \times 10^{-5}}{1.754 \times 10^{-7}}$$

$$= 6.545 \times 10^{-12} \text{ sec}$$

$$= 65.45 \times 10^{-13} \text{ sec } (\because 1\ S = 10^{-13} \text{ sec})$$

$$= 65 \text{ Svedberg units}$$

Thus, the Sedimentation coefficient of SCMV-RNA was found to be 65 Svedberg units.

4.9.4 Determination of molecular weight of the SCMV-RNA

The molecular weight of the SCMV-RNA was determined using following formula:

$$M = \frac{2\ RT}{(1 - \bar{V}p) \cdot w^2} \cdot \frac{d\ln r}{(dt^2)}$$

$$= \frac{2x(8.315 \times 10^{-7}) \times 272}{(1-0.55x1) \times 1.754 \times 10^{-7}} \times 5.92x\ 10^{-5}$$

$$= 3.455 \times 10^{-6} \text{ daltons}$$

Thus, the molecular weight of the sugarcane mosaic virus ribonucleic acid was found to be 3.455×10^{-6} daltons.

FIG.36. UV SCAN PATTERN OF SCMV-RNA OBTAINED AFTER 3,5 AND 9 MINUTES OF CENTRIFUGATION IN AN-A ROTOR AT 40,000 RPM.

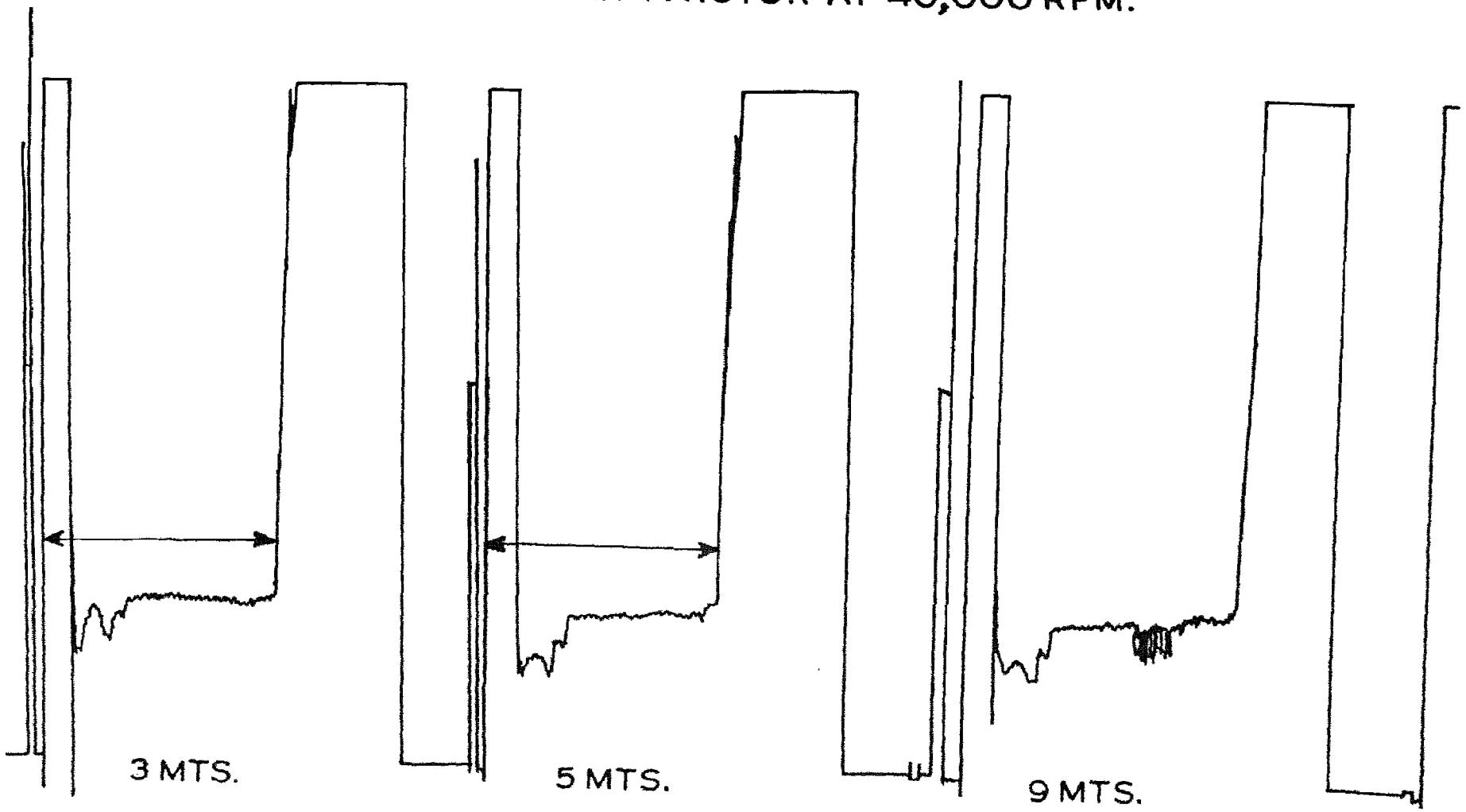


FIG. 37. UV SCAN PATTERNS OF SCMV–RNA OBTAINED AFTER 12, 14 AND 16 MINUTES RUN TIME AT 40,000 RPM IN AN-A ROTOR.

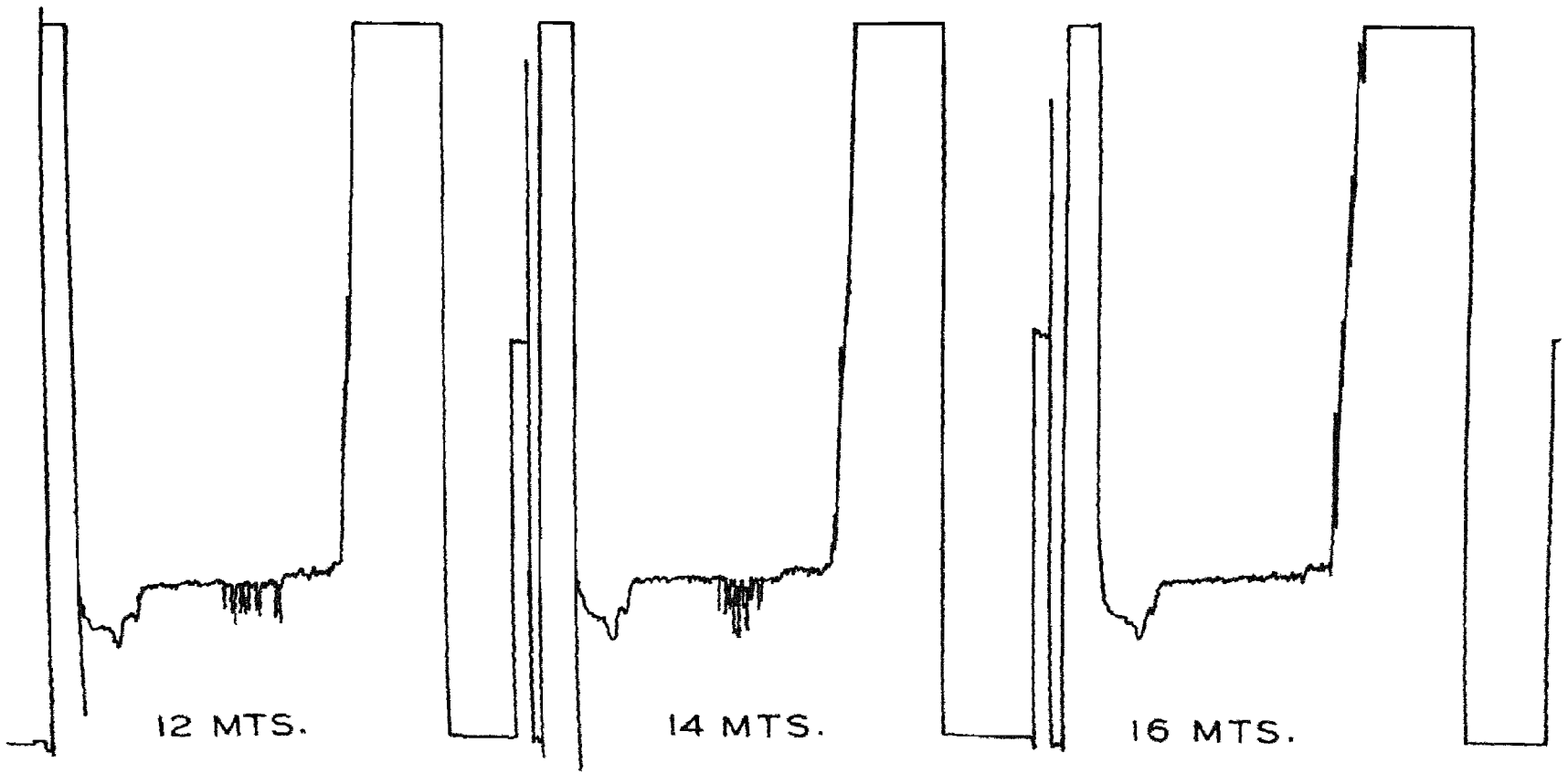


FIG.38. UV SCAN PATTERN OF SCMV-RNA OBTAINED AFTER 22,26 AND 31 MINUTES OF CENTRIFUGATION IN AN-A ROTOR AT 40,000 RPM.

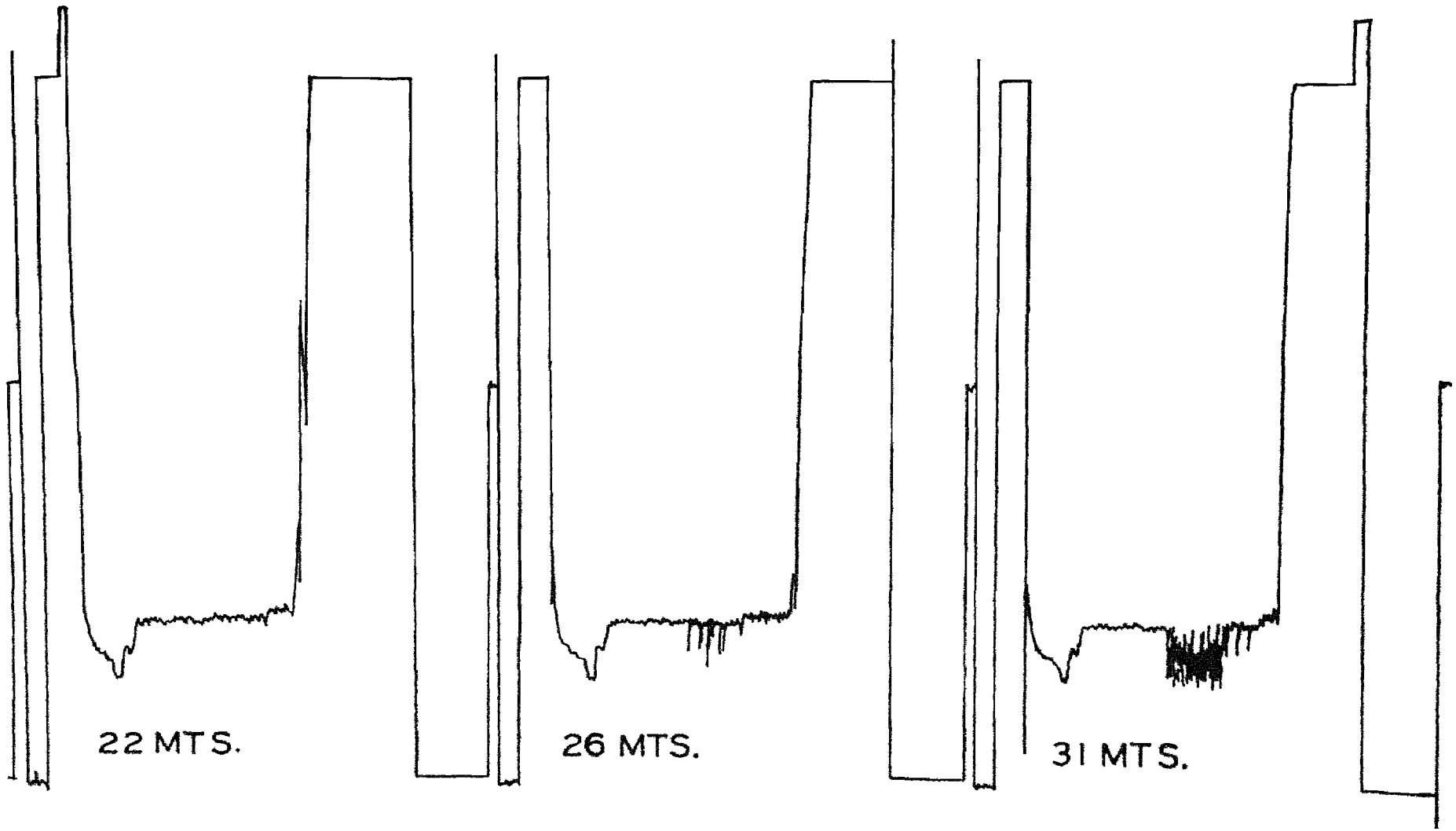
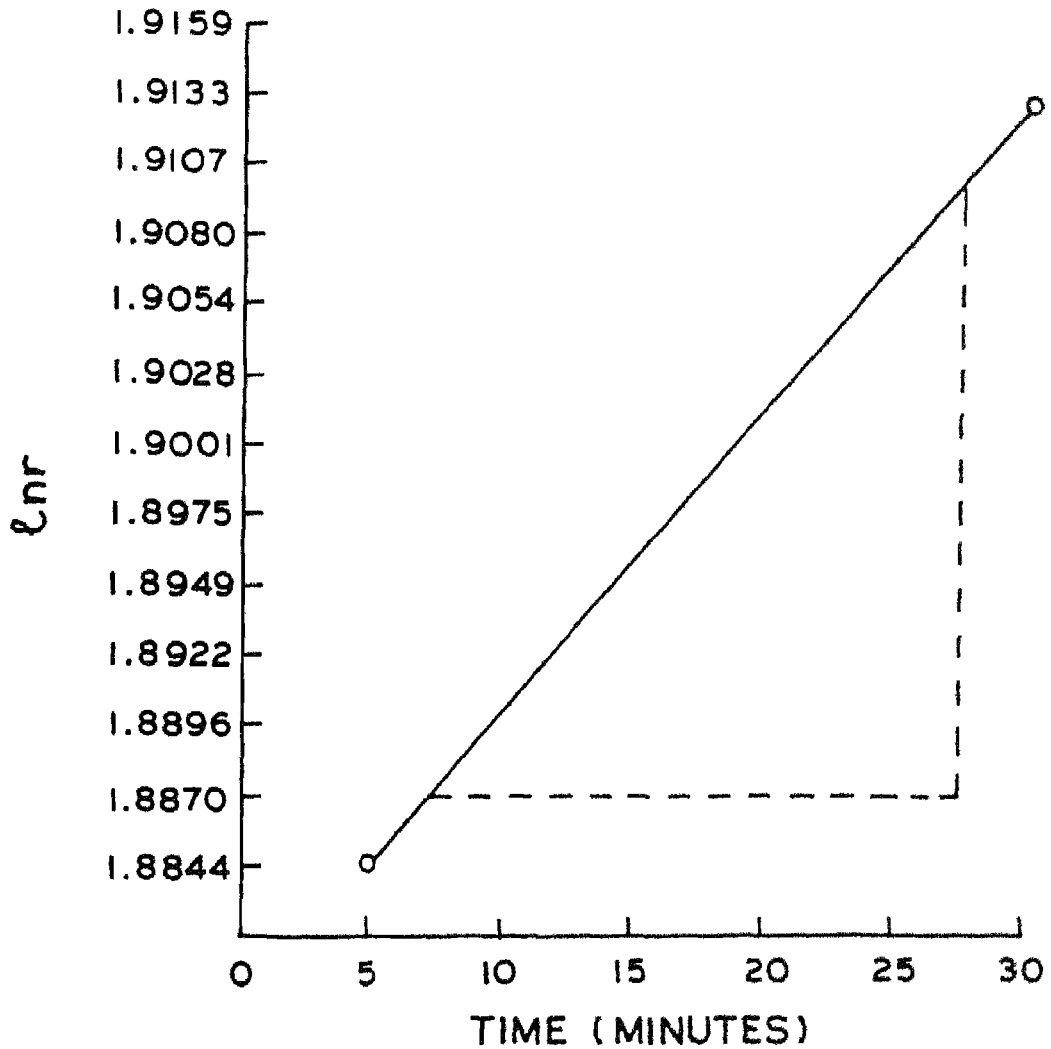


Table 19. Calculation, 'x', 'r' and 'lnr' values from scans of the run for measurement of the sedimentation coefficient of the SCMV-RNA

Time (min)	'd' (cm)	$F = \frac{d}{1.62}$	'x' (cm)	$r = \frac{x}{F} + 5.7$	$lnr = 2.303 \log r$
3	9.2	5.67901	5.10	6.598043849	1.8871132
5	9.2	5.67901	5.00	6.580435146	1.88444037
9	9.2	5.67901	5.80	6.72130477	1.905625614
12	9.2	5.67901	5.85	6.730109121	1.90693491
14	9.2	5.67901	5.90	6.738913473	1.90824249
16	9.2	5.67901	5.60	6.686087364	1.900371224
22	9.2	5.67901	5.50	6.668478661	1.897733642
26	9.2	5.67901	5.90	6.738913473	1.908242494
31	9.2	5.67901	6.10	6.774130878	1.913455802

FIG. 39. PLOT OF ℓnr VERSUS TIME, FOR ESTIMATION OF SEDIMENTATION COEFFICIENT OF THE PURIFIED SUGARCANE MOSAIC VIRUS RIBONUCLEIC ACID



4.10 ELECTROPHORETIC ANALYSIS OF SCMV-RNA

The purified SCMV-RNA was loaded on a 0.6 per cent agarose gel at 10 and 20 µg concentration, in native state did not appeared to move, as no bands developed after an electrophoresis run for 1.5 h, with 50 volts electromotive force, and gel stained with ethidium bromide. However, under similar electrokinetic conditions, the purified SCMV-RNA when denatured by heating at 60°C, moved 3.7 cm from edge of the loading wells (Table 20, Plate 21). As compared to heat treatment, other denaturing treatments i.e. by 3M urea, and 36 per cent formamide, when administered, did not change the electrophoretic mobility of purified SCMV-RNA under the similar electrokinetic conditions (Table 20, Plate 22).

The glyoxal treated SCMV-RNA in 1 per cent agarose gel only moved 4.7 cm from edge of the well in 2 h at 30 volts electromotive force. The gel was stained with acridine orange, which indicated an orange coloured single band of SCMV-RNA (Plate 23).

SCMV-RNA was also electrophoresed in vertical polyacrylamide slab gel in native and denatured states. No band was visualized in 2.6 and 4 per cent gel (Table 21) loaded with untreated native SCMV-RNA (Plate 24). After the denaturation of SCMV-RNA at 60°C for 5 min, the SCMV-RNA was electrophoresed on 5 per cent polyacrylamide gel. It travelled as single band 3 cm from the bottom of the loading well in 37 min, when stained with Silver nitrate (Plate 25). In 2.5 h, the SCMV-RNA appears to have ranout of the gel as no band was visible (Table 21).

Table 20. Electrophoretic analysis of SCMV-RNA in agarose horizontal slab gel

Sl No.	Treatment to SCMV-RNA	% agarose used	Electrokinetic conditions	Gel staining	Distance migrated in from edge of wall (cm)
1.	Native State	0.6	1.5 h, 50 V	EB*	0.0
2.	Denatured State				
	60°C	0.6	1.5 h, 50 V	EB	3.7
3.	60°C	0.6	1.5 h, 50 V	EB	3.8
	60°C + 3M urea	0.6	1.5 h, 50 V	EB	3.8
	60°C + 36% formamide	0.6	1.5 h, 50 V	EB	3.8
4.	53°C + Glyoxal	1.0	2 h, 30 V	AO**	4.7

* EB = Ethidium bromide

**AO = Acridine orange

Plate 21. Heat denatured (60°C) SCMV-RNA, electrophoresed in 0.6% agarose gel and stained with ethidium bromide, moved as a single fluorescent band, photographed under ultraviolet light. The two traks were loaded with 10 and 20 μg of SCMV-RNA

Plate 22. Denatured SCMV-RNA with heat (Track 1) 3 M urea (Track 2), and 36% formamide (Track 3), electrophoresed in 0.6% agarose gel stained with ethidium bromide. The denatured SCMV-RNA moved as a single band, in each track. The gel was photographed under ultraviolet light.

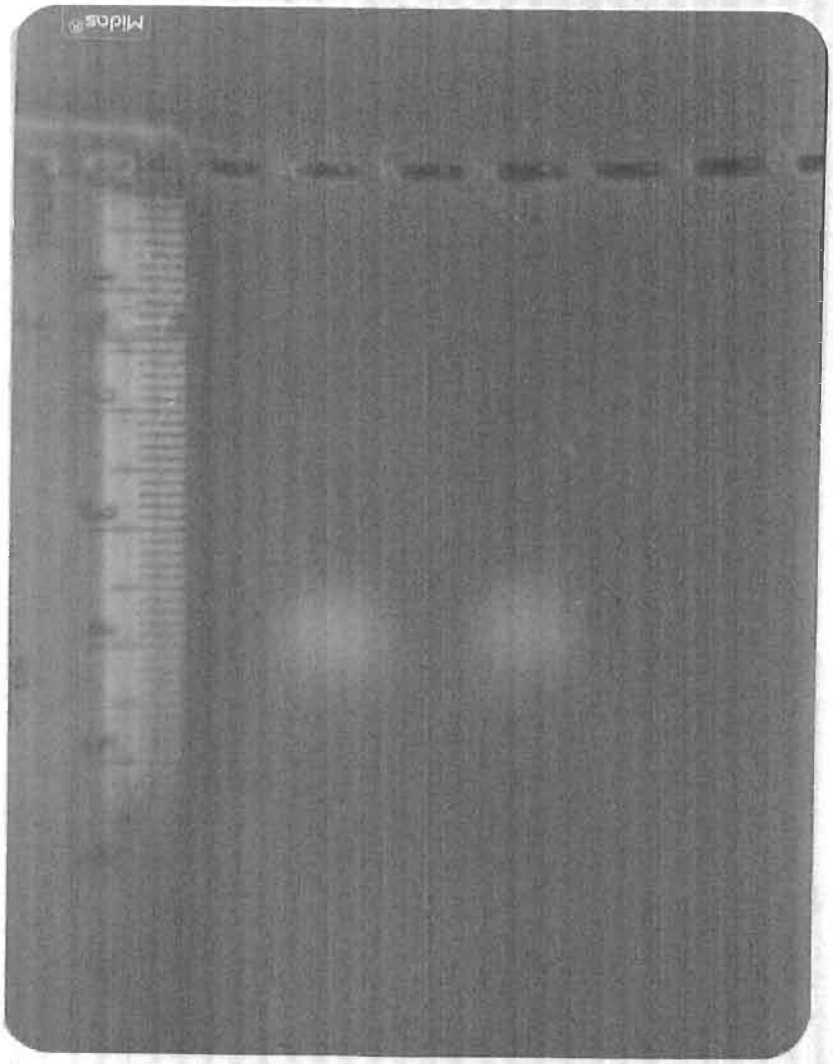


PLATE-21

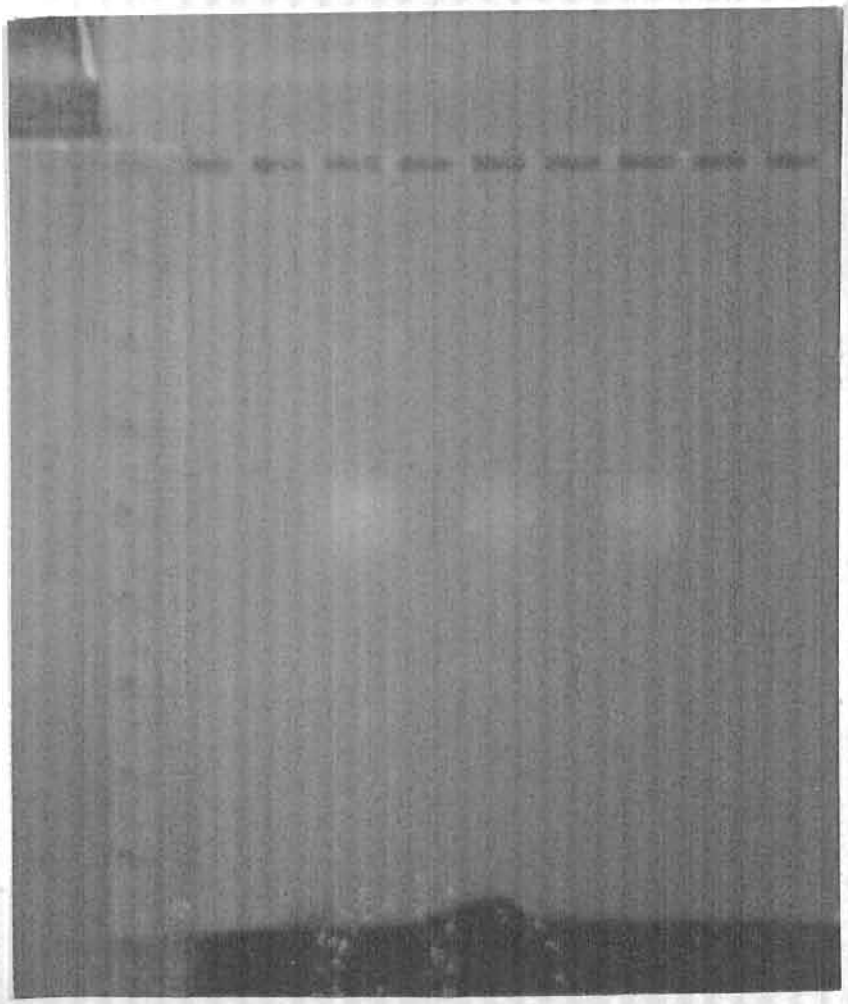


PLATE-22

Plate 23. Glyoxal denatured SCMV-RNA, electrophoresed in 1% agarose gel, moved as a single band. The gel was stained with acridine orange.

PLATE-23

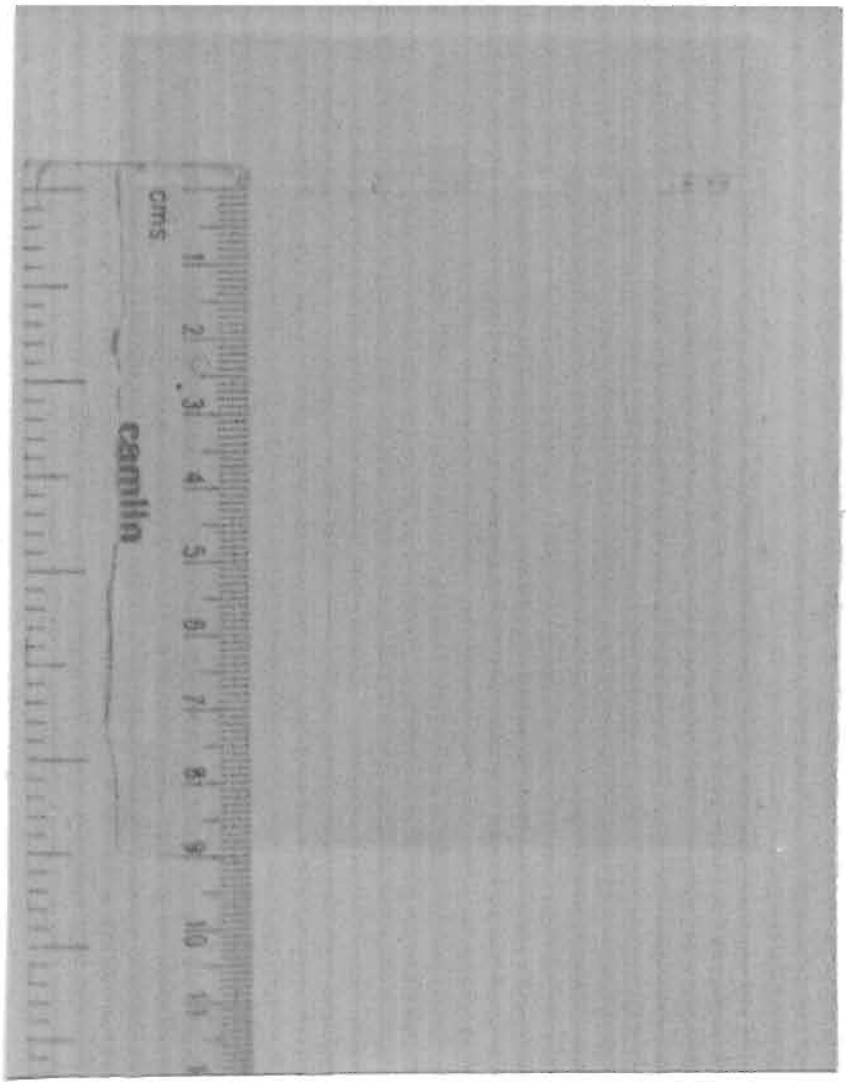


Plate 24. PAGE of SCMV-RNA in 2.7% gel, which was stained with silver nitrate. The SCMV-RNA ran out of the track

Plate 25. PAGE of SCMV-RNA in 5% gel. The RNA moved as a single band. The electrophoresed was carried for 37 min and gel stained with silver-nitrate

PLATE-24

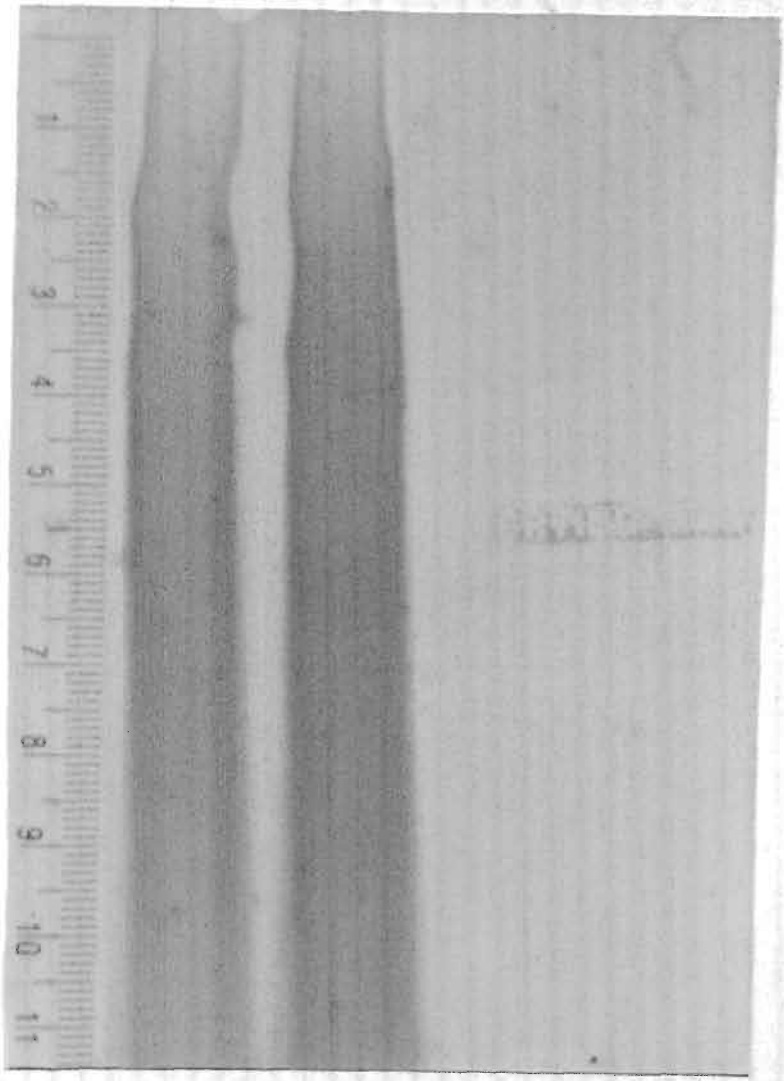


PLATE-25

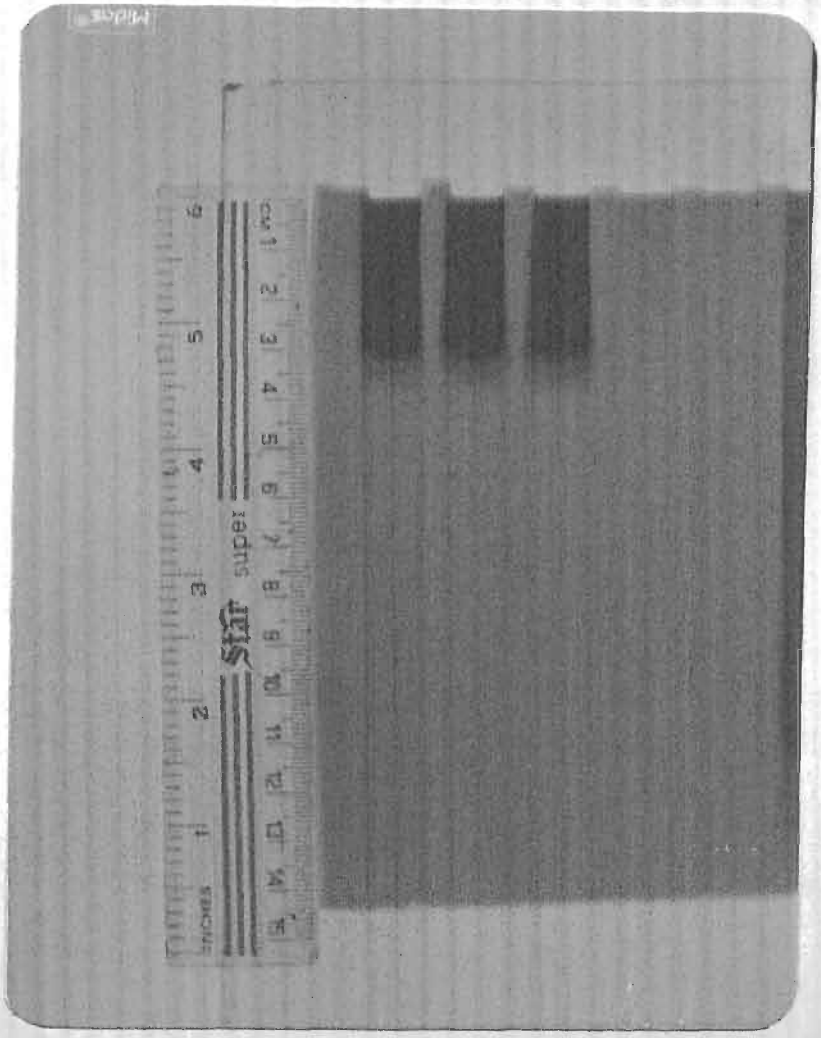


Table 21. Electophoretic analysis of SCMV-RNA (denatured State) in vertical polyacrylamide slab gel

Sl No.	Treatment to SCMV-RNA	% PAGE	Electrokinetic conditions	Gel staining	Distance migrated from edge of wall (cm)
1.	Native state	2.6	2.5 h, 50 V	S*	Ran out
		4.0	3.5 h, 70 V	S	Ran out
2.	Denatured at 60°C	5.0	2.5 h, 70 V	S	Ran out
		5.0	37 min, 70 V	S	3.0 cm

* S = Silver stained

4.11 DIFFERENTIATION OF SCMV STRAINS

The ten different sorghum lines as suggested by Gillispie and Mock (1984), [Table 22] were used to differentiate different strains of SCMV from three isolates. Johnsongrass [*Sorghum halepense* (L.) Pers.] which has been used by Persley et al. (1985) for designating A strain of SCMV, was also included for these studies. The reaction of these differentials on inoculation with the three isolates of SCMV recorded one month after inoculation (Tables 23, 24 and 25) were compared with the standard reactions of individual SCMV strains given by Gallispie and Mock (1984). Since each isolate appeared to be a mixture of many strains, only characteristic distinctive symptoms on individual differential were taken in consideration to identify different strains (Table 26). Generally, the SCMV-A strain was distinguished on the basis of severe mosaic symptoms on johnsongrass (Plate 26). SCMV-E strain was identified by the necrosis and chlorotic streaks on rio (Plate 27). The SCMV-H strain was recorded on the basis of severe mosaic and necrotic streaks on atlas (Plate 28), whereas SCMV-I and K strains were recognised on the basis of severe mosaic symptom on BTx398 (Plate 29), and chlorotic flecks on NM 31 (Plate 30), respectively. The SCMV-F strain which Gillaspie and Mock (1984) had distinguished into 4961 and 4975 isolates which were also identified from three isolates of SCMV, on the basis of necrotic flecks and mild mosaic symptoms on QL 11 (Plate 31), and mosaic symptomson Q 7539 (Plate 32) and SC 0097-14E, respectively.

Four sorghum lines namely Martin, Martin-40, Martin-5330 and QL-3-Tx obtained from ICRISAT, Hyderabad, were also inoculated from 3 SCMV isolates. They produced different symptoms with each

Table 22. Reaction of ten sorghum lines to SCMV strains from sugarcane under controlled growth conditions (Gillaspie and Mock, 1984)

Strains/ isolates	OKY 8	SA 8735	BTx3197	Atlas	NM 31	Rio	BTx398	SC0097-14E	Q 7539	QL 11
A	nil	n, Cst(n)	n, Nst-D	n, D	n	M_1	nil	nil	nil	nil
B	Cst	n,M_1,Nfl	n, D, S	gn, M_2, Nfl	n,M_2,N	M_2	Cfl	nil	nil	nil
D	Cst	gn,Cst(n)	n,Nst-D	gn,D	n,Nst	M_1	nil	nil	nil	nil
E	nil	n	n	n, Cfl	n	n, Cst	nil	nil	nil	nil
H	M_1	n, D	n, D	n,M_2,Nst	n, D	M_1	M_1	nil	nil	nil
I	M_2	n,D,S	n,D	n,D,S	n,D,S	M_2Nmv	M_2	nil	nil	nil
K	nil	n,Cfl & Nfl-Nst	n, Nfl-Nst	n, Cfl,Nst	n, Cfl	M_2,Nfl	nil	nil	nil	nil
M	nil	n,Nst,D	n, D	n, M_2-D	n, D	M_1	M_1	nil	nil	nil
4961	Cfl	M_2,Nfl	M_2Nmv	M_1	M_1	M_1	M_1,Nfl	Cst	Cfl	M_1, Nfl
4975	M_1	M_1	M_2	M_1	n,M_2,Nfl	M_2,Nfl	M_1	M_1	M_1	nil

Key to symbols:

Inoculated leaf symptoms: n= Necrotic or red lesions; v= Veinal reddening; gn= General necrosis

Systemic symptoms: C= Chlorosis; $M_{(1 \text{ or } 2)}$= $\text{Mosaic}_{(\text{mild or severe})}$; N= Necrosis; D= Death of plant; S= Severe stunting; fl= Flecks; st= Streaks; Nmv= Necrotic midvein; Cst(n)= Chlorotic streak with necrotic centres; nil= No symptoms observed

4961 and 4975 are isolates from Pakistan sugarcane, probably containing SCMV-F

Table 23. Reaction of sorghum lines to SCMV from Co 671 sugarcane variety collected from Gujarat under controlled growth conditions

Sl No.	Sorghum lines	Symptoms		SCMV strains identified
		Local	Systemic	
1.	NM 31	Nil	Cfl	SCMV-K
2.	QL 11	Nspt	M_1,Nfl, Nmv	SCMV-F*(4961)
3.	BTx398	Nmv, Nspt	M_2	SCMV-I
4.	SA 8735	Nil	Nil	-
5.	OKY 8	Nil	Nil	-
6.	BTx3197	Nil	Nmv, Nspt	-
7.	Johnsongrass	Nil	M_2	SCMV-A
8.	SC0097-14E	Nspt	M_2,Cst,Ivc,Cspt	-
9.	Atlas	NLL, Nmv	M_2, Nst	SCMV-H
10.	Rio	NLL, Nspt, Nmv	Cst, M_2	SCMV-E
11.	Q 7539	Nil	Nil	-

Key to symbols:

Cspt= Chlorotic spots; Cst= Chlorotic streaks; Cfl= Chlorotic flecks; Ivc= Interveinal chlorosis; Nst= Necrotic streaks; Nspt= Necrotic spots; Nfl= Necrotic flecks; Nmv= necrotic midvein; M_1= Mild mosaic; M_2= Severe mosaic, NLL= Necrotic local lesions; Nil= No symptoms

* SCMV-F isolate 4961

Table 24. Reaction of sorghum lines to SCMV from Co 671 sugarcane variety collected from Coimbatore under controlled growth conditions

Sl No.	Sorghum lines	Symptoms		SCMV strains identified
		Local	Systemic	
1.	NM 31	Nil	Cfl	SCMV-K
2.	QL 11	Nspt	M_1,Nfl,Nmv	SCMV-F (4961)*
3.	BTx398	Nspt	M_2	SCMV-I
4.	SA 8735	Nil	Nil	-
5.	OKY 8	Nil	Nil	-
6.	BTx3197	Nil	M_1	-
7.	Johnsongrass	Nil	M_2	SCMV-A
8.	SC0097-14E	Nspt	M_1	SCMV-F(4975)*
9.	Atlas	NLL, Nmv	M_2, Nst	SCMV-H
10.	Rio	Nmv, Nspt, Gn	M_2, Cst	SCMV-E
11.	Q 7539	Nil	Nil	-

* Isolate 4961 and 4975 of SCMV-F strain

Key to symbols:

Cspt= Chlorotic spots; Cst= Chlorotic streaks; Cfl= Chlorotic flecks; Nfl= Necrotic flecks; NLL= Necrotic local lesions; Nst= Necrotic streaks; Nsp= Necrotic spots; Nmv= Necrotic mid vein; Gn= General necrosis; M_1= Mild mosaic; M_2= Severe mosaic

Table 25. Reaction of sorghum lines to SCMV from Co 859 sugarcane variety, collected from Coimbatore, under controlled growth conditions

Sl No.	Sorghum lines	Symptoms		Strain identified
		Local	Systemic	
1.	NM 31	Nil	Nil	-
2.	QL 11	Nspt	M_1, Nfl	SCMF-F(4961)*
3.	BTx398	Nil	Nil	-
4.	SA 8735	Nil	Nil	-
5.	OKY 8	Nil	Nil	-
6.	BTx3197	Nil	Cdt, NSpt	-
7.	Johnsongrass	Nil	M_2	SCMV-A
8.	SC0097-14E	Nil	Nil	-
9.	Atlas	NLL, Nmv	M_2, Nst	SCMV-H
10.	Rio	Nmv	M_1	-
11.	Q 7539	Nil	M_2	SCMV-F(4975)*

* Isolates 4961 and 4975 of SCMV-F strain

Key to symbols:

Cdt= Chlorotic dots; Nfl= Necrotic flecks; NSpt= Necrotic spots; Nmv= Necrotic mid vein; NLL= Necrotic local lesion; Nst= Necrotic streaks; M_1= Mild mosaic; M_2= Severe mosaic

Table 26. Strainal differentiation of three SCMV isolates collected from Gujarat and Coimbatore

Sl No.	Differential host	Characteristic symptoms	SCMV isolates		
			Co 671 (Gujarat)	Co 671 (Coimbatore)	Co 859 (Coimbatore)
1.	Johnsongrass	Severe mosaic	SCMV-A	SCMV-A	SCMV-A
2.	Rio	Necrosis on inoculated leaf and chlorotic streaks	SCMV-E	SCMV-E	-
3.	QL 11	Mild mosaic and necrotic flecks	SCMV-F (4961)	SCMV-F (4961)	SCMV-F (4961)
4.	Q 7539	Mild mosaic	-	SCMV-F (4975)	SCMV-F (4975)
5.	Atlas	Severe mosaic and necrotic streaks	SCMV-H	SCMV-H	SCMV-H
6.	BTx398	Severe mosaic	SCMV-I	SCMV-I	-
7.	NM 31	Chlorotic flecks and necrosis on inoculated leaves	SCMV-K	SCMV-K	-
Total no. of strains			6	7	4

Plate 26. SCMV-A strain causing severe mosaic symptoms on johnsongrass

Plate 27. The Rio sorghum reacted by producing chlorotic streaks, characteristic of SCMV-E strain

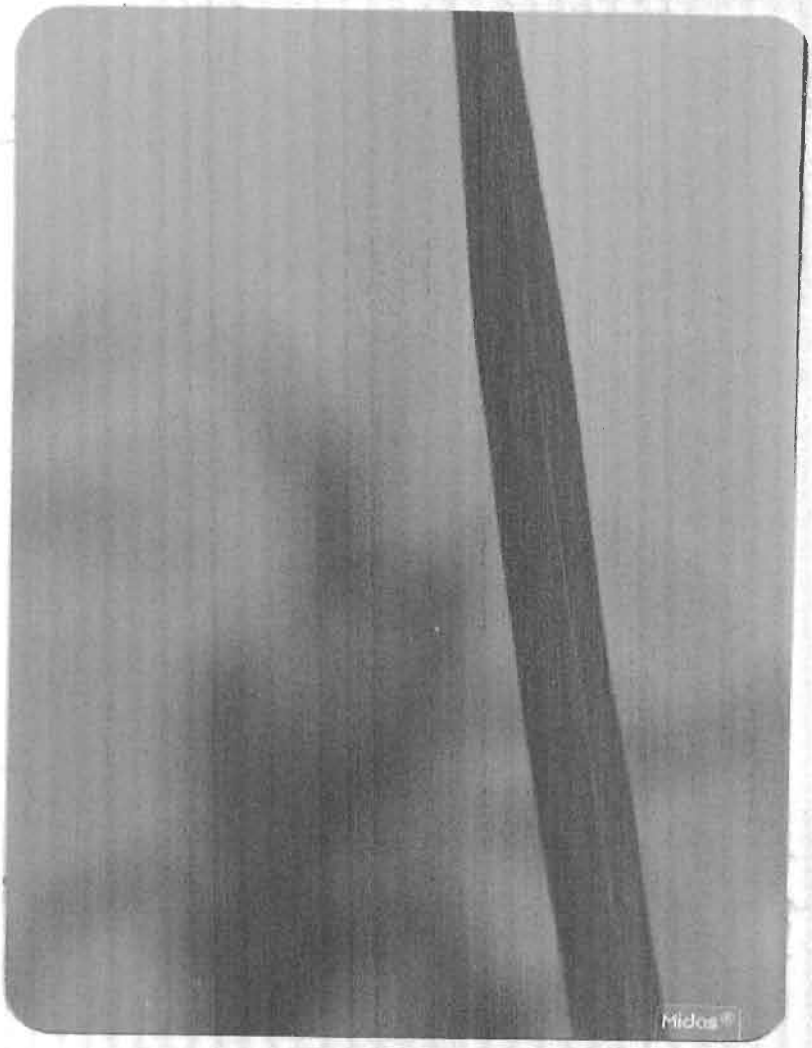


PLATE-26

PLATE-27

Plate 28. SCMV-H strain produced severe mosaic and nectrotic streaks symptoms on Atlas sorghum

Plate 29. SCMV-I strain caused severe mosaic symptoms on BTx398 sorghum line

PLATE-28

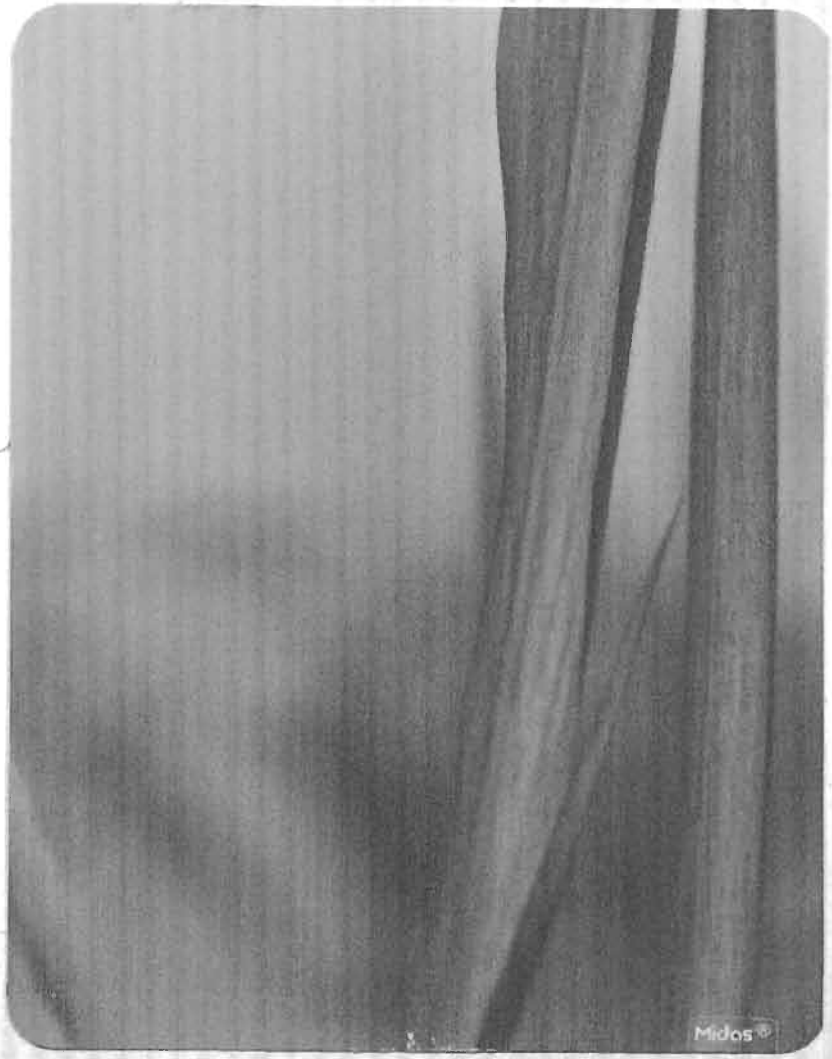


PLATE-29

Plate 30. SCMV-K strain produced chlorotic flecks on NMx31 sorghum line

Plate 31. SCMV-F 4961 isolate produced mild mosaic and necrotic flecks on QL-11 sorghum line

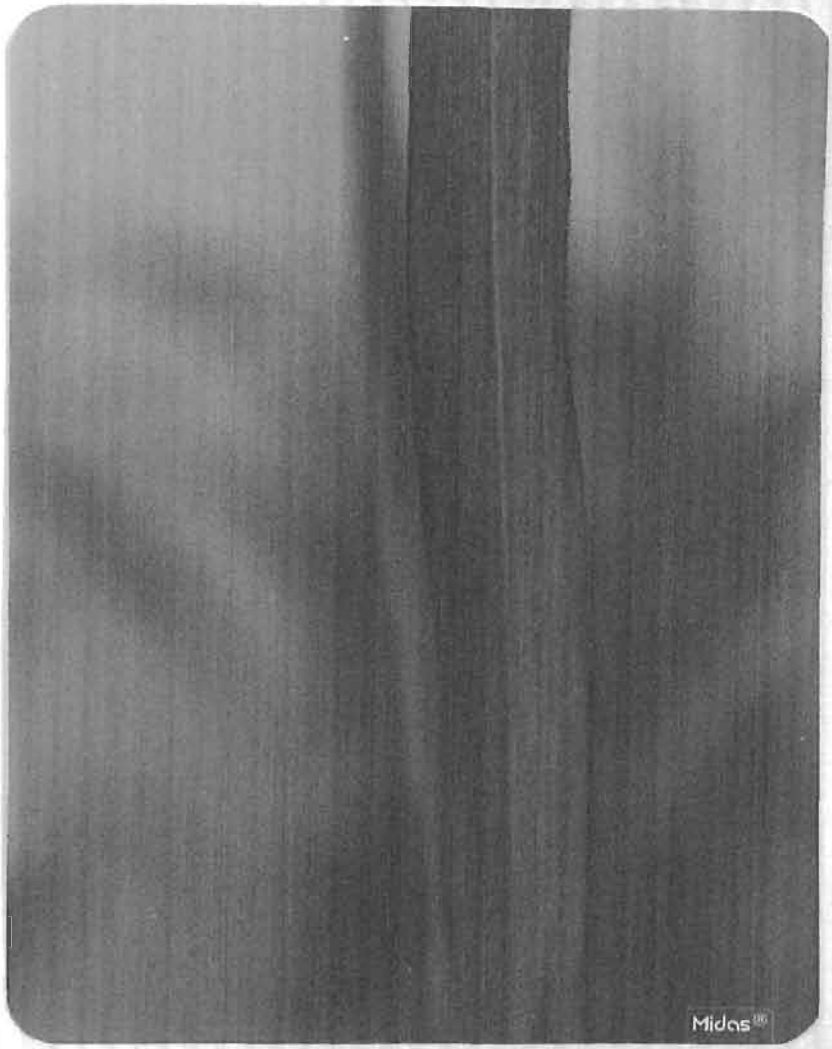


PLATE-30

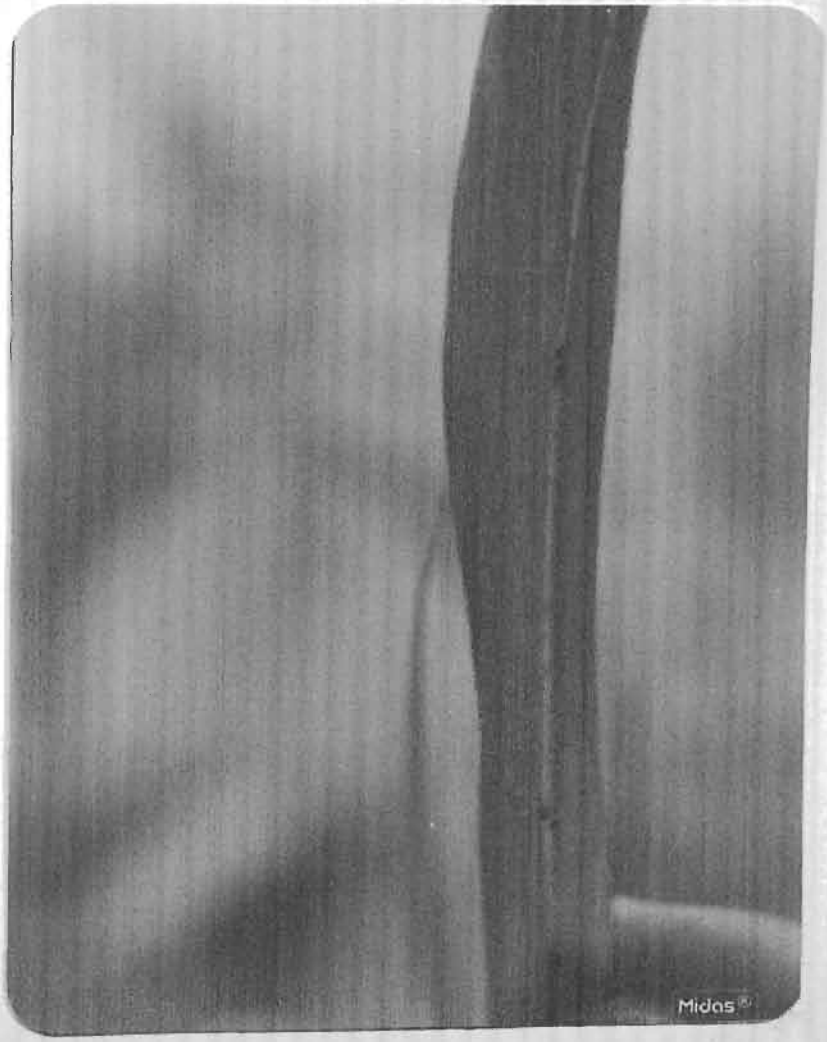


PLATE-31

Table 27. Reaction of three different SCMV isolates on four additional sorghum lines

Sl No.	Differential host	Symptoms produced by 3 SCMV isolates		
		Co 671 (Gujarat)	Co 671 (Coimbatore)	Co 859 (Coimbatore)
1.	Martin	NSpt, M_2, Nst	M_1	M_2, NSpt, Cdt
2.	Martin-40	CSpt, M_2	M_2, NSpt	Nil
3.	Martin-5330	NSpt, IVc	M_1	M_2, NSpt, Cdt
4.	QL-3-Tx	NSpt, M_2	NSpt, M_2, Nmv	Nil

Key to symbols:

Cdt= Chlorotic dots; CSpt= Chlorotic spots; IVc= Interveinal chlorosis; NSpt= Necrotic spots; Nst= Necrotic streaks; Nmv= Necrotic mid vein; M_1= mild mosaic; M_2= Severe mosaic; Nil= No symptoms

Plate 32. SCMV-F 4975 isolate produced mild mosaic symptoms on Q 7539 sorghum line

Plate 33. Martin-40 sorghum line produced severe mosaic when inoculated with SCMV-Guj. isolate

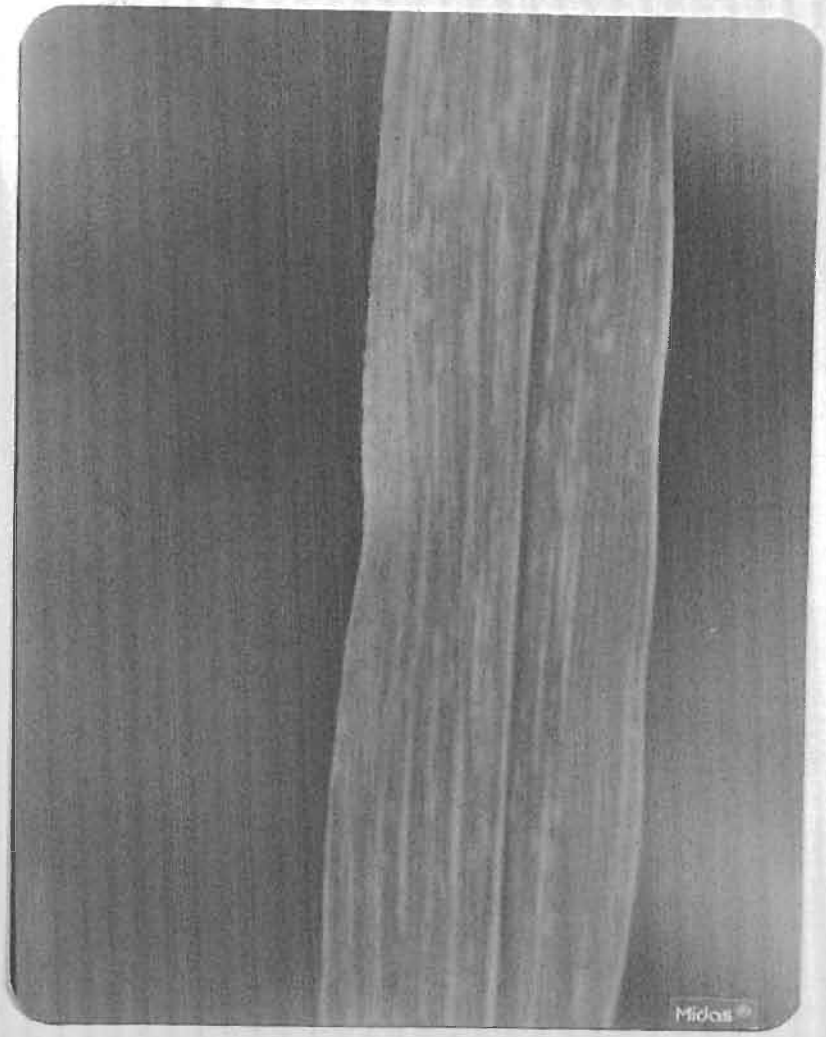


PLATE-32

PLATE-33

isolates (Table 27; Plates 33 and 34). It may be noted that Martin-40 and QL-3-Tx did not produced any symptoms when inoculated with SCMV Co 859 Coimbatore isolate. The two other SCMV isolate infecting Co 671 variety from Gujarat and Coimbatore induced mosaic symptoms on all the four sorghum lines, except Martin-5330 inoculated with Co 671 infecting, SCMV isolate from Gujarat.

From Co 671 variety six SCMV strains namely, SCMV-A, E, F 4961, H, I and K were identified from Gujarat and Coimbatore. In addition, the SCMV isolate from Coimbatore also gave indication of presence of SCMV-F 4975 strains. The Co 859 SCMV isolate from Coimbatore indicated the presence of only 3 strains i.e. A, F and H including both 4961 and 4975 isolates of F strain.

Plate 34. QL-3-Tx reacted by producing severe mosaic and necrotic spots,when inoculated with SCMV-Gujarat isolate

PLATE-34

5. DISCUSSION

The cultivation of 'ikshu' or 'ikh' or sugarcane in India dates back to 1500 B.C. to 1000 B.C. Bhargava (1975) pointed out that these plants have been depicted in the paintings of Ajanta and Ellora. These facts indicate that sugarcane has been a important plant, cultivated in India for centureis from prehistoric times. Besides other historical evidences to show that India is the home of sugarcane, the sugar ('Sharkara') making process is known to have originated here and travelled eastward towards Malaysia and China and Westward to Persia and beyond.

Sugarcane is a favoured host of a large number of diseases caused by Fungi, bacteria, mycoplasma, and viruses. Amongst these diseases, sugarcane mosaic is the most widewpread. It has all the potentialities of being a threat to the sugarcane cultivation, through the development of more pathogenic strains (Chona, 1958). At one time, between 1920-27, it has threatened the sugarcane industry in Lousiana, U.S.A., inflicting heavy yield losses to the extent of 30 to 46 per cent.

In fact, in absence of prominent symptoms the disease remains unnoticed, as Dean (1974) during a tour to Pakistan observed very high percentage of SCMV infection as against the opinion of the officials of the Ministry of Agriculture that mosaic does not occur in Pakistan. Dean (1974) pointed out that the percentage of loss due to mosaic may be very small but when applied to the total cane area of the country, the loss is enormous.

The sugarcane mosaic disease, which has been identified in almost all the major sugarcane growing regions of the country, is characterized by such symptoms as elongated yellow chlorotic streaks interspersed with light and dark green patches, as noticed on susceptible cultivars like Co 671, Co 740, Co 859 and Co 1158.

In a recent survey of the South Gujarat, sugarcane cultivar Co 671 had more than 80 per cent plants infected with sugarcane mosaic virus (SCMV) causing severe chlorotic symptoms, widely prevalent during premonsoon and monsoon period. The new tillers from infected plants may also display milder symptoms, that may disappears on older leaves in winters.

The virus culture was originally collected on Co 671 sugarcane from Malanpur area of South Gujarat for the present investigations. The other mosaic infected sugarcane varieties namely Co 671, Co 859 and Co 1158 were also obtained from Coimbatore for the occurrence of strains. All these samples indicated similar severe symptoms and affected most of the susceptible varieties in different regions. According to Bhargava (1975) such symptoms are most prominent during monsoon season only. The SCMV culture was maintained on Co 671 cultivar in the insect proof glasshouse and also in microplots outside, all along the course of these investigations. These plantings indicated severe symptoms, all the year around except in winters. This isolate of SCMV is referred as SCMV-Gujarat isolate.

The loss of chlorophyll, generally, as a result of SCMV infection leads to the loss of effective area for photosynthesis resulting in the stunted growth. The overall damage due to SCMV infection varies

greatly according to variety and the strain of SCMV. In general, clones of *Saccharum officinarum* L. varieties are highly susceptible to mosaic; resulting in total failure of the crop as in U.S.A. in 1920, when noble canes were widely grown. *S. barberi* Jesw. clones are equally susceptible but suffer less severe damage. *S. spontaneum* L. and *S. sinense* (Roxb.) Jesw., on other hand, are highly resistant, but most of the hybrids of *S. sinense* (Roxb.) Jesw. possess undesirable traits. *Saccharum acclarum* and *S. spontaneum* L. therefore, has long been the gene bank for resistance genes to the mosaic disease in commercial varieties (Blackburn, 1984).

Sugarcane mosaic virus in addition to sugarcane also infects a variety of cultivated crops such as maize (Ordosgoitty and Malaguti, 1969; Lastra and Trujillo, 1978; Klein et al., 1973; Rishi, 1973; Louie, 1980; Allam et al., 1981; Wechmer et al., 1984; Martin et al., 1984), sorghum (Rishi et al., 1973; Klein et al., 1973; Benigno and Veqgard, 1977; Stokes et al., 1982; Henzell et al., 1982; Garud and Mali, 1983; Derrick et al., 1984), wheat, barley, rye and rice (Abbott and Tippett, 1964), bajra (Rishi et al., 1973), johnsongrass (Klein et al., 1973), ragi (Subbayya and Raychaudhuri, 1970) and bamboo (Benda, 1970). In addition, numerous other cultivated and wild grasses are also the host for the sugarcane mosaic virus.

During the course of the present study, all the ten varieties of sorghum namely, Surat-1, M-234, GJ-35, GJ-37, BP-53, Tukadi, Desi white, Chhasathio, Solapuri, CSH-9 were found to be susceptible and reacted by producing severe mosaic symptoms. Six varieties of maize namely, Ageti-76, Bari local, Deccan-103, MDR-1 and inbred lines CM-110 and CM-600 were found totally immune to the SCMV-Gujarat isolate.

Also the three bajra varieties viz. WCC-75, BK-560-280 and MBH-110 with four rice varieties viz. Saket-4, IR-8, TN-1 and Jaya were also found to be immune to SCMV-Gujarat isolate. Sorghum, therefore, is the principal host affected by the Gujarat isolate of SCMV.

Sugarcane mosaic virus has been reported to be transmitted by number of aphid species. During present investigations *Aphis craccivora* (Koch), *Rhopalosiphum maidis* (Fitch.),and *Melanaphis sacchari* (Znht.), which were abundant in nature during the October sugarcane planting season were tested for their efficiency as SCMV vector. All the three aphid species were found to be potent vectors with percentage of transmission as 48, 40 and 30 per cent with *A. craccivora*, *R. maidis* and *M.sacchari*, respectively.

In Australia, Teakle and Grylls (1973) found that *A. craccivora* (Koch) and *Rhopalosiphum maidis* (Fitch) transmitted all the four SCMV strains viz., Johnsongrass strain, sugarcane strain, *Urochloa mosambicensis* strain and *Digitaria didyctyla* strain. Young et al. (1984) found *Aphis craccivora* (Koch) as one of the seven species of aphid that were most abundant on sugarcane. The colonization of the *Rhopalosiphum maidis* (Fitch) into the protected central whorl of leaves of sorghum and other hosts, makes it an important vector of SCMV. The aphid species *Melanaphis sacchari* (Znht.) was observed to colonize on leaf surface of the sorghum only superficially, and therefore did not have the advantage as that of *R. maidis*. Most of the aphids may not be important as regular pests of sugarcane, but they play an important role in transmission of mosaic disease. Aphid transmission of potyviruses in general is influenced by many factors, and different aphid species

transmit a potyvirus with different levels of efficiency (Hollings and Brunt, 1981).

During the course of present investigations, five different purification schedules were tested for isolating SCMV particles to study their morphology and biochemical and biophysical properties besides preparing the antiserum. Only two of these procedures gave excellent yield of purified SCMV. The other purification procedures, those of Lagenberg (1973), Gough and Shukla (1981) and a modification of Gough and Shukla (1981) did not yield purified SCMV. The procedure of Gough and Shukla (1981) used 2 cycles of differential centrifugation with overnight suspension of the high speed pellets, and density gradient centrifugation, before putting the suspension over 10-40 per cent sucrose gradients. In the modified protocol, the second cycle of differential centrifugation was eliminated altogether as no virus containing bands were detected in the sucrose gradients layered of the second cycle.

The procedure of Rishi and Rishi (1985), carried out in its original format, also did not yield purified SCMV. The most likely reason being the inadequate centrifugation time during 2 cycles of high speed centrifugation for pelleting of the sugarcane mosaic virus (25,000 rpm for 1.5 h). The centrifugation time was extended to 3 h ($t = \frac{K}{S} = \frac{346.7}{176} = 1.87$ h) considering reported sedimentation coefficient (176±5S) of SCMV, and the K factor of 346.7 at the rotor speed of 25,000 rpm. Besides, some other minor changes were introduced in the modified protocol. The SCMV yield of the final preparation was 9.81 mg of SCMV/kg of infected tissue. The best results were, however, obtained (a yield of 22.6 mg of SCMV/kg of infected tissue), in the

procedure-V, wherein preformed cesium sulphate density gradients were used. SCMV suspension after polyethyleneglycol precipitation was floated on 15 to 30 per cent Cs_2SO_4 gradients, prepared by dissolving appropriate quantities of Cs_2SO_4 in 10 per cent sucrose solution. The higher density of the gradients prevented the SCMV to settle down at the bottom as observed in 10 to 40 per cent sucrose gradients. A clear distinct virus peak was observed, well separated from the host component (Figure 19). The second cycle of preformed Cs_2SO_4 density gradient centrifugation yielded even purer SCMV preparation (Figure 19) which was used to inject rabbits for the production of the antiserum. By using preformed cesium chloride density gradient during second cycle, SCMV travelled further to form a band at a lower depth in the column as compared to that of cesium sulphate gradients (Figure 20). This is perhaps because of the lower density of cesium chloride solution. The preformed cesium sulphate gradients have also been used for the purification of citrus tristeza virus - aphloem limited closterovirus (Lee et al., 1987).

On the top of the Cs_2SO_4 gradient column, layering of 2 ml pad of 10 per cent sucrose resulted in considerable clarification of the SCMV containing sap layered at the top of the entire column.

The SCMV was also recovered from the reprocessed supernatant of polyethyleneglycol precipitation, step no. 9 of the protocol procedure-V (Figure 21). It is, therefore, advantageous to reprocess the supernatant so as to avoid substantial loss of SCMV at this stage of the schedule.

The difficulty in purifying potyviruses are often attributed solely to the aggregation of particles after their extraction from infected plant tissues and their subsequent loss in low speed centrifugation (Brunt, 1988). In fact, there are two major groups of potyviruses-one, that is easy to purity and second, dificult to purify with low yield. The SCMV belongs to later group (Hollings and Brunt, 1981).

Serology of plant viruses has contributed immensely to our knowledge of diversity of viruses, and have played major role in rapid and specific detection (Bos, 1983). Antisera have been produced for several strains and isolates of sugarcane mosaic virus (Pirone, 1972) with varying titers. The antiserum was also produced against the SCMV-Gujarat isolate by administering one intravenous and three intramuscular injections at weekly intervals. The SCMV-Gujarat isolate is a good immunogen. The titer of the SCMV antiserum was estimated by employing ring interface, tube precipitin, microprecipitin, and IEM-decoration tests. The titer of the antiserum was 1:512 as determined by ring interface, and tube precipitin tests at the antigen dilution of 1:4 and 1:8 and 1:16, respectively. In microprecipitin test the precipitin reaction was clearly detectable under binocular microscope up to 1:1024 dilution of SCMV antiserum, at 1:16 and 1:32 dilution of the antigen-diseased plant extract.

Immune electron microscopy combines techniques of electron microscopy with serology, involving detection of complexes of virus antigen and specific antibodies reaction at particulate level. Three basic techniques widely used are 'trapping' on serum activated grids, 'decoration' and 'clumping'. Decoration refers to coating of virus particles already on the grid with specific antibodies and is used to confirm serological

identity of unknown viruses or separating morphologically identical viruses in mixture (Hill, 1984).

During the present investigations the virus particles on the grid were decorated using different dilutions of antiserum. Electron microscopic examinations of these grids indicated decoration of SCMV particles up to antiserum dilution of 1:512. This IEM-decoration technique is considered to be a sensitive technique for detection of virus present in low concentration, usually one or two fold step higher than gel diffusion or precipitin titer. For this test, 1:4 dilution of crude extract from SCMV infected Co 671 sugarcane leaves were taken to layer the virus on the grid for further decoration treatment. Gough and Shukla (1980) observed that precoating electron microscope grids with protein-A before coating them with antiserum enhanced the number of trapped SCMV particles by 25.7 fold, over those on the grids treated with specific antiserum alone.

Enzyme-Linked Immunosorbent Assay (ELISA) is an extremely sensitive technique. The technique has now been used increasingly for detecting SCMV (Jarjees and Uyemoto, 1984; Gillaspie and Harris, 1987). Devergne et al. (1982) detected 1 ng/ml concentration of SCMV-D strain.

For the present studies, DAS-ELISA technique was employed for detection of SCMV in different tissues of sugarcane and sorghum. A conjugate with alkaline phosphatase was prepared from partially purified y-globulin fraction of the antiserum. In order to optimize test conditions, four dilutions of SCMV antibody-enzyme conjugate and three concentrations of coating antibodies were tested. For the standard test conditions

0.1 µg/ml concentration of SCMV antibodies for coating, and 1:2000 dilution of conjugate were used to detect the relative titer of the SCMV in different parts of infected Co 671 sugarcane and CSH-9 sorghum plants at three different 10-fold dilutions of the diseased plant extract.

The relative absorbance of each well at 405 nm indicated invariably significantly higher titer of SCMV in all the tissues of Co 671 sugarcane as compared to that of sorghum. The SCMV titer was estimated in roots, leaves and stem tissues of both the plant species. The sugarcane, roots invariably indicated significantly higher titer at all the three dilutions tested. It is followed by stem and leaves. These findings are contrary to Rishi and Rishi (1985). They indicated minimum concentration in roots of old mature plants and maximum in youngest leaves. However, as they have not furnished details of ELISA data it is difficult to compare the results.

In the case of CSH-9 sorghum, though roots and leaves gave significantly higher SCMV titer, at times the SCMV in stem remained undetected.

Gillaspie and Harris (1987) studied six SCMV strains, viz., SCMV-A, B, D, E, H and I using ELISA and suggested four groups from their tests and also data from Jarjees and Uyemoto (1984). Their groupings are: (1) SCMV-J, (2) SCMV-A, B,D & K, (3) SCMV-E and (4) SCMV-H, I, M. However, they were of opinion, that serological methods cannot be used independent of differential host test to identify SCMV strains.

The blocking of well surface of ELISA microtiter plates with caesin were generally attempted to prevent nonspecific adsorption of

conjugate to well surface. In the present investigation milk powder at 5 per cent solution in PBS-Tween was used. It has proportionately reduced the absorbance of wells under each treatment, so much so that the absorbance of wells having samples from diseased and unknown were at par. Since blocking has uniformly affected, by reducing the absorbance of diseased and the unknown at 405 nm, it seems to have no advantage in this particular test situation of DAS-ELISA.

The cross absorption with healthy plant extract is usually employed to prevent nonspecific serological reactions. During the present investigation attempts have been made to assay an unknown sugarcane plant, with no apparent SCMV symptoms; by using its extract for cross adsorption of SCMV-conjugate. Scanning of wells at 405 nm indicated significant elemination of absorbance in case of wells loaded with both diseased and the unknown plant extracts to the zero absorbance as obtained with PBS-tween control. These results indicate the presence of SCMV antigen in the symptomless sugarcane plant. The absence of symptoms may be attributed to the cold weather conditions of Delhi, as symptoms were later observed on the same very plants with the advent of warmer climatic conditions.

The morphology of SCMV particles as revealed by electron microscopy indicated long, flexous, thread like particles typical of potyviruses with length varying from 600-880 nm and an average width of 15.4 nm. The modal length of these flexous rod shaped particles was 750 nm. In most of the preparations the particles were intertwined, clumped and aggregated.

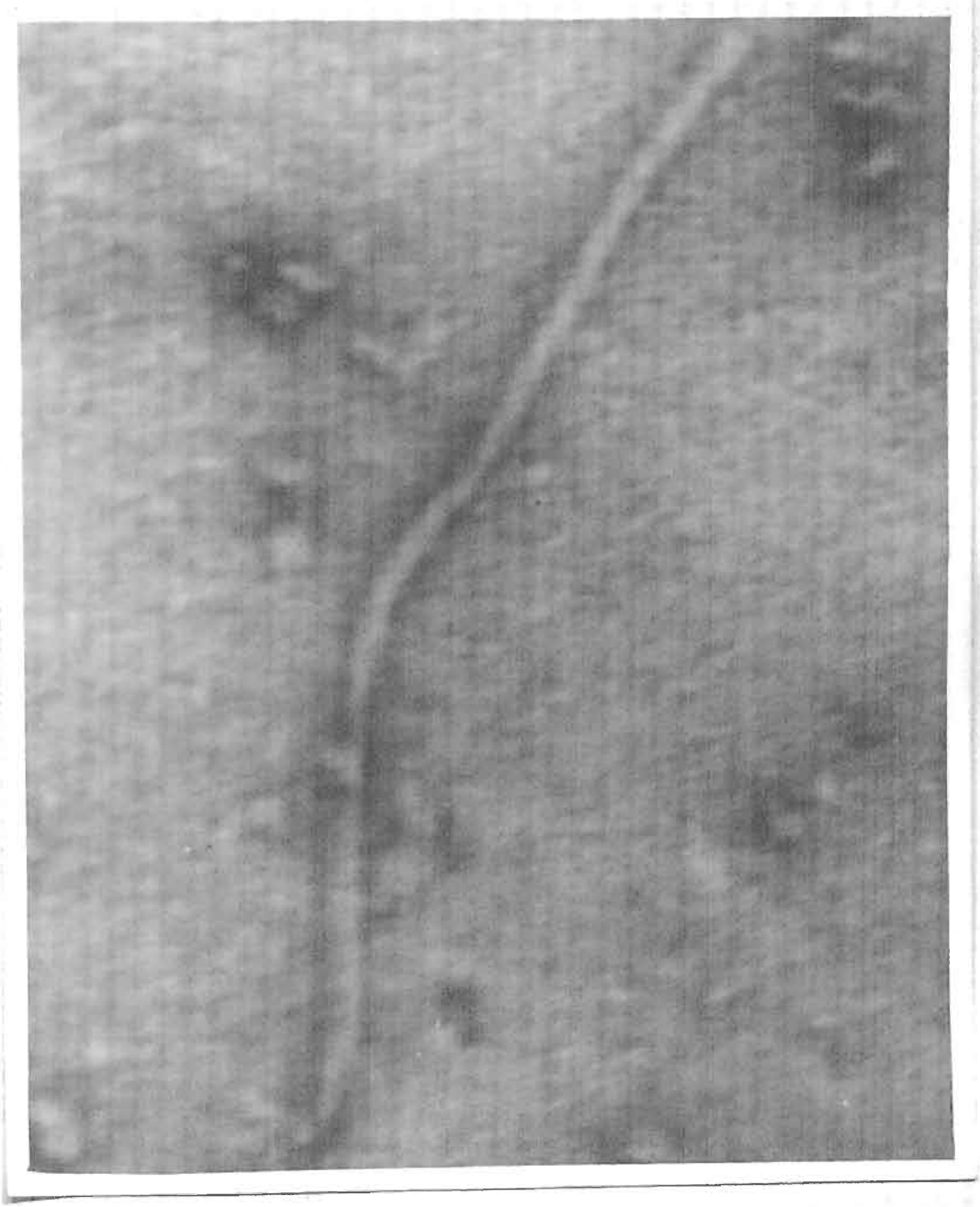
These particles were stained with uranyl acetate which is known to swell or partially desrupt the particles. Wide variation in length of the virus particles may be due to breakage of these particles during different purification stages. The earlier published reports regarding the particle morphology of the sugarcane mosaic virus indicated SCMV to be rodshaped(Gold &Martin,1955; Paliwal and Raychaudhuri, 1966), although subsequently published reports record the flexous rod shaped morphology with typical 750 nm potyviral length (Anonymous, 1968; Tosaic et al., 1978; Chen, 1978; Liu, 1979; Moghal and Francki, 1981; Rishi and Rishi, 1985; and Giorda, 1986). The width of the particles recorded on CMI/ A.A.B. discriptions of plant viruses No. 88, 1972, was 13 nm. Rishi and Rishi (1985) used 2 per cent PTA and recorded 14 nm width. The measurement of the width during present investigations indicate some degree of swelling of the particles as a result of the use of 2 per cent UA.

Though the details of the helical substructure was not clearly made out, the individual capsomeres were discernible (Plate 35). The central canal was also indistinguishable, probably because the capsomeres were so compact as not to allow stain to penetrate the particle.

The physicochemical properties of the purified sugarcane mosaic virus-Gujarat isolate, were studied during the course of this investigation. The ultraviolet absorbance profile of the purified SCMV had the maximum absorbance at 260 nm and minimum at 250 nm with little bump at 280 nm. The UV absorbance profile is characteristic of nucleoprotein. The A260/A280 ratio of purified SCMV (absorbance corrected for light scattering) was 1.18, and A max./A min. ratio as

Plate 35. The SCMV particle showing capsomeres and have no central canal (x 1,87,068)

PLATE-35



1.09. These values are in conformity with the range, characteristic of nucleoproteins containing 5-6 per cent nucleic acid. Rishi and Rishi (1985) published the range of variation in 260/280 ratio as 1.2-1.4 in case of sucrose gradient purified material, which appears to be obtained from absorbance values not corrected for light scattering. The uncorrected 260/280 ratio, obtained during the present investigation was 1.329, close to Rishi and Rishi's (1985).

The SCMV particles had 5.5 per cent RNA contents as determined by two-different methods (Layne, 1957 and Gibbs and Harrison, 1976). This calculated RNA percentage of SCMV is similar to the one reported for potato virus Y (Veerisetty, 1978), a type member of the potyvirus group.

The extinction coefficient ($E260_{1}^{0.1\%}_{cm}$) of the purified SCMV was also calculated on the basis of RNA% and found to be 2.658, on the basis of absorbance values corrected for light scattering. The extinction coefficient (E260 mg cm^{-3}) of only a few potyviruses has been determined experimentally, but all reported values are within the range of 2.4 to 2.9. By drying and weighing samples of known absorbance at 260 nm, tobacco etch virus (Purcifull, 1966) was found to have extinction coefficient of 2.40. From the aminoacid composition and assumed value of nucleic acid content of 5 per cent, extinction coefficient of 2.86 was established for PVY by Stace-Smith and Tremaine (1970).

The sedimentation coefficient of the SCMV was determined by band sedimentation velocity run of the SCMV particles in analytical ultracentrifuge (Sussman, 1975). The SCMV particles sedimented as single nondispersed component. The sedimentation coefficient value of SCMV

was found to be 166 svedberg units. The sedimentation coefficient (Svedbergs) values, earlier published for SCMV are 168±6 (Shepherd, 1965), 155±3 (Bancroft et al., 1966), 148±2 (Sehgal, 1968) and 160-162 (Jones and Tolin, 1972), seems to vary with different strains. The sedimentation coefficient of other potyviruses ranged from 142-154 S (Hollings and Brunt, 1981). The lower values within this range are reported to be produced as a result of relatively high concentration of broken particles during purification (Barnet and Alper, 1977).

The purified SCMV Gujarat isolate, particles had a density of 1.2988 g/ml which is also in close conformity of 1.3245 g/ml value, published for maize dwarf mosaic virus (Sehgal and Jean, 1970), a serologically very closely related virus to SCMV.

The partial specific volume ($\bar{V}$) of 0.76 obtained for purified SCMV, is indicative of the fact that SCMV nucleoprotein particles contains ssRNA (Gibbs and Harrison, 1976).

The coat protein subunit of SCMV obtained by disrupting virus particles with 4M LiCl and precipitation with 5 volumes of cold acetone containing 10 mM of 2-mercaptoethanol. The capsid protein migrated as a single band when electrophoresed in SDS-polyacrylamide gel, indicating it to be a single polypeptide. By coelectrophoresis with pharmacia's low molecular weight protein markers, its molecular weight was measured to be 40,500 daltons. Gough and Shukla (1981) reported the molecular weight for the four Australian Sugarcane mosaic virus strains, ranging from 33,700 to 40,300 daltons. Jensen et al. (1986) from USA estimated the molecular weight of the capsid proteins of SCMV-A, B, H and M strains, as 36,900, 36,700, 39,100 and 37,400 daltons, respectively. The

40,500 daltons weight of capsid protein of SCMV-Gujarat isolate is close to that of Sabi strain (Mol. wt. 40,300 daltons) reported from Australia (Gough and Shukla, 1981).

The SCMC-RNA was also isolated from purified SCMV using Kirby's modified procedure. The ultraviolet absorbance profile indicated maximum absorbance at 265 nm and minimum at 235 nm. As the A 260/A 280 ratio was 1.34, the SCMV-RNA preparation appeared to be uncontaminated by protein. The sedimentation coefficient of SCMV-RNA was determined by boundary sedimentation velocity run in Beckman L8-60M ultracentrifuge, and found to be 65 Svedberg units.

In the case of PVY-RNA, Pring and Lagenberg (1972) and Makkouk and Gumpf (1974), the sedimentation coefficient was recorded as 39S and 24S respectively. Such lower values as 24S, 32S and 34S of viral-RNA species have been reported as in the case of TMV by Loening (1969) depending on the medium he used for suspending the TMV-RNA. During present investigations SCMV-RNA was suspended in sterile distilled water and the higher value of sedimentation coefficeints in this case may in particular be due to large number of intact particles which is generally difficult to achieve in the case of such a long particles as those belongs to potyvirus groups. It appears from the extremely low sedimentation coefficient values of PVY-RNA that their preparation of viral nucleic acid may have been degraded or RNA was isolated from broken particles.

The molecular weight of purified SCMV-RNA from its sedimentation coefficient using Sussman's formula was found to be 3.455×10^{-6} daltons. The sedimentation coefficient or molecular weight

of any other strain of SCMV-RNA has so far not been reported. For example, PVY-RNA has sedimentation coefficient of 39 and 24 S respectively (Pring and Lagenberg, 1972; Makkouk and Gumpf, 1974). However, Loening (1969)estimated the 24S, 32S and 34S for the TMV.

All the potyviruses so far examined have been shown to contain single stranded RNA. The nucleic acid of several of these viruses have been isolated. From amongst these tobacco etch virus is most thoroughly studied.

The SCMV-RNA was further analysed by electrophoresis in 0.6 per cent agarose horizontal slab gel. The purified SCMV-RNA did not move at all in the native state when electrophoresed at 50 Volts for 1.5 h. On denaturation by treatments with heat (60°C), urea (3 M), and formamide (36%), the SCMV-RNA moved 2.8 cm from the edge of loading wells as a single band. The denaturation treatment with glyoxal has resulted in further movement of SCMV-RNA as a single band. The denaturation treatment with glyoxal has resulted in further movement of SCMV-RNA as a single band to 4.7 cm from edge of the loading well, when electrophorsed at 30 Volts for 2 h, in 1% agarose gel. Electrophorsis of the denatured SCMV-RNA also moved as a single band in verticle polyacrylamide slab gel.

As the electrophoretic mobility of nucleic acids in agarose gel is affected by secondary and tertiary structures, the effect of denaturing agents on the electrophoretic mobility suggest that the SCMV-RNA molecule have certain degree of secondary structures involving base pairs. Glyoxal which is reported to react with all the bases of the nucleic acids in probably more effective in linearizing the RNA molecule, and hindering the formation of GC pairs leading to denaturation of native forms as demonstrated by Hutton and Wetmur (1973).

Ethidium bromide and silver stains are commonly used as sensitive stains for detecting nucleic acid in the gels, but these dyes cannot distinguish between single and double stranded RNA. Acridine orange which has been employed as cytochemical stain has shown to differentiate single and double stranded RNA and DNA, in situ (Mc Master and Carmichael, 1977). The acrydine orange stain was used to visualize the SCMV-RNA bands in the 1 per cent agarose gel. The band flouresced red colour when viewed with incident UV illumination indicative of its being ssRNA.

Almost all the member of potyvirus groups induce formation of proteinaceous inclusion bodies (Holling and Brunt, 1981). Edwardson (1974) separated potyviruses into 3 groups according to the inclusions induced by them. Crystalline nuclear inclusions are one of such inclusions which are stable in vitro and could be readily extracted and clarified from the infected plants (Knuhtsen et al.,1974). Attempts were made to isolate such inclusions from Co 671 sugarcane infected by SCMV-Gujarat isolate. SCMV induced rectangular crystalline formation measuring from 150-350 nm in width x 500-1050 nm in length as observed in vitro under electron microscope after their isolation from sugarcane. These were coelectrophoresed with Pharmacia's low molecular weight marker in SDS-PAGE for estimating the molecular weight of the SCMV crystalline inclusion protein which had the molecular weight of 62,000 daltons. In addition, another protein species of molecular weight 38,000 daltons was also visualised in the gels. Jensen et al. (1986) also obtained a single species of protein. The estimated molecular weight of such inclusion protein species, isolated from different sugarcane specimens

infected with SCMV-A, B, H and M strains were recorded as 66,500; 66,400; 64,200; 64,200 daltons, respectively.

The SCMV-Gujarat isolate, crystalline inclusion protein had the molecular weight lower than the ones reported by Jensen et al. (1986) for the four strains of SCMV. They have, however, not given the electron micrograph for the size and shape of the proteinaceous inclusions. However, McDonald and Hiebert (1975) have shown such inclusions in the electron micrographs of the purified inclusion preparations as induced by turnip mosaic virus. They have shown long, narrow, rectangular, triangular shaped inclusion bodies negatively stained with 2 per cent uranyl acetate. These inclusions were reported to appear as long, flat, laminated aggregates in ultrathin sections alongwith pinwheels and scrolls. The rectangular inclusions induced by SCMV-Gujarat isolate were morphologically different in being more rectangular than long and narrow. The crystalline inclusions of tobacco etch virus appeared as flat crystals, 6-8 μm square, when viewed from above, but slightly curved plates in side view. The substructure of crystalline inclusions induced by tobacco etch virus, which were stained with 0.2 per cent uranylacetate (pH 4) have clearly displayed periodic substructure with strainations spaced at 10.2 nm and having primary axes that intersects at 90° (Knuhtsen et al., 1974).

The strains of sugarcane mosaic virus were differentiated by the Summers in 1934, using sugarcane clones CP 31-294, CP 31-588, Co 281 and sweet sorghum cultivar 'Rio'. Sugarcane, being vegetatively propagated, is less convenient to use as a differential host. At the 1978 International Workshop on sorghum diseases, held at the International

Crops Research Institute for semi-Arid Tropics, Hyderabad, India, recommendations to develop a set of sorghum inbred lines to differentiate strains of SCMV were made. Gillaspie and Mock (1984) evolved set of 10 sorghum lines as differentials for SCMV strains, by studying their reactions after inoculating them with a set of 10 SCMV strains under controlled environmental conditions. Their results indicated that entire set of differential sorghum inbred lines can be used to differentiate strains of SCMV by themselves or as supplement to other differentials under controlled environmental conditions. For the present studies, a number of other differential hosts namely, Johnsongrass, and sorghum lines viz., Martin, Martin-40, Martin-5330 and QL-3-Tx were added to the 10 sorghum lines of Gallaspre & Mock (1984), and inoculated with the one isolate obtained from Gujarat (Co 671) and the two isolates from Coimbatore (Co 671 and Co 859) under controlled environments.

Since each of these isolate appeared to be a mixture of strains, only characteristic distinctive symptoms on the individual differentials were taken into consideration for identifying strains. The SCMV-A strain was distinguished on the basis of severe mosaic symptoms of Johnsongrass as demonstrated by Persley et al. (1985). Similarly, other strains SCMV-E, F- 4961, F- 4975 H, I and K were identified on the basis of such symptoms as chlorotic streaks on Reo, mild mosaic and necrotic flecks on QL-11, mild mosaic on Q 7539, severe mosaic and on BTx398, and chlorotic flecks and necrosis on inoculated leaves of NM 31, respectively, as determined by Gillaspie and Mock (1984).

The samples of SCMV infected sugarcane Co 671 were collected from Gujarat and Coimbatore, the two different agroclimatic locations, and indexed. Six SCMV strains viz., SCMV-A, E, F 4961, H, I and K were identified from both the locations i.e. Gujarat and Coimbatore. In addition, SCMV-F 4975 was also present in samples of Co 671 from Coimbatore.The Co 859 sugarcane variety infected with SCMV, collected from Coimbatore, yielded only 3 strains namely SCMV-A, F and H including both 4961 and 4975 isolates of F strain. Thus, these exists similarity in types of SCMV strains observed in same variety of sugarcane (Co 671), collected from two different agroclimatic zones of the India. However, variations exist as for number and types of strains infecting different varieties of sugarcane in the same agroclimatic zones.

Sugarcane mosaic virus disease is an important malady not only of sugarcane but also of sorghum causing mosaic or red stripe disease (Chatterjee and Singh, 1973; Garud and Mali, 1983), and maize causing mosaic (Chona and Seth, 1960). Whereas the SCMV was reported to cause not more than 10 per cent loss in yield in sugarcane (Chona, 1944) and 32 per cent loss in grain yield in maize (Chona and Seth, 1960), the potentiality of infection particularly in the sugarcane was emphasized by Chona himself in 1958 by emphasizing the effect of development and spread of more pathogenic strains on the crop yield. Olalla Mercades et al. (1982) reported from Spain the loss of 0.4-0.5 tons of sugarcane yield for every 1 per cent infection indicating the extent of reduction in yield even with low incidence of disease. A current outbreak of SCMV on cultivar CP-52-68 was recorded in Texas, U.S.A., which caused 36 per cent yield reduction of cane/acre (Villalon, 1980).

Though SCMV infects three important crops and a number of grasses as recorded from all over the India, much of the information on SCMV is fragmentary. The present investigation are attempts to fill up certain gaps in our knowledge of this virus in the country. The SCMV which is sapinoculable, transmitted by aphid in nonpersistent manner and is a flexous rod of 600-880 nm x 15.4 nm measurement when viewed under electron microscope, stained with 2 per cent uranyl acetate. It belongs to potyvirus group. The sedimentation coefficient of purified SCMV was calculated as 166 S, with density as 1.2988 g/ml and partial specific volume as 0.76. The SCMV-RNA, which was calculated to be 5.5 per cent by 280-260 ratio, on isolation indicated a sedimentation coefficient of 65 S and molecular weight of 3.455×10^{-6} daltons indicative of being a single species with a certain degree of secondary structure. The fluorescent red colour of SCMV-RNA band in gel is indicative of single standedness of the RNA. The SCMV capsid protein consisted of single species of polypeptide with molecular weight 40,500 daltons, close to SCMV-Sabi strain. The SCMV is moderately antigenic, with the titer of antiserum as 1:512 or 1:1024. DAS-ELISA could detect the virus in vivo, in different tissues with highest titer of SCMV in roots.

The SCMV induced proteinaceous crystalline inclusion, measured 150-350 x 500-1050 nm with molecular weight as 62,000 daltons. An additional protein species of molecular weight 38,000 daltons was also detected in absorbance profile of gel.

As the SCMV Gujarat isolate infected 10 varieties of sorghum, this isolate of SCMV appears to be important for sorghum as well. The indexing of different isolate from Gujarat and Coimbatore on entire set of sorghum inbred lines indicated these isolates to be a mixture of strains, the number and type of which depend on sugarcane cultivar and agroclimatic zones. Therefore, besides the percentage of infection, severity of disease and serological recognition of the disease, indexing on set of differentials is important for assessing the overall potentiality of the disease in inflicting the losses. The information on strainal profile in a particular agroclimatic zone and on a particular cultivars are important to the breeders and pathologist to intercept the threat in sugarcane cultivation in time.

6. SUMMARY

1. The sugarcane mosiac disease induced such symptoms as elongated chlorotic streaks interspersed with light and dark green patches on susceptible sugarcane (*Saccharum officinarum* L.) cultivars Co 671, Co 740, Co 859, Co 1158 etc.

2. An isolate of sugarcane mosaic virus, collected from Gujarat (SCMV-Gujarat) was studied for characterization.

3. The SCMV by sap inoculation induced severe mosaic to the 10 varieties of sorghum [*Sorghum bicolor* (L.) Moench] namely, Surat-1, M-234, GJ-35, GJ-37, BP-53, Tukadi, Desi white, Chhasathio, Solapuri and CSH-9 only, while four varieties viz., Ageti-76, Bari local, Deccan-103, MDR-1 and two inbred lines CM-110, CM-600 of maize (*Zea mays* L.) were all found to be immune to the sugarcane mosaic virus. Three varieties of bajra [*Pennisetum americanum* (L.) Leek] namely, WCC-75, BK-560-280 and MBH-110, and four varieties of rice (*Oryza sativa* L.) viz., Saket-4, IR-8, TN-1 and Jaya, were also immune to the sugarcane mosaic virus.

4. The sugarcane mosaic virus (SCMV), the isolate collected from Gujarat was transmitted by three different aphids and the transmission efficiencies were 48, 40 and 34 per cent for the *Aphis craccivora* (Koch), *Rhopalosiphum maidis* (Fitch.) and *Melanaphis sacchari* (Znht.), respectively. Since *R. maidis* (Fitch) colonized the inside of whorl of central leaf of sorghum, these protected places makes *R. maidis* (Fitch.) potentially an important vector of SCMV.

5. The original purification schedules of Lagenberg (1973), Gough and Shukla (1981), and modified schedule of Gough and Shukla (1981), Rishi and Rishi (1985), were not effective procedures for purification of SCMV-Gujarat isolate. While the SCMV-Gujarat was effectively purified using modified procedure of Rishi and Rishi (1985), and another standardized procedure, with use of Cs_2SO_4 density gradient centrifugation, yielding 9.81 mg and 22.6 mg of SCMV/kg of infected tissues. In general, Cs_2SO_4 preformed density gradients (15-30% in 10% sucrose) were found more suitable over 10-40 per cent sucrose density gradients.

6. An antiserum to SCMV-Gujarat isolate was produced in the New Zealand white rabbit with titer of 1:512 as determined by ring interface and tube precipitation serological test at 1:8 dilution of diseased plant extract. Using microprecipitin test positive reaction with 1:16 and 1:32 dilution of SCMV diseased plant leaf extract was observed at 1:1024 dilution of an antiserum produced against Gujarat isolate of the SCMV. The Decoration technique of Immuno Electron Microscopy (IEM) effectively decorated the SCMV up to 1:512 dilution of the antiserum.

The 0.1 µg/ml y-globulin concentration of SCMV partially purified antibodies, and 1:2000 dilution of SCMV antibody-enzyme conjugate, were found to be optimum for use with double antibody sandwich form of Enzyme-Linked Immunosorbent Assay (DAS-ELISA). Blocking of well surface with 5 per cent milk powder had no specific advantage. While cross absorption of conjugate with 1:100 dilution of SCMV infected plant sap resulted in mean absorbance at 405nm

to that obtained with buffer controls, thus the procedure can be used for indexing for the plants materials for SCMV.

7. The electron microscopic studies clearly indicated that the sugarcane mosaic virus (SCMV) is a member of the potyvirus with flexous rod shaped particles. The length of particles ranged from 600-880 nm with 750 nm as modal length. The width of SCMV particles was observed to be 15.4 nm.

8. The ultraviolet profile of purified sugarcane mosaic virus was that of typical nucleoprotein. The maximum absorbance was observed at 260 nm. The A 260/A 280 ratio of purified SCMV corrected for light scattering was found to be 1.18. The purified SCMV particles contained 5.5 per cent nucleic acid (RNA), had extinction coefficient ($E\ 260^{0.1\%}_{1\ cm}$) of 2.685 and 166 Svedberg unit sedimentation coefficient. The density of SCMV-Gujarat isolate particles was found to be 1.2988 g/ml, and had 0.765 partial specific volume($\bar{V}$). The $\bar{V}$ value indicated the fact that SCMV nucleoprotein particles contains ssRNA.

9. The SCMV-coat protein consists of single polypeptide which migrated as single band during SDS-PAGE. The molecular weight of SCMV coat protein was determined to be 40,500 daltons.

10. The SCMV-RNA isolated from purified SCMV preparation had typical ultraviolet absorbance profile that of nucleic acid with maximum absorbance at 265 nm and minimum at 235 nm. The A 260/A 280 ratio of purified SCMV-RNA was 1.34 and the preparation appeared uncontaminated by proteins. The sedimentation coefficient of SCMV-

RNA was 65 Svedberg units and had the molecular weight of 3.455×10^{-6} daltons.

11. The purified SCMV-RNA did not move at all in native state when electrophoresed at 50 volts in 0.6 per cent agarose horizontal slab gel for 1.5 h. While upon denaturation with heat (60°C), urea (3M), and formamide (36%), the SCMV-RNA moved 2.8 cm from edges of the loading wells as a single band. Denaturation of the SCMV-RNA in presence of glyoxal at 53°C for 1 h caused it to move still farther at even lower voltages employed for the SCMV in 1 per cent agarose gel.

    In the verticle polyacrylamide slab gel electrophoresis the SCMV-RNA also moved as a single band when denatured, and silver stained.

12. The crystalline inclusion isolated from SCMV infected leaves of Co 671 sugarcane variety measured 150-500 nm x 350-1050 nm in width and length, respectively. The molecular weight of major crystalline inclusion protein determined by SDS-PAGE was 62,000 daltons. In addition, a second protein species of molecular weight 38,000 daltons was also observed.

13. Based on their reaction on a set of sorghum differential lines, six strains of SCMV were identified from Co 671 variety collected from Gujarat and Coimbatore. The strains identified were SCMV-A, E, F 4961, H, I, and K. In addition, SCMV-F 4975 was present in samples of Co 671 sugarcane variety from Coimbatore. From the Co 859 SCMV infected sugarcane variety, SCMV-A, F and H, including both 4961 and 4975 isolates of F strain were identified.

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