1954

Ultraviolet light scattering by tobacco mosaic virus nucleic acid

George R. Hopkins
Iowa State College

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UMI
ULTRAVIOLET LIGHT SCATTERING BY
TOBACCO MOSAIC VIRUS NUCLEIC ACID

by

George R. Hopkins

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Physics

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Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College

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I. INTRODUCTION

Tobacco mosaic virus (TMV) consists, as far as is known, of protein and ribose nucleic acid (RNA), with the RNA constituting about 5 to 6% by weight of the whole virus. Little is known about the biological mechanisms of viruses and it is hoped that a study of the chemical constituents of viruses may eventually give some insight into this problem. Light scattering techniques provide a means of studying the properties of large molecules or particles in solution, in this case the TMV RNA.

One of the purposes of this work was to design and construct an instrument capable of measuring the light scattered by solutions in the visible and near ultraviolet regions of the spectrum. The use of two or more wavelengths should be helpful in eliminating ambiguity in interpretation of light scattering data. The second purpose was to determine some of the physical properties, accessible by light scattering techniques, of the RNA prepared from TMV. Evidence indicated that all of the RNA in a virus may be separated in one piece, thus the separated RNA should be homogeneous, assuming identical virus particles. The state of the separated RNA may be similar to that of the biologically active material in the virus, but little interpretation beyond a description of the properties of the RNA is possible with what is known to date.
II. THEORY OF LIGHT SCATTERING BY SOLUTIONS

The theory of light scattering by solutions has been presented by Debye (1, 2) in a form permitting determination of molecular weight, shape, and size of large molecules in solution. There are several good review articles (3, 4, 5, 6, 7) which adequately cover the theory and the areas of investigation, so that a complete duplication will not be presented here. The literature demonstrating the usefulness of light scattering as a tool for studying high polymers and macromolecules is becoming extensive. It is of interest, however, to discuss the phases of the theory pertinent to the problem and interpretation of the data.

The general boundary value problem of scattering from a plane electromagnetic wave incident upon a particle of arbitrary size, shape, orientation, and index of refraction (real or complex) has not yet been solved because of mathematical difficulties. Nevertheless, with various restrictions on the size, shape, etc., useful results can be obtained. The special cases of interest that will be discussed are: small isotropic particles, large isotropic particles with relative index of refraction near unity, and large anisotropic particles with relative index of refraction near unity.

Rayleigh in 1871 (8) was the first to recognize scattering as an electromagnetic phenomena and solved the problem for independent isotropic particles small compared to the wavelength of light. The equation giving the intensity of scattered light is derived in detail.
in treatises on electromagnetic theory (9, 10) and is given by

\[ R(\theta) = \frac{I(\theta) r^2}{I_0 v} = \frac{8 \pi ^4 \nu ^2}{\lambda ^4} (1 + \cos^2 \theta) \quad (1) \]

where \( R(\theta) \) is Rayleigh's ratio, \( I(\theta) \) is the intensity of light scattered at angle \( \theta \), \( I_0 \) is the incident intensity, \( v \) is the scattering volume at a distance \( r \) from the observer, \( \nu \) is the number of particles per unit volume, \( \alpha \) is the polarizability, and \( \lambda \) is the wavelength of light in the surrounding medium. The polarizability may be related to conveniently measured optical quantities by an equation due to Maxwell (See Jeans (11)).

\[ \frac{n^2 - n_0^2}{n_0^2} = 4 \pi \nu \alpha \quad (2) \]

\( n \) and \( n_0 \) are the refractive indices of the particles and the surrounding medium respectively. \( \nu \) can be written in terms of Avogadro's number \( N \), the molecular weight of solute \( M \) and the weight concentration \( c \) as \( \nu = \frac{Nc}{M} \). Substitution for \( \nu \) and \( \alpha \), and rearrangement gives the useful form of equation (1) for molecular weight measurement.

\[ \frac{Kc(1 + \cos^2 \theta)}{R(\theta)} = \frac{1}{M} \]

where \( K = \frac{2 \pi ^2 n_0^2 (dn/dc)^2}{\lambda ^4 N} \).
\( \lambda \) is the vacuum wavelength of light and \( \frac{dn}{dc} \) is the usual refractive increment, and is an approximation of \( \frac{(n - n_0)}{c} \).

If the particles have a linear dimension greater than \( \frac{\lambda}{10} \) or if the index of refraction is complex, the single dipole radiator theory of Rayleigh is not valid. The scattering for a single particle will then consist of a superposition of wavelets from all parts of the particle, taking into account the phase and intensity relationships of the wavelets. The scattering of many particles in solution will be an average over all orientation of the particles. Mie (12) has solved the problem for spheres of any size and relative index of refraction. The solution is given as a summation of the electric and magnetic multipole radiation terms as functions of the relative index of refraction \( \frac{n}{n_0} \) and the relative size of the particle compared to the wavelength \( \frac{R}{\lambda'} \). \( R \) in this case is the radius of the spheres. If \( \frac{R}{\lambda'} \) is less than one and \( \frac{n}{n_0} \) is near unity, the distortion by the particle of the incident plane wave and the scattered wavelets may be neglected. That is, radiation of order higher than electric dipole will be negligible. Each volume element in a single large particle will then scatter light according to the Rayleigh law. The many wavelets from a given particle will interfere with each other, the phase relationships being determined by optical path differences due to geometry only. In general with \( R < \lambda \) the phase differences between volume elements will be larger in backward directions than in forward directions, resulting in reduced intensity in the backward direction.
The method of evaluating the scattering pattern for this case is identical mathematically to that of X-ray and electron scattering and is treated in detail, for example, by Compton and Allison (13) and Zimm, Stein and Doty (7). The right hand side of equation (3) must be multiplied by the particle scattering factor denoted \( P(\Theta) \) and defined by

\[
P(\Theta) = \sum_{i} \sum_{j} \frac{\sin(h\pi/\lambda') I_{ij}}{(h\pi/\lambda') I_{ij}}
\]

where \( I_{ij} = r_{ij} \sin \Theta /2 \).

\( r_{ij} \) is the distance between volume elements \( i \) and \( j \) and \( \Theta \) and \( \lambda' \) are as defined previously. Closed forms of \( P(\Theta) \) have been obtained for rigid rods, random coils, and spheres.

The random coil is considered as a chain of many links. Each link makes a definite angle with the preceding link and is free to rotate about a cone-shaped locus with the preceding link as axis. The probability function of finding two particular links a distance \( r \) apart will be Gaussian.

The rigid rod is defined as having a diameter small enough so that each element of length, \( dl \), may be considered as a Rayleigh dipole scatterer. It is the limiting case of the random coil with the valence angles equal to zero.
The resulting expressions for $P(\Theta)$ are:

a) Sphere $\frac{1}{x^3} (\sin x - x \cos x)^2$ where $x = kSD/2$

b) Rod $\frac{1}{x} \sin 2x - (\sin x/x)^2$ $x = kSL/2$ (5)

c) Coil $\frac{2}{x^2} [e^{-x} - (1 - x)]$ $x = k^2s^2R^2/6$

where $D$ is the diameter of the sphere, $L$ the length of the rod, and $R$ the root mean square distance between ends of the random coil, $K = 2\pi/\lambda'$ and $S = 2 \sin \Theta/2$. $\sin 2x$ is the sine integral function:

$$\sin 2x = \int_0^{2x} \sin w/dw.$$ 

Doty and Steiner (14) have published numerical values of these functions for $x$ ($\sqrt{x}$ for coil) ranging from 0.1 to 5.0. $P(0^\circ) = 1$ for all models since there is no phase shift in the forward direction.

P. Debye and P. P. Debye (see Oster (5)) have pointed out that for a given model, the size is uniquely determined by the scattered intensity at two angles symmetrical about $90^\circ$. That is, the $P(\Theta)$ dependency of the radiation envelope is given by these two intensities. Measurements are usually made at $45^\circ$ and $135^\circ$ with the dissymmetry $Z$ being defined as

$$Z = \frac{I(45^\circ)}{I(135^\circ)}.$$ (6)

Curves for dissymmetry vs $S/\lambda$ are shown in Figure 1. $S$ is $D$, $L$, or $R$ for the appropriate model.

If the particles are anisotropic, that is the polarizability varies with direction in the particle, an additional correction must
Figure 1. Dissymmetry as a Function of Particle Size.

Figure 2. Effect of Anisotropy on Rod Dissymmetry.
be made to equation 3. In the case of small anisotropic particles
where \( P(\Theta) = 1 \), Cabannes (15) has shown that the \( 90^\circ \) scattering must
be multiplied by \( \frac{6 + 6\rho_u}{6 - 7\rho_u} \). The depolarization \( \rho_u \) is defined as
the ratio at \( 90^\circ \) scattering of the horizontally to vertically
scattered components for unpolarized incident light. The subscript
on \( \rho \) indicates the state of polarization of the incident light. The
angular dependence of the scattering on the anisotropy is considerably
more elaborate and has been studied by Martin (16) and King (17) and
more recently by Horn and Benoit and Oster (18) and Northrop and
Sinsheimer (19). For small particles in solution, the right side of
equation (3) must be multiplied by a function \( F(\Theta, \rho_v) \) where

\[
F(\Theta, \rho_v) = \frac{(3 - \rho_v) (1 + \cos^2 \Theta)}{3 \left[ 1 + 3 \rho_v + (1 - \rho_v) \cos^2 \Theta \right]}
\]  

(7)

\( \rho_v \) is the depolarization for vertically polarized incident light at
\( \Theta = 90^\circ \). Two general properties of this function that may be noted
are that it is symmetrical about \( 90^\circ \) and it is not unity at \( \Theta = 0^\circ \).

The conditions of interest for this work are when \( P(\Theta) \neq 1 \) for
all \( \Theta \) and \( \rho_v \neq 0 \). That is the particles have a dimension in the order
of the wavelength of light and are anisotropic. In general, the
effect of size and anisotropy are not separable and independent, so
that the exact correction that must be made to equation 3 is not simply
the ratio \( P(\Theta)/F(\Theta, \rho_v) \). This non-separability of \( P(\Theta) \) and \( F(\Theta, \rho_v) \)
should not, however, affect molecular weight measurements appreciably.
The left side of equation 3 is usually extrapolated to zero where
Application of the correction \( F(\theta, \gamma) \) should be a good approximation for all models. Thus, the general equation for molecular weight measurement may be written:

\[
R(\theta) = \frac{KcN(\theta)(1 + \cos^2 \theta)}{F(\theta, \gamma)}.
\]

The data are plotted as \( c(1 + \cos^2 \theta) \) vs \( \sin^2 \theta/2 \). Such graphs will be referred to as scattering curves. The intercept at \( \theta = 0^\circ \) is equal to \( F(0, \gamma)/KN \). The size and shape of the particle may be determined by a comparison of the experimental curve with curves calculated from the theoretical analysis.

Horn, Benoît, and Oster (18) have carried out the exact calculation for the case of a rigid rod where the polarizability along the axis of the rod is different from that perpendicular to the axis. The resulting expressions are rather lengthy and cumbersome. Figure 2 is taken from their paper and shows the effect of anisotropy on dissymmetry. The parameter \( S \), characterising the anisotropy, is defined as \( S = (\alpha - \beta)/(\alpha + 2\beta) \) where \( \alpha \) is the polarizability in the axial direction and \( \beta \) perpendicular. The approximate correction \( F(\theta, \gamma) \) would have no effect on the dissymmetry since it is symmetrical about \( 90^\circ \). Thus this approximation would not be valid if the length were calculated from dissymmetry measurements and \( \gamma \) were not small.
Hom and Benoît (20) have also shown that $S$ can be calculated from depolarization measurements by the following equation:

$$\lim_{\theta \to 0} \mathcal{R}_v(\theta) = \frac{3 S^2}{5 + 4 S^2}. \quad (9)$$

This equation determines the magnitude of $S$, but not the sign. The sign may be determined from birefringence measurements or other knowledge of the particle.

The reason for the inseparability of the anisotropy and size effect in the rod is that the depolarization of the wavelet scattered from a small volume element of the rod depends on the spatial orientation of the rod. It does not, however, depend on the position of the volume element in the rod. Since the $P(\Theta)$ term includes an integration over all orientations, the two effects are not separable.

Consider a Gaussian coil made of small links which are anisotropic. A wavelet scattered from a volume element in the coil will then show depolarization, depending on the axial direction of the link from which the wavelet is scattered. There will be no preferred axial direction in a given volume element, so that the average depolarization will be independent of position of the volume element in the molecule. Since there is no orientation associated with a random coil, the anisotropy and size effects are separable. Thus, anisotropy can not affect this dissymmetry and thus the size, if the dissymmetry is used as a measure.
III. MATERIALS AND APPARATUS

A. Light Scattering Instrument

Figure 3 shows a schematic diagram of the light scattering apparatus and differential refractometer. All of the components were firmly mounted on a rigid optical bench. Except for the monochromator and source the entire apparatus was enclosed in a light-tight box.

The instrument may be considered as consisting of three main parts: 1) a scattering cell and holder 2) a source and optical system to illuminate the contents of the cell and 3) a detector to measure the scattered radiation. Several instruments for measuring the light scattered from solutions are described in the literature (21, 22, 23, 24, 25, 26), this one being essentially similar to Northrop's with modification to fit the particular problem and available equipment.

The scattering cell is shown in Figure 4 and is a sinter fused optical glass cell of the Doty type. The solution whose scattering is to be measured is placed in compartment A and illuminated through the plane windows as shown by the arrow $I_0$. The minimum volume of sample required for this cell is about 3.5 ml. The detector then views the light scattered by a small volume of solution in the center of the cell, the direction of the scattered light being shown for a particular case by the arrow $I_s$ making an angle $\Theta$ with the
incident beam. The hemicylindrical compartment (B) is filled with buffer of the same salt concentration as the sample compartment (A). Thus all scattered rays leaving the cell pass through the glass-air interface with approximately normal incidence. If this were not the case, it would be necessary to make a correction to the scattering angle because of refraction at the glass-air interface. It is assumed that the difference in index of refraction of the glass partition between compartments A and B and that of the solutions is small so that the change in reflection with a change in $\Theta$ is negligible. The back surface of the scattering cell is painted with black paint to reduce reflection from the glass-air interface.

Since measurements were made at 3131 Å it would have been desirable to have a cell made of quartz instead of glass. Two 2 mm. thickness of this glass cell had an optical density of about 0.46 at 3131 Å. This figure includes losses due to reflections for four air and glass interfaces. The necessary correction to the scattering curve because of this optical density will be discussed later. An attempt to procure a quartz cell was made but none were available in time. It is suggested that perhaps a quartz bubble cell of the Zimm (26) type would be easier to fabricate. Wittnauer and Scherr (27) have described a cylindrical cell which has plane windows ground at each end of a diameter to admit the illuminating beam. This type has the disadvantage of requiring larger volumes of solution than a comparable cell of the Doty type. In general the glass Doty type cell was adequate.
M, MONOCHROMATOR; L₁, ILLUMINATING LENS; L₂, REFRACTOR;
J, WATER JACKET; T, LIGHT TRAP; RC, REFRACTOR.
STOP SIZES: S₁, 1.1 x 3.4 cm; S₂, 0.35 x 1.0 cm; S₃, 0.35 cm.

Figure 3. Scattering
3. Scattering Instrument and Refractometer
Figure 4. Scattering Cell.

Figure 5. Refractometer Cell.
While in the scatterer, the cell rested in a slot accurately milled in a brass base which was in turn fastened to the optical bench. Since it was necessary to keep the contents of the cell as close to 0°C as possible at all times, the holder was cooled with ice water circulated through a copper tube soldered on the under side of the base. Except for an opening to allow illumination and passage of the scattered light, the cell was surrounded with a water jacket through which ice water was also circulated. With ice water circulating through the jacket and holder, the equilibrium temperature of the cell and its contents was about 5°C. This was adequate for all measurements.

Measurements at these temperatures required some means for dehumidification to prevent condensation on the scattering cell if the dew point of the air were above 0°C. Several methods of dehumidification by cooling were proposed but it was found that the dew point of the air from the compressed air jet in the laboratory was low enough to prevent condensation in its atmosphere. All that was necessary on humid days was to flush the scattering box with the compressed air for 20 to 30 minutes before placing the cell in the instrument. Since some condensation accumulated on the cell in the transfer from the cold room to the scattering instrument, provision was made for spraying the cell walls with commercial compressed nitrogen. This quickly removed any condensate and could then be turned off.
Since measurements were to be made at 4358 Å and 3131 Å, a General Electric G-133 mercury arc lamp was used as a source. The arc was operated through a constant voltage transformer and was positioned as near as possible to the entrance slit of the monochromator (See Figure 3). A monitor photocell (929) was used to further eliminate variations in arc light intensity. The monochromator employed a 60° quartz prism in a Wadsworth mounting, quartz collimating and camera lenses, and adjustable entrance and exit slits. Both the prism table-and lenses had calibrated adjustments for a wavelength range from 5000 Å to 2000 Å. The monochromator was calibrated with a mercury arc.

The maximum entrance and exit slit widths usable on a monochromator are determined by the maximum permissible bandwidth for isolation of the desired line in the spectrum. The closest bright line to 4358 Å in the mercury spectrum is 4047 Å, giving a bandwidth of about 600 Å. At 3131 Å the bandwidth is about 200 Å. The calculated maximum slit widths for both of these cases is about 2 mm. For all scattering measurements the entrance and exit slits were set at 1.5 mm, allowing an adequate safety factor for slight misadjustments of the instrument.

The exit slit of the monochromator was used as an object for the illuminating lens (L1) and was imaged in the center of the scattering cell. The lens was made of fused quartz and had focal lengths of 12.3 cm. at 4358 Å and 11.7 cm. at 3131 Å. The path of the illuminating beam through the cell could be seen easily if a fluorescent solution
were put in the cell. Thus, the focusing and centering of the image in the cell were done by visual means. An average setting of the lens between the optima for 4358Å and 3131Å was used so that it was not necessary to change the lens position with a change in wavelength. It was found that movement of the lens 1 cm. from this average in either axial direction did not appreciably change the scattered light intensity.

A rectangular aperture stop (S₁) was used on the lens to limit the angular distribution of the light entering the scattering cell. The maximum angle an incident ray could make with the axis in the cell was 3.5 degrees in a vertical direction and 1.1 degrees in a horizontal direction. This number includes a correction for refraction of the light as it enters the cell. Stop S₂, positioned as close to the scattering cell as possible, helps eliminate stray radiation. As a further precaution, the incident beam was surrounded by a light-tight black tube and was trapped in another tube (T) upon leaving the cell.

It is desirable to have as large an amount of incident intensity as practical to keep the detection problems as simple as possible. For a given detector the practical lower limit to the amount of light passing through the cell is determined by the amount of light scattered from the solvent in which the particles are dissolved. This scattering is the background which must be subtracted from all readings and is the limit which the solution approaches as the
concentration of scattering particles approaches zero. The following analysis gives the conditions for maximum light in the cell.

Consider the area \( A \) of the exit slit of the monochromator as that area which is used for illumination and the solid angle \( \Omega \) that which is accepted by the optical system for each point in the area \( A \). Then for maximum total light in the scattering cell, the product \( A \times \Omega \) should be maximum. The conditions that must be satisfied in the cell are: 1) the image should be less than 3 mm. wide to prevent rays from striking the sides of the cell and less than 5 mm. high to keep the volume of solution necessary small, 2) the angular divergence from the axis of a ray in the beam should not be greater than 1.1° in the cell in a horizontal direction and 3.5° in a vertical direction. These two conditions determine \( A' \) and \( \Omega' \) for the image of \( A \) and \( \Omega \).

Let \( A_n \) be the area of the stop \( S_1 \) on lens \( L_1 \) and \( p \) and \( q \) be the object and image distances respectively for this lens. Then \( \Omega = \frac{A_n}{p^2} \), \( A_n = q^2 \Omega' \), and \( \Omega = \Omega' m^2 \), where \( m \) is the linear magnification.

Since the maximum image size may be 3 mm. wide while the exit slit is 1.5 mm. wide, the entire width of the exit slit will be effective up to a magnification of two. The height of the exit slit effective in illumination will, however, depend on the magnification of lens \( L_1 \) since a total height of 1 cm. was available. Thus, \( A = A'/m = A'p/q \), and \( A\Omega = mA'\Omega' \) for a magnification less than 2. For an image larger than 3 mm. using stop \( S_2 \) to limit the size in the cell, \( A = A'/m^2 \) and \( A\Omega = A'\Omega' \). Thus the lens should be adjusted for a magnification
of two or more. In this case, the solid angle of light from the exit slit was limited by the focal length and diameter of the camera lens in the monochromator, so that nothing would be gained beyond the point where the stop $S_1$ accepts all the light from the monochromator. With the particular stop $S_1$ used and a magnification of 1.75, practically all the available light from the monochromator was utilized.

The optimum condition for illumination of the monochromator is that the collimator lens be fully illuminated. This lens had a focal length of approximately 20 cm. and a diameter of 2 cm. With no condensing lens between the source and entrance slit, the source should be as close to the entrance slit as possible, in this case 2 cm. Thus, with the source width of about 2 mm., the collimator would be fully illuminated assuming a very narrow entrance slit width. This was not the case, however, since the entrance slit width used was 1.5 mm., increasing the width of source necessary to fill the effective aperture by a factor of about two. A properly used condenser lens would have approximately doubled the amount of light in the cell, assuming no additional losses due to reflection. A condensing lens was not used because of the additional mounting difficulties.

A polarizer could be inserted in the incident beam to provide either vertically or horizontally polarized light. A Foucault prism with an aperture of 2.5 cm. was mounted in a sleeve with adjustable mechanical stops so it could be easily rotated. The polarizer had to be mounted after the quartz lens because of rotations of the plane of polarization by the optical imperfections in the fused quartz.
The detector system consisted of a 1P28 photomultiplier with two stops \( S_3 \) and \( S_4 \) for limiting the field of view. The entire system could be rotated about the cell through an angle of \( 150^\circ \) from the incident beam by means of a worm drive extending outside of the light-tight box. Stop \( S_4 \) was placed as close to the photomultiplier as practical while Stop \( S_3 \) was placed close to the hemicylindrical section of the scattering cell. These two stops determine the area of the illuminating beam that is viewed and the angular divergence from the nominal viewing angle of the light rays entering the photomultiplier. The maximum area that may be viewed is determined by the geometry of the cell and the desired minimum scattering angle. That is, the photomultiplier must not see any of the glass or glass, liquid, or air interfaces through which the illuminating beam passes. Since the scattered intensity from particles small compared to the wavelength is a slowly varying function of angle, the angular divergence is not critical. In general, it should be as small as a reasonable output signal will allow.

With the dimensions shown in Figure 3, the angular divergence of rays entering the photomultiplier was about \( \pm 2.5^\circ \) in a horizontal direction and a half angle of divergence of \( 4^\circ \) in a vertical direction. The entire height of the illuminated volume (5 mm.) was viewed with an effective horizontal distance of about 5 mm. at \( \Theta \) equal to \( 90^\circ \).

Systems employing lenses and stops have been described in the literature. These have the advantage of giving less total angular divergence for the detected rays. Without lenses the cones of
radiation from different points in the scattering volume do not have the same vertex angles nor are their axes all exactly parallel. The difference with or without lenses should be small. No apparent difficulty was encountered using only stops.

An analyzer ($P_2$) could be inserted as shown for measuring the horizontally and vertically polarized components of the scattered radiation. This was a Glan-Thompson calcite prism with a 9 mm aperture and ratio of width to length of 1 to 3. Glycerine rather than Canada balsam was used between the prisms for transmission of ultra violet light. The prism was mounted in a sleeve with adjustable mechanical stops so it could be easily rotated to measure either vertically or horizontally polarized light. The extinction of the crossed polarizer and analyzer was about $5 \times 10^{-4}$.

A 929 phototube was used to monitor the source similar to the method given by Hadlow, Sheffer and Hyde (24). By changing the gain of the photomultiplier, it eliminated variations in the output due to variations in arc light intensity. The output of the photomultiplier tube was fed through an Abyrton shunt into a Rubicon spotlight galvanometer of sensitivity 0.00016 μA/mm. In addition, a D.C. amplifier (See Olson (28) for current diagram) of the bridge type employing two Victoreen VX-4LA tubes could also be used for depolarization measurements where the scattered intensity was small.

Measurement of molecular weights by means of equation (8) requires a knowledge of the absolute value of $R(\theta)$. Thus the quantity $Q(\theta)$ relating $R(\theta)$ to galvanometer current ($i(\theta)$) must be
determined, and is defined by: \( R(\theta) = Q(\theta) I(\theta) \). \( Q(\theta) \) is expressed in cm\(^{-1}\) of turbidity per centimeter galvanometer deflection. If the cell is non-absorbing \( Q(\theta) = [V(90^\circ)/V(\theta)] \).

\( Q(90^\circ) \) where \( V(\theta) \) represents the volume of solution viewed by the detector at the scattering angle \( \theta \). \( V(90^\circ)/V(\theta) \) was determined with a dilute fluorescent solution. An additional factor is needed at 3131Å where the glass wall in the cell is absorbing. The path of the scattered radiation through the glass partition between the solution compartment and the hemicylindrical compartment is a minimum at 90° and increases as \( \csc \theta \) for other angles. Thus: \( Q(\theta) = [V(90^\circ)/V(\theta)] \cdot A(\theta) \cdot Q(90^\circ) \) where \( A(\theta) = e^{-x(\csc \theta - 1)} \). \( \alpha \) is the absorption coefficient of the glass and \( x \) the thickness of the partition. \( \alpha \) was determined experimentally and had a value of 0.465 at 3131Å for this cell. \( A(\theta) \) equals one at 4358Å.

The calibration constant \( Q(90^\circ) \) was determined by a method described by Northrop and Sinsheimer (19) using "Ludox"\(^1\). The equation is:

\[
Q(90^\circ) = \frac{3}{16\pi A \sigma}.
\]

(10)

\( A \) is defined by the equation relating turbidity \( (T) \) to concentration \( (c) \) of "Ludox" solutions:

\[
\frac{c}{T} = A + Bc.
\]

(11)

\(^1\) A 30% colloidal dispersion of silica kindly furnished by Dr. H. H. Snyder of E. I. du Pont de Nemours & Company.
\( \sigma \) is defined by the equation relating the scattered intensity in centimeters galvanometer deflection to concentration of "Ludox":

\[ i(90^\circ) = \sigma c. \]

Table 1 gives the values for these constants.

### Table 1

**Calibration Constants**

<table>
<thead>
<tr>
<th>( \lambda (\text{cm. - }^\circ) )</th>
<th>( \sigma (\text{cm.}/% )</th>
<th>( Q (90^\circ)(\text{cm.}^{-1}/\text{cm.}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda = 4358^\circ )</td>
<td>10.9</td>
<td>2.56 \times 10^3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.06 \times 10^{-6}</td>
</tr>
<tr>
<td>( \lambda = 3131^\circ )</td>
<td>2.81</td>
<td>9.70 \times 10^2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.05 \times 10^{-5}</td>
</tr>
</tbody>
</table>

The usual scattering equations are derived with the condition that the incident radiation is unpolarized. That is, that the vertical component equals the horizontal component. If this is not true a correction must be made in the \( (1 + \cos^2 \theta) \) term of the scattering. A difference in these two components may arise from the source, transmission in the optical system or differences in sensitivity of the photomultiplier cathode. Thus it was necessary to test the instrument for this effect.

It was found that a 1/16" thick piece of "Teflon" acted as a "perfect" diffuser within 2%, allowing the polarization of the incident
beam and the sensitivity of the photomultiplier to the two polarizations to be tested separately. The difference in the two components for both the incident beam and the photomultiplier was less than 2\% at 4358\AA\ and was neglected in molecular weight measurements. At 3131\AA\ the ratio of horizontal to vertical polarization in the incident beam was 0.939. The ratio of sensitivity of the photomultiplier to horizontally and vertically polarized light was 1.077. The net effect would be the product of these two numbers or 1.01, giving a correction of 1\%. This was again negligible for molecular weight measurement.

A standard scatterer was used to check the instrument for drift. It consisted of dust-free toluene sealed in a cylindrical glass tube. This was mounted on a base and could be reproducibly placed in the cell holder. A reading was taken both at 3131\AA\ and 4358\AA\ as close as possible to the time that data were taken. The data were then corrected accordingly. The sensitivity of the instrument had a tendency to decrease with time. This decrease may have been due to "aging" of the mirror in the monochromator. A recalibration with "Ludox" was made after several months and agreed with the drift shown by the standard. In all cases the correction was less than 10\%.

B. Differential Refractometer

Molecular weight determination requires that the refractive increment, dn/dc, at the scattering wavelength be known. Several instruments for measuring this quantity are described in the literature
(25, 29, 30, 31). Measurements at 4358 Å were made on an instrument constructed earlier in this laboratory by T. G. Northrop (25). Since this instrument employed a visual detector, another instrument was constructed for use at 3131 Å and is shown schematically in Figure 3. This refractometer was essentially similar to that of Brice and Halwer (30) and Northrop with a photoelectric detector similar to that used by Schulz, Bodmann and Cantow (31). For convenience the same source, monochromator, and optical bench as the scattering apparatus were used. All that was necessary to make refractive increment measurements was to remove lens L₂, polaroid P₂, the scattering cell and the light trap T. Adjustment of the exit and entrance slits of the monochromator was also necessary.

Figure 5 shows a diagram of the cell with two typical light rays. Ray 1 always goes through compartment A of the cell and is formed into image 1 by the lens L₂ (Figure 3) with focal lengths 33.1 cm. and 31.8 cm. at 4358 Å and 3131 Å respectively. Ray 2 traverses parts of compartments A and B as shown and forms image 2. It may be assumed that the source is far enough away from the cell so that rays 1 and 2 are initially parallel. The deviation of these two rays and hence the linear distance between images 1 and 2 should be proportional to the difference in index of refraction (Δn) between the solutions in the two compartments of the cell. The two images were not coincident, however, with the same solution in each compartment due to the thickness of the partition. In this case Δn is proportional to the change in distance (Δd) between images 1 and 2.
for a given change in index of refraction. The experimental relationship relating \( \Delta n \) to \( \Delta d \) for a given change in index of the solution in compartment B was linear for \( \Delta n \) up to \( 2.2 \times 10^{-3} \). The calibration constant \( K \), defined by \( \Delta n = K \Delta d \), had a value of \( 2.29 \times 10^{-1}/\text{rev.} \) at 31318 and was determined with accurately known concentrations of \( \text{CoSO}_4 \). There is some variation in the literature for the index of refraction of water at 31318, but the relative accuracy of Roberts' work should be sufficient since it is only the difference in index of refraction between water and the \( \text{CoSO}_4 \) solutions that is used.

The distance between the images was measured with a 1P28 photomultiplier tube and a movable slit \( S_7 \) (Figure 3). The slit width was permanently adjusted to 0.003 cm. and mounted on a movable frame. The frame was spring mounted and could be moved by means of a screw of 80 threads to the inch, the distance between the images being measured in terms of the number of revolutions of this screw. The output of the photomultiplier was fed directly into a galvanometer, thus the slit and image were coincident when the galvanometer showed a maximum reading. The images were focused on the slit by moving the slit and photomultiplier system axially on the optical bench. For a given setting on the optical bench, the slit was moved across the focal plane and the distribution of light in the plane of the slit determined. The optimum setting on the optical bench was that which gave a minimum width to this distribution. Stops \( S_5 \) and \( S_6 \) were

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\(^1\)Anhydrous \( \text{CoSO}_4 \) was kindly furnished by Dr. H. C. Diehl, Chemistry Department, Iowa State College, Ames, Iowa.
adjusted to give approximately the same maximum reading on the
galvanometer for each image.

The distance between the two images was taken as the distance
between the two maxima. The distance with water or buffer in each
side of the cell was subtracted from this, giving \( \Delta d \). Schulz,
Bochmann and Cantow plotted the intensity distribution for each
image and measured the distance between the half width point at half
maximum intensity. While it may be theoretically better to do this,
large enough \( \Delta n \)'s were obtainable that any additional accuracy by
this method did not warrant the additional time necessary to take
the data and plot curves for each image. It is also probable that
slight movement of the detector system due to thermal effects and
jarring would have partially offset any gain since it would have
taken 10 to 15 minutes to gather sufficient data for curves of each
image, while the two maxima could be determined in less than 1
minute. Calibration with a solution rather than geometry does not
require a knowledge of the absolute distance between the images. It
is consistency in measuring from the same point on each image rather
than the position of this point that is most important in this case.

Five or more readings on each of the two maxima were taken and
an average used to determine \( \Delta d \). The probable error in \( \Delta d \) was in
the order of \( \pm 10^{-2} \) revolutions, corresponding to a probable error in
\( \Delta n \) of \( \pm 2 \times 10^{-6} \).
C. Tobacco Mosaic Virus

The tobacco mosaic virus used throughout this work was prepared earlier in this laboratory by Northrop (25). Since preparation in 1952, the virus was stored at 2°C in 0.033 M K₂HPO₄ buffer at pH 7.2. A layer of n-hexane covered the virus suspension to prevent bacterial and fungal growth. A molecular weight of 4.0 x 10⁷ was obtained by light scattering techniques. This is in agreement with the generally accepted value. The infectivity of the virus was measured on n glutinosa and was in agreement with that of other workers for the crystalline virus.

The concentration of the virus solutions was related to the optical density (D) at 2650μ from data given by Schramm and Dannenburg (33). The concentration in mg./ml. is given by:

\[ c = \frac{D_{2650\mu}}{2.88} \]

This figure was obtained from nitrogen analyses. For comparison, a sample of the virus was evaporated to dryness in a vacuum over P₂O₅ and its weight measured, the initial optical density and volume having been previously determined. From this a figure of 2.83 was obtained, in good agreement with that based on nitrogen analyses.
IV. DATA ON TMV RNA

A. Preparation of the RNA

The RNA was separated from the virus protein by the method of Cohen and Stanley (34) and Knight (35). It consists essentially of a denaturation of the protein by heating. Since the preparation procedure was found to be quite critical, it is described below.

Several milliliters of the stock virus suspension were first centrifuged in a Sorvall angle head centrifuge at 40,000 g. One hour and fifteen minutes was sufficient to sediment almost all the virus. The 0.033 M phosphate buffer was then poured off and discarded. The pellet of TMV and the tube were rinsed several times with the buffer in which the heating was to be done. The original buffer for this was 0.1M sodium acetate pH 5.7 plus 0.2M NaCl per liter. This will be referred to as the "scattering buffer". Usually 1 ml. of the desired buffer was added to the pellet and the TMV resuspended by squirting the buffer and sedimented virus through a small pipette in the order of 50 times. After the virus was well dispersed, enough additional buffer was added to obtain the desired concentration of TMV. The virus suspension in the heavy wall pyrex centrifuge tube was then removed from the cold room and immersed in boiling water for 60 to 80 sec. with constant stirring. After this heating it was immediately immersed in a bath of ice water with continued stirring.
and returned to the cold room. Northrop and Sinsheimer (19) reported a degradation of the RNA if it were allowed to warm up after initial preparation, so care was taken to keep the preparation below 5°C at all times. The protein was then spun down by several 2 or 3 minute spins in a clinical centrifuge at 700 g. Any remaining visible particles of protein could then be removed by passing the solution through a Corning fine porosity sintered-glass filter.

The final cleaning of a solution for scattering measurements was done by forcing it through a 1 cm. diameter ultrafine filter with compressed nitrogen. The scattering cell was first washed free of dust with filtered buffer. The RNA solution was then filtered directly into the cell. Filtration was repeated by pouring the solution back into the filter directly from the cell and forcing it through again. Three of four such filtration cycles were generally sufficient to remove all visible large particles and to mix the RNA solution with the buffer that remained in the cell and filter from the initial washing.

Eventually the ultrafine filters became plugged. The filters were cleaned by soaking in concentrated nitric acid for a few hours followed by at least 12 hours in a 1N NaOH solution. The filter was then rinsed thoroughly with scattering buffer to remove the NaOH.
B. Refractive Increment

The refractive increment, \( \text{dn/dc} \), of RNA was measured at 5°C since this is the temperature at which scattering measurements were made. Several of the RNA preparations were concentrated by pervaporation at 2°C through cellulose tubing over \( \text{CaCl}_2 \), since the preparation yielded RNA concentrations too low for accurate refractive increment measurements. Dialysis for several days against scattering buffer equilibrated the salt concentrations. Table 2 shows the results.

Table 2

<table>
<thead>
<tr>
<th>Conc. (mg./ml.)</th>
<th>( \frac{\text{dn}}{\text{dc}} )</th>
<th>ml. at 3131Å</th>
<th>( \frac{\text{dn}}{\text{dc}} )</th>
<th>ml. at 4338Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.71</td>
<td>0.246</td>
<td>0.197(^a)</td>
<td>1.64</td>
<td>0.259</td>
</tr>
<tr>
<td>1.64</td>
<td></td>
<td>0.189</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.39</td>
<td>0.261</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.688</td>
<td>0.252</td>
<td>0.232</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Av.</td>
<td>0.254</td>
<td>0.206</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) The values at 4338Å are in agreement with 0.194 ml./gm. reported by Northrop and Sinsheimer (19).
C. Molecular Weight

Molecular weight measurements for TMV RNA have been reported by Northrop and Sinshelmer (19), along with their preparation procedure. In all of their preparations, about 80 mg. of TMV suspended in 4 ml. of scattering buffer were heated for times of 60 seconds to 80 seconds in boiling water. The preparations were then treated as outlined above. Consistent values of molecular weights averaging about $2 \times 10^6$ with a depolarization correction were obtained. Some preparations were centrifuged for 1½ hours at 40,000 g before filtration while others were filtered directly, the value being independent of this procedure.

Following this method a total of six preparations were made in an attempt to duplicate their molecular weights. Reproducible results were not obtained, with molecular weights ranging from $2.15 \times 10^6$ to $3.68 \times 10^6$ without depolarization correction. The dissymmetries for the six preparations ranged from 1.33 to 1.55, with no apparent correlation between dissymmetry and molecular weight. Northrop and Sinshelmer's average molecular weight without depolarization correction was $2.14 \times 10^6$ with an average dissymmetry of 1.5. One of the characteristics of these six preparations was that they would not pass through an ultrafine filter after the centrifugation at 700 g. The filter would usually become completely plugged after a few milliliters had passed through. Centrifugation at 40,000 g removed enough of the large particles so that filtration and scattering
measurements were possible.

Further investigation of the preparation procedure revealed at least two factors which effected the resulting RNA. One was the concentration of the TMV at the time of heating and the other was the presence of phosphate. Knight (35) did not measure molecular weights, but reported that the presence of 0.001 molar phosphate aided protein coagulation. He also stated that a concentration of 20 mg./ml. TMV gave the greatest yield of RNA.

The results of 21 preparations are shown in Table 3, and are arranged in the chronological order in which they were prepared. The first six preparations represent the initial attempts described earlier. All were prepared as described previously unless otherwise indicated in the second column, the scattering data being taken in the buffer in which the RNA was prepared (0.3M salt). The usual practice is to extrapolate the intercept (θ = 0) of the scattering curve to zero RNA concentration. This was not done since the concentrations measured were very low and it had been shown (19) that the intercept was independent of RNA concentrations up to 0.3 mg./ml. The effect, if any, would be small. A typical scattering curve for \( \lambda = 4358 \AA \), from which molecular weights were determined, is shown in Figure 6. The linearity of this curve may be used as a rough criterion for homogeneity of the scattering particles.

It was thought that the high molecular weights obtained from the 20 mg./ml. preparations were due to aggregation of the virus before and at the time of heating. It was impossible to test this by
scattering measurements because measurements could not be made on these concentrated virus suspensions. The maximum virus concentration on which scattering measurements could be made was about 0.1 mg./ml. Several dilution experiments were tried, all giving the correct weight for TMV. It may be that dilution dispersed the TMV aggregates.

The preparation procedures that did not follow that outlined previously will now be discussed. In preparations 14, 15, and 16 the TMV was not initially centrifuged and resuspended. The 0.033 M phosphate TMV buffer was changed to scattering buffer by dialysis for several days. This should eliminate effects of aggregation due to poor resuspension after centrifugation. The molecular weights are shown, but the curves were not as straight as obtained with centrifugation. This may have been due to foreign material in the original TMV suspension which was eliminated with centrifugation in the other preparations.

Preparation 13 was vigorously squirted through the pipette 350 times in resuspension of the virus. The low molecular weight indicates that the virus may have been broken up by this procedure.

Preparation 9, a and b, show that the phosphate had no effect if removed after the time of heating. The phosphate was dialysed out with no significant change in scattering. Addition of phosphate to an aggregated 20 mg./ml. preparation after heating would not disperse the RNA. Enough phosphate was added to preparation 14 to make it 0.001M. Again there was no significant change in scattering.
Table 3. Molecular Weights of RNA (*358*)

<table>
<thead>
<tr>
<th>Prep. no.</th>
<th>Prep. procedure (Virus Conc., buffer(^a), heating time, centrifugation(^b), etc.)</th>
<th>Conc. measured in mg./ml.</th>
<th>Dissymmetry</th>
<th>Intercept</th>
<th>Mol. wt. (x 10^{-6})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20 mg./ml., 70&quot;</td>
<td>0.435</td>
<td>1.55</td>
<td>0.42</td>
<td>3.88</td>
</tr>
<tr>
<td>2 a)</td>
<td>20 mg./ml., 70&quot;, before cent.</td>
<td>0.351</td>
<td>---</td>
<td>0.224</td>
<td>7.3</td>
</tr>
<tr>
<td>b)</td>
<td>after 40,000 g. 1 hr.</td>
<td>0.309</td>
<td>1.40</td>
<td>0.765</td>
<td>2.15</td>
</tr>
<tr>
<td>3</td>
<td>20 mg./ml., 70&quot;, 40,000 g., 1 hr.</td>
<td>0.290</td>
<td>1.42</td>
<td>0.630</td>
<td>2.59</td>
</tr>
<tr>
<td>4</td>
<td>20 mg./ml., 90&quot;</td>
<td>0.435</td>
<td>1.48</td>
<td>0.750</td>
<td>2.17</td>
</tr>
<tr>
<td>5</td>
<td>20 mg./ml., 90&quot;, 40,000 g., 1 hr. 15 min.</td>
<td>0.237</td>
<td>1.33</td>
<td>0.565</td>
<td>2.88</td>
</tr>
<tr>
<td>6</td>
<td>20 mg./ml., 60&quot;</td>
<td>0.360</td>
<td>1.39</td>
<td>0.652</td>
<td>2.50</td>
</tr>
<tr>
<td>7</td>
<td>10 mg./ml., 90&quot;</td>
<td>0.212</td>
<td>1.31</td>
<td>0.940</td>
<td>1.73</td>
</tr>
<tr>
<td>8</td>
<td>10 mg./ml., 0.001 M phos., 60&quot;</td>
<td>0.184</td>
<td>1.32</td>
<td>0.92</td>
<td>1.77</td>
</tr>
<tr>
<td>9 a)</td>
<td>20 mg./ml., 0.001 M phos., 60&quot;</td>
<td>0.221</td>
<td>1.36</td>
<td>0.92</td>
<td>1.77</td>
</tr>
<tr>
<td>b)</td>
<td>dialyse out phosphate</td>
<td>0.154</td>
<td>1.40</td>
<td>0.925</td>
<td>1.76</td>
</tr>
</tbody>
</table>

(Continued on next page)

\(^a\)Concentration of salt in addition to scattering buffer. Scattering buffer only if none indicated.

\(^b\)Other than the centrifugation at 700 g.

\(^c\)Using \(dn/dc = 0.194\) ml./gm. at *358*. 


Table 3. (Continued)

<table>
<thead>
<tr>
<th>Prep. no.</th>
<th>Prep. procedure (Virus Conc., buffer(a), heating time, centrifugation,(b) etc.)</th>
<th>Conc. measured in mg./ml.</th>
<th>Dissymmetry</th>
<th>Intercept</th>
<th>Mol. wt. (c) x 10(^{-6})</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10 mg./ml., 0.01 m phos., 70(^n)</td>
<td>0.186</td>
<td>1.41</td>
<td>0.98</td>
<td>1.66</td>
</tr>
<tr>
<td>11</td>
<td>10 mg./ml., 0.0005 m phos., 90(^n)</td>
<td>0.158</td>
<td>1.37</td>
<td>0.995</td>
<td>1.64</td>
</tr>
<tr>
<td>12</td>
<td>10 mg./ml., 0.001 m phos., 60(^n)</td>
<td>0.190</td>
<td>1.31</td>
<td>0.96</td>
<td>1.70</td>
</tr>
<tr>
<td>13</td>
<td>10 mg./ml., Squirt 350 times, 70(^n)</td>
<td>0.143</td>
<td>1.32</td>
<td>1.13</td>
<td>1.45</td>
</tr>
<tr>
<td>14</td>
<td>6.4 mg./ml., (TMV buffer changed to scattering buffer by dialysis) 70(^n), 70(^n), 40,000 g 1 hr.</td>
<td>0.090</td>
<td>1.34</td>
<td>0.88</td>
<td>1.86</td>
</tr>
<tr>
<td>15</td>
<td>6.4 mg./ml., (TMV buffer changed to scattering buffer by dialysis) 70(^n)</td>
<td>0.120</td>
<td>1.38</td>
<td>0.94</td>
<td>1.73</td>
</tr>
<tr>
<td>16</td>
<td>6.4 mg./ml., (TMV buffer changed to scattering buffer plus 0.001 m phos. by dialysis), 70(^n)</td>
<td>0.101</td>
<td>1.24</td>
<td>1.10</td>
<td>1.49</td>
</tr>
<tr>
<td>17</td>
<td>10 mg./ml., 60(^n)</td>
<td>0.174</td>
<td>1.29</td>
<td>0.94</td>
<td>1.74</td>
</tr>
<tr>
<td>18</td>
<td>5 mg./ml., 0.001 m phos., 60(^n)</td>
<td>0.206</td>
<td>1.30</td>
<td>0.98</td>
<td>1.66</td>
</tr>
<tr>
<td>19</td>
<td>5 mg./ml., 60(^n)</td>
<td>0.225</td>
<td>1.28</td>
<td>0.98</td>
<td>1.66</td>
</tr>
<tr>
<td>20</td>
<td>10 mg./ml., 0.001 m phos., 70(^n) (scattering curves obtained in water and 0.075 m NaCl)</td>
<td>0.212</td>
<td>1.30</td>
<td>0.985</td>
<td>1.65</td>
</tr>
</tbody>
</table>

\(a\) Excluding preparations 1 thru 6.

\(b\) Excluding preparations 1 thru 6.
Figure 6. Scattering Curve at 4358 Å.
In summary, the apparent molecular weight of RNA prepared in scattering buffer only is the same for initial concentrations of TMV of 5 and 10 mg./ml., but increases when the TMV concentration is 20 mg./ml. The molecular weight of a 20 mg./ml. preparation can be decreased to that of the 5 and 10 mg./ml. preparations by addition of 0.001M phosphate before heating. The molecular weight for an initial TMV concentration of 10 mg./ml. is independent of the phosphate concentration for a range of zero to 0.01 molar.

The reason for the dependence of molecular weight on preparation procedure is not entirely clear. The procedure giving reproducible molecular weights with the varying conditions mentioned was used for determination of the size of the RNA. That is, the RNA had a nominal molecular weight of $1.7 \times 10^6$. About 2.7% of the virus weight remained in solution as RNA for these preparations. All concentrations were calculated from optical density measurements and data given by Northrop and Sinsheimer (19).

The depolarisation correction is negligible in this case, a depolarisation, $\rho_v(90^\circ)$, of 0.005 decreasing the molecular weight by only 1%. The molecular weight was also calculated from the scattering data at 3131Å. Table 4 summarizes the data for the preparations that were measured at this wavelength. The value of $dn/dc$ at 3131Å given previously was used in the molecular weight calculation. The average molecular weight is reasonably close to that determined at 4358Å, but the spread is much larger.
A typical scattering curve at 3131° is shown in Figure 7. As can be seen, the data points form two straight lines which are joined with a discontinuity in slope at \( \sin^2 \theta/2 = 0.5 \), corresponding to 90° scattering. This indicates that the cell absorption correction \( A(\theta) \) was not large enough. The correction used was initially applied to preparation 4, giving a straight line. An approximate graphical correction may be made as shown by the dashed line in Figure 7. This is drawn so that it makes equal angles with the two parts of the original curve. The intercepts and molecular weights given in Table 4 were corrected in this manner.

**Table 4**

Molecular Weights of RNA (3131°)

<table>
<thead>
<tr>
<th>Prep. no.</th>
<th>RNA Conc. mg./ml.</th>
<th>Dissymmetry</th>
<th>Intercept</th>
<th>Molecular wt. x 10^-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.348</td>
<td>1.89</td>
<td>0.122</td>
<td>1.98</td>
</tr>
<tr>
<td>12</td>
<td>0.190</td>
<td>1.64</td>
<td>0.154</td>
<td>1.57</td>
</tr>
<tr>
<td>17</td>
<td>0.174</td>
<td>1.66</td>
<td>0.150</td>
<td>1.61</td>
</tr>
<tr>
<td>18</td>
<td>0.206</td>
<td>1.67</td>
<td>0.128</td>
<td>1.88</td>
</tr>
<tr>
<td>20</td>
<td>0.212</td>
<td>1.70</td>
<td>0.165</td>
<td>1.46</td>
</tr>
</tbody>
</table>

\( \text{Av.}^c 1.67 \quad \text{Av.}^c 1.63 \)

\( ^a \) See Table 3 for further details of preparation.

\( ^b \) In 0.3M salt.

\( ^c \) Excluding preparation 4.
Two additional corrections that may be applied are the absorption of the RNA solutions at 3131A, and the depolarization correction. The RNA had an optical density of about 0.016 at 3131A for a path length of 1 cm and a concentration of 0.2 mg./ml. The average path length of light through the RNA solution was about 2.2 cm. for 90° scattering. This correction would shift the curve downward about 3.5% at \( \sin^2 \theta/2 = 0.5 \). The resultant intercept would be 0.142.

The depolarization correction would have an opposite effect. The average depolarization in 0.3M salt was 0.019. This would increase the above corrected intercept by about 9.5% giving 0.155. These two corrections would result in about a 3% decrease in molecular weight from that shown in Table 4. Thus, these molecular weights are not as precise or accurate as those determined at 4358A, but are within reason.

It is of interest to note the effect of the inherent enzyme on the RNA. Northrop and Sinsheimer (19) report that after a removal of protein by Sevag extraction, the disintegration at 20°C of their RNA stopped at a molecular weight of about \( 1.65 \times 10^6 \). A similar extraction was performed on two of the preparations (7 and 9b) of molecular weight \( 1.7 \times 10^6 \) in the hope that a stable product would be obtained. The extracted RNA, however, was not stable. In 1½ hours the 90° scattering decreased by 40% and the dissymmetry decreased to 1.25. This would correspond approximately to a 40% decrease in molecular weight.
Figure 7. Scattering Curve at 3131Å.
D. Effect of Salt Concentration

As shown in Tables 3 and 4, the RNA had an average dissymmetry of 1.33 at 4358Å and 1.68 at 3131Å. These figures are for the RNA in scattering buffer, equivalent to a salt concentration of 0.3M. Rowen (36) reported that the dissymmetry, hence shape, of sodium desoxyribonucleate depended on the salt concentration of the solvent. A similar effect is observed with this RNA and is shown in Figure 6. The limiting dissymmetry for zero salt concentration is 1.74 at 4358Å and 1.98 at 3131Å. The constant values that are obtained at salt concentrations higher than 0.3M are 1.31 at 4358Å and 1.69 at 3131Å. These numbers are based on data from preparations 18, 19, and 20.

Complete scattering data were obtained on several of the different salt concentrations, and are shown in Table 5. It will be noted that the molecular weight is approximately constant between zero and 0.3M salt concentration, but increases as the salt concentration is increased from 0.3 to 0.5M. This increase in molecular weight may be interpreted as a side by side aggregation of the RNA. If it were an end to end aggregation, it would have been evidenced by a change in dissymmetry. That this aggregation is reversible may also be seen in Table 5. The order in which the concentrations are listed corresponds to the order in which the salt concentrations were changed. The details follow.

Preparations 18 and 19 were mixed and one half of the mixture set aside as a control. Enough 1.25M NaCl solution was then added to make
the total salt concentration 0.5M, and scattering data obtained. The RNA was then dialyzed against distilled water overnight giving a total salt concentration 0.02M. The molecular weight of this final product was approximately the same as the initial preparation. Data on the control were taken at the end. More complete disymmetry data were obtained on preparation 20. In this case the RNA preparation was first dialyzed to water. After data were taken in water, 1.25M NaCl solution was added to obtain the data given in Table 5 and Figures 8, 9, and 10.

Figures 9 and 10 show the change in the scattering curves for different salt concentrations.

### Table 5

<table>
<thead>
<tr>
<th>Prep. no.</th>
<th>Salt Conc.</th>
<th>$\lambda = 4358^\circ$ Intercept</th>
<th>Mol. Wt.</th>
<th>$\lambda = 3131^\circ$ Intercept</th>
<th>Mol. Wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 &amp; 19</td>
<td>0.3M</td>
<td>0.98</td>
<td>1.66</td>
<td>0.125</td>
<td>1.93</td>
</tr>
<tr>
<td></td>
<td>0.5M</td>
<td>0.77</td>
<td>2.11</td>
<td>0.105</td>
<td>2.30</td>
</tr>
<tr>
<td></td>
<td>0.02M</td>
<td>1.01</td>
<td>1.61</td>
<td>0.155</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.89</td>
<td>1.82</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>20</td>
<td>Water</td>
<td>0.99</td>
<td>1.64</td>
<td>0.170</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td>0.075M NaCl</td>
<td>1.02</td>
<td>1.59</td>
<td>0.165</td>
<td>1.46</td>
</tr>
<tr>
<td></td>
<td>0.5M NaCl</td>
<td>0.76</td>
<td>2.14</td>
<td>0.11</td>
<td>2.19</td>
</tr>
</tbody>
</table>
Figure 8. Effect of Salt on Dissymmetry.
Figure 9. Scattering Curves at Different Salt Concentration (λ = 1358 Å).
Figure 10. Scattering Curves at Different Salt Concentration (3131Å).
An earlier attempt to increase the salt concentration to 1 molar resulted in extreme aggregation of the RNA. In this case, however, NaCl crystals were added directly to the solution. The resulting solution plugged the ultrafine filter, and 70% of the RNA was sedimented upon centrifugation at 40,000 g. for one hour. The supernatant could be filtered and data obtained. The scattering curve was very irregular and uninterpretable with an intercept corresponding to a molecular weight of $5.6 \times 10^6$. No further attempts were made in this direction.

E. Depolarisation

Only the depolarisation for vertically polarised incident light was measured, since this quantity may be used for both molecular weight and size correction. Two typical plots of $\rho_v$ vs $\sin^2 \theta/2$ for 4358 Å and 3131 Å are shown in Figures 11 and 12, respectively. The rest of the data are shown in Table 6. The values for $\rho_v(0^\circ)$ were obtained by extrapolation of curves such as shown in the Figures. All of these curves had shapes and slopes like the two curves shown, the different values of $\rho_v$ corresponding to an upward or downward shift of these curves.

The scattered horizontally polarised light intensity was very small in both cases, the signal being near the lower limit of sensitivity of the instrument with the D.C. amplifier. The horizontal scattering due to buffer alone was about 30% of that due to the RNA solutions at both wavelengths. These two factors may account for the large variations
in the values of $\rho_v$. Since the depolarization due to the RNA is very small, it may be that impurities would affect the depolarization more than say the scattering curves which were linear, indicating homogeneity.

Table 6

Depolarization Data

<table>
<thead>
<tr>
<th>Prep. no.</th>
<th>Equiv. Salt Conc.</th>
<th>$\lambda = 4358 \AA$</th>
<th>$\rho_v(0^\circ)$</th>
<th>$\rho_v(90^\circ)$</th>
<th>$\lambda = 3131 \AA$</th>
<th>$\rho_v(0^\circ)$</th>
<th>$\rho_v(90^\circ)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0.3M</td>
<td>0.0050</td>
<td>0.0056</td>
<td>0.021</td>
<td>0.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>0.3</td>
<td>0.0037</td>
<td>0.0045</td>
<td>0.013</td>
<td>0.015</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 &amp; 19</td>
<td>0.3</td>
<td>0.0030</td>
<td>0.0033</td>
<td>0.021</td>
<td>0.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.0040</td>
<td>0.0050</td>
<td>0.0094</td>
<td>0.012</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.0027</td>
<td>0.0034</td>
<td>0.018</td>
<td>0.020</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>zero</td>
<td>0.0059</td>
<td>0.0068</td>
<td>0.037</td>
<td>0.046</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.075</td>
<td>0.0052</td>
<td>0.0058</td>
<td>0.033</td>
<td>0.040</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5M</td>
<td>0.0033</td>
<td>0.0040</td>
<td>0.024</td>
<td>0.030</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 11. Depolarization as a Function of Angle (4358 Å).
Figure 12. Depolarization as a Function of Angle (3131 Å).
V. RESULTS AND DISCUSSION

A. Molecular Weight

Phosphorus analyses on TMV (19) give an upper limit to the molecular weight of RNA of about $2.2 \times 10^6$. The assumptions are that 1) all the RNA is separated in one piece, 2) all the TMV phosphorus is in the RNA, and 3) there is one and only one nucleoside per phosphorus atom. Molecular weights above this value must be attributed to either aggregation of the RNA or incomplete protein separation and/or denaturation. Since a molecular weight $1.7 \times 10^6$ was obtained under a fairly wide range of preparation conditions, it appears that this may correspond to all the RNA in one virus, in disagreement with phosphorus analyses. It is worthwhile to look at some of the possible sources of this difference.

It should first be pointed out that the detailed mechanism of the separation of the protein and RNA and denaturation of the protein is not understood, thus little can be said about assumption 1 above.

If all of the TMV phosphorus were not in the RNA, the apparent molecular weight calculated from phosphorus analysis would be too high. Bawden and Pirie (37) report that the heat preparation leaves all the phosphorus in the supernatant liquid. This suggests, but does not assure, that it is all in the RNA. Thus assumption 2 perhaps needs further verification.
Even if the assumption of one nucleoside per phosphorus were not true, the difference would not be explained. The molecular weight, determined from scattering, depends on the measured concentrations of RNA as

$$\frac{1}{M} \propto c \left( \frac{dn}{dc} \right)^2,$$

giving $M \propto c$. The RNA concentrations were related to optical density by phosphorus analyses as given by Northrop and Sinsheimer (19) assuming also that there was only one nucleoside per phosphorus. For example, if there were less than one nucleoside per phosphorus, the upper limit determined from TMV phosphorus would be too large. But, the weight concentration used in scattering calculations would also be too large since it is based on the same assumption. Thus, this assumption could not affect the molecular weight determined from scattering relative to that determined from TMV phosphorus analysis.

Another possible source may be in the enzyme present in these RNA preparations. It has been shown (19) that this enzyme is not active at 100°C or 0°C, but its possible effects during cooling from 100°C to 0°C immediately after preparation are not clear. The time for cooling in these preparations was about 1 to 1½ minutes. Two experiments were performed with rapid cooling of the RNA, accomplished by pouring the hot preparation over pieces of copper
precooled to -80°C with dry ice. The solution reached 10°C in about 10-15 sec., reaching 0° in another 20 sec. One preparation was heated for 70° and the other 100°. The resulting molecular weights were 3.3 x 10^6 and about 4 x 10^6, much higher than would be expected.

Thus, while samples with consistent values of molecular weight of 1.7 x 10^6 could be prepared, it is not certain that each particle of this weight corresponds to all the RNA in one virus.

B. Shape and Size

The shape and size of the RNA may be determined from the dissymmetry as discussed above (Section II). Consider first the limiting dissymmetries with water as the solvent and no depolarization correction. The average dissymmetry of 1.73 at 4350Å corresponds to a rod with L/λ' = 0.495 or a random coil with S/λ' = 0.335. L is the length of the rod, S the mean distance between ends of the coil and λ' the wavelength of light in water (3280Å for λ = 4350Å and 2310Å for λ = 3131Å). Since L and S are independent of wavelength, the ratios L/λ' and S/λ' may be calculated for λ = 3131Å as 0.734 and 0.496 respectively. From these, the expected dissymmetries at 3131Å would be 2.14 for the rod and 2.51 for the coil. The observed value at 3131Å had an average of 2.0 from Figure 8. Thus the coil as a model is eliminated, the data corresponding most nearly to an isotropic rigid rod of length 1600Å. The sphere as a model may be eliminated in a similar manner.
Now consider the effect of anisotropy. As pointed out earlier, the dissymmetry of a perfectly random coil is independent of any anisotropy in the fiber making up the coil. Thus, only the rigid rod would fit the data. The value of $\mathcal{P}_y(0^\circ)$ of about 0.0055 at 4358Å corresponds to value of the anisotropy function $S$ (Equation 9) of $\pm 0.1$. Using $\mathcal{P}_y(0^\circ) = 0.035$ at 3131Å gives a $S$ of $\pm 0.25$.

Since curves were not given by Horn and Benoit for these values of $S$ and the necessary calculations would have been quite lengthy, a graphical interpolation of their curves was made to determine the curves corresponding to $S = \pm 0.1$ and $S = \pm 0.25$. The values of $L$ determined in this way are shown in Table 7. $S = 0$ corresponds to the isotropic rod.

### Table 7

**Lengths of an Anisotropic Rod**

<table>
<thead>
<tr>
<th>$\lambda = 4358\text{Å}$ and $Z = 1.73$</th>
<th>$\lambda = 3131\text{Å}$ and $Z = 2.0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S = -0.1$</td>
<td>$S = -0.25$</td>
</tr>
<tr>
<td>$L = 1470\text{Å}$</td>
<td>$L = 1120\text{Å}$</td>
</tr>
<tr>
<td>$S = 0$</td>
<td>$S = 0$</td>
</tr>
<tr>
<td>$L = 1620\text{Å}$</td>
<td>$L = 1420\text{Å}$</td>
</tr>
<tr>
<td>$S = \pm 0.1$</td>
<td>$S = \pm 0.25$</td>
</tr>
<tr>
<td>$L = 1880\text{Å}$</td>
<td>None$^a$</td>
</tr>
</tbody>
</table>

$^a$The "none" means that a rod of this anisotropy can not have a dissymmetry of 2.0 for any length (including $\infty$).
It may be seen that the experimental values of either \( \phi \) do not correspond to a consistent length for the rod at both wavelengths. Horn and Benoit (20) have measured \( \phi \) for TmV and calculate a value of \( \phi = 0.3 \) (wavelength not given). They have also calculated the expected value of \( \phi \) from index of refraction measurements as \( \phi = 0.06 \). Thus for TmV, light scattering gives a value of \( \phi \) four times too large. It appears that this is also the case for RNA. It may be that the theory is not complete and that light scattering is not a good way to measure anisotropy.

Since the 3131\( \AA \) wavelength is approaching an absorption band from the long wavelength side, this anisotropy should be larger than that at 4358\( \AA \). Referring again to Table 7, the data would be explained if the rod were isotropic at 4358\( \AA \) and \( \phi \) approximately equal to \( \pm 0.1 \) at 3131\( \AA \). The length would then be 1620\( \AA \). An estimate of the error would be \( Z = 1.73 \pm 0.03 \) or \( L = 1620 \pm 100 \AA \).

Now consider the RNA in concentrated salt (0.3M) where it is at a minimum length. There is no a priori reason to say the shape must still be a rigid rod, so an analysis similar to that in water is necessary. The dissymmetries are 1.31 at 4358\( \AA \) and 1.69 at 3131\( \AA \). The expected dissymmetries at 3131\( \AA \), based on the 4358\( \AA \) value, are identical for both the isotropic rod and coil, namely 1.62. For the dissymmetry of 1.31, the anisotropy correction (for \( \phi = \pm 0.1 \)) is less than the limits of error of the dissymmetry. This would correspond to an isotropic rigid rod of length 1050
A rigid rod of this length and a dissymmetry of 1.69 at 3131° would have an anisotropy \( \delta \) of -0.1 and not \( \pm 0.25 \) as the data would indicate. Another possible model would be a bent or kinked rod, only not to the extent that it would be a perfectly random coil. Calculations of length are not available for such a model.
VI. CONCLUSIONS

(1) The apparent molecular weight of TMV RNA depends on the conditions of preparation by the heat method. However, a molecular weight of $1.7 \times 10^6$ can be obtained under a fairly wide range of conditions.

(2) This molecular weight may correspond to all the RNA in one virus, that is the RNA is separated in one piece, but the evidence is not conclusive.

(3) The RNA molecule is a rigid rod of length $1600 \pm 100\AA$ in water solution. In salt solution its longest dimension decreases, reaching a minimum at 0.3M. Two possible interpretations of this decrease are contraction in length of the rigid rod (helical structure) or bending and kinking of the rod fiber. The interpretation of a rigid rod gives a minimum length of $1050 \pm 100\AA$.

(4) Reversible side by side aggregation of the RNA takes place at 0.5M salt concentration.

(5) The rod shape indicates that TMV consists of an inner core of RNA with the protein forming an outer shell.
VII. BIBLIOGRAPHY


VIII. ACKNOWLEDGMENTS

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