Molecular Weights, Dispersion of Refractive Index Increments, and Dimensions from Transmittance Spectrophotometry. Bacteriophages R17, T7, and PM2, and Tobacco Mosaic Virus†

Rafael D. Camerini-Otero,‡ Richard M. Franklin,§ and Loren A. Day*

ABSTRACT: In this study we have shown that accurate molecular weights of macromolecules can be conveniently obtained from the turbidity per centimeter of light path, τ, measured with commonly available ultraviolet–visible spectrophotometers. The molecular weights obtained in this manner for R17 bacteriophage are $3.95 \pm 0.22 \times 10^6$ and $3.85 \pm 0.28 \times 10^6$ at 436 and 546 nm, respectively. Molecular weights obtained using other methods are: $3.64 \pm 0.18 \times 10^6$ by high-speed equilibrium centrifugation, $3.80 \pm 0.23 \times 10^6$ by light scattering, and $3.98 \pm 0.19 \times 10^6$ by sedimentation–viscosity. The average of all these values of M for R17 is 3.85×10^6 which is in agreement with M calculated from the viral composition. The molecular weights obtained by the transmittance method at 436 nm for three other viruses are: for lipid-containing bacteriophage PM2, $43.5 \pm 2.1 \times 10^6$; for bacteriophage T7, $53.1 \pm 2.8 \times 10^6$; and for tobacco mosaic virus (TMV), $41.3 \pm 1.7 \times 10^6$. We have also shown that for many nucleoproteins the wavelength dependence of turbidity in the visible region can be completely accounted for by intraparticle interference and a Cauchy-like dispersion of the refractive index increment. From measurements of τ as a function of λ_0 and the exact equation relating τ and M, the wavelength dependence of $(\partial n/\partial c)_\mu$ has been determined for these viruses and could be described by a single dispersion relationship. This information has been used to obtain virus dimensions and molecular weights from wavelength scans of τ. A simpler version of the transmittance method which involves only the turbidity at 436 nm and an assumed value for $(\partial n(436)/\partial c)_\mu$ (also used to determine c) is shown to yield values of M accurate to about 10%.

Light scattering by dilute solutions (Debye, 1944; Zimm *et al.*, 1945) is a widely used method for the determination of the molecular weight and radius of gyration of macromolecules and colloid particles. Usually the intensity of the scattered light is measured at different angles to the incident beam with a light scattering[1] photometer. It is a well-known problem with such photometers that virtually all the methods used to determine the ratio of the intensity of the scattered light to the incident beam, of the order of 10^{-4} or lower, involve some uncertainties. Another way of measuring the light scattered by solutions of macromolecules is to determine the loss of intensity from the incident beam in a transmittance spectrophotometer. This kind of measurement directly gives intensity ratios and involves the use of a more easily accessible and less experimentally demanding instrument, a spectrophotometer.

Although the use of transmittance to measure molecular weights of macromolecules has long been recognized (Debye, 1944; Zimm *et al.*, 1945; Oster, 1946; Cashin and Debye, 1949) the method has been seldom used (Doty and Steiner, 1950; Billmeyer, 1954). There is one major aspect of transmittance measurements which has made them less popular than their simplicity and accuracy would warrant. With visible light (λ_0 400–700 nm), destructive interference occurs for particles having at least one dimension larger than ~200 Å. Thus, although solutions of scatterers having molecular weights greater than 10^6 scatter strongly at moderate concentrations (0.1–1.0 mg/cm^3) and are therefore more amenable to transmittance measurements, it is precisely the scattering from these large particles that requires correction for destructive interference.

To our knowledge, only one procedure to correct transmittance data for destructive interference has hitherto been used to obtain the molecular weight of a biological macromolecule (Doty and Steiner, 1950). The limited success of this early application is perhaps a further reason for the virtual neglect of the transmittance method for determining the molecular weights of biological macromolecules. In this paper it is shown that transmittance measurements can yield accurate molecular weights for biological macromolecules of different mass, shape, and composition. In order to test the experimental accuracy of the method we have compared the molecular weight by the transmittance method for the RNA-containing bacteriophage R17 with the molecular weights obtained by more commonly used methods. Because of the lack of agreement in the literature we have determined molecular weights by sedimentation–diffusion (Camerini-Otero *et al.*, 1974), sedimentation–viscosity, equilibrium sedimentation, and light scattering. We also used transmittance measurements to determine the molecular weights of bacteriophages PM2 and T7, spherical viruses larger than R17, and the highly asymmetric tobacco mosaic virus.

† From The Public Health Research Institute of the City of New York, Inc., New York, New York 10016. *Received February 12, 1974.* This research was supported by Grant No. AI-07645 (R. M. F.) and Grants AI-09049 and 1-K04-GM70363 (L. A. D.) from the U. S. Public Health Service. One of us (R. D. C.-O.) was an M.D.–Ph.D. Predoctoral Fellow at the New York University School of Medicine, supported by U. S. Public Health Service Medical Scientist Training Grant No. 5-T05-GM0-1668-06.

‡ Present address: Laboratory of Molecular Biology, National Institute of Arthritis, Metabolic and Digestive Diseases, National Institutes of Health, Bethesda, Md. 20014.

§ Present address: Biozentrum der Universität, Klingelbergstrasse 70 CH-4056 Basel, Switzerland.

[1] The term light scattering will be applied to measurements of scattered light intensities in a given direction, whereas the terms turbidity and transmittance will be applied to measurements of the total loss of intensity from the incident beam by scattering in all directions. The turbidity, τ, is related to the fractional transmittance per centimeter, T, by $\tau = -2.303 \log T$.

The transmittance data have also been used to obtain the wavelength dependence of the refractive index increment through the introduction of the known molecular weights and shapes into the turbidity expression. It has been possible to obtain constants for a dispersion relationship for the refractive index increment of these viruses of different chemical compositions: R17, containing 30–32% RNA (Hoffmann-Berling *et al.*, 1966); PM2, containing 13% supercoiled DNA and 13% lipid (Espejo and Canelo, 1968; Camerini-Otero and Franklin, 1972); T7, containing 50% linear DNA (Bancroft and Freifelder, 1970); and TMV,[2] a rod-shaped virus containing 5% RNA (Bawden and Pirie, 1937). These constants were sufficiently close for the viruses tested that an average dispersion relationship could be used to obtain molecular weights and radii of gyration from the transmittance data in the manner suggested by Cashin and Debye (1949).

Transmittance by Monodisperse Solutions of Macromolecules; Preliminary Considerations.

Intraparticle Interference. If $-\mathrm{d}I$ is the amount of light lost along an element $\mathrm{d}x$ in the path of the beam, and the turbidity, τ, is defined as the proportionality constant in

$$-\mathrm{d}I = \tau I \mathrm{d}x \qquad (1)$$

where I is the incident intensity, then (Doty and Steiner, 1950)

$$\tau = HcMQ(\lambda) \qquad (2)$$

where H is as given below, c is the concentration, and (Doty and Steiner, 1950)

$$Q(\lambda) = (3/8)\int_0^{\pi} P(K)(1 + \cos^2\theta)\sin\theta \mathrm{d}\theta \qquad (3)$$

$P(K)$ is a particle scattering factor defined as the ratio of the scattered intensity in the presence of interference to that in its absence. It is a function of the magnitude of the scattering vector, $K = (4\pi/\lambda)\sin(\theta/2)$, where λ is the wavelength of the incident light in the scattering medium and θ is the angle of observation. $Q(\lambda)$, a function of λ, is the transmittance equivalent of the particle scattering factor and is a positive dimensionless number between 0 and 1 (Doty and Steiner, 1950).

By rearranging terms we obtain the main expression used in this study

$$H(\lambda_0)cQ(\lambda)/\tau = 1/M \qquad (4)$$

where

$$H(\lambda_0) = 32\pi^3(n_0(\lambda_0))^2(\partial n(\lambda_0)/\partial c)_\mu{}^2/3N_A\lambda_0{}^4 \qquad (5)$$

where the refractive index of the solvent, n_0, the refractive index increment at constant chemical potential of diffusible components, $(\partial n/\partial c)_\mu$, and H are all functions of λ_0, the *in vacuo* wavelength of the incident light, and N_A is Avogadro's number.

When the various experimental parameters at a given wavelength are known, and $Q(\lambda)$ can be obtained by one of the procedures described below, eq 4 can be used to obtain the molecular weight from τ. This treatment of intraparticle interference is strictly valid only in the limit of infinite dilution or in the absence of interparticle interference.

Before discussing methods to evaluate $Q(\lambda)$ it is important to know some of the properties of $Q(\lambda)$ and its counterpart $P(K)$: (1) regardless of the size or shape of the scatterers, $Q(\lambda)$ and $P(K)$ approach 1 from below as $\lambda \to \infty$ $(1/\lambda \to 0)$ and $K \to 0$; (2) for scatterers with their largest dimension $< \lambda/20$ or $< K^{-1}/3$, $Q(\lambda)$ and $P(K)$ have values between 0.99 and 1.00; (3) for the scatterers we are concerned with in this paper, $Q(\lambda)$ and $P(K)$ are monotonically decreasing functions of $1/\lambda$ and K, for which the larger the scatterer, the stronger the functional dependence of $Q(\lambda)$ and $P(K)$ on $1/\lambda$ and K.

Evaluation of the Magnitude of Intraparticle Interference Effects on Transmittance Measurements. There are several ways to correct transmittance measurements for intraparticle interference in the absence of interparticle interference. If the shape and dimensions of the scatterer are known, Q can be calculated or obtained from existing tables (Doty and Steiner, 1950). This value can be combined with c, τ, and $(\partial n(\lambda_0)/\partial c)_\mu$, measured at *one* wavelength, to obtain M (eq 4). With the availability of negative staining techniques for the electron microscope (Brenner and Horne, 1959), obtaining the shapes and dimensions for macromolecular structures large enough to require this correction is relatively simple.

Another method for evaluating Q is that of Doty and Steiner (1950). It is based on the relationship

$$\frac{-\mathrm{d}\log\tau}{\mathrm{d}\log\lambda_0} = 4 - \frac{\mathrm{d}\log Q}{\mathrm{d}\log\lambda_0} = 4 - \beta \qquad (6)$$

where the derivative $\mathrm{d}\log Q/\mathrm{d}\log\lambda_0$ is denoted by β. For solutions of biological macromolecules, where the refractive index of the solute is higher than that of the solvent, β is a positive number (Kerker, 1969). Put more simply, τ will be proportional to $1/\lambda_0{}^{4-\beta}$. Experimentally, τ is measured as a function of λ_0 and β can then be evaluated from a double logarithmic plot of τ *vs.* λ_0, where the slope of this plot is $\beta - 4$, typically a number between -3 and -4. Once β is known, Q can be evaluated from tables published by Doty and Steiner (1950) and M can be calculated by substitution into eq 4. It is important to recognize, however, that over a wide wavelength range τ has to be corrected for the wavelength dependence of $(\partial n(\lambda_0)/\partial c)_\mu$ and $n_0(\lambda_0)$ (Cashin and Debye, 1949; Doty and Steiner, 1950; Cancelleri *et al.*, 1974) and thus β will not be a constant but a monotonically increasing function of $1/\lambda_0$ (Camerini-Otero, 1973; Camerini-Otero, R. D., and Day, L. A., manuscript in preparation).

The third method to correct for intraparticle interference is that suggested by Cashin and Debye (1949), and involves extrapolation of τ to infinite λ_0 to obtain the molecular weight and is the counterpart of the extrapolation to zero angle commonly used in light scattering (Zimm, 1948). The first and third methods have been used in this study and are presented in more detail below.

Materials and Methods

Preparation of Viruses. Bacteriophages R17 and T7 were grown and purified according to a combination of published procedures (Vasquez *et al.*, 1966; Yamamoto *et al.*, 1970; Camerini-Otero, 1973; Camerini-Otero *et al.*, 1974). Bacteriophage PM2 was grown on *Pseudomonas BAL-31* and purified according to a previously published procedure (Salditt *et al.*, 1972). Partially purified TMV was the kind gift of Dr. F. A. Anderer. Further purification was carried out according to the method of Boedtker and Simmons (1958). The final step in the preparation of all the viruses was extensive dialysis against the buffers listed in Table I; these were the buffers used throughout this investigation.

Measurements of Virus Concentrations, Refractive Index Increments, and Density Increments. Virus concentrations were measured spectrophotometrically at 260 nm. For bacteriophage R17 an extinction coefficient of 7.93 $\mathrm{cm}^2\ \mathrm{mg}^{-1}$ was used, which is an average of the two literature values available, 7.66 $\mathrm{cm}^2\ \mathrm{mg}^{-1}$ (Gesteland and Boedtker, 1964) and 8.20 cm^2

[2] Abbreviation used is: TMV, tobacco mosaic virus.

mg^{-1} (Enger *et al.*, 1963; also see Discussion). The other extinction coefficients used were 4.60 cm^2 mg^{-1} for bacteriophage PM2,[3] 12.90 cm^2 mg^{-1} for bacteriophage T7 (Bancroft and Freifelder, 1970), and 3.06 cm^2 mg^{-1} for tobacco mosaic virus (Boedtker and Simmons, 1958). Dilutions of virus solutions were made by weight; corrections were applied for the difference between solution and solvent densities when necessary.

Refractive index increments for bacteriophages R17, PM2, and T7 were measured with a Brice-Phoenix differential refractometer at 546 and 436 nm at 25 ± 0.1°. Virus solutions were dialyzed against the appropriate solvent with at least three changes of outer solution in closed containers, the final dialysis lasting at least 48 hr. The undiluted virus solutions, in a concentration range of 20–30 mg cm^{-3}, were measured against the outer dialysate. This procedure gives an apparent refractive increment at constant chemical potential. The refractive index increment at 436 nm for tobacco mosaic virus was obtained from Boedtker and Simmons (1958).

The apparent density increment at constant chemical potential is defined by

$$(\Delta\rho/c)_\mu = (\rho_s - \rho_o)/c$$

where ρ_s is the density of the virus solution taken undiluted from the dialysis bag and ρ_o is the density of the outer solution at equilibrium. For many purposes it is convenient to express $(\Delta\rho/c)_\mu$ in terms of an apparent specific volume ϕ' (Casassa and Eisenberg, 1964)

$$(\Delta\rho/c)_\mu = 1 - \phi'\rho_o$$

We have assumed, as is usually done, that ϕ' determined at the virus concentration used here is equal to that in the limit of infinite dilution. The densities of the R17 solution and the outer dialysate were determined at 25.0 ± 0.05° with a 5.2-cm^3 Lipkin pycnometer (Lipkin *et al.*, 1944). Weights were known to be within ±0.02 mg and volumes were within ±0.0002 cm^3, to give $\rho_s - \rho_o$ = 7.84 ± 0.08 mg cm^{-3}. For both the virus solution and the solvent the pycnometer was filled and weighed and its volume measured; three separate determinations were made.

Viscosity. The specific viscosity of R17 solutions was measured with a Cannon-Ubbeholde semimicro dilution viscometer having a volume of 1.5 ml and flow times of about 230 sec for the solvent. The temperature was controlled at 25.0° with an accuracy of ±0.05° and with a relative deviation of less than ±0.01°. The flow times for the virus solutions were 12–35 sec longer than those for the solvent.

Analytical Ultracentrifugation. Equilibrium sedimentation of bacteriophage R17 was carried out according to the high-speed technique developed by Yphantis (1964). The virus solutions, ranging in initial concentration from 10 to 40 μg cm^{-3}, were centrifuged to equilibrium in an An-J rotor in a Beckman Model E ultracentrifuge equipped with ultraviolet optics, photoelectric scanner, electronic speed control, and temperature control unit.

Temperature regulation at 20° was used and the rotor speed (3014 rpm) was determined by means of the revolution counter and a stopwatch. The distributions, recorded with photoelectric scans at 265 nm, were independent of time for over the last 60 hr of the centrifugation. The scanner tracings were recorded such that a 1-cm deflection corresponded to 0.2 optical density unit (OD unit); the estimated uncertainty in reading these tracings was ±0.0035 OD unit. The weight-average molecular weight at radius r, $M_w(r)$, was calculated from the slope of a least-squares straight line through six adjacent data points in a plot of ln OD *vs.* r^2; overlapping regions with four common points were evaluated throughout the concentration distribution at optical densities from 0.05 to 1.0. For the range of concentration used in the experiments with R17, $M_w(r)$ was independent of the virus concentration. $\bar{M}_w$ was calculated from the slope of a least-squares straight line through all the points tabulated for a cell sector.

[3] R. D. Camerini-Otero and R. M. Franklin, unpublished results.

TABLE I: Solvents Used throughout This Investigation.

Virus	Solvent	pH[a]	n_0^{23} (546)[b]	n_0^{23} (436)[b]
R17	0.15 M NaCl 0.05 M Tris-HCl	7.4	1.336	1.342
PM2	1 M NaCl	6.2–6.6[c]	1.343	1.349
T7	0.5 M NaCl 0.01 M Tris-HCl 0.001 M $MgCl_2$	7.8	1.339	1.345
TMV	0.01 M NaH_2PO_4 0.001 M EDTA	7.2	1.334	1.340

[a] Measured at room temperature (~23°). [b] Solvent refractive indices at 546 and 436 nm; values are at room temperature (23°) and estimated to within ±0.001 from values in the International Critical Tables. For the solvent refractive index as a function of wavelength, $n_0^{23}(\lambda)$, we have used the relationship $n_0^{23}(\lambda_0) = [n_0^{23}(546)/n_w^{23}(546)][n_w^{23}(546)/n_w^{18}(546)][n_w^{18}(\lambda_0)]$, where the subscript w denotes water, and according to the International Critical Tables $[n_w^{18}(\lambda_0)]^2 = -0.013414\lambda_0^2 + 1.76148 + [0.0065438/(\lambda_0^2 - (0.11512)^2)]$. [c] Measured on the virus solutions used for the measurements.

Angular Dependent Light Scattering. Light scattering experiments with unpolarized light were performed at a temperature of 25.0°, at angles of observation, θ, from 30 to 150°, at λ_0 546 nm with a light scattering photometer (Wippler and Scheibling, 1954) manufactured by Fica (formerly Sofica), Paris. Calibration of the instrument was based on a Rayleigh ratio of benzene of 16.4 × 10^{-6} cm^{-1} (Doty and Steiner, 1950). Other experimental details have been presented elsewhere (Camerini-Otero, 1973).

Turbidity Measurements. All spectrophotometric measurements for determining molecular weights were made at room temperature (~23°) with a Cary Model 14 double-beam recording spectrophotometer. The amplitudes were calibrated to within ±0.5%. Wavelengths were calibrated to within approximately 0.2 nm. Since the measured optical densities per centimeter of pathlength (OD units/cm) were usually below 0.02, most of the measurements were made with a pair of matched cylindrical cells with a 10-cm pathlength (volume ~ 30 cm^3) and with an instrumental sensitivity capable of recording 0.2 OD unit as a 20-in. pen deflection. The scanning speed was 1 nm/sec and the display was 15 nm/in. In the wavelength range from 350 to 450 nm the spectral band width was less than 1.5 nm; from 400 to 600 nm, less than 0.4 nm; and from 600 to 700 nm, less than 3.5 nm. Since according to our calculations based on the optical design of the Cary 14 less than 0.5% of the total scattered light from the 30-ml sample could reach the photodetector, we have taken the apparent optical densities displayed on the recorder as the absolute extinction of light by the virus solution relative to the solvent.

The experimental procedure we used for all the transmit-

tance measurements was the following. A concentrated virus solution was extensively dialyzed against buffer and all dilutions were made with the outer dialysate. The dialysate buffer was filtered into both cells, with the aid of a glass syringe, using 25-mm Millipore filters having pore sizes used for the corresponding viral solutions: 0.22 μ for R17 and 1.2 μ for PM2, T7, and TMV. The solvent base line was recorded from 700 down to 350 nm. The sample cell was then emptied, rinsed with a small amount of filtered virus solution, and filled with filtered virus solution and repositioned in the instrument. The optical density was recorded from 700 nm down to the point where the optical densities were off scale, usually 350–400 nm. The sample cell was then thoroughly rinsed with filtered dialysate, and a second base line was recorded. Only when the two base lines were superimposable within ±0.0004 OD unit (±1 mm in pen position) was the data used for molecular weight determinations.

Considering the uncertainties in the signal and the base line for the turbidity measurements, we estimate a total uncertainty of ±0.0010 OD unit (±0.00023 turbidity unit/cm path). The relative error ranged from ±12% for the lowest recorded amplitude to ±0.5% for the highest amplitude.

For sufficiently low concentrations, a convenient relationship for measurements at 436 nm is

$$M = 8.44 \times 10^3 \text{ cm}^4(\text{OD units/cm})/Q(\lambda)(\partial n/\partial c)_\mu{}^2 c$$

where we have used a value of n_0 of 1.341 for 0.15 M NaCl at 436 nm.

Error Analysis. Whenever possible, the uncertainty, δx, associated with the mean value, $\bar{x}$, of a set of n observations, x_i, has been estimated as the 95% confidence interval for $\bar{x}$. From the standard error of the mean 95% confidence intervals were obtained from tables for the distribution of t (see, for example, Freund, 1962, Table IV). On those occasions when it was not possible to calculate 95% confidence intervals, we have attempted to estimate δx at this level of confidence.

The uncertainty in a derived quantity, M, a function of experimental variables, $x, y, \ldots$, was estimated from the uncertainties $\delta x, \delta y, , \ldots$, according to the law of propagation of errors (see, for example, Colquhoun, 1971). In applying this relationship to quantities which we have measured and that are a function of concentration, such as $(\Delta n/c)_\mu$, $(\Delta\rho/c)_\mu$, and η_{sp}/c, we have been careful to consider the numerator and denominator as independent variables such that, for example, the uncertainty, δq, in a derived quantity $q = (\Delta n/c)_\mu{}^2 c$ is $q[(\delta c/c)^2 + (2\delta(\Delta n)/\Delta n)^2]^{1/2}$.

Computational Methods. All slopes and intercepts were based on least-squares analysis of the data. Values of $Q(\lambda)$ as given by eq 3 were obtained by numerical evaluation of the integrals by Simpson's rule. The values were invariant to within ±0.02% for 12 or more integration subintervals; 16 subintervals were used routinely to ensure numerical accuracy to better than ±0.01%. Comparisons between the values of $Q(\lambda)$ so obtained with those evaluated by graphical integration and published by Doty and Steiner (1950) showed agreement to be within about ±1.5%.

Results

Molecular Weight of Bacteriophage R17. The apparent specific volume, ϕ', was 0.689 ± 0.012 cm^3 g^{-1}. The reduced viscosity, η_{sp}/c, was plotted *vs.* the virus concentration. Expressing the concentration dependence in the usual manner (Huggins, 1942; see for example, Tanford, 1961), $\eta_{sp}/c = [\eta] + k_0[\eta]^2 c$, where c is the virus concentration in grams per deciliter, then $[\eta]$ is 0.0417 dl/g with an uncertainty of 4.1%; k_0, the Huggin's constant, is 1.59. The latter is to be compared with the theoretical value of 2.0 for unhydrated spheres (Simha, 1952). Values of 0.044 dl/g for $[\eta]$ and 1.2 for k_0 for the virtually identical RNA-bacteriophage fr were obtained by Marvin and Hoffmann-Berling (1963).

The value for $s_{20,w}{}^0$ that we have used, 78.9 S (Enger *et al.*, 1963), is close to the average of six literature values of $s_{20,w}{}^0$ (see Table I in Hoffmann-Berling *et al.*, 1966; also, Overby *et al.*, 1966), 79.2 S; we have used as an estimate of the uncertainty in $s_{20,w}{}^0$ the 95% confidence interval for this mean, ±1.5 S (±1.9%). Using the Scheraga–Mandelkern relationship and assuming a spherical shape for the virus, hence a value for the constant β of 2.12 × 10^6 (Scheraga and Mandelkern, 1953), we have calculated for R17 a sedimentation–viscosity molecular weight of 3.98 ± 0.19 × 10^6.

The molecular weight of R17 was also determined by the technique of high-speed equilibrium sedimentation (Yphantis, 1964). The values of $\bar{M}_w$ obtained for three samples were (in millions): 3.60 ± 0.18, 3.65 ± 0.16, and 3.68 ± 0.17. The average molecular weight is 3.64 ± 0.18 × 10^6. The quoted uncertainty in each case was estimated from an uncertainty of 4.0% in the density increment (see Materials and Methods) and the corresponding 95% confidence intervals (±3.3, ±2.2 and ±2.4%, respectively, for the slopes of ln OD_{265} *vs.* r^2, and ±2.8% for the mean of the three samples).

We have also measured the molecular weight of R17 by light scattering. No concentration dependence was observed over the range from 0.08 to 0.9 mg cm^{-3}. The average molecular weight obtained from measurements on six different solutions is 3.80 ± 0.23 × 10^6, where the uncertainty is calculated from a 3.9% uncertainty in the concentration, a 1% uncertainty in the refractometer measurement, and the 95% confidence interval for the mean of the intercepts at $\theta = 0°$ of the measured intensities (±4.2%).

Finally, the molecular weight of R17 was determined by the transmittance method. The experimental data for this determination are given in Figure 1. Since, within the significance of the data, there were no indications of a concentration dependence (Figure 1), a line has been drawn through the average of the measurements at both of these wavelengths. These average values were converted to molecular weights by using the data shown in Tables I and II. In calculating $Q(\lambda)$ according to eq 3, we have used a diameter of 26.6 nm for R17; this value obtained by X-ray scattering (Fischbach *et al.*, 1965) is in excellent agreement with the more recent value of 26.4 nm obtained using the same method (Zipper *et al.*, 1971) and with other values obtained by other methods (see Camerini-Otero *et al.*, 1974).

The 95% confidence intervals for the means of the measured turbidities at 436 and 546 nm were ±3.2 and ±5.7%, respectively. The molecular weights by the transmittance method were 3.95 ± 0.22 × 10^6 for the data collected at 436 nm and 3.85 ± 0.28 × 10^6 for that obtained at 546 nm. The uncertainties were calculated as for the light scattering data.

Molecular Weights of PM2, T7, and TMV from Transmittance at 436 and 546 nm. Plots of c/τ *vs.* c for several transmittance measurements on solutions of PM2, T7, and TMV are also presented in Figure 1. Again, since there is no detectable concentration dependence, the averages of the values for each of these viruses are taken as the values at infinite dilution. The additional data used in calculating the molecular weights from these values according to eq 4 are given in Tables I and II.

In calculating Q we have used the viral dimensions indicated

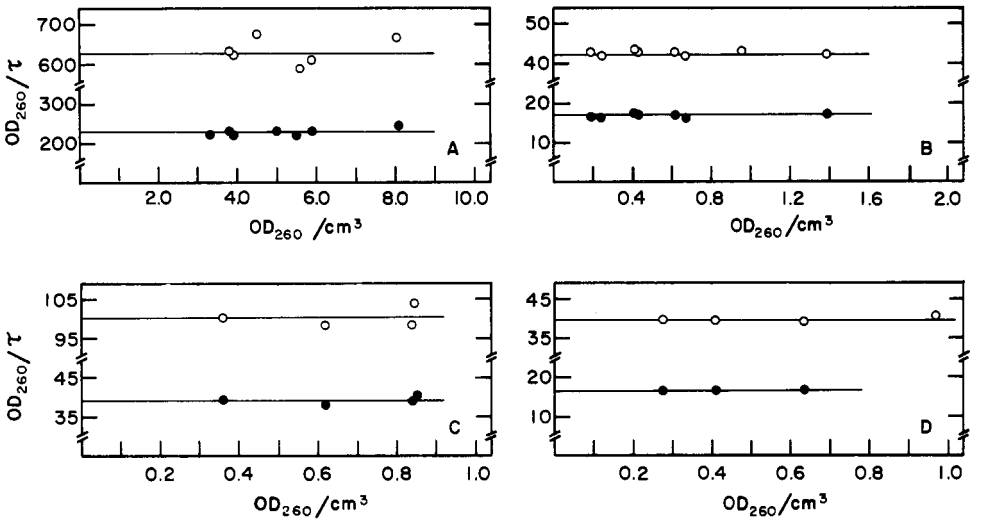

FIGURE 1: Plots of c/τ *vs.* c, where c is given as OD_{260}/cm^3 and c/τ is given as OD_{260}/τ per centimeter pathlength. The extinction coefficients are given in Table II. Filled circles are for τ measured at 436 nm and empty circles are for τ measured at 546 nm. (A) R17 bacteriophage; maximum concentration, 1.02 mg cm^{-3}. (B) PM2 bacteriophage; maximum concentration, 0.303 mg cm^{-3}. (C) T7 bacteriophage; maximum concentration, 0.066 mg cm^{-3}. (D) TMV; maximum concentration, 0.315 mg cm^{-3}.

TABLE II: Summary of the Data Used in the Calculation of Molecular Weights from Transmittance Measurements.

Virus	$E_{1cm}^{0.1\%}$[a] (cm^2 mg^{-1})	$[\partial n(\lambda_0)/\partial c]_\mu$[b] ($cm^3$ g^{-1})		d[c] (nm)	$Q(\lambda)$[d]	
		λ_0 436 nm	λ_0 546 nm		λ_0 436 nm	λ_0 546 nm
R17	7.93 ± 0.30	0.180	0.176	26.6 ± 0.3	0.974 ± 0.001	0.984 ± 0.001
PM2	4.60 ± 0.10	0.162	0.156	63.0 ± 6.3	0.866 ± 0.029	0.913 ± 0.018
T7	12.90 ± 0.07	0.163	0.157	65.0 ± 6.5	0.856 ± 0.032	0.906 ± 0.020
TMV	3.06 ± 0.10[e]	0.194[e]		300.0 ± 10.0	0.439 ± 0.010	0.517 ± 0.011

[a] Extinction coefficients; literature references are listed in Materials and Methods. The uncertainty in $E_{1cm}^{0.1\%}$ for R17 has been estimated from the 95% confidence interval for five literature values (Camerini-Otero, 1973). The uncertainty in the values for PM2 and T7 is that quoted by the original workers; that for TMV has been estimated from a comparison of published values (Bawden and Pirie, 1938; Fraenkel-Conrat and Williams, 1955; Stevens and Lauffer, 1965). [b] This study, unless otherwise indicated. The uncertainty in the values for R17, PM2, and T7 can be estimated from the uncertainties in Δn (~1%) and in $E_{1cm}^{0.1\%}$. [c] Largest dimension: diameter for R17, PM2, and T7, rod length for TMV. Literature references are given in the text. [d] The uncertainty in $Q(\lambda)$ was calculated from the uncertainty in d (note: $\lambda = \lambda_0/n_0$). [e] Boedtker and Simmons, 1958; 0.5% is the quoted uncertainty.

in Table II. Although both PM2 and T7 can be considered as spheres for the present purposes, they appear in the electron microscope as icosahedra (Silbert *et al.*, 1969; Harrison *et al.*, 1971; Dubin, 1970) for which face-to-face distances rather than radii are more appropriate dimensions. For PM2 this distance, 60.0 ± 3.0 nm, was measured on unpublished electron micrographs of negatively stained specimens obtained by Dr. J. A. Silbert (*cf.* Silbert *et al.*, 1969); for T7 the face-to-face distance, also measured on negatively stained specimens, is 62.0 ± 3.0 nm (Dubin, 1970). For both of these viruses the diameter used in calculating Q can be estimated from the fact that the face-to-face distances are ~0.95 times the diameter of a sphere with the same volume as the iscosahedron. Although the uncertainties in the icosahedral dimensions are ±5%, the uncertainties in the equivalent diameters have been taken as ±10%. In the case of TMV, the rod length measured by electron microscopy is generally accepted as 300.0 nm with an uncertainty of ±5.0 to ±10.0 nm (Williams and Steere, 1951; Hall, 1958; Finch, 1969).

The molecular weights calculated from the transmittance measurements at 436 and 546 nm, respectively, are (in millions): 43.5 ± 2.1 and 43.8 ± 1.6 for PM2 and 53.1 ± 2.8 and 52.2 ± 2.7 for T7. For these values of M the uncertainties were calculated from the uncertainties in c (that for the extinction coefficient in Table II), Δn(~1%), Q (see Table II), and the 95% confidence intervals for the means of the measured turbidities (±1.5 and ±1.2% for PM2 and ±3.2 and ±4.1% for T7 at 436 and 546 nm, respectively).

For TMV the molecular weight calculated from τ at 436 nm was 41.3 ± 1.7 × 10^6. From the values of τ at 546 nm two molecular weights can be calculated. A molecular weight of 41.1 ± 2.0 × 10^6 is that obtained from the value of $(\partial n/\partial c)_\mu$ at 546 nm, 0.182 ± 0.004 cm^3 g^{-1}, calculated from the dispersion of the refractive index increment of TMV and the value of $(\partial n/\partial c)_\mu$ at 436 nm given in Table II (see below and Table III). Using a value of $(\partial n/\partial c)_\mu$ of 0.1856 ± 0.001 cm^3 g^{-1} (Stevens and Lauffer, 1965), the value of M is 40.0 ± 1.7 × 10^6. All the above uncertainties were calculated from the uncertainties in c, $(\partial n/\partial c)_\mu$, and Q, and from the 95% confidence intervals for the means of the measured turbidities (±1.0 and ±1.8% at 436 and 546 nm, respectively).

Dispersion of the Refractive Index Increment, and the Radi-

TABLE III: Dispersion Constants for the Refractive Index Increment Obtained from Transmittance Measurements.

Virus	No. of Spectra Analyzed	A^a	B^a ($\times 10^4$ nm^2)	$(\partial n/\partial c)_\mu{}^b$ (cm^3/g)		$(\partial n/\partial c)_\mu{}^c$ (cm^3/g)	
				436 nm	546 nm	436 nm	546 nm
R17	5	0.920 ± 0.047 (5.1%)	2.12 ± 0.39 (18.4%)	0.181	0.174	0.183	0.175
PM2	7	0.930 ± 0.005 (0.5%)	1.91 ± 0.15 (7.8%)	0.161	0.155	0.162	0.155
T7	4	0.914 ± 0.041 (4.5%)	2.52 ± 0.51 (20.2%)	0.164	0.157	0.163	0.156
TMV	4	0.901 ± 0.010 (1.1%)	2.91 ± 0.44 (15.1%)	0.192	0.182	0.189	0.181
Av from all spectra ($\bar{A}$ and $\bar{B}$)		0.916	2.37				
Proteins[d]		0.933	1.99				

[a] Average values from spectra for each virus, where A and B are constants in $(\partial n(\lambda_0)/\partial c)_\mu = (\partial n(546)/\partial c)_\mu(A + (B/\lambda_0{}^2))$ (see text). ± = 95% confidence interval for the mean. [b] Calculated using A and B for corresponding virus. [c] Calculated using $\bar{A}$ and $\bar{B}$ [d] Calculated from value of Perlman and Longsworth (1948).

us of Gyration and the Molecular Weight from Transmittance Measurements as a Function of Wavelength. Measurements of τ as a function of wavelength provide more information than measurements at a single wavelength. In this section we show how to evaluate from such data not only the dimensions and the molecular weight of the scatterer (Cashin and Debye, 1949; Doty and Steiner, 1950) but also the dispersion of the refractive index increment of the scatterer. In the next few paragraphs we present some of the necessary background.

Guinier (1939) suggested that in the low K limit, and strictly speaking at infinite dilution also, the particle scattering factor for any shape can be approximated by

$$P(K) = 1 - (R_G{}^2K^2/3) \quad (7)$$

where R_G is the radius of gyration, the convenient shape-independent measure of the dimensions of the scatterer which can be easily related to the radius of a solid sphere, R, by $R_G{}^2 = (\frac{3}{5})R^2$ and to the length of a solid rod, L, by $R_G{}^2 = L^2/12$. Substitution of eq 7 into eq 3 gives

$$Q(\lambda) = 1 - (8\pi^2R_G{}^2/3\lambda^2) \quad (8)$$

For values of $P(K)$ and $Q(\lambda) > 0.90$ the error involved in using these approximations is about 1%. (Equation 8, in terms of the radius of a sphere, was first obtained by Cashin and Debye (1949).) Using the approximation that $1/(1 - x) \simeq (1 + x)$ for small x, substituting eq 8 into eq 4, defining a constant $H' = 32\pi^3/3N_A\lambda_0{}^4$, and rearranging terms, we obtain

$$(H'c/\tau)(n_0(\lambda_0))^2(\partial n(\lambda_0)/\partial c)_\mu{}^2 = (1/M)(1 + (8\pi^2R_G{}^2/3\lambda^2)) \quad (9)$$

We can then see that, if the dispersion of $n_0(\lambda_0)$ and of $(\partial n(\lambda_0)/\partial c)_\mu$ (their dependence on λ_0) is known and τ is measured as a function of wavelength, a plot of the left-hand side of this equation *vs.* $1/\lambda^2$ will have $1/M$ and $8\pi^2R_G{}^2/3M$ as its intercept and slope, respectively. For the solvents used here, the dispersion of $n_0(\lambda_0)$ for solutions can be calculated from the dispersion of $n_w(\lambda_0)$, the refractive index of water, and the value of the ratio n_0/n_w at any wavelength in the visible region. The relationships used (see Table I, footnotes) give $n_0(\lambda_0)$ to within ±0.1% of data listed by Huglin (1972).

If we represent the dispersion of the refractive index increment for each virus by a relationship similar to that used by Perlman and Longsworth (1948)

$$(\partial n(\lambda_0)/\partial c)_\mu = a' + (b'/\lambda_0{}^2) \quad (10)$$

where a' and b' are constants for each virus in a given solvent, then eq 9 involves four unknowns, a' and b' in addition to M and R_G. Only two of these unknowns can be evaluated in terms of the other two, and we have chosen to evaluate a' and b' from M and $Q(\lambda)$ (or R_G).

First, using a more exact form of eq 9

$$(\partial n(\lambda_0)/\partial c)_\mu{}^2 = \tau/H'cMQ(\lambda)(n_0(\lambda_0))^2 \quad (11)$$

we have calculated $(\partial n(\lambda_0)/\partial c)_\mu$, from τ and $Q(\lambda)$ at 10-nm intervals and the molecular weight. For each different virus, the dimensions used to calculate $Q(\lambda)$ were those used to calculate Q at 436 and 546 nm and the values for M were the averages of those obtained at 436 and 546 nm (except for TMV where only the value at 436 nm was used; see above). Once all the appropriate substitutions are made, eq 11 for TMV, for example, takes on the following appearance

$$(\partial n(\lambda_0)/\partial c)_\mu{}^2 = \left(\frac{\tau_{\lambda_0}}{\tau_{436}}\right)\left(\frac{Q_{436}}{Q_{\lambda_0}}\right)\left(\frac{n_0(436)}{n_0(\lambda_0)}\right)^2 \times \frac{H'_{436}}{H'_{\lambda_0}}(\partial n(436)/\partial c)_\mu{}^2 \quad (12)$$

where the subscripts λ_0 and 436 denote values measured or evaluated at those wavelengths, all the turbidities have been normalized to the same concentration, and τ_{436} is the mean value of all the values of the normalized turbidity at 436 nm. The expressions for the other viruses which involve the average of two values for M are essentially the same.

For each virus several τ *vs.* λ_0 "spectra" were recorded over the concentration ranges shown in Figure 1. A typical wavelength scan of τ is shown in Figure 2. The calculated values of $(\partial n(\lambda_0)/\partial c)_\mu$ for one representative scan of the "spectrum" of each virus are plotted *vs.* $1/\lambda_0{}^2$ in Figure 3. That the proper weighting factor is related to τ in some fashion can be seen from the data. By plotting these values on a highly expanded

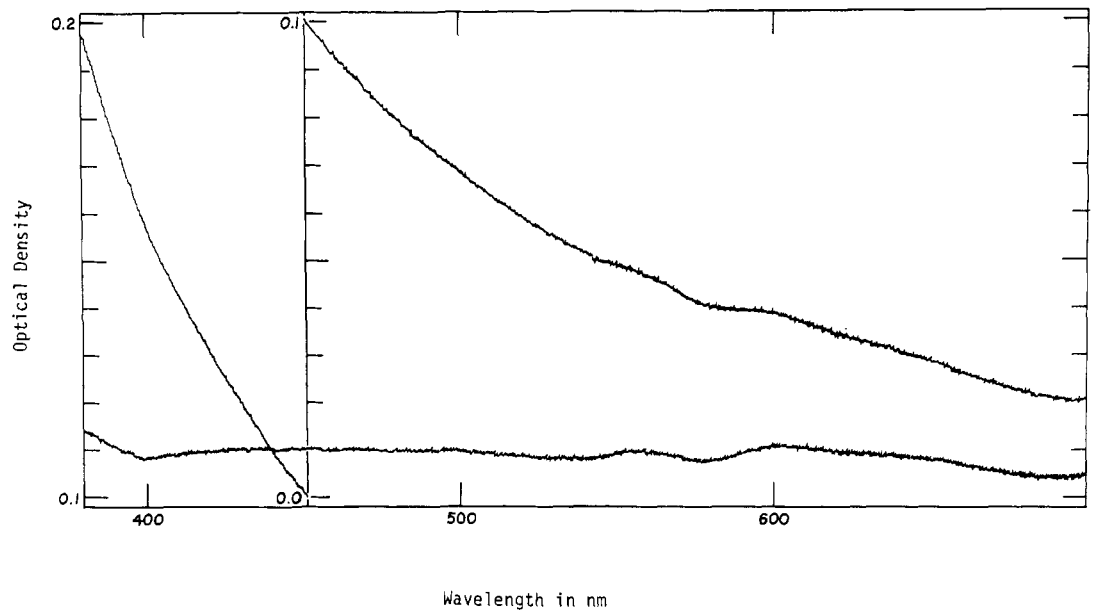

FIGURE 2: Direct tracing of a representative wavelength scan. The sample was a solution of PM2 at a concentration of 0.0893 mg cm^{-3} in a 10-cm pathlength cell. The turbidity per centimeter equals 2.303 × OD/pathlength.

scale we can see that the deviations from the linear relationship "die out" quickly as the wavelength is decreased (or as τ increases). The use of equal weighting in the least-squares analysis of this kind of data yields a fit (dashed line in Figure 3) that, while not significantly different from that obtained with τ weighting, tends to disregard the obvious linear relationship in the data.

For the purpose of comparing the values for each virus to each other and to the data for proteins (Perlman and Longsworth, 1948) eq 10 has been rewritten as

$$(\partial n(\lambda_0)/\partial c)_\mu = (\partial n(546)/\partial c)_\mu (A + (B/\lambda_0^2)) \quad (13)$$

where $A = a'/(\partial n(546)/\partial c)_\mu$, $B = b'/(\partial n(546)/\partial c)_\mu$, and $(\partial n(546)/\partial c)_\mu$ is the refractive index increment for each virus measured at 546 nm. For TMV we have used a value of 0.182 $cm^3\ g^{-1}$ for $(\partial n(546)/\partial c)_\mu$ evaluated using eq 12 where $(\partial n(436)/\partial c)_\mu$ is the value of Boedtker and Simmons (1958) measured on virus samples prepared in the same manner as ours.

For all the viruses the variability from scan to scan (Table III) was greater than that expected from the uncertainties in the recording of each scan. Apart from this variability, part of which can be considered as the contribution of $\delta\bar{\tau}_{436}$ and $\delta\bar{\tau}_{546}$ to the uncertainty in $(\partial n(\lambda_0)/\partial c)_\mu$, the uncertainties in A and B are also a function of δQ_{λ_0}, δQ_{436}, δQ_{546}, $\delta[(\partial n(436)/\partial c)_\mu]$, and/or $\delta[(\partial n(546)/\partial c)_\mu]$. Once these errors are considered (Camerini-Otero, 1973) there are no significant differences in the values of A and B from virus to virus despite the large differences in their chemical compositions and in the solvents used (see Tables I, II and III).

Using the finding that, for at least this group of viruses (and several proteins; Perlman and Longsworth, 1948; also, see Table III), $\bar{A}$ and $\bar{B}$ can be used to describe the wavelength dependence of $(\partial n(\lambda_0)/\partial c)_\mu$, we have been able to evaluate M and R_G from eq 9. For each virus we have calculated for each of the recorded "spectra" the left-hand term of this equation, Hc/τ, at each wavelength (every 10 nm) from the values of τ at the same wavelength, the value of $(\partial n(546)/\partial c)_\mu$ used in eq 13, and these two "universal" constants, $\bar{A}$ and $\bar{B}$. The calculated values of Hc/τ for one typical scan of R17, PM2, and T7 are shown in Figure 4. For all three viruses similar plots for the scans at different virus concentrations indicated that the values for the intercepts and slopes were independent of virus concentration.

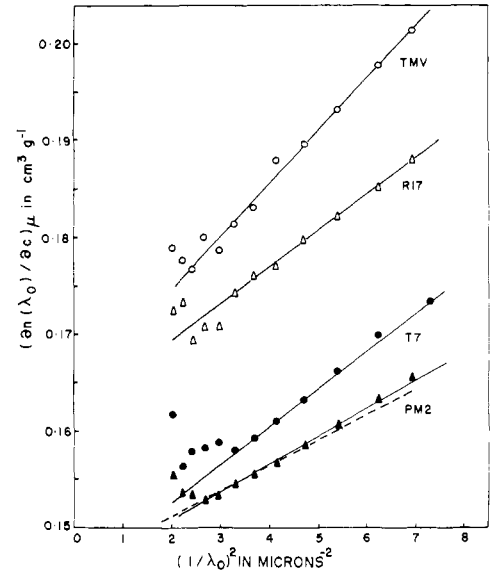

FIGURE 3: Representative plots of $(\partial n(\lambda_0)/\partial c)_\mu$ *vs.* $1/\lambda_0^2$. Starting at 700 nm, the values plotted are those at every 30 nm and that corresponding to the last wavelength recorded. The solid lines represent the τ weighted linear least-squares fits to *all* the values from each scan (every 10 nm). The values of A and B and their 95% confidence intervals for these particular scans were (in the units given in Table III): 0.920 (±0.8%) and 2.11 (±9.6%) for R17 (c = 0.488 mg cm^{-3}); 0.929 (±0.7%) and 1.86 (±8.3%) for PM2 (c = 0.0893 mg cm^{-3}); 0.936 (±1.1%) and 2.29 (±10.0%) for T7 (c = 0.0647 mg cm^{-3}); and 0.901 (±0.7%) and 2.98 (±5.5%) for TMV (c = 0.207 mg cm^{-3}). The dashed line represents an equal weighted fit for the data for PM2 (A = 0.936 (±0.9%) and B = 1.67 (±10.2%)). The values of $(\partial n(\lambda_0)/\partial c)_\mu$ for PM2 were those calculated from the scan shown in Figure 2.

For each scan the values of Hc/τ as a function of wavelength were then fit in least-squares fashion to eq 9 with τ as the weighting factor. The values of M and R obtained from these

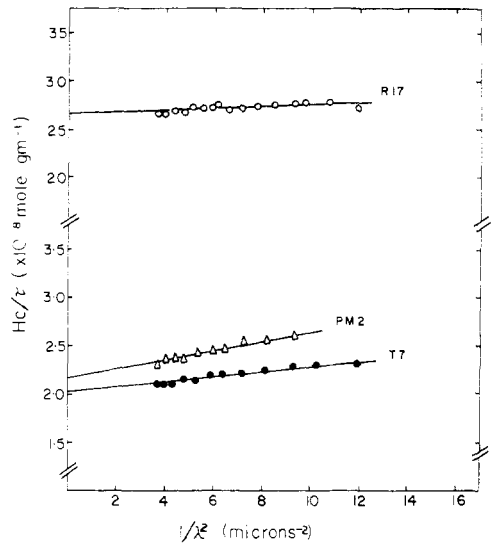

FIGURE 4: Representative plots of (Hc/τ) *vs.* $1/\lambda^2$. The value at 700 nm is the first value plotted, and subsequent values are those at 30-nm intervals (these represent approximately a third of the points used in each least-squares analysis). The virus concentrations were: 0.479 mg cm^{-3} for R17, 0.149 mg cm^{-3} for PM2, and 0.0657 mg cm^{-3} for T7.

fits were: 3.86 ± 0.37 × 10^6 and 12.3 ± 15.8 nm for R17; 45.4 ± 1.5 × 10^6 and 38.4 ± 1.5 nm for PM2; and 51.2 ± 3.6 × 10^6 and 29.7 × 9.5 nm for T7. For each virus the uncertainty in M was calculated from the uncertainties in c and Δn, and from the 95% confidence interval for the mean of the intercepts at λ equal to ∞; the uncertainty in R was evaluated from the 95% confidence interval for the mean of the slopes of Hc/τ *vs.* $1/\lambda^2$.

Since for TMV the values of Hc/τ are not a linear function of $1/\lambda^2$ (the approximation of eq 8 does not hold) we have analyzed these data in a different manner. First, in order to simplify our arguments and to show how information about dimensions can be extracted from this kind of data we have used A and B for TMV rather than $\bar{A}$ and $\bar{B}$ to calculate values of Hc/τ for each scan of TMV. These values were then plotted *vs.* $1/\lambda^2 + 10(OD_{260})$, where OD_{260} is the measure of virus concentration for each scan (these are similar to plots of angular dependent data as a function of $\sin^2(\theta/2) + kc$; Zimm, 1948). The values at $OD_{260} = 0$ were then evaluated from the intercepts of least-squares straight lines through the values of Hc/τ corresponding to the same wavelengths, as shown in Figure 5. For the purposes of demonstration only a third of the data points (every 30 nm) is shown.

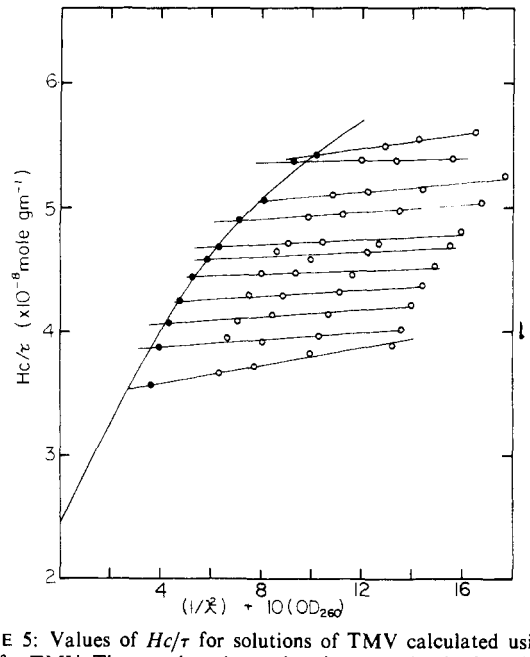

FIGURE 5: Values of Hc/τ for solutions of TMV calculated using A and B for TMV. The wavelengths are in microns and only the values at 0.03-μ (30 nm) intervals are plotted. The empty circles are the experimental values; the filled circles are the $OD_{260} = 0$ intercepts of straight lines through the values of Hc/τ for a given wavelength. The curve is that which can be calculated for a rod of a molecular weight of 42 × 10^6 and a length of 290 nm.

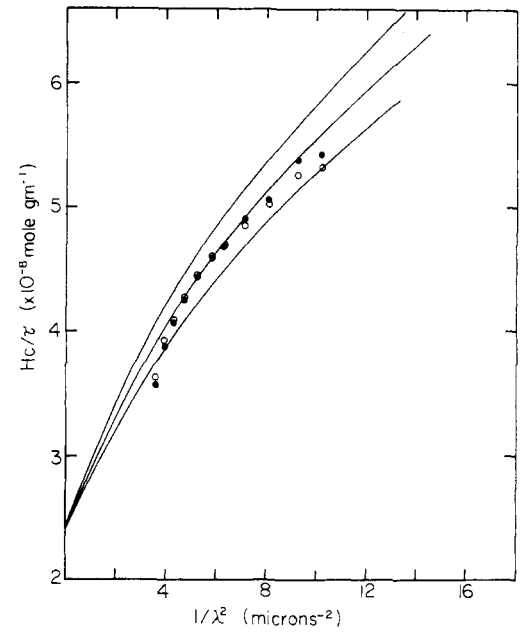

FIGURE 6: Comparison of experimental and calculated reciprocal turbidity as a function of $1/\lambda^2$. The three solid curves are for rods of 42 × 10^6 daltons with lengths 280 nm (bottom), 300 nm (middle), and 320 nm (top). The filled circles are the $OD_{260} = 0$ intercepts for H calculated with A_{TMV} and B_{TMV} (Figure 5; Table III) and the open circles are the intercepts from a plot similar to that in Figure 5 where H was calculated using $\bar{A}$ and $\bar{B}$ (Table III).

As this plot shows, for our data, extrapolations to $\lambda = \infty$ are difficult. We could, however, in principle evaluate M and L (the rod length) from a weighted least-squares analysis of these data. For this purpose we would have to use either the full form of $Q(\lambda)$ or include more terms in the series expansion than in eq 8 (Camerini-Otero, 1973; Camerini-Otero, R. D., and Day, L. A., manuscript in preparation). A simpler, and as valid, approach is to calculate from sets of values of M and L different curves which can be compared to those for our values of Hc/τ. Although for our purposes we have calculated these curves by numerical integration of the expressions for Q, such curves can be constructed from published values of Q (Doty and Steiner, 1950).

In Figure 6 we show three calculated curves for $M = 42 \times 10^6$ and values of L of 280, 300, and 320 nm superimposed on the $OD_{260} = 0$ intercepts of Figure 5. Since the values corresponding to the shorter wavelengths (higher values of τ) should be weighted more than those at longer wavelengths we see that a curve corresponding to this molecular weight and to a length between 280 and 300 nm fits the data well. In Figure 6 we show that we can use $\bar{A}$ and $\bar{B}$ to calculate values of Hc/τ and still obtain a similar fit.

The slight deviations at the shorter wavelengths from the calculated curve in Figure 6 are due to the approximation in-

TABLE IV: Summary of Molecular Weights for R17.

Method	M (millions)
From transmittance	
at 436 nm	3.95 ± 0.22
at 546 nm	3.85 ± 0.28
extrapolated to infinite wavelength	
av of five spectra	3.86 ± 0.37
Light scattering based on	
$R_{benzene}$ at 90° = 16.4 × 10^{-6} [a]	3.80 ± 0.23
Sedimentation equilibrium	3.64 ± 0.18
Sedimentation–viscosity	3.98 ± 0.19
Sedimentation–diffusion[b]	4.02 ± 0.17

[a] Doty and Steiner, 1950. [b] The diffusion coefficient was measured by intensity fluctuation spectroscopy on the same virus preparations used in this study (Camerini-Otero *et al.*, 1974).

TABLE V: Molecular Weights of PM2, T7, and TMV by the Transmittance Method.

	Values of M (×10^6) from Transmittance Measurements[a]		
Virus	At λ_0 436 nm	At λ_0 546 nm	Extrap. to Infinite Wavelength
PM2	43.5 ± 2.1 (7)	43.8 ± 1.6 (8)	45.4 ± 1.5 (7)
T7	53.1 ± 2.8 (4)	52.2 ± 2.7 (4)	51.2 ± 3.1 (4)
TMV	41.3 ± 1.7 (3)	41.1 ± 2.0 (4)[b]	42.0[c] (4)

[a] The number of measurements at several virus concentrations is indicated in parentheses (see Figure 1 for concentrations and text for discussion of concentration dependence). [b] Obtained using the value of $(\partial n(546)/\partial c)_\mu$ evaluated from the dispersion analysis (see text). [c] From fit to calculated curves; the uncertainty for this value is at least that for those at 436 and 546 nm (±2.0 × 10^6).

volved in using $\bar{A}$ and $\bar{B}$ and not to polydispersity in the sample (Boedtker and Simmons, 1958). The deviations at the longer wavelengths (values of τ that are too high) have also been observed for some of the data of the other viruses (for example, for PM2 and T7 in Figure 3). Since in the longer wavelength region of the visible range one would expect an even better adherence to eq 8 and 13 (*i.e.*, no anomalous dispersion or absorption from ultraviolet absorption bands), it is most likely that "interfering substances" larger than the viruses (for example, dust, or, more likely, aggregates of native or denatured virus), which would account for a higher proportion of the "signal" at longer wavelengths, are responsible for this behavior.

Finally, we should point out that the results of this section, that the wavelength dependence of the transmittance of solutions of all four of these viruses can be accounted for by the interplay of the intraparticle interference and the Cauchy-like dispersion of the refractive index increment, are perhaps the strongest evidence that for nucleoproteins in the wavelength region investigated all the loss in transmitted intensity is due to scattering and none due to true absorption or anomalous scattering.

Discussion

Transmittance Method. In the process of calibrating our light-scattering photometer by transmittance measurements on solutions of R17 we came to appreciate the feasibility and ease of obtaining an absolute measure of the scattering of light by transmittance spectrophotometry (Doty and Steiner, 1950). The early success with R17 led us to try to resurrect and refine the transmittance method for determining molecular weights and to show that it is as precise and accurate a method as others in common use and the easiest to use. It is satisfying to note here that for R17, for example, the mean of values of M from transmittance, 3.89 × 10^6 daltons, is the one value that is closest to either the mean of all values, 3.87 × 10^6 daltons, or to the mean of the nontransmittance values, 3.86 × 10^6 daltons (Table IV). In general, however, the different methods have comparable precision and accuracy (Tables IV and V), and all are affected to the same extent by the uncertainties in ϕ', $(\partial n/\partial c)_\mu$, and concentration.

Most of the instrumental precautions that must be taken in making turbidity measurements with a spectrophotometer have been carefully delineated by Heller and Tabibian (1957); the most significant sources of error include the effect of scattered stray light, the "corona effect" (due to secondary scattered light), and the effect of the solid angle of scattered light reaching the photodetector. In general, these sources of error are only important for highly scattering solutions of large particles; Heller and Tabibian (1957) observed such effects with latex spheres of diameters greater than 100 nm.

The principal disadvantage of the transmittance method is its low sensitivity. Nevertheless, the advantages of absoluteness, ease, and economy may well make transmittance measurements the method of choice for determining molecular weights in many instances.

Obtaining and Using Turbidity Data. The most convenient, and in general, the most accurate way of using the transmittance method is to make measurements at a single wavelength (eq 4). The only experimentally determined parameters in addition to τ are c, $(\partial n(\lambda_0)/\partial c)_\mu$, and the dimensions of the scatterers. Since rather large uncertainties in dimensions give rise to considerably smaller uncertainties in $Q(\lambda)$,[4] hence M, dimensions available from electron microscopy are usually accurate enough for this purpose (Table II). If we restrict ourselves to measurements at ionic strengths of 0.1–0.2 M we can adopt 0.190 $cm^3\ g^{-1}$ as a useful mean value for $(\partial n(436)/\partial c)_\mu$ for most "proteins." [5] Since $(\partial n/\partial c)_\mu$ and c appear in the expression for M in a manner such that their errors are negatively correlated, $(\partial n/\partial c)_\mu$ can also be conveniently used to measure concentration with a diffractometer without significantly affecting the overall error in M. In fact, for relatively large errors in $(\partial n/\partial c)_\mu$ of, *e.g.*, 8–10%, the overall error in M would be approximately the same as in $(\partial n/\partial c)_\mu$, but in the opposite direction.

Another application of the method is to determine both M and R_G from measurements of τ as a function of wavelength; for this purpose we have derived from the data in this study av-

[4] For a sphere of radius of R for which we can use eq 8 as an expression for $Q(\lambda)$, $\delta Q(\lambda)/Q(\lambda) = (k_0/[(\lambda_0/R)^2 - k_0])(\delta R)/R$, where $k_0 = 8\pi^2(n_0(\lambda_0))^2/5$.

[5] Of over 20 values of $dn(436)/dc$ at ionic strengths between 0.1 and 0.2 M reported for 13 simple proteins (*cf.* Timasheff, 1970) approximately 75% were within 5% of this value, as are the values for R17, TMV, and DNA (Cohen and Eisenberg, 1968).

erage dispersion constants that can be used to correct τ for the wavelength dependence of the refractive index increment (Table III).

Molecular Weight of R17. The molecular weight of R17 bacteriophage[6] has been generally accepted as 3.6×10^6 (*cf.* Hohn and Hohn, 1970). This value could be calculated from the sum of the best available molecular weights of the phage components and was in agreement with determinations by light scattering (Strauss and Sinsheimer, 1963; Gesteland and Boedtker, 1964; Overby *et al.*, 1966) and X-ray scattering (3.7×10^6; Zipper *et al.*, 1971). Despite this apparent agreement, there have been several measurements of the molecular weight of these viruses by sedimentation–diffusion and sedimentation–viscosity that have yielded values from 4.2×10^6 to 5.3×10^6 (Enger *et al.*, 1963; Marvin and Hoffmann-Berling, 1963; Möller, 1964). The molecular weights that we have obtained by equilibrium sedimentation (M_{ES}), transmittance (M_τ), light scattering (M_{LS}), and sedimentation–viscosity ($M_{S,\eta}$) are in close agreement with each other and with that obtained by sedimentation–diffusion ($M_{S,D}$; Camerini-Otero *et al.*, 1974) but, except for M_{ES}, deviate from the values of 3.6×10^6 (Table IV). Because of this apparent discrepancy it is important to briefly discuss the uncertainties in some of the parameters that were used to calculate M.

The major contributions to the uncertainty in M are from the uncertainties in the values for ϕ' and $(\partial n/\partial c)_\mu$. The principal uncertainty in these parameters is the uncertainty in $E_{1\,cm}^{0.1\%}$ (*i.e.*, concentration) which we estimate to have a 95% confidence interval of 3.8% (Table II). To our knowledge the present values of ϕ' and $(\partial n/\partial c)_\mu$ are the only ones reported for R17 which were clearly measured under these conditions of dialysis equilibrium. With the exception of Overby *et al.* (1966), there are no reports on values for both ϕ' and $(\partial n/\partial c)_\mu$ so that direct comparisons with our data are difficult.

The best criterion for assessing the reliability of the values of S, Q, $[\eta]$, ϕ', $(\partial n/\partial c)_\mu$, and $E_{1\,cm}^{0.1\%}$ is in the consistency of the values of M calculated from them. The mean of our values of M obtained from light scattering (the first four values in Table IV) is in almost exact agreement with the mean of the three "hydrodynamic" methods, 3.88×10^6. Furthermore, the mean value of all seven values in Table IV is 3.87×10^6 with a 95% confidence interal of 0.12×10^6. Since the uncertainty in the concentration ($E_{1\,cm}^{0.1\%}$) affects all our values of M in the same fashion, the total uncertainty for this mean value is $\pm((0.12)^2 + [(0.038)(3.87)]^2)^{1/2} \times 10^6$ or $\pm 0.19 \times 10^6$.

How does this accurately measured value of M ($3.87 \pm 0.19 \times 10^6$) compare with that calculated from the apparent composition? The value for the molecular weight of the RNA has generally been taken as 1.1×10^6 to yield a compositional weight of 3.61×10^6 (*cf.* Hohn and Hohn, 1970). Recently, however, Boedtker (1971) and Slegers *et al.* (1973) have obtained values for the RNA ranging from 1.15×10^6 to 1.3×10^6. These values give a compositional molecular weight of $3.74 \pm 0.1 \times 10^6$ in agreement with the value obtained in this investigation. A very recent report (Fukuma and Cohen, 1973) of the presence of 770–1020 molecules of spermidine (contributing ~100,000) as a still fourth component of R17 virions suggests that our value of the molecular weight of the virion is very close to a more realistic compositional molecular weight than had been envisioned when this investigation started. It appears that all of the mass of the virus can be accounted for by the known viral components.

Molecular Weights of PM2, T7, and TMV. Although compositional molecular weights cannot yet be calculated for the relatively complex virions of PM2 and T7, independent measures of the viral molecular weights are available. For the lipid-containing bacteriophage PM2 the values of the molecular weight obtained by the transmittance method (Table V) are in agreement with those obtained by equilibrium sedimentation,[3] $44.0 \pm 2.5 \times 10^6$, and by sedimentation–diffusion, $47.9 \pm 1.7 \times 10^6$ (Camerini-Otero *et al.*, 1974). Our value for T7 is in agreement with recent values obtained by equilibrium sedimentation, $49.9 \pm 2.0 \times 10^6$ (Bancroft and Freifelder, 1970), and by sedimentation–diffusion, $50.4 \pm 1.8 \times 10^6$ (Dubin *et al.*, 1970) and $50.9 \pm 1.1 \times 10^6$ (Camerini-Otero *et al.*, 1974).

The molecular weight of TMV has for many years been accepted as somewhere between 39 and 40×10^6 (Schramm and Bergold, 1947; Boedtker and Simmons, 1958; Beams *et al.*, 1963). The molecular weight calculated from the virus composition is $39.2 \pm 1.2 \times 10^6$ (Klug and Caspar, 1960). Our value (Table V) obtained by the transmittance method is in agreement with the generally accepted value.

The molecular weight of TMV has played an interesting role in the development of the transmittance method. Doty and Steiner (1950) in their paper first describing the transmittance method obtained a molecular weight for TMV of $49.0 \pm 3.0 \times 10^6$ daltons in disagreement with what became the generally accepted value. Although they used different values than those we have used for $(\partial n(\lambda_0)/\partial c)_\mu$ (9% lower than the one we have used at 436 nm) and $Q(\lambda)$ (29% higher than ours), correcting for these differences would only *increase* their value for the molecular weight to 52.0×10^6 daltons ($M = \tau/H'c(\partial n(\lambda_0)/\partial c)_\mu^2 Q(\lambda)$). On the other hand the fact that the value they obtained by light scattering was 51.0×10^6 daltons suggests either an error in their concentrations or possibly dust or virus aggregates, rather than any error inherent to the transmittance method, was responsible for their high value.

Acknowledgments

It is a pleasure to thank Margarete Klein, Jutta Simon, and Marianne Salditt for their assistance during the course of this study.

References

Bancroft, F. C., and Freifelder, D. (1970), *J. Mol. Biol. 54*, 537.

Bawden, F. C., and Pirie, N. W. (1937), *Proc. Roy. Soc., Ser. B 123*, 274.

Bawden, F. C., and Pirie, N. W. (1938), *Brit. J. Exp. Pathol. 19*, 251.

Beams, J. W., Kupke, D. W., Weber, F. N., Jr., Kim, H. G., Rose, R. D., and Elton, R. M. (1963), *Chem. Eng. News 41*(7), 50.

Billmeyer, F. W. (1954), *J. Amer. Chem. Soc. 76*, 4636.

Boedtker, H. (1971), *Biochim. Biophys. Acta 240*, 448.

Boedtker, H., and Simmons, N. S. (1958), *J. Amer. Chem. Soc. 80*, 2550.

Brenner, S., and Horne, R. W. (1959), *Biochim. Biophys. Acta 34*, 103.

Camerini-Otero, R. D. (1973), Ph.D. Thesis, New York University, New York, N. Y.

Camerini-Otero, R. D., and Franklin, R. M. (1972), *Virology 49*, 385.

Camerini-Otero, R. D., Pusey, P. N., Koppel, D. E., Schaefer, D. W., and Franklin, R. M. (1974), *Biochemistry 13*, 960.

[6] R17 has been the most extensively characterized of the small RNA bacteriophages, but since it is well known that the differences in the virions of R17, MS2, f2, and fr are very minor (*cf.* Hohn and Hohn, 1970), we shall use data for all four viruses in this discussion.

Cancelleri, A., Frontali, C., and Gratton, E. (1974), *Biopolymers 13,* 735.

Casassa, E. F., and Eisenberg, H. (1964), *Advan. Protein Chem. 19,* 287.

Cashin, W. M., and Debye, P. (1949), *Phys. Rev. 75,* 1307.

Cohen, G., and Eisenberg, H. (1968), *Biopolymers 6,* 1077.

Colquhoun, D. (1971), Lectures on Biostatistics, London, Oxford University Press.

Debye, P. (1944), *J. Appl. Phys. 15,* 338.

Doty, P., and Steiner, R. F. (1950), *J. Chem. Phys. 18,* 1211.

Dubin, S. B. (1970), Ph.D. Thesis, Massachusetts Institute of Technology, Cambridge, Mass.

Dubin, S. B., Benedek, G. B., Bancroft, F. C., and Freifelder, D. (1970), *J. Mol. Biol. 54,* 547.

Enger, M. D., Stubbs, E. A., Mitra, S., and Kaesberg, P. (1963), *Proc. Nat. Acad. Sci. U. S. 49,* 857.

Espejo, R. T., and Canelo, E. S. (1968), *Virology 34,* 738.

Finch, J. T. (1969), *Virology 38,* 182.

Fischbach, F. A., Harrison, P. M., and Anderegg, J. W. (1965), *J. Mol. Biol. 13,* 638.

Fraenkel-Conrat, H., and Williams, R. C. (1955), *Proc. Nat. Acad. Sci. U. S. 41,* 690.

Freund, J. E. (1962), Mathematical Statistics, Englewood Cliffs, N. J., Prentice-Hall.

Fukuma, I., and Cohen, S. S. (1973), Abstracts of the Annual Meeting of the American Society of Microbiology, V86.

Gesteland, R. F., and Boedtker, H. (1964), *J. Mol. Biol. 8,* 496.

Guinier, A. (1939), *Ann. Phys. 12,* 161.

Hall, C. E. (1958), *J. Amer. Chem. Soc. 80,* 2556.

Harrison, S. C., Caspar, D. L. D., Camerini-Otero, R. D., and Franklin, R. M. (1971), *Nature (London), New Biol. 229,* 197.

Heller, W., and Tabibian, R. M. (1957), *J. Colloid Sci. 12,* 25.

Hoffmann-Berling, H., Kaerner, H. C., and Knippers, R. (1966), *Advan. Virus Res. 12,* 329.

Hohn, T., and Hohn, B. (1970), *Advan. Virus Res. 16,* 43.

Huggins, M. L. (1942), *J. Amer. Chem. Soc. 64,* 2716.

Huglin, M. B. (1972), *in* Light Scattering from Polymer Solutions, Huglin, M. B., Ed., New York, N. Y., Academic Press, p 165.

Kerker, M. (1969), The Scattering of Light and Other Electromagnetic Radiation, New York, N. Y., Academic Press, p 339.

Klug, A., and Caspar, D. L. D. (1960), *Advan. Virus Res. 7,* 225.

Lipkin, M. R., Davison, J. A., Harvey, W. T., and Kurtz, S. S. (1944), *Ind. Eng. Chem. 16,* 55.

Marvin, D. A., and Hoffmann-Berling, H. (1963), *Z. Naturforsch. B 18,* 884.

Möller, W. J. (1964), *Proc. Nat. Acad. Sci. U. S. 51,* 501.

Oster, G. (1946), *Science 103,* 306.

Overby, L. R., Barlow, G. H., Doi, R. H., Jacob, M., and Spiegelman, S. (1966), *J. Bacteriol. 91,* 442.

Perlman, G. E., and Longsworth, L. G. (1948), *J. Amer. Chem. Soc. 70,* 2719.

Salditt, M., Braunstein, S. N., Camerini-Otero, R. D., and Franklin, R. M. (1972), *Virology 48,* 259.

Scheraga, H. A., and Mandelkern, L. (1953), *J. Amer. Chem. Soc. 75,* 179.

Schramm, G., and Bergold, G. H. (1947), *Z. Naturforsch. B 2,* 108.

Silbert, J. A., Salditt, M., and Franklin, R. M. (1969), *Virology 39,* 666.

Simha, R. (1952), *J. Appl. Phys. 23,* 1020.

Slegers, H., Clauwaert, J., and Fiers, W. (1973), *Biopolymers 12,* 2033.

Stevens, C. L., and Lauffer, M. A. (1965), *Biochemistry 4,* 31.

Strauss, J. H., and Sinsheimer, R. L. (1963), *J. Mol. Biol. 7,* 43.

Tanford, C. A. (1961), Physical Chemistry of Macromolecules, New York, N. Y., p 392.

Timasheff, S. N. (1970), *in* Handbook of Biochemistry, 2nd ed, Cleveland, Ohio, Chemical Rubber Publishing Co., p C67.

Vasquez, L., Granboulan, N., and Franklin, R. M. (1966), *J. Bacteriol. 92,* 1779.

Williams, R. C., and Steere, R. L. (1951), *J. Amer. Chem. Soc. 73,* 2057.

Wippler, C., and Scheibling, G. (1954), *J. Chim. Phys. 51,* 201.

Yamamoto, K. R., Alberts, B. M., Benzinger, R., Lawhorne, L., and Treiber, G. (1970), *Virology 40,* 734.

Yphantis, D. A. (1964), *Biochemistry 3,* 297.

Zimm, B. H. (1948), *J. Chem. Phys. 16,* 1099.

Zimm, B. H., Stein, R. S., and Doty, P. (1945), *Polym. Bull. 1,* 90.

Zipper, P., Kratky, O., Herrmann, R., and Hohn, T. (1971), *Eur. J. Biochem. 18,* 1.