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Intensity Fluctuation Spectroscopy of Laser Light Scattered by Solutions of Spherical Viruses: R17, Qβ, BSV, PM2, and T7. II. Diffusion Coefficients, Molecular Weights, Solvation, and Particle Dimensions†

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ABSTRACT: The diffusion coefficients (D) of several spherical viruses have been determined by the method of intensity fluctuation spectroscopy. The values for $D^0_{20,w}$ for bacteriophages R17, Qβ, PM2, and T7 are (in units of 10^{-7} cm^2 sec^{-1}): 1.534 ± 0.015, 1.423 ± 0.014, 0.650 ± 0.007, and 0.644 ± 0.007, respectively; that for the plant virus, tomato bushy stunt virus (BSV), is 1.246 ± 0.012. The molecular weights were calculated from these values of D combined with literature values for the sedimentation coefficients (s) and values for the apparent specific volumes determined by us (R17, Qβ, and PM2) or obtained from the literature (BSV and T7). The calculated molecular weights for R17, Qβ, BSV, PM2, and T7 are (in millions of daltons): 4.02 ± 0.17, 4.55 ± 0.16, 8.81 ± 0.17, 47.9 ± 1.7, and 50.9 ± 1.1, respectively. These values are in good agreement with literature values and those estimated from composition. Using the Stokes–Einstein equation, we have calculated hydrodynamic radii, R_h, for these viruses from our values of $D^0_{20,w}$. These radii are 140 ± 2, 151 ± 2, 172 ± 2, 330 ± 3, and 333 ± 3 Å for R17, Qβ, BSV, PM2, and T7, respectively, and are in agreement with the dimensions obtained by such techniques as low-angle X-ray scattering, light scattering, and electron microscopy. Values for the solvation of these viruses, derived from the data, are (in cm^3 of solvent/g of virus): 1.02 ± 0.09, 1.22 ± 0.08, 0.75 ± 0.04, 1.11 ± 0.08, and 1.18 ± 0.06, in the same order as above. The diffusion coefficients for all the viruses were studied as functions of virus concentration. In addition, D for R17 and PM2 was studied as a function of ionic strength, and D for R17 was also measured as a function of temperature and pH.

The method of intensity fluctuation spectroscopy of scattered coherent (laser) light (Pecora, 1964; Dubin *et al.*, 1967, 1970; Cummins *et al.*, 1969; Foord *et al.*, 1970; Pusey *et al.*, 1972, 1974) has been used to determine the translational diffusion coefficients[1] of several nearly spherical viruses: the bacteriophages R17, Qβ, PM2, and T7 and the plant virus,

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[1] Throughout we shall assume *translational* diffusion coefficients to be understood.

tomato bushy stunt virus (BSV).[2] These viruses have been chosen as representatives of a wide range of groups with varying chemical composition. PM2 contains 13.5% (w/w) supercoiled circular DNA (Espejo and Canelo, 1968; Espejo *et al.*, 1969); T7 has 50% (w/w) linear DNA (Bancroft and Freifelder, 1970); R17, Qβ, and BSV are RNA-containing viruses ranging in RNA content from 16.5% (w/w) for BSV (de Fremery and Knight, 1955) to about 30% (w/w) for bacteriophages R17 and Qβ (Hoffmann-Berling *et al.*, 1966). For all except PM2 the remainder of the particle mass is protein. PM2 has, in addition to protein and nucleic acid, 13% (w/w) lipid (Camerini-Otero and Franklin, 1972). This group of viruses includes two which are ideally suited for the kind of study undertaken here, R17 and BSV. The physical properties of R17 and BSV have been the best characterized among those for spherical viruses (Bawden and Pirie, 1938; Carlisle and Dornberger, 1948; Leonard *et al.*, 1953; de Fremery and Knight, 1955; Klug and Caspar, 1960; Enger *et al.*, 1963; Marvin and Hoffmann-Berling, 1963; Kupke, 1966; Overby *et al.*, 1966; Vasquez *et al.*, 1966; Harrison, 1969; Butler, 1970; Finch *et al.*, 1970; Crowther and Amos, 1971; Zipper *et al.*, 1971). This enabled us to compare and relate the results obtained by IFS to the large extant body of biophysical data.

The present paper considers three topics. The first topic concerns the determination of viral molecular weights (M) using the Svedberg equation which relates M to the diffusion coefficient (D), the sedimentation coefficient (s), and the partial specific volume ($\bar{v}$). Although the biophysical properties of the small R17-like RNA bacteriophages have been studied for several years their molecular weights have not, however, been unequivocably established. These viruses have played a central role in molecular biology (Hohn and Hohn, 1970) and a knowledge of their value of M is extremely important in studies of RNA replication and sequencing, and virus structure, to mention just a few examples. For the lipid-containing bacteriophage PM2, a recently discovered virus (Espejo and Canelo, 1968), the knowledge of M is crucial in studies of the structure of the virion and its lipid bilayer (Harrison *et al.*, 1971; Datta *et al.*, 1971; Camerini-Otero and Franklin, 1972).

The second topic of this paper concerns the relation of viral dimensions, surface structure, and solvation to hydrodynamic behavior. A major emphasis of the present investigation has been on the meaning of the hydrodynamic radius, R_h, calculated from D by the Stokes–Einstein equation and its relation to radii obtained by other techniques, such as electron microscopy, and X-ray and light scattering.

The third topic of this paper concerns the effect of temperature, pH, ionic strength, and virus concentration on D. For example, one of our aims has been to investigate under what circumstances the diffusion of macromolecules in multicomponent (macromolecule, water and electrolyte) systems, as measured by IFS, can be represented by an "ideal" *two-component* (macromolecule and solvent) system. The validity of such a representation is implicitly assumed in most of the published reports of diffusion measurements by IFS.

Materials and Methods

Preparation of Viruses. Bacteriophages R17 and Qβ were grown and purified according to a combination of published procedures (Vasquez *et al.*, 1966; Yamamoto *et al.*, 1970). In summary, the procedure involves the preparation of a viral lysate by infecting *Escherichia coli* with either R17 or Qβ, concentration of the viral lysate by phase separation with poly(ethylene glycol), extraction of the virus from the lower phase, an extraction with Genosolv-D (trifluorotrichloroethane), and high-speed centrifugation to pellet the virus. The resuspended virus was then first treated with ribonuclease, then with deoxyribonuclease, and finally with Pronase. The virus was then pelleted again and the final step in the purification involved two cycles of isopycnic centrifugation in CsCl. The exact details of this purification procedure are available elsewhere (Camerini-Otero, 1973).

The preparations of virus purified in the manner described above appeared homogeneous in subsequent CsCl equilibrium density gradients and in the electron microscope; the diffusion coefficient did not vary by more than 1% in over 100 separate determinations on samples from three separate R17 virus preparations. When these preparations of R17 and Qβ were subjected to sodium dodecyl sulfate gel electrophoresis (kindly performed by Dr. S. N. Braunstein) and the gels stained with Coomasie Brilliant Blue, there was no evidence of nonviral proteins. Not only were the viral particles prepared in this manner homogeneous and pure, but highly infective. Approximately 40% of the R17 particles and 12% of the Qβ particles were infective; these values exceed or equal those obtained by others (Strauss and Sinsheimer, 1963; Hoffmann-Berling *et al.*, 1963; Vasquez *et al.*, 1966; Yamamoto *et al.*, 1970).

Bacteriophage T7 was grown and purified by essentially the same procedure used for R17 and Qβ (Camerini-Otero, 1973), with the following exceptions: (a) the host bacterium was *E. coli* B; (b) the Genosolv extraction was not carried out; (c) the second CsCl density gradient was omitted; and (d) after the CsCl centrifugation, the virus was collected, dialyzed three to six times against 0.5 M NaCl–0.001 M $MgCl_2$–0.01 M Tris-HCl (pH 7.8), and stored in this buffer at 4°. Approximately 40% of the T7 particles were infective; this value is equal to that obtained by Yamamoto *et al.* (1970).

Bacteriophage PM2 was grown and purified according to a procedure published elsewhere (Salditt *et al.*, 1972). The homogeneity, purity, and high plating efficiency of PM2 prepared in this manner has been investigated and reported elsewhere (Datta *et al.*, 1971; Salditt *et al.*, 1972).[3]

Purified BSV was the generous gift of Dr. S. C. Harrison (Children's Cancer Research Foundation, Boston, Mass.) to whom it had been kindly provided by Professor C. A. Knight (University of California, Berkeley, Calif.).

Measurements of Virus Concentrations and Density Increments. Previously reported extinction coefficients ($E^{0.1\%}_{1\,cm}$) were used to convert measured optical densities of viral solutions at 260 nm to viral concentrations. A value of 7.93 $cm^2\ mg^{-1}$ was used for bacteriophage R17; this is an average of the two values available in the literature (see Discussion), 8.20 $cm^2\ mg^{-1}$ (Enger *et al.*, 1963) and 7.66 $cm^2\ mg^{-1}$ (Gesteland and Boedtker, 1964). The other extinction coefficients used were 8.02 $cm^2\ mg^{-1}$ for Qβ (Overby *et al.*, 1966), 5.0 $cm^2\ mg^{-1}$ for BSV (Bawden and Pirie, 1938), 4.60 $cm^2\ mg^{-1}$ for PM2[3] and 12.90 $cm^2\ mg^{-1}$ for T7 (Bancroft and Freifelder, 1970).

For a monodisperse multicomponent maromolecular solution it is important to consider interactions between pro-

[2] Abbreviations used are: BSV, tomato bushy stunt virus; IFS, intensity fluctuation spectroscopy; pfu, plaque-forming units; $M_{s,D}$, sedimentation–diffusion molecular weight.

[3] R. D. Camerini-Otero and R. M. Franklin, manuscript in preparation.

TABLE I: Solvents used in the Determination of the Diffusion Coefficients.

Virus	Solvent	pH[a]	Viscosity $(\eta/\eta^0)_{25}$
R17	0.015 M NaCl	6.2–6.8[b]	1.001[c]
	0.15 M NaCl[d]	6.3–6.5[b]	1.013[c]
	1.0 M NaCl	6.2–6.6[b]	1.095[e]
	0.15 M NaCl[f] + 0.015 M Tris-HCl	7.2	1.016[e]
	0.15 M NaCl + 0.02 M glycine	8.0, 9.0, 10.0	1.020[e,g]
	0.15 M NaCl + 0.02 M NaH_2PO_4	6.0, 7.0	1.019[c]
Qβ	Same as for BSV		
BSV	0.15 M NaCl + 0.05 M Tris HCl	7.4	1.022[e]
PM2	0.5 M NaCl	6.2–6.6[b]	1.046[c]
	1.0 M NaCl[d]	6.2–6.6[b]	1.095[e]
	2.0 M NaCl	6.2–6.6[b]	1.217[c]
	5.0 M NaCl	6.2–6.6[b]	1.855[e]
T7	0.5 M NaCl + 0.001 M $MgCl_2$ + 0.01 M Tris HCl	7.8	1.048[e]

[a] At 25°. [b] Range of pH values measured on the different virus solutions used for the measurements of D. [c] From Svedberg and Pedersen (1940). [d] Solvents used for data in Figure 1. [e] Determined with the use of a Cannon-Ubbeholde viscometer. [f] Used for one measurement at a virus concentration of 0.209 mg cm^{-3}, see Figure 1. [g] Measured at pH 8.0 and assumed not to vary with pH.

tein and solvent; the net effect of these interactions on the apparent molecular weight is termed preferential interactions. The effect of these interactions on molecular weight determinations may be eliminated by defining an apparent specific volume ϕ' related to the apparent density increment at constant chemical potential of all diffusible components $(\Delta\rho/c)_\mu$ (Casassa and Eisenberg, 1964)

$$(\Delta\rho/c)_\mu = 1 - \phi'\rho_0$$

where c is the concentration of macromolecule and $\Delta\rho$ is the difference between the density of the macromolecular solution taken undiluted from the dialysis bag (ρ_s) and that of the dialysate (ρ_0). We have assumed, as is usually done, that the value of ϕ' obtained here is equal within experimental error to its value at infinite dilution. The density increment of bacteriophage Qβ was determined with a viral solution dialyzed with three changes against 0.15 M NaCl and 0.05 M Tris (pH 7.4) buffer, the last change lasting at least 48 hr. The densities of the undiluted Qβ solution and the dialysate were measured at 25.0 ± 0.05° with a 5.2-cm^3 Lipkin pycnometer (Lipkin *et al.*, 1944; Scientific Glass Apparatus, Bloomfield, N. J.). For both the virus solution and the solvent the pycnometer was filled and weighed, and its volume was measured three separate times. Weights were reproducible to ±0.02 mg and volumes to ±0.0002 cm^3; the difference in the densities was 4.85 ± 0.08 mg cm^{-3}. The concentration was determined spectrophotometrically on a gravimetric dilution of the virus solution; corrections were applied for the difference between solution and solvent densities.

The density increments for PM2 and R17, in solvents very similar to those used to measure $D^0_{20,w}$ and $s^0_{20,w}$, were determined pycnometrically in the same manner and are reported elsewhere (Camerini-Otero, 1973).[3,4] The value of ϕ' for T7 was taken from published work (Bancroft and Freifelder, 1970). For BSV we have taken the value for a not well defined specific volume (Kupke, 1966).

Sample Preparation, Solvents, and Viscosities. Since IFS experiments are extremely sensitive to the presence of high molecular weight contaminants (Pusey *et al.*, 1974), a careful sample filtration procedure was followed. A 1 cm × 1 cm sample cuvet was first soaked in chromic acid cleaning solution and then scrubbed in detergent solution. The cell was then attached to a closed filtration system and flushed with about 500 cm^3 of twice-filtered distilled water (a 0.1 μ Millipore filter, followed by a 0.1–0.45 μ GA Gelman filter). Solvent appropriate to the system under study was then injected into the system before the Gelman filter and the cell was flushed until refractive index gradients were not evident. The solvent was then ejected with filtered air. Finally, about 1 cm^3 of sample was introduced before the Gelman filter. The sample was followed by a small change of solvent to clear the filter and entrance tubing of sample (0.2 cm^3). This filling procedure resulted in a sample dilution of about 15%. Sample concentrations were determined after the measurement of D.

The virus solutions at different initial virus concentrations were prepared by dilution from a stock solution dialyzed against the appropriate solvent with three changes. For PM2 and R17 concentrated virus solutions were prepared for each different solvent used.

A list of solvents used throughout this study and their respective viscosities relative to water at 25°, $(\eta/\eta^0)_{25}$, is presented in Table I (see Results). Solvent viscosities were determined from data in the tables provided by Svedberg and Pedersen (1940); when these were not available, kinematic viscosities were determined with a Cannon-Ubbeholde semimicro four-bulb variable shear viscometer (0.5% precision, Cannon Instrument Co., State College, Pa.). In some cases an assumption was made as the additive nature of the constituent specific viscosities, $[(\eta/\eta^0)_{25} - 1]$. Solvent densities either taken from the International Critical Tables or determined in a Lipkin pycnometer (Lipkin *et al.*, 1944) were used to convert kinematic viscosities to relative viscosities. Those solvent densities required to convert the buoyancy term in the Svedberg equation (Svedberg and Pedersen, 1940) to standard conditions were measured pycnometrically.

IFS Apparatus. The IFS apparatus is described in detail in the accompanying paper (Pusey *et al.*, 1974). Except for the temperature dependence run of R17 all sample measurements were carried out at 25° and corrected to $D_{20,w}$ in the standard manner (see Pusey *et al.*, 1974). For the temperature dependence run the sample was held for 1–2 hr at each temperature, except for about 4.5 hr at 45°. After cooling to 25° the sample stood overnight and $D_{20,w}$ was determined the next day.

Results

Diffusion Coefficients. For a monodisperse solution of noninteracting particles of diameter small compared to the wavelength of light and/or spherically symmetric, the autocorrela-

[4] R. D. Camerini-Otero, R. M. Franklin, and L. A. Day, manuscript in preparation.

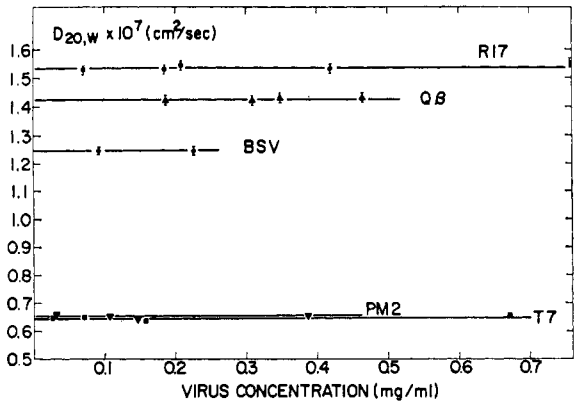

FIGURE 1: Dependence of $D_{20,w}$ on low virus concentration for R17, BSV, Qβ, PM2, and T7. For solvents see Table I; bars = ±1%.

tion function of the scattered electric field is a single exponential with a decay rate, Γ, related to D by (Pecora, 1964)

$$D = \Gamma/K^2 \quad (1)$$

where $K = 4\pi(n/\lambda_0)\sin(\theta/2)$ is the magnitude of the scattering vector, and n, λ_0, and θ are, respectively, the refractive index of the scattering medium, the *in vacuo* wavelength of the incident light, and the scattering angle. The data-collecting and handling techniques used by us in the measurement of the autocorrelation function are described in the accompanying paper (Pusey *et al.*, 1974). For each sample at least two runs were made for values of K corresponding to θ = 75, 90, and 105° or θ = 70, 90, and 110°; typically then, the values from 6 to 10 runs lasting 1–3 min were averaged to obtain D for each sample. The observed single-clipped photocount correlation function could be described by a single exponential as evidenced by the fact that the "quality parameter," Q, was zero within experimental error (±0.02, see Pusey *et al.*, 1974); occasionally some runs did not fulfill this condition, these were discarded. In addition, Γ was found to be proportional to K^2 within experimental error. Figures for a typical run showing both the single exponential nature of the autocorrelation function and the linear dependence of Γ on K^2 are shown in the accompanying paper (Pusey *et al.*, 1974). Both the exponential nature of the correlation function and the linear dependence of Γ on K^2 are strong evidence that a single diffusion coefficient describes the Brownian motion of the virus particles (Debye, 1965; Dubin *et al.*, 1967; Pusey *et al.*, 1972).

The diffusion coefficient of each virus was measured in a solvent identical with or very similar to that commonly used in the measurement of most of its physical parameters, including both s and ϕ'. The results of these measurements, expressed as $D_{20,w}$, are plotted as a function of concentration (Figure 1). The diffusion coefficients of R17 and PM2 have also been measured as a function of concentration in solvents of different ionic strengths (Figures 2 and 3), and that for R17 has also been measured as a function of pH (Figure 4) and temperature (Figure 5).

Because of interparticle interactions the diffusion coefficients of spherical viruses can vary with virus concentration (Figure 2). In the presence of such a concentration dependence, the values of D calculated from eq 1 and plotted in Figure 2 must be considered *effective* values. The data presented in this figure are briefly disussed below and have been more extensively discussed elsewhere (Pusey *et al.*, 1972). At low concentrations (<1 mg/ml), however, even for ionic strengths as low as 0.015, the values of $D_{20,w}$, in agreement with theoretical

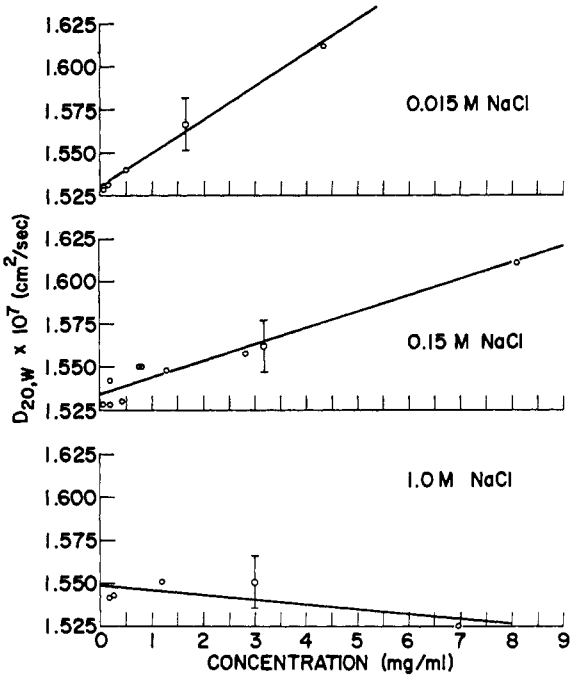

FIGURE 2: Dependence of $D_{20,w}$ on virus concentration and ionic strength for R17. Bars indicate an uncertainty of 1%; lines were obtained from a linear least-squares fit to the data.

expectations (see, for example, Goldstein and Zimm, 1971, and Pusey *et al.*, 1972), show no detectable concentration dependence. Thus, the averages of the values in Figure 1 are taken as the zero concentration values, $D^0_{20,w}$, and are listed in Table II. This $D^0_{20,w}$ value for R17, obtained in this fashion, is in agreement with that obtained by a linear extrapolation of the concentration-dependent data (Figure 2).

Virus Molecular Weights. The molecular weights were calculated using the relationship (Svedberg and Pedersen, 1940)

$$M = RTs^0_{20,w}/D^0_{20,w}(1 - \rho\phi') \quad (2)$$

where R is the gas constant, T is 293°K, and ρ is the density of water at 20°.

In calculating these molecular weights the values used for $s^0_{20,w}$ are listed in Table II. With the exception of the value for Qβ, all of these were taken directly from the literature. For Qβ we have corrected the data of Overby *et al.* (1966), who have determined $s^{0.5\%}_{20,w}$ to be 84.3 S; using the concentration dependence of the sedimentation coefficient of R17 (Enger *et al.*, 1963) we have estimated a value of 88.4 ± 2 S for the $s^0_{20,w}$ of Qβ. There is good reason to believe that this correction procedure should not introduce a greater uncertainty than that indicated (see Goldstein and Zimm, 1971). The values of ϕ' used (Table II) are all taken from the litera-

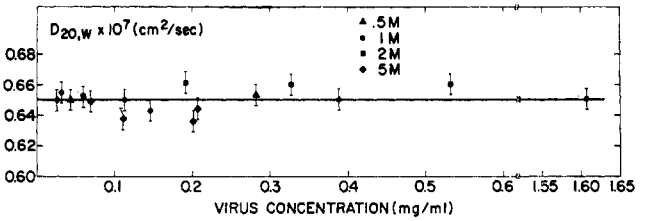

FIGURE 3: Dependence of $D_{20,w}$ on virus concentration and ionic strength for bacteriophage PM2 (in varying molarities of NaCl). Bars indicate an uncertainty of 1%.

TABLE II: Summary of Results on Diffusion Coefficients and Molecular Weights of Viruses.

Virus	$D^0_{20,w}$ ($\times 10^{-7}$ cm^2 sec^{-1})	$s^0_{20,w}$ ($\times 10^{-13}$ sec)	ϕ' (cm^3 g^{-1})	$E^{0.1\%}_{1\,cm}$ [a] (cm^2 mg^{-1})	M ($\times 10^6$)
R17	1.534 ± 0.015	78.9 ± 1 [b]	0.689 ± 0.012 [c]	7.93 ± 0.30 [c]	4.02 ± 0.17
Qβ	1.423 ± 0.014	88.4 ± 2 [d]	0.668 ± 0.009	8.02 ± 0.16 [d]	4.55 ± 0.16
BSV	1.246 ± 0.013	133 ± 2 [e]	0.706 ± 0.002 [f]	5.00 [g]	8.81 ± 0.17
PM2	0.650 ± 0.007	294 ± 3 [h]	0.771 ± 0.007 [h]	4.60 ± 0.10 [h]	47.9 ± 1.7
T7	0.644 ± 0.007	487 ± 5 [i]	0.639 ± 0.006 [j]	12.90 ± 0.07 [j]	50.9 ± 1.1

[a] Extinction coefficients at 260 nm. [b] Enger *et al.* (1963). Error estimated from distribution of values in the literature; for review of these, see Overby *et al.* (1966). [c] See footnote 4. [d] Overby *et al.* (1966); errors estimated. $s^0_{20,w}$ calculated from $s^{0.5\%}_{20,w}$ (see text). [e] Dorne and Hirth (1970); error estimated. [f] Kupke (1966); this is not a well-defined apparent specific volume. [g] Bawden and Pirie (1938). [h] See footnote 3. [i] Davison and Freifelder (1962). [j] Bancroft and Freifelder (1970).

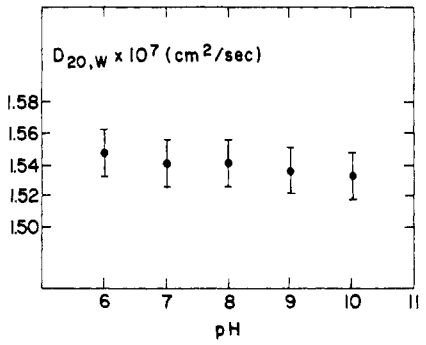

FIGURE 4: Dependence of $D_{20,w}$ on pH for R17 at a concentration of ~0.1–0.2 mg cm^{-3} and ionic strength of 0.15; for details on buffers, see Table I. Bars indicate an uncertainty of 1%.

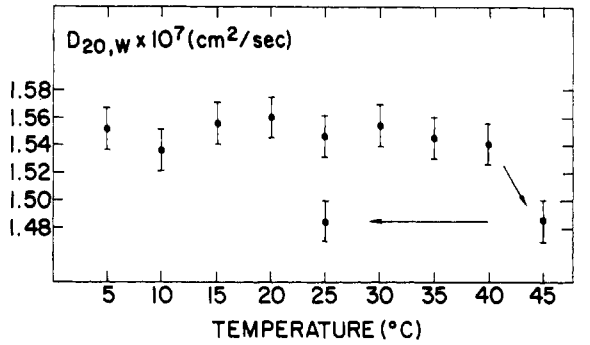

FIGURE 5: Dependence of $D_{20,w}$ on temperature for R17 at a concentration of ~0.324 mg cm^{-3} in 0.15 M NaCl and 0.015 M Tris (pH 7.2). Bars indicate an uncertainty of 1%.

ture with the exception of that for Qβ, which was determined by us to be 0.668 cm^3 g^{-1}.

The molecular weights for the different viruses are 4.02 ± 0.17 × 10^6 for R17, 4.55 ± 0.16 × 16^6 for Qβ, 8.81 ± 0.17 × 10^6 for BSV, 47.9 ± 1.7 × 10^6 for PM2, and 50.9 ± 1.1 × 10^6 for T7. The quoted uncertainty of these molecular weights was obtained by the procedure described in the Appendix.

Hydrodynamic Radii and Solvation. For any macromolecule we can define the dry radius, R_d, as

$$R_d = (3M\bar{v}/4\pi N)^{1/3} \quad (3)$$

where $\bar{v}$ is the partial specific volume of the macromolecule in the absence of preferential interactions and N is Avogadro's number. Since for our purposes the difference between ϕ' and $\bar{v}$ (Cohen and Eisenberg, 1968; Reisler and Eisenberg, 1969; Inoue and Timasheff, 1972) will not result in a serious error, we have used ϕ' in our calculations. R_d can be regarded as that of an equivalent unsolvated sphere. The radius of a hydrodynamically equivalent sphere, R_h, can be defined using the Stokes–Einstein equation

$$R_h = kT/6\pi\eta D^0 \quad (4)$$

where k is the Boltzman constant, η is the viscosity of the solvent, T is the absolute temperature, and D^0 the zero concentration limit of D. The ratio of these radii is a function of both the solvation and the degree by which the shape deviates from a sphere (Tanford, 1961)

$$R_h/R_d = (f/f_0)([\bar{v} + \delta]/\bar{v})^{1/3} \quad (5)$$

where f/f_0 represents the effect of shape on the frictional coefficient, the second term in parentheses is the ratio of the solvated to dry volume, and δ is the volume (in cm^3) of solvent associated with each gram of dry macromolecule. Since for these viruses the information required to estimate the density (composition) of the solvate (*i.e.*, both $\bar{v}$ and ϕ') is not available, we have used δ defined in units of cm^3 of solvent per gram of virus instead of the more conventional definition in units of gram of solvent per gram of virus. The shapes of all of these viruses have been studied by electron microscopy and those of BSV, R17, and PM2 by X-ray diffraction (Harrison, 1969; Fischbach *et al.*, 1965; Zipper *et al.*, 1971; Harrison *et al.*, 1971). BSV, R17, and Qβ virus particles appear under the electron microscope as spheres (Finch *et al.*, 1970; Vasquez *et al.*, 1966; Overby *et al.*, 1966). Although T7 and PM2 have icosahedral shapes, with a small tail in the case of T7 (Davison and Freifelder, 1962; Dubin, 1970) and small spikes for PM2 (Silbert *et al.*, 1969; Harrison *et al.*, 1971), their values of f/f_0 cannot deviate by more than 1% from 1.0 (Tanford, 1961). For $f/f_0 = 1$, $F \equiv 1 - (R_d/R_h)^3$ is the fraction of the virus hydrodynamic volume occupied by solvate. Values for δ and F are shown in Table III. It should be emphasized that these values are independent of any assumption about the viruses other than their spherical shape. The relationship of this solvation to other measurements of bound solvent and to particle size and structure is discussed below.

Discussion

Virus Molecular Weights. R17. Although several attempts have been made to determine the molecular weight of R17, M has not been established unequivocably.[5] There have been

[5] As it is well known that the difference in the virions of MS2, R17, f2, and fr are very minor (Hohn and Hohn, 1970), we shall use data for these four viruses in this discussion.

TABLE III: Summary of Virus Solvation and Dimensions.

Virus	δ (cm³ of H_2O/g of Dry Virus)	$F \times 100$ (% H_2O by Vol)	R_d[a] (Å)	R_h[a] (Å)	*EM* Radii (Å)[b] Individual Particles	*EM* Radii (Å)[b] Interparticle Half-distance in Arrays	Other Radii (Å)
R17	1.02 ± 0.09	60 ± 2	103 ± 2	140 ± 2	115[c] 125[d]	135 ± 1[d]	132 (X)[e,f] 133 (X)[h] 132 (R)[e]
Qβ	1.22 ± 0.08	65 ± 2	106 ± 2	151 ± 2	125[d]		
BSV	0.75 ± 0.04	52 ± 1	135 ± 1	172 ± 3			154 (X)[i] 155 (X)[j] 170 (C)[k] 167 ± 5 (C)[l]
PM2	1.1 ± 0.08	59 ± 2	245 ± 3	330 ± 3	300 ± 15[m,n] (319 ± 16)		300 (X)[o]
T7	1.18 ± 0.06	65 ± 1	235 ± 2	333 ± 3	310 ± 15[m,p] (330 ± 16)		325 (R)[q]

[a] R_d and R_h are defined by eq 3 and 4, respectively. [b] Only values obtained from negatively stained specimens are presented; phosphotungstic acid was used in all cases. [c] Vasquez *et al.* (1966). [d] See footnote 6. [e] Zipper *et al.* (1971). [f] The capital letters in parentheses refer to radii evaluated from the radial electron density distribution (X), the radius of gyration obtained from light (T7) and X-ray (R17) scattering (R), and the X-ray measurement of interparticle distance in wet crystals (C). [g] Overby *et al.* (1966). [h] Fischbach *et al.* (1965). [i] Harrison (1969). [j] Leonard *et al.* (1953). [k] Bernal *et al.* (1938). [l] Carlisle and Dornberger (1948). [m] These experimental values are icosahedral face-to-face distances; the values in parentheses are corrected to the equivalent radius (see text). [n] Measured face-to-face distance from unpublished electron micrographs of negatively stained specimens obtained by Dr. J. A. Silbert (Silbert *et al.*, 1969). [o] Harrison *et al.* (1971). [p] Dubin (1970). [q] Davison and Freifelder (1962).

many reports of M for R17, fr, f2, and MS2, but the values obtained using the sedimentation–diffusion method have consistently differed from those obtained by other means. Whereas other techniques have given values in the range from 3.6×10^6 to 4.0×10^6 (Strauss and Sinsheimer, 1963; Gesteland and Boedtker, 1964; Overby *et al.*, 1966; Zipper *et al.*, 1971; Camerini-Otero, 1973),[4] those from sedimentation–diffusion (with one exception, Overby *et al.*, 1966) have ranged from 4.2×10^6 to 5.3×10^6 (Enger *et al.*, 1963; Marvin and Hoffmann-Berling, 1963; Möller, 1964).

The three values of D corresponding to these values of M are (in units of 10^{-7} cm² sec^{-1}): $D^0_{20,w} = 1.16$ (Möller, 1964), $D^{0.20\%}_{20,w} = 1.328$ (Enger *et al.*, 1963), and $D^0_{20,w} = 1.45$ (Marvin and Hoffmann-Berling, 1963). Since the discrepancy in the value of M cannot be ascribed to errors in the literature values of s and $\bar{v}$ (see Overby *et al.*, 1966),[4] it should be traceable to errors in these values of D. We have confirmed this to be the case. The molecular weight we have obtained using $D^0_{20,w} = 1.534 \times 10^{-7}$ cm² sec^{-1} not only agrees well with those determined by a variety of other methods on the same virus preparations (Camerini-Otero, 1973),[4] but also with that calculated from the viral composition using the latest value for the molecular weight of the RNA (Boedtker, 1971).

Our sedimentation-diffusion molecular weight, for R17, $M_{s,D} = 4.02 \pm 0.17 \times 10^6$, agrees well with those determined on the same preparations by light scattering, $3.80 \pm 0.23 \times 10^6$, turbidity, $3.90 \pm 0.25 \times 10^6$, sedimentation–viscosity, $3.98 \pm 0.19 \times 10^6$, and sedimentation–equilibrium, $3.64 \pm 0.18 \times 10^6$ (Camerini-Otero, 1973).[4]

The quoted uncertainty of about 5% in the value for $M_{s,D}$ includes estimates of both systematic and random errors in the determination of D, s, and ϕ'. This estimate was calculated by using the following as the uncertainties in D, s, and ϕ'. The uncertainty in $D^0_{20,w}$ is ±1% as mentioned above and discussed elsewhere (Pusey *et al.*, 1974). The majority of the literature values for $s^0_{20,w}$ (Gesteland and Boedtker, 1964; Marvin and Hoffmann-Berling, 1963; Overby *et al.*, 1966) are all within 1 S (1.3%) of 78.9 S (Enger *et al.*, 1963), the value used here. As can be seen from eq 2, the major contribution to the uncertainty in $M_{s,D}$ is from the uncertainty in ϕ' and the estimated uncertainty here is 1.7%,[4] or a contribution to the uncertainty in M of ~4%. In turn, the major uncertainty in ϕ' is the uncertainty in $E^{0.1\%}_{1\,cm}$. The value for $E^{0.1\%}_{1\,cm}$ of R17 used here (Table II) is within 1% of the mean of five literature values for the extinction coefficients of R17-like RNA bacteriophages; we have used the 95% confidence interval for this mean to estimate the uncertainty in $E^{0.1\%}_{1\,cm}$ for R17.[4] Finally, we should point out that to our knowledge the value of ϕ' for R17 used here is the only one reported for R17 which is clearly a specific volume at constant chemical potential, the correct value to use in multicompoent systems (Casassa and Eisenberg, 1964).

Until recently the molecular weight of R17-like viruses that was estimated from the viral composition was $3.6 \pm 0.1 \times 10^6$ (Hohn and Hohn, 1970); since this value is in apparent disagreement with the value of $4.02 \pm 0.17 \times 10^6$ reported here, some discussion of the origin and reliability of the compositional value is necessary. The contribution of the coat protein is reliably known. From structural considerations there are estimated to be 180 subunits (Caspar and Klug, 1962; Vasquez *et al.*, 1966) of mol wt 13,750 (known from the amino acid sequence, see Hohn and Hohn, 1970). A minor contribution comes from a small but uncertain number of subunits of A-protein each with a molecular weight of 37,500 daltons (Steitz, 1968). The major uncertainty is from the molecular weight of the RNA. Until recently this value was given as $1.1 \pm 0.1 \times 10^6$ (Gesteland and Boedtker, 1964; Sinha *et al.*, 1965). Assuming, as is usual, that only one subunit of the minor A protein is present (Strauss and Kaesberg, 1970), then the molecular weight of R17 would be $3.61 \pm 0.1 \times 10^6$.

This value is lower than those obtained by us by practically all the other methods except sedimentation–equilibrium[4] but is in good agreement with the molecular weights obtained by others with light scattering (Strauss and Sinsheimer, 1963; Gesteland and Boedtker, 1964; Overby *et al.*, 1966). This agreement has been the mainstay for this generally accepted value (see Hohn and Hohn, 1970), and the discrepancy between the value reported here and that obtained from composition has been a matter of concern to us.

Recently, however, Boedtker (1971) has reinvestigated the molecular weight of the RNA of R17 by the technique of polyacrylamide gel electrophoresis and has revised her earlier value to $1.3 \pm 0.1 \times 10^6$ daltons. Using this figure, the molecular weight calculated from the composition is $3.81 \pm 0.1 \times 10^6$, which is in agreement with the value obtained in the present study. This agreement confirms the higher molecular weight of the viral RNA, and shows that all the mass of the virus can be accounted for by the known viral components.

Qβ. Assuming, as is usually done, that each virion contains 180 coat protein subunits of mol wt 14,000 (Konigsberg *et al.*, 1970) and there are four to six subunits of a minor A-protein of mol wt 38,000 (Strauss and Kaesberg, 1970), these values combined with the new value of $1.5 \pm 0.1 \times 10^6$ for the molecular weight of the RNA (Boedtker, 1971) yield a compositional molecular weight of $4.26 \pm 0.2 \times 10^6$, in agreement with our value of $4.55 \pm 0.16 \times 10^6$.

Although Overby *et al.* (1966) have also obtained values for $M_{s,D}$ which are in good agreement with the compositional molecular weights, 3.87×10^6 and 4.29×10^6 daltons for MS2 and Qβ, respectively, in general, their values of s, D, and apparent specific volume are, however, considerably different than those used here. In particular, their values of s and D were measured at concentrations from 5 to 10 mg cm^{-3} and are uncorrected for concentration dependence; their values of D obtained by boundary spreading were 7–9% higher than those measured by us.

BSV. From structural considerations there is good reason to believe that the BSV virion contains 180 mol of a major coat protein (Finch *et al.*, 1970; Crowther and Amos, 1971) of mol wt 38,000 (Butler, 1970); this protein constitutes 93.1% of the total virus protein. In contrast to the RNA bacteriophages, the molecular weight of the RNA of BSV is not known and we must rely on the per cent of the viral weight known to be nucleic acid (16.5% by weight, de Fremery and Knight, 1955) to estimate its contribution to the molecular weight of the virion. Summing all these contributions, one can obtain a compositional molecular weight of 8.8×10^6 which is in agreement with the value reported here, $8.81 \pm 0.17 \times 10^6$.

This confirmation of the structural and compositional data has also been reported by Weber *et al.* (1970) who reported an $M_{s,D}$ of 8.9×10^6 using a value for D of 1.26×10^{-7} cm^2 sec^{-1}, using the technique of boundary spreading in the centrifuge.

PM2. A compositional molecular weight cannot yet be calculated for the relatively complex virion of PM2, but independent measures of the viral molecular weight are available. The value for $M_{s,D}$, $47.9 \pm 1.7 \times 10^6$ obtained by us is in agreement with that obtained by equilibrium–sedimentation, $44.0 \pm 2.5 \times 10^6$ [3] and by turbidity, $45.4 \pm 2.0 \times 10^6$ (Camerini-Otero, 1973).[4]

T7. Dubin *et al.* (1970) using essentially the same method used here have also measured the diffusion coefficient of T7, obtaining $D^0_{20,w} = 0.603 \pm 0.006 \times 10^{-7}$ cm^2 sec^{-1} in disagreement with our value of $0.644 \pm 0.007 \times 10^{-7}$ cm^2 sec^{-1}. Dubin *et al.* (1970) also reported a value of 453 ± 8 S for $s^0_{20,w}$. This value is not in accord with two previously published values for $s^0_{20,w}$: 480 S (Putman, 1954) and 487 ± 5 S (Davison and Freifelder, 1962). Thus, there are basically two combinations of these figures which yield identical values for $s^0_{20,w}/D^0_{20,w}$. Using the data of Dubin *et al.* (1970) the value of this ratio is 7.52×10^{-4} cm^{-2} sec^2; using the more recent other value for $s^0_{20,w}$, 487 S, and our value for $D^0_{20,w}$ the value of this ratio is 7.57×10^{-4} cm^{-2} sec^2. The molecular weights calculated from these two values for $s^0_{20,w}/D^0_{20,w}$, using the same value for ϕ' (Table II), are 50.6×10^6 and 50.9×10^6. The latter value is the one reported here (Table II). Both of these values are in agreement with the value of M obtained by equilibrium–sedimentation, $49.4 \pm 1.5 \times 10^6$ (Bancroft and Freifelder, 1970) and by turbidity, $52.2 \pm 2.2 \times 10^6$ (Camerini-Otero, 1973).[4] Other possible combinations of the values for $s^0_{20,w}$ and $D^0_{20,w}$ do not yield values of M which are in such agreement. Furthermore, although the value of M using the data of Dubin *et al.* (1970) and the data used here, including our value of $D^0_{20,w}$, agree, there are several reasons why we feel the data we have used to be correct.

The values of $s^0_{20,w}$ and $D^0_{20,w}$ obtained by Dubin *et al.* (1970) are both 7% smaller than the values we have used; this implies an increase in the frictional coefficient without a loss of mass. Denaturation of the virus would lead to an equal decrease in both s and D. On the other hand, barring unusual circumstances, it is hard to explain an increase in both s and D. On this basis alone it is possible that the samples of T7 studied by Dubin *et al.* (1970) were partially denatured. This conclusion is in fact supported by the following. First, our value of $D^0_{20,w}$ agrees almost exactly with that which has been calculated from the viral diminesions obtained from electron micrographs, $0.638 \pm 0.015 \times 10^{-7}$ cm^2/sec (Dubin, 1970); as we shall point out below, such an agreement is expected. Secondly, Dubin and his colleagues reported erratic and unreproducible results in the measurement of D if the T7 sample were not allowed to stand for about 10 hr after dilution. We searched for such an effect, but found no significant difference in the diffusion coefficient measured minutes, hours, and days after sample preparation.

Finally, it should be pointed out that the value of ϕ' used by Dubin *et al.* (1970) and us to calculate M was measured at a low ionic strength, about 0.1 (Bancroft and Freifelder, 1970), in contrast to the ionic strength of $\sim$0.5 used in the measurement of D and s (see Table I; Dubin *et al.*, 1970). Since in a multicomponent system the value of ϕ' can be a function of ionic strength (Casassa and Eisenberg, 1964; Cohen and Eisenberg, 1968), it is possible that the uncertainty in M is greater than that quoted based on a 1% uncertainty of ϕ'.

Particle Dimensions. A further aspect of the present investigation is the study of the correspondence between the hydrodynamic radii obtained by IFS of solutions of viruses and the particle dimensions obtained by other techniques, such as electron microscopy. Spherical, or nearly spherical viruses are ideally suited for such a study; their shape factors are largely inconsequential and their relatively large dimensions can be measured by several physical methods.

There have been at least two published reports that have suggested that electron microscopic results underestimate the radii measured by IFS by a factor of about two, presumably because of a dehydrating effect (French *et al.*, 1969; Lin *et al.*, 1971). Even the value of $D^0_{20,w}$ for T7 obtained by Dubin *et al.* (1970) raises the possibility of such a discrepancy. For the case of the casein micelles reported by Lin *et al.* (1971) the difference in radii might have a structural basis; these are structures that are sponge like and show dramatic changes in

size as a function of solvent composition (Lin *et al.*, 1971). We shall show that for the viruses we have studied the radii measured by IFS are in agreement with those obtained by electron microscopy and other methods.

Since there is a wealth of information on the dimensions of the R17-like RNA bacteriophages, it will be worthwhile to examine this case in some detail. French and his colleagues measured the radius of MS2 by IFS as 205.5 ± 5.5 Å. French *et al.* attributed the difference between this value and that which they determined with the electron microscope, 125 Å, to dehydration. Were IFS the only method for measuring radii of spherical particles in solution, such a conclusion might go undisputed. At this point it is perhaps appropriate to review some of the other methods that can be used to measure the dimensions of spherical particles in solution using R17 and its relatives to illustrate the case in point. First, the radius of gyration for such a sphere can be easily calculated from the central maximum obtained from the low-angle X-ray scattering of a solution of these particles (Guinier, 1939). Although in order to obtain a radius from such a value one must assume a certain mass distribution, this has not in general been a great limitation. Using the common assumption of a uniform mass density, the value for the R17 radius in solution obtained from the radius of gyration is 132 Å (Zipper *et al.*, 1971) in good agreement with the value obtained by electron microscopy and by us (140 Å, Table III). A second method for measuring the radii of macromolecular structures in solution, closely related to the first, is light scattering. Since by this method it is difficult to obtain an accurate value for the radius of gyration of a scatterer of the size of bacteriophage R17, a value for its radius using light scattering has not been reported. This method has been used to measure the radius of T7 (Davison and Freifelder, 1962; see Table III). A third method is from the calculated radial electron density distribution obtained by a Fourier transformation of the X-ray scattering amplitudes. The two literature values for the radius of R17 by this method are 132 (Zipper *et al.*, 1971) and 133 Å (Fischbach *et al.*, 1965). This method has also been used in calculating the spherically averaged radius of BSV and PM2 (Harrison, 1969; Harrison *et al.*, 1971; see Table III). Thus, all the methods that have been used to measure the radius of R17 in solution, including our own by IFS, are in agreement with the electron microscopic radius, in contrast to the value reported by French and his colleagues.

For the other viruses, the radii measured by the several techniques, including those mentioned above, are shown in Table III. Two of the viruses we have studied, PM2 and T7, appear in the electron microscope as icosahedra (Silbert *et al.*, 1969; Harrison *et al.*, 1971; Dubin, 1970); for these, face-to-face distances, rather than radii, are more appropriate viral dimensions. Since the diffusion coefficient of a regular icosahedron is 0.94 times that of a sphere with a diameter equal to the face-to-face distance (Dubin, 1970), this volume correction has been applied to convert the electron microscope measurements of PM2 and T7 to radii which can be compared with R_h.

An examination of Table III shows there is agreement between the radius measured by the different techniques, including IFS. In general, however, the hydrodynamic radii are always slightly larger than those measured by the other techniques. Barring any systematic error in our measurements, which we consider unlikely (Pusey *et al.*, 1974), we believe that certain recent findings regarding the surface structure of viruses can account for this trend. There is some reason to believe that much of the surface and outer shell of perhaps all viruses is not occupied solely by protein but is heavily fenestrated by large solvent spaces that extend from the outer radius of the sphere inward. For some of the viruses studied here, BSV, PM2, and R17, this hypothesis can be made on the basis of indirect experimental evidence or arguments (Harrison, 1969; Harrison *et al.*, 1971; Datta *et al.*, 1971; Hohn and Hohn, 1970; Zipper *et al.*, 1971), and in the case of BSV it has been confirmed by three-dimensional reconstructions of electron micrographs (Crowther *et al.*, 1970; Crowther and Amos, 1971) as well as by X-ray diffraction structure analysis (Harrison, 1971). Obviously, any large solvent spaces on the periphery of these viral structures will lead to underestimates of the maximum radius of a sphere when techniques based on spherical averaging, negative staining, or an assumption of uniform mass density are used. What is not as clear is the effect these solvent spaces will have on the relationship between the outer radius and the hydrodynamic radius. One might suppose that such solvent spaces might be free draining resulting in hydrodynamic radii somewhat smaller than the maximum radius. This, however, is probably unlikely. Given a hollow sphere with a surface made up of smaller spheres of one-tenth the larger radius, a size not unlike that of the morphological subunits of viruses, Bloomfield *et al.* (1967) have calculated that up to 60% of the small spheres can be removed without reducing the frictional coefficient by more than 4%. Furthermore, when a hollow macroscopic model of a phage had more than 50% of its surface removed by drilling holes in it, there was no difference in its frictional resistance (Douthart and Bloomfield, 1968). With these results in mind, it would seem that the hydrodynamic radius of a fenestrated sphere with a nonhollow center, such as a virus, is at least as large as the outer radius.

Having set a minimum for R_h, what we must now ask is how much larger than the outer radius can it be. In order to address this problem we must have an independent measure of the outer radius. A good approximation to this value should be half the inter-particle distance of closely packed virus particles. This distance is available for two of the viruses we have studied, R17 and BSV. For R17 the half-interparticle distance in negatively-stained paracrystalline arrays is 135 ± 1 Å.[6] For BSV, an interparticle distance of 167 ± 5 Å was measured by X-ray diffraction of wet crystals (Carlisle and Dornberger, 1948; a recent analysis of this data is presented in Klug and Caspar, 1960). This is in agreement with our value for R_h of 172 ± 2 Å.

In the absence of strong experimental evidence proving the existence and defining the properties of extensive external layers of solvation (beyond the maximum particle radius) we have taken the view that closely packed particles are not separated by such layers. In fact, Klug and Caspar (1960) have argued strongly that the BSV particles must be in contact in the crystal lattice and that it is not necessary to postulate layers of external solvation to account for the difference observed between half the interparticle distances and radii obtained from "spherically averaging" techniques. Furthermore, for the paracyrstalline array of R17 the small difference, within experimental uncertainty, between this distance (135 Å) and the spherically averaged value (132–133 Å) speaks against such an external solvation beyond one or two layers of water molecules.

In summary, we believe that as more data become available, the kind of agreement shown above will be found for other

[6] H. Frank and T. Hohn, unpublished data; quoted by Hohn and Hohn (1970) and Zipper *et al.* (1971).

spherical macromolecular structures and that the following general conclusions can be made: (1) the hydrodynamic radius is a very good approximation to the particle outer radius; (2) half-interparticle distances obtained from paracrystalline or crystalline arrays can be used to estimate, within an error of a few percent, the hydrodynamic radius and diffusion coefficient of spherical particles; (3) internal and external solvation should be defined in reference to these interparticle half-distances; and (4) if spherical macromolecular structures have outer layers of hydrodynamically bound solvent, these do not extend for more than a bilayer of water ($\lesssim$6 Å).

Solvation. Our last conclusion brings us to the topic of solvation, the hydrodynamically "bound" solvent as it is defined by eq 5. This is not to be confused with preferential hydration (Casassa and Eisenberg, 1964; Cohen and Eisenberg, 1968) or the other forms of hydration measured by nuclear magnetic resonance (Kuntz *et al.*, 1969). Our values for the solvation of the five viruses studied here are presented in Table III. We have also calculated the solvation of R17 from recent data on its intrinsic viscosity (Camerini-Otero, 1973)[4] and found it to be 0.98 cm^3 of solvent/g of dry R17, in good agreement with the value reported here. For all the viruses studied, BSV excepted, the solvations are virtually identical; this in spite of the wide range in viral chemical composition. At this point we do not know what significance, if any, to attribute to the difference in δ for BSV compared to the other viruses. It is tempting to suppose that it might be related to the plant origin of this virus; recently a value for δ of 0.68–0.71 g of H_2O/g (dry) for turnip yellow mosaic virus (TYMV) has been reported (Kupke *et al.*, 1972).

Among the viruses studied R17 has the largest ratio of surface area to volume and in this case an external layer of solvation of 6 Å would account for only 12% of the hydrodynamic volume; *i.e.*, at most 20% of the solvation could be considered external to the domain of the virus particle. Considering that for small to medium size proteins (*e.g.*, lysozyme and hemoglobin), structures much more compact than viruses and with less elaborate quaternary structures, most of the solvation is in the form of an external (surface) layer 1–2 molecules of water thick (Dubin *et al.*, 1971; Grant and South, 1972), this kind of solvation on the solvent exposed surfaces of the protein subunits in the virus would account for some fraction of their internal solvation; the remainder, presumably, is solvent entrained or hydrodynamically "trapped."

Effect of Virus Concentration, Ionic Strength, pH, and Temperature on D. The values for the single exponential decay rate, Γ, for this group of spherical viruses show no significant concentration dependence at concentrations below 1 mg/ml (Figures 1, 2, and 3). At these concentrations Γ can be related to D according to eq 1. Only the concentration dependence of R17 has been studied at higher concentrations; here the values for Γ showed a dependence on virus concentration, assumed to to be linear, which increased with decreasing solvent ionic strength. We have calculated *effective* values for $D_{20,w}$ from Γ *assuming* that the concentration dependence we have observed is that of D, or in other words that eq 1 is valid (Figure 2); we must emphasize however that this is an assumption since there is at present no theory that relates Γ to D in the presence of interparticle interactions.

Proceeding with this assumption and also the assumption that the *apparent* concentration dependence of D measured by IFS corresponds to the concentration dependence of D measured across *macroscopic* concentration boundaries, this dependence can be ascribed to two factors: (a) the concentration dependence of the chemical potential gradient, and (b) that of the frictional coefficient (see, for example, Tanford, 1961; Herbert and Carlson, 1971; Pusey *et al.*, 1972). That is, if, for small c, $D(c) \cong kT(1 + 2BMc)/f(c)$ and $f(c) \cong f_0(1 + B'c)$ describe these two effects, then the apparent concentration dependence of D is such that $D(c) \cong kT[1 + (2BM - B')c]/f_0$. Since the coefficient B' can be evaluated from the concentration dependence of $s([s(c) \cong s^0(1 - B'c)])$, B, the thermodynamic second virial coefficient, can be evaluated by determining $D(c)$ and $s(c)$. Carrying out the kind of analysis shown above, using $s(c)$ for R17 determined by Enger *et al.* (1963), we have shown, by comparing B as a function of ionic strength, that probably the major contribution to the concentration dependence of D is from screened interparticle electrostatic interactions (Pusey *et al.*, 1972). Since this kind of analysis cannot be justified in the absence of a theoretical treatment for the light-scattering intensity fluctuations from interacting particles, we will not elaborate on these electrostatic interactions any further.[7] In the same vein, the effect on Γ of other interactions in multicomponent systems which affect the concentration dependence of D, such as interacting flows (Gosting, 1956), Donnan effects (see, for example, Tanford, 1961), and preferential interactions (Scatchard, 1946), cannot even be surmised in the absence of such a theory. What is perhaps important to point out is that in spite of our inability to understand in molecular terms the behavior of Γ in multicomponent systems of varying ionic composition, a value for D can be obtained which at least empirically can be described in terms of a *two-component* system with different interparticle interactions, and, at low concentrations, in terms of an "ideal" *two-component* system (Figures 1, 2, and 3).

The ionic dependence of Γ for PM2 (Figure 3) was measured for a range of ionic strengths corresponding to a large change in water activity (a_w from 0.98 to 0.80; Hearst, 1965). As the water activity decreases over this range the preferential hydration of nucleic acids has been shown to decrease by ~70% (Hearst, 1965; Cohen and Eisenberg, 1968). Since this preferential hydration is perhaps akin to our "surface" solvation (rather than that solvation due to passive solvent "trapping"), a fraction of which is probably external, it was considered possible, especially if the external layer of solvation was larger than we supposed (see above), that changes in a_w might be accompanied by significant changes in R_h and $D_{20,w}$ (*i.e.*, greater than the experimental uncertainty by ~1% or greater, and reflecting the loss of one (~3 Å) or more layers of water molecules). We did not observe such a change (Figure 3).

Over the range of pH we examined, R17 remained a rather compact and rigid structure (Figure 4). Until R17 was irreversibly denatured (40–45°), the effect of temperature on D could be accounted for by the Stokes–Einstein relationship (for exceptions in biological systems see Gosting, 1956). The denatured species could range from slightly denatured virions to viral components; in this regard we should point out that $D^0_{20,w}$ for R17 RNA can be calculated to be ~1×10^{-7} cm^2/sec (Figure 5).

In closing, although much work has been published on IFS of solutions of biological samples, to this date few data have been reported on the influence of the parameters investigated here. Thus, although variation of these solution parameters appear not to prevent us from extracting useful values for

[7] In referring to the proceedings of a light-scattering colloquium (Pusey *et al.*, 1972) where these calculations were presented, the interested reader should bear in mind that, although the conclusions presented are in no way affected, the constant we then loosely called the second virial coefficient is in fact 2BM.

$D^0_{20,w}$, for particles of the size investigated here, this might not be the case for smaller macromolecules, for which the solvent environment might be more important (Gosting, 1956).

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Appendix

We have calculated the uncertainty in M, ΔM, in the following manner. Since $M = f(s,D,\bar{v})$, the propagation of experimental errors can be determined from

$$\Delta M = \sqrt{\left(\frac{\partial M}{\partial s}\right)^2 (\Delta s)^2 + \left(\frac{\partial M}{\partial D}\right)^2 (\Delta D)^2 + \left(\frac{\partial M}{\partial \bar{v}}\right)^2 (\Delta \bar{v})^2} \quad (6)$$

where we have assumed that the individual errors are independent. By differentiating eq 2 and substituting into eq 6 we obtain

$$\Delta M = \sqrt{M^2\left[\left(\frac{\Delta s}{s}\right)^2 + \left(\frac{\Delta D}{D}\right)^2 + \left(\frac{\Delta \bar{v}}{1-\bar{v}}\right)^2\right]} \quad (7)$$

where we have assumed the solvent density is 1. In a similar manner the uncertainty in R_d, F, and δ can be determined from

$$\Delta R_d = \sqrt{\frac{R_d^2}{9}\left[\left(\frac{\Delta s}{s}\right)^2 + \left(\frac{\Delta D}{D}\right)^2 + \left(\frac{\Delta \bar{v}}{\bar{v}(1-\bar{v})}\right)^2\right]} \quad (8)$$

$$\Delta F = \sqrt{F^2\left[\left(\frac{2\Delta D}{D}\right)^2 + \left(\frac{\Delta s}{s}\right)^2 + \left(\frac{\Delta \bar{v}}{\bar{v}(1-\bar{v})}\right)^2\right]\left[\frac{R_d^3}{R_h^3 - R_d^3}\right]^2} \quad (9)$$

and, assuming $f/f_0 = 1$

$$\Delta\delta = \sqrt{\delta^2\left(\left[\left(\frac{2\Delta D}{D}\right)^2 + \left(\frac{\Delta s}{s}\right)^2\right]\left[\frac{\delta+\bar{v}}{\delta}\right]^2 + \left[\frac{\Delta \bar{v}}{1-\bar{v}}\right]^2\left[\frac{\delta+1}{\delta}\right]^2\right)} \quad (10)$$

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Conformation of Retinal Isomers†

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ABSTRACT: The solution conformations of the polyene chain portions of *all-trans*-retinal and 11-*cis*-retinal have been investigated by ^{1}H nuclear magnetic resonance (nmr) spectroscopy, including measurements of long-range nuclear spin coupling constants, chemical shifts, spin–lattice relaxation times, and nuclear Overhauser enhancements. Theoretical calculations of the minimum energy conformations and torsional potentials of these molecules are also presented. The results show that the polyene chain of *all-trans*-retinal has a planar conformation in solution with all of the single bonds from 7-C to 15-C in the s-trans conformation. The polyene chain of 11-*cis*-retinal is shown to be essentially planar in the regions 7-C to 10-C and 13-C to 15-C, but is twisted slightly from planarity around the 10–11 single bond and exists as an equilibrium between two low-energy conformers, distorted s-cis and distorted s-trans, about the 12–13 single bond. In acetone at low temperature the distorted s-trans conformation appears to be preferred.

The conjugated polyene aldehyde 11-*cis*-retinal is the chromophore of the visual pigment. It is isomerized to the 11-trans compound in the photochemical reaction of vision. Subsequent dark reactions, involving both the chromophore and the protein opsin to which it is attached through an imine linkage, result ultimately in a nervous impulse and the sensation of vision (Wald, 1968). The solution conformation of the retinal isomers (see Figure 1) is therefore of considerable interest for an understanding of the nature of the visual pigment. Two features of the conformation of the retinal isomers require particular attention: the orientation of the β-ionone ring relative to the polyene chain and the conformation of the polyene chain itself, especially with respect to the stereochemistry about the 10–11 and 12–13 single bonds in the 11-cis isomer. The torsional angle of the β-ionone ring in *all-trans*-[1] and 11-*cis*-retinal has been determined in the crystal by X-ray analysis (Hamanaka *et al.*, 1972; Gilardi *et al.*, 1972), measured in solution by use of nuclear magnetic resonance (nmr) spectroscopy (Honig *et al.*, 1971), and calculated theo-

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[1] The term "*all-trans*" is used in accord with the current convention for this isomer, which is predominantly distorted s-cis with respect to the 6–7 bond (Figure 1) (Honig *et al.*, 1971).