ents for polystyrene, and both gave results characteristic of non-solvents.[18] With acetic acid, the intrinsic viscosities of the polymers *increased* with the concentration of solvent. In polymerization at 60° at acetic acid:styrene ratios of 0, 0.5, 1.0 and 5.0, the polystyrenes obtained had intrinsic viscosities of 3.4, 4.2, 4.5 and 5.3. Analogous results were obtained at 100 and 132°. The polymers were not always reproducible and the rates were erratic with a tendency to be low. Separation of polystyrene from solution apparently permits some molecules to grow to unusual size. Carbon analyses of one purified polymer made at an acetic acid:styrene ratio of 1.01 showed only 91.8% carbon (theoretical, 92.28%) indicating incorporation of solvent in the polymers. Ethanol at low concentrations leads to a polymer of lower intrinsic viscosity, but above an ethanol–styrene ratio of about 5, further addition of ethanol gives polymers of *higher* intrinsic viscosity. Dichloroacetic acid combines a non-solvent effect with high transfer activity. As the acid–monomer ratio increases, the intrinsic viscosity of the polymer continues to decrease, but not as much as expected. Malonic, phenoxyacetic and benzoic acids also gave abnormal polymers, but experiments were too limited for further comment.

The behavior of paraformaldehyde was unique and unaccounted for. At concentrations of 0, 0.05 and 0.3% by weight, polystyrenes of intrinsic viscosities 3.4, 4.3 and 5.4 were obtained. The paraformaldehyde appeared to be insoluble in the monomer, and was recovered from the partially polymerized styrene. The high intrinsic viscosity of the polymer was unchanged by shaking a benzene solution with either water or mineral acid but was reduced from 6 to 2.8 by extracting a carbon tetrachloride solution of the polystyrene with water at approximately 40° for one week. Mixing or heating solutions of normal polystyrene with paraformaldehyde produced no increase in viscosity.

PASSAIC, N. J.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND VIRUS LABORATORY, UNIVERSITY OF CALIFORNIA]

Analysis of a Concentration Anomaly in the Ultracentrifugation of Mixtures[1]

BY W. F. HARRINGTON[2] AND H. K. SCHACHMAN

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Dilute solutions of two macromolecular components have been studied in the ultracentrifuge. The boundary area of the slow component of the mixture is found to increase as much as 300% over that of a comparable concentration of that same component alone. Further, the area due to the slow component, corrected for the geometry of the cell and the centrifugal field, decreased as much as 30% as the boundary moved through the cell. By comparison of the experimental results with a theoretical treatment based on the ideas of Johnston and Ogston, it is shown that the magnitude of these anomalies depends on the proximity of the sedimentation constants of the individual components. It is shown that a reasonable prediction of the magnitude of the anomaly can be made from knowledge of the physical properties of the fast component. The existence of convection during the ultracentrifugation of mixtures is discussed.

Introduction

In the course of a study in this Laboratory on the degradation of tobacco mosaic virus using the ultracentrifuge to follow the appearance and disappearance of decomposition products, it became clear that errors, not heretofore realized, can be made by analyzing the ultracentrifuge patterns in terms of concentrations in the conventional manner. It has been known for some time that, in contrast to the study of a single monodisperse protein, the determination of the concentrations of components of a mixture from ultracentrifugal patterns often yields erroneous values.[3–6] Specifically, the concentration of the slower component of a mixture, as obtained from analysis of the ultracentrifuge patterns, is too high, and the concentration of the faster component is too low. Since there has been some discussion[7–9] as to the explanation for this anomaly and because of the unprecedented magnitude of the effect observed in mixtures containing tobacco mosaic virus, detailed ultracentrifugal analyses were conducted with known mixtures. This communication presents the results of such studies and a theoretical interpretation of the results along the lines originally suggested by Johnston and Ogston.[6]

Materials and Methods

Several preparations of tobacco mosaic virus (TMV) and tomato bushy stunt virus (BSV) purified by a series of cycles of alternate high and low-speed centrifugation according to the method of Stanley[10] were used in this study. Bovine plasma albumin (ALB) and β-lactoglobulin (β-lact) obtained from Armour and Company were used in mixture studies with bovine plasma fibrinogen (FIB), also obtained from Armour and Company (fraction I). Some experiments were conducted with a degradation product (DP) of tobacco mosaic virus obtained by treating the virus with sodium dodecyl sulfate.[11] Electron micrographs showed the DP particles to be the same diameter as TMV with lengths about $^3/_4$ those of the virus particles. Ultracentrifuge patterns of this preparation showed a single sharp boundary.

Sedimentation analyses were performed in a Spinco Model E ultracentrifuge equipped with a Philpot–Svensson optical system.[12–14] Centrifuge runs were made at room temperature and sedimentation constants were corrected to a stand-

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(2) Presented in partial fulfillment for the Ph.D. degree in Biochemistry at the University of California, 1952.

(3) A. S. McFarlane, *Biochem. J.*, **29**, 407, 660 (1935).

(4) K. O. Pedersen, *Nature*, **138**, 363 (1936).

(5) K. O. Pedersen, *Compt. rend. Lab. Carlsberg*, **22**, 427 (1938).

(6) J. P. Johnston and A. G. Ogston, *Trans. Faraday Soc.*, **42**, 789 (1946).

(7) B. Enoksson, *Nature*, **161**, 934 (1948).

(8) K. O. Pedersen, *Ann. Rev. Biochem.*, **17**, 187 (1948).

(9) R. Cecil, J. P. Johnston and A. G. Ogston, *Nature*, **163**, 919 (1949).

(10) W. M. Stanley, THIS JOURNAL, **64**, 1804 (1942).

(11) H. K. Schachman, *ibid.*, **73**, 4808 (1951).

(12) J. Thovert, *Ann. Phys.*, **2**, 369 (1914).

(13) J. St. L. Philpot, *Nature*, **141**, 283 (1938).

(14) H. Svensson, *Kolloid Z.*, **87**, 181 (1939).

ard state corresponding to water at 20° by the method of Svedberg and Pedersen.[15] Since the temperature rise in most ultracentrifuge runs was less than 1°, the sedimentation constants were calculated from plots of $\log_{10} x$ *vs.* t, where x is the distance in cm. from the axis of rotation to the boundary and t is the time in seconds.

To obtain the concentration of a given material from the ultracentrifuge pattern, the photographs were enlarged from four to six times by projection upon white paper and the curves were traced according to the method of Cecil and Ogston.[16] Owing to diffraction at the edges of the bar in the plane of the image of the light source slit, the curves as photographed are somewhat indistinct and errors could occur if only the upper or lower outlines are used. Accordingly, areas from the upper and lower outlines were averaged in each case to correct for exposure errors. In many patterns the areas obtained from the upper and lower edges agreed to within a few per cent., although in some runs the area of the upper curve was about 35% greater than that of the lower. Even in the latter cases the average area was within 5% of the expected area for runs on one sample of TMV and one preparation of β-lactoglobulin kindly supplied by Dr. T. L. McMeekin. In all but a few analyses the boundaries were completely separated so that graphical resolution of the patterns was unnecessary.

True protein concentrations were assumed to be those calculated from Kjeldahl nitrogen determinations. The Markham[17] distillation apparatus was employed in conjunction with a Scholander[18] micro-buret for all nitrogen analyses.

Viscosity measurements were made in an Ostwald type viscometer specially designed to produce a low average shear gradient of about 300 sec.$^{-1}$.

Theory

Consider a cell with sector angle, ϕ (in degrees), and thickness, b (distance perpendicular to direction of sedimentation), filled with a solution of two sedimentable components. Let the initial concentration of slow component be c_S^0. The total mass of slow component between the meniscus in the cell, x_0, and some arbitrary level, x_F, before sedimentation starts, is

$$m_S^0 = c_S^0 \frac{\pi b\phi}{360} (x_F^2 - x_0^2) \qquad (1)$$

and the amount of slow component passing through the plane at x_F in time, dt, is

$$\mathrm{d}m = c_S^t \frac{\phi b}{360} 2\pi x_F \omega^2 x_F s_{S,mixt}\, \mathrm{d}t \qquad (2)$$

where ω is the angular velocity in radians per sec., c_S^t is the concentration of slow component at time, t, at the plane x_F, x values are measured from the axis of rotation and $s_{S,mixt}$ is the sedimentation constant of the slow component in the presence of the fast. During the time, t, the fast boundary moves up to x_F, the slow boundary to x_S, and the total amount of slow component passing across the x_F level is

$$M = \int_0^M \mathrm{d}m = 2\pi x_F^2 \frac{\phi b}{360} \omega^2 \int_0^t c_S^t s_{S,mixt}\, \mathrm{d}t \qquad (3)$$

If, for the present, we assume $s_{S,mixt}$ is constant we can substitute

$$c_S^t = c_S^0 e^{-2\omega^2 s_{S,mixt} t}$$

and integrate equation 3 to give

$$M = c_S^0 x_F^2 \frac{\pi b\phi}{360}\left[1 - e^{-2\omega^2 s_{S,mixt} t}\right] \qquad (4)$$

(15) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford University Press, New York, N. Y., 1940.

(16) R. Cecil and A. G. Ogston, *Biochem. J.*, **43**, 592 (1948).

(17) R. Markham, *ibid.*, **36**, 790 (1942).

(18) P. F. Scholander, *Science*, **95**, 177 (1942).

Subtracting equation 4 from equation 1 and rearranging gives m_S^t, the mass of slow component behind the x_F level after the time, t.[19]

$$m_S^t = c_S^0 \frac{\pi b\phi}{360} [x_F^2 e^{-2\omega^2 s_{S,mixt} t} - x_0^2] \qquad (5)$$

Since all of m_S^t is confined to the volume, $v_S^t = (\pi b\phi/360)(x_F^2 - x_S^2)$, between the two boundaries, we can write for the concentration, c_S^{obs}, of slow component observed at time, t

$$c_S^{obs} = c_S^0 \frac{x_F^2 e^{-2\omega^2 s_{S,mixt} t} - x_0^2}{x_F^2 - x_S^2} \qquad (6)$$

This equation can be rewritten in the following forms making use of the definition of the sedimentation constant, $x_t = x_0 e^{\omega^2 s t}$, to give

$$\frac{c_S^{obs}}{c_S^0} = \frac{e^{2\omega^2 t(s_F - s_{S,mixt})} - 1}{e^{2\omega^2 s_F t} - e^{2\omega^2 s_S t}} \qquad (7)$$

where s_F and s_S are the sedimentation constants of the fast and slow components, respectively.[20]

$$\frac{c_S^{obs}}{c_S^0} = e^{-2\omega^2 s_{S,mixt} t}\left[\frac{e^{2\omega^2 s_F t} - e^{2\omega^2 s_{S,mixt} t}}{e^{2\omega^2 s_F t} - e^{2\omega^2 s_S t}}\right] \qquad (7a)$$

If $\omega^2 st << 1$, we can substitute $e^x = 1 + x$ to give

$$\frac{c_S^{obs}}{c_S^0} = (1 - 2\omega^2 s_{S,mixt} t)\left[\frac{s_F - s_{S,mixt}}{s_F - s_S}\right] \qquad (7b)$$

At the beginning of the run when $t \to 0$, equation 7b reduces still further to equation 8 which is the result of Johnston and Ogston.[21]

$$\frac{c_S^{obs}}{c_S^0} = \frac{s_F - s_{S,mixt}}{s_F - s_S} \qquad (8)$$

It is important to note that the term in equations 7a and 7b which accounts for the dilution of the slow component during the run depends on $s_{S,mixt}$ which is less than s_S; *i.e.*, the slow component decreases with time less rapidly than would be expected on the basis of the treatment of Svedberg and Pedersen.[15] The time dependent term in equation 7a can be rewritten alternatively as

$$[(e^{-2\omega^2 s_S t})(e^{2\omega^2(s_S - s_{S,mixt})t})]$$

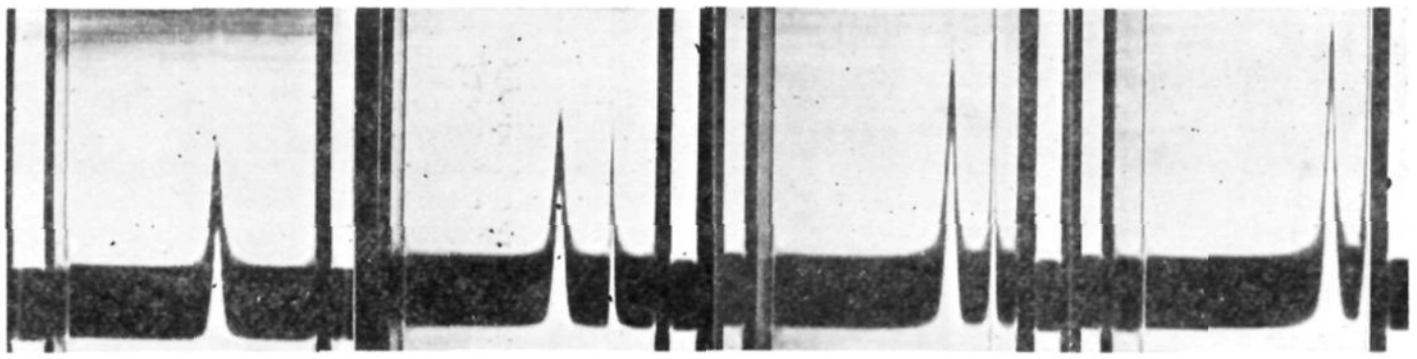
Therefore, if we correct the concentration of the slow component according to the square law of Svedberg and Pedersen, by $e^{2\omega^2 s_S t}$, the corrected concentrations should increase with time according to $e^{2\omega^2(s_S - s_{S,mixt})t}$. This increase, it should be noted, will be smaller than the usual dilution term

(19) This treatment is directly analogous to that of Svedberg and Pedersen[15] and our equation 5 is similar to their equation 192. We, however, are considering a plane defined by the position of the fast boundary whereas they considered a fixed plane such as the barrier in a separation cell.

(20) s_F is directly measurable from the optical pattern since sedimentation of the fast component produces a boundary. It should be noted that s_F really corresponds to the sedimentation constant of the fast component in the presence of the slow component and will generally be different from the sedimentation constant of the fast component when studied alone. Since the constant is directly measurable in the mixtures, we use the nomenclature s_F for the sake of simplicity rather than the perhaps more logical $s_{F,mixt}$.

$s_{S,mixt}$ will be less than s_S since sedimentation constants generally decrease with increasing concentration. Whereas s_S can be measured directly from the optical patterns, $s_{S,mixt}$ cannot be so measured since no boundary results from sedimentation of the slow component in the presence of the fast.

(21) This limitation amounts to considering $c_S^t = c_S^0$ which is equivalent to substituting a rectangular cell in a homogeneous field for a sector shaped cell in a centrifugal field. For this special case, equation 8 can be derived in a variety of ways.



Effect of TMV on area of BSV boundary in ultracentrifuge.

Fig. 1.—Representative ultracentrifuge diagrams for mixtures of BSV and TMV. Concentrations above are in g./100 cc. and all pictures were taken with the same magnification but at different times after reaching speed.

due to the sector shape of the cell and the inhomogeneity of the field since $(s_S - s_{S,mixt}) << s_S$. As will be seen later (Fig. 4) the concentration corrected in the conventional way does not increase during the run, but rather it decreases because of another effect (the change in the Johnston–Ogston term) which will be discussed later.

It must be emphasized that the treatment developed above does not take into account any change in the sedimentation constants of the components as they undergo radial dilution. For many macromolecules the dependence of sedimentation constant on concentration is sufficiently small so as to warrant integration of equation 3 with $s_{S,mixt}$ taken as a constant. In solutions containing asymmetric particles such as TMV we would expect a progressive increase in sedimentation rate as the particles move through the cell. Indeed, Lauffer[22] has shown for TMV that the sedimentation constant of a 1.3% solution of virus increased about 18% as the boundary moved from a position 5.83 to 7.11 cm. from the axis of rotation. For solutions containing such particles equation 4 would not obtain, since $s_{S,mixt} = f(c_S, c_F) = f'(t)$. In view of the lack of an adequate theory for this function, no attempt at integrating equation 3 with $s_{S,mixt}$ as a variable will be made.

If we were to assume that $s^t_{S,mixt} = s^0_{S,mixt}(1 + At)$ we would have an additional term in equation 4; this modification shows that the mass of slow component crossing the plane at x_F increases with time even faster than is calculated from equation 4. Pursuing this reasoning we find that c_S^{obs} would decrease with time. Equation 8 is valid for conditions at the beginning of the run when the boundaries are still very close to the meniscus. Under such circumstances there has been so little dilution of the components in the cell that we can integrate equation 3 assuming $s_{S,mixt}$ is a constant. Alternatively, the perturbing term needed to modify equation 3 for the lack of constancy of $s_{S,mixt}$ throughout the cell vanishes as time approaches zero. Thus a quantitative test of the above theory involves measurements of c_S^{obs} at different positions in the cell and extrapolation back to the meniscus to obtain the hypothetical value of the enhanced concentration at zero time.

Results and Discussion

Analysis of Known Mixtures.—Figure 1 shows representative ultracentrifuge patterns from four different analyses of BSV–TMV mixtures. In all runs the initial concentration of BSV (slow component) was 0.3% whereas the TMV (fast component) concentration was varied from 0 to 1%. Optical factors are identical for each pattern so that areas of the BSV boundary would have been the same had the anomaly discussed above not occurred. It is readily seen from Fig. 1 that the area of the BSV boundary in the mixtures containing TMV increases markedly as the TMV concentration is increased. Quantitative measurements of these patterns show that the area of the BSV boundary is increased more than 200% when the TMV concentration in the mixture is only 1%. This enhancement of concentration of the slow component is much greater than any reported by Johnston and Ogston, presumably due to the asymmetric nature of the fast component in our mixtures, as contrasted to the more symmetrical components employed by these workers.

No attempt was made to measure the area of the TMV boundary in the above experiments since the sharpness of the boundary precluded accurate area measurements. In mixtures of DP and TMV, however, the sharpness of the TMV boundary was much less than that for the TMV boundary alone[23] and area measurements could be made. Johnston and Ogston found that the total area of the patterns of mixtures was about equal to the expected area as judged by the patterns of the components when studied alone. This has been confirmed in studies of DP and TMV. Since the DP particles are highly asymmetric, an enhancement of their concentration behind the TMV boundary can be demonstrated in another way. Figure 2 shows how the sedimentation constant of DP decreases with concentration. Some of the points on this graph are from studies of DP alone, but included in the figure are data from mixtures in which the sedimentation constant was determined directly and the concentration was either the initial concentration or the concentration calculated from the boundary area. When the initial concentration is used, the points,

(22) M. A. Lauffer, THIS JOURNAL, **66**, 1195 (1944).

(23) For a single highly asymmetric material the particles at the trailing edge of the boundary are travelling in a medium of relatively low viscosity compared to the leading edge of the boundary where the concentration is much higher. Thus the trailing edge will be continually tending to overtake the leading edge, resulting in a boundary which is sharper than one would expect as a result of diffusion. In a mixture, however, the trailing edge will be in a region of higher concentration of slow component than will the leading edge. This will tend to reduce the viscosity change across the fast boundary, and the fast boundary will consequently be broader in mixtures.

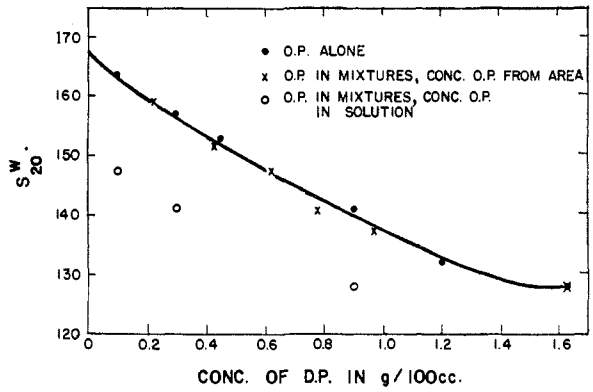


Fig. 2.—Sedimentation of DP alone and in mixtures with TMV: ●, DP alone; ×, DP in mixtures where DP concn. is obtained from area; O, DP in mixtures where DP concn. is initial concn. in solution.

as shown for only a few mixtures, fall to the left of the curve. However, if the data are plotted according to calculated concentration, all of them, as shown in the figure, fall on the curve. Since the sedimentation rate is a function of concentration, these results suggest that the concentration of the slow component is indeed that calculated from the area. It must be emphasized that this conclusion can be drawn for only the slow component since, in the region of its boundary, no other components are present and the sedimentation constant is, therefore, a function of its true concentration in that region. The results on mixtures of BSV and TMV, as summarized in Table II, also show the same effect since the sedimentation constant of the slow component was found to decrease from 133 to 126 *S* by the addition of fast component. The decrease in these experiments is much less than that shown in Fig. 2 presumably due to the spherical shape of BSV as compared to the rod-like shape of DP. Lauffer[24] found a much smaller decrease in sedimentation constant with concentration for BSV than that reported here. These results are not in conflict with one another, however, since the work reported here was at a much lower ionic strength where the primary charge effect[15] would cause an appreciable decrease in sedimentation rate at high concentrations of virus.

Viscosity Studies on the Mixtures.—According to Einstein[25] the viscosity of a dilute solution of spherical macromolecules is a function of the volume fraction of the solute so that in a dilute solution containing two different macromolecules, each component should contribute to the viscosity of the solution depending on its concentration and its viscosity increment. Treffers[26] and Bingham and Roepke[27] have provided experimental confirmation of the additivity of viscosity contributions in their studies of the fluidity of solutions of several macromolecular components.

Since the fast component in our studies possesses a high intrinsic viscosity, it seems likely that viscosity measurements on the mixtures should provide evidence of any dissociation of the fast component in the presence of slow component as originally suggested by Pedersen.[5] In view of this possibility, a large number of viscosity measurements were made on mixtures of TMV and BSV and TMV and DP as well as on the individual components of the mixtures at their appropriate concentrations. In all cases the specific viscosities of the mixtures were equal to the sum of the specific viscosities of the individual components at corresponding concentrations. That the viscosities did prove to be additive confirms the results of Johnston and Ogston with other materials that there is no justification in invoking dissociation as an explanation for the mixture anomaly obtained in the ultracentrifuge.[28]

Determination of the Sedimentation Constant of the Slow Component in the Presence of the Fast.—In order to predict the concentration of the slow component in a mixture according to equation 8, it is evident that $s_{S,mixt}$ must be known. Johnston and Ogston[6] approximated this value by assuming that the effect of the fast component on the rate of sedimentation of the slow "is the same as would be produced by the same concentration of (slow) alone." For the systems examined by these workers, this assumption seems reasonable since the components have similar *s vs. c* dependencies. Further, Cecil, Johnston and Ogston[9] claimed that the faster component sediments in a mixture at the same rate as it would travel through the same total concentration of itself alone, and they presented data to substantiate this claim. If, however, the mixture is composed of components of differing *s vs. c* dependence, the properties and concentration of each component must be considered. The results summarized in Table I, showing the variation in the sedimentation constant of the faster sedimenting component in different pairs of experiments, demonstrate the importance of considering the environment in which individual particles are sedimenting rather than just the total protein concentration.

TABLE I

SEDIMENTATION CONSTANT OF TMV IN TMV-BSV MIXTURES

TMV, concn., g./100 cc.	BSV, concn., g./100 cc.	Total concn., g./100 cc.	S^{w}_{20}
0.3	1.0	1.3	162
1.3	0.0	1.3	124
0.1	.9	1.0	177
1.0	.0	1.0	137

Many workers[22,29-35] have suggested on both theoretical and experimental grounds that the sedimentation constant, *s*, in dilute solutions can be

(24) M. A. Lauffer, *J. Phys. Chem.*, **44**, 1137 (1940).
(25) A. Einstein, *Ann. Physik*, **19**, 289 (1906); **34**, 591 (1911).
(26) H. P. Treffers, THIS JOURNAL, **62**, 1405 (1940).
(27) E. C. Bingham and R. R. Roepke, *ibid.*, **64**, 1204 (1942).
(28) That some dissociating systems may occur which would lead to this type of anomaly is, of course, not excluded by our work.
(29) W. O. Kermack, A. G. M'Kendrick and E. Ponder, *Proc. Roy. Soc. Edinburgh*, **49**, Part II, No. 15, 170 (1929).
(30) J. M. Burgers, *Proc. Acad. Sci. Amsterdam*, **44**, 1045, 1177 (1941); **45**, 9, 126 (1942).
(31) I. Jullander, *Arkiv Kemi, Mineral, Geol.*, **A21**, No. 8 (1945).
(32) G. Kegeles and F. J. Gutter, THIS JOURNAL, **73**, 3770 (1951).
(33) J. H. Fessler and A. G. Ogston, *Trans. Faraday Soc.*, **47**, 667 (1951).
(34) N. Gralen, "Sedimentation and Diffusion Measurements on Cellulose and Cellulose Derivatives," Inaugural Dissertation, Uppsala (1944).
(35) H. K. Schachman and W. J. Kauzmann, *J. Phys. Colloid Chem.*, **53**, 150 (1949).

expressed in terms of the sedimentation constant, s_0, at infinite dilution by an equation of the form

$$s = s_0/(1 + kc) \qquad (9)$$

where c is the concentration of the macromolecules and k is a constant which is related in a complicated way to the viscosity of the solution and the backward flow of solvent caused by the transport of material toward the bottom of the cell. In view of the results shown in Table I and other results which show that the k values are different for different macromolecules, it would seem that an expression for the sedimentation constant of the slow component in the presence of the fast must include k terms for each component. We may then write as an approximation

$$s_{S,mixt} = s_{S,0}/(1 + k_S c_{S,mixt} + k_F c_F) \qquad (10)$$

Thus the availability of data relating sedimentation constant to concentration for the two components permits the approximate calculation, by means of equations 10 and 8, of the buildup of the slow component in a mixture. It will be shown later how such calculated results compare with the observed concentration of the slow component.

The sedimentation constant of the slow component in the presence of the fast can also be obtained directly in the ultracentrifuge by the use of a synthetic boundary technique.[36] If a solution of fast component is carefully layered over a solution of a mixture of fast component, at the same concentration, and slow component, then a boundary due to the slow component (in the presence of the fast) is created in the middle of the cell. Measurement of the rate of movement of this boundary before the fast component overtakes it permits a determination of the sedimentation constant of the slow in the presence of the fast.[37] Preliminary experiments in the ultracentrifuge cell, though qualitatively successful, indicated that the boundary must be formed in a rotating cell, so as to exploit the stabilizing effect of the centrifugal field in layering the lighter solution over the heavier one and to eliminate the disturbances caused by handling the cell and the rotor after the boundary had been formed. In order to achieve this purpose a new type ultracentrifuge cell was used.[39,40]

(36) M. A. Lauffer and N. W. Taylor, *Arch. Biochem. Biophys.*, **37**, 457 (1952), have created boundaries in an ultracentrifuge cell by means of layering one solution over another solution of slightly higher density.

(37) Although it is not possible to obtain $s_{S,mixt}$ in an ultracentrifuge cell in the conventional manner, it should be possible, theoretically, to determine it through the use of the separation cell of Tiselius, Pedersen and Svedberg[15,38] whereby the sedimentation constant of a material can be determined from a comparison of the amount of substance left above the barrier after time, t, with that originally present (see equation 5). If the sedimentation of a mixture were confined to periods of time such that the fast boundary did not reach the barrier, then all of the slow component disappearing from the upper compartment travels in the presence of the fast component with the sedimentation constant, $s_{S,mixt}$. Analyses of the contents of the upper compartment for slow component would then yield the desired sedimentation constant. Preliminary experiments with this cell suggested that the barrier in the cell interferes with convection-free sedimentation, and in view of the development of the synthetic boundary cell, this approach was abandoned.

(38) A. Tiselius, K. O. Pedersen and T. Svedberg, *Nature*, **140**, 848 (1937).

(39) G. Kegeles, THIS JOURNAL, **74**, 5532 (1952).

(40) E. G. Pickels, W. F. Harrington and H. K. Schachman, *Proc. Nat. Acad. Sci.*, **38**, 943 (1952).

Figure 3 shows a plot of the data obtained in the new synthetic boundary cell for the sedimentation constant of BSV in the presence of TMV as a function of total concentration of material.[41] On the same graph are plotted the sedimentation constant of BSV *versus* concentration in solutions of BSV alone. The differences between the two curves is evidence showing the importance of considering the nature of the components in a mixture as well as the concentrations in predicting the sedimentation constant of either one of the components.

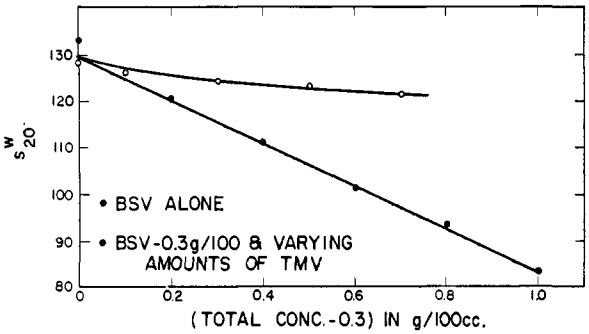


Fig. 3.—Sedimentation constant of different preparations of BSV as a function of concentration: ●, BSV (A) alone; ○, BSV (B) at 0.3 g./100 cc. and varying amounts of TMV.

Decrease in the Area of the Slow Boundary as it Moves Through the Cell.—In an earlier section it was pointed out that the radial dilution of the faster sedimenting component of a mixture would result in a progressive decrease in the slow component area during sedimentation if the sedimentation constant increases appreciably with dilution. That this effect is obtained can be seen from Fig. 4 where the areas of the slow boundary corrected for different optical factors, the shape of the cell, and the effect of the field are plotted against the position of the boundary in the cell. It should be noted that, in accord with theory and the work of others,[15] the corrected area for the BSV boundary in the run of that material alone is constant through the cell.

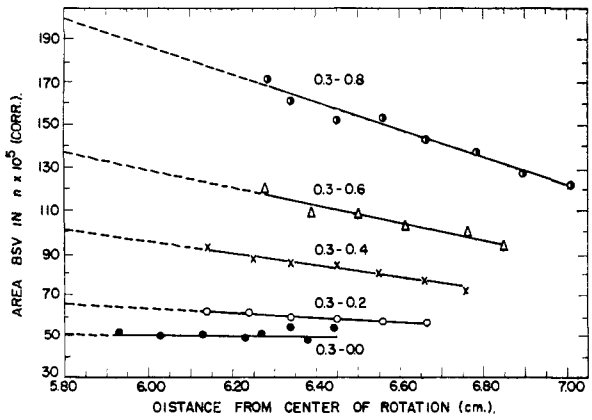


Fig. 4.—Decrease in area (multiplied by $e^{2\omega^2 s_S t}$) of slow component. Areas are in refractive index units.

(41) The measurements of $s_{S,mixt}$ are complicated by the differential sedimentation of TMV caused by the difference in sedimentation rate of the TMV in the presence and absence of BSV. Since the BSV concentration is low, this effect is probably very small.

Figure 4 emphasizes the striking changes in the area of the BSV boundary in the mixture and shows that the effect is greater the larger the concentration of TMV in the mixture.

In order to test equation 8 the areas plotted in Fig. 4 were extrapolated back to the meniscus to give hypothetical values for the area of the slow component in the various mixtures. These hypothetical values represent the areas that would have been observed if the boundaries had been resolved at the meniscus and measurements made. It should be noted that the extrapolated values may be in error for the mixtures containing large amounts of TMV where the extrapolation is especially hazardous. In these mixtures the sedimentation constants of the two components were so close that resolution of the boundaries sufficient to permit accurate area measurements did not occur until the boundaries had moved half the distance to the bottom of the cell. Table II presents the analysis of a series of mixtures of BSV and TMV. Column 8 gives buildup in concentration of the slow component as calculated from equation 8 using values for $s_{S,mixt}$ obtained directly in the synthetic boundary cell. Column 6 gives the same buildup calculated from equations 10 and 8, and column 9 gives the observed value of the buildup at the meniscus as obtained by extrapolation from the experimental data.

TABLE II

ANALYSIS OF MIXTURES OF BSV AND TMV

BSV concn., g./100 cc.	TMV, concn., g./100 cc.	s_S S	s_F S	$s_{S,mixt}$ S	c_S^{obs}/c_S^0 Calculated Eq. 10 + 8	Visc. + eq. 8	Eq. 8	Observed
0.3	0	133						
.3	0.2	132	172	120	1.3[41a]	1.2	1.2	1.3
.3	.4	132	162	111	1.7	1.6	1.7	2.1
.3	.6	129	152	101	2.1	2.0	2.2	2.8
.3	.8	128	144	93	3.0	2.7	3.2	4.1
.3	1.0	126	137	83	4.3	3.8	4.9	5.6

The agreement between the observed and calculated buildups shown in Table II must be considered satisfactory when we see that in equation 8 we are employing the small difference between large numbers. Any such calculation, to be reliable, requires extreme precision in the determination of each of the large numbers. In the systems which we have studied, errors of the order of 3% in either s_S or s_F will produce large changes in the denominator of equation 8. On the other hand, the same error in s_F or a corresponding error in $s_{S,mixt}$ will result in only a minor change in the numerator of the equation. If, for example, in the mixture of 0.3% BSV and 1.0% TMV the sedimentation constant of TMV were 133 *S* instead of 137 *S* the calculated buildup would be 7.1 instead of 4.9.

Since the sedimentation constant of TMV increases upon dilution, as determined from studies at different concentrations, we should expect an increase in the sedimentation constant of TMV during any one run in the ultracentrifuge. Indeed, as mentioned earlier, Lauffer[22] demonstrated that the sedimentation constant does increase and it seems reasonable to conclude that the sedimentation constants reported for TMV in Table II are average values for the sedimentation through the cell and correspond to lower concentrations than those originally present. Therefore, we should use a slightly lower value of s_F for calculations of the buildup at the meniscus. This would increase the calculated value of the buildup.[42]

(41a) Values of k_F in equation 10 were obtained from plots of $1/s$ *vs.* c for TMV alone.

Discussion

Enoksson[7] and Pedersen[8] have attributed the enhancement of concentration of slow component in mixtures to the sedimentation volume of the fast component. Removal of the fast component by sedimentation leads to a diminution of volume of solution and, hence, to an increase in concentration of slow component. Their equation

$$c_S^{obs} = c_S \frac{1}{1 - c_F\Phi_F} \qquad (11)$$

where Φ_F is the specific sedimentation volume of the fast component in cc./g., takes no cognizance of the relative sedimentation constants of the components in the mixture. To account for the buildup of slow component obtained in our experiments in terms of Enoksson's theory requires that we postulate a sedimentation volume of TMV as great as 90 cc./g. This amount seems wholly unreasonable since it is difficult to imagine the type of forces which could hold that much solvent to the virus particles.

In order to test equation 11, analyses were performed on mixtures of the same fast component with each of two slow components which differ in sedimentation constant. Table III shows the results of two sets of experiments using TMV as the fast component in one set of mixtures and FIB in the other. Even though the concentration of the fast component, and presumably the sedimentation volume, is the same in each set of experiments the observed buildup of the slow component is very different. These results show that the buildup cannot be accounted for by consideration of the sedimentation volume alone. On the other hand, the results are in accord with equation 8 which stresses the importance of the relative sedimentation constants of the components in the mixture.

TABLE III

EFFECT OF FAST COMPONENT ON DIFFERENT SLOW COMPONENTS

Mixture	Fast concn., g./100 cc.	s_F	Slow concn., g./100 cc.	s_S	c_S^{obs}/c_S^0
TMV-BSV	1.0	137	0.5	126	2.7
TMV-FIB	1.0	117	.5	8.1	1.0
FIB-ALB	2.0	5.4	.5	4.1	2.5
FIB-β-LACT	2.0	5.3	.5	2.7	1.5

As Cecil, Johnston and Ogston[9] have pointed out, their theoretical explanation for the concen-

(42) The progressive decrease in area through the cell for mixtures of TMV and BSV follows from equation 8 since s_F and $s_{S,mixt}$ would increase about the same amount as the boundaries move through the cell whereas s_S would hardly increase. Thus the numerator of equation 8 would be relatively constant and the denominator would increase. Further a slight increase in s_F as it moves through the cell will have a greater effect on the absolute value of $(s_F - s_S)$ the smaller the value of $(s_F - s_S)$. For this reason we should expect the decrease in area of the slow boundary with distance traveled to be greater at higher concentrations of TMV as shown in Fig. 4.

tration anomaly is not dependent on any particular assumption as to the cause of the sedimentation constant–concentration dependence. Though there seems to be no adequate theoretical explanation for the marked dependence observed for asymmetric particles like TMV, the viscosity contribution of the virus particles themselves is an important factor.[22,37] The results of the present investigations showing much larger effects with asymmetric molecules as the fast component as compared to the results obtained by earlier workers with more globular proteins suggests that the increase in viscosity caused by the fast component is a major factor in causing the buildup. If, as a first approximation, we were to assume that $s_{S,mixt}$ is less than s_S because of the viscosity contribution of the fast component in the mixture, we can calculate $s_{S,mixt}$ by dividing s_S by the relative viscosity derived from the fast component. Column 7 in Table II shows the buildup of the slow component calculated from equation 8 where $s_{S,mixt}$ was in turn calculated from the measured viscosities of the fast component.[43] Comparison of columns 7 and 9 show that a reasonable prediction of the buildup can be made in this way.

It should be noted that the decrease in area of the slow boundary through the cell, beyond that attributable to the geometry of the cell and the effect of the centrifugal field, implies the existence of convection in the region between the slow and fast boundaries. Since the buildup of slow component decreases throughout the run we should expect a negative density gradient due to the changing concentration of slow component immediately behind the fast boundary. This negative density gradient apparently would be greater than the positive gradient resulting from compression of the fluid in the cell. Such a negative density gradient would be unstable under the high centrifugal field and we would expect convection to occur. Since the optical patterns show two separate boundaries separated by a base line region (corresponding to a plateau of constant concentration) it would appear that convection does occur to eliminate the negative density gradient. Other examples of convective systems are being investigated in the synthetic boundary cell to study the effect of convection on the shape and movement of boundaries in the ultracentrifuge.[44]

(43) Theoretical considerations[31,32] indicate that s varies with c partly because of the backward flow of solvent caused by the sedimenting particles. We should, therefore, expect that the buildup of slow component should be due in part to backward flow. Preliminary measurements of backward flow caused by the sedimentation of TMV indicate that the viscosity effect is a more important factor in the mixtures reported on in this paper.

(44) H. K. Schachman and W. F. Harrington (unpublished).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE POLYTECHNIC INSTITUTE OF BROOKLYN]

Studies on Ion Exchange Resins. VIII. Activity Coefficients of Diffusible Ions in Various Cation-exchange Resins

BY HARRY P. GREGOR AND MELVIN H. GOTTLIEB

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Mean ionic activity coefficients of several electrolytes in divinylbenzene–polystyrenesulfonic acid resins of different degrees of cross-linking and in equilibrium with various concentrations of electrolytic solutions were determined. With concentrated external equilibrating solutions, activity coefficients in the resin phase approached those for the solution phase Activity coefficients for all electrolytes decrease sharply with increasing dilution.

The mean activity coefficient of a diffusible electrolyte in the resin phase is an important parameter in ion-exchange reactions.[1] A previous paper in this series[2] described phase equilibria for the system: sulfonated polystyrene–divinylbenzene cation-exchange resin–aqueous electrolytic solution containing a single uni-univalent electrolyte. The resin composition varied from 2 to 17% divinylbenzene (DVB) and non-exchange electrolyte was determined for various concentrations of ammonium chloride. The mean activity coefficient of ammonium chloride in the resin phase was calculated from Gibbs–Donnan considerations, neglecting the pressure–volume term. This paper describes phase equilibria of hydrochloric acid, lithium chloride, potassium chloride, potassium acetate, potassium sulfate and ammonium chloride solutions with resins having a DVB content ranging from 0.4 to 26%. Activity coefficients for these salts in the resin phase are calculated.

(1) H. P. Gregor, THIS JOURNAL, **73**, 642 (1951).

(2) H. P. Gregor, F. Gutoff and J. I. Bregman, *J. Coll. Sci.*, **6**, 245 (1951).

Experimental

A series of sulfonated polystyrene–divinylbenzene cation-exchange resins were used; the manner of their preparation and conditioning is described in a previous paper.[2] Unless specified, the resins are not identical with those previously described. Wet weights were determined by centrifugation, non-exchange electrolyte by elution.

All of the solutions used and calculations made are on a molal (m) basis. The temperature is 24-26°. Data are expressed in the following terms: We, specific wet weight of the centrifuged resin; N.E., specific quantity in millimoles of non-exchange or diffusible electrolyte in the resin phase; m_+ and m_-, molalities of cations and movable anions in the resin phase; $\gamma^i_{\pm}$, mean activity coefficient of the electrolyte in the resin phase, where

$$\gamma^i_{\pm} = \left[\frac{\gamma^{o\nu}_{\pm}\ \nu_+^{\nu_+}\ \nu_-^{\nu_-}\ m^{\nu}}{m_+^{\nu_+}\ m_-^{\nu_-}}\right]^{\frac{1}{\nu}}$$

the superscripts i and o refer to resin and solution phases, respectively; ν_+ and ν_- are the number of cations and anions, respectively, formed on dissociation of a molecule of the neutral salt ($\nu_+ + \nu_- = \nu$). All data thus refer to a standard resin sample, namely, one gram of dry hydrogen resin.

A tabulation of the experimental values of the external molality (m), We and N.E. for resins DVB 0.4, 2, 10 and