Differential Sedimentation Coefficients. I. Precise Measurement. Determination of Concentration Dependence for IgG-Immunoglobulin*

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ABSTRACT: In this communication are discussed the techniques we have employed to measure changes in sedimentation coefficients, δs, to within ± 0.016 S (1 std dev). Paired experiments are performed using two cells (with positive and negative wedge windows) in the same rotor. Thus fluctuations in temperature, rotor speed, optical alignment and magnification, and rotor precession are minimized.

The data are processed to automatically compensate for the change in concentration dependence with radial dilution and are then plotted to yield directly the change in sedimentation coefficient. Resulting values are corrected to the viscosity and density of water at 20°. From a series of such experiments at different concentrations the quantity $\delta s^0_{20,w}$ is finally obtained. In order to assess the precision of this technique, a series of experiments were performed using rabbit IgG-immunoglobulin at a variety of concentrations to obtain a precise value for the concentration dependence of the sedimentation coefficient.

Sedimentation coefficients may be measured during a single experiment with a precision considerably greater than $\pm 1\%$ (Kegeles and Gutter, 1951). The accuracy which is obtained between separate experiments, however, is about 1%. By repeated measurements on the same film, we find that only one-tenth of this error can be attributed to inaccuracies in measuring boundary positions. Most of the error must be caused by such variables as thermal gradients within the rotor and inability to regulate and measure the temperature of the ultracentrifuge cell with precision. Other factors may also contribute, such as fluctuations in rotor speed, precession of the rotor, optical misalignment, and, perhaps, changes in line-current frequency.

Regardless of the source of these variations, however, they may be almost entirely eliminated by the technique of differential sedimentation. We have employed the simple expedient of using two cells in the same rotor, with positive and negative wedge windows, and placing in one cell a reference sample of protein. The schlieren optical system has been used to record boundary positions. The difference in the sedimentation coefficients between the two samples is measured directly. Reproducibility seems limited only by the ability to locate the center of the schlieren peak, which we are able to do within $\pm 4\ \mu$ on the film when the protein concentrations are equal to or greater than 4 mg/ml. The results are sufficiently good so that it is desirable to take into account the change in the concentration dependence of the sedimentation coefficient as it occurs while the run is in progress. For this purpose, we propose a special method of plotting the data. As an initial test, we have used IgG-immunoglobulin[1] in both cells at the same concentration. In six experiments, the measured differences were +0.001, −0.003, −0.005, +0.011, −0.013, and +0.014 S.

Differential sedimentation experiments may have been first proposed by Richards and Schachman (1957, 1959) who investigated the suitability of using interferometric techniques for the measurement of small differences in s. Later Lamers *et al.* (1963) and Schachman (1963) showed that the scanning absorption system could be successfully employed for these measurements. Recently, Gerhardt and Schachman (1968) have used the schlieren optical system with excellent results to measure a 0.4S difference in sedimentation coefficient of aspartic transcarbamylase in the presence and absence of the ligands succinate and carbamyl phosphate. Their beautiful paper clearly demonstrates the power of the differential technique.

In this communication, we have applied differential sedimentation techniques to determine the dependence of the sedimentation coefficient upon concentration for rabbit IgG-immunoglobulin. We describe in detail the precautions taken in performing the experiments and our methods of plate reading and data analysis which improve the precision by about a factor of 5 over that readily obtainable in separate runs.

Computations

First, we shall describe how the data from paired runs may be processed and plotted to yield values of δs corrected for radial dilution. The differential equa-

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[1] Fleischman (1966).

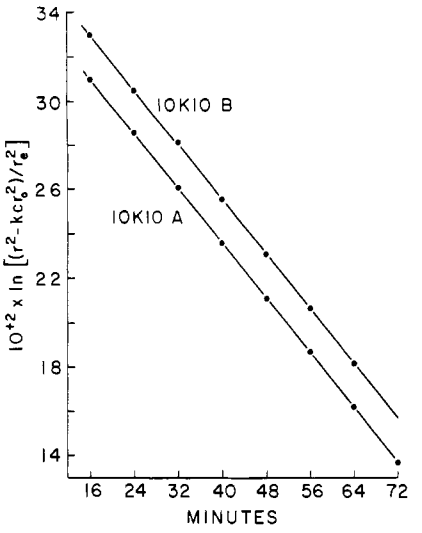

FIGURE 1: Two plots of $\ln ((r^2 - kc_0r_0^2)/r_e^2))$ *vs.* t for runs 10K10 A and B. This method of plotting the data is suggested in the text, except for the division by r_e^2. The latter quantity is a constant and equal to the square of the distance from the axis of rotation to the far reference edge (hole drilled in rotor). Since it is a constant, the inclusion of r_e^2 does not affect the slope of the line nor the value computed for the sedimentation coefficient. It greatly speeds up the computation of the logarithmic term on our computer, however, since it makes the ratio close to one. The run conditions are 60,000 rpm, 20.0°, 0.15 M KCl–0.01 M phosphate buffer (pH 6.5); 4 mg/ml of IgG-immunoglobulin in each cell.

tion relating radial distance, r, the time, t, the rotor speed, ω, the sedimentation coefficient at infinite dilution (superscript 0) at temperature t in solution (subscript s), $s^0_{t,s}$ the concentration, c, and the concentration dependence parameter, k, may be written[2] as

$$\frac{d \ln r}{dt} = \omega^2 s^0_{t,s} (1 - kc) \qquad (1)$$

According to the square law of radial dilution, the concentration may be related to the initial *boundary position*, r_0, and initial concentration, c_0

$$\frac{c}{c_0} = \left[\frac{r_0}{r}\right]^2 \qquad (2)$$

Substituting eq 2 into eq 1 and integrating yields

$$\ln \left[\frac{r^2 - kc_0r_0^2}{r_0^2 - kc_0r_0^2}\right] = 2\omega^2 s^0_{t,s} t \qquad (3)$$

Therefore, a plot of $\ln (r^2 - kc_0r_0^2)$ as a function of time, t, will give a straight line with a slope of $2\omega^2 s^0_{t,s}$ (see Figure 1).

(In Figure 1 is actually plotted log $((r^2 - kc_0r_0^2)/r_e^2)$, where r_e is the distance to the reference edge. Dividing by this constant does not change the slope, but it increases the accuracy of calculating the logarithmic term by our desk-top computer.)

For practical purposes, a value of k may be used in eq 3 that is not accurately known; however, even if approximately correct, the lines will be quite straight, and a least-squares treatment may be employed to yield additional precision. Still, the value of s^0 obtained by eq 3 is slightly in error if k is not known with precision. This error may now be almost eliminated by recalculating the sedimentation coefficient at the initial concentration, c_0, of the experiment by use of the expression $s^{c_0} = s^0(1 - kc_0)$. This recalculated value of the sedimentation coefficient will now be almost independent of the value chosen for k.

We wish to emphasize that eq 3 will yield the wrong value of $s^0_{t,s}$ if a bad guess is made for k. In spite of this, the error in s^{c_0} is almost canceled out because the same k (possibly erroneous) is used twice, once in eq 3 and once in the expression $s^{c_0} = s^0(1 - kc_0)$.

[2] Equations 1 and 2 are rigorously correct in the absence of convection only if values of $\bar{r}$, the second moment of the concentration gradient curve, are employed. For our purposes, however, peak maxima appear to obey these equations well enough.

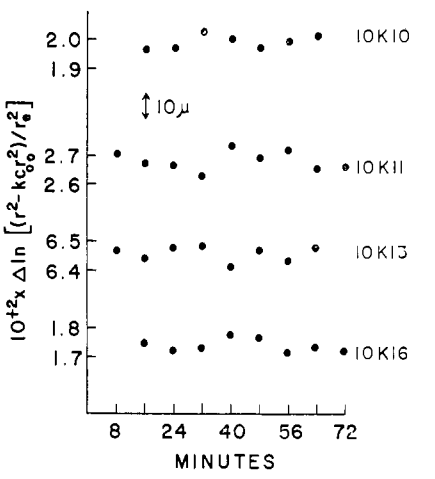

FIGURE 2: Difference plots for four of the experiments described in Table I. The slope of the least-squares line through the points yields values of $\delta s^0_{t,s}$ directly. These values are then converted into values of $\delta s^0_{20,w}$ by eq 5, and finally to $\delta s^{c_1,c_2}_{20,w}$ by eq 6. The latter quantities are used to construct Figure 4. The short arrow drawn in Figure 2 above shows the variation in location of a point which would be caused by an error of 10 μ in the distance between the schlieren peaks on the photographic plate.

The Difference Plot. So far we have discussed determination of the absolute sedimentation coefficient. In paired experiments, however, additional accuracy may be obtained by plotting a difference term, a procedure which minimizes errors due to fluctuations in temperature, rotor speed, etc. Denoting the sedimenting boundaries in the two cells by the subscripts 1 and 2, the difference term to be plotted is the quantity $\ln (r_1^2 - k_1c_{0,1} \cdot r_{0,1}^2) - \ln (r_2^2 - k_2c_{0,2}r_{0,2}^2)$. This quantity is plotted as a function of time, and from the slope is obtained directly $2\omega^2\delta s^0_{t,s}$ (see Figure 2).

Calculation of $\delta s^0_{20,w}$ *from* $\delta s^0_{t,s}$. Values of $\delta s^0_{t,s}$ computed from the difference plot will be erroneous unless the correct values of k are used in the logarithmic terms. But the best estimate of k depends upon the slope of a δs *vs.* c line. In order to make such a plot, the values of $\delta s^0_{t,s}$ are first corrected to values of $\delta s^0_{20,w}$. If we define the quantity F

$$F = \frac{(1 - \bar{v}\rho^0_{t,s})\eta^0_{20,w}}{(1 - \bar{v}\rho^0_{20,w})\eta^0_{t,s}} \qquad (4)$$

where $\bar{v}$, ρ, and η are the partial specific volume of the macromolecule and the density and the viscosity of the solvent, then the desired correction is

$$\delta s^0_{20,w} = \frac{\delta s^0_{i,s} + (F_2 - F_1)(s^0_{20,w})_2}{F_1} \quad (5)$$

where F_1 and F_2 are values of F for the two solutions used in the paired run. (When the same protein and same solvent are used in both cells, $F_2 - F_1 = 0$, and eq 5 simplifies.)

Computation of $\delta s^{c_1,c_2}_{20,w}$ from $\delta s^0_{20,w}$. To correct back to the value of δs at the concentrations actually employed during the experiment, the proper equation is

$$\delta s^{c_1,c_2}_{20,w} = \delta s^0_{20,w} - k_1 c_{0,1}(s^0_{20,w})_1 + k_2 c_{0,2}(s^0_{20,w})_2 \quad (6)$$

Here again, the error in $\delta s^{c_1,c_2}_{20,w}$ is almost canceled out because the same k's (possibly erroneous) are used twice.

Determination of k. The values of $\delta s^{c_1,c_2}_{20,w}$ which are calculated by eq 6 may then be plotted as a function of concentration to yield a better estimate of k. In a series of paired runs, the concentration of one solution, say 1, is kept constant while that of the other is varied. Therefore, k_2 is obtained from a plot of $\delta s^{c_1,c_2}_{20,w}$ *vs.* c_2.[3]

Materials and Methods

Temperature Control during Ultracentrifugation. A Spinco–Beckman Model E analytical ultracentrifuge equipped with a rotor temperature indicator control system was employed for all experiments. Certain routine checks were found to be essential since a defective thermister can become sensitive to large changes in air pressure, and a vacuum leak into the thermister assembly is then equivalent to a change in temperature of $+1°$. To ensure a good seal, the "O ring" inside the bottom plate of the rotor was occasionally regreased, and the bottom plate was screwed tightly in place. In order to routinely check for the absence of this problem, the indicator reading was observed for the first few minutes after starting the vacuum pump. A large change immediately occurs if even a small leak is present.

The cup of mercury in the bottom of the vacuum chamber is another source of constant trouble. A seriously misleading error is caused by a thin film of silicone oil getting beneath the mercury and partially insulating the cup. Cleaning the cup and adding fresh mercury eliminates the trouble. It is essential to check this system at the beginning of each run, however. This is readily accomplished by seeing that the thermister reading is identical either with the cup or with the external jack. To do this easily, the rotor is first suspended in the vacuum chamber, the thermister needle is inverted, and then, holding the jack assembly in place by hand at the bottom of the rotor, the indicator is read. The chamber is then closed and the indicator read again. Since the rotor has not been touched by hand, and since the readings are taken only a few seconds apart, they will be identical if the system is functioning properly. Of course, the chamber must be reopened and the needle reinverted before starting the run.

Rotor Speed during Ultracentrifugation. Rotor speeds were determined by timing the odometer with an electric stop watch for the duration of the run and calculating the average speed. An error in the size of ± 0.03 S easily can be caused in the absolute sedimentation coefficient if the speed control mechanism is not functioning properly, unless the average value of ω is employed. The value of δs, however, is not much affected.

Cell Balance and Alignment during Ultracentrifugation. It is possible that some small errors may be caused by imbalance of the two cells in the rotor. Although the manufacturer states that they may be allowed to be out of balance as much as 0.5 g, we routinely filled the cells to within 3 mg. But in one experiment in which we deliberately applied 0.5-g imbalance, results of excellent precision were still obtained. Cell alignment was also performed as accurately as possible with an aligning tool, and deliberate misalignment of one cell by 8° caused a significant change of -0.035 S in δs. A small misalignment of 2° did not affect precision.

Method of Reading Centrifuge Plates. The Nikon two-dimensional microcomparitor was employed to measure distances on the centrifuge plates. After reading the far reference edge (image of knife edge in the side hole of the rotor), the peak positions for each boundary in the superimposed images were recorded. The plate was not shifted on the stage between reading the two peaks, which greatly minimizes errors in δs due to alignment of the plate on the stage, location of this rather fuzzy reference edge, etc. Each peak was read four times in succession, backing off the micrometer screw one-half turn between readings. (Only the last two or three digits are recorded when repeating.) From an examination of sets of data of four readings each, we estimate that the mean location of the peak has a standard deviation of 4 μ on the film. Somewhat poorer values may be obtained late in the run, or at the lower concentrations employed. (It is important to guard against the natural tendency to pick a minor flaw in the emulsion when repeating the measurements.) Kegeles and Gutter (1951) have suggested that the cross formed by the interference fringes flanking the peak be used to indicate the peak center. We prefer to split the peak visually, focusing the eye on the small dark area immediately below the maximum ordinate. This area is elliptical in shape, and can be bisected visually with the indicated precision. A clean optical system greatly facilitates precise measurement.

We have used an Olivetti-Underwood desk-top computer (Programma 101, Olivetti-Underwood Corp., New York, N. Y.) to average the four readings and compute ln $((r^2 - c_0 r_0^2)/r_e^2)$ in one operation. (The division by r_e^2, which is the square of the reference edge location, does not affect the slope of the line, but makes the numbers close to one which speeds up our computer logarithm computation.) Then the difference between two such quantities is determined by subtraction for each pair of readings. The result is plotted

[3] The treatment which we have developed in this communication is for a linear concentration dependence. An analogous treatment may be derived from the integrated form of the equation for the linear concentration dependence of $1/s$.

TABLE I

Run	Source	Cell Window (deg)	Concn (mg/ml)	$s^c_{20,w}$ [a,b]	$s^0_{20,w}$	$\delta s^{c_1,c_2}_{20,w}$ [a]
10K6	Preparation I	+2	4	6.762	6.88	−0.103 ± 0.015
10K6	Preparation I	−1	8	6.663	6.90	
10K7	Preparation I	+2	1	6.771	6.80	
10K7	Preparation I	−1	2	6.751	6.81	
10K10	Preparation I	+2	4	6.595 ± 0.009	6.710	+0.011 ± 0.013
10K10	Preparation I	−1	4	6.599 ± 0.021	6.714	
10K11	Preparation I	+2	4	6.591 ± 0.015	6.706	−0.003 ± 0.013
10K11	Preparation I	−1	4	6.595 ± 0.018	6.710	
10K12[c]	Pentex	+2	4	6.554 ± 0.007	6.669	−0.035 ± 0.011
10K12	Pentex	−1	4	6.601 ± 0.011	6.717	
10K13[d]	Pentex	0	4	6.487 ± 0.016	6.601	+0.001 ± 0.012
10K13	Pentex	−1	4	6.505 ± 0.019	6.619	
10K16[e]	Pentex	+2	4	6.525 ± 0.011	6.639	−0.005 ± 0.010
10K16	Pentex	−1	4	6.529 ± 0.021	6.643	
10K19	Pentex	+2	8	6.400 ± 0.006	6.628	−0.104 ± 0.020
10K19	Pentex	−1	4	6.531 ± 0.019	6.645	
10K20	Pentex	+2	4	6.559 ± 0.008	6.674	−0.013 ± 0.005
10K20	Pentex	−1	4	5.529 ± 0.017	6.643	
10K21	Pentex	+2	2	6.657 ± 0.028	6.715	+0.070 ± 0.008
10K21	Pentex	−1	4	6.617 ± 0.012	6.733	
10K23	Pentex	+2	1	6.724 ± 0.015	6.753	+0.100 ± 0.015
10K23	Pentex	−1	4	6.607 ± 0.015	6.723	
10K30	Preparation II	+2	8	6.559 ± 0.007	6.793	−0.116 ± 0.013
10K30	Preparation II	−1	4	6.676 ± 0.019	6.793	
10K31	Preparation II	+2	4	6.775 ± 0.010	6.894	+0.014 ± 0.011
10K31	Preparation II	−1	4	6.762 ± 0.010	6.881	
10K32	Preparation II	+2	2	6.761 ± 0.021	6.820	+0.087 ± 0.009
10K32	Preparation II	−1	4	6.668 ± 0.022	6.785	
10K33	Preparation II	+2	1	6.769 ± 0.032	6.798	+0.108 ± 0.034
10K33	Preparation II	−1	4	6.703 ± 0.011	6.820	

[a] The plus and minus variations listed in this table represent 67% confidence limits on the slopes of the least-square lines through the experimental data. [b] Values of $s^c_{20,w}$ are measured with the indicated precision. They are not accurate beyond the first decimal place, however. [c] 8° misalignment. [d] 0.5 g heavier. [e] 2° misalignment.

as a function of time. (We strongly recommend plotting at this point, since it gives an excellent "feel" for the preciseness of the experimental data and allows obviously erroneous points to be rechecked.) Next, the least-squares line is computed and the difference in the sedimentation coefficient determined. Finally, an estimate of the standard deviation of the slope of the least-squares line (Dixon and Massey, 1951) is computed, and converted into a value of $\delta s^0_{l,s}$.

IgG-Immunoglobulins. These were prepared from rabbit blood by the method of Kekwick (1940), or purchased from Pentex Inc., Kankakee, Ill. Prior to centrifugation, the preparation was dialyzed overnight against several changes of 0.15 M KCl–0.01 M phosphate (pH 6.5). Concentrations were determined by ultraviolet absorption at 280 mμ using an extinction coefficient of 1.36 (Small and Lamm, 1966).

Condition of Runs. In all experiments charcoal-filled-Epon centerpieces were used in an anodized-black rotor at 60,000 rpm. A 1° negative and a 2° positive wedge were used to separate the patterns, and the cells were filled to within 3-mg constant weight. Menisci were separated by about 2 mm on the film, but this did not affect the results or computations. The temperature of the rotor was initially lowered to about 18° prior to insertion of the cell and screwing the rotor onto the drive shaft. The RTIC was set to regulate at 20° while pumping vacuum and during acceleration, but upon reaching 60,000 rpm it was readjusted to control the rotor at its current temperature (usually within a few tenths of a degree of 20°). For IgG-immunoglobulin eight to ten pictures were taken at 8-min intervals after a readable peak had developed.

Results

In Table I are given the results of 30 ultracentrifuge runs (15 paired runs) on 3 different preparations of IgG.

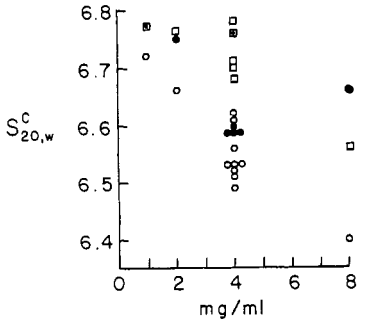

FIGURE 3: A plot of $s^c_{20,w}$ *vs.* c for all of the runs described in Table I except 10K12, which was deliberately misaligned. The symbols employed represent the different preparations of IgG-immunoglobulins: (○) Pentex, (●) our preparation I, and (□) our preparation II. These runs were all made at 60,000 rpm at temperatures close to 20.0° and in 0.15 M KCl–0.01 M phosphate (pH 6.5).

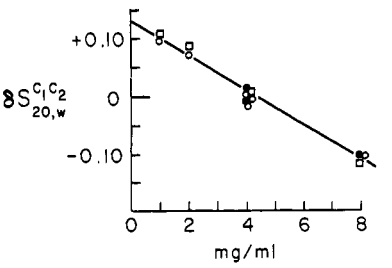

FIGURE 4: A plot of $\delta s^{c_1,c_2}_{20,w}$ *vs.* c_2 for all of the runs described in Table I except 10K12 which was deliberately misaligned, and 10K7 for which a 4-mg/ml control was not employed. In each case, the reference protein was taken as the sample, 1, in the cell with the 1° negative wedge window which was kept at a uniform concentration of 4 mg/ml. The difference, $\delta s^{c_1,c_2}_{20,w}$, between this sample and the other sample, 2, in the cell with the 20° positive wedge, was computed and plotted directly as a function of concentration, c_2. These data are the same as those plotted in Figure 3, and the symbols have the same meaning as described in the preceding legend. The slope of the least-squares line through the experimental points yields the equation $s^c_{20,w} = s^0_{20,w} - (0.030 \pm 0.001)c$, where c is concentration in milligrams per milliliter of rabbit IgG-immunoglobulin.

These data were computed from plots of ln $((r^2 - kc_0r_0^2)/r_e)$ *vs.* time and of ln $((r^2 - kc_{0,2}r_{0,2}^2)/r_e)$ − ln $((r_1^2 - kc_{0,1}r_{0,1}^2)/r_e)$ *vs.* time as shown in Figures 1 and 2. Such plots yield values of $s^0_{t,s}$ and $\delta s^0_{t,s}$ directly, and these are then converted into $s^c_{20,w}$ and $\delta s^{c_1,c_2}_{20,w}$ as previously described.

From an examination of the data in Table I, it can be seen that the values of $s^0_{20,w}$ are not in good agreement, ranging from 6.60 to 6.90 S.

The values of $s^c_{20,w}$ listed in Table I were recalculated by the expression $s^c_{20,w} = s^0_{20,w}(1 - kc)$. These values are plotted in Figure 3. The data in Figure 3 are badly scattered. At first, we thought this was partially caused by intrinsic differences in the globulin preparations studied. Later, we discovered the sensitivity of the thermister to air pressure, and we now realize the gross variation was due to inaccuracies in the recorded temperature as well as variation in thermal gradients between the cells and the thermister.

A far superior method of obtaining an accurate value for k employs the slope of a plot of $\delta s^{c_1,c_2}_{20,w}$ *vs.* c_2. Such a plot is presented in Figure 4. The slope of the least-squares line through the experimental points is 0.030 ± 0.001 S/mg per ml. Note that the data from the same runs were used for both Figures 3 and 4.

Discussion

In order that the method for the determination of differential sedimentation coefficients be reliable, it is essential that negligible difference be found when the same solution is studied in both cells. Averaging the values obtained for all of the experiments at 4 mg/ml (except for run 10K12 where an 8° twist was deliberately given one cell) we find that the mean value of $\delta s^0_{20,w} = +0.001$ S. The standard deviation of this mean value is ±0.0046 S; therefore, the deviation of the mean from zero is not significant. We conclude that any consistent difference attributable to the choice of cells and position in the rotor is negligible. The estimate of the standard deviation of the population which is calculated from the results of these six pairs is ±0.010 S.

The standard deviation in δs can be largely attributed to the error in locating peak center by eye on the enlarged image on the microcomparitor screen. As mentioned above, this can be done to only about ±4 μ for a "good" peak. The scatter of the points on the difference plots, such as Figure 2, are almost entirely due to the difficulty of reading the peak positions, as can be seen from the short line drawn to show how much a point is shifted by a 10-μ error. For each least-squares line, we have computed the standard deviation in the slope, due to the scattering of the points, and these are listed in the last column of Table I. The square root of the average value of the sum of the squares of these deviations is ±0.016 S. This value is the one we state to be the precision by which δs may be measured with this technique. We conclude that the principle source of error of this technique is the estimation by eye of the peak center position.

The computation of the slope of the concentration dependence line as shown in Figure 4 can be performed with considerable accuracy. For rabbit IgG-immunoglobulin, we find the expression $s^c_{20,w} = s^0_{20,w} - (0.030 \pm 0.001)c$, where concentration units are milligrams per milliliter.

Finally, we suggest that while this highly accurate technique only provides a direct measurement of the differences in sedimentation coefficients, absolute values could be obtained if a reference protein were used for which an absolute value were known with great precision. For best accuracy of the absolute sedimentation coefficient, the s of the standard should be as close to that of the unknown as possible. It is also desirable to prepare solutions for differential runs by dialyzing them in separate bags immersed in the same container of buffer to avoid a slight mismatch in the solvents which could possibly arise, for example, by evaporation from the solvents outside the bag.

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Differential Sedimentation Coefficients. II. Interpretation in Terms of Changes in Size and Shape*

Verne Schumaker

ABSTRACT: Simple equations are developed for the interpretation of changes in sedimentation coefficient in terms of changes in frictional ratio. These equations are particularly suitable for the study of macromolecules which change shape and volume when binding a low molecular weight ligand, but do not change their state of aggregation. The equations are applied to a theoretically constructed model protein system to see how sensitive the results become to variations in parameters other than shape and volume.

Conformational changes in protein structure may be accompanied by changes in molecular volume and shape which are detectable by sedimentation analysis. With the development of techniques for accurate measurement of small differences in sedimentation coefficient (Richards and Schachman, 1959; Lamers *et al.*, 1963; Gerhart and Schachman, 1968; Schumaker and Adams, 1968), the interpretation of these differences becomes important.

First, to gain an appreciation of the magnitude of expected changes, we may use the X-ray data of Perutz for hemoglobin. The unoxygenated molecule is a spheroid of dimensions 50 × 55 × 69 Å, while the oxygenated molecule is smaller by 50 × 55 × 64 Å (Muirhead and Perutz, 1963). This corresponds to a volume decrease of about 8%. From this, it may be calculated that the sedimentation coefficient would increase by about 3% upon oxygenation.

Changes of sedimentation coefficient of this magnitude have been observed with other proteins under conditions where conformational changes are expected. Changes of 4% have been observed for the enzyme aspartate transcarbamylase upon ligand binding (Gerhart and Schachman, 1968), and we have detected changes of +3% when a purified antibody combines with hapten (C. Warner, V. N. Schumaker, and F. Karush, unpublished data).

Observation and measurement of a small difference in sedimentation coefficient do not necessarily mean that the protein itself has undergone alterations in size and shape, however, For example, the simple addition to the molecular weight and volume of the macromolecule which accompanies ligand binding would be reflected in a change in the sedimentation properties, or more subtle effects, such as alteration in salt and water binding affinities, could affect the buoyant density (Katz and Schachman, 1955) and sedimentation coefficient. Should a conformational change in protein structure actually occur, the partial specific volume may be changed which would also affect the sedimentation coefficient.

In this paper we first develop equations to take into account the various parameters which could influence the sedimentation coefficient. Then, we use these equations together with a theoretically constructed model protein system to see how sensitive the results become to small variations in all the parameters. From this study, it is concluded that interpretation of sedimentation coefficient changes in terms of alterations in size, shape, and molecular weight is reasonable. One very sensitive parameter does exist, however, and this is the partial specific volume of the protein. If conformational change is accompanied by a large change in partial

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