formation of a chelate, of the amino group in the cysteine methyl ester complex is therefore decreased, with a consequent decrease in the stability of the complex. In the case of lead ion complexes, an additional factor comes into play: it will be recalled in our previous discussion that the carboxylate ion becomes a binding site in the lead–cysteine complex. In going from cysteine to cysteine methyl ester, the negatively charged carboxylate ion is replaced by an uncharged $-COOCH_3$ group, thus causing a further decrease in stability of the lead complex, in addition to that caused by the decrease in pK's discussed above.

We have not identified the pK's of cysteine methyl ester with the dissociation of any particular group. Although most workers would assign the higher pK value to the –SH group and the lower pK value to the $-NH_3^+$ group, Calvin[14] has expressed the opinion that the assignment should be reversed. We are however in agreement with Edsall's interpretation that, since the intrinsic affinity of the amino and the sulfhydryl ion for proton is so similar, the two dissociation constants are really of a composite nature.

It is seen in Table V that for lead complexes of cysteine and 2-mercaptoethylamine, the formation constants depend on the anion of the lead salt. The order $ClO_4^- > NO_3^-$ is in agreement with the well known fact that lead forms the least possible amount of complex with perchlorate ion and a firmer complex with nitrate ion.

A comparison of the formation constants of glycine and methionine complexes of zinc leads to the conclusion that the sites of binding in methionine are the same as in glycine, namely, $-NH_2$ and $-COO^-$. Gonnick, Fernelius and Douglas[15] have given the following values

$$Cu^{++}\text{–}CH_3SCH_2CH_2NH_2,\ \log k_1 = 5.58\ (30°)$$
$$Cu^{++}\text{–}NH_2CH_2CH_2NH_2,\ \log k_1 = 10.55\ (30°)$$

which show that the amino group binds far more strongly than a $-SCH_3$ group. Inasmuch as the highest order zinc complex is two for both glycine and methionine as ligands and the characteristic coördination number of zinc ion is four, we may state that the $-SCH_3$ group in methionine is not involved in binding. Our interpretation is that since no sulfhydryl ion can be produced by the reaction of methionine with metal ion, no chelation with zinc ion can take place which would involve sulfur. In the case of the cysteine complex, therefore, the sulfhydryl ion rather than the –SH group is taken as the actual binding site.

Acknowledgment.—The authors wish to thank the National Science Foundation and the American Philosophical Society for the research grants which made this work possible.

(14) M. Calvin in "Glutathione, a Symposium," Academic Press, Inc., New York, N. Y., 1954, p. 3.

(15) E. Gonnick, W. C. Fernelius and B. E. Douglas, THIS JOURNAL, **76**, 4672 (1954).

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Ultracentrifuge Studies with a Synthetic Boundary Cell.[1] II. Differential Sedimentation

BY R. HERSH[2] AND H. K. SCHACHMAN

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Through the use of the recently developed synthetic boundary ultracentrifuge cells it is possible to form concentration boundaries between two solutions of the same sedimenting substance. The movement of such boundaries gives differential sedimentation coefficients. A theoretical treatment of such experiments is presented and it is shown that the theory based on the conservation of mass accounts satisfactorily for the results observed for different types of systems commonly studied in the ultracentrifuge. Not only are the observed sedimentation coefficients in agreement with values calculated from the theory but the areas of the boundaries also demonstrate the validity of the theory. Also there is presented a practical application of the differential sedimentation method to the determination of both the dependence of sedimentation coefficient on concentration and the value of the sedimentation coefficient at infinite dilution, and it is shown that the differential method gives reliable results with less effort than is required by conventional ultracentrifuge experiment.

Introduction

Ultracentrifugal studies by the sedimentation velocity method[3] have heretofore involved analyses of the movement and shape of the boundaries originally formed by the migration (either sedimentation or flotation) of solute molecules from a region in the centrifuge cell in which they were originally present into another region in which there were already molecules of the same solute. By this process there is created a region devoid of a particular solute. If the system is composed of only two components, then the resulting boundary represents the transition zone between a pure solvent and a solution of uniform concentration of the solute. For three component systems,[4] or multicomponent systems in general, each boundary developed as a result of the sedimentation process is really a compound boundary across which there is, in addition to the disappearance of one of the components, a change in concentration of the other components present in the solution.

There are different types of ultracentrifuge bound-

(1) These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the University of California, NR-121-175; and by grants from Lederle Laboratories and the Rockefeller Foundation.

(2) National Science Foundation Predoctoral Fellow, 1954–1955. Some of this work is submitted in partial fulfillment of the requirement for the Ph.D. degree in Biophysics at the University of California.

(3) T. Svedberg and K. O. Pederson, "The Ultracentrifuge," Clarendon Press, Oxford, Eng., 1940.

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

aries which cannot be obtained by the migration of solute molecules by sedimentation or flotation in an initially homogeneous solution; and it is now possible routinely to form such boundaries through the use of the recently developed synthetic boundary ultracentrifuge cells.[5,6] As long as the solutions differ in density by about 0.0002 g./cc., initially sharp boundaries can be formed in the rotating ultracentrifuge cell and the movement and shape of these boundaries can be followed as a function of time by the use of conventional optical methods.

There are three principal types of boundaries which may be formed: between a pure solvent and a solution of some solute in that solvent; between a solution of some sedimenting material at a fixed concentration and another solution of the same substance at a higher concentration; and finally between a solution of a sedimenting material and another solution of the same material at the same concentration to which is added another, either more or less rapidly, sedimenting component. Of the latter two types of boundaries, the simpler system is that containing only a single sedimenting component and such boundaries are termed concentration boundaries or differential boundaries[7] by analogy with the nomenclature used in transference studies.[8] A differential boundary is then a boundary between two solutions of the same substance at different concentrations, and ultracentrifugal studies of such boundaries are termed differential sedimentation studies.

In this article we shall discuss the theory and experimental results of differential sedimentation studies on the different types of macromolecules commonly examined in the ultracentrifuge.

Materials and Methods

All ultracentrifuge studies were performed on the substances tomato bushy stunt virus (BSV), chymotrypsin and deoxyribonucleic acid (DNA). Bushy stunt virus was grown in *Datura Stramonium* Linn. plants and the virus isolated and purified according to the procedure of Stanley[9] with but slight modifications regarding the solvents used to resuspend the pellets of virus obtained by high speed centrifugation. The chymotrypsin was obtained from Armour Laboratories. We are indebted to Dr. Sidney Katz of this Laboratory for the gift of the DNA preparation which was isolated by the method of Signer and Schwander.[10]

Ultracentrifuge experiments were performed in a Model E ultracentrifuge constructed by the Specialized Instruments Corp. The synthetic boundary ultracentrifuge cell described by Pickels, *et al.*,[6] was used for some of the experiments, and a modified version of that cell was used for the remainder of the work. In the original synthetic boundary cell the cup, which originally contained the less dense liquid, had a small hole in the bottom which was always placed in contact with a shoulder of the duralumin centerpiece. The speed of break through of the liquid against the forces of surface tension was only qualitatively controlled by applying a thin film of vacuum grease to the bottom of the cup. In order to control more precisely the speed at which the cup begins to empty and the rate at which the liquid flows from the cup the cell was modified slightly by the Specialized Instruments Corp. to provide for a valve like action. A hole about $^3/_{32}$" in diameter and $^5/_{32}$" in length was drilled into one of the metal shoulders of the centerpiece, and a rubber plug of length only slightly greater than $^5/_{32}$" was inserted into this hole in the centerpiece. In assembling the cell for operation, the cup is placed in the cell so oriented that the pin hole in the cup is directly above the rubber plug. The rubber plug acts as a valve which prevents liquid from leaving the cup until the centrifugal field is sufficiently great to depress the plug, thus allowing the liquid to flow freely from the cup into the sectoral cavity of the centerpiece. Furthermore, the cup is provided with a stand pipe to allow air originally in the sectoral opening to escape into the cup as the liquid flows down. With this modified synthetic boundary cell, boundaries can be formed at about the same speed from run to run, and it is possible with different plugs to form boundaries between speeds of 5,000 to 30,000 r.p.m.

In the course of this work, it was frequently observed that convection occurred during the ultracentrifuge runs. Study of this phenomenon suggested that the convection, which caused the formation of hypersharp, spurious boundaries, was the result of slight misalignment of the ultracentrifuge cell in the rotor. In collaboration with the Specialized Instrument Corporation an alignment instrument was designed and constructed.[11] This alignment tool facilitated the precise alignment of the centrifuge cell so that the walls of the cell were lying along true radii from the center of rotation of the rotor. Most of the work described was performed with cells aligned with this instrument.

The photographic plates were read in a microcomparator making use of the fringe pattern resulting from diffraction caused by the bar in the schlieren optical system.[12-14] Due to the symmetry and sharpness of the boundaries, the maximum ordinate was used. In some studies the areas corresponding to the different boundaries were measured from tracings of the patterns enlarged about eight times.

Systems for Which Sedimentation Coefficient Varies Only Slightly with Concentration

Theory.—Figure 1 shows the concentration distribution in a hypothetical differential sedimentation study. If we imagine a volume element in the cell bounded by a plane at x_1 (measured from the axis of rotation) in the upper solution of con-

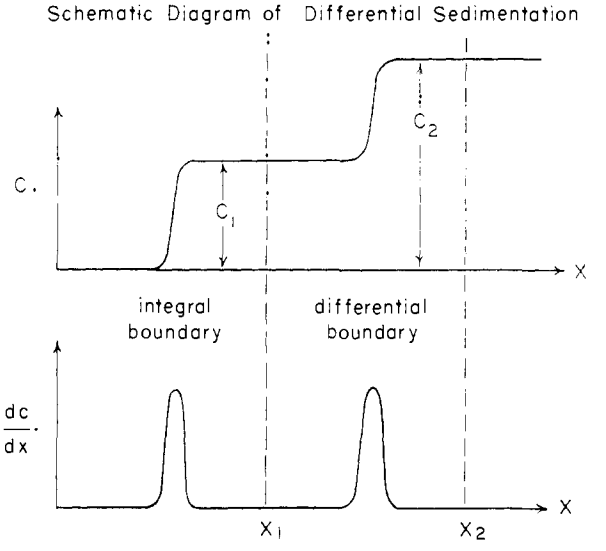


Fig. 1.—Schematic diagram of differential sedimentation showing the distribution of concentration, c, and concentration gradient, dc/dx, in the ultracentrifuge cell.

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

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

(7) H. K. Schachman and W. F. Harrington, *J. Polymer Sci.*, **12**, 379 (1954).

(8) L. G. Longsworth, THIS JOURNAL, **65**, 1755 (1943).

(9) W. M. Stanley, *J. Biol. Chem.*, **135**, 437 (1940).

(10) R. Signer and H. Schwander, *Helv. Chim. Acta*, **33**, 1522 1950).

(11) E. G. Pickels, H. K. Schachman and R. T. Hersh (unpublished).

(12) G. Kegeles and F. J. Gutter, THIS JOURNAL, **73**, 3770 (1951).

(13) H. Wolter, *Ann. Physik*, **7**, 182 (1950).

(14) R. Trautman and V. W. Burns, *Biochim. Biophys. Acta*, **14**, 26 (1954).

centration, c_1, and another plane at x_2 in the lower solution of concentration, c_2, we can write for the total mass, m, of material in that volume element

$$m = q[c_2(x_2^2 - x_D^2) + c_1(x_D^2 - x_1^2)] \quad (1)$$

where x_D represents the distance to the differential boundary and q is a constant depending on the geometry of the ultracentrifugal cell. We assume that the plane at x_1 moves at a rate corresponding to the sedimentation coefficient s_1, of molecules in that region and similarly the plane at x_2 moves at a rate, s_2, which could be obtained if the material at the concentration c_2 were run in a conventional ultracentrifuge cell. Since no material is entering or leaving the volume element defined by the moving planes and the walls of the cell, we can consider m equal to a constant and differentiation of equation 1 with respect to time leads to

$$\frac{dc_2}{dt}(x_2^2 - x_D^2) + 2c_2\left(x_2\frac{dx_2}{dt} - x_D\frac{dx_D}{dt}\right) + \frac{dc_1}{dt}(x_D^2 - x_1^2) + 2c_1\left(x_D\frac{dx_D}{dt} - x_1\frac{dx_1}{dt}\right) = 0 \quad (2)$$

Due to the radial inhomogeneity of the centrifugal field and the sector shape of the cell, the contents in each region are being continually diluted and we can write, following Svedberg and Pedersen[3] and also Trautman and Schumaker[15]

$$\frac{dc_1}{dt} = -2c_1\omega^2 s_1; \quad \frac{dc_2}{dt} = -2c_2\omega^2 s_2 \quad (3)$$

where ω is the angular speed of the rotor. From the definition of sedimentation coefficient

$$\frac{dx_1}{dt} = x_1 s_1\omega^2; \quad \frac{dx_2}{dt} = x_2 s_2\omega^2 \quad (4)$$

Combination of equations 2, 3 and 4 and rearranging leads to the differential sedimentation coefficient, s_D

$$\frac{1}{\omega^2 x_D}\frac{dx_D}{dt} = s_D = \frac{c_2 s_2 - c_1 s_1}{c_2 - c_1} \quad (5)$$

Since the lower solution must be more dense than the upper solution in order to have gravitational stability, we can write

$$c_2 = c_1 + \Delta c \quad (6a)$$

where Δc is always a positive quantity. Also

$$s_2 = s_1 + \Delta s \quad (6b)$$

where Δs can be either a positive or a negative quantity. Substitution of (6a) and (6b) into equation 5 gives

$$s_D = s_2 + c_1\frac{\Delta s}{\Delta c} = s_2 + c_2\frac{\Delta s}{\Delta c} \quad (7)$$

As the difference in concentration, Δc, between the upper and lower solutions approaches zero and c_1 equals c_2, the differentials in equation 7 can be rewritten as derivatives and equation 8 results

$$s_D = s + c\frac{ds}{dc} \quad (8)$$

where s is the sedimentation coefficient obtained in a conventional ultracentrifuge experiment at the concentration, c.

For most systems (ds/dc) is a negative quantity and equation 8 shows that the sedimentation coefficient of the differential boundary is smaller than the sedimentation coefficient of either of the two solutions when examined independently. Similarly equation 8 shows that the s_D will be greater than either of the two integral sedimentation coefficients if (ds/dc) is positive.

In the theoretical development of equation 5 it was assumed that the contents of the cell in both plateau regions were being diluted with time due only to the geometry of the cell and the radial inhomogeneity of the centrifugal field. This is implied in the use of equation 3. If this is the case, the area of the differential boundary should follow the inverse square law of ultracentrifugation.[14] This can be shown in the following manner.

Equation 1 can be written in the following form since m is independent of time.

$$c_{2,t}(x_{2,t}^2 - x_{D,t}^2) + c_{1,t}(x_{D,t}^2 - x_{1,t}^2) = c_{2,0}(x_{2,0}^2 - x_{D,0}^2) + c_{1,0}(x_{D,0}^2 - x_{1,0}) \quad (9)$$

where the subscripts t and 0 refer, respectively, to a time, t, and zero time, and the other symbols have the same meaning as in equation 1. By means of the inverse square law (a combination of equations 3 and 4), $c_{1,t}$ and $c_{2,t}$ can be related to $c_{1,0}$ and $c_{2,0}$, respectively; and substitution of these relationships into equation 9 and rearrangement of terms leads to

$$(c_{2,0} - c_{1,0})\frac{x_{D,0}^2}{x_{D,t}^2} = c_{2,0}\frac{x_{2,0}^2}{x_{2,t}^2} - c_{1,0}\frac{x_{1,0}^2}{x_{1,t}^2} \quad (10)$$

or

$$\frac{\Delta c_{D,0}}{\Delta c_{D,t}} = \frac{x_{D,t}^2}{x_{D,0}^2} \quad (10a)$$

where $\Delta c_{D,0}$ and $\Delta c_{D,t}$ are the concentration changes across the differential boundary at zero time and time, t, respectively.

It should be pointed out that the boundary positions throughout this discussion are the positions obtained by taking the square root of the second moment of the gradient curves.[15,16]

For many systems which show little dependence of sedimentation coefficient on concentration, we can write

$$s = s_0(1 - kc) \quad (11)$$

where k is a constant, s_0 is the sedimentation coefficient at infinite dilution and s is the sedimentation coefficient at the concentration, c. Combination of equations 5 and 11 leads directly to

$$s_D = s_0[1 - k(c_1 + c_2)] \quad (12)$$

Thus the theory shows that the differential boundary has a sedimentation coefficient equal to that of an integral boundary at a concentration which is the sum of the concentrations on the upper and lower solutions. It should be noted that we are restricting ourselves here to systems which obey equation 11 and for which k is sufficiently small that the sedimentation coefficients in the two plateau regions can be considered constant throughout the experiment. A more general treatment is presented later.

Results and Discussion

In order to test the theory a series of runs were performed with BSV in which the bottom solution

(15) R. Trautman and V. N. Schumaker, *J. Chem. Phys.*, **22**, 551 (1954).

(16) R. J. Goldberg, *J. Phys. Chem.*, **57**, 191 (1953).

was constant in concentration from run to run and the concentration of the upper solution was varied. Typical ultracentrifuge patterns from such a study have been published elsewhere[7] and there was no evidence of convection in any of these runs. Figure 2 shows the results of these studies. In the upper curve are plotted the values of the integral sedimentation coefficients and the lower curve contains the data for the differential sedimentation coefficients. As can be seen from the graphs, the value of s_D in all experiments is less than the value of s_2 in accordance with the theory. Also it is observed, in accord with equations 7 and 12, that the curve of s_D *vs.* c_1 has approximately the same slope as that obtained from the integral sedimentation coefficients. Finally, if the data of Fig. 2 for s_D and the values of the sedimentation coefficient of the upper solution are used to calculate s_2 from equation 5, an average value of 117 S is obtained, in excellent agreement with the value, 120 S, found experimentally for the bottom solution when studied separately.

When ds/dc is negative it would be expected that there would be gravitational stability in the region of the differential boundary. As molecules in the dilute solution are moving more rapidly than those in the lower solution, there will be a crowding together of the molecules from the upper solution in the region of the differential boundary. This accumulation will continue until a concentration equal to that in the lower phase is achieved. In this way, as the particles in the lower solution near the initial differential boundary move through the cell, material at the same concentration is constantly being added behind them; and, for this reason, the differential boundary moves at a lower rate than do the molecules in the concentrated solution. Following this line of reasoning we might expect that instability and, consequently, convection would result if the molecules in the lower solution move more rapidly than those in the upper solution, *i.e.*, ds/dc is positive. In such an experiment, after the differential boundary is formed, the molecules below will move more rapidly than those above can fill up the region at the differential boundary and a region of density less than that of the upper solution would result. This, of course, is gravitationally unstable, and convection would result. Since simple systems showing a positive value for ds/dc are not available, model experiments were performed[7] with different proteins in the top and bottom of the cell, and convection was seen whenever the sedimentation coefficient of the bottom material was greater than that of the top.

There are examples of reversibly associating substances[17] for which the rate of interconversion of monomers to polymers and *vice versa* is very rapid, and consequently ds/dc for those systems is positive. As a further test of equation 5 studies were performed on chymotrypsin under conditions of concentration, ionic strength and *p*H for which the fraction of polymers increases as the concentration increases. Figure 3 shows several ultracentrifuge patterns from a typical study and there appears to be no convection during the run. At 0.4 g./100 ml.

(17) G. W. Schwert, *J. Biol. Chem.*, **179**, 655 (1949).

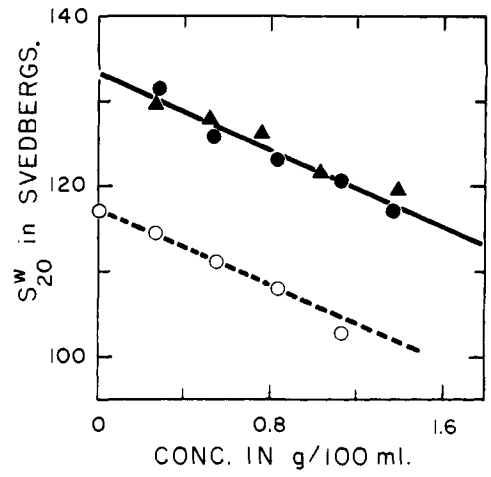
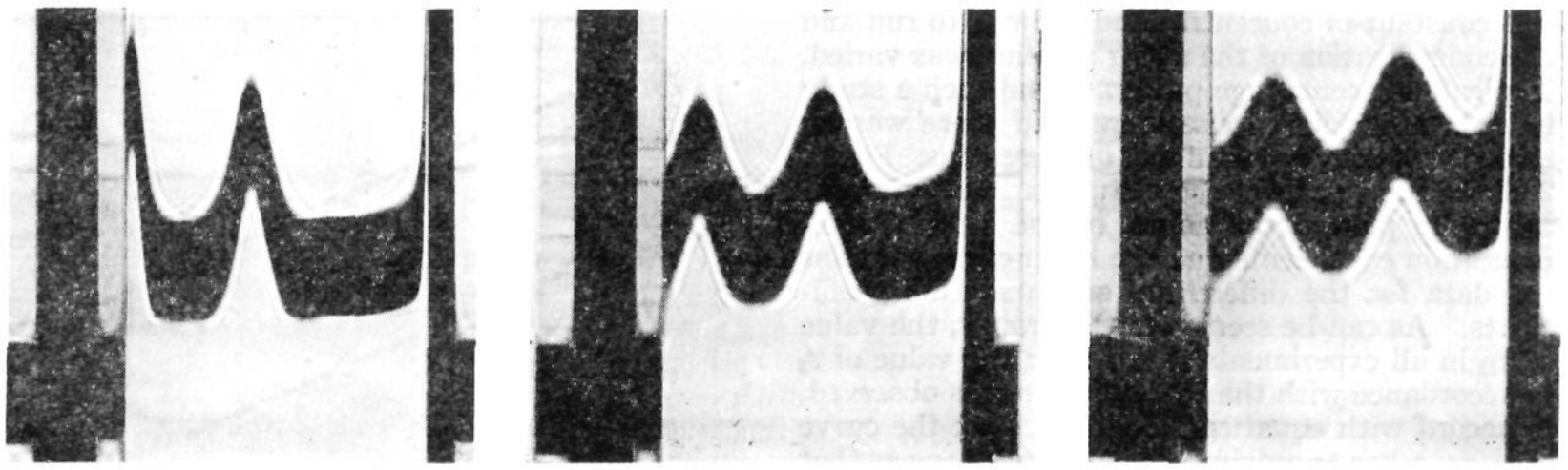


Fig. 2.—Plot of sedimentation coefficient *versus* concentration of BSV. The upper curve contains values of the integral sedimentation coefficients. Experimental points designated by $-\Delta$ are taken from conventional runs, and the points designated by ● are the values obtained from the integral sedimentation rates in the differential runs. The lower curve is the differential sedimentation rate *versus* the concentration of BSV in the upper solution where the bottom solution is held fixed at an initial concentration of 1.5 g./100 ml. The dotted line is the expected curve based on the differential boundary moving at the sedimentation rate at a concentration $(c_1 + c_2)$ and the experimental points are designated by 0. The value at $c_1 = 0$ for the lower curve is the average of the values obtained for s_2 from the differential data.

and 1.0 g./100 ml. the integral sedimentation coefficients are 2.83 and 2.93 S, respectively, and the differential sedimentation coefficient for a boundary between those two solutions was 3.03 S. Not only was this differential sedimentation coefficient greater than either of the integral sedimentation coefficients but also the value, 2.95 S, for the bottom solution calculated according to equation 5 from the differential boundary and the integral boundary was in excellent agreement with the value mentioned above which was obtained from an independent study. Although all of the data on chymotrypsin do not agree quantitatively with the theoretical values, it was found almost invariably that $s_D > s_2$ when the experiments were performed in a concentration region for which $ds/dc > 0$. The absence of convection in these runs is strong evidence for the view that the chymotrypsin polymers dissociate rapidly to the lower molecular weight forms as soon as the concentration is decreased. This is in marked contrast to the behavior with insulin,[7] another reversibly associating protein, for which convection was observed in the concentration region where $ds/dc > 0$.

The theory developed above also requires that the area of the differential boundary follows the inverse square law of ultracentrifugation. Therefore, measurements of the areas of the boundaries of some of the ultracentrifuge studies on BSV were made; and the results, shown in Table I, demonstrate satisfactorily the validity of the radial dilution law for both the integral and differential boundaries. In some studies it was observed that the



4 min. 28 min. 56 min.
50° 45° 40°

Fig. 3.—Ultracentrifuge patterns of a differential run of chymotrypsin in 0.2 μ, pH 6.1 phosphate buffer containing 0.18 M sodium chloride. The concentrations of the upper and lower solutions were 0.4 and 1.0 g./100 ml., respectively. The ultracentrifuge was operated at 59,780 r.p.m. and pictures were taken at 4, 28, 56 minutes, respectively, with bar angles 50, 45, 40°, respectively.

area of the integral boundary was slightly greater (about 5%) and the area of the differential boundary was correspondingly less than expected from the known concentrations of the two solutions.

TABLE I

AREA MEASUREMENTS FOR INTEGRAL AND DIFFERENTIAL BOUNDARIES OF BSV

Upper solution 0.48 g./100 ml.[a]; lower solution 0.96 g./100 ml.[b]

	Integral boundary		Differential boundary		Total
t, min.	$(x_{1,t}/x_{1,0})^2$	$A_{cor.}$, arbitrary units	$(x_{D,t}/x_{D,0})^2$	$A_{cor.}$, arbitrary units	$A_{cor.}$, arbitrary units
8	..	..	1.021	25	..
24	1.0.3	25	1.062	24	49
44	1.133	26	1.119	25	51
52	1.159	26	1.142	23	49
68	1.198	27	1.180	24	51
80	1.257	25			

[a] This solution in a conventional run had a corrected area of 27 ± 1. [b] This solution in a conventional run had a corrected area of 51 ± 1, and the expected area for the differential boundary is, thus, 24.

This may have resulted from a slight mixing of the solutions at the time the boundary was formed in the synthetic boundary cell; or it may have been due to slight pipetting errors in the making up of solutions. Even for those studies in which the two areas were not exactly as expected, the decrease in area of both boundaries during the run was precisely as expected from the inverse square law.

Area measurements on the chymotrypsin runs were in general not very satisfactory; and it was not possible to perform as critical a test of the radial dilution hypothesis for differential sedimentation as was the case for the studies with BSV. For chymotrypsin there is more difficulty in making accurate measurements because of cell distortions at high speeds. Also, the high diffusion rate and relatively low sedimentation rate leads to the loss of the base line relatively early in the centrifuge run. Finally there is so little movement of the boundaries during the run that the total dilution is too small to allow an adequate test of the theory. None of these disadvantages exist for the studies with BSV where a dilution of as much as 20% was observed.

Systems for which the Sedimentation Coefficient Varies Greatly with Concentration

Theory.—For many systems the dependence of sedimentation coefficient on concentration is so great that the theory developed above must be modified so as to account for the changes in sedimentation coefficients during the run as a result of dilution of the macromolecule with time. Considerable attention has been given to this dilution effect in conventional ultracentrifugation, and different procedures have been suggested for calculating sedimentation coefficients from the experimental data showing boundary position as a function of time.[18-21]

If the dependence of sedimentation coefficient on concentration can be expressed by equation 11, then the expression relating the position of an integral boundary, x_t, as a function of time becomes

$$x_t^2 = kc_0x_0^2 + x_0^2(1 - kc_0)^{2\omega^2 s_0 t} \qquad (13)$$

Combination of equations 10 and 13 and rearrangement of the resulting equation leads to an expression relating the position of the differential boundary to time.

$$\frac{x_{D,t}^2}{x_{D,0}^2} = \frac{k^2c_{1,0}\,c_{2,0}\,(1 - e^{2\omega^2 s_0 t})^2 + k(c_{2,0} + c_{1,0})(1 - e^{2\omega^2 s_0 t})\,e^{2\omega^2 s_0 t} + e^{4\omega^2 s_0 t}}{e^{2\omega^2 s_0 t}} \qquad (14)$$

The value of the differential sedimentation coefficient can be expressed as a function of time by differentiation of equation 14 and then dividing it by equation 14. This leads to

$$s_{D,t} = s_0\left\{1 - \frac{2k^2c_{1,0}\,c_{2,0}\,(1 - e^{2\omega^2 s_0 t}) + k(c_{1,0} + c_{2,0})\,e^{2\omega^2 s_0 t}}{k^2c_{1,0}\,c_{2,0}\,(1 - e^{2\omega^2 s_0 t})^2 + k(c_{1,0} + c_{2,0})(1 - e^{2\omega^2 s_0 t})e^{2\omega^2 s_0 t} + e^{4\omega^2 s_0 t}}\right\} \qquad (15)$$

At $t = 0$ equation 15 reduces to equation 12 which was derived earlier for systems which show so lit-

(18) M. A. Lauffer, THIS JOURNAL, **66**, 1195 (1944).
(19) R. H. Golder, *ibid.*, **75**, 1739 (1953).
(20) Robert A. Alberty, *ibid.*, **76**, 3733 (1954).
(21) R. Trautman, V. N. Schumaker, W. F. Harrington, H. K. Schachman, *J. Chem. Phys.*, **22**, 555 (1954).

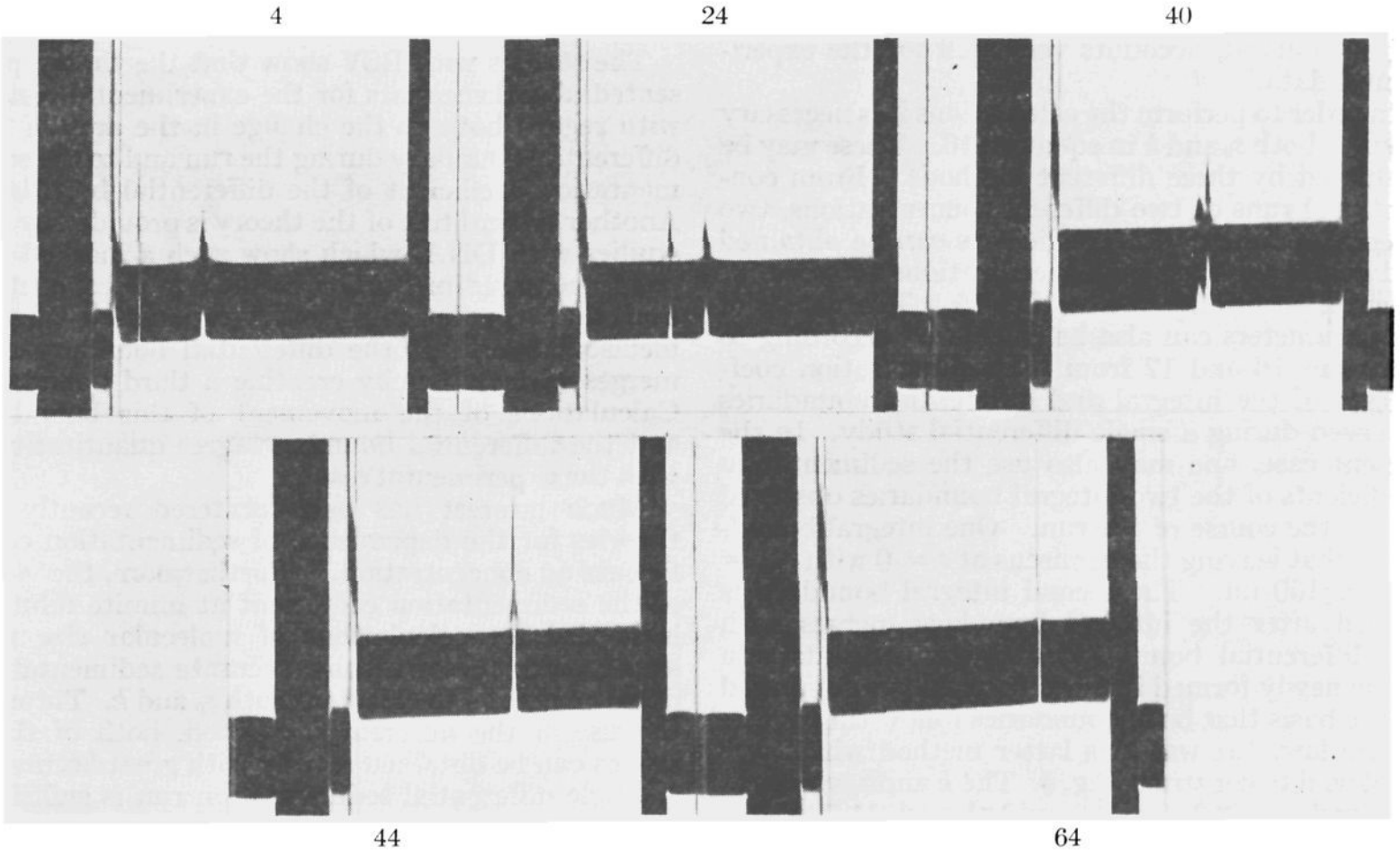


Fig. 4.—Ultracentrifuge pattern of a differential sedimentation run of DNA. The concentrations of the upper and lower solutions were 0.05 and 0.1 g./100 ml., respectively. The ultracentrifuge was operated at 59,780 r.p.m. and pictures were taken at 4, 24, 40, 44, 64 minutes, respectively.

tle dependence of sedimentation coefficient on concentration that the effect of dilution during a single run can be neglected.

Thus the theory shows that the differential boundary has a sedimentation coefficient at zero time equal to that of an integral boundary at a concentration equal to the sum of the upper and lower solutions. Furthermore, it is readily shown for systems in which the terms containing k^2 are small compared to those with k that the sedimentation coefficient of the differential boundary changes with time in exactly the same way as would an integral boundary with concentration, $(c_1 + c_2)$.

Although equation 11 does express adequately the concentration dependence of the sedimentation coefficient for some materials it is found more generally that

$$s = \frac{s_0}{1 + kc} \tag{16}$$

Equations analogous to 14 and 15 cannot be obtained readily for these systems, however, since the position of the boundary (either integral or differential) cannot be written in explicit form with regard to time and, as a result, it is necessary to solve the equations numerically. It should be noted that combination of equations 5 and 16 leads to

$$s_{D,0} = \frac{s_0}{(1 + kc_{1,0})(1 + kc_{2,0})} \tag{17}$$

Results and Discussion.—The dependence of s on c for DNA is very large, and therefore DNA is a good model system to test the theory developed above. It was especially interesting to determine how accurately we could predict the curvature of the plot of log x *vs.* t for the differential boundary. Figure 4 shows the ultracentrifuge patterns from a typical differential sedimentation study with DNA. The integral boundary moves so much more rapidly than the differential boundary that the two boundaries finally merge to form a new integral boundary. Thus, three sedimentation rates can be calculated in this experiment. In an attempt to see whether the position of the differential boundary as a function of time could be accounted for in terms of the theory, curves for the change in position with time were calculated, and it

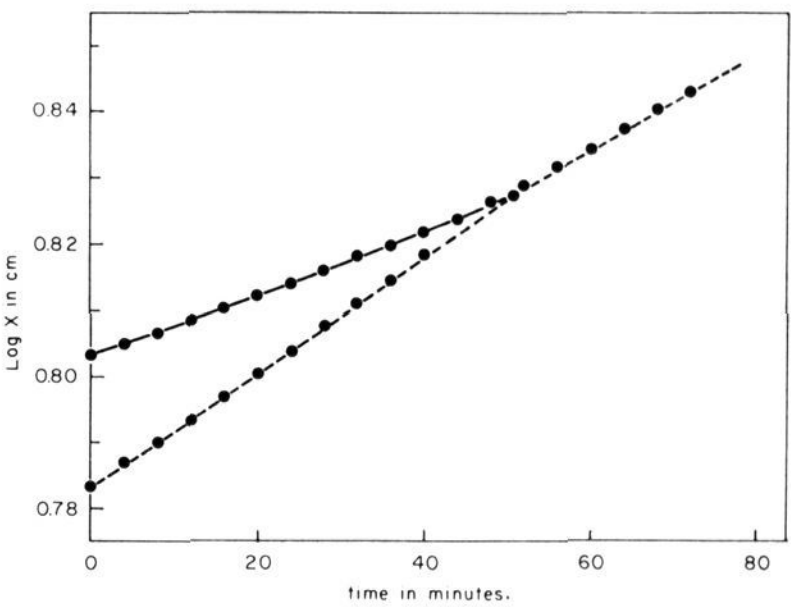


Fig. 5.—Plot of data from a differential sedimentation study on DNA in which the upper solution was at 0.05 g./100 ml. and lower solution was at 0.1 g./100 ml. Experimental points are shown by ● and the dashed lines (---) are calculated from equation 18 based on the parameter, s_0 and k, evaluated from the experimental points. The solid line (—) for the differential boundary is obtained theoretically from the same parameters.

is seen in Fig. 5 that the theoretical line, which is slightly curved, accounts very well for the experimental data.

In order to perform the calculations it is necessary to know both s_0 and k in equation 16. These may be evaluated by three different methods. From conventional runs at two different concentrations, two integral sedimentation coefficients can be obtained and combined with the concentrations to solve for s_0 and k according to equation 16. The values of the parameters can also be calculated according to equations 16 and 17 from the sedimentation coefficients of the integral and differential boundaries observed during a single differential study. In the present case, one may also use the sedimentation coefficients of the two integral boundaries observed during the course of the run. One integral boundary is that leaving the meniscus at $t = 0$ with $c_{1,0} =$ 0.05 g./100 ml. The second integral boundary is formed after the integral boundary merges with the differential boundary, and the concentration of the newly formed integral boundary is computed on the basis that both boundaries follow the inverse square law. It was this latter method which was employed to construct Fig. 5. The k and s_0 values so obtained are 8.3 (g./100 ml.)$^{-1}$ and 11.7 S. It should be pointed out that these compare favorably with the values 8.7 (g./100 ml.)$^{-1}$ and 11.8 S obtained from the combination of the differential and integral sedimentation coefficients. The test of the theory is then to see if the position of the differential boundary computed from equation 10 accounts satisfactorily for the experimental data.

The computed positions are obtained by the following procedure. In order to calculate $x_{D,t}$, according to equation 10, values of both $x_{1,t}$ and $x_{2,t}$ are needed as a function of time. These latter values are obtained from curves plotted according to the equation derived previously[21] for systems which obey equation 16.

$$\frac{x_t}{x_0} = e^{s_0\omega^2 t + 1/2 c_0\left[k(x_0^2 - x_t^2)/x_t^2\right]} \qquad (18)$$

Thus values of k and s_0 enable us to determine $x_{1,t}$ and $x_{2,t}$ as a function of t. For a given value of t, the values of $x_{1,t}$ and $x_{2,t}$ can be read off the curves, and then these values are substituted into equation 10 to give $x_{D,t}$ for the time, t. The results of these calculations, shown in Fig. 5, show that the three experimental curves can be accounted for theoretically from two parameters.

Discussion

The studies with BSV show that the theory presented above accounts for the experimental results with regard both to the change in the area of the differential boundary during the run and to the sedimentation coefficient of the differential boundary. Another critical test of the theory is provided by the studies with DNA, which show such a marked dependence of sedimentation coefficient on concentration that the integral boundary created at the meniscus overtakes the differential boundary and merges with it thereby creating a third boundary. Calculations of the movement of this boundary and the differential boundary agree quantitatively with the experimental results.

Much interest has been centered recently on theories for the dependence of sedimentation coefficients on concentration.[22] Furthermore, the value of the sedimentation coefficient at infinite dilution is needed for calculations of molecular size and shape. For these reasons, accurate sedimentation data are needed to calculate both s_0 and k. Through the use of the differential method, both of these values can be obtained readily with great accuracy. A single differential sedimentation run is sufficient for calculations of both s_0 and k, whereas two runs, at least, are required when conventional ultracentrifuge runs are made. Furthermore, the calculations involve the small difference between two large numbers each of which is subject to experimental error. By the differential method such errors are in part obviated since factors such as speed and temperature of the rotor are the same for both of the experimentally required measurements. The differential method has a further advantage in that effectively higher concentrations, $c_1 + c_2$, are employed when solutions of concentration no greater than c_2 are available. For materials in short supply and of a limited concentration, differential sedimentation analysis would appear to be the method of choice for the determination of s_0 and k.

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