[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY]

Measurement of Sedimentation Velocity in Simple Air-driven Tops as Ultracentrifuges

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The ultracentrifuge is any centrifuge of low or high power in which convection does not occur, and in which it is possible to measure any redistribution of the contents. It has become one of the most important tools for investigations in colloid systems, non-aqueous systems, immunology, biochemistry, and even in simple molecular solutions. It is therefore gratifying that simple air-driven spinning tops have now been developed as ultracentrifuges, at such low cost that they may be made available in every laboratory. The very great advantage of these opaque ultracentrifuges is that they possess no optical system and require no accessories other than those everywhere to be found. They yield accurate results. Direct analysis is made by any appropriate physical, chemical or biological method.

The time would seem to be approaching when experiments with the ultracentrifuge may be included in practical courses in biochemistry, colloid and physical chemistry.

The essential feature of our ultracentrifuges is the immobilization of the liquid, usually between horizontal surfaces placed sufficiently close together that friction inhibits convection.[1] Elsewhere[1] we have reviewed the methods adopted by other investigators and also have pointed out the great possibilities of the original one-piece spinning top.

The present communication describes a simple opaque ultracentrifuge of general applicability.

Two problems had to be solved: first, the design of a liquid-tight rotor, and, second, the design of suitable immobilizing inserts to place in the rotor.

The Two-Piece Rotor with Tight Seal

The rotor, shown in Fig. 1, consists of two pieces made of 4 UMA steel, a chrome manganese alloy made by the Republic Steel Corporation. The steel is oil-quenched from 1525°F. and drawn at 850°F. after making up. The top part, A, is 32.5 mm. in outside diameter and 11.5 mm. high. Its inner well into which the various inserts for immobilization are fitted is 28 mm. in diameter and 9 mm. deep.

The shell A screws into the rotor cone B to a depth of 4 mm. Thus in the centrifugal field, when the rotor is assembled, the thinner longer annular walls of the upper part bend elastically outward, engaging still more tightly into the lower part. The angle of the cone walls is 100°. The largest diameter is 38 mm. A cylindrical space 14 mm. in diameter is cut in the bottom of the cone, with screw threads to fit onto a holding jig and handle serving as a wrench for assembling the rotor, the upper shell being held by a strap wrench, or better a split ring tightened by a screw and held in a vise. In experiments of longer duration the screw threads should be kept dry, or be oiled to avoid rusting or seizing. The flutes or grooves in the conical walls of the rotor cone against which the driving air is directed are cut with one side vertical, the other sloping, and are about 8–10 mm. long. All threads on the rotor are 32 per inch (2.54 cm.).

The degree of polish on the rotor greatly affects the speed. It was found that, after a high polish had been obtained, keeping the rotor in any convenient oil, or petroleum ether, was effective in preserving the polish. If kept in oil, the rotor was washed with petroleum ether before use. The insides of both parts of the rotor are made indifferent to the materials studied by several coats of Bakelite lacquer, applied unthinned, allowed to dry in air for forty-five minutes and then baked at 135° for thirty minutes. In applying the lacquer care must be taken that none of the lacquer touches the lower edge of the upper part of the rotor that will make contact in the joint. This Bakelite coating must be replaced whenever damaged in any way.

Assembly of the Rotor.—To achieve a seal which is always leak proof, even in petroleum systems, a pliofilm or other plastic disk C, shown in Fig. 1, a loose metal disk D, 0.020 in. (0.5 mm.) thick, and a loose metal ring E, 0.0125 in. (0.3 mm.) thick, are used. The pliofilm, 250 gage P5A supplied by Goodyear Rubber Company, or even cellophane, is initially cut slightly larger than the outer diameter of the top part of the rotor. The rotor is assembled upside down. After the insert is placed in the upper shell, the plioform disk is put in place. Then while holding the cellophane by one edge, the metal disk is gently slid over the top of it, squeezing out the excess liquid. After the metal disk is carefully centered, the cellophane is trimmed to exact size with a razor blade. The metal ring is then put in place and the rotor cone screwed on. This seal has been shown to be perfectly tight, the filled rotor weighing the same within ±0.0001 g. before and after a run of many days. One or more grooves are sometimes cut the entire way around the bottom surface of the top half of the rotor, allowing the pliofilm gasket to be forced into the depressions and possibly making the seal even more ideal.

(1) McBain and O'Sullivan, THIS JOURNAL, **57**, 780 (1935), last paragraph 4a and 4b; McBain and Stuewer, *Kolloid-Z.*, **74**, 10 (1936); McBain and Tostado, THIS JOURNAL, **59**, 2489 (1937); McBain, *Science*, **87**, 93 (1938); 15th Colloid Symposium, Cambridge, *J. Phys. Chem.*, **42**, 1063 (1938).

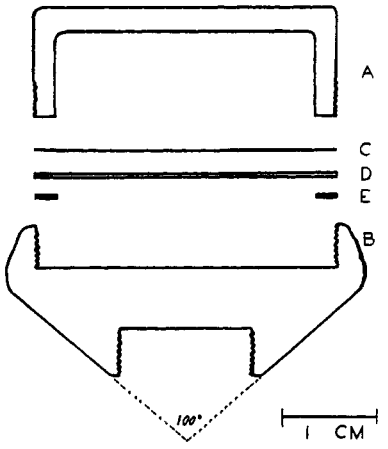

Fig. 1.—Cross section through rotor showing: A cover, B rotor cone, C film of plastic material, D loose metal disk, and E loose metal washer to facilitate slipping when assembling.

The Stator.—The stator, A, shown in Fig. 2, is made of Duralumin 17ST with a brass insert at the top edge of the inner walls for longer wear. The cone walls make an angle of 90°. The thickness of the walls may vary, being usually from about 2 to 4 mm. The air ports are so placed that they will strike at approximately the mid-point of the grooves or flutes cut in the lower part of the rotor. It is essential that the grooves do not reach the outer edge of the stator cone. The orientation of the air ports may be described by two angles, the angle with the horizontal plane through the center of the outlet of the air port and the axis of the stator being 35°, and that with the vertical plane 65°. Six to twelve of these air ports may be cut at equal distances apart around the stator.

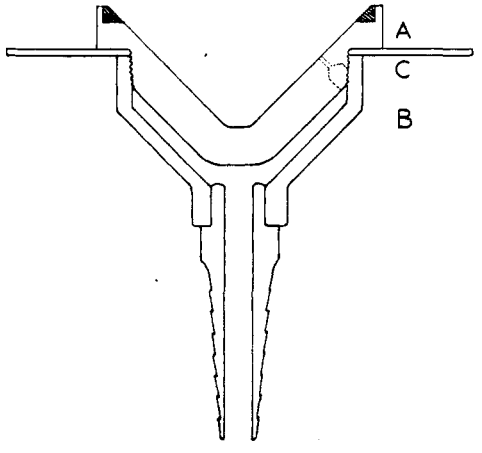

Fig. 2.—Simple form of stator showing: A the stator, B the manifold or air box, and C the steadying disk; also cross section of an air-jet and a brass inset to minimize chance wear on upper part of cone.

The stator is held in the manifold or air box B with a disk C placed in the joint. This assembly is placed in a metal guard, the disk C being steadied by sponge rubber (that used for bath mats has been found most convenient) to dampen vibration, and the central air inlet of the manifold connected to the air supply by means of heavy pressure tubing securely clamped on.

The manifold was also made of 17ST Duralumin. Its walls are about 2 mm. thick, the angle of the conical walls being 90°. The supporting disk was made of Duralumin, or Bakelite, or any convenient metal or fiber.

Whereas the stator shown in Fig. 2 is usually satisfactory, the form shown in Fig. 3 is sometimes better, especially for taller rotors, since adjustment of the air sucked in through the central tube (partly closed) with a thin rubber tube and screw clamp prevents "blooping" and sometimes increases the speed by 10% for a given pressure of driving air. The latter is fed in through the side tube.

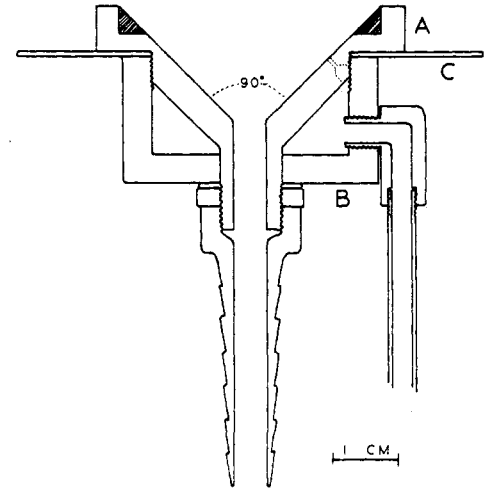

Fig. 3.—Alternative stator with adjustable central inlet for allowing air partially to relieve the vacuum below the rotor.

Temperature Control

The temperature control of these opaque ultracentrifuges is unique in that it is positive and is as accurate as a thermostat. It is that designed for the McBain-O'Sullivan transparent ultracentrifuge[2] and consists simply of passing the constant pressure driving air through a copper coil immersed in a thermostat at any desired temperature. The rotor maintains itself at the temperature of the slip-stream.

The Immobilizing Inserts

While for measurement of sedimentation equilibrium it does not matter what the size or shape of the container for the liquid may be, provided that convection is suppressed and that no mixing occurs, before the samples are withdrawn for analysis, the additional conditions for sedimentation velocity are more stringent. In the first place, the radial movement of the sedimenting particles in the line of the centrifugal force must

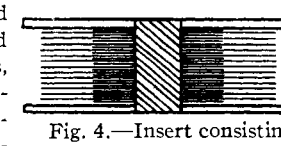

Fig. 4.—Insert consisting of piles of disks for general use in sedimentation velocity or equilibrium.

(2) McBain and O'Sullivan, THIS JOURNAL, **57**, 2631 (1935).

be unobstructed. This is achieved by using horizontal surfaces unobstructed over the complete range of 360°, utilizing circular disks or annular washers spaced uniformly apart by others of different diameter.

In the second place, the mechanical baffles must be so far apart that there are no wall effects upon the motion of the particle. Spaces 0.08 mm. wide are 20,000 times the diameter of the smaller proteins. Experience has shown that the sedimentation velocity observed within these spaces is the same as that given by the transparent ultracentrifuges. Much wider spacings are probably satisfactory, certainly so for the more viscous petroleum systems. Narrower spacings have not yet been studied.

A further requirement for the mechanical baffles is that they must be of material, such as plastic or metal, that will not flow in the centrifugal field. For this reason pure silver, platinum, or monel metal are satisfactory for smaller diameters, but must be replaced by stainless steel or coin silver or platinum-iridium for diameters approaching three centimeters. The insert if made of metal should be all of one kind of metal to avoid electric couples.

Insert 1, Generally Useful for Velocity or Equilibrium.—The liquid to be studied may be immobilized most simply by using as insert a central pile of disks, alternately wide and narrow with a larger disk just fitting inside the rotor at top and bottom to keep them central. A vertical axial pin runs through the central solid pile of metal, holding it together. The axial pin may be replaced by a screw permitting a change of the spacing pieces to obtain a wider sedimenting column.

This insert as used for the present measurements is shown diagrammatically in Fig. 3. The disks are made of coin silver (or stainless steel), the larger ones of metal 0.004" (0.102 mm.) thick, the smaller ones 0.003" (0.076 mm.) thick. The larger disk at top and bottom was of metal 0.030" (0.762 mm.) thick. The insert is made so that it just fills the space left in the top section of the rotor, as many disks being used as can be fitted in. A convenient diameter for the larger disks is 24.6 mm. and 13.6 mm. for the smaller ones. To make it possible to obtain for analysis a sample at the bottom of the sedimenting column without disassembling the rotor, a hole 1 mm. square is cut on opposite sides of the larger top disk of the insert, matching holes being cut in the bottom disk. A sample is then taken with a hypodermic pipet or syringe after the rotor is stopped.

A convenient method of filling the baffles with the solution to be studied is to place them in a small metal case filled with solution and placed in an ordinary centrifuge for a few minutes. Preliminary work on various nonaqueous solvents and stainless steel (-18-8-) baffles has given excellent results in filling by capillarity.

Insert 2, for Monodisperse Sedimentation Equilibrium Only.—This is fully described by McBain and Tostado,[1] the immobilized sedimenting liquid lying between horizontal annular washers of coin silver spaced at uniform known distances apart by using alternately narrow and wide washers. Convection is permitted in the liquid in contact and in equilibrium with the innermost part of the sedimentation column. Analysis of this liquid before and after gives the molecular weight of any monodisperse substance. For sucrose 341 was found (theory, 342). A window[1] is a convenience but by no means a necessity. It is obvious that the same method of calculation of sedimentation equilibrium is applicable to Insert 1, which is also generally more convenient.

Insert 3, for Equilibrium Only, Including Polydisperse Systems.—This is a modification of 2, permitting analysis of the liquid above and below the sedimenting column, especially for use in polydisperse systems. Here the annular washers are all alike and are merely piled loosely upon each other. They are kept centered by perforated buttresses or bosses, or by pillars, on the container. The rotor is stopped for analysis, and the liquid from the middle and the exterior withdrawn at approximately the same rate to avoid mixing.

Other Inserts.—Several other inserts have been designed and briefly referred to,[1] and they will be described in more detail when the corresponding measurements are published.

The Sedimentation Velocity of Egg Albumin

Salt-free isoelectric egg albumin was prepared by Dr. Eloise Jameson, who followed Hopkins and Pinkers' method, using acetic instead of sulfuric acid. The material was twice crystallized and furnished us in a more or less solid form mixed with ammonium sulfate. This sample was dialyzed in running distilled water during the daytime and set aside overnight in the refrigerator in distilled water saturated with toluene. The first batch dialyzed was in the process perhaps a day and a half, at the end of which it gave no test for sulfate with barium chloride. The second batch was dialyzed in a similar manner but the process lasted about two and a half days, after which it gave no test for sulfate. The first batch tested 1.36% egg albumin as first prepared and was used only on run 1. Most of it was wasted in attempts to fill the baffles by capillarity. The second batch tested 5.4%. Portions of this were diluted and used in all the remaining runs. One portion approximately 1% was used on runs 2–8 inclusive, and another similar portion was used in runs 9–15 inclusive. All 15 runs on egg albumin were completed well within a month after the samples had been prepared.

Concentrations were determined refractometrically. The refraction of proteins (at least in the concentrations and concentration range studied) follows a linear law. The relation is given by

$$(n - n_1) = ac$$

where $(n - n_1)$ is the difference in refractive index of the solution and solvent, c is the grams of protein per 100 cc. of solution, and a the constant giving the change in refractive index of solvent on the addition of 1% protein. As to the nu-

merical value of a for egg albumin in 0 to 2% solutions, Haas[3] obtained a value of 0.00177 ± 0.00006 with a Pulfrich refractometer. Barker[4] using a Zeiss dipping refractometer obtained a value of 0.001851 ± 0.000020, and this result was used.

In assembling, care was taken to avoid entrapping air bubbles. Any liquid that was in the threads was wiped off before the rotor cone was screwed on. The assembled rotor was weighed before and after the run. This was a check not only upon the completeness of the filling, but also a proof that no evaporation occurred. The small volume of liquid above and below the insert was included in calculations in the outer non-immobilized liquid. Whether it was immobilized or not during the run was of no consequence for it ran together with the rest of the non-immobilized liquid when the rotor was taken apart for sampling.

The rotor was started at approximately 20 lb. (1.3 atm.) pressure and gradually speeded up to about 2000 r. p. s. This is easily done within a minute. In stopping the rotor, the pressure is gradually reduced to 20 lb., after which it was allowed to remain there and by placing the fingers around the rotor and exerting a slight pressure it was stopped quite smoothly. An estimated correction for the time in starting and stopping was made. An essential detail in stopping is not to allow the last few revolutions to end too abruptly. If this happens the non-immobilized liquid may have a tendency to swirl into the immobilized space and displace some of this liquid. However, only a moderate amount of care in this respect is needed.

Method of Calculation.—In the opaque ultracentrifuge all that is measured is the number or weight of molecules or particles that pass through a given radius during a given time. In complete contrast to the requirements for the transparent ultracentrifuge, it is a matter of indifference whether or not a boundary is formed or whether it is sharp or blurred by diffusion, as long as its influence does not extend to the external radius of the immobilizing disks.

Two methods of calculation were used. The first is a relation given by Tiselius, Pedersen, and Svedberg[5] requiring a knowledge of the initial concentration of the solution.

$$s_{(obsd.)} = -\frac{1}{2\,\omega^2 t}\ln\left(1 - \frac{2\Delta}{q\,x\,c_0}\right)$$

where ω = angular velocity = 2π (r. p. s.), t is the time in seconds, Δ is the change in amount of substance above or below the level at which the separation is made, x is the distance in centimeters of this level from the center of rotation (radius of larger disks), q is the cross-sectional area of the cell at this level, and c_0 is the original concentration within the cell. ω, t, x, and q are obtained readily from the dimensions of the baffles and observations during the run. c_0 is determined by analysis. The density of the dilute solutions used was considered unity within the experimental error. Δ in grams is then given by the following relation

$$\frac{|n_f - n_i|}{0.00185} \times \frac{V_1}{100}$$

where n_f and n_i are the refractive indices of the final and initial samples of outer liquid, respectively, and V_1 is the volume of the outer convecting liquid.

After sampling the outer liquid, the rotor was thoroughly cleaned and dried. The excess solution on the outside of the baffles was wiped off. The rotor was then reassembled and allowed to spin for a minute or so to force out the liquid in the baffles to give a second sample. Then a similar formula was applied to the change in concentration of the immobilized liquid.

The second method involves calculation of the theoretical position of the idealized boundary within the immobilized liquid, as if there were no effect of diffusion. Here the boundary will be at a position x_2 between the radii a and b of the smaller and larger disks, respectively. The average concentration c_2 as measured over the whole of the immobilized liquid, V_2, between the radii a and b is determined by direct analysis of that liquid. It is likewise determinable by analysis of the outer convective liquid c_1, of total volume V_1, for

$$c_2 = \frac{(V_1 + V_2)c_0 - V_1 c_1}{V_2}$$

where c_0 is the original uniform concentration.

Since the concentration decreases with time in centrifugal sedimentation, the concentration c_t at the time t beyond the sedimenting boundary x_2 is related to the original concentration c_0 by the equation

$$c_t = \frac{a^2}{x_2^2}\,c_0$$

but the total volume of the immobilized liquid

(3) Haas, *J. Biol. Chem.*, **35**, 119 (1918).
(4) Barker, Dissertation, Stanford University, 1933.
(5) Tiselius, Pedersen, and Svedberg, *Nature*, **140**, 848 (1937).

TABLE I

SEDIMENTATION VELOCITY OF ISOELECTRIC EGG ALBUMIN IN WATER

(Method of Calculation 1)

Run	Speed, r. p. s.	Time, sec.	Temp., °C.	$(n - n_{H_2O}) \times 10^5$ Initial	Final	Δ, g.	$s_{obsd.} \times 10^{13}$	Correction factor	$s_{20} \times 10^{13}$
11—outer[a]	1855	4680	20.5	174	248	0.0056	3.73	0.988	3.69
12—outer	1920	4740	23.0	173	255	.0062	3.96	.926	3.67
—inner					53	.0058	3.61		3.34
13—outer	1953	3930	20.5	178	245	.0051	3.44	.988	3.41
—inner					67	.0053	3.66		3.62
14—outer	1997	4440	22.4	175	256	.0061	3.79	.940	3.56
—inner					52	.0059	3.62		3.40
15—outer[b]	1950	4140	20.5	178	258	.0056	3.69	.988	3.65
—inner					56	.0058	3.94		3.89

Average s_{20} from outer liquid 3.60

Average s_{20} from inner liquid 3.56

Average 3.58×10^{-13}

[a] No sample from inner liquid was taken. [b] In this run a metal disk was placed in the rotor to fill up the space between the top of the baffles and the pliofilm disk. In the other runs this space was taken up by solution. Volume of liquid in outer space in this run was 1.29 cc.

below the boundary is proportional to $b^2 - x_2^2$. Therefore the total amount of material present at the time t is proportional to

$$c_0(b^2 - x_2^2)\, a^2/x_2^2 = c_2(b^2 - a^2)$$

where c_2 is again the average concentration that is measured over the whole of the immobilized liquid. From this equation the position of the boundary

$$x_2 = \sqrt{b^2H/(P + H)}$$

where H is $a^2/(b^2 - a^2)$, and $P = c_2/c_0$.

The sedimentation constant

$$s = \frac{dx}{dt}\frac{1}{\omega^2 x} = \frac{2.303\ (\log x_2 - \log a)}{39.48\ (\text{r.p.s.})^2\ t}$$

Results were given the usual correction of multiplying the observed value of s by

$$\frac{\eta}{\eta_{20}} \times \frac{1 - \bar{V}\rho_{20}}{1 - \bar{V}\rho}$$

where η and η_{20} are the viscosities of the solvent and of pure water at the observed temperature and at 20°, respectively, and ρ and ρ_{20} are the corresponding densities. $\bar{V}$ for egg albumin is taken as 0.749.

The following are the numerical constants of the cell used: $V_1 = 1.40$ cc., $V_2 = 0.89$ cc., $a = 0.797$ cm., $b = 1.20$ cm., $H = 0.789$, $q = 2.626$ cm.^{2}.

The Results.—The results are given in Tables I and II as calculated by methods 1 and 2, respectively, for runs 11 to 15 in which temperature control was used. It should be noted that in method 1 the concentration is explicitly derived from the refractive index, whereas in method 2 the observed increment of refractive index is used directly with no reduction to concentration.

TABLE II

SEDIMENTATION VELOCITY OF ISOELECTRIC EGG ALBUMIN IN WATER

(Method of Calculation 2)

Run	P	x_2	$s_{obsd.} \times 10^{13}$	$s_{20} \times 10^{13}$
11—outer	0.329	1.008	3.70	3.66
12—outer	.252	1.047	3.92	3.63
—inner	.306	1.019	3.56	3.30
13—outer	.410	0.975	3.40	3.36
—inner	.376	0.987	3.62	3.58
14—outer	.276	1.033	3.71	3.43
—inner	.297	1.023	3.57	3.36
15—outer	.347	1.000	3.65	3.61
—inner	.315	1.014	3.88	3.83

Average s_{20} from outer liquid 3.55

Average s_{20} from inner liquid 3.52

Average 3.54×10^{-13}

Summary

1. Simple opaque ultracentrifuges capable of measuring sedimentation velocity or equilibrium in all systems with accuracy are described. They require no optical accessories, and run at constant temperature if the driving air passes through a thermostat.

2. A simple two-piece rotor with liquid-tight seal is used with any suitable immobilizing insert of which several are described.

3. The sedimentation velocity of egg albumin in water at *p*H 4.63 was measured as $s_{20} = 3.56 \times 10^{-13}$ in agreement with Svedberg's most recent result[6] 3.55×10^{-13}.

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(6) Svedberg, *Ind. Eng. Chem., Anal. Ed.*, **10**, 125 (1938).