Magnetically Suspended Equilibrium Ultracentrifuge

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approximately 20 A/sec. The system can accommodate 18 3-×1-in. slides or more than 200 ½-×½-in. substrates for microelectronic circuitry.

Distribution of the Sputtered Deposit

In thin film experimental work, it is desirable to have a film of uniform thickness; in microelectronics work, it is mandatory.

The distribution of the sputtered deposit below the cathode is determined by measuring the sheet resistivity at various points on the sputtered film. This is accomplished by etching the dumbbell-shaped pattern, shown in Fig. 4(b), into a thin film of copper on 6-×1-in. slides (see Fig. 4). Accurate geometries and dimensions are achieved with photolithographic techniques. The slide then is positioned accurately under the tantalum cathode. After the films have been sputtered, the substrates are etched with a strong copper etch. The etch removes all the metal except the tantalum complementary pattern of the original copper pattern [see Fig. 4(c)]. The sheet resistivity distribution of the sputtered film below the cathode was determined and plotted (see Fig. 5) from the tantalum "resistor" measurements. These data indicate that distribution is uniform in the central 4-×4-in. area below the cathode.

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Magnetically Suspended Equilibrium Ultracentrifuge*

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An improved magnetically supported equilibrium ultracentrifuge is described for the determination of molecular weights and molecular weight averages. The rotor is magnetically suspended in a vacuum. The rotor is driven to operating speed by an electric motor or by an air turbine. The rotor is allowed to "coast" freely during the course of the sedimentation and loses about 1 rps per day. An improved Jamin interferometer is used to measure the concentration in the ultracentrifuge cell both before and after equilibrium has been established. A precision of the order of 0.1% is obtained for monodisperse substances over a very wide molecular weight range.

In the case of a large number of complex organic substances many of which are of importance in biology and medicine, it is difficult to measure their molecular weights with sufficient accuracy to characterize them properly. Among the several methods now in use for measuring these molecular weights, the centrifuge method has promise of considerable improvement of accuracy. This is especially true in the case of the equilibrium ultracentrifuge method which yields absolute values that are based upon equilibrium thermodynamic theory.[1] In this paper an improved magnetically suspended equilibrium ultracentrifuge[2] is described where the measured quantities in the ultracentrifuge have an over-all presicion of about one part in 10^3. In this equilibrium method for dilute solutions, the molecular weight M is given by the relation[1]

$$M=\frac{2RT\ln(C_2f_2/C_1f_1)}{4\pi^2N^2(1-\rho\bar{V})(r_2^2-r_1^2)}, \qquad (1)$$

where C_1 and C_2 are the concentrations at the radial distances r_1 and r_2 in the ultracentrifuge cell, N is the rotational speed in rps, T is the absolute temperature, R is the gas constant, f_1 and f_2 are the activity coefficients, ρ is the density of the solution, and $\bar{V}$ is the partial specific volume. The quantities f_1, f_2, and $(1-\rho\bar{V})$ are measured outside the centrifuge while all of the others are determined during the operation of the centrifuge. The equilibrium ultracentrifuge must be so constructed that the temperature can be held extremely constant for long periods of time and N must be constant or very slowly decreasing. It is especially important that "hunting" or other irregular speed variations in the rotor are absent.

Figure 1 is a diagram of the ultracentrifuge showing the magnetic support coils, the rotor, the centrifuge housing or vacuum chamber, and the air turbine drive. Figure 2 shows an electrical drive which is easily interchangeable with the air turbine drive. The magnetic support coil consists of three "doughnuts," each containing 12 000 turns of No. 22 insulated copper wire. A section is 24 cm o.d., 7 cm i.d., and 8 cm high. The sections are separated by brass plates 0.2-cm thick, each of which is soldered to circumferential copper coils which carry a cooling liquid.

* Supported by N.I.H. of P.H.S. Grant.

[1] T. Svedberg and K. O. Pedersen, *The Ultracentrifuge* (Oxford University Press, New York, 1940).

[2] J. W. Beams, J. D. Ross, and J. F. Dillon, Rev. Sci. Instr. **22**, 77 (1951); J. W. Beams, H. M. Dixon, A. Robeson, and N. Snidow, J. Phys. Chem. **59**, 915 (1955); J. W. Beams, Proc. Am. Phil. Soc. **101**, 43 (1957).

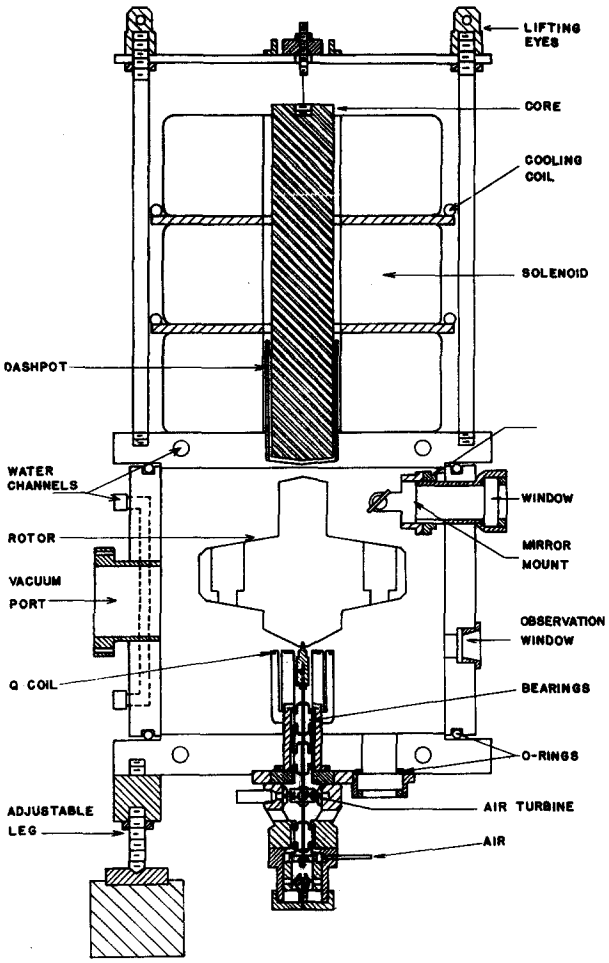

FIG. 1. Diagram of magnetically supported equilibrium ultracentrifuge.

The bottom coil surrounds a dash pot containing S.A.E. type 30 motor oil. The core of the solenoid is a soft iron rod 4.5 cm in diameter and 30 cm long suspended by a 0.0623 piano wire. The lower end of the core fits into the dash pot and is about 0.15 cm above the top of the vacuum chamber. This damps any horizontal motion of the rotor.

The nickel plated rotor, machined from a single piece of steel, is 19.02 cm in diameter and 14.3 cm high. It weighs 13.5 kg. There are four 2.7-cm channels cut 90° apart which contain the ultracentrifuge cell, the "chopper slit," and their counterbalances. Since most sedimentation equilibrium experiments are run at comparatively low speeds, the stress developed within the rotor usually is well below the bursting strength.

The brass vacuum chamber is both nonmagnetic and a good heat conductor. It is 22.2 cm high, 23 cm i.d., and has a wall thickness of 1.76 cm. Channels are cut in the sides of the chamber through which a cooling liquid is circulated to maintain a constant temperature. The top plate is 1.6 cm thick, 30.5 cm in diameter, with a well machined out of the surface in order to bring the iron core down close to the top of the chamber. Cooling liquid is circulated around this plate for temperature control. The bottom plate is 31.5 cm in diameter, 2.7 cm thick, with cooling liquid also circulating through it. This plate has two circular channels 90° apart, through which light passes. Glass windows, 4.1 cm in diameter and 0.8 cm thick, of excellent optical quality, are placed in these holes and sealed with Neoprene O-rings. The cylindrical housing contains a 6.2-cm vacuum port and five additional windows. Two of these are situated so as to permit light to enter and leave the vacuum chamber. Two others are placed so as to permit the operator to see both the top and bottom of the rotor when it is magnetically supported. The fifth window is used to provide lighting of the chamber when desired. The top and bottom plates are sealed to the cylindrical housing by 10-in. i.d. $\frac{1}{4}$-in. round Neoprene O-rings which are placed in grooves cut into the cylindrical housing. The vacuum chamber is evacuated with an oil diffusion and fore pump combination.

The air turbine drive is mounted on and below the bottom plate of the vacuum chamber. This drive is connected to the rotor through a small (0.25-cm diam) flexible steel shaft which passes through vacuum-tight oil gland bearings. During the acceleration period, the shaft is connected to the rotor by an Allen-type socket arrangement. When the desired operating speed is reached, compressed air is forced into the air piston which separates the shaft from the rotor, stops the turbine, and tightens the vacuum seal around the shaft. With the pressure in the vacuum chamber approximately 10^{-6} mm Hg, the centri-

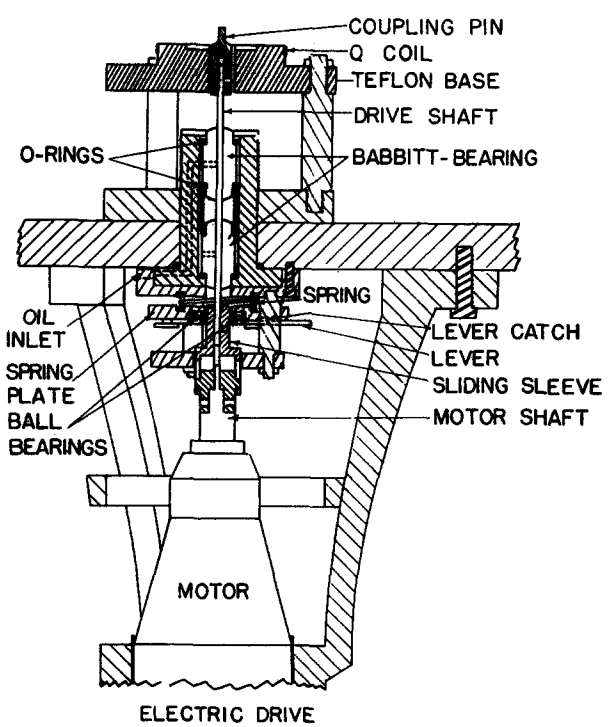

FIG. 2. Electric drive.

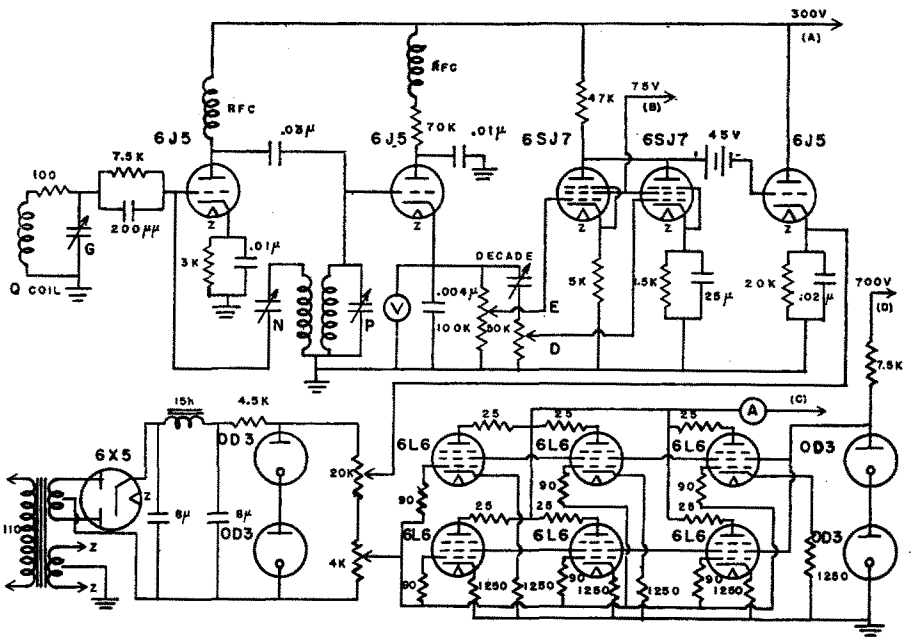

FIG. 3. Support circuit.

fuge rotor, when coasting freely, will lose only about one rps per day. It is, therefore, allowed to "coast" freely during the sedimentation period. This air turbine drive contains only minor modifications of one previously described in detail.[2] Although this drive serves the purpose very effectively, it is "noisy" and requires auxiliary air compressors, etc. Consequently, for most experiments it has been replaced by an electric motor drive shown in Fig. 2. The ½-hp electric motor operates from the standard 110-v 60-cycle power lines and its speed can be regulated by a simple Variac arrangement from 0 to 45 000 rpm. Both the motor and speed regulator may be obtained commercially[3] from firms which sell very high speed rotary grinders. A thin flexible shaft couples the motor to the rotor during acceleration and is decoupled upon attainment of the desired speed. A 1.45-cm shaft is fitted to the drive shaft of the motor and held by a set screw. A sliding sleeve 1.45 cm i.d. and 1.83 cm o.d. fits over the motor shaft and controls the vertical action of a thin flexible steel drive shaft which drives the rotor. The flexible shaft, 0.25 cm in diameter, has a hardened steel "coupling pin" attached to its upper end with a set screw. This coupling pin is hexagonally shaped and fits into a modified Allen set screw in the base of the rotor. This prevents any slipping in the coupling of the drive shaft to the rotor. A Teflon base plate holding the Q coil and a 0.15-cm stainless steel disk are mounted about 7 cm above the bottom plate of the chamber. The rotor, when not in support, sits on this base plate. The Q coil contains 10 turns of No. 22 wire and is connected to the electronic support system (Fig. 3) outside the chamber through Kovar seals and a low loss coaxial cable. The drive shaft turns in hard babbitt-lined bearings mounted on Neoprene O-rings. Low vapor pressure vacuum pump oil is used as a lubricant and provides sealing against air leaks. A disk-type support, which suspends the iron core, is used to move the rotor around until it is supported directly over the coupling pin. To engage the rotor, the spring plate is pushed up by hand against the spring and locked with the lever catch. The sliding sleeve is then slipped up over the motor shaft and the coupling pin is slipped into the rotor. The motor is then used to accelerate the rotor until the desired operating speed is attained. A decoupling arm trips the lever out of the catch and the spring action forces the coupling pin down and out of the rotor.

The rotor speed is measured by placing a photomultiplier tube in the path of the light as in Fig. 4. This tube receives a burst of light during each revolution of the rotor. The signal is amplified and applied to the vertical plates of an

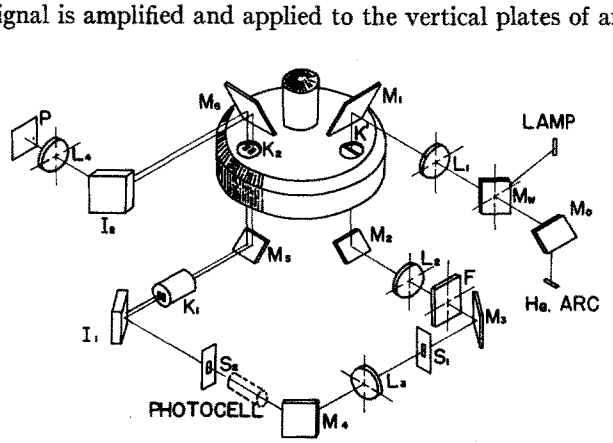

FIG. 4. Schematic diagram of optical system for measuring sedimentation.

[3] Obtained from Precise Instrument Company, Racine, Wisconsin.

oscilloscope. A signal from a generator, calibrated and monitored by WWV, is applied to the horizontal plates of the oscilloscope. The frequency of the rotor is obtained by matching the motion of the resulting Lissajous figures. The rotor frequency is chosen to be near one of the multiples or submultiples of the WWV broadcast frequency. The angular speed of the rotor is routinely measured to the nearest 0.01 of a cycle. The vacuum chamber pressure is measured by a discharge vacuum gauge.

Figure 3 is a diagram of the electronic support circuit which has been described previously.[2] The Q coil is in the grid circuit of a tuned grid-tuned plate oscillator which has a frequency of about 3 Mc. A downward movement of the rotor changes the impedance of the coil such that the current in the solenoid increases and the rotor rises. When in position, no vertical movement of the rotor can be detected, thus giving a very stable support position. Following the tuned plate-tuned grid oscillator there is a detector stage from which an error signal and its time derivative are obtained. These are amplified separately and combined on the grid of a cathode follower which controls the current to the power tubes, whose load is the support solenoid. The very stable support obtained at both high and low speeds is particularly advantageous in this work. For example, in the case of large macromolecules speeds as low as 3000 rpm are required and with this support system very smooth operation is obtained.

The absolute temperature can be obtained to less than one part in 10^4. Calibrated thermometers are used at exit and entrance of the water into the chamber walls and the temperature in the water bath is controlled by a precision temperature controller. This has a control accuracy of better than 0.01°C.

Figure 5 is a diagram of the ultracentrifuge double sector

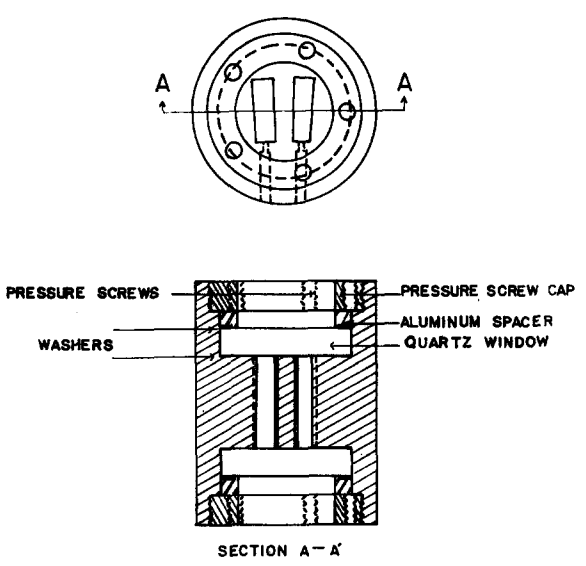

FIG. 5. Ultracentrifuge cell.

cell which contains the material to be centrifuged. The cells are 2.7 cm in diameter and 3.7 cm high machined out of Duraluminum. After construction, the cells are anodized or gold plated to prevent interaction between the solutions and the compartment walls. The compartments holding the solution under test are sector shaped subtending an angle of 2.4° at the axis of the rotor. The cell depth is about 1 cm and the radial length of the cell varies according to the solution under test. Radial cell lengths of 0.3, 0.5, and 0.8 cm have been used in this Laboratory. For some compounds, especially proteins, the shorter time required to attain equilibrium with the short cells prevents denaturation of the compound. However, if the compound does not decompose in a day or so, the longer the cell the better the accuracy in determining the molecular weight. The sector shaped cells prevent convective flow in the solutions during sedimentation and make possible more accurate measurements of concentrations. Viton A washers provide tight seals between the grooved (0.004 cm in depth) cell ends and the crystal quartz windows which have their optic axis perpendicular to their optically flat parallel faces. The Viton washers also seal the solution cell compartment from that of the solvent. Aluminum spacers and pressure screw caps transmit pressure to the quartz windows. Pressure screws are used to apply the final pressure to the windows. It has been found that in this way distortion of the quartz windows can be eliminated effectively. The optical system first is aligned without the quartz windows, the solution and quartz windows next are added to the cell and the pressure screws adjusted to give the same optical pattern as obtained previously. The Viton A washers have provided excellent sealing of the solution compartments.

Figure 4 is a diagram of the optical system using the Jamin interferometer plates which also has been modified considerably since the last report.[2] The Jamin plates used are optically flat glass plates 30% aluminized on the front surface and 100% aluminized on the back surface. Interference fringes are formed by superposition of the two split beams. In addition to the 1200-w mercury arc lamp, which has been used with a filter for a monochromatic source, a 1500-w projection lamp is used as a white light source. The two light sources can be alternated during an experiment by moving mirror Mw. The white light source and the mercury arc source are situated at equal distances from lens L_1, which focuses the light on the "chopper slit" K_1, mounted in the rotor, following reflection from mirror M_1, mounted on the centrifuge housing. After passing through the rotor the light is reflected by M_2 and focused on the first of two adjacent perpendicular slits S_1. This provides a small intense point source of light. A collimating lens L_3 renders parallel the light which is reflected from M_4 and which is further limited by slit S_2 before incidence on the first Jamin plate I_1. This beam splitter provides two parallel beams, each of which passes through a sector

section of a compensating ultracentrifuge cell K_1 identical in all respects to the cell in the rotor, except that the compartments containing the solution and solvent are reversed. This gives identical optical paths for the two beams of light which pass through the compensating cell and the cell in the rotor. Upon reflection from M_5 one beam of light passes through the solvent and the other through the solution in K_2. After passing through the cell the light is reflected from M_6 and impinges on the second Jamin plate, where interference of the light takes place. The light is then focused by L_4 on Kodak spectroscopic plates 103 AG which are sensitive to the green light of mercury of wavelength 5460.7 A. The concentration gradient formed by the centrifugal field is interpreted as an index of refraction gradient by the optical system. Since $\Delta n\lambda = \Delta ua$, where Δn is the number of fringes formed in the cell, λ the wavelength of the monochromatic light, Δu the change in the index of refraction of the solution under test, and a the depth of the cell compartments, whenever the product Δua changes by a wavelength an additional fringe is formed in the cell. With the Jamin interferometer now in use, one adjusts the fringes initially, before the rotor is accelerated, so that the fringes are parallel to the radius of the cell. Since the light is parallel, adjustment of the Jamin plates will bring into view a white light fringe which can be made to cover the entire field of view. In this way all fringes formed in the cell upon centrifugation are due to the concentration gradient and the fringes lie along lines of constant radial distance. The concentration at each point in the cell is obtained by knowing the refractive increment $k = \Delta u/\Delta c$, where c is the concentration. This ratio is measured outside the cell using an Abbe refractometer, a differential refractometer, or an interferometer. Work is currently under way in this laboratory on the development of a Michelson-type interferometer for determination of this refractive increment with increased precision. The relation $\Delta c = (\lambda/ka)\Delta n$ gives the change in concentration from point to point in the cell.

For a monodisperse ideal solution, Eq. (1) becomes

$$C_r = \frac{C_0 AM(b^2-a^2)\exp\langle -AM(b^2-r^2)\rangle}{1-\exp\langle -AM(b^2-a^2)\rangle},$$

where

$$A = (1-\bar{V}\rho)4\pi^2N^2/2RT.$$

This relation expresses the concentration at any point r within the cell as a function of the original uniform concentration c_0.

Archibald[4] and others[5] have shown, and data obtained in this Laboratory[2] indicate, that after a period considerably less than the equilibrium time the concentration remains essentially constant at a radius in the cell called the "hinge" point. This concentration is equal to the initial uniform concentration. Since by initially using the white light source a single white light fringe covers the entire field of view, the location of this white light fringe when the ultracentrifuge is spinning will give the radial distance r in the above formula, where $c_r = c_0$. The molecular weight can then be found by solving the equation

$$1-\exp\langle -AM(b^2-a^2)\rangle = AM(b^2-a^2)\exp\langle -AM(b^2-r^2)\rangle$$

for M. This, of course, does not require the attainment of equilibrium before obtaining the molecular weight. A special microphotometer is under development to measure accurately the position of this white light fringe with respect to the ends of the cell. Upon completion of this, the above method of using white light fringes to obtain very precise data appears promising.

Currently monochromatic fringes are used to measure the concentration gradient within the cell. From the photographs the position of each fringe is plotted against the number of fringes. By extrapolation to the ends of the cell, the change in the number of fringes between any two points in the cell can be determined. From these data the change in concentration between any two points in the cell is obtained. It should be noted that with this type of interferometer there is a point-to-point relation between the cell and the photographs. Numerical integration using the relation

$$\int_a^b c_r r dr = \int_a^b c_0 r dr,$$

which expresses the fact that the mass of the solution in the cell does not change upon centrifugation, gives the point in the cell along the radius where the concentration is equal to the initial uniform concentration. Once this is known, a plot of $\ln c$ vs r^2 can be made. This yields a straight line for a monodisperse solution at equilibrium.

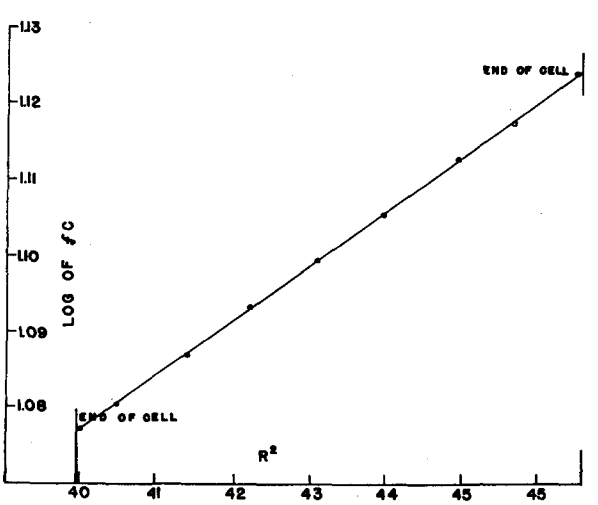

FIG. 6. Sucrose. Log of fc vs R^2.

[4] W. J. Archibald, J. Phys. Chem. **51**, 1602 (1947).
[5] D. F. Waugh and D. A. Yphantis, J. Phys. Chem. **57**, 312 (1953).

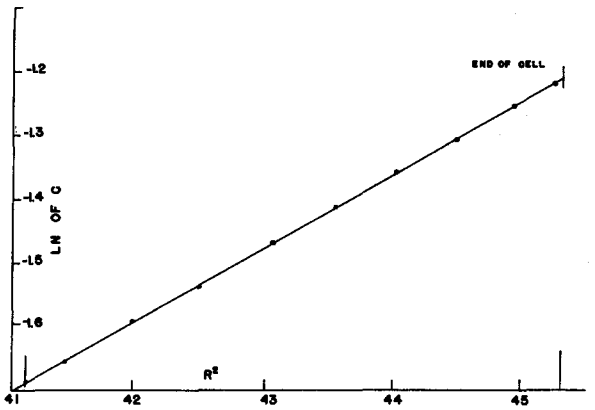

FIG. 7. Ribonuclease. Log of concentration vs R^2.

The rotor speed is known to about one part in 10^5, the temperature to at least one part in 10^4 and C_1/C_2 in Eq. (1) to better than one part in 10^3. The quantities $(1-\rho\bar{V})$ and the activity coefficients are measured outside the centrifuge and usually are the least precise quantities which are measured. As examples of the data obtained, Fig. 6 shows a plot of the values of $\ln fC$ vs R^2 for sucrose (N.B.S. Lot No. 5706) and Fig. 7 gives a similar plot for chromotographically pure ribonuclease obtained from Sigma Chemical Company. It will be observed that these plots are straight lines which is in accord with the theory [Eq. (1)] for a monodisperse substance at equilibrium. From the slope of this line the molecular weight can be determined using the known values of the activity coefficients and $(1-\rho\bar{V})$. Typical results and conditions of experiments are listed in Table I. c_0 is the original uniform concentration in g/dl, t is the time in hours for duration of the experiment, N is the frequency of the rotor in rps, $\bar{V}$ is the partial specific volume, r_2-r_1 is the radial cell length, and M_{av} the molecular weights obtained.

When the plot of $\ln fc$ vs r^2 is not straight but concave upward polydispersity in the solution is indicated. If concave downward it indicates that the solution is not ideal.

TABLE I. Results and conditions of experiments.

Substance	c_0	t	N	$\bar{V}$	r_2-r_1	M_{av}
Sucrose	3	24	400	0.618	8	342.02±0.58
Ribonuclease	0.3	14	220	0.695	3	13,665±23

It should be noted that from the above type of data, in addition to the molecular weight of monodisperse substances, weight average and so called Z values can be obtained for polydispersed systems. It will be noted that the ribonuclease solutions, although probably polydisperse, are essentially monodisperse solutions as indicated by the straight line in Fig. 7.

Experience with this magnetically suspended vacuum-type ultracentrifuge indicates that the time required for equilibrium to occur is much shorter than that given by Weaver's now classical rule.[6] This results from the fact that the rotor speed is decreasing very slowly and from other factors in the experiments which differ from those assumed by the theory. Archibald[7] has treated the theory for the case where the speed is very slowly decreasing and found a considerable decrease in the time required to reach within less than 0.1% of equilibrium, while van Holde and Baldwin[8] have discussed the case where the speed is constant. These theoretical considerations predict much shorter times for attainment of equilibrium which is in accord with our data. For macromolecules such as ribonuclease and insulin, equilibrium is attained in less than a day for a 3-mm cell and in approximately 4 hr for sucrose. Previously a principal objection to the equilibrium method was the long time required for equilibrium to take place. Now with the reduced time required for equilibrium and the increased reliability of the ultracentrifuge the magnetically supported equilibrium ultracentrifuge described above should find wide use.

[6] W. Weaver, Phys. Rev. 27, 499 (1926).
[7] W. J. Archibald (private communication).
[8] K. E. Van Holde and R. L. Baldwin, J. Phys. Chem. 62, 734 (1958).