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Equilibrium Ultracentrifuge for Molecular Weight Measurement

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The equilibrium centrifuge method of determining molecular weights[1] of many substances has several inherent advantages when accuracy is required. In the first place, the method is based upon equilibrium thermodynamic theory and, therefore, yields reliable values. Second, it gives absolute values of the molecular weights directly which essentially are independent of the shapes of the molecules. Third, it does not require a knowledge of the diffusion constant, viscosity, etc. Fourth, it gives precise values over a very wide range of molecular weights (50 to 10^8 molecular weight units) and fifth, the centrifugal fields and rotor speeds required are comparatively low. This simplifies the rotor design and makes it possible to practically eliminate window strains and other effects that otherwise would reduce the precision of the optical system, with which the sedimentation is determined. On the other hand, the method has not been widely used because it required centrifuging for very long times at a uniform known temperature and with a uniform or very slowly decreasing rotor speed that is free from "hunting." In this paper, an improved magnetically suspended equilibrium ultracentrifuge[2] is described which is essentially free of the above objections.

In the equilibrium method, for dilute substances which obey Henry's Law, 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 respectively, N is the rotational speed in rps, T the absolute temperature, R the gas constant, ρ is the density of the solution, $\bar{V}$ the partial specific volume, and f_2 and f_1 are activity coefficients. The above quantities are determined during the operation of the centrifuge except $(1 - \rho\bar{V})$, f_1, and f_2, which are measured outside the centrifuge.

Figure 1 is a diagram of the ultracentrifuge showing the rotor, the vacuum chamber, the magnetic support solenoid, and the connections to the motor drive. This apparatus has some important improvements over that previously reported[2] and will be described somewhat in detail although many of the parts remain unchanged. The nickel plated rotor machined

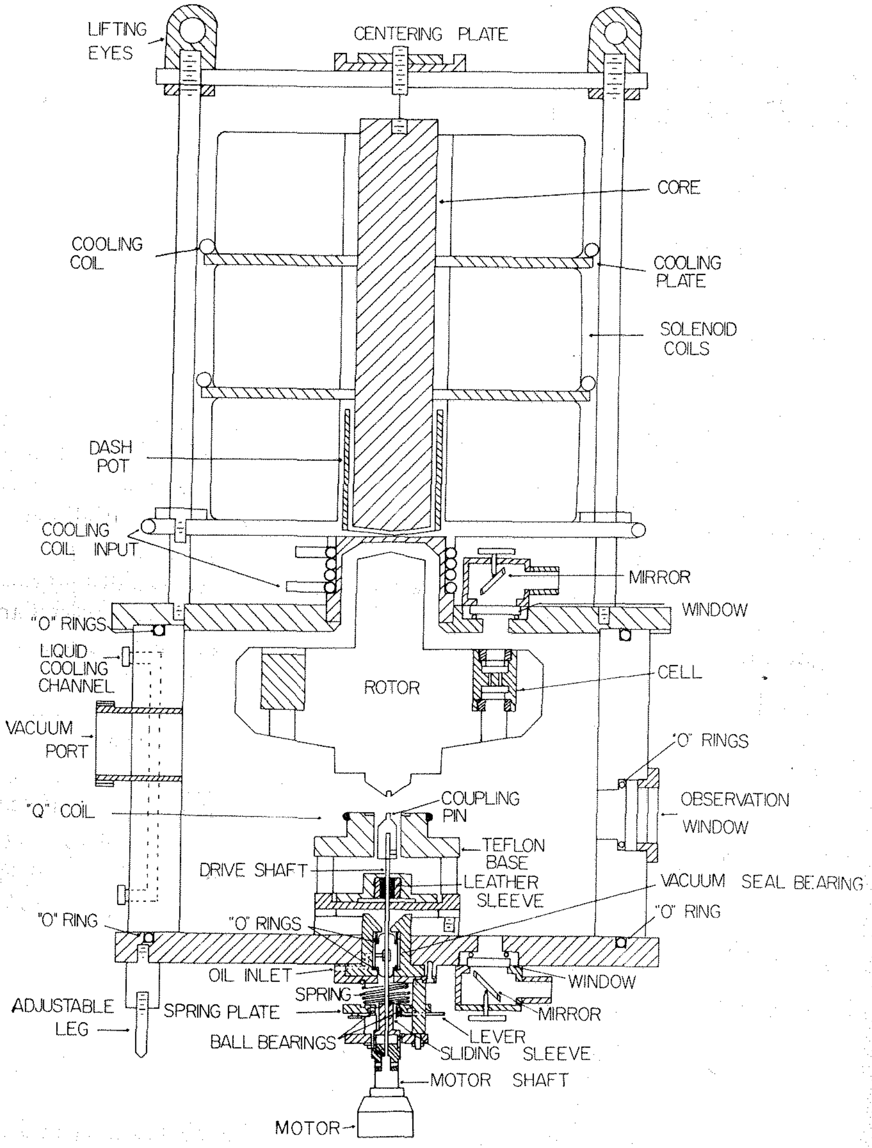

Fig. 1. Cross-sectional diagram of equilibrium ultracentrifuge.

from a single piece of high strength alloy steel is 19.02 cm. in diameter, 14.3 cm. high, and weighs 13.5 kg. It contains four 2.7-cm. channels parallel to and 6.5 cm. from the axis of rotation and spaced 90° apart which contain the ultracentrifuge cell, the chopper slit, and their respective counterbalances. The vacuum chamber is made of brass because it is both nonmagnetic and a good heat conductor. The base is 23 cm i.d., has a wall thickness of 1.76 cm., and is 19.5 cm. high. The upper cylindrical neck is 6.5 cm i.d., wall thickness of 1.8 cm., and 4.5 cm. high. The bottom plate is 32 cm. in diameter, 2.7 cm. thick. It has two circular channels 90° apart closed by flat optical glass windows 4.1 cm. in diameter and 0.8 cm. thick through which the light passes. The upper plate of the neck is 1.7 cm. thick, with wells machined out of the surface in order to bring the iron core down closer to the top of the rotor. The top plate of the base has the same thickness as the bottom plate and contains plane optical glass windows vertically aligned with those in the lower plate. The temperature of the chamber is maintained at the desired value by cooling liquid flowing through channels in good thermal contact with all of the walls of the chamber as indicated in Figure 1. The cylindrical chambers are sealed to the end plates with $^{1}/_{4}$ in. round neoprene O-rings which are placed in grooves cut into the housing. The windows also are sealed with neoprene O-rings. The cylindrical chamber contains a 6.5-cm. vacuum port through which it is evacuated by a liquid nitrogen or solid CO_2–acetone cold trap, diffusion and rotary forepump combination. The pressure in the chamber is measured by an ionization gage or a gas discharge vacuum gage. The cylindrical chamber also contains two windows used for observing the rotor when desired. The magnetic support solenoid coil is made of three insulated doughnuts, each containing about 12,000 turns of No. 22 copper wire placed one above the other. The doughnuts are separated by brass plates, 0.2 cm. thick, each of which is soldered to circumferential copper tubes which carry the returning cooling liquid. The bottom doughnut 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. It is suspended by a 0.0623 piano wire. The lower end of the core can swing like a pendulum in the oil dashpot and thus damps any horizontal motion of the rotor. The rotor is driven by a $^{1}/_{2}$-hp. electric motor which operates from the standard 110-v. 60 cycle power lines. Its speed can be regulated by a Variac arrangement from 0 to 60,000 rpm. These motors are used to drive high speed rotary grinders, and both the motor and speed regulator can be obtained commercially (Precise Instrument Company, Racine, Wisconsin). A thin (0.25 cm.), flexible shaft which passes through a vacuum tight oil gland bearing couples the motor to the rotor during acceleration. It is decoupled when the desired speed is attained, and the rotor "coasts" freely during the process of sedimentation. A hexagonal coupling pin fastened to the upper end of the flexible shaft fits into a modified Allen-type set screw in the base of the rotor while the rotor is being accelerated. To connect the shaft to the rotor, the clamp spring is compressed by hand and locked with the lever catch. The sliding sleeve is then slipped up over the motor shaft and the coupling pin is fitted into the rotor. This is all done from below the chamber. When the rotor reaches the desired speed, the decoupling arm is used to pull the lever out of the catch and the compressed spring forces the coupling pin down and out of the rotor.

When not supported by the solenoid, the rotor rests on a 0.15-cm. thick, stainless steel disk which is mounted on a Teflon base as shown in the figure. This Teflon base also supports the sensing or Q coil and a loose-fitting

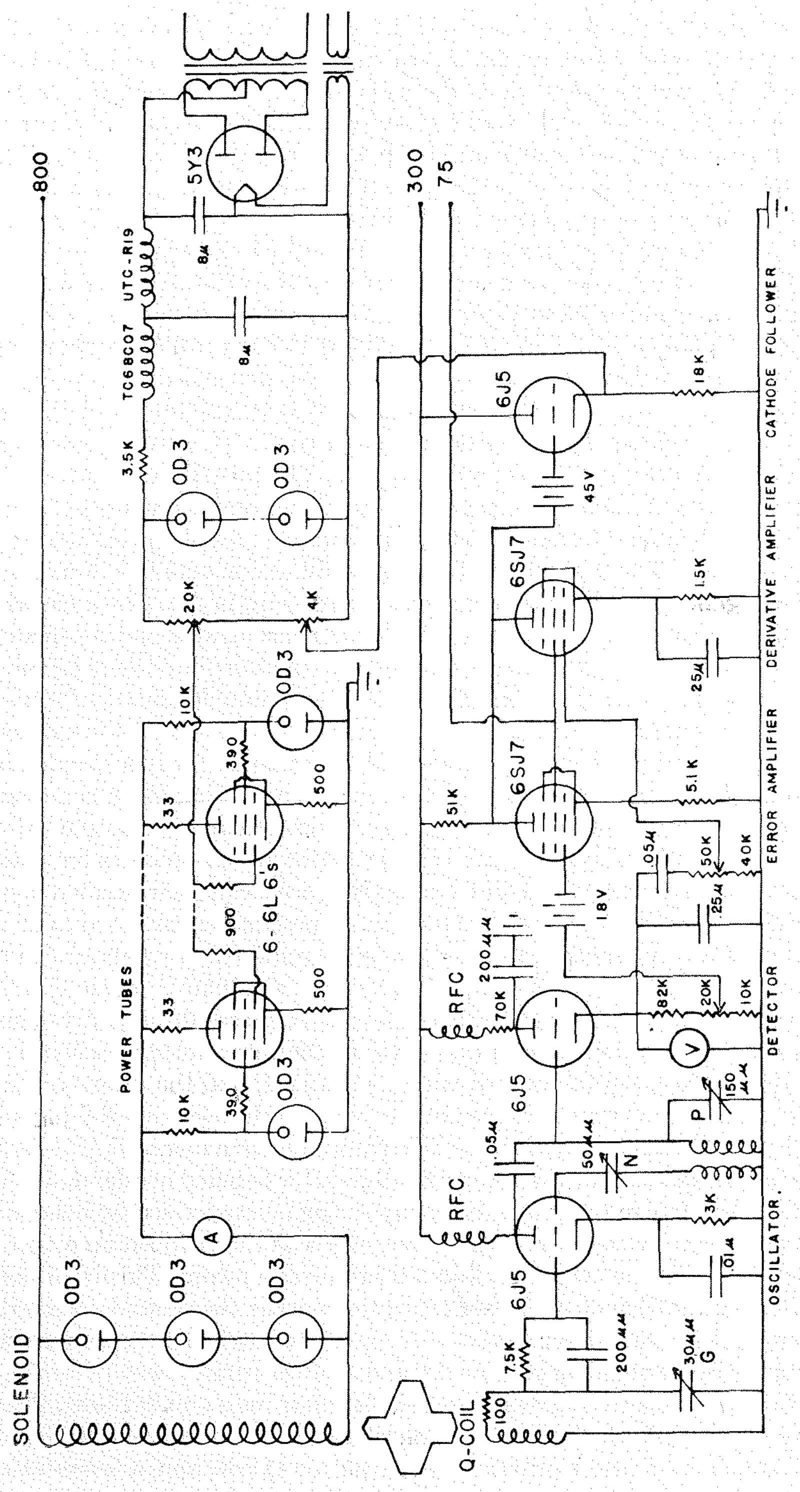

Fig. 2. Support circuit.

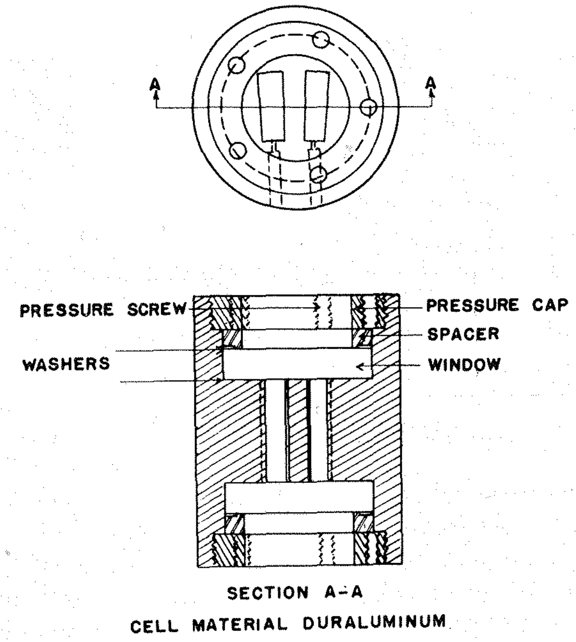

Fig. 3. Ultracentrifuge cell.

leather collar sleeve mounted upon a sliding plate which serves to damp any vibrations of the shaft. The Q coil contains 10 turns of No. 22 wire and is connected to the electrical support system through Kovar seals and a low loss coaxial cable. The electrical support system is shown in Figure 2. The sensing coil is in the grid circuit of a tuned-grid tuned-plate oscillator which has a frequency of from 2 to 5 Mcycles/sec. Following the oscillator is a detector stage which produces an electrical signal if the rotor moves up or down with respect to the sensing coil. This signal together with its time derivative are amplified and applied to the grid of a cathode follower which in turn controls the power tubes whose load is the support solenoid. When properly adjusted, this electronic circuit maintains the rotor at the desired vertical position. The circuit has been found to be most reliable and no vertical motion of the rotor can be observed with a microscope. A detailed description of this circuit has been given previously.[2]

The absolute temperature is maintained to less than one part in 10^4 by cooling liquid from a thermostated bath flowing through the cooling coils in the walls of the vacuum chamber. The temperature in the bath is controlled to at least 0.01°C. and the temperature measured by thermometers at the entrance and exit of the liquid to the chamber.

The ultracentrifuge cell is shown in Figure 3. It is 2.7 cm. in diameter and 3.7 cm. high and made from Duralumin. It contains two sector-shaped compartments, each of which subtends an angle of 2.4° at the axis of the rotor, has a depth of approximately 1 cm. and a radial length which may vary from 1 mm. to 12 mm. One of these compartments contains the

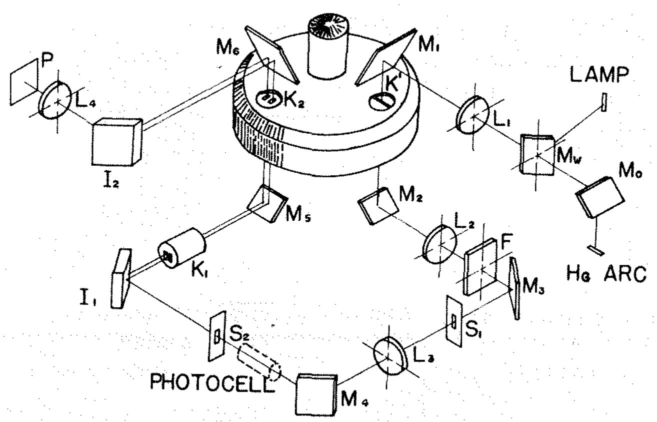

Fig. 4. Diagram of optical system for measuring sedimentation.

solvent and the other the solution of the substance to be centrifuged. The metal parts of the cell are anodized or gold-plated to prevent interaction between the solutions and the compartment walls. In some cases, Kel-F cells are used instead of the Duralumin. The two cell compartments are sealed on each end by a crystal quartz window which has its optic axis perpendicular to its optically flat parallel surfaces. Viton A washers provide tight seals between the windows and the grooved (0.004 cm. in depth) cell ends as well as between the two compartments. The pressure is applied to the windows by screw caps and pressure screws.

The rotor speed is measured by a photomultiplier tube placed (as shown dotted in Fig. 4) so that it receives a light signal during each revolution of the rotor. The resulting electrical signal is amplified and applied to one pair of plates of an oscilloscope while a signal from a generator calibrated and monitored by National Bureau of Standards Station WWV is applied to the other pair of the oscilloscope plates. The angular speed of the rotor is determined by the Lissajous figures formed on the oscilloscope screen and the known frequency of the generator. The rotational speed may be measured to one part in 10.[6]

The sedimentation in the cell is measured by a Jamin interferometer which is used with parallel light as shown in Figure 4. Light from a 1200-w. mercury capillary arc lamp is focused by the lens L_1 on the chopper slit as *K'* mounted in the rotor with its length along the radius. This chopper slit allows the light to pass through the optical system only while the rotor turns through a very small angle. The light is then focused on the slit S_1, rendered parallel by the lens L_3 and limited by the wide slit S_2 before incidence upon the Jamin plate I_1. The Jamin plates are matched, optically flat glass interferometer plates 30% aluminized on the front surface and 100% aluminized on the back surface. I_1 splits the light beam into two parallel beams, each of which traverses one of the sector compartments of the compensating centrifuge cell K_1. The two beams are next reflected by the optically flat front surface mirror M_5 through the centrifuge cell K_2 mounted in the rotor. K_1 and K_2 are identical in every respect except that the beam which traverses the compartment containing the solution in K_1 passes through the compartment containing the solvent in K_2 and vice versa. After leaving K_2, the two beams are reflected by the optically flat front surface mirror M_6 to the second Jamin interferometer plate I_2, where they are recombined to produce interference. The interference fringes are focused on a photographic plate by the lens L_4. The photographic plates used are Kodak spectroscopic plates 103 AG which are sensitive to the green light of mercury. The light filter F which removes all of the light from the mercury arc except the wavelength 5460.7 A. is placed in the beam only when monochromatic light is required. In addition to the mercury arc, a 1500-w. incandescent projection light is used as a source of white light. The two sources are equal optical distances from the lens L_1 and can be alternated during an experiment by moving M_w into or out of its position.

The optical system is adjusted initially while the rotor is at rest. The Jamin plates are adjusted with monochromatic light so that the fringes are accurately parallel to the radius of the cell and very broad. White light is then substituted for the monochromatic light and the Jamin plates adjusted to give a single broad white light fringe which covers the entire field of view on the plate P. Since the light is parallel between the Jamin plates, the centrifuge cell and the fringes are in focus on the photographic plate. The centrifuge is then started, and any concentration gradient produced by the centrifugal field in the compartment of K_2 containing the solution will give fringes which lie along lines of constant radial distance. Since a change in concentration produces a change in the refractive index, if Δn is the number of fringes produced on the plate P, Δu the change in refractive index of the solution under test, and L the depth of the cell, then $\Delta n\lambda = \Delta uL$. The change in concentration Δc in the cell is given by the relation $\Delta c = (\lambda/Lk)\Delta n$, where λ is the wavelength of the light and $k = \Delta u/\Delta c$. k is measured outside of the centrifuge by an Abbe refractometer, a differential refractometer, or a Michelson-type interferometer and in most cases is approximately constant at least over comparatively small ranges of concentrations. The position of the fringes on the plate are measured both by a Mann comparator and a special microphotometer. If the position of each fringe produced by monochromatic light on the photographic plate P is plotted against the number of fringes and the curve is extrapolated to the ends of the cell, the change in the number of fringes between any two points in the cell is determined. This gives the change in concentration between any two points in the cell. Although the Hg arc is satisfactory as a source of intense monochromatic light and good fringes can be photographed in a few seconds, recently investigations of the possibility of using a LASER as a source have been undertaken in our laboratory. Such a source gives very intense monochromatic coherent light and the photographs probably could be taken with a single flash. The optical system shown in Figure 4 is used because, first, it gives good precision. Second, all of the

TABLE 1

Substance	c_0, g./dl.	t, hr.	N, rps	$1 - \bar{V}\rho$	$b-a$, cm.	M_{av}
Sucrose	3.0000	24	400	0.376	0.8118	342.02 ± 0.58
Ribonuclease	0.3000	14	220	0.301	0.3025	13,665 ± 23
Raffinose	3.0000	25	400	0.378	0.8118	503 ± 2
Insulin (dimer)	0.2000	12	250	0.260	0.3025	11,762 ± 25
Insulin (monomer)	0.4000	9	300	0.267	0.3025	5,800 ± 18

components are available commercially at a reasonable cost. Third, the fringes and the cell are in good focus and there is a point to point correspondence between the cell and fringes; i.e., the fringe pattern gives the concentration gradient in any direction in the cell and not just along the radius as in the Raleigh type interferometer. This is most useful in detecting any convection in the cell due to such things as rotor "hunting" or misalignment of the cell. Fourth, any strains in the windows of the cell introduced by the centrifugal field are automatically compensated to a first approximation by the double sector compartment cell; and fifth, when the adjustment has been carried through a few times it can be made in a few minutes. Also, if properly mounted, the system remains in adjustment indefinitely.

As mentioned above, the centrifuge rotor is first spun to operating speed and then allowed to coast freely during the period of sedimentation. When the gas pressure in the vacuum chamber is about 10^{-6} mm. of Hg, the rotor will lose only about one part in 10^8 of its speed per second, which is independent of its speed. This, in addition to the complete absence of "hunting," makes it possible to get excellent precision even at very low rotor speeds where the sedimentation is easily disturbed. We have used as low as 20 rps in the molecular weight measurement of very heavy molecules. Equilibrium in the centrifuge cell is determined when the position of the observed fringes stop changing. When equilibrium is established for a monodisperse ideal solution, the concentration c_r at a radius r is, from eq. (1),

$$c_r = \frac{c_0 AM(b^2 - a^2) \exp\left\{-AM(b^2 - r^2)\right\}}{1 - \exp\left\{-AM(b^2 - a^2)\right\}} \tag{2}$$

where

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

and c_0 is the original concentration in the cell, b is the peripheral radius of the cell, and a is the radius of the top or the meniscus in the cell. Since the mass of the solution does not change upon centrifuging

$$\int_a^b c_r r dr = c_0 \int_a^b r dr \tag{3}$$

Consequently, numerical integration of the experimental values gives the radial distance in the cell where the concentration is equal to the initial uniform concentration c_0. With this information, a plot of ln cf versus r^2 can be made. This is a straight line for a monodisperse solution at equilibrium. From the slope of this line, the value of the molecular weight can be determined from known values of $(1 - \bar{V}\rho)$ and the activity coefficients. As examples, Figure 5 shows a graph of ln c versus r^2 for chromatographically pure ribonuclease and Figure 6 a similar plot for pure sucrose (N.B.S. lot No. 5706). It will be noted that these graphs are straight lines. Results for a few substances are listed in Table I, where c_0 is the initial uniform concentration, t is the time of the duration of the experiment, N is the rotor speed, $b - a$ is the radial length of the cell, and M_{av} the molecular weights obtained.

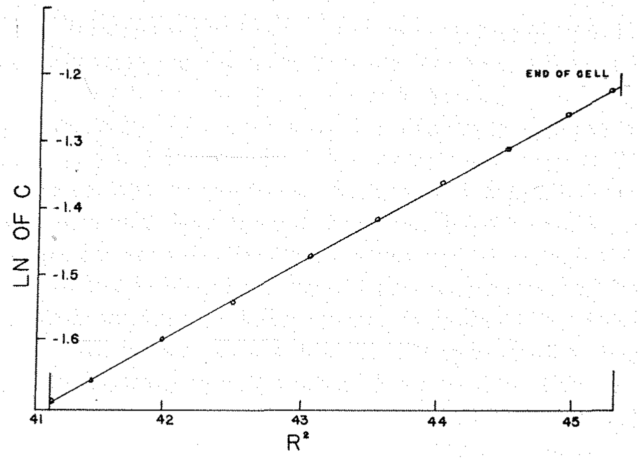

Fig. 5. Experimental values for ribonuclease.

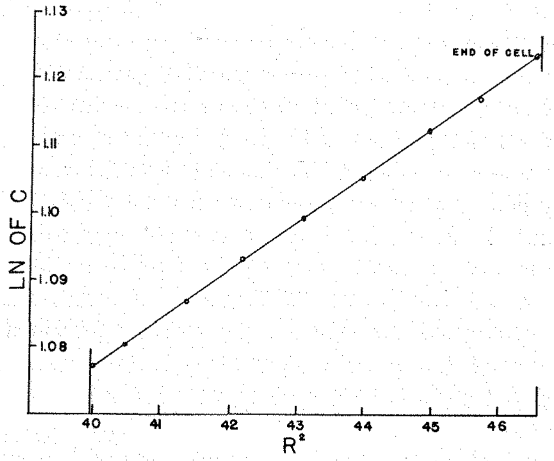

Fig. 6. Experimental values for sucrose.

The observed times required for equilibrium to be established in this magnetically supported vacuum type ultracentrifuge are much less than those predicted by the classical equation of Weaver.[3] This arises mostly from the fact that the rotor speed is decreasing very slowly. Both experiments and the theory show that the concentration distribution in the cell approaches a curve which differs by only a comparatively small amount from that of the equilibrium distribution in a relatively short time. It is the final approach to equilibrium which requires a very long time. As a result, the slowly decreasing rotor speed greatly reduces the time required for effective equilibrium to occur. Archibald[4] has shown that if

$$(1/N)(dN/dt)/4\pi^2N^2S \ll 1$$

where S is the sedimentation constant, then the equilibrium condition will remain within the limits of the precision of the experiment once it is established in the centrifuge cell.

When it becomes impractical to centrifuge for times as long as those shown in Table I, there are several methods which are adaptable to the equilibrium centrifuge described above which will drastically reduce the centrifuging time. Archibald[5] and Waugh and Yphantis[6] have shown and data obtained with the centrifuge described above confirm that after a time short in comparison with the equilibrium time the concentration at a radius in the cell called the hinge point remains essentially constant. This concentration is equal to c_0, the initial uniform concentration. Consequently, if white light is used in the optical system, a white fringe will occur at the radial distance r where $c_0 = c_r$. Equation (2) can be then written

$$1 - \exp\{-AM(b^2 - a^2)\} = AM(b^2 - a^2) \exp\{-AM(b^2 - r^2)\}$$

and the molecular weight found by solving this equation for M. This procedure, of course, does not require the establishment of equilibrium and can be carried out in a short centrifuging time. With the optical system shown in Figure 3 the position of the hinge point can in most cases be determined with precision so that the method is useful especially where the material being centrifuged deteriorates with time. Van Holde and Baldwin[7] have pointed out that the time required to attain equilibrium is roughly proportional to the square of the length of the cell and have proposed the use of short cells. Comparison of data obtained with the magnetically suspended equilibrium centrifuge described above with 8 mm., 5 mm., and 3 mm. cells shows this to be essentially true. The principal disadvantage in the use of short cells is the large reduction in precision as the cell length is reduced. Pasternak et al.[8] have considered the saving in time which may be achieved by starting with a step distribution of concentration. This initial step distribution is achieved by using a synthetic boundary cell. It was found to reduce the time required for equilibrium, but required the preparation of two solutions and some sacrifice of accuracy.

We have found that the time to reach equilibrium can be very much reduced by introducing a step function in the rotor speed early in an experiment, provided the rotor speeds and the times at which the change in velocity is made are properly chosen in accordance with a mathematical or experimental analysis of the approach to equilibrium. The theory of Mason and Weaver and of Weaver for settling of particles in a uniform gravitational field[3] is used in these calculations instead of the more exact Archibald theory[5] because it gives a precision sufficient for the purpose and does not require as extensive numerical calculations. The experimental procedure with the centrifuge shown in Figure 1 is to spin the rotor up to an angular velocity (ω_1) well above that (ω_2) where the attainment of equilibrium is desired. The rotor is then allowed to coast for a time roughly determined by theory or a previous experiment, its speed then reduced to (ω_2) where the sedimentation is allowed to come to equilibrium. As an example of the use of the above method, ribonuclease was centrifuged at a temperature $T = 293$°K. in a 3-mm. cell. At 220 rps the time required to reach effective equilibrium was 14 hr., as shown in Table I. However, when the rotor was spun at 350 rps for 1 hr. and then slowed down to 220 rps, the sedimentation reached equilibrium in about 65 min., i.e., about one eighth of the time shown in Table I, and without reducing the precision. The reduction in speed of the rotor must be accomplished very smoothly and without "hunting." It is best carried out by introducing helium at a pressure of a few millimeters of Hg into the vacuum chamber. This decelerates the coasting rotor rapidly and at the same time conducts the heat generated to the walls very quickly so that the rotor temperature at the lower speed is precisely known. If helium or hydrogen is not available, dry air serves the purpose if silicone oil is used in the diffusion pump. When the lower speed is reached, the gas inlet to the vacuum chamber is closed and the pumps allowed to evacuate the chamber very quickly so that the rotor coasts freely at the reduced speed. This method is precise and requires a relatively short centrifuging time. In addition to the above methods, the well known Archibald method[5,9] may be used which permits molecular weight determinations to be made during the transient states in the approach to equilibrium which, of course, reduces the centrifuging time.

The relatively high precision with which the molecular weights may be determined with the centrifuge described above makes it a sensitive instrument for detecting and measuring polydispersity in molecular weights. In fact, except in a relatively few cases, it has not been possible to obtain large molecular weight substances pure enough not to show some polydispersity. If the plot of ln fc versus r^2 is not straight but concave upward, polydispersity is indicated. If the curve is concave downward, there is a suggestion that the solution is not ideal.[9] Methods of correcting for nonideal solutions in a centrifuge have been discussed by Svedberg and Pedersen[1] and by Williams, Wales, and their collaborators.[10,11] Methods of determining the polydispersity of substances in solution from ultracentrifuge data have been discussed by Lansing and Kramer,[12] Svedberg and Pedersen,[1] Schachman,[9] and many others. If the different molecular weight species do not interact with each other in the solution, a concentration gradient for each particular species given by eq. (2) is set up in the centrifuge cell. Consequently the resultant concentration gradient is the superposition of all of the gradients of the different species. Since, in general, neither the molecular weights nor the concentrations of the different species are known, the solution is usually characterized by weight averages, number averages, and Z averages.[1,9,12] However, often by operating the centrifuge for different times and at different speeds, the molecular weight distributions can be roughly indicated. Because of the increased precision with which the resultant concentration across the cell can be determined with the centrifuge described above at various rotor speeds and times of centrifuging and because of the availability of fast computers, we believe that reliable values of molecular weight distributions can be found. The procedure is to estimate the values of the molecular weights and relative concentrations of each component from the various centrifuging data. Then substitution is made in the theoretical relations by use of the computer and the values obtained compared with the experimental data. It is necessary to employ successive approximations if only to check accuracy of the results. As an example, we have studied the relative concentrations of the monomer and dimer of insulin at various concentrations and various pH values and have found that it changes from roughly a monomer in 30% acetic acid containing $0.15M$ NaCl to approximately a dimer at pH 2.8 for a 0.2% solution in phosphate buffer. As checks on the method, we have tested various known mixtures of substances with known molecular weights, such as sucrose and raffinose in water solution, and found that experiment and theory are in excellent agreement. With the recently developed, very high intensity coherent LASER light sources now becoming commercially available, it may become possible in the future to determine the light scattering in a specially designed centrifuge cell as a function of the radius simultaneous with the sedimentation measurements discussed above. If so, this will add further information on the molecular weight distributions.

Finally, it should be noted that the magnetically suspended ultracentrifuge described above may, of course, be used for sedimentation velocity measurements as well as for the equilibrium determinations.

We wish gratefully to acknowledge our indebtedness to Dr. Bruce V. English for valuable assistance in the development of the interferometer, to Mr. F. W. Linke who constructed the ultracentrifuge, and to L. A. Sluga and C. E. Williams for assistance with some of the experiments.

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References

1. Svedberg, T., and K. O. Pedersen, *The Ultracentrifuge*, Oxford Press, London, 1940.
2. Beams, J. W., J. D. Ross, and J. F. Dillon, *Rev. Sci. Instr.*, **22,** 77 (1951); J. W. Beams, H. M. Dixon, III, A. Robeson, and N. Snidow, *J. Phys. Chem.*, **59,** 915 (1955); J. W. Beams, *Proc. Am. Phil. Soc.*, **101,** 43 (1957); J. W. Beams, R. D. Boyle, and P. E. Hexner, *Rev. Sci. Instr.*, **32,** 645 (1961).

3. Mason, M., and W. Weaver, *Phys. Rev.*, **23,** 412 (1924); W. Weaver, *Phys. Rev.*, **27,** 499 (1926).
4. Archibald, W. J., private communication.
5. Archibald, W. J., *J. Phys. Chem.*, **51,** 1602 (1947); *Ann. N. Y. Acad. Sci.*, **43,** 221 (1942).
6. Waugh, D. F., and D. A. Yphantis, *J. Phys. Chem.*, **57,** 312 (1953).
7. Van Holde, K. E., and R. L. Baldwin, *J. Phys. Chem.*, **62,** 734 (1958).
8. Pasternak, R. A., G. M. Nazarian, and J. R. Vinograd, *Nature*, **179,** 92 (1957).
9. Schachman, H. K., *Ultracentrifugation in Biochemistry*, Academic Press, New York, 1959.
10. Wales, M., J. W. Williams, J. O. Thompson, and R. H. Ewart, *J. Phys. & Colloid Chem.*, **52,** 983 (1948).
11. Wales, M., *J. Phys. & Colloid Chem.*, **52,** 235 (1948).
12. Lansing, W. D., and E. O. Kramer, *J. Am. Chem. Soc.*, **57,** 1369 (1935).

Synopsis

The magnetically supported vacuum-type equilibrium ultracentrifuge previously described has been improved and used for the measurement of the molecular weights of a number of substances. The modifications include an electric motor drive which accelerates the rotor to operating speed. The rotor coasts freely during the sedimentation, and the operating speed may have any desired value from a few revolutions per second to the maximum safe speed of the rotor. Improvements have been made in the ultracentrifuge cell, and both white light and monocromatic light fringes can be used interchangeably in the interferometer. These modifications make possible the determination of the molecular weight quickly either without waiting for equilibrium to take place or after equilibrium has been established. The overall precision is the order of one part in a thousand for the centrifuge measurements themselves. Molecular weight data are given for a number of substances, including sucrose, ribonuclease, and insulin. The application of the centrifuge to the determination of molecular weight distributions is discussed.

Résumé

L'ultracentrifuge à équilibre fonctionnant sous vide par support magnétique, qui a été précédement décrite, a été perfectionnée et utilisée pour des mesures de poids moléculaires d'un certain nombre de substances. La modification comprend un moteur électrique qui accélère le rotor jusqu'à la vitesse opérationnelle. Le rotor tourne librement pendant la sedimentation et la vitesse d'opération peut avoir une valeur quelconque depuis un petit nombre de révolutions par seconde jusqu'à la vitesse maximum de sécurité du rotor. La cellule de l'ultracentrifuge a été perfectionnée, et on peut utiliser dans l'interféromètre indifféremment les franges obtenues en lumière blanche et en lumière monochromatique. Ces modifications rendent possible la détermination rapide du poids moléculaire sans devour attendre que l'équilibre soit progressivement atteint ou que l'équilibre se soit déjà établi. La précision globale est de l'ordre d'un pour mille pour les mesures de centrifugation elles-mêmes. Les poids moléculaires seront donnés pour un certain nombre de substances comprenant le sucrose, la ribonucléaset, l'insuline. L'application de la centrifugeuse à la détermination des distributions de poids moléculaires est discutée.

Zusammenfassung

Die früher beschriebene, magnetisch gelagerte Vakuum-Gleichgewichtsultrazentrifuge wurde verbessert und zur Messung des Molekulargewichts einer Anzahl von Substanzen verwendet. Die Änderungen beinhalten einen Elektromotorantrieb, der den Rotor auf die Arbeitsgeschwindigkeit bringt. Der Rotor bewegt sich während der Sedimentation frei und die Arbeitsgeschwindigkeit kann jeden beliebigen Wert von einigen wenigen Umdrehungen pro Sekunde bis zur maximal zulässigen Geschwindigkeit des Rotors annehmen. Auch die Ultrazentrifugenzelle wurde verbessert und es können Interferenzstreifen von weissem und monochromatischem Licht abwechselnd im Interferometer verwendet werden. Diese Änderungen ermöglichen eine rasche Bestimmung des Molekulargewichts, entweder ohne die Gleichgewichtseinstellung abzuwarten oder nach erfolgter Gleichgewichtseinstellung. Die Gesamtgenauigkeit liegt in der Grössenordnung von. 1% für die Zentrifugenmessungen selbst. Die Molekulargewichte werden für eine Anzahl von Substanzen, Rohrzucker, Ribonuclease und Insulin. Die Anwendung der Zentrifuge zur Bestimmung von Molekulargewichtsverteilungen wird diskutiert.

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Shear Dependence in Solutions of Fractionated Dextran: A Variable-Shear Capillary Viscometer for Use with Aqueous Solutions*

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I. INTRODUCTION

If two samples of the same polymer are identical except in respect to the extent of branching, the one that is more branched will have the lower intrinsic viscosity, $[\eta]$. This fact has been made the basis of a method for detecting and estimating branching in polymers.[1-3] The experimental data required are values of $[\eta]$ and of molecular weight M, the latter usually obtained from light-scattering measurements.

For a linear polymer and for a branched polymer all of whose fractions are similarly branched, a plot of log $[\eta]$ versus log M yields a straight line. Senti et al.[4] found, however, that the data they obtained with fractions of so-called "linear" dextran (B-512) gave a line showing a pronounced downward curvature in the high molecular weight region, the divergence from linearity being greater the higher the molecular weight. They attributed this anomaly solely to branching that increased in extent with increasing molecular weight.

Now it has frequently been observed that solutions of high polymers can exhibit non-Newtonian flow, and that the shear dependence of the viscosity becomes more noticeable as the molecular weight of the polymer increases. The measured intrinsic viscosity of such polymers depends on the mean rate of shear in the viscometer and is smaller at higher shear rates. Such shear dependence has been observed[5] with polystyrenes, for example, at molecular weights as low as 5×10^5.

The molecular weights of the dextran fractions investigated by Senti et al.[4] were as high as 9.5×10^6, and the measurements of viscosity were presumably made at finite shear rate. It might well be, then, that the decrease in intrinsic viscosity (below that expected for truly linear fractions), which they attributed to branching, could be at least partly due to a neglected shear dependence. Patat and Hartmann[6] have, in fact, found evidence for pronounced shear dependence of the viscosity in solutions of native dextran

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* Based in part on a thesis submitted by H. van Oene in October, 1958, in partial fulfillment of the degree M.Sc. of McMaster University.

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