Reports on Progress in Physics

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To cite this article: J W Beams 1941 *Rep. Prog. Phys.* **8** 31

HIGH-SPEED CENTRIFUGING

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§ 1. INTRODUCTION

In addition to its long use as a means of separating or purifying materials of different density, the centrifuge in recent years has become a powerful analytical tool for measuring sizes, weights and shapes of particles. Since, in general, the latter use is more exacting on the apparatus than the former, careful studies, both theoretical and experimental, of the centrifuging process have been made ; these have resulted in several fundamental advances. The principal theoretical advances have consisted in the derivation of the relations between the amount of sedimentation in dilute idealized solutions and the physical and chemical properties of the solvent and solute, together with the derivation of the relations for the separation of gases. Experimentally, the advances have been made mainly by increasing the effective centrifugal field and in preventing re-mixing of the material during the centrifuging process.

§ 2. THEORY OF SEDIMENTATION

The solution of the differential equation for sedimentation in an ideal dilute solution in a uniform gravitational field was first worked out by Mason and Weaver (1924). This theory, while not strictly applicable to the sedimentation in a centrifuge, where the field varies as the radius, may be used to give approximate results in a centrifuge where the radius of the centrifuge is large and the length of the sedimenting column small. The extension of this theory by Weaver (1926) is of considerable use in calculating the time required for equilibrium between sedimentation and diffusion to be established in a centrifuge.

The general differential equation for the sedimentation of uncharged particles or molecules in an ideal, dilute, incompressible solution enclosed in a sector-shaped centrifuge cell rotating with an angular velocity ω is, according to Lamm (1929 a, 29 b, 37),

$$\frac{1}{r}\frac{\partial}{\partial r}\left\{\left(D\frac{\partial c}{\partial r}-\omega^2 rsc\right).r\right\}=\frac{\partial c}{\partial t}, \qquad \ldots\ldots(1)$$

where r is the distance of the material from the axis of rotation, c is the concentration, t the time, D the diffusion constant, and s the sedimentation constant or the velocity of settling of the solute (or particles) in unit (gravitational) field. Faxen (1929, 36) and Soyten (1936, 37) have each given approximate solutions to equation (1) which, although indicating the possible error introduced into certain measurements, are usually not suited for numerical calculations. More recently Archibald (1938 a, 38 b) has obtained a complete solution to equation (1) and has been able to put it into a usable form. He first (1938 a) treated the case of a sector-shaped centrifuge cell extending to the axis of rotation, and afterwards (1938 b) worked out the case where the sector cell was bounded by any two coaxial cylindrical surfaces. Fortunately, in most centrifuging experiments it is possible to utilize the simple solutions of equation (1), derived many years ago by Svedberg (1928–39) * for special cases, rather than the more elaborate and detailed general solutions of Archibald. Svedberg has shown that if the centrifuging process is continued until equilibrium between sedimentation and diffusion is established, i.e., $\partial c/\partial t = 0$ in equation (1), and the change in free energy throughout the cell vanishes, then

$$M_e = \frac{2RT \log_e (c_1/c_2)}{(1 - V\rho)\omega^2(r_1^2 - r_2^2)}, \qquad \ldots\ldots(2)$$

$$v_e = \frac{2RT \log_e (c_1/c_2)}{N(\rho_p - \rho_d)\omega^2(r_1^2 - r_2^2)}, \qquad \ldots\ldots(3)$$

where M_e is the molecular or particle weight, v_e is the volume of the particle or molecule, c_1 and c_2 are the concentrations at distances r_1 and r_2 from the axis of rotation respectively, V is the partial specific volume of the substance, ρ, ρ_p and ρ_d are the densities of the solution, particle and solvent respectively, R is the gas constant, T is the absolute temperature, and N is the Avogadro number.

Since the quantities entering into equation (2) are measured for equilibrium conditions in a dilute ideal solution, the M_e obtained is independent of the shape of the molecule (assuming that any small orientation of non-spherical particles by the field does not affect diffusion). In practice V is usually measured outside the centrifuge and consequently does not take account of hydration or solvation effects. Kraemer (1941), and his collaborators † have shown how these solvation effects can be estimated, and conclude that usually they are small, though not in all cases. If the solution contains more than one solute, the concentration will be given, by equation (2), as a function of the radius for each, independently of the others. In instances where the concentrations in the centrifuge cell are measured by optical or other methods which do not distinguish directly between the different molecular species, the mixture manifests itself by a drift in M_e for different respective c and r values. It is possible not only to determine the molecular weights of the different homogeneous molecular species in a solution, but also to get the distribution of particle or molecular weights. However, in cases where the molecular weights are not too small and the molecular shapes are not thread-like, it is usually more convenient to use the rate-of-sedimentation method, to be described later, for this latter case. Equations (1), (2) and (3) were derived on the assumption that the particles or molecules were uncharged.

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* See also Svedberg and Pedersen, 1940.

† See Kraemer and Lansing, 1934–36 ; Svedberg and Pedersen, 1940.

Frequently in cases where the solutions are not at the isoelectric point, or when the particles are charged, the electrostatic effect of the ions changes the sedimentation. If the molecules or particles are large, these charges can be repressed by the addition of a low molecular weight electrolyte such as NaCl (Svedberg, 1928–39). For an electrolyte which dissociates into m n-valent cations and n m-valent anions, where the numbers n and m contain no whole-number factor, Pedersen (Svedberg and Pedersen, 1940) has shown that

$$M_e = \frac{2(m+n)RT \log_e (c_1 f_1^{\pm}/c_2 f_2^{\pm})}{(1-V\rho)\omega^2(r_1^2 - r_2^2)}, \qquad \ldots\ldots(4)$$

where $f_1^{\pm}$ and $f_2^{\pm}$ are the " activity coefficients ".

Although a minimum of measurements is required to obtain M_e by equations (2) and (4) and the equations hold with high approximation, it is often necessary to centrifuge for prohibitively long times before equilibrium is established. This is especially true in the case of the large molecular weight substances, which may decompose with time. Archibald (1938 a, 38 b) has given a method of calculation for the equilibrium time, but the criterion developed by Weaver (1926) for a uniform field can usually be applied to the centrifuge cell to give the approximation desired. Weaver showed that, in a uniform gravitational field, equilibrium is established in a time $t_{\max}$ required for the particles to settle through twice the vertical length of the sedimenting column. Applied to the centrifuge (see Svedberg and Pedersen, 1940) this gives

$$t_{\max} = \frac{(r_1 - r_2)^2}{1800 D_{20} \log_e (c_1/c_2)} \text{ hours}, \qquad \ldots\ldots(5)$$

where D_{20} is the diffusion constant at 20° c. Thus it is seen that, for some of the biologically important substances whose molecular weights are of the order of 10^5 to 10^6, $t_{\max}$ is almost prohibitively long.

Fortunately, a second method of determining molecular weights and sizes, also due to Svedberg (1928–39), and known as the rate-of-sedimentation method, supplements the equilibrium method discussed above, i.e., it is especially applicable to the case of large molecular weight compounds. If the solution is ideal and dilute and the particles or molecules are uncharged, also if the reflection of the molecules or particles from the ends of the sedimenting column can be neglected, then by equating the sedimentation per mole to the frictional force per mole,

$$M_s(1-V\rho)\omega^2 r = f . dr/dt, \qquad \ldots\ldots(6)$$

where $f = RT/D$ from the diffusion theory, and

$$M_s = \frac{RT}{D(1-V\rho)} \frac{dr/dt}{\omega^2 r} = \frac{RT}{D(1-V\rho)} s, \qquad \ldots\ldots(7)$$

where s is the sedimentation constant or velocity of sedimentation in a unit field. Since each homogeneous molecular species has its characteristic s, it is convenient to reduce sedimentation constants to a standard solution at a standard temperature (20° c.) by the equation

$$\frac{s_t}{s_{20}} = \frac{\eta_{20}}{\eta_t} \frac{1 - V_t \rho_t}{1 - V_{20} \rho_{20}}, \qquad \ldots\ldots(8)$$

where η_t, V_t and η_{20}, V_{20} are the viscosities of the medium and the partial specific volumes of the solute in the solution at t° and 20° c., and ρ_t and ρ_{20} are the densities of the medium at t° c. and of the reference medium at 20° c.

If the particles are spherical and Stokes's law is assumed, then the radius a of the particle or molecules is given by

$$a = \left[\frac{9}{2} \frac{\eta \log_e(r_2/r_1)}{(\rho_p - \rho_d)\omega^2 t}\right]^{\frac{1}{2}}, \qquad \ldots\ldots(9)$$

where η is the coefficient of viscosity and t is the time of centrifuging. If the particles are non-spherical, corrections must be made to equation (9). It will be observed that in order to obtain M_s in equation (7), one must measure the rate of settling of the material dr/dt in the centrifuge cell and the diffusion constant D in addition to T, V, ρ and ω. In most cases the rate-of-sedimentation method is useful only when the centrifugal force is high enough or the molecular weight large enough to form an easily distinguishable boundary between the clear solvent and the sedimenting substance. Hence, by observing the rate at which this sedimenting boundary moves in the centrifugal cell, the sedimentation constant can be calculated. As the boundary moves outward, a blurring due to diffusion takes place. Svedberg (1928–39) has shown that the concentration c_x at a distance x (positive toward the axis) from the sharp sedimenting boundary (which would have been formed if diffusion were absent) is given by

$$c_x = \left(1 - 2\pi^{-\frac{1}{2}} \int_0^y e^{-y^2} dy\right) c_t/2, \qquad \ldots\ldots(10)$$

where $y = x/2(Dt)^{\frac{1}{2}}$ and $c_t/2$ is the concentration at $x = 0$ after the time of centrifuging t. Hence, from the measurements of the concentration in the centrifuge cell as a function of the distance from the axis of rotation, both s and D can be obtained. However, it has been found by experience that it is often advisable to measure D by a separate experiment. Methods of measuring the partial specific volume while the centrifuging is taking place have not proved satisfactory, so it is necessary to obtain V by experiments done outside the centrifuge. Consequently, as in the equilibrium method of equation (2), the effect of hydration is difficult to estimate. As a matter of fact, the uncertainties due to hydration effects are more serious in the case of the rate-of-sedimentation method than in the sedimentation equilibrium method. The effects of solvation on the M_s values obtained by equation (7) have been critically discussed by Kraemer and his colleagues.* The shape of the particle or molecule also affects the sedimentation constant s and the values of M_s obtained by equation (7). Experiment shows that in the case of those substances where both M_e and M_s values have

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* See Kraemer and Lansing, 1934–36 ; Svedberg and Pedersen, 1940.

been measured, they often differ by appreciable amounts. In those substances where this difference occurs, the evidence points either to the existence of hydration or non-spherical molecules or both. In those cases where the possible effects of hydration are known to be small, Svedberg has indicated how the shape of the molecules can be deduced from centrifuging data (Svedberg, 1928–39 ; Svedberg and Pedersen, 1940).

When the solution contains more than one sedimenting substance, each homogeneous molecular species forms its characteristic sedimenting boundary, so that the rate-of-sedimentation method analyses the solution by giving the molecular weights of all the substances it contains. When the solution contains a large number of molecular species or a distribution of molecular sizes or weights, the rate-of-sedimentation method gives the molecular distribution.

As pointed out before, the theory discussed above holds strictly only for ideal dilute solutions. In practice these conditions are sometimes not fulfilled with sufficiently close approximations, and it is necessary to estimate the necessary corrections. Obviously there is need for a general theory of sedimentation in liquids in a centrifuge which is applicable to all cases, i.e., to concentrated non-ideal solutions. Such a theory, if obtained, might be difficult to use in practice and would probably depend to a certain extent upon the structure of the liquid ; but it would undoubtedly give valuable information as to the magnitude of the corrections necessary to the present theory.

§3. THEORY FOR THE SEPARATION OF GASES AND VAPOURS IN A CENTRIFUGE

The separation of gases in a centrifuge has been treated theoretically for special cases by Chapman (1919), Lindemann and Aston (1919), Joly and Pool (1920), Joly (1921) and Mulliken (1922 a, 22 b). For a gas that follows the ideal gas laws

$$\rho_r = \rho_0 \exp\,(M\omega^2 r^2/2RT), \qquad \ldots\ldots(11)$$

where ρ_0 is the density of the gas near the axis and ρ_r is the density at a distance r from the axis of rotation. If the gas or vapour contains two isotopes of masses M_1 and M_2 and equilibrium is established in the centrifuge, Lindemann and Aston showed that

$$S = K_0/K = \exp\,(M_2 - M_1)\omega^2 r^2/2RT, \qquad \ldots\ldots(12)$$

where K_0 is the ratio of the quantities of the light and heavy isotopes at the axis and K is the same ratio at a distance r from the axis. They defined S as the separation factor.

This theory has been extended by Mulliken to include the separation of the isotopes in substances containing several isotopes. Also, he has shown that the separation factor for any two isotopes of an element in a centrifuge is independent of the state of combination of the element. Chapman treated the problem as one of separation by pressure diffusion, and arrived at essentially the same results as Lindemann and Aston and Mulliken. It will be observed that according to equation (12) the separation factor increases very rapidly with increasing mass-differences and peripheral speeds. Recently the separation of the isotopes in a centrifuge when equilibrium does not exist has been investigated by Beams (1937, 38b, 40), Beams and Skarstrom (1939), Humphreys (1939), Urey (1940) and Wilson (1940). They of course find that equation (12) must be modified when equilibrium does not exist, but the corrections depend upon the particular method of centrifuging. In addition to the separation of isotopes, equation (12) can be used to compute the separation of any two gases which approximately obey the ideal gas laws.

§ 4. EXPERIMENTAL TECHNIQUE

The modern technique of high-speed centrifuging has been developed almost completely in the last decade and a half. Beginning in 1924, Svedberg first succeeded in obtaining convection-free sedimentation in centrifugal fields as high as 5000 *g*. The results obtained with this machine proved to be so interesting and important that Svedberg and his collaborators were led to undertake an extensive development of centrifuging technique. This work has resulted in the development of their two general types of centrifuges which have played an indispensable rôle in their classical experiments on the determination of molecular weights and sizes. Both types of Svedberg ultracentrifuges were designed primarily for analytical work, i.e., the determination of molecular weights and sizes.

The first type develops comparatively low centrifugal fields (500 to 15,000 *g*). It is driven by an electrical motor and supported in ball bearings. The rotor carries a cell which contains the material to be centrifuged. The cell is usually sector-shaped and has glass or quartz windows through which the concentration of the material being centrifuged at various radial distances can be determined by optical means while the rotor is spinning. The rotor spins in hydrogen at atmospheric pressure and is surrounded by a water-cooled casing. This machine has been used principally for sedimentation-equilibrium measurements (equation (2)). The second type of Svedberg ultracentrifuge is driven by oil turbines and is used to produce fields in the range between 15,000 and 750,000 *g*. The rotor of this machine is made of a special alloy steel and is designed to give a maximum centrifugal field inside the centrifuge cell without explosion. The cell which carries the material to be centrifuged is sector-shaped and is enclosed by crystal quartz windows for observing optically the concentration of the sedimenting material while the rotor is spinning. The rotor is surrounded by a heavy steel case through which hydrogen at about 20 mm. of mercury pressure is continuously circulated. The purpose of the hydrogen is to conduct the heat generated by the friction in the bearings, by the gaseous friction on the rotor, and by the oil impinging on the turbine, to the casing. By proper adjustment this makes it possible to obtain a uniform temperature throughout the centrifuge cell. The rotor and its contents are carefully balanced, both statically and dynamically, before running the machine. This ultracentrifuge has been used primarily for rate-of-sedimentation measurements (equation (7)). For detailed descriptions of these machines, reference should be made to the papers by Svedberg (1928–39) and his collaborators (see Svedberg and Pedersen, 1940).

§ 5. AIR-DRIVEN CENTRIFUGES

There are several different kinds of high-speed air-driven centrifuges, but they can be classified somewhat arbitrarily into two general types. In the first type the centrifuge rotor spins in air at atmospheric pressure, while in the second type the centrifuge rotor is enclosed in an air-tight chamber which may be evacuated. The first type is primarily suited to centrifuging small amounts of material and where accurate temperature control is not essential. The second type has a wider use and can be applied to practically every type of high-speed centrifuging problem. Also, it is provided with precise temperature control. With either type high rotational speeds are obtained.

A significant advance in the first type of air-driven centrifuge was made in 1925 by Henriot and Huguenard (1925, 27), when they succeeded in supporting and spinning small rotors at very high speeds (11·7 mm. rotor to 11,000 r.p.s.) on properly directed jets of air. The rotor was cone-shaped and spun a fraction of a millimetre above the surface of a cone-shaped stator into which it fitted. This design of Henriot and Huguenard has been modified, improved, and stabilized by Beams (1930 a–40), by Garman (1933), by Girard and Chukri (1933) and by McBain (1939). Thus, at the present time, the centrifuges of this type are remarkably stable and are also simple and easy to construct (Beams, 1937, 38 b, 40). Since this type of centrifuge is driven and supported by expanding jets of air, the lower part of the rotor is cooled, while the top part and the periphery are heated by air friction. Consequently, unless special care is taken, temperature gradients large enough to produce re-mixing or convection of the material being centrifuged may exist in the centrifuge cell. Incidentally, convection due to differences in density resulting from temperature variations are greatly magnified in a centrifuge because of the large centrifugal field (Beams, 1937, 38 b, 40).

This type of centrifuge has been used widely for centrifuging small biological tissues or cells which have such minute volumes that the temperature gradients in the centrifuge have no appreciable effect upon the sedimentation inside the biological cell. The relative movements of the various components of the biological cell in the centrifugal field may be determined by microscopic examination after the centrifuge is stopped, or under comparatively low-power magnification while the rotor is spinning (Beams (H. W.) and King, 1934–38 ; Harvey, 1932–38 ; Pickels, 1936, 38). Also, many workers have used this type of centrifuge for the sedimentation of small particles and molecules ; it has also been successfully applied to analytical work by Beams, Weed and Pickels (1933), by McIntosh and Selbie (1937), by Beams (1937, 38 b, 40), by Bawden and

Pirie (1938), by Elford (1938) and by McBain (1939). McBain (1939) and McBain and Leyda (1938 a, 38 b) especially have found this type of centrifuge useful in their molecular-weight studies. Incidentally, these air-supported air-driven turbines have provided means of spinning small mirrors to very high rotational speeds (Henriot and Huguenard, 1925, 27; Beams, 1930 a–40; Chiles, 1937), and have been used to study the light emitted in the various stages of condensed electrical discharges, to demonstrate the existence of the velocity of light (rough measurements), and to produce short flashes of light. Unfortunately, as the size of the mirror is increased, the air friction becomes so great that it is difficult to obtain high rotational speeds with this type of turbine drive. For the rotor shapes used for the above types of centrifuges, the air friction increases very rapidly with increasing radius of rotor for a given peripheral velocity. In practice this limits the diameter of the rotors, which can be spun to high speeds, to an inch or two unless, of course, large high-pressure air supplies are utilized. As a result, in addition to troublesome temperature variations in the rotor noted above, the centrifugal field must vary rapidly along the small radius and thus reduces the precision of the experiments. If hydrogen is used for the driving and supporting gas instead of air, higher peripheral velocities are obtained. For example, a 9 mm. rotor driven and supported by air attained about 12,500 r.p.s., while the same rotor spun by hydrogen at the same pressure as the air attained a rotational speed of about 22,000 r.p.s. (Beams, Weed and Pickels, 1933; Beams, 1937, 38 b, 40).

§6. VACUUM-TYPE AIR-DRIVEN CENTRIFUGE

The troublesome effects of air or gaseous friction on high-speed rotors led to the development of the air-driven vacuum-type ultracentrifuge (Beams and Pickels, 1935; Pickels and Beams, 1935; Beams, 1937, 38 b, 40 ; Beams, Linke and Sommer, 1938). The various designs of these machines differ somewhat, but essentially they consist of a large (centrifuge) rotor situated inside a vacuum-tight chamber, a smaller, air-driven, air-supported turbine located above or below the chamber, and a small-diameter flexible shaft which fastens them together and is coaxial with their common vertical axis of rotation. The flexible shaft passes through a vacuum-tight oil gland which seals the vacuum chamber. This oil gland has comparatively little friction since the shafts are small, so that a properly designed air turbine will reach a rotational speed of the same order of magnitude with the attached rotor inside the evacuated chamber as outside it. The vacuum attainable in the chamber is limited only by the vapour pressure of the oil, which may be very low (10^{-6} mm. for some oils used). The flexible shaft allows the large rotor to " seek its own axis of rotation " and permits extremely steady rotation, i.e., the rotor is self-balancing. With these machines the only factor which limits the rotational speed attainable is the mechanical strength of the large rotor. This, coupled with the fact that the rotor spins in a vacuum where no appreciable heat, which might cause re-mixing, can be generated makes this vacuum-type machine an ideal high-speed centrifuge for most purposes. The vacuum-tight glands may be thermostated in such a way that material can be forced into and out of the spinning rotor through hollow shafts without appreciably changing its temperature. In addition to rotors whose diameters are greater than their length, the same technique has been used to spin long tubular rotors (Beams, 1930 a–40). In these tubular vacuum-type air-driven centrifuges, the material to be centrifuged (liquid, gaseous, or vapour) enters through a hollow shaft at the top of the vertical spinning tube and passes out at the lower end in a heavy and a light fraction through a shaft with two partitions, or through two coaxial hollow tubes which serve as the shaft. A number of methods of determining the rotational speed of these high-speed centrifuges, as well as means for holding their speed constant, have been devised, but usually some form of the stroboscopic method is used (Beams, 1930 a, 30 b, 33 a, 33 b, 35, 38 a, 39 ; Davis, 1936 ; Kahler, 1938 ; Pickels, 1936, 38 ; Shapiro and Butt, 1937 ; Snoddy and Beams, 1937).

The vacuum-type air-driven ultracentrifuge has been used extensively as an analytical tool for the determination of molecular weights and sizes. It is applicable both to the sedimentation-equilibrium method (equation (2)) and the rate-of-sedimentation method (equation (7)). The cell containing the material to be centrifuged is sector-shaped * and enclosed by crystal quartz windows for observing the sedimentation. The rotor is designed to give a maximum resolving power throughout the cell, where resolving power is qualitatively defined as the ability to distinguish between two homogeneous molecular species with slightly different sedimentation constants or molecular weights. When the centrifuge is used for rate-of-sedimentation measurements, the resolving power is approximately proportional to the product of the centrifugal force and the length of the sedimenting column, i.e., to $\omega^2 xh$, where x is the distance of the sedimenting boundary from the axis of rotation, and h the length of the sedimenting column (Svedberg, 1928–39 ; Svedberg and Pedersen, 1940). It has been shown both by theory and experiment that the resolving power is highest for values of x between 5 and 10 cm. It will be observed that the maximum value of $\omega^2 xh$ for rotors of a given shape is roughly proportional to the square of the peripheral velocity, which in turn is proportional to the strength of the rotor. It might be assumed from this that the maximum resolving power had been reached for a given rotor material. However, the resolving power has recently been considerably increased for the case of large molecular weight compounds by effectively increasing the length of the sedimenting column (Beams, 1930 a–40). Many interesting bio-

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* Svedberg (1928–39 ; see also Svedberg and Pedersen, 1940) has shown that for rate-of-sedimentation measurements the centrifuge cell should be sector-shaped. However, for sedimentation-equilibrium measurements this is not so important. As a matter of fact, it has been shown that the time required for equilibrium to be established is longer in the case of sector-shaped cells than for some other shapes.

logical substances have sedimentation constants so large that they settle through an appreciable length of the sedimenting column in a convection-free ultracentrifuge before it can reach full speed. Artificial convection can be introduced to prevent this, but unless special care is taken, undesirable " false " boundaries may develop. Consequently the full resolving power of the centrifuge cannot be used. In the new high-resolving power vacuum-type ultracentrifuge (Beams, 1930 a–40), the solvent is made to flow from the periphery toward the axis at the same rate as the material settles out toward the periphery. This allows sedimenting boundaries to be observed for long periods of time. Hence sedimenting boundaries of homogeneous molecular species with nearly the same sedimentation constants can be observed to separate.

Various optical schemes have been used for measuring the density of the sedimenting material as a function of the distance from the axis of rotation while the centrifuge is spinning (Svedberg, 1928–39 ; Svedberg and Pedersen, 1940). Generally they depend upon the light absorption of the material or upon its refractive index. The light-absorption methods depend upon the fact that in some cases it is possible to find a convenient absorption band of the sedimenting material which is not absorbed by the solvent. By measuring the light absorption in the cell and knowing the relation between this and the amount of absorbing material, the density is determined. In practice it is often necessary to use ultra-violet light in order to find a satisfactory absorption band, and this requires quartz-fluorite lenses (which should have long focal lengths if precision is desired). In some cases the absorption method yields inaccurate results because of Tyndall scattering (Brohult, 1940 ; Svedberg and Pedersen, 1940).

The refractive-index methods are applicable to any transparent solution. Probably the most precise index-of-refraction method is that developed by Lamm (1929 a–37). This consists in photographing a scale through the transparent centrifuge while the centrifuge is spinning. If the scale or its real image is placed just in front of the cell, the image of the scale formed by a long-focus lens on a photographic plate or in the field of view of a telescope is distorted in those regions where the rays of light pass through gradients in refractive index (density gradients) in the centrifuge cell. From these distortions of the scale, the density gradients in the centrifuge cell can be calculated. The Schlieren method is the most direct and convenient index-of-refraction method. It has taken several forms and has been made automatic by Philpot (1938), Andersson (1939) and others. Other refractive-index methods, such as the interferometer method, have not as yet been extensively used. There is a need for a convenient precise optical method, which is inexpensive, for observing sedimentation in a centrifuge cell.

In some cases it is impossible or impracticable to measure the concentration in the centrifuge cell by optical means. In such instances it is necessary to measure the concentration of the material taken from different distances from the axis of rotation by the ordinary methods of analytical chemistry. Different methods have been devised for collecting these samples without re-mixing (Svedberg, 1928–39; Beams, 1937, 38 b, 40; McBain, 1939). Tiselius, Pedersen and Svedberg (1937), and others have provided the sector-shaped centrifuge cell with a partition perpendicular to the radius which divides it into approximately equal parts. Usually this partition consists of a thin bakelite plate perforated with a large number of small holes and covered with special filter paper. Such a partition does not disturb sedimentation during the centrifuging process, but prevents re-mixing while the centrifuge is decelerated. If Δ is the total change in amount of the material above or below the partition, x the distance from the axis of rotation of the partition, q the area of the partition, ω the angular velocity, c_0 the concentration of the material at the beginning, and t the time of centrifuging, the sedimentation constant s is given by

$$s = -\frac{1}{2\omega^2 t}\log_e\left(1-\frac{2\Delta}{q\times c_0}\right). \qquad \ldots\ldots(13)$$

It is difficult to calculate ω and t for the acceleration and deceleration period in the arrangement described above, and it is not as precise as the optical methods. However, a vacuum-type ultracentrifuge rotor has recently been designed in which the sector-shaped cell is filled after the rotor reaches full speed (Beams, 1937, 38 b, 40; Stauffacher, Beams and Snoddy, 1941). When the desired centrifuging time t at a constant ω has elapsed, the cell is effectively divided into two approximately equal parts by a displacement method and the centrifuge then brought to rest. From a chemical analysis of one of the two fractions and the original uncentrifuged sample, the sedimentation constant is determined from equation (13). In order to facilitate the chemical analysis by providing larger specimens for analysis, a large number of identical sector-shaped cells instead of one are constructed in the same centrifuge. This method gives good precision, especially in the case of homogeneous molecular species.

In addition to its use as an analytical tool, the vacuum-type centrifuge has made possible the purification of materials in appreciable quantities (Bauer and Pickels, 1936–40; Wyckoff, 1937 a–38 b). When used chiefly for concentrating materials, it is sometimes referred to as the quantity-type vacuum-type ultracentrifuge (Wyckoff, 1937 a–38 b; Beams, Linke and Sommer, 1938). Many of the most interesting substances in biology and medicine often occur naturally in extreme dilution. They are difficult to concentrate by chemical means because in general they are deactivated by high temperature or large changes in the pH values. Fortunately, high centrifugal fields do not deactivate them, so that the high-speed centrifuge may be used for their purification. The rotor in the quantity-vacuum-type centrifuge is made from a large block of metal in which are holes bored at an angle to the vertical. These holes carry Lusteroid (plastic) test tubes which, in turn, hold the material to be centrifuged. One or more vacuum-tight caps seal the centrifuge (Bauer and Pickels, 1936–40; Beams, Linke and Sommer, 1938). This construction permits the use of high centrifugal fields on comparatively large quantities of material under conditions ideal for concentration. The smaller the angle made by the test tubes with the vertical, the greater is the efficiency down to angles of about ten degrees (Masket, 1940 ; Wyckoff, 1937 a–38 b).

The above quantity-type centrifuge, while very useful for centrifuging volumes of material up to the order of one-half litre per day (depending upon the sedimentation constant of the material), is not convenient for centrifuging greater quantities. Larger quantities can be centrifuged by means of the tubular vacuum-type ultracentrifuge (Beams, 1930 a–40). In this machine the material to be centrifuged enters the top of a vertical spinning tube through a hollow shaft at a continuous rate. The separation takes place as the material flows from top to bottom of the vertical spinning tubular rotor and emerges in a light and a heavy fraction through two coaxial tubes which serve as the lower shaft. The tubular rotor contains a solid core to confine, to the strongest part of the field, the material to be centrifuged. Since the material enters and leaves the rotor along the axis and the rotor is spun in a vacuum, the power required to spin it is very small. With this machine the amount of a given material that can be concentrated per unit of time is about one hundred times greater than in the case of the quantity-type centrifuge.

The air-driven vacuum-type centrifuge has also been applied to the concentration of isotopes (Beams, 1937, 38 b, 40; Beams and Skarstrom, 1939; Humphreys, 1939). In general, two methods have been used in this work. The first is the evaporative centrifuge method suggested by Mulliken (1922). In this method the material in the liquid state is contained in a layer around the cylindrical inside wall of a hollow vacuum-type rotor. This material slowly evaporates and diffuses in the vapour state to the axis against the centrifugal field. During this diffusion the separation takes place. The material flows out of the rotor through a hollow shaft and is collected in a series of fractions. The first fractions show a concentration of the lighter isotopes, while the last fractions show a concentration of the heavy isotopes. The experiments show that the theory (equation (12)) holds unless, of course, the material is removed from the rotor too rapidly for approximate equilibrium to be established. The second centrifuge method of concentrating isotopes employs the tubular vacuum-type centrifuge (Beams, 1937, 38 b, 40). The material in the gaseous or vapour state enters at the top of the vertical spinning tubular centrifuge through a hollow shaft. As it flows down along the length of the tube, the separation occurs, with the heavier fraction moving toward the periphery and the lighter fraction toward the axis. Each of these fractions flows out of the machine through one of two channels formed by two coaxial tubes comprising the lower shaft. This method also gives the approximate separation expected by the theory when the material is not forced through the machine too rapidly. In addition to the concentration of isotopes, this type of machine has been used for the separation of N_2 and CO_2, N_2 and O_2, etc. (Beams, 1937, 38 b, 40).

§ 7. ELECTRICALLY DRIVEN MAGNETICALLY SUPPORTED VACUUM-TYPE ULTRACENTRIFUGE

The electrically driven magnetically supported vacuum-type ultracentrifuge was developed primarily to give accurate automatic speed control over long periods of time and to avoid the necessity for a supply of compressed air (Beams and Snoddy, 1937 ; Beams, 1937, 38 b, 40 ; Skarstrom and Beams, 1940). This centrifuge is self-balancing and utilizes the same mechanical principles as the vacuum-type air-driven air-supported centrifuge. The larger rotor spins in a vacuum and hence has the same temperature control as the vacuum-type centrifuges previously described. Only the drive and support are different. The rotating members are driven by an induction motor, the field of which is actuated by a high-frequency power supply. The rotating members are supported (except for a very minute part of the weight which is taken by a small thrust bearing) by the upward pull of an electromagnet on an iron or steel rod fastened to the shaft and coaxial with it. This type of magnetic support is almost friction-free if constructed properly, because the magnetic field is symmetrical across the end of the rod and no appreciable electromagnetic drag exists. The armature of the induction motor is a cylindrical rod of strong steel and is water-cooled, especially during the starting period, when the " slip " is large. The motor and support are situated either above or below the vacuum chamber, i.e., they are situated in the same position as the air turbine in the machines described above. The rotational speed is held constant (±0·5 r.p.s. for days at a speed of 1100 r.p.s.) by a speed control operated by the " slip ", i.e., the difference between the driving frequency and the centrifuge speed. The electrical motor is more efficient than the air drive, has a good starting torque, is almost entirely automatic, and operates from the 110-volt a.c. lines (Skarstrom and Beams, 1940). Since the armature of the motor is small in diameter in comparison with the large rotor which it spins inside the vacuum chamber, the only factor which limits the rotor speed is the strength of the rotor material. These electrical motors have been made to operate up to about 3000 r.p.s., but this is not by any means the upper limit.

Recently an effective method of spinning rotors to high speeds *in vacuo,* which utilizes the magnetic suspension of F. T. Holmes, has been developed. (Holmes, 1937 ; Holmes and Beams, 1937 ; Beams, 1937, 38 b, 40 ; MacHattie, 1941 ; Smith, 1941). The rotating members are attached to a vertical steel rod or ball which is suspended in the coaxial field of one or more solenoids. When two solenoids are used, the magnitude of the steady d.c. current passed through the larger solenoid is not quite sufficient to lift the rotating members. The remaining weight is supported by the second solenoid, the current through which is controlled by the amount of light falling upon a photoelectric cell. The intensity of the incident light striking the photoelectric cell is increased as the centrifuge rotor moves upward and decreased as it moves downward, in such a way that the rotor is maintained at a predetermined height. This stabilizes the vertical motion while the geometry of the field stabilizes the horizontal motion of the rotor. In some arrangements the current in the supporting solenoid (and hence the height of the rotor) is regulated by making the rotor one plate of a condenser (Smith, 1941). The change in capacity of this condenser changes the current in the solenoid. In another arrangement by MacHattie (1941) the change in characteristics of a small coil carrying a radio-frequency current, as the rotor approaches or recedes, is made to regulate the current in the supporting solenoid. The supporting magnetic field is symmetrical across the spinning rod so that there is no appreciable electromagnetic drag or friction opposing axial rotation.. Also, the rotating members are not mechanically connected to anything and can be completely surrounded by a glass container. This allows the rotor to be degassed by heating and makes it possible to obtain a very high vacuum. The rotors have been spun by rotating electrostatic and by rotating magnetic fields. A small rod which was spun to 1000 r.p.s. in a good vacuum, and the driving torque removed, required about 1000 sec. to lose one r.p.s. A steel ball 3/32″ diameter was spun to $6{\cdot}5 \times 10^6$ r.p.m., giving a centrifugal field of about $57 \times 10^6\, g$ (MacHattie, 1941).

In practically all of the types of centrifuges described above, the mechanical strength of the rotor limits the rotational speed attainable. For a smooth solid rotor of a given shape (for example, one of uniform strength) the bursting speed depends upon the yield-point divided by the density of the material. If the rotor carries a load or has been bored or pierced with channels, as is required in many experiments, local stresses may rise to very high values over comparatively small regions. If the material is plastic (has a large elongation), when the local stresses exceed the yield-point of the material, it flows and distributes the local stresses over a larger volume (as well as increases the strength of the material locally) and the rotor does not explode. On the other hand, if the material has a small elongation, the local stresses start cracks which cause the rotor to explode. For this reason some investigators have had disastrous results with rotors made of very strong hard steel (Pollock and Collie, 1937 ; Beams, 1937, 38 b, 40 ; Svedberg and Pedersen, 1940). For many purposes, the alloy steels such as SAE 4130 X, heat-treated to a Brinell hardness between 400 and 450 (yield-point 200,000 lb./in.^{2}), or the tough nickel alloy steels, etc., are preferable to the stronger but harder steels. Some of the aluminium and magnesium alloys have been used extensively for centrifuge rotors. They have a yield-point/density ratio almost as large as the alloy steels mentioned above. Duralumin ST 14 has had very wide use because of its favourable elastic properties and its ready machinability.

§ 8. APPLICATIONS

High-speed rotors have been applied to many different fields of research. As a matter of fact they should be classed with that rapidly growing group of physical apparatus which has made possible so much recent borderline research between the sciences. Already the applications of high-speed centrifuges are too numerous to permit a reasonably complete review in this *Report*. Instead, a few results will be mentioned to illustrate the types of problems that have been undertaken and briefly to call attention to some data usually considered outside the province of physics, yet which may be of interest to physicists.

From the standpoint of biology and medicine, one of the most important applications of the ultracentrifuge is in the purification in quantities of the naturally occurring substances such as viruses, hormones, etc., without deactivating them (Elford, 1938; Severinghaus, Levin and Chiles, 1938; Wyckoff, 1937 a–38 b; Bauer and Pickels, 1936–40). These substances, which often occur in very dilute solution, are known to influence critically the well-being of plants and animals. In fact, their existence was usually determined by their biological effects. Consequently their purification and concentration by the high-speed centrifuge has put into the hands of the experimenter concentrated specimens whose properties can be investigated. For example, Bauer and Pickels (1936–40) obtained a ten-thousand-fold change of concentration by one centrifuging of yellow-fever virus, while Wyckoff (1937 a–38 b) and his associates have concentrated several of the larger viruses until they crystallized by one centrifuging. Incidentally, it might be of interest to note that crystallization of some of these viruses any number of times apparently does not affect their biological activity.

In their earlier work Svedberg (1928–39) and his collaborators, contrary to the belief at that time, discovered that certain proteins such as haemoglobin, egg albumen, etc., consisted of homogeneous molecular species with high molecular weights rather than colloids (particles of various masses made up of ordinary small molecules). Subsequently a large number of substances composed of these giant molecules has been studied in the ultracentrifuge, by many different workers, and their molecular weights, sizes and shapes determined. Not all of these naturally occurring substances, such as keratin, fibroin, and myosin, are composed of homogeneous molecular species, but at least most of the easily soluble proteins have definite molecular weights. Consequently the high-speed centrifuge has made available pure substances with molecular weights which may exceed one hundred million (Wyckoff, 1937 a–38 b). Clearly such substances should possess novel physical properties, especially in cases where the molecules are elongated. Several physical properties of a few of the viruses, such as streaming double refraction, viscosity, Kerr electro-optical effect, spontaneous double refraction, etc., have been studied, and most interesting results obtained (Lauffer, 1939 ; Lauffer and Stanley, 1939 ; Svedberg and Pederson, 1940). Also some of these giant molecules show somewhat surprising chemical properties (Wyckoff, 1937 a–38 b ; Brohult, 1940 ; Svedberg and Pedersen, 1940). The analytical centrifuge shows that many of the protein molecules are dissociated or associated, usually reversibly, by such weak agents as small changes in pH, changes in salt concentration, or even changes in concentration of the protein itself. In some cases, such as the respiratory proteins, which have been extensively studied, giant molecules dissociate into sub-multiples. For example, when a solution of haemoglobin is made very dilute, the molecules split into halves. In the case of haemocyanin (*Helix Pomatia*) solution near the isoelectric point, whole molecules (molecular weight = $8{\cdot}9 \times 10^6$) exist alone provided the salt concentration is not too high. When the solution is made weakly acid, half-molecules appear. If it is made alkaline, half-molecules first appear, followed by molecules of one-eighth the original molecular weight, etc. Within the region of *p*H 4 to *p*H 9 the dissociation is reversible, and the whole molecules will be re-formed at the isoelectric point. Outside the *p*H region (4–9) non-homogeneous components appear and will not completely re-associate. It is interesting to note that in this case the whole, the half, and the one-eighth molecules all have approximately the same length (Brohult, 1940). Svedberg (1928–39) has pointed out that the molecular weights of the soluble proteins which are composed of homogeneous molecular species are integral multiples of a unit weight (17,600) ; he has, in fact, divided most of these proteins into eleven classes with molecular weights equal to integers containing some power of 2 or 3 multiplied by 17,600. The rule does not fit exactly in all cases, but undoubtedly indicates a regularity in the masses of the very large virus proteins, which he thinks cannot be due to chance.

The fact that many substances have molecular weights well over 5×10^7 raises interesting theoretical questions as to why they are stable or why they are all exactly the same size. Usually they are assumed to have the same mass because each molecular species sediments in the ultracentrifuge with a sharp sedimentation boundary. However, at present too much confidence should not be placed in this conclusion because possible variations in mass of 10^6 which are not observable may occur in some instances. There is need for more precise observations on the homogeneity of some of these giant or macro-molecules.

McFarlane (1935, 38), Pedersen (Svedberg and Pedersen, 1940) and others have used the ultracentrifuge to study differences between normal and pathological sera and have found in many cases that the changes are pronounced. In some diseases new sedimentation boundaries appeared along with changes in the amounts of the various components such as the globulin or albumen.

In addition to the proteins mentioned above, the molecular weights and sizes of many other substances have been studied by means of the ultracentrifuge. Lamm (1929 a–37) and Beckmann and Landis (1939) have determined the masses of the starch molecules under various conditions. Nichols, Kraemer, and others * have studied a large number of materials used industrially, such as neoprene, rubber and cellulose. In some cases they have been able to correlate the sizes and shapes of the molecules of the substance with its physical properties. Many of the synthetic compounds show no sharp sedimenting boundaries, indicating that they are polydisperse, i.e., they are made up of particles whose weights vary over considerable ranges. Fortunately, the ultracentrifuge data give not only the masses of the particles but their number or concentration as well. In some solutions the rate of sedimentation in the centrifuge is influenced rather

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* See Svedberg (1928–39) and Svedberg and Pederson (1940).

critically by the concentration. This is true especially in the case of the polystyrenes dissolved in organic solvents, where the rate of change of the sedimentation constant increases as the dilution is increased. Such behaviour is thought to be due to the entanglement, or interference with each other, of the long thread-like molecules. Many natural substances, as well as those made synthetically, which have long thread-like molecules, are polydisperse. It should be noted that in addition to giving the particle-weight distribution, the ultracentrifuge can distinguish between mixtures of molecules and chemical equilibrium (Pedersen, 1934; Svedberg, 1928–39).

When electrolytes are placed in a gravitational field, electrical potentials are developed between two electrodes at different heights (MacInnes, 1939). The phenomenon is magnified in the centrifuge, as has been shown by Tolman (1911), MacInnes, and others. According to theory, the potential difference between centre and periphery of the centrifuge should be a maximum when the centrifuge first reaches full speed and then decrease with time until equilibrium is established in the centrifuge cell. From these measurements it is possible to determine the transference numbers. By comparing the molecular weight of an electrolyte obtained by the equilibrium method of centrifuging with that derived from chemical data, Pedersen (1934), Drucker (1937) and others have been able to obtain the activity coefficients.

Interesting investigations have been carried out with the high-speed centrifuge on the sedimentation occurring inside a living cell (Harvey, 1932–38; Beams (H.W.) and King, 1934–38). Estimates have been made of the relative density of the various components of the cell, the " viscosity," and " surface tension " of the protoplasm and the elasticity of the cell membranes. In some cases certain components of the cell were thrown through the cell wall, yet the cell continued to function. In other cases, after milder centrifuging, the cell developed abnormally or died. H. W. Beams and King (1934–38) subjected fertilized eggs of *Ascaris suum* to fields of 4×10^5 gravity for an hour. The eggs were then removed from the centrifuge and observed under a microscope. At first the contents of the cell were stratified into definite layers. However, twelve hours later they had lost their stratification and many of them continued to live.

As mentioned earlier, the high-speed centrifuge has been used to concentrate the isotopes of several elements. Most of this centrifuging was done with the substances in the vapour or gaseous state. The separation of gaseous mixtures such as O_2 and N_2 or N_2 and CO_2 has also been studied. When the rate of flow through the centrifuge was not too great, the simple theory (equation (12)) was found to hold. In addition to the centrifuging method, the separation of gaseous mixtures has been accomplished by the use of a high-speed rotor of special design used as a velocity selector (Beams, 1937, 38 b, 40).

Fortunately, most of the high-speed rotors discussed in this article are stable enough to carry a polished mirror. Since the bursting strength of the rotor is the only factor which limits rotational speed, the construction of very high-speed rotating mirrors is possible. Such high-speed mirrors have been mostly used to study phenomena which occur in very short intervals of time (Chiles, 1937 Beams, 1930 a–40).

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