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High Speed Centrifuging

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THE METHOD of separating materials by centrifuging was used so long ago that apparently there is no definite record of its origin. Certainly for many years its use has been very wide, both in industry and in the laboratory. Consequently, new improvements in the technique of centrifuging not only should stimulate research activity, but should eventually modify those industrial procedures to which it can be applied. For these reasons it may be of interest to review briefly some of the recent advances in the experimental technique and theory of centrifuging. In a review of so broad a subject, no attempt can be made to cover all of the recently published material, but it is hoped that the description of a few typical experimental arrangements and some of the results obtained may be worth while.

A. EXPERIMENTAL TECHNIQUE

The recent advances in centrifuging technique in general have been in two main directions: first, the rotational speed, and hence the centrifugal force throughout the centrifuge bowl, has been greatly increased; and, second, stirring in the centrifuge has been eliminated. The first improvement increases the rate of sedimentation, while the second raises the efficiency of separation to the theoretical value. It is obvious that the first would be of little value without the second. The increase in the rotational speed and centrifugal forces has been brought about by more suitable machine design, and especially by radical changes in the methods of mounting and driving the centrifuge, while the stirring or convection in the centrifuge bowl has been prevented by the elimination of vibrations, accelerations and decelerations of the centrifuge and by maintaining the material being centrifuged at a very uniform temperature.

1. Svedberg ultracentrifuges

The beginning of the recent rapid advances in centrifuging technique dates back to the pioneering work of Professor Svedberg and his students in the early nineteen twenties.[1–3] Their interest in centrifuging apparently arose from a desire to determine the size and mass of particles in the region between colloidal dispersions and true solutions. In their first centrifuge,[2, 3] which gave fields of about 1000 gravity, they studied, among other things, the settling of gold sols of ultramicroscopically determined size. The results obtained so clearly demonstrated the importance of eliminating stirring that a systematic study of the conditions for convection-free sedimentation in the centrifuge was undertaken. As a consequence, in 1924 they were able to obtain convection-free sedimentation in centrifugal fields of 5000 times gravity and proposed the name "ultracentrifuge" for their machine.[3-5] The results obtained with this apparatus,[3-7] especially in the study of particle mass and size of hemoglobin, were of such great interest and importance that much time and effort have been spent in the development of their ultracentrifuges.

At the present time there are two general types of Svedberg ultracentrifuges:[3] One is used in the region 500 to 15,000 times gravity and the other in the range from 15,000 to 750,000. The first, or low speed machine, is provided with ball bearings and is driven by an electrical motor. The rotor spins in hydrogen at atmospheric pressure and is surrounded by a water-cooled casing. The cells containing the material to be centrifuged are sector-shaped and have either glass or quartz windows for observing the concentration of the material as a function of the distance from the axis of rotation. It is used principally for sedimentation equilibrium measurements (which will be described later) in solutions of compounds of very high molecular weight and for observing the settling of particles. The second, or high speed, machine is the "oil turbine" ultracentrifuge, with which Professor Svedberg has measured molecular weights from 40 to 20,000,000. Although for a detailed description of the oil turbine ultracentrifuge, which is quite elaborate, reference must be made to the original papers,[3] yet a few details should be given here, for the machine in the hands of Svedberg and his students has produced epoch-making results. The rotor is made of chrome-nickel steel and given the proper shape to carry a cell containing the material to be centrifuged at as high a rotational speed as possible. The cell is sector-shaped and is provided with plane parallel crystal quartz windows for observing optically the sedimentation of the materials. The rotor is supported in horizontal bearings and is spun by twin oil turbines, one on each end of the rigid shaft. The oil under pressure which drives the turbines is furnished by a special oil compressor and is cooled and thermostated at a suitable temperature by a special refrigeration system. The rotor is surrounded by a heavy steel casing which is maintained at the desired temperature by circulating oil or water. Hydrogen is continuously admitted at the periphery and constantly pumped off at the center so as to maintain a pressure of about 20 mm surrounding the rotor. The function of the hydrogen is to conduct the heat generated in the bearings, turbines and rotor to the casing and thus prevents the temperature of the cell in the rotor from changing or becoming appreciably non-uniform. Hydrogen at 20 mm is used because of the high ratio of its heat conductivity to its viscosity at this pressure. The rotor containing the cell and material to be centrifuged must be very carefully balanced, both statically and dynamically, before running the machine. Rotors with a mean radius of 6.5 cm and with a height of column of solution in the cell of 1.2 cm have been used to give centrifugal fields of 400,000 g, while rotors with mean radius of 3.25 cm and sample height of 0.8 cm have been used to make measurements in fields of 710,000 g. This machine has been used principally for velocity of sedimentation measurements in solutions of high molecular weight compounds and for sedimentation equilibrium measurements on low molecular weight substances.

2. Air-driven centrifuges*

There are in general two types of air-driven centrifuges.[8] In the first the centrifuge "bowl" spins in air or other gases at approximately atmospheric pressure, while in the second, the centrifuge "bowl" spins inside an air-tight chamber which may be highly evacuated. The first type is suitable primarily to problems which require high rotational speed but in which it is unnecessary to control accurately the temperature of the centrifuge bowl. Also, its use is limited to problems which can be carried out with a centrifuge bowl of comparatively small size. On the other hand, the second or vacuum-type centrifuge gives very high centrifugal forces and the centrifuge bowl may be as large as desired and provided with precise temperature control. The only advantage in the first type is its extreme simplicity of construction, for the second, or vacuum-type, machine is clearly superior for almost any type of centrifuge problem.

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* All of the centrifuges described in this paper are entitled to the name ultracentrifuge. While the prefix "ultra" is useful, it will be dropped in the remainder of this paper for the sake of brevity.

In 1925 Henriot and Huguenard[9] succeeded in constructing a centrifuge or "top" of the first type, which was both driven and supported by a system of directed air jets. The rotor was cone-shaped and spun a fraction of a millimeter above a stator into which it fitted. Subsequently the method has been modified and improved by a number of workers[10–16] until at the present time the rotors are very stable.[8] That is, the rotor may be loaded or unloaded while at full speed or be made to carry a superstructure which need not be dynamically balanced with much care. Fig. 1 is a diagram of an apparatus that operates very satisfactorily and is simple to construct.[8, 16] The stator consists of three parts: a so-called stator cone A shown in Fig. 1(a), an air box B, and a tube C through which the compressed air enters the box. The three are screwed together as shown in Fig. 1(b). They are usually made of Duralumin or brass, although any easily machinable material will serve equally well. The rotor should be made of as strong material as possible, such as alloy steel or Duralumin ST14. To operate the centrifuge, flexible pressure tubing (rubber) is first joined to C. The stator is then flexibly supported from C by a clamp fitting over the rubber tubing and mounted in a clamp stand. Compressed air is next admitted to the stator cup. This air flows through the straight channels LL' and forms into jets which impinge upon the flutings of the rotor. The motion of the air between the rotor and stator gives rise to Bernoulli forces which prevent the rotor from flying out of the stator and hence forces it to spin on a thin cushion of air between them. The stability of this air cushion is conditioned upon the proper proportions of the stator and rotor, the correct position of the impinging air jets, and by the free flow of air through the channel S from the atmosphere. In Fig. 1, $P=90°$; $Q=45°$ to $46°$; angle of stator cone $=91°$ to $92°$; angle of rotor cone $=102°$ to $103°$; and S is bored with drill size No. 43. The upper openings of the channels LL' (7 or 8 in number bored with a No. 62 drill for the dimensions of Fig. 1(b)) lie in a circular section of the stator cone midway between its vertex and base. The diameter of the base of the stator cone in Fig. 1 is $1\frac{1}{8}$ inches. The number and size of the channels LL' are not critical and can be varied over wide ranges. If the number of channels is even, the number of flutings on the rotor should be odd and vice versa. The stator can be built to fit any size of rotor by changing all dimensions proportionately. The rotor will spin either in the position shown in Fig. 1(b) or inverted. Rotors of almost any size or shape may be spun successfully in air or other gases at atmospheric pressure by the above method, but it is necessary to increase greatly the power input as the size of the rotor increases, because of the greater air friction.[8] The $1\frac{1}{8}''$ rotor in Fig. 1 will spin 180,000 r.p.m. with 50 lb./in.^{2} driving air pressure and 14.5 cu. ft. of air (reduced to standard conditions) per minute air flow. The highest speed so far reported[8, 16] (1,300,000 r.p.m.) was made with a 9-mm rotor driven by hydrogen at 160 lb./in.^{2}.

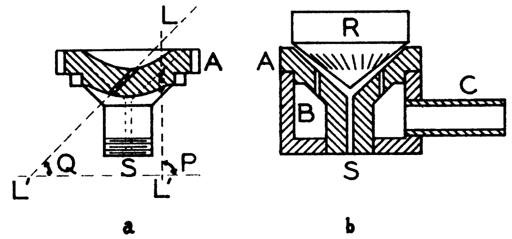

FIG. 1. Diagram of simple air-driven centrifuge. (a) A section (noncentral) through stator cone. (b) Complete machine.

The above type of apparatus has been utilized for spinning small mirrors to very high speeds for the purposes of studying the light emitted in the different stages of sparks, both in gases at various pressures and in a vacuum;[17, 18] also for producing short flashes of light[17, 19, 20] and for demonstrating and roughly measuring the velocity of light. In many types of biological experiments where accurate temperature control or thermal equilibrium throughout the whole rotor is not essential, the apparatus has been used as a centrifuge,[21–26] as, for example, in the determination of the relative displacement of the different components of plant or animal cells by centrifuging. Simple optical schemes for viewing biological material with a microscope while it is being centrifuged have been used successfully by Harvey[21] and Pickels.[27] These optical methods are of special interest, as they should be applicable to any type of rotor or centrifuge.

Several successful experiments have been carried out on the sedimentation of small particles and molecules from solution,[15, 25, 26, 28, 29] and a few workers[25, 28, 29] have reported success in the determination of sedimentation constants and molecular weights. However, in these experiments it is essential to take great care to maintain temperature equilibrium[*] throughout the centrifuge "bowl" in order to prevent stirring.[8, 16, 28–31]

It is especially difficult to maintain temperature equilibrium in the air-driven centrifuge which spins in air at atmospheric pressure. The air friction at high surface speeds is so great that the periphery and top of the centrifuge are heated. On the other hand, the expanding air jets striking the under surface of the centrifuge cool it in such a way that temperature gradients are set up and stirring results. The centrifuge bowl itself has been held at approximately uniform temperature by surrounding it with a layer of a liquid having a high temperature coefficient of expansion.[28] Stirring has also been much decreased by dividing the centrifuge bowl into a large number of very thin compartments made by stacking thin coaxial circular plates one above the other, with a fraction of a millimeter spacing between,[28, 29] or by filling the centrifuge with a jelly-like substance,[29] etc. However, such procedure is somewhat troublesome and not to be recommended where precise measurements are to be made. Furthermore, unless the power input to these centrifuges is increased to high values (which requires costly air-compressor equipment), rotors of more than an inch or so in radius cannot be spun to high rotational speeds because of the air friction.[8] It has been shown by Svedberg and his students[3] that the power of a centrifuge to resolve a mixture of different kinds of molecules is proportional to $\omega^2 Xh$, where ω is the angular velocity of the centrifuge, X the mean distance from the center of rotation, and h the radial length of the column of the solution. Also in some centrifuging problems it is undesirable to have the field vary rapidly along the radius.[3, 4] Hence, for many types of problems, precision measurements are difficult to make with small rotors.

3. Vacuum-type air-driven centrifuges

The vacuum-type air-driven centrifuge originally was devised[8, 16, 33] to overcome the difficulties encountered with the air-driven centrifuges described above. It consisted of a large rotor or "centrifuge" situated inside a vacuum-tight chamber which was driven and supported by an air turbine, similar to that shown in Fig. 1, located outside and vertically above the chamber. The turbine and centrifuge were connected by a flexible shaft (piano wire) which was coaxial with their axis of rotation and passed through a vacuum-tight oil gland which sealed the vacuum chamber. With this arrangement the centrifuge was self-balancing, since the flexible shaft allowed it to seek its own axis of rotation, and hence was extremely stable. Also, since the vapor pressure of vacuum pump oil in the gland, which limited the vacuum attainable, may be very low (10^{-5} to 10^{-7} mm for some Apiezon and Litton oils), the gaseous friction on the centrifuge was made negligible. Furthermore, it was found that

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[*] While, as far as the writer knows, the complete mathematical theory of the stirring or remixing of a liquid because of non-uniformity of temperature in a centrifuge has never been worked out, it is easy to see qualitatively that it is essential to maintain approximate uniformity of temperature if stirring is to be absent. For example, it is common observation that if a vessel or test tube is filled with water, say, at room temperature and the liquid in the lower part is heated a degree or so hotter than that in the upper part, stirring will take place. One of the principal factors in this stirring is the product of the difference in density of the cooler liquid at the top and the warmer liquid at the bottom, multiplied by the force of gravity. In a high speed centrifuge, instead of gravity there is an acceleration at least several hundred thousand times gravity, so that the temperature must be indeed very uniform if this factor is to be negligible. (Of course, if the denser (usually cooler) liquid is near the periphery, no stirring will occur; but in practice this is seldom the case.) Fortunately, there are other small factors which tend to counterbalance the above force which produces stirring. One of these arises from the fact that the density of a liquid increases with the pressure. In a centrifuge the pressure on the liquid increases from the center toward the periphery, so that any differences of density caused by non-uniformity of temperature, which are in the right direction to produce stirring, are counteracted to a certain extent. The maximum stable or adiabatic radial temperature gradient for a substance in a centrifugal field can be calculated[8, 32] from the well-known equation $(\partial T/\partial p)_s = (T/c_p)(\partial v/\partial T)_p$, where the symbols have the usual meaning. For an ideal gas $(\partial T/\partial r)_s = 10^{-7}\omega^2 r$ where ω is the angular velocity and r the radius, or for centrifugal fields of 10^6 gravity, about 100°C per cm. For water $(\partial T/\partial r)_s = 1.7 \times 10^{-9}\,\omega^2 r$, or for fields of 10^6 g, about 1.7°C per cm. It is clear that this stabilizing effect is small except in gases or in very intense centrifugal fields. A second small counterbalancing factor results from the fact that the denser materials in a mixture move toward the periphery making the resultant mixture denser there. This factor also is small except where the substance being centrifuged out is much denser than the liquid in which it is dissolved or suspended.

the efficiency of the air-driven turbine was not lowered appreciably by the weight of the centrifuge or by the friction of the flexible shaft in the vacuum-tight oil gland. Since a small air turbine can support a large diameter rotor, the maximum rotational speed, and hence the maximum centrifugal force, are limited entirely by the bursting strength of the centrifuge. It was found that the centrifuges could be made as large or as small as desired and that their temperatures remained very uniform throughout the centrifuges. Their temperatures could be accurately controlled and varied over large ranges.[8] Consequently, convection-free sedimentation was easily attained and the experimental results were in agreement with theory. Since the air turbine in Fig. 1 required some care to be taken in stopping the centrifuge, a driving turbine was devised[16] in which the cushion of air which supported it was independent of the air jets which spun it. This allowed the centrifuge to coast smoothly to rest on the air cushion support after the driving jets were turned off. For cases where it was necessary to stop the centrifuge in a comparatively short time, reverse air jets were provided. Several workers, especially in biology and medicine, have adapted and improved the above vacuum-type centrifuge to suit their special problems.[31, 34–38] However, the principal features of the machine are shown in Fig. 2, which has been used for the separation of isotopes by centrifuging.[8, 39, 40]

The rotating members consist of the air-driven air-supported turbine T, the rotor or centrifuge C, and the flexible shaft A which lies in their vertical axis of rotation and connects them together. A, which may be either a rod or a tube, passes through the vacuum-tight oil glands G_1 (which seals the vacuum chamber) and G_2 (which is added so that C may be filled with liquid or gas or evacuated while spinning). G_2 is also necessary if the very stable "flat type" of air bearing is used. The vacuum-tight oil glands are mounted in round flexible rings made of oil-resistant material, such as Duprene, so that they have a small amount of flexibility. Vacuum pump oil is forced into G_1 and G_2 under a pressure not less than that of the air which is applied through I and supports T. With this arrangement the bearings in the glands are both lubricated and cooled by the oil. The collar B is usually made of Bakelite and rests upon a round Duprene or rubber ring. The free air space between B and T is reduced to a practical minimum. (For a description of the different parts of the apparatus, reference should be made to the more detailed articles.[8, 38, 41]) To operate the centrifuge, C is first clamped by M to A at the proper distance (found by trial to be $\frac{3}{8}''$ to $\frac{3}{4}''$ for the centrifuge of Fig. 2) below G_1 and the axis of rotation is made vertical. Air is then admitted through I and the pressure increased until the rotating parts are freely supported upon the air film formed between B and T (6 to 8 lb./in.^{2} for rotor C of Fig. 2). This same air pressure is usually also applied to the oil tank to force the oil into G_1 and G_2. The vacuum-tight chamber surrounding C is next evacuated (if C is to remain at constant temperature, the pressure must be well below a micron). The driving air is then applied through D to the air box S, which directs air jets upon the flutings of T. As the rotor accelerates, it passes through a few speeds at which vibration takes place, but fortunately these are not violent and exist for only narrow bands of frequencies of rotation. At all other speeds the

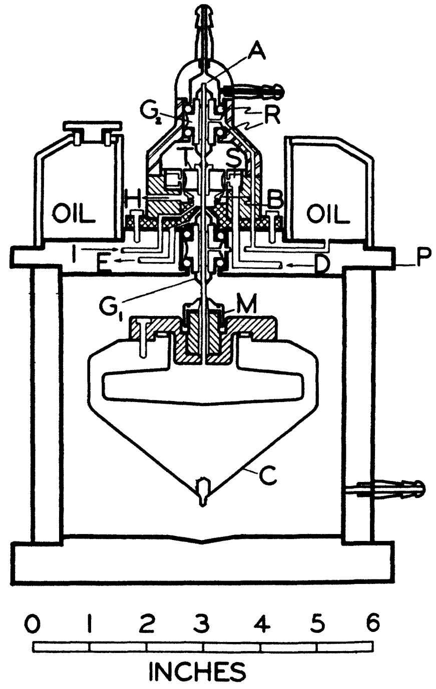

FIG. 2. Vacuum-type air-driven centrifuge.

centrifuge spins so smoothly that, when examined with a microscope or by reflected light at a distance of a meter or so, no observable wobble or vibration can be found. Care must always be taken to keep a careful check upon the rotational speed in order to prevent the centrifuge from exceeding its bursting speed.*

For the hollow 4″ Duralumin ST14 rotor *C* of Fig. 1, the bursting speed is about 1650 r.p.s., making a working speed of about 1550 r.p.s. possible. It is maintained at this speed by a driving air pressure of about 10 lb./in.^{2} and air flow of 2 ft.^{3}/min. NTP, although about 35 lb./in.^{2} is used in accelerating it. To carry out the experiment of "evaporative" centrifuging on the isotopes of chlorine,[39, 40] 15 cc of CCl_4 is placed in *C* (which holds about 65 cc when full). The centrifuge is then brought up to the working speed and *C* evacuated through the hollow shaft *A*. The CCl_4 evaporates at the periphery, diffuses against the centrifugal field, passes out through *A*, and is collected in three equal fractions in dry ice traps. The different fractions, when very carefully purified, show a difference in density in very rough accord with that predicted by the theory for the separation of isotopes in centrifugal fields (to be discussed later). For this particular experiment the highest vacuum surrounding *C* is not used. Enough air (a few microns) is left in the chamber so that the heating caused by air friction will furnish the energy necessary to evaporate the CCl_4. However, in most experiments a high vacuum is used.

The type of rotor or centrifuge *C* has been varied over wide ranges to suit the particular problem. For the concentration of rather heavy materials, a so-called "quantity" type of centrifuge has been used.[8, 34–38] It consists of a block of metal with channels bored at an acute angle with the vertical to hold (Lusteroid) test tubes. The material to be centrifuged is contained in the test tubes and sealed from the vacuum chamber by an air-tight cap. The heavy materials collect at the bottom, leaving the lighter fractions at the top of the test tubes. In some of the rotors as much as 120 cc may be centrifuged at one time. Rotors containing a sector-shaped cell, with quartz windows for optical observation of the rate of sedimentation of different molecular species, or so-called analytical rotors have also been used by a number of workers.[16, 31, 35, 42] The rotors have been constructed in various shapes to increase their bursting speed. The precision obtained at the present time in the determination of sedimentation velocities and molecular weights is the same as with the Svedberg oil turbine ultracentrifuge.[31, 42] Triangular and other variously shaped rotors have been used for various purposes, such as the velocity selection of molecules,[39] rotating mirrors, discharge tubes, determining the strength of materials, etc. The vacuum-type centrifuge has also been spun at both low and high temperatures.

Vacuum-type air-driven centrifuges have been constructed in which the driving mechanism is placed below, rather than above, the vacuum chamber.[8, 41] This allows observing apparatus to be placed over the rotor. Also with this arrangement, the rotor is like a top spinning on a point and thus will right itself if violently disturbed while spinning.

In most problems it is not only important to know the rotational speed of the centrifuge with precision; but it must be maintained constant. Several different methods have been described for measuring the speed[8, 11, 43, 44] and a few have been also used for controlling it.[8, 44–46] Stroboscopic methods have been the most popular, but with these trouble is sometimes caused by harmonics. A method which avoids this difficulty consists in measuring the frequency of the alternating current induced in a coil by a small magnet mounted upon one of the rotating members. The current is amplified and sent through a

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* As mentioned before, in all of the air-driven vacuum-type centrifuges the bursting strength of the centrifuge is the only factor which limits the rotational speed attainable. For homogenous rotors the bursting speeds are roughly proportional to ω^2r^2 where ω is the angular velocity and r the radius, so for problems which require high centrifugal forces or ω^2r only, the rotors should be made small. However, for a great many centrifuging problems, ω^2r^2 should be large. The importance of developing better materials for the rotors in these centrifuges cannot be overemphasized because it is possible to drive the rotors much faster than their present bursting speed. Also a more exact and usable theoretical analysis which would predict the best shapes of rotors, made from actual rather than idealized materials, would be helpful. The best material found so far for general use probably is the strong alloy steels, although Duralumin ST14, which is almost as good for many purposes, has been quite widely used. The smaller strength of the Duralumin is almost compensated for by its smaller density. An essential precaution is to surround the apparatus with a barricade with sufficient strength to stop the flying pieces should a rotor explosion occur.

frequency bridge.[44] After a little experience most observers can pick out the frequency of sound which is always given off equal to the rotational frequency. This frequency can then be measured by beating it with that given off by, say, a calibrated harmonica siren disk or tuning fork.

4. Steam and electrically-driven centrifuges

The steam-driven vacuum-type centrifuge[8, 47] is essentially the same as the air-driven type except that steam is used to support and drive the turbine instead of air. Special heat shields and cooling coils are necessary to insulate thermally the hot turbine, steam box, etc., from the top of the vacuum chamber containing the centrifuge. Fortunately, in practice this is easy to do. Unless air pressure is not available, the air-driven machine is usually to be preferred to the steam-driven one.

In many laboratories air pressure is not available in sufficient amounts to operate the air-driven centrifuge. Also there is great need for a vacuum-type centrifuge that is "fool-proof." That is, an apparatus should be available which would automatically accelerate to a given speed, remain at that speed for the desired time, and then slowly stop without the attention of an operator. The electrically-driven vacuum-type centrifuge[48, 49] holds promise of fulfilling these needs. In this apparatus a small electrical motor is used to drive the centrifuge instead of the air turbine. Otherwise it is practically the same as the air-driven vacuum-type centrifuge. Both induction and synchronous motors have been used. The high frequency alternating current has been produced either by variable frequency thyraton inverter circuits or by variable frequency oscillator circuits employing high vacuum tubes. Both of the above types of circuits operate on the 110-volt a.c. lines. The latter type of circuit has been found somewhat preferable for the centrifuges used so far. In the first electrically-driven machines the rotating members were supported upon an air cushion almost identical with that used in the air-driven machine (Fig. 2). However, recently S. A. Black and the writer have used a magnetic support which has an extremely small frictional torque. The flexible shaft which connects the armature of the motor and the centrifuge and passes through the vacuum-tight oil glands similar to G_1 and G_2 of Fig. 2 is made long enough to extend above the upper gland. A short piece of round iron rod with a channel bored along its axis, into which the flexible shaft fits, is anchored on the shaft so that the shaft extends beyond the center of its circular flat upper surface about $\frac{1}{8}''$. The rotating parts are supported by the attraction of a solenoid for the iron rod, the solenoid is coaxial and directly above the shaft. The end of the shaft fits into a small hole bored in the end of the solenoid of such a depth that a small air gap remains between the upper end of the rod and the lower end of the solenoid—that is, the end of the shaft fits into a stop which prevents the iron rod from touching the face of the solenoid. The current through the solenoid is adjusted so that its magnetic attraction for the rod is slightly greater than the weight of the rotating parts, so that the pressure of the end of the shaft on the "stop" is very small. Since the shaft is oiled, its friction is less than one of the oil glands, which is small. These magnetic supports have been used to support rotors weighing several pounds and there is no reason why very heavy rotors cannot be supported. Also rotors may be spun to as high a rotational speed as with the air bearing support. It will be observed that, since the magnetic field is symmetrical over the circular end of the rod, there is practically no drag caused by eddy currents in the rod. With this magnetic support and electrical drive, the need for compressed air can be completely eliminated. It is run from 110-volt lines. Six-inch rotors have been driven to over 1000 r.p.s. and 4″ to 1200 r.p.s. in this manner, with only 275 watts input to the field of the motor. Over a trial period of six hours no variation in the rotational speed could be observed with the usual stroboscopes available, with no attention from the operator. It is probable that for most work the electrically-driven magnetically-supported centrifuge, when fully developed, will supplant the air-driven air-supported types.

5. Tubular vacuum-type centrifuge

The vacuum-type machines described above are practically ideal centrifuges, since the separation observed is in accord with that predicted by the theory, which assumes no stirring. Also the maximum centrifugal force attainable is determined by the strength of the material and could be much increased if better rotor material could be obtained. Furthermore, the centrifuging can be carried out at any temperature over a wide range. Unfortunately, however, the quantity of material which can be centrifuged per unit of time is limited because of the size and shape of the centrifuge. In order to overcome this limitation, the vacuum-type tubular centrifuge has been developed.[50–52] In this apparatus the material to be centrifuged enters at a continuous rate at the top and is collected in two fractions at the bottom of a spinning tube. No upper limit has as yet been found for the length or size of the centrifuge, hence, the rate of centrifuging may be made correspondingly large. Separation of materials in the liquid, gaseous and vapor states have been carried out with essentially the same design of centrifuge, the difference being only in the internal construction of the spinning tube.

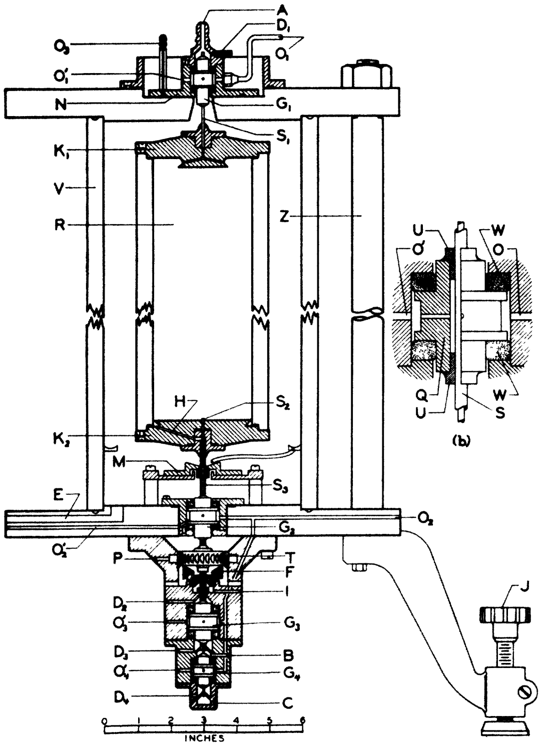

FIG. 3. Vacuum-type tubular centrifuge for the separation of gases and vapors.

Figure 3 shows a scale diagram of a vacuum-type tubular centrifuge used for the separation of gases and vapors. The rotating members include the air-driven air-supported turbine T, the tubular rotor R, and the flexible tubular shafts S_1, S_2, and S_3. S_1 passes through the vacuum-tight oil gland G_1. S_2 and S_3 pass through the vacuum-tight oil glands G_2 and G_3, while S_2 passes through the vacuum-tight oil gland G_4 also. G_1 and G_2 seal the vacuum chamber V, while G_3 and G_4 make it possible to separate the lighter fraction of the material which flows out through S_2 from the heavier fraction which flows out between the coaxial tubes S_2 and S_2. The gland G_1 is mounted on a movable metal disk N, the under surface of which is ground to fit the upper surface of the top plate of the vacuum chamber. Vacuum pump oil is forced in at O_3 under a pressure just sufficient to float N upon a thin film of oil. It was found that only a few cc of oil leaked into the vacuum chamber per hour through this floating bearing. As in all the vacuum-type machines, the vacuum pump oil is collected, filtered, and used again. The air turbine drive is similar to that in Fig. 2. (For a detailed description of the different parts of this machine, reference must be made to the original papers.[50–52])

To operate the machine, the axis of rotation is made vertical, the supporting air applied to I at a pressure which will freely support the rotating parts, the pressure applied to the oil in the glands, the vacuum chamber evacuated to less than a micron, and the driving pressure applied through P. As the rotating members speed up the tubular centrifuge, R, passes through certain comparatively sharp frequencies where the floating plate N is observed to wobble freely upon its oil cushion. At all other rotational speeds N remains stationary and seeks a position which makes the axis of rotation vertical. The centrifuge is, of course, always operated at a speed well removed from any critical frequency. The large moment of inertia of the long alloy steel cylinder R makes the speed easy to keep constant. Again the maximum speed is set only by the strength of R. When gases or vapors are centrifuged, the material enters R at the top through A and separation takes place as it flows downward. The lighter fraction passes down through S_2 and is collected at C, while the heavier fraction flows out between the loosely fitting circular flange and the inner wall of *R*, then through the channels *H* into the space between the coaxial tubes S_2 and S_3 and is collected at *B*. The ratio of the quantity of material collected at *B* to that collected at *C* can be regulated by adjustable valves at *B* and *C*. It should be noted that it has been possible to place the air turbine above as well as below the vacuum chamber in the case of the tubular centrifuge, but this requires certain modifications in the above design.

When the apparatus is used for centrifuging liquids, the internal construction of the tube is modified by mounting a solid steel rod inside and coaxial with it, whose radius is less than the inner radius of *R*. The presence of this rod forces the material being centrifuged through the stronger part of the centrifugal field and thus increases the rate of separation.

The vacuum-type tubular centrifuge was originally designed and used for the separation of isotopes, but it has also been employed to separate gases of different molecular weight (N_2 and CO_2), constant boiling-point mixtures,[53] and substances of high molecular weight in liquid solutions.[50–52] It should be especially suited for the purification and separation of biological substances. In addition to the purification of materials, the above apparatus may be used for the determination of the rate of sedimentation of comparatively heavy molecules from which, as will be described later, the molecular weight and particle size can be estimated.

6. Axially magnetically suspended centrifuge

Recently a new type of centrifuge has been made possible by the Holmes magnetic suspension.[54–56] This consists of a vertical rod of iron suspended by the coaxial field of one or more solenoids. The "centrifuge" is attached to the iron rod. A steady direct current in one of the solenoids is not quite sufficient to support the rotating members, while the current through the other solenoid is controlled by a photoelectric cell arrangement. The amount of light incident on the photo-cell is partly intercepted by the centrifuge or vane attached to it in such a way as automatically to maintain the rod at a predetermined height. Such a suspension is macroscopically stable and, in a vacuum, theoretically may have infinitesimal torque against axial rotation. Rotors have been driven by induction from a rotating magnetic field outside the vacuum chamber and by "rotating" electrostatic fields inside the vacuum chamber. Rotors have been spun to about 180,000 r.p.m. and the maximum speed attainable is limited apparently only by the bursting strength of the rotors. Tests have shown that the frictional torque of the axial magnetic suspension can be made so small that several seconds are required for the rotor to lose one-millionth of its speed. Smith[56] has developed a capacity coupling arrangement to actuate the current in the second solenoid instead of the photo-cell, which makes the support equally as stable and avoids the necessity of the light beam. Because of the low frictional torque, rotors can be spun inductively by rotating magnetic fields controlled by piezoelectric circuits. Hence very constant rotational speed should be attained. This should make them adaptable to a great many problems in physics outside of centrifuging, including the measurement of the velocity of light or of high speed particles. Also, since the rotor is freely supported without mechanical connections and can be driven by induction, it can be completely sealed in glass, thus making a very high vacuum possible. Also this same property makes it possible to spin rotors at extremely low or at high temperature.

B. Theory of Sedimentation in Ideal Dilute Solutions

The quantitative theory for the separation of mixtures of substances in a centrifugal field in general is in a rather unsatisfactory state mathematically. As a matter of fact, there is no complete theory with which it is possible to calculate the concentration of nonidealized mixtures as a function of the radius of the centrifuge and of the time of centrifuging. However, accurate calculations can be made for certain special cases like the equilibrium state, which occurs after an infinite time, the case of dilute ideal liquid solutions which can be assumed to be incompressible, etc. These theories are of great value, as they make it possible to determine the molecular or particle size and mass of the materials centrifuged, as well as serve as valuable guides in practically all centrifuging problems. The importance of more quantitative theoretical work on the separation in centrifuges cannot be overemphasized because of its obvious value for the interpretation and stimulation of experimental work.

The problem of sedimentation in ideal solutions in a uniform gravitational field has been solved by Mason and Weaver.[57, 58] This theory may be used to give approximate results for sedimentation in a centrifuge when the radius of the latter is large and the height of the sedimenting column is small, i.e., where the field is approximately uniform over the length of the column.

The general differential equation which represents the concentration c at a point as a function of the time t in a centrifugal field of force for a dilute ideal solution in a sector-shaped cell rotating at constant angular velocity w, as given by Lamm,[59] is

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

where r is the distance from the center of rotation, D the diffusion constant, and s the sedimentation constant or the velocity of settling of the dissolved substance in a unit (gravitational) field. This equation has been solved by Archibald.[60] Faxen[61] and Sôyten[62] had previously obtained approximate solutions for Eq. (1) which, although of mathematical interest, could not conveniently be used for numerical computation. Archibald was able to put his complete solution in a usable form for both the case of a sector cell extending to the center of rotation as well as for one which does not. Reference to his articles[60] must be made for details of his solutions and numerical curves.

The differential equation (1) is readily solved for the limiting or equilibrium case where $t=\infty$ or $\partial c/\partial t=0$ and $dF=0$, where F is the free energy at every point in the cell;* i.e., when the sedimentation and diffusion are in equilibrium. Svedberg[1] gives the solution in the following form:

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

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

where M is the molecular or "micellar weight," R the gas constant, T the absolute temperature, c_1 and c_2 the concentrations at the points r_1 and r_2 distant from the axis of rotation, respectively, ρ the density of the solution, V the partial specific volume of the substance, v the volume of the molecule or micell, N the Avogadro number, ρ_p and ρ_d the densities of the particle and solvent, respectively.

For cases where the equilibrium method can be used, it is probably superior to any centrifuging method so far devised for determining the molecular weights and volumes. It is based upon somewhat sounder theory and avoids the necessity of measuring the diffusion constant. However, it is found from Archibald's theory and from experiment that in many cases an inconveniently long time is required for the equilibrium state to be approached closely enough for Eqs. (2) and (3) to be employed. This is especially true for large molecular weight substances. Also a great many interesting substances decompose or otherwise change before the centrifuging can be completed with the above method. Fortunately, another method of measuring the molecular weights and sizes is available. It is known as the velocity of sedimentation method and the measurements can be carried out in a comparatively short time, provided the centrifugal field is sufficiently large. Also it is strictly applicable only to cases where the liquid can be considered infinite in extent, i.e., when the cell is long enough so that no reflection of the particles takes place from the ends of the sedimenting column while the measurements are being carried out. Under these conditions the centrifugal force per mole can be set equal to the frictional force per mole,[1] so that

$$M(1-V\rho)\omega^2 r=f(dr/dt), \tag{4}$$

where $f=RT/D$ from Einstein's diffusion equa-

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* The change in free energy with position in the solution can be written (reference 63) as $dF=(\partial F/\partial r)dr+(\partial F/\partial p)dp+(\partial F/\partial c)dc$ where p is the pressure. After the centrifuging has been continued long enough for the equilibrium state to be established in the case of a dilute solution, it is possible to substitute for the terms in the above equation as follows: $dF=0=-M\omega^2 rdr+MV\rho\omega^2 rdr+(RT/c)dc$, the integration of which gives Eq. (2).

tion, hence

$$M=\frac{RT(dr/dt)}{D(1-V\rho)\omega^2 r}=\frac{RTs}{D(1-V\rho)}\tag{5}$$

or in the integrated form

$$M=\frac{RT\log r_2/r_1}{D(1-V\rho)\omega^2(t_2-t_1)}.\tag{6}$$

Hence, by measuring the sedimentation constant* s ($s=$ sedimentation velocity per unit field) and the diffusion constant D, the molecular weight of the material in solution may be obtained. The experimental methods of measuring s have consisted either of observing the concentration of the material as a function of the radius and time optically or by so-called analytical methods. The optical methods which have been developed by Svedberg and his students consist of determining the concentration by the amount of light absorbed[1, 3, 6, 7] or by the index of refraction.[3, 64] The former method is not so satisfactory as the latter except when the substance being centrifuged has convenient absorption bands. It often happens that the absorption bands of the solvent overlap those of the material being centrifuged, which makes the determination of the concentration difficult by absorption methods. On the other hand, the refraction methods can be used in the visible region of the spectrum except where the solution is opaque throughout the entire visible region, thus obviating the necessity of using expensive quartz-fluorite optical equipment. The projected scale method of Lamm[64] has been used for the most precise measurements, although the Toepler *schlieren* method has found wide use in many problems.[3, 65] The writer has found a modification of the Twyman interferometer useful for this work.

In a great many cases the analytical are preferable to the optical methods for determining the concentrations at different points in the centrifuge. This is especially true for many biological materials such as antibodies, enzymes, hormones, etc., which often occur in such small concentrations and impure states that the optical methods are not readily applicable.[66] Also, in the case of many biological substances the usual tests for activity consist in injecting the material into an animal and noting its reaction. The analytical method consists in making analyses of samples taken from the centrifuge while it is spinning, or after it has been brought to rest. In the latter procedure it is customary to place permeable membranes or other devices in the rotor which will not disturb the sedimentation but will prevent stirring or remixing while stopping the centrifuge. These schemes can be worked either with the equilibrium method, in which Eqs. (2) and (3) are used, or the velocity of sedimentation method of Eq. (5). A convenient method[3, 67] of using the latter for the case of large molecules, which gives good results, is as follows: A thin Bakelite partition divides a sector-shaped cell into two compartments of almost equal volume in such a way that the direction of sedimentation is perpendicular to the partition. The partition is perforated with a great many fine holes and is covered with a piece of hard filter paper. This arrangement does not disturb the sedimentation but prevents remixing upon stopping. It can be shown[67] that if the cell is long enough and the time t is not too large, the sedimentation constant

$$s=-\frac{1}{2\omega^2 t}\log_e\left(1-\frac{2\Delta}{qxc_0}\right),$$

where Δ is the total change in amount of the material above or below the partition, x the distance of the partition from the axis of rotation, q the area of the partition, and c_0 the concentration of the substance at the beginning.

Schemes for removing samples from the rotor while it is at full speed, such as are used in the tubular vacuum-type centrifuge of Fig. 3, may be employed for the determination of the sedimentation constant s or the molecular weight and size. To calculate either s or the speed of separation in the tubular centrifuge, it is necessary to use the theory of Archibald.[60] However, for the case of particles or substances with very

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* If the sedimentation velocity is referred to standard conditions and unit field,

$$s=\frac{dr/dt}{\omega^2 r}\frac{\eta}{\eta_0}\frac{1-V\rho_0}{1-V\rho}$$

where η and ρ are the viscosities and densities of the solution and η_0 and ρ_0 are the same quantities for the standard solvent under standard conditions.

large molecular weight in liquid solution, where diffusion can be neglected during the centrifuging, the rough calculations are very simple. For this condition, if R_1 is the inner radius of the tubular centrifuge, R_2 the radius of the solid rod inside and coaxial with it, a the clearance between the flange and the inner wall of the tube through which the heavier fraction emerges, C_0 the concentration of the material to be centrifuged, which is introduced into the spinning tube at the top, and C is the concentration of the heavy fraction, then for most efficient operation,

$$\frac{C_0}{C}=\frac{a(2R_1-a)}{R_1^2-R_2^2}.$$

For this to hold, the volume of the heavy fraction collected per sec. should be approximately C_0/C of the volume per sec. entering the centrifuge at the top. The remainder is, of course, collected in the light fraction. If T is the time the material remains in the centrifuge and L the length of the tube,

$$T=\pi(R_1^2-R_2^2)L/(\text{volumetric rate of flow}),$$

$$S=\frac{1}{2\omega^2T}\log_e\left\{\frac{R_1^2}{R_2^2}\left(1-\frac{C_0}{C}\right)+\frac{C_0}{C}\right\}.$$

Volumetric rate of flow

$$=\frac{\pi L\omega^2s(R_1^2-R_2^2)(R_1+R_2-a)}{2(R_1-R_2-a)}$$

$$=\frac{\pi L\omega^2s(R_1+R_2)^2}{2}$$

if a is very small.

It is clear from the above approximations that the rate of centrifuging is directly proportional to the length of the spinning tube and to the square of the angular speed. Also, when a is much smaller than R_1-R_2, the rate is proportional to $(R_1+R_2)^2$. Obviously, there is a practical lower limit to the magnitudes of (R_1-R_2) and of a.

The diffusion constant may be measured either during the centrifuging[1] or in separate experiments.[3, 64] During the centrifuging the boundary between the sedimenting molecules and the solvent is blurred by diffusion, so that the diffusion constant D as well as s can be obtained from an analysis of the same radial-concentration-time curves.[1] In practice it is usually better to check the value of the diffusion constant with a separate experiment.[3] It can be shown[1, 68] that, if the centrifuging is fast enough to give a zone of pure solvent close to the meniscus of the sedimenting molecules so that reflection of particles from the ends of the sedimenting column can be neglected, approximately

$$C_x=\frac{C_0}{2}\left(1-\frac{2}{(\pi)^{\frac{1}{2}}}\int_0^y e^{-y^2}dy\right), \tag{5a}$$

where $y=x/2(Dt)^{\frac{1}{2}}$ where C_0 is the initial concentration at $t=0$, C_x the concentration at a distance x (positive) toward the axis from the sharp boundary which would have been formed if there had been no diffusion. If x is set equal to zero, $C_x=\frac{1}{2}C_0$ so that the position of the boundary, which would have resulted had there been no blurring by diffusion, is where the concentration is one-half that at the beginning of the centrifuging. This method is generally used only for comparatively large molecules or micells and in high centrifugal force fields. When the centrifugal force is too small to produce a clear zone of liquid at the top of the sedimenting column, the equations of Archibald[60] must be used for determining the sedimentation constant.

Under the assumption of Stokes law and that the particles are spherical, the radius R of the particles is found to be[1, 68]

$$R=\left(\frac{9}{2}\frac{\eta s}{\rho_p-\rho_d}\right)^{\frac{1}{2}}=\left(\frac{9}{2}\frac{\eta\log_e r_2/r_1}{(\rho_p-\rho_d)\omega^2(t_2-t_1)}\right)^{\frac{1}{2}}, \tag{7}$$

where η is the coefficient of viscosity. Since the previous equations (3) and (5) do not require the assumption of spherical particles, it is, of course, possible, by a comparison of values, to determine whether or not the molecules are spherical.*[1, 64, 69]

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* The molar frictional constant is given by

$$f=M(1-V\rho)/s.$$

For a spherical molecule with the same specific volume, the molar frictional constant $f_0=6\pi\eta N(3MV/4\pi N)^{\frac{1}{3}}$ where N is the Avogadro number. The ratio f/f_0 is unity for a spherical particle, but greater than unity when the particle is not spherical. It is called the Svedberg dissymmetry constant.

Or if the particles are known to be spherical, Stokes law can be tested.

The above equations are obviously based upon the hypothesis that the sedimenting particles are all of the same size and are uncharged. If the solution being centrifuged contains a mixture of substances with different molecular weights, each different substance will have a characteristic sedimentation velocity s which can be measured. If several molecular species are present, their volumes and molecular weight values can be determined with the sedimentation equilibrium method by an analysis of the different values determined for M by substituting a number of values for the concentration c at different distances from the axis of rotation into Eq. (2). In the velocity of sedimentation method they are determined by sharp changes in the concentration gradient at various distances along the radius. If the material contains particles with continuously varying masses or sizes, the analysis of the radial variation in concentration gives a determination of the distribution of sizes and masses present by both methods.[4, 64, 68, 89–94] The presence of charged particles or ionized molecules in a solution disturbs the sedimentation, since the centrifuging may produce a partial separation of the charges, thus setting up an electrical potential in opposition to the centrifugal potential. This effect is of utmost importance in the study of molecular or micellar weights and sizes of colloids because many of them are charged. The dissociation of a molecule into a positive and a negative ion, one of which is larger or heavier than the other, considerably disturbs the sedimentation measurements, because the larger heavier ions settle toward the periphery of the centrifuge at a greater rate than the lighter ones, so that an opposing electrical potential is set up. In practice this potential can be repressed experimentally for the cases of large molecular substances such as the proteins by the addition of a small molecular weight electrolyte and by working at the so-called proper pH value. The theory for the sedimentation in a colloid electrolyte has been given by Svedberg.[68] The general theory of sedimentation in electrolytes has been developed by a number of different workers and the results have been checked experimentally.[70–73] If a is the activity coefficient, for the condition of sedimentation equilibrium in a centrifugal field

$$\frac{d \log_e a}{dr} = M(1 - V\rho)\frac{\omega^2 r}{RT}. \tag{8}$$

This equation has been used to determine M when a is known[71–73] and a when M is known.* In the case of a nonelectrolyte, a can be taken equal to c so that Eq. (8) reduces to (2) upon integration.

C. Applications

The methods described above for measuring the size and mass of particles and molecules have been used most extensively up to the present time in the fields of medicine and biology. The discovery by Svedberg and his students[3] by centrifugal analysis that proteins, which are of fundamental importance to all living things, usually possess perfect molecular homogeneity and thus have a definite molecular weight, stimulated great activity in these fields. Also it has been found possible to study the aggregation, association, dissociation, etc., of these protein compounds. Although a critical discussion of the biological and medical applications of the ultracentrifuge must be left to specialists in the field, the behavior of some of these large protein molecules might be mentioned because of their possible interest in connection with recent physical theory. Svedberg[3] lists a table of almost fifty measured molecular weights of proteins which vary from about 17,000 to about 10,000,000. In this group he has pointed out a remarkable regularity which he thinks is a clue to the manner in which the protein molecules are built. The measurements are precise enough to show that

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* It is probable that results on the centrifuging of inorganic electrolytes in a water solution can be repeated to considerably less than one percent. This suggests the interesting possibility of using the vacuum-type centrifuge to measure the molecular or atomic weights of the radioactive isotopes formed by nuclear bombardment. By calibrating the apparatus with materials of known molecular weights which have the same chemical properties as the radioactive isotope, it should be possible to determine which isotope is radioactive. Of course, the mass could not be obtained with high accuracy but the isotope certainly could be identified. It would be necessary only to have a very small amount of the artificially radioactive isotope, since its ionizing radiations may be utilized to obtain the concentration of the isotope as a function of the radius.

the various molecular weights of the proteins probably are multiples of a definite unit which, for the substances listed, he takes as 17,600. Svedberg suggests that the protein molecule is built up by successive aggregations of definite units, but that only a few aggregates are stable and that the greater the molecular weight, the fewer are the possibilities of stable aggregation. In other words, the gaps between the existing molecular weights become greater as the weight increases. Furthermore, it is found that some of the molecules are profoundly changed by a slight variation in *p*H, by high dilution, by extremely small amounts of foreign matter, etc. For example, by changing the *p*H by a small amount, haemocyanin *Helix promatia* of molecular weight 6,740,000 dissociates stepwise into halves, eighths, and sixteenths, each dissociation product representing homogeneous components. Such changes are often reversible. A solution of *Helix promatia* at *p*H 6.8 ($s_1=98.9\times10^{-13}$, $M_1=6{,}740{,}000$) was changed to *p*H 8.0 where it was split up into three components with sedimentation constants and molecular weight values $s_1=98.9\times10^{-13}$, $M_1=6.740{,}000$; $s_2=62.0\times10^{-13}$, $M_2=3{,}370{,}000$; and $s_3=16.0\times10^{-13}$, $M_3=842{,}000$. The *p*H was then brought back to 6.8 and a sedimentation analysis showed that the different parts had united to form the original substance of molecular weight of 6,740,000. It is also interesting to note that many substances show definite sedimentation constants which reveal molecular weights much above ten million. According to Wyckoff[42] the sizes of the different viruses range up to microscopically visible bodies. Beard, Finkelstein and Wyckoff[74] have reported elementary bodies of vaccinia which give sharp sedimentation boundaries in the centrifuge, indicating a definite molecular weight with a sedimentation constant $S_{20^\circ}=5400\times10^{-13}$. The analysis of normal and pathological human sera in the centrifuge shows some distinct differences.[3, 75] In most cases new components appear in the pathological sera which are characteristic of the disease. Also certain substances such as the globulin content increases during a disease. The use of the centrifugal analysis is now being tried out as a means of diagnosis in medicine.

Another extremely important use of the high speed centrifuge has been in the concentration and purification of biological materials in quantities large enough for experimentation.[34, 76–88] Many of the most important biological materials apparently cannot be purified in any other way. As in the cases mentioned above, a slight change in the *p*H value causes the molecules to dissociate. In many cases even a change in temperature will destroy the material. Consequently chemical methods of purification are powerless. In many instances the materials exist in such dilute solution that it would be impossible to study them without concentration. Bauer and Pickels[34] in their original experiments reported a concentration of the yellow fever virus of over 10^4 times, while Wyckoff and Corey[77] found that the tobacco mosaic viruses were crystallized from the solution while it was being centrifuged. The number of materials concentrated recently has multiplied so rapidly that complete reference to the literature cannot well be given here. It is almost a foregone conclusion that the high speed centrifuge must become an integral part of research laboratories which propose to study proteins or other macromolecular compounds. Fortunately, the vacuum-type centrifuges are inexpensive enough to make this possible. If it becomes possible to separate the isotopes in large enough quantities by centrifuging, the high speed centrifuge automatically should become also an integral part in the many biological experiments that can be carried out with them.

The high speed centrifuge has also been utilized for studying the living cell.[21–24] From the relative movement of the different parts of the cell it is possible to estimate such things as the "viscosity" and "surface tension" of the protoplasm (consistency), as well as the relative density of all the materials found in the cell and the elasticity of the cell membrane. Especially has it been helpful in cytological studies of the cell, as it affords a method of distinguishing between real structures and so-called artifaxes. The relative importance of the different structures in the cell has been determined by centrifuging them out[22] and observing the effects on the life and growth of the cell. Several structures which were previously thought to be of vital importance to the process of living have been thrown out of the cell without killing it. In many cases after the cell has been centrifuged, abnormal development occurs, but this is not always the case. It is rather surprising the high fields some cells will withstand. H. W. Beams and R. L. King[22] centrifuged fertilized eggs of *Ascaris suum* at 400,000 times gravity for an hour. The eggs were then removed from the rotor and observed under a microscope. At first they were found to be stratified into distinct layers. Twelve hours later they had lost their stratification and a large percentage of them lived. However, many other kinds of cells are killed very easily by centrifugal fields.

In addition to proteins, other types of high molecular weight compounds have been studied by means of the centrifuge: for instance, the so-called linear macromolecules, such as carbohydrates and hydrocarbons, as well as a number of synthetic compounds. For example, Signer[89] has investigated solutions of polystyrenes in different organic solvents and has interpreted his results as showing that free movement of the long molecules could take place only in very dilute solutions, and that the movement became less with increasing molecular weight. Lamm[64] has shown that the molecular weights of the starches depend upon their previous history and in general are polydisperse. Nichols,[90] Kraemer[91] and others[92–94] have applied the ultracentrifuge to the study of a great many materials made industrially, such as rubber, Neoprene, cellulose and cellulose derivatives. They have found important relationships between the weight and shape of the molecules, on the one hand, and macroscopic properties of the material such as viscosity, on the other. Also they conclude that, at the present time, the centrifuge method is the most satisfactory one known for the study and measurement of the non-uniformity of large molecular weight materials showing large diffusivity.[90] These same workers have applied comparatively low speed centrifuges to the measurement of particle size in powders and pigments which can be suspended in a liquid, as well as to the study of emulsions, the mechanism of which they were able to trace. As mentioned before, the particle size and mass of both organic and inorganic colloids[89–92] may be studied over wide ranges. It has been especially useful[90] over the range of particle radius from 0.7 mμ to 10 mμ.

In addition to the determination of particle size, shape and weight, the ultracentrifuge can distinguish between a simple mixture of molecules and a chemical equilibrium; as a matter of fact, the relation between the equilibrium constant, centrifugal field, pressure and temperature can be studied.[90] It is possible to study the hydration of molecules in solution and to determine the ratio of the radius of the unsolvated particle to that of the solvated one.[68, 94] The constituents of alloys[95] in the liquid form have been separated and it is probable that a number of rare elements or compounds could be purified by centrifuging. Valuable information on the structure of liquids should be obtained by centrifuging, especially after the general theory of centrifuging is further developed.

D. Separation of Gases and Vapors

The theory for the separation of gases and vapors in a centrifuge has been given quantitatively only for the case of an ideal gas when the equilibrium state is reached, i.e., $\partial c/\partial t=0$ at every point in the centrifuge. This case was first worked out by Lindemann and Aston,[96, 97] later elaborated by Mulliken[98] and Chapman.[99] If ρ is the density of the gas and p the pressure, then the change in pressure along the radius r of the centrifuge is given by

$$dp=\rho\omega^2 r\,dr \quad \text{and} \quad p=(RT/M)\rho, \tag{9}$$

whence

$$\frac{d\rho}{\rho}=\frac{M\omega^2}{RT}r\,dr$$

and

$$\rho=\rho_0\exp\left(M\omega^2r^2/2RT\right)=\rho_0\exp\left(Mv^2/2RT\right), \tag{10}$$

where v is the peripheral velocity, M the molecular weight of the gas, and ρ_0 the density of the gas at the center and ρ the density at a distance r from the center. If the gas contains two isotopes of masses M_1 and M_2, and if K_0 is the ratio of the quantities present at the center, the ratio at the periphery K is

$$K=K_0\exp\left(M_2-M_1\right)v^2/2RT. \tag{11}$$

The so-called separation factor S is usually defined as

$$S=\frac{K}{K_0}=\frac{(X_1)_p/(X_2)_p}{(X_1)_0/(X_2)_0},$$

where $(X_1)_p$ and $(X_1)_0$ represent the mole fraction of the first gaseous isotope at the periphery and center of the centrifuge, respectively, while $(X_2)_p$ and $(X_2)_0$ represent the corresponding quantities for the second isotope. The theoretical value of S given in Eq. (11) is independent of the state of combination of the elements,[98] provided the isotopes have identical chemical properties. Further, it has been proved[98] that Eq. (11) holds for the separation of ideal liquid isotopic mixtures as well as for gases. The difference in the atomic weights ΔM of the mixture of two isotopes after equilibrium is reached at distances r_1 and r_2 from the axis of rotation is given by Mulliken[98] as approximately

$$\Delta M = \frac{(M_2 - M_1)^2 X_1 X_2 \omega^2 (r_2^2 - r_1^2)}{2RT}. \qquad (12)$$

Mulliken also generalizes this equation to give ΔM for a mixture of several isotopes. For the case known as "evaporative" centrifuging, suggested by Mulliken, the procedure consists in drawing out vapor from the axis of a hollow spinning rotor containing the liquid in its periphery,—that is, evaporation takes place and the vapor diffuses from the periphery to the center of the rotor. If this process occurs slowly enough so that equilibrium can be considered to be established, and if the vapor obeys the ideal gas laws, then the separation factor S is given by Eq. (11) and the change in molecular weight between the residue remaining in the rotor and the original material ΔM for a mixture of two isotopes[98] is, approximately,

$$\Delta M = \frac{(M_2 - M_1)^2 X_1 X_2 \omega^2 r^2}{2RT} \log_e C, \qquad (13)$$

where C is the so-called "cut," or the ratio of the total amount of the material initially to the amount of residue remaining in the centrifuge.

It will be noted from Eq. (11) that the separation coefficient S depends upon the difference in masses and not upon their absolute values. For this reason, considerable effort has been spent in attempts to separate the isotopes by centrifuging because, if the method could be made to work, it would be as effective for the heavier elements as for the lighter ones. This would be a distinct advantage over most of the other methods, which depend upon some power of the absolute masses. Furthermore, the separation coefficient S is independent of the state of combination of the element[98] so that it should be possible to find some compound of almost any element, either organic or inorganic, which would have the proper physical properties such as vapor pressure, etc., to adapt it to the process of centrifuging. Unfortunately, the early attempts[98, 100] to separate the isotopes experimentally were uniformly unsuccessful and the method was generally abandoned as impractical.[98]

TABLE I. *Separation coefficient for various mass differences and temperatures.*

M_2-M_1	300° K	200° K	90° K	20° K
1	1.05	1.08	1.18	2.13
2	1.11	1.16	1.40	4.53
3	1.16	1.25	1.65	9.68
4	1.22	1.35	1.95	20.7

However, the development of the vacuum-type air-driven centrifuges previously described in this review again raised the question whether or not the method might be made practical. It will be observed from Eq. (11) that S increases with the square of the peripheral velocity $\omega^2 r^2$ in the exponent of e and with decreasing temperature in the denominator of the exponent of e. The vacuum-type centrifuges may be spun to any speed which the material of the rotor will stand without exploding under its own centrifugal forces. Also it may be spun at either high or low (liquid-air) temperatures, is convection and vibration free, and may be adapted to either gases, vapors, or liquids. Hence it should afford an ideal means for the test. The highest peripheral velocity so far attained with the rotor materials available is about 8×10^4 cm/sec., and there is no great difficulty in obtaining an effective peripheral velocity of 5×10^4 cm/sec. under working conditions. Table I shows the separation coefficient S to be expected for various mass differences and at temperatures of the room, dry ice, liquid air, and liquid hydrogen for a peripheral velocity of 5×10^4 cm/sec.

Although the work is still in its preliminary stages, a definite separation of the isotopes of chlorine has been obtained.[39, 40] In these experiments the general method of evaporative centrifuging was used. The apparatus was that shown in Fig. 2, and a brief description of the experiment was given following that of the apparatus. Unfortunately, there is no theory available which will quantitatively give the exact separation to be expected in the experiment, because the vapor was drawn off from the center so rapidly that equilibrium was probably not established. The vapor was drawn off at the rate of 2 cc of liquid CCl_4 per hour (which was a convenient rate for the experiments) and the centrifuge chamber in which the diffusion could take place was only 6.4 mm in height. At this rate of drawing vapor out, the results showed a separation of 40 percent of that calculated by Eq. (13). When the rate of drawing out vapor was decreased or increased, the separation was increased or dedecreased, respectively. In order to determine whether or not the concentration of the isotopes would continue with successive centrifugings, the heavy fractions were recentrifuged and the increase in density of the material determined. This increase was also approximately 40 percent of the theoretical value.[40] Recently, using long tubular chambers spun by apparatus similar to that shown in Fig. 3, much more efficient separation with greater speed of drawing off of vapor was obtained by C. Skarstrom and the writer. While the long vacuum-type tubular centrifuges give promise of making a usable method of separating the isotopes, especially of the heavier elements, it is probable that other centrifuging methods can be made more efficient. For example, Professor Urey has suggested to the writer that if an apparatus analogous to his fractionating columns could be mounted in a long tubular centrifuge in such a manner that the separation would result from the centrifugal field rather than the difference in vapor pressure, etc., as in his apparatus, then the separation could be greatly increased. For example, the design might be such that the material would first diffuse through the pressure gradient set up by the centrifugal field, then be thrown (or allowed to flow without diffusion) back again. This cycle would take place many times. If this can be done, the separation would be multiplied by the number of cycles which could be made large because tubes of almost any size and length can be spun with the tubular vacuum-type centrifuge. Attempts to increase the separation of the isotopes by centrifuging in the above manner are in progress at the present time at the University of Virginia.

With the vacuum-type centrifuge, the separation has been carried out mostly with vapors and gases because of their smaller density. With the material in the liquid state, the pressure developed in the rotor is so great that the rotational speed must be decreased to prevent the centrifuge from exploding. Since $\omega^2 r^2$ enters into S in the exponent of e, the separation is much cut down. However, separation in the liquid state may become practical at very low temperatures. For example, the difference in densities of the two isotopes of neon in the liquid state is ten percent, in helium it is about twenty-five percent, while in liquid hydrogen the difference should approach fifty percent.

The preliminary experiments discussed above are sufficient to emphasize the need for a complete theoretical solution to the separation of gases and vapors in a centrifuge. Although from Eq. (11) S is independent of the absolute value of the mass and has been shown to be independent of the state of combination of the element, this is only for the equilibrium state. Physically it would seem that the time required for this equilibrium state to be reached should be dependent upon the diffusion constant D and the viscosity, which in turn does depend upon the absolute value of the mass and the state of combination. Hence a knowledge of the transient terms of the solution should prove of great value in guiding the experimental work.

In addition to the tests upon the separation of isotopes, Eq. (11) has been shown to hold approximately in the separation of N_2 and CO_2 gases,[39] as well as in the separation of certain constant boiling point mixtures.[53] The separation of gases by the principle of velocity selection has also been accomplished successfully.[39] A vacuum-type machine equipped with a special rotor was used. The rotor, triangular in cross section, had openings in the sides near the vertices, from which channels led to the axis and connected there with a hollow shaft, through which the gas was drawn off. The openings of the channels are so placed that when the rotor is spinning, only those molecules which are traveling faster than the periphery of the rotor can enter the openings. Since, if necessary, the peripheral velocity of the rotors can be made around twice the average velocity of the molecules at room temperature, then, in most gases, the rotor acts as a velocity selector. The velocity of the molecules depends upon their mass so that the rotor also acts as a mass selector of molecules. The experiments were carried out with CO_2 and N_2 at a few hundredths of a mm pressure and the theory was approximately verified.

The above examples were given to illustrate some of the many uses that have been made of high speed centrifuges. There is no doubt but that much wider use will be made of them in the future, both as analytical tools and as instruments for the purification of materials. Furthermore, at the present time the experimental technique has been simplified and the costs reduced to a point where efficient and precise methods are within the reach of almost any laboratory that has a need for high speed centrifuging.

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