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Daniel R. Raichel[a]; Raymond B. Schaefer[a]
[a] ELECTRO-NUCLEONICS, INC., FAIRFIELD, NEW JERSEY

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Performance Parameters of Centrifuges

DANIEL R. RAICHEL and RAYMOND B. SCHAEFER*

ELECTRO-NUCLEONICS, INC.
FAIRFIELD, NEW JERSEY 07006

Summary

A systematic approach for selecting centrifuges and rotors is explained on the basis of their performance parameters. While a parametric number $\Delta\overline{ST}$, essentially a function of sedimentation coefficient and rotor speed, is used to characterize batch centrifugation, an analogous parametric number $\overline{STY}$ is introduced to specify continuous centrifugation and modified to describe semicontinuous centrifugation. A graphic technique is explained for determining the maximum possible output from a semicontinuous flow centrifuge; this procedure permits convenient selection of the optimal core for a given rotor bowl.

INTRODUCTION

A considerable number of centrifuge models exists in the market; it therefore behooves the researcher or the production analyst to select the proper unit for specific purposes. The aim of this paper is to present a simple and systematic technique for the optimal selection of a centrifuge and to present a new manner of specifying performance of "semicontinuous" units.

BASIC TYPES OF CENTRIFUGES

Two principal categories of centrifuges exist, viz., the batch unit and the continuous flow unit. The latter can be made to function as a

* Presently Teaching Fellow, Department of Physics, Boston University, Boston, Massachusetts.

batch centrifuge by simply closing off the throughput lines. Continuous flow centrifuges can be further classified into two subcategories, i.e., the "fully" continuous flow, in which means is provided for removing the centrifuged suspension while the unit is running, and the "semicontinuous" unit, which is turned off when the spinning rotor is filled to a predetermined thickness with separated particles. The contents of the rotor is then emptied and the centrifuge restarted, thereby resuming the separation process.

The continuous method of centrifugation obviously lends itself to commercial production of separated viruses and suspension particles. The batch method, with its loading and unloading steps, finds greater use in laboratory and pilot production procedures. Semicontinuous operation generally requires less centrifugation time than the batch method. In fact, semicontinuous units are available with separation capabilities exceeding those of any purely continuous flow models.

PARAMETERS FOR CENTRIFUGE SELECTION

An important parameter indicative of centrifuge performance is the *sedimentation rate* or *sedimentation coefficient,* which describes the radial movement of particles subject to a centrifugal field. The sedimentation rate, defined as the velocity of a suspended spherical particle per unit centrifugal field, is described by

$$S = 2a^2(\rho' - \rho)/9\eta \tag{1}$$

where S = sedimentation rate, sec; a = particle radius, cm; ρ' = density of a soliated particle, g/cm^3; ρ = density of solution, g/cm^3; and η = viscosity of suspending medium, P.

Equation (1), based on Stokes' law of motion for spherical particles in a viscous medium, constitutes a fair approximation for particles of other than spherical forms. The particles can be separated according to size; also, a radial density gradient of the solution will generate a radial variation of the sedimentation coefficient S. At some specific value of radius where the particle density equals the density of the solution, sedimentation ceases, and all particles of an assigned density tend to congregate or "band" about that radius.

From the definition of Eq. (1) the sedimentation coefficient may be written (*1*)

$$S = v/F \propto (1/N^2)(1/r)(dr/dt)$$

where v = radial velocity of a particle, cm/sec; r = radial position of a particle, cm; t = time, sec; F = centrifugal field (radial acceleration), cm/sec^2; and N = rotor speed, rpm. While the sedimentation coefficient of a particle is difficult to measure, per se, the *average* sedimentation rate of a particle moving from one radial position r_1 to another r_2 during a time interval t' is more readily observable and is given by

$$S^* = (10^{13}/4\pi^2)[\ln (r_1/r_2)/t'N^2] \tag{2}$$

where S^* denotes the average sedimentation rate in Svedberg units (1 Svedberg = 10^{-13} sec). Here it is assumed that t' is given in units of hours. From the presence of inner and outer radii in Eq. (2), it is apparent that the geometry of the rotor system plays an important role.

In addition to the time required to separate particles of given *average sedimentation coefficient* from a solution, the amount of solution that is processed during that time interval constitutes another parameter. In the discussion following this section, complete separation of particles from the suspending medium is assumed for comparison purposes. While over a dozen parameters can be listed to describe centrifugation systems (*2*), it is preferable to minimize such details for most practical purposes.

BATCH CENTRIFUGATION

For a given rotor a relationship exists between the average sedimentation coefficient S^*, separation time t', operating rotor speed N, and maximum allowable (usually due to structural limitations) rotor speed N_{max}

$$\Delta\overline{ST} = S^*t'(N/N_{max})^2 \tag{3}$$

which results from the definition

$$\overline{ST} = (10^{13}/4\pi^2)[\ln (r_b/r)/N^2] \tag{4}$$

where r_b is the rotor bowl radius. In Eq. (3), Δ has been defined to avoid a negative sign. From the above definitions, the minimum apparent S-rate of a particle moving between any two levels is

$$S^*_{min} = (\Delta\overline{ST}/t')(N_{max}^2/N^2)$$

where t' is expressed in hours. The parameter $\overline{ST}$ defined in Eq. (4) can be tabulated for a tubular bowl as a function of volume fill; it is dependent on rotor geometry. Assumption of 100% cleanout assigns the value r_c of the core radius to the variable r in Eq. (4). The parameter $\Delta\overline{ST}$ can be used to choose a rotor speed on the basis of separation time and sedimentation coefficient.

CONTINUOUS FLOW CENTRIFUGATION

Rotor geometry plays an important role in continuous flow centrifugation. In treating the process of clarification whereby the particles are continuously removed from a solution, we shall deal only with the tubular-bowl rotor systems which are the most prevalent and are quite simple structurally.

Referring to Fig. 1, the important geometric parameters of a tubular-bowl rotor are apparently the core radius r_c, the bowl inner radius r_b, and the inside bowl length L. Moreover, it is assumed that the through-

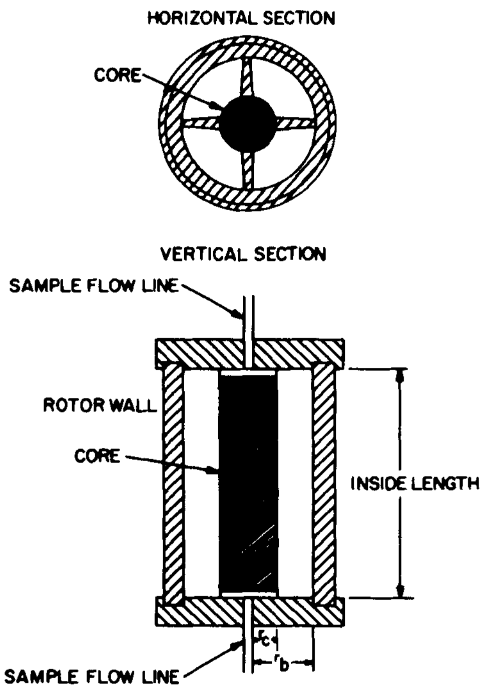

FIG. 1. Cylindrical rotor.

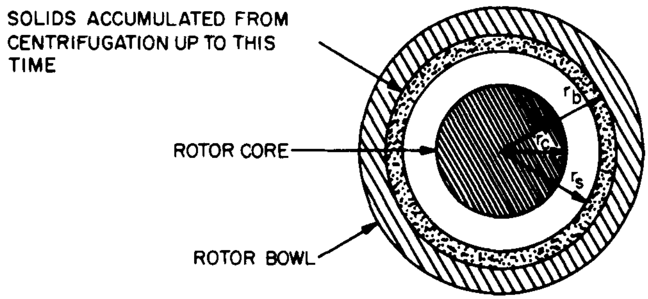

FIG. 2. Rotor cross section at some instant after centrifugation has begun.

put flow occurs axially in the annular region between the core radius r_c and the bowl inner radius r_c (cf. Fig. 2). The region of flow initially occupies the entire annular cross section; but as the particles sediment out of the solution to accumulate on the inner wall of the bowl, the effective outer radius r_s continually decreases from the initial value r_b, and the flow cross section effectively decreases. The flow rate at the onset is determined by the requisite that 100% of the particles be separated out of the solution by the time the fluid leaves the rotor system. The fluid entering the centrifuge must therefore dwell a minimum amount of time (*residence time*) for the separation to be fully accomplished. Assuming that the flow rate remains constant, the decrease in the flow area necessitates a higher flow velocity, thereby resulting in a shorter residence time. But perusal of Eq. (2), which reads for this case,

$$t' = (10^{13}/4\pi^2)[\ln (r_s/r_c)/S^*N^2]$$

indicates that the residence time required for 100% separation decreases but not as rapidly as the available flow volume in the rotor decreases. The volumetric flow must be lessened so that the solution dwells long enough for all particles to separate out. The maximum permissible flow rate for a given sedimentation coefficient, rotor length, bowl inner radius is plotted as a function of the effective annular radius $(r_b - r_s)$ in Fig. 3 for different values of the core radius.* A family of curves is useful whenever a series of cores is available for a rotor bowl of assigned length and bowl inner radius.

* All of the graphs give typical plots—not necessarily actual ones. They are, however, representative of the curves obtained for centrifuges manufactured by Electro-Nucleonics, Inc.

Rotor bowl performance is also affected by the fraction (by volume) of the solution occupied by the particles. A greater degree of volumetric presence of the particles enables the rotor to be filled with them more quickly than would be the case with a solution containing fewer particles. The maximum permissible flow rate as a function of time for a specific value of sedimentation coefficient depends upon the fraction of the particles in the solution. The family of curves in Fig. 3 can be converted into a new set of curves of maximum permissible flow vs. time, each curve representing a stated percentage of solids (cf. Fig. 4). From Fig. 3, the maximum permissible flow evidently depends upon the values of core radius, bowl inner radius, and rotor length (this last quantity being held fixed).

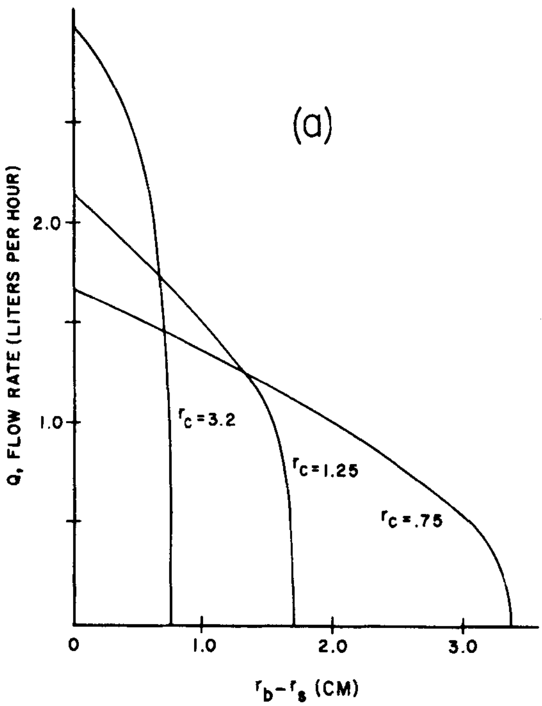

FIG. 3. Maximum permissible flow rate vs. $(r_b - r_s)$ for different values of the core radius, given an inside bowl radius of 4 cm, for different fractions of particles in solution. The inside length of the rotor is fixed and 100% clean-out is assumed. The curves are for some given average sedimentation rate.

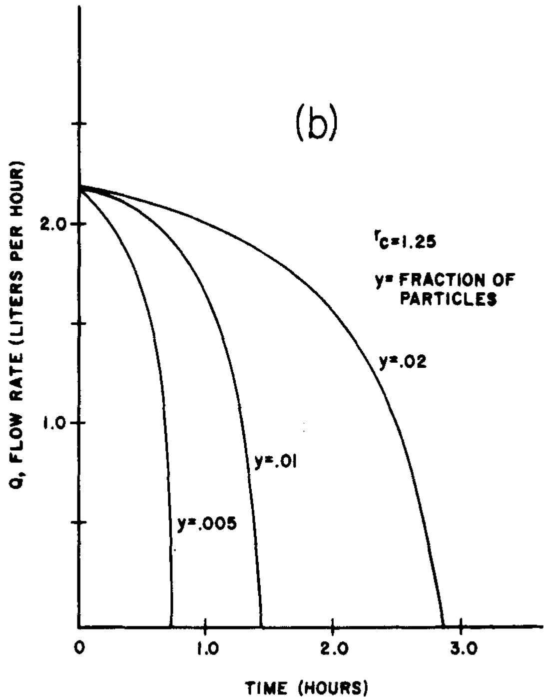

FIG. 4. Maximum permissible flow rate vs. time, given an inside bowl radius of 4 cm and a core radius of 1.25 cm, for different fractions of particles in solution. The inside length of the rotor is fixed and 100% cleanout is assumed. The curves are for some given average sedimentation rate.

A parameter which is a function of rotor geometrv and analogous to the $\overline{ST}$ number defined by Eq. (4) for batch centrifugation can be established to describe continuous flow centrifugation, whether fully continuous or semicontinuous. We consider a solution flow through the rotor in keeping with the flow variation scheme of Fig. 3. The centrifuge is kept operative for a time of duration T, from the beginning of separation to the instant the rotor is replete with solids. The performance capability of the rotor is characterized by a parameter $\overline{STY}$ which depends only upon rotor geometry and rotational speed. The $\overline{STY}$ number correlates to the centrifugation parameters as follows (cf. Appendix for details)

$$\Delta\overline{STY} = S^*Ty(N/N_{\max})^2 \tag{5}$$

where y = volumetric fraction of solids present in unprocessed solution. The run time T can be calculated from Eq. (5) on the basis of the values of y, $\overline{STY}$, and N_{max}.

Until now no distinction had to be made between the fully continuous and the semicontinuous centrifuges. The $\overline{STY}$ number may suffice to define a continuous flow centrifugation process for most purposes, but further inferences based upon the distinction between fully continuous and semicontinuous processes can be drawn.

In a fully continuous process the sedimented particles can be removed from the rotor during operation while centrifugation continues. Neglecting the time required to flush out the particles from the rotor as compared with the time of run T, the average flow $\bar{Q}$ is defined as

$$\bar{Q} = V_p/T = V_a/yT \tag{6}$$

where $\bar{Q}$ = average flow, cm^3/sec; V_p = volume of solution processed during the run; and V_a = volume available in rotor for particles separated from sample fluid. The value of $\bar{Q}$ provides the maximum possible output per unit time from a fully continuous centrifuge.

SEMICONTINUOUS CENTRIFUGATION

The semicontinuous centrifuge receives its throughput supply in intervals, between which the centrifuge is stopped, the rotor is emptied of its centrifuged contents, and the unit restarted. This interval is not negligible and should be included in defining a total average flow rate $\bar{Q}_t$, which is the volume V_p of solution processed divided by the cycle time

$$\bar{Q}_t = V_p/\tau = V_a/y(T + C) \tag{7}$$

where C denotes the time interval between runs.

The average flow $\bar{Q}_t$ partially depends upon rotor geometry. In establishing the efficacy of the $\overline{STY}$ number, it is convenient to assume constant values for the inside bowl length L and the bowl inner radius r_b and to vary the rotor dimensions by altering the core radius r_c. For each value of S^* there exists a value of core radius that yields the *maximum* total average flow rate $\bar{Q}_{t_{max}}$. This information is presented in the plot of Fig. 5, and the mathematical details are covered in the Appendix. A family of curves occurs for different rotor speeds and for each value of the product Cy of the time between batches multiplied

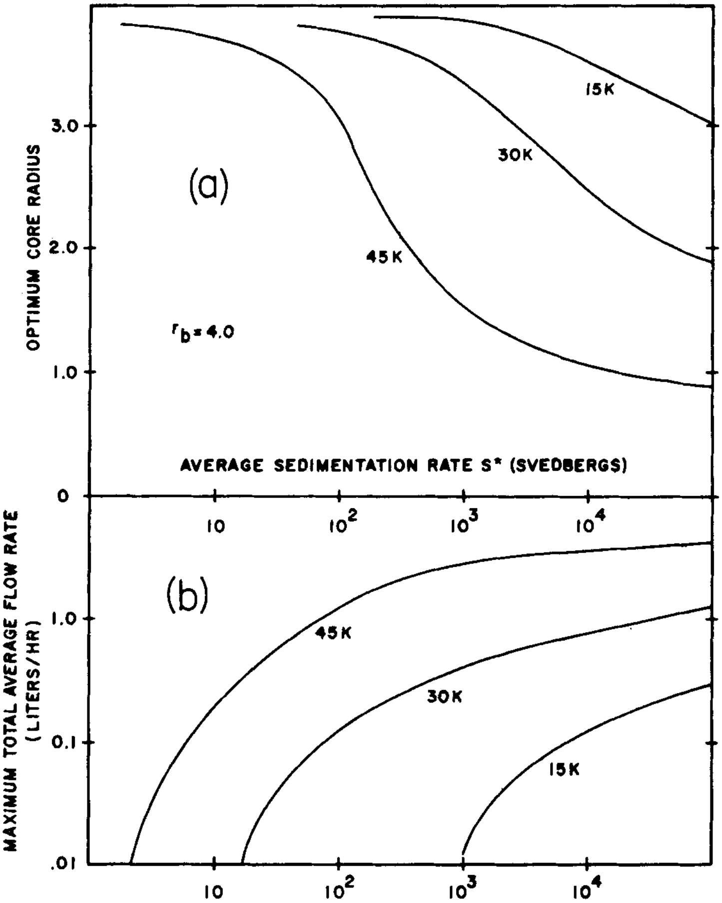

FIG. 5. Average sedimentation rate S^* vs. (a) optimum core radius and (b) maximum total average flow rate. Curves for different rotor speeds are indicated in krpm. The product of time between runs and fraction of solids in the solution is assumed to be 0.6.

by the volumetric fraction of particles in the solution. For a given average sedimentation rate the optimum core radius is obtained from the upper chart of Fig. 5 and the associated $\bar{Q}_{t_{max}}$ from the lower chart. As the value of $\bar{Q}_{t_{max}}$ allows comparison with that of $\bar{Q}$ of the fully continuous case, the semicontinuous centrifuge, as a result of its possessing greater separation capabilities, may outperform a continuous centrifuge.

After the optimum core radius is ascertained from the plots of Fig. 5, the total average flow is found from the $\overline{STY}$ rating of the rotor and applying Eq. (7). The graphical methods thus circumvents the necessity for finding separately the values of τ and $\bar{Q}_t$ for each available core in order to determine the optimal design for a particular application.

APPENDIX: PARAMETRIC MATHEMATICAL DETAILS

In the process of clarification, particles sediment out of a solution that flows axially through a centrifugating rotor. The solids separate out of the solution to accumulate on the inner wall of the rotor bowl. Referring to Fig. 2, the flow rate through the rotor is given by

$$Q = V_a/t = \pi L(r_s^2 - r_c^2)/t \qquad (8)$$

where Q = volumetric flow, cm³/hr; t = residence or dwell time of solution in rotor, hr; V_a = empty volume of rotor, cm³; L = inner length of rotor bowl, cm; r_s = radius extending from core center to layer of particles accumulated on the inner wall of the bowl, cm; and r_c = core radius, cm.

The residence time should be sufficiently long that nearly all particles are removed from the solution. Rearranging Eq. (2), with the appropriate radial notation, we obtain

$$t' = 10^{13} \ln (r_s/r_c)/4\pi^2 N^2 S^* \qquad (9)$$

The time t' is the interval necessary for a particle initially at the core to sediment to r_s. Identifying t in Eq. (8) as being equal to t' so that 100% clarification occurs and eliminating t' between Eqs. (8) and (9) yields

$$Q = (4\pi^3/10^{13})[LN^2S^*(r_s^2 - r_c^2)/\ln (r_s/r_c)]$$

Since r_s varies in the course of centrifugation, Q also varies. If separation is carried out until the rotor is filled with solids, the average flow rate is

$$\bar{Q} = \int_{rb}^{r_c} Q(r_s)\, dr_s \Big/ \int_{rb}^{r_c} dr_s$$

$$= (4\pi^3/10^{13})[LN^2S^*/(r_b - r_c)]r_c^3[Li(r_b/r_c)^3 - Li(r_b/r_c)] \qquad (10)$$

Here $Li(x)$ is a special function which is related to the exponential integral $Ei(y)$ as follows

$$Li(x) = Ei(\ln x)$$

The $Ei(y)$ function being tabulated (4), the integral in Eq. (10) may be evaluated directly.

We now modify the above theory to apply to semicontinuous centrifuges. Denoting T as the time of separation from the beginning to the end, Eq. (7) for the average flow rate occurs

$$\bar{Q} = V_p/T = V_a/yT = \pi L(r_b^2 - r_c^2)/yT \tag{11}$$

where V_p = volume of solution processed, cm^3; and y = volumetric fraction of solution occupied by particles. Eliminating $\bar{Q}$ between Eqs. (9) and (10) with some rearrangement results in

$$S^*Ty = \frac{[(r_b/r_c) - 1]^2[(r_b/r_c) + 1]}{[Li(r_b/r_c)^3 - Li(r_b/r_c)]} \frac{10^{13}}{4\pi^2 N^2} \tag{12}$$

Because the right side of Eq. (11) is dependent only upon the dimensions and the rotational speed N of the rotor, S^*Ty is a number characteristic of the rotor and is therefore termed the $\overline{STY}$ *number*

$$\overline{STY} = S^*Ty$$

Since a time lapse occurs between runs, the total average flow is defined by

$$\bar{Q}_t = V_p/\tau \tag{13}$$

where $\tau = T + C$. $\bar{Q}_t$ denotes the total average flow rate in cm^3/hr, while τ represents the cycle time which includes T and the interval C between runs. Using Eqs. (11) and (12), Eq. (13) may be transformed to

$$\bar{Q}_t = \frac{\pi L(r_b^2 - r_c^2)r_c^3}{\dfrac{(r_b - r_c)^2(r_b + r_c)10^{13}}{4\pi^2 N^2 S^*[Li(r_b/r_c)^3 - Li(r_b/r_c)]} + Cy}$$

It should be stated here that the sedimentation coefficient is customarily referred to a standard which, for biological systems, is usually water at 20°C

$$S_{20,w} = S_{T,m}[\eta_{T,m}(\rho_p - \rho_{20,w})/\eta_{20,w}(\rho_p - \rho_{T,m})]$$

where $S_{20,w}$ = sedimentation coefficient of particle in water at 20°C,

$S_{T,m}$ = sedimentation coefficient of particle through medium at temperature T of centrifugation, $\eta_{T,m}$ = viscosity of medium at temperature T of centrifugation, $\eta_{20,w}$ = reference viscosity of water at 20°C, ρ_p = density of particle, $\rho_{T,m}$ = density of medium at temperature T of centrifugation, and $\rho_{20,w}$ = reference density of water at 20°C.

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