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The Nonsynchronous Flow-Through Coil Planet Centrifuge without Rotating Seals Applied to Cell Separation

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Abstract

A new nonsynchronous flow-through coil planet centrifuge allows continuous elution through a coiled separation column without the use of rotating seals. Rates of rotation and revolution of the column are independently adjustable to meet the requirements for the separation of cell particles. The capability of the present apparatus was demonstrated by the separation of human and sheep erythrocytes using an isotonic saline solution.

INTRODUCTION

In the past, various types of flow-through centrifuges have been developed for the separation of biological materials (*1–4*). In most of these devices the use of rotating seals is eliminated by choosing a synchronous planetary motion of the coiled column where the ratio of the rate of rotation (about its own axis) to the rate of revolution (around the central axis of the apparatus) is fixed at 1. This particular mode of planetary motion, however, limits the versatility of the methods, especially

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in the separation of cells where slower rotational speeds are required for the sedimentation of cell particles. Recently, a nonsynchronous flow-through coil planet centrifuge has been developed and successfully applied to the separation of cells in a physiological solution (*5*). In that scheme the ratio of rotation and revolution in the planetary motion of the coiled column is adjustable. A slow rotation combined with a high revolutional rate facilitates more rapid sedimentation of cells in the coiled column. However, the apparatus is equipped with a dual rotating seal which is a potential source of complications such as cell damage, cross contamination, leakage, and corrosion.

The present paper introduces a nonsynchronous flow-through coil planet centrifuge without the use of a rotating seal device. The capability of the apparatus has been demonstrated in the separation of human and sheep erythrocytes in isotonic saline.

DESIGN PRINCIPLE OF THE APPARATUS

Figure 1A illustrates the principle of the rotating-seal free design of the apparatus. A cylindrical column holder undergoes a planetary motion as indicated by a pair of arrows (ω_{II}). A bundle of flow tubes led from the holder first exits the imaginary Compartment C shown by a dotted line at Point B located on the central axis of the apparatus and then makes a loop to reach Point A located on the central axis of the apparatus on the opposite side. At A the bundle is tightly supported by a stationary member of the apparatus.

For ease in the description of the system, the total pathway of the bundle is divided into two parts, i.e., A to B and within Compartment C. Between A and B the loop of the bundle rotates in a circle around the central axis of the apparatus at angular velocity ω_I and at B it rotates about its own axis at $2\omega_I$ in the same direction. The system is identical to that in the flow-through centrifuge reported earlier (*3*) in that the bundle is free from

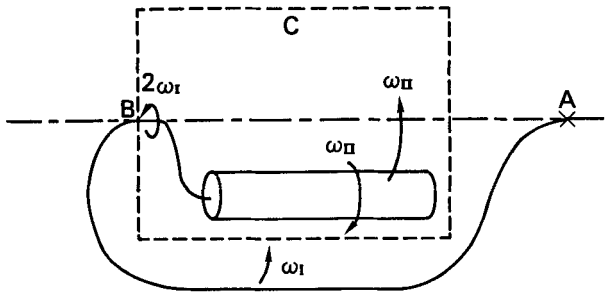

FIG. 1A. Principle of design.

twisting. Within Compartment C, which rotates at $2\omega_I$, the holder undergoes a synchronous planetary motion with respect to the rotating Compartment C, i.e., the holder counterrotates about its own axis at ω_{II} and simultaneously revolves around the central axis of the apparatus also at ω_{II}. This system becomes identical to the flow-through coil planet centrifuge system (*1*). The direction of this synchronous planetary motion can also be reversed with the same effect. In either case, the bundle is free from twisting within Compartment C. The entire length of the bundle, therefore, becomes free of twisting at any arbitrary, independent values of ω_I and ω_{II}.

Under the above mode of planetary motion, the net revolutional rate of the holder with respect to the outside observer is given by $2\omega_I+\omega_{II}$, the sum of the rotational rate of Compartment C ($2\omega_I$) and the revolutional rate of the holder relative to Compartment C (ω_{II}). The total acceleration acting on the axis of the holder is given by

$$\alpha = R(2\omega_I + \omega_{II})^2 \tag{1}$$

where α denotes the total acceleration and R is the radius of revolution or the distance from the holder axis to the central axis of the apparatus. The rotational rate of the holder about its own axis is given by ω_{II} and is entirely dependent upon the synchronous planetary motion of the holder within Compartment C. Therefore, the ratio of the rotational rate to the revolutional rate of the holder is expressed as $\omega_{II}/(2\omega_I+\omega_{II})$. Because ω_I and ω_{II} are made independently variable, the present system permits any value of the above ratio under a desired acceleration field acting on the axis of the holder. When the coiled separation column is mounted around the holder, the rotation of the holder induces an additional acceleration which acts radially from the axis of the holder to modify the total acceleration expressed in Eq. (1), However, the effect is practically negligible due to the fact that the optimum rotational speed (ω_{II}) for cell separation usually ranges from 3 to 20 rpm (at 3 cm radius) compared with the revolutional rate ($2\omega_I + \omega_{II}$) of 500 to 1200 rpm (at 15 cm radius). Rotation of the coiled column also produces an undulating centrifugal force field acting on the individual column units mounted eccentrically around the holder as shown in Fig. 1B. Under this arrangement each column unit is subjected to a greater force field at the position remote from the central axis of the apparatus and a smaller force field at the position close to the same axis. While the actual effect of this undulation of the centrifugal force field on cell separation is unknown, it may be desirable to minimize this effect by mounting the column units close to the axis of the holder. This will tend to decrease the eccentric motion caused by the rotation of the column holder.

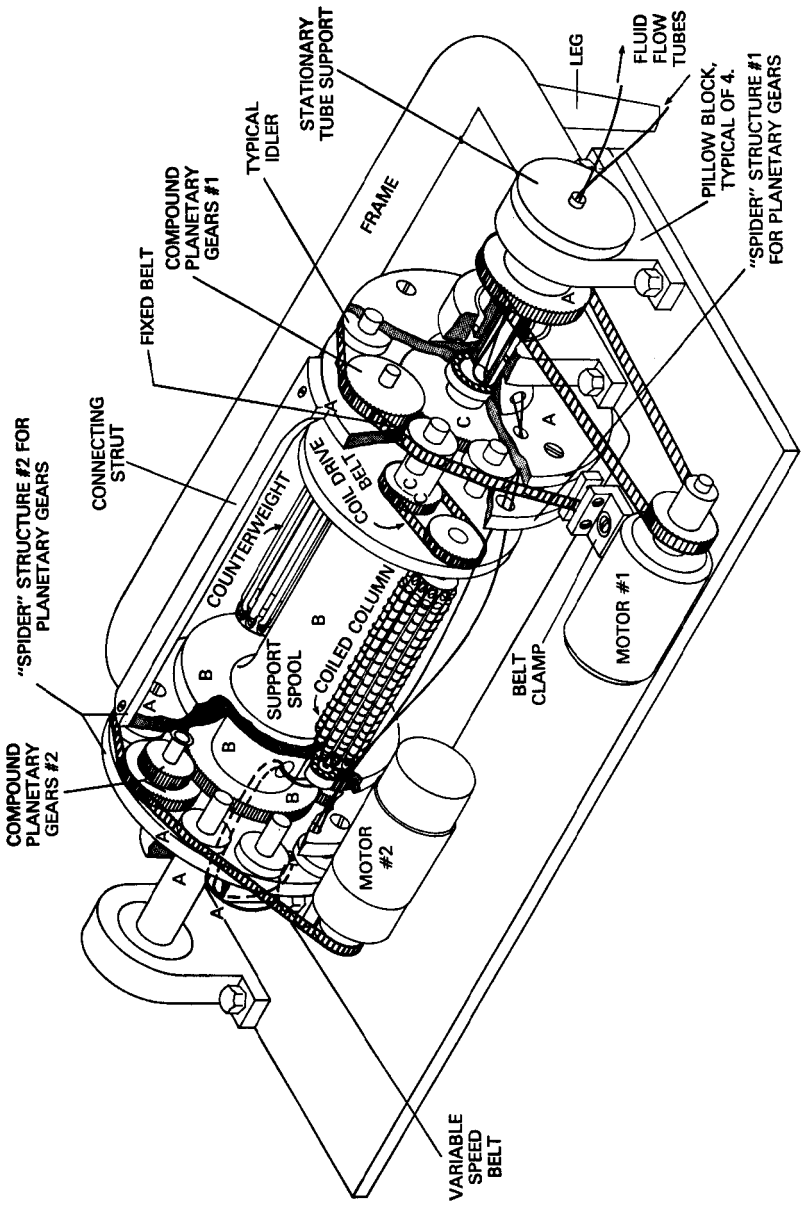

FIG. 1B. Sketch of implementation.

DESIGN OF THE APPARATUS

The required independent rotations of the coils are provided by two motors and a system of planetary gears to transmit the motions while guiding and not interfering with the motions of the tube bundles.

As shown in Fig. 1B, Motor I drives gear supports (spiders) A at both ends of the apparatus at ω_I. The motor drives one spider via a timing belt, and the other spider is connected to the first via two connecting struts. The spiders support the outer loop of tubing and cause the planetary gears to orbit at ω_I.

The internal ring gears of traditional planetary sets have been replaced by timing belts supported by idlers. At one end of the apparatus this belt is fixed, causing the Sun Gear C to rotate at $2\omega_I$. The effective pitch diameter of the fixed belt is 12 in., that of the sun gear 6 in., and those of the compound gears 2 and 4 in. Since there are two diametrically opposed sets of planet gears at each end, at least one is in mesh at all times, even when the other one is free wheeling as it passes the area where the belt is fixed.

Sun Gear C drives the coiled column support via an auxiliary shaft on the centerline and a "coil drive belt" (a timing belt). Thus, with respect to the laboratory frame, the coiled column rotates always at exactly $2\omega_I$. (Study of the kinematics of an orbiting pulley driven by a belt from a central pulley will show that angular rotational velocity with respect to the laboratory frame is independent of orbital velocity.)

At the other end of the apparatus Sun Gear B also rotates at $2\omega_I$ if Motor II is stopped, because there is an identical set of planetary gears. However, when Motor II imposes an effective angular velocity to the belt acting as the internal gear for the planetary set at that end, then that angular velocity ω_{II} will be superimposed on Sun Gear B which will revolve at $2\omega_I + \omega_{II}$. Sun Gear B is rigidly attached to the support spool, and the coiled columns must orbit at $2\omega_I + \omega_{II}$.

The present device is still undergoing mechanical refinement. Standard timing belts (Browning) seem to behave in a trouble-free manner as opposed to a proprietary "sprocket cable" (Winfred M. Berg, Inc.) which was tried first. There is noticeable vibration due to the polygonal shape of the belts as supported by the finite number of idlers, but it is believed that this could be greatly reduced in another model by using a larger number (perhaps double) of smaller idlers. This system does avoid the necessity of custom internal gears which could be substituted.

To avoid the demanding tolerances of alignment of the various bearings for rotating members, the position of each spider assembly is determined by two coaxial bearings, and the support spool is then supported by one

bearing in each spider assembly. Thus small amounts of angular misalignment can be tolerated as the system is not overdetermined.

A rectangle formed of 2 in. diameter, 0.125 in. wall aluminum tubing surrounds the rotating elements and supports the bearing assemblies at each end and the motors to the side. Thus rigidity, light weight, and excellent access to all parts are achieved. This stiffness is required to withstand the forces and vibrations produced by 1200 rpm operation (164 *g* at the 4 in. radius of the axis of the coils).

PRINCIPLE OF PARTICLE SEPARATION

The principle of particle separation with a rotating coiled tube has been described earlier (*5–7*). When a liquid-filled coiled tube is rotated in an acceleration field acting perpendicularly to the coil axis, particles present in the coil are subjected to an Archimedian screw force which causes the particles to move toward one end of the coil. This end is called the head of the coil and the other end the tail of the coil. When the rotational rate of the coil is relatively slow, all particles move at the same rate of one helical turn per one rotation of the coil. In this case no separation is observed. As the rotational speed of the coil is increased, particles having low sedimentation velocities fail to maintain the above rate and are retarded in their movement toward the head of the coil. This results in the separation of these particles from the rest. When the rotational speed of the coil is further increased, all particles reduce their traveling rates toward the head of the coil, but the degree of their retardation depends upon the sedimentation velocity of the particles. As a result the particles are separated along the length of the coil according to their relative size and density.

In order to facilitate the fractionation of samples, the coiled tube is eluted with liquid from the head end to collect separated particles at the tail end of the coil. In this case particles with greater sedimentation velocities are retained within the coil for longer periods of time while the particles with lower sedimentation velocities are eluted earlier. The present apparatus permits continuous elution without complications arising from the use of rotating seals and allows optimization of the rotational speed of the coil relative to the acceleration field according to the sedimentation velocities of the sample particles.

MATERIALS AND METHODS

The separation column was prepared from a piece of 0.55 mm i.d. PTFE tubing by winding it onto six units of stainless steel tubes (0.68 cm

o.d. and 20 cm long) in series in such a way that the tail of the first unit bridges to the head of the second unit, the tail of the second to the head of the third, and so forth. The entire column consisted of 1200 helical turns with a total capacity of about 6 mL. The column was symmetrically arranged around the holder shaft and tightly screwed to the column support through a hole made at each end of the column units.

Isotonic buffered saline solution (pH 7.4) was prepared by dissolving NaCl, 90 g; Na_2HPO_4, 13.65 g; and $NaH_2PO_4 \cdot H_2O$, 2.15 g in 1 L of distilled water and diluting 85 mL of this stock solution with distilled water to bring the final volume to 1 L. In order to prevent the adhesion of cells to the tube wall, bovine serum albumin (BSA) 35% solution (Miles Laboratories) was added to the above solution to make the concentration of BSA at about 0.5%.

The human erythrocyte suspension was prepared from EDTA-treated, fresh, adult blood by washing with the buffered saline solution three times, repeating centrifugation, and decanting the supernatant. One part of the loosely packed cells thus obtained was finally mixed with 4 parts of the same solution. The sheep erythrocyte suspension was similarly prepared from ACD-treated blood stored at 4°C. Equal volumes of human and sheep erythrocyte suspensions were combined and 0.2 mL of this mixture was used for each separation.

The separation was performed as follows: The coiled column was first filled by pumping with the buffered saline solution. This was followed by injection of the sample, with a syringe, through a sample port which is located in the flowline between the pump and the inlet flow tube. Then the column was eluted with the buffered saline solution at a flow rate of 5 mL/h with a pump while the apparatus was spun at 800 rpm combined with 10 rpm column rotation. The eluate was collected into test tubes at 10 min intervals (0.8 mL/tube).

Fractionated samples were analyzed microscopically with a blood counting chamber to obtain the total cell population together with the percentages of human and sheep erythrocytes. Original samples and some fractions were also analyzed with a Coulter size distribution analyzer (Electro Zone/Celloscope).

RESULTS AND DISCUSSION

A typical separation of human and sheep erythrocytes with the present method is illustrated in Fig. 2A where the total cell population for each group determined by microscopic analysis is plotted against the fraction number. The first peak represents the sheep cells and the second the human cells. An overlapping of the distribution curves occurs near Fraction 18.

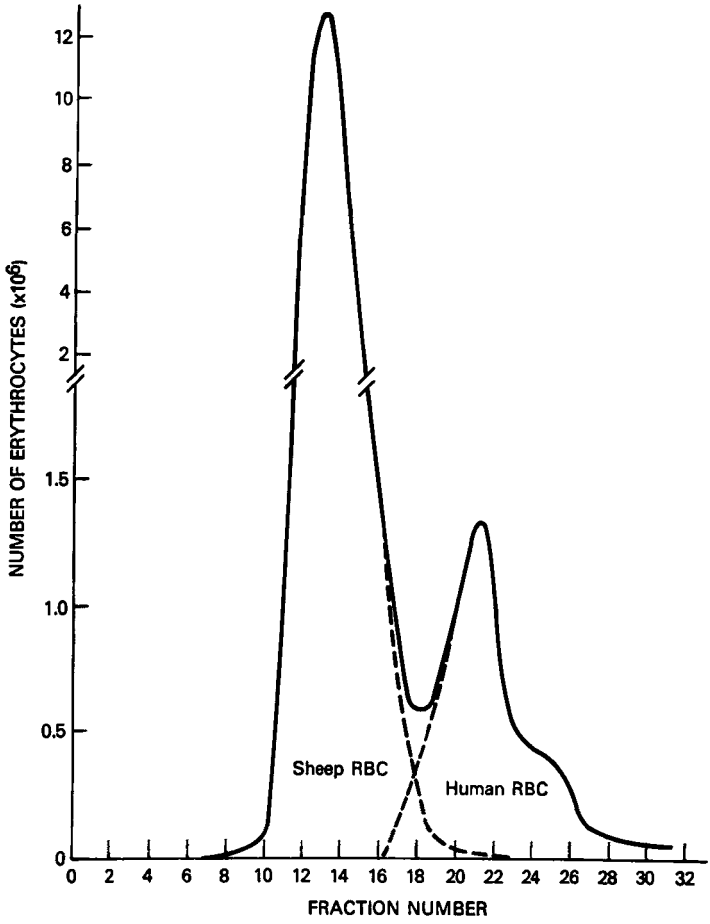

FIG. 2A. Separation of human and sheep erythrocytes.

The curve closely resembles the size distribution curve of the original sample mixture obtained by the Coulter analyzer shown in Fig. 2B. Also, analysis of the fractions by the Coulter analyzer shows that the cell size increases with the fraction number. These findings strongly indicate that the present method separates cell particles according to their size difference. Microscopically, the cells were well preserved and no evidence of hemolysis was observed during separation.

The potential capabilities of the present method were demonstrated in the separation of human and sheep erythrocytes using a physiological saline solution. Although the separation of these cells is not practically

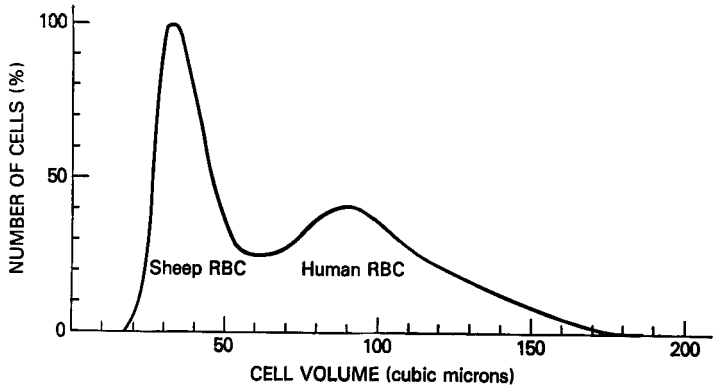

FIG. 2B. Size distribution of original sample mixture.

useful, the optimization of various parameters involved in the present method is easily established by the aid of these samples. The optimal operational conditions thus determined will be directly applied to the separation of other types of cells with minor modification. During separation, the behavior and distribution of the erythrocytes are visible through the wall of the column under stroboscopic illumination. When the rotational speed of the coil and/or the applied flow rate are below the optimal range, cells are retained at the beginning portion of the column, forming segments of densely packed cells which occupy about half the space in each turn of the coil. Though these segments of packed cells are continuously mixed by the rotation of the coil and gradually disappear, the results of separation under these conditions usually show contamination of small cells among the large cells, suggesting insufficient mixing of cells in the coil. Poor recovery of the samples in the present method is caused by the adhesion of cells to the internal surface of the tube, which tends to retain the cells in the column almost permanently. The degree of cell adhesion can be measured by eluting the column with distilled water, the amount of hemoglobin released from lysed cells being analyzed colorimetrically. A small amount of bovine or human serum albumin added to the buffered saline solution effectively prevents this detrimental phenomenon under otherwise identical conditions.

The present scheme offers great advantages over the previous nonsynchronous flow-through coil planet centrifuge which uses a dual rotating seal (*5*). Potential complications, such as cell damage, cross contamination, and leakage at the seal, are entirely eliminated. Because the present scheme uses a second motor which independently controls the slow rotation of the column, the desired operational conditions are easily set and maintained

with a wide range of high accuracy. The capability of the present scheme will be extended to the partition of cells and macromolecules with polymer phase systems (*8*).

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REFERENCES

1. Y. Ito, R. E. Hurst, R. L. Bowman, and E. K. Achter, *Sep. Purif. Methods*, *3*, 133 (1974).
2. Y. Ito and R. L. Bowman, *Anal. Biochem.*, *65*, 310 (1975).
3. Y. Ito, J. Suaudeau, and R. L. Bowman, *Science*, *189*, 999 (1975).
4. Y. Ito and R. L. Bowman, *Anal. Biochem.*, *82*, 63 (1975).
5. Y. Ito, P. Carmeci, and I. A. Sutherland, *Ibid.*, *94*, 249 (1979).
6. Y. Ito, M. A. Weinstein, I. Aoki, R. Harada, E. Kimura, and K. Nunogaki, *Nature (London)*, *212*, 985 (1966).
7. Y. Ito and R. L. Bowman, *Anal. Biochem.*, *61*, 288 (1974).
8. P. Å. Albertsson, *Partition of Cell Particles and Macromolecules*, Almqvist and Wiksell, Stockholm/Wiley, New York, 1971.

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