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K-Series Centrifuges

III. Effect of Core Taper on Particle Capture Efficiency

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Large-scale continuous-flow centrifuges for the K-series previously described (1–3) have been employed for commercial vaccine purification (4–6) and for the isolation of viral inclusion bodies for insect control (7). The K-II centrifuge combines particle concentration by continuous-flow centrifugation with purification by isopycnic banding, a technique previously developed with smaller rotors of the Oak Ridge B-series (8–12). A suspension is fed continuously into one end of the rotor, flows as a thin layer centripetal to a liquid density gradient, and leaves the rotor at the opposite end. Particles which sediment from the stream within the residence interval remain in the rotor and are banded.

The K-II core previously described (2, 3) had a ½-degree taper which was useful for dynamic gradient recovery and also served to define the taper volume which has been considered to be equal to the rotor stream holdup volume. This core employed six radial channels at each end face to carry liquid into and out of the rotor during gradient loading, continuous-flow, and gradient recovery. These grooves are equally spaced, with one groove at each end for each of the six sectors.

In vaccine purification it is important to develop as efficient a process as possible. The most useful definition of capture or cleanout efficiency is:

$$\text{fraction cleanout} = (C_i - C_o)/C_i$$

where C_i is the particle concentration in the inflowing stream and C_o is the concentration in the effluent. In practice, the centrifuge is usually operated at the maximum safe speed, and the flow rate is kept at the

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highest rate giving acceptable recovery. In previous studies it has been difficult to measure and compare cleanout efficiencies between experiments because (*a*) the viruses commonly used, such as influenza, cannot be counted with high precision, (*b*) there occur bath-to-batch variations in the physical properties of suspending media, such as chorioallantoic fluid; and (*c*) the temperature of the rotor and its contents was only approximately known and partially controlled. With the development of a temperature measurement and control system for the K-II centrifuge (13), the problem of rotor temperature measurement and control is largely solved.

Because the cleanout efficiency of the K-II has been significantly below that predicted from theory (3), we wished to explore design modifications which might improve efficiency. Polystyrene beads of viral dimensions have been found to be a useful substitute for virus as a test material in these studies.

EXPERIMENTAL

Two modifications were made in an experimental rotor and compared with the ½°-taper K-II rotor previously described. First, the taper was eliminated and the rotor core walls were made parallel. Second, up to 60 radial grooves were cut in the core end caps to produce better distribution and lower stream velocity at the entrance where the liquid flowing stream begins to flow across the gradient. These modifications are designated K-II (0°,6) and K-II (0°,60). The influent and effluent concentrations of 0.109 μ polystyrene beads[3] were measured at 244 nm using a 1 cm path cell. The absorbance at 244 nm was linearly proportional to the particle concentration up to 0.05 gm/liter with a proportionality constant of 40 absorbancy units/gm/liter. The per cent cleanout (% Cl.) was calculated from the formula:

$$\%\ \text{Cl.} = \left(1.00 - \frac{A_{\text{effluent}}}{A_{\text{influent}}}\right) \times 100$$

Operating Procedure. The rotors were loaded at rest with 1500 ml of 2% w/w sucrose, followed by sufficient 55% sucrose to fill the rotor. The total internal volume of the K-II (½°) at rest is 3600 ml. (Expansion due to wall deformation at 30,000 rpm increases the fluid capacity to about 3760 ml.) The straight core [K-II (0°)] was made with the core radius equal to the radius of the larger end of the K-II (½°) core, thus eliminating 700 ml of "taper volume" and reducing the capacity at rest to 2900 ml.

[3] Obtained from Dow Chemical Company, Midland, Michigan.

The rotor was accelerated to 2100 rpm using an automatic electronic control device (14) to give an acceleration rate of 2 rpm/sec from rest to 500 rpm, and 4 rpm/sec from 500 to 2100 rpm. Flow of water at room temperature (24°C ± 1°) was established at 2100 rpm and was adjusted to a rate of 10 liters/hr, as measured by a calibrated flow meter on the effluent stream. After flow of water was established, the chosen flow rate was maintained while the centrifuge rotor was accelerated to 30,000 rpm using 45 psig air to the turbine. This results in an acceleration rate of approximately 50 rpm/sec. The experimental system is shown diagrammatically in Figure 1.

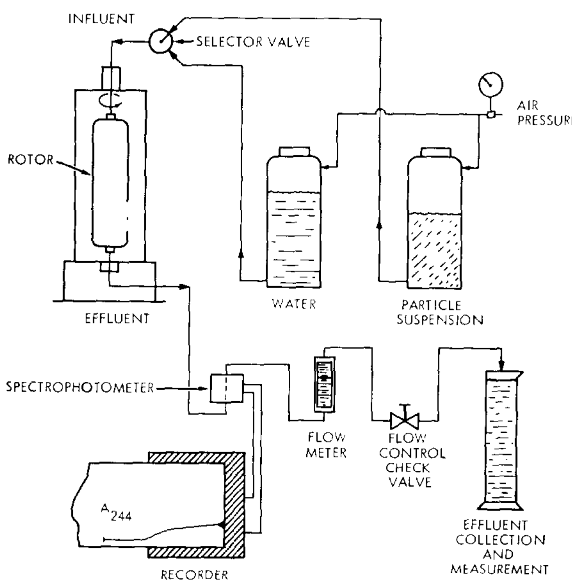

FIG. 1. Diagram of experimental apparatus for cleanout efficiency measurements with continuous flow centrifugation.

Continuous flow of water or particle suspension at 10 liters/hr was maintained for 3 hr and 20 min from the time at which the operating speed was reached. Ten minutes after operating speed was reached, the influent stream was switched from water to the particle suspension for 10 min. A 100 ml sample of effluent was collected during the last minute of particle suspension flow, after which the flow of water was resumed.

Intermittent sampling of the effluent during longer (20 min) periods of particle flow showed there was no significant increase in cleanout efficiency after the first 10 min of suspension flow. The reported values are therefore very close to the highest attainable under steady-state conditions. The 10 min particle suspension flow periods with effluent sampling were repeated three times at 1 hr intervals. This method was used to see if flow conditions in the rotor change appreciably during operation.

Continuous monitoring of effluent absorbance gave a trace of effluent response to a step function increase in particle concentration in the input stream. This is a common experimental method of flow analysis (15). However, most of the particles are removed during passage through the rotor, giving a low absorbance reading, when read against a baseline which rose slowly because a small number of polystyrene particles adhered to the quartz flow cell walls. The recording potentiometer curves were therefore not suitable for the usual analysis for mean residence time and dispersion values. However, the delay time and general shape of the curves allowed qualitative comparison of flow conditions at intervals during the experiment. Definitive measurements were made on the collected samples using a Gilford model 220 spectrophotometer and 1 cm path cells.

In each experiment, flow was terminated after the fourth effluent sample was taken. Centrifugation was continued for 1 hr at 30,000 rpm to band the trapped particles at their isopycnic position, after which reverse air-flow braking was used to decelerate the rotor to 2000 rpm. This was followed by controlled deceleration at 4 rpm/sec to 500 rpm, and 2 rpm/sec to rest. The rotor was unloaded statically from the bottom at a rate of 65 ± 5 ml/min. A continuous UV absorbance trace was made of the rotor contents during unloading and the peak width at half-height measured for use in resolution studies.

RESULTS

Cleanout Efficiency. Per cent cleanout as a function of operating time is shown in Figure 2. The values reported were measured on 100 ml samples collected during the last minute of each 10 min bead suspension flow interval.

Cleanout measurements made at 5 min intervals during longer bead-flow periods in the tapered core rotor are given in Figure 3. Figure 4 gives similar data for the straight core rotor. The data show that cleanout efficiency approaches its steady-state value within 5 to 10 min and that sampling at later times would not produce significantly different values.

Residence Time. After feed into the centrifuge is switched from water to the particle suspension, there is a delay of approximately a minute (at feed flow rate of 10 liters/hr) before particles begin to appear in the effluent. The absorbance traces from a continuous effluent monitor allow

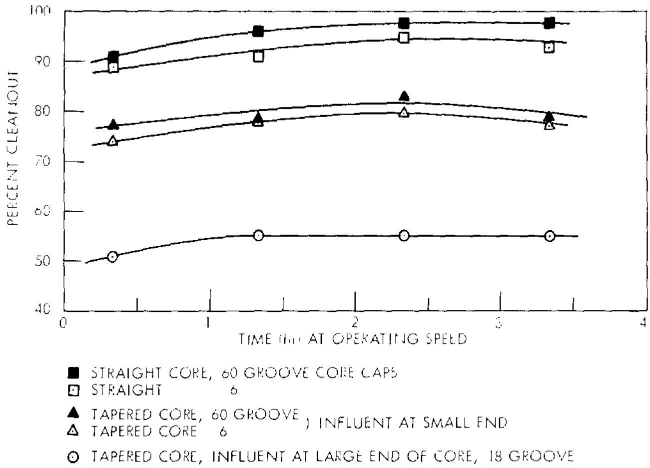

FIG. 2. Cleanout efficiency as a function of operating time for all experiments. *Conditions:* K centrifuge rotors at 30,000 rpm; influent partice suspension was 0.025 gm/liter of 0.109 μ diameter. Dow polystyrene latex spheres, $s_{20,w}$ (calculated) = 320; influent temperature 24°C; effluent temperature 33°C ± 2.

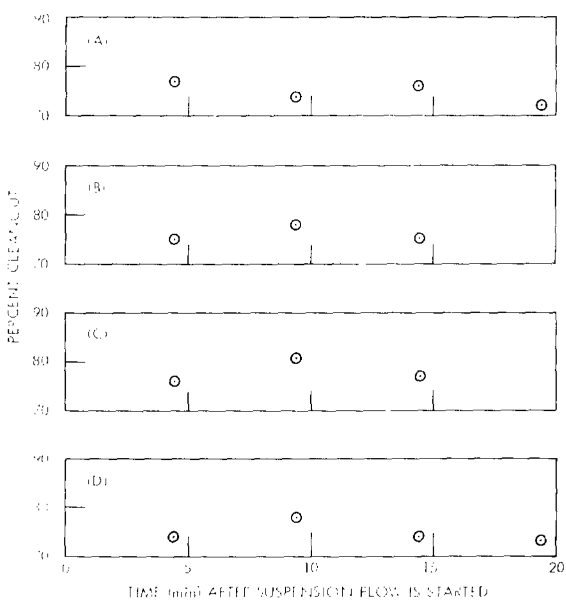

FIG. 3. Cleanout efficiency as function of time during 20 min intervals beginning when flow is switched from water to particle suspension. Tapered core K-II rotor with 6-groove core caps. Speed = 30,000 rpm. (A) For first suspension flow period beginning 10 min after operating speed was attained; (B) at 1 hr 10 min; (C) at 2 hr 10 min; (D) at 3 hr 10 min.

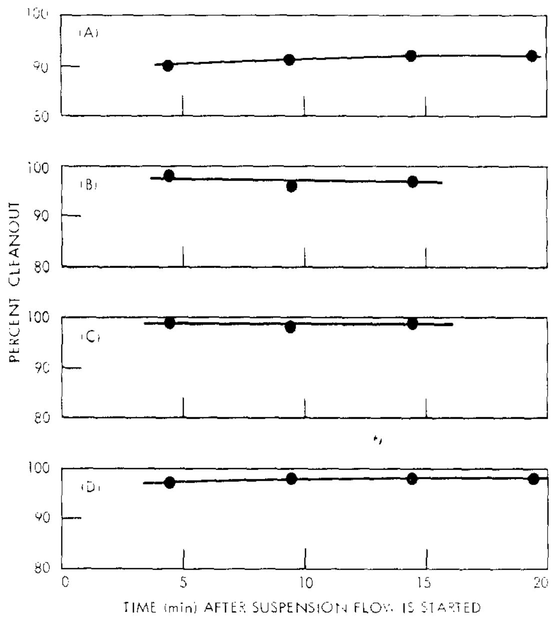

FIG. 4. Cleanout efficiency as function of time for straight (nontapered) core rotor. Other conditions as in Figure 3.

measurement of the delay time for each suspension flow interval, and these times are compiled in Table 1, along with other temperature and cleanout data for each run.

Gradient Profiles. The resulting sucrose gradient profiles for tapered and nontapered cores are shown in Figure 5. Neither the number of core

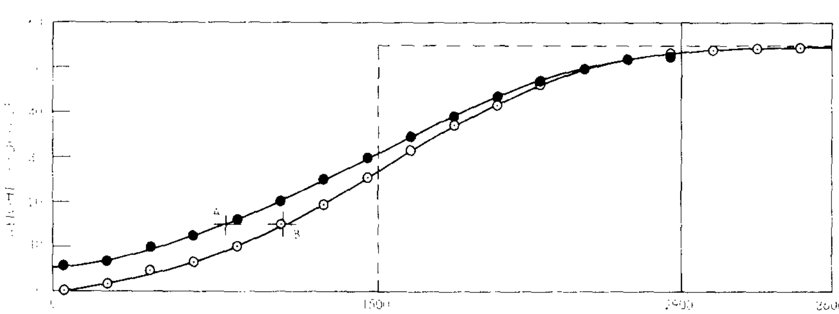

FIG. 5. Sucrose gradient profiles for tapered and nontapered core. Open circles apply to tapered K-II with 3600 ml fluid volume. Closed circles for nontapered core rotor with 2900 ml fluid volume. Dashed line shows step gradient loaded into rotor. Point A shows isopycnic banding position of polystyrene beads in sucrose gradient of nontapered rotor; B shows banding position in tapered rotor.

TABLE 1

Compilation of Delay Times, Temperature, and Cleanout Data

Rotor type (T = tapered, N = nontapered)	T[a]	T	T	N	N
Number of grooves in core cap	18	6	60	6	60
Cleanout efficiency (%)[b]	55	79	80	93	97
Rotor temperature (°C)[b]	34.8	33.8	34.5	34.5	34.5
Effluent temperature (°C)[b]	34.3	33.5	32.0	32.7	31.3
Feed delay times (min)[c] first suspension flow period	0.56	0.68	0.85	0.68	0.70
second	0.25	0.67	0.94	0.60	[d]
third	0.20	0.75	0.70	0.60	[d]
fourth	0.18	0.61	0.60	0.50	[d]

[a] Reverse flow operation with influent at large end of core.

[b] Average of three values taken at 1 hr intervals during the tenth minute of the last three suspension flow periods.

[c] Elapsed time between entrance of bead suspension into the rotor and first appearance of beads in the effluent.

[d] Not measurable because of high cleanout.

grooves nor flow direction affected the resulting sucrose concentration values by more than 1% sucrose. The concentration values, measured by refractometry, show that solute concentration near the core wall (at the left side of the graph) is higher for the nontapered core. The two curves converge at the upper end of the density gradient.

DISCUSSION

Cleanout Efficiency. Highest cleanout was attained with a nontapered core with 60 grooves in each core cap. Lowest cleanout occurred with the tapered core with feed entering at the large end of the core. The tapered core fed from the narrow end produced intermediate values. The apparent superiority of the nontapered core is due primarily to difference in radius. The tapered K-II core has a radii of 4.29 cm at the small end and 4.94 cm at the large end. The *mean* radius is thus smaller than that of the nontapered core whose radius is 4.94 cm at both ends. Cleanout efficiency is proportional to the square of the radius because a larger radius produces a higher centrifugal field and also decreases the sedimentation distance for escape.[4] The escape distance is less because a larger radius produces a greater core perimeter and thinner feed film

[4] In previous theoretical studies using narrow-gap rotors, such as the B-V (16), change in the stream thickness in the rotor with radius was small and was neglected. It was noted that as the stream thickness was decreased, residence time decreases proportionally, cancelling out the advantage of a shorter sedimentation path. Here the change in average stream radius is sufficient to be considered.

layer at any given flow rate. The ratio of the larger radius to the arithmetic mean of the tapered core radii is 1.07, and the ratio of the squares is 1.15.

A tapered core with mean radius equal to 4.94 cm would be expected, therefore, to show a cleanout efficiency of 1.15 times that attained in the rotor of 4.615 mean core radius. The actual values, radius-corrected values, and straight core values are compiled in Table 2. The cleanout efficiencies with straight cores retain a slight superiority over the radius-corrected tapered core values; thus, it seems safe to conclude that cleanout performance of nontapered-core rotors is equal to or somewhat better than that of tapered-core rotors.

The cleanout efficiency in each experiment did not vary greatly as the run progressed. Measured cleanout values were somewhat lower during the first minutes, but this is probably due to the lower rotor temperatures prevailing at the time. As the rotor warmed from room temperature (24°) to 34.5°C during the first hour of operation, the cleanout efficiency increased due to lower density and viscosity of the feed stream flowing through the rotor. The cleanout remained fairly constant until the experiment was terminated 3 hr later.

Theoretical Cleanout. The theoretical cleanout values were calculated for the two cores used in these experiments. The Berman (16) equations were used with the assumptions and results shown in Table 3. For the tapered core, the equations predicted 100% cleanout at 17.3 liters/hr and 80% cleanout at 19.5 liters/hr. The experimental value was 79% at 10 liters/hr. 100% cleanout was predicted at 20 liters/hr with the nontapered core, but the highest experimental value was only 95% at 10 liters/hr. Thus, attainment of the given cleanout levels required that the flow rate be lowered to about one-half the theoretical value.

Residence Time. The only trend evident in delay time behavior, as shown in Table 1, is the decrease in delay times with a tapered core fed

TABLE 2
Comparison of Cleanout Efficiencies with Tapered and Nontapered Rotors

Number of grooves	Cleanout efficiencies (%)		
	Tapered core	Tapered core radius corrected[a]	Nontapered core
6	79	91	93
60	80	92	97

[a] Radius correction factor is $1.15 = (4.94/4.615)^2$.

TABLE 3
Physical Property Values and Results of Theoretical Cleanout Calculation

Particles: Dow uniform polystyrene latex spheres.
Particle diameter = 0.0000109 cm.
Particle density = 1.050 gm/cm³.
Suspending medium: Water (at 30°C in the rotor).
Density = 0.966 gm/cm³.
Viscosity = 0.0080 poise.
Centrifuge speed: 30,000 rpm.
Feed zone dimensions (cm):

Core	Inner radius	Outer radius
Tapered	4.61[a]	4.94
Nontapered	4.94	5.14[b]

Results:

	Predicted 100% clean-out at flow rate of
Tapered core	17.3 liters/hr
Nontapered core	20 liters/hr

[a] Arithmetic mean of the radii at large and small ends of the core.
[b] Thickness of the flowing feed layer assumed to be 0.20 cm. The value is not critical and has little effect on results as long as it is small compared to the radius of the core.

from the larger end. Since the cleanout efficiency did not change appreciably during the experiment, the thickness of the flowing film must have decreased in proportion to the residence time. The lower, over-all performance of the reverse-fed tapered core may be attributed to shorter average residence time for the feed suspension.

Delay time sequences in the other experiments do not show any clear trend. This indicates that the gradient concentration near the flowing feed stream was about the same under starting conditions and under later flow conditions. The starting concentration at the light end of the density gradient was 2% sucrose, with a density (1.0038 gm/cm³) higher than the water entering as feed suspension. The heavy end of the step gradient was 55% sucrose.

The delay times with tapered and nontapered cores are not very different, and, assuming comparable angular feed distribution, it appears that the thickness of the flowing feed stream is about the same in the two cases. The difference in gradient profiles seen in Figure 5, however, implies that the flow area is further outboard from the core in the tapered rotor. If the thickness of the flowing layer is about the same with tapered and nontapered cores, one must conclude that the taper volume is partially or largely nonfunctional during continuous flow operation. The taper volume may actually contain light, particle-free fluid during operation, with the feed suspension flowing between it and the gradient, thus partially mimicking a zero taper core.

Cleanout efficiencies of tapered and nontapered rotors are approximately equal when mean radius corrections are included in the comparison. The nontapered core performed somewhat better in the experiments reported here, but both were only about half as efficient as the available model predicts they should be. Three possible explanations have been offered: (*a*) back diffusion of the sedimenting particles, (*b*) channeling of feed fluid through the rotor, and (*c*) turbulence, including end effects, in the rotor.

Appreciable back diffusion of the sedimenting particles is not considered possible because of the brief time intervals involved. Channeling or uneven feed distribution over the core may account for part of the inefficiency, but an attempt to improve the distribution with 60-groove core caps produced only slight improvement, not approaching the factor of two discrepancy which was observed. Also, the high centrifugal force field at operating speeds would tend to smooth the flow patterns and eliminate angular density variations. The possibility of turbulence remains, but no evaluation of its effects can be attempted without detailed knowledge of the flow patterns in the rotor.

SUMMARY

The taper in the K-II core was designed to aid in maintaining resolution during dynamic unloading. Static unloading has proved to be superior in practice however, thereby eliminating the requirement for a taper. Since, as shown here, superior recovery is obtained with a nontapered core, the latter design has been adopted for the K-III titanium continuous-sample-flow-with-banding rotor (17).

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