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K-Series Centrifuges

II. Performance of the K-II Rotor

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The K-II centrifuge (1-3) was designed for large-scale virus isolation using the continuous-sample-flow-with-banding technique (CFB) (4-8), and has been used successfully for the commercial production of influenza vaccine (9, 10). The rotor was originally designed for dynamic unloading; however, the resolution obtained was less than expected. The gradient has therefore been allowed to reorient (11, 12) during deceleration to the configuration obtained at rest, and the rotor contents recovered. The over-all configuration with a tapered core employed for dynamic unloading was retained, however.

In this paper, results of studies, largely with polystyrene beads as model particles, are described, together with data on loss of gradient solutes during continuous flow through the rotor. The results indicate that loss of resolution during reorientation is surprisingly small.

EXPERIMENTAL STUDIES

Both aluminum and plastic versions of the K-II rotors previously described (3) were used for experimental studies. Most of the work was carried out in K-A and K-B armor. A plastic door was provided for the K-A machine to allow direct observation of flow through the rotor during rotation.

The loading procedure was as follows. The rotor at rest was partially filled through the lower seal and shaft with either distilled water or a

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dilute buffer. A sucrose solution was then pumped in through the same line, displacing the water or buffer upward, until the rotor was completely filled (total volume 3600 ml). The rotor was then accelerated according to the schedule given in Table 1. Flow through the rotor was

TABLE 1
K-II Centrifuge Acceleration and Deceleration Programs

Speed range, rpm	Acceleration or deceleration rate, rpm/sec
Acceleration	
0–500	2
500–2100	4
2100–operating speed[a]	~51
Deceleration	
Operating speed–2000 rpm[b]	~58
2000–500	4
500–0	2

[a] Full air pressure (45 psig) to turbine.
[b] Full pressure reverse air flow (45 psig) to turbine for braking.

started at approximately 2000 rpm and was continued during acceleration as illustrated diagrammatically in the previous paper in this series (3). When the continuous-flow portion of the experiment was completed, the rotor was in some instances run for an additional interval at speed to allow the last material sedimented out of the stream to band isopycnically. The rotor was then decelerated according to the schedule in Table 1, and the gradient recovered through the bottom of the rotor. Air pressure (~7 psig) was applied through the upper line, and unloading rate was controlled by a roller pump[4] with a silicon controlled rectifier (SCR) speed control.

When more precise control of particle concentration was required in experiments on the effect of reorientation on resolution, the particles to be banded were suspended in the water or buffer placed in the rotor initially, and the continuous-flow portion of the experiment was omitted.

Polystyrene Beads

A stable spherical particle of uniform size and shape having sedimentation properties in the range characteristic of viruses is required

[4] Model 7020V-14 obtained from Cole Parmer, Chicago, Illinois.
[5] Dow Uniform Latex Particles obtained from Diagnostic Products, Dow Chemical Company, Midland, Michigan. Form No. 340-047-65.

for studies on rotor performance. Uniform latex particles[5] fulfill these requirements. The particles used were characterized by the manufacturer as having a mean diameter of 0.091 μ, with a standard deviation of 0.0058 μ. The density was given as 1.05 gm/cm^3, and the beads banded isopycnically at 12.7% w/w sucrose at 20°C, for a measured density of 1.049.

The sedimentation coefficient of the beads calculated from their diameter and density was 230 S in water at 20°C. The experimental values, measured at a concentration of 1.0 mg/ml in a Spinco model E analytical ultracentrifuge, were $s_{20,w} = 210$ in distilled water and $s_{20,w} = 237$ in Miller-Golder buffer (pH 7.4, $\mu = 0.2$).

Particle Assay

Absorption of the effluent stream was monitored continuously at 244 nm during unloading with a 0.2 cm light path quartz flow cell (13) and a Gilford absorbance indicator,[6] optical density converter, and recording potentiometer. An event marker on the recorder allowed close correlation between absorbance and the collected fractions. Rotor contents were collected manually in 100 ml fractions. The observed absorbance is due to both true light absorption at the wavelength used and light scattering, and may be related to particle concentration through the calibration curve given in Figure 1. The sucrose concentration of the collected fractions was determined with a refractometer[7] calibrated directly in weight per cent sucrose.

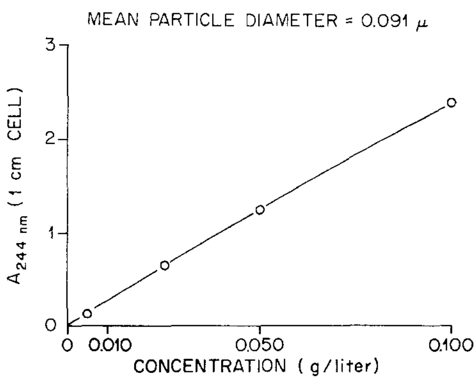

FIG. 1. UV absorbance as function of particle concentration for suspensions of Dow uniform polystyrene latex spheres in water. Mean particle diameter = 0.091 μ.

[6] Model 220 obtained from Gilford Instrument Laboratories, Inc., Oberlin, Ohio.
[7] Model "Abbe-3L" refractometer obtained from Bausch & Lomb, Rochester, New York.

Resolution

The first question to be asked is: Can a sharp zone existing in the rotor at speed be recovered with high resolution by using the reorienting gradient technique? To answer this question, 10 mg of polystyrene latex in 2 liters of water was loaded into the rotor followed by 1600 ml of 55 w/w per cent sucrose solution. After 2 hr at 30,000 rpm the rotor was decelerated and unloaded at rest. The average band width at half-height for five experiments was 64 ml (Fig. 2), corresponding to a

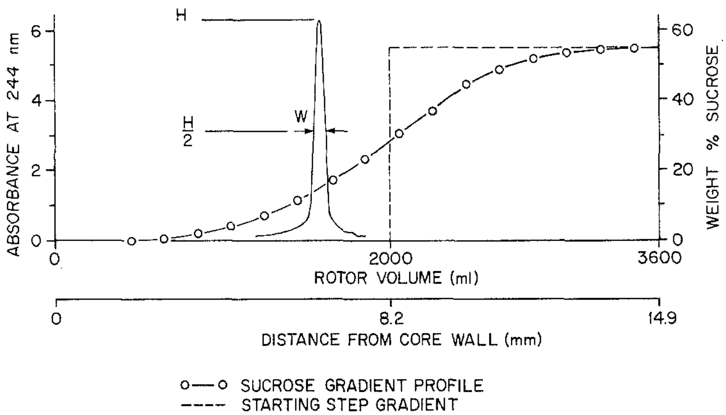

FIG. 2. Band width of polystyrene spheres in a sucrose gradient unloaded statically. Particle diameter = 0.091 μ; particle density = 1.05 gm/cm^3; banding time = 2 hr at 30,000 rpm. Results, average of five runs: $H = 6.33$ OD units; $W =$ 64 ml or 0.27 mm; standard deviation of W was $s = 4.2$ ml.

band thickness of 0.27 mm in the rotor during centrifugation. (The average distance from core to wall is 14.9 mm.) In the best experiment, the half-height width was 59 ml, corresponding to a 0.24 mm width in the rotor. The resulting density gradient profiles were essentially identical in all these experiments. Rotor core caps with eighteen radial grooves were used in this series.

A specially designed core cap permits dynamic unloading of the K-II rotor, but the best resolution attained with this technique was a band width of 79 ml at an unloading rate of 56 ml/min, compared with 59 ml band width from static unloading at 65 ml/min. Static unloading, therefore, gave somewhat better resolution with this system, is more convenient, and may be quicker than dynamic unloading.

The actual band width in the rotor could not be measured directly with the foregoing techniques. Additional experiments were necessary to evaluate the loss of resolution from reorientation and unloading, and to estimate the true band width during rotation. The problem was approached by repeating the latex banding experiment with an additional reorientation at the end of the experiment. That is, after the rotor was stopped once, it was reaccelerated up to 2000 rpm and then immediately decelerated back to rest. The total elapsed time during the extra acceleration and deceleration steps was approximately 20 min. Since the centrifugal force in the rotor at 2000 rpm was only 240*g* at the radius of the band, and since deceleration was started as soon as this speed was reached, the extra centrifugal force would produce negligible narrowing of the band.

The results are shown in Table 2. The average band width observed in three experiments was only 4% greater than that previously observed for only a single reorientation. If we assume that band spreading during acceleration and deceleration were equal, then the spreading due to the single reorientation during deceleration would correspond to only a 2% increase in band width at half-height.

TABLE 2
Effect of Gradient Reorientation on Resolution in the K-II Rotor

Treatment	Resulting band width at half-height, ml	
	Results of separate experiments	Mean
Normal operation (one reorientation during deceleration)	65, 69, 59, 66, 60	64
Two extra reorientations (normal operation plus one extra reorientation during acceleration to 2000 rpm, and a second during deceleration back to rest)	67, 68, 65	67

The effect of unloading alone was measured more directly by unloading the rotor through the upper shaft by displacement from below with 60 wt per cent sucrose, through the spectrophotometer, and directly into the bottom of a second K-II rotor suspended above the first. The entire gradient was loaded into the second rotor. Then the upper rotor was unloaded from the bottom shaft back through the spectrophotometer flow cell. Again, only the summed effects of loading and unloading could be observed, but the effect of reorientation was absent. Thus the perturbation of a true isopycnic band of known shape by flow into and out of the rotor could be observed through comparison of the two absorbance tracings.

The band width after the second unloading was, on the average, 36% greater than after the first. In the four experiments averaged, the widening effect was greatest on a narrow 60 ml band and lowest (only 13%) on a wider 96 ml band.

Using half of the percentage widening found from the added effect of treatments described above, one can estimate that gradient reorientation and deceleration effects cause about a 2% widening, and static unloading contributes about 18% band spread, for a total of 20%. With the small number of experiments considered, the scatter of the data does not allow statistical significance for the exact percentages reported. The important point is that the combined effects of diffusion and bulk mixing during reorientation, deceleration, and flow through the centrifuge (annular section, feed channels, shaft, and connecting tubing) do not grossly destroy resolution during the band recovery process.

Gradient Decay

The gradients used in the K-II rotor are rapidly formed by diffusion in the thin annular space in the rotor. During continuous-flow operation, the feed solution stream passes over the entire inner surface of the gradient before leaving the rotor. Sucrose may be expected to mix with the stream, as a result of both bulk fluid mixing and diffusion, resulting in gradual removal of sucrose from the rotor and a decay of the gradient. If this proceeds to the point at which the maximum density in the gradient is below that of the particles being banded, the particles will sediment to the wall and will be neither separated according to density nor recovered as a discrete band. The rate of gradient decay therefore limits the length of time the rotor may be run, and the volume of fluid that may be passed through it.

The initial loading of the rotor was with two solutions: water and a concentrated sucrose solution. The first reorientation and concurrent

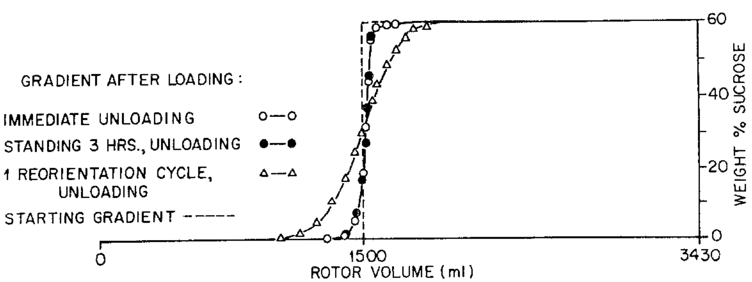

FIG. 3. Gradient decay from loading and unloading and from one reorientation cycle.

diffusion would tend to produce a very short, steep gradient. The possibility therefore exists that the first material banded might band too sharply in the rotor and aggregate, or that two closely spaced particle bands might not be resolved. This is demonstrated by loading water and 60% sucrose into the rotor as shown in Figure 3. Only a very short, steep gradient was observed if the rotor was unloaded immediately or after standing for 3 hr at rest. As expected from the greater surface area which obtains after reorientation to the spinning configuration, a short gradient was rapidly formed as the rotor was brought up to speed. Note that no evidence of anomalous mixing through the gradient was observed.

The effect of multiple reorientations on gradient shape was next examined as shown in Figure 4. (The following terminology is used:

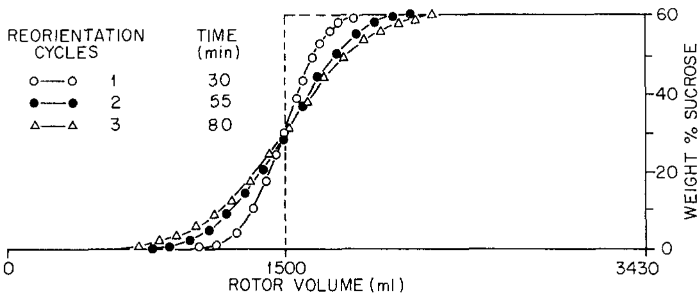

FIG. 4. Gradient decay as function of number of reorientation cycles.

a "reorientation" means one change in direction of the density gradient, either from axial to radial, as during acceleration, or from radial to axial, as during deceleration; a "reorientation cycle" will include both the acceleration and deceleration steps, that is, two reorientations.) The length of time between loading and unloading was 30 min for one reorientation cycle, 55 min for two cycles, and 80 min for three. It is apparent that more gradient spreading appeared between the first and second reorientation cycles than between the second and third, i.e., that less decay is seen with less steep gradients, suggesting that part of the effect observed was due to the longer time required for multiple reorientations and not entirely due to reorientation itself.

This conclusion is amply supported by the experiments, shown in Figure 5, in which one and three reorientation cycles were arranged to extend over equal time intervals of 80 min. Greater gradient broadening was observed with one cycle than with three. With a single reorientation cycle, the gradient was left in the spin or maximum area configuration a larger fraction of the time than was the case with

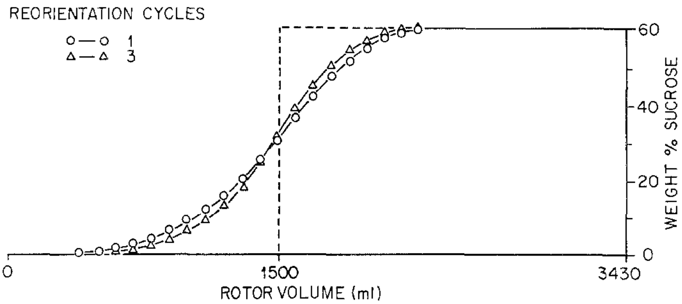

FIG. 5. Gradient decay as function of reorientations with time constant. Elapsed time for each experiment was 80 min.

the triply reoriented gradient. These results confirm diffusion as the major source of solute movement in a reorienting-gradient K-II rotor using sucrose density gradients.

The change in gradient shape as a function of time due to diffusion alone was calculated and compared with experimental measurements. Figure 6 shows experimentally found diffusion of a sucrose step gradient as a function of specified spinning times of 0, 1, and 4 hr at 2000 rpm with no flow. (Zero time at 2000 rpm means that the rotor was decelerated immediately after reaching 2000 rpm. The curve is the same as that for one gradient reorientation cycle in Figure 4.) Gradient decay with no flow was found to be nearly independent of rpm, and is primarily a function of operating time. Diffusion is relatively rapid at operating speeds because the annular space is narrow and the resulting radial concentration gradient dc/dr, which is the diffusion driving force, is large. Also, the available diffusion area is large, about 2500 cm^2.

A theoretical gradient profile resulting from diffusion alone (no feed washout) was calculated by using a flat plate model bounded at $Z = \pm L$ (14). The original concentration profile is $C = 0$ for

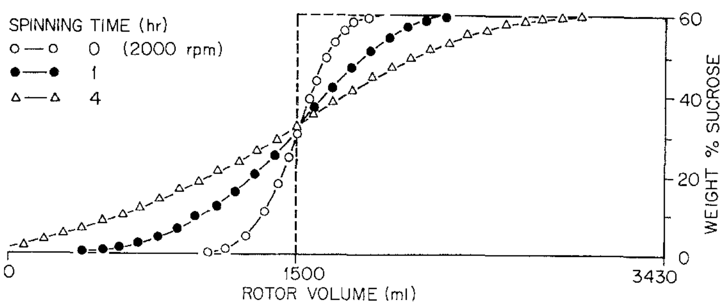

FIG. 6. Gradient decay as function of time with no feed.

$-L \leq Z < 0$, and $C = C_A^+$ for $0 \leq Z \leq +L$. The solution for the model is:

$$C_A = \frac{C_A^+}{2}\left[1 + \frac{2}{\pi}\sum_{n=0}^{\infty}\left(\frac{\sin\left(n+\frac{1}{2}\right)\frac{\pi Z}{L}}{n+\frac{1}{2}}\right)\exp\left[-\left(n+\frac{1}{2}\right)\frac{\pi}{L}\right]^2 D_{AB}t\right]$$

where

C_A = concentration at any distance Z and time t,
C_A^+ = starting concentration for $t = 0$, $0 < Z < +L$ = 60 wt % sucrose,
L = boundary distance from origin = 0.65 cm, and
D_{AB} = average diffusivity = 3.0×10^{-6} cm²/sec, from Sato (15).

The actual geometry is that of an annular space of constant outside radius of 6.10 cm, with the inside radius of 4.29 cm at one end and 4.94 cm at the other. For a flat plate approximation, the average distance between the boundaries would be 1.49 cm. For this model, however, the distance of interest was taken to be 1.30 cm, with the original concentration discontinuity at the center plane. The distance and rotor volume scales then have the correspondence given in Table 3.

TABLE 3
Relation between Centrifuge Dimensions and Diffusion Model Distance

Rotor volume, ml	Average distance from core wall, cm	Model distance, Z for diffusion equations, cm
0	0	−0.65
1500	0.65	0
3000	1.30	+0.65
3430	1.49	

The origin $Z = 0$ then corresponds to a volume of 1500 ml, the volume of water loaded for actual experiments.

Comparison of the theoretical curves with the experimental in Figure 7 again demonstrates diffusion to be the major cause of gradient decay.

Gradient Washout during Continuous Flow

The removal of sucrose during continuous flow was studied initially under relatively adverse circumstances, namely, at low rotor speed, high flow rate, and room temperature using a plastic rotor. The results of three experiments using a flow rate of 20 liters per hour for 2, 6, and 10 hr are shown in Figure 8. After 200 liters flow, the concentration at the high end of the gradient had fallen from 60 to 56% w/w sucrose,

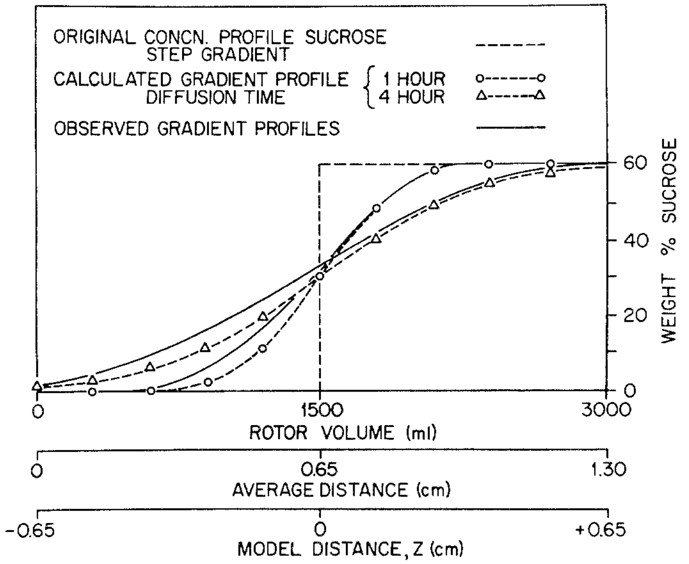

FIG. 7. Comparison of theoretical and observed gradient profiles in K-11 rotor. Observed gradient profiles are from Figure 6.

indicating that a gradient could be maintained for 10 hr and 200 liters flow with only a small loss in concentration at the wall. This study indicates the K-II may be useful for the recovery and isopycnic banding of relatively large particles sedimented at low speed as well as particles of viral dimensions.

The effect of flow rate on gradient decay at low speed is illustrated in Figure 9—time and speed were kept constant but flow rate was varied from 0 to 40 liters per hour. With increased flow rate, gradient decay is increased at the lower end. All curves appear to converge, how-

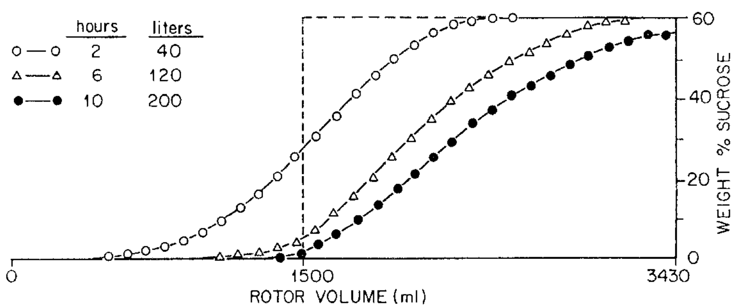

FIG. 8. Gradient decay as function of time at low rotor speed and high flow rate. Speed = 2000 rpm; flow rate = 20 liters/hr.

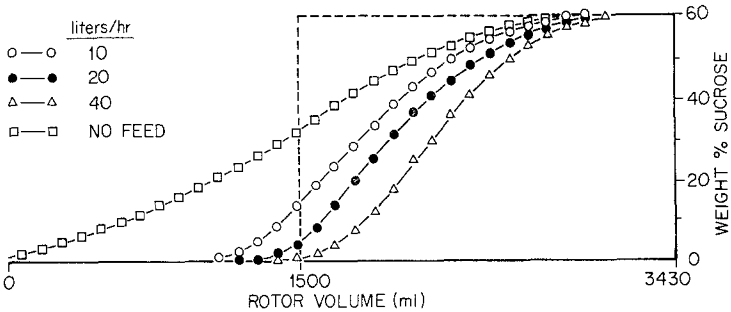

FIG. 9. Gradient decay as function of feed rate with total time kept constant. Total continuous flow time = 4 hr; speed = 2000 rpm.

ever, at the high end of the profile, indicating that diffusion is controlling in this region, while the flow rate is controlling in the low sucrose region. Experiments in which the volume of fluid fed through the rotor was kept constant at 40 liters, but the flow rate was varied from 10 to 40 liters per hour, are shown in Figure 10. Greatest gradient decay occurred at a flow rate of 10 liters per hour because of the long time (4 hr) involved. Although diffusion is controlling at the upper end of the profile, the feed rate markedly affects the slope and the rate of loss from the gradient toe, especially for the relatively short operating time at the higher flow rates. A marked change in slope occurs for the 40 liter/hr run, indicating that this high feed rate tends to wash the gradient more than the others. There is little difference, however, between the slopes of the 10 and 20 liter/hr profiles.

These studies indicate that the gradients may be maintained during 100–200 liter experiments at low speed. Less washout may be expected at high speed at which the higher centrifugal force would tend to minimize turbulent flow. This is borne out experimentally as shown in

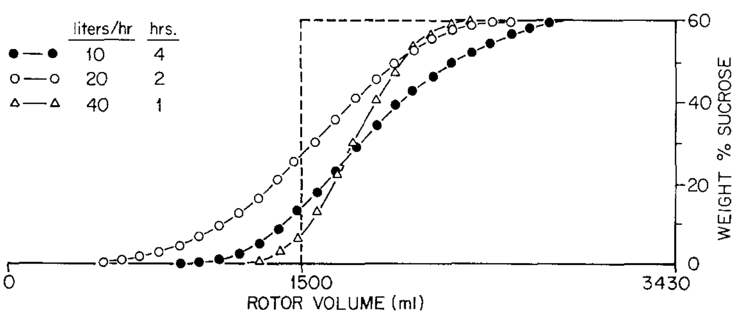

FIG. 10. Gradient decay as function of feed rate with feed volume constant. Total feed volume = 40 liters; speed = 2000 rpm.

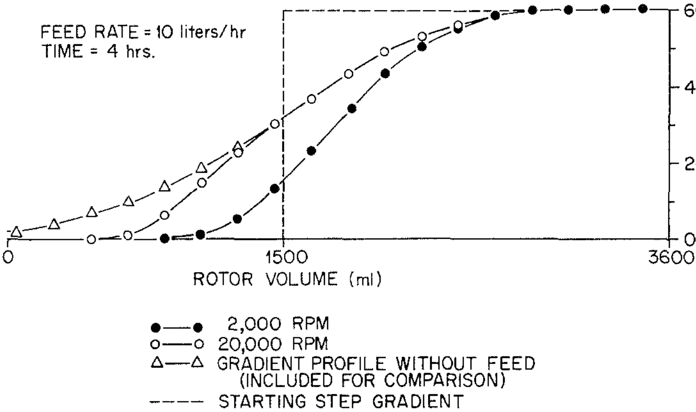

FIG. 11. Effect of rotor speed on gradient washout at constant flow rate. Total continuous flow time = 4 hr; flow rate = 10 liters/hr.

Figure 11 where gradient decay was studied at 2000 and 20,000 rpm at a constant flow rate of 10 liters per hour for 4 hr. The gradient profile obtained with no flow is shown for comparison. The high concentration end of the gradient is identical to that resulting from diffusion alone, and gradient washout at the lower end is much less at high speed. The point along the ordinate at which the sucrose concentration equals that of the feed stream is different at the two speeds, indicating that at low

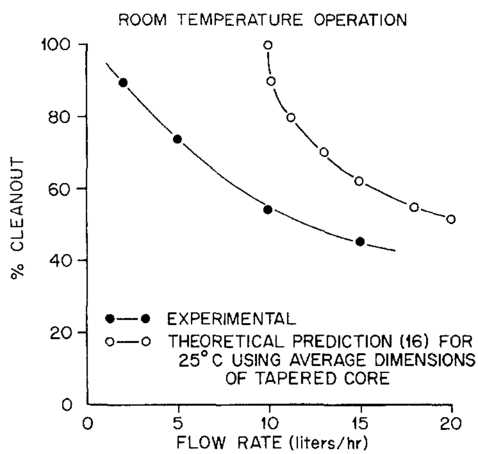

FIG. 12. Per cent removal of 230 S polystyrene latex particles in the K-II centrifuge as function of flow rate. Speed = 30,000 rpm; room temperature operation. For comparison a theoretical plot obtained with the Berman equation (16) is also shown.

speed the streams sweep much farther out away from the core than at high speed.

Efficiency of Particle Removal from the Stream

The percentage removal of polystyrene particles as a function of flow rate was measured with the K-II rotor operating at 30,000 rpm at room temperature. The results are shown in Figure 12, together with theoretical values obtained by using the Berman equation (16). At high flow rates up to 73% of the theoretical value was obtained, while at low flow rates there was marked deviation from the expected value. Similar incomplete cleanout has been observed with influenza virus (10). This effect is only partially explained by inhomogeneity of the particle suspension. Greater discrepancy would appear if the theoretical curve were calculated for 30°C; and the temperature of the feed stream in the rotor may be closer to 30° than to 25° because there is some warming effect as the flowing stream picks up heat dissipated from the bearing surfaces. The actual temperature of the rotor contents is not known at this time.

Residence Time in the Rotor

The Berman equation was originally developed for the B-V rotor (17). In experiments with this rotor, which had a core with no taper, the experimental results were in close agreement with theory (18) using poliovirus as the test object. This suggests that, in the K-II, which has a tapered core and feed zone defined only by a liquid density gradient on the outboard side, flow patterns may be quite different from the flow assumed by the Berman model. Experimental studies of residence time were carried out in which the dynamic response to a step change in absorbance of the input stream was measured at flow rates of 10, 20, and 40 liters per hour in the aluminum K-II rotor operated at 20,000 rpm using a gradient formed between 1500 ml of water and 2100 ml of 60% w/w sucrose. Buffalo Black was used as the dye in a trace concentration which did not produce a measurable change in the physical density of the stream. After flow was established using water, the stream was changed quickly to the dyed water. The absorbance of the effluent stream was monitored at 610 nm and the results are shown in Figure 13 as a function of reduced time, θ, and of real time in minutes.

It is evident that flow through the rotor is not accurately described as plug flow or as back-mix flow, but has elements of each. It is interesting that the relative delay in emergence of the dye increased as the flow rate was increased. The change at higher flow rates may be due

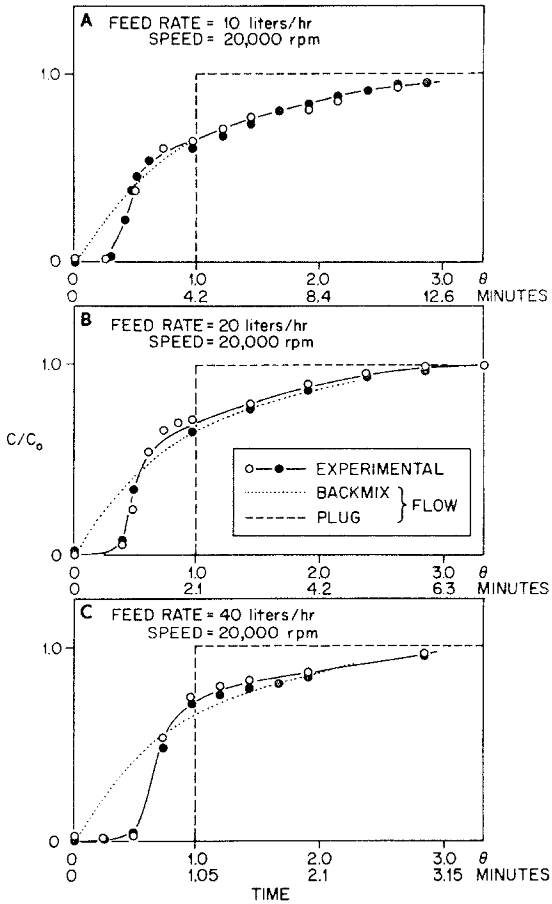

FIG. 13. Effluent response to step change in absorbance of input stream. Speed = 20,000 rpm. Flow rate was 10 liters/hr in A, 20 liters/hr in B, 40 liters/hr in C. Theoretical curves for plug flow and complete back-mix flow are included for comparison. C_0 = absorbance of input stream; C = absorbance of effluent. Reduced time θ = real time/calculated residence time. Calculated residence times for holdup volume of 700 ml were 4.2 min in A, 2.1 min in B, and 1.05 min in C.

to an earlier entry into a transitional or turbulent flow regime at the wide (exit) end of the core, where the flow channel is thinner and the Reynolds Number would increase. The velocity profile would flatten during turbulent flow and would more closely approximate plug flow. The results are partially described as being due to the existence of two separate areas in the rotor, the first characterized by laminar flow with parabolic velocity profile, the second by back-mixing.

DISCUSSION

The K-II continuous-sample-flow-with-banding zonal rotor (3) has been successfully used to concentrate influenza virus for vaccine production (9, 10). The design was based largely on previous experience with the smaller B-VIII, B-IX, and B-XVI rotors. However, detailed information was lacking on the effect of reorientation, and of unloading on resolution, on the factors affecting gradient decay, and on the so-called "cleanout efficiency" of this rotor.

The effect of gradient reorientation on the width of isopycnic bands in the rotor is negligible when the bands are near the center of the gradient during rotation, and the center of the rotor during rest, and when unloading is done through the bottom of the rotor. In additional experiments not described here, it was found that sample zones near the dense end of the gradient were broadened less when unloaded through the top than through the bottom.

The efficiency of the reorienting process is surprising. It may be partially explained by the very slow reorientation which occurs during controlled deceleration, or when such massive rotors coast to rest on very low friction bearings. The air turbines also lack the frictional drag variations sometimes found with commutators.

The stability of a very narrow sucrose gradient positioned to cover the entire inner surface of the rotor wall is also somewhat surprising. Removal of sucrose by the flowing stream is faster at low rotor speeds than at high speed, and is slow enough in all instances to allow useful gradients to be retained in the rotor for 10 hr during which 200 liters of water passed through the rotor. Movement of sucrose toward the stream-gradient interface was almost entirely due to diffusion, while movement across the interface and the position of the interface was due to both diffusion and bulk flow.

The efficiency of removal of particles from the flowing stream was less than expected from the Berman theory. However, studies on residence time in the rotor indicated a combination of laminar flow and back-mixing occurred in the rotor, and that a large fraction of the fluid spent less time in the rotor at a given flow rate than is assumed in the Berman theory.

We conclude that the K-II centrifuge's performance is, in many respects, close to theory. However, the low cleanout suggests that other core configurations be considered, as described in a subsequent paper.[8]

[8] The K-II zonal centrifuge is available from Electro-Nucleonics, Inc., 368 Passaic Avenue, Fairfield, New Jersey 07006.

SUMMARY

Performance of the K-II continuous-sample-flow-with-banding zonal centrifuge rotor was evaluated using sucrose gradients and polystyrene latex beads having a mean diameter of 0.091 μ and a banding density of 1.049 gm/cm³ in sucrose at 20°C. Narrower particle zones were recovered with static unloading as compared with dynamic unloading. With the former, the average band width at half-height was 64 ml in the total volume of 3600 cm³, corresponding to a width of 0.24 mm in the rotor. Multiple reorientations of the gradient produced only a small loss of resolution. Measurements of gradient decay and sucrose loss during continuous flow indicate that gradients suitable for influenza virus recovery may be retained in the rotor for 10 hr, while 200 liters of fluid passes through the rotor. The particle capture efficiency of the rotor is slightly less than predicted by the Berman theory.

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