ANALYTICAL BIOCHEMISTRY 26, 415–419 (1968)

Analytical Techniques for Cell Fractions

XI. Rotor B-XXIII—A Zonal Centrifuge Rotor for Center or Edge Unloading[1]

N. G. ANDERSON, C. T. RANKIN, JR., D. H. BROWN, C. E. NUNLEY, AND H. W. HSU[2]

The Molecular Anatomy Program, The Oak Ridge National Laboratory,[3] and The Technical Division, The Oak Ridge Gaseous Diffusion Plant,[3] Oak Ridge, Tennessee 37830

Received April 26, 1968

It is of advantage to be able to recover gradients from zonal centrifuges either from the rotor center (light end of the gradient) or from the rotor edge (heavy end of the gradient). In swinging buckets, gradients are recovered in either direction at rest. In zonal centrifuge rotors unloaded during rotation, however, recovery from the rotor edge without large losses of resolution presents certain difficulties. The area perpendicular to the radius occupied by a particle zone increases with the radius until the zone reaches the rotor wall. Horizontal flow is then required to move the zone to exit ports. In isopycnic separations in which particle sedimentation during flow along the wall does not occur, good zone recovery has been achieved by using a vertical wall which is slightly eccentric to the axis (1). Unloading from the edge during rate-zonal sedimentation appears to offer special problems, however. A rotor having a tapered wall (B-XXIII) has been constructed with a removable seal identical to that previously described for the B-XIV and -XV rotors (2). In the present paper, the B-XXIII rotor and its application to the problem of model particle separation are described. In a subsequent paper, the application of this rotor to cell fractionation will be presented.

[1] Research supported by the Joint NIH-AEC MAN Program sponsored by the National Cancer Institute, The National Institute of General Medical Sciences, the National Institute of Allergy and Infectious Diseases, and the U. S. Atomic Energy Commission.

[2] University of Tennessee.

[3] Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.

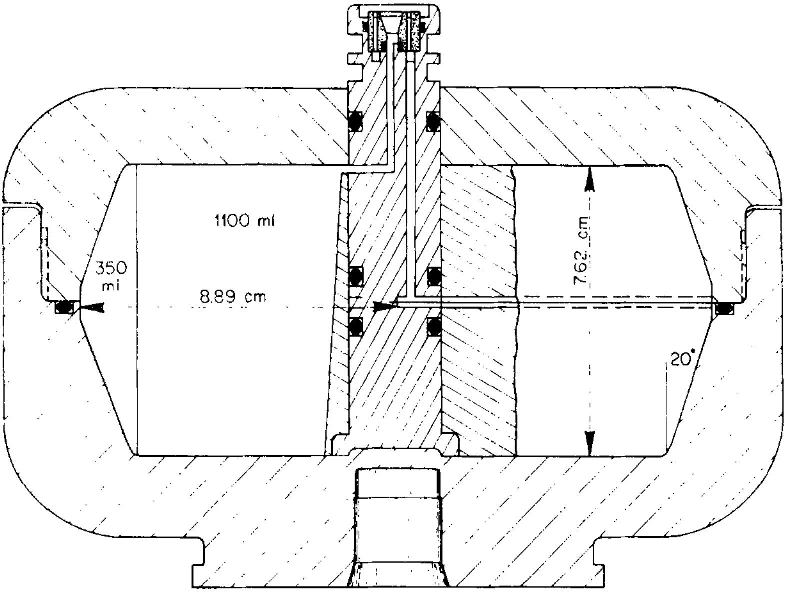

FIG. 1. Side section drawing of assembled B-XXIII rotor showing internal tapered wall surfaces.

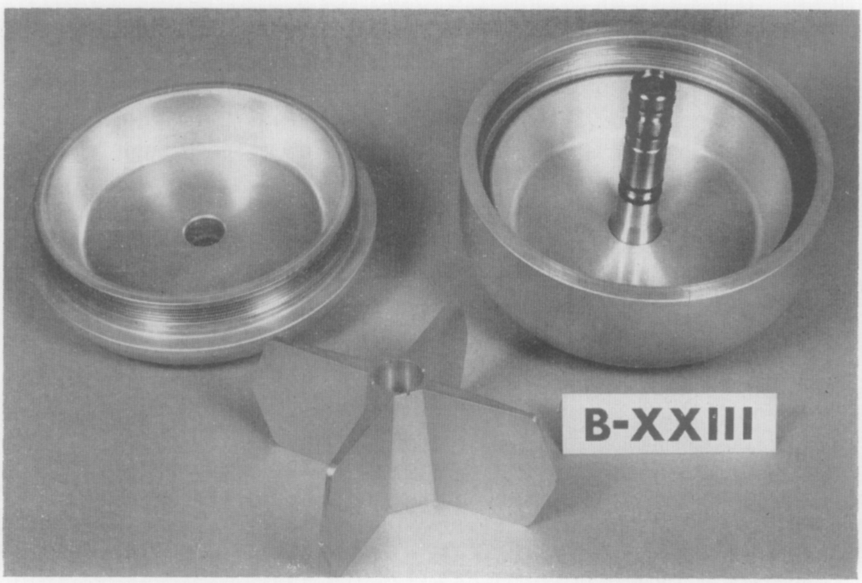

FIG. 2. Photograph of partially disassembled B-XXIII zonal centrifuge rotor.

ROTOR DESIGN

The B-XXIII rotor is shown diagrammatically in Figure 1 and partially disassembled in Figure 2. The general configuration is similar to that of the B-XV aluminum rotor previously described (2), except that the edge of the fluid chamber is tapered 20° and the septa are tapered to match. The position of the B-XXIII threads differs from that of B-XV threads in that the closure point internally is midway between the top and bottom end surfaces of the rotor. As shown in Figure 1, the volume in the chamber centripetal to the edge taper is 1100 ml, and a volume of 350 ml is included in the tapered region near the wall (total volume 1450 ml). The rotor is constructed of 7075 Duraluminum with a T-6 heat treatment, and was anodized before use. The rotor was operated in a modified Spinco model L (3) with a digital ω^2t indicator attached (4).

ROTOR EVALUATION

The rotor was operated at 5°C and was loaded during rotation at 2500 rpm with an unbuffered sucrose gradient (1000 ml total volume) extending from 17 to 55% w/w sucrose followed by an underlay of 55% sucrose to fill the rotor completely. A sample consisting of 10 ml of 1% bovine serum albumin (BSA) in 8.5% sucrose and of 2 ml of alcohol-washed ragweed pollen was introduced through the center line. An overlay of 50 ml of 4% sucrose was added to move the sample out clear of the core. When the gradient, sample, and overlay were in position in the rotor, it was accelerated to 10,000 rpm for approximately 10 min, and then decelerated to 2500 rpm for unloading. When $\omega^2t = 66 \times 10^7$, unloading was begun. The test mixture of BSA and pollen was chosen

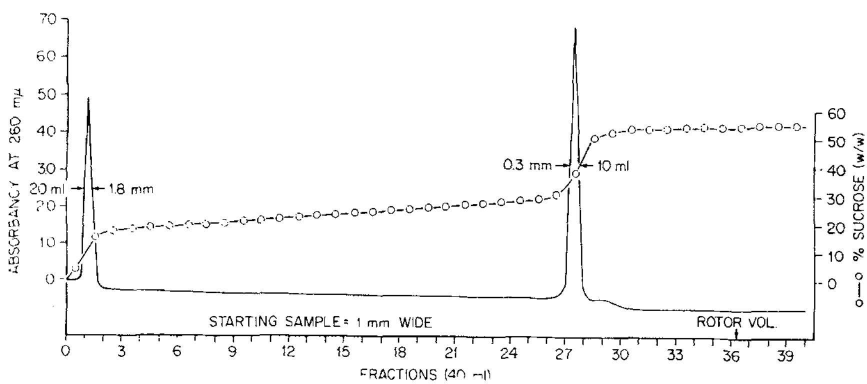

FIG. 3. Recovery of bovine serine albumin (left) and ragweed pollen (right) zones in a sucrose gradient unloaded through the center line. Calculated width of sample zone initially (assuming no mixture) was 1 mm.

because it provides two narrow zones, one near the core face and the other near the rotor wall. Anomalies occurring during unloading, or due to gradient instability or mixing, may be easily observed.

The gradients were recovered from the rotor center (Fig. 3) by displacement by using 55% sucrose pumped to the rotor edge, and from the rotor edge (Fig. 4) by using distilled water pumped to the rotor center for displacement. The rotor contents were monitored at 260 nm by using a 0.2 cm flow cell (5) and were collected in 40 ml fractions.

From the experimental data, the starting sample was calculated to be 1 mm wide when positioned in the rotor. When recovered through the center line, the width of the BSA peak was calculated to be 1.8 mm wide at half height. In the same experiment the pollen peak was calculated to have a width at half height of 0.3 mm in the position it occupied in the rotor.

When the rotor was unloaded from the edge, the pollen peak had a calculated width at half peak height of 1.5 mm, whereas the BSA peak was 2.25 mm wide at half height.

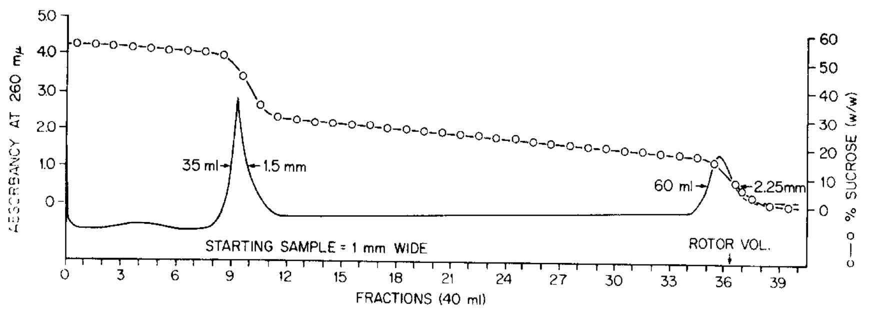

FIG. 4. Recovery of ragweed pollen (left) and bovine serine albumin (right) zones in a sucrose gradient unloaded from the rotor edge. Note that the plot is the reverse of that shown in Figure 3.

DISCUSSION

These results show that a small amount of band spreading occurs during edge unloading with the configuration described. While the decrement does not appear to be sufficient to interfere with most separations as actually performed, it appears of interest to examine additional modifications which would provide better flow to the edge or septal lines. Work on these modifications is now in progress.

As previously noted (6) unloading of rotors from either the center or the edge makes possible a series of new separation techniques, including sequential removal of subcellular particles which are approaching their isopycnic points. Thus, liver cell membrane fragments may be removed while mitochondria are still sedimenting. It is also possible to remove a large fraction of the mitochondria while the lysosomes are still trailing behind them. All of these particles have very similar isopycnic positions but different sedimentation rates. Experiments demonstrating this technique will be described in a subsequent paper.

A second possibility is rate separations based on flotation. Here reasonably sharp starting bands at the edge of the rotor are essential, and may be obtained by using the B-XXIII rotor.

A third possibility is the use of a continuously moving gradient flowing through the rotor at a rate calculated to keep the particles being purified at the same radius during the entire experiment. The advantage of this is that the particles may be kept in a higher centrifugal force field near the wall at all times, and a longer effective path used by employing a large volume gradient. In the practice, the gradient volume may exceed that of the rotor.

SUMMARY

A new zonal centrifuge rotor—the B-XXIII rotor—has been constructed that has tapered edge surfaces allowing a density gradient to be recovered either from the rotor edge or the rotor center. Experiments with a test sample containing bovine serum albumin and ragweed pollen grains showed only a small decrement in resolution when the rotor was unloaded from the edge instead of the center as is usually done. The removal of the outer portions of a gradient and the particles which it contains, and the reinsertion of a fresh gradient allow separations based on rate and on isopycnic banding density to be made sequentially in one rotor.

REFERENCES

1. Beaufay, H., "La centrifugation en gradient de densité" (Thèse d'Agregation de l'Enseignement Supérieur, Univ. Catholique de Louvain, Louvain, Belgium), p. 132. Ceuterick S. A., Louvain, Belgium, 1966.
2. Anderson, N. G., Waters, D. A., Fisher, W. D., Cline, G. B., Nunley, C. E., Elrod, L. H., and Rankin, C. T., Jr., *Anal. Biochem.* **21,** 235 (1967).
3. Anderson, N. G., Barringer, H. P., Babelay, E. F., Nunley, C. E., Bartkus, M. J., Fisher, W. D., and Rankin, C. T., Jr., *in* "The Development of Zonal Centrifuges and Ancillary Systems for Tissue Fractionation and Analysis" (N. G. Anderson, ed.), *Natl. Cancer Inst. Monograph* **21,** 137 (1966).
4. Anderson, N. G., "Analytical Techniques for Cell Fractions. VIII," *Anal. Biochem.* **23,** 72 (1968).
5. Anderson, N. G., *Anal. Chem.* **33,** 970 (1961).
6. Anderson, N. G., "Preparative Particle Separation in Density-Gradients," *Quart. Rev. Biophysics,* in press.