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Analytical Techniques for Cell Fractions

VII. A Simple Gradient-Forming Apparatus[1]

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For rate-zonal centrifugation in zonal centrifuge rotors, smooth gradients of large volume are required. In most instances the shape of the gradient in the rotor should be convex, i.e., the rate of change of concentration should decrease with radius (1). This follows from: (*a*) The capacity of a gradient increases as the difference between the particle and the solvent density decreases; therefore, to obtain identical particle capacity at all levels, the steepness of the gradient may be decreased at higher densities. (*b*) Greatest particle capacity is required just below the sample zone to support the particle population before it becomes widely spread throughout the gradient. (*c*) Radial dilution and diffusion combine to diminish the particle concentration in a zone as it sediments, thus diminishing the steepness of the gradient required to support it. In addition, since the gradients are spun in sector-shaped compartments, the gradient must be convex in a concentration vs. volume plot even when the gradient is to be linear-with-radius in the rotor.

These considerations suggest that a simple exponential gradient-making device that uses a constant-volume mixing chamber could be adapted to zonal centrifuge rotor loading.

CONSTRUCTION OF GRADIENT DEVICE

A convex gradient may be made by introducing a dense solution into a constant-volume mixer which initially contains a dilute solution corresponding to the light end of the gradient, and by withdrawing a stream

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

from the mixing chamber continuously. The concentration of the stream withdrawn at any given time is given by the equation (2–4):

$$C_t = C_2 - (C_2 - C_1)e^{-v_t/v_1}$$

where C_t is the concentration at time t, C_2 is the concentration of the denser solution, C_1 is the concentration of the light solution originally in the mixer, v_1 = the volume of the mixer, and v_t = the volume withdrawn to time t. If the mixer concentration is taken to be zero, and the concentration of the dense solution is set equal to one, then the equation reduces to:

$$C_t = 1 - \frac{1}{e^{v_t/v_1}}$$

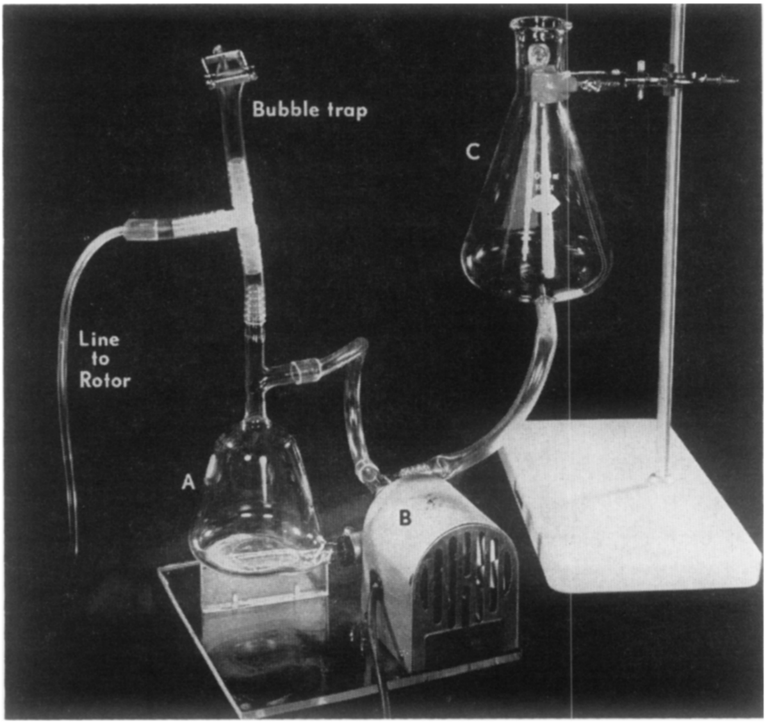

FIG. 1. Gradient mixer with (A) mixing chamber, (B) centrifugal pump, and (C) reservoir for dense gradient solution. Initially, the mixing chamber and the centrifugal pump are filled with the solution used for the light end of the gradient. As fluid is removed through line leading to rotor, an equal volume of dense fluid flows into the gradient mixer. In actual use, reservoir C is mounted several feet above mixer B and the connection into B is through a small ascending loop which prevents back convection.

and C_t is the composition of the fluid in the mixer expressed as volume fraction of denser fluid. This equation assumes that the contents of the mixer are homogeneous and that the rate of mixing is rapid relative to the rate of fluid withdrawal. When viscous sucrose solutions are being used, considerable energy must be expended to satisfy this requirement, and magnetic mixers or propeller stirrers have not been found adequate.

A small centrifugal pump[3] connected to a conical reservoir by very large tubing as shown diagrammatically in Figure 1 has therefore been used.

The mixing flask was constructed from a heavy-walled suction flask with 0.5 in. openings to the pump. Dense solution is introduced into the line leading from the flask to the pump. Initial mixing therefore occurs in the impellor chamber of the pump. The fluid then flows rapidly back to the mixing chamber through a tangentially attached tube to produce

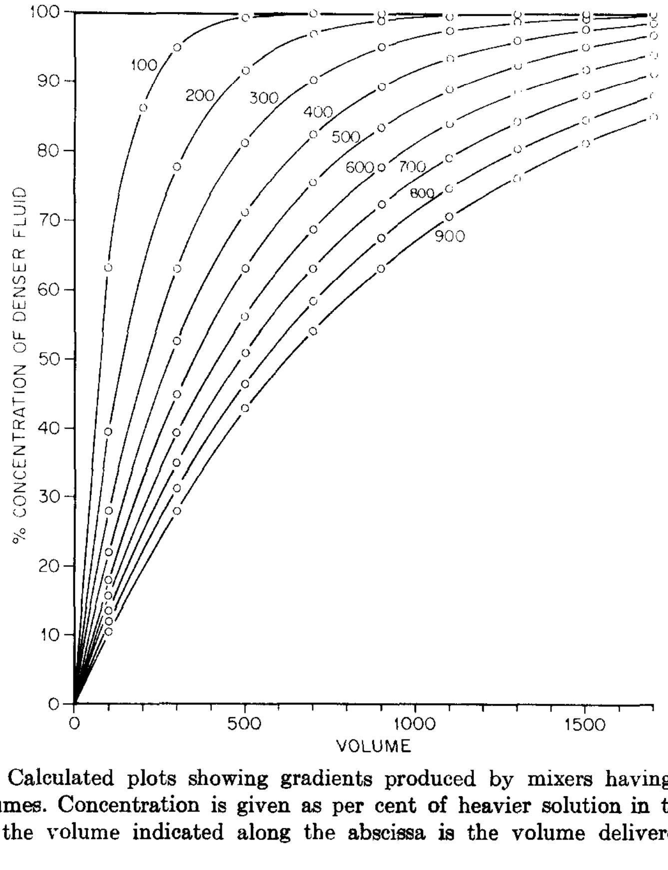

FIG. 2. Calculated plots showing gradients produced by mixers having different total volumes. Concentration is given as per cent of heavier solution in the lighter one, and the volume indicated along the abscissa is the volume delivered to the rotor.

[3] Obtained from Cole-Parmer Instrument and Equipment Co., 7330 North Clark Street, Chicago. Cat. No. 7000.

maximum spinning of the mixing chamber contents. Any unmixed dense solution tends to be centrifuged to the lower peripheral portion of the flask where it would mix with the incoming stream. Outflow is from a point at the vertex of the mixing chamber cone to ensure that only fluid equal in density to, or very slightly lighter than, the bulk of the fluid is removed. In addition, air bubbles may be removed initially through the delivery (to rotor) line. Mixing in the chamber should be so fast that a tiny air bubble will be drawn out to form a very fine line extending almost the full length of the flask.

PERFORMANCE

The theoretical curves for mixers of various sizes are shown in Figure 2. It is evident that nearly linear gradients can be produced if only the very first portion of the gradient is used. Note that the volume of the mixer includes the volume of solution in the conical reservoir, in the pump, and in the lines connecting the two. A number of mixers were

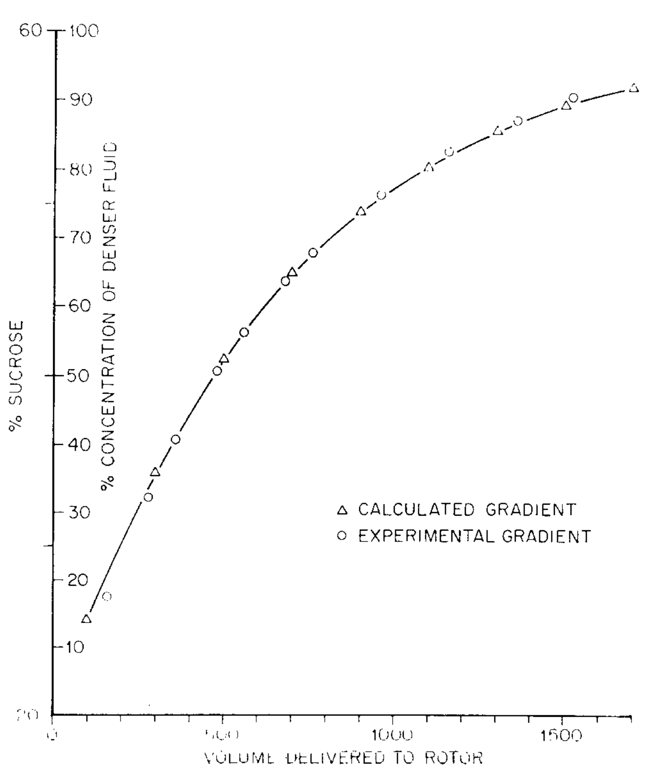

FIG. 3. Theoretical and experimental plots for a mixer having a total volume of 668 ml.

tested by measuring the sucrose concentration as delivered by the pump, and as recovered from a B-XV zonal rotor (5). Theoretical and experimental curves obtained are shown in Figure 3 for a 668 ml mixer. Only very small departures from the predicted curves were seen when

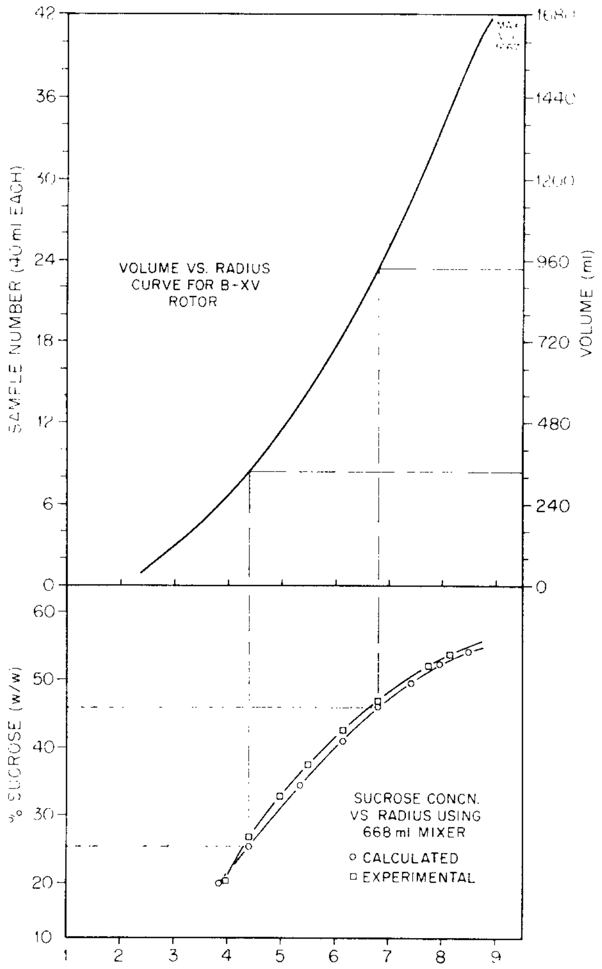

FIG. 4. Method for plotting shape of gradient in rotor. Upper plot relates volume to radius in the B-XV rotor. Data on concentration vs. volume for a gradient mixer are plotted in the lower part of the figure as follows: The radius for a particular volume is found by locating the volume on the upper right-hand scale and then drawing a horizontal line to intersect the rotor volume-vs.-radius curve. A vertical line is dropped from this intersection to intersect a horizontal line indicating the sucrose concentration at that volume (or radius). In this experiment the gradient was formed from 20 and 60% (w/w) sucrose.

gradients prepared from 20 and 60% (w/w) sucrose were used. Higher concentrations will require the use of heavier pumps than have been used here. When the sample volume plus the overlay equals 200 ml, 1460 ml remains for the gradient in the B-XV rotor (6). By using a 668 ml gradient mixer, the fraction of the dense solution in the less dense when the rotor was full would be 0.89. Thus, if the gradient were constructed from 20 and 60% sucrose, the gradient in the rotor would actually extend from 20 to 55.6% (w/w) sucrose. If necessary, a dense underlay can be used to make the very end of the gradient steeper.

SHAPE OF GRADIENT IN ROTOR

The relationship between volume and radius in a zonal rotor is a complex one since the chambers are only approximately sector-shaped due to the shape of the vanes attached to the core, and the curvature of the chambers near the edge. A plot of volume as a function of radius for the B-XV rotor is shown in Figure 4 (upper curve). In the lower portion of the diagram, the curve relating radius to concentration for a 668 mi mixer is given. The vertical and horizontal lines allow concentration to be related to rotor radius in a simple graphic manner. The plot given assumes a 40 ml sample and a 200 ml overlay. An actual experimental plot is also included which was made by loading the B-XV rotor at 2500 rpm, inserting an overlay of distilled water, and then recovering the gradient by displacement. Good agreement between the theoretical and experimental plots was obtained.

Very little pressure is required to load B-XV zonal rotors, provided flow into the rotor is started at about 1000 rpm. Near the end of the loading period, additional pressure may be required. This may be produced by either raising the reservoir for dense sucrose or using low-pressure air to pressurize the reservoir.

SUMMARY

A simple gradient device is described which allows convex gradients to be formed for use in zonal centrifuge rotors. The configuration chosen produces very rapid mixing of the very viscous sucrose solutions. By varying the total volume of the mixer, the shape of the gradient may be altered within rather wide limits. With large volume mixers, gradients may be obtained which are very nearly linear with radius when placed in the sector-shaped compartments of the rotor. With smaller volume mixers, gradients that are convex with respect to radius when in position in the rotor may be generated which are more useful where high-capacity high-resolution separations are required.

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