Effects of Flow Cells on Sample Resolution
in Density Gradient Fractionation

Overview

This application note demonstrates that raising the gradient through a bulk-flow cell minimizes broadening of zones of separated particles in density gradient fractionation. Repetitive scans of the same sample showed little effect on sample resolution.

By employing bulk flow it is possible to achieve flow in which the center velocity and the edge velocity are the same, and no differential movement (laminar flow) is detected. Optimal conditions for high-resolution gradients were described by Allington, *et al.*[1] Their study demonstrated the advantages of bulk flow over laminar flow for gradient analysis.

Experiment Parameters

Bean plant ribosomal RNA is extracted using ammonium carbonate buffer at pH 9.4. Sucrose gradients are generated using the Isco Programmable Density Gradient Fractionation System. Starting gradient is 30% sucrose, ending gradient is 7.5% sucrose.

Table 1: Experiment Parameters

Instrumentation:	Isco Programmable Density Gradient Fractionation System
Wavelength:	254 nm
Mobile Phase:	7.5—30% sucrose in Ultra centrifuge tube
Flow Rate:	1 mL/min
Injection:	0.5 mL

Brakke and Van Pelt[2] demonstrated how gradient-stabilized bulk flow can be utilized. By repeatedly moving the same centrifuged gradient through a bulk-flow cell to measure the absorbance at different wavelengths, they found the mixing due to the movement of the gradient to be so low that more sensitive testing methods need be employed to estimate the small amounts of diffusion that may be occurring.

Results

The gradient was pushed upwards through the flow cell and into a reservoir tube after the scan was complete (see Figure 1). The sample was then lowered back into the centrifuge tube. There was little to no evidence of zone broadening between the initial scan and the sixth scan (see Figure 2).

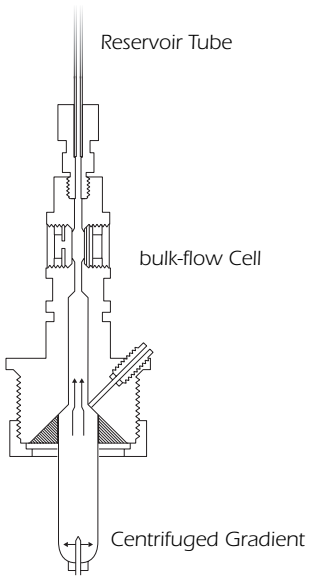

Figure 1: Gradient Flow Diagram

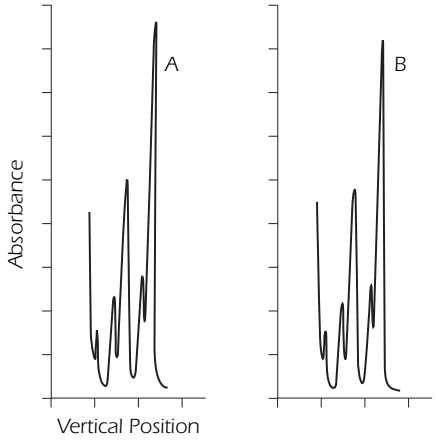

Figure 2: Example demonstrating that raising the gradient through the bulk-flow cell does not result in loss of resolution. These absorbance scans are of ribosomal RNA zones from a bean plant. A sucrose gradient tube had been centrifuged 15 hours at 30,000 rpm and 4° C. The tissue was extracted in ammonium carbonate buffer at pH 9.4. The scans were made by pumping the gradient upwards through the flow cell and into a reservoir tube. After each scan the gradient was lowered back into the centrifuge tube. A remarkable absence of zone-broadening is evident after repeated scans. A, original scan. B, sixth scan. (Courtesy M.K. Brakke.)

Figure 3 illustrates the effects of distortion of well-resolved bands using laminar flow through a 4 µl illuminated volume conventional flow cell (Case B). Identical gradient passed through a bulk-flow cell shows little distortion (Case A). Both gradients were repeatedly raised and lowered through the flow cells; each utilized a straight through design.

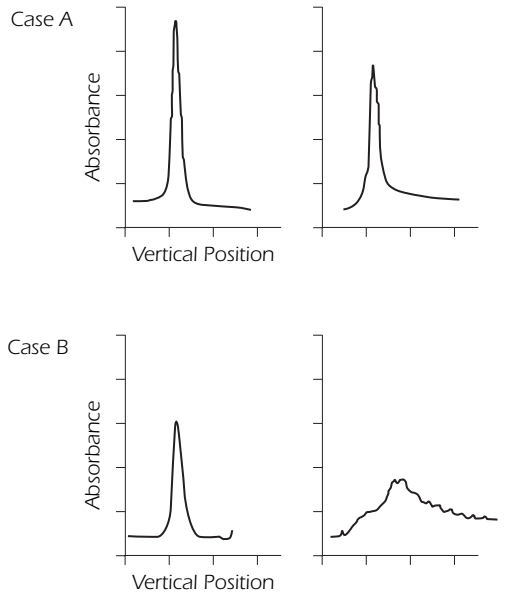

Figure 3: *Experiment comparing bulk-flow cell with a minimum volume, chromatography-type cell. Scans of identical gradients repeatedly raised and lowered through flow cells with reservoir cylinders attached to the tops. Case A, first (left and eighth (right) scans using an Isco bulk-flow cell. Illuminated volume is 14 µl. Case B, first (left) and eighth (right) scans using a 4 µl illuminated volume, straight-through (vertical) chromatography cell and small bore tubing and laminar flow significantly degrade resolution of gradients.*

Summary

Repetitive scans on a single sample have shown little to no effect on peak resolution. Initial scans using the Isco bulk-flow cell will not disturb the placement of analytes in the gradient solution.

References

1. Allington R.W., Brakke M.K., Nelson J. W., Aron C.G., and Larkins B.A. "Optimum Conditions for High Resolution Gradient Analysis." *Analytical Biochemistry* **73**, 78-92 (1976). Reprinted as Isco Applications Bulletin.

2. Brakke M. K., and Van Pelt N., "Photometric Scanning of a Centrifuged Density Gradient Column at several wavelengths." *Analytical Biochemistry* **26**, 242-250 (1968).

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