Comparison of Methods to Retrieve Samples

from Density Gradient Solutions

Overview

Various methods for the analysis of density gradient samples have been investigated, including:

- puncturing the tube from the side and using a sipper to remove the analytes from either the bottom or the top of the tube,
- sample removal by piercing the bottom of the tube and allowing the sample to drip out using gravitational forces,
- scanning the tube directly out of the centrifuge,
- piercing the bottom of the tube and injecting a chase solution.

General Method

As methods for retrieving density gradient samples from centrifuge tubes have been investigated, it has become clear that many methods had limitations due to band broadening resulting from inconsistencies in flow. Flow velocities at the center of the tube are generally greater than the flow velocities at the outer edges, generally where solutions contact sidewalls.

When tubes are pierced from the side significant limitations occur. To be able to remove the bands with this method they must be visible or diffuse light when it is placed under the tube. Brakke[1,2] investigated a similar method in which a bent hypodermic needle is lowered through the gradient to withdraw the band directly; this method is subject to the same flaws as the previous method.

Sample drip out methods have significant limitations, pelleted material tends to be carried out with the drops. Drops vary in size and frequency as the gradient level lowers in the tube. Loss of resolution has been seen and is directly correlated to non-uniform flow just above the orifice. Martin[3] found that these flaws make it difficult, if not impossible, to pinpoint closely positioned or partially separated zones.

Resolution is inferior in designs that remove gradients from the centrifuge tube using a needle or small tube. To prevent band-spreading, material must enter the needle at the same time. This is true whether the gradient is removed through the bottom of the tube or from the top, as illustrated in Figure 1.

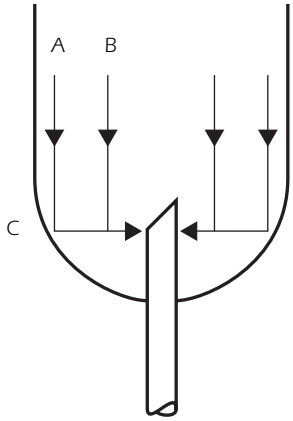

Figure 1: Non-ideal flow when removing gradient through a needle

To prevent band spreading, material from cylindrical lamina “A” must enter the needle at the same time as material from lamina “B.” In order for this to happen, lamina “A” must move down to the level of the discontinuity in the gradient at “C” (this discontinuity has been formed by partial withdrawal of the gradient), turn a right angle, and move with a higher velocity to the point of intersection of lamina “B.” Material from both laminae then rapidly proceeds together and enters the needle. Two difficulties thus arise which cause band spreading. First, in free flowing conditions, it is impossible that either of the laminae can turn a perfect right angle when changing direction from vertical to horizontal. Secondly, and more importantly, the horizontal velocity of lamina “A” after it turns the corner will cause mixing with lamina “B” because of the effect of viscous shear forces.

Morton and Hirsch[4] presented data demonstrating the inferior resolution obtained when the gradient is removed through a needle (with either upward or downward flow) instead of by displacement through a conical cap above the centrifuged tube. However, they attempted to decrease the band spreading due to laminar flow by minimizing the volume between the centrifuge tube and flow cell rather than by employing bulk flow.

Bulk or “plug” flow is achieved when a section of the density gradient is slowly raised through a relatively large (6–8 mm), vertical bore in such a way that the center velocity is the same as the edge velocity and no differential movement (laminar flow) is detectable. Refer to Figure 2.

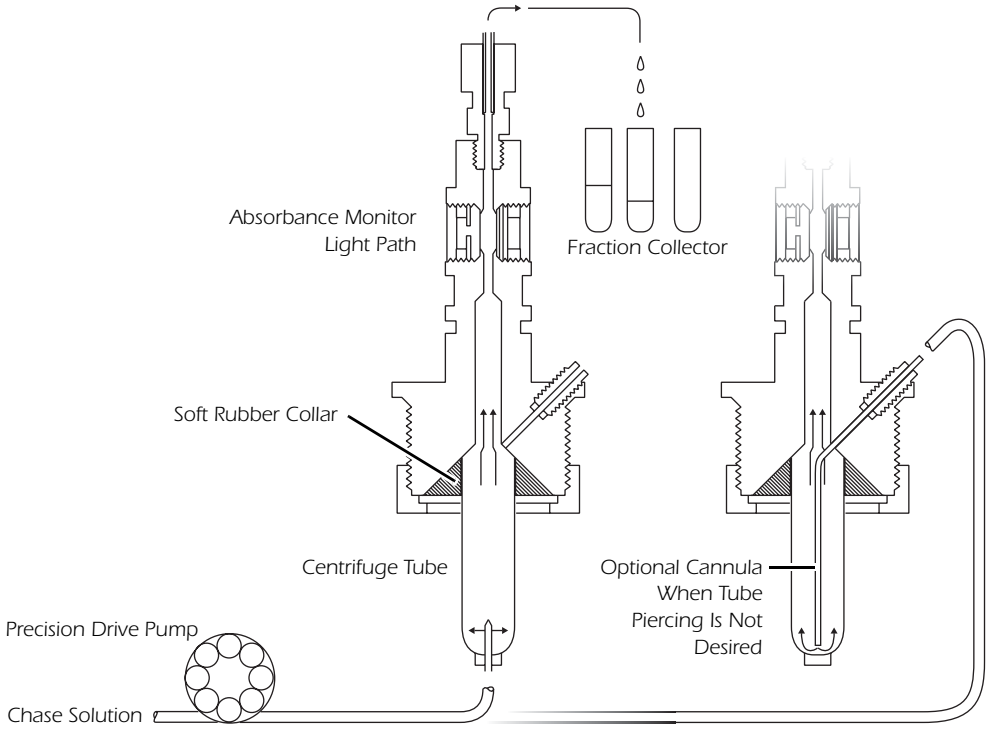

Figure 2: Raising the gradient by bulk flow through a scanning cell

Summary

By avoiding the problematic effects of laminar flow, bulk flow cells have a distinct advantage over other gradient or sample removal techniques. Bulk flow improves resolution by preserving the strata, thereby increasing the ability to isolate and analyze the samples.

References

1. Brakke, M.K., "Zonal Separations by Density-Gradient Centrifugation", *Archives of Biochemistry and Biophysics* **45**, 275-290 (1953).
2. Brakke, M.K., "Stability of Potato Yellow Dwarf Virus", *Virology* **2**, 463-476 (1956).
3. Martin, R.G., and Ames, B.N., "A Method for Determining the Sedimentation Behavior of Enzymes: Application to Protein Mixtures", *Journal of Biological Chemistry* **236**, 1372-1379 (1961).
4. Morton, B.E. and Hirsch, C.A., "A High-Resolution System for Gradient Analysis", *Analytical Biochemistry* **34**, 544-559 (1970).

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