The Production of a Linear Density Gradient by Means of a Proportioning Pump[1]

We have utilized a proportioning pump to construct a simple apparatus (Fig. 1) for making gradients of sucrose for the sedimentation of nuclei

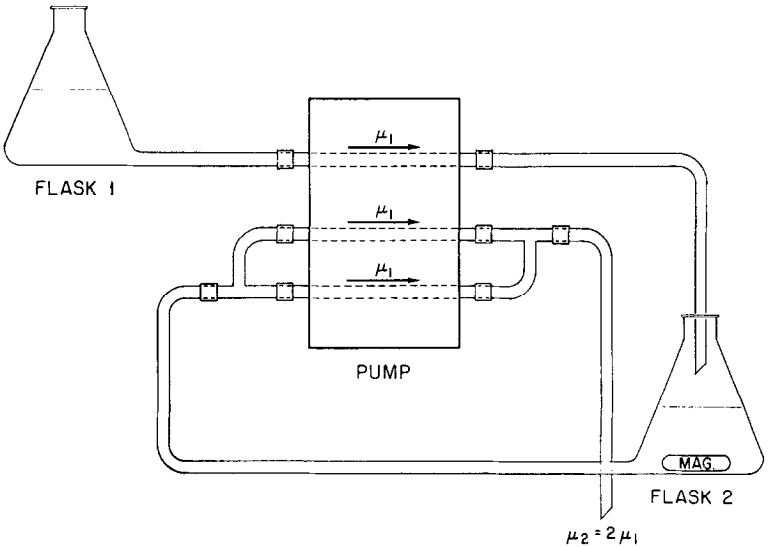

FIG. 1. A given volume of solvent of density ρ_1 is placed in flask 1, and the same volume (V_2^0) of solvent of density ρ_2^0 is placed in flask 2, and the solution is mixed with a magnetic stirrer. Three channels of an AutoAnalyzer proportioning pump[2] are fitted with clear Tygon tubing of identical diameter. Two lengths of tubing are fitted together with a type DO "h" fitting.[2] The tubing comprising μ_2 may be substituted by a single tube having a flow rate twice μ_1, as described in the text.

(1), macromolecules, etc. A gradient is produced according to the equation of Oster and Yamamoto (2):

$$\frac{\rho_2 - \rho_1}{\rho_2^0 - \rho_1} = \left[\frac{\mu_1 - \mu_2 + V_2^0}{V_2^0}\right]^{\frac{\mu_1}{\mu_2 - \mu_1}}$$

The density of the gradient produced in the mixing chamber is ρ_2 and, when $\mu_2 = 2\mu_1$ as in Fig. 1, a linear gradient is produced. In our experience, with 50 ml of 30% sucrose in flask 1, and 50 ml of 7.5% sucrose

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[2] Technicon Instruments Corp., Chauncey, New York.

in flask 2, the device produced a uniform, linear gradient. In this instance μ_1 was established by 0.045″ i.d. clear Tygon tubing (T-109),[2] and μ_2 by a single length of 0.065″ i.d. tubing (T-111)[2] which produces twice the flow rate. A 100-ml gradient is produced in about 1 hr. The gradient was tested by adding methylene blue to the reservoir solution, and connecting the outflow to the flow-through cell[3] of a Beckman DU monochromator with a Gilford recording attachment.[3] A further check was given by isopycnic sedimentation of droplets of chloroform-toluene of known density. The use of a proportioning pump appears to have several advantages over the various specialized gradient-producing devices (3). Proportioning pumps are generally available, and no additional new equipment is needed. The method is versatile: a gradient of any volume can be produced. The rate of gradient formation can be altered by changing either the diameter of tubing or the number of parallel lengths of tubing used. Some commercially available pumps have a variable speed. A variety of nonlinear gradients can be produced by varying μ_1/μ_2. The use of a pump eliminates errors in gradients found in gravity-fed devices due to differences in densities of two solutions in adjoining columns. Since the gradient is produced under pressure at a fixed flow rate, the output seems ideal for gradient column chromatography in which a fixed rate of flow through the column is desired Solvent-resistant tubing such an Acidflex[2] should be used for chloroform-methanol gradients.

REFERENCES

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