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An Inexpensive Device to Facilitate Preparing Density Gradients of Viscous Solutions for the Ultracentrifuge

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NOTE

An Inexpensive Device to Facilitate Preparing Density Gradients of Viscous Solutions for the Ultracentrifuge

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Summary

We have developed an inexpensive device which, when used with a Technicon proportioning pump, will prepare reproducible density gradients of viscous solutions.

Equilibrium ultracentrifugation in a density gradient was originally used by Meselson (*1*) for the study of macromolecules that banded at a specific density of cesium chloride solution corresponding to the density of the macromolecule. Procedures for such separations as described by Vinograd (*2*) allow the formation of the density gradient in a centrifugal field. More recently other materials such as sucrose or Ficoll have been used in such density gradients, and it is necessary to pre-form the gradient in the centrifuge tube prior to the application of sample to the top.

Those devices that form concentration gradients for various elution patterns in column chromatography (*3*, *4*) can also be used to form density gradients for the ultracentrifuge. Such devices usually incorporate two vessels connected at the bottom with a mixing device and an exit opening from one of them. If the device is formed of two cylinders of equal diameter, the gradient will be a linear one (*4*). Such

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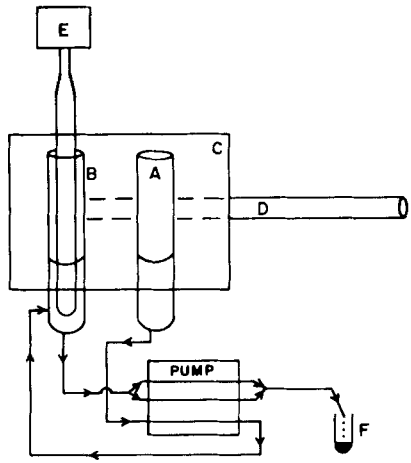

FIG. 1. A diagram of the gradient forming apparatus.

devices are commonly used in laboratories to prepare density gradients for the ultracentrifuge. Their chief disadvantage is being gravity fed. When one solution is much denser than the other, or when either solution is highly viscous, either the height of the two columns is different or the feed becomes impaired. This tends to give gradients which are either nonlinear or nonreproducible.

While fractionating subcellular components of skin we found that unusually dense and viscous solutions had to be used in the density gradient procedures that were necessary for complete separation. The densest solution we used consisted of 16% Ficoll and 1.4 *M* sucrose. The apparatus we had been using to fashion the gradients consisted of two cylindrical holes bored in a block of Lucite; the holes connected at the bottom and one cylinder was fitted with an exit tube. The cylinder fitted with the exit tube also had a mixing device in it. When the new density gradient solutions were used in this device, the gradients formed were not reproducible.

We were able to overcome the problems by fashioning a simple and inexpensive device that, used in conjunction with a Technicon pump, has produced uniform gradients which are completely reproducible and can be formed with speed.

A diagram of the complete device is shown in Fig. 1. It consists of two chambers, A and B, for holding solutions used to form the density gradient, connected through the Technicon pump by thin bore polyethylene tubing. One of the chambers is fitted with a paddle-type stirring device which gives very efficient mixing. The blade is quite

thin, but is almost as wide as the chamber. The individual chambers were formed from plastic centrifuge tubes of 15 ml volume. One centrifuge tube was cut off with a pair of scissors quite close to the bottom and pressed into the top of the other one. The two were sealed together by using a Sears Roebuck hot melt, hot glue gun which acts by melting polyethylene sticks and forcing the fluid out through a very small aperture. Small holes were punched in the bottom or side of the chambers, as shown, using hypodermic needles. Polyethylene tubing was then forced through the hole from the bottom until it came out of the top. The end was then flared by heating with a lighted match. When the flared end had cooled it was pulled down to the site of the puncture and held there tightly while the hot melt glue was cautiously

FIG. 2. A photograph of the gradient forming apparatus.

applied to the other side. Care has to be taken at this point because the polyethylene tubing will also tend to soften under heat. The tubing is arranged as shown in Fig. 1. A Technicon pump fitted with three identical pumping chambers is used. Two of these are connected in parallel and this is connected to the exit tube of the left-hand chamber B. The other is connected from the exit tube of the right hand chamber A to the input tube of the left-hand chamber. This must be done because the pumping rate out of the left-hand chamber should be twice that of the pumping rate into it. We sealed the two chambers onto a small sheet of Lucite (C) using the hot glue gun and fitted an aluminum alloy bar (D) to the Lucite so that it could be clamped onto a standard laboratory scaffolding.

When we form a linear gradient of 0.88 to 1.4 *M* sucrose and 16% Ficoll, we use the following procedure. First, 1 ml of the dense solution is pipetted into B. The pump is turned on and all the fluid is pumped out until no more emerges from the exit tube which would normally empty into a 30 cc centrifuge tube (F). This cleans out the plumbing of any previous washings and makes sure that the initial solution to pass through will be the most dense. Now 15 ml of 0.88 *M* sucrose is pipetted into A and the pump turned on. Just before the fluid is about to enter B the pump is turned off and 15 ml of the dense solution is added to B. The pump and the stirrer (E) are now turned on. The initial fluid entering the exit tube of B is now only the dense solution. As the less dense solution enters it is completely mixed, the gradient is formed continuously and pumped out into the centrifuge tube. Figure 2 is a photograph of the actual apparatus.

Acknowledgments

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