

## Analytical Techniques for Cell Fractions: I. Simplified Gradient Elution Programming

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### INTRODUCTION

Many methods have been devised for producing gradients in elution fluids for ion-exchange chromatography (1-9). While many of these are invaluable for special purposes, none appears to allow: (a) the production of a complex multicomponent gradient directly from a graphic presentation, and (b) the possibility of changing from gradient to stepwise elution with the same system. A simple method for producing complex gradients or stepwise elutions is described.

### METHOD

The device used is shown schematically in Fig. 1. Identical glass balls are connected through 1-mm i.d. capillary tubing to a common line that leads to a mixer and a chromatographic pump. Sufficient Tygon tubing is allowed between the vertical and horizontal capillaries for clamps (indicated in Fig. 1 by X's) used during filling. The vessels are open to air from above through capillary tubing. If different solutions (lettered *a* to *e*) are placed in the glass balls, virtually pure solution *a* will be pumped as the level of fluid falls from 0 to 0.5, indicated by the broken lines in Fig. 1A. Between level 0.5 and 1.5 the composition of the stream entering the mixing chamber will change smoothly from that of solution *a* to that of *b*. When the solution level is at the center of a given ball, the composition of the solution entering the mixing chamber will be essentially that of the particular ball.

As the composition changes between two ball centers, the gradient observed will not be linear but will be slightly sigmoidal according to the equation:

$$C = \frac{2Rh - h^2}{R^2 + 2Rh - 2h^2} \times 100 \quad (1)$$

<sup>1</sup> Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.

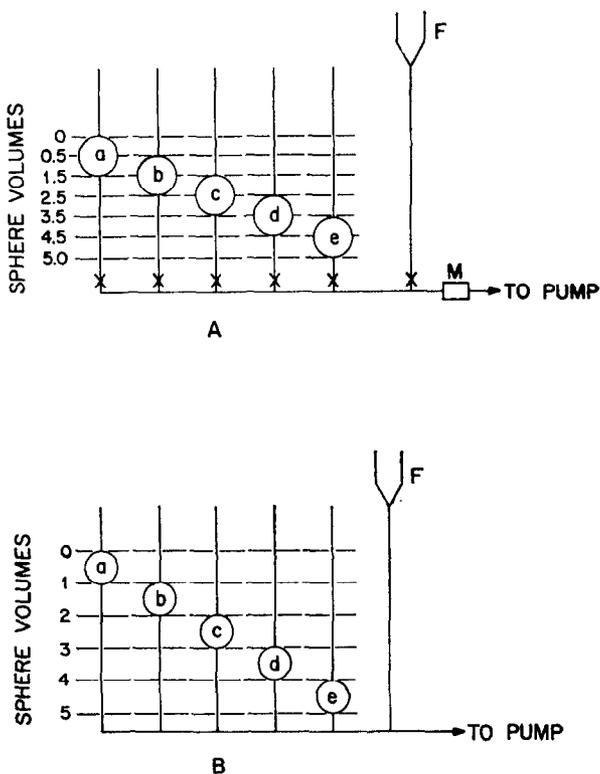


FIG. 1. (A) Schematic diagram of gradient-producing system consisting of five bulbs (*a-e*), filling funnel *F*, and mixing vessel *M*. (B) Schematic diagram of five-bulb system arranged for stepwise elution using five buffers. The composition of effluent fluid flowing into the mixer at the levels indicated by the broken lines is shown in Fig. 2.

where *C* is the per cent of buffer from the second of the two balls in question, *h* the distance from the center of the first ball (or the top of the second), and *R* the radius of the ball. To plot concentration as a function of volume, the volume may be calculated from the formula:

$$V = \pi(Rh^2 + R^2h - 2/3h^3) \quad (2)$$

This equation describes the volume between the centers of two consecutive balls. The volume to the center of the first sphere is  $2/3\pi R^3$ , and the volume to the center of any given ball *N* is  $4/3\pi R^3(N - 1/2)$ .

It is evident that, if a gradient can be represented graphically with concentration or pH plotted along the ordinate and the volume along the abscissa, then the solutions to be used will be those having compositions corresponding to the volumes equal to  $(V/2) + V(N - 1)$ , where

$N$  is the number of a given ball. This is illustrated in Fig. 2A, where the vertical broken lines indicate the pH and ionic strength of solutions placed in similarly lettered spheres in Fig. 1A. The gradient can include reversals in slope (if this should prove useful) and alterations in pH

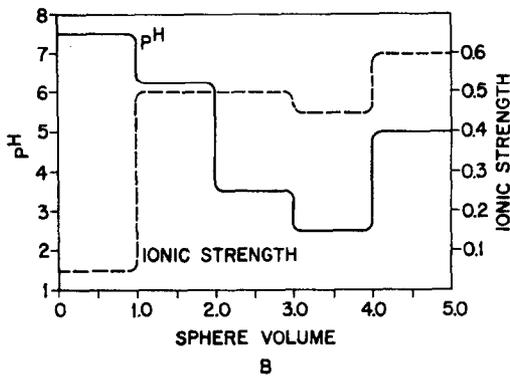
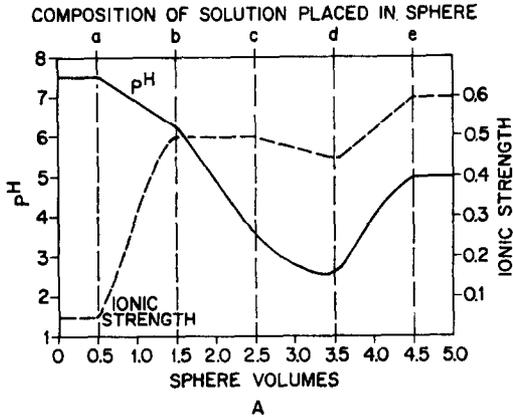


FIG. 2. (A) Hypothetical gradient illustrating (1) independent manipulation of two variables, in this case pH and ionic strength, and (2) reversible variation of one or two variables, i.e., the pH and (or) ionic strength may, for example, increase, decrease, and then increase. Letters at the top of vertical broken lines correspond to liquid level positions shown in Fig. 1. (B) Hypothetical step elution curve resulting when spheres and solutions used in Fig. 1A are changed to the configuration shown in Fig. 1B.

and ionic strength (or composition), which may be in opposite directions in one part of a gradient and in the same direction in another part.

To fill the apparatus all clamps except those on ball  $e$  and on the loading funnel are closed and ball  $e$  and its capillary tubing are filled to level 0. The tubing below ball  $e$  is then clamped, and the funnel and

tubing into ball *e* drained through mixing flask *M*, and rinsed with the solution to be placed in flask *d*, which is then filled. In like manner all remaining balls are filled with appropriate solutions.

To produce discontinuous step gradients, the balls are spaced as shown in Fig. 2B, with the top of each ball slightly below the bottom of its im-

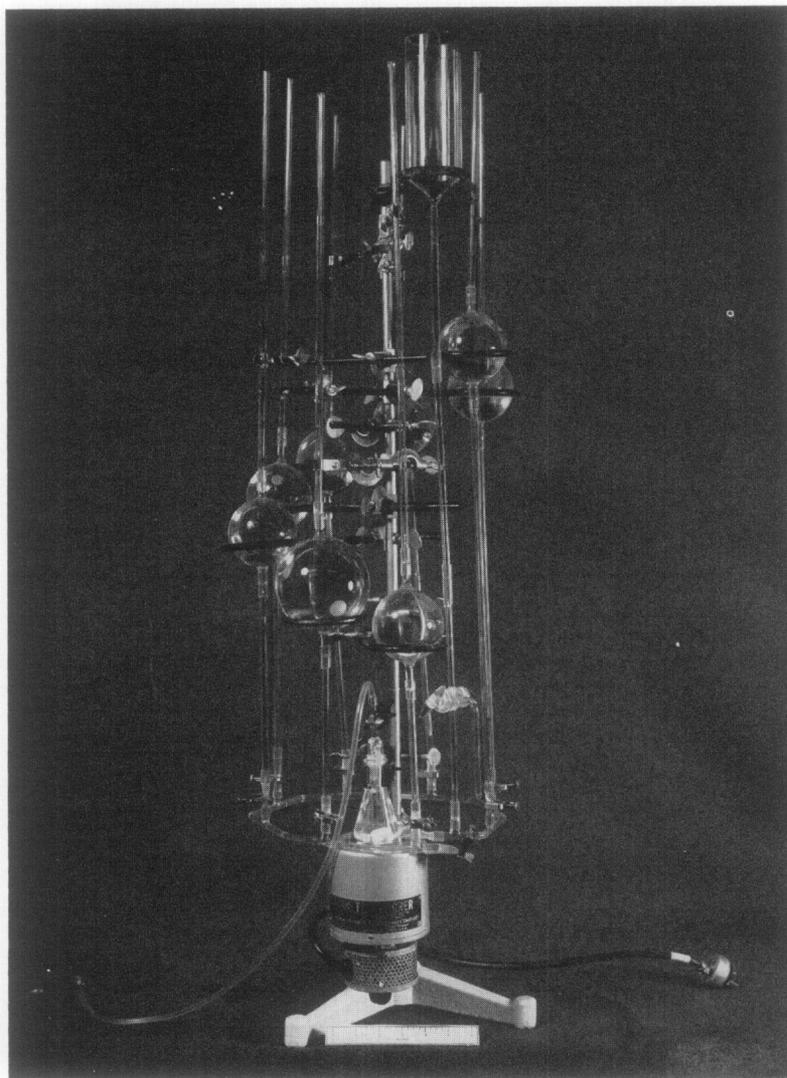


FIG. 3. Eight-sphere gradient arranged around central support used for elution of rat liver-soluble proteins from DEAE (Anderson and Bond, in preparation). All parts are glass except for short plastic interconnections.

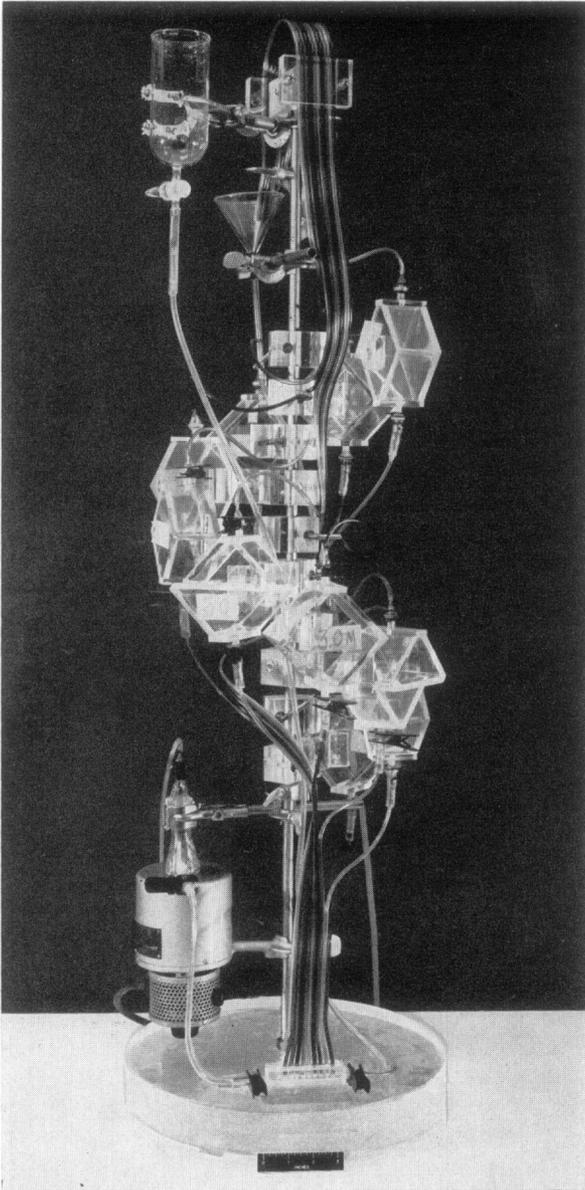


FIG. 4. Gradient system using rectangular Lucite boxes to achieve a completely linear gradient. Variation in capacity is achieved by changing the length of the boxes while keeping the vertical cross section constant. When the capacity of the boxes is to be changed, boxes having upper or lower halves of different length are used.

mediate predecessor. If the same solutions used in Fig. 2A are used, the step elution pattern shown in Fig. 2B will result.

A gradient system using glass balls, a circular glass manifold, glass capillary connecting tubing, and Tygon tubing joints is shown in Fig. 3. Leakage due to density inversions may be prevented by constructing mixing manifolds in such a manner that they are always fed from below.

To obtain approximately linear gradation from one solution to another, a system having rectangular plastic chambers is used (Fig. 4). Connections are made through plastic tubing<sup>2</sup> to a small suitably drilled Lucite block that connects with the mixing chamber.

The volume of the mixer used affects the shape of the gradient, a negligible effect being observed when the volume of the mixer is small in proportion to the volume of a sphere or rectangular chamber. While large density differences may occur between the beginning and the end of the gradient, the difference in density between adjacent vessels is generally small and may be neglected.

#### DISCUSSION

The art of chromatography is not sufficiently advanced to allow one to predict the exact shape and composition of an elution gradient suitable for separating a complex mixture. In practice, therefore, what is required is a method for producing gradients that is both flexible and reproducible. In our experience, it is often necessary to make small changes in several parts of a gradient to separate closely spaced components, or to move widely spaced ones together. This requires that portions of the gradient be manipulated independently. In the present system, for example, the gradient can be flattened at any point by inserting an extra ball containing fluid of the same composition as the part of the gradient to be extended.

By merely changing the levels of the balls so that the bottom of each is above the top of its successor, the gradient can be changed to a step elution system. By suitable arrangements of level, both step elution and gradient elution can be included in the same chromatograph. Vessels may be arranged to provide not only an elution gradient, but the complete column regeneration and equilibration cycle as well.

It should be emphasized that very small amounts of fluid from the capillary lines attached to either full or empty balls drain into the mixing chamber. This amount is proportional to the meniscus area in each ball or line. In actual practice where very large changes in pH or

<sup>2</sup> Strip-A-Tube, SAT/V/903/1, color coded, available from Jessall Plastic Co., Kensington, Connecticut.

ionic strength occur, we find it convenient to monitor continuously the pH and conductivity of the gradient.

#### SUMMARY

A simple system, based on a series of vessels arranged so that the top of each is at the midlevel of its predecessor, and all draining by gravity into a common line, has been shown to be capable of producing very complex, predictable gradients. By simply changing the levels, so that the top of each chamber is below the level of its predecessor, a step elution system may be obtained.

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