

NOMOGRAMS FOR GRADIENT ELUTION CHROMATOGRAPHY

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Gradient elution chromatography, as reported by ALM *et al.*¹ and DONALDSON *et al.*² has provided new facilities for the separation of mixtures and is widely used. The concentration, ionic strength, or other characteristics of the eluting agent, are continually changing in the course of the experiment. The change is usually nonlinear and the determination of the particular characteristic at a given moment of the experiment, for instance at the peak, is difficult. The theoretical approach to this problem^{1,3,4} has led to equations that make possible the computation of the relationship between the characteristics studied and the volume of the eluting agent, under given experimental conditions and in given solutions. This elaborate computation has to be repeated after every change in the working conditions.

In this paper it is shown that some relations can be replaced directly by nomograms. However, in some cases the quantities that are necessary for drafting the nomogram have to be obtained from auxiliary nomograms. The application of nomograms is always time-saving and facilitates the choice of optimal experimental conditions.

This paper deals with the gradients of concentration and ionic strength, the latter being proportional to the concentration at constant pH value.

THEORETICAL

I. A single closed mixing chamber

This simple case is widely used although it gives a less advantageous convex gradient. The equation^{1,4,5} giving the dependence of the concentration on the volume of the eluting agent may be written as follows:

$$\frac{c_1 - c}{c_1 - c_2} = e^{-v/V_2} \quad (1)$$

and transformed to a determinant

$$\begin{vmatrix} 0 & \alpha c_1 & 1 \\ \delta & \alpha c_2 & 1 \\ \frac{1}{D} [\alpha \delta (e^{-v/V_2} - 1)] & \frac{1}{D} [-\alpha \beta c] & 1 \end{vmatrix} = 0$$

where: $D = (\alpha - \beta)e^{-v/V_2} - \alpha$, c_1 is the concentration of the agent in the reservoir, c_2 the initial concentration of the agent in the mixing chamber, V_2 the volume of the mixing chamber, c the concentration and v the volume of the agent flowing into the column. α, β, δ are scale moduli, conveniently chosen as 10, 10, and 20 cm, respectively. By comparing this determinant with a general one

$$\begin{vmatrix} \xi_1 & \eta_1 & 1 \\ \xi_2 & \eta_2 & 1 \\ \xi & \eta & 1 \end{vmatrix}$$

the design equations can be given. For example, the design equation for c_1 is derived from the first row of the determinant: $\xi_1 = 0, \eta_1 = 10c_1$. The scale for c_2 can be computed from the second row. The third row contains the quantity c as well as the ratio v/V_2 and is represented by a network, situated between the scales c_1 and c_2 .

The nomogram is represented in Fig. 1. It is used as follows: given values for the initial concentration in the reservoir, c_1 , and in the mixing chamber, c_2 , are connected by a straight line. The intersection of this straight line with a vertical line cutting the corresponding mark on the scale for v/V_2 gives the actual concentration. When repeating a series of experiments with identical starting conditions, the connecting line between c_1 and c_2 is drawn in pencil.

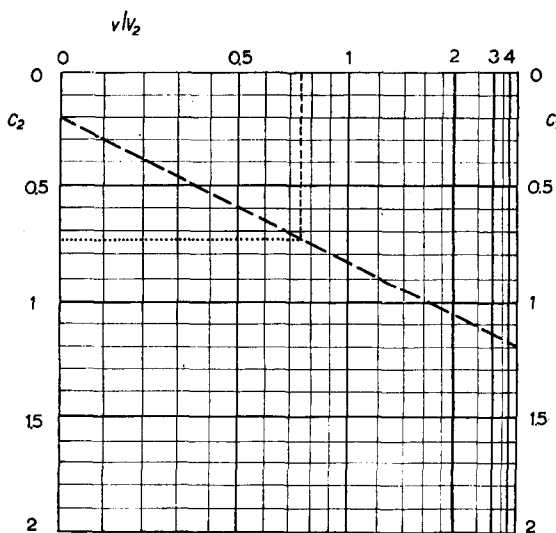


Fig. 1. Nomogram for a closed mixing chamber.

As an example, the computation of one case with starting conditions $c_1 = 1.2 M$, $c_2 = 0.2 M$ and $v/V_2 = 0.75$, is given in Fig. 1. The result is read on the vertical scale as $c = 0.73 M$.

II. Reservoir and mixing chamber as communicating vessels

(a) *The difference in the densities is small.* For this relatively simple and universal method of forming a gradient, equations have been derived⁵, that are similar in

form to eqn. (1) for a closed mixing chamber. With cylindrical vessels we have

$$\frac{c_1 - c}{c_1 - c_2} = \left(1 - \frac{v}{V_t}\right)^\kappa \tag{2}$$

where V_t is the sum of the starting volumes of reservoir and mixing chamber and $\kappa = q_1/q_2$ is the ratio of the cross-sections of both vessels. Compared with eqn. (1),

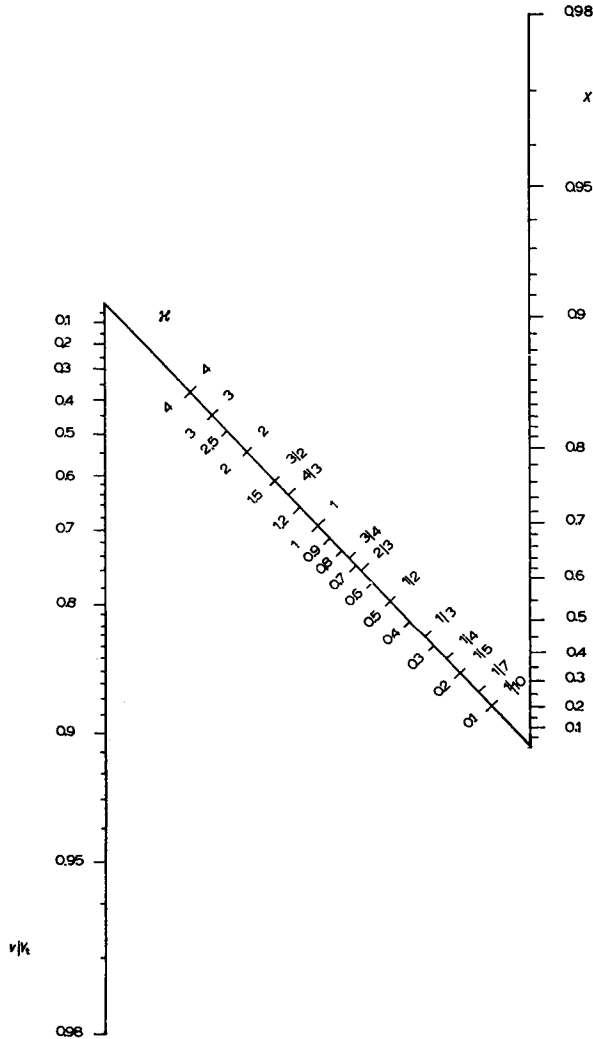


Fig. 2. Nomogram for computing the function $\kappa = 1 - (1 - v/V_t)^\kappa$.

eqn. (2) differs in the function of volume. Hence the computation of the nomogram is the same, except that the horizontal scale for v/V_t is different. Let this scale be denoted as x and calibrated uniformly from zero to 1 in the direction of the scale from c_1 to c_2 .

The nomogram in Fig. 2 gives the relation of x and v/V_t and κ . The value of κ for

a given experimental arrangement, read on the inclined scale, is connected with the particular value for v/V_t by a straight line. When extended, this line, subtends the corresponding value for v/V_t on the scale x . Fig. 3 is an auxiliary diagram for marking the scale v/V_t with whole tenths. On a strip of paper placed horizontally at the height

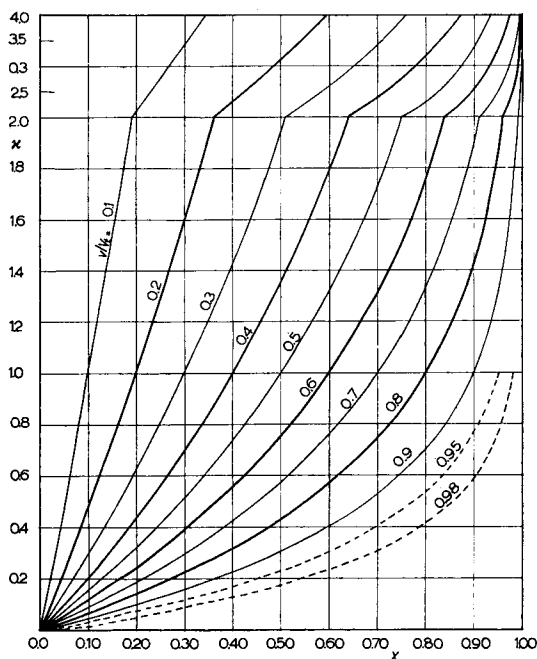


Fig. 3. Graph of the function $x = 1 - (1 - v/V_t)^x$ for definite values of v/V_t .

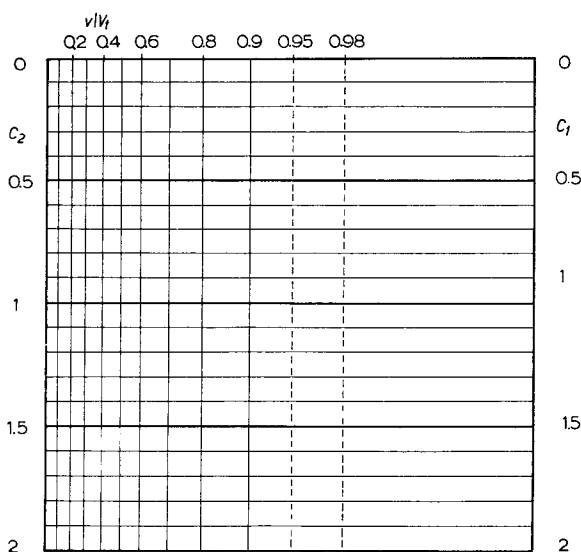


Fig. 4. Nomogram for communicating vessels. Ratio of cross-sections 1:4. The solutions in both vessels are of the same density.

of the corresponding value for κ are drawn the intersections with the curved scales for particular values of v/V_t . From this auxiliary strip the scale is transferred to the nomogram. Fig. 4 is an example of this technique for $\kappa = 1/4$.

The nomogram is used in the same way as the nomogram in Fig. 1.

(b) *The densities differ widely.* If the solution in the reservoir is fairly concentrated, then after it has communicated with the dilute solutions in the mixing chamber, the level in the reservoir will be lower than in the mixing chamber, owing to the difference in density of the liquids in the two vessels. Consequently, the relative volume of the more concentrated solution flowing into the mixing chamber will be smaller. During the course of the experiment the densities approach each other and the relative volume of the solution flowing out of the reservoir converges to the value κ . Let us compute the relation between the volume and the concentration of the solution flowing out for the latter case.

Let c_1 be the initial concentration in the reservoir, c_2 the initial concentration in the mixing chamber, V_t the sum of the initial volumes in the reservoir and in the mixing chamber, s_1 and s_2 the initial densities of both solutions, v the total volume of agent that has flowed out into the column, c its concentration, and s its density. For the actual volumes v_1 in the reservoir and v_2 in the mixing chamber we have

$$v_1 + v_2 = V_t - v$$

and

$$v_1 : v_2 = \frac{q_1}{s_1} : \frac{q_2}{s} = \kappa : \frac{s_1}{s}$$

Whence

$$v_1 = \frac{\kappa}{\kappa + s_1/s} (V_t - v), \quad v_2 = \frac{s_1/s}{\kappa + s_1/s} (V_t - v), \quad dv_1 = -\frac{\kappa \cdot dv}{\kappa + s_1/s}$$

The change of concentration of the solution in the mixing chamber may be represented by a differential equation

$$dc = \frac{c_1 - c}{v_2} (-dv_1)$$

Substitution gives

$$\frac{dc}{(c_1 - c) \cdot s/s_1} = \frac{\kappa \cdot dv}{V_t - v}$$

In order to integrate this equation the density is expressed as a linear function of the concentration $s = 1 + kc$. This equation is almost exactly satisfied.

After substitution the following differential equation is obtained

$$\frac{dc}{c_1 - c} + \frac{k \cdot dc}{1 + kc} = \frac{\kappa \cdot dv}{V_t - v}$$

which can be integrated. For the starting conditions $v = 0$, $c = c_2$ we have

$$\frac{c_1 - c}{c_1 - c_2} \cdot \frac{1 + kc_2}{1 + kc} = \left(1 - \frac{v}{V_t}\right)^\kappa$$

or after introducing the densities

$$\frac{c_1 - c}{c_1 - c_2} \cdot \frac{s_2}{s} = \left(1 - \frac{v}{V_t}\right)^\alpha \tag{3}$$

This equation differs from eqn. (2) merely by the factor s_2/s on the left-hand side. In order to transform this equation into a nomogram let the expression on the right-hand side of the equation be designed as x . Then we transform to the determinant

$$\begin{vmatrix} 0 & \alpha c_1 & 1 \\ \beta s_2 & \alpha c_2 & 1 \\ \beta s x & x c & 1 \end{vmatrix} = 0$$

The scale is marked by means of the design equations, the resulting nomogram being of the same type as that in Fig. 5. It is a deformed nomogram like that in Fig. 4. The scale c_1 is identical to that in Fig. 4, while the scale for c_2 is in general curved,

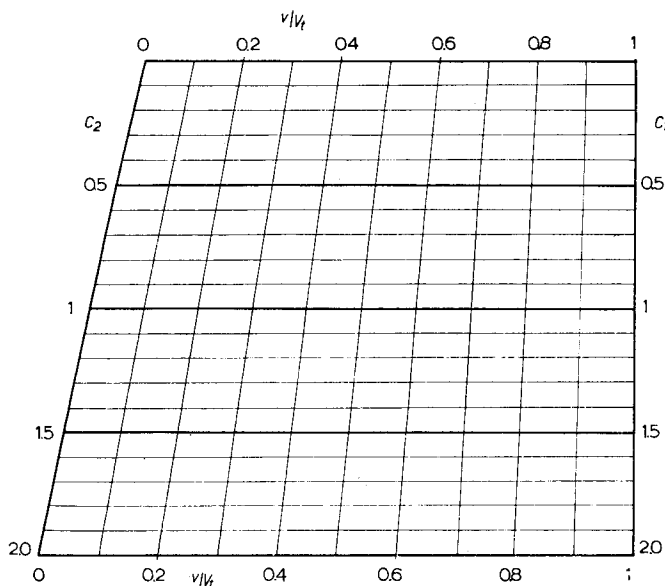


Fig. 5. Nomogram for communicating vessels with the same cross-section, containing phosphate buffer solutions of different densities.

but almost linear. At every point of the scale c_2 the distance from the scale c_1 is proportional to the density corresponding to the concentration c_2 . The binary scale for c and v/V_t is given by a network, the inclined lines of which are geometrically similar to the scale c_2 . The similarity constant x is read either from the nomogram in Fig. 2 or from the diagram in Fig. 3.

The nomogram must be constructed, not only for every new value of the ratio x (*i.e.* when changing the experimental arrangement), but also when a new buffer is used, since in general the dependence of the density on the concentration changes.

So far as these dependences are linear or at least mutually proportional a correction factor for the concentration scale may be introduced and the nomogram may be used for minor series.

III. The reservoir and mixing chamber communicate by means of a micropump

If the reservoir and the mixing chamber communicate by means of micropumps, then neither the density of the solutions nor the form of the vessels are of consequence and it is only important to maintain a uniform velocity of flow. Let r_1 be the velocity of the liquid flowing out of the reservoir into the mixing chamber, r_2 the velocity of the liquid flowing out of the mixing chamber into the column, and ρ the ratio of these velocities. The relationship between ρ and κ can be expressed as follows

$$\rho = \frac{r_1}{r_2}, \quad \kappa = \frac{\rho}{1 - \rho}$$

For the concentration of the solution flowing out of the mixing chamber at a given moment, eqn. (2) holds. By introducing directly measurable quantities the equation is transformed to

$$\frac{c_1 - c}{c_1 - c_2} = \left[1 - (1 - \rho) \frac{v}{V_2} \right] \rho / (1 - \rho) \quad (4)$$

where V_2 is the initial volume of the liquid in the mixing chamber.

The concentration c may be determined from the nomogram in Fig. 4. In order to draft this nomogram it is necessary to compute the quantity κ from the above equation and V_t from the formula $V_t = V_2 / (1 - \rho)$. The nomogram in Fig. 6 is, however, more convenient. By using micropumps the ratio ρ can be easily changed and hence the network of perpendicular lines for the volume function is not drawn. The quantity κ for given values of ρ and v/V_2 is read in the annexed auxiliary diagram and then transferred to the network.

An example, with starting conditions $c_1 = 1.2 M$, $c_2 = 0.2 M$, $\rho = 0.25$, $V_2 = 200$ ml, is given in Fig. 6 with broken lines. For $v = 150$ ml and 220 ml respectively, the concentration $c = 0.46$ and 0.66 respectively results.

IV. Comments

1. When determining the volume v it is necessary to subtract the volume of the column and tubing between the mixing chamber and column from the volume that has flowed out of the column since the beginning of the gradient elution.

2. The computation of the relative volume is facilitated by choosing a simple value for the volume in the denominator of the fraction. In practice this can be very easily done for the cases treated in Sections I and III, *i.e.* in eqns. (1) and (4).

3. The volume V_t in eqns. (2) and (3) is correct only in the case when the bottom of the reservoir is exactly at the same level as the bottom of the mixing chamber. Since this condition is by no means necessary for the production of a given gradient,

it is not necessary to observe it when assembling the apparatus. The quantity V_t is computed from the formula

$$V_t = V_2 \left(1 + \kappa \frac{s_2}{s_1} \right)$$

where V_2 is the initial volume of the solution in the mixing chamber.

4. Nomograms can also be conveniently used in the preparation of phosphate buffer solutions of a given ionic strength^{6,7}.

EXPERIMENTAL

Experiments for the verification of eqn. (3) were performed in an apparatus similar to that reported by ŠEBESTA AND SCHNEIDER⁸. We do not recommend effecting the communication of the reservoir with the mixing chamber by means of a siphon, for this deforms the course of the gradient, especially at the beginning of the experiment.

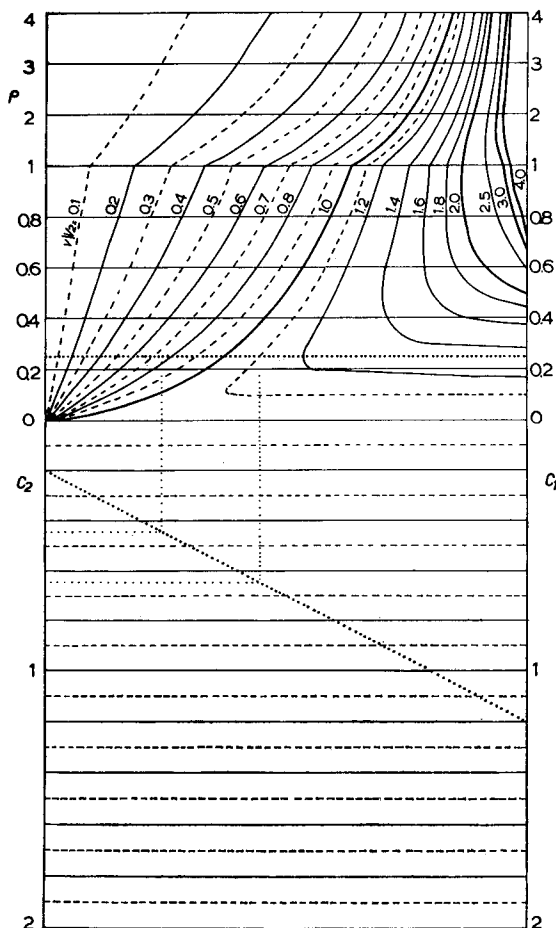


Fig. 6. Nomogram for vessels connected by means of a micropump.

The reservoir was filled with a 2 M phosphate buffer solution, coloured with acid fuchsine, and the mixing chamber was filled with a 0.1 M buffer solution prepared by diluting the first mentioned buffer. The concentration of the effluent buffer solution was determined photometrically using a calibration curve. There was good agreement between the experimental and the calculated values of the concentration (Fig. 7).

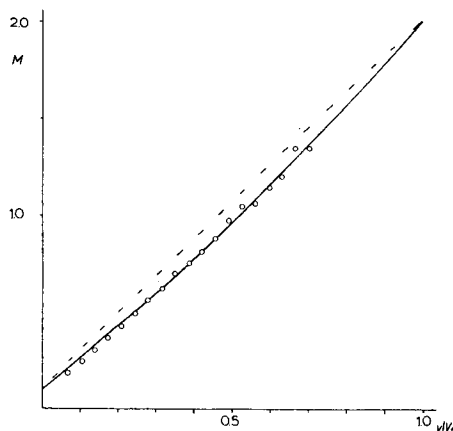


Fig. 7. Verification of the nomogram in Fig. 5. — theoretical values; - - - theoretical values when the differences in the densities are neglected; o = experimental readings.

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SUMMARY

Nomograms are suggested that facilitate the determination of the concentration of the eluates at a given moment in gradient elution chromatography. Among the various experimental arrangements the following were considered: a single mixing chamber, a reservoir and a mixing chamber connected by means of a micropump, and a reservoir connected with a mixing chamber as communicating vessels. In the latter case the relationship between volume and concentration with respect to density differences was established.

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