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GENERATION OF NON-LINEAR pH GRADIENTS FOR CHROMATOGRAPHY BY USING ONLY SIMPLE APPARATUS

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SUMMARY

An improved theory is presented for predicting the solute gradients that can be generated with vessels of variable or constant cross-sectional area together with flexible connecting tubing. Formulae are given for two possible arrangements using four such vessels and, where there is the restriction of identical starting volumes, equations are given for n compartments. The general principles behind the use of these equations for predicting pH gradients are considered, and the special case of two vessels is shown to be capable of generating eight different pH profiles as a function of volume delivered. Experiments are reported which support the usefulness of the theoretical treatment.

INTRODUCTION

In a previous paper¹ we discussed the general principles underlying the generation of solute gradients using only a series of vessels and a peristaltic pump. We mentioned that the concentration *versus* time profile could be calculated assuming that the pump delivered at a constant rate, and that this could then be expressed as a function of volume delivered. The present study arose out of the need to use a pump of variable flow-rate or even to dispense with a pump altogether and also from the requirement to calculate pH gradients for use in ion-exchange chromatography. In this paper we present a simpler theory of solute gradients which only considers the concentration profile as a function of volume delivered. Then we extend the theory to the prediction of pH gradients.

THE GENERAL THEORY OF GRADIENTS POSSIBLE WITH ALL VESSELS OPEN TO THE ATMOSPHERE AND OF CONSTANT CROSS-SECTIONAL AREA

Consider a series of open vessels connected as in Fig. 1a. There is a starting vessel, 0, followed by n further vessels, all well-stirred so that mixing is instantaneous. Hence as long as the flow-rate is sufficiently small we have no need to consider time as an independent variable, and the vessels may be drained by gravi-

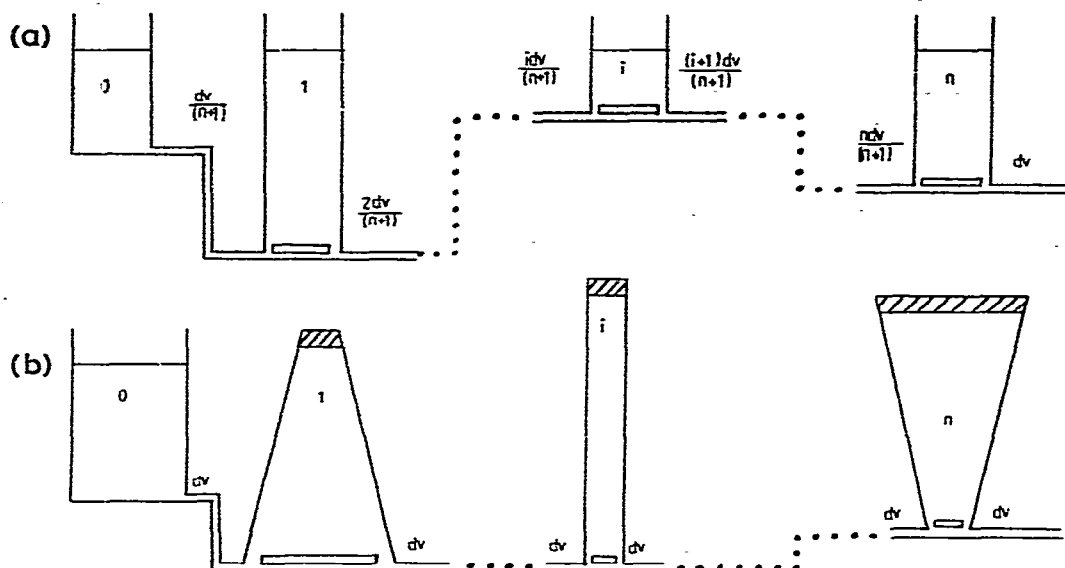


Fig. 1. The assemblies of vessels used to generate solute and pH gradients. Vessel number 0 is always open to the atmosphere and is not stirred. Vessels number 1, 2, ..., n are well stirred by magnetic followers. The fluid constituting the gradient may flow from vessel n under gravity or may be controlled by a pump. The flow-rate is irrelevant within reason, *i.e.*, providing that mixing is instantaneous in all vessels. The gradient may be stopped at any point and restarted or the flow-rate may be altered as required since the equations given only consider the volume delivered from vessel number n . (a) All open vessels. In this arrangement, all of the vessels have the same cross-sectional area and are at constant hydrostatic pressure. Clips preventing flow between vessels are removed at the commencement of the experiment. As a volume element dv flows out of vessel n , a volume element $idv/(n+1)$ flows into vessel i and an element $(i+1)dv/(n+1)$ flows out. The net flow is thus $-dv/(n+1)$ for each vessel. The gradient will continue until that vessel containing the least starting volume is exhausted, *i.e.* the valid experimental range is $0 \leq v \leq (n+1) \min(V_{i0}, V_{j0})$. (b) Closed vessels. In this arrangement, the vessels $i = 1, 2, \dots, n$ are closed and so $V_i(v) = V_{i0}$, $i = 1, 2, \dots, n$. Because of this, the shape of vessels $i = 1, 2, \dots, n$ and the volumes contained are irrelevant. As a volume element dv is removed from vessel number n , a volume element dv transferred from number 0 into number 1. Each vessel gains dv and loses dv and the gradient is realised until vessel number 0 empties, *i.e.* $0 \leq v \leq V_{00}$. The calculation can then be continued using C_1 ($v = V_{00}$) as the concentration in a new starting vessel and using the appropriate set of equations for one fewer compartment. In this method of generating gradients, it is advisable to reduce the dead volume of air as much as possible by having vessels 1, 2, ..., n filled.

tational flow or by an intermittent or variable-speed pump. Let the mass of solute in the i th compartment be M_i and the volume be V_i and consider the element of mass dM_i added to the compartment when an element of volume dv flows out of vessel n . This is

$$dM_i = \frac{i M_{i-1}}{(n+1) V_{i-1}} dv - \frac{(i+1) M_i}{(n+1) V_i} dv$$

or

$$\frac{dM_i}{dv} = (iC_{i-1} - (i+1)C_i)/(n+1)$$

where

$$C_i = M_i/V_i$$

Now since

$$\frac{dV_i}{dv} = -1/(n + 1)$$

then

$$V_i = V_{i0} - v/(n + 1)$$

where V_{i0} is the volume in vessel i at $v = 0$, *i.e.* the start of the gradient. The problem is now to derive a general formula for $C_i(v)$, the concentration of solute in the i th compartment, as a function of volume delivered, v . To do this we note that

$$\begin{aligned} \frac{dC_i}{dv} &= \left(\frac{dM_i}{dv} V_i - M_i \frac{dV_i}{dv} \right) / V_i^2 \\ &= i(C_{i-1} - C_i) / [(n + 1) V_{i0} - v] \end{aligned}$$

using the previous expressions for C_i , V_i , dM_i/dv and dV_i/dv .

It is now clear that to obtain the required expression for $C_i(v)$ we have to solve the first-order differential equation

$$\frac{dC_i}{dv} + iC_i / [(n + 1) V_{i0} - v] = iC_{i-1} / [(n + 1) V_{i0} - v]$$

with integrating factor $[(n + 1) V_{i0} - v]^{-i}$. In order to integrate the equation to obtain $C_n(v)$, the gradient applied to the column, we need the expression for $C_{n-1}(v)$ and so we simply have to integrate systematically the expressions for $C_i(v)$, $i = 1, 2, \dots, n$.

THE SPECIAL CASE OF FOUR OPEN VESSELS

To explain the general technique we systematically solve the equations for $C_i(v)$, $i = 1, 2, 3$. This will illustrate the general procedure to be followed if more compartments are necessary and will lead to formulae that should cover most cases required in practice.

For $i = 1$ we have

$$\frac{dC_1}{dv} + C_1 / [(n + 1) V_{10} - v] = C_{00} / [(n + 1) V_{10} - v]$$

leading to

$$C_1(v) = C_{10} + (C_{00} - C_{10}) v / (n + 1) V_{10}$$

where C_{i0} is the concentration of solute in the i th compartment when $v = 0$, i.e. $C_{i0} = C_i(0)$ and of course $C_0(v)$ is constant at $C_{00} = C_0(v)$ for the duration of the experiment.

For $i = 2$ we have

$$\frac{dC_2}{dv} + 2C_2/[(n+1)V_{20} - v] =$$

$$2(C_{10} + (C_{00} - C_{10})v/(n+1)V_{10})/[(n+1)V_{20} - v]$$

leading to

$$\frac{C_2}{[(n+1)V_{20} - v]^2} = 2 \int \left\{ \frac{C_{10} + (C_{00} - C_{10})V_{20}/V_{10}}{[(n+1)V_{20} - v]^3} + \frac{(C_{10} - C_{00})}{(n+1)V_{10}[(n+1)V_{20} - v]^2} \right\} dv$$

and giving the final expression

$$C_2(v) = C_{20} + 2(C_{10} - C_{20})v/(n+1)V_{20} + (C_{20} - C_{10} + (C_{00} - C_{10})V_{20}/V_{10})(v/(n+1)V_{20})^2.$$

For $i = 3$ we have, after some algebra,

$$\frac{dC_3}{dv} + 3C_3/[(n+1)V_{30} - v] =$$

$$3\{\alpha + \beta[(n+1)V_{30} - v] + \gamma[(n+1)V_{30} - v]^2\}/[(n+1)V_{30} - v]$$

where

$$\alpha = C_{20} + 2(C_{10} - C_{20})V_{30}/V_{20} + (C_{20} - C_{10} + (C_{00} - C_{10})V_{20}/V_{10})(V_{30}/V_{20})^2$$

$$\beta = 2[C_{20} - C_{10} + (C_{20} - C_{10} + (C_{00} - C_{10})V_{20}/V_{10})V_{30}/V_{20}]/(n+1)V_{20}$$

and

$$\gamma = (C_{20} - C_{10} + (C_{00} - C_{10})V_{20}/V_{10})/(n+1)^2V_{20}^2$$

After using the integrating factor we find

$$\frac{C_3}{[(n+1)V_{30}-v]^3} = 3 \left\{ \left\{ \frac{\alpha}{[(n+1)V_{30}-v]^3} + \frac{\beta}{[(n+1)V_{30}-v]^2} + \frac{\gamma}{[(n+1)V_{30}-v]} \right\} dv \right. \\ \left. = \frac{\alpha}{[(n+1)V_{30}-v]^3} + \frac{3\beta/2}{[(n+1)V_{30}-v]^2} + \frac{3\gamma}{[(n+1)V_{30}-v]} + \delta \right.$$

and the integration constant, δ , can be calculated from

$$C_{30} = \alpha + 3(n+1)V_{30}\beta/2 + 3(n+1)^2V_{30}^2\gamma + (n+1)^3V_{30}^3\delta$$

to be given by the expression

$$\delta = [C_{30} - C_{20} + (C_{10} - C_{20})V_{30}/V_{20} + \\ (C_{10} - C_{20} + (C_{10} - C_{00})V_{20}/V_{10})(V_{30}/V_{20})^2]/(n+1)^3V_{30}^3.$$

So the final equation for $C_3(v)$ is.

$$C_3(v) = C_{30} + 3(C_{20} - C_{30})(v/(n+1)V_{30}) \\ + 3(C_{30} - C_{20} + (C_{10} - C_{20})V_{30}/V_{20})(v/(n+1)V_{30})^2 \\ + [C_{20} - C_{30} - (C_{10} - C_{20})V_{30}/V_{20} \\ + (C_{20} - C_{10} + (C_{00} - C_{10})V_{20}/V_{10})(V_{30}/V_{20})^2](v/(n+1)V_{30})^3$$

Note that these equations for $C_1(v)$ and $C_2(v)$ differ from those given previously¹ since, in the former paper, the symbol q was mistakenly used in some places to represent the flow out of compartment number zero, *i.e.* $q/(n+1)$, rather than the rate of delivery of the pump. The equations become consistent on replacing q by $q/(n+1)$ in the previous paper in the equations for $C_1(t)$, $C_2(t)$ and t on page 992 and that for v by $v/(n+1)$ in the legend to Figure 3 on page 995.

Finally we emphasise that this theory is only valid for vessels of constant identical cross-sectional area with all compartments at the same hydrostatic pressure. The equations are collected together in the Appendix for convenience; there are no special problems when $V_{i0} = V_{j0}$ for any $i \neq j$. The integrated solutions are valid in the interval

$$0 \leq v \leq (n+1)V_{j0}, V_{j0} \leq V_{i0}, i = 1, 2, \dots, n.$$

THE SPECIAL CASE WHEN ALL n OPEN VESSELS HAVE THE SAME VOLUME

It is evident from the symmetry involved in these calculations that a general formula can be given for $C_n(v)$ in terms of the C_{i0} and V_{i0} . However, this formula takes a very simple form when all the starting volumes are identical, say $V_{i0} = V$, $i =$

1, 2, ..., n . This is a situation of considerable experimental value and the general formula is

$$C_n(v) = C_{n,0} - \binom{n}{1} (C_{n,0} - C_{n-1,0}) (v/(n+1) V) \\ + \binom{n}{2} (C_{n,0} - 2C_{n-1,0} + C_{n-2,0}) (v/(n+1) V)^2 \\ - \binom{n}{3} (C_{n,0} - 3C_{n-1,0} + 3C_{n-2,0} - C_{n-3,0}) (v/(n+1) V)^3 + \dots$$

Using the substitution $v/(n+1)V = w$ the formula may also be written as

$$C_n(w) = C_{n,0}(1-w)^n + \binom{n}{1} C_{n-1,0} w(1-w)^{n-1} \\ + \binom{n}{2} C_{n-2,0} w^2(1-w)^{n-2} + \binom{n}{3} C_{n-3,0} w^3(1-w)^{n-3} + \dots + C_{00} w^n$$

To prove this we observe that the general differential equation relating dC_k/dv , C_k and C_{k-1} , $k = 2, 3, \dots, n$, is

$$\frac{(1-w)}{k} \frac{dC_k}{dw} + C_k = C_{k-1}$$

Now for $C_k(w)$ defined by

$$C_k(w) = \sum_{i=0}^k \binom{k}{i} C_{k-i,0} w^i (1-w)^{k-i}$$

it follows that

$$\frac{(1-w)}{k} \frac{dC_k}{dw} + C_k = \frac{(1-w)}{k} \sum_{i=0}^k \binom{k}{i} C_{k-i,0} \times \\ [i w^{i-1} (1-w)^{k-1-i} - k w^i (1-w)^{k-1-i}] + \sum_{i=0}^k \binom{k}{i} C_{k-i,0} w^i (1-w)^{k-1-i} \\ = \sum_{i=1}^k \binom{k}{i} C_{k-i,0} (i/k) w^{i-1} (1-w)^{k-i} \\ = \sum_{j=0}^{k-1} \binom{k-1}{j} C_{k-1-j,0} w^j (1-w)^{k-1-j} = C_{k-1}$$

THE GENERAL THEORY OF GRADIENTS POSSIBLE WITH ONE OPEN VESSEL AND ALL OTHER COMPARTMENTS CLOSED TO THE ATMOSPHERE

Consider the arrangement depicted in Fig. 1(b), where vessel number zero is open and vessels $i = 1, 2, \dots, n$ are closed. For $i \neq 0$ the element of mass dM_i added to the i th compartment during the transfer of an element of volume dv is given by

$$dM_i = (C_{i-1} - C_i) dv$$

and since

$$\frac{dV_i}{dv} = 0$$

then

$$\frac{dC_i}{dv} = (C_{i-1} - C_i)/V_i$$

and it follows that the general differential equation to be solved is

$$\frac{dC_i}{dv} + C_i/V_{i0} = C_{i-1}/V_{i0}$$

This time, since the volumes V_i are constant for $i \neq 0$, we have a linear system of simultaneous differential equations to solve, namely

$$\begin{bmatrix} \frac{dC_1}{dv} \\ \frac{dC_2}{dv} \\ \frac{dC_3}{dv} \\ \dots \\ \frac{dC_n}{dv} \end{bmatrix} = \begin{bmatrix} -1/V_{10} & 0 & 0 & \dots & 0 \\ 1/V_{20} & -1/V_{20} & 0 & \dots & 0 \\ 0 & 1/V_{30} & -1/V_{30} & \dots & 0 \\ \dots & \dots & \dots & \dots & \dots \\ 0 & 0 & 0 & \dots & -1/V_{n0} \end{bmatrix} \begin{bmatrix} C_1 \\ C_2 \\ C_3 \\ \dots \\ C_n \end{bmatrix} + \begin{bmatrix} C_{00}/V_{10} \\ 0 \\ 0 \\ \dots \\ 0 \end{bmatrix}$$

with characteristic equation:

$$|A - \lambda I| = (-1)^n \prod_{i=1}^n (1/V_{i0} + \lambda)$$

THE SPECIAL CASE OF FOUR COMPARTMENTS, THREE BEING CLOSED

We presume first that none of the three closed vessels contain equal volumes *i.e.* $V_{i0} \neq V_{j0}$, $i \neq j$ and note that this leads to three distinct eigenvalues. Calculating the eigenvectors corresponding to these eigenvalues and adding a particular integral to the complementary function, we obtain the general solution

$$\begin{bmatrix} C_1 \\ C_2 \\ C_3 \end{bmatrix} = \begin{bmatrix} 1 \\ \frac{V_{10}}{V_{10} - V_{20}} \\ \frac{V_{10}^2}{(V_{10} - V_{20})(V_{10} - V_{30})} \end{bmatrix} (A + f)e^{-v/V_{10}} + \begin{bmatrix} 0 \\ 1 \\ \frac{V_{20}}{V_{20} - V_{30}} \end{bmatrix} (B + g)e^{-v/V_{10}} + \begin{bmatrix} 0 \\ 0 \\ 1 \end{bmatrix} (C + h)e^{-v/V_{30}}$$

where A , B and C are arbitrary constants and f , g and h are functions of v . Now we use the variation of parameters technique to evaluate f , g and h which must satisfy the system

$$\begin{bmatrix} 1 \\ \frac{V_{10}}{V_{10} - V_{20}} \\ \frac{V_{10}^2}{(V_{10} - V_{20})(V_{10} - V_{30})} \end{bmatrix} f e^{-v/V_{10}} + \begin{bmatrix} 0 \\ 1 \\ \frac{V_{20}}{V_{20} - V_{30}} \end{bmatrix} g e^{-v/V_{10}} + \begin{bmatrix} 0 \\ 0 \\ 1 \end{bmatrix} h e^{-v/V_{30}} = \begin{bmatrix} C_{00}/V_{10} \\ 0 \\ 0 \end{bmatrix}$$

Performing the integration leads to the following equations:

$$f = C_{00} e^{v/V_{10}}$$

$$g = [C_{00} V_{20} / (V_{20} - V_{10})] e^{v/V_{10}}$$

$$h = [C_{00} V_{30}^2 / (V_{10} - V_{30})(V_{20} - V_{30})] e^{v/V_{30}}$$

and it now only remains to evaluate A , B and C . This can be done since $v = 0$ requires that

$$\begin{bmatrix} C_{10} \\ C_{20} \\ C_{30} \end{bmatrix} = \begin{bmatrix} 1 \\ \frac{V_{10}}{V_{10} - V_{20}} \\ \frac{V_{10}^2}{(V_{10} - V_{20})(V_{10} - V_{30})} \end{bmatrix} A + \begin{bmatrix} 0 \\ 1 \\ \frac{V_{20}}{V_{20} - V_{30}} \end{bmatrix} B + \begin{bmatrix} 0 \\ 0 \\ 1 \end{bmatrix} C + \begin{bmatrix} 1 \\ 1 \\ 1 \end{bmatrix} C_{00}$$

We find the expressions for A , B and C to be

$$A = C_{10} - C_{00}$$

$$B = C_{20} + (C_{00}V_{20} - C_{10}V_{10})/(V_{10} - V_{20})$$

$$C = C_{30} - C_{20}V_{20}/(V_{20} - V_{30}) + (C_{10}V_{10} - C_{00}V_{30})V_{30}/(V_{10} - V_{30})(V_{20} - V_{30})$$

and this completes the integration for $C_i(v)$ using up to four compartments. The general solution for n compartments can be obtained by symmetry from the equations just given for four compartments. However, a complication arises when any of the starting volumes are equal since this leads to repeated eigenvalues. As this complication would often occur in the laboratory, we now deal with the case $V_{10} = V_{j0}$ for all i, j .

THE SPECIAL CASE WHEN THE n CLOSED VESSELS HAVE THE SAME VOLUME

It would often be convenient to the experimentalist to have a series of closed vessels of the same volume, V , and, in this situation, the general formula for $C_n(v)$ is quite simple. In fact

$$C_n(v) = C_{00} + \{(C_{n0} - C_{00}) + (C_{n-1,0} - C_{00})(v/V) + [(C_{n-2,0} - C_{00})/2](v/V)^2 + \dots + [(C_{10} - C_{00})/(n-1)!](v/V)^{n-1}\}e^{-v/V}$$

To see this we recall the fact that the recurrence relationship is

$$V \frac{dC_n}{dv} + C_n = C_{n-1}$$

and use $0! = 1! = 1$.

Now for $k = 2, 3, \dots, n$ the general formula

$$C_k(v) = C_{00} + e^{-v/V} \sum_{i=0}^{k-1} [(C_{k-i,0} - C_{00})/i!](v/V)^i$$

leads to

$$V \frac{dC_k}{dv} = e^{-v/V} \left\{ \sum_{i=0}^{k-2} [(C_{k-i-1,0} - C_{00})/i!](v/V)^i - \sum_{i=0}^{k-1} [(C_{k-i,0} - C_{00})/i!](v/V)^i \right\}$$

and so

$$V \frac{dC_k}{dv} + C_k = C_{00} + e^{-v/V} \sum_{i=0}^{k-2} [(C_{k-i-1,0} - C_{00})/i!](v/V)^i = C_{k-1}$$

as required.

THE GENERAL PRINCIPLES UNDERLYING THE CALCULATION OF pH GRADIENTS

The equations given previously all refer to the concentration of solute emerging from the n th vessel, i.e., C_n as a function of the volume delivered to the column. We showed earlier (Solano-Muñoz and Bardsley, 1981) that the gradients realised experimentally are insensitive to specific-gravity effects even up to molarities of around unity. Therefore, if the starting vessels contain several different, non-interacting solutes, it is possible to calculate the profiles for individual components. Suppose, for instance, that the vessels contained solutes S_1, S_2, \dots, S_j and there were n vessels plus a starting vessel, then the equations describing the concentration of S_1, S_2, \dots, S_j would be $C_n^{S_1}(v), C_n^{S_2}(v), \dots, C_n^{S_j}(v)$ as long as S_1, S_2, \dots, S_j did not react chemically. This is exactly the situation with buffers. Although ionic equilibria are set up, we can treat each ionising solute as if it were an independent component. To be more specific, consider the case of sodium acetate and acetic acid. Suppose that each vessel had a certain molarity, C_i^S , of sodium acetate and another molarity, C_i^A , of acetic acid, then we could calculate the pH in vessel i using the well-known approximation

$$\text{pH}(v) = \text{p}K_a + \log_{10} (C_i^S/C_i^A)$$

to give the pH in the i th vessel as a function of the volume delivered. This follows since, although the salt and acid ionise, conservation of mass and electrical neutrality mean that we can always use $C_i^S(v)$ and $C_i^A(v)$ to calculate the sodium acetate and acetic acid concentrations and hence the pH in the i th vessel as v varies. There is no objection in principle to using the more exact equations for pH or calculating the pH

by this method for a mixture of buffer salts. The only question is whether the extra labour is justified, for instance if a pH range of more than two units, one either side of the pK_a , is required. When this approach is used to calculate $pH(v)$ we can not only predict the gradient but, by analysis of dpH/dv and d^2pH/dv^2 we can classify the graphs of $pH(v)$ that are possible for any given arrangement. We now do this for a polynomial and exponential solute gradient.

THE SPECIAL CASE OF pH GRADIENTS GENERATED BY TWO OPEN VESSELS OF IDENTICAL CROSS-SECTIONAL AREA

Suppose that there are two vessels in all as in Fig. 1a with $i = 1$. If we adopt the convention that the concentrations of salt at $v = 0$ are given by

$$C_{00}^S = S_0$$

$$C_{10}^S = S_1$$

while those of acid at $v = 0$ are defined to be

$$C_{00}^A = A_0$$

$$C_1^{A0} = A_1$$

then the expression for the pH gradient emerging from vessel number one as a function of the volume, v , delivered to the column is

$$pH(v) = pK_a + \log_{10} \left\{ \frac{2S_1V_{10} + (S_0 - S_1)v}{2A_1V_{10} + (A_0 - A_1)v} \right\}$$

In order to analyse the $pH(v)$ profiles we need the expressions for the derivatives which are

$$\frac{dpH}{dv} = \frac{2V_{10} (A_1S_0 - A_0S_1)}{2 \cdot 303(2A_1V_{10} + (A_0 - A_1)v) (2S_1V_{10} + (S_0 - S_1)v)}$$

$$\frac{d^2pH}{dv^2} = \frac{-4V_{10}(A_1S_0 - A_0S_1) [V_{10}(A_0S_1 + A_1S_0 - 2A_1S_1) + (A_0 - A_1)(S_0 - S_1)v]}{2 \cdot 303(2A_1V_{10} + (A_0 - A_1)v)^2 (2S_1V_{10} + (S_0 - S_1)v)^2}$$

Note that there can be no turning points but

$$A_1S_0 - A_0S_1 > 0 \Leftrightarrow dpH/dv > 0$$

$$A_1S_0 - A_0S_1 = 0 \Leftrightarrow dpH/dv = 0$$

$$A_1S_0 - A_0S_1 < 0 \Leftrightarrow dpH/dv < 0$$

Just one inflexion is possible when

$$(A_0 S_1 + A_1 S_0 - 2A_1 S_1)(A_0 - A_1)(S_0 - S_1) < 0$$

but this can only be realised at $v = v_I$ if

$$v_I = \frac{V_{10}(2A_1 S_1 - A_1 S_0 - A_0 S_1)}{(A_0 - A_1)(S_0 - S_1)} < 2 \min(V_{00}, V_{10})$$

where $\min(V_{00}, V_{10})$ is the smallest starting volume of V_{00} and V_{10} . Hence, not counting the trivial case $A_1 S_0 - A_0 S_1 = 0$, there are just eight gradients possible. These are shown in Fig. 2, and the signs of the expressions required to classify these pH profiles are summarised in the legend.

THE SPECIAL CASE OF pH GRADIENTS GENERATED BY TWO VESSELS, ONE BEING CLOSED TO THE ATMOSPHERE

Using the arrangement of Fig. 1(b) with two vessels in all, the pH profile is given by

$$\text{pH}(v) = \text{p}K_a + \log_{10} \left\{ \frac{S_0 + (S_1 - S_0)e^{-v/V_{10}}}{A_0 + (A_1 + A_0)e^{-v/V_{10}}} \right\}$$

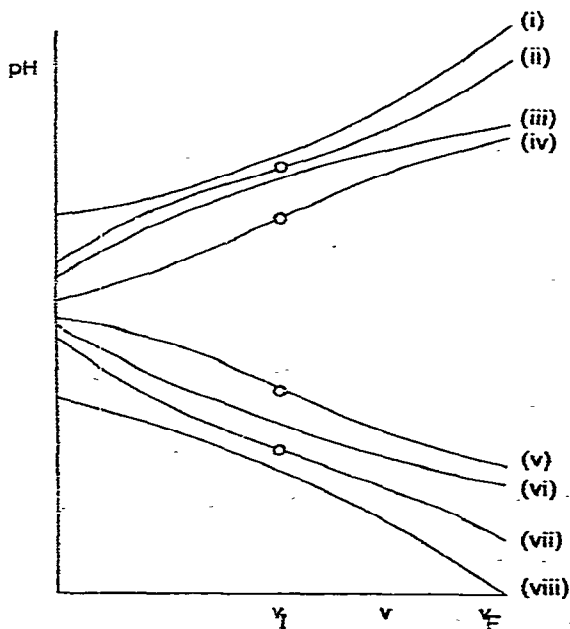


Fig. 2

Fig. 2. pH gradients possible with just two vessels. Concentrations of salt and acid in the two vessels at $v = 0$ are S_0, S_1, A_1, A_0 ; the starting volumes are V_{00} and V_{10} . The eight pH(v) profiles labelled (i)–(viii) are possible with both vessels open or one open and one closed. (a) Inequalities involved with both vessels open. Classification of the gradients according to the arrangement of Fig. 1(a) requires the sign of the following four functions:

$$\begin{aligned}\varepsilon_1 &= A_1 S_0 - A_0 S_1 \\ \varepsilon_2 &= A_1 S_0 + A_0 S_1 - 2A_1 S_1 \\ \varepsilon_3 &= (A_0 - A_1)(S_0 - S_1) \\ \varepsilon_4 &= 2 \min(V_{00}, V_{10}) - V_{10} (2A_1 S_1 - A_0 S_1 - A_1 S_0) / (A_0 - A_1)(S_0 - S_1)\end{aligned}$$

The equation of the gradient is

$$\text{pH}(v) = \text{p}K_a + \log_{10} [(2S_1 V_{10} + (S_0 - S_1)v) / (2A_1 V_{10} + (A_0 - A_1)v)]$$

and the appropriate signs are now listed:

Shape	ε_1	ε_2	ε_3	ε_4
(i)	+	–	+	–
(ii)	+	–	–	+ or –
(iii)	+	+	–	–
(iv)	+	+	+	+ or –
(v)	–	–	+	+
(vi)	–	+	+	+ or –
(vii)	–	+	–	–
(viii)	–	–	–	+ or –
	–	–	+	–

The end of the gradient occurs at $v_F = 2 \min(V_{00}, V_{10})$ and the inflexion point, where present, occurs at $v_I = V_{10}(2A_1 S_1 - A_0 S_1 - A_1 S_0) / (A_0 - A_1)(S_0 - S_1)$. (b) Inequalities involved with one vessel closed. Classification of the gradient according to the arrangement of Fig. 1b requires the sign of the following four functions:

$$\begin{aligned}\theta_1 &= A_1 S_0 - A_0 S_1 \\ \theta_2 &= A_0 S_0 - (A_0 - A_1)(S_0 - S_1) \\ \theta_3 &= (A_0 - A_1)(S_0 - S_1) \\ \theta_4 &= 2V_{00}/V_{10} - \ln [(A_0 - A_1)(S_0 - S_1) / A_0 S_0]\end{aligned}$$

The equation of the gradient is

$$\text{pH}(v) = \text{p}K_a + \log_{10} [(S_0 + (S_1 - S_0)e^{-v/V_{10}}) / (A_0 + (A_1 - A_0)e^{-v/V_{10}})]$$

and the appropriate signs are now listed:

Shape	θ_1	θ_2	θ_3	θ_4
(i)	+	–	+	–
(ii)	+	+	+	+
(iii)	+	+	+	–
(iv)	+	+	–	not defined
(v)	+	–	+	+
(vi)	–	+	+	–
(vii)	–	+	–	not defined
(viii)	–	–	+	+

The end-point of the gradient occurs at $v_F = V_{00}$ and the inflexion point, where present, occurs at $v_I = (V_{10}/2) \ln [(A_0 - A_1)(S_0 - S_1) / A_0 S_0]$

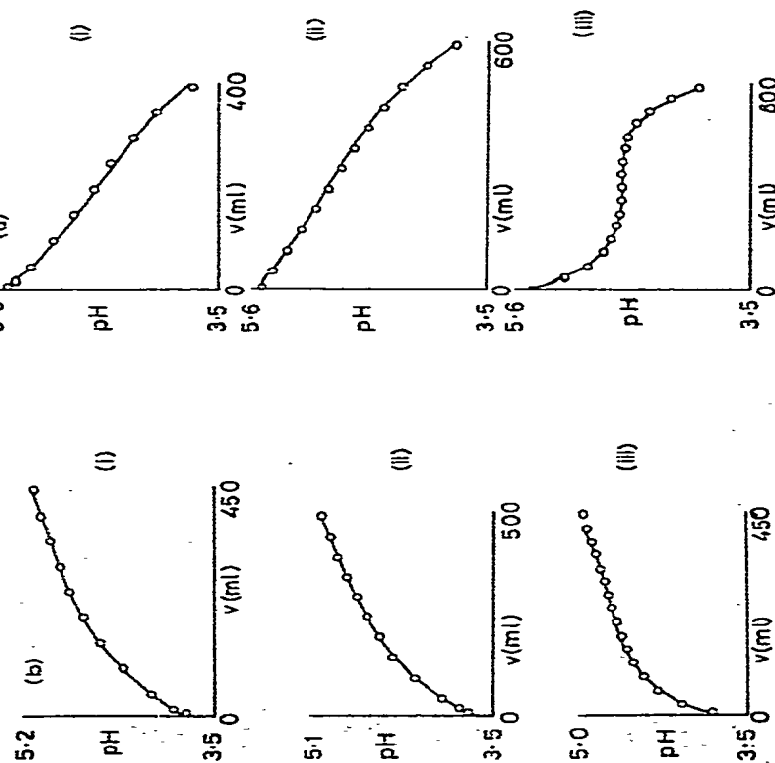


Fig. 3. Experimentally generated pH gradients. The pH(v) profiles illustrated were obtained with a flow-rate of 25 ml/h using vessels of 35 mm diameter. Flexible connecting tubes were used and these had a diameter of 4 mm each containing a plug of 1 mm I.D. to act as a flow restrictor. The solid lines represent profiles calculated using the equations listed in the Appendix as discussed in the text, i.e. $\text{pH}(v) = \text{p}K_a + \log_{10} (C_n^s(v)/C_n^A(v))$, where $C_n^s(v)$ is the molarity of sodium acetate in vessel n and $C_n^A(v)$ is the molarity of acetic acid in vessel n and $\text{p}K_a$ was taken to be 4.65. The symbols (O) represent experimental points. (a) All vessels were open to the atmosphere as shown in Fig. 1a; (b) n vessels were closed to the atmosphere as shown in Fig. 1b. Details are now listed.

Diagram	n	Concentrations of sodium acetate/acetic acid (M/M)			Volumes (ml)			
		C_{10}	C_{20}	C_{30}	V_{00}	V_{10}	V_{20}	V_{30}
(a)(i)	1	0.01/0.09	0.0805/0.0115	—	200	200	—	—
(a)(ii)	2	0.01/0.09	0.0645/0.0355	—	200	200	200	—
(a)(iii)	3	0.01/0.09	0.0885/0.0115	0.0885/0.0115	200	200	200	200
(b)(i)	1	0.0885/0.0115	0.01/0.09	—	450	250	—	—
(b)(ii)	2	0.0885/0.0115	0.0645/0.0355	—	500	250	250	—
(b)(iii)	3	0.0885/0.0115	0.0885/0.0115	0.01/0.09	750	250	250	250

and the first and second derivatives are

$$\frac{dpH}{dv} = \frac{(A_1 S_0 - A_0 S_1) e^{-v/V_{10}}}{2 \cdot 303 V_{10} (A_0 + (A_1 - A_0) e^{-v/V_{10}}) (S_0 + (S_1 - S_0) e^{-v/V_{10}})}$$

$$\frac{d^2pH}{dv^2} = \frac{-(A_1 S_0 - A_0 S_1) e^{-v/V_{10}} [A_0 S_0 - (A_0 - A_1) (S_0 - S_1) e^{-2v/V_{10}}]}{2 \cdot 303 V_{10}^2 (A_0 + (A_1 - A_0) e^{-v/V_{10}})^2 (S_0 + (S_1 - S_0) e^{-v/V_{10}})^2}$$

As with the open vessels there are no turning points and the sign of the first derivative depends only on the sign of $A_1 S_0 - A_0 S_1$. An inflexion can occur at a positive zero, $v = v_I$, of

$$A_0 S_0 - (A_0 - A_1) (S_0 - S_1) e^{-2v_I/V_{10}} = 0$$

providing this can be realised experimentally, *i.e.*

$$v_I = (V_{10}/2) \ln [(A_0 - A_1) (S_0 - S_1)/A_0 S_0] < V_{00}$$

The eight possible shapes for $pH(v)$ are shown in Fig. 2. They are the same shapes as those possible for open compartments except that different inequalities are involved, as shown in the legend to Fig. 2.

THE EXPERIMENTAL TESTING OF PREDICTED GRADIENTS

Any theoretical studies of the type presented in this paper should be tested to determine the validity of the equations given. Since a large number of pH profiles are possible even with only a few compartments, it was not thought profitable to generate gradients of all possible types. Instead, we decided to generate typical gradients using two, three and four vessels, *i.e.* $n = 1, 2$ and 3 , for the situations where either all of the vessels were open to the atmosphere or n were closed. We generated gradients using sodium acetate and acetic acid and the results are to be seen in Fig. 3. Details of the solutions and apparatus used are to be found in the legend to the figure and the equations used are to be found in the Appendix.

In Fig. 3a are the results for (i) $n = 1$, (ii) $n = 2$ and (iii) $n = 3$, all vessels being open to the atmosphere and giving a descending pH gradient covering the range of one pH unit each side of the pK_a value. Similarly, Fig. 3(b) shows the results for (i) $n = 1$, (ii) $n = 2$ and (iii) $n = 3$, with n vessels closed, the concentrations being arranged so that the gradient increases. The agreement between the calculated $pH(v)$ points (solid line) and experimental points (O) is excellent in all cases.

CONCLUSIONS

It is valuable to know what solute and pH gradients are possible using only containers, connecting tubes and magnetic stirrers. In this paper and the previous one¹ we have calculated the equations necessary to predict such gradients under the assumption that mixing is instantaneous in each vessel and neglecting gravitational

effects. The experimental findings are that, under the sort of flow-rates required for ion-exchange chromatography, these equations are extremely accurate. It is reasonable to end by giving some guide as to the procedure to be adopted by an experimentalist who wished to generate a particular pH gradient.

Analysis of the equations for predicting pH(v) gradients when $n > 1$ show that there is a very large number of possible pH(v) shapes including profiles with several turning points and inflexions. It is likely that $n = 2$ or 3 and open containers would be sufficient for most requirements, a typical gradient being that of Fig. 3a (iii). The experimentalist wishing to generate such a gradient would have to draw a sketch of the required gradient spanning a range of up to one pH unit either side of the pK_a value of the selected buffer. A number of such idealised pairs of coordinates would then be chosen sufficient to obtain enough numerical sets of pH and v values to solve for the coefficients of the pH(v) equations appropriate for the system selected. The equations necessary for this purpose are collected together in the Appendix.

APPENDIX: EQUATIONS USED FOR PREDICTING SOLUTE AND pH GRADIENTS

Symbols: v = volume delivered to the column from vessel number n . $C_i(v)$ = concentration of solute in the i th vessel. $C_{i0} = C_i(0)$, i.e. the concentration in the i th vessel when $v = 0$. $V_i(v)$ = volume of solution in the i th vessel. $V_{i0} = V_i(0)$, i.e. the volume in the i th vessel when $v = 0$.

Assembly as in Fig. 1a, $n + 1$ open compartments

Valid for $0 \leq v \leq (n + 1)$ smallest V_{i0}

Vessel 0, $C_0(v) = C_{00}$, a constant

Vessel 1, $C_1(v) = C_{10} + (C_{00} - C_{10})(v/(n + 1)V_{10})$

Vessel 2, $C_2(v) = C_{20} + 2(C_{10} - C_{20})(v/(n + 1)V_{20}) + (C_{20} - C_{10} + (C_{00} - C_{10})V_{20}/V_{10})(v/(n + 1)V_{20})^2$

Vessel 3, $C_3(v) = C_{30} + 3(C_{20} - C_{30})(v/(n + 1)V_{30}) + 3(C_{30} - C_{20} + (C_{10} - C_{20})V_{30}/V_{20})(v/(n + 1)V_{30})^2 + \{C_{20} - C_{30} - (C_{10} - C_{20})V_{30}/V_{20} + (C_{20} - C_{10} + (C_{00} - C_{10})V_{20}/V_{10})(V_{30}/V_{10})^2\}(v/(n + 1)V_{30})^3$

The special case when $V_{10} = V$, $i = 1, 2, \dots, n$

$$C_n(v) = C_{n0} - \binom{n}{1}(C_{n,0} - C_{n-1,0})(v/(n + 1)V) + \binom{n}{2}(C_{n,0} - 2C_{n-1,0} + C_{n-2,0}) \times (v/(n + 1)V)^2 - \binom{n}{3}(C_{n,0} - 3C_{n-1,0} + 3C_{n-2,0} - C_{n-3,0})(v/(n + 1)V)^3 + \dots$$

$$= \sum_{i=0}^n \binom{n}{i} C_{n-i,0} (v/(n + 1)V)^i (1 - v/(n + 1)V)^{n-i}$$

Assembly as in Fig. 1b, i open and n closed vessels

Valid for $0 \leq v \leq V_{00}$, $V_{i0} \neq V_{j0}$

Definitions: $A = C_{10} - C_{00}$; $B = C_{20} + (C_{00}V_{20} - C_{10}V_{10})/(V_{10} - V_{20})$; $C = C_{30} + C_{20}V_{20}/(V_{20} - V_{30}) + (C_{10}V_{10} - C_{00}V_{30})V_{30}/(V_{10} - V_{30})(V_{20} - V_{30})$

Vessel 0, $C_0(v) = C_{00}$, a constant

Vessel 1, $C_1(v) = C_{00} + Ae^{-v/V_{20}}$

Vessel 2, $C_2(v) = C_{00} + [AV_{10}/(V_{10} - V_{20})]e^{-v/V_{10}} + Be^{-v/V_{20}}$

Vessel 3, $C_3(v) = C_{00} + [AV_{10}^2/(V_{10} - V_{20})(V_{10} - V_{30})]e^{-v/V_{10}} + [BV_{20}/(V_{20} - V_{30})]e^{-v/V_{20}} + Ce^{-v/V_{30}}$

The special case when $V_{10} = V, i = 1, 2, \dots, n$

$$C_n(v) = C_{00} + \{(C_{n,0} - C_{00}) + (C_{n-1,0} - C_{00})(v/V) + [(C_{n-2,0} - C_{00})/2](v/V)^2 + \dots + [(C_{10} - C_{00})/(n-1)!](v/V)^{n-1}\}e^{-v/V} = C_{00} + e^{-v/V} \sum_{i=0}^{n-1} [(C_{n-i,0} - C_{00})/i!](v/V)^i$$

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