[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF STANFORD UNIVERSITY]

The Theory of Chromatography

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In chromatography a solution is run through a column packed with a suitable adsorbing material and the solutes are deposited on the surface of the material in bands. If afterwards a pure solvent is run through the column the bands are caused to move along at various rates. Treatises have been written by Zechmeister and Cholnoky and by Strain.[1] These works may be consulted for further references.

Martin and Synge[2a] have developed a theory of chromatography in a form applicable to cases of linear adsorption isotherms in which effects of diffusion and of non-attainment of equilibrium are taken into account by dividing the column into "theoretical plates" similar to the discussion of a distillation column. Their results indicate that the solute originally present in one theoretical plate becomes smeared out over a band having a half width (measured at $1/e$ of the maximum concentration in the band) which is approximately equal to $\sqrt{r}$ theoretical plates after it has traveled r theoretical plates down the column. They then present experimental evidence that the height equivalent to one theoretical plate, at least with their rate of flow and with their type of column, is about 0.002 cm. Therefore, with such conditions, diffusion and lack of equilibrium will tend to smear out a chromatographic boundary roughly 1 mm. as it travels 5 cm. or 2 mm. in a distance of 20 cm., etc. If no greater accuracy is required one may, therefore, neglect these factors.

Wilson[3] has attempted a theoretical treatment neglecting diffusion and non-attainment of equilibrium. The purpose of this paper is to point out certain important results given by a treatment similar to Wilson's but not discussed by him. An example of application to some experimental data on lauric acid[4] will also be given.

Single Solute

The Differential Equation.—We treat first the case in which only a single solute is adsorbed. We use the same symbols as Wilson:

α = pore volume per unit length of the column
M = amount of adsorbing material per unit length of the column
V = volume of solution at any time under consideration that has been poured into the column since the initial time. V is also the volume of solution that has passed any given point since the initial time if the pores of the column were initially filled with solution. V may thus be thought of as a convenient measure of time
x = distance of any point in the column under consideration from the beginning of the column
$x_d = x_d(V)$ = value of x at which a discontinuity (sharp boundary) occurs
$Q = Q(V,x)$ = amount of solute adsorbed per unit length, as a function of time and position in the column
$c = c(V,x)$ = amount of solute in solution per unit volume of solution, as a function of time and position in the column
c_0 = concentration of solution entering column
$f = f(c)$ = adsorption isotherm of the solute on the adsorbent such that $Q = Mf$

We will assume instantaneous equilibrium at all points and will neglect diffusion.

To set up the differential equation for these conditions consider a column which has in it any desired distribution of adsorbed solute and filled with solution in equilibrium with it. Consider a cross sectional layer of the column of thickness, δx, which is small enough so that $\partial c/\partial x$, $\partial c/\partial V$ and $\partial Q/\partial V$ may be considered substantially constant throughout the section. The difference between the concentration at the front of the section and that at the rear is $(\partial c/\partial x)\delta x$. Pass into the column an infinitesimal volume of solution δV. A portion of solution of volume δV will enter the layer under consideration at its rear boundary and, simultaneously, a different portion of solution of equal volume will leave the section across its front boundary. The amount of solute carried out of the section with the solution leaving it exceeds the amount carried in with the solution entering by: $\left(\frac{\partial c}{\partial x}\,\delta x\right)\delta V$. The amount of solute in solution in the section increases by the amount:

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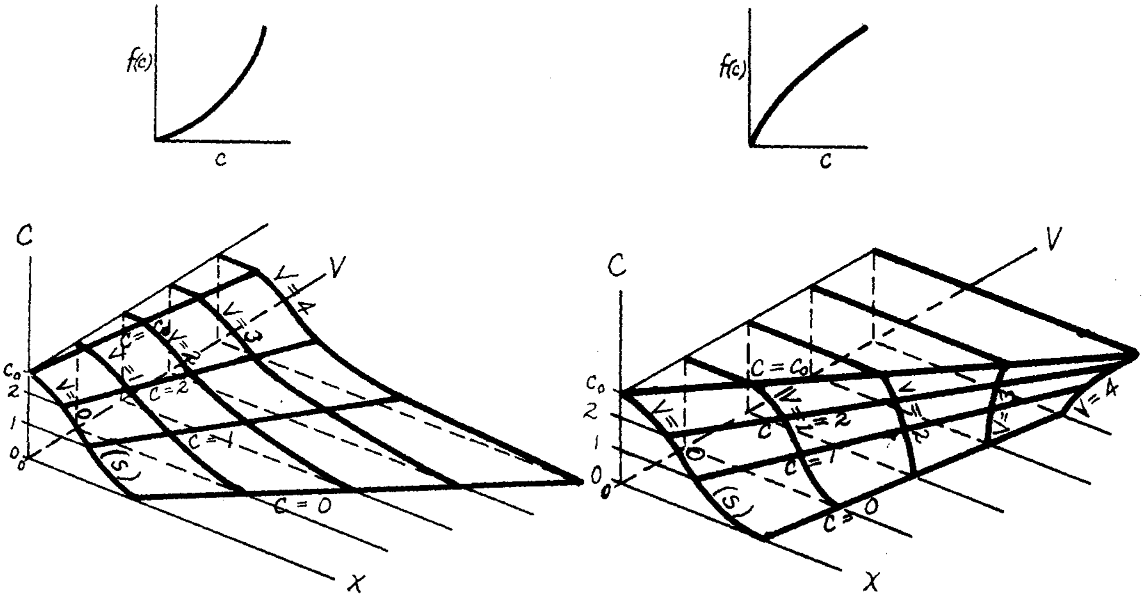
(1) (a) Zechmeister and Cholnoky, "Die chromatographische Adsorptionsmethode," Verlag Julius Springer, Vienna, 1938; "Principles and Practice of Chromatography," Chapman and Hall, London, 1941. (b) Harold H. Strain, "Chromatographic Adsorption Analysis," Interscience Publishers, Inc., New York, N. Y., 1942.

(2a) A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, **35**, 1358–1368 (1941). (b) A. L. LeRosen, THIS JOURNAL, **64**, 1905–1907 (1942), has also begun an experimental study of chromatographic theory.

(3) J. N. Wilson, THIS JOURNAL, **62**, 1583–1591 (1940).

(4) H. G. Cassidy and S. E. Wood, *ibid.*, **63**, 2628–2630 (1941).



a. b.

Fig. 1.—Graphical representation of equation (5) for two cases, together with their corresponding adsorption isotherms: Case (a), f' increases with c; case (b), f' decreases with c. The lines of constant c are the straight lines whose slopes with respect to V and x are given by equation (3). Some solution of varying concentration was assumed to be in the column initially as shown by the curves S.

$\alpha\delta x\left(\frac{\partial c}{\partial V}\delta V\right)$. The amount of solute adsorbed in the section increases by the amount: $\delta x\left(\frac{\partial Q}{\partial V}\delta V\right)$. Setting the sum of these expressions equal to zero in order to conserve matter and canceling δx and δV give

$$\partial c/\partial x + \alpha\partial c/\partial V + \partial Q/\partial V = 0 \qquad (1)$$

This may also be written

$$\partial c/\partial x + [\alpha + Mf'(c)]\partial c/\partial V = 0 \qquad (2)$$

where f' is the first derivative of f.

Integrated Equation for Single Solute.—Substituting $-(\mathrm{d}V/\mathrm{d}x)_c\ (\partial c/\partial V)$ for $\partial c/\partial x$ one obtains still another form for equation (2)

$$(\mathrm{d}V/\mathrm{d}x)_c = \alpha + Mf'(c) \qquad (3)$$

Since equation (3) is the differential equation of a straight line in variables V and x, the general solution is merely a family of straight lines in V and x of slopes $\alpha + Mf'$, and positions defined by the initial conditions.

The usual initial condition is an empty column, but no trouble is encountered if we keep the discussion broad enough to include any initial distribution of solute through the column, providing that at all points adsorbed solute is in equilibrium with adjacent solution. The initial conditions ($V = 0$) may be defined by

$$x = S(c) \qquad (4)$$

Among other conditions it can be seen that S will have the value 0 and all negative values when $c = c_0$. If the column is initially empty S has the value 0 and all positive values when $c = 0$, and for any value of c between 0 and c_0, S has the value 0. The family of straight lines in x and V which constitute the general solution can then be written

$$x = S(c) + \frac{V}{\alpha + Mf'(c)} \qquad (5)$$

This can easily be rearranged to

$$c = \varphi(V - x[\alpha + Mf'(c)]) \qquad (6)$$

where φ is any function determined partly by the initial conditions.

Equations (2) and (6) reduce to Wilson's equations (4) and (5) for the case that $\alpha = 0$.

Figure 1 gives three dimensional plots illustrating the behavior of equation (5). In the case of "development" where pure solvent is poured into the column the behavior is the same. One needs only to put in the appropriate function for S so as to obtain a trailing boundary as well as a leading one.

The Discontinuous Solution.—The overhanging region of Fig. 1b obviously represents a physical impossibility. Only in such a case is it necessary to consider Wilson's discontinuous solution to the equation (2).

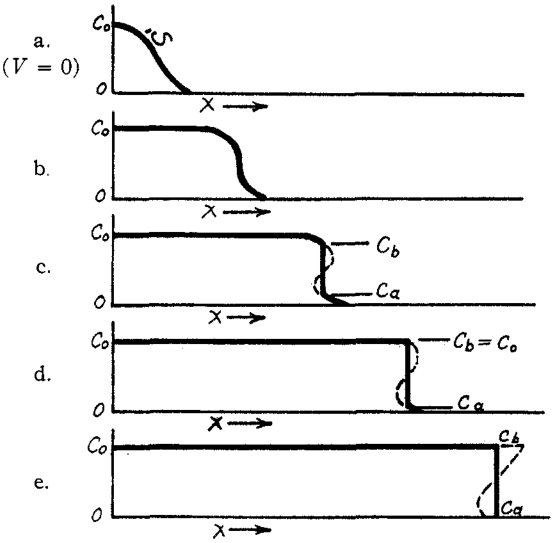


Fig. 2.—The sharpening of a boundary in a special case. The successive diagrams are plots of c against x for successively larger values of V. Dotted lines indicate unsuitable portions of the general solution replaced by the discontinuity according to equation (8). Although c is plotted, the amount of material adsorbed at a given x bears a direct relation to c and so the boundaries of the adsorbed material will look about the same.

The discontinuity will occur wherever $\partial c/\partial x$ becomes positive in a leading boundary or negative in a trailing boundary. Looking into this matter further we see that

$$\left(\frac{\mathrm{d}x}{\mathrm{d}c}\right)_V = S'(c) - \frac{[VMf''(c)]}{[\alpha + Mf'(c)]^2} \tag{7}$$

where S' is the first derivative of S and f'' is the second derivative of f. Since in a leading boundary S' will always be negative and in a trailing one positive, we may state that *the condition for the occurrence of a discontinuity* is that the second term be larger than the first *and that in a leading boundary f'' must be negative or in a trailing boundary f'' must be positive.*

The equation for a boundary in such a discontinuity will be $x = x_\mathrm{d}(V)$ for all values of c between c_a and c_b where c_a and c_b are two concentrations at which x has the same value, x_d, in the general solution as in the discontinuity and where x_d varies with V alone and has the value necessary for conservation of matter. The conservation condition may be expressed as follows

$$\alpha x_\mathrm{d}(c_\mathrm{b} - c_\mathrm{a}) + Mx_\mathrm{d}(f(c_\mathrm{b}) - f(c_\mathrm{a})) = \int_{c_\mathrm{a}}^{c_\mathrm{b}} [\alpha + Mf'(c)]\left\{S(c) + \frac{V}{[\alpha + Mf'(c)]}\right\}\mathrm{d}c$$

in which the left-hand member represents conditions in the discontinuity and the right-hand member conditions in the unsatisfactory part of the general solution.

Solving for x_d gives

$$x_\mathrm{d} = \frac{V(c_\mathrm{b} - c_\mathrm{a}) + \int_{c_\mathrm{a}}^{c_\mathrm{b}} [\alpha + Mf'(c)]S(c)\mathrm{d}c}{\alpha(c_\mathrm{b} - c_\mathrm{a}) + M(f(c_\mathrm{b}) - f(c_\mathrm{a}))} \tag{8}$$[5]

In the case that c_a and c_b are 0 and c_0, respectively, and $S(c) = 0$ this reduces to Wilson's solution

$$x_\mathrm{d} = Vc_0/(\alpha c_0 + Mf(c_0)) \tag{9}$$

Figure 2 shows graphically how the discontinuity operates in a particular complicated case.

General Discussion of Single Solute.—As suggested above the treatment for "development" is the same, providing S is given an appropriate form and that an adsorption isotherm suitable to the solvent used in "developing" is used. It is, however, convenient mathematically to break the equations (5) and (8) (whichever apply) each into separate equations for front and rear boundaries. If one wished to consider also a case in which the initial distribution of solute in the column had one or more *maxima* at given values of x, separate equations may be used for the leading and the trailing boundaries of each maximum.

It should be noted that although the equations are given in terms of c, the amount of adsorbed material is found immediately from c through the relation, $Q = Mf(c)$. If a case is to be treated in which a change of solvents occurs suddenly, the concentration, c, in the column will have to be changed also at the same moment. If the amount of adsorbed material is much greater than the amount in solution this is easily done by assuming that Q does not change and that c changes by whatever amount will give the same Q on changing to the new value for f.

Figure 3 illustrates the behavior of equations (5) and (8) [or (9)] during "development" of a band with initially sharp boundaries. (a) and (b) are for two different types of isotherms. The usual type of isotherm is that shown in (a) where f'' is negative. *In this case the front boundary will tend to be sharp and the rear boundary "diffuse."* Furthermore, even though diffusion and nonattainment of equilibrium may tend to smear out the front boundary, this effect is counteracted by that of equation (5) which tends constantly to sharpen it. This latter effect was deduced by Tiselius[6] on the basis of qualitative arguments.

If the adsorption isotherm is linear ($f'' = 0$), then both boundaries will tend to keep their original shape, except for diffusion, etc. *This condition might be achieved in practice by using concentrations so low that f is approximately linear for the range involved if f is such that it has a finite slope*

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(5) The simplification of this integration is the chief reason for writing the equations with x the explicit function.

(6) A. Tiselius, *Arkiv Kemi., Mineral. Geol.*, **14B**, No. 22 (1940).

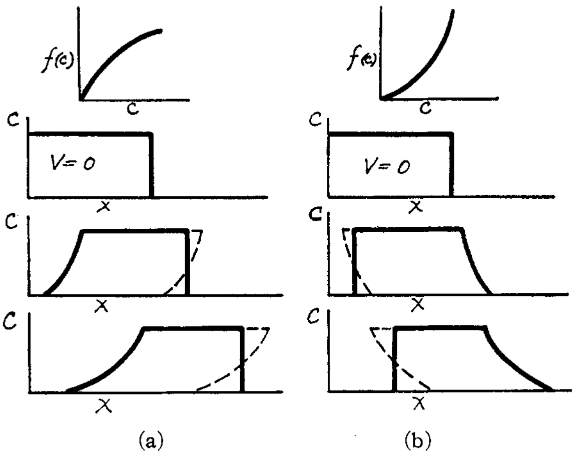

Fig. 3.—Appearance of the boundaries at successive values of V during development for two different types of isotherms, assuming initially sharp boundaries. See also remarks under Fig. 2.

at zero concentration. This would not be possible however, with substances like the fatty acids on various adsorbents studied by Cassidy[7] where the adsorption isotherms appear to have infinite slope at zero concentration.

Application to Some Experimental Data.—Cassidy and Wood[4] have constructed "liquid chromatograms" for the adsorption of lauric acid on charcoal from petroleum ether solution for the purpose of testing Wilson's theory. Since they measure the concentration of solution flowing out of the bottom of the column as a function of the amount that has flowed out and since c_a and c_b will be 0 and c_0, respectively, it will be more convenient to write equations (5) and (8) as follows: For the general solution

$$V = (x - S(c))(\alpha + Mf'c)) = V_x + \alpha x \qquad (10)$$

For the discontinuity, assuming that S is constant between 0 and c_0 (*i. e.*, that the boundary is initially sharp)

$$V = (x_d - S)\left(\alpha + M\frac{f(c_0)}{c_0}\right) = V_{x_d} + \alpha x_d \qquad (11)$$

V_x is defined by the above equations and is equal to the amount of solution that has passed the point x, the column being initially empty. If x (or x_d) is set equal to the length of the column, then Mx (or Mx_d) becomes the total amount of adsorbent present and the equations give the values of the quantities V and V_x (or V_{x_d}) at which solution of concentration c (or at which the discontinuity) leaves the bottom of the column. V_{x_d} has, in the case where $S = 0$, been defined by Tiselius[5] as the "retardation volume" and V_{x_d}/Mx_d as the "specific retardation volume."[8]

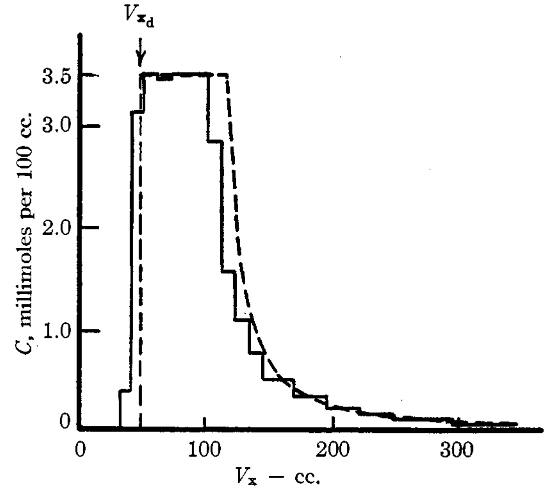

Fig. 4.—Comparison of the theory with data given by Cassidy and Wood for lauric acid on charcoal: solid line, experimental data; dashed line, theory according to equations (10) and (11).

Figure 4 is a reproduction of Cassidy and Wood's Fig. 1a, showing the data from one of their experiments (solid line) and the predictions of the above theory (dotted line). In this experiment the column contained 1 g. of carbon; 100 cc. of petroleum ether containing 3.5 millimoles of lauric acid was poured through and then several hundred cubic centimeters of pure petroleum ether. The concentration of solution dripping out below the column was measured from time to time and plotted in Fig. 4 against the amount that had dripped out.

To make the theoretical calculations for the front boundary, let $S = 0$ and use equation (11). For the rear boundary use equation (10). S can again be made 0 if the point $V = 0$ is now shifted to the point at which the last solution entered the column and pure solvent began to follow. 100 cc. is then added on to the resulting value for V_x before plotting. The following empirical adsorption isotherm given by Cassidy and Wood was used:

$$\log f = 0.324 \log c + 0.081$$

where f is in millimoles of lauric acid per gram of charcoal and c is in millimoles of lauric acid per 100 cc. of petroleum ether.

The agreement seems within the experimental error, considering also the possible error in the empirical expression for the adsorption isotherm. The slight deviation from sharpness noted in the front boundary could easily be attributed to "channelling" in the column. The agreement

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(7) H. G. Cassidy, THIS JOURNAL, **62**, 3073, 3076 (1940).

(8) A. Tiselius, *Arkiv. Kemi, Mineral. Geol.*, **15B**, No. 6 (1941).

is equally good for the rest of the data of Cassidy and Wood. We must conclude then that, at least in these experiments, non-attainment of equilibrium and diffusion are indeed negligible in *both* boundaries.

One might even venture to reverse the procedure and obtain the adsorption isotherm from the shape of the "diffuse" (rear) boundary, at least in cases where the sharpness of the front boundary indicates channelling to be negligible. The equation to be used would be:

$$f'(c) = V_x/Mx \tag{12}$$

The quantity on the right is experimentally determined as a function of (c) and, after Tiselius, might be called a "generalized specific retardation volume" or a "specific retardation volume in the diffuse boundary." f' can then be integrated over c to obtain f. The quantity $V_{xd}/Mx = f(c_0)/c_0$, obtained from the sharp boundary, gives one point of the isotherm directly. It can be used either as a check on the integration or to obtain a constant of integration in case V_x becomes too large to handle near $c = 0$ as in Fig. 4 and makes it difficult to integrate over the whole range.

Multiple Solutes

As Wilson pointed out, this case is complicated by the fact that the adsorption isotherms are usually functions of the concentrations of all the solutes that are adsorbed. That is

$$Q_i(V,x) = Mf_i(c_1, c_2, \ldots, c_i, \ldots c_n) \tag{13}$$

where Q_i is the amount of solute i adsorbed per cm. of column length and there are n solutes present. Because of this we may make the preliminary observation that wherever or whenever one of the solutes undergoes a change in concentration, so will all the other solutes and when this change is sudden or discontinuous with one it will also be sharp with the others and when gradual with one it will be gradual with the others. c_i (V, x) represents the concentration in solution of solute i as a function of V and x.

Sharp Boundaries.—In the case of multiple solutes, as with the single solute, sharp boundaries are probably to be expected under some conditions. Without saying what conditions are necessary for their formation, the rest of their behavior can be derived with quite simple considerations.

Take a section of the column which includes within it one and only one of these sharp boundaries. By "sharp" we mean that the concentrations of all the solutes remain constant until the solution reaches the boundary and also remain constant at their new values after leaving the boundary. For the purposes of this treatment the actual width of the boundary may be greater than zero if the error introduced by the unsharpness in measuring either the "position" of the boundary or the amounts of material involved are not too great to be tolerated. Let c_{ai} represent the concentration of solute i entering the section and c_{bi} represent its concentration after passing the boundary. Let the solution flow for such a length of time that the volume which flows past a given point is ΔV. During this time the boundary moves a distance Δx_d, and is assumed still to be within the section of column under consideration. Conservation of matter gives a set of n simultaneous equations, like the following, one for each of the solutes

$$(c_{ai} - c_{bi})\Delta V = \alpha(c_{ai} - c_{bi})\Delta x_d + M[f_i(c_{a1}, \ldots, c_{an}) - f_i(c_{b1}, \ldots, c_{bn})]\Delta x_d \tag{14}$$

Rearranging gives

$$\frac{\Delta V}{\Delta x_d} = \alpha + M\frac{f_i(c_{a1}, c_{a2}, \ldots, c_{an}) - f_i(c_{b1}, \ldots, c_{bn})}{(c_{ai} - c_{bi})} \tag{15}$$

The fraction in the right-hand member is the only quantity dependent on the particular solute and must therefore have the same value for all solutes at the same boundary. Using the abbreviations, $f_i(c_{a1}, \ldots, c_{an}) = f_{ai}$, etc., this observation may be written

$$\frac{f_{a1} - f_{b1}}{c_{a1} - c_{b1}} = \frac{f_{a2} - f_{b2}}{c_{a2} - c_{b2}} = \ldots\ldots = \frac{f_{an} - f_{bn}}{c_{an} - c_{bn}} \tag{16}$$

If the boundary results from one of the solutes, say j, becoming exhausted then the rate of movement of the boundary will be

$$\frac{\Delta x_d}{\Delta V} = \frac{1}{\alpha + M\dfrac{f_{aj}}{c_{aj}}} \tag{17}$$

and the values of the ratios in equation (16) will be determined by that for j

$$\frac{f_{a1} - f_{b1}}{c_{a1} - c_{b1}} = \frac{f_{a2} - f_{b2}}{c_{a2} - c_{b2}} = \ldots = \frac{f_{aj}}{c_{aj}} = \ldots = \frac{f_{an} - f_{bn}}{c_{an} - c_{bn}} \tag{18}$$

In general for a given f_{aj}/c_{aj} only a definite value of c_{bi} can give the proper $(f_{ai} - f_{bi})/(c_{ai} - c_{bi})$ to satisfy the equations (18) and also the equations (13). However, the equations must be solved simultaneously since the value of a given f_{bi} depends upon all of the c_{bi}. This conclusion differs from Wilson's because his equation (17) neglects the contribution to c_i' (our c_{bi}) of the previously adsorbed material which is de-adsorbed (so to speak) by the advancing boundary.

If the boundary were only partially sharp as in Figs. 2c and 2d equation (15) may still be made to apply by making a and b refer to the concentrations at the limits of the sharp part and by making the section of the column considered in its derivation vanishingly small so that $\Delta V/\Delta x_d$ becomes dV/dx_d. It will be noticed also that equations (15) and (16) apply to development as well as to formation of the chromatogram.

It can be proved in the following way that in a chromatogram formed in the ordinary manner the substance, j, whose concentration goes to zero in a particular boundary will be the one having the largest ratio of adsorption on the adsorbent to concentration in solution on the side of the boundary from which the solution comes. That, is $i = j$ for the solute having the largest value of f_{ai}/c_{ai}.

Assume for the sake of argument that the theorem is not true. Let $i = k$ for the solute that does have the largest value of f_{ai}/c_{ai}. After passing the boundary the ratio f_{bk}/c_{bk} will still be larger than f_{aj}/c_{aj} if equation (18) is followed.

$$\left(\frac{f_{bk}}{c_{bk}} = \frac{f_{ak} + \Delta f_k}{c_{ak} + \Delta c_k} > \frac{c_{ak}\,(f_{aj}/c_{aj}) + \Delta f_k}{c_{ak} + \Delta c_k} = \frac{c_{ak}(\Delta f_k/\Delta c_k) + \Delta f_k}{c_{ak} + \Delta c_k} = \Delta f_k/\Delta c_k = f_{aj}/c_{aj}\right)$$

By similar arguments it can be shown that eventually when solute k does come to a boundary in which c_k goes to zero the ratio f_k/c_k will still be larger than the ratio f_{ah}/c_{ah} of some other solute, h, which previously went to zero, the ratio f_{ah}/c_{ah} referring to the boundary at which c_b goes to zero. (h might be the same as j.) Then according to equation (17) the boundary at which c_k went to zero will move more slowly than the one at which c_h went to zero. This would be impossible in a chromatogram formed in the ordinary way because then all boundaries originate at a common point at a common time and a slower-moving boundary will not be found in front of a faster one.

The General Case.—In the same way that equation (1) was derived we may derive

$$\frac{\partial c_i}{\partial x} + \alpha\frac{\partial c_i}{\partial V} + \frac{\partial Q_i}{\partial V} = 0 \qquad (19)$$

There are n such equations, one for each value of i. The equations may also be written

$$\frac{\partial c_i}{\partial x} + \alpha\frac{\partial c_i}{\partial V} + M\left(\frac{\partial f_i}{\partial c_1}\frac{\partial c_1}{\partial V} + \frac{\partial f_i}{\partial c_2}\frac{\partial c_2}{\partial V} + \dots + \frac{\partial f_i}{\partial c_n}\frac{\partial c_n}{\partial V}\right) = 0 \qquad (20)$$

It is no longer possible to write down a simple solution to these differential equations as Wilson's equation (13b) might suggest because it is no longer possible to hold the functions f constant by holding only one of the concentrations, c_i, constant. Also an equation analogous to equation (3) would contain the function:

$$\left(\frac{df_i}{dc_i}\right)_x = \frac{\partial f_i}{\partial c_1}\left(\frac{dc_1}{dc_i}\right)_x + \frac{\partial f_i}{\partial c_2}\left(\frac{dc_2}{dc_i}\right)_x + \dots$$

and not simply $(\partial f_i/\partial c_i)$ alone.

More explicit information about the behavior of the chromatogram can be obtained, however, by solving the equations (20) simultaneously for the variations in the concentrations that will occur at each point as the solution passes it and expressing them in terms of presumably known quantities. The solutions in determinant form are

$$\frac{\partial c_k}{\partial V} = \frac{-\mathbf{D}_k}{M\mathbf{D}} \qquad (21)$$

where $\mathbf{D}$ is the determinant:

$$\begin{vmatrix} \left(\frac{\alpha}{M} + \frac{\partial f_1}{\partial c_1}\right) & \frac{\partial f_1}{\partial c_2} & \frac{\partial f_1}{\partial c_3} & \dots & \frac{\partial f_1}{\partial c_n} \\ \frac{\partial f_2}{\partial c_1} & \left(\frac{\alpha}{M} + \frac{\partial f_2}{\partial c_2}\right) & \frac{\partial f_2}{\partial c_3} & \dots & \frac{\partial f_2}{\partial c_n} \\ \frac{\partial f_3}{\partial c_1} & \frac{\partial f_3}{\partial c_2} & \left(\frac{\alpha}{M} + \frac{\partial f_3}{\partial c_3}\right) & \dots & \frac{\partial f_3}{\partial c_n} \\ \cdot & \cdot & \cdot & \dots & \cdot \\ \cdot & \cdot & \cdot & \dots & \cdot \\ \cdot & \cdot & \cdot & \dots & \cdot \\ \frac{\partial f_n}{\partial c_1} & \frac{\partial f_n}{\partial c_2} & \frac{\partial f_n}{\partial c_3} & \dots & \left(\frac{\alpha}{M} + \frac{\partial f_n}{\partial c_n}\right) \end{vmatrix}$$

and $\mathbf{D}_k$ is the same as $\mathbf{D}$ except that the kth column is replaced by the column (in order from top to bottom)

$$\frac{\partial c_1}{\partial x}, \frac{\partial c_2}{\partial x}, \frac{\partial c_3}{\partial x}, \dots, \frac{\partial c_n}{\partial x}$$

If one knows the distribution of the various solutes throughout the chromatographic column at a given time, and also the adsorption isotherms (equations (13)), then all of the quantities in the two determinants are known. The changes which the movement of the solution will cause in the concentrations found at a point, x, can then be calculated from this information and the equations (21).

In order to obtain any more specific information from these equations it seems necessary to substitute actual quantities into them and work out the results. The next section will attempt to extract some qualitative ideas as to what behavior may be expected by studying some special cases that are probably typical.

Application to Specific Cases.—Typical adsorption isotherms would be expected to have the properties shown in Table I.

TABLE I

EXPECTED PROPERTIES FOR ADSORPTION ISOTHERMS

Function	Sign	Value approaches zero as
f_i	+	$c_i \rightarrow 0$; (or $c_l \rightarrow \infty$)
$\dfrac{\partial f_i}{\partial c_i}$	+	$c_i \rightarrow \infty$; (or $c_l \rightarrow \infty$)
$\dfrac{\partial f_i}{\partial c_k}$	−	$c_i \rightarrow 0$; or $c_k \rightarrow \infty$; (or $c_l \rightarrow \infty$)
$\dfrac{\partial^2 f_i}{\partial c_i^2}$	−	$c_i \rightarrow \infty$; (or $c_l \rightarrow \infty$)
$\dfrac{\partial^2 f_i}{\partial c_i \partial c_k}$	−	$c_i \rightarrow \infty$; or $c_k \rightarrow \infty$ (or $c_l \rightarrow \infty$)
$\dfrac{\partial^2 f_i}{\partial c_h \partial c_k}$	+	$c_i \rightarrow 0$; or $c_h \rightarrow \infty$; $c_k \rightarrow \infty$; (or $c_l \rightarrow \infty$)

Subscripts *h*, *k* and *l* can have any value except *i* and can equal each other. The predictions enclosed in parentheses are perhaps not justified, but no use will be made of them anyway.

Each boundary in a chromatogram formed in the usual way results from the concentration of one solute, say the *j*th decreasing to (or, in rear boundaries, rising from) zero. At the point in the column where c_j is nearly equal to zero, the terms, $\partial f_j/\partial c_k$, will vanish and the equation (20) for solute *j* becomes

$$\frac{\partial c_j}{\partial x} + \left(\alpha + M\frac{\partial f_j}{\partial c_j}\right)\frac{\partial c_j}{\partial V} = 0 \qquad (22)$$

Further

$$\left(\frac{dx}{dV}\right)_{c_j} = -\frac{(\partial c_j/\partial V)}{(\partial c_j/\partial x)} = \frac{1}{\alpha + M(\partial f_j/\partial c_j)} \qquad (23)$$

which gives the rate of travel of the boundary as measured by the point at which c_j becomes zero. This rate will remain constant as long as all the concentrations at this point are constant, as they should ordinarily be, since they are equal to the concentrations found in the uniform adjacent region of the column throughout which $c_j = 0$.

It would be interesting to find out under what conditions the assumption made above that the general solution is applicable is valid, and under what conditions equation (17) for the discontinuous solution should be used instead. If the rate of movement given by equation (23) tended to *increase* as c_j *increases* then the boundary would tend to become sharp and *discontinuous* in the manner illustrated in Figs. 1b, 2, etc. The question cannot be answered immediately because whether $(\partial f_j/\partial c_j)$ increases or decreases with increasing c_j depends upon what changes the concentrations of the other solutes undergo simultaneously. It will be simpler to find out what factors influence this behavior by studying the case in which only two solutes are involved.

The method will be to assume certain simple conditions to exist in the column and then to see what changes will tend to come about. The changes should point out what conditions tend to prevail and would therefore be expected from the beginning. For the case of two solutes there are four boundaries to be considered: (1) the front boundary of the faster moving solute, (2) the front boundary of the slower moving solute involving simultaneously a change in the concentration (and amount adsorbed) of the faster one, (3) in the case of development, the rear boundary of the faster moving solute, with accompanying change in the concentration of the slower one, and (4) also in development, the rear boundary of the slower moving solute.

The first boundary involves only one solute and, with an isotherm of the ordinary type, will obey equation (9). Instead of being the concentration in the original solution before entering the column, c_0 will be whatever concentration the faster moving solute changes to as it passes over boundary (2).

Assume first, for the sake of argument, that boundary (2) obeys the general solution. Let subscript 1 refer to the faster moving solute, and 2 to the slower one. In this boundary c_2 changes from its value in the original solution to zero. From equation (21), neglecting α

$$\frac{\partial c_1}{\partial V} = \frac{\dfrac{\partial c_2}{\partial x}\dfrac{\partial f_1}{\partial c_2} - \dfrac{\partial c_1}{\partial x}\dfrac{\partial f_2}{\partial c_2}}{M\dfrac{\partial f_1}{\partial c_1}\dfrac{\partial f_2}{\partial c_2} - M\dfrac{\partial f_1}{\partial c_2}\dfrac{\partial f_2}{\partial c_1}} \qquad (24)$$

If α were not to be neglected, add the term α/M to every $(\partial f_i/\partial c_i)$. The qualitative conclusions of this discussion would not be changed. The denominator of the right-hand side is expected to be positive because the absolute value of the second term in it is ordinarily expected to be less than that of the first. This is especially true as c_2 approaches zero because then $(\partial f_2/\partial c_1)$ approaches 0 according to Table I. In the numerator $(\partial c_2/\partial x)$ is being assumed negative. Let us assume first that the concentration of the faster moving solute is constant throughout this boundary, *i. e.*, $(\partial c_1/\partial x) = 0$. Since $(\partial f_1/\partial c_2)$ is negative (Table I), $(\partial c_1/\partial V)$ is found to be positive. Therefore c_1 will tend to increase in this boundary. This boundary will thus tend to feed into the region beyond it, where $c_2 = 0$, solution of increased c_1, and the increase will be carried forward with the speed of boundary (1). We conclude that $(\partial c_1/\partial x)$ will tend to be positive in boundary (2).

The same considerations used in deriving equation (18) predict that the total increase in c_1 will not vary much during the whole course of formation or development of the chromatogram and will approximately equal the value given by equation (18) *if* the width of the boundaries are small compared to the widths of the bands between the boundaries. However, there is no guarantee in this treatment that such will be the case or even that the boundaries will not sometimes overlap.

Next we should try to see whether boundary (2) will actually tend to follow the general solution or be discontinuous. The boundary will tend to sharpen and become discontinuous if

$$\frac{\partial}{\partial V}\left(\frac{\partial c_2}{\partial x}\right) = \frac{\partial^2 c_2}{\partial x \partial V}$$

is negative. From equation (21) neglecting α

$$\frac{\partial c_2}{\partial V} = \frac{\frac{\partial c_1}{\partial x}\frac{\partial f_2}{\partial c_1} - \frac{\partial c_2}{\partial x}\frac{\partial f_1}{\partial c_1}}{M\frac{\partial f_1}{\partial c_1}\frac{\partial f_2}{\partial c_2} - M\frac{\partial f_1}{\partial c_2}\frac{\partial f_2}{\partial c_1}} \qquad (25)$$

For simplicity we confine ourselves to the region in which c_2 is nearly zero and certain terms vanish according to Table I. Partially differentiating equation (25) with respect to x at constant V and discarding all terms containing $(\partial f_2/\partial c_1)$ and $(\partial^2 f_2/\partial c_1^2)$ gives

$$\frac{\partial^2 c_2}{\partial V \partial x} = M\left(\frac{\partial c_2}{\partial x}\right)^2 \frac{\partial f_1}{\partial c_1}\left[\frac{\partial^2 f_2}{\partial c_2^2}\frac{\partial f_1}{\partial c_1} - \frac{\partial^2 f_2}{\partial c_1 \partial c_2}\frac{\partial f_1}{\partial c_2}\right] + M\frac{\partial c_1}{\partial x}\frac{\partial c_2}{\partial x}\frac{\partial f_1}{\partial c_1}\frac{\partial^2 f_2}{\partial c_1 \partial c_2}\left[\frac{\partial f_1}{\partial c_1} + \frac{\partial f_2}{\partial c_2}\right] - M\frac{\partial^2 c_2}{\partial x^2}\frac{\partial f_2}{\partial c_2}\left(\frac{\partial f_1}{\partial c_1}\right)^2 \qquad (26)$$

In determining the tendency of the boundary to sharpen we are not interested in the special effect of the curvature in the boundary and therefore disregard the term containing $(\partial^2 c_2/\partial x^2)$. According to Table I the coefficients of both $(\partial c_2/\partial x)^2$ and $(\partial c_1/\partial x)(\partial c_2/\partial x)$ are negative. It was found above that $(\partial c_1/\partial x)$ should be positive while $(\partial c_2/\partial x)$ is negative in this boundary. This circumstance makes the sign of $(\partial^2 c_2/\partial V \partial x)$ dependent upon the relative sizes of the first two terms on the right of equation (26) and leaves question of whether boundary (2) will tend to be sharp or not undetermined until more facts are known or assumed.

Even if boundary (2) is discontinuous it can still be proved that c_1 must increase at this boundary in the case of isotherms of the type presented in Table I. Making use of equation (18) and the argument at the end of the same section we see that $\Delta f_1/\Delta c_1 = f_{02}/c_{02} > f_{01}/c_{01}$. (Subscript 0 refers to initial values and b to values after crossing boundary (2).) Δf_1 and Δc_1 are either both positive or both negative. If negative then a little algebra shows that f_{b1}/c_{b1} is smaller than f_{01}/c_{01}. But it is impossible to find any value of c_1 less than c_{01} at which f_1/c_1 is smaller than f_{01}/c_{01} as long as $(\partial^2 f_1/\partial c_1^2)$ is always negative and the values of f_1 for $c_2 = 0$ always lie above those for $c_2 = c_{02}$. Therefore only a positive value for Δc_1 can satisfy all requirements.

At boundary (3), which will be found in development, it is c_1 that goes to zero, or, since it is a rear boundary, "rises from zero" $(\partial c_1/\partial x)$ is positive. If we first let $(\partial c_2/\partial x)$ be zero and examine equation (25) in the same way that equation (24) was discussed we conclude that c_2 will tend to decrease in boundary (3). c_2 in the region between boundaries (2) and (3) is determined by the concentrations that would be in equilibrium with the adsorbed solutes already present in this region when in contact with the solvent being used for development. The ever-widening new region between boundaries (3) and (4) will therefore have the lowered value of c_2. Thus $(\partial c_2/\partial x)$ in the third boundary tends to be positive. This information put into the equation analogous to equation (26) with subscripts reversed and discussed in the same way shows that $(\partial^2 c_1/\partial V \partial x)$ will be negative, at least in the region where c_1 is very small. Since this is a rear boundary it means that *boundary* (3) *will tend to be "diffuse" and not discontinuous.*

Boundary (4) involves only solute 2 and, with the type of isotherm postulated, will obey equation (5). The function S would ordinarily be 0 if $V = 0$ at the beginning of development and c_0 would have the lowered value of c_2 left behind by the faster moving third boundary.

It is acknowledged that this treatment leaves much unfinished business and much to be desired in the discussion of multiple solutes. It should be pointed out especially that no guarantee has been given that queer things will not happen under a wide variety of conditions not discussed here.

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Summary

The simple theory of chromatography (assuming instantaneous equilibrium and no diffusion) proposed by J. N. Wilson,[3] if carried out completely, predicts both sharp and "diffuse" bound-

aries to the bands formed and "developed" in the chromatographic column.

When only one solute is present, if the adsorption isotherm is of the usual type, the front boundary of the band will be sharp and will tend constantly to sharpen itself in spite of diffusion, etc., while the rear boundary will be broad and gradual. The quantitative agreement with experimental data, at least in the case of lauric acid on charcoal, is sufficiently good to justify the assumptions made. The equations developed (equations (2), (5) and (8)) can easily be applied to a wide variety of conditions besides those ordinarily encountered. Examples are given.

The partial differential equations for multiple solutes are given (equations (20) and (21)) and the discontinuous solutions for multiple solute (equations (15) to (18)) are discussed separately, but no general solution is arrived at. Some evidence that here also the boundaries may usually tend to be sharp in front and diffuse in the rear was obtained by closer examination of a case involving two solutes, but the conclusion is not definite. In the typical two-solute case the concentration of the faster moving solute in the band where it is pure tends to be larger than in the original solution. Under similar conditions during development the concentration of the slower moving solute in the rear band where it is pure tends to be smaller than in the intermediate (overlapping) band.

It is suggested that more information about adsorption isotherms can be obtained from the diffuse boundaries of chromatograms than from the sharp ones. The chief characteristic difference is the presence of a (df/dc) in the equations for the former type in place of ($f(c_0)/c_0$) found in the equations for the latter.

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Ionization Constants of Several Substituted Phenylarsonic Acids

BY DAVID PRESSMAN AND DAVID H. BROWN

In the course of our immunological studies[1] it became desirable to know the ionization constants of several substituted phenylarsonic acids. For this reason the constants at 22° for nineteen phenylarsonic acids were determined and are reported here. The values were obtained under identical conditions by the use of a glass electrode and are good only to about 15%. No correction for activities was made.

Experimental

Preparation of Compounds.—The arsonic acids were prepared by the methods of the references in Table I.

Potentiometric Titration of Arsonic Acids.—Solutions of the dipotassium salts (or the tripotassium salts in the cases of *p*-hydroxy- and *p*-carboxyphenylarsonic acids) were made by weighing out accurately 0.00200 mole of the acid, adding the calculated amount of carbonate-free 0.0822 N potassium hydroxide solution, and making up to a total volume of 100 ml. The solution was then titrated with 0.0979 N hydrochloric acid solution. The *p*H was measured by use of a Beckman *p*H meter after every addition of 1 to 2 ml. of the acid. The titrating system was maintained in a bath at 22.0° which was also room temperature.

In the case of some very insoluble acids, the acid precipitated before the titration was complete. This prevented the calculation of the first ionization constant in these cases. The second constant was determined in all cases.

Calculation of Ionization Constants.—The graphical point from a plot of *p*H against the volume of hydrochloric acid added which corresponds to half neutralization of the first, second, or in some cases the third hydrogen ion is taken as the ionization constant; it is good to within 0.1 *p*H unit, limiting the value of the dissociation to an error of about ±15%.

Correction was necessary in some cases for the ionization of the first hydrogen ion and for the hydrolysis of the di-ionized arsonate ion. This was done by the use of equation 1

$$K = (\mathrm{H}^+)m\left[\frac{C/2 + (\mathrm{H}^+)m - K_\mathrm{w}/(\mathrm{H}^+)}{C/2 - (\mathrm{H}^+)m + K_\mathrm{w}/(\mathrm{H}^+)}\right] \quad (1)$$

where K is the dissociation constant, C is the total

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(1) D. Pressman, D. H. Brown and L. Pauling, THIS JOURNAL, **64**, 3015 (1942).