undoubtedly was present, the actual average molecular weights for the vapor must be somewhat lower. The theoretical molecular weights for the molecules Ga_2Cl_6, $GaCl_3$, Ga_2Cl_4, and $GaCl_2$ are 352.2, 176.1, 281.2, and 140.6, respectively. The molecular weights in Table IV can be accounted for by postulating that the vapor was a mixture of molecules of $GaCl_2$ with heavier molecules, undoubtedly Ga_2Cl_6 and $GaCl_3$ and perhaps Ga_2Cl_4.

Klemm and Tilk found that solid gallium dichloride is diamagnetic and concluded that the solid is composed of Ga_2Cl_4 molecules and not $GaCl_2$. The present investigation indicates that considerable quantities of $GaCl_2$ molecules exist in the vapor above 400° under the conditions of the experiments. There seems to be no chance of determining the configuration of the gallium dichloride molecules by an electron diffraction study of its vapor because of the complex mixture of molecules present.

Summary

1. The equilibrium vapor pressures of gallium trichloride have been studied from 50 to 200° and its thermal properties have been determined. Vapor density studies from 117 to 498° give no evidence for association beyond the dimer. The saturated vapor is more than 98.5% dimeric up to 200°, and above this temperature reversible dissociation to the monomer becomes marked, the vapor being 88% dissociated at 498°.

2. Gallium dichloride has been prepared and a method for purifying it has been devised. The properties of the pure compound have been examined. Decomposition to gallium trichloride and gallium becomes appreciable at 200°. Vapor pressure measurements from 219 to 459° indicate that the system is divariant, although it apparently involves three phases and two components. Vapor density measurements in the range 400 to 470° indicate that the vapor contains some $GaCl_2$ molecules, in which gallium must show the anomalous valence of two.

3. No evidence for the existence of gallium monochloride was observed.

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A Theory of Chromatography

BY J. NORTON WILSON

The so-called chromatographic adsorption method of analysis was originated by the Russian botanist M. Tswett.[1] He discovered that if a solution containing a mixture of colored solutes is allowed to run through a vertical glass tube filled with a suitable powdered adsorbing material, the material adsorbed in the column appears as a series of colored bands, indicating that a partial separation of the components of the solution has taken place. This series of colored bands is known as a chromatogram. The separation can be completed by a procedure known as development of the chromatogram: there is poured through the column a suitable solvent which washes the colored bands down the tube at different rates, the lowest-lying band moving the fastest. If this process is carried out in a sufficiently long tube with the use of a sufficiently large volume of solvent it is possible to effect complete separation of the original components of the solution into a series of discrete bands separated by clear spaces of adsorbent.

The technique just outlined has become in recent years an important method for the separation, purification and identification of small amounts of complex naturally-occurring organic compounds; these developments have been described at length by Zechmeister and Cholnoky.[2] The method also has been applied to some extent in inorganic chemistry[3]; the most spectacular application in this field has been to the separation of lithium isotopes.[4]

Although the nature of the phenomenon seems to be roughly understood,[2] there has appeared so

(1) M. Tswett, *Ber. deut. botan. Ges.*, **24**, 234, 316, 384 (1906); *Ber.*, **41**, 1352 (1908); **43**, 3199 (1910); **44**, 1124 (1911).

(2) Zechmeister and Cholnoky, "Die chromatographische Adsorptionsmethode," Springer, Vienna, 1938; see also P. M. Jensen, "Die Bedeutung der chromatographischen Adsorptionsanalyse usw.," Dissertation, Technische Hochschule, Zurich, 1936; Zechmeister and Cholnoky, *Monatsh.*, **68**, 68–80 (1936).

(3) G. M. Schwab and co-workers, *Naturwiss.*, **25**, 44 (1937); *Angew. Chem.*, **50**, 546 (1937).

(4) T. I. Taylor and H. C. Urey, *J. Chem. Phys.*, **6**, 429 (1938).

far in the literature no quantitative treatment by means of which even an approximate prediction can be made of the width or rate of development of the bands to be expected in a given chromatogram. There is presented here a theoretical treatment which is based on the assumption of instantaneous equilibrium between the solution and the adsorbed material. The chromatography of a solution containing a single solute will be discussed first; it is evident that this simple problem must be solved before the more complicated problem of the chromatography of mixtures can be attacked successfully.

The Chromatography of a Solution Containing One Solute.—Let us consider a long column which contains M grams of adsorbent per centimeter of its length; we wish to calculate the distribution of adsorbed material in the column after a volume v of a solution containing solute at an initial concentration c_0 has passed through it. We shall assume that equilibrium between adsorbed material and the solution in contact with it is always maintained and that the volume of the interstices between the particles of adsorbent per unit length of the column is negligible. Let the isotherm which represents the adsorption of the given solute on the adsorbent be

$$q/m = f(c) \qquad (1)$$

where q is the number of millimoles of solute adsorbed on m grams of adsorbent in equilibrium with a solution whose concentration is c moles per liter. Then under equilibrium conditions at any point in the column where the concentration of solute is c there will be adsorbed Q millimoles of solute per centimeter length of the column, where

$$Q = Mf(c) \qquad (2)$$

Let the distance from the top of the column to any lower point in the column be x, and consider the changes in Q and c which occur at x when an element of volume dv of solution, whose concentration at x is c, passes through a thin cross-sectional layer of the column, of thickness dx, on which is adsorbed initially an amount $Q dx$ of solute. The number of millimoles of solute adsorbed on this layer will change by an amount $(\partial Q/\partial v)_x\, dv\, dx$, and the concentration of the solution will change by $(\partial c/\partial x)_v dx$. The number of millimoles of solute contained in the volume dv of solution will therefore change by an amount $(\partial c/\partial x)_v\, dx\, dv$. But since the total amount of solute is unchanged, we have

$$\left(\frac{\partial Q}{\partial v}\right)_x dv\, dx + \left(\frac{\partial c}{\partial x}\right)_v dv\, dx = 0 \qquad (3)$$

Substituting for Q from equation 2 we obtain as the differential equation for the chromatography of a single solute

$$\left[\frac{\partial}{\partial v}(Mf(c))\right]_x + \left(\frac{\partial c}{\partial x}\right)_v = 0 \qquad (4)$$

The general solution of this equation is

$$c = \varphi\{v - Mxf'(c)\} \qquad (5)$$

where φ is an arbitrary function and $f'(c)$ is the first derivative of $f(c)$. This equation will apply either to the process of formation of the chromatogram or the process of development. Different boundary conditions will apply to the two cases.

Let the chromatogram be formed by pouring a volume v of a solution whose initial concentration is c_0 through the column. The appropriate boundary conditions are:

$$\left.\begin{array}{ll} (1)\ \text{When } v = 0, & f(c) = 0 \text{ for } x \geqslant 0 \\ (2) \qquad v > 0, & f(c) = f(c_0) \text{ at } x = 0 \\ (3) \qquad vc_0 = \displaystyle\int_0^\infty Q dx = \int_0^\infty Mf(c) dx & \end{array}\right\} \qquad (6)$$

The last is a conservation condition.

It has been found that the first two boundary conditions are satisfied only by a discontinuous solution: $\varphi = const. = c_0$ up to a certain value of x; $\varphi = 0$ beyond that value. Application of conditions (2) and (3) leads to the solution:

$$\left.\begin{array}{l} \text{For } 0 \leqslant x < \dfrac{vc_0}{Mf(c_0)},\ Q = Mf(c_0) \\ \text{For } x > \dfrac{vc_0}{Mf(c_0)},\ Q = 0 \end{array}\right\} \qquad (7)$$

The discontinuous nature of the solution 7 accounts in a satisfactory way for the sharpness of the bands observed in chromatographic experiments; the result is also in accordance with the experimental observation that the intensity of color is approximately uniform throughout the band ($Q = Mf(c_0) = \text{const.}$).

We may now consider the case in which a band already formed is developed by passage through the column of a volume V of some solvent not necessarily the same as the solvent used in forming the band. Let the adsorption isotherm for the solute in the developing solvent be

$$q/m = F(c) \qquad (8)$$

We assume that the band was formed in accordance with equations 7, *i. e.*, that up to some point $x = x_1$, $Q = Q_0 = \text{const.}$; beyond x_1, $Q = 0$. We may also write

$$Q_0 = Mf(c_0) = MF(c_0') \qquad (9)$$

where c_0' is the concentration of solute which would be in equilibrium with Q_0 in the new solvent, and is not necessarily equal to c_0. The boundary conditions to be applied are

$$\left.\begin{array}{l}(1)\ \text{When } V = 0,\ 0 \leqslant x \leqslant x_1,\ Q = Q_0 = MF(c_0') = const. \\ (2)\ \text{When } V > 0, \text{ at } x = 0,\ Q = MF(c) = 0 \\ (3)\ \text{For all values of } V,\ \int_0^\infty MF(c)\mathrm{d}x = \int_0^\infty Q_0\mathrm{d}x = const.\end{array}\right\} \quad (10)$$

The solution of equation 4 for these conditions is

$$\left.\begin{array}{l}Q = 0 \text{ for } 0 \leqslant x < \dfrac{Vc_0'}{MF(c_0')} \text{ and for } x > x_1 + \dfrac{Vc_0'}{MF(c_0')} \\ Q = Q_0 \text{ for } \dfrac{Vc_0'}{MF(c_0)} < x < x_1 + \dfrac{Vc_0'}{MF(c_0')}\end{array}\right\} \quad (11)$$

The conclusion is thus reached that the band should remain sharp during the process of development and that its width should remain constant as it moves down the column. Experimentally these conclusions are found to hold only approximately.

The solutions 7 and 11 have been verified for the special case of the isotherm $f(c) = Kc$, where K is a constant, by an independent method in which the column was considered as being divided up into a large number of thin layers perpendicular to the wall of the tube. The process of formation or development of the chromatogram was treated as a succession of steps; in each step a small element of volume of solution was allowed to pass through the column layer by layer, coming into equilibrium with each layer in turn. The over-all effect was obtained by summing the effects of the individual steps in the process; a limiting value was then found for the summation by letting the thickness of the layers and the magnitude of the elements of volume become infinitesimal. The calculation will not be presented here in detail since it is tedious and treats only a special case. The method just outlined does, however, have the advantage of making clear the physical reason for the discontinuous nature of the bands: as an infinitesimal element of volume of solution passes over an infinitesimal element of length of the column just beyond the leading edge of the band, it deposits an infinitesimal amount of solute. The concentration of solute in the element of volume decreases in this process however by a *finite* amount, so that the concentration of the solution becomes zero in a small number of infinitesimal steps, *i. e.*, within an infinitesimal distance from the leading edge of the band.

In the development of equations 7 and 11 it has been assumed that the volume of the interstitial space between the particles of adsorbent is negligible, and that the volume v of solution or V of developing solvent is passed completely through the column before the measurement of the width and position of the band is made. In actual practice the interstitial volume is not negligible, and the developing solvent is added as soon as all of the original solution has entered the column, and is added continuously until the development has been carried to a convenient stage. If these facts are taken into account, the treatment of the problem is little altered.

Let the volume of interstitial space per centimeter length of the column be α cc. Then it is seen readily that the initial width of the band when a volume v of solution has entered the column is $vc^0/(\alpha c^0 + Mf(c^0))$. The edges of the band are sharp, as before, and the amount of solute per centimeter length of the band has the uniform value $\alpha c^0 + Mf(c^0)$ millimoles.

Let us now consider in detail the development of this band, assuming that the same solvent as was used in forming the band is added as soon as the solution has all entered the column. When a small volume ΔV of solvent enters the column, the liquid within the column will be displaced a distance $\Delta V/\alpha$. A volume ΔV of solution whose concentration is c^0 will leave the lower edge of the band and will form an extension to the band of width $\Delta Vc^0/(\alpha c^0 + Mf(c^0))$. Suppose the upper edge of the band meanwhile has migrated a distance Δx. Since the liquid between this point and the point $x = 0$ has not passed through any part of the band, the volume of solvent which has been used to dissolve the solute formerly adsorbed between $x = 0$ and $x = \Delta x$ is $\Delta V - \alpha\Delta x$. If the concentration of solute in this volume of solvent has reached the value c^0, then

$$(\Delta V - \alpha\Delta x)c^0 = Mf(c^0)\Delta x$$

Whence

$$\Delta x = \frac{\Delta Vc^0}{\alpha c^0 + Mf(c^0)}$$

We conclude that the band should maintain the constant width $vc^0/(\alpha c^0 + Mf(c^0))$, and should migrate at a rate $c^0/(\alpha c^0 + Mf(c^0))$ cm. per cc. of developing solvent added to the column, provided the same solvent is used in developing the band as was used in forming it. A similar analysis

may be used to discuss the case in which a new solvent is used for development, whose adsorption isotherm is $q/m = F(c)$. As in the previous treatment (equation 9) let $f(c^0) = F(c^{0\prime})$. Then it is shown readily that the width of the band will change to $vc^0/(\alpha c^{0\prime} + Mf(c^0))$; after it has reached this value the width will remain constant and the band will migrate at a rate $c^{0\prime}/(\alpha c^{0\prime} + Mf(c^0))$ cm. per cc. of developing solvent added to the column. In practice the width of the bands varies widely with changes in the nature of the developing solvent. A discussion will now be given of the formation and development of chromatograms formed from solutions containing several solutes. In this discussion the volume of the interstitial space between the particles of adsorbent will for convenience be neglected. The treatment can easily be extended to take the interstitial volume into account.

The Chromatography of Mixtures.—The treatment of the formation and development of a chromatogram containing a mixture of solutes is very simple if the solutes are independently adsorbed, *i. e.*, if the adsorption isotherm of each solute is a function of the concentration of that solute alone. In that case each of the solutes will form a uniform band of width $vc_i^0/Mf_i(c_i^0)$, where v is the initial volume of the solution, c_i^0 is the initial concentration of the i'th solute, and $f_i(c_i)$ is the corresponding adsorption isotherm. These bands, each of which has its origin at $x = 0$, will overlap; if the solutes are colored, the resulting chromatogram will appear as a series of contiguous color zones, throughout each of which the color is constant. On development with the original solvent, each of the bands will move independently of the others at a rate equal to $c_i^0(Mf(c_i^0)$ cm. per cc. of developing solvent drawn through the column. In general these rates will be different; after sufficient development the bands will be completely separated, and will have, according to the present treatment, their original width and intensity.

In most actual cases, however, the adsorption of one solute is affected by all other solutes present, so that the adsorption isotherm of the i'th solute in a solution containing n solutes is a function of the concentrations of all the solutes. We thus write for the i'th solute

$$q_i/m = f_i(c_1, c_2, c_3, \ldots c_n) \qquad (12)$$

There appear to exist no general rules for predicting the form of the functions f_i in terms of the adsorption isotherms which apply to pure solutions of the individual solutes.[5] It is usually true that the adsorption of one solute from a solution of given concentration is less when a second solute is present than when only the first solute is present, and that the decrease in the adsorption of the first solute is greater, the greater the adsorption tendency of the second solute. It is well known, however, that the reverse effect, co-precipitation, sometimes occurs, and that in other cases two solutes may adsorb independently of each other. The difficulty of finding an explicit expression for the f_i's will not affect our general considerations, however, and we shall see that the results obtained from the treatment of the chromatography of mixtures, with the interdependence of the adsorption isotherms taken into account, are qualitatively similar to the results for the case in which all solutes adsorb independently of one another.

The Formation of a Mixed Chromatogram.—Let a chromatogram be formed from a volume v of a solution containing n solutes whose initial concentrations are $c_1^0, c_2^0, \ldots c_n^0$. Let the adsorption isotherms be defined by equation 12. For the i'th solute the differential equation, its general solution, and the boundary conditions governing the formation of the chromatograms are, in analogy with equations 4, 5 and 6:

$$\left.\begin{aligned} &\left[\frac{\partial}{\partial v}\{Mf_i(c_1, c_2, \ldots c_n)\}\right]_x + \left[\frac{\partial c_i}{\partial x}\right]_v = 0 \\ &c_i = \varphi\left[v - xM\frac{\partial f_i(c_1, c_2, \ldots, c_n)}{\partial c_i}\right] \end{aligned}\right\} \qquad (13)$$

Boundary conditions

$$\left.\begin{aligned} &(1)\ \text{When } v = 0,\ x \geqslant 0: f_i(c_1, c_2, \ldots, c_n) = 0 \\ &(2)\qquad v > 0,\ x = 0: f_i(c_1, c_2, \ldots, c_n) = f_i(c_1^0, c_2^0, \ldots, c_n^0) \\ &(3)\quad vc_i^0 = \int_0^\infty Q_i dx = \int_0^\infty Mf_i(c_1, c_2, \ldots, c_n)dx \end{aligned}\right\} \qquad (14)$$

The solution for a given value of v is similarly $Q_i = Mf_i(c_1^0, c_2^0, \ldots, c_n^0)$ between $x = 0$ and $x = x_j$ at which point one of the solutes, say the j'th solute, has become exhausted. At this point there will be a discontinuity. Evidently

$$x_j = \frac{vc_j^0}{Mf_j(c_1^0, c_2^0, \ldots, c_n^0)} \qquad (15)$$

The solute which is exhausted at the first discontinuity is thus the one for which the quantity on the right side of equation 15 is lower than that for any other component in the solution. It should be noted that each of the solutes is adsorbed to

(5) Freundlich, "Colloid and Capillary Chemistry," Methuen, London, 1926, p. 200 ff.

some extent between $x = 0$ and the first discontinuity at $x = x_j$.

The amount of the i'th solute adsorbed between 0 and x_j is

$$Mf_i(c_1^0, c_2^0, \ldots, c_n^0)x_j = \frac{f_i(c_1^0, c_2^0, \ldots, c_n^0)}{f_j(c_1^0, c_2^0, \ldots, c_n^0)} vc_j^0 \quad (16)$$

The amount of the i'th solute originally present in the solution was vc_i^0. Hence the concentration of the i'th constituent in the solution which traverses the column beyond the first discontinuity is

$$c_i' = c_i^0 - \frac{f_i(c_1^0, \ldots, c_n^0)}{f_j(c_1^0, \ldots, c_n^0)} c_j^0 \quad (17)$$

The concentrations of all constituents change in this way at the first discontinuity; beyond this point the adsorption of the i'th solute is given by the equation

$$Q_i = Mf_i(c_1', c_2', \ldots, c_n'); c_j = 0 \quad (18)$$

This solution will hold between x_j and the point x_k where the k'th constituent is exhausted and the next discontinuity occurs. The changes in concentration at this discontinuity can be calculated in a similar way, and the process continued until all the solutes are exhausted. The equations representing the formation of a chromatogram from a volume v of a solution containing a mixture of solutes may then be summarized thus:

$$\begin{aligned}
&\text{For } 0 \leq x < x_j, \quad Q_i = Mf_i(c_1^0, c_2^0, c_3^0, \ldots)\\
&\qquad x_j = \frac{vc_r^0}{Mf_j(c_1^0, c_2^0, \ldots)}\\
&\text{For } x_j < x < x_k, \quad Q_i = Mf_i(c_1', c_2', c_3', \ldots)\\
&\qquad c_i' = c_r^0 - \frac{f_i(c_1^0, c_2^0, c_3^0, \ldots)\, c_r^0}{f_j(c_1^0, c_2^0, c_3^0, \ldots)} \quad (19)\\
&\qquad x_k = x_j + \frac{vc_k'}{Mf_k(c_1', c_2', c_3', \ldots)}\\
&\qquad Q_j = 0\\
&\text{For } x_k < x < x_l \quad Q_i = Mf_i(c_1'', c_2'', c_3'', \ldots)\\
&\qquad c_i'' = c_i^0 - \frac{f_i(c_1^0, c^0, c^0, \ldots)c_j^0}{f_j(c_1^0, c_2^0, c_3 \ldots)} - \frac{f_i(c_1', c_2', c_3', \ldots)c_k'}{f_k(c_1', c_2', c_3', \ldots)}\\
&\qquad x_l = x_k + \frac{vc_l''}{Mf_l(c_1'', c_2'', c_3'', \ldots)}\\
&\qquad Q_j = Q_k = 0
\end{aligned}$$

In Fig. 1A is shown a graphical representation of the results obtained from the application of these equations to the hypothetical case of a chromatogram formed from 10 cc. of a solution containing three solutes, each at an initial concentration of 0.02 mole per liter. It has been assumed that the adsorption isotherms are of the type proposed by Langmuir[6] to account for the sorption of dilute gases on solid surfaces:

$$q_i/m = A \frac{a_i c_i}{1 + \sum_j a_j c_j} \quad (20)$$

where A is a constant characteristic of the adsorbent and a_i is a constant characteristic of the i'th solute. The summation is taken over all solutes. This isotherm was chosen because it affords a convenient representation of the effect of one solute in lowering the adsorption of another. For convenience A and M have been taken equal to unity. The value $a_1 = 40$ has been assigned to solute number 1, with $a_2 = 20$ and $a_3 = 5$. For comparison in Fig. 1B, 1C and 1D are shown the chromatograms which would be formed from 10 cc. of a solution containing only one of these solutes at a concentration of 0.02 mole per liter. In connection with Fig. 1A it should be noted that whether the adsorption of a given solute increases or not after passing a discontinuity in the chromatogram depends on the total amount of the given solute which has been adsorbed up to that point, as well as on the solute which is exhausted at the discontinuity.

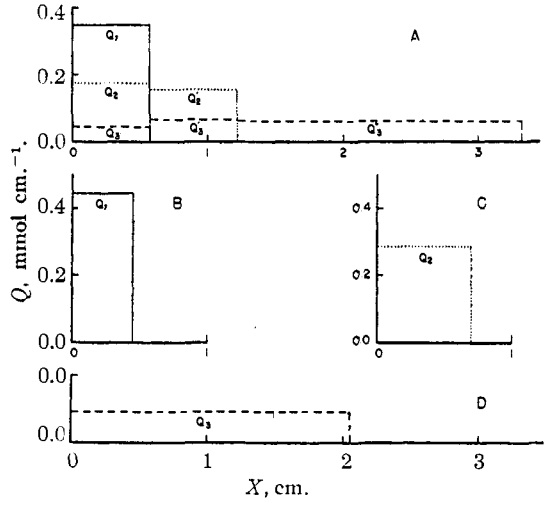

Fig. 1.—A: Chromatogram formed from a solution containing three solutes. B, C, D: Chromatograms formed from the same volume of solution containing only one solute (Solutes 1, 2 and 3, respectively). X is the vertical distance measured from the top of the column.

The Development of Mixed Chromatograms.—Since the discussion of the development of a mixed chromatogram becomes very complicated in the general case, the present treatment will be

(6) I. Langmuir, *Phys. Rev.*, **2**, 331 (1913); **6**, 79 (1915); THIS JOURNAL, **38**, 2221 (1916).

limited to the case of a chromatogram formed from a solution containing two solutes. Let the chromatogram formed from a volume v of a solution of initial concentrations c_1^0, c_2^0, be represented as follows:

$$\left.\begin{aligned} 0 < x < x_1\text{: } & Q_1 = Mf_1(c_1^0, c_2^0);\ Q_2 = Mf_2(c_1^0, c_2^0) \\ & x_1 = \frac{vc_1^0}{Mf_1(c_1^0, c_2^0)} \end{aligned}\right\} \text{Band 1}$$

$$\left.\begin{aligned} x_1 < x < x_2\text{: } & Q_1 = 0;\ Q_2 = Mf_2(c_2') \\ & x_2 = x_1 + \frac{vc'}{Mf_2(c_2')} \\ & c_2' = c_2^0 - \frac{f_2(c_1^0, c_2^0)\, c_1^0}{f_1(c_1^0, c_2^0)} \end{aligned}\right\} \text{Band 2} \qquad (21)$$

If this chromatogram is developed with a small volume V of the same solvent as was used in forming the chromatogram, the two solutes will migrate at different rates. The origin of the adsorbed solute 2 will migrate to a point $x_{20} = Vc_2^0/Mf_2(c_1^0, c_2^0)$. The solution as it reaches the point x_1 will have acquired the concentrations c_1^0, c_2^0; as it passes this point solute 1 will be completely, and solute 2 partially adsorbed. The discontinuity at x_1 will therefore migrate to a point $(v + V)c_1^0/Mf_1(c_1^0, c_2^0) = x_1' = (v + V)x_1/v$. The amount of solute 2 adsorbed between this point and $x = 0$ is then

$$\left\{\frac{(v + V)c_1^0}{Mf_1(c_1^0, c_2^0)} - \frac{Vc_2^0}{Mf_2(c_1^0, c_2^0)}\right\} Mf_2(c_1^0, c_2^0) = (v + V)c_1^0 \frac{f_2(c_1^0, c_2^0)}{f_1(c_1^0, c_2^0)} - Vc_2^0$$

The concentration c_2'' of solute 2 which passed the point x_1' is then given by the expression

$$Vc_2'' = vc_1^0 \frac{Mf_2(c_1^0, c_2^0)}{Mf_1(c_1^0, c_2^0)} - (v + V)c_1 \frac{f_2(c_1^0, c_2^0)}{f_1(c_1^0, c_2^0)} + Vc_2^0$$

Whence

$$c_2'' = c_2^0 - c_1^0 \frac{f_2(c_1^0, c_2^0)}{f_1(c_1^0, c_2^0)} = c_2' \qquad (22)$$

Thus the concentration of solute 2 in the solution traversing band 2 during this development is the same as went into the formation of the second band. The lower end of band 2 will therefore migrate to the point

$$x_2' = x_1' + \frac{(v + V)c_2'}{Mf(c_2')} = \frac{V + v}{v} x_2$$

The development will proceed in this way until the point x_{20}, the origin of solute 2, overtakes the point x_1', the discontinuity between bands 1 and 2. This will occur at a volume

$$V = v \left\{\frac{c_2^0 f_1(c_1, c_2^0)}{c_1^0 f_2(c_1^0, c_2^0)} - 1\right\}^{-1}$$

When V exceeds this value, the bands will separate. The solute 2 will all be contained in the second band which on further development will migrate at the rate $c_2'/Mf_2(c_2')$, and will maintain the constant width

$$\frac{vc'}{Mf_2(c_2')} \left\{\frac{c_2^0 f_1(c_1^0, c_2^0)}{c_2^0 f_1(c_1^0, c_2^0) - c_1^0 f_2(c_1^0, c_2^0)}\right\}$$

It should be noted that the volume V required for separation of the bands is infinite if the ratio $c_2^0 f_1(c_1^0, c_2^0)/c_1^0 f_2(c_1^0, c_2^0)$ is equal to unity. The departure of this ratio from unity is thus a measure of the ease of separation of two substances by chromatography on a given adsorbent. If the ratio is equal to unity the separation is impossible unless a different adsorbent is used or the value of the ratio is changed by altering the concentration of the initial solution.

The development of solute 1 is somewhat more complicated. As the origin of solute 2 moves through the first band, it will be accompanied by a discontinuity in the adsorption of solute 1. Suppose the adsorption of solute 1 between the beginning of the first band and the origin of solute 2 in the first band is given by $Q_1 = Mf_1(c_1')$. When the volume V of developing solvent has passed through the system, the origin of solute 1 will have moved to $x_{10} = Vc_1'/Mf_1(c_1')$. Between x_{20} and x_1' the adsorption of solute 1 is given by $Q_1 = Mf_1(c_1^0, c_2^0)$. Application of the conservation condition gives

$$vc_1^0 = Mf_1(c_1')(x_{20} - x_{10}) + Mf_1(c_1^0, c_2^0)(x_1' - x_{20}) \qquad (23)$$

Substituting the expressions previously derived for x_{20}, x_{10} and x_1', we obtain

$$c_2^0\{f_1(c_1') - f_1(c_1^0, c_2^0)\} = f_2(c_1^0, c_2^0)\{c_1' - c_1^0\} \qquad (24)$$

This equation can be solved for c_1' if the initial concentrations and the adsorption isotherms are known. When the two bands have separated, the solute 1 will be all contained within the first band, whose rate of migration will then be $c_1'/Mf_1(c_1')$, and whose width will maintain the constant value $vc_1^0/Mf_1(c_1')$. In Fig. 2 there is shown a graphical representation of several stages in the development of a hypothetical chromatogram which is assumed to have been formed from 10 cc. of a solution in which $c_1^0 = c_2^0 = 0.02$ mole liter^{-1}. The adsorption isotherms have been assumed to be of the Langmuir type, with

$$Q_1 = MA \frac{a_1 c_1}{1 + a_1 c_1 + a_2 c_2};\quad Q_2 = MA \frac{a_2 c_2}{1 + a_1 c_1 + a_2 c_2}$$

For convenience M and A have been given the value unity, and the values $a_1 = 40$, $a_2 = 10$ have

been assumed. Q_1, Q_2 are in units of millimoles per centimeter.

The treatment evidently can be extended to more complex situations involving a greater number of solutes, but the computations become much more involved. The development of such a complex chromatogram may take place in any one of a number of ways depending on the initial concentrations and the adsorption isotherms. The initial development will lead to broadening of the bands. At some point in the development the initial band system of contiguous bands will split into two sub-systems, each of which may later split again. This process will continue until each of the solutes initially present occupies a separate band, provided that the condition for separability of the solutes is fulfilled.

Discussion of the Theory

The theory presented here is in essence a very simple one. Since the processes of diffusion, adsorption and desorption proceed at finite rates, however, the present theory, in which it is assumed that the effects of diffusion can be neglected and that equilibrium between solute and adsorbed material is instantaneously reached, can provide only an approximate description of the phenomena of chromatography. The theory does account for the qualitative features of chromatography in a satisfactory way and provides a convenient basis from which a more precise theory may be developed.

The theory predicts that a chromatogram formed from a solution containing only one solute should form a single sharp band, and that if this chromatogram is developed with the same solvent as was used in its formation, the band should maintain a constant width and sharply defined edges. Experimentally none of these predictions is quantitatively verified. Solutes which adsorb strongly do in many cases form surprisingly sharp bands whose width does not vary rapidly on development and whose edges become diffuse only slowly. On the other hand, a solute which is not strongly adsorbed often forms a band whose lower edge is somewhat diffuse when the chromatogram is formed. The width of such a band and the diffuseness of its edges may increase quite rapidly as development proceeds. These observations are readily explained when the factors which have been neglected in the present treatment are taken into account. The width of a band may increase

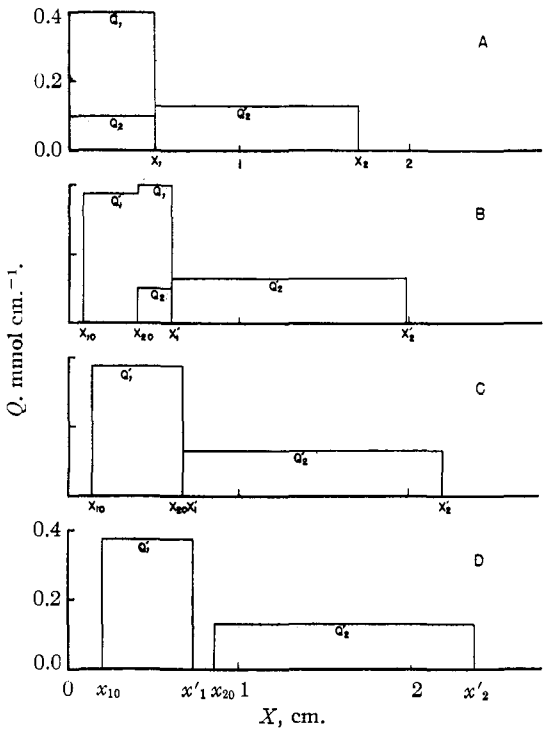

Fig. 2.—Stages in the development of a two-solute chromatogram, after various volumes V of developing solvent have passed through the column: A, V = 0; B, V = 2.00 cc.; C, V = 3.33 cc.; D, V = 5.00 cc. X is the vertical distance measured from the top of the column.

because of diffusion, or because the leading edge of the band migrates too rapidly on account of a low rate of adsorption, or because the trailing edge of the band migrates too slowly on account of a low rate of desorption. All these effects will lead the edges of the band to become diffuse. The process of diffusion is probably the least important of the factors leading to broadening of the bands since its effects are automatically corrected to a considerable extent as development proceeds. It is likely that in many cases a solute which is adsorbed but slightly will also have a low rate of adsorption, so that the leading edge of its chromatogram should migrate too rapidly and become more and more diffuse. The effects arising from low rates of adsorption and desorption can be diminished by decreasing the rate at which liquid flows through the column, but as the rate of flow decreases the importance of diffusion effects increases.

A further complication is introduced by the fact that the flow of liquid through the column is not uniform. The liquid usually flows more slowly at

the center of the column than near the walls of the tube. It is difficult to eliminate this effect completely, though its importance can be reduced somewhat by careful packing of the column. As a result of the varying rate of flow the development of a chromatogram proceeds more rapidly near the wall of the tube than at the center, so that an initially uniform and approximately horizontal band becomes dome-shaped (concave downward). Diffusion can then occur in a radial direction in the column, and the effect of this diffusion is not corrected for in the process of development. As a consequence the colored band as it is observed through the wall of tube appears to become wider and both edges to become diffuse. When the column is removed from the tube and carefully sliced it is often found that the band is narrower and more sharply defined near the center of the column than near the periphery.

Some preliminary experiments have shown that the band widths and rates of development predicted by means of the theory from adsorption measurements are correct as to order of magnitude. In these experiments all adsorption data used were determined directly with the same adsorbent as was used in the chromatographic experiments. The value of α, the volume of voids per centimeter length of the column, was determined by measuring the length of the packed column filled by a measured volume of liquid. The experiments will not be described in detail because of the wide variation in adsorbent properties among samples of a given adsorbent obtained from different sources. The experiments were moreover of only an exploratory nature; a systematic study of the extent to which the predictions of the theory approximate the results of experiment has yet to be made.

The following results were obtained. The initial width of a band formed on alumina from a solution of picric acid in benzene was 10% greater than the width predicted from adsorption measurements; on the other hand, a band formed on alumina from an aqueous solution of methylene blue had a width three times the predicted value. In a test of the relation between the width of the band and its rate of migration on development, the observed rate of development was found to be about 80% of the predicted rate. In this experiment a solution of lycopene in a mixture of benzene and petroleum ether was used; the adsorbent was calcium carbonate. The width of the lycopene band did not remain constant, but increased from 2 mm. to 4 mm. while the band was migrating 15 mm.

The theory presented here differs qualitatively from earlier ideas about the subject in one important respect. The belief has been expressed by some authors that the first color zone of a mixed chromatogram contains, before development occurs, essentially only the most strongly adsorbed solute. According to the present treatment, on the other hand, the first color zone should contain some of each solute initially present, in proportions determined by the initial concentrations and the adsorption isotherms. That the latter conclusion is correct was verified by the following experiment. A chromatogram was prepared by pouring a large volume of an extract of dried grass in a mixture of petroleum ether and benzene into a large column packed with powdered sugar. Seven bands of various widths and colors were formed; the first was quite wide. The liquid was forced completely through the column, which was then dried and removed from the tube. The first color zone was cut from the column and repacked on top of a second narrower column freshly packed with powdered sugar. When the same solvent as was used in preparing the first chromatogram was poured through this new column, the same sequence of six color zones appeared below the top band as occurred below the top band in the original chromatogram; the intensities of color and the relative widths of the bands were moreover roughly the same as in the original band system, in accordance with the present theory.

The present theory is also applicable to capillary analysis. In capillary analysis the lower end of a vertical strip of porous paper is allowed to dip into a solution of colored solutes. The solution rises in the paper strip because of capillarity; if the solutes are adsorbed by the paper a chromatogram is formed at the lower end of the paper in accordance with equations 19. This chromatogram may then be developed by dipping the lower end of the strip of paper into pure solvent and allowing solvent to rise in the paper through the initial chromatogram.[7]

The present treatment also may be applied to the chromatographic technique developed by W. G. Brown.[8] In this technique the chromatogram is formed on a sheet of blotting paper pressed be-

(7) Schwab and Jockers, *Angew. Chem.*, **50**, 546 (1937).
(8) W. G. Brown, *Nature*, **143**, 377 (1939).

tween glass plates, the upper one of which has a small hole bored through the center. The solution is allowed to run into the blotting paper from a glass tube whose fine capillary tip passes through the hole in the upper plate and presses against the paper. The chromatogram spreads radially from the point of contact as center. If M' is the mass of blotting paper per square centimeter, Q' the number of millimoles adsorbed per square centimeter, and r the radius measured from the point of contact, the equations analogous to equation 7 and 11 become, for formation of the chromatogram

$$\left.\begin{aligned} 0 \leqslant r_2 < \frac{vc^0}{\pi M'f(c^0)} \quad & Q' = M'f(c^0) \\ r^2 > \frac{vc^0}{\pi M'f(c^0)} \quad & Q' = 0 \end{aligned}\right\} \qquad (25)$$

and for development of the chromatogram with a volume V of the same solvent

$$\left.\begin{aligned} 0 < r^2 < \frac{Vc^0}{\pi M'f(c^0)}, \quad & Q' = 0 \\ \frac{Vc^0}{\pi M'f(c^0)} < r^2 < \frac{(V+v)c^0}{\pi M'f(c^0)}, \quad & Q' = M'f(c^0) \\ r^2 > \frac{(V+v)c^0}{\pi M'f(c^0)}, \quad & Q' = 0 \end{aligned}\right\} \qquad (26)$$

Thus it is predicted that the area of the ring will remain constant, and that the band will become narrower as development proceeds. This prediction is usually not verified experimentally because of diffusion and the finite rates of adsorption and desorption. The equations 25 and 26 may be readily corrected to take into account the interstitial volume between the fibers of the paper, or extended to the case of a mixed chromatogram. If the interstitial volume is taken into account it readily can be shown that the area of the band should remain constant if the same solvent is used for development as was used in forming the chromatogram, but should change if a different solvent is used for development.

It remains to discuss the possibility of using the present theory to predict on the basis of known sorption isotherms whether or not a given adsorbent may be used for the chromatographic separation of a given set of solutes. In view of the approximation involved in the neglect of diffusion and of rates of adsorption and desorption, it is evident that only an approximate prediction can be made; the complete application of the theory requires moreover a knowledge of the dependence of the sorption isotherms on the concentrations of all solutes present. In most cases it is more difficult and tedious to acquire this knowledge than to determine empirically which adsorbents are useful for the separation. In case adsorption isotherms for solutions containing only single solutes are available, a rough prediction can be made by assuming that the solutes are independently adsorbed; the separations actually obtained will in general be better than those predicted on the basis of this assumption. A better approximation may be made by fitting the adsorption isotherm for each solute, using measurements made on solutions containing that solute alone, to a curve of the Langmuir type: $q_i/m = Ka_ic_i/(1 + a_ic_i)$. It is usually possible to fit adsorption data to such a curve over small ranges of concentration, especially at low concentrations, say from 0 to 0.01 mole per liter. A very rough approximation to the adsorption isotherms which apply to a solution containing a mixture of solutes may then be obtained by using the a_i's obtained in this way, assuming that in mixed solutions $q_i/m = Ka_ic_i/(1 + \Sigma_i a_ic_i)$. Calculations made in this way, in spite of their approximate nature, might be useful for instance in deciding on a suitable adsorbent with which to attempt the separation of amino acids resulting from protein hydrolysis.

Summary

A theory of chromatographic analysis has been developed on the basis of the assumption that equilibrium between solution and adsorbent is instantaneously established and that the effects of diffusion can be neglected. The theory accounts qualitatively for the separations effected in chromatographic analysis, for the uniformity of color in the adsorption bands, and for the sharpness of the edges of the bands. Some experimental evidence is presented to show that the theory provides a semi-quantitative description of chromatographic adsorption. A brief discussion is given of the factors which make quantitative agreement between the theory and experiment unlikely.

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