241. *Theory of Chromatography. Part IV. The Influence of Incomplete Equilibrium on the Front Boundary of Chromatograms and on the Effectiveness of Separation.*

By E. Glueckauf and J. I. Coates.

It is shown that in the chromatographic column, under the usual working conditions, complete local equilibrium is not obtained. A theory is developed for the effect of incomplete equilibrium on chromatographic boundaries, and the form of observed boundaries agrees with those calculated. On decrease of grain size and flow velocity, equilibrium becomes more complete, and then some chromatographic separations can be effected with ease which are impossible by the usual methods; *e.g.*, the separation of nickel and cobalt ions on alumina.

(a) *Chromatographic Boundaries.*—The influence of incomplete local equilibrium is very similar to a diffusion effect and so shows itself most markedly at the boundaries of the chromatograms where high concentration gradients exist. Normal binary chromatograms when fully developed have, in the absence of disturbing effects, the form shown in Fig. 1 of the preceding paper. We may distinguish here two types of boundaries : (i) sloping rear boundaries such as AB, CD, and $C'D'$, and $L'Q$, and (ii) sharp front boundaries such as the threshold boundary HG or PQ and the inter-boundary EF, $E'F'$ or LK, $L'K'$ which is the front of solute 2. As has been shown by De Vault (*J. Amer. Chem. Soc.*, 1943, **65**, 532) the two front boundaries have normally (*i.e.*, for isotherms concave against the c-axis) self-sharpening properties. This means that, even if by some artificial means HG were made sloping, it would again become vertical during the process of development—at least theoretically. This self-sharpening tendency increases with the curvature of the isotherm and it is nil for linear isotherms (see De Vault, eqn. 7).

In the case of the threshold boundary HG, the self-sharpening effect is usually very considerable, as linear single-solute isotherms are rare. In the case of the inter-boundaries EF the corresponding function depends essentially on the " exchange " isotherm between the two solutes. If the two solutes are very different and easily separable, this boundary, too, usually shows considerable self-sharpening, but if the two solutes are very similar, the exchange isotherm is practically linear and then this effect is negligible. The inter-boundary is then referred to as a non-sharpening front boundary.

(b) *Attainment of Complete Equilibrium.*—In order to study the effect of non-equilibrium and diffusion phenomena, chromatographic elution curves were measured, using adsorbents of different grain size. The adsorbents were ground to a diameter as low as 0·01 mm. (as compared with the commercial grain sizes of about 0·08 mm.), and a uniform grain size was obtained by elutriation in air streams of varying velocity.

The flow rate of the solvent in the chromatographic column was varied from 0·001 cm./sec., which means 1 drop in 2 mins. for a tube of 1 cm. diameter, to the usual flow rates of 10—60 drops per min.

Systematic experiments showed that the quality and sharpness of non-sharpening inter-boundaries, *e.g.*, such as between copper and manganese ions on Zeo-karb H.I. or H.I.P., continuously increased with reduction of grain size and flow rate. There must be an optimum flow rate, as the latter can obviously not be infinitely decreased without introducing disturbances on account of diffusion. But this latter effect is so small compared with the non-equilibrium effect and other disturbances that even at the lowest flow rates used an improvement was still noticeable compared with faster flow rates.

Under the conditions mentioned above ($d = 0·01$ mm., $F = 0·001$ cm./sec.), we were able to obtain, for a column of 1 m. length, non-sharpening inter-boundaries between 0·6 and 1 cm., and self-sharpening front boundaries extending on one occasion over only 0·10 cm., the former corresponding to 0·5—1%, the latter to 0·1% of the threshold volume.

That these conditions are not obtained with the usual practice can be seen from Fig. 10*a* of Weil-Malherbe (*J.*, 1943, 303), which contains about the best chromatographic experiments published. Here a non-sharpening front (benzpyrene on silica gel) extends over 25% of the threshold volume, and a strongly self-sharpening one (benzpyrene on alumina) over 12% of the threshold volume. A check on the form of non-sharpening front boundaries is therefore essential in all experimental work connected with the study of elution curves and, in particular, in such cases where elution curves are used for the calculation of adsorption equilibria (see Glueckauf, *Nature*, 1945, **156**, 748, and Part II).

Although it is apparent that non-equilibrium phenomena can easily occur in the case of exchange materials, it is not usually realised that the same, though perhaps to a lesser degree, also applies to the so-called " adsorbents " like alumina and calcium carbonate, as adsorption takes place not only on the surface, but also inside the grains. The reason for this is that most experimenters investigating the form of bands have worked with single solutes which usually have strongly self-sharpening fronts. These maintain a reasonable sharpness even if considerably disturbed by non-equilibrium phenomena. Consequently, observers are easily deceived by this appearance of moderately sharp fronts, which are usually taken as proof of the absence of non-equilibrium phenomena.

How far the process of wishful thinking can go is shown, *e.g.*, by Jacobs and Tompkins (*Trans. Faraday Soc.*, 1945, **41**, 401, Fig. 1, Curve A), who convinced themselves—with the help of the equilibrium curve applied to a diffuse non-equilibrium boundary—that, in spite of the very diffuse eluted front which they found, the front of the adsorbate in the column
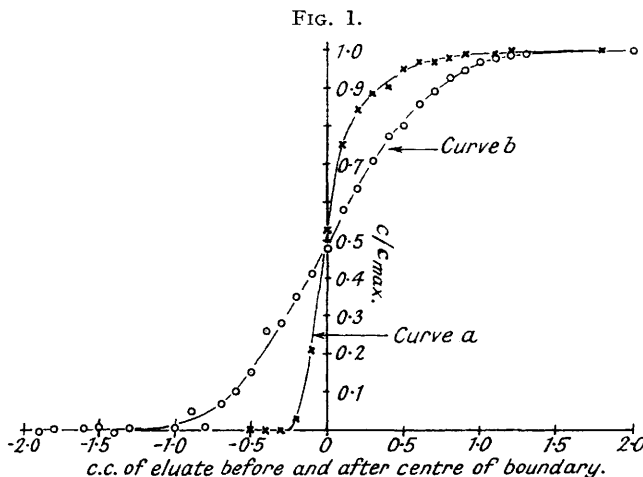
FIG. 1.

c.c. of eluate before and after centre of boundary.

Experimentally measured boundaries. (a) *Self-sharpening threshold boundary of* N-$CoCl_2$ *solution in column of acid Zeo-karb.* ($\times$ — $\times$ — $\times$) *(compare with curve calculated for* $bc^0 = 10$ *in Fig. 4).* (b) *Non-sharpening inter-boundary between frontal band of* $^{59}CoCl_2$ *and mixed band of* $^{59}CoCl_2$ *and* $^{60}CoCl_2$ *in same column of Zeo-karb* (○— ○—○) *(symmetrical S shape).*

must have been very sharp. By doing so, they overlooked the fact that a sharp front of adsorbate moving through the column must deliver the solute at the end in an equally sharp-fronted elution curve—simply by reason of the conservation of matter (for details see eqn. 5).

The non-equilibrium phenomena, however, become very obvious when fronts are concerned which have little or no self-sharpening properties, *e.g.*, the boundary of one solute against another of similar adsorption affinity. As it is just such a boundary which occurs at the point of separation of two similar solutes (boundary *EF* in Fig. 1 of Part III), these non-equilibrium effects greatly reduce the efficiency of the separation.

(c) *Form of the Disturbed Self-sharpening Front Boundary.*—No theory has so far been given for the theoretical form of a front boundary disturbed by non-equilibrium phenomena in the case of a non-linear isotherm. Weiss (*J.*, 1943, 301; eqns. 59—62 and Fig. 5) has produced a schematical representation for the case of a linear isotherm which, however, apart from the diffuseness, does not really represent the experimental facts (see our Fig. 1, curve *b*, showing that the measured elution curve of a front boundary for a strictly linear isotherm has a symmetrical sigmoid form. A similar conclusion has also been reached by Klotz, *Chem. Rev.*, 1946, **39**, 243). A satisfactory theory for the case of the linear isotherm has been produced by Dr. H. London which, however, has not yet been published.

In the case of a self-sharpening front boundary caused by a non-linear isotherm, the general differential equations become very complicated, but, after the front has travelled some distance, a final form is attained which can be represented by a simple equation. Here the effects of non-equilibrium and self-sharpening just balance each other. For simplicity the calculations are carried out for the " undeveloped " chromatogram where the front has the original concentration c^0.

In the case of non-equilibrium, the usual chromatographic equation of mass conservation (see Part II, eqn. 2), *viz.*,

$$[\Delta v/\Delta x]_c = df^*_{(c)}/dc + \alpha \qquad (1)$$

changes to

$$[\Delta v/\Delta x]_c = dq^*/dc + \alpha \qquad (2)$$

where q^* is the amount actually adsorbed, the missing amount $(f^*_{(c)} - q^*)$ being due to disequilibrium. When at a given point (x) of the front boundary the concentration rises during the passage of the front through this point, q^* is, with good approximation, represented by the equation

$$dq^*/dt = K(f^*_{(c)} - q^*) \qquad (3)$$

which means that the rate of diffusion into the grains is essentially proportional to the amount still required to produce equilibrium. Here K is a diffusion factor (the inverse of the time in which a grain of adsorbent reaches $1/e$ of its equilibrium adsorption), and $K = D/(\eta d)^2$, where D is the diffusion constant of the solute in the solid material, d is the grain diameter, and $\eta(<1/2)$ depends on the geometry of the grains.

FIG. 2.
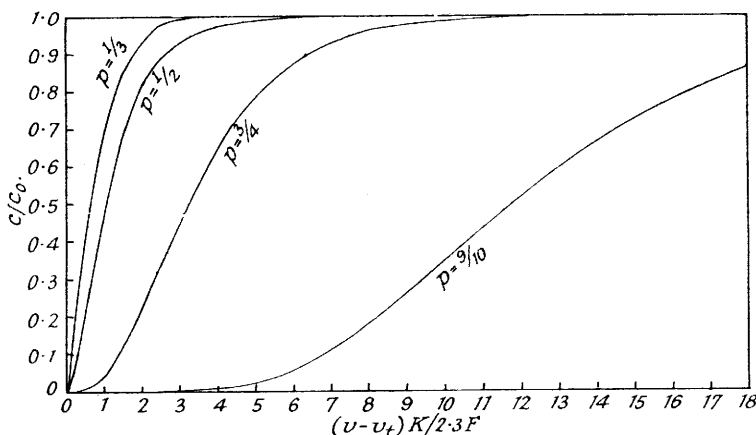
Self-sharpening front boundaries for solutes obeying Freundlich isotherms $q = ac^p$ *for different values of* p.

After competition between the self-sharpening and the non-equilibrium effects has resulted in a final form of the front boundary, the whole front boundary and thus also the point of concentration c move with the usual velocity of the sharp undeveloped front boundary

$$[\Delta v/\Delta x]_c = f^*_{(c^0)}/c^0 + \alpha \qquad (4)$$

It follows from equations (2) and (4) that in this final state

$$dq^*/dc = f^*_{(c^0)}/c^0 \qquad (5)$$

This means that, no matter what the form of the equilibrium isotherm, q^* must always be a linear function of c. Eliminating q^* by means of (5) in (3) and replacing t by v/F (where F = flow velocity of solvent, in c.c./sec.), we get

$$dc/dv = (K/F)[f^*_{(c)} \cdot c^0/f^*_{(c^0)} - c] \qquad (6)$$

Equation (6) has a particularly simple form in the case of the Freundlich isotherm

$$f^*_{(c)} = A \cdot c^p \qquad (7)$$

Integration of (6) then leads to the equation for the elution curve of a disturbed self-sharpening front (final state) :

$$v - v_t = \frac{-F}{K(1-p)} \cdot \log\left[1 - (c/c^0)^{(1-p)}\right] \qquad (8)$$

Similarly one obtains for the frontal distribution in the column

$$x_f - x = \frac{-F}{K(1-p)} \cdot \frac{c^0}{f_{(c^0)}} \cdot \log\left[1 - (q^*/q^0)^{(1-p)}\right] \qquad (9)$$

where x_f is the most advanced point of the front boundary. Equation (8) requires that the length of the eluted front is independent of the concentration c^0, which is perfectly shown in Jacobs and Tompkins's Fig. 2 (*loc. cit.*, p. 402). Equation (8) also shows the effect of p on the slope and extension of the front boundary, which is demonstrated in the experiments of Weil-Malherbe (*loc. cit.*, Fig. 10a), though not quantitatively, since, with the short columns used, the final state is not attained for values of p near unity.

Fig. 2 gives the theoretical curves of eluted front boundaries in the stationary state for various values of p. It shows that for small values of the Freundlich exponent p (*e.g.*, $p = \frac{1}{3}$) the front rises sharply but reaches the maximum (c^0) only asymptotically. For comparatively large values of p it has a sigmoid form, which for $p = \sim 1$ reaches both the values $c = 0$ and $c = c^0$ asymptotically.

<div align="center">FIG. 3.</div>
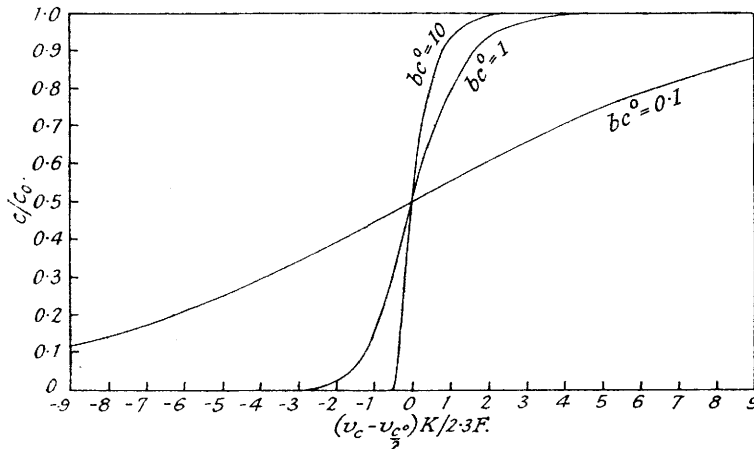
Self-sharpening front boundaries for solutes obeying Langmuir isotherm $q = ac/(1 + bc)$ *for different values of* bc^0.

Equation (8) also shows that the question of grain size and flow rate comes into play to a very marked extent only when very similar substances have to be separated, where the inter-boundary is due to an exchange isotherm which, for very similar substances, is almost linear $(1 - p = \sim 0$; thus $v - v_t = \sim \infty$). No final state is attained by the front boundary when $p = 1$ (linear isotherm), under which conditions there is no self-sharpening effect, and, consequently, the slope of such a front boundary continues to decrease indefinitely.

Very similar conditions arise in the case of the Langmuir isotherm

$$f^*_{(c)} = ac/(1 + bc) \qquad \cdots \cdots \cdots (10)$$

Here integration of eqn. (6) leads to the equation for the elution curves :

$$v_c - v_{\frac{c^0}{2}} = -\frac{K}{F}\left[\frac{1}{bc^0} \cdot \log (c^0 - c)/c + \log 2 (c^0 - c)/c^0\right] \qquad \cdots \cdots (11)$$

The form of the front boundary here depends on bc^0 which, according to the physical interpretation of the Langmuir isotherm, is the ratio of covered to free adsorbing surface. As can be seen from Fig. 3, the self-sharpening tendency increases with bc^0, *i.e.*, with increasing surface saturation. A comparison with Fig. 2 shows that there is a complete parallelism in the form of the front boundaries for the two isotherms, small values of p corresponding to large values of bc^0 and *vice versa*.

The diagrams of two accurately measured front boundaries are shown in Fig. 1. Curve *a* is the eluted front boundary of a 1N-$[CoCl_2]$ band after passing $3g$ of acid permutite. The isotherm is approximately of the Langmuir type with a value of $b \simeq 3$—4 (thus $bc^0 = 3$—4). A comparison with the corresponding calculated curves in Fig. 3 shows a good agreement. Curve *b* shows an inter-boundary between a mixture of ^{59}Co and ^{60}Co against a front-band of pure ^{59}Co, where the rise in the concentration of ^{60}Co has been measured. As the exchange isotherm between the two isotopes is strictly linear, there is no self-sharpening to be expected, and the experiment shows, in agreement with the theory, a symmetrical S-form for this front boundary.

The elution curve of the boundary of the two isotopes was measured by means of the β activity of ^{60}Co ($\tau = 5.5$ years) in single drops of eluate which were dried on filter-paper.

(d) *Effect of Non-equilibrium on Separation of Two Solutes.*—A typical example of the importance of such considerations is shown in the separation of copper and nickel on commercial alumina by Jacob and Tompkins (*loc. cit.*, p. 398, Fig. 3), using a linear flow velocity of 0·07 cm./sec. Even after the band has travelled several times the distance which they expect to be required for complete separation, the mixed part of the band still contains about 70% of the nickel and about 30% of the total copper.

We obtained very similar results with copper and manganese (the latter being used instead of nickel owing to its easy volumetric determination). Using a flow velocity of 0·027 cm./sec. and 0·25N-sulphuric acid for elution on 2·2 g. of commercial alumina, we obtained the elution curve of Fig. 4a, which shows the retention of a mixed band with 47% of the manganese and about 25% of the copper. (Figs. 4a and b were obtained by analysing single drops of eluate.)

FIG. 4.
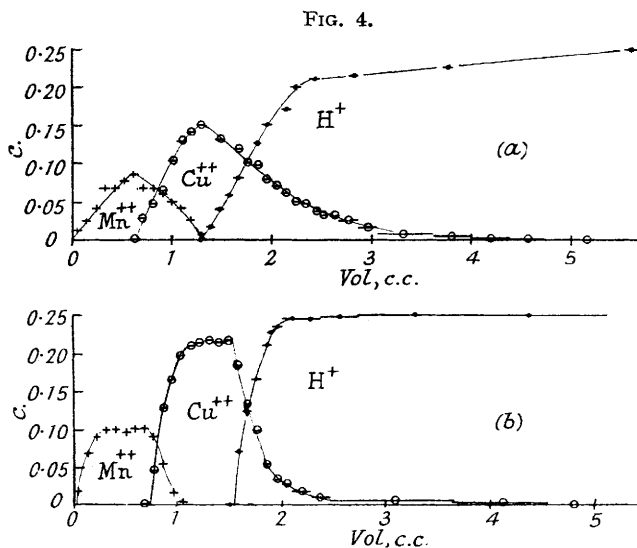
Elution curves of mixture of $CuSO_4$ *and* $MnSO_4$ *chromatographed on 2·2 g. of* Al_2O_3. *Development with* 0·25N-H_2SO_4.

Abscissa : volume of eluate after appearance of solute in eluate.
Ordinate : concentration of solutes in eluate (*milliequiv./c.c.*).
(a) *Diameter of* Al_2O_3 = 0·08 *mm.*; *flow velocity* = 0·027 *cm./sec.*
(b) *Diameter of* Al_2O_3 = 0·01 *mm.*; *flow velocity* = 0·0014 *cm./sec.*

By reducing the grain size from 0·08 to 0·01 mm. diameter and the flow velocity to 0·0014 cm./sec., and otherwise using identical conditions, the effect of the diffusion and non-equilibrium phenomena was much reduced (see Fig. 4b). The mixed part of the band now contained only 20% of the manganese and 14% of the eluted copper, and in some experiments still less. (Actually, separation appeared to be much more complete in the column, and the small amount of mixing observed in the eluate seemed to be due to the mixing of 2 or 3 drops at the bottom of the tube.) Also, in other respects, the two experiments show marked differences. In the second experiment (Fig. 4b) all the front boundaries of the Mn^{++}, Cu^{++}, and H^+ are fairly steep, and the flat levels of the manganese and copper bands show clearly that these bands are not being " developed " by a diffuse rear boundary, a feature which is lost completely through non-equilibrium phenomena in Fig. 4a, and in the copper elution curves of Jacob and Tompkins (*loc. cit.*, p. 402, Fig. 2).

Although, under ordinary working conditions (commercial grain size and usual flow rate), some separation is still shown in cases where the differences in adsorption affinity and the self-sharpening tendencies of the inter-front are comparatively large, as is the case for Cu–Ni and Cu–Mn, it becomes impossible to separate solutes of very similar adsorption affinity, which consequently have fairly linear exchange isotherms, and whose inter-front is practically non-self-sharpening.

Such solutes as, *e.g.*, the group Ni, Co, Cd, Fe^{II}, are usually considered as non-separable (see Schwab and Jockers, *Z. angew. Chem.*, 1937, **50**, 646), but their inseparability is entirely due to the disturbing influence of diffusion and non-equilibrium phenomena which almost completely undo any chromatographic separation. No difficulty is experienced in effecting an almost complete separation—with adjoining bands, of course—between any two of these solutes if a smaller grain size and a lower rate of flow is employed. To give an example : 0·15 c.c. of 0·5N-NiSO$_4$ + 0·5N-CoSO$_4$ were developed with 0·25N-H$_2$SO$_4$. The original band, in a column of alumina of 8 mm. diameter, had a length of 4·5 mm. The band at first broadened on development with the more dilute acid and, by the time the just visible rear band of nickel had travelled 6 mm., separation was complete, the nickel occupying a position between 6 and 12 mm., and the cobalt that from 12 to 18 mm. Treatment with dimethylglyoxime and ammonia showed that, if there were any overlap between the nickel and the cobalt zone, it must have been less than 1 mm. A quantity of 0·3 g. of alumina thus proved to be sufficient to separate almost quantitatively 0·15 milliequiv. of the " inseparable " cobalt–nickel sulphate mixture.

It should also be mentioned that, as a result of using finer grain, the quality of packing was much improved. The boundaries obtained were absolutely straight. Fringes—only observable under the microscope—did not exceed 3—4 grain diameters, *i.e.*, 0·04 mm. on any of the boundaries of Fig. 4*b*.

Fig. 5.
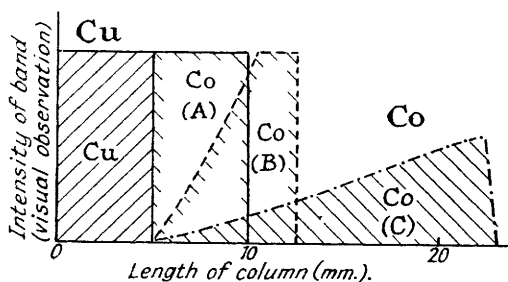
Chromatogram of mixture of CuSO$_4$ *and* CoSO$_4$ *on* Al$_2$O$_3$. *Development with* MnSO$_4$ *solution.* Cu *band remains at the top of the column. Three stages in the " development " of the* Co *band are shown.*

(e) *Separation into Isolated Bands.*—As the slow separation of metal ions on fine-grain alumina proved so successful, an attempt was made to obtain a complete separation into isolated bands. With acid as developing agent, this is of course not possible, as hydrogen ions are the most strongly adsorbed ions and, consequently, the bands cannot be expected to separate (see Glueckauf, Part I, *Proc. Roy. Soc.*, 1946, *A*, **186**, 50, case 4). If a complete separation, *e.g.*, of copper and cobalt bands is required, development must be attempted with a less adsorbed solute, such as Mg^{++} or Mn^{++} solutions.

Fig. 5 shows the approximate distributions of the separated bands observed during a chromatographic separation of 0·15 c.c. of 0·5N-CuSO$_4$ + 0·5N-CoSO$_4$, developed with 1, 1·5, and 4·5 c.c. of 0·5N-MnSO$_4$ solution. Already after 1 c.c. of developing solvent had been added, the separation of copper and cobalt seemed almost complete, resulting in adjoining bands of about equal width. Further development hardly altered the position of the copper band. The front of the cobalt band continued to move down the column, but the tail end, though greatly decreasing in concentration, never moved away from the front of the copper band, a typical sign that cobalt ions follow a Freundlich isotherm even in the presence of 0·5N-Mn^{++}. (During the development it looked as if the bands were actually completely apart, but subsequent treatment with ammonium sulphide showed that the cobalt band reached right back to the copper front.)

Thus, if separation into two entirely separated bands were required, it would in this case be necessary either to develop with a solute of intermediate adsorption affinity (*e.g.*, zinc), or to add such a solute to the mixture and develop with acid.

Somewhat similar is the separation of nickel and cobalt with 0·5N-MnSO$_4$ solution as developing agent. Here both bands attained a triangular shape of approximately equal length, the nickel band beginning at the top of the column, and the cobalt band at the fairly sharp front of the nickel band. (The cobalt band was here not actually a triangle, but began

with a definite concentration, as must be expected for Freundlich isotherms. This is shown in detail in Figs. 4, 5, and 6 of Part V.)

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