

240. *Theory of Chromatography. Part III. Experimental Separation of Two Solutes and Comparison with Theory.*

By J. I. COATES and E. GLUECKAUF.

The results obtained in Part I (*Proc. Roy. Soc.*, 1946, *A*, **186**, 35) for chromatograms of two solutes are summarised in Tables I—V, giving the features and movements of the important parts of binary chromatograms (see Fig. 1) at various stages of the development. The results are given both for the general case and for the special case of the Langmuir isotherm.

The agreement of these calculations with quantitative elution experiments is shown for a chromatographic separation of Cu^{++} and Mn^{++} on Zeo-karb H.I. (see Fig. 2, 3, and 4), the exchange of which follows approximately an isotherm of the Langmuir type.

(a) *Summary of Part I.*—A normal binary chromatogram has, according to whether it is fully developed or not, a distribution of solutes as shown in Fig. 1*b* and 1*a*, respectively. (Some

(see Part IV). The displacements Δx_1 and Δx_2 have to be determined in the absence of the third solute. It is obvious that development with a third solute gives the advantage of requiring shorter columns only if $(\Delta x_1/\Delta x_2 - 1) \ll 1$. Other advantages of using a third solute, like the greater mobility in the case of very strongly adsorbed solutes, are independent of this consideration.

EXPERIMENTAL

The experiments were carried out with columns containing 60 g. of acid Zeo-karb H.I. The attainment of equilibrium (see subsequent paper) is of particular importance in the case of chromatography with ion exchangers, for the diffusion in these is somewhat slower than, e.g., in alumina. However, by using a grain size of 0.01 mm. diameter and a flow velocity of 0.001 cm./sec. (corresponding to one drop in two minutes), elution curves were obtained which were even better than those obtainable with the apparently faster alumina of equal grain size. This seems to be due to a different effect altogether. Acid Zeo-karb when taking up larger ions like copper or manganese, undergoes a noticeable

TABLE I.

Fundamental equations.

	General isotherm.	Langmuir isotherm.
Adsorption isotherm	$q_1 = f_1(c_1, c_2)$ $q_2 = f_2(c_1, c_2)$	$q_1 = a_1 c_1 / (1 + b_1 c_1 + b_2 c_2)$ $q_2 = a_2 c_2 / (1 + b_1 c_1 + b_2 c_2)$. . . (1)
Condition for coexistent concentrations in the mixed band	$df_1(c_1, c_2)/dc_1 = df_2(c_1, c_2)/dc_2$ can be integrated graphically for a given case of c_1^0 and c_2^0 (see Part V)	$a_2 b_1 c_1 = a_1 b_2 c_2 \lambda - (a_2 - a_1) \lambda / (1 + \lambda)$, where λ can be obtained from the same equation with $c_1 = c_1^0$ and $c_2 = c_2^0$. In the following, λ is considered a known constant (2)
Pseudo-adsorption isotherms in mixed band	$q_1 = F_1(c_1)$ $q_2 = F_2(c_2)$ $\left\{ \begin{array}{l} \text{obtainable from} \\ \text{relationship} \\ \text{between coex-} \\ \text{istent values} \\ \text{of } c_1 \text{ and } c_2 \end{array} \right.$	$q_1 = A_1 c_1 / (1 + B_1 c_1)$ $q_2 = A_2 c_2 / (1 + B_2 c_2)$ } where $A_1 = a_1(1 + \lambda) / (1 + \lambda + \delta)$ $B_1 = b_1(1 + \lambda) / \lambda$ $A_2 = a_2(1 + \delta) / (1 + \lambda + \delta)$ $B_2 = b_2(1 + \lambda)$ $\delta = (a_2 - a_1) / a_1$ (3)
Movement of a given mass-point of solute 1 (and <i>vice versa</i> for solute 2) in the mixed band	$\left[\frac{\Delta x}{\Delta v} \right]_{m_1} = c_1 / f_1(c_1, c_2) = c_1 / F_1(c_1)$	$= (1 + b_1 c_1 + b_2 c_2) / a_1$ $= (1 + B_1 c_1) / A_1$ (4)
Movement of point of given concentrations c_1, c_2 in a diffuse boundary	$\left[\frac{\Delta x}{\Delta v} \right]_{c_1, c_2} = 1 / F'_1(c_1) = 1 / F'_2(c_2)$	$= (1 + B_1 c_1)^2 / A_1 = (1 + B_2 c_2)^2 / A_2$ (5)
Amount of solute in the chromatogram between the points of concentrate c and $c = 0$ (c within the mixed band)	$m_{1(a-x)} = x f_1(c_1, c_2) - v c_1$ $m_{2(a-x)} = x f_2(c_1, c_2) - v c_2$	Same (6)

TABLE II.

Important concentrations in the chromatogram (see Fig. 1a, b).

	General isotherm.	Langmuir isotherm.
c_{2o}	Obtainable from graphical integration	$c_{2o} = \delta / B_2$ (7)
c_{1y}	Obtainable from $\frac{c_{1y} - c_1^0}{c_2^0} = \frac{f_1(c_{1y}) - f_1(c_1^0, c_2^0)}{f_2(c_1^0, c_2^0)}$	$c_{1y} = c_1^0(1 + \lambda) / \lambda$ $\approx c_1^0 + c_2^0 - c_{2o}$ (8)
c_{2w}	Obtainable from $v = m_2 \frac{F'_2(c_{2w})}{F_2(c_{2w}) - c_{2w} F'_2(c_{2w})}$	$c_{2w} = \sqrt{(m_2 / B_2 v)}$ (9)
c_{1w}	Obtainable from c_{2w} by means of the graphically obtained $c_1 - c_2$ relation	$c_{1w} = \lambda(a_1 b_2 / a_2 b_1) c_{2w} - \lambda(a_2 - a_1) / a_2 b_1 (1 + \lambda)$ $\approx \lambda(c_{2w} - c_{2o})$ (10)
c'_{1w}	Obtainable from c_{1w} by $\frac{c'_{1w} - c_{1w}}{c_{2w}} = \frac{f_1(c'_{1w}) - F_1(c_{1w})}{F_2(c_{1w})}$	$c'_{1w} = c_{1w}(1 + \lambda) / \lambda$ $\approx c_{1w} + c_{2w} - c_{2o}$ (11)
c_{1z}	Obtainable only by graphical evaluation according to	$\int_{x_0}^x q_1 dx = m_1$ (12)

The corresponding values of q are obtainable from c by means of eqn. (1).

TABLE III.

Equations for the different chromatographic boundaries (see Fig. 1a, b).

Type of boundary.	Ref. for Fig. 1 a, b.	General isotherm.	Langmuir isotherm.
Rear boundary of pure solute 2	A - B	$v/x = [\delta f_{2(c_2)} / \delta c_2]_{c_1=0}$	$v/x = a_2 / (1 + b_2 c_2)^2$ (13)
Rear boundary of solute 2 in mixed band (developed)	C - D and C' - K	$v/x = F'_{2(c_2)}$	$v/x = A_2 / (1 + B_2 c_2)^2$ (14)
Rear boundary of solute 1 in mixed band (developed)	C' - D' and C'' - K'	$v/x = F'_{1(c_1)}$	$v/x = A_1 / (1 + B_1 c_1)^2$ (15)
Rear boundary of pure solute 1 in fully developed frontal band	L' - Q	$x = \frac{v}{f_{1(c_1)} + f'_{1(c_1)} (F_{2(c_2)} - F'_{2(c_2)})} + \frac{m_2 (f'_{1(c_1)} - F'_{2(c_2)})}{m_2 (f'_{1(c_1)} - c_{2w} F'_{2(c_2)}) - c_{2w} F'_{2(c_2)}}$	$x = v(1 + b_1 c_1)^2 / a_1 + \delta m_2 b_2 (1 + b_1 c_1)^2 / a_1 (\delta + b_2 c_1)^2$ (16)
Front of mixed band (undeveloped)	E - F	$x_u = (v + v^0) c_2^0 / f_{2(c_2)}^0$	$x_u = (v + v^0) (1 + b_1 c_1^0 + b_2 c_2^0) / a_2$ (17)
Front of mixed band (developed)	E' - F'	$x_w = m_2 / (F_{2(c_2w)} - c_{2w} F'_{2(c_2w)})$ (see c_{2w})	$x_w = [\sqrt{(v/A_2)} + \sqrt{(m_2 B_2 / A_2)}]^2$ (18)
Front of pure solute 1 (undeveloped)	K' - L'	$x_y = (v + v^0) c_{1w} / f_{1(c_1w)}$ (see c_{1w})	$x_y = (v + v^0) (1 + b_1 c_1) / a_1$ (see c_{1w}) (19)
Front of pure solute 1 (developed)	G - H	Graphical solution only (see c_{1w})	— (20)

Where c_{2w} is a function of c_1 such that if $c_1 = c'_{1w}$ then c_{2w} is the corresponding c_{2w}

TABLE IV.

Amount of solute in different parts of the chromatogram.

Part of chromatogram.	Ref. to Fig. 1a, b.	General isotherm.	Langmuir isotherm.
Pure rear band of solute 2 (developed or undeveloped)	Area ABC'	$v \frac{(F_{2(c_2)} - c_{2w})}{F'_{2(c_2)}}$	$v \delta^2 / B_2$ (21)
Pure front band of solute 1 (undeveloped)	Area FE'F'GH	$(v + v^0) c_1^0 (1 - c_2^0 \cdot f_{1(c_1)}^0 / c_1^0 \cdot f_{2(c_2)}^0)$	$(v + v^0) c_1^0 (a_2 - a_1) / a_2$ (22)
Solute 1 in mixed band (developed)	Area C'K'L	$v (F'_{1(c_1w)} - c_{1w} F'_{1(c_1w)}) / F'_{1(c_1w)}$ (see c_{1w})	$B_1 v c_{1w}^2 w$ (see c_{1w}) (23)

The other parts are obtainable by difference from m_1 or m_2 .

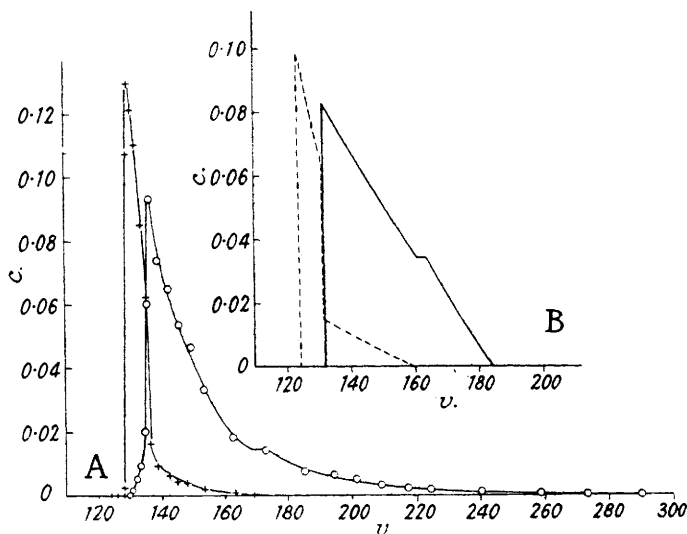
TABLE V.

Conditions for complete separation.

	General isotherm.	Langmuir isotherm.
Minimum amount of adsorbent required	$X_0 = m_2/(F_{2(c_{20})} - c_{20}F'_{2(c_{20})})$	$X_0 = m_2B_2/A_1\delta^2 \dots (24)$ $\approx (m_1 + m_2)b_2/a_1\delta^2$
Minimum volume of developing solvent required	$V = m_2/(F_{2(c_{20})}/F'_{2(c_{20})} - c_{20})$	$V = m_2B_2/\delta^2 \dots (25)$ $\approx (m_1 + m_2)b_2/\delta^2$

swelling, which has a stabilising effect on the front boundary of the ion band. For instance, if one side of the boundary should get in advance of the rest, the flow resistance on this side increases on account of the swelling, so that the other parts of the boundary can catch up. The effect is so marked that an ion band can be passed through a 1 m. column of 1" diameter, without showing a noticeable tilt in the circular front boundary.

FIG. 2.



Eluted chromatogram (fully developed) for partial separation of (1) Mn^{++} and (2) Cu^{++} on 60 g. of Zeo-karb H.I. Development with 1N- H_2SO_4 . Experimental conditions: $m_1 = 0.6$ milliequiv., $m_2 = 2.0$ milliequivs., $c_1^0 = 0.1N$, $c_2^0 = 0.4N$. Isotherm constants: $a_1 = 2.12$, $b_1 = 2.3$, $a_2 = 2.35$, $b_2 = 2.56$. $\alpha = 0.7$ c.c./g. Grain size of Zeo-karb 0.01 mm. Flow velocity ~ 100 c.c./24 hours. Note the exceedingly sharp front boundary due to reduction of non-equilibrium phenomena.

(B) Elution curve calculated with the equations of Tables I—III for a Langmuir isotherm with the above constants.

The columns used had a diameter of 1 cm. and were filled with suspended acid Zeo-karb H.I., the usual method of tapping and stuffing being employed. Equally good results were obtained if the tubes were filled with large quantities of material at a time, these being allowed to settle while the whole tube was subjected to constant vigorous vibrations by means of a bell movement clamped to the tube. This method saves much labour with large tubes and produces a very uniform sedimentation of the particles.

The elution curves were obtained by collecting, with an automatic device, fractions of the eluted solution, and analysing these by titration. Within the limits of the analytical error, the recovery of the solutes from 60 g. of Zeo-karb appeared to be 100%, but varying losses were obtained when working with alumina (see also Jacobs and Tompkins, *Trans. Faraday Soc.*, 1945, **41**, 403, Fig. 4).

Results.—Considerable difficulty was experienced in finding a binary system which follows a Langmuir isotherm (see eqn. 1). The nearest approach was found in the exchange isotherms of cupric, manganese, and hydrogen ions on exchange resins (Zeo-carb H.I. and H.I.P) for 1N. and higher total concentrations, and then only when more than 50% of hydrogen ions were present. For a lower hydrogen-ion ratio, the isotherm attains a sigmoid form, and for lower total concentration it changes increasingly towards the Freundlich type (see Part II, Fig. 5). However, within the region of

$$[Cu] + [Mn] < [H]$$

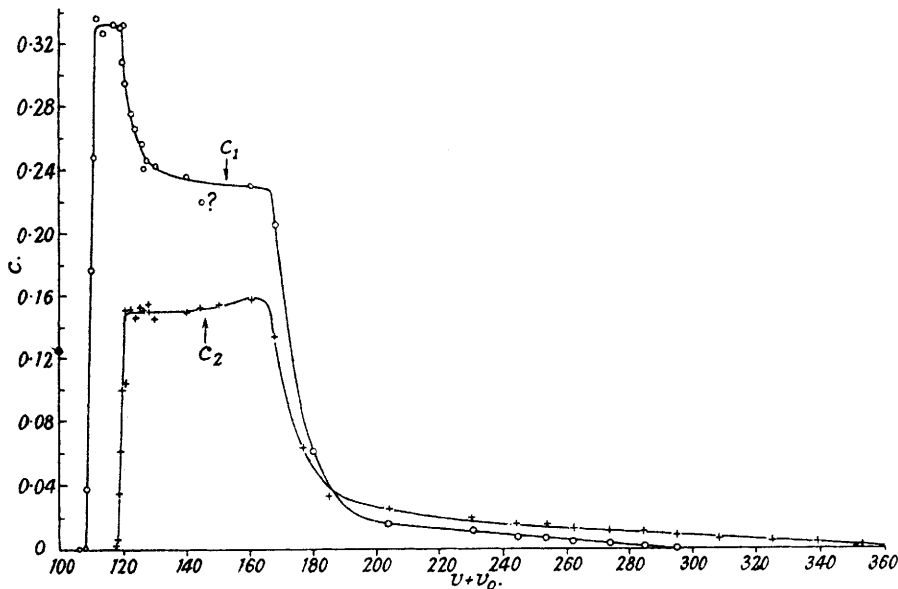
and

$$[Cu] + [Mn] + [H] = 1 \text{ milliequiv./c.c.} \dots (26)$$

the exchange isotherm can be well represented over the greater part of its course by equations of the type

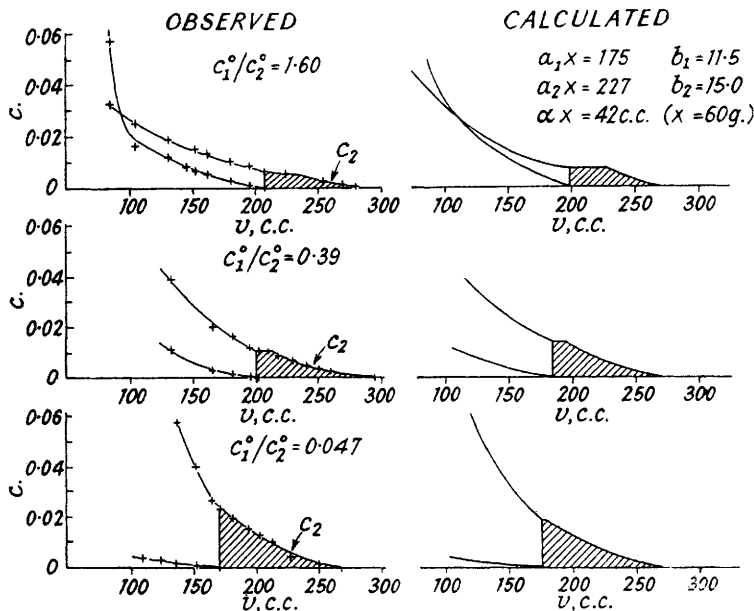
$$q_{Mn} = q_1 = K_1[Mn]/([H] + K_2[Mn] + K_3[Cu]) \dots (27)$$

FIG. 3.



Elution curve for two solutes (development incomplete).
 Eluted chromatogram ("development" incomplete) of (1) Mn^{++} and (2) Cu^{++} on 60 g. acid Zeo-karb H.I.
 Development with 1N- H_2SO_4 . $c_1^0 = 0.23N$, $c_2^0 = 0.16N$.

FIG. 4.



Observed and calculated rear boundary effects for different ratios of the initial concentrations c_1^0/c_2^0 . Note that the form of and amount separated in the pure rear band depends on c_1^0/c_2^0 . Calculation acc. to Tables I-IV.

and vice versa for q_{Cu} . Eliminating $[H]$ by means of the condition of constant total concentration (eqn. 26) we obtain Cu-Mn isotherms

$$q_{Mn} = K_1[Mn]/\{1 + (K_3 - 1)[Mn] + (K_4 - 1)[Cu]\} \quad . . . \quad 28)$$

and similarly for q_{Ca} . These equations are formally identical with the Langmuir isotherm (eqn. 1).

At the very lowest metal concentrations [$(c_1 + c_2) < 0.03N$], rather more metal ions are taken up than correspond to the rest of the isotherm, which means that at the lowest concentrations all the constants a and b become larger.

Apart from reasons of their simple determination, copper and manganese had been chosen because they represent solutes which are, on this adsorbent, very difficult to separate. Their exchange affinities differ less than those of nickel and cobalt on alumina and they thus afford a particularly stringent test for both theory and practice.

Fig. 2A shows the measured elution curve of a manganese-copper separation in a column 1 m. long containing 60 g. of acid Zeo-karb H.I. In spite of the considerable length of column and the consequent increase of all disturbing factors, the theoretically vertical front boundaries were found to be exceedingly sharp—not only did the self-sharpening front boundary of pure manganese rise up within 1 c.c. of eluate, but even the practically non-self-sharpening front boundary of the copper against the frontal band of manganese is almost completely contained within 2 c.c. of eluate. It can therefore be assumed that equilibrium is almost complete at every point of the column. (For a detailed discussion on non-equilibrium phenomena at boundaries, see Part IV.)

Fig. 2B gives the elution curve calculated on the basis of the given constants (see legends of Fig. 2) by means of the equations deduced in Part I (*loc. cit.*) which are summarised at the beginning of this paper. In these calculations the pore space $a = 0.7$ c.c./g. of adsorbent, which had been determined by direct measurement, has been taken into consideration.

The agreement between experiment and calculation is quite good except at the tail end of the chromatogram where the concentrations are very low and where, as mentioned before, the constants of the isotherm become larger. As a result, the tail is considerably longer than would be the case for a "pure" Langmuir isotherm and the concentration (c_{20}) at the front of the pure rear band of solute 2 is consequently reduced. Agreement is particularly good for the front part of the chromatogram, in particular for the threshold volume, and the sharp rise in the concentration of solute 1 at the inter-front (at $v = 36$ c.c.), which is to be expected for solutes of very similar adsorption affinity, is clearly shown.

The not inconsiderable disagreement in the details of the rear boundaries is of particular interest, because it shows the enormous sensitivity of the form of the rear boundary against minor variations in the form and shape of the isotherm.

If the isotherm is calculated from the rear boundaries of both the experimental and the calculated elution curve on Fig. 2, the two isotherms are practically indistinguishable between the concentrations of 0.02 to 0.09 milliequiv./c.c., as is seen from the following data:

[Cu].	q_{Ca} (milli-equiv./g. of Zeo-karb).	
	Calc. from exp. chromatogram.	Calc. for Langmuir isotherm.
0.093	0.176	0.175
0.065	0.130	0.1315
0.038	0.083	0.0835
0.019	0.046	0.044
0.006	0.017	0.014
0.002	0.007	0.005

The difference at the very lowest concentrations, which are practically inaccessible to ordinary equilibrium measurements, can thus with the greatest ease be recognised and calculated from the rear boundary of a chromatogram.

It is apparent therefore that, with the exception of threshold values, there is little hope of being able to calculate the shape of chromatograms if only the approximate forms of the isotherms are known. The value of the theory lies rather in the opposite direction in that it provides a convenient tool for the calculation of the isotherms, in particular of multiple isotherms from chromatographic data.

An example of a chromatogram where "development" has not been completed is shown in Fig. 3. Here the two front boundaries rise up steeply within about 3 c.c. of eluate. There follows the flat non-developed section, after which a rapid fall in the concentrations of both solutes takes place which, owing to small deviations in the isotherm, is again sharper than one would expect for the Langmuir type.

Fig. 4 shows the details of a few measured rear boundaries. Though relatively less important for the technical separation, these rear parts form a good test of the theory. Both c_{20} and the amount μ_2 separated at the rear are, according to the theory, dependent on the ratio c_1^0/c_2^0 of the initial concentrations, which in these three experiments have been varied over a wide ratio. The agreement between the calculated and the observed elution curves is as good as can be expected. Not only c_{20} and μ_2 , but also the form of the rear band of pure solute 2 and the position of the point where solute 1 becomes zero, clearly show the trend demanded by theory.