

### 239. Theory of Chromatography. Part II.\* Chromatograms of a Single Solute.

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Chromatograms of single solutes, in particular the case of sigmoid isotherms, are treated theoretically and measured experimentally. The form of the chromatograms resulting from the different isotherms and the movement of their characteristic points are shown and calculated (see Figs. 1—6). Adsorption and exchange isotherms can be calculated from chromatographic elution curves (see Figs. 7 and 9). Comparison of an isotherm, determined in this way, with directly determined equilibrium values shows a good agreement (see Fig. 5), and the chromatographic method gives a much larger number of points for less experimental work.

#### Symbols :

$c$ , concentration in milliequivs. per c.c.

$q = f_{(c)}$ , amount of solute taken up by 1 g. of adsorbent (including pore space) in equilibrium with  $c$  (m.-equiv./g.).

$f^*_{(c)} = q - ac$ , the amount actually adsorbed on the adsorbent.

$a$ , pore space per g. of adsorbent (c.c./g.).

$v$ , volume of developing solution used (c.c.).

$v^0, c^0, q^0$ , volume and concentration of original solution, and amount adsorbed per g. in equilibrium with  $c^0$ .

$x$ , distance from the top of the column, measured in g. of adsorbent.

$m = v^0 c^0$ , amount of solute used (m.-equiv.).

$\mu_{\bar{x}}$ , amount of solute in the column behind a given point  $\bar{x}$  of concentration  $\bar{c}$  on the rear boundary.

$\mu'_{\bar{x}}$ , amount of solute in front of a given point  $\bar{x}$  of concentration  $\bar{c}$  on the front boundary.

Indices in italics refer to characteristic points of the chromatogram, shown in the diagrams :

$u$  to the undeveloped front (see Fig. 2, A, B).

$w$  to the front of a fully developed chromatogram (see Figs. 2, C and 6, D).

$s$  to the sharp undeveloped rear boundary (see Fig. 4, A).

$r$  to the sharp rear boundary of a fully developed band (see Fig. 4, B).

$o$  to the point of concentration 0 in a diffuse boundary (see Figs. 2, 4, 6).

$d$  to the point of concentration  $c^0, q^0$  in a diffuse boundary (see Figs. 2, 4, 6).

$e, e', f$ , and  $\epsilon$  are used for points on a partially diffuse boundary (see Fig. 6).

(a) *Chromatographic Equations for Single Solute Isotherms.*—Chromatography is the name given to the process of separation by continuous fractional adsorption, because of its earlier applications to the separations of coloured substances, though this limitation no longer obtains. The normal procedure is to pour a solution of the solutes to be separated on to the top of a column filled with adsorbent and allow it to percolate into the column, a process which may be accelerated by applying suction at the bottom or, better, pressure at the top. After this original solution is taken up by the column, and has formed a band, further amounts of pure solvent are added at the top, dissolving the band of adsorbed solutes from the rear and slowly carrying it through the column, a process called "development." During the development stage the band usually spreads, whereby its local concentration decreases and, as the individual solutes move with different speed through the column, partial or complete separation takes place.

The regions in the front and rear of the adsorption band are called "boundaries," and these may be "sharp" or "diffuse" according to the type of adsorption isotherm obtaining for the particular case of adsorbent, adsorbate, and solvent. (The boundaries are always diffuse if the flow of solvent is too fast to permit local equilibrium to be reached; see Part IV.) In the case of multiple solutes the word "boundary" is applied to all parts where concentration gradients exist.

The movement of any type of boundary is governed by the principle of mass-conservation, which means that, if we consider the solute content of a very thin section of the chromatographic band, its increase (or decrease) is given by the difference of the inflowing and the outflowing solute.

The movement of the band can be considered in two ways.

We can mentally fix our eyes on a given particle of the solute. Then this point of fixed mass within the band of a single solute, the concentration of which may, e.g., be  $c^0$ , moves with a given amount of solvent  $v$  according to (1) (see Wilson, *J. Amer. Chem. Soc.*, 1940, **62**, 1583) :

$$[\Delta x / \Delta v]_m = c^0 / f'_{(c^0)} \quad \dots \quad (1)$$

Alternatively, we may follow the movement of a point of constant concentration in a diffuse boundary (see Fig. 2, C, curve A—B), which is described by equation (2) (see De Vault, *ibid.*, 1943, **65**, 532; Weiss, *J.*, 1943, 297) :

$$[\Delta x / \Delta v]_c = dc / df_{(c)} = 1 / f'_{(c)} \quad \dots \quad (2)$$

\* For Part I, see *Proc. Roy. Soc.*, 1946, *A*, **186**, 35.

Finally, we have the integrated equations for the amount of solute in the chromatogram between the concentrations  $c$  and  $c = 0$ , which in the case of a diffuse rear boundary (see Fig. 2) is

$$\mu = \bar{x}f(\bar{c}) - v\bar{c} \quad \dots \quad (3)$$

(Glueckauf, *Nature*, 1945, 156, 748) and for a diffuse front boundary (see Fig. 4) :

$$\mu' = (v + v^0)\bar{c} - \bar{x}f(\bar{c}) \quad \dots \quad (4)$$

FIG. 1.

FIG. 2.

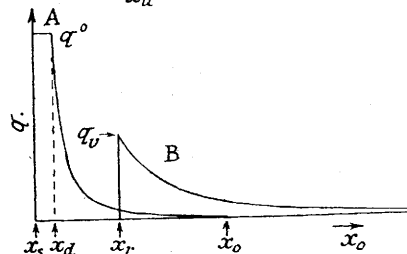
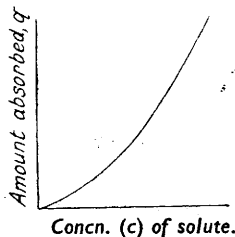
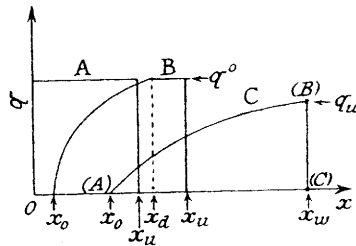
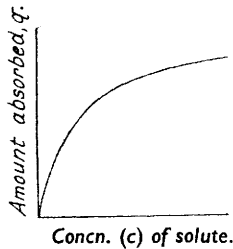


FIG. 3.

FIG. 4.

FIG. 1.—Normal isotherm concave against  $c$ -axis.

FIG. 2.—Development of a band of solute with isotherm as in Fig. 1.

Abscissa = distance from top of column.

Ordinate = amount absorbed per g. of column material.

(A) Original band. (B) Incomplete development. (C) Complete development. (A)–(B) Diffuse rear boundary, (B)–(C) sharp front boundary.

FIG. 3.—Isotherm convex against  $c$ -axis.

FIG. 4.—Development of a band of solute with isotherm as in Fig. 3.

(A) Original band. (B) Complete development.

Equation (3) actually comprises equation (1).

The combinations of these equations represent all the relevant conditions of the various parts of the chromatograms (some of which have already been given by Weiss, *loc. cit.*).

For an adsorption isotherm concave towards the  $c$ -axis (see Fig. 1) we get (for subscripts see Figs. 2, A, B, C) :

$$x_0 = v/f'_{(0)} \quad \dots \quad (5a)$$

$$x_d = v/f'_{(c^0)} \quad \dots \quad (6a)$$

$$x_u = (v + v^0)c^0/f'_{(c^0)} \quad \dots \quad (7a)$$

$$x_w = m/(f'_{(c_w)} - c_w f'_{(c_w)}) \quad \dots \quad (8a)$$

where  $c_w$  is defined by

$$v = mf'_{(c_w)}/(f'_{(c_w)} - c_w f'_{(c_w)}) \quad \dots \quad (9a)$$

For an adsorption isotherm convex towards the  $c$ -axis (see Fig. 3) we get (for subscripts see Figs. 4, A, B) :

$$x_0 = (v + v^0)/f'_{(0)} \quad \dots \quad (5b)$$

$$x_d = (v + v^0)/f'_{(c^0)} \quad \dots \quad (6b)$$

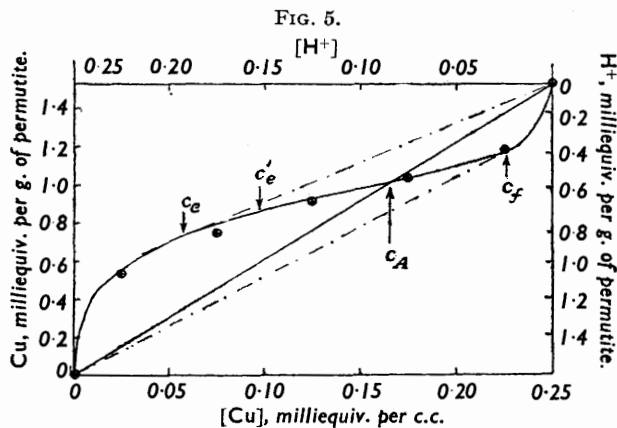
$$x_s = vc^0/f'_{(c^0)} \quad \dots \quad (7b)$$

$$x_r = m/(c_r f'_{(c_r)} - f'_{(c_r)}) \quad \dots \quad (8b)$$

where  $c_r$  is defined by

$$v + v^0 = mf'_{(c_r)}/(c_r f'_{(c_r)} - f'_{(c_r)}) \quad \dots \quad (9b)$$

Slightly more complicated are the conditions in the case of sigmoid isotherms. These are particularly frequent in ion-exchange systems on zeolites, permutites, and exchange resins, in cases where both ions are taken up with similar affinity (see, e.g., Rothmund and Kornfeld,



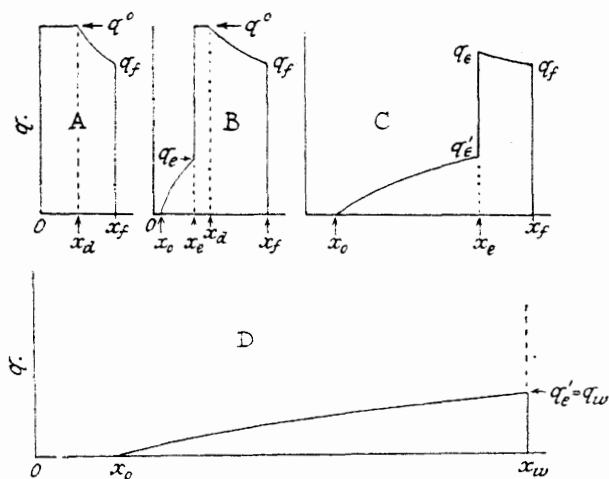
Example of sigmoid isotherm.

Exchange isotherm of  $H^+$  and  $Cu^{++}$  on Zeo-carb H.I. for a total concentration in solution of 0.25N.

Abscissa :  $Cu^{++}$  concentration in solution (milli-equiv. per c.c.).  
 Ordinate : Cu content of zeo-carb (milli-equiv. per g. of zeo-carb).  
 Curve : isotherm obtained from chromatographic data.  
 Points : isotherm points from direct equilibrium measurements.

*Z. anorg. Chem.*, 1918, **103**, 129, for the case of  $Na-NH_4$  and  $Ag-Tl$ ). Though two ions are involved in any isotherm, they can nevertheless be treated as simple systems, for, under the conditions of constant total concentration existing during the elution process, there is only

FIG. 6.



Development of a band of solute with sigmoid isotherm.

- (A) Original band. (C) First stage of development completed.  
 (B) Development incomplete. (D) Complete development.

one freely variable concentration. Bands in every respect identical with those produced by adsorption phenomena are obtained by using a solution of the original cation of the permutite as developing agent.

In the case of sigmoid isotherms, chromatograms are obtained which, at first, have partly diffuse and partly sharp boundaries both in front and rear. In the later stages of the develop-

ment, however, their form changes to one of the cases treated above, according to the curvature of the isotherm at low concentrations.

If the isotherm is concave towards the  $c$ -axis at low concentration (see Fig. 5), which is usual in exchange equilibria on permutites, the original band after formation has a form shown in Fig. 6A, assuming the form of Fig. 6B after development.

The frontal concentration  $c_f$  shown in Fig. 6A, B, C, is defined by the two conditions of movement for the front boundary :

$$[x_f]_{c_f \text{ const.}} = (v + v^0)/f'(c_f) \dots \dots \dots (10)$$

(see equation 1) for the diffuse edge, and

$$[x_f]_{\mu=0} = c_f(v + v^0)/f(c_f) \dots \dots \dots (11)$$

(see equation 1 or 7) for the sharp front. The combination of these two equations leads to the condition for  $c_f$  :

$$f'(c_f) = f(c_f)/c_f \dots \dots \dots (12)$$

The rear concentration  $c_e$  is defined by the conditions of mass conservation :

$$v/x = \Delta f(c)/\Delta c = (f(c^0) - f(c_e))/(c^0 - c_e) \dots \dots \dots (13)$$

(corresponding to equation 1), and by the movement of a point of the diffuse rear boundary of constant concentration  $c_e$  :

$$v/x = f'(c_e) \dots \dots \dots (2')$$

resulting in

$$f'(c_e) = (f(c^0) - f(c_e))/(c^0 - c_e) \dots \dots \dots (14)$$

It follows from (12) and (14) that the concentrations  $c_e$  and  $c_f$  can be obtained very simply from the adsorption isotherms. They represent the points where the tangents from the points  $c = 0$  and  $c = c^0$  touch the adsorption isotherm (see Fig. 5).

The first stage of development, when the original concentration  $c^0$  disappears, is completed when  $x_e$  overtakes  $x_d$ . Thus

$$v_{d,e} = v^0 f'(c_e)/(f'(c^0) - f'(c_e)) \dots \dots \dots (15)$$

With further development the diffuse rear band continues to increase in quantity at the expense of the rest. With the fall of  $c_e$  (see Fig. 6C) is connected a slow rise of  $c'_e$ . When  $x_e$  catches up with  $x_f$  (see Fig. 6D),  $c'_e$  is given by the condition

$$f'(c'_e) = (f(c_f) - f(c_e))/(c_f - c'_e) \dots \dots \dots (16)$$

From this point onwards the chromatogram behaves like a fully developed band in case (a), and equations (2), (2a), (3), (4a), and (4b) apply. The position of the band at this point is given by (4a) and (4b) with  $c_w = c'_e$ . If the isotherm is known,  $c'_e$  can be obtained by drawing a tangent (not shown in Fig. 5) from  $c_f$  which touches the isotherm in  $c'_e$ .

In the case of sigmoid isotherms which are concave at low concentrations (as found for some dyes in aqueous solution when adsorbed on alumina), similar conditions arise but with the bands in a reversed position.

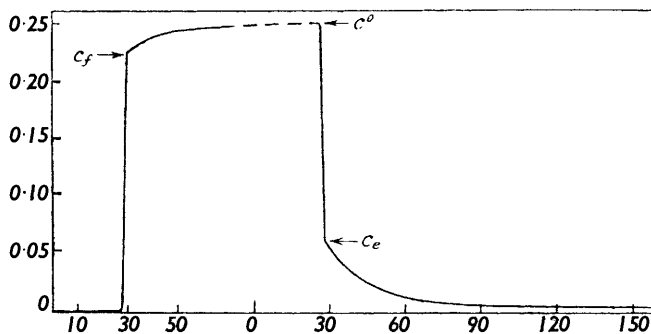
An interesting feature attaches to the point  $c_A$  of the exchange isotherm (see Fig. 5), where the latter is intersected by the diagonal of the phase rectangle. At this point  $c_1/c_2 = q_1/q_2$  and, consequently, this composition shows all the characteristics of an azeotropic mixture. This has the result that if, e.g., a solution of 0.165N-CuSO<sub>4</sub> + 0.085N-H<sub>2</sub>SO<sub>4</sub> is poured on Na-Zeo-karb H.I., no separation of either copper sulphate or sulphuric acid takes place at the front boundary until full development has taken place.

(b) *Determination of Sigmoid Adsorption Isotherms from Chromatographic Data.*—It is apparent that, in both types of sigmoid isotherms, the chromatogram nowhere contains concentrations between  $c'_e$  and  $c_f$ , so in this case it is not possible to calculate the whole of the adsorption isotherm from a single chromatographic experiment. Fig. 7 shows the concentrations in the eluate obtained when 0.25N-CuSO<sub>4</sub> solution is passed through a column containing 5 g. of acid Zeo-karb H.I. and subsequently eluted with 0.25N-HCl.

The elution curves were registered automatically by measuring the conductivity of the solutions with a recording microammeter. The arrangements of the circuit used are shown in Fig. 8. The only operations necessary were occasional measurements of the quantity of eluate, so as to correlate it with the time scale of the recording instrument, and the determination of the end concentration and of the residual cupric or hydrogen ion in the column at the end of the elution process when the concentrations had become too low to be measured accurately with

the recorder. (The residual cupric ion was eluted with  $2N\text{-H}_2\text{SO}_4$ , which removed it quickly, and then titrated.) To avoid non-equilibrium phenomena, the permutite material was ground to a grain size of 0.02 mm. diameter.

FIG. 7.



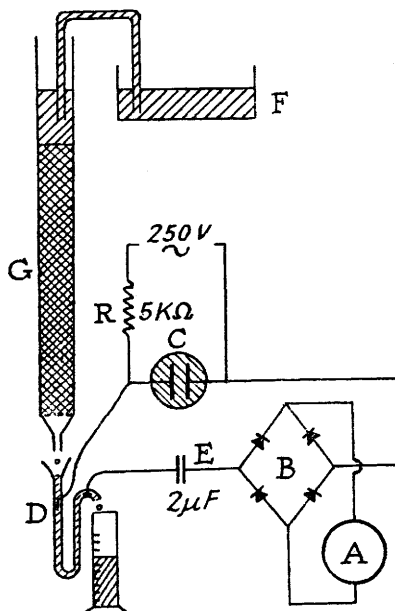
Measured elution curve of a solute with sigmoid isotherm.

Abscissa : volume of eluate (c.c.).

Ordinate : concentration of eluate, milliequiv./c.c.

(Compare with the band shown in Fig. 6B.)

FIG. 8.



Arrangement for the automatic registration of concentrations in the eluate.

(A) Recording  $\mu$ -ammeter (0—100  $\mu\text{A}$ .)

(B) Full-wave rectifier.

(C) Neon voltage-stabiliser.

(D) Capillary with Pt electrodes for resistance measurement of eluate.

(E) Condenser  $2\mu\text{F}$ .

(F) Constant level for constant dropping speed.

(G) Column filled with 5 g. of Zeo-carb.

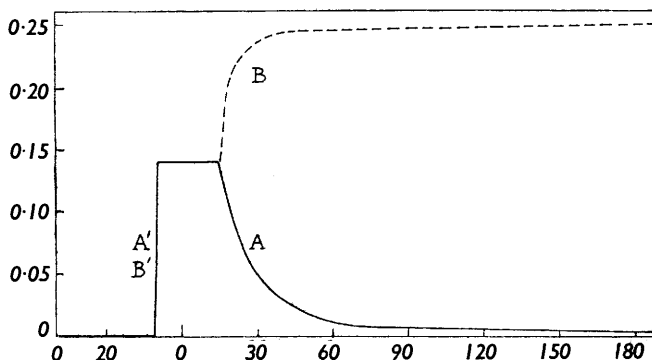
(R)  $5000\ \Omega$  resistance.

The eluate consists at first of  $0.25N\text{-H}_2\text{SO}_4$ . The front of the copper arrives at a threshold volume  $v_t = 29$  c.c. Its concentration rises sharply to a value of  $c_f = 0.225$ , as one would expect from the theory (eqn. 12) which fixes this concentration as the point where the tangent from the origin ( $c = 0$ ), touches the isotherm (see Fig. 5). Thence the concentration rises asymptotically to the original value  $c^0 = 0.25$  milliequiv./c.c.

After passing a sufficiently large amount of copper solution through the column to convert the zeo-karb completely into Cu-zeo-karb, this "undeveloped band" was now "developed" with 0.25N-H<sub>2</sub>SO<sub>4</sub>, whereby the rear boundary of the copper band was obtained. Here, too, the concentration fell at first sharply to a value of  $c_e = 0.060$  milli-equiv./c.c. [which again is in agreement with the theory (eqn. 14), representing the point in Fig. 5 where the tangent from the point  $c^0 = 0.25$  milliequiv./c.c. touches the exchange isotherm]. With continued elution, the copper concentration exhibits the normal behaviour and falls asymptotically to zero (see Fig. 7).

Thus the greater part of the isotherm, between  $c_e = 0.060$  and  $c_f = 0.225$ , cannot be calculated from the chromatographic data of this experiment, as these concentrations do not appear in the chromatogram. Boundaries covering the whole concentration range can, however, be obtained by saturating the column with solute of the concentration ( $c_i$ ) existing at the point of inflexion (or near by), and then (i) eluting with pure solvent (acid) and (ii) saturating the column with the concentration  $c^0$ . From the first experiment (elution of a copper band) results a diffuse rear boundary which permits the calculation of the adsorption data between  $c_i$  and 0; in the second case (elution of an acid band) is obtained a diffuse boundary between  $c_i$  and  $c^0$ .

FIG. 9.



Measured "elution curves" of a solute with sigmoid isotherm, suitable for determination of the isotherm by means of eqn. (3) or (4).

TABLE I.

(From curve A, Fig. 9.)

$v$ (obs.).	$c$ (obs.).	$\Delta\mu$ .	$\mu$ .	$(v - \alpha x)c$ .	$xf^*_{(c)}$ .	$f^*_{(c)}$ .
14.5 = $V_i$	0.140	0.56	3.62	1.19	4.81	0.962
19.1	0.103	0.49	3.06	1.35	4.41	0.882
24.9	0.066	0.31	2.57	1.25	3.82	0.764
30.6	0.044	0.22	2.26	1.08	3.34	0.668
36.4	0.033	0.17	2.04	1.00	3.04	0.608
42.1	0.028	0.17	1.87	1.01	2.88	0.576
63.1	0.010	0.40	1.47	0.57	2.04	0.408
166	0.005	—	0.73	0.80	1.53	0.306

TABLE II.

(From curve B, Fig. 9.)

$v + v^0$ .	$c$ .	$\Delta\mu$ .	$\mu$ .	$(v + v^0 - \alpha x)c$ .	$xf^*_{(c)}$ .	$f^*_{(c)}$ .
15.3 = $V$	0.140	0.18	2.14	1.30	4.81	0.962
16.5	0.166	0.37	2.32	1.74	5.08	1.016
18.5	0.200	0.41	2.68	2.50	5.47	1.094
2.04	0.213	1.17	3.07	3.07	5.65	1.130
25.6	0.227	2.04	4.19	4.45	5.91	1.182
34.0	0.237	—	6.15	6.71	6.21	1.242
$\infty$	0.250	—	—	—	7.50	1.500

Italicised values are measured directly. Other values of cols. 1, 2, 3 are taken from the registered elution curves. Col. 6 in Table I is the sum of cols. 4 + 5. Col. 6 in Table II is (5.65 + col. 5 - col. 4). The figure 5.65 represents  $(f^*_{(c^0)} + ac^0)x$ , i.e., the amount of solute in the column at the time of the change-over.  $\Delta\mu$  is the eluate between two evaluated points, i.e., the product of  $\Delta v$  and the average concentration. The amount  $\mu$  left in the column is obtained by adding all the  $\Delta\mu$ 's to the amount finally left in the column and measured directly.

In either case no discontinuous "rear" boundaries are obtained, because from this point ( $c_i$ ) no tangent can be drawn to any other part of the isotherm.

The elution curves shown in Fig. 9 A, B are obtained in the following way: Through a column of acid Zeo-karb (5 g.) was passed a solution of 0.14N-CuSO<sub>4</sub> + 0.11N-H<sub>2</sub>SO<sub>4</sub> which approximately corresponds to the mixture at the point of inflexion of the isotherm. The copper arrived in the eluate with a sharp front boundary ( $v_i = 40$  c.c.). Then the solution was changed, 0.25N-H<sub>2</sub>SO<sub>4</sub> (see curve A) being used for the elution of the copper, and (see curve B) in a second experiment 0.25N-CuSO<sub>4</sub> for the elution of the hydrogen ions. In either case a diffuse rear boundary was obtained beginning at about 14.5 and 15.3 c.c. respectively after the change-over.

From these two curves the complete exchange isotherm can be calculated. Curve A can be evaluated according to eqn. (3):

$$f^*(\bar{c}) = [\mu_{\bar{c}} + (v - \alpha x)\bar{c}]/x \text{ (see Table I).}$$

Curve B can be evaluated in two ways: It can be considered as the rear boundary of a hydrogen-ion band, and then eqn. (3) applies, with  $c$  referring to the hydrogen-ion concentrations. Alternatively, it can be considered as the partial front of a pure copper-ion band moving against a mixed solution, in which case eqn. (4) applies [though with some modification on account of the chromatographic tube containing originally some Cu ( $c_0$ )]. Integration for these conditions leads to:

$$f^*(\bar{c}) = f^*(c_0) + [(v + v^0 - \alpha x)\bar{c} - \mu_{\bar{c}}]/x$$

where  $f^*(c_0)$  is obtained from Table I, or from the threshold volume  $v_i = 40$  (frontal line A'B' of Fig. 9) by means of

$$f^*(c_0) = v_i c_0/x - \alpha c_0 \text{ (see eqn. 1a).}$$

From the experimental data ( $v_i = 40.0$  c.c.,  $c_0 = 0.140$ ,  $x = 5$  g.,  $\alpha = 1.20$  c.c./g.) follows a value for  $f^*(c_0) = 0.953$  milli-equiv./g., in good agreement with 0.962 found from Table I. The agreement of the equilibrium values of the exchange isotherm calculated in Tables I and II is shown in Fig. 5. Here the continuous curve represents the isotherm obtained by the chromatographic method, and circles mark points determined by direct equilibrium measurements. The obvious advantage of the chromatographic method of measuring isotherms, especially if the concentration of eluate can be measured continuously, is that a single experiment gives an almost unlimited number of equilibrium points of the isotherm.

(c) *Gas Adsorption Columns* (added in proof, August, 1947).—The considerations about the distribution of solutes in an adsorption column apply equally to the case of gases adsorbed from an air stream in adsorption columns. In the recently published experimental observations of Barrow, Danby, Davoud, Hinshelwood, and Staveley (this vol., p. 401), the different cases of Figs. 1 and 2, A and of Figs. 3 and 4, A are found when air containing carbon tetrachloride and water vapour respectively is passed through a charcoal column. The removal of the adsorbates from the column by passing clean air through (see their Figs. 7, 8, and 11, 12) corresponds to a development with pure solvent and is represented by Figs. 2B, C and 4B of this paper and by the corresponding equations. The above-mentioned experiments with gases also show clearly the effect of non-equilibrium phenomena, which are more fully discussed in Part IV.