82. *An Experimental Study of Chromatography.*

By H. Weil-Malherbe.

Simple chromatographic systems consisting of one adsorbent, one adsorptive, and one solvent were studied by plotting the quantity of adsorptive in the filtrate against the total volume of the filtrate. The resulting "elution curve" is of sigmoid form. The volume of filtrate collected up to the beginning of elution is called the threshold volume, V_t.

It was found that the shape of the elution curve depends on a, the exponential coefficient of Freundlich's adsorption isotherm. Where a is very near to 1, the elution curve is almost symmetrical, whereas a "tail" develops if a is less than 1.

The mean concentration of the eluate was found to be inversely proportional to V_t.

The variations of V_t with factors such as volume and concentration of the original solution or quantity of adsorbent were studied and shown to conform to simple expressions.

The results are discussed on the basis of the theory presented in the preceding paper.

In spite of the great importance of chromatographic adsorption, it has not yet been systematically studied from a quantitative point of view, and therefore its practical application is still largely empirical. The effect produced by variation of such factors as concentration or quantity of adsorptive, volume of solvent, quantity of adsorbent, etc., upon adsorption and elution is only understood in crude qualitative terms. A quantitative knowledge of these effects is essential for the application of chromatography to quantitative analysis, especially where absence of colour makes direct observation of the band impossible.

The problems which led to the present study arose during the development of a method for the micro-estimation of 3 : 4-benzpyrene and other hydrocarbons in extracts containing the non-saponifiable matter of animal tissues. In the interest of quantitative recovery the procedure had to be devised in such a way that it was capable of coping with all the concentrations of hydrocarbon likely to occur, down to the limits of accurate measurement; on the other hand, any undue extension of this margin had to be avoided to insure the greatest possible purification. With the aid of the results obtained, it is possible approximately to predict which procedure will best meet these requirements.

One of the reasons for the scarcity of quantitative data on chromatography is the difficulty of measuring the phenomena taking place in the column itself, such as width and concentration of the band and the rate of its advance. All the necessary information can, however, be obtained by measurements carried out on the filtrate. The quantity of solvent necessary to move the band through the whole column is a direct expression of the rate of its progress, and the concentration of the eluate must depend on the concentration of the band in the bottom layer of the column. These two factors thus accurately reflect the structure of the band.

Results.—(1) *The basic experiment: nomenclature.* The simplest chromatographic system has three components: adsorbent, solvent, and substance to be adsorbed. The last will be called the "adsorptive," whether it is in the adsorbed state or in solution. Three phases can be distinguished during the chromatographic process: initial adsorption, final elution, and those continuous processes of elution and readsorption during the advance of the band through the column, which are usually called the phase of development. The solution of the adsorptive *before* adsorption will be called the "initial solution" of initial volume v_0 and initial concentration c_0. The liquid which leaves the lower end of the column will be called the "filtrate," whereas the term "eluate" will refer to only that part of the filtrate that contains adsorptive. The quantity of filtrate which can be collected, before the band has passed the bottom layer of the column and elution proper begins, is termed the "threshold volume," V_t.*

Only simple systems containing a single adsorptive (usually benzpyrene) have been investigated in this study. The solvent used in preparing the initial solution was always the same as that used for development and elution. The adsorbents used were silica gel and aluminium oxide.

(2) *Elution curves.* If the amount of adsorptive in the filtrate is plotted against the total volume of the filtrate, a curve of typical sigmoid shape results, the elution curve. The concentration of the eluate is given by

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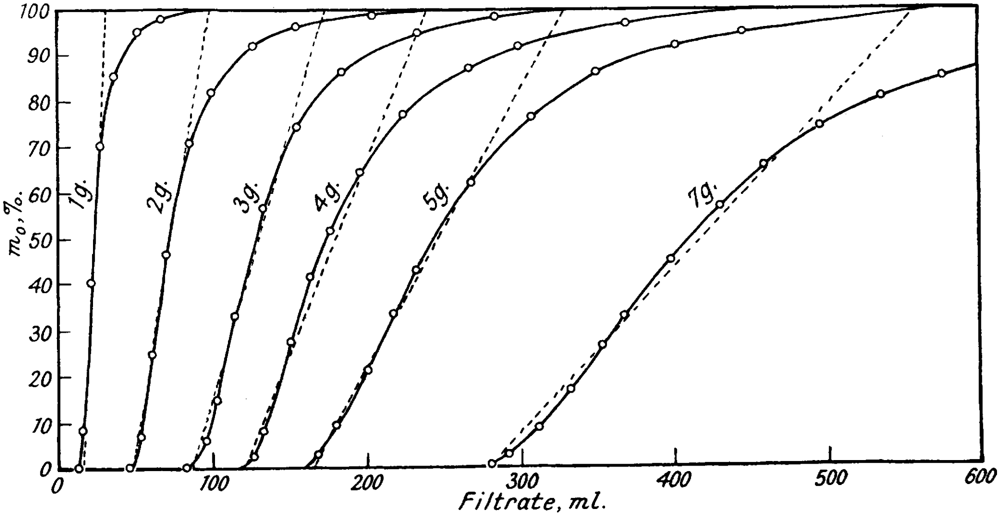
* According to this definition, V_t differs from Weiss's term v_t (preceding paper), which equals $V_t - v_0$. As v_0 is usually small compared with V_t, V_t and v_t can be regarded as practically identical in most cases.

the slope of the curve. Roughly, the elution curve may be divided into three parts : a flat part at the beginning and another at the end of the curve, and a steep part in the centre. Actually, there is a gradual transition, the concentration increasing steadily to a maximum and then decreasing again. The rate of elution is never strictly linear, although the curvature is slight in the steep part of the curve. It can therefore be closely approximated by a straight line the slope of which indicates the mean concentration over this particular section of the eluate.

FIG. 1.

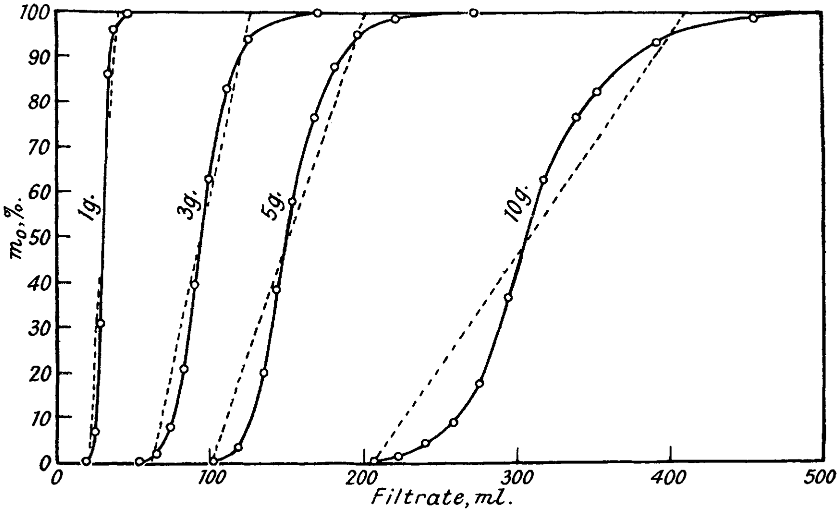
Typical elution curves obtained with columns containing various amounts (1—7 *g.*) *of alumina.*



Solvent: benzene–light petroleum (1 : 4). Initial solution: 300 μg. of benzpyrene in 1 ml. Ratio of length: diameter of columns = 10—15.
The broken lines are m_0/V_t lines.

FIG. 2.

Typical elution curves obtained with columns of silica gel (1—10 *g.*).



Solvent: light petroleum containing 3% of benzene. Initial solution: 300 μg. of benzpyrene in 1 ml. Ratio of length: diameter of columns = 10—18.
The broken lines are m_0/V_t lines.

The more detailed shape of the elution curve depends on the adsorbent used. The curves obtained with alumina (Fig. 1) differ from those obtained with silica gel (Fig. 2) in several respects : in the first case the initial flat part of the curve is so small that it is often hardly measurable. The upper flat part of the curve, on the other hand, is drawn out into a long " tail." The point of inflexion, *i.e.*, the maximum of concentration, is reached after the elution of about 30% of the adsorptive. The steep part of the elution curve may be said to extend over the part showing the elution between 3 and 60% of the adsorptive.

The silica gel curves are more symmetrical. The lower flat part is not much shorter than the upper flat part.

The point of inflexion is slightly below the point marking 50% elution. The section showing the elution of 20—80% of adsorptive represents the steep part of the curve.

(3) *Connection of eluate concentration and threshold volume.* If a number of elution curves with different threshold volumes are compared (Figs. 1 and 2), it appears that the slope, *i.e.*, the mean concentration, of the steep part of the elution curve decreases proportionally to the increasing threshold volume. Let the straight line approximating the steep part of the elution curve be extended up to its points of intersection with the lower and the upper abscissa (the upper one indicating the level of the total quantity of adsorptive, m_0). If the volume of filtrate between the ordinate and the lower point of intersection is called a_1, and that between the ordinate and the upper point of intersection a_2, then the mean concentration over the steep part is given by $m_0/(a_2 - a_1)$. In the case of alumina (Fig. 1) this line is close to a line for which $a_2 - a_1 = a_1 = V_t$. The slope of this line, which will be called the m_0/V_t line, gives the concentration of a solution containing the total amount of adsorptive (m_0) dissolved in the threshold volume (V_t). If this line is superimposed on the elution curve in such a way that it goes through the point of inflexion, it cuts the elution curve at two further points: first at the beginning of the elution curve on the abscissa, *i.e.*, at the point marking V_t, and secondly, at a point marking the elution of about 65% of the adsorptive. It follows that the mean concentration of that part of the eluate containing the first 65% of adsorptive equals m_0/V_t, *i.e.*, that, reckoned from the start of elution, a volume of the eluate $= 0{\cdot}65V_t$ will contain $0{\cdot}65m_0$. The slope of the elution curve indicates that the highest concentration reached during elution is of the order of $m_0/0{\cdot}65V_t$ to $m_0/0{\cdot}8V_t$.

If the curves obtained with silica gel are now considered (Fig. 2), it will be noticed that the divergence between the slope of the steep part of the curve and the m_0/V_t line is greater, the former indicating a maximum concentration of $m_0/0{\cdot}4V_t$ to $m_0/0{\cdot}6V_t$. If now the m_0/V_t line is again superimposed on the elution curve so that it passes through the point of inflexion, it crosses the abscissa at the point marking V_t, as was also the case with the alumina curves. The second point of intersection, however, marks the elution of about 95% of the adsorptive. Up to this point the mean concentration of the eluate $= m_0/V_t$, so $0{\cdot}95V_t$ of the eluate will contain $0{\cdot}95m_0$.

These differences in the shape and slope of the elution curves obtained with alumina on the one hand and with silica gel on the other can be related to the exponential coefficient α of Freundlich's adsorption isotherm, which was found to be 0·67 for the alumina system and 0·96 for the silica gel system. The evidence thus strongly suggests that, in general, the first part, αV_t, of the eluate will contain a fraction, αm_0, of adsorptive.

(4) *Determination of the threshold volume.* The threshold volume has been defined on p. 303. The beginning of elution is marked by the first drop of filtrate containing adsorptive. Owing to the low concentration of the eluate at the beginning of elution, this point is often difficult to ascertain accurately; sometimes it is displaced owing to disturbing influences, such as irregularities of flow. All the values of V_t reported in this study have therefore been determined by a standard method of extrapolation based on the data of a complete elution curve. It has been shown above that an m_0/V_t line joins two points on the lower and upper abscissæ, the latter point twice as far from the ordinate as the former. For any given elution curve that m_0/V_t line is selected which passes through the point of inflexion of the elution curve. Its point of intersection with the abscissa marks the threshold volume. Usually, it coincides with the point where elution has been actually observed to start.

It is obviously much easier accurately to determine by direct observation a point somewhere in the middle of the elution curve. In Table I the values obtained by direct observation for the elution of half the adsorptive are compared with those obtained by extrapolation for the threshold volume. There is a constant ratio between the two sets of values, evidence that the method of extrapolation adopted is not arbitrary but yields correct results; confirmation, too, of the dependence of eluate concentration on V_t.

TABLE I.

Ratio of threshold volume (by extrapolation) to volume necessary for elution of $m_0/2$ *(by observation).*

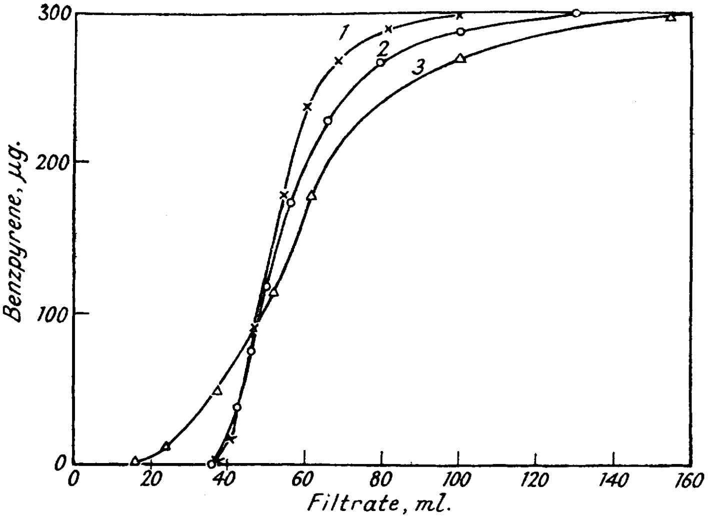
Alumina.						Silica gel.					
V_t (ml.).	$v_{1/2m_0}$ (ml.).	$V_t/v_{1/2m_0}$.	V_t (ml.).	$v_{1/2m_0}$ (ml.).	$V_t/v_{1/2m_0}$.	V_t (ml.).	$v_{1/2m_0}$ (ml.).	$V_t/v_{1/2m_0}$.	V_t (ml.).	$v_{1/2m_0}$ (ml.).	$V_t/v_{1/2m_0}$.
16	23·5	0·681	99	143	0·692	19·5	29·5	0·664	115	172	0·669
49	72	0·680	120	175·5	0·684	40·5	60·5	0·670	117	175·5	0·667
60	89	0·675	122	178	0·685	63	94·5	0·666	145	217	0·669
63	93	0·678	130	191	0·680	101	150	0·674	159	237	0·671
82·5	120	0·688	160	235	0·681	102·5	153	0·670	205	304	0·674
86	126·5	0·680	235	345	0·682	109	162·5	0·671	218	320	0·681
92	133	0·692	280	411	0·682						
97	141	0·688									

(5) *The "typical" elution curve and its limitations.* It follows from Section 3 that knowledge of V_t and α permits the prediction of the approximate shape of the elution curve with the aid of the following three points: (1) origin of the elution curve (abscissa V_t; ordinate 0); (2) upper point of intersection with m_0/V_t line (abscissa $V_t + \alpha V_t$; ordinate αm_0); (3) point of inflexion (abscissa approx. $V_t + \frac{1}{2}\alpha V_t$; ordinate approx. $\frac{1}{2}\alpha m_0$). An elution curve conforming to this general shape may be called a typical elution curve. In order to obtain such a typical curve several conditions must be fulfilled:

(a) Shape of column. This is not a very critical factor. The ratio of length to diameter may be considerably varied (between about 5 and 20) without the elution curve's being affected, provided the quantity of adsorbent be kept constant. If, however, one uses either higher or lower ratios, certain deviations from the shape of the typical elution curve appear (Fig. 3): a very short column leads to flattening of the elution curve, no doubt owing to irregularities of flow caused by decrease of friction towards the centre of the stream, whereas an extremely thin and long column gives some compression of the elution curve, probably owing to the extremely slow rate of filtration, which makes it unsuitable for use.

FIG. 3.

Effect of shape of column on elution curve.

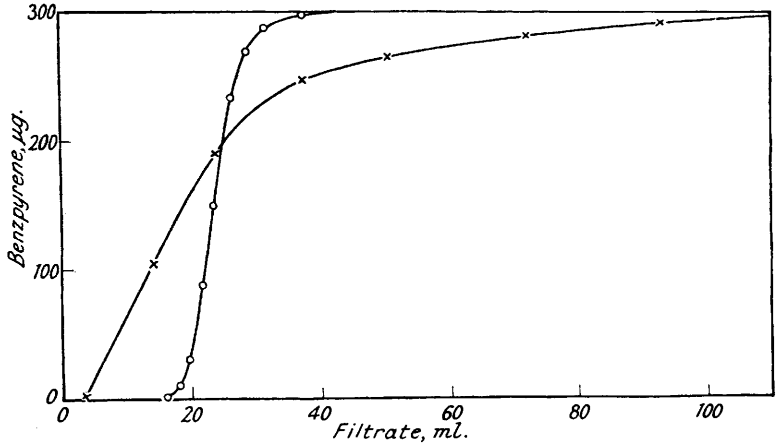


Solvent: benzene–light petroleum (2 : 3). Initial solution: 300 μg. of benzpyrene in 1 ml. Adsorbent: 5 g. of alumina.

Dimensions: column 1 (×——×), 204 × 5 mm.; column 2 (○——○), 70 × 9 mm.; column 3 (Δ——Δ), 20 × 18 mm.

FIG. 4.

Effect of particle size on elution curve.



Solvent: decalin. Initial solution: 300 μg. of benzpyrene in 1 ml. Adsorbent: 3 g. of silica gel.
Column 1 (×——×): 20—30 mesh; 100 × 8 mm. Column 2 (○——○): 100—160 mesh; 80 × 8 mm.

(b) Particle size of adsorbent. The use of an adsorbent of large particle size has an effect on the elution curve similar to that of using a short column, and probably for the same reasons (Fig. 4).

(c) Ratio of v_0 to V_t. The typical elution curve does not contain any part where the rate of elution is linear. The concentration of the eluate may reach a maximum of about $m_0/0{\cdot}4V_t$ with silica gel and $m_0/0{\cdot}65V_t$ with alumina. If this maximum concentration is denoted by m_0/V_t, then the initial concentration c_0 must be $\geq m_0/\delta V_t$. If c_0 is smaller than this, then the elution curve will contain a linear part of concentration c_0 and may be described as incompletely developed. To obtain a fully developed or " typical " elution curve it is therefore necessary that $v_0 \leq \delta V_t$.

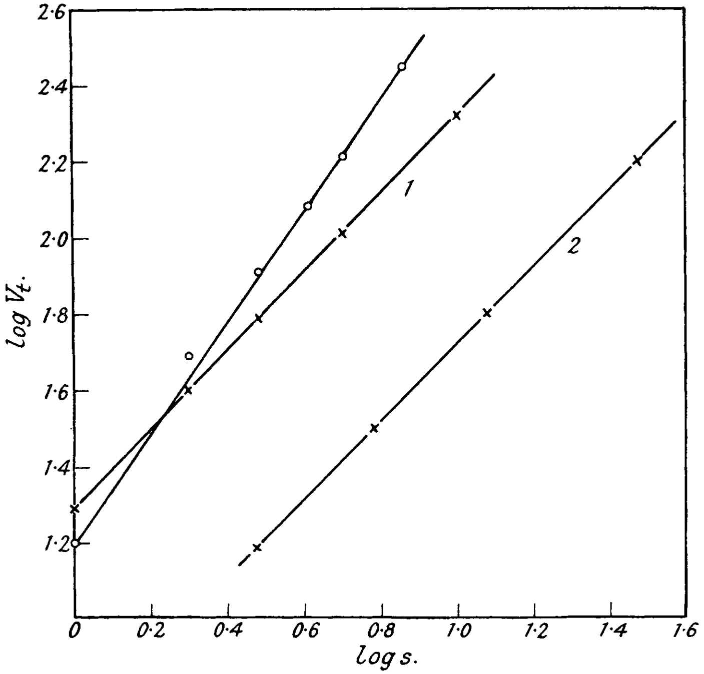
(6) *Variation of* V_t *with the quantity of adsorbent.* As, for any given system of adsorptive, solvent, and

adsorbent, α is constant, the factor which determines the course of elution is V_t. We consider how it is influenced by the variation of some other factors.

If the quantity of adsorbent (s) is varied while other factors are kept constant, again a significant difference between silica gel and alumina is found. If V_t is plotted against s, the relationship is almost linear for the former but parabolic for the latter. On a log scale, straight lines result in both cases (Fig. 5). The silica-gel line has a slope of 1·03 and the alumina line a slope of 1·5. It is immediately obvious that these values are the reciprocal of α for the respective systems. We therefore arrive at the equation :

FIG. 5.

Variation of V_t *(ml.) with quantity of adsorbent,* s *(in g.).*

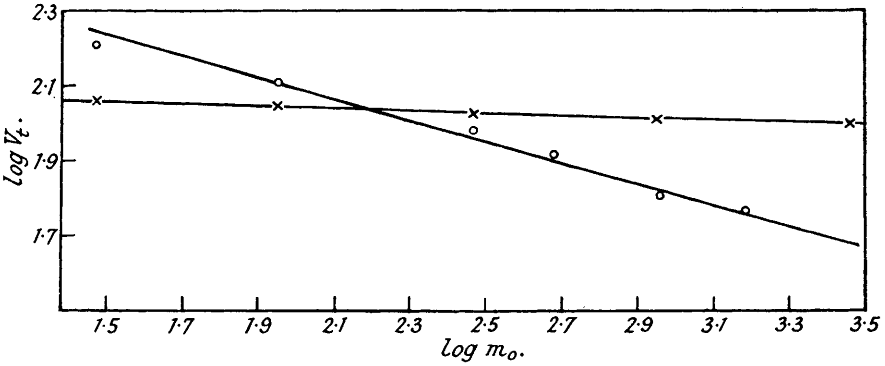


○——○ : Alumina experiments. Solvent : benzene–light petroleum (1 : 4). Initial solution : 300 μg. of benzpyrene in 1 ml.

×——× : Silica gel experiments. Line 1 : solvent light petroleum containing 3% of benzene. Line 2 : solvent decalin. Initial solution : 300 μg. of benzpyrene in 1 ml.

FIG. 6.

Variation of V_t *with quantity of adsorptive,* m_0 *(in* μ*g.).*



○——○ : Adsorbent 3·5 g. of alumina. Solvent : light petroleum containing 20% of benzene.

×——× : Adsorbent 5 g. of silica gel. Solvent : light petroleum containing 3% of benzene. Initial volume : 15 ml. in each case.

$$V_t = ks^{1/\alpha} \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (1)$$

(7) *Variation of* V_t *with the quantity of adsorptive.* The results of the experiments performed to test this point are presented in Fig. 6, where log V_t is plotted against log m_0. Straight lines are obtained in both sets of

experiments. The line found with alumina has a slope of 0·28, whereas the slope of the silica gel line is 0·03. Denoting this exponent by β, we obtain :

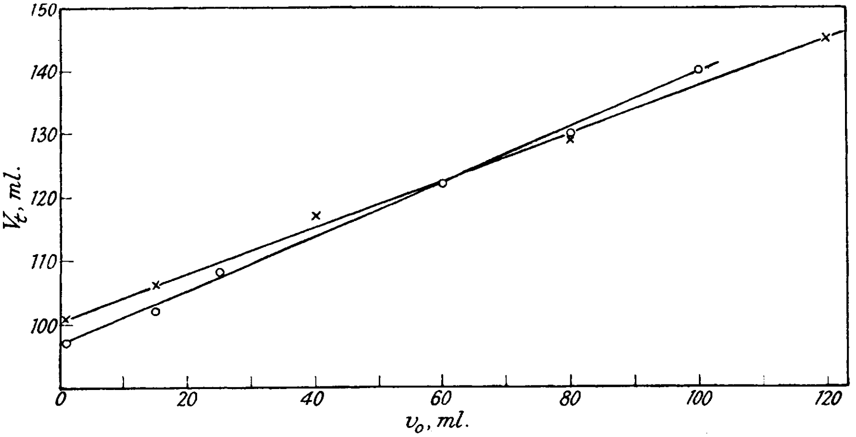
$$V_t = k'/m_0^{\beta} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \qquad (2)$$

(8) *Variation of* V_t *with the initial volume.* The threshold volume was found to increase to some extent with increasing v_0, all other factors including m_0 being kept constant. The relationship is linear, and the slope of the v_0–V_t curve has been determined as 0·43 for alumina and 0·37 for silica gel (Fig. 7). If this factor is given the symbol γ, we may write :

$$V_t = k'' + \gamma v_0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \qquad (3)$$

FIG. 7.

Variation of V_t *with initial volume,* v_0, *both in ml.*



○——○ : Adsorbent 3·5 g. of alumina. Solvent : light petroleum containing 20% of benzene.
×——× : Adsorbent 5 g. of silica gel. Solvent : light petroleum containing 3% of benzene. m_0 = 300 μg. of benzpyrene in each case.

In some of these experiments v_0 exceeded the limit set for the achievement of full development. In spite of this, the values of V_t observed lie on the same line as those where development was complete; γ is thus not affected by the degree of development.

(9) *General expression for* V_t. By combining equations (1), (2), and (3) we obtain

$$V_t = Ks^{1/\alpha}/m_0^{\beta} + \gamma v_0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \qquad (4)$$

and

$$K = m_0^{\beta}(V_t - \gamma v_0)/s^{1/\alpha} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \qquad (5)$$

TABLE II.

Calculation of threshold volume.

(1) *Alumina.*

Solvent : light petroleum containing 20% of benzene. Adsorptive : benzpyrene, $1/\alpha = 1·5$, $\beta = 0·28$, $\gamma = 0·43$, $K = 70·5$.

			V_t (ml.).					V_t (ml.).	
m_0 (μg.).	v_0 (ml.).	s (g.).	Found.	Calc.	m_0 (μg.).	v (ml.).	s (g.).	Found.	Calc.
30	1	3·5	155	178·5	300	1	2	49	40·8
30	15	3·5	160	184·5	300	1	3	86	74·7
90	15	3·5	126	137·5	300	1	4	120	114·5
120	4	3·5	117	122·7	300	1	5	165	160·0
300	1	3·5	97	94·0	300	1	7	280	264·5
300	5	3·5	99	95·6	480	16	3·5	82·5	89·0
300	30	3·5	108	106·4	900	15	3·5	63	75·2
300	60	3·5	122	119·3	1500	15	3·5	60	66·0
300	80	3·5	130	127·9	1500	60	3·5	87	85·4
300	100	3·5	140	136·5	1920	64	3·5	80·5	83·1
300	1	1	16	14·7					

(2) *Silica gel.*

Solvent : light petroleum containing 3% of benzene. Adsorptive : benzpyrene, $1/\alpha = 1·03$, $\beta = 0·03$, $\gamma = 0·37$, $K = 22·9$.

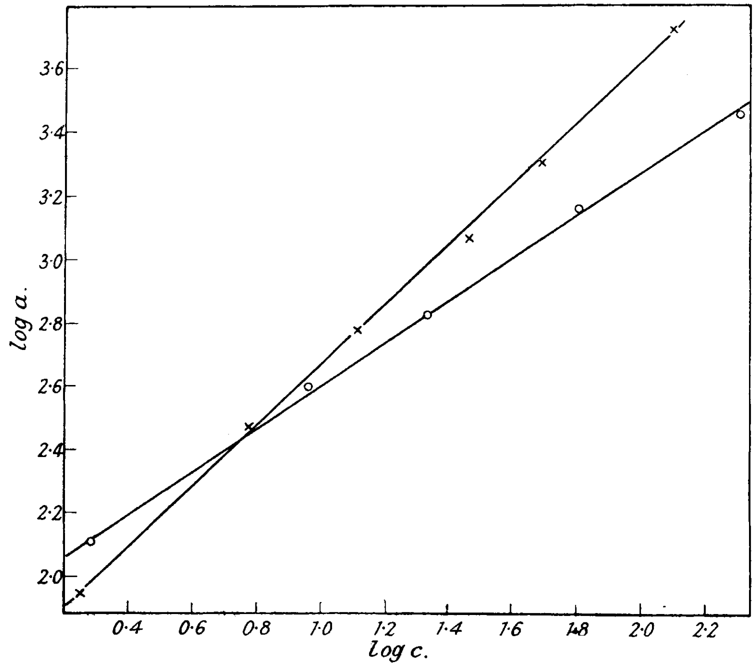
30	15	5	115	114·1	300	15	5	106	106·8
90	15	5	109	110·5	300	40	5	117	116·1
300	1	1	19·5	19·7	300	80	5	129	130·9
300	1	2	40·5	39·9	300	120	5	145	145·6
300	1	3	63	60·3	900	15	5	103	103·9
300	1	5	101	101·7	3000	15	5	102	100·0
300	1	10	205	207·9					

In a system for which the constants α, β, and γ are known, it is sufficient to determine K in a single experiment; from this the approximate value of V_t can be calculated for any possible values of m_0, V_0, and s. Table II shows the degree of agreement between calculation and observation. This is more satisfactory with silica gel than with alumina, which may partly be due to the fact that the alumina experiments were done with long time intervals, so that different batches of solvents had to be used and, although the adsorbent was from the same batch, it had to be dried and stored in different portions.

(10) *Freundlich's adsorption isotherm.* The results already reported are based on the experimental data contained in Fig. 8. Here, a = amount adsorbed per unit weight of adsorbent (μg./g.) and c = the final concentration of the supernatant solution (μg./ml.). The correlation between a and c is given by $a = k'''c^{a}$.

FIG. 8.

Adsorption isotherms (concns. in μg. of benzpyrene/ml.).



○——○ : Adsorbent, alumina. Solvent : light petroleum containing 20% of benzene.
×——× : Adsorbent, silica gel. Solvent : light petroleum containing 3% of benzene.

(11) *Results with other adsorptives.* The general validity of the results obtained with benzpyrene was checked in a series of experiments in which two other adsorptives, *p*-dimethylaminoazobenzene and acridine, were used. Alumina was the adsorbent, and benzene the solvent. The correlation between V_t and the shape and slope of the elution curve was the same as in the experiments with benzpyrene and alumina. Also the variation of V_t with the quantity of adsorbent was investigated, and the exponential coefficients found (1·45 and 1·48) were about the same as with benzpyrene (1·5). Details of these experiments are omitted for the sake of economy.

Some Practical Applications.—(1) *Determination of eluant power.* If in a chromatographic system the only factor to be varied is the solvent, the value of V_t found may serve as a measure of the eluant power for which so far no quantitative data are available. The relative eluant powers of light petroleum, benzene, and mixtures of both are recorded in Fig. 9, where $k = V_t/s^{1/a}$. The ×——× line shows the values to be expected from a purely additive behaviour of the mixtures; they considerably diverge from the observed values, showing that the addition of light petroleum decreases the eluant power of benzene far beyond what can be attributed to mere dilution.

(2) *Standardisation of adsorbents.* Two different batches of the same adsorbent may differ appreciably with respect to their adsorbing qualities. A problem which frequently arises on changing over from one batch of adsorbent to another is to find out how much of the new adsorbent will make up a column of adsorbing power equal to a given column of the old adsorbent. Provided m_0 and v_0 be kept constant, formula (1) is valid, from which it follows that $k = V_t/s^{1/a}$. A series of adsorbents can be standardised by determining their k values, *i.e.*, the threshold volumes per unit weight of adsorbent. For two different adsorbents having k values of k_1 and k_2 we obtain $k_1/k_2 = (s_2/s_1)^{1/a}$ and $s_2 = s_1(k_1/k_2)^{a}$ where s_1 and s_2 are the respective quantities of adsorbent.

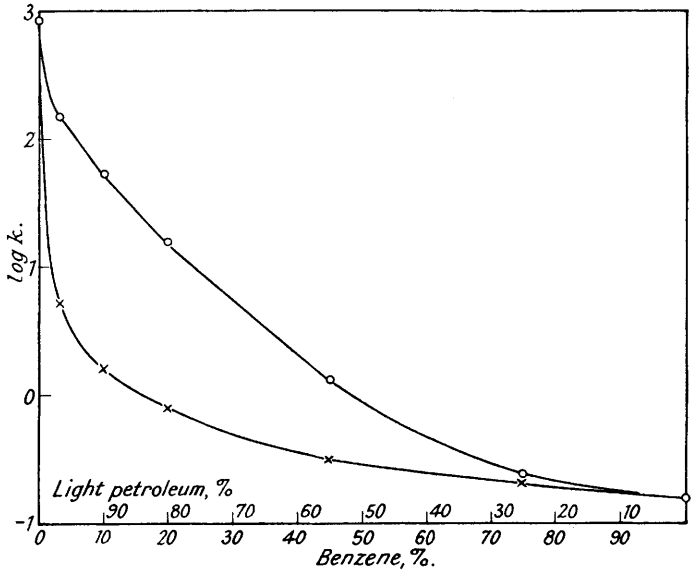
(3) *To determine the minimum size of a column necessary to ensure full development of an initial solution of* v_0, m_0. As previously found [Section 5(c)], the condition for full development is that $v_0 < 0{\cdot}65 V_t$ in the case

of alumina, and $v_0 < 0{\cdot}4V_t$ in that of silica gel. Allowing a margin of safety, we may put $V_t = 3v_0$ for silica gel and $V_t = 2v_0$ for alumina as the limiting condition for full development. From equation (5) it follows that, for silica gel, $s = [m_0{}^{\beta}v_0(3 - \gamma)/K]^{\alpha}$, and for alumina we have the same expression but with $(2 - \gamma)$ instead of $(3 - \gamma)$.

(4) *To determine the fraction of the filtrate containing the eluate.* We now give the solution of some problems connected with the micro-estimation of benzpyrene. The method adopted consisted of the following steps : adsorption on a column of silica gel from light petroleum, and elution with light petroleum containing 20% of benzene; re-adsorption on a column of alumina from this mixture, followed by development with the same solvent; elution with benzene. The method had to be so devised that it allowed quantitative recovery of 0·5—300 μg. of benzpyrene. Both columns contained 2 g. of adsorbent.

FIG. 9.

Variation of k *(threshold volume per g. of adsorbent) with composition of light petroleum–benzene mixtures.*



Adsorbent, alumina. Initial solution, 300 μg. of benzpyrene in 1 ml.

The first adsorption took place from 5—10 ml. of light petroleum on a column of silica gel; v_0 is here only 2·5—5% of V_t and can therefore be neglected in the calculation of the next step, *viz.*, elution by benzene–light petroleum (1 : 4). From formula (4) we obtain for $m_0 = 300$ μg. and $K = 2{\cdot}41$

$$V_{t(300\ \mu g.)} = 2{\cdot}41 \times 2^{1{\cdot}03}/300^{0{\cdot}03} = 4{\cdot}15 \text{ ml.}$$

and for $m_0 = 0{\cdot}5$ μg. :

$$V_{t(0{\cdot}5\ \mu g.)} = 2{\cdot}41 \times 2^{1{\cdot}03}/0{\cdot}5^{0{\cdot}03} = 5{\cdot}02 \text{ ml.}$$

With silica gel, elution is completed after the total filtrate has reached a volume of about $2{\cdot}5V_t$. The portion of the filtrate containing the eluate is therefore between 4·15 and 12·6 ml. A margin of safety being allowed, the first 15 ml. of the filtrate were collected, and passed through the alumina column. For $K = 78{\cdot}8$ and $m_0 = 300$ μg. we find :

$$V_{t(300\ \mu g.)} = 78{\cdot}8 \times 2^{1{\cdot}5}/300^{0{\cdot}28} + 0{\cdot}43 \times 15 = 51{\cdot}8 \text{ ml.}$$

and for $m_0 = 0{\cdot}5$ μg. :

$$V_{t(0{\cdot}5\ \mu g.)} = 78{\cdot}8 \times 2^{1{\cdot}5}/0{\cdot}5^{0{\cdot}28} + 0{\cdot}43 \times 15 = 277 \text{ ml.}$$

With alumina a volume of filtrate of at least $4V_t$ has to be collected before elution is completed; the required fraction is therefore that between 50 and 1200 ml. of filtrate. After the collection of the first 50 ml. of filtrate, which were rejected, elution was carried out with pure benzene. For $K = 0{\cdot}79$, the required fraction is that between 0·4 and 12 ml.

Such calculations are, of course, only an approximation, subject to the absence of any appreciable displacement effects due to impurities. They have to be verified by direct observation.

DISCUSSION.

The results obtained show that a number of features of chromatography conform to simple empirical expressions. The question arises how these compare with the theory developed on the assumption of immediate and complete adsorption equilibrium (Weiss, preceding paper).

The variation of the threshold volume V_t with the amount of adsorbent s is given by $V_t = ks^{1/\alpha}$, where α is the exponential coefficient of Freundlich's adsorption isotherm. In this case the experimental results were so unequivocal ($1/\alpha$ for alumina : calc. 1·5; found, 1·5; for silica gel : calc., 1·04; found, 1·03) that the correlation with the adsorption isotherm immediately suggested itself. It also follows directly from the theory (Weiss, equation 47). For the case of the linear adsorption isotherm V_t is directly proportional to the quantity of adsorbent.

On the other hand, the factor γ, which determines the variation of V_t with v_0, was found to be almost identical for both adsorbents despite the difference of their adsorption isotherms, and it might therefore be assumed that γ is independent of α. This, too, is confirmed by the theory (Weiss equations 47 and 48) which postulates $\gamma = 1$ for complete development ($v_0 < \delta V_t$) and $\gamma <1> 0$ for incomplete development ($v_0 > \delta V_t$). Actually, γ was found to be about 0·4 regardless of the degree of development. If v_0 is very small compared with V_t, then the effect of v_0 on V_t becomes insignificant.

Fig. 10a.

Effect of the adsorption isotherm on the elution curve on the basis of the experimental findings.

Fig. 10b.

Effect of the adsorption isotherm on the elution curve on the basis of the theory.

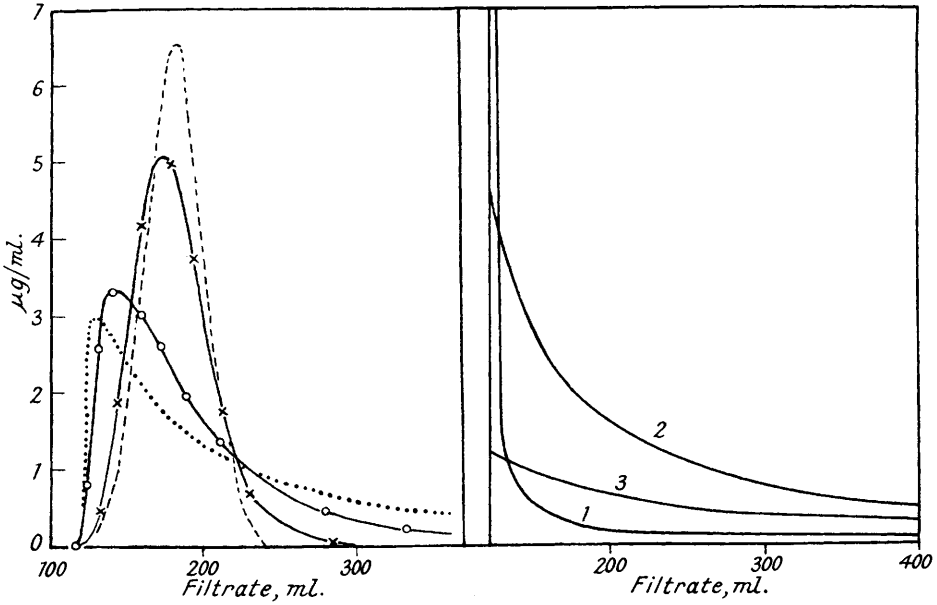


Fig. 10a.—○——○ : Elution curve with 4 g. of alumina; $v_0 = 1$ ml., $m_0 = 300$ μg. of benzpyrene. Solvent : benzene–light petroleum (1 : 4).

×——× : Elution curve with 6 g. of silica gel; $v_0 = 1$ ml., $m_0 = 300$ μg. of benzpyrene. Solvent : light petroleum containing 3% of benzene.

Broken line : Elution curve extrapolated for $a = 1$.
Dotted line : Elution curve extrapolated for $a = \frac{1}{3}$.

Fig. 10b.—Curve 1 : Elution curve for a near 1.
Curve 2 : Elution curve for $a = \frac{2}{3}$.
Curve 3 : Elution curve for $a = \frac{1}{3}$.

The exponential coefficient, β, giving the dependence of V_t on the quantity of adsorptive (m_0), is different in the two cases, and it is therefore probable that it is a function of α. According to the theory (Weiss, equation 47), $\beta = (1 - \alpha)/\alpha$. The agreement is good in the case of silica gel, where $\beta = 0{\cdot}03$ and $(1 - \alpha)/\alpha$ is 0·04, but less satisfactory for alumina, where $\beta = 0{\cdot}28$ and $(1 - \alpha)/\alpha = 0{\cdot}50$. For a system with a linear adsorption isotherm, $(1 - \alpha)/\alpha$ would be 0; here V_t is independent of m_0 or $c_{0,\ \mathrm{const.}\ v_0}$. This is practically realised with silica gel, where V_t was found to be 115 and 103 ml. with initial concentrations of 30 μg. and 3000 μg./ml., respectively, *i.e.*, a decrease of 10·5% for a hundredfold increase of concentration.

The shape of the elution curve to be expected on the basis of the equilibrium theory can be predicted with the aid of equations (50 and 52, Weiss), which only apply to those cases where development has been complete For the linear adsorption isotherm, $c_{\mathrm{el.}} = c_0$; this means that in this case no development takes place : the concentration of the eluate is that of the original solution, m_0/v_0.

If α is near 1, the factor $[v_t/(v_t + v_{\mathrm{el.}})]^{a/(1-a)}$ (Weiss, equation 50) is negligibly small. In such a case, the effect of development will be small, and the concentration of the eluate should not differ greatly from that of the original solution; also, the " tail " at the end of elution will be small. Such a case is illustrated by curve 1, Fig. 10b. Curve 2 shows a theoretical elution curve for $\alpha = \frac{2}{3}$, and curve 3 one for $\alpha = \frac{1}{3}$. It will be noticed that the " tail " of the elution curve increases, and the change of concentration during elution becomes less marked with decreasing α. The maximum of concentration is always reached at the very beginning of elution.

On the experimental side, two points were found up to which the mean concentration of the eluate was m_0/V_t, *viz.*, those marking volumes of eluate of $\frac{1}{2}\alpha V_t$ and αV. In spite of the considerable difference of their adsorption isotherms, the elution curves of both silica gel and alumina conformed to this rule. It is therefore perhaps permissible to assume that this relation holds for all values of α. For $\alpha = 1$, it follows on this assumption that the mean concentration of the entire eluate is m_0/V_t, or that the quantity of solvent required for complete elution equals $2V_t$, if reckoned from the beginning of the adsorption phase, or V_t, if reckoned from the beginning of the elution phase.

The maximum of concentration was found to lie near the point corresponding to a volume of eluate $= \frac{1}{2}\alpha V_t$, for both the silica gel and the alumina curve. For the case of $\alpha = 1$, it might be expected to coincide with the point where $\frac{1}{2}m_0$ are eluted. Here the elution curve would be strictly symmetrical.

Fig. 10a shows elution curves obtained by experiments with alumina and silica gel, and also curves for $\alpha = 1$ and $\alpha = \frac{1}{3}$ which were extrapolated on the basis of the assumptions just outlined. To facilitate comparison, the curves of Fig. 10b were calculated by assuming the same experimental conditions as those under which the observations recorded in Fig. 10a were made. Agreement between theory and observation is qualitatively satisfactory for the case of alumina where $\alpha = \frac{2}{3}$; here the mean concentration of the eluate for $\frac{1}{2}\alpha V_t$ is calculated as $1{\cdot}3m_0/V_t$ and for αV_t as $0{\cdot}96m_0/V_t$. The theory is, however, no longer fulfilled in the case of silica gel, where α is near 1. According to the theory the concentration of the eluate is independent of V_t and is equal to c_0, if $\alpha = 1$. The experimental results show for a case where α is very near 1 that the concentration of the eluate is still inversely proportional to V_t. As shown by Weiss (p. 302), a better approximation to the experimental findings is obtained by introducing certain non-equilibrium factors.

The volume of v_0 which must not be exceeded if full development is to be attained has been defined by the formula $v_0 = \delta V_t$. According to the theory (Weiss, equation 44), δ, like β, is equal to $(1 - \alpha)/\alpha$; δ was found to be approximately 0·65 in the case of alumina and 0·4 in that of silica gel. The values calculated from the theory are 0·5 and 0·04 respectively; there is thus reasonable agreement in the first example, but again the theory is no longer obeyed when α is near 1. The theory postulates that in the case of the linear adsorption isotherm $\delta = 0$, *i.e.*, that the volume necessary to bring about development is infinite. The experimental results, however, suggest that here, too, this volume is finite.

EXPERIMENTAL.

Adsorbents.—Silica gel (B.D.H.) was ground, the 100—160 mesh fraction screened out, and dried at 110° for 24 hours without any further purification. It was stored in a glass-stoppered bottle kept in a desiccator over phosphoric oxide. Aluminium oxide (B.D.H., "for chromatographic adsorption analysis") was dried at 300° for 3 hours and stored as above. During operations, such as weighing or filling the columns, exposure to atmospheric moisture was reduced to a minimum.

Solvents.—High purification of solvents proved indispensable for obtaining reproducible results.

Light petroleum. The commercial liquid (b. p. 40—60°) was purified by the following successive extractions, each carried out with several minutes' vigorous shaking: nitrating mixture (4 times), fuming sulphuric acid (3 times), water, and 25% sodium hydroxide. After being dried over calcium chloride, it was rectified through an efficient column of the Clarke and Rahrs type (*J. Ind. Eng. Chem.*, 1923, **15**, 349).

Benzene. Extraction of A.R. benzene with concentrated sulphuric acid (3—4 times), followed by washing with water and alkali, drying, and rectification.

Decalin. Purified as for benzene. Decalin solutions of benzpyrene must be re-extracted with sulphuric acid before recovery, as benzpyrene is slightly volatile with decalin.

Estimation of Benzpyrene.—3 : 4-Benzpyrene was estimated fluorimetrically. A G.E.C. Osira lamp served as U.V. light source. Fluorescence was measured by a Westinghouse "Green Sensitive" photoelectric cell set at right angles to the incident beam and connected to a mirror galvanometer. It was mounted on the blackened inside of a closed box. The U.V. beam entered the box through a window of Wood's glass, in front of which was a flat-bottomed spherical quartz flask of about 250 ml. capacity, filled with distilled water, which served as condenser. The solution to be examined was placed in a thin-walled test-tube of soft glass, which was passed through a closely fitting hole in the centre of the lid of the box and held in position by a shallow receptacle at a level with the lower end of the photoelectric cell. Before entering the latter, the fluorescence light was filtered through a layer of 5% sodium nitrite. A stream of oxygen-free nitrogen was bubbled through the fluorescent solution for 2 mins. before a reading was taken in order to eliminate the quenching effect of atmospheric oxygen (Bowen and Williams, *Trans. Faraday Soc.*, 1939, **35**, 765; Weil-Malherbe and Weiss, *Nature*, 1942, **149**, 471). During the reading, the nitrogen stream was shut off by a tap. The solution to be examined contained up to 3 μg. of benzpyrene in 5 ml. of solvent. For this range of concentration the deflection curve was almost linear. With 1 μg. a deflection of about 65 mm. was obtained. The zero-reading was perfectly stable. A blank, determined with 5 ml. of pure solvent, showed a deflection of 1—2 mm. At least 3 points of a reference curve were established before each series of estimations.

Chromatographic Experiments.—The technique of tapping and compressing generally recommended for the filling of columns (*e.g.*, Zechmeister and von Cholnoky, "Die Chromatographische Adsorptionsmethode," Springer, Wien, 1937) was observed. The rate of filtration was usually 20—60 drops/min. Where volatile solvents were used, filtration was always carried out under pressure to minimise evaporation losses. The progress of the band was watched in the light of the U.V. lamp. As soon as elution had started, fractions of the eluate were collected at frequent intervals, and their volumes recorded.

Adsorption Isotherm Experiments.—These were carried out in sealed test-tubes which were shaken at room temperature for 5—6 hours.

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CANCER RESEARCH LABORATORY, NORTH OF ENGLAND COUNCIL OF THE BRITISH EMPIRE CANCER CAMPAIGN,
ROYAL VICTORIA INFIRMARY, NEWCASTLE-UPON-TYNE.
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