[CONTRIBUTION FROM THE COATES LABORATORY OF LOUISIANA STATE UNIVERSITY]

The Rate of Movement of a Chromatographic Zone as a Function of Column Position, Initial Concentration and Initial Volume

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Two methods have been developed for the experimental study of chromatographic phenomena; one is based on the variations in the concentration of the substance chromatographed in the filtrate from the column; the other depends on the movement of a zone on the column. The first method has been used by Cassidy,[1,2] Claesson,[3] and Weil-Malherbe.[4] Jacobs and Tompkins[5] have used both methods, while the second procedure has been employed in investigations by Martin and Synge,[6] Austin and Shipton,[7] and the author.[8]

In the experimental study and theoretical development of chromatography a considerable number of quantities have been defined and represented by letters. In order to restrict this tendency where possible the terms suggested by other authors will be used here. The terms used, their numerical value in this work, their source and definition are listed below:

α	Pore volume per unit column length (0.039 ml./mm.).	Wilson[9]
M	Weight of adsorbent per unit length (0.0366 g./mm.).	Wilson
Q	Weight of adsorbed solute per unit length (2.97 Mc).	Wilson
$f = f(c)$	Adsorption isotherm, $Q = Mf$ (f = 2.97 for a linear isotherm).	Wilson
x	Distance of any point in the column from the beginning of the column.	Wilson
x_d	Value of x, measured from the top of the column, at which a discontinuity occurs (sharp boundary).	Wilson
c	Concentration of the solution.	Wilson
c_0	Initial concentration of the solution.	Wilson
v_0	Initial volume of solution poured on column.	Weiss[10]
A	Solute adsorbed on the adsorbent in volume of column containing 1 ml. solution (Q/α).	LeRosen
k	Proportionality constant in a linear isotherm so that $A = kc$ (2.76).	LeRosen
R	Displacement of zone on column/displacement of solvent in column, $R = dx/dD$. R_t for trailing, R_l for leading boundary of zone. (A similar term, R, has been suggested by Martin and Synge.)	LeRosen
W	Weight of adsorbed solute per gram of adsorbent.[11]	LeRosen
D	Distance the developing solvent has moved in the column.[11]	LeRosen
V_c	Rate of flow of the developing solvent through the column when a constant value is attained.	LeRosen
T_{50}	Time required for the solvent to penetrate 50 mm. under the vacuum given by the water pump.	LeRosen
S	Length of adsorbent column containing 1 unit volume of solvent/length of unfilled tube required to hold the same volume of solvent (packing measure).	LeRosen
P	Capacity of column per gram of adsorbent. (This term could also be defined as capacity per gram or per weight of adsorbent equivalent to one unit interstitial volume.)	Walter[12]
K	Equilibrium constant: $K = \dfrac{Q}{(P - Q)(C)}$ (if units are in mg./g., P = 172, K = 0.014)	Walter
a	Constant in Langmuir isotherm ($a = KP$) $W = ac/(1 - bc)$	
b	Constant in Langmuir isotherm ($b = K$)	

This investigation was undertaken in order to determine quantitatively the way in which R depends on the variables, column position (x), initial volume (v_0), and initial concentration (c_0). Martin and Synge[6] have already indicated that R is independent of column position, and depends to some extent on v_0 and c_0. Austin and Shipton[7] have shown that R is independent of V_c in the limits of their study. Weil-Malherbe[4] has carried out the corresponding study using V_t (the volume of filtrate collected before the solute first appears in the filtrate) as a measure of the rate of movement of a zone.

When the factors influencing R are known it will be possible to use this term to describe the strength of an adsorbent, to compare developers, to determine the optimum conditions for chromatographic separations, and possibly to use it for the identification of some substances.

The system silicic acid–benzene–*o*-nitroaniline was selected for study for the following reasons: for organic compounds silicic acid is a good adsorbent with a broad application, it packs uniformly in the column, however, it does not give very sharp boundaries; benzene is a good developer and is easily obtainable in relatively pure condition; *o*-nitroaniline shows about the correct rate of movement for a study of this system and is available in a high degree of purity; it is colored so it may be followed visually on the column.

The value of R was determined for both the leading and trailing edges of the zone. The latter measurement was difficult and not very

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(1) H. G. Cassidy, THIS JOURNAL, **62**, 3073, 3076 (1940).

(2) H. G. Cassidy and S. E. Wood, *ibid.*, **63**, 2628 (1941).

(3) S. Claesson, *Arkiv Kemi Mineral. Geol.*, **23A**, [1] (1946).

(4) H. Weil-Malherbe, *J. Chem. Soc.*, 303 (1943).

(5) P. W. M. Jacobs and F. C. Tompkins, *Trans. Faraday Soc.*, **41**, 395 (1945).

(6) A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, **35**, 1358 (1941).

(7) C. R. Austin and J. Shipton, *J. Council Sci. Ind. Research*, **17**, 115 (1944).

(8) A. L. LeRosen, THIS JOURNAL, **64**, 1905 (1942); **67**, 1683 (1945).

(9) J. N. Wilson, *ibid.*, **62**, 1583 (1940).

(10) J. Weiss, *J. Chem. Soc.*, 297 (1943).

(11) Defined for the first time in this paper.

(12) John E. Walter, *J. Chem. Phys.*, **13**, 229, 332 (1945).

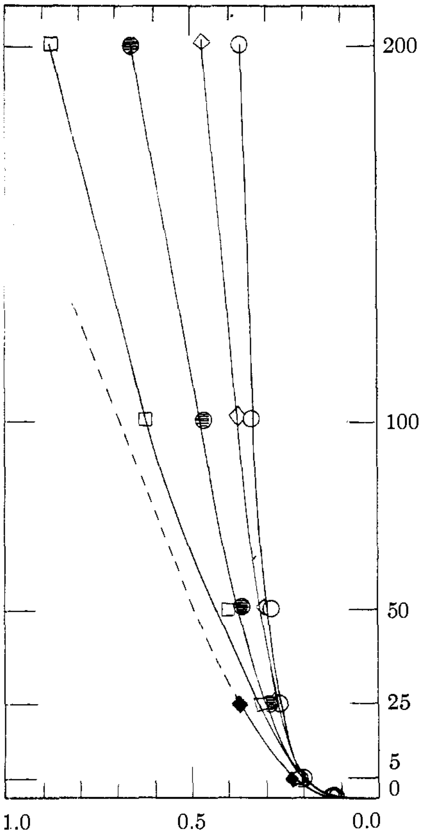

Fig. 1.—The variation of R_l with concentration and initial volume; the various curves represent different initial volumes: 0.2 ml., ○; 0.5 ml. ◇; 1.0 ml. ●; 2.0 ml. □; 4 ml. ◆.

accurate because of the diffuse nature of this section of the zone. In the 75 mm. columns used the value of R was independent of column position, this was also true when a much longer column was used (535 mm.) in agreement with data given by Martin and Synge for a different system.

For the trailing boundary R was practically constant for all values of c_0 and v_0, within the limits of accuracy of the determinations. The value was $R = 0.1 \pm 0.03$; if a few widely varying figures are dropped the variation can be reduced to about ± 0.01.

The value of R_l was determined over the entire concentration range possible in solution, 0.005 to 200 mg./ml. (3.3×10^{-5} to 1.3 M), and with initial volumes of 0.2 to 4.0 ml. where practical to do so. The empirical equation given below agrees reasonably well with the experimental values over the entire range studied.

$$R_l = (1 + 0.0042[v_0 - 0.1]c_0)(0.14 + 0.1 \log[c_0 + 1])$$

The method used to obtain this equation was as follows: a plot of the value of R against the concentration gave a series of curves shown in Fig. 1; these indicated that R is a logarithmic function of c_0. It is reasonable to assume that R_l approaches R_t as a limit as c_0 approaches zero, so that with $c_0 = 0$, $R_l = 0.1$. The function, $R_l - 0.1 = \log (c_0 + 1)$ has the correct properties for $c_0 = 0$; this equation was evaluated for the values of c_0 used and the R_l values calculated were plotted against those determined experimentally. Figure 2 shows a linear relation between the calculated and experimental values for $v_0 = 0.2$ ml.; for higher values of v_0 there is a divergence which is a function of both v_0 and c_0. The ratio $R_l v_0/R_l(v_0 = 0.2) = Z$ was evaluated for different c_0 values in each v_0 group, and Z was found to be a linear function of c_0 for each v_0 value, of the form $Z = nc_0 + 1.0$. Now n was evaluated as a function of v_0, with the result: $n = 0.0042 (v_0 + 1)$; when the parts are collected and assembled the result is the equation given above. It is noteworthy that in the final equation R_l approaches 0.14, instead of the R_t value, as c_0 approaches zero.

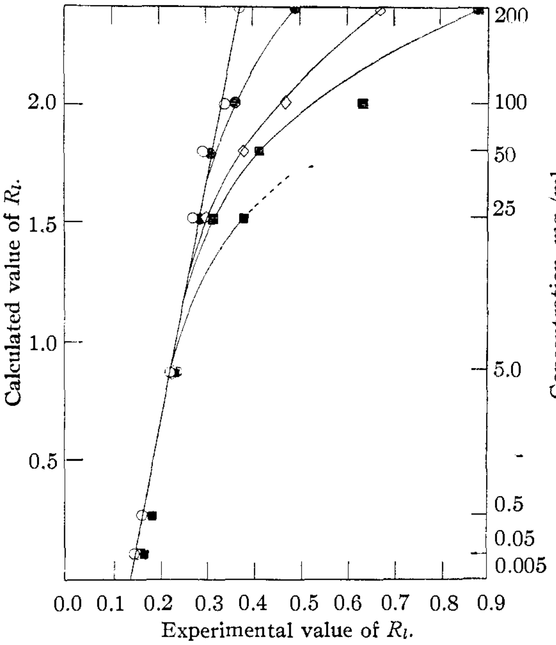

Fig. 2.—The relation between values at R_l calculated from: $R_l = 0.1 + \log(c + 1)$ and the experimental values of R_l for certain initial volumes: 0.2 ml. ○; 0.5 ml. ●; 1.0 ml. ◇; 2.0 ml. ▣; 4.0 ml. ■.

Table I gives the experimental values for R_t, R_l, and the initial value for R_l, determined by plotting the initial zone width against the D value for the initial volume (mm./ml. × ml.). This table also includes the values of R_l calculated from the empirical equation, and the Langmuir isotherm.

Discussion

The value of R may be calculated from the adsorption isotherm according to the theory of chromatography given by several authors. For the linear isotherm all theoretical equations[5,7,8,9,12] may be reduced to the common form $R_l = 1/(k +$

TABLE I

EXPERIMENTAL AND THEORETICAL VALUES OF R

c_0, mg./ml.	v_0, ml.	R_t	R_l	Initial R_l	Calculated R_l: Empirical equation	Calculated R_l: Langmuir
200	2		0.88		0.96	
200	1		.67	0.570	.65	0.625
200	0.5		.48		.50	
200	.2	0.087	.37		.37	
100	2		.63		.61	
100	1		.47		.47	
100	0.5	.053	.36	.362	.40	.510
100	.2	.087	.34		.35	
50	2		.41		.41	
50	1	.098	.38	.258	.37	.427
50	0.5	.074	.30		.34	
50	.2	.105	.29		.32	
25	4		.38		.40	
25	2	.061	.31		.34	
25	1	.061	.29	.196	.31	.370
25	0.5	.077	.28		.29	
25	.2	.119	.27		.28	
5	4	.088	.23		.24	
5	2	.142	.23		.23	
5	0.6	.124	.22	.191	.22	.319
5	.2	.120	.22		.22	
0.5	4	.104	.19	.149	.16	.305
.5	0.2	.098	.16		.16	
.05	4	.106	.17	.130	.14	.304
.05	0.2	.107	.15		.14	
.005	4	.119	.16	.142	.14	.304
.005	0.2	.108	.14		.14	

1), where $k = Mf/\alpha$. Weiss[10] and DeVault[13] give equations which may be used with the Langmuir or Freundlich isotherms, giving, respectively: $R = 1/(1 + Ma/\alpha(1 + bc))$; $R = 1/(1 + (M/\alpha)(Kc^{a-1}))$. In passing it seems worthwhile to note that the term $Mf/\alpha(c)$ is the amount of substance adsorbed on the adsorbent in contact with a unit volume of solution and that the (M/α) may be eliminated by expressing the isotherm in different units.

The adsorption isotherm was determined for the system studied and was found to be best fitted by a Langmuir type equation, although it was nearly linear over a large concentration range. For the linear range the equation is $W = 2.95\ c$, this gives a value of 0.27 for R. The Langmuir isotherm gives values in the range 0.30–0.62 for the concentration range studied. The Freundlich isotherm cannot be used because the wrong kind of concentration dependence is shown. Zones suitable for chromatographic work are obtained in the concentration range 1–0.02 mg./ml. for the columns used; in this interval the R values are 0.22–0.14. It is apparent that the calculation from the linear isotherm gives a rough approximation to the rate of movement of the front edge of the zone. Table I shows the relation between the experimental values and those calculated from the Langmuir isotherm; the calculated values agree reasonably well for the largest volume in each concentration down to 5 mg./ml., below this the calculated values are too large.

None of the calculations includes the initial volume of solution as a variable, and this leads to considerable uncertainty in the rate value. DeVault has in his equation the term S for the initial distribution of solute in the column, however, this does not seem suitable for use in the present case.

In the closely related field of ion-exchange Thomas[14] has given a mathematical treatment using a second order reaction between the adsorbent and solute as the basis for his calculations; Walter,[12] using the same approach, has published two interesting papers on the chromatographic behavior of zeolites. The first led to the result $R = 1/(1 + M/\alpha(KP/c\alpha))$. This agrees well with the experiment for large values of c, but approaches 0 for small ones; however, the Langmuir equation may be derived from the same line of reasoning, leading to agreement with other equations for non-ionic substances. In the second paper the results of non-attainment of equilibrium are discussed, but a closed solution is reached only for irreversible adsorption, which is not useful in chromatography. In this work two quantities worthy of note are mentioned, P, the capacity of the adsorbent per unit length (or per g.) and K, the equilibrium constant for the reversible reaction of solute and adsorbent. These fundamental qualities have been evaluated in the present work for this system.

The empirical equation derived from the present work cannot be discussed in terms of the adsorption isotherm until data for other samples of silicic acid, and for other adsorbents are available.

This equation leads to some useful results; it is evident that in all cases zones must spread on the column, since R_l does not approach R_t when c becomes small. The spreading will be greater the larger the R_l value, and hence will increase with the concentration and with v_0 to a less extent; it follows that for a given amount of solute a more compact zone is obtained by using, within limits, a large volume, than by using a smaller volume and a high concentration.

The independence of R_t from concentration and initial volume is probably a result of the condition that R_t is determined by the time necessary to remove the solute from the adsorbent at the trailing edge where the concentration is practically constant, and if there is any effect due to concentration it should be such that R_t decreases with concentration. Such an effect in the present work could not be distinguished from the experimental error.

One reason for the difference between the calculated and experimental values for R_l is the fact that c_0 values were used instead of c values, there-

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(13) D. DeVault, THIS JOURNAL, **65**, 532 (1943).

(14) H. C. Thomas, *ibid.*, **66**, 1664 (1944).

fore too fast a rate was obtained, nevertheless the limiting value at 0 concentration is still too high, indicating a deficiency of the theory.

The increase of R_l with concentration raises the question as to what will happen if the slower of two chromatographic zones is increased in concentration. A preliminary experiment has shown that when the slower zone is increased in concentration it speeds up the zone below it in the column, acting as an eluting agent for the lower zone. This action is similar to the displacement development described by Tiselius.[15]

Experimental

Methods and Apparatus.—The methods used in this work have been described elsewhere.[8] The chromatographic tube was 130 mm. long with a 9 mm. inside diameter. A water pump was used to draw the developing solvent through the column; the pressure difference between the ends of the column was maintained at about 665 ± 5 mm. by means of the pressure regulating device described by Gilmont and Othmer.[16]

Adsorbents and Solvents.—The benzene used was not specially treated, the acetone was redistilled, and the ether was dried over sodium wire. Merck Reagent Silicic Acid was used as the adsorbent. This was pre-washed with 3.3 ml. of acetone, 3.3 ml. of ether and 6.6 ml. of benzene. The unwashed adsorbent showed the following characteristics: $V_0 = 10.7$, $T_{50} = 115$, $S = 1.62$; after the pre-wash V_0 showed a marked increase to an average of 17.0.

For use in the experiment with the long column, and for the isotherm determination it was desirable to use a dry adsorbent without a pre-wash and of the same strength as the pre-washed material. One sample was pre-washed in the usual way, followed by a portion of 28–38° b. p. petroleum ether, then dried over calcium chloride in a vacuum desiccator; a second sample was dried at 110° for twenty-four hours. The properties were as follows.

TABLE II

THE RATE OF MOVEMENT OF *o*-NITROANILINE ON TREATED SILICIC ACID

	R_t	R_l
Prewashed	0.12	0.22
Prewashed and dried	.10	.21
Dried at 110°	.07	.13

On this basis the pre-washed and dried sample seemed suitable for use.

Some other information which might be needed in later calculations based on the data in this paper are listed below.

Water removed in acetone wash (based on volume removed from column after extraction with water saturated benzene.)	0.0032	ml./mm.
Void in chromatographic tube used	.064	ml./mm.
Adsorbent volume in column	.025	ml./mm.
Adsorbent volume corrected for water	.021	ml./mm.
Adsorbent volume from dried adsorbent	.020	ml./mm.
Adsorbent/ml. interstitial solution	.94	g.
Bulk density of packed adsorbent	.58	g./ml.
Volume of benzene carried by adsorbent pre-washed and sucked dry	.034	ml./mm.
Calculated density of adsorbent—		
—untreated	1.49	
—prewashed	1.72	
Density of silicic acid (Lange's "Handbook") H_2SiO_3	2.1–2.3	
H_4SiO_4	1.576	

Adsorption Isotherm.—For this determination 2 g. of prewashed and dried adsorbent was weighed out to the nearest mg. and mixed with 5 ml. of solution. The adsorbent was centrifuged off and the concentration of the supernatant solution was determined spectrophotometrically. The adsorbent substance was determined by difference.

The data is listed in Table III.

TABLE III

ADSORPTION OF *o*-NITROANILINE ON SILICIC ACID FROM BENZENE AT 25°

	W, mg./g.	
c, mg./ml.	Experimental	Calcd.
0.0025	0.0046	0.0061
.026	.0592	.063
.035	.088	.085
.050	.125	.121
.155	.364	.374
.235	.665	.58
.473	1.33	1.15
12.9	30.2	26.5
30	49.8	51.0
66	86.1	83.9
154	117	118

These data were well fitted by the Langmuir type isotherm $W = 2.42c/(1 + 0.014c)$. The values of W calculated from this equation are given in Table III.

Summary

In agreement with qualitative statements by other workers the rate of movement, relative to the solvent, of the front edge of a chromatographic zone on the columns studied was found to be independent of the column position and to vary with the initial concentration and to a less extent with the initial volume. The results are summarized in an empirical equation. Theoretical calculations are in rough agreement with the observed rate of movement of these zones.

The rate of movement of the trailing edge of the zones, relative to the solvent, is practically independent of column position, initial concentration, or initial volume; however, this boundary is difficult to measure because of its diffuse nature.

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[15] A. Tiselius. *Arkiv. Kemi Mineral. Geol.*, **15A**. 9 (1941).

[16] R. Gilmont and D. F. Othmer. *Ind. Eng. Chem.*, *Anal. Ed.*, **15**. 641 (1943).