been proposed for these reactions.

2. The α-hydroxy-β-aminoketones are readily converted to the corresponding α-chloro and α-acetoxy-β-aminobenzylacetophenones. The latter are identical with the products from the reactions of α-bromo-β-aminobenzylacetophenones with acetate salts.[1]

3. The α-hydroxy-β-aminobenzylacetophenones react with lithium aluminum hydride and with phenylmagnesium bromide to produce aminopropyleneglycols.

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An Experimental Study of Chromatography on Silicic Acid–Celite. The Applicability of the Theory of Chromatography[1,2]

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Introduction

Although the theory of the simple adsorption chromatogram has been discussed extensively during the past decade,[5–10] comparatively few experimental tests of the predictions of the proposed theories have been reported. Furthermore, those experimental studies which have been described present a somewhat incomplete and conflicting picture. The importance of the simple adsorption chromatogram, not only as a practical laboratory tool in its own right, but also as the fundamental chromatographic experiment, prompted the present investigation, which was designed to provide quantitative experimental data suitable for testing the various theories which may be applied to the simple Tswett chromatogram, in particular in their application to chromatograms on columns of silicic acid–Celite.

The first careful experimental study of the fundamental factors involved in simple adsorption chromatography was made by Weil-Malherbe[11] who investigated a few simple systems on alumina and silica gel by means of analyses of the filtrate from the column and found in general fairly good agreement with the theory proposed by Weiss.[7] Likewise, DeVault[6] was able to explain reasonably well on the basis of his theory the rather limited data of Cassidy and Wood[12] on the development of lauric acid on carbon. On the other hand, LeRosen[13] could obtain no more than partial qualitative agreement with theory in his experiments with *o*-nitroaniline in benzene on silicic acid, and similarly Thomas[10] found at best only rough qualitative agreement with his kinetic theory of chromatography in experiments with anthracene in cyclohexane on alumina. Jacobs and Tompkins[14] encountered some difficulty in reconciling with theory the results of their studies of the development of certain inorganic ions on alumina, but the more recent experiments of Glueckauf and Coates have almost completely resolved these difficulties. Indeed, as a result of the excellent work of the latter investigators and of Boyd and his coworkers,[15] and Mayer and Tompkins,[16] the theoretical treatment of the special field of ion-exchange chromatography now rests on a firm experimental foundation. Similarly, the theories which have been developed for the special adsorption analysis techniques of Tiselius have been tested extensively and found entirely adequate.[17] Although much of the theory applicable to ion-exchange columns may be considered to apply also to the closely analogous Tswett adsorption chromatogram, the theories of frontal analysis and displacement development[17] are of no direct aid in consideration of the simple adsorption chromatogram; however, their success does demonstrate that straightforward reasoning on the basis of measured adsorption isotherms can for many systems lead to the correct predictions of the chromatographic behavior of mixtures under particular conditions. Several earlier attempts[18] to find a correlation between the adsorption isotherms and relative

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[5] J. N. Wilson, THIS JOURNAL, **62**, 1583 (1940).

[6] D. DeVault, *ibid.*, **65**, 532 (1943).

[7] J. Weiss, *J. Chem. Soc.*, 297 (1943).

[8] A. C. Offord and J. Weiss, *Nature*, **155**, 725 (1945); **156**, 570 (1945).

[9] E. Glueckauf, *Proc. Roy. Soc.*, **A186**, 35 (1946); *J. Chem. Soc.*, 1302, 1321 (1947); E. Glueckauf and J. I. Coates, *ibid.*, 1308, 1315 (1947).

[10] H. C. Thomas, *Ann. N. Y. Acad. Sci.*, **49**, 161 (1948).

[11] H. Weil-Malherbe, *J. Chem. Soc.*, 303 (1943).

[12] H. G. Cassidy and S. E. Wood, THIS JOURNAL, **63**, 2628 (1941).

[13] A. LeRosen, *ibid.*, **69**, 87 (1947).

[14] P. W. M. Jacobs and F. C. Tompkins, *Trans. Faraday Soc.*, **41**, 388, 395, 400 (1945).

[15] (a) G. E. Boyd, J. Schubert and A. W. Adamson, THIS JOURNAL, **69**, 2818 (1947); (b) G. E. Boyd, A. W. Adamson and L. S. Myers, Jr., *ibid.*, 2836; (c) G. E. Boyd, L. S. Myers, Jr., and A. W. Adamson, *ibid.*, 2849; (d) B. H. Ketelle and G. E. Boyd, *ibid.*, 2800.

[16] S. W. Mayer and E. R. Tompkins, *ibid.*, **69**, 2866 (1947).

[17] S. Claesson, *Arkiv. Kemi Mineral. Geol.*, **23A**, No. 1 (1946).

[18] See for example (a) A. Lottermoser and K. Edelmann, *Koll. Z.*, **83**, 262 (1938); (b) H. G. Cassidy, THIS JOURNAL, **62**, 3073, 3076 (1940).

chromatographic positions of a group of compounds in a simple adsorption chromatogram led to the general conclusion that a study of adsorption isotherms alone is of uncertain value in the prediction of chromatographic behavior.

The present investigation comprised two distinct parts. First the relation between the adsorption isotherm and the rate of development was investigated experimentally and the results were compared with theoretical predictions. For this study six compounds were chosen; five of these substances, 4-nitroaniline and its N-ethyl, N-phenyl, N,N-diethyl, and N,N-diphenyl derivatives, are closely related in structure but vary widely in adsorption affinity, while the sixth, *sym*-diethyldiphenylurea (ethyl centralite) differs in structure from the others and has an intermediate adsorption affinity. This selection of compounds was made in order that any effect which the adsorption affinity or chemical nature of the test substance might have on the results could be detected. Several different types of developing mixtures were used and a tenfold range of developing rates was investigated.

In the second phase of the work an investigation was made of the distribution of adsorbed material in chromatographic zones on the column. The only detailed studies of the shapes of zones that have hitherto been reported have been made by examination of the filtrate from the column. However, it seemed likely that a study of the distribution of material in zones in various positions on the column might yield more directly interpretable information about the processes taking place on the column. The method developed in the present work permits the direct measurement of the distribution of the substance in a zone on the column (the "profile" of the zone) by cutting the column[19] into thin uniform slices perpendicular to its axis and eluting the adsorbed material from each of these slices. Since the thickness of the slices, usually about 2 mm., can be made constant within 1 or 2%, the method yields a true picture of the differential distribution of the material in the zone. With the aid of this method a few selected zones were studied in some detail and considerable insight was obtained into the phenomena occurring on the column.

The selection of silicic acid (mixed with Celite) as the adsorbent in the present experiments was prompted by the uniformly satisfactory results which have been obtained in the chromatography of a wide variety of organic compounds on this medium.[20] It appeared that chromatographic phenomena might be more simply studied on this adsorbent than on most others, and it also seemed probable that an understanding of the chromatographic process on columns of silicic acid–Celite would be of particular value as the merits of this adsorbent become more generally recognized.

Predictions of the Theories

The chromatographic theories which can be applied to the simple adsorption chromatogram may conveniently be considered in three categories: first, the idealized or classical theory, with the assumption of equilibrium between the adsorbed and unadsorbed material throughout the column at all times[5–9]; second, the theories which allow for non-idealities by use of an analogy with the operation of a distillation column, with the assumption of constant distribution coefficient of the solute between the two phases throughout the column[21,16]; and third, the theories in which the phenomena causing departures from ideal behavior are considered explicitly.[9,10,15] Although the theories in the second of these categories were developed specifically for application to the partition chromatogram and the ion-exchange chromatogram, they are equally valid for a chromatogram in which there is adsorption according to a linear isotherm. It is demonstrated below that the adsorption isotherms of all of the systems investigated in the present work were essentially linear in the range of concentrations important in practical chromatography on silicic acid–Celite.

For systems with a linear isotherm, all of the theories lead to essentially the same simple prediction concerning the relation between the rate of movement of a compound during development and the adsorption isotherm of the compound in the developer, provided that the departures from equilibrium behavior are not too great. For the linear isotherm, $f(c) = ac$, this rate is independent of concentration and may be expressed as $R = 1/(1 + Ma/\alpha)$, where R is the ratio of the linear rates of movement of the midpoint of the zone and the developer through the column,[22] M is the weight of adsorbent per unit length of column and α is the interstitial volume per unit length of column. The expression for R in the symbolism of Mayer and Tompkins[16] is $R = 1/(1 + C)$, where C represents the distribution of the solute between the solution and the adsorbent or resin in a plate of the column. Thus their C is just equal to Ma/α, and their distribution coefficient, K_d, is equal to a in the expression for the linear isotherm, $f(c) = ac$.

Although the classical theory leads to the prediction that with a linear isotherm the zone will remain constant in width and maintain sharp boundaries during development, these boundaries would have no intrinsic self-sharpening tendency[23] and consequently any non-idealities might be expected to cause the boundaries of the zone to become diffuse and the zone to spread in a more or

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[19] J. W. Hickey and E. O. Wiig, This Journal, **70**, 1574 (1948), employed a somewhat similar technique in a study of the distribution of arsine on a column of charcoal.

[20] See for example W. A. Schroeder, *Ann. N. Y. Acad. Sci.*, **49**, 204 (1948), and references given there.

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

[22] A. LeRosen, This Journal, **64**, 1905 (1942).

[23] Such a tendency is shown by the lower boundary of the zone in the case of the usual form of non-linear isotherm, expressed by the Freundlich or Langmuir equation; see ref. 6.

less symmetrical fashion. The various non-idealized theories lead to this prediction quantitatively; with the aid of the theory of Martin and Synge[21] or Mayer and Tompkins,[16] which predict that the zone will be approximately Gaussian in shape, the height equivalent to a theoretical plate may be calculated from the distribution of adsorbed material in a zone developed under known conditions. Such a calculation is described in Part II of this paper, where it is applied to certain of the present data.

It has not been possible to consider our data in the light of the non-equilibrium theories of Glueckauf[9] and Thomas[10] because these theories do not apply to the systems which we have used. As Glueckauf points out,[23a] his treatment applies only to the stationary state condition of a zone obeying a *non-linear* isotherm, when the effects of "self-sharpening" and non-equilibrium just balance each other; since the boundaries of a zone formed by a system obeying a linear isotherm have no self-sharpening tendency, they cannot be described by Glueckauf's equations. The kinetic theory of chromatography of Thomas[10] is designed for application only to effluents from the column and not to the structure of bands in the tube, and thus cannot be tested with our data.

Part I. Relation between Adsorption Isotherms and Rates of Development

Experimental

Adsorbent.—The adsorbent used in all of the present experiments was a mixture of two parts by weight of Merck reagent silicic acid 40446[24] with one part of Celite 545, a product of the Johns-Manville Corporation. This adsorbent was activated before use by removing the "free" water which it contained[24] in one of two ways, either by heating in an oven at 180–200° for two hours, or by prewashing with suitable solvents. The usual prewash was 0.2 V ml.[25] of ether, V ml. of 1:1 acetone–ether, 0.8 V ml. of ether, and V ml. of ligroin. It was necessary for the measurement of adsorption isotherms to have a dry adsorbent with the same adsorptive properties as prewashed adsorbent; this was readily prepared in a manner similar to that used by LeRosen,[13] by prewashing a large column of adsorbent in the usual manner and then washing it with 2 V ml. of 28–38° ligroin and drying it *in vacuo* over Drierite. Since both heated and prewashed-and-dried adsorbent are very hygroscopic, it is necessary to minimize exposure of these adsorbents to the laboratory air at all times; adsorption of even a fraction of a per cent. of moisture decreases the adsorptive strength of treated adsorbent significantly.

Solvents.—All solvents with the exception of the absolute ethanol used for spectrophotometry were distilled in an all-glass still before use. Reagent grade anhydrous ether was used in most of the experiments without further drying. Although the small amount of water present in this ether caused a slight diminution in the adsorptive strength of prewashed adsorbent, it was demonstrated in experiments in which ether dried over sodium was used that these traces of water had no other noticeable effect.

Special Compounds.—Samples of 4-nitro-N-ethylaniline,[26] 4-nitro-N,N-diethylaniline,[27,28] and 4-nitrotriphenylamine[29] were prepared by standard methods. Traces of 4-nitroso-N,N-diethylaniline were readily removed from the 4-nitro-N,N-diethylaniline by chromatography on silicic acid–Celite with ether–ligroin development; similarly, higher nitration products were removed from the 4-nitrotriphenylamine by successive benzene-ligroin and ether–ligroin development. Commercial samples of 4-nitroaniline, 4-nitrodiphenylamine, and ethyl centralite (*sym*-diethyldiphenylurea) were recrystallized until their melting points became constant. The chromatographic homogeneity of the purified samples of the five nitro compounds was investigated; each contained less than 0.1% of detectable impurity.

Spectrophotometry.—All of the quantitative estimations upon which this work is based were made spectrophotometrically on solutions in absolute ethanol with a Beckman Quartz Ultraviolet Spectrophotometer. The usual corrections,[29,30] were made for cell transparency and cell thickness, and in the estimation of ethyl centralite a blank correction was applied since the absorption maximum of this compound occurs at so short a wave length (247 mμ) that impurities from the adsorbent and solvents may contribute several per cent. of the absorption at low dilutions.

Precise measurements were made of the extinction coefficient of each of the six compounds at its absorption maximum. Unpublished studies in these laboratories by Dr. A. O. Dekker have shown that the molar extinction coefficient for ethyl centralite at 247 mμ is 8740 and that the compound obeys Beer's law satisfactorily in the useful range of optical densities.

For each of the five nitro compounds a 2.5-fold concentration range was studied, from an optical density of about 0.36 to one of about 0.90 in a 1-cm. cell, in order to discover whether Beer's law was obeyed in this range. No variation in the extinction coefficient with concentration was found for any of the compounds. The spectrophotometric data are summarized in Table I.

TABLE 1

SPECTROPHOTOMETRIC DATA FOR SOLUTIONS OF 4-NITROANILINE AND FOUR RELATED COMPOUNDS IN ABSOLUTE ETHANOL

Compound	Wave length of maximum absorption, mμ	Molar extinction coefficient
4-Nitroaniline[a]	371	15840
4-Nitro-N-ethylaniline[b]	385–386	19390
4-Nitrodiphenylamine[c]	390–391	21590
4-Nitro-N,N-diethylaniline[d]	393–394	21460
4-Nitrotriphenylamine	392–393	18530

[a] Lit. values 375, 15,450 for a solution in 95% ethanol, W. D. Kumler, THIS JOURNAL, **68**, 1184 (1946). [b] Lit. values 390, 19,000 for a solution in 95% ethanol, *ibid.* [c] Lit. values *ca.* 393, *ca.* 20,000, P. Ramart-Lucas and M. Grumez, *Compt. rend.*, **211**, 120 (1940). [d] Lit. values 400, 21,550 for a solution in 95% ethanol, Kumler, *loc. cit.*

Determination of Adsorption Isotherms.—In the measurement of adsorption isotherms, a suitable volume (14 to 20 ml.) of a solution of appropriate composition and concentration was pipetted into a glass-stoppered flask which contained a weighed sample of adsorbent (0.6 to 3 g.). After the adsorbent and solution had been mixed thoroughly by cautious swirling, the flask was allowed to

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(23a) Ref. 9, p. 1316.

(24) For a discussion of the properties of this adsorbent and amplification of some other details of experimental procedure see K. N. Trueblood and E. W. Malmberg, *Anal. Chem.*, **21**, 1055 (1949).

(25) V ml. has been defined[20] as the volume of solvent necessary to wet completely a column of adsorbent; see Table III.

(26) E. Nolting and A. Collin, *Ber.*, **17**, 267 (1884).

(27) W. C. Davies, *Bull. soc. chim.*, [5] **2**, 295 (1935).

(28) "Organic Syntheses," Coll. Vol. II, p. 224, John Wiley and Sons, New York, N. Y., 1943.

(29) R. Herz, *Ber.*, **23**, 2536 (1890).

(30) W. A. Schroeder, E. W. Malmberg, L. L. Fong, K. N. Trueblood, J. D. Landerl and E. Hoerger, *Ind. Eng. Chem.*, **41**, 2818 (1949).

stand for fifteen to thirty minutes. It was shown that equilibrium was established in less than ten seconds after the adsorbent and solution were mixed, and that this steady state persisted for at least one hour. The initial and final concentrations of the solution were measured spectrophotometrically and the amount adsorbed was calculated from the difference. The proportion of adsorbent to solution was so chosen in all of the experiments that never less than 30% nor more than 60% of the compound present was adsorbed; the equilibrium concentrations varied from about 0.01 to 0.6 mg. per ml. (*ca.* 5×10^{-5} to 3×10^{-3} M).

When an adsorption experiment is made with a mixed solvent, there is usually a preferential adsorption of one of the components of the solvent. Hence, in a static experiment, such as the measurement of an adsorption isotherm in the usual way as described above, the final composition of the solvent will be slightly different from the original composition. In a chromatographic experiment on the other hand, the developer retains its original composition insofar as its effect on the compound being developed is concerned if the components of the developer are less strongly adsorbed than the compound being developed; this is always the case except in displacement development,[17] which is, however, not pertinent to the present discussion.

Experiments were performed to determine the extent of this preferential adsorption with benzene–ligroin, ether–ligroin, and ether–benzene mixtures; about 1.5 g. of prewashed-and-dried adsorbent and 10 ml. of solvent was used in each experiment, and a wide range of compositions was investigated for each solvent pair. Estimations of the changes in composition of the solvent mixture were made refractometrically; it was found that the effect was negligible with benzene–ligroin but was significant for the other two mixtures, amounting to as much as a 15% diminution in the concentration of the polar component at low concentrations of this component. Corrections were made by preparing the solvent mixtures for the adsorption isotherm experiments with a concentration of polar component slightly higher than that desired at equilibrium, the difference being just equal to the diminution expected because of preferential adsorption of one component.

Chromatographic Procedure.—All chromatograms, except as otherwise noted, were carried out in Pyrex tubes of the type described by Zechmeister and Cholnoky.[31] Two sizes of tube were used, 13 mm. and 19 mm. in inside diameter, and about 200 mm. long; the columns of adsorbent were 150 ± 5 mm. in height.

The success of the entire investigation, and especially of that phase of it in which zone profiles were measured, depended on minimizing the distortions normally found in chromatographic zones. Each phase of the chromatographic operations was therefore studied in an effort to eliminate these effects; although the details of these investigations cannot be presented here, the technique which was finally adopted will be described briefly.

Although the full suction of a water-aspirator was used as the driving force in all chromatograms, the packing of the columns was done without suction. The adsorbent was allowed to flow into the tube with the receiving flask open to the atmosphere until the column was about 15% higher than the final desired height. The full suction of the aspirator was then applied and the column contracted into position within about thirty seconds.

This packing procedure differs in several respects from that which was used in the work described by Schroeder, *et al.*,[30] and which has also been used by LeRosen.[32] The packing of silicic acid–Celite has previously been done under full suction and tapping of the side of the tube during packing has been recommended. Although this technique is entirely adequate for all normal chromatographic work, it was observed in the present investigation in which uniform zones were of utmost importance that columns packed in this fashion tended to give distorted zones and erratic flow rates more often than columns packed in the manner described above. The flow rates through columns packed in the manner here recommended were about 10 to 15% greater than those through columns packed under full suction; the reproducibility of the present method of packing is indicated by the fact that the flow rates in successive identical chromatograms seldom varied by more than 1 or 2%.

Since it is imperative that the upper surface of the column be flat and regular if undistorted zones are to be formed, this surface was first levelled very carefully and then a flat circular piece of fine-mesh wire screen of approximately the same diameter as the inside of the tube was placed on the surface before any solvent was added. This screen effectively prevented any erosion by the solvents which were poured into the tube during the process of chromatography.[33]

After the usual prewash with acetone–ether and ether, the column was washed with V ml. of ligroin (60–70°) and about 0.3 V ml. of the solvent to be used as developer. The sample to be chromatographed was then placed on the column from a solution in the same solvent used for development; the volume of sample solvent was usually 0.1 V ml. and the concentration of the solution approximately 0.25 mg. per ml. Extreme care in the transfer of the sample to the column was essential for the production of a uniform zone.

Measurement of Development Rates.—The values of *R* were determined by measuring the distance through which the midpoint of the zone moved while V ml. of developer passed through the column, and dividing this distance by the length of the column. The reproducibility in duplicate experiments was excellent.[34] *R* was referred to the midpoint of the zone because of the linearity of the isotherms in the systems investigated. The constancy of development rate during each experiment was checked by measuring the position of the zone after every 0.5 V ml. of developer. A correction was made for the initial position of the zone, by subtracting it from the measured position, since the volume of sample solvent was not included in the calculation of the number of V ml. of developer used. It was found that, with very few exceptions, the rate of development of the zone was independent of the position on the column within the accuracy of the measurements, a few per cent. The few exceptions to this generalization involved rapid benzene–ligroin development of certain of the less strongly adsorbed compounds; a very slight increase in the rate of development was sometimes observed as the zone moved down the column. It is probable that this effect was related to the fact that, as discussed in Part II, a column which has been prewashed in the usual way no longer has a completely uniform adsorptive strength for benzene–ligroin developers.

Coning.—As a consequence of the downward coning of zones which is almost always observed on prewashed silicic acid–Celite columns in glass tubes,[24] the actual center of gravity of the zone is somewhat below the apparent midpoint of the zone as observed on the outer surface of the column. This disparity between the surface position and the interior position of the zone is generally due in part to an effect which we shall term "surface spreading," that is, the spreading of a zone in a *very thin* layer on the outer surface of the column to a width significantly greater than its width in the interior of the column. For zones which are coned downward on silicic acid–Celite, surface spreading almost invariably occurs at the upper boundary of the zone. The term is here used to refer to a cylindrically

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[31] L. Zechmeister and L. Cholnoky, "Principles and Practice of Chromatography," John Wiley and Sons, New York, N. Y., 1943, pp. 62 and 63, Figs. 19 and 20.

[32] A. LeRosen, This Journal, **67**, 1683 (1945).

[33] W. Trappe, *Biochem. Z.*, **306**, 316 (1940), proposed a different solution for this problem; he covered the upper surface of his silicic acid columns with a layer of diatomaceous earth which could be firmly tamped down and which had negligible adsorptive power.

[34] For a discussion of the measurement of zone positions and the reproducibility of chromatographic experiments on silicic acid–Celite, see reference 24.

TABLE II

CORRELATION OF ADSORPTION ISOTHERMS AND RATES OF DEVELOPMENT

System no.	Compound[a]	Developer[b]	Constants of the isotherm: Freundlich k	Freundlich n	Langmuir a	Langmuir b	$R_{calcd.}$	$R_{obs.}$	$R_{obs.}/R_{calcd.}$
			A. Heated Adsorbent						
1	NitroDPA	60% B in L	24	1.16	34	1.2	0.049	0.052	1.05
2	NitroDPA	87% B in L	8.6	1.04	10.4	0.5	.139	.125	0.9
			B. Prewashed Adsorbent						
3	EC	14% E in L	12	1.03	13.2	.7	.102	.10	1.0
4	EC	19% E in L	5.2	1.03	5.2	.0	.222	.22	1.0
5	EC	30% E in L	3.0	1.01	3.05	.01	.325	.33	1.0
6	Nitro DPA	14% E in L	8.2	1.00	8.2	.0	.156	.155	1.0
7	Nitro DPA	20% E in L	4.5	1.00	4.5	.0	.251	.25	1.0
8	NitroEtAn	24% E in L	5.2	c	5.2	c	.226	.20	0.9
9	NitroAn	54% E in L	4.8	c	4.8	c	.240	.21	.9
10	NitroTPA	35% B in L	4.0	1.00	4.0	.0	.273	.22	.8
11	NitroTPA	50% B in L	1.73	1.02	2.00	.4	.43	.37	.85
12	Nitro DPA	60% B in L	18	1.02	19.4	.5	.072	.038	.5
13	NitroDPA	d	10.5	1.01	10.8	.3	.123	.115	.95
14	NitroDPA	75% B in L	7.3	1.05	8.4	.7	.153	.10	.65
15	NitroDPA	87% B in L	4.1	1.00	1.1	.1	.268	.20	.75
16	NitroDPA	Benzene	2.5	1.00	2.5	.1	.365	.37	1.0
17	NitroDiEtAn	89% B in L	3.9	1.00	3.9	.0	.280	.20	0.7
18	NitroDiEtAn	Benzene	2.45	1.00	2.45	.0	.382	.35	.9
19	NitroEtAn	Benzene	7.8	1.02	8.8	.5	.146	.085	.55
20	NitroEtAn	0.5% E in B	4.25	1.02	4.6	.1	.245	.21	.85
21	NitroAn	10.7% E in B	4.4	1.04	5.1	.3	.225	.21	.95

[a] Abbreviations: NitroAn for 4-nitroaniline, NitroEtAn for 4-nitro-N-ethylaniline, NitroDPA for 4-nitrodiphenylamine, NitroDiEtAn for 4-nitro-N,N-diethylaniline, NitroTPA for 4-nitrotriphenylamine, EC for ethyl centralite. [b] Abbreviations: E for ether, L for ligroin (60–70°), B for benzene. [c] In systems 8 and 9 measurements were made at only two concentrations, *ca.* 0.08 and 0.6 mg. per ml. [d] The developer for System 13 was 60% B plus *ca.* 0.1% E in L.

symmetrical condition; the irregular streaks of a compound which are occasionally found on the surface of a column are simple distortions which can usually be eliminated by precautions in technique.

Although many experiments were done in the present investigation in an effort to eliminate coning and surface spreading, no practical method for preventing these effects completely has yet been found. It has been established that the general features of coning and surface spreading are common to different commercial samples of silicic acid mixed with Celite.[24] The effects vary somewhat with the pretreatment of the adsorbents; however, on adsorbents prewashed in the usual manner, the extent and direction of coning does not seem to vary appreciably for different adsorbed compounds or different developers. Many experiments were done in chromatographic tubes of special design in order that the more obvious possible causes of coning, such as the geometry of the lower joint of the tube, could be eliminated or controlled. The surprising observation was made that the direction and extent of coning, and especially of surface spreading, appeared to depend on the *nature* and *history* of the chromatographic tube used; these effects appeared to be almost negligible in metal tubes and in new Pyrex glass tubes but were quite appreciable in Pyrex glass tubes which had been used several times. It seems possible that these facts can best be explained as manifestations of electrokinetic effects; however, further investigation was postponed until the primary object of the present work had been fulfilled. Although coning and surface spreading were not completely eliminated, a measure of control and reproducibility adequate for successful completion of the experiments was achieved. The observed development rates were corrected for the effect, which caused the true midpoint of the zone to be from 5 to 10% below the surface midpoint.

Results and Discussion

The isotherms of all of the twenty-one systems investigated were found to be linear, or very nearly so, within the range of concentrations studied, a result which is in full qualitative agreement with the theory of chromatography in view of the observed symmetry[24] of zones on silicic acid–Celite columns. The results of the experiments designed to test the correlation of adsorption isotherms with rates of development are summarized in Table II. The constants of the adsorption isotherms are presented there, together with the value of R calculated from the data of the isotherm, the observed value of R, and the ratio of these two quantities. The constants of the equations of the isotherms, both in the Freundlich formulation, $f(c) = kc^{1/n}$, and the Langmuir formulation, $f(c) = ac/(1 + bc)$, were evaluated graphically in the usual manner; because all of the isotherms are essentially linear, they can be fitted equally well by

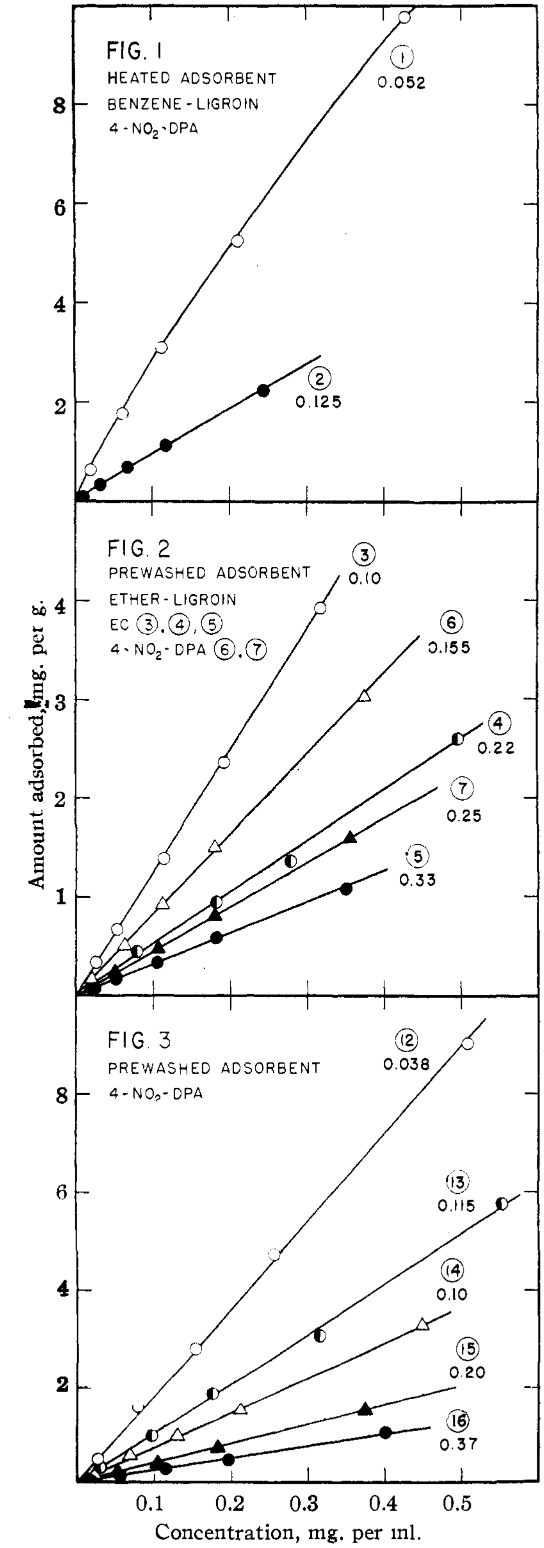
TABLE III

CHARACTERISTICS OF PACKING FOR DIFFERENT TUBES AND ADSORBENTS

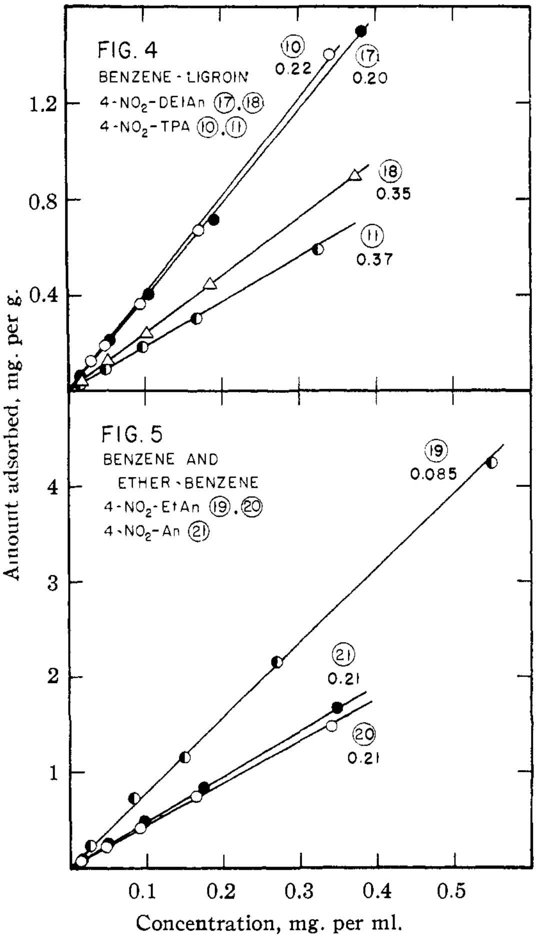
Inside diam. of tube mm.	Adsorbent	M,[a] g./mm.	α, ml./mm.	α/M, ml./g.	V,[b] ml./150 mm.
13	Prewashed	0.058	0.088	1.52	13.2
	Heated	.057	.093	1.63	14.0
19	Prewashed	.127	.192	1.51	28.8

[a] M is the weight of adsorbent per unit length of column and α the pore volume per unit length of column. [b] Defined in footnote 25.

either of these equations. Data pertaining to the packing of the adsorbents, which were necessary for the calculation of the theoretical values of R from the constants of the isotherms, are summarized in Table III. The adsorption isotherms have been plotted in Figs. 1 to 5.



Figs. 1–3.—Adsorption isotherms on silicic acid–Celite. The encircled number beside each curve is the system no. (Table II). The other number is the value of R_{obs}.



Figs. 4–5.—Adsorption isotherms on prewashed silicic acid–Celite. The encircled number beside each curve is the system no. (Table II). The other number is the value of R_{obs}.

The ratio of the observed value of R to that calculated on the basis of the theory of chromatography provides a convenient measure of the usefulness of the theory in the prediction of development rates from adsorption isotherms; the closer this ratio to unity, the greater the success of the theory. The values of the ratio in Table II indicate that for the majority of the systems studied the agreement of theory with experiment was very satisfactory; however, for certain of the systems the observed rate was significantly less than the predicted rate. It will be noted that the most significant departures from theoretical behavior occurred with slow benzene–ligroin development on prewashed adsorbent (Systems 12, 14, and 19). When heated adsorbent was used in similar experiments (Systems 1 and 2) the agreement with theory was satisfactory. Furthermore, in the experiments in which the developer contained ether, the observed development rate on prewashed adsorbent was generally equal to the predicted rate, even at low rates of development (Systems 3, 6, and 13). This is of particular interest in System 13 for here the developer differed from that of System 12 only by the addition of approximately 0.1% of ether.

An interpretation of the anomalously low development rates observed with benzene–ligroin mixtures on prewashed adsorbent is suggested by the fact that, as revealed by the studies of zone profiles described in Part II, a prewashed column is not uniform in adsorptive strength with benzene–ligroin developers. Rather, when such developers are used the upper part of a prewashed column is initially a stronger adsorbent than would be expected from the adsorption isotherms measured with prewashed and dried adsorbent; as development proceeds the remainder of the column gradually becomes stronger also. On the other hand, with developers which contain even a trace of ether the column appears to be quite uniform and to have just that adsorptive strength which would be expected from an isotherm measured with prewashed and dried adsorbent. With heated adsorbent the column is uniform in adsorptive strength with all of the developers investigated.

An explanation of these facts is presented with the pertinent experimental data in Part II below. However, their significance for the present experiments is apparent: if the adsorbent in the column is actually stronger than the isotherm indicates that it is, the observed development rate should be less than that predicted from the isotherm. Furthermore, since only the upper few centimeters of the column is initially stronger, a zone which is developed rapidly might be expected to accelerate very slightly as it moved down the column, whereas a slowly developed zone would move at a more constant rate inasmuch as the adsorptive strength of the remainder of the column apparently gradually increases also as development proceeds. Thus the more rapidly developed zone would move at a rate which was more nearly equal to the predicted rate than was that of the zone developed more slowly; this is precisely the behavior which was observed.

These considerations afford a reasonable explanation of the results of the six experiments in Table II in which the observed development rate was appreciably different from the predicted rate. That these discrepancies are due to peculiarities of the prewashed adsorbent rather than to a defect in the theory is substantiated by the fact that they were not observed in similar experiments with heated adsorbent, nor with developers which contained ether.

The linear isotherm has heretofore been considered of little practical importance in adsorption chromatography,[7,9,15d] presumably because the isotherms of most of the systems studied experimentally have been markedly nonlinear.[11,12,14,17,18] In contrast to such other adsorbents as alumina and carbon, however, silicic acid and silica gel apparently frequently yield adsorption isotherms which are essentially linear, at least in the range of concentrations usually employed in practical chromatography.[35] Not only are the chromatograms of single compounds on silicic acid columns correspondingly simpler than those on adsorbents with non-linear isotherms, but the behavior of mixtures of compounds is just the sum of the behaviors of the compounds separately, *i. e.*, there is negligible interaction between the components of a mixture when chromatographed on silicic acid columns. Experimental evidence[36] for this statement is presented in Table IV; two binary mixtures and one ternary mixture were selected as examples and the rates of development of the components were measured when they were developed both individually and together. No significant difference in rates of development could be detected.

TABLE IV

RATES OF DEVELOPMENT OF COMPOUNDS DEVELOPED SINGLY AND IN MIXTURES

Mixture	Developer	Component[a,b]	Rate of development, R[c] Singly	In mixture
1	11% ether in ligroin	NitroDPA	0.17	0.17
		EC	.12	.115
2	17% ether in ligroin	NitroDPA	.29	.30
		EC	.23	.23
		NitroEtAn	.23	.23
3	Benzene	NitroDiEtAn	.43	.42
		NitroEtAn	.19	.195

[a] 1.0 mg. of each compound developed on a prewashed column. [b] For abbreviations see Table II, footnote *a*. [c] These experiments were carried out about two and one-half years after those reported in Table II. The adsorptive strength of the adsorbent had decreased significantly during this time.

The simplicity of the experimentally observed relation between adsorption isotherms and development rates is further illustrated qualitatively in Figs. 1 to 5. In each figure the observed development rate corresponding to each of the isotherms has been placed beside the curve to which it refers. In theory, the greater the slope of the isotherm, the smaller the development rate; the validity of this relation is illustrated most strikingly in Fig. 2, wherein are plotted isotherms of two different compounds, ethyl centralite and 4-

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(35) Thus Weil-Malherbe[11] found that the adsorption of benzpyrene on silica gel from benzene-petroleum ether followed a nearly linear isotherm and we have found that the adsorption isotherm of 2,4-dinitrodiphenylamine on unprewashed silicic acid–Celite is strictly linear at concentrations below at least 2 mg. per ml. Likewise the data of LeRosen[18] show that the adsorption isotherm of 2-nitroaniline in benzene on prewashed silicic acid is essentially linear at concentrations below several mg. per ml.

(36) Further evidence is provided by a mass of chromatographic data accumulated in these laboratories by a number of workers under the direction of Dr. W. A. Schroeder and reported in part in references 20 and 30.

nitrodiphenylamine, in a variety of ether–ligroin developers. The steepest isotherm corresponds to the lowest rate of development, and the rates increase regularly as the slopes of the isotherms decrease. This same simple relation is evident in Fig. 1 and Fig. 5; in addition, in Fig. 5 the isotherms of 4-nitroaniline and 4-nitro-N-ethylaniline in different ether–benzene developers which develop them at the same rate are seen to be almost coincident, as theory demands that they should be.

All of the isotherms in Fig. 3 which correspond to a given *type* of developer (curves 12, 14, 15, and 16) bear the proper relation to one another. The apparent exception to the theoretical requirement (curves 13 and 14) is a consequence of the inhomogeneity in adsorptive strength produced in a prewashed column by benzene–ligroin developers. The developer for System 13 contained ether, so that the observed rate was about equal to that predicted from the isotherm, whereas the developer in System 14 contained no ether, so that the corresponding development rate was appreciably less than that expected on the basis of the isotherm measured with prewashed-and-dried adsorbent inasmuch as the adsorbent in the column was actually stronger. The slight difference in observed developed rates for the two essentially coincident isotherms in Fig. 4 (Systems 10 and 17) may likewise be considered a consequence of the inhomogeneity of a prewashed column with benzene–ligroin developers.

It appears safe to conclude from the results of the experiments in Tables II and IV that for silicic acid–Celite columns a knowledge of the adsorption isotherms of a series of compounds will usually permit the prediction of the rate of development of each of the compounds on a column of uniform adsorptive strength not only when it is developed separately but also when it is present in a mixture of not widely disproportionate quantities of all of them. Although no attempt was made in the present work to investigate the isotherms of the compounds in mixtures with each other, it seems reasonable to assume, on the basis of the experimental observations of the chromatographic behavior of mixtures, that they would be insignificantly different from the isotherms of the same compounds separately.

Part II. Measurement and Interpretation of Profiles of Zones

The measurement of the "profiles" of zones, that is, of the differential distribution of material in the zones, was carried out by careful sectioning of the columns and subsequent elution. Both developed and undeveloped zones were investigated; the profiles of the latter were of particular value in the clarification of the special properties of prewashed adsorbent.

Experimental

The sectioning of chromatographic columns was effected as follows: first, the column of adsorbent was loosened in the chromatographic tube by tapping the end of the tube gently against the palm of the hand or rapping the side of the tube sharply once or twice; second, the chromatographic tube was fixed in a horizontal position: next, a flat glass plate was set vertically, perpendicular to the axis of the tube, at a distance beyond the end of the tube just equal to the width of the section to be cut, usually 2 to 3 mm., and the column was extruded gently until it came into contact with the plate; then the plate was removed and the 2- to 3-mm. section of column protruding from the end of the tube was sliced off with a razor blade, care being taken not to chip the fresh flat surface which was just flush with the end of the tube; finally, the glass plate was replaced in precisely its original position and the operations of extrusion and cutting were repeated.

This method differs significantly from that of Hickey and Wiig[19] in that they controlled the size of the extruded portion by means of a screw at the end of the column opposite to that from which sections were cut. Although we tried a similar method, we found that variations in the thickness of the sections were more likely than in the method which we finally adopted because the column often tended to stick in the tube and to be compressed slightly and irregularly as it was extruded. On the other hand, in the method adopted the thickness of the section is governed solely by the distance from the near end of the tube to the vertical glass plate; this distance can be made precisely reproducible by firmly fixing the position of the tube and of the supports against which the glass plate rests.

In a control experiment a column of adsorbent was packed and prewashed in the usual manner and was then washed with 2 V ml. of 28–38° ligroin. Six sections about 2.5 mm. thick were cut from the column and were weighed after they had been allowed to dry in air for one-half hour. The average deviation in weight was 1.5% and the maximum deviation only 3%. A similar experiment was done in which the sections were about 1.6 mm. thick; the average deviation was again 1.5% and the maximum deviation 2%. Thus the reproducibility in the weight, and hence the thickness, of successive slices is entirely satisfactory.

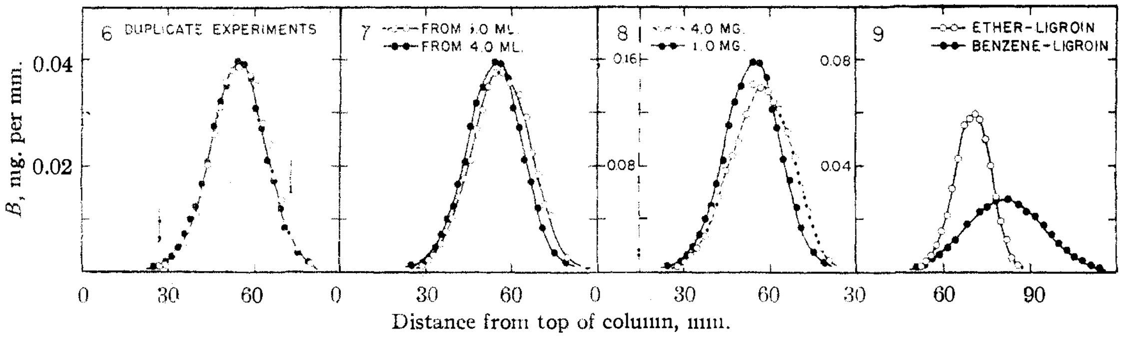
After each of the sections had been cut, it was placed in a small sintered glass funnel and the adsorbed material was eluted with absolute ethanol. It was possible to make an essentially quantitative elution with as little as 3 to 4 ml. of alcohol; in all of the experiments to be described the recovery of the compound in the zone was between 96 and 100%.

All of the profiles reported here were made from zones of 4-nitrodiphenylamine; the experimental conditions are summarized in Table V. Although the volume of solvent used for placing the undeveloped zones on the column, generally 64 ml., was considerably greater than would be used in an ordinary chromatographic experiment on a column 19 mm. in diameter, a large volume was desirable in the present experiments in order that undeveloped zones of sufficient width for fruitful investigation might be obtained. The developed zones, except as noted, were placed on the column from 4.0 ml. of the developer.

In the preparation of zones for sectioning, all distorted zones were rejected. Although there was generally little coning in the undeveloped zones, there was some downward coning in most of the zones developed with benzene–ligroin; the extent of this effect is illustrated in Fig. 6. Only one profile of a zone developed with ether–ligroin is shown; this zone was developed in a new glass tube and was apparently not coned or surface-spread.

Results and Discussion

Since there is always an appreciable quantity of solvent left in the column at the conclusion of the chromatogram, even after the usual fifteen or twenty seconds of draining, the above-described method for determining the profile of a zone provides a measure not of the amount of *adsorbed* material alone in each section, but rather of the sum



Figs. 6–9.—Profiles of developed zones. See Table V for experimental details.

Table V

Experimental Conditions in Formation of Zones for Measurement of Profiles[a]

Fig. No.	Pretreatment of adsorbent	Solvent[b,c] or developer
	A. Developed zones[d]	
6, 7, 8	Usual prewash	2 V ml. 87% B in L
9	Usual prewash	3 V ml. 87% B in L
9, 10	Usual prewash	2 V ml. 20% E in L
	B. Undeveloped zones[e]	
11	Usual prewash	20% E in L
12	Usual prewash	87% B in L
13	Heated; then washed with V ml. 87% B in L	87% B in L
14	Usual prewash	87% B plus 0.15% E in L
15	Usual prewash	87% B, which had been passed through a prewashed column, in L[f]
16	Usual prewash followed by 3 V ml. B	87% B in L
17	Usual prewash	87% B in L[c]
18	Usual prewash	60% B in L

[a] Each zone contained 1.00 mg. of 4-nitrodiphenylamine, except as noted in Fig. 8. [b] Abbreviations: E for ether, L for ligroin (60–70°), B for benzene. [c] All benzene was dried over sodium before use except that used for the profile shown in Fig. 17. [d] Each sample was placed on the column from 4.0 ml. of developer except as noted in Fig. 7. [e] The sample was placed on the column from 64 ml. in each experiment except that in Fig. 15, in which 32 ml. was used. [f] See discussion for details.

of the amount of material adsorbed in the section and that dissolved in the solvent still present in the interstices of the adsorbent. If the quantity of solvent per unit length of column is constant throughout the sectioned zone, then, for a linear isotherm, the measured profile will be identical in shape with the profile of the adsorbed material alone, differing only by a scale factor. Thus, if the assumption is made that the volume of solution per unit length in the extruded column is α', where α' is slightly less than α, the pore volume per unit length,[37] and that the isotherm is $Q = Mac$, then the total amount of compound per unit length of the extruded column, a quantity which we shall term B, will be given by the expression

$$B = \alpha' c + Mac = (\alpha' + Ma)c = Q(\alpha' + Ma)/Ma$$

Thus, since M and a are constants for a particular system, B will be proportional to the amount adsorbed per unit length, Q, provided that α' is independent of position on the column.

Although no investigation was made of the constancy of α' throughout the column, it seems reasonable that even if it does vary slightly it will vary in a gradual and regular fashion so that the measured profile will be only slightly distorted from the true profile of the adsorbed material. Furthermore, in all of the profiles which are reported here, the quantity Mac constitutes between 70 and 93% of B so that any slight uncertainty in α' would mean at most an uncertainty of a few percent in B.

Developed Zones.—The profiles in Figs. 6 through 9 illustrate some applications of this technique to the study of developed zones. The reproducibility of the method is illustrated in Fig. 6, where the results of duplicate experiments have been plotted; the profiles are exactly coincident. The effect of downward coning is also illustrated in Fig. 6; the arrows denote the position of the extreme limits of the zone as detected visually on the surface of the column.

All of the profiles of developed zones show clearly that the distribution of material in these zones was very nearly symmetrical and, qualitatively at least, approximately Gaussian. Although the slight downward coning of the zones developed with benzene–ligroin probably tends to accentuate the Gaussian character of profiles measured in this manner, the profile of the unconed zone which was developed with ether–ligroin (Fig. 9) also closely resembles a normal error curve.

The latter profile was selected for a test of the applicability of the chromatographic theory of Mayer and Tompkins.[16] From the measured adsorption isotherm (System 7, Table II) and the maximum observed value of B, the height equivalent to a theoretical plate (HETP) was calculated for this system to be 0.85 mm. This calculation was made as follows: The maximum is at 71.1 mm., $B_{\max} = 59.5 \times 10^{-3}$ mg./mm., and for this system $\alpha/Ma = 1.51/4.5 = 0.336$ (*cf.* Tables II and III). Thus $C = Ma/\alpha = 2.98$. We shall use the symbols of Mayer and Tompkins:

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[37] LeRosen[13] found that the quantity which we have termed α' was equal to about 87% of α for a column of silicic acid.

$S_{\max}$ = fraction of the solute adsorbed in the plate at which the maximum occurs
$L_{\max}$ = fraction of the solute in solution in the plate at which the maximum occurs
$p_{\max}$ = serial number of the plate at which the maximum occurs

and also introduce two new terms

p^* = number of plates per mm.
$S^* = Sp^*$ = fraction of the solute adsorbed per mm.

To convert $B_{\max}$ to $S^*_{\max}$ we must multiply by $Ma/(\alpha + Ma) = 1/1.336 = 0.75$ since only this fraction[38] of the total solute in the zone is adsorbed, and then divide by the weight of solute in the zone, 1.00 mg. Thus

$$S^*_{\max} = \frac{59.5 \times 10^{-3}}{1.00 \times 1.336} = 0.0445 \text{ mm.}^{-1}$$

Since the maximum occurs at 71.1 mm., $p_{\max} = 71.1p^*$, and thus

$$S_{\max} = S^*_{\max}/p^* = (0.0445)(71.1)/p_{\max}$$

Making use of equations (1) and (6) of Mayer and Tompkins, $L_{\max} = S_{\max}/C$ and $L_{\max} = 1/\sqrt{2\pi p_{\max}C(1 + C)}$, we get

$$S_{\max} = C^{1/2}/(p^{1/2}_{\max}\sqrt{2\pi(1 + C)}) = 1/(p^{1/2}_{\max}\sqrt{8.37})$$

in which we have substituted $C = 2.98$. If now we equate our two expressions for $S_{\max}$, we have $p^{1/2}_{\max} = 3.16\sqrt{8.37}$ and thus $p_{\max} = 83.7$. Consequently

$$\text{HETP} = 1/p^* = 71.1/83.7 = 0.85 \text{ mm.}$$

This result was then used in the calculation of a theoretical profile for the zone, by means of equations (1) and (5) of Mayer and Tompkins. A constant value of $n + p$, corresponding to a fixed position of the zone on the column, was used in this calculation; since $n_{\max} = Cp_{\max} = 249.4$, the value of $n + p$ chosen was 333 (= 249.4 + 83.7). This theoretical profile is plotted, together with the experimental points, in Fig. 10. The agreement of theory with experiment is excellent. Thus, the theory of Mayer and Tompkins seems entirely adequate not only for the prediction of development rates (as are all of the theories) but also for the prediction of the shapes of developed zones. The classical theory fails completely in the latter respect; for example, according to the classical theory, the zone in Fig. 10 should be 5.2 mm. wide, with a constant density of 0.19 mg. per mm.

The HETP of 0.85 mm. found here may be compared with that of about 0.02 mm. reported by Martin and Synge[21] for their partition chromatograms on silica gel, and those of 1 to 5 mm. found by Tompkins, *et al.*, for certain ion-exchange systems.[39,16]

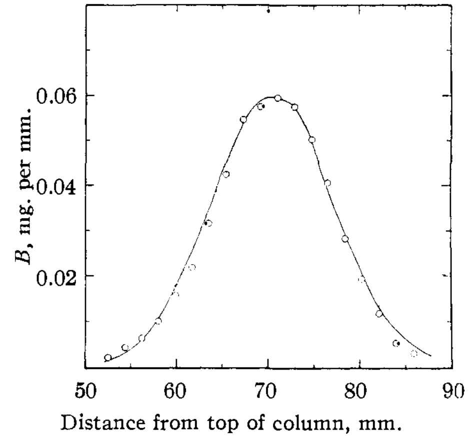

Fig. 10.—Correlation of experimental and theoretical profiles. The circles represent experimental points from a zone of 4-nitrodiphenylamine developed with ether–ligroin (System 7, Table II). The full curve was calculated according to the theory of Mayer and Tompkins with HETP = 0.85 mm.

When the theory of Martin and Synge was used to calculate the HETP from the width of the zone at a height = $1/e$ of the maximum height, the HETP was found to be approximately 0.4 mm. This result must be considered only a rough approximation since the equations of Martin and Synge for zone width apply to a "zone" at a constant position on the column and varying time or volume of developer, that is, an effluent zone, whereas we were applying it to a zone spread over the column at a particular stage of development. The observed agreement in order of magnitude, within a factor of 2, with the result obtained using Mayer and Tompkins' theory, is all that could be expected.

The great disparity in the widths of zones developed with benzene–ligroin and ether–ligroin on prewashed adsorbent is illustrated in Fig. 9. Although downward coning of the zone developed with benzene–ligroin exaggerates slightly the width of that zone in the measured profile, the broadening due to this effect constitutes only a small fraction of the total width of the zone. The greater width of the zone developed with benzene–ligroin apparently is a consequence of the fact that prewashed adsorbent is not uniform in adsorptive strength with this developer. This inhomogeneity in adsorptive strength was discovered in the course of the investigation of the profiles of undeveloped zones; the pertinent experiments and their significance are reported below in connection with the discussion of undeveloped zones.

The effects of varying the size of the sample and the volume of solvent used to place the sample on the column are illustrated in Figs. 7 and 8 and provide further qualitative evidence in favor of the non-idealized theories. The profiles in Fig. 7, which represent zones containing equal quantities of material introduced from different initial volumes, are essentially identical; according to

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[38] If we use $\alpha' = 0.87\alpha$ in place of α in the conversion factor, this factor would then be 0.78 instead of 0.75, and the HETP so calculated would be 0.80 mm., which does not differ significantly from the value 0.85 mm. obtained as described above.

[39] D. H. Harris and E. R. Tompkins, THIS JOURNAL, **69**, 2792 (1947).

the classical theory that introduced from 4 ml. should be four times as wide and one-fourth as high as that introduced from 1 ml. On the other hand, according to the theory of Martin and Synge or Mayer and Tompkins, these profiles should differ in width only by the difference in the widths of the initial zones, about 3 mm., which is almost negligible inasmuch as the developed zones are more than 40 mm. wide. The zone introduced from 4 ml. may be considered to be formed from four overlapping chromatograms, each displaced about 1 mm. from the next and each similar in shape to that from 1 ml. but only one-fourth as high.

In Fig. 8 are depicted the profiles of two zones containing respectively 1 mg. and 4 mg. of 4-nitrodiphenylamine; each was placed on the column from 4 ml. of developer. In this figure, the vertical scale for the 4-mg. sample is four times as great as that for the 1-mg. sample. The profiles are very similar in shape, differing essentially only by the vertical scale factor of four; this result is to be expected according to both the classical and non-idealized theories. However, the densities and widths of the zones of course differ widely from the values predicted on the basis of the classical theory.

In connection with the spreading of zones on development on ion-exchange columns, Boyd, *et al.*,[15c,d] report that both theoretically and experimentally the degree of spreading increases as the particle size increases but, for a given sample of adsorbent, changes only very slowly with change in flow rate. We have found this behavior with silicic acid–Celite columns also. With different samples of adsorbent[24] zones similarly developed are wider on the adsorbents with larger particle size; on the other hand, for a given sample of adsorbent, a four-fold variation in flow rate has been found to have no discernible effect on either the width or rate of development of a zone.

Undeveloped Zones.—As pointed out in Part I, rates of development are anomalously low with benzene–ligroin development on prewashed adsorbent. However, when heated adsorbent is used, or when developers containing even traces of ether are used with prewashed adsorbent, the development rates are normal. Furthermore, as illustrated in Fig. 9, a zone developed on prewashed adsorbent with benzene–ligroin is nearly twice as wide as one developed similarly with ether–ligroin. In order to clarify these anomalies, studies were made of the profiles of very broad zones placed on columns under varying conditions but not developed away from the top of the column.

The profiles of zones placed on prewashed adsorbent from ether–ligroin and benzene–ligroin are shown in Figs. 11 and 12, respectively, together with the shapes expected according to the classical theory on the basis of the isotherms reported in Table II (Systems 7 and 15). Just as in the experiments on rates of development, the agreement with theory is good for ether–ligroin and unsatisfactory for benzene–ligroin with this prewashed adsorbent. The profile depicted in Fig. 12 suggests that, except perhaps for the top few millimeters of the column, the adsorbent in this column behaves as if it were progressively weaker in adsorptive strength as the distance from the top of the column increases. In an experiment in which heated adsorbent was used with benzene–ligroin, the shape of the zone (Fig. 13) agreed reasonably well with that expected on the basis of the isotherm (System 2, Table II), indicating that this column was of approximately uniform adsorptive strength. When 0.15% of ether was added to the benzene–ligroin developer, the profile had the shape shown in Fig. 14, rather than the abnormal shape shown in Fig. 12. Although no isotherm was measured for the system of Fig. 14, it is apparent that, qualitatively at least, the zone has the shape expected theoretically, and that the addition of a trace of ether has removed the anomaly.

Thus, the results shown in Figs. 11, 12, 13, and 14 parallel closely those presented in Table II concerning rates of development in that benzene–ligroin development on prewashed adsorbent leads to anomalous results, whereas with heated adsorbent, or with developers containing even traces of ether on prewashed adsorbent, the behavior is close to that predicted theoretically. This suggests that a common cause of the anomalies in development rate and zone shape is to be sought.

It was established experimentally that the anomalous zone shape with benzene–ligroin on prewashed adsorbent was not caused by the acetone in the prewash or by an impurity in the benzene. In one experiment 1:1 absolute ethanol–ether was substituted for the 1:1 acetone–ether in the prewash and in others, different samples of benzene were used, including several commercial C.P. samples and one purified with concentrated sulfuric acid.[40] The profiles were identical with that in Fig. 12.

Some information concerning the anomalies with benzene–ligroin developers may be obtained from a consideration of the nature of the prewashing process and from the experiments reported in Figs. 15 and 16. Since the column is completely wet with ether immediately before the ligroin is poured on during the prewash, and since ligroin is an extremely poor developer, a portion of the ether presumably remains adsorbed on the column after the ligroin has passed through. Benzene, a stronger developer than ligroin, removes the ether much more completely but evidently even it removes ether from the column fairly slowly. This fact has been demonstrated experimentally.

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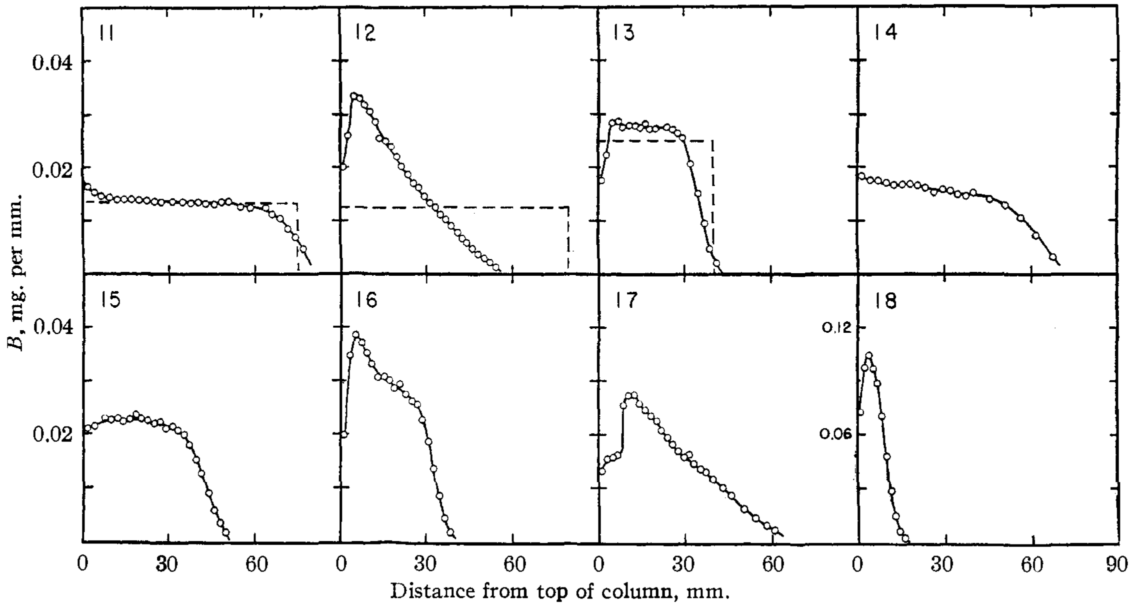
(40) See L. F. Fieser, "Experiments in Organic Chemistry," second edition, D. C. Heath and Co., New York, N. Y., 1941, Part II, p. 363.



Figs. 11–18.—Profiles of undeveloped zones. See Table V for experimental details.

About 125 ml. of benzene was poured through a prewashed column; after the first 20 ml. of the effluent benzene had been rejected because of possible contamination with the ligroin which had just preceded the benzene, the remainder was collected in several fractions. The ether content of the benzene in each of these fractions was determined in an approximate manner, both refractometrically and by tests of developing strength; it was found that the first fraction contained somewhat more than 1% of ether and that the last still contained a trace of ether,[41] too small to be detected with an Abbé refractometer, but nevertheless demonstrable in its effect on the development rate.

When benzene–ligroin prepared with some of the benzene which had flowed from the column in the above experiment was used to place a zone on a prewashed column (Fig. 15), the profile was similar to that of the zone introduced from benzene–ligroin containing a trace of ether (Fig. 14) and markedly different from that of a zone introduced from ordinary benzene–ligroin (Fig. 12). The shape of the profile of Fig. 15 is strong confirmatory evidence of the presence of a few tenths of a per cent. of ether or some similar substance in the benzene which flowed from the prewashed column. That the removal of the adsorbed ether from the column by this washing with benzene altered the adsorptive properties of the silicic acid–Celite is shown by the profile of a zone chromatographed from benzene–ligroin on a prewashed column which had been washed with benzene in the manner described (Fig. 16). Comparison of Fig. 16 with Fig. 13 indicates that the removal of the adsorbed ether tends to make the adsorbent more uniform with this developer and more like heated adsorbent, although there is still a difference from heated adsorbent, manifested by the pronounced maximum in the profile of the zone on the prewashed column.

The progressive decrease in adsorptive strength of a prewashed column with benzene–ligroin developers thus may be attributed to the gradual removal by the non-polar developer of the ether which is adsorbed, rather weakly, throughout the column at the conclusion of the prewash. When the developer itself contains ether, or any relatively polar compound, the adsorbed ether will be replaced essentially as fast as it is removed from the adsorbent and consequently there is no inhomogeneity in adsorptive strength. Similarly, with heated adsorbent, which presumably has no easily elutable compounds adsorbed on it, the compound being developed will in general be more strongly adsorbed than every component of the developer (except in displacement development, a special situation not pertinent to the present discussion) and therefore it will move in a region behind the front of the developer, that is, in a region where the composition of the chromatographic solvent will remain always the same.

The inhomogeneity which we have found with non-polar developers on prewashed adsorbent was in all probability present in the experiments of LeRosen[13] with 2-nitroaniline and benzene on prewashed silicic acid. Consequently, the empirical equation which he proposed, on the basis of these experiments, to relate the rate of development to the initial concentration and initial volume of the solution of the sample is of doubtful significance.

Two other features of the profiles of undeveloped zones remain to be discussed. The profile

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(41) Neither of these methods of analysis provides a unique test for ether, of course; it is possible that minor impurities in the ether such as peroxides or hydroperoxides might be more strongly adsorbed than the ether and might be present in the last fractions of benzene. It is difficult to see what other substances could be present under the circumstances; any compound not eluted by the ether of the prewash could scarcely be removed by benzene. In any event the qualitative explanation of the observed facts is the same whatever the substances responsible for them may be.

of Fig. 17 is of especial interest in demonstrating the sensitivity of the method of measuring profiles, as well as in emphasizing the need for rigorous drying of solvents to be used in the study of undeveloped zones. This zone was chromatographed under the same conditions as that in Fig. 12 except that the benzene which was used had been dried only over calcium chloride rather than over sodium. The presence of traces of water in this benzene is manifest; the initial plateau and sharp boundary in the profile indicate the presence of a front of water moving very slowly down the column and diminishing the adsorption of 4-nitrodiphenylamine near the top of the column where both are present. With developed zones rigorous drying of the developer is less important because traces of water will be adsorbed at the top of the column and almost invariably will move much more slowly than the zone of interest.

One characteristic of the profiles of undeveloped zones chromatographed from benzene–ligroin (Figs. 12, 13, 16, 17 and 18) remains unclear. In each of these profiles, including that measured on heated adsorbent, *B* was smaller in the top few millimeters than in succeeding portions of the column. Since the solution which is present in the interstices of the adsorbent contributes to *B*, a slight decrease in *B* in the top few millimeters of the column would occur if some of the solution were drawn out of this region of the column during the period of draining at the conclusion of development. However, the magnitude of the initial decrement in *B* with benzene–ligroin is appreciably greater than would be expected even if all of the solvent had been drawn out of the top few millimeters of the column; for example, in Fig. 18, the initial decrement is about four times as great as could be accounted for by the volume of interstitial liquid present. Furthermore, the phenomenon can actually be detected visually while solvent is flowing through the chromatogram. When the solvent contained ether (Figs. 11, 14 and 15) the initial portion of the zone was about normal or perhaps contained even slightly more than the expected amount of 4-nitrodiphenylamine. The initial decrement with benzene–ligroin does not appear to be due to any impurity in the benzene for it is identical with different samples of sodium-dried redistilled technical and C.P. benzene.

Conclusion

Our results indicate that both the classical and non-idealized theories of the simple adsorption chromatogram are satisfactory for predicting rates of development on columns of silicic acid–Celite, provided that the peculiarities of prewashed adsorbent are recognized and taken into account. On the other hand the shapes of developed zones can be explained qualitatively and quantitatively only by the non-idealized theories. The anomalies in the rates of development and the shapes of zones chromatographed on prewashed adsorbent from non-polar developers may be interpreted in terms of the gradual removal of adsorbed ether from this adsorbent and a consequent progressive variation in the adsorptive strength along the column.

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Summary

A quantitative experimental study has been made of the applicability of chromatographic theory to chromatograms on silicic acid–Celite. Six compounds of varying structure and adsorption affinity were chromatographed and a variety of developing mixtures and rates of development were investigated.

Adsorption isotherms on silicic acid–Celite were found to be essentially linear in the range of concentrations most important in practical chromatography on this adsorbent. Development rates predicted from these isotherms on the basis of simple chromatographic theory generally agreed well with those observed experimentally on adsorbents activated both by prewashing and by heating. Rates of development of compounds in mixtures were demonstrated to be the same as those of the same compounds developed separately.

A technique has been developed for determining precisely the distribution of a compound in a zone on a column (the "profile" of the zone). With the aid of this technique it was demonstrated that the theory of Mayer and Tompkins accounts quantitatively for the approximately Gaussian distribution of material in a developed zone on silicic acid–Celite, provided that the height equivalent to a theoretical plate is about 0.85 mm. for this system. This theory also accounts, at least qualitatively, for the variation of zone shape with the initial volume and concentration of the sample.

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