I. The Coordination of Silver Ion with Ethylene and Propylene

II. The Development of a Chromatographic-Spectrophotometric Method for the Isolation and Estimation of Minor Impurities in RDX

III. An Experimental Study of Chromatography on Silicic Acid-Celite Columns

IV. A Method for the Study of the Absorption Spectra of Adsorbed Compounds

Thesis

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Part I

The Coordination of Silver Ion with Ethylene and Propylene

Part I. The Coordination of Silver Ion with Ethylene and Propylene

The coordination complexes which are formed by silver ion with a variety of unsaturated compounds have been extensively studied in this laboratory[1]. All of the butenes[1b] and several of the higher olefins have been investigated previously. The present study of the complexes formed by silver ion with ethylene and propylene completes the series of investigations of the simpler unsaturated hydrocarbons.

The method which was employed in the present work was almost identical with that used previously, only slight modification being necessary because of the greater volatility of the simplest olefins. The distribution ratio of the unsaturated substance between carbon tetrachloride and a 1 N aqueous solution of potassium nitrate was first determined and was then combined with data obtained from an investigation of the distribution of the compound between carbon tetrachloride and a 1 N aqueous solution of silver nitrate or a solution of silver nitrate and potassium nitrate of total ionic strength unity. The notation used is the same as that which was used previously except that since only one silver ion and one olefin molecule are involved in the complex there is no need to distinguish K_1, the first argentation constant, from K, the evaluated constant. These constants are here identical and are denoted by the symbol K_E.

μ = ionic strength

B = the olefin

(Bt) = total concentration of the olefin, both free and complexed, in the aqueous phase, moles per liter

(B) = concentration of the uncomplexed olefin in the aqueous phase, moles per liter

(BAg^+) = concentration of the silver ion-olefin complex, BAg^+ in the aqueous phase, moles per liter

(Agt) = total silver concentration in the aqueous phase, moles per liter

(Ag^+) = concentration of free silver ion in the aqueous phase, moles per liter

$(B)_c$ = concentration of the olefin in carbon tetrachloride, moles per liter

$K_D = (B)_c/(B)$ = distribution ratio of the olefin between carbon tetrachloride and 1 N potassium nitrate

$$K_E = \frac{(BAg^+)}{(B)(Ag^+)} = \frac{[(Bt)-(B)]}{(B)[(Agt)-(Bt)+(B)]} =$$

$$= \frac{K_D[(Bt)-(B)_c/K_D]}{(B)_c[(Agt)-(Bt)+(B)_c/K_D]} =$$

= equilibrium constant for reaction of the uncomplexed olefin in the aqueous phase with silver ion to form the complex BAg^+

$K_b = K_E/K_D = \frac{(BAg^+)}{(B)_c(Ag^+)}$ = equilibrium constant for reaction of the olefin in carbon tetrachloride with silver ion in the aqueous phase to form the complex BAg^+

K_W = distribution ratio of the olefin between carbon tetrachloride and pure water

The Analytical Procedure

In the earlier work, satisfactory analyses for the higher olefins and other unsaturated substances were made by bromination with acidified standard bromate-bromide solution; the excess of bromine was determined by the addition of potassium iodide and titration of the liberated iodine with thiosulfate. Since it has been reported that bromine adds less rapidly to ethylene than to the higher olefins in water[2] as well as in dry carbon tetrachloride[3] and methylene chloride[4], the suitability of the bromination procedure for the determination of ethylene required some consideration and study. Because, as shown below, the method was found to be satisfactory for the determination of ethylene it was assumed likewise to be suitable for the determination of propylene and no explicit study of the rate of bromination of propylene was made.

Suida and Wesely[2] studied the rate of absorption of ethylene when mixtures of ethylene and air were shaken with 0.005 N bromine water. They showed that all of the ethylene was absorbed in less than six minutes from a mixture which contained initially 10 percent of the olefin and that all of the ethylene disappeared in less than four minutes from a mixture containing originally only 7 percent of this compound. In all of our experiments in which the bromination mixture was homogeneous, that is, in the analyses of aqueous solutions of ethylene and of ethylene and the readily dissociated silver ion-ethylene complex, the excess concentration of bromine was 0.003 N or greater and the bromination time was about fifteen minutes. It would

appear that there should be no difficulty in obtaining quantitative bromination of the ethylene under these homogeneous conditions.

Several studies have been made of the rate of bromination of ethylene in the dark in carbon tetrachloride[3,5,6] and although none of the results can be applied directly to the present work several observations are pertinent. The addition of bromine to ethylene is reported to be relatively slow in pure dry carbon tetrachloride but it is markedly accelerated by the presence of moisture[3] and hydrogen bromide[5]. Davis claims that the greater part of the reaction takes place in the aqueous phase; he also states that "in solutions of carbon tetrachloride saturated with water and containing equimolar amounts of bromine and ethylene the bromine color disappears in a few minutes although in the absence of water the color may persist for a long time."

In order to test the applicability of the previously used bromination procedure to the determination of ethylene in carbon tetrachloride several experiments were done in which the conditions were varied somewhat. In each of these experiments an aliquot portion from one of two different freshly prepared solutions of ethylene in carbon tetrachloride was added to an excess of acidified aqueous bromate-bromide solution in an evacuated flask which was wrapped in a dark cloth. The time of bromination was varied and in addition certain of the mixtures were shaken mechanically during all or part of the period of bromination while the others were allowed to stand essentially undisturbed. At the end of the period of bromination an excess of a 2 N aqueous solution of potassium iodide was added, the

mixture was shaken for a few minutes *in vacuo*, and the iodine which had been liberated was titrated at once with thiosulfate.

The results of these experiments are summarized in Table 1. It is apparent from Experiment A that mechanical shaking is essential and a consideration of both Experiments A and B indicates that essentially complete bromination takes place within 15 minutes under the conditions used. In the actual analyses of distribution mixtures, the bromination mixture was shaken for 15 to 20 minutes in the dark.

Table 1

The Effect of Variations in the Time of Bromination and in the Time of Shaking on the Extent of Bromination of Ethylene in Carbon Tetrachloride in the Dark with Acidified Aqueous Bromate-Bromide Solution

	Aliquot portion No.	Time of bromination, min.	Time of shaking, min.	Molar concentration of ethylene equivalent to bromine absorbed
Experiment A	1	11	0	0.038
	2	34	0	.045
	3	6	6	.048
	4	12	10	.050
	5	30	12	.052
	6	37	17	.051
Experiment B	1	16	8	.024
	2	24	22	.024
	3	46	35	.024

The Distribution Ratios

The distribution ratios at 25° of ethylene and propylene between carbon tetrachloride and water and between carbon tetrachloride and a 1 N aqueous solution of potassium nitrate are given in Table 2.

Table 2

Distribution Ratios of Ethylene and Propylene between Carbon Tetrachloride and Water and Carbon Tetrachloride and 1 N Aqueous Potassium Nitrate at 25°

	Water			1.000 N KNO_3		
	$(B)_c$	(B)	K_W	$(B)_c$	(B)	K_D
Ethylene	0.0210	0.00060	35.0	0.0155	0.00038	40.8
	.0119	.00036	33.1	.0058	.000144	40.2
	.0200	.00059	33.8	.0154	.00039	39.5
	.0117	.00036	32.5	.0097	.00024	40.4
	.0147	.00046	32.0			
	.0096	.00028	34.3			
					Average	40.2
		Average	33.5			
Propylene	0.0882	0.00061	144	0.280	0.00122	229
	.0775	.00055	141	.284	.00130	218
	.0530	.00036	147	.267	.00115	232
	.0755	.00051	148	.181	.00081	224
	.0624	.00042	148	.173	.00079	220
	.0715	.00047	152	.175	.00077	228
		Average	147		Average	225

The Argentation Constants

Following the procedure which was previously adopted[1a] we shall consider two equilibrium constants:

K_E for the reaction

$$B\ (aq.) + Ag^+(aq.) \rightleftharpoons (BAg^+)\ (aq.)$$

and K_o for the reaction

$$B\ (c) + Ag^+(aq.) \rightleftharpoons (BAg^+)\ (aq.).$$

As pointed out earlier there are several advantages in considering K_o instead of K_E, the chief among these being that the measured value of K_E depends on the observed value of K_D whereas K_o is generally almost independent of the value of K_D. K_D is often difficult to measure precisely because the concentrations in the aqueous phase are too small; furthermore it is subject to error because of the effect of partial miscibility of the carbon tetrachloride and water, which tends to make the measured value smaller than the true value. Finally the measured value of K_D or of K_E is subject to error because of the use of concentrations instead of activities in its formulation. Although there will also be some uncertainty in the value of K_o arising from the same source, it will in general probably be considerably smaller since the concentrations which enter into the expression for K_o are usually far below the saturation values and since in addition the solution of olefin in carbon tetrachloride might be expected to be more nearly ideal than that of the olefin in the aqueous phase.

Table 3

Distribution at 25° of Ethylene between Carbon Tetrachloride and Solutions of Silver Nitrate plus Potassium Nitrate of Total Ionic Strength Unity

(Agt)	(B)	(Bt)	K_o	
1.000	0.00496	0.01198	2.42	
	.00403	.01000	2.48	
	.00375	.00906	2.41	
	.00277	.00681	2.45	
	.00371	.00920	2.48	
	.00266	.00645	2.42	
		Average	2.44	K_E = 98.1
0.500	0.0032[a]	0.00365	2.22[a]	
	.00185[a]	.00214	2.28[a]	
	.00267[a]	.00328	2.42[a]	
	.00186[a]	.00209	2.21[a]	
		Average	2.28	K_E = 91.7

a The precision of these values is relatively low because only a small volume of the carbon tetrachloride phase was taken for analysis

Table 4

Distribution at 25° of Propylene between Carbon Tetrachloride and Solutions of Silver Nitrate and Potassium Nitrate of Total Ionic Strength Unity

(Agt)	$(B)_c$	(Bt)	K_o	
1.000	0.0358	0.01565	0.440	
	.0325	.01386	.428	
	.0371	.01562	.423	
	.0354	.01510	.429	
	.0297	.01268	.429	
	.0264	.01134	.430	
	.0727	.0311	.437	
	.0651	.0272	.425	
		Average	0.430	$K_E = 96.8$
0.500	0.0778	0.01560	0.405	
	.0763	.0152	.403	
	.0415	.00879	.422	
	.0653	.01320	.406	
		Average	0.409	$K_E = 92.0$

It is to be noted from the data of Tables 3 and 4 that, just as with the higher olefins, the argentation constants of both ethylene and propylene are independent of the concentration of the olefin over a somewhat greater than two-fold range in concentration, and are also relatively insensitive to a two-fold variation in the silver ion concentration, indicating that only one olefin molecule and one silver ion are present in each molecule of complex. For both ethylene and propylene a 50-percent decrease in the silver ion concentration, at constant ionic strength, leads to a 5- or 6-percent drop in K_o. This trend has been noticed before[1a,7] and has been

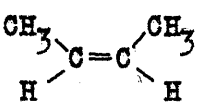
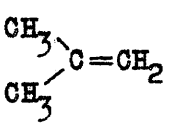
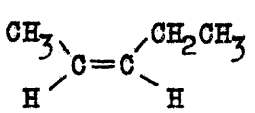
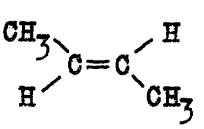
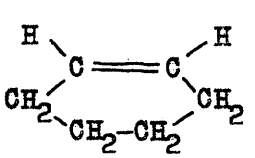
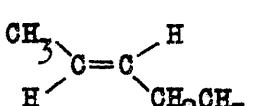
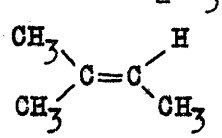
observed to reach a value of about 10 percent with the olefins of higher molecular weight. There are two possible explanations for this phenomenon: (a) it is possible that a small quantity of the di-silver complex, $AgBAg^{++}$, is formed; (b) there may be an error in the assumption that the ratio of the activity coefficients of the complex (BAg^{+}) and of the silver ion is constant as silver nitrate is replaced by potassium nitrate. This assumption is implicit in the formulation of K_O or K_E with concentrations instead of activities of the silver ion and the complex.

Discussion

In Table 5 are summarized the various constants obtained for the simple mono-olefins which have been studied.

Table 5

Summary of Constants for Silver Ion-Olefin Complexes at 25°

Olefin Name	Formula	K_W	K_D	K_E	K_o
Ethylene	$CH_2=CH_2$	33.5	40.2	98.1	2.44
Propylene	$CH_3CH=CH_2$	147	225	96.8	0.430
1-Butene[1b]	$CH_3CH_2CH=CH_2$		898	140.9	.1568
1-Hexene[1a]	$CH_3CH_2CH_2CH_2CH=CH_2$		9050	860	.095
<u>cis</u> 2-Butene[1b]			890	72.0	.0808
Isobutene[1b]			806	61.2	.0758
<u>cis</u> 2-Pentene[1c]		2590	3607	112.5	.0312
<u>trans</u> 2-Butene[1b]			1019	29.2	.0286
Cyclohexene[1d]		3610	4760	91.0	.0191
<u>trans</u> 2-Pentene[1c]		3320	5620	62.4	.0111
Trimethylethylene[1d]		2920	3460	23.3	.00674

There is apparently a definite trend in the values of K_o with variation in the structure of the olefin. The lack of regularity in the values of K_E may perhaps best be interpreted in terms of the large and varying disparity between activities and concentrations of the different olefins in the aqueous phase.

Consideration of the K_o values for the different olefins shows that the more deeply the double bond is buried in the chain the smaller is the value of K_o, that is, the lower the stability of the complex of the olefin with silver ion. It is to be noted that in each of the two pairs of cis-trans isomers studied the cis compound has much the higher argentation constant. The data summarized in Table 5 seem to demonstrate quite conclusively that the influence of the structure of the olefin upon the stability of these silver ion-olefin complexes is essentially steric in nature. This conclusion is in agreement with that of Anderson[8] regarding the stability of complexes of homologues of Zeise's salt, $K[PtCl_3, CH_2=CH_2]H_2O$. The structure of the silver ion-olefin complex may, as pointed out earlier[1a,1c], be considered as a resonance hybrid of several contributing forms.

Although tetramethylethylene has not as yet been studied we can conclude with reasonable safety from the results on ethylene and the various methyl-substituted ethylenes that it would display an exceedingly low value of K_o, indicating a relatively small amount of complex formation.

Experimental

Materials. - The potassium iodide which was used for the work with ethylene was of USP grade; all other inorganic chemicals were of CP or reagent grade. The standard solutions of silver nitrate and potassium nitrate and of bromate-bromide were made up by weight. The concentration of the bromate-bromide was about 0.06 N, that is, one liter of this solution contained approximately 0.01 moles of potassium bromate and 0.06 moles (a 20-percent excess) of potassium bromide. The concentration of the solution of sodium thiosulfate was about 0.02 N; it was checked frequently against the standard bromate-bromide solution.

Carbon tetrachloride was purified by passing into it a large excess of chlorine, irradiating the mixture with sunlight for forty-eight hours, and removing the chlorine by washing with dilute sodium hydroxide. After several washings with water the product was dried over calcium chloride and distilled. A blank test on the purified material showed that it neither absorbed nor liberated bromine or iodine.

Propylene bromide was prepared from isopropanol by dehydration and subsequent bromination. The crude product was distilled and then fractionated twice, b.p. 139.3-139.8° (uncorr.) at 746 mm., $n_D^{25} = 1.5173$, $n_D^{20} = 1.5200$. These values for the boiling point and refractive index agree satisfactorily with those reported by Kahlbaum[9], b.p. 141.0° at 760 mm. and $n_D^{20} = 1.5198$ and by von Zawidzki[10], $n_D^{25.4} = 1.51745$.

Ethylene bromide was prepared from ethanol by dehydration and subsequent bromination; the crude product was recrystallized from methanol in a dry ice-isopropyl ether bath. It was then washed twice with 80-percent sulfuric acid and with water until the wash water was neutral, and was subsequently dried over potassium carbonate and distilled. The column and condenser were carefully dried after the few remaining drops of water had distilled over. The purified ethylene bromide distilled at 129.7-129.8° (uncorr.) at 746 mm. and the refractive index of the distillate was found to be $n_D^{25} = 1.5359$. These figures agree well with those reported by Anschutz[11], b.p. 129.5° at 745 mm. and by von Zawidzki[10], $n_D^{25.4} = 1.53601$. When the recrystallization and subsequent washings, drying, and distillation were repeated the boiling point and refractive index were found to be unchanged.

Preparation of Propylene and Ethylene. - Ethylene and propylene were prepared in an identical fashion, by the reaction of strip zinc with the corresponding dibromide diluted with an equal volume of 95-percent ethanol. The reaction was carried out in a 500-ml. three-neck flask fitted with a Friedrich's condenser, a thermometer, and a vertical safety tube extending well below the surface of the reaction mixture and leading to an open Erlenmeyer flask about 30 inches above the reaction flask. The safety tube served as a convenient pressure gauge during the generation of the gas. From the outlet of the Friedrich's condenser the gas passed to a 20-cm. one-piece spiral wash bottle containing a 1:1 mixture of ethanol and water and then to another similar wash bottle containing pure water.

Finally the gas was absorbed in two Friedrich's-type gas wash bottles each of which contained about 200 ml. of carbon tetrachloride.

After the dibromide and the ethanol had been placed in the reaction flask the whole system was swept out with nitrogen. Then a small portion of zinc was added and the mixture was heated very gently for about thirty seconds in order to start the reaction. Once started it proceeded quite rapidly without further heating; a cooling bath was usually necessary in the early stages of the reaction in order to prevent the evolution of gas from becoming too vigorous. The remainder of the zinc was added as needed, and the reaction was allowed to continue at room temperature for several hours until the carbon tetrachloride appeared to be saturated with the olefin. The concentration of this nearly saturated solution appeared to be for propylene very approximately 0.5 molar and for ethylene between 0.05 and 0.10 molar; however no weight should be attached to these values. The solutions of the olefins in carbon tetrachloride were stored under an atmosphere of nitrogen in order to prevent the formation of peroxides which might interfere seriously with the analytical procedures[1a].

Distributions. - The distributions were carried out in a 500-ml. three-neck flask fitted with a mercury-sealed stirrer and a two-hole rubber stopper*, one hole of which was used for the introduction of the sampling pipette and was kept closed at other times,

* Since the values of the constants were not observed to vary appreciably with time of standing of the distribution mixture it was not thought necessary to use an all-glass system. Earlier it had been found necessary to eliminate the rubber stopper in order to obtain reproducible results in the work with 2-pentene, although it was not necessary with 2-butene or isobutene. See reference 1c.

and the other of which was fitted with a glass tube carrying a three-way stopcock[12]. After introduction of the aqueous phase, the flask was swept out with nitrogen. The solution of the olefin in carbon tetrachloride was then added and nitrogen was passed through the flask for an additional few minutes. The flask was immersed in a thermostat at 25.00 ± 0.03° and the contents were stirred mechanically for about one hour. After the mixture had been allowed to stand quietly for another hour an appropriate sample of each phase was removed by forcing it into a sampling pipette by means of a stream of nitrogen. The sampling pipettes were fitted with three-way stopcocks in order to facilitate rinsing of the sample into the bromination flask[13].

Analyses. - The method of analysis was essentially that which has been described previously[7,14]. The sample was added to a measured excess of a previously acidified bromate-bromide solution in a 300-ml. evacuated standard-taper Erlenmeyer flask through a stopcock in the stopper and the flask was shaken mechanically for 15 to 20 minutes, a time sufficient for the complete addition of bromine to the olefin. An excess of a solution of potassium iodide was then passed into the flask and the liberated iodine was titrated at once with thiosulfate, using starch as an indicator. In all runs black cloth was placed around the bromination flask before the addition of the olefin and was removed only after the addition of the potassium iodide. During the analysis of aqueous solutions containing silver ion an amount of potassium bromide more than equivalent to the silver ion present was added to the acidified bromate-bromide before the addition of the sample; an excess of potassium iodide sufficient to metathesize all of the silver bromide formed was also used.

Summary

A description is given of the investigation by means of a distribution technique of the complexes which are formed by silver ion with ethylene and propylene. A summary of the argentation constants of all of the simple mono-olefins which have been studied is presented.

The influence of the structure of the olefin upon the stability of the silver ion-olefin complex appears to be essentially steric in nature.

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Part II

The Development of a Chromatographic-Spectrophotometric Method for the Isolation and Estimation of Minor Impurities in RDX

Part II. The Development of a Chromatographic-Spectrophotometric Method for the Isolation and Estimation of Minor Impurities in RDX*

A. Introduction

The work which is described in the following pages is intended to serve as an illustration of the power and scope of a combination of chromatographic and spectrophotometric methods in implementing the analysis of a particularly intractable mixture containing as many as ten very similar organic compounds in widely disproportionate amounts. The particular analytical procedure which will be described was developed for the isolation and estimation of the minor impurities which may occur in samples of RDX. The work was carried out under the general supervision of Dr. Robert B. Corey and Dr. W. A. Schroeder; the experimental work was done chiefly by Mr. Earl W. Malmberg, Mr. Thomas D. Waugh, and the author of this Thesis. Preliminary experiments had been conducted by Dr. Arthur L. LeRosen and Mr. Waugh.

RDX, or cyclonite, as it is often called, is cyclotrimethylene-1,3,5-trinitramine, and all of the compounds which were

* The work described in this part of the Thesis comprises a portion of the scientific investigations carried out under Contract OEMsr-881 between the Office of Scientific Research and Development and the California Institute of Technology.

considered to be possible impurities in RDX are very closely related cyclic or linear nitramines. Although chromatographic-spectrophotometric methods had earlier been applied with marked success[1],* to the qualitative and quantitative analysis of other mixtures of closely related organic compounds, most of the techniques which had been employed earlier could be applied to the present compounds only with modification, often to a considerable degree. Special problems arose because the compounds are insoluble in all but a few very polar solvents, because these compounds are colorless and so inert chemically that it was difficult to find suitable means of locating many of them on the chromatographic column, and because they are so similar in structure that they can not in general be distinguished spectrophotometrically.

The general plan which was followed in the development of the analytical scheme is described briefly in the following paragraphs.

(1) A rather complete chromatographic study was made of the ten compounds separately. This study included the development of a prewashing treatment which greatly improved the adsorptive properties of the silicic acid-Celite columns, the investigation of suitable solvents for placing the compounds on the column and for developing them, the finding of suitable streak reagents for the location of the developed zones, and the investigation of the recoverability of each of the possible impurities on elution from a column of

* This work was carried out under Contracts OEMsr-702 and OEMsr-881 between the Office of Scientific Research and Development and the California Institute of Technology.

silicic acid-Celite. In addition, procedures were devised by which each of the ten compounds might be separated from others of similar adsorption affinity; since many of the compounds were very similar in structure and chromatographic properties, considerable experimentation was necessary in order to find adequate procedures for these separations.

(2) On the basis of the information gathered in the study of the compounds separately and in binary mixtures a tentative chromatographic procedure was devised for the complete separation of a mixture of all of the compounds. This procedure was then tested with a synthetic mixture of all of these substances and a few necessary revisions were made in it.

(3) It was necessary to devise a procedure for concentrating the impurities before chromatography because it was desired to detect traces of these compounds in the RDX and because the extreme insolubility of this substance severely limited the quantity which could be chromatographed. The preliminary concentration was effected by fractional precipitation of the major portion of the RDX. The recovery of each of the possible impurities from a synthetic mixture with a large excess of RDX was tested by use of this fractional precipitation procedure.

It must be emphasized that in its early stages the development of the procedures described herein was a cooperative enterprise. As mentioned above, Dr. LeRosen and Mr. Waugh carried out preliminary

experiments and, with the aid of Mr. Malmberg, devised the "modified" prewashing procedure which greatly improved the adsorptive properties of silicic acid-Celite mixtures and made it possible to obtain satisfactory zones of the nitramines on this adsorbent. Dr. LeRosen and Mr. Waugh also made preliminary measurements of the ultraviolet absorption spectra of many of the compounds. The investigation of the detailed chromatographic properties of four of the ten compounds was done by Mr. Waugh and Mr. Malmberg, who also worked out many of the details of the procedure for the determination of the two compounds which are less strongly adsorbed than RDX.

I would like to express my sincere appreciation to Mr. Malmberg for his invaluable advice and cooperation throughout all of the work which is discussed below.

B. A Brief Description of the Chromatographic-Spectrophotometric Method and of the Structure and Properties of RDX and Certain Related Compounds

1. The chromatographic-spectrophotometric method

A description of the chromatographic-spectrophotometric method which served as a basis for the present work has been given elsewhere[1]. Only a brief discussion of some of the more important features of the method as they applied in this work will be presented here.

The adsorbent which was used throughout this investigation was a mixture composed of two parts by weight of Merck reagent silicic acid and one part of Celite 535, a product of the Johns-Manville Corporation. Number 2 chromatographic tubes, which are 19 to 20 mm. in inside diameter, were used for most of the chromatograms; the columns of adsorbent were usually 150 mm. in length, although occasionally longer columns were used.

The Beckman Quartz Photoelectric Spectrophotometer was used extensively for qualitative and quantitative analyses in the earlier work[1]; however in the work with RDX and related compounds its use was restricted chiefly to quantitative estimations since, as mentioned before, the spectra of many of the compounds are very similar. A quantity of the order of magnitude of one milligram is quite sufficient for the quantitative determination of any of the compounds in a chromatographic eluate.

Because most commercial grades of organic solvents were found to contain non-volatile impurities which interfered with

spectrophotometric analyses at wave lengths below about 300 mμ the practice was adopted of distilling all chromatographic solvents in an all-glass still before use. In this way most of the foreign substances which might have been introduced by the solvents into the residues from the evaporation of chromatographic eluates were eliminated. It was generally unnecessary to distil the absolute ethanol which was used as a spectrophotometric solvent if the same stock of ethanol was used for solutions and comparison solvent in the spectrophotometric measurements, since the quantity of impurity which the available ethanol contained was usually relatively minor. Certain of the solvents, particularly the nitroparaffins, required special purification because they contained substances which interfered with streak tests. The nitroparaffins were purified by percolation through a column of silica gel and subsequent distillation in an all-glass still.

Early in the development of quantitative chromatographic-spectrophotometric methods it was discovered that the adsorbent contained significant quantities of readily-elutable non-volatile impurities which showed appreciable absorption of ultraviolet radiation at wave lengths below about 300 mμ. Therefore, in order to prevent contamination of eluates by these substances the practice was adopted of washing all columns with a strong eluent and then with ligroin before introducing the sample to be chromatographed. At first this "prewash" consisted of ether followed by ligroin; it was found that this procedure increased somewhat the adsorptive strength of the silicic acid-Celite. Later, as mentioned in the

Introduction, a modification of this prewashing procedure was developed in which a 1:1 mixture of acetone and ether was used in addition to the ether and ligroin. This procedure increased the adsorptive strength considerably, made the adsorbent more uniform, and decreased the amount of spectrophotometric "background" absorption in eluates; thus it was in all ways more satisfactory than the first prewash.

For convenience a convention was adopted for specifying the volumes of solvent employed in the washing or development of a chromatogram. That volume which is necessary to wet completely a column of adsorbent is referred to as "V" milliliters; for a 15-cm. No. 2 column of 2:1 silicic acid-Celite, V is about 29 ml. Using this convention, the modified prewash may be described as follows: the column is washed first with 0.2 V ml. of ether, then with V ml. of 1:1 acetone-ether, next with 0.8 V ml. of ether and finally with 2 V ml. of ligroin. This prewash was used in all of the work which is to be described.

The compounds which were investigated in our earlier chromatographic studies[1] included a wide variety of organic substances many of which are colorless or nearly so. Zones of almost all of these substances can be located on the column by streaking the column with some reagent which gives a characteristic color with the compound. Streak tests are also often valuable in work with colored substances; for example, they provide a very simple method for distinguishing between two zones of similar color and chromatographic behavior. The streak tests which were used throughout the present work are described in Section C 2 (c) below.

2. The structures and properties of RDX and certain related compounds

Since RDX and the compounds which are closely related to it belong to a rather unusual class of organic compounds a brief discussion of their physical and chemical properties is necessary. This discussion will be very limited, however, and only those properties which were of especial importance in the present work will be mentioned.

RDX is generally prepared by the nitration of hexamethylenetetramine, either with nitric acid alone or with a mixture of nitric acid, acetic anhydride, and ammonium nitrate. The chemical names, common names, and structural formulas of most of the important compounds which have been found in the reaction mixtures under widely varying conditions are presented in Tables 1 and 2. All of the compounds contain the methylenenitramino group, $-CH_2-\underset{\underset{NO_2}{|}}{N}-$, and some also contain other functional groups, in particular N-acetyl and acetoxy. Some of the compounds, including RDX itself, retain a part of the cyclic structure of hexamethylenetetramine, while others are linear. RDX contains a 6-membered ring of alternate methylene and nitramino groups; HMX is the next higher analogue of RDX and contains an 8-membered ring. It will be noted that there are several pairs of compounds the members of which are related in the same fashion as are HMX and RDX, that is, one merely contains an additional methylenenitramino group in the ring or chain. Thus AcAn is so related to BSX and SEX is a higher analogue of TAX.

The close similarity of the compounds in each of these pairs

Table 1

Chemical Names, Common Names, and Structural Formulas of Some Cyclic Nitramines Derived from Hexamethylenetetramine

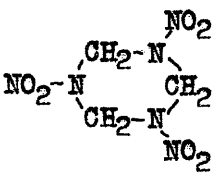
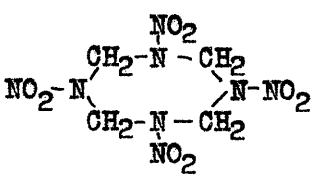
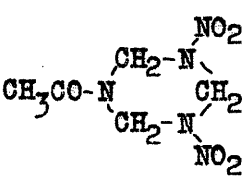
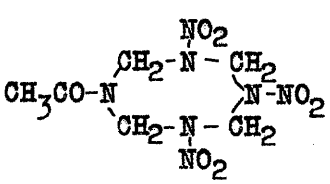
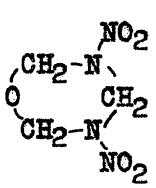
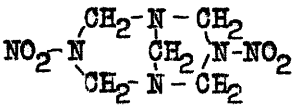
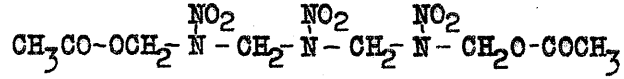
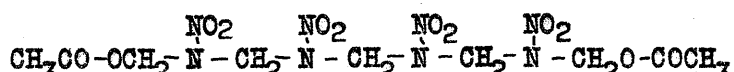
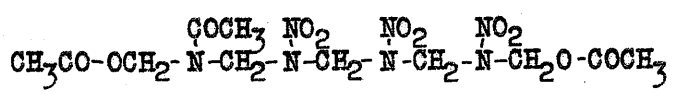
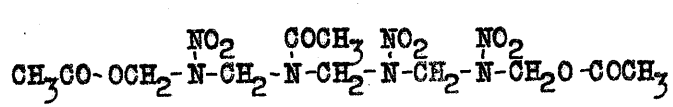
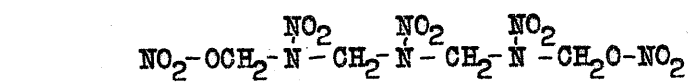
Chemical Name	Common Name	Formula
cyclo-trimethylene-1,3,5-trinitramine	cyclonite, RDX	
cyclo-tetramethylene-1,3,5,7-tetranitramine	HMX	
1-acetyl-3,5-dinitrocyclo-trimethylene-1,3,5-triamine	TAX	
1-acetyl-3,5,7-trinitrocyclo-tetramethylene-1,3,5,7-tetramine	SEX (QDX)	
cyclic ether of 1,5-dihydroxy-trimethylene-2,4-dinitramine	cyclonite oxide	
1,5-dinitro-bicyclopenta-methylenetetramine	DPT (DNPT)	

Table 2

Chemical Names, Common Names, and Structural Formulas of Some Linear Nitramines Derived from Hexamethylenetetramine

Chemical Name	Common Name	Formula
(linear) 1,7-diacetoxytetramethylene-2,4,6-trinitramine	BSX	
(linear) 1,9-diacetoxypentamethylene-2,4,6,8-tetranitramine	AcAn	
(linear) 1,9-diacetoxypentamethylene-2(or 4?)-acetyl-4(or 2?),6,8-tetranitramine	H-16	 or 
(linear) 1,7-dinitroxytetramethylene-2,4,6-trinitramine	ATX	

was reflected in their chromatographic behavior and streak reactions. Although the near identity of these properties for the closely related pairs simplified somewhat the investigation of the chromatographic properties of each of the compounds separately it introduced additional complications into the problem of devising procedures for the separation and identification of all of the compounds from a mixture. However, as discussed in Section C 4 below, it was eventually possible to devise procedures for the satisfactory chromatographic separation of even the most closely related compounds.

As a class, these methylenenitramino compounds are rather stable from the standpoint of ordinary chemistry. For example, as of 1943 at least, it was not possible to prepare a single derivative from RDX; the compound is slowly decomposed by cold concentrated sulfuric acid but all other acid and alkaline solutions affect it only when hot. Certain strong oxidizing agents attack RDX slowly in the cold and the nitro groups can be fairly readily reduced under some conditions. Although certain of the compounds, and in particular the linear ones, are more reactive than RDX in specific reactions there is little uniformity in their behavior. Consequently the task of finding suitable streak reactions for the compounds proved a formidable one. Two useful streak tests were eventually developed; one of these was a modification of Schryver's test for formaldehyde[2] and can be used to locate zones of those compounds which are rapidly decomposed by concentrated sulfuric acid. The other test, which is applicable to all of the compounds, was based primarily on the Franchimont test for nitramines[3] although in its final form this test

involved also the procedure devised by Griess for the detection of nitrites[4].

The ultraviolet absorption spectra of these nitramines were measured in order that they could be used for qualitative and quantitative analysis. The insolubility of most of the compounds in ethanol made quantitative work difficult until a procedure was devised for measuring the spectrum in a mixed solvent. The compounds were dissolved in a small but definite quantity of acetone or dioxane and the resulting solution was diluted 100-fold with absolute ethanol. The comparison solvent was of course prepared in an identical fashion.

It had previously been reported only that some of the compounds exhibited "characteristic nitramine spectra", that is, maxima in the neighborhood of 230 mμ with relatively high extinction. Hence it was necessary for us to measure the ultraviolet absorption spectra of all of the compounds which we were investigating; no discussion of the results can be presented here but it should be mentioned that some interesting correlations of features of the spectra with structure were found. Because, as mentioned earlier, the spectra of many of the compounds are rather similar and because appreciable absorption occurs only at wave lengths below about 240 mμ where the effects of minor impurities from the adsorbent and solvent on the position of the absorption maximum are most pronounced, it was generally not possible to use the spectra for reliable qualitative analysis.

The spectrophotometric data which were used in the quantitative analyses are presented in Table 3. The "spectrophotometric factor" is a convenient expression of the extinction of the compound;

it is equal to the concentration in milligrams per 100 milliliters which will produce an optical density of 1.000 in a cell 1.000 cm. thick.

Table 3

Spectrophotometric Data for RDX and Certain Related Nitramines

Compound	Position of absorption maximum, mμ	Spectrophotometric factor, mg. per 100 ml. for 1.000 cm.
RDX	213	2.02 1.41 at 235 mμ
HMX	228-9	2.42
TAX	231-4	ca. 3.36
CyOx	211	ca. 2.10 ca. 3.47 at 240 mμ
SEX	227	ca. 1.85
DPT	242-3	ca. 2.23
BSX	227	ca. 2.19
ATX	224-5	ca. 2.2
AcAn	227-8	ca. 2.10
H-16	229-30	ca. 2.72

The samples of all of the compounds which were investigated except ATX were supplied to us by Dr. W. E. Bachmann of the University of Michigan, Dr. A. T. Blomquist of Cornell University, and Dr. Marvin Carmack of the University of Pennsylvania. The sample of ATX was prepared in this laboratory by Mr. Philip E. Wilcox from a sample of BSX which had been sent to us.

C. The Chromatographic Properties of RDX and Certain Related Compounds

1. Introduction

The first step in the development of a procedure for the determination of minor impurities in RDX was the detailed investigation of the chromatographic behavior of RDX and of the compounds which were considered to be the most probable impurities. It is not possible to present here a complete description of the chromatographic properties of each of the ten compounds which were investigated. Instead the general method by which these properties were studied will be described briefly and the most useful results which were obtained will be considered, with emphasis placed on the behavior of the compounds with different types of developers and on the separation of compounds of similar adsorption affinity. Then a brief general discussion will be presented, and mention will be made of certain interesting correlations which were found between structure and chromatographic properties. Finally a short discussion of the elution and recovery of these compounds will be given.

2. The method which was used in the systematic study of chromatographic behavior

(a) General features of the method

The investigation of the chromatographic properties of the individual compounds followed a fairly standard pattern after the initial complications due to the extreme insolubility and relative chemical inertness of the compounds had been resolved. About 5 mg. of the compound was developed on a No. 2 column which had been prewashed according to the modified procedure. The final position of

the zone was determined by vertical streaks at several different places along the circumference and along the axis of the column in order that coning and distortions and other unfavorable effects which characterize certain developers could be detected.

A brief discussion is given in Sections (b) and (c) of the solvents which were used to place the samples on the column and of the streak reagents which were employed to locate the developed zones. A discussion of the chromatographic behavior of the individual compounds with different developers is given in Section 3.

(b) Sample solvents

The solubility of these nitramines is appreciable only in such solvents as the nitroparaffins, ethyl acetate, pyridine, and acetone, all of which are very strong developers or eluents for the compounds. Fortunately however most of these nitramines are relatively strongly adsorbed on silicic acid and therefore these solvents can, by suitable admixture with benzene or ligroin, be used to obtain compact zones of most of the compounds at the very top of the column. The most generally used sample solvents were mixtures of the nitroparaffins with benzene although many other mixtures were used for special purposes. A 1:4 mixture of nitromethane and benzene is satisfactory for most of the compounds at a concentration of about one milligram per milliliter; however for the relatively weakly adsorbed compounds ATX and cyclonite oxide ("CyOx") it is necessary to avoid the use of such strong developers. Fortunately these compounds are more soluble than the others and such sample solvents as 1:4 dioxane-ligroin or 1:9 ethyl acetate-ligroin are satisfactory. Some precautions

must be observed with the more strongly adsorbed compounds also; for example, ethyl acetate cannot be used in the sample solvent for AcAn or BSX because it tends to spread zones of these compounds badly. Similarly pyridine is unsatisfactory for certain of the compounds although it is an extremely good sample solvent for others.

(c) Streak reactions

The first streak reactions which were used to detect the nitramino compounds on the chromatographic column were rather insensitive and somewhat specialized and consequently were not very satisfactory. Modifications and improvements were however introduced from time to time and in their present form two of the tests are extremely sensitive and valuable. Only these two tests, the benzene-Franchimont test and the Schryver test, have more than specialized applicability, and they are sufficient for almost all purposes. Hence only these tests will be described. Special reagents can sometimes be used for compounds with particular functional groups; for example, ATX, which contains two nitrate ester groups, can be located with the hydroxide-diphenylamine reagent[1].

(i) The benzene-Franchimont test

The Franchimont test for nitramines[3] consists in treating the suspected nitramine with any of several aromatic amines in the presence of zinc dust and acetic acid. A positive reaction is indicated by the production of a characteristic color, which varies somewhat with the aromatic amine which is used. The reaction, which is specific for the nitramino group, can be used in various ways as a chromatographic streak test. In one method a colorless solution of

chromatographically purified α-naphthylamine in glacial acetic acid is applied to the column and then the streak is lightly sprinkled with zinc dust from a camel-hair brush. A pink color is produced in the zone of the nitramine.

An extremely sensitive modification of this test, which can be used for the detection of any of the nitramine compounds studied, was eventually developed as the result of a series of empirical discoveries. In this form of the test, usually called the "benzene-Franchimont test", a thin streak of zinc dust is first applied along the column with a camel-hair brush; then the column is streaked with benzene and finally with the Griess reagent[4], a solution of 0.5 percent of sulfanilic acid and 0.15 percent of α-naphthylamine in a 30-percent solution of acetic acid in water. A positive test is indicated by the development of a deep red-pink color within the limits of the zone. As little as 0.01 to 0.02 mg. of most of the nitramines which were investigated can be detected with this test when the compound has been chromatographed so that it forms a zone in the upper part of a No. 1.5 column. It is generally best to postwash the column with ligroin before applying the benzene-Franchimont test since certain of the usual developing solvents are known to interfere with the sensitivity of the test.

It should be noted that when the Griess solution is used the test is no longer specific for nitramines since the Griess reagent produces a pink color with nitrous acid. Hence in the benzene-Franchimont test nitrosamines produce a pink color which is similar to that formed by nitramines although it develops more slowly and is

somewhat less sensitive. Nothing is known about the mechanism of the benzene-Franchimont test with nitramines. Omission of any of the reagents used in the test decreases the sensitivity of the test considerably or makes it fail completely. For example, if the streak with benzene is not used the pink color is much less intense and the sensitivity of the test is diminished by a factor of at least three or four. It has been demonstrated that the thiophene which is present in commercial benzene is not responsible for the intensification which benzene produces. The color produced in this test is apparently far more intense than the sum of the rather weak colors produced in the Franchimont test (that is, zinc dust plus α-naphthylamine in acetic acid) and the Griess test separately.

(ii) The Schryver test

Several of the nitramines derived from hexamethylenetetramine are readily decomposed by concentrated sulfuric acid to give formaldehyde. Hence it is possible to use the Schryver test for formaldehyde as a streak reaction for these compounds. This test consists in the formation of the phenylhydrazone and then oxidation of this compound to a red pigment; the streak reaction is effected by streaking first with a 5-percent solution of phenylhydrazine in 90-percent sulfuric acid and then with a 5-percent aqueous solution of potassium ferricyanide. The test is very insensitive in the presence of benzene; for best results the column should be postwashed with 30-60° ligroin.

The Schryver test is about as sensitive as the benzene-Franchimont test for cyclonite oxide, DPT, BSX, and ATX, and is only

slightly less sensitive for AcAn and H-16. On the other hand RDX, HMX, SEX, and TAX do not react with the Schryver reagent sufficiently rapidly for the test to be practical as a chromatographic streak for these compounds. About 0.02 mg. of a compound which reacts with the Schryver reagent can be detected with this test when the compound is in a zone in the upper part of a No. 1.5 column.

3. The chromatographic behavior of RDX and related compounds with different types of developers

The chromatographic properties of these nitramines were studied in a series of separate investigations of the individual substances. At first many different developers were investigated, including binary mixtures with benzene of varying proportions of ether, ethyl acetate, 1-nitropropane, 2-nitropropane, nitromethane, acetic acid, dioxane, acetone, and ethanol, and binary mixtures of many of these solvents with ligroin. On the average, about 40 or 50 separate chromatograms were run for each of the compounds in order that its developmental behavior could be well characterized. The first few chromatograms were generally used for determining the most suitable sample solvents and streak reagents. Two or three columns were then run with each of about ten or twelve different types of developing mixtures. Finally those developers which seemed most promising from the standpoint of affording the possibility of separation of the compound from other similar compounds were studied in somewhat more detail. As experience was gained in the chromatography of these nitramino compounds it became apparent that certain developers would be most generally useful, others would be satisfactory

for particular separations, and still others, although quite adequate for the individual compounds, would be of no particular value in aiding in the solution of the problem of separating a mixture of all of the compounds. Parenthetically it might be added that these detailed studies of developmental behavior could have been accomplished with about one-fifth as many chromatograms if the compounds had been colored.

In the following section each of the different types of developers which had some general utility is discussed briefly and the approximate order of adsorption affinity of the different compounds with this developer is given. Not all compounds were studied with each type of developer but complete data were accumulated for those developers which seemed to be most generally useful. In order that a general idea may be gained of the relative adsorption affinities of the different compounds, a representative development is given for each of the compounds, namely that volume and concentration of developer which will move the zone into the middle third of the column. The width of developed zones was generally equal to about one half of the distance through which the lower edge of the zone had moved. Thus if the lower edge was about 60 mm. below the top of the column the zone was about 30 mm. wide and thus was situated approximately between 30 mm. and 60 mm., or if the lower edge was at 100 mm. the zone was about 50 mm. wide and occupied approximately the middle third of the 150-mm. column.

It must be emphasized that the volume and strength of developer necessary to achieve a particular development may vary somewhat from one lot of adsorbent to another. In general, however, it has been

our experience that any adjustments which must be made in the strength or volume of developer will be about the same in magnitude and direction for all of the compounds. Hence the quantities specified in the "characteristic developments" of each of the compounds in the tables which follow provide a frame of reference which can be expanded or contracted for another lot of adsorbent with somewhat different adsorptive properties. A further brief discussion of the variation in adsorptive properties between different lots of silicic acid is presented in Part III of this Thesis.

(a) Characteristics of benzene as a developer

Pure benzene does not have an appreciable developing action on any of the nitramines except the least strongly adsorbed compounds. The compounds for which it may be used are listed in Table 4. As mentioned above, benzene was used very generally as a diluent for the more polar solvents in the preparation of the mixed developers which are discussed below.

Table 4

Development by Benzene: Order of Decreasing Adsorption Affinity

Characteristic development*	Compound	Comment
10 V ml. of benzene	RDX	All of the other nitramines except CyOx and ATX are adsorbed above RDX
3 V ml. of benzene	CyOx	
V ml. of benzene	ATX	Zone was spread down from the top of the column rather than developed

* Volume of developer which is required to move the zone into the middle third of the column

(b) Characteristics of the ether-benzene developers

The cyclic nitramino compounds (excepting cyclonite oxide) have the rather unusual property of being only very slowly developed by pure ether while the linear compounds are essentially eluted by this solvent. In mixture with benzene, therefore, ether can be used for certain preliminary group separations. The detailed characteristics of ether-benzene developers are presented in Table 5.

Table 5

Development by Ether-Benzene: Order of Decreasing Adsorption Affinity

Characteristic development*	Compound	Comment
	SEX, TAX	Not separable. Concentrations of ether up to 50 percent develop them very slowly
4 V ml. of 50 percent 4 V ml. of 33 percent 3 V ml. of 30 percent	HMX, DPT, RDX	Not separable. High concentrations of ether spread DPT
V ml. of 30 percent	CyOx	
2 V ml. of 20 percent	H-16	
3 V ml. of 8 percent 3 V ml. of 6 percent	AcAn, BSX	Not completely separable

* Volume and concentration of ether in the developer which is required to move the zone into the middle third of the column

(c) Characteristics of the ethyl acetate-benzene developers

Ethyl acetate seems to possess greater developing power than ether when both are used in high concentrations in benzene although at lower concentrations the two are somewhat similar. As shown in Table 6 the order of the adsorption affinities is essentially the same as for ether-benzene.

Table 6

Development by Ethyl Acetate-Benzene: Order of Decreasing Adsorption Affinity

Characteristic development*	Compound	Comment
3 V ml. of 50 percent	SEX	Not completely separable
2 V ml. of 50 percent	TAX	
2 V ml. of 25 percent	HMX	Not separable
2 V ml. of 25 percent	DPT	
2 V ml. of 15 percent	H-16	Not separable
2 V ml. of 12 percent	RDX	
2 V ml. of 8 percent	AcAn	Not separable
2 V ml. of 7 percent	BSX	

* Volume and concentration of ethyl acetate in the developer which is required to move the zone into the middle third of the column

(d) Characteristics of the nitroparaffin-benzene developers

Nitromethane and the two nitropropanes were used as developers in mixtures with benzene. Although at equivalent concentrations in benzene nitromethane is a much stronger developer than are the nitropropanes the different nitroparaffin-benzene mixtures behaved qualitatively very similarly and may all be considered together. The order of adsorption affinities with these mixtures is presented in Table 7; since nitromethane was used more widely than the nitropropanes the characteristic developments are given for nitromethane-benzene mixtures.

The most important feature of these developers is the relative chromatographic sensitivity of RDX toward them, that is, the ease with which RDX may be developed by small percentages of nitroparaffin in benzene. Only ATX and cyclonite oxide are developed more rapidly and all of the remaining compounds are developed much more slowly. This circumstance was of great importance in the final analytical scheme since it provided an effective means of removing the large excess of RDX from most of the possible impurities.

Table 7

Development by Nitromethane-Benzene: Order of Decreasing Adsorption Affinity

Characteristic development*	Compound	Comment
	SEX, H-16, TAX	Not separable. Concentrations up to 50 percent develop them very slowly
2 V ml. of 40 percent	AcAn	
2 V ml. of 40 percent	BSX	Not separable
2 V ml. of 30 percent	DPT	Not completely separable from BSX
2 V ml. of 12 percent	HMX	Not completely separable from DPT
V ml. of 4 percent	RDX	
V ml. of 1 percent	CyOx	

* Volume and concentration of nitromethane in the developer which is required to move the zone into the middle third of the column

(e) Characteristics of miscellaneous developers which contain benzene

The developing action of binary mixtures of acetic acid, dioxane, acetone, and ethanol with benzene was investigated with certain of the compounds. The results will not be described in detail. Mixtures of dioxane with benzene were the most useful, and gave an order of adsorption affinities much like that with ether or ethyl acetate in benzene, although dioxane is a considerably stronger developer than either of these substances. Acetic acid-benzene was of some special value in the separation of BSX from AcAn and of TAX

from SEX but had little general applicability; furthermore it sometimes gave "double zones"* with certain of the compounds and thus was in general avoided. Mixtures of ethanol with benzene were unsatisfactory for a variety of reasons, chiefly because they often caused anomalous spreading or double zone formation. Mixtures of acetone and benzene sometimes behaved similarly, especially with the less strongly adsorbed compounds. Acetone-benzene mixtures can be used quite successfully however with a few of the more strongly adsorbed compounds, and were especially valuable in the separation of DPT and HMX.

(f) Characteristics of developers which contain ligroin

Although many mixtures of polar solvents with ligroin were investigated as developers for the nitramines most of these proved unsatisfactory and therefore they will not be discussed in detail. Development could generally be achieved only with rather high concentrations of the polar solvent in ligroin and complications such as spreading or double-zone formation often accompanied such development. The ligroin-based developers were however very useful in certain isolated cases and in particular they were essential in the chromatography of ATX since this compound is spread badly by benzene. Among the best developers for ATX are a 10-percent solution of ethyl acetate in ligroin and a 10-percent solution of acetone in ligroin.

* We have used the expression "the double zone effect" to describe the formation, from a homogeneous compound, of two well separated zones which cannot be distinguished by other than chromatographic methods and each of which, when eluted and rechromatographed, again produces two zones. See reference 1 for further details.

4. The separation of compounds with very similar chromatographic properties

A consideration of the data presented in Section 3 above will show that there are only a few binary mixtures of any of the nitramines for which a satisfactory chromatographic separation cannot immediately be specified, provided that the chromatographic behavior of each of the individual compounds is not significantly altered by the presence of the other compounds. This important provision appears to be entirely fulfilled in all normal chromatographic work on silicic acid-Celite, not only with these nitramines but also with all of the other compounds which we have studied, at least when the column is not seriously overloaded.

Means of effecting several of the most difficult separations have already been mentioned; these and some others are summarized in Table 8.

It is apparent that if all binary mixtures can be separated a complete separation of a mixture of all of the compounds is theoretically possible although it might prove a tedious and impractical procedure. In actuality, once the developmental properties of all of the compounds had been investigated and the binary separations established it was possible to outline a reasonably simple scheme for the separation of a mixture of all of the compounds. This procedure and the tests which were made of it are described in Section E below.

Table 8

Separation of Some Pairs of Compounds with Very Similar Adsorption Affinities

Satisfactory developer	Compound more strongly adsorbed	Compound less strongly adsorbed
2 percent of acetic acid in benzene	AcAn	BSX
5 percent of ethyl acetate in 1:2 ether-ligroin	AcAn	BSX
6 percent of acetone in benzene	HMX	DPT
1:1 nitropropane-ligroin	DPT	HMX
10 percent of ether in benzene	H-16	AcAn
2 percent of dioxane in benzene	H-16	AcAn
14 percent of acetone in benzene	SEX	TAX
14 percent of dioxane in benzene	SEX	TAX
5 percent of nitromethane in benzene	HMX	RDX
5 percent of acetone in benzene	HMX	RDX

5. Discussion

(a) The threshold effect

A rather striking phenomenon which has been termed the "threshold effect" was first noticed during a series of chromatographic experiments with AcAn in which ethyl acetate-benzene development was used. It was found that as the concentration of ethyl acetate in the developer was increased slowly from one experiment to the next the rate at which the zone of AcAn moved increased fairly slowly and regularly for a given percentage increase in the

concentration of ethyl acetate in benzene up to a concentration of about 9 percent of ethyl acetate. Thereafter the rate of development of the zone of AcAn increased much more rapidly for a given increment in the concentration of ethyl acetate. Thus, an 8-percent solution of ethyl acetate in benzene developed AcAn only about 15 or 20 percent more rapidly than did a 7-percent solution but a developer consisting of 9 percent of ethyl acetate in benzene developed the zone about 50 or 60 percent more rapidly than did the 8-percent solution. Similar though somewhat less striking effects were noted for certain other developers and with some of the other nitramines. During the studies which are reported in Part III of this Thesis several additional examples of the threshold effect were noted, the most striking being those with benzene-ligroin development of 4-nitrotriphenylamine and 4-nitrodiphenylamine.

(b) Generalizations about the chromatographic properties of the nitramines

Certain general relations between the chemical structures and the chromatographic properties of these nitramines can be demonstrated. The most valuable of these will be presented here; they help to summarize and systematize the information which has been presented in this section and they may be of some value in the study of similar compounds of uncertain structure.

On the basis of their general chromatographic behavior RDX and the compounds related to it fall into several classes. One of these classes, that of the linear nitramino acetate esters, includes BSX, AcAn, and H-16. This group is characterized by a relatively high sensitivity to ether-benzene developers (that is, small

percentages of ether produce rapid development) and a relatively low sensitivity to the nitroparaffins. Within this group the adsorption affinity increases as the chain length increases, and also increases when an acetyl group is substituted for a nitro group on a nitrogen atom or on the oxygen atom that is esterified. The latter conclusion follows when ATX is considered in the group.

The increase in adsorption affinity which accompanies the replacement of a nitramino group by an N-acetyl group is evidenced also by the fact that TAX is more strongly adsorbed than RDX and that SEX is more strongly adsorbed than HMX.

In the cyclic nitramino compounds which contain no acetyl group the adsorption affinity increases with increase in the number of nitramino groups or the molecular weight. The compounds in this group, CyOx, RDX, and HMX, are characterized by a sensitivity to developers which contain the nitroparaffins and by a relative insensitivity to those which contain ether. TAX and SEX, in which one of the nitramino groups has been replaced by an N-acetyl group, are extremely strongly adsorbed and show little or no sensitivity to particular types of developers. Little can be said about DPT because it has a rather unique structure; however it does show the strong adsorption affinity of the cyclic compounds.

The above generalizations are of course of limited validity but they can serve to confirm other evidence. For example, the linear structure of H-16 has been considered probable but not definitely established. The developmental characteristics of this compound, and to a lesser extent its behavior with the streak reagents, afford

strong confirmatory evidence of the correctness of the linear formulation.

6. The elution and recovery of the compounds from silicic acid-Celite

The chromatographic investigation of the impurities in RDX is based on the assumption that these substances can be recovered from the silicic acid adsorbent. Therefore the recovery of each of the compounds on elution with 1:1 acetone-ether was studied in at least a roughly quantitative fashion. In addition the recovery of RDX and HMX was investigated quantitatively. The estimation of the quantity of each compound which was recovered was done spectrophotometrically.

The size of the sample which was used varied from 1 to 8 mg. for the different compounds; the details of the experiments will not be given here. It was found that on the lot of adsorbent which was in use at the time of the experiments all of the compounds except SEX and HMX could be recovered essentially quantitatively; the recovery of these two compounds was only about 90 percent. There is some evidence that the percentage of these compounds which is recoverable varies somewhat from one lot of adsorbent to another.

A rather surprising and unusual phenomenon was discovered in the experiments with DPT; the recovery of this compound if the column is eluted immediately after the completion of development is greater than 95 percent but if the compound is allowed to remain on the adsorbent for as long as 10 hours the percentage which can be recovered falls to zero. At intermediate times the fraction which

can be recovered is between these limits. Although such an effect was sought for each of the other compounds (except ATX) in no other case did a detectable dimimution in the recoverable fraction occur when elution was delayed for several hours.

D. The Development of a Procedure for the Concentration of Possible Impurities in RDX

1. Introduction

There are certain limitations in the application of chromatographic-spectrophotometric techniques to the isolation of minute quantities of a compound from large quantities of accompanying material. In particular, if the main constituent of the mixture has a very limited solubility, it may be difficult to chromatograph satisfactorily a large enough sample to permit the isolation of more than a trace of the desired compound. This consideration applies to the present problem, for, as mentioned earlier, the solubility of RDX is very limited in all but a few of the most polar solvents and these solvents are just those which are the strongest developers for RDX and the compounds related to it on columns of silicic acid-Celite.

Preliminary experiments with the samples of RDX which were to be investigated for minor impurities showed that these impurities constituted considerably less than one percent of the samples. Hence it was apparent that some special procedure for concentrating these substances before chromatography was necessary. It was discovered that by fractional precipitation of the major portion (93 to 97 percent) of the RDX from acetone by the addition of 30-60° ligroin the proportion of the impurities in the mixture could be increased to such a degree that these compounds could then be directly separated and estimated. It was of course necessary to test this precipitation procedure with synthetic mixtures of RDX with the other nitramines in order that any losses of the impurities which might occur during the

precipitation procedure could be detected and allowed for. Such losses might be expected because of coprecipitation with the RDX or perhaps merely because the impurity itself had an extremely low solubility in the precipitating mixture.

In the following section are described first a few preliminary considerations and experiments which were necessary in devising the fractional precipitation procedure, then the final form of this procedure, and finally some of the tests which were used in checking the procedure.

2. Preliminary considerations and experiments on the concentration of the impurities

A logical scheme for the classification of the nitramines which were considered as possible impurities in RDX is on the basis of their adsorption affinities relative to RDX. Such a classification was of practical importance in connection with the development of a concentration procedure because the position of the zone of a compound to be detected relative to that of the zone of RDX determined the amount of RDX which could be tolerated in the sample to be chromatographed; this was true because chromatographic zones normally spread as they develop and because the degree of spreading is in large part a function of the quantity of the compound which is on the column. When the compound to be detected was adsorbed above the RDX a relatively large quantity of RDX could be chromatographed because it could all be developed into the lower part of the column or into the filtrate while the other substance remained in the upper part of the column. When however the impurity was less strongly adsorbed than the RDX generally a smaller quantity of RDX could be tolerated

because if too much were present it might spread down into the zone of the impurity.

Consideration of the data on relative adsorption affinities with different developers led us to the conclusion that nitromethane-benzene was the most satisfactory developer for the general separation of the large excess of RDX from the other compounds. This developer not only gave better separations of RDX from most of the compounds but it also placed seven of the nine compounds above RDX; only cyclonite oxide and ATX were less strongly adsorbed. Fortunately these two compounds are appreciably more soluble than the others so that a larger fraction of the RDX could be precipitated without danger of precipitating either of these substances.

It was found that a satisfactory chromatographic separation of several milligrams of any of the more strongly adsorbed compounds from 30 mg. of RDX could readily be achieved with nitromethane-benzene development on a No. 2 column. An appreciably larger quantity of RDX could not in general be permitted because the sample solvent which this would require had too strong a developing action on HMX. In the separation of the two less strongly adsorbed compounds from RDX it was found that the best results were obtained when the sample to be chromatographed contained no more than about 10 mg. of RDX. Consequently two concentration procedures were devised, one for the determination of the more strongly adsorbed compounds and one for the determination of the less strongly adsorbed compounds.

As mentioned above, the fractional precipitation of the RDX was accomplished by the addition of 30-60° ligroin to a solution of

RDX in acetone. This combination of solvents seemed especially suitable since it afforded a wide range of solubilities with almost no complications. The chief problem lay in choosing the proper volumes and conditions for the precipitation; this in turn necessitated a preliminary study of the solubility of RDX and of the other nitramines in acetone and in mixtures of acetone with ligroin. It was found that at room temperature the solubility of RDX in acetone was about 60 mg. per ml.; the addition of three volumes of 30-60° ligroin reduced the solubility to about 1.8 mg. per ml. In 1:4 acetone-ligroin the solubility was decreased to about 0.7 mg. per ml., in 1:6 acetone-ligroin it was about 0.4 mg. per ml. and in 1:8 acetone-ligroin it was only about 0.25 mg. per ml. In addition it was determined that one milligram of SEX, the least soluble of the entire group of compounds, remained entirely in solution when it was dissolved in 3 ml. of acetone and the resulting solution was diluted with 10 ml. of ligroin, and further that both cyclonite oxide and ATX are soluble at least to the extent of 6 mg. in 27 ml. of 1:8 acetone-ligroin.

The quantity of RDX which must be taken originally for any concentration procedure obviously is determined by the percentage of impurities which the RDX contains. Since the total quantity of impurities actually present was known to be appreciably less than one percent, and since about one milligram of any of these compounds is an amount entirely sufficient for positive identification and estimation, the preliminary experiments with the concentration procedures were done with a series of binary mixtures of 500 mg. of RDX with 0.5 mg. of each of the more strongly adsorbed compounds. The

fractional precipitation was effected by dissolving the sample in 11 ml. of acetone and precipitating most of the RDX by the addition of 35 ml. of 30-60° ligroin. The supernatant solution was then decanted from the crystals of RDX and was evaporated to dryness. The residue was chromatographed, the RDX was separated, and the impurity was eluted and estimated spectrophotometrically. When it was found that HMX could not satisfactorily be separated from the amount of RDX in that concentrate, a second precipitation was carried out from 3 ml. of acetone with 10 ml. of ligroin. The results of the experiments are summarized in Table 9; the compounds are grouped in three classes according to the percentage which was recovered. A brief discussion of this grouping is presented later.

Table 9

Recovery of Strongly Adsorbed Compounds from Mixtures with a 1000-fold Excess of RDX

Compound	Quantity added, mg.	Quantity recovered, mg.	Percentage recovered
BSX	0.52	0.45	86
H-16	.55	.47	85
AcAn	.52	.43	83
DPT	.49	.33	68
SEX	.52	.32	61
TAX	.51	.21	41
HMX	.56	.23	41

Several experiments were done on the recovery of cyclonite oxide from mixtures with RDX. Some were concerned with the

reproducibility of results and some with the effect of small variations in the procedure on the percentage which could be recovered. In general the experiments were surprisingly reproducible; the results of duplicate experiments usually agreed within several percent. A recovery of 60 percent was obtained when a mixture of one gram of RDX and one milligram of cyclonite oxide was subjected to a double fractional precipitation, first from 15 ml. of acetone with 60 ml. of ligroin and then from 3 ml. of acetone with 24 ml. of ligroin.

3. The final form of the concentration procedures

In order to increase the sensitivity of the entire analytical procedure so that as little as a few hundredths of a percent of any of the impurities could be detected the precipitation procedures were revised so that a 10-g. sample of RDX could be investigated. For practical reasons a multiple precipitation procedure was necessary; the solubility of RDX decreases sharply when the first ligroin is added to the acetone solution but decreases almost negligibly after about four volumes of ligroin have been added. With 10-g. samples of RDX it was found most satisfactory to employ a three-stage precipitation procedure in order that the final concentrate should contain 10 or 30 mg. of RDX.

(a) Procedure for concentration of the more strongly adsorbed compounds

A 10-g. sample of RDX was dissolved in 150 ml. of warm acetone and partially precipitated by the rapid addition of 600 ml. of 30-60° ligroin. After a short time the supernatant liquid was decanted and evaporated to dryness. The resulting residue was again

fractionally precipitated by dissolving it in 15-20 ml. of warm acetone and adding four volumes of ligroin. The decantation and evaporation were repeated and the residue was dissolved in 4 ml. of acetone and precipitated once more with 13 ml. of ligroin. The final supernatant solution was decanted and evaporated and the resulting crystalline residue was investigated chromatographically by the procedures which are described in Section E.

(b) Procedure for concentration of the less strongly adsorbed compounds

The procedure of fractional precipitation in the preparation of the concentrate for the determination of the compounds less strongly adsorbed than RDX was exactly the same as that for the more strongly adsorbed compounds except that the final of the three precipitations was done using 3 ml. of acetone and 24 ml. of ligroin. This proportion of ligroin to acetone was not used with the concentrate of the more strongly adsorbed compounds because of the possibility of precipitating some of the less soluble compounds.

4. Tests of the final concentration procedures

The preliminary experiments demonstrated the need for testing the concentration procedures. Since losses of the impurities did occur it was necessary to show that substantial fractions of the quantities in the original sample were present in the final concentrates. Furthermore, a knowledge of the fraction which was lost proved to be of value in permitting estimation of the quantity of each compound originally present since it was found that the fraction of a compound which was lost appeared to be approximately independent of the concentration of the compound in the original sample, at

least when this original concentration was no greater than one-tenth of one percent, the maximum concentration investigated.

(a) Tests of the concentration procedure for the less strongly adsorbed compounds

The recovery of cyclonite oxide in the concentration procedure for the less strongly adsorbed compounds was investigated for a series of samples in which various amounts of cyclonite oxide were present. The results are summarized in Table 10.

Table 10

Recovery of Cyclonite Oxide from Mixtures with a 2000- to 100,000-fold Excess of RDX

Original mixture	Quantity of CyOx recovered, mg.	Percentage recovered
10 g. RDX plus 5.0 mg. CyOx	2.7 2.8*	54 56*
10 g. RDX plus 3.0 mg. CyOx	1.6	53
10 g. RDX plus 0.1 mg. CyOx	ca. 0.05	ca. 50

* Duplicate experiment

These results demonstrate that the percentage recovered is reproducible and approximately independent of the original quantity of "impurity" in the mixture.

Two corresponding experiments were done with ATX; 60 percent was recovered from a mixture of 1.8 mg. with 10 g. of RDX and about 50 percent from a mixture of 0.1 mg. with 10 g. of RDX. Incidentally it was demonstrated conclusively that ATX is entirely stable in

acetone solution and can be recrystallized from acetone without change in spectrophotometric or chromatographic properties. It had been claimed by other workers that ATX is changed into a substance of unknown composition on recrystallization from acetone but the sole evidence for the reported transformation was a lowering of the melting point. Since we have found that the melting point of ATX is not sharp and varies appreciably with the rate of heating, it does not appear to us that any weight can be attached to this evidence alone.

(b) Tests of the concentration procedure for the more strongly adsorbed compounds

The final concentration procedure was not tested for all seven of the more strongly adsorbed compounds since when it was tested with three of these substances, one from each of the three categories represented in Table 9, the recoveries were just about those to be expected on the basis of the preliminary experiments summarized in Table 9. The results of the investigations are presented in Table 11.

Table 11

Recovery of AcAn, DPT, and HMX from Binary Mixtures with a Large Excess of RDX

Compound	Original amount, mg.	Amount recovered, mg.	Percentage recovered
(a) Mixture of about 2.5 mg. of the compound with 10 g. of RDX			
AcAn	2.54	1.96	78
DPT	2.68	1.50	56
HMX	2.46	0.71	29
(b) Mixture of about 0.5 mg. of the compound with 0.5 g. of RDX (from Table 9)			
AcAn	0.52	0.43	83
DPT	.49	.33	68
HMX	.56	.23	41

In the new experiments reported in Table 11 the three compounds showed respectively the good, fair, and poor recovery that characterized the three groups that they were chosen to represent. Furthermore, the fact that the recoveries were slightly lower than in the earlier experiments is to be expected since the 10-g. samples required three precipitations instead of the one or two which were used in the earlier tests. The good agreement of all of these experiments constitutes a satisfactory background for interpreting the results of a qualitative analysis and demonstrates as well that the procedure is satisfactory.

(c) Discussion of the causes of the losses of impurities in the concentration procedure

The results of the tests of the preliminary forms as well as of the final forms of the concentration procedures have been

presented because of their interesting implications. Additional experiments, which will not be described, showed that although the work was done with quantities as small as one-half milligram, mechanical losses, losses due to insolubility of the compound, and losses in the chromatographic procedure were not responsible for the incomplete recovery.

The three recovery-groups into which the more strongly adsorbed compounds fall differ significantly with regard to the structures of the compounds which comprise them. The three compounds which were recovered in highest yield, BSX, AcAn, and H-16 are linear and do not resemble RDX closely in structure, while, of the other four compounds, all cyclic, the two recovered in lowest yield are the most similar to RDX structurally. These facts suggest that perhaps mixed crystal formation with the RDX was chiefly responsible for the loss.

E. Chromatographic-Spectrophotometric Procedures for the Isolation and Estimation of Minor Impurities in RDX

1. Introduction

The information and experience which were gained in the detailed study of the chromatographic behavior of the individual compounds served as a basis for the development of a systematic procedure for the analysis of a mixture of all of the compounds. This procedure was devised so that all seven of the compounds adsorbed more strongly than RDX could be isolated from a single concentrate and likewise so that the two compounds adsorbed less strongly could be isolated from a second concentrate. Although a chromatographic procedure for the isolation of all nine of the compounds from one concentrate could have been worked out it would have been less practical; furthermore, as discussed in Section D, it was desirable to employ slightly different precipitation procedures for the preparation of the final concentrates for the two classes of compounds so that there was no need for a single analytical scheme for the determination of all nine of the compounds from a single sample.

After the isolation of an impurity, an approximate estimation of the amount which was present was made spectrophotometrically or chromatographically as described below. This datum, together with the estimated loss of the compound during the concentration procedure, permitted a calculation of the approximate amount of the compound in the original sample.

The following section contains descriptions of the chromatographic procedures which were used for the isolation and estimation

of the impurities in the concentrates, together with a brief description of the tests which were made of these procedures with known mixtures, and a discussion of the sensitivity of the entire analytical scheme.

It must be emphasized again that the amounts and concentrations of developer which are specified in the detailed descriptions of the chromatographic procedures may need to be varied somewhat for other lots of adsorbent. Even when the same lot of adsorbent is used for all of the work the strength of this adsorbent should be checked at intervals by chromatographing known mixtures in order to make certain that a specified zone will contain only the expected compound.

2. Chromatographic analysis for compounds which are adsorbed more strongly than RDX

(a) Procedures for the isolation and detection of the compounds

SEX, TAX, DPT, HMX, H-16, AcAn, and BSX are more strongly adsorbed than RDX with nitromethane-benzene development. The procedure which was finally adopted for the analysis of a mixture of these compounds with RDX involved five chromatograms. The procedure is depicted schematically in Figure 1. In the first chromatogram, Column A, the RDX was completely removed with nitromethane-benzene development and the other compounds were separated into two groups, the more strongly adsorbed of which (Zone A-1) contained SEX, TAX, H-16, and part of the AcAn, while the other (Zone A-2) contained HMX, DPT, BSX, and the remainder of the AcAn. Zone A-2 was rechromatographed and separated into two groups of two compounds each, the more strongly adsorbed zone containing HMX and DPT and the other BSX and

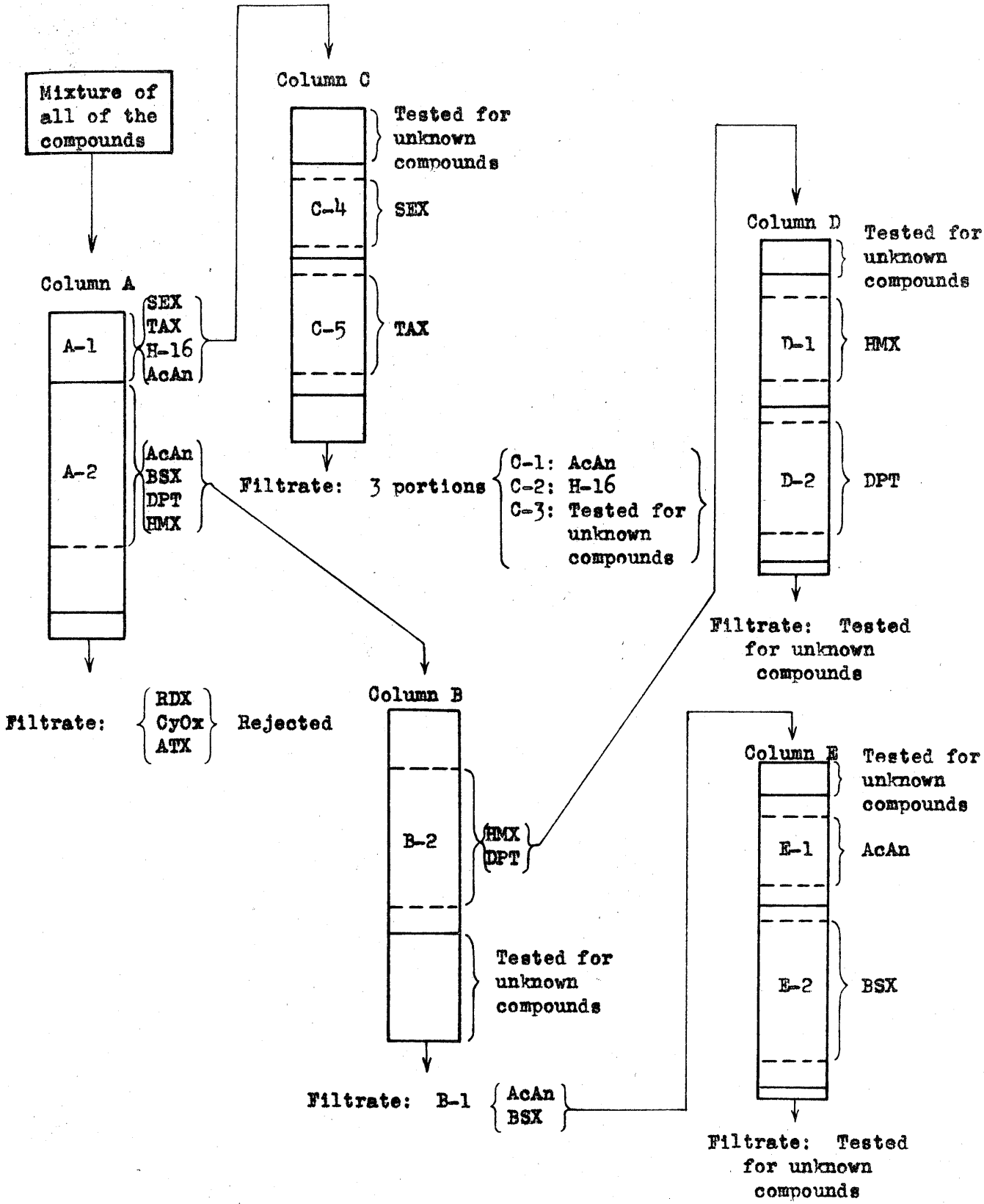


Figure 1 Schematic Diagram of the Chromatographic Procedure for the Isolation of the Compounds Adsorbed More Strongly than RDX

– – – – Limits of Zone ——— Positions of Cuts

AcAn. These two mixtures were then chromatographed separately with developments appropriate for the separation of their constituents. Meanwhile Zone A-1 was rechromatographed and by a "liquid chromatogram" was separated into its four components in one operation.

(i) Column A. Removal of RDX and preliminary separation of other constituents

The residue from the fractional precipitation procedure which was described in Section D 3 (a) was dissolved in 2 ml. of nitromethane and the solution was diluted with 8 ml. of benzene just before it was placed on a 15-cm. No. 2 column. After the chromatogram had been developed with 3 V ml. of a 7-percent solution of nitromethane in benzene and postwashed with 1.5 V ml. of ligroin, the column was cut at a point 30 mm. from the top and again at 135 mm. The uppermost section contained all of the SEX, TAX, H-16, and part of the AcAn (Zone A-1); the portion of the column between 30 mm. and 135 mm. contained all of the BSX, DPT, and HMX, and the remainder of the AcAn (Zone A-2). The bottom 15-mm. section of the column was rejected.

Zones A-1 and A-2 were eluted at once with 1:1 acetone-ether and the eluent was then removed by evaporation under reduced pressure. Approximately 5 ml. of redistilled absolute ethanol was added to each of the residues and was then removed by evaporation <u>in vacuo</u>. The residues were rinsed into the bottom of the flasks with acetone and after the acetone had been evaporated in a slow stream of air, the residues were subjected to the full suction of an aspirator for about five minutes.

The procedure described in the preceding paragraph was used throughout the investigation in the preparation of zones for rechromatography or spectrophotometric examination.

(ii) Column B. Separation of BSX and AcAn from DPT and HMX

The material which was present in Zone A-2 was dissolved in one milliliter of nitromethane and diluted with 4 ml. of benzene just before it was placed on a 15-cm. No. 2 column. The chromatogram was developed with 2 V ml. of 1:2 ether-benzene; after 0.6 V ml. of the developer had entered the column the receiver was changed and the filtrate was collected in a clean flask. The column was postwashed with 1.5 V ml. of ligroin; after 1.2 V ml. of the postwash had entered the column the filtrate was no longer saved. The filtrate which was collected contained all of the AcAn and BSX which were present in Zone A-2; it was evaporated to dryness and prepared for rechromatography in the usual fashion. The residue so obtained was referred to as sample B-1.

Meanwhile the material adsorbed in the portion of the column above a point 110 mm. from the top (Zone B-2) was eluted with 1:1 acetone-ether and prepared for rechromatography. The lower 40-mm. section of the column was streaked with the benzene-Franchimont reagent. Although none of the known compounds would be expected to be present in this portion of the column the possibility remained that some compound which had not previously been encountered might be adsorbed there.

Zone B-2 was eluted as soon as possible after completion of the chromatogram because the percentage recovery of DPT on elution is

known to decrease when an adsorption complex which contains this compound is allowed to stand[5]. This precaution was observed throughout the investigation in the elution of all of the zones which might have contained DPT.

(iii) Column C. Isolation of SEX, TAX, H-16, and part of the AcAn

The material which was present in the residue from the eluate of Zone A-1 was dissolved in one milliliter of nitromethane and diluted with 4 ml. of benzene just before it was placed on an 18-cm. No. 2 column. The chromatogram was developed with 1.7 V ml. of a 15-percent solution of ethyl acetate in benzene and 2.5 V ml. of a 14-percent solution of acetone in benzene, and was postwashed with 1.3 V ml. of ligroin. The filtrate from this chromatogram was collected in three separate but consecutive portions. The first portion, designated as C-1, was collected from the time when 0.6 V ml. of the ethyl acetate-benzene solution had entered the column until 0.7 V ml. of the acetone-benzene solution had entered; this filtrate contained all of the AcAn present in Zone A-1. The second portion of the filtrate was collected until 0.2 V ml. of the postwash had entered the column; this second fraction, referred to as C-2, contained all of the H-16 present. The remainder of the filtrate from the chromatogram was collected in order that any unknown compound which might be present in it would not escape detection; this fraction was called C-3.

The chromatogram was cut at 30 mm., 80 mm., and 160 mm.; the section from 30 to 80 mm. (C-4) contained all of the SEX present while that between 80 and 160 mm. (C-5) contained all of the TAX. These

portions of the column were eluted and the eluates were evaporated in the usual manner. The chromatographic filtrates were evaporated similarly.

The upper 30-mm. section of the column was streaked with the benzene-Franchimont reagent so that the presence of any unknown nitramine which was more strongly adsorbed than SEX could be detected.

(iv) Column D. Isolation of DPT and HMX

The material which had been adsorbed in Zone B-2 was chromatographed on a 15-cm. No. 2 column from 5 ml. of 1:4 nitromethane-benzene. After development with 2 V ml. of a 6-percent solution of acetone in benzene the chromatogram was postwashed with ligroin. The column was cut at 15 mm. and 75 mm. from the top and the portion between these cuts (Zone D-1) was eluted and evaporated; it contained any HMX which was present. The remainder of the column, with the exception of the bottom 5 mm., was eluted and the eluate was prepared for rechromatography or spectrophotometry in the usual way; it contained any DPT which was present, and was designated sample D-2.

The filtrate from column D was collected and evaporated to dryness in the usual fashion; the upper 15 mm. of the column was streaked with the benzene-Franchimont reagent in order that any unknown compounds present would not escape detection.

(v) Column E. Isolation of BSX and the remainder of the AcAn

The residue from the evaporation of the filtrate from chromatogram B (sample B-1) was chromatographed on a No. 2 column from 5 ml. of 1:4 nitromethane-benzene. The sample was rinsed onto the column with an additional 5 ml. of 1:4 nitromethane-benzene and was

then developed with 1.8 V ml. of a one-percent solution of acetic acid in benzene. After the chromatogram had been postwashed as usual with 1.5 V ml. of ligroin, it was cut at three points, 15 mm., 65 mm., and 145 mm. from the top. The portion of the column between 15 and 65 mm. (Zone E-1) contained all of the AcAn which was present in sample B-1 and the section from 65 to 145 mm. (Zone E-2) contained all of the BSX. Both sections were eluted and the eluates were evaporated in the usual fashion.

The filtrate from column E was collected and evaporated; the upper 15 mm. of the column was streaked with the benzene-Franchimont reagent in order that any unknown nitramines which were present would not escape detection.

(b) Methods of estimating the amounts of the compounds isolated; sensitivity of the analytical method

Spectrophotometry can be used for the estimation of these nitramines in the residues from the chromatographic procedures only if more than about one-half milligram of any of the compounds is present, because at lower concentrations background absorption due to impurities from the adsorbent usually obscures the absorption maxima, which occur at very short wave lengths. If spectrophotometric estimation is applicable the methods mentioned in Section B 2 can be used; a blank correction should be applied.

If however less than about one-half milligram of the impurity is present chromatographic methods must be used for the detection and rough estimation of the compound. The residue from the evaporation of the eluent is dissolved in 10 ml. of 1:10 nitromethane-benzene, and is then placed on a 12-cm. No. 1.5 column and washed with ligroin. The

benzene-Franchimont or Schryver streak is applied to the column and the presence or absence of any compound which reacts with these reagents is thus demonstrated in a remarkably sensitive and reliable manner. Tests have shown that 0.1 mg. of any of the compounds produces a strong color under these conditions and that as little as 0.02 mg. can readily be detected. The actual estimation of the quantity of a given compound which has been isolated may be made colorimetrically by comparison of the intensity and extent of the streak color which it produces with those produced by various known quantities of the same compound under identical conditions.

The problem of deciding whether or not a given residue from the evaporation of a chromatographic eluate or filtrate contains sufficient material for reliable spectrophotometric estimation is one which can be solved only with experience. Traces of silicic acid are sometimes carried into the chromatographic filtrate and give the residue the appearance of containing more crystalline material than is actually present. On the other hand some of the compounds crystallize only with difficulty; a few tiny drops remaining after evaporation of the solvent sometimes contain appreciable amounts of one of these compounds. When there is doubt about the feasibility of spectrophotometric estimation the most satisfactory procedure is to measure the optical density of a solution of the residue in the ordinary fashion and then, if the optical density is too low, to evaporate the solution to dryness and estimate the residue by the colorimetric streaking procedure.

After the amount of the impurity which has been isolated has

been estimated, the proper corrective factor may be used to calculate the approximate amount of the substance in the original sample. The value of the factor for each compound is listed in Table 12. It must be emphasized that these factors are only approximate; they are, however, entirely adequate for the estimation of trace quantities.

Table 12

Approximate Factors by Which the Quantity of Each Compound Actually Isolated Should Be Multiplied in Order to Obtain an Estimate of the Amount Present in the Original 10-g. Sample

Compound	Factor
H-16	1.3
BSX	1.4
AcAn	1.6
SEX	2.2
DPT	2.2
TAX	3.5
HMX	3.5

The sensitivity of the entire analytical method can be deduced from the sensitivity of detection of the individual compounds with the streak reagents and the approximate losses in the concentration procedures. The overall sensitivity of the method calculated in this manner ranges from about 0.07 mg. for HMX to about 0.03 mg. for H-16 in the original 10-g. sample of RDX. Such a very high sensitivity does have real significance as numerous tests and checks on the procedures demonstrated, although it is perhaps considerably higher than is necessary or even of practical interest.

(c) Tests of the reliability of the analytical procedure

The chromatographic procedures are the essential part of

the analytical methods for both the more strongly and less strongly adsorbed impurities. These compounds are colorless, of course, and moreover, columns which are used for analytical purposes are normally best left unstreaked. The quantitative isolation of the compounds is dependent upon the uniform chromatographic behavior of the given impurities. When these impurities occur only in traces, in insufficient amounts for confirmation of the identification by routine methods, the fact that a substance is isolated from a given chromatographic zone is the only evidence available as to its identity.

A test of the entire chromatographic procedure with a mixture of 26 mg. of pure RDX and 6 mg. of pure HMX showed that neither of these substances, usually the two most abundant, contaminated any of the regions occupied by the zones of the other compounds. Furthermore each step of the entire procedure was tested with mixtures of the compounds which were to be separated in that particular chromatogram and the final specifications allowed a margin of safety for slight variations in the positions of the zones.

Actually, for the qualitative procedure to be reliable, the procedure must be roughly quantitative. The recovery of the compounds when they were present in quantities of about a milligram was tested experimentally by analyzing a mixture of 30 mg. of RDX and 1 mg. of each of the strongly adsorbed compounds. The results of the experiment are presented in Table 13.

Table 13

Results of the Analysis of a Synthetic Mixture Which Contained 30 mg. of RDX and 1 mg. of Each of the More Strongly Adsorbed Compounds

Compound	Percentage recovered
SEX	82
TAX	91
H-16	96
AcAn	76
BSX	86
DPT	84
HMX	102

On the whole, the percentage of each compound which was recovered was about what would have been expected if one considers the sizes of the samples and the length of the procedure which was involved. The low recovery of AcAn is not at all anomalous; the procedure of column E, part (a) of this section, was not entirely satisfactory but the AcAn which was lost did not interfere with the other tests. The loss of AcAn was taken into account in calculating the approximate factors listed in Table 12.

3. Chromatographic analysis for compounds which are adsorbed less strongly than RDX

(a) Procedures for the isolation and detection of the compounds

A schematic diagram of the chromatographic procedure for the isolation of the compounds adsorbed less strongly than RDX is shown in Figure 2.

(i) Isolation and detection of ATX

Column I A Isolation of ATX. - For the "liquid chromatogram" by which ATX was isolated, the residue from the final step in

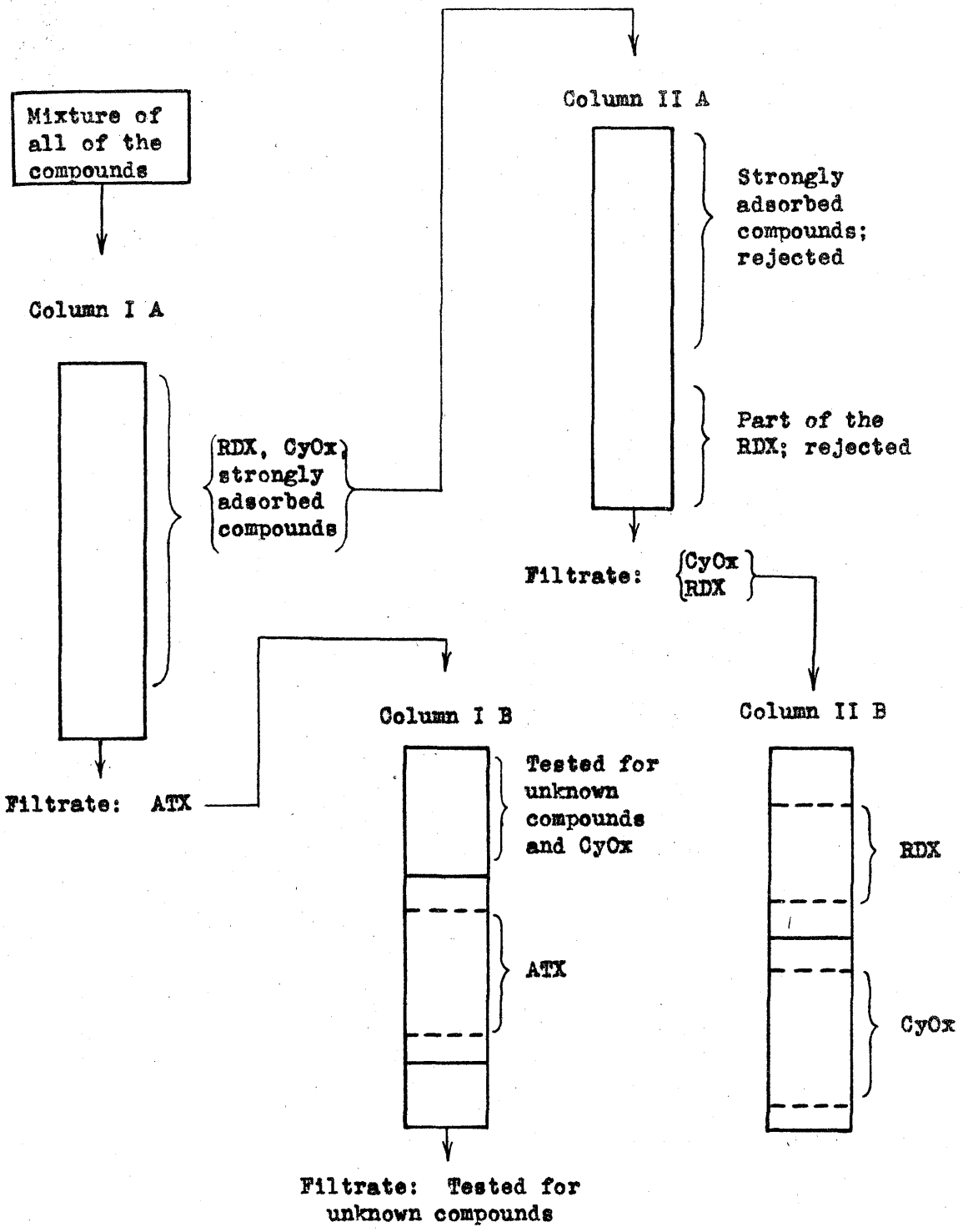


Figure 2 Schematic Diagram of the Chromatographic Procedure for the Isolation of the Compounds Adsorbed Less Strongly than RDX

_ _ _ _ Limits of Zone ——— Positions of Cuts

the concentration procedure of Section D 3 (b) was dissolved in one milliliter of warm 2-nitropropane; this solution was diluted with 4 ml. of benzene and chromatographed on an 18-cm. No. 2 column. The sample was developed with a total of 1.5 V ml. of a 6-percent solution of ethyl acetate in benzene and 1.5 V ml. of 30-60° ligroin. Since the ATX was washed through the column with the sample solvent and the first portions of the developer, the receiver was changed after the first 10 ml. of the developer had passed into the column, and the rest of the filtrate was collected in a clean flask. The filtrate was evaporated under reduced pressure, and the residue was rechromatographed on Column I B.

RDX, cyclonite oxide, and the strongly adsorbed impurities remained on the column when the ATX was washed into the filtrate. The column was therefore eluted with 150 ml. of 1:1 acetone-ether, the eluent was evaporated, and the residue was used in the analysis for cyclonite oxide, Column II A.

If the sample contains an unusually large amount of cyclonite oxide, a trace of the compound may be washed into the filtrate with the ATX. Its presence in the filtrate makes no difference because it will be removed when the ATX is purified in Column I B; this trace of cyclonite oxide could be recovered from the upper part of Column I B, but the fraction of the total amount is so small that it is of no consequence as far as the analysis for cyclonite oxide is concerned.

Column I B Purification of ATX. - The ATX isolated in the filtrate of Column I A was purified by rechromatographing the residue on an 18-cm. No. 2 column by the following procedure. The residue,

dissolved in 5 ml. of 1:4 dioxane-ligroin, was developed with 2.2 V ml. of an 11-percent solution of ethyl acetate in ligroin and 1.5 V ml. of 30-60° ligroin. As in the first chromatogram, the filtrate was collected after the first 10 ml. of the developer had entered the column because if any very weakly adsorbed impurities had been present in the original sample, they would have been washed through Column I A with the ATX, and therefore would have been present with the ATX in the present sample. They presumably would have been washed through Column I B also, and therefore the residue from this filtrate was examined for any substance which might give a positive nitramine test (Column I C).

With the above development, the ATX was found in the zone from 70 to 135 mm. from the top of the column; the treatment to which this zone was subjected was determined by the amount of the compound which was present. A one- to two-milligram sample was sufficiently large that spectrophotometric measurements could be used to confirm the identity of the substance and to make a quantitative estimation of the amount which was present. If less than a milligram of ATX was present, an approximate estimation was made by a colorimetric streaking procedure similar to that used for the more strongly adsorbed compounds. The approximate amount of ATX on Column I B was determined by means of a special method of streaking; the procedure was designed to avoid loss of adsorbent and contamination by streak reagent. The upper 60 mm. of the column was streaked in the usual fashion; in the lower section, where ATX was expected to be present, small flakes of adsorbent were taken from the column at 90, 110, and

170 mm. from the top, removed to a porcelain spot plate, and treated with the usual benzene-Franchimont streak. Approximately one milligram on the column would be required to give a positive test under these conditions; therefore the results of these tests indicated the proper subsequent procedure for treating the residue which was obtained by elution of this zone. Sometimes a positive reaction was obtained in the upper section, about 20 mm. from the top of the column. This zone was shown to be cyclonite oxide and was present only when large amounts of cyclonite oxide were present on Column I A. As mentioned above, under these conditions a trace of cyclonite oxide was washed into the filtrate with the ATX.

Column I C Detection of any very weakly adsorbed compounds. - Any very weakly adsorbed nitramines which might have been present in the filtrate from Column I B were detected as follows. The residue from evaporation of the filtrate was dissolved in 2.5 ml. of 1:4 dioxane-ligroin, placed on a No. 1.5 column, and washed with 0.6 V ml. of 28-38° ligroin. The column was streaked with the benzene-Franchimont reagent. The sections into which the sample solvent had moved as well as the upper part of the column were examined closely for any positive nitramine reaction.

(ii) Isolation and detection of cyclonite oxide

Column II A Preliminary purification of cyclonite oxide. - The mixture of cyclonite oxide, RDX, and the more strongly adsorbed compounds which was eluted from Column I A was first treated to remove the more strongly adsorbed compounds. The residue was dissolved in 5 ml. of 1:4 nitromethane-benzene and placed on a 10-cm. No. 2 column.

The development which was used, 1.5 V ml. of a 5-percent solution of nitromethane in benzene and 1.5 V ml. of 30-60° ligroin, washed the cyclonite oxide and most of the RDX into the filtrate. The procedure was therefore run as a "liquid chromatogram", and the filtrate was collected after the first 5 ml. of developer had entered the column. The residue from evaporation of the filtrate was rechromatographed on Column II B.

Column II B Final purification of cyclonite oxide. - The cyclonite oxide was separated from the last contaminant, RDX, by rechromatographing the residue from the filtrate in Column II A. The sample was dissolved in 8 ml. of 1:4 pyridine-ligroin; the initial solution in pyridine was warmed before the ligroin was added in order to prevent precipitation of any of the sample. The separation was accomplished by development with 1.3 V ml. of 1:1 ether-benzene and 1.3 V ml. of 30-60° ligroin. The cyclonite oxide was found in the zone 105 to 175 mm. from the top of the column; the zone of RDX extended down to about 80 mm. from the top. The subsequent procedure used for the investigation of cyclonite oxide depended upon the absolute amount which was present, just as in the case of the ATX, and therefore the same procedure of streaking was used. The usual benzene-Franchimont streak was applied down to 85 mm. from the top of the column so that the bottom of the zone of RDX was indicated. The cut for the separation was made 10 mm. below the RDX, and the entire cross-section at this point was tested to detect any possible distortion. Flakes of adsorbent were removed at 95, 125, 150, and 175 mm. from the top of the column and tested on a spot plate. A

positive reaction indicated the presence of a milligram or more of cyclonite oxide, and in that case the cyclonite oxide was identified and estimated spectrophotometrically. If the spot tests were negative, the very sensitive chromatographic method was used to detect and roughly estimate the amount of cyclonite oxide that was present. In either case, the zone below the RDX was eluted with 150 ml. of 1:1 acetone-ether, the eluent was evaporated, and the residue was either taken up in alcohol for spectrophotometric examination or prepared for rechromatography.

(b) Methods of estimating the amounts of the compounds isolated; sensitivity of the analytical method

The problem of estimating the amounts of the less strongly adsorbed impurities which are isolated is essentially the same as for the more strongly adsorbed compounds. Amounts too small to be estimated spectrophotometrically can be chromatographed on a No. 1.5 column from 2.5 ml. of 1:4 dioxane-ligroin and washed with 0.6 V ml. of 28-38° ligroin. The column is streaked with the benzene-Franchimont reagent, and the color produced by the zone of ATX or cyclonite oxide at the very top of the column is compared with that produced by known quantities of the same compounds under identical conditions.

After a rough quantitative estimation of the amount of the impurity which is isolated has been obtained, an approximate calculation of the original amount of the compound in the sample may be made because the loss during concentration is known. As with the strongly adsorbed compounds, the fact that in experiments with artificial mixtures the fraction recovered was constant over wide variations in

the proportion of the minor constituent to RDX makes the calculation reasonably reliable. In order to calculate the approximate original content of ATX or cyclonite oxide in the 10-g. sample the amount isolated should be multiplied by 1.8.

The limits on the sensitivity of detection of cyclonite oxide and ATX can be deduced from the limiting amount which is detectable with the benzene-Franchimont streak reaction. The smallest quantity of cyclonite oxide which can be detected is about 0.01 mg.; for ATX the minimum detectable quantity is about 0.008 mg. Thus when the loss during concentration is considered these figures mean that 0.02 mg. of either of these compounds can be detected in the original 10 g. of RDX.

4. Identification of the compounds which are isolated

A number of experimental methods can be used to advantage in establishing the identity of the compounds which are isolated. These methods include investigation of the chromatographic behavior, streak reactions, melting points and mixed melting points, and ultraviolet absorption spectra. Of course these techniques are only useful in demonstrating that the isolated nitramine is identical with or different from one of the known compounds.

These methods may be divided into two classes: all of them may be used when the amount of the compound isolated is of the order of one milligram or more; only the first two are of practical value when only a few hundredths of a milligram is isolated. It is highly probable that only in the former case can the impurity be considered to be present in significant quantity in the original 10-g. sample,

and in such a case the identification can be established unambiguously.

A certain degree of specificity is possessed by the Schryver streak test. The sensitivity of H-16, AcAn, BSX, and DPT is approximately the same for both the Schryver streak and the benzene-Franchimont streak, but HMX, RDX, SEX, and TAX do not react with the Schryver reagent. This specificity of reaction to the Schryver test may be used to check that a particular nitramine which is isolated has the reactions of the class to which it presumably belongs.

The most reliable evidence for the identification of any of the substances which are present only in traces is the chromatographic behavior. It is for this reason that it was necessary to check the chromatographic procedures rigorously before they were actually applied to the analysis of samples of RDX so that a particular region of one of the chromatograms which was supposed to contain only a specific compound or group of compounds could definitely be assumed to contain all of the specified compound or compounds and no other known substances. Of course the possibility exists that some unknown nitramine with essentially the same properties as one of the known compounds might appear in the zone supposed to contain only that known compound, but the probability of this occurrence is greatly decreased by the fact that several successive chromatograms are used in the isolation of each compound and that a different developer is used in each of these chromatograms.

Summary

The application of chromatographic-spectrophotometric methods to the development of a procedure for the isolation and estimation of about ten minor impurities in RDX is described. This development included a detailed study of the chromatographic behavior of RDX and of nine similar nitramino compounds which were considered to be possible impurities in RDX, and also of methods for the isolation of the components of a mixture of any or all of these substances. In addition a method was devised for concentrating the impurities when they occurred in amounts too small to be detected directly in the original sample.

Rigorous tests and checks were applied to all phases of the analytical procedure. The procedure is suitable for the detection and approximate estimation of as little as a few parts in a million of any of the known compounds. In addition provision has been made for the detection of hitherto unknown substances.

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5. See page 50 of this Thesis

Part III

An Experimental Study of Chromatography on Silicic Acid-Celite Columns

Part III. An Experimental Study of Chromatography on Silicic Acid-Celite Columns

A. General Introduction

Despite the extremely widespread use of and interest in Tswett's chromatographic method[1], the fundamental factors which are responsible for the remarkable successes of this technique are still only partially understood. One of the chief reasons for the lack of understanding is the paucity of pertinent experimental data. During the past seven years, since Wilson[2] first proposed a theory of chromatography, there have been many papers[3-7] dealing with the theory of chromatography, but there have been comparatively few more than casual experimental investigations of the theories which have been proposed. The general aim of the work which will be described here was the collection of data which could aid in obtaining an understanding of the chromatographic process, in particular as applied to columns of silicic acid-Celite.

The most careful and thorough experimental study of the fundamental factors involved in chromatography was that of Weil-Malherbe[8], who studied a few simple systems of one adsorbent, one solvent, and one adsorbed substance by means of analyses of the filtrate from the column, and found in general fairly good agreement with the theory proposed by

Weiss[4] (which is almost identical with that of DeVault[3]). Jacobs and Tompkins[9] investigated in some detail the relation between adsorption isotherms and the position, rate of advance, width, and elution curves of zones of some inorganic substances on alumina and found fair agreement in some respects with the extant theories of chromatography, although there were many complications. Cassidy and Wood[10] and LeRosen[11c] have also attempted to correlate experimental data with the theories in two rather specialized cases. In addition, several attempts have been made to find a correlation between the adsorption isotherms and relative chromatographic positions of a group of compounds; for example, Lottermoser and Edelmann[12] studied the chromatography and the adsorption isotherms of aliphatic amines and amino acids on alumina from aqueous solution and Cassidy[13] made a similar investigation of four fatty acids on a variety of adsorbents from petroleum ether and ligroin. Each of these authors reached the conclusion that a study of adsorption isotherms alone was of uncertain value in the prediction of the behavior of a compound on the chromatographic column.

The discussion which has been presented so far has been limited to those papers which deal with the usual form of chromatography, essentially as originally proposed by Tswett, and has not included a consideration of the two chief modifications which have been made in this technique. The "partition chromatogram" of Martin and Synge[14], which utilizes differences in the partition coefficients between some pair of solvents of the substances to be separated, has already achieved remarkable success. A theory of this method was developed and tested by Martin and Synge and was found to give good agreement with experiment.

It should be noted that although no adsorption process is involved in this form of chromatogram, the assumption by Martin and Synge that the partition coefficient was independent of concentration is formally identical with the assumption of a linear adsorption isotherm. The expression which they derived for the rate of movement of the zone can be shown to be identical with that which is obtained for a linear isotherm in the usual form of chromatography[2,3,4]. I believe that its success in predicting the rate of movement of zones in partition chromatograms may be taken as much as a justification of the theory for the usual form of chromatography with adsorption according to a linear isotherm (and idealized conditions) as a verification of the theory of Martin and Synge, which was derived from a quite different, and not entirely unquestionable, approach.

Probably the most brilliant advances in the technique of adsorption analysis have been those made by Tiselius and his collaborators, notably Claesson[15,16]. The elegant methods which they have developed, namely frontal analysis and displacement development, differ in some essential details from the usual Tswett method. Both techniques depend upon an analysis of the filtrate. In frontal analysis the solution to be analyzed is poured continuously through the column until a "front" of each of the components present has appeared in the filtrate; that is, no development, in the usual sense, is ever achieved. In displacement development the sample to be analyzed is developed down the column in such a way that the zones of the individual components remain always contiguous although not overlapping. These methods are especially suited for the qualitative and quantitative analysis of mixtures of the

members of a homologous series or other closely related substances. The theories of the methods are on a very satisfactory basis; they can be derived in a fairly simple and straightforward fashion and have been tested extensively and found entirely adequate[16]. Because of the nature of these methods, the theories are considerably simpler than those of ordinary chromatography, which is termed by Tiselius "elution analysis"; hence, unfortunately, these theories offer no direct aid in the evaluation of the theories of the Tswett chromatographic procedure except insofar as they demonstrate that simple reasoning on the basis of measured adsorption isotherms can under certain circumstances lead to the correct picture.

Silicic acid-Celite mixtures have proved to be eminently satisfactory for the chromatography of a wide variety of organic substances, as described in Part II of this Thesis and the first reference given there. Silicic acid or silica gel has of course been used as a chromatographic adsorbent by many other workers and it is our belief that its use will increase in the future as its merit becomes more generally recognized. For this reason it was felt that studies which would lead to an understanding of the chromatographic processes as applied to columns of silicic acid-Celite would be of particular value.

The first problem which was considered was that of obtaining an adsorbent of uniform and reproducible adsorptive strength; for this purpose some means of characterizing the adsorptive properties of the silicic acid-Celite mixtures was needed. Several methods have been suggested for the characterization of chromatographic adsorbents. They may conveniently be classified as follows:

(a) determination of the approximate positions of certain dyes when chromatographed under specified conditions (Brockmann and Schodder[17]);

(b) determination of the rate of advance of a zone of a suitable compound when chromatographed under specified conditions (LeRosen[11a,b]); or, similarly, the determination of the volume of developer just necessary to develop a given compound into the filtrate (the "threshold volume") under specified conditions (Weil-Malherbe[8]);

(c) determination of the heat of wetting of the adsorbent with a suitable solvent (Müller[18]);

(d) determination of the percentage of some compound which is adsorbed by a given quantity of adsorbent under specified conditions (Trappe[19b]);

(e) determination of certain fundamental constants involved in the relation between the constants of the adsorption isotherms of the members of a homologous series (Claesson[16]).

The methods listed under (b) above are really merely an elaboration of the method of Brockmann and Schodder but since they provide a more reasonable quantitative criterion they are listed separately. A method similar to that of LeRosen was adopted as the simplest and most practical technique for the characterization of adsorbent throughout the present work.

It has long been established that the adsorptive strength of silicic acid or silica gel, as well as of alumina[17] and some other adsorbents, is a function of the water content[20,19]. Because the

prewashing treatment which had been devised in connection with the work described in Part II of this Thesis was known to increase markedly the adsorptive strength of silicic acid-Celite and to render this adsorbent more uniform it was suspected that this prewash removed water from the adsorbent. An investigation was therefore carried out of the comparative activating effects of prewashing and of heating at 200° for one hour and it was found that these two procedures were generally nearly identical in their effect although for certain weakly adsorbed substances the heated adsorbent was somewhat stronger; this phenomenon is discussed briefly in Section D below. It was demonstrated, in an experiment carried out by Mr. Earl W. Malmberg, that the amount of water removed by the prewash (as determined by titration with Karl Fischer reagent) was identical with that removed by heating at 200° for one hour; this generally amounted to about 10 or 15 percent of the weight of the silicic acid.

A brief investigation, whose results will not be reported in detail here, was made of the relation between the water contents and the adsorptive strengths of seven samples of silicic acid obtained from different commercial sources. It was found that prewashed columns of 2:1 silicic acid-Celite prepared from five of the seven samples of silicic acid had remarkably similar adsorptive properties; the other two samples were about 25 to 40 percent weaker. A definite correlation existed between the adsorptive strength of the silicic acid and the content of "bound" or "structural" water[21,22] in the sample, that is, the water which could be removed completely only by ignition at about 1100° (as contrasted with the "unbound" or "free" water which

could be removed at temperatures of 100-200°, or evidently, by the prewash). For the seven samples studied the content of structural water varied from about 3.3 to about 5.7 percent; the sample with the highest content of structural water had the greatest adsorptive strength.

Despite the near identity in the adsorptive properties of the different samples of silicic acid it was decided that it would be safest to carry out all of the present work on silicic acid-Celite prepared from a single sample of silicic acid and therefore this procedure was followed, as indicated in Section B below.

Before the fundamental measurements necessary to test the theories of chromatography were made it was thought desirable to make a brief study of the phenomena of coning and distortion* on silicic acid-Celite columns in the hope that these undesirable effects might

* For the present purposes I shall attach the following meanings to the terms coning, surface spreading, and distortion:

Coning shall be used to refer to that situation in which the boundary of a zone on an axial section of the column, that is, a section cut along a diameter and parallel to the axis, has the shape of a V or U or an inverted V or U.

Surface spreading shall be used to refer to the spreading of a zone in a thin layer on the outer surface of the column to a width appreciably greater than its width in the interior of the column. This phenomenon can occur at either the upper or lower boundary of a zone, although only rarely at both simultaneously. The term shall be reserved for typifying a cylindrically symmetrical condition; the irregular streaks of a compound which are occasionally found on the surface of a column are considered to be distortions (see below). Surface spreading is closely related to coning; it is, in fact, an extreme form of U-shaped coning.

Distortion shall be used to describe any distribution of material in a zone in a manner which is not cylindrically symmetrical.

be minimized or perhaps completely eliminated. It was found that distortions could be fairly completely eliminated merely by observing certain precautions in matters of technique, such as the method of transfer of the sample to the column, the flatness of the upper surface of the column, and so on.

The elimination of coning was however a much more difficult problem and one which has not yet been solved in a completely practical manner. A considerable number of experiments was done in the study of coning; these experiments were carried out in tubes of identical shape and 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. It was discovered that the direction and extent of coning, and especially of that phenomenon which may be described as "surface spreading", appear to depend on the nature and history of the chromatographic tube used; these effects appeared to be almost negligible in metal tubes and new glass tubes but were quite appreciable in glass tubes which had been used several times. Although it seemed likely that a detailed study of these effects might yield fundamental information concerning the processes which occur in a chromatographic column, it was apparent that a considerable amount of time would be required for this study. Furthermore the knowledge which had been gained was adequate to permit a measure of control sufficient for the successful completion of the present experiments and therefore no more detailed study was made of coning and related phenomena. Further experiments are contemplated however.

After the preliminary work which has been mentioned had been

completed it was possible to obtain data which could be used to test the relation between the adsorption isotherm and the rate of development which is predicted by the idealized theory of chromatography[2,3,4] (that is, the simple theory including the assumptions of instantaneous equilibrium, negligible diffusion, continuous structure of the adsorbent, etc.). For this study six different compounds were chosen. Five of these substances, 4-nitroaniline and its N-ethyl, N-phenyl, N,N-diethyl, and N,N-diphenyl derivatives, were similar in structure but covered a wide range of adsorption affinities, while the sixth, sym-diethyldiphenylurea (ethyl centralite), was quite different in structure from the others and had an intermediate adsorption affinity. By this selection of compounds it was hoped to obtain results whose validity would be independent of the adsorption affinities and the chemical nature of the substances used. Several different types of developing mixtures were investigated and a wide range of developing rates was studied. The results of these studies are presented in Section C below: it was found that all of the isotherms investigated were linear, or approximately so, in the range of concentrations studied and that in general the agreement between the predicted and observed development rates was very good.

The only detailed studies of the shapes of chromatographic zones that have been reported to date have been made by examination of the filtrate from the column[8,9,10,16]. It seems apparent that a study of the distribution of the material in a zone while it is in various positions on the column would yield more direct and unambiguous data about the processes taking place on the column. In the present

work a method was developed by which the distribution of the adsorbed substance in the zone on the column (the "profile" of the zone) could be directly measured by cutting the column in a direction perpendicular to its axis into thin slices of uniform thickness and eluting the adsorbed material from each of these slices. Since the thickness of the slices, usually about 2 mm., could be made constant within about one percent, the method yielded 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. These studies are described in Section D.

In Section E are described a few experiments which were done in order to determine at least the maximum time necessary for the attainment of adsorption equilibrium. No detectable departure from equilibrium was found for any of the systems studied in the shortest time (between ten and fifteen seconds) in which a measurement could be made after mixing the adsorbent and solution. No strictly comparable experiments could be found in the literature, although for adsorption on silica gel or alumina from aqueous solutions, periods of time ranging from a few minutes to many hours have usually been reported to be necessary for the establishment of equilibrium[23,12].

In order that needless repetition of experimental details might be avoided, descriptions of those materials and procedures which were common to the different phases of the present work have been collected in Section B below.

B. Introduction to the Experimental Work

Most of the materials which were used in this work and many of the experimental procedures which were employed were common to the different phases of the investigation. These experimental details will be described in the following section; the special experimental features pertinent to each one of the other sections will be discussed in that particular section.

1. Materials

(a) Adsorbent

The adsorbent which was used throughout all of the work, except where specifically stated otherwise, was a mixture of two parts by weight of Merck reagent silicic acid, code number 40446*, and one part of Celite 545, a product of the Johns-Manville Corporation.

(b) Solvents

All solvents with the exception of the absolute ethanol used for spectrophotometry were distilled in an all-glass still before use. Commercial benzene was dried over calcium chloride before distillation, and most of the water which remained after this treatment was removed during the distillation. C. P. benzene which had been dried over sodium was used in some of the experiments discussed in Section D and it was demonstrated that the results obtained with this solvent were in general indistinguishable from those found with ordinary redistilled

* The code numbers on Merck products refer only to the packing date and do not necessarily designate different production lots; however, they offer the only convenient means of distinguishing between different shipments of materials, which may have different properties, and therefore will be used for that purpose here.

benzene, although in certain special tests the traces of water remaining in the latter solvent had a slight demonstrable effect. U. S. P. anhydrous ether was used without further drying in most of the work; it was shown that when this material was dried over sodium before use the adsorptive strength of prewashed adsorbent became very slightly greater but otherwise there was no significant difference in results.

(c) Chromatographic tubes

Most of the chromatograms were carried out in No. 2 tubes of the type described by Zechmeister*; these tubes are made of pyrex glass and are 20 cm. long with an inside diameter of 19 mm. Some of the rates of development used in connection with the adsorption isotherms were measured in No. 1.5 tubes, about 18 cm. long and 13 mm. in inside diameter. In the preparation of prewashed and dried adsorbent for use in the measurement of adsorption isotherms (Section C) a No. 5 tube, 30 cm. long and about 50 mm. in inside diameter, was used.

All columns of adsorbent were 150 ± 5 mm. in height unless otherwise stated.

(d) Special compounds

The preparation of pure samples of the six compounds which were used as test substances in this work will be described very briefly. The chromatographic homogeneity of the purified samples of each of the five nitro compounds was investigated and each of these substances was found to contain less than 0.1 percent of detectable impurity. Spectrophotometric data for these compounds are presented in part 3 of this section.

* Reference 1a, pages 62-63, Figures 19 and 20

All melting points have been corrected.

4-Nitroaniline. - Eastman White Label 4-nitroaniline was recrystallized from absolute ethanol; the product melted at 148-149°. Previously reported values for the melting point of 4-nitroaniline range from 145° to 147° [24a].

4-Nitro-N-ethylaniline. - This compound was prepared by the nitration of N-ethylacetanilide according to the directions of Nölting and Collin[25] and Weller[26]. The flat brilliant yellow needles which were obtained after two recrystallizations from 50-percent ethanol melted at 95.6-96.0°, in good agreement with the reported values of 95° [24b] and 96° [26].

4-Nitrodiphenylamine*. - Eastman White Label 4-nitrodiphenylamine was freed of the large amount of green impurity which it contained by treatment of a solution in hot benzene with an amount of Norite equal to one-half the weight of 4-nitrodiphenylamine used. Golden platelets of pure 4-nitrodiphenylamine were obtained by recrystallization from 2:1 benzene-ligroin. The product melted at 135-135.5°; the melting points reported in Beilstein[24c] are 132° and 133°.

4-Nitro-N,N-diethylaniline. - A four-times recrystallized sample of 4-nitro-N,N-diethylaniline which had been prepared* by the oxidation[27] of 4-nitroso-N,N-diethylaniline[28] with potassium permanganate was available, but it still had a faint green cast due to the presence of a trace of 4-nitroso-N,N-diethylaniline. This impurity was readily removed by chromatographing the material on silicic acid-

* The purification of 4-nitrodiphenylamine and ethyl centralite and the preparation of 4-nitro-N,N-diethylaniline were done by Mr. Philip E. Wilcox.

Celite with ether-ligroin development. The purified material was recrystallized from 60-70° ligroin; the lemon-yellow crystals melted at 77-78°, the same temperature reported in Beilstein[24c] as the melting point of 4-nitro-N,N-diethylaniline.

4-Nitrotriphenylamine. - A sample of 4-nitrotriphenylamine was prepared according to the method of Herz[29], by the controlled nitration of Eastman White Label triphenylamine in glacial acetic acid. Purification of the mustard-colored product was accomplished by chromatographing it twice on silicic acid-Celite with benzene-ligroin and ether-ligroin development. A significant quantity of higher nitration products was removed in this manner. The final product was recrystallized from 60-percent ethanol; the fine orange crystals which were formed melted at 141-142°. Herz[29] reported the melting point of 4-nitrotriphenylamine as 139-140°; Gambarjan[30] stated that his preparation melted at 144°.

Ethyl centralite (sym-diethyldiphenylurea)*. - A sample of commercial ethyl centralite was recrystallized four times, alternately from ethanol and ligroin. The final product consisted of fine white odorless crystals which melted at 72.6-73.2°; the melting point of this compound has previously been reported as 74° [31] and 79° [32].

(e) Volumetric apparatus

All volumetric apparatus was calibrated before use. Pipettes were calibrated with absolute ethanol since they were to be used almost exclusively for organic solvents.

* See the footnote on the previous page.

2. Chromatographic procedures

(a) The procedure for packing the tube

The procedure which was used for preparing columns of adsorbent will be described in some detail because it has been found to be important in eliminating distortions of zones. Although the full suction of a water-aspirator was used as the driving force in all chromatograms, the packing was done with a minimum of suction. The aspirator connected to the receiving flask was turned on before packing, but a stopcock in the line was opened; hence there was a flow of air through the tube before the packing was started but as soon as a layer of adsorbent of any appreciable thickness had been formed the resistance to flow was sufficiently great so that there was little subsequent flow of air through the tube during packing. The adsorbent was allowed to flow into the tube until the column was about 15 percent higher than the final desired height. Then, after the top of the column had been very gently levelled, the full suction of the aspirator was applied and the column contracted lengthwise into position within about 30 seconds. The upper surface of the adsorbent was again levelled with extreme care; since the adsorbent was very easy to pack it was not necessary to apply any appreciable pressure to the upper surface. It was found to be imperative that the upper surface of the column be flat and regular if an even zone was to be formed; in order that the original flatness of the upper surface might be preserved, a flat circular piece of fine-mesh wire screen of approximately the same diameter as the inside of the tube was placed on this surface before any solvent was added. This wire screen served admirably to

prevent any erosion by the solvents which were poured into the tube during the process of chromatography.

The procedure which has been described differs in several respects from that which was used in the work described in Part II of this Thesis and which is also used by LeRosen[11b,d]. First of all, the packing of silicic acid-Celite has previously been done under full suction. Although this method is entirely adequate for all normal chromatographic work it was observed in the present investigations that, on columns packed under full suction, zones tend to be distorted more often than on columns packed in the manner described above.

The second respect in which the present method differs from that used earlier is that the tube is never tapped at any time. Tapping is specifically recommended by LeRosen for columns packed under full suction; it was avoided here because it was observed to cause a variation in the flow rate of solvents through the solumn. The flow rates through columns packed under full suction are about 10 to 15 percent less than those through columns packed in the manner recommended above; when, however, a column packed in the latter fashion is tapped during the packing the rate of flow decreases somewhat irregularly but tends to approach that through columns packed under full suction. In order that conditions should be as reproducible as possible in the present experiments tapping was therefore avoided; the flow rates in successive identical chromatograms seldom varied more than one or two percent.

Finally, the procedure for protecting the top surface of the column with a piece of wire gauze was a new departure and one which

proved most successful. It is to be noted that Trappe[19b] proposed a different solution for the same problem: he covered the upper surface of his silicic acid columns with a layer of diatomaceous earth which could be firmly stamped down and which had negligible adsorptive power.

(b) The prewash

The prewash which was used throughout this work was, except where otherwise noted, very similar to that which has been described earlier (Part II, p. 25) as the "modified" prewash. It consisted in washing the column, before introduction of the sample, with 0.2 V ml.* of ether, V ml. of 1:1 acetone-ether, 0.8 V ml. of ether, V ml. of ligroin, and finally about 0.2 V ml. of the developer. This prewash differs from that described earlier in the use of only V ml. of ligroin (in place of 2 V ml.) and in the use of the small volume of developer as the final stage of the prewash. The latter procedure served to make the conditions under which the compound to be chromatographed was adsorbed and developed as well-defined as possible, inasmuch as the sample solvent was also the same as the developer.

It is now believed that it might be of some advantage to eliminate the use of the V ml. of ligroin in the prewash and merely to follow the ether by V ml. of the developer.

One matter of technique must be mentioned. In the preparation of the 1:1 acetone-ether for the prewash, or of any mixed solvent to be used in the chromatogram, it is necessary to avoid shaking the mixture (which might be done in order to ensure thorough

* The term "V ml." was defined in Part II, p. 25, as the volume of solvent required to wet completely a column of adsorbent.

mixing) just before placing it on the column since the many tiny air bubbles which are thereby introduced into the solvent will be removed in the upper region of the column and may eventually cause a serious disturbance of the upper surface of the column.

LeRosen[11b,c] uses a prewash similar to the one described here although he uses pure acetone in place of the 1:1 acetone-ether. We never adopted this practice because it appeared to cause increased coning and offered no advantage.

(c) The introduction of the sample and of the developer

The exact procedure which was employed for transferring the sample to the column was critical because of the importance of obtaining absolutely uniform zones for the measurement of the zone-shapes or "profiles". As mentioned earlier, the solvent in which the sample was dissolved and the final solvent in the prewash were always identical with the developer in order that the adsorption conditions might be as simple as possible. The volume of sample solvent was usually 0.1 V ml. and the concentration of the solution was approximately 0.25 mg. per ml.

It was found after some experimentation that a satisfactory procedure for the transfer of the sample to the column was to pour the solution of the sample quickly down a small glass rod into the center of the tube while the level of the final solvent of the prewash was still one to two millimeters above the top of the column of adsorbent. The initial portion of the developer was placed on the column in a similar fashion. This attention to minor details may seem trivial but it must be emphasized again that the problem of

obtaining consistently uniform zones was an extremely important one and the method of introducing the sample was one of the several critical factors.

(d) The measurement of the position of a zone

One of the chief reasons why these studies of chromatographic phenomena on silicic acid-Celite were undertaken was that the zones of the more than 100 compounds whose chromatographic properties we had investigated on this adsorbent appeared, without exception, to have the general shape of a probability distribution function, apparently quite symmetrical with both the upper and lower boundaries diffuse. This was true for zones of colorless substances, as delineated by streak reagents, as well as for visible zones. The only theory which expressly predicts such a zone shape is that of Martin and Synge[14] although one might infer from the idealized theories of chromatography that for a linear isotherm such a shape would be expected since these theories predict the formation of a uniform zone with both boundaries sharp and the lack of ideality in an actual experiment might tend to blur both boundaries equally since neither has any intrinsic stability. However, for the usual form of isotherm, which departs from linearity in the manner expressed by the Freundlich or Langmuir equation, the theories predict that the zone will have a sharp lower boundary and a diffuse upper one and that a mechanism exists by which this sharp lower boundary will maintain its sharpness despite non-ideality[3,4].

The measurement of the position of a zone with diffuse boundaries is necessarily a partially subjective process. The

procedure which I adopted was to measure and record both the extreme limits of the zone, that is, the position of the first visible material on each boundary of the zone, and the limits of the more concentrated portion of the zone, that is, that part of the zone which appeared to contain about 90 percent of the adsorbed material. Since the gradation of color (of the zone or of the streak) was always quite uniform it was only by the adoption of an essentially subjective convention that any reproducibility in the measurement of the position of the "concentrated" part of the zone could be obtained. Nevertheless it was felt that it was important to record the positions of both the "extreme limits" and the "concentrated limits" so that the proper center of gravity of the zone could better be located and a measure of the zone's symmetry obtained. The studies of the "profiles" of zones have fully confirmed the apparent symmetry of zones as deduced visually (Section D).

Each of the five nitro compounds which were used in these studies forms a yellow or yellow-orange zone on a column of silicic acid-Celite. The colorless zones of ethyl centralite were located by means of the red-pink color which they produce when streaked with a one-percent solution of ceric sulfate in 85-percent phosphoric acid.

The position of each zone was measured (to the nearest millimeter) in three or four places on the surface of the column; in general the separate measurements for undistorted zones which had been developed 100 mm. below the top of the column did not vary by more than about 2 mm. The average of these measurements was recorded as the "surface position" of the zone. Then the column was sliced in

half axially (along a diameter) and a measurement was made of the "axial position" of the zone, that is, the position on the exposed plane surface of either half of the column. For zones which are coned or surface-spread this position is of course different from the surface position.

In order to demonstrate the sort of reproducibility that can be obtained in successive identical chromatographic experiments there are listed in Table 1 the observed zone positions in a series of experiments in which every effort was made to maintain strictly identical conditions. This set of experiments involved the development of 4-nitrotriphenylamine with 2 V ml. of a 3-percent solution of ether in ligroin. The results are typical of those which were obtained with all of the compounds and developers studied; it is apparent that the reproducibility is very good.

In recording the positions of zones I have adopted the convention of placing the extreme limits of the zone in parentheses. Thus the position of a zone might be recorded as (70)76-109(116). This would mean that the extreme limits were 70 and 116 mm. below the top of the column while the limits of the more concentrated portion were 76 and 109 mm. from the top.

Table 1

Reproducibility of Zone Position in Consecutive Identical Experiments: 4-Nitrotriphenylamine Developed with 2 V Ml. of a 3-Percent Solution of Ether in Ligroin

Experiment	Surface limits	Surface midpoint	Axial limits	Axial midpoint
35C	(72)76-107(111)	92	(88)92-117(122)	105
35D	(73)79-110(114)	94	(82)90-110(117)	100
35E	(70)75-108(113)	92	(87)95-111(117)	103
35F	(71)77-106(110)	91	(84)93-116(121)	104
35G	(70)76-109(116)	93	(85)94-115(121)	104

3. 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[33] 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 such a short wave length (247 mμ) that impurities from the adsorbent and solvents may contribute several percent of the absorption at low dilutions.

In general, in the preparation of samples for spectrophotometric estimation the solution which contained the sample was evaporated to dryness <u>in vacuo</u> at 30-40° and after the residue had been dissolved in absolute ethanol it was transferred quantitatively to a volumetric flask of such a size that the final concentration of the solution was suitable for spectrophotometry. In the study of the profiles of zones however, the adsorbed material was eluted with absolute ethanol directly from each of the sections of the column

into a suitable volumetric flask, usually a 10-ml. one.

Precise measurements were made of the spectrophotometric factors* of each of the six compounds at its absorption maximum in ethanolic solution. Unpublished studies by Dr. A. O. Dekker have shown that the spectrophotometric factor, C/D, for ethyl centralite at 247 mμ is 3.07 mg. per 100 ml. for a cell thickness of 1.000 cm. (that is, an ethanolic solution of that concentration in a cell of that thickness would have an optical density of 1.000) 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 order to discover whether Beer's law was obeyed in this range. No variation in the spectrophotometric factor with concentration was found for any of the compounds; the maximum spread in the values at four concentrations was 0.6 percent and there was no trend in the values with changing concentration. Each of the determinations was done in duplicate; the agreement was in all cases excellent and it is believed that the final values are accurate to within a few tenths of one percent. They are summarized in Table 2.

* See Part II, pp. 30-31

Table 2

Spectrophotometric Data for Solutions of 4-Nitroaniline and Four Related Compounds in Absolute Ethanol

Compound	Wave length, mμ		C/D, mg. per 100 ml. for 1.000 cm.	Molecular extinction coefficient
	Absorption maximum	C/D measured at		
4-Nitroaniline	370-372	371	0.872	15840
4-Nitro-N-ethylaniline	385-386	385	.857	19390
4-Nitrodiphenylamine	389-392	390	.992	21590
4-Nitro-N,N-diethylaniline	392-395	393	.905	21460
4-Nitrotriphenylamine	391-394	393	1.567	18530

C. The Relation between Adsorption Isotherms and Rates of Development

1. Introduction

Any serious attempt to evaluate experimentally the merits of existing theories of chromatography must involve measurements of adsorption isotherms in the range of concentrations of interest in chromatography since according to the idealized theory the isotherm is the sole determinant of chromatographic behavior and even in those theories in which an attempt is made to include such effects as finite rate of establishment of equilibrium, the isotherm retains a prominent position. In this Section a discussion is given of the experiments which were done to test the predicted relation between adsorption isotherms and rates of development. In Section D a brief discussion is presented of the measurement of "profiles" of zones on silicic acid-Celite columns and of the interpretation of these results in terms of the measured adsorption isotherms. These investigations of profiles were found to be extremely helpful in explaining certain anomalies which had been found in the relation between the rates of development and the isotherms.

The compounds which were used as test substances in these investigations of chromatographic phenomena have already been described. The selection of these substances was not entirely arbitrary. In addition to the obvious requirement that the compounds form satisfactory zones on silicic acid-Celite, it seemed desirable that most of them be colored, not only to facilitate the chromatographic work but also because any study of the shapes of zones on the column almost precludes

the use of colorless compounds and such studies were contemplated. Furthermore, as mentioned in the Introduction, it seemed desirable to choose a group of compounds which were similar in structure but of widely varying adsorption affinities so that the number of variables might be kept to a minimum and at the same time some elementary relations between structure and chromatographic properties might be illuminated. On the other hand it was recognized that any results obtained in a study of a group of closely-related compounds would require substantiation with one or more substances of quite different structure. The six compounds which have been mentioned, namely 4-nitroaniline and its N-ethyl, N-phenyl, N,N-diethyl, and N,N-diphenyl derivatives, and ethyl centralite (sym- diethyldiphenylurea), fulfilled all of these requirements.

Before the quantitative tests of the relation between adsorption isotherms and development rates can be discussed it will be necessary to digress briefly on the theory of chromatography. Because the idealized theory of chromatography as developed by Wilson[2], DeVault[3], and Weiss[4] was found to be entirely adequate for the interpretation of the present results the discussion will be limited to this theory. Furthermore no detailed derivations will be given since these are presented fully in the original papers. In general the symbols which will be used are the same as those used by Wilson and DeVault; the terms R, k', and A, as here defined, were first used by LeRosen[11].

x = distance of any point in the column from the top of the column

x_o = distance of the lower edge of the initial zone from the top of the column; thus x_o is equal to the width of the initial zone

x_d = value of x at which a discontinuity (sharp boundary) occurs

c = concentration of the solute in the solution in the column

c_o = concentration of the solute in the solution initially poured on the column

v = volume of solvent which, at any time under consideration, has been poured into the column since the initial time; the initial time will here be assumed to be the beginning of the development. v is also the volume of solvent that has passed any given point since the initial time if the pores of the column were initially filled with solvent; this situation obtained in all of the experiments to be described. v may thus be considered as a convenient measure of time*.

v_o = volume of the sample solvent, that is, the volume of the solution of the solute initially poured on the column

α = pore volume per unit length of column

M = amount of adsorbent per unit length of column

Q = amount of solute adsorbed per unit length of column

$f(c)$ = adsorption isotherm of the solute on the adsorbent such that $Q/M = f(c)$

k, n = constants in the Freundlich equation for the adsorption isotherm, $f(c) = kc^{1/n}$

a, b = constants in the Langmuir equation for the adsorption isotherm, $f(c) = \frac{ac}{1 + bc}$

* I have used here a lower-case symbol, v, as did Wilson and Weiss, instead of a capital letter, as did DeVault, in order to avoid confusion with the expression "V ml."

A = amount of solute adsorbed on the quantity of adsorbent in the volume of column which contains a unit volume of solvent; thus $A = Q/\alpha$

k' = proportionality constant* in a linear isotherm such that $A = k'c$; thus $k' = A/c = Q/\alpha c$

R = ratio of the linear rate of movement of a zone to the linear rate of movement of the solvent in the column; it is apparent that R is a dimensionless quantity and also that $0 \leqslant R \leqslant 1$

For the linear isotherm, $f(c) = ac$, the theory predicts that the initial zone will have a width, x_0, given by the relation

$$x_0 = \frac{v_0 c_0}{\alpha c_0 + Mac_0} = \frac{v_0}{\alpha + Ma} \quad , \qquad (1)$$

and that on development with the same solvent, it will maintain this same width and will move at a constant rate, which, expressed as a linear rate of movement per unit volume of solvent added, will be given by the expression

$$\frac{1}{\alpha + Ma} \quad . \qquad (2)$$

When the rate of movement is expressed in terms of the quantity R, the expression (2) must be divided by $1/\alpha$, since a given volume of solvent, v, occupies a length of column equal to v/α. Thus,

$$R = \frac{1}{1 + Ma/\alpha} = \frac{1}{1 + Q/\alpha c} = \frac{1}{1 + k'} \quad , \qquad (3)$$

which is the expression used by LeRosen.

* Le Rosen has defined this simply as k, but this seems needlessly confusing because of the widespread use of the symbol k in the Freundlich expression,

$$f(c) = kc^{1/n} \quad .$$

For the more general form of isotherm, the width of the initial zone will be

$$x_o = \frac{v_o c_o}{\alpha c_o + Mf(c_o)} = \frac{v_o}{\alpha + Mf(c_o)/c_o} \quad . \tag{4}$$

As shown by DeVault and Weiss, a zone adsorbed according to the usual form of isotherm, as expressed for example by the Freundlich or Langmuir equation, will have a sharp leading boundary, whose sharpness will tend to be maintained during development in spite of the effects of diffusion and lack of attainment of equilibrium. As long as there is some solution of concentration c_o still present in the zone, the linear rate of movement of the discontinuous boundary per unit volume of solvent will be expressed by the relation

$$\frac{x_d}{v_o + v} = \frac{1}{\alpha + Mf(c_o)/c_o} \tag{5}$$

and the rate of movement of this boundary in terms of R will be

$$R = \frac{1}{1 + Mf(c_o)/\alpha c_o} \quad . \tag{6}$$

For a Freundlich isotherm this expression becomes

$$R = \frac{1}{1 + \frac{Mk}{\alpha} c_o^{(1/n) - 1}} \quad , \tag{7}$$

and for a Langmuir isotherm it is

$$R = \frac{1}{1 + \frac{M}{\alpha} \frac{a}{1 + bc_o}} \quad . \tag{8}$$

It is apparent that for an approximately linear isotherm, that is, for $1/n \approx 1$ in (7) or for $b \approx 0$ in (8), these expressions will be approximately equal to (3), which, it will be noted, is independent of the concentration. Since all of the isotherms which were investigated in the present work were found to be linear or very nearly so, the relation expressed in (3) is entirely satisfactory for the calculation of the expected value of R and it was therefore used for this purpose.

2. Experimental procedures

(a) Measurement of adsorption isotherms

In order that the adsorbent used in the measurement of adsorption isotherms should be the same as that used in the measurement of development rates it was necessary to devise a procedure for the preparation of a dry adsorbent which would have the same properties as prewashed adsorbent. It was found that this could be done quite satisfactorily by prewashing a large batch of adsorbent in the usual manner, and then washing it with 2 V ml. of 28-38° ligroin and transferring it as quickly as possible to a desiccator containing Drierite; after evacuation with a water pump (through a calcium chloride tube) for about one hour, the small quantity of ligroin still present was removed by evacuation with a Hyvac pump for several hours*. The weight of the final dried adsorbent was within about one percent of that expected on the basis of the known water content. Certain of the experiments to be described were made with adsorbent which had been activated by heating at 150-200° for two hours; it was, of course,

* LeRosen has used a similar procedure for the same purpose[11c].

stored over Drierite just as was the prewashed and dried adsorbent.

Both heated and prewashed and dried adsorbent are very hygroscopic; a thin layer of either was found to gain weight at a rate of about 0.02 percent per minute in air of about 40 percent relative humidity. Since even a fraction of a percent of moisture decreases the adsorption affinity of the treated adsorbent significantly it was necessary to minimize exposure of these adsorbents to the laboratory air at all times. However, even when such precautions were observed it was found that occasionally a particular batch of adsorbent was weaker than usual, or would decrease slightly in adsorptive strength during storage, due probably to adsorption of water vapor during sampling. Because of these effects, most of the development rates were actually measured or checked on prewashed and dried (or heated) adsorbent from the same batch which was used in the measurement of the corresponding isotherm at the same time that the isotherm was measured. In this way it was possible to make certain that the development rates and the corresponding isotherms were actually measured under equivalent conditions.

The technique of measuring the isotherms was fairly straightforward. As mentioned earlier, the present study was limited to the range of concentrations of practical interest in chromatography on silicic acid-Celite; the highest equilibrium concentration in most of the isotherms was not above about 0.5 mg. per ml. although some were extended to concentrations of about 3 mg. per ml. The lowest concentration was usually about 0.02 mg. per ml.

Five or six points were determined on each of the isotherms.

About 150 ml. of the developer and two stock solutions (of different concentrations) of the compound in the developer were first prepared; then suitable samples of adsorbent, about 0.6 g. to about 3 g., depending on the extent of adsorption, were weighed quickly into glass-stoppered Erlenmeyer flasks. Suitable volumes of the developer and of one of the stock solutions of the compound were at once pipetted into each of the flasks; the total volume of solution was usually 14 to 20 ml. The contents of the flask were mixed thoroughly by careful swirling and were then allowed to stand with occasional swirling at room temperature for about one-half hour*. Meanwhile an aliquot portion of each of the stock solutions was taken for analysis in order that its concentration might be checked. An aliquot portion of appropriate size was taken from each of the equilibrium mixtures; the solvent from each of these aliquots was removed by evaporation and the quantity of the residue was estimated spectrophotometrically, as discussed in Section B, part 3. A blank correction was determined and applied in the measurement of isotherms for ethyl centralite; it was almost negligible at all but the lowest concentrations.

Since the amount which was adsorbed was calculated from the difference between the initial and final concentrations of the solutions, it was essential for accuracy that the relative quantities of solution and adsorbent be chosen so that this difference was appreciable. The quantity of adsorbent which could be used was limited because of the difficulty of pipetting a sample of the

* Equilibrium was established essentially instantaneously; this matter is discussed further in Section E.

equilibrium solution when the proportion of adsorbent was large. The proportion of adsorbent to solution was so chosen in all of the present experiments that even in extreme cases never less than 30 percent nor more than 60 percent of the compound was adsorbed.

One final point must be mentioned. When a mixed developer is used, there is of course generally a selective adsorption of one of the components of the developer by the adsorbent. Thus when a static experiment is performed, such as the measurement of an adsorption isotherm, the final composition of the "developer" will not be the same as the original composition if such an effect takes place. 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 "active component" of the developer is less strongly adsorbed than the compound being developed, and this is almost always the case (except in displacement development, which is, however, not pertinent to the present discussion). The reason for this may be made clearer by the following argument. If the active component of the developer is less strongly adsorbed than the compound being developed, the "front" of the zone formed by this component of the developer will move down the column faster than the zone of interest. Furthermore, behind this front, the column will be saturated, or nearly so, with respect to the particular concentration which the active component has in the original developer. Hence, in the region behind the front, which is the region where the zone of interest will be situated, the developer will move through the column unchanged.

Now it is apparent that a correction must be made for this effect if the adsorption isotherms measured for mixed developers are to have any real significance. In order that such a correction could be made experiments were performed to determine the magnitude of the effect with benzene-ligroin, ether-ligroin, ether-benzene, and ethyl acetate-benzene developers. Estimations of the changes in concentration were made refractometrically; the details of the experiments will not be described. It was found that the effect was negligible with benzene-ligroin mixtures, but was significant for the other three mixtures; it was approximately the same for ether-benzene as for ethyl acetate-benzene. The results are summarized in Table 3; it must be emphasized that they are only approximate. However they were adequate for present purposes.

Table 3

Approximate Changes in Composition of the Developer in Adsorption Isotherm Experiments*

Type of developer	Percent of ether or ethyl acetate	
	Original	Final
Ether in ligroin	5.0	ca. 4.3
	10	9
	18	17
	25	24
	40	39
	57	56
Ether or ethyl acetate in benzene	1.0	ca. 0.85
	2.5	2.1
	5.0	4.5
	10.0	9.3

* These experiments were done with 10 ml. of developer and about 1.5 g. of prewashed and dried adsorbent.

The way in which a correction was made for the effect illustrated in Table 3 is of course obvious. The developer was prepared with a slightly higher concentration of the "active component" than that desired, the difference being just equal to the diminution expected because of adsorption. This correction is of greatest importance for weakly adsorbed compounds, since the percentage diminution in the concentration of the active component of the developer is greatest at low concentrations.

(b) Measurement of development rates

The measurement of the quantity R, that is, the ratio of the rate of movement of the zone to the rate of movement of the solvent, can be done very simply without measuring either rate separately. Since "V ml." is defined as the quantity of solvent necessary to wet completely a column of adsorbent, it is apparent that during the time which is required to pour V ml. through a column the solvent will move a distance equal to the length of the column. If, then, one merely measures the distance which the zone has moved in the same period one can obtain R simply by dividing this distance by the length of the column. This procedure was used throughout the present work.

As discussed in part (a) of this Section most of the development rates were measured or checked on the same batch of treated adsorbent which was used in the measurement of the isotherm, at the same time that the isotherm was measured. In the few instances in which duplicate determinations were made the agreement of the duplicates was excellent.

Since zones invariably spread as they move down a column of

silicic acid-Celite under normal chromatographic conditions (that is, except when displacement development is taking place), a problem arises as to just how to define the rate of movement of the zone because the different parts of the zone are moving at slightly different rates. Since zones on silicic acid-Celite are very symmetrical, it seems most reasonable to define the rate of movement as the rate of movement of the midpoint of the zone. This procedure is theoretically sound for a linear isotherm, because the idealized theory predicts that the zone will move with constant width and sharp boundaries, and hence any non-idealities which might operate to cause spreading of the zone in a symmetrical fashion might be expected to leave the midpoint unaltered.

It should be noted that the downward coning of zones which is almost always observed on silicic acid-Celite columns in glass tubes makes the actual center of gravity of the zone somewhat below the apparent midpoint of the zone as observed on the surface of the column. The measurements of zone profiles which are reported in Section D will illustrate this point; for the present it is enough to say that a correction was made for the effect, which usually was such as to cause the true midpoint to be about 10 percent below the surface midpoint.

In order that some idea might be gained of the constancy of development rate during each experiment, the position of the zone was measured after every 0.5 V ml. of developer and the development rate corresponding to each position was calculated. A correction was made for the initial position of the zone, by subtracting it from the

measured position, because 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 percent. 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 Section D, a column which has been prewashed in the usual way no longer has a completely uniform adsorptive strength for benzene-ligroin developers; rather, the adsorbent in the upper portion of the column appears initially to be somewhat stronger than the remainder of the column, although as development progresses this inhomogeneity is probably gradually removed.

3. Results and discussion

As mentioned earlier, all of the isotherms which were measured were found to be linear, or very nearly so, in the range of concentrations investigated, a result which is in full qualitative agreement with the theory of chromatography in view of the observed symmetry of zones on silicic acid-Celite columns. Because of the near-linearity of all of the isotherms the data for all can be fitted well to either the Freundlich or the Langmuir equation. In Table 4 are summarized the data for each of these isotherms, together with the value of R calculated from the data of the isotherm, the corresponding observed value of R, and the ratio of these two quantities. The

definitions of R and of the constants of the isotherms have been presented on pages 109 and 110. The constants of the isotherms were evaluated graphically in the usual manner.

The data which were necessary for the calculation of the values of R are presented in Table 5; the quantities α, M, and V have been defined earlier (pages 110 and 25).

Table 4

Summary of the Results of the Investigation of the Relation Between Adsorption Isotherms and Rates of Development

Abbreviations: E = Ether; L = Ligroin, 60-70°; B = Benzene
NitroDiEtAn = 4-Nitro-N,N-diethylaniline; NitroDPA = 4-Nitrodiphenylamine; NitroEtAn = 4-Nitro-N-ethylaniline
NitroAn = 4-Nitroaniline; EC = Ethyl centralite; NitroTPA = 4-Nitrotriphenylamine

Expt. No.	Compound	Developer	Constants of the isotherm: Freundlich k	Freundlich n	Langmuir a	Langmuir b	Calculated R	Observed R	$R_{obs.}/R_{calc.}$	Figure
(a)	Heated adsorbent									
1	NitroDPA	60% B in L	21	1.10	31	1.2	0.049	0.052	1.05	1, 10
2	"	87% "	8.6	1.04	10.1	0.5	.139	.125	0.9	1, 10
(b)	Prewashed adsorbent									
3	EC	14% E in L	12	1.03	13.2	.7	.102	.10	1.0	2, 11
4	"	19% "	5.2	1.01	5.2	.0	.222	.22	1.0	2, 11
5	"	30% "	3.0	1.01	3.05	.0	.325	.33	1.0	2, 11
6	NitroDPA	14% "	8.2	1.00	8.2	.0	.156	.155	1.0	2, 11
7	"	20% "	4.5	1.00	4.5	.0	.251	.25	1.0	2, 11
8	NitroEtAn	24% "	5.2	*	5.2	*	.226	.20	0.9	8
9	NitroAn	54% "	4.8	*	4.8	*	.240	.21	0.9	8
10	NitroTPA	35% B in L	4.0	1.00	4.0	.0	.273	.22	.8	5, 9
11	"	50% "	1.73	1.02	2.00	.4	.43	.37	.85	5
12	NitroDPA	60% "	18	1.02	19.4	.5	.072	.038	.5	3, 12
13	"	**	10.5	1.01	10.8	.3	.123	.115	.95	3, 12
14	"	75% "	7.3	1.05	8.4	.7	.153	.10	.65	3, 12
15	"	87% "	4.1	1.00	4.1	.1	.268	.20	.75	3, 9, 12
16	"	Benzene	2.5	1.00	2.5	.1	.365	.37	1.0	3, 4, 12
17	NitroDiEtAn	89% B in L	3.9	1.00	3.9	.0	.280	.20	0.7	6, 9
18	"	Benzene	2.45	1.00	2.45	.0	.382	.35	.9	4, 6
19	NitroEtAn	"	7.8	1.02	8.8	.5	.146	.085	.55	4, 7
20	"	0.5% E in B	4.25	1.02	4.6	.1	.245	.21	.85	7
21	NitroAn	10.7% "	4.4	1.04	5.1	.3	.225	.21	.95	8

* In experiments 8 and 9 measurements were made at only two concentrations, ca. 0.08 and 0.6 mg. per ml.
** The developer for experiment 13 was 60% B plus ca. 0.1% E in L.

Table 5

Summary of the Values of M, α, and V

Designation of tube	Inside diameter of tube, mm.	Adsorbent	M, g. per mm.	α, ml. per mm.	α/M, ml. per g.	V, ml. per 150 mm.
No. 1.5	13	Prewashed	0.058	0.088	1.52	13.2
		Heated	.057	.093	1.63	14.0
No. 2	19	Prewashed	.127	.192	1.51	28.8

The ratio of the observed value of R to the calculated value of R provides a very convenient measure of the usefulness of the theory of chromatography in the prediction of development rates from adsorption isotherms. Obviously the criterion of complete success would be to have the ratio, $R_{obs.}/R_{calc.}$, equal to unity. The values of this ratio for the present experiments are listed in the next-to-last column of Table 4; they have been calculated only to the nearest five hundredths, since the overall precision and accuracy of the experiments does not warrant expressing the ratios more precisely than this.

An inspection of the values of this ratio in Table 4 indicates that in general the agreement of theory with experiment was very satisfactory although there are some apparent exceptions to this statement. It will be noted that a ten-fold range of development rates was investigated, and that the largest deviations of the observed rate from the predicted rate occurred with slow benzene-ligroin development on prewashed adsorbent (Experiments 12, 14, and

19). When heated adsorbent was used in similar experiments (Experiments 1 and 2) the agreement with the theory was excellent. Furthermore, in all of the experiments in which the developer contained ether the observed development rate on prewashed adsorbent was generally equal to the predicted rate for all of the compounds tested, even at low rates of development (Experiments 3, 6, and 13). This is of particular interest in Experiment 13 for here the developer was the same as that used in Experiment 12 except for the addition of approximately 0.1 percent of ether.

The explanation of the anomalously low development rates with benzene-ligroin developers on prewashed adsorbent seems to be related to the fact that, as mentioned earlier, a prewashed column apparently no longer has a uniform adsorptive strength with benzene-ligroin developers. The studies of zone profiles which are described in Section D revealed that with benzene-ligroin developers the upper part of a prewashed column is initially a stronger adsorbent than would be expected from adsorption isotherm measurements with prewashed and dried adsorbent, and that 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 apparently uniform in adsorptive strength even with benzene-ligroin developers.

An interpretation of the facts outlined in the previous paragraph will be presented with the pertinent data in Section D.

However the significance of these facts for the present experiments is at once apparent: if the adsorbent in the column is actually stronger than the isotherm indicates that it is, then the observed development rate should be less than that predicted from the isotherm. Furthermore, since only the upper centimeter or two 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 remainder of the column apparently gradually increases in adsorptive strength 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.

The considerations of the preceding paragraph appear to afford a reasonable explanation of the results of the six experiments in Table 4 in which the observed development rate was appreciably different from the predicted rate. That the discrepancies are due not to a defect in the theory but rather to peculiarities of the prewashed adsorbent is shown by the fact that they were not observed in the experiments with heated adsorbent.

The simplicity of the relation between adsorption isotherms and development rates is illustrated quite strikingly in a qualitative fashion in Figures 1 to 9, in which the data of the isotherms have been plotted. In each Figure the observed development rate corresponding to each of the isotherms has been placed next to the curve to which it refers. Representative data have been plotted

logarithmically in Figures 10, 11, and 12 in order to demonstrate that the data fit the Freundlich equation well. For a truly linear isotherm this is of course trivial, but in a plot of f(c) versus c it is not always easy to determine to what extent the data depart from linearity if the different equilibrium concentrations differ by a constant factor (rather than a constant amount) as they did in the present experiments.

Inspection of Figures 1 to 9 shows clearly that a prediction of relative development rates on silicic acid-Celite columns can be made very simply if the adsorption isotherms are known; the sole apparent exception (Figure 3, curves 13 and 14) is caused by the fact that the developer in Experiment 13 contained ether, so that the observed rate was about equal to that predicted from the isotherm, whereas the developer in Experiment 14 contained no ether and the corresponding development rate was appreciably less than that expected on the basis of the measured isotherm.

Figures 2, 4, 8, and 9 are particularly illustrative. A comparison of curves 3 and 6 in Figure 2 shows that the relative chromatographic positions of two compounds, here ethyl centralite and 4-nitrodiphenylamine, in a given developer, in this case a 14-percent colution of ether in ligroin, can be successfully deduced from the isotherms. The same conclusion is reached from Figure 4; the isotherms of 4-nitro-N,N-diethylaniline (curve 18) and 4-nitrodiphenylamine (curve 16) in benzene are essentially identical and the development rates for the two compounds in this solvent are likewise almost identical. 4-Nitro-N-ethylaniline (curve 19) moves at an appreciably

Legend for Plate I

The numbers on the curves in the Figures refer to the experiment numbers in the column on the extreme left in Table 4.

Figure 1 Adsorption isotherms on heated silicic acid-Celite: 4-nitrodiphenylamine at two different rates of development with benzene-ligroin mixtures

Figure 2 Adsorption isotherms on prewashed silicic acid-Celite: ethyl centralite (3, 4, 5) and 4-nitrodiphenylamine (6, 7) at different rates of development with ether-ligroin mixtures

Figure 3 Adsorption isotherms on prewashed silicic acid-Celite: 4-nitrodiphenylamine at four different rates of development with benzene-ligroin mixtures (12, 14, 15, 16) and at one rate of development with an ether-benzene-ligroin mixture (13)

Figure 4 Adsorption isotherms in benzene on prewashed silicic acid-Celite: 4-nitro-N-ethylaniline (19), 4-nitrodiphenylamine (16), and 4-nitro-N,N-diethylaniline (18)

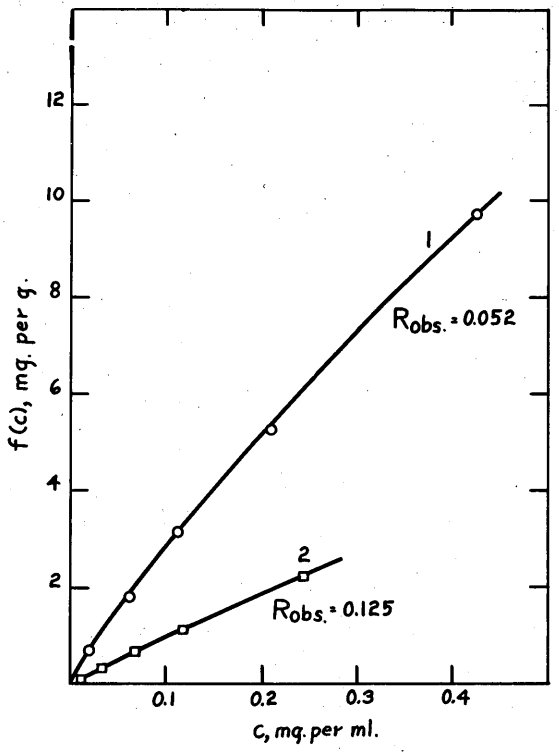

FIGURE 1

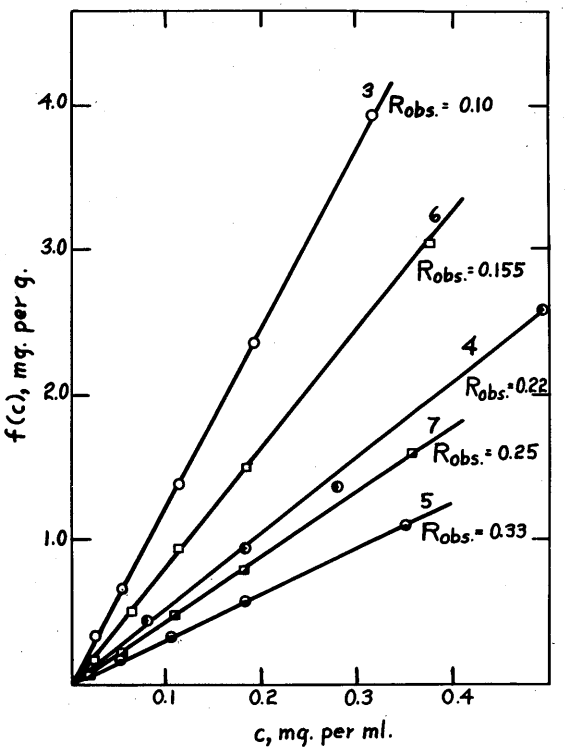

FIGURE 2

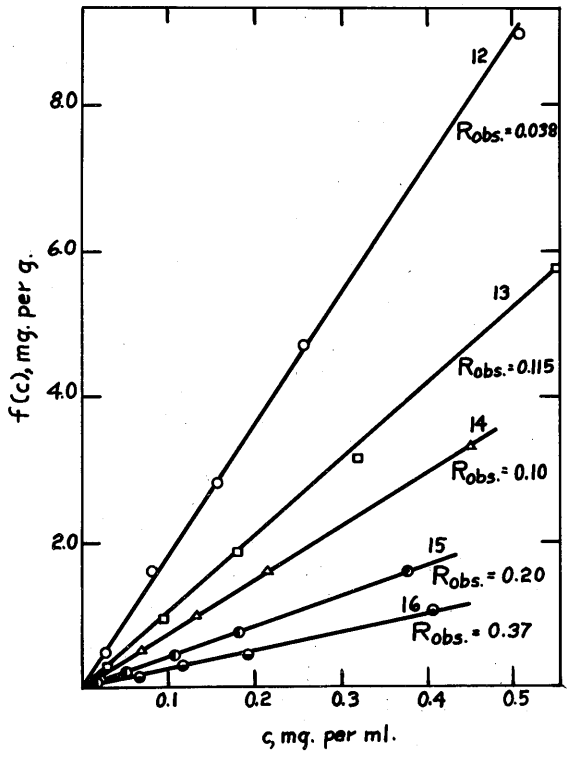

FIGURE 3

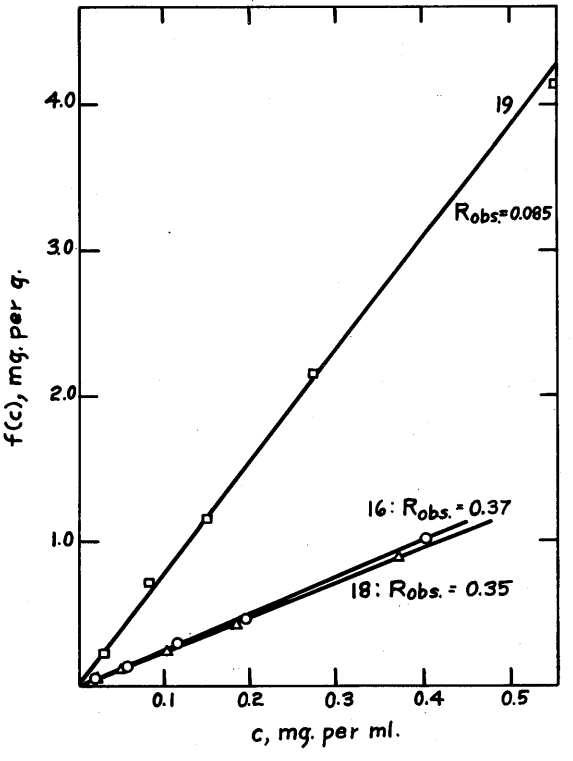

FIGURE 4

PLATE I

Legend for Plate II

The numbers on the curves in the Figures refer to the experiment numbers in the column on the extreme left in Table 4.

Figure 5 Adsorption isotherms on prewashed silicic acid-Celite: 4-nitrotriphenylamine at two different rates of development with benzene-ligroin mixtures

Figure 6 Adsorption isotherms on prewashed silicic acid-Celite: 4-nitro-N,N-diethylaniline at two different rates of development, with benzene-ligroin and benzene

Figure 7 Adsorption isotherms on prewashed silicic acid-Celite: 4-nitro-N-ethylaniline at two different rates of development, with benzene and ether-benzene

Figure 8 Adsorption isotherms on prewashed silicic acid-Celite: equal development rates for 4-nitro-N-ethylaniline in ether-ligroin (8) and 4-nitroaniline in both ether-ligroin (9) and ether-benzene (21)

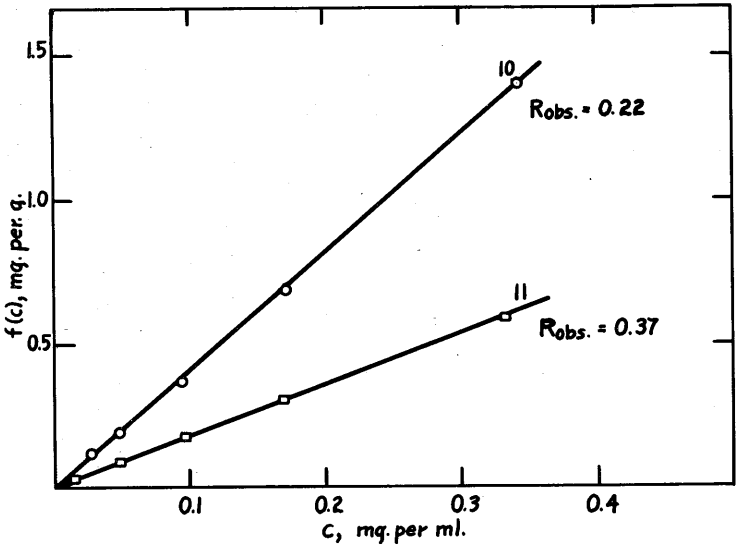

FIGURE 5

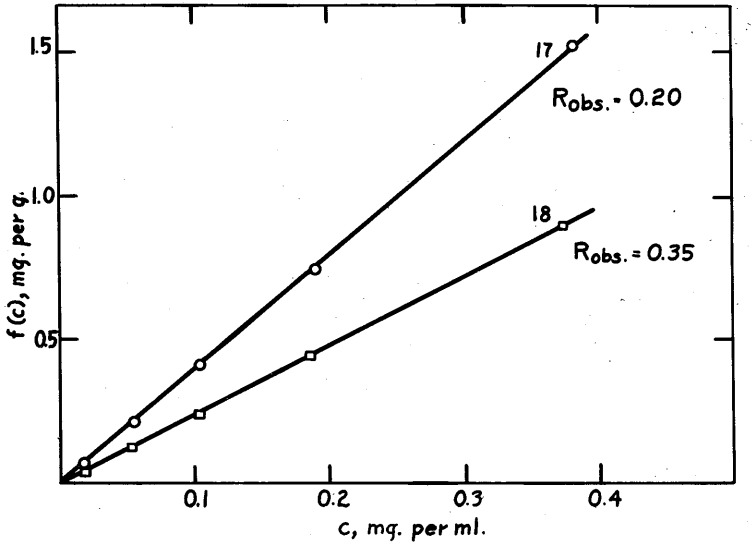

FIGURE 6

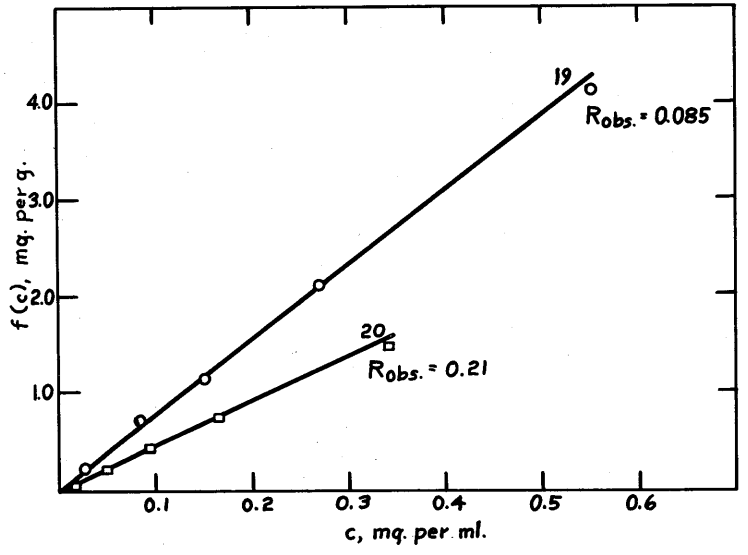

FIGURE 7

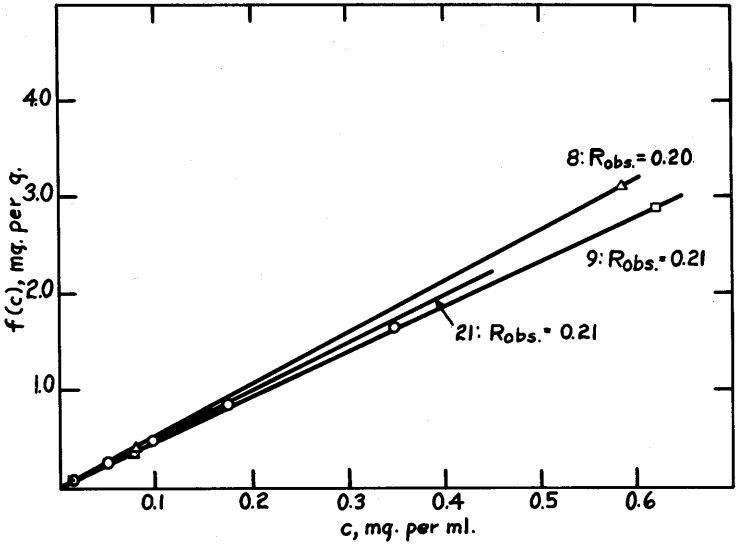

FIGURE 8

PLATE II

Legend for Plate III

The numbers on the curves in the Figures refer to the experiment numbers in the column on the extreme left in Table 4.

Figure 9 Adsorption isotherms on prewashed silicic acid-Celite: equal development rates for 4-nitrotriphenylamine (10), 4-nitrodiphenylamine (15), and 4-nitro-N,N-diethylaniline (17) in benzene-ligroin mixtures

Figure 10 Adsorption isotherms on heated adsorbent: logarithmic plot of the data of Figure 1, showing that the data fit the Freundlich equation well

Figure 11 Adsorption isotherms on prewashed adsorbent: logarithmic plot of the data of Figure 2, showing that the data fit the Freundlich equation well and that the value of 1/n, here equal to the slope, is very nearly the same for all of these isotherms

Figure 12 Adsorption isotherms on prewashed adsorbent: logarithmic plot of the data of Figure 3, showing that the data fit the Freundlich equation well and that the value of 1/n, here equal to the slope, is approximately the same for all of these isotherms

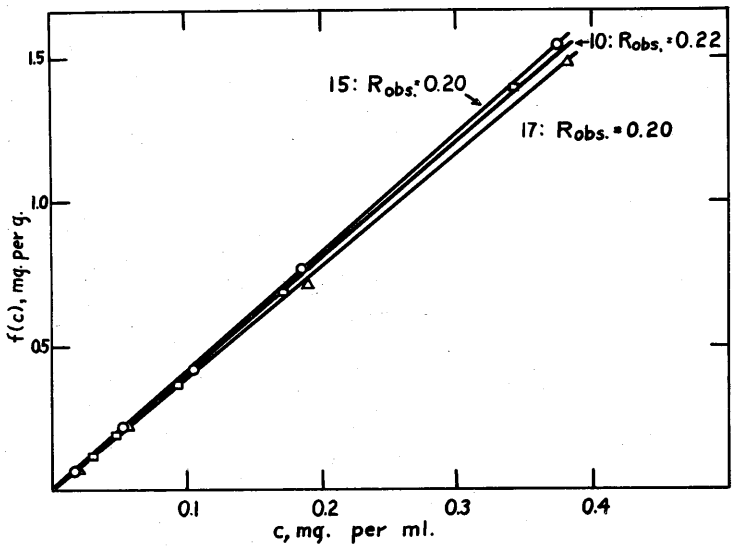

FIGURE 9

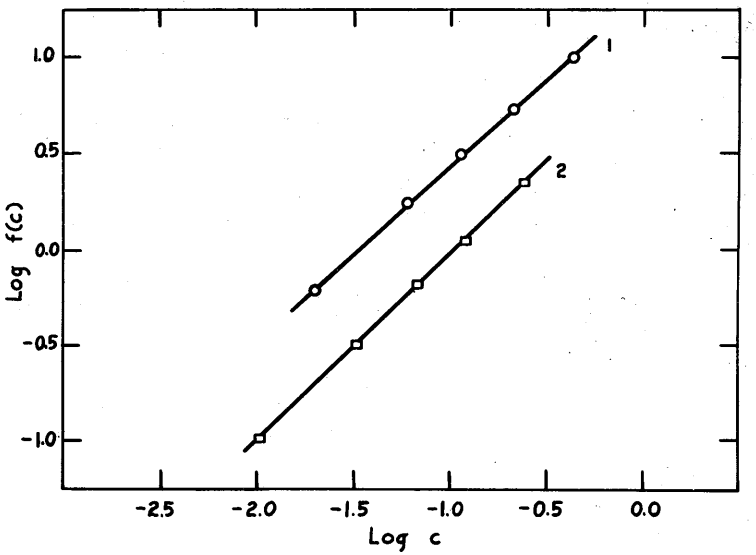

FIGURE 10

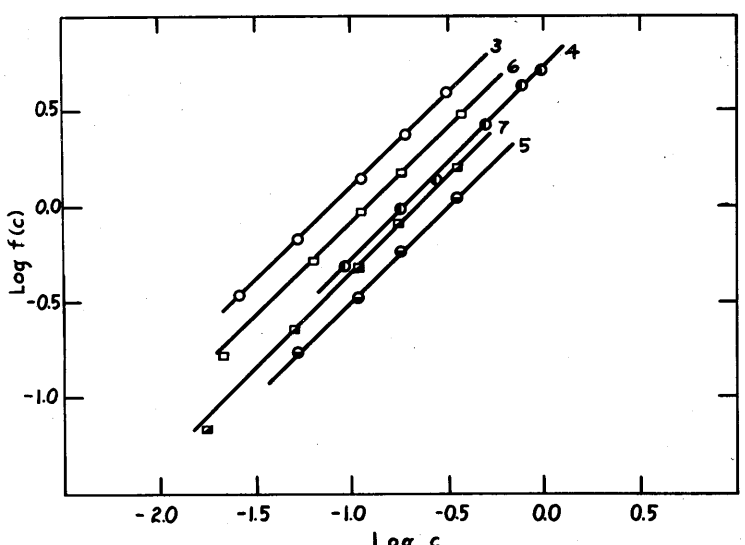

FIGURE 11

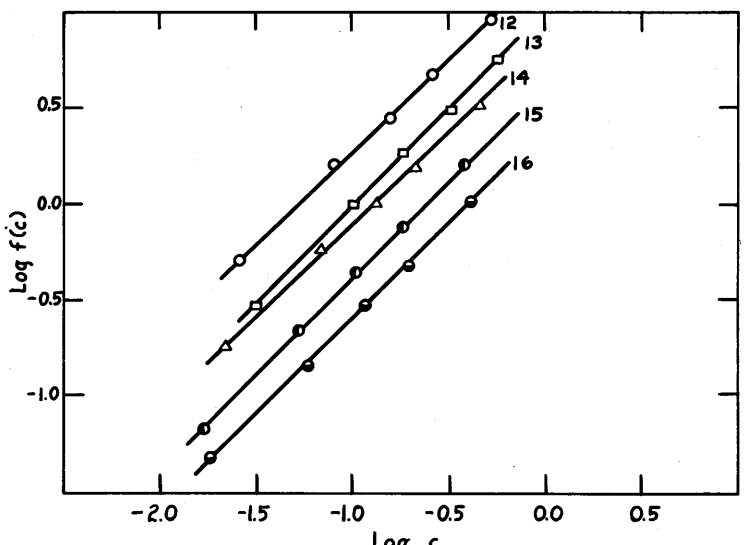

FIGURE 12

PLATE III

slower rate, as its isotherm indicates that it should.

In Figure 8 are plotted the isotherms of 4-nitroaniline and 4-nitro-N-ethylaniline in different ether-ligroin and ether-benzene developers which move the compounds at the same rate; the isotherms are practically coincident. A similar situation is depicted in Figure 9, where are plotted the isotherms of 4-nitrotriphenylamine (curve 10), 4-nitrodiphenylamine (curve 15), and 4-nitro-N,N-diethylaniline (curve 17) in different benzene-ligroin developers which move the compounds at the same rate. It is particularly interesting that although none of these compounds moves at the rate predicted from its isotherm, the actual rates of development are all less than the theoretical by about the same amount. Thus the qualitative prediction from a comparison of the isotherms that the development rates will be essentially identical is fulfilled, although the quantitative predictions are not completely correct.

It will be recalled* that other workers have made the statement that adsorption isotherms are of uncertain value in the prediction of chromatographic behavior. I believe that it can safely be said that for silicic acid-Celite columns a knowledge of the adsorption isotherms of a series of compounds will usually suffice to permit the prediction not only of the chromatographic behavior of each of the compounds separately but also of that of a mixture of not widely disproportionate quantities of all of them.

It would be well to restate here (in slightly different words) the very important generalization which was stated on page 46

* See page 84 of this Thesis.

of this Thesis: we have found that the chromatographic behavior of a compound on a silicic acid-Celite column under normal conditions is not significantly altered in the presence of reasonable quantities of other similar substances. Apparent exceptions to this rule may occur when the column is seriously overloaded or when even small amounts of very strongly adsorbed substances, such as water or ethanol, are present, but these could not be considered as normal conditions. The validity of the generalization is apparent from the fact that all of the work which was predicated upon it, as described in Part II of this Thesis and in other similar discussions[33], was entirely successful. Thus, our procedure has always been first to study separately the chromatographic behavior of each of the compounds to be investigated and then to assume that this behavior will be essentially unaltered in mixtures. This assumption has been fully justified by experience.

The pertinence of the discussion of the preceding paragraph to the present work lies, of course, in the justification which it gives to the prediction of the relative chromatographic positions of the compounds in a mixture from an examination of the isotherms measured separately. 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 above-stated generalization, that they would be insignificantly different from the isotherms of the same compounds separately. Incidentally, it would seem that the almost innumerable pages of complicated multiple-solute chromatographic theory propounded by Glückauf[7] are

quite unrelated to practical chromatography on silicic acid-Celite columns, although of course they may have some significance for other adsorbents.

It is interesting to note that heretofore it seems to have been generally accepted that the linear isotherm is of little or no importance in practical chromatography. Weiss[3], for example, made a statement to this effect although he had access to the data of Weil-Malherbe[8], who found that the adsorption of benzpyrene on silica gel from a 3-percent solution of benzene in petroleum ether followed a nearly linear isotherm ($n = 1.04$ in the Freundlich expression). Apparently silicic acid or silica gel characteristically gives linear isotherms; Mr. Earl Malmberg, in some unpublished studies in these laboratories, found that the adsorption isotherm of 2,4-dinitrodiphenylamine on unprewashed silicic acid-Celite from 1:1 benzene-ligroin was strictly linear.

The results which have been presented in this section may be summarized briefly as follows. The adsorption isotherms of all of the systems studied were linear, or approximately so, in the concentration range investigated. Furthermore the simple idealized theory of chromatography seems to be entirely satisfactory for the prediction of development rates on silicic acid-Celite columns under controlled conditions. The apparent anomalies at low development rates on prewashed adsorbent with benzene-ligroin developers appear to be due to peculiarities of the prewashed adsorbent rather than to a defect of the theory of chromatography.

D. The Profiles of Zones on Silicic Acid-Celite Columns

1. Introduction

It has been mentioned earlier (Section A, page 91) that the only detailed studies of the shapes of chromatographic zones that have been reported to date have been made by examination of the filtrate from the column[8,9,10,16]. The method which will be described here was designed to permit the direct study of the distribution of material in the zone while it is on the column; this method has proved remarkably successful. I have, for convenience, adopted the term "profile of the zone" to describe the differential distribution of the compound in the zone as a function of position on the column.

The results which are described here deal chiefly with the profiles of undeveloped zones under a variety of conditions, for it was by studies of such zones that the most insight into chromatographic phenomena was gained. However, some applications of the method to the study of developed zones are also illustrated and discussed, and some possibilities for future experiments are indicated.

2. The method

The technique which has been employed for measuring the profiles of zones on the column is very straightforward. The column is sliced in a direction perpendicular to its axis into thin sections of uniform thickness and the material present in each of these sections is recovered by elution. The device which is used for sectioning the column is illustrated in Figure 13 and its operation is described there. Only a few further comments will be necessary.

Legend for Figure 13

The Operation of the Device for Sectioning a Chromatographic Column

The chromatographic tube, A, is held in a fixed position by the blocks B and B' and the springs C and C', which hold the tube firmly against the block D. The column, E, is extruded cautiously against a glass plate, F, which is held in a vertical position, about 2 mm., or whatever thickness of section is desired, beyond the end of the tube at G. The glass plate rests against two vertical supports, H and H', which are fixed to the ends of the sliding members I and I'. The position of the glass plate can be varied at will by sliding I and I', and thus H and H', along the scales J and J'; I and I', which are slotted along about one-third of their lengths, can be clamped in any desired position with the aid of the wing nuts at K and K'.

After the column has been extruded against the glass plate, F, the plate is removed and the small section of the column which protrudes from the tube is carefully cut off with a razor blade, until the end of the column is just flush with the end of the tube. Then the glass plate is returned to position against H and H' and the operation of extrusion and cutting is repeated.

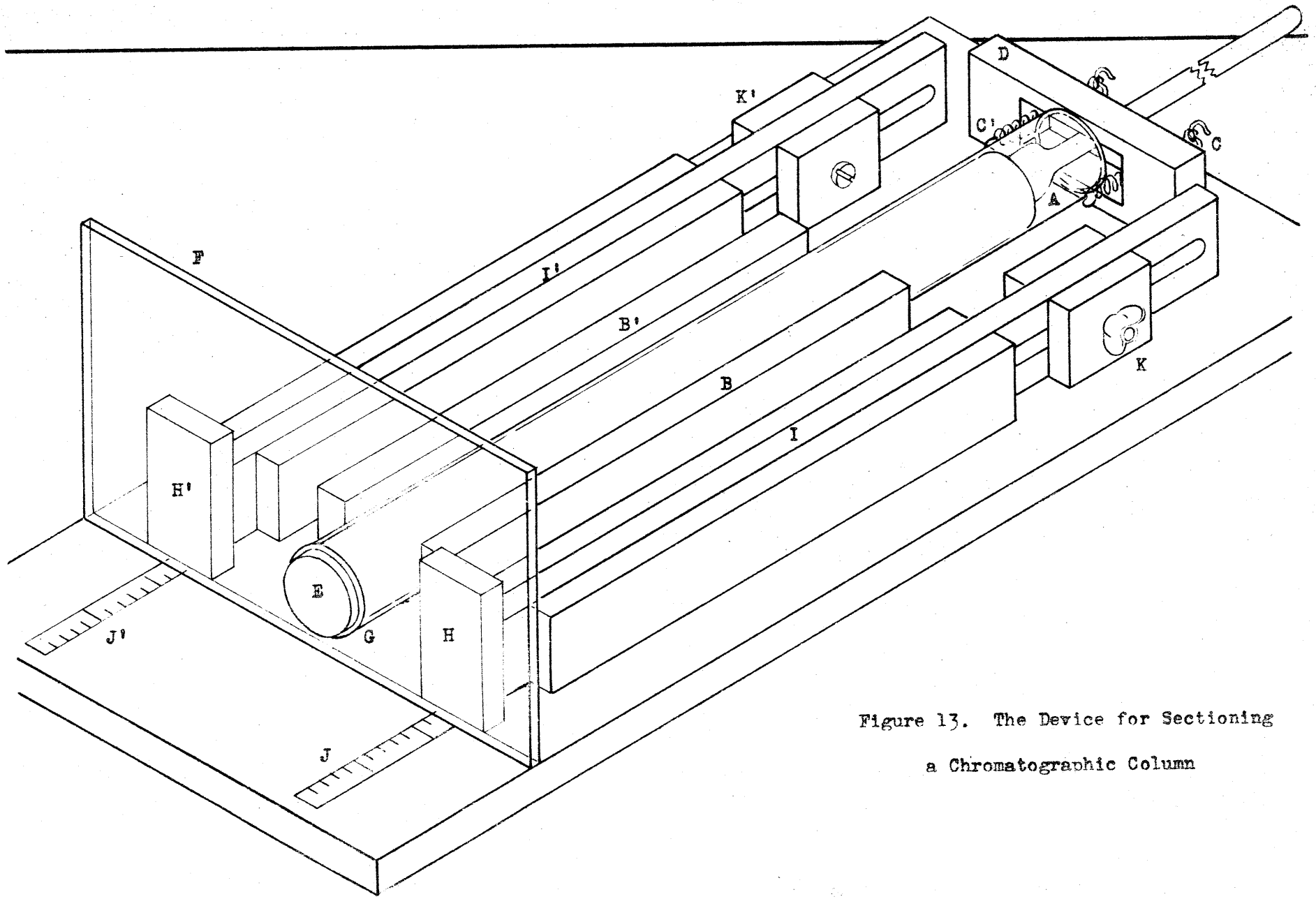

Figure 13. The Device for Sectioning a Chromatographic Column

It is generally desirable that the column of adsorbent be fairly loose in the tube so that it can be extruded with a minimum of pressure. The column can generally readily be loosened by tapping either end of the tube gently against the palm of the hand or rapping the side of the tube sharply once or twice.

In order to test the precision of the method of cutting, a control experiment was done. A column of unprewashed adsorbent was packed in the usual manner and then was washed with 2 V ml. of 28-38° ligroin. Sections about 2.5 mm. thick were cut from the column and after they had been allowed to dry in air for about one-half hour they were weighed in tared beakers. A similar experiment was done in which the sections were about 1.6 mm. thick. The results of these experiments are presented in Table 6. It is apparent that the method is quite satisfactory; it should be mentioned that considerable care is necessary in cutting the sections in order to prevent chipping the freshly cut surface.

Table 6

Weight After Drying of Successive Sections Cut from a Column of 2:1 Silicic Acid-Celite Which Had Been Washed with 28-38° Ligroin

Section No.	Weight, g.	Deviation from average weight, percent
(a) 2.5-mm. sections		
1	0.365	+ 1.5
2	.366	+ 1.5
3	.366	+ 1.5
4	.349	- 3
5	.359	0
6	.356	- 1.5
Average	.360	
(b) 1.6-mm. sections		
1	.231	+ 1.5
2	.226	- 1
3	.223	- 2
4	.232	+ 1.5
Average	.228	

In the measurement of the profiles of undeveloped zones, which, of course, extended to the top of the column, it was generally necessary to smooth the upper surface of the first section because the top of the column was never perfectly flat. However it was seldom necessary to shave off more than about 0.1 to 0.2 mm. of the column in this process.

In all experiments the approximate width of the sections was estimated with the aid of the scales (J and J' in Figure 13), which are graduated in millimeters. However, a more precise measure of the width was obtained by measuring the distance of the top of the

first section and the bottom of the last section from the bottom of the column and dividing the difference by the number of sections cut.

After each of the sections had been cut, it was placed in a small sintered glass funnel and the adsorbed material was eluted directly with absolute ethanol into a small volumetric flask or glass-stoppered graduated cylinder. It was possible to make an essentially quantitative elution with as little as 3 to 4 ml. of absolute ethanol. In all of the experiments to be described the recovery of the compound in the zone was between 96 and 100 percent.

Since there is always an appreciable amount of solvent left in the column at the end of the chromatogram, even after the usual fifteen or twenty seconds of draining, it is apparent that the above-described method for measuring the profile of the zone does not measure just the amount of <u>adsorbed</u> material in each section, but rather the sum of the amount of material adsorbed in the section and the amount in solution in the solvent still present in the interstices of the adsorbent. If the amount 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. This may be shown readily as follows. Let us assume 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*), and that the isotherm is $Q = Mac$. Then the total amount of material per unit

* LeRosen[11c] found that the quantity which I have termed α' was equal to about 87 percent of α for a column of silicic acid.

length of the extruded column, a quantity which I shall call B, will be given by the expression

$$B = \alpha' c + Mac = (\alpha' + Ma)c \quad ; \qquad (9)$$

the amount of adsorbed material alone per unit length is

$$Q = Mac \quad . \qquad (10)$$

It is apparent that if α' is independent of position on the column and concentration,

$$B = \left(\frac{\alpha' + Ma}{Ma}\right)Q = \text{constant} \times Q \quad . \qquad (11)$$

No investigation was made of the constancy of α' throughout the column; however, it seems reasonable that even if it does vary slightly it will vary in a very gradual and regular fashion so that the measured profile will be only very slightly distorted from the true profile of the adsorbed material. Furthermore in all of the profiles which will be reported here, the quantity Ma constitutes between 70 and 93 percent of B so that the slight uncertainty in α' will mean at most an uncertainty of a few percent in the value to be expected for B.

In the preparation of zones for sectioning it was of course extremely important to avoid any distortion of the zone whatsoever. All of the precautions which have been described earlier (Section B) were rigorously observed; if by mischance the zone was, nevertheless, distorted, the column was rejected and the experiment repeated.

On the other hand coning was not always eliminated. There was generally little coning in the undeveloped zones which were investigated but there was some downward coning in most of the zones developed with benzene-ligroin mixtures. This will be mentioned in

connection with the profiles of these zones. Only one profile of a zone developed with ether-ligroin will be shown here; this zone had been developed in a new glass tube of special design and was apparently not coned or surface-spread*.

3. Results and discussion

The profiles which will be discussed have been plotted in Figures 14 through 25, Plates IV and V. All of these profiles are of zones of 4-nitrodiphenylamine on No. 2 columns of 2:1 silicic acid-Celite. In all of the experiments except one of those shown in Figure 24 the size of the sample was 1.00 mg.; in that experiment it was, as indicated, 4.00 mg. Most of the columns were activated by prewashing in the usual way, but, as discussed later, one was prewashed according to another procedure, and in one experiment heated adsorbent was used. The benzene which was used for all of the profiles except that pictured in Figure 21 was dried over sodium before use.

(a) Undeveloped zones

All of the undeveloped zones whose profiles will be shown were placed on the column from a volume of 64 ml. except that represented in Figure 19, for which only 32 ml. was used. Although these volumes of sample solvent are far greater than would ever be used in a practical experiment on a No. 2 column, they were used in the present experiments in order that undeveloped zones of sufficient width for fruitful investigation might be obtained. As is evident from equation (1), page 110, the width of an undeveloped zone for a

* A brief discussion of coning and related phenomena has been presented on pp. 89-90.

Legend for Plate IV

Profiles of Undeveloped Zones Which Contain 1.00 Mg. of 4-Nitrodiphenylamine

In each Figure, B, the sum of the amounts of material adsorbed and in solution in the zone per unit length is plotted against x, the distance from the top of the column.

Figure 14 The sample was placed on a prewashed column from 64 ml. of a 20-percent solution of ether in ligroin; both the experimental result and that predicted on the basis of the isotherm measured with prewashed and dried adsorbent are shown.

Figure 15 The sample was placed on a prewashed column from 64 ml. of an 87-percent solution of dry benzene in ligroin; both the experimental result and that predicted on the basis of the isotherm measured with prewashed and dried adsorbent are shown.

Figure 16 The sample was placed on a column of heated adsorbent which had been washed with V ml. of an 87-percent solution of dry benzene in ligroin; the sample solvent was 64 ml. of the same mixture. Both the experimental result and that predicted on the basis of the isotherm measured with heated adsorbent are shown.

Figure 17 The sample was placed on a column which had been prewashed in the usual way except that the V ml. of ligroin of the usual prewash had been replaced by 3 V ml. of dry benzene. The sample solvent was 64 ml. of an 87-percent solution of dry benzene in ligroin.

Figure 18 The sample was placed on a prewashed column from 64 ml. of a solution of 87-percent of dry benzene and 0.15 percent of ether in ligroin.

Figure 19 The sample was placed on a prewashed column from 32 ml. of an 87-percent solution of "benzene" in ligroin; the benzene had previously been passed through a prewashed column. See the text for details.

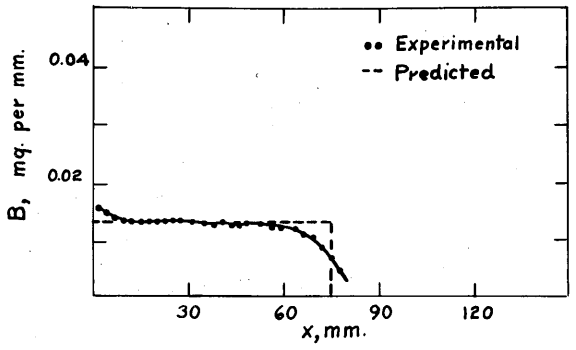

FIGURE 14

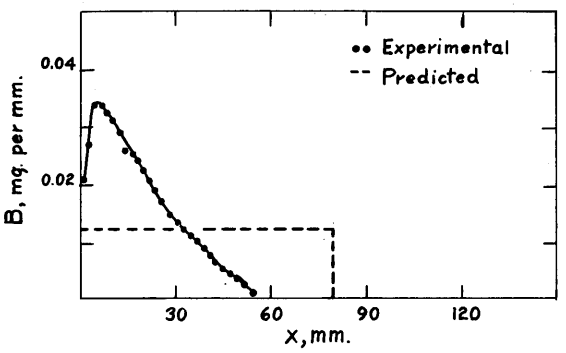

FIGURE 15

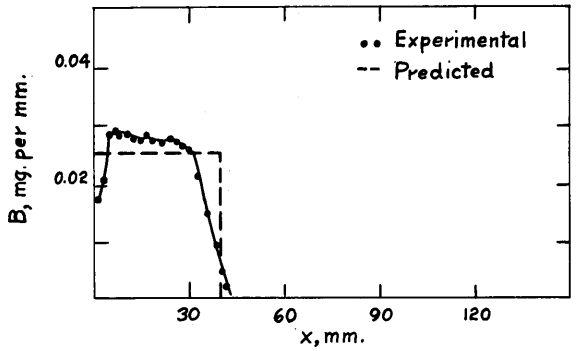

FIGURE 16

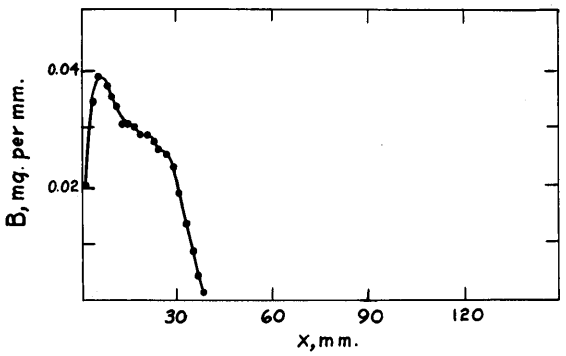

FIGURE 17

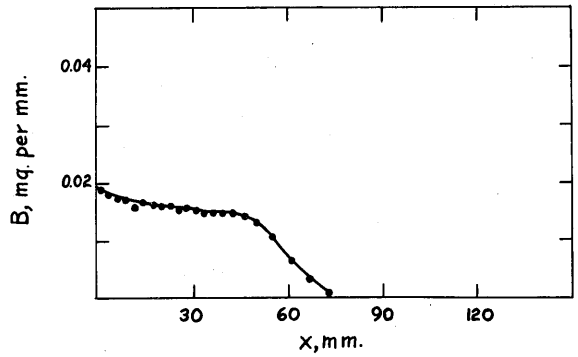

FIGURE 18

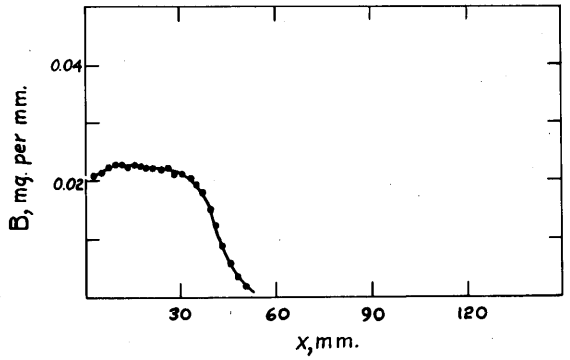

FIGURE 19

PLATE IV

Legend for Plate V

Profiles of Zones of 4-Nitrodiphenylamine

In each Figure, B, the sum of the amounts of material adsorbed and in solution in the zone per unit length, is plotted against x, the distance from the top of the column.

Figure 20 One milligram was placed on a prewashed column from 64 ml. of a 60-percent solution of dry benzene in ligroin.

Figure 21 One milligram was placed on a prewashed column from 64 ml. of an 87-percent solution of benzene in ligroin; the benzene had not been dried over sodium.

Figure 22 Duplicate experiments: one milligram was placed on a prewashed column from 4 ml. of an 87-percent solution of benzene in ligroin and developed with 2 V ml. of the same mixture. The arrows indicate the approximate positions of the "extreme limits" and the "concentrated limits" of the zone on the surface of the column. This Figure illustrates three things: the approximate error-function shape of developed zones, the reproducibility of the profiles of developed zones, and the effect of coning on the position of the maximum of the zone.

Figure 23 Comparison of the profiles of zones which contain 1.00 mg. placed on the column from different initial volumes: prewashed adsorbent was used and the zones were developed with 2 V ml. of an 87-percent solution of benzene in ligroin.

Figure 24 Comparison of the profiles of zones which contain different quantities of 4-nitrodiphenylamine placed on a prewashed column from 4.0 ml. of an 87-percent solution of benzene in ligroin and developed with 2 V ml. of the same mixture.

Figure 25 Comparison of the profiles of zones developed with 2 V ml. of a 20-percent solution of ether in ligroin and 3 V ml. of an 87-percent solution of benzene in ligroin on prewashed adsorbent: each zone contained 1.00 mg. and was placed on the column from 4.0 ml. of the developer.

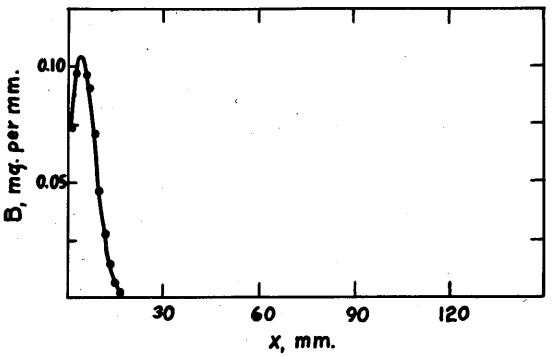

FIGURE 20

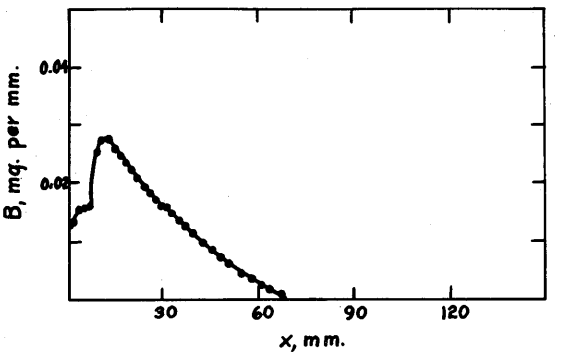

FIGURE 21

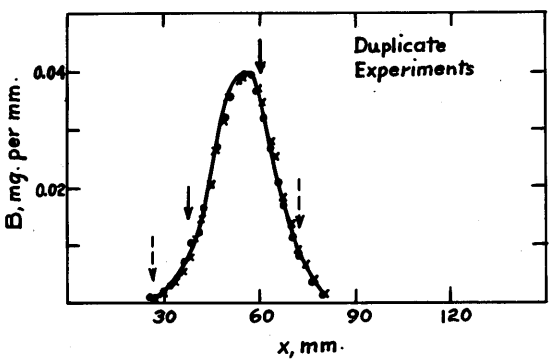

FIGURE 22

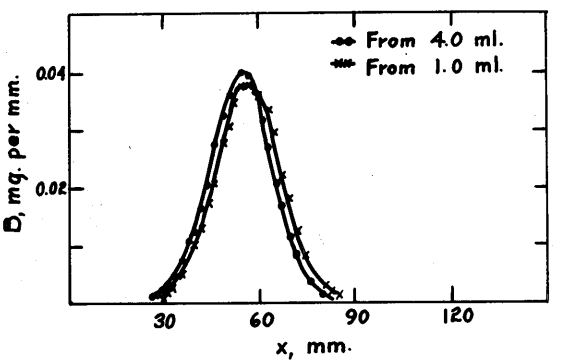

FIGURE 23

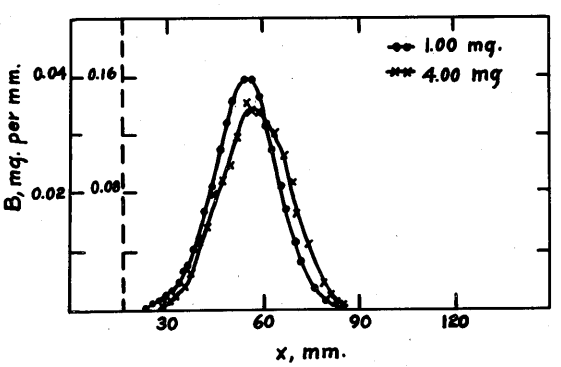

FIGURE 24

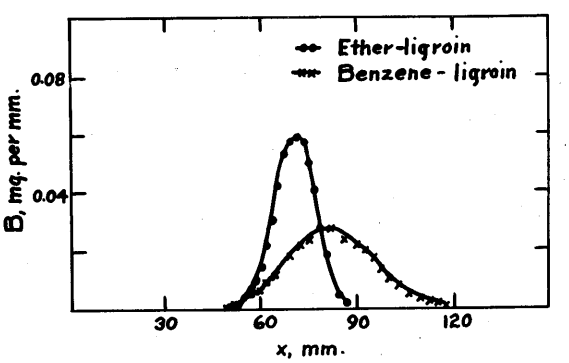

FIGURE 25

PLATE V

linear isotherm is, according to the theory, directly proportional to the volume of the sample solvent (and independent of the concentration).

Figures 14 and 15 show, respectively, the shapes of zones introduced from a 20-percent solution of ether in ligroin and an 87-percent solution of benzene in ligroin, together with the shapes expected on the basis of the isotherms reported in Table 4 (Experiments 7 and 15). The adsorbent in these experiments had been prewashed in the usual way. It is apparent that, just as in the development rate experiments, the agreement is excellent for ether-ligroin but, for this prewashed adsorbent, unsatisfactory for benzene-ligroin. Of course, even for the ether-ligroin the front of the zone is not sharp but this is entirely reasonable, for with a linear isotherm no discontinuous boundary would be expected to have any intrinsic stability.

The most reasonable interpretation of the result pictured in Figure 15 is that, except perhaps for the top few millimeters of the column, which will be discussed later, the adsorbent in this prewashed column effectively becomes progressively weaker in adsorptive strength as the distance from the top increases, and therefore the amount adsorbed per millimeter decreases progressively with increasing distance. This interpretation has been mentioned earlier, in connection with the apparently anomalous development rates with benzene-ligroin development on prewashed adsorbent. In the experiment reported in Figure 16, heated adsorbent was used with a prewash of V ml. of an 87-percent solution of benzene in ligroin; otherwise the conditions were identical with those in the experiment whose results are pictured in Figure 15. The shape of the initial zone agreed

reasonably well with that expected on the basis of the isotherm measured in Experiment 2, Table 4, indicating that in this system the column was of uniform adsorptive strength.

Several experiments were done in an attempt to find some explanation of the postulated non-uniformity of prewashed adsorbent with benzene-ligroin developers. In addition to the experiment with heated adsorbent (Figure 16), the experiments whose results are pictured in Figures 17 through 21 were done, together with several other experiments the results of which have not been plotted here because the profiles were identical with that shown in Figure 15. Among the latter were one in which a 1:1 mixture of absolute ethanol and ether was substituted for the 1:1 acetone-ether in the prewash, and several experiments in which other samples of benzene were used, including one with Baker's C. P. benzene and one with benzene purified with concentrated sulfuric acid according to a procedure similar to that described by Fieser[34]. These experiments established that it was not some peculiarity of the acetone in the prewash or an impurity in the benzene which was responsible for the anomalous zone shape.

In Figure 17 is shown the profile of a zone which was chromatographed under conditions identical with those used for the zone depicted in Figure 15 except that 3 V ml. of benzene was used in the prewash in place of the usual V ml. of ligroin. It appears that the benzene tends to make the adsorbent in the column more uniform and more like heated adsorbent, although there is still a marked difference between this prewashed adsorbent and heated adsorbent, manifested

particularly by the pronounced maximum in the zone on the prewashed adsorbent.

The zone whose profile is shown in Figure 18 was chromatographed under the same conditions as that shown in Figure 15 except that 0.15 percent of ether was added to the 87-percent solution of benzene in ligroin. Although no isotherm was measured for this system, it is apparent that, qualitatively at least, the zone has the shape expected on the basis of the theory of chromatography. Thus here again, as in the development rate experiments, especially Experiment 13, Table 4, and in the profile shown in Figure 14, it appears that when the solvent on the column contains ether, even in traces, the behavior of the zone approaches the ideal.

In considering this phenomenon, it must be remembered that the column is completely wet with ether just before the ligroin is poured on during the prewash. Since ligroin is an extremely weak developer, a portion of the ether presumably remains adsorbed on the column after the ligroin has been poured through. Benzene, which is a stronger developer than ligroin, will remove the ether much more completely but even it evidently removes ether from the column fairly slowly. This was demonstrated in the following manner. About 125 ml. of benzene was poured through a prewashed column; after the first 20 ml. of the effluent benzene had been rejected the remainder was collected in several fractions. The ether-content of the benzene in each of these fractions was determined in an approximate manner refractometrically and also by tests of its developing strength; it was found that the first fraction contained somewhat more than one

percent of ether while the last still contained traces of ether*, too small to be detected with an Abbé refractometer but nevertheless demonstrable in development rate experiments. In Figure 19 is plotted the profile of a zone which was placed on a prewashed column from 32 ml. of a mixture which contained 13 percent of ligroin, and 87 percent of "benzene" from some of the intermediate fractions in the above experiment. The shape of this profile is strong confirmatory evidence of the presence of a few tenths of a percent of ether or some similar substance in the benzene.

With the aid of the experimental facts which have been discussed in the past few pages it seems to me that a reasonable explanation can be advanced for the fact that prewashed columns appear to be non-uniform in adsorptive strength with benzene-ligroin or benzene developers but quite uniform with developers which contain even traces of ether, and the related fact that a column of heated adsorbent seems quite uniform even with developers which contain no ether. A prewashed column normally contains a small quantity of adsorbed ether**, although this compound is probably rather weakly adsorbed. When a

* Neither of the methods used for 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 thus it may be that these substances, rather than ether itself, are present in the last fractions of benzene. It is difficult to see what other substances could be present under the circumstances; any compound which was not eluted from the column by the ether of the prewash could scarcely be removed by benzene.

** As mentioned above it is possible that impurities in the ether rather than the ether itself are responsible for the effects described here; this possibility has not been tested experimentally but in any event the qualitative explanation of the observed facts would not be altered.

compound whose adsorption affinity is comparable to or greater than that of the ether is placed on the column it will tend at least partially to displace the ether. If, however, this compound has a linear isotherm it will have no tendency to form a sharp-fronted zone behind the ether; rather, there will be a region, perhaps fairly wide if the adsorption affinities are of comparable orders of magnitude, where both compounds are present and where the presence of the ether may have a significant effect on the adsorption of the other compound, provided that the latter is not very strongly adsorbed. Furthermore, throughout this region the relative amounts of ether and the other compound will in general vary; in the usual case the second compound will be more strongly adsorbed than the ether and therefore in any region in which both are present the ether will tend to move somewhat more rapidly. As development proceeds or an undeveloped zone is widened, the upper part of the column, from which most of the ether has now been removed, will behave as a stronger adsorbent that it did earlier.

When, on the other hand, the chromatographic solvent itself contains ether, the environment of the compound being chromatographed will not vary appreciably during the experiment since a continual supply of ether is available to replace any which may be removed from the adsorbent. Consequently no especial non-uniformity is to be expected. Similarly, with heated adsorbent, which presumably has no readily removable compounds adsorbed on it, the compound being developed will in general be more strongly adsorbed than every component of the developer (except of course in displacement development)

and therefore will move in a region behind the front of the developer, that is, in a region where conditions remain always the same.

This interpretation of the observed facts could be tested in a fairly simple though not completely conclusive way by investigating the profile of a zone which was placed on the column after a similar zone had first been developed through the column. One difficulty of the analysis which has been presented is the explanation of the reason why ether, if indeed it is ether and not some minor impurity from the ether, is removed from the column only slowly by benzene in view of the fact that ether is under some conditions appreciably less strongly adsorbed than many compounds (e.g., 4-nitrotriphenylamine) which are essentially eluted by benzene.

It should be noted that the above argument, together with the experimental results, indicates that prewashed and dried adsorbent, even after evacuation on a Hyvac pump for several hours, still contains a small quantity of ether or some similar substance.

There is another effect illustrated in these profiles of introduced zones whose explanation is still unclear. It will be noticed that the quantity B was less in the top few millimeters for each of the zones introduced from a benzene-ligroin mixture; this was true even when heated adsorbent was used. However, when the solvent contained ether (Figures 14, 18, and 19), the initial portion of the zone was about normal or perhaps contained even slightly more than the expected amount of 4-nitrodiphenylamine.

Since the solution which is present in the interstices of the adsorbent contributes to the quantity B it might be expected

that there would be a slight decrease in B in the top few millimeters of the column if some of the solution were drawn out of this region of the column during the "sucking dry" of the column. 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; furthermore, the phenomenon can actually be detected visually before the column has "run dry". The effect does not appear to be due to any impurity in the benzene for it is identical with sodium-dried redistilled technical benzene, with sodium-dried Baker's C. P. benzene, and with sodium-dried benzene which had been purified with concentrated sulfuric acid according to the directions given by Fieser[34]. It is at least as pronounced with a 60-percent solution of benzene in ligroin (Figure 20) as with the 87-percent solution used in most of the experiments; it is to be noted that with this 60-percent solution of benzene in ligroin the initial decrement is about four times as great as could be accounted for by the volume of interstitial liquid present.

In Figure 21 is pictured the profile of a zone chromatographed under the same conditions as the zone in Figure 15 except that the benzene had not been dried over sodium before use. The benzene which was used had been dried over calcium chloride before distillation; evidently, however, it still contained a significant quantity of water. The initial plateau and sharp boundary in the profile presumably indicate the presence of a front of water which was moving very slowly down the column and diminishing the adsorption of 4-nitrodiphenylamine in the region where both were present. This profile is

of especial interest in emphasizing the need for rigorous drying of solvents at all times in the study of undeveloped zones; with developed zones this is less important because traces of water will be removed at the top of the column and will almost invariably move much more slowly than the zone of interest.

It seems doubtful that the presence of traces of water in the solvents even after drying over sodium could account for the diminution in B in the upper few millimeters of the column in the experiments whose results are pictured in Figures 15, 16, 17, and 20. If this were the explanation of the effect it would be difficult to understand why the addition of a trace of ether should cause it to be inoperative.

(b) Developed zones

The application of this method of measuring profiles to the study of developed zones has so far been hardly more than preliminary. The profiles which will be discussed (Figures 22 through 25) merely serve to illustrate the sort of uses to which the method can be put.

In Figure 22 is shown the profile of a zone which was placed on a prewashed column from 4.0 ml. of an 87-percent solution of benzene in ligroin and developed with 2 V ml. of the same mixture. The experimental points which have been plotted were taken from duplicate experiments; it is apparent that the reproducibility was excellent. Figure 22 serves to illustrate also the effect of downward coning on the position of the midpoint of the zone; the arrows

denote the position of the extreme and concentrated limits* of the zone as observed on the surface of the column, and show that the true midpoint of the zone lay somewhat below the apparent midpoint as measured on the surface.

All of the profiles which are pictured in Figures 22 through 25 show clearly that the distribution of material in these zones was very symmetrical and fitted approximately a probability distribution curve. The slight downward coning of the zones developed with benzene-ligroin probably tends to make even more pronounced the resemblance of the profiles measured in this way to a probability distribution function; however, it is to be noted that the profile of the unconed** zone which was developed with 2 V ml. of a 20-percent solution of ether in ligroin (Figure 25), was similar in shape.

The great disparity in the widths of a zone developed with benzene-ligroin and a similar zone developed with ether-ligroin is illustrated quite strikingly in Figure 25; 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. It seems reasonable to suppose that a significant factor, perhaps the chief factor, in the greater width of zones developed with benzene-ligroin lies in the progressive non-uniformity of a prewashed column with this developer. In this regard it would be

* See page 102.

** See page 134 and pages 89-90.

enlightening to study the profiles of zones developed on heated adsorbent; qualitative evidence already available shows that similarly developed zones are appreciably narrower on heated adsorbent than on prewashed adsorbent.

In Figures 23 and 24 are illustrated other examples of the application of this method to the study of developed zones. Figure 23 shows the profiles of zones containing equal quantities of material introduced from different initial volumes; it is interesting that these profiles are practically identical, for, according to the idealized theory, the zone introduced from 4 ml. should be four times as wide and one-fourth as high as that introduced from 1 ml. Figure 24 shows the profile of a zone containing 4 mg. as well as that of one containing 1 mg.; it is to be noted that the vertical scale for the 4-mg. sample is four times as great as that for the 1-mg. sample. Each zone was placed on a prewashed column from 4.0 ml. of an 87-percent solution of benzene in ligroin and was developed with 2 V ml. of the same mixture. The profiles are very similar except for the vertical scale factor of 4; this result, together with that shown in Figure 23, indicates that the chief factor which determines the distribution of material in such developed zones is the quantity of material on the column rather than the volume or concentration of the initial solution. However it must be emphasized that a much more detailed study, covering a wider range of amounts, concentrations, and volumes, would be necessary in order to substantiate this statement. Although LeRosen,[11c] using o-nitroaniline, benzene, and prewashed silicic acid, has made some experiments which might bear on this point most of the data which

he presents are of doubtful significance in view of the fact that his columns in all probability exhibited the same sort of progressive non-uniformity which was observed in the present work; he apparently was not aware of this fact. Furthermore many of his conclusions seem unwarranted even on the basis of the data which he presents.

E. Preliminary Investigations of the Rate of Adsorption on Silicic Acid-Celite from Organic Solvents

1. Introduction

It was necessary in connection with the measurement of adsorption isotherms that some idea be obtained of the length of time necessary for the attainment of adsorption equilibrium in the systems studied. Furthermore it was realized that if equilibrium was not established essentially instantaneously in the chromatographic process any agreement between the experimental results and those predicted by the theory based on an isotherm measured under equilibrium conditions would be mainly fortuitous. Therefore some experiments were made in order to investigate the length of time necessary for the attainment of equilibrium in a few systems typical of those which were used in the studies of development rates and profiles; these experiments will be described briefly here.

It was mentioned in the Introduction that no strictly comparable experiments could be found in the literature, although for adsorption on silica gel or alumina from aqueous solutions periods of time ranging from a few minutes to many hours have usually been reported to be necessary for the establishment of equilibrium[23,12]. In the present experiments the shortest time in which a measurement could be made after mixing the adsorbent and solution was between ten and fifteen seconds; no detectable departure from equilibrium could be found for any of the systems studied.

2. The method

Preliminary experiments which were made when the adsorption

isotherms were investigated revealed that in all of the systems studied the concentration of the solution which was present in the flask with the adsorbent reached a constant value within a very few minutes; the concentration was the same, within a fraction of one percent, after one hour as it had been after five minutes. Thus in the adsorption isotherm experiments, in which the solution and adsorbent were mixed merely by swirling the flask cautiously several times, it would have been entirely adequate to pipette samples of the solution within a few minutes after mixing the solution and adsorbent. Actually, for reasons of expediency, the mixtures were allowed to stand for about one-half hour, with occasional swirling. It was necessary of course to allow the adsorbent to settle after the final swirling before a sample of solution could be removed; settling was always essentially complete in less than one minute.

It was apparent that ordinary pipetting techniques would be inadequate for making a series of measurements of the concentration of the solution during the first few minutes after it had been mixed with the adsorbent, chiefly because of the excessive time required for filling and draining a pipette. In addition, the possibility of entrapping particles of suspended adsorbent would be very great during the removal of the first samples of solution by such a method; to wait for the adsorbent to settle would, of course, defeat the purpose of the experiment.

The method which was used in the present experiments was to remove samples of solution with a 1.00-ml. Yale Tuberculin Syringe

in a specially-designed holder* which permitted the rapid and reproducible withdrawal of any desired volume of solution up to 1.00 ml. A thin strip of filter paper was clipped over the short protruding tip of the barrel of the syringe so that it just covered the small hole in the tip; this strip of paper served reasonably well to keep adsorbent from entering the syringe when a sample was being withdrawn from a slurry which had not yet settled.

After a few preliminary experiments had been done, the following procedure was chosen as the most satisfactory. About 20 ml. of a solution of the compound in the developer was placed in a 100-ml. beaker and was stirred gently with the aid of a mechanical stirrer. Then 1 to 3 g. of prewashed and dried adsorbent was added over a period of a few seconds in a thin stream so that it would not form clumps. A sample of solution was withdrawn from the mixture at once; it was ejected from the syringe into a clean flask and another sample of solution was withdrawn immediately. Four or five samples were taken in this manner; the solvent was evaporated from each and the quantity of the residue was estimated spectrophotometrically. In all experiments the proportions of adsorbent and solution were so chosen that at least 30 percent of the quantity of the compound present would be adsorbed at equilibrium, and thus an appreciable diminution in the concentration of the solution would occur.

It was necessary to perform several control experiments first in order that some idea might be gained of the precision of the method. In the first of these experiments a series of samples was

* This holder had been designed by Mr. William Schuelke and Dr. Reuben E. Wood.

taken from a solution which had not been mixed with adsorbent; the solution which was used contained about 0.16 mg. of 4-nitrotriphenylamine per ml. of 1:2 benzene-ligroin. Seven one-ml. aliquot portions were taken and the quantity of 4-nitrotriphenylamine in each was determined; the maximum variation from the average was less than one percent and most of the results agreed to within about 0.5 percent. Thus the syringe itself was entirely satisfactory for sampling.

Next an experiment was done with a slurry which had been stirred for about ten minutes and which, therefore, presumably had reached equilibrium. The slurry had been prepared from a solution containing about 0.3 mg. of 4-nitroaniline per ml. of a 5-percent solution of ether in benzene. Four samples were taken at 30-second intervals; the results of the analyses of these samples are presented in Table 7.

Table 7

The Content of 4-Nitroaniline in Successive One-Ml. Samples Withdrawn from a Slurry in a Control Experiment

Sample	Time after preparation of slurry, seconds	Amount of 4-nitroaniline, mg.
1	560	0.166
2	590	.166
3	620	.171
4	650	.165

The results shown in Table 7 were substantiated in a similar experiment with 4-nitrotriphenylamine and 1:1 benzene-ligroin; these experiments indicated that the method of sampling from the slurries

was in general precise to within one percent but that individual variations of as much as 3 or 4 percent might be expected. These occasional erratic variations appeared to be caused, at least in part, by the occasional entrapment of small clumps of moist adsorbent between the tip of the syringe and the filter-paper strip which covered the tip and prevented the entrance of adsorbent into the syringe. An attempt was made to dispense with the strip of filter paper entirely but severe difficulties were encountered with particles of adsorbent which entered the syringe. No further effort was made to improve the method since the chief purpose of the present studies was merely to determine whether there was any appreciable effect.

3. Results and discussion

Four systems were selected for study: 4-nitrotriphenylamine in 1:1 benzene-ligroin and in a 1.7-percent solution of ether in ligroin, 4-nitroaniline in a 5-percent solution of ether in benzene, and ethyl centralite in a 20-percent solution of ether in ligroin. The results of the experiments with these systems are summarized in Table 8.

Table 8

Results of Experiments in Which an Attempt Was Made to Determine the Rate of Establishment of Adsorption Equilibrium on Prewashed and Dried Silicic Acid-Celite from Organic Solvents

Compound	Developer	Original concentration, mg. per ml.	Time of sampling, seconds after preparation of slurry	Concentration in sample, mg. per ml.
4-Nitrotriphenylamine	1.7% Ether in ligroin	0.33	10	0.173
			30	.178
			60	.178
			110	.161
			160	.171
4-Nitrotriphenylamine	1:1 Benzene-ligroin	.48	15	.322
			45	.354
			80	.355
			110	.356
4-Nitroaniline	5% Ether in benzene	.30	12	.185
			45	.178
			65	.186
			110	.187
			160	.180
Ethyl centralite	20% Ether in ligroin	4.6	12	2.54
			35	2.34
			55	2.56
			120	2.47

Although the results which are shown in the Table display some erratic variations, they do seem to demonstrate reasonably well that the equilibrium concentration is reached in these systems in less than fifteen seconds. It should be noted that in a chromatographic experiment the contact between adsorbent and solution will be even more efficient than it was in these experiments and therefore equilibrium should be established at least as rapidly as it was in the present experiments.

I believe that perhaps an even more sensitive test of whether equilibrium is really "instantaneous" might be made by studies of the relative spreading of a zone on development at different flow rates. A preliminary experiment of this sort has already been made; it was found that decreasing the flow rate from the normal by a factor of four had no perceptible effect on the width of, or the value of R for, a zone of 4-nitrodiphenylamine developed on prewashed adsorbent with benzene. A more careful study of this effect could be made using the technique of measuring the profiles of zones which was described in Section D. It would probably be best to avoid using benzene-ligroin or benzene developers on prewashed adsorbent since in such systems the progressive non-uniformity of the column might complicate the situation.

Summary

An experimental study has been made of some features of the process of chromatography on silicic acid-Celite columns, and in particular of the degree to which chromatograms on this adsorbent conform to the simple predictions of the idealized theory of chromatography.

It has been found that adsorption isotherms on silicic acid-Celite are essentially linear in the range of concentrations most important in practical chromatography on this adsorbent and that development rates predicted from these isotherms generally agree very well with those observed experimentally. Furthermore, for the few systems for which the development rates seem to be anomalous a reasonable explanation can be offered in terms of the special properties of silicic acid-Celite columns which have been activated by prewashing.

A technique has been developed for determining the distribution of a compound in a zone on a column (the "profile" of the zone) by cutting the column into many thin sections and determining the amount of the compound in each. Some applications of this technique are described; it has proved especially valuable in the study and clarification of certain phenomena which at first appear to be anomalous.

A few experiments are described in which it was demonstrated that equilibrium is established in less than fifteen seconds in the adsorption of certain compounds on silicic acid-Celite from dilute solutions in organic solvents.

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Part IV

A Method for the Study of the Absorption Spectra of Adsorbed Compounds

Part IV. A Method for the Study of the Absorption Spectra of Adsorbed Compounds

A. Introduction

The light-absorbing properties of many compounds frequently change markedly when the compound passes from the unadsorbed to the adsorbed state. Thus, for example, when a pale yellow compound is adsorbed on silicic acid it usually acquires a golden yellow color; similarly a compound which is normally yellow-orange in color may appear orange-red when adsorbed, and so on. As a preliminary to the investigation of the changes which take place in the spectra of compounds on adsorption a method has been devised for the measurement of the absorption spectra of adsorbed compounds. It will be described briefly here; to date, it has been used only in the study of the near-ultraviolet and visible spectra of colored compounds on silicic acid. However, it seems likely that the method can be extended to certain other adsorbents and perhaps also to the investigation of spectra in the region below 300 mμ.

No effort will be made here to discuss all of the previous investigations of changes in color on adsorption which have been reported; rather, reference will be made only to some of the more important work in this field. De Boer and his collaborators have made a number of investigations of the absorption spectra of adsorbed

compounds; generally they used a vacuum-sublimed film of an alkaline-earth fluoride as the adsorbent. For example, de Boer and Custers have studied the spectra of iodine[1] and of p-nitrophenol[2] adsorbed on a film of calcium fluoride. The spectra of dyes adsorbed on colloids have been investigated by Fodiman and Kargin[3] and Michaelis and Granick[4]. Several Russian investigators have studied the spectra of a few selected substances (e.g., iodine, ammonia, acetone, and benzene) adsorbed on transparent silica gel, alumina, and other similar substances[5,6,7,8,*]. Schwab and Issidoridis[9] measured the reflection spectra of certain inorganic ions on chromatographic columns of alumina, although the technique was not very sensitive. Weitz, Schmidt, and Singer[10] have investigated qualitatively the changes in color of a variety of organic substances when adsorbed on silica gel and similar adsorbents, and Meunier[11] has discussed briefly a few similar systems.

In connection with the detailed investigations of chromatographic phenomena which are reported in Part III of this Thesis it seemed to me that a simple and practical method for studying the spectra of compounds adsorbed on silicic acid might prove valuable in helping to elucidate the nature of the adsorbed state. However, no simple method for the investigation of these spectra seemed available until an observation which had been made by Trappe[12] was noted; he found that a column of silicic acid became semi-transparent when it was wet with chloroform and no pressure was applied. This observation led me to believe that perhaps the absorption spectrum of a compound

* Since only abstracts of most of these papers are available at present I am not familiar with the details of the experiments.

adsorbed on silicic acid could be measured simply in a slurry prepared from silicic acid and a solution of the compound in chloroform (provided, of course, that the compound was adsorbed from chloroform). A little experimentation showed that this idea was correct. Furthermore, other solvents and solvent mixtures of refractive index similar to that of chloroform proved to be as good as, and in some cases better than, chloroform in producing transparency of the silicic acid which was used. In particular, carbon tetrachloride and certain benzene-ligroin mixtures were about as good as chloroform, methylene chloride seemed superior to it, and a mixture of benzene and dioxane which contained about 70 percent of dioxane produced almost complete transparency. The disadvantage of the latter mixture was of course that few compounds are strongly adsorbed in the presence of so much dioxane.

Since mixtures of benzene and ligroin are common in chromatography, it seemed of most practical interest to investigate first the spectra of compounds adsorbed from such mixtures. The spectra of 4-nitroaniline and 4-nitrotriphenylamine adsorbed on both unheated and heated silicic acid from benzene-ligroin mixtures will be reported here; the spectra of these same compounds in a variety of solvents are included for the purpose of comparison. All of the measurements of spectra were made with a Beckman spectrophotometer.

B. The Experimental Method

1. Preliminary experiments

It was necessary to determine first the composition of the mixture of benzene and 60-70° ligroin which would produce the maximum transparency of the silicic acid to be used. With unheated silicic acid*, it was found that the maximum transparency was produced when the mixture contained between 40 and 50 percent of benzene by volume ($n_D \approx 1.43$ to 1.44); since the mixture which contained 40 percent of benzene, that is, 2:3 benzene-ligroin, had the advantage of being less of an eluent than 1:1 benzene-ligroin, it was used in the experiments with unheated silicic acid. With heated silicic acid* the maximum transparency occurred with a mixture containing about 65 to 70 percent of benzene ($n_D \approx 1.46$ to 1.465); a 2:1 mixture of benzene and ligroin was therefore used in the experiments with heated silicic acid.

Since these slurries of maximum transparency were still somewhat cloudy it was felt that an absorption cell thinner than the 1-cm. cells which are supplied with the Beckman instrument would be desirable. Therefore two small cells were constructed; each of these cells is 3.0 mm. thick, 25 mm. wide and 38 mm. high, and was constructed from a U-shaped piece of brass plate 3 mm. thick and two flat 25 x 38-mm. glass plates. The glass plates, which serve as the windows of the cell, were cut from two 25 by 76-mm. microscope slides; because these two slides had different transparencies to near-ultraviolet radiation, one

* Merck reagent silicic acid, code number 40446, was used; see page 93 of this Thesis.

of the two windows on each cell is from one of the slides and the second is from the other slide. Thus the transparencies of the cells themselves are very nearly identical throughout the range of wave lengths for which they can be used; they cannot be used below about 300 mμ. Each of the cells has a small brass cover which fits fairly securely and minimizes evaporation of the solvent from the slurry in the cell. A special cell-holder, of the proper size to fit in the cell compartment of the Beckman spectrophotometer, was constructed to hold these two cells during spectrophotometry.

In order that the cells might be cleaned readily it seemed desirable that the windows not be cemented to the brass body of the cell. Hence it was necessary to find some sort of wax or grease that could be used to prevent the solvent from leaking out at the glass-brass interface, and also a means of holding the cell together firmly but in such a manner that it could readily be disassembled. The latter requirement was easily met; small spring clips of phosphor-bronze are slipped over the side edges of the cell and serve very well to hold the glass windows firmly against the brass plate. It proved somewhat more difficult to find a satisfactory means of preventing leakage from the cell. A thin film of silicone grease between the glass and brass surfaces prevents leakage from a slurry of silicic acid and benzene-ligroin, although if benzene alone is in the cell it leaks out slowly. Silicone grease cannot be used with chloroform however. I believe that the Carbowaxes might be the most satisfactory greases for use with chlorinated hydrocarbons and benzene; at the time that these experiments were done however, no samples of these materials

were immediately available. Such things as paraffin, sealing wax, and the ordinary stopcock greases proved unsatisfactory. Since the silicone lubricant was adequate, although not ideal, it was used in these preliminary experiments, in all of which the solvent was a mixture of benzene and ligroin.

It was next necessary to devise a standard method for the preparation of slurries, one that would yield a slurry which would not settle on standing but which would have a low enough viscosity so that it could readily be poured from the vessel in which it was prepared into the absorption cell. It was also necessary to determine whether such slurries could be prepared reproducibly, that is, whether two slurries prepared simultaneously in an identical fashion were equally transparent at all wave lengths. The latter point was a most important one for upon it depended the sensitivity of this method of measuring the spectra of adsorbed compounds.

A little experimentation showed that a slurry of the optimum viscosity could be prepared from 3.15 g. of unheated silicic acid and 7.0 ml. of 2:3 benzene-ligroin, or from 2.90 g. of heated silicic acid and 7.0 ml. of 2:1 benzene-ligroin. The relative transparency of duplicate slurries was investigated several times and it was found that the reproducibility in the preparation of slurries was excellent. The optical density, or extinction, of one slurry relative to another similar one was seldom more than 0.01, and was usually less than this; furthermore it varied only very slowly with wave length so that its effect on the shape of the spectral curve was almost completely negligible, while its effect on the height of the spectral curve

amounted to only 1 or 2 percent at an absorption maximum. There was some indication that reproducibility was a little harder to attain with heated silicic acid than with the unheated adsorbent; however too few experiments were done to permit a definite conclusion to be reached. In any event the error arising from this cause was completely negligible in the present preliminary experiments.

In order that some idea could be obtained of the error which might arise from a slight variation in the proportions taken for the slurry, a slurry was prepared with 10 percent more silicic acid than usual. The optical density of this slurry was about 0.07 greater than normal at 320 mμ and decreased slowly with increasing wave length to about 0.03 at 440 mμ. Thereafter it remained fairly constant with increasing wave length. This experiment showed that an error of a few percent in the proportions taken for the slurry would have an almost negligible effect on the shape of the absorption curve.

2. The procedure for the measurement of the spectra of adsorbed compounds

In the preparation of slurries for the measurement of absorption spectra it was first necessary to prepare a solution of suitable concentration in 2:3 or 2:1 benzene-ligroin; for 4-nitroaniline, the concentration was about 0.03 mg. per ml. and for 4-nitrotriphenylamine about 0.055 mg. per ml. Two samples of silicic acid were then weighed into 25-ml. beakers; as mentioned earlier, 3.15 g. of unheated silicic acid or 2.90 g. of heated silicic acid was used. Exactly 7.0 ml. of the appropriate solution was then added to one of the samples of silicic acid, the resulting mixture was carefully

stirred for one to two minutes until it was thoroughly homogeneous, and was then poured into one of the cells. Care was taken to remove any air bubbles that might be trapped in the cell. The covers were placed on the cells after the windows had been cleaned carefully with a piece of absorbent tissue. A second slurry was prepared at once in an identical manner except that the benzene-ligroin mixture which was used contained no dissolved compound; this slurry was poured into the second of the two cells and was used as a blank in the usual manner in the operation of the Beckman spectrophotometer.

It was found that the optical density of a slurry prepared from unheated silicic acid did not change with time, at least during the first hour, a period of time more than sufficient for a complete measurement of the spectrum. With heated silicic acid there seemed to be a slight drift in the optical density at low wave lengths (below about 350 mμ) during the first ten minutes but thereafter the density remained constant; this effect was observed with the blank slurries as well as with those which contained adsorbed compounds. Because of this effect, slurries which contained heated adsorbent were allowed to stand for about fifteen minutes before measurements were taken.

C. Results and Discussion

The results which are reported here are intended merely to illustrate the application of this method of measuring the absorption spectra of adsorbed compounds. These results are summarized in the accompanying Tables and Figures. In addition to the spectra of 4-nitroaniline and 4-nitrotriphenylamine adsorbed on both unheated and heated silicic acid from benzene-ligroin mixtures, spectral data for each of these compounds in a variety of solvents are presented for purposes of comparison.

Duplicate experiments were made in each of the measurements of the spectrum of an adsorbed compound. The agreement in the shapes of the absorption curves and the positions of the maxima and minima was in all cases excellent in these duplicate experiments. The agreement in the value of the spectrophotometric factor*, C/D, was generally quite good also. It must be noted that there is not complete adsorption in any experiment conducted in the manner described here. In order that the percentage of a compound adsorbed in any system might be calculated a point on the adsorption isotherm at an appropriate concentration was determined for each of the combinations of compound, adsorbent, and solvent which was used. From the known ratio of adsorbent to solvent in the slurry it was then possible to calculate the fraction of the compound which was adsorbed and the fraction which was still in solution. Then, with the aid of the known spectrum of the compound in the benzene-ligroin mixture, it was possible to correct

* See pp. 30-31 for a definition of this term.

the data observed with the entire slurry for the contribution of the unadsorbed compound and thus to obtain the spectrum of the adsorbed material.

In order that the spectrophotometric factors could be calculated for the adsorbed compounds in the slurries, it was necessary that the concentration of the compound be known; therefore an approximate measurement of the final volume of each of the types of slurries was made by preparing the slurry in a graduated cylinder. It was found that with unheated silicic acid the original 7.0 ml. of liquid and 3.15 g. of silicic acid yielded a final volume of about 8.5 ml., whereas with heated silicic acid the final volume of the slurry was about 8.3 ml. Thus the concentration of the adsorbed compound was calculated by multiplying the original concentration of the solution by 7/8.5 or 7/8.3 and by the fraction of the compound which was adsorbed. Since the spectrophotometric factor is defined for an optical path of 1.000 cm. and the cells here were only 0.30 cm. thick it was necessary to multiply the observed maximum optical density by 10/3 in order to get the proper optical density for the calculation of the spectrophotometric factor.

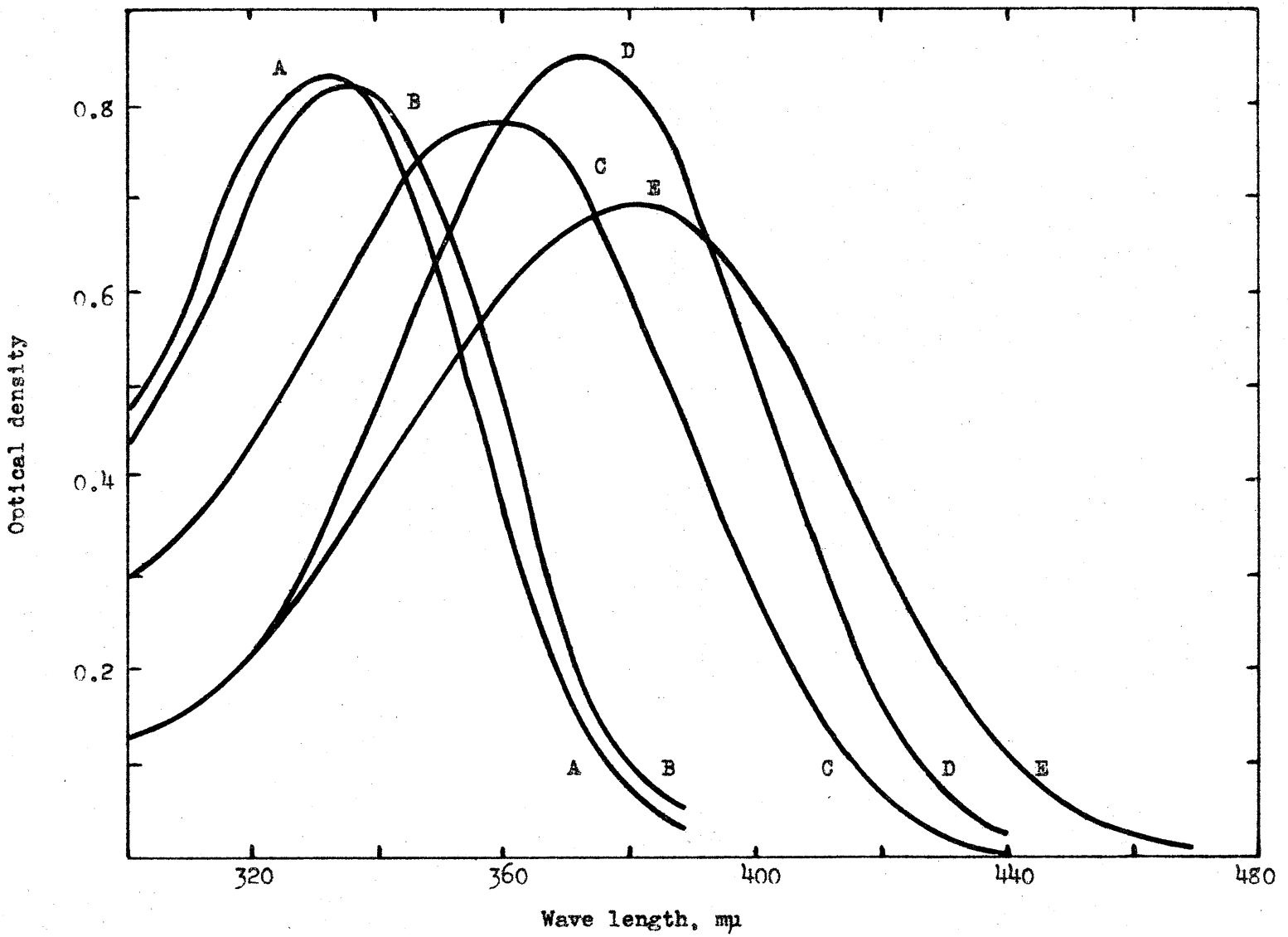

Figure 1 Optical densities of solutions of 4-nitroaniline in a 1.00-cm. cell: A, ca. 0.8 mg. per 100 ml. in 2:3 benzene-ligroin; B, ca. 0.8 mg. per 100 ml. in a one-percent solution of ethanol in 2:3 benzene-ligroin; C, ca. 0.8 mg. per 100 ml. in a 20-percent solution of ethanol in 2:3 benzene-ligroin; D, ca. 0.73 mg. per 100 ml. in absolute ethanol; E, ca. 0.72 mg. per 100 ml. in a 5-percent solution of ethanol in water.

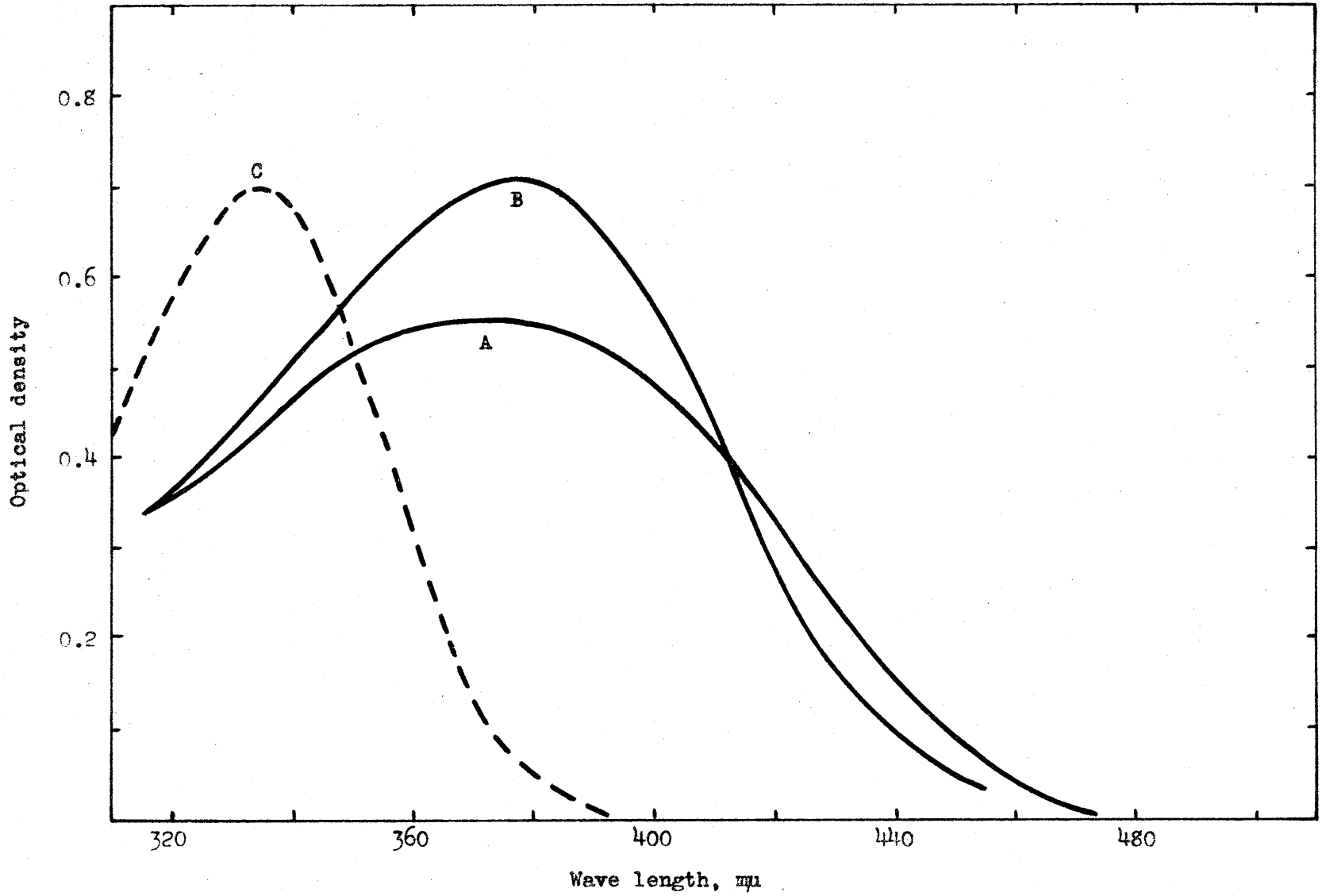

Figure 2 Optical densities of 4-nitroaniline: A, in a slurry prepared from heated silicic acid and 2:1 benzene-ligroin, total concentration ca. 2.3 mg. per 100 ml., cell thickness 0.30 cm.; B, in a slurry prepared from unheated silicic acid and 2:3 benzene-ligroin, total concentration ca. 2.3 mg. per 100 ml., cell thickness 0.30 cm.; C, in solution in 2:3 benzene-ligroin, ca. 0.70 mg. per 100 ml., cell thickness 1.00 cm.

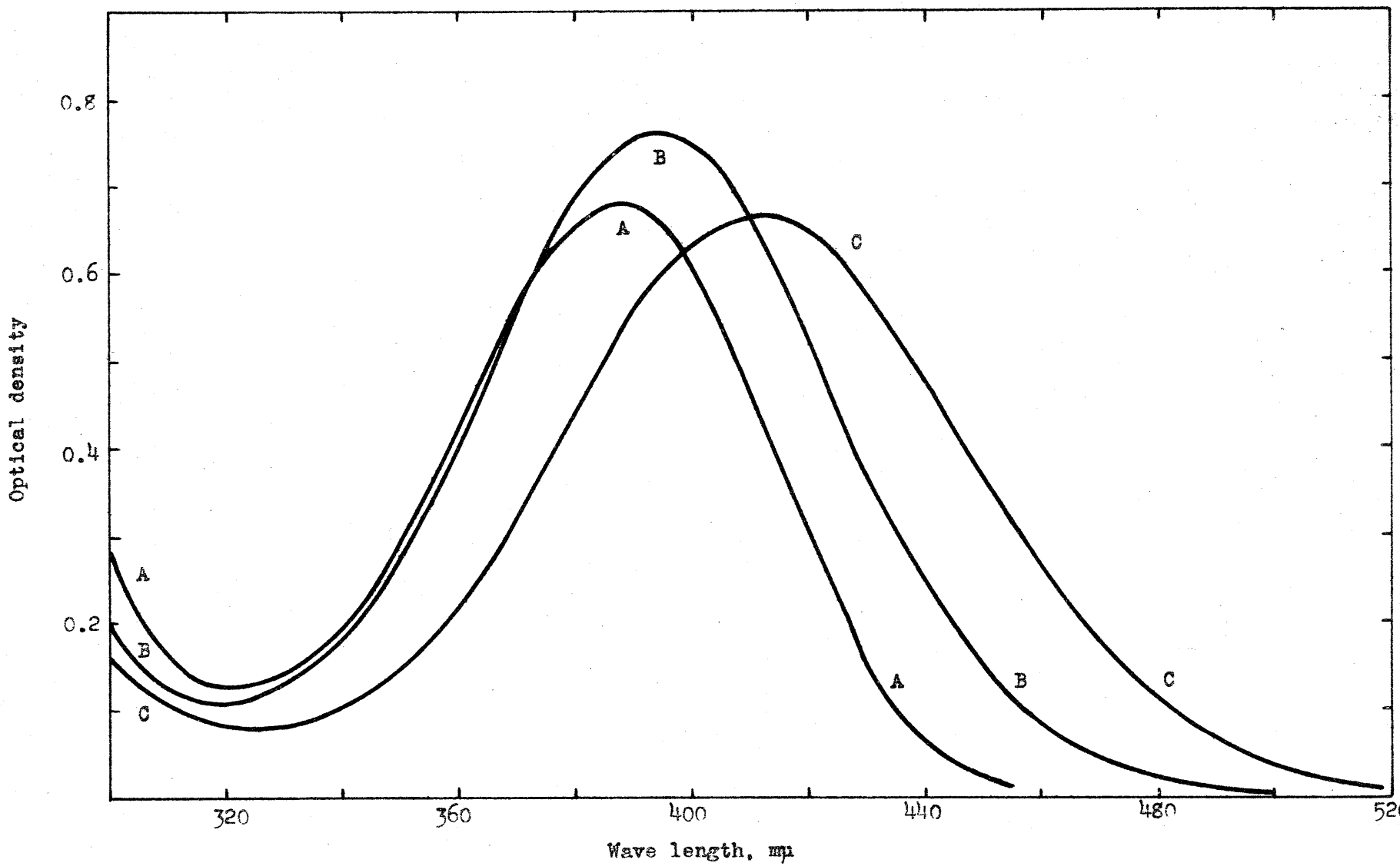

Figure 3 Optical densities of solutions of 4-nitrotriphenylamine in a 1.00-cm. cell: A, *ca.* 1.1 mg. per 100 ml. in 2:3 benzene-ligroin; B, *ca.* 1.2 mg. per 100 ml. in absolute ethanol; C, unknown concentration in 1:2 ethanol water

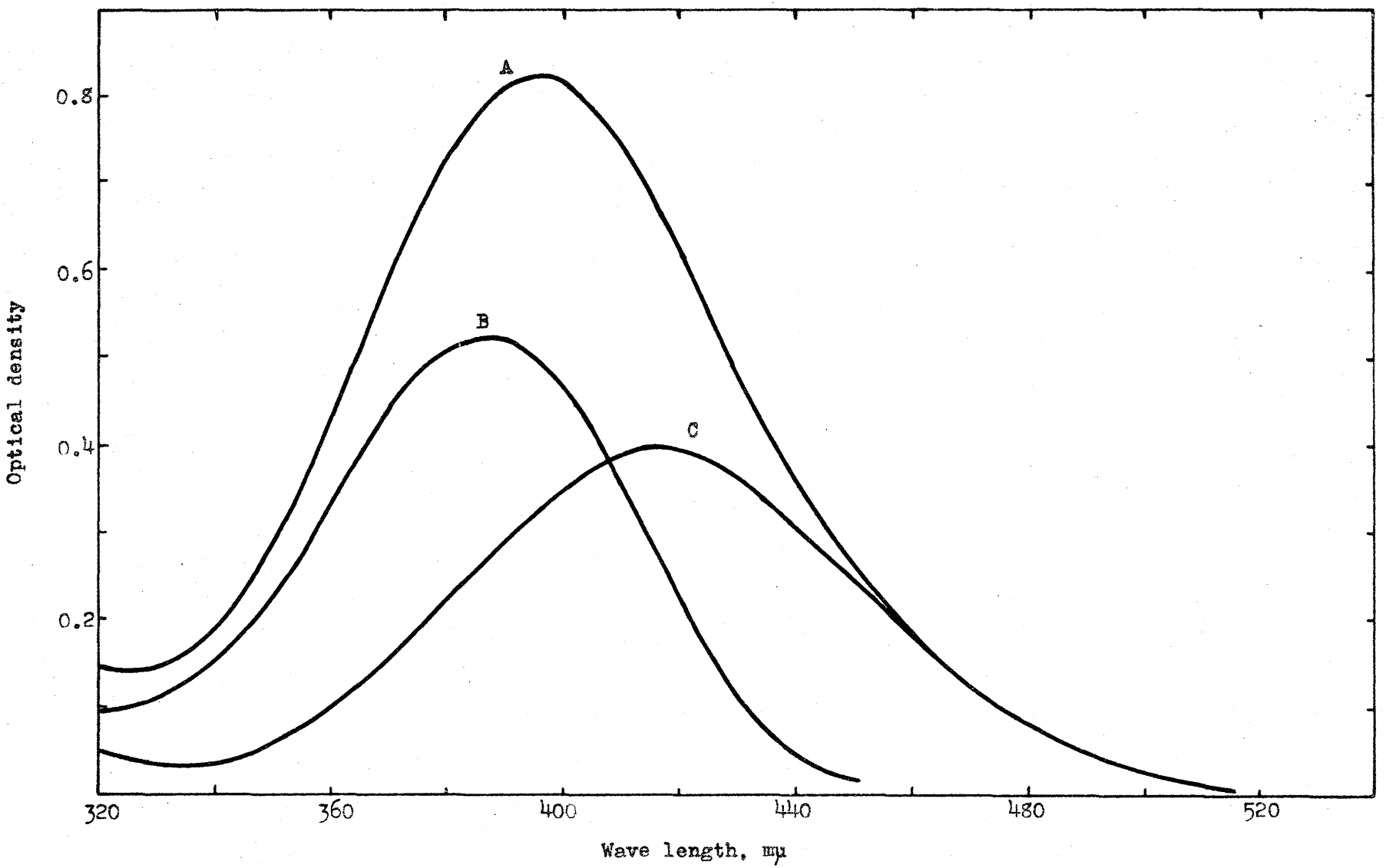

Figure 4 Optical densities of 4-nitrotriphenylamine in a slurry prepared from unheated silicic acid and 2:3 benzene-ligroin, total concentration ca. 4.5 mg. per 100 ml., cell thickness 0.30 cm.: A, total slurry; B, contribution of the 62 percent of the compound which is in solution; C, contribution of the 38 percent of the compound which is adsorbed

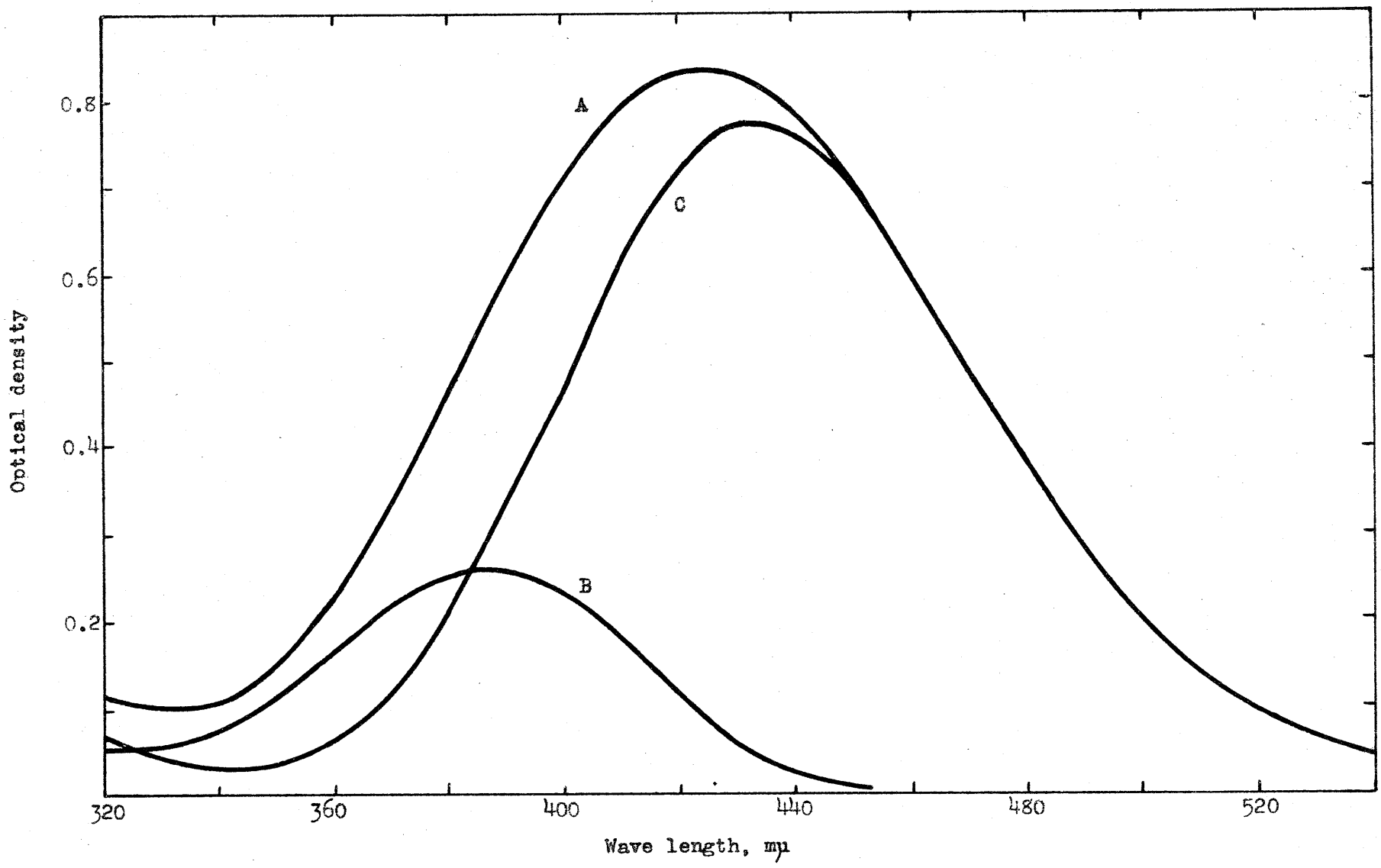

Figure 5 Optical densities of 4-nitrotriphenylamine in a slurry prepared from heated silicic acid and 2:1 benzene-ligroin, total concentration ca. 4.5 mg. per 100 ml., cell thickness 0.30 cm.: A, total slurry; B, contribution of the 30 percent of the compound which is in solution; C, contribution of the 70 percent of the compound which is adsorbed

Table 1

Results of the Determinations of the Quantity Which Was Adsorbed in Each of the Slurries

Compound	Type of silicic acid	Solvent, ratio of benzene to ligroin	Percentage adsorbed
4-Nitroaniline	Unheated	2:3	93
	Heated	2:1	99
4-Nitrotriphenylamine	Unheated	2:3	38, 39*
	Heated	2:1	70

* Duplicate determination

Table 2

Spectrophotometric Data for 4-Nitroaniline and 4-Nitrotriphenylamine Adsorbed on Unheated and Heated Silicic Acid from 2:1 and 2:3 Benzene-Ligroin

Compound	Type of silicic acid	Wave length of maximum absorption, mμ	C/D for adsorbed compound, mg. per 100 ml. for 1.00 cm.
4-Nitroaniline	Unheated	374-379	ca. 1.0
	Heated	366-378	ca. 1.29
4-Nitrotriphenylamine	Unheated	415-420	ca. 1.28
	Heated	430-435	ca. 1.24

Table 3

Spectrophotometric Data for 4-Nitroaniline and 4-Nitrotriphenylamine in Solution in Various Solvents

Compound	Solvent	Wave length of maximum absorption, mμ	C/D, mg. per 100 ml. for 1.00 cm.
4-Nitroaniline	2:3 Benzene-ligroin	330-333	ca. 1.00
	1% Ethanol in 2:3 benzene-ligroin	334-336	
	20% Ethanol in 2:3 benzene-ligroin	358-363	
	Ethanol	370-372	0.872
	5% Ethanol in water	378-382	ca. 1.1
4-Nitrotriphenylamine	2:3 Benzene-ligroin	386-389	ca. 1.6
	Ethanol	391-394	1.567
	1:2 Ethanol-water	410-412	

The results given in Table 2 for 4-nitroaniline on unheated silicic acid may be slightly in error because no correction was made for the small portion (7 percent) of the compound which remained in solution in the slurry. Inspection of the curves in Figure 2 will show that this correction would have been almost negligible at the absorption maximum; the curves to be considered are those labeled B and C. If 7 percent of the height of curve C at the wave length

at which B shows a maximum is subtracted from B, the shift in the position of the absorption maximum in curve B will be at most a very few millimicrons, and since the maximum appears to be about 5 mμ wide this shift will be quite unimportant.

It will be noted from Figures 4 and 5 that for 4-nitrotriphenylamine on both unheated and heated silicic acid the shift in the position of the maximum from that observed in the curve for the total slurry to that for the adsorbed compound alone is appreciable. For 4-nitroaniline, on the other hand, the shift is very slight with unheated silicic acid, as discussed above, and with heated silicic acid only about 1 percent of the compound remains in solution so that the observed curve is essentially that due to the adsorbed compound.

The qualitative differences in the form of the spectral curves for the adsorbed compounds are quite marked; the curves are much broader and flatter for 4-nitroaniline than for 4-nitrotriphenylamine. For 4-nitroaniline there is a large bathochromic shift in the position of the absorption maximum as ethanol is added to the solution in benzene-ligroin; for 4-nitrotriphenylamine this shift is only a few millimicrons. On the other hand, for 4-nitrotriphenylamine there is a great difference between the position of the absorption maximum for a solution in ethanol and that for the adsorbed compound, whereas for 4-nitroaniline this difference is very small. For each of the compounds there is a very pronounced bathochromic shift of this entire region of the spectrum when adsorption takes place from benzene-ligroin. For each of the compounds there appears to be a distinct difference between the absorption curve for adsorption on unheated silicic acid

and that for adsorption on heated silicic acid. With 4-nitroaniline this is manifested by a difference in the form of the curve but not particularly in the position of the maximum; the absorption of 4-nitroaniline adsorbed on heated silicic acid extends further into the longer wave length region and the curve is generally flatter and broader than that for this compound adsorbed on unheated silicic acid. With 4-nitrotriphenylamine the absorption maximum on heated silicic acid is shifted about 15 mμ toward the red from that on unheated silicic acid, and the absorption extends about 50 mμ further towards the red.

No further discussion of these results will be presented here because this report is intended merely to illustrate the application of the method. In its present form the method is not suitable for the investigation of spectra at wave lengths below about 300 mμ; an extension to this region would necessitate the use of quartz windows and solvents other than benzene and 60-70° ligroin, which are not sufficiently transparent below about 280 mμ. There seems no reason why the method could not be used with other adsorbents which have suitable optical properties (negligible absorption in the region of the spectrum to be investigated and fairly uniform refractive index). In any event the method as developed here will permit the study of the spectra of a wide variety of compounds adsorbed on silicic acid and should yield information which may aid in an understanding of the nature of the adsorbed state.

Summary

A method has been developed for the study of the absorption spectra of adsorbed compounds. Applications of this method to the measurement of the spectra of 4-nitroaniline and 4-nitrotriphenylamine adsorbed on unheated and heated silicic acid from mixtures of benzene and ligroin are described.

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10. E. Weitz and F. Schmidt, Ber. 72B, 1740, 2099 (1939); E. Weitz, F. Schmidt, and J. Singer, Z. Elektrochem. 46, 222 (1940); ibid., 47, 65 (1941)

11. P. Meunier, Compt. rend. 217, 449 (1943)

12. W. Trappe, Biochem. Z. 306, 316 (1940)

Summary of the Thesis

Part I of this Thesis contains a description of an investigation of the coordination complexes which are formed by silver ion with ethylene and propylene.

In Part II is presented a description of the application of chromatographic-spectrophotometric methods to the development of a procedure for the isolation and estimation of any or all of about ten nitramino compounds related to RDX when these compounds occur as minor impurities in RDX.

An experimental study of chromatography on columns of silicic acid-Celite is described in Part III. It is demonstrated that adsorption isotherms on this adsorbent are essentially linear in the range of concentrations important chromatographically and that under controlled conditions chromatograms on silicic acid-Celite conform very well to several of the simple predictions of the idealized theory of chromatography. Certain anomalous properties of columns which have been activated by prewashing are illustrated and an explanation of these anomalies is offered.

A description of a method for the study of the absorption spectra of adsorbed compounds comprises Part IV of this Thesis. A few applications of this technique are illustrated.

Propositions

1. The phenomena of coning and "surface spreading" of a chromatographic zone on a column of silicic acid-Celite are related to the nature of the material of which the chromatographic tube is made and, for the tubes made of pyrex glass at least, to the previous history of the tube (this Thesis, pp. 89-90). It is proposed that these facts are best explained as manifestations of electrokinetic effects.

2. (a) A simple and practical method is proposed for the study of the absorption spectra of compounds adsorbed on silicic acid and similar adsorbents.

(b) The suggestion of Roncato (1) that examination of the ultraviolet absorption spectrum would aid in distinguishing between (physical) adsorption and chemical processes is demonstrably valueless in view of the pronounced changes which frequently take place in the absorption spectra of molecules upon adsorption.

(1) A. Roncato, Arch. ital. biol. 74, 146 (1924); C. A. 19, 2446 (1925)

3. I propose the widespread adoption of the convenient term "ron" in place of the awkward and confusing "and/or" for the expression of the concept "either or both".

4. (a) I propose that Martin and Synge's derivation of a theory of chromatography (1) is basically unsound in assumptions ron mathematical details, in spite of the fact that it yields an apparently reasonable result for the shape of a zone and a verifiable expression for the rate of movement of a zone.

(b) Since the expression which Martin and Synge obtain for the rate of movement of a zone can be shown to be formally identical with that predicted by the idealized theory of chromatography of Wilson, DeVault and Weiss (2, 3, 4), the experimental verification of this relation in partition chromatograms is no indication of the merit of the theory of Martin and Synge. Rather it merely indicates that the mechanism by which the solute is distributed between the two phases in a chromatographic experiment is unimportant (as long as equilibrium is established reasonably rapidly).

(1) Biochem. J. 35, 1358 (1941)
(2) J. Am. Chem. Soc. 62, 1583 (1940)
(3) ibid., 65, 532 (1943)
(4) J. Chem. Soc. 1943 297

5. No single explanation suffices for the various examples of the double zone effect (this Thesis, p. 45) which have been observed to date. At least three distinct mechanisms can be proposed for true examples of double zone formation and several others for the spurious examples which can and do arise.

6. It has apparently not heretofore been recognized that the conjugated double bond system of the naturally occurring carotenoid rhodoxanthin (1) is unique among those of the natural C_{40} carotenoids, in that it contains no central stereochemically effective double bond. Rhodoxanthin contains only 4 stereochemically effective double bonds, rather than the 5 which have been attributed to it (2), and consequently should have only 10 isomeric forms rather than 20. It might be expected that cis-trans isomerism would occur less readily for this compound than for other carotenoids with conjugated systems of similar length.

(1) R. Kuhn and H. Brockmann, Ber. 66, 828 (1933)
(2) L. Zechmeister, Chem. Rev. 34, 278 (1944)

7. A detailed investigation of the possible complex between silver ion and benzene has not yet been made because of the difficulty of analyzing for benzene rapidly and reliably. I propose that this analysis be made spectrophotometrically.

8. The work of Cassidy (1) on the relation between the adsorption isotherms and chromatographic sequence of fatty acids from ligroin and petroleum ether on various adsorbents is usually cited in support of the claim that a comparison of adsorption isotherms is of uncertain value in the prediction of relative positions in a chromatographic experiment. I submit that most of Cassidy's comparisons were made under extremely unfavorable circumstances and that they can not be considered to constitute a satisfactory test of the value of adsorption isotherms in such predictions.

(1) J. Am. Chem. Soc. 62, 3073, 3076 (1940)

9. I propose a simple modification in the conditions of the Goldberg synthesis (1, 2) of mono- and poly-nitrodiphenylamines in order to improve the yield and greatly simplify the procedure for the isolation and purification of the product.

(1) I. Goldberg, Ber. 40, 4541 (1907)
(2) T. L. Davis and A. A. Ashdown, J. Am. Chem. Soc. 46, 1054 (1924); Ind. Eng. Chem. 17, 674 (1925)

10. (a) Simple qualitative reasoning leads to the conclusion that for a given adsorbent the relative eluting powers of a series of solvents should be predictable from a consideration of their adhesion tensions. Predictions made on such a basis are, at least approximately, in accord with experimental facts.

(b) Much confusion and many untenable interpretations of adsorption phenomena have arisen through failure to consider all of the possible interactions between the three components in even the simplest systems of one solvent, one solute, and one adsorbent. In particular, the importance of inter- and intra-molecular hydrogen bonding in adsorption on adsorbents such as magnesia, alumina, and silicic acid has usually (although not always (1)) been overlooked. Thus, for example, Arnold (2) failed to understand why picric acid was adsorbed more strongly than o-nitrophenol on alumina, and Williams (3) offered a needlessly complex and rather unreasonable explanation for the fact that the adsorption of fatty acids and alcohols on polar adsorbents decreases with increasing unsaturation, while for the similar hydrocarbons and esters the order is reversed. Simple explanations can be offered for these and similar phenomena.

(1) A. L. Elder and R. A. Springer, J. Phys. Chem. 44, 943 (1940)
(2) R. T. Arnold, J. Am. Chem. Soc. 61, 1611 (1939)
(3) K. A. Williams, Analyst 71, 259 (1946)

11. The values which Adkins (1) presents for the percentages of conversion of various carbonyl compounds to the cyanohydrin have been calculated in a fashion which is meaningless insofar as comparisons of one compound with another are concerned (although it would have been possible to calculate such data in a significant fashion).

(1) H. Adkins in Gilman's "Organic Chemistry", Second Edition, Volume I, pp. 1036-8, John Wiley and Sons, N. Y., 1943

12. The chemistry library at Cal Tech is universally recognized as being in the sorriest condition of any comparable library anywhere. I propose that a full-time guard, or librarian, be hired to enforce the library regulations and that a one-story structure be extended from the present library into the unexplored region between Gates and Crellin, with provision for the addition of underground stacks as needed. No shelves should be higher than 8 feet above the floor, and adequate provision should be made for lighting and heating, even at night.