

LINEAR ELUTION ADSORPTION CHROMATOGRAPHY

VIII. GRADIENT ELUTION PRACTICE. THE EFFECT OF ALKYL SUBSTITUENTS ON RETENTION VOLUME

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INTRODUCTION

The technique of gradient elution has been used frequently in the adsorption chromatographic separation of complex organic mixtures, with the intention of optimizing sample resolution, maximizing sensitivity in the detection of trace components, and minimizing separation time. The theory of gradient elution adsorption chromatography (GEAC) has been treated in the preceding paper¹ of the present series. In this theoretical study it was found that eluent gradients of the so-called *linear strength* form, eqn. (1), are generally optimum in GEAC separation:

$$\alpha\varepsilon^{\circ} = a + bV \quad (1)$$

Here, $\alpha\varepsilon^{\circ}$ is the *effective* eluent strength (adsorbent activity function, α , times eluent strength, ε°) of the GEAC binary eluent after the passage of V ml of eluate through the adsorbent column, and a and b are constants for a particular gradient. In GEAC separations with gradients obeying eqn. (1), and for sample sizes within the linear capacity² of the column (linear elution adsorption chromatography, LEAC), a simple relationship between solute retention volume R_g , solute structure, and experimental separation conditions is predicted:

$$R_g = \frac{\log (2.31 A_s b W \underline{R}_p 10^{-a A_s} + 1)}{b A_s} \quad (2)$$

The constants a and b are defined by eqn. (1), A_s refers to the effective solute surface volume, W to the adsorbent weight, and \underline{R}_p to the linear equivalent retention volume of the solute for elution from the same adsorbent by pentane. The calculation of values of A_s and the prediction of \underline{R}_p for a variety of separation systems (varying solute, adsorbent, adsorbent activity) have been detailed in preceding papers of this series and summarized in Part VI³.

Having demonstrated the theoretical advantages of a linear or near-linear strength gradient in GEAC separation, it remains to consider how such gradients may be attained in practical separations. Additionally, the theory we have derived

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for GEAC separation¹, and particularly eqn. (2), needs to be verified experimentally. Finally, in the course of other studies of the adsorption chromatographic process, the role previously assigned⁴ to solute alkyl groups in determining retention volume in strong eluent systems (and in GEAC separation) has been revised. These various items form the basis of the present communication.

EXPERIMENTAL

The GEAC separations described in the present paper were carried out using glass-
teflon units similar to that of Fig. 1. Solid glass rods of varying diameter were placed
in the strong eluent (B) reservoir to permit variation of its effective cross sectional

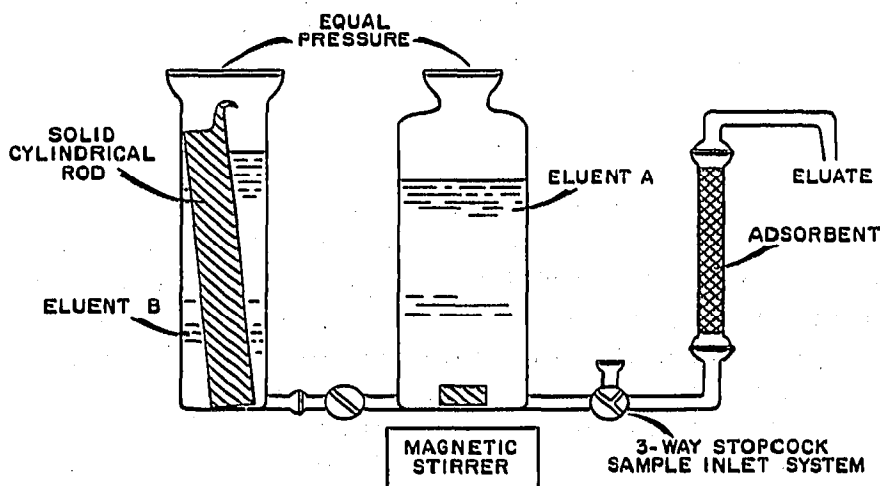


Fig. 1. Gradient elution apparatus used in present study.

area. A predetermined amount of weak eluent (A) was added to its reservoir, and the adsorbent column prewet or left dry, as desired. Sufficient strong eluent (B) was added to the second eluent reservoir to give hydrostatic equilibrium between the two reservoirs:

$$h_A/h_B = d_B/d_A$$

h_A and h_B refer to the height of each liquid head, and d_A and d_B to the respective eluent densities. Sample was introduced through the three-way teflon bore stopcock, all stopcocks opened for flow into the adsorbent column, and elution begun with stirring of the weak eluent reservoir. Measurement of sample retention volume R_g was carried out as in the case of fixed eluent separation⁵, being equal to the measured retention volume in ml with a correction for the dead volume of the column in the case of prewet columns. Linear capacity in the systems studied had been previously evaluated^{6,7}, and the sample sizes used ($< 5 \cdot 10^{-5}$ g/g) insured isotherm linearity.

The use of equipment of the type in Fig. 1 for the GEAC separation of samples of interest is facilitated by the following design considerations. First, the overall size of the apparatus required is roughly proportional to the sample sizes that must be dealt with. However, provision should be made for varying the ratio of cross sectional areas (A_A/A_B) between 0.5 and 16. As the cross sectional area of the rod in vessel B approaches that of vessel B, capillary entrainment of solvent becomes a problem,

so that more than one diameter vessel B is desirable. If the two vessels of Fig. 1 are detachable, as shown, only the strong eluent vessel requires duplication. It is recommended that four sizes of the vessel B be available, giving relative cross sectional areas (A_A/A_B) equal 0.5, 1, 5 and 10.

To prevent non-equilibrium in the solvent mixer, the bore of the stopcock between vessels A and B should be large (at least 2 mm). For ease in introducing the control rod of vessel B, the inside diameter of the top spherical joint of that vessel should be approximately as large as the diameter of vessel B. The stirring bar used in vessel A should be no longer than $2/3$ of the diameter of the vessel, to restrict the backflow of eluent A into vessel B. Finally, standard glassware sizes restrict the possible values of (A_A/A_B) to certain discrete values. For most work, the values of this ratio thus available will approximate desired values sufficiently closely. An optimum arrangement might use machined stainless steel or teflon rods, in order to vary the cross sectional area ratio precisely as desired.

LINEAR STRENGTH GEAC SEPARATION

The specific advantages of linear strength gradient elution (*i.e.*, using gradients which obey eqn. (1)) have been derived previously¹. They include (i) optimum peak spacing (weakly adsorbing components sufficiently separated to be resolved, strongly adsorbing components eluted within convenient separation times), (ii) elution bands of approximate equal width (for maximum sensitivity of detection), and (iii) no band splitting with development of spurious peaks. Eluent strength gradients which are mildly convex, rather than linear, are generally acceptable in GEAC separation, and in many cases are predicted to give a slightly better separation system. The major disadvantage of convex eluent strength gradients is the difficulty of accurately predicting R_f values, due to the inapplicability of eqn. (2) in non-linear separations. For many GEAC systems, particularly in the separation of samples whose constituents are initially unknown and for which R_f values cannot therefore be predicted, this consideration will be unimportant. *Concave gradients are generally undesirable in every respect.*

To determine the form (linear, concave, etc.) of the eluent strength gradient in a given GEAC separation, we must be able to relate the composition of the eluent to the eluate volume V , and the effective eluent strength $\alpha\varepsilon^\circ$ to eluent composition. The latter relationship for a simple binary eluent gradient (pure solvents A and B) has been derived⁴:

$$\alpha\varepsilon^\circ = \alpha\varepsilon^\circ_A + \frac{\log (X_B 10^{an_b(\varepsilon^\circ_B - \varepsilon^\circ_A)} + 1 - X_B)}{n_b} \quad (3)$$

Here, α is the adsorbent activity function, ε°_A and ε°_B are eluant strengths, respectively, of A and B, n_b is the value of A_s for B, and X_B is the mole fraction of B in the binary eluent. The values of these various parameters have been reported in previous papers of this series and indexed in Part VI³.

X_B in eqn. (3) may be related to the corresponding volume fraction V_B through the molecular weights M_A and M_B of A and B,

$$X_B = \frac{V_B}{(1 - V_B) (d_A/M_A) (M_B/d_B) + V_B} \quad (3a)$$

so that the necessary relationship between V_B and V for a linear (or other) strength gradient can be specified. A number of experimental devices have been described recently⁸⁻¹³ which are capable of producing any predetermined eluent gradient or V versus V_B relationship (primarily for application to ion exchange gradient elution). These devices are moderately complex and were not investigated in the present study, although they should prove useful where the number of separations contemplated justifies the fabrication of the necessary equipment and the time to become familiar with its use. The application of these devices to GEAC separation appears straightforward in principle, and requires no further comment. Alternately, it is possible to carry out linear strength GEAC separation in simpler devices, particularly of the kind shown in Fig. 1. The derivation of the relationship between V_B and V for gradient devices of this type has been given by at least two authors^{14, 15}, for the case of equal eluent densities d_A and d_B . For the general case of unequal densities an approximate extension of prior derivations for the device of Fig. 1 gives¹⁶:

$$V_A = [1 - (a^* V/V^{\circ}_A)]^{(1 - a^*)/a^*} \quad (4)$$

where:

$$a^* = (A_A/A_B)/[(A_A/A_B) + (d_A/d_B)] \quad (4a)$$

V°_A is the volume of solvent in reservoir A prior to the beginning of elution. The variation of V_A with the fractional depletion of *both* solvent reservoirs V/V° (V equal V° when both reservoirs empty) is illustrated in Table I for several values of the parameter a^* .

Figs. 2 and 3 illustrate the dependence of $\alpha\epsilon^{\circ}$ on V for several hypothetical chromatographic systems using the apparatus of Fig. 1. In Fig. 2, the eluent parameters n_b and $\alpha(\epsilon^{\circ}_B - \epsilon^{\circ}_A)$ are assumed equal to 2 and 0.30, respectively, while in Fig. 3 these eluent parameters are given values of 6 and 0.30 (*i.e.*, the strong eluent B in Fig. 2 is a small molecule and in Fig. 3 a large molecule). The different curves

TABLE I

 COMPOSITION OF ELUENT FOR GRADIENT DEVICE OF FIG. 1 *versus* TOTAL ELUATE VOLUME AND a^*

V/V°	V_B for various values of a^*								
	0.50	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95
0.05	0.05	0.034	0.027	0.022	0.017	0.013	0.009	0.006	0.003
0.10	0.10	0.068	0.055	0.044	0.035	0.026	0.018	0.012	0.006
0.20	0.20	0.138	0.112	0.091	0.072	0.054	0.038	0.024	0.012
0.30	0.30	0.212	0.174	0.142	0.112	0.085	0.061	0.039	0.019
0.40	0.40	0.289	0.239	0.196	0.156	0.120	0.086	0.055	0.027
0.50	0.50	0.370	0.311	0.257	0.206	0.159	0.115	0.074	0.036
0.60	0.60	0.457	0.389	0.324	0.263	0.205	0.149	0.097	0.047
0.70	0.70	0.552	0.477	0.403	0.330	0.260	0.191	0.125	0.062
0.80	0.80	0.658	0.579	0.498	0.415	0.331	0.247	0.164	0.082
0.90	0.90	0.785	0.710	0.627	0.536	0.438	0.333	0.226	0.115
0.95	0.95	0.864	0.800	0.728	0.632	0.527	0.410	0.283	0.146

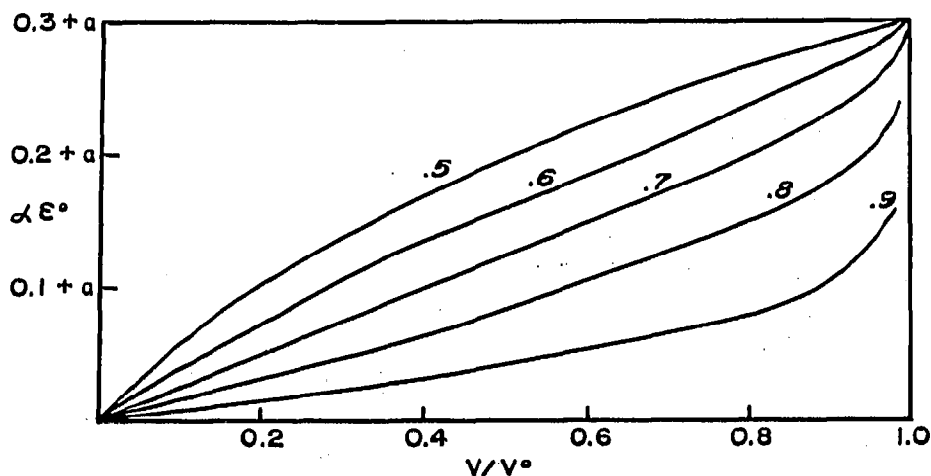


Fig. 2. Eluent strength *versus* eluate volume curves for device of Fig. 1. $\alpha\epsilon^{\circ}_A = a$; $\alpha\epsilon^{\circ}_B = 0.3 + a$; $n_b = 2$; $(d_A/M_A)/(d_B/M_B) = 1.0$. Values of a^* on curves.

in Figs. 2 and 3 correspond to different values of the gradient apparatus parameter a^* . In Fig. 2, for B small, values of a^* between 0.6 and 0.7 give a reasonably linear gradient for values of V/V° less than 0.9 (*i.e.*, for the first 90% of the separation). In Fig. 3, where the strong eluent is a large molecule, reasonably linear gradients occur for larger values of a^* (0.90–0.95). For values of a^* less than the linear gradient value, the gradient is convex, and for larger values, concave. As a general observation, *gradient devices of the present type should not be used for V greater than 0.9 V°* , because the gradient frequently becomes severely concave during the last 10% of the separation. It should also be noted that the larger the strong eluent (value of n_b), the smaller the effective range in eluent strength covered during a single GEAC separation ($0 \leq V \leq V^{\circ}$), and the smaller the number of sample components conveniently separable.

As the difference in strength between the two eluents ($\epsilon^{\circ}_B - \epsilon^{\circ}_A$) is increased, the family of $\alpha\epsilon^{\circ}$ *versus* V curves tends to resemble the case of larger n_b . Increasing $\alpha(\epsilon^{\circ}_B - \epsilon^{\circ}_A)$ in Fig. 2 would tend to give curves of shape similar to those in Fig. 3, with higher values of a^* required for gradient linearity. The curves of Figs. 2 and 3

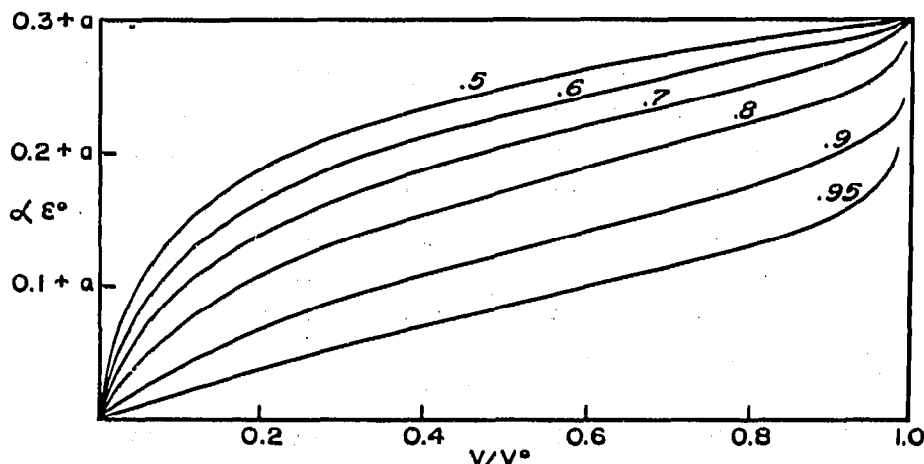


Fig. 3. Eluent strength *versus* eluate volume curve for device of Fig. 1. $\alpha\epsilon^{\circ}_A = a$; $\alpha\epsilon^{\circ}_B = 0.3 + a$; $n_b = 6$; $(d_A/M_A)/(d_B/M_B) = 1.0$. Values of a^* on curves.

assume $(d_A/M_A)/(d_B/M_B) = 1$. When this ratio is increased, the effect on the $\alpha\epsilon^\circ$ versus V curves is similar to decreasing n_b , but less pronounced.

The curves of Figs. 2 and 3 in conjunction with the data of Table II offer a qualitative guide to the selection of optimum a^* values in practical GEAC separation work. The calculation of similar curves for specific situations is not difficult, using

TABLE II
PROPERTIES OF SOME COMMON ELUENTS FOR USE IN DESIGNING GEAC SEPARATIONS

Eluent	d_A/M_A	d_A	n_b	ϵ°	
				Al_2O_3	SiO_2
Pentane	0.00861	0.626	5	0.00	0.00
Isooctane	0.00606	0.692	8	0.01	
Cyclohexane	0.00926	0.780	6	0.04	
Carbon tetrachloride	0.01037	1.595	4	0.18	0.14
Benzene	0.01127	0.879	6	0.32	0.25
Methylene chloride	0.01574	1.336	3	0.42	0.32
Acetone*	0.01363	0.792	4	0.56	
Methyl acetate*	0.01253	0.927	5	0.60	
Ethyl acetate	0.01023	0.901	5	0.60	
Dioxan	0.01176	1.035	6	0.63	
Pyridine*	0.01242	0.982	6	0.71	

* ϵ° values and supporting data to be described in a following communication.

eqn. (3) and Tables I and II. A number of specific linear strength systems are itemized in Table III for the three adsorbents so far studied. These systems are restricted to weak or moderately strong eluents, since very strong eluents such as the alcohols have not yet been studied in the present series of investigations.

At first glance, it might appear particularly useful in GEAC separation to have the weak and strong eluents as widely different as possible (e.g., pentane A, isopropanol B). From Figs. 2 and 3 and the related discussion, however, this would require values of a^* quite close to 1.00, and at V equal 0.9 V° the effective eluent strength would be only a fraction of that of pure B. Consequently, substances eluted readily by pure B will not necessarily be eluted from the column in the linear gradient region ($V \leq 0.9 V^\circ$), although this would generally be true of GEAC systems where the eluent strengths of the two constituent solvents are closer in value. Consequently, much of the expected advantage of a very strong second eluent will be lost inasmuch as many strongly adsorbing solutes will not be eluted in the course of the separation. An additional objection to gradient eluents of widely different strengths exists by virtue of the displacement effect¹, which becomes especially marked in such cases. Displacement causes poor separation of weakly adsorbing solutes, because the initial stages of separation serve only to saturate the column adsorbent with the strong eluent B, all weakly adsorbing compounds being displaced as a single band by the advancing strong eluent front. Gradient systems based on eluents of moderately different strengths (as in Table III) appear optimum from the standpoint of all but very weakly and very strongly held solutes, and it seems likely that most samples of interest can be adequately separated by GEAC systems of this type.

TABLE III
SUMMARY OF SEVERAL BINARY LINEAR STRENGTH GRADIENT ELUTION SYSTEMS

Weak eluent <i>A</i>	Strong eluent <i>B</i>	Adsorbent		A_A/A_B	$h_A^{h_B} = \frac{d_A}{d_B/d_A}$	$\alpha \varepsilon^{\circ} A$	bV°		
		Type	% H_2O						
<i>n</i> -Pentane	Carbon tetrachloride	Al_2O_3	1	1.1	2.55	0.000	0.130		
			2	1.1					
			4	0.9					
<i>n</i> -Pentane	Benzene	Al_2O_3	1	1.3	1.41	0.000	0.161		
			2	2.8					
			4	2.1					
		SiO_2^*	1	2.8	0.000	0.156			
			4	1.6					
			16	1.3					
			Flor. **	1	1.3	0.000	0.110		
		<i>n</i> -Pentane	Methylene chloride	Al_2O_3	1	4.2	2.13	0.000	0.250
					2	3.5			
4	2.7								
SiO_2^*	1			3.5	0.000	0.233			
	4			2.3					
	16			1.9					
	Flor. **			1	4.2	0.000	0.168		
				4	1.9	0.000	0.165		
Carbon tetrachloride	Benzene			Al_2O_3	1	4.3	0.55	0.151	0.108
		2	4.3		0.135	0.092			
		4	3.4		0.113	0.084			
Carbon tetrachloride	Methylene chloride	Al_2O_3	1	3.4	0.84	0.151	0.176		
			2	3.1		0.135	0.163		
			4	2.6		0.113	0.140		
Benzene	Dioxan	Al_2O_3	1	16	1.08	0.269	0.148		
			2	10		0.240	0.157		
			4	7.7		0.202	0.110		
Methylene chloride	Dioxan	Al_2O_3	1	5.2	0.77	0.353	0.142		
			2	4.2		0.315	0.122		
			4	3.0		0.265	0.102		

* Davison Code 12 or equivalent.

** See ref. 3.

In the case of unusually complex samples which are suspected to contain a number of components, some easily eluted and some strongly adsorbed, no simple gradient system of the type so far described will prove wholly satisfactory. Two alternative procedures are useful in this connection, however. Thus, the sample may be separated first by conventional elution chromatography into several fractions by successive elution with a series of eluents $j, k, m \dots$ of increasing strength ($\varepsilon^{\circ}_j < \varepsilon^{\circ}_k < \varepsilon^{\circ}_m \dots$). Each of the resulting fractions may then be further separated in an optimum GEAC separation as above, since the range of compounds in each of

these fractions should be properly spaced in the right GEAC system. As a general rule, if a sample is eluted by 2-3 column volumes of some eluent j , it will be readily eluted by a GEAC system using j as strong eluent (B). Similarly, all compounds eluted after 2-3 column volumes of the eluent j will tend to be separated in a GEAC separation using j as the weak eluent (A). Consequently, an optimum *overall* separation might be accomplished by elution with 2-3 column volumes of each eluent j , k , etc., in the initial preparation of the various fractions, with the second fraction being re-separated in a GEAC system using eluents j (weak) and k (strong). The third fraction would be re-separated in a GEAC system based on eluents k and m , and so on for the rest of the initial fractions. The various eluents j , k , etc., need not be pure solvents, but can be binary eluents if this is required or convenient. The only difference is the use of eqn. (5) in the calculation of eluent strength in the resulting *ternary* solvent systems¹, rather than eqn. (3) for binary solvents:

$$\alpha\epsilon^{\circ} = \alpha\epsilon^{\circ}_B + \frac{\log X_C 10^{an_c(\epsilon^{\circ}_C - \epsilon^{\circ}_B)} + X_B}{n_c} \quad (5)$$

X_C is the mole fraction of C in the eluent, n_c is the value of A_s for the eluent C (same as n_b for eluent B), and ϵ°_C is the eluent strength of C.

A second solution to the problem of separating complex samples by GEAC is through the use of additional eluent after the initial emptying of the eluent reservoirs. Simple elution with the strong eluent B at this point will serve to further elute sample from the column, but the advantages of GEAC separation in separating additional sample components are rapidly lost; separation time for the most strongly adsorbing components mounts, solute bands broaden with loss in sensitivity of detection, and quite possibly a spurious peak would appear soon after change to pure eluent.

A third and more reasonable approach is the use of a new eluent pair which will continue the original eluent gradient described by eqn. (1), thus extending the range of compounds which may be separated under the original separation conditions, and incidentally permitting the continued prediction of solute R_D values through eqn. (2). As we have seen, the first step of the GEAC separation will normally terminate around $V = 0.9 V^{\circ}$, at which point the eluent entering the column will be of some composition $x\%$ A-B. Now if the latter *binary* eluent ($x\%$ A-B) is used in place of the original weak eluent A in the *second* step of the overall GEAC separation, and a stronger eluent C ($\epsilon^{\circ}_C > \epsilon^{\circ}_B$) is used as the strong eluent, a value of a^* for the second part of the separation can be calculated for linear strength elution, and a value of V° determined so as to make the slope b in the second stage of the separation equal to that in the first stage. Equation (5) must of course be substituted for eqn. (3) in the resulting calculations. One such ternary eluent GEAC separation of this type is described in the following section. Other ternary eluent separations can be readily formulated.

In Table III are listed values of bV° , which corresponds approximately to the effective eluent strength range available during separation. If the eluent strength gradient were precisely linear throughout the whole separation, bV° would equal $\alpha(\epsilon^{\circ}_B - \epsilon^{\circ}_A)$. By varying V° , b can be varied at will, and the question of an optimum value of b must be considered. The analysis of such a question is reasonably complex, and dependent upon factors (column efficiency) which are not yet fully

understood. However, as a rough guide, W/V° should be small (less than 0.1), and the size of W will be dictated by the sample size required to be separated, the linear capacity of the adsorbent, etc. The longer the column and the smaller the eluent flow rate (mm/min), the better will be the resulting separation.

THE EXPERIMENTAL VERIFICATION OF EQUATION (2) AND SOME COROLLARY RELATIONSHIPS

The validity of the theoretical analysis of GEAC presented in the previous paper¹ of this series, and of the discussion in the preceding section of this communication, ultimately rests on the accuracy of eqn. (2). For these reasons, as well as to test the applicability of eqn. (2) *per se* in the prediction of GEAC separability, it is important to compare experimental and calculated values of R_g in some representative separations. Experimental R_g values are tabulated in Table IV for four different linear strength GEAC systems (linear isotherm loadings). These four separations include data for two adsorbents and three binary eluent pairs. The 39 solutes include examples from most of the compound types previously studied in fixed eluent separation: hydrocarbons, substituted hydrocarbons, and heterocyclic compounds of various types. With experimental values of R_g ranging from 3 to 96 ml, the average deviation between these 51 experimental and calculated R_g values is only ± 2.5 ml, fully confirming the accuracy of eqn. (2) in binary eluent linear strength GEAC separation, and verifying our analysis of the experimental characteristics of gradient devices of the type of Fig. 1 (which were used in measuring the experimental values). A few comments on the calculation of these R_g values are in order. Systems II, III and IV of Table IV are linear in strength over the range $V \leq 0.9$, as recommended in the preceding section. System I of Table IV is approximately linear over the entire range, deviating only at the lower end ($V < 10$ ml). For this reason, the best approximation to eqn. (1) for system I has a equal to 0.03, rather than to the value of $\alpha\epsilon^\circ_A$ for the weaker eluent (0.00).

Values of R_p used in eqn. (2) may be calculated either from fundamental molecular parameters as reviewed in Part VI³, or interpolated from R° values for other eluents, and even other adsorbent activities. Where the adsorbent is the same or closely similar, the latter procedure is potentially the more accurate, particularly where R_p is extrapolated from a retention volume R_A for elution by the weak eluent A. The latter procedure, where values of R_A had previously been measured, underlies the calculation of R_g values in Table IV. The alternative of calculating R_p from tabulated parameters has been illustrated for system I in the previous paper of this series¹.

Table V presents some data for an extended GEAC separation. Following the termination of linearity in the eluent strength gradient in an initial binary eluent system, the gradient was extended by ternary eluent elution using the gradient eluents (A-B), C. The initial binary eluent system was that described for system III of Table IV, for which eluent strength linearity terminates at V equal $0.91 V^\circ$. At that point, the composition of eluent entering the column is 65.4 mole % benzene-carbon tetrachloride. A continuation of the original eluent gradient using the new system, 65.4 mole % benzene-carbon tetrachloride (weak), dioxan (strong), was then considered. For α^* equal 0.86 and V° equal 196 ml, it was calculated that b would have

TABLE IV
 EXPERIMENTAL TEST OF EQUATION 2; BINARY ELUENT SYSTEMS

Solute	R_D (ml) in system							
	I		II		III		IV	
	Exptl.	Calc.	Exptl.	Calc.	Exptl.	Calc.	Exptl.	Calc.
Naphthalene	12	8						
Acenaphthylene	19	16						
Phenanthrene	24	27					8	7
Anthracene	27	27						
Fluoranthene	35	40						
Triphenylene	51	53						
Chrysene	48	53						
Benzantracene	51	53	3	4				
Perylene	63	65						
3:4-Benzopyrene	63	65	6	7				
Benzperylene	76	76						
Picene			15	13			16	22
1:2;4:5-Dibenzopyrene	96	84						
Coronene	86	84						
1:2-Benzocoronene			42	43	41	41		
1,2-Diphenylethane							8	10
Phenyl ethyl sulfide							9	9
Nitrobenzene							30	37
Methyl benzoate							52	53
2-Methoxynaphthalene							22	25
1-Nitronaphthalene			7	5				
1-Cyanonaphthalene			8	7				
1-Acetonaphthalene			23	20	18	18		
2-Acetonaphthalene			20	22				
<i>p</i> -Diethoxybenzene			6	4			65	67
<i>o</i> -Nitroanisole					18	16		
<i>m</i> -Nitroanisole			6	7				
<i>p</i> -Nitroanisole							71	71
<i>m</i> -Dinitrobenzene							82	84
<i>o</i> -Nitroaniline			75	69				
3:4-Benzacridine			8	8	7	8		
7:8-Benzquinoline					9	8		
Quinoline			30	29				
β -Naphthoquinoline			36	38	34	35		
Phenanthridine			38	37	37	35		
1-Azapyrene			40	40				
6-Nitroquinoline			53	58	51	53		
Isoquinoline			67	60	61	56		
Carbazole							50	55

System	Adsorbent	Eluent A	Eluent B	a^*	a	b	V^0 (ml)	W (g)
I	3.7 % $H_2O-Al_2O_3$	Isooctane	Ethyl ether	0.75	0.03	0.00190	133	10.0
II	4.0 % $H_2O-Al_2O_3$	Carbon tetrachloride	Benzene	0.69	0.113	0.00070	109	2.0
III	4.0 % $H_2O-Al_2O_3$	Carbon tetrachloride	Benzene	0.69	0.113	0.00078	95	2.0
IV	17 % H_2O-SiO_2	Pentane	Methylene chloride	0.80	0.000	0.00125	124	1.7

TABLE V
EXPERIMENTAL TEST OF EQUATION 2; EXTENDED TERNARY ELUENT SYSTEM*

Solute	R_g (ml)	
	Exptl.	Calc.
1,2,4-Tricarbomethoxybenzene	105	109
2,4-Dinitroaniline	137	130
1-Azacarbazole	175	181
7-Azaindole	177	179
2-Aminoanthraquinone	204	206

* First stage conditions. System III of Table IV to $V = 87$ ml. Second stage conditions. Weak eluent: 65.4 mole % benzene-carbon tetrachloride. Strong eluent: dioxan. $\alpha^* = 0.86$; $V^0 = 196$ ml.

the same value (0.00078) as in the initial GEAC separation using carbon tetrachloride and benzene. R_g values for five solutes eluting in the second stage of this extended gradient (ternary eluent) separation are given in Table V, and these data show good agreement with calculated values from eqn. (2) (average deviation ± 4 ml).

For the four gradient elution systems of Table IV, the importance of displacement was estimated by means of Fig. 2 of the previous paper¹. In all cases, V_s/V^0 (the initial fraction of the separation not at equilibrium) was estimated at less than 0.01, or column equilibrium is predicted for V greater than 1 ml. In agreement with this calculation, there is no tendency for the first eluted solutes of Table IV to have larger than calculated R_g values, with the apparent exception of system I. In the latter case, the deviation in the experimental R_g values in the first eluted solutes may actually be shown to be due to the modest failure of eqn. (1) to approximate the true eluent strength *versus* eluate volume relationship at small values of V . In agreement with theory¹, which predicts an absence of band splitting in linear strength GEAC separation, none of the solutes described in Table IV showed any tendency toward giving double peaks.

TABLE VI
BAND WIDTH IN GEAC SEPARATION; DATA FOR SYSTEM II OF TABLE IV

Solute	R_g (ml)	Band width (ml)	w/R_g
1:2-Benzanthracene	2.5	6	1.5
3:4-Benzpyrene	5.7	11	1.3
<i>p</i> -Diethoxybenzene	6.1	22	6.1
1-Nitronaphthalene	7.3	30	7.2
3:4-Benzacridine	7.5	11	1.4
1-Cyanonaphthalene	8.3	17	2.8
Picene	15	37	3.4
2-Acetonaphthalene	20	14	0.7
Quinoline	30	29	1.0
β -Naphthoquinoline	36	34	1.2
Phenanthridine	38	22	0.6
1-Azapyrene	41	49	1.2
6-Nitroquinoline	52	31	0.7
Isoquinoline	67	44	1.1
<i>o</i> -Nitroaniline	75	28	0.7

It is desirable that the widths of solute bands remain approximately constant throughout GEAC separation. While no GEAC system will meet this requirement exactly, linear strength separation is theoretically predicted¹ to give reasonably constant band widths under certain conditions: (i) solutes whose sizes (A_s) do not differ greatly; (ii) column separation efficiency constant throughout separation; (iii) solute R_f values reasonably large. Table VI presents a number of data on solute band width from system II of Table IV. Column separation efficiency (measured by band width in fixed eluent separation) normally varies with eluent flow rate and the viscosity of the eluent; the viscosities of the two eluents of system II (carbon tetrachloride and benzene) are approximately equal, and eluent velocity was held constant during the acquisition of the band width data of Table VI, so column separation efficiency was presumed near constant for these data. The solute sizes are not widely different ($8 \leq A_s \leq 14$). While the individual band widths vary from 6 to 49 ml in Table VI, band width is relatively independent of R_f for R_f greater than 10:

R_f range (ml)	No. of solute	Average band width (ml)
0-10	6	16 \pm 7
10-30	3	28 \pm 8
30-50	3	34 \pm 10
50-80	3	34 \pm 6

Theoretically, the ratio of band width w to "instantaneous retention volume" R should be constant for R_f large¹. As seen in Table VI this is only very approximately true, with several striking exceptions. This suggests that column separation efficiency may be a function of the solute.

ALKYL SUBSTITUENTS AND SOLUTE RETENTION VOLUME

The role played by alkyl substituents in determining the retention volume of the solute has been examined briefly in earlier papers^{4,5}. For solutes eluted by weak eluents such as pentane, the solute alkyl substituent will be adsorbed along with the remainder of the solute. For elution by stronger eluents, theoretical considerations suggest that most of the alkyl group will lie in the solution phase when the solute adsorbs, since the weakly adsorbing alkyl carbons cannot effectively compete with the more strongly adsorbing eluent for a place on the adsorbent surface. Limited data⁶ for elution of some alkyl-substituted solutes from alumina suggest that in strong eluent systems only one of the alkyl carbons of each alkyl substituent lies in the adsorbed phase, and that the area required for adsorption of the solute (A_s) is independent of the length of its alkyl substituents. Because the calculation of A_s for a solute enters into the prediction of R_f values in GEAC separation, some recently acquired data relevant to this point are summarized in Tables VII and VIII.

The data of Table VII (for alumina) and VIII (for silica) illustrate the variation of retention volume with eluent strength for several solute types possessing alkyl substituents of varying length. The general relationship between retention volume \bar{R}^0 (ml/g) and eluent strength is given by eqn. (6):

TABLE VII

EFFECT OF ALKYL SUBSTITUENTS ON SOLUTE RETENTION VOLUME FOR ELUTION BY STRONG ELUENTS FROM ALUMINA

Solute	n ^a	% H ₂ O-Al ₂ O ₃	Eluent ^b	log R_p	α^c	log R^o	
						Exptl.	Calc. eqn. (6)
Naphthalene	0	1.3	P	0.83	0.000	0.83	—
		1.3	10% C-P		0.029	0.60	—
		1.3	50% C-P		0.084	0.16	—
Methyl benzoate	1	1.3	B	2.53 ^d	0.259	0.20	—
		3.7	P	1.58	0.000	1.58	—
		3.7	1.5% E-P		0.056	1.08	—
		3.7	5% E-P		0.100	0.68	—
		3.7	15% E-P		0.148	0.24	—
Methyl 3,5-dinitrobenzoate	1	1.3	B	4.46 ^d	0.259	0.84	—
		1.3	M	4.72 ^d	0.340	—0.04	—
		3.7	25% M-P		0.149	1.22	—
		3.7	B	3.30 ^d	0.208	0.30	—
Dimethyl phthalate	1	3.7	25% M-P		0.157	1.48	—
		3.7	B	3.36 ^d	0.208	0.86	—
		3.7	70% M-P		0.253	0.32	—
1-Ethyl naphthalene	2	1.3	10% C-P	0.92	0.029	0.66	0.65
		1.3	50% C-P		0.084	0.13	0.13
1-n-Butyl naphthalene	4	1.3	10% C-P	0.92	0.029	0.64	0.63
		1.3	50% C-P		0.084	0.07	0.07
1-n-Hexyl naphthalene	6	1.3	10% C-P	0.97	0.029	0.66	0.66
		1.3	50% C-P		0.084	0.10	0.08
n-Butyl benzoate	4	1.3	B	2.58 ^e	0.259	—0.04	—0.04
		3.7	1.5% E-P	1.65	0.056	1.06	1.08
		3.7	5% E-P		0.100	0.61	0.64
		3.7	15% E-P		0.148	0.09	0.15
n-Decyl benzoate	10	1.3	B	2.68 ^e	0.259	—0.22	—0.32
		3.7	1.5% E-P	1.72	0.056	1.05	1.07
		3.7	5% E-P		0.100	0.56	0.56
		3.7	15% E-P		0.148	—0.07	0.00
n-Hexadecyl benzoate	16	1.3	B	2.78 ^e	0.259	—0.55	—0.41
		3.7	1.5% E-P	1.72	0.056	1.03	1.03
		3.7	5% E-P		0.100	0.49	0.49
		3.7	15% E-P		0.148	—0.14	—0.10
n-Butyl 3,5-dinitrobenzoate	4	1.3	B	4.64 ^e	0.259	0.69	0.73
		1.3	M		0.340	—0.38	—0.49
		3.7	25% M-P	3.34 ^e	0.153	1.09	1.03
		3.7	B		0.208	0.20	0.20
n-Hexadecyl 3,5-dinitrobenzoate	16	1.3	B	4.83 ^e	0.259	0.55	0.35
		3.7	25% M-P	3.49 ^e	0.153	0.80	0.84
		3.7	B		0.208	—0.17	—0.11
Di-n-butyl phthalate	4	3.7	25% M-P	3.44 ^e	0.153	1.22	1.27
		3.7	B		0.208	0.54	0.49
		3.7	70% M-P		0.253	0.07	—0.15
Di-n-hexadecyl phthalate	16	3.7	25% M-P	3.75 ^e	0.153	0.89	0.90
		3.7	B		0.208	0.21	—0.12

^a Number of alkyl carbon atoms in substituent.^b % refers to per cent by volume; P = pentane; C = carbon tetrachloride; B = benzene; M = methylene chloride; E = ethyl ether.^c Measured through eqn. (6) from R^o for unsubstituted or methyl-substituted solutes for binary eluents; pure eluents calculated as usual.^d Calculated from eqn. (6).^e Calculated from value of R_p for unsubstituted or methyl-substituted solute as described in text.

$$\log \underline{R}^{\circ} = \log \underline{R}_p - \alpha \varepsilon^{\circ} A_s \quad (6)$$

The solute surface volume A_s can generally be expressed as the sum of individual contributions a_i from each solute group i :

$$A_s = \sum_i a_i \quad (7)$$

The data of Tables VII and VIII permit the calculation of A_s for each solute type by means of eqn. (6). Comparison of values of A_s for a solute substituted by an alkyl group C_nH_{2n+1} with the A_s value of the unsubstituted or methyl-substituted solute then permits the calculation of values of a_i for alkyl substituents of varying length. Some of the \underline{R}_p values of Tables VII and VIII are experimental values for elution by pentane. Other \underline{R}_p values for unsubstituted or methyl-substituted solutes are extrapolated from \underline{R}° values in strong eluent systems, by means of eqn. (6). Still other \underline{R}_p values for alkyl-substituted solutes related to the latter unsubstituted or methyl-substituted solutes were calculated from these \underline{R}_p values by adding 0.02 α for each additional methylene group (alumina⁶), or subtracting 0.05 α for each such group (silica⁷).

TABLE VIII

EFFECT OF ALKYL SUBSTITUENTS ON SOLUTE RETENTION VOLUME FOR ELUTION BY STRONG ELUENTS FROM DAVISON CODE 12 SILICA

Solute	n^a	$\frac{\%}{H_2O-SiO_2}$	Eluent ^b	$\log \underline{R}_p$	$\alpha \varepsilon^{\circ c}$	$\log \underline{R}^{\circ}$	
						Exptl.	Calc. eqn. (6)
Methyl benzoate	1	16.0	P	2.13	0.000	2.13	—
		16.0	5% M-P		0.029	1.69	—
		16.0	15% M-P		0.065	1.14	—
		16.0	25% M-P		0.088	0.78	—
<i>n</i> -Butyl benzoate	4	16.0	5% M-P	2.04 ^d	0.029	1.59	1.56
		16.0	15% M-P		0.065	0.92	0.98
		16.0	25% M-P		0.088	0.59	0.60
<i>n</i> -Decyl benzoate	10	16.0	5% M-P	1.87 ^d	0.029	1.41	1.35
		16.0	15% M-P		0.065	0.68	0.71
		16.0	25% M-P		0.088	0.23	0.29

^a Number of alkyl carbon atoms in substituent.

^b % refers to per cent by volume; P = pentane; M = methylene chloride.

^c Measured through eqn. (6) from \underline{R}° for methyl-substituted solute.

^d Calculated from value of \underline{R}_p for methyl-substituted solute as described in text.

Table IX summarizes values of a_i calculated for alkyl substituents of varying lengths from the data of Tables VII and VIII. These a_i values are seen to be constant for substituents of a given length, even though derived from a variety of solutes eluted by several eluents from two adsorbents of varying activity (α). A complete tabulation of a_i values for alkyl substituents containing from 1 to 20 carbon atoms is shown in Table X. As a final check on the derived values of a_i , Tables VII and VIII compare calculated values of $\log \underline{R}^{\circ}$ for the alkyl-substituted solutes with experimental values. The average deviation between calculated and experimental data is only ± 0.05 log units, which represents quite satisfactory agreement.

TABLE IX
EXPERIMENTAL VALUES OF a_i FOR ALKYL SUBSTITUENTS

Group	Solute	Adsorbent	A_s	a_i	Au. a_i
-C ₂ H ₅	1-Ethynaphthalene	Al ₂ O ₃	9.3	1.3	1.3
-C ₄ H ₉	1- <i>n</i> -Butylnaphthalene	Al ₂ O ₃	10.1	2.1	2.1 ± 0.1
	<i>n</i> -Butyl benzoate	Al ₂ O ₃	10.3	2.3	
	<i>n</i> -Butyl 3,5-dinitrobenzoate	Al ₂ O ₃	15.0	2.0	
	Di- <i>n</i> -butyl phthalate	Al ₂ O ₃	13.8	1.9	
	<i>n</i> -Butyl benzoate	SiO ₂	14.8*	2.3	
-C ₆ H ₁₃	1- <i>n</i> -Hexylnaphthalene	Al ₂ O ₃	10.4	2.4	2.4
-C ₁₀ H ₂₁	<i>n</i> -Decyl benzoate	Al ₂ O ₃	11.6	3.6	3.7 ± 0.1
	<i>n</i> -Decyl benzoate	SiO ₂	16.3*	3.8	
	<i>n</i> -Hexadecyl benzoate	Al ₂ O ₃	12.6	4.6	
-C ₁₆ H ₃₃	<i>n</i> -Hexadecyl benzoate	Al ₂ O ₃	17.5	4.5	4.3 ± 0.3
	<i>n</i> -Hexadecyl 3,5-dinitrobenzoate	Al ₂ O ₃	17.5	4.5	
	Di- <i>n</i> -hexadecyl phthalate	Al ₂ O ₃	17.7	3.9	

* A_s values for some solutes are different on SiO₂ relative to Al₂O₃ (ref. 7).

The data originally used⁴ in drawing the conclusion that only one alkyl carbon of a substituent is adsorbed (and that a_i for all alkyl groups equal one, regardless of size) are included in Table VII. The present modification in this original conclusion

TABLE X
VALUES OF a_i FOR ALKYL SUBSTITUENTS OF VARYING LENGTH

No. of alkyl carbons	a_i	No. of alkyl carbons	a_i
1	1.0	11	3.8
2	1.4	12	3.9
3	1.7	13	4.0
4	2.1	14	4.1
5	2.3	15	4.2
6	2.6	16	4.3
7	2.9	17	4.4
8	3.1	18	4.4
9	3.4	19	4.5
10	3.6	20	4.5

reflects certain limitations on the use of eqn. (6) in the experimental determination of A_s values. Unless the range of eluent strengths covered in such a determination is large (as was not the case in the original study⁴), inaccurate values of A_s can result.

GLOSSARY OF TERMS

- a, b Constants in eluent strength *versus* eluate volume relationship, eqn. (1).
 a_i Contribution of group i to solute surface volume A_s .
 a^* GEAC separation parameter, for device of Fig. 1, defined by eqn. (4a).
A, B, C Refer to solvents used as eluents in GEAC separation.
 A_A, A_B Cross sectional areas of gradient device of Fig. 1.
 A_s Solute surface volume, see eqn. (6).

- d_A, d_B Densities of eluents A and B.
 h_A, h_B Initial heights of eluent in two chambers of device of Fig. 1.
 M_A, M_B Molecular weights of solvents A and B.
 n Number of alkyl carbons in alkyl substituent.
 n_b, n_c Value of A_s for eluents B and C.
 \underline{R}° Solute linear equivalent retention volume (ml/g).
 \underline{R}_p Value of \underline{R}° for elution by pentane.
 \underline{R}_A Value of \underline{R}° for elution by eluent A.
 R_g Retention volume (ml) of a solute in GEAC separation.
 R_t Instantaneous retention volume (see Part VII¹).
 V Eluate volume (ml).
 V_s Initial GEAC eluent volume during which displacement occurs (see Part VII¹).
 V° Total volume of eluent (A and B) stored in gradient device of Fig. 1 prior to beginning of separation.
 V°_A Total volume of eluent A stored in its chamber prior to beginning of separation.
 V_A, V_B Volume fraction of eluents A or B in binary eluent mixture.
 W Weight of adsorbent (g) in column.
 w Solute band width (ml), measured from 4 to 96 % solute elution from column.
 X_B, X_C Mole fraction of eluents B and C in a binary or ternary solvent mixture.
 α Adsorbent activity function.
 $\varepsilon^\circ, \varepsilon^\circ_A, \varepsilon^\circ_B, \varepsilon^\circ_C$ Eluent strength parameters for indicated solvents.

SUMMARY

The technique of *linear strength* gradient elution has been studied experimentally under linear isotherm conditions. A previously derived theoretical relationship between solute retention volume (in linear strength gradient elution), solute molecular structure, and experimental separation conditions has been verified. The unique advantages of linear strength separation have been confirmed. Simple experimental devices for carrying out linear strength gradient elution separation are described, and a number of specific linear strength separations cataloged for easy duplication. The effect of solute alkyl groups on retention volume in strong eluent systems has been re-examined on the basis of new experimental data, and previous conclusions with respect to the retention volumes of these solutes have been modified.

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