

## LINEAR ELUTION ADSORPTION CHROMATOGRAPHY

## VII. GRADIENT ELUTION THEORY

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## INTRODUCTION

Gradient elution chromatography, first developed by ALM *et al.*<sup>1</sup>, has found application to almost every form of elution chromatography which uses a liquid moving phase<sup>2</sup>. Conceptually, gradient elution bears a marked resemblance to temperature programming in gas chromatography<sup>3</sup>, and offers the same experimental advantages. In the separation of broad-boiling mixtures by gas chromatography, temperature programming permits the separation of low-boiling sample components at low column temperatures with maximum resolution, while high-boiling components emerge from the column at higher temperatures within convenient analysis times. Similarly, gradient elution chromatography begins with a weakly displacing eluent that will separate the least strongly retained solutes, and ends with a sufficiently strong eluent for the convenient elution of the most strongly held substances. A further advantage in each of these two chromatographic techniques is that the last eluted sample bands have widths comparable to those of the first eluted bands. In conventional elution chromatography, band width increases with increasing band retention volume, and traces of strongly held substances frequently escape detection entirely because the very broad bands are lost within the noise or drift of the base line. Both temperature programming and gradient elution greatly increase sensitivity in the measurement of strongly held sample components. A final advantage of these two chromatographic techniques, which in fact supplied the major incentive for the original invention of gradient elution, is of importance primarily in adsorption chromatography. Excessive tailing of elution bands may occur either as the result of column overloading or solute chemisorption (Part VI)<sup>4</sup>, and the use of gradient elution has been recommended for the reduction of such tailing.

The theory of gradient elution chromatography has been treated by several authors<sup>5-7</sup>. Previous theoretical work specific to adsorption chromatography has been of little practical value, since the required general relationships between eluent, solute structure, and retention volume have been unknown until recently. Preceding papers in the present series (Parts II<sup>8</sup>, III<sup>9</sup>, V<sup>10</sup> and VI<sup>4</sup>) have discussed the interacting roles of eluent and solute in determining retention volume for elution from alumina, silica and Florisil. Quantitative correlational equations have been derived for the prediction of retention volume in various linear elution adsorption chromatographic (LEAC) systems based on these three adsorbents. It is therefore appropriate to ex-

tend our preceding analysis of fixed eluent LEAC separations to include the case of gradient elution. This should serve to expand the usefulness of our original correlational equations, and perhaps clarify certain of the principles basic to the technique of gradient elution adsorption chromatography (GEAC).

The intention of the present communication is the development of a theory of GEAC, the exploration of some related theoretical problems, and the application of these results to a general discussion of the technique. A following paper will discuss some more practical considerations related to the use of GEAC, and provide experimental data on several GEAC systems for the confirmation of the theory given in the present paper.

#### OPTIMIZING SEPARATION IN GRADIENT ELUTION SYSTEMS

Following sections will explore in detail the theory of GEAC separation. The intention of the present section is a discussion of the results of this theoretical study and its application to some practical problems in the design of optimum GEAC separations. A GEAC system customarily involves the elements in the schematic diagram of Fig. 1. Prior to the beginning of separation, a weak eluent A and a strong eluent B

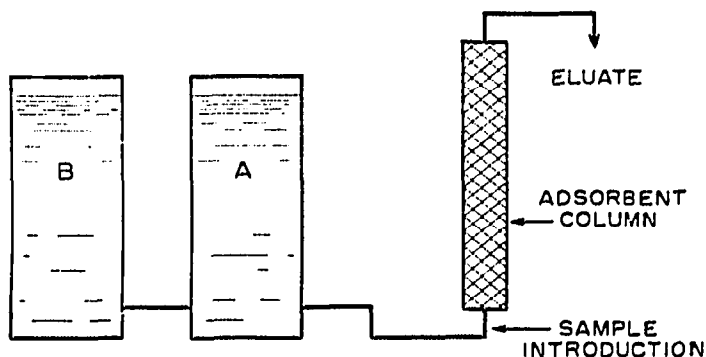


Fig. 1. Schematic diagram of GEAC separation.

are contained in separate units A and B. Unit A provides for the continuous mixing of its contents. Sample is introduced to the column, and elution begun. Eluent B flows into unit A, and the mixed eluent from A enters the column. At the beginning of separation, the eluent to the column is essentially pure A. As elution proceeds, the eluent becomes progressively richer in B and hence progressively stronger. The volume fraction of B in the combined eluent to the column ( $V_B$ ) is determined by the geometry of units A and B, and their mode of connection. Exact relationships between  $V_B$  and  $V$ , the total eluate volume, have been derived for various gradient elution systems<sup>5,11</sup>. Gradient devices for the production of any desired eluent gradient ( $V_B$  versus  $V$ ) have also been described<sup>12</sup>.

As developed in preceding papers of the present series, the effectiveness of an eluent in the elution of a solute from an adsorbent column is measured by its eluent strength parameter  $\epsilon^\circ$ . Thus, if  $\underline{R}_p$  is the linear equivalent retention volume (ml/g) of a solute eluted by pentane from a given adsorbent, its retention volume  $\underline{R}^\circ$  for elution from the same adsorbent by an eluent of strength  $\epsilon^\circ$  is:

$$\underline{R}^\circ = \underline{R}_p 10^{-aA\epsilon^\circ} \quad (1)$$

$\alpha$  is the adsorbent activity function<sup>8</sup>, and  $A_s$  is the effective area of the solute<sup>4</sup>. For binary eluents, as occur in GEAC separation, the eluent strength is related to the strength of the two eluents,  $\varepsilon^\circ_A$  and  $\varepsilon^\circ_B$ , to the mole fraction of the stronger eluent  $X_B$ , to the adsorbent activity  $\alpha$ , and to  $n_b$ , the value of  $A_s$  for the eluent B (considered as a solute)<sup>9</sup>:

$$\varepsilon^\circ = \varepsilon^\circ_A + \frac{\log (X_B 10^{\alpha n_b (\varepsilon^\circ_B - \varepsilon^\circ_A)} + 1 - X_B)}{\alpha n_b} \quad (2)$$

The eluate volume  $R_g$  (ml) required to elute a solute from a particular GEAC column can be calculated as discussed in the next section. For this calculation, we must know the dependence of  $\varepsilon^\circ$  on  $V$ , as well as the values of certain solute parameters ( $R_p$ ,  $A_s$ ) and of the adsorbent activity ( $\alpha$ ). Values of the fundamental parameters  $\alpha$ ,  $\varepsilon^\circ_A$ ,  $\varepsilon^\circ_B$ , and  $n_b$  have been tabulated elsewhere<sup>4</sup> for a number of solvents and adsorbents, and  $R_p$  and  $A_s$  are calculable for many solutes from data summarized previously<sup>4</sup>. For eluent strength gradients of the so-called linear form,

$$\alpha \varepsilon^\circ = a + bV, \quad (3)$$

where  $a$  and  $b$  are constant throughout a GEAC separation, calculation of  $R_g$  takes a relatively simple form:

$$R_g = \frac{\log (2.31 A_s b W R_p 10^{-a A_s} + 1)}{b A_s} \quad (4)$$

$W$  is the total adsorbent weight, and  $R_g$  assumes sample charged to dry column. An example of the calculation of  $R_g$  in a GEAC system is offered at the close of this section.

Qualitatively, it would appear desirable to have  $\varepsilon^\circ$  increase linearly with  $V$ . Thus, eluent strength gradients ( $\varepsilon^\circ$  versus  $V$ ) severely concave to the  $V$  axis will show  $\varepsilon^\circ$  increasing very rapidly with  $V$  at high values of  $V$ , and all strongly adsorbing solutes will be eluted as a single band. Alternately, convex gradients tend to level off to a constant value of  $\varepsilon^\circ$  at large  $V$ , and strongly adsorbing solutes are then eluted very slowly. By calculating  $R_g$  for different solutes in GEAC systems employing one or more of these three gradient types, it should be possible to establish unequivocally which gradient type is indeed optimum. Prior to such a calculation, however, it is necessary to distinguish between the various sample types which it may be desired to separate.

A major application of gradient elution chromatography is in the initial study of mixtures of unknown composition and complexity. Thus, in a short time such sample types may be completely eluted from a column with near optimum resolution at each point in the separation. If we lump *all* the solute types thus far studied together and arrange them in order of increasing adsorption energy  $S^\circ$  (from pentane), it is found that  $S^\circ$  increases continuously while  $A_s$  tends to vary more or less randomly. Alternately, within individual sample groups (*e.g.*, the hydrocarbons) there is the tendency for  $A_s$  to increase with  $S^\circ$  as additional adsorbing groups are added to the solute molecule. As a general rule, simple mixtures of known compositional range (*e.g.*, the product of nitrating a single parent molecule, mixtures of aromatic hydro-

carbons, etc.) will involve one or two individual sample types, and  $A_s$  for the solutes in such a sample will *increase* with  $S^\circ$ . It is appropriate to distinguish between what we will call *simple* (known) and *complex* (unknown) sample types,  $A_s$  increasing with  $S^\circ$  for solutes in the former sample type and independent of  $S^\circ$  for solutes in the latter sample type. Obviously, some samples of known compositional range will prove to be "complex" with respect to the dependence of  $A_s$  on  $S^\circ$ , and *vice versa*, but the coupling of "simple" with "known" samples and "complex" with "unknown" is a useful generalization.

The solute retention volume for elution by pentane  $\underline{R}_p$  is related to  $S^\circ$  by

$$\underline{R}_p = V_a 10^{\alpha S^\circ} \quad (5)$$

where  $V_a$  is the surface volume of the adsorbent<sup>8,9</sup>. For *complex* sample types,  $A_s$  may be treated as approximately constant, and for linear eluent strength gradients at *large values* of  $V$  and of  $R_g$  eqn. (4) simplifies to

$$\begin{aligned} R_g &\approx \log (2.31 A_s b W 10^{-a A_s} V_a) + (\alpha/b A_s) S^\circ \\ &\approx C + D S^\circ \end{aligned} \quad (6)$$

after substituting eqn. (5) for  $\underline{R}_p$  into eqn. (4) and ignoring the unity term. The coefficients  $C$  and  $D$  are constant for various solutes where  $A_s$  is constant. Equation (6) states that after the beginning of elution of a *complex* sample from a linear eluent strength GEAC system, the  $R_g$  values for various solutes vary linearly with their adsorption energies. *This spacing of solute retention volumes is a generally desirable one.* In contrast, fixed eluent separations show solute retention volumes varying exponentially as in eqn. (5), leading to the difficulties gradient elution is designed to overcome.

Table I presents some calculated  $R_g$  values for a series of solutes in a *complex* sample ( $A_s$  constant,  $S^\circ$  varying). In the first column of  $R_g$  values, normal gradient elution using a linear eluent strength gradient is assumed, with the separation parameters at the top of Table I. Both  $R_g$  values and the spacing between adjacent solute peaks ( $\Delta R_g$ ) are shown. As predicted above, the spacing of solute peaks of regularly increasing adsorption energy  $S^\circ$  becomes constant after  $R_g$  becomes reasonably large (391). There is some compression of the peaks at low  $R_g$  values, but this is basic to all elution chromatographic systems, as illustrated by data (second column of  $R_g$  values) in Table I for elution of the same sample by pentane, a very weak eluent ( $\varepsilon^\circ = 0.00$ ). Obviously, *no* gradient shape could improve the spacing of the first two solutes of Table I. Alternately, initial elution by pentane (200 ml) followed by normal gradient elution (column 3) provides all the advantages of both fixed weak eluent and GEAC separation. Finally, in the last column of Table I normal gradient elution with  $V_a$  increased tenfold shows improved resolution of the lesser adsorbing solutes. Increasing adsorbent area is obviously the best solution to band compression in the initial part of either GEAC or fixed eluent separation.

Examination of eqn. (6) for the case of  $A_s$  increasing with  $S^\circ$  (*simple* samples) suggests that band spacing ( $R_g$  values) will tend to be slightly compressed at high values of  $R_g$  (as well as at low). This is confirmed in Table II for a model calculation

TABLE I  
CALCULATED GRADIENT ELUTION SEPARATION OF A *complex* SAMPLE  
( $a = 0.0$ ,  $b = 0.0003$ ,  $(WV_a) = 0.20$ ,  $A_g$  assumed equal 10.0)

$\alpha S^\circ$	$R_g$ (ml) and band spacing ( $\Delta R_g$ )						Normal gradient elution ( $WV_a$ ) equal 2.0	
	Normal gradient elution		Pentane elution		Initial pentane elution, followed by gradient elution			
	$R_g$	$\Delta R_g$	$R_g$	$\Delta R_g$	$R_g$	$\Delta R_g$	$R_g$	$\Delta R_g$
1.0	2.0	17	2.0	18	2.0	18	19	107
2.0	19	107	20	180	20	180	126	265
3.0	126	265	200	1800	200*	377	391	324
4.0	391	324	2000	18000	577	335	715	335
5.0	715	335	20000	180000	908	340	1048	335
6.0	1048		200000		1248		1383	

\* Gradient elution begun.

based on a *simple* sample type. The  $R_g$  values are seen to be compressed with both low and high eluate volumes, with the band spacing  $\Delta R_g$  a maximum at intermediate values of  $V$ . The compression of solutes bands at low  $V$  values cannot be avoided by changing the gradient shape (just as in the case for *complex* samples). The compression of the more strongly adsorbing solute bands, although relatively modest, can be avoided by using a mildly convex gradient. This is illustrated in the second case of Table II for a convex gradient approximated by two linear gradients:  $b = 0.0003$  for  $V \leq 347$  ml;  $b = 0.0002$  for  $V \geq 347$  ml.

TABLE II  
CALCULATED GRADIENT ELUTION SEPARATION OF A *simple* SAMPLE  
( $a = 0.0$ ,  $(WV_a) = 0.20$ )

$\alpha S^\circ$	$A_g$	$R_g$ (ml) and band spacing ( $\Delta R_g$ )			
		Linear gradient, $b = 0.0003$ throughout		Convex gradient*	
		$R_g$	$\Delta R_g$	$R_g$	$\Delta R_g$
1.0	6	2	17	2	17
2.0	8	19	109		19
3.0	10	126	221	126	109
4.0	12	347	198	347	221
5.0	14	545	152	590	243
6.0	16	697		820	230

\*  $V \leq 347$ ,  $b = 0.0003$ ;  $V \geq 347$ ,  $b = 0.0002$ .

It is desirable to keep solute band width  $w_g$  in GEAC separation relatively constant throughout the separation. As developed in a following section,  $w_g$  depends upon two independent aspects of the separation: the plate number  $p$  or separation efficiency of the column, and the instantaneous retention volume  $R_t$  of a solute at the time it leaves a GEAC column.  $R_t$  is the value of the retention volume ( $R^\circ W$ ) for the solute assuming fixed eluent elution from the same column by eluent of that composition passing through the GEAC column at the time when  $V = R_g$ . Column efficiency in normal LEAC systems has not yet been investigated in detail. It is known that band width (inverse of efficiency) increases with increasing eluent viscosity<sup>9</sup> and flow rate<sup>13,14</sup>. If these latter two aspects of the separation can be maintained reasonably constant throughout a GEAC separation, column efficiency may be approximately constant and  $w_g$  values will be proportional to  $R_t$  values, at least at large values of  $R_g$ . Table III compares calculated  $R_t$  values for the separation systems of Tables I and II. It is seen that  $R_t$  becomes constant in the linear gradient

TABLE III  
CALCULATED BAND WIDTH IN GEAC SEPARATION  
( $a = 0.0$ ,  $(W/V_a) = 0.20$ )

$\alpha S^\circ$	$A_g$	$\log(R_t/V_a)^*$	
		Linear gradient $b = 0.0003$ throughout	Convex gradient**
<i>Complex</i>			
1.0	10	0.99	—
2.0	10	1.94	—
3.0	10	2.62	—
4.0	10	2.83	—
5.0	10	2.85	—
6.0	10	2.86	—
<i>Simple</i>			
1.0	6	1.00	1.00
2.0	8	1.95	1.95
3.0	10	2.62	2.62
4.0	12	2.75	2.75
5.0	14	2.70	2.86
6.0	16	2.64	2.82

\*  $(R_t/V_a)$  is proportional to band width.

\*\*  $V \leq 347$ ,  $b = 0.0003$ ;  $V \geq 347$ ,  $b = 0.0002$ .

separation of *complex* samples at large values of  $R_g$ , and this can be shown to follow from the fundamental GEAC equations (see a following section). At low  $R_g$  values,  $w_g$  tends to become constant and independent of  $R_t$ , so that the GEAC separation of *complex* samples appears to give optimum band widths with a linear gradient. The corresponding GEAC separation of *simple* samples shows a pattern reminiscent of the  $R_g$  data. Band width goes through a maximum (maximum  $R_t$ ) at intermediate values of  $R_g$ . Use of convex gradients tends to correct for this effect as seen. Again, moderately convex gradients appear best in the separation of *simple* samples with respect to band width as well as band spacing.

Another aspect of GEAC separation which deserves comment is the phenomenon of band splitting<sup>2,15</sup>. If, after elution of a band maximum in a GEAC system, the eluent strength increases sufficiently rapidly, a portion of the original band tail may be *displaced* from the column to give an apparently new band. A similar phenomenon is encountered in the stepwise elution of a sample by a series of progressively stronger eluents<sup>16</sup>; after elution of the major part of a band by one eluent, changing to a stronger eluent elutes the band tail sufficiently rapidly to develop a new band maximum and give the appearance of a second solute band. Intuitively, it would seem unlikely that band splitting would ever occur with convex or linear gradients, and this is shown in a later section to be true for normal bands of the theoretical Gaussian shape. In the case of chemisorbed solutes<sup>4</sup>, where a non-Gaussian tail is developed, it is possible to get band splitting with all three gradient types.

A final consideration in designing optimum GEAC separations is the phenomenon of *displacement*. By displacement is meant the tendency, in fixed binary eluent systems involving small concentrations of a strong eluent B, of the strong solvent to selectively adsorb at the beginning of the column and leave essentially pure A to elute the balance of the column<sup>17</sup>. In the case of solutes readily eluted by pure eluent B but not by A, the solute may advance along the column in front of the advancing zone of adsorbed B until displaced from the column. Table IV shows some experimental data illustrating this effect for a *fixed eluent* system. In the first column, experimental  $\bar{R}^\circ$  values are reported for elution of several solutes from a particular LEAC

TABLE IV

DISPLACEMENT IN THE CHROMATOGRAPHIC SYSTEM 4.6% H<sub>2</sub>O-SiO<sub>2</sub> (DAVISON CODE 12),  
2% v ETHYL ETHER-PENTANE

Solute	$\bar{R}^\circ$ (ml/g)	
	No equilibration*	Equilibrated (true)
Naphthalene	5.4	0.4
Phenanthrene	5.9	1.4
Triphenylene	5.8	2.5
Phenctole	5.8	1.2
Nitrobenzene	8.9	7.0

\* Column pre-wet with one column volume prior to sample introduction.

system, without first passing sufficient eluent through the column to equilibrate the adsorbent and eluent. In the second column, the *true* experimental  $\bar{R}^\circ$  values for *equilibrated* columns (initial passage of a large eluent volume through the column) are given.

A theoretical analysis of displacement in GEAC systems is offered in a later section. Displacement, or column non-equilibrium must always occur in the first stages of a GEAC separation. The volume of eluent required to bring the column to initial equilibrium may be defined as  $V_s$ . If  $V_0$  is the total eluate passed through the column during separation (equal  $\alpha(\epsilon^\circ_B - \epsilon^\circ_A)/b$  for total elution of contents of units A and B), then  $V_s/V_0$  represents the fraction of the separation during which column non-equilibrium exists. Solute normally eluted (assuming instantaneous

column equilibrium and no displacement) within the eluate volume  $V_s$  may be poorly separated in practice so that this fraction  $V_s/V_0$  of the separation can be considered as possibly wasted or ineffective. The ratio  $V_s/V_0$  obviously increases with the difference in eluent strengths ( $\varepsilon^{\circ}_B - \varepsilon^{\circ}_A$ ) and the size of the strong eluent  $n_b$ , since these two factors promote selective adsorption of the strong eluent. Similarly,  $V_s/V_0$  will increase with the capacity of the column ( $WV_a$ ), since the more B that can be adsorbed, the longer non-equilibrium will persist. Fig. 2 summarizes the calculation of  $V_s/V_0$  as a function of the separation conditions. The ratio  $d_B M_A / d_A M_B$  is a mole correction factor converting percent volume of B to  $X_B$ ;  $d_B$  and  $d_A$  are the densities of B and of A,  $M_B$  and  $M_A$  are the molecular weights of B and A. Normally, the ratio  $WV_a/V_0$  will vary from about 0.001 to 0.03 in GEAC separation, and the mole correction factor will lie between 0.8 and 2.0.  $V_s/V_0$  is therefore usually unimportant (less than 0.1 from Fig. 2) as long as  $\alpha n_b (\varepsilon^{\circ}_B - \varepsilon^{\circ}_A)$  is less than 1.0. For GEAC eluent combinations where this latter term is greater than 1.0,  $W/V_0$  or  $V_a$  may be varied so as to control the maximum value of  $V_s/V_0$  by means of Fig. 2.

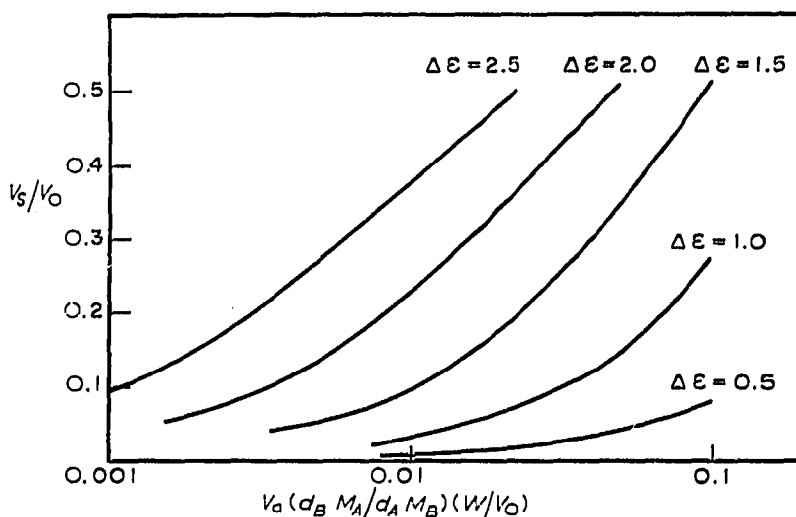


Fig. 2. Dependence of displacement or column non-equilibrium linear strength in GEAC systems on separation variables;  $\Delta\varepsilon = n_b \alpha (\varepsilon^{\circ}_B - \varepsilon^{\circ}_A)$ .

An alternate means of reducing the importance of displacement in GEAC separation, particularly where a wide range of  $\varepsilon^{\circ}$  values is desired during elution, is through the use of more than two eluents. Thus, elution may be begun with a weak eluent A and an eluent B of intermediate strength. At the conclusion of the first elution, the eluent combination B (intermediate strength)-C (strong) may be used in tandem to achieve in the overall GEAC separation the  $\varepsilon^{\circ}$  range between eluents A and C, but without the corresponding displacement. Frequently, for practical reasons, it will prove convenient to switch eluents prior to the end of the first elution, so that the tandem eluent system will be A-B (weak), C (strong). In this case, it is necessary to relate the strength  $\varepsilon^{\circ}$  of a ternary eluent A-B-C to its composition, analogously to eqn. (2) for binary eluents. A later section provides a derivation of this relationship, eqn. (7), between  $\varepsilon^{\circ}$  and the strength of the constituent solvents,  $\varepsilon^{\circ}_A$ ,  $\varepsilon^{\circ}_B$ , and  $\varepsilon^{\circ}_C$  ( $\varepsilon^{\circ}_A < \varepsilon^{\circ}_B < \varepsilon^{\circ}_C$ ), the mole fractions of B and C,  $X_B$  and  $X_C$ , and the value of  $A_s$  for C,  $n_c$ :



$$\varepsilon^\circ = \varepsilon^\circ_B + \frac{\log [X_C 10^{\alpha n_c (\varepsilon^\circ_C - \varepsilon^\circ_B)} + X_B]}{\alpha n_c} \quad (7)$$

Equation (7) will prove useful in other applications, as discussed in the following paper of this series.

To summarize, linear eluent strength gradients provide the best separation of so-called *complex* sample types, with respect to both band width and band spacing. For *simple* samples, where  $A_s$  increases with  $S^\circ$ , a moderately convex gradient is preferred, although it is not markedly superior to the linear gradient. The linear gradient case has the additional advantage that solute  $R_f$  values can be easily predicted. Linear or convex gradients are also preferred from the standpoint of band splitting and the development of spurious bands. Only concave gradients are normally capable of band splitting. Displacement in GEAC separation can be a problem whenever the strengths of the eluents A and B differ widely and/or  $n_b$  is large. Fig. 2 permits the problem to be anticipated in a given separation system and avoided through control of  $W$  or  $V_a$ , or by changing to a ternary eluent system.

As one example of the application of eqn. (4), consider the elution of the solute triphenylene from a GEAC system where  $a = 0.03$ ,  $b = 0.00187$ ,  $W$  equal 10 g, and the adsorbent is 3.7%  $H_2O-Al_2O_3$ .  $R_p$  for this system can be calculated from eqn. (5), providing  $S^\circ$  is first calculated as described previously<sup>1</sup>.

$$\begin{aligned} S^\circ &= \sum^i Q^\circ_i + \sum^j q^\circ_j - f(Q^\circ_k) \sum^{i \neq j} Q^\circ_i \\ &= 18 \times 0.31 + 0 - 0 \\ &= 5.58 \end{aligned}$$

Substituting the above value of  $S^\circ$  into eqn. (5), with  $\alpha = 0.63$  and  $V_a = 0.016^8$  gives  $R_p = 51$ . Finally,  $A_s$  for triphenylene may be calculated<sup>9</sup> as 12, and substituting into eqn. (4):

$$\begin{aligned} R_R &= \frac{\log (2.31 \times 12 \times 0.00187 \times 10 \times 51 \times 10^{-0.03 \times 12} + 1)}{0.00187 \times 12} \\ &= 52.2 \text{ ml} \end{aligned}$$

An experimental value of 51 ml was observed with a GEAC system based on isooctane (A)-ethyl ether (B) and characterized by the above separation parameters.

#### SOLUTE RETENTION VOLUME

Solute retention volume plays a key role in determining the separation capabilities of any elution chromatographic system, and it is of interest to relate the retention volume of a solute in a GEAC separation to its molecular structure and to the conditions of separation. At some time  $t$  after the introduction of a solute to a GEAC column as in Fig. 1 and the beginning of elution, the solute will have traversed some fractional distance  $x$  along the column longitudinal axis. To a first approximation, the composition of the eluent both entering and within the column at time  $t$  can be assumed

identical (if displacement is unimportant), and the instantaneous retention volume  $R_t$  can be defined as previously. At any time during the separation, a differential flow of eluate  $dV$  will induce a corresponding differential flow of the solute band  $dx$ , equal to  $dV/R_t$ . The retention volume of the solute is then given by:

$$\int_0^{R_\theta} dx = 1$$

and

$$\int_0^{R_\theta} dV/R_t = 1 \quad (8)$$

That is, the total length of column is traversed ( $\sum dx = 1$ ) when the eluate volume  $V$  equals  $R_\theta$  (by definition). FREILING<sup>6</sup> has provided a similar derivation of eqn. (8) in his treatment of gradient elution in ion exchange systems. The quantity  $R_t$  is equal to  $\underline{R}^\circ W$ , and  $\underline{R}^\circ$  is related to the eluent strength  $\varepsilon^\circ$  through eqn. (1).  $\varepsilon^\circ$  can in turn be expressed as a function of eluent composition through eqn. (2) or by experimental determination<sup>6</sup>. Finally, the arrangement and geometry of the gradient elution system permits eluent composition and hence  $R_t$  to be known as a function of  $V$ . Substitution of this function of  $V$  into eqn. (8) permits in principle the integration of eqn. (8) and the evaluation of  $R_\theta$ . In actual fact, however, the resulting expression under the integral sign of eqn. (8) is so complex in the general case as not permit an explicit algebraic solution.

It will be seen profitable to sidestep this difficulty in the general integration of eqn. (8) by restricting our attention to the special case of linear eluent strength systems, as defined by eqn. (3). Substitution of eqn. (3) into eqn. (1) gives:

$$\underline{R}^\circ = \underline{R}_p 10^{-A_s(a + bV)} \quad (9)$$

and substitution of  $\underline{R}^\circ$  from eqn. (9) into eqn. (8), with  $R_t$  equal  $\underline{R}^\circ W$ , provides:

$$\int_0^{R_\theta} \frac{10^{A_s(a + bV)} dV}{W \underline{R}_p} = 1 \quad (10)$$

which upon integration gives eqn. (4). Equation (4) is the fundamental equation of linear strength GEAC separation.

The calculation of  $R_\theta$  for non-linear strength gradients (those not given by eqn. (3)) can be done using numerical integration of eqn. (8) with trial and error estimates of  $R_\theta$ . A similar, approximate, procedure is worth mentioning. A general non-linear  $\varepsilon^\circ - V$  relationship as illustrated in Fig. 3 can be approximated by several line segments (dashed lines in Fig. 3) of the form:

$$\text{(segment 1) } \alpha \varepsilon^\circ = a + b_1 V$$

$$\text{(segment 2) } \alpha \varepsilon^\circ = a + b_1 V_1 + b_2 (V - V_1), \text{ etc.}$$

$V_1, V_2$ , etc., are the eluate volumes  $V$  at which the approximating line segments intersect. Now define the function  $T$ :

$$T = \int_0^V dV/R_t$$

$$= \int_0^{V_1} dV/R_t + \int_{V_1}^{V_2} dV/R_t + \dots + \int_{V_t}^V dV/R_t$$

Each of the integrals above can be evaluated as in the development of eqn. (4):

$$\int_0^V dV/R_t = \frac{10^{a A_s}}{2.31 R_p W A_s b_1} (10^{A_s b_1 V} - 1) \tag{11a}$$

$$\int_{V_1}^V dV/R_t = \frac{10^{A_s(a + b_1 V_1 - b_2 V_1)} (10^{A_s b_2 V} - 10^{A_s b_2 V_1})}{2.31 R_p W A_s b_2} \tag{11b}$$

and so forth.

For  $V < V_1$ , the resulting calculation of  $R_g$  is exactly equivalent to the linear case, with  $R_g$  given by eqn. (4). For  $V_1 < V < V_2$ :

$$T = \int_0^{V_1} dV/R_t + \int_{V_1}^V dV/R_t$$

$$= \frac{10^{a A_s}}{2.31 R_p W A_s b_1} (10^{A_s b_1 V_1} - 1)$$

$$+ \frac{10^{A_s(a + b_1 V_1 - b_2 V_1)} (10^{A_s b_2 V} - 10^{A_s b_2 V_1})}{2.31 R_p W A_s b_2}$$

$T$  is obtained for trial values of  $V$  and the solution of  $V = R_g$  obtained at  $T = 1$ . It should be noted that  $T$  is not linear in  $V$  so that interpolation between values of  $T$  greater and less than 1 requires care. The procedure for calculating  $T$  when  $V > V_2$  is essentially similar.

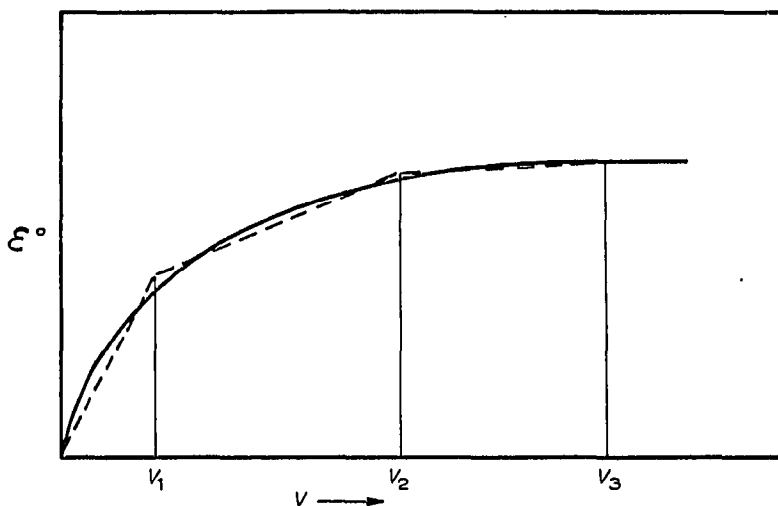


Fig. 3. Approximation of a non-linear  $\epsilon^\circ$ - $V$  curve by line segments for calculation of  $R_g$  values.

## SOLUTE BAND WIDTH

The equivalent plate model of elution chromatography<sup>18</sup> predicts that, in a given chromatographic system which may be approximated by  $p$  equivalent equilibrium plates, band width  $w$  (measured in the column eluate) is proportional to solute retention volume  $R'$ . In fact,  $p$  is customarily calculated from the ratio  $R'/w$ . As discussed previously, a major advantage of GEAC is its tendency to reduce this normal increase of solute band width with increasing retention volume. Ideally, all solute bands would have comparable widths in GEAC separation, and it is of interest to investigate the theoretical effect of the conditions of separation on solute band width.

Band width as measured in the eluate is determined both by the width of the *adsorbed* solute band immediately prior to elution from the column, and by the retention volume  $R'$ . Fig. 4 illustrates this relationship, an adsorbed band at the end of a long column being shown just prior to elution from the column, along with the resulting eluate band. By the time the band has arrived at the end of a *long* column,

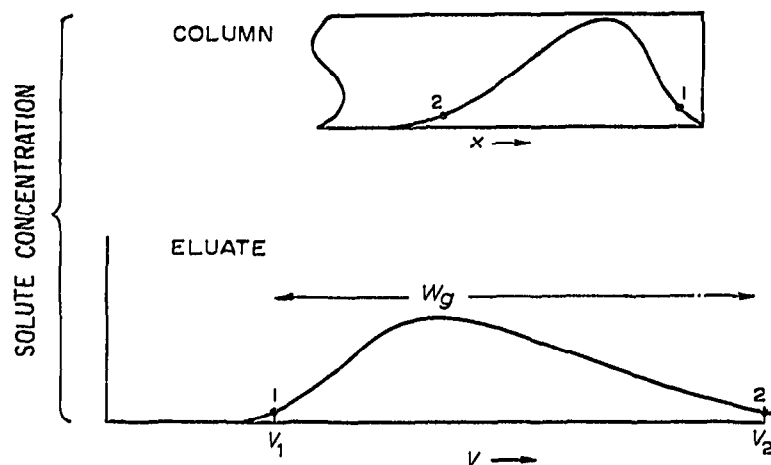


Fig. 4. Dependence of band width in eluate on adsorbed band width.

band shape has to a first approximation been determined, and the band appearing in the eluate may be regarded as arising from the simple elution of each element of the adsorbed band  $x$  by a corresponding eluent volume  $V$ .  $x$  in Fig. 4 represents the fractional distance along the column length, with a value of 1.0 to the column end. The eluent volume required to elute the band element at  $x$  is approximately  $R'(1 - x)$  when  $R' \gg 1$ . If  $(x_1 - x_2)$  represents the width of the adsorbed band as measured between the arbitrary points 1 and 2, then the volumes required to elute these two corresponding points are  $v_1$  and  $v_2$ , equal  $R'(1 - x_1)$  and  $R'(1 - x_2)$ , respectively. The width of the eluted band is  $(v_2 - v_1)$ , equal  $R'(x_1 - x_2)$ . That is, the eluted band width is  $R'$  times the adsorbed band width. Since in the simple plate model theory  $R'/w$  is constant, the adsorbed band width of all solutes immediately prior to elution must also be constant (independent of  $R'$ ).

In the case of GEAC separation, the above considerations similarly apply, as long as the plate number  $p$  of the column is constant throughout separation. That is, adsorbed band widths of all solutes are comparable, and eluate band width  $w_g$  is proportional to  $R_t$ , the instantaneous retention volume at the time the band is eluted

from the column ( $V = R_g$ ). For large values of  $R_g$ ,  $R_t$  may be calculated as follows. First, eqn. (1) reduces to:

$$R_g \approx (\log 2.31 A_s b W R_p 10^{-a A_s}) / b A_s$$

and substitution of eqn. (5) for  $R_p$  gives:

$$R_g \approx (\log 2.31 A_s b W V_u) / b A_s - (a/b) + \alpha S^\circ / A_s$$

At the time of band elution,  $V = R_g$ , and from eqn. (3):

$$\alpha \varepsilon^\circ = a + b R_g$$

$$\alpha \varepsilon^\circ \approx a + (\log 2.31 A_s b W V_u) / A_s - a + S^\circ / A_s$$

$R_t$  equal  $W R^\circ$  is now obtained from eqns. (1) and (5) with substitution of the above expression for  $\alpha \varepsilon^\circ$ :

$$\begin{aligned} R_t &\approx W V_u 10^{a S^\circ - A_s (\log 2.31 A_s b W V_u) / A_s - a S^\circ} \\ &\approx \frac{1}{2.31 A_s b} \end{aligned} \quad (12)$$

For solutes of constant or approximately constant  $A_s$  values, eqn. (12) predicts constant band widths at large  $R_g$  values for linear strength gradient separations.

#### BAND SPLITTING

Fig. 5(a) provides an illustration of what is meant by band splitting. Toward the end of elution, an initially normal elution band appears suddenly to develop a second maximum, giving the appearance of two rather than one eluted bands. The origin of band splitting is easily understood in terms of our previous discussion of band width. For fixed eluent elution, as in Fig. 4, or a band that has reached the end of a column, it was assumed that  $R_t$  was approximately constant during the course of final elution, and that  $R_t x$  ml of eluent were required to elute the solute from a

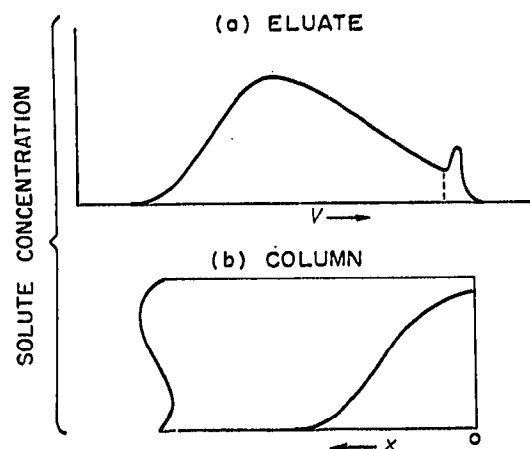


Fig. 5. Band splitting in GEAC separation.

column element at  $x$ . This process assumes transfer of all solute from a given column element into a corresponding volume element  $dV$ . Since  $V$  equals  $R_t x$ ,  $dV/dx$  equals  $R_t$ , and the ratio of solute concentrations in the eluent element relative to the original column element is  $1/R_t$ . The solute concentration in the eluate is then related to the concentration in the original adsorbed band  $C_x$  by:

$$C_v = C_x/R_t \quad (13)$$

In GEAC separation,  $R_t$  actually is assumed to decrease during the final elution of the band, although this normally insignificant effect was ignored in our preceding discussion of band width.  $C_v$  must therefore increase up to the point  $x_m$  corresponding to the original adsorbed band maximum. Past the elution of the band element originally at  $x_m$ ,  $C_x$  decreases while  $1/R_t$  continues to increase. If the decrease in  $C_x$  is large relative to the increase in  $1/R_t$ ,  $C_v$  will begin to decrease and the eluted band will have developed a band maximum. If at some later time,  $1/R_t$  suddenly increases more rapidly than  $C_x$  is decreasing,  $C_v$  will then increase, and a second band maximum in the eluate will have developed. It is of interest to establish under what experimental conditions band splitting can occur.

Fig. 5(b) shows the adsorbed solute band eluted to the original point  $x_m$ ; *i.e.*, with the adsorbed band maximum at the column end ( $x_m = 0$ ). The  $x$  axis is now reversed for convenience in Fig. 5(b), the column end corresponding to  $x = 0$ , and the front end of the column having  $x = 1.0$ . The preceding discussion indicates that no band maximum will have developed in the eluate prior to the arrival of the adsorbed band maximum at the column end ( $x = 0$ ), so that the shape of the eluate band past this point ( $V = R_0$ ) will determine the occurrence of band splitting. Theoretically, the adsorbed elution band in Fig. 5(b) should be a Gaussian curve<sup>18</sup>, and:

$$C_x = C e^{-Dx^2} \quad (14)$$

where  $C$  and  $D$  are constants for a given column of  $p$  equivalent plates. Analogously to eqn. (8), we can write for the elution of the band in Fig. 5(b):

$$\int_0^x dx = \int_{R_0}^{V_x} dV/R_t$$

where  $V_x$  is the eluate volume  $V$  at which the column element  $dx$  is eluted. In terms of eqns. (1) and (3):

$$R_t = R_0 10^{-b(V_x - R_0)} \quad (15)$$

for a linear eluent strength gradient, and substitution of this into the previous integral with integration gives:

$$x = \frac{10^{-bR_0}}{2.31b R_0} (10^{bV_x} - 10^{bR_0})$$

or:

$$x = G (10^{bV_x} - 10^{bR_0}) \quad (16)$$

Substitution of eqns. (14) and (15) into eqn. (13) gives:

$$C_v = \frac{C e^{-Dx^2} 10^{b(V_x - R_g)}}{R_g} \quad (17)$$

The conditions for  $C_v$  being a maximum at some eluate volume  $V$  is  $\partial C_v / \partial V = 0$ , or differentiating eqn. (17) and rearranging gives:

$$x \partial x / \partial V = b/2 CD \quad (18)$$

Substituting eqn. (16) and its derivative with respect to  $V$  into eqn. (18) then gives:

$$10^{bV_x} (10^{bV_x} - 10^{bR_g}) = 1/2 CDG^2 \quad (19)$$

The term within the brackets of eqn. (19) is zero when  $V = R_g$ . For  $V_x > R_g$ , both  $10^{bV_x}$  and the bracketed factor in eqn. (19) increase continuously with  $V_x$ , as must their product. Therefore, at one and only one value of  $V_x$ , the product on the left must equal the positive constant on the right, or there is only one band maximum in the eluate. *Consequently, it has been proved that linear strength gradients do not permit band splitting.*

An exception to the above conclusion may result whenever the adsorbed band is non-Gaussian, as in the case of solute chemisorption<sup>4</sup>. Chemisorbed solutes show pronounced, very flat tailing of the elution band with fixed eluent elution, and it seems quite likely that GEAC separation under such conditions will lead to band splitting even with linear eluent strength gradients.

#### DISPLACEMENT

The phenomenon of displacement is illustrated in Fig. 6. A fixed eluent chromatographic system is assumed, with elution by a dilute solution of a strongly adsorbing eluent B in a weak adsorbing eluent A. The eluent issuing from the column will be initially depleted of B, because of mass transfer to the adsorbed phase. At some time

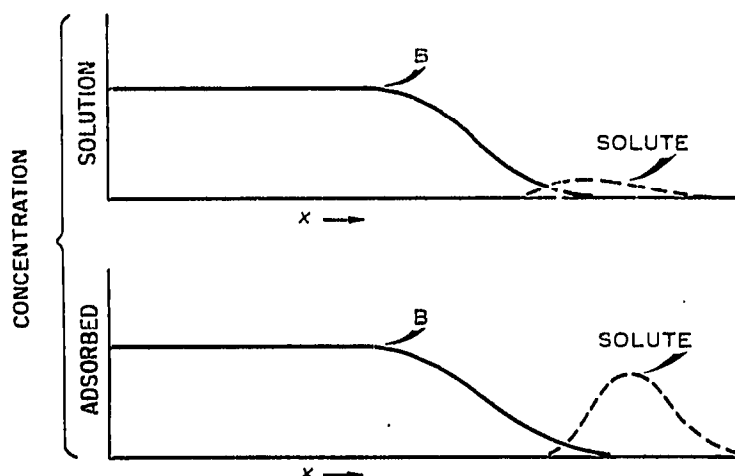


Fig. 6. Displacement in a fixed eluent adsorption chromatographic system. Concentration profiles within the column.

during elution, the concentration profile of B in adsorbed and solution phases within the column will be as represented by Fig. 6. Solutes that are readily eluted (small  $R^{\circ}$ ) by the given binary eluent from *equilibrated* columns (entering eluent in equilibrium with leaving eluent) will tend to move down the column ahead of the adsorbed B zone by displacement, as illustrated in Fig. 6 (dashed solute band). The data of Table IV for that fixed eluent chromatographic system suggest, after taking column void volume into account, that 6.8 ml/g of eluent must flow through this system before the adsorbent is saturated with ethyl ether, since all weakly adsorbed solutes (true  $R^{\circ} < 3$ ) have identical retention volumes in the non-equilibrated column. The adverse effect of displacement on separation is also illustrated in the data of Table IV. Whereas reasonable separation occurs between phenanthrene and triphenylene in the equilibrium column, no separation exists in the non-equilibrium column.

The mathematically precise formulation of the displacement effect in GEAC separation is obviously a formidable task. The reasonably complex equilibria associated with analogous fixed binary eluent systems are further complicated in GEAC by the rapidly changing composition of eluent entering the column. Consequently, it is necessary to seek a less exact, mathematically tractable, approximation of displacement in GEAC systems.

Because displacement will be most serious for strongly adsorbing eluents B, a reasonable initial assumption is that the adsorbent surface will remove all B from the eluent, up to the point where the adsorbent surface is completely covered by adsorbed B. After deriving the relevant equations for displacement based on this approximation, we will show how to correct for the case where the adsorbent surface is *not* largely covered by B after equilibrium is reached within the column. With the assumption of complete surface coverage by B, the eluate volume  $V_s$  required to saturate the adsorbent surface (whose total volume capacity is  $WV_a$ ) is defined by:

$$WV_a = \int_0^{V_s} V_B dV \quad (20)$$

where  $V_B$  refers to the column fraction of B in the entering eluent at a given time. For small values of  $V_B$ , where displacement is of most importance, the mole and volume fractions of B in the entering eluent are related by:

$$X_B \approx (d_B M_A / d_A M_B) V_B \quad (21)$$

where  $d$  and  $M$  refer to the density and molecular weight, respectively, of the subscript species. Now, for convenience, define the symbols:  $\Delta\varepsilon \equiv n_b \alpha(\varepsilon^{\circ}_B - \varepsilon^{\circ}_A)$  and  $\varepsilon \equiv n_b \alpha(\varepsilon^{\circ} - \varepsilon^{\circ}_A)$ , which may be regarded, respectively, as the effective eluent strength *range* of the separation, and the relative eluent strength at a given time. For a linear strength gradient:

$$\varepsilon = \Delta\varepsilon V/V_0 \quad (22)$$

where  $V_0$  is the total eluate volume associated with the GEAC separation (equal  $V$  when  $\varepsilon^{\circ}$  equal  $\varepsilon^{\circ}_B$ ). From eqn. (2):

$$\begin{aligned} X_B &= (10^{\varepsilon} - 1)/(10^{\Delta\varepsilon} - 1) \\ &= (10^{\Delta\varepsilon V/V_0} - 1)/(10^{\Delta\varepsilon} - 1) \end{aligned} \quad (23)$$



Solution for  $V_B$  by eliminating  $X_B$  from eqns. (21) and (23), substituting into eqn. (20), and integrating gives:

$$(WV_a d_B M_A \Delta \varepsilon / d_A M_B V_0) (10^{\Delta \varepsilon} - 1) = 10^{\Delta \varepsilon V_s / V_0} - 1 - \Delta \varepsilon V_s / V_0 \quad (24)$$

Tables of the function  $(10^x - 1 - x)$  permit the solution of  $V_s/V_0$  as a function of  $WV_a d_B M_A \Delta \varepsilon / d_A M_B V_0$  and  $\Delta \varepsilon$ . At the time  $V$  equal  $V_s$ , the adsorbent surface may be only fractionally covered by solute, so that the above expression (24) generally overestimates the magnitude of  $V_s/V_0$ . If the fraction of the surface covered by B is  $S_B$ , this is equivalent, in terms of the amount of B required for surface saturation, to a lower value of  $W$  or  $V_a$ . Consequently, tables of  $V_s/V_0$  can be prepared as a function of  $WV_a d_B M_A \Delta \varepsilon / d_A M_B V_0$  for various values of  $\Delta \varepsilon$ , assuming  $S_B = 1.0$ ,  $S_B$  actually calculated for each value of  $V_s/V_0$  obtained from the table assuming an equivalent reduction in the  $(WV_a, \text{etc.})$  term by a factor of  $S_B$ . A new value of  $S_B$  can be calculated, a further reduction in  $WV_a$  estimated, and further correction of  $V_s/V_0$  obtained. By this iterative procedure, final tabulated values of  $V_s/V_0$  can be obtained corrected for the actual value of  $S_B$ . This is the basis of the calculated values of Fig. 2. The required function  $S_B$  has been derived previously in connection with the derivation of eqn. (2), and is [eqn. (6), Ref. (9)]:

$$S_B = \frac{X_B 10^{\Delta \varepsilon}}{X_B 10^{\Delta \varepsilon} + 1 - X_B}$$

Substitution of  $X_B$  from eqn. (23) and simplification gives finally:

$$S_B = \frac{10^{\Delta \varepsilon (1 + V_s/V_0)}}{10^{\Delta \varepsilon (1 + V_s/V_0)} + 10^{\Delta \varepsilon} + 1 - 10^{\Delta \varepsilon V_s/V_0}}$$

#### THE ELUENT STRENGTH OF TERNARY SOLVENTS

It is desired to calculate  $\varepsilon^\circ$  for ternary solvents A-B-C as a function of the ternary compositions,  $X_A$ ,  $X_B$ ,  $X_C$ , and the eluent strengths of the pure constituents,  $\varepsilon^\circ_A, \varepsilon^\circ_B, \varepsilon^\circ_C$  ( $\varepsilon^\circ_A < \varepsilon^\circ_B < \varepsilon^\circ_C$ ). For the usual case of interest to GEAC separation, it may be assumed that  $\varepsilon^\circ_C \gg \varepsilon^\circ_A$  and  $X_A$  does not approximate unity (the eluent has an appreciable concentration of B and/or C). Under these conditions, the adsorbent surface may be considered to be covered almost exclusively by B and C. Referring back to the original derivation of eqn. (2) in Ref. 9, it is seen that the ternary case under the above conditions almost exactly duplicates the binary case: the mole fraction of adsorbed B and C for the ternary is the same as A and B for the binary, and the solution mole fractions of B and C differ from those of A and B for the binary only in that the sum of  $X_B$  and  $X_C$  are not unity in the ternary case, as  $X_A$  and  $X_B$  were for the binary. The derivation of the ternary  $\varepsilon^\circ$  relationship now follows that of the previous binary derivation exactly, differing only in that the term  $X_B$  (instead of  $1 - X_C$ ) is retained in the final expression (7).

The accuracy of eqn. (7) is checked in Table V for a series of ternary eluents

TABLE V

COMPARISON OF EXPERIMENTAL ELUENT STRENGTHS OF TERNARY SOLVENTS WITH VALUES CALCULATED FROM EQUATION (7); 3.7% H<sub>2</sub>O-Al<sub>2</sub>O<sub>3</sub>

Solvent composition % (vol.)				log R <sup>o</sup> *	$\alpha\epsilon^o$ (expt.)**	$\epsilon^o$	
Pentane	Benzene	Methylene chloride	Dioxan			(Expt.)**	(Calc.)***
0	100	0	0	1.06	—	—	0.32
0	0	100	0	0.39	—	—	0.42
60	30	10	0	1.72	0.141	0.22	0.20
30	30	40	0	1.09	0.204	0.32	0.35
60	38	0	2	0.90	0.223	0.35	0.31
60	35	0	5	0.61	0.252	0.40	0.37
60	30	0	10	0.36	0.277	0.44	0.43
60	0	38	2	0.66	0.247	0.39	0.38
60	0	35	5	0.42	0.271	0.43	0.40
60	0	30	10	0.16	0.297	0.47	0.44
60	0	20	20	—0.12	0.325	0.52	0.50
20	0	75	5	0.08	0.305	0.48	0.44
20	0	70	10	—0.10	0.323	0.51	0.47
20	0	60	20	—0.35	0.348	0.55	0.50

\* For 6-methoxyquinoline.

\*\* From  $R^o$  and eqn. (1), assuming  $A_s = 10$  and  $\log R_p = 3.11$ .\*\*\* From eqn. (7), assuming  $\epsilon^o$  dioxan = 0.65; a previously reported value<sup>9</sup> (0.63) was in error.

using pentane, benzene, methylene chloride, and/or dioxan. Measurement of the retention volume  $R^o$  for a solute of known  $A_s$  value in each of these ternary solvents and in two pure solvents of known  $\epsilon^o$  value permits the derivation of experimental  $\epsilon^o$  values, which are seen in Table V to agree with the calculated values with an average precision of  $\pm 0.03$  unit. Equation (7) is not expected to be a good approximation when the various constituent eluents are of roughly comparable strength, or when the mole fraction of A is close to one. Neither of these cases, however, is of practical interest in the use of ternary solvent systems in GEAC separation.

## ACKNOWLEDGEMENT

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## GLOSSARY OF TERMS

$A_s$	Solute effective area.
A, B, C	Refer to solvents A, B, C.
$a, b$	Coefficients of eqn. (3).
$b_1, b_2$	Values of $b$ occurring in various parts of a GEAC separation.
C, D, G	Constants.
$C_v, C_x$	Concentration of solute in eluate at volume $V$ , and on column at point $x$ .
$d_A, d_B$	Density of solvents A, B.

$M_A, M_B$	Molecular weight of A, B.
$n_b, n_c$	Value of $A_s$ for solvents B, C.
$\bar{p}$	Number of theoretical equivalent plates in a column.
$\underline{R}^\circ$	Solute linear equivalent retention volume (ml/g).
$\underline{R}_p$	Value of $\underline{R}^\circ$ for elution by pentane (ml/g).
$R_g$	Retention volume in a GEAC separation (ml), corrected for column volume.
$\Delta R_g$	Difference in $R_g$ values for adjacent solutes.
$R_t$	Instantaneous retention volume; see text (ml).
$R'$	Uncorrected solute retention volume (ml).
$S^\circ$	Solute adsorption energy; pentane solvent.
$S_B$	Mole fraction in adsorbed phase of B.
$T$	Fractional movement of a solute along a GEAC column; see equations I1a, I1b
$v_1, v_2, v_x$	Eluate volume required to elute point 1, 2, $x$ on adsorbed solute band.
$V_a$	Adsorbent surface volume (ml/g); proportional to surface area.
$V$	Eluate volume.
$V_0$	Total volume of eluate for a given GEAC separation.
$V_s$	Eluent volume required to bring GEAC column into equilibrium with entering eluate; volume at which displacement effect ends.
$V_B$	Volume fraction of B in eluent.
$V_1, V_2$	Eluate volume at which eluent strength gradient $b$ changes; see treatment of non-linear eluent strength GEAC separation.
$W$	Weight of adsorbent in column.
$w$	Band width in eluate (ml).
$w_g$	$w$ in GEAC separation.
$x, x_1, x_2$	Fractional distance along the column, with origin ( $x = 0$ ) taken variously at either column end.
$x_m$	Value of $x$ at band maximum.
$X_B, X_C$	Mole fraction of B, C in eluent.
$\alpha$	Adsorbent activity function.
$\Delta\varepsilon$	$n_b \alpha(\varepsilon^\circ_B - \varepsilon^\circ_A)$ .
$\varepsilon^\circ$	Eluent strength; particularly the eluent strength in a GEAC separation at time $t$ .
$\varepsilon$	$n_b \alpha(\varepsilon^\circ - \varepsilon^\circ_A)$ .

## SUMMARY

The fundamental correlational equations previously developed for normal, linear elution from alumina, silica, and Florisil have been extended to include the technique of gradient elution. It has been shown that in general the eluent gradient should be of the *linear strength* form. The theoretical advantages of linear strength gradient elution include: (1) approximately equal spacing of solute peaks according to increasing solute adsorption energy, providing maximum resolution between both weakly and strongly adsorbing solutes with minimum separation time; (2) approximately equal band widths for maximum detection sensitivity; (3) no band splitting with development of spurious double peaks; (4) easily calculable retention volumes for solutes whose adsorption parameters have been measured or can be estimated. For some sample types a mildly convex eluent strength gradient is predicted to give

a better separation system, although the advantages over the corresponding linear strength case will normally be small. The use in gradient elution of solvents of widely differing strengths can lead to displacement, rather than elution, with resulting loss of resolution for weakly adsorbing solutes. The experimental conditions required for displacement to occur are discussed, as is the avoidance of this phenomenon through various means including the use of ternary solvent mixtures. The theoretical strength of such ternary eluents has been derived as a function of the eluent composition and the strengths of the pure constituent solvents.

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