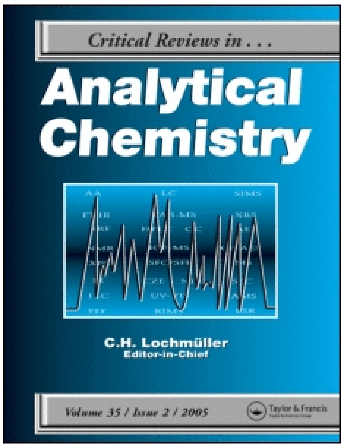

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SOME RECENT ADVANCES IN THE THEORY OF GAS CHROMATOGRAPHY

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I. INTRODUCTION

It is true to say that the vast majority of scientists using gas chromatography as an everyday technique for the separation and analysis of complex mixtures are relatively unfamiliar with the elements of basic chromatographic theory, and know even less about the underlying physics and chemistry of the system. This state of affairs may in part be justified, in light of the enormous literature in chromatography–over 2250 publications in 1969 in gas chromatography alone.[1] Furthermore, a successful analysis need not depend upon an understanding of the fundamental processes. Manufacturers' literature and custom service facilities make it possible to obtain separations on an 'ad hoc' basis.

Giddings[2] posed the question, "How many theories of chromatography can you write?" In spite of the efforts of the workers active in this field we are very little nearer than in 1966 to finding the one true theory. We may be even farther away from the goal, for the process of chromatography, apparently so simple in practice, is in reality a complex amalgamation of phenomena including hydrodynamics, kinetics, solution thermodynamics, surface chemistry, and diffusion which by themselves have no single or exact theory. It is not surprising then that no such single and exact theory exists for chromatography. Chromatographic theory has only advanced by a series of judicious approximations, making the mathematical treatment tractable.

Giddings[2] has recognized three levels of gas chromatographic theory. The first deals with the structural concepts and encompasses the thermodynamics of retention, the structure of the flow space, the flow hydrodynamics, and the kinetics of mass transfer. The first of these is proving to be a profitable field of study since gas chromatography allows for the rapid accumulation of thermodynamic data with which to test theories of solution and adsorption. The use of gas-liquid chromatography (G.L.C.) for the determination of thermodynamic properties has been reviewed by Young.[3] A corollary of this is that we are now in a much better position to predict the type of system likely to produce a required separation.

The structure of the flow space, flow hydrodynamics, and kinetics of mass transfer are everyday problems to the chemical engineer and the physicist,[4] but the considerable knowledge in this field has been applied only infrequently to chromatographic problems. Although several different types of columns are in use in chromatography and have been evaluated for efficiency,[5] the void geometry of these columns and the flow profiles have not been analyzed, except in the simple case of open tube columns. This is not surprising since the typical chromatography column is a highly disordered system, giving rise to complex flow patterns around irregular, chemically non-uniform surface with complex mass transport in and between the phases, and rigorous mathematical treatments are not yet possible.

The use of glass bead loaded columns, while not being the first choice of the analyst in spite of certain favorable properties, has advantages in theoretical studies; for example, the effluent profile width may be predicted within 5 to 20% using only non-chromatographic parameters.[6] This may not appear to be a very high level of prediction, but it does represent a real advance which can be attributed to the favorable properties of glass bead columns where (a) the rate controlling factor of mass transfer is diffusion in the liquid phase and (b) the geometry of the liquid phase is well defined, contrary to the conventional porous support materials where liquid distributution is controlled by the pore size distribution. This approach has been used by Giddings[7] to show that turbulent flow will increase the rate of sorptive-desorptive transfer of solute molecules and more lately to extend the non-equilibrium theory of chromatography to the situation involving complex laminar flow.[8]

The second level of chromatographic theory relates the solute zone to the underlying parameters. We require the theory to give information regarding the location and shape of a solute zone when injected onto the chromatographic column. The sum total of the relative zone displacements and widths of two solutes is the resolution. The zone location is determined by the sorptive properties of the system and the zone width by the kinetic and mass transport processes related to the equilibrium process. The position of the zone may be described in terms of the retention ratio R, i.e., the ratio of the passage time of a non-sorbing zone to that of the given component or, equivalently, the fraction of solute in the mobile phase.

The retention equation of Martin and Synge,[9] generalized by Keller and Stewart[10] for several mechanisms of retention, gives

$$R = V_m/(V_m + \Sigma K_i V_s) \qquad (1)$$

The recognition of mixed mechanisms of retention proposed by Martin[11] has important consequences both to the theory and practice of gas chromatography.

Since $$R = t_M/(t_M + t_s) \qquad (2)$$

where t_M and t_s are the retention times for an unresolved peak and a retained peak, Equation 1 may be written in the more familiar form

$$V_R = V_m + \Sigma K_i V_s \qquad (3)$$

A point overlooked by some workers is that only at the center of the zone does true equilibrium exist so that Equations 1 and 2 relate the peak maximum retention time or, more correctly, the first statistical moment, to the thermodynamic distribution coefficients.

The width of the zone is determined by the complex mass transfer processes in the column. These phenomena are not amenable to strict mathematical description and again approximations must be made. One of the most important assumptions is the "long-time approximation" which allows the location and profile of the zone to be approximated by a limiting form which is exact when the elution time is infinitely longer than the time for equilibration between the phases. With equilibration times probably of the order of milliseconds this is a fair approximation. Further approximations involve the solute zone having a Gaussian distribution and require departures from equilibration to be small. These set fairly strict limits on the Equation 1; for instance, a Gaussian distribution implies a linear relationship between the concentration of the solute in the gas and liquid phases; i.e., sample sizes must be small enough for the isotherm to be in the Henry's Law region.

The retention equation has been extended by Everett[12] and by Cruikshank, Windsor, and Young[13] to allow for non-ideal behavior in the gas phase and other effects, and by Conder and Purnell[14] to take into account a finite solute concentration.

The width of the solute zone is characterized by the plate height which depends upon the various structural and dynamic parameters. Since the plate height is a measure of the efficiency of a column and this reflects the ability of a column operated under given conditions to perform a separation, the dependence of plate height on these parameters is of great importance to the chromatographer and has received much attention.

The third level of chromatographic theory involves the optimization of the operating conditions to give maximum resolution in the minimum time. In writing a review on a topic with such varied facets one must be selective. The fact that some works have been ignored is no reflection on their importance to the overall development of chromatographic theory. I have largely neglected those topics which are of more limited interest and have endeavored to review those facets which I feel will be of most use to the analyst; namely, selectivity, efficiency, the optimization of column conditions and the correlation of retention behavior with structure. If in ignoring some of the more academic problems I am guilty of perpetrating still further the analysts' ignorance, I can only justify this on the grounds of expediency.

II. THE RETENTION EQUATION

The link between practice and theory in gas liquid chromatography is provided by the basic retention equation which relates the net retention volume V_N to the partition coefficient K and the volume of the liquid phase V_L

$$V_N = V_R - V_M = KV_L \qquad (4)$$

V_R and V_M are the retention volumes and gas-holdup of the column, respectively, (both corrected for gas compressibility) and K is defined by:

$$K = \frac{\text{concn. of solute in liquid phase}}{\text{concn. of solute in gas phase}} = \frac{q}{c} \qquad (5)$$

Neglecting gas imperfections the retention volume is related to the activity coefficient at infinite dilution γ^∞ by the relation

$$V_N = \frac{n_L RT}{\gamma^\infty p^o} \qquad (6)$$

where n_L is the number of moles of liquid phase, p^o is the saturated vapor pressure of the solute at the column temperature T. The development of the theoretical description of the retention volume is contained in the review by Young.[3] These equations refer to the case of a volatile solute at infinite dilution in a non-volatile solvent. The general finite concentration case has been treated in a series of papers by Conder and Purnell[14-17] by accounting for gas compressibility and gas imperfection and variation of the mobile phase velocity due to the flux of solute molecules across the interphase boundary.

Retention Equation with a Finite Solute Concentration

Conder and Purnell have related the activity coefficient of the solute in the solvent at zero pressure to the finite concentration of the solute in the liquid phase.

$$\begin{aligned} \ln \gamma(o) = {} & \ln \frac{RT(qv_1 + n_L)}{p_2^o Kv_1} \\ & + \frac{p_o J_3^4}{RT} \Big[2y_o J_3^4 B_{22} \\ & + 2\left(1 - y_o J_4^3\right) B_{23} - \bar{v}_2 \Big] \\ & - \frac{p_2^o}{RT} \left[B_{22} - v_2^o \right] \qquad (7) \end{aligned}$$

p_o is the outlet pressure and y_o the solute concentration in the gas phase so that the terms $p_o J_3^4$ and $y_o J_4^3$ give the mean values of the column pressure and the solute concentration and take into account the pressure drop. B_{22} and B_{23} are the virial coefficients for the solute-solute and solute-solvent interactions and $\bar{v}_2$ and v_2^o are the partial molar and molar volumes of the solute, respectively. At infinite dilution $y \rightarrow O$ and $q \rightarrow O$ so that

$$\begin{aligned} \ln \gamma^\infty(o) = {} & \ln \frac{n_L RT}{Kv_1 p_2^o} \\ & + \frac{1}{RT} \Big[p_o J_3^4 \left(2B_{23} - v_2^\infty \right) \\ & - p_2^o \left(B_{22} - v_2^o \right) \Big] \qquad (8) \end{aligned}$$

Equations 4 and 5 are only valid if $p_0J_3^4 \lesssim 10$ atm.

It should be noted that these equations do not take into account the solubility of the carrier gas in the solvent.

As shown by Sewell and Stock[18] there is no measurable solution of nitrogen or carbon dioxide in squalane and dinonylphthalate below vapor pressures of one atmosphere, and Conder and Langer[19] have shown that this is also true for helium and nitrogen in tetrachlorophthalate and benzoquinoline. Sie, van Beersum, and Rijnders[20] also concluded that the dissolution effect is trivial. In contradiction to this Stalkup and Kobayshi[21] show that this is an important effect with methane at high pressure. Both of these latter works ignored chromatographic non-ideality and gas-phase non-ideality involving higher order terms than the first. Also, they were complicated by adsorption at the gas-liquid and liquid-solid interfaces. However, Cruikshank et al.,[22] in the most thorough theoretical description of the retention equation at infinite dilution, which considers second-order gas phase imperfection terms, carrier gas dissolution, chromatographic non-ideality, and adsorption at the liquid-gas interphase, have shown that carrier gas dissolution may be substantial in some systems, the effect increasing in the order hydrogen < nitrogen < argon. They conclude that in order to avoid this effect it is not only necessary for the carrier gas to be insoluble in the solvent but the solute must also be almost ideally soluble in the solvent. Besides the direct dissolution of the carrier gas in the solvent, the carrier gas is also involved in a ternary system solute-solvent-carrier gas which means that when the carrier gas is appreciably soluble in the solvent it is not possible to determine the cross-term second virial coefficient unambiguously.

It may be concluded that the solubility of the carrier gas is unlikely to be important except in studies of thermodynamic data or in high pressure chromatography in the supercritical fluid range.

Consideration of the parameters in Equations 7 and 8 shows that the gas imperfection terms (i.e., the non-logarithmic terms) will contribute between 0 and 20% to $\log \gamma(o)$.[15] The gas imperfection term is the sum of two terms, the second of which is always positive since B_{22} is always negative. Depending upon the relative values of B_{23} and $\bar{v}_2^\infty$ the two terms will either complement each other, as for instance when helium is the carrier gas since $2B_{23} > \bar{v}_2^\infty$. or they will tend to cancel. This occurs as the carrier changes in the sequence helium → hydrogen → nitrogen toward a more imperfect carrier gas. At infinite dilution the correction would only be negative with the most imperfect carrier gases, but at a finite concentration the correction must go negative as y increases because of the y_0B_{22} terms.

It is normally assumed that the partition coefficient K is constant throughout the length of the chromatographic column. Everett[12] first corrected for the pressure dependence of K, assuming a linear relationship, and this was extended by Cruikshank, Windsor, and Young.[13] Conder and Purnell[15] have developed an expression for the variation of $(\delta q/\delta c)p$ as a polynomial in p which is convergent if x (the mole fraction of solute in solution) is in the range 0.8 to 0.9 while p is in the range 5 to 10 atm.

The retention Equation 4 is only applicable when the concentration of the solute in the solvent is infinitely small. The extension into the finite concentration range gives a more convenient and rapid means of obtaining data on solute-solvent and adsorbate-adsorbent interactions, since in order to satisfy Equation 4 very small sample sizes have to be used or results obtained using finite solute concentrations have to be extrapolated to zero concentration, a time consuming operation.

As distinct from G.C. at infinite dilution with a finite solute concentration, the variation of the distribution coefficient with solute concentration and the change in flow rate of the mobile phase due to the flux of solute molecules between the stationary and mobile phase (the sorption effect) must both be taken into account. Allowance should also be made for non-ideality in the gas phase and for the pressure dependence of the activity coefficient of the solute in the solvent.

The equations have only been derived for the case of 'ideal' chromatography, i.e., when band broadening from diffusion, slow mass transfer, and other kinetic effects are negligible.

The rate of movement of a chromatographic zone of constant concentration c is then given by

$$\left(\frac{\partial v}{\partial \ell}\right)_c = v_m + v_\ell \left[\frac{1 - y}{1 - y \left(\frac{\partial C}{\partial c}\right)_p}\right] \left(\frac{\partial q}{\partial c}\right)_p \tag{9}$$

where v_m and v_l are the volumes of the gas free space and stationary phase per unit length

C(mole.ml^{-1}) is the total concentration of gas (solute + carrier) in the gas phase.

y = mole fraction of solute in the gas phase (= c/C)

To obtain an expression for the retention volume Equation 9 must be integrated over the column length (ℓ). This may be simplified by considering the practical limits of the various parameters when the following assumptions can then be made.

(i) The equation of state of the gas phase requires only first power terms and the deviations from ideal gas behavior may be represented by the expression $B = y^2 B_{22}$.

(ii) Second and higher order terms ($y^2 B_{22} p/RT$) are negligible. This is justified if the normal values of B_{22} in G.C. are considered.

(iii) Following Everett[12] and writing

$$J_n^m = \frac{n}{m}\left[\frac{(p_i/p_o)^m - 1}{(p_i/p_o)^n - 1}\right] \qquad (10)$$

where p_i and p_o are the inlet and outlet pressures, respectively; when m = 2 or 3, J_m^{m-1} and J_{m+1}^m may be equated with negligible error as long as $p_i/p_o < 5$.

(iv) The change in the partition coefficient with pressure may be represented by a polynomial. This is true if x and y are limited to maximum values of 0.8 to 0.9 and p to a maximum value in the range 2 to 20 atm.

(v) It is assumed that the variation in the viscosity of the mixed gas with y is small and can be assumed constant over the distance ℓ.

(vi) The variation in the flow rate due to the sorption effect can be represented by

$$\frac{F(y)}{F(o)} = \frac{1 + k}{1 + k(1 - y)} \qquad (11)$$

where F(y) is the volume flow of carrier + solute vapor at the outlet pressure, k is the mass distribution coefficient and y is the mole fraction of solute in the gas phase. Although k is also dependent on the pressure this has little effect on F(y) since k appears in both the numerator and denominator.

Since in G.C. the retention time is measured and from this the retention volume is calculated, then

$$\left(\frac{\partial t}{\partial \ell}\right)_c \qquad \frac{1}{F(y)}\left(\frac{\partial V}{\partial \ell}\right)_c \qquad (12)$$

and it is more convenient to evaluate the integral

$$\int_o^{\ell_o} \left(\frac{\partial t}{\partial \ell}\right)_c d\ell = \frac{1}{jF(y_o J_3^2)}\left[V_g + V_l(1 - ajy_o)\left(\frac{\partial q}{\partial c}\right)_{p=P}\right] \qquad (13)$$

At low values of y_o (say = 0.1) and $p_i/p_o \approx 1.2$

$$j = J_3^2\,, \quad a = 1 \quad \text{and} \quad P = p_o J_3^4$$

The generalized retention equation for a zone of constant concentration c at a mean column pressure P becomes

$$V_R^o = jV_R = V_M^o + V_l\ (1 - ajy_o)\left(\frac{\partial q}{\partial c}\right)_P \qquad (14)$$

where V_M^o is the fully corrected gas hold up.

At infinite dilution $j = J_3^2$, $y_o \rightarrow 0$ and $(\delta q/\delta c) \rightarrow q/c = K$ and the equation becomes

$$V_R^o = J_3^2 V_R = V_M^o + KV_l \qquad (15)$$

It should be noted that since the generalized Equation 14 has been derived taking into account non-ideality in the gas phase the infinite dilution expression also remains valid when non-ideality is considered even though it was originally derived assuming perfect gas behavior.

Mixed Mechanisms of Retetnion

The contribution to retention from adsorption at the liquid-gas interface, first recognized by Martin,[11] has been the subject of a recent review by Martire.[23] If partition coefficients are to be measured by G.C. and used in the understanding of retention behavior, the means by which mixed retention mechanisms may be recognized and corrected for must be determined. It has been suggested[24,25] that in any system with an activity coefficient greater than 5.0 surface adsorption effects are in operation. Martire[26] has further shown that even for certain polar solute-polar solvent systems with an activity coefficient around unity surface adsorption effects may still be observed.

Assuming that the various sources of retention operate independently, the net retention volume V_N may be written

$$V_N = K_LV_L + K_AA_L + K_SA_S + K'_SA'_S + \phi(K'_L\ K'_A\ V'_L\ A'_L) \qquad (16)$$

where the K's represent the particular distribution coefficients, the V's the volumes, and the A's the active surface areas, so that K_LV_L, K_AA_S, and K_SA_S are the sorptions due to bulk liquid, liquid-gas interface and gas-solid interface, respectively, $K'_SA'_S$ is the sorption due to the solid surface modified by a thin layer of coating liquid[27] and $\varphi\ (K'_L\ K'_A \cdot V_L\ A_L)$ is a term introduced to allow for the fact that the coating liquid may have a structure and surface which is different from the bulk structure due to the influence of the underlying support material.

The work of Urone et al.[27-29] on the effect of an adsorbent surface modified by the coating liquid deserves further comment as there are discrepancies between this and other work. That other workers have found no difference between isotherms measured in the bulk liquid and when the liquid is coated on solid could be due to a difference in the sensitivity of the balance used. However, Sewell and Stock[30] used a McBain balance with a sensitivity of 10^{-5}g, and obtained identical isotherms for chlorinated hydrocarbons on squalane and dinonylphthalate using the bulk liquid and 20% liquid coated supports at relative pressures $p/p^0 = 0.05 \rightarrow 0.1$. As shown in Urone's paper adsorption due to the modified adsorbent may be as much as half the sorption due to the bulk liquid at $p/p^0 \sim 0.05$ and it seems surprising that a difference of this magnitude was not observed by other workers. Sewell and Stock[18] also observed marked changes in the isotherms for CCl_4 in squalane and dinonylphthalate when N_2 or CO_2 was introduced into the system, although there was no measurable uptake of these gases by the solvents themselves, this being a fugacity effect. Conder and Purnell[15] have calculated that the gas imperfection correction is greater for helium than for nitrogen yet Urone finds no effect on the isotherm when helium is present. Urone assumes that the contribution from adsorption at the gas-liquid interface is generally of too small magnitude to be observed except for non-polar solutes on a polar solvent. It is difficult then to reconcile the very high value for the activity coefficient γ_2^∞ found by Pecsok and Gump[31] for the methanol/squalane system ($\gamma_2^\infty = 24.4$).

The activity coefficients determined by Urone et al.[29] compare very well with the bulk liquid values and this argues in favor of their interpretation, and further studies are required to resolve the discrepancies.

Not all the retention mechanisms represented in Equation 16 would necessarily operate in every situation. For example, Urone[27] considers that K_SA_S will disappear when $K'_SA'_S$ is present. Further, Berezkin[32] has shown that the K_SA_S is relatively small for benzene and n-heptane in $\beta\beta'$thiodipropionitrile, a polar solvent. The K_AA_L term would achieve maximum importance in the situation of a non-polar solute on a highly polar solvent. The function $\varphi(K'_L\ K'_A\ V_L\ A_L)$ would only be expected to be important in thin films. Conder, Locke, and Purnell[33] have considered the general situation where the first three retention mechanisms contribute to retention.

The retention volume V_N can then be written

$$V_N/V_L = K_{obs} = K_L + K_AA_L/V_L + K_SA_S/V_L \qquad (17)$$

If measurements of V_N are made for columns containing different amounts of stationary phase, a plot of V_N/V_L against $1/V_L$ gives K_L as the intercept; and if A_L is known, both K_A and K_SA_S may be determined by the method of Conder.[34]

Since V_L is changing, if a constant sample size is used, the concentration will vary; i.e., measurements are being made on different points of the sorption isotherm. If, on the other hand, the sample size is varied, the concentration can be kept constant. This latter mode of operation is more useful and enables the distribution coefficients to be varied. Working at infinite dilution would, of course, be equivalent to using a constant concentration. It must be remembered, however, that the condition of infinite dilution must apply to all the sources of retention. In the case of adsorption this level may be several orders of magnitude lower than in partition. The surface area of a porous solid is known to fall rapidly as the percentage of coating liquid is increased. This dependence will be different for a coating liquid which wets the surface than for one which does not. Figure 1[33] shows the relationship between V_N and V_L for different cases at constant sample

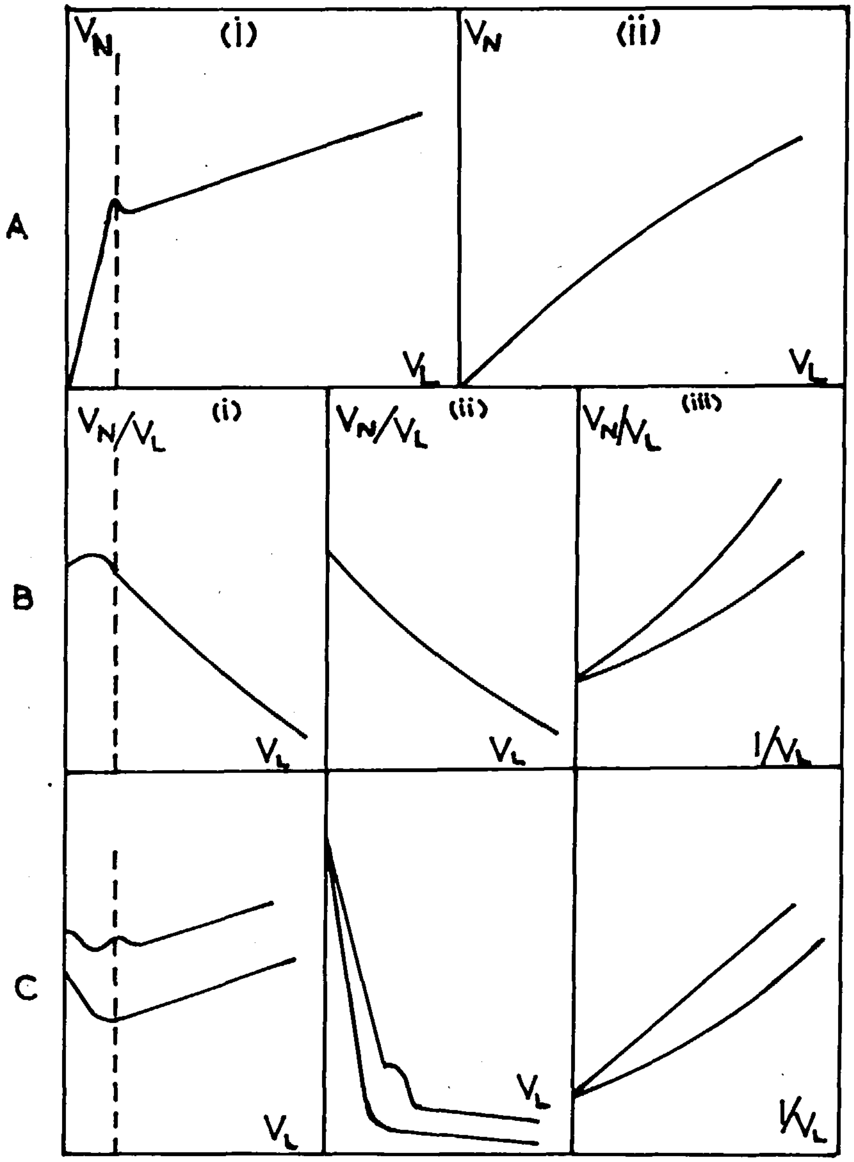

FIGURE 1. A. Variation of net retention volume, V_N, with V_L when $K_LV_L \simeq K_AA_L \gg K_SA_S$ for (i) wetted and (ii) non-wetted solid supports. B. Variation of V_N/V_L with V_L for (i) wetted and (ii) non-wetted solid supports and (iii) variation of V_N/V_L with $1/V_L$--upper curve larger sample size. C. V_N for polar solute with $K_LV_L \simeq K_AA_L \simeq K_SA_S$ for wetted support. Upper curve: non-polar stationary phase; lower curve: stationary phase more polar than solute. Constant sample size throughout. Vertical broken line: point of monolayer formation.

size. It should be noted that the plots of V_N/V_L against $1/V_L$ for differing sample size (Figure 1B iii) extrapolate to the same point, allowing an unambiguous determination of K_L. Figure 2 shows the same relationships at constant concentration.

The exact forms of Figures 1 and 2 will depend on the relative contributions from the retention mechanisms, and these must be carefully considered before drawing conclusions from the overall retention diagrams.

If a system exhibits no change in retention volume with V_L it cannot be assumed that liquid surface adsorption is absent. Such behavior is shown[35] by chlorobenzene and fluorobenzene on water coated Porasil D but these solutes have K_A values of 2.6×10^{-4} cm and 1.1×10^{-4} cm and K_L values of 13.5 and 6.8 at 12.5°C and the contributions from adsorption to retention are 40% and 28%, respectively, on a 26% water column.

As pointed out by Martire[23] the reporting of retention data should take into account the possibility of mixed mechanisms. Both the weight percent of the liquid phase and the nature of the support material together with its surface area should be reported. This also applies to the presentation of relative retention data as Table 1 shows.

The specific retention volume V_g is, of course, the retention volume normalized to one gram of stationary phase and satisfies the above requirement. Rohrschneider[36] has shown that the spe-

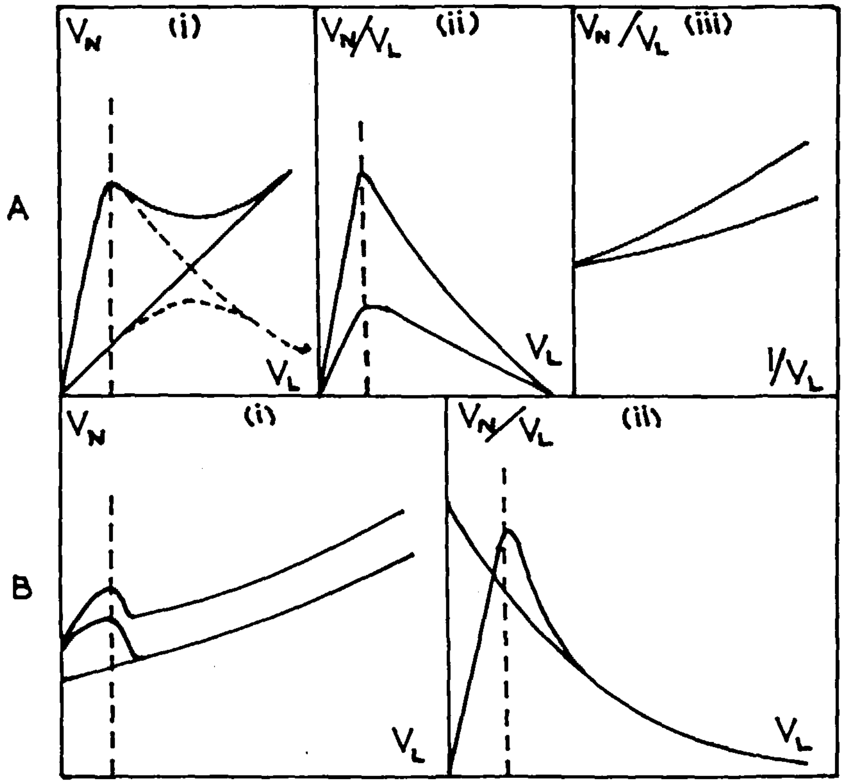

FIGURE 2. A. V_N at constant solute concentration, when $K_LV_L \simeq K_AA_L \gg K_SA_S$. In (i) and (ii): upper curves: wetted support; solid curves: $K_LV_L > K_AA_L$. Upper curve in (iii) larger sample size. (curves do not intersect at $V_{L=\omega}$) B. $K_LV_L \simeq K_AAL$ (i) Variation of V_N with V_L. Upper curve: non-polar stationary phase which wets support; middle curve: polar stationary phase which wets support; lower curve: non-polar stationary phase which does not wet support.(ii) Variation of V_N/V_L with V_L. Upper curve: non-polar stationary phase which wets support, curve for polar wetting stationary phase is similar. Lower curve: non-polar stationary phase which does not wet the support. Vertical broken line: point of monolayer formation.

TABLE 1

Variation of Retention Relative to n-Octane with Liquid Loading at 12.5° C[(a)]

Solute Percent water	n-C_7	ϕH	ϕCH_3	ϕC_2H_5
40.0	0.44	–	4.79	–
37.6	0.43	2.14	3.78	6.92
35.6	0.43	1.74	3.37	6.46
25.3	0.44	1.14	2.73	5.63
18.1	0.44	1.00	2.60	5.73
0.1	0.43	0.91	2.49	5.40

(a) V_N^o n-octane = 1 on all columns
(Taken from Ref. 48. Courtesy of Lehigh University)

cific retention volume can be calculated from relative retention volumes. A linear correlation exists between the logarithm of the specific retention volume of an n-alkane (Rx) and the retention ratio of adjacent paraffins. An even better correlation is obtained if two pairs of retention ratios are used. The correlation may then be written

$$\log V_g^{RX} = K + a \log(t'_{decane}/t'_{octane}) + b \log(t'_{benzene}/t'_{octane}) + c \log(t'_{RX}/t'_{octane}) \quad (18)$$

The average error in the determination of V_g for a large number of solutes on a wide range of solvents is 14%. Certain stationary phases, e.g., diglycerol, give anomalous results. Thus, the correlation must be used with extreme caution, but in view of the common practice of expressing retention data in the form of relative retentions such an approximation can be useful.

The analytical implications of the possibility of mixed retention mechanisms should not be overlooked. By changing the percentage loading of the stationary phase the surface area/volume ratio will be changed, and according to whether partition or adsorption at the gas-liquid interface is the main separating mechanism the retention order may be changed.

III. SOLVENT SELECTIVITY

The development of highly sophisticated retention equations has been brought about by the use of gas chromatography as a technique for making physicochemical measurements and for the study of solute-solvent interactions. As our knowledge and understanding of the partition and adsorption processes increase the results of these studies can be applied to the separation of more closely related substances.

In high resolution chromatography there are two distinct problems. The first is to separate the components of a mixture into discrete, though not necessarily unique, zones. This is dependent upon the relative retardation of the components in the chromatographic column, i.e., upon the sum of the intermolecular forces between the sorbate (component) molecules and the sorbent (or stationary phase) molecules. Any modification which will alter these forces may introduce selectivity into the system and bring about the necessary degree of separation.

Having once separated the components into discrete zones they must be prevented from remixing in the column before they pass through the detection system. This second problem is equally important and a considerable amount of work has been carried out on the effects of various column parameters on the height equivalent to a theoretical plate (H.E.T.P.) which is a measure of the efficiency of a column and the optimization of these parameters.

The sum of the solvent selectivity and column efficiency determines the resolution of the chromatographic column, and only by paying due attention to both can maximum advantage be taken of the tremendous separating power available to the analyst.

By its very nature selectivity tends to be a unique problem for any desired separation. Thus, a stationary phase which offers selectivity for one class of compound may be very poor for another class of compound. Column efficiency, on the other hand, is universal although, for example, a highly efficient capillary column may not be suitable for trace analysis due to the restriction on the loadability of this type of column. Further, some types of columns are more difficult than others to prepare reproducibly, so that in practice it is not always possible to work with the column which theoretically would be the first choice. Just as with selectivity then each case must to some extent be approached as a unique problem.

A measure of the ability of a solvent to bring about a separation of two components (1 and 2) is the relative retention α where

$$\alpha = \frac{t_{R2} - t_M}{t_{R1} - t_M} = \frac{t'_{R2}}{t'_{R1}} = \frac{\gamma_1^{\infty} p_1}{\gamma^{\infty} p^o} \qquad (19)$$

where t_{R2}, t_{R1} and t_M are the retention times of the two components (2 and 1) and an unretained species, γ^{∞} is the activity coefficient at infinite dilution of the component in the solvent, and p^o the saturated vapor pressure of the component at the column temperature. It should be noted here that when α is defined in terms of the activity coefficients and saturated vapor pressures, the effect of gas phase non-ideality is being ignored. For closely related solutes these effects will cancel out, but for widely differing chemical types the gas phase non-ideality term should be taken into account.

The relative retention may also be written as the ratio of the relevent distribution coefficients (K_2 and K_1)

$$\alpha = K_2/K_1 = \frac{(C_s/C_m)_2}{(C_s/C_m)_1} \qquad (20)$$

where C_s and C_m are the solute concentrations in the stationary and moving phases, respectively.

Karger[37] has discussed the meaning of the distribution coefficient K in relation to gas chromatography. The difference in the standard molar free energies of gas-liquid partition for two components $\Delta(\Delta G^\circ)$ is related to the distribution coefficients and to the relative retention by the expression

$$\Delta(\Delta G^o) = - RT \ln \frac{K_2}{K_1} = - RT \ln \alpha \qquad (21)$$

or from (28)

$$\Delta(\Delta G^o) = RT \ln \frac{p_2^o}{p_1^o} + RT \ln \frac{\gamma_2^{\infty}}{\gamma_1^{\infty}} \qquad (22)$$

It is therefore possible to use $\Delta(\Delta G^\circ)$ rather than α as a measure of the relative retentions. The use of $\Delta(\Delta G^\circ)$ has the advantage that the free energy differences may be directly compared to other types of distribution.

Herlicek[38] in studies on the separation of diastereoisomers by G.C. found it impossible to determine $\Delta(\Delta G^\circ)$ values at the same temperature in a reasonable time because of wide variations in retention behavior. Although $\Delta(\Delta G^\circ)$ values can be obtained by extrapolating values measured at higher temperatures they do not reflect the time required to elute the components. In this case a value of $\Delta(\Delta G^\circ)$ may be defined at a constant capacity factor (k_2) for the second component where

$$k_2 = \frac{t_{R_2} - t_m}{t_m} = K_2 \frac{V_L}{V_m} \qquad (23)$$

and K_2 is the partition coefficient of the second component and V_L and V_m are the volumes of the stationary and mobile phases, respectively. By varying the column temperature k_2 may be kept constant for any given diastereoisomeric pair. In practice a given value of k_2 is selected ($k_2 = 2$) and a plot of ln k_2 against 1/T is made to determine the temperature at which $k_2 = 2$. Further, a plot of ln α against 1/T, also being linear, the appropriate value of α at the same temperature may be determined and $\Delta(\Delta G^\circ)$ calculated from Equation 21.

If V_lV_m is assumed to be independent of temperature, then from Equation 23, K_2 can be considered as a constant in the above mode for the diastereoisomeric series. Since the retention volume of the second component can also be assumed to be constant, $\Delta(\Delta G^o)$ may be considered as being measured under normalized conditions and to be a measure of the practical separation factor under reasonable retention conditions. The use of $\Delta(\Delta G^o)$ values in this sense is justified when unduly differing molecular weight species are chromatographed, but its determination depends upon two graphical computations so that the values obtained will not have the precision of values determined directly from the relative retention.

From Equation 22 it can be seen that there are two possible approaches to changing the relative retention of two components: by changing the relative saturation vapor pressures or by changing the relative activity coefficients. The s. v. p. ratio has only slight temperature dependence but it may be changed by the formation of derivatives of varying volatility. This introduces an additional complication, especially if the original substances are to be recovered, and should be avoided where possible.

Changing the ratio of the activity coefficients is much more productive in obtaining separations. The activity coefficient represents deviations from ideal solution behavior and therefore reflects the intermolecular forces and entropy relationships within the system. Any modification which will change these forces will change the activity coefficients; and if the change is selective, this will increase the separation. However, the most difficult separations to achieve are between closely related species, e.g. isomers, and the force fields of two closely related molecules may well be modified to the same amount resulting in no change in the activity coefficient ratio. Fortunately, with high efficiency columns only small changes, as for instance those brought about by steric differences, may be necessary.

Some of the recent advances in this field include the use of complexing agents, liquid crystals, optically active liquid phases, volatile solvents, and mixed solvents.

In a parallel way, in gas-solid chromatography modifications to the adsorbent surface may increase its selectivity. This topic has been the subject of a review by Phillips and Scott[39] and there is a chapter on analytical uses of gas adsorption chromatography in the book by Kiselev.[40]

Complexation Equilibria

The use of reversible complexation between solute and solvent molecules or between solute and a complexing additive has been used to increase selectivity by several workers (e.g., Bradford et al.,[41] and Phillips[42]).

Much of this work has been carried out in order to study the complexation process or for thermodynamic studies of hydrogen-bond formation. However, there is a direct feedback from this type of measurement to the analyst since, from a knowledge of formation constants for various complexation reactions, the analyst may select the best additive or solvent to achieve separation. For closely related compounds complexation may well occur for all the components, but selectivity may still be achieved by varying the amount of additive in the solvent.

Purnell[43] has discussed the classification of complexation reactions and the selectivity which they can bring into gas chromatography. Four broad categories of reaction are recognized, involving solute, solvent, and additive:

(i) Solute reacts with additive,
(ii)Solute reacts with solvent,
(iii) Solute reacts with itself,
(iv) Additive reacts with solvent.

In a recent paper[44] systems involving reactions of type 1 have been studied, where a volatile solute (X) interacts with the additive (A) in an inert solvent (S) to yield 1:1 AX adducts. The stoichiometric formation constants K_1 may be determined from the relationship

$$K_R = K_R^o \left(1 + K_1C_A\right) \tag{24}$$

where K_R is the apparent partition coefficient of X between the liquid and gas phase and is obtained from the fully corrected retention volume (V_R^o) since

$$V_R^o = K_RV_L \tag{25}$$

K_R^o is the partition coefficient of uncomplexed X between liquid S and the gas phase, and C_A is the concentration of the additive A in solvent S. The actual meaning of the stoichiometric formation constant K_1 may be obscure in some cases and, except where the complexing reaction is determined unambiguously, it is best looked upon as an empirical constant. It is determined indirectly from the apparent partition coefficient K_R and therefore is subject to the errors and requirements for determining an accurate value of the retention volume, such as carrier-gas dissolution and chromatographic non-ideality.

The formation constants were measured for alkyl benzene complexes with 2.4.7.-trinitro-9-fluorenone in six alkyl-ester solvents and in $\beta\beta'$ thiodipropionitrile(TDPN). It was found that in TDPN adsorption as well as partition occurred, as indicated by the peak asymmetry and by the change of retention volume with sample size. In this case the results must be treated using the method of Conder.[8]

Plots of K_R against C_A are linear and the intercept at C_A=0 corresponds with the value of K_R obtained using the pure solvent. A useful check on the validity of the K_R values is to elute a non-complexing solute, such as an n-alkane, when a plot of K_R against C_A should be a line of zero slope.

The activity coefficient at infinite dilution γ_f^∞ can be obtained from the well known relationship

$$\ln \gamma_f^\infty = \ln \gamma_p^\infty - \frac{p^o}{RT}\left(B_{22} - v_2^o\right) + \frac{\bar{p}}{RT}\left(2B_{12} - \bar{v}_2\right) \qquad (26)$$

where B_{22} and B_{12} are the solute and mixed virial coefficients, respectively, v_2^o is the solute molar volume and $\bar{v}$ its partial molar volume, $\bar{p}$ is the average column pressure and

$$\gamma_p^\infty = \frac{RT}{K_R^o \bar{v}_s p^o} \qquad (27)$$

where $\bar{v}_s$ is the molar volume of the solvent and p^o is the saturated vapor pressure of the solute at the column temperature T.

The formation constants obtained show that, except for TDPN, irrespective of the solvent, the complexing power increases in the order ethyl benzene < toluene < m-xylene < p-xylene ≃ o-xylene. In TDPN the order is the same except that m and p-xylene had the same K_1, but the complicating effect of the mixed retention mechanism may have masked any real difference.

The possible analytical uses of such complexing reactions may be seen from the following:[44]

For two components a and b, both capable of forming a 1:1 complex with the additive A, it can be shown that

$$K_1^a = (\alpha\beta - 1)/C_A + \alpha\beta K_1^b \qquad (28)$$

where α is the retention ratio K_R^b/K_R^a in the base solvent S, β is the retention ratio in the mixed A + S solvent, and K_1^a and K_1^b are the respective stoichiometric formation constants. Equation 28 will give the required value of K_1^a/K_1^b for any given value of β in terms of K_1^b and C_A.

In an inert solvent the separation of m and p-xylene has an $\alpha \approx 1.03$, the p-isomer being eluted first. Complexing additives tend to reverse the order of elution by complexing more strongly with the p-isomer. If β is taken as $K_R^a/K_R^b = 1.25$, about 1000 theoretical plates would be required for the separation, a number easily obtainable. Table 2 gives the values of K_1^a/K_1^b needed to achieve β = 1.25.

TABLE 2

Values of $K_1{}^a/K_1{}^b$ needed to achieve β = 1.25 as a function of $K_1{}^b$ and c_A with α = 1.03.

	K_1b (l mole^{-1})				
c_A	0.2	0.5	1.0	10	∞
0.2	8.55	4.20	2.74	1.44	1.29
0.5	4.17	2.44	1.86	1.35	1.29
2.0	2.01	1.58	1.43	1.30	1.29
5.0	1.58	1.40	1.34	1.29	1.29

(Courtesy of University of Houston)

From Equation 28 it is seen that the minimum value of K_1^a/K_1^b necessary to achieve the separation ratio β is $\alpha\beta$. For α = 1.03 and β = 1.25 $(K_1^a/K_1^b)_{min}$ = 1.29. Also, from Table 2 it can be seen that this would correspond to a concentration of additive of 5 moles.liter^{-1} and a K^b of 10 liter.mole^{-1}, and such high values are not normally associated with alkyl-benzenes in π- complexes. As K_1^b is reduced C_A must be increased to keep down K_1^a/K_1^b. A reasonable limit on C_A would be 1 mole.liter^{-1}, so that K_1^b should exceed unity.

These requirements place a severe limitation on the practicability of using such systems for separating isomeric alkyl benzenes unless stronger π-acids or complexing agents of a different type are used.

The choice of complexing agent may be made on the bases of the following criteria:

(i) High solubility in some readily available, non-volatile and non-complexing solvent

(ii) Stoichiometric formation constants in the range 1 to 10 liter.mole^{-1}

(iii) Sufficient selectivity for the two components to yield a K_1^a/K_1^b ratio of at least 1.5.

The first two of these would lead to long retention times, and higher flow rates and shorter columns than are conventionally used may be necessary. This of course would lead to a reduction in column efficiency.

Queignic and Wojtkowiak[45] have studied the complexation of alkine-1 and substituted alkine-1 compounds with N-N diethylacetamide as the additive and squalane as the solvent. A comparison of the K_1 values obtained by G.C. and from spectroscopic data confirms the validity of gas chromatography as a method for obtaining complexation equilibrium coefficients.

Castells and Cataggio[46] have studied the interactions between metallic ions and aliphatic amines in Quadrol [N.N.N'N'-tetrakis (2 hydroxypropyl) ethylenediamine]. In pure Quadrol the primary amines are eluted in order of increasing boiling points; secondary amines are eluted before the primary ones with the same boiling point and a tertiary amine is eluted before a secondary. Addition of Ca ions as the metal stearate produces no change in the order of retention, though the retention volumes are increased slightly. Ni ions change the order of elution and still greater changes are found with Zn and Cd ions. The retention volumes of the primary amines increase still further but there is little change in retention of the secondary and tertiary amines. Interpretation of the results is complicated by the fact that the metallic ions probably complex with the Quadrol, but it can be shown that the interactions of the primary amines decrease in the order cadmium >> nickel > calcium. One feature of this system was the very poor column efficiencies achieved which makes it difficult to assess the system for the separation of amines, but similar systems may well prove useful in these separations.

The use of silver nitrate in organic solvents is well known in gas chromatography, particularly for the analysis of olefins where well defined Ag^+-olefin complexes are formed. Wasik and Tsang[47] have recently studied the elution of some unsaturated hydrocarbons and their deuterated isomers from aqueous silver nitrate solutions. To prevent a change in the concentration of the silver ion by depletion of water from the column the carrier gas must first be bubbled through a solution of silver nitrate at the same silver ion concentration as the column. A novel feature is the elution of trans-butene-2, isobutene, and propylene before ethylene. The column efficiencies are reasonable but decrease with decreasing silver ion concentration in the water and with increasing temperature. The olefins show a marked improvement on the separation obtained on a $AgNO_3$-ethylene glycol column.

Compared to the usual methods, using graphitized carbon black, etched glass, Poropak, or squalane, normal and deuterated benzene can be separated with an enhanced isotope effect so that shorter columns may be used.

The advantage of using water rather than ethylene glycol would appear to be in the strong ionizing properties of the water, increasing the silver ion activity. From the point of view of selectivity it is probably true that water is the preferred solvent for metal ions, but this author feels that as a general analytical method the use of volatile solvents is best avoided. Even with presaturation there are serious limitations on the operating temperatures of the column. The use of water in the solvent system also complicates theoretical studies since, as shown by Karger et al.,[35] even quite strongly polar molecules adsorb at the gas-water interface, and for hydrocarbons adsorption and not solution is the sole retention mechanism.[48] However, from an analytical standpoint the possibility of mixed mechanisms of retention adds another factor in the search for selectivity, although the presence of an adsorption mechanism may lead to a lower column efficiency if sample sizes are too large; because of the relatively low concentrations that overload effects occur in adsorption systems.

Complexation as a means to increase column selectivity is a real possibility. Just how useful it will prove to be remains to be seen since the numerical limitations set out by Purnell are very restrictive. There have been relatively few analytical studies in this field and what is required is a much broader based search for suitable additives and solvent systems within the framework set by Purnell.

Salt Modified Adsorbents

As previously mentioned modification of the solid surface in G.S.C. may be used to enhance selectivity. Sawyer et al.[49-52] have been notably active in this field. The thermodynamics of adsorption and chromatographic retention behavior of various alkanes and alkenes has been studied on salt modified aluminas and porous silica beads. Brookman and Sawyer [49] studied hydrocarbons on modified alumina columns and found that the retention volume was affected by both specific and non-specific interactions between the solute molecules and the modified alumina surface, as one would expect from a polar adsorbent. The relative retentions of a variety of compounds are independent of the nature of the salt modifier, but the absolute values of the retention vary considerably, probably due to changes in the surface area (Table 3) and although selectivity is not improved the time of analysis may be reduced. For aromatic hydrocarbons substitution effects the retention order, chlorobenzene eluting before

TABLE 3

Relative Retention Volumes[a] and Surface Areas[b] for Modified Alumina Columns
Adsorbents: 10% salt on acid-washed F-1 alumina

Compound	NaCl	Na_2SO_4	Na_2MoO_4	Na_3PO_4	NaOH
Hexane	1.85	1.78	1.99	1.58	1.38
Cyclohexene	2.66	2.78	2.71	2.38	1.86
Benzene	6.13	6.63	5.91	4.32	2.86
Octane	6.31	5.41	7.63	4.72	3.62
Octene-1	9.60	7.86	10.5	6.75	4.64
Toluene	14.5	13.4	13.4	8.30	4.95
Surface Area($m^2 gm^{-1}$)	269	254	279	210	233

(a) V_X Pentane = 1 on each column.
(b) Surface area of unmodified adsorbent = $278 m^2 .gm^{-1}$.
(Courtesy of *Analytical Chemistry*)

ethylbenzene and bromobenzene before cumine. In both these cases the retention order is the opposite to that predicted from dipole-dipole interaction considerations and it is suggested that this is due to the electron withdrawal effect from the aryl ring, which would reduce the interaction between adsorbate and adsorbent. For the isomeric chlorotoluenes the retention order does follow the order of polarity. For alkenes, the cis isomer is retained more strongly than the trans isomer, steric factors allowing closer approach of the cis isomer to the surface.

The effect of column temperature is important in determining the maximum selectivity, lower temperatures giving better resolution by enhancing the specific interactions.

The particular choice of salt modifier can be made by reference to plots similar to Figure 3 which shows the variation of the retention volume with boiling point of the solute for a 10% Na_2SO_4 modified column. The greater the slope of the lines the more selective will be the column. Reference to Figure 6 also allows the prediction of the elution order for various molecules; e.g., aromatic molecules with bulky side groups are eluted before planar aromatic molecules.

Hargrove and Sawyer[50] studied the thermodynamics and efficiencies of Na_2PO_4 and Na_2SO_4 salt modified alumina columns and found that the magnitude of the non-specific interactions is dependent upon the amount and type of the modifier, but that the specific interactions are independent of these factors. However, above a loading of 10% wt/wt both the specific and non-specific interactions are relatively independent of the loading. This then introduces additional selectivity, for by varying the amount and nature of the modifier the relative retention of two molecules such as pentane and pentene-1 may be changed.

Brookman and Sawyer[51] extended this work to porous silica surfaces when linear relation-

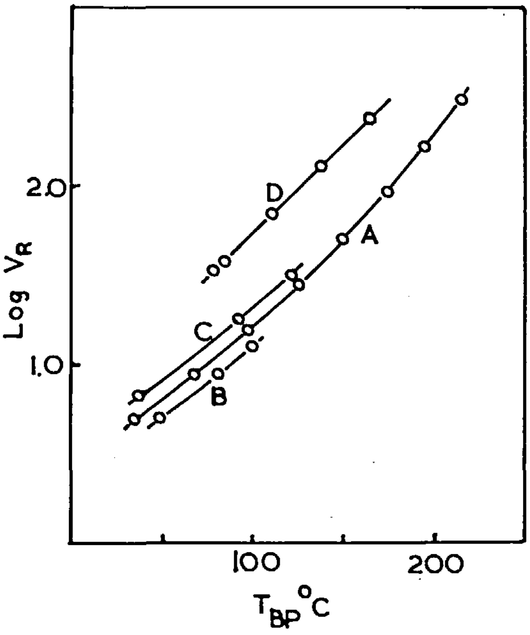

FIGURE 3. Variation of retention volume with boiling point for a 10% Na_2SO_4 modified alumina column at 250°C. A. normal alkanes; B. cyclic alkanes; C. normal alkanes; D. benzene and methylated aromatics.

ships again were found between various types of hydrocarbon molecules and their boiling points.

With cyclic hydrocarbons specific interaction, due to the π-electron system, is very dependent on geometric and steric factors, especially when Na_2SO_4 is the modifier, e.g., in the 1.3.diene C_8 isomer only one π-bond can interact but in the 1:5 configuration both π-bonds may interact. Further, cyclic alkanes are retained longer than their straight chain analogues although the straight chain alkanes have the larger enthalpy of adsorption. The alkanes, however, undergo a greater entropy change on adsorption than do the cycloalkanes and this term is the controlling factor.

It is clear that modification of the adsorbent surface by salts is a very useful technique for improving selectivity. The actual part played by the additive in the retention, however, is not so clear. For example, as shown in Table 3 the relative retention bears no relation to the charge or size of the anion. Sodium phosphate and sodium hydroxide are known to react with alumina and these also cause the largest change in the surface area of the alumina. Scott and Phillips[53] have also used salt modified alumina and have varied the nature of the cation of the modifier, for example, using the alkyl halides, but again the trends in retention followed no particular pattern. In an attempt to resolve some of the problems Cadogan and Sawyer[52] studied thermally activated and chemically modified silicas.

The retention volumes of various adsorbates were measured at several temperatures after the adsorbents had been preheated in the range 250° to 700°C so that enthalpies and entropies of adsorption for the various functional groups could be evaluated and from this it is possible to determine the optimum activation temperature for a given separation. The importance of the correct activation temperature is illustrated by Figure 4 where resolution is obtained when the activation temperature is 500°C but not when activation is carried out at 400° or 600°C. These changes are associated with the nature of the sites on the adsorbent surface at different activation temperatures. As the temperature is raised to 200°C water H-bonded to surface silicon atoms and to surface OH groups is removed, and H-bonds form between adjacent OH groups. At 600°C the hydrogen bonded OH groups appear to condense to give surface siloxane linkages. Heating above 700°C removes the remaining single OH groups but also reduces the surface area.

Liquid Crystals

It would appear that liquid crystals, because of their rod-like form and therefore selectivity on the basis of molecular shape, would be strong candidates for study in the search for selective stationary phases. However, this is a field of study which is rather neglected. An excellent review on liquid crystals has been written by Kelker and Schivizhoffen.[54] The requirements for a liquid crystal stationary phase are much the same as for a normal stationary phase; the main limitation is the temperature range of the liquid-crystalline phase and its high viscosity, which gives rise to poor mass transfer characteristics for the columns. To over-

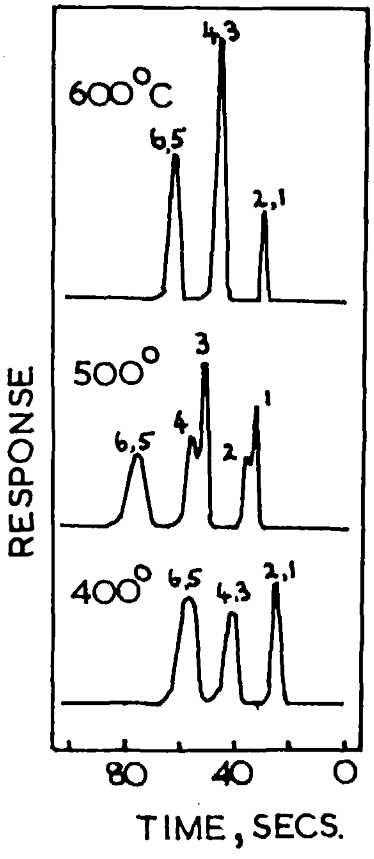

FIGURE 4. Gas chromatograms for six-component mixture with 10% NaCl-silica gel column activated at three different temperatures. 1. Fluorobenzene, 2. n-octane, 3. bromobenzene, 4. toluene, 5. iodobenzene, 6. ethylbenzene.

come this mixed phases may be used; however, this has been known to reduce the selectivity as compared with the pure phase.

The ability of liquid crystals to show great selectivity may be seen by the base line separation of the meta and para isomers of divinylbenzene and ethylvinylbenzene by Martire et al.[55] when 4-4′-dihexoxyazoxybenzene in the nemantic range (81 to 128°C) is used as the stationary phase. The meta isomers are eluted first. The partial molar enthalpies and entropies of solution have been calculated (Table 4), the values for the para isomers being more negative than for the meta isomers. Since more negative enthalpies are indicative of stronger solute-solvent interactions and more negative entropies represent a more ordered state, the values are consistent with the hypothesis that a more rod-like molecule will interact more strongly because they are a better 'fit' in the rod-like liquid crystal. Thus, in going from the disordered vapor state the p-isomer loses more degrees of freedom but this lowering in entropy is more than compensated by the gain in enthalpy, making it more soluble than the m-isomer. Naphthalene, which was also eluted from the 4.4′dihexoxyazoxybenzene, had the largest retention time, due in part to its lower vapor pressure and in part due to its un-rodlike structure. In the isotropic region the liquid crystals become far less selective since the molecules are disorientated.

Mixed Retention Mechanisms

It has been mentioned previously that the possibility of more than one sorption mechanism contributing to the retention may add a new dimension in the field of selectivity. Because of the large volume of solubility data, from independent methods of determination, for aqueous solutions water would appear to be a particularly suitable stationary phase on which to study the effects of mixed sorption mechanisms on retention. The idea of using water as a stationary phase is not new. For example, Purnell and Spencer[56] wrote a preliminary report on the separation of chloromethanes on water and Pollard and Hardy[57] also used water to separate chloromethanes. More recently Karger and Hartkopf,[58] and Karger, Hartkopf, and Posmanter[59] have shown that water may be used to good effect for the separation of hydrocarbons. Although the importance of adsorption to the retention mechanism in these cases was recognized it has since been shown that retention in these cases is due only to adsorption at the gas liquid interface. However, if more soluble compounds are used, e.g., benzene, toluene ethylbenzene, both *partition and adsorption are important.*[35] By varying the percentage of water on the column it is possible to change the retention order of some of these compounds. On a porous silica column containing less than 20% w/w water, benzene is eluted before n-octane; above this value the order is reversed. Figure 5 shows the variation of the retention volume per gram of packing V_N^o with liquid loading for a number of compounds on a water column. While these examples do not represent outstanding separations, a broad spectrum of differing classes of compounds could be separated in this way.

TABLE 4

Solute Partial Molar Enthalpy[a] ($\Delta H_{soln,2}^{\infty}$) and Entropy[b] ($\Delta H_{soln,2}^{\infty}$) of Solution in 4.4'-DHAB Neumatic Phase.

	$\Delta H_{soln,2}$	$\Delta S_{soln,2}$
m-EVB	-31.36	-85.1
p-EVB	-32.80	-87.2
m-DVB	-34.00	-89.0
p-DVB	-36.16	-93.2
Naphthalene	-34.41	-90.4

(a) Kilojoules per mole.
(b) Joules per mole-deg.

Courtesy of *Anal. Chem.*

Mixed Solvents

Pure liquids are normally used as the stationary phase in gas-liquid chromatography. Selectivity, however, may be improved by using mixed stationary phases. These may be used in three different ways:

(i) coupled columns - each column containing its own stationary phase,

(ii) each stationary phase coated on its own support and the supports mixed before packing,

(iii) the stationary phases mixed before coating the support.

The equivalence or otherwise of these three processes has been considered by Young[60] in the case of zero pressure drop. The experimental results indicate that the processes are not necessarily equivalent.

In the benzene-glycerol system. Cruiskhank et al.[22] found that there was a linear depen-

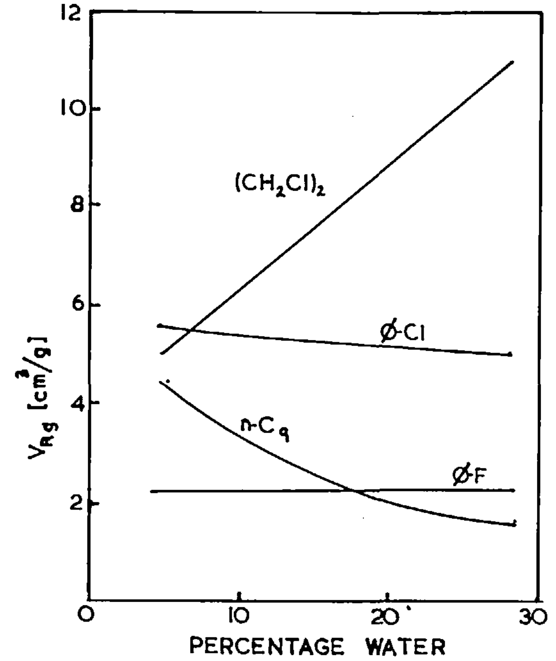

FIGURE 5. Variation of the retention volume per gram of packing with percentage stationary phase (H_2O). Column temperature 12.5° C.

dence of the net retention value on the flow rate. This indicates that there is a large "resistance to mass transfer" in the stationary liquid, and there is no reason to suppose that columns of type (2) and (3) should have the same mass transfer characteristics, except possibly at very low flow rates.

It can be shown that a necessary thermodynamic condition for type (1) and (2) columns to be the same as type (3), gas phase imperfections being neglected, is that

$$\gamma^{\infty}_{i(1+2)} = \frac{n_{(1+2)}\, \gamma^{\infty}_{i1}\, \gamma^{\infty}_{i2}}{n_1\gamma^{\infty}_{i2} + n_2\gamma^{\infty}_{i1}} \qquad (29)$$

where n_1, n_2 are the number of moles of stationary liquid on the types (1) and (2) columns and $n_{1\,2}$ is the total number of moles on a type (3) column, γ^{∞}_{i1}, γ^{∞}_{i2}, and $\gamma^{\infty}_{i(1+2)}$ are the activity coefficients at infinite dilution of a component i in solvents 1 and 2 and the mixture (1+2), respectively.

For ideal solutions γ^{∞}_{i1}, i2, and $\gamma^{\infty}_{i(1+2)}$ are all unity and Equation 29 would be valid. However, because of large size differences between solute and solvent molecules the activity coefficients encountered in G.C. systems often show large deviations from unity.

For regular solutions the activity coefficients may be approximated thus

$$\ln \gamma^{\infty}_{i_1} = K(\delta_1 - \delta_i)^2$$

$$\ln \gamma^{\infty}_{i_2} = K(\delta_2 - \delta_i)^2$$

$$\ln \gamma^{\infty}_{i(1+2)} = K(\phi_1\delta_1 + \phi_2\delta_2 - \delta_i)^2 \qquad (30)$$

where K is a constant for a given solute, δ_i, δ_1, δ_2 are the respective solubility parameters and φ_1 and φ_2 are the mole fractions of component i. The relationship (3) does not generally satisfy Equation 29. Since regular solution theory is only applicable to non-polar molecules this argument is of limited applicability.

If surface adsorption effects are important, these would not be expected to be the same on a type (2) and type (3) column.

The main problem in using a mixed solvent is in choosing a column which, while improving the separation of one or more pairs of solutes, will not decrease the separation of others. This problem can be particularly bad if a wide variety of chemical types are being chromatographed. A very elegant solution has been proposed by Molera et al.[61] using a computer program which selects the best two, three, or four simple phases for the separation of up to 50 solutes. Using standard columns, the computer determines the optimum mixed phase and the minimum column length to achieve a 98% resolution of all components. The number of phases has been limited to four to keep the computer time to a reasonable figure (50 min for a 15 component mixture).

The effectiveness of the computer program can be shown by comparing the chromatograms in Figure 6 obtained from four single phases with that obtained on the four phase column (Figure 6.5) consisting of

25% Di-2-ethylhexylsebacate
10% Ucon oil LB 550X
35% Squalane
30% Carbowax 1540

The four phases were mixed together and dissolved in the same solvent. Table 5 shows the relative retention times referred to M.E.K., as

TABLE 5

Relative Retention Times Referred to Methyl Ethyl Ketone

Number	Compound	Calculated	Experimental	Error
1	Acetaldehyde	0.206	0.198	-3.8
2	Methyl Formate	0.273	0.279	+2.2
3	Diethyl Ether	0.312	0.312	0
4	Vinyl ethyl ether	0.345	0.353	-2.3
5	Methylal	0.406	0.402	-0.4
6	Acetone	0.465	0.458	-1.5
7	Methyl acetate	0.535	0.538	+0.5
8	Methyl alcohol	0.601	0.579	-3.6
9	Dimethyl acetal	0.733	0.732	-0.2
10	Ethyl alcohol	0.841	0.827	-1.6
11	Methyl ethyl ketone	1	1	
13	Acetone dimethyl acetal	1.174	1.190	+1.3
15	sym-Dimethoxyethane	1.4700	1.438	-2.5
17	2.2.Dimethyl-1.3-dioxolane	1.939	1.941	+0.1
18	Diethylketone	2,148	2,174	+1.2

(Courtesy *J. Chromatogr. Sci.*)

determined experimentally and calculated from the computer program.

The usefulness of such an approach to increasing selectivity is self-evident in the chromatograms presented.

The use of cryogenic temperatures and phase changes as an aid to selectivity has received little attention, but as shown by Claeys and Freund[62] this approach can be used to increase selectivity. The best results are obtained when a truly crystalline stationary phase is used, e.g., cetyl alcohol. One disadvantage is that column efficiencies fall markedly at the freezing point though they may increase again as the temperature is further decreased. An unavoidable consequence of the lower temperature is, of course, the longer retention time.

High Pressure Gas Chromatography and the Use of Supercritical Fluids

Until recently it was tacitly assumed that the carrier gas in G.C. played no role in the separation other than to provide the means of transporting the mixture through the column. Klesper, Corwin, and Turner[63] had observed that the migration rate of porphyrins increased as the pressure increased up to 140 atm. Giddings[64] reviewed the possible advantages of pressure induced equilibrium shifts and pointed out the possibility of increasing selectivity in this manner.

Changes in pressure may affect the partition coefficient (K) by altering the gas phase solute-carrier gas interactions, causing the solute equilibrium vapor pressure to increase, or by changing the nature of the stationary phase by solution of the carrier gas thereby changing the activity coefficient of the solute in solution.

Sie, Beersum, and Rijnders[65] studied the effect of pressure on partition coefficients and related these to the gas phase solute-carrier interactions (crossed virial coefficient term B_{12}) using carbon dioxide as the carrier gas. There was a tenfold gain in volatility (decrease in K) at pressures up to 80 atm and the change was greatest near the critical temperature of the carrier gas. This means that substances can be chromatographed at lower temperatures than in normal G.C. and introduces the possibility of chromatographing 'involatile' solutes. The enhanced volatility is relatively greater for heavier substances; i.e., there is a decrease in 'light/heavy' selectivity as shown by the lower slope of the log K vs. carbon number plot as the pressure increases, and the separation of members of a homologous series will decrease as the pressure increases. However, the changes in K increase the possibility of separating different classes of compounds especially with less volatile compounds, separations not easily accomplished under normal G.C. conditions because of the low thermal stability of many solvents and the fact that selectivity usually decreases with increasing

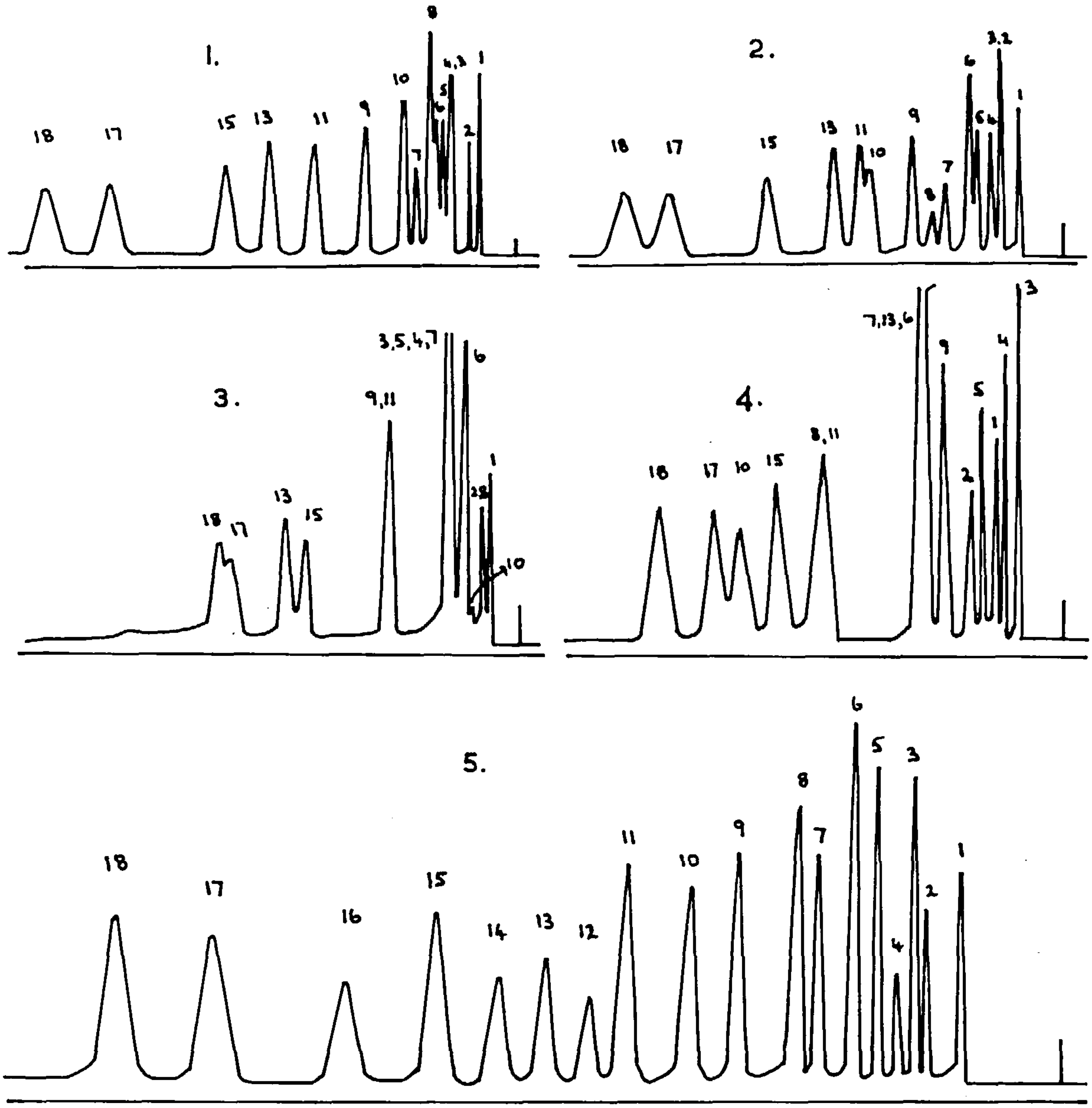

FIGURE 6. Chromatograms 1, 2, 3, 4, obtained on columns B(Di-2-ethylhexyl-sebecate), R (Ucon oil LB 550X), U (Squalane), K (Carbowax 1540) Column lengths 2m. Chromatogram 5 obtained on mixed stationary phase column BRUK-25-10-35-30. Column length 4m. Key to solutes in Table 5.

temperature. An example of time saving using high pressure G.C. is illustrated by Figure 7.[65]

Giddings et al.,[66] using pressures up to 2000 atm, have migrated substances with molecular weights up to 40,000 and have successfully chromatographed ergosterol, cholesterol, and lanosterol at only 40°C and have separated α and β carotene, an impossible separation under normal conditions because of their low stability.

The possibility of pressure programming with supercritical fluids was suggested by Giddings.[67] The pressure at which a species becomes soluble enough to detect and measure is well defined. It should therefore be possible to pressure-program the system and to increase the solvent power of the gas by degrees, eluting the components as each becomes soluble. This technique has been used successfully by Jentoft and Gouw[68] for the separation of polynuclear aromatic hydrocarbons, polyphenyl ethyl ether isomers of molecular weight ~ 538, and to fractionate a 'monodisperse' polystyrene of molecular weight up to 3000.

The development of high pressure-supercritical fluid G.C. will partly depend on a solution to the operational difficulties. For example, neoprene gaskets have been migrated at very high pressures, the Joule-Thomson effect at the pressure reducing valve can cause the valve to close spontaneously and there are sample introduction problems associated with the high pressures and also the time required for the sample and carrier fluid to come to equilibrium. When the very real danger of working with pressures up to 2000 atm is added to these one is led to the conclusion that advances in this field will probably be made using much lower pressures as, for example, the 40 to 70 atm used by Jentoft.

The use of supercritical fluids with greater chemical selectivity is another possible approach. Sie, Bleumer, and Rijnders[69] have used porous polymer stationary phases with several different supercritical fluids. An example using helium (P_{crit} = 2.26 atm) and n-pentane (P_{crit} = 33.0 atm) as the mobile phases is given in Figure 8, both fluids being near their critical pressures. As Figure 9 shows, the relative retention of two components, a small polar molecule and a heavy non-polar molecule, may be reversed by suitable choice of the super-critical fluid and the pressure. As the pressure increases non-specific interactions (n-pentane as the mobile fluid) will shift the equilibrium of the non-polar molecule more rapidly than the polar molecule and the retention order can be reversed. If the mobile fluid is capable of

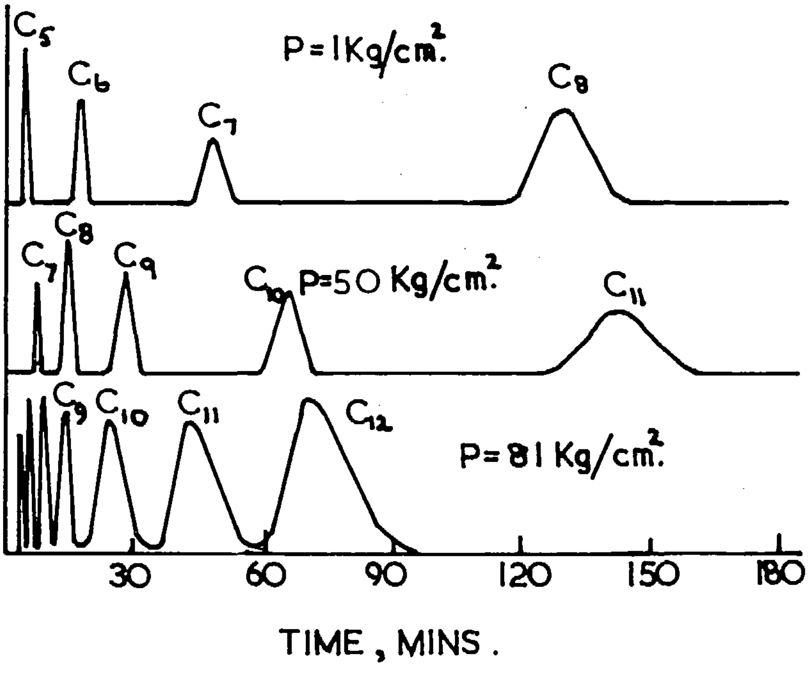

FIGURE 7. Effect of increasing carrier gas pressure on the separation of n-alkanes on squalane at 40°C with CO_2 as carrier gas.

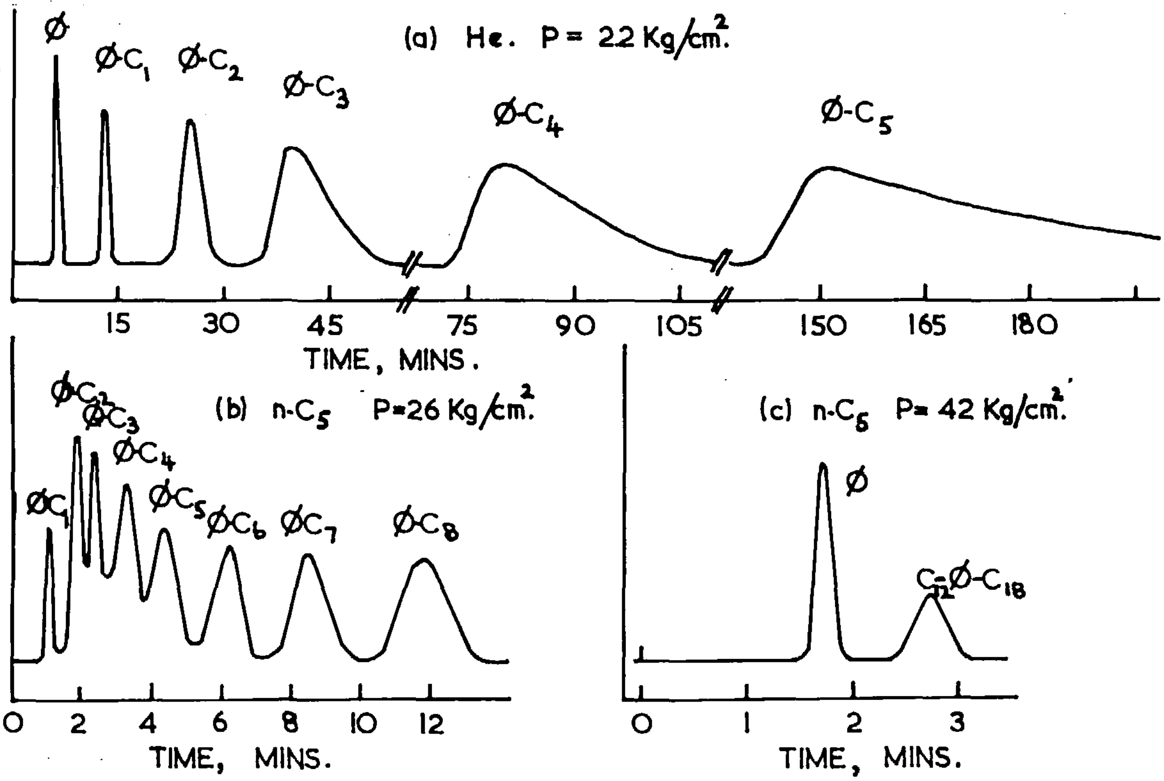

FIGURE 8. Elution of alkylbenzenes as a function of the nature of the mobile phase.

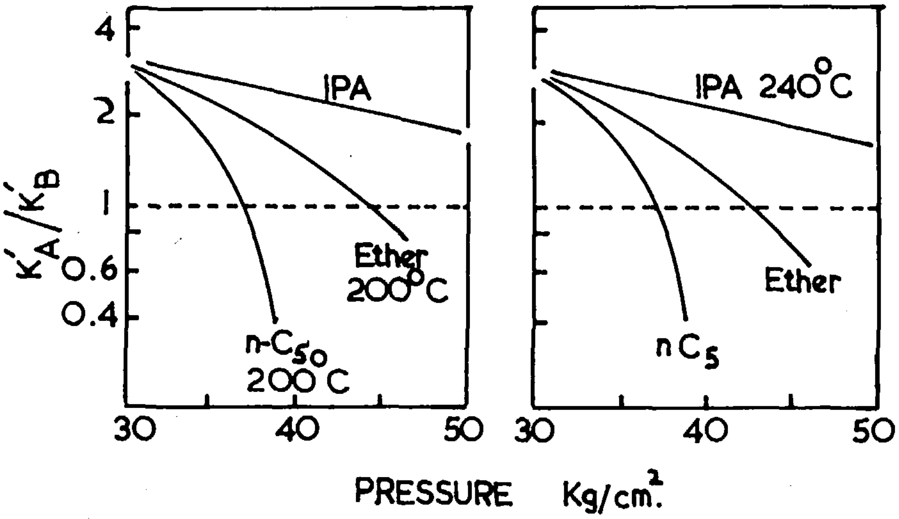

FIGURE 9. Effect of nature of the mobile phase and pressure on the elution order. A=n-octyl benzene, B=benzonitrile, C=aniline.

specific interactions, the change in equilibrium for the two solutes may be more nearly equal so that no change in retention order is observed. Such is the case when isopropyl alcohol (I.P.A.) is used. A combination of moderate pressures and mobile fluid selectivity is most likely to be the trend for the future.

IV. COLUMN EFFICIENCY

Solvent selectivity is only one aspect of the resolution problem. The efficiency of the column is related to the degree of band spreading and can be expressed in terms of the height equivalent to a theoretical plate (H.E.T.P.)

$$H = \frac{L}{16}\left(\frac{w}{t_R}\right)^2 \qquad (31)$$

where L is the column length, w the basal peak width, and t_R the retention time. The definition of H.E.T.P. includes the gas hold up time t_M. This is the time that a non-adsorbed component spends in the column and has no bearing on the resolution of the column. A more useful parameter since it is directly related to the resolution is the "height equivalent to an effective theoretical plate" (H.E.E.T.P.) which is given by

$$H_{eff} = \frac{L}{16}\left(\frac{w}{t'_R}\right)^2 \qquad (32)$$

where t'_R is the adjusted retention time and $t'_R = t_R - t_M$. The two expressions are related thus

$$H_{eff} = [(1 + k)/k]^2\ H \qquad (33)$$

where k is the capacity factor $(=K\frac{V_L}{V_m})$, V_L and V_m being the stationary and mobile phase volumes, respectively. H_{eff} has the further advantage that the number of peaks which may be separated between the inert peak and the peak which is characterized by H_{eff} is approximately inversely proportional to $H_{eff}^{1/2}$. As seen from Equation 33 H_{eff} is a function of k (or retention time) and the efficiency of a column refers to a particular solute. This point is often overlooked and to be exact the solute used in the determination should be quoted along with the H.E.T.P.

Plate Height Equations–Theory and Practice

The justification for the van Deemter equation is more practical than theoretical. It adequately describes the experimental results in terms of a number of empirical constants the physical meaning of which, however, is often obscure. In terms of the H.E.E.T.P. (H_{eff}) which is the more fundamental term for practical analytical purposes the van Deemter equation may be written

$$H_{eff} = A + B/u_o + C_m u_o + C_s \bar{u} \qquad (34)$$

or since $\bar{u} = j\ u_o$

$$H_{eff} = A + B(j/\bar{u}) + C_m(\bar{u}/j) + C_s \bar{u} \qquad (35)$$

where

A is the eddy diffusion term

B accounts for longitudinal gas phase diffusion

C_m and C_s are the mobile and stationary phase mass transfer times, respectively

u_o is the outlet mobile phase velocity and $\bar{u}$ is the time averaged linear velocity.

To relate these constants to the normal van Deemter constants the terms on the right hand side of Equation 35 must be multiplied by $[k/(1 + k)]^2$.

The variance of theory with experiment has been demonstrated by Halasz.[70] The eddy diffusion term has been found to depend on the nature of the solute and the carrier, the pressure and the temperature whereas theory predicts it should be independent of these. Similarly, theory and practice do not agree with respect to the other terms either.

Takacs[71] has developed an expanded form of the van Deemter equation which in simple terms may be written

$$\text{H.E.T.P.} = A + B/u + Cu + D/u^2 + Eu^2 \qquad (36)$$

This is in effect a McLaurin Series.

The coefficient D takes into account diffusion and E the mass transfer conditions. The A term differs from the van Deemter eddy diffusion term by containing a constant depending upon the nature of the solute in accordance with the findings of Halasz. The functional curves of the van Deemter equation, Figure 10, are compared with those for the new constants in Takacs' equation, Figure 11. Several points of interest arise from the comparison of the two curves. Thus,

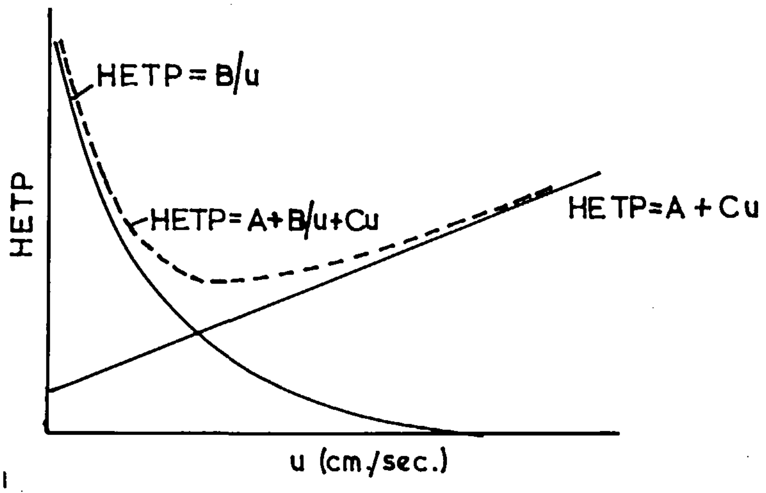

FIGURE 10. H.E.T.P. versus linear gas velocity (u). van Deemter equation.

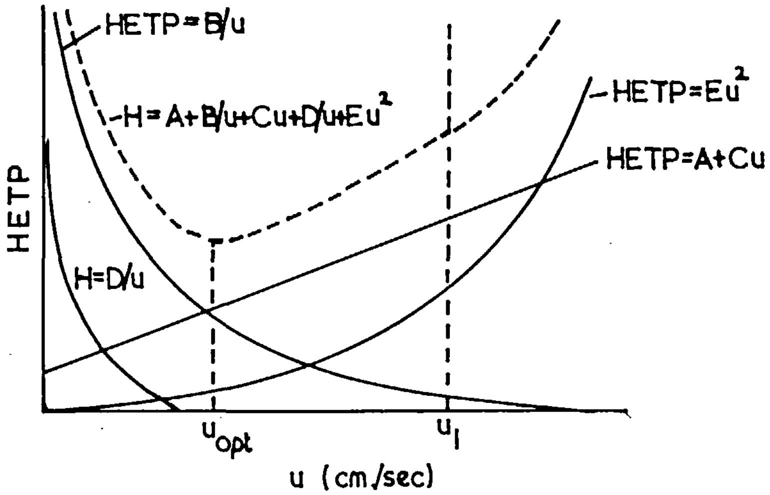

FIGURE 11. Functional curves for the approximation by Equation 36.

although u_{opt} is approximately the same for each case, H_{MIN} for the Takacs' curve is higher than that of the van Deemter equation. This is in agreement with experiment where values are generally higher than predicted from the van Deemter equation. The proposed rapid increase in the H.E.T.P. at high linear velocities is apparently due to the E u^2 term.

The increase in H at high linear gas velocities predicted by this equation is at variance with experiment. Sie and Rijnders[72] and more recently Landault and Guiochon[73,74] on packed capillary columns have found that the column efficiency decreases more slowly than predicted from the van Deemter equation as the carrier velocity increases.

The studies of Landault and Guiochon again show up the limitations of the simple van Deemter equation. The eddy diffusion term is found to depend upon the nature of the solute and the carrier gas, generally decreasing in the order hydrogen > carbon dioxide > argon > nitrogen. Above a linear flow rate of 30 cm sec^{-1} the experimental results do not fit the simple van Deemter curve. These results may be interpreted as due to a decrease in the resistance to mass transfer in the gas phase, as predicted by the coupling theories of Giddings[75] and of Littlewood.[76]

Vergnaud[77] has developed a theory of the mean values of pressure, carrier gas linear velocity, and H.E.T.P. The two variables considered are the length of the column and the time. Relationships are then developed, starting with the Boyle-

Mariotte law assuming perfect gases, for the pressure as a function of length, and for the carrier gas velocity. The James-Martin function J_3^2 is then obtained for the mean column pressure.

$$\bar{P}_x = \frac{2}{3} P_o \left[\frac{P^3 - 1}{P^2 - 1}\right] \qquad P = \frac{P_i}{P_o} \qquad (37)$$

As a function of relative time (t/T) the pressure is given by

$$P_t = \left[P_i^3 - \frac{t}{T}\left(P_i^3 - P_o^3\right)\right]^{1/3} \qquad (38)$$

and the mean column pressure

$$\bar{P}_{(t)} = \frac{3}{4} P_o \left[\frac{P^4 - 1}{P^3 - 1}\right] \qquad (39)$$

The differences in the mean pressure calculated from Equations 37 and 39 only become significant at P values of 1.5 or greater.

The linear velocity can otherwise be represented as a function of length or of time.

$$\bar{u}_x = 2u_o \frac{P - 1}{P^2 - 1} \qquad (40)$$

$$\bar{u}_t = \frac{3}{2} u_o \frac{P^2 - 1}{P^3 - 1} \qquad (41)$$

Again these velocity differences are not significant below P values of about 1.5.

Since the linear gas velocity will vary with the retention time and length of the column, assuming the simpler form of the van Deemter equation, H will also vary with these parameters thus

$$H_x = A + \frac{B}{u_o} \cdot \frac{2}{3} \cdot \frac{P^3 - 1}{P^2 - 1} + Cu_o \frac{2}{P+1} \qquad (42)$$

$$H_t = A + \frac{B}{u_o} \cdot \frac{3}{4} \cdot \frac{P^4 - 1}{P^3 - 1} + Cu_o \frac{3}{2} \left[\frac{P^2 - 1}{P^3 - 1}\right] \qquad (43)$$

Variation of H.E.T.P. with Column Length

The H.E.T.P. according to current theories of gas chromatography should be independent of column length. However, Halasz, Deininger, and Gerlach[78] found a marked increase in H.E.T.P. when two supposedly identical columns are coupled together.

The effect of increasing the column length may be shown from the van Deemter equation. For conventional open tube columns (C.O.T.) $C_m >> C_s$ and we may write

$$H = A + \frac{B}{u_o} + C_m u_o \qquad (44)$$

Therefore, if H is to remain constant, u_o must be constant. Rewriting 44 in terms of the linear velocity $\bar{u}$ (= $u_o j$)

$$H = A + \frac{Bj}{\bar{u}} + \frac{C_m \bar{u}}{j} \qquad (45)$$

where $j = \frac{3}{2}\left[\frac{P2 - 1}{P3 - 1}\right]$, and $p = p_i/p_o$, is the James-Martin compressibility factor. If $\bar{u}$ is kept constant the H vs. $\bar{u}$ curve will change with column length because of the change in j. As the column length increases $\bar{u}_{min}$ decreases, and the right hand branch ascends more steeply.

With packed columns $C_s >> C_m$ and we may write

$$H = A + \frac{B}{u_o} + C_s \bar{u} \qquad (46)$$

and $H \propto \bar{u}$. If $\bar{u}$ is kept constant and the column is lengthened, the H vs. u curve will not change. This is difficult to achieve in practice because of the inherent difficulty of obtaining columns of different length but with the same mass of packing per unit length, although it may be achieved using porous layer beads. It is then found that H is independent of $\bar{u}$ for columns of length 0.5 to 2.0 meters but for longer columns H is found to be dependent on $\bar{u}$, probably due to packing difficulties with the longer columns.

Similar conclusions have been reached by Novak[79] using a more complete form of the van Deemter equation containing a term to allow for longitudinal diffusion in the stationary phase $B_s\sqrt{\bar{u}}$ Thus,

$$H = A + \frac{B_s}{\bar{u}} + \frac{B_m}{\bar{P}\bar{u}} + C_m\bar{P}\bar{u} + C_s\bar{u} \qquad (47)$$

Approximating the mean column pressure $\bar{P}$ to

$$\bar{P} \simeq P_o + \frac{\eta L\bar{u}}{2K} = P_i - \frac{\eta L\bar{u}}{2K} \qquad (48)$$

we have

$$H = A + \frac{B_s}{\bar{u}} + \frac{B_m}{\bar{u}P \pm (\eta L/2K)\,\bar{u}^2}$$

$$+ C_m P\bar{u} \pm C_m\left(\frac{\eta L}{2K}\right)\bar{u}^2 + C_s\bar{u} \qquad (49)$$

When $P = P_o$ the permeability terms are added and when $P = P_i$ these terms are subtracted.

At low pressures $B_m / \bar{P} >> B_s$, and there are two cases to be considered as before when (i) $C_m\bar{P} >> C_s$ and (ii) when $C_s >> C_m\bar{P}$.

In case (i)

$$\bar{u}_{opt} = \left(\frac{1}{\bar{P}_{opt}}\right)\left(\frac{B_m}{C_m}\right)^{1/2}$$

$$= \left(\frac{K}{\eta L}\right)\left\{P_o^2 + \left(\frac{2\eta L}{K}\right)\left(\frac{B_m}{C_m}\right)^{1/2}\Bigg]^{1/2} - P_o\right\} \qquad (50)$$

and

$$H_{opt} = A + 2(B_m C_m)^{1/2} \qquad (51)$$

In case (ii)

$$\bar{u}_{opt} = \left[\left(\frac{1}{\bar{P}_{opt}}\right)\left(\frac{B_m}{C_s}\right)\right]^{1/2}$$

$$= \left(\frac{K}{\eta L}\right)\left\{P_i^2 - \left[P_i^2 - \left(\frac{2\eta L}{K}\right)\left(\frac{B_m}{C_m}\right)^{1/2}\right]^{1/2}\right\} \qquad (52)$$

$$H_{opt} = A + 2(B_m C_s/\bar{P}_{opt})^{1/2} \qquad (53)$$

The variation of H with u can be shown by differentiating Equations 51 and 53 when

$$\frac{dH}{d\bar{u}} = C_s + C_m\left(\frac{\eta L}{K} \cdot \bar{u} + P_o\right) \qquad (54)$$

$$\frac{dH}{d\bar{u}} = C_s + C_m\left(P_i - \frac{\eta L}{K}\bar{u}\right) \qquad (55)$$

and column elongation will increase the slope with increasing carrier gas velocity, though the increase will be less working at a constant P_i than at a constant P_o.

$\bar{u}_{opt}$ will decrease as η L/K increases if P_o is kept constant but will increase if P_i is kept constant.

Grob and Grob[80] have confirmed the increase in H.E.T.P. with increasing column length experimentally.

Coupled Columns

One way of investigating experimentally the change in H.E.T.P. with column length is to couple together several columns. Kwok, Snyder, and Sternberg[81] have studied the validity of this and have shown that the overall plate number $N \leq \sum_i N_i$ where N_i is the plate number of the i single columns. The equality is only found if the different columns are of the same efficiency per unit length (H_i), have the same diameters or cross-sectional areas, have equal phase volume ratios and the same stationary phase and there is negligible pressure drop across the connecting tubes. Furthermore, the retention volumes and variances of the single columns must be determined or corrected to the same average pressures as they have in the combined columns. These limitations make it difficult to verify the relationships experimentally.

The plate number for a combination of columns is given by

$$N = \frac{\left[\sum_{i=1}^{n}\left(\frac{V_i}{L_i}\right) \cdot L_i\right]^2}{\sum_{i=1}^{n}\left(\frac{V_i}{L_i}\right)^2 \cdot H_i L_i} \qquad (56)$$

where V_i, H_i, and L_i are the retention volumes and plate heights for the i columns of length L.

The resolution R_s is given by

$$R_s = \frac{1}{4}\ 1 - \left(\frac{K_1}{K_2}\right)\left[\frac{K_2}{K_2 + \sum^{n} V_i^o / \sum^{n} W_i}\right]\sqrt{N} \quad (57)$$

where K_1 and K_2 are the weighted quantities $\sum^{n} K_i W_i$ for each component, K_i and W_i being the distribution coefficients and weights of stationary phase for each separate column i, and $\sum^{n} V_i^o$ is the sum of the void volumes for the individual columns.

Since the overall plate number cannot be equal to $\sum^{i} N_i$ if there are different stationary phases in the various columns, such columns can never give the efficiency of a single column, the overall efficiency being determined by the column with the largest K_i value. This suggests that if mixed solvents are used, they should be used as a mixed-bed column. As seen from Equation 57 the resolution on R_s is proportional to $\sqrt{N}$, and mixed solvents give lower values of N; but if K_2 can be made large, there will still result an overall increase in the resolution.

The use of coupled columns of decreasing diameter, or what is equivalent, a tapered column, also cannot give as high an efficiency as a system of columns of equal diameter, the efficiency being determined by the diameter of the widest column.

A criterion for rejecting or retaining a given column in a group of columns is that a column with an H_i value more than three times the average value will increase the overall H, and a column whose H_i is less than twice the average will decrease the overall H.

Gradient Loaded Columns

A gradient loaded column, according to Kwok et al.,[81] should have a lower plate number than one with a constant liquid loading per unit length. Duty[82] has confirmed this on gradient loaded preparative columns for small sample sizes (0.03 ml benzene on a 12' long 5/8" i.d. column), but at larger sample sizes (1.0 ml) the gradient loaded column had a higher plate number than an equivalent constant loaded column (i.e., one with the same total mass of stationary phase). A very full treatment of an exponentially loaded column (E.L.C.G.C.) has been given by Bunting and Meloan.[83] They concluded that for solutes of low or intermediate retention the column efficiency is comparable to that of an equivalent constant loaded column but that the resolution was better than on a column with a linear gradient or on a constant loaded column. A study of the liquid phase mass transfer terms shows that although the peak front is moving faster than the rear because of the lower concentration of solute, and hence the peal is broadening and giving a lower efficiency, the peak is always moving into a zone of less significant resistance to mass transfer. The first effect is controlling for solutes of high K while the latter effect is more important at small K. Further, the gas phase mass transfer is more favorable to the exponentially loaded column at low overall liquid loadings.

In terms of the resolution the E.L.C.G.C. is theoretically intermediate between the constant loaded column and the linearly loaded column. However, solutes of not too high K values will spend relatively more time in the regions of low liquid loading, and in practice the resolution will be at least as good as a regular column, if not better. Furthermore, the analysis time will be shorter. The column capacity is also greater, an important factor in preparative columns. With volatile solvents or temperature unstable solutes E.L.C.G.C. offers an alternative to temperature programming.

Effect of Gas Flow Rate on the H.E.T.P.— Laminar and Turbulent Flow

Chromatography is normally carried out at fairly low carrier gas flow rates (5 to 10 cm sec^{-1}) when the flow of gas can be described by parallel flow lines. As the flow rate increases the gas flow passes from this laminar flow region, through an intermediate stage where the flow lines become increasingly disordered, until the flow lines are completely disordered and the flow is said to be turbulent.

The degree of turbulence is given by the Reynold's number R_c which for granular materials is given by

$$R_c = \rho\ v\ d_p / \eta \qquad (58)$$

where

ρ is the density of the gas

v is the velocity

η is the viscosity

d_p is the particle or channel diameter

Since the change from laminar flow to turbulence is a continuous one it is not possible to say at what point turbulence sets in. For packed columns the Reynolds number can be in the range 1 to 100. This corresponds to a N_2 flow rate $\sim$ 4 to 400 cm sec^{-1} and for $H_e \sim$ 30 to 3000 cm sec^{-1} for porous supports. In contrast, turbulence sets in more sharply in open tubular columns when $R_e \gtrsim$ 2000, and this would require a velocity $\gtrsim 10^4$ cm sec^{-1}, so that turbulence is not easily observed in open tubes. However, because the flow profiles can only be described by analytical equations in this case theoretical studies have all been made on open tube columns.

It has been suggested by several workers[7,84,85] that the efficiency may be increased by using gas velocities in the turbulent flow region.

Sie and Rijnders[72] have studied column efficiencies at various pressures on packed columns. The average nominal velocity is given by $\bar{u} = K\ \Delta P/\eta L$ where K is the permeability, ΔP the pressure drop, L the column length, and η the carrier gas viscosity. As linear flow gives way to turbulence the linear dependence of $\bar{u}$ on ΔP decreases. At low pressures the van Deemter relation holds but the C term increases rapidly with pressure and is the controlling factor on efficiency, the B term being negligible (see Figure 12).

At normal G.C. pressures of one atmosphere the liquid phase mass transfer term C_s is the important one, but above 10 atm the gas phase mass transfer term C_m becomes the more important. A schematic representation of the various contributions to the plate heights in the laminar flow region is shown in Figure 13.[72]

Interparticle diffusion is an important factor at high pressure and C_m becomes a linear function of the square of the particle diameter (d_p). The use of smaller particles would decrease C_m and since gases at high pressure have low viscosities compared with liquids the necessary pressure drop is not impossible to achieve.

Smuts, Clerk, and Pretorius[86] have developed a plate height expression applicable to both laminar and turbulent flow in open tube columns. The theory predicts that the plate height will decrease with increasing Reynolds number. It is also found that the decrease is strongly dependent on the mass distribution coefficient (K), the decrease in H being less pronounced as K increases until H may also increase. This decrease in H is due to the greater lateral mass transfer and also to the flatter velocity profile which in effect gives a less diffuse solute zone.

The theory has been tested experimentally[87] and the broad outlines of the theory confirmed. The major problem in relating theory to practice is the variation in the mass distribution coefficient K with increasing column pressure, e.g., for n-hexane at $R_e \approx$ 16000 the deviation of K during a run $\sim$ 25%. This makes a quantitative test of the H versus R_e behavior impossible. The liquid phase mass transfer remains more or less constant as the R_e

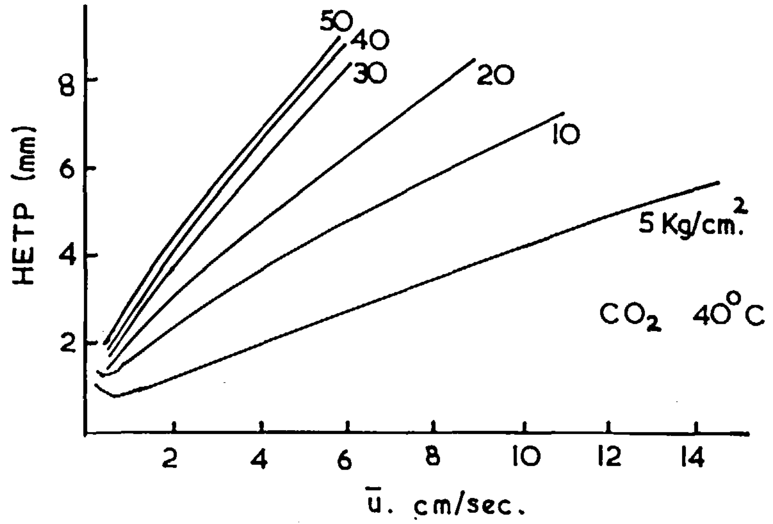

FIGURE 12. Effect of carrier gas pressure on the plate-height curve, with n-pentane as solute.

values vary from 484 to 16,372 where as the mobile phase mass transfer decreases by an order of two. The decrease in C_m in the secondary flow region $400 < R_e < 2000$ may be attributed to the lack of true streamline flow in this region.

A similar study has been made by Doue and Guiochon.[88] An equation is derived to show that if turbulence is fully developed, then the time average velocity and hence retention time is constant independent of the inlet pressure, depending only on the nature of the carrier gas, column length, and radius. The assumptions in the derivation are that the carrier gas is ideal and the walls of the tube are rough enough to give a high friction factor (f) and that the tube is not coiled; this latter assumption being impossible from a practical point of view. The independence of flow rate on inlet pressure arises from the fact that under turbulent flow conditions the mechanical energy is converted into thermal energy by the turbulence. Since the viscosity of gases increases with increasing temperature the flow rate increases less with turbulent flow than with laminar flow. Thus

$$t_R = \frac{L}{u}(1 + k) = (1 + k)\sqrt{\frac{8fL^3M}{9r_oRT}} \qquad (59)$$

Equation 59 may be combined with the expression of Purnell which gives the number of theoretical plates required for the separation α

$$n = 16\,R^2\left(\frac{\alpha}{1 - \alpha}\right)^2\left(\frac{1 + k^1}{k^1}\right)^2 \qquad (60)$$

so that

$$t_R = \frac{128}{3}\,R^3\left(\frac{\alpha}{\alpha - 1}\right)^3\left(\frac{1 + k}{k}\right)^4\sqrt{\frac{2f\,ML^3}{9r_oRT}} \qquad (61)$$

Hence, the analysis time increases with the cube of both the separation of the two peaks α and (α-1). Inspection of Equation 61 shows that K should be fairly small, especially as H increases rapidly with K; r_o should also be small and the molecular weight of the carrier gas M should be low. (However, as shown by Giddings,[7] H for pentane in helium is 3.5 times larger than in nitrogen.)

The experimental results show a small change in retention time with Reynolds number even in the turbulent flow region. This is probably due to a dependence of the roughness factor on the Reynolds number. The H value for methane is found to decrease rapidly in the turbulent flow region and then to level off to a limit which is approximately $6r^o$ in excellent agreement with the theory.

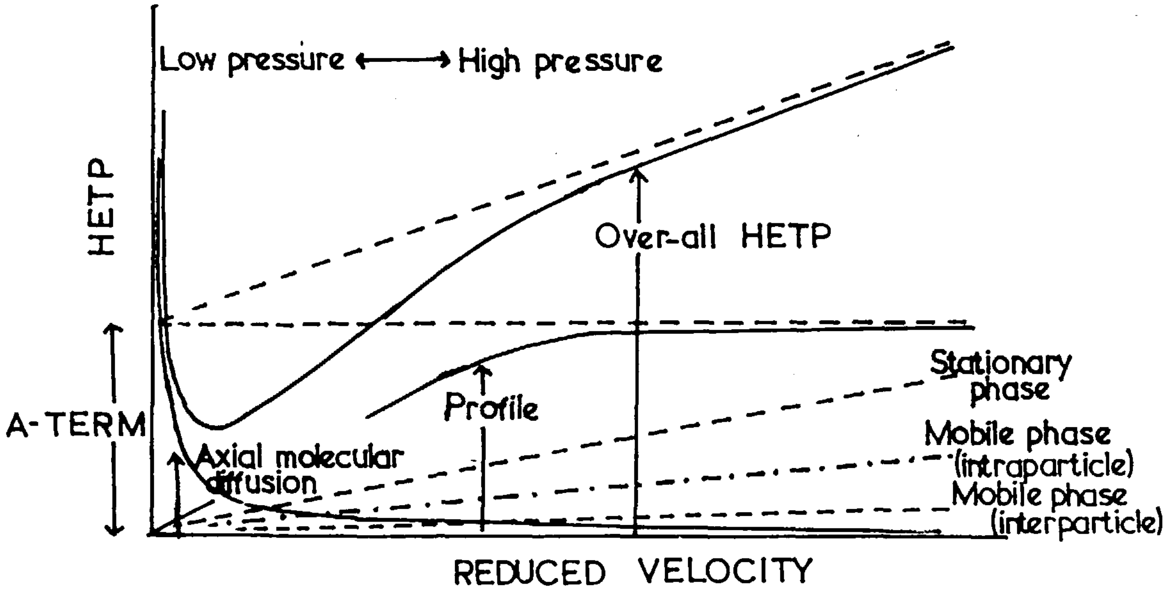

FIGURE 13. Schematic representation of plate-height contributions as a function of mobile fluid velocity (laminar flow).

Analytically, chromatography in the turbulent flow region offers no advantage over conventional chromatography. The observed decrease in H is considerably reduced when $k > 0$, since although gas phase mass transfer is increased by the mixing in the turbulent flow region the unfavorable liquid phase mass transfer term due to the high gas velocity results in an overall low efficiency. To achieve a given separation much longer columns would be required and the increased retention time due to the additional column length cannot be offset by using a higher flow rate.

V. RESOLUTION AND PEAK CAPACITY

We have so far looked at solvent selectivity and column efficiency as two separate column functions. From a theoretical point of view this is the simplest way, but in the practical situation they are brought together and the combined effects of the two give the resolution of the column, which is the parameter that analysts are most concerned with.

The resolution of a pair of components (1 and 2) is defined by

$$R_s = \frac{2(t_{R_2} - t_{R_1})}{w_1 + w_2} \qquad (62)$$

where t_{R_2}, t_{R_1} and w_2, w_1 are the respective retention times and basal peak widths.

In terms of the standard deviations σ_{t_2} and σ_{t_1} this can be written

$$R_s = \frac{1}{2}(t_{R_2} - t_{R_1}) \Big/ (\sigma_{t_2} + \sigma_{t_1}) = \frac{\Delta t}{4\sigma_t} \qquad (63)$$

Δt is a measure of the solvent selectivity and σ_t, the mean standard deviation, a measure of the column efficiency.

It should be remembered that almost complete basal separation is obtained at $R_s = 1.5$ (not at $R_s = 1$) because of the Gaussian distribution of the solute bands.

The resolution can also be expressed as

$$R_s = \frac{1}{4}\left(\frac{\Delta k}{k}\right)\left\{\frac{k}{1 + k}\right\}\sqrt{N} \qquad (64)$$

where the capacity factor $k = K.V_L/V_m$, so that $\Delta k/k$ is the fractional difference in solubility of the two substances to be separated and is a thermodynamic quantity, depending upon the nature of the stationary and mobile phases and the column temperature, while N can be considered as a kinetic factor, depending upon carrier flow rate, diffusion, and mass transfer. Assuming that the value of R_s applies to the pair of solutes in a mixture with the smallest relative retention, a larger value of R_s will only result in an unnecessary increase in analysis time. Since resolution is defined for a pair of solutes it does not in theory give information about the separation of the components of a mixture as a whole. If the resolution applies to that pair of solutes with the smallest separation, however, the resolution is practically defined for other pairs of components as being at least as good.

Giddings[89,90] has proposed the concept of the peak capacity 'n', as being a useful description of the overall performance of a column. The peak capacity is the maximum number of components that can be resolved completely between an unretained peak and the longest retained peak. Assuming that the number of theoretical plates generated by the column is the same for all solutes and that R = 1.0 for adjacent peaks the peak capacity is given by

$$n = 1 + \frac{\ln t_n/t_A}{\ln\left(\frac{\sqrt{N}}{2} + 1\right) - \ln\left(\frac{\sqrt{N}}{2} - 1\right)} \qquad (65)$$

where t_A and t_n are the retention times for a non-retained component (e.g., air) and for the nth retained component, respectively, and N is the plate number. Equation 65 can be shown[91] to approximate to

$$n = 1 + \frac{\sqrt{N}}{4} \cdot \ln\left(\frac{t_n}{t_A}\right) \qquad (66)$$

Grushka[92] has applied the concept of the peak capacity to the various methods of chromatography. Equation 66 has the lower limit

$$\lim n \longrightarrow 1$$
$$\frac{t_n}{t_A} \longrightarrow 1 \qquad (67)$$

In Figure 14 the number of resolvable peaks, n, is plotted against the number of plates N for different values of t_n/t_a and in Figure 15 n is plotted as a function of t_n/t_a for $N = 10^5$ (upper curve) and $N = 10^2$ (lower curve).

The limit $t_n/t_a \rightarrow 1$ means that all components would move with the same velocity as the unretained peak, i.e., no separation would be achieved. As Figure 14 shows, as t_n/t_a increases the peak capacity increases for a given value of N, but a small t_n/t_a value requires a larger N for the same peak capacity, i.e., the column must be longer. Since the ratio $t_n/t_a = 1 + k$ where k is the capacity factor, a conventional open tube column (C.O.T.) must have a larger value of N than a conventional packed column (C.P.) to achieve the same peak capacity, because k for a C.O.T. column is less than k for a C.P. column.

Equation 66 may be rearranged to give

$$- \frac{t_n}{t_A} = \exp[(n - 1)\ 4N^{-\frac{1}{2}}] \qquad (68)$$

This expression is plotted in Figure 16 and allows an estimate of the number of theoretical plates required to separate 'n' components. Alternatively, it could be used to calculate the time required to produce the desired separation of the 'n' components.

The capacity factor n is proportional to the plate number N and the ratio t_n/t_a, and its dependence on various column parameters will depend upon the variation of these two terms with these parameters. The ratio t_n/t_a is independent of the mobile phase velocity and column length but is dependent upon column temperature and amount of stationary phase. The maximum number of resolvable peaks occurs, therefore, at the minimum of the H vs. $\bar{u}$ plot when N is a maximum. Close to u_{opt} an increase in length will increase N and improve peak capacity.

Both N and t_n/t_a are dependent upon temperature. An approximate dependence of n for a temperature change from T_1 to T_2 is given by

$$\Delta n_{T_2-T_1} = \frac{\sqrt{N}}{4}\left[\ln\cdot\frac{t_n}{t_A}(\sqrt{x} - 1) - \sqrt{x}\ \ln y\right] \qquad (69)$$

where N, t_n, and t_a apply to the lower T, $x = n_{T_2}/n_{T_1}$ and

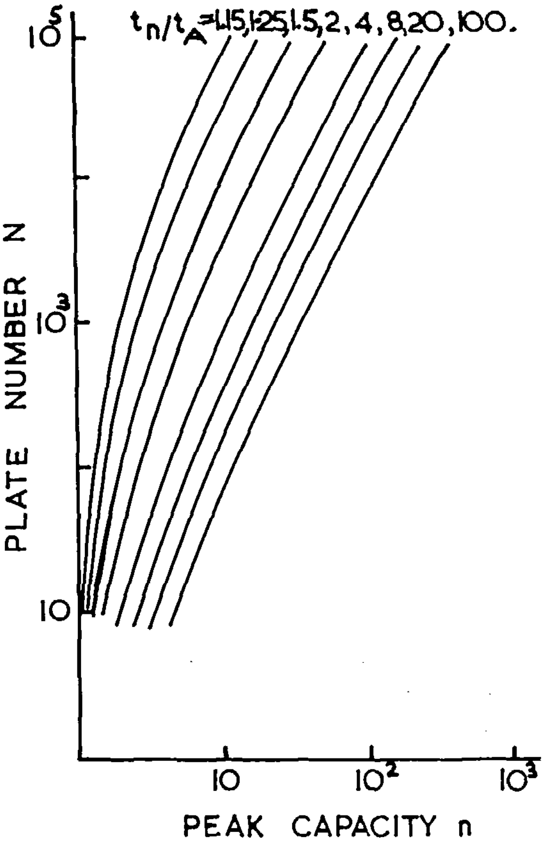

FIGURE 14. Peak capacity as a function of plate number at constant t_n/t_a.

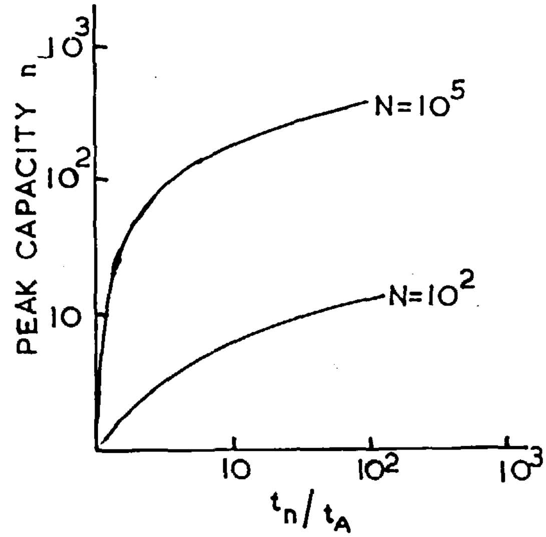

FIGURE 15. Peak capacity as a function of t_n/t_a at constant N.

$$y = \left(\frac{t_n}{t_A}\right)_{T_1} \Bigg/ \left(\frac{t_n}{t_A}\right)_{T_2} \qquad (70)$$

If $x < 1$, an increase in T will decrease n, since in most cases y is greater than unity.

The dependence of peak capacity on the amount of stationary phase is given by

$$n = 1 + \frac{\sqrt{N}}{4} \ln (1 + k) \qquad (71)$$

since $k = K. V_L/V_m$ increasing V_L will increase n, but since N is also decreasing the actual amount of increase in n is dependent upon the relative values of k and N.

If temperature programming is used, it should be possible to keep the width of the peaks constant and hence improve the peak capacity. In this case

$$t_A + (n - 1)\ w = t_n \qquad (72)$$

where n is the number of peaks of width w. Rearranging (72) and solving

$$n = 1 + \frac{\sqrt{N_A}}{4} \left(\frac{t_n}{t_A} - 1\right) \qquad (73)$$

where N_A is the number of plates obtained from the inert peak. Hence, n depends linearly on t_n/t_a and temperature programming will increase the peak capacity.

From the foregoing discussion it can be seen that the peak capacity is indeed a useful measure of the efficiency of the column and since it can be related in a fairly simple way to various column parameters, a study of the effect of varying these parameters on the efficiency enables the analyst to operate his column under optimum conditions.

VI. OPTIMUM COLUMN CONDITIONS FOR SEPARATION

Having selected the stationary phase to perform a given separation, the analyst must consider the column variables such as flow rate, column length, support material, and liquid loading and adjust these to give not only the optimum efficiency but often the optimum time for analysis as well. Since the column parameters are often interdependent the final set of conditions is usually a compromise.

As stated by Halasz and Heine,[5] the van Deemter equation is best regarded as a short McLaurin series which more or less adequately describes the H.E.T.P. - $\bar{u}$ relationship. It does, however, provide a working framework for studies

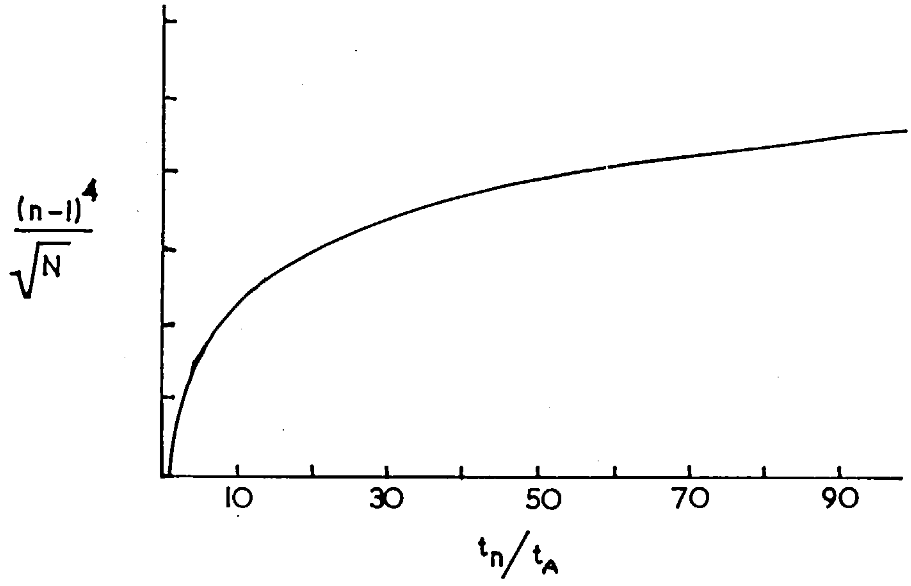

FIGURE 16. The relationship between t_n/t_a, N and n.

on the optimization of column conditions for maximum efficiency.

This topic has been the subject of three reviews and the reader is referred to these for a detailed study. Halasz and Heine,[5] and Guiochon[93] have discussed the efficiency and resolution of various types of columns while Scott[94] has described an experimental procedure for finding the optimum conditions for conventional packed columns.

We shall discuss here some of the more recent studies. Glass beads, because of their excellent packing properties and low porosity, are ideal materials for such studies. The 'stationary' mobile phase which occurs in porous supports is absent, and resistance to mass transfer is reduced. However, from a practical point of view they are not a good support material because of their low surface area and the 'puddling effect' whereby the solute accumulates at the contact points of the beads leaving the remaining bead with only a thin coating of liquid. These difficulties can be overcome by using either porous glass beads, the porosity of which can be carefully controlled in the manufacture, or by the use of controlled surface porosity supports, which consist of a spherical siliceous particle with a porous surface of controlled thickness and pore size.[95] In comparing the performance of porous and non-porous supports the mobile phase velocity must be carefully defined. The linear mobile phase velocity may be given by the retention time of a non-adsorbed peak when $u_1 = L/t_0$, or by the volume flow rate when $u_2 = F/q$, where q is the free cross section of the column.

For non-porous supports $q \triangleq d^2/3$ in a 'regular' packed column (defined as one in which the ratio of the column to particle diameter is greater than ten) and is independent of the particle size. For porous supports $q \triangleq 2d^2/3$.

From the Kozeny-Carman equation it may be shown that for a porous support, assuming the true density to be ~ 2.2g.cm.$^{-3}$, although 60% of the column is occupied by the support, 40% consists of the pore volume and only 20% is solid, and the mobile phase occupies 40% of the column. Since there will be 'static' mobile phase in the pores and the solute molecules will not be transported during the time spent in the pores, the two velocities u_1 and u_2 will not be the same. In fact, for porous beads $u_1 = ½ u_2$ since the molecules only spend half their time in the moving phase.

It follows that to achieve high speeds in chromatography non-porous supports should be used, especially if the column inlet pressure is limited. Porous layer beads, because of their high solid core to porous layer ratio, are nearer to the non-porous materials in this respect.

Filbert and Hair[96] investigated the effect of pore size and liquid loading on H.E.T.P. using porous glass beads and compared these with Chromosorbs P and W. In a porous material band broadening may occur due to differences in the path lengths, and also to differences in velocities in pores of differing size (gases move more rapidly in small pores and move slowly in large pores), and this obviously accounts in part for the fact that comparable H.E.T.P. values are found on the porous glasses (4000 to 1200 Å pore diameter) and for Chromosorb P (10^4 Å) but that these values were lower than for Chromosorb W (9 x 10^4 Å), and porous glasses with smaller pore diameters had still higher H values. Another factor is the different rate of liquid phase mass transfer due to differences in the liquid film thickness which is greatest with Chromosorb W (low surface area to volume ratio). The fact that Chromosorb W and the smaller pore size glasses have H values which show a dependence on the liquid loading is of interest. With Chromosorb W, H decreases as the liquid loading decreases; the reason obviously lies in the change in the mass transfer term with change in film thickness. With the small pore size glass beads H *decreases* as the liquid loading *increases;* this must be due to a wall effect and the film thickness.

The estimate of the extent of the ordered liquid layer (33 Å) due to the solid surface in this paper is unlikely to be valid because of the assumption that the liquid surface area can be calculated from a knowledge of the dry support surface area and the volume of liquid. Martire et al.[97] have shown that the surface area of 20% β.β'oxydipropionitrile on Chromosorb P is 1.0 m^2.g^{-1} as compared with the dry surface area of ~ 4.0 m^2.g^{-1}. Further, Karger et al.[48] have shown that the surface area of 16% H_2O on Porasil D is ~ 16 m^2.g^{-1}, a reduction of one half from the dry material.

Hawkes and Nyberg[98] have used Ballotini glass beads to study the effect of using ultra-low liquid loadings. The minimum liquid loading would appear to be ~ 0.02%; lower than this adsorption effects come into play. Marked increases in efficiency are obtained by dropping the liquid loading from 0.1 to 0.05%.

N. P. Knox and Salem[99] have studied the kinetic conditions for optimum speed and resolution in both gas and liquid chromatography, taking into account the pressure drop in the column. It is found that the maximum speed of analysis occurs at a finite velocity and column length. The limiting factor on the speed of analysis is the available pressure drop. This is dependent on the particle diameter (d_p) of the packing material and the column length (L). There is a critical particle diameter and column length which gives a maximum speed of analysis for a given pressure drop.

Optimization of the so-called kinetic parameters in the resolution Equation 64 implies optimization in terms of the separation number S (or N_{ne}) defined by Purnell[100] and given by

$$S = N_{ne} = \left(\frac{k}{1 + k}\right)^2 N = (1 - R)^2 N \qquad (74)$$

where R is the retention ratio. If the ratio $\Delta k/k$ is optimized, a specific value of N_{ne} must be obtained to give the required degree of resolution. Since it is a specific value of N_{ne}, not N, which is required, when k is close to unity or R close to zero, the number of plates required to produce a given separation may be very large.

The problem is to resolve the most difficult pair of components and to complete the analysis in a reasonable time.

To optimize conditions for an analysis the relationships which must be considered are:

(i) the time required to carry out the analysis, given by

$$t_{ne} = L(1 + k)/ju_o \qquad (75)$$

(ii) the necessary number of plates for a given resolution given by Equation 74, and

(iii) the relationship between the plate height and the column parameters; and of linear mobile phase velocity to ΔP, L, dp and η.

The reduced plate height $\zeta = \frac{h}{dp}$ and reduced velocity $\nu = u_o d_p/D_m{}^o$, where $D_m{}^o$ is the diffusion coefficient of the solute in the mobile phase at the column outlet, simplify the discussion of these parameters since the ζ vs. ν relationship is independent of the particle diameter and the nature of the mobile phase, if d_p is large (it is often assumed $> 100\mu$, but this is not necessarily so). There is no theoretical explanation for this limit and it probably arises from the difficulties associated with packing small particles.

The parameters for minimum time of analysis are conveniently summarized in Table 6 for the two carrier gases He and N_2[99]

It is interesting that the minimum time t_{min} is similar for both gases although the N_2 column is only approximately one third the length of the He column.

If the inlet pressure is limited, the analysis time is critically dependent upon the ζ value for the column; but if pressure is not the limiting factor, analysis time is reduced by reducing the particle size.

The particular form of the H vs. $\bar{u}$ function depends on the type of column considered, e.g.,

Open tube columns (C.O.T.) $H = \frac{B}{u} + Cu \qquad (76)$

Conventional packed (C.P.C.) $H = A + \frac{B}{u} + Cu \qquad (77)$

Packed capillary (P.C.C.) $H = \frac{B}{u} + C_s u$

$$+ \frac{1}{\frac{1}{A} + \frac{1}{C_m u}} \qquad (78)$$

This latter relationship will be recognized as the simple form of the coupling equation and has been shown by Landault and Guiochon[74] to describe packed capillary columns.

At high velocities it may be assumed, though with some caution, that both the B/u term and the A term are negligible. Equation 77 may then be written[101]

$$H = Cu \qquad (79)$$

Since u is a function of L which may be expressed as $u = \zeta/L$ Equation 79 may then be written

$$H = C\xi/L \qquad (80)$$

TABLE 6

Parameters for Minimum Time of Analysis

	He	N_2
$\eta D_m{}^o$	1.0×10^{-4}	1.8×10^{-5}
t_{min}(sec)	340	300
d_p(cm)	0.027	0.0115
column diam/particle diam	15.	35
column length	1300	550
emergent bandwidth 4σ	50	20
Max. injection vol cm^3	0.5	0.2
Max. detector vol cm^3	2.5	1.0

The value of L will be given by

$$L^2 = C\xi \cdot N_{ne} \tag{81}$$

and the time (t_z) for the elution of the last component by

$$t_z = C(1 + k_z)\ N_{ne} \tag{82}$$

The effects of various column parameters on the retention time can now be discussed on the basis of these three equations.

Since $C = C_s + C_m$, and C_m is a function of the outlet pressure p_o the analysis time may be minimizee if p_o is zero when C_m has its smallest value. The effect is minimal, though, since the pressure drop occurs mainly over the last part of the column, and the advantage to be gained does not warrant the use of an evacuated column outlet.

Interparticle effects would become negligible with ν values ~ 1000 so that coupling and turbulence effects add little advantage in high speed analysis. However, with present G.C. equipment these values are not yet attainable.

Intraparticle effects are not subject to coupling. If C_m is the dominant plate height term, since $C_m \propto d_p{}^2$ it would be an advantage to reduce d_p until $C_m < C_s$. Particularly in G.S.C. where C_s terms are small, this would involve using unsuitably small particle diameters, with an increased pressure drop and packing irregularities. Further, the increase in pressure will increase C_m and will affect the decrease in retention time.

The optimum conditions for a C.O.T. column have been calculated using a computer.[101] Conditions were optimized for a stationary phase having an assumed D_l value of 5×10^{-6}, which was independent of temperature. Under optimum conditons when $C_m \gg C_s$ the analysis time is found to be almost independent of the value of D_l. The results of this analysis lead to the following conclusions:

The nature of the stationary phase will effect the relative retentions of pairs of solutes, but since increasing the relative retention for one pair may decrease the retention for another pair this effect is of little importance in the time of analysis. Strong solvent retention power, implying a large value of $(k_2 \text{-} k_1)$ and hence of $1/N_r$, will allow a decrease in column length and hence shorter analysis time. Alternatively, the k values could be decreased by decreasing V_L giving a lower C_s value, or by increasing the temperature to give a lower C_m value.

The optimum temperature is found to be lower than the temperatures usually used in G.C. However, at the optimum temperature, optimum column length and peak width are too small to make this an attractive practical proposition. The cost of equipment for low temperature working is also higher than for normal temperature work. The optimum pressure is a function of the resolution; at constant temperature it is independent of the nature of the last component; but changes in temperature affect resolution and hence the optimum pressure.

Although the speed of analysis is limited by the available pressure the use of high inlet pressures creates problems with the injection system.

Furthermore, the readout system must also be capable of handling fast analyses. The width of the first peak must be greater than a certain minimum value, of the order of 1 sec. When this limitation is considered it is found that for a high liquid column loading such that all K values are $\gg 1$ the analysis time(t) is independent of loading. As the loading decreases t decreases, passes through a minimum, and then increases. If the C_ℓ term is too high, resolution will not be possible with the minimum peak width. The nature of the solid support is then important as it must be capable of carrying the required loading without causing the C_ℓ term to be too high. If the temperature were lowered, the liquid loading would have to be reduced to as little as a monolayer or less. Hawkes and Nyberg[98] have also observed this. If the surface area of the support is small, less material may be put onto it without approaching monolayer effects.

If column length is limited, as it is in many commercial instruments, the inlet pressure must be increased until the peak narrows to the minimum allowable within the limitations of the instrument. If, the column does not then generate sufficient plates for resolution, the peak width limitation cannot be applied. If resolution is very high, one can accept the unnecessarily long retention time, or the speed can be increased by increasing the C term, or the temperature could be raised which would both decrease resolution and increase speed. This latter is the easiest experimentally, but the same effect could be achieved by using, say, N_2 instead of He as carrier gas. This would have the added advantage of a saving in operational costs.

In the absence of adequate pressure the highest possible pressure should be used; failure to do this will make for the inefficient use not only of the column but also of the rest of the instrument. The existence of the peak width limitation suggests that the use of higher inlet pressures than normally used should be carefully considered.

The specific problems associated with the optimization of preparative scale chromatography columns have been investigated experimentally by Craven[102] using 4-inch diameter columns. Efficiency and high throughput are both required on the preparative scale, but the two are incompatible and compromise conditions must be used. The H.E.T.P. increases with the temperature, sample size, and carrier gas flow. The relationship between resolution, column efficiency (H), and throughput (Q) (ml/hr) is shown in Figure 17 which clearly shows that a high throughput is not compatible with high efficiency and resolution. The throughput line for 125°C is noticeably higher than that for the other temperatures. This shows that at this temperature a higher throughput can be achieved for a given resolution. Figure 18 shows the variation of the production efficiency (Q/H) with temperature, and again shows a maximum at 125°C. It also shows that Q/H is greatest at this temperature for the larger injection size but if minimum H is required, the maximum throughput is obtained using a small sample at higher temperature. Q/H can also be related to the linear gas velocity when a maximum is obtained between 3.5 and 4.0 $cm.sec.^{-1}$ There is, therefore, for a given selected value of R, a corresponding maximum throughput and injection size specific for that Q value.

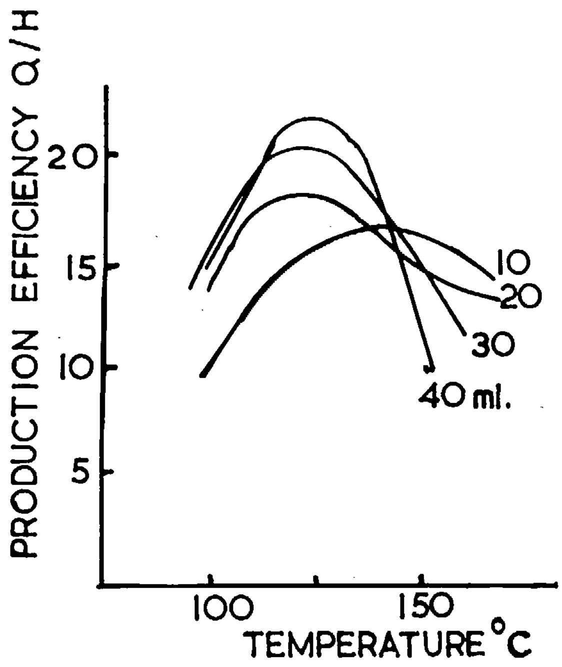

FIGURE 18. Effect of column temperature on the production efficiency (Q/H) for different sample sizes.

The optimization of column conditions is essential for the efficient use of the chromatographic column. However, since this involves many depen-

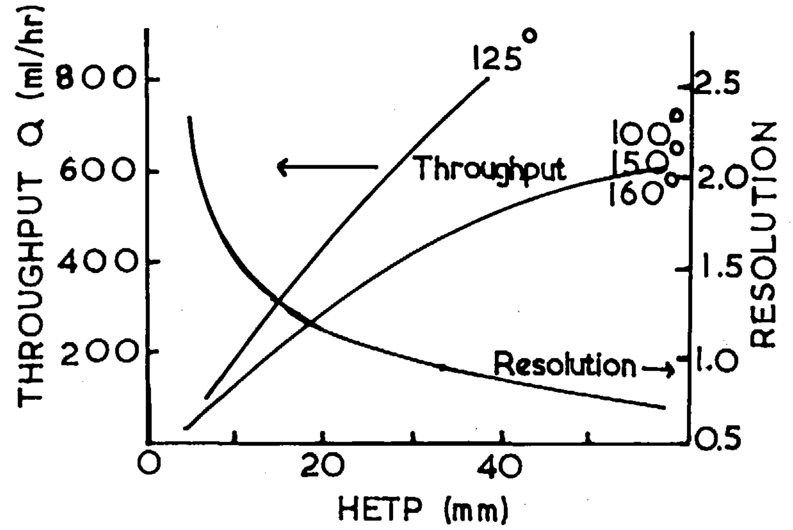

FIGURE 17. Effect of throughput (Q) on the efficiency (H.E.T.P.) and throughput for a 'prep-scale' column at various temperatures.

dent factors it is difficult to draw too many general conclusions. The type of column under consideration is all important. For example, for columns with a large phase ratio (i.e., V_m/V_s) e.g., C.O.T. columns, the C_s term may be neglected whereas for packed columns the C_m term may be negligible. However, the choice of column usually depends upon the phase ratio required to give the optimum capacity ratio (k = 1.0 - 5.0). Furthermore, since H_{min} is more or less independent of the column type (if the columns are well packed), with H_{min} and k determined by the choice of column $\bar{u}_{min}$ and H_{eff} are also determined. It is then only the shape of the H vs $\bar{u}$ curve which must be determined. The determination of this relationship should be the first duty of the analyst, and yet, invariably, chromatograms are obtained at the same flow rate under widely differing conditions without any reference to the van Deemter relationship.

In the past little attention has been paid to the nature of the support material, the main concern being that it should be non-adsorptive. Recent work has shown that this can have a serious effect on the efficiency of the column. The solid support should be the one with the largest surface area compatible with the loadability required and a near optimum pore diameter. This, however, will lead to longer analysis times; but as long as the efficiency of the column permits, this can be offset by a higher mobile phase flow rate.

Finally, the design of the equipment should be considered. The free gas volume in the injection port, detector, and connecting pipes should be small compared to the free gas volume of the column itself.

It is a credit to the efficiency of the chromatographic column that so many successful analyses are carried out in conditions so removed from the optimum.

VII. SOLUTE STRUCTURE – RETENTION RELATIONSHIPS

The importance of the relation between the structure of a compound and its retention behavior is twofold. From an analytical point of view, having once measured the retention, it is desired to identify the compound. This is usually done by comparing the retention data, obtained on stationary phases of differing specificity, with reference materials. Even then an unambiguous identification is not always possible and additional information in the form of infrared (ir) and nuclear magnetic response (nmr) spectra must be used. Even the mass spectrometer cannot always provide the answer and is a very expensive form of detector.

Secondly, these relationships can be used as a probe for determining unknown structures.

The Retention Index

One of the earliest and the most useful relationship between retention and structure is the Kovats retention index (I)[103] which in effect relates the retention behavior of an unknown compound to that of an n-alkane (C_n) eluted just prior to it, and to the next higher n-alkane homologue (C_{n+1}) which is eluted after the unknown compound. The retention index I is then given by

$$100 \left\{ n + \frac{\log V'_{Rx} - \log V'_{Rn}}{\log V_{Rn+1} - \log V'_{Rn}} \right\} \qquad (83)$$

where V'_{Rx} is the net retention volume of the unknown x, and ($V'_{R\,n+1}$- V'_{Rn}) re the retention volumes of the two homologous hydrocarbons of carbon number n and n+1. There has been renewed interest in the Kovats retention index as a parameter for structure-retention correlations. Closely related to this has been development in the measuremnt of very precise retention data, since if subtle structural changes are to be picked up by differences in retention behavior a high precision of retention volume measurement is required. The high precision of retention indices, ± 2 index unit (i.u.), arises because the measurement uses a double standard and is a relative measurement so that if unknown and standards are run together, as long as (V_{Rn+1} - V_{Rn}) is small, changes in the column conditions will affect unknown and standards alike. The running of sample and standard concurrently, however, is not convenient for a multicomponent mixture or if the peaks overlap. Nevertheless, as shown by Takacs and Krolik[104] this is highly desirable since "the deviation of the average retention index, calculated from a number of replicate measurements, from the correct index value does not give information about the reproducibility and accuracy of the index measurement, since relatively high positive and negative deviations of single index values from the correct one may compensate each other." They develop an equation for the estimation of the error in determining the retention index in terms of partial derivatives involving the retention times of the unknown and the hydrocarbons. Some results from Reference 104 are shown in Table 7. These show that the total error is in some cases considerably higher than the reported precision, the worst situation being when component retention times are small compared to the retention time of an unretained peak t_n. If the interpolation is to be made between non-neighboring homologues or an extrapolation is made, the logarithmic linearity of the reference scale must be checked.

TABLE 7

Component	Error (index unit) caused by				Total error (index units)
	$t_R(x)$	$t_R(z)$	$t_R(z\text{-}1)$	t_M	
Ethene	15.8	9.5	77.5	0.7	33.5
Cyclobutane	2.3	1.4	0.9	0.1	4.7
Styrene	0.6	0.2	0.3	0.0	1.1

(Courtesy *J. Chromatogr.*)

The retention index system and correlations with molecular structure has been the subject of a review by Kovats.[105] In this review the dangers of using systems which may give rise to mixed mechanisms of retention are clearly pointed out. It is assumed, however, that if there is a close similarity between the reference substance and the sample to be chromatographed, the adsorptive effects of retention will automatically cancel out. This assumes that the adsorption coefficients of, say, the homologous n-alkanes are the same as the branched chain alkanes. For this to be true would be quite fortuitous. It also makes interpolation and extrapolation procedures more dangerous.

The effects of adsorption on the retention index have been shown by Bonastre et al.[106] On a 2.5% P.E.G. 20,000 column benzene has an I value of 832, i.e., it is eluted between n-octane and n-nonane, but on a 30% P.E.G. 20,000 column it is eluted just before decane. Even comparing a 15% and a 30% column the I value shifts from 943 to 970.

Variation of Retention Index with Temperature

Retention indices can be presented in two ways. The first involves determining the I value at different temperatures on a single column. These values can then be presented as the ratio $\delta I/10^\circ C$ or by a relationship of the type[107]

$$I(T) \simeq a'\,T + b \tag{84}$$

where T is the column temperature. This relationship is linear for a wide variety of solutes both polar and non-polar on a non-polar solvent, but on a polar solvent the relationship may no longer be linear.

This form of presentation has several advantages. For example, intermediate temperatures may be interpolated and the optimum column temperature for the separation obtained.

Studies of the variation in the slopes of the lines allow some general correlations with structural features to be made; e.g., for the isoparaffins in squalane the slope of the lines increases with the degree of branching; for isomers with the same degree of substitution, e.g., 2.2. and 3.3dimethylpentane, substitution nearer to the end of the chain gives a smaller slope. Both peak shifts and co-elution temperatures may be predicted from the I vs. T relationship. In addition to their interpretive value peak shifts are useful in separative procedures; for example, if the concentration of two peaks differs considerably, it is better to have the smaller peak eluted first.

Since the retention index value is a function of temperature, values obtained under isothermal conditions are not applicable when the temperature is programmed, although as shown by Guiochon[108]

$$I_\theta(x) \simeq I_T(x) \quad \text{if} \quad T = 0.92\theta\ [^\circ K] \tag{85}$$

where $I\theta(x)$ is the retention index of the component x eluted at temperature θ in the temperature program.

Takacs, Rockenbauer, and Olacssi[109] have used the relationship

$$I(T) = A + \frac{B}{T + C} \tag{86}$$

to better represent the variation of I with temperature T. If there is no initial or final isothermal period in the temperature program, I (PTGC) is given by[110]

$$I(\mathrm{P.T.G.C.}) = A + \frac{2.3\ B \log\left(\frac{T_r + C}{T_o + C}\right)}{T_r - T_o} \qquad (87)$$

where T_r and T_o are the elution and initial temperatures of the program.

The validity of this equation has been successfully tested on a wide range of stationary phases both polar and non-polar.

Saha and Mitra[111] have used the temperature coefficients of the Kovats retention index for peak identification. Excellent linearity for plots of I vs. T°C was found for saturated and unsaturated hydrocarbons on S.E. 30, but on a polar solvent the randomness increased.

These plots can be used to classify hydrocarbons since it is found that the value of the temperature coefficient divided by n (the carbon number of the lower n-alkane standard) can be associated with the hydrocarbon type; e.g., in S.E. 30, the value for aromatics lies between 30 and 50 and between 16 and 26 for trialkyl paraffins.

Retention indices can be related to resolution, plate number, and other fundamental parameters. Ettre and Billeb[112] have suggested a simple treatment. For two peaks n and m both eluting between the same pair of n-alkanes (relative retention $r_{Cn+1}/_{Cn}$) on the same stationary phase and at the same temperature we can write:

$$i = I_n - I_m = 100\,\frac{\log t'_{R_n} - \log t'_{R_m}}{\log R_{C_{n+1}/C_n}}$$

$$= \frac{100 \log \alpha_{n/m}}{\log r_{C_{n+1}/C_n}} \qquad (88)$$

For a given temperature and liquid phase $r_{Cn+1}/_{Cn}$ is a constant so that we have

$$i = \mathrm{const} \times \log \alpha_{n/m} \qquad (89)$$

Hence, knowing the relative retentions for the two n-alkanes if the I values for the two components n and m are known, their relative retention can also be found.

Furthermore, since

$$i = \mathrm{const} \times \log \frac{\sqrt{N}_{ne}}{\sqrt{N}_{ne} - 4R} \qquad (90)$$

the difference in retention index is related to the resolution and the necessary number of theoretical plates to achieve this.

For parallel or intersecting plots of I vs. T, there will be two temperatures where $i_{T1} = i_{T2}$; i.e., the necessary number of effective theoretical plates for the desired separation will be the same at the two temperatures However, the k value will be smaller at the lower temperature so that more theoretical plates are needed at the higher temperature since

$$N = N_{eff}\left(\frac{1 + k}{k}\right)^2 \qquad (91)$$

Solvent Classification Using the Retention Index

Retention indices may also be expressed as the difference between the index as determined on a polar and non-polar solvent ($\Delta I_{p\text{-}np}$). Such values are a measure of the polarity of the stationary phase and have been used in attempts to classify solvent systems according to their polarity.

Comparison of results obtained at different temperatures is complicated by the effect of temperature on the specific interactions between solute and solvent. To overcome this and to enable a direct comparison Takacs and Erdy[113] have proposed a zero point index – a retention index difference for a compound on a polar and non-polar phase at 0°K, the $\Delta I_{p\text{-}np}$ values being measured over a temperature range and the correction to 0°K being made by Equation 86. This procedure is only strictly valid if the I vs. T° relationship for the polar solvent is truly linear.

As pointed out by Rohrschneider[114] the polarity of the column is also a function of the substances chromatographed. It is therefore better to determine the retention index difference with several types of solutes. Rohrschneider used benzene, ethanol, methyl ethyl ketone, nitromethane, and pyridine. ΔI is then given by

$$\Delta I = ax + by + cz + du + es \qquad (92)$$

where x is the polarity of the column when benzene is chromatographed and is equal to ΔI/100 for benzene. Similarly, y is ΔI/100 for ethanol, z is $\Delta I/100$ for MEK, u is $\Delta I/100$ for nitromethane, and s is $\Delta I/100$ for pyridine.

To characterize a given column it is necessary, therefore, to determine the retention indices of the selected solutes on, say, a squalane column and on the column to be characterized, under identical conditions. Rohrschneider constants for a large number of stationary phases have been tabulated by Supina and Rose.[115] The x values increase in an orderly manner with the polarity of the stationary phase since the dispersion interaction is proportional to the polarizability of the molecule, but the other terms vary somewhat. A high Rohrschneider constant indicates that the probe solute molecule will be strongly retained in the given solvent. Hence, QF.1. has a high z value and indicates that this solvent will selectively retain ketones.

The Rohrschneider constant could also be used to predict retention if the values of a,b,c,d, and e in Equation 92 are known. These can be determined (but the determination requires at least five ΔI measurements each on a different column) and as shown by Rohrschneider give reasonable agreement with experimental values.

At the moment there is no extensive catalogue of Rohrschneider constants and the a,b,c,d, and e values are known for only a few organic compounds, but if sufficient data could be collected, the application of this technique to retention problems could be advanced.

Structural Effects

Walraven and co-workers[116,117] have shown that very subtle structural effects can be correlated with retention indices determined on two different stationary phases, one polar and one non-polar. If the retention indices are plotted so that a point represents the retention index of the compound on the two different stationary phases, it is found that groups of isomers are then classified according to the number of primary, secondary, tertiary, and quaternary carbon atoms in the molecule, e.g., 2-methyl hexane, 3-methyl hexane, and 3-ethyl pentane would constitute one group designated 3.3.1.0.; compounds in the same group give straight lines, and all such lines are parallel. Also, the code numbers within each isomer group are in numerical order. This is the so-called "roofing-tile effect" and has been found to exist for a variety of classes of organic compounds. The extension of these studies to alkenes allows the effects of cis-trans isomerism and the position of the double bond to be investigated. Results for heptenes on octadecene-1 and dimethyl sulphalane are shown in Figure 19.[117] From this it is seen that compounds with the same group code give parallel lines, but these lines are split by cis and trans configurations, the trans configurations being on the upper lines. Compounds which are neither cis nor trans may be classed as alkenes-1, found upon or near the 'cis' line, and alkenes-2 which are found upon or near to the 'trans' line.

There are sufficient examples of the 'roofing tile' effect to show that a real correlation may be made in this manner, e.g., the configurations assigned to 3 methyl hexene-2 and 3 methyl hexene-3 have been changed partly on the basis of these plots.

Closely related to the Kovats retention index scale is the "gas chromatographic molecular weight" introduced by Evans and Smith[118] whence

$$M_e = M_n + 14.026\, n \left\{ \frac{\log R_x - \log R_N}{\log R_{N+n} - \log R_N} \right\} \tag{93}$$

where M_n is the molecular weight of an n-alkane with n carbon atoms, and R_x, R_N and R_{N+n} are the retentions of the unknown, and two n-alkanes with carbon numbers N and N+n. This index does not give such simple numbers as the Kovats index nor does it so clearly show the elution position with respect to the n-alkanes. It is convenient, however, for the prediction of retention data from group contributions since the characteristic values assigned to various functional groups are related to their molecular weights and it is possible[118] to predict the elution of simple linear molecules. When extended to more complex molecules the method fails badly[119] unless corrections are made for steric effects within the molecule. It is also found that the retention increments for double bonds are very sensitive to the substitution pattern and to stereoisomerism.

The use of retention index-structure correlations can be a useful aid to the analyst, but because of possible surface effects the requirements previously discussed for the reporting of retention data should be observed. In the case of non-polar solutes in non-polar or weakly polar stationary phases at high support loadings it is probably sufficient to report the specific retention

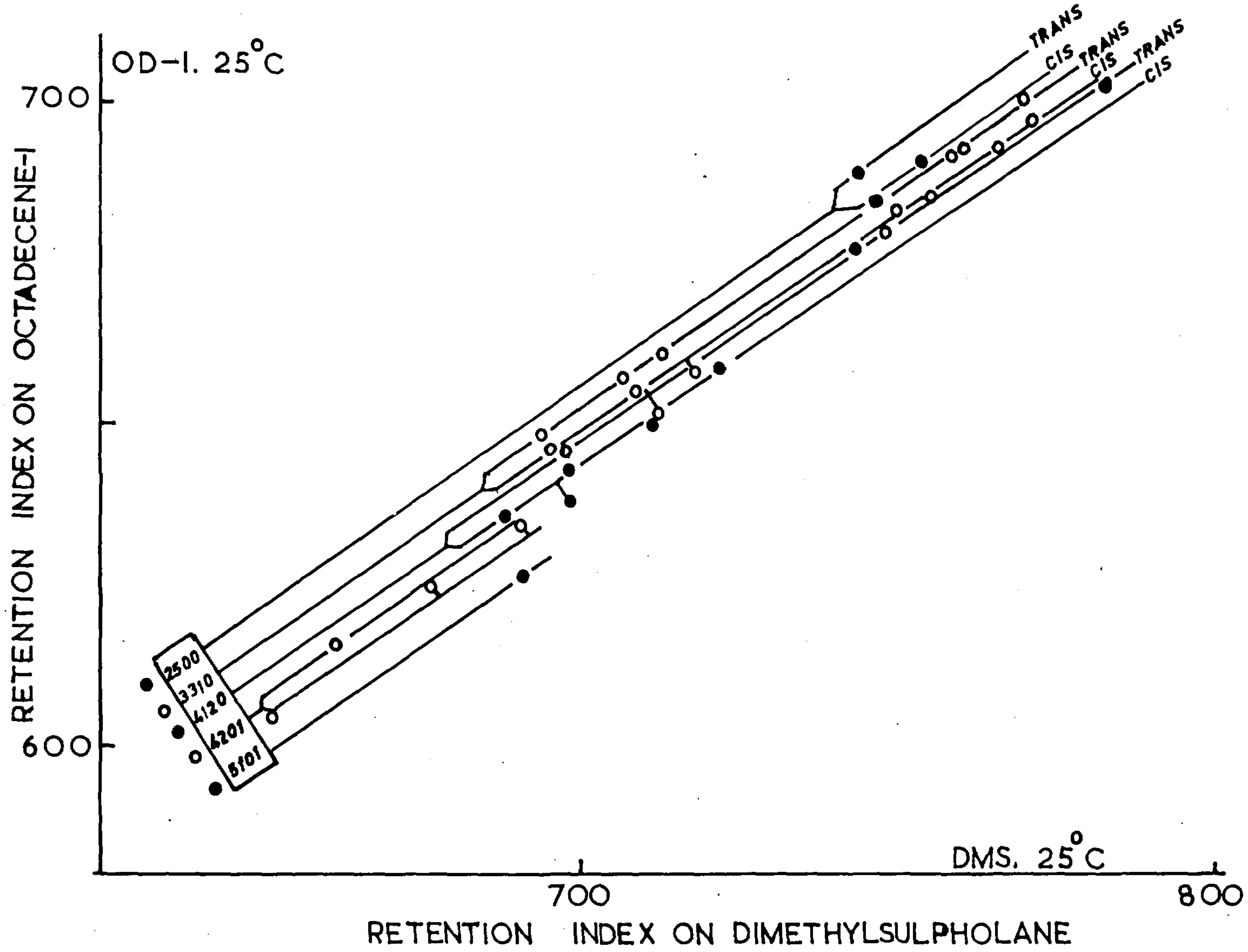

FIGURE 19. 'Roofing-tile' effect for heptenes, showing the cis and trans lines.

volume of one of the reference n-alkanes along with the retention indices. When polar solvents are used it is necessary to correct for the polarity of the stationary phase by converting the data to standard polarities using the Rohrschneider approach as suggested by Schomberg.[120]

Energy Parameter Relationships

We have seen that the difference in the standard molar free energies of gas liquid partition for two components $\Delta(\Delta G^o)$ may be used as a measure of the relative retention (Equation 21). Rohrschneider[121] has shown that $\Delta(\Delta G^o)$ is related to the resolution R_s by

$$R_s = \frac{\Delta(\Delta G^o)}{RT}\sqrt{\frac{N}{16}} \qquad (94)$$

There have been several attempts to relate energy parameters to retention behavior.

The Hammett Equation

The Hammett equation,[122] used as a predictive tool in organic chemistry, relates the equilibrium or rate behavior of a benzene derivative and a series of corresponding meta and para monosubstituted benzene derivatives. The relationship may be written

$$\log \frac{K'_x}{K'_o} = \sigma\rho \qquad (95)$$

where K'_x and K'_o are the equilibrium (or rate) constants for a chemical reaction involving the substituted and unsubstituted benzene derivations, respectively. The constants on the R.H.S. of Equation 95 are 'σ'-dependent only upon the substitutent and not the reaction, and ρ dependent upon the reaction type and conditions but independent of the substitutent. The sign and magnitude of the substitutent constant σ are measures of the electronic environment at the reaction center (functional group) and the reaction constant ρ reflects the sensitivity of the specific reaction and conditions to changes in the electronic environment at the functional group.

Karger and co-workers[123,124] have applied the Hammett equation to gas chromatography. The relative volatility α being given by

$$\alpha = \frac{K_x}{K_o} = \frac{t_{R_x} - t_m}{t_{R_o} - t_m} = \frac{\gamma_o^\infty P_o^\infty}{\gamma_x^\infty P_x^\infty} \qquad (96)$$

neglecting gas phase imperfections, where K_x, K_o, t_{Rx}, t_{Ro} are the partition coefficients and retention times of a m or p substituted phenol and phenol itself, it is possible to determine the product $\sigma\rho$ from the chromatogram. Furthermore, since

$$\Delta(\Delta\bar{G}^o)_e = -RT \ln \frac{\gamma_o^\infty}{\gamma_x^\infty} \qquad (97)$$

where $\Delta(\Delta G^{-o})_e$ is the difference in the excess partial molar free energy of mixing for the two components the Hammett relationship may be written in the form

$$\log \frac{\gamma_o^\infty}{\gamma_x^\infty} = \sigma\rho + b \qquad (98)$$

where b is a constant independent of the electronic factors considered in the product $\sigma\rho$.

Values of $\gamma_o^\infty/\gamma_x^\infty$ can be obtained from Equation 111 and there is a linear relationship between log $\gamma_o^\infty/\gamma_o^\infty$ and the Hammett substituent constant σ.

The Hammett equation empirically measures the change in the electronic environment caused by addition of substituents to the ring, and it is not surprising that a relationship exists between the substituent constant and the activity coefficient ratio, since this ratio is a measure of the differences in the solute-solvent interactions for the two components (phenol:substituted phenol). The ratio, therefore, is measuring the effect of the substituent on the amount of specific interaction between the phenolic H atom and the solvent. Therefore, any other specific interactions between solute and solvent, e.g., dipole-induced dipole interactions involving the π electrons or the benzene ring, will tend to obscure the Hammett effect.

The slope of the line ρ is a measure of the solvent selectivity, since the larger the value of ρ the more sensitive is the solvent to changes in the electronic environment at the phenolic oxygen which will change the degree of specific interaction and hence selectivity.

If the log $\gamma_o^\infty/\gamma_x^\infty$ σ relationship truly represents specific interactions at the phenolic group it would be expected that the correlation coefficients would increase if the conditions are favorable to these interactions to the exclusion of others. This is found to be true; for example, the CH_3O-group gives rise to a low correlation coefficient presumably due to interactions with the solvent. Also, the correlation coefficients increase linearly as the temperature decreases, a situation favorable to specific interactions of the H-bonded type.

As well as giving an insight into the separation mechanism the Hammett relationship can also be used to estimate relative volatility (α).

The correlation between log $\gamma_0^\infty/\gamma_x^\infty$ and σ is improved, if the substituent constants σ_c are calculated using the method of Jaffe,[125] except when the solvent is non-polar. This is not surprising since specific interactions with this solvent will be very weak.

Empirically we see that the Hammett equation can be applied to G.L.C. systems when 1. there are strong specific interactions, e.g., H-bonding between solute and solvent molecules and 2. the substituent group itself does not interact with the solvent. This result has been put on a more theoretical basis by Karger et al.[124] assuming that a 1:1 hydrogen-bonded complex between the solute and solvent molecule is formed, and that the solvent molecules are not self-associated.

An interesting application of this work is in the use of mixed solvent systems. Figure 20 shows the variation in ρ with mole fraction of tris-(4-t-butyl phenyl) phosphate in dotriacontane and Figure 21 the change in specific retention volume with mole fraction of phosphate. No hydrogen bonding would be expected from the hydrocarbon which would act as a diluent for the phosphate which will hydrogen bond. The selectivity as measured by ρ remains substantially constant as the mole fraction of phosphate increases from 0.25 → 1.00, whereas the retention volume increases linearly. Hence, to use a mixed solvent containing more than 0.25 mole fraction of phosphate while adding little to the selectivity causes a large and unnecessary increase in retention. The resolution of 4-chlorophenol and 3-ethylphenol was also found to fall off rapidly below a mole fraction of phosphate of 0.25. This must be due to a change in the activity coefficient ratio since the vapor pressure ratio is constant.

Hammett substituent constants show that in G.S.C. of salt modified alumina[126] the substituent effects appear to be due to electron transfer to or from the aromatic ring and that purely inductive forces have little effect on retention. Hence, modified adsorbents can be used to separate molecules where the substituent group can conjugate with the ring from those in which it cannot.

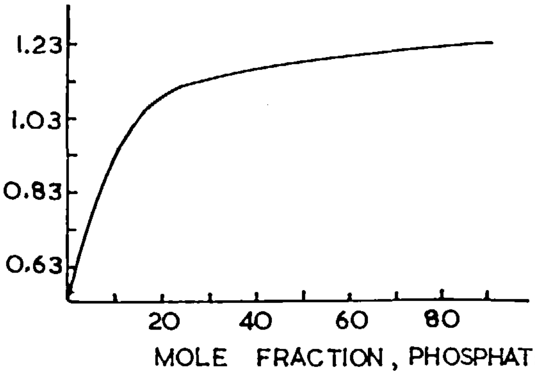

FIGURE 20. Plot of ρ vs. mole fraction of tris-(4-t-butylphenyl) phosphate in dotriacontane for monosubstituted phenols.

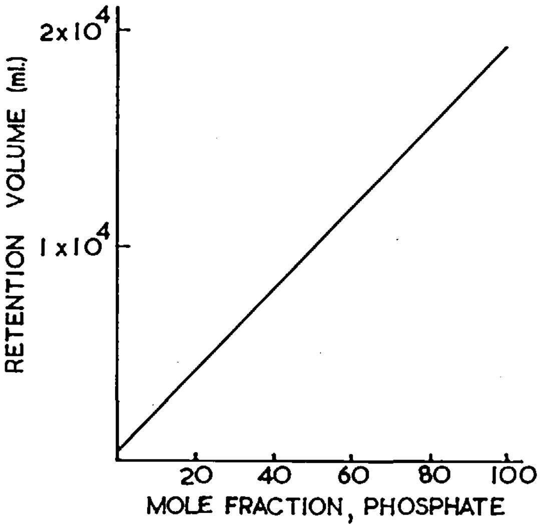

FIGURE 21. Plot of retention volume of 4-chlorophenol vs. mole fraction of tris-(4-t-butylphenyl) phosphate in dotriacontane.

As pointed out by Karger, the Hammett relationship fails if the solvent is non-polar. Krupeik, Liska, and Sojak[127] have found an empirical relationship for the C_6 to C_{10} alkyl aromatic hydrocarbons on squalane which gives a high correlation coefficient, using toluene as the reference

$$\log \frac{\gamma_t^\infty}{\gamma_x^\infty} = \rho T_T + b_2 \qquad (99)$$

where the chromatographic substituent coefficient σ has been replaced by $T_T = \log(T_t/T_x)$ where T_t and T_x are the boiling points in °K of toluene and the substituted alkyl aromatic. Krupcik et al. also correlate the retention index with the substituent constant

$$\log \frac{I_t}{I_x} = \rho T_T + b_3 \qquad (100)$$

Such a correlation is not unexpected since the ratio of Kovats retention index is a measure of the relative retention. Knowing ρ for a series of compounds it can be used for the identification of compounds if the retention indices are measured.

Theoretical Prediction of Retention Behavior and Solvent Classification

The correlations discussed so far have been empirical ones based on actual chromatographic measurements. The degree of correlation depends upon the model chosen and the accuracy with which the retention data can be measured. Since the accuracy of measurement is high, good correlation can be achieved if the model is a correct one.

However, the model as applied to one system is not necessarily the correct model for a different system so that the correlations are often of limited applicability, e.g., the Hammett equation.

Predictions based on more fundamental parameters while perhaps not giving such a good correlation might be more generally applicable.

The Solubility Parameter

The solubility parameter, δ , defined by $\delta = (\Delta E^v)^{1/2}$ where ΔE^v is the energy of vaporization and v is the molar volume is widely used in solution theory.[128]

Giddings[67] has used the solubility parameter in place of the virial equation of state to relate the enhancement in volatility (lower k) with the pressure, temperature, carrier gas, and size and nature of the solute at pressures above 50 atm, since the solubility parameter is related to the critical pressure P_c and to the liquid (ρg)and gas (ρ_l densities by the expression

$$\delta = 1.25\, P_c^{1/2} \left(\frac{\rho_g}{\rho_\ell}\right) \qquad (101)$$

The range of δ values for dense gases is from 0 to 10 whereas liquids normally fall in the range 5 to 10, so that dense gases cover a range not normally available to G.C.

The volatility enhancement I_{12} on going isothermally from state 1 to state 2 is

$$I_{12} = \frac{K_1}{K_2} \qquad (102)$$

where K_1 and K_2 are the distribution coefficients. and

$$\ln I_{12} = \left(\frac{V_o\delta_o^2}{RT}\right) (\Delta_2 - \Delta_1)\ (2 - \Delta_2 - \Delta_1) \qquad (103)$$

where V_o is the molar volume of the solute and $\Delta = \delta/\delta_o$ (the reduced solubility parameter).

From this equation it is possible to predict the effects of molecular size and polarity on volatility enhancement and the possible effects of pressure, temperature, and the nature of the carrier gas. At this stage in the development the results should only be considered as guidelines.

Rohrschneider[129, 130] has applied the solubility parameter concept to solutions which are assumed to be ‘regular’. Since regular solution theory only applies to non-polar molecules it is again of only limited application.

The relative retention in regular solution is determined solely by the solubility parameters of the stationary liquid δ_s and solute δ_2 and the respective molar volumes.

$$RT \ln \left(\frac{t'_2}{t'_1}\right) = (V_1 - V_2)\, \delta_s^2 - 2(V_1\delta_1 - V_2\delta_2)\, \delta_s \qquad (104)$$

Retention ratios calculated for the n-alkanes from this equation do not agree with experimental values, but it is known that n-alkanes give anomalous solubility parameters.[128] Only if the solubility parameters are calculated from known retention values can Equation 104 be used to predict retention ratios for other hydrocarbons.

Rohrschneider concludes that observations attributed to the “polarity of the stationary liquid,” are caused in regular solutions by the internal pressure of the liquid. This would appear to be merelv substituting one name for another.

The solubility parameter (internal pressure) is a measure of the cohesive energy between like molecules which reflects the force field surrounding the molecule. In such considerations it is not sufficient to consider the overall dipole moment of the molecule, but the individual bond dipole moments must be taken into consideration. Thus, a molecule normally considered as being non-polar may possess certain polar functions.

An attempt to widen the scope of the usefulness of the solubility concept has been made by Keller, Karger, and Snyder.[131] The internal pressure (δ^2) is taken to be the sum of contributions from dispersive, δ_d, polar δ_p, and hydrogen bonding δ_h components of the solubility parameter such that

$$\delta^2 = \delta_d^2 + \delta_p^2 + \delta_h^2$$

The problem arises in determining the values of δ for the various contributions. Keller et al. have used the Lorentz-Lorenz function for the dispersion contribution

$$\delta_d = C\left(\frac{n^2 - 1}{n^2 + 2}\right) \qquad (105)$$

where n is the refractive index. The linear relationship breaks down when $\frac{n^2-1}{n^2+2} \geqslant 0.28$ which corresponds to a δ_d value ~ 8.8. Above this value the δ values calculated from the Hildebrand rule fall on a smooth curve. Previous estimates of δ_d have been based on the homomorph principle, δ_d for a polar molecule being assumed equal to its homomorph, i.e., a non-polar molecule of roughly the same size and shape.

The polar contribution to δ when no hydrogen bonding occurs can be calculated from the relationship

$$\delta^2 = \delta_d^2 + \delta_p^2 \qquad (106)$$

if δ and δ_d are determined.

The hydrogen bond contribution can be divided into contributions from the acid and base strength of the proton donor and acceptor, respectively, such that

$$\delta_h = \delta_a \delta_b \qquad (107)$$

Calculation of δ_a and δ_b depends upon the determination of the strength of the hydrogen bond.

Some values for the various contributions are shown in Table 8. Four of the solutes, C_6H_6, C_2H_5OH, CH_3NO_2, and C_6H_5N, are solutes used in the Rohrschneider solvent classification.

It is not anticipated that this approach will allow the accurate prediction of retention parameters, but the selection of solutes used for solvent classification may be put on a logical basis.

TABLE 8

Solubility Parameter Components

Solvent					
n-C_5H_{12}	7.0	7.0	0	0	0
C_6H_6	9.1	9.1	0	0.9	0
Pyridine	10.4	9.0	2.7	5.0	0
CH_3CN	11.8	6.5	6.2	2.2	0
CH_2I_2	11.9	11.3	1.2	0.4	0
CH_3NO_2	12.0	8.4	6.2	1.7	0
CH_3OH	12.9	6.2	4.2	5.2	5.2

(Courtesy of the Institute of Petroleum, London)

Statistical Mechanical Energy Functions

As shown by Kwa, Korver, and Boelhouwer[132] it should be possible to relate the increments in retention indices or the logarithm of the relative retention volumes to the statistical mechanical partition functions. Since the partition coefficient is the equilibrium constant for the phase transition reaction of the solute between the gas and liquid phase it can be related to the partition functions of the component groups (i.e., parent and substituent groups) which themselves consist of rotational and vibrational group contributions. The energy functions are found to be very sensitive to chemical structure. This approach is unlikely to be applied very widely but is interesting as representing a very fundamental approach to the retention-structure problem.

VIII. HIGH PRECISION IN GAS CHROMATOGRAPHY

We have seen how G.C. is being used to discern structural differences between molecules which may involve only very small free energy differences and these structural differences are being correlated with retention behavior. The success of these correlations depends upon the precision with which the retention data can be measured. Retention volumes are commonly only measured with a precision of 2% or worse, though a precision of 1% is obtainable. The magnitude of the task in obtaining a much higher precision than this may be seen from the data of Guiochon[133] (Table 9). These data give the precision required for various column parameters if a precision of 1% or 0.01% in t_R is required.

Such precision will not be achieved without recourse to the use of computer control and evaluation. Goedert and Guiochon,[134] using carefully controlled column temperature, pressure, and carrier-gas flow rate, automatic control of the injection, and digital integration for peak area and retention measurement, obtained a relative precision of ±0.05% in retention time.

Oberhaltzer and Rogers[135, 136] have determined retention times with a precision of better than ± 0.02% using carefully chosen chromatographic components, digital control of the injection, and digital data acquisition and computer calculation. For the experimental details the reader is referred to the original papers.

In order to obtain these high precisions use must be made of statistical moments analysis. The "long-time" assumption in G.C. leads to the solute zone being described by the Gauss distribution curve. In practice a truly Gaussian distribution is extremely rare. In such a case, the distribution curve may be defined by the Gaussian distribution corrected by terms which are functions of the so-called statistical moments. The property of the solute zone which is usually used in chromatography is the break-through time of the peak maximum although no simple physical interpretation can be given to this property. The property which has a definite physical meaning is the breakthrough time of the centroid of the area under the curve.[137] The first statistical moment μ_1' indicates the position of the centroid which is the position of the arithmetic mean of the distribution on the time axis and may be written

$$\mu_1' = \frac{\Sigma\, C_i t_i}{\Sigma\, C_i} \tag{108}$$

where C_i is the height of the abscissa at the point t_i, and $\Sigma\, C_i$ is the peak area.

Higher moments are defined by

$$\mu_k' = \frac{\Sigma\, C_i t_i^k}{\Sigma\, C_i} \qquad k = 2,3,\ldots. \tag{109}$$

These higher moments are more usually written as deviations from the mean so that

$$\mu_k = \frac{\Sigma\, C_i (t_i - t_o)^k}{\Sigma\, C_i} \qquad k = 2,3,4,\ldots. \tag{110}$$

and t_o is the mean value μ_1'.

The first statistical moment about the mean is 0, and the second statistical moment is the variance. The third moment measures the asymmetry of the distribution curve and the fourth statistical moment measures the flattening of the distribution.

In determining isotherms by G.C. using the E.C.P. method,[16] a criterion often applied is that the diffuse boundaries of chromatograms obtained using varying sample sizes should superimpose one upon the other. This implies that the various peak maxima fall on the envelope of the superimposed chromatograms. As stated above this has no true physical meaning since it is the position of the centroid of the distribution curve which is of concern, and it is this that is dependent upon the equilibrium constant for the distribution mechanism.

Statistical moments theory has been applied by Grubner[137] to diffusion controlled kinetics in gas-solid chromatography, and by Grushka, Myers, and Giddings[138] to the problems of peak contamination and peak overlap, this latter application being important in qualitative analysis.

The advantage to be gained in structure-retention correlations when retention data are measured with a higher degree of precision than is usual has been illustrated several times. The retention index scale assumes that the relationship between the logarithmic retention value and the carbon number of the n-alkanes is strictly linear.

TABLE 9

	Precision in t_R	
Column Parameter	1%	0.01%
Outlet pressure	0.25%	0.25%
inlet pressure drop	0.005%	0.005%
temperature	0.04%	0.04%
error in peak measurement	0.005%	0.005%

This premise has been questioned several times. Using very precise retention index measurements Kemenade and Groenendijk[139] studied this relationship and concluded that if the retention of methane is recognized and the dead volume is not obtained from the methane retention time, but from three 'standard' normal alkane retention times, then the relationship is strictly linear even for the lowest members of the n-alkane series. Oberholtzer and Rogers, however, came to the conclusion that the relationship is somewhat nonlinear for 'n' in the range 6 to 10.

Oberholtzer and Rogers,[135] in a further study using a high precision gas chromatograph, have studied the effect of micropores on peak shape and retention volume in gas-solid chromatography using 4A and 5A molecular sieves. For the first time in gas-solid chromatography changes in retention time with flow rate were observed and the H.E.T.P. was found to increase with temperature. Methane and ethane, which are both small enough to enter into the crystallites, give tailing peaks irrespective of the sample size. These observations can be explained by assuming that these molecules undergo only restricted diffusion in the intraparticle space since a larger molecule which does not enter the pores does not show these phenomena. Such measurements would not have been possible without the high precision.

Kemenade and Groenendijk[140] have measured the retention indices of branched chain alkanes on squalane and find an increase in I with temperature showing they are selectively retarded with respect to the n-alkanes. Using the four digit code for grouping compounds introduced by Walraven, it is found that within a group the retention index increases with an increasing number of secondary methyl groups; e.g., 2 Me hexane $<$ 3 Me hexane $<$ 3 Et-pentane. If the chain length of the stationary phase is varied, an increase in I is found as the chain length ($-CH_3$) content increases. These results are explained by assuming a van der Waals type interaction between solute and solvent CH_3 groups when their rotational frequencies correspond. The preferential interaction of a 'secondary' methyl group is then due to the lower internal potential barrier of this configuration.

FUTURE TRENDS

Gas chromatography is basically a simple technique requiring relatively unsophisticated equipment operated near to atmospheric pressure. Within this simple framework the column is expected to perform near miracles of separation.

The number of stationary phases available to the analyst will continue to increase, although about ten phases, liquid and solid, are all that are required to perform the majority of separations. Selectivity can usually be achieved by the judicious choice of the stationary phase. The use of liquid crystals and complexation equilibria, although very interesting as a means of increasing selectivity, are not in general likely to prove as useful as mixed stationary phases or the use of salt modified adsorbents. A renewed interest in G.S.C. may well prove to be the most fruitful path to column selectivity. A two-dimensional solid surface is better suited to take advantage of subtle structural differences in the solute molecules than is the three-dimensional environment of a solvent where structural differences may be obscured.

Gas chromatography is a mature technique. No doubt the retention equations will be further modified and refined but this is of more academic interest. Major advances in column efficiency are unlikely. When one has played about with the various column types, support materials, type and amount of stationary phase. and the other column parameters the final conclusion is always that to increase the number of theoretical plates the column length must be increased. In order to maintain the gas flow the permeability of the column must be increased, the inlet pressure must be increased, or the outlet pressure must be decreased. Such operations immediately take away some of the advantage of simplicity and are unacceptable to the majority of analysts at the present time. Moderate inlet pressures, however, can be used to good effect without too much difficulty and there will be increased use of these higher pressures.

As better resolution is achieved, more complex mixtures are being analyzed and it is becoming increasingly tedious to identify the components. The use of other physical methods of identification will be coupled with high precision retention index data to solve this problem. Computers will not only be needed to handle the retention data but also to control the chromatograph and to analyze the component peaks in order to obtain the high precision necessary. With this higher precision will come more valid structural correlations and more accurate thermodynamic data so that our understanding of sorbate-sorbent interactions will be put on a firmer basis and this may lead to more valid models for prediction of retention behavior.

It has been said that "gas chromatographic analysis is today partly a science but, to a greater degree an art."[5] The very meaning of the word chromatography -- "color writing" suggests this dual nature. The placing of the emphasis is in the eyes of the beholder; the physical chemist is more likely to regard it as a science, the anlyst as an art. It is true to say that as a science it is not a very exact one, and obtaining the correct ingredients requires considerable art.

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