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SOLUTE INTERACTIONS WITH THE MOBILE AND STATIONARY PHASES IN LIQUID-SOLID CHROMATOGRAPHY

R. P. W. SCOTT and P. KUCERA

Chemical Research Department, Hoffmann-La Roche, Nutley, N.J. 07110 (U.S.A.)

SUMMARY

A dynamic equation for the distribution coefficient is proposed that accounts for the different solute-phase interactions in liquid chromatography. The validity of the basic form of the equation is experimentally verified and the effect of solvent composition on retention is shown to be dependent on the probability of solute-solvent interaction and thus the concentration of polar solvent in the mobile phase. Using the equation, the change in retention volume with polar solvent concentrations can be accurately predicted. Where polar forces between solute and solvent are weak, it is shown that dispersive interactions are proportional to the density of the dispersing solvent. The magnitude of the polar interactive effects of both solute and solvent are shown to be proportional to the exponent of the polarizability per milliliter of the respective solute or solvent for a limited number of solutes and solvents.

INTRODUCTION

Many of the problems that arise in the practice of liquid chromatography are associated with the selection of a suitable phase system to effect the separation of the sample presented for analysis. These problems often result from the fact that insufficient knowledge is available on the manner in which the solute interacts with the two phases. There have been several approaches made to provide a theoretical explanation of these interactions in order to aid in phase selection.

The most common approach¹ is to use the thermodynamic relationship that describes the partition coefficient as a function of the exponent of the partial molar excess free energy associated with the removal of the solute from one phase to another. However, the thermodynamic approach has severe limitations from the practical point of view.

Thermodynamic properties are bulk properties and are a measure of the net interactive effects and, although thermodynamic functions can be assigned to the different interactions², if there is more than one effect present the individual interactions cannot easily be separated or identified. Another disadvantage of the thermodynamic approach results from the fact that there is a very limited amount of thermodynamic data available on solute-phase systems and further, that such data are difficult and time consuming to obtain.

In this paper an alternative method of describing the distribution coefficient is given, which, for want of a better term, is called the dynamic equation for the distribution coefficient and which allows each specific interaction to be described separately and accounted for individually.

The equation given is experimentally verified with respect to interactions in the mobile phase and when fully developed could predict the relative magnitude of the interactions between different solutes and solvents in liquid-solid chromatography. The precise nature of the interactions of the solute with the stationary phase, however, remains to be identified and substantiated.

THEORY

The distribution of a solute between two phases results from the balance of forces between the molecules of the solute and the molecules of each phase. These forces can be polar in nature, arising from permanent or induced electric fields associated with both solute and solvent molecules, or they can be due to London's dispersion forces (Van der Waals forces). The exact nature of these forces is not, at present, known and the so-called polar and dispersive forces may be essentially the same in character, but of different magnitude. For the purpose of this paper, however, it will be assumed that the two forces are different and each requires to be accounted for separately. This approach will also simplify the theoretical arguments necessary to describe interactions in mobile phases that consist of polar solvents, such as ethyl acetate, in dispersive solvents, such as *n*-heptane. In ion-exchange chromatography the forces on the solute molecules will be substantially ionic in nature but will include the so-called polar and non-polar forces as well.

It is, therefore, possible to define the distribution coefficient (K) of a solute between the two phases in a chromatographic system in the following way:

$$K = \frac{\text{total forces acting on the solute in the stationary phase}}{\text{total forces acting on the solute in the mobile phase}}$$

$$= \frac{\text{forces between solute and stationary phase} \times \text{probability of interaction}}{\text{forces between solute and mobile phase} \times \text{probability of interaction}}$$

At this stage it should be pointed out that a specific value for K or the corrected retention volume V' can be realized in many ways. Assume that a given solute has a distribution coefficient of unity for a given chromatographic system. From the above equation it is seen that for such a case the numerator and the denominator must take the same value, but this can be large, intermediate or small, depending on the nature of the interactions. Thus, for two solutes eluting at a k' of unity, one may have strong interactions with the two phases and the other weak interactions with both phases. However, because the ratios of the forces in each phase are the same for each solute, they will both be eluted at the same k' value. It will be seen later that the absolute values of the interacting forces determine whether the dispersion forces can be employed to enhance separation in the chromatographic system or not.

The forces between solute and either phase will depend largely on the tempera-

ture, the nature of the solute and that of the two phases, whereas the probability of interaction will vary with such factors as concentration of the interacting moieties of each phase and temperature.

Considering the situation with respect to liquid chromatography in the general case where solvent mixtures are employed as the stationary phase, there may be three types of intermolecular forces involved, *viz.*, ionic forces, polar forces, and dispersive forces. Thus, the equation for K can be put in the form

$$K = \frac{F'_i(P'_i) + F'_p(P'_p) + F'_d(P'_d)}{F_i(P_i) + F_p(P_p) + F_d(P_d)}$$

where F'_i , F'_p and F'_d are the ionic, polar and dispersive forces, respectively, between the solute molecule and the stationary phase, F_i , F_p and F_d are the ionic, polar and dispersive forces, respectively, between the solute molecule and the mobile phase, and P'_i , P'_p , P'_d and P_i , P_p , P_d are the probabilities of the solute molecule interacting with the ionic, polar and dispersive moieties of the stationary and mobile phases, respectively.

If the special case of liquid–solid chromatography is considered where the adsorbent is silica gel and non-ionic mobile phases are employed, there will be no ionic forces effective in the separation, and the equation for K becomes

$$K = \frac{F'_p(P'_p) + F'_d(P'_d)}{F_p(P_p) + F_d(P_d)}$$

It is now necessary to rationally expand the individual functions in the above equation to contain variables that are pertinent to liquid chromatography. In so doing, the change in K that will result from a change in the magnitude of those variables should be quantitatively predictable. The probability of interaction of a solute with one of the phases will be some function of the absolute temperature and be proportional to the concentration of the interacting moieties in each of the respective phases.

Thus

$$K = \frac{F'_p f_1(T) c'_p + F'_d f_2(T) c'_d}{F_p f_3(T) c_p + F_d f_4(T) c_d}$$

where c'_p , c'_d and c_p , c_d are the concentrations of polar moieties and dispersive moieties in the stationary and liquid phases, respectively, and T is the absolute temperature.

If the hypothesis is made that the dispersive forces result from mass interaction, then c_d will be proportional to the density of the dispersing medium, which can be expressed as a concentration in terms of the mass per unit volume. Thus

$$c_d = Ad$$

where A is a constant and d is the density of the dispersing medium. It follows that

$$K = \frac{F_p'f_1(T)c_p' + F_d'f_2(T)c_d'}{F_p'f_3(T)c_p + F_d'f_4(T)Ad}$$

Now the corrected retention volume V' is equal to $K\Phi_s$, where Φ_s can be the volume of stationary phase or the total number of active moieties on the surface of a solid depending on the chromatographic system and the manner in which the concentration of solute in or on the stationary phase is defined.

Thus

$$V' = K\Phi_s = \left(\frac{F_p'f_1(T)c_p' + F_d'f_2(T)c_d'}{F_p'f_3(T)c_p + F_d'f_4(T)Ad} \right) \Phi_s \quad (1)$$

Eqn. 1 is a novel form of describing the corrected retention volume of a solute and has considerable practical significance. For example, the use of such an equation would allow the prediction of retention volumes and separation ratios for specific concentrations of a polar solvent in the mobile phase and for non-polar dispersing solvents of specific density providing suitable reference data were available. The validity of this equation can be tested in a relatively simple way using silica gel liquid-solid chromatography, but, before such data are presented, it is of interest to consider the nature of the retentive forces associated with silica gel. From the above argument these forces can be polar or dispersive in nature or a combination of both.

Scott and Kucera³ have shown that the dispersive forces on silica gel, if present at all, do not have a significant effect on solute retention (this will be particularly true when polar solvents are employed, as the polar forces will be vastly greater than the relative effect of any dispersive forces that may be present). Thus eqn. 1 can be simplified. It follows that for separations on silica gel using a polar or semi-polar mobile phase the dispersive factor for the stationary phase can be eliminated. Thus

$$V' = \left(\frac{F_p'f_1(T)c_p'}{F_p'f_3(T)c_p + F_d'f_4(T)Ad} \right) \Phi_s$$

which for practical convenience can be put in the inverse form

$$\frac{1}{V'} = \frac{1}{\Phi_s} \frac{F_p'f_3(T)c_p + F_d'f_4(T)Ad}{F_p'f_1(T)c_p'} \quad (2)$$

The validity of eqn. 2 can be established as follows:

(1) Employing different concentrations of a polar or semi-polar solvent in a dispersion medium such as *n*-heptane, then—providing the minimum concentration of polar solvent is kept above 3% (w/v)^{4,5}—the activity of the silica gel will be constant and eqn. 2 reduces to

$$\frac{1}{V'} = A + Bc_p$$

Further, if the same solute is chromatographed under constant conditions but using a range of values for c_p for each of a number of solvents, the linearity constant (B) can be related for each solvent to the physical properties of the polar solvent employed. In a like manner, if the same solvent is used and a series of different solutes are chromatographed for a series of different c_p values, the values obtained for the linearity constant B for each of the solutes can be correlated with the physical properties of the solutes. Combining the correlated linearity constants for both solutes and solvents, the form of the constant F_p can be determined.

(2) Employing a constant concentration of a polar solvent in a series of different dispersion solvents and again maintaining the concentration of polar solvent above 3% (w/v), so that the activity of the silica gel will remain constant, the polar interactions in the mobile phase will be constant and thus eqn. 2 reduces to

$$\frac{1}{V'} = A + Bd$$

where d is the density of the dispersing solvent.

In this paper these two sets of experiments will confirm the validity of the form of eqn. 2 and determine the nature of the constant F_p . However, the determination of the constant F_d must await the results of further work.

EXPERIMENTAL

The apparatus used consisted of a Waters Model 6000 pump with the mobile phase supplied from a glass reservoir which was fitted with a drying tube. The injection systems used were either a Valco high-pressure sample valve or a Varian injection system, designed to operate at high pressures, for use with a hypodermic syringe. Several columns were employed, all packed with Partisil 10 using the slurry packing technique⁶. For solutes of high retention, a 10-cm \times 4.6-mm-I.D. column was used and for those solutes of medium and short retention a 25-cm \times 4.6-mm-I.D. column was employed. The column was thermostated in a water jacket at $25^\circ \pm 0.5^\circ$. The detector employed was either the LDC UV monitor operated at 254 nm or the LDC refractometer detector, the choice depending on the extinction coefficient or refractive index of the solute being detected. The outlet from the detector was connected directly to a 25-ml or 50-ml grade A burette, thus permitting the absolute measurement of retention volume. The flow-rates employed ranged between 1 and 3 ml/min, the high flow-rate being used to measure retention volumes of solutes that were well retained to economize on time. The volume of sample employed ranged between 2 and 10 μ l and the concentration of solutes was adjusted to suit the sensitivity of the detecting system employed for the particular solutes being chromatographed. All solvents were carefully dried by passing over activated silica gel and the various solvent mixtures were made up on a weight volume basis by weighing a known amount of solvent into a 500-ml standard flask and making up to the mark with the dispersive solvent, which was usually heptane. Prior to any measurements being taken, 30–35 column dead volumes of solvent were passed through the system to ensure equilibrium. All retention volume measurements were made in duplicate and if the duplicates agreed to within $\pm 0.5\%$, the average of these duplicates was taken. If agreement was outside

TABLE I

RETENTION DATA FOR DIFFERENT SOLUTES CHROMATOGRAPHED IN MOBILE PHASES OF DIFFERENT COMPOSITION

Solute	Solvent	Solvent composition (% w/v)	V' (ml)	1/V' (ml ⁻¹)	Index of determination
Benzyl alcohol	Tetrahydrofuran in <i>n</i> -heptane	8.8	23.90	0.041	1.000
		13.3	12.90	0.077	
		17.7	8.50	0.117	
		22.2	6.40	0.156	
		26.6	5.10	0.196	
3-Phenyl-1-propanol	Tetrahydrofuran in <i>n</i> -heptane	8.8	38.10	0.026	0.998
		13.3	20.30	0.049	
		17.7	12.90	0.077	
		22.2	9.60	0.104	
		26.6	7.40	0.135	
Desoxycorticosterone alcohol (C ₂₁ H ₃₀ O ₃)	Isopropanol in <i>n</i> -heptane	15.7	25.21	0.039	0.998
		23.5	13.15	0.076	
		31.4	9.13	0.109	
		39.2	7.14	0.140	
		47.1	5.48	0.182	
		54.9	4.80	0.208	
		62.8	3.95	0.253	
70.6	3.63	0.275			

the 0.5%, a third replicate was repeated and that pair that agreed within 0.5% was averaged and this average value was taken as the retention volume.

Proof of the relationship $1/V' = A + Bc_p$ (where c_p is the concentration of the polar solvent in the dispersive medium)

Two polar solvents were chosen, tetrahydrofuran and isopropanol; the solutes employed were benzyl alcohol, 3-phenyl-1-propanol and desoxycorticosterone alcohol.

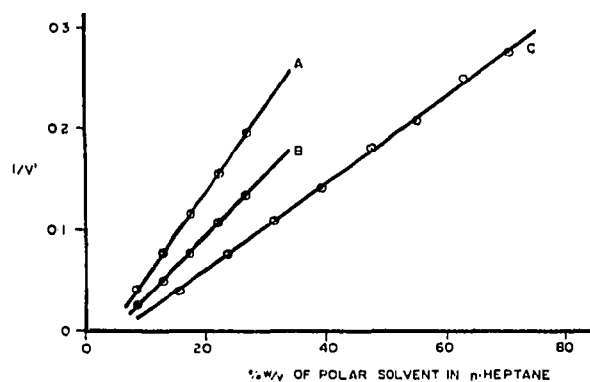


Fig. 1. Graphs relating the reciprocal of the corrected retention volume to the % (w/v) of polar solvent in *n*-heptane for different solutes. Column, 25 cm × 4.6 mm I.D., packed with Partisil 10. (A) Solute, benzyl alcohol; polar solvent, tetrahydrofuran. (B) Solute, 3-phenyl-1-propanol; polar solvent, tetrahydrofuran. (C) Solute, desoxycorticosterone alcohol; polar solvent, isopropanol.

The dispersive solvent employed was *n*-heptane and the solutes were chromatographed using mobile phases containing between 8 and 30% (w/v) and 16 and 76% (w/v) of tetrahydrofuran and isopropanol, respectively, in *n*-heptane. A range of concentrations was employed for each mobile phase for the three solutes. A summary of the results obtained is shown in Table I and the curves relating $1/V'$ to c_p are shown in Fig. 1.

RESULTS AND DISCUSSION

It is seen that the correlation of the function $1/V' = A + Bc_p$ is completely confirmed from the value of the indices of determination. It should be noted that the results obtained for the solute desoxycorticosterone alcohol chromatographed using the isopropanol-*n*-heptane mixture has shown complete correlation up to a mobile phase composition of 90% (v/v) of isopropanol in *n*-heptane.

*Examination of the relationship of the function $1/V' = A + Bc_p$ at low concentrations of polar solvent in *n*-heptane*

It has been previously shown^{4,5} that small concentrations of a polar solvent in a non-polar dispersive medium behave quite differently due to the silica gel being incompletely deactivated until the polar solvent is present at concentrations higher than 1 or 2% (w/v). It was, therefore, of interest to examine the change in the function given in eqn. 2 over that range of solvent concentrations where the silica gel was being deactivated.

A series of different solutes was chromatographed using several polar solvents in *n*-heptane, the concentrations ranging from about 0.2 to 7.0% (w/v). The solutes and solvents were as follows: phenyl methyl carbinol/isopropanol; phenanthrene/chloro-

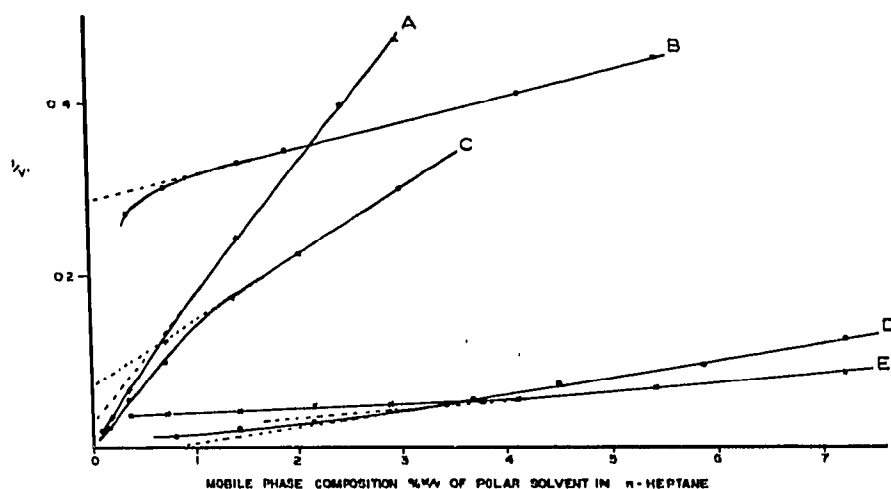


Fig. 2. Graphs relating the reciprocal of the corrected retention volume of different solutes to the % (w/v) composition of the mobile phase. Column, 10 cm \times 4.6 mm I.D., packed with Partisil 10. (A) Solute, phenyl methyl carbinol; polar solvent, isopropanol. (B) Solute, phenanthrene; polar solvent, chloroform. (C) Solute, benzyl acetate; polar solvent, ethyl acetate. (D) Solute, phenyl methyl carbinol; polar solvent, ethyl acetate. (E) Solute, nitrobenzene; polar solvent, chloroform.

roform; benzyl acetate/ethyl acetate; phenyl methyl carbinol/ethyl acetate; nitrobenzene/chloroform. The retention volume of each solute was measured at each concentration of the polar solvent in *n*-heptane. The results obtained are shown in Fig. 2.

It is seen that where the concentration of polar solvent is insufficient to deactivate the silica gel, the linear relationship between $1/V'$ and polar solvent concentration breaks down. It is also seen that for solutes that have relatively low retention,

TABLE II

RETENTION DATA FOR THE SOLUTE PHENYL METHYL CARBINOL CHROMATOGRAPHED IN MOBILE PHASES OF DIFFERENT COMPOSITION

Solvent	Solvent composition (% w/v)	V' (ml)	$1/V'$ (ml ⁻¹)	Index of determination	Linearity constants*	
					A	B
Isopropanol in <i>n</i> -heptane	3.14	4.55	0.219	0.997	0.0506	0.0549
	4.71	3.23	0.309			
	6.28	2.48	0.403			
	7.86	2.10	0.476			
Butanol in <i>n</i> -heptane	3.24	5.94	0.168	1.000	0.0173	0.0463
	4.86	4.14	0.241			
	6.48	3.17	0.315			
	8.10	2.54	0.393			
Pentanol in <i>n</i> -heptane	3.26	8.36	0.119	0.998	0.0077	0.0383
	4.89	5.62	0.177			
	6.52	4.20	0.238			
	8.15	3.25	0.307			
Dioxane in <i>n</i> -heptane	6.20	20.29	0.049	0.997	-0.0236	0.0116
	8.27	14.37	0.069			
	10.34	10.28	0.097			
	12.40	8.35	0.119			
Tetrahydrofuran in <i>n</i> -heptane	7.11	18.36	0.054	0.998	-0.0247	0.0111
	8.89	13.41	0.074			
	10.67	10.85	0.092			
	12.45	8.73	0.114			
Methyl acetate in <i>n</i> -heptane	7.47	21.57	0.046	1.000	-0.0270	0.0099
	9.34	15.40	0.064			
	11.21	11.92	0.083			
	13.08	9.85	0.101			
Ethyl acetate in <i>n</i> -heptane	7.21	29.16	0.034	0.998	-0.0153	0.0068
	9.01	22.05	0.045			
	10.81	16.84	0.059			
	12.61	14.15	0.070			
Propyl acetate in <i>n</i> -heptane	5.33	41.89	0.023	0.999	-0.0076	0.0059
	7.11	29.20	0.034			
	8.88	22.75	0.044			
	10.66	18.02	0.055			
Butyl acetate in <i>n</i> -heptane	3.53	58.37	0.017	0.999	-0.0039	0.0059
	5.29	36.93	0.027			
	7.05	26.85	0.037			
	8.81	20.69	0.048			

* Linearity constants of the function $1/V' = A + Bc_p$.

that is where the forces in both phases are of the same order, activation of the adsorbent, as the concentration of polar solvent is reduced, produces a rapid increase in retention volume and this increase occurs between 0.8 and about 1.2% (w/v) of polar solvent in *n*-heptane. It is seen that with the mildly polar solvent chloroform, deactivation of the adsorbent for less retained solutes is complete at about 1.3% (w/v) chloroform in *n*-heptane. This is contrary to the suggestion by Snyder⁷, who assumed that for mildly polar solvents a high concentration was necessary for complete deactivation of adsorbents.

However, if the solutes are well retained, that is, where the forces between the solutes and the adsorbent are significantly greater than the forces between the solutes and the solvent, activation of the adsorbent at low concentrations of polar solvent has a relatively small effect on the solute retention. It is seen, however, that the curve now deviates from linearity at between 3 and 4% (w/v) of the polar solvent in *n*-heptane and the rate of increase in retention as the solvent concentration is reduced becomes slightly smaller. Therefore, it can be generally stated that for solutes that are eluted at *k'* values up to about 5, activation of the adsorbent by reduction of the polarity of the solvent will cause a rapid increase in retention. Conversely, solutes that are already well retained experience little change in retention as the adsorbent becomes activated and this is true for both low- and high-polarity mobile phases.

Examination of the function $1/V' = A + Bc_p$ for different polar solvents using a single solute

Using the standard 25-cm × 4.6-mm-I.D. column packed with Partisil 10, the corrected retention volumes were determined for the solute phenyl methyl carbinol in mobile phases containing a range of concentrations of the following solvents in *n*-heptane: isopropanol, *n*-butanol, *n*-pentanol, dioxane, tetrahydrofuran, methyl acetate, ethyl acetate, butyl acetate.

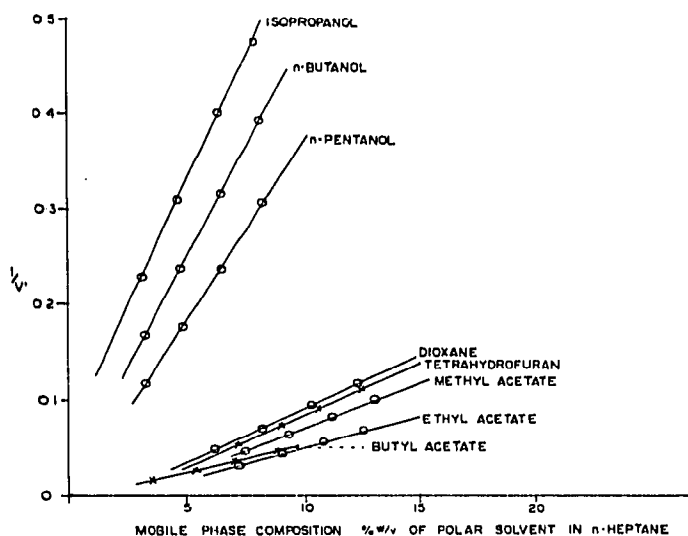


Fig. 3. Graphs relating the reciprocal of the corrected retention volume of phenyl methyl carbinol to the composition of the mobile phase containing different polar solvents in *n*-heptane. Column, 25 cm × 4.6 mm I.D., packed with Partisil 10.

TABLE III

DATA FOR THE CORRELATION OF $\log_{10}d(1/V')/dc_p$ AGAINST SOLVENT POLARIZABILITY PER MILLILITER FOR PHENYL METHYL CARBINOL

Index of determination = 0.965. Linearity constants of the function $\log_{10}d(1/V')/dc_p = A + B \frac{(\epsilon - 1)}{(\epsilon + 2)}$
 $A = -4.43$, $B = 3.65$.

Solvent	$d(1/V')/dc_p$	$\log_{10}d(1/V')/dc_p$	Dielectric constant [ϵ]	Polarizability/ml $(\epsilon - 1)/(\epsilon + 2)$
Isopropanol in <i>n</i> -heptane	0.0549	-1.260	19.92	0.863
Butanol in <i>n</i> -heptane	0.0463	-1.334	17.51	0.846
Pentanol in <i>n</i> -heptane	0.0383	-1.417	13.90	0.811
Dioxane in <i>n</i> -heptane	0.0116	-1.936	8.67*	0.719
Tetrahydrofuran in <i>n</i> -heptane	0.0111	-1.955	7.58	0.687
Methyl acetate in <i>n</i> -heptane	0.0099	-2.004	6.68	0.654
Ethyl acetate in <i>n</i> -heptane	0.0068	-2.168	6.02	0.626
Propyl acetate in <i>n</i> -heptane	0.0059	-2.229	6.002	0.625
Butyl acetate in <i>n</i> -heptane	0.0059	-2.229	5.01	0.572

* The dielectric constant for dioxane was taken as twice the dielectric constant for ether.

acetate, ethyl acetate, propyl acetate, and butyl acetate. Under these conditions, as the same solute is employed, changes in retention between different solvents will reflect the contribution of the solvent to solute-solvent interaction.

The results obtained are shown in Table II and the curves relating $1/V'$ to the mobile phase composition as % (w/v) of polar solvent in *n*-heptane are shown in Fig. 3. It is seen that excellent correlation is obtained for all solvents and this is confirmed by the indices of determination which are shown in Table II. It is also seen from Fig. 3 that the interactions of the solute with the mobile phase given by the slopes of these curves appear to be related to the so-called polarity of the polar solvent employed. An attempt was made to correlate the slopes given in Table II to the different physical parameters of the polar solvent. In Table III the polarizability per milliliter taken from the dielectric constant values⁸, using the simple formula $(\epsilon - 1)/(\epsilon + 2)$, is given, together with the slopes and the logarithm of the slopes for the curves shown in Fig. 3.

The logarithm of the slopes and the solvent polarizability values were curve fitted to the linear function $y = A + Bx$ and the results obtained from this correlation are also given in Table III. The curves relating the logarithm of the slopes against the solvent polarizability are shown in Fig. 4.

It is seen that good correlation is obtained but it should be pointed out that the polarizability per milliliter for dioxane was not taken directly from the dielectric constant. The two dipoles in dioxane are in opposition and, therefore, cancel and pro-

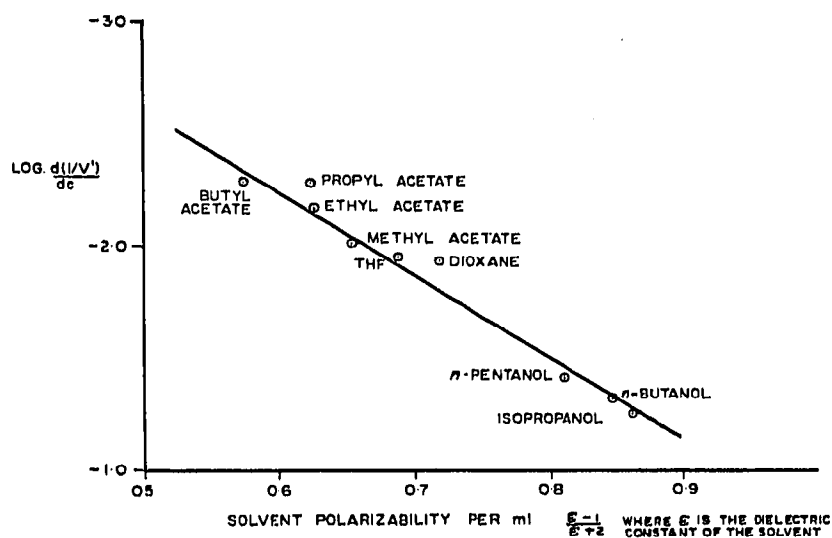


Fig. 4. Graph of $\log d(1/V')/dc$ against solvent polarizability per unit volume for phenyl methyl carbinol eluted from silica gel employing different solvents.

vide dielectric constants that do not reflect the polarity of the solvent with respect to solute-solvent interaction. This is another example of the disadvantage of using bulk properties of the system to describe molecular effects. The value for the dielectric constant of dioxane was, therefore, taken as twice that of diethyl ether. It is seen that using this value places the point for dioxane close to the regression line through the other polarizability values for the other solvents. It follows that the contribution to retention from the solvent-solute interactions would appear to be proportional to the exponent of the polarizability per milliliter and thus can be put in the following form

$$\text{Solvent interaction} = Ae^{ap} = Ae^{\alpha\left(\frac{\epsilon-1}{\epsilon+2}\right)}$$

where p is the polarizability per unit volume of the solvent, ϵ is the dielectric constant of the solvent determined at 25°, and A and α are constants.

*Examination of the function $1/V' = A + Bc_p$ for different solutes using the same polar solvent over a range of concentrations in *n*-heptane*

Using a standard 25-cm \times 4.6-mm-I.D. column packed with Partisil 10, the corrected retention volumes were determined for the solutes shown in Table IV using mobile phases containing concentrations of ethyl acetate in *n*-heptane ranging from 7.2 to 12.6% (w/v). Under the conditions where the same polar solvent is employed, the relationship between the corrected retention volume and the concentration of polar solvent will be a function of the interactive force between ethyl acetate and the different solutes. The results obtained are shown in Table IV and the curves relating $1/V'$ to the mobile phase composition are shown in Fig. 5. It is seen that again excellent correlation is obtained for all solutes and this is confirmed by the indices of determina-

TABLE IV
RETENTION DATA FOR DIFFERENT SOLUTES CHROMATOGRAPHED IN ETHYL ACETATE-*n*-HEPTANE MOBILE PHASES OF DIFFERENT COMPOSITION

Solute	Solvent composition (% w/v)	V' (ml)	$1/V'$ (ml ⁻¹)	Index of determination	Linearity constants*	
					A	B
Benzyl acetate	7.2	3.96	0.252	0.982	0.0571	0.02815
	9.0	3.09	0.323			
	10.8	2.79	0.358			
	12.6	2.44	0.409			
Methyl acetate	7.2	4.39	0.227	0.998	0.0886	0.01945
	9.0	3.78	0.264			
	10.8	3.31	0.302			
	12.6	3.01	0.332			
Tetrahydrofuran	7.2	7.26	0.137	0.998	0.0485	0.01243
	9.0	6.22	0.160			
	10.8	5.41	0.184			
	12.6	4.89	0.204			
<i>n</i> -Octanol	7.2	20.81	0.048	1.000	-0.0170	0.00900
	9.0	15.60	0.064			
	10.8	12.46	0.080			
	12.6	10.33	0.096			
α -Phenethyl alcohol	7.2	22.77	0.043	0.995	-0.0185	0.00877
	9.0	16.07	0.062			
	10.8	13.37	0.074			
	12.6	10.83	0.092			
Benzyl alcohol	7.2	31.64	0.031	0.995	-0.0133	0.00630
	9.0	22.37	0.044			
	10.8	18.63	0.053			
	12.6	15.06	0.066			
<i>n</i> -Pentanol	7.2	25.39	0.039	1.000	-0.0108	0.00696
	9.0	19.26	0.051			
	10.8	15.54	0.064			
	12.6	17.69	0.077			
<i>n</i> -Butanol	7.2	28.27	0.035	1.000	-0.0066	0.00588
	9.0	21.49	0.046			
	10.8	17.69	0.056			
	12.6	14.75	0.067			
Acetophenone	7.2	5.40	0.185	0.992	0.0410	0.02050
	9.0	4.31	0.232			
	10.8	3.83	0.261			
	12.6	3.35	0.298			
Methyl ethyl ketone	7.2	7.45	0.134	0.999	0.0238	0.01543
	9.0	6.09	0.164			
	10.8	5.24	0.190			
	12.6	4.59	0.217			
Acetone	7.2	14.40	0.069	1.000	0.0120	0.00799
	9.0	11.83	0.084			
	10.8	10.20	0.098			
	12.6	8.86	0.112			

* Linearity constants of the function $1/V' = A + Bc_p$.

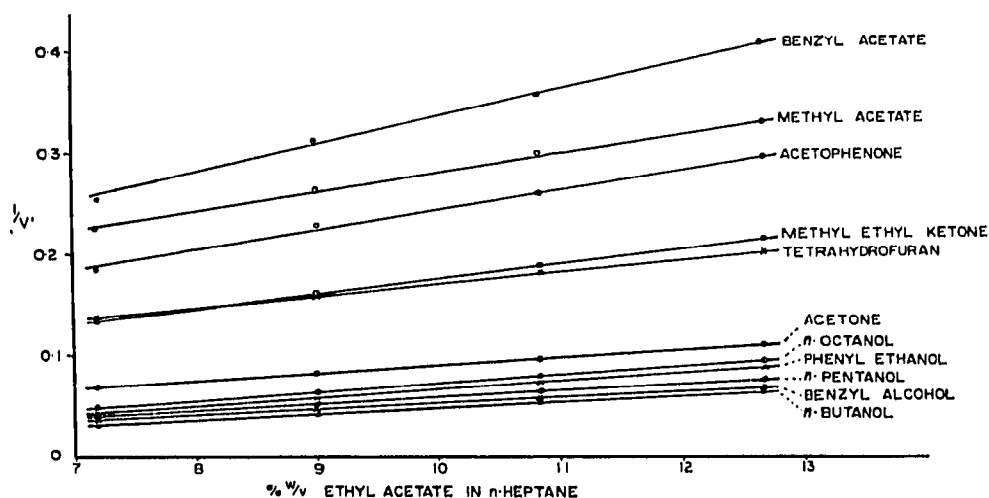


Fig. 5. Graphs relating the reciprocal of the corrected retention volume to the % (w/v) of ethyl acetate in *n*-heptane for different solutes.

tion which are shown in Table IV. In a similar manner to the results from experiments using different polar solvents, the different interactions of the solutes with ethyl acetate appear to be related to the polarity of the solute.

In Table V the polarizability per milliliter, again calculated from the dielectric constant for each solute⁸, is given together with the slopes and the logarithm of the slopes for the curves shown in Fig. 5. The logarithm of the slopes and the corresponding solute polarizability values were curve fitted to the linear function $y = A + Bx$

TABLE V

DATA FOR CORRELATION OF $\log_{10}d(1/V')/dc_p$ AGAINST SOLUTE POLARIZABILITY PER MILLILITER FOR THE SOLVENT SYSTEM ETHYL ACETATE-*n*-HEPTANE

Index of determination = 0.968. Linearity constants of the function $\log_{10}d(1/V')/dc_p = A + B \frac{(\epsilon - 1)}{(\epsilon + 2)}$

$A = -0.052$, $B = -2.61$. The correlation excluded the values for acetophenone, methyl ethyl ketone, and acetone.

Solute	$d(1/V')/dc_p$	$\log_{10}d(1/V')/dc_p$	Dielectric constant [ϵ]	Polarizability/ml $(\epsilon - 1)/(\epsilon + 2)$
Benzyl acetate	0.0281	-1.550	5.10	0.577
Methyl acetate	0.0194	-1.711	6.68	0.654
Tetrahydrofuran	0.0124	-1.906	7.58	0.687
<i>n</i> -Octanol	0.0090	-2.046	10.34	0.757
α -Phenethyl alcohol	0.0087	-2.057	12.00	0.786
Benzyl alcohol	0.0063	-2.201	13.10	0.801
<i>n</i> -Pentanol	0.0069	-2.157	13.90	0.811
<i>n</i> -Butanol	0.0058	-2.231	17.51	0.846
Acetophenone	0.0205	-1.688	17.39	0.845
Methyl ethyl ketone	0.0154	-1.812	18.51	0.854
Acetone	0.0079	-2.097	20.70	0.868

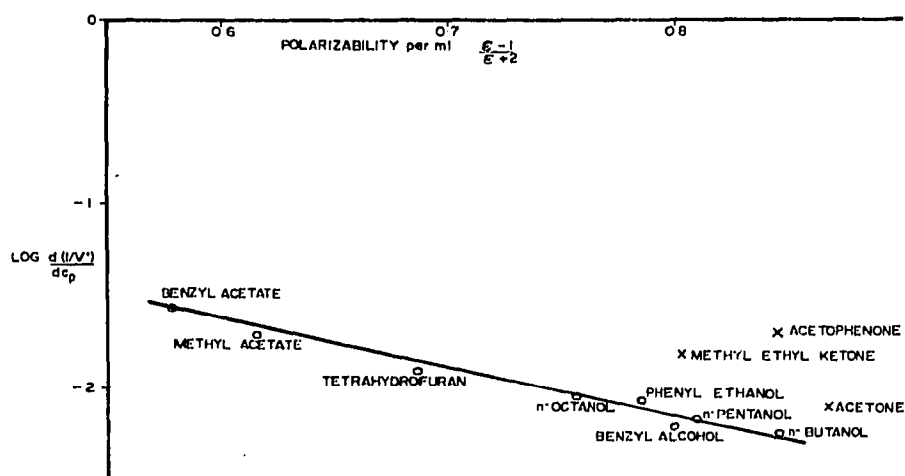


Fig. 6. Graph of $\log d(1/V')/dc_p$ against solute polarizability per unit volume.

and the results obtained from this correlation are given in Table V and Fig. 6. It is seen that good correlation is obtained for the solutes containing ester, ether and alcohol groups, but the solutes with ketonic moieties fall a significant distance from the regression curve for the other solutes. Thus, solute interaction will also be described for the expression $Be^{\beta p}$, where p is the polarizability per unit volume of the solute and B and β are constants. The cause of the anomalous behavior of the ketones is uncertain. It is likely, however, that the ketones are liable to enolize on the acidic surface of the silica gel, which could be conditioned by the concentration of ethyl acetate in the mobile phase. From the curve it is seen that the chromatographic behavior of the ketones appears to lie between that of the ethers and the alcohols. This would be expected, but the polarizability per milliliter, which is derived from the dielectric constant, indicated that they should behave more like alcohols. Therefore, an alternative explanation for the anomalous behavior of the ketone solutes would be that in the measurement of the dielectric constant and as a result of the applied electric field, the values obtained are reflecting the enolic properties of the ketone as opposed to the ketonic properties.

Examination of the function $1/V' = A + Bc_p$ for different solutes using the same polar solvent ethyl acetate mixed with different dispersing media as the mobile phase

Using the standard 25-cm \times 4.6-mm-I.D. column packed with Partisil 10, the corrected retention volumes were determined employing a 4.51% (w/v) of ethyl acetate in different hydrocarbons for the following solutes: 2-ethyl-anthraquinone, β -methyl-anthraquinone, anthraquinone, phenyl methyl carbinol, benzyl alcohol, and 3-phenyl-1-propanol. Under these conditions, where both the solutes and the polar solvent are the same and the polar solvent is maintained at the same weight volume concentration in the mobile phase, the relationship between $1/V'$ and d , the density of the dispersing solute, indicates the degree and nature of the dispersive interactions between the solute and the diluting solvent. The results obtained are shown in Table VI and the curve relating $1/V'$ to the density of the dispersing solvent is shown in

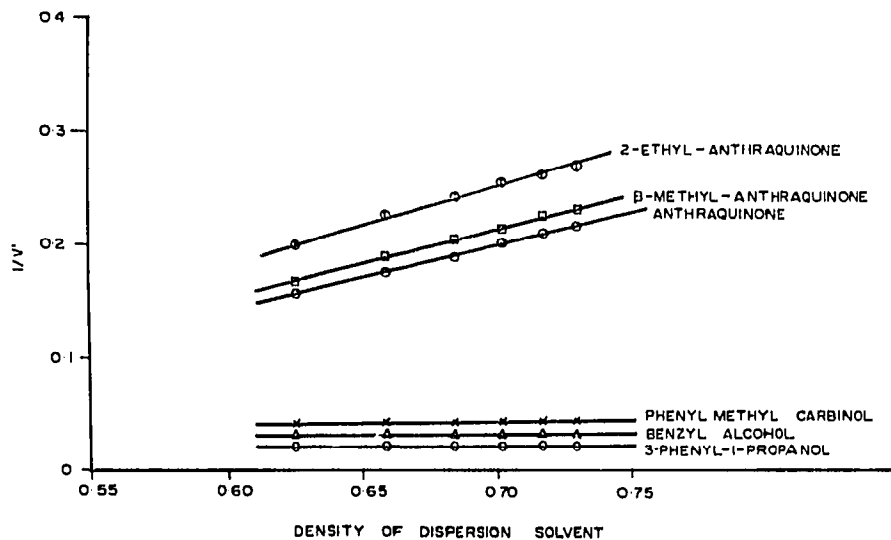


Fig. 7. Graphs of the reciprocal of the corrected retention volume against the density of the dispersion solvent. Column, 25 cm \times 4.6 mm, packed with Partisil 10. Mobile phases, different *n*-hydrocarbons containing 4.51% (w/v) [5.0% (v/v)] ethyl acetate.

Fig. 7. It is seen that excellent correlation is obtained between $1/V'$ and the dispersing solvent density which is confirmed by the indices of determination seen in Table VI for the anthraquinone solutes. The low value for the indices of determination for the more polar solutes is due to the fact that the change in retention volume with density of dispersing solvent is almost negligible and is, in fact within repeatability of the measurements. It is seen, however, from Fig. 7 that a straight line is produced, which, however, is horizontal to the density axis, indicating no significant dispersive interaction with the different hydrocarbon solvents. It is interesting to note that solutes of the anthraquinone type are often used to demonstrate the dispersive selectivity of the so-called non-polar bonded phases presently available. It has been generally accepted that the absolute or relative dispersive interactions in or between the two phases are considered weaker than polar interactions and, therefore, for them to significantly affect the retention characteristics of a solute the polar interaction must be small and at least of the same order as the net dispersive interactions. This could be an example, as mentioned earlier, where the forces on the solute both in the stationary phase and in the mobile phase are relatively weak and, therefore, changes in the dispersive interactions can have a profound effect on solute retention. The more polar solutes will exhibit little or no dispersive interaction, as they will be strongly held by polar forces in both phases and, therefore, their retention will change little with the density of the dispersive medium. Where the polar forces are weak and dispersive forces can be exploited, changes in density of the dispersing media can result in profound changes in the elution order of a series of solutes. An example of this is given in Fig. 8. Changing the dispersing solvent from *n*-pentane to *n*-decane can rearrange the order of eluted peaks. The first peak in each of the chromatograms in Fig. 8 is tocopherol acetate, the second benzyl acetate, and the third anthraquinone,

TABLE VI
RETENTION DATA FOR A RANGE OF SOLUTES CHROMATOGRAPHED IN DIFFERENT DISPERSION SOLVENTS

<i>Solute</i>	<i>Dispersion solvent</i>	<i>Density of dispersion solvent</i>	<i>V'</i> (ml)	<i>1/V'</i> (ml ⁻¹)	<i>Index of determination</i>
Anthraquinone	<i>n</i> -Pentane	0.626	6.41	0.156	0.994
	<i>n</i> -Hexane	0.659	5.59	0.179	
	<i>n</i> -Heptane	0.683	5.19	0.193	
	<i>n</i> -Octane	0.702	4.93	0.203	
	<i>n</i> -Nonane	0.717	4.67	0.214	
	<i>n</i> -Decane	0.730	4.61	0.217	
β -Methyl-anthraquinone	<i>n</i> -Pentane	0.626	5.91	0.169	0.996
	<i>n</i> -Hexane	0.659	5.22	0.192	
	<i>n</i> -Heptane	0.683	4.80	0.208	
	<i>n</i> -Octane	0.702	4.61	0.217	
	<i>n</i> -Nonane	0.717	4.36	0.229	
	<i>n</i> -Decane	0.730	4.27	0.234	
2-Ethyl-anthraquinone	<i>n</i> -Pentane	0.626	4.97	0.201	0.993
	<i>n</i> -Hexane	0.659	4.37	0.229	
	<i>n</i> -Heptane	0.683	4.08	0.245	
	<i>n</i> -Octane	0.702	3.87	0.258	
	<i>n</i> -Nonane	0.717	3.78	0.265	
	<i>n</i> -Decane	0.730	3.65	0.274	
Phenyl methyl carbinol	<i>n</i> -Pentane	0.626	24.07	0.0415	0.382
	<i>n</i> -Hexane	0.659	23.40	0.0427	
	<i>n</i> -Heptane	0.683	23.49	0.0426	
	<i>n</i> -Octane	0.702	23.39	0.0428	
	<i>n</i> -Nonane	0.717	23.37	0.0428	
	<i>n</i> -Decane	0.730	23.65	0.0423	
Benzyl alcohol	<i>n</i> -Pentane	0.626	32.70	0.0306	0.261
	<i>n</i> -Hexane	0.659	31.55	0.0317	
	<i>n</i> -Heptane	0.683	31.61	0.0316	
	<i>n</i> -Octane	0.702	30.81	0.0325	
	<i>n</i> -Nonane	0.717	32.64	0.0306	
	<i>n</i> -Decane	0.730	30.59	0.0327	
3-Phenyl-1-propanol	<i>n</i> -Pentane	0.626	49.60	0.0202	0.750
	<i>n</i> -Hexane	0.659	47.95	0.0209	
	<i>n</i> -Heptane	0.683	48.37	0.0207	
	<i>n</i> -Octane	0.702	47.27	0.0212	
	<i>n</i> -Nonane	0.717	47.43	0.0211	
	<i>n</i> -Decane	0.730	47.45	0.0211	

and the small peaks are impurities. It is seen that the significant change in resolution and order of elution results almost exclusively from the movement of the anthraquinone (the third major peak) but the effect on resolution is quite dramatic.

More work is necessary to determine the nature of the constant F_d but at this stage it is clearly indicated that the use of dispersive media of different density can be significantly effective, particularly where the polar interactions of the solute and the two phases are relatively weak and the dispersive forces, although also weak, can still contribute significantly to solute retention. The results presented show a correlation

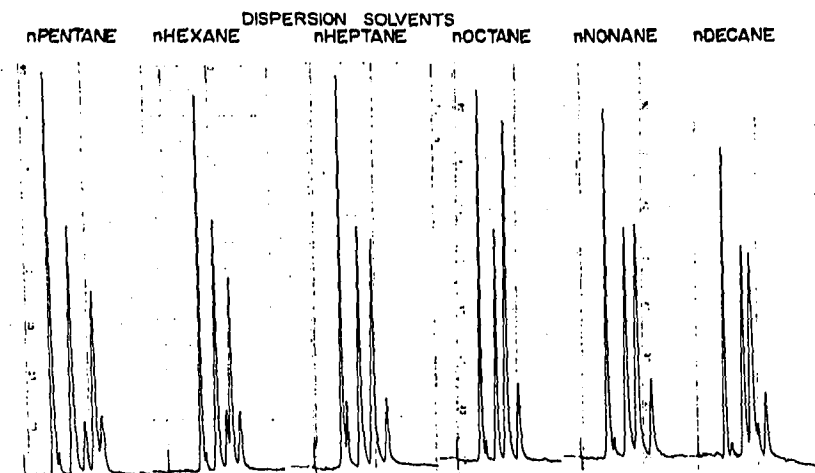


Fig. 8. Chromatograms showing the effect of changes of dispersion solvent when using a mobile phase containing 4.5% (w/v) of ethyl acetate.

between $1/V'$ and d , the density of the dispersive solvent. However, it should be noted that for a homologous series of hydrocarbons the densities of the solvents correlate with the solvent molecular weight, the solvent carbon number and, to a less extent, with the polarizability per milliliter of the solvent. Therefore, at this stage the precise property of the dispersing solvent that correlates with the dispersion interactions should be left open to question. However, from a practical point of view for a homologous series of hydrocarbons, density can be taken as the effective parameter controlling dispersive interactions until the time when other parameters are shown to be more appropriate.

CONCLUSION

The general form of the dynamic equation for the distribution coefficient has been substantiated but more work is necessary to determine the precise nature of the individual functions that describe the different interactions. The role played by the concentration of the polar solvent in determining the probability of interaction in the mobile phase is completely confirmed. From a practical point of view, retention values for solutes at two concentrations of a polar solvent in the mobile phase will permit the prediction of retention data at any other concentration of that polar solvent providing the concentration remains above that level at which the silica gel is deactivated (ca. 2% w/v). There is also a strong indication that under the defined conditions given for the equation the solute and solvent polar interactions are a function of the polarizability per milliliter of the respective solute and solvent.

If the polarizability per milliliter of solute, the polar solvent and the interactive moieties of the silica gel (probably simple hydroxyl groups³) are p_1 , p_2 , and p_3 , respectively, then polar interactions in the mobile phase have been shown to be exponential functions of p_1 and p_2 . At this stage it can be reasonably assumed that the polar interactions with the silica gel will also be an exponential function of p_3 .

A possible expansion of eqn. 2 in the following form can be suggested for isothermal conditions

$$\frac{1}{V'} = \frac{1}{\phi_s} = \left(\frac{Ae^{f_1(p_1, p_2)} c_p + Bd}{Ce^{f_2(p_1, p_2, p_3)}} \right)$$

where A , B and C are constants. Further, from the results given in the paper it is likely that

$$f_1(p_1, p_2) = \alpha p_1 + \beta p_2$$

where α and β are constants.

Where polar interactions are weak, dispersive interactions can be exploited to modify solute retention and under such circumstances it has been shown that the dispersive interactions are linearly related to the density of the dispersing solvent.

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