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### Effect of binary eluent composition on retention of solutes in high-performance liquid chromatography

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With two exceptions<sup>1,2</sup> there is no published work providing systematic studies in high-performance liquid chromatography (HPLC) of retention volumes as a function of composition of the eluent over the whole composition range for binary solvents. In virtually all previous publication on this theme<sup>3-15</sup> the authors have presented some form of theory and have included some experimental data with which the theory is compared. In every case some degree of success is reported. It appears important to have a general view that is more firmly based on experimentation and so, here, a study has been undertaken of a wide variety of HPLC systems over the whole composition range (volume fractions varying from 0 to 1) in order to determine what empirical (but experimentally based) relationship is required for describing binary solvent systems. The results of a substantive study are described below.

#### EXPERIMENTAL

The several systems used each included a Rheodyne injection valve connected to a column immersed in a thermostatically controlled water bath. Liquid was pumped either by an Altex 100A or 110A and UV-visible detection was either via a Pye Unicam LC-3 detector or a Cecil spectrophotometric detector. The flow-rate was monitored throughout all experiments by determining the time taken for the eluent to fill an accurately calibrated volume. All samples were the best available quality commercial compounds and solvents were all of AnalaR quality or the equivalent. Samples for injection were made up by dissolving the minimum amount of solute in prepared binary eluent mixture which was ultrasonically outgassed before use and was stored in the same thermostat as was used for the column. Multiple samples were injected to establish each experimental point. This was especially important for conditions where the elution volume was similar in magnitude to the void volume of the column which was determined by injecting an appropriate unretained solute, after a small sample of a hydrocarbon.

#### RESULTS

In Figs. 1-6, one example of each type of observed behaviour is shown as a plot of  $1/(\text{corrected retention volume})$  against volume fraction. In most of the pre-

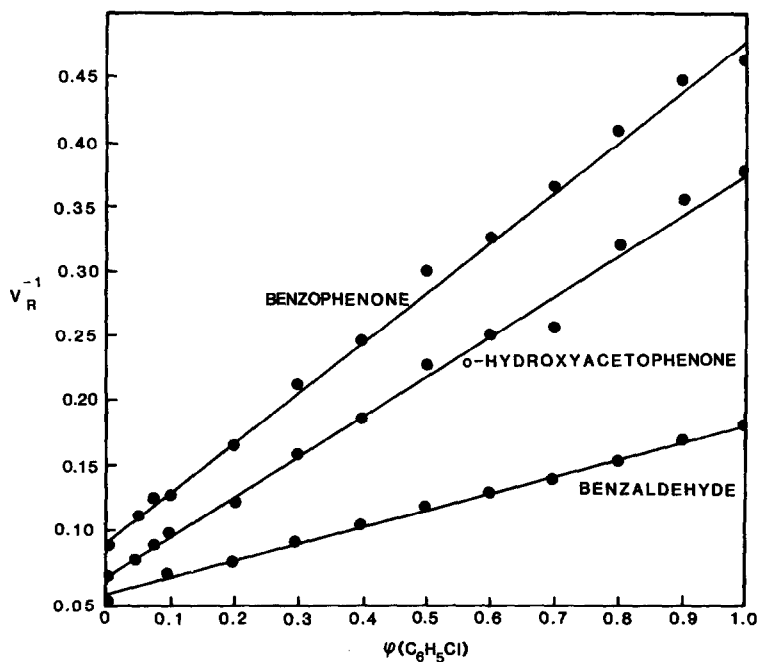


Fig. 1. Elution of benzophenone, *o*-hydroxyacetophenone or benzaldehyde from Hypersil by tetrachloromethane (A)-chlorobenzene (B) mixtures. Type I curves.  $V_R$  = Retention volume in  $cm^3$ .

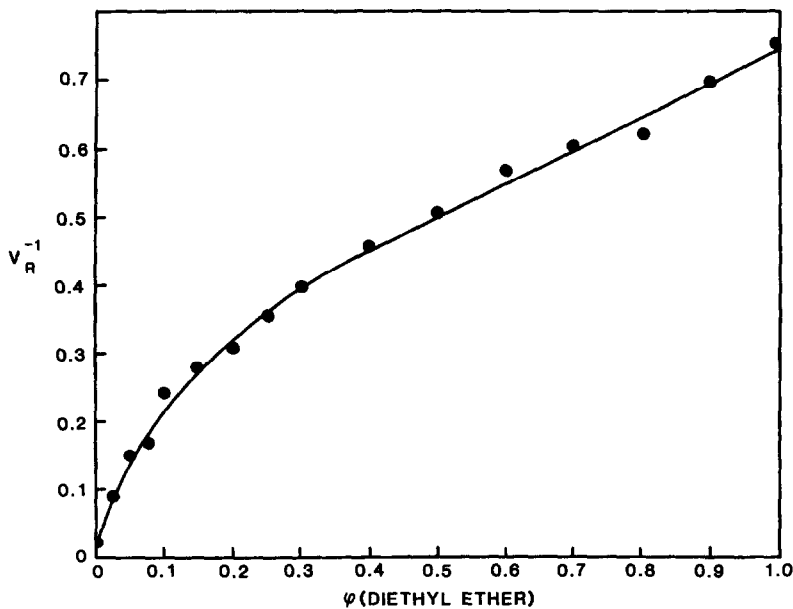


Fig. 2. Elution of acetone from Hypersil by light petroleum (b.p. 60-80°C) (A)-diethyl ether (B) mixtures. Type II curve.

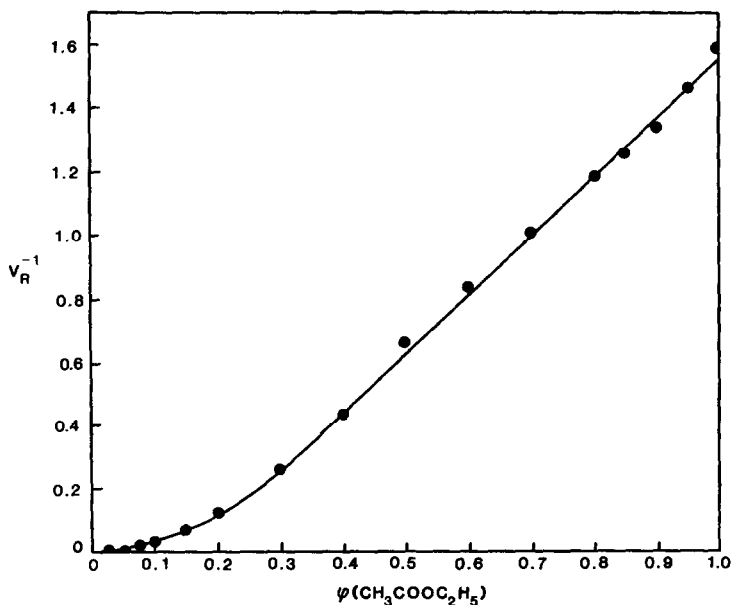


Fig. 3. Elution of tetrahydrofuran from Hypersil by *n*-heptane (A)-ethyl acetate (B) mixtures. Type III curve.

viously published work it has been the convention to represent the data graphically in the form of  $1/(\text{corrected retention volume})$  versus molar concentration or mole fraction and there are basic theoretical reasons why this procedure is not followed here. In all the results of this work, and in subsequent experiments, six differently shaped plots have been identified, but it is noteworthy that no systems yielding plots in which there is either a maximum or a minimum have yet been identified. Many systems showed a linear relationship of inverse retention with volume fraction and

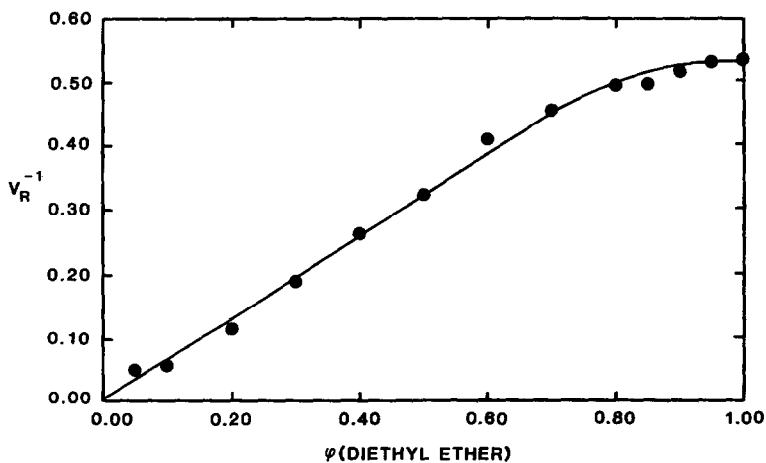


Fig. 4. Elution of 3-phenyl propan-1-ol from Hypersil by tetrachloromethane (A)-diethyl ether (B) mixtures. Type IV curve.

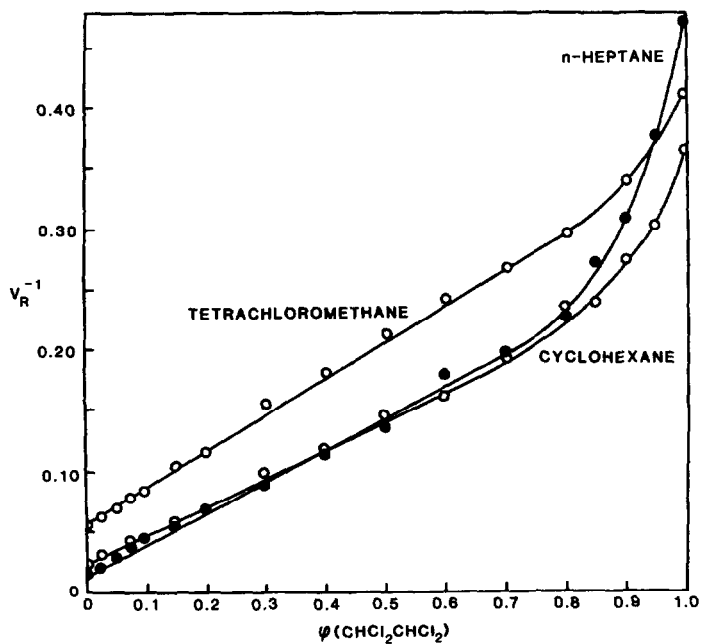


Fig. 5. Elution of benzaldehyde from Hypersil by *n*-heptane, tetrachloromethane or cyclohexane. (A) tetrachloroethane (B) mixtures. Type V curve.

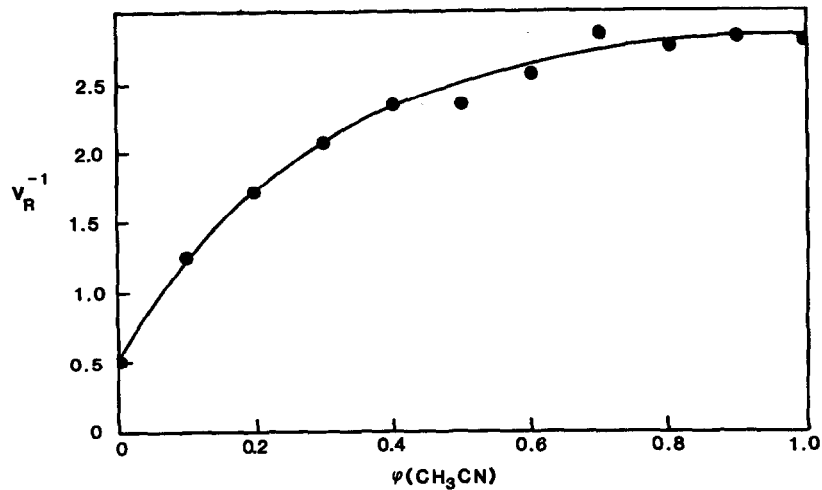


Fig. 6. Elution of 3-phenyl propan-1-ol from Hypersil by diethyl ether (A)-acetonitrile (B) mixtures. Type VI curve.

TABLE I  
SYSTEMS: TYPES I-VI

Type	Details		
I	Systems showing strictly linear dependence of $V_R^{-1}$ on either solvent component over whole range $\varphi = 0 \rightarrow 1$ .		
	(i) Column packing: Hypersil	(ii) Column packing: Hypersil ODS	
	Solvent pair	Solvent pair	Solutes
	D + F	7, 8, 9	A + B
	C + G	9	A + C
	D + H	10	B + C
			F + A
			F + B
			F + C
			C + E
			E + F
	<i>Solvent</i>	<i>Solutes</i>	
	<i>n</i> -Hexane (A)	Acetone (1)	
	<i>n</i> -Heptane (B)	Butanone (2)	
	Cyclohexane (C)	2-Methylbutanone (3)	
	Tetrachloromethane (D)	Pentan-2-one (4)	
	Toluene (E)	Pentan-3-one (5)	
	Chlorobenzene (F)	Benzyl alcohol (6)	
	Bromobenzene (G)	Benzaldehyde (7)	
	Diethyl ether (H)	Benzophenone (8)	
		<i>o</i> -Hydroxyacetophenone (9)	
		Phenol (10)	
II-VI	Systems exhibiting non-linearity of $V_R^{-1}$ plots.		
II	<i>Solute</i>	<i>Solvent pair</i>	<i>Adsorbent</i>
	Acetone	Light-petroleum (b.p. 60-80°C)-diethyl ether	Silica
	Nitromethane	Diethyl ether-tetrachloromethane	Silica
	Nitroethane	Diethyl ether-tetrachloromethane	Silica
	Nitrobenzene	Diisopropyl ether-light petroleum (b.p. 60-80°C)	Alumina
	Benzonitrile	Diisopropyl ether-light petroleum (b.p. 60-80°C)	Alumina
	2-Methyl-butanone	Diisopropyl ether-light petroleum (b.p. 60-80°C)	Alumina
III	Tetrahydrofuran	<i>n</i> -Heptane-ethyl acetate	Silica
	Benzophenone	Cyclohexane-bromobenzene	Silica
	Nitrobenzene	Cyclohexane-tetrachloroethane	Silica
IV	3-Phenyl propan-1-ol	Diethyl ether-tetrachloromethane	Silica
V	Benzaldehyde	Cyclohexane-tetrachloroethane	Silica
	Benzaldehyde	<i>n</i> -Heptane-tetrachloroethane	Silica
	Benzaldehyde	Tetrachloromethane-tetrachloroethane	Silica
	Isobutyl methyl ketone	<i>n</i> -Pentane-diethyl ether	Silica
VI	2-Phenyl-propan-1-ol	Diethyl ether-acetonitrile	Silica
	3-Phenyl-butan-1-ol	Diethyl ether-acetonitrile	Silica

we term such systems as of Type I with those yielding non-linear shapes designated Types II–VI. All are listed in Table I.

#### DISCUSSION

Despite the substantially different behaviour displayed by these HPLC systems, as exemplified above, the following equation has been found satisfactorily to describe the data derived for all the systems yet studied.

$$\frac{1}{V_{AB}} = \varphi_A \left\{ \frac{1}{V_A} + \frac{b\varphi_B}{1 + b'\varphi_B} \right\} + \frac{\varphi_B}{V_B} \quad (1)$$

$V_{AB}$  is the corrected elution volume for the solute (experimental elution volume – void volume) in the binary eluent A + B, with volume fractions  $\varphi_A$  and  $\varphi_B$ , respectively.  $V_A$  is the corrected elution volume for the solute with pure A as eluent and  $V_B$  is that with pure B.  $b$  and  $b'$  are constants and the term which includes them is reminiscent of the Langmuir adsorption isotherm.

Values of  $1/V_A$  and  $1/V_B$  were determined by the respective ( $\varphi_A = 1$  and  $\varphi_B = 1$ ) intercepts while  $b$  and  $b'$  were determined by the best fit to the experimental points. It is important to recognise that eqn. 1 can be written in terms of the alternative binary component:

$$\frac{1}{V_{AB}} = \varphi_B \left\{ \frac{1}{V_B} + \frac{a\varphi_A}{1 + a'\varphi_A} \right\} + \frac{\varphi_A}{V_A} \quad (2)$$

If this is done then the constants in eqn. 2 are related to those in eqn. 1 by:

$$a = b/(1 + b') \text{ and } a' = b'/(1 + b)$$

Thus comparison can be made if the data are plotted using eqn. 2 rather than eqn. 1.

It is of course perfectly possible to write eqn. 1 in the following form:

$$\frac{1}{V_{AB}} = \frac{\frac{1}{V_A} + \left\{ \frac{1}{V_B} - \frac{1}{V_A} + \frac{b'}{V_A} + b \right\} \varphi_B + \left\{ \frac{b'}{V_B} - \frac{b'}{V_A} - b \right\} \varphi_B^2}{(1 + b'\varphi_B)} \quad (3)$$

and for some calculations this form might be more convenient. However in another sense it is less revealing since we have shown<sup>16</sup> that in models which involve Langmuir behaviour with ideal behaviour in the solvent (eluent), or alternatively with a surface of constant properties and with diachoric behaviour in solution, the form of the derivable equation in either case, is

$$\frac{1}{V_{AB}} = \frac{\varphi_A}{V_A} + \frac{\varphi_B}{V_B} \quad (4)$$

TABLE II  
VALUES OF COEFFICIENTS IN EQUATION

$$\frac{1}{V_{AB}} = \varphi_A \left\{ \frac{1}{V_A} + \frac{b\varphi_B}{1 + b'\varphi_B} \right\} + \frac{\varphi_B}{V_B}$$

TCE = 1,1,2,2-tetrachloroethane. All elutions from Hypersil at 24.5°C.

Solute	Solvents		$V_A^{-1}$	$V_B^{-1}$	$b$	$b'$
	A	B				
Benzaldehyde	<i>n</i> -Heptane	TCE	0.011	0.473	- 0.235	-0.92
Benzaldehyde	Cyclohexane	TCE	0.021	0.364	- 0.110	-0.92
Benzaldehyde	Tetrachloromethane	TCE	0.054	0.412	- 0.058	-0.92
Nitrobenzene	Cyclohexane	TCE	0.055	1.300	- 0.100	-0.92
Benzophenone	Cyclohexane	Bromobenzene	0.006	0.216	- 0.115	+1.38
Acetone	Light petroleum (b.p. 60-80°C)	Diethyl ether	0.040	0.745	+ 2.18	+8.00
Isobutylmethylketone	<i>n</i> -Pentane	Diethyl ether	0	5.25	- 1.78	-0.75
Tetrahydrofuran	<i>n</i> -Heptane	Ethylacetate	0	1.57	- 1.90	+2.79
2-Phenylbutan-1-ol	Diethyl ether	Acetonitrile	1.16	6.91	+16.2	+3.12
3-Phenylpropan-1-ol	Diethyl ether	Acetonitrile	0.50	2.83	+ 6.56	+2.02

This is, of course, the form that eqn. 1 reduces to when  $b = 0$ . Systems which give the linear Type I plot evidently conform to this condition.

For the non-linear systems, Types II-VI, the parameters listed in Table II were used to produce the excellent fit produced by eqn. 1 to the experimental data. The goodness of the fit can be judged by comparing the solid lines (calculated on the basis of eqn. 1 and the parameters in Table II and the experimental points in Figures 1-6 above.

It is then seen that the form of eqn. 1 is closely related to our earlier analysis<sup>16</sup>, while eqn. 3 indicates only an empirical relationship.

We believe we have established a general equation for HPLC binary solvent retention behaviour and that, as a result, theoretical effort should now be directed to establishing a chromatographic retention model which generates a result in the form of eqns. 1 or 3.

#### ACKNOWLEDGEMENTS

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