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STUDY OF THE INFLUENCE OF COMPETITION AND SOLVENT INTERACTION ON RETENTION IN LIQUID-SOLID CHROMATOGRAPHY BY MEASUREMENT OF ACTIVITY COEFFICIENTS IN THE MOBILE PHASE

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SUMMARY

By measuring the activity coefficients of solutes and moderator in the mobile phase and the capacity ratios, it was possible to study separately the dependence of the competition term and the solvent interaction term on the composition of the mobile phase. This was done for mobile phases consisting of 2-propanol and ethyl acetate as moderators in *n*-heptane, in combination with silica with a high surface area. It was found that both the competition and solvent interaction effects contribute significantly to the change in retention with changing composition of the mobile phase.

For a considerable concentration range of the moderator, the stationary phase effects can be understood as a competition for the surface between the moderator and the solutes, while assuming an adsorbed layer of fixed size consisting of moderator and solute. For higher moderator concentrations the competition effect diminishes, and it seems that in this instance solutes can enter the layer without displacing the moderator.

The measurement of activity coefficients was also applied to the mobile phases acetonitrile-water and methanol-water used in reversed-phase adsorption chromatography. Comparisons are made with retention data obtained with octyl-modified silica. Preliminary results suggest that the assumption of constant activity of solutes in the stationary phase is not tenable.

INTRODUCTION

In normal-phase adsorption chromatography there are two different theories, namely the competition phenomena theory and the solvent interaction theory, and these have been reviewed by Snyder¹.

Snyder² and Soczewiński and Golkiewicz^{3,4} developed the competition theory, and stated that there is competition between the solute and the polar solvent for the occupation of the polar surface. Snyder treated the surface as a homogeneous layer, in which the solvent and solute molecules occupy certain areas, depending on their molecular structures. Soczewiński postulated localized adsorption sites, for instance silanol groups, interacting with functional groups of the adsorbed molecules.

For a polar solvent B with a molar fraction $X_B < 0.1$ and an non-polar solvent A, the two systems of Snyder and Soczewiński are about equivalent. We can represent this by the exchange reaction



A solute molecule I initially present in the mobile phase adsorbs by displacing a number (N) of polar solvent molecules B from the adsorbent surface. According to this theory, the concentration of the polar solvent B will influence directly the activity of the solute within the adsorbed phase.

The other theory is due to Scott and Kucera⁵, who stated that the change of retention above a certain percentage of the polar solvent B is due primarily to changing interactions in the mobile phase. At higher percentages of polar solvent B, the interactions in the mobile phase increase, and this effect will diminish the retention of the solute. Scott and Kucera found a linear relationship between the percentage of B and the inverse of the capacity ratio, $1/\kappa$.

In the next section, a theoretical framework is given in which both effects are accounted for.

THEORETICAL

The relationship between the capacity ratio and the thermodynamic distribution constant is given by

$$K_{thI} = \frac{X_{Ia}}{X_{Im}} \quad (2)$$

and

$$K_{cI} = \frac{c_{Ia}}{c_{Im}} = \frac{\bar{v}_m}{\bar{v}_a} \cdot K_{thI} \quad (3)$$

so that

$$\kappa_I = \frac{V_a W}{V_m} \cdot \frac{\bar{v}_m}{\bar{v}_a} \cdot K_{thI} \quad (4)$$

where K_{thI} is the thermodynamic equilibrium constant of solute I, X_{Ia} and X_{Im} are the molar fractions of solute I in the adsorbed and the mobile phase, respectively, K_{cI} is the distribution coefficient, c_{Ia} and c_{Im} are the concentrations of I in the adsorbed layer and the mobile phase, respectively, \bar{v}_m and \bar{v}_a are the molar volumes of the mobile phase and the adsorbed phase, respectively, W is the weight of adsorbent in the column, V_m is the volume of the mobile phase in the column and V_a is the volume of the adsorbed phase per unit weight of adsorbent. For a distribution equilibrium, we have

$$\frac{X_{Ia}\gamma_I^a}{X_{Im}\gamma_I^m} = 1$$

and

$$K_{thI} = \frac{\gamma_I^m}{\gamma_I^a} \tag{5}$$

where γ_I^m and γ_I^a are the activity coefficients. We chose the pure solute in the liquid state as the standard state for both phases. The result is

$$\kappa_I = \frac{V_a W}{V_m} \cdot \frac{\bar{v}_m}{\bar{v}_a} \cdot \gamma_I^m \cdot [\gamma_I^a]^{-1} \tag{6}$$

The various factors on the right-hand side of this equation correspond to different aspects of work on retention in liquid–solid chromatography (LSC). The first factor corresponds to the phase ratio and would be appropriate for discussions on the influence of the specific area and pore volume of adsorbents. The second factor is only slightly variable and is not much discussed. The third factor corresponds to the solvent interaction model. In this model, especially developed for an understanding of the change in retention in a binary solvent mixture of variable composition, it is assumed that the influence of other factors is negligible as soon as the surface is covered with a layer of the strongest component in the mixture. The assumptions of this model can therefore be expressed as

$$\frac{1}{\bar{v}_m \gamma_I^m} = A + B c_B \tag{7}$$

where A and B are constant and c_B is the concentration of the more polar solvent B.

The fourth factor in eqn. 6 focuses on the competition model. Snyder², for example, gives the expression

$$\log K_{th} = \alpha' [S_0 - A_s^0 \varepsilon^0] \tag{8}$$

where α' = parameter for the activity of the adsorbent, S_0 = dimensionless free energy of adsorption of a sample on an adsorbent of standard activity ($\alpha' = 1$), A_s^0 = the solute molecular area occupied on the surface and ε^0 = solvent strength parameter.

Because in this treatment it is argued that the influence of γ_I^m will be small, the activity coefficient γ_I^a is assumed to have a dependence parallel with that given in eqn. 8.

This equation was derived by considering an exchange reaction that occurs when a solute molecule adsorbs, at the same time displacing a number (N) of solvent molecules.

For a binary solvent system AB with a non-polar solvent A (*n*-heptane) and a polar solvent, the solvent strength parameter is

$$\varepsilon_{AB}^0 = \varepsilon_A^0 + \frac{1}{\alpha' n_b} \cdot \log(X_B \cdot 10^{\alpha' n_b (\varepsilon_B^0 - \varepsilon_A^0)} + 1 - X_B) \tag{9}$$

where ε_{AB}^0 , ε_A^0 and ε_B^0 are the solvent strength parameters of the mixture AB, pure A and pure B, n_b is the molecular area occupied by B on the surface and X_B is the molar fraction of B in the solvent.

The relationship between the capacity ratio and the molar fraction of B can be derived by combining eqns. 4, 8 and 9:

$$\log \kappa_1 = \log\left(\frac{V_a W}{V_m} \cdot \frac{\bar{v}_m}{\bar{v}_a}\right) + \alpha' S_0 - \alpha' A_s^0 \varepsilon_A^0 - \frac{A_s^0}{n_b} \cdot \log(1 + C_{AB} X_B) \quad (10)$$

in which

$$C_{AB} = 10^{\alpha' n_b (\varepsilon_B^0 - \varepsilon_A^0)} - 1$$

This results in

$$\log \kappa_1 = \text{constant} - \frac{A_s^0}{n_b} \cdot \log(1 + C_{AB} X_B) \quad (11)$$

The competition effect in a binary mixture with changing composition, as expressed in eqn. 9, can be derived generally in terms of the activity coefficients. Considering the relationship

$$d(G^a - \mu_B^a n_B^a) = \mu_I^a \cdot dn_I^a - n_B^a d\mu_B^a \quad (12)$$

where G is the free enthalpy, μ is the chemical potential, n is the number of moles, superscript a refers to the adsorbed layer and subscripts B and I refer to the moderator and solute, respectively, and by applying the properties of an exact differential it can be derived that

$$\left(\frac{\partial \mu_I^a}{\partial \mu_B^a}\right) n_I^a = - \left(\frac{\partial n_B^a}{\partial n_I^a}\right) \mu_B^a = N \quad (13)$$

in which N is the exchange ratio of I and B when displacing each other from the layer. The second part of eqn. 13 is valid for a layer with a saturation effect, *i.e.*, $N n_B^a + n_I^a = \text{constant}$. As $\mu_B^a = \mu_B^m$ and $n_I^a = \text{constant}$, *i.e.*, equivalent to $X_I^a = \text{constant}$ and $X_B^a = \text{constant}$, eqn. 13 can be rearranged to

$$\frac{d \ln \gamma_I^a}{d \ln X_B^m \gamma_B^m} = N \quad (14)$$

or

$$\gamma_I^a = D (X_B^m \gamma_B^m)^N \quad (15)$$

The conditions for the validity of eqn. 13 (and eqns. 14 and 15 derived from it) are: (a) the adsorbent is covered with a fixed amount of B, not dependent on X_B ; the amount of A adsorbed is negligible. (b) when a solute adsorbs, a certain number (N) of B

molecules leave the layer, *i.e.*, $n_1^q + Nn_B^q = \text{constant}$. This equation shows that the competition effect with varying concentration of B is necessarily present if a saturation effect (fixed total volume, mass or area of adsorbed species) occurs in the adsorbed layer. The existence of a competition effect in this kind of system is not a hypothesis, but a thermodynamic necessity. Using eqns. 5 and 15 to obtain an expression for K_{th} :

$$K_{th} = \frac{\gamma_1^m}{D(X_B^m \gamma_B^m)^N} \quad (16)$$

with the help of eqn. 4 an expression for the capacity ratio can be derived:

$$\kappa_1 = \frac{V_a W}{V_m} \cdot \frac{1}{D} \cdot \frac{\bar{v}_m}{\bar{v}_a} \cdot \gamma_1^m \cdot (X_B^m \gamma_B^m)^{-N} \quad (17)$$

As was shown convincingly by Soczewiński⁶, it is almost impossible to distinguish between the solvent interaction and competition models on the experimental basis of retention data only. This can be seen by comparing eqns. 7 and 9; when $N = A_s^0/n_b = 1$, they are equivalent.

As γ_1^m is accessible experimentally via the vapour pressure of solutes (at least for those solutes which in a dilute solution exert a vapour pressure high enough for quantitation with a flame-ionization detector (FID)), we decided to use headspace analysis for determining the dependence of γ_1^m on X_B separately. This allows us to discriminate between effects operating via γ_1^q (competition) and those operating via γ_1^m (solvent interaction).

EXPERIMENTAL

Apparatus

Gas chromatographic (GC) part (Fig. 1). A syringe of 20 ml capacity (Hamilton 1020 LL) was used as a variable-volume vessel⁷ in which liquid was equilibrated with the gas phase. By moving the piston of the syringe, saturated vapour was forced into the sample loop (1 ml) of the injection valve (Micro volume valve, Type 2021, Carle Instruments, Fullerton, Calif., U.S.A.) fitted into the gas chromatograph (GC L 350, Siemens, Karlsruhe, G.F.R.) equipped with an FID. The connection consisted of heated glass-lined tubing (GLT, 1/16 and 1/8-in. O.D., 0.5-mm I.D.; SGE, Melbourne, Australia). The syringe was surrounded by a water-jacket, connected to a circulating water thermostat (FS, Haake, Karlsruhe, G.F.R.). The PTFE top of the piston was covered with a layer of mercury in order to prevent sorption of components by the PTFE. Gas chromatographic separation columns were constructed from stainless-steel tubing of different lengths. The detector output was connected to an electronic integrator (Autolab Minigrator, Spectra Physics, Santa Clara, Calif., U.S.A.).

Liquid chromatographic (LC) part. The liquid chromatograph was constructed from custom-made and commercially available components and consisted of a thermostated eluent reservoir, a high-pressure pump (Orlita, Giessen, G.F.R., Type ZB-DE-024n), a flow-through Bourdon-type manometer, serving as a damping device, a high-pressure sampling valve (Valco; CV-6-UHPa-C20) and a thermostated (25.0°) stainless-steel column of dimensions 250 × 3.0 mm I.D. with a thermostated water-

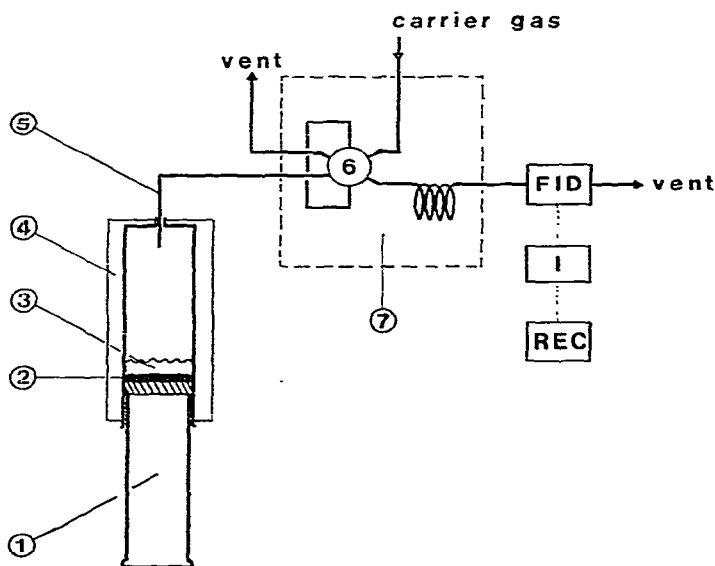


Fig. 1. Schematic diagram of the apparatus for the measurement of the activity coefficients in the mobile phase. 1 = Plunger of syringe; 2 = mercury layer; 3 = solution; 4 = thermostated water-jacket; 5 = heated sample tube; 6 = injection valve; 7 = GC oven.

bath (Haake, Type FS). Two detectors were used, a spectrophotometer (Zeiss, Oberkochen, G.F.R., Type PM 2 ALC) and a home-made thermostated permittivity detector⁸.

The chromatograms were recorded on a linear and logarithmic potentiometric recorder (Kipp, Delft, The Netherlands, Type BDH Lin-log).

Chemicals and materials

Chromosorb 101, 100–120 mesh (Johns-Manville, Denver, Colo., U.S.A.) and Porapak QS, 100–120 mesh (Waters Assoc., Milford, Mass., U.S.A.) were used as column packing materials in GC. The column material in high-performance liquid chromatography (HPLC) was Partisil 10 (Reeve Angel Scientific, London, Great Britain) and RP-8 (Merck, Darmstadt, G.F.R.). All chemicals used were of at least analytical-reagent grade and were dried over activated molecular sieve 5 A (Merck).

Procedures

LC capacity ratios. Capacity ratios were obtained from retention times and estimates of the retention times of truly unretarded peaks. These estimates were obtained from estimates of the mobile phase volume and the flow-rate. The mobile phase volume was estimated from the total amount of liquid present in the column, V_0 , and that in the external critical parts, but was corrected for the volume of the adsorbed layer on the silica surface (V_s). V_0 was obtained by weighing a column dried with a stream of dry nitrogen and the same column filled with tetrachloromethane. The volume of the layer, V_s , was determined by means of breakthrough curves⁹, as described by Paanakker *et al.*¹⁰.

Activity coefficients. These were calculated from the equation

$$\gamma_I^m = \frac{S' \cdot c_m}{S^0 \left(c_I^0 - \frac{V_g}{V_l} \cdot c_g \cdot \frac{S'}{S^0} \right)} \quad (18)$$

where S' is the signal (integral) obtained with a solution of I of initial concentration c_I^0 (generally about 0.05–0.06 mole/l), S^0 is the signal obtained with pure I, c_m is the total concentration of solvent, c_I^0 is the initial concentration of I, V_g and V_l are the volumes of gas and liquid phase, respectively, in the equilibrium vessel and c_g is the concentration of the solute in saturated vapour of I.

In some instances, corrections for non-linearity of the measurement system had to be applied; c_g was mostly calculated from vapour pressure data^{11,12}. In most instances the correction term in eqn. 18 was less than 5%.

RESULTS AND DISCUSSION

Normal-phase adsorption chromatography

As already mentioned, it was the intention to investigate the validity of the different theories on the solvent effect in LSC. In our experimental approach we used three independent sources of information:

(i) measurement of the retention of solutes in the adsorption column as a function of the binary mobile phase composition;

(ii) measurement of the activity coefficients of the solutes and the polar moderators in the solvent by means of GC headspace analysis;

(iii) measurement of the coverage of the adsorbent surface with the polar moderator, by means of breakthrough curves^{9,10}; responses in the output concentration, when a stepwise change in the polar moderator concentration is applied at the input of the column.

The inverse of the measured capacity ratios against the volume percentage of the polar moderator B (2-propanol and ethyl acetate, respectively) is plotted in Figs. 2a and 3a, which gives the results in terms of the solvent interaction model.

It can be seen that above about 2% (v/v) of moderator, approximately linear relationships are observed. At lower percentages of the moderator, considerable deviations from this linear dependence are found.

This interpretation of retention data is dependent on the assumption that no changes in the stationary phase effects occur; when such changes do occur, the resulting plots will give a misleading impression of the solvent interaction effects. A more preferable method of studying solvent interaction effects in the mobile phase is to plot the expression $(\gamma_I^m \bar{v}_m)^{-1}$ for different solutes against the volume fraction of the polar solvent B according to eqn. 7 (Figs. 2b and 3b). Although an approximately linear dependence is observed, some curvature of the lines is unmistakable. This is especially noticeable in these plots because the available concentration range extends down to the pure non-polar liquid.

A closer comparison of Figs. 2b and 3b shows that specific solute-moderator interactions occur. In Fig. 2b, phenylethanol has the steepest dependence, while benzyl acetate and nitrobenzene show only a minor increase, especially at higher concentra-

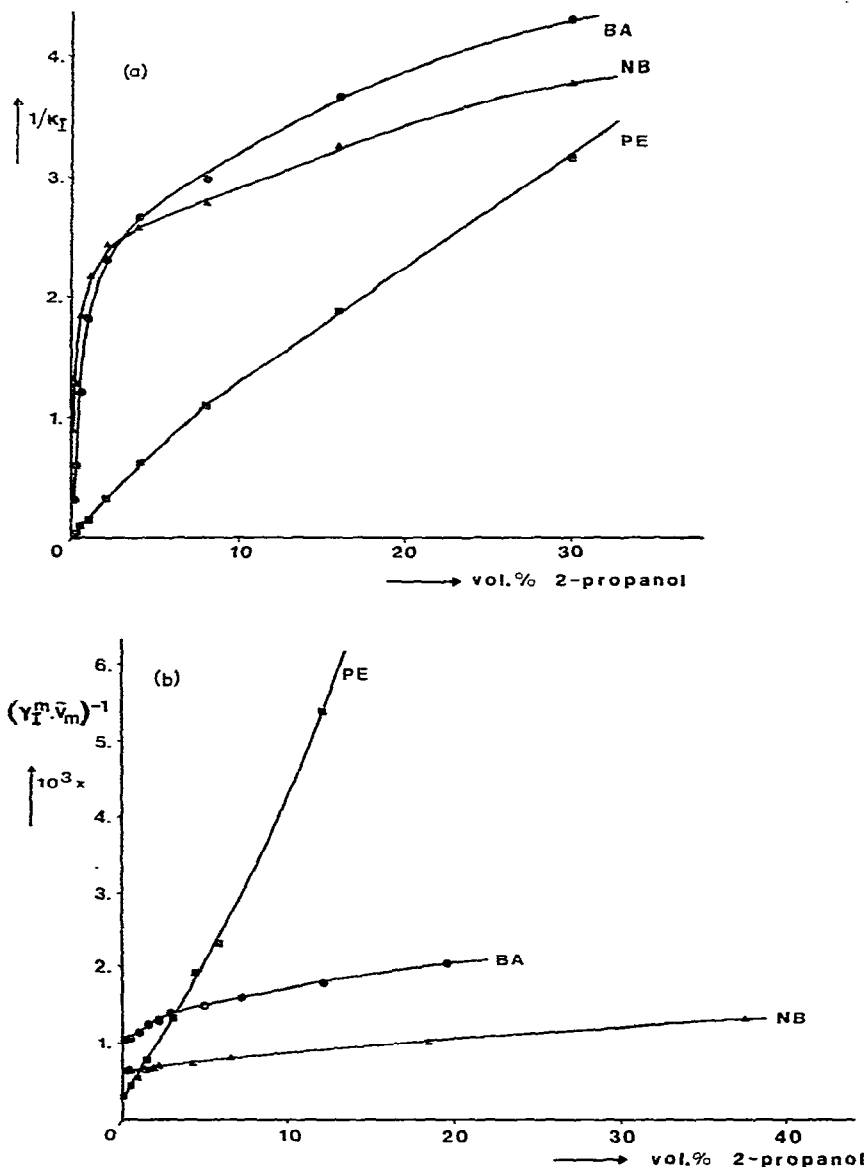


Fig. 2. (a) Inverse of the capacity ratio, $1/\kappa_T$, versus concentration of 2-propanol in *n*-heptane. Adsorbent, Partisil 10; temperature, 25°. NB = nitrobenzene; BA = benzyl acetate; PE = 1-phenylethanol. (b) $1/\gamma_1^m \bar{v}_m$ versus concentration of 2-propanol as moderator. Conditions as in (a).

tions. In contrast, Fig. 3b shows about the same slope for benzyl acetate and phenylethanol, while that of nitrobenzene is much lower. Multifactor descriptions of these interactions, as developed by Rohrschneider¹³ and Snyder¹⁴ and more recently by Karger *et al.*¹⁵ and Tijssen *et al.*¹⁶, are obviously necessary.

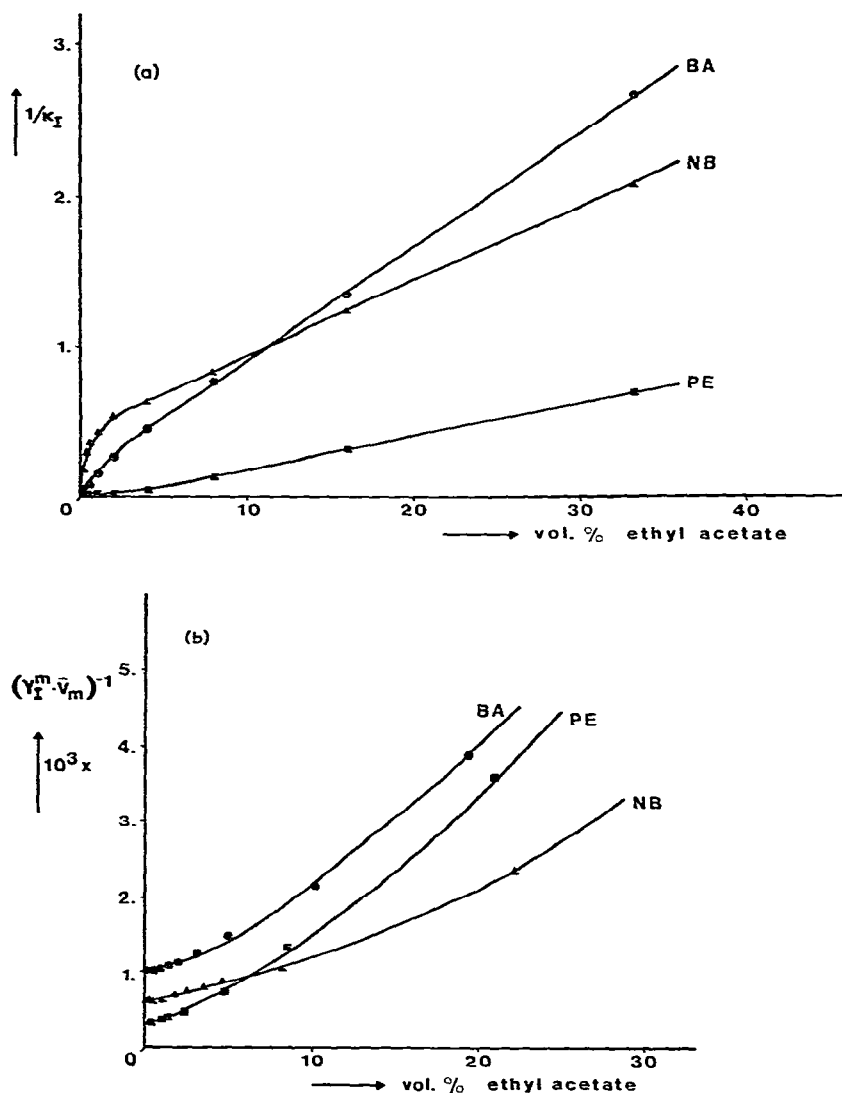


Fig. 3. (a) Inverse of the capacity ratio, $1/\kappa_I$, versus concentration of ethyl acetate in *n*-heptane. Other conditions and symbols as in Fig. 2a. (b) $1/\gamma_I^m \bar{v}_m$ versus concentration of ethyl acetate. Conditions and symbols as in Fig. 2a.

Tables I–III list the the experimental data of the activity coefficients and capacity ratios.

In Fig. 4 capacity ratios (κ) are plotted logarithmically against the molar fraction of the moderator. This gives the relationship in terms of the competition theory (eqn. 11), neglecting the solvent interaction effect. The plots obtained suggest a close adherence to the dependence as predicted by the competition theory, especially when the solute and moderator contain the same functional group. The dashed lines in Fig. 4 give the adsorption isotherms of the moderators used.

TABLE I

ACTIVITY COEFFICIENTS AT DIFFERENT CONCENTRATIONS OF 2-PROPANOL IN *n*-HEPTANE

The activity coefficients of the moderator and the solutes are interpolated to the same solvent conditions. 2-P = 2-propanol; NB = nitrobenzene; BA = benzyl acetate; PE = 1-phenylethanol. Temperature, 25.0°.

2-P (vol.-%)	2-P (mole-%)	γ_{2-P}^{17}	γ_{NB}	γ_{BA}	γ_{PE}
0.00	0.00	36.0	11.4	6.69	21.9
0.26	0.50	31.8	11.4	6.67	20.3
0.52	1.00	27.4	11.4	6.60	16.4
1.59	3.00	14.4	11.3	5.59	8.85
2.68	5.00	10.0	10.4	5.30	5.75
5.49	10.0	5.87	9.50	5.18	3.22
11.55	20.0	3.36	8.54	4.29	1.5
18.3	30.0	2.41	7.97	4.02	—
25.8	40.0	1.90	7.57	—	—
34.3	50.0	1.58	7.15	—	—

TABLE II

ACTIVITY COEFFICIENTS AT DIFFERENT CONCENTRATIONS OF ETHYL ACETATE IN *n*-HEPTANE

The activity coefficients of the moderator and the solutes are interpolated to the same solvent conditions. EA = ethyl acetate; other symbols as in Table I.

EA (vol.-%)	EA (mole-%)	γ_{EA}	γ_{NB}	γ_{BA}	γ_{PE}
0.00	0.00	—	11.4	6.69	23.4
0.10	0.15	4.24	11.4	6.72	—
0.25	0.37	4.20	11.4	6.72	22.9
0.50	0.75	4.06	11.4	6.71	21.8
1.00	1.49	3.82	11.3	6.59	18.4
2.50	3.70	3.30	9.55	6.13	14.4
5.00	7.31	3.02	8.40	4.55	9.2
10.0	14.3	2.58	6.27	3.37	4.5
20.0	27.2	2.01	3.59	1.93	2.2

TABLE III

CAPACITY RATIOS OF NITROBENZENE (NB), BENZYL ACETATE (BA) AND 1-PHENYLETHANOL (PE) IN *n*-HEPTANE WITH AS MODERATORS 2-PROPANOL AND ETHYL ACETATE AT 25° ON PARTISIL 10

Column, 25 cm × 3 mm I.D.; adsorbent weight, 0.9 g.

Vol.-%	2-Propanol			Ethyl acetate		
	K_{NB}	K_{BA}	K_{PE}	K_{NB}	K_{BA}	K_{PE}
0.00	40.85	—	—	—	—	—
0.10	1.12	3.12	45.4	5.05	34.6	—
0.20	0.79	1.65	23.5	—	—	—
0.25	—	—	—	3.50	18.2	—
0.50	0.54	0.73	9.36	2.87	11.4	100.3
1.00	0.49	0.55	6.26	2.32	6.43	61.3
2.00	0.41	0.43	2.92	1.89	3.75	34.5
4.00	0.39	0.38	1.60	1.59	2.24	18.2
8.00	0.36	0.34	0.91	1.20	1.29	8.22
16.00	0.31	0.27	0.54	0.82	0.75	3.29
30.00	0.26	0.23	0.32	0.50	0.37	1.45

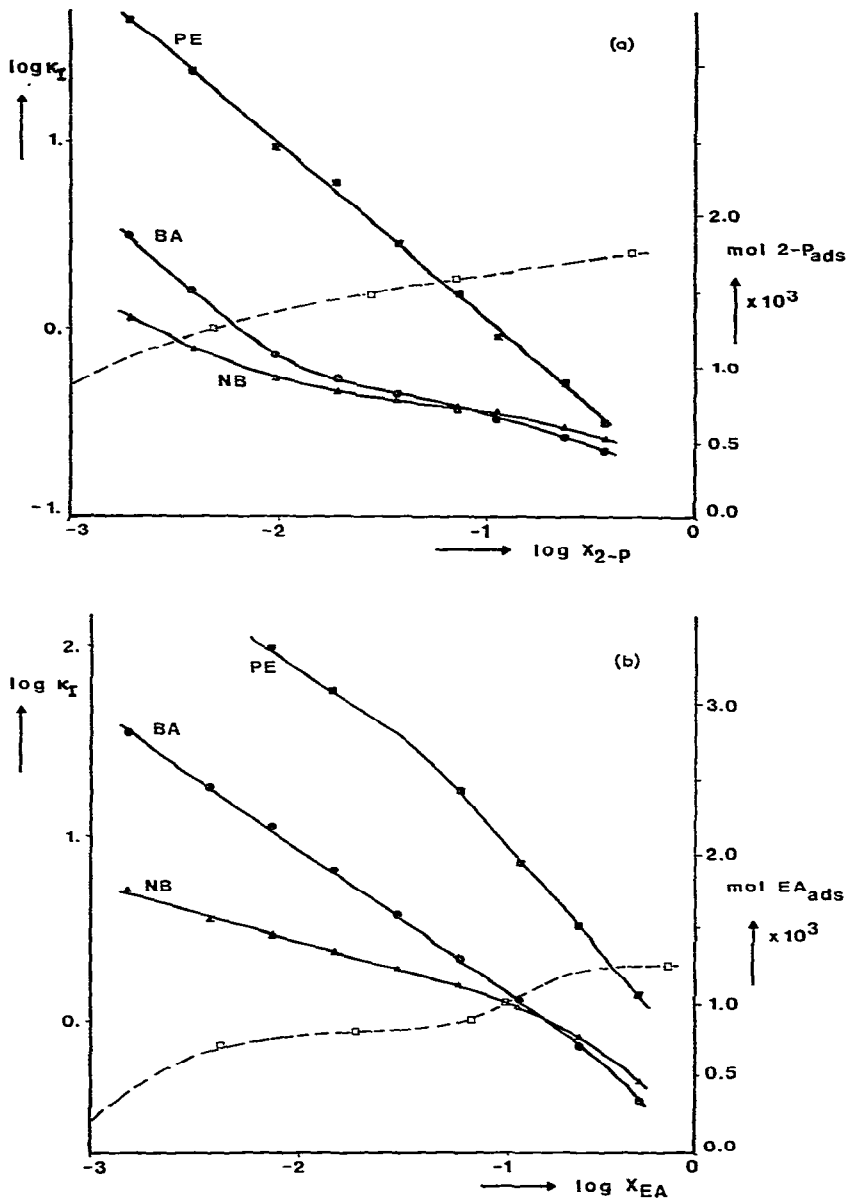


Fig. 4. (a) Simple competition plot and the adsorption isotherm of 2-propanol (2-P) (dashed curve). Conditions and symbols as in Fig. 2. (b) As (a) with the moderator ethyl acetate (EA).

In order to obtain an undistorted picture of the influence of the competition effect, one has to correct the capacity ratios according to eqn. 17 with the variable factor $(\gamma_I^m \bar{v}_m)^{-1}$ and the molar fraction X_B^m of the moderator with the factor γ_B^m . The other factors in eqn. 17 can be assumed to be virtually constant for the situation as-

sumed in competition theory that a layer of constant composition (pure moderator) and thickness is present on the surface (Table IV). From the isotherms given in Fig. 4, it can be seen that this is a reasonable approximation in view of the wide range of moderator concentrations used.

TABLE IV

EXPERIMENTAL DATA FOR THE ADSORPTION ISOTHERM OF 2-PROPANOL AND ETHYL ACETATE IN *n*-HEPTANE ON PARTISIL 10 AT 25°

Column, 25 cm × 3 mm I.D.; adsorbent weight, 0.9 g.

<i>2-Propanol</i>			<i>Ethyl acetate</i>		
<i>Vol.-%</i>	<i>Total amount adsorbed (mol)</i>	<i>Amount adsorbed per gram adsorbent (g/g)</i>	<i>Vol.-%</i>	<i>Total amount adsorbed (mol)</i>	<i>Amount adsorbed per gram adsorbent (g/g)</i>
40.0	$1.80 \cdot 10^{-3}$	0.120	50.0	$1.27 \cdot 10^{-3}$	0.124
3.85	$1.59 \cdot 10^{-3}$	0.107	9.09	$1.01 \cdot 10^{-3}$	0.099
1.48	$1.45 \cdot 10^{-3}$	0.097	4.58	$0.88 \cdot 10^{-3}$	0.084
0.30	$1.26 \cdot 10^{-3}$	0.084	1.31	$0.82 \cdot 10^{-3}$	0.080
0.01	$0.12 \cdot 10^{-3}$	0.008	0.28	$0.72 \cdot 10^{-3}$	0.070
			0.06	$0.09 \cdot 10^{-3}$	0.009

The plots resulting from these corrections are given in Fig. 5. As expressed in eqn. 15, the slopes of these plots give an estimate of N , the exchange ratio of the moderator and solute. A constant non-zero value of N corresponds to a perfect competition mechanism; a value of zero for N would indicate a situation comparable to liquid-liquid partition, where transfer of one solute molecule can take place without displacing any other molecule.

Fig. 5a shows a constant slope for the three solutes at lower moderator concentrations, of the order of 0.5–1.0, indicating competition. For all three solutes, however, at higher moderator concentrations ($X_1^m > 0.025$; > 1.4 vol.-%), a decrease in the slope is very obvious, which indicates that the competition effect diminishes at these higher moderator concentrations. Apparently the solute can enter the interfacial layer under these conditions without displacing a moderator molecule. Formation of multiple layers is suggested by these results, and other confirmatory evidence is also available¹⁰.

The results obtained so far give a ready explanation for the fact that both the solvent interaction and competition theories could be defended with success for a number of years. Both mechanisms are operative, in different concentration ranges, and in such a way that it is difficult to distinguish between them from retention data only. In fact, for systems such as phenylethanol-propanol, benzyl acetate-ethyl acetate and, to a lesser extent, nitrobenzene-ethyl acetate, a direct plot of $\log \kappa_1$ versus $\log X_B$ yields a straight line over a large range of X_B , suggesting one mechanism, while in fact the two mechanisms operate alternately.

Reversed-phase adsorption systems

In addition to the investigations with normal-phase adsorption systems, we also considered the solvent and stationary phase effects in reversed-phase systems.

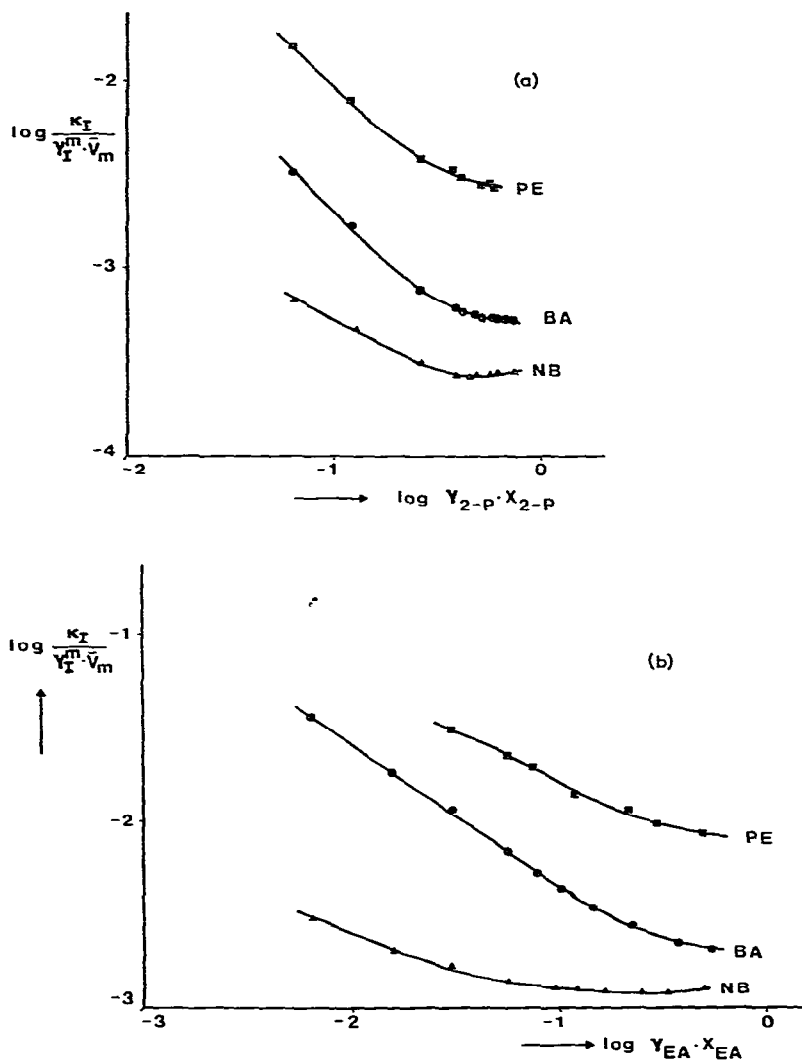


Fig. 5. (a) Capacity ratio, κ , corrected for the solvent interaction, $(\kappa_I/\bar{v}_m \gamma_I^m)$, versus activity $(X_{2-P} \cdot \gamma_{2-P}^m)$. Conditions and symbols as in Fig. 2a. (b) As (a) with the moderator ethyl acetate (EA).

This was done because of the postulate by Locke¹⁸ that in reversed-phase systems the selectivities are mainly determined by the mobile phase effects (a solvent interaction model). If this was true, then logarithmic plots of the capacity ratio and the product $\gamma_I^m \bar{v}_m$ in the mobile phase versus the solvent composition should be parallel. Such a plot is given for RP-8 in Fig. 6, where it can be seen that the curves diverge. This means that interactions in the stationary phase also change significantly with changing eluent composition.

Another means of relating the mobile phase interactions and the capacity ratio is to plot the activity coefficient against the capacity ratio. Locke's postulate would

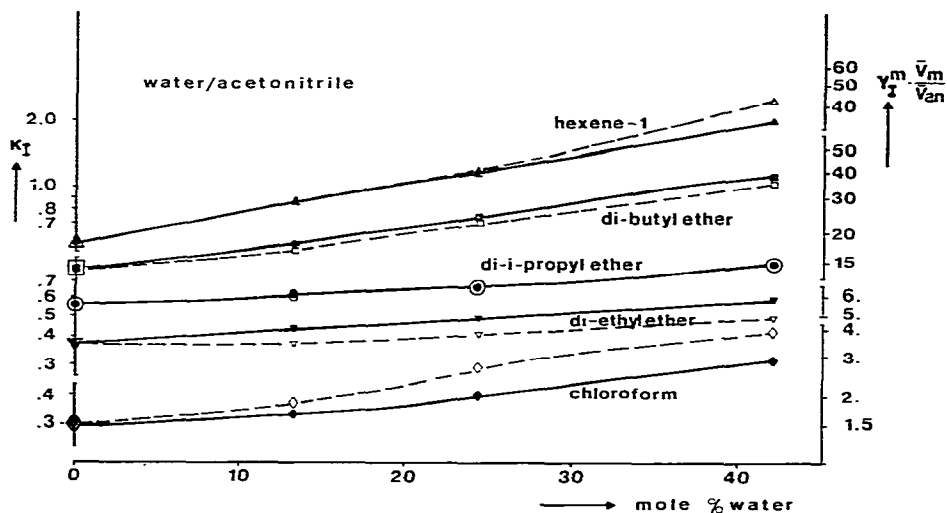


Fig. 6. Logarithmic plots of the expression $\gamma_{1m} \bar{v}_m / \bar{v}_{an}$ and the capacity ratio (κ_T) for different solutes versus the water content in a water-acetonitrile system. \bar{v}_{an} = molar volume of pure acetonitrile. Open symbols correspond to the activity coefficients and closed symbols to the capacity ratios. \diamond , Chloroform; \triangle , diethyl ether; \circ , diisopropyl ether; \square , dibutyl ether; Δ , hexene-1. Adsorbent, RP-8; temperature, 25°.

predict a straight line passing through the origin. When we plotted this relationship for six solutes in four different compositions of an acetonitrile-water mixture (Fig. 7a), an approximate linear dependence was observed, but there was a significant intercept on the ordinate. This intercept cannot be due to an incorrect estimate of the mobile phase volume within the column, as the value we used (the total amount of liquid within the column) is larger than any other estimate.

Fig. 7b shows a similar experimental plot for methanol-water mixtures using the same solutes. In this instance a simple dependence of the capacity ratio on mobile phase effects is not observed. As activity coefficients in the stationary phase appear to be a function of the mobile phase composition it can be inferred that the physical nature of the stationary phase changes with the mobile phase composition.

CONCLUSION

As this work has shown that in LSC retention-determining factors reside in both the mobile and stationary phases, future work on the understanding of these phase systems should be carried out under conditions such that either the two effects can be separated or it is known with certainty that one effect is constant. The latter condition, in our opinion, can be fulfilled only at very low moderator concentrations, where the solvent interaction effect can be neglected. Meaningful studies on the competition effect on the basis of retention data can therefore be expected only in the low moderator concentration range, or with a method such as that described in this paper, in which solvent interaction effects are corrected for. More extensive measurements of activity coefficients, of course, will also contribute to the understanding of

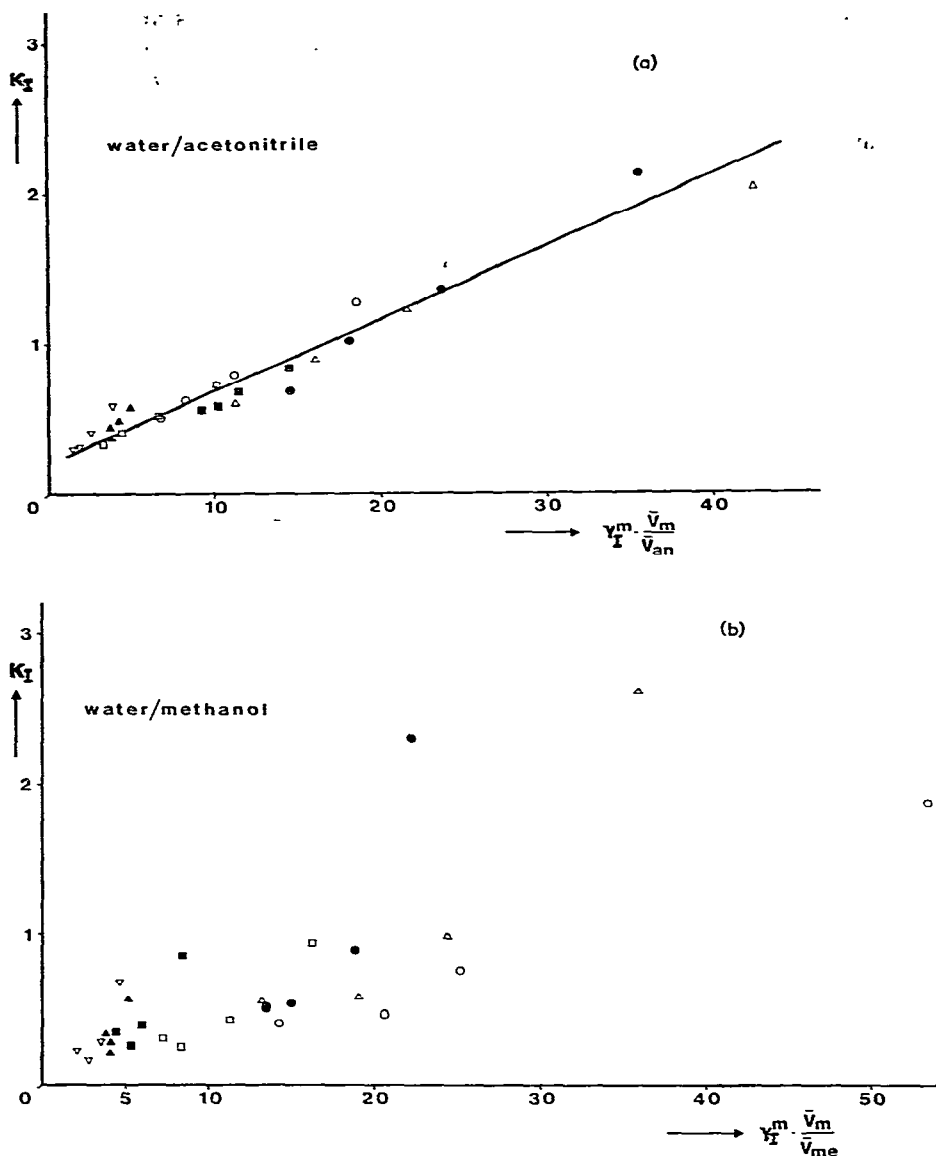


Fig. 7. (a) Capacity ratios (κ_I) versus $\gamma_I^m \bar{v}_m/\bar{v}_{an}$. \bar{v}_{an} = molar volume of pure acetonitrile. Four phase systems, in which the mobile phase consisted of acetonitrile-water, were used. Δ , Hexene-1; \circ , ethyl benzene; \square , benzene; \blacktriangle , diethyl ether; \blacksquare , diisopropyl ether; \bullet , dibutyl ether, \triangle , chloroform. The activity coefficients increase with the water content. Other conditions as in Fig. 6. (b) As (a) with a water-methanol system. \bar{v}_{me} = molar volume of pure methanol.

liquid interactions and to improvements and requirements in the multi-parameter models developed for this purpose¹⁴⁻¹⁶.

The dependence of solute activity on composition in solvent mixtures has been given particularly little attention in these models. The anomalies with respect to the

competition effects at higher moderator concentrations, observed by us and also by Paanakker *et al.*¹⁰, deserve further attention, especially the formation of multiple layers, binary layers or even layers with the moderator only. Careful study of adsorption isotherms and the stoichiometric determination of exchange ratios¹⁹ will give valuable information.

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