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SOME ASPECTS OF THE INFLUENCE OF THE MOBILE PHASE COMPOSITION IN NORMAL-PHASE LIQUID–SOLID CHROMATOGRAPHY, WITH SPECIAL ATTENTION TO THE ROLE OF WATER PRESENT IN BINARY ORGANIC MIXTURES

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SUMMARY

The influence of the mobile phase composition in normal-phase liquid–solid chromatography (LSC) was investigated for binary mixtures of 2,2,4-trimethylpentane with *n*-butanol and ethyl acetate. The effect of varying water content in these mixtures on the retention was also measured. Carotenoids and simple aromatic solutes with different functional groups were used as solutes and silica Si 60 was used as an adsorbent.

The results are discussed in terms of the competition concept for LSC retention, and are related to experimentally observed adsorption isotherms and measured activities of the moderators used. The results show that in all of the mobile phase composition ranges investigated, low water contents influence retention and selectivity. The behaviour of water as a moderator is anomalous in terms of the competition concept, and suggests binary multilayer formation.

INTRODUCTION

The water contents of the mobile phase and stationary phase in liquid–solid chromatography (LSC) play a crucial role in the adjustment of absolute retention and selectivity of different solutes, as was investigated by many authors[1–7]. Therefore it is important to control the water content of solvents in order to obtain reproducible retention data[8,9]. From these papers it can be concluded that, in principle, water can be used for adjustment of isotherm linearity and retention. In practice, however, its use is less attractive because equilibration times are excessively long because of its low solubility in hydrocarbons[10]. The use of organic modifiers such as acetonitrile and dichloromethane is therefore recommended[11]. However, also when using this method, the problems associated with the unintended presence of water in the mobile phase will persist in practice.

From this point of view it is interesting to know the composition ranges within which low water contents still influence the retention and the selectivity in binary mixtures. Few papers, however, paid attention to this problem. Snyder[12], while using ternary mixtures of pentane, dichloromethane and water, investigated the change of

retention when varying water content at fixed dichloromethane content and *vice versa*. In another paper by the same author[13] the effect of water in an ethyl ether–isopropanol mixture of fixed composition was reported. These papers give valuable information on the subject, but from neither paper can it be inferred how the water and the strongest organic moderator operate together. It seemed useful to us to make a systematic experimental investigation on this point.

Such work could also contribute to the understanding of mechanisms that control retention in LSC, especially the competition effect[1,14]. For this purpose *n*-butanol (BuOH) and ethyl acetate (EtA), both in conjunction with 2,2,4-trimethyl-pentane (i-Oct) were chosen as organic modifiers, as these solvents allow for a large variation in solute retention because they are strong moderators and are miscible with i-Oct in different proportions. Solutes with widely differing sizes and functional groups were found in a number of simple aromatic compounds and carotenoids[15].

EXPERIMENTAL

Apparatus

The liquid chromatograph was constructed from a high-pressure reciprocating membrane pump (Orlita, Type AE 10-4), a Bourdon-type flow-through manometer (custom-made), an injection valve (Chromatronix, HPSV 20) and a variable-wavelength spectrophotometer (Zeiss, PM2D), equipped with a linear potentiometric recorder (Goertz, Servogor RE 512). In all experiments stainless-steel 316 columns of length 250 mm and I.D. 3.0 mm were used. Both the eluent reservoir and the column were thermostated at 21°.

Chemicals

The carotenoids (β-carotene, ethyl β-apocarotenate, β-apocarotenal, echinenone, canthaxanthin, luteine and zeaxanthin) were donated by Dr. H. Thommen of Hoffmann-La Roche (Basle, Switzerland). All other chemicals were of analytical-reagent grade. All solvents were dried by pumping them through columns filled with molecular sieve 5A (0.2–0.5 mm) that had been activated at 350° overnight, *in vacuo* (Merck, Darmstadt, G.F.R.).

Procedures

In all experiments silica gel (Merck Si 60) was used, ground and classified in a specific particle size range (8–9 μm) by means of an air classifier (Alpine MZR, Augsburg, G.F.R.) and subsequently sedimented in order to remove fines.

The columns were filled by a balanced-density slurry technique, as described elsewhere[16]. The columns were activated by eluting them successively with dry acetone (150 ml) and dry (10 ppm, w/w, of water) i-Oct (300 ml). A test mixture of benzene, naphthalene, anthracene and 3,4-benzpyrene was injected in order to check the activation state of the column.

The solutes were dissolved in the mobile phase and 15-μl samples were injected with the valve. In all experiments β-carotene was used as the unretained component, except in very weak solvents, in which the known volume of mobile phase in the column (1106 μl) and the flow-rate were used to calculate t_{R0}.

The water content of the different solvent mixtures was determined by means

of an automatic Karl Fischer titrator (Metrohm, Herisau, Switzerland). Experiments with varying water contents were carried out as follows. After addition of a known amount of water to the eluent, the phase system was equilibrated overnight by re-cycling the eluent. The water content of the eluent was subsequently determined and the capacity ratios were measured and checked for constancy.

Adsorption isotherms for BuOH and EtA in i-Oct on Si 60 were measured by means of breakthrough curves[17], responses in the output concentration when a step-wise change in the moderator concentration was applied at the inlet of the column, monitored with a custom-made permittivity detector[18]. The amount of moderator adsorbed on the adsorbent was calculated as follows:

$$(V_t - V_0)\, C_B = V_{B,ads}$$

where

V_t = breakthrough volume, measured at 50% of the observed stepwise change (μl);
V_0 = volume of mobile phase in the column (μl);
C_B = concentration of moderator B (%, v/v);
$V_{B,ads}$ = volume of adsorbed B (μl).

Activity coefficients of moderators in i-Oct were measured by means of gas chromato-graphic headspace analysis, as described elsewhere[19]. The solubilities of water in i-Oct–BuOH and i-Oct–EtA were determined by mixing 50 ml of different i-Oct–BuOH (or i-Oct–EtA) mixtures with 5 ml of water for 8 h in a series of thermostated (21°) vessels, de-mixing for 8 h and titrating an aliquot (10 ml) of the equilibrated organic phase by means of Karl Fischer titration.

TENTATIVE INTERPRETATION OF RETENTION DATA IN TERNARY SYSTEMS

For normal-phase adsorption chromatography, two theoretical models are available: (i) the competition model as proposed by Snyder[1] and Soczewiński and Golkiewicz[14] and (ii) the solvent interaction model as proposed by Scott and Kucera[20].

The solvent interaction model assumes that when the surface is completely covered by the moderator [ca. 3% (v/v) of moderator], the retention is mainly deter-mined by solute–solvent (i.e., moderator) interactions in the mobile phase (i.e., no significant changes in the stationary phase interactions with increasing moderator percentage). Scott showed that at complete coverage of the surface a linear relation-ship exists between $1/\kappa_i$ and the percentage of moderator in the eluent. At incomplete coverage, however, this relationship fails to describe the retention.

The competition model assumes that there is competition between the solvent and solute molecules in occupying active sites on the surface. With binary solvents the moderator molecules are the competitive ones.

For a binary mixture, the elution strength (ε_{AB}) is given by

$$\varepsilon_{AB} = \varepsilon_A + \frac{1}{a n} \log \left(X_B \cdot 10^{a n (\varepsilon_B - \varepsilon_A)} + 1 - X_B \right) \tag{1}$$

where

ε_A, ε_B = solvent strength parameters of A and B, respectively;
α = adsorbent activity parameter;
X_B = molar fraction of moderator B.

It is assumed that $n = n_B = n_A$ = molecular area of the solvent. With $10^{\alpha n (\varepsilon_B - \varepsilon_A)} - 1 = C_{AB}$, it follows that

$$\log \kappa_i = \log\left(V_a \cdot \frac{W}{V_0}\right) + \alpha S_i^0 - \alpha A_{s_i}\, \varepsilon_A - \frac{A_{s_i}}{n} \log (1 + C_{AB} \cdot X_B) \qquad (2)$$

where

κ_i = capacity ratio of solute i;
V_a = volume of adsorbed monolayer per unit weight of adsorbent;
W = weight of adsorbent;
V_0 = volume of mobile phase;
S_i^0 = adsorption energy of solute i;
A_{s_i} = molecular area of solute i.

or

$$\log \kappa_i = C_i - \frac{A_{s_i}}{n} \log (1 + C_{AB} \cdot X_B) \qquad (3)$$

where

$$C_i = \log\left(V_a \cdot \frac{W}{V_0}\right) + \alpha S_i^0 - \alpha A_{s_i}\, \varepsilon_A$$

According to the competition model, the plot of $\log \kappa_i$ versus $\log X_B$ should have a shape as shown in Fig. 1. At very small X_B (i.e., $1 \gg C_{AB}\, X_B$), $\log \kappa_i = C_i$ = constant. At very large X_B (i.e. $1 \ll C_{AB}\, X_B$), a linear relationship must be found between $\log \kappa_i$ and $\log X_B$ with a slope of A_{s_i}/n, the exchange ratio between solute and solvent molecules. In the intermediate X_B range, a curved dependence of $\log \kappa_i$ on $\log X_B$ will exist. If the competition model is correct the retention should be predictable, when the different parameters are known, over the whole range of moderator concentrations. Soczewiński[21] later showed that the competition and solvent interaction models are indistinguishable at larger moderator concentration and when the exchange ratio is unity.

When adding a third component, such as water, to a binary mixture, eqn. 2 can be applied in principle when extending eqn. 1 analogously to the treatment described by Snyder[22]. This results in

$$\varepsilon_{abc} = \frac{1}{\alpha n} \log (X_A\, 10^{\alpha n\, \varepsilon_A} + X_B\, 10^{\alpha n\, \varepsilon_B} + X_C\, 10^{\alpha n\, \varepsilon_C}) \qquad (4)$$

and

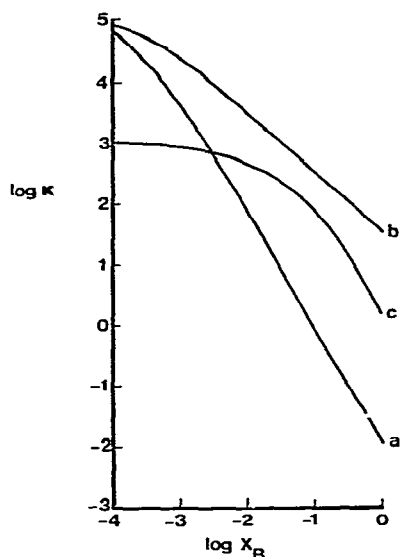$$\log \kappa_i = C_i - \frac{A_{s_i}}{n} \cdot \log (1 + C_{AB}\, X_B + C_{AC}\, X_C) \qquad (5)$$

Fig. 1. Theoretical dependence of the logarithm of the capacity ratio on the molar fraction of the moderator according to eqn. 3. (a) $C_t = 5$, $C_{AB} = 3160$, $A_{S_i}/n = 2$; (b) $C_t = 5$, $C_{AB} = 3160$, $A_{S_i}/n = 1$; (c) $C_t = 3$, $C_{AB} = 31.6$, $A_{S_i}/n = 2$.

with

$$C_{AC} = 10^{an(\varepsilon_C - \varepsilon_A)} - 1$$

The relative magnitude of the terms in the logarithmic expression of eqn. 5 reflects the relative effectiveness of the two moderators. Especially at very low values of, e.g., X_C (water) and high values of X_B, the influence of the moderator should be negligible, according to this model.

For a constant ratio of B and A, and low values of X_C, eqn. 5 can be transformed into

$$\log \kappa_i = C_i' - \frac{A_{si}}{n} \log (1 + C_{ABC} X_C) \tag{6}$$

where C_i' includes ε_A, ε_B and X_B/X_A and

$$C_{ABC} = \frac{10^{an\varepsilon_C}}{X_A 10^{an\varepsilon_A} + X_B 10^{an\varepsilon_B}}$$

RESULTS AND DISCUSSION

Influence of the moderator

For a large number of compounds of different nature, the capacity ratios were measured as a function of two organic moderators, BuOH and EtA, containing different amounts of water. The results are given in Tables I and II. In order to verify

TABLE I

CAPACITY RATIO AS A FUNCTION OF THE PERCENTAGE OF BuOH AND WATER IN i-OCT

BuOH concentration (%, v/v)	H$_2$O (ppm)	Anthracene	Anisole	Nitrobenzene	Benzonitrile	Ethyl β-apocarotenoate	1,4-Dinitrobenzene	Acetophenone	β-Apocarotenal
0.1	14	0.43	0.74	1.49	3.51	4.25	5.50	6.99	15.73
	26	0.31	0.58	1.21	2.92	3.41	4.27	5.69	13.24
	40	0.25	0.47	1.10	2.43	2.81	3.46	4.67	11.80
0.3	17	0.30	0.38	0.78	1.49	0.83	1.75	2.56	2.84
	25	0.29	0.37	0.73	1.37	0.78	1.63	2.28	2.69
	38	0.28	0.36	0.67	1.29	0.74	1.44	1.95	2.52
0.4	15	0.27	0.34	0.66	1.19	0.52	1.35	2.21	1.93
	25	0.26	0.34	0.62	1.05	0.49	1.16	1.95	1.81
	42	0.26	0.33	0.58	1.05	0.48	1.16	1.73	1.74
0.5	14	0.25	0.32	0.60	0.97	0.41	1.18	2.08	1.42
	41	0.24	0.31	0.57	0.94	0.40	1.11	1.89	1.32
1.0	49	0.20	0.24	0.47	0.84	0.29	0.78	1.28	0.82
	60	0.19	0.24	0.45	0.83	0.28	0.74	1.23	0.80
	95	0.18	0.23	0.49	0.86	0.27	0.75	1.15	0.83
	142	0.20	0.25	0.56	0.95	0.31	0.93	1.24	0.89
2.5	118	0.18	0.22	0.43	0.64	0.21	0.58	1.00	0.50
	204	0.19	0.23	0.45	0.67	0.21	0.60	0.94	0.51
	258	0.22	0.24	0.48	0.72	0.21	0.64	0.94	0.51
	310	0.22	0.24	0.49	0.75	0.21	0.67	0.92	0.51
3.5	107	0.17	0.22	0.37	0.56	0.17	0.52	0.87	0.40
	201	0.18	0.22	0.53	0.70	0.18	0.68	0.97	0.42
	305	0.17	0.23	0.60	0.72	0.18	0.71	0.98	0.41
	365	0.17	0.23	0.61	0.73	0.19	0.72	0.98	0.42
5.0	98	0.16	0.21	0.35	0.47	0.14	0.44	0.68	0.36
	245	0.17	0.23	0.37	0.54	0.17	0.50	0.70	0.37
	394	0.18	0.22	0.44	0.57	0.18	0.52	0.71	0.39
7.5	90	0.17	0.21	0.34	0.43	0.13	0.38	0.67	0.29
	200	0.17	0.21	0.35	0.44	0.13	0.39	0.67	0.29
	435	0.18	0.19	0.37	0.45	0.14	0.41	0.65	0.29
10.0	106	0.16	0.18	0.32	0.40	0.11	0.37	0.63	0.23
	238	0.16	0.18	0.30	0.39	0.10	0.37	0.58	0.22
	348	0.17	0.18	0.31	0.41	0.11	0.35	0.56	0.21
	646	0.16	0.16	0.32	0.41	0.11	0.36	0.58	0.23
	979	0.18	0.19	0.34	0.44	0.13	0.38	0.62	0.27

the competition model (*i.e.*, eqn. 3), the capacity ratios of a number of solutes were plotted double-logarithmically against the moderator concentration with the smallest attainable water content (Figs. 2a and 3a). The broken lines in these figures show the adsorption isotherm of the moderator as measured by means of breakthrough curves. For BuOH a clear saturation curve is obtained. The saturation value of the adsorbed amount of BuOH is about 70 mg per gram of silica. With a surface area of 400 m²/g for the adsorbent used, this corresponds to a mean layer thickness of about 2.2 Å;

Echinenone	Canthaxanthin	Phenol	Aniline	Benzyl alcohol	Catechol	Resorcinol	Hydroquinone	Luteine	Zeaxanthin
4.23	68.10								
4.07	58.05								
3.72	45.62								
2.90	26.14	11.89	15.05	28.85					
2.70	22.13	11.64	13.84	25.66					
2.58	18.89	10.94	11.89	21.93					
1.66	14.49	9.55	11.30	20.43					
1.54	13.18	9.11	9.21	17.19					
0.98	5.94	4.00	7.72	11.01					
0.94	5.67	3.88	7.45	10.14					
0.96	5.70	4.32	6.63	10.20					
0.97	5.75	5.80	6.14	10.75					
0.61	2.36	1.86	5.53	4.35	4.94	10.15	16.58	16.56	18.24
0.63	2.50	1.91	5.00	4.46	5.42	11.75	19.07	18.32	20.52
0.64	2.60	2.12	4.86	4.72	6.35	14.66	20.55	19.10	21.78
0.66	2.61	2.25	4.76	4.76	7.21	17.31	21.44	19 44	22 30
0.54	1.82	1.14	5.07	3.35	3.04	5.30	8.77	9.24	10.04
0.55	1.92	1.34	5.05	3.55	3.10	6.02	9.22	10.19	10.70
0.57	2.03	1.50	4.85	3.62	3.45	7.10	10.58	10.64	10.91
0.57	2.12	1.57	4.60	3.64	3.72	7.76	11.21	10.70	11.12
0.44	1.28	0.71	4.46	2.32	2.01	2.89	4.48	5.06	5.53
0.47	1.35	0.88	3.90	2.41	1.99	3.32	5.14	5.85	6.02
0.49	1.44	0.89	3.65	2.44	2.14	3.60	5.61	5.94	6.20
0.38	0.90	0.48	3.96	1.62	1.19	1.44	2.06	2.36	2.55
0.38	0.91	0.49	3.47	1.60	1.18	1.45	2.08	2.42	2.60
0.39	0.95	0.53	3.22	1.54	1.14	1.44	2.15	2.52	2.69
0.33	0.64	0.37	3.29	1.23	0.77	0.82	0.86	1.36	1.46
0.33	0.64	0.35	2.77	1.12	0.68	0.74	1.13	1.40	1.50
0.34	0.70	0.34	2.77	1.11	0.70	0.81	1.18	1.45	1.54
0.35	0.75	0.37	2.47	1.11	0.74	0.89	1.27	1.55	1.62
0.38	0.79	0.46	2.47	1.18	0.91	1.08	1.53	1.67	1.73

and to a molecular area occupied by one molecule of 67 Å^2. These values and the distinct saturation strongly suggest the existence of a monolayer.

With EtA, a completely different curve is found. The highest level observed, 123 mg per gram of silica, corresponds to a layer thickness of 3.4 Å and a molecular area of 48 Å^2. These values suggest that at high concentrations adsorption in excess of monolayer formation occurs. The S-shape of the curve suggests a similar interpretation.

TABLE II

CAPACITY RATIO AS A FUNCTION OF THE PERCENTAGE OF EtA AND WATER IN i-OCT

| EtA concentration (%, v/v)	H_2O (ppm)	Benzene	Anthracene	Anisole	Nitrobenzene	Ethyl β-apocarotenoate	Phenol	Aniline	Catechol	Canthaxanthin	Resorcinol
1.0	18	0.49	0.72	1.02	2.58	8.10					
	34	0.49	0.72	0.96	2.59	7.68					
	68	0.49	0.71	0.93	2.58	7.20					
2.5	20	0.31	0.60	0.70	2.02	2.85	12.69	24.68			
	61	0.31	0.60	0.69	2.02	2.71	14.42	18.21			
	102	0.31	0.58	0.68	1.98	2.50	16.10	15.97			
10.0	68	0.15	0.35	0.36	1.09	0.49	2.31	7.69	9.46	14.23	26.34
	287	0.14	0.36	0.36	1.10	0.49	2.60	5.92	10.44	13.51	31.07
	403	0.15	0.36	0.36	1.11	0.49	2.91	5.47	12.43	12.71	38.55
25.0	198	0.07	0.21	0.20	0.54	0.10	0.70	2.40	1.99	1.70	3.38
	368	0.08	0.21	0.21	0.52	0.10	0.74	2.09	2.05	1.70	3.51
	710	0.08	0.20	0.20	0.53	0.10	0.76	1.95	2.21	1.66	3.84
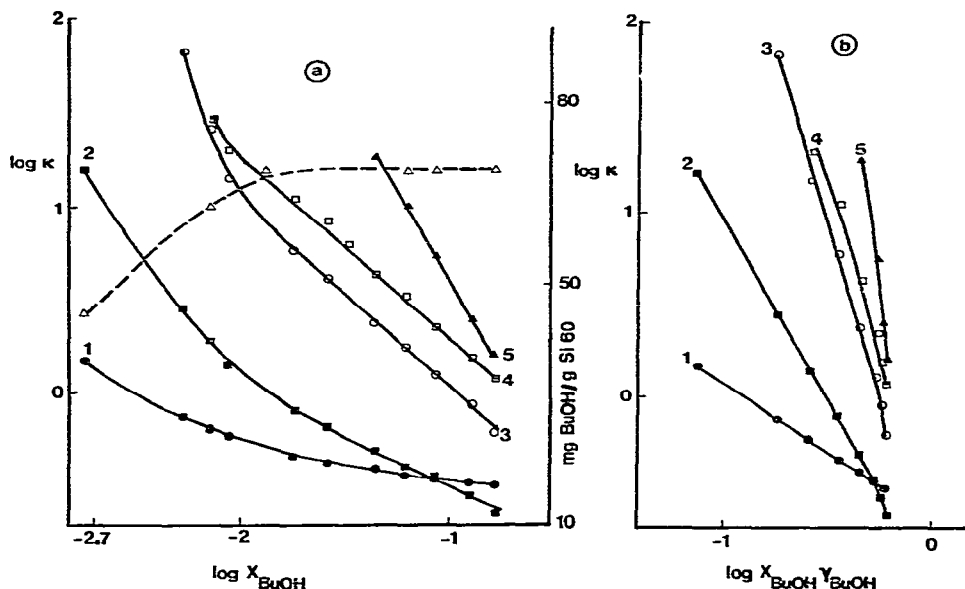
Fig. 2. (a) Dependence of log κ on log molar fraction of BuOH in i-Oct. 1 = Nitrobenzene; 2 = β-apocarotenal; 3 = canthaxanthin; 4 = benzyl alcohol; 5 = zeaxanthin. △– – –△. Amount of BuOH adsorbed per gram of silica. (b) Dependence of log κ on log activity of BuOH. Symbols as in (a).

The retention data in these figures show a linear relationship between log κ_i and log X_B at higher moderator concentrations, as expected on the basis of the competition theory. At low moderator concentrations, however, for a number of solutes deviations from linearity are observed. The direction of these deviations is opposite to that expected from the theory as shown in Fig. 1. This deviation coincides with the steep part of the adsorption isotherms.

For the proper interpretation of retention data one has to include the activity coefficients of the solutes as well as those of the moderators in both phases. This was shown theoretically, e.g., by Snyder[1], Locke[23] and Eon[24]. In practice, however, this concept is not applied in the majority of the discussions on LSC equilibria. In the present work the activity coefficients of the solutes were not available and were difficult to determine. For the moderator, however, the activity coefficient can easily be determined by headspace analysis. The activity coefficients of BuOH in i-Oct at different concentrations as determined by headspace analysis are given in Table III. For EtA, the values of Slaats et al.[19] were used. In terms of activity coefficients, eqn. 3 must be written as

$$\log\left(\frac{\kappa_i}{\gamma_{im}\,\bar{v}_m}\right) = C_1' - \frac{A_{si}}{n}\cdot\log C_2\,\gamma_B\,X_B \tag{7}$$

where $\bar{v}_m$ is the molar volume of the mobile phase, γ_{im} is the activity coefficient of component i in the mobile phase and γ_B is the activity coefficient of the moderator.

Figs. 2b and 3b show the correlation between the capacity ratio and the moderator concentration corrected for the actual activity coefficients. It can be seen
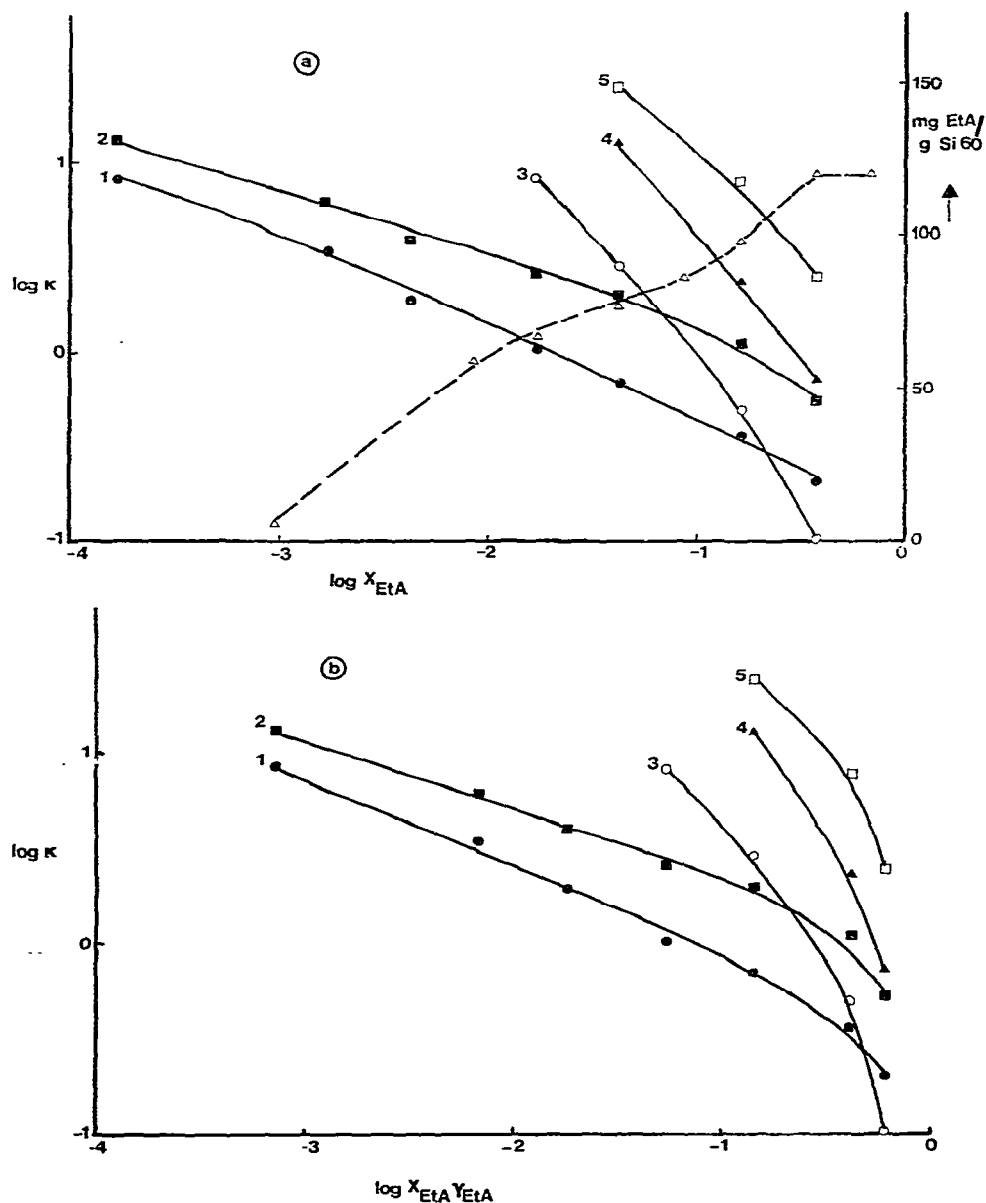
Fig. 3. (a) Dependence of log κ on log molar fraction of EtA in i-Oct. 1 = Nitrobenzene; 2 = anisole; 3 = ethyl β-apocarotenoate; 4 = phenol; 5 = aniline. $\triangle$---$\triangle$, Amount of EtA adsorbed per gram of silica. (b) Dependence of log κ on log activity of EtA. Symbols as in (a).

that for BuOH the linear correlation for a number of solutes fits over a much wider moderator range. Nearly all solutes, however, now show a deviation from linearity at larger moderator concentrations. This deviation can be corrected for when correcting for the activity coefficient of the solute in the mobile phase, as was shown by Slaats et al.[19].

TABLE III

ACTIVITY COEFFICIENTS OF BuOH IN i-OCT

BuOH (%, v/v)	X_{BuOH}	γ_{BuOH}
0.1	0.00179	42.66
0.3	0.00541	34.67
0.5	0.00900	28.71
1.0	0.0179	20.08
2.5	0.0443	10.41
5.0	0.0869	6.35
7.5	0.1279	4.57
10.0	0.1673	3.63

It should also be noted that the linear ranges at the lower concentration ends in Figs. 2b and 3b are significantly larger than expected on the basis of the competition model for a homogeneous surface, which would predict a tendency of the retention to become constant as soon as the monolayer of the moderator breaks down. For the moment we assume that the heterogeneity of the adsorbent is responsible for this deviation from theory. This heterogeneity would not necessarily obviate the whole interpretation, as could be suggested, because at higher moderator concentrations and with more complete coverage of the surface, Snyder's linearization effect for strong moderators would certainly restore "normal" behaviour to the system. Detailed tests of the homogeneity of silica gels are certainly needed.

Influence of addition of water

Table IV summarizes the solubilities of water in the solvent systems used. A major question that arises when water is present, on purpose or unintentionally, in a binary mixture, is whether water displaces the organic modifier or whether it is competitive with this modifier in the adsorption on the silica gel.

A column was equilibrated with a wet mixture with a fixed percentage of organic modifier, at such a low value that a partly covered surface would result in a dry system. The amount of adsorbed water was determined by calculation from the total amount introduced and the amount found in the mobile phase by Karl Fischer

TABLE IV

SOLUBILITY OF WATER IN BINARY MIXTURES OF BuOH AND EtA IN i-OCT

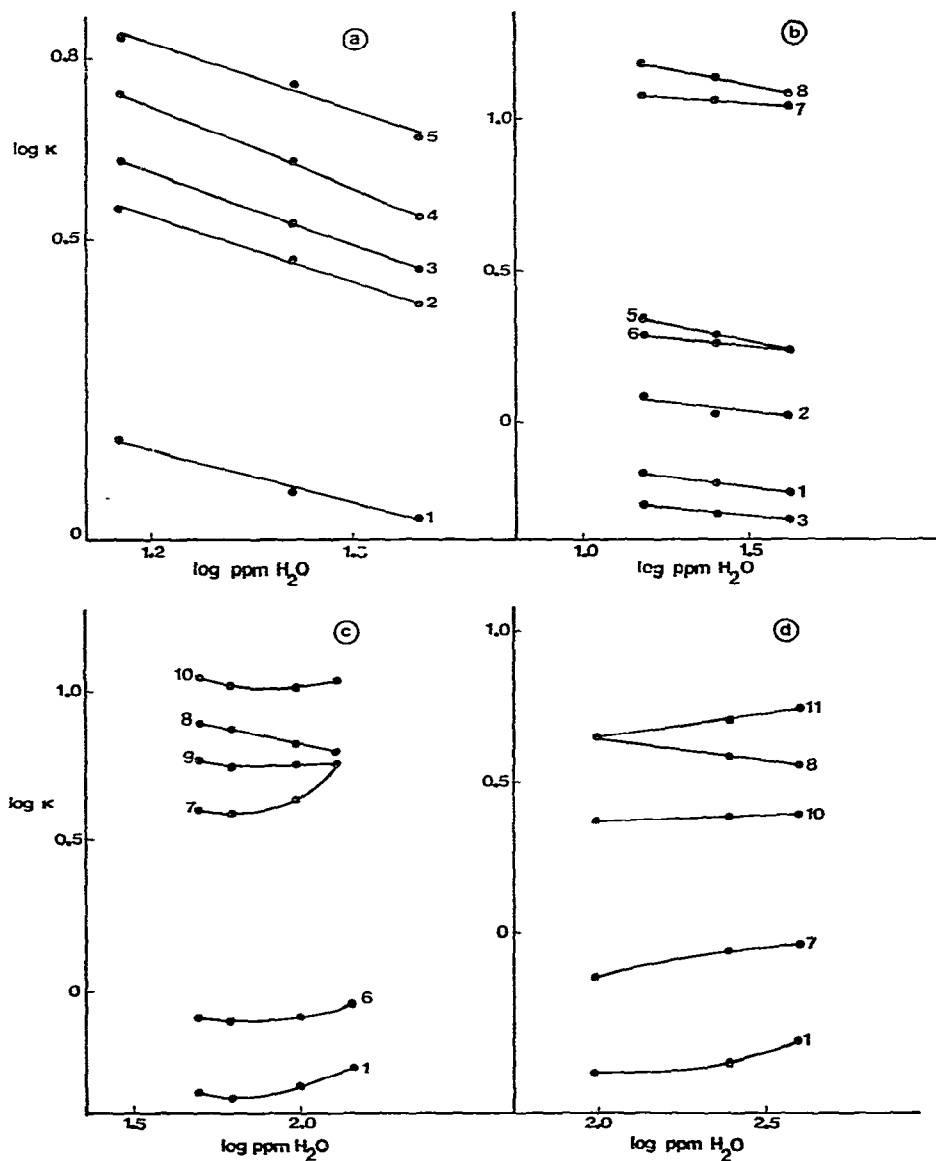| Concentration of moderator (%, v/v)	i-Oct–BuOH	i-Oct–EtA
	ppm H_2O	ppm H_2O
0.1	49	43
0.5	60	54
1.0	148	76
2.5	571	148
5.0	1374	212
10.0	3810	451
25.0	—	1685
50.0	—	6264

Fig. 4. Double-logarithmic plot of the capacity ratio *versus* the water content in the mobile phase at different molar fractions of BuOH in *i*-Oct: (a) 0.00179; (b) 0.00721; (c) 0.0179; (d) 0.0869. 1 = Nitrobenzene; 2 = benzonitrile; 3 = ethyl β-apocarotenoate; 4 = 1,4-dinitrobenzene; 5 = acetophenone; 6 = β-apocarotenal; 7 = phenol; 8 = aniline; 9 = canthaxanthin; 10 = benzyl alcohol; 11 = hydroquinone.

titration, after equilibration. The amount of adsorbed organic modifier was determined from a breakthrough curve obtained after switching to a dry 10% solution of the modifier.

The results obtained in these experiments coincided with those obtained in a

dry system (see Figs. 2a and 3a); in other words, adsorption of BuOH and of EtA is independent of the water content, at least in the concentration ranges investigated (0–500 ppm of water). Water seems to be a non-competitive adsorbate with respect to BuOH and EtA. Even the adsorption of 30 mg of water on the column results in the same adsorbed amount of BuOH (79 mg), at least within limits of experimental error.

Figs. 4a–4d show the influence of water on retention in BuOH mixtures. Considering first percentages of BuOH up to 1 % (Figs. 4a–4c), the slope of the curves is in agreement with the competition model, as formulated in eqns. 5 and 6, with high negative slopes for low BuOH contents, decreasing to zero with increasing BuOH content. However, this explanation does not agree with the observations mentioned above on the simultaneous adsorption of BuOH and water. For instance, the slopes in Fig. 4 are in the region of 0.3–0.4. Those of the plots of log κ_i versus log X_B (Fig. 2a) are in the region of 0.2–0.5. This would be in agreement with competition theory only if substantial displacement of BuOH by water occurs. As mentioned above, no such displacement can be observed experimentally.

When the slopes of the retention data plots are used for the calculation of ε_{H_2O}, by considering the relationships between these slopes and the relative coverages of the two moderators, we find values in the region of 0.7–0.9 for ε_{H_2O}, which is surprisingly low. Such a value can only be considered as an empirical correlation constant, valid within a narrow range of conditions.

The complexity of the phenomena is even more accentuated in Fig. 4d, where an increase in retention with increasing water content is observed for nearly all compounds.

For EtA–i-Oct mixtures with different water contents, similar effects can be observed (Figs. 5a–5d).

From these results, it can be inferred that the role of water in a normal LSC system with mixed organic solvents cannot be described by simply considering water as a competitor in the adsorption equilibrium. The observations rather lead to conjectures into the following directions:

(i) Formation of adsorbed layers exceeding the size of a monolayer, either by one moderator (EtA) or by two moderators (water + BuOH and water + EtA) forming a binary layer. There is only one instance studied here (BuOH without water) where a distinct monolayer is observed.

(ii) Where multilayer formation occurs, the simple competition description is invalidated, because adsorption of a solute no longer involves an exchange reaction.

(iii) Binary multilayer formation yields an extra deviation from the simple picture, because the physical environment of an adsorbed molecule would change with changing conditions, as the composition of the adsorbed layer is not constant. This effect might be held responsible for the increase in retention observed with higher water contents observed in some instances.

From a practical point of view, the results given above lead to the important conclusion that control of the water content is also very important when strong moderators such as BuOH and EtA are used in high concentrations. Insufficient control of water content would lead to a loss of reproducibility of retention, and especially to changing selectivities.

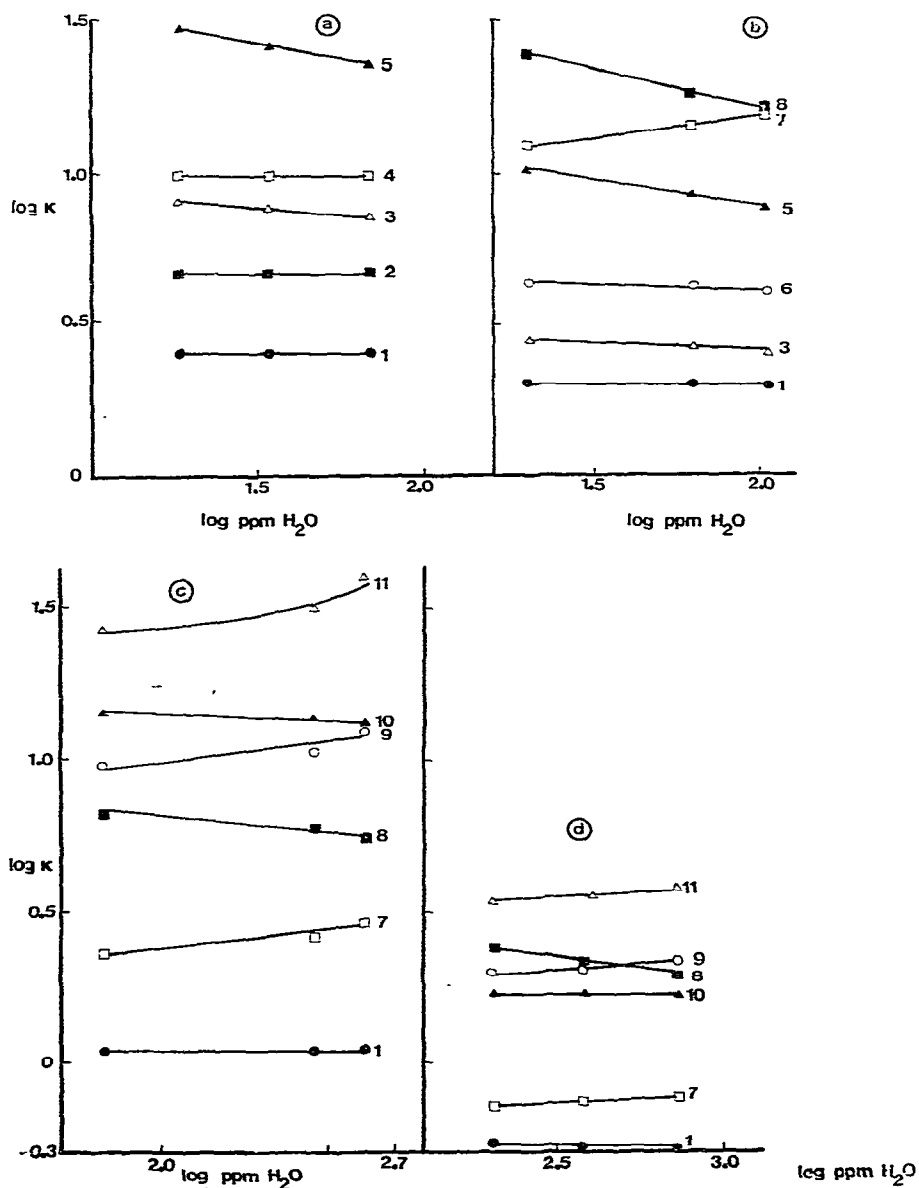Column efficiency was noticed to be practically independent of the composition

Fig. 5. Double-logarithmic plot of the capacity ratio *versus* the water content in the mobile phase at different molar fractions of EtA in i-Oct: (a) 0.0169; (b) 0.0418; (c) 0.1589; (d) 0.3617. 1 = Nitrobenzene; 2 = benzonitrile; 3 = ethyl β-apocarotenoate; 4 = 1,4-dinitrobenzene; 5 = β-apocarotenal; 6 = acetophenone; 7 = phenol; 8 = aniline; 9 = catechol; 10 = canthaxanthin; 11 = resorcinol.

of the mobile phase. Also peak shapes, with respect to symmetry, were found to be rather constant. Especially effects as observed by Kirkland[25], who found anomalous peak shapes when using alcohols as moderators at low percentages, were not noticed. Some typical chromatograms are given in Fig. 6.
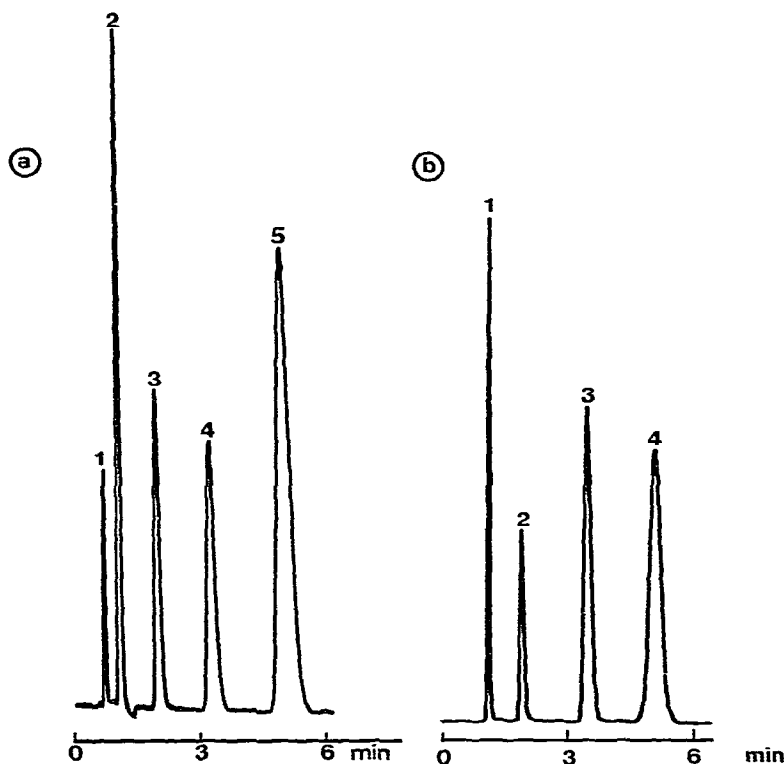
Fig. 6. Chromatograms illustrating peak shape on Si 60. Column: 25 cm × 3.0 mm I.D. (a) Eluent: i-Oct–EtA (1 %, v/v), 68 ppm H_2O; pressure, 150 atm; flow-rate, 1.94 ml/min; detection at 254 nm. 1 = Benzene; 2 = anisole; 3 = nitrobenzene; 4 = benzonitrile; 5 = acetophenone. (b) Eluent: i-Oct–BuOH (0.3 %, v/v), 38 ppm H_2O; pressure, 80 atm; flow-rate, 0.98 ml/min; detection at 460 nm. 1 = β-Carotene; 2 = ethyl β-apocarotenoate; 3 = β-apocarotenal; 4 = echinenone.

CONCLUSION

Correlation of retention data in normal-phase adsorption chromatography within a simple competition model, although significantly improved by accounting for the change in the activity coefficient of the moderator, has been shown to be poor. Future work on the interpretation of these equilibrium data, in our opinion, should involve more extensive measurements of liquid phase activity coefficients, an investigation of the homogeneity of silica surfaces and measurement of the adsorption isotherms of moderators on these surfaces. Experimental work on these aspects is in progress.

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