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THEORETICAL BASIS FOR SYSTEMATIC OPTIMIZATION OF MOBILE PHASE SELECTIVITY IN LIQUID–SOLID CHROMATOGRAPHY

SOLVENT–SOLUTE LOCALIZATION EFFECTS

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SUMMARY

The optimization of retention in liquid–solid chromatography (LSC) is explored in the present paper. Previously it was shown possible to calculate solvent strength (ε^0 values) for multi-component mobile phases, and specifically for quaternary solvent mixtures A–B–C–D. With ε^0 held optimum and constant for a particular sample, the composition of A–B–C–D can be further varied for optimization of separation factors α (solvent selectivity) for various solute-pairs in the sample of interest. The selection of optimum pure solvents A–D for this purpose and the systematic variation in the proportions of these solvents for optimum separation are approached here in terms of a fundamental description of how solvent selectivity arises in LSC. In this paper we discuss two major contributions to solvent selectivity: solvent/solute localization and solvent-specific localization. In a later paper we apply these findings for the development of a systematic approach to the optimization of retention in LSC separation.

INTRODUCTION

Optimization in liquid chromatography (LC) refers to the selection of experimental conditions for adequate separation and acceptable elapsed time per sample. Most optimization strategies are based on eqn. 1 for resolution, R_s[1]:

$$R_s = \frac{1}{4} \cdot (\alpha - 1) \cdot \sqrt{N} \cdot [k'/(1 + k')] \qquad (1)$$

Here, α is the separation factor (k_X/k_Y), N is the plate number of the separation system (column or bed) and k' is the average capacity factor for bands X and Y (k_X

and k_V). It is customary to separately optimize the terms α, N and k' of eqn. 1 for a given separation. The optimization of N in column chromatography (so-called high-performance liquid chromatography, HPLC) is now on a sound theoretical basis[1–5], which allows calculations of preferred conditions for the best compromise between large N and short separation time, t. The optimization of retention (k' and α) is less well understood, and is usually approached more empirically.

Strategies for retention optimization in LC fall into one of three groups:

(1) empirical (trial-and-error) approaches guided by experience and whatever theory is available[1]

(2) statistical-design or computer-search routines which allow intelligent guesses for successive trial-and-error changes in conditions[6–8]

(3) development of an overall theory of retention as a function of separation conditions; this would then allow the development of optimization schemes based on preselection of a small number of well-chosen LC systems, followed by interpolation to an optimum system for a given sample.

Several LC variables are discontinuous in nature (*e.g.*, selection of mobile phase solvents A, B, C, ..., choice of a particular adsorbent, etc.) so that only the third approach above offers the possibility of absolute optimization, *i.e.*, choosing conditions that provide the best possible separation of a given sample.

In this paper we consider the third approach to optimizing LC separation. Earlier papers[5,9] have illustrated how optimization in this fashion might proceed, based on partial theories of retention for reversed-phase LC. However, adequately complete theories of retention –particularly as regards sample α values— have not yet been presented for any of the LC methods. Here we examine one particular LC method: liquid–solid (adsorption) chromatography (LSC). Retention for LSC is better understood at present than for the remaining LC methods[10–14]; LSC therefore offers a better opportunity for exploring the possibility of the third approach to optimization. Aside from being of value in its own right, optimization in LSC may offer guidance for a similar approach to the other LC methods.

A PRACTICAL SUMMARY OF THE PRESENT PAPER

A general approach to optimizing retention in LSC is as follows:

(1) determine the best solvent strength ε^0 of the mobile phase for optimum k' values ($0.5 \leqslant k' \leqslant 20$) of the given sample; this is done by varying the composition (% v/v B) of a mobile phase A–B which consists of a weak solvent A and a strong solvent B.

(2) while holding ε^0 constant, further vary conditions for an optimum spacing of sample bands within the chromatogram (maximum α values); this can be done in various ways: (a) vary the mobile phase composition by substituting other strong solvents (C, D, ...) for solvent B (Ch. 9 of ref. 1); (b) vary the adsorbent chosen as column packing; silica is the usual first choice, but alumina offers different selectivity for some samples[10]; (c) vary the temperature of the column; often temperature has no significant effect on α values; however, exceptions have been noted[15]; (d) take advantage of special chemical effects via change in mobile phase pH or the use of complexing agents (*e.g.*, silver ion for olefins); this approach is limited to samples that are acidic or basic, and/or can undergo complexation. In this paper we consider only the

theory of LSC solvent strength as a function of mobile phase composition (1, above), and the variation in α values as a result of change in mobile phase composition (2a). This will in turn allow a major improvement in our ability to develop final LSC methods with a minimum of experimental effort. Further improvement in this approach (options 2b–d above) can best be attempted after we achieve a good understanding of steps 1 and 2a. An optimization scheme based on steps 1 and 2a is presented in a later paper[16].

Once a mobile phase composition (A–B) has been found that has the optimum ε^0 value for the sample of interest, we need to be able to calculate other mobile phase compositions (A–C, A–D, ...) that give the same value of ε^0. These new mobile phases will then allow us to vary separation selectivity for improved resolution. It has been found elsewhere[9,17–19] that mobile phases containing more than two solvents (*e.g.*, A–B–C) are required for the maximum exploitation of selectivity in reversed-phase LC, when the sample contains several components (this is not the case for a two-component sample). This is true of LSC as well. Therefore, a general theory is needed for the calculation of ε^0 for any multi-component mobile phase. In practice, ternary and quaternary-solvent mobile phases will be used. Previous papers[13,14] have shown that it is possible to calculate the strength of multicomponent mobile phases in LSC with adequate precision.

Changes in α which result from a change in the mobile phase composition are presumably due to various physico-chemical phenomena that affect sample retention. These changes in α are very much affected by the particular sample (mixture of solutes) selected for study, and there are an almost unlimited number of possible mobile phase compositions from which to choose. Consequently, the empirical study of mobile phase selectivity will be quite complex, and there is little hope that the results obtained will apply to all possible samples. On the other hand, there are a much smaller number of these discrete physico-chemical effects that can contribute significantly to sample α values. If we can identify these effects and relate them to mobile phase composition in a way that is independent of the nature of the sample to be separated, we can bypass much of the potential difficulty in understanding and using mobile phase selectivity.

Several of these physico-chemical effects have already been identified in LSC systems[10,11]:

solvent/solute localization
solvent strength
hydrogen-bonding in the stationary phase
hydrogen-bonding in the mobile phase

In this paper we examine solvent–solute localization. At this time we believe that their contribution to sample α values is generally more important than are hydrogen-bonding effects. Later papers will discuss hydrogen-bonding and its exploitation in a total-optimization scheme for LSC. Table I summarizes such an approach.

Solvent strength and mobile phase composition

Preceding papers[13,14] have described a general model of solvent strength in LSC for the case of mobile phases that contain two to four solvent components. This model is based upon a displacement mechanism of solute retention in LSC. Thus, for adsorption of a solute X from a mobile phase B, it is assumed that one molecule of X

TABLE I

RETENTION OPTIMIZATION IN LSC: CLASSIFICATION OF A TOTAL APPROACH

hex = Hexane; MC = methylene chloride; MTBE = methyl *tert.*-butyl ether; ACN = acetonitrile; TEA = triethylamine; M = methanol.

Effect		*Solvent variables*	
1	Optimize solvent strength in terms of k'	A–B	hex–MC
2a	Optimize selectivity in terms of polar solvents (B, C, B–C, etc.) selected for the mobile phase		
	Optimize solvent/solute localization (value of m)	A–B–C	hex–MC–MTBE
	Optimize solvent-specific localization	A–B–C–D	hex–MC–MTBE–ACN
	Fine-tune solvent strength	A–B–C–D	vary N_A
	Create solvent–solute hydrogen-bonding in stationary phase		
	Use proton-acceptor (basic) solvent	A–B–C–E	hex–MC–MTBE–TEA
	Use proton-donor (acidic) solvent	Less effective	
	Create solvent–solute hydrogen-bonding in mobile phase		
	Use proton-donor (acidic) solvent	A–B–C–F	hex–MC–MTBE–M
	Use proton-acceptor (basic) solvent	Less effective	
2b	Optimize column packing (adsorbent type)	Silica, alumina; repeat steps 1 and 2a*	
2c	Optimize separation temperature	After step 2a**	
2d	Optimize pH, add complexing agents	After 2a***	

* Solvent strength and selectivity must be reoptimized when adsorbent is changed.

** Increase N_A to compensate for lower k' at higher temperatures; maintain other N values in same ratio (*e.g.*, N_C/N_B constant).

*** Do not change N_A, N_B, etc.

in the mobile phase (X_n) displaces some number, n, of solvent molecules B from the stationary phase (B_a)

$$X_n + n\,B_a \rightleftharpoons X_a + n\,B_n \tag{2}$$

to give a molecule of adsorbed X (X_a) and n molecules of B in the mobile phase (B_n). The effect of solvent strength, ε^0, on sample retention is then given as

$$\log (k_1/k_2) = \alpha' A_s (\varepsilon_2 - \varepsilon_1) \tag{3}$$

where for a given solute X, k_1 and k_2 refer to k' values for mobile phases 1 and 2, ε_1 and ε_2 refer to solvent strength (ε^0) values for mobile phases 1 and 2, α' is an adsorbent activity parameter and A_s refers to the cross-sectional area of the molecule X. Values of ε^0 for multicomponent mobile phases can be related to the mole fractions, N_i, of each solvent component i in the mobile phase, and to the ε^0 values (ε_i) of each pure solvent i. For so-called "localizing" solvents j (see next section), the value of ε_j varies with the mole fraction, θ_j, of j in the stationary phase. The stationary phase is assumed to consist of a monolayer of adsorbed solvent molecules. Values of ε_j are relatively constant ($\varepsilon_j = \varepsilon'$) at low values of θ_j, but ε_j decreases as θ_j approaches a

value of 0.75 (toward a lower limiting value ε'' for $\theta_j > 0.9$). The condition $\theta_j \approx 0.75$ corresponds to the approximate completion of a "localized" layer of solvent molecules j in the stationary phase. Further filling of the monolayer by j ($\theta_j > 0.75$) corresponds to adsorption of non-localized j molecules. The treatment of refs. 13, 14 yields values of θ_i for all mobile phase components i. The next section suggests that solvent selectivity is also dependent upon values of θ_i for different mobile phase components i.

Fig. 1a plots experimental values of ε^0 *vs.* values calculated according to ref. 13, for 98 different binary-solvent mobile phase compositions and 22 different strong solvents (B, C, ...). Similarly, Fig. 1b plots data calculated according to ref. 14 for mobile phases consisting of ternary and quaternary-solvent mixtures. The experimental data are taken from the review[13] as well as from ref. 14 and the present paper. These data suggest that the procedures of refs. 13, 14 allow the prediction of ε^0 in LSC within ± 0.02 units. This is adequate for selecting optimum-strength mobile phase compositions, since resolution, R_s, is not a sensitive function of k', when $k' > 1$.

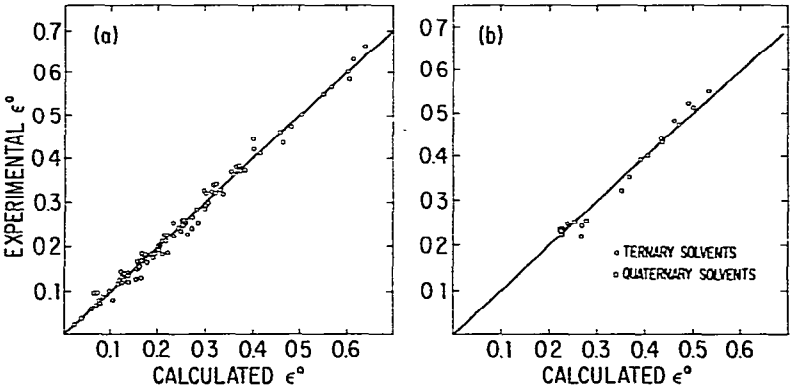

Fig. 1. Comparison of experimental solvent strength values for multi-component mobile phases with calculated values as in refs. 13, 14 for alumina and silica as adsorbents. a, Binary-solvent mobile phases 13; b, mobile phases containing three (○) or four (□) solvents (from ref. 14 and present study).

Solvent selectivity: solvent/solute localization

This selectivity effect has been discussed previously[12,20], but not developed to the degree required for optimization as in a later paper[16]. Localization refers to the direct interaction of the most polar substituent group, k, in a molecule of solute or solvent with a corresponding polar adsorption site which forms part of the adsorbent surface. Localization of a polar solvent molecule, C, is illustrated in Fig. 2a–d. In the case of silica as adsorbent, the polar surface sites are silanols (≡Si–OH), as illustrated in Fig. 2a for a side view of the silica surface. These silanol groups are shown as asterisks in Fig. 2b from an overhead view of the silica surface; they are randomly distributed across the surface, which accords with the current belief that porous silicas are non-crystalline[10]. Adsorbing solvent molecules C are shown in Fig. 2c, d as discs, with the polar group k centered on one face of the disc (this assumed molecular shape is arbitrary). Fig. 2c shows a side view of the adsorbed monolayer of solvent C, with two of the three C molecules (i), shown centered over adsorbent sites,

i.e., these molecules (i) are adsorbed with localization. The third molecule of C in Fig. 2c (ii) cannot assume the optimum configuration required for localization and is therefore delocalized. Fig. 2d shows an overhead view of the monolayer of adsorbed solvent C. Molecules of delocalized C are shown as broken curves, while localized molecules are shown as full circles. Delocalization can be the result of either (a) imperfect alignment of the solvent group k with a surface site (ii in Fig. 2c, iia in Fig. 2d), or (b) crowding of adsorbed C by adjacent molecules in the monolayer (iib in Fig. 2d, *i.e.*, steric hindrance to adsorption). Localized molecules (i) are held much more strongly to the adsorbent surface than are delocalized molecules (ii).

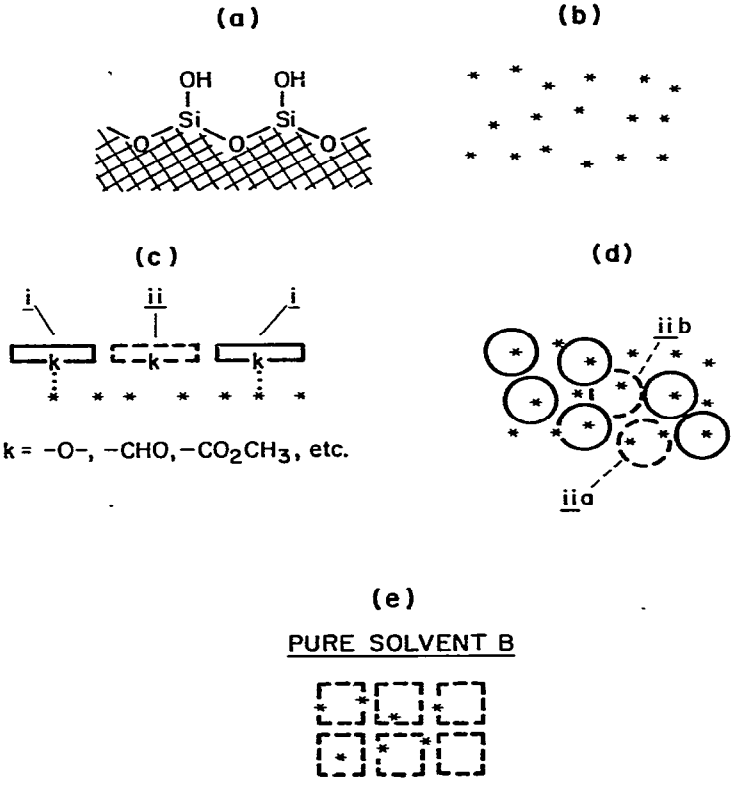

Fig. 2. Visualization of adsorbed solvent monolayer on silica, showing the localization of a polar solvent C. a, Side view of silica surface, showing silanol groups; b, overhead view of silica surface, with silanols shown as *; c, side view of monolayer of adsorbed solvent molecules C, with polar solvent-group k shown; d, overhead view of solvent monolayer in c; e, overhead view of adsorbed monolayer of solvent B (non-localizing). See text.

When a molecule of solute or solvent possesses no strongly polar group k, there is no reason for that molecule to prefer a specific position or configuration within the adsorbed monolayer, *i.e.*, all molecules will be delocalized. This is illustrated for a less polar solvent molecule, B, in Fig. 2e, where molecules of B in the adsorbed monolayer are shown as squares. Since all molecules of B are delocalized, they are shown as broken squares with no tendency toward centering of the molecule with respect to surface sites (*vs.* Fig. 2d for the localizing solvent C). Polar groups k which can cause

the localization of either solvent or solute molecules include such substituents as –O– (ether), –COR (ketone), $-CO_2R$ (ester), $-NR_2$ (amine) and other functional groups of similar polarity. Non-localizing solvents B are less polar compounds such as chloroform, dichloromethane, benzene and RCl, RBr or RI (monohaloalkanes). Localizing solvents C include ethers, esters, nitriles and alcohols.

In LSC separation, moderately polar solvents B and/or polar solvents C are generally used in admixture with a non-polar solvent A such as hexane, heptane or isooctane. Fig. 3 shows the resulting arrangement of adsorbed solvent molecules within the monolayer, for the solvent system A–B (Fig. 3a) and A–C (Fig. 3b). Adsorbed molecules A are shown as broken (*i.e.*, delocalized) triangles. The adsorption of a solute molecule X (localizing) or Y (non-localizing) from the mobile phase A–B is illustrated in Fig. 3c. In either case, because B is non-localizing, the adsorbing solute molecule displaces a non-localized molecule of B (or A). In the case of the adsorption of X or Y from a mobile phase A–C that is localized (Fig. 3d), X adsorbs with localization and must therefore displace a preadsorbed molecule of localized C. Because Y is non-localizing, it adsorbs by displacing a non-localized molecule of A (or C). If we assume that X and Y have the same k' values ($\alpha = 1$) in the system A–B (Fig. 3c), then the k' value of X must be less than that of Y ($\alpha \neq 1$) in the system A–C (Fig. 3d). The reason is that the free energy required to displace a solvent molecule in Fig. 3c is the same for both X and Y, because for each solute the displaced solvent molecule (A or B) is delocalized. In Fig. 3d, however, solute X (but not Y) must displace a localized molecule of C during adsorption, and the energy required for this will be greater than for displacement of a delocalized molecule of C (or A) by an adsorbing molecule of Y.

As a result of solvent/solute localization, a change from a localizing mobile phase (A–C) to a non-localizing mobile phase (A–B) can create large differences in solvent selectivity and the α values of various solute-pairs. The effect is limited to solutes which show some degree of localization, and is therefore more pronounced for more polar samples and the stronger mobile phases that are required for their optimum separation.

A quantitative model: binary-solvent mobile phases. In the general case, both solutes and solvents will exhibit varying tendencies toward localization, rather than being characterizable as simply "localizing" or "non-localizing". Thus, the effects of solute/solvent localization will increase with increasing tendencies toward localization of solute and solvent (*i.e.*, increase in polarity of the localizing group k in each molecule). The effect of solvent localization will also be more pronounced for higher mole fractions, N_C, of the localizing solvent in the mobile phase A–C, as illustrated in Fig. 4. Here, for a lower value of N_C and a resulting value of $\theta_C = 0.5$, a molecule of X can adsorb with localization by displacing a delocalized molecule of A. However, for a higher value of N_C, such that $\theta_C = 0.75$, localized adsorption of X requires displacement of a localized molecule of C. The reason is that with increase in θ_C from zero to 0.75, all adsorbing molecules C can localize onto the surface; when θ_C exceeds a value of 0.75, additional molecules of C adsorb without localization[13]. Therefore, the effect of solvent localization on selectivity will increase in magnitude with increase in N_C and θ_C, until $\theta_C \geqslant 0.75$.

Eqn. 3 already recognizes the localization of the solvent and solute. Thus, localization of the solvent leads to a predictable change in the value of ε^0 (ref. 13, 14),

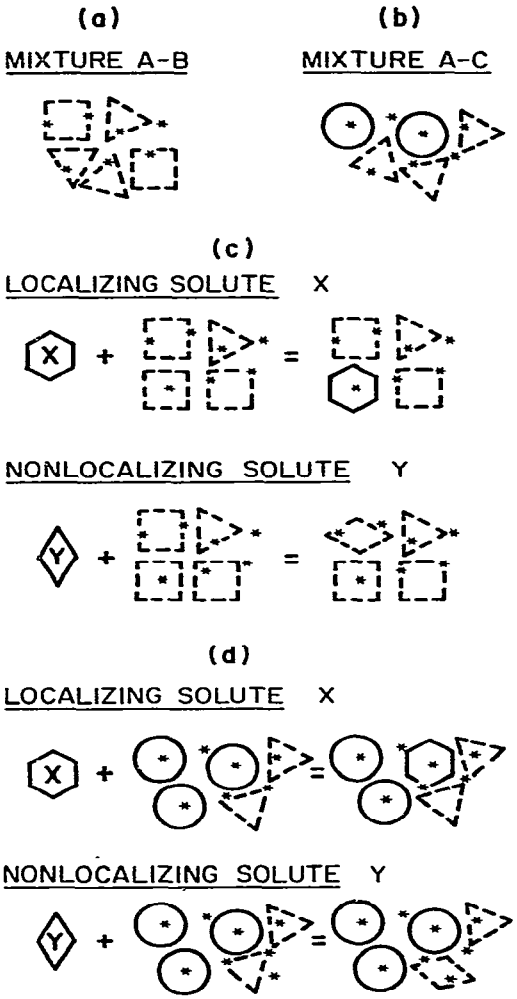

Fig. 3. Visualization of adsorption of localizing solute X and non-localizing solute Y from mobile phases A–B (non-localizing) and A–C (localizing); overhead view in each case. a, Solvent monolayer A–B (non-localizing); b, solvent monolayer A–C (localizing); c, adsorption of X and Y from mobile phase A–B (with displacement of adsorbed solvent molecule); d, adsorption of X and Y from mobile phase A–C (with displacement of solvent molecule). See text.

while localization of the solute leads to a change in its apparent A_s value (silica as adsorbent[10]). However, eqn. 3 does not take into account the *interaction* of these two effects as in Fig. 3c, d. Therefore, for the case of polar (*i.e.*, localizing) solutes and solvents, a term A_1 must be added to eqn. 3:

$$\log (k_1/k_2) = \alpha' A_s (\varepsilon_2 - \varepsilon_1) + A_1 \quad (4)$$

The term A_1 corrects eqn. 3 for the interaction of solute and solvent localization, and its effect on k'. From our discussion of Fig. 3 (see also ref. 20), it is clear that A_1

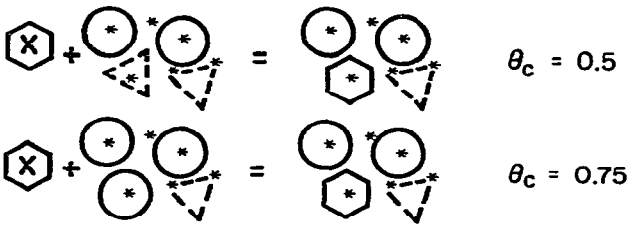

Fig. 4. Visualization of adsorption of solute X from localizing mobile phase A–C. Effect of surface coverage, θ_C, on solvent molecule displaced by X.

should depend upon both the nature of the solute (X) and the mobile phase (i); A_1 will become larger for increasing localization of both X and i. We therefore expect that A_1 will be a function of parameters A_X (solute) and m_i (mobile phase); A_X measures the relative localization of X, and m_i increases with both the degree of localization of some mobile phase solvent j and with its relative coverage of the adsorbent surface (θ_j). A linear-free-energy relationship between A_1 and the parameters A_X and m_i is expected, because A_1 is a free-energy term which is the result of the *interaction* of effects produced by solute localization and solvent localization; such a linear-free-energy relationship was verified experimentall[20]:*

$$A_1 = -A_X m_i \tag{5}$$

Because solute/solvent localization leads to decreased retention of the solute, the term A_1 is negative.

The expected increase of A_X with increasing localization of X has been observed[20]. Thus, solute localization increases with the polarity or adsorption energy, Q_k^0, of the most strongly adsorbing group k in the solute molecule, and A_X is found to increase with Q_k^0.

The solvent selectivity parameter, m, of eqn. 5 is of primary interest in terms of controlling separation. Thus, the sample components in a given LSC separation (and values of A_X for those solutes) are fixed, but we can vary mobile phase composition so as to change m and sample α values. As described in a following section, values of m can be related to mobile phase composition as follows. For the case of a mobile phase A–j, where the weak solvent A cannot localize and the strong solvent j can, the value of m is determined by the polarity of pure j (m°) and by the mole fraction, θ_j, of j in the adsorbed monolayer

$$m = m^0 \, f(\theta_j) \tag{6}$$

where $f(\theta_j)$ varies from zero for $\theta_j = 0$ to one for $\theta_j = 1$. Eqn. 6 is tested in Fig. 5a, where values of (m/m^0) are plotted *vs.* θ_j (data of ref. 20 for alumina) for several polar solvents. For a total of 35 mobile phases listed in Table II, it is found that m is predicted by eqn. 6 with an accuracy of ± 0.07 units (1 S.D.), for $-0.29 \leqslant m \leqslant 1.16$. The function $f(\theta_j)$ *vs.* θ_j used in this calculation is shown as the solid curve in Fig. 5a, b, and is listed in Table III. This function was determined as a best fit to the data of Fig. 5.

* Eqn. 5 is expressed in ref. 20 as $A = A^0 m$; we have changed the terminology here (see Glossary).

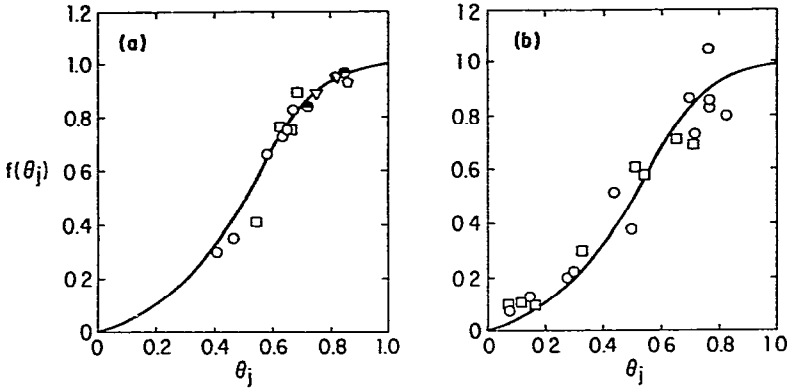

Fig. 5. Verification of eqn. 6 for dependence of solvent-selectivity function, m, on surface coverage, θ_j, by a localizing solvent j. a, Constancy of $m/m^0 = f(\theta_j)$ *vs.* θ_j for different solvents C and mobile phases A–C (alumina, data of Table II). ○, Solvent j is acetonitrile; , pyridine; □, acetone; ▽, tetrahydrofuran; ◓, ethyl acetate (see Table II). b, Dependence of $f(\theta_j)$ on θ_j for multi-component mobile phases and silica (data of this study and ref. 14, see Table V). ○, Solvent j is MTBE; □, acetonitrile. Solid curve in each case (a, b) is the function taken from Table III.

As expected, the solvent parameter m^0 increases with the adsorption energy Q_k^0 of the most polar solvent group k. Thus, as discussed in a later section, m^0 is 0.6 or larger for solvents with $Q_k^0 > 3.5$. For solvents with $Q_k^0 < 1.8$, m^0 is less than 0.4. Therefore, more polar solvents j with large values of m^0 (and ε^0) can provide larger values of Δ_1 and greater solvent selectivity variation. With such solvents, eqns. 5, 6 allow us to vary Δ_1 in continuous fashion over wide limits, by varying m via change in the concentration of j (N_j).

Consider next two solutes X and Y, with α referring to k_X/k_Y. Assume two mobile phases 1 and 2 which have equal strengths ($\varepsilon_1 = \varepsilon_2$), let α_1 and α_2 refer to α values in each mobile phase, and let m_1 and m_2 refer to their solvent selectivity (m) values. Further assume that the solvent selectivity m_2 for mobile phase 2 is equal to zero. From eqns. 4, 5 we can write

$$\log \alpha_1 = \log \alpha_2 + (\Delta_Y - \Delta_X)\, m_1$$

or

$$\log \alpha = C_1 + C_2\, m \qquad (7)$$

The constants C_1 and C_2 are now defined by the particular pair of solutes (X, Y) selected. Eqn. 7 is tested for representative data from ref. 20 in Fig. 6. The agreement of experimental data with the best fit of eqn. 7 in these plots is ± 0.05–0.06 log units (1 S.D.), for a range in log α of -0.2 to $+0.5$. Other solute-pairs from ref. 20 show comparable agreement with eqn. 7.

A quantitative model: mobile phases containing more than two solvents. Assume a mobile phase composed of solvents A, B, C, ..., where A is non-localizing ($m^0 = 0$) and solvents B, C, ... exhibit increasing localization —and therefore increasing values of m^0. The coverage of adsorption sites, with localization of the solvent molecule, can be pictured as proceeding in steps: initial adsorption of the strongest solvent j until its equilibrium surface coverage θ_j is attained, then adsorption of the next strongest

TABLE II

ANALYSIS OF SOLVENT-SELECTIVITY PARAMETER, m, FOR DATA FROM REF. 20

Alumina as adsorbent, binary-solvent mobile phases. Solvent A is pentane.

Mobile phase A–B (% v/v)		m*		θ_B**	m^0	Q_k^0
		Exptl.	*Calc.*			
1	Acetonitrile				1.31	5.0
	0.1	0.39	0.45	0.31		
	0.14	0.46	0.57	0.47		
	0.3	0.87	0.85	0.59		
	0.4	0.95	0.91	0.64		
	0.6	0.98	0.94	0.66		
	0.7	1.09	1.04	0.68		
2	Pyridine				1.22	4.8
	2	1.16	1.14	0.83		
	5	1.14	1.17	0.87		
3	Acetone				1.02	5.0
	0.2	0.42	0.58	0.55		
	0.4	0.79	0.74	0.64		
	0.6	0.77	0.80	0.67		
	0.8	0.91	0.83	0.70		
4	Tetrahydrofuran				0.82	3.5
	2	0.73	0.72	0.76		
	5	0.77	0.77	0.84		
5	Triethylamine				0.82	4.4
	5	0.77	0.77	0.83		
6	Ethyl acetate				0.77	5.0
	1	0.65	0.65	0.73		
	4	0.72	0.72	0.85		
7	Diethyl ether				0.62	3.5
	2	0.32	0.26	0.47		
	5	0.55	0.47	0.63		
	9	0.47	0.53	0.73		
	23	0.43	0.58	0.85		
8	1,2-Dichloroethane				0.35	1.8
	15	0.33	0.33	0.85		
9	Chloroform				0.34	0.7
	15	0.23	0.30	0.76		
	30	0.41	0.33	0.90		
10	Dichloromethane				0.29	0.8
	13	0.25	0.25	0.73		
	23	0.26	0.27	0.85		
	35	0.33	0.28	0.93		
	60	0.22	0.29	0.97		
	100	0.30	0.29	1.00		
11	Ethyl sulfide				0.29	2.6
	8	0.18	0.20	0.62		
	15	0.27	0.25	0.76		
12	Chlorobenzene				0.12	0.3
	30	0.12	0.12	0.91		
13	Bromoethane				0.08	2.0
	40	0.08	0.08	0.87		

(Continued on p. 310)

TABLE II *(continued)*

	Mobile phase A–B (% v/v)	*m**		θ_B**	m^0	Q_k^0
		Exptl.	*Calc.*			
14	2-Chloropropane				0.02	1.8
	35	0.02	0.02	0.79		
	60	0.02	0.02	0.91		
15	Perchloroethylene				0.03	0.3
	100	0.03	0.03	1.00		
16	Carbon tetrachloride				−0.09	0.3
	50	−0.08	−0.08	0.78		
17	Benzene				−0.15	0.3
	15	−0.04	−0.13	0.72		
	28	−0.02	−0.14	0.86		
	50	−0.25	−0.15	0.95		
	80	−0.29	−0.15	0.99		
18	Toluene				−0.16	0.3
	30	−0.15	−0.15	0.90		

* Data of ref. 20; calculated values from eqn. 6.
** Calculated as described[13].

solvent i until a surface coverage equal to $(\theta_i + \theta_j)$ is reached, and so on until completion of adsorption of the weakest solvent A so that $\Sigma\theta = 1$ (completed monolayer of solvent). At some point during the successive adsorption of weaker solvents (j, i, h, ...) a value of $\Sigma\theta = 0.75$ will be reached, beyond which localization of later solvents is not possible, and their contribution to m will be miror (Fig. 5).

Based on the foregoing discussion, we can infer that m for a four-solvent mobile phase A–B–C–D will be given as:

$$m = m_D^0\, f(\theta_D) + m_C^0[f(\theta_C + \theta_D) - f(\theta_D)] + m_B^0[f(\theta_B + \theta_C + \theta_D) - f(\theta_C + \theta_D)] \quad (8)$$

For a three-solvent mobile phase A–B–C, eqn. 8 can be used with θ_D and $f(\theta_D)$ set equal to zero. In eqn. 8, m_B^0, m_C^0 and m_D^0 ref to values of m^0 for solvents B, C and D; $f(\theta_C + \theta_D)$ refers to the value of $f(\theta_j)$ for $\theta_j = (\theta_C + \theta_D)$; $f(\theta_B + \theta_C + \theta_D)$ refers to the

TABLE III

VALUES OF THE SOLVENT-LOCALIZATION FUNCTION, $f(\theta_j)$, FROM EQN. 6 *VS.* THE FRACTIONAL COVERAGE, θ_j, OF THE ADSORBENT SURFACE BY LOCALIZING SOLVENT j (SEE FIG. 5)

θ_j	$f(\theta_j)$	θ_j	$f(\theta_j)$
0.0	0.00	0.6	0.68
0.1	0.04	0.7	0.83
0.2	0.11	0.8	0.92
0.3	0.20	0.9	0.97
0.4	0.32	1.0	1.00
0.5	0.47		

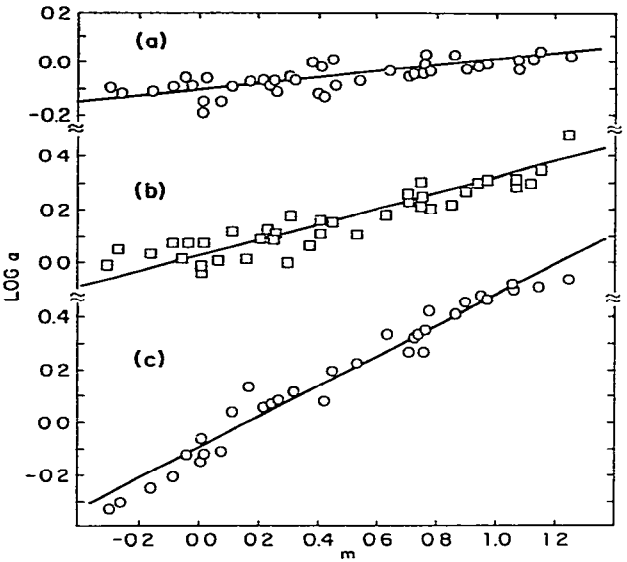

Fig. 6. Variation of log α values with m, and verification of eqn. 7; data for binary-solvent mobile phases and alumina[20]. a, X = 1-naphthaldehyde, Y = 1-cyanonaphthalene; b, X = 1-nitronaphthalene, Y = 1,7-dimethoxynaphthalene; c, X = 1,5-dinitronaphthalene, Y = 1-acetylnaphthalene.

value of $f(\theta_j)$ for $\theta_j = (\theta_B + \theta_C + \theta_D)$. In a later section we will see that eqn. 8 provides calculated values of m that agree well with experimental values for both ternary-solvent and quaternary-solvent mobile phases.

The applicability of eqn. 7, which describes α as a function of m, is illustrated in Fig. 7 for silica as adsorbent and several mobile phases which consist of ternary- and quaternary-solvent formulations. Representative plots for three different solute-pairs are shown, based on experimental data from ref. 14 and the present study. The scatter of the data points in Fig. 7 around the best fit to eqn. 7 ($\pm$0.05 log units, 1 S.D.) is comparable to that for the plots in Fig. 6 for binary-solvent mobile phases and alumina. There are eighteen different polar solvents j represented in Fig. 6, and four such solvents in Fig. 7.

Based on the foregoing discussion, we can now select a pair of polar solvents (B and C) with different values of m_B^0 and m_C^0. These can be blended with a non-polar solvent A in ternary formulations to allow the continuous and independent variation of both ε^0 and m for the mobile phase. This then allows the simultaneous optimization of both solvent strength and solvent selectivity (based on solvent/solute localization) as described in ref. 16.

Solvent selectivity vs. mobile phase composition: solvent-specific solvent/solute localization

Eqn. 7 allows us to predict values of α for given solute-pairs as a function of the mobile-phase m value, once we know the values of C_1 and C_2 for that solute-pair. However, although the correlations of Fig. 6 confirm the importance of m in determining solvent selectivity, there is still significant scatter of experimental data around these plots ($\pm$0.05–0.06 log units, 1 S.D.). Consequently, two mobile phases

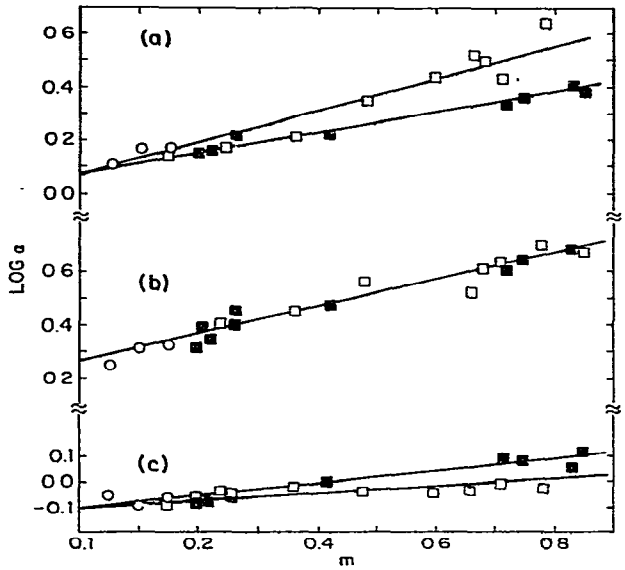

Fig. 7. Variation of log α values with m; data for multi-component mobile phases and silica (this study and ref. 14). a, X = 1-nitronaphthalene, Y = 2-methoxynaphthalene; b, X = 1,5-dinitronaphthalene, Y = 1,2-dimethoxynaphthalene; c, X = methyl 1-naphthoate, Y = 2-naphthaldehyde. ○, Solvent j is chloroform or dichloromethane; □, solvent j is MTBE; ■, solvent j is acetonitrile.

which have the same value of m can still exhibit somewhat different selectivities toward different solute-pairs. Thus, once we have optimized selectivity in terms of choosing the best mobile phase m value, the use of different mobile phases of similar m value may lead to further improvement in selectivity. It is therefore of interest to explore the basis of these deviations from eqn. 7 so that we can use them to practical advantage.

Deviations from eqn. 7 of the type discussed above suggest similar deviations from eqn. 4. This additional selectivity effect —which we will call *solvent-specific solvent/solute localization* (see below)— requires the addition of a further term (A_2) to eqn. 4:

$$\log (k_1/k_2) = \alpha' A_s (\varepsilon_2 - \varepsilon_1) + A_1 + A_2 \qquad (4a)$$

We have found that A_2 is a function of both the mobile phase composition and of the two solutes (X, Y) used to measure α.

Deviations from eqn. 4 (non-zero values of A_2) were found in ref. 20 and the present study to occur for sample–solvent combinations that do not include proton-donor compounds. Therefore, hydrogen-bonding in the stationary or mobile phase can be ruled out as a possible cause of this effect. An alternative explanation is provided by the solvent/solute localization model *per se*. So far we have considered the degree of localization of solute and solvent molecules (values of A_X and m_i), but have ignored the molecular details of the configuration of adsorbed solute and solvent molecules; that is, how do localized molecules of solvent or solute "fit" into the monolayer in relationship to surrounding molecules and to the adjacent silanol group

of the silica surface? Differences in configuration between solvent and solute molecules would be expected to lead to differences in the net adsorption energy of the solute, which translate into a further contribution to α (*i.e.*, Δ_2).

The configuration of localized molecules within the adsorbed monolayer is probably a function of the nature of the bonding between silanol groups and adsorbed molecules. A later section in fact suggests that the relative basicity of the solvent is a major factor in determining the magnitude of Δ_2 as a function of mobile phase composition. This in turn leads to a criterion for selecting two localizing solvents C and D, such that maximum differences in Δ_2 (and in solvent selectivity) can be achieved: the two solvents should have different relative basicities as defined by the selectivity triangle[23]. Thus, solvent C can be a less basic solvent from group VI of ref. 23, such as acetonitrile or ethyl acetate. The second solvent D should then be selected from solvent groups I or III; *e.g.*, tetrahydrofuran, methyl *tert.*-butyl ether, triethylamine, etc. The weakly localizing, moderately polar solvent B is ideally a solvent such as dichloromethane, with a small m^0 value. In this way, by varying the proportions of B, C and D in the mobile phase, ε^0 can be held constant while values of α are continuously varied over wide limits through change in both solvent/solute localization (value of m) and solvent-specific localization (Δ_2); see ref. 16.

The above hypothesis for solvent-specific localization suggests that the molecular structures of the localizing solutes (X, Y) and solvent C will *together* determine the values of Δ_2 in eqn. 4a. This in turn implies that eqn. 4 (which ignores Δ_2) should be more accurate when m is varied by changing the concentration of a strongly localizing solvent C (in a mixture A–B–C, where B is weakly localizing), rather than by changing to another localizing solvent D*. That is, for a given solvent C and solute X, the value of Δ_2 will remain constant while N_C is varied. This conjecture is tested in Fig. 7 for several solute-pairs from the present study and ref. 14. In the case of each solute-pair in Fig. 7, data for the two strongly localizing solvents used (methyl *tert.*-butyl ether, MTBE, and acetonitrile) are differentiated in these plots (□, MTBE; ■, acetonitrile). It is clear that separate straight-line plots for each of these latter two solvents are generally better fit by eqn. 7 ($\pm$0.02 log units, 1 S.D.) than are the composite plots ($\pm$0.05 log units) for each solute-pair. This is expected in terms of the above discussion. That is, significant change in Δ_2 and consequent failure of eqn. 7 (with larger S.D.s) should occur when changing the localizing solvent C, rather than when the concentration of C in the mobile phase is simply varied. The form of the experimental plots of Fig. 7 suggests that C_2 in eqn. 7 is only approximately independent of the localizing solvent j in the mobile phase. Thus, for maximum accuracy, C_2 in eqn. 7 will be a function of the localizing solvent j in the mobile phase A–j (or A–B–j).

EXPERIMENTAL

All measurements were done on a DuPont Model 850 liquid chromatograph (DuPont, Wilmington, DE, U.S.A.) equipped with a Model 870 pump, a UV absorbance detector operated at 254 nm and a Model 845 refractive index detector. Samples were introduced with a Model 725 Micromeritics Auto-Sampler (Micromeritics,

* Note that the somewhat scattered plots of Fig. 6 are based on a number of different localized solvents (Table II), with differing values of Δ_2.

TABLE IV

EXPERIMENTAL DATA FOR THREE-SOLVENT AND FOUR-SOLVENT MOBILE PHASES WITH SILICA AS ADSORBENT

Solutes are substituted naphthalenes; see Experimental for details. N_A = Mole fraction of hexane; N_B = mole fraction of dichloromethane; N_C = mole fraction of MTBE; N_D = mole fraction of acetonitrile.

	N_A =	0.881	0.849	0.818	0.958	0.870	0.686	0.565	0.555	0.553	0.680	0.710	0.7675	0.920
	N_B =	0.096	0.094	0.092	–	0.100	0.300	0.4305	0.442	0.440	0.310	0.280	0.2200	0.048
	N_C =	–	–	–	0.042	–	–	–	–	–	0.006	–	0.0125	0.0156
	N_D =	0.023	0.057	0.090	–	0.030	0.014	0.0045	0.003	0.007	0.005	0.010	–	0.0164

Solute	A_S	*Mobile phase**												
		14	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*
2-Methoxy	12.7	0.58	0.43	0.39	0.59	0.57	0.65	0.55	0.63	0.67	0.53	0.48	0.54	0.67
1-Nitro	15.6	1.32	1.04	0.97	1.62	1.20	1.10	0.80	0.91	0.93	0.81	0.77	0.90	1.36
1,2-Dimethoxy	17.3	0.91	0.62	0.54	1.00	0.82	1.02	0.97	1.21	1.02	0.90	0.84	0.91	0.95
1,5-Dinitro	23.1	3.99	2.93	2.58	3.70	3.27	2.98	2.14	2.55	2.44	2.40	2.35	2.63	3.62
1-Formyl	16.4	2.01	1.17	0.95	2.45	1.69	2.22	2.37	2.74	2.45	2.11	2.15	2.27	2.11
2-Methoxycarbonyl	16.2	3.05	1.65	1.16	2.83	2.22	3.00	2.81	3.29	2.91	2.55	2.60	2.78	2.99
1-Methoxycarbonyl	16.2	3.44	1.92	1.47	3.07	2.71	3.13	2.83	3.38	2.94	2.79	2.68	2.85	3.33
2-Formyl	16.4	2.83	1.47	1.31	3.25	2.49	3.19	3.34	3.95	3.34	2.81	2.97	3.12	2.83
1-Cyanomethyl	17.8	6.03	3.55	2.74	7.23	4.73	4.86	3.86	4.51	4.04	4.17	4.28	4.83	6.09
1-Hydroxy	15.7	9.89	5.47	3.83	6.65	8.17	6.77	5.22	5.85	5.52	7.36	5.81	6.27	7.14
1-Acetyl	17.3	3.22	1.62	1.19	3.54	2.58	3.71	4.15	4.95	4.23	3.53	3.60	3.72	3.25
2-Acetyl	17.3	4.36	2.07	1.44	4.76	3.33	5.14	5.88	7.03	5.91	4.89	5.04	5.16	4.39
2-Hydroxy	15.7	15.60	7.76	4.11	11.35	11.86	10.69	9.49	10.55	9.62	10.35	10.72	11.57	11.58
ε^0 (exptl.)**		0.218	0.242	0.254	0.226	0.227	0.224	0.227	0.220	0.224	0.225	0.229	0.225	0.221
ε^0 (calc.)***		0.203	0.272	0.302	0.223	0.224	0.222	0.223	0.215	0.222	0.222	0.223	0.221	0.225

* Data for mobile phases 1–13 in ref. 14.

** Calculated according to ref. 14, eqn. 15 with $\alpha = 0.57$.

*** Calculated according to ref. 14, Appendix I, assumes ε^0 (hexane) = 0.00; ε^0 (CH_2Cl_2) = 0.30; ε' (MTBE) = 0.87, ε'' (MTBE) = 0.58; ε' (ACN) = 0.9, ε'' (ACN) = 0.4.

Norcross, GA, U.S.A.) using a 25-μl sampling loop. A 15 × 0.46 cm column packed with Zorbax-SIL chromatographic packing was used for all studies.

All solvents were distilled-in-glass grade (Burdick & Jackson Labs., Muskegon, MI, U.S.A.) except *n*-hexane, which was Spectrograde (Phillips Petroleum Co., Bartlesville, OK, U.S.A.). The mobile phases were 50% water-saturated using the procedure described in ref. 1. The solvents were all degassed individually, and then mixed before the water-saturation procedure. The substituted naphthalenes were dissolved in hexane.

All retention measurements and k' calculations were carried out with a PDP-10 computer system[22]. Other calculations were performed on a PDP-11/60 minicomputer (Digital Equipment, Maynard, MA, U.S.A.) programmed in FORTRAN. The t_0 measurements for accurate determination of k' were done by injecting a sample aliquot of the mobile phase which had already passed through the column, but which had been diluted slightly with hexane. This had the effect of injecting a sample which was slightly weaker than the mobile phase into the system. Both short- and long-term reproducibility measurements of k' values were shown to have a standard deviation of less than 2%. The k' data for the mobile phases with silica as adsorbent discussed in this paper are shown in Table IV.

RESULTS AND DISCUSSION

Solvent/solute localization

Binary-solvent mobile phases and alumina. The study of ref. 20 examines solvent/solute localization for alumina as adsorbent in considerable detail. The retention of 20 different solutes was studied in 44 different binary-solvent mobile phases A–B, involving 20 different polar solvents B. The m values found[20] are summarized in Table II. The measurement of these m values and the verification of eqn. 5 for these LSC systems is further described in ref. 20.

Best values of m^0 for each B solvent of ref. 20 were derived in the present study, along with the function $f(\theta_j)$ of eqn. 6. Values of m^0 are given in Table II; $f(\theta_j)$ is plotted in Fig. 5 and listed in Table III. Table II also provides values of m calculated from eqn. 6, using the m^0 values of Table II plus values of $f(\theta_j)$ from Table III. These experimental and calculated values of m agree within ± 0.07 units (1 S.D.), for $-0.29 \leqslant m \leqslant 1.16$.

Values of m^0 correlate roughly with Q_k^c for the solvent as required by theory. Thus solvents 1–9 of Table II have $Q_k^0 > 3$, and their m^0 values are greater than 0.6. Solvents 10–20 of Table II have $Q_k^0 < 2$, and their m^0 values are less than 0.4. An exact correlation of m^0 *vs.* Q_k^0 is not observed, possibly because of secondary effects of the type involved in solvent-specific localization.

The shape of the $f(\theta_j)$ *vs.* θ_j curve in Fig. 5 is reasonable in terms of theory. Thus, we expect m/m^0 to increase only slowly with increase in θ_j, until completion of a localized solvent layer at $\theta_j = 0.75$ is approached. The reason is that prior to completion of the localized solvent layer, a localizing solute molecule can adsorb with displacement of a non-localized solvent molecule. Under these conditions, solvent/solute localization effects are less important in affecting solute α values. However, as θ_j approaches and exceeds a value of 0.75, a rapid increase in m/m° is expected. The ratio m/m° should then level out at a value of ≈ 1 for $\theta_j > 0.75$.

TABLE V

VALUES OF SOLVENT-SELECTIVITY PARAMETER, m, FOR MULTI-COMPONENT MOBILE PHASES AND SILICA

Data from ref. 16 and present study.

Mobile phase	*Composition (mole fractions)*					*Surface coverage, 0**				*m*	
	Hexane	*Chloroform*	*Dichloromethane*	*MTBE*	*ACN*	*Chloroform*	*Dichloromethane*	*MTBE*	*ACN*	*Exptl.*	*Calc.***
1	0.422	–	0.578	–	–	–	0.87	–	–	0 09	0.10
2	0.248	0.752	–	–	–	0.94	–	–	–	0.05	0.10
3	0.956	–	–	0.044	–	–	–	0.77	–	0.81	0.74
4	0.7165	–	0.270	0.0135	–	–	0.38	0.44	–	0.44	0.36
5	0.5875	0.399	–	0.0135	–	0.39	–	0.50	–	0.32	0.44
6	0.331	0.399	0.270	–	–	0.49	0.43	–	–	0.16	0.10
7	0.502	0.286	0.204	0.008	–	0.30	0.28	0.30	–	0.24	0.24
8	0.574	0.244	0.176	0.0064	–	0.29	0.27	0.28	–	0.24	0.22
9	0.474	0.053	0.470	0.0017	–	0.06	0.73	0.08	–	0.15	0.13
10	0.542	0.053	0.402	0.0033	–	0.06	0.64	0.15	–	0.20	0.15
11	0.841	0.0713	0.0488	0.0389	–	0.06	0.06	0.72	–	0.60	0.71
12	0.978	–	–	0.022	–	–	–	0.70	–	0.71	0.68
13	0.913	–	–	0.087	–	–	–	0.83	–	0.66	0.77
14	0.881	–	0.096	–	0.023	–	0.15	–	0.51	0.75	0.62
15	0.849	–	0.094	–	0.057	–	0.11	–	0.66	0.85	0.93
16	0.818	–	0.092	–	0.090	–	0.09	–	0.71	0.83	1.02
17	0.958	–	–	0.042	–	–	–	0.77	–	0.68	0.73
18	0.870	–	0.100	–	0.030	–	0.14	–	0.55	0.68	0.72
19	0.686	–	0.300	–	0.014	–	0.44	–	0.33	0.43	0.35
20	0.565	–	0.4305	–	0.0045	–	0.67	–	0.12	0.17	0.15
21	0.555	–	0.442	–	0.003	–	0.70	–	0.08	0.16	0.13
22	0.553	–	0.440	–	0.007	–	0.68	–	0.17	0.21	0.19
23	0.680	–	0.310	0.006	0.005	–	0.47	0.16	0.15	0.34	0.28
24	0.710	–	0.280	–	0.010	–	0.45	–	0.27	0.33	0.26
25	0.7675	–	0.220	0.0125	–	–	0.34	0.44	–	0.37	0.37
26	0.920	–	0.048	0.0156	0.0164	–	0.07	0.32	0.32	0.69	0.70

* Calculated as in ref. 14.

** Eqn. 6 or 8, see text, m^0 values are: chloroform, 0.10; dichloromethane, 0.10; MTBE, 0.82; acetonitrile, 1.19.

Ternary and quaternary-solvent mobile phases and silica. Table V summarizes m values for several multicomponent mobile phases and silica as adsorbent. The approach used in ref. 20 to determine values of A_1 cannot be used for silica as adsorbent, because the reference hydrocarbon solutes (for which $A_1 = 0$) have very small k' values on silica, when $\varepsilon^0 \gg 0$. We therefore used eqn. 7 to extract values of m from the data of ref. 14 and the present study. Since the product $C_2 m$ of eqn. 7 is obtained from plots such as those of Fig. 6, the ratio of C_2 to m is arbitrary. We have chosen a value of this ratio such that m^0 values for silica and alumina are of similar size for the same solvents. The detailed procedure used by us to obtain m values in this study is given in Appendix I.

The agreement of present data with eqn. 7 is illustrated in Fig. 7 for representative solute-pairs. Eqn. 7 calculates values of log α for silica and multi-component mobile phases with an accuracy of ± 0.05 units (1 S.D.)*, which is comparable to that found for alumina and binary-solvent mobile phases.

Values of m for multi-component mobile phases. Calculated values of m from eqn. 8 are compared with experimental values in Table V, for silica as adsorbent. The agreement of these two sets of values (± 0.08, 1 S.D.) is comparable to that found in Table II (± 0.07) for alumina and binary-solvent mobile phases. For ternary-solvent or quaternary-solvent mobile phases with only a single strongly-localizing solvent D, the term $[m - m_D^0 f(\theta_D)]$ of eqn. 8 is small, so that accurate values of $f(\theta_D)$ can be calculated from eqn. 8 for each value of m. Resulting values of $f(\theta_D)$ for different localizing solvents D are plotted in Fig. 5b with the solid curve from Table III superimposed. Thus, the general relationship of $f(\theta_j)$ *vs.* θ_j from Table III applies for both alumina and silica as adsorbents, and for binary-, ternary- and quaternary-solvent mobile phases. This in conjunction with the similar plots of Figs. 6 and 7 (eqn. 7) for these various systems suggests that solvent/solute localization occurs in essentially the same manner in these various LSC systems. This similarity of effects and their quantitative adherence to a small number of simple mathematical relationships (eqns. 6, 7) serves as additional evidence for the correctness of the present model and of the displacement mechanism (eqn. 2) on which it is based.

The calculation of m for any mixture of solvents is described in Appendix II.

Solvent-specific localization

Binary-solvent mobile phases and alumina. Values of A_1 for the mobile phases of Table II were reported[20] for nine standard solutes that do not include proton-donor compounds (compounds I–IX of Table III[20]). These A_1 values were determined experimentally, using eqn. 4 and assuming that $A_2 = 0$. In fact, the values of A_1 reported[20] are actually equal to $(A_1 + A_2)$. We will refer to these latter values as "apparent A_1 values" equal to A_1'. These A_1' values can be used to further analyze the rôle of the solvent in affecting solvent-specific selectivity.

Consider first the case where A_2 is in fact zero, and solvent-specific localization is unimportant. Further assume two different mobile phases p and q, with values of m equal to m_p and m_q. From eqn. 5 we then have

$$(A_1')_p = (m_p/m_q)(A_1')_q \tag{9}$$

* A single (average) value of C_2 is used for all solvents j in Fig. 7, corresponding to fitting data for each solute-pair to a *single* curve (rather than two curves as in Fig. 7).

where $(\Delta t'_1)_p$ and $(\Delta t'_1)_q$ are apparent Δt_1 values for mobile phases p and q, and a given solute X. If we now plot values of $(\Delta t'_1)_p$ *vs.* $(\Delta t'_1)_q$ for different solutes, all points should fall on a straight line, according to eqn. 9. Similarly, if a least squares correlation is carried out, a correlation coefficient, r, near unity should be obtained.

The situation is altogether different if values of Δt_2 for either mobile phase p or q are not zero. In this case, different solutes experience varying degrees of localization, depending on the polar solvent C or D which forms part of the mobile phase. That is, values of k' and α (Fig. 7a, c) depend upon the exact solvent (C or D) used in the mobile phase, as well as on the m value of that mobile phase. Therefore, we should be able to determine the relative importance of solvent-specific localization among different mobile phases studied in ref. 20, by carrying out correlations of apparent Δt_1 values among different pairs of mobile phases, according to eqn. 9. Those mobile phase-pairs exhibiting r values near unity can be presumed to be relatively free of solvent-specific localization, while poor correlation and smaller values of r must be associated with the presence of relatively large Δt_2 values for one or both mobile phases. *Furthermore, maximum differences in solvent selectivity among mobile phases of similar strength and m value should likewise occur for mobile-phase pairs that exhibit poor correlation* (see related discussion[23]).

Correlations of $\Delta t'_1$ values from ref. 20 were carried out among various pairs of mobile phases, according to eqn. 9. Since a high degree of correlation is expected for mobile phases containing the same polar solvent (C or D), the mobile phases chosen were binaries A–C where the solvent C was different in each mobile phase*. Furthermore, only relatively polar solvents C (large m^0 values) were tested. The results of these correlations are summarized in Table VI. It is seen that the first four solvents of Table VI (nitromethane, acetonitrile, acetone and ethyl acetate) show excellent correlations with each other ($0.98 \leqslant r \leqslant 0.99$) implying an absence of solvent-specific localization for these mobile phases. The last five solvents of Table VI (dimethyl sulfoxide, triethylamine, tetrahydrofuran, diethyl ether and pyridine) show poorer correlation with the first four solvents ($0.87 \leqslant r \leqslant 0.97$), and not much better correlation within this group of five solvents ($0.91 \leqslant r \leqslant 0.97$). Therefore, it appears that solvent-specific localization is most important for mobile phases containing one of these latter five solvents.

The ranking of various polar solvents in Table VI according to solvent-specific localization effects closely parallels the classification of solvents in ref. 23 according to the relative importance of hydrogen-bonding *vs.* dipole-interaction tendencies. Thus, the first four solvents of Table VI show little solvent-specific localization, and all belong to selectivity groups VI or VII of ref. 23. These latter solvent-groups (VI, VII) are characterized by strong dipole interactions and lesser proton-donor or acceptor strength. The last five solvents of Table VI show significant solvent-specific localization, and belong to groups I or III of ref. 23. The latter selectivity groups (I, III) comprise solvents that are good proton-acceptors, but show little proton-donor strength or dipole-interaction. Since both alumina and silica behave as acidic adsorbents[10,24], this suggests that solvent-specific localization occurs mainly for solvents that strongly hydrogen-bond to the adsorbent surface. This is confirmed in the

* Several mobile phase compositions were reported[20] for each C solvent; *e.g.*, 2, 5, 9 and 23% (v/v) diethyl ether–pentane. In these cases, values of $\Delta t'_1$ were averaged for each C solvent and a given solute-pair.

TABLE VI

MAXIMALLY DIFFERENT SOLVENTS IN TERMS OF SOLVENT-SPECIFIC LOCALIZATION

Correlation, r, of solvent selectivity among different solvent pairs (i–j) from study of ref. 20 for alumina as adsorbent in terms of eqn. 9.

Solvent i	*Solvent j*								
	NM	*ACN*	*ACT*	*EA*	*DMSO*	*TEA*	*THF*	*EE*	*PYR*
Nitromethane (NM)	1.00	0.98	0.99	0.98	0.97	0.93	0.92	0.92	0.90
Acetonitrile (ACN)	0.98	1.00	0.98	0.98	0.96	0.96	0.90	0.90	0.87
Acetone (ACT)	0.99	0.98	1.00	0.99	0.96	0.94	0.94	0.95	0.92
Ethyl acetate (EA)	0.98	0.98	0.99	1.00	0.97	0.95	0.96	0.96	0.94
Dimethyl sulfoxide (DMSO)	0.97	0.96	0.96	0.97	1.00	0.96	0.97	0.90	0.94
Triethylamine (TEA)	0.93	0.96	0.94	0.95	0.96	1.00	0.92	0.89	0.91
Tetrahydrofuran (THF)	0.92	0.90	0.94	0.96	0.97	0.92	1.00	0.93	0.97
Diethyl ether (EE)	0.92	0.90	0.95	0.96	0.90	0.89	0.93	1.00	0.94
Pyridine (PYR)	0.90	0.87	0.92	0.94	0.94	0.91	0.97	0.94	1.00

next section for silica, where large values of Δ_2 (and poor correlation) are observed for the solvents MTBE (group I) *vs.* acetonitrile (group VI).

Maximum differences in solvent selectivity as a result of solvent-specific localization are predicted for solvent-pairs with small r values in Table VI. Pyridine–acetonitrile has the poorest correlation of these solvents ($r = 0.87$), but pyridine is not a suitable solvent for LC with photometric detection. Diethyl ether–acetonitrile and tetrahydrofuran–acetonitrile have correlations that are almost as poor ($r = 0.90$), and appear to be suitable solvents (C, D) for use in an optimization scheme based on maximizing differences in localization selectivity. Since MTBE should be quite similar to diethyl ether in terms of solvent selectivity, and is better suited for routine application in LSC[1], we recommend the use of MTBE and acetonitrile as optimum localizing solvents C and D. Because of the poor correlation among the last five solvents of Table VI, we anticipate that additional solvent selectivity is achievable by using more than one of these solvents, *i.e.*, use of additional solvents E, F, ... in a retention-optimization scheme for LSC.

Multi-solvent mobile phases and silica. We have seen in this paper and in ref. 20 that solvent/solute localization effects are similar for silica and alumina, and for mobile phases that contain two, three or four solvent components. We might therefore infer that solvent-specific localization effects are also similar for these various LSC systems. Limited data from the present study suggest that this is the case. We should therefore expect to see significant differences in selectivity between mobile phases A–B–C (hexane–dichloromethane–MTBE) and A–B–D (hexane–dichloromethane–acetonitrile) on the basis of Table VI and the related discussion in a preceding section. Using the solute-pairs defined in Table VII, this premise is confirmed in Table VIII.

The seven solvent systems shown in Table VIII were selected for use in a systematic optimization scheme[16]. Their compositions are selected to provide roughly equal changes in selectivity within a "selectivity triangle". We refer to them as "selectivity-spaced" mobile phases. These mobile phases are believed to cover the range of

TABLE VII

SOLUTE-PAIRS USED FOR CALCULATION OF m VIA EQN. i-1

Solute-pair	*X*	*Y*	C_1	C_2	
				MTBE	*ACN*
A	1-Nitro	2-Methoxy	0.08	0.56	0.36
B	1-Nitro	1,2-Dimethoxy	−0.21	0.76	0.53
C	1,5-Dinitro	1,2-Dimethoxy	0.25	0.56	0.50
D	1,5-Dinitro	1-Formyl	−0.06	0.30	0.53
E	2-Methoxycarbonyl	1-Formyl	0.06	0.00	0.13
F	1-Methoxycarbonyl	2-Methoxycarbonyl	0.01	0.03	0.07
G	1-Methoxycarbonyl	2-Formyl	−0.06	0.03	0.20
H	1-Cyanomethyl	2-Formyl	−0.04	0.50	0.50
I	1-Cyanomethyl	1-Acetyl	−0.15	0.56	0.59
J	2-Acetyl	1-Acetyl	0.13	0.00	−0.03

possible α values, for mobile phases of $\varepsilon^0 = 0.22$, and exclusion of proton-donor solvents and solutes (*i.e.*, localization-selectivity effects only). Of interest in the present discussion are the mobile phase groupings (a) 5, 23 and 19 and (b) 17, 26 and 18. The first group has m constant at 0.37 ± 0.06 while for the second group m is 0.68 ± 0.01. Within each of these two groups, the localizing-solvent concentration varies from pure MTBE (5, 17) to pure acetonitrile (19, 18), with an intermediate composition that is roughly 50% of each (23, 26).

The relative importance of various contributions to selectivity can be evaluated from these data. First, for a change in m from 0.1 (mobile phase 1 of Table VIII) to 0.7 (either 17 or 18), we can calculate the change in log α for a given solute-pair, *e.g.*,

TABLE VIII

SEPARATION FACTORS, LOG α, FOR STANDARD SOLUTE-PAIRS OF TABLE VII AND SEVEN "SELECTIVITY-SPACED" MOBILE PHASES OF PRESENT STUDY AND REF. 14

For identification of mobile phases see Table V. C = MTBE; D = ACN.

Solute-pair	*Mobile phase*	*1*	*5*	*23*	*19*	*17*	*26*	*18*
	m	0.09	0.32	0.37	0.43	0.68	0.43	0.68
	$N_C/(N_C + N_D)$	−*	1.00	0.45	0.00	1.00	0.49	0.00
A		0.17	0.21	0.18	0.23	0.44	0.31	0.32
B		−0.12	−0.03	−0.05	0.03	0.21	0.16	0.17
C		0.31	0.45	0.43	0.47	0.57	0.58	0.60
D		−0.08	0.08	0.06	0.13	0.18	0.23	0.29
E		0.06	0.06	0.08	0.13	0.06	0.15	0.12
F		0.00	0.03	0.04	0.02	0.04	0.05	0.09
G		−0.09	−0.02	0.00	0.01	−0.02	0.07	0.04
H		0.01	0.13	0.17	0.18	0.35	0.33	0.28
I		−0.11	0.05	0.07	0.12	0.31	0.27	0.26
J		0.15	0.11	0.14	0.14	0.13	0.13	0.11

* No MTBE or acetonitrile present in mobile phase.

solute-pair A, 0.32 units for 17 and 0.16 units for 18. We can then average these changes in log α for all ten solute-pairs and mobile phases 17 and 18 (twenty values): equal 0.21 log units. Since the localizing solvent (MTBE or acetonitrile) is not changed during each of these comparisons of the effect of m, the average change in log α (0.21 units) is due only to solvent/solute localization (A_1 effect).

Second, with m held constant (mobile phase 17 *vs.* 18), we can compare log α values for a given solute-pair and MTBE *vs.* acetonitrile as localizing solvent. For solute-pair A and m equal 0.7, the difference in log α values is 0.16 units. The average change in log α values for all ten solute-pairs is 0.07 units, and this corresponds to the relative contribution of solvent-specific localization (A_2 effect) to mobile phase selectivity for the systems of Table VIII.

Finally, consider the agreement of experimental values of log α for mobile phases 17 and 18 with values calculated from eqn. 7 using values of C_1 and C_2 from Table VII. The average difference between experimental and calculated log α values is 0.02 units. The latter value comprises other selectivity contributions plus the effects of experimental imprecision in measured values of log α. Imprecision contributes about 0.01 log unit, so that other selectivity contributions must be small.

Similar calculations of these various selectivity contributions can be made for alumina using the data of ref. 20. For the same solute-pairs, but substituting the solvent ethyl acetate for acetonitrile, we obtain the data summarized below:

Selectivity	*alumina*	*silica*
A_1 (av.)	0.09	0.21
A_2 (av.)	0.05	0.07
residual (av.)	–	0.02

The overall conclusions appear as follows. Mobile-phase selectivity is similar for both adsorbents, but more important for silica. Solvent/solute localization, A_1, is significantly more important than solvent-specific localization, A_2, in affecting selectivity. Other contributions to selectivity are minor.

Returning to Table VIII, it is of interest to examine the log α values of various solute-pairs for the mobile phases which have $N_C/(N_C + N_D) \approx 0.5$. These are roughly intermediate in value between the corresponding log α values (m constant) for $N_C/(N_C + N_D) = 0$ and 1. That is, to a first approximation these log α values for $N_C/(N_C + N_D) = 0.5$ can be calculated from eqn. 7, using a value of C_2 that is the average of C_2 values (Table VII) for MTBE and acetonitrile. However, the latter relationship is sufficiently imprecise (S.D. = ± 0.03 log units) to suggest that a simple linear interpolation of log α values between $N_C/(N_C + N_D) = 0$ and 1 is not recommended.

Solvent strength and solvent-solute localization. We have seen that the value of m for the mobile phase can be varied while solvent strength ε^0 is held constant. However, the maximum possible value of m increases with increasing ε^0. This is illustrated in Fig. 8 for alumina as adsorbent. In Fig. 8, we plot m *vs.* ε^0 for different solvents B as N_B is varied. The resulting plots are experimentally indistinguishable for solvents B of similar polarity, *i.e.*, where the pure solvents have similar ε^0 values. Therefore, each plot in Fig. 8 is for a group of similar B solvents, *e.g.*, group IV is for $\varepsilon^0 = 0.38$ (diethyl ether); group VI is for solvents with ε^0 values greater than 0.56

(acetonitrile, nitromethane, etc.). For localizing B solvents, which comprise groups IV, V and VI in Fig. 8, there are two characteristic features of these plots. At low ε^0 values (small values of N_B), all B solvents give about the same value of m for a given value of ε^0. However, at higher values of ε^0 for the mobile phase, the less polar solvents (*e.g.*, group IV) approach a limiting value of m beyond which no increase in N_B or ε^0 will give greater solvent-selectivity.

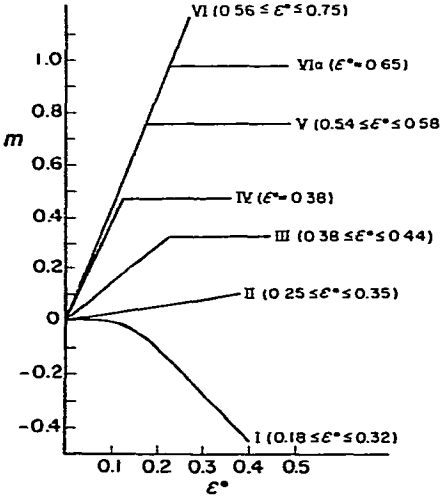

Fig. 8. Dependence of mobile phase m values on solvent strength for binary-solvent mobile phases and alumina; different plots (I, II, ...) refer to different groups of B solvents. From ref. 20.

The practical significance of the plots of Fig. 8 is as follows. First, for a given value of mobile phase strength ε^0, there is a maximum possible value of m. This value increases with ε^0 so that larger m values are possible for the separation of more polar samples which require larger ε^0 values for optimum k' values. In the case of MTBE–hexane, the maximum value of m is almost reached for 2% (v/v) MTBE and $\varepsilon^0 = 0.22$ ($m = 0.75 = 90\%$ of m^0 for MTBE). For stronger mobile phases ($\varepsilon^0 > 0.22$), it is possible to increase m significantly ($m > 0.8$), but only by substituting a more polar solvent for MTBE. On this basis, it is tempting to select the most polar solvents possible for the solvents C and D used to control localization selectivity in LSC separation. However, for weaker mobile phases this will mean very small concentrations of these solvents, which may be experimentally inconvenient in some applications.

CONCLUSIONS

In this paper we have further examined the nature of solvent selectivity in LSC separation. It has been confirmed that localization of solvent and solute molecules can lead to large changes in the α values of different solute-pairs. Detailed mathematical relationships have been derived to describe this dependence of sample separation sequence on mobile phase composition. These equations have been verified by numer-

ous data for alumina and silica as adsorbents, with mobile phases containing two to four solvent components.

We also describe the basis for an overall optimization strategy in LSC separation, as summarized in Table I. A later paper[16] describes the reduction of this scheme to practice using a computer-optimization program. Another paper[25] will offer a simplified version of retention-optimization for use without detailed calculations or access to a computer.

We propose that sample retention be varied for maximum resolution using a four-solvent mobile phase A–B–C–D. Each of these four solvents A–D is selected for its ability to contribute in a unique and independent way to optimum retention. The optimum solvent strength, ε^0, for the sample of interest is first established empirically (as in ref. 1) using a binary-solvent mobile phase A–B. Compositions A–B–C–D of equivalent strength, for optimum separation, are then calculated. The solvent A will be a saturated hydrocarbon such as hexane or isooctane. Variation of its concentration allows ε^0 to be held constant, while the proportions of B, C and D are varied for change in separation selectivity.

Solvent B should be a weakly localizing compound (small value of m^0) such as methylene chloride. Varying the proportions of B *vs.* (C + D) allows change in the relative localization (m value) of the mobile phase. This in turn allows continuous variation in solvent/solute localization over wide limits, which generally results in substantial changes in the α values of various solute-pairs within the sample. An acceptable spacing of solute bands within the chromatogram can often be achieved by simple variation of the mobile phase m value.

The localizing solvents C and D are chosen for their differing contributions to solvent-specific localization. Each solvent will have a large value of m^0, which means that C and D will be relatively polar solvents. Solvent C should be relatively basic (*e.g.*, an ether such as MTBE), while D should be strongly dipolar (*e.g.*, acetonitrile). Varying the concentration ratio of C and D (N_C/N_D) results in a further change in α values. The possible variation of α with change in N_C/N_D is about 1/3 as great as for variation of the total concentration of C plus D ($N_C + N_D$). Therefore, N_C/N_D should be varied only after the optimum value of ($N_C + N_D$) has been determined. Additional solvent selectivity can be achieved by exploring other basic solvents in place of C (solvents from groups I or III of ref. 20).

APPENDIX I

Derivation of m values for mobile phases used with silica as adsorbent

The various mobile phases reported here and in ref. 14 were characterized as to their m values as follows. First, the procedure described[20] for alumina as adsorbent could not be used. The latter approach is based on k' values for reference aromatic-hydrocarbon solutes, but with silica these compounds have generally small k' values whenever ε^0 exceeds about 0.1. Therefore, a different procedure was used here.

For the various reference solutes of Table IV and ref. 14 we can select adjacent solute-pairs and determine their α values for various mobile phases. We selected ten such pairs as summarized in Table VIII. The solutes 1- and 2-naphthol were excluded because of possible hydrogen-bonding effects that would complicate the interpre-

tation of Δ_1 values and related values of m. For a given mobile phase i, we can sum the values of log α_i for the ten solute-pairs A–J of Table VIII to give:

$$\sum^{10} \log \alpha_i = \sum^{10} C_1 + m_i \sum^{10} C_2 = D_1 + D_2 m_i \quad \text{(i-1)}$$

The values of D_1 and D_2 are functions only of the ten solute-pairs selected (Table VIII), and moreover their absolute values are arbitrary. We arbitrarily define $D_1 = 0$ and $D_2 = 3.3$, so that:

$$m_i = 0.3 \sum^{10} \log \alpha_i \quad \text{(i-2)}$$

As example, consider mobile phase 8 of ref. 14. The values of log α_i for solute-pairs A–J are: 0.17, −0.07, 0.40, 0.03, 0.06, 0.02, −0.04, 0.10, 0.00, 0.13, and their sum is 0.80. The value of m is then $0.3 \times 0.80 = 0.24$. The present convention of assigning $D_1 = 0$ and $D_2 = 3.3$ gives m^0 values for silica which are similar in magnitude to values for alumina.

Values of m for other mobile phases can be calculated from eqn. 7, using average values of C_1 and C_2 for any of the solute-pairs of Table VIII. However, it is seen that the complication of solvent-specific effects and varying values of Δ_2 can be minimized by selecting solute-pairs which have similar (relatively large) values of C_2 for both MTBE and acetonitrile (*e.g.*, solute-pairs C, H, I of Table VIII).

APPENDIX II

Examples of the calculation of m for different mobile phases

Binary solvent mobile phase. Consider mobile phase 17 of Table V, MTBE–hexane (A–B) with $N_B = 0.042$. The value of θ_B (or θ_j) for the polar solvent MTBE is calculated as 0.77 (refs. 13, 14). The value of $f(\theta_j)$ from Table III is then 0.89. From eqn. 6, with $m^0 = 0.82$, we then have $m = 0.89 \times 0.82 = 0.73$. The experimental value determined as in Appendix I is 0.68.

Ternary solvent mobile phase. Consider mobile phase 5 of Table V, MTBE–chloroform–hexane (C–B–A) with $N_C = 0.0135$ and $N_B = 0.399$. The values of θ are calculated as in ref. 14; $\theta_B = 0.39$, $\theta_C = 0.50$. Eqn. 8 is now used to calculate m, with $m^0 = 0.10$ for chloroform ($= m_B^0$) and $m^0 = 0.82$ for MTBE ($= m_C^0$). Note that θ_D and $f(\theta_D)$ are zero.

First, determine the function $f(\theta_C + \theta_D) = f(\theta_C) = 0.47$ (Table III). Similarly, $f(\theta_B + \theta_C + \theta_D) = f(0.89) = 0.97$. Now inserting these values into eqn. 8.

$$m = 0 + 0.82\,(0.47 - 0) + 0.10\,(0.97 - 0.47) = 0.44$$

The experimental value was 0.36 determined in Appendix I.

Quaternary solvent mobile phase. Consider mobile phase 23 of Table V, acetonitrile–MTBE-dichloromethane-hexane (D–C–B–A). Values of θ for each polar solvent (B–D) are given in Table V determined according to the procedure of ref. 14. The value of m^0 are 0.10 (B), 0.82 (C) and 1.19 (D). These data with Table III allow solution for m in terms of eqn. 8:

$$m = 1.19 \times 0.075 + 0.82 \times (0.21 - 0.075) + 0.10\,(0.96 - 0.21)$$
$$= 0.28$$

The experimental value was 0.37.

GLOSSARY

A, B, C, D	refers to specific solvents comprising the mobile phase; see Table I
A_s	the cross-sectional area of a solute molecule as required on the adsorbent surface during adsorption; one unit is equal to 0.08 nm^2
B_a, B_n	a solvent molecule B in the adsorbed (a) or non-sorbed (n) phase
C_1, C_2	constants in eqn. 9; C_2 varies with the localizing solvent j in the mobile phase, as shown in Table VII
D_1, D_2	constants in eqn. i-1
$f(\theta_B)$, $f(\theta_j)$	a solvent-localization function (Table III) which varies with the fractional coverage θ of the adsorbent surface by a localizing solvent B or j; eqns. 6, 8
i, j	solvent components of a mobile phase; j is always a localizing solvent
k'	solute capacity factor, equal to fraction of solute molecules in stationary phase divided by fraction in mobile phase
k_X, k_Y	values of k' for solutes X and Y in a given LC system
k_1, k_2	value of k' for a solute X in mobile phases 1 and 2; eqn. 3
m^0	solvent-localization parameter for pure solvent; *e.g.*, m^0 = 0.1, 0.1, 0.82 and 1.19 for dichloromethane, chloroform, MTBE and acetonitrile (silica); see Table II for alumina
m_B^0, m_C^0, m_D^0	values of m^0 for solvents B, C and D
MTBE	methyl *tert.*-butyl ether
n	number of solvent molecules B displaced by an adsorbing solute molecule X; eqn. 2
N	column plate number
N_A, N_B, N_C, N_D	mole fraction of solvents A, B, etc., in mobile phase
m	mobile-phase-localization parameter
m_i	value of m for mobile phase i; eqn. 5
m_1, m_2	values of m for mobile phases 1 and 2; eqn. 7
Q_k^0	dimensionless free energy of adsorption ($\Delta G/RT$) for a substituent group k on a solute molecule; k is normally the most polar or strongly-adsorbing group in the molecule
r	correlation coefficient for least-squares regression analysis, as of eqn. 9 in Table VI

R_s	resolution function, equal to difference in retention times for two solute bands, divided by average band width
X_a, X_n	a solute molecule X in the adsorbed (a) or non-sorbed (n) phase
α	separation factor for two solutes X and Y; equal to k_x/k_Y
α_1, α_2	value of α for mobile phases 1 and 2; eqn. 7
α'	adsorbent activity function; eqn. 3
Δ_x, Δ_Y	solute localization parameters for solutes X and Y; eqns. 5, 7
Δ_1	contribution to k' from solvent/solute localization (eqn. 4); difference in log k' values for two mobile phases 1 and 2 when $\varepsilon_1 = \varepsilon_2$
Δ_2	contribution to k' from solvent-specific localization; eqn. 4a
Δ'	equal to $\Delta_1 + \Delta_2$ for a particular mobile phase (relative to a mobile phase with $\Delta' = 0$)
Δ'_p, Δ'_q	values of Δ' for mobile phases p and q, or localizing solvents P and Q; eqn. 9
ε^0	solvent strength parameter; eqn. 3; also, values of ε° for pure solvents A, B, C, etc.
ε_i, ε_j	values of ε^0 for pure solvents i and j
ε_1. ε_2	values of ε^0 for mobile phases 1 and 2
ε', ε''	value of ε^0 for localizing solvent j with $\theta_j = 0$ or 1, respectively; see discussion in refs. 13, 14

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