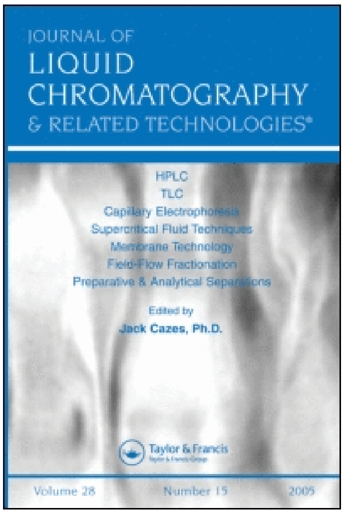

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Solute Retention in Column Liquid Chromatography. I. Binary Non-electrolyte Mobile-Phase Additives at High Dilution with Silica Sorbent

A. -J. Hsu[a]; R. J. Laub[a]; S. J. Madden[a]
[a] Department of Chemistry, San Diego State University, San Diego, CA

To cite this Article Hsu, A. -J. , Laub, R. J. and Madden, S. J.(1984) 'Solute Retention in Column Liquid Chromatography. I. Binary Non-electrolyte Mobile-Phase Additives at High Dilution with Silica Sorbent', Journal of Liquid Chromatography & Related Technologies, 7: 3, 599 — 614
To link to this Article: DOI: 10.1080/01483918408073989
URL: http://dx.doi.org/10.1080/01483918408073989

JOURNAL OF LIQUID CHROMATOGRAPHY, 7(3), 599-614 (1984)

SOLUTE RETENTION IN COLUMN LIQUID CHROMATOGRAPHY. I. BINARY NON-ELECTROLYTE MOBILE-PHASE ADDITIVES AT HIGH DILUTION WITH SILICA SORBENT

A.-J. Hsu, R. J. Laub*, and S. J. Madden

Department of Chemistry
San Diego State University
San Diego, CA 92182

ABSTRACT

The two principal models purporting to describe elution behavior are presented, and it is argued that they cannot be distinguished solely on the basis of chromatographic data. A recent modification (so as to describe all known isotherm types) of one of these is fitted in this work to the retentions of five test solutes with silica sorbent and mobile phases comprised of hexane + the additive pairs: THF/MC; ACN/MC; IPA/THF; IPA/MC; ACN/THF; and ACN/IPA over the compositional range 0-0.12% v/v, for which advantages in routine analysis are said to obtain. The fits describe without exception all systems studied to within experimental error, although there is no immediately-apparent correlation of the resultant parameters with the bulk properties either of the solutes or solvents. Nevertheless, the ability to forecast retentions with blended mobile phases from data pertaining to at most four or five measurements is claimed to represent a considerable advance over all other models in use in liquid chromatography at the present time.

INTRODUCTION

There can be little doubt that considerable interest is currently focussed on "high-performance" liquid chromatography. Indeed, the technique has already found extensive application in organic and

---

*Author to whom correspondence may be addressed.

0148-3919/84/0703-0599$3.50/0

biochemistry, and is rapidly becoming a major analytical tool in biological and medicinal studies. However, the number of system parameters which may influence lc separations is greater than that e.g., in gc, due primarily to the presence of condensed-state mobile phase. Moreover, and as is true in gc, the sorption process (be this solution or adsorption) remains only poorly understood. For example, Locke (1) re-stated in simplified terms a model of sorption originally developed by Everett (2), and concluded that differences in retentions can be attributed, at least in part, to solute solubility in the mobile phase. It was then argued that log(retention) should be inversely proportional to -log(solute solubility) for homologous series of compounds on the assumption that the support is covered with a monolayer or so of adsorbed solvent, and that solutes desorbed from the mobile phase compete with solvent for adsorption sites during the elution process. However, no account was taken of the undoubted differences between bulk and adsorbed solvent and so, only modest agreement was found when solute bulk solubilities were contrasted with those deduced from lc experiments.

Since the original report by Locke, there have evolved three principal models of retentions in liquid chromatography. The first is that by Snyder (3), whose assumptions parallel those of Locke. He arrived ultimately at the expression:

$$\log K^{o} = \log V_{a} + \alpha(S^{o} - A_{(1)}\epsilon^{o}) \qquad (1)$$

where K^{o} is taken as the solute sorption isotherm ($cm^{3}\ g^{-1}$), defined as the ratio of moles of adsorbed solute per gram adsorbent to moles of solute per cm^{3} of mobile phase; V_a is the volume of adsorbed mobile phase per gram adsorbent; α is a function of the adsorbent surface activity; $A_{(1)}$ is the surface area occupied by adsorbed solute; and S^{o} and ϵ^{o} are said to represent energies of adsorption of solute and solvent per unit area of adsorbent at some "standard" activity. Thus, V_a and α are properties of the adsorbent; S^{o} and $A_{(1)}$ pertain to the solute; while ϵ^{o} relates to the solvent.

Extension of eqn. 1 to include binary mobile phases requires expansion only of the ϵ^{o} term. The result, for solvents A and S, is given by:

$$\epsilon^{o}_{(M)} = \epsilon^{o}_{(S)} + \frac{\log\left[x_A \; 10^{\alpha A_{(A)}(\epsilon^{o}_{(A)} - \epsilon^{o}_{(S)})} + 1 - x_A\right]}{\alpha A} \tag{2}$$

where as before $A_{(i)}$ refers to a molecular size and x_i (i = A or S) is the mole fraction of the ith component in the mobile phase. Substitution of this relation for ϵ^o into eqn. 1 then yields an approximate description of the variation of K^o with mobile-phase composition.

It is fair to say that despite the considerable assumptions utilized in simplifying the development of this model, and aside from its limited success in practice, it represents nevertheless the first attempt at a comprehensive description of retentions as a function of mixed solvents in liquid chromatography.

Snyder's model is essentially thermodynamic in nature in that it describes the partition coefficient as a function of the exponent of the partial molar excess free energy associated with transfer of solute from the mobile phase to the stationary phase. However, this approach is limited in that the (differential) thermodynamic properties as formulated pertain only to the **net** interactive forces acting upon the solute when it is distributed between phases. The alternative is description of individual interactions occurring in each phase, which leads to the model proposed initially by Scott and Kucera (4):

$$K^{o}_{R(M)} = \frac{\left[\sum_{i=1}^{n} \Theta_i \; F_i \; P_i \; f(T)\right]_S}{\left[\sum_{i=1}^{n} \Theta_i \; F_i \; P_i \; f(T)\right]_M} \tag{3}$$

where Θ is a constant which incorporates the probability of contact with stationary S or mobile M phases, F is the magnitude of the force between the solute and the respective phase, P is the probability of molecular interaction, and f(T) is the thermal energy of the molecule at the moment of contact. So long as the stationary phase remains at some state of constant activity, this arising as a result of sorbed mobile-phase component, the numerator of eqn. 3 will be fixed and so,

$$\frac{1}{K^{o}_{R(M)}} = A + B\ C_A \tag{4a}$$

This can also be cast in terms of volume fractions, viz.,

$$\frac{1}{K^{o}_{R(M)}} = \frac{\phi_A}{K^{o}_{R(A)}} + \frac{\phi_S}{K^{o}_{R(S)}} \tag{4b}$$

where A and B are constants. Eqn. 4 thus is the liquid-chromatographic equivalent of the diachoric solutions hypothesis of Laub and Purnell (5,6):

$$K^{o}_{R(M)} = \phi_A\ K^{o}_{R(A)} + \phi_S\ K^{o}_{R(S)} \tag{5}$$

Eqn. 4 is found to hold for very many systems so long as the concentration of "polar" component of the mobile phase is greater than about 5-10%, i.e., such that the surface of the adsorbent is completely deactivated. In addition, eqns. 3 and 4 are equivalent to eqn. 1 if the latter is cast in terms of fractional surface coverage of the adsorbent by the mobile phase, as brought out clearly in the work of Jandera and Churacek (7), Soczewinski and Golkiewicz (8), Jaroniec and his coworkers (9), and Slaats and colleagues (10). The three models represented by eqns. 1-5 cannot generally be distinguished, therefore, solely on the basis of retention data.

As a result of considerable testing of the above retention relations, very many solute-sorbent systems have now been documented in the literature. All bear strong resemblance to the shapes of (curved) BET adsorption isotherms and, consequently, McCann, Purnell, and Wellington (11), followed by Madden, McCann, Purnell, and Wellington (12), proposed a modification of eqn. 4 with which all six isotherm shapes could be represented. Their result, applicable strictly only to nonionic systems at this time, is given by:

$$\frac{1}{K^{o}_{R(M)}} = \phi_A \left\{ \frac{1}{K^{o}_{R(A)}} + \frac{b\ \phi_S}{1 + b'\phi_S} \right\} + \frac{\phi_S}{K^{o}_{R(S)}} \tag{6}$$

where values of b and b' are derived from an analysis of the experimental data. In every case tested thus far the fits have been excellent (13). However, it must be pointed out that the fitted parameters have yet to be rationalized either from one solute to the next or from one solvent system to another. Nevertheless, the ability to represent generally (hence predict) the variation of solute retentions as a well-defined function of mobile-phase composition represents a very considerable advance.

From the standpoint of analysis, the most useful carrier compositions self-evidently are those which provide the "best" separation in the fastest time. However, the "best" separation frequently is defined in terms of local criteria established by the analyst and which may or may not correspond to this or that general principle. For example, it is often the case that a particular solute elution order is mandated in order to retrieve quantitative data for trace components whose retentions have been adjusted to fall well removed from some major analyte constituent. As a result, the mobile-phase compositions of overriding interest in chemical analysis comprise those with which solute retentions and retention order can most readily be altered. Experience with silica sorbent has shown that these amount in practice to high (ca. 0-0.1%) dilution of this or that **individual** mobile-phase additive such that the surface of the stationary phase is far from completely covered. As a result, it would seem that adjustment of the concentrations of **binary, ternary, ...** additives over this limited range could provide an even more powerful means of control of elution behavior. We have therefore carried out in this work a study of the utility of ternary mobile phases, where two of the components were varied only over the range 0-0.12% v/v. The opportunity was taken as well to explore the limits of application of eqn. 6 with such systems.

EXPERIMENTAL

Solvent Purification. Reagent-grade hexane (wet; a blend of several hexane isomers) was found to contain numerous aromatic impurities which were removed as follows. Concentrated sulfuric acid was added (1:10 v/v) to the hexane and the mixture stirred overnight. The organic layer was then drawn off and treated again if the resultant acid layer was yellow in color. A solution of 5% sodium bicarbonate was next added (Caution!) to the separated organic layer to neutralize any residual acid and the hexane was then washed to neutrality with water distilled from dichromate solution. Fresh Drierite was added next to remove most of the water, following which dried nitrogen (molecular sieves; calcium chloride or magnesium perchlorate) was passed through the solvent to which had been added metallic sodium and a small amount of benzophenone. (The latter acts in this instance as an indicator and changes from light yellow to dark blue upon consumption of the last traces of moisture). Finally, the organic solvent was refluxed for several hours under nitrogen until it turned dark blue, following which it was distilled and stored over molecular sieves.

Mobile-Phase Preparation. Solutions of hexane as treated above were mixed with the six distilled and pre-dried (molecular sieves) additive pairs: tetrahydrofuran (THF)/methylene chloride (MC), acetonitrile (ACN)/MC, iso-propyl alcohol (IPA)/THF, IPA/MC, ACN/THF, and ACN/IPA over the volume-percent range of each of 0-0.12% in steps of 0.024%.

Test Solutes. The test samples were polycyclic aromatic hydrocarbons (PAH) dissolved in hexane and comprised naphthalene (1), anthracene (2), fluoranthene (3), pyrene (4), and benzo(a)anthracene (5). Injection of these individually or in admixture confirmed that retention times were independent of the manner of sample introduction.

Instrumentation. The liquid chromatograph used throughout this work was a Varian Model 5000. The detector was an LDC UV Monitor (254 nm) or a Varian Vari-Chrom variable wavelength UV monitor set at 254 nm, very little difference being found between the two units. The column was a Varian Micro-Pak Si-5 (5 um silica), and was 30 cm by 4 mm id. It was thermostated at constant temperature (25°C) with a water-jacket. The injector was a Valco valve with a 10-ul external loop.

Procedure. The column was allowed to equilibrate with each new mobile phase (3 cm^3 min^{-1} flow rate) until retention times were constant. Dead volumes were then measured by injection of 10-ul amounts of pentane or of iso-octane, a small blip being obtained in the recorder trace in each case.

TABLE 1

Equilibration Times/min for Listed Additive (A + B) Systems[a]

		% v/v B					
A	B	0.0	0.024	0.048	0.072	0.096	0.120
THF	MC	15	30	30	30	30	60
ACN	MC	30	30	30	50	60	60
IPA	MC	15	30	30	50	50	70
ACN	THF	15	20	20	30	45	95
IPA	THF	10	20	20	30	45	95
ACN	IPA	50	55	45	45	40	35

[a] tetrahydrofuran (THF), methylene chloride (MC), acetonitrile (ACN), and iso-propyl alcohol (IPA)

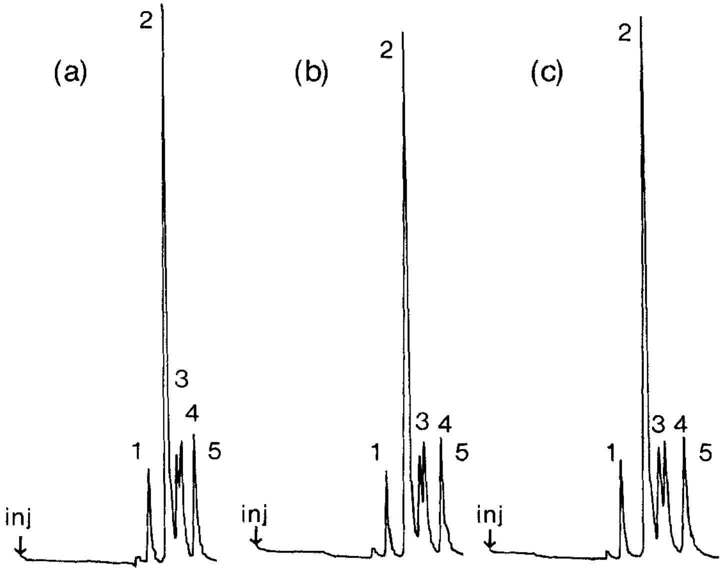

FIGURE 1

Illustration of the variation of solute retention times (ca. 5 min for the last-eluting compound) prior to equilibration of stationary phase with new mobile phase (here, 0.024% v/v IPA + 0.096% THF).

RESULTS AND DISCUSSION

Equilibration Time. Our first concern was with the time required to establish equilibrium surface coverage of the silica sorbent with each combination of mobile phases, this being of particular importance in obtaining reproducible results in gradient elution. The consequences of slow kinetics of displacement of one component by another have been studied and commented upon at length by Scott and Kucera (4), and our results establish that their findings hold equally true in the instance of high dilution of mobile-phase additives. Table 1 presents the equilibration times for all binary-additive/hexane mixtures used in this work. The first five sets of these required increasing times in accordance with solvent "strength" while, in contrast, the pair ACN/IPA gave the opposite result (where the equilibration time decreased from 50 min to 35 min on passing

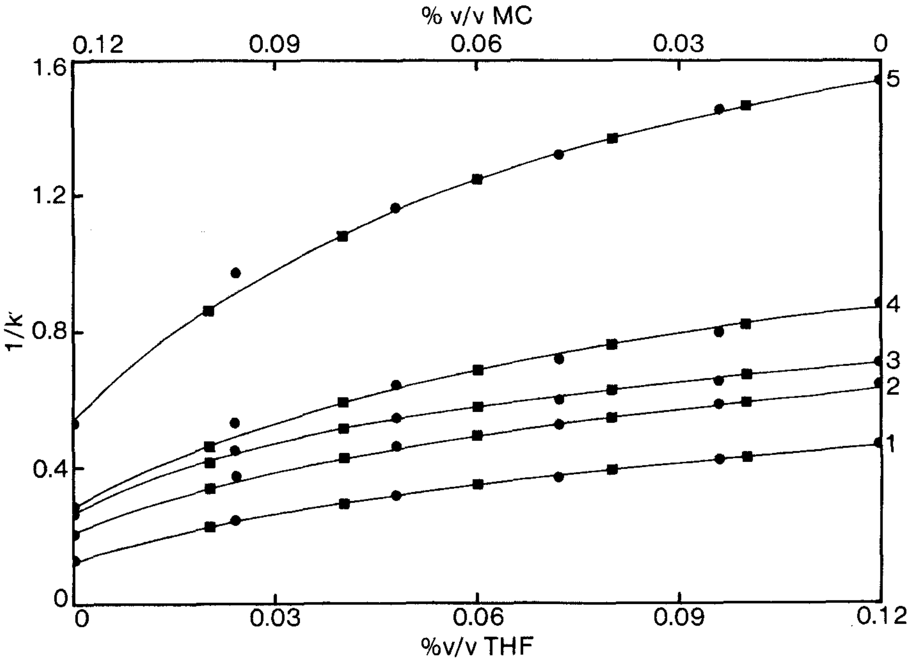

FIGURE 2

Plots of inverse capacity factor against composition (volume percent) of the, indicated mobile-phase additive pairs for the solutes: (1) naphthalene, (2) anthracene, (3) pyrene, (4) fluoranthene, and (5) benzo(a)pyrene. Circles: experimental data; squares and solid curves: eqn. 6.

from 0.12% ACN to 0.12% IPA). The situation is illustrated further in Figure 1, where the chromatograms resulting from three successive injections of the test mixture prior to the establishment of column equilibrium are shown (0.024% IPA + 0.096% THF). There is a noticeable improvement in the separation of solutes 3 and 4 on passing from (a) to (c), while the absolute retention time for the last-eluting compound increased roughly by 10%. We also observed that the equilibration times in Table 1 were independent of whether or not the solute test mixture was injected during the course of a particular determination, which would seem to lend weight to a model (4) of solute interaction with sorbed mobile-phase constituent as opposed to one of displacement (3).

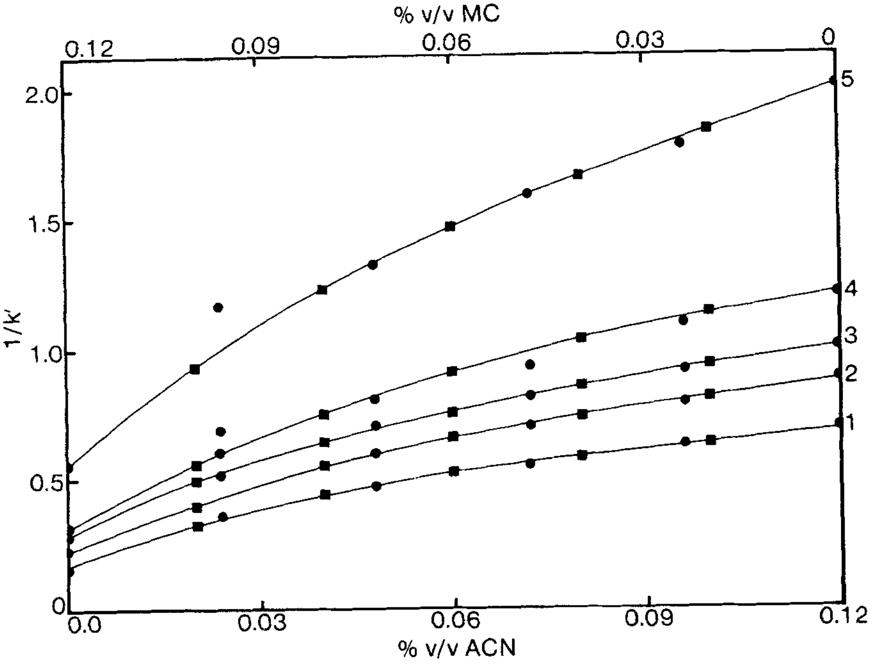

FIGURE 3. As in Figure 2.

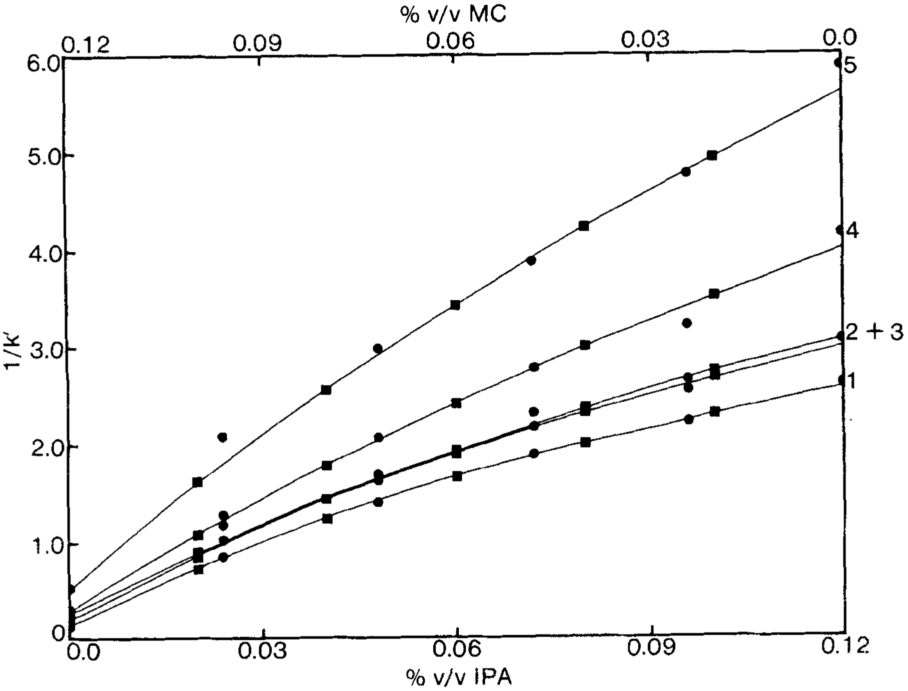

FIGURE 4. As in Figure 2.

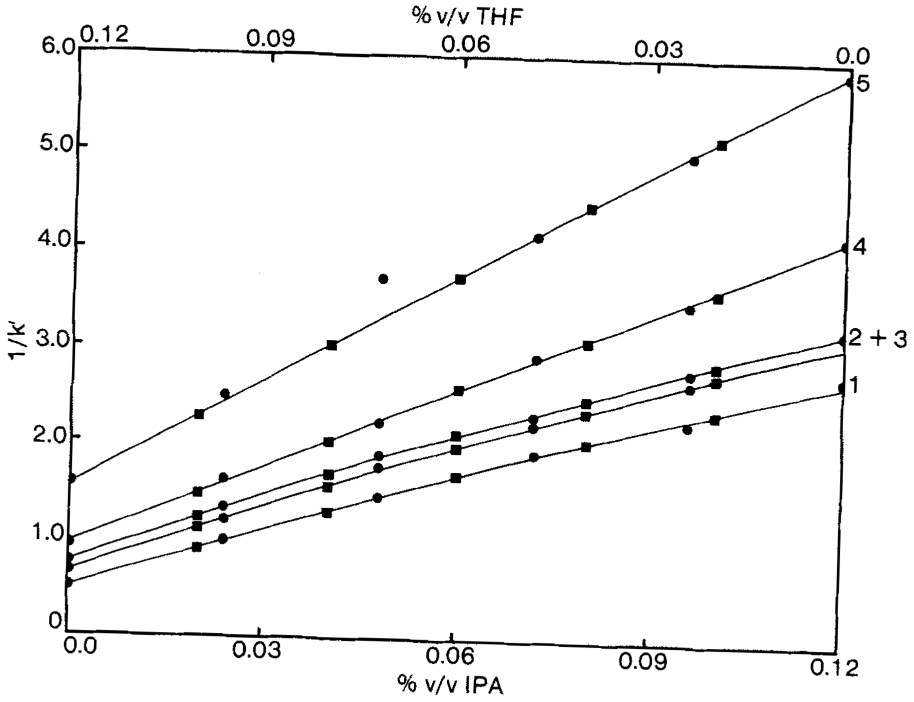

FIGURE 5. As in Figure 2.

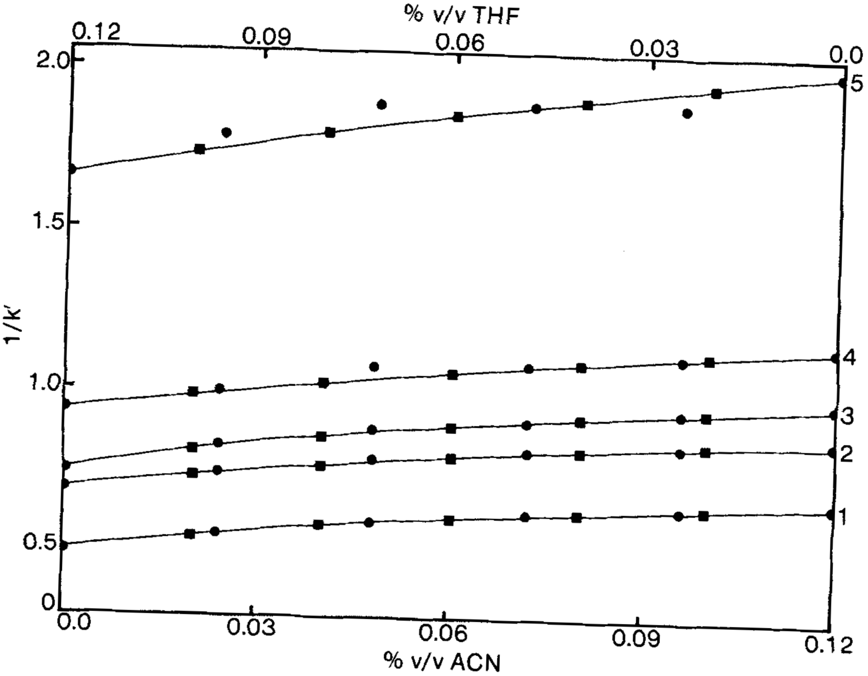

FIGURE 6. As in Figure 2.

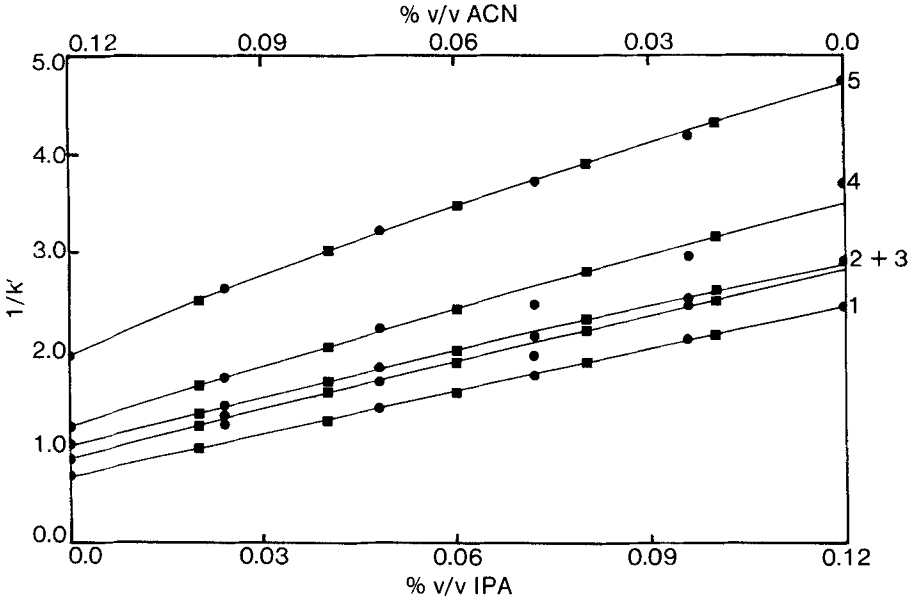

FIGURE 7. As in Figure 2.

Graphical Presentation and Interpretation of Retention Data. Figures 2-7 provide plots of reciprocal solute capacity factor against ϕ for the indicated mobile-phase additive pairs over the range of each of 0-0.12%. The filled circles represent the experimental data ($\pm$ ca. 3%) while the squares were generated from best-fits of eqn. 6 with which were constructed also the solid curves.

The plots in Figures 2-4 exhibit substantial curvature, while those in Figures 5-7 are very nearly linear. Moreover, there is in some instances a considerable improvement in the resolution of pairs of solutes upon alteration of the binary-additive composition, as found for example in Figures 2 and 3. In contrast, the separation of solutes 2 and 3 is consistently poor with IPA/MC (Figure 4), improved somewhat with IPA/THF (Figures 1 and 5), and decreases from left to right on passing from ACN to IPA modifier (Figure 7). Thus, retentions are indeed sensitive to minute changes in the type and make-up of mobile-phase additive even at levels of the latter of 0.1%.

Table 2

Best-Fit Parameters of Eqn. 6

		Solute				
Solvent Pair		Naphthalene	Anthracene	Pyrene	Fluoranthene	Benzo(a)pyrene
THF/MC	b	2103.	1087.	720.2	700.5	526.8
	b'	1247.	969.0	940.3	810.4	770.2
ACN/MC	b	2176.	1500.	1190.	1057.	928.0
	b'	696.0	800.0	828.0	791.0	984.0
IPA/MC	b	5741.	4237.	3329.	3758.	3449.
	b'	297.2	302.6	337.3	516.7	586.3
ACN/THF	b	406.1	252.3	364.8	278.5	295.1
	b'	352.6	366.0	823.9	854.3	914.9
IPA/THF	b	3754.	2731.	2510.	2397.	2121.
	b'	30.26	17.39	177.2	147.5	129.5
IPA/ACN	b	3020.	2222.	1738.	1767.	0.
	b'	251.9	123.2	104.1	80.40	0.

We note in passing that the capacity factors (hence times of analysis) are substantially altered in each figure. For example (Figure 2), k' for benzo(a)anthracene (no. 5) is 1.9 with 0.12% MC yet is reduced to 0.65 with 0.12% THF, an improvement of a factor of 3. On the other hand, this change induces a higher number of theoretical plates required to effect a separation (14), the increase demanded amounting also to a factor nearly of 3. The gain achieved in analysis time may well then be lost if the system efficiency is incapable of the higher required resolving power. The use of highly-dilute binary mobile-phase additives thus permits optimization in terms both of resolution and analysis time even in the case of the very simple example presented here (15). (The optimum turns out in fact to be 0.12% ACN.)

Fitted Parameters of Eqn. 6. We present in Table 2 the derived values of b and b' for all systems studied. We note at the outset that these are moderately insensitive on a relative basis, that is, if for a particular system b is increased by 10% b' can then be decreased by the same amount without affecting to any great extent the goodness of fit of the experimental data. Even so, we have thus far been unable to formulate a correlation of the values in terms either of solute or solvent bulk properties. Nevertheless, there is no question that eqn. 6 provides an accurate fit of all retention data with each of the six binary-additive pairs, hence the means of prediction of separations, which we intend taking up further in subsequent reports.

ACKNOWLEDGMENT

We gratefully acknowledge support received for this work from the Department of Energy.

REFERENCES

1. Locke, D. C., **J. Chromatogr. Sci.**, 12, 433, 1974.
2. Everett, D. H., **Trans. Faraday Soc.**, 61, 2478, 1965.
3. Snyder, L. R., **Principles of Adsorption Chromatography**, Marcel Dekker, New York, 1968.

4. Scott, R. P. W., and Kucera, P., **Anal. Chem., 45**, 749, 1973; **J. Chromatogr. Sci., 12**, 473, 1974; **13**, 337, 1975; **J. Chromatogr., 112**, 425, 1975; **122**, 35, 1976; **149**, 93, 1978; **171**, 37, 1979.

5. Laub, R. J., and Purnell, J. H., **J. Am. Chem. Soc., 98**, 30,35, 1976; Purnell, J. H., and Vargas de Andrade, J. M., **J. Am. Chem. Soc., 97**, 3585,3590, 1975.

6. Of special note is the peculiar notion put forth by Snyder and Poppe (**J. Chromatogr., 184**, 363, 1980) that eqn. 5 (hence, 3 and 4) violates the Gibbs-Duhem relation and that it cannot therefore be considered to be rigorously valid under any circumstances. Self-evidently, this argument is specious on several grounds, as pointed out by Laub (in **Physical Methods in Modern Chemical Analysis**, Vol. **3**, T. Kuwana, Ed., Academic Press, New York, 1983, Ch. 4). First of course, since the Gibbs-Duhem relation is formulated in terms of mixed **solvents**, it offers no information regarding the variation of infinite-dilution activity or partition coefficients of probe **solutes** as a function of blended-phase composition. Secondly, since in the diachoric solutions formulation the solvent components are assumed to be immiscible the activity coefficients of each must be taken to be unity over all compositions. The Gibbs-Duhem relation is thereby obeyed exactly although, since $d \ln \gamma_i = 0$, little further insight is gained. The point thus emphasizes that for the purposes of eqns. 4 and 5, there is in fact no difference between completely ideal mixtures and those whose (diachoric) components are completely demixed.

7. Jandera, P., and Churacek, J., **J. Chromatogr., 91**, 207,223, 1974; **93**, 17, 1974; **104**, 257, 1975; Chandera, P., Churacek, J., and Janderova, M., **J. Chromatogr., 115**, 9, 1975.

8. Soczewinski, E., **Anal. Chem., 41**, 179, 1969; Soczewinski, E., and Golkiewicz, W., **Chromatographia, 6**, 269, 1973.

9. Jaroniec, M., Narkiewicz, J., and Borowko, M., **Chromatographia, 11**, 581, 1978; Narkiewicz, J., Jaroniec, M., Borowko, M., and Patyrkiejew, A., **J. Chromatogr., 157**, 1, 1978.

10. Slaats, W. M., Kraak, J. C., Brugman, W. J., and Poppe, H., **J. Chromatogr., 149**, 255, 1978.

11. McCann, M., Purnell, J. H., and Wellington, C. A., in **Chromatography, Equilibria, and Kinetics**, Faraday Society Symposium No. 15, D. A. Young, Ed., The Royal Society of Chemistry, London, 1980, p. 82.

12. Madden, S. J., McCann, M., Purnell, J. H., and Wellington, C. A., paper presented at the 184th National Meeting of the American Chemical Society, Kansas City, Missouri, 1982; Madden, S. J., Ph.D. Thesis, University College of Swansea, Swansea, Wales, 1983.

13. It is important to note that in the studies cited the abscissa covered the entire compositional range, pure S to pure A. It is also significant

that in all but two of the six types (V and VI) linearity of the plots extended over a considerable range; any theoretical attempt to derive eqn. 6 hence must take this into account.

14. Purnell, J. H., **J. Chem. Soc.**, 1268, 1960.

15. There is the additional practical advantage that since the modifiers are used at such low concentration the column efficiency and pressure drop remain nearly invariant.