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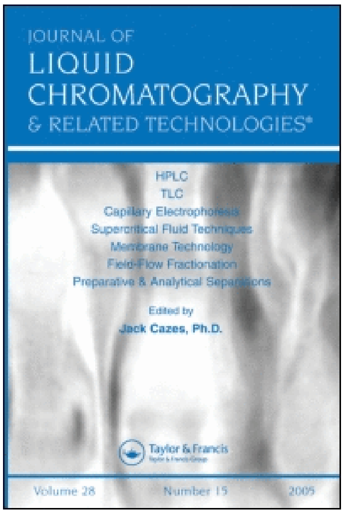

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SOLUTE RETENTION IN COLUMN LIQUID CHROMATOGRAPHY. VII. REDUCTION OF MASS-TRANSFER NON-EQUILIBRIUM AND ANALYSIS TIME

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ABSTRACT

Retentions are reported for the test solutes benzene and the polycyclic aromatic hydrocarbons (PAH) naphthalene, biphenyl, anthracene, and fluoranthene with columns containing mechanical mixtures of (inert diluent) Spherisorb silica + (active sorbent) Spherisorb octadecylsilyl (ODS) derivatized silica with methanol:water mobile phase. All solutes elute in one column volume with neat silica diluent packing, while both the extent of separation as well as analysis time increase as the amount of ODS phase is increased. Plots of the reduced plate height h against the reduced linear carrier velocity v show that the column efficiency with pure diluent phase is a factor of two superior to that containing neat active sorbent at v_{opt}. However, an anomalous rise in h is found at v less than v_{opt}. The capacity factors of the solutes increase linearly from zero as the amount of active phase is increased, hence limiting the permissible sample size at high packing dilution; there also occur some inversions of retention order. Dilution of lc sorbents with inert packings is thereby shown to provide quantitative control over column efficiency, analysis time and, potentially, selectivity. The technique is claimed to offer particular advantage in microbore-column liquid chromatography.

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INTRODUCTION

Two of the major drawbacks to gas-solid (gsc) and gas-liquid-solid chromatography (glsc) in comparison with gas-liquid chromatography (glc) are slow kinetics of mass transport (resulting in poor column efficiency), and generally elevated column temperature due to high heats of adsorption. Al-Thamir, Laub, and Purnell (1,2) overcame these difficulties by dilution of sorbent stationary phases (e.g., alumina) with inert packings (e.g., Chromosorb G). Plots of the theoretical plate height H against linear carrier velocity u for their systems then showed that observed plate heights approached the limits of the longitudinal diffusional term of the van Deemter equation (see below), $H = B/u$ at $u \simeq 0$, and $H = 0$ at $u >> 0$, flow inhomogeneity and mobile-phase mass-transfer being negligible for the solutes considered. Upon the addition of some sorbent to the column, plots of H against u assumed the usual hyperbolic shape as a result of some stationary-phase mass-transfer non-equilibrium, where the slope beyond u_{opt} (hence, column efficiency at the optimum practical velocity u_{opv}) approximated a linear function of the weight percent of the amount of sorbent present in the column.

The rationale for these results derives from the abbreviated form of the van Deemter relation:

$$H = A + \frac{B}{u} + (C_S + C_M)u \tag{1}$$

where the plate height H is said to be a function of flow-path inhomogeneity (A), longitudinal diffusion (B), and mobile- and stationary-phase mass-transfer non-equilibrium (C_M, C_S, respectively); and where u is measured either at the column outlet u_o or calculated as an average value $\bar{u}$ over the column length L. For columns containing pure inert (that is, non-sorbing) diluent packing, there is no contribution from the C_S term since the solutes are not retarded:

$$H = A + \frac{B}{u} + C_M u \tag{2}$$

In addition, solute capacity factors k' should tend to zero. The consequent practical advantages of the use of diluted packings in gsc and glsc therefore include quantitative control over column efficiency as well as solute retentions.

There is, of course, in principle, no difference between the thermodynamic aspects of partitioning that govern separations achieved either in gc or in lc and, in practice, diffusion and mass-transfer in the respective gaseous or condensed-state mobile and stationary phases account entirely for whatever kinetic distinctions are commonly drawn. Thus, plots of column efficiency against average linear carrier velocity for gc and lc are predicted a priori to be identical in general form although, self-evidently, the various parameters deduced from each (e.g., longitudinal diffusion) must be markedly different. [Recent estimable studies of a quantitative nature (3) bear out this proposition and demonstrate with little ambiguity that heretofore-reported data at variance with the van Deemter relation in fact arose in lc largely as a consequence of extra-column band dispersion.] Dilution of sorbent packings with inert media thus would be expected to be as beneficial in lc as in gc. Moreover, packing dilution should prove to be of immediate use in microbore-column lc (4), one of the major drawbacks to which is extended analysis time.

In this work, we test the extent to which dilution of sorbent lc stationary phases enhances column efficiency and also, investigate the variation of solute capacity factors as a function of the extent of dilution. We then examine the possibility that there exists an optimal degree of dilution in terms of system selectivity and analysis time.

EXPERIMENTAL

Materials. The hplc columns, 15 cm x 4.6 mm i.d. (Jones Chromatography, Columbus, Ohio), were packed with 5µ Spherisorb silica in admixture with 5µ Spherisorb ODS silica in weight ratios as indicated. The polycyclic aromatic hydrocarbon (PAH) solutes were supplied by Chem Service, while carbon tetrachloride and methanol were ACS-grade from Fisher Scientific.

Apparatus. The columns were packed with a laboratory-fabricated apparatus comprised of a Haskel pump (Burbank, California) capable of delivering slurry from a reservoir to an lc column at up to 70,000 psig. The chromatographic system consisted of a Waters Model 6000A pump coupled to a Jasco UV spectrophotometric detector, or a Varian Model 8500 liquid chromatograph coupled with a Varian Vari-Chrom variable UV/Vis detector. All injections were made from a Valco valve fitted with a 0.2-µl internal loop.

Column-Packing Procedures. Columns were packed at pressures of 12,000-15,000 psig with degassed methanol as the displacement solvent. Thus, 2.5 g of packing material were slurried in 11 cm^3 of carbon tetrachloride, chosen because it gave well-dispersed slurries both with normal and reverse-phase silicas. Subsequent to depressurizing, an additional 100 cm^3 of methanol was passed through the columns. Immediately prior to experimental use, each column was conditioned with 150 cm^3 of mobile phase.

Chromatographic Procedures. Benzene (1), naphthalene (2), biphenyl (3), anthracene (4), and fluoranthene (5) comprised the test solutes, while the mobile phase was in all instances methanol:water (72.5:27.5 v/v) at a nominal flow rate of 1 cm^3 min^{-1}, measured with a calibrated and thermostated (25°C) pipet.

RESULTS AND DISCUSSION

Testing of the Inert Diluent Packing. A representative chromatogram of the test solutes with 100% w/w silica, the diluent phase, is shown in Figure 1(a). There is clearly no separation of the five PAH, a single peak being observed that corresponds to the column void volume, ca. 1.4 cm^3. Thus, there is no interaction between the stationary phase and the solutes with this solvent system, i.e., k' = 0. The packing therefore fulfills the requirement of an inert diluent medium.

Columns Containing Some Sorbent Packing. Chromatograms (b)-(d) present the results obtained with mixed-bed packings comprised of various weight-percent amounts of silica + ODS silica (the latter acting as the sorbent stationary phase). That in (b) was achieved with an active/diluent ratio of 1:3; (c) corresponds to a ratio of 1:1; and chromatogram (d) shows the results with a packing ratio of 3:1 ODS silica:silica. Chromatogram (e) was obtained with 100% of the active phase, ODS silica.

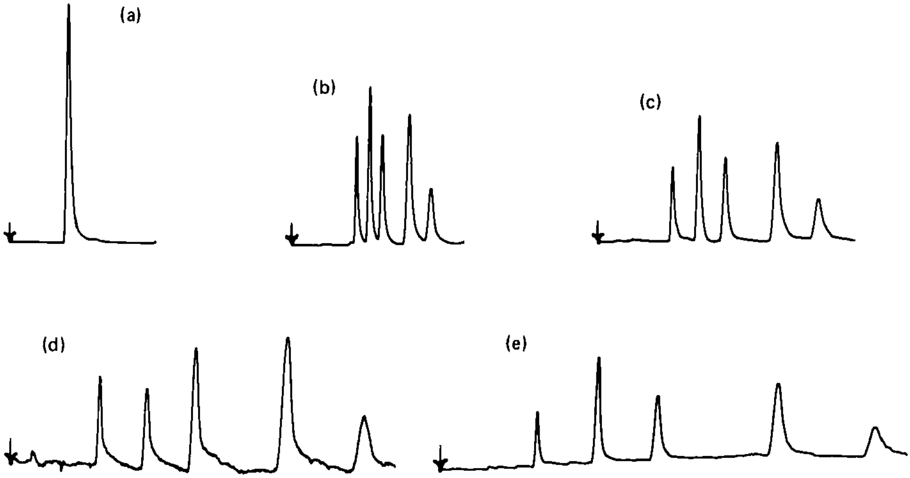

FIGURE 1. Chromatograms of the test solutes (cf. Experimental Section and Table 1) with columns containing (a) Spherisorb silica (diluent packing); (b)-(d) 1:3, 1:1, and 3:1 w/w mechanical mixtures of Spherisorb ODS:Spherisorb; and (e) Spherisorb ODS silica (active sorbent).

The separation is obviously more than adequate with the pure ODS column, although the time of analysis is somewhat extended. To remedy this, the organic concentration in the mobile phase could be increased, as could the flow rate, each resulting in a reduction of retentions, but each of these measures would be at the expense of resolution. An alternative of potential utility is use of a shorter column of reduced particle size whereby system efficiency is maintained, yet where retentions are reduced. However, this, too, may not be practicable in certain situations, e.g., where the particle size is already 3μ or so, or in instances where separation of the solute mixture requires more than a few hundred theoretical plates (5).

In contrast, chromatograms (b)-(d) of Figure 1 show that progressive addition of an inert diluent packing reduces accordingly the analysis times from those in (e) while, at the same time, preserving the column efficiency. Moreover, the analysis in (b) appears to exhibit the optimal extent of dilution such that baseline separation is achieved in the shortest analysis time.

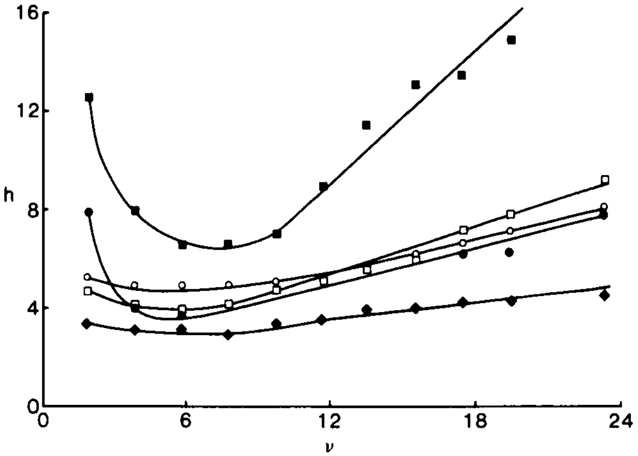

FIGURE 2. Plots of reduced plate height h against reduced linear mobile-phase velocity v for biphenyl solute with columns containing Spherisorb silica diluent packing (◆); 1:3 (●), 1:1 (□), and 3:1 (○) w/w mechanical mixtures of Spherisorb ODS:Spherisorb; and (■) Spherisorb ODS silica sorbent.

Graphical Representation of the Variation of Column Efficiency. van Deemter plots representative of the results of this portion of the work for biphenyl solute with each of the five columns are illustrated in Figure 2, where we have made use of the semi-empirical fitting relation that involves the so-called "reduced" plate height h (6,7):

$$h = Av^{1/3} + B/v + (C_S + C_M)v \tag{3}$$

where $h = H/d_p$ and $v = u\, d_p/D_M$, d_p being the particle diameter and D_M the solute diffusion coefficient in the mobile phase (ca. 10^{-5} cm^2 sec^{-1}). The column efficiency with 100% diluent phase is more than twice that found with 100% active phase at v_{opt}. Moreover, the portion of the curve to the right of v_{opt} for the pure diluent packing does not lie horizontally to the abscissa, that is, since C_S is presumed to be zero, there appears to be a small but finite contribution from the C_M term, mobile-phase mass-transfer non-equilibrium. We see also that as some active sorbent is added, the slopes of the lines to the right of the optimum increase substantially, although not linearly, with the largest being found with 100% ODS phase.

The situation is altered somewhat in regions of the plots where v is less than v_{opt}. In general, the magnitude of h at low v can be attributed primarily to the contribution from the B/v term, which becomes increasingly important as solute residence times become longer. Thus, at v_{opt} (ca. 5-8 in Figure 2), h increases as the amount of active phase in the column is increased since, if nothing else, solute retentions are thereby also extended. The column with only 25% active phase hence exhibits a lower plate height at v = 6 than does that with 50% silica. However, there is an anomalous rise in h found for the former at v less than 4, for which we have no explanation at this time. Further, there is no question that the divergence is real, and that the efficiency of this column very nearly deteriorates to that containing 100% active phase at v less than 2.

Regression of Retentions Against Degree of Dilution. Table 1 provides the k' values for each of the solutes with the various stationary phases utilized in this work. The linear relation found between the solute capacity factors and weight-percent composition of active phase is illustrated in Figure 3. The lines clearly pass through the origin, which provides additional supportive evidence of negligible retentions with packings of neat silica for these systems.

Optimal Dilution. The question arises at this point regarding an optimal extent of dilution. In order to assess whether this might be the case for the systems at hand, we show in Figure 4 the window diagram (8) of relative separations (values of alpha) plotted against the extent of

TABLE 1

Capacity Factors of PAH Eluted from Mixed-Bed Systems

	Percent w/w Active Phase			
Solute	25%	50%	75%	100%
Benzene	0.131	0.250	0.488	0.637
Naphthalene	0.339	0.726	1.32	1.68
Biphenyl	0.548	1.14	2.16	2.66
Anthracene	1.02	2.04	3.70	4.69
Fluoranthene	1.38	2.75	5.01	6.38

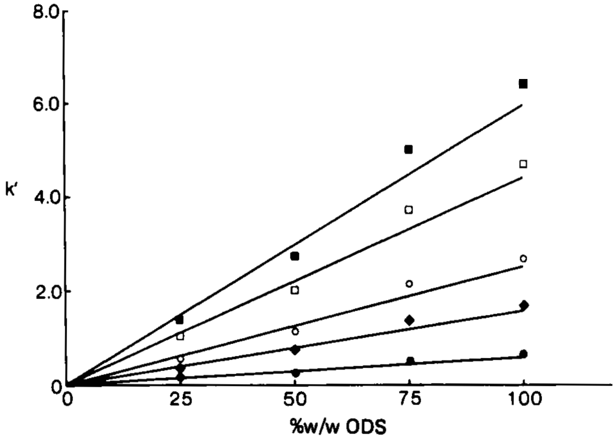

FIGURE 3. Plots of capacity factors of PAH of Table 1 against extent of dilution of Spherisorb ODS by Spherisorb silica: (■) fluoranthene; (□) anthracene; (○) biphenyl; (◆) naphthalene; and (●) benzene.

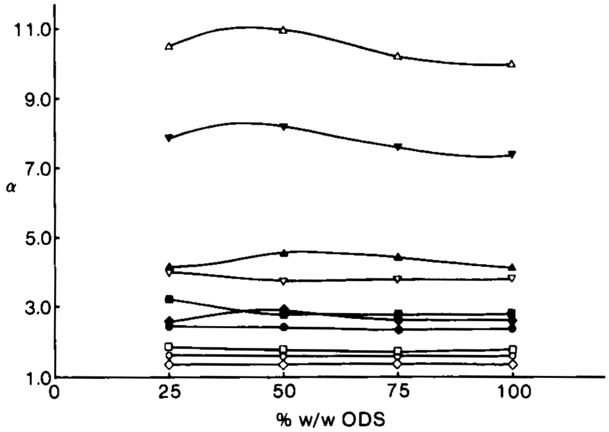

FIGURE 4. Window diagram (8) of relative retentions (alpha) of all possible pairs of solutes against extent of dilution of Spherisorb ODS by Spherisorb silica: (△) fluoranthene/benzene; (▼) anthracene/benzene; (▲) biphenyl/benzene; (▽) fluoranthene/naphthalene; (■) anthracene/naphthalene; (◆) naphthalene/benzene; (●) fluoranthene/biphenyl; (□) anthracene/biphenyl; (○) biphenyl/naphthalene; and (◊) fluoranthene/anthracene.

dilution for all pairs of solutes. Moderation of the active packing appears to have little effect on the separations in these instances, the three most-difficult solute pairs being virtually unaffected by the stationary-phase composition. Thus, the advantage of dilution of the stationary phase amounts here to reduction of the analysis time. We note in passing, however, that there are in fact crossovers of lines of alpha for several solute-pairs and so, in other situations, there may well exist optimal degrees of dilution in terms also of the system selectivity.

SUMMARY AND CONCLUSIONS

Inert dilution of lc stationary-phase media can be of use in decreasing analysis times as well as enhancing system efficiency in instances where other strategies might be of limited practicality. It is also clear that inert dilution will be of benefit with microbore systems (3), which has in fact been tested by us elsewhere and found to hold in practice (9). In addition, our findings are of course not limited to column lc but should also find application in various modes of thin-layer (e.g., rotating-disc tlc) (10), ion-exchange, and ion-chromatographic techniques.

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