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SOLVENT STRENGTH STUDIES ON POLYSTYRENE-DIVINYLBENZENE COLUMNS

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SUMMARY

The retention behavior of a series of non-polar solutes on two polystyrene-divinylbenzene high-performance liquid chromatography supports was studied. The solvent strength of several organic solvents was measured over a range of solvent compositions. Poor peak symmetry was observed for some solvents, and this appears to be correlated with the ability of the solvent to swell the polymer. Addition of a solvent that can swell the polymer to another solvent which cannot usually improves peak shape. The observed improvement in peak symmetry also correlates with the ability of the ternary mixture to decrease the void volume of the column.

INTRODUCTION

Although alkyl-bonded silica-based stationary phases dominate the field of reversed-phase chromatography, hydrophobic polymer columns are increasing in popularity as their retention characteristics and applications become better understood. The lack of silanol groups in the polymeric matrices as well as their broad pH stability range would be significant advantages in some separations. Most of the literature on polymeric stationary phases has focused on their application to a specific problem rather than on developing an understanding of their adsorption characteristics. The properties of polymers, the effects of solvents on the polymer itself, and the surface non-homogeneity of the polymer matrix combine to make a simple description of the retention process an impossibility. In the present paper, we have characterized some of these interactions for a series of non-polar solutes.

The theory of retention upon alkyl-bonded silica support materials has been well studied¹⁻⁴. Theoretical models, based on solubility parameters², predict that the logarithm of the capacity factor, k' , varies quadratically with the fraction of organic modifier in the mobile phase, φ . When the retention of a solute is examined over an extended range of solvent composition, the relationship is given by

$$\log k' = A\varphi^2 + B\varphi + C$$

where A , B and C are dependent on the solubility parameter of the solute, stationary

phase, and mobile phase. Experimental evidence shows significant deviations from quadratic behavior at low organic solvent concentrations^{1,5,6}. Over a limited range of solvent composition, the relationship between $\log k'$ and φ is approximately linear, *i.e.*,

$$\log k' = \log k'_0 - S\varphi$$

In this equation, k'_0 is the extrapolated retention of the solute in a totally aqueous phase and S is a representation of the solvent strength for a given system. There has been some discussion about the fundamental solute-solvent interactions responsible for the linearity of the relationship. It has frequently been shown that the solvent strength term is a function of the size and functionality of the test solute. Nevertheless, the simplicity of this linear approach makes it quite useful for the qualitative comparison of solvent strengths and selectivities for various alkyl bonded stationary phases¹.

The relative retention of a solute, i , in two different solvent systems, j and k , is given by the relation

$$\log (k'_{i,j}/k'_{i,k}) = \log (V_j A_k / A_j V_k) + \log (\gamma_{i,j}^b \gamma_{i,k}^s / \gamma_{i,j}^s \gamma_{i,k}^b) + A_i (\varepsilon_k^0 - \varepsilon_j^0)$$

where k' is the capacity factor of solute i in solvents j and k , A and V are the molar surface area and volume of each of the species, $\gamma_{i,j}^b$ and $\gamma_{i,j}^s$ are the activity of solute i in solvent j in the bulk of solution and on the surface, respectively, and ε^0 is the eluotropic strength of solvents j and k ⁷. The eluotropic strength has been shown to be related to the energy of adsorption of the solvent onto the adsorbent in adsorption chromatography on polar phases. Neglecting the activity coefficient term, a rough approximation of the eluotropic strength of the solvent can be obtained from a homologous series of compounds⁸. It should be emphasized that neglecting the activity coefficient term is not rigorously correct in mobile phases containing water. As a result, the eluotropic solvent strength does not accurately reflect the energetics of adsorption. It can, however, represent the relative elution strength of a solvent mixture at constant composition, and it is in this regard that it is used here.

Most recently, Geng and Regnier⁹ have extended the above theory to the case where multiple adsorbed solvent species are displaced from the surface of this stationary phase on adsorption of the solute. The retention of a solute is then given by

$$\log k' = \log I - z \log [D_0]$$

where I is a factor related to the phase ratio, the partition coefficient, and the concentration of organic displacer bound to the surface, z is the number of solvent molecules displaced, and $[D_0]$ is the concentration of organic solvent in the mobile phase. Note that the form of these equations is similar and thus differ only in the physical interpretation assigned to the coefficients. This reflects the inability of an equilibrium technique such as chromatography to elucidate the mechanism of the chromatographic process. Nevertheless, it is useful to have a measure of relative sorbent and solvent strength to allow systematic optimization of separation systems.

The retention mechanism on polymeric, pyrocarbon, and porous graphite

phases is probably quite different from that on silica-based bonded phases. In much of the previously reported work on the former sorbents, both selectivity differences for specific functional groups and unusual "adsorption" and tailing were observed¹⁰⁻¹³ in contrast to the separations achieved on alkyl-bonded stationary phases. It is quite likely that retention on these materials is a combination of partitioning and adsorption to specific sites on the polymer. Colin and Guichon¹² have summarized the difficulty in distinguishing between the two separation mechanisms. There have been several solvent strength studies on polystyrene-divinylbenzene (PSDVB) matrices¹⁴⁻¹⁷. Robinson *et al.*¹⁶ reported an eluotropic series for XAD-2 and XAD-4, based on distribution of benzo[*a*]pyrene and benzo[*a*]anthracene. One interesting feature of this series is the relatively high strength assigned to dimethylsulfoxide (DMSO), which is a stronger solvent than acetonitrile. Also of note is the relatively weak solvent strength of the alcohols, in contrast to their behavior with bonded phase packings. Since DMSO and methoxyethanol have been shown to be excellent solvents for protein separation, we have evaluated their solvent strength with a series of non-polar solutes.

In addition to exploring the relationship between $\log k'$ and ϕ for a PSDVB high-performance liquid chromatography (HPLC) packing, we will examine the effects of the solvent composition on the polymer itself. The dependence of peak symmetry on the type of organic solvent used will also be discussed.

EXPERIMENTAL

Instrumentation

The chromatograph used consisted of an Altex-Beckman (Berkeley, CA, U.S.A.) 110A pump, a Rheodyne (Cotati, CA, U.S.A.) 7125 injection valve, and a Perkin Elmer (Norwalk, CT, U.S.A.) LC-5 fixed-wavelength UV detector modified with a Max-N flow cell and related electronics (Laboratory Data Control, Riviera Beach, FL, U.S.A.). Detection was at 280 nm for DMSO-containing solvent systems and at 254 nm for all other mobile phase modifiers. Two 150 × 4.6 mm polymeric columns, a 5 μm , 100 Å PLRP-S packing and a 8 μm , 300 Å PLRP-S 300 packing, were supplied by Polymer Laboratories (Amherst, MA, U.S.A.). The octadecylsilane-bonded phase column was a 150 × 4.6 mm column packed by us with 5 μm Hypersil ODS (Shandon, Chelshire, U.K.).

Materials

The HPLC solvents included methanol, acetonitrile (Fisher Scientific, Fairlawn, NJ, U.S.A.), DMSO, methoxyethanol (Burdick & Jackson, Muskegon, MI, U.S.A.), and unstabilized tetrahydrofuran (MCB Reagents, Gibbtown, NJ, U.S.A.). The dead volume markers were uracil (MCB), ²H₂O (Aldrich, Milwaukee, WI, U.S.A.) and Blue Dextran (Sigma, St. Louis, MO, U.S.A.). The test solutes were phenol, *p*-cresol, anisole, phenetole, ethylbenzene, naphthalene (Aldrich), benzene, and toluene (Fisher). The solutes were of reagent grade or better and were used without further purification. The water used was deionized in a Milli-Q system (Millipore, Bedford, MA, U.S.A.).

Methods

The capacity factors for eight small aromatic solutes were calculated from the time elapsed to the peak maximum. The void volume of each column and solvent system was determined with $^2\text{H}_2\text{O}$ -enriched mobile phase and with uracil. The excluded volume was determined with Blue Dextran (MW $2 \cdot 10^6$). Slopes, intercepts, their standard deviations, and the correlation coefficient were obtained from linear regression analysis of the $\log k'$ vs. ϕ plots for each solute. Each solute-solvent composition data point was the average of a least two replicate injections. The test solutes were prepared in the mobile phase or a solvent mixture weaker than the mobile phase. The solute solutions had a concentration of about $1 \mu\text{g}/\mu\text{l}$, and 5–10 μl were injected. All experiments were performed at room temperature (22°C). Peak asymmetry was measured as the B/A ratio as defined by Snyder and Kirkland¹⁸. The A and B measurements were made at 10% of the peak height.

RESULTS AND DISCUSSION

Column retention and solvent strength

The retention behaviour for a series of solvents on the PLRP-S columns as well as an alkyl-bonded phase is summarized in Table I. The slopes and intercepts shown are the average calculated from the behavior of a set of solutes in each solvent. This results in the relatively large standard deviations observed in the table and precludes discussion of specific interactions between the stationary phase and solute. The data on Hypersil ODS agree quite well with the data of Dolan *et al.*¹. Note that the intercepts for all of the solvents are essentially the same on the alkyl-bonded phase. On the polymeric column, the solvents seemed to fall into two different classes. The slopes and the intercepts of the $\log k'$ vs. ϕ curves were approximately the same for acetonitrile and tetrahydrofuran (THF) on both the bonded phase and the

TABLE I
SOLVENT STRENGTH DETERMINED FROM $\log k'$ vs. ϕ CURVES

N.D. = Not determined.

Organic solvent	Curve parameter	Stationary phase		
		PLRP-S 100	PLRP-S 300	Hypersil ODS
Methanol	Slope	3.3 ± 0.35	3.4 ± 0.40	3.2 ± 0.60
	Intercept	2.8 ± 0.44	2.7 ± 0.50	2.2 ± 0.70
Acetonitrile	Slope	2.4 ± 0.28	2.6 ± 0.20	3.2 ± 0.35
	Intercept	2.1 ± 0.63	2.0 ± 0.60	1.7 ± 0.50
THF	Slope	N.D.	3.5 ± 0.25	4.0 ± 0.54
	Intercept	N.D.	2.0 ± 0.36	1.8 ± 0.60
Methoxyethanol	Slope	N.D.	4.2 ± 0.60	3.1 ± 0.60
	Intercept	N.D.	3.7 ± 1.0	2.1 ± 0.70
DMSO	Slope	N.D.	5.3 ± 0.50	N.D.
	Intercept	N.D.	4.1 ± 0.57	N.D.

PLRP-S materials. However, these solvents had substantially smaller intercepts on the polymer materials than did the other solvents investigated. Methanol, methoxyethanol, and DMSO had large slopes on the PLRP-S, but had very large intercepts as well (Fig. 1). Graphic representation of the data indicated significant non-linearity for all of the solvents in the region of less than 30% organic solvent. The non-linearity was particularly striking for THF. The slopes were measured in the region 50–90% strong solvent for acetonitrile, 40–70% for THF, 60–80% for methanol, 40–90% for methoxyethanol, and 70–90% for DMSO to insure that the k' values were 0.5–10. It is interesting to note that if solvent strength were based solely on the slope of the $\log k'$ vs. ϕ curve for the polymeric phases, it could be quite misleading.

To compare our data with those of Robinson *et al.*¹⁶, conversion of the solvent strength to an elutropic series was necessary. It should be pointed out that extension of the elutropic series concept of Snyder to reversed-phase systems is fraught with problems. Because of the small retention in pure solvents, we used a organic solvent–water (4:1) mixture to determine the solvent strength. Methanol–water was used as the reference solvent. The order of solvent strength of the pure solvents may be different from that determined from a solvent mixture with a constant volume fraction of water. Nevertheless, this approach should be useful for comparing column and solvent strength behavior. Table II shows the ϵ^0 values calculated for each of the solvents. Our data is significantly different from those of Robinson *et al.*¹⁶. It is

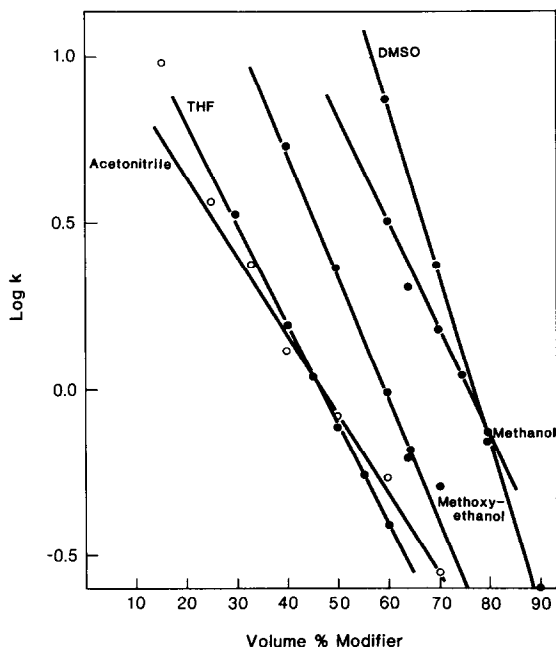


Fig. 1. $\log k'$ of phenol as a function of solvent composition for several solvents and a PLRP-S 100-Å column. Note that the slopes for a number of the solvents is similar, but unlike the observed case for alkyl-bonded stationary phases, the k'_0 for the solvents on the polymer column fail into two distinct groups. Accordingly, the solvent strength, derived from retention data for the pure solvent may be misleading.

TABLE II

ELUOTROPIC SERIES OF WATER-ORGANIC SOLVENT MIXTURES ON POLYSTYRENE-DI-VINYLBENZENE SORBENTS

<i>Organic modifier</i>	<i>PLRP-S*</i>	<i>Robinson**</i>
Methanol	0.000	0.000
DMSO	-0.009	0.097
Methoxyethanol	0.072	—
Acetonitrile	0.093	0.072
THF	0.137	Large

* Measured with alkylbenzenes in a water-organic solvent (1:4) mixture.

** Measured with benzo[*a*]pyrenes in pure solvents.

apparent that DMSO is a relatively poor solvent for use with PSDVB materials. Since DMSO is a good solvent for benzo[*a*]pyrenes, it would appear that the results of Robinson *et al.* were influenced by the large solubility of the solute in the mobile phase. Alcohols were also found to be relatively poor solvents, in accord with earlier work.

In addition to the relatively weak solvent strengths of the alcohols and DMSO, these solvents gave rise to very asymmetrical peaks, as might be expected for the interaction of the solute with a strong binding site. These interactions were more exaggerated as the aromatic nature of the solute increased. For example, the anthracene peak was more tailed than the naphthalene peak, which was much more tailed than that of benzene. This problem extended to other types of compounds such as the tricyclic antidepressant drug family, which possesses polar substituents. The tailing did not seem to be correlated with the k' of the solute, and there were no particular values of k' at which the tailing suddenly became worse. This peak asymmetry is probably the main limitation of polystyrene-divinylbenzene matrices for reversed-phase chromatography. Benson recently reported a "deactivated" polymer, which had been modified with C₁₈ groups bound to the aromatic ring to decrease π - π interactions¹⁰. The alternate approach to the problem seems to be the use of "strong" solvents in the mobile phase mixture to deactivate the strongest binding sites in a manner analogous to the use of water in adsorption chromatography (Fig. 2).

Solvent-dependent stationary phase changes

The peak shapes attained with "strong" solvents were correlated with a significant variation in the void volumes, measured with both ²H₂O-enriched solvent mixtures and uracil, as shown in Table III. For acetonitrile and THF, the void volumes were significantly smaller than for the alcohols and DMSO. This decrease in volume may arise as a result of swelling of the resin by the solvents. There appear to be two discrete swelled states, since the volumes measured for acetonitrile and THF are similar despite the fact that their solvent strength, and thus their energetics of binding to the polymer, are different.

The possibility that the polymer might swell is not surprising considering the methods used to produce the materials. The macroporous polymers are synthesized using a "porogen" which solubilizes the monomer but is not soluble in the polymer^{10,19}. The resulting material contains both macroporosity, due to pores among

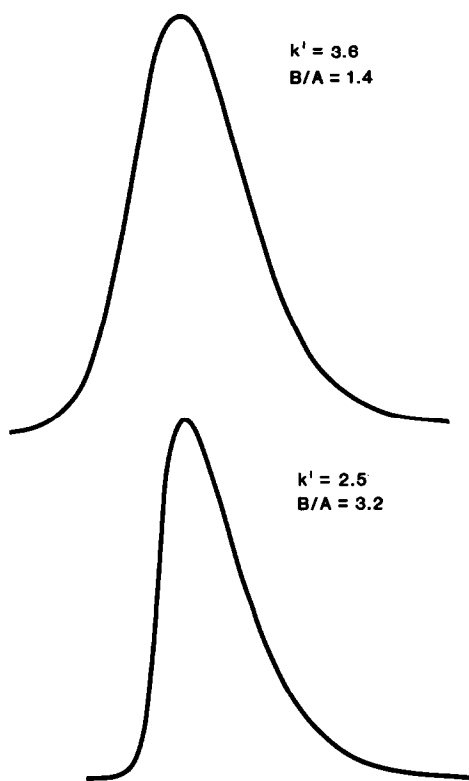


Fig. 2. Peak shape as a function of the presence or absence of THF. The mobile phase yielding the upper tracing was THF-methanol-water (1:3:6) while for the lower tracing a methanol-water (1:1) mobile phase was used. The mobile phases were selected to match k' to avoid any effect of k' on the peak shape.

TABLE III

VOID VOLUMES WITH VARIOUS WATER-ORGANIC SOLVENT MIXTURES

Column	Solvent	2H_2O	Uracil	Blue Dextran
PLRP-S 100	Water-methanol (2:3)	1.74	1.74	0.64
	Water-methoxymethanol (1:2)	1.72	1.74	0.65
	Water-acetonitrile (3:2)	1.43	1.44	0.59
	Water-THF (3:2)	1.41	1.51	—
PLRP-S 300	Water-methanol (2:3)	1.86	1.93	0.72
	Water-methoxymethanol (1:2)	1.89	1.87	—
	Water-DMSO (1:4)	1.89	1.91	—
	Water-acetonitrile (3:2)	1.74	1.79	0.78
	Water-THF (1:1)	1.77	1.77	—
	Water-methanol-THF (93:106:1)	—	1.87	—
	Water-methanol-THF (8:9:1)	—	1.81	—
Water-methanol-THF (6:3:1)	—	1.77	—	

the microspheres and their agglomerates, and microporosity, due to pores within the microspheres¹⁹. The latter may be inaccessible to solvent or solute. It is also quite reasonable to presume that the transition from microsphere to pore structure is not discontinuous, but rather exists as a continuum from heavily crosslinked polymer chains to non-crosslinked polymer chains²⁰. This region of less heavily crosslinked chains could possibly engage in multiple interactions with solute molecules that possess π electrons, resulting in the observed tailing. Studies with solvent swelling of polymers and subsequent thermal desorption of the solvent indicate that several types of "bound" solvent exist²¹, lending support to our hypothesis. Nevejans and Verzele²² have also reported a swelling of the microporous structure of PRP-1 on the basis of a comparison of surface area and pore volumes, measured by two techniques. The differences in chromatographic behavior observed are presumably the result of specific interactions between the solutes and the stationary phase, as has also been observed for pyrocarbons¹². It is interesting to note that transitions occur in the solvent content and structure of alkyl-bonded phases as well²³⁻²⁷.

If swelling of the microporous structure were associated with an improvement in chromatographic peak shape, one would predict that the addition of a small amount of acetonitrile or THF to a mixture of water and methanol would result in such an improvement. In fact, the improvement in peak shape occurred as expected (Fig. 2), and the observed void volume was also altered as expected (Table III). Several interesting observations can be drawn from the void volume data. First, the swelling that occurs within the particle as demonstrated by the constant volume explored by the Blue Dextran. Second, the difference between the abilities of the 100-Å pore and the 300-Å pore material to swell is significant and striking, the smaller pore material being able to change volume by about 20%. A final connection between the swelling phenomenon and the improvement in chromatographic peak shape can be surmised from the results of altering the amount of THF in the methanol-water mixture while keeping the k' constant. The addition of 0.5% THF does not improve peak shape and has no effect on the void volume of the column. The addition of 5.6% THF improves the peak asymmetry (B/A) from 3.2 to 2.1 and decreases the void volume somewhat. When 10% THF is added to the mobile phase, the resin is completely swollen (see Table III), and the asymmetry has improved to 1.4. This is the same asymmetry as that observed with pure THF, but variation of the methanol content of the mobile phase can yield a solvent strength slope similar to methanol. Thus, it is possible to exploit the advantageous properties of methanol while obtaining reasonable column performance.

CONCLUSIONS

The solvent strengths of a number of water-miscible organic solvents were evaluated with a PLRP-S, a polystyrene-divinylbenzene copolymer, column. Acetonitrile and THF were found to be good reversed-phase solvents, in contrast to methanol, methoxyethanol, and DMSO. The use of a ternary mixture of water and a "good" and poor solvent were found to improve the observed peak shape substantially without a change in relative solvent strength. This improvement was correlated with the swelling of the microporous structure observed with larger volume fractions of the "good" solvent.

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