

CHROMOSYMP. 1658

## COMPARISON OF RETENTION BEHAVIOR ON POLYMERIC RESINS AND AN ALKYL-BONDED SILICA PHASE IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

SUSAN PEDIGO and LARRY D. BOWERS\*

*Department of Chemistry and Department of Laboratory Medicine and Pathology, University of Minnesota, 420 Delaware St. S.E., Minneapolis, MN 55455 (U.S.A.)*

---

### SUMMARY

The two most obvious differences between alkyl-bonded silica phases and polystyrene–divinylbenzene resins as reversed-phase chromatographic supports are the aromaticity and the lack of hydrogen bonding ability in the polymeric resin. The effect of these differences on the selectivity for a set of small solutes was studied through the use of a solvatochromic comparison method. For retention on a polymeric phase for mobile phases with the modifiers methanol and acetonitrile, the linear solvation energy relationship indicated an increased dependence on the polarizability/dipolarity of solutes. For the modifier tetrahydrofuran, retention on the polystyrene–divinylbenzene resin was indistinguishable from that on the alkyl-bonded silica phase. The hydrogen bonding ability of a solute was found to play a greater role in retention on alkyl-bonded silica than on the polymeric resin for all three modifiers. Since the mobile phase compositions were chosen such that the Hildebrand solubility parameters were equal, the dependence of retention on molar volume was found to be the same for all mobile phase–stationary phase combinations examined.

---

### INTRODUCTION

Silica-based bonded phases currently dominate the high-performance chromatographic field as stationary phase materials. In order to be chromatographically useful, stationary phases must meet many requirements, such as mechanical stability at high pressure and chemical inertness toward chromatographic solvents. Although these characteristics are met by siliceous stationary phase materials, problems inherent in the silica support material present limitations. Instability at high pH and the presence of residual silanol groups, which have been implicated as a source of poor protein recovery<sup>1</sup> and poor peak symmetry in amine chromatography<sup>2</sup>, are most frequently mentioned. These problems have generated interest in exploring non-siliceous alternatives as reversed-phase sorbents.

Polystyrene–divinylbenzene (PS–DVB) polymeric materials meet the requirements of mechanical stability and chemical inertness. Since they are stable in alkaline solution, they present an alternative to alkyl-bonded silica as a reversed-phase

material. There are two obvious structural differences between alkyl-bonded silica phases and PS-DVB resins as reversed-phase materials. These are the aromaticity of the polymeric phase and its lack of silanol groups. These differences are significant because they may influence selectivity. Because of the potential impact of polymeric stationary phases, it is important to characterize their chromatographic behavior, particularly in comparison with alkyl-bonded silica phases.

The behavior of PS-DVB as a reversed-phase sorbent has been studied under a variety of mobile phase conditions including pH<sup>3-7</sup>, electrolyte concentration<sup>3,4</sup> and mobile phase modifier<sup>4,8</sup>. Of these factors the role of the mobile phase modifier has been studied most extensively. Mori<sup>8</sup> and Robinson *et al.*<sup>4</sup> discussed the influence of solvent polarity on the chromatographic separation mode. They found that as the solvent became more polar, the separation mode changed from adsorption to gel permeation and then to reversed-phase partition. Several authors have used a retention index scale to compare solvent-dependent selectivity differences for the elution of solutes from PS-DVB resins<sup>9-11</sup>.

In a previous paper<sup>12</sup>, we reported the solvent-dependent differences between the performance of a hydrophobic polymeric resin and an alkyl-bonded silica phase. A distinct correlation exists between peak symmetry and the swelling of the polymeric stationary phase. The observed changes in the polymer beads appear to be intraparticulate. Some solvents, such as tetrahydrofuran (THF), swell the polymer with a concomitant improvement in the peak symmetry. Conversely, a more hydrophilic solvent, such as methanol, shrinks the polymer and asymmetric peaks are observed. This suggests a significant difference in the nature of the stationary phase or the retention mechanism depending upon the mobile phase modifier. Although interesting, none of the work done so far elucidates the role of aromaticity and hydrogen bonding in the retention process.

Several recent reports in the literature on the comparison of solvatochromic polarity measurements with chromatographic retention<sup>13,14</sup> will allow us to address these issues directly. These research workers have utilized a solvatochromic comparison technique to isolate several properties of a solvent previously dealt with under a collective "polarity" value. The solvent properties include the hydrogen bond accepting (HBA) basicity<sup>15</sup>, hydrogen bond donating (HBD) acidity<sup>16</sup> and the polarizability/dipolarity<sup>17</sup> of a solvent. In a linear solvation energy relationship (LSER), these solvatochromic parameters are useful for discussing solute-solvent interactions<sup>18</sup>. Taft *et al.*<sup>19</sup> used LSER to predict octanol-water partition coefficients. Sadek *et al.*<sup>13</sup> used the same approach for examining retention in reversed-phase high-performance liquid chromatography (RP-HPLC). The combination of the solvatochromic parameters of the solute with the molar volume of the solute in a LSER were shown to correlate with log *k'* (capacity factor) values, determined experimentally. We chose this method for the present study because of the information it can yield about the stationary phases involved.

According to the solvatochromic comparison method, a solubility property (SP) of a solute is given by the following equation.

$$SP = SP_0 + M(\delta_1^2 - \delta_2^2)\bar{V}_3/100 + S(\pi_2^* - \pi_1^*)\pi_3^* + B(\alpha_2 - \alpha_1)\beta_3 + A(\beta_2 - \beta_1)\alpha_3 \quad (1)$$

Previously established<sup>19</sup> rules for the symbols will be followed: *i.e.* 1, 2, and 3 denote

the mobile phase, stationary phase and the solute, respectively; the molar volume, dipolarity/ polarizability, hydrogen bond donating acidity and accepting basicity are denoted by  $V$ ,  $\pi^*$ ,  $\alpha$ , and  $\beta$ , respectively. The last term in eqn. 1 will be omitted for reasons discussed elsewhere<sup>18</sup>. Eqn. 1 may be represented as follows

$$SP = SP_0 + mV/100 + s\pi^* + b\beta \quad (2)$$

The magnitudes of the coefficients  $m$ ,  $s$ , and  $b$  are due to a *difference* between mobile phase and stationary phase properties. The sign of the coefficient is determined by whether the term represents an exoergic or endoergic factor in the retention process. In the case of reversed-phase chromatography, SP is the logarithm of the capacity factor.

In the present study, two types of stationary phase were examined, an octadecyl-bonded silica (ODS) and two PS-DVB polymeric resins. The retention of twelve test solutes was compared for the two stationary phase materials under the same mobile phase conditions. We correlated retention data for the solutes with their solvatochromic parameters. In identical mobile phases, differences in the coefficients in eqn. 2 indicate differences in the stationary phases. In addition, we looked at overall selectivity differences between the phases under the same conditions.

## EXPERIMENTAL

Table I lists the test solutes and their solvatochromic parameter values. All of the solutes are small aromatic compounds, which were chosen to span a wide range of  $V$ ,  $\pi^*$  and  $\beta$  values. This is important in order to have a statistically meaningful correlation in eqn. 2 with a limited number of solutes. All of the solutes were obtained from Aldrich (Milwaukee, WI, U.S.A.), and diluted in the mobile phase prior to injection. Samples contained 1 to 2  $\mu\text{g}$ , depending upon the solute. Because of apparent problems with isotherm non-linearity at relatively low solute concentrations (5  $\mu\text{g}/\text{injection}$ ), a study was performed for each test solute to ensure that data were taken in a linear portion of the isotherm. The non-linearity was observed on all polymeric columns tested.

Mobile phases were prepared with water purified by the Milli Q system (Millipore, Milford, MA, U.S.A.). Unless otherwise noted, the mobile phase modifiers methanol, acetonitrile and THF (Fisher Scientific, Fairlawn, NJ, U.S.A.) are in the concentrations 70%, 50% and 40%, respectively. At these volume fractions, the Hildebrand solubility parameters<sup>20</sup> are nearly equal for the mixtures. The system dead-volume was determined with either uracil or <sup>2</sup>H<sub>2</sub>O (Aldrich).

Three columns were used in this study. They included a PLRP-S column (40 × 4.6 mm I.D., 300 Å pores, 8- $\mu\text{m}$  particle diameter) from Polymer Labs. (Amherst, MA, U.S.A.), a PRP-1 column (150 × 4.6 mm I.D., 10- $\mu\text{m}$  particle diameter) from Hamilton (Reno, NV, U.S.A.), and a Hypersil ODS column (20 × 4.6 mm I.D., 5- $\mu\text{m}$  particle diameter) from Shandon (Sewickley, PA, U.S.A.). The last column was upward-slurry-packed in methanol in our laboratory.

The chromatographic system consisted of a Beckman 110A pump (Fullerton, CA, U.S.A.), Rheodyne Model 7125 valve (Cotati, CA, U.S.A.) with a 20- $\mu\text{l}$  loop, and a Perkin Elmer LC-15 detector (Norwalk, CT, U.S.A.) at 254 nm. The detector was equipped with a Max-N flow-cell and associated electronics (LDC/Milton Roy,

TABLE I  
SOLVATOCHROMIC PARAMETERS<sup>a</sup> FOR SELECTED SOLUTES AND SOLVENTS

	$\pi^*$	$\beta$	$V/100$	$\alpha$
<i>Solutes</i>				
Benzene	0.59	0.10	0.989	
Toluene	0.55	0.11	1.139	
<i>tert.</i> -Butylbenzene	0.49	0.12	1.649	
Chlorobenzene	0.71	0.07	1.118	
Iodobenzene	0.81	0.05	1.215	
Benzyl alcohol	0.80	0.55	1.169	
Anisole	0.73	0.22	1.186	
2-Phenyl-2-propanol	0.75	0.61	1.305	
Acetophenone	0.90	0.49	1.269	
Nitrobenzene	1.01	0.30	1.129	
3-Nitrotoluene	0.97	0.31	1.285	
Benzonitrile	0.90	0.37	1.130	
<i>Solvents</i>				
Methanol	0.60			0.93
Acetonitrile	0.75			0.19
Tetrahydrofuran	0.58			0.00
Water	1.09			1.17
Aliphatics	0.00			0.00

<sup>a</sup> Taken from ref. 25. Solvatochromic parameters defined in text.

Riviera Beach, FL, U.S.A.). All experiments were performed at room temperature. Quadruplicate experiments were performed for all solutes. The data were taken on a strip-chart recorder. When the peaks were asymmetrical ( $B/A > 1.6$ )<sup>21</sup>, the data were acquired with an Apple IIe computer equipped with an Adalab board (Interactive Microwave, State College, PA, U.S.A.) and stored with the Vidichart program. The data were subsequently analysed for the peak centroid via a moments program. The experimental  $k'$  values were regressed against the solvatochromic parameters via a standard multivariable least-squares linear regression program.

## RESULTS AND DISCUSSION

As an initial test of our solute set, we compared values obtained on a ODS-bonded phase with values in the literature for similar conditions. Table II contains the regression results for eqn. 2 for alkyl-bonded silica data sets. These include the present data, the data of Smith<sup>22</sup> and the data of Haky and Young<sup>23</sup>. The regression results of the data of Smith and of Haky and Young were previously reported by Sadek *et al.*<sup>13</sup>. The present data and Smith's data were obtained with identical mobile phase systems and columns (Hypersil ODS). Even though only three of the test solutes were common to the two data sets, the correlations were statistically equivalent. Likewise, the regression results were the same for our data and the data of Haky and Young, even though the mobile phase compositions and the size of the solute data sets were different. We take this agreement as evidence that our selection of solutes is valid for evaluating the solvatochromic parameters. Table III contains the regression results for

TABLE II  
SOLVATOCHROMIC PARAMETERS FOR OCTADECYL-BONDED PHASE COLUMNS

SP<sub>0</sub>, *m*, *b*, and *s* are defined by eqn. 2. The correlation coefficient is given by *r*; *n* represents the number of solutes studied.

Solvent	Methanol		Acetonitrile		Tetrahydrofuran		
	Present <sup>a</sup>	Smith <sup>b</sup>	Haky <sup>c</sup>	Present <sup>a</sup>	Smith <sup>b</sup>	Present <sup>a</sup>	Smith <sup>b</sup>
SP <sub>0</sub> <sup>d</sup>	-1.10 ± 0.39	-0.48 ± 0.014	-0.27 ± 0.09	-0.71 ± 0.32	-0.38 ± 0.17	-0.28 ± 0.26	-0.13 ± 0.17
<i>m</i>	1.57 ± 0.36	0.90 ± 0.09	1.53 ± 0.07	1.15 ± 0.19	1.11 ± 0.15	1.03 ± 0.16	0.97 ± 0.11
- <i>b</i>	1.49 ± 0.18	1.28 ± 0.14	1.97 ± 0.12	1.83 ± 0.18	1.77 ± 0.17	1.63 ± 0.15	1.80 ± 0.17
<i>s</i>	-0.55 ± 0.19	-0.13 ± 0.13	-0.54 ± 0.10	0.12 ± 0.22	0.28 ± 0.16	-0.09 ± 0.18	0.17 ± 0.16
<i>r</i>	0.972	0.981	0.992	0.976	0.984	0.981	0.983
<i>n</i>	10	9	28	12	9	12	9
Av. res. <sup>d</sup>	0.08	0.08	0.07	0.10	0.06	0.08	0.04

<sup>a</sup> The chromatographic system consisted of a Hypersil ODS column and 70:30 MeOH-H<sub>2</sub>O, 50:50 MeCN-H<sub>2</sub>O, and 40:60 THF-H<sub>2</sub>O.

<sup>b</sup> Recalculated from data presented in ref. 29. Chromatographic system same as above.

<sup>c</sup> Recalculated from data presented in ref. 30 as in ref. 28. The chromatographic system consisted of an Alltech RP-18 column and methanol-0.05 M (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> buffer (55:45).

<sup>d</sup> Av. res. = Average residual observed in the fit of eqn. 2 to log *k'* of the solute retention data.

TABLE III

LOG  $k'$  DATA AND SOLVATOCHROMIC PARAMETERS FOR A PLRP-S STATIONARY PHASE

Chromatographic conditions and terms defined in Table II.

<i>Solute</i>	<i>Methanol</i>	<i>Acetonitrile</i>	<i>Tetrahydrofuran</i>
Benzene	1.139	0.769	0.611
Toluene	1.559	0.999	0.820
<i>tert.</i> -Butylbenzene	—	1.458	1.163
Chlorobenzene	1.547	1.056	0.801
Iodobenzene	—	1.387	0.963
Benzyl alcohol	0.093	-0.197	-0.247
Anisole	1.381	0.755	0.576
2-Phenyl-2-propanol	0.400	0.071	0.075
Acetophenone	0.959	0.382	0.134
Nitrobenzene	1.218	0.649	0.482
3-Nitrotoluene	1.405	0.886	0.709
Benzonitrile	0.850	0.446	0.247
SP <sub>0</sub>	-1.75 ± 0.63	-0.65 ± 0.29	-0.41 ± 0.39
<i>m</i>	2.80 ± 0.59	1.31 ± 0.18	1.04 ± 0.20
- <i>b</i>	3.39 ± 0.30	2.60 ± 0.16	2.06 ± 0.19
<i>s</i>	0.73 ± 0.31	0.64 ± 0.20	0.31 ± 0.23
<i>r</i>	0.979	0.988	0.976
Average residual	0.12	0.09	0.10

the data obtained with the two polymeric stationary phases. The differences between the two phases will be discussed as they are reflected in the individual coefficients.

Generally, the molar volume coefficient ( $m$ ) is always large and positive. The cavity-forming process is energetically unfavorable in the aqueous mobile phase. As a result, an increase in the molar volume of a solute results in an increase in retention. For the sake of simplicity, the specific forces determining the magnitude of  $m$  are neglected, except for its dependence upon the Hildebrand solubility parameter (see eqn. 1). Since the  $\delta^2$  values for the solvent systems are nearly equal and  $\delta^2$  for the non-polar stationary phases is small, the value of  $m$  (molar volume coefficient) should be nearly equal for the data correlations involving all solvent-stationary phase combinations tested in this experiment (see Tables II and III). The single obvious exception is methanol-water (70:30). In this case the organic modifier is also a hydrogen-bond donor, as will be discussed below.

The dependence of retention upon the hydrogen bond forming abilities of the stationary and mobile phases is a complex issue. One would expect that if a solute can form a hydrogen bond, it will do so with the mobile phase, thereby decreasing its retention relative to a non-hydrogen bond-forming solute. For this reason, the coefficient  $b$  is negative and always significant in the correlation of  $\log k'$  with the solvatochromic parameters in eqn. 2. Hydrogen bonding in the mobile phase can occur with water and, in some situations, with the modifier, depending upon the nature of the modifier. Generally, the water in the mobile phase would be expected to dominate the hydrogen bond donating character of that phase. A mobile phase mixture with less water will show less of a dependence upon the hydrogen bond accepting ability of a solute. Our data yield two examples of this. For acetonitrile-water mobile phases on

PLRP-S, the magnitude of  $b$  (coefficient of hydrogen bond-donating acidity) decreases as the water content changes from 50% to 30%. The same decrease is seen for methanol–water mixtures. The  $-b$  for 70% methanol is  $3.39 (\pm 0.30)$  (see Table III), and for 85% methanol it is  $2.56 (\pm 0.37)$ . This decrease in the magnitude of the coefficient of hydrogen bond-donating acidity ( $b$ ) does not indicate that an increased modifier concentration increases retention; rather it indicates a decrease in the importance of the hydrogen bond-accepting basicity of a solute as a factor in the retention process as the concentration of water in the mobile phase is decreased.

As mentioned earlier, the ability of the modifier to form a hydrogen bond will influence the hydrogen bond donating acidity of the mobile phase. Of the three modifiers used in this study, methanol is the strongest hydrogen bond donor (see Table I). For equal modifier concentrations in the mobile phase—and assuming the stationary phase does not have significant hydrogen bond-donating character—the  $b$  coefficient for the methanol should have the greatest magnitude. This is seen for the polymeric resin in Table III [ $|b(70\% \text{ methanol})| > |b(70\% \text{ acetonitrile})|$ ].

The assumption that the polymeric phase is a poor hydrogen bond donating acid is probably valid. There are no hydrogen bond donating functionalities in the polymeric phase itself, but some researchers have reported oxidation of the PS–DVB or traces of initiator or catalyst. The absence of hydrogen bond donating ability in our studies suggests that this was not a problem, although we did not specifically test for their presence. In addition, PS–DVB shows poor solvent uptake of water or methanol<sup>24</sup>, either of which could lend hydrogen bond donating character to the phase. ODS, on the other hand, is likely to have significant hydrogen bond donating character. Yonker *et al.*<sup>25</sup> have shown that methanol and a significant amount of water are adsorbed on alkyl-bonded silica phases which, in addition to the residual silanol groups, further increases their hydrogen bond donating (HBD) character. As mentioned earlier, the coefficient,  $b$ , for PLRP-S (poor HBD acidity) in methanol–water mobile phases (good HBD acidity) is greater than  $b$  for the other two modifiers. Because of the enhanced HBD character of ODS in methanol (*e.g.*, modification of the stationary phase), this is not seen for the ODS phase, where  $(b(\text{methanol}) = b(\text{acetonitrile}) = b(\text{THF}))_{\text{ODS}}$ .

The greatest difference in the behavior of the polymeric and alkyl-bonded phases is seen in  $s$ , the coefficient for the dipolarity/polarizability term in eqn. 2. Because of the aromaticity of the polymeric phase, the coefficient  $s$  for the correlations is always positive. This indicates that a solute with a large  $\pi^*$  is relatively more retained on a polymeric phase than a solute with a low  $\pi^*$ . In contrast to PLRP-S, alkyl-bonded silica phases show  $s$  values close to zero (THF and acetonitrile) or negative (methanol), indicating that the polarizability and dipolarity of a solute are less important in retention on ODS phases.

The values of  $s$  on PLRP-S are all positive. However, they are not equal. There appears to be a relationship between the mobile phase modifier and the dependence of retention on the solute  $\pi^*$ . In a study performed by Pietrzyk<sup>24</sup>, THF was found to be adsorbed on a polystyrene resin to a greater extent than acetonitrile. Methanol and water are also adsorbed on the aromatic resin, but to a lesser extent than either THF or acetonitrile. There is an inverse correlation between the amount of adsorbed solvent and the dependence of retention upon the solute  $\pi^*$  ( $s(\text{THF}) < s(\text{acetonitrile}) < s(\text{methanol})$ ). The relatively small  $s$  value for PLRP-S in a THF environment indicates

that there is less of a difference in the  $\pi^*$  parameters between the PS-DVB stationary phase and THF-water mobile phase. We postulate that the adsorbed THF creates an environment on the adsorbent which is more similar to the environment in the mobile phase, and therefore the influence of the polarizability of the polymeric phase is reduced. In contrast, the relatively small amount of methanol adsorbed on the polymeric phase from methanol-water mobile phases will not significantly alter the polarizability of the polymeric phase. As a result, the  $s$  value for PLRP-S in methanol-water is large and positive.

Alkyl-bonded silica phases also exhibit a solvent dependent  $s$  value. Our ODS data show that methanol yields an  $s$  value that is significantly different from that with the other two modifiers. One possible explanation for this involves the amount of modifier adsorbed on ODS and the polarizability of that modifier. Since all of the modifiers are more polarizable than the aliphatic phase, the adsorption of modifier will increase the polarizability of ODS, thereby decreasing the value of  $s$ . Yonker *et al.*<sup>26</sup> found methanol to be adsorbed to a lesser extent than either THF or acetonitrile. The relatively low amount of adsorbed methanol and the low  $\pi^*$  of methanol indicate that it will not enhance the polarizability of the ODS phase to the same extent as either THF or acetonitrile. This results in a large negative  $s$  value for methanol-water mixtures.

In order to determine whether the behavior observed for PLRP-S was characteristic of PS-DVB phases in general, a similar set of experiments was performed on PRP-1 at 70% acetonitrile (Table IV). The correlation for the test solutes was identical on the two polymeric phases.

TABLE IV

## COMPARISON OF TWO POLYSTYRENE-DIVINYLBENZENE HPLC SORBENTS

Columns as described in Experimental; mobile phase, 70:30 MeCN-H<sub>2</sub>O. Solvatochromic terms defined in Table II.

Solute	PLRP-S	PRP-1
<i>log k'</i>		
Benzene	0.195	0.467
Toluene	0.485	0.617
<i>tert.</i> -Butylbenzene	0.786	0.885
Chlorobenzene	0.550	0.664
Iodobenzene	0.884	0.917
Benzyl alcohol	-0.445	-0.264
Anisole	0.308	0.439
2-Phenyl-2-propanol	-0.325	0.172
Acetophenone	-0.017	-0.078
Nitrobenzene	0.154	0.348
3-Nitrotoluene	0.348	0.512
Benzonitrile	0.018	0.211
<i>Solvatochromic parameters</i>		
SP <sub>0</sub>	-0.87 ± 0.26	-0.45 ± 0.23
<i>m</i>	1.03 ± 0.16	0.83 ± 0.14
- <i>b</i>	2.13 ± 0.15	1.91 ± 0.13
<i>s</i>	0.58 ± 0.18	0.47 ± 0.16
<i>r</i>	0.985	0.985
Ave. residual	0.08	0.07



The modulus concept, as introduced by Melander *et al.*<sup>27</sup>, compares differences in retention between two stationary phases. The modulus, or the selectivity for a solute between two columns in identical mobile phase systems, is given by:

$$\mu = \frac{k'_{A-1}}{k'_{A-2}} = \frac{\varphi_1}{\varphi_2} \exp[(\Delta G_{A-2}^0 - \Delta G_{A-1}^0)/RT] \quad (3)$$

The solute is denoted by A and the two columns by 1 and 2. In identical mobile phases, assuming that only solvophobic interactions<sup>6</sup> are responsible for retention, the modulus cancels out the mobile phase effects. Retention differences would then be due to preferential interactions with either phase 1 or phase 2. If  $\mu$  is not constant, there is a difference in the selectivity between the two columns.

Using the modulus technique for examining the selectivity differences between stationary phase materials, Melander *et al.*<sup>27</sup> compared several aromatic bonded phases with alkyl-bonded silica phases. Even though the selectivity differences were much smaller than those reported for the present data on a PS-DVB phase, they found differences which were due to more than just a difference in the phase ratio ( $\varphi$ ). There was greater selectivity for alkyl benzenes on the alkyl-bonded phases and greater selectivity for polyaromatic compounds on the aromatic bonded phases. This would clearly be the situation if we considered the differences in the relative  $\pi^*$  values of polyaromatic molecules (more polarizable) and alkylbenzenes (less polarizable). Other authors have discussed the unique selectivity afforded by the polarizable phenyl groups in phenyl-bonded silica phases<sup>27-30</sup>. However, the role of the aromatic groups in retention on phenyl-bonded phases is not clear, owing to the variation of phase ratio and the effect of the residual silanol groups<sup>31</sup>.

As Fig. 1 shows, methanol produces very drastic differences in relative retention for the test solutes on PS-DVB in comparison with alkyl-bonded phases. The large average modulus ( $\mu = 16.8$ ) indicates how much more strongly the solutes are retained on the polymer. The large variation in the modulus values shows the wide range of relative retention between the solutes. It is interesting to note that in MeOH solutions,

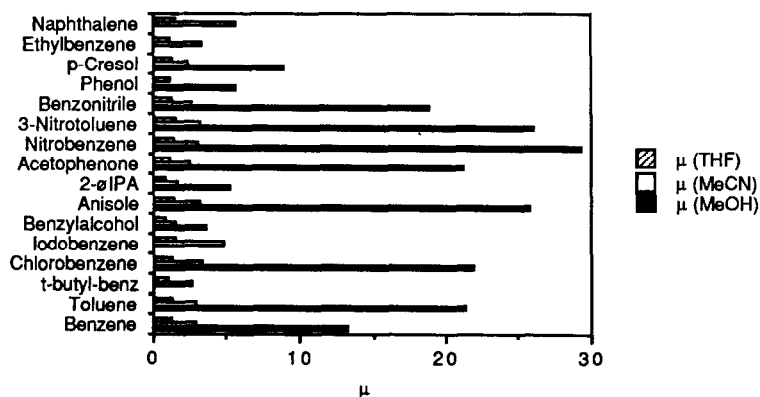


Fig. 1. The modulus for the retention of fourteen test solutes on PLRP-S in comparison with their retention on ODS. The mobile phases were 40% THF, 70% methanol and 50% acetonitrile, 2-φIPA = 2-phenyl-2-propanol; t-butyl-benz = *tert.*-butylbenzene.

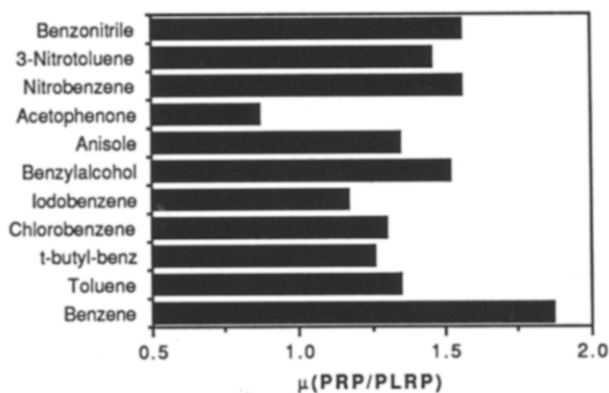


Fig. 2. The modulus for the retention of twelve test solutes on PRP-1 in comparison with their retention on ODS. The mobile phase was acetonitrile-water (70:30, v/v).

alcohols (HBD acids) are more retained on ODS relative to the mean modulus (Fig. 1, solutes 1–4). This is additional confirmation that the ODS phase has enhanced HBA basicity in methanol–water mobile phases. Fig. 1 also shows that solutes with high  $\pi^*$  values are more retained on PLRP-S relative to the mean modulus for a methanolic mobile phase. This agrees with the results from the solvatochromic comparison technique study discussed previously ( $s(\text{methanol})_{\text{PLRP-S}}$  is large and positive). With methanol–water mixtures, PLRP-S offers an advantage in selectivity over ODS, the potential limitations are lengthy analysis times and asymmetrical peaks ( $B/A = 3$ ) (ref. 12).

The mean modulus for acetonitrile is 3.1. Acetonitrile–water mobile phases afford significant selectivity when a polymeric column is used. THF has a mean modulus very close to unity ( $\mu = 1.2$ ), which indicates a similarity between the ODS and polymeric phases when in the THF environment. Little selectivity enhancement is obtained by using an aromatic phase with this organic modifier in comparison with alkyl-bonded silica sorbents.

For comparison purposes, data taken for a different polymeric column (PRP-1) are shown (Fig. 2). The retention of test solutes on the two columns is very similar. The fact that the mean modulus is greater than unity may reflect a difference in the phase ratio. Because of a smaller pore size (60–100 Å pore diameter), the PRP-1 has a larger surface area than the PLRP-S column.

## CONCLUSIONS

Significant differences exist between polymeric phases, such as PS–DVB, and alkyl-bonded silica phases. In addition to the extended usable pH range of polymeric supports, unique selectivity is observed. For organic solvents, such as methanol, which are not adsorbed on the support, the aromatic nature of the backbone is clearly expressed, as shown by the increase in the  $s$  term of the solvatochromism studies. For solvents such as THF, which are adsorbed on the polymer, a similarity exists between the polymeric and bonded silica phases. This finding confirms the importance of solvent modification of the stationary phase as a major factor in reversed-phase

chromatography. It also presents the chromatographer with two additional advantages. If a separation at high pH is desired, but the aromatic nature of the solutes is not significantly different, the use of THF will be the best organic co-solvent, since the overall hydrophobicity of the solutes will be the major factor in the separation. The unique selectivity of the polymeric phase for halogen-substituted compounds and other compounds with polarizable groups presents additional means of separation.

In contrast to the differences observed with alkyl-bonded phases, polymeric phases from different manufacturers seem to show more similarities than differences. It remains to be seen whether the polymeric phases are more reproducible from lot-to-lot than the silica-based chromatographic materials.

#### ACKNOWLEDGEMENTS

The authors thank Frank Warner of Polymer Laboratories, Ltd., for the donation of the polymeric supports used in this work. The project was supported in part by the Industry/University Cooperative Research Center for Biocatalytic Process Technology of the University of Minnesota.

#### REFERENCES

- 1 E. C. Nice, M. W. Capp, N. Cooke and M. J. O'Hare, *J. Chromatogr.*, 218 (1981) 569.
- 2 P. C. Sadek and P. W. Carr, *J. Chromatogr. Sci.*, 21 (1981) 314.
- 3 D. J. Pietrzyk and C. H. Chu, *Anal. Chem.*, 49 (1981) 757.
- 4 J. L. Robinson, W. J. Robinson, M. J. Marshall, A. D. Barnes, K. J. Johnson and D. S. Salas, *J. Chromatogr.*, 189 (1980) 145.
- 5 D. P. Lee, *J. Chromatogr. Sci.*, 20 (1982) 203.
- 6 J. Bontemps, L. Bettendorff, J. Lombet, C. Grandfils, G. Dandrifosse, E. Schoffeniels, F. Nevejan and J. Crommen, *J. Chromatogr.*, 295 (1982) 486.
- 7 H. Takahagi and S. Seno, *J. Chromatogr.*, 108 (1975) 354.
- 8 S. Mori, *Anal. Chem.*, 50 (1977) 745.
- 9 R. M. Smith, *J. Chromatogr.*, 291 (1984) 372.
- 10 F. Smejkal, M. Popl, A. Cihova and M. Zazvorkova, *J. Chromatogr.*, 148 (1980) 147.
- 11 M. Popl, V. Dolansky and J. Fahrnich, *J. Chromatogr.*, 148 (1978) 195.
- 12 L. D. Bowers and S. Pedigo, *J. Chromatogr.*, 371 (1986) 243.
- 13 P. C. Sadek, P. W. Carr, R. M. Doherty, M. J. Kamlet, R. W. Taft and M. H. Abraham, *Anal. Chem.*, 57 (1986) 2971.
- 14 P. W. Carr, R. M. Doherty, M. J. Kamlet, R. W. Taft, W. Melander and Cs. Horvath, *Anal. Chem.*, 58 (1986) 2674.
- 15 M. J. Kamlet and R. W. Taft, *J. Am. Chem. Soc.*, 98 (1976) 377.
- 16 R. W. Taft and M. J. Kamlet, *J. Am. Chem. Soc.*, 98 (1976) 2886.
- 17 M. J. Kamlet, J. L. Abboud and R. W. Taft, *J. Am. Chem. Soc.*, 99 (1977) 6027.
- 18 M. J. Kamlet, J. L. M. Abboud, M. H. Abraham and R. W. Taft, *J. Org. Chem.*, 48 (1983) 2877.
- 19 R. W. Taft, M. H. Abraham, G. R. Famini, R. M. Doherty, J. L. M. Abboud and M. J. Kamlet, *J. Pharm. Sci.*, 74 (1985) 807.
- 20 B. L. Karger, L. R. Snyder and Cs. Horvath, *An Introduction to Separation Science*, Wiley, New York, 1973, p. 55.
- 21 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 1979, p. 222.
- 22 R. M. Smith, *Anal. Chem.*, 56 (1984) 256.
- 23 J. E. Haky and M. A. Young, *J. Liq. Chromatogr.*, 7 (1984) 675.
- 24 D. J. Pietrzyk, *Talanta*, 16 (1969) 169.
- 25 C. R. Yonker, T. A. Zweier and M. F. Burke, *J. Chromatogr.*, 241 (1982) 257.

- 26 C. R. Yonker, T. A. Zweier and M. F. Burke, *J. Chromatogr.*, 241 (1982) 269.
- 27 W. R. Melander, J. X. Huang, Cs. Horvath, W. R. Stout and J. J. DeStefano, *Chromatographia*, 20 (1985) 641.
- 28 W. T. Cooper and Y. T. Lin, *Chromatographia*, 21 (1986) 335.
- 29 T. Hanai and J. Hubert, *J. Chromatogr.*, 291 (1984) 81.
- 30 R. M. Smith, *J. Chromatogr.*, 237 (1982) 144.
- 31 P. E. Antle, A. P. Goldberg and L. R. Snyder, *J. Chromatogr.*, 321 (1985) 1.