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SOLVATOCHROMIC SOLVENT POLARITY MEASUREMENTS AND SELECTIVITY IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

BRUCE P. JOHNSON*, MORTEZA G. KHALEDI** and JOHN G. DORSEY*

Department of Chemistry, University of Florida, Gainesville, FL 32611 (U.S.A.)

SUMMARY

The $E_T(30)$ polarity values of binary acetonitrile–water and methanol–water mobile phases, used in reversed-phase liquid chromatography, were measured and compared with methylene selectivity (α_{CH_2}) for both traditional siliceous bonded phases and for a polystyrene–divinylbenzene resin reversed-phase material. The variation in methylene selectivity for both was found to correlate best with percent organic solvent in methanol–water mixtures, while the $E_T(30)$ polarity provided the best correlation in acetonitrile–water mixtures. The polymeric resin column was found to provide higher methylene selectivity than the siliceous bonded phase at all concentrations of organic solvent.

INTRODUCTION

The description of methylene selectivity in reversed-phase liquid chromatography (RPLC) has traditionally been more difficult than that of retention. In a sense, the methylene selectivity (α_{CH_2}) is quite similar to the retention of normal alkanes, since it is based on the measurement of the capacity factors for a homologous series. It has also been referred to as the "hydrophobic selectivity", or "non-specific selectivity", owing to the large hydrophobicity of the methylene group. Methylene selectivity serves as a convenient measure of elution strength. Thus, knowledge of this for a given system is quite useful, since the mobile phase strength can be held constant for different modifiers, while the selectivity of other interactions is exploited to maximize the separation between two or more solutes.

From an energetic standpoint, log α_{CH_2}, is directly related to the change in the free energy of transfer, caused by adding or subtracting a methylene group to a molecule, by the following equation

$$\log \alpha_{CH_2} = -\Delta\Delta G \tag{1}$$

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* Present address: Eastman-Kodak Company, Eastman Chemicals Division, Kingsport, TN 37662, U.S.A.
** Present address: Department of Chemistry, University of New Orleans, New Orleans, LA 70148, U.S.A.

Of course, eqn. 1 applies to any form of selectivity; any two solutes that possess different free energies of transfer will be differentially retained. Methylene selectivity is thus only one aspect of chromatographic separations. In terms of research on retention mechanisms, there are at least two distinct advantages to the study of chromatographic selectivity.

First, it can be seen that log α values are not affected by the phase ratio of the column. Different phase ratios lead to changes in capacity factors (k'). These phase ratios are a function of the bonded-group chain length, the degree of surface coverage, the pore structure of the original silica, as well as the manner in which the column was packed. Thus, drawing conclusions about the variation in k' for different columns is hindered by the number of variables to be considered. Since the methylene selectivity (or any selectivity, for that matter) is not affected by the phase ratio, any differences seen between columns are due to actual differences in the nature of the bonded-phase structure. Moreover, the log α_{CH_2} values are generally independent of the specific column used, so that all C_{18}-type bonded phases will exhibit similar behavior, implying that only the most fundamental aspects of the retention process are being probed.

A second advantage, obtained only in the study of methylene selectivity, is that of lack of sensitivity to the presence of residual silanols on the bonded-phase surface. Residual silanols lead to anomalous retention behavior of many solutes which possess highly polar and/or hydrogen-bond donor–acceptor groups. While the individual solutes which comprise the homologous series may be susceptible to these effects, the change in k' caused by additional methylene groups will still be measurable, and largely unaffected by specific interactions with the stationary phase.

Many papers have been published in regard to methylene selectivity. For example, Karger et al.[1] reported a linear relationship between log α_{CH_2} and percent methanol for a C_{18} column, while the behavior of acetonitrile–water mixtures was found to be more complex. This behavior was ascribed to the differing natures of the two modifiers, in that methanol is a proton acceptor–donor, which leads to less disruption in the overall solvent mixture structure as the concentration is varied. The structure of acetonitrile–water mixtures is of much greater complexity, since acetonitrile does not associate with water to any extent in comparison with methanol.

Colin et al.[2] carried out an extensive investigation of methylene selectivity, in which a total of seven binary and one ternary systems were explored. These measurements were then used to derive an eluotropic scale of solvent strength. These new values were found to correlate quite well with Snyder's eluotropic values for RPLC solvents[2,3].

It is also worth noting here that homologous series have been used in the determination of dead volumes[4]. In essence, this method involves the adjustment of the elution times for an unretained species (t_0) used to calculate k' values, such that the highest correlation is obtained in a plot of log k' versus carbon number for the homologous series when the "true" t_0 is reached.

We have previously reported on correlation between chromatographic retention and empirical solvent polarity measurements[5]. A total of 332 retention data sets (of log k' versus percent organic solvent) were examined, and, in general, a linear relationship was found between log k' and the $E_T(30)$ polarity for the same solvent mixture. That is, plots of log k' versus $E_T(30)$ polarity were generally found to be

better descriptors of chromatographic retention than the more commonly used plots of log k' *versus* percent organic solvent. For the 332 retention data sets, the average squared correlation coefficients for log k' *versus* percent organic solvent or $E_T(30)$ polarity were 0.9783 and 0.9910, respectively. The $E_T(30)$ solvent polarity measure used is a single-parameter probe that is sensitive to dipole interactions as well as hydrogen-bond donating and accepting interactions. The analytical uses of this probe and the synthetic procedure have recently been reported[6]. For the sake of clarity, we will refer to the polarity value as $E_T(30)$ and the probe molecule itself as ET-30.

In the experiments described herein, the methylene selectivity was evaluated for a C_{18} bonded-phase column (Ultrasphere ODS), and a styrene–divinylbenzene (polymeric; Hamilton PRP-1) reversed-phase column, with methanol and acetonitrile as the organic solvent. Also, a large body of methylene selectivity data has been extracted from the literature, either directly from tabulated log α_{CH_2} values, or calculated from the slope of log k' *versus* carbon number. Here we report correlations between methylene selectivity, percent organic solvent, mole fraction organic solvent, and $E_T(30)$ polarity.

EXPERIMENTAL

Retention measurements (other than those reported in the literature), were obtained with a Spectra-Physics (San Jose, CA, U.S.A.) SP8700 ternary proportioning LC system. Columns used were an Altex (San Ramon, CA, U.S.A.) Ultrasphere ODS (5 μm particle size) and a Hamilton (Reno, NV, U.S.A.) PRP-1 (10 μm) and both were 15 cm × 4.6 mm I.D. Test solutes were obtained from Aldrich (Milwaukee, WI, U.S.A.) and Eastman-Kodak (Rochester, NY, U.S.A.). Sample introduction was achieved with either an Altex Model 210 injector, equipped with a 5-μl sample loop, or a Rheodyne (Cotati, CA, U.S.A.) Model 7125 injector, equipped with a 20-μl sample loop. Flow-rates were either 1.0 or 2.0 ml/min. The column was thermostated at 40 ± 0.1°C with a Haake (Saddle Brook, NJ, U.S.A.) Model D1 water bath. HPLC-grade methanol and acetonitrile (Fisher Scientific, Fair Lawn, NJ, U.S.A.) were used as received. Water was first purified with a Barnstead (Boston, MA, U.S.A.) Nanopure system and then irradiated with UV light in a Photronix (Medway, MA, U.S.A.) Model 816 HPLC reservoir for at least 24 h. The water was then filtered through a 0.45-μm nylon-66 membrane filter (Rainin Instruments, Woburn, MA, U.S.A.) prior to use. A fixed-wavelength, 254-nm, Beckman Model 153 UV detector (Altex) was used.

The t_0 values were evaluated with injections of the pure organic solvent (either methanol or acetonitrile). For the Hamilton PRP-1 column, this proved to be difficult at low organic solvent concentrations due to actual retention of the acetonitrile or methanol. Other supposedly unretained solutes (such as urea and uracil) exhibited similar behavior. Therefore, as t_0, obtained from injections of pure organic solvent, the t_0 at 60% organic solvent concentration was used, since at this concentration the retention time reached a minimum in each of the two solvent systems.

Simple linear regression calculations were performed with the "Curve Fitter" program (Interactive Microware, State College, PA, U.S.A.), run on an Apple (Cupertino, CA, U.S.A.) II Plus 48K microcomputer. The program was modified to allow calculation of 95% confidence intervals for slope and y-intercept values. This

program was also used to interpolate $E_T(30)$ values for solvent compositions that had not been measured (*e.g.*, methanol–water, 45:55).

The sensitivity of the probe and the measurement of the polarity values have been described previously[5]. $E_T(30)$ energy values can be converted to kJ/mole by multiplying the value in kcal/mole by 4.184.

RESULTS

Ultrasphere ODS column

The methylene selectivity was measured by using the homologous series of benzene, toluene, ethylbenzene and *n*-butylbenzene. In Figs. 1 and 2, the results of experiments with the Ultrasphere ODS column are shown for methanol–water mixtures. The selectivity data are plotted in two ways: percent organic solvent (Fig. 1) and $E_T(30)$ polarity (Fig. 2). For methanol as an organic modifier, methylene selectivity decreases in a linear manner as the percentage of organic modifier is increased ($r^2 = 0.9972$ for a straight-line fit of the data in Fig. 1). The squared correlation coefficient does decrease to 0.9884 in Fig. 2 (*versus* $E_T(30)$ polarity), though this may be due in part to an increase in scatter; it is likely that the $E_T(30)$ values have contributed some extra variance to the linear regression. The regression line drawn through the data in Fig. 2 is given by

$$\log \alpha_{CH_2} = -4.82 \pm 0.37 + 0.08817 \pm 0.0065 \cdot E_T(30) \tag{2}$$

$(n = 5, s = 0.0107)$

In Figs. 3 and 4, the methylene selectivity results for acetonitrile–water mixtures are plotted with respect to percent acetonitrile; and $E_T(30)$ polarity, respectively. As shown in Fig. 3, for percent organic solvent, the methylene selectivity varies in a non-linear manner; r^2 for a straight line regression is 0.9655. Plotting with respect to the $E_T(30)$ polarity (Fig. 4) yields the best linear correlation ($r^2 = 0.9919$). The regression equation for the line in Fig. 4 is given by

$$\log \alpha_{CH_2} = -3.24 \pm 0.16 + 0.06116 \pm 0.0028 \cdot E_T(30) \tag{3}$$
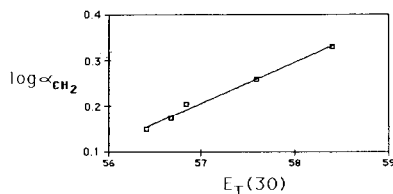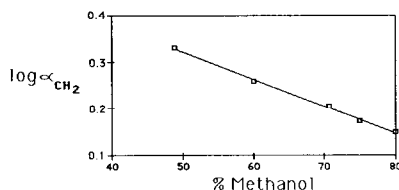
$(n = 6, s = 0.0068)$

Fig. 1. Chromatographic selectivity measurements as a function of percent methanol. Ultrasphere ODS column, 150 × 4.6 mm; 5 μl injection volume; flow-rate 1.0 ml/min. Alkylbenzenes used as the homologous series.

Fig. 2. Chromatographic selectivity measurements as a function of $E_T(30)$ polarity of methanol–water mixtures. For conditions, see Fig. 1.
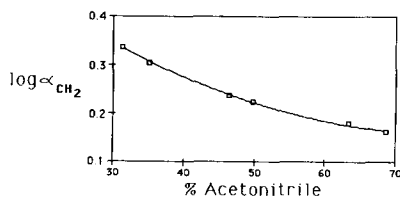
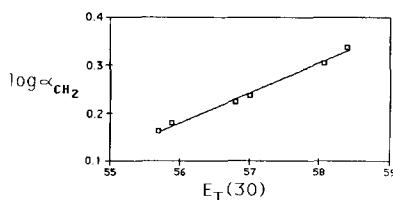Fig. 3. Chromatographic selectivity measurements as a function of percent acetonitrile. For conditions, see Fig. 1.

Fig. 4. Chromatographic selectivity measurements as a function of $E_T(30)$ polarity of acetonitrile–water mixtures. For conditions, see Fig. 1.

The ratio of the two slopes found in eqns. 2 and 3 is 1.44. That is, selectivity in the methanol–water system is more affected by overall changes in mobile phase polarity than with acetonitrile as the organic solvent. It should also be noted that the value of this ratio is the same as that found for slopes of log k' versus $E_T(30)$, as we have previously reported[5]. This is not surprising, considering the effect of solvation of the stationary phase alkyl chains by the organic solvent. Acetonitrile has been shown to solvate the stationary phase to a much greater extent than methanol[7], so that in the methanol–water system, changes in overall mobile phase polarity have more effect on retention or selectivity, since the stationary phase polarity is not changing significantly. The two lines defined by eqns. 2 and 3 intersect at an $E_T(30)$ value of 58.5 kcal/mole. This corresponds to approximate organic solvent concentrations of 45 and 28% for methanol and acetonitrile, respectively. That is, at these concentrations the methylene selectivity would be equivalent in the two systems. This also means that, except at this one intersection point, iso-$E_T(30)$ polarity values would not correspond to equivalent methylene selectivity in the two systems.

Literature data

A wealth of methylene selectivity data have been published in the literature by various workers[1,2,8–11]. In some cases, the log α_{CH_2} values have been tabulated (with respect to percent organic solvent), while in other cases the α_{CH_2} values can be calculated from the reported k' values for a homologous series. Linear regression was carried out for data reported in these six references.

The results of the various correlations with all data sets discussed are shown in Table I. Squared correlation coefficients are reported for each of the three comparisons (*vs.* percent organic solvent, *vs.* mole fraction and *vs.* $E_T(30)$ polarity). This provides a way in which the general trends of the data may be viewed. In Fig. 5 the results of Table I are shown graphically. Squared correlation coefficients for log α_{CH_2} versus percent organic solvent are plotted with respect to those found for log α_{CH_2} versus $E_T(30)$ polarity. The line drawn through Fig. 5 corresponds to "iso-r^2" values. That is, all points would fall along this line if all correlation coefficients were equivalent for the two comparisons. Thus, a point appearing above the line denotes a better correlation when the log α_{CH_2} data are plotted with respect to percent organic solvent. Of the eleven data sets, in seven cases the correlation is clearly equal to or better than the "*vs.* $E_T(30)$ polarity" comparisons.

There are a number of conclusions that can be reached regarding the data in

TABLE I

SQUARED CORRELATION COEFFICIENTS (r^2) FOR LOG α_{CH_2} DATA WITH RESPECT TO PERCENT ORGANIC SOLVENT, MOLE FRACTION ORGANIC SOLVENT AND $E_T(30)$ POLARITY

Ref.	Organic solvent	$n^\star$	Concentration range (%)	r^2 versus		
				Percent organic solvent	Mole fraction organic solvent	$E_T(30)$ polarity
This work	Methanol	5	50–80	0.9972	0.9897	0.9884
This work	Acetonitrile	6	32–68	0.9655	0.9173	0.9919
1	Methanol	13	0–100	0.9943	0.9337	0.9655
1	Acetonitrile	8	5–80	0.9186	0.7777	0.9921
10	Methanol	7	40–100	0.9945	0.9501	0.9858
11	Methanol	6	10–100	0.9903	0.9488	0.9902
11	Acetonitrile	7	10–80	0.9102	0.8032	0.9859
2	Methanol	11	0–100	0.9923	0.9592	0.9486
2	Acetonitrile	9	0–80	0.9492	0.8829	0.9776
8	Methanol	10	55–100	0.9983	0.9738	0.9936
9	Acetonitrile	6	20–70	0.9855	0.9684	0.9439

$\star$ n is the number of individual α values used for the correlations.

Table I. One interesting pattern that is apparent is that for every data reference where the methylene selectivity was measured with both organic modifiers, the correlation coefficients for "vs. percent organic solvent" and "vs. $E_T(30)$" mirror each other. That is, in methanol–water mixtures, the methylene selectivity varies most closely with the percent organic solvent, while in the acetonitrile–water system, $E_T(30)$ polarity values yield the highest correlation. In contrast to the excellent correlation seen between log k' and $E_T(30)$ polarity[5], here there is a clear-cut distinction between the two organic modifiers.

Of course, the variation with respect to percent organic solvent is also quite complex, especially with acetonitrile–water mixtures. Colin et al.[2] found that, except for methanol–water mixtures, every system studied exhibited a non-liner variation in log α_{CH_2} with respect to percent organic solvent.

Hamilton PRP-1 column

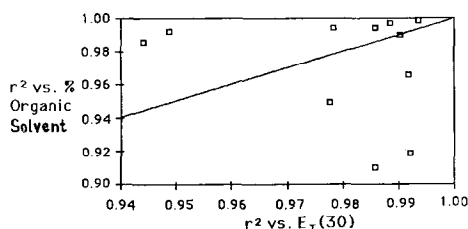While RPLC is typically performed with chemically bonded silica, there are

Fig. 5. Comparison between r^2 values for plotting methylene selectivity data with respect to either percent organic solvent or $E_T(30)$ polarity.

other materials that may serve as suitable stationary phases. In recent years, a number of polymeric reversed-phase columns have become commercially available. Polymer-based RPLC columns offer a number of potential advantages over their more traditional counterparts. Unlike silica-based columns, polymeric columns are stable to pH levels of 1–13 and high concentrations of buffer salts, with no degradation in performance.

Another aspect of these columns that can be of use is their preferential retention of aromatic compounds. Apparently, the presence of aromatic moieties on the stationary phase leads to preferential retention of aromatic solutes, leading to a different chromatographic selectivity than that observed with silica-based RPLC columns.

A final advantage is the lack of any silanol groups whatsoever. The presence of these groups on the surface of conventional bonded phases can be a problem in the chromatography of highly polar solutes, which will, in effect, be retained by a dual adsorption–partitioning mechanism. Thus, polymeric columns are, by virtue of their composition, entirely free of these troublesome residual silanols.

The experiments carried out with a Hamilton PRP-1 column involved measuring the methylene selectivity over 0–100% methanol and 0–80% acetonitrile. Owing to the high retentivity of the polymer matrix with respect to aromatic compounds, it was found that the homologous series of alkylbenzenes is unsuitable at low concentrations of organic solvent (*i.e.*, less than 60%). Therefore, nitroalkanes (nitromethane through nitrohexane) were used to measure log α_{CH_2} values, and were found to be usable over the entire range of organic solvent concentrations.

Since the polymeric column has a preferential retentivity toward aromatic compounds, it was necessary to insure that the use of a different homologous series would not significantly affect the measurement of log α_{CH_2}. Both nitroalkanes and alkylbenzenes were used to measure selectivity values at three different concentrations of each of the two organic solvents. Concentrations were so chosen that the alkylbenzenes could still be used to measure the methylene selectively (> 60% organic solvent). The results of these comparisons are found in Table II, and are plotted in Fig. 6. The slope of the line drawn through the data was 1.02 ± 0.17, with a y-intercept of -0.005 ± 0.04. Based on these results, it does not appear that the measurement of

TABLE II

COMPARISON OF LOG α_{CH_2} VALUES AS MEASURED BY NITROALKANES AND ALKYLBENZENES

Hamilton PRP-1 column.

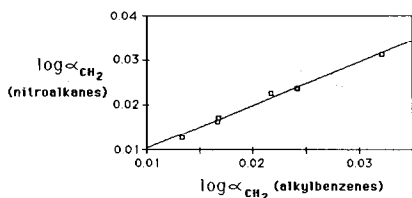| Mobile phase	log α_{CH_2}	
	Alkylbenzenes	Nitroalkanes
Acetonitrile–water (50:50)	0.2167	0.2274
Acetonitrile–water (65:35)	0.1667	0.1645
Acetonitrile–water (80:20)	0.1328	0.1288
Methanol–water (70:30)	0.3215	0.3130
Methanol–water (80:20)	0.2425	0.2361
Methanol–water (90:10)	0.1677	0.1727

Fig. 6. Comparison between methylene selectivity results obtained with either 1-nitroalkanes or alkylbenzenes as the homologous series. Hamilton PRP-1 (polymeric) column 150 × 4.6 mm; 20 μl injection volume; flow-rate 1.0 ml/min.

methylene selectivity is significantly biased by the homologous series used to measure it, and is consistent with results previously published for conventional bonded phases[2].

One distinct disadvantage of the use of nitroalkanes is their generally low absorption of light in the UV region. For example, the molar absorptivity of nitromethane is only 18.6 l mol^{-1} cm^{-1} in ethanol, with a $\lambda_{max.}$ of 271 nm. Nevertheless, it was found that by increasing the concentration of nitroalkanes in the injected standards to approximately 5 mg/ml (20 μl sample volume; 100 μg injected), a 254-nm UV detector could still be used for the nitroalkanes. The peak shapes did not appear to be distorted by the large amount of injected solute; this may be a reflection of the nature of the polymeric stationary phase, since a higher load level should (in theory) be tolerated.

The results of the mesurements of α_{CH_2} for methanol–water mixtures appear in Figs. 7 and 8. Fig. 7 shows that the methylene selectivity decreases in a highly linear fashion as the percent methanol is increased. The regression line corresponds to

$$\log \alpha_{CH_2} = -5.24 \pm 0.34 + 0.0970 \pm 0.0058 \cdot E_T(30) \qquad (4)$$

$$(n = 11, s = 0.0468)$$

Plotting with respect to the $E_T(30)$ polarity (Fig. 8) results in curvature, though at higher $E_T(30)$ polarities (lower methanol concentrations) the dependence is nearly linear.

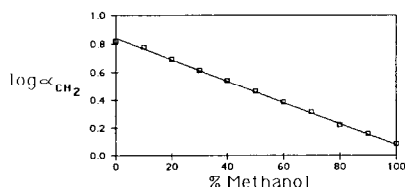The results for the same measurements with acetonitrile as the organic solvent

Fig. 7. Chromatographic selectivity measurements as a function of percent methanol. Nitroalkanes used as the homologous series. For conditions, see Fig. 6.
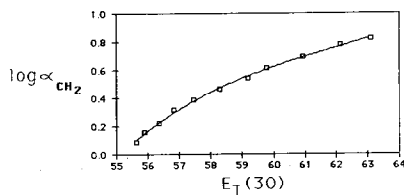
Fig. 8. Chromatographic selectivity measurements as a function of $E_T(30)$ polarity of methanol–water mixtures. For conditions, see Fig. 7.
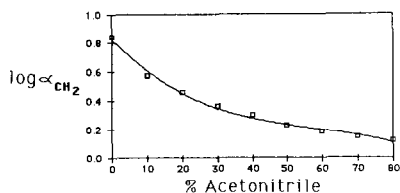
Fig. 9. Chromatographic selectivity measurements as a function of percent acetonitrile. For conditions, see Fig. 7.
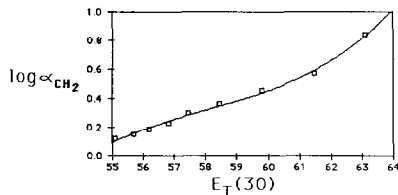
Fig. 10. Chromatographic selectivity measurements as a function of $E_T(30)$ polarity of acetonitrile–water mixtures. For conditions, see Fig. 7.

appear in Figs. 9 and 10. Here, the curve shapes are distinctly different from those seen with methanol. Instead of linearity *versus* percent organic solvent, strong curvature is seen (Fig. 9); this behavior in the two organic solvents is quite similar to that seen with standard bonded-phase columns[1,2]. The change in log α_{CH_2} is most pronounced at low concentrations of organic solvent. In Fig. 10, the selectivity increases in a nearly linear manner with respect to $E_T(30)$, with strong curvature seen at high $E_T(30)$ polarity values (low acetonitrile concentration). If the data in Fig. 10 are fitted to a straight-line model, the resultant regression line is given by

$$\log \alpha_{CH_2} = -4.58 + 0.31 + 0.0847 + 0.0053 \cdot E_T(30) \qquad (5)$$

$(n = 9, s = 0.0411)$.

Correlation coefficients for the three comparisons discussed herein appear in Table III.

The ratio of slopes of eqns. 4 and 5 is 1.15. As discussed previously, the ratio of the slopes of eqns. 2 and 3 (α_{CH_2} for methanol and acetonitrile with the C_{18} column) is 1.44. That these ratios are different is further evidence that these slope ratios are a measurement of stationary phase solvation effects. The difference in the solvation structure between methanol and acetonitrile is less for the polymeric resin column than for the traditional C_{18} column. These ratios may prove useful for characterizing

TABLE III

CORRELATIONS BETWEEN LOG α_{CH_2} AND PERCENT ORGANIC SOLVENT, MOLE FRACTION ORGANIC SOLVENT OR $E_T(30)$ POLARITY FOR A HAMILTON PRP-1 POLYMERIC COLUMN

Organic solvent	n^*	Concentration range (%)	r^2 versus		
			Percent organic solvent	Mole fraction organic solvent	$E_T(30)$ polarity
Methanol	11	0–100	0.9989	0.9498	0.9692
Acetonitrile	9	10–90	0.8816	0.7352	0.9731

* n is the number of individual α values used for the correlations.

reversed-phase columns. We are further investigating these values for different mobile phases and columns.

At 100% organic solvent concentration, the log α_{CH_2} values are 0.0850 and 0.0754 for methanol and acetonitrile, respectively. This is as one might expect, owing to the greater "strength" of acetonitrile as a modifier in RPLC. Lastly, it should be noted that log α_{CH_2} values for this column are significantly higher at a given organic solvent concentration than that of the Ultrasphere ODS bonded phase-column. This shows that the polymeric surface is even more "hydrophobic" than the bonded phases, since the free energy of transfer of a methylene group is larger.

CONCLUSIONS

The variation in methylene selectivity for both traditional bonded phases as well as a polymeric column was found to correlate best with percent organic solvent in methanol–water mixtures, while the $E_T(30)$ polarity variation provided the best correlation in acetonitrile–water mixtures. Also, the polymeric column was found to provide higher methylene selectivity at all concentrations of organic solvent. Thus, this type of column appears to have great utility in the separation of molecules of very similar structure.

ACKNOWLEDGEMENTS

REFERENCES

1 B. L. Karger, J. R. Gant, A. Hartkopf and P. H. Weiner, *J. Chromatogr.*, 128 (1976) 65–78.
2 H. Colin, G. Guiochon, Z. Yun, J. C. Diez-Masa and P. Jandera, *J. Chromatogr. Sci.*, 21 (1983) 179–184.
3 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968.
4 G. E. Berendsen, P. J. Schoenmakers, L. de Galan, G. Vigh, Z. Varga-Puchony and J. Inczedy, *J. Liq. Chromatogr.*, 3 (1980) 1669–1686.
5 B. P. Johnson, M. G. Khaledi and J. G. Dorsey, *Anal. Chem.*, 58 (1986) 2354–2365.
6 B. P. Johnson, B. Gabrielsen, M. Matulenko, J. G. Dorsey and C. Reichardt, *Anal. Lett.*, 19 (1986) 939–962.
7 R. M. McCormick and B. L. Karger, *Anal. Chem.*, 52 (1980) 2249–2257.
8 P. Dufek, *J. Chromatogr.*, 299 (1984) 109–117.
9 T. Hanai and J. Hubert, *J. Liq. Chromatogr.*, 8 (1985) 2463–2473.
10 S. Petrović, S. Lomić and I. Šefer, *J. Chromatogr.*, 348 (1985) 49–65.
11 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 218 (1981) 261–284.