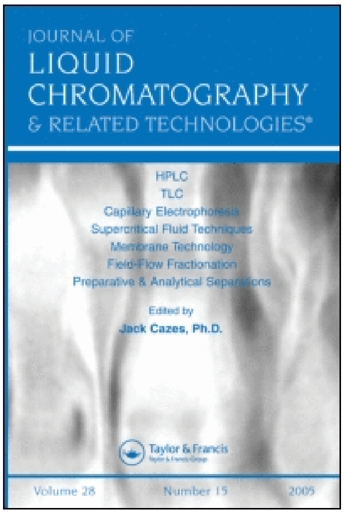

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John G. Dorsey[a]; Bruce P. Johnson[a]
[a] Department of Chemistry, University of Florida, Gainesville, Florida

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SOLVATOCHROMIC MEASUREMENTS OF MOBILE PHASE COMPOSITION IN REVERSED PHASE LIQUID CHROMATOGRAPHY: POLARITY VALUES, RETENTION, AND STATIONARY PHASE INFORMATION

John G. Dorsey* and Bruce P. Johnson

Department of Chemistry
University of Florida
Gainesville, Florida 32611

ABSTRACT

The $E_T(30)$ polarity values of binary methanol-water, acetonitrile-water and tetrahydrofuran-water mobile phases used in reversed phase liquid chromatography are reported, and equations are presented for the calculation of polarity values for any composition of these solvents. We summarize previously reported comparisons of these measured polarities with both chromatographic retention and methylene selectivity. Plots of log k' vs. $E_T(30)$ polarity were generally found to be better descriptors of retention than commonly used plots of log k' vs. percent organic modifier. For 332 data sets examined, the average r^2 value for plotting log k' vs. percent organic modifier was 0.9783 while plotting vs. $E_T(30)$ polarity gave a value of 0.9910. Retention in acetonitrile-water mixtures is also compared with theory developed by Dill using binary interaction parameters. Variation in methylene selectivity was found to correlate best with percent organic modifier in methanol-water mixtures, while the $E_T(30)$ polarity provided the best correlation in acetonitrile-water mixtures. Solvatochromic solvent polarity measurements allow an independent examination of the effect of changing mobile phase polarity on chromatographic retention, and give useful information about the solvation structure of the stationary phase.

INTRODUCTION

Retention in reversed phase liquid chromatography is a complex function of the solute, mobile phase and stationary phase. Elucidation of the exact retention mechanism, however, is a goal that still remains elusive. The solution of this problem may be near, as it has attracted the interest of chromatographers, surface scientists, spectroscopists and statistical mechanicians. An understanding of the mechanism would allow the *a priori* prediction of separations, computer based optimization of complex separations, and the development of a rational retention index scheme.

It was first thought that the stationary phase was inert, playing no active role in the separation. In fact, the solvophobic theory of Horvath (1) treats only the partitioning of solutes from the mobile phase into an assumed amorphous disordered oil stationary phase. It does not predict any effect of surface density of the bonded alkyl chains or effects of chain length. It is now known that the structure and composition of the stationary phase plays a very active role in the separation process, and that the stationary phase orientation and solvation structure can actually change with changing temperature and mobile phase composition. This is best witnessed by the lengthy reequilibration necessary after an organic concentration gradient. Two excellent reviews of stationary phase structure and the effect on retention have recently appeared (2,3).

There have also been many investigations into the effect of the mobile phase on retention. Solubility parameter theory (4,5), interaction indices (6), and even statistical mechanical theory (7) have all been applied in trying to better understand the mechanism of retention. However, none of these approaches yet allow quantitative predictions to be made.

It is often assumed that there exists a linear relationship between the log of the capacity factor for a solute and the percent by volume of organic modifier in the mobile phase. Gradient elution theory as well as many chromatographic optimization schemes and physicochemical measurements are based on this assumed linearity. However, if the relationship is viewed over a wide enough range of organic modifier concentrations, a quadratic fit is almost always found. This is particularly true when acetonitrile is used as the modifier instead of methanol. Figures 1 and 2 show retention plots, taken from (8), for pyrene on a C_4 column, with methanol and acetonitrile mobile phases, respectively.

Retention in reversed phase chromatography should be proportional to the difference in polarity between the mobile and stationary phases, as this controls the partition coefficient of a solute between the two phases. Unfortunately, polarity is difficult to describe quantitatively, as it is a complex combination of many different interactions, including hydrogen bonding, dispersion forces, dipole interactions, etc. What is needed then is a convenient measure of the polarity of the mobile phase.

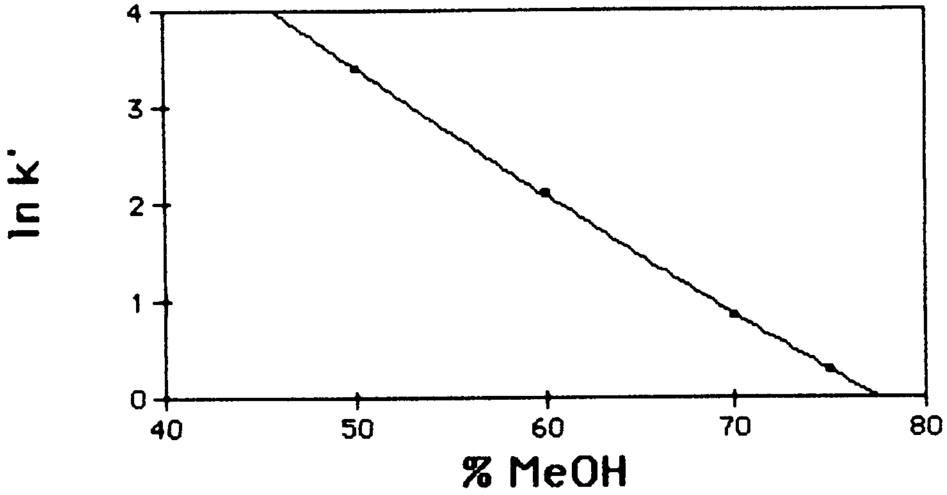

FIGURE 1. Comparison of log k' vs. % methanol for pyrene on a C_4 column. Data from (8).

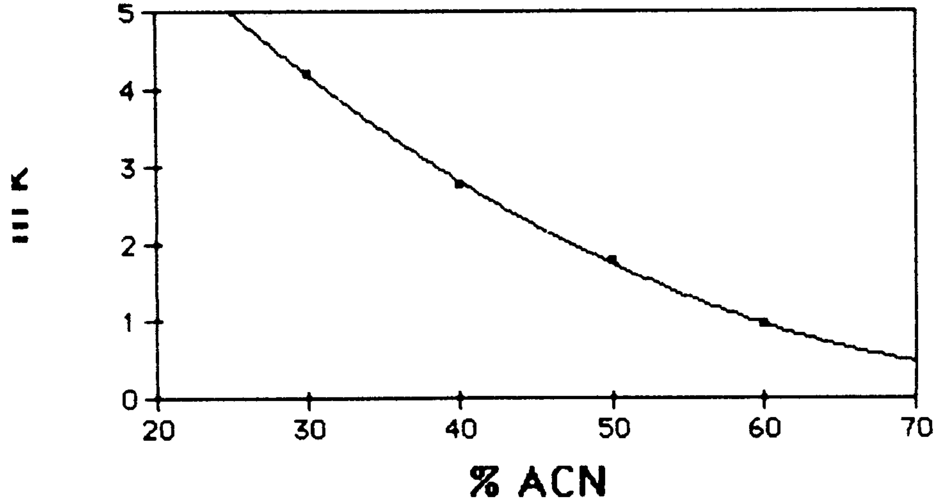

FIGURE 2. Comparison of log k' vs. % acetonitrile for pyrene on a C_4 column. Data from (8)

SOLVENT POLARITY MEASUREMENTS

Many empirical scales exist for describing solvent polarity. An excellent review of the polarity of binary liquid mixtures has recently appeared (9) which discusses many of the scales available. One of the most widely used scales is known as the $E_T(30)$ scale, and is based on the charge transfer absorption of 2,6-diphenyl-4-(2,4,6-triphenyl-N-pyridinio)phenolate, also known as ET-30. This molecule, shown in Figure 3, exhibits one of the largest observed solvatochromic effects of any known molecule, as the charge transfer absorption maximum shifts from 453 nm in water, a very polar solvent, to 810 nm in diphenyl ether, a very nonpolar solvent. This molecule is then a very sensitive probe of subtle changes in solvent polarity, and it has been shown to be sensitive to both solvent dipolarity/polarizability as well as solvent hydrogen bond donor ability. The analytical applications and synthesis of this molecule have recently been reported (10).

We have made correlations between the $E_T(30)$ polarity values of binary aqueous/organic mobile phases and both retention (11) and selectivity (12) in reversed phase liquid chromatography. Tables 1-3 show the $E_T(30)$ polarity values for methanol/water, acetonitrile/water and tetrahydrofuran/water mixtures respectively. The $E_T(30)$ values are reported as the energy value of the charge transfer absorption, and are calculated as

$$E_T(30)\ (\mathrm{kcal/mole}) = 28{,}592/\lambda_{max} \qquad (\mathrm{Eqn.}\ 1)$$

where the constant is the product of Avogadro's number, the speed of light, and Planck's constant.

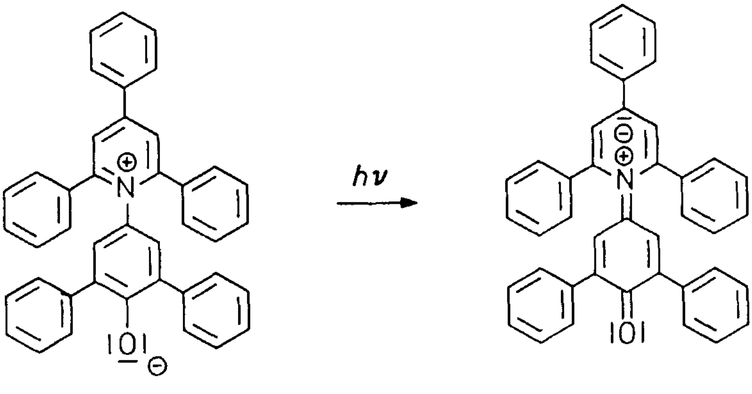

FIGURE 3. ET-30 solvent probe.

TABLE 1.
Solvatochromic Solvent Polarity Measurements of Methanol-Water Mixture

% (v/v) Methanol	$E_T(30)$ Polarity (kcal/mole)
0	63.11
10	62.15
20	60.94
30	59.78
40	59.17
50	58.30
60	57.46
70	56.84
80	56.37
90	55.89
100	55.62

TABLE 2.
Solvatochromic Solvent Polarity Measurements of Acetonitrile-Water Mixtures.

% (v/v) Acetonitrile	$E_T(30)$ Polarity (kcal/mole)
0	63.11
10	61.43
20	59.81
30	58.44
40	57.46
50	56.82
60	56.19
70	55.71
80	55.09
90	53.80
100	45.97

Table 3.
Solvatochromic Solvent Polarity Measurements of Tetrahydrofuran-Water Mixtures.

% (v/v) Tetrahydrofuran	$E_T(30)$ Polarity (kcal/mole)
0	63.11
10	60.97
20	58.54
30	55.54
40	53.83
50	52.62
60	51.64
70	50.86
80	49.61
90	47.91
100	39.14

Tables 2 and 3 show that the measured polarity of both acetonitrile/water and tetrahydrofuran/water mixtures decrease rapidly above concentrations of 80% organic modifier. The actual solvent polarity does not change this dramatically, rather this is an artifact of the ET-30 probe molecule, as the remaining water becomes specifically associated with the ET-30 due to the presence of the negatively charged phenoxide group. This represents a weakness of this specific polarity probe, and for this reason we have included no data for acetonitrile/water mixtures above 80% in our retention and selectivity correlations.

If $E_T(30)$ values for compositions other than those reported are desired, they can be calculated from the equation resulting from a second degree polynomial fit of the experimental points. For methanol-water over the 0→100% range, the equation is

$$E_T(30) = 63.1927 - 0.1222(\%\ \text{methanol}) + 0.0005\ (\%\ \text{methanol})^2 \quad \text{(Eqn. 2)}$$

$$r = 0.9994$$

For acetonitrile-water over the 0→80% range, the equation is

$$E_T(30) = 63.0412 - 0.1773(\%\ \text{acetonitrile}) + 0.0010(\%\ \text{acetonitrile})^2 \quad \text{(Eqn. 3)}$$

$$r = 0.9986$$

And for tetrahydrofuran over the 0→80% range, the equation is

$$E_T(30) = 63.3642 - 0.2915(\%\ \text{THF}) + 0.0015(\%\ \text{THF})^2 \quad \text{(Eqn. 4)}$$

$$r = 0.9974$$

RETENTION AND SOLVENT POLARITY

We have reported correlations between log k' and both % organic modifier and $E_T(30)$ polarity for 332 different retention data sets (11). These involved many different solutes on eight different stationary phases of chain lengths C_2, C_4, C_8, and C_{18} with mobile phases of both methanol-water and acetonitrile-water. For the 332 data sets, plotting log k' vs. $E_T(30)$ polarity gave significantly better linearity, with an average r^2 value of 0.9910 as opposed to an average r^2 value of 0.9783 when plotted vs. % organic modifier. The retention data for pyrene shown in Figure 2 is shown replotted vs. $E_T(30)$ polarity in Figure 4.

It is interesting, however, to investigate the correlations of retention with methanol-water and acetonitrile-water mobile phases separately. For the methanol-water retention data, which involved 92 individual data sets, there was little difference in plotting log k' vs. $E_T(30)$ or vs. % methanol, with r^2 values of 0.9907 and 0.9956, respectively. However, with acetonitrile-water retention data, the plots of log k' vs. $E_T(30)$ polarity gave significantly

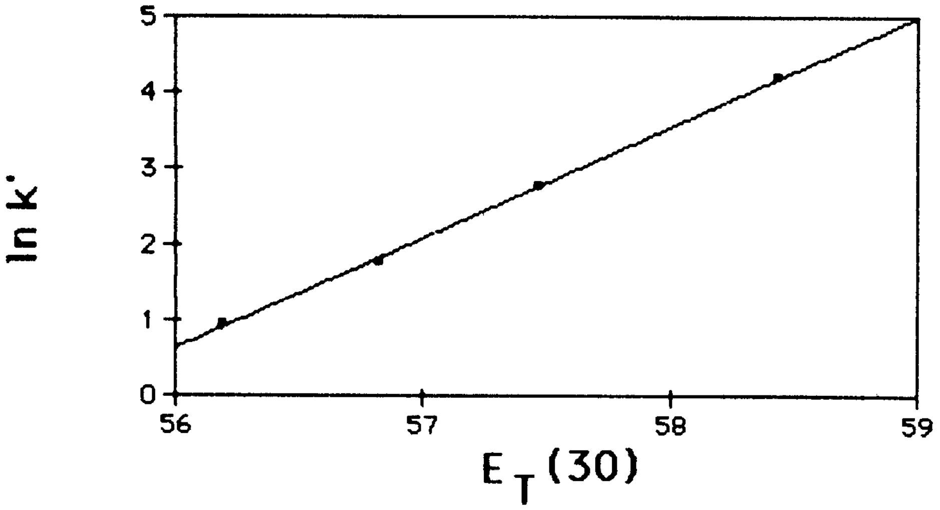

FIGURE 4. Comparison of log k' values from Figure 2 vs. $E_T(30)$ polarity.

better linearity, with an average r^2 value of 0.9914 for 240 different data sets, as opposed to an average r^2 of 0.9733 for plots of log k' vs. % acetonitrile. The quadratic dependence of plots of log k' vs. % acetonitrile has been predicted both by Schoenmakers et al. (5) using the Hildebrand solubility parameter approach, and by Dill (13) using statistical mechanical theory.

The linearization of these retention plots is then quite useful. This will hopefully lead to an improved understanding of retention processes, as well as improved optimization schemes and physicochemical measurements. Chromatographic retention measurements themselves provide information about the combined nature of the mobile and stationary phases. In contrast, solvatochromic polarity measurements are made independently of the stationary phase, thus allowing the effect of changes in mobile phase composition to be examined *independently* of chromatographic retention measurements. This then allows useful deconvolution of subtle changes in stationary phase structure (vide infra).

Dill (13) has recently predicted the quadratic dependence of plots of log k' vs. % acetonitrile by the use of statistical mechanical theory. He showed that the affinity of a solute for the bonded alkyl chains of a reversed phase stationary phase is determined by the entropy of mixing of the solute, the configurational entropy of the grafted chains, and the contact interactions among solute, solvent and chains. Using binary interaction parameters, χ, he showed that

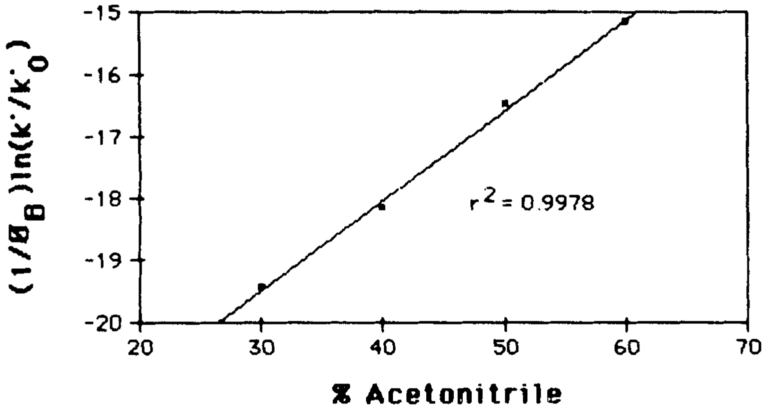

FIGURE 5. Comparison of $(1/\phi_B)$ ln (k'/k'_0) vs. % acetonitrile for pyrene data from Figure 2.

$$(1/\phi_B)\ln(k'/k'_0) = (\chi_{SB} - \chi_{SA} - \chi_{AB}) + \phi_B(\chi_{AB}) \qquad \text{(Eqn. 5)}$$

where ϕ_B is the volume fraction of the organic modifier, k'_0 is the value of k' when $\phi_B = 0$, and $\chi_{SB} - \chi_{SA} - \chi_{AB}$ are the binary interaction parameters among the solute (S), water (A) and organic modifier (B). Then a plot of $(1/\phi_B)$ ln (k'/k'_0) vs. ϕ_B should be linear for any value of χ_{AB}, provided the regular solution approximation holds. Figure 5 shows the pyrene data replotted in this form, with the value of k'_0 determined from extrapolating a quadratic fit of ln k' vs. ϕ_B to zero organic modifier. The excellent agreement of linearity of the plots of retention vs. $E_T(30)$ and the form derived by Dill suggest that the ET-30 dye may actually be providing a measure of the binary interaction parameters for the measured solvents. We are presently investigating this possibility with the use of the retention data reported in (1 1).

SELECTIVITY AND SOLVENT POLARITY

Methylene selectivity is the difference in retention between two molecules of a homologous series differing only by one methylene unit, and is measured from the slope of a plot of log k' vs. carbon number of the homologous series. Methylene selectivity provides much information about reversed phase columns that can be useful both for the elucidation of mechanistic information and for optimization of separations. For example, methylene selectivity serves as a convenient measure of elution strength. Then for a difficult separation, mobile phase strength can be held constant for different organic modifiers, while the selectivity of other interactions is exploited to maximize the separation between two or more solutes. Jandera has recently described methods whereby selectivity data can be used to aid in the identification of functional groups in simple organic compounds (14) as well as for the prediction of retention data (15).

The use of selectivity data for the elucidation of mechanistic information has some distinct advantages over the use of retention data alone. First, selectivity values are not affected by chain ordering, whereas retention values will reflect this effect. Since what is being probed is the *difference* in free energy change as a methylene unit is added to a molecule, both molecules will see the same stationary phase structure. This means that selectivity values should be a very useful probe of real stationary phase polarity, reflecting changes in stationary phase solvation. Selectivity values are further not affected by the presence of residual silanols which can lead to anomalous retention behavior of solutes with highly polar or hydrogen bond donor/acceptor groups.

The measurement of methylene selectivity is made at a fixed mobile phase composition, and the value of the selectivity changes with mobile phase composition, reflecting changes in the polarity of the mobile phase. Traditionally the description of the selectivity as a function of organic modifier has been found to be quite complex. Colin et al. (16) investigated selectivity for binary aqueous-organic mixtures of five different solvents, as well as a ternary solvent, and two different non-aqueous binary mixtures. They reported that except for methanol-water, every system studied exhibited a nonlinear variation of log α with respect to percent organic modifier.

We have reported correlations between selectivity and $E_T(30)$ solvent polarity for both methanol-water and acetonitrile-water mobile phases on a variety of columns (12). For 11 different data sets of measurements of log α as a function of mobile phase composition, there was little overall difference in plotting log α vs. % organic modifier or vs. $E_T(30)$, with r^2 values of 0.9724 and 0.9785 respectively. However, again it is useful to break this data set into methanol and acetonitrile components. For methanolic mobile phases, plots of log α vs. % organic modifier gave an r^2 value of 0.9945, while plotting vs. $E_T(30)$ gave an r^2 value of only 0.9787. For acetonitrile mobile phases, however, the situation is reversed, with plots of log α vs. % organic modifier yielding an r^2 value of 0.9458, while plotting vs. $E_T(30)$ gave an r^2 value of 0.9783. While the selectivity correlations are nowhere near as impressive as the retention correlations, for acetonitrile they are still a significant improvement over traditional plots utilizing % organic modifier.

STATIONARY PHASE SOLVATION

The solvatochromic polarity measurements of mobile phase strength are quite information rich. The excellent linearity of the large body of retention data is proof that the measured polarity is in fact a good measure of elution strength of reversed phase mobile phases. This then gives a measure of elution strength that is made *independently* of actual chromatographic retention measurements, which may allow for deconvolution of subtle contributions from changes in stationary phase structure. Both the retention and selectivity studies have provided such information.

From the 332 data sets used in the retention correlations, there were 89 instances where retention data was available for the same solute and column in both methanol and acetonitrile mobile phases. If the stationary phase were truly inert, the slope of the plots of log k' vs. $E_T(30)$ should be equal for the two solvents. The slopes were found to be different, however, for the same solute and column when the mobile phase was changed from methanol to acetonitrile. For the 89 instances, the average ratio of the slope with methanol to the slope with acetonitrile was 1.43, with a standard deviation (s) of only 0.06. Initially, the greater slope found with methanol-water mixtures would appear to be at odds with the general notion of acetonitrile being a "stronger" solvent for reversed phase liquid chromatography. However, this observation is entirely reasonable, considering the effect of solvation of the stationary phase.

That the slope is different for the two organic modifiers is evidence of the importance of the stationary phase in the retention process. If the stationary phase was truly "inert", the slopes should be equal, as the $E_T(30)$ polarity measures the actual solvating power of the mobile phase, and iso-$E_T(30)$ values should have equivalent elution strengths. Then different slopes of log k' vs. $E_T(30)$ must indicate that the solute is experiencing a different environment in the stationary phase as the organic modifier is changed from methanol to acetonitrile. The greater slope in methanol-water shows that for an equal change in mobile phase polarity, retention is affected to a greater extent in methanol-water systems. This is consistent with the results of McCormick and Karger (17, 18) who measured the distribution isotherms for organic modifiers used in reversed phase chromatography, and showed that at all concentrations of organic modifier, the stationary phase contained a higher concentration of acetonitrile than methanol. Then the greater slope (vs. $E_T(30)$ polarity) found in methanol-water systems is a reflection of the fact that there is less methanol in the stationary phase, so a change in the mobile phase polarity will influence chromatographic retention to a greater extent than in acetonitrile-water systems, where a change in overall mobile phase polarity is compensated by more or less solvation of the stationary phase.

Confirmation of the theory that the difference in slope is a stationary phase effect is found from the selectivity measurements. The methylene selectivity for a series of alkylbenzenes was measured in both methanol-water and acetonitrile-water mobile phases, and the selectivity was plotted vs. $E_T(30)$ polarity. The ratio of the slopes from regression lines of the two plots was also 1.44! That is, selectivity in the methanol-water system is more greatly affected by overall changes in mobile phase polarity than with acetonitrile as the organic modifier, and the ratio of the slopes of the two mobile phases is the same as found with the retention data! We are further investigating whether this is indeed a stationary phase effect by plotting retention of several compounds vs. $E_T(30)$ polarity of methanol-water, ethanol-water, propanol-water and acetonitrile-water mobile phases, where the slopes should change in a progressive fashion.

CONCLUSIONS

Solvatochromic solvent polarity measurements offer a unique view of the retention process in reversed phase liquid chromatography. Retention measurements provide information about the nature of both mobile and stationary phases, while solvatochromic measurements are made independently of the stationary phase. This then allows deconvolution of the two effects so that changes in mobile phase compositon can be examined independently of retention measurements. It is likely that these measurements will then provide a useful way of classifying the "strength" of stationary phases through appropriate ratioing of retention plots. The solvatochromic indicator may also provide a useful means of directly measuring the polarity of reversed phases through diffuse-reflectance measurements. These future extensions are presently under investigation in this laboratory.

ACKNOWLEDGEMENTS

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