Characterizing the Selectivity of Stationary Phases and Organic Modifiers in Reversed-Phase High-Performance Liquid Chromatographic Systems by a General Solvation Equation Using Gradient Elution

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Abstract

Retention data for a set of 69 compounds using rapid gradient elution are obtained on a wide range of reversed-phase stationary phases and organic modifiers. The chromatographic stationary phases studied are Inertsil (IN)-ODS, pentafluorophenyl, fluoro-octyl, *n*-propylcyano, Polymer (PLRP-S 100), and hexylphenyl. The organic solvent modifiers are 2,2,2-trifluoroethanol (TFE); 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP); isopropanol; methanol (MeOH); acetonitrile (AcN); tetrahydrofuran; 1,4-dioxane; *N*,*N*-dimethylformamide; and mixed solvents of dimethylsulfoxide (DMSO) with AcN and DMSO with MeOH (1:1). A total of 25 chromatographic systems are analyzed using a solvation equation. In general, most of the systems give reasonable statistics. The selectivity of the reversed phase-high-performance liquid chromatographic (HPLC) systems with respect to the solute's dipolarity–polarity, hydrogen-bond acidity, and basicity are reflected in correspondingly large coefficients in the solvation equation. We wanted to find the most orthogonal HPLC systems, showing the highest possible selectivity difference in order to derive molecular descriptors using the gradient retention times of a compound. We selected eight chromatographic systems that have a large range of coefficients of interest (s, a, and b) similar to those found in water–solvent partitions used previously to derive molecular descriptors. The systems selected are IN-ODS phases with AcN, MeOH, TFE, and HFIP as mobile phase, PLRP-S 100 phase with AcN, propylcyano phase with AcN and MeOH, and fluorooctyl phase with TFE. Using the retention data obtained for a compound in the selected chromatographic systems, we can estimate the molecular descriptors with the faster and simpler gradient elution method.

Introduction

Following the work of Kamlet, Taft, and Abraham (1,2), the general solvation equation of Abraham (equation 1) has been widely used to describe chromatographic retentions (3–7), partitions (8,9), solubility (10,11), and biological transport (12,13) processes. The linear free energy equation uses five molecular descriptors to characterize the interactions of a solute in a bulk solvent.

$$SP = c + rR_2 + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^0 + vVx \qquad \text{Eq. 1}$$

where SP is a solute property such as the logarithm of partition coefficients (logP), chromatographic retention parameters, t_{Rg}, logk, and $logk_w$. The explanatory variables are solute descriptors as similarly described elsewhere (14): R_2 is an excess molar refraction that can be obtained from a compound's measured refractive index or can easily be calculated, π_2^H is the solute dipolarity–polarisability, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^0$ are the solute overall or effective hydrogen-bond acidity and basicity, respectively, and V_x is the McGowan characteristic volume (in $cm^3/100$ mol) that can be calculated for any solute simply by the molecular structure using a table of atomic constants (15). It should be noted that for reversed phase (RP)-high-performance liquid chromatographic (HPLC) processes, the $\Sigma\beta_2^0$ parameter is used instead of $\Sigma\beta_2^H$.

The equation constants (c, r, s, a, b, and v) are obtained by multiple linear regression analyses and describe a measure of differences in properties between the two phases of the system. The r constant gives a measure of the propensity of the solvent that interacts with solute π- and *n*-electron pairs, the s constant is a measure of the dipolarity–polarisability, the a constant measures the hydrogen-bond basicity (because an acidic solute will interact with the basic phase), the b constant measures hydrogen-bond acidity, and v is a measure of the hydrophobicity. The sign of the coefficients shows the phase that the solute favors. To be statistically valid, a set of known solute properties should be widely varied to probe all the interaction parameters in equation 1 with a sufficient number of data points.

Application of the solvation equation to chemical and biomedical processes is restricted by the availability of the molecular descriptors for compounds, because these have to be

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obtained from experimental data. To determine the parameters (π_2^H, α_2^H, and β_2^0) for any given compound, data from gas–liquid chromatography (16,17) for simple volatile compounds or from water–solvent partitions (18) (logP) are usually required. Partition measurements by the shake flask method are very time-consuming and can be very difficult in certain solvent–water systems. RP-HPLC has been employed to determine the descriptors, being that HPLC is a simpler and much faster method. Most of the stationary phases that were studied have been based on *n*-alkyl chains (C8 and C18) made by various manufacturers with mainly acetonitrile (AcN) or methanol (MeOH) as the organic modifier (19,20). There are some differences in properties between the systems of the different *n*-alkyl chain stationary phases and the solvents that were studied, but the difference or selectivity is not large enough for the determination of descriptors. Valko et al. (21,22) extended the stationary phases to include Cyclodextrin and immobilized artificial membrane bonded phases in addition to the Inertsil (IN)-ODS phases using a faster method of gradient elution instead of the more common isocratic method. However, the organic modifier was limited to AcN.

In this study, we carried the search further using gradient elution in HPLC by extending the range of stationary phases and more importantly the organic solvents used as modifiers. We report a set of solvation equations generated from RP-HPLC systems that show specific selectivity towards solute hydrogen-bond acids, hydrogen-bond bases, dipolarity, polarisability, and size (i.e., the coefficients in equation 1 are as orthogonal to each other as possible). If RP-HPLC systems with sufficiently different coefficients in equation 1 were obtained, they were then considered capable of being used to determine solute descriptors as an alternative or an additional method to that using the water–solvent partition.

Table I. The Linear Gradient Program Used for Gradient Mixing

Time (min)	% Organic solvent	Flow rate (mL/min)
0–1.5	0	1
1.5–10.5	0–100	1
10.5–11.5	100	1
11.5–12	100–0	1
12–15	0	1

Table II. The HPLC Columns Used in the Study and Their pH Tolerance Range

Column	Dimension	Supplier	pH stability range
ODS2-IK5 Inertsil	150 × 4.6 mm	Capital HPLC	2–7.4
PFP	150 × 4.6 mm	Capital HPLC	2–7.4
Hexylphenyl	150 × 4.6 mm	Phenomenex	2–7.4
FO	150 × 4.6 mm	ES Industries	2–8
DCN, 5µg	150 × 4.6 mm	Phenomenex	2–11
PLRP-S 100	150 × 4.6 mm	Polymers Laboratories	1–13
Novapak CN	70 × 4.6 mm	Waters Chromatography	2–7.4

Table III. List of Solvents Used and Their Kamlet–Taft Solvatochromic Parameters

Solvent	π^*_1	α_1	β_1
Water	1.09	1.17	0.18
MeOH	0.60	0.93	0.62
EtOH	0.54	0.78	0.83
IPA	0.48	0.76	0.95
TFE	0.73	1.51	0
HFIP	0.65	1.96	0
AcN	0.75	0.19	0.31
THF	0.73	0	0.22
1,4-Dioxane	0.55	0	0.37
DMF	0.88	0	0.69
DMSO	1.00	0	0.76

Experimental

Gradient retention data were measured on a Hewlett-Packard (Amsterdam, Netherlands) 1090 series HPLC. Data acquisition and processing was performed on a Viglen IBM compatible PC with HP Chemstation software (Hewlett-Packard). Data analysis was carried out using the JMP statistical software package.

Gradient mixing was carried out by a low-pressure gradient mixer built into the HP 1090 and was controlled by the Chemstation program. The linear gradient program can be found in Table I.

The aqueous mobile phase was 50mM ammonium acetate obtained from Fisons (Loughborough, U.K.) and adjusted to buffer pH 9.5 by adding a concentrated ammonia solution. For acids or neutral compounds, the aqueous phase was prepared from 0.1% phosphoric acid, pH 2. The organic solvents were AcN; MeOH; ethanol (EtOH); isopropanol (IPA); 2,2,2-trifluoroethanol (TFE); 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP); dimethylsulfoxide (DMSO); tetrahydrofuran (THF); 1,4-dioxane; and *N,N*-dimethylformamide (DMF). These solvents were supplied as follows: MeOH and THF (Rathburn Chemicals Ltd., Walkerburn, Scotland); EtOH, DMF, DMSO, and IPA (Romil Ltd., Cambridge, U.K.); 1,4-dioxane (Aldrich, Gillingham, U.K.); and TFE and HFIP (Fluorochem, Whaley Bridge, U.K.).

The reversed-phase columns used in this study are listed in Table II with their main properties and the suppliers. These stationary phases were chosen to show different selectivity and for their tolerance of a wide range of pH (from pH 2 to 9.5). The reason for a wide range of pH was to be able to measure the retention of solutes in their nonionized state. The IN-ODS column was an octadecyl bonded silica stationary phase with Silanol endcapping. The Polymer stationary phase that was based on the copolymerization of styrene and divinylbenzene and bonded by alkyl functionality (PLRP-S 100) (23) had a

high surface area and extreme pH tolerance. On the recommendation of the manufacturer, for the PLRP-S 100 phase, the aqueous mobile phase always contained 1% organic modifier; therefore, the gradient was started at 1% organic solvent as opposed to 0%. We found that this 1% organic solvent addition drastically improved the quality of the phase and extended its lifetime.

The solutes used in this study are all commercially available and their descriptors are in the UCL database. The solutions were prepared by adding approximately 0.2 mg of the solid to 1 mL buffer–AcN mixtures (1:1) at pH 7.4. The gradient system was calibrated with the test mixture containing octanophenone, heptanophenone, hexanophenone, valerophenone, butyrophenone, propiophenone, acetophenone, acetanilide, and paracetamol. The test mixture was injected at the start and the end of the run in order to ensure that the physical conditions during the measurements were the same. The test mixture was also used as a check on the condition of the column; therefore, a significant change in retention times (0.1–0.2 min) indicated that the column had deteriorated and a replacement was necessary.

Different solvents have different elution powers for a given stationary phase. Found in Table III is a list of solvents that were used in this study. They were selected because of their properties in terms of the Kamlet and Taft (24,25) solvatochromic parameters: dipolarity–polarisability (π^*_1), hydrogen-bond acidity (α_1), and hydrogen-bond basicity (β_1). The solvents DMF and DMSO provide the capacity of dipole-type interactions (π^*_1 = 0.88 and 1.0). The DMSO was mixed with AcN (1:1) as a mobile phase in order to reduce the high viscosity of DMSO and consequently the high back pressure during a gradient elution. Both solvents had very high hydrogen-bond basicity (β_1 = 0.69 and 0.76). The alcohols MeOH, EtOH, IPA, TFE, and HFIP were selected for their hydrogen-bond acidity (α_1 = 0.76 – 1.96). The fluorinated alcohols not only exhibited very strong hydrogen-bond acidity (significantly stronger than water), but also very low hydrogen-bond basicity (β_1 = 0)—a property that is different from the normal alcohols. AcN is a commonly used solvent in HPLC. It is important when selecting a solvent to ensure that it is completely miscible with water (buffer) and that it does not have substantial ultraviolet (UV) absorbance when an UV detector is used.

Results and Discussion

System characterization

The gradient retention times were measured for a set of 69 solutes in 25 RP-HPLC systems. The gradient retention times of the solutes studied in different RP-HPLC systems were linearly regressed against the five descriptors in the solvation equation. The coefficients obtained are summarized in Tables IV and V with the statistics for the fit. Overall, the solvation equations for the 25 stationary phase solvent systems gave reasonable correlation coefficients.

Using equation 1, the gradient retention times could be cor-

Table IV. The Coefficients of the Solvation Equation for ODS-Inertsil Column with Different Organic Solvent Modifiers

Column	Assigned Number	Solvent	c	r	s	a	b	v	Compounds included in the regression	Standard error of the estimate	Multiple correlation coefficienct
IN-ODS	1	MeOH	7.23 ± 0.23	0.57 ± 0.25	–1.04 ± 0.24	–1.07 ± 0.26	–4.68 ± 0.28	5.15 ± 0.27	67	0.67	0.954
	2	IPA	4.35 ± 0.28	0.83 ± 0.26	–1.32 ± 0.27	–1.00 ± 0.28	–3.48 ± 0.31	3.81 ± 0.33	41	0.55	0.949
	3	TFE	6.94 ± 0.25	0.67 ± 0.33	–1.96 ± 0.25	–3.10 ± 0.28	–3.94 ± 0.31	5.67 ± 0.28	68	0.68	0.965
	4	HFIP	7.87 ± 0.31	0.64 ± 0.37	–1.37 ± 0.31	–4.72 ± 0.34	–3.98 ± 0.38	4.47 ± 0.36	55	0.77	0.953
	5	AcN	7.09 ± 0.14	0.41 ± 0.15	–1.06 ± 0.15	–1.59 ± 0.15	–4.88 ± 0.17	4.80 ± 0.16	68	0.38	0.984
	6	THF	7.59 ± 0.18	–0.32 ± 0.17	–0.25 ± 0.18	–0.45 ± 0.20	–3.59 ± 0.22	2.87 ± 0.20	62	0.47	0.94
	7	DMF	6.67 ± 0.38	–0.10 ± 0.47	–0.50 ± 0.37	0.35 ± 0.38	–5.10 ± 0.40	5.52 ± 0.39	53	0.84	0.930
	8	1,4-Dioxane	6.68 ± 0.25	0.064 ± 0.34	–0.41 –± 0.27	–1.08 ± 0.29	–5.07 ± 0.35	4.63 ± 0.29	56	0.66	0.951
	9	MeOH–DMSO (1:1)	7.55 ± 0.30	–0.268 ± 0.31	–0.91 –± 0.32	–0.72 ± 0.33	–4.43 ± 0.37	5.55 ± 0.38	61	0.84	0.925
	10	AcN–DMSO (1:1)	7.28 ± 0.21	–0.16 ± 0.21	–0.90 ± 0.21	–1.30 ± 0.23	–4.87 ± 0.25	5.59 ± 0.25	62	0.56	0.967

Table V. The Coefficients of the Solvation Equation for Assorted Stationary Phases with AcN, MeOH, TFE, THF, and DMF Organic Modifiers

Column	Assigned Number	Solvent	c	r	s	a	b	v	Compounds included in the regression	Standard error of the estimate	Multiple correlation coefficienct
PFP	11	AcN	7.22 ± 0.15	0.38 ± 0.16	–0.56 ± 0.16	–1.20 ± 0.17	–4.21 ± 0.19	3.64 ± 0.18	67	0.43	0.970
	12	MeOH	7.94 ± 0.20	0.48 ± 0.20	–0.64 ± 0.20	–0.89 ± 0.22	–3.29 ± 0.25	3.75 ± 0.23	68	0.55	0.936
	13	THF	6.83 ± 0.25	0.03 ± 0.32	–0.52 ± 0.27	–0.20 ± 0.29	–3.92 ± 0.31	3.71 ± 0.29	62	0.67	0.906
FO (perfluorinated)	14	AcN	7.21 ± 0.20	–0.48 ± 0.21	–0.25 ± 0.21	–1.62 ± 0.21	–4.10 ± 0.24	3.74 ± 0.23	66	0.54	0.960
	15	MeOH	7.29 ± 0.33	–0.62 ± 0.36	–0.54 ± 0.38	–1.74 ± 0.38	–3.37 ± 0.41	4.50 ± 0.39	67	0.93	0.894
	16	TFE	7.45 ± 0.23	–0.12 ± 0.24	–0.57 ± 0.25	–3.67 ± 0.26	–1.89 ± 0.30	3.11 ± 0.28	65	0.64	0.950
	17	DMF	6.71 ± 0.35	–1.21 ± 0.35	–0.77 ± 0.47	–0.73 ± 0.41	–4.21 ± 0.46	6.04 ± 0.55	50	0.89	0.902
Novapak CN	18	AcN	–0.83 ± 0.21	1.07 ± 0.23	–1.55 ± 0.22	–0.43 ± 0.27	–1.88 ± 0.26	4.47 ± 0.24	63	0.57	0.953
	19	MeOH	–1.27 ± 0.21	0.78 ± 0.22	–1.37 ± 0.23	–0.68 ± 0.23	–1.49 ± 0.27	4.59 ± 0.27	64	0.60	0.950
DCN	20	AcN	5.67 ± 0.23	0.20 ± 0.22	–0.28 ± 0.21	–0.55 ± 0.26	–4.15 ± 0.26	3.68 ± 0.25	60	0.57	0.936
	21	MeOH	3.93 ± 0.29	0.79 ± 0.30	–1.05 ± 0.29	–0.72 ± 0.32	–4.50 ± 0.35	5.42 ± 0.33	69	0.82	0.924
	22	TFE	4.65 ± 0.20	0.97 ± 0.20	–0.77 ± 0.22	–0.63 ± 0.22	–3.21 ± 0.24	3.22 ± 0.23	65	0.53	0.934
PLRP-S 100 phase	23	AcN	8.19 ± 0.20	–0.41 ± 0.35	–0.44 ± 0.21	–2.50 ± 0.25	–5.64 ± 0.28	4.38 ± 0.23	66	0.58	0.969
Hexylphenyl phase	24	AcN	7.31 ± 0.16	0.21 ± 0.17	–0.52 ± 0.16	–1.41 ± 0.18	–4.06 ± 0.20	3.72 ± 0.18	69	0.45	0.967
	25	MeOH	7.72 ± 0.26	0.07 ± 0.27	–0.57 ± 0.26	–1.00 ± 0.29	–4.08 ± 0.32	4.65 ± 0.30	69	0.74	0.928

Table VI. The Coefficient Ratios of the Solvation Equations for Selected Partition Systems

Solvent–water	r/v	s/v	a/v	b/v
Octanol–water	0.15	–0.28	0.01	–0.91
Cyclohexane–water	0.18	–0.37	–0.81	–1.06
Dichloromethane–water	0.00	0.00	–0.76	–0.97

related with the fundamental molecular properties. The regression coefficients r, s, a, b, and v reflect the differences in the complimentary properties of the stationary and mobile phases. A detailed explanation for the meaning of the coefficients in terms of chromatographic separation can be found in recent work by Reta et al. (26). This provides a measure of molecular property for the bulk system. Thus, if a particular coefficient is numerically very large, then any solute with the complimentary property will interact very strongly with either the mobile phase (negative coefficients) or stationary phase (positive coefficients). For example, if the a coefficient is a large negative value, then solutes that are hydrogen-bond acids will have a low retention whenever the interaction between the solute–solvent is stronger than the solute–stationary phase. For good selectivity, it is desirable that in addition to a large coefficient for the interaction of interest, the other coefficients in the regression should also be relatively small. In general, most systems will have multiple interactions, thus selectivity is a matter of degree (27,28).

IN-ODS phase

The IN-ODS phase studied with different aqueous–solvent mobile phases showed that in all cases the dominant parameters were the large positive v constant and negative b constant, which is commonly observed in RP-HPLC systems.

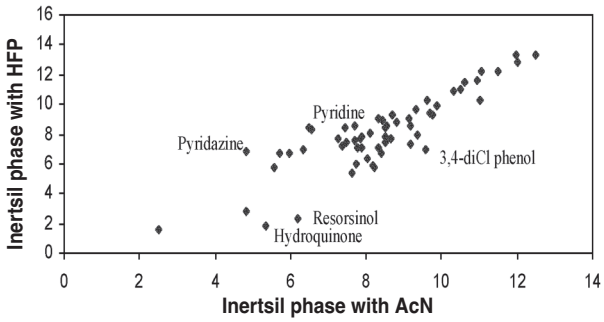

Figure 1. The plot of the solutes's retention times (min) obtained on an IN-ODS column with HFIP and AcN as the organic modifiers.

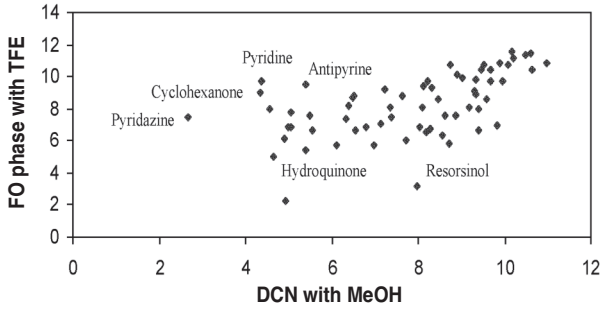

Figure 2. The plot of the solutes's retention times (min) obtained on an FO column with TFE and DCN phase with MeOH.

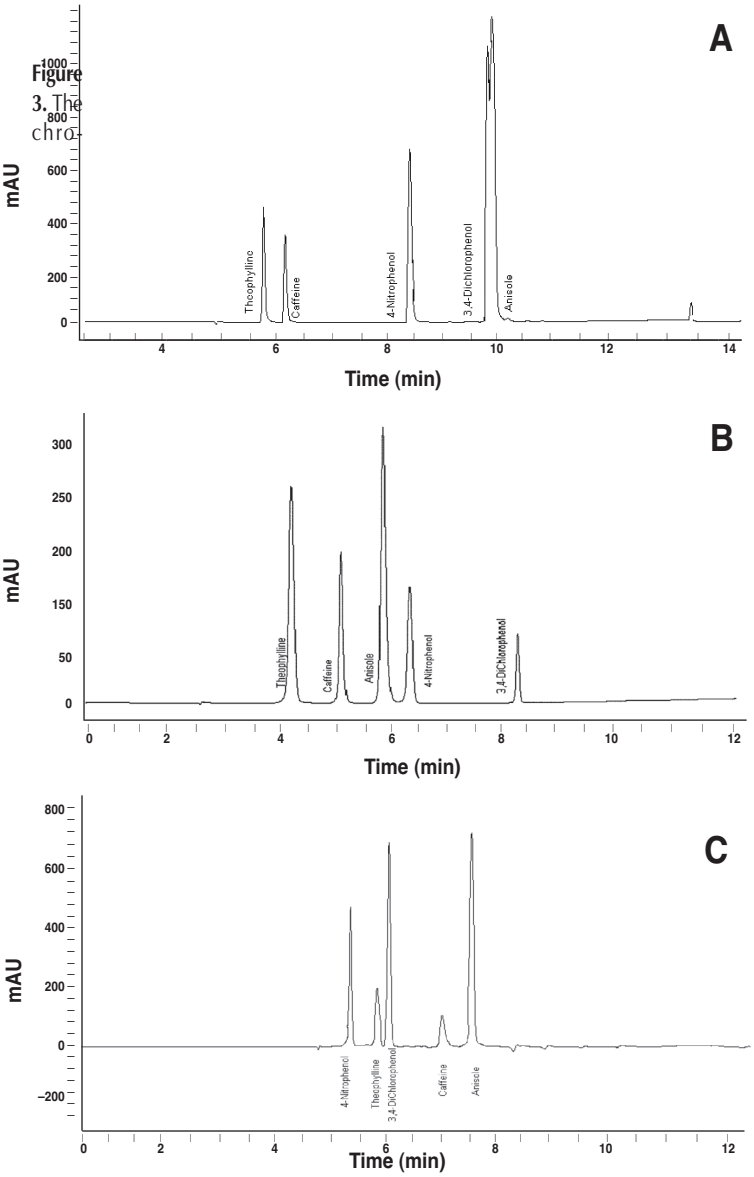

Figure 3. The chro

obtained on IN-ODS column (4.6 × 150 mm) with AcN gradient (A), DCN column (4.6 × 150 mm) with MeOH gradient (B), and FO column (4.6 × 150 mm) with TFE gradient (C). The flow rate was 1.5 mL/min, and the gradient steps were as described in the Experimental section.

Statistics for the THF, DMF, and mixed MeOH–DMF modifiers were not as good as usual; therefore, we considered these systems no further. Of the remaining systems, we chose those with the organic modifiers MeOH, TFE, HFIP, and AcN as representative systems for a classical octadecyl silica stationary phase. The b coefficient values ranged from –3.48 with IPA to –5.07 with dioxan organic solvents, both indicating that the mobile phase was a much stronger hydrogen-bond acid than the stationary phase. It also meant that hydrogen-bond donor compounds would have much longer retention on ODS phases with *i*-propanol than with dioxan as an organic modifier. It would be expected that the two fluorinated alcohols (TFE and HFIP possessing the largest solvent hydrogen-bond acidity, as shown in Table III) would have the largest negative b coefficient, but that was not observed. One possible explanation for this is that the fluorinated alcohols were significantly sorbed on the stationary phase (28). Also, it should be noted that the mobile phases were all aqueous organic mixtures; the Kamlet–Taft solvatochromic parameters (Table III) were for the pure organic solvents and thus would only give an indication of the properties of the aqueous organic mobile phase.

The a coefficient for most organic modifiers with an ODS stationary phase are usually small (a = –0.2 to –1.59). For the fluorinated alcohols TFE and HFIP, we found a large a constant (a = –3.10 and –4.72 for TFE and HFIP, respectively). This meant that the mobile phase was much more hydrogen-bond basic than the stationary phase. From the Kamlet–Taft solvatochromic parameter (Table III), the β_1 value for these two solvents was zero; therefore, the hydrogen-bond basicity character we observed for these two HPLC systems must have came mainly from water (29). The s constant did not vary much between solvents; the biggest difference was between 1,4-dioxane (s = –0.41) and TFE (s = –1.96). The negative s coefficients indicate that the dipolarity–polarisability of the mobile phase was larger than the stationary phase. It meant that highly dipolar compounds with

the same hydrogen-bond donor–acceptor character would show short retention on the ODS phase for whatever solvent used.

Fluorinated stationary phases

The coefficients of the solvation equation for both the alkyl fluorooctyl (FO) and the aromatic pentafluorophenyl (PFP) phases are in Table V. The equation obtained for PFP did not show big differences to the IN-ODS phase with the same solvents. The donor–acceptor property differences of the PFP phases and their selectivity towards polycycliclaromatic hydrocarbons have been shown by Felix et al. (30). However, the equation and thus the selectivity of the FO phase with TFE as the organic modifier was quite different from the ODS phase with TFE. The a coefficient had a very large negative value, and the b coefficient was much less negative. These coefficients marked out the FO phase with TFE as highly selective towards solute hydrogen-bond acidity; therefore, hydrogen-bond donor compounds retained much less.

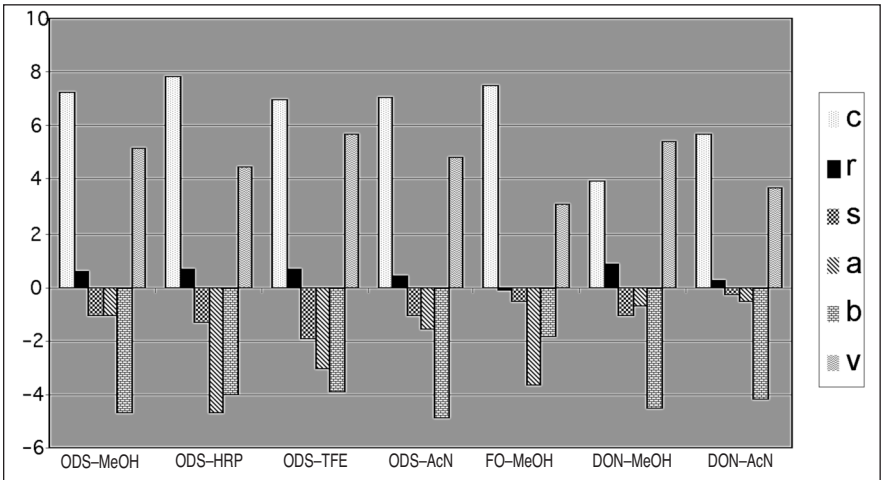

Figure 4. Bar graph showing the ranges of the coefficients in the solvation equation on the selected RP-HPLC systems.

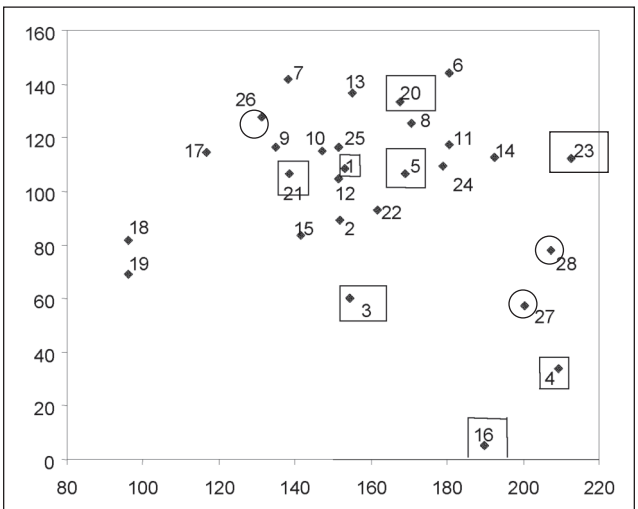

Figure 5. The nonlinear map showing the positions of the normalized coefficients (r/v, s/v, a/v, and b/v) of the solvation equation of the RP-HPLC systems (represented by squares) in relation to coefficients for water–solvent partitions (represented by circles). The HPLC systems are represented by the same numbers as the assigned numbers for the systems found in Tables IV and V except for: octanol–water partition, 26; cyclohexane–water partition, 27; and dichloromethane–water partition, 28.

Propylcyano phases

The propylcyano (CN) phases were obtained from two different manufacturers (Phenomenex and Waters Chromatography, as shown in Table II). The Develosil CN (DCN) phase was stable up to pH 11 and the Novapak CN phase up to pH 8. As shown in Table V, there were some differences in the coefficients of the solvation equation for these two phases using the same solvents. It is accepted that stationary phases from different manufacturers do have a certain degree of difference in selectivity. It should also be noted that direct comparison of the two should only be made using the normalized coefficients (constants, v) as the two columns were not of equal length, as shown in Table II. The most significant differences between the CN phase compared with the other phases were the very negative b coefficients for DCN with AcN and MeOH modifiers and the extraordinarily large v coefficient for DCN with MeOH. These effects were very similar to those found by Poole et al. (31) determined isocratically. The DCN systems with AcN and MeOH were included in our preferred selection of systems. It should be mentioned that the CN phases behaved as normal phases whenever higher than 70% organic solvent was used in the mobile phase. We wanted to use the same gradient profile with all columns and solvents studied; therefore, we reached the 100% organic concentration on these columns. However, all of the studied compounds eluted before the organic solvent concentration reached 70%. This ensured that only a partition mechanism was involved during the chromatography.

PLRP-S 100 phase

For the PLRP-S 100 phase (with AcN modifier, Table V), the very negative b coefficient and the relatively small negative a coefficient made this system attractive, especially in view of the wide range of tolerated pH values (as shown in Table II). We also examined PLRP-S 100 with MeOH, but found that very lipophilic solutes could not be eluted, even at 100% MeOH.

Hexylphenyl phase

The Hexylphenyl phase with an AcN modifier did not show very different selectivity from the straight alkyl-chain ODS phase.

Selection of phases

Finally, we selected eight different RP-HPLC systems from the 25 that we screened, as shown in Table VI. These systems showed the widest practically possible range of selectivity to the solute's hydrogen-bond acidity , basicity, and dipo-

larity–polarisability. The obtained solvation equations showed good fit between the observed and predicted retention data (R and small standard error of the estimate). The selected columns had reasonably good stability at high pHs, and the viscosity and UV transparency of the solvents were acceptable in a practical point of view. IN-ODS with MeOH and AcN and PLRP-S 100 phases were selected for interaction with solutes that had a large hydrogen-bond basicity and dipolarity–polariability. IN-ODS with TFE and HFIP were chosen for their interaction with solutes that were hydrogen-bond acids and also dipolar. FO with TFE system was also useful for its selectivity with solute hydrogen-bond acids. The DCN phase with AcN and MeOH were selected for their weak interaction with solute acids. To show the different ranks of retention that could be obtained with a phase towards a particular solute, retention times obtained from the phases were plotted against each other. A plot of the gradient retention times obtained on IN-ODS with HFIP against the gradient retention times on IN-ODS with AcN (Figure 1) showed that for very strong hydrogen-bond acid solutes, retention was much weaker with HFIP compared with AcN, but the reverse was the case for basic solutes. Figure 2 shows a plot of retention times for a set of compounds measured on the FO phase with TFE against the CN phase with MeOH. Again, there were very considerable selectivity effects for hydrogen-bond acidic and hydrogen-bond basic solutes. To show the differences in selectivity towards the solute hydrogen-bond character between the DCN phase with MeOH and the FO phase with TFE, a mixture of solutes were analyzed: caffeine, 4-nitrophenol, anisole, theophylline, and 3,4-dichlorophenol. Figure 3 shows the obtained chromatograms on IN-ODS with AcN, DCN column with MeOH, and FO column with TFE. It can be seen that not only the gradient retention times but also the elution order of the five components were very different in the three chromatographic systems. The ODS phase with AcN did not retain either the hydrogen-bond donor or acceptor compounds like theophylline and caffeine or both at once. The aromatic compounds retained more on the column. Anisole and 3,4-dichlorophenol could not be separated under these conditions. On the other hand, the CN stationary phase showed less retention to anisole in comparison with the other components. The exceptionally low retentive property of the FO phase with TFE organic modifier towards the hydrogen-bond donor compounds can be observed on the chromatogram in Figure 3C. The hydrogen-bond donor compounds eluted first, and the only hydrogen-bond acceptor (caffeine) and not the hydrogen-bond donor–acceptor anisole eluted last. Figure 4 shows the range of the coefficients in the solvation equation for the selected systems.

The solvation equations for a number of different solvent–water partitions have been used to obtain molecular descriptors when the corresponding logP values for a given solute are known. The success of the logP equations in the determination of the descriptors is because of their very selective properties in regards to the hydrogen-bond acidity and basicity and to a lesser extent the dipolar-type interactions. To ascertain whether any of our selected systems in RP-HPLC represented as wide a range in properties as the solvent–water partitions, a representative selection of the normalized coefficients for solvent–water partitions are given in Table VI. In the partition equations, the s/v ratio ranged from 0 to –0.37, the a/v ratio ranged from 0 to –0.8, and the b/v ratio ranged from –0.9 to –1.15. It is clear that the same range was also observed for the RP-HPLC systems. A comparison is shown by the nonlinear map on the relative coefficients (Figure 5) for all the RP

Table VII. The Coefficients of the Selected RP-HPLC Systems Normalized by Dividing Each of the Coefficients by the v Coefficient

Assigned Number	Solvent	c	r	s	a	b	v	Compounds included in the regression	Standard error of the estimate	Multiple correlation coefficienct
1	IN-ODS/MeOH	1.40 ± 0.23	0.11 ± 0.25	–0.20 ± 0.24	–0.21 ± 0.26	–0.91 ± 0.28	5.15 ± 0.27	69	0.67	0.954
3	IN-ODS/TFE	1.22 ± 0.25	0.12 ± 0.33	–0.35 ± 0.25	–0.55 ± 0.28	–0.69 ± 0.31	5.67 ± 0.28	68	0.67	0.965
4	IN-ODS/HFIP	1.76 ± 0.31	0.14 ± 0.37	–0.31 ± 0.31	–1.06 ± 0.34	–0.89 ± 0.38	4.47 ± 0.36	55	0.77	0.953
5	IN-ODS/AcN	1.48 ± 0.14	0.09 ± 0.15	–0.22 ± 0.15	–0.33 ± 0.15	–1.02 ± 0.17	4.80 ± 0.16	68	0.38	0.984
16	FO/TFE	2.40 ± 0.23	–0.04 ± 0.24	–0.18 ± 0.25	–1.18 ± 0.26	–0.61 ± 0.30	3.11 ± 0.28	65	0.64	0.950
20	DCN/AcN	1.54 ± 0.23	0.05 ± 0.22	–0.08 ± 0.21	–0.15 ± 0.26	–1.13 ± 0.26	3.68 ± 0.25	60	0.57	0.936
21	DCN/MeOH	0.73 ± 0.29	0.15 ± 0.30	–0.19 ± 0.29	–0.13 ± 0.32	–0.83 ± 0.35	5.42 ± 0.33	69	0.82	0.924
23	PLRP-S 100/AcN	1.94 ± 0.20	–0.15 ± 0.35	–0.10 ± 0.21	–0.57 ± 0.25	–1.29 ± 0.28	4.38 ± 0.23	66	0.58	0.969

systems we have examined together with a number of the most common partition systems used to derive the descriptors. The nonlinear map showed the relative positions of the four normalized coefficients (r/v, s/v, a/v, and b/v) projected into a two-dimensional space, thus the coordinates of the map represented arbitrary distance units in the four-dimensional space projected into two dimensions. A short distance between points suggested close similarity of the systems, and a long distance suggested dissimilarity (at least one of the coefficients were very different). We were not interested in looking for a match in any RP-HPLC systems to solvent–water partitions, as this has been proved difficult (32), but it is worth mentioning that the IN-ODS phase with HFIP was the closest to the water–cyclohexane partition. It can be seen that our chosen eight systems spanned the area of space that included solvent–water partition systems with very different characteristics to each other.

Conclusion

We have been able to find a reasonable number of RP-HPLC systems that are very selective in terms of the constants in the solvation equation (1). In large part, this is because of the variety of solvents with very different characteristics that we have used as the organic modifier. We have shown previously that the factors that influence retention in ODS and other columns with isocratic mobile phase (33,34) are the same as those that influence retention in the gradient method. We believe that we have found eight RP-HPLC solvation equations (Table VII) that have a large range of coefficients for the interactions of interest (similar to those in solvent–water partitions) that could be used to derive molecular descriptors from retention times obtained through gradient elution. This in turn means that molecular descriptors can be obtained much more quickly and easily than by using traditional methods such as water–solvent partitions.

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