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Column selectivity in reversed-phase liquid chromatography III. The physico-chemical basis of selectivity

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Abstract

Reversed-phase liquid chromatography (RP-LC) retention data for 23 additional solutes have been acquired to further test and evaluate a general relationship from part I:

$$\log \alpha = \log (k/k_{ref}) = \underset{(i)}{\eta'}\mathbf{H} + \underset{(ii)}{\sigma'}\mathbf{S} + \underset{(iii)}{\beta'}\mathbf{A} + \underset{(iv)}{\alpha'}\mathbf{B} + \underset{(v)}{\kappa'}\mathbf{C} \quad (1)$$

The physico-chemical origin of terms *i*–*v* above is examined here by comparing values of (a) the solute parameters of Eq. (1) (η', σ', etc.) vs. solute molecular structure, and (b) the column parameters (**H**, **S**, etc.) vs. column properties (ligand length and concentration, pore diameter, end-capping). We conclude that terms *i*–*v* correspond, respectively, to hydrophobic (*i*), steric (*ii*), hydrogen bonding (*iii*, *iv*) and ionic (*v*) interactions between solute and stationary phase. While steric interaction (term *ii*) is superficially similar to what previously has been defined as ''shape selectivity'', the role of the solute and column in determining steric selectivity (term *ii*) appears more complex than previously proposed for ''shape selectivity''. Similarly, what has previously been called hydrogen bonding between donor solutes and an acceptor group in the stationary phase (term *iv*) is very likely an oversimplification.

Keywords: Column selectivity; Selectivity; Steric selectivity; Hydrophobic interaction; Soluble parameters; Shape selectivity; Hydrogen bonding; Stationary phases, LC; Ion-exchange interaction

1. Introduction

The two preceding papers [1,2] have described the variation of solute retention as a function of the solute, column and experimental conditions in terms of Eq. (1) (all symbols are in the Glossary of part I [1]). A solute retention factor k is related to the value of k for ethylbenzene (k_{ref}) and to parameters that depend on the solute (η', σ', β', α', κ') and column (**H**, **S**, **A**, **B**, **C**). In the present paper, we consider the possible origin of terms *i*–*v* of Eq. (1) in terms of different physico-chemical interactions that are believed to determine reversed-phase HPLC (RP-LC) retention and column selectivity. Our conclusions in the present paper are inferential, based largely on the relationship of the solute and column

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parameters of Eq. (1) to the structure of the solute molecule and certain characteristics of the column. The goals of this ongoing project include (a) a more detailed and quantitative characterization of the primary factors responsible for differences in column selectivity (parts I and II [1,2]), (b) a further verification of the physico-chemical processes that determine these factors (the present paper), and (c) the application of this information to several practical goals (work in progress).

2. Experimental

2.1. Equipment, materials and procedures

These are described in parts I and II [1,2]. Solutes #1–67 and #1a–87a referred to in the present paper are defined in Tables 6 and 7 of part I [1]. Additional solutes #68–90 whose retention has been determined in the present study are listed in Table 1. Retention data for the solutes of Table 1 are given in Table 2 for nine columns from Table 2 of Ref. [1]. Structures for some of the less familiar compounds of the present study (#1–90) are shown in Fig. 1. The experimental conditions for the data of Table 2 are the same as in Table 3 of Ref. [1].

Limited retention data were obtained for four additional columns: ''monomeric'' columns (a) a production Symmetry C_{18} column (#11) and (b) a research column (#12) that was otherwise identical to (a), except for an absence of end-capping (#12); columns #11 and 12 were gifts from Waters Corp.; ''polymeric'' columns (c) Vydac 201 TP (Separations Group, Hesperia, CA) (#13) and (d) Hypersil ''Green'' PAH (Thermo-Keystone, State College, PA) (#14) were purchased. From retention data for these columns, approximate values of **H**, **S**, etc. could be derived in similar fashion as described in Ref. [1], but using a smaller number of test solutes (#3, 31, 36, 45, 46 and 60).

3. Results and discussion

Terms *i*–*v* of Eq. (1) have been examined for their physico-chemical significance, based on (a) our analysis of the variation of each solute parameter (η', σ', etc.) with solute molecular structure, and (b) similar comparisons of the column parameters **H**, **S**, etc. with various column properties. The dependence of retention on temperature and mobile phase composition was also studied for further insight into the origin of terms *i*–*v*. Apart from the role of mobile phase pH (discussed in Ref. [2]), no new and significant findings resulted from the latter attempt; this is unsurprising, since column selectivity was

Table 1
Additional solutes of present study. Solute classifications indicated here correspond approximately to the dominant interaction in Eq. (1) (shown in parentheses)

''Ideal'' solutes (η'**H**)	Acceptor solute (β'**A**)
68. 1,2-Dinitrobenzene	81. *N*,*N*-Diethylacetamide
69. 1,3-Dinitrobenzene	
	Donor solutes (α'**B**)
''Shape-selective'' solutes (σ'**S**)	82. 3-Nitrophenol
70. Nitrocyclohexane	83. 4-Nitrophenol
71. Biphenyl	84. 2,4-Dinitrophenol
72. 2-Nitrobiphenyl	85. 2,5-Dinitrophenol
73. 3-Nitrobiphenyl	86. Picric acid
74. 2-Biphenylmethanol	87. Fisetin hydrate
75, 2,2′-Biphenol	88. Biochanin A
76. 4,4′-Biphenol	
77. Diphenylbutyrolactone	Basic solutes (β'**A**, κ'**C**)
78. Fluorescamine	89. 4-Phenylpyridine
79. Camphorquinone	90. *N*-Butylaniline
80. Ferrocene	

Table 2
Retention data for compounds of Table 1 for nine columns of Table 2 of Ref. [1]. Conditions: 50% ACN–buffer, buffer is 31.2 m*M* potassium phosphate buffer (pH-2.8); 35 °C; 1.5 ml/min

Solute	Log *k* for indicated solute and column (numbering as in Tables 1 and 2 of Ref. [1])								
	1	2	3	4	5	6	7	8	10
68	0.460	0.316	0.272	0.291	−0.184	0.280	0.322	0.295	0.034
69	0.479	0.343	0.295	0.312	−0.168	0.306	0.347	0.322	0.055
70	0.692	0.582	0.522	0.530	0.029	0.540	0.565	0.543	0.277
71	1.247	1.159	1.052	1.047	0.513	1.097	1.119	1.105	0.824
72	0.988	0.854	0.805	0.815	0.306	0.827	0.857	0.833	0.550
73	1.170	1.047	0.982	0.989	0.469	1.010	1.042	1.021	0.734
74	0.567	0.449	0.399	0.412	−0.068	0.411	0.450	0.429	0.166
75	0.395	0.275	0.213	0.229	−0.231	0.228	0.275	0.252	0.001
76	0.007	−0.161	−0.175	−0.150	−0.572	−0.180	−0.124	−0.155	−0.396
77	0.870	0.742	0.702	0.712	0.210	0.718	0.746	0.721	0.444
78	0.901	0.748	0.719	0.737	0.235	0.733	0.768	0.739	0.453
79	0.531	0.415	0.377	0.388	−0.102	0.384	0.412	0.389	0.123
80	1.177	1.087	0.992	0.985	0.446	1.036	1.048	1.036	0.751
81	−0.333	−0.440	−0.296	−0.266	−0.758	−0.466	−0.453	−0.478	−0.681
82	0.184	0.048	−0.003	0.017	−0.431	0.003	0.052	0.027	−0.218
83	0.133	0.000	−0.054	−0.033	−0.475	−0.051	0.000	−0.025	−0.267
84	0.278	0.232	0.082	0.102	−0.340	0.070	0.128	0.102	−0.158
85	0.372	0.257	0.189	0.205	−0.262	0.197	0.241	0.215	−0.047
86	0.400	0.342	−0.210	−0.137	−0.515	−0.191	0.084	0.055	−0.320
87	−0.335	−0.497	−0.509	−0.491	−0.858	−0.521	−0.458	−0.489	−0.714
88	0.679	0.518	0.488	0.509	0.035	0.497	0.556	0.527	0.241
89	1.213	1.106	1.038	1.039	0.523	1.074	1.098	1.078	0.791
90	0.766	0.664	0.582	0.579	0.063	0.621	0.639	0.629	0.343

shown in part II [2] to be approximately independent of separation conditions other than pH.

In support of the following analysis, retention data for the 23 additional solutes (≠68–90) of Table 1 were correlated by means of Eq. (1) (as in step ≠8 of Table 4 of Ref. [1]) with values of the column parameters **H**, **S**, etc. from Table 5 of Ref. [1], so as to yield the solute parameters of Table 3. Note that solute numbering in Table 3 (≠68–90) is a continuation of the numbering of the 67 solutes reported in Ref. [1]. The selection of the solutes of Table 1 was suggested by our preliminary analysis of data reported in Ref. [1]. In addition, the new solutes of Table 1 provide a further test of the ability of Eq. (1) to accurately describe RP-LC retention. With the exception of solutes ≠84 (SD = 0.016) and 86 (SD = 0.026), the average standard deviation (SD) for the fit of Eq. (1) to the data of Table 2 (SD = 0.004 log units) was the same as for the original 67 test solutes of Ref. [1]. This supports the applicability of Eq. (1) for a wide range of solute structures; i.e. in our opinion, most samples likely to be submitted for RP-LC separation.

3.1. Analysis of solute parameters in terms of solute molecular structure

3.1.1. Term (i): $\eta'\mathbf{H}$ (''hydrophobic'' interaction)

On the basis of data presented and discussed in part I [1], we believe that the $\eta'\mathbf{H}$ term of the equation arises from ''hydrophobic'' interaction between the solute and stationary phase. In the absence of other contributions to retention, Eq. (1) becomes

$$\log k = \log k_{\mathrm{ref}} + \eta'\mathbf{H} \quad (2)$$

Eq. (2) provides an accurate description of the retention of 22 ''ideal'' solutes defined in Ref. [1] and is a reasonable approximation for most other solutes as well (cf. Fig. 2 of Ref. [1] and related discussion). RP-LC retention has often been used as an approximate measure of solute hydrophobicity η'

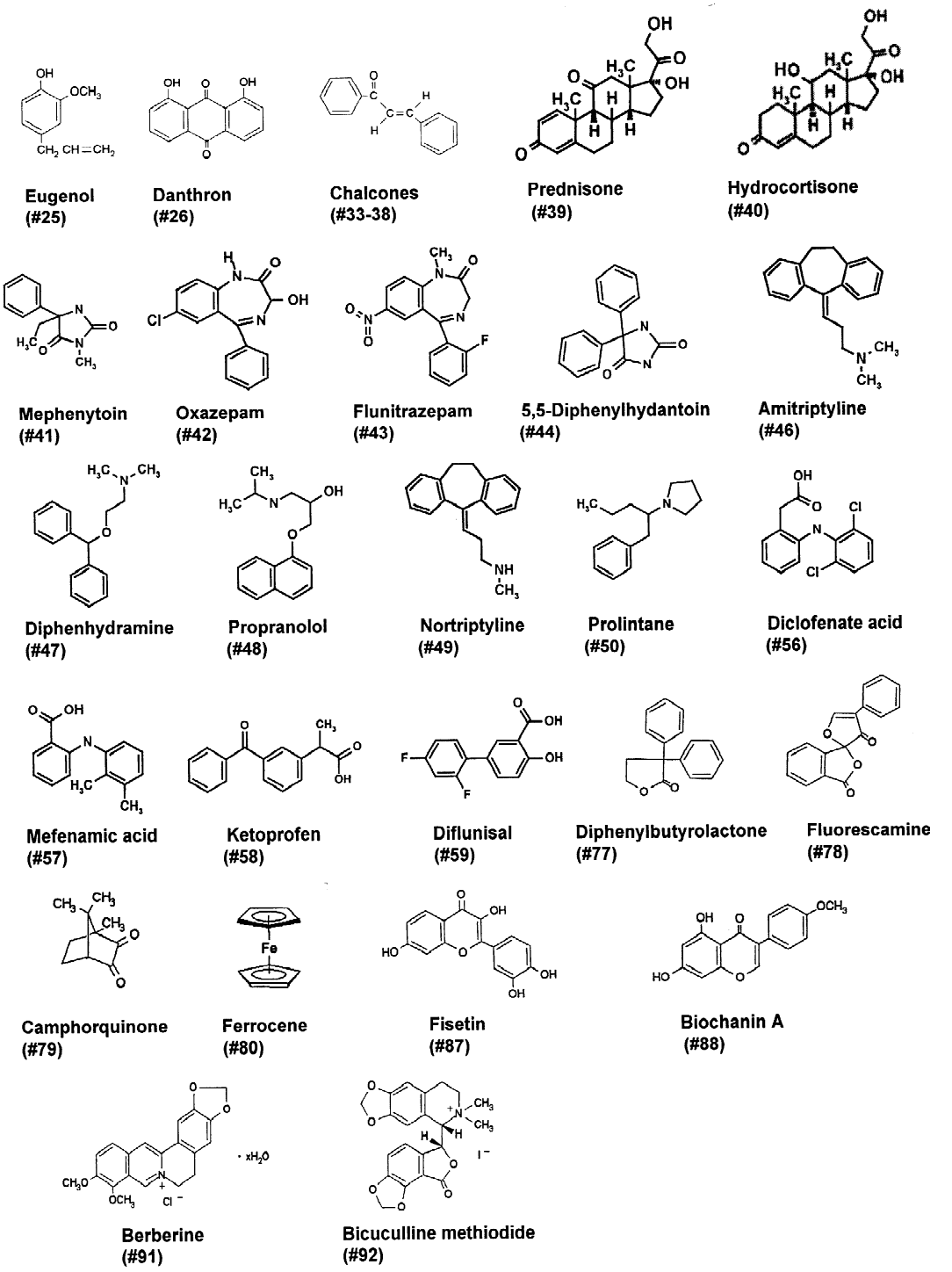

Fig. 1. Structures of ‘‘less obvious’’ compounds from the present study.

Table 3
Solute parameters for compounds of Table 1 and conditions of Table 2

Solute	η'	σ'	β'	α'	κ'
68. 1,2-Dinitrobenzene	−0.637	0.475	−0.031	0.117	−0.037
69. 1,3-Dinitrobenzene	−0.614	0.460	−0.030	0.021	−0.026
70. Nitrocyclohexane	−0.393	0.309	0.001	−0.025	−0.003
71. Biphenyl	0.153	0.225	−0.041	0.100	−0.017
72. 2-Nitrobiphenyl	−0.107	0.798	−0.039	0.137	−0.036
73. 3-Nitrobiphenyl	0.075	0.748	−0.053	0.219	−0.039
74. 2-Biphenylmethanol	−0.511	0.371	−0.030	0.079	0.009
75, 2,2′-Biphenol	−0.687	0.153	−0.051	0.169	0.005
76. 4,4′-Biphenol	−1.082	0.491	−0.064	0.201	−0.012
77. Diphenylbutyrolactone	−0.215	0.735	−0.021	0.093	−0.016
78. Fluorescamine	−0.197	1.047	−0.041	0.146	−0.051
79. Camphorquinone	−0.545	0.422	0.009	−0.094	0.014
80. Ferrocene	0.085	0.286	−0.022	−0.105	−0.002
81. *N*,*N*-Diethylacetamide	−1.341	0.402	0.409	0.097	0.065
82. 3-Nitrophenol	−0.906	0.136	−0.039	0.156	−0.012
83. 4-Nitrophenol	−0.956	0.057	−0.034	0.217	−0.017
84. 2,4-Nitrophenol[a]	*−0.82*	*−1.06*	*0.13*	*1.06*	*−0.16*
85. 2,5-Nitrophenol	−0.718	0.096	−0.002	0.180	−0.039
86. Picric acid[a]	*−0.93*	*−3.19*	*−0.09*	*4.04*	*−0.98*
87. Fisetin hydrate	−1.413	0.141	−0.074	0.412	0.009
88. Biochanin A	−0.417	0.998	−0.089	0.236	−0.052
89. 4-Phenylpyridine	0.131	0.687	−0.050	0.167	−0.006
90. *N*-Butylaniline	−0.325	0.237	−0.031	−0.355	0.015

[a] These solutes correlate more poorly with Eq. (1) (S.E. = 0.02, 0.03, respectively, for solutes ≠84 and 86), suggesting that the derived values of η', σ', etc. are less reliable; except for ≠84, 86, SE = 0.004 log units for the correlation of log *k* values (Table 2) with Eq. (1).

[3], based on relationships similar to Eq. (2) for a given column (where **H** is constant). Also (see later discussion), values of η' increase linearly with homolog carbon number, as expected for a quantity that is related to hydrophobicity. The main conclusion to be drawn from values of η' is their close correlation with values of *k* for a given column (Eq. (2) with **H** constant), suggesting that η' is primarily a measure of solute hydrophobicity [1]. For example, for the Symmetry column and solutes ≠1–67,

$$\eta' = -0.92 + 0.92 \log k;\ r = 0.996,\ \mathrm{SE} = 0.05 \quad (3)$$

*3.1.2. Term (ii): σ'**S** (steric selectivity)*

Compared to other solutes, retention data on different columns for solutes ≠32–40 and ≠43–44 of Ref. [1] exhibit significant, highly correlated deviations from Eq. (2) that were used in part I [1] to define values of the column parameter **S**. An examination of the structures of these solutes (Fig. 1) suggests that their molecular shapes differ in many respects from those of the substituted alkanes and benzenes that comprise most of the solutes studied in Ref. [1] or reported in previous investigations of column selectivity. Because solutes ≠32–40 and ≠43–44 are generally larger or more ''bulky'' molecules, we initially assumed that their retention would be reduced because of greater difficulty in penetrating the stationary phase—similar to the case of column ''shape selectivity'' discussed below. If this assumption is correct (and it appears to be so), a few initial comments regarding the σ'**S** term are in order. If σ' and **S** are each positive, then the value of σ'**S** is positive. This suggests a possible *increase* in retention due to steric interaction, which cannot be the case; i.e. steric hindrance to the insertion of a solute into the RP-LC stationary phase must always reduce retention. The explanation of this apparent paradox (positive values of σ'**S**) is that values of σ' and **S** are *relative* to values for an *average* solute and column, each of which contributes some steric interaction and a corresponding reduction of retention. For a column that for reasons given below (varying ligand length and concentration, pore diam-

eter) can experience a minimum of steric interaction, the value of **S** will be larger than for any of the columns so far studied (Table 5 of Ref. [1]). Relative to a hypothetical, non-sterically-interacting column (for which the absolute value of $\mathbf{S}=0$), values of $\sigma'\mathbf{S}$ for all other solutes and columns will be smaller (i.e. negative), corresponding to a decrease in retention due to steric interaction. Similarly, values of σ' are relative to the solute ethylbenzene; compared to a hypothetical solute that experiences no steric interaction, all σ' values would be positive (and larger than the values reported here by about one unit; cf. Eq. (4) below). Columns that are less penetrable to larger, bulkier molecules will have lower values of **S**, and solutes that are larger and bulkier will have larger values of σ'.

As noted above, we initially assumed that the $\sigma'\mathbf{S}$ term of Eq. (1) represents contributions to retention from so-called column ''shape selectivity'' [4,5]. Shape selectivity effects were first reported for the retention of planar vs. nonplanar polycyclic aromatic hydrocarbons (PAHs), where it was found that certain columns exhibit preferential retention of planar molecules. This was attributed to the presence of openings or ''slots'' in the stationary phase that restrict the access of (thicker) nonplanar molecules. Rigid stationary phases with narrow ''slots'' (which exhibit greater shape selectivity) are more likely to result from a synthesis that uses di- or tri-functional silanes (yielding ''polymeric'' phases with high bonding density), rather than the more common monofunctional silanes (resulting in less-rigid ''monomeric'' phases).

Columns #2–10 of the present study (Table 2 of Ref. [1]) are ''monomeric'' phases made from monofunctional silanes, as opposed to so-called ''polymeric'' phases (e.g. columns #13, 14) that provide maximum ''shape selectivity''. Furthermore, previous examples of ''shape selectivity'' have been restricted to relatively hydrophobic solutes, which require mobile phases of high organic content (80–100% B). This is in contrast to our use of solutes that have convenient retention ($k<20$) for mobile phases of 40–50% ACN–buffer. Finally, most of the solutes used in the current study (solutes #1–90, 1a–87a) have considerable conformational freedom, as a result of rotation about single bonds; PAHs which exhibit ''shape selectivity'' are much more rigid. For these and other reasons, it might be anticipated that steric selectivity as described here (i.e. for solute molecules and stationary phases that are less rigid) could differ in important respects from previously described ''shape selectivity'' [4,5].

3.1.3. Term (ii): $\sigma'\mathbf{S}$ (steric selectivity): experimental results

The following discussion supports a dependence of values of σ' on molecular size and shape, with additional contributions from polar functional groups within the solute molecule. Thus, molecules of similar size, shape and functionality are predicted to have similar values of σ', which is observed for several groups of related compounds (Table 4). The average standard deviation of σ' values for ''similar'' solutes in Table 4 is ± 0.09 σ'-units. Values of σ' exhibit a strong correlation with solute molecular length L, as seen in Fig. 2 for the neutral solutes #1–45 (excluding solute #44, discussed below). In Fig. 2, molecular length L is approximated by the number of atoms (excluding hydrogen) in the longest connected series that does not double back on itself (examples in Fig. 3). For example, L equals 4 for benzene or *n*-propanol, 6 for naphthalene, nitrocyclohexane or *p*-chlorophenol, and 8 for biphenyl or 1-nitrohexane. An additional support of a correlation of σ' with molecular length is provided by the *trans* vs. *cis* chalcones (#33–38). The more extended conformation of the *trans* isomers should result in slightly greater lengths (even though values of L are identical for the two isomers) and larger values of σ'. This is observed for the three chalcone isomer-pairs; the average value of $[\sigma'(trans)-\sigma'(cis)]$ is $+0.22\pm0.12$ (1 SD).

Very limited attempts were made to correlate values of σ' with other solute properties, such as molar volume or the total number of atoms in the molecule apart from hydrogens. The resulting correlations gave slightly greater values of SD than the correlation of σ' with length. It seems likely that more exact measurements of molecular length and width might lead to an improved mathematical prediction of values of σ', but we have not explored this option.

Given the correlation of Fig. 2, values of σ' can be estimated for any of the solutes studied here or in Ref. [1]:

Table 4
Similar values of the solute parameter σ' for solutes of near-identical size and shape

Solutes	Structure	Avg. σ'	SD
Nonpolar compounds			
#40a, 42a	Dihalomethanes	−0.21	0.07
#61a, 62a	*p*-Nitrobenzylhalides	0.65	0.15
#63–66a, 72a	Monohalobenzenes, toluene	−0.15	0.05
#68–70a, 77a	*p*-Halotoluenes, dichlorobenzene, *p*-xylene	−0.18	0.05
#67a, 73a	Benzylbromide, ethylbenzene	0.20	0.29
Polar compounds			
#39, 40	Steroids	0.97	0.01
#46, 49	Amitriptyline, nortriptyline	0.05	0.01
#56, 57	Diclofenate acid, mefenamic acid	0.33	0.10
Average			0.09

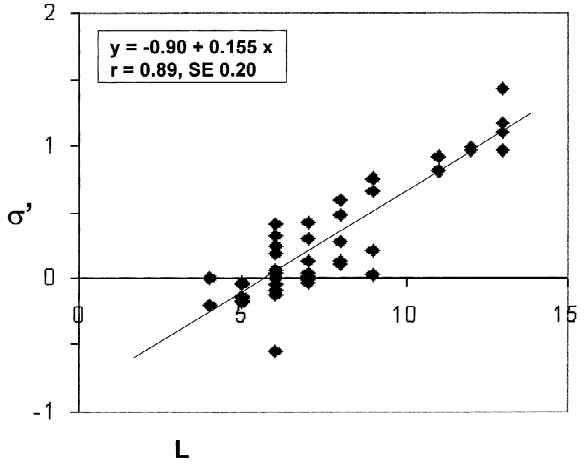

Fig. 2. Correlation of solute parameter σ' with molecular length L (see examples of the calculation of L in Fig. 3).

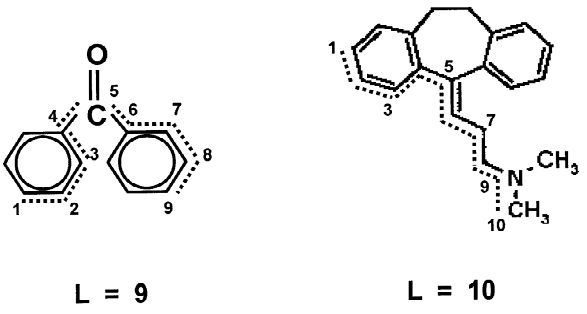

Fig. 3. Examples of the calculation of molecular length L, equal to the minimum number of connected atoms (except hydrogen) between the furthest separated parts of the molecule.

$$\sigma'(\text{predicted}) = -0.90 + 0.155\,L \quad (4)$$

Eq. (4) implies that ''absolute'' values of σ' should be increased by about 0.9 units (which we have not done). It is useful to define the *difference* $\delta\sigma'$ between experimental and predicted values of σ':

$$\sigma'(\text{expt.}) - \sigma'(\text{predicted}) = \delta\sigma' \quad (5)$$

Values of $\delta\sigma'$ can be compared with solute structure to infer molecular contributions to σ' *other* than those arising from solute length. Positive values of $\delta\sigma'$ mean larger values of σ and greater steric interaction with the stationary phase (and vice versa for negative $\delta\sigma'$). For neutral solutes #1–43 and 45 plotted in Fig. 2, an examination of values of $\delta\sigma'$ does not suggest any consistent contribution of molecular shape (other than length) or functionality to σ'. Similarly, compounds #1a–87a (Table 6 of Ref. [1]), consisting mainly of different homologous series, yield an average value of $\delta\sigma'$ equal to 0.00 ± 0.27 (excluding the deviant solute anthracene [#81a]).

Values of σ' are also affected by whether the solute is ionizable; i.e. an acid or a base. Thus, for ionizable solutes #46–67, average values of $\delta\sigma'$ were -0.67 ± 0.28 for the strong bases (#46–50), -0.75 ± 0.40 for the weak bases (#51–55), and -0.50 ± 0.35 for the weak acids (#56–67). While the scatter in values of σ' for each of these groups of solutes (SE equal $\pm0.3-0.4$ σ'-units) is greater than found for the neutral compounds of Fig. 2 (SE = 0.2),

it is apparent that acids and bases have σ' values that are significantly lower than values for neutral solutes, by an average of 0.6 units. There is not, however, a good correlation between values of $\delta\sigma'$ and the relative ionization of the solute molecule (see data of Table 8 of Ref. [2], ''average charge on solute molecule''): $\delta\sigma' = -0.52 - 0.19$[ionization]; $r = 0.21$, SE = 0.37.

Finally, there is some indication that increased molecular ''thickness'' (as opposed to length or width) moderately increases values of σ', mainly for the case of very ''thick'' molecules. Cyclohexane rings are ''puckered'', so that molecular ''thickness'' will be somewhat greater for these molecules vs. corresponding benzene derivatives. Similarly, biphenyl derivatives (especially those substituted in the 2-position) are nonplanar and even ''thicker''. However, neither of these two structural factors changes values of σ' significantly, presumably because the molecules are not ''thick'' enough to affect steric interaction. Thus, values of σ' differ but little for nitrocyclohexane (#70; 0.31) vs. nitrobenzene (#13; 0.32), or cyclohexanol (#5a; −0.34) vs. phenol (#82a; −0.18). Similarly, values of $\delta\sigma'$ for two steroids (#39, 40) are close to zero (0.02 ± 0.01). Likewise, the average value of $\delta\sigma'$ for the various biphenyls of Table 1 (#71–76) is also small: 0.05 ± 0.26. More significantly, the introduction of a substituent at the 2-position of biphenyl favors greater nonplanarity of the two rings, but does not lead to a consistent increase in σ': for biphenyls unsubstituted in the 2-position (#71, 73, 76), $\delta\sigma' = -0.01 \pm 0.23$ (1 SD); for biphenyls substituted in the 2-position (#72, 74, 75), $\delta\sigma' = 0.10 \pm 0.33$ (1 SD). On average, 2-substituted biphenyls have only modestly elevated values of σ' (by about 0.1 unit).

From Fig. 1, it can be seen that compounds #44 and #77–80 are each quite ''thick'' or ''3-dimensional''. Values of $\delta\sigma'$ for these compounds are summarized in Table 5: $\delta\sigma'$ (avg.) $= 0.48 \pm 0.26$. Thus, molecules that are quite thick appear to have generally larger values of σ', apart from molecular length (a cautionary note: the estimation of molecular length L as in Fig. 2 for the compounds of Table 5 is somewhat subjective, as are related values of $\delta\sigma'$). To summarize, σ' increases with greater molecular length and to a lesser extent with increased molecular ''thickness'', while acids and bases have significantly smaller values of σ' than predicted based on their lengths. That is, acids and bases exhibit less steric interaction with the column, other factors equal.

Table 5
Values of σ' and $\delta\sigma'$ for ''thicker'' solutes #44 and #77–80

Solute	σ'	L	$\delta\sigma'$
44. 5,5-Diphenylhydantoin	1.284	9	0.79
77. Diphenylbutyrolactone	0.735	9	0.24
78. Fluorescamine	1.047	11	0.24
79. Camphorquinone	0.422	6	0.39
80. Ferrocene	0.286	3[a]	0.72

[a] A ''common sense'' definition of L is substituted for the procedure of Fig. 2.

3.1.4. Term (ii): σ'S (steric selectivity): a proposed model

The above observations for σ' vs. solute structure suggest a similarity with retention vs. structure in size-exclusion chromatography (SEC [6]), namely decreased retention for increased molecular length. In the latter form of chromatography (illustrated in Fig. 4a, ''SEC''), retention is determined by the access of solute molecules to particle pores—longer molecules have larger hydrodynamic (''Stokes'') diameters d and are excluded from narrower pores, those with diameters less than d. However, the exclusion of long molecules from small pores by an SEC retention process does not prevent retention when the molecule is attracted to the stationary phase as in RP-LC [7]. In the latter case, long molecules with lengths greater than the pore diameter can still enter the pore (unlike SEC), much as a snake enters a narrow hole in the ground, even when the average length of the moving snake (its ''hydrodynamic diameter'') is longer than the diameter of the hole. Nevertheless, the RP-LC retention of long molecules in narrow pores should be reduced (but not eliminated) as a result of a greater decrease in entropy upon retention; i.e. from the *constraint* imposed on the conformation of the retained solute molecule by adjacent C_8 or C_{18} ligands. A similar, tentative explanation for steric interaction (based on the dependence of σ' on structure) is that the *spaces* between the alkyl ligands of the stationary phase provide the same restricted access to solute molecules that is provided in SEC by *pores* within the

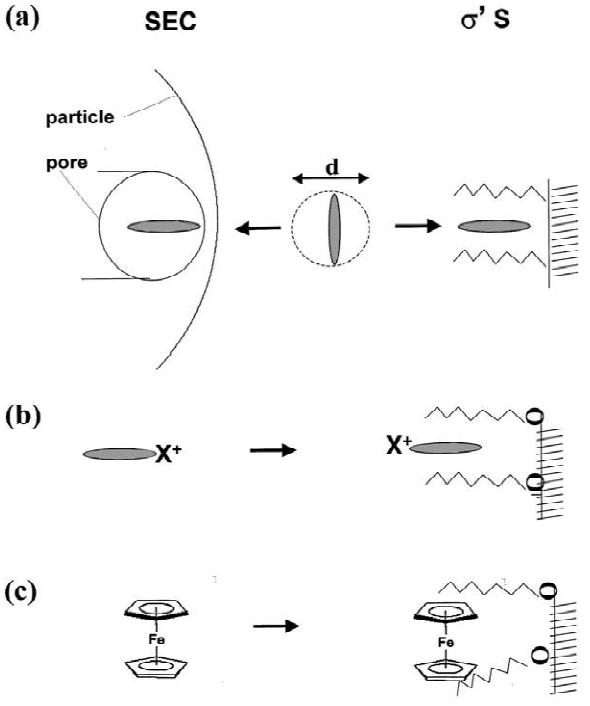

Fig. 4. SEC retention and the contribution of the $\sigma'\mathbf{S}$ term to RP-LC retention. (a) Comparison of SEC and RP-LC retention with respect to solute molecular length; (b) shortening of the effective molecular length in RP-LC retention; (c) partial exclusion of ''thick'' molecules from an RP-LC stationary phase. See text for details.

particle (see Fig. 4a, ''$\sigma'\mathbf{S}$''). An important distinction between these two retention processes in Fig. 4a is that particle pores are rigid, whereas the spaces between ligands are not. Also, SEC retention does not involve an attraction between the solute molecule and the stationary phase, whereas RP-LC does.

Acidic or basic solutes possess a hydrophilic group that resists penetration into the hydrophobic stationary phase, resulting in a decrease in steric interaction and smaller values of σ' (Fig. 4b). Similar to the case for acids and bases, alcohols (solutes #1a–4a, 43a–45a) with a hydrophilic end-group have an average value of $\delta\sigma'$ that is negative ($\delta\sigma' = -0.4\pm0.2$ [1 SD]).

Fig. 4c illustrates the role of solute ''thickness'' in RP-LC retention. Molecules that are sufficiently ''thick'' will experience greater resistance (more constraint) in penetrating the stationary phase, and this resistance will increase for less penetrable stationary phases. To summarize, steric selectivity and the $\sigma'\mathbf{S}$ term of Eq. (1) can be described in terms of the interaction of less-rigid solute molecules with less-rigid (''monomeric'') stationary phases. Solute molecules whose size and shape results in a greater constraint by surrounding alkyl ligands, will experience greater steric interaction effects, resulting in larger values of σ'. ''Shape selectivity'', on the other hand, involves more rigid solute molecules and stationary phases, with consequent differences when compared with ''steric selectivity''. Quantitative differences between ''steric'' and ''shape'' selectivity are demonstrated in the immediately following section, where these two kinds of column selectivity are compared experimentally.

3.1.5. ''Steric'' vs. ''shape'' selectivity

''Shape selectivity'' can be characterized by a widely used test [8] that measures the separation factor $\alpha_{\mathrm{TBN/BaP}}$ for the two solutes tetrabenzonaphthalene (TBN) and benzo(a)pyrene (BaP). Larger values of $\alpha_{\mathrm{TBN/BaP}}$ for a column mean less shape selectivity and a less rigid stationary phase. Since values of **S** also increase for reduced steric interaction, if ''shape'' and steric selectivity reflect a similar process, values of $\alpha_{\mathrm{TBN/BaP}}$ should increase with **S**. Table 6 provides experimental data to test the similarity of steric and ''shape'' selectivity (values of **S** and $\alpha_{\mathrm{TBN/BaP}}$ for columns #1–8, 10, 13 and 14). For these data, a linear regression of values of **S** vs.

Table 6
Data for a comparison of ''shape'' and ''steric'' selectivity

Column #[a]	$\alpha_{\mathrm{TBN/BaP}}$[b]	**S**[c]
1. Inertsil	2.00	−0.013
2. Symmetry	1.52	−0.059
3. SB-100	1.86	0.021
4. SB-90	1.81	0.042
5. SB-300	1.52	0.043
6. Eclipse	1.85	−0.008
7 YMC 15	1.89	0.002
8 YMC 16	1.87	−0.008
10. Discovery	1.55	−0.023
13. (Vydac 201TP)	0.65	0.031
14. (Hypersil ''Green'' PAH)	0.80	0.026

[a] Columns described in Table 5 of Ref. [1] and Experimental.
[b] Values measured as in Ref. [8].
[c] Values from Ref. [1] for columns #1–8 and 10; values for columns #13 and 14 determined as described in the text.

$\alpha_{TBN/BaP}$ gives the following result: $\mathbf{S} = 0.036 - 0.020\ \alpha_{TBN/BaP}$; $r = 0.29$, SE = 0.031. The correlation is quite weak, and (more important) in the wrong direction. A significant difference between shape and steric selectivity is strongly indicated by the data of Table 6.

*3.1.6. Term (iii): β'**A** (hydrogen bonding of basic solutes to silanols)*

Hydrogen bonding of neutral proton acceptors to stationary phase donors (assumed to be silanols) has been noted as a significant contributor to column selectivity in several studies that have been interpreted in terms of the Abraham–Carr solvation equation [9]. The relative retention of the basic solute caffeine vs. that of the acidic solute phenol has also been used as a measure of stationary phase donor strength [10].

The N,N-dialkyl amides of Table 7 of Ref. [1] have large values of β', with other solutes exhibiting generally smaller values. These amides (≠10a–14a) have similar acceptor strengths β_2 in solution (0.74–0.80), but values of β' vary markedly (0.26–0.99) with the degree of steric hindrance around the nitrogen (Table 7). Thus, for these solutes, there is only a poor correlation of values of β' vs. β_2 (but in the right direction): $\beta' = -1.2 + 2.4\ \beta_2$, $r = 0.41$, SE = 0.36. Intramolecular steric hindrance of an acceptor functional group X seems to be much more important in RP-LC retention than for hydrogen bonding in solution; it appears that the C_{18} (or C_8) ligands that surround a silanol group can enhance steric hindrance between the silanol and a hydrogen-bond acceptor group X. Other workers have also noted that steric hindrance around hydrogen-bonding sites can be of greater importance in the RP-LC stationary phase than in solution [11].

The effect of steric hindrance on values of β' (as above) should be similar (but not exactly the same) for aliphatic homologs with comparable alkyl substitution. We have therefore chosen various butyl (or diethyl) substituted homologs C_4–X and related compounds from Table 7 of Ref. [1] for a comparison of values of β' vs. β_2 (solute acceptor strength in solution). Fig. 5a compares values of β'

Table 7
Values of β' and acceptor strength β_2 for various amide solutes

Solute	β' [a]	β_2 [b]
10a. *N,N*-Dimethylformamide	0.89	0.74
13a. *N,N*-Dimethylacetamide	0.99	0.78
11a. *N,N*-Diethylformamide	0.49	0.76
14a. *N,N*-Diethylacetamide	0.53	0.78
12a. *N,N*-Dibutylformamide	0.20	0.80
47a. *N*-Benzylformamide	0.10	0.63

[a] Data of Table 7 of Ref. [1].
[b] Hydrogen-bond acceptor strength; values from hydrogen bonding in solution [11].

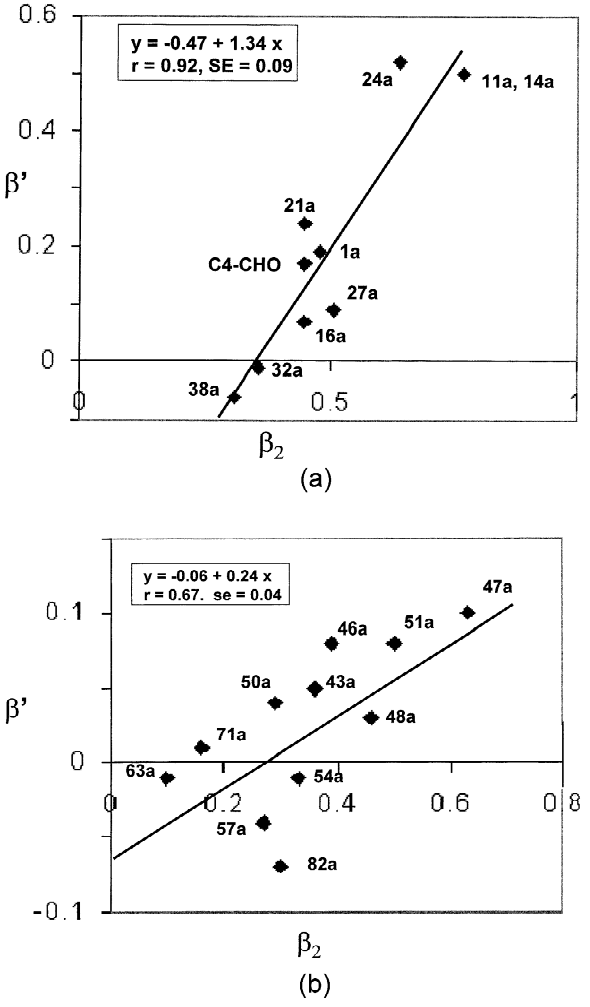

Fig. 5. Correlation of the solute parameter β' with solution values of β_2 (hydrogen bond acceptor strength from Ref. [12]). (a) Aliphatic solutes with comparable inter-molecular hindrance of the acceptor group (C_4 or diethyl derivatives); (b) aromatic solutes.

for the latter solutes with their corresponding acceptor values β_2 from Ref. [12]. A significant correlation is observed ($r=0.92$), as expected. The scatter of data points in this plot is likely due to differences in steric hindrance for individual compounds; our selection of the C_4–X derivatives in an attempt to equalize these steric effects is at best a crude approximation. A similar plot is shown in Fig. 5b for corresponding aromatic solutes. As indicated by Fig. 5 and the data of Table 7, values of β' are generally much smaller for aromatic vs. aliphatic solutes. Smaller β' values for aromatics are likely due to (a) the greater steric hindrance around the acceptor group X and (b) electron transfer from X to the aromatic ring (thereby decreasing the basicity of X). The correlations of Fig. 5 are consistent with our belief that the $\beta'\mathbf{A}$ term of Eq. (1) arises from hydrogen bonding between proton acceptor solutes and non-ionized silanols (proton donors) in the stationary phase.

3.1.7. Term (iv): $\alpha'\mathbf{B}$ (hydrogen bonding of acidic solutes to basic groups within the stationary phase?)

This possible contribution to RP-LC column selectivity has been reported to be less important [8,10] and has received only occasional attention in the literature [13]. Assuming the presence of proton-acceptor groups in the stationary phase (e.g. silanols, siloxane groups, preferentially sorbed organic solvent), hydrogen bonding of these groups with proton-donor solutes would be anticipated, similar to the hydrogen bonding of basic solutes by silanols. Large values of α' are associated primarily with weak acids (#56–67; $0.36 \leq \alpha' \leq 3.10$), suggesting that these compounds are hydrogen bonding to a basic group in the stationary phase. However, a further examination of α' values for other donor solutes contradicts this hypothesis. Table 8 compares values of α' for different groups of donor solutes with their hydrogen-donor values α_2^H in solution (last column of Table 8). Whereas donor strength in solution ($0.32 \leq \alpha_2^H \leq 0.6$) suggests that alcohols and phenols should have large values of α', this is not the case ($0.1 \leq \alpha' \leq 0.2$). Nor can the non-correlation of values of α' and α_2^H be readily explained by means of steric hindrance, as in the example of Table 7 for hydrogen bonding between acceptor solute molecules and column silanols.

There are other difficulties with hydrogen-bonding as an explanation of the $\alpha'\mathbf{B}$ term of Eq. (1). As discussed below, end-capping the stationary phase is expected to greatly reduce the number or accessibility of silanol or siloxane groups as potential stationary-phase donor groups, yet end-capping has little effect on values of **B**. Apart from solvent molecules in the stationary phase, this result leaves no other stationary phase acceptor group as a reasonable candidate. On the other hand, if solvent molecules in the stationary phase serve as proton acceptors, we can expect that the concentration of ACN should increase with **H**, while the concentration of water should decrease. Therefore, if ACN is serving as an acceptor, there should be a positive correlation between **B** and **H**, whereas if water is providing the acceptor group, there should be an inverse correlation of values of **B** vs. **H**. We in fact observe an inverse correlation between **B** and **H** ($\mathbf{B} = -0.70 - 0.70\ \mathbf{H}$; $r=0.95$, SE = 0.01), suggesting that sorbed water could be responsible for the $\alpha'\mathbf{B}$ term. Also puzzling, however, is that if the $\alpha'\mathbf{B}$ term is due to hydrogen bonding, values of α' for an *n*-alkylben-

Table 8
The solute parameter α' as a function of solute donor strength

Solute type	Solutes	Avg. α'	SD	α_2^H [14]
Neutral non-donors	#1–45 except R-OH	0.02	0.12	0.00
Alcohols	#18–20, 39, 40	0.10	0.17	0.32–0.39
Phenols	#21, 22, 24–26, 75, 76, 82, 83, 88[a]	0.17	0.09	0.60
Activated-OH	#23, 87 (vicinal diol)	0.52	0.13	
	#42	0.58		
Weak acids	#56–58, 60–63, 65–67	0.88	0.33	0.59
Strong acids	#59, 64	2.28	1.16	

[a] Solutes #84 and 86 not included, because of poor agreement with Eq. (1).

zoic acid should not change when the alkyl group is lengthened; in fact, values of α' increase significantly for higher homologs of the alkylbenzoic acids (see following discussion). Finally, it should be noted that experimental values of $\alpha'\mathbf{B}$ (i.e. Δ values; see discussion of steps #5 and 6 of Table 4 of Ref. [1]) are not as highly correlated among those solutes (#56–58, 60–65) used for the calculation of **B** values ($r = 0.92$), compared to similar correlations of the other terms of Eq. (1) ($\kappa'\mathbf{C}$, $r = 1.00$; $\sigma'\mathbf{S}$, $r = 0.96$; $\beta'\mathbf{A}$, $r = 0.95$). This suggests, as examined further in Appendix A that the $\alpha'\mathbf{B}$ term of Eq. (1) may be the result of more than one solute–column interaction.

Other possible explanations of the $\alpha'\mathbf{B}$ term include anion exchange with some (unknown) cationic group in the stationary phase, or ion repulsion of negatively charged solutes from the negatively charged column. The present study provides no better support for either of these possibilities than for hydrogen bonding. We can say only that columns with larger values of **B** provide preferential retention of acidic solutes, with a tendency toward stronger retention (larger values of α') for more highly ionized acids; e.g. solutes #59 and 63, with average α' values of 2.28, vs. an average $\alpha' = 0.88$ for the weaker acids of Table 8 (see Table 7 of Ref. [2] for the relative ionization of acidic solutes #56–67).

The possible interaction of acidic solutes with trace metal impurities in the stationary phase as a contribution of the $\alpha'\mathbf{B}$ term was suggested by two reviewers. However, all of these columns are based on highly pure silica, with relatively small concentrations of trace metal [1]. Furthermore, the presence of trace metals in the stationary phase generally results in peak tailing, because of slow sorption–desorption kinetics of those solutes which interact with the metal. In the present study, with a few exceptions (notably column #2; for columns #1 and 3–10, the tailing factor for butybenzoic acid was <1.5), acidic solutes were characterized by symmetrical peaks and normal plate numbers. Furthermore, there was no correlation between acidic-solute band tailing and values of **A**.

3.1.8. Term (v): $\kappa\mathbf{C}$ (ion-exchange retention of protonated bases by ionized silanols)

The RP-LC separation of protonated bases by means of alkyl-silica columns has received considerable attention in the literature, primarily because of increased peak tailing for these compounds [15]. Considerable evidence has been reported [16] that suggests this problem is related to the interaction of protonated bases with ionized silanols in the stationary phase; i.e. an ion-exchange process. An interaction between protonated bases and ionized silanols should also result in the increased retention of these solutes.

Table 9 summarizes values of the parameter κ' as a function of solute functionality (for a mobile phase pH = 2.8). Neutral solutes exhibit κ'-values close to zero, while strong (fully protonated) bases have large values of κ' ($\kappa' = 1.00 \pm 0.17$). The *n*-alkylanilines (partially protonated weak bases) have small, but significant values of κ' ($\kappa' = 0.09 \pm 0.00$), while κ'-values for the pyridines resemble those of neutral solutes ($\kappa' = -0.01 \pm 0.00$). Finally, acidic solutes tend to have negative values of κ' ($\kappa' = -0.05 \pm 0.14$), with more negative values for the more highly ionized acids #59 and 64 (cf. Table 9 footnote). The data of Table 9 therefore support the origin of the $\kappa'\mathbf{C}$ term as an ionic interaction (positive for cations, negative for anions) between charged solutes and ionized silanols in the stationary phase. Values of κ' for the ionizable solutes #46–67 (acids and bases) are highly correlated with the estimated average charge on these solute molecules (values of Table 7 of Ref. [2]): $\kappa' = 0.01 + 0.90$ (avg. charge); $r = 0.94$, SE = 0.16 (Fig. 6). The latter correlation implies that any ionic repulsion of ionized acids from the negatively charged stationary phase is accounted for by the $\kappa'\mathbf{C}$ term of Eq. (1); therefore, there is no need of other terms in Eq. (1)

Table 9
Values of κ' for different solute groups

Solute group	Solutes	κ'	
		Avg.	SD
Neutrals	#1–45	−0.01	0.02
Strong bases	#46–50	1.00	0.17
4-*n*-Alkylanilines	#51–53	0.09	0.00
N-Alkylanilines	#54, 90	0.06	0.05
Pyridines	#55, 89	−0.01	0.00
Acids[a]	#56–67	−0.05	0.14

[a] The two, more highly ionized acids (#59, 64) have $\kappa' = -0.31 \pm 0.16$.

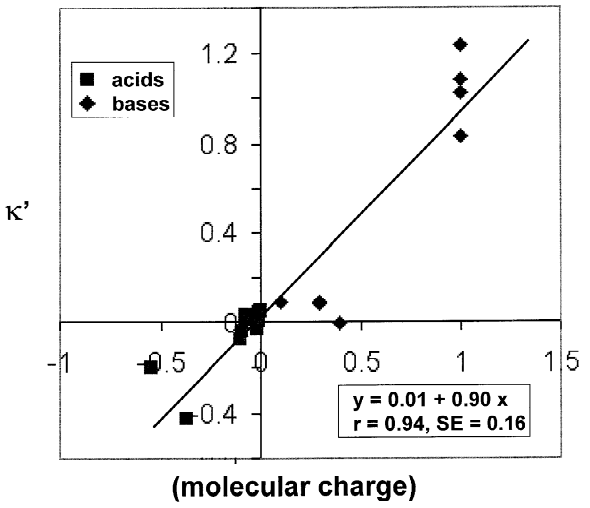

Fig. 6. Correlation of values of the parameter κ' for basic and acidic solutes (≠46–67) with the average charge on the solute molecule (as a result of varying degrees of ionization).

(e.g. α'**B**) to account for any contributions to retention as a result of ion repulsion.

3.2. Homolog values of the solute parameters η', σ', etc.

The addition of a methylene group to an alkyl substituent in the solute molecule results in an approximately constant change in values of each solute parameter per added methylene (Fig. 4 of Ref. [1]), the so-called ''methylene increment''. If our present picture of the origin of the various terms of Eq. (1) is correct, values of the methylene increment for η', σ', etc. should be qualitatively predictable. Thus, we expect that hydrophobicity and values of η' will increase as carbon number n increases in a homologous series. Similarly, since σ' increases with the length of the solute molecule, σ' should increase with increasing n. In the case of parameters β', α' and κ', the interactions responsible for these solute parameters are presumed to involve specific groups in the solute molecule and the stationary phase (i and j, respectively). The enlargement of an alkyl chain attached to a distant part of the solute molecule (so as to avoid steric hindrance in the interaction of i with j) should not affect the i–j interaction or values of the related solute parameters. Therefore, our initial expectation was that the methylene increment values for β', α' and κ' should equal zero within experimental error.

Table 10 summarizes average values of the methylene increment for each solute parameter and different groups of solutes. The solutes of Table 10 are divided into three sets: neutrals, acids plus bases, and (discussed separately) aromatic alcohols. In the case of the hydrophobicity parameter η', the methyl-

Table 10
Incremental contribution of a homolog methylene group to various solute parameters η', σ', etc.

Solutes	Effect of an added methylene group on parameter				
	η'	σ'	β'	α'	κ'
Non-polar neutrals					
≠1–3, 5, 6—avg.	0.22	0.12	−0.01	0.03	0.01
—SD	0.01	0.03	0.00	0.03	0.01
Polar neutrals					
≠1a–3a, 6a–9a, 16a–18a, 26a–29a, 31a–36a, 37a–39a—avg.	0.24	0.18	−0.02		
—SD	0.02	0.07	0.05		
Acids and bases					
≠51–53, 60–62—avg.	0.23	0.01	−0.01	0.16	0.00
—SD	0.01	0.03	0.00	0.01	0.01
Aromatic alcohols[a]—avg.	0.16	0.09	0.01	0.12	0.00
≠18–20—SD	0.03	0.02	0.00	0.01	0.00

[a] α-Hydroxy-ψ-phenylalkanes.

ene increment equals 0.22–0.24 for neutrals, acids and bases. As expected, there is a regular increase in η' as n increases. Similarly, the methylene increment for σ' is 0.12–0.18 for neutral solutes, and again this is expected. On the other hand, the σ' methylene increment is close to zero for acids and bases—which may be related to our proposal that these solutes are retained primarily near the outer surface of the stationary phase (but is still surprising).

The methylene increments for β' and κ' are generally small (0.00 to −0.02), as expected. Small values of the methylene increment for α' are observed for neutral compounds, but not for acids or bases (methylene increment = 0.16). The latter observation is unexpected and suggests that the attribution of the α'**B** term to hydrogen bonding of acidic solutes with a basic stationary phase group is not correct. Methylene increments for the aromatic alcohols (Table 10) are equal to about 2/3 of values for ''normal'' homologs. Interestingly, the average value of the aromatic-alcohol methylene-increments for α' (0.12) resembles that for ionic solutes (0.16) more closely than for the alkyl benzenes (0.03).

3.3. Column parameters and the origin of terms i–v of Eq. (1)

3.3.1. Methylene selectivity and values of H

Values of so-called ''methylene selectivity'' α_{Me} have been used previously as a measure of column hydrophobicity [10]; α_{Me} equals the ratio of k-values for adjacent homologs (n-butyl and n-propyl benzenes in the present case). As expected, there is a reasonable correlation of **H** vs. log α_{Me},: **H** = −0.298 + 5.62 log α_{Me}; $r = 0.933$; SE = 0.026 (columns ≠1–10, 1a–5a). However, since values of **S** also increase with increasing homolog number, this suggests that log α_{Me} should be a function of both **S** and **H**. A linear regression of values of log α_{Me} vs. values of **H** and **S** yields log $\alpha_{Me} = 0.053 + 0.178$ **H** + 0.083 **S**, with $r = 0.958$ and SD = 0.003 (vs. an experimental uncertainty in values of log α equal ±0.002 [1]; i.e. a very good fit).

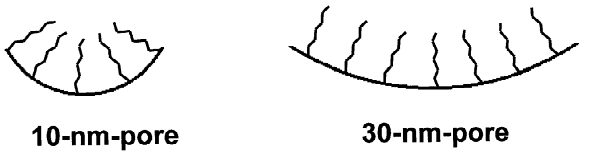

Fig. 7. Illustration of the greater crowding of ligand groups for columns with narrow pores (a) vs. columns with wide pores (b). See text for details.

3.3.2. Hydrophobic and steric selectivity vs. column characteristics

Table 11 summarizes values of **H** and **S** for four pairs of columns that each differ in one major respect: ligand length, ligand concentration, ligand type (dimethyl- vs. di-*i*-butyl-silane), or pore diameter. Values of **H** measure column hydrophobicity, so it is unsurprising to see **H** increasing with increased ligand length and concentration. Columns bonded with a di-*i*-butyl silane (StableBond) have a lower ligand concentration than conventional (dimethyl silane) C_{18} packings, so lower values of **H** for the StableBond column (≠1a) vs. the conventional column ≠2a are also expected (and found). **H** decreases as pore diameter increases (when ligand concentration ($\mu mol/m^2$) remains constant), apparently because a wider pore leads to less bunching of the ends of the ligands (Fig. 7) and an effective lowering of the average ligand concentration ($\mu mol/ml$) within the volume comprising the stationary phase [17].

Table 11
Dependence on column characteristics of the column parameters **H** and **S**

	Ligand length[a]		Ligand coverage[b]		Ligand type[c]		Pore diameter[d]	
	C_{18}	C_8	100%	90%	di-C_1	di-iC_4	10 nm	30 nm
H	0.963	0.854	0.998	0.967	1.065	0.990	0.998	0.894
S	−0.006	0.017	0.021	0.042	−0.056	0.012	0.021	0.043

[a] Columns ≠3a and 4a.
[b] Columns ≠3 and 4.
[c] Columns ≠1a (dimethyl silane) and 2a (di-*i*-butyl silane).
[d] Columns ≠3 and 5; note that ligand coverage is the same for these two columns (2.08–2.09 $\mu mol/m^2$).

Resistance to the penetration of solute molecules into the stationary phase (stationary phase impedance) leads to decreased retention. Therefore, smaller values of **S** signify a stationary phase that is more resistant to penetration (see above). The data of Table 11 suggest that stationary phase impedance is increased for longer ligands and higher ligand concentrations, which again is reasonable. Likewise, an increase in pore diameter (equivalent to decreased ligand concentration per unit volume; Fig. 7) results in an increase in **S**, or less restriction to penetration by the solute into the stationary phase (as expected).

*3.3.3. Effect of end-capping on values of **A**, **B** and **C***

If silanols are involved in the solute–column interactions that determine values of a given column parameter (i.e., **A**, **B** or **C**), end-capping should reduce the value of that parameter. Two Symmetry C_{18} columns (≠11, 12) that differed only in the presence or absence of end-capping (same silica, same bonding batch) were obtained as a gift from Waters Corp. Based on retention data for a small number of appropriate test solutes, the following *changes* in column parameters were determined as a result of end-capping: **H**, 0.01; **S**, −0.03; **A**, −0.34; **B**, 0.01; **C**, −0.24. There is a large decrease in **A** and **C** as a result of end-capping (and removal of silanols), confirming a role for silanols in the β'**A** and κ'**C** terms of Eq. (1). The effect of end-capping on **B** is negligible, which suggests that silanols are *not* involved in the α'**B** term of Eq. (1). It should also be noted that end-capping both removes silanols *and* covers the surface below the trimethylsilyl (TMS) end-capping groups. Thus, if potential donor or acceptor groups other than silanols were part of the silica surface (e.g. siloxanes), they could also be shielded from hydrogen-bonding solutes by the TMS layer.

Similar, less conclusive conclusions as above can be drawn from values of **A**, **B** and **C** for columns ≠1–10 (data in Table 5 of Ref. [1]). Thus, columns ≠3–5 are not end-capped, while remaining columns ≠1, 2, 6–10 are. Values of **A** are uniformly higher for the end-capped columns ($0.11 \leq \mathbf{A} \leq 0.27$) compared to the non-end-capped columns ($-0.14 \leq \mathbf{A} \leq 0.01$). Values of **B** are little different for end-capped ($-0.03 \leq \mathbf{B} \leq 0.02$) vs. non-end-capped ($0.01 \leq \mathbf{B} \leq 0.08$) columns. Values of **C** are generally lower for the end-capped columns ($-0.35 \leq \mathbf{C} \leq 0.04$), compared to the non-end-capped columns ($0.05 \leq \mathbf{C} \leq 0.22$), except in the case of end-capped column ≠10 (**C** = 0.18). Possibly column ≠10 is based on a more acidic silica and/or is less fully end-capped. An increase in mobile phase pH results in a much larger increase in **C** for non-end-capped columns (0.73 to 0.76) vs. the case for end-capped columns (−0.05 to 0.16) (see Table 10 of Ref. [2]), as expected. That is, as mobile phase pH approaches a value of 7, all silica-base columns will experience some ionization of the silanols (silica $pK_a \approx 7$ [19]), but ionized silanols are less accessible for end-capped columns.

3.3.4. Comparison of column selectivity parameters for Eq. (1) vs. the solvation equation [9,12]

Another means for testing the nature of the column parameters **H**, **S**, etc. of Eq. (1) is to compare these parameters with corresponding parameters from the application of the solvation equation (Eq. (8) [11]) to the same solutes and columns (data reported in Ref. [18]):

$$\log k = C_1 + rR_2 + s\pi_2^{\mathrm{H}} + a\sum\alpha_2^{\mathrm{H}} + b\sum\beta_2 + \nu V_x \quad (8)$$

Eq. (8) has been discussed in part I [1], where the column parameters for this relationship are the quantities *r*, *s*, *a*, *b* and ν. Because of the fundamental nature of Eq. (8) (as confirmed by a large number of different applications), these latter column parameters can be assigned to specific solute–column interactions with relatively little ambiguity. Based on our preliminary interpretation of terms (*i*), (*iii*) and (*iv*) of Eq. (1) in terms of certain contributions to retention, positive correlations between the column parameters **H**, **A** and **B** and their counterparts in Eq. (8) are therefore expected.

For a fixed mobile phase, the parameter ν of Eq. (8) should vary in accordance with the free energy for insertion of the retained solute molecule. We therefore expect a correlation between values of ν and **H**; this is indeed the case: $\mathbf{H} = -0.44 + 0.90\ \nu$; $r = 0.95$, SE = 0.014. Similarly, if the parameter *b* of Eq. (8) corresponds to column hydrogen-bond acidi-

ty, b should correlate with the column parameter **A**. A marginal correlation is found, **A** increasing with b as expected: $\mathbf{A} = 2.29 + 1.31\ b$, $r = 0.58$, SE = 0.14. The latter, relatively poor correlation likely reflects differences between values of β_2 in solution and in the RP-LC stationary phase (see above discussion of Table 7). Finally, the parameter a of Eq. (8) corresponds to column hydrogen-bond basicity, which correlates with the column parameter **B**: $\mathbf{B} = 0.52 + 1.04\ a$, $r = 0.81$, SE = 0.02. Given the more approximate nature of Eq. (8), we conclude that the latter correlations are consistent with our interpretation of the origin of terms $\eta'\mathbf{H}$ and $\beta'\mathbf{A}$.

4. Conclusions

The accuracy (±1–2% in k) of Eq. (1)

$$\log \alpha = \log (k/k_{\text{ref}}) = \underset{(i)}{\eta'\mathbf{H}} + \underset{(ii)}{\sigma'\mathbf{S}} + \underset{(iii)}{\beta'\mathbf{A}} + \underset{(iv)}{\alpha'\mathbf{B}} + \underset{(v)}{\kappa'\mathbf{C}} \quad (1)$$

for its application to a broad range of solute structures (151 different compounds; Refs. [1,2] and Table 1) suggests that all important contributions to column selectivity have been identified for the case of monomeric C_8 or C_{18} silica columns. Eq. (1) therefore provides a basis for the study of other column types (e.g. columns with embedded polar groups, cyano or phenyl columns, etc.) as a means of uncovering any additional contributions to column selectivity. Work in this direction is underway in our laboratory.

Terms i–v of Eq. (1) and their corresponding solute and column parameters can be related to previously described solute–column interactions, but with interesting differences in some cases. Term (i) (η [illegible]

increases for columns with increased ligand length and concentration, and with decreased pore diameter.

Term (ii) ($\sigma'\mathbf{S}$) appears to reflect steric impedance to insertion of the solute molecule into the stationary phase. The solute parameter σ' increases with molecular length ($r = 0.90$) and to a lesser extent with molecular ''thickness''; σ' decreases for molecules substituted by hydrophilic groups such as –OH, –COOH, $-NH_2$ or $-NR_2H^+$. A tentative explanation for term (ii) is that it reflects the entropy increase (i.e. constraint of the solute molecule) that attends insertion of the solute molecule between the ligands of the alkyl-silica stationary phase. Larger, more ''bulky'' solute molecules are entropically more difficult to insert into the stationary phase and have larger values of σ'. Molecules with hydrophilic substituents will be (on average) less completely inserted into the stationary phase and therefore experience less steric interaction. See the simplified illustrations of Fig. 4.

Note that values of $\sigma'\mathbf{S}$ are *relative* values, obtained with respect to average values of $\sigma'\mathbf{S}$ for solutes #1–67 and columns #1–10. A column in which there is no resistance to solute penetration would have a larger value of **S** than any of the present columns. Values of **S** measure the resistance by the stationary phase to penetration by a solute molecule, larger **S** meaning *less* resistance. As expected, **S** decreases with increased ligand length and concentration, and with decreased pore diameter. Values of σ' are also relative to the solute ethylbenzene; ''absolute'' values of σ' are probably larger by about a unit, compared to values given here and in Ref. [1].

Values of **S** do not correlate with a common measure of ''shape selectivity'' ($\alpha_{\text{TBN/BaP}}$), suggesting that these two examples of restricted retention (''shape'' *vs.* ''steric'' selectivity) are significantly

solute or stationary phase rigidity. However, it appears to us that steric interactions may be a more important contribution to column selectivity in most RP-LC separations.

Term (*iii*) (β'**A**) appears to be the result of hydrogen bonding between solute acceptors and stationary phase silanols. Values of β' decrease sharply with increasing intramolecular steric hindrance of the solute acceptor group. This steric hindrance to hydrogen bonding of acceptor solutes by column silanols is much more pronounced than for corresponding hydrogen bonding in solution. For aliphatic solutes with similar hindrance of the acceptor group, the solute parameter β' correlates well with compound acceptor strength β_2 in solution ($r=0.92$). The column parameter **A** correlates with stationary phase donor strength *b* from the solvation equation ($r=0.58$); the latter, poorer correlation is likely due to variable steric hindrance that is not recognized by the solvation equation. End-capping results in a marked decrease in **A**, suggesting that column donor strength is due to unreacted silanol groups.

Term (*iv*) (α'**B**) appears at first glance to involve hydrogen bonding between a donor solute and an unidentified acceptor group in the stationary phase. Thus, large values of α' (0.4–3.1) occur for various acidic solutes. Also, the column parameter **B** correlates with stationary phase acceptor strength *a* from the solvation equation ($r=0.81$). However, other strong donors (alcohols, phenols) have generally smaller values of α' (0.0–0.4), which implies that hydrogen bonding is not the sole explanation for the α'**B** term of Eq. (1). End-capping does not result in a change in values of **B**, suggesting that neither silanols or siloxane groups are responsible for the solute–column interaction(s) responsible for the α'**B** term. However, an inverse correlation of values of **B** and **H** ($r=0.95$) supports a role for sorbed water from the mobile phase as the source of column hydrogen-bond basicity.

Term (*v*) (κ'**C**) appears to arise from the electrostatic interaction of charged solute molecules with ionized (negatively charged) silanols. Fully protonated bases have large, positive values of κ', partially protonated bases have small, positive values, and partially ionized acids have small negative values. There is a good correlation ($r=0.94$) between values of κ' and the charge on the solute molecule (mobile phase pH = 2.80). Values of **C** decrease sharply when a column is end-capped, corresponding to a reduction in silanols. The retention of cationic solutes and **C**-values for a given column vary with mobile phase pH and buffer concentration, as expected for the ionic interaction of these solute cations with ionized silanols in the stationary phase.

The widely-applied solvation equation [9,12] has been used to predict values of *k* and characterize column selectivity, similar to the application of Eq. (1). However, whereas the accuracy of Eq. (1) for predictions of *k* is ±1–2%, the corresponding accuracy of the solvation equation is typically ±10–20%; i.e. about an order of magnitude less reliable. Data presented in this series of papers suggests that the lower accuracy of the solvation equation can be attributed in part to its failure to recognize that typical RP-LC stationary phases restrict or constrain the solute molecule, by limiting possible conformations of the retained molecule. Solutions (e.g. the mobile phase) are homogeneous, and therefore do not constrain the solute molecule in this way. As a result, the shape and polarity of the solute molecule can affect its ease of insertion into the stationary phase (σ'**S** term). Furthermore, a constraining stationary phase can cause the interaction of solute acceptors with column silanols to be much more sensitive to steric hindrance around the solute acceptor group (β'**A** term). Consequently, solute acceptor strength as determined in solution (β_2) is only roughly related to acceptor strength in the stationary phase (β').

Because the solvation equation assumes constant values of the various solute parameters, this equation has proved to be approximately applicable to a very wide range of physicochemical systems—not just chromatography. Furthermore by its use of chemically well-defined parameters such as those related to hydrogen bond donor acidity and hydrogen bond acceptor basicity the various fitting coefficients are readily chemically interpretable. However, what the solvation equation gains in terms of its universality, it loses in terms of precision. By recognizing that solute parameters are somewhat system specific, especially in RP-LC, Eq. (1) offers greater potential accuracy, but at the expense of being applicable to only one narrowly-limited system.

Acknowledgements

Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose. See also Acknowledgements in part I [1]. For Glossary of Terms, see part I [1].

Appendix A. Evidence for multiple contributions to the α'B term of Eq. (1)

In the derivation of Eq. (1) (see Table 4 of Ref. [1]), values of log α were first corrected for hydrophobic interactions, and residual contributions Δ_{ij} of the column to retention were calculated for solutes i and columns j. It was found that values of Δ_{ij} for certain solutes were highly correlated (see examples of Fig. 3 of Ref. [1]). On the basis of these correlations, different groups of ''similar'' solutes could be defined; e.g. Δ_{ij} values for all of the strong bases (#46–50) were highly correlated and assumed to arise from a single contribution to column selectivity (κ'**C**). The correlation of Δ_{ij} values for solutes #46–50 are illustrated in Table A.1a. Because of the uniformly high correlation of these Δ_{ij} values for the strong bases (avg. $r=0.998$), it appears that these Δ_{ij} values are indeed the result of a single solute–column interaction (ionic interaction of cationic solutes with the anionic column).

Correlations as in Table A.1a for the case of other solute groups (and especially the weak acids, #56–67) and solute–column interactions are generally weaker: steric interaction (σ'**S**; #32–40, 43, 44), $r=0.96$; acceptor solute/donor column hydrogen bonding (β'**A**; #1a, 4a–6a, 10a, 11a, 13a, 14a, 21a, 24a–26a), $r=0.95$; donor solute/acceptor column

Table A.1
Correlation of Δ values for solutes of related structure

Solute	Correlation coefficient r for indicated solute pairs											
	#46	#47	#48	#49	#50							
(a) Strong bases (#46–50)												
#46	1.000	0.999	0.996	1.000	0.994							
#47	0.999	1.000	0.998	0.999	0.997							
#48	0.996	0.998	1.000	0.996	0.998							
#49	1.000	0.999	0.996	1.000	0.993							
#50	0.994	0.997	0.998	0.993	1.000							
(b) Weak acids (#56–67)[a]												
	#56	#57	#58	#60	#61	#62	#63	#66	#67	#59	#64	#65
#56	**1**	**0.99**	**0.99**	**0.89**	**0.88**	**0.87**	**0.98**	**0.89**	**0.87**	0.78	0.8	0.96
#57	**0.99**	**1**	**0.99**	**0.94**	**0.94**	**0.93**	**0.97**	**0.94**	**0.9**	0.72	0.75	0.95
#58	**0.99**	**0.99**	**1**	**0.89**	**0.88**	**0.88**	**0.94**	**0.91**	**0.88**	0.67	0.71	0.91
#60	**0.89**	**0.94**	**0.89**	**1**	**1**	**0.99**	**0.92**	**0.92**	**0.88**	0.62	0.63	0.89
#61	**0.88**	**0.94**	**0.88**	**1**	**1**	**1**	**0.91**	**0.92**	**0.88**	0.62	0.63	0.89
#62	**0.87**	**0.93**	**0.88**	**0.99**	**1**	**1**	**0.91**	**0.92**	**0.86**	0.6	0.62	0.88
#63	**0.98**	**0.97**	**0.94**	**0.92**	**0.91**	**0.91**	**1**	**0.89**	**0.87**	0.84	0.85	1
#66	**0.89**	**0.94**	**0.91**	**0.92**	**0.92**	**0.92**	**0.89**	**1**	**0.91**	0.59	0.71	0.85
#67	**0.90**	**0.88**	**0.88**	**0.88**	**0.86**	**0.87**	**0.91**	**0.81**	**1**	0.61	0.64	0.85
#59	0.78	0.72	0.67	0.62	0.62	0.6	0.84	0.59	0.61	**1**	**0.95**	**0.88**
#64	0.8	0.75	0.71	0.63	0.63	0.62	0.85	0.71	0.64	**0.95**	**1**	**0.87**
#65	0.96	0.95	0.91	0.89	0.89	0.88	1	0.85	0.85	**0.88**	**0.87**	**1**
κ'	−0.03	−0.01	0.01	0.04	0.05	0.06	−0.04	0.01	0.03	**−0.43**	**−0.20**	**−0.07**

[a] Bolded values define sub-groups B-1 and B-2 (see text).

hydrogen bonding (α'**B**, #56–58, 60–65), $r=0.92$. The particular solutes represented in these groupings were selected as described in part I [1], except for the case of those representing the β'**A** term. Only one solute among compounds #1–67 was found to be representative of acceptor solute/donor column hydrogen bonding, but several such solutes (#1a, 4a–6a, 10a, 11a, 13a, 14a, 21a, 24a–26a) were present in the study of Ref. [12]. The weaker average correlations of Δ_{ij} values for solutes in these latter three groups (σ'**S**, β'**A**, α'**B**) suggest that these three terms in Eq. (1) are the result of contributions from more than one solute–column interaction.

The average correlation ($r=0.92$) for the solutes comprising the α'**B** group suggests that this term of Eq. (1) is the least ''pure''. It is interesting to observe the correlation matrix for all of the weak acids (#56–67) used in the derivation of Eq. (1) (Table A.1b). Two, reasonably distinct sub-groupings can be seen: solutes #56–58, 60–63, 66 and 67 (group B-1), and solutes #59, 64 and 65 (group B-2). Whereas the average correlation for all of these solutes (#56–67) is $r=0.87$, the correlation for each of the sub-groups is significantly better: group B-1, $r=0.928$; group B-2, $r=0.933$. Solute groups B-1 and B-2 are further differentiated by the extent of ionization of each compound, which can be approximated by the solute parameter κ' (last row of Table A.1b). Thus, $-0.04 \leq \kappa' \leq 0.06$ for the solutes of group B-1, and $-0.43 \leq \kappa' \leq -0.07$ for the solutes of group B-2. This relationship is perhaps better shown in a correlation of values of r vs. solute #59 against κ' (Fig. A.1). As values of κ' increase, corresponding to a decrease in the ionization of the acidic solute, there is a decrease in values of r, meaning a poorer correlation of Δ_{ij} values for solute i vs. values of Δ_{ij} for solute #59.

The results of Table A.1 and Fig. A.1 suggest that the α'**B** term of Eq. (1) can be subdivided into two separate solute–column interactions, one of which is determined by the extent of ionization of the acidic solute, and one which is determined by the interaction of the neutral acid with the column. The first interaction should be taken into account by the κ'**C** term of Eq. (1), but may not be because of the way in which the terms of Eq. (1) were derived. In any case, the average deviation of experimental and calculated values of log α (Eq. (1)) for solutes #56–58 and 60–67 is SD = 0.004, which is the same as for remaining solutes #1–55.

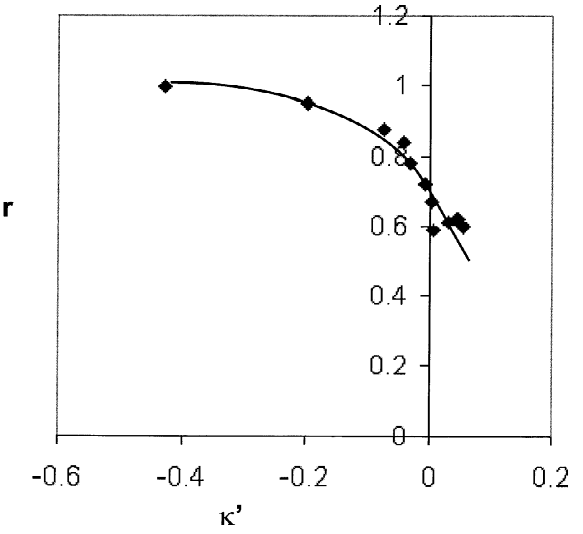

Fig. A.1. Correlation of ''hydrophobic-interaction-corrected'' values of log α for various acidic solutes (#56–67) vs. corresponding values for diflunisal (#59) as a function of the solute parameter κ'. See text for details.

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