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FACTOR ANALYSIS AND EXPERIMENT DESIGN IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

VII. CLASSIFICATION OF 23 REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC PACKINGS AND IDENTIFICATION OF FACTORS GOVERNING SELECTIVITY

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SUMMARY

The chromatographic behaviour of 63 solutes was investigated in reversed-phase high-performance liquid chromatographic (RP-HPLC) systems with the same mobile phase and 23 different commercially available packings.

The results provide a new insight into variations of selectivity with column type. The factors affecting solute selectivity in RP-HPLC systems, emerging from correspondence factor analysis (CFA), are grouped into types: hydrophobic factor and chemical and/or steric factors. The relative importance of these factors is considered. Physical and chemical properties of packings, expected to affect the solute selectivity, such as the nature of the organic ligand, carbon loading, end-capping procedure, shape of silica, monomeric or polymeric layer and compression technique, are compared. It is shown that only the three first influence the selectivity. The CFA results also permit a classification of the packings on a relative scale of "hydrophobicity". To position any new RP-HPLC packing on this scale and to estimate its "hydrophobicity", a test with sets of only four or five test compounds is proposed.

INTRODUCTION

Retention in reversed-phase high-performance liquid chromatographic (RP-HPLC) systems is influenced by both solvophobic and chemical interactions between

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solute molecules and reactive sites of the stationary phase (ligand, functional group of the ligand, silanol group of the support, etc.)[1–3]. The relative importance of these two classes of effects depends on the characteristics of the stationary phase[4]: (a) the nature of the organic ligand chemically bonded on the surface of silica, (b) the specific surface area and the pore size distribution of the silica and (c) the method of preparation of these phases.

Chemical interactions are frequently regarded as undesirable because, when both solvophobic and chemical interactions are significant, irregular retention behaviour occurs[5] and construction of a retention index system[6] and the identification of sample components become exceedingly difficult. Silanophilic interactions, for example, can be responsible for excessive peak tailing, for very low absolute protein recoveries and for the extremely long retention times often observed for amine solutes and many other polar substances[5,7–11]. However, in chromatographic practice this type of interaction may be useful for a given separation, and it can be used to optimize the separation[12–16], *e.g.*, in preparative-scale chromatography. In order to exploit the full potential of RP-HPLC, a knowledge of the relative importance of solvophobic and chemical interactions is needed.

The aim of this paper is to classify the packings most often used in RP-HPLC according to the relative importance of solvophobic and chemical interactions and to identify the main factors governing selectivity. As direct physico-chemical measurements are difficult, we have attempted to achieve this aim by an indirect method. This method combines a systematic study of the influence of the nature of the packing on variations in the selectivity of a large series of compounds with powerful data processing techniques. We thus intended to gain a deeper insight into the numerous factors that influence the selectivity of the test compounds and to determine the relative importance of these factors.

This work completes our systematic studies in HPLC data processing[17–22]. To compare the chromatographic selectivity we selected the same set of compounds as used previously[17]. This set includes 63 compounds varying widely in terms of their physico-chemical properties: 47 chalcones and 16 test compounds most often used in the literature. The behaviour of these compounds was analysed in the 23 RP-HPLC systems we have used to study the selectivity of *Z* or *E* configurational isomers[22]. These 23 systems include the 14 ODS packings in ref. 17 and packings with C_8, C_6, TMS, CN and phenyl ligands to test the importance of the length of the alkyl ligand or the importance of its nature (alkyl, phenyl, nitrile). Correspondence factor analysis (CFA)[23–25] was used to extract a set of "abstract" factors that affect the selectivity in the chromatographic systems. A testing procedure[26] was applied to transform the abstract factors into "hydrophobic" and "non-hydrophobic" factors. Thus, a classification of the packings according to their "hydrophobic" and "non-hydrophobic" properties can be proposed. In addition, some test compounds were chosen to recreate the proposed scale and to classify new commercially available packings. Further, the influence of the potential main factors governing selectivity was tested.

EXPERIMENTAL

The chromatographic procedures and instruments have been described pre-

viously[22]. Reagents were presented in Table I in ref. 17. Columns were specified in Table I in ref. 22 and the packings will be indicated later in the tables and figures.

Data processing

A set of "abstract" factors affecting selectivity in RP-HPLC systems was extracted from the data matrix, the elements of which are the capacity factors, k'_{ij}, by factor analysis (FA)[17–26].

From data free from experimental error, FA would yield n eigenvectors, one for each of the n factors controlling retention. Because of the error, FA invariably yields m eigenvectors ($m > n$), but only n eigenvectors of this set have a meaning and create the primary set of eigenvectors; the remaining ($m - n$) eigenvectors create the secondary set. The determination of n, the correct factor "size", is a particularly important step. As a result of this step, we obtain an estimate of the complexity of the data space, information which is normally lacking even for the simplest chemical problems.

With principal component analysis (PCA), the most widely known method of FA, different indicators, the imbedded error function (IE)[26] or factor indicator function (IND)[26], can be used to deduce this true number of factors, without any *a priori* knowledge of the experimental error.

Both IE and IND are functions of the secondary set of eigenvalues, of the number of rows and columns in the data matrix and of the number of PCA factors. By examining the behaviour of these indicators as the number of factors varies, the true number of factors, n, can often be deduced. Both indicators ought to reach a minimum when the correct number of factors is employed. However, for the IND function, the minimum is much more pronounced and more important than for the IE function and it often occurs in situations in which IE exhibits no minimum.

As we are not interested in the absolute retention of the compounds but in their selectivity, the choice of another method of FA, CFA, is more fruitful. In this analysis, two proportional rows or columns of the data matrix are represented by the same point in the reduced factor space. However, to deduce the size of the factor space without any *a priori* knowledge of the experimental error, no indicator, as far as we know, exists for CFA. Nevertheless, it is known[25] that the number of significant CFA factors is equal to the number of significant PCA factors minus 1.

The "abstract" factors (axes) extracted from FA are not recognizable as physical or chemical parameters, as they are generated to yield a purely mathematical solution. To evaluate ideas concerning the nature of these factors, we tested potential physical or chemical parameters of the chromatographic systems or of the solutes, using multiple linear regression analysis in a stepwise procedure. This operation involved the CFA results (*i.e.*, coordinates of systems or solutes on CFA axes) and the individual parameter to be tested. This procedure can be summarized by

$$P_{\text{test}} \stackrel{?}{=} P_{\text{predicted}} = |C|_{\text{CFA}}\, T \qquad (1)$$

where $|C|_{\text{CFA}}$ is the matrix of systems or solute coordinates on the n CFA axes, P_{test} is the vector of parameters being tested and T is the transformation vector. If the test vector, P_{test}, is a real factor, the predicted vector, $P_{\text{predicted}}$, obtained from the

operation will be reasonably similar to the test vector, confirming the idea embodied in the test vector.

Comparison of retention mechanisms in the RP-HPLC systems

Log k'_{ij} versus log $k'_{ij'}$, approach. The separating power of chromatographic systems depends on the phase ratio, ψ_j, of the system and on its polarity. As the separation mechanism was the main aim of this study, the stress is put not on absolute retention but on selectivity. The phase ratio of the systems can then be eliminated from consideration, as it is constant for a given chromatographic system and does not influence the separation mechanism and relative retention of solutes (selectivity).

The logarithmic retention factors, $\log k'_{ij}$ and $\log k'_{ij'}$ for the two columns (jth and j'th) can be written as

$$\log k'_{ij} = \log \psi_j - \Delta G^0_j/2.3\ RT \tag{2}$$

$$\log k'_{ij'} = \log \psi_{j'} - \Delta G^0_{j'}/2.3\ RT \tag{3}$$

Subtraction and rearrangement of these equations yield

$$\log k'_{ij} = \log k'_{ij'} + \log \psi_j - \log \psi_{j'} - \frac{\Delta G^0_j - \Delta G^0_{j'}}{2.3\ RT} \tag{4}$$

where k'_{ij} is the capacity factor of the ith solute in the jth chromatographic system; $\psi_j = n_s/n_m$ is the ratio of the number of moles, n, of the mobile (m) and stationary (s) phases in the jth chromatographic system; and ΔG^0_j is the Gibbs free energy of the solute transport from the mobile to the stationary phase in the jth chromatographic system. Depending on the difference in free energy, $\Delta G^0_j - \Delta G^0_{j'}$, between the jth and j'th systems, three cases are possible:

if $\Delta G^0_j = \Delta G^0_{j'}$ then

$$\log k'_{ij} = \log k'_{ij'} + \log \psi_j - \log \psi_{j'} \tag{5}$$

if $\Delta G^0_j = a_{jj'}\ \Delta G^0_{j'}$ then

$$\log k'_{ij} = a_{jj'} \log k'_{ij'} + \log \psi_j - a_{jj'} \log \psi_{j'} \tag{6}$$

if $\Delta G^0_j \neq a_{jj'}\ \Delta G^0_{j'}$ then no linear dependence exists between $\log k'_{ij}$ and $\log k'_{ij'}$.

If the slope, $a_{jj'}$, and the correlation coefficient, $r_{jj'}$, of the $\log k'_{ij}$ *versus* $\log k'_{ij'}$ plots are considered, one can compare and classify the retention mechanisms on the jth and j'th packings[27].

The retention is called homoenergetic (the same) if $r_{jj'} > 0.95$ and $a_{jj'} = 1 \pm 0.1$. The retention is called homeoenergetic (similar) if $r_{jj'} > 0.95$ but $a_{jj'} \neq 1 \pm 0.1$. The retention is called heteroenergetic (different) if $r_{jj'} < 0.95$.

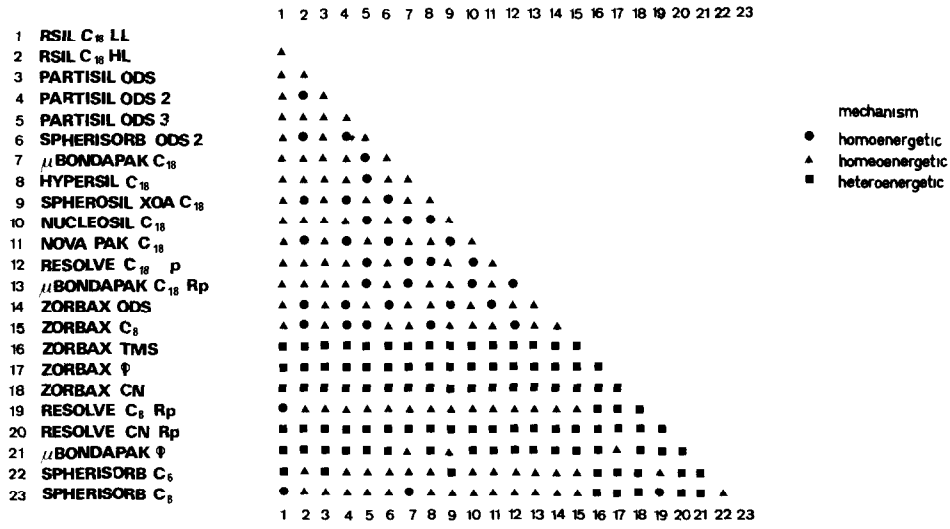

Fig. 1. Comparison of retention mechanisms for 253 pairs of packings, based on the Horváth approach (log k'_{ij} *versus* log $k'_{ij'}$).

A comparison of the retention mechanisms, for all the packing pairs, based on the log k'_{ij} *versus* log $k'_{ij'}$ plots and the above-mentioned criteria is presented in Fig. 1.

From Fig. 1, it appears that for all system pairs including ODS phases or C_8 phases, the retention is homo- or homeoenergetic. For the pairs of phases with TMS, phenyl or CN ligands (packings 16, 17, 18, 20 and 21) the retention is mainly heteroenergetic. Such an approach gives an analysis of trends, and some of the classifications are on the borderline between the two classes of mechanisms. For example, phases 10 (Nucleosil C_{18}) and 7 (μBondapak C_{18}) are homoenergetic, whereas phases 7 and 21 (Bondapak Phenyl) are homeoenergetic. A simple deduction would suggest that 10 and 21 would be homeoenergetic, but in practice they are heteroenergetic. This is due to the limitation of a classification criterion based on regression analysis. To avoid this limitation, a complementary approach by CFA is needed.

Correspondence factor analysis approach. To reduce the hyperspace of phases and solutes to the space of the main factors affecting the retention mechanism, the retention data were analysed by CFA.

With this CFA method, as in the log k'_{ij} *versus* log $k'_{ij'}$ approach, the influence of the system phase ratio on the chromatographic data is eliminated from consideration. This permits the study of only the differences in system selectivity due to the differences in the thermodynamic properties of the retention process. It appears directly from the theory of CFA[23–25] that the homoenergetic systems, *i.e.*, the systems in which proportionality between the capacity factors is observed ($k'_{ij} = ak'_{ij'}$ or $\log k'_{ij} = \log a + \log k'_{ij'}$) have the same representative point in the space of extracted CFA factors, whereas this is not the case in canonical PCA. Further, the factorial axes in CFA are common for both lines and columns of the data

TABLE I

RESULTS OF PCA OF THE CAPACITY FACTORS OF 63 SOLUTES IN 23 RP SYSTEMS

n = Number of factors; *IE* = imbedded error function; *IND* = factor indicator function.

n	*Eigenvalue*	*IE*	*IND*	n	*Eigenvalue*	*IE*	*IND*
1	131801.703	0.252169	0.002499	7	22.609	0.110795	0.000785
2	1397.414	0.203439	0.001564	8	19.142	0.088990	0.000671
3	298.182	0.185248	0.001282	9	7.164	0.079773	0.000651
4	166.768	0 154707	0.001028	10	3.609	0.075489	0.000677
5	68.140	0.136078	0.000901	11	2.890	0.070442	0.000707
6	33.322	0.124137	0.000841	12	2.126	0.065616	0.000751

matrix. Nevertheless, the PCA method was applied in our data processing to determine the number of factors and to demonstrate that our data matrix is factor analysable. We decided to do so because the determination of the number of "true" physical factors influencing raw data is one of the most important steps in the FA process. Unfortunately, most of the methods proposed in the literature require a knowledge of the experimental error. If this knowledge is incorrect, it can lead to erroneous conclusions. The only methods that permit the deduction not only of the size of the factor space but also of the experimental error without any *a priori* knowledge of the error are the methods using the IE and IND, proposed by Malinowski and Howery[26] for PCA. For CFA, such indicators have not yet been developed. We decided to determine the number of factors, based on the behaviour of IE and IND functions in PCA, and to transpose these results to our CFA study. The results of PCA carried out on the raw retention data are presented in Table I.

The IND function reaches a minimum value for the number of factors $n = 9$. This means that there are nine important factors governing retention. Thus, only eight important factors influence the solute selectivity and ought to be kept in the CFA of the RP-HPLC systems studied.

The results of CFA limited to this set of eigenvectors are presented in Table II. Projection of the chromatographic systems on to the planes defined by the first and second or second and third main axes of inertia are shown in Fig. 2a and b, respectively.

According to what has been outlined above, the distance between two chromatographic systems, the jth and j'th, in the eight-dimensional space of factors is a measure of the deviation from proportionality of the capacity factors of the compounds on these packings. The greater is the distance, the greater are the differences in solute selectivity. Even when the distances for two pairs of packings are similar, in this space, the contribution of particular factors can be completely different. Packings that have similar coordinates on one axis can be distinguished by the remaining factors. For example, proximity of the Partisil ODS and Zorbax TMS phases on the plane determined by axes 1 and 2 (Fig. 2a) shows that these packings are not different when factors 1 and 2 are considered, but Fig. 2b shows that the differences in selectivity for this phase pair are due to factor 3.

Relationship between the above two approaches. The extracted factors are called "abstract" because, although they have a mathematical meaning, they have no evi-

TABLE II

COORDINATES OF THE 23 RP-HPLC SYSTEMS ON THE MAIN CFA AXES

Chromatographic system (j)	*Coordinates*							
	Axis 1	*Axis 2*	*Axis 3*	*Axis 4*	*Axis 5*	*Axis 6*	*Axis 7*	*Axis 8*
1	0.139	− 0.014	0.053	−0.040	−0.020	−0.033	0.003	0.002
2	− 0.122	0.006	−0.019	0.032	0.015	−0.016	0.027	0.007
3	0.230	0.050	0.118	−0.039	−0.028	−0.029	0.020	−0.009
4	− 0.084	− 0.044	0.012	−0.021	−0.009	0.000	0.007	0.010
5	− 0.029	0.048	−0.026	0.002	0.009	−0.010	−0.003	0.001
6	− 0.123	0.000	−0.017	0.023	0.037	0.017	0.005	−0.013
7	0.056	0.013	−0.048	−0.012	0.025	−0.019	−0.018	0.003
8	− 0.071	0.045	−0.014	0.021	0.019	0.003	0.006	−0.008
9	− 0.068	− 0.090	0.010	−0.025	0 015	0.003	0.000	0.007
10	0.013	− 0.019	0.040	−0.024	−0 011	−0.004	0.005	−0.002
11	− 0.192	0.055	0.022	0.034	−0.022	−0.008	0.005	0.013
12	− 0.010	− 0.031	0.058	−0.011	0.011	0.019	0.002	−0.017
13	0 032	0.019	−0.020	−0.015	−0.001	−0.030	−0.035	0.000
14	− 0.139	− 0.008	0.030	0.021	−0.015	0 010	0.021	−0.012
15	− 0 051	0.071	−0.074	−0.013	−0.031	0.006	−0.001	0.015
16	− 0.247	0.041	−0.086	−0.026	0.088	−0.003	−0.012	0.001
17	0.256	− 0.100	−0.095	0.037	−0.033	0.007	0.004	0.006
18	0.449	0.006	0.127	0.071	0.024	−0.060	0.029	−0.007
19	0.095	0.049	−0.019	−0.056	−0.003	0.035	0.023	0 004
20	0.469	0.059	0.180	0.073	0.034	0.067	−0.037	0.073
21	0.288	− 0.015	−0.020	0.034	−0.016	0.014	0.001	−0.036
22	0.176	0.151	0.006	−0.023	−0.010	0.017	0.009	−0.015
23	0.065	0.099	−0.016	−0.018	−0.015	0.013	0.003	−0 008
Contribution to cluster inertia	73.66	10.03	8.01	2.85	1.76	1.18	0.84	0.62

dent direct physical or chemical meaning. Nevertheless, these factors can be converted into physically significant parameters, *e.g.*, into differences in Gibbs free energies of the solute transport from the mobile to the stationary phase. Such a relationship is useful in giving a thermodynamic meaning to the factorial coordinates of the chromatographic system. To obtain such a relationship, the intermediate parameter $\bar{a}_j$ is created, which corresponds to the slope of the log k'_{ij} *versus* log $\bar{k}'_i$ plots, where $\bar{k}'_i = \sum_{j=1}^{23} k'_{ij}/23$ is the average capacity factor of the ith solute. Using a stepwise multiple linear regression procedure, it was found that $\bar{a}_j$ is strongly correlated with factorial coordinates. To achieve the required precision of $\bar{a}_j$ values (and $a_{jj'}$ values, see text below) only five of the eight factors are needed, and then the $\bar{a}_j$ parameters can be expressed in the following form (Table VI):

$$\bar{a}_j = c + a_1 x_{1j} + a_2 x_{2j} + a_3 x_{3j} + a_4 x_{4j} + a_5 x_{8j} \quad (7)$$

where x_{ij} is the coordinate of the jth chromatographic system on the ith CFA axis; coefficients $a_1, \ldots a_5$ and constants c are listed in Table III.

Fig. 2. Results of correspondence factor analysis of capacity factors of 63 solutes in 23 RP-HPLC systems. Projection of 23 RP-HPLC systems on to the plane defined by (a) 1 and 2 main axes of inertia; (b) 2 and 3 main axes of inertia. ●, Systems with ODS packings; ■, systems with C_8, C_6, TMS, CN and phenyl packings.

Based on the $\bar{a}_j$ parameters, the ratio of the Gibbs free energies, $a_{jj'} = \Delta G_j^0/\Delta G_{j'}^0$, for all phase pairs ($j$th and j'th) can be expressed in terms of the extracted factors as

$$a_{jj'} = \Delta G_j^0/\Delta G_{j'}^0 = a_j/a_{j'} = \frac{c + a_1x_{1j} + a_2x_{2j} + a_3x_{3j} + a_4x_{4j} + a_5x_{8j}}{c + a_1x_{1j'} + a_2x_{2j'} + a_3x_{3j'} + a_4x_{4j'} + a_5x_{8j'}} \quad (8)$$

TABLE III

RELATIONSHIP BETWEEN HORVÁTH's APPROACH AND THE CFA APPROACH

Multivariant relationship for predicting $\bar{a}_j$ parameters based on the CFA coordinates of chromatographic systems. Parameters in eqn. 7, with correlation coefficient and standard deviation.

Parameter	*Value*	*Parameter*	*Value*
a_1	−1.3869	c	1.0179
a_2	−0.8553	mr	0.9973
a_3	−0.3578	s	0.0227
a_4	0.3456	N	23
a_5	0.5587		

It should be noted that only five and not eight factors are necessary to recreate the $\bar{a}_j$ parameters. This can be elucidated in the following manner. The $\bar{a}_j$ parameters are the slopes of the log k'_{ij} *versus* log $\bar{k}'_i$ plots, emerging from the least-squares regression procedure. They are not sensitive to some specific interactions of some compounds with the phases; these interactions are accounted in the 5–7 factorial axes of AFC. The more precise way to estimate the differences between the selectivity of packings is, in our opinion, to compare the packing distance, χ^2, in the true factor space[17], but this distance cannot be interpreted in terms of thermodynamic parameters. As the concept of the differences in the Gibbs free energy is very useful from a chromatographic point of view, we decided to exploit it. Eqn. 8 gives the relationship between the χ^2 factorial distance and the thermodynamic data.

Hydrophobicity information content from CFA results. The five factors used to recreate the $a_{jj'}$ parameters can be arranged into two factor groups describing the influence of the hydrophobic effect and the other effects on the chromatographic selectivity of the test compounds. The "test vector", which reflects the influence of the hydrophobic effect, can be built from the data concerning the "hydrophobicity" of compounds, expressed by the Rekker constants[28]. Such a procedure is possible because each factor can be evaluated independently, even if a multitude of other factors simultaneously influence the data. The remaining factors can further be treated as responsible for the chemical or/and steric effects affecting solute selectivity.

As it appears from a stepwise procedure, the "hydrophobicity" of compounds, F, correlates well with only the first CFA factor, and the remaining factors do not improve the correlation, as indicated in Table IV.

Table IV permits the evaluation of the relative importance of the hydrophobic and non-hydrophobic effects in differentiating the solute selectivity on the packing pairs. All the discussed effects cause deviations of the $a_{jj'}$ parameters from unity. This deviation is denoted by $\Delta a_{jj'}$:

$$\Delta a_{jj'} = 1 - a_{jj'} \tag{9}$$

The differences in the phase "hydrophobicity" only cause the following deviation:

$$\Delta a^h_{jj'} = 1 - a^h_{jj'} \tag{10}$$

TABLE IV

CORRELATION BETWEEN THE SOLUTE HYDROPHOBICITY, F, AND THE SOLUTE COORDINATES ON THE CFA AXES

Correlation	*Axis (j)*							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
F/jth axis	0.964	−0.354	−0.355	−0.063	−0.324	0.114	−0.173	−0.237
Residual of (F *vs.* axis 1)/jth axis	–	−0.051	−0.124	0.069	−0.133	0.049	−0.001	−0.100

where $a^h_{jj'}$ is based on eqn. 8, limited to the factor $x_{1j'}$ and can be expressed as

$$a^h_{jj'} = (1.02 - 1.39x_{1j})/(1.02 - 1.39x_{1j'}) \tag{11}$$

Then the relative importance of the "hydrophobic" effect in the $\Delta a_{jj'}$ parameters can be estimated as $\Delta a^h_{jj'}/\Delta a_{jj'}$.

Scale of "hydrophobicity" of the packings

Creation of the hydrophobicity scale. The previous results can be used to propose a hydrophobicity scale of the packings (Fig. 3). To do this, the a^h_j parameter is defined by using the general regression (eqn. 7) and considering only the contribution of the first factorial axis, related to hydrophobicity:

$$a^h_j = 1.02 - 1.39x_{1j} \tag{12}$$

The relative "hydrophobicity" of any two phases, j and j', can be calculated from the ratio of the a^h_j parameters.

If, for example, we are interested in the relative "hydrophobicity" of the RSIL C_{18} LL and Nova Pak C_{18} stationary phases, then, according to eqn. 11,

$$a^h_{\text{RSIL } C_{18} \text{ LL/Nova Pak } C_{18}} = \frac{1.02 - 1.39 \cdot 0.139}{1.02 - 1.39 \cdot (-0.192)} = 0.64$$

This scale gives the relative hydrophobicity of the packings tested. The a^h_j values range from 1.28 for Nova Pak C_{18} to 0.37 for the less hydrophobic Resolve CN. Some very well known similarities may be noticed, *e.g.*, between Zorbax ODS, Spherisorb ODS 2 and RSIL C_{18} HL.

To elucidate the meaning and usefulness of such a scale, the following example may be considered. In the chemical literature, the selectivity, *i.e.*, the ratio of the capacity factors (k') of naphthalene (n) and 1-nitronaphthalene (nn), $\alpha_{n/nn}$, is proposed to reveal the degree of activation or of deactivation by the end-capping of stationary phases. Verzele and Dewaele[29] demonstrated that a properly deactivated, end-capped or trimethylsilylated octadecylsilica gel would yield an $\alpha_{n/nn}$ value of about 1.4 or higher. For non-deactivated phases, this value is lower and usually around 1.1–1.2. For octylated silica gels the $\alpha_{n/nn}$ value is always higher for end-

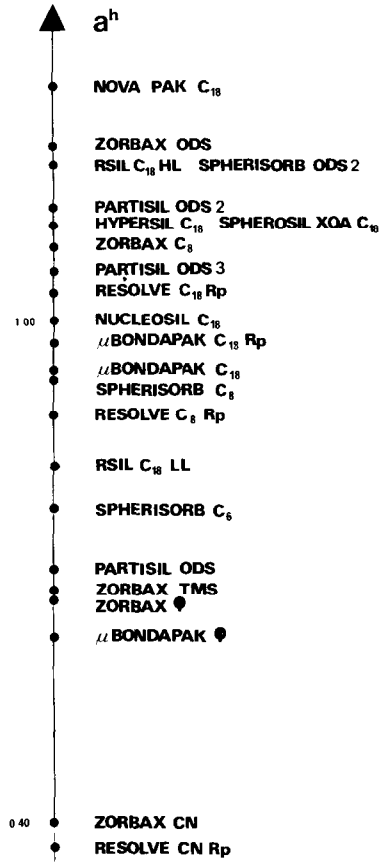

Fig. 3. "Hydrophobicity" scale for 23 RP-HPLC packings.

capped materials, but the difference between the two types is smaller. For a phenylsilica gel phase the $\alpha_{n/nn}$ value is always lower than 1, even on a deactivated phase. In other words, the retention time sequence on phenylsilica gel for naphthalene and 1-nitronaphthalene is inverted relative to that on octadecylsilica gel phases.

In fact, the $\alpha_{n/nn}$ value is determined not only by the nature of organic ligand and the degree of deactivation by end-capping of the phase, but also by the carbon loading, pore size distributions and other properties, which overlap and determine the non-specific properties of the chromatographic system. We must emphasize that only the difference of an ideal $\alpha_{n/nn}$ value, predicted on the basis of the "hydrophobicity" of packings and the experimental $\alpha_{n/nn}$ value, can reflect the specific properties of the packings.

Relative hydrophobicity of a new column, determined by using a minimal series of test compounds. To position a new packing on the proposed scale, a few solutes from the set of test compounds (Nos. 48–63, ref. 17) were chosen by a stepwise multiple linear regression procedure. The a_j^h parameters can be estimated within experimental error according to the following equation:

TABLE V

TESTS OF "HYDROPHOBICITY" OF PACKINGS

Multivariant relationship for predicting a_j^h parameters of chromatographic systems based on the log k' of test solutes.

Parameter	*Eqn. 13a*	*Eqn. 13b*	*Eqn. 13c*
a_1	0.67450	0.88418	0.78071
a_2	− 0.41604	− 1.87393	−1.46127
a_3	0.87633	0.93532	1.70518
a_4	− 1.09227	0.21904	−2.02640
a_5			1.03146
c	0.33234	0.44176	0.37001
mr	0.9940	0.9904	0.9923
s	0.031	0.039	0.036
N	23	23	23

$$a_j^h = a_1 \log k'_{1j} + a_2 \log k'_{2j} + a_3 \log k'_{3j} + a_4 \log k'_{4j} + a_5 \log k'_{5j} + c \qquad (13)$$

where k'_{ij} is the capacity factor of the ith solute in the jth chromatographic system.

With the first set of test compounds selected, (1) 9-phenyl-1-nonanol, (2) 4-phenyl-1-butanol, (3) naphthalene and (4) α-nitronaphthalene, the coefficients in eqn. 13a were obtained and are given in Table V.

As 9-phenyl-1-nonanol can be retained very strongly in a particular chromatographic system (*e.g.*, in systems with Nova Pak C_{18} or Zorbax ODS phases), two additional tests are proposed with other sets of compounds: (1) biphenyl, (2) α-nitronaphthalene, (3) 6-phenyl-1-hexanol and (4) anthracene, evaluated by eqn. 13b (Table V); and (1) biphenyl, (2) α-nitronaphthalene, (3) 6-phenyl-1-hexanol, (4) 4-phenyl-1-butanol and (5) methyl benzoate, evaluated by eqn. 13c (Table V). The a_j^h parameters, determined according to eqns. 12 and 13a–c, are presented in Table VI.

There is good agreement between the original a_j^h hydrophobicity parameters, extracted from the CFA study, and the calculated values, determined with the three different equations. To confirm the validity of the tests presented above, the a_j^h parameters for two new packings were determined according to eqn. 13a–c. The capacity factors of the test compounds, chromatographed in systems with Chromspher C_{18} and LiChrosorb RP-8 5 μm as stationary phases and with methanol–water (7:3) as the mobile phase, are presented in Table VII.

The $a^h_{\text{Chromspher C}_{18}}$ parameters are 1.19, 1.24 and 1.16 according to eqn. 13a, b and c, respectively.

These values are within the experimental error and show that these different sets of compounds give concordant results when recreating the hydrophobicity scale. The average value of 1.20 indicates the position of Chromspher C_{18} on the hydrophobicity scale of packings. This hydrophobicity is similar to those observed for the RSIL C_{18} HL ($a^h_{\text{RSIL C}_{18}\text{ HL}} = 1.19$) and Zorbax ODS ($a^h_{\text{Zorbax ODS}} = 1.21$) packings. The $a^h_{\text{LiChrosorb RP-8}}$ parameters are 0.90, 0.90 and 0.90 according to eqn. 13a, b and

TABLE VI

RECREATION OF THE "HYDROPHOBICITY" SCALE

a_j^h parameters accounted for according to eqns. 12 and 13a–c.

No.	*Packing*	a_j^h *according to eqn.*			
		12	*13a*	*13b*	*13c*
1	RSIL CL LL	0.82	0.85	0.84	0.81
2	RSIL C_{18} HL	1.19	1.18	1.18	1.18
3	Partisil ODS	0.70	0.75	0.72	0.72
4	Partisil ODS 2	1.13	1.13	1.12	1.09
5	Partisil ODS 3	1.06	1 05	1.07	1.07
6	Spherisorb ODS 2	1.19	1.16	1.13	1.16
7	μBondapak C_{18}	0.94	0.94	0.94	0.95
8	Hypersil C_{18}	1.12	1.10	1.11	1.08
9	Spherosil XOA 600 C_{18}	1.11	1.12	1.11	1.10
10	Nucleosil C_{18}	1.00	0.98	0.97	0.95
11	Nova Pak C_{18}	1.28	1.29	1.31	1.30
12	Resolve C_{18} Rp	1.03	1.03	1.00	1.04
13	μBondapak C_{18} Rp	0.97	1.00	1.02	0.99
14	Zorbax ODS	1.21	1.24	1.24	1.26
15	Zorbax C_8	1.09	1.09	1.10	1.11
16	Zorbax TMS	0.68	0.59	0.56	0.60
17	Zorbax Phenyl	0.66	0.65	0.66	0.69
18	Zorbax CN	0.40	0.42	0.43	0.42
19	Resolve C_8 Rp	0.88	0.86	0.85	0.88
20	Resolve CN Rp	0.37	0.38	0.41	0.37
21	μBondapak Phenyl	0.62	0.62	0.64	0.64
22	Spherisorb C_6	0.77	0.80	0.81	0.82
23	Spherisorb C_8	0.93	0.92	0.91	0 92

c, respectively. Thus, the hydrophobicity of this packing is similar to that of Resolve C_8 Rp and Spherisorb C_8.

A more general indication of the validity of our procedure of using the ratio of the Gibbs free energy, $a_{jj'}^h$ for any two chromatographic systems is that the results presented in our previous paper[17] for ODS phases only are in good agreement with the present results. The equations connecting the $\bar{a}_j$ parameters with the CFA coordinates are different, and the CFA results, values of the phase coordinates for the 14 ODS and for the 23 ODS, C_8, C_6, TMS, CN and phenyl phases, are different, but the relative positions of the 14 ODS packings are the same in both scales.

Parameters of the packings influencing solute selectivity

The packing materials and the mode of packing both contribute to the chromatographic performance. The discussion here deals only with the chemical factor, α, affecting the resolution of peaks.

Some unique properties of the packings were expected to be the real factors influencing the solute selectivity. A "test vector", corresponding to a property, was defined in the following manner. The value 1 is assigned to the phases having the tested property and the value 0 to those lacking this property. Stepwise multiple

TABLE VII

CAPACITY FACTORS, k', FOR THE COMPOUNDS DETERMINED IN TWO NEW ADDITIONAL SYSTEMS WITH (A) CHROMSPHER C_{18} AND (B) LICHROSORB RP-8, 5 μm, AS STATIONARY PHASES AND WITH METHANOL–WATER (7:3) AS THE MOBILE PHASE

Test compound	k'	
	A	*B*
9-Phenyl-1-nonanol	20.12	14.43
4-Phenyl-1-butanol	1.98	2.29
Naphthalene	5.41	4.38
α-Nitronaphthalene	3.14	3.79
Biphenyl	8.90	6.48
6-Phenyl-1-hexanol	4.53	4.50
Anthracene	17.88	9.54
Methyl benzoate	1.80	2.22

linear regression was used to recreate the "tested vector" from the phase coordinates on the CFA axes. The success of the test procedure is determined by observing the agreement between the tested and the predicted vector. The property in question can only be considered as a real factor affecting solute selectivity if this agreement is good and if there are no important differences between the tested and predicted values.

The following unique properties of the stationary phases were tested: (a) nature of the organic bristles (C_{18}, C_8, C_6, TMS, CN and phenyl); (b) source of silica gels (Waters Assoc., DuPont, Phase Separations); (c) type of bristle layers (mono- or multilayer); (d) shape of silica gel particles (regular or irregular); and (e) compression technique (axial or radial). The "tested" and "predicted" vectors are presented in Table VIII.

Let us consider the "tested" and "predicted" vectors Nos. 1, 2, 3 and 4. In this instance, the unique property in question is the nature of the organic bristles. The test vectors are defined in the following manner.

Vector 1: the C_{18} phases are defined as unity, the remaining ones (C_8, C_6, TMS, CN and phenyl) as zero;

Vector 2: the C_8 phases are defined as unity, the remaining ones (C_{18}, C_6, TMS, CN and phenyl) as zero;

Vector 3: the CN phases are defined as unity, the C_{18}, C_8, C_6, TMS and phenyl phases as zero;

Vector 4: the phenyl phases are defined as unity, the C_{18}, C_8, C_6, TMS and CN phases as zero.

The predicted values for vector 1 vary between 1.2 and 0.7 for the C_{18} phases and between 0.4 and -0.1 for the other phases. The predicted values for vector 3 vary between 0.8 and 1.0 for the CN phases and between 0.2 and -0.2 for the remaining ones. The predicted values for the vector 4 vary between 0.8 and 1.0 for the phenyl phases and between -0.1 and 0.2 for the other phases. Hence, the predicted values for the phase property being tested is considerably greater then zero, and there are no other relatively high values on the predicted vectors. The nature of organic bristles (C_{18}, CN and phenyl) can be considered as the real factor influencing solute selectivity.

The test of vector 2, which is calculated to reflect the unique property of C_8 phases, was not successful. The predicted values for C_8 phases vary between 0.4 and 0.7 and for the remaining phases between –0.4 and 0.5. A relatively high predicted value (0.5) is observed for the Spherisorb C_6 phase, which suggests that the C_6 phases behave similarly to the C_8 phases. This is proved by the test of vector 2′, in which phases of both types (C_8 and C_6) of organic bristles are defined as unity.

The negative results of tests of vectors 5–10 indicate that the type of bristle layer (vector 5, multilayer = 1, monolayer = 0), the shape of silica gel particles (vector 6, irregular shape = 1, regular shape = 0), the compression technique (vector 7, radial = 1, axial = 0) and, what seems to be the most interesting and important conclusion, the source of silica gels (see test of vector 8; DuPont packings = 1; vector 9, Phase Separations packings = 1; and vector 10, Waters Assoc. packings = 1) are not factors affecting selectivity in the investigated RP-HPLC systems. Thus, for example, the packings from DuPont or from Phase Separations have no unique properties to differentiate them from the remaining packings when selectivity only is considered.

The other parameters expected to influence solute selectivity are the pore size, surface area, carbon loading, percentage of derivatization, etc. Unfortunately, the influence of these factors is limited because the exact details concerning the silica substrate and the bonded-phase syntheses are unavailable.

Derivatization of silica is often described only qualitatively. The packing is, *e.g.*, characterized as end-capped or uncapped. From the test of vector 11, which is calculated only with 0 and 1 elements (0 for end-capped and 1 for uncapped phases), it appears that there is no sharp division between the predicted values for the two types of phases. This is not surprising, because it is known[30–32] that a sizable percentage of the total number of silanol groups originally present on silica surfaces remain underivatized, even after "exhaustive" silanization, and even end-capped packings do not all behave similarly. This is evidenced by the fact that many bonded C_{18} columns from various suppliers are "end-capped", but exhibit excessive peak tailing with amine samples. It seems that even the percentage of derivatization can be misleading when the end-capping status of packings is considered, because the accessibility of silanol groups may also depend on the nature of the organic bristles or on the carbon loading. To reflect the end-capping status of packings, we calculated the test vector (No. 11′) on the basis of our experience, *i.e.*, on the selectivity of naphthalene and α-nitronaphthalene and on the shape of the chromatographic peaks. A value of 0 is attributed to the packings that seem to be deactivated and a value of 1 is attributed to the remaining packings (vectors 11′). the test of this vector is successful and suggests that, *e.g.*, uncapped Zorbax ODS behaves as deactivated, but end-capped Zorbax TMS or RSIL C_{18} LL as not fully deactivated. More gernerally, the packings RSIL C_{18} HL, Partisil ODS 3, Spherisorb ODS 2, μBondapak C_{18}, Hypersil C_{18}, Nova Pak C_{18}, Zorbax C_8, Zorbax Phenyl, μBondapak Phenyl, Spherisorb C_8 and Spherisorb C_6, described in the suppliers' sourcebooks as end-capped, and Zorbax ODS, described as uncapped, form a single group of packings, which behave effectively as deactivated. The remaining uncapped phases, Partisil ODS 2, Spherosil XOA C_{18}, Resolve C_{18}, Zorbax CN, Resolve C_8, Resolve CN and end-capped Zorbax TMS and RSIL C_{18} LL, belong to a second group of packings, which behave effectively as not fully deactivated.

TABLE VIII

TESTS OF PACKING PROPERTIES EXPECTED TO INFLUENCE SOLUTE SELECTIVITY IN RP-HPLC SYSTEMS

Tested (t) and predicted (p) vectors:

1 ODS = 1
2 C_8 = 1
3 CN = 1
4 Phenyl = 1
5 Multilayer = 1
6 Irregular shape of silica = 1
7 Radial-Pak = 1
8 DuPont = 1
9 Phase Separations = 1
10 Waters Assoc. = 1
11 Uncapped
12 Carbon loading (%)

No.	*Packing*	*Vector*											
		1		*2*		*2'*		*3*		*4*		*5*	
		t	*p*	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*
1	RSIL C_{18} LL	1	0.9	0	0.1	0	0.0	0	0.1	0	0.0	1	0.7
2	RSIL C_{18} HL	1	0.9	0	0.2	0	0.0	0	0.1	0	0.0	1	0.5
3	Partisil ODS	1	0.7	0	0.2	0	0.4	0	0.2	0	−0.1	1	0.8
4	Partisil ODS 2	1	1.1	0	0.2	0	0.0	0	0.0	0	0.0	1	0.5
5	Partisil ODS 3	1	0.7	0	0.2	0	0.2	0	0.0	0	0.0	1	0.3
6	Spherisorb ODS 2	1	0.9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
7	μBondapak C_{18}	1	0.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.2
8	Hypersil C_{18}	1	0.7	0	0.1	0	0.2	0	0.0	0	0.0	0	0.2
9	Spherosil XOA C_{18}	1	1.2	0	0.0	0	−0.2	0	0.0	0	0.0	0	0.5
10	Nucleosil C_{18}	1	1.0	0	0.1	0	0.1	0	0.0	0	0.0	1	0.5
11	Nova Pak C_{18}	1	1.1	0	0.2	0	0.2	0	0.1	0	−0.1	0	0.4
12	Resolve C_{18} Rp	1	1.0	0	−0.1	0	0.0	0	0.0	0	0.0	0	0.2
13	μBondapak C_{18}	1	1.0	0	−0.1	0	−0.1	0	0.0	0	0.0	0	0.2
14	Zorbax ODS	1	1.2	0	−0.1	0	−0.1	0	0.0	0	0.1	0	0.0
15	Zorbax C8	0	0.4	1	0.6	1	0.7	0	−0.2	0	0.1	0	0.3
16	Zorbax TMS	0	0 1	0	0.0	0	0.1	0	0.1	0	0.0	0	0.0
17	Zorbax Phenyl	0	−0.1	0	0.2	0	0.1	0	0.1	1	1.0	0	−0.2
18	Zorbax CN	0	0.4	0	−0.4	0	−0.4	1	0.8	0	0.2	0	0.5
19	Resolve C_8 Rp	0	0.1	1	0.7	1	0.8	0	−0.1	0	0.0	0	0.4
20	Resolve CN	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	0	−0.1
21	μBondapak Phenyl	0	−0.1	0	0.0	0	0.2	0	0.1	1	0.8	0	−0.4
22	Spherisorb C_8	0	−0.1	0	0.5	1	1.0	0	0.0	0	0.0	0	0.1
23	Spherisorb C_8	0	0.2	1	0.4	1	0.7	0	−0.1	0	0.1	0	0.2

A phase factor successfully identified with physically significant parameters is the carbon loading (see test of vector 12). The tested vectors contain only approximate data from the sourcebooks of the suppliers. The carbon loading, *e.g.*, for the Spherosil C_{18} XOA 600 packing, is given as 20–23%, for Nucleosil C_{18} as 15–16%, etc. Hence, the agreement between tested and predicted values can be considered to be good.

To summarize, from the analysis presented it appears that the nature of the bristles, the carbon loading and the end-capping status are the real unique properties of packings that influence solute selectivity. The type of bristle layer, the shape of silica gel particles, the effect of axial or radial compression and the origin of silica gel do not seem to influence the solute selectivity.

6		*7*		*8*		*9*		*10*		*11*		*11'*		*12*	
t	*p*	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*
1	0.7	0	0.2	0	0.1	0	−0.1	0	0.2	0	0.55	1	1.0	10	10
1	0.3	0	−0.2	0	0.3	0	0.0	0	0.0	0	0.05	0	0.2	16	13
1	0.6	0	0.2	0	0.0	0	0.2	0	0.0	1	0.76	1	1.2	5	7
1	0.3	0	0.2	0	0.0	0	−0.1	0	0.2	0	0.50	1	0.8	15	15
1	0 4	0	0.1	0	0.3	0	0.2	0	0.3	0	0.02	0	0.1	10	9
0	0.0	0	0.1	0	0.2	1	0.2	0	0.3	0	0.23	0	0.2	12	13
1	0.6	0	0.1	0	0.4	0	0.0	1	0.4	0	−0.02	0	0.3	10	9
0	0.2	0	0.1	0	0.2	0	0.3	0	0.3	0	0.09	0	0.0	9	10
0	0.3	0	0.2	0	0.0	0	−0.2	0	0.3	1	0.60	1	1.1	20	16
0	0.4	0	0.2	0	0.0	0	0.0	0	0.2	0	0.58	1	0.8	15	12
0	0.5	0	0.1	0	0.1	0	0.1	1	0.2	0	0.18	0	−0.2	7	12
0	0.1	1	0.4	0	−0.1	0	0.2	1	0.4	1	0.76	1	0.9	12	13
1	0 9	1	0.2	0	0.3	0	0.0	1	0.5	0	0.07	0	0.0	8	10
0	0.4	0	0.4	1	0.0	0	0.2	0	0.5	1	0.45	0	−0.1	15	14
0	0.4	0	0.1	1	0.3	0	0.2	0	0.3	0	−0.17	0	−0.2	10	8
0	0.2	0	0.1	1	0.5	0	0.2	0	0.4	0	−0.17	1	0.7	4	5
0	0.4	0	−0.1	1	0.8	0	−0 2	0	0.3	0	−0.03	0	0.1	8	10
0	0.7	0	−0.2	1	0.6	0	−0.1	0	−0.2	1	0.52	1	0.8	6	6
0	−0.1	1	0.3	0	0.0	0	0.3	1	0.2	1	0.37	1	1.0	6	8
0	−0.1	1	1.0	0	0.1	0	0.0	1	0.9	1	1.18	1	1.1	4	4
1	0.3	0	0.1	0	0.5	0	0.4	1	0.4	0	0.21	0	0 0	8	7
0	0 2	0	0.3	0	0.2	1	0.7	0	0.3	0	0.14	0	0.1	6	2
0	0.3	0	0.2	0	0.2	1	0.5	0	0.3	0	0 10	0	0.0	6	6

CONCLUSION

As a direct determination of the main characteristics of packings (hydrophobicity, specific interactions, etc.) influencing chromatographic selectivity was impossible, an indirect method was developed. This work was based on factor analysis by CFA of a large number of k' data. PCA was also used to define the number of significant factors governing retention. To test the meaning of these factors or to select the important chromatographic characteristics governing selectivity, different multilinear regressions were employed.

This procedure allows the progressive delineation of the factors governing selectivity in RP-HPLC. The results presented can be summarized under four headings, as follows.

Thermodynamic and CFA relationships

According to Horváth[1], plots of log k'_{ij} values obtained on one stationary phase, j, *versus* log $k'_{ij'}$ values obtained on another stationary phase, j', with the same mobile phase, can serve as a useful tool for comparing the energetics of solute retention on different packings. The slope of the log k'_{ij} *versus* log $k'_{ij'}$ plot is equal to the ratio of the Gibbs free energy of solute transport from the mobile to the stationary phase in the jth and j'th chromatographic systems, $a_{jj'} = \Delta G^0_j / \Delta G^0_{j'}$. This ratio can be expressed as a linear combination of HPLC system coordinates x_{ij} on CFA axes. Thus, according to the Horváth[1] approach, log k'_j *versus* log $k'_{j'}$ give the $a_{jj'}$ parameters with $a_{jj'} = \Delta G^0_j / \Delta G^0_{j'}$; also, according to the CFA approach, these $a_{jj'}$ parameters may be calculated from the system coordinates x_{ij} on the factorial axes with $a_{jj'} = f(x_{ij})$.

Hydrophobicity scale of RP-HPLC packings

Hydrophobicity is the main factor governing selectivity. A general scale is proposed, which should be useful to the chromatographer in selecting packings according to hydrophobic or non-hydrophobic considerations. Even if the procedure followed to establish this scale seems complicated, the coordinate on this scale for a new packing can easily be calculated from the retentions of one among three sets of standard compounds. Thus, a new RP-HPLC packing can be easily classified.

Classification and/or illustration of the differences between packings

The coordinates of the packings on the main factorial axes give a practical classification of the differences of the selectivity of the packings. If two packings have the same hydrophobicity (*i.e.*, the same coordinate axis 1 in CFA), any differences will be due to the contribution of at least one of the other specific factors. The successive different projection planes of the eight-dimensional reduced space is more easily visualized than the set of coordinates in the 23-dimensional space of the raw data matrix.

Packing characteristics governing selectivity

With a target testing procedure it was demonstrated that (a) the real characteristics that affect solute selectivity are the carbon loading, the nature of the organic ligand and the accessibility of the silanol groups and (b) the characteristics that have no influence on selectivity are the source of the silica gel, the shape of the silica, the compression technique and the type of organic layer. This separation into two groups only means that the last characteristics (the source of the silica gel, etc.) are not important parameters with respect to selectivity, even if it is well known that they are all important in the overall chromatographic process.

Work is in progress to develop the practical applications of this systematic characterization of the packings, which is fundamental to analysts and manufacturers of new packings.

SYMBOLS

k'	Capacity factor.
j	Stationary phase or chromatographic system with the jth phase.
i	Compound chromatographed.

ΔG_j^0 Gibbs free energy for the solute transport from the mobile to the stationary phase in the jth chromatographic system.

$a_{jj'}$ Slope of log k'_{ij} *versus* log $k'_{ij'}$ plots, where k'_{ij} is the capacity factor of the ith compound in the jth system, $a_{jj'} = \Delta G_j^0/\Delta G_{j'}^0$ *i.e.*, the ratio of the Gibbs free energy for two (jth and j'th) systems.

$\overline{a_j}$ Slope of log k'_{ij} *versus* log $\overline{k'_i}$ plots, where $\overline{k'_i}$ is defined as $\overline{k'_i} = \sum_{j=1}^{23} k'_{ij}/23$.

a_j^h "Hydrophobicity" parameter of the jth packing.

$a_{jj'}^h$ Parameter of relative hydrophobicity of the jth and j'th packings, $a_{jj'}^h = a_j^h$ equal to the ratio of the Gibbs free energies of the packing pair due to the "hydrophobic" effect.

$\Delta a_{jj'} = 1 - a_{jj'}$ Deviation of the $a_{jj'}$ parameters from 1 due to all types of effects affecting solute behaviour.

$\Delta a_{jj'}^h = 1 - a_{jj'}^h$ Deviation of the $a_{jj'}$ parameters from 1 due to the hydrophobic effect only.

x_{ij} Coordinate of the jth system on the ith CFA axis.

mr Correlation coefficient for multiple linear regression.

s Standard deviation.

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