ELSEVIER

Journal of Chromatography A, 668 (1994) 301–312

JOURNAL OF CHROMATOGRAPHY A

Characterization of stationary phases used in reversed-phase and hydrophobic interaction chromatography

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Abstract

Nine prepacked columns used in reversed-phase and in hydrophobic interaction chromatography were studied. The stationary phases investigated were widely different with respect not only to the type and surface concentration of the ligands but also to the material and geometry of the support and the extent of previous usage. In order to characterize the columns, measurements were made under isocratic conditions with three different homologous series. From the retention behaviour of the components some descriptors were calculated according to four characterization methods described in the literature. On the basis of the intercept of the ln k–methanol content relationship, a rough estimate of the hydrophobicity of the stationary phases could be given. More realistic results were obtained with the polar–apolar characterization suggested by Hetem and co-workers, but some discrepancies were also found. The use of the isopotential eluent composition as a descriptor resulted in the most acceptable characterization. Two-dimensional mapping along the parameters of the slope–intercept relationships summarizes the results obtained with the other techniques.

1. Introduction

With continuing developments in high-performance liquid chromatography (HPLC), an increasing variety of stationary phases are commercially available. Improvements are aimed at increasing the selectivity and the efficiency of the columns, but the number of the tailor-made stationary phases suitable for specific separations is also increasing [1,2]. The most commonly used technique in routine laboratory practice is reversed-phase liquid chromatography (RPLC), which is usually performed with n-octadecyl-modified silica packings. As the ligands are chemically bonded to the surface of the support, these packings are stable with time, providing selective and reproducible separations. With a better understanding of the phenomenon of "selectivity" in RPLC practice and the need for sophisticated and fully optimized solutions to separation problems [3–5], interest in RP stationary phases with various type of ligands and geometry has grown. Even if only these "reversed-phase-like" packings are considered, the number of the applicable columns is tremendous and the choice amongst them is not at all straightforward.

The separation of biologically active compounds, especially proteins, needs stationary phases of different types. The ligands in RPLC packings are very hydrophobic and are distributed very densely. The interaction between the proteins and the stationary phase is strong, and addition of an organic modifier is therefore

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0021-9673/94/$07.00
SSDI 0021-9673(93)E1274-4

needed for elution. As a consequence of these conditions, dehydration, conformational changes, partial or complete unfolding of the components and significant losses of amount and biological activity could occur [6–8]. For the separation of proteins, hydrophobic interaction chromatography (HIC) is an alternative to RPLC. The functional groups in HIC packings are less hydrophobic and their surface concentration is low, producing moderately hydrophobic stationary phases and mild hydrophobic interactions. Protein-stabilizing salts are used to promote the retention, and protein purity can therefore be obtained while retaining the biological activity [6,9–13]. More recently, a wide variety of stationary phases suitable for HIC have become available [1,2,14]. The selection of the most applicable packing material for a certain protein separation is even more difficult than in RPLC as the HIC columns have greater diversity as regards type and geometry of the support and the ligands.

It is well known that the retention of a solute measured at a specific mobile phase composition varies widely between stationary phases having the same type of ligands but originating from different manufacturers [15,16]. Even packings from the same source differ between batches of the same material [17]. Several reasons for these batch-to-batch variations have been given. One of the major causes is the regions of the support uncovered by the ligands which can also interact with the solutes, *i.e.*, the retention can be greatly affected by the properties of the accessible parts of the support [18]. This means that preliminary characterization of the stationary phases is necessary when new columns are to be used or when the possibilities of new types of columns are investigated.

To study the properties of chemically bonded phases, a broad range of characterization techniques have been reported. These procedures can be divided into two categories: non-chromatographic and chromatographic methods. The techniques belonging to the first group were summarized, discussed and investigated by Claessens *et al.* [19] and Hetem and co-workers [20–22], so here only a brief summary is given. These methods can be further subdivided.

(i) The determination of bulk properties such as specific surface area, volume and size distribution of the pores and of the particles. The procedures applicable for the purpose (*e.g.*, BET and Coulter Counter techniques) are well known.

(ii) The characterization of the surface structure of the packing materials considering the concentration, conformation and mobility of the ligands and the structure, chemistry and accessibility of the support. The procedures could be destructive or non-destructive. In the former instance, the packing material is subjected to some modification, such as titration, chemical reactions or solvolysis, pyrolysis and fragmentation, and the reaction products are analysed so as to obtain information about the properties investigated. The latter group involves instrumental analysis such as fluorescence and infrared spectroscopy or NMR techniques of different kinds. These methods leave the packing material intact and are also suitable for direct chromatographic characterization.

The chromatographic characterization of the stationary phases utilizes the information obtained from the retention behaviour of some selected components. These methods can also be subdivided.

(i) The characterization of the efficiency properties of the packings, which reflect mainly the kinetics of the separation in a packed chromatographic bed but some physico-chemical aspects such as secondary interactions must also be considered. For commercially available columns a test chromatogram is usually provided from which some efficiency characteristics (*e.g.*, theoretical plate number, asymmetry factor) can also be calculated. However, in most instances these token chromatograms are not applicable to comparing different columns as neither the sample components nor the eluent composition are the same.

(ii) The characterization of some specific properties of the stationary phase such as silanophilic or polar capacity and hydrophobicity. On the basis of the type and number of the test components and the eluent conditions, three groups of methods can be distinguished. (a) Only a few solutes with different molecular properties

are applied, usually at one eluent composition. From the retention order of the components the polar–apolar character of the packing could be qualified [23]. (b) Homologous series are used and the behaviour of the components is modelled over a wider range of eluent composition. From the constants of the regression equations the above characteristics can be calculated (see Results and Discussion). (c) Application of retention indices: the idea of using retention indices calculated from the retention behaviour of homologous series also in RPLC originates from the well known Kováts retention indices used in gas chromatography. The aim is to obtain "phase-system-independent" retention parameters. Most of these techniques have been reviewed [24]. However, it is also revealed that the retention indices vary with the properties of the stationary phase [17] and of the eluent [25]. (Note that these procedures are transient from method (b) above to the following methods.)

(iii) Calibration of the stationary phase. Here usually a set of components are chromatographed at several eluent compositions and an abstract model of retention is built by using the techniques of multivariate data analysis such as factor analysis [26], principal component analysis [27] and related techniques [28,29]. The basic aim of this type of investigation is the accurate prediction of retention. When columns with the same or similar stationary phases are used these techniques give adequate results [26–29] but the applicability for different packing materials has not been completely revealed.

As outlined above, RPLC and HIC are the same in nature. In both techniques hydrophobic functional groups are bound to the support and the driving force of the separation, hydrophobic interaction, is considered identical. However, the features of the packings can be unique. The use of homologous series for the characterization of RPLC phases has been extensively investigated [19–22,30–34] on *n*-alkyl-modified silica substrates but we are not aware of any application for comparison of stationary phases with widely different characteristics.

In this work, homologous series were used to characterize stationary phases used in RPLC and HIC with different characteristics. First, the validity of the underlying retention models of the methods was checked, then the characterization of the columns was performed on the basis of some calculated descriptors.

2. Experimental

2.1. Materials

The test components used for characterization were all of analytical-reagent grade and were obtained from different sources: set I = benzene homologues (I): benzene (B), toluene (T), *m*-xylene (mX) and *p*-isopropyltoluene (*p*-cymene, pC); set II = dialkyl phthalates (II): dimethyl (DMP), diethyl (DEP), dipropyl (DPP) and dibutyl phthalate (DBP); set III = *n*-alkyl *p*-hydroxybenzoates (III): methyl (MP), ethyl (EP), *n*-propyl (PP) and *n*-butyl *p*-hydroxybenzoate (BP). Methanol was of reagent grade (Reanal, Budapest, Hungary). Water was freshly prepared by double distillation in the laboratory.

2.2. Columns

The characteristics of the columns used are listed in Table 1. The symbols RPLC and HIC in parentheses reflect the operational mode recommended by the manufacturers.

2.3. Chromatography

A Merck–Hitachi (Merck, Darmstadt, Germany) fully automated chromatograph was used, consisting of an L-4250 UV–Vis programmable detector operated at 254 nm, L-6200 programmable pumps and an autoinjector (Rheodyne, Cotati, CA, USA) with a 10-μl loop. System control, data acquisition and evaluation were performed with HPLC Manger software (Merck) running on an IBM PC AT-compatible computer.

Measurements were made under isocratic conditions with methanol–water mixtures. The composition of the eluent was always adapted to the components, *i.e.*, it was varied so as to obtain 5–7 retention values in the range $-1 < \ln k < 3$ for all the test components on all the

Table 1
Characteristics of the columns used

Material	Source	Ligand type	Support material	Column dimensions (mm × mm I.D.)	Particle size (μm)	Pore size (Å)	Abbreviation
LiChrospher 100 RP-8	Merck (Darmstadt, Germany)	C_8	Silica	125 × 4.0	5	100	MC8 (RPLC)
Bakerbond WP C18	J.T. Baker (Deventer, Netherlands)	C_{18}	Silica	250 × 4.6	5	300	BWP (RPLC)
LiChrospher 500 CH-8	Merck	C_8	Silica	250 × 4.0	10	500	MHB (RPLC)
Synchropak RP-4	Synchrom (Lynden, IN, USA)	C_4	Silica	250 × 4.1	6.5	300	SC4 (RPLC)
Hypersil C_8 WP-300	Shandon Southern Products (Runcorn, UK)	C_8	Silica	120 × 4.6	5	300	HPS (RPLC)
Separon Hema-BIO 1000 Phenyl	Tessek (Prague, Czech Republic)	Phenyl	HEMA[a]	80 × 8.0	10	1000	HEMA (HIC)
TSK PHENYL 5-PW	Beckman Instruments (San Ramon, CA, USA)	Phenyl	SDVB[b]	75 × 7.5	5	1000	TSK (HIC)
OR C_3-OH	Dr. Ohmacht POTE (Pécs, Hungary)	C_3-OH	Silica	250 × 4.6	6	300	OR (HIC)
Synchropak propyl	Synchrom	C_3	Silica	250 × 4.1	6.5	300	SC3 (HIC)

[a] Hydroxyethyl methacrylate.
[b] Styrene–divinylbenzene copolymer.

columns. The hold-up time was also measured at all compositions with an aqueous solution of sodium nitrite. All the measurements were repeated at least twice and the average values were used for the calculations.

3. Results and discussion

After the completion of the measurements it was immediately revealed that the RPLC and HIC columns were similar in nature. On the basis of the raw data, the columns cannot be divided into two definite sets as is done by the manufacturers, but rather they form a series regarding strength. The retention values obtained on the HEMA and TSK columns were nearly the same as those on some RPLC columns.

If the same components are used in the same eluent but on different columns, the deviations in retention should reflect the alteration of the properties of the stationary phases and therefore at a reference eluent composition comparison of the columns can be performed. In our case direct comparison cannot be performed without further evaluation as the compositions of the eluents used were not the same for the columns (see section 2.3). For such a comparison, the most straightforward selection is ln k_w (ln k extrapolated to 100% water), as this parameter is not affected by the type of organic modifier used and additionally water is the eluent that usually provides the largest differences in the retentions

of the components. Although this parameter cannot be measured in most instances, it can be calculated from the data measured.

The retention of a solute under RPLC conditions can be described as a function of the eluent composition [19–22,30–34]

$$\ln k = \ln k_w - Sx \quad (1)$$

where x is the methanol content of the eluent, ln k_w and S (constants) are the intercept and the slope of the profile, characteristic of the components and the type of the stationary and mobile phases used. Eq. 1 was fitted to the measured retention data and a very good correlation was obtained for all the components on all columns ($r > 0.998$ for RPLC and $r > 0.992$ for HIC columns), *i.e.*, the retention can be described by Eq. 1 also on the HIC columns operating under RPLC conditions.

Comparison of the columns on the basis of the calculated ln k_w values is shown in Fig. 1a–c (note that the lines in this and other figures of the same type have no physical meaning; they are used only to connect the related points). The overall picture corresponds well with the order expected from the retention data measured. The RPLC and HIC columns cannot be divided sharply, rather they form a set with gradually decreasing strength. The HEMA and TSK columns were very similar to the RPLC columns. It was also surprising that SC4 was as strong as most of the C_8 columns.

The order of the set for the RPLC columns is II > I > III, which fits the hydrophobicity of these components. For the HIC columns the order is III > II > I, *i.e.*, the retention of the most polar set is the highest, which could be an indication of the higher polarity of these stationary phases. It is also clear that the ranges spanned by the ln k_w values obtained are always wider for the RPLC columns. This means that the characterization of the columns with only this parameter is ambiguous as the order of the columns depends not only on the type of set used but also on the member of the set which is taken into account. On the other hand, the information relating to the nature of the components (homologous series) is neglected.

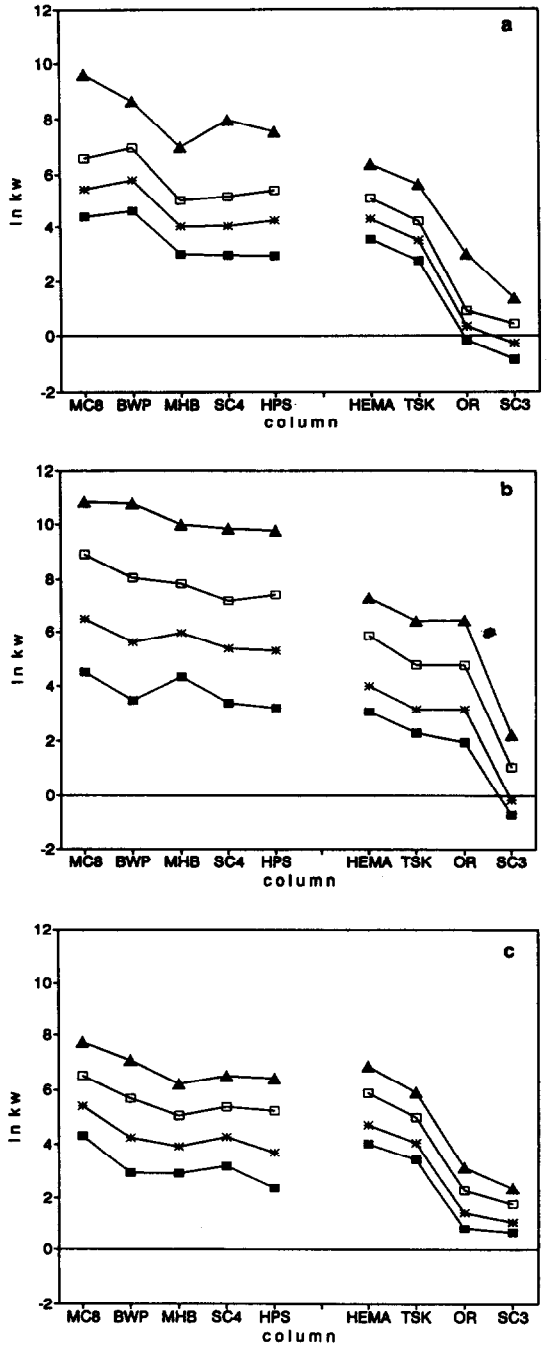

Fig. 1. Ln k_w values of the test components obtained on the different columns with (a) benzene homologues, (b) dialkyl phthalates and (c) *n*-alkyl *p*-hydroxybenzoates. (a) ■ = B; * = T; □ = mX; ▲ = pC. (b) ■ = DMP; * = DEP; □ = DPP; ▲ = DBP. (c) ■ = MP; * = EB; □ = PB; ▲ = BB (for abbreviations, see Experimental).

The characterization of apolar phases with homologous series is based on the work of Jandera [30–32] and Smith [33,34]. For such components

$$\ln k_w = a_0 + a_1 n_c \quad (2a)$$

$$S = m_0 + m_1 n_c \quad (2b)$$

and further

$$p = m_1/a_1 \quad (3a)$$

$$q = m_0 - a_0 p \quad (3b)$$

where n_c is the incremental carbon number of the homologous series and a_0, a_1, m_0 and m_1 are constants. The parameters m_0 and a_0 are the characteristic constants (slope and intercept) of the molecular residue of the homologous series ($n_c = 0$). After elimination of n_c from Eqs. 2a and 2b, we obtain

$$S = m_0 - a_0(m_1/a_1) + a(m_1/a_1) = q + p \ln k_w \quad (4)$$

The parameters of Eqs. 2–4 are not purely formal and an interpretation can be given on the basis of interaction indices [30,31].

The validity and applicability of the above model has been extensively investigated [19–22] on *n*-alkyl-modified silica phases with water–methanol eluents and the following conclusions were drawn. The selectivity of the separation system can be readily described by the parameters m_0 and q; m_0 reflects the apolar/hydrophobic selectivity and q gives the polar selectivity of the stationary phases. Hetem *et al.* [21] used *n*-alkylbenzenes, *n*-alkyl aryl ketones and *n*-alkyl *p*-hydroxybenzoates as test components on *n*-alkyl (C_1–C_{18}) phases with known surface characteristics. It was found that m_0 calculated for *n*-alkylbenzenes showed a good correlation with the hydrophobicity of the packings, *i.e.*, with the increasing carbon number of the ligands at identical surface coverage. As a measure of polar selectivity, q calculated for *n*-alkyl *p*-hydroxybenzoates was recommended because it showed a good correlation with the uncovered silica surface.

Eq. 2 was also fitted to our data and good correlations were found ($r > 0.963$) even for the HIC columns. The largest deviations were obtained for Set I, which was caused by *p*-cymene (pC). This component is not a "real" member of the homologous series; the apparent n_c is between 3 and 4. After re-evaluating the data of this set without pC, the correlation was significantly improved ($r > 0.984$).

As the model proved to be valid on the columns investigated, the characteristics were calculated according to Eqs. 2 and 3. The hydrophobicity and polarity of the stationary phases calculated for all the three sets are shown in Fig. 2a and b. It is clear that the parameters are highly dependent on the type of test components

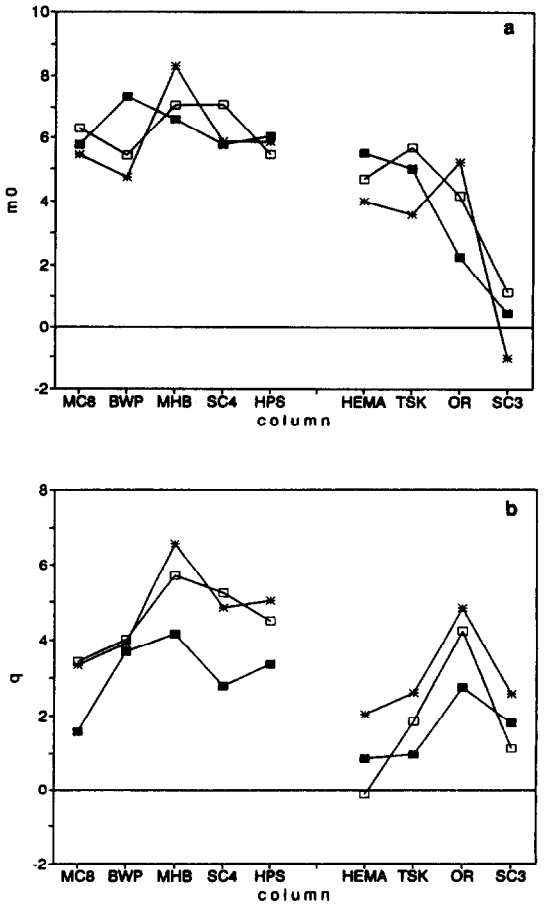

Fig. 2. Values of (a) hydrophobic and (b) polar selectivity obtained on the different columns. ■ = Set I; * = Set II; □ = Set III.

used, which is in agreement with the literature [19–22,30–34]. The hydrophobicity of the stationary phases measured by m_0(I) (m_0 calculated for Set I) corresponds well with that expected from the retention data and that indicated by the order of ln k_w values. (All further parameters derived from or relating to measurements with Sets I–III will be designated by the number of the set in parentheses.) This parameter is the slope in Eq. 1 for the molecular residue of Set I, which is benzene. As this component was included, the measured and calculated values were compared and a very good correlation was obtained ($r = 0.991$). The result is shown in Fig. 3. This means that this parameter can be measured directly with good accuracy.

Judging the polarity indicated by q(III) is not straightforward because the information concerning the surface properties of the stationary phases is deficient; nevertheless, the values obtained for the RPLC packings are in the range reported in the literature [19–22,30–34]. It is interesting that the order of polarity is more or less the same for all sets. This is also in accordance with the literature [21], where deviations were only obtained for columns having very short alkyl ligands (C_1, C_2). This means that for comparison any of these sets (or those used in the studies cited) could be used, and the polarity order of the columns is not affected as much by the type of the compounds as is the hydrophobicity.

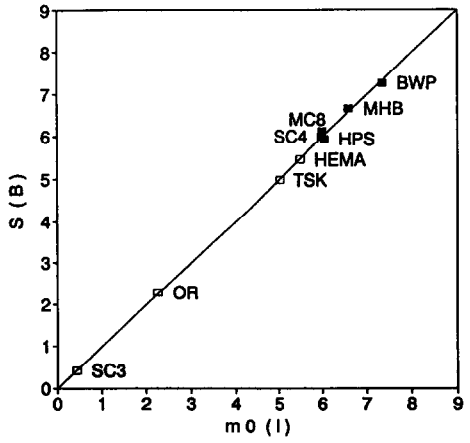

Fig. 3. Comparison of the calculated [m_0(I)] and measured [S(B)] slopes for benzene. ■ = RPLC; □ = HIC.

However, the overall picture seems to be slightly contrary to the recommendations of the manufacturers and to the order that could be expected from the ligand type and geometry of the packings. The largest deviations appear with HEMA, TSK and SC4. The HIC columns are intended for use in protein separations but stationary phases as strong as calculated cannot be operated under HIC conditions. A possible explanation of this behaviour may be the nature of the support. The stationary phases of these columns are formed by moderately hydrophobic phenyl (Ph) groups with low surface concentrations suited to protein separations and therefore low-molecular-mass components could have access to the uncovered part of the support having a similar hydrophobic character. Increased retention could result from this composite interaction. Another reason for this behaviour could be the specific affinity of these phases towards the aromatic components, *i.e.*, the increased retention could have been caused by Ph–Ph interactions. However, none of these explanations can hold true for SC4. In this case, possibly the lower hydrophobicity of the ligand was compensated for by an increased surface coverage. However, it seems that this sort of characterization of the hydrophobicity is applicable only for stationary phases with longer alkyl ligands (C_8 or larger) and for other types of ligand or support material it tends to overestimate the apolar character of the packings.

Another equation describing the behaviour of the homologous series under RPLC conditions was given by Bidlingmeyer *et al.* [35]:

$$\ln k = c_0 + c_1(c_2 - x)(c_3 - n_c) \quad (5)$$

where c_0–c_3 are constants. Although this equation was purely empirical, it described well the retention of some *n*-alkylsulphonates. In Eq. 5, c_2 was called the isopotential eluent composition (x_{ip}) because in this eluent ($x = c_2$) there is no difference between the retentions of the components. The ln k–x profiles converge to this point, *i.e.*, at this composition the retention is

independent of n_c. The retention corresponding to x_{ip} is c_0 (ln k_{ip}). It was presumed [35] that the isopotential eluent composition could be a measure of the overall polarity of the stationary phases because the higher is x_{ip} the lower is the polarity of the stationary phase.

As Eqs. 1–5 are consistent, the parameters of Eq. 5 can be readily formulated by the parameters of Eqs. 1–4:

$$c_0 = a_0 - m_0(a_1/m_1) = -q/p = \ln k_{ip} \quad (6a)$$

$$c_1 = m_1 \quad (6b)$$

$$c_2 = a_1/m_1 = 1/p = x_{ip} \quad (6c)$$

$$c_3 = -m_0/m_1 = n_{c,ip} \quad (6d)$$

The polarity of the stationary phases (x_{ip}) calculated for all sets is shown in Fig. 4. The values obtained for the RPLC columns are not realistic (the isopotential eluent content is >100%) but are applicable for comparison purposes. A value >1 means that even pure methanol is not strong enough to reach isopotential conditions. It is seen that the value of x_{ip} is dependent on the type of components used. The order indicated by x_{ip}(I) is very similar to that obtained with m_0(I) but the presumed lower hydrophobicity of the HIC and SC4 columns is clearly shown and the differences between the columns seem to be more acceptable. On the other hand, the order given by x_{ip}(II) and x_{ip}(III) is similar to that of q(II) and q(III) but, again, the values are more realistic. This means that x_{ip} acts as m_0 and q, and it is applicable to measure the polar–apolar character of the stationary phases. A two-dimensional mapping along x_{ip}(I) as hydrophobic and x_{ip}(III) as polar descriptors is shown in Fig. 5. The RPLC and HIC columns are clearly separated (they spread over different regions) but it is also seen that the HIC columns are, from a practical point of view, "weakened" RPLC columns, as indicated above, and the main difference is in the overall strength of the columns.

Inserting x_{ip} into Eq. 1 and rearranging gives

$$\ln k_w = \ln k_{ip} + x_{ip}S \quad (7)$$

which is the inverse of Eq. 4. Earlier, Eq. 4 was considered as a general function [36], valid for all RPLC stationary phases. Further studies [37] revealed some deviations from the suggested values. The differences were high for q but low for p. This seems to be in agreement with the literature [20,31], where p was postulated as a constant. The variations of the parameters were explained by the error of measurement and/or by the "incompleteness" of the sample set used, and less attention was paid to these results, because they do not cause serious errors in the

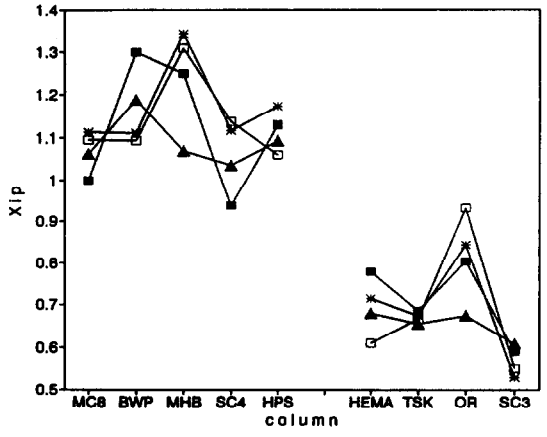

Fig. 4. Isopotential eluent composition obtained on the different columns with (■) (I) benzene homologues, (*) (II) dialkyl phthalates, (□) (III) *n*-alkyl *p*-hydroxybenzoates and (▲) (all) all the components.

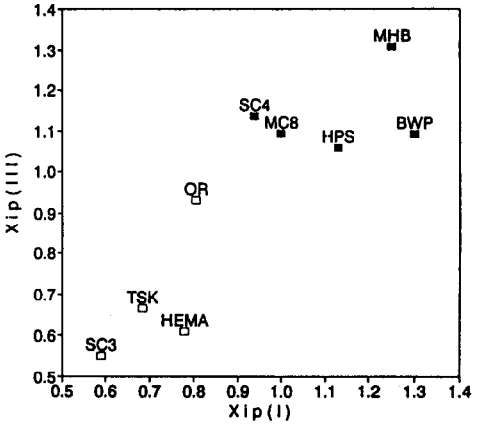

Fig. 5. Two-dimensional mapping of the columns. The axis x_{ip}(I) corresponds to the hydrophobicity and x_{ip}(III) to the polarity of the stationary phases. ■ = RPLC; □ = HIC.

prediction of retention on the most commonly used C_{18} columns [38,39]. However, on the basis of the model described, the parameters of the ln k_w–S or the S–ln k_w function should be characteristic for the stationary phases.

The slope and intercept values calculated for the RPLC columns are shown in Fig. 6a and those for the HIC columns in Fig. 6b. When the data were fitted to Eqs. 4 and 7, the correlations for Sets I–III were good ($r > 0.967$) on all columns but the parameters (q, p) were different. When all the data obtained on a column were fitted the correlation was lower ($r > 0.921$). This is the result of the diverse behaviour of Sets I–III, which is shown in Fig. 7a (RPLC) and 7b

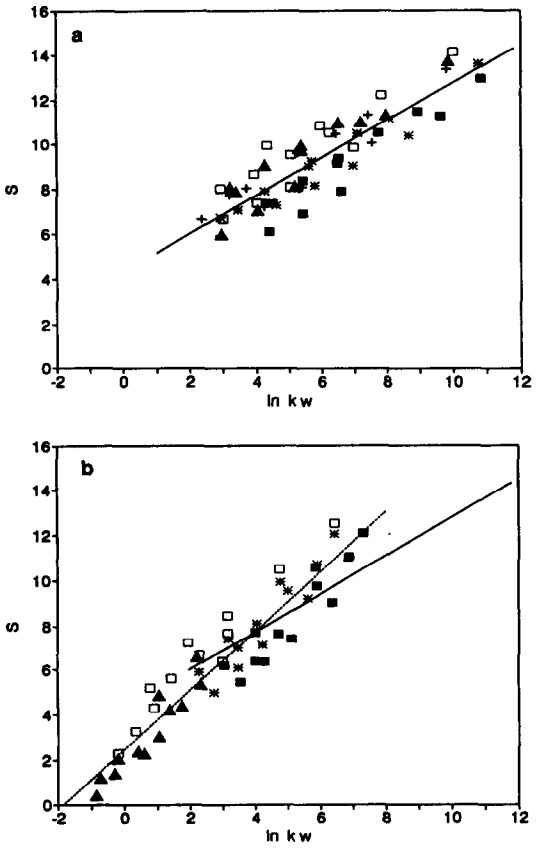

Fig. 6. Slope (S) and intercept (ln k_w) values obtained on the different (a) RPLC and (b) HIC columns. (a) ■ = MC8; * = BWP; □ = MHB; ▲ = SC4; + = HPS. (b) ■ = HEMA; * = TSK; □ = OR; ▲ = SC3. Solid line = RPLC; dashed line = HIC.

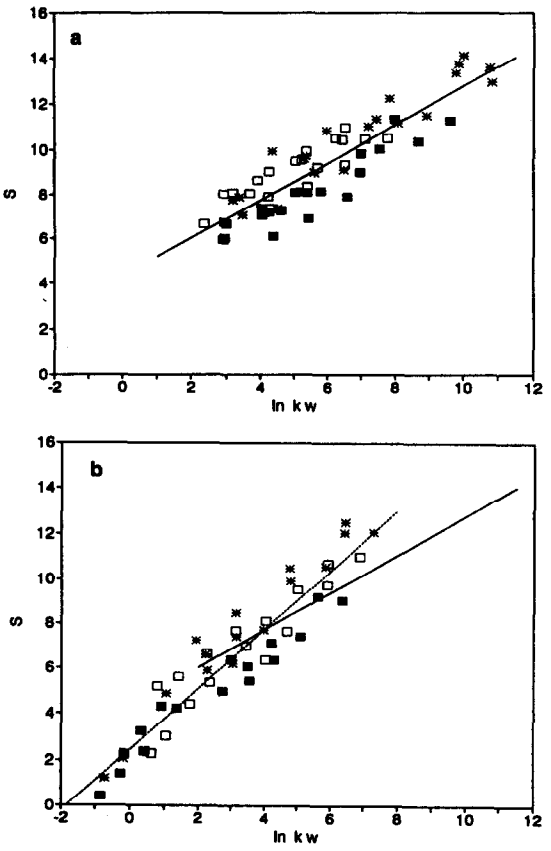

Fig. 7. Slope (S) and intercept (ln k_w) values obtained with (■) (I) benzene homologues, (*) (II) dialkyl phthalates and (□) (III) n-alkyl p-hydroxybenzoates on the (a) RPLC and (b) HIC columns. Solid line = RPLC; dashed line = HIC.

(HIC). In this instance p corresponds to a general x_{ip} value (see Eq. 6c), which is also shown in Fig. 4 [x_{ip}(all), triangles]. The order obtained with this parameter is the same as with x_{ip}(I), but the differences between the columns are smaller. For the HIC columns this order seems to be the most realistic. In our earlier studies [40,41] it was shown that the difference between TSK and SC3 when used under HIC conditions is small, which corresponds to the relationship indicated by x_{ip}(all) or x_{ip}(I) and x_{ip}(III) or q(III).

It is also seen in Figs. 6 and 7 that the ranges spanned by the values are different for the RPLC and HIC columns. When all the data obtained on the RPLC and HIC columns were

fitted to Eq. 4, two different functions were obtained. These are indicated by the lines in Figs. 6 and 7. The correlation was acceptable ($r > 0.941$ for RPLC and $r > 0.901$ for HIC). The "RP-like" behaviour of HEMA and TSK is clearly indicated. The parameters obtained on these columns fall in the range for RPLC columns but the direction of their spread is that of HIC columns. These results indicate that the discrimination of RPLC and HIC columns is arbitrary.

In Fig. 8 all the S–ln k_w values are shown. It is seen that the overall function relating to all points is curved, *e.g.*, logarithmic or square root type. Within this profile the columns span different regions (the strongest RPLC columns are located at the top right and the weakest HIC columns at the bottom left) which can be approximated with a linear function. The two-dimensional mapping of the columns along the parameters of these functions (p, q) is shown in Fig. 9. The order of the stationary phases along the q axis reflects polarity and the order along the p axis is inversely proportional to hydrophobicity. The values suggested in the literature [36,37] are also included for comparison. The small variation in p mentioned above can be attributed to the similar hydrophobicities of the stationary phases investigated (all were well conditioned C_{18} packings).

Possibly the scale of hydrophobicity would be

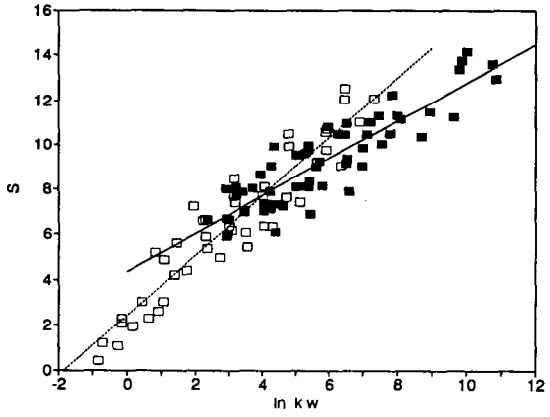

Fig. 8. Slope (S) and intercept (ln k_w) values obtained on the different columns. ■ (solid line) = RPLC; □ (dashed line) = HIC.

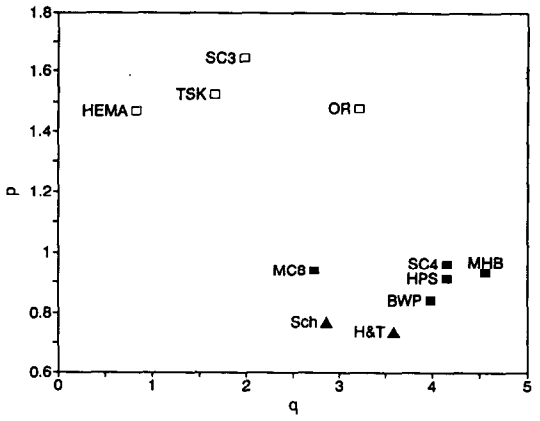

Fig. 9. Two-dimensional mapping of the stationary phases using the parameters of Eq. 4 obtained on the different columns and suggested in the literature [37–39]. ■ = RPLC; □ = HIC; ▲ = literature.

more realistic with x_{ip}(I) and that of the polarity with q(III) or x_{ip}(III) (*cf.*, Fig. 5), but we think that a more appropriate selection of the test components is needed. The investigation of some possible test mixture is in progress. On the other hand, these parameters [p(all), q(all)] are very practicable because they can be directly inserted into an optimization process [38,39,42] while this type of application of m_0(I), q(III) or any of the x_{ip} values is not trivial. Further, thorough characterization of the HIC columns under HIC conditions is also needed to judge the applicability of these types of descriptors for the design and optimization of HIC separations. This work has begun [40,41,43] and the available data seem to be in accordance with the results outlined above.

4. Conclusions

Nine commercially available prepacked columns used in RPLC and HIC with different characteristics concerning the type of ligands and the type and geometry of the support material were studied. Three homologous series were chromatographed under isocratic conditions with water–methanol as eluent. From the retention behaviour of the components, different characterizations of the stationary phases were performed according to the literature.

On the basis of ln k_w (the intercept of the ln

k–methanol content relationship), a rough estimate of the hydrophobicity of the stationary phases could be given. Some conclusions could also be drawn concerning the polarity of the packings, but the characterization was ambiguous.

More realistic results can be obtained with the polar–apolar characterization suggested by Hetem and co-workers [20–22], but some discrepancies were also found. It seems that this sort of characterization is applicable only for n-alkyl-modified silica packings with ligand chain length greater than C_4 and with a relatively high surface concentration. For columns of different types this method could give misleading results.

The use of the isopotential eluent composition as a descriptor resulted in the most acceptable characterization. From the parameters obtained for the different type of homologous series the polar–apolar nature of the stationary phases can be described.

Two-dimensional mapping along the parameters of the slope–intercept relationship seems to summarize the results obtained with other techniques. This type of representation reveals the polar and hydrophobic character of the stationary phases and also has practical relevance, *i.e.*, these parameters can be used directly in the design and optimization of separations.

5. Acknowledgements

This work was supported by the Hungarian Academy of Sciences under grant OTKA-1998. Special thanks are due to Dr. Jan Plicka (UVVVR, Prague), Dr. Robert Ohmacht (Medical University of Pécs) and Mr. Dietrich Brügel (Baker Chemikalien, Gross-Gerau) for donating the Separon HEMA, OR C_3-OH and Bakerbond WP columns, respectively.

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