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STATIONARY PHASE EFFECTS IN REVERSED-PHASE CHROMATOGRAPHY

I. COMPARISON OF ENERGETICS OF RETENTION ON ALKYL-SILICA BONDED PHASES

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SUMMARY

A large number of retention data on various hydrocarbonaceous bonded phases was examined in order to shed light on the energetics of retention in reversed-phase chromatography. Plots of logarithmic retention factors measured on column pairs with different eluites but with the same eluent were used in the analysis that was extended also to retention data obtained at different temperatures. Linear correlation with unit slope indicates identical intrinsic thermodynamic behavior for the two columns, *i.e.*, *homoenergetic* retention, as predicted by the solvophobic theory. Linear correlation with slope different from unity suggests similar physico-chemical basis for retention on the two columns that is termed *homeoenergetic*. No correlation is taken as proof that, besides solvophobic interactions with the stationary phase ligates, the eluites interact with residual silanol groups. Such heteroenergetic behavior implies the use of experimental conditions outside of solvophobic chromatography. Most bonded phases with medium- or long-chain alkyl ligates showed homoenergetic retention when water-rich eluents and relatively non-polar eluites without amino functions were employed, whereas those packed with stationary phases containing short alkyl chain or adamantyl ligates revealed homeoenergetic behavior. In contradistinction, only with eluents rich in organic solvent and/or with basic eluites was heteroenergetic behavior observed. It is concluded therefore that under conditions of solvophobic chromatography the main source of the differences between columns packed with hydrocarbonaceous bonded phases is a difference in phase ratio and possibly pore size distribution. In fact, if several columns exhibit homoenergetic retention behaviour it may indicate that the experimental conditions are those for solvophobic chromatography. Nevertheless the analysis clearly demonstrates that when eluites having polar groups are chromatographed in water-lean eluents the interaction with surface silanols can be significant. The approach presented can serve not only as a diagnostic tool for column and chromatographic conditions but also as a means to obtain information on the relative magnitude of column phase ratios and to predict retention data in solvophobic chromatography.

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INTRODUCTION

Alkyl-silicas are the most commonly used stationary phases in high-performance liquid chromatography (HPLC) and the corresponding technique employing polar eluents is called reversed-phase chromatography (RPC). When silanol groups at the surface of such stationary phases play a negligible role in the chromatographic process, the solvophobic theory has been found to provide a framework for the interpretation of retention and selectivity in RPC[1,2] and the technique can be appropriately termed solvophobic chromatography.

This work presents a phenomenological investigation of various hydrocarbonaceous bonded phases by using a statistical comparison of the energetics of eluite retention on selected column pairs. Most experimental results analysed were obtained with relatively non-polar eluites and with hydro-organic eluents rich in water, conditions under which the solvophobic theory is expected to hold. Nevertheless, columns were compared also under conditions where silanol groups at the stationary phase give rise to retention behavior different from that characteristic for solvophobic chromatography. In order to evaluate a large set of data, published experimental results from other laboratories have been examined in addition to our own chromatographic data.

THEORETICAL

Use of κ–κ plots

The retention factor k in RPC is given by

$$k = \varphi K \tag{1}$$

where φ is the phase ratio and K is the thermodynamic equilibrium constant for the reversible binding of the eluite to the stationary phase. We recall that the logarithm of the retention factor, which has been given the symbol κ[3,4] is directly related to the Gibbs free energy attributed to the retention process, ΔG^0, according to the following relationship

$$\kappa = \log \varphi + \log K \tag{2a}$$

$$\kappa = \Phi - \Delta G^0/2.3RT \tag{2b}$$

where R, T and Φ are the gas constant, temperature and the logarithm of the phase ratio, respectively.

The solvophobic theory[1,2] predicts that with the same eluent and in the absence of silanophilic interactions many alkyl-silica stationary phases exhibit identical Gibbs free energies for the binding of a particular eluite. Consequently the variations observed in retention behavior are believed to arise from differences in the phase ratios for the hydrocarbonaceous ligates.

Plots of κ values obtained on one stationary phase, A, *versus* those obtained on another, B, with the same mobile phase can serve as a useful tool for comparing

the energetics of eluite retention on different columns. The logarithmic retention factors κ_A and κ_B for the two columns can be written as

$$\kappa_A = \Phi_A - \Delta G_A^0/2.3RT \quad (3)$$

$$\kappa_B = \Phi_B - \Delta G_B^0/2.3RT \quad (4)$$

Subtraction and rearrangement of these equations yield

$$RT\kappa_A = RT\kappa_B + RT(\Phi_A - \Phi_B) + (\Delta G_B^0 - \Delta G_A^0)/2.3 \quad (5)$$

For any eluite, the second term on the right hand side of eqn. 5 is constant because it is the quotient of the phase ratios for columns B and A. The third term is also constant, but unlike the second term, its value may depend on the eluite used and will in fact depend systematically on κ_A (or κ_B) if the Gibbs retention energies for the two columns are not identical for all eluites when for each eluite the retention energy is the same for both phases, although the energies need not be the same for all eluites. In the case of special interest here, the second and third terms of eqn. 5 are constant for all eluites and linear κ–κ plots are obtained. The phenomenon of identical retention energies for a given eluite on two stationary phases is called homoenergetic retention ($\delta\mu o\varsigma$ = same). Homoenergetic behavior is expected to occur only when isothermal retention factors are compared. However, the underlying physical concomitants may persist at different temperatures and as shown below κ–κ plots of data taken at two different temperatures can be readily interpreted if the retention is homoenergetic under isothermal conditions.

The use of κ–κ plots as diagnostic tools is not novel to chromatography. In gas chromatography, double logarithmic plots of corrected retention volumes obtained on different phases are linear for a homologous series and the slopes are very similiar[5]. If retention is mainly due to vaporization from the stationary phase, Trouton's rule suggests such behavior. Differences in the intercepts of plots for various families of homologues is due in part to eluite interaction with stationary phases.

Retention at the same column temperature

Homoenergetic behavior. Eqn. 5 expresses the relationship between retention factors obtained on two different stationary phases, A and B. If the difference in the Gibbs retention energies of the two phases is zero for all eluites, eqn. 5 can be simplified.

$$\kappa_B = \kappa_A - \Phi_A + \Phi_B \quad (6)$$

In the case of homoenergetic retention, the logarithmic retention factor, κ, for one stationary phase is directly proportional to that for another with the same eluite and eluent, the slope of the plots is unity and the intercept is given by the logarithm of the quotient of the two phase ratios.

Homeoenergetic retention. If the corresponding Gibbs energies for the two chromatographic phases are not identical at a fixed temperature, they may be proportional so that

$$\Delta G_A^0 = \alpha \Delta G_B^0 \quad (7)$$

where α is a constant. Eqn. 7 can be combined with eqns. 3 and 4 to yield

$$\kappa_A = \alpha\kappa_B + \Phi_A - \alpha\Phi_B \tag{8}$$

Eqn. 8 shows that when the ratio of the Gibbs retention energies in two chromatographic systems is constant, linear κ–κ plots with slope α are obtained. In this case, the retention on a column pair can be termed homeoenergetic ($\delta\mu o\iota o\varsigma$ = like). It is seen that eqn. 6 can be considered a special case of eqn. 8 when α is unity.

Retention at different column temperatures

Enthalpy–entropy compensation. Retention on two stationary phases at different temperatures will not be homoenergetic in general. However, enthalpy–entropy compensation[6–10] has been found to exist between the retention enthalpies and entropies and this effect can give rise to homoenergetic retention at identical column temperatures.

In systems exhibiting enthalpy–entropy compensation a series of related chemical reactions may have large and widely different enthalpies and entropies, yet the corresponding Gibbs free energies will be nearly invariant across the series. This is so because differences in the enthalpies of reaction are partially offset by the corresponding entropy changes. At one temperature, called the compensation temperature, the compensation is exact. Adsorption to a surface or partitioning between two solvents can be considered chemical reactions and, in chromatography, compensation behavior may be observed among different eluites, eluents or stationary phases having similiar properties[6–9].

In the simple case, the enthalpy change, ΔH^0, for the chromatographic retention process is related to the corresponding entropy change, ΔS^0, by

$$\Delta H^0 = \Delta H_\beta^0 + \beta\Delta S^0 \tag{9}$$

where ΔH_β^0 and β are constants. The dimension of β is that of temperature and therefore it is called compensation temperature. It has been found that β is approximately 625°K in solvophobic chromatography[6].

Since for retention of an eluite on column A the binding enthalpy and entropy are related to the Gibbs energy by

$$\Delta G_A^0 = \Delta H_A^0 - T\Delta S_A^0 \tag{10}$$

eqns. 3, 9 and 10 can be combined to express the logarithmic retention factor for column A at temperature T_1

$$\kappa_A = \Phi_A - \frac{\Delta H^0}{2.3R}\left(\frac{1}{T_1} - \frac{1}{\beta}\right) - \frac{\Delta H_\beta^0}{2.3R\beta} \tag{11}$$

Linear κ–κ plots for data obtained on two columns operated at different temperatures can be explained by enthalpy–entropy compensation. The two cases examined here are related to homoenergetic and homeoenergetic retention behavior when the column pairs are at the same temperature. For convenience they are called isothermally homoenergetic and isothermally homeoenergetic retention behavior.

Isothermally homoenergetic retention. The logarithmic retention factor, $\kappa_{A,1}$, of an eluite on stationary phase A at temperature T_1 is related to the corresponding Gibbs free energy, ΔG^0_A, as

$$\kappa_{A,1} = \Phi_A - \Delta G^0_{A,1}/2.3RT_1 \tag{12}$$

A similar relationship can be written for $\kappa_{B,2}$ of an eluite on stationary phase B at temperature T_2 with the corresponding Gibbs free energy, $\Delta G^0_{B,2}$ as

$$\kappa_{B,2} = \Phi_B - \Delta G^0_{B,2}/2.3RT_2 \tag{13}$$

Whereas combination of eqns. 12 and 13 to obtain an expression of the form of eqn. 6 was not successful, enthalpy–entropy compensation observed in RPC[6–9] can be used to establish a relationship between $\kappa_{A,1}$ and $\kappa_{B,2}$ (ref. 3).

When the corresponding enthalpy and entropy are the same for systems A and B, $\kappa_{B,2}$ can be expressed as

$$\kappa_B = \Phi_B - \frac{\Delta H^0}{2.3R}\left(\frac{1}{T_2} - \frac{1}{\beta}\right) - \frac{\Delta H^0_n}{2.3R\beta} \tag{14}$$

Combinations of eqns. 11 and 14 with concomitant elimination of ΔH^0_B yields

$$\kappa_B = m\kappa_A + \Phi_B - m\Phi_A - \frac{\Delta H^0_n}{2.3R\beta}(1 - m) \tag{15a}$$

and

$$m = \frac{T_1}{T_2}\left(\frac{\beta - T_2}{\beta - T_1}\right) \tag{15b}$$

In view of eqns. 15a and 15b, $\kappa_{A,1}$–$\kappa_{B,2}$ plots of retention data will be linear with slope given by eqn. 15b if the Gibbs free energy is invariant for the two chromatographic systems and enthalpy–entropy compensation occurs.

Isothermally homeoenergetic retention. When the retention enthalpies in phase systems A and B are not equal but proportional

$$\Delta H^0_A = \alpha \Delta H^0_B \tag{16}$$

we can rewrite eqns. 11 and 14 to obtain the relationships

$$\kappa_B = m\kappa_A + \Phi_B - m\Phi_A - \frac{\Delta H^0_n}{2.3R\beta}(1 - m) \tag{17a}$$

and

$$m = \frac{T_1}{\alpha T_2}\frac{(\beta - T_2)}{(\beta - T_1)} \tag{17b}$$

Examination of eqns. 17a and 17b shows that two special cases yield linear $\kappa_{A,1}$–$\kappa_{B,2}$ plots. In the first case the entropy difference is zero, or the same for all eluites and

the enthalpy change for a given eluite is the same with both columns so that we obtain the relationship

$$\kappa_B = \frac{T_1}{T_2}\kappa_A + \Phi_B - \frac{T_1}{T_2}\Phi_A \quad (18)$$

This limiting case is obtained in view of eqn. 17 when the compensation temperature is infinitely large and the slope of the linear κ–κ plot is given by T_1/T_2.

The other limiting case occurs when the entropy change for the retention of a given eluite is identical with both columns and the enthalpy change is zero. In this particular situation eqn. 17 reduces to eqn. 6.

EXPERIMENTAL

Chromatographic data were obtained using a Perkin-Elmer (Norwalk, CT, U.S.A.) liquid chromatograph equipped with a Model 7105 (Rheodyne, Berkeley, CA, U.S.A.) sampling valve with a 20-μl injection loop. The column effluent was monitored at 210 or 254 nm with a Model LC-65T variablewavelength photometric detector (Perkin-Elmer) and the chromatograms were obtained with a Model SR 204 strip chart recorder (Heath, Benton Harbor, MI, U.S.A.).

The eluent was aqueous 0.1 *M* phosphate buffer, pH 2.15, plain or mixed with methanol (1:1). The flow-rate was 1.5 ml/min and the column temperature was maintained at either 25 or 45°C by using a constant temperature bath (Messgeraete Werke, Lauda, G.F.R.). The mobile phase was preheated by passage through a 10 m × 0.25 mm I.D. heat exchanger before entering the column.

Experimental results and literature data were analyzed by using a linear regression program written in BASIC on a PDP 11/10 computer (Digital Equipment Co., Maynard, U.S.A.). Standard error of parameter estimate was evaluated by the algorithm of Blaedel and Iverson[11] and deviations between experimental values and regressed values were also calculated and used to identify digressing data points. Benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, naphthalene and anthracene were obtained from Aldrich (Milwaukee, WI, U.S.A.). Progesterone, testosterone, amino acids, niacinamide, 4-hydroxypyrimidine, adenosine and xanthosine were from Sigma (St. Louis, MO, U.S.A.). Methanol was obtained from Fisher (Pittsburgh, PA, U.S.A.).

Both home-made and commercial columns, all 250 × 4.6 mm I.D., were used. Cholestanyl and adamantyl silica were prepared in our laboratory from 5-μm Spherisorb supplied by Phase-Sep (Hauppage, NY, U.S.A.) whereas 5-μm LiChrosorb RP-2, RP-8 and RP-18 stationary phases were obtained from Knauer (Berlin, G.F.R.). Columns with the above stationary phases were packed in our laboratory. Commercial columns also employed in the study were: Partisil ODS-3 (Whatman, Clifton, NJ, U.S.A.), Hypersil ODS (Shandon Southern, Sewickley, PA, U.S.A.), Zorbax ODS (DuPont, Wilmington, DE, U.S.A.) and Supelcosil LC-1, LC-8 and LC-18 (Supelco, Bellefonte, PA, U.S.A.).

RESULTS AND DISCUSSION

According to the solvophobic theory that assumes eluite binding to an ideal hydrocarbonaceous surface, the differences in the retention behavior of a given eluite on various columns by using the same eluent are essentially due to the

different phase ratios, at least when the alkyl ligates are the same. Silanol groups at the stationary phase surface, however, may give rise to a mixed mechanism for the retention of eluites with polar, particularly amino, functions. Here we are primarily concerned with the correlation of retention energies on different columns under otherwise identical conditions in order to compare the intrinsic properties of various columns used in RPC.

As discussed in the theoretical section, plots of κ values for a set of eluites obtained on one stationary phase *versus* those of the same set obtained on another stationary phase can serve as diagnostic tools for homoenergetic or homeoenergetic binding. In our study the linearity of the plots was measured by the correlation coefficient of the regressed line. Slopes and intercepts as well as the 95% confidence intervals for their estimates were also determined. The two criteria were used to examine the difference between two packing materials from the energetic point of view: linearity and the slope of the κ–κ plots. Homoenergetic retention results in linear κ–κ plots with unit slope according to eqn. 6. For our purpose, a correlation coefficient greater than 0.95 was used as an index of linearity and the slope was considered unity when the absolute difference between the observed slope and unity was less than the 95% confidence limit. Intracolumn comparisons of the data taken with identical columns, *e.g.* LiChrosorb RP-18, under identical conditions served to estimate the error in the analysis due to experimental uncertainty.

Retention behavior of various eluite types

Non-polar and moderately polar eluites. Řehák and Smolková[12] prepared various hydrocarbonaceous bonded phases by reacting LiChrosorb SI-100 silica with *n*-butyltrichlorosilane, octadecyltrichlorosilane, 2,4,4-trimethylpentylmethyldichlorosilane, *n*-octylmethyldichlorosilane, 4-butyloctyltrichlorosilane, 1-ethyladamantyltrichlorosilane and *n*-dodecyltrichlorosilane, and measured the retention of phenylalkanes, *n*-alcohols, dialkyl phthalates, benzene, toluene and *p*-xylene on such columns. Plots of the logarithmic retention factors *versus* the carbon number were linear for homologous eluites and branched hydrocarbons were more strongly retarded on stationary phases with branched ligates than on stationary phases with linear alkyl ligates of similar carbon number. Benzene, toluene and *p*-xylene were also more strongly retarded on stationary phases containing branched rather than non-branched ligates. Experiments were carried out on with three eluents, plain methanol for alkyl benzenes, methanol–water (70:30) for dialkyl phthalates, benzene, toluene and *p*-xylene, and methanol–water (30:70) for *n*-alcohols.

Retention factors obtained on various column pairs were subjected to linear regression (Table I). Slopes and correlation coefficients significantly different from unity are marked with asterisks. The data were pooled to include all three solvent conditions. However, in no case were retention data measured under two different solvent conditions compared, and all data points used in the regression represented the retention of a certain eluite with one particular eluent on two stationary phases. As the retention mechanism for RPC with non-aqueous eluents (NARP) may not be the same as that for RPC under normal conditions with plain aqueous (PARP) and mixed aqueous (MARP) eluents, sets of data with and without NARP points, *i.e.* those obtained with plain methanol as the eluent, were subjected to regression analysis.

The first six entries for Analysis I in Table I show that the retention values

TABLE I

COMPARISON OF THE RETENTION BEHAVIOR REPORTED BY ŘEHÁK AND SMOLKOVÁ[12] FOR RPC WITH VARIOUS HYDROCARBONACEOUS BONDED PHASES

Methanol and methanol–water mixtures (30:70) and (70:30) were the eluents. Retention data reported with eluites on column A and column B under otherwise identical conditions were used for κ_A–κ_B plots. The slopes, their 95% confidence limits (CI) and the correlation coefficients are listed. The first two sets I and II, represent slopes obtained with data sets without the alkylphthalates and neat methanol, respectively, and III represents the entire data pool. Asterisks indicate slopes or *r* values statistically different from unity.

Hydrocarbonaceous ligate		*Analysis I*		*Analysis II*		*Analysis III*	
Column A	*Column B*	*Slope ± CI*	*r*	*Slope ± CI*	*r*	*Slope ± CI*	*r*
Octadecyl	Dodecyl	1.07 0.16	0.9746	0.96 0.27	0.9556	0.99 0.08	0.9882
	Octyl	1.13 0.18	0.9732	1.05 0.32	0.9497*	1.26 0.08*	0.9923
	Adamantyl	0.98 0.18	0.9693	1.09 0.26	0.9782	1.30 0.09*	0.9904
	4-Butyloctyl	1.13 0.24	0.9546	0.97 0.39	0.9187*	1.32 0.09*	0.9910
	2,4,4-Trimethylpentyl	1.21 0.30	0.9358*	1.07 0.55	0.8716*	1.56 0.13*	0.9881
	Butyl	2.18 0.83*	0.8850*	2.16 1.15*	0.8653*	2.18 0.83*	0.8850*
Dodecyl	Octyl	1.05 0.05*	0.9977	1.11 0.05*	0.9986	1.25 0.11*	0.9851
	Adamantyl	0.85 0.19	0.9565	1.05 0.18	0.9884	1.27 0.13*	0.9798
	4-Butyloctyl	1.07 0.13	0.9835	1.02 0.26	0.9638	1.30 0.12*	0.9829
	2,4,4-Trimethylpentyl	1.15 0.19	0.9697	1.15 0.41	0.9339*	1.57 0.10*	0.9928
	Butyl	2.21 0.66*	0.9252*	2.28 0.93*	0.9148*	2.21 0.66*	0.9252*
Octyl	Adamantyl	0.82 0.15*	0.9710	0.95 0.13	0.9924	1.02 0.03	0.9982
	4-Butyloctyl	1.01 0.12	0.9845	0.93 0.20	0.9748	1.04 0.02*	0.9993
	2,4,4-Trimethylpentyl	1.10 0.16	0.9769	1.06 0.32	0.9508	1.15 0.04*	0.9981
	Butyl	2.07 0.55*	0.9386*	2.09 0.77*	0.9288*	2.07 0.55*	0.9386*
Adamantyl	4-Butyloctyl	1.17 0.29	0.9466*	1.00 0.09	0.9969	1.02 0.04	0.9973
	2,4,4-Trimethylpentyl	1.30 0.27*	0.9601	1.16 0.14*	0.9937	1.13 0.05*	0.9966
	Butyl	2.22 0.57*	0.9530	2.29 0.69*	0.9649	2.22 0.57*	0.9530
4-Butyl octyl	2,4,4-Trimethylpentyl	1.09 0.07*	0.9949	1.15 0.13*	0.9928	1.10 0.02*	0.9995
	Butyl	2.22 0.35*	0.9770	2.30 0.45*	0.9779	2.22 0.35*	0.9770
2,4,4-Trimethylpentyl	Butyl	1.95 0.29	0.9795	1.98 0.41*	0.9755	1.95 0.30*	0.9785

obtained on the various stationary phases, except butyl-silica and possibly 2,2,4-trimethylpentyl-silica and on octadecyl-silica yield linear κ–κ plots with slopes that are statistically indistinguishable from one. This strongly suggests that the intrinsic behavior of these phases is identical under the conditions investigated as far as the energetics of eluite retention is concerned. Butyl-silica, which contains the smallest hydrocarbonaceous ligate, appears to be different from all other stationary phases as indicated by the slope. Adamantyl-dodecyl, adamantyl-octyl and adamantyl-2,4,4-trimethylpentyl-silica stationary phase pairs failed to satisfy the test of unit slope and linearity and a possible explanation for the divergent behavior of adamantyl-silica is discussed later.

When retention data obtained with plain methanol as the eluent are also included in the analysis however, only the octadecyl-octyl, 4-butyloctyl-octyl, 4-butyl-octyl-adamantyl and octyl-adamantyl dyads passed the two tests for homoenergetic behavior. The poor correlation of binding energies measured with plain methanol is believed to be due to the effect of residual silanol groups at the stationary phase surface on retention in the absence of water in the eluent. On the other hand, in solvophobic chromatography, *i.e.* with hydro-organic eluent rich in water, silanophilic interactions are expected to contribute negligibly to retention of relatively non-polar eluite. Indeed, the results of this analysis suggest that the energetics of binding for non-polar eluites is essentially the same for almost all of these hydrocarbonaceous bonded phases as long as hydro-organic eluents are used in conventional RPC. Under such conditions the solvophobic theory[1] may facilitate a semiquantitative treatment of retention behavior.

The finding by Řehák and Smolková[12] that adamantyl-silica binds "branched" eluites, *e.g.* the dialkyl phthalates, stronger than expected on the basis of retention data for other systems has also been examined. Retention data obtained with aqueous methanol were reanalyzed but those for the dialkyl-phthalates were removed. For most column pairs with the exception of butyl-silica–adamantyl-silica the results were similar to those calculated originally and support the observation that adamantyl-silica stationary phase preferentially retains branched hydrocarbons. We shall discuss the effect after examining data obtained on other stationary phases with ligates of unusual geometry.

Eluites of wide ranging polarity. In order to compare retention energies for polar eluites in RPC the following substances were chromatographed in our laboratory on LiChrosorb RP-18, RP-8 and RP-2 as well as on a homemade adamantyl-silica stationary phase: adenosine, xanthosine, 4-hydroxypyrimidine, niacinamide, tryptophan, tyrosine, methionine, phenylalanine, leucine, isoleucine, phthalic acid, trihydroxybenzoic acid, dihydroxyphenylacetic acid, vanilmandelic acid, aniline and acetone. Plain aqueous 0.1 *M* sodium phosphate buffer, pH 2.2, was used as the eluent. The eluites, except leucine, isoleucine and 3,4,5-trihydroxybenzoic acid, were also chromatographed by using a mobile phase which contained 5 m*M* butylamine in 0.1 *M* H_3PO_4–NaH_2PO_4 buffer pH 2.2, to mask the surface silanols, thereby simultaneously identifying and eliminating their effect on retention.

Results obtained by analyzing the appropriate κ–κ plots are given in Table II. Data from different runs on the same column were not pooled; the multiple entries for each column give an indication of the reliability of analysis. The correlation coefficients and slopes of such analyses are expected to be constant unless either the

TABLE II

COMPARISON OF THE RETENTION BEHAVIOR OF POLAR ELUITES ON VARIOUS HYDROCARBONACEOUS BONDED PHASES

Eluent was 0.1 *M* phosphate buffer, pH 2.15, and contained also 0.05 *M* butylamine in the measurement of data compared in III. Slopes of κ_A *vs.* κ_B plots with the appropriate 95% confidence limits (CI) are listed for various column pairs. I contains the data pool without niacinamide whereas the retention data for all eluites including niacinamide obtained without and with butylamine in the eluent were used in II and III, respectively. Asterisks represent slopes or *r* values statistically different from unity.

Stationary phase		*Analysis I*		*Analysis II*		*Analysis III*	
Column A	*Column B*	*Slope* ± *CI*	*r*	*Slope* ± *CI*	*r*	*Slope* ± *CI*	*r*
RP-18*	RP-8*	0.92 0.12	0.9672	0.95 0.11	0.9701		
		0.94 0.14	0.9577	0.95 0.13	0.9628		
		0.93 0.12	0.9682	0.96 0.11	0.9705		
RP-18*	Adamantyl**	0.86 0.13*	0.9571	0.78 0.16	0.9204*		
		0.81 0.13*	0.9490*	0.74 0.16*	0.9094*		
RP-18*	RP-2*	0.73 0.30	0.7675*	0.68 0.28*	0.7499*		
		0.82 0.27	0.8787*	0.67 0.29*	0.7973*		
RP-8*	Adamantyl**	0.91 0.13	0.9642	0.79 0.17*	0.9107*	1.01 0.23	0.9698
		0.87 0.12*	0.9637	0.75 0.16*	0.9088*	0.83 0.13*	0.9693
		0.88 0.13	0.9576	0.79 0.17*	0.9127*	0.86 0.30	0.9166*
		0.84 0.12*	0.9604	0.75 0.16*	0.9128*	0.78 0.14*	0.9554
		0.90 0.13	0.9589	0.79 0.17*	0.9062*		
		0.85 0.12*	0.9500	0.74 0.17*	0.9041*		
RP-8*	RP-2*	0.83 0.28	0.8252*	0.73 0.26*	0.7929*	0.41 0.28*	0.6694*
		0.90 0.20	0.9398*	0.70 0.27*	0.8312*	0.37 0.26*	
		0.83 0.27	0.8293*	0.73 0.26*	0.8021*		
		0.91 0.21	0.9308*	0.71 0.27*	0.8326*		
		0.80 0.27	0.8237*	0.73 0.26*	0.8027*		
		0.90 0.21	0.9341*	0.70 0.28*	0.8212*		
Adamantyl**	RP-2*	0.83 0.32	0.7884*	0.83 0.31	0.7901*	0.39 0.48*	0.6051*
		0.92 0.28	0.8847*	0.90 0.29	0.8735*	0.44 0.32*	0.6183*
		0.84 0.38	0.7557*	0.84 0.34	0.7562*		
		0.94 0.34	0.8557*	0.84 0.35	0.8388*		

* LiChrosorb alkyl-silicas.

** Prepared from Partisil silicas gel.

experimental conditions are varied between the experimental runs under comparison or column properties change. Table II shows this to be the case. Intracolumn comparisons, *e.g.* LiChrosorb RP-8 *vs.* LiChrosorb RP-8, not shown in the table, also lent credence to this analysis as unit slopes within the 95% confidence limits which were usually less than 0.05, and correlation coefficients exceeding 0.99 were always obtained.

Niacinamide was stronger retained on adamantyl-silica than would be expected on the basis of the linear regression equation for adamantyl-silica columns and its retention on other stationary phases, *e.g.* LiChrosorb RP-8. On the premise that specific interactions between niacinamide and the adamantyl moiety might occur, the linear regression was repeated without niacinamide data, and the results of both the analyses are given in Table II.

Retention factors obtained on LiChrosorb RP-18 are highly correlated with those obtained on LiChrosorb RP-8 and the slope of κ–κ plots is indistinguishable from unity. On the other hand comparison of LiChrosorb RP-18 with adamantyl-silica did not yield unambiguous results. When the complete data set is considered, the linearity of the plots was not satisfactory since $r < 0.95$. Moreover, the confidence interval for the slope was so broad that the slope may indeed be different from one.

Upon excluding niacinamide data from the analysis, correlation coefficients increase and the confidence limits for the slope estimate become narrow. The unit slope of κ–κ plots suggests that the retention of most eluites on adamantyl-silica is homoenergetic with the retention on LiChrosorb RP-18 even if niacinamide deviates markedly from the rule.

The results are summarized in the third column of Table II. Comparison of the slopes of κ–κ plots for data obtained in the presence and absence of butylamine show little difference. Therefore, we cannot ascribe the anomalous behavior of niacinamide to participation of silanol groups in the retention mechanism, *i.e.* to silanophilic interactions.

Analysis of retention data obtained on LiChrosorb RP-2 demonstrates that this stationary phase differs fundamentally from LiChrosorb RP-18, RP-8 and adamantyl-silica. In no case are κ–κ plots linear as measured by the correlation coefficient and, although the 95% confidence intervals of the slope estimate usually encompass unity, the intervals are so broad as to moot the point.

In another set of experiments homemade adamantyl- and cholestanyl-silica and six commercial hydrocarbonaceous bonded phases were compared on the basis of the retention of benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, anthracene, naphthalene, testosterone and progesterone with methanol–water (1:1) as the eluent. The four different κ–κ plots, the linear regression of which is given in Table III, were made by using the following retention data: I, benzene and alkylbenzene only; II, all nine eluites; III, steroid data excluded; IV, data for anthracene and naphthalene excluded. This approach was taken in order to shed light on the role of steric factors causing unusual retention differences.

When data obtained with all eluites are included in the analysis, the values for cholestanyl-silica are collinear only with those for Zorbax ODS and Supelcosil LC-18. However, the slopes in all cases are nearly unity so that homoenergetic retention can be assumed with these eight stationary phases under the conditions investigated.

Whereas the analysis of alkylbenzene data shows excellent linearity for cho-

TABLE III

COMPARISON OF THE RETENTION BEHAVIOR OF STEROIDS, BENZENE, ALKYLBENZENES AND POLYNUCLEAR AROMATICS IN RPC WITH VARIOUS HYDROCARBONACEOUS STATIONARY PHASES

Methanol–water (50:50) was used as the eluent. Slopes of the κ_A *vs.* κ_B plots with the appropriate 95% confidence limits and correlation coefficients are listed for various column dyads. In I the retention factors of only benzene and alkylbenzene are compared whereas the entire data set is represented in II. On the other hand, retention data of steroids and polynuclear aromatics are excluded from the comparison in III and IV, respectively. Asterisks indicate slopes and *r* values statistically different from unity.

Stationary phase		*Analysis I*			*Analysis II*			*Analysis III*			*Analysis IV*		
Column A	*Column B*	*Slope*	± *CI*	*r*	*Slope*	± *CI*	*r*	*Slope*	± *CI*	*r*	*Slope*	± *CI*	*r*
Cholestanyl-silica	Supelcosil LC-1	0.97	0.13	0.9957	0.63	0.41	0.9472*	0.61	0.29*	0.8854*	1.13	0.40	0.9328*
	Zorbax ODS	1.22	0.11*	0.9979	1.14	0.27	0.9547	1.12	0.13	0.9924	1.39	0.39	0.9565
	Adamantyl-silica	1.08	0.16	0.9947	0.79	0.37	0.8410*	0.77	0.26	0.9401*	1.23	0.39	0.9451*
	Supelcosil LC-18	1.08	0.16	0.9948	1.04	0.24	0.9523	1.02	0.11	0.9936	1.24	0.39	0.9458*
	Supelcosil LC-8	1.18	0.25	0.9888	0.92	0.32	0.9046*	0.90	0.22	0.9659	1.31	0.38	0.9548
	Hypersil ODS	1.11	0.18	0.9936	0.79	0.23	0.9253*	0.77	0.27	0.9364*	1.12	0.11*	0.9944
	Partisil ODS-3	1.17	0.35	0.9792	0.98	0.41	0.8678*	0.95	0.21	0.9713	1.40	0.59	0.9099*
Supelcosil LC-1	Zorbax ODS	1.25	0.17*	0.9956	1.24	0.49	0.8779*	1.48	0.68	0.8949*	1.19	0.11*	0.9946
	Adamantyl-silica	1.12	0.02*	0.9999	1.10	0.13	0.9875	1.18	0.15*	0.9904	1.07	0.05*	0.9987
	Supelcosil LC-18	1.10	0.24	0.9881	1.13	0.46	0.8733*	1.33	0.65	0.8836*	1.08	0.13	0.9917
	Supelcosil LC-8	1.22	0.11*	0.9979	1.14	0.29	0.9444*	1.31	0.38	0.9540	1.13	0.11	0.9949
	Hypersil ODS	1.14	0.04*	0.9997	0.92	0.30	0.9116*	1.20	0.15*	0.9914	0.87	0.29	0.9390*
	Partisil ODS-3	1.22	0.20*	0.9936	1.28	0.29	0.9550	1.35	0.44	0.9421*	1.26	0.10*	0.9960
Zorbax ODS	Adamantyl-silica	0.88	0.13	0.9945	0.74	0.20*	0.9366*	0.69	0.22*	0.9448*	0.89	0.09*	0.9946
	Supelcosil LC-18	0.88	0.10*	0.9970	0.92	0.04*	0.9985	0.91	0.05*	0.9983	0.91	0.06*	0.9978
	Supelcosil LC-8	0.96	0.21	0.9888	0.84	0.13*	0.9790	0.81	0.15*	0.9796	0.94	0.11	0.9922
	Hypersil ODS	0.90	0.15	0.9936	0.67	0.17*	0.9426*	0.69	0.23*	0.9422*	0.74	0.21*	0.9567
	Partisil ODS-3	0.96	0.27	0.9805	0.91	0.17	0.9670	0.85	0.15	0.9816	1.04	0.18	0.9836
Adamantyl-silica	Supelcosil LC-18	0.98	0.24	0.9859	1.09	0.31	0.9318*	1.18	0.41	0.9359*	1.00	0.14	0.9894
	Supelcosil LC-8	1.09	0.08*	0.9987	1.07	0.16	0.9804	1.12	0.20	0.9824	1.06	0.06	0.9979
	Hypersil ODS	1.02	0.02*	0.9999	0.87	0.20	0.9535	1.01	0.01	0.9999	0.82	0.25	0.9514
	Partisil ODS-3	1.09	0.16	0.9949	1.18	0.16*	0.9822	1.17	0.24	0.9764	1.17	0.12*	0.9937
Supelcosil LC-18	Supelcosil LC-8	1.07	0.34	0.9766	0.91	0.16	0.9715	0.88	0.21	0.9695	1.03	0.17	0.9837
	Hypersil ODS	1.01	0.26	0.9841	0.73	0.20*	0.9329*	0.75	0.27	0.9325*	0.80	0.26	0.9421*
	Partisil ODS-3	1.07	0.40	0.9663	0.99	0.21	0.9617	0.92	0.21	0.9720	1.14	0.22	0.9784
Supelcosil LC-8	Hypersil ODS	0.93	0.05*	0.9992	0.81	0.16*	0.9653	0.87	0.15	0.9821	0.79	0.20*	0.9649
	Partisil ODS-3	1.00	0.08	0.9983	1.10	0.10	0.9928	1.04	0.06	0.9980	1.11	0.13	0.9916
Hypersil ODS	Partisil ODS-3	1.07	0.14	0.9958	1.24	0.34	0.9353*	1.15	0.24	0.9759	1.27	0.48	0.9248*

lestanyl-silica and other stationary phases, plots of data obtained with steroids and polynuclear aromatics exhibited poor linearity. The observed heteroenergetic behavior may be be due to the low energy for binding eluites with rigid molecular structure, such as polycyclic aromatics, to rigid stationary phase ligates, such as cholestanyl functions, with respect to the energy of binding to the alkyl ligates of other siliceous stationary phases. Indeed, upon exclusion of retention data for rigid eluites the linearity of κ–κ plots markedly increases.

Somewhat different results are seen when adamantyl-silica data are compared to those obtained on octyl-silica or octadecyl-silica. Although the poor linearity obtained with the whole data set improves significantly when only data of the alkylbenzenes are analyzed, the slopes are different from unity. Therefore, the results suggest that the retention on adamantyl-silica is not homoenergetic but homeoenergetic with that on octyl and octadecyl silicas.

The energetics of retention on Supelcosil LC-1 is different from that on most other hydrocarbonaceous bonded phases as seen from the generally poor linearity of κ–κ plots. When they are linear, the slopes differ from unity. Supelcosil LC-1 is similar to LiChrosorb RP-2 as both stationary phases contain short chain alkyl ligates. Therefore the interpretation of the behavior of LiChrosorb RP-2 probably serves in this case also.

Eluites containing basic functions. Use of κ–κ plots can reveal specific eluite–stationary phase interactions as shown by the following analysis of the data obtained by Sokolowski and Wahlund[13]. Those authors chromatographed propranolol, pentobarbital, diphenylacetic acid, disipramine, imipramine, trimipramine and N-methylimipramine on Nucleosil C_{18} and C_8, LiChrosorb RP-18 and RP-8, μBondapak C_{18}, as well as Hypersil-ODS using a mixture of 0.1 *M* phosphate buffer, pH 3.0, and methanol (1:1). Only data obtained with the column pairs LiChrosorb RP-8 and Nucleosil C_{18}, μBondapak C_{18} and LiChrosorb RP-8, μBondapak C_{18} and Nucleosil C_{18} as well as Hypersil-ODS and LiChrosorb RP-18 gave linear plots and only for the pair LiChrosorb RP-8 and μBondapak C_{18}, was the slope unity.

In another series of experiments 50 m*M* dimethyloctylamine was added to the eluent in order to mask the silanol groups at the stationary phase surface. As seen in Table IV, κ–κ plots were now in all cases linear with essentially unit slope. Thus the widely different retention behavior of the alkyl-silica stationary phases became homoenergetic upon addition of dimethyloctylamine to the eluent. The dramatic homoenergizing effect of the octylamine is believed to arise from its strong binding to the silanol groups at the surface of the stationary phase. As a result, silanophilic interactions with eluites are so attenuated that retention occurs via solvophobic interactions only.

Different column temperatures

We have shown in the theoretical section that due to enthalpy–entropy compensation in RPC the use of κ–κ plots for comparison of stationary phases may be meaningful also when the columns are operated at different temperatures. Chen and Horváth[4] reported that κ–κ plots were linear for data obtained on Partisil ODS, Spherisorb treated with octadecyltrichlorosilane and LiChrosorb RP-18 columns operated at 333, 296 and 343°K, respectively. The eluent was plain aqueous 0.1 *M* phosphate buffer, pH 2.1. We analyzed their data by the method described above.

TABLE IV

COMPARISON OF RETENTION BEHAVIOR OBSERVED BY SOKOLOWSKI AND WAHLUND[13] IN RPC OF POLAR SAMPLE COMPONENTS WITH DIFFERENT NON-POLAR BONDED STATIONARY PHASES WHEN DIMETHYLOCTYLAMINE WAS ADDED TO THE ELUENT

In the absence of this additive no correlation of the κ_A and κ_B values could be obtained. Slopes of the κ_A *vs.* κ_B plots obtained with their data as well as the appropriate 95% confidence limits and correlation coefficients are listed for various column pairs.

Stationary phase		*Slope* ± *CI*		*r*
Column A	*Column B*			
μBondapak C_{18}	Hypersil ODS	1.16	0.12	0.9934
	LiChrosorb RP-18	1.20	0.12	0.9938
	Spherisorb ODS	0.86	0.51	0.8420
Hypersil ODS	LiChrosorb RP-18	1.03	0.02	0.9997
	Spherisorb ODS	0.73	0.43	0.8436
LiChrosorb RP-18	Spherisorb ODS	0.70	0.43	0.8315
LiChrosorb RP-8	μBondapak C_{18}	0.83	0.09	0.9925
	Hypersil ODS	0.98	0.07	0.9966
	Spherisorb ODS-18	0.69	0.47	0.8040
	LiChrosorb RP-18	1.01	0.06	0.9981

Assuming that the energetics of retention is identical on these stationary phases we calculated theoretical slopes by using eqn. 15 and listed them in Table V. Slopes were also calculated by using eqn. 18 for the special cases when enthalpies are constant or negligible compared to binding entropies. The 95% confidence limits of the slopes for the plot of experimental data were estimated by the *t*-test[15].

The plots of experimental data yield slopes statistically different from those predicted on the basis of constant retention enthalpy with the exception of the Partisil ODS–LiChrosorb RP-18 column pair. The observed slopes and those predicted by assuming homoenergetic retention, however, are statistically indistinguishable in all three cases. Thus, data obtained at different temperatures also suggest that the energetics of eluite binding is essentially the same for the above phases at least when they are used with plain aqueous eluents.

Effects of stationary phase properties

Chemical nature of the hydrocarbonaceous ligates. As discussed above, the solvophobic theory predicts homoenergetic behavior as long as the eluent composition, the molecular contact area upon eluite binding and the dispersion forces exerted by the stationary phase on the eluite and mobile phase are the same. Indeed, under conditions investigated retention on all octadecyl-silica phases was found homoenergetic by using κ–κ plots as test. Comparison of octadecyl- and octyl-silicas also revealed homoenergetic retention behavior. In many cases octadecyl- and octyl-silicas exhibited retention behavior identical to that of adamantyl-silica.

In contradistinction, no short chain alkyl-silica such as LiChrosorb RP-2 or Supelcosil LC-1 has been found to be identical to octadecyl-, octyl-, or adamantyl-silica, which have much bulkier hydrocarbonaceous ligates. This is in agreement with the suggestion that an increase in the chain length of alkyl ligate above C_{10} (ref. 16) does not alter the properties of the stationary phase drastically and is sup-

ported by the observation that no increase in retention is observed for most eluites upon increasing chain the length of the ligate over 5–9 methylene groups[17].

The differences observed between alkyl-silicas and adamantyl-silica may be accounted for by the solvophobic theory which postulates that an important contribution to the energy of eluite binding to the ligate arises from van der Waals interactions. The energy of this contribution is proportional to the eluite size and opposite in sign to the other major term, that of cavity formation, that is also proportional to eluite size[1]. As a result, the overall retention energy is usually proportional to eluite size for most hydrocarbons. Whereas the Van der Waals potentials are quite similar for most hydrocarbons[18], peculiar steric effects associated with the binding of the eluite to the stationary phase ligate may account for either unusually large or anomalously low contact area. Consequently, retention in such systems will not be homoenergetic with respect to another regularly behaving phase yet it may still be homeoenergetic. As shown in the tables, the results of the data analysis lend support to this explanation.

Another interpretation is required, however, to account for the observed differences in the behavior of long chain and short chain alkyl-silicas. With ethyl- or methyl-silica poorly shielded siloxane and silanol groups will change the depth and position of the minimum of the Van der Waals force curve with respect to that for stationary phases having bulky hydrocarbonaceous ligates and the magnitude of Van der Waals interactions will be markedly different. As a result the contribution of the Van der Waals interactions to retention energy is not the same for hydrocarbonaceous bonded phases having very small and sufficiently bulky ligates so that κ–κ plots yield poor correlations when heteroenergetic behavior occurs, as shown by the pertinent correlation coefficients in Tables I–III.

Surface coverage. The influence of surface coverage on retention has been investigated by Tanaka *et al.*[19] by using Hypersil reacted to different extent with octyldimethylchlorosilane. The surface concentration of octyl ligates were 2.4 and 3.4 μmol/m^2 for the lightly loaded and the heavily loaded octyl-silicas, respectively. The latter was also subjected to aftertreatment with trimethylchlorosilane and hexamethyldisilazane in order to cap-off residual silanols of the stationary phase surface. Three solvent systems were used: methanol–water (50:50), acetonitrile–water (30:70) and tetrahydrofuran–water (25:75). The κ–κ plots of the two highly loaded stationary phases, show excellent linearity (Table VI) and the correlation coefficients are higher than 0.99. In contradistinction the slopes are different from unity when the lightly loaded octyl-silica is compared to the two other stationary phases. Closer examination of the retention data reveals that the absolute value of the retention free energy is lower for lightly loaded than for heavily loaded stationary phase.

Silanophilic interactions. In most cases hydrocarbonaceous bonded phases show homoenergetic behavior as long as water-rich eluents are used in the chromatographic experiments. Homoenergetic behavior does not necessarily imply that retention is governed by a single mechanism. If silanophilic interactions are also involved, such as in the case of dual mechanism[20], and both pathways are utilized to the same extent, the free energy of retention for different phases will be identical and homoenergetic behavior is observed. This would usually occur when the concentration ratio of accessible surface silanols and hydrocarbonaceous functions is the same in the two stationary phases under investigation.

TABLE V

ANALYSIS OF RETENTION DATA MEASURED BY CHEN AND HORVÁTH[4] AND MOLNÁR AND HORVÁTH[14] WITH OCTADECYLSILICA COLUMNS AND PLAIN AQUEOUS ELUENTS AT DIFFERENT TEMPERATURES

Slopes of κ_A *vs.* κ_B plots and the appropriate confidence intervals are listed. Besides slopes obtained from the comparison of experimental data, theoretical slopes calculated from eqns. 15 and 18 are also shown. Asterisks denote theoretically calculated slopes different from those given by the pertinent plots of experimental data.

Stationary phase		*Experimental data: Slope ± CI*	*Data calculated by eqn. 15: Slope*	*Data calculated by eqn. 18*	
Column A	*Column B*			*Slope*	*r*
Partisil ODS	Spherisorb ODS	1.27 ± 0.05	1.27	1.13*	0.9946
Partisil ODS	LiChrosorb RP-18	1.01 ± 0.07	0.94	0.97	0.9793
Spherisorb ODS	LiChrosorb RP-18	0.78 ± 0.07	0.74	0.86*	0.9701

TABLE VI

COMPARISON OF RETENTION DATA MEASURED BY TANAKA *et al.*[19] WITH "MONOMERIC" OCTYLSILICA STATIONARY PHASES PREPARED IN VARIOUS WAYS FROM HYPERSIL SILICA

The surface coverage of octylsilicas I and II is the same, 3.4 μmol/m^2, but in I the residual silanols are capped off in aftertreatment by TMS. The surface coverage of III is 2.4 μmol/m^2. Slopes and intercepts shown are those of κ_A *vs.* κ_B plots of data obtained with different eluents and column dyads.

Stationary phases		*Mobile phases*		
Column A	*Column B*	*Water–methanol (1:1)*	*Water–acetonitrile (7:3)*	*Water–tetrahydrofuran (3:1)*
		Slope ± CI	*Slope ± CI*	*Slope ± CI*
I	II	0.94 0.04	0.95 0.01	1.02 0.01
II	III	0.82 0.05	0.84 0.02	0.95 0.02
II	III	0.88 0.02	0.89 0.01	0.93 0.02
		Intercept ± CI	*Intercept ± CI*	*Intercept ± CI*
I	II	0.04 0.02	0.05 0.01	0.06 0.01
I	III	−0.07 0.02	0.06 0.02	0.17 0.01
II	III	−0.10 0.01	0.01 0.01	0.12 0.02

It is unlikely, however, that in any two bonded phases prepared by different methods or from different silica supports, the proportion of the two phase ratios for the two chromatographically active functions would be the same. Consequently, in such cases we may not expect the κ–κ plots to be linear or to have unit slopes, but to observe heteroenergetic behavior. We analyzed the data reported by Hansson *et al.*[21] for DOPA derivatives on Partisil ODS, Spherisorb ODS, Nucleosil C18 and LiChrosorb RP-18 by using water-rich eluents at low pH with or without 50 m*M* Na_2SO_4. The results showed clearly heteroenergetic retention for the dyads Partisil ODS–Spherisorb ODS and Spherisorb ODS–Nucleosil C_{18}. For the other column pairs, the confidence intervals of the slope estimate were larger than 0.4 and 0.2 for data obtained in the absence and presence of salt, respectively. Thus the retention behavior observed with the columns cannot be considered either homo- or homeoenergetic. The discrepancy is readily explained by the interaction of the ionized amino functions of the amino acids with the silanol groups at the surface and the attenuation of this effect at elevated ionic strength of the eluent.

Indeed, when the eluite has a charged amino group and/or the eluent is rich in organic solvent, the physicochemical basis of retention may significantly differ from that underlying the solvophobic theory because of specific eluite interactions with the silanol groups at the stationary phase surface. Since the balance of the surface concentrations of the accessible hydrocarbonaceous ligates and silanol group is unlikely to be the same for different bonded phases, comparison of retention data obtained under the above conditions ought to reveal heteroenergism.

We also analyzed the data of Goldberg and Wilson[22] who investigated the relative retention of five eluite pairs on twenty stationary phases employed in RPC, fifteen of which were octyl- or octadecyl-silicas and the results are shown in Table VII. In agreement with other findings reported here the selectivity differences between the columns increase with increasing organic solvent concentration in the eluent and/or

with increasing eluite polarity. Relative retentions for caffeine–theophylline show a large scattering that can be explained by the strong tendency of the nitrogens in these eluites to enter into silanophilic interactions even at relatively low acetonitrile concentrations. The magnitude of the effect is expected to depend on the concentration of accessible silanol groups in the various stationary phases employed in the study. The results strongly suggest that silanophilic interactions are mainly responsible for selectivity differences observed in the various alkyl-silica columns.

TABLE VII

SELECTIVITY STUDIES ON ALKYL-SILICA COLUMNS FOR FIVE ELUITE PAIRS

The results of Goldberg and Wilson[22] were analyzed to establish the scatter in selectivity as measured by the relative standard deviation (R.S.D.).

Chromatographic conditions		*Relative retention*		
Eluite pair	*Eluent*	*Mean*	*Range*	*R.S.D.*
Caffeine–theophyline	Acetonitrile–water (20:80; pH 4.5)	2.17	3.41–1.65	0.204
Toluic acid–benzoic acid	Acetonitrile–water (20:80; pH 4.5)	2.68	3.33–2.71	0.110
Diethyl phthalate–dimethylphthalate	Methanol–water (65:35)	2.22	2.52–1.58	0.122
Anthracene–naphthalene	Methanol–water (80:20)	1.99	2.67–1.61	0.145
Terphenyl–biphenyl	Methanol–water (90:10)	2.51	3.45–1.67	0.194

Phase ratio. The phase ratios of columns packed with alkyl silicas having long chain ligates are mainly determined by the packing density, the amount of hydrocarbonaceous ligate bound and the pore structure of the column material. The surface coverage by the hydrocarbonaceous ligate in stationary phases prepared from the same silica support increases with carbon load until a maximum is reached. The phase ratio is expected to have a similar dependence on the carbon load. However, the pores of the silica support may be comparable in size to the ligates and the alkyl groups in the bonded phases prepared from such silica may span the width of the pore and block passage of eluites through the pore. In this case a high carbon content can be undesirable because clogging of the pores may occur so that a large part of the particle interior becomes inaccessible to eluites.

Measurement of phase ratio is beset with great difficulty in chromatography with bonded phases as neither the effective volume nor the accessible surface area of the chromatographically active organic moiety can be clearly circumscribed. Furthermore, in the case of a mixed mechanism[20] phase ratios for both the organic ligates and the accessible silanol groups at the surface have to be defined. The difficulties may be exacerbated by changes in the effective volume or accessible surface area of the ligates with eluent composition and perhaps with the size of eluite molecules. As the mechanistic details of the interaction between eluite and stationary phase ligates *viz.* adsorption *vs.* partition, are still subject to controversy the definition of phase ratio remains ambiguous. Nevertheless for hydrocarbonaceous bonded phases the carbon load, the surface area of the silica matrix and the mobile phase volume in the

column allow a crude estimate of the phase ratio. When the same silica support is used the quotient of phase ratios for hydrocarbonaceous bonded phases might be estimated by the ratio of their carbon loads. Further work is needed, however, to examine the correlation between the numbers so obtained and the retention behavior for a variety of columns used in RPC.

In chromatographic practice the composition of the eluent is adjusted in order to obtain retention factors ranging between 0.5 and 20. Change in eluent strength, however, is usually accompanied by a change in relative retention of the eluites since solvent interactions with different substituents and other molecular subunits of the eluites are not identical[1,2,19]. Consequently selectivity differences frequently observed between alkyl bonded phases often derive from differences in eluite–eluent interactions, rather than from differences in the energetics of retention on columns having different phase ratios. As suggested by the results of the present analysis, most of the differences observed with hydrocarbonaceous bonded phases when water-rich eluents

TABLE VIII

PHASE RATIO QUOTIENTS FOR VARIOUS ALKYL-SILICA COLUMN DYADS

The quotient is evaluated as the antilog of the intercept of κ_A *vs.* κ_B plot and given by the ratio of the corresponding phase ratios, φ_A/φ_B. The ranges representing the 95% confidence limits of the quotients are given as appropriate multiplicands.

Stationary phase		*Quotient*		*Ref.*[*]
Column A	*Column B*	*Mean*	*Range*	
μBondapak C_{18}	Hypersil ODS	1.63	0.96–1.04	19
	LiChrosorb RP-18	1.60	0.96–1.04	19
	Spherisorb ODS	2.32	0.85–1.18	19
Hypersil ODS	LiChrosorb RP-18	0.97	0.98–1.02	19
	Spherisorb ODS	1.63	0.74–1.36	19
	Partisil ODS-3	0.77	0.74–1.36	
Zorbax ODS	Supelcosil LC-18	0.91	0.80–1.26	
	Hypersil ODS	1.03	0.71–1.41	
	Partisil ODS-3	1.35	0.52–1.91	
Supelcosil LC-18	Hypersil ODS	1.16	0.61–1.64	
	Partisil ODS-3	1.55	0.46–2.15	
LiChrosorb RP-18	Spherisorb ODS	1.67	0.74–1.35	
LiChrosorb RP-8	μBondapak C_{18}	0.78	0.95–1.05	19
	LiChrosorb RP-18	1.18	0.97–1.03	19
	LiChrosorb RP-18	1.22	0.96–1.04	
	Hypersil ODS	1.21	0.96–1.04	19
	Spherisorb ODS	1.91	0.77–1.30	19
Supelcosil LC-8	Zorbax ODS	1.85	0.61–1.63	
	Supelcosil LC-18	1.62	0.53–1.89	
	Hypersil ODS	1.84	0.94–1.07	
	Partisil ODS-3	2.49	0.90–1.11	
LiChrosorb RP-2	LiChrosorb RP-18	3.68	0.90–1.11	
	LiChrosorb RP-8	3.14	0.91–1.10	
Supelcosil LC-1	Zorbax ODS	7.42	0.95–1.06	
	Supelcosil LC-18	5.32	0.92–1.08	
	Supelcosil LC-8	3.69	0.96–1.04	
	Hypersil ODS	6.26	0.99–1.01	
	Partisil ODS-3	9.23	0.94–1.07	

[*] Data from this laboratory unless otherwise indicated.

and relatively non-polar eluites having no basic functions are employed can be attributed to differences in the phase ratio and pore size distribution, rather than to differences in intrinsic thermodynamic properties.

For column pairs exhibiting homoenergetic retention the intercept of κ–κ plots yield the quotient of the phase ratios of two columns, according to eqn. 6. Consequently homoenergetic κ–κ plots allow us to estimate the relative magnitude of the phase ratios for various columns.

Table VII shows that the ratios or their reciprocals range between one and ten, *i.e.* the phase ratio values of all columns encompass less than an order of magnitude. The differences are sufficiently great to mandate significantly different operating conditions for attaining practical retention values. For instance the phase ratio for Supelcosil LC-8 is about 2.5 times lower than that for Partisil ODS-3. Consequently, retention factors on ODS-3 are expected to be 2.5 times greater than on Supelcosil LC-8 under identical mobile phase conditions and at the same temperature. In order to obtain identical retention factors for a given eluite on the two phases, eluents of different composition have to be used. A hydro-organic eluent of intermediate composition should contain about 10% more organic solvent for Partisil ODS-3 rather than for Supelcosil LC-8 in order to obtain about the same retention values on both columns. As a result the apparent selectivity of the two stationary phases will be different because polar groups of eluites interact differently with mobile phases having such variation in composition[3,19].

When silanophilic interactions also play a significant role in determining the energetics of retention, phase ratio differences can no longer account for the observed variation in retention behavior. Under such conditions the phase ratio quotient for a given column pair may depend on the eluent composition and the eluite as well. Indeed, analysis of retention data obtained by Tanaka *et al.*[19] with lightly and heavily loaded stationary phases show a dependence of the phase ratio quotient on the mobile phase proper. With methanol–water, the quotient for the heavily to lightly loaded stationary phases is about 0.8 whereas with aqueous acetonitrile or tetrahydrofuran the quotients were found to be 1.14 and 1.4, respectively. The result may indicate differential solvation of the stationary phase ligates but more likely it reflects differences in masking of surface silanols by the eluent. A reduction of silanophilic interactions by tetrahydrofuran in the eluent has been already been put forward by Tanaka *et al.*[19]. It should be noted perhaps that according to our analysis the retention behavior on all stationary phases is closest to homoenergetic when aqueous tetrahydrofuran is used in the eluent. Moreover, the phase ratio quotient is essentially the same as that estimated from the carbon load of the stationary phases in question (1.41 = 3.4/2.4).

CONCLUSIONS

Results obtained by analyzing a large number of κ–κ plots suggest that the retention on medium and long chain alkyl-silica stationary phases is homoenergetic as long as the eluite does not contain silanophilic functions or the surface silanol groups are masked and the eluent is sufficiently rich in water. These conditions circumscribe solvophobic chromatography, *i.e.* "genuine" RPC, in which the solvophobic theory can account semi-quantitatively for the factors governing the observed re-

tention behavior and differences between columns arise from differences in their phase ratios. On the other hand, heteroenergetic behavior in RPC is believed to indicate mixed retention mechanism due to silanophilic interactions.

Homoenergetic κ–κ plots allow the transformation of retention factors from one column to another for the same elution condition by using the quotient of the phase ratios. Consequently, κ–κ plots may serve not only as useful diagnostic tools but also as means to predict the optimal column choice for a required retention factor.

Moreover the intrinsic identity of columns in solvophobic chromatography might make possible the tentative identification of unknown sample components from normalized retention factors which are calculated by using an appropriate phase ratio with respect to an arbitarily chosen "standard (reference) column". The use of retention data obtained under different conditions as far as eluent composition and temperature are concerned might be made possible by recent finding that the retention factors of alkylbenzenes obtained under different mobile phase conditions and/or temperatures are interrelated in a simple fashion and, as a consequence, retention at a given eluent composition and column temperature can be predicted from those obtained under other conditions[3]. The approach has shown promise for extension to more complicated molecules.

Further work is required to exploit the potential of the method proposed here although κ–κ plots may have an immediate use as analytical tools for the energetics of retention in RPC. Whereas they reveal the complexity of the chromatographic process over the broad range of conditions characteristic for this technique, they also suggest a common physico-chemical basis for retention in solvophobic chromatography.

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