

CHROM. 10,972

COMPARISON OF SOME PACKINGS FOR REVERSED-PHASE HIGH-PERFORMANCE LIQUID-SOLID CHROMATOGRAPHY

II. SOME THEORETICAL CONSIDERATIONS

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SUMMARY

The retention mechanism in reversed-phase chromatography (RPC) with bonded phases has been studied. It is shown that the variation of the logarithm of the capacity factor with the inverse of the temperature and with the carbon number in homologous series does not give reliable information on the retention mechanism. The comparison between equilibrium isotherms of *n*-octylbenzene in methanol and either ODS-modified silica or liquid *n*-octadecane, and also the structure of the modified packing, suggest an adsorption rather than a partition process.

The efficiency of RPC columns is high but attention must be paid to the importance of the error of measurements when trying to estimate Knox's *C* parameter in HETP equations.

The solvent strength of water-methanol has been studied over the whole range of concentrations and the effects of various organic water modifiers are compared.

Elutions were performed at different temperatures and the enthalpies of transfer were measured.

INTRODUCTION

Reversed-phase liquid chromatography (RPC) is playing an increasingly important role in high-performance liquid chromatography (HPLC). This is essentially due to the development of chemically bonded phases (BP), which are much easier to use than mechanically held packings. Grafting various organic moieties on 5- or 10- μ m silica particles allows the preparation of very efficient columns and provides rapid analyses.

The main characteristics of this technique have been reviewed and discussed¹. A comparative analysis of the analytical properties of different RPC packings was made in Part I of this work², which deals with *n*-C₁₈ silicas of various origins, phenyl-silica [where a C₆H₅-(CH₂)₂ moiety is bonded to the silica surface] and carbon black, as well as silica gel modified by pyrocarbon. Part II is concerned with the retention mechanism, efficiency, solvent eluotropic strength and the effect of column temperature.

RETENTION MECHANISM IN RPC

If the mechanism of the retention of solute molecules on pyrocarbon packings is due to an adsorption process, then the true mechanism on bonded reversed phases would become even more debatable¹. Is it an adsorption on the non-polar bonded moieties or a partition in a stationary phase composed either of the chemically grafted molecules, of solvent molecules or of a mixture of both?

One must first define precisely the difference between adsorption and partition. Adsorption means the interactions of the stationary phase with the solute or solvent molecules covering the external molecular layer of the "adsorbent". In the simplest case, adsorption is characterized by a monolayer in which the solute and solvent molecules are in competition to cover the external surface of the packing. More often, the interfacial region between the adsorbent and the bulk mobile phase can be composed of several layers. The separation surface results from an arbitrary choice and is generally selected in a position such that the Gibbs' surface excess for the solvent is zero³. In a partition process, the sole role of the support is to create a large surface area where the stationary liquid phase will be deposited. The phenomena in the interfacial region are then generally neglected and the solute molecules partition between the two liquid phases. Each phase contains two components: the solvent and the solute for the mobile phase and the stationary liquid and the solute for the stationary phase.

Difficulty of distinguishing between a partition and an adsorption process

Nakae and Muto⁴ suggested that the retention on Hitachi gel (a styrene-divinylbenzene copolymer) is due to a partition process because of the linear variation of the logarithm of the capacity factor with the inverse of the temperature and with the number of carbon atoms in homologous series. This cannot be considered as a characteristic of partition systems¹ because this kind of retention pattern is very similar for both liquid-liquid (LLC) and liquid-solid chromatography (LSC).

In their systematic studies of LLC, Locke and Martire⁵ established expressions for the variation of the logarithm of the net retention volume (V_g) per gram of packing with the inverse of the temperature (eqn. 1) and with carbon number (eqn. 2) for series of homologous compounds:

$$\frac{\partial \ln V_g}{1/T} = \Delta \bar{h}_2^e + RT^2 \alpha_m \quad (1)$$

$$\partial \ln V_g = \alpha + \beta n \quad (2)$$

where $\Delta \bar{h}_2^e$ is the partial molar enthalpy of transfer of the solute between the mobile and the stationary phases, α_m is the thermal expansion coefficient of the mobile phase, α and β are constants, n is the carbon number of the linear alkyl chain of the molecules and T is the absolute temperature. Using the equation

$$V_g = \frac{k' V_M}{W_s} \quad (3)$$

where V_M is the mobile phase hold-up of the column and W_s the weight of stationary

phase, it is possible to obtain equations similar to eqns. 1 and 2 that account for the variation in the logarithm of the capacity factor, k' .

The most complete work on LSC has been carried out by Snyder⁶. The variation in the thermodynamic adsorption equilibrium constant with temperature (K_0) is given by

$$\log K_{0(T)} = \log V_a + \alpha \left(1 - \frac{1 - 297/T}{1 - 297/a} \right) f(x, s)_{T_0} \quad (4)$$

where V_a is the volume of a monolayer of solvent adsorbed on unit mass (1 g) of packing, α is the activity of the adsorbent and a and $f(x, s)_{T_0}$ are thermodynamic constants. If the activity, α , is kept constant on changing the temperature, then

$$\log K_{0(T)} = A - \frac{B}{T} \quad (5)$$

Using the following equation, which relates k' and K_0 , a linear dependence of $\log k'$ on $1/T$ is obtained:

$$k' = K_0 \cdot \frac{A_s}{V_M} \quad (6)$$

where A_s is the total surface area of adsorbent in the column.

It is possible to derive an equation similar to eqn. 2 by using the theory of Locke⁷ for LSC:

$$\ln K_0 = \frac{a_2 (\sigma_1^0 - \sigma_2^0)}{RT} + \frac{a_2}{a_1} \cdot \ln \left(\frac{V_1^M}{V_1^S} \right) + \ln \left(\frac{\gamma_2^{M,\infty}}{\gamma_2^{S,\infty}} \right) \quad (7)$$

where subscripts 1 and 2 represent the solvent and the solute, respectively, a_i , V_i^M and V_i^S are the molar area and the molar volumes of species i in the mobile and stationary phases, respectively, $\gamma_2^{i,\infty}$ the activity coefficient of solute in phase i under conditions of infinite dilution and σ_i^0 is the interfacial tension between species i and the adsorbent (0).

As did Locke and Martire⁵, we assume that activity coefficients in homologous series can be expressed by using the first two terms of the Pierotti *et al.*⁸ development:

$$\ln \gamma_2^{i,\infty} = a_i + b_i n \quad (8)$$

where a_i and b_i are constants depending of the nature of the solute and/or solvent for the phase i .

According to Fowkes⁹, the interfacial tension, σ_i^j , is given by

$$\sigma_i^j = \gamma_i + \gamma_j - 2\sqrt{\gamma_i^d \gamma_j^d} \quad (9)$$

where γ_i is the surface tension of species i and γ_i^d the dispersive contribution of surface tension. When the solvent is kept unchanged, the first term of the right-hand side of eqn. 7 is given by

$$\frac{a_2}{RT} (\sigma_1^0 - \sigma_2^0) = a_2 (A - \gamma_2 - 2\sqrt{\gamma_2^d \gamma_0^d}) \quad (10)$$

where A is a constant depending on the solvent; $\gamma_2 - \sqrt{\gamma_2^d \gamma_0^d}$ has been estimated for compounds in homologous series (n -alkanes and n -alkylbenzenes) on graphite, γ_0^d is 123 erg/cm² (ref. 9) and γ_2 and γ_2^d are obtained from data in ref. 10. Because the value of a_2 is 8.5 Å², then $2.3RT$ is 114 at 20°C (ref. 11). Values of $\gamma_2 - 2\sqrt{\gamma_2^d \gamma_0^d}$ are given in Table I (note that $\gamma_2 - 2\sqrt{\gamma_2^d \gamma_0^d}$ is $-\tau_A$, where τ_A is the work of adhesion). It appears that the work of adhesion is virtually constant for homologous compounds. The dispersion of the data is larger for n -alkylbenzenes because the introduction of one CH₂ group has a much more important effect in short than in long chain, which explains why the data for benzene, toluene and ethylbenzene are different.

TABLE I
WORK OF ADHESION IN HOMOLOGOUS SERIES

Solute	a_2 ($l \equiv 8.5 \text{ \AA}$)	$\tau_A/2.3 RT$
<i>n</i> -Hexane	6.8	0.66
<i>n</i> -Heptane	7.7	0.65
<i>n</i> -Octane	8.6	0.67
<i>n</i> -Decane	10.4	0.69
<i>n</i> -Dodecane	12.2	0.69
<i>n</i> -Tetradecane	14.0	0.71
<i>n</i> -Hexadecane	15.8	0.70
		Average: $0.68 \pm 3\%$
Benzene	6	1.11
Toluene	6.8	1.06
Ethylbenzene	8.5	0.93
Propylbenzene	9.4	0.89
Butylbenzene	10.3	0.89
		Average: $0.98 \pm 10\%$

If we assume that a_2 is proportional to the carbon number (n) in the molecule for homologous solutes, then eqn. 10 becomes

$$\frac{a_2}{RT} (\sigma_1^0 - \sigma_2^0) = k_1 + k_2 n \quad (11)$$

Combination of eqns. 6, 7, 8 and 11 gives a linear variation of $\ln k'$ with n .

Equilibrium isotherms

It seems difficult to make a distinction between adsorption and partition mechanisms on the basis of the variation in k' with either temperature or carbon number. Adsorption or partition isotherms can give more useful information. If we assume that unreacted silanol groups have no effect on the retention of non-polar solutes, it is reasonable to compare the equilibrium isotherms on ODS-silica and on n -octadecane (n -C₁₈). Because the melting point of n -C₁₈ is about 30°C, it is *a priori* possible to use this material in the liquid state (partition) or in the solid state (adsorption). However, it has been impossible to measure the adsorption isotherms as we could not prepare a powder with a large enough specific surface area.

Isotherms are determined by the static method. A given amount of packing material is left in contact with a known volume of a standard solution (with a solute

molar fraction of x_0). Once equilibrium has been attained, the actual solute molar fraction (x) is measured. The quantitative analysis of the solution is carried out by HPLC, using an ODS column and methanol as solvent. The solute peak area is measured with a digital integrator; the molar fractions are determined by the external standard technique. The static method can be used for both liquid–solid and liquid–liquid systems; we used the systems methanol–ODS bonded particles (LiChrosorb RP18; Merck, Darmstadt, G.F.R.) and methanol–liquid n -C₁₈, the solute being n -octylbenzene (n -C₈Bz).

The partition isotherm represents the variation in the solute concentration (moles per gram) in n -C₁₈ with that in methanol at equilibrium. The concentration in n -C₁₈ is given by

$$C = n \left(\frac{x_0}{1 - x_0} - \frac{x}{1 - x} \right) / m \quad (12)$$

where n is the number of moles of methanol and m the weight of liquid n -C₁₈. For dilute solutions:

$$C = n(x_0 - x) / m \quad (13)$$

$$1 - x_0 \approx 1 - x \approx 1 \quad (13b)$$

Fig. 1 shows (curves 1 and 2) that the isotherm is nearly linear in the concentration range 0 – 10^{-4} mole/g (in methanol). The corresponding value of the equilibrium partition constant (ratio of the concentration in n -C₁₈ to that in methanol) is 6. The deviation from linearity at a methanol concentration of $7 \cdot 10^{-5}$ mole/g is -6% , the equilibrium partition constant being 5.64 instead of 6.0. The isotherms do seem to be markedly modified when changing the temperature.

Considering the data obtained with ODS-silica particles, we can assume either a partition or an adsorption process.

In an adsorption process, the adsorbed layer contains both n -C₈Bz and methanol molecules. As it is impossible to know the volume of the adsorbed layer, we use the Gibbs' excess (Δ), given by

$$\Delta = n_0(x_0 - x) / m \quad (14)$$

where n_0 is the total number of moles of the liquid system (methanol plus n -C₈Bz) and m (weight of adsorbing material) is equal to $M \cdot \% \text{ODS}$, where M is the weight of modified silica introduced into the vessel and $\% \text{ODS}$ is the percentage of bonded ODS on that silica. The adsorption isotherms (variation of Δ with x) are given in Fig. 2. As usual, the adsorption increases as the temperature decreases. Note that the dependence of the isotherm with temperature is much more important for ODS-modified silica than for pure n -C₁₈. The tangent at the origin of lines in Fig. 2a was obtained by assuming linear isotherms at low molar fractions (Fig. 2b).

If retention is due to a partition process, the data are handled in the same way as in the study of the system liquid n -C₁₈–methanol. The isotherm obtained at 40.5°C is shown in Fig. 1. The difference in the isotherm obtained for the pure partitioning system is important, the equilibrium constant being only 1.25.

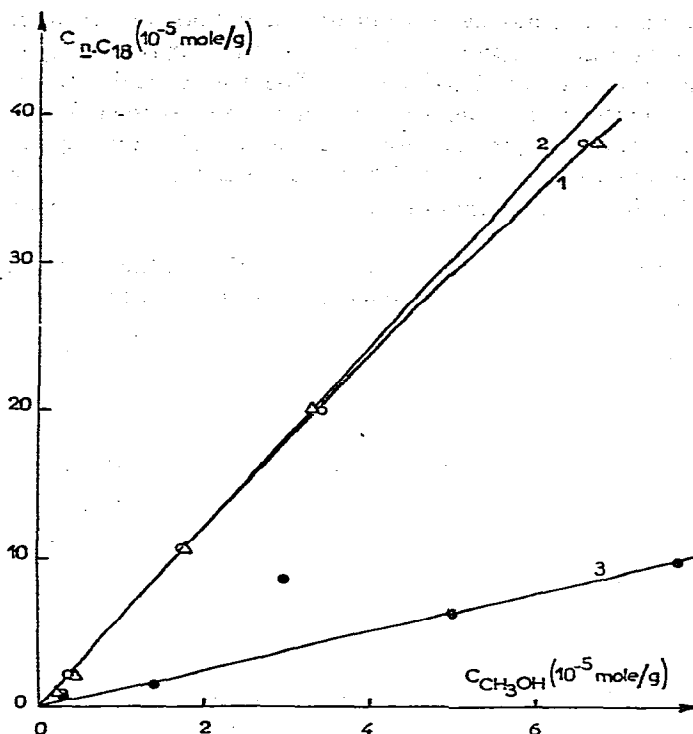


Fig. 1. Equilibrium isotherm for *n*-octylbenzene. 1, Partition isotherm between liquid *n*-octadecane and methanol; 2, tangent at the origin of line 1; 3, equilibrium isotherm between ODS-coated silica, (LiChrosorb RP 18) and methanol. The data were treated assuming a partition process. ● and ○, 40.5 °C; △, 55.5 °C.

It is not possible to conclude from these data that the mechanism responsible for retention with an ODS packing is pure adsorption; nevertheless, in view of the very low "partition" constant, it does not seem that it can be a pure partition mechanism.

Discussion

An ODS packing cannot be regarded as a solid support coated with a liquid phase. The structure of a monomeric phase is of the "brush" type: a monolayer of organic species is bonded to the surface of silica particles without any cross-linking polymerization. All of the organic groups are attached by one end, and their degrees of freedom are reduced compared with those of similar molecules in a liquid where internal movements are allowed. This results in a marked decrease in the phase entropy. In addition, the density of bonded groups is larger than for pure *n*-octadecane. Molecular volumes (V^0) of bonded ODS, bulk ODS and *n*-octadecane are compared in Table II. Assuming that the chains are straight, the thickness of the layer is 20 Å, corresponding to $V^0 = 500 \text{ \AA}^3$ for a surface concentration of 4 molecules per 100 \AA^2 (ref. 1). As the free end of the chains can be slightly bent, 500 \AA^3 is the maximum value. The difference between V^0 values for bonded and bulk ODS is im-

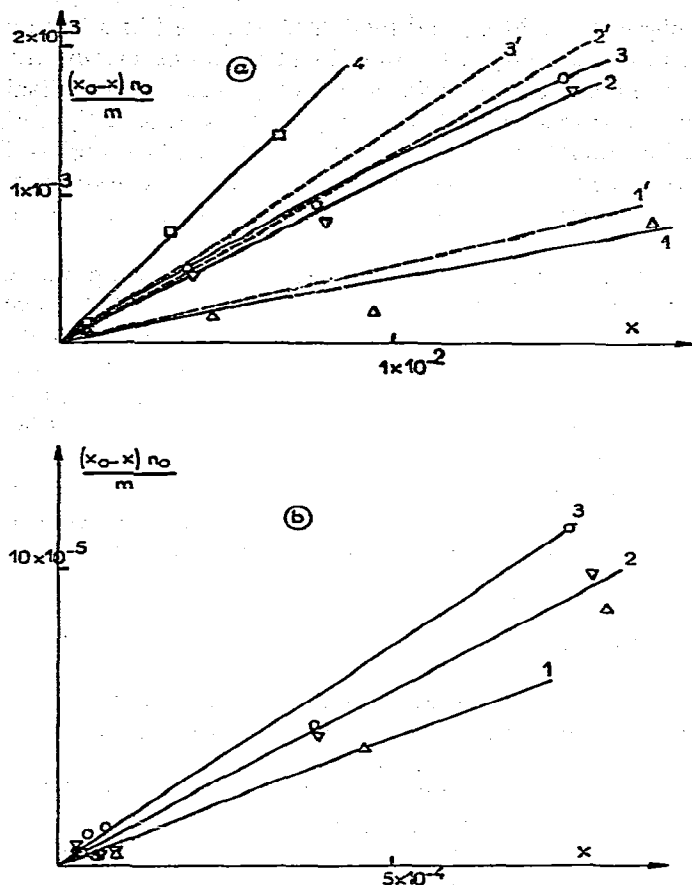


Fig. 2. (a) Plot of the Gibbs' excess for *n*-octylbenzene in the system ODS-coated silica (LiChrosorb RP 18)-methanol versus the molar fraction in methanol. (b) As (a) but for the low molar fraction range. \square , 0 °C; \circ , 24 °C; ∇ , 32 °C; \triangle , 40 °C.

TABLE II
MOLECULAR VOLUMES OF PHASES

Species	Molecular weight	Density (g/cm ³)	V^0 (Å ³)
<i>n</i> -Octadecane	254.5	0.777	544
ODS (bulk)	387.6	0.931	691
ODS (grafted)	—	—	500*

* Assuming that the thickness of the brush is 20 Å and the surface concentration is 4 molecules per 100 Å².

portant, suggesting that the grafted phase cannot be considered as a layer of liquid ODS (V^0 ratio about 0.7). There is a better agreement with the value for bulk *n*-octadecane, although it is larger than that for bonded ODS.

The monolayer of ODS in the chromatographic system ODS-silica-polar solvent can be compared with the interfacial layer between the two immiscible liquids

n-octadecane (at temperature higher than 30°C) and methanol. It is well known that complementary adsorption phenomena occur in the interfacial region separating the two liquids. If we imagine that the *n*-octadecane bulk phase is replaced with modified silica gel, where the OH groups are transformed into inactive groups, it is likely that the adsorption process at the interfacial layer will continue to take place and will remain similar in nature. Hence it will be possible to find a region where stationary molecules of solute and solvent are interacting with the packing: this is the main characteristic of adsorption systems.

In conclusion, we believe that the retention mechanism with *n*-C₁₈ monomeric bonded silica is an adsorption process. However, there is an important difference with pyrocarbon packings. In this instance there is a particular affinity between the solvent, solute and adsorbent. With non-polar bonded packings the polarity of the solvent is responsible for solute adsorption: it is the solvophobic effect described by Horváth and co-workers^{12,13} (we assume that the equations previously given for LSC can be used with this packing material).

Note that the argument on adsorption or partition is not new. Martin discussed the mechanism of partition in paper chromatography and concluded that water held by cellulose cannot be considered as liquid water. Similar comparisons have also been made in anion-exchange chromatography.

COMPARATIVE STUDY OF THE PROPERTIES OF RPC COLUMNS

This work deals with efficiency, retention and solvent strength in RPC. The different columns that we compared are listed in Table III. The μ Bondapak fatty acids column is especially recommended by the manufacturer for the separation of fatty acids. However, good results are also obtained for other solutes as well (see later and ref. 2). The densities of coverage of the material used for columns 1 and 2 are different, the carbon percentages being 4 and 15%, respectively. The pyrocarbon-modified silica column was prepared as described earlier^{14,15}.

TABLE III
COLUMNS USED IN THE COMPARATIVE STUDY OF RPC

<i>Bonded species</i>	<i>Name</i>	<i>Origin</i>	<i>Column length (cm)</i>	<i>Particle diameter (μm)</i>	<i>Column No.</i>
<i>n</i> -C ₁₈	ODS-1 Partisil	Whatman	25	10	1
<i>n</i> -C ₁₈	ODS-2 Partisil	Whatman	25	10	2
<i>n</i> -C ₁₈	μ Bondapak C ₁₈	Waters Assoc.	30	10	3
-(CH ₂) ₂ -C ₆ H ₅	μ Bondapak fatty acids	Waters Assoc.	30	10	4
Pyrocarbon (coated on silica)		Home-made with Spherosil XOB-75 (Rhône-Poulenc)	50	15-20	5
Pyrocarbon (55%) (coated on graphite)		Home-made with carbon black (Black Pearls L)	53	25-31.5	6

Column efficiency

The HETP curve gives useful information on the quality of the packing and the mass transfer processes. This curve represents the variation of the reduced HETP ($h = \text{HETP}/d_p$) with the reduced solvent velocity ($v = ud_p/D_m$), where d_p is the average particle size, D_m the diffusion coefficient, and u the mobile phase velocity¹⁶⁻¹⁸. The h versus v curves are fitted as previously¹⁹ with the Knox equation:

$$h = \frac{2}{v} + A v^{0.33} + C v \quad (15)$$

The values of A and C are summarized for some columns in Table IV. The data for column 3 suggest that very efficient column packing can be achieved, as shown previously by Knox and Pryde¹⁸. The bonding of silica gel particles with ODS does not cause a decrease in column efficiency, as has been often claimed. The coefficient A (quality of the packing) is less than 2, which characterizes well packed columns: with this column we obtained more than 11,800 theoretical plates (ca. 40,000 plates/m for 10- μm particles). The h versus v curves obtained on column 3 for different solutes are given in Fig. 3. Note that these results were obtained under "normal" conditions, that is with syringe injection carefully carried out, but without injection directly into the head of the column (this increases the efficiency but leads to rapid destruction of the top of the packing). No attempt was made to reduce the dead volumes between the column and detector and the column and injector below values that were convenient. Table IV shows that the efficiencies of the other columns tested are lower. Column 1 was old when we obtained it, and in general ageing causes the efficiency of a column to deteriorate.

As pointed out by Karch *et al.*²⁰, the packing efficiency depends on the packing procedure for RPC supports. Columns with low-density packings are generally more

TABLE IV
PARAMETERS OF THE HETP EQUATION (EQN. 15)

Column	Solvent**	Solute	k'	A	C	h_{mta}	v_{mta}
1	Methanol-water (1:1)	2,3,5,6-Tetramethylphenol	2.15	3.98	—*	5.88	1.40
		Phenol	0.83	5.04	—*	7.02	1.10
2	Methanol-water (1:1)	Phenol	1.20	2.67	—*	4.52	1.70
		Benzene	5.0	2.17	—*	3.73	2.20
3	Methanol-water (1:1)	Phenol	0.81	1.83	$18.23 \cdot 10^{-2}$	3.67	1.90
		2,3,5,6-Tetramethylphenol	5.7	1.27	$9.96 \cdot 10^{-2}$	2.77	2.50
		2,4,5-Trimethylphenol	3.9	1.07	$17.89 \cdot 10^{-2}$	2.70	2.30
5	Methanol	Benzene	0.08	4.14	$6.42 \cdot 10^{-2}$	6.14	1.30
		<i>o</i> -Xylene	0.44	4.37	$8.64 \cdot 10^{-2}$	6.41	1.20
		1,2,3,4-Tetramethylbenzene	3.45	5.64	—*	7.64	1.10

* Insufficient data available for meaningful estimation.

** Composition in v/v.

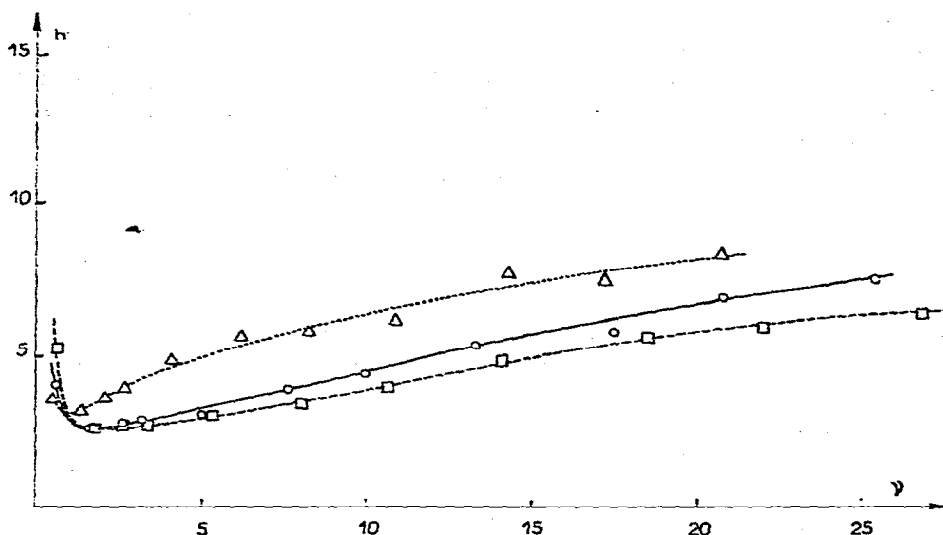


Fig. 3. Plot of reduced HETP versus reduced velocity for column 3 (cf., Table III). Solvent: water-methanol (1:1, v/v). Solutes: \triangle , phenol ($k' = 0.82$); \circ , 2,4,5-trimethylphenol ($k' = 3.90$); \square , 2,3,5,6-tetramethylphenol ($k' = 5.40$).

efficient but have shorter lifetimes. It would have been interesting to study the deterioration in service of columns 2 and 3.

The poor column efficiency of the pyrocarbon column may be due in part to its length (50 cm): we observed that it is more difficult to pack correctly or well a 50-cm column with 20- μm particles than a 25-cm column with 10- μm particles. Work is now in progress using 10- μm particles modified with pyrocarbon.

Comparison of the C values (cf., Table IV) is interesting as C accounts for the contribution of the resistance to mass transfer. The lower the C value, the better is the system. The smallest C values are obtained with the pyrocarbon-coated silica column ($C \approx 7 \cdot 10^{-2} - 8 \cdot 10^{-2}$). Knox and Pryde¹⁸ measured very small C values for SAS-silica (a short-chain RPC packing). The C term seems to be large for column 3, which would result in poor efficiencies at high solvent reduced velocities. However, the use of such large velocities ($v > 50$) is not necessary with 5- or 10- μm particles because of the already large solvent speed at the minimum of the h versus v curve (optimal condition of use).

The dependence between C and the capacity factor, k' , is complex^{21,22,29}. The column efficiency varies with k' as well as the optimum velocity (cf. Fig. 3). Some results are reported in Fig. 4. For each column two curves are given, corresponding to alkylphenols and alkylbenzenes (polar and non-polar solutes). The solvent is the same for the four CB columns (methanol-water, 1:1).

With bonded-phase columns, the efficiency is better for non-polar than polar compounds, especially at small k' . For non-polar solutes, the HETP of the three best columns decreases linearly with increasing k' , at least for $k' < 15$. For polar solutes, the data are rather scattered, showing a weaker correlation. However, for the four columns, the HETP decreases very quickly with increasing k' up to 5 and

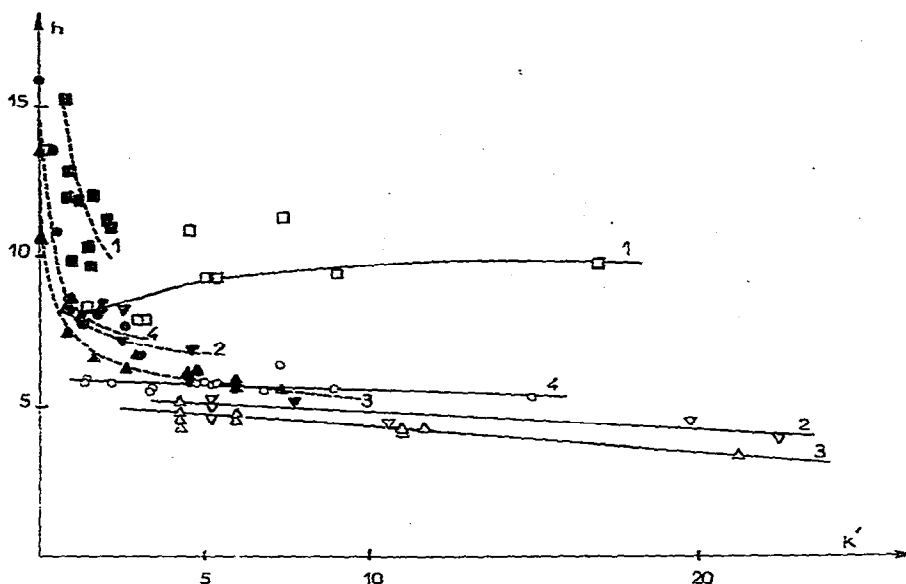


Fig. 4. Variation of the reduced HETP with k' for several columns. Solvent: water-methanol (1:1, v/v) at a constant flow-rate of 1 cm³/min. Open symbols, apolar solutes; closed symbols, polar solutes. ■, □ = Column 1; ▼, ▽ = column 2; ▲, △ = column 3; ●, ○ = column 4 (for column characteristics, see Table III).

then more slowly and almost linearly. Contrary to the situation with carbon adsorbents, better results are obtained with bonded phases when separations are performed with k' in the range 3–10. Values of k' larger than 10 result in analysis times that are too long.

As previously reported, an important drawback when using carbon black is the substantial decrease in efficiency with increasing retention. This is particularly important for k' in the range between 0 and 1 or 2. Fig. 5 represents the variation of the relative reduced HETP, h/h_0 (where h_0 is the value of h for an unretained solute) with k' . Measurements were performed at different mobile phase velocities with the solutes benzene ($k' = 0$), *m*-xylene ($k' = 0.44$), 1,2,4-trimethylbenzene ($k' = 0.95$) and 1,2,3,4-tetramethylbenzene ($k' = 3.50$); the solvent was methanol. The h/h_0 values are not markedly affected by the eluent flow-rate but, at a given k' , the larger the flow-rate the smaller is h/h_0 . The increase in h is the most important at small k' . For instance, at a solvent velocity of 0.33 cm/sec, the increase in h/h_0 between $k' = 0$ and 0.44 is 11% and between $k' = 0.44$ and 3.5 it is 23%. It is possible that the use of "large" particles contributed to this phenomenon. Using 31.5–40- μ m particles of the same material we found that $h/h_0 = 1.78$ for $k' = 2$. Done²³ obtained a similar result when comparing HETP values for Spherisorb 20W (20 μ m) and for 5- or 10- μ m particles.

The values of C obtained by fitting experimental data should be used with care¹⁴, especially if the HETP measurements have been performed at v values where the contribution of mass transfer is small. For instance, assuming that the theoretical equation

$$h = 2/v + 2v^{0.33} + 5 \cdot 10^{-2}v \quad (16)$$

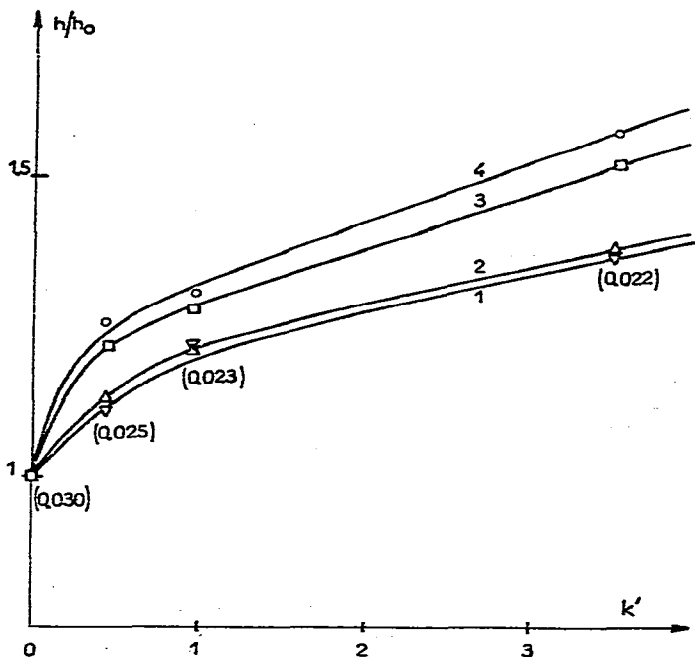


Fig. 5. Variation of the relative reduced HETP (h/h_0) for column 5 (cf., Table III). h_0 = value of h for an unretained compound; solvent, methanol. Solutes: Δ , benzene; ∇ , *m*-xylene; \circ , 1,2,4-trimethylbenzene; \square , 1,2,3,4-tetramethylbenzene. Flow velocities: 1 = 0.33; 2 = 0.122; 3 = 0.078; 4 = 0.020 cm/sec. Figures in parentheses are the optimal solvent velocities (cm/sec) for each compound assuming that $v_{opt} = 2.5$.

TABLE V

CONTRIBUTION OF MASS TRANSFER TO COLUMN EFFICIENCY

ν	$5 \cdot 10^{-2} \nu/h^*$
5	0.06
10	0.10
20	0.15
30	0.19
50	0.25
75	0.31
100	0.35
1000	0.72

$$* h = 2/\nu + 2\nu^{0.33} + 5 \cdot 10^{-2}\nu$$

is valid, the fraction of h due to the last term was calculated at different reduced velocities (Table V). Because of unavoidable dispersion of the experimental measurements, an accurate determination of C requires measurements to be carried out at $\nu > 50$ (the contribution of C to h is 25%). Unfortunately, it is very difficult with small particles to make meaningful measurements of C when ν is larger than 50. Table VI gives the values of the time constant of the detector (τ_{max}) and of the in-

TABLE VI
CONTRIBUTIONS OF DETECTOR TIME CONSTANT AND INJECTION TIME TO COLUMN EFFICIENCY

Column	ν	h	N	t_R (sec)	$\tau_{max.},$ 1%*	$\tau_{max.},$ 25%**	$t_{max.},$ 1%*	$t_{max.},$ 25%**
$L = 10$ cm, $d_p = 5$ μ m	50	9.81	2039	6.67	0.015	0.086	0.051	0.300
	75	12.09	1654	4.44	0.011	0.063	0.038	0.220
	100	14.16	1412	3.33	0.009	0.051	0.031	0.180
$L = 25$ cm, $d_p = 10$ μ m	50	9.81	2548	33.33	0.066	0.380	0.229	1.330
	75	12.09	2068	22.22	0.049	0.285	0.169	0.980
	100	14.16	1766	16.67	0.040	0.230	0.137	0.800
SAS-silica ¹⁷	50	3.63	4591	23.75	0.035	0.200	0.121	0.700
	75	4.46	3734	15.83	0.026	0.150	0.090	0.520
	100	5.24	3181	11.88	0.021	0.120	0.073	0.420

* $\Theta = 0.10$; $\Theta^2/1 + \Theta^2 = 10^{-2}$; relative decrease in efficiency, 1%.

** $\Theta = 0.58$; $\Theta^2/1 + \Theta^2 = 25 \cdot 10^{-2}$; relative decrease in efficiency, 25%.

jection time ($t_{max.}$) that produce a contribution to band broadening that is equal at 1 and 25% with that given by the column. The calculations were made for unretained solutes on hypothetical columns 10 and 25 cm long, packed with 5- and 10- μ m particles. Eqn. 16 was assumed to describe the h versus ν relationship and D_m was taken to be $1.5 \cdot 10^{-5}$ cm²/sec, an average value for solutes in RPC. We also used data obtained by Knox and Pryde¹⁸ with an SAS-silica column for which the h versus ν equation is

$$h = 4/\nu + 0.7\nu^{0.33} + 2 \cdot 10^{-2}\nu \quad (17)$$

and the solute was acetone ($k' = 0.9$).

$\tau_{max.}$ and $t_{max.}$ were estimated according to Martin *et al.*²⁴:

$$\tau_{max.} = \Theta \cdot \frac{t_R}{\sqrt{N}} \quad ; \quad t_{max.} = \Theta K \cdot \frac{t_R}{\sqrt{N}} \quad (18)$$

where Θ^2 is the increase in peak variance due to the extra-column effects and K is the injection constant (*ca.* $\sqrt{12}$). The change in the number of theoretical plates is then

$$\Delta N/N = \Theta^2/1 + \Theta^2 \quad (18')$$

The data in Table VI suggest that a minimal loss of efficiency of 20% is unavoidable in most instances because the time constant of most commercial detectors is between 0.3 and 3 sec. The contribution of the injection time is less important because it is possible to make injections within 0.5 sec with a syringe; however, further studies and much care need to be devoted to the shape of the injection function. The determination of C is very difficult, even if possible at all, for classical analytical columns packed with 5- or 10- μ m particles. The values of C that can be derived from

h versus v curves must be used with caution. Our opinion is that current techniques can give reliable information about the quality of the packing (A) but values of C only to within an order of magnitude. Moreover, this assumes that the amount of solute injected does not contribute to peak broadening, which is generally incorrect^{2,23}.

Retention and solvent strength

It is generally considered that retention on RP systems varies in an opposite manner to that on normal systems and that the larger the solubility of a solute in the mobile phase, the smaller is its retention. This must be considered merely as a general trend, as illustrated by Table VII, where the capacity factors (k') of some phenols measured on columns packed with ODS-silica, silica gel and carbon black hardened by pyrocarbon are compared. These data suggest that, in terms of k' , the molecular size of the solute plays an important role with ODS-silica. We have shown that the molecular size is very important for packings that contain carbon or pyrocarbon: methylphenols are eluted first, according to their molecular weight. For positional isomers, the order of elution is the reverse of that obtained on silica. In such a situation, the solubility of solutes in the solvent has the greatest effect in determining the elution order.

TABLE VII
COMPARISON BETWEEN THE VALUES OF k' IN RPC AND NORMAL CHROMATOGRAPHY (NC)

Solute	RPC*	NC**	RPC***
<i>p</i> -Cresol	1.64	11.13	0.26
2,6-Xylenol	2.68	4.81	0.60
2,4-Xylenol	2.95	7.88	0.69
2,3,4-Trimethylphenol	4.50	7.81	2.39
2,4,5-Trimethylphenol	4.80	7.06	1.81

* Column, μ Bondapak C₁₈; solvent, methanol-water (1:1)

** Column, Partisil 5; solvent, *n*-hexane-ethyl acetate (95:5).

*** Column, carbon black Sterling FT.FF with 15% pyrocarbon; solvent, acetonitrile.

The stationary phase-solute interactions are weaker with non-polar bonded silica than with pyrocarbon. For instance, assuming that γ^d for ODS-modified silica is similar to γ^d for bulk *n*-octadecane (which is a rough approximation), the term related to the interfacial tension ($\sigma_1^0 - \sigma_2^0$) in eqn. 11 was calculated for two solutes (*n*-tetradecane and *n*-butylbenzene) on ODS-silica and graphite with methanol as solvent. In the first instance (silica) we obtained values of 2.77 and 14.60 erg/cm², respectively, and in the second 9.81 and 40.45 erg/cm² (roughly three times more important for graphite than for ODS). Graphite and carbon blacks are strong, non-specific adsorbents, which explains why the net retention volumes are much larger on graphite than an ODS-silica, although the specific surface areas of the carbon packings are much smaller (less than 50 m²/g). Larger packing-solute interactions involve the use of stronger (less polar) solvents. The result is a lower solubility of polar compounds in the mobile phase and consequently the retentions of these polar solutes do not decrease as much as expected with increasing solvent strength. Methyl-

phenols are more retained than methylbenzenes on carbon adsorbents, while the opposite is true on ODS-silica (because of the stronger adsorption on pyrocarbon a less polar solvent is required than with ODS-silica). This suggests that, depending on the balance between the contributions of the polarity and the polarizability of the molecules to their retention, changes in the order of elution of polar and non-polar solutes may sometimes occur. The ratio (α) of the capacity factors of benzene and phenol was measured on columns 3, 4 and 5 with various water-methanol mixtures. In Fig. 6, α is plotted against the proportion of water in the mobile phase. On column 5, benzene is more retained than phenol only if the water content is between 15 and 65%: there is an inversion in the elution order. A similar behaviour of α occurs on column 3, but α is always larger than unity and no inversion can be observed. Such convex profiles show that for very polar solvents the most important effect on the retention time is the decrease in the adsorbent-solvent interactions, while for moderately polar solvents it is the solute solubility. The curve obtained with column 3 (μ Bondapak C₁₈) is more skewed than that for pyrocarbon silica (column 5) because the interactions with the mobile phase play a more important role for *n*-C₁₈ phases (solvophobic effect) whereas adsorption on the support is likely to be very important for carbon packings.

The results obtained with column 4 are surprising. The curve is concave, which

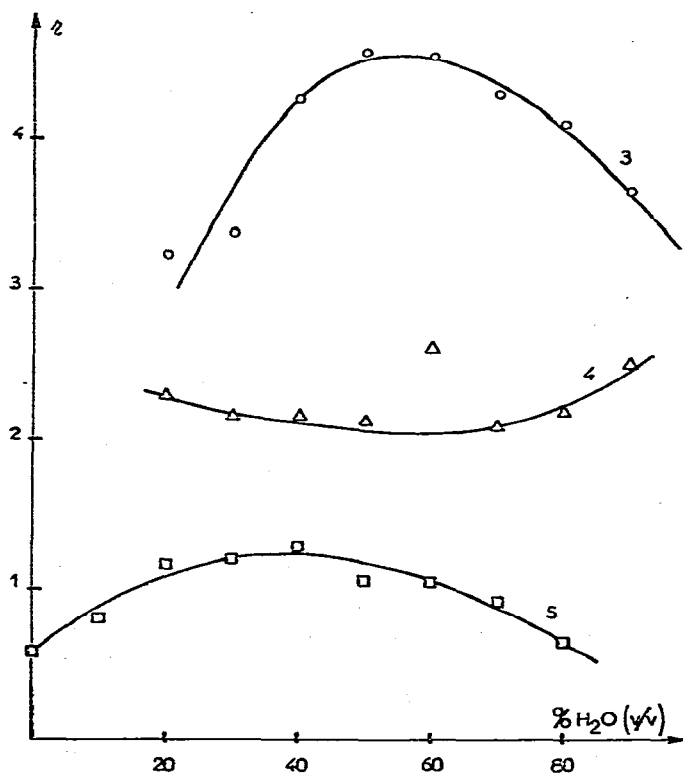


Fig. 6. Variation of the ratio α ($k'_{\text{benzene}}/k'_{\text{phenol}}$) for different columns with the composition of the water-methanol mixture used as eluent. \circ , Column 3; \triangle , column 4; \square , column 5 (see Table III).

means that the main effect with polar solvents is the solubility in the mobile phase, although the interactions between the stationary phase on the solute molecules are rather weak: we indeed observed that the retention volumes are much smaller on the phenylsilica packing than on ODS-silica. This may be due to the smaller number of carbon atoms in the organic groups bound to silica and to the larger polarity of the surface.

The eluotropic strengths (ϵ^0) of methanol-water mixtures were compared for columns 2, 4 and 5 using polymethylphenols and alkylbenzenes (C_nBz with $n < 12$) as solutes. ϵ^0 values were calculated by using the equation

$$\log\left(\frac{k'_{i,j}}{k'_{i,k}}\right) = A_i(\epsilon_k^0 - \epsilon_j^0) + \log\left(\frac{V_j}{A_j} \cdot \frac{A_k}{V_k}\right) \quad (19)$$

where A_i and V_i are the molar surface area and volume, respectively, of the species i , whose capacity factor in solvent j is $k'_{i,j}$ (ref. 19). Eqn. 19 is an approximation and the values of ϵ^0 we calculated are thus semi-quantitative estimates, but are nevertheless useful for comparisons between column performances.

For each column we chose as the reference solvent the mixture with which it was possible to elute the maximal number of alkylbenzenes and polymethylphenols. The molar volumes and surface areas of the solvent mixtures were assumed to vary linearly with the molar fractional composition. For a mixture in which the molar fraction of water is x_w , then the molar volume V is given by:

$$V_{\text{Mixt.}} = V_w \cdot x_w + (1 - x_w)V_{\text{MOH}} \quad (20)$$

where the subscripts W, MOH and Mixt. represent water, methanol and the mixture, respectively. ϵ^0 was calculated from the data corresponding to each solute studied and average values are reported. There were generally small differences between ϵ^0 values calculated for polar and non-polar solutes. The data pertaining to these two kinds of solutes have been dealt with separately. However, Fig. 7 shows, for instance, the data obtained with column 2, where the water content of the reference mixture ($\epsilon^0 = 0$) was 20%. It appears that the behaviour of the substituted benzenes and the polymethylphenols are slightly different. The comparison between the three columns is shown in Fig. 8. As solvent strengths are additive, it is easy to change the reference: in Fig. 8 we chose pure methanol as the common reference for all columns.

Except for polar solutes on column 2, the behaviours of the different chromatographic systems are very similar for mixtures with water contents less than 50% (v/v). The curves corresponding to bonded packings have a slight upwards curvature, whereas those obtained with pyrocarbon silica have a downwards curvature. For mixtures containing less than 50% of water, the change in ϵ^0 is about -0.05 per 10% increase in the water content. Using a solute with a molecular surface area of 10 units (85 Å), this corresponds to a decrease in k' by a factor of $10^{0.5} = 3$, which is in excellent agreement with the average value obtained by different workers¹.

As the solvent strength is a characteristic of only the system solvent-stationary phase, ϵ^0 must have the same value when determined from the elution of polar or non-polar solutes. The differences we observed are thus due to the fact that we did not take into account the activity coefficients term in eqn. 19. The difference between

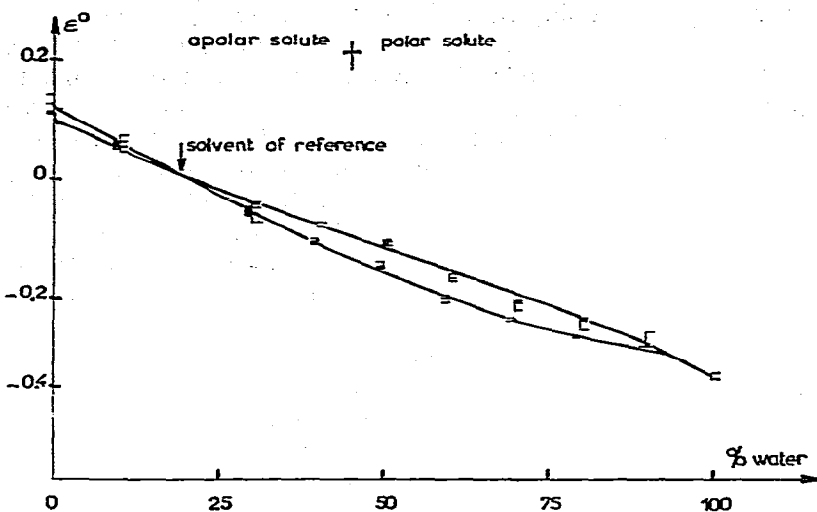


Fig. 7. Eluotropic strength (ϵ^0) of water-methanol mixtures for column 2 (see Table III). The vanes to the right indicate data for polar solutes and those to the left indicate data for apolar solutes.

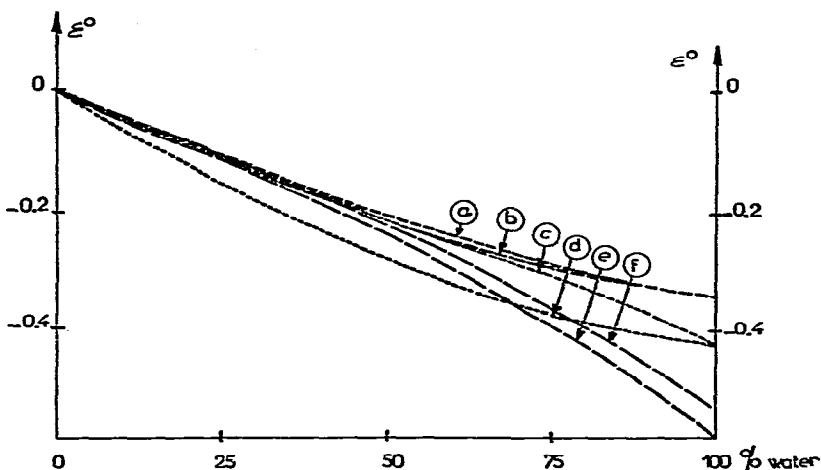


Fig. 8. Eluotropic strengths of water-methanol mixtures on different columns: column 4, polar (a) and apolar (b) solutes; column 2, polar (c) and apolar (d) solutes; column 5, polar (f) and apolar (e) solutes (for column numbers, see Table III).

the results obtained for the two kinds of solutes is particularly large when working with column 2. An estimate of the error made can be derived from the *a priori* calculation of the value of ϵ^0 for water. From the work of Eon¹¹, it appears that for adsorbents with an activity of unity

$$\epsilon_w^0 = \frac{\sigma_{MOH}^0 - \sigma_w^0}{114} \tag{21}$$

with the usual units and $\varepsilon_{\text{MOH}}^0 = 0$. Using eqn. 20 and the values $\gamma_{\text{w}}^{\text{d}} = 21.8$, $\gamma_{\text{w}} = 72.0$, $\gamma_{\text{MOH}}^{\text{d}} = 19.10$, $\gamma_{\text{MOH}} = 22.6$, $\gamma_{\text{ODS}}^{\text{d}} = 25$ and $\gamma_{\text{graphite}}^{\text{d}} = 123 \text{ erg/cm}^2$ (cf., ref. 9), we obtain $\varepsilon_{\text{w}}^{\text{ODS}} = -0.41$ and $\varepsilon_{\text{w}}^{\text{graphite}} = -0.38$. The agreement with experimental data is very good for an ODS packing (measured value -0.43) and less good for a pyrocarbon column (measured value between -0.5 and -0.6). This may be due to the fact that we used pyrocarbon-modified silica instead of pure carbon. Indeed, working with pyrocarbon-modified carbon black (with or without further graphitization)²¹ we measured $\varepsilon_{\text{w}}^0 = -0.41$, which is in good agreement with the value of -0.38 calculated previously. This proves that the role of the underlying silica can be important with pyrocarbon-modified silica, probably because of the discontinuous structure of the carbon film¹⁴.

Other water-soluble organic solvents can be used in RPC. Work has been carried out to determine ε^0 for aqueous solutions of ethanol, isopropanol, propanol and acetonitrile. Because of the small retentions, experiments have not been performed with the pure solvents. We used a 3:1 (v/v) mixture of organic solvent and water. The order of the eluotropic strengths of the pure solvents may be different from that of their mixtures with water at constant volume composition. Hence, we must consider whether it is more meaningful to work with constant volume composition or constant molar fraction mixtures. The corresponding data can be significantly different. Table VIII shows, for instance, the molar composition of mixtures of various solvents with a constant volume fraction, and the converse. We have assumed that the excess volume of mixing is zero. In this work, a constant volume composition has been preferred because it is simpler from the experimental point of view.

TABLE VIII

COMPARISON BETWEEN THE COMPOSITIONS OF WATER-ORGANIC SOLVENT MIXTURES IN VOLUME FRACTION AND MOLAR FRACTION

<i>Solvent</i>	<i>Molar fraction for 75:25 (v/v) mixtures</i>	<i>Volume fraction for 1:1 mole/mole mixtures (50% molar)</i>
Methanol	57.1	69.2
Ethanol	48.1	76.4
Propanol	41.2	81.1
Isopropanol	41.4	80.0
Acetonitrile	51.4	73.9

Results are reported in Table IX with methanol-water as the reference solvent. The classification for column 2 (ODS) is slightly different from that obtained by Karch *et al.*²⁵ and Schmitt *et al.*²⁶: we found acetonitrile to be a weaker modifier than ethanol. These differences can be explained by the fact that Schmitt *et al.* made their measurements using aqueous solutions with 30% of modifier and Karch *et al.* used the pure organic solvents. The orders of eluotropic strength are the same on columns 2 and 4 (alkyl and phenyl chemically bonded groups), the figures being smaller with column 4. This is in agreement with the general trend of a lower selectivity for the fatty acids column². The results obtained with the pyrocarbon column (column 5) are very similar to those obtained with the ODS packing except for the increased

TABLE IX

ELUOTROPIC STRENGTHS OF 1:3 (v/v) WATER-ORGANIC SOLVENT MIXTURES (MEASURED WITH ALKYL BENZENES)

Organic modifier	Column 2	Column 4	Column 5
Methanol	0.000	0.000	0.000
Ethanol	0.050	0.041	0.058
Isopropanol	0.085	0.059	0.090
Propanol	0.095	0.068	0.095
Acetonitrile	0.041	0.026	0.060

strength of acetonitrile. Note that for pure organic solvents, the ϵ^0 values measured with carbon black are¹⁹ methanol 0.000, acetonitrile 0.038 and ethanol 0.049.

For these reasons and the following, we feel that acetonitrile is a good modifier. Its elution strength is larger than that of methanol and is comparable to that of ethanol. Its viscosity is low, and the pressure drop necessary to sustain a given flow-rate is much lower than for the other organic solvents. For 3:1 (v/v) organic solvent-water mixtures, the ratio of pressure drops referred to methanol as modifier are 1.72 for ethanol, 2.42 for isopropanol, 2.10 for *n*-propanol and 0.48 for acetonitrile. In fact, in order to achieve the same capacity factors, we can either use acetonitrile-water (3:1) or methanol-water (4.9:1). The pressure ratio needed to achieve the same flow-rate then becomes about 0.65. Another advantage of acetonitrile is its low UV absorption. For instance, at 220 and 254 nm, the UV transmissions of acetonitrile and methanol are 92.5 and 66% (220 nm) and 99.5 and 97% (254 nm), respectively. This can be useful in gradient elution applications when a high sensitivity of detection is required.

Effect of temperature

It has been seen that in both LLC and LSC, $\log k'$ varies linearly with the inverse of the temperature. The Van 't Hoff plots ($\log k'$ versus $1/T$) can be used to determine the enthalpy of transfer, ΔH^0 , between the stationary and the mobile phases. K_0 , the equilibrium constant of the retention mechanism, is related to the Gibb's free energy, ΔG^0 , by:

$$\ln K_0 = \frac{\Delta G^0}{RT} \quad (22)$$

Eqns. 22 and 6 give

$$\log k' = \frac{\Delta H^0}{2.3RT} - \frac{\Delta S^0}{2.3R} + \alpha \quad (23)$$

where α is the logarithm of the phase ratio and ΔS^0 is the standard entropy of transfer assuming that the standard state is unit molar concentration in either phase. The plots yield straight lines provided that ΔH^0 is constant.

We studied the effect of temperature on the retention on modified carbon black, pyrocarbon-coated silica and bonded silica [*n*-C₁₈ and -(CH₂)₂-C₆H₅]. The results are summarized in Table X. In order to elute the same solutes on columns 2, 4 and 5 in reasonable time, it was not possible to keep constant the solvent composition.

TABLE X
ENTHALPIES OF TRANSFER IN DIFFERENT CHROMATOGRAPHIC SYSTEMS

Solute	Column 2, methanol-water (65:35, v/v)		Column 4, methanol-water (60:40, v/v)		Column 5, methanol-water (75:25, v/v)		Column 6, acetonitrile	
	ΔH° (kcal/ mole $\cdot^{\circ}K$)	C*	ΔH° (kcal/ mole $\cdot^{\circ}K$)	C*	ΔH° (kcal/ mole $\cdot^{\circ}K$)	C*	ΔH° (kcal/ mole $\cdot^{\circ}K$)	C*
Bromobenzene	2.92	0.9899	2.81	0.9984	3.11	0.9823	—	—
Toluene	2.60	0.9870	2.53	0.9890	2.98	0.9787	—	—
Benzene	1.84	0.9842	2.22	0.9744	2.13	0.8754	—	—
Anisol	2.30	0.9796	2.55	0.9866	3.07	0.9965	—	—
Phenol	1.70	0.8311	1.96	0.9199	1.81	0.9256	—	—
<i>n</i> -Hexylbenzene	—	—	—	—	—	—	2.83**	0.9955
<i>n</i> -Octylbenzene	—	—	—	—	—	—	2.92***	0.9739
<i>n</i> -Nonylbenzene	—	—	—	—	—	—	2.97 [†]	0.9919

* Correlation coefficient for linear regression.

** Value from ref. 27 = 3.01.

*** Value from ref. 27 = 2.66.

[†] Value from ref. 27 = 3.44.

The solvent mixtures are not very different, but we must bear in mind that ΔH° depends on the stationary phase, the solvent and the solute.

For the systems studied, the linearity of the Van 't Hoff plots is fairly good: the correlation coefficients for linear regressions are very close to unity (*cf.*, Table X).

The behaviours of columns 2, 4 and 5 seem to be similar. The ΔH° values obtained by Locke²⁷ in pure partition chromatography, using the squalane-acetonitrile system (which is not *a priori* very different from that used here) were 0.435 and 0.952 kcal/mole $\cdot^{\circ}K$, respectively, for benzene and toluene. This suggests that the retention process on BP might be more similar to adsorption than to partition, as the values of ΔH_0 obtained on the BP columns are much closer to those on column 5 (adsorption on carbon) than to Locke's values. On the other hand, the data obtained for *n*-hexyl-, *n*-octyl- and *n*-nonylbenzene on column 6 (adsorption from acetonitrile on carbon black) are similar to those given by Locke (see Table X). The conclusion is that comparisons between enthalpy of transfer must be used carefully in any attempt to elucidate a retention mechanism.

It is difficult to make measurements of the entropy of transfer (ΔS°). Theoretically, ΔS° can be obtained by using eqn. 23, but it is very difficult to estimate A_s and consequently α . For bonded phases, for instance, if an adsorption mechanism is assumed, the total surface area of the packing available for the adsorption of solute and solvent molecules is different from that of the silica before bonding²⁸. On the other hand, if it is possible to measure the specific surface area of the bonded material as for any other material, it is difficult to account for the measurements. Indeed, the underlying silica is likely to interfere in the determination of the surface area, and it is probable that this surface area is different at the temperature of liquid nitrogen (using the BET technique) and at 20°C, because of the mobility of the *n*-C₁₈ bonded groups. With pyrocarbon-coated silica, the main problem is the determination of the percentage of the surface covered with the pyrocarbon film. The BET measurement

provides only the total surface area. However, we can estimate the pyrocarbon surface area by comparing the capacity factors of non-polar solutes with those obtained on a column of pure carbon black, using the same solvent (methanol). The contribution of the silica surface is then expected to be negligible. The calculated changes in entropy are listed in Table XI (A_s is measured in square metres and V_M in cubic centimetres).

The ΔS° values are fairly constant and high, suggesting that the molecules are strongly oriented on the pyrocarbon surface. The lower value for phenol may be due to the important role of the interactions with the polar mobile phase, which probably modifies the orientation of the molecules at the solid surface. Indeed, these interactions are very strong because, with solvents of lower polarity, the capacity factor of phenol is similar to that of toluene while with the mixture methanol-water (75:25) the ratio of capacity factors is about 3.

TABLE XI

ENTROPY OF TRANSFER FOR THE SYSTEM PYROCARBON-COATED SILICA AND METHANOL-WATER (75:25)

<i>Solute</i>	<i>Capacity factor</i>	<i>S° (cal/°K)</i>
Bromobenzene	1.59	15.71
Toluene	0.92	16.36
Benzene	0.32	15.58
Anisole	1.13	16.26
Phenol	0.36	14.25

The use of relatively high temperatures can be very useful in RPC because of the large viscosity of polar solvents. Increasing the temperature decreases the pressure drop that is necessary to achieve a constant flow-rate and increases the diffusion coefficients. As a consequence, the column efficiency is improved provided that the reduced velocity is larger than that at the minimum of the h versus v curve. The decrease in pressure drop is very important. For instance, the relative viscosities of the mixture methanol-water (75:25) at 20.5°, 40° and 60 C° are 1.00, 0.68 and 0.52, respectively. A more detailed study of temperature effects with pyrocarbon materials will be published soon³⁰.

CONCLUSION

If the retention mechanism in RPC using pyrocarbon-coated silica (or carbon black) is likely to be an adsorption process, there are some doubts with non-polar monomeric bonded phases. No conclusion can be drawn from relationships such as the variations of the logarithm of the capacity factor with the inverse of the temperature or with the number of carbon atoms in homologous series. However, the theory of the solvophobic effect^{12,13} and the comparison with pyrocarbon seem to indicate an adsorption rather than a partition process.

Several conclusions can be drawn from the comparative study of different reversed-phase materials (Parts I and II). On the one hand, the different origins of the interactions responsible for solute adsorption (solvophobic effect for chemically bonded silica and "natural adsorption" by particular affinity for pyrocarbon) results

in the use of more polar solvents with bonded silica. This often involves a good selectivity of this packing for compounds with similar polarities. The adsorption energy being acutely sensitive to the geometry of the adsorbed molecule, pyrocarbon is generally useful for the separation of compounds with similar geometries (*cis-trans* isomers, positional isomers on aromatic rings, etc.).

On the other hand, if the choice of solvents is larger with pyrocarbon (possibility of using chloroform, carbon tetrachloride and *n*-heptane) the strong adsorption potential of this material can result in irreversible adsorption of compounds with large and flat molecules (conjugated polyaromatics, for instance).

Pyrocarbon-modified silica (and carbon black) and non-polar chemically bonded silica have common characteristics. However, we believe that these packings are complementary and both offer interesting advantages. It must be pointed out that the reproducibility of pyrocarbon is excellent, with respect to both the preparation of the material and the analytical properties. We hope that the use of 5- and 10- μ m particles able to withstand the high temperatures necessary for the deposition of pyrocarbon (without substantial modifications to the porous structure) will provide columns as efficient as those packed with bonded silica.

ACKNOWLEDGEMENTS

We acknowledge the French representatives of Whatman, Waters Assoc. and Merck for the generous loan of columns and gifts of packing material. We thank LTT (Paris, France) for the loan of an ICAP 5 digital integrator and Coultronics France, representative of Micromeritics, for the loan of a Model 7000 liquid chromatograph. We are greatly indebted to Norman Ward for his help with the experimental work.

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EDITOR'S NOTE

This manuscript discusses a very scholastic question: can bonded phases be classified under adsorption or partition? The word partition (A. J. P. Martin, personal communication) has its origin in the first paper on the topic, when its authors wanted to call it simply liquid-liquid chromatography and the Editor of the *Biochemical Journal* changed it to liquid-liquid partition chromatography. If this had not been done, today we would have only liquid-solid chromatography and liquid-liquid chromatography and the discussion of this paper would have no point because a bonded phase is a solid although it may have liquid-like properties.

The whole situation, furthermore, lacks novelty. In ion-exchange chromatography, the first materials used extensively were synthetic resins with various functional groups (in HPLC terms they would be called sulphonic bonded phases, etc.). Later, much work was carried out with cellulose and silica gel impregnated with liquid ion exchangers. Many pages were filled exhorting the advantages of liquid exchangers over resins (here the bonded phases did not win!) and comparing liquid extraction with so-called extraction chromatography and liquid exchangers with resins. Prof. Guiochon is convinced that his paper is news to HPLC chromatographers (I agree with him). If it is, some reading on liquid exchangers, etc., will also be good for them.

Editor of J. Chromatogr.