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COMPARISON OF OCTADECYL-BONDED SILICA AND STYRENE-DIVINYLBENZENE COPOLYMER SORBENTS FOR TRACE ENRICHMENT PURPOSES

FUNDAMENTAL ASPECTS I. CALIBRATION OF THE STATIONARY PHASES IN PURE WATER

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SUMMARY

An octadecyl-bonded silica (Partisil ODS-3) and a styrene-divinylbenzene copolymer (PRP-1) with comparable particle sizes, average pore diameters and specific pore volumes were investigated for trace-enrichment purposes. The organic resin leads to systematically higher values of the logarithm of the capacity factor, $\log k'$, whatever the composition of the methanol-water eluent and whatever the solute investigated. Lower retention differences were observed for two alcohols. Accordingly, the correlation of experimental $\log k'$ values obtained for a given eluent composition with calculated hydrophobic $\log P$ coefficients leads to one regression line for the C_{18} material and two lines (for hydroxylated and non-hydroxylated solutes respectively) for the organic resin. This also applies to the calibration graphs of $\log P$ with the weighted linearly extrapolated $\log k'_w$ values in pure water. A weaker affinity of the copolymer for hydroxylated compounds can generally account for all the experimental results.

INTRODUCTION

In the past fifteen years, great progress has been made in both the practice and theory of reversed-phase liquid chromatography (RPLC). Alkyl-bonded silica packings have become the most popular stationary phases for liquid chromatography as

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they allow the investigation of a great variety of compounds covering a wide polarity range. However, these extraordinary developments must not be allowed to obscure the interesting possibilities offered by RPLC as an extraction technique.

This so-called trace-enrichment chromatography takes advantage of the retentive power of non-polar sorbents to extract compounds from dilute aqueous solutions and includes two steps. The first one consists in passing a large volume of water sample through an extraction column. During the second step, the retained compounds are eluted with a small volume of an organic solvent.

Until now, trace-enrichment experiments have been performed almost exclusively in order to study the organic pollution of potable^{1,2}, river-^{3,4} or wastewaters^{5,6}. Many papers also reported on the recoveries of test solutes under a given set of experimental conditions and on the influence of some parameters upon the experimental results⁷⁻¹⁸. Most of them deal with organic copolymers of the well known polystyrene divinylbenzene (PS-DVB) type as extracting sorbents. Until a few years ago, organic resins were available as cheap coarse particles suitable for extraction purposes. On the contrary, alkyl-based stationary phases were less often used and the great majority of published papers concerned analytical investigations of the retention mechanism(s) on those bonded supports. To our knowledge these two kinds of non-polar media have rarely been compared^{19,20}.

Moreover, as the various studies of trace-enrichment chromatography involved different experimental conditions^{14,20-24}, no quantitative conclusions can be drawn. Finally, very few authors²⁵ tackled the problem of the prediction of breakthrough volumes for extraction purposes and the published results apply only to the solutes studied.

The present study is therefore designed to compare both kinds of non-polar stationary phases for the recovery of aroma compounds contained in the effluents from food industries. We also intend to define an "easy-to-apply" calibration procedure for RPLC systems in pure water so that the breakthrough volume of any solute may be estimated.

To achieve this twin goal, one must bear in mind that, when the spreading of the migrating front in the column is neglected, the maximum sample volume that can be passed through a given column before leakage occurs corresponds to the breakthrough volume determined by frontal analysis in pure water. Whether trace-enrichment experiments lead to quantitative recovery of the solutes thus mainly depends on the magnitude of the capacity factor in a totally aqueous eluent, k'_w . As k'_w values are hardly accessible experimentally because of the excessive retention of the solutes in totally aqueous eluents, a reliable method must be employed to estimate them. This generally consists in linear²⁶⁻²⁹ or quadratic³⁰⁻³³ extrapolation of the variations in the logarithm of the capacity factor, $\log k'$, with the mobile phase composition to pure water.

Lastly, one must find a proper parameter such as a physico-chemical constant that can be correlated to the capacity factor of the compounds and enable the *a priori* estimation of k'_w . Among the parameters usually advocated as being useful for the prediction of retention in RPLC³⁴, the most appropriate is undoubtedly the octanol-water partition coefficient, P (or $\log P$). This constant, which characterizes the molecular behaviour governed by solute distribution between two hydrophilic and hydrophobic regions, is accessible in two main ways. First is the experimental

determination, known as the conventional shake-flask method, which has several disadvantages^{35,36}. Second is the *a priori* calculation according to the method developed by Rekker³⁷ and elaborated from the earlier works of Hansch and co-workers³⁸⁻⁴⁰. The many reported experiments using alkyl-bonded silica and various organic solvents mixed with water enable the following conclusions to be drawn.

The characteristics of the linear $\log k' = A \log P + B$ correlation may vary with the nature and the composition of the chromatographic eluent as well as the nature of the solutes, but the number of outliers in the regressions are much less numerous when:

calculated $\log P$ values are involved rather than those measured by the traditional method^{41,42} or even those calculated according to Hansch's method⁴³;

methanol is used as co-solvent rather than any other organic modifier such as ethanol, acetonitrile or tetrahydrofuran^{35,42,44,45};

the water content in the eluent increases⁴⁶⁻⁴⁹, the highest correlation coefficients being obtained when retentions in pure water, whether estimated or measured, are involved^{27-29,35,48}.

This first part of the study therefore concerns the retention of model aroma compounds in various methanol-water eluents, the estimation of the corresponding k'_w in pure water and the correlation of all retention values to the hydrophobic octanol-water coefficient.

EXPERIMENTAL

Apparatus

All experiments were carried out using a liquid chromatograph assembled from a Model 6000 A pump (Waters Assoc., Milford, MA, U.S.A.), a Valco six-port sampling valve with a 20- μ l injection loop, a Spectromonitor III variable wavelength UV spectrophotometer (LDC, Riviera Beach, FL, U.S.A.) and a R401 differential refractometer (Waters Assoc.). The column, mobile phase and refractometer cells were thermostatted at $25.0 \pm 0.1^\circ\text{C}$ using a circulating water-bath.

Solvents and chemicals

HPLC-grade methanol was obtained from Prolabo (Paris, France). Ultra-pure water was obtained from a purification line consisting of RO-4 and Milli-Q systems (Millipore, Bedford, MA, U.S.A.) mounted in series. Mixtures of methanol and water were degassed by filtration through a 0.45- μ m membrane (Millipore) before use.

Chemicals, chosen among hundreds of aroma compounds in order to represent various chemical classes, were tested for purity by gas chromatography. 2,3-Butanedione (> 99.5%), ethyl butanoate (> 99.5%), 2-nonanone (> 99.5%), methyl octanoate (> 99%) were obtained from Fluka (Buchs, Switzerland), *cis*-3-hexen-1-ol (> 99.5%), *trans*-2-hexenal (> 99%), 1-nonanol (> 99.5%) from Interchim (Montluçon, France) and dibutyl sulphide (> 99.5%) from K & K (Plainview, N.Y., U.S.A.). The formulae of these model aroma compounds are shown in Table I.

Samples were prepared as individual solutions from 10^{-5} to 10^{-3} M whenever possible in each mobile phase under investigation or in a water-leaner eluent.

TABLE I
STRUCTURES OF THE AROMA COMPOUNDS STUDIED

$\text{CH}_3-\overset{\text{O}}{\parallel}{\text{C}}-\overset{\text{O}}{\parallel}{\text{C}}-\text{CH}_3$	$\text{C}_2\text{H}_5-\underset{\text{H}}{\text{C}}=\underset{\text{H}}{\text{C}}-(\text{CH}_2)_2\text{OH}$	$\text{CH}_3(\text{CH}_2)_2-\overset{\text{H}}{\text{C}}=\underset{\text{H}}{\text{C}}-\overset{\text{H}}{\text{C}}=\text{O}$	$\text{CH}_3(\text{CH}_2)_2-\overset{\text{O}}{\parallel}{\text{C}}-\text{O}-\text{C}_2\text{H}_5$
1 2,3-Butanedione	2 <i>cis</i> -3-Hexen-1-ol	3 <i>trans</i> -2-Hexenal	4 Ethyl butanoate
$\text{CH}_3(\text{CH}_2)_6-\overset{\text{O}}{\parallel}{\text{C}}-\text{CH}_3$	$\text{CH}_3-(\text{CH}_2)_8-\text{OH}$	$\text{CH}_3(\text{CH}_2)_6-\overset{\text{O}}{\parallel}{\text{C}}-\text{O}-\text{CH}_3$	$\text{CH}_3(\text{CH}_2)_3-\text{S}-(\text{CH}_2)_3\text{CH}_3$
5 2-Nonanone	6 1-Nonanol	7 Methyl octanoate	8 Dibutyl sulphide

Columns

The stainless-steel columns were 15 cm × 4.1 mm I.D. pre-packed with PRP-1 polystyrene-divinylbenzene copolymer (Hamilton, Reno, NV, U.S.A.) and 15 cm × 4.7 mm I.D. packed with commercially available Partisil ODS-3 octadecyl-bonded silica (Whatman, Clifton, NJ, U.S.A.) according to the well known slurry technique. The Partisil ODS-3 and PRP-1 packings have comparable particle sizes, average pore diameters and specific pore volumes (Table II). The C₁₈ silica was bonded with trichlorooctadecylsilane then end-capped with trimethylchlorosilane. As stated by the supplier, this leads to a material of polymeric nature with 10.5% (w/w) carbon.

Chromatographic procedure

Provided that the extracting support is not overloaded, which is *a priori* the case because of the usually very low concentration of aroma compounds in natural products and therefore much lower concentration in food industry effluents, the retention volume determined by the S-shaped curve obtained by frontal analysis exactly corresponds to that of the Gaussian-shaped peak observed in the conventional injection technique. Consequently, the experiments reported in this study were performed according to the latter method.

The methanol-water eluent was pumped isocratically at a flow-rate of 2 ml/min, which was continuously measured. The absorbance of the effluent was monitored at the wavelength of maximum absorbance for each compound.

TABLE II
MAIN CHARACTERISTICS OF THE STATIONARY PHASES STUDIED

Characteristic	Partisil ODS-3	PRP-1
Nature	Octadecyl silica	PS-DVB copolymer
Particle size (μm)	10 ± 1	10 ± 2
Specific surface area (m ² /g)	350	415
Average pore diameter (Å)	85	75
Specific pore volume (ml/g)	0.85	0.79
pH range	[2; 8]	[1; 13]

Capacity factors were calculated from the retention volume, V_R , of each solute:

$$k' = (V_R - V_0)/V_0$$

The dead volume, V_0 , was obtained for each eluent composition and both columns by measuring the elution time of $10^{-2} M$ potassium nitrate. Each value is the mean of at least three replicate determinations.

RESULTS AND DISCUSSION

Comparison of retention on the two sorbents

Capacity factors of aroma compounds were determined on each stationary phase. The volume content of water in the eluent, x , varied from 0 (pure methanol) to 1 (pure water). Fig. 1 presents the variations in $\log k'$ with x for each solute and both sorbents. The graphs are numbered from 1 to 8 according to the elution order of the solutes on the octadecyl bonded silica.

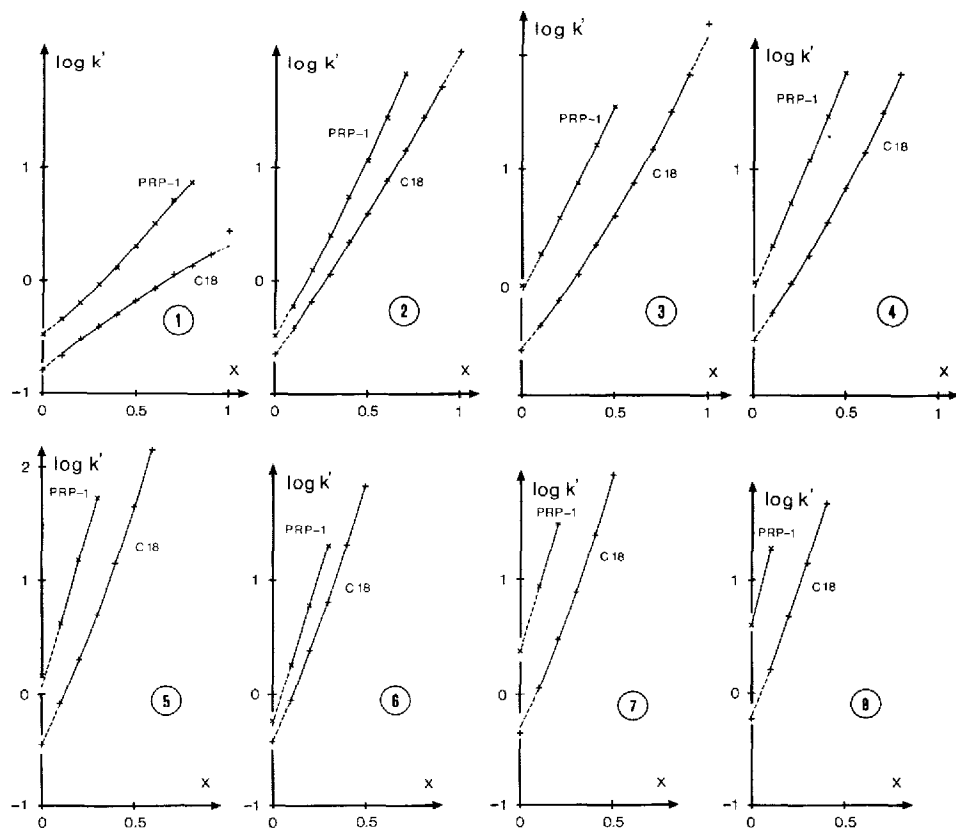


Fig. 1. Change in the capacity factor with increased volume content of water in the eluent, x , for eight model aroma compounds on two hydrophobic sorbents. Columns: (C₁₈) 10- μ m Partisil ODS-3 and 10- μ m PRP-1. Mobile phase: methanol-water; flow-rate: 2 ml/min. Detectors: UV absorbance and refractive index at 25°C. Volume injected: 20 μ l. Solute identification: see Table I.

Experimental retentions on the organic polymer are higher than those on the C₁₈ material, whatever the eluent composition and the solute studied:

$$k'_{\text{PRP-1}} > k'_{\text{C}_{18}} \quad (1)$$

However, the difference $\Delta k' = k'_{\text{PRP-1}} - k'_{\text{C}_{18}}$ is smaller for compounds 2 and 6 in comparison with the other solutes. These two compounds belong to the same chemical class, that of alcohols:

$$\Delta k'(\text{alcohols}) < \Delta k'(\text{non-alcohols}) \quad (2)$$

The first observation clearly shows the superiority of the PRP-1 material for trace-enrichment purposes as larger sample volumes can be treated before breakthrough occurs from columns of comparable dimensions. In other words, a given compound is quantitatively extracted by a smaller quantity of resin compared to bonded silica. This remark is of course valid only for the two stationary phases investigated in this study. The differences in k' would undoubtedly have been smaller if an octadecyl-bonded silica with a higher carbon loading or an organic copolymer with a lower specific surface area had been studied. Nevertheless, we investigated intermediate packings in both classes of reversed-phase media. The specific surface area for PRP-1 is between those of the well known XAD-2 (300 m²/g) and XAD-4 (780 m²/g) resins and so is the average pore diameter (75 Å, between 50 and 90 Å respectively). The specific surface area for alkyl-bonded silica usually varies from 150 to 600 m²/g and carbon loading from 3 to 20%. Partisil ODS-3 is an intermediate packing with 10.5% carbon loading and a specific surface area greater than 350 m²/g. Besides, (much) higher retentions on PS-DVB resins than on octadecyl-bonded silica with different characteristics than those studied here have already been reported for various compounds and eluents^{19,20,25,49-51}. Though it cannot be concluded that PS-DVB copolymers exhibit an higher retentive power than octadecyl-bonded silica as a rule, we are inclined to regard this statement as a general tendency.

Another helpful retention parameter is the selectivity of an LC system, defined for two solutes 1 and 2 by:

$$\alpha_{1,2} = k'_2/k'_1$$

As high retention and high selectivity often go together, higher selectivity is expected, under identical conditions, for the organic resin. Table III lists the selectivities of the two stationary phases, assessed relative to 2,3-butanedione for various water contents in the mobile phase. Except for alcohols, the higher selectivity of PRP-1 is verified for all values of x . For the two alcohols (compounds 2 and 6) PRP-1 exhibits an higher selectivity than C₁₈ only at higher water contents ($x > 0.7$ for compound 2 and $x > 0.2$ for compound 6). Fig. 2 compares the selectivities, relative to ethyl butanoate, of both stationary phases for all solutes tested and the eluent with $x = 0.1$. All but the hydroxylated model solutes lie on a straight line. The characteristics of the corresponding lines are almost independent of which compound (except alcohols) is involved as reference in the selectivity calculations, as shown below:

TABLE III

LOGARITHMS OF THE SELECTIVITIES, RELATIVE TO 2,3-BUTANEDIONE, OF BOTH RPLC SYSTEMS FOR VARIOUS WATER CONTENTS IN THE ELUENT, x

	x										
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Partisil ODS-3											
<i>cis</i> -3-Hexen-1-ol	0.15	0.26	0.34	0.47	0.65	0.78	0.95	1.11	1.32	1.49	1.60
<i>trans</i> -2-Hexenal	0.20	0.29	0.36	0.48	0.64	0.77	0.95	1.13	1.37	1.59	1.84
Ethyl butanoate	0.29	0.40	0.51	0.65	0.83	1.01	1.22	1.44	1.70		
2-Nonanone	0.40	0.59	0.82	1.11	1.45	1.83	2.21				
1-Nonanol	0.38	0.62	0.90	1.22	1.61	2.01					
Methyl octanoate	0.45	0.72	0.99	1.30	1.69	2.10					
Dibutyl sulphide	0.57	0.88	1.20	1.56	1.98						
PRP-1											
<i>cis</i> -3-Hexen-1-ol	0.01	0.12	0.30	0.45	0.64	0.75	0.93	1.12			
<i>trans</i> -2-Hexenal	0.44	0.59	0.77	0.92	1.11	1.24					
Ethyl butanoate	0.47	0.67	0.90	1.12	1.36	1.53					
2-Nonanone	0.63	0.96	1.38	1.77							
1-Nonanol	0.23	0.60	0.99	1.34							
Methyl octanoate	0.84	1.28	1.69								
Dibutyl sulphide	1.07	1.62									

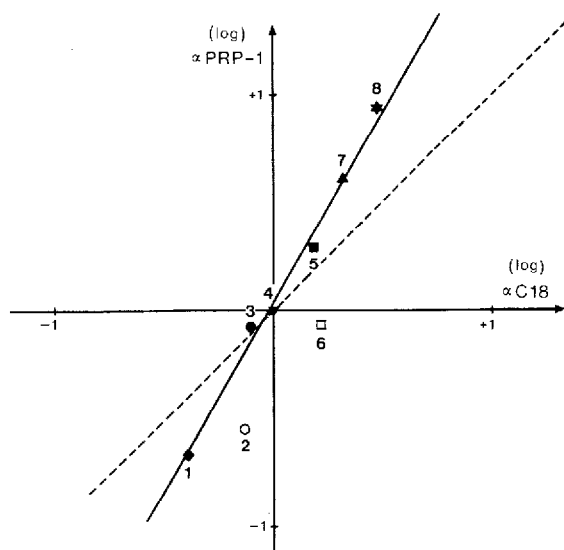


Fig. 2. Comparison of the selectivities of the octadecyl-bonded silica (αC_{18}) and of the PS-DVB copolymer ($\alpha PRP-1$) relative to ethyl butanoate; co-ordinates are logarithmic. The broken line represents equal selectivity. Mobile phase: methanol-water (90:10, v/v). See Table I for solute identification.

Reference compound	Slope	Intercept	Correlation coefficient
Dibutyl sulphide	1.78	-0.05	0.994
Ethyl butanoate	1.78	0.04	0.994
2,3-Butanedione	1.78	0.00	0.994

Accordingly, the selectivities of both sorbents for methanol-water (90:10, v/v) as the eluent and all but hydroxylated aroma compounds are related according to:

$$\alpha_{\text{PRP-1}} \approx (\alpha_{\text{C}_{18}})^{1.8}$$

The high selectivity of a sorbent may not be satisfactory for some particular trace enrichment purpose, *e.g.* when both the quantitative recovery of a given compound and the lowest distortion in the distribution of the solutes present in the sample before extraction are required. Then the C_{18} material may be preferred. In most cases, however, this shortcoming exhibited by the PRP-1 packing is economically counterbalanced by its higher retentive power.

From the open symbols for solutes 2 and 6 in Fig. 2 it is seen that both alcohols exhibit a particular chromatographic behaviour, *i.e.*, they are too strongly retained by the octadecyl-bonded silica or too weakly by the organic resin. Though the specific interaction of the hydroxyl moiety of these solutes with residual silanol sites of the C_{18} material can never be excluded, it is probably of limited importance, however, as the Partisil ODS-3 is an end-capped packing and the number of such sites is minimized. Furthermore, the silanols are covered with water molecules as soon as the water content in the eluent exceeds a few per cent, which prevents their direct interaction with the alcohols. The behaviour of compounds 2 and 6 must therefore be attributed in large part to the organic resin.

Relationship between $\log k'$ and $\log P$

The $\log P$ coefficients for model aroma compounds were calculated according to Rekker's method (see Table VI). As the hydrophobic contribution of the $-\text{CO}-\text{CO}-$ fragment is unknown, $\log P$ for 2,3-butanedione could not be estimated. Accordingly, the regression lines in Fig. 3 correlate the $\log P$, $\log k'$ data points for compounds 2-8 and various mobile phase compositions. The characteristics of the least-squares linear regressions are given in Table IV. The regression coefficients, r , are quite satisfactory for retention on the C_{18} -bonded silica. For the PS-DVB copolymer, solutes 2 and 6 are outliers. Indeed, when all compounds are considered in the regressions, the r values are very poor ($0.7 < r < 0.8$) but they are satisfactory values when compounds 2 and 6 are excluded. Separate lines, for alcohols (broken lines in Fig. 3b) and non-alcohols (full lines in Fig. 3b) respectively, must therefore be drawn.

The results argue in favour of a weaker interaction of alcohols with the PS-DVB in comparison to the C_{18} material and to other solutes, no particular role being attributed to the mobile phase. We also studied the retention of alcohols having an aliphatic skeleton, and Smith⁵² previously reported on aromatic compounds and two PS-DVB resins with methanol-water and acetonitrile-water eluents. Using retention indices based on an homologous series of alkyl aryl ketones, it was clearly that, in contrast to previous studies on silica-based columns, two hydroxylated compounds

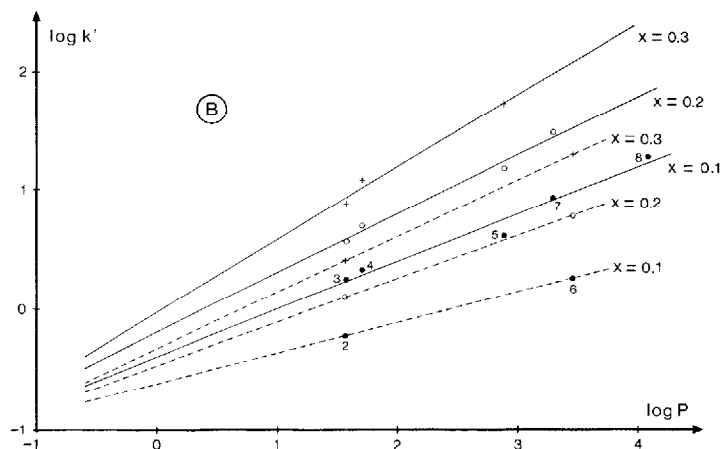
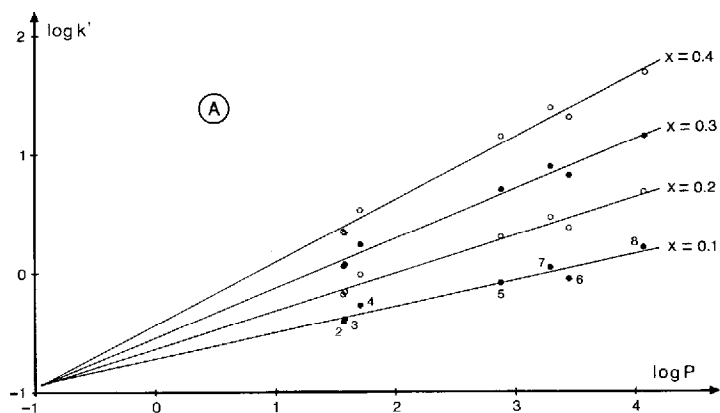


Fig. 3. Correlation between the octanol-water partition coefficient $\log P$ and $\log k'$ determined on both RPLC systems; (A) Partisil ODS-3; (B) PRP-1. The full lines in (B) correspond to all but hydroxylated solutes and the broken lines to alcohols. See Table I for solute identification.

TABLE IV

RESULTS OF THE LEAST-SQUARES LINEAR REGRESSION $\log k' = a \log P + b$ FOR BOTH RPLC SYSTEMS AND VARIOUS WATER CONTENTS IN THE ELUENT, x

x	Partisil ODS-3				PRP-1*			
	a	b	N^{**}	r	a	b	N^{**}	r
0.1	0.22	-0.71	7	0.97	0.40	-0.39	5	0.98
0.2	0.32	-0.63	7	0.985	0.50	-0.18	4	0.992
0.3	0.42	-0.54	7	0.990	0.61	-0.02	3	0.991
0.4	0.53	-0.43	7	0.992				

* Alcohols 2 and 6 are excluded.

** Number of data points.

(*p*-cresol and 2-phenylethanol) were less strongly retained on the polymer columns compared to the other solutes. It therefore makes no difference whether the hydrocarbonaceous skeleton of alcohols is aliphatic or aromatic, and the particular chromatographic behaviour of these solutes is due to their hydroxyl moiety. A possible explanation of the observed phenomenon lies in the different nature of the two hydrophobic materials investigated. PS-DVB copolymers are totally hydrocarbonaceous media and thus exhibit an homogeneous chemical structure. Non-polar bonded silica is more complex because alkyl chains as well as $\equiv\text{Si}-\text{O}-\text{Si}\equiv$, $\equiv\text{Si}-\text{O}-\text{C}\equiv$ and $\equiv\text{Si}-\text{OH}$ heteroatom-containing moieties co-exist in the material. Though one can assume octadecyl chains to have a somewhat lower solubility parameter ($\delta_{\text{octadecane}} = 8.04$)⁵³ than PS-DVB resins ($\delta \in [8.5; 9]$)⁵⁴; δ for octadecyl-bonded silica is therefore definitely greater than $8 \text{ cal}^{1/2}/\text{cm}^{3/2}$ and mixtures of methanol ($\delta = 14.5$) and water ($\delta = 23.4$) solvate the silica-based material better than the copolymer. Accordingly, direct interactions between solutes and the PS-DVB resin are favoured in comparison with the C_{18} packing, which may explain the systematically higher retentions observed for all compounds investigated. If one regards the weaker affinity of the resin for methanol as evidence of a general tendency, the lower retention of hydroxylated solutes may be accounted for as well.

Relationship between extrapolated log k'_w and log P

The major interest in the octanol–water partition coefficient is that it quantifies the hydrophobicity of various compounds. Therefore, $\log P$ is *a priori* a proper descriptor of the phenomena which govern solute retention in RPLC systems using totally aqueous mobile phases. In the scope of this work, focusing on such a consideration implies that the capacity factors of the solutes in pure water are known and raises the question of their estimation from the $\log k' = f(x)$ graphs in Fig. 1.

Each set of experimental x , $\log k'$ data was tested for least-squares regressions over the $0.1 \leq x \leq 0.9$ range. As $\log k'$ variations with x (or $1 - x$) are often reported to be linear when using methanol–water eluents with alkyl-bonded materials, the function $\log k' = ax + b$ was first investigated and the results were quite satisfactory ($r \geq 0.998$). However, the graphs in Fig. 1 become slightly curved as x increases and the quadratic function $\log k' = ax^2 + bx + c$ fits the experimental data better with $r \geq 0.9996$, or at least as well as the linear one. Finally, the variations in retention for 2,3-butanedione on PRP-1 are more accurately fitted by the power equation $\log k' = ax^n + b$ which accounts for the concavity of the somewhat S-shaped graph (curve 1).

Whatever the best mathematical function $\log k' = f(x)$, k'_w may significantly differ from the value predicted by the corresponding equation. For 2,3-butanedione, *cis*-3-hexen-1-ol and *trans*-2-hexenal on the C_{18} -bonded silica, positive differences are noticeable between the experimental k'_w and the quadratically extrapolated value for $x = 1$ (broken lines of graphs 1–3 in Fig. 1). Positive as well as negative differences may generally be observed³¹ but neither their sign nor their magnitude can be predicted. The accuracy of the estimation of k'_w by extrapolation of the $\log k' = f(x)$ curves may therefore be very poor as shown by several examples in Table V. For some solutes (aniline, biphenyl, dimethyl phthalate), the quadratic functions lead to retention volumes in better agreement with the experimental values than are those obtained by the regression lines. Nevertheless, these quadratic extrapolations may be

TABLE V

COMPARISON OF RETENTION VOLUMES IN PURE WATER

Mobile phase: methanol-(acidified)water. Stationary phase: 5- μ m LiChrosorb RP-18. Predicted data which satisfactorily match with the data measured are underlined. From ref. 25 (© American Chemical Society).

Compound	Retention volume (ml)		
	Calculated*		Experimental**
	Quadratic	Linear	
Aniline	<u>0.7</u>	0.5	1.0
Benzophenone	1370	22	62
Biphenyl	365	154	390
Dimethyl phthalate	<u>32</u>	4.3	35
Ethylbenzene	150	<u>43</u>	25
Naphthalene	2320	<u>64</u>	37
Phenol	0.9	<u>0.6</u>	0.4
Toluene	70	<u>14</u>	7.5

* Extrapolated using a quadratic and a linear relationship for $\ln k'$ vs. eluent composition. Column: 5 cm \times 4.6 mm I.D.

** Determined on a 2.2 mm \times 4.6 mm I.D. column.

the cause of the most dramatic differences from the experimental retention volumes (predicted volume for benzophenone is 22-fold and that for naphthalene 63-fold greater than the experimental volume). Generally, experimental data for k'_w are unavailable and the choice of an appropriate estimation has to be made. In order to reduce the possible difference between extrapolated and true k'_w values, and as an "easy-to-apply" calibration procedure was sought, we decided to linearly extrapolate the retention observed for the two higher x values experimentally investigated for

TABLE VI

EXTRAPOLATED LOGARITHMS OF CAPACITY FACTORS IN PURE WATER

Compound	$\log P$	x^*		$\log k'$	
		ODS-3	PRP-1	ODS-3	PRP-1
2,3-Butanedione	0.16**	1.0	0.7-0.8	0.44***	1.19
cis-3-Hexen-1-ol	1.56	1.0	0.6-0.7	2.04***	3.00
trans-2-Hexenal	1.57	1.0	0.4-0.5	2.28***	3.20
Ethyl butanoate	1.70	0.7-0.8	0.4-0.5	2.51	3.69
2-Nonanone	2.88	0.5-0.6	0.2-0.3	4.10	5.58
1-Nonanol	3.45	0.4-0.5	0.2-0.3	4.43	4.87
Methyl octanoate	3.29	0.4-0.5	0.1-0.2	4.57	5.89
Dibutyl sulphide	4.07	0.3-0.4	0.0-0.1	4.86	7.40

* Water content corresponding to the data points involved in the linear extrapolation (see text).

** Mean of seven estimations (see text).

*** Measured data.

each solute. For instance, data points characterized by $x = 0.7$ and 0.8 were considered for ethyl butanoate and $x = 0.3$ and 0.4 for dibutyl sulphide on the Partisil packing. The estimated capacity factors for all model solutes in pure water in both stationary phases are given in Table VI together with the corresponding x values.

This procedure is *a priori* more accurate as the water content in the eluent is higher. As for the correlation between $\log P$ and the extrapolated k'_w values, we weighted each $\log P$, $\log k'_w$ point according to the highest x value which enabled the estimation of the corresponding k'_w . For the examples given above on the bonded silica, ethyl butanoate was assigned a weight of 8 and dibutyl sulphide one of 4. Whenever available, experimental k'_w values were of course considered in the regression and the highest weight, 10, was assigned to the corresponding points. Finally, 2,3-butanedione was considered as well as other solutes thanks to the octanol-water coefficient determined from the experimental retentions and the various regression characteristics listed in Table IV (mean value : $\log P = 0.16$). The correlation lines

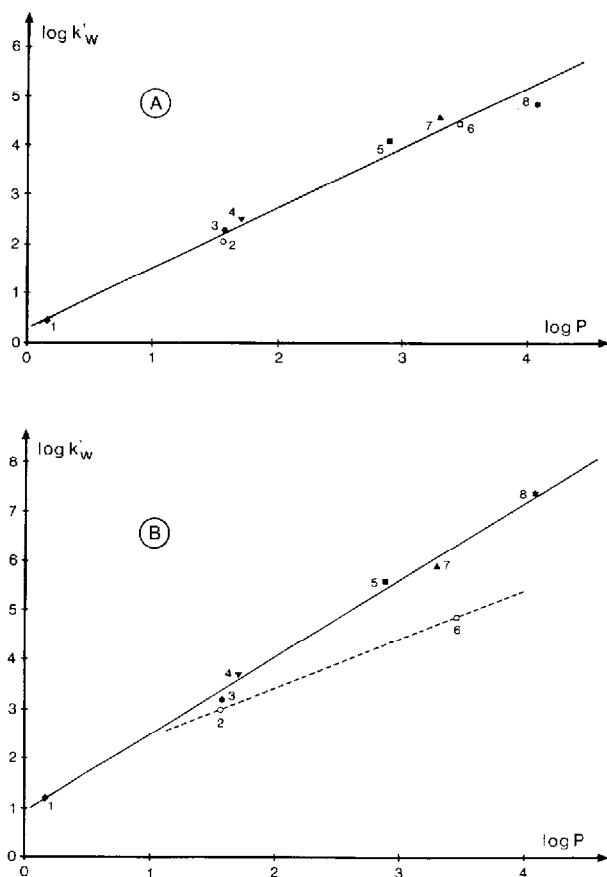


Fig. 4. Calibration graphs for both RPLC systems in pure water; (A) Partisil ODS-3; (B) PRP-1. The full line in (B) corresponds to all but hydroxylated solutes and the broken line to hydroxylated compounds. See Table I for solute identification.

obtained on both non-polar materials are presented in Fig. 4 and can be written as follows:

$$C_{18}: \log k'_w = 1.22 \log P + 0.30 \quad (r = 0.992)$$

PRP-1: non-hydroxylated solutes

$$\log k'_w = 1.57 \log P + 0.92 \quad (r = 0.998)$$

hydroxylated solutes

$$\log k'_w = 0.99 \log P + 1.46 \quad (r = 1)$$

Comparison of the figures in Table IV shows that the correlation coefficient for the octadecyl-bonded packing is as high as those corresponding to lower x values. As for the PRP-1 copolymer, two separate lines (for alcohols and non-alcohols respectively) must be drawn and the correlation coefficient for non-hydroxylated solutes is greater than those obtained for other eluent compositions.

These regression lines calibrate both RP materials in pure water. No additional experiment is needed for the estimation of the retention of any compound. The sole limitation lies in the chemical structure of the solutes considered which must not be too complicated so that the $\log P$ calculations be as accurate as possible. The mean error associated with the estimation of k'_w can be calculated by:

$$\bar{e} = \frac{\sum_{i=1}^N \frac{k'_{w \text{ lsr } i} - k'_{w \text{ ext } i}}{k'_{w \text{ ext } i}}}{N}$$

\bar{e} is therefore defined as the percentage difference between the $k'_{w \text{ lsr } i}$ value given by the least-squares regression function and the extrapolated $k'_{w \text{ ext } i}$ value among the N aroma compounds. It equals 47% for the Partisil material and 30% for non-hydroxylated solutes retained on the PRP-1 resin respectively. It must be emphasized that these \bar{e} values only quantify the mean error associated with the use of the calibration lines for both chromatographic systems in pure water. They do not provide an unequivocal evidence for the accuracy of the k'_w extrapolation method suggested above. Such evidence, which requires (more) experimental k'_w values, will appear later, together with that of the $\log P$, $\log k'_w$ ponderal regression, in another article on the trace enrichment of aroma-containing food plant waste-waters⁵⁵.

CONCLUSION

Retention experiments performed with methanol-water mobile phases and model aroma compounds showed that the PRP-1 polystyrene-divinylbenzene copolymer exhibits an higher retentive power than the Partisil ODS-3 octadecyl-bonded silica and is therefore better suited for trace-enrichment purposes.

An "easy-to-apply" calibration procedure for both RPLC system has been presented, which correlates the hydrophobic $\log P$ coefficient calculated according to Rekker's method with the extrapolated logarithm of the capacity factor for solutes in pure water, $\log k'_w$. This procedure led to one calibration line for the C_{18} material and two lines (for hydroxylated and non-hydroxylated solutes respectively) for the

organic resin. Such a standardization enables the estimation of the retention of any solute in pure water, *i.e.*, the corresponding breakthrough volume as far as dilute samples are concerned. Further investigations which help define the scope of application of such a calibration thanks to frontal analysis experiments as well as its use for the trace enrichment of industrial waste-waters will be presented in forthcoming papers^{5,56}.

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REFERENCES

- 1 H. P. M. van Vliet, Th. C. Bootsman, R. W. Frei and U. A. Th. Brinkman, *J. Chromatogr.*, 185 (1979) 483.
- 2 R. Shinohara, A. Kido, S. Eto, T. Hori, M. Koga and T. Akiyama, *Water Res.*, 15 (1981) 535.
- 3 Y. Kumar, *J. Environ. Sci. Health*, B18 (1983) 757.
- 4 B. Crathorne, M. Fielding, C. P. Steel and C. D. Watts, *Environ. Sci. Technol.*, 18 (1984) 797.
- 5 L. Renberg and K. Lindström, *J. Chromatogr.*, 214 (1981) 327.
- 6 B. T. Mori and K. J. Hall, *J. Environ. Sci. Health*, A12 (1977) 341.
- 7 W. A. Saner, J. R. Jadamec, R. W. Sager and T. J. Killeen, *Anal. Chem.*, 51 (1979) 2180.
- 8 J. Pempkowiak, *J. Chromatogr.*, 258 (1983) 93.
- 9 P. van Rossum and R. G. Webb, *J. Chromatogr.*, 150 (1978) 381.
- 10 R. A. Moore and F. W. Karasek, *Int. J. Environ. Anal. Chem.*, 17 (1984) 187.
- 11 A. K. Burnham, G. V. Calder, J. S. Fritz, G. A. Junk, H. J. Svec and R. Willis, *Anal. Chem.*, 44 (1972) 139.
- 12 G. R. Aiken, E. M. Thurman, R. L. Malcolm and H. F. Walton, *Anal. Chem.*, 51 (1979) 1799.
- 13 R. L. Gustafson, R. L. Albright, J. Heisler, J. A. Lirio and O. T. Reid, Jr., *Ind. Eng. Chem. Prod. Res. Dev.*, 7 (1968) 107.
- 14 A. Tateda and J. S. Fritz, *J. Chromatogr.*, 152 (1978) 329.
- 15 V. C. Block, G. P. Slater and E. M. Giblin, *Water Sci. Technol.*, 15 (1983) 149.
- 16 G. A. Junk, J. J. Richard, M. D. Grieser, D. Witiak, J. L. Witiak, M. D. Arguello, R. Vick, H. J. Svec, J. S. Fritz and G. V. Calder, *J. Chromatogr.*, 99 (1974) 745.
- 17 D. Levesque and V. N. Mallet, *Int. J. Environ. Anal. Chem.*, 16 (1983) 139.
- 18 R. L. Smith and D. J. Pietrzyk, *J. Chromatogr. Sci.*, 21 (1983) 282.
- 19 B. Zygmunt, J. Visser, U. A. Th. Brinkman and R. W. Frei, *Int. J. Environ. Anal. Chem.*, 15 (1983) 263.
- 20 C. E. Werkhoven-Goewie, W. M. Boon, A. J. J. Praat, R. W. Frei, U. A. Th. Brinkman and C. J. Little, *Chromatographia*, 16 (1982) 53.
- 21 J. A. Leenheer, *J. Res. U.S. Geol. Survey*, 4 (1976) 737.
- 22 F. A. Maris, R. B. Geerdink, R. W. Frei and U. A. Th. Brinkman, *J. Chromatogr.*, 323 (1985) 113.
- 23 A. R. Oyler, D. L. Bodenner, K. J. Welch, R. J. Llukkonen, R. M. Carlson, H. L. Kopperman and R. Caple, *Anal. Chem.*, 50 (1978) 837.
- 24 U. Niederschulte and K. Ballschmitter, *Fresenius' Z. Anal. Chem.*, 269 (1974) 360.
- 25 C. E. Werkhoven-Goewie, U. A. Th. Brinkmann and R. W. Frei, *Anal. Chem.*, 53 (1981) 2072.
- 26 W. Golkiewicz, C. E. Werkhoven-Goewie, U. A. Th. Brinkmann, R. W. Frei, H. Colin and G. Guiochon, *J. Chromatogr. Sci.*, 21 (1983) 27.
- 27 W. E. Hammers, G. J. Meurs and C. L. de Ligny, *J. Chromatogr.*, 247 (1982) 1.
- 28 W. Butte, C. Fooker, R. Klussmann and D. Schuller, *J. Chromatogr.*, 214 (1981) 59.
- 29 J. L. G. Thus and J. C. Kraak, *J. Chromatogr.*, 320 (1985) 271.
- 30 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 185 (1979) 179.
- 31 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 282 (1983) 107.
- 32 M. J. M. Wells and C. R. Clark, *J. Chromatogr.*, 235 (1982) 31.

- 33 M. J. M. Wells, C. R. Clark and R. M. Patterson, *J. Chromatogr.*, 235 (1982) 43.
- 34 S. Bitteur, *Thèse de Doctorat d'Etat*, Université Pierre et Marie Curie, Paris, 1986.
- 35 T. Braumann and L. H. Grimme, *J. Chromatogr.*, 206 (1981) 7.
- 36 L. Renberg and G. Sundström, *Chemosphere*, 7 (1979) 449.
- 37 R. F. Rekker, *The Hydrophobic Fragmental Constant*, Elsevier, Amsterdam, 1977.
- 38 C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, 86 (1964) 1616.
- 39 C. Hansch, A. Leo and D. Nikaitani, *J. Org. Chem.*, 37 (1972) 3090.
- 40 A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 71 (1971) 525.
- 41 H. Könemann, R. Zelle, F. Busser and W. E. Hammers, *J. Chromatogr.*, 178 (1979) 559.
- 42 T. M. Xie, B. Hulthe and S. Folestad, *Chemosphere*, 13 (1984) 445.
- 43 R. E. Koopmans and R. F. Rekker, *J. Chromatogr.*, 285 (1984) 267.
- 44 M. C. Hennion, *Thèse de Doctorat d'Etat*, Université Pierre et Marie Curie, Paris, 1982.
- 45 N. Tanaka, H. Goodell and B. L. Karger, *J. Chromatogr.*, 158 (1978) 233.
- 46 M. Harnisch, H. J. Möckel and G. Schulze, *J. Chromatogr.*, 282 (1983) 315.
- 47 M. J. M. Wells, C. R. Clark and R. M. Patterson, *J. Chromatogr. Sci.*, 19 (1981) 573.
- 48 M. J. M. Wells and C. R. Clark, *J. Chromatogr.*, 284 (1984) 319.
- 49 D. J. Pietrzyk, E. P. Kroeff and T. D. Rotsch, *Anal. Chem.*, 50 (1978) 497.
- 50 J. Bontemps, L. Bettendorff, J. Lombet, C. Grandfils, G. Dandrifosse, E. Schoffeniels, F. Nevejans and J. Crommen, *J. Chromatogr.*, 295 (1984) 486.
- 51 R. I. Greyson and A. M. Patch, *J. Chromatogr.*, 242 (1982) 349.
- 52 R. M. Smith, *J. Chromatogr.*, 291 (1984) 372.
- 53 A. S. Kertes, *J. Inorg. Nucl. Chem.*, 26 (1964) 1764.
- 54 S. Mori, *Anal. Chem.*, 50 (1978) 745.
- 55 S. Bitteur and R. Rosset, *J. Food Sci.*, submitted for publication.
- 56 S. Bitteur and R. Rosset, *Chromatographia*, in press.