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# Comparison of reversed-phase extraction sorbents for the on-line trace enrichment of polar organic compounds in environmental aqueous samples

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## ABSTRACT

On-line sample handling coupling the enrichment of trace organic compounds on reversed-phase sorbents packed in small-size precolumns and their liquid chromatographic separation was investigated. Methods allowing the prediction of breakthrough volumes are reviewed and it is pointed out that the capacity factor of solutes in water,  $k_w'$ , is a convenient parameter for predicting the sample volume which can be handled for the preconcentration. A comparison between the main LC-grade sorbents available for the preconcentration of organic compounds in water, alkyl-bonded silicas, non-polar styrene-divinylbenzene copolymers and porous graphitic carbons, is presented. The potential of porous graphitic carbon for trace enrichment of very polar compounds is shown.

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## INTRODUCTION

The use of liquid chromatography (LC) for environmental monitoring has gained in popularity in recent years owing to its suitability of the determination of polar and/or thermodegradable compounds without any derivatization step [1]. Screening for a large group of pesticides over a wide range of polarity can be easily achieved in one run by combining gradient elution reversed-phase LC and sensitive diode-array detection [2–4]. Such a screening cannot be performed by gas chromatography, which requires specific derivatization for each polar group of pesticides.

Determination of trace amounts of organic compounds in environmental aqueous samples requires a preconcentration step before LC analysis. Solid-phase extraction (SPE) techniques have grown in interest as an alternative to laborious and time-consuming liquid-liquid extraction (LLE) methods.

SPE is often described as an off-line sample preparation technique. Trace organics are trapped by a suitable sorbent packed in disposable columns or cartridges or enmeshed in an inert matrix of a membrane-based extraction disc, while the water passes through, and are later recovered by elution with a small volume of organic solvent. Compared with LLE-based sample preparation methods, off-line SPE offers reduced processing time and substantial solvent savings. Percolation of samples can be performed in the field, avoiding the problem of transport and storage of voluminous samples. Automation is possible using robotics or special sample preparation units that sequentially extract samples and clean them up for automatic injections. Nevertheless, a certain amount of tedious labour remains and off-line procedures have the inherent disadvantages of a loss in sensitivity owing to the injection of an aliquot, losses in the evaporation step and some risks of contamination, so that internal standards are required.

Many of these drawbacks can be avoided by using on-line trace enrichment on a so-called precol-

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umn. After the sample percolation, the precolumn is coupled to a reversed-phase analytical column via switching valves. The concentrated analytes are then directly desorbed and transferred to the analytical column by a water–organic solvent gradient at the same time as they are separated. As there is no sample manipulation between preconcentration and analysis, no loss or contamination risk can occur and the whole of the concentrated analytes are analysed, allowing the handling of a smaller volume in comparison with off-line techniques where usually only an aliquot is analysed. If adsorption and desorption are efficient, one can expect more accurate quantitative results. Automation can be easily achieved [5,6] and two automatic devices for the on-line coupling of SPE to LC are now commercially available, *i.e.*, the Prospekt (Spark Holland) and the OPS-2 (Merck). In order to avoid band broadening of analytes during their transfer from the precolumn to the analytical column, the dimensions of the precolumn have to be small compared with those of the analytical column [7] and the precolumn has to be packed with HPLC-grade sorbents. Disposable precolumns used with the Prospekt are 10 mm × 2 mm I.D. and several cartridge versions containing 30–80 mg of sorbent are available for the OPS-2. The recent commercialization of these automatic devices and of sensitive diode-array detectors will certainly help in the development of on-line trace enrichment methods in environmental analysis. It is then important to be able to select the parameters of the preconcentration step depending on the detection level required and on the solutes to be determined.

SPE can be described to a first approximation as a simple chromatographic process and retention of organics occurs provided that the organic compounds are not eluted by the water of the aqueous sample itself. A significant parameter in SPE is the sample volume that can be handled for the preconcentration without any breakthrough. There is a need to determine many organic pollutants at concentrations below the  $\mu\text{g/l}$  level. Monitoring drinking water in Europe requires detection limits as low as 50 ng/l, as the maximum allowed concentration limit for each pesticide is 0.1  $\mu\text{g/l}$ . The sample volume that has to be handled for detection at this level should be at least 100–200 ml and depends on the detection mode. In contrast with off-line SPE, one cannot increase the breakthrough volume by

increasing the amount of sorbent and therefore the retention ability of the sorbent is an important parameter in on-line trace enrichment. In this work, prediction of the breakthrough volumes was examined as a function of the nature of the sorbent and of the characteristics of the pollutant. Predictions are based on retention data.  $\text{C}_{18}$  silicas, the copolymer-based PRP1 and porous graphitic carbon, suitable for HPLC, were investigated and were compared for the trace enrichment of polar compounds.

#### PREDICTION OF BREAKTHROUGH VOLUMES

The breakthrough volume is the key parameter in SPE because it indicates the sample volume and therefore the amount of analyte that can be preconcentrated and that is available for detection. Two factors can be responsible for breakthrough: insufficient retention of the analytes by the sorbent and overloading of the sorbent. It is unlikely that in practical environmental analyses of organic pollutants where concentrations are typically of the order of  $\mu\text{g/l}$  that breakthrough will occur owing to overloading of the sorbent capacity [5,8,9].

#### Recording breakthrough curves

Fig. 1 presents a breakthrough curve obtained when monitoring continuously the UV signal of the effluent from an extraction precolumn. Water spiked with an organic compound at the trace level and having a UV absorbance  $A_0$  is percolated through the precolumn. Provided that the compound is retained by the sorbent of the precolumn, the effluent does not contain it and its UV absorbance is

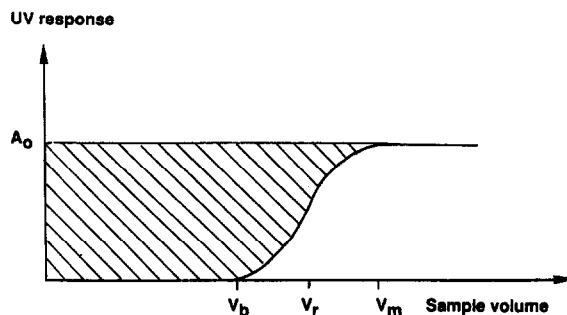


Fig. 1. Theoretical breakthrough curve obtained by percolation of a spiked sample (UV absorbance  $A_0$ ) through a precolumn. See text for definition of  $V_b$ ,  $V_r$  and  $V_m$ .

zero. For a volume  $V_b$ , usually defined at 1% of the initial absorbance, a frontal or breakthrough curve is recorded, and after a volume  $V_m$ , defined at 99% of the initial absorbance, the effluent has the same composition as that of the entering solution. Under ideal conditions, this curve has a bi-logarithmic shape and the inflection point is the retention volume,  $V_r$ , of the solute eluted by pure water (when the precolumn is not overloaded). Recovery is defined as the ratio between the amount extracted and the amount percolated. As can be seen in Fig. 1, a theoretical 100% recovery can be obtained only for sample volumes equal to or lower than  $V_b$ . The  $V_b$  values are therefore important because a simple calculation indicates if the concentration in the sample can be detected by comparing the absolute detection limit of the detection system (in nanograms injected) and the amount that can be preconcentrated in the volume  $V_b$ . The difficulty is that recording breakthrough curves is time consuming and other methods for determining or predicting  $V_b$  are necessary.

#### Calculations of breakthrough volumes

Werkhoven-Goewie *et al.* [10] have shown that  $V_b$  can be calculated easily from retention data. The retention volume is related to the breakthrough volume by

$$V_b = V_r - 2 \sigma_v \quad (1)$$

where  $\sigma_v$  is the standard deviation depending on the axial dispersion of analyte along the bed of particles in the precolumn.  $V_r$  can be calculated easily from the knowledge of the capacity factor in pure water,  $k'_w$ , and the dead volume of the precolumn,  $V_0$ , according to

$$V_r = V_0 (1 + k'_w) \quad (2)$$

$\sigma_v$  can be calculated if the number of theoretical plates  $N$  of the precolumn is known by the equation

$$\sigma_v = \frac{V_0}{\sqrt{N}} (1 + k'_w) \quad (3)$$

Both  $V_0$  and  $N$  values were determined from one experimental breakthrough curve and it was assumed that these values were the same for other solutes.  $V_b$  values can be calculated if  $k'_w$  can be predicted or easily measured.

#### Determination and prediction of $k'_w$

Several means have been proposed for determining  $k'_w$  values. Direct experimental measurement is difficult and sometimes impossible owing to high values of the order of magnitude of 100 and above, depending on the size and polarity of the solute.

With alkylsilicas as reversed-phase sorbents, quadratic relationships have been found for the variation of the capacity factor with the volume fraction of organic solvent,  $\phi$ , in binary mobile phases, according to theories based on the solubility parameter concept or on interaction indices [11–13]:

$$\log k' = a - b\phi + d\phi^2 \quad (4)$$

It has been shown that for some solutes this equation does not provide an accurate description of the solute retention in water-rich mobile phases [14]. Nevertheless, over a limited range of binary composition, a linear relationship between  $\log k'$  and  $\phi$  can often be used as a good approximation, especially with methanol–water mobile phases. This linear relationship allows graphical extrapolation of  $k'_w$  by measuring  $\log k'$  for at least three different mobile phase compositions. However, as pointed out by Jandera and Kubat [15], the accuracy of the extrapolated value depends strongly on the range of the experimental  $k'$  and on the range of methanol concentrations used for the determination of the experimental data points. They have shown that when using a short microcolumn (30 × 1 mm I.D.), for some solutes, it was possible to measure  $k'_w$  directly by elution methods and that for more apolar herbicides extrapolations are more rapid and more accurate because the experimental range for extrapolation is water-rich.

Jandera and Kubat [15] have also proposed a rapid sorption method;  $k'_w$  is defined as the ratio between the amount of solute in the stationary phase and in the mobile phase. A sample is pumped through a microcolumn until equilibrium and the amount of solute adsorbed on the stationary phase is measured by desorption to an analytical column via a switching valve, similarly to the on-line preconcentration device. They found that experimental values determined by the sorption method are higher than those determined by elution methods.

Another method for predicting  $k'_w$  is based on the use of the water–octanol partition coefficient, characterizing the hydrophobicity of a compound and

playing an important role in phenomena of physico-chemical, biological and environmental interest [16]. These partition coefficients,  $P_{\text{oct}}$ , can be calculated by taking into account the molecular fragment and intramolecular effects [17]. Braumann [18] has clearly shown the good relationship between extrapolated  $\log k'_w$  values from the linear  $\log k'$ -methanol volume fraction relationship and  $\log P_{\text{oct}}$ .

#### *Experimental determination of breakthrough volumes*

An experimental method for determining both breakthrough volumes and recoveries has been developed [8,19]. It is easily performed with the on-line set-up. A small volume,  $V_p$ , spiked with a known concentration,  $C_p$ , of each analyte of interest is percolated through the precolumn. The chromatogram corresponding to the on-line elution of the precolumn is then recorded and peak areas are measured. This first volume is chosen in order that breakthrough does not occur for any solute (5 or 10 ml, for instance) and this can be easily verified by comparing the chromatogram with that obtained by loop injection of the same amount directly into the analytical column. Then, the sample volume is increased and the concentration decreased in order to have the same amount,  $C_p V_p$ , of analytes in each sample volume  $V_p$  percolated. Provided that breakthrough does not occur for any solute, the amount concentrated remains constant and the peak areas obtained on the on-line chromatogram are constant. When breakthrough occurs, the amount extracted decreases, as also do the peak areas. Breakthrough volumes can thus be estimated by three or four percolations for all the solutes of interest simultaneously. Corresponding recoveries can also be calculated by dividing peak areas obtained when percolating a volume  $V_p$  by those obtained for 10 ml. By noting the peak area  $A_i$  of one analyte in the first chromatogram without breakthrough, and the peak area  $A_p$  of the same analyte when percolating  $V_p$ , there is a relationship between  $A_i$ ,  $A_p$  and  $V_b$  as follows:

$$\text{when } V_p < V_b: A_p/A_i = 1 \quad (5)$$

$$\text{when } V_p > V_m: A_p/A_i = V_r/V_p \quad (6)$$

The relationship between  $V_b$  and  $V_m$  is not simple, but it has been shown that eqn. 6 allows a good estimation of  $V_m$  values, as defined in the break-

through curve [8]. An advantage of this method is that these determinations are performed via the whole on-line system using the same operating conditions as for the quantitative analyses.

## EXPERIMENTAL

### *Apparatus*

On-line percolation of water was performed with a Chromatem (Touzart et Matignon, Paris, France) or a Milton Roy (LDC, Riviera Beach, FL, USA) pump. Precolumn elutions and analyses were carried out with a Model 5060 liquid chromatography equipped with a UV 200 variable-wavelength spectrophotometer (Varian, Palo Alto, CA, USA) and a Coulochem Model 5100 electrochemical detector (ESA, Bedford, MA, USA). Precolumns and analytical column switching were connected with two Rheodyne (Berkeley, CA, USA) valves. Quantitative measurements of peak areas were provided by a CR3A integrator-computer from Shimadzu (Kyoto, Japan). The on-line experimental set-up is described, *e.g.*, in ref. 5, the stainless-steel precolumn being placed in the sample-loop position of the six-port liquid switching valve. The recording of breakthrough curves is described in ref. 8.

### *Stationary phases and columns*

A commercially available column packed with Hypercarb porous graphitic carbon (100 × 4.6 mm I.D., 7- $\mu\text{m}$  particle size) (Shandon, Runcorn, UK), a laboratory-packed column with the PRP-1 copolymer (100 × 4.6 mm I.D., 10- $\mu\text{m}$  particle size) (Hamilton, Reno, NV, USA) and a laboratory-packed column with LiChrosorb RP-8 or RP-18 (100 × 4.6 mm I.D., 5- $\mu\text{m}$  particle size) (Merck, Darmstadt, Germany) or a 250 × 4.6 mm I.D. column prepaced with spherical 5- $\mu\text{m}$  Zorbax octylsilica (Interchim, Paris, France) were used for retention measurements. Stainless-steel precolumns (21 mm × 10 mm I.D.) available from Chrompack (Middelburg, Netherlands) and laboratory-made stainless-steel precolumns (22 mm × 4.6 mm I.D. or 27 mm × 4.6 mm I.D.) were used for high retention measurements in water-rich mobile phases and for on-line preconcentration. They were laboratory-packed using a thick slurry and a microspatula. The void volume was determined by injection of a 2 M solution of sodium nitrate.

### Chemicals

HPLC-grade acetonitrile was obtained from Rathburn (Walkerburn, UK) and methanol from Prolabo (Paris, France). LC-grade water was prepared by purifying demineralized water in a Milli-Q filtration system (Millipore, Bedford, MA, USA). Other chemicals were obtained from Prolabo, Merck or Fluka (Buchs, Switzerland). Stock solutions of selected solutes were prepared by weighing and dissolving them in methanol. LC-grade water samples were spiked with these solutions at the ppb or ppt level. The final standard solutions did not contain more than 0.5% of methanol.

### RESULTS AND DISCUSSION

#### Alkyl-bonded silica sorbents

**Experimental measurements of  $V_b$ .** The different methods for predicting breakthrough values were first investigated with *n*-alkylsilica stationary phases, as many data have been published using these sorbents. Fig. 2a shows experimental breakthrough curves obtained for three herbicides with a 10 mm × 2.1 mm I.D. precolumn packed with  $C_{18}$  silica. These curves have a different shape and are spread over a larger volume when the compounds are more strongly retained, owing to the low plate number in the precolumn. The front corresponding to linuron is spread over nearly 100 ml from a  $V_b$  value of 70 ml to a  $V_m$  value of 165 ml. First, the determination of  $V_b$  at 1% of the initial absorbance on the front curves cannot be accurate when the front is not sharp. Also, if one wants a 100% recovery, the percolated volume has to be lower than 70 ml. Nevertheless, it can be seen that increasing the percolated volume to 165 ml increases considerably the amount preconcentrated, up to nearly 50%. The corresponding recovery at that point is below 100%, but overcoming the breakthrough volume is often of interest when traces of organic compounds have to be determined in water samples with relatively low organic contamination. When many solutes are to be determined together, this situation occurs, of course, for some analytes. The volume  $V_r$  is then a good indication of the sample volume that can be preconcentrated with good recoveries. It is important to note that with on-line methodologies, once the experimental conditions have been selected,

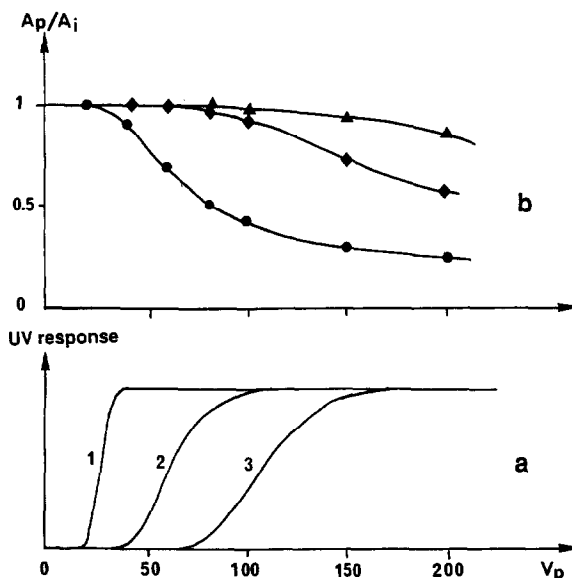


Fig. 2. (a) Experimental breakthrough curve recorded with a 1 cm × 0.21 cm I.D. precolumn packed with RP-18 silica. Sample: solution spiked with 100 µg/l of (1) simazine, (2) atrazine and (3) linuron. (b) Experimental variations of the ratio between peak area obtained for the on-line preconcentration and elution of a sample volume  $V_p$  spiked at a concentration  $C_p$  and peak area obtained for a 10-ml percolation volume spiked with 50 µg/l of each of the three herbicides (amount preconcentrated = 0.5 µg). The product  $C_p V_p$  is constant and equal to 0.5 µg. Solutes: ● = simazine; ◆ = atrazine; ▲ = linuron.

quantitative analyses are carried out with spiked samples that are preconcentrated and analysed on-line via the whole system. Knowing accurately the recoveries of analytes is therefore useless.

The experimental method derived from the percolation of spiked samples with increasing volumes and decreasing concentration is represented in Fig. 2b for the three herbicides together. For simazine similar  $V_b$  values are found, whereas for atrazine and linuron higher  $V_b$  values are estimated on the curves representing the variations of  $A_p/A_i$  with the percolated volume than those measured on breakthrough curves recorded for each compound separately. This can be explained by the fact that  $V_b$  values can be slightly different when analytes are percolated together on the precolumn. This is why we consider it is better to estimate both  $V_b$  and recovery values by spiking samples and using the whole on-line system under similar conditions to those used for the analyses of real samples.

TABLE I

$V_r$  VALUES MEASURED ON BREAKTHROUGH CURVES AND CALCULATED USING EXPERIMENTAL  $\log k'_w$  VALUES OR EXTRAPOLATED  $\log k'_w$  VALUES

See text for calculations.

Solutes	Breakthrough curves			Experimental values		Extrapolated values	
	$V_b$ (ml)	$V_r$ (ml)	$V_m$ (ml)	Log $k'_w$	$V_r$ (ml)	Log $k'_w$	$V_r$ (ml)
2-Nitrophenol	1.5	2.5	7	1.9	1.8	1.9	1.8
Toluene	2.5	5	9	2.45	$7 \pm 1$	2.75	$13 \pm 1$
Simazine	19	26	36	3.1 <sup>a</sup>	$30 \pm 3$	2.7 <sup>a</sup>	$12 \pm 2$
Atrazine	37	60	110	3.55 <sup>a</sup>	$80 \pm 7$	3.2 <sup>a</sup>	$33 \pm 3$

<sup>a</sup> Values taken from ref. 15.

*Calculations of  $V_r$  from  $\log k'_w$ .* From a practical point of view, the curves above indicated that  $V_r$  is a good indication of the sample volume that can be handled. As many values of  $k'_w$  have been published, the practical problem is to relate this value to  $V_r$ , which can be easily done by the equation  $V_r = (1 + k'_w)V_0$ . The void volume is the product of the geometric volume of the precolumn by the porosity  $\epsilon$  of the sorbent. An average value between 0.65 and 0.70 was taken for the porosity value of *n*-alkyl-silicas. Table I reports the experimental values measured on breakthrough curves and the calculated values from experimental measurements of  $k'_w$ . The agreement is correct if one takes into account the fact that for simazine and atrazine breakthrough curves have been recorded with RP-18 silica and  $k'_w$  values are taken from ref. 15 and have been measured with Silasorb SPH C<sub>18</sub> silica. It is well known that retention varies with the characteristics of the C<sub>18</sub> silica, *i.e.*, the number of octadecyl groups bonded at the surface [20]. Nevertheless, experimental values of  $k'_w$  are seldom available and, as seen earlier, only extrapolated values from the  $\log k'$  vs. methanol content relationship or from the  $\log k'_w$ – $\log P_{oct}$  relationship are found. For the four compounds reported in Table I, it is evident that agreement is obtained from experimental values and that calculations with extrapolated values can be different depending on the difference between the experimental and predicted values. One example of the difference in these two values is reported in Fig. 3, showing the variation of the experimental

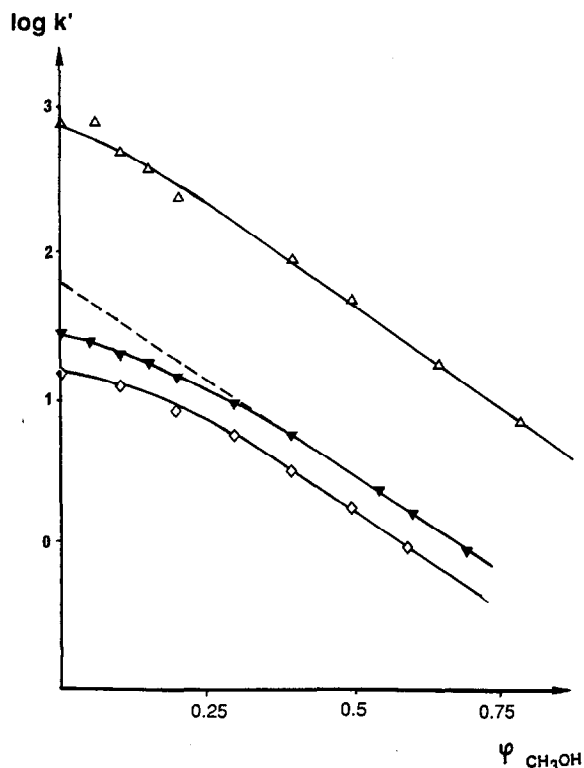


Fig. 3. Variation of the capacity factor of benzene with the volume fraction of methanol contained in the water-methanol mobile phase.  $\blacktriangledown$  = Measured on a 10 cm  $\times$  0.46 cm column laboratory-packed with RP-8 silica;  $\triangle$  = measured on a 5 cm  $\times$  0.46 cm column laboratory-packed with PRP-1 copolymer;  $\diamond$  = measured on a 10 cm  $\times$  0.46 cm column packed with Hypercarb.

TABLE II  
EXPERIMENTAL AND EXTRAPOLATED  $\log k'_w$  VALUES AND THE DIFFERENCE ( $\Delta$ ) BETWEEN THEM

Extrapolations were made in the linear range of methanol volume fraction indicated. Log  $P_{\text{oct}}$  values are mainly from ref. 18.

Solute	Log $k'_w$		Linear range	$\Delta$ (log $k'_w$ ) (exp. – extrap.)	Log $P_{\text{oct}}$
	Experimental	Extrapolated			
Chlorobenzene	2.1	2.4	0.3–0.7	–0.3	2.84
Fluorobenzene	1.6	1.8	0.2–0.7	–0.26	2.27
Benzene	1.45	1.75	0.3–0.7	–0.3	2.14
4-Nitrophenol	1.65	1.60	0.05–0.7	0.05	1.91
Nitrobenzene	1.77	1.68	0.1–0.7	0.09	1.84
4-Chloroaniline	1.63	1.60	0.05–0.7	0.03	1.83
Acetophenone	2.08	1.70	0.3–0.7	0.38	1.70
Benzonitrile	1.72	1.65	0.05–0.7	0.07	1.56
Phenol	1.14	1.09	0.05–0.7	0.03	1.48
Benzaldehyde	1.71	1.53	0.05–0.7	0.25	1.45
4-Nitroaniline	1.49	1.33	0.1–0.7	0.06	1.39
Benzyl alcohol	1.27	1.20	0.1–0.7	0.07	1.10
Aniline	0.85	0.85	0–0.7	0	0.91

capacity factor of benzene with the methanol volume fraction of the mobile phase. The extrapolated  $\log k'_w$  is higher than the experimental value. The extrapolated value was obtained from the linear range for methanol contents above 30%, which corresponds to the way that data are usually extrapolated in the literature. In order to investigate the differences between real and extrapolated values, experimental retention volumes were measured in water using short precolumns packed with  $C_8$  silica instead of  $C_{18}$  silica in order to obtain shorter and measurable values. Table II reports the experimental  $\log k'_w$  values, the extrapolated values  $\log k'_w$  values from the  $\log k' - \phi$  relationship and the  $\phi$  range used for the graphical extrapolation, the difference between experimental and extrapolated  $\log k'_w$  and the water–octanol partition coefficient. As pointed out by Schoenmakers *et al.* [14], the difference between experimental and predicted  $\log k'_w$  values can be positive or negative, depending on the solutes. For benzenes monosubstituted by halogen or alkyl groups, the experimental values are found to be lower than the predicted values and there is a factor of two between the two values, as indicated by the average difference of 0.3 in logarithmic units. For other compounds, the predicted values are lower

than or similar to the experimental values. Jandera and Kubat [15] found lower values for the nine herbicides they examined. From our results and other published data [14,15], the difference between predicted and experimental values are small and lower than 0.10–0.15 logarithmic units for solutes having experimental  $\log k'_w$  values below 1.5–1.6. The more polar and water-soluble are the compounds, the less retained they are by *n*-alkylsilicas and the closer are the extrapolated and experimental  $\log k'_w$  values.

The water–octanol partition coefficients are widely used in the biological field and efforts should be made to collect these data for many environmental pollutants. Table III reports some results for a few moderately polar ( $\log P_{\text{oct}}$  between 1 and 3) and weakly apolar ( $\log P_{\text{oct}}$  between 3 and 4) compounds. The variation of extrapolated  $\log k'_w$  from the  $\log k'_w$ –methanol content relationship are reported as a function of the  $\log P_{\text{oct}}$  of all the compounds in Table III in Fig. 4. The good correlation obtained between these two values confirms that  $\log k'_w$  can be estimated for any compounds having a  $\log P_{\text{oct}}$  value between 1 and 4. It has been observed that the relationship is not verified for  $\log P_{\text{oct}}$  values lower than 1. Therefore, extrapolated

TABLE III

OCTANOL-WATER PARTITION COEFFICIENTS, LOG  $k'_w$  VALUES EXTRAPOLATED FROM THE LOG  $k'$ -METHANOL VOLUME FRACTION RELATIONSHIP AND CORRESPONDING CALCULATED RETENTION VOLUME IN WATER,  $V_r$ , USING 1 cm  $\times$  0.2 cm I.D. PRECOLUMNS PACKED WITH C<sub>18</sub> SILICA AND PRP-1

The difference between extrapolated log  $k'_w$  values measured on PRP-1 and on C<sub>18</sub> silica is given in the last column.  $V_r$  was calculated assuming a porosity of 0.7 for C<sub>18</sub> and 0.65 for PRP-1.

Solute	Log $P_{oct}$	C <sub>18</sub> silica		PRP-1		$\Delta$ (log $k'_w$ ) (PRP-1 - C <sub>18</sub> )
		Extrapolated log $k'_w$	$V_r$ (ml)	Extrapolated log $k'_w$	$V_r$ (ml)	
<i>Monosubstituted</i>						
Ethylbenzene	3.15	3.4	55	4.8	1390	1.4
Toluene	2.76	2.75	12	4.14	304	1.39
Benzene	2.14	2.2	3.5	3.52	73	1.32
Phenol	1.48	1.55	0.8	2.45	6.2	0.9
Benzyl alcohol	1.10	1.40	0.6	2.45	6.2	1.05
Aniline	0.91	1.08	0.3	2.48	6.6	1.40
Benzoic acid	1.77	1.90	1.4	3.3	44	1.40
Benzaldehyde	1.45	1.73 <sup>a</sup>	1.2	2.94	19	1.2
Benzonitrile	1.56	1.80 <sup>a</sup>	1.4			
Nitrobenzene	1.84	2.05	2.5	3.62	92	1.57
Acetophenone	1.70	1.8	1.4	3.1	28	1.3
<i>Di- and trisubstituted</i>						
4-Nitroaniline	1.39	1.5	0.7	2.8	14	1.3
4-Chloroaniline	1.83	1.84 <sup>a</sup>	1.5	2.8	14	0.96
2-Chlorophenol	2.16	2.11 <sup>a</sup>	3	3.2 <sup>b</sup>	35	1.09
2,6-Dichlorophenol	2.84	2.76 <sup>a</sup>	12	3.92 <sup>b</sup>	180	1.16
3,5-Dichlorophenol	3.56	3.49 <sup>a</sup>	68			
2,4,5-Trichlorophenol	4.1	3.96 <sup>a</sup>	200			
2-Methylphenol	1.93	1.8	1.5	3.3	44	1.5
4-Nitrophenol	1.91	1.84	1.5	2.8	11	0.96
1,3-Dinitrobenzene	1.49	1.6	0.9	3.26	40	1.66

<sup>a</sup> Value from ref. 18.

<sup>b</sup> Value estimated from curves in ref. 19.

values from the general log  $k'_w$ -log  $P_{oct}$  relationship are very useful for  $V_r$  calculations which indicates rapidly the order of magnitude of the sample volume that can be handled without any breakthrough. No  $k'$  measurement is then required for these estimations. The calculations of  $V_r$  indicate that for moderately polar compounds breakthrough occurs rapidly.  $V_r$  is lower than 5 ml for compounds such as phenol and methyl- and chlorophenol, about 20 ml for dichlorophenols and 200 ml for the more apolar trichlorophenols. If the minimum sample volume required for detection is about 50 ml, then an on-line pre-concentration using small cartridges packed with C<sub>18</sub> silica can only be applied to organic compounds

with log  $P_{oct}$  values above 3. Nevertheless, it is always possible to use larger precolumns than that used for calculations in Table III. This size was selected because it is the size of disposable cartridges that have been selected by manufacturers for automatic on-line enrichment-LC apparatus. However, for a classical 15 cm  $\times$  0.46 cm I.D. analytical column, a precolumn up to 15 mm  $\times$  3.2 mm I.D. can be employed without introducing band broadening in the on-line enrichment chromatogram. With such a precolumn, retention volumes  $V_r$  are more than three times higher than those calculated in Table III. It can be seen that by increasing the size of the analytical column to 25 cm, the length of the precolumn can be up to 2 cm.



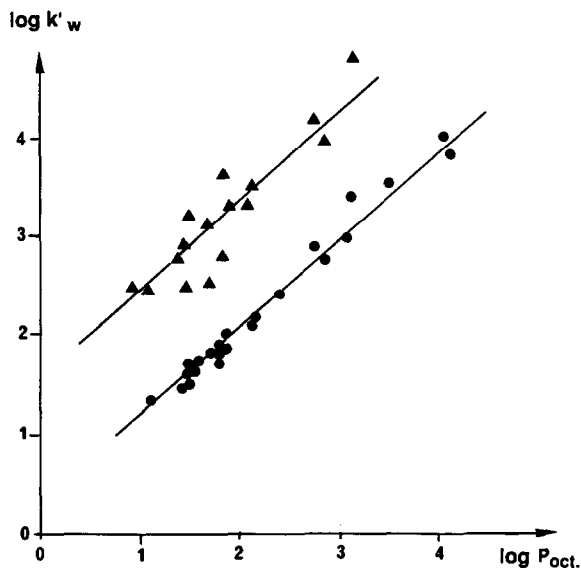


Fig. 4. Variations of extrapolated  $\log k'_w$  values from the  $\log k'$ -methanol volume fraction relationship with the water-octanol partition coefficients of solutes. Values obtained for (●) RP-18 silica and (▲) PRP-1.

#### Styrene-divinylbenzene non-polar copolymers

XAD-type styrene-divinylbenzene copolymers have been widely used for the off-line extraction of analytes from environmental samples, as pointed out in some reviews [21–23]. The ability of these types of sorbents to concentrate polar organic compounds has been demonstrated. These sorbents cannot be used in on-line methodologies because they are not pressure resistant. Porous, rigid and pressure-resistant styrene-divinylbenzene copolymers are commercially available for LC under trade names PRP-1 (Hamilton) and PLRP-S (Polymer Laboratories). They also have the advantage of being stable over the pH range 1–14, in contrast to silica-based materials which are stable only in the pH range 3–8. Frei and co-workers, who were the pioneers and promoters of on-line methodologies, have shown that precolumns packed with PRP-1 retained moderately polar compounds such as chlorophenols much more than  $C_{18}$  silicas [5,8,19,24]. Precolumns packed with PLRP-S have also been employed for the on-line trace enrichment of polar pesticides with suitable breakthrough volumes [2,25]. The retention behaviour of analytes is governed by

hydrophobic interactions similarly to  $C_{18}$  silicas but, owing to the aromatic rings in the network of the polymer matrix, one can expect strong electron-donor interactions ( $\pi$ - $\pi$ ) with aromatic rings of the solutes. It should be also sensitive to changes in the solute electron density caused by the electron-withdrawing ability of solute substituents. The retention behaviour of analytes on PRP-1 has not been studied in depth and we have tried to compare  $\log k'_w$  values obtained with PRP-1 with those obtained with  $C_{18}$  silicas. In Fig. 3, we also reported the variations of the capacity factor of benzene with the methanol content of the mobile phase. First, whatever the mobile phase composition, benzene is about 25–30 times higher more retained by PRP-1 than it is by RP-8. It can be also seen that the variations of  $\log k'$  with the mobile phase are parallel, indicating that the change in retention observed is mainly due to the mobile phase effects, specially to changes in the solubility of benzene in the methanol-water mixture, as was demonstrated for *n*-alkylsilicas [21]. A similar linear relationship is obtained in the same  $\varphi$  range. Similar observations have been made for many other compounds and similar differences to those obtained with  $C_8$  silica have been measured, positive or negative. A consequence is that similar differences exist between extrapolated and predicted values and that  $\log k'_w$  can also be extrapolated from the  $\log k'$ -methanol content relationship. However, we can expect the  $\log k'_w$ - $\log P_{oct}$  relationship to be less linear owing to the  $\pi$ - $\pi$  interactions being different from one solute to another. In Table III are also reported the extrapolated  $\log k'_w$  values obtained with PRP-1 sorbent. All these solutes are much more retained by PRP-1 than they are by RP-18 and the corresponding  $V_r$  values are consequently higher. The difference between the logarithm of the retention on the two sorbents is reported in the last column of Table III and varies between 0.9 and 1.66 with an average value of 1.27, indicating that  $V_r$  values obtained with PRP-1 are about twenty times higher than those obtained with  $C_{18}$  silica. The difference is the highest for benzene derivatives substituted by nitro groups having a strong electron-withdrawing effect, and the smallest for derivatives substituted by hydroxy groups with an electron-donating effect. The extrapolated  $\log k'_w$ - $\log P_{oct}$  relationship is, of course, less linear than that with  $C_{18}$  silica, as shown in Fig. 4, and cannot

be used for accurate enough values of  $\log k'_w$ . Nevertheless, the average values of  $25 \pm 12$  times higher verified for many compounds are important and the prediction of the retention volume can be derived to a first approximation from predictions carried out with  $C_{18}$  silicas.

Moderately polar compounds can be determined at trace levels using this sorbent in on-line methodologies, but early breakthrough is still observed for polar compounds such as phenol or aniline. For

polar compounds, we tried to increase as far as possible the size of the precolumn. A longer analytical column (25 cm  $\times$  0.46 cm I.D.) was selected for the chromatographic separation and the size of the precolumn was increased to 2.2 cm  $\times$  0.46 cm I.D. These dimensions are high for optimization of the coupling. An application to the on-line preconcentration of some triazines and degradation products was carried out using this precolumn [26]. This size was necessary for preconcentration of deisopropyl-

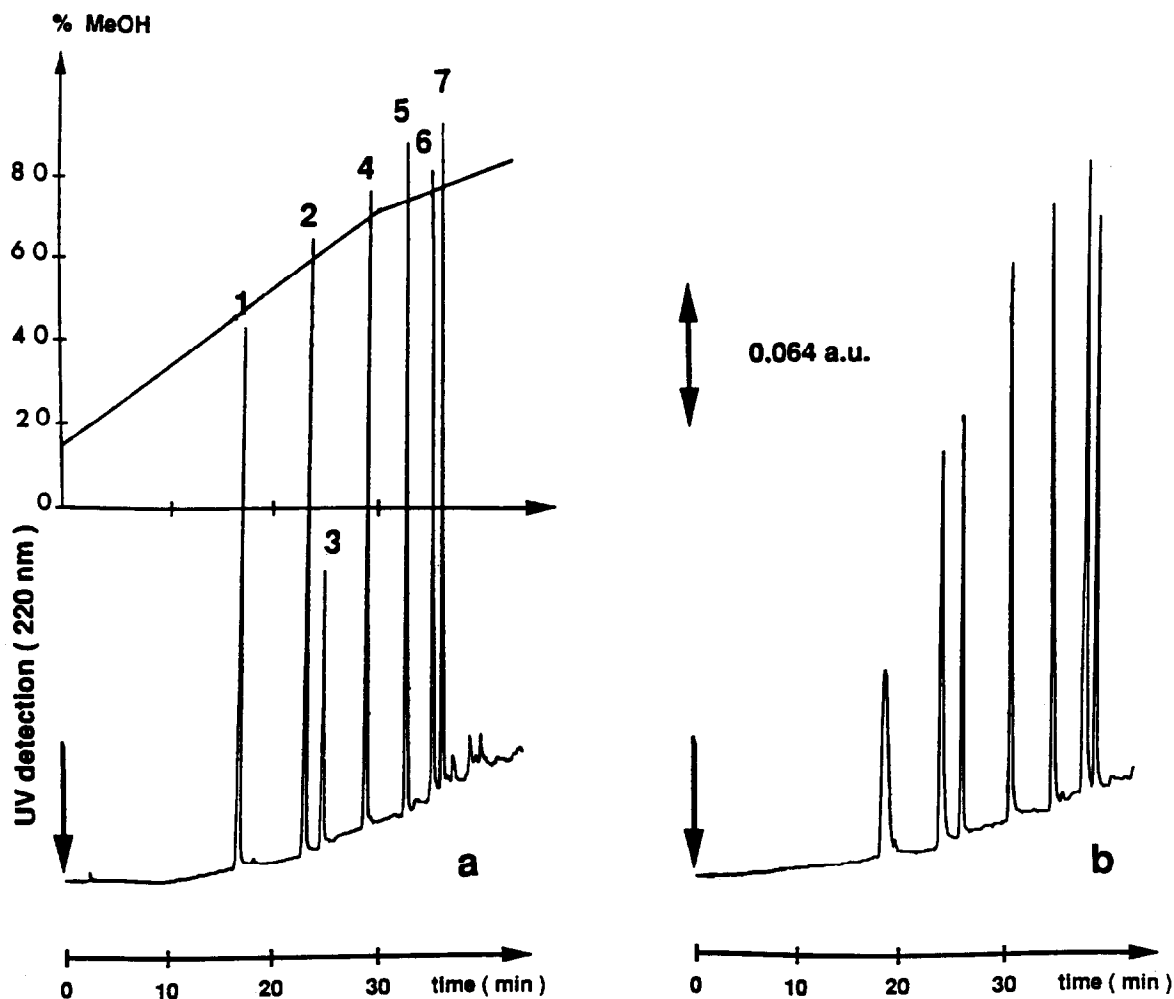


Fig. 5. Efficiency of the on-line coupling of a large-size precolumn packed with PRP-1 with a 25-cm long analytical column packed with  $C_{18}$  silica. Comparison between (a) direct 20- $\mu$ l loop injection and (b) on-line preconcentration of 50 ml of LC-grade water spiked with 5  $\mu$ g/l of each compound. Solutes: 1 = deisopropylatrazine; 2 = deethylatrazine; 3 = hydroxyatrazine; 4 = simazine; 5 = atrazine; 6 = propazine; 7 = terbutylazine. Analytical column: 25 cm  $\times$  0.46 cm I.D. prepacked with 5- $\mu$ m  $C_8$  Zorbax silica. Precolumn: 2.2 cm  $\times$  0.46 cm I.D. packed with PRP-1 copolymer. Mobile phase: methanol gradient with potassium phosphate buffer (pH 7) as shown. Flow-rate: 1 ml/min. UV detection at 220 nm.

atrazine, deethylatrazine and hydroxyatrazine, which are more polar than atrazine with breakthrough volumes of 80, 100 and 150 ml, respectively, measured with this 2.2-cm long precolumn. The chromatogram shown in Fig. 5b corresponds to the preconcentration of 50 ml of LC-grade water spiked with 5  $\mu\text{g/l}$  of each analyte. On comparison with the chromatogram obtained with direct loop-injection of 20  $\mu\text{l}$  into the analytical column in Fig. 5a, slight band broadening can be observed for the two first peaks and none for the other products. The large dimensions of the precolumn and the fact that compounds are more retained by the sorbent in the precolumn than by the sorbent in the analytical column should cause band broadening, which was visible when using isocratic elution. In that event, isocratic conditions should be avoided and, when applying a rapid gradient, band broadening does not occur, as can be seen in Fig. 5. Increasing the size of the precolumn to these dimensions allows the  $V_r$  values in Table III to be more than ten times higher.

Another advantage of the PRP-1 and PLRP-S sorbents is their high stability in the pH range 1–14. These sorbents retained analytes in their neutral form but not in their ionic form. As an example, the breakthrough volume of aniline measured with 9 cm  $\times$  0.46 cm I.D. PRP-1 column was 180 ml at pH 6 whereas it was 6 ml at pH 2. Polar and ionizable compounds have been determined at trace levels using on-line methodologies with two precolumns. The first, packed with PRP-1, traps the compounds in their neutral form. They are later desorbed and reconcentrated on a second precolumn packed with an ion exchanger in their ionic form by eluting the two precolumns in series with a small volume of deionized water adjusted to a convenient pH. The first PRP-1 precolumn is never analysed on-line and this is why its dimensions can be increased considerably for retention of very polar compounds. This methodology allows the use of the selective ion-exchange sorbents which cannot be used directly with natural water samples because these water samples contains many inorganic ions at the mg/l level and the ion exchanger is rapidly overloaded. With the combination of the two precolumns, the water sample is never percolated through the precolumn containing the ion exchanger. This method has been applied to trace levels of aniline derivatives, chlorotriazines, phenols and phenoxy acids [27–30].

#### Porous graphitic carbon

Carbonaceous sorbents were certainly the first materials used for off-line extraction of medium- to low-polarity organic compounds from water. Graphitized carbon black has been successfully employed for the preconcentration of chlorophenols and chloroanilines and other moderately polar pesticides [31–33], but its practical use in LC is prevented by its poor mechanical properties. Pyro-carbon-modified silicas and pyromodified carbon black [34,35] were synthesized by Colin *et al.* as LC stationary phases. Although these materials were not commercialized, their suitability for on-line trace enrichment of chlorophenols [10] and other medium-polarity compounds such as nitrophenol and nitrobenzene [36] has been reported. Recently a porous graphitic carbon became available as an LC stationary phase [37] under the trade name Hypercarb. This sorbent shows a reversed-phase behaviour, as can be seen in Fig. 3, where the variation of the capacity factor of benzene with the methanol content of the mobile phase is also reported. It is a reversed-phase behaviour in the sense that retention decreases when the methanol content of the mobile phase increases.

The curve corresponding to the porous graphitic carbon (PGB) is also parallel to those obtained with the RP-8 and the PRP-1 sorbents. This indicates that  $\log k'_w$  can be extrapolated from the linear  $\log k' - \phi$  relationship as for the other reversed-phase sorbents. Owing to its crystalline structure, made of large graphitic sheets held together by weak Van der Waals forces, it is often presented as a more retentive reversed-phase sorbent than  $\text{C}_{18}$  silica [38], requiring mobile phases with higher organic/aqueous ratios to achieve elution comparable to  $\text{C}_{18}$  silica-based sorbents. Our results indicates that it depends greatly on the solute polarity and, for instance, Fig. 3 shows clearly than benzene is less retained by PGB than it is by RP-8 silica. It has been pointed out that one basic difference between these reversed-phase sorbents is that solute–stationary phase interactions play an important role with carbon-based sorbents whereas they can be neglected to a first approximation with  $\text{C}_{18}$  silicas. The affinity of PGC towards very polar and water-soluble polyhydroxybenzenes has been shown [39]. The capacity factor in water of the very polar 1,3,5-trihydroxybenzene (phloroglucinol) is about 1000 with PGC whereas it

TABLE IV  
COMPARISON OF EXTRAPOLATED LOG  $k'_w$  VALUES  
OBTAINED WITH RP-18 SILICA, PRP-1 AND PGC

Solute	$k'_w$		
	RP-18	PRP-1	PGC
<i>Monosubstituted</i>			
Benzene	2.2	3.5	1.45
Aniline	1.08	2.5	1.35
Phenol	1.55	2.4	1.8
Benzoic acid	1.9	3.2	2.4
Nitrobenzene	2.05	3.6	2.45
<i>Polysubstituted</i>			
4-Aminophenol		1.1	2.05
1,4-Diaminobenzene		1.2	2.4
4-Aminobenzoic acid		2	2.85
4-Hydroxybenzoic acid		2.3	2.7
3,5-Dihydroxybenzoic acid		1.35	3
1,3-Dihydroxybenzene		1.35	2.35
1,4-Dihydroxybenzene		0.83	2.15
1,3,5-Trihydroxybenzene		0.5	2.7

was found to be 3 with PRP-1. This compound is not retained by  $C_{18}$  silica and it was even proposed as an experimental probe for determining the void volume of  $C_{18}$  columns [40].

Other extrapolated or real log  $k'_w$  values have been measured for mono- and polysubstituted benzene derivatives with RP-18, PRP-1 and PGC. Results are reported in Table IV. First, when comparing the values for monosubstituted benzenes, compounds are more retained by PRP-1 than they are by PGC. It is interesting to compare the retention behaviours of these two sorbents because they both can involve  $\pi$ - $\pi$  interactions. Nevertheless, the difference in specific area should be taken into account as there is a factor of three between the two areas. The comparison between RP-18 and PGC indicates that benzene and nitrobenzene are less retained by PGC than they are by RP-18 and that aniline, phenol and benzoic acid are more retained. In contrast to results on PRP-1, indicating that the retentions of all the solutes were higher with PRP-1 than that with  $C_{18}$  silicas, no correlation was found between the retention of monosubstituted benzenes on PGC and that on  $C_{18}$  silicas. The disubstituted benzenes in Table IV are polar compounds and are not, or only

slightly, retained by  $C_{18}$  silicas, explaining why log  $k'_w$  values have not been reported. The comparison between the retentions obtained on PRP-1 and on PGC are interesting. With PRP-1, the values of log  $k'_w$  obtained with two polar substituents are always lower than those measured for each corresponding monosubstituted benzene, whereas the opposite is observed with PGC. For instance, log  $k'_w$  of aminophenol is 1.1 with PRP-1 and is lower than those of both phenol (2.4) and aniline (2.5). With PGC, log  $k'_w$  of aminophenol is 2.05 and is higher than those of both phenol (1.8) and aniline (1.35). The retention mechanism is therefore very different for the two sorbents.

The capacity factor is defined as the ratio between the sum of solute-stationary phase interactions and the sum of solute-mobile phase interactions. The addition of a second substituent to the benzene ring of the solute increases the polarity of the molecule and therefore increases the solubility of the molecule in water and the overall solute-mobile phase interactions. As the retention time decreases on adding the second substituent, the increase in polarity is higher than the modification of the solute-stationary phase ( $\pi$ - $\pi$ ) interactions induced by this second substituent. The same effect is observed with  $C_{18}$  silicas and the retention mechanism with PRP-1 sorbent is mainly governed by hydrophobic interactions. In contrast with PGC, the retention increases on adding a second polar substituent and this indicates that the increase in the solute-stationary phase interactions involved is higher than the increase in the mobile phase interactions which occurs. The separation mechanism involved is obviously different from a hydrophobic mechanism and is more similar to an adsorption process, as already pointed out [36]. The understanding of this process and the possibility of predicting log  $k'_w$  are under study [41].

The important practical point is the high retention in water for some polar compounds that cannot be analysed in aqueous samples at present because they are too water soluble to be extracted by liquid-liquid extraction and not retained by classical sorbents. Using PGC as a reversed-phase sorbent is therefore useful for trace analysis in on-line methodology but the coupling with analytical  $C_{18}$  columns is impossible when the analytes of interest are very polar. The difference in retention is too strong and it is impossible to separate the compounds by  $C_{18}$  silica

owing to their high polarity. Their separation requires water-rich mobile phases unable to desorb and transfer analytes from the PGC precolumn to the C<sub>18</sub> analytical column. We have reported previously an on-line determination of pyrocatecol, resorcinol and phloroglucinol in a 50-ml drinking water sample with detection limits as low as 50 ng/l using a PGC precolumn and a commercial 10-cm long analytical column prepacked also with PGC [39].

## CONCLUSIONS

A significant parameter of solid-phase extraction coupled on-line to an analytical separation is the sample volume that can be handled without breakthrough of analytes. This volume is related to the knowledge of the retention volume of analyte in waters, and predictions can be made if the retention mechanism involved is known. Prediction of retention volume can be easily carried out with C<sub>18</sub> silicas from the water–octanol partition coefficients. A collection of these values for priority pollutants and pesticides would be useful, because calculation of these constants rapidly becomes difficult for non-simple molecules. The advantage of using styrene–divinylbenzene copolymers for the preconcentration of moderately polar compounds is obvious, but the prediction of the retention of solutes depending on their characteristics is less accurate. The potential of porous graphitic carbon for both trace enrichment and on-line elution of very polar compounds has been demonstrated even though the prediction of the sample volume to handled has not yet been achieved.

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