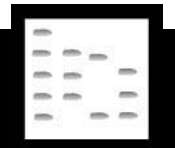
rivatization with *o*-phthaladehyde. *Analytical Biochemistry* 204: 215–219.
Chaplin MF and Kennedy JF (eds) (1994) *Carbohydrate Analysis: A Practical Approach*, 2nd edn, pp. 15, 41, 66, 152, 209. Oxford: IRL Press.
Charlwood J, Birrell H, Tolson D and Camilleri P (1998) Two-dimensional chromatography in the analysis of complex glycans from transferrin. *Analytical Chemistry* 70: 2530–2535.
Guille GR, Rudd PM, Wing DR, Prime SB and Dwek RA (1996) A rapid high-resolution high-performance liquid chromatographic method for separating glycan mixtures and analyzing oligosaccharide profiles. *Analytical Biochemistry* 240: 210–226.
Karger BL and Hancock WS (ed.) (1996) *Methods in Enzymology* 270: 67, 119, 190.
Lim CK (ed.) (1986) *HPLC of Small Molecules: A Practical Approach*, pp. 49. Oxford: IRL Press.
Ohsuga H (1996) The carbohydrate moiety of the bermuda grass antigen BG60. New oligosaccharides of plant origin *Journal of Biological Chemistry* 271: 26653–26658.
Robles MD, Matés JM and Niell X (1995) Determination of the degree of polymerization of agar-type polysaccharides by a high-performance liquid chromatography method. *Journal of Liquid Chromatography* 18: 3175–3178.
Wunschel D, Fox KF, Fox A *et al.* (1997) Quantitative analysis of neutral and acidic sugars in the whole bacterial cell hydrolysates using high-performance anion-exchange liquid chromatography-electrospray ionization tandem mass. *Journal of Chromatography* 776: 205–219.
Zhang Y, Cedergren RA, Nieuwenhuis TJ and Hollingsworth RI (1993) *N,N*-(2,4-dinitrophenyl)octylamine derivatives for the isolation, purification, and mass spectrometric characterization of oligosaccharides. *Analytical Biochemistry* 208: 363–371.

POROUS GRAPHITIC CARBON: LIQUID CHROMATOGRAPHY



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Introduction

Various carbonaceous sorbents have been used successfully since the early days of gas chromatography (GC), but for many years their application to liquid chromatography (LC) was unsuccessful. Active carbons with high specific surface areas were shown to be microporous and to contain polar groups at their surface, which provided poor LC performance. Graphitized carbon blacks (GCBs) lacked sufficient mechanical strength to withstand high LC pressures, in addition to having polar surface groups. In the 1970s, bonded silicas were extensively developed, but they had some disadvantages including solubility in the eluents, hydrolysis of the bonded chain at low or high pH, and the effects of the unavoidable unreacted silanol groups. Several attempts were made to prepare graphite-based sorbents that would not suffer from the disadvantages of bonded silica sorbents, but it was not until 1979 that Knox and Gilbert patented a method for making a robust porous carbon that possessed the required properties for use in LC. An improved version of this material became commercially available in 1988 under the tradename Hypercarb®. Although one or two other carbons made by Japanese workers are sometimes mentioned, most of the studies and applications described in the literature utilize the porous graphitized carbon (PGC) Hypercarb®.

The properties of PGC come from its highly ordered crystalline structure composed of large flat layers of carbon atoms. It has proven to be unique, behaving as a stronger reversed-phase sorbent than any other existing reversed-phase packing or as a normal-phase sorbent. Separations of both nonpolar and highly polar mixtures can be performed that are impossible with other sorbents. Resolution of anionic and cationic analytes can be achieved in one run. These properties are partly explained by a retention mechanism that is quite different from that of other LC stationary phases. The properties of PGC are discussed here together with some selected applications.

Structure and Characteristics of PGC

PGC is obtained by impregnating a porous silica with a phenol–formaldehyde mixture. This mixture is polymerized within the pores of the silica gel and carbonized at 1000°C. The silica is then removed by dissolution in a concentrated ($5\ \mathrm{mol\ L^{-1}}$) sodium hydroxide solution. Graphitization is performed in the temperature range 2000–2800°C to remove the micropores. The resulting macroporous material has a flat crystalline surface.

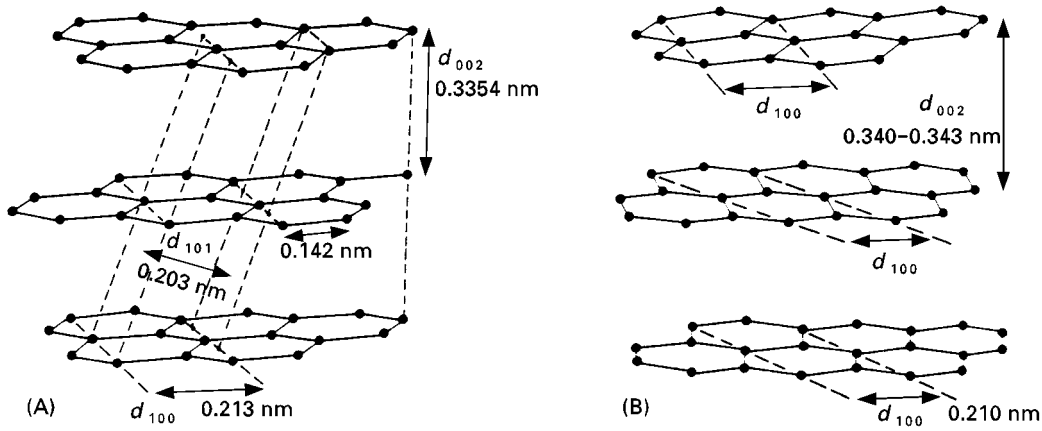

Figure 1 Structure of (A) 3D graphite and (B) 2D porous graphitic carbon.

PGC does not have the true graphite structure, in which layers are well organized in three dimensions, as shown in **Figure 1A**. Rather it has a two-dimensional (2D) graphite structure composed of layers of hexagonally arranged carbon atoms in the sp^2 hybridization. The close intertwining of the graphitic sheets provides the rigidity and mechanical stability. The layer spacing is slightly larger than in 3D graphite, as shown in **Figure 1B**.

PGC meets the required properties for a good LC sorbent. It is available in a narrow particle-size distribution with mean particle diameter of 5 or 7 μm, it has an average specific surface area of 120 $m^2\,g^{-1}$, a uniform pore structure with mean pore diameter of 25 nm, and a porosity of 75%. Designed to withstand pressures of more than 400 bar, PGC is geometrically stable and free from any swelling or shrinking. It is inert to the common organic eluents and to extremes of pH. In the early stage of commercialization, some columns packed with the 7 μm material lacked efficiency, but significant improvements have been made and the 5 μm packing now available has a guaranteed efficiency of 60 000 plates m^{-1} and offers batch-to-batch reproducibility.

The flat homogeneous surface of PGC is responsible for its unique selectivity to geometrical isomers. The extensive layers of carbon atoms containing delocalized π electrons and the high polarizability are responsible for its unique retention mechanism.

Retention Mechanism

Mobile-Phase Effects: Reversed-Phase Behaviour for Polar and Nonpolar Analytes

The reversed-phase behaviour of PGC towards nonpolar and polar analytes has been observed in many applications. **Figure 2** shows plots of the logarithm of the retention factor of phenol, 1,3-dihydroxybenzene (resorcinol) and 1,3,5-trihydroxybenzene (phloroglucinol) against the methanol volume fraction (ϕ) in a water/methanol mobile phase for PGC and two other reversed phases, C_{18} silica and the styrene–divinylbenzene porous copolymer PRP-1. Two important features of PGC are highlighted in this figure. First, the addition of methanol to the mobile phase results in a similar decrease in retention for all three sorbents, thus showing a classical reversed-phase dependence. However the retention values, and in particular the retention order, indicate that the behaviour of PGC is different from that of the other two materials for the analytes considered in this figure.

Retention of Nonpolar Analytes: A Strong Hydrophobic Sorbent

Since PGC was produced with the objective of being an improved reversed-phase material compared with the widely used C_{18} silicas, the first applications were made with nonpolar analytes, and PGC is described as a stronger hydrophobic sorbent than C_{18} silica. A common way of comparing the hydrophobicity of sorbents is to measure the effect of the addition of a methylene group in an homologous series. The methylene increment is 4.5 and 3.8 for the *n*-alkanols on PGC and C_{18} silica, respectively, with pure water as mobile phase. Kriz and co-workers compared the retention of 52 aromatic hydrocarbons, mostly alkylbenzenes, on PGC, C_{18} silica, phenylsilica, silica and alumina. **Figure 3** shows the variation of the retention factor on PGC according to the number of carbon atoms for both the series of *n*-alkylbenzenes and the series of methylbenzenes. The methylene increment measured for the *n*-alkylbenzenes was 0.22 using PGC with pure methanol, compared with

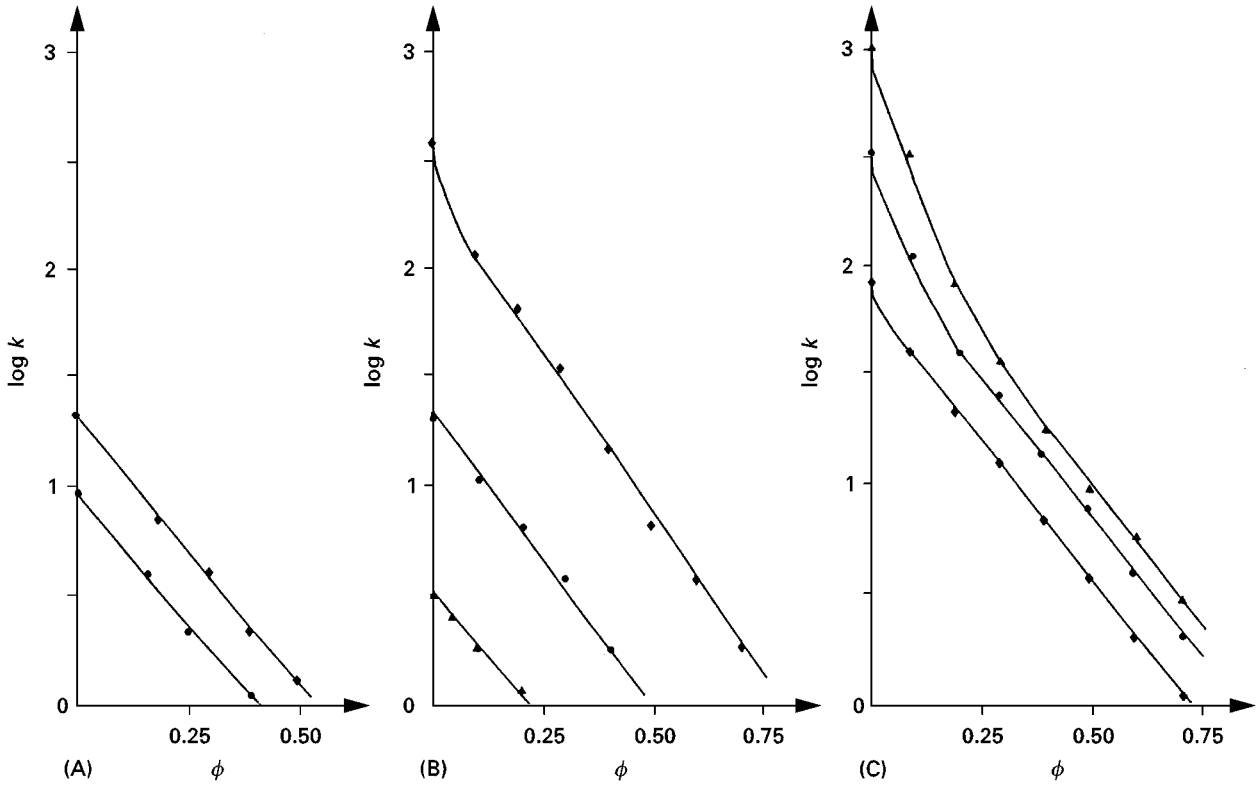

Figure 2 Variation of the retention factors (log *k*) with the mobile phase composition obtained on (A) LiChrosorb RP-18, (B) PRP-1 and (C) PGC. Solutes: ◆, phenol; ●, resorcinol; ▲, phloroglucinol. ϕ is the volume fraction of the mobile phase. (Reprinted from Coquart V and Hennion M-C (1992) Trace-level determination of polar phenolic compounds in aqueous samples by HPLC and on-line preconcentration on porous carbon. *Journal of Chromatography* 600: 195–201. Copyright (1992) with permission from Elsevier Science.)

values of 0.17 and 0.10 with C_{18} silica and phenylsilica using a methanol/water mobile phase (80 : 20 v/v). Another important feature that can be seen from **Figure 3** is the possibility given by PGC for discriminating between the two series. In the same study it was shown that C_{18} silica is unable to differentiate between the addition of a methylene group to an alkyl chain and the addition of methyl group to the benzene ring. A very slight difference could be observed on phenylsilica. Silica and alumina with pentane as mobile phase could discriminate between the two series, but with a methylene increment close to zero for the *n*-alkylbenzene series. This study illustrates well with the higher hydrophobicity of PGC over C_{18} silica as a reversed-phase sorbent for these nonpolar analytes, as well as its superior selectivity towards isomeric compounds.

Retention of Very Polar Analytes: Comparison with a Hydrophobic Reversed-Phase Mechanism

Several studies have shown that PGC does not behave as a perfect reversed-phase sorbent. **Figure 2** shows that the same order is observed for C_{18} silica and PRP-1 and that the retention factor decreases with the analyte's polarity from phenol to resorcinol and phloroglucinol. Plots of phloroglucinol for C_{18} silica have not been reported because this analyte is too polar to be retained with a methanol-rich mobile phase; it has been proposed as an experimental probe for the determination of the void volume of C_{18} columns. This corresponds to a retention mechanism based on hydrophobic interactions. The higher retention observed with PRP-1 is explained by additional π–π interactions between these aromatic analytes and the styrene–divinylbenzene matrix of the PRP-1 sorbent.

On PGC, the retention increases with the number of hydroxy groups. There is a great difference in retention for phloroglucinol with retention factors in water, k_W, of 1050 with PGC, 3 with PRP-1 and 0.3 with C_{18} silicas. Values of log k_W, have been extrapolated from the variation of the retention factors in water/methanol mixtures from curves similar to those in Figure 1. These values, which allow comparison with C_{18} silicas and the apolar copolymer PRP-1, are reported in **Table 1** for some mono-, di- and tri-substituted benzene derivatives.

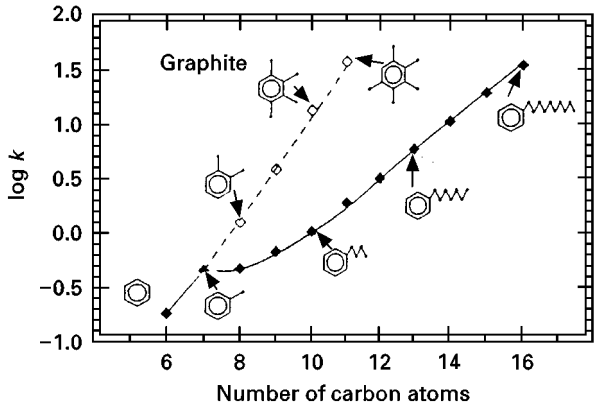

Figure 3 Dependence of log *k* values for polymethylbenzenes and *n*-alkylbenzenes on carbon numbers. Packing material: graphite; eluent: methanol; temperature 20°C. Broken line drawn through points for the polymethylbenzenes; full line drawn through points for *n*-alkylbenzenes. (Reprinted from Kriz J, Adancova E, Knox JH and Hora J (1994) Characterization of adsorbents using aromatic hydrocarbons. Porous graphite and its comparison with silica gel, alumina, octadecylsilica and phenylsilica. *Journal of Chromatography A* 663: 151–161. Copyright (1994) with permission from Elsevier Science.)

First, when comparing values for monosubstituted benzenes, compounds are more retained by PRP-1 than they are by PGC or C_{18} silica. The comparison between C_{18} silica and PGC indicates that some solutes are less retained and some more retained by PGC than they are by C_{18}. The di- and tri-substituted benzenes listed in **Table 1** are rather polar compounds and are either not, or only slightly, retained by C_{18} silicas, which explains why the log k_W values have not been reported. The comparison between the retentions on PRP-1 and on PGC are informative.

Table 1 Comparison of reversed-phase sorbents using extrapolated log k_W values obtained with C_{18} silica, PRP-1 and PGC

Solute	*C_{18}*	*PRP-1*	*PGC*
Monosubstituted			
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
Di- and tri-substituted			
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

With PRP-1, the log k_W values obtained with two polar substituents are always lower than those measured for each corresponding monosubstituted benzene, whereas the opposite is observed with PGC. For example, log k_W of aminophenol is 1.1 with PRP-1 and is lower than both log k_W of phenol (2.4) and aniline (2.5). With PGC, log k_W of aminophenol is 2.05 and is higher than log k_W of both phenol (1.8) and aniline (1.35). Since on C_{18} silicas and PRP-1, the retention order is correlated with the polarity of the molecules, but not on PGC, the retention mechanisms must, therefore, be very different.

Many studies have been carried out on the retention mechanism on C_{18} silica, which has been shown to be primarily governed by hydrophobic interactions between the analytes and the carbonaceous moieties of the alkyl chain bonded at the silica surface. The octanol–water partition coefficient (K_{OW}) has been shown to be a good measure of the hydrophobicity of compounds. To a first approximation, the retention order is linked to this octanol–water partition coefficient as shown in **Table 2**, which lists the log k_W values obtained for the three reversed-phase materials and the log K_{OW} values for the very polar degradation products of triazines. The log k_W values decrease with log K_{OW} values for both C_{18} silicas and PRP-1 and analytes are no longer retained when compounds become very polar and more soluble in water than in octanol, as shown by negative log K_{OW} values. On PGC, the log k_W values also decrease with log K_{OW}, but only slightly and they are still very high for the water-soluble analytes.

An extensive study has measured or estimated log k_W values for 46 polar benzene derivatives. The correlation between log K_{OW} and log k_W is shown in **Figure 4**. The relationship is good on C_{18} silica. With PRP-1, log k_W values are more scattered but the relationship is still acceptable. On PGC, there is no relationship at all except for *n*-alkylbenzenes, and a high retention is observed for very polar compounds with log K_{OW} values between 0 and 1.5.

The high retention of very polar analytes, particularly those in an ionized form, is a key property that has been explored for extraction purposes. The trace analysis of water-soluble pollutants requires an extraction step before analysis, the parameters of which can be predicted from LC data, especially log k_W values. PGC is the only sorbent able to extract from water very polar degradation products, such as those reported in **Table 2**.

The delocalization of the π electrons in the large graphitic bonds and the high polarizability of the carbon are responsible for strong induction interactions in addition to solvophobic interactions. The result is that the presence of hydrophilic groups in the

Table 2 Relationship between the retention factor obtained for triazines and some very polar degradation products on reversed-phase materials and the corresponding octanol–water partition coefficient

Compound	*log K_{OW}*	*log k_W^a*		
		C_{18}	*PRP-1*	*PGC*
Atrazine	2.7	3.4 ± 0.2	> 4	> 4
Simazine	2.3	3.0 ± 0.2	> 4	> 4
Deethylatrazine	1.6	2.6 ± 0.1	3.5 ± 0.3	3.2 ± 0.2
Hydroxyatrazine	1.4	2.5 ± 0.1	3.0 ± 0.2	3.4 ± 0.2
Deisopropylatrazine	1.2	2.1 ± 0.1	3.1 ± 0.2	> 3.5
Hydroxydeethylatrazine	0.2	1.5 ± 0.1	1.8 ± 0.1	2.8 ± 0.2
Deethyldeisopropylatrazine	0	1.3 ± 0.1	1.2 ± 0.1	2.8 ± 0.2
Hydroxydeisopropylatrazine	-0.1	1.0 ± 0.1	1.0 ± 0.1	3.0 ± 0.2
Cyanuric acid (2,4,6-trihydroxy-1,3,5-triazine)	-0.2	< 0.5	< 0.5	2.6 ± 0.1
Ammelide (2-amino-4,6-dihydroxy-1,3,5-triazine)	-0.7	< 0.5	< 0.5	2.5 ± 0.1
Ammeline (2,4-diamino-6-hydroxy-1,3,5-triazine)	-1.2	< 0.5	< 0.5	2.4 ± 0.1

[a] The values of log k_W are experimental or extrapolated from measurements in water/methanol mobile phases.

solute molecule does not cause as great a retention decrease on carbon as on C_{18} silica. Any molecular mass increase in the solute, be it in hydrophobic or dipolar moieties, tends to cause a retention increase. The term 'hydrophobic adsorption' was introduced to characterize the positive interaction between the PGC and the solute, as opposed to the 'hydrophobic partitioning' observed with C_{18} silica. When polar

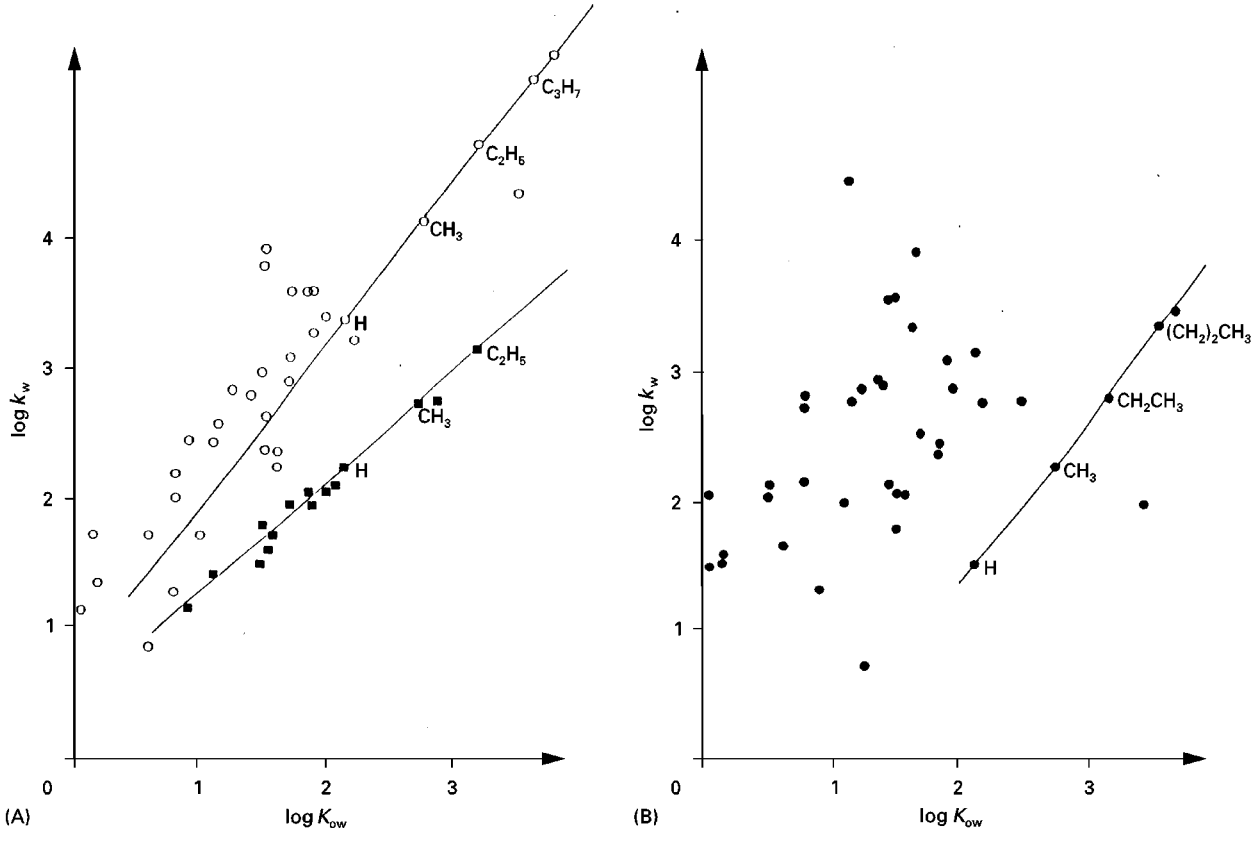

Figure 4 Relationship between log k_W obtained for various mono-, di- and tri-substituted benzene derivatives and log K_{OW}. (A) log k_W obtained on (■) C_{18} silica and (○) PRP-1 copolymer; (B) log k_W values on PGC. (Reprinted from Hennion M-C, Coquart V, Guenu S and Sella C (1995) Retention behaviour of polar compounds using porous graphitic carbon with water-rich mobile phases. *Journal of Chromatography A* 712: 287–301. Copyright (1995) with permission from Elsevier Science.)

analytes are of interest, the electronic interactions have been shown to be more important than the hydrophobic interactions in the retention mechanism. The exploitation of data shown in Figure 4 has demonstrated that the relative position of the substituents on the ring is important and that retention on PGC is very sensitive to the electronic density of the solute molecule. The effect of the polarity of the solutes, taking into account field and resonance effects, has been studied using local dipolar moments and the overall electron excess charge density. The analyte retention factors of polar analytes can be predicted through correlation between log k_W and the electron excess charge density.

Selection of the Mobile Phase: Solvent Strength

Few attempts have been made to establish the eluotropic strengths (ε^0) of commonly used organic solvents with PGC. Using the Snyder equation, the results indicate that the range of solvent strengths for any eluent is small compared with that of silica base sorbents (about 0.2 unit compared with 1 unit). There is no PGC eluotropic series similar to that of silica or alumina. Another result is that the ε^0 value for any eluent may depend upon the solutes or the group of solutes used to determine it. However, the evidence confirms that water is undoubtedly the weakest solvent. Methanol and acetonitrile are shown to be of equivalent weakness, while dioxan, tetrahydrofuran (THF) and dichloromethane have the strongest elution strengths. The elution strength of hexane has been shown to depend strongly on the polarity of the analytes, but is generally intermediate between methanol or acetonitrile and THF.

The retention factors of some pesticides and other organic pollutants measured using a PGC column eluted with methanol, THF and methylene chloride are reported in **Table 3**. The results show that retention can be very high with methanol and that THF and methylene chloride are stronger eluents. Measurements performed with acetonitrile indicate that the retention factors are similar to those obtained with methanol. Once more, there is no apparent relationship between polarity and retention of compounds. A polar pesticide such as metamitron is highly retained in pure methanol, and to a less extent by THF and methylene chloride.

It is important to realize that methanol or acetonitrile are similar weak solvents and compounds that will not elute with methanol will not elute with acetonitrile. Unlike C_{18} silica, the PGC column should not be washed with acetonitrile, and a stronger solvent such as dioxan or THF is required. If the retention of polar, ionizable analytes is too low, then ion-pairing agents can be used in the same way as in reversed-phase LC to increase the retention. When compounds are strongly retained, the PGC surface can be modified by adsorption of various molecules such as trifluoroacetic acid (TFA) in order to reduce the retention of polar analytes. When looking at some of the values in **Table 3**, the high retention of phenanthrene in pure THF indicates that nonpolar analytes can be totally retained. The addition of a surfactant such as Tween 80 has been shown to reduce the retention of hydrophobic molecules by 15–20%.

Table 3 Retention factors of various analytes measured in pure organic solvent (methanol, tetrahydrofuran and methylene chloride)

Compound	*log* k_{OW}	*log* K		
		MeOH	*THF*	CH_2Cl_2
Oxamyl	− 0.4	− 0.51	− 1.42	− 1.22
Methomyl	1.2	0.04	− 0.92	− 1.01
Metamitron	0.8	> 1.4	0.23	0.26
Fenuron	1.0	0.28	− 0.56	− 0.66
Deisopropylatrazine (DIA)	1.1	0.57	− 0.71	− 0.28
Deethylatrazine (DEA)	1.5	0.22	− 1.01	− 1.04
Metoxuron	1.6	1.28	0.05	− 0.02
Metribuzin	1.6	− 0.35	− 1.42	− 1.04
Aminocarb	1.7	− 0.25	− 1.35	− 1.01
Carbendazim	1.5	1.4	0.79	nd
Chloridazon	1.2	0.96	− 0.13	− 0.05
Simazine	2.3	0.97	− 0.49	− 0.39
Atrazine	2.5	0.62	− 0.82	− 0.85
Diuron	2.8	> 1.4	0.17	nd
Linuron	2.8	1.38	− 0.10	− 0.16
3,5-Dichlorophenol	3.6	0.52	− 0.73	− 0.51
2,4,5-Trichlorophenol	4.1	0.99	− 0.12	0.20
Anthracene	4.7	> 1.66	1.21	nd
Pentachlorophenol	5	> 1.4	0.81	nd

nd, not determined.

Selected Applications

Geometrical Isomers and Diastereoisomers

PGC allows unique selectivity for geometrical and diastereoisomers owing to its flat structure. A typical example is the separation of phenol and *o*-, *m*- and *p*-cresol which can be achieved in less that 10 min, whereas it is impossible to separate *m*- and *p*-cresol on C_{18} silica. Other examples include the isomers of xylene, some ionizable isomers such as anisidic, toluic, bromobenzoic and nitrobenzoic acids, and the isomers of the corresponding basic forms (anisidine, toluidine, etc.).

Another interesting application from the environmental field is the pre-separation of the less toxic

PCBs from the more toxic non-*ortho* PCBs using hexane/dichloromethane (70 : 30 v/v) as an extraction solvent. A 50 × 4.6 mm column has been specifically developed for the purpose.

Enantiomers

PGC is not a chiral phase but it can be used as a support to separate enantiomers by the addition of chiral discriminators in the mobile phase. The advantages over silica-based sorbents is that the surface of PGC is homogeneous, which allows rapid equilibration. The separation of the enantiomers of a benzodiazepine can be achieved by the addition of *β*-cyclodextrin to the mobile phase. Another chiral discriminator, carbobenzoxygycil-L-proline, was used for the separation of the optical isomers of hydrophilic amino alcohols strongly retained on silica-based materials. The enantiomers were eluted in 5 min on PGC. Another advantage is that the range of chiral compounds can be extended to the whole pH range.

Instead of adding the chiral modifier to the mobile phase, Knox and Wan have coated the PGC with a near-monolayer of an adsorbed enantiomeric modifier (L- or D-isomers of *N*-(2-naphthalenesulfonyl)phenylalanine), which then acts as an adsorbed stationary phase. Separations of the enantiomers of amino- and hydroxy acids were obtained in this way.

Basic and Acidic Compounds

Because of their inertness, PGC columns can be used over the entire pH range without deterioration of column efficiency and are therefore well suited to the separation of basic or acid compounds. Separation of basic compounds on C_{18} silicas is sometimes difficult and often poor peak shapes are observed, owing to strong secondary ionic interactions between residual silanol groups and the basic analytes. **Figure 5** illustrates the difference that was observed for the separation of monochloroanilines and hydroxychloro- or methylchloroanilines. On C_{18} silica (Figure 5A), the more polar 2-hydroxy-5-chloroaniline is eluted first and separation of the monochloroanilines requires a mobile phase containing 33% acetonitrile. On PGC (Figure 5B), monochloroanilines are eluted before 2-hydroxy-5-chloroaniline and the initial mobile phase contains 68% methanol. Little tailing is observed on PGC as compared to C_{18} silica. This has also been observed for the separation of aromatic amines and pyridine.

The use of extreme pH is illustrated by the separation of aromatic amino acids with a mobile phase at pH 1, or the separation of benzodiazepines carried out at pH 10.6. Another example is the separation of water-soluble sugars, which can be ionized at high pH and retained by PGC through the use of ion pairing.

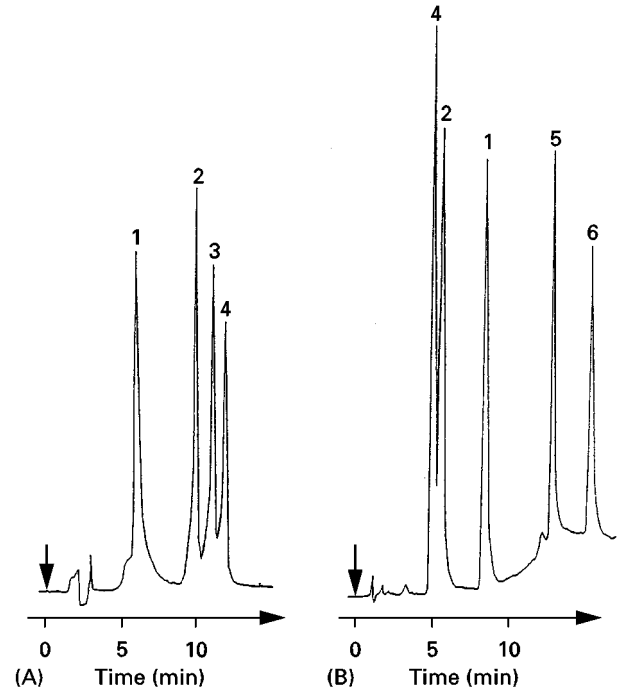

Figure 5 Separation of aniline derivatives (A) using a C_{18} column and (B) using a PGC column.
(A) Spherisorb ODS, 25 cm × 0.46 cm i.d.); mobile phase, 33% acetonitrile with 67% of a 0.05 mol L^{-1} sodium acetate/acetic acid solution at pH 4.6. (B) Hypercarb column, 10 cm × 0.46 cm i.d. packed with 7 μm particles; mobile phase, 68% methanol with 32% of a 0.05 mol L^{-1} sodium acetate/acetic acid solution at pH 4.6 from 0 to 6 min and then gradient up to 91% methanol at 9 min.
Analytes: 1, 2-hydroxy-5-chloroaniline; 2, 2-chloroaniline; 3, 3-chloroaniline; 4, 3-chloroaniline; 5, 5-chloro-2-methylaniline; 6, 2, 3-dichloroaniline. UV detection at 240 nm.

Ionic Compounds

In most cases, ions can be retained on PGC without the help of ion-pairing agents. Remarkable separations of small ionizable compounds of biomedical interest have been published by Lim *et al*. The retention of anionic compounds is explained by electronic interactions occurring between the lone pair electrons of the anionic analyte and the delocalized π bonds of the PGC. Examples include the determination of oxalic acid in human urine; this analyte is totally ionized in water and its separation from the other organic compounds in urine cannot be achieved using a C_{18} silica column. The combination of a sample preparation step using a C_{18} pre-column, which retains hydrophobic interferences but not oxalic acid, and a separation step with a PGC column, allows

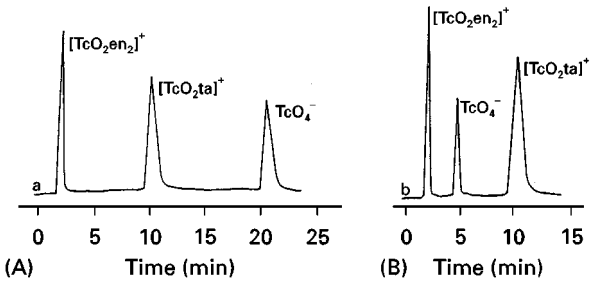

Figure 6 Separation of pertechnetate (TcO_4^-), dioxo(bis-ethylenediamino)technetium, $[TcO_2en_2]^+$, and dioxo(1,5,8,12-tetraazadodecane)technetium, $[TcO_2ta]^+$, on PGCs (A) with 2% (v/v) acetonitrile in 0.1% TFA as eluent; (B) with 2% acetonitrile in 1% TFA as eluent; flow rate, 1 mL min^{-1}; detector, radiometric. (Reprinted from Gu G and Lim CK (1990) Separation of anionic and cationic compounds of biomedical interest by HPLC on porous graphitic carbon. *Journal of Chromatography* 515: 183–192. Copyright (1990) with permission from Elsevier Science.)

oxalic acid to be separated from other polar compounds using a mobile phase containing 0.08% TFA. Creatine and creatinine can also be determined in urine and in serum within 8 min using 3% (v/v) acetonitrile and 0.1% TFA.

Anionic and cationic compounds can be separated simultaneously with a mobile phase containing an electronic mofidier such as TFA and an organic modifier (e.g. acetonitrile). PGC has been used to separate pertechnetate anions (TcO_4^-) from other monocationic amine complexes as shown in **Figure 6**A with a mobile phase containing 2% (v/v) acetonitrile in 0.1% TFA. TcO_4^- is retained exclusively by electronic interactions while the cationic complexes are retained by hydrophobic reversed-phase interaction. The separation can be controlled precisely according to the nature of the interactions. Increase in the concentration of TFA had a significant effect on the retention time of TcO_4^-, as shown in **Figure 6**B. The TFA acts as an 'electronic modifier'. Modifying the acetonitrile content affected the retention of the two cationic complexes, but not that of the anionic TcO_4^-.

Ion exchange chromatography can be achieved by modifying the surface of the PGC polyethyleneimine, which is rendered insoluble by flushing the column with a phosphate buffer. This column provides good separation of simple anions and it has been verified that retention is by a simple ion exchange mechanism.

Highly Polar and Water-Soluble Analytes

The use of PGC for the separation of very polar analytes is an important area that is far from being fully explored. Some pertinent examples include the analysis of highly water-soluble carbohydrates, drug metabolites and polar pesticides and their degradation or transformation products. Many of the separations that have been performed in this area are not possible with other reversed-phase materials.

Monosaccharides can be directly eluted with water from PGC and disaccharides with water containing either 15% methanol or 4% acetonitrile, each peak being split into anomers. A range of alditols containing up to five monosaccharides has been separated using an acetonitrile gradient with 0.05% TFA by Davies *et al*. These workers concluded from the order of retention that the retention mechanism involved interaction of the polar segments of the carbohydrate with the delocalized electrons of the PGC layers. Other examples have been presented for the separation of mono- and disaccharides, sugar acids and sugar amines using mobile phases at pH up to 13.

Figure 7 shows an example of the separation of highly polar pesticides using an acetonitrile/water gradient with an initial content of 10% acetonitrile.

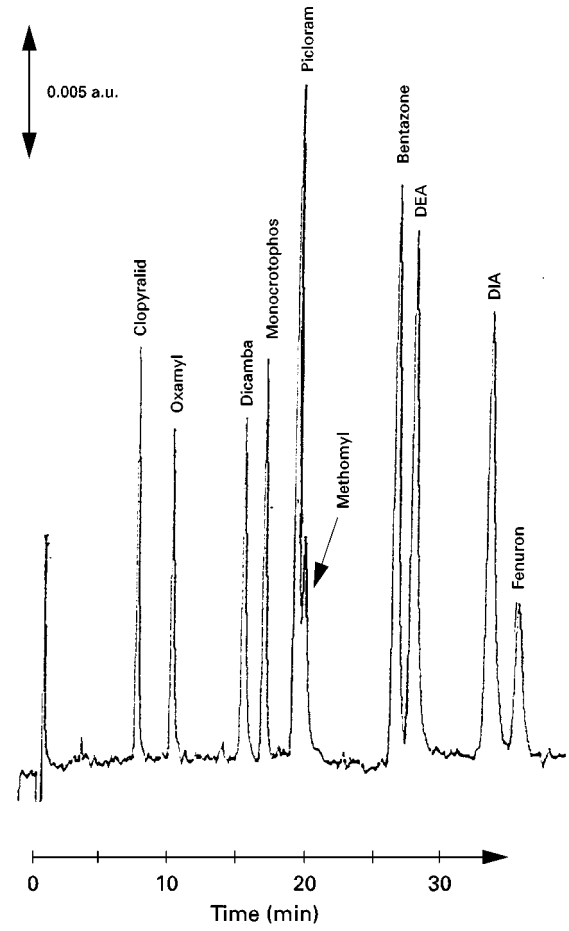

Figure 7 Analytical separation of polar pesticides using 10 cm × 0.46 cm i.d. Hypercarb column packed with 5 μm particles; mobile phase: 0.005 mol L^{-1} phosphate buffer at pH 7 and acetonitrile, gradient from 10% acetonitrile to 15% from 0 to 5 min and up to 40% and 40 min. UV detection at 220 nm.

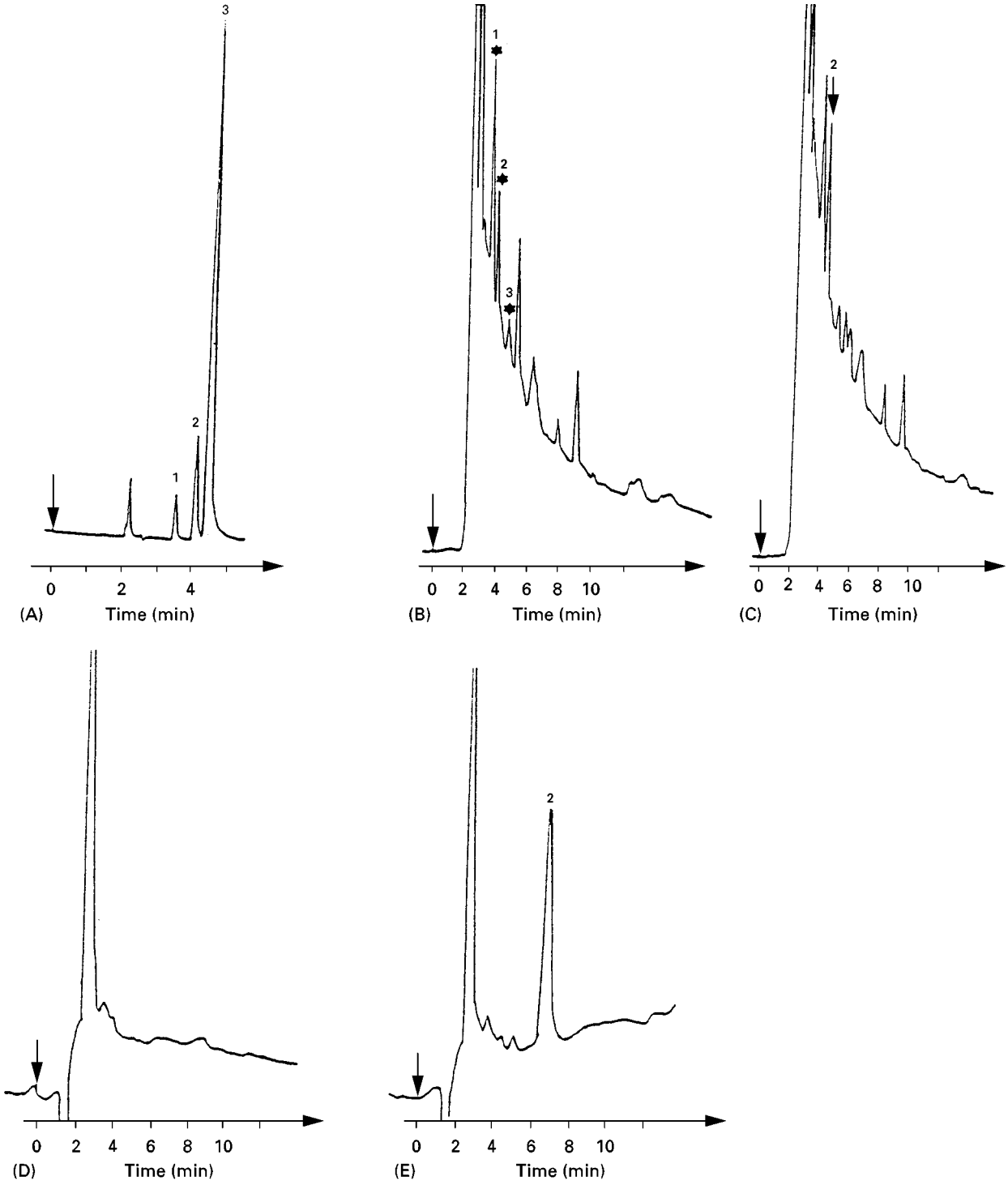

Figure 8 (A) Separation of (1) ammeline, (2) cyanuric acid and (3) ammelide using a 25 cm × 0.46 cm i.d. C_{18} column (Spherisorb ODS) eluted with a 10^{-3} mol L^{-1} perchloric acid solution. (B) Analysis of a water extract from 250 mL of drinking water not spiked and (C) spiked with 5 μg L^{-1} of cyanuric acid. (D) and (E) Analysis of the same samples as in (B) and (C) using a Hypercarb analytical column eluted with methanol/0.05 mol L^{-1} phosphate buffer at pH 7 (30 : 70 v/v). UV detection at 220 nm. Extract from water obtained using a commercial solid-phase extraction cartridge packed with PGC.

With a similar gradient applied to a C_{18} column, the retention order is different and oxamyl and deisopropylatrazine (DIA) are only slightly retained. Another example is the separation of the polar degradation products of atrazine. Ammeline, ammelide and cyanuric acid (see **Table 2**) can be separated with an

aqueous mobile phase but with retentions close to the void volume of the column, as shown in **Figure 8**A. Therefore, when these analytes are to be determined in real water at trace levels, the extraction and concentration step generates many interfering compounds that hinder the detection of early eluted analytes, as shown in **Figure 8**B where peaks marked with the stars may correspond to the three analytes. **Figure 8**C corresponds to the extract spiked with cyanuric acid, but no sound conclusion can be made. It was verified that the water did not contain cyanuric acid because, using a PGC column, cyanuric acid is eluted within 8 min with a mobile phase containing 30% methanol. With this column and eluent there is no problem with the separation between the analyte of interest and the interfering coextracted analytes, which are eluted in the peak close to the void volume of the column with such a mobile phase (**Figure 8**D and E).

Future Trends

Designed to be the 'perfect reversed-phase material', PGC has been shown to possess a unique chromatographic behaviour, different from other reversed-phase materials. It makes possible the separation of many solutes over a wide range of polarities and is well suited for the separation of positional isomers. Its inert structure allows a complete pH range of eluents to be used, permitting a greater exploitation of the functionality of analytes. The possibility for modification of its surface has opened new areas of applications.

Its unusual retentive behaviour for polar analytes has led to separations that cannot be achieved with other available stationary phases. More work is undoubtedly necessary to achieve a better understanding of the unique behaviour of PGC.

Further Reading

Forgacs E and Cserhati T (1995) Retention strength and selectivity of a porous graphitized carbon column: theoretical aspects and practical applications. *Trends in Analytical Chemistry* 14: 23–29.

Gu G and Lim CK (1990) Separation of anionic and cationic compounds of biomedical interest by HPLC on porous graphitic carbon. *Journal of Chromatography* 515: 183–192.

Guenu S and Hennion M-C (1994) On-line sample handling of water-soluble organic pollutants in aqueous samples using porous graphitic carbon. *Journal of Chromatography A* 665: 243–251.

Hennion M-C, Coquart V, Guenu S and Sella C (1995) Retention behaviour of polar compounds using porous graphitic carbon with water-rich mobile phases. *Journal of Chromatography A* 721: 287–301.

Knox JH and Ross P (1997) Carbon-based packing materials for liquid chromatography, structure, performance and retention mechanism. In: Brown PR and Grushka E (eds) *Advances in Chromatography*, vol. 37, pp. 74–119. New York: Marcel Dekker.

Knox JH and Wan QH (1995) Chiral chromatography of amino- and hydroxy-acids on surface modified porous graphite. *Chromatographia* 40: 9–14.

Kriz J, Adamcova E, Knox JH and Hora J (1994) Characterization of adsorbents by HPLC using aromatic hydrocarbons: porous graphite and its comparison with silica gel, alumina, octadecylsilica and phenylsilica. *Journal of Chromatography A* 663: 151–161.

Pichon V, Chen l, Guenu S and Hennion MC (1995) Comparison of sorbents for the solid-phase extraction of the highly polar degradation products of atrazine (including ammeline, ammelide and cyanuric acid). *Journal of Chromatography A* 711: 257–267.

Ross P and Knox JH (1997) Carbon-based packing materials for liquid chromatography: applications. In: Brown PR and Grushka E (eds) *Advances in Chromatography*, vol. 37, pp. 122–162. New York: Marcel Dekker.

Tanaka N, Tanigawa T, Kimata K, Hosoya K and Araki T (1991) Selectivity of carbon packing materials in comparison with octadecylsilyl- and pyrenylethylsilylsilica gels in reversed-phase liquid chromatography. *Journal of Chromatography* 549: 29–41.

Wan QH, Davie MC, Shaw PN and Barrett DA (1995) Chromatographic behaviour of positional isomers on porous graphitic carbon. *Journal of Chromatography A* 697: 218–227.

Wan QH, Davie MC, Shaw PN and Barrett DA (1996) Retention behaviour of ionizable isomers in reversed-phase liquid chromatography: a comparative study of porous graphitic carbon and octadecyl bonded silica. *Analytical Chemistry* 68: 437–446.