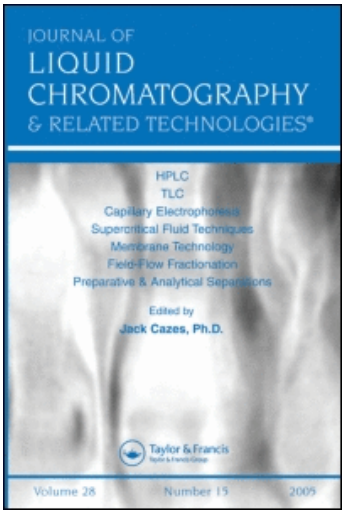

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Porous Graphitic Carbon as a Stationary Phase in HPLC: Theory and Applications

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Abstract: This paper gives an overview of the properties and behaviour of porous graphitic carbon (PGC) as a stationary phase in HPLC. Key applications areas that illustrate the capabilities of this chromatographic material to solve problem separations are reviewed. The retention mechanisms on this stationary phase for hydrophobic and polar solutes are described. PGCs' physical and chemical properties and the development of this unique chromatographic material are discussed. Over 140 references of applications of PGC in pharmaceutical, environmental, food safety, natural products, biochemical, clinical, and chemical warfare analysis are reviewed covering a range of solute properties.

Keywords: Electronic interaction chromatography, Hypercarb, Isomers, Polar retention effect on graphite, Polar solutes, Retention mechanisms

INTRODUCTION

Porous graphitic carbon (PGC) has unique properties as a stationary phase in high performance liquid chromatography (HPLC). Its chemical surface properties distinguish PGC from more conventional LC packings such as bonded-silica gels and polymers.

PGC behaves as a strongly retentive alkyl-bonded silica gel for non-polar analytes, however its retention and selectivity behaviour toward polar and structurally related compounds is very different. In a

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comprehensive review of the structure, performance and retention mechanism of PGC, published by Knox and Ross in 1997,[1] PGC's chromatographic behaviour was summarised as showing:

- a polar retention effect (PREG – polar retention effect on graphite) whereby solutes of increasing polarity showed a high affinity towards the graphite surface. With conventional alkyl-bonded silicas, the addition of a polar group to a molecule will normally reduce retention in the reversed-phase mode whereas with PGC retention is reduced to a much smaller extent or may even increase. This behaviour makes PGC well suited to the separation of very polar and ionized solutes such as carbohydrates and compounds with several hydroxyl, carboxyl, amino and other polar groups.
- increased retention of non-polar compounds (based on dispersive interactions) compared with conventional alkyl-bonded silicas. Increasing the hydrophobicity of a structure by the additions of $-CH_2-$ or other non-polar groups increases retention.
- increased selectivity towards structurally related compounds (geometric and diastereoisomers) due to the flat and highly adsorptive surface of the graphite.
- unique and complex retention mechanism; the strength of interaction depends on both the molecular area of an analyte in contact with the graphite surface and upon the nature and type of functional groups at the point of interaction with the flat graphite surface.
- stability at extreme mobile phase pH conditions (10 M acid to 10 M alkali), salt and temperature, due to its extreme chemical stability.

These chromatographic properties have allowed PGC to become, since its commercial introduction in 1988, a stationary phase to solve what may be considered problematic LC separations, and which complements the applications of typical reversed-phase packings. There have been two major application areas for PGC, one being the retention and separation of highly polar and ionized compounds. Such analytes are not normally retained on typical reversed-phase packings even those which contain some polar character; examples include the analysis of carbohydrates, sugars, carrageenans, glucuronides, nucleotides, hydrophilic peptides, charged organometallic complexes and inorganic ions. The second area is in the separation of structurally similar solutes and isomers for which alkyl-bonded silicas or bare oxide sorbents do not provide adequate selectivity; such compounds include positional isomers and diastereoisomers. PGC has also been used in the separation of enantiomers with a chiral selector in the mobile phase. Ross and Knox performed a comprehensive review of all published applications of PGC up to 1995.[2] The applications were categorized in: a) geometric isomers and closely

related compounds; b) enantiomers; c) sugars, carbohydrates and glucuronides; d) residue analysis; e) ionized and other highly polar solutes. In 1992, Lim reviewed biomedical applications of PGC[3] and more recently in 2003, Hanai reviewed the separation of polar compounds using carbon columns.[4] PGC also has advantages when used in solid phase extraction, especially for very polar residual pollutants from water. This application area was reviewed by Hennion in 2000.[5]

In this paper the properties and behaviour of porous graphitic carbon as a stationary phase in HPLC are discussed, with emphasis on the retention mechanisms responsible for the separation of polar compounds. Some of the more recent application areas which illustrate this stationary phase's unique properties are reviewed.

BRIEF HISTORY OF THE DEVELOPMENT OF PGC

The advantages of graphitized carbons as chromatographic supports were recognised very early, in 1976, by Colin, Eon and Guichon,[6] however it was not until 1981 that Ciccioli and co-workers reported that graphitized carbon black (GCB) provided satisfactory chromatographic peaks in both gas and liquid chromatography.[7] However, at that time, the particles were too fragile to be used routinely in LC. In addition to particle fragility, other difficulties with the use of graphitized carbons in chromatography were retention capacity and poor mass transfer. In 1982 Knox and Gilbert[8–10] made a breakthrough by developing a method for making mesoporous glassy carbon using silica gel as a template. Their process created a packing material with the required mechanical stability and surface area. Heating of this material to temperatures greater than 2000°C removed micropores and the material become graphitized. The porous graphitic carbon produced by this process provided the indispensable chromatographic column performance. Other manufacturing processes for porous graphite have been developed since then and these, along with its history, have been reviewed in the past.[1,11–12] Initially PGC was manufactured, using the method of Knox and Gilbert, by the Wolfson Liquid Chromatography Unit in the Chemistry Department at the University of Edinburgh. In 1988 the manufacture was transferred to Shandon HPLC in Runcorn, Cheshire (now Thermo Fisher Scientific) and the company started to market the material under the trade name Hypercarb™, still in use today. In the years between 1988 and the present time, further optimization of the material has taken place with the introduction of 5 and 3 μm particles. Today, Hypercarb compares well with bonded silica gels in terms of chromatographic performance.

PHYSICAL AND CHEMICAL PROPERTIES

PGC particles are spherical and fully porous with a porosity of approximately 75%. The surface of PGC is crystalline and highly reproducible and does not contain micropores. At the molecular level, PGC is made up of sheets of hexagonally arranged carbon atoms linked by the same conjugated 1.5-order bonds which are present in any large polynuclear aromatic hydrocarbon.[1] In principle there are no functional groups on the surface since the aromatic carbon atoms have fully satisfied valencies within the graphitic sheets. High resolution transmission electron microscopy[13] indicates that PGC consists of intertwined ribbons of carbon, where each ribbon consists of around 30 layers of sheets approximately 3.4 Å apart, as illustrated in Figure 1. This structure has similarities with GCB, however in PGC the ribbons are interconnected, this accounts for its high mechanical strength compared with GCB. The individual sheets of carbon atoms are held together by London dispersion interactions (instantaneous-dipole-induced-dipole interactions between the carbon atoms in adjacent sheets). The spacing between the graphitic layers is typical of a three dimensional graphite (3D), however, unlike the 3D graphite there is no ordering of the atoms between different layers in PGC. This is demonstrated in Figure 2a, the X-ray diffraction pattern[13] for PGC, where there is a well defined (l)-spacing between layers, and well defined (h,k)-spacings of the atoms within the layers, but no registration of one layer relative to those above and below as in a 3D graphite (Figure 2b). PGC is, therefore, a two dimensional graphite.

No crystal structure is perfect and that of PGC is no exception. There are undoubtedly carbon atoms at the edges of the graphitic sheets which

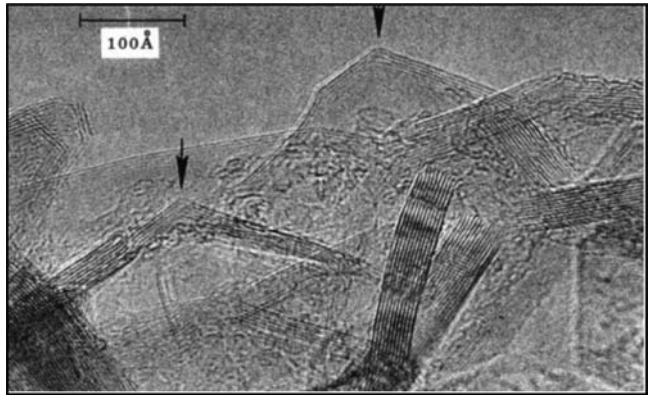

Figure 1. High resolution electron micrograph of PGC section (originally published by Knox, Kaur, Millward[13]).

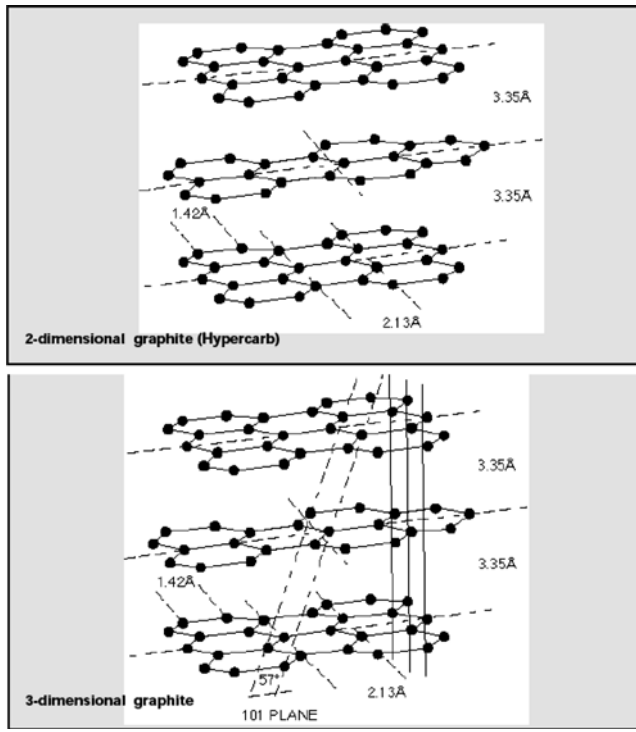

Figure 2. Crystal structure of graphite: (a) 2-dimensional graphite with no layer registration (Warren structure); (b) 3-dimensional graphite with ABAB (Bernal structure).

must have valency satisfying functional groups attached to them such as hydroxyl, carbonyl, carboxylic or perhaps amino functions. These functional groups and lattice imperfections constitute less than 1% of the surface: as a result their deleterious effects on chromatography are minimal.

Table 1 lists the more important physical properties of PGC. The requirements placed on its physical properties are similar to other HPLC supports where factors such as narrow particle size distribution are essential to the ultimate performance of the phase if good bed uniformity and low operating pressures are to be achieved. A specific surface area which falls in the range of 50 to 400 m^2/g ensures good chromatographic capacity and suitable porosity allows for good mass transfer within the particles. PGC also has a tight pore size distribution with a mean value around 250 Å, allowing for good mass transfer of a wide range of analyte shapes and sizes. Surface homogeneity and absence of highly adsorptive sites are essential for good peak symmetry. PGC meets all the conventional operating criteria of a chromatographic support.

Table 1. Physical properties of PGC

Property		To meet requirement of
Particle shape	Spherical, fully porous	No micropores
Specific surface area	$120\,m^2/g$	Retention linearity and loading capacity
Median pore diameter	250 Å	Mass transfer for wide range of analyte's shapes and sizes
Pore volume	$0.7\,m^3/g$	
Mean particle diameters	3, 5, 7 μm (30 μm)	Packing bed uniformity
Porosity	75%	Mass transfer within particles
% C	100%	Chemical stability
Mechanical strength	>400 bar	Operational particle stability; pressure gradients in packing process

MECHANISMS OF RETENTION

The surface of the graphite is flat and highly crystalline unlike that of alkyl-bonded silicas, which possess a brush type surface with bonded phase and residual silanols. Consequently, the PGC mechanism of interaction is very different. The retention by graphite from aqueous/organic eluents is determined by the balance of two factors: (1) hydrophobicity, which is primarily a solution effect that tends to drive analytes out of solution and (2) the interaction of polarizable or polarized groups in the analyte with the graphite (these are additional to the normal dispersive interactions). The strength of interaction depends on both the molecular area of an analyte (and, therefore, shape of the analyte) in contact with the graphite surface and upon the nature and type of functional groups at the point of interaction with the flat graphite surface. The more planar the analyte, the closer its alignment to the graphite surface, and so the greater the number of points of interaction possible–hence, maximum retention. Retention is reduced for highly structured and rigid molecules that can contact the surface with only a small part of their surface, compared with planar molecules with the same molecular mass. This is illustrated in Figure 3.

There have been a number of studies over the past twenty years to elucidate the retention mechanisms at the surface of the graphite. Studies up to 1997 are reviewed in detail by Knox and Ross in reference 1. In this review the factors that determine retention on graphite were summarized as follows:

a) Eluent-analyte interaction (dispersive, dipole-dipole and hydrogen bonding) which occur in the eluent. These discourage retention.

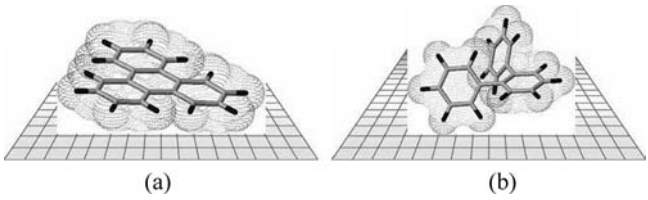

Figure 3. Effect of the solute shape on the strength of the interaction with the graphite surface: (a) Good alignment of planar molecule to the flat graphite surface; (b) Poor alignment of non-planar molecule to the flat graphite surface.

b) Hydrophobic eluent-analyte repulsions (arising from resistance to the disruption of the structure of hydrogen-bonded solvents by non-hydrogen-bonding analytes). These occur between a hydrophilic eluent and any non-polar segments of the analytes, and encourage retention.
c) Dispersive interactions of the London type between the graphite surface and the analytes. These are largely balanced by similar interactions between the graphite surface and the eluent which is displaced by the analyte. Their net effect may either encourage or discourage retention, but they may have an important effect on selectivity.
d) Charge-induced interactions of the analyte with the graphite which promote retention of polar molecules (PREG). These interactions are compensated to a greater or lesser extent by polar interactions of the analyte with the eluent (type a) above. These charged-induced interactions are strongest when the polar groups of the analyte are forced into direct contact with the graphite surface by the stereochemistry of the analyte molecule. In such cases, the additional interactions resulting from substitution of –H by a polar group can be so strong that they more than compensate for the increased analyte-solvent interactions. When stereochemistry does not force direct contact of the polar group with the surface, the effect is less strong but still significant.

The overall effect of these competing interactions is that increasing the hydrophobicity of the analyte, for instance by adding alkyl groups into a molecule, always increases retention, as expected in a typical reversed-phase mode. However, increasing the polarity of the analyte by adding groups which can either donate or accept electrons or can polarize the graphite surface may also increase retention, particularly if these groups are constrained to be in close contact with the graphite surface.

Retention of Non-Polar Compounds

Möckel and co-workers[14] compared the retention of a series of homologous alkane derivatives on PGC and a C_{18}-silica phase, using methanol as the eluent. By measuring the gradient of the plots of log k (where k is the capacity factor) against carbon number for the different homologous series $CH_3(CH_2)_{n-1}X$ (where X is a substituent such as $-CH_3$, $-CN$ or -Phenyl), they found that PGC shows much greater discrimination for the methylene groups than does the C_{18}-silica phase.

Tanaka et al.[15] have also published a study of the selectivity of carbon- and silica-based packings using homologous alkanes and derivatives thereof. Their results are in good agreement with those of Möckel and co-workers, in that graphite shows a higher affinity for homologous series than either C_{18}-silica or pyrenylethyl-silica (PYE) over a wider range of methanol/water compositions (0-80%). Tanaka and co-workers also confirmed the high stereoselectivity of graphite especially for cis- and trans-isomers of di-substituted cyclohexanes. The more planar the isomer, the more it is retained. The observed selectivity for C_{18} and PYE-silica is less. This feature of PGC is attributed to the flat graphite surface, which allows for stronger dispersive interactions with those molecules which can align themselves better to the flat surface.

The study of Kříž and co-workers compared plots of a $CH_3(CH_2)_{(n-1)}$-aryl homologous series with a series of ortho-substituted polymethylbenzenes, aryl-$(CH_3)_{(n+1)}$, on a ODS phase, PGC, silica and alumina. Their results showed that PGC is better able to discriminate compounds based on the number of methyl substituents present than C_{18}-silica. These results are briefly summarized in Table 2: no discrimination between methylene and methyl groups is observed for C_{18}-silica and very little discrimination for silica or alumina. Retention is considerably increased for PGC and this effect becomes more apparent as the carbon number increases through the addition of either a methylene or methyl group.

Table 2. Comparison of $\alpha(CH_3)$ and $\alpha(CH_2)$ selectivity on different stationary phases

Phase	$\alpha(CH_3)$	$\alpha(CH_2)$	Eluent
PGC	0.46	0.22	Methanol
C_{18}-silica	0.17	0.17	Methanol/water (80:20)
Silica	0.046	0.1	Pentane
Alumina	0.195	0.00	Pentane

α, the expected linear gradient, is calculated from equation: $\log_{10} k = \alpha + \beta n$, where k is the retention factor, β is the theorectical log k for benzene and n is the number of carbons.

The improved discrimination of PGC for both methylene and methyl substitution provides information about how PGC achieves improved resolution and selectivity of complex mixtures of analytes.

Retention of Polar Compounds

It soon became apparent that the elution profile on PGC is not in order of solute hydrophobicity, as for typical reversed-phase packings, but in the order of their polarity, i.e., more polar compounds can be more strongly retained than less polar ones. Tanaka and co-workers[15] plotted log k for the various stationary phases against log P (where P is the octanol-water partition ratio). For C_{18} the correlation was very good, but this was not the case for PGC. The retention of polar compounds on PGC was much higher than expected, exhibiting k values 4 to 15 times higher than expected on the basis of their log P.

Coquart and Hennion correlated log k and log P for PGC, C_{18}-silica and polystyrene divinylbenzene (PS-DVB), as part of their study of the use of Hypercarb as an adsorbent for removing polar contaminants from water samples.[17–19] The correlation with C18-silica was very good, for PS-DVB the data points were more scattered and for PGC the data points for polar compounds lay well above the line for the alkyl benzenes or alkanes. They measured the log k values for a range of compounds in their mono-, di- and tri-substituted forms at different concentrations of methanol-water eluents. The data was then extrapolated to generate log k in pure water, log k_w (intercept at the y axis) in equation 1:

$$\text{Log k} = log\ k_w + A\text{C}_{\text{org}} \tag{1}$$

where A is the gradient of the line and C_{org} is the percentage of organic solvent in the water-organic solvent mixture.

These results (summarized in Table 3) showed that the retention of monosubstituted benzenes is similar for C_{18}-silica and PGC and lower than on PS-DVB. The log k_w values obtained when solutes have 2 polar substituents using PS-DVB are always lower than those measured for each corresponding monosubstituted benzene, whereas the contrary is observed with PGC. The di-substitued benzenes are not retained on C_{18}-silica. These results also highlight potential applications areas where PGC offers selectivity and retention where other chromatographic supports cannot (further detailed in the Applications section). The changes in retention of opiates at acidic and alkaline pH were investigated by Barret et al.[20] and they concluded that the elution order was not related to the log P values of the solute. They also observed strong retention of the fully ionized solutes, especially of those with acidic functional groups,

Table 3. Comparison of values of log k_w on reversed phase stationary phases

Compound	C_{18}-silica	PS-DVB	PGC
Benzene	2.20	3.50	1.45
Monosubstituted			
Aniline	1.10	2.50	1.35
Phenol	1.55	2.40	1.80
Benzoic acid	1.90	3.20	2.40
Nitrobenzene	2.05	3.60	2.45
Polysubstituted			
4-Aminophenol	–	1.10	2.05
1,4-Diaminobenzene	–	1.20	2.40
4-Aminobenzoic acid	–	2.00	2.85
4-Hydroxybenzoic acid	–	2.30	2.70
3,5-Dihydroxybenzoic acid	–	1.35	3.00
1,3-Dihydroxybenzene	–	1.35	2.35
1,4-Dihydroxybenzene	–	0.85	2.15
1,3,5-Trihydroxybenzene	–	0.50	2.70

–: not retained.

suggesting that hydrophobic interactions are present in addition to the polar retention effects.

Wan and co-workers studied the retention of 36 positional isomers of substituted benzenes on PGC and C_{18}-silica and confirmed the greater steric selectivity of PGC.[21] Forgács and co-workers carried extensive studies of the retention of various classes on compounds, including barbiturates, amines, and phenols by PGC.[22–30] They found that the more polar or hydrophilic analytes eluted later, and that, therefore, the increased affinity of a solute for water within these series was more than balanced by the increased affinity for the graphite surface. The retention behaviour of polyethoxylated alcohols on PGC and C_{18}-silica was studied by Chaimbault et al.[31] They concluded that PGC offers stronger retention than C_{18}-silica and that retention on PGC increased with hydrocarbon chain length and increasing ethylene oxide number.

In 1995 Hennion et al.[32] analysed the solute polarity, using local dipole moments and the overall electron-excess charge density, and concluded that electronic interactions are more important for the retention of polar compounds than hydrophobic interactions. More recently in 2002, Jackson and Carr showed that any polar functional group added to the benzene ring induces an increase in retention, regardless of its electron-donor or electron-acceptor properties.[33] This behaviour was explained by the polarizability of the carbon surface due to the overlapping of the hybridized orbitals, allowing dipole type and electron lone pair donor-acceptor interactions.

In 2003, Hanai used computational chemical analysis with model polycyclic aromatic hydrocarbons as models of graphitic carbon to study retention mechanisms of ions and saccharides.[4] He concluded that the electrostatic effect is negligible but that of hydrophobic effect related to van der Waals energy change is predominant for retention. West et al. used subcritical fluid chromatography with carbon dioxide/methanol mobile phases to describe the retention on PGC.[34] They used the solvation parameter model to provide a better understanding of the interactions developed between the solute, the stationary phase and the mobile phases. Their results showed that the dominant contribution to retention was given by the solute polarizability and the solute volume, while the hydrogen-bond basicity was not selected in the retention model. An increase in the methanol content favours a decrease in retention, through the volume for hydrophobic compounds and through hydrogen-bond acidity for polar compounds.

Polar Retention Effect on Graphite (PREG)

The polar retention effect on graphite defines the ability of molecules having lone-pair or aromatic-ring electrons to apparently interact with graphite by an electron transfer mechanism to the electronic cloud of the graphite. PREG is particularly pronounced when the polar groups are attached to a benzene ring and other larger aromatic systems. It is thought to be some type of orbital overlap between the conductivity electrons in graphite and lone pair and/or π electrons in analytes.[1] Attempts to correlate solute properties with retention behaviour on graphite were unsuccessful until Ross and Knox measured the PREG by comparing the retention behaviour of test polar compounds with those of equivalent hydrocarbons.[35,36] They defined an equivalent hydrocarbon as the hydrocarbon obtained by replacing atoms such as oxygen and nitrogen with CH_2 and CH groups, respectively. The retention factor of the polar solute (x) on graphite can then be compared with that of the equivalent hydrocarbon (hc), in typical eluents and its partition coefficient (D) into a non-polar eluent (hexane) from the same eluent. They defined the polar retention effect on graphite as:

$$2.303RT\log_{10}(k_x/D_x) - 2.303RT\log_{10}(k_{hc}/D_{hc}) \quad (2)$$

where R is the gas constant, T is the temperature in Kelvin, k_x and D_x are the retention factor and partition coefficient respectively of the polar compound and k_{hc} and D_{hc} are the retention factor and partition coefficient respectively of the equivalent hydrocarbon.

The values of the PREG were determined to be as high as 42 kJ/mol; typical van der Waals interactions associated with reversed-phase LC are

approximately 2 kJ/mol. Ross and Knox considered the correlation of the PREG and the heats of formation for each group of analytes and the energy of interaction with a polarizable graphitic surface. The correlation found was reasonable but did not explain the polar retention effect on graphite. The polarization of the graphite surface is based on the premise that graphite can donate as well as accept electrons. Calculated values for the energies associated with an ion-induced dipole at the graphite surface for a range of analytes were of the correct order. The energy of a charge approaching a polarizable surface is given by Equation (3):

$$E = S\alpha/(4\pi\varepsilon_0\varepsilon_r)r^4 \quad (3)$$

where E is the energy of an ion-induced dipole, S is the charge distribution on the analyte, α is the polarizability constant for the medium (1.5×10^{-10}cm^3 for graphite), $4\pi\varepsilon_0$ is the electrostatic unit for energy, r is the distance between the analyte charge and the surface of the graphite (Ross and Knox used a value of 3.5×10^{-10} m, which represents approximately the distance between graphite layers) and ε_0 is the electric permitivity in a vacuum. The polarizable properties of the graphite hold the key to understanding the mechanism by which polar molecules are retained at the surface (Figure 4).

APPLICATIONS

Reference 2 provides an excellent review of applications on PGC up to mid-1995. This paper reviews key applications published since then. Table 4 provides an overview of the applications reviewed in section 5 which are organised in the following subsections: isomers (geometrical, positional and diastereoisomers), polar compounds (nucleotides/nucleosides/nucleobases, amino acids and peptides, carbohydrates and

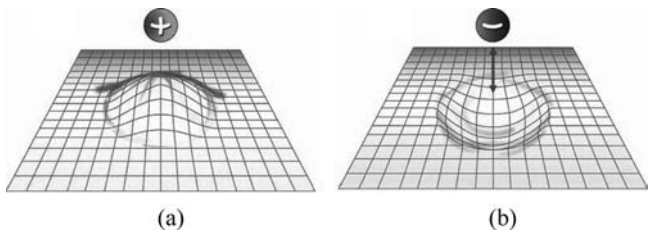

Figure 4. Schematic representation of polar analyte retention in which (a) positive charge and (b) negative charges approach the graphite surface, resulting in a charge-induced dipole at the graphite surface.

Table 4. Quick reference guide of applications reviewed

Application by solute type Application area	Analyte group	Reference number
Isomers	Ionizable substitued benzenes	37, 38
	Cresol	39
	Hippuric acids	Authors lab
	Substituted aromatics	40
	p-Nonylphenol	41
	Sulfobutyl ether-β-cyclodextrin	42
	Schumannificine	43
	Tropane alkaloids	44
	Estrogens	45
	F2-Isoprostanes	46, 47
	Branched oligosaccharides	48
	Leucine, isoleucine, allo-isoleucine	49
	Cis/trans related substances of a drug	50
Diastereoisomers	Xylose derivative	51
	Oligomers of methylidene malonate	52
	Metabolites II, III, and VII, VIII	53
Nucleotides / Nucleosides	Nucleobases, nucleosides, nucleotides	54
	Purine bases	55
	Nucleosides and their mono-, di- and triphosphates	56
	Uracil, dihydrouracil	57
	Tegafur, 5-fluorouracil, 5-fluorodihydrouracil	58
Amino acids and peptides	Underivatized amino acids	60
	Taurine derivatives	62
	Di-, tri-, tetra-peptides	61, 65
	Small peptides in wine	64
	Phosphopeptides	65, 66
	Glycopeptides	67
Carbohydrates and sugars	Mono-, disaccharides	67, 71, 82
	Cyclodextrins	67, 86, 87, 42
	Oligosaccharide alditols	67, 74, 75
	N-linked oligosaccharides	67, 72, 74, 78, 81
	Oligosaccharides	67, 75, 77, 79, 80, 82
	Sugar phosphates	68
	Sulphated disaccharides	69
	Glycosaminoglycans	70
	Carrageenans	83, 84, 85

(*Continued*)

Table 4. Continued

Application by solute type Application area	Analyte group	Reference number
Other polar species	Catecholamines / neurotransmiters	88, 89, 90, 91
Biochemical	Glutathione and conjugates	92, 93
	Oligonucleotides	94
	Creatinine / creatine	95
Pharmaceutical	Clopidogel and metabolites	96
	Acarbose and metabolites	97
	Metabolites in Escherichia coli K12	98
	Arabinoside-CMP, cytarabine	99, 100
	Cimetidine	101
	Levetiracetam	104
	Oxaliplatin	103
	EDTA impurities	102
Food safety	Methylamines	105
	Polar Phenolic	106
	Acrylamide	107, 108
	Acromelic acid A	109
Environmental	Aniline	110
	Glyphosate and Ampa	111
	Cyanuric acid	112
Surfactants	Alkylglycoside detergents	113
	Polyethoxylated alcohols	114
	Oligoglycerols	115
	Oligomers of nonylphenyl ethylene	116
	Polyglycerol fatty esters and fatty ethers	117
Natural products	Cyanoglycosides	118
	Glucosinolates	119
Chemical warfare	Phosphonic acids	120
Other	Guanidino compounds	121, 122
	Diphosphine-bridged complexes	123
Charged solutes	Pertechnetate and perrhenate ions	124
	Organometallic-charged complexes	125, 126
	Inorganic anions	127– 129, 131,132
	Copper (II), copper (III)	130

(*Continued*)

Table 4. Continued

Application by solute type Application area	Analyte group	Reference number
	Diquat, paraquat and difenzoquat	133
Non-polar solutes	Wax esthers	134
Lipids	Ceramides	134–137
	Fatty acids methyl esters	134, 138–140
	Glycosphingolipids	141
	Triacylglycerols	147
Natural products	Digalactosyldiacylglycerol	142
	Triterpenic acids	143
	Taxol	144, 145
	Non-flavonoid polyphenols	146
	Ferrichrome and ferricrocin	148
Pharmaceutical	Morphine and metabolites	149
	Pharmaceuticals and related substances	150–154
	Steroids	155
	Cyclosporins A and U	156
	Tetracycline antibiotics	157
Clinical	Phthalate metabolites	158
	Boron-containing compounds	159
	Flame retardants hydrolysis products	160
Environmental	Benzo[α]pyrene	161
	PCBs	162–164
	Halogenated contaminants and PAHs	165
Food safety	Betamethasone and dexamethasone	166, 167
	Glucocorticoids and corticosteroids	168–170
	Fenbutin oxide	171
Explosives	Nitroaromatic and organic explosives	172–176
	Nitrate ester, nitramine and nitroaromatic explosives	177

sugars, and other polar molecules), charged solutes and miscellaneous. The miscellaneous and other polar molecules subsections are further categorised into application areas such as food safety, environmental, pharmaceutical, etc.

Applications areas for PGC which are outside the scope of this review and therefore not covered include high temperature applications, chiral separations, PGC as a sorbent in solid phase extraction, electrically modulated chromatography and electrochromatography.

Isomers

Geometrical and Positional Isomers

Wan et al.[37] studied the chromatographic behaviour of six sets of ionisable substituted benzene isomers on PGC. They found that the elution orders varied with the different type of substituents on the benzene ring and were not readily predictable from the structures of the isomers. The mobile phase pH range of 2 to 9.4 was investigated and the retention of the solutes was correlated directly with their degree of ionization, with the ionised form being the least retained. This study was indicative of the importance of the orientation of polar solutes with respect to the planar graphite surface on the PGC. The following year, 1996, the same group published a comparative study of the retention of the substituted benzene isomers on PGC and octadecyl-bonded silica.[38] PGC demonstrated a higher selectivity towards positional isomers than ODS, which was attributed to the greater steric discriminating ability arising from the flat surface of the PGC compared with the more fluid nature of the ODS bonded phase. The usefulness of PGC to separate positional isomers was explored by Schlatter in a method to analyse the isomers of cresol in urine of workers exposed to toluene.[39] Figure 5 illustrates an example from the author's laboratory for the separation of the metabolites of xylene and styrene (hippuric acid and o-, m-, p-methyl hippuric acids, mandelic acid and phenyl gyloxylic acid). West et al. published the separation of substituted aromatic isomers with PGC using subcritical fluid chromatography.[40] They studied the effect of both nature and percentage of the modifier, and observed two types of selectivity behaviour. The first related to steric recognition and the second to the interaction between the polar moieties of the solutes and the stationary phase. The separations observed were greatly improved compared with those obtained with HPLC. The methodology was applied to BTEX (benzene, toluene, ethylbenzene and xylenes) analysis and flavour molecules separations.

PGC was used to separate a technical mixture of p-nonylphenol into 12 peaks or groups of isomers.[41] p-Nonylphenol, a degradation product of nonylphenol polyethoxylate surfactants, is composed of numerous structural isomers resulting from the various branching patterns of the C-9 group. Typically, in HPLC analyses, p-nonylphenol elutes as a single,

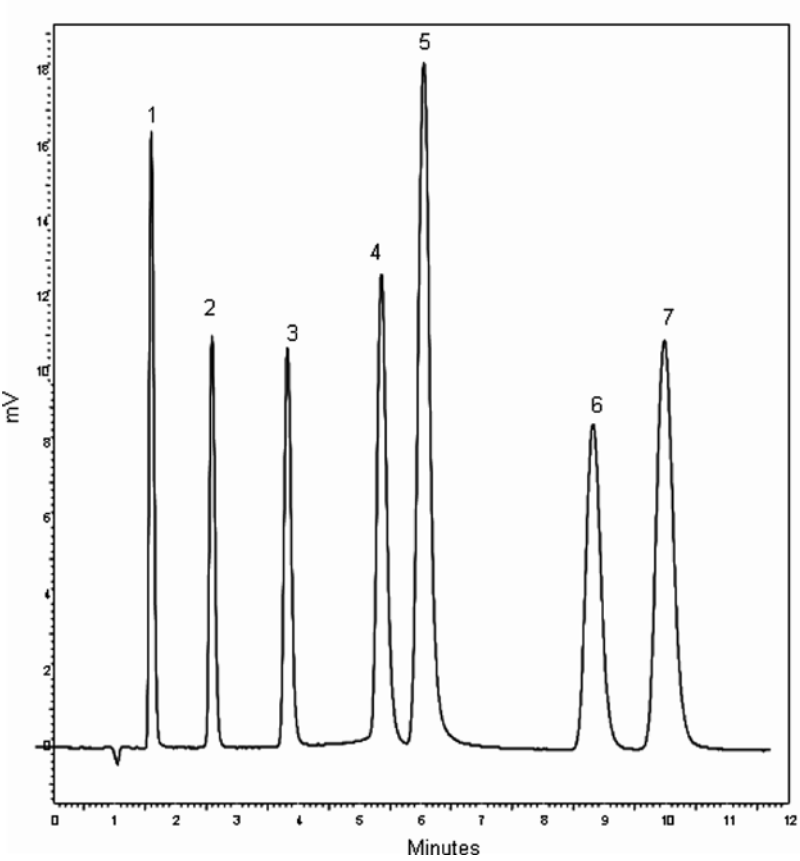

Figure 5. Separation of metabolites of xylene and styrene on PGC. Experimental conditons: Column–Hypercarb 3 μm, 100 × 4.6 mm; Mobile phase H_2O + MeOH (1:1) + 0.1%TFA; Flow rate–1 mL/min; Detection–UV at 240 nm. Analytes: 1. Mandelic acid; 2. 2-Methylhippuric acid; 3. Hippuric acid; 4. Phenyl gyloxylic acid; 5. 4-Hydroxybenzoic acid (internal standard); 6. 3-Methylhippuric acid; 7. 4-Methylhippuric acid.

broad peak due to lack of discrimination for the structural isomers by the stationary phase. The method on PGC can potentially be used to fractionate nonylphenol isomers based on structure to assess the potential for different isomers to act as endocrine disrupters. PGC was also the stationary phase of choice for the separation of positional isomers of monosubstituted sulfobutyl ether-β-cyclodextrin.[42] These isomers cannot be separated on conventional bonded phases but were well resolved on PGC. The retention of these hydrophilic and anionic compounds is thought to be a reversed-phase mechanism with electronic

interactions playing a secondary role. The proposed methodology using ELSD provides an efficient chromatographic fingerprint for each crude mixture of monosubstituted sulfobutyl ether-β-cyclodextrin and allows rapid quantification of each isomer within a mixture. The isocratic conditions (aqueous ammonium acetate/acetonitrile) suggest an easy scale up to semi-preparative chromatography of synthetic mixtures.

The separation of two isomers of schumannificine and N-methylschumannificine, which are piperidino-type chromone alkaloids with anti-viral activity, was developed on PGC and compared with other chromatographic unsuccessful methods.[43] The two pairs of isomers could be separated although the interconvertion of the isomers in aqueous medium precluded the separation of each isomer for separate biological assay. A second type of alkaloids, also with pharmacological activity, tropane alkaloids from the stem-bark of *Schizanthus grahamii* (Solanaceae), were analysed by LC/MS with a capillary PGC column.[44] A rapid and simple LC method was developed for the separation of four tropane (hydroxytropane esters) isomers by the systematic investigation of LC conditions. At an elevated temperature of 60°C, an outstanding selectivity was obtained towards all alkaloids with the PGC column.

Two physiologically active steroid sulfate geometric isomers, equilin-3-sulfate and $\Delta^{8,9}$-dehydroestrone-3-sulfate, which differ structurally only in the location of an olefinic bond in a steroid ring, typically exhibit a single peak during analysis by reversed-phase HPLC on C_{18}- or other alkyl-bonded silica gel stationary phases. Partial separations were reported on a diphenyl phase with a resolution of 1.5, however, separation with resolution of 19 was achieved on a porous graphitic carbon column.[45] In 2003 Bohnstedt et al. described a convenient HPLC–tandem MS method for the determination of isoprostanes in human urine involving robotized liquid–liquid extraction and separation on a PGC column followed by tandem MS detection.[46] More recently they published a method for the separation and determination of all four classes of F2-isoprostanes in human cerebrospinal fluid (CSF).[47] This methodology involved the use of a 1×10mm PGC guard column functioning as a trap for the analytes which allowed the injection of a relatively large sample volume (300 μL) for improved detection limits, and a 1×150 mm PGC analytical column with detection by triple quadrupole mass spectrometry in negative-ion electrospray mode. Insight of isoprostane levels in CSF from various disease states where lipid peroxidation and oxidative stress are suspected can be gained by using this very selective methodology.

Other isomeric separations achieved on PGC include branched oligosaccharides produced by enzymatic degradation,[48] leucine, isoleucine, allo-isoleucine and hydroxy-proline[49] and the cis/trans isomeric related

substances of a non nucleoside reverse transcriptase inhibitor drug for the treatment of HIV infections.[50]

Diastereoisomers

The ability of PGC with its flat surface to discriminate between closely related compounds has also been utilized to separate diastereoisomers. Four diastereoisomers of a xylose derivative were separated on PGC using a mobile phase of water/methanol.[51] This method allowed for the collection of milligram quantities of each diastereoisomer to study their relative biological activity. In another publication the major diastereoisomeric oligomers of methylidene malonate were separated on PGC.[52] The PGC material was successfully employed in the quantitation of diastereomeric metabolites in plasma using crude acetonitrile precipitation extraction.[53] The column showed excellent resolution between the diastereomeric metabolites II and III, which could not be separated using silica-bonded phases, such as C_{18}, C_{30}, phenyl, phenyl ether, perfluorinated, polar embedded and polar end-capped phases. Excellent resolution was also achieved for another set of diastereomeric metabolites, VII and VIII. Good column ruggedness was reported as evidenced by the consistency of the retention times, peak symmetry, resolution and response during a period of more than 400 injections of plasma acetonitrile precipitated extract onto a single column. This work indicates that the PGC column could be an ideal choice for the separation and quantification of diastereomeric metabolites in biological samples.

Polar Compounds

The chromatographic analysis of very polar compounds is of interest in many biological, pharmaceutical and environmental studies but challenging due to the lack of retention in typical reversed-phase systems. Conventional reversed-phase columns do not provide enough retention and therefore elution occurs near or at the solvent front, with very low capacity factors. Addition of an ion pair reagent is necessary, or alternatively use of ion exchange chromatography or HILIC (hydrophilic interaction liquid chromatography). The mobile phases used in ion exchange and ion-pair RP are generally not compatible with detection techniques such as MS and ELSD. In spite of the recent popularity of HILIC, its interaction mechanisms are still not fully understood. The PREG allows for retention of very polar compounds on PGC using standard RP mobile phases.

The use of a PGC column was found to be efficient for achieving the separation of various mixtures of normal and modified nucleobases, nucleosides and nucleotides resulting from the enzymatic hydrolysis of photo-oxidized oligonucleotides, in the study of oxidative damage to DNA.[54] In 2004, Lofti developed a method for the simultaneous determination of hypoxanthine, xanthine, guanine and adenine in shellfish.[55] The author concluded that the isocratic elution method was simple, rapid and could be employed, as an alternative to existing methods, in sea food quality control. The resolution of mixtures of nucleosides and their mono-, di- and triphosphates was achieved using a PGC stationary phase, under conditions suitable for liquid chromatography/mass spectrometry (LC/MS).[56] Different organic mobile phases and modifiers were evaluated for the gradient elution separation of 16 nucleosides and nucleotides. The separation was attempted on silica-based columns designed for the retention of polar compounds but these could not provide suitable separation for accurate quantitation of mixed nucleosides and their phosphates. Figure 6 illustrates the separation of cyclic monophosphate nucleotides obtained in the authors' laboratory.

G. Remaud and co-workers developed a HPLC method for measuring uracil and dihydrouracil simultaneously in plasma.[57] These compounds were poorly retained on silica gel based columns even with fully aqueous conditions, whilst PGC provided good retention and separation. This method is now used in clinical practice to detect patients at

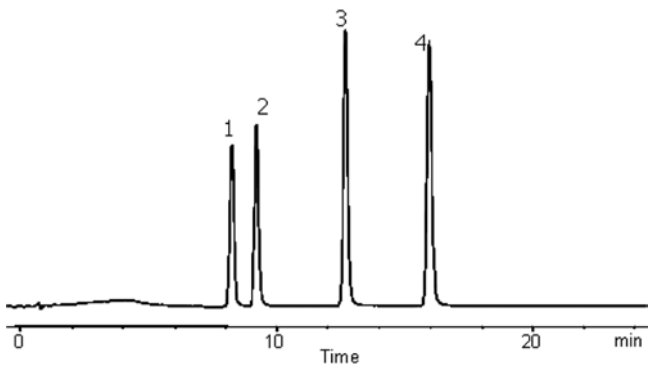

Figure 6. Separation of 3′,5′-cyclic monophosphates on PGC column. Experimental conditons: Column – Hypercarb 3 µm, 100 × 0.32 mm; Mobile phase – A: ammonium acetate 20 mM, pH 5.5, B: CAN; Gradient – 10 to 30%B in 15 min; Flow rate – 6 µL/min; Detection – UV at 254 nm. Analytes: 1. 3′,5′-cCMP; 2. 3′,5′-cUMP 3. 3′,5′-cGMP; 4. 3′,5′-cAMP.

risk of fluoropyrimidine toxicity and to individually adapt the dosage. The same authors reported a LC/MS/MS method for the analysis of Ftorafur (an oral pro-drug of 5-fluorouracil used in the treatment of digestive cancer), 5-fluorouracil and 5-fluoro-5,6-dihydrouracil in human plasma. This method can be applied to pharmacokinetic studies in patients treated with oral UFT.[58]

Amino Acids and Peptides

Underivatized amino acids have weak hydrophobic character which makes the retention of the more polar species difficult in RP-LC, unless an ion pair reagent is used.[59] Chaimbault and co-workers achieved the separation of twenty underivatized amino acids on a PGC column, using nonafluoropentanoic acid as an ion pair reagent and evaporative light scattering detection (ELSD).[60] They concluded that this chromatographic system had a faster equilibration time than the previous system based on a C_{18}-silica stationary phase. The elution order observed on the PGC column was different from that on a C_{18}-silica stationary phase, making the two chromatographic systems complementary for the identification of trace amino acids. Similar methodology was has been used for the separation of small peptides (di-, tri-, tetrapeptides) on a PGC column with ELSD.[61] Chaimbault et al. described a method for the simultaneous determination of the sulfur amino acids taurine, hypotaurine and thiotaurine in tissues of some marine invertebrates by LC/MS/MS.[62] The PGC column allowed this group to use isocratic conditions (with ammonium acetate 10 mM, pH 9.3) and negative electrospray detection for quantification of these metabolites in biological matrices in less than 10 minutes, without derivatization.

In 1997, Nèmeth-Kiss and co-workers studied the retention of fourteen peptides on PGC using acetonitrile–water mixtures as mobile phase.[63] They reported that retention decreased with increasing concentration of acetonitrile in the lower concentration range, reached a minimum and increased again with increasing concentration of acetonitrile in the higher concentration range. Calculations indicated that the PGC column shows mixed retention mechanisms for peptides in which hydrophobic, steric, and electronic forces are equally involved.

The analysis of small hydrophilic peptides such as mono-, di-, tri-, tetrapeptides or phosphopeptides in RP-LC can be problematic due to lack of retention, but retention can be achieved on PGC as illustrated on Figure 7. Desportes et al. reported the analysis of small peptides in wine using fractionation on a PGC stationary phase.[64] Chin and Papac used PGC for desalting flow-through fractions from a C_{18}-silica column, containing di-, tri-, tetra and penta-peptides, and the separation of phosphorylated from nonphosphorylated peptides.[65] Similar application

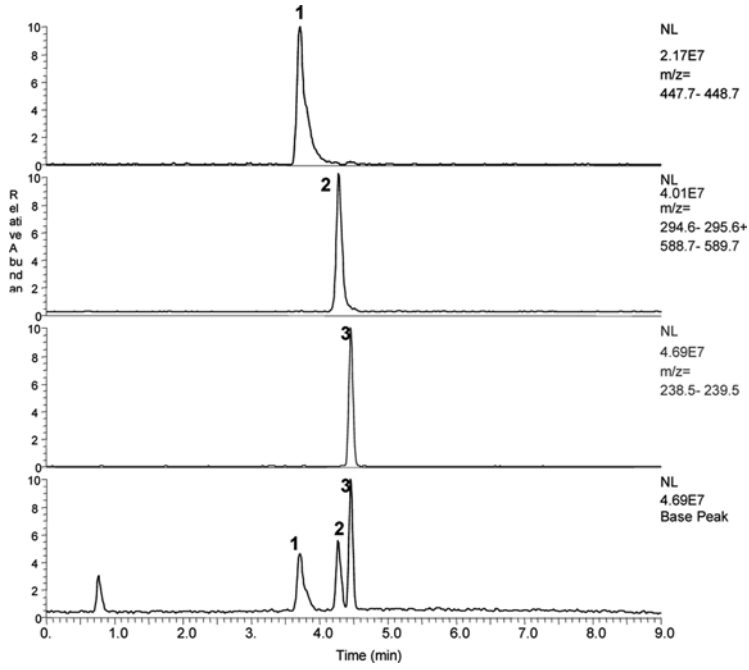

Figure 7. Retention of hydrophilic peptides on PGC. Experimental conditons: Column – Hypercarb 5 mm, 50×2.1 mm; Mobile phase: A – H_2O, + 0.1% formic acid; B – ACN + 0.1% formic acid; Gradient : 5 to 100% B in 10 min; Flow rate: 0.2 mL/min; Temperature: 30°C; Detection: + ESI. Analytes: 1. Arg-Gly-Glu-Ser (RGES); 2. Asp-Ser-Asp-Pro-Arg (DSDPR); 3. Gly-Tyr (GY).

of the PGC stationary phase for the retention and resolution of phosphopeptides eluted in the flow-through fractions from a C_{18}-silica column along with non-volatile salts and buffers was reported by Vacratsis et al.[66]

Carbohydrates and Sugars

Carbohydrate separations are challenging because of the great diversity of structure and water solubility which makes retention on bonded-silica and polymeric phases difficult. PGC has the advantage that the PREG can be exploited to retain and separate them and its flat surface allows for differentiation of closely related structures. In 1996 Koizumi[67] published a review on the HPLC analysis of carbohydrates using graphitized carbon columns, more specifically mono- and disaccharides, cyclodextrins, oligosaccharide alditols, N-linked oligosaccharides, chitooligosaccharides and glycopeptides. Their chromatographic behaviour

was studied and it became apparent that the elution patterns are based on the size and the planarity of the molecule, namely position and configuration of linkage.

Recently Antonio et al., reported a LC-ESI-MS/MS method for the sensitive targeted analysis of key glycolytic intermediates, sugars and sugar phosphates from plants, using a PGC stationary phase and an MS compatible mobile phase.[68] The method performance was demonstrated for the analysis of biological samples by applying it to the simultaneous quantitation of changes in soluble sugars and sugar phosphates in A. thaliana Columbia-0 and its starchless phosphoglucomutase mutant over a 12-h light/12-h dark growth cycle. Begona et al., reported a method for the identification and quantitation of sulphated disaccharides derived from chondroitin sulphate and dermatan sulphate chains attached to proteoglycans. After digestion with Chondroitinase ABC, the pool of disaccharides was separated by liquid chromatography on a PGC column and identified by on-line electrospray mass spectrometry under negative ionization conditions.[69] Karlsson et al., developed a LC/MS method with negative electrospray and a capillary PGC column for the analysis of enzymatically digested glycosaminoglycans.[70] This separation and identification method was used to successfully analyse digests of keratan sulphate (keratanase) and heparin (heparinase) standards, and hyaluronic acid (hyaluronidase) from synovial fluid samples. A liquid chromatography–tandem mass spectrometry method for simultaneous analysis of acrylamide and the precursors, asparagine, fructose, glucose and sucrose in bread was developed by Nielsen and co-workers, using the ability of PGC to retain very polar analytes.[71]

PGC has been extensively used in the analysis of oligosaccharides. Typical applications include the desalting / purification[72,73] and the profiling of these compounds released from glycoproteins using LC/MS analysis.[74–82] The desalting of oligosaccharides is necessary for the success of differential chemical, separation and spectroscopic techniques used for the preparation, isolation and analysis of the glycan component of glycoconjugates. PGC has been used for the purification of oligosaccharides from solutions containing salts, detergents, proteins, and reagents used for the release of the oligosaccharides from glycoconjugates, with complete recoveries reported.[72] LC–MS with a PGC column was found to be successful for rapid, sensitive, and simultaneous analysis of high-mannose-type, desialylated fucosyl complex-type, sialylated complex-type, and sialylated fucosyl complex-type oligosaccharide alditols.[74] The authors of these work also demonstrated that the method could be used to characterize high-mannose-type, hybrid-type, and complex-type oligosaccharides in tissue plasminogen activator produced from human melanoma cells in a single analysis.

Carrageenans are a class of sulphated polysaccharides extracted from red seaweeds. PGC has advantages over ion exchange chromatography for the separation of oligomers of carrageenans since the simultaneous hydrophobic and electronic interaction mechanisms enable better regulation of the resolution between peaks, especially with the use of an organic eluent modifier.[83,84] Figure 8 illustrates a separation of oligosachcarides of κ-carrageenan. The liquid chromatography–electrospray ionisation mass spectrometry analysis of enzymatically digested oligosaccharides of κ-carrageenan with a PGC column has been reported.[85] The advantage of this method is that the mobile phase (ammonium hydrogencarbonate, MeCN) used for the separation permits at the same time their MS detection as fully deprotonated ions (valid for oligosaccharides with more than six sulphate groups) which gives information concerning the position of the sulphate group on a selected oligosaccharide. According to the authors of this study this is an advantage compared with ion-pair LC/ESI–MS where the sulphate position is based on assumptions that cannot always be verified.

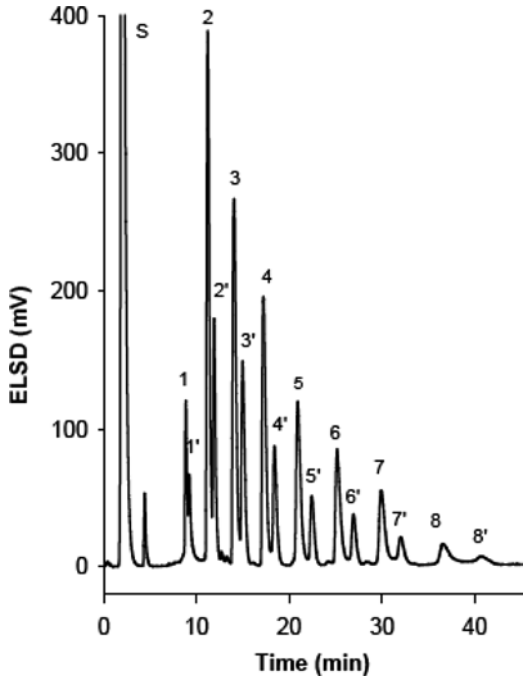

Figure 8. Separation of oligomers of k-carrageenan on PGC column. Experimental conditons: Mobile phase: A-water, B-ammonium acetate 700 mM, C-15% CAN; Gradient: 0–45 min, 0–70% of B. Flow-rate: 0.2 mL/min; S designates salt. *Source*: Pending permission from journal of chromatography, Ref. [83].

The retention behaviour of cyclodextrins on PGC has been studied and compared to the retention on silica-C_{18} phases.[86,87] It was interpreted as a balance between mobile phase-cyclodextrin solute interactions and London dispersion forces involved in PGC-cyclodextrin interactions.[86]

Other Polars

PGC has been used in many applications for the retention of polar species of biochemical interest such as catecholamines,[88,89,90] neurotransmitters,[91] oxidized glutathione and selenium-containing glutathione S-conjugates,[92,93] photooxidized oligonucleotides[94] and urinary creatine/creatinine (review).[95] In pharmaceutical analysis of polars PGC has been used for clopidogrel and its metabolite,[96] acarbose and its metabolite,[97] intracellular metabolites in Escherichia coli K12,[98] 5′-monophosphate cytosine arabinoside in cell extracts[99] (Figure 9), cytarabine in mouse plasma,[100] cimetidine and related compounds,[101] EDTA and impurities,[102] oxaliplatin in blood,[103] and levetiracetam in human

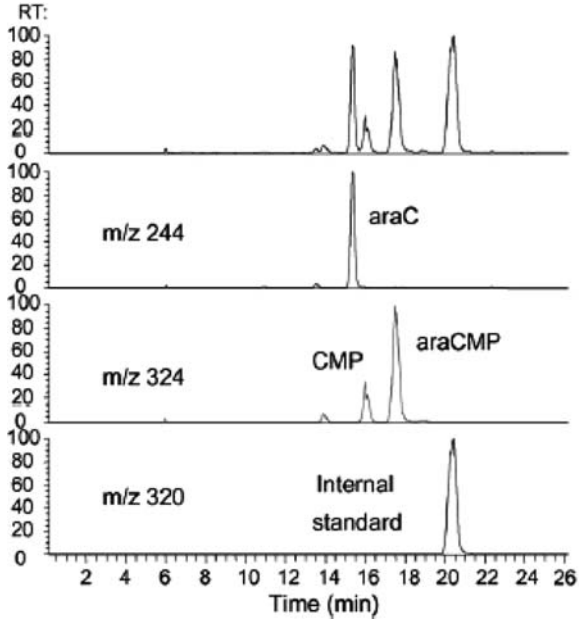

Figure 9. TIC and SIM chromatograms from cell extracts spiked with 5′-monophosphate cytosine arabinoside (Ara-CMP), AraC and internal standard. *Source*: Pending permission from analytical chimica acta, Ref. [99].

plasma.[104] Another application area where very polar compounds need to be analysed is food safety; examples of the use of PGC include methylamines in fish,[105] polar phenolics in olive oil,[106] acrylamide in cooked foods and bakery products,[107,108] and acromelic acid A in poisonous mushrooms.[109] In environmental analysis aniline in fresh water,[110] glyphosate and aminomethyl phosphonic acid[111] and cyanuric acid in swimming pool waters[112] are examples of applications where PGC allowed determination at trace levels. The analysis of non-ionic surfactants can be very challenging due to the polarity of these molecules and also the complexity of such mixtures caused by different degrees of polymerization and branching of the chains. The PREG and stereoselectivity of PGC has been utilized to assist in studies of physicochemical properties and environmental impact of non-ionic surfactants: alkylglycosides detergents,[113] polyethoxylated alcohols,[114] industrial oligoglycerols,[115] oligomers of nonylphenyl ethylene[116] and polyglycerol fatty esters and fatty ethers.[117] PGC has also been used to analyse naturally occurring products such as cyanoglycosides[118] and glucosinolates,[119] chemical warfare agents such as phosphonic acids (Figure 10)[120] and other polar species such as guanidino compounds,[121,122] and diphosphine-bridged complexes.[123]

Charged Solutes

In the late eighties, early nineties, Lim et al., achieved some interesting separations of inorganic ions and complex ions on PGC.[124–126] They showed that TcO_4^- and RhO_4^- ions (pertechnetate and perrhenate ions) could be retained and separated by PGC using aqueous TFA as the eluent,[124] and that organometallic-charged complexes of technetium and rhenium could also be separated using TFA solutions containing 2–10% acetonitrile.[125] Lim coined the term "electronic interaction chromatography" for this type of separation through an electron-transfer mechanism with the graphite surface. Elfakir and co-workers demonstrated the separation of inorganic anions (hydrogenophosphate, sulphate, nitrate, perclorate) with a volatile electronic interaction additive (formic, acetic, or perfluoro-carboxylic acid) in the aqueous mobile phase.[127,128] Takeuchi et al. used sodium sulphate in the mobile phase to retain and separate iodate, bromide, nitrite, bromate, nitrate and iodide on a PGC column.[129] Cations such as copper (II) and copper (III) can also be retained on PGC as demonstrated by Merly et al.[130]

Ion interaction chromatography with tetrabutylammonium hydroxide has also been used to separate inorganic ions on PGC.[131] The same authors dynamically coated the surface of graphite with cetyltrimethylammonium (CTA) ions for the separation of seven common anions in

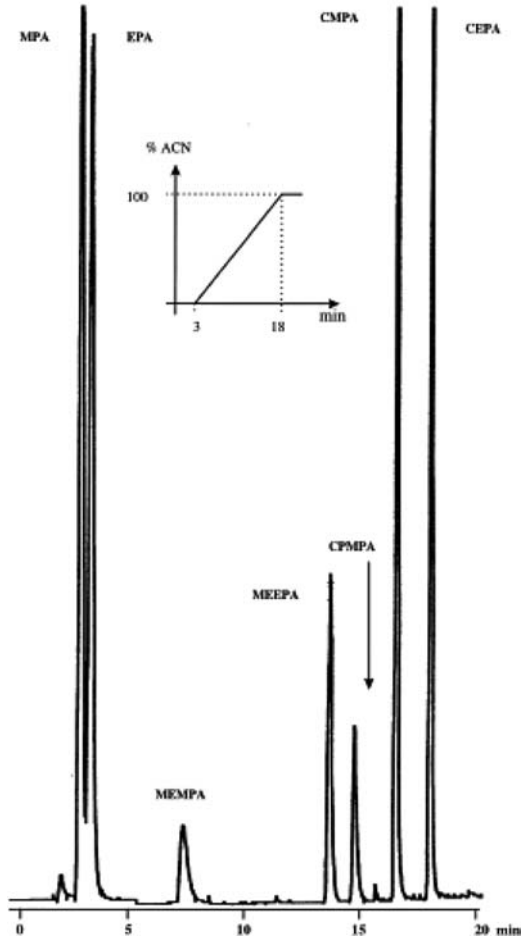

Figure 10. LC–ELSD separation of phosphonic acids on PGC column. Experimental conditions: Column – Hypercarb,150 × 2.1 mm I.D., 7 μm; Mobile phase gradient: 0–3 min, 0.1% (v/v) trifluoroacetic acid in water; 3–18 min, 0.1% (v/v) trifluoroacetic acid in water to 0.1% (v/v) trifluoroacetic acid in CAN; Flow rate: 0.2 mL/min. Detection: ELSD. *Source*: Pending permission from analytical chimica acta, Ref. [120].

water: F^-, Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{2-}, SO_4^{2-}.[132] Using CTA-bromide as the coating agent, a permanently coated ion-exchange column was obtained which allowed separations of the anions without any coating agent in the mobile phase. A separation obtained in the authors' lab under similar conditions is illustrated in Figure 11.

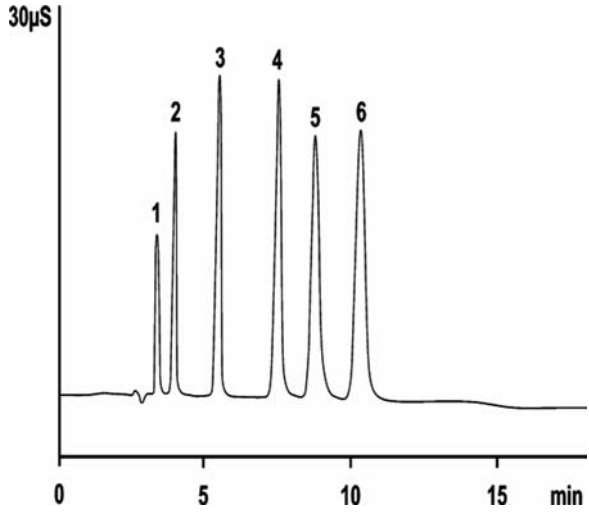

Figure 11. Separation of inorganic anions on a cetyltrimethylammonium bromide dynamically coated PGC column. Experimental conditons: Column–Hypercarb 5 μm, 100 × 4.6 mm; Mobile phase: 2 mM Na_2CO_3/1 mM $NaHCO_3$ + 2.5%ACN; Flow rate: 1.2 mL/min; Detection: suppressed conductivity. Analytes: 1. Fluoride, 2. Chloride, 3. Bromide, 4. Nitrate, 5. Phosphate, 6. Sulphate.

Bipyridylium herbicides (diquat, paraquat and difenzoquat) have been analysed in water down to levels lower than 0.1 μg/L with a mobile phase solution of tetramethylammonium hydroxide, ammonium sulphate and methanol, on a PGC column.[133] PGC was also used for solid phase extraction of the analytes from water. The PGC column was found to give a low probability of false positives for bypiridylium herbicides. A simplified separation method obtained by the author is illustrated in Figure 12, where the TFA in the mobile phase behaves as an electronic modifier which competes with the analytes to the surface of the graphite, forcing elution.

Miscellaneous

PGC has been used in many other applications for its ability to separate solutes with very similar structures or for providing alternative and complementary selectivity to other typical reversed-phase column packings. The applications reviewed in this section do not necessarily fit into the categories reviewed above.

Non-aqueous liquid chromatography with a PGC column has been used for the analysis lipids such as wax esters, ceramides and fatty-acid

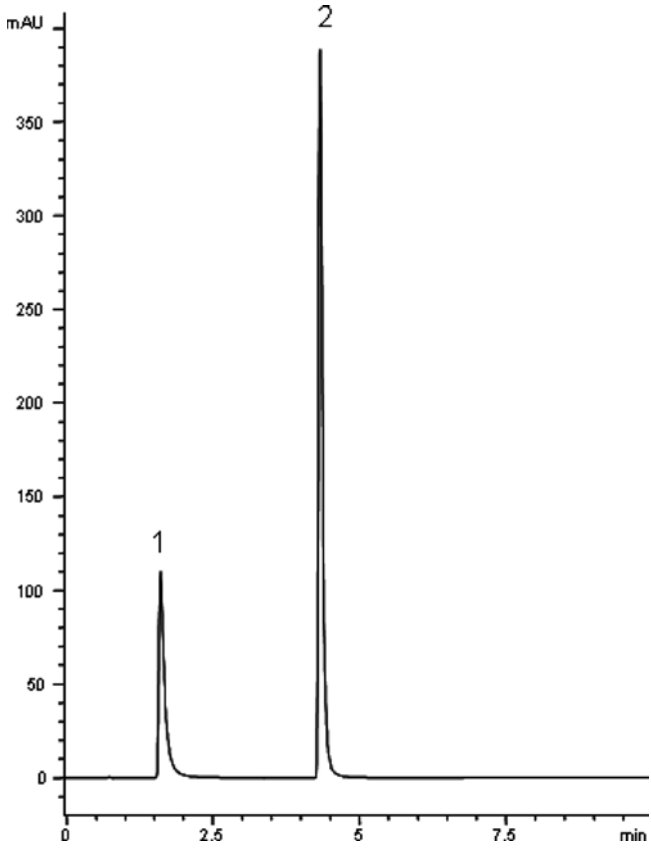

Figure 12. Separation of diquat and paraquat. Experimental conditons: Column – Hypercarb 5 mm, 50×4.0 mm; Mobile phase: A – H_2O, +0.05% TFA; B –ACN + 0.05%TFA; Gradient : 5 to 35% B in 10 min; Flow rate: 0.8 mL/min; min; Detection: UV at 295 nm, 0 to 3 min, 245 , 3 to 10 min. Analytes: 1. Diquat; 2. Paraquat.

methyl esters.[134] The study of the mechanism of interaction of ceramides with PGC revealed that there is a linear relationship between retention and the structure of ceramides.[135,136] A two-dimensional system comprising a polyvinyl alcohol-silica column to collect ceramide fractions on the first dimension and a PGC column to separate the molecular species on the second dimension was successfully used to analyse stratum corneum ceramides.[137] PGC has been compared with octadecyl bonded phases for the analysis of fatty acid methyl esters.[138] Retention is greater on PGC and greater selectivity is observed for high number of double bonds. An additional advantage of PGC is the separation of

hexadecatrienoic and hexadecadienoic acids.[138,139] For polyunsaturated fatty acids methyl esters the isomers differing only in the position of the carbon double bond on the alkyl chain can be separated on PGC.[140] A liquid chromatography on porous graphitic carbon with atmospheric pressure photo-ionization tandem mass spectrometry method was developed for the analysis of glycosphingolipids.[141] The study of several structural variations (the length, the degree of unsaturation and hydroxylation of the alkyl chains, the number and nature of osidic residues) helped understand the behaviour of neutral glycosphingolipids. Other studies of naturally occurring compounds on PGC include the profiling of wheat digalactosyldiacylglycerol molecular species,[142] triterpenic acids (betulinic, ursolic, oleanolic, 18α- and 18β-glycyrrhetinic acids),[143] the anticancer drug taxol,[144,145] complex mixtures of non-flavonoid polyphenols in a two-dimensional system with a C_{18} column,[146] triacylglycerols[147] and structurally similar hydroxamate siderophores (ferrichrome and ferricrocin) in podzolic forest soils.[148]

Pharmaceutical methodology developed on PGC columns include the analysis of morphine-based opiates and its glucuronide and sulphate conjugates,[149] metoprolol and relates substances,[150] paracetamol and related substances,[151] naproxen and related substances,[152] haloperidol and degradation products,[153] non-nucleoside reverse transcriptase inhibitors and degradation products,[154] 11 steroidal drugs,[155] cyclosporins A and U in liposomes[156] and tetracycline antibiotics.[157]

PGC has also been used in clinical diagnostics applications; examples are the bio-monitoring of phthalate metabolites (monoethyl phthalate, monobutyl phthalate, monobenzyl phthalate, and monoethylhexyl phthalate) in human urine,[158] determination of boron-containing compounds in urine and plasma from boron neutron capture therapy patients,[159] and the determination of a flame retardant (triphenyl phosphate)hydrolysis products in human urine.[160]

Other application areas where the selectivity of PGC has proved useful are environmental, food safety analysis, and the analysis of explosives. PGC has been used for the selective determination of benzo[α]pyrene in soil at ppb levels,[161] the fractionation of polychlorinated biphenyl (PCBs) congeneres into classes as a preliminary step prior to GC analysis,[162–164] and it features in methodology for the determination of halogenated contaminants and polycyclic aromatic hydrocarbons reviewed in reference.[165] The simultaneous separation, identification, quantification and confirmation of betamethasone and dexamethasone in equine plasma,[166] and the determination of residues of these two drugs in bovine liver[167] both methods using liquid chromatography/tandem mass spectrometry with PGC columns have been reported. PGC has also been used for the LC/MS analysis of glucocorticoids in bovine liver[168] and cattle tissue,[168] and corticosteroids in bovine liver.[170] A method to

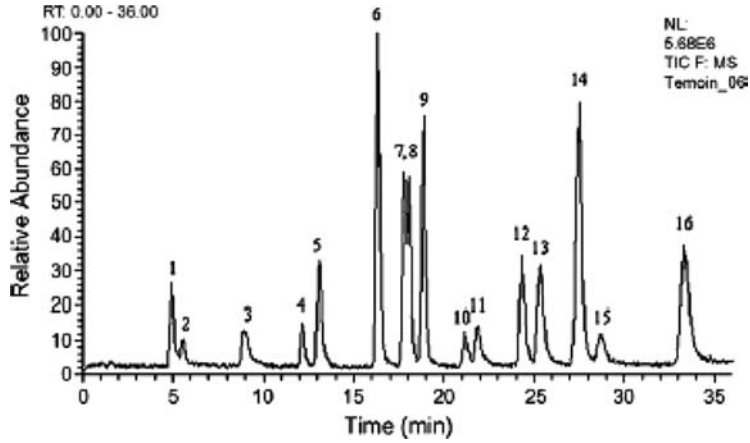

Figure 13. Total ion chromatogram from LC/APCI-MS analysis of 10 ng/μl standard mixture (1 = RDX; 2 = NG; 3 = HMX; 4 = PETN; 5 = 1,2-DNB; 6 = Tetryl; 7 = 2,6-DNT; 8 = 3,4-DNT; 9 = 2,3-DNT; 10 = 1,3-DNB; 11 = 1,4-DNB; 12 = 2,5-DNT; 13 = 2,4-DNT; 14 = TNT; 15 = 3,5-DNT; 16 = TNB). *Source*: Pending permission from journal of chromatograph, Ref. [177].

monitor the pesticide fenbutin oxide in tomatoes, cucumbers and bananas by LC/APCI/MS with a PGC column reported limits of detection from 0.06–0.12 ng/L (equivalent to 0.01–0.02 mg/kg in the crop).[171] Finally, Crescenzi and co-workers have demonstrated the ability of PGC in separating structurally similar compounds such as nitroaromatic and organic explosives,[172–176] and the use of PGC for the analysis of nitrate ester, nitramine and nitroaromatic explosives and by-products by LC/APCI/MS has also been reported recently.[177] The method reported in reference 177 was for sixteen analytes including the most common organic explosives encountered in forensic investigations and showed the separation of DNTs and DNBs isomers (Figure 13).

CONCLUSION

PGC is a crystalline and highly reproducible material which meets all the conventional operating criteria of a chromatographic support in terms of surface homogeneity, pore size, surface area, and particle size distribution. At the molecular level, PGC is made up of sheets of hexagonally arranged carbon atoms arranged as in a large polynuclear aromatic hydrocarbon. It is generally accepted that retention on graphite from aqueous/organic eluents is determined by the balance of two factors: (1) hydrophobicity, which is primarily a solution effect that tends to drive

analytes out of solution and (2) the interaction of polarizable or polarized groups in the analyte with the graphite, additional to the normal dispersive interactions. The strength of interaction depends on both the molecular area of an analyte (and, therefore, shape of the analyte) in contact with the graphite surface and upon the nature and type of functional groups at the point of interaction with the flat graphite surface. PGC shows high stereoselectivity, which is attributed to its flat, unmodified surface, allows the resolution of isomers (including diastereoisomers) and structurally related compounds. The polarizability of the graphite surface makes PGC well suited for the retention and resolution of very polar and ionized solutes, which are difficult to retain in typical reversed-phase LC. Its chemical stability facilitates the use of extremes of pH and temperature. PGC's strength is in solving problematic LC separations, and this has been illustrated by the variety of applications reviewed in this paper.

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