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Porous graphitic carbon sorbents in biomedical and environmental applications

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Abstract This review is written as a privilege of the work of Professor Mietek Jaroniec on surface phenomena, adsorption, chromatographic separations, chemistry of conventional and ordered nanoporous materials.

The problems of the porous graphitic carbon (PGC) application in analytical field are presented. Special attention is paid on possibilities of use PGC as a specific sorbent and packing material for selective isolation and analytes enrichment from complex matrices by means of liquid-solid extraction techniques (SPE, SPME, MSPD) and as particular stationary phase in analytical chromatographic columns. Surface and adsorption properties as well as a unique mechanism of retention on porous graphitized carbon sorbents are described. As supplement the examples of application in biomedical and environmental area are added.

Keywords Porous graphitic carbon · HPLC · Stationary phase · Environmental and biomedical application

1 Introduction

For the solution of analytical problems in trace analysis, particularly in the analysis of multicomponent mixtures, various preconcentration and/or preseparation steps have been utilized dependent on the sample matrix. The application of carbon materials for these purposes is related with the development of sorbents with (a) physicochemical properties, and (b) adsorption-desorption properties which provide effective sample enrichment. Carbon sorbents have been applied in various enrichment techniques, particularly in solid-phase extraction (SPE), the purge-and-trap technique and headspace analysis. Chemical separations and chemistry of materials with a special emphasis placed on physical adsorption at activated carbons are also described (Choma et al. 1990, 1999; Jaroniec and Choma 1986, 1987; Kruk et al. 1996; Li et al. 1998).

In environmental samples the target of the qualitative and quantitative analysis may be the complete multicomponent trace analysis in various matrices, or only the analysis of individual trace component(s). The whole preconcentration/preseparation procedure differs accordingly. The need for enrichment of trace analytes generally depends on a combination of:

- the volume injected for the analysis,
- the nature and concentration of the compounds analyzed,
- the nature of the sample matrix,
- the minimum detectable amount characteristic for the selected sample introduction, separation and detection system.

When analyzing e.g. endogenous compounds in biological tissues there is a need for a separation technique by which the analytes of interest can be separated from each other and from the sample matrix. One of the most important separation techniques used today is liquid chromatography (LC). To succeed with a liquid chromatographic separation, it is important to both choose the right column (i.e. to choose an appropriate stationary phase) and the right mobile phase. The combination of a relatively non-polar stationary phase,

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as octadecylsilane (ODS, i.e. C18 on silica particles), and a relatively polar mobile phase, e.g. a mixture of methanol and water, represents today the most common liquid chromatographic mode. Although ODS enables a high separation performance of a majority of chemical analytical samples, the stationary phase has limitations for which powerful alternative separation media are needed. In addition, the silica based stationary phases (including ODS) commonly restricts the choice of mobile phases to within the pH range of 2–8 due to limited pH stability of the bulk silica particles. If, for same reasons, there are restrictions on the use of ion-pairing agents in the mobile phase, or if there is a desire to use high contents of organic modifiers, it will become difficult to retain and thereby separate small polar analytes on these columns. When LC is coupled to the selective and often sensitive mass spectrometric detector, it is well known that these restrictions and desires are incompatible. Volatile mobile phase additives and eluents of relatively high content of organic modifier are then preferred, and thereby difficulties arise with insufficient retention when analyzing polar compounds on e.g. ODS separation media.

Porous graphitic carbon (PGC) is a stationary phase with interesting properties that was developed during the 1980's. It has been adopted for several applications, including separation of polar analytes and closely related substances, where e.g. ODS materials are a less successful alternative. The choice of pH is not restricted since PGC offers stability over the entire pH range. Moreover, PGC makes it possible to use relatively high contents of organic modifiers and still retain even very polar analytes. The flat layered surface characteristics of PGC have found to be advantageous for separation of closely related substances. Due to the hydrophobicity of this two-dimensionally ordered surface, PGC may also be successfully used as a support for a dynamically adsorbed selector for chiral separations. More detailed descriptions of the carbonaceous materials in general are available in a review articles previously and recently appeared in the literature (Jankowska et al. 1991; Buszewski and Michel 2008; Ligor et al. 1998).

2 Porous graphitic carbon (PGC) as packing material in separation technology

Carbon has historically proven to be a difficult stationary phase to develop for liquid chromatography. Different procedures for preparation of the packing material have been described (Knox and Kaur 1989; Knox and Ross 1997; Knox et al. 1983; Leboda et al. 1998) but problems with fragility, retention capacity and poor mass transfer have been encountered.

In principle four consecutive stages may be distinguished in the formation of carbons from naturally occurring or synthetic precursors: homogenization, carbonization, volatilization of inorganic impurities, and graphitization (Knox et al. 1983; Mattson and Mark 1971).

The term "homogenization" covers all operations which lead to an improved ordering of the structure of any solid or liquid carbonaceous starting material. It usually consists of a thermal treatment of the starting material at 450–700 °C in an inert atmosphere. It is well known that the degree of structural order of the carbon precursor essentially determines the extent to which the penultimate material is converted into a graphitic or an amorphous carbon, which represent the two limiting cases.

Carbonization covers a number of processes including cooking, charring and reaction with oxidizing gases such as oxygen, carbon dioxide and water vapor. It is carried out between 700 and 1200 °C. Carbonization increases the percentage of carbon content and introduces pores. The products so formed are termed active carbons and possess a high adsorptive capacity. Carbonization also covers processes whereby a gaseous hydrocarbon is pyrolyzed between 1000 and 1700 °C to yield dense non-porous layers of pyrolytic carbon (Bokros 1969).

Active carbons may still contain inorganic impurities such as sulphur and silica depending upon their origin. These can be removed by volatilization at 1200–1700 °C. This process leaves a large number of defect sites in the structure and causes a disordering of the mutual arrangement of layers. Microscopic holes may even be formed within the particles.

Graphitization covers the subsequent heat treatment in an inert atmosphere at 1700–3000 °C. Such heat treatment brings about densification with concurrent removal of structural defects, and forms a three-dimensionally ordered graphitic structure. The degree of graphitization of any carbon brought about by high temperature treatment depends strongly on its initial structure. Thus treatment of some active carbons at a temperature as low as 1200 °C can greatly reduce or even completely eliminate the porosity of the material, whereas some glassy carbons may not convert to graphite even on heating to 3000 °C.

In 1982, however, Knox and co-workers published a method for making a mesoporous glassy carbon material (i.e. a structure with a pore size of a few nanometers (nm) to tens of nm's) with the required physical and chemical stability Gilbert et al. 1982). They used silica gel as a template and when the mesoporous glassy carbon product was further graphitized using temperatures above 2000 °C a crystalline product without micropores (with a pore size less than a few nm) was obtained. This procedure resulted in a breakthrough for the production of porous graphitic carbon material (of 7 μm particles) for liquid chromatography. In 1988, the PGC manufacturing process developed at the Wolfson Unit in Edinburgh University, UK, was transferred

to Thermo Hypersil-Keystone (formerly Hypersil and Shandon, Runcorn, Cheshire, OK). The company further optimized the medium's performance by introducing a 5 μm particle diameter material in 1994.

Today, PGC (Hypercarb™) is a complementary stationary phase to e.g. octadecylsilane (ODS, i.e. C18), and it has found use in a diverse range of applications (Ross 2000; Ross and Knox 1997; Bassler and Hartwick 1989; Forgacs 2002) e.g. for the separation of closely related substances (Tanaka et al. 1991; Knox et al. 1986; Kriz et al. 1994; Wall et al. 1995, 1996) and separation of polar analytes (Tanaka et al. 1991; Lim 1989; Lim 1989; Gu and Lim 1990; Hennion et al. 1995; Elfakir et al. 1998; Forgacs et al. 1992; Mercier et al. 1999; Takeuchi et al. 2000; Michel et al. 2006).

3 Characteristics of PGC

The PGC material is manufactured by impregnating a high porosity LC silica gel (to provide the desired pore size) with a phenol-formaldehyde resin. The material is carbonized at 1000 °C in nitrogen and the silica is dissolved in alkali. Finally, it is heated to above 2000 °C to get the material graphitized. The resulting porosity of the PGC material is approximately 75% and the specific surface area is about 120 m^2/g (Hypersil guide 2009) (Table 1).

PGC behaves as a strong reversed-phase stationary phase, even stronger than C18 silica phases (Tanaka et al. 1991; Knox et al. 1986; Kriz et al. 1994) which represents the most hydrophobic of the commonly used alkyl substituted silica phases. The hydrophobic property of PGC bas been investigated by *inter alia* Tanaka et al. (1991) who compared the retention of mono substituted alkanes on PGC and C18 silicas. With an increased number of methyl groups to the alkane molecule they found that the retention on PGC increased more than on C18. Their conclusion was thus that PGC is the strongest retarding stationary phase of the two. Hence, stronger mobile phases with higher concentrations of an organic modifier are normally required to elute the solutes from PGC than from C18.

Table 1 Physical specifications of PGC

Particle sizes (μm)	5, 7, 30
Pore size (Å)	250
Pore volume (cc/g)	0.7
Surface area (m^2/g)	120
Mechanical strength (bar)	>400
% carbon	100

The surface of PGC is crystalline and is composed of flat sheets of hexagonally arranged carbon atoms (Knox et al. 1986). The surface of PGC differs from the brush type surface associated with C18 silicas, which is illustrated by a simple schematics in Fig. 1. The flat rigid surface of PGC and the fact that it is highly adsorptive allows for unique stereoselectivity of solutes (Tanaka et al. 1991; Knox et al. 1986; Leboda and Sokołowski 1977). The strength of interaction depends on the molecular area of the solute in contact with the surface of PGC. Kriz and co-workers (Kriz et al. 1994) investigated the retention characteristics of aromatic hydrocarbons and found that PGC showed higher selectivity for methyl substituted benzenes than C18 bonded silica.

PGC is extremely unreactive. Its high chemical stability over the full pH range allows separation with strongly acidic (Wall et al. 1995, 1996; Emery and Lim 1989; Lim 1989; Gu and Lim 1990) and basic (Merly et al. 1998; Stefansson and Lu 1993) mobile phases. Gu and Lim (1990) separated pertechnetate anion and cationic technetium-amine complexes with a mobile phase containing 1% trifluoroacetic acid. Barrett and co-workers (Barrett et al. 1998) studied the retention behavior of morphine and its metabolites at pH 2–12.

The retention mechanism of PGC is different from that observed of reversed-phase silicas. Apart from hydrophobic interaction, also polar interaction can dominate the analyte-stationary phase interaction. Some groups have observed an increase in retention on PGC with an increased number of polar groups to the analyte (Tanaka et al. 1991; Hennion et al. 1995; Möckel et al. 1991). The presence of underivatized silanol groups on alkyl bonded silica phases may also cause polar interactions e.g. for amines. However, these interactions are much less significant than on PGC. Retention of polar substances on PGC will be discussed in more detail below.

As can be seen in Fig. 1, the PGC surface consists of sheets in a surface architecture that is very different from that of e.g. ODS materials. The regular and two-dimensionally ordered surface of PGC (Fig. 2) is composed

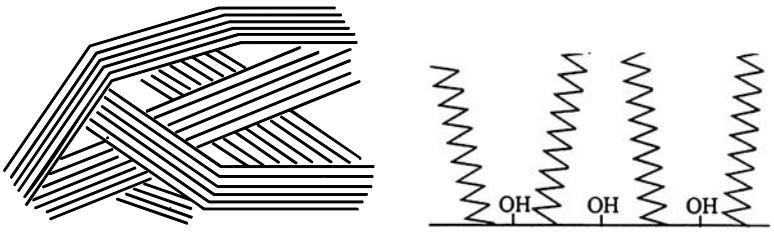

Fig. 1 Surface of PGC and C18 silica

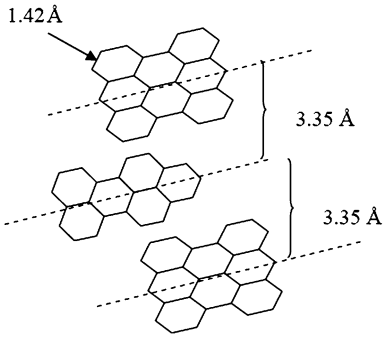

Fig. 2 The crystal Structure of PGC, showing the two-dimensional graphite arrangement (Knox et al. 1986)

of flat sheets of hexagonally arranged carbon atoms showing sp^2 hybridization (Gilbert et al. 1982; Knox et al. 1986). Within the sheets, the carbon atoms are vacancy satisfied and the surface is homogeneous. However, there are undoubtedly carbon atoms at the edges of the graphite sheets which must have vacancy satisfying functional groups attached to them such as hydroxyl, carbonyl, carboxylic or amino functions (Knox and Ross 1997; Tanaka et al. 1991; Hypersil guide 2009).

In Fig. 2, the distances between the carbon atoms and between the carbon layers are shown. The spacing of the carbon atoms within the sheets is very close to that in large polycyclic aromatic molecules and the individual graphite sheets might be regarded as gigantic aromatic molecules composed of almost entirely carbon.

3.1 Retention behavior of polar substances

Bassler et al. (1989) were the first who show that the retention behavior on PGC was different from ordinary reversed-phase materials. They studied the retention of substituted aromatic molecules of different polarity using heptane as eluent. It was found that the retention increased with polarity contrary to what has been observed on other reversed-phase materials. They explained the dominant retention mechanism as being an electron-pair donor-acceptor interaction in contrast to the dispersive interactions normally obtained with reversed-phase materials. The retention of polar analytes has been studied by others (Emery and Lim 1989; Lim 1989; Gu and Lim 1990; Hennion et al. 1995; Mercier et al. 1999; Forgacs and Cserhati 1992; Elfakir and Dreux 1996) and has been described as the "polar retention effect on graphite" or PREG by Knox and Ross (1997). In a recent paper, Ross (2000) suggested that the retention mechanism of polar analytes on PGC is a charged-induced interaction of the polar analyte with the polarizable surface of graphite.

"The polarizable properties of the graphite surface clearly hold the key to understanding the mechanism by which polar molecules are retained at the surface… However, full molecular modelling calculations are necessary to explain the polar retention effect on graphite."

It has, however, been concluded that PREG cannot be caused by simple electrostatic interaction in which one regards PGC as a conductor (Knox and Kaur 1989) and that the PREG involves energies as high as 42 kJ/mole (Ross 2000). The latter value can be compared with the energy of molecular interactions usually associated with reversed-phase LC, e.g. van der Waals interactions of approximately 5 kJ/mole (Knox et al. 1983).

3.2 Retention of non-polar analytes and geometric isomers

The strength of interaction between a hydrophobic analyte molecule and the PGC surface largely depends on how well the molecule fit onto the flat graphite surface (Ross 2000). PGC has been found to be particularly selective with respect to geometrical isomers and closely related substances (Tanaka et al. 1991; Knox et al. 1986; Kriz et al. 1994; Wall et al. 1995, 1996; Elfakir and Lafosse 1997). It has been concluded that highly structured and rigid molecules generally will be less retained on PGC than flexible molecules of the same molecular weight (Ross and Knox 1997).

The retention of homologous series of hydrophobic species (e.g. alkylbenzenes) has been studied on PGC by many researchers, e.g. Kriz et al. (1994) and Möckel et al. (1991). It was found that non-polar analytes were strongly retained on PGC. Compared to ODS materials, more organic modifier was required to elute a solute from a PGC column. In general, the authors found that PGC showed a linear relationship between $\log_{10} k'$ and carbon number, in accordance with (1) below:

$$\log_{10} k' = \log_{10} k'_0 + \gamma n \tag{1}$$

where k' is the retention factor, $\log_{10} k'_0$ is the theoretical $\log_{10} k'$ for benzene, n is the number of carbons and γ is the expected linear gradient which hence is a measure of how selective the material is with respect to addition of e.g. methylene groups. PGC has been found to yield greater discrimination for methylene addition and methyl substitution than ODS, silica and alumina. Kriz et al. (1994) have reported y values of 0.22–0.25 for addition of a methylene group to a carbon chain and 0.46 for addition of a methyl group into an *ortho* position of the benzene ring. The corresponding values for the comparable ODS stationary phase were reported to be 0.17 in both cases.

3.3 PGC and mass spectrometric detection

Using PGC as stationary phase often enables separations of small and polar analytes with a high content of organic modifier and with mobile phases containing no ion-pairing agents. Additionally, the chemical stability of PGC allows

for a free choice of mobile phase pH. These degrees of freedom given to the choice of mobile phase conditions should greatly simplify the coupling to mass spectrometric detection (Ehrsson et al. 1995). Several researchers (Voyksner 1997; Puig et al. 1996) coupled PGC columns to mass spectrometric (MS) detection. However, as PGC is a conducting stationary phase, it is not recommended to connect the packed capillary column directly to a high voltage electrospray ionization interface. When using this type of interface, it is highly recommended to insert a transfer-line composed of e.g. fused silica with small internal diameter (e.g. 50 μm i.d.) between the column and the mass spectrometric interface. The conducting PGC material will then be partly decoupled from the high ion spray voltage.

The importance of grounding the conducting PGC stationary phase (i.e. grounding the stainless steel union) was illustrated. Removing the ground point resulted in a potential drop over the column and a current passing through the chromatographic stationary phase. Shifts in retention times for 3-O-methyldopa and tyrosine as well as peak splitting was observed (for 3-O-methyldopa). However, the less retained dopamine and noradrenaline did not show either shift in retention or peak splitting.

3.4 Effect of organic modifier

The relationship between the retention and the concentration of organic modifier is generally linear in reversed-phase LC in accordance with the equation

$$\log k' = \log k'_w + mC \tag{2}$$

where k' is the retention factor, k'_w is the retention of the analyte in water as the mobile phase, m is a constant and C is the concentration of the organic modifier. Hennion et al. investigated the relationship between $\log k'$ and the concentration of methanol on PGC for polar substituted aromatic derivatives (Hennion et al. 1995; Hennion and Coquart 1992). They found a linear decrease in the logarithm of the retention factor with the concentration of the organic modifier. Bassler et al. (1989) and Forgacs and coworkers studied the effect of the concentration of the organic modifier on retention for different classes of compounds (Cserhati and Forgacs 1993; Forgacs and Cserhati 1995, 1996, 1997, 1998a, 1998b, 1998c; Nemeth-Kiss et al. 1997; Balcan et al. 1997) such as aniline derivatives (Bassler et al. 1989) phenol derivatives (Forgacs et al. 1992) phenoxy acetic acid derivatives (Cserhati and Forgacs 1993) and steroids (Forgacs and Cserhati 1998a, 1998b, 1998c). They also found a linear relationship between $\log k'$ and the concentration of the organic modifier for all those chemical classes. All the above results are in accordance with (1).

Similar results were obtained when methanol was used, except that the retention minimum occurred at higher levels (70%, v/v). Nemeth-Kiss et al. (1997) have also observed retention minima on PGC when analyzing peptides. Retention minima at alkyl bonded silica phases are explained by the interaction of basic or cationic solutes with the silanols present in the stationary phase (Karlsson et al. 1998). With PGC, the minima might be due to dispersive interactions being dominant at low concentrations of organic modifier while charge-induced dipolar interactions are dominant at high concentrations of organic modifier.

3.5 Functional group contribution

In reversed-phase LC, retention usually increases with the number of methyl groups in accordance with the equation

$$\log k' = \log k'_o + \alpha n \tag{3}$$

where k'_o is the retention for an unsubstituted solute molecule, α is a constant and n is the number of groups (e.g. methyl groups) in a homologous series. This linear increase in $\log k'$ with the number of methyl groups has been demonstrated on PGC as well (Tanaka et al. 1991; Kriz et al. 1994; Möckel et al. 1991). However, a linear relationship between $\log k'$ and number of sulphate groups on sulphated disaccharides was noted. For a reversed-phase material, it is expected that the retention would increase with the hydrophobicity of the analyte. Here, the opposite was true with the more polar analytes being most retained. Similar results have been observed by Hennion et al. (1995) who found a linear increase in $\log k'$ with the number of hydroxyl or carboxylic acid groups on the benzene molecule. These results indicate that the polar functional group interacts with the PGC material.

3.6 Effect of buffering agent

Several groups (Kriz et al. 1994; Wall et al. 1996; Hennion et al. 1995; Möckel et al. 1991; Bassler et al. 1989) have reported that it is possible to retain negative ions on PGC which are not, or only slightly, retained on C18. They have all found that the concentration and/or type of buffering agent affects the retention to a much larger extent than on e.g. silica based C18. Lim and co-workers separated pertechnate (TcO_4^-) and perrhenate (ReO_4^-) (Lim 1989). The ions were totally retained with water as the mobile phase but eluted when TFA (0.1%) was added. The more TFA added (0.05–2%), the shorter the retention time. When using acetic acid as an additive, higher concentrations (5%) of the acid was needed to elute the ions. Gu and Lim (1990) used PGC to separate anionic and cationic compounds of biomedical interest. Oxalic acid was not eluted when acetonitrile was added to the mobile phase. TFA was needed to

elute the acid. Elfakir and Dreux (1996) analyzed intact and desulphated glucosinolates on PGC. To elute the intact glucosinolate (containing a sulphate group), both TFA and an organic modifier were needed. The more TFA, the shorter the retention time for the intact glucosinolate. However, the retention of the desulphated compound was not affected by the concentration of TFA. Elfakir et al. separated inorganic anions (F^-, Cl^-, Br^-, I^-, NO_3^-, $H_2PO_4^-$, SO_4^{2-}, ClO_3^-, BrO_3^-, IO_3^-, ClO_4^-, IO_4^-) on a PGC column (1998). They were totally retained with deionized water as the mobile phase. The authors investigated different carboxylic acids and found that the elution strength decreased in the following order: heptafluorobutyric acid > TFA > formic acid > acetic acid. They also found that the higher the concentration, the shorter the retention time. Moreover, the more polarizable the inorganic ion, the more it was retained. Mercier et al. (1999) analyzed phosphonic acids in tap water and found that the acids were totally retained when water was used as the mobile phase. TFA was selected as additive after examining different carboxylic acids. The elution strength order of those acids were found to be the same as in the study of Elfakir et al. (1998).

The influence of various buffering agents on the solute retention was studied. The retention of the sulphated disaccharides was strongly affected by the choice of buffer components, contrary to the behavior of the neutral polar reference substance ISMN. Much stronger retention was obtained with 200 mM formic acid (pH 2.2) as the buffer additive than with 10 mM sulphuric acid (pH 2.0). When 10 mM sodium chloride was added to the formic acid mobile phase, the retention decreased dramatically. In addition, a study was performed where the concentration of formic acid was varied between 0 and 200 mM in 40% acetonitrile. The retention of the sulphated disaccharides decreased with increasing concentrations of formic acid and the more sulphate groups on the disaccharides the more the retention was affected.

3.7 Temperature effects

The retention correlates with the temperature in accordance with

$$\ln k' = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \phi \tag{4}$$

where ΔH^0 and ΔS^0 are the standard molar enthalpy and entropy, respectively, R is the gas constant, T is the temperature (in Kelvin) and Φ is the phase ratio. A plot of $\ln k'$ versus $1/T$ (van't Hoff plot) has a slope of $-\Delta H^0/R$ and an intercept of $\Delta S^0/R + \ln \Phi$. Usually, the retention decreases with temperature, an effect that has also been observed on PGC by e.g. Koimur et al. (1996) who got linear van't Hoff plots when analyzing disaccharides. However, in some case the retention of the sulphated disaccharides increased with temperature. On the other hand, the retention of the neutral reference substance (ISMN) decreased with temperature, as one would have expected. Linear van't Hoff plots were obtained. Enthalpy and entropy values were calculated from the slope and intercept of the van't Hoff plot and it was found that the retention of the sulphated disaccharides were entropically driven. Koizumi et al. (1991) have also observed that the retention increased with temperature when they analyzed cyclomaltaoses and their glucosyl derivatives.

4 Applications of PGC columns

In developing a separation on PGC it is important to take into account both the hydrophobic and electronic properties of the packing. A higher ratio of organic modifier is needed for elution of given solutes, and the electronic interactions between the surface of the PGC support and the solute may markedly modify the retention and must also be considered.

4.1 Pharmaceuticals

The neutral surface of PGC makes it specially suitable for the separation of basic solutes, as was demonstrated by Gu and Lim (1990). The authors carried out the separation of remoxipride and FLA-981, two potential neuroleptic agents. The authors compared two different methods: separation with ion suppression at pH 10 and ion pairing with TFA. The superiority of the eluent system containing TFA was established; remoxipride and FLA-981 eluted with convenient retention times. The separation of antihypoxia drug tiaconazole from its closely related impurities was studied by Berridge (1998). The PGC with an alkaline eluent consisting of tetrahydrofuran–water (7:3 v/v) with 1% ammonia gives excellent separation.

Forgacs and co-worker determined the retention behavior of 45 barbituric acid derivatives in various unbuffered eluent systems: methanol–water, ethanol–water (Forgacs and Cserhati 1998a, 1998b, 1998c), dioxane–water (Forgacs and Cserhati 1998a) and acetonitrile–water (Forgacs and Cserhati 1997). Linear correlations were calculated between the logarithm of the capacity factor and the concentration of organic modifier in the eluents. Various multivariate mathematical statistical methods such as stepwise regression analysis (Barrett et al. 1998), canonical correlation analysis (Nazir et al. 1997), principal component analysis (Forgacs 1994)) and Free–Wilson analysis (Nemeth-Kiss et al. 1996a, 1996b), were used to elucidate the role of individual substituents and to elucidate the similarities and dissimilarities in the information content of various calculation methods. Authors concluded that electronic and steric factors of derivatives governed the retention on PGC. Their conclusions support that a polar retention effect (PREG) exists with

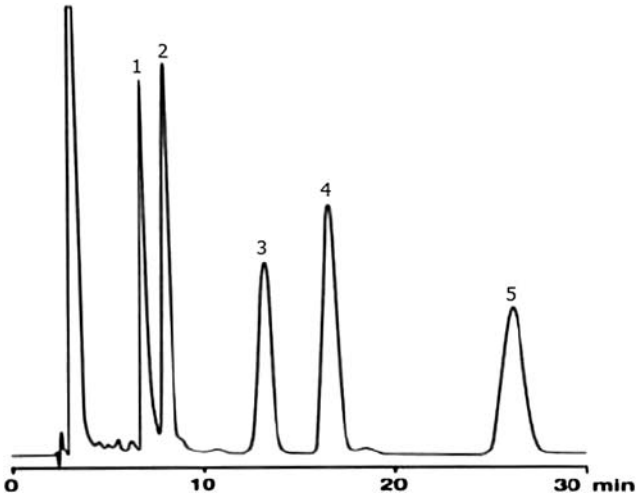

Fig. 3 Chromatogram of morphine and metabolites on Hypercarb™ 5 μm 100 × 4.6 mm column; mobile phase: acetate buffer pH 9–methanol (40:60, v/v). Analytes: *1* normorphine, *2* morphine-3-glucuronide, *3* morphine-6-glucuronide, *4* morphine, *5* codeine (Barrett et al. 1998)

graphite; this effect is absent from typical reversed-phase packings. PREG appears to arise from an interaction of the conductivity electrons of graphite with p or lone-pair electrons of analytes. Also it may be assumed that the additional ring structures of barbiturates also interact with the hexagonal graphitic structures on the PGC surface by stacking interactions affected by the retention behavior of these derivatives.

The chromatographic behavior of a series of morphine based opiates has been investigated by Barrett et al. (1998) using PGC methanol–water eluent systems at acid and alkaline pH. The effects of mobile phase pH, mobile phase organic percentage, column temperature and ion-pairing agents were studied.

The authors described that the retention order of morphine based opiates was not related to the log *P* values of the derivatives, and strong retention of the fully ionized compounds was observed, particularly those with acidic functional groups. The effect of pH on the retention of the compounds indicated that the degree of ionization of the compounds was important in the separation mechanism, suggesting that hydrophobic interactions were present in addition to the polar retentive effects.

Using a Hypercarb column a simple validated method was developed by Nazir et al. (1997) for analysis of the immunosuppressant Cyclosporin A and Cyclosporin U entrapped liposomes. For optimum selectivity and resolution of cyclosporins, the temperature of the PGC column was adjusted to 70 °C and *tert*.-butylmethylether-methanol (50:50 v/v) was employed as the mobile phase. Linearity was maintained at a concentration range of 2–20 mg/ml. The limit of quantification (LOQ) was 200 ng/ml. The study has shown the PGC column to be stable for over 2500 injections, which is an improvement over previous assays for cyclosporin analysis.

The anticancer drug taxol was determined on a PGC column in the extract and needles of *Taxus baccata* (Nemeth-Kiss et al. 1996a, 1996b). The method has been successfully used for the determination of the taxol content in various *Taxus* species (Nemeth-Kiss et al. 1996a, 1996b) and for the elucidation of the effect of vegetative period on the taxol yield (Ting and Porter 1997).

Electrochemically modulated liquid chromatography (EMLC) has been applied to the separation of a mixture of structurally similar corticosteroids (prednisolone, prednisone, cortisone and hydrocortisone) using a porous graphitized carbon stationary phase (Forgacs and Cserhati 1998a, 1998b, 1998c). The authors stated that the retention of these analytes can be markedly and effectively manipulated through alterations in the value of E_{appl}. These changes are realized through the dependence of the strength of donor–acceptor interactions between the samples and PGC on E_{appl} which is modified to different extents by the competitive interaction from the ionic species that make up the supporting electrolyte and PGC.

The chromatographic parameters ($\log k_0$ and b values) for 11 steroidal drugs with unbuffered tetrahydrofuran–water eluent mixtures were also published (Karlsson et al. 1998).

Two different LC methods for the quantification of alprenolol and estimation of related substances were compared (Cserhati and Forgacs 1993). In the first LC method a silica based material (Hibar LiChrosorb RP-8) was used as the stationary phase, and the mobile phase consisted of a counter-ion dissolved in acidic buffer and acetonitrile. The mobile phase in the other method consisted of alkaline methanol, and the stationary phase was porous graphitized carbon (Hypercarb). The robustness of the methods was investigated and evaluated with multivariate calculations.

Authors stated that the porous graphitized carbon system was far more robust than the silica system. The retention order of alprenolol and three related substances were the same, within the experimental design, when using the Hypercarb column.

4.2 Agrochemicals

The separation capacity of PGC columns for the important agrochemicals, chlorophenoxyacetic acid congeners, has also been explored using dioxane–water as eluent without additives and with added sodium acetate, acetic acid or lithium chloride. The results indicated that acetic acid had the greatest effect on retention on the PGC column. Retention parameters ($\log k_0$ and b) have been given for each chlorophenoxyacetic congener and eluents (Forgacs

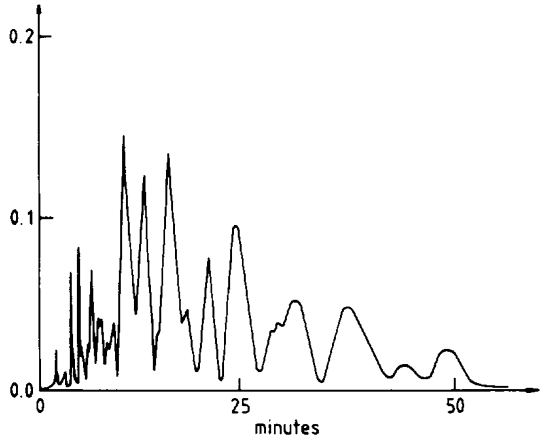

Fig. 4 Chromatogram of nonylphenyl ethylene oxide oligomers on PGC column; mobile phase: methanol–water

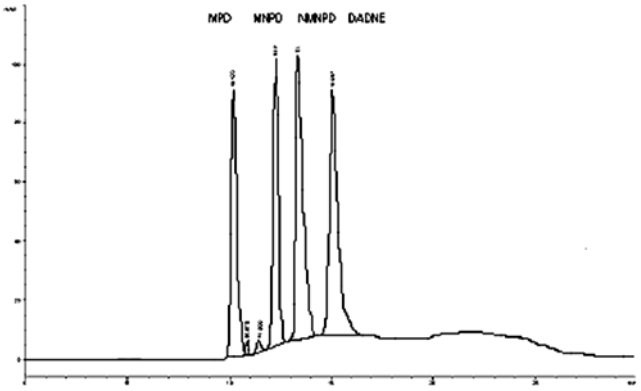

Fig. 5 Chromatogram of a novel explosive *1* DADNE (1,1-diamino-2,2-dinitroethene, FOX-7) and its intermediate products: *2* MPD, *3* MNPD, *4* NMNPD on Hypercarb™ 5 μm 100 × 4.6 mm column; mobile phase: water–acetonitrile-0.1% NH_3 (Buszewski et al. 2008)

and Cserhati 1995). The retention of 30 commercial pesticides and herbicides was determined on a PGC column using dioxane–water mixture as eluent.

The constants of $\log k_0$ and b were tabulated. Both $\log k_0$ and b values showed high variations, suggesting that PGC can be successfully used for the separation and quantitative determination of these agrochemicals (Cserhati et al. 1997).

Ibanez et al. (1997) published a selective on-line solid phase extraction and liquid chromatography determination of diquat, paraquat and dibenzoquat herbicides from environmental water samples. The method involved passing 50 mL of water through a cartridge filled with Carbograph. In the elution step, the herbicides were transferred from the cartridge to the Hypercarb column. Gradient elution was used; the eluent contained methanol, water, tetramethylammonium hydroxide and ammonium sulphate (pH 3). Authors described that Hypercarb columns give a low probability of false positives for these herbicides and are very selective for polar compounds. The limits of quantification of the method were lower 21 than 0.1 μg/L.

4.3 Xenobiotics

A PGC column has also been successfully employed for the separation of nonylphenyl ethylene oxide oligomers according to the length of the ethyleneoxide chain (Balcan et al. 1997).

Ethylene oxide surfactants containing α-(1,1,3,3-teramethylbutyl)phenyl hydrophobic moiety have also been separated on PGC columns. The retention of surfactants increased linearly with increasing number of ethylene oxide groups per molecule, indicating hydrophilic interactions between the solutes and the surfaces of the graphite support. It was also published that the character of the organic modifier exerted a considerable impact on the separation capacity of PGC columns. This phenomenon was explained by the supposition that the bulky organic modifier occupies the active adsorption centers on the surface of PGC, resulting in the decreased separation efficacy of the column (Balcan et al. 1997).

Elfakir and Dreux (1996) investigated the chromatographic behavior of seven alkylglycosides on a Hypercarb column under isocratic and gradient elution modes and compared it to that on an ODS column. Authors stated that using acetonitrile as organic modifier reinforces alkylglycoside separation depending on the alkyl chain length, whereas methanol favors the separation of alkylglycosides according to their polar head. The authors described the excellent separation capacity of PGC columns in the case of five closely related octylglycosides in a methanol–water eluent system.

The environmental pollutants 3,4-dimethoxybenzaldehyde and 3,4-dimethoxyphenylacetone were separated and quantitatively determined in waste waters using PGC columns and acetonitrile–water eluents (Fan et al. 1994). The measurements proved that both compounds can be easily decomposed by both aerobic and anaerobic treatments.

4.4 Natural products

Graphitized carbon has great potential for separation and purification of glycopeptides and oligosaccharides (Koizumi et al. 1991) in applying acetonitrile–water with NH_4OH. Oligosaccharides and glycopeptides with few amino acids are barely retained on reverse-phase columns even under high salt or low pH conditions, but can be retained effectively on PGC column.

The retention order of glycopeptides on PGC suggested that both the carbohydrate moiety and amino acids chain exert a considerable influence on the retention, and the retention time observed is the result of the interplay of various interactions between the surface of PGC and the various substructures of glycopeptides. The authors stated that

although the resolution and the capacity of ODS are still superior to those of graphitized carbon, the graphitic carbon is a valuable alternative for the separation and preparation of glycopeptides and oligosaccharides. PGC columns were employed for the separation and quantitative determination of disaccharides using post-column derivatization with benzamide (Lipniunas et al. 1996). The influence of the organic modifier in the mobile phase and that of column temperature were studied in the publication. The detection limits were 20 and 10 picomoles for melobiose and glucose. The relative standard deviation varied between 1 and 3%, indicating the good reproducibility of the method. It was stated that this method is suitable for the separation of disaccharides at low detection limit and the PGC support can be successfully used in the analysis of this type of compounds.

Oligosaccharide branch isomers have also been separated on a porous two-dimensional graphite stationary phase and with high pH anion exchange chromatography (HPAEC) (Kiniwa et al. 1989).

The results prove that this combination is a successful tool for the analysis of oligosaccharide branch isomers. The authors described that PGC has a unique capacity for the separation and quantitative determination and microscale preparation of neutral branch-isomer mixtures.

The L- and D-isomers of *N*-(2-naphtalenesul-phophyl)-phenylalanine (*N*S-Phe) have also been used as chiral selectors for the chiral separation of amino- and hydroxy-acids. The chiral selectors were adsorbed on the surface of PGC and the enantiomers were eluted by aqueous 2.0 mM copper acetate at pH 5.6. The PGC column coated with NS-Phe separations showed excellent chiral separation capacity; the order of retention of enantiomers depended on the configuration of the chiral selectors. The retention and selectivity of this system was compared with ODS support (Katoh et al. 1989). Due to its efficiency and long term stability PGC was proposed as a good alternative to silica based stationary phases for the chiral separation of amino acids and other compounds (Reh and Kapfer 1990).

Enantiomeric separations using chiral ion-pair chromatography have been investigated (Karlsson and Charron 1996) with porous graphitized carbon column. The enantiomers of several aminoalcohols were successfully separated as diastereomeric ion pairs with *N*-benzyloxycarbonyl-glycyl-L-proline or *N*-benzyloxycarbonylglycylglycyl-L-proline dissolved methanol as the mobile phase. The influence of the solute structure as well as the counter ion structure on the chiral recognition was examined. The position of substituents in the aromatic ring, type of alkyl group attached to the nitrogen and the number of methylene groups between the asymmetrical carbon atom and the nitrogen atom were studied. This study showed that a column temperature below 0 °C improved the enantioselective resolution. A stable and robust chromatographic system with a short equilibration time was presented.

The separation capacity of PGC was compared with other RP columns using 39 peptides as model compounds. Peptides were eluted from the PGC column by gradient elution. The retention of peptides on the PGC column considerably depended on the pH of the mobile phase. The data indicated that no ideal support can be found for the separation of each peptide and that the efficacy of the support highly depends on the type of peptides (Buszewski et al. 2007; Michel et al. 2007). The anomalous retention behavior of peptides on PGC columns has also been reported (Buszewski and Michel 2008). The retention increased 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.

5 Conclusion

Carbonaceous sorbents have effectively unique properties in retention. They behave as normal-phase, reversed-phase and ion exchanger sorbents. It is clear that more work is necessary for a full understanding of the interactions involves with PGC or GCB.

The development of carbon sorbents for the preconcentration of analytes utilizes the results of the long-term research and development of stationary phases for HPLC and GC. Recent developments in the production of thermally modified carbon blacks, carbon molecular sieves and porous carbons for quantitatively trapping of organic components are reflected in their superior performance over “traditional” sorbents such as activated charcoal and porous polymers which tend to lack uniform sorbent characteristics for the adsorption and retention of the different compounds encountered in different environments. The application of such adsorbents has minimized the problems of contamination and artifact formation, and hence they are suitable for the sampling of VOCs in quite different environments and preconcentration and isolation of semi-volatile and non-volatile compounds from various matrices.

In spite of the broad application of sorbents in trace analysis for several tens of years, the results of tests of new sorbents, synthesis of tailored sorbents, or combination of sorbents with various physico-chemical properties with the aim of special and very broad application, research on the influence of various factors on the efficiency of preconcentration, show that the developments in this field with the aim to obtain an inert sorbent for ultra-trace analysis will continue.

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