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Journal of Chromatography A, 975 (2002) 229–243

JOURNAL OF CHROMATOGRAPHY A

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Review

Retention characteristics and practical applications of carbon sorbents

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Received 27 May 1999; received in revised form 12 November 1999; accepted 16 November 1999

Abstract

Reversed-phase separations provides a versatile technique in high-performance liquid chromatography. Porous graphitized carbon (PGC) support shows unique retention characteristics. Separations on PGC columns use typical reversed-phase eluents (water and organic modifiers miscible with water), however, the retention order of solutes generally does not follow their hydrophobicity order. Molecular hydrophobicity influences but not determines the elution order of any set of solutes. The properties of these supports, mechanisms of retention and application are discussed, along with correlations which can guide the choice of solvent combinations for typical separations.

Keywords: Reviews; Retention characteristics; Stationary phases, LC; Carbon sorbents; Porous graphitized carbon

Contents

1. Introduction

During the past few years there has been an intense search for new adsorbents because of the realization of the disadvantages of silica-based columns.

Carbon was frequently used in classical liquid chromatography [1–3], but the active carbons then available showed nonlinear adsorption isotherms which made them unsuitable for elution chromatography as now practised. DiCorcia and Liberti [4] widely used unmodified graphitized carbon black (GCB) in gas chromatography.

GCBs are composed of colloidal particles of

0021-9673/02/$ – see front matter
PII: S0021-9673(99)01250-9

graphitized carbons with an average diameter between 0.01 and 0.05 μm.

Unfortunately this support was too fragile for HPLC, therefore Colin et al. [5,6] had attempted to improve the strength of GCB by depositing pyrolitic carbon on its surface.

The mechanical strength of this material was much greater than that of the carbon aggregates but was still insufficient for LC. Knox and Gilbert [7] described a novel method of making a robust porous carbon. A high porosity HPLC silica gel was impregnated with a phenol–formaldehyde mixture. The impregnated materials were heated gradually to 150°C to form phenol–formaldehyde resin within the pores of the silica gel.

The resin was then carbonized by heating slowly to 900°C in nitrogen. After dissolution of the silica template with aqueous KOH, the carbon was heated to 2500°C in an oxygen–argon atmosphere. This material was called ''porous graphitized carbon'' (PGC).

Recently manufacture was taken over by Hypersil, a division of ThermoQuest Corporation, using the method of Knox and Gilbert. The support is now marketed under the trade name Hypercarb.

In 1991 two Japanese groups (Obayashi, Ozawa and Kawase of the Tonen Corporation and Ichikawa, Yokoyama, Kawai, Moriya, Komiya and Kato of the Nippon Carbon Company and Tosoh Corporation) disclosed porous graphites made by entirely different procedures. Table 1 indicates the typical physical and chromatographic characteristics of the three carbon columns: PGC, Tonen carbon and Nippon/Tosoh carbon [8].

In confirmation of the view that graphite, however produced, should show the same chromatographic characteristics, it is encouraging to note that in spite of widely different methods of production and the somewhat different physical and chromatographic properties of the three porous graphites, the absolute and relative retention factor (k) values for test phenols are similar.

Table 1
Comparison of physical and chromatographic properties of porous graphites[a]

	PV	SA	PD	E″	k value
PGC	0.85	120	350	95%	0.25
Tonen	0.44		300	95%	0.50
Nippon/Tosoh	0.95	24	355	100%	0.30

[a] PV = pore volume in ml/g; SA = surface area in m^2/g; PD = mean pore diameter in Å; E = percentage methanol in eluent, remainder water; k values = bracketed values relative to k for *p*-cresol. (Reprinted with permission from Ref. [8]).

The following parameters characterize the porous graphitized carbon support [9,10]:

- sufficient hardness to withstand high pressures;
- a well reproducible and stable surface that shows no change during chromatographic work or storage;
- specific surface area, to give adequate retention of solutes and maintain a reasonably linear sample capacity over a large concentration range;
- a mean pore diameter >10 nm and the absence of micropores to ensure rapid mass transfer of solutes into and out of the particles;
- uniform surface energy to give a linear adsorption isotherm;
- unaffected by aggressive eluents – no swelling, shrinkage or dissolution;
- stable across the entire pH range and extreme conditions of salt concentration, temperature;
- used with normal and reverse phase eluents;
- unrivalled stereo-selective surface with unique ability to resolve isomeric and closely related compounds;
- unique retention mechanism and selectivity.

PGC is practically inert to acids and bases, so eluents of any pH can be used. Many experiments indicate [10,11] that the apparent hydrophobicity of the surface is higher than other traditional reversed-phase supports such as octadecylsilica (ODS) or phenyl-bonded silica, and the users sometimes say that the PGC is a super-reversed phase. This implies that a higher percentage of organic modifier is required for elution of a given solute from a PGC column, the increase generally being 20–40 vol.%.

With selected solute samples, an attempt has been made to establish the eluotropic strengths ($\Sigma°$) of some organic solvents on PGC. The results showed that the actual value of $\Sigma°$ for a particular solvent considerably depends on the chemical character of the solute or solute group used to determine it, and the differences in the eluotropic strength are usually small. In general, methanol and acetonitrile showed low, while dioxane and tetrahydrofuran showed

higher eluotropic strengths for the majority of solutes. It was further established that ethyl acetate, dichloromethane, butylchloride, and hexane have eluotropic strengths that vary the most strongly with solute. It has been concluded that an eluotropic series comparable to that of silica and alumina is difficult, if not impossible, to establish for PGC support. A further complication is that peak shapes are also solvent dependent. A solvent that provides the highest strength does not necessarily also give the best peak shape [12].

2. Retention behavior of PGC columns

The retention mechanisms of PGC supports and the role of various interactions (hydrophobic, dispersive, charge-induced etc.) of solutes with the PGC surface have been vigorously discussed, although the conclusions are sometimes contradictory. The earlier studies considered PGC to be a 'pure' reversed-phase support because it has no free silanol groups which are not covered by the hydrophobic ligand or other adsorptive centers on the surface. Kaliszan and co-workers were first to describe that PGC did not behave as 'pure' reversed-phase material [13,14]. They studied the retention of 24 benzene derivatives using heptane as solvent. By contrast to the expectations of a reversed-phase column, more polar samples were more strongly retained than less polar samples. Log k values of samples showed a poor correlation with the estimated energy of overlap between the π electrons of the graphite surface and those of the adsorbate, but showed a good correlation with polarity parameters. It was demonstrated by a statistical approach that the PGC primarily behaves as an electron-pair acceptor for substituted aromatic solutes which are capable of n-electron donation under non-polar conditions. The retention order of the samples closely followed the basicity of their lone-electron pair.

Lim et al. [15,16] suggested a strong attractive interaction between the charged centers in the analyte and the graphitic surface leading to the observed retention of pertechnetate and perhennate anions from an aqueous eluent. Authors concluded that PGC may well be an important stationary phase for the separation of ionized solutes by a mechanism other than ion exchange, and they showed that positively and negatively charged ions could be separated in the same chromatogram.

Tanaka and co-workers [17] compared the selectivity of carbon phases (Hypercarb, Carbonex) and silica-based packings octadecylsilica (ODS) and 2-(1-pyrenyl)ethylsilated silica (PYE) using homologous alkanes and other simple derivatives.

They described the carbon atoms in n-hexane samples as assuming a completely planar arrangement, whereas those in cyclohexane were unable to adopt a stable conformation. Decalin can have more points of contact with a flat surface than adamantane at a similar molecular mass. Graphitized carbon packing showed retention characteristics based on the major contribution of dispersion forces, and hence steric factors of solutes. Solute-stationary phase interactions on the carbon phase were much greater than on any silica based packing materials, resulting in the preferential retention of planar compounds. The results show the utility of carbon and PYE stationary phases with rigid, planar surfaces to provide steric selectivity for the separation of compounds with similar hydrophobicities, which are difficult to separate with C_{18} phase. Tanaka et al. [17] also stated that over a wide range of methanol/water concentrations, graphite shows a higher selectivity for homologous series of alkanes than either ODS or PYE.

Other studies [18–20] using different sets of solutes and eluents confirmed the unique stereoselectivity of PGC.

This stereoselectivity is very different: CH_2 addition and CH_3 substitution mirrors that of the adsorbents silica and alumina.

Quantitative structure-retention relationship (QSAR) calculations were used to elucidate the retention mechanism of PGC.

The retention of ring-substituted phenol [21], ring-substituted aniline [22], and barbituric acid derivatives [23] were determined in unbuffered methanol–water and acetonitrile–water eluent systems and the relationship between log k and the concentration of organic modifier (C vol.%) was calculated separately for each solute and eluent system:

$$\log k = \log k_0 - bC \quad (1)$$

The significant relationships between the retention

parameters (log k_0 and b) and the Hammett's constant (characterizing the electron-withdrawing power), proton–donor capacity and steric effects of substituents proved that the retention behaviour of investigated compounds are mainly governed by these polarity parameters. The results suggest that the graphite surface is sensitive to changes in the solute electron density caused by the electron-donating and -withdrawing ability of solute substituents and the number and position of electron dense bonds in the solute. Using these results it can be confirmed that PGC is highly sensitive to steric changes that disturb the electron density of the solute molecule and the resulting interaction of the solute with the graphite surface. The position of the substituents on the solute ring determines how the solute can approach the interaction with the graphite surface. The lipophilicity of the compounds did not affect significantly the measured retention parameters log k_0 and b.

This finding, and the fact that the retention order of solutes (Fig. 1) on PGC and ODS columns was not correlated emphasizes again the marked differences between the retention characteristics of PGC and the traditional reversed-phase (ODS) columns, although the eluents used on PGC columns are typical reversed-phase eluents generally used on ODS columns [24].

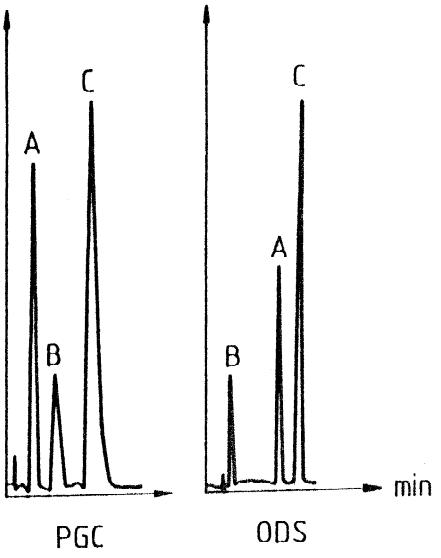

Fig. 1. Separation of phenol derivatives on PGC and ODS column. Eluents: PGC column, methanol–water (90:10 v/v), ODS column, methanol–0.025 *M* KH_2PO_4 (80:20 v/v) flow rate 1 ml/min; detection 254 nm, A=2,4 di-*tert*.-butylphenol, B=4-bromophenol, C=2,6-di-*tert*.-butylphenol. (Reprinted with permission from Ref. [21].)

Wan and co-workers [25] measured the retention characteristics of ionizable substituted benzene isomers on PGC and ODS column buffered acetonitrile/water eluent systems, over the range of mobile phase between pH 2 and 7.

The retention as a function of pH was modeled using equations based on solute ionization. In the case of PGC, the theoretical equations fitted the observed retention data for each class of solute, indicating that the retention mechanism was uniform over the whole pH range. However, for the ODS only the acidic solutes showed agreement with the theoretical model; for the amine-containing compounds, serious deviations from the theory were observed with strongly acidic silanols giving added retention at low mobile phase pH.

To summarize, the retention by PGC from an aqueous/organic eluent system is determined by a balance of the following interactions:

1. Interactions of polarized or polarizable functional groups in samples with a graphite surface (charge-induced interaction) – stereochemistry of the sample molecule forces the polar group to be close to the graphite surfaces. This interaction is called ''polar retention effect by graphite'' (PREG) [26].
2. London type dispersive interaction between the graphite surface and the sample. There are similar interactions between the PGC surface and the eluent which is displaced by the sample. They may have an important effect on the selectivity of PGC.
3. Hydrophobic interaction, which tends to drive samples out of solution.

3. 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.

3.1. Pharmaceuticals

The neutral surface of PGC makes it specially suitable for the separation of basic solutes, as was demonstrated by Gu and co-worker [27]. 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 anti-hypoxia drug tiaconazole from its closely related impurities was studied by Berridge [28]. The PGC with an alkaline eluent consisting of tetrahydrofuran–water (7:3 v/v) with 1% ammonia gives excellent separation.

Forgács and co-worker determined the retention behaviour of 45 barbituric acid derivatives in various unbuffered eluent systems: methanol–water [23], ethanol–water [29], dioxane–water [30] and acetonitrile–water [31]. 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 [23,30,31], canonical correlation analysis [29], principal component analysis [29,31] and Free–Wilson analysis [30] were used to elucidate the role of individual substituents [23,29–31] and to elucidate the similarities and dissimilarities in the information content [30,31] 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 graphite; this effect is absent from typical reversed-phase packings. PREG appears to arise from an interaction of the conductivity electrons of graphite with π 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 behaviour of these derivatives.

The chromatographic behaviour of a series of morphine based opiates has been investigated by Barrett et al. [32] 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 (Fig. 2) of the derivatives, and strong retention of the fully ionised compounds was observed, particularly those with acidic functional groups. The effect of pH on the retention of the compounds indicated that the degree of ionisation 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. [33] for analysis of the immunosuppressant Cyclosporin A and Cyclosporin U entrapped liposomes. For op-

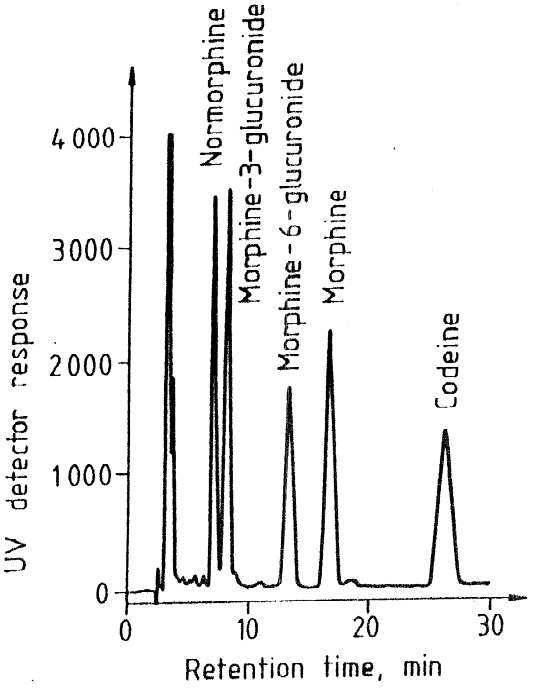

Fig. 2. Typical chromatogram of the separation of morphine-based opiates on the PGC column. Eluent: aqueous buffer–methanol (40:60 v/v) pH 10.6, flow rate 1 ml/min; detection 220 nm (Reprinted with permission from Ref. [32].)

timum 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 μg/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* [34]. The method has been successfully used for the determination of of the taxol content in various *Taxus* species [35] and for the elucidation of the effect of vegetative period on the taxol yield [36].

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 [37]. The authors stated that the retention of these analytes can be markedly and effectively manipulated through alterations in the value of E_{appl} (see Fig. 3). 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 [38].

Two different LC methods for the quantification of alprenolol and estimation of related substances were compared [39]. 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

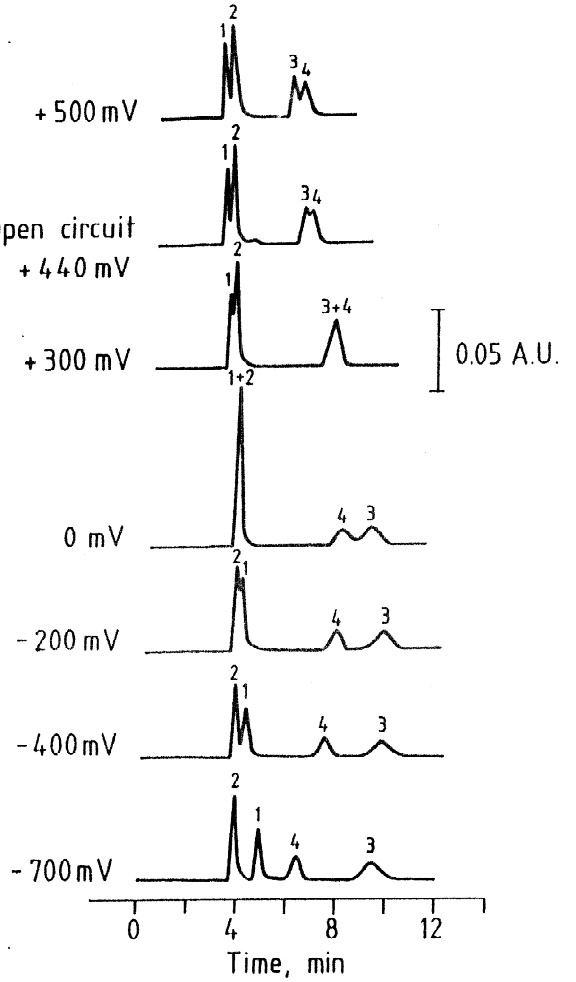

Fig. 3. Separations using EMLC of a mixture of prednisone (1), prednisolone (2), cortisone (3) and hydrocortisone (4) at a PGC stationary phase as a function of applied voltage: $E_{appl} = +500$, open circuit (i.e +440), +300, 0, −200, −400, and −700 mV. All applied voltages given with respect to an Ag/AgCl/saturated NaCl electrode. The mobile phase was composed of two components: (0.1 *M* $HClO_4$) acetonitrile (0.1 *M* $LiClO_4$)(50:50 v/v). The flow rate was 0.90 ml/min. (Reprinted with permission from Ref. [37].)

substances were the same, within the experimental design, when using the Hypercarb column.

3.2. 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 [40]. 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 [41].

Ibanez et al. [42] 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 than 0.1 μg l^{-1}.

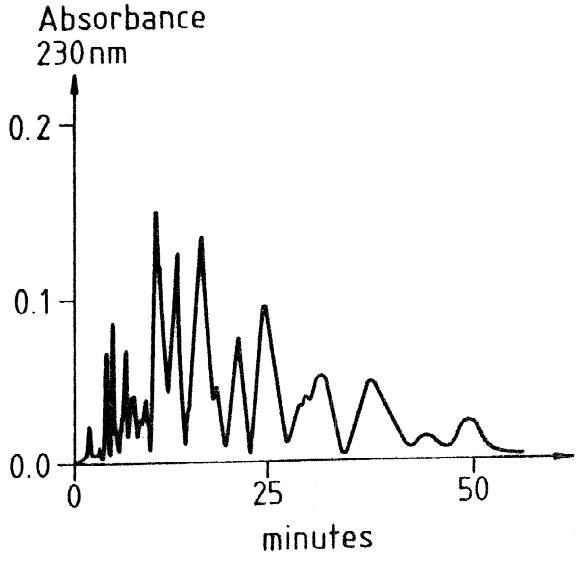

Fig. 4. Chromatograms of nonylphenyl ethylene oxide oligomers on PGC column. Eluent: methanol–water (97.5:2.5 v/v) detection 230 nm: flow rate 1 ml/min. (Reprinted with permission from Ref. [43].)

3.3. 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 [43].

Ethylene oxide surfactants containing α-(1,1,3,3-teramethylbutyl)phenyl hydrophobic moiety have also been separated on PGC columns (see Fig. 4). The retention of surfactants increased linearly with increasing number of ethylene oxide groups per molecule, indicating hydrophylic 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

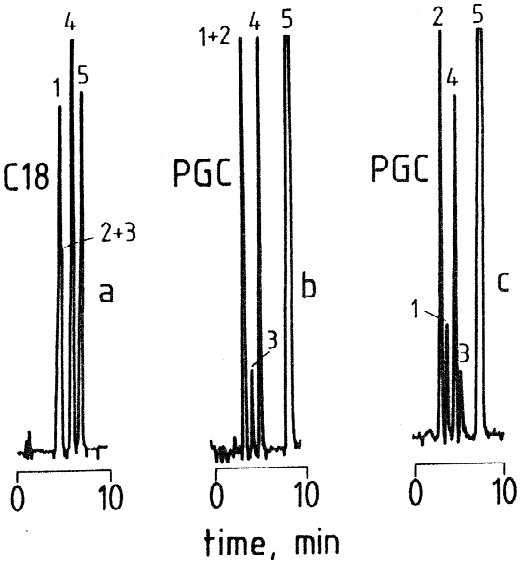

Fig. 5. Separation of five octylglycosides. Column, mobile phase: (a) Zorbax ODS (150×4.6 mm I.D.) ACN–water (40:60); Hypercarb S (100×4.6 mm I.D.); ACN–water (40:60); (c) Hypercarb S (100×4.6 mm I.D.) MeOH–water (95:5); Flow rate: 1 ml/min; evaporative light scattering detection. Elution order: 1. C_8–Oβgal; 2. C_8–Oαglu; 3. C_8–Oβglu; 4. C_8–Cβglu; 5. C_8–SCβglu. (Reprinted with permission from Ref. [45].)

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 [44].

Elfakir and co-worker [45] investigated the chromatographic behaviour 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 favours 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 (Fig. 5).

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 [46]. The measurements proved that both compounds can be easily decomposed by both aerobic and anaerobic treatments (Fig. 6).

3.4. Natural products

Graphitized carbon has great potential for separation and purification of glycopeptides and oligo-

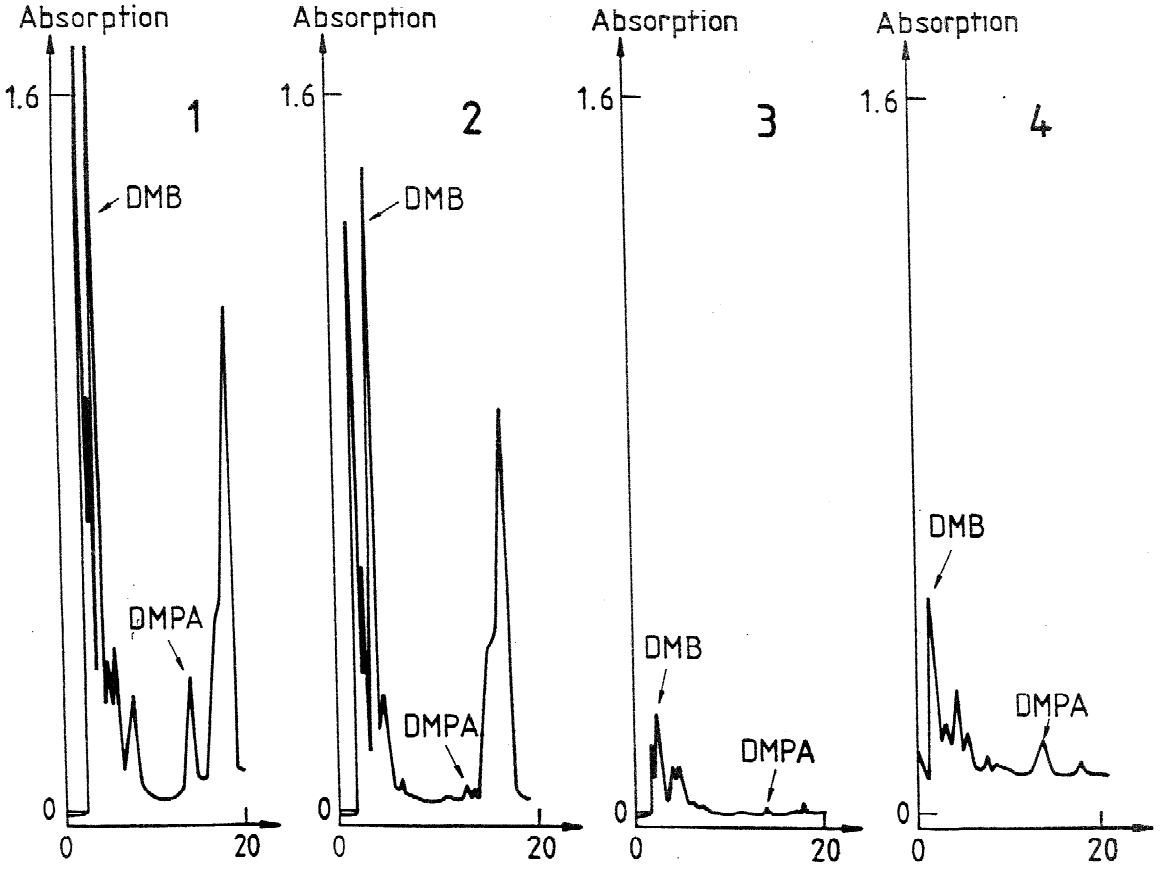

Fig. 6. Chromatograms of 3,4-dimethoxybenzaldehyde (DMB) and 3,4-dimethoxyphenylacetone (DMPA) extracted from industrial waste waters on PGC columns. 1 = waste water influx; 2 = waste efflux after 15 h treatment; 3 = waste water efflux after 24 h aerobic treatment; 4 = waste water efflux after anaerobic treatment. Eluent: acetonitrile–water (8:2 v/v%). (Reprinted with permission from Ref. [46].)

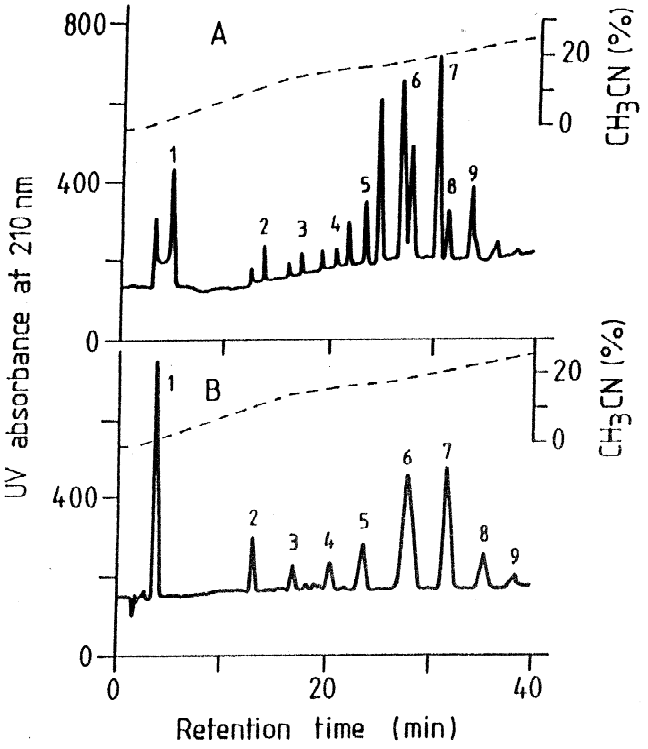

Fig. 7. The separation of chito-oligosaccharides on graphitized carbon column.The column was kept at 50°C. The numbers above the peaks denote the degree of polymerization. The sensitivity of the detector at 210 nm was set at 0.1 absorbance unit as full scale. A. Elution with water and acetonitrile. B. Elution with 10 m*M* NH_4OH and acetonitrile. (Reprinted with permission from Ref. [47].)

saccharides [47] in applying acetonitrile–water with NH_4OH (see Fig. 7). 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 composition of glycopeptides and

Table 2
Composition of glycopeptides and oligosaccharides purified by PGC

Compounds[a]	Putative structures	Man[b]	GlcN[c,d]	Asp[d]	Phe[d]	Leu[d]
S-I	$Man_9GlcNAc_2Asn$	8.94	2	0.87	0.00	0.00
S-II	$Man_9GlcNAc_2Asn$-Phe	8.89	2	1.10	1.23	0.00
R-I	$Man_6GlcNAc_2Asn$	5.94	2	0.87	0.00	0.00
R-II	$Man_5GlcNAc_2Asn$	5.03	2	1.06	0.00	0.00
R-III	$Man_6GlcNAc_2Asn$-Leu	5.99	2	1.10	0.00	1.09
R-IV	$Man_5GlcNAc_2Asn$-Leu	4.91	2	1.00	0.00	0.77
O-I	$Man_6GlcNAc$	5.77	1	–	–	–
O-II	$Man_5GlcNAc$	4.63	1	–	–	–

[a] Acid hydrolyses were performed with 2 *M* TFA or *4* M HCl at 100°C. The residues of sugars and amino acids were calculated based on 2 GlcN (glusoamine) in glycopeptide and 1 GlcN in oligosaccharide. (Reprinted with permission from Ref. [48].)
[b] 2 *M* TFA hydrolysis for 4 h.
[c] 4 *M* HCl hydrolysis for 6 h.
[d] 4 *M* HCl hydrolysis for 18 h.

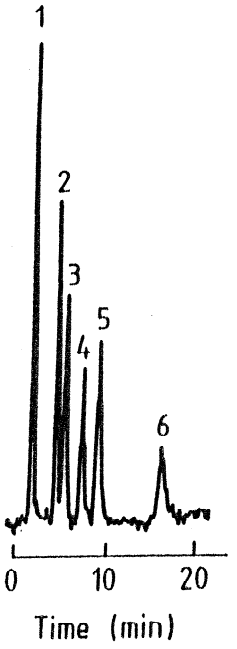

Fig. 8. Separation of a mixture of reducing sugars (0.1 m*M*) on Hypercarb by isocratic elution with 1% v/v acetonitrile at 95°C column temperature. Mobile phase flow rate at 1 ml/min. Peaks: 1 = glucose, 2 = melobiose, 3 = maltose, 4 = lactose, 5 = gentibiose, 6 = cellobiose (Reprinted with permission from Ref. [48].)

oligosaccharides separated by PGC is shown in Table 2.

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 [48]. The influence of the organic modifier in the mobile phase and that of column temperature were studied in the publication. The separation of six reducing sugars under optimal conditions is shown in Fig. 8. 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) [49].

The results prove that this combination is a successful tool for the analysis of oligosaccharide branch isomers as demonstrated in Fig. 9. 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-naphtalenesulphophyl)-phenylalanine (NS-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 m*M* copper acetate at pH 5.6. Some typical chiral separations are shown in Fig. 10. 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 [50,51]. The data are compiled in Table 3. 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 [52].

Enantiomeric separations using chiral ion-pair chromatography have been investigated [53] 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*-benzyloxycarbonyl-glycylglycyl-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 sub-

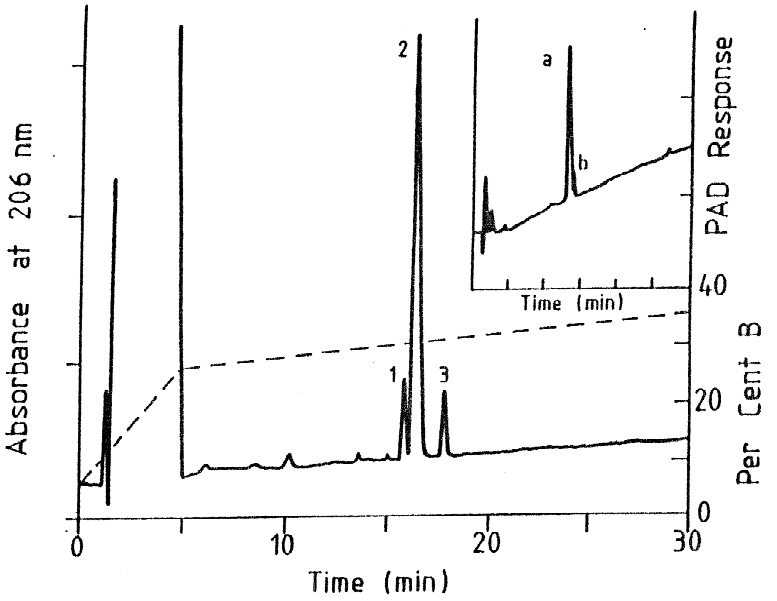

Fig. 9. HPAEC and PGC–HPLC of $Man_{10}GlcNac_1$ isomers from invertase expressed in *Pichia pastoris*. The reduced oligosaccharides (24 nmol) were eluted from PGC column (4.6×100 mm) and equilibrated in 5% of solvent B (0.05% TFA in 40% aqueous acetonitrile). After the percentage of the solvent B increased to 26% over 5%, the oligosaccharides were eluted with the indicated gradient (dashed line), a 0.4% increase in B per minute. All fractions were collected, dried, and analyzed using HPAEC analysis of the same oligosaccharide mixture prior to PGC–HPLC. The unnumbered fractions were found to give no detectable electrochemical response. The full-scale attenuation was 300 nA for PAD and 0.1 for UV detection. Numbers refer to oligosaccharide. (Reprinted with permission from Ref. [49].)

stituents 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 [52]. 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 (Fig. 11). 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. The anomalous retention behaviour of peptides on PGC columns has also been reported [54]. The retention increased with increasing concentration of ACN in the lower concentration range, reached a minimum, and increased again with increasing concentration of ACN in the higher concentration range.

4. Conclusions

Porous graphitized carbon (PGC) support shows unique retention characteristics. Separations on PGC columns use typical reverse-phase eluents (water and organic modifiers miscible with water), however, the retention order of solutes generally does not follow their hydrophobicity order. Molecular hydrophobicity influences, but does not determine, the elution order of any set of solutes. It has been many times proved that electrostatic interactions may occur between the planar ring substructures of solutes and the hexagonal graphite molecules on the PGC surface. These interactions have a considerable impact on the retention capacity and selectivity of PGC.

Porous graphitized carbon support makes possible the separation of many solutes (both polar and apolar

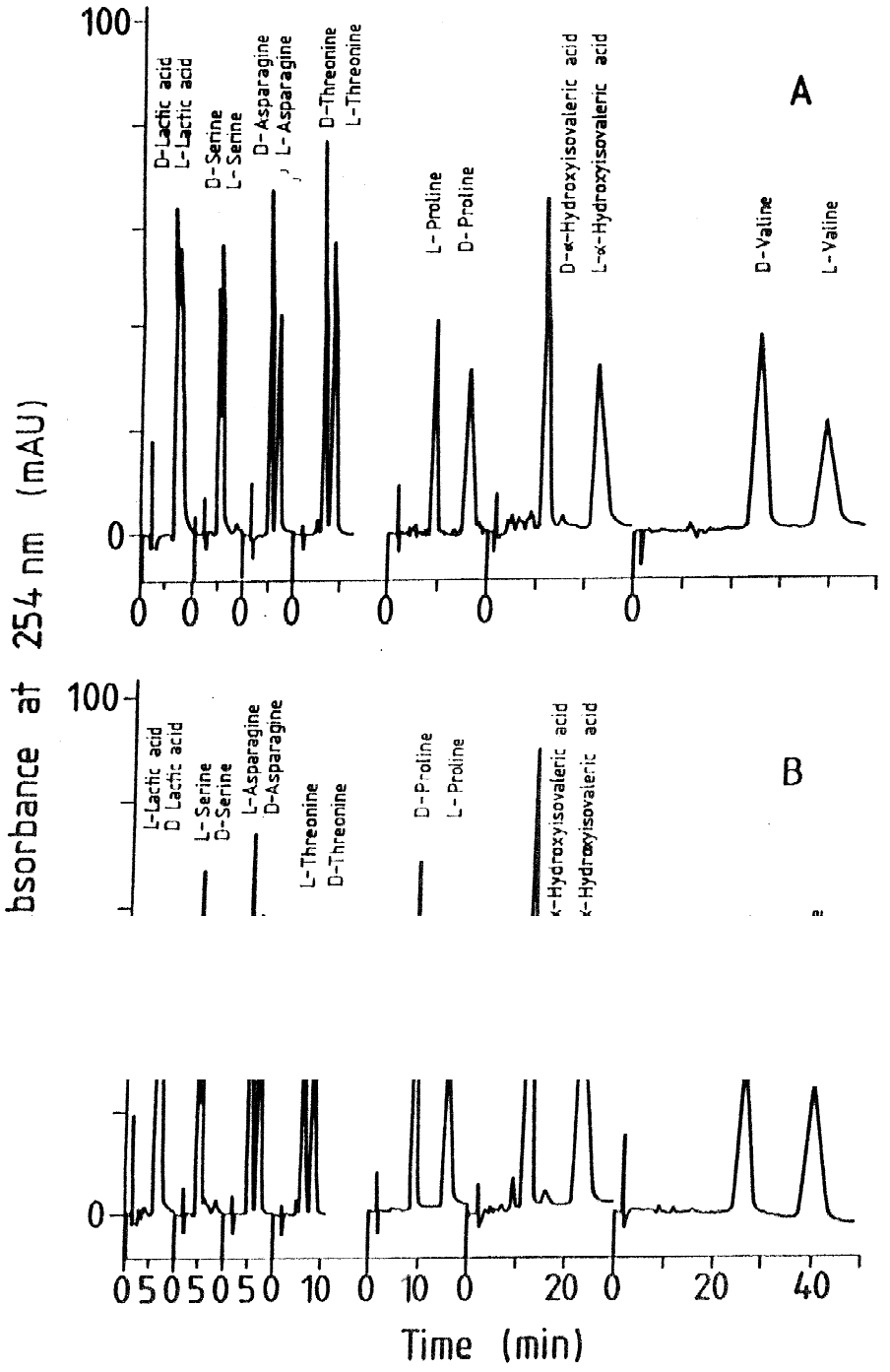

Fig. 10. Chromatograms of enantiomers of amino acids and hydroxy acids separated on porous graphite coated with (a) L-isomer and (b) D-isomer of *N*-(2-naptalenesulphonyl)-phenylalanine. 2.0 m*M* copper acetate, pH 5.6; detection UV 254; flow rate 0.5 ml/min, (Reprinted with permission from Ref. [50].)

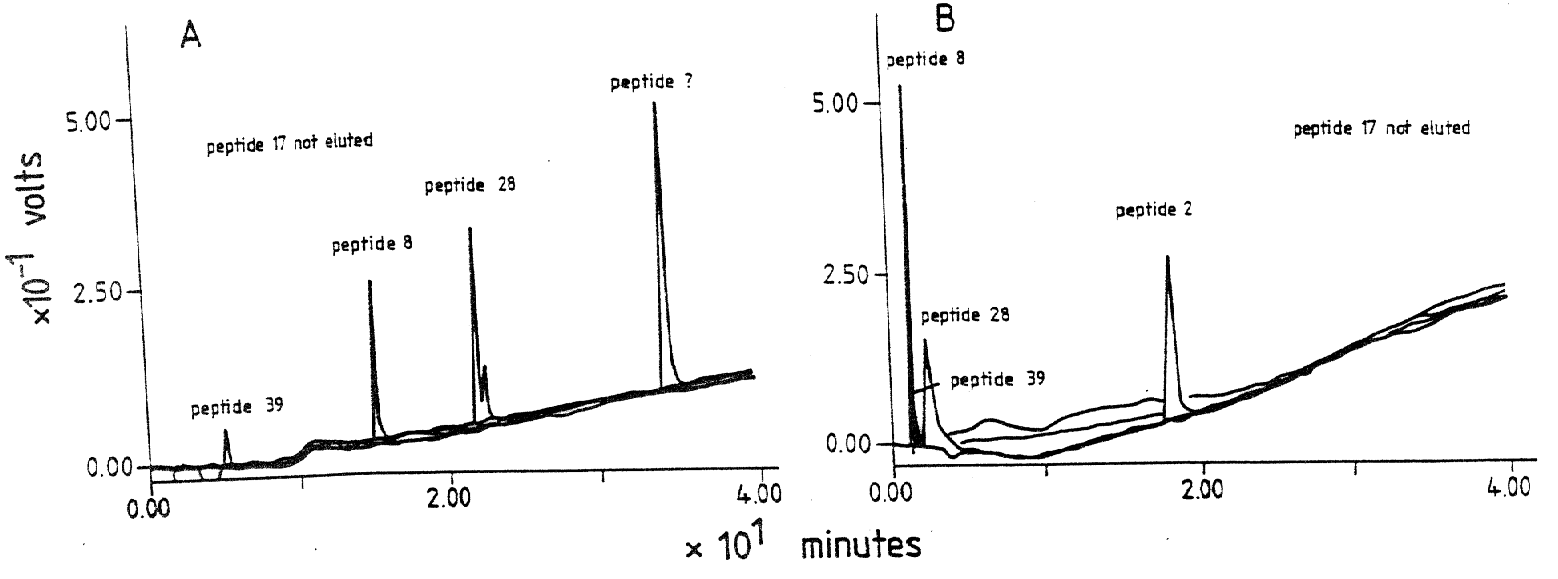

Fig. 11. Elution of peptides 2 (Leu-Leu-Val-Tyr), 8 (Ala-Gly-Ser-Glu), 17 (Cys-Gly-Asn-Leu-Ser-Thr-Cys-Met-Leu-Gly-Thr-Tyr-Thr-Gln-Asp-Phe-Asn-Lys-Phe-His-Thr-Phe-Pro-Gln-Thr-Ala-Ile-Gly-Val-Gly-Ala-Pro-amid) 28 (Thr-Tyr-Ser-Lys) 39 (Trp-Gly-Gly-Tyr) from PGC column, (a) gradient elution, eluent: 2-propanol and aqueous buffer at pH 2.2, (b) gradient elution, eluent: 2-propanol and aqueous buffer at pH 6.8. (Reprinted with permission from Ref. [52].)

Table 3
Retention and selectivity for enantiomers separated on porous graphite coated with NS-L-Phe

Analyte	Porous graphite[a]			Reversed-phase silica[b]		
	k_D'	k_L'	α	k_D'	k_L'	α
Serine	1.58	1.91	1.21	6.45	8.04	1.25
Asparagine	1.86	2.92	1.57	–	–	–
Threonine	2.63	3.86	1.47	7.07	9.13	1.29
Proline	7.91	3.96	2.00	5.64	12.04	2.13
Glutamine	5.08	5.78	1.14	–	–	–
Valine	13.39	21.33	1.60	7.32	14.93	2.04
Lactic acid	2.80	3.17	1.13	12.5	15.90	1.27
α-Hydroxyisocaproic acid[c]	5.04	10.75	2.13	–	–	–
α-Hydroxyisocaproic acid[d]	18.82	21.67	1.15	–	–	–

[a] Column, PGC 94F coated with NS-L-Phe, 50×4.6 mm; eluent, 2.0 m*M* copper acetate, pH 5.6; detection, UV 254 nm; temperature, 30°C.

[b] Column, MCI GEL S10W (ODS-silica coated with *N*,*N*-dioctyl-L-alanine, 50×4.6 mm; eluent, 0.1 to 2.0 m*M* $CuSO_4$, pH 5.50; detection, UV 24 nm.

[c] α-Hydroxyvaleric acid is $(CH_3)_2CHCH(OH)COOH$.

[d] α-Hydroxyisocaproic acid, also known as leucic acid, is (CH3)2CHCH2CH(OH)COOH. (Reprinted with permission from Ref. [51].)

in character); it seems to be specially suitable for the separation of positional isomers containing polar substructures. Due to its highly inert surface the PGC column can be used throughout the complete pH range without deterioration of the column efficiency, therefore it is well suited for the separation of both basic and acidic solutes. The influence of the buffering of eluent on the retention is not clearly understood. Sometimes good separation of solutes with highly dissociative polar groups has been achieved, in other instances the pH of the eluent exerted a considerable impact on the retention. Similarly to other reversed-phase support, TFA has been proved to be an excellent general-purpose mobile phase additive for the separation of anions and other electron-rich species and it can be used as an ion-pairing agent for cations. Enantiomer separations can also be carried out on PGC columns using slight modifications of separation methods developed for traditional supports.

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