Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 989 (2003) 183–196
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Review

Separation of polar compounds using carbon columns

Toshihiko Hanai*

Health Research Foundation, Institute Pasteur 5F, Tanaka-Monzencho, Sakyo-ku, Kyoto 606-8225, Japan

Received 28 May 1999; received in revised form 28 October 2002; accepted 29 November 2002

Abstract

The objective of this review with 122 references is to provide structure and retention mechanisms of porous graphitic carbon by chromatographic analysis and computational chemical analysis of retention mechanisms. Synthesis methods of porous graphitic carbon are described. Applications for use as matrix for dynamic coating on porous graphitic carbon and direct separation of polar compounds on porous graphitic carbon demonstrated that the physical and chemical stability of graphitic carbons performed in both chromatography and extraction, especially for polar compounds, those are difficult on both silica-based and organic polymer-based packing materials. The disadvantage is difficult desorption of non-polar compounds adsorbed on the surface. The development of 3.5-μm particles improves the separation power of graphitic carbon columns with the high theoretical plate number.

Keywords: Reviews; Carbon columns; Retention mechanism; Polar compounds

Contents

1. Introduction

Polar compounds are usually separated by ion-exchange liquid chromatography using ion-exchangers or reversed-phase ion-pair liquid chromatography using octadecyl-bonded silica gel or organic polymer gels. Reversed-phase ion-pair liquid chromatography is a very powerful separation method, but the separation of very polar compounds is still difficult.

*Tel.: +81-757-124-158; fax: +81-757-022-141.
E-mail address: thanai@attglobal.net (T. Hanai).

0021-9673/02/$ – see front matter
doi:10.1016/S0021-9673(02)02017-4

Improvement of the chemical stability of silica-based columns expands the application of polar compounds in reversed-phase ion-pair liquid chromatography. However, ion-exchange liquid chromatography is predominant for separation of very polar compounds, such as saccharides, various ionized compounds and ions even though the handling of ion-exchange resins is not easy compared to silica-based packing materials. A new ion-exchange resin was developed [1–3] in which the guanidino-phase was made from the amino-phase of vinylalcohol copolymer gel, and used for chromatography of saccharides [1] and acidic drugs [2]. Saccharides were quantitatively recovered from the guanidino-phase but not from the original amino-phase. The guanidino-phase was used to study drug–albumin interactions [3]. Other new organic polymer based-phases have not been developed for chromatography of polar compounds in the last few years. On the other hand, carbon columns have unique properties compared to silica-based packing materials and ion-exchangers, the matrixes of which are organic polymers. Carbon columns are physically and chemically stable. They can resist strong acidic and alkaline conditions, and no swelling is observed in various organic solvents. Furthermore, they are very hydrophobic compared to chemically modified silica gels and porous organic polymer gels.

According to Hosoya [4], a graphitic carbon molecule should consist of over 10^5 carbons, and the localized electrons can be observed at the edge of graphitic carbons after analysis by Hückel's calculation. This may mean that the center of graphic carbon has no localized electrons and is neutral except for induced effects. Several polycyclic aromatic hydrocarbon phases were constructed using the molecular editor of the CAChe™ program, and their electron densities were calculated to study the retention mechanism on graphitic carbon using Extended-Hückel and MOPAC calculation after optimization of their structure using the CAChe™ program [5].

Previously, Lim reviewed biomedical applications of porous graphitic carbon (PGC). He described the structure and chromatographic properties of PGC, development of separation on PGC, biomedical applications and maintenance of the PGC column [6]. Knox and Ross reviewed carbon-based packing materials for liquid chromatography: development and production of carbon-based packing materials for HPLC, structure and performance of PGC, retention mechanisms, and collected data of separation of geometric isomers, enantiomers, sugars, carbohydrates and glucuronides, ionized and highly polar compounds [7,8].

This review focuses on the retention mechanisms and recent applications of porous graphitic carbon.

2. Structure and retention mechanisms of graphitic carbon

2.1. Chromatographic analysis of retention mechanisms

Since a novel synthesis method of porous graphitic carbon (PGC) of small particle-size was developed by Knox et al. [9–11], PGC has been applied for chromatography of various kinds of compounds. The structure of PGC is homogenous compared to silica-based packing materials and ion-exchange resins of organic polymers, but the retention mechanisms are complex. The retention mechanisms have been discussed based on chromatographic results of a variety of compounds. The retention mechanism and the selectivity of solutes on porous glassy carbon in HPLC and SFC have been studied [12–22]. Glassy carbon, like graphite, is a hydrophobic, highly-polarizable solid. On reversed-phase HPLC, it can be classified as an adsorbent where the glassy carbon surface acts as a Lewis base toward polar solutes and is involved in π–π interactions and dispersive interactions with aromatic solutes. Porous glassy carbon has the advantage of extreme pH stability. The homogeneous surface has fewer chromatographic active sites and is good for chromatography of basic compounds. According to Gu and Lim, the retention mechanism is a mixture of hydrophobic and electrostatic interactions [23] because π-electron recognition increases with a higher degree of graphitization, although there is no change in the selection for a methylene group.

On porous graphitic carbon, the retention factor was found to increase with increases in the number

of polar substituents, and was shown to depend on both the field and mutual resonance effects of the different substituents on the aromatic ring. Hennion et al. concluded that electronic interactions are more important than hydrophobic interactions in the retention of polar compounds. The various parameters of the polarity of solutes, taking into account the field and resonance effects, were analyzed using local dipole moments and the overall electron-excess charge density [24]. The retention of polar benzene derivatives was correlated directly with their degree of ionization [25]. Forgacs and Cserhati described the effects of physicochemical parameters on the retention of some monoamine oxidase inhibitory drugs on a porous graphitized carbon column. They concluded that both steric and electronic parameters influenced the retention of 16 monoamine oxidase inhibitory drugs [26]. Barrett et al. investigated the chromatographic behaviour of morphine-based opiates using a porous graphitic carbon column at acid and alkaline pH. The retention order was not related to the log *P* values of the opiates, and strong retention of the fully ionized compounds was observed, particularly those with acidic functional groups, suggesting that hydrophobic interactions were present in addition to the polar retentive effects [27]. Chaimbault, Elfakir and Lafosse studied the retention behavior of polyethoxylated alcohols on porous graphitic carbon and polar as well as apolar bonded-silica phases. They found that PGC support offers stronger interactions with these compounds than ODS packing. The retention increased with increasing hydrocarbon chain length and increasing ethylene oxide number [28]. Wan et al. also performed a comparison study of PGC and octadecyl bonded silica for the retention of 36 positional isomers of ionizable substituted benzenes. They found the greater steric discriminating ability from the flat surface of PGC [29]. Leira et al. used a PGC and an octadecyl bonded silica column for an effective separation of non-flavonoid polyphenols [30]. Knox and Ross summarized the retention by graphite from aqueous/organic eluents is apparently determined by a balance of two factors: (1) hydrophobicity, which is primarily a solution effect tending to drive analytes out of solution and (2) the interaction of polarizable or polarized functional groups in the analyte with graphite, which is in addition to the normal dispersive interactions. The second effect is particularly strong when the stereochemistry of the analyte molecules forces the polar group to be close to the graphite surface. They called this the polar retention effect by graphite, and it appears to be an effect which is additional to the normal hydrophobic and dispersive effects found with conventional reversed-phase materials [7]. PGC has the disadvantage of being very retentive, and higher molecular mass solutes may not be eluted from columns of this material. Large mobile phase additives such as *p*-terphenyl have been used to reduce retention and improve peak shapes [31,32]. Eluotropic strength in non-aqueous liquid chromatography was studied using 10 organic solvents from the fatty acid methyl ester homologous series. A modeling of the eluotropic strength for binary mobile phases was envisaged in order to provide a prediction tool [33].

A low temperature glassy carbon (LTGC) stationary phase coated on porous glassy carbon and zirconium oxide was chromatographically evaluated. Glassy carbon is microscopically amorphous but contains the regional microstructures of graphite. Glassy carbon is less dense than graphite, which implies structural voids. Glassy carbon is particularly well suited as an adsorbent or stationary phase material for HPLC because of its excellent mechanical and chemical stability and high surface area (150–200 m^2/g) [34]. LTGC-coated PGC had less reversed-phase retentivity than the uncoated PGC through decreased solute-stationary phase dispersive interactions. Both LTGC-coated phases had faster mass transfer kinetics than PGC. LTGC-coated zirconia had the lowest reversed-phase retentivity and produced the highest efficiency columns with good mass transfer characteristic of its pellicular-like structure. The selectivity of the LTGC-coated zirconia was most like that of conventional reversed-phase HPLC [31,35].

2.2. Computational chemical analysis of the retention mechanisms

The possibility of computational chemical analysis of molecular interactions can be understood from analysis of simple model compounds. The ion–ion

interaction was first studied for combination of a tetrabutyl ammonium (cation) and a methylphosphate (anion) by molecular mechanics calculation using the CAChe™ program. The ion-pair formation and their energy values calculated using molecular mechanics were as follows.

	$(CH_3)_4N^+$	+ $CH_3HPO_4^-$	→ $(CH_3)_4CH_3HPO_4$
Stretch	0.458	0.968	1.420
Stretch bend	0.149	−2.018	−1.873
Improper torsion	0	0	0
Electrostatic	0	2.193	−0.327
Angle	0.634	6.596	7.215
Dihedral angle	0	0.055	0.017
Van der Waals	3.114	0.962	2.038
Hydrogen-bond	0	−0.622	−0.609
Final	4.3562	8.1337	7.8803

unit: kcal/mol

The structure of tetramethylammonium and methylphosphate ions and the ion-pair are shown in Fig. 1, and their electron density is shown in Fig. 2.

The above results indicated that Van der Waals energy contributes to their ion–ion interaction, and electrostatic energy contributes to interactions, too. Then, the retention mechanisms of these cations and anions were studied using a model carbon-phase. Graphitic carbon should be constructed using more than 10^5 carbon atoms [4]. However, the calculation capacity of a computer is limited. First a polycyclic aromatic hydrocarbon (PAH7) consisting of 7 rings (coronene) was constructed, the structure was optimized using molecular mechanics, and the net atomic charge was calculated using the Extended-Hückel calculation. Furthermore atomic charge and atom electron density were calculated using MOPAC of CAChe program. The electron density is shown in Fig. 3.

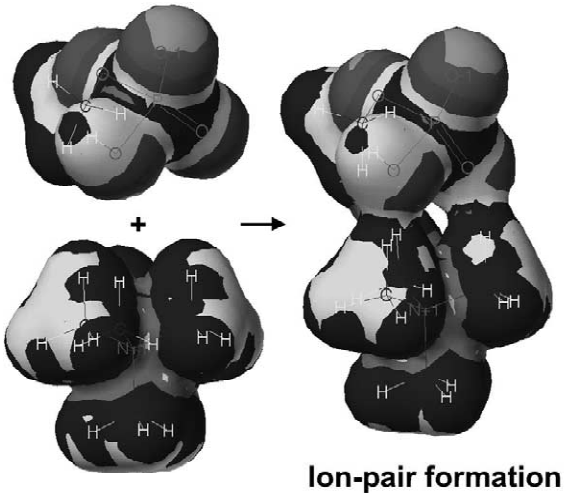

Fig. 2. Electron density of tetramethylammonium ion, methylphosphate ion and tetramethylammonium-methylphosphate ion-pair optimized by MOPAC.

Net atomic charge of the center carbons of PAH7 was positive (0.1610 au), and that of the outer carbons was negative (−0.2375 au) by Extended-Hückel calculation. Atomic charge of the center

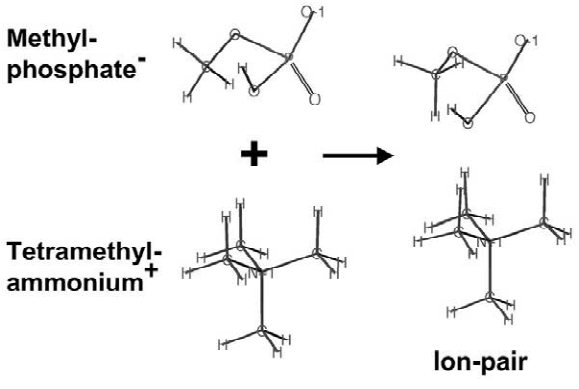

Fig. 1. Ion-pair formation between tetramethylammonium and methylphosphate ions.

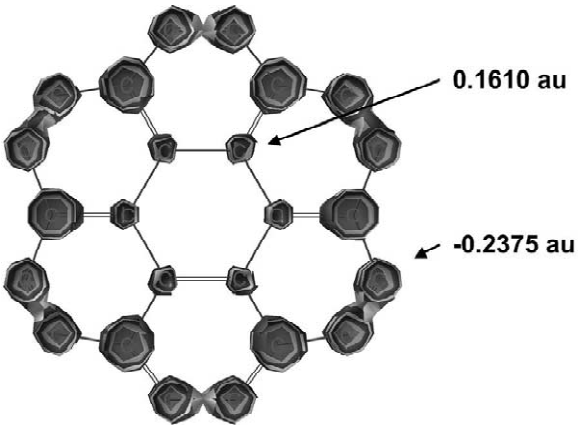

Fig. 3. Electron density of PAH7 optimized by MOPAC.

carbons was −0.0250 and those of outer carbons was −0.0661 according to MOPAC. These values were slightly changed after molecular interactions were calculated. Net atomic charge and atomic charge of center carbons were 0.1612 and −0.0272 au by Extended-Hückel and MOPAC calculations, respectively. Those of outer carbon atoms were −0.2391 and −0.2361 au, −0.0644 and −0.0646 au, respectively, for the pairs of molecules. No significant differences were observed between the two molecules. However, the formation of a pair reduced electronic energy and ionization potential, and increased core–core repulsion energy as summarized in Table 1.

Doubling the size of the molecule (PAH14) reduced the mean net atomic charge of center atoms from 0.1610 to 0.0978 au and of outer atoms from −0.2375 to −0.3125 au according to Extended-Hückel calculation. The net atomic charge of center atoms was further reduced by increasing the number of rings. The value was affected by the shape of the final molecule, (symmetry or asymmetry). The value was 0.0189 au for PAH22, and 0.0281 au for PAH31, and was dependent on the length and width ratio. The electrostatic potentials are shown in Fig. 4. This structure indicated the existence of two types of molecular interactions; hydrophobic interaction at the center of large molecules and electrostatic interaction at the edge of graphitic carbon. This also indicated that electron density is low at the center, and high at the edge of the molecule. The electron charge of center atoms of larger molecules was lower than that of small molecules. These observations suggested that the electron charge of the center of graphitic carbon is close to zero and neutral. Similar phenomena were also observed in a saturated carbon-layer of 22 rings the structure of which is similar to that of PAH22 with a more homogenous net atomic charge

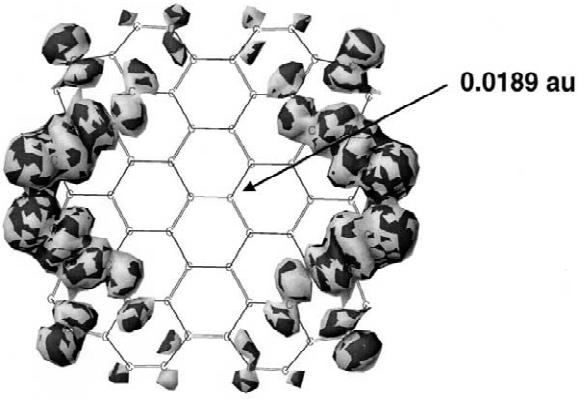

Fig. 4. Electrostatic potential of PAH22 optimized by MOPAC.

distribution. The net atomic charge of the center carbon was 0.0168 and that of the outer carbon was −0.1987 au. Therefore, a saturated carbon layer with a smaller size can be used as a model of graphitic carbon phase which requires more than 10^5 carbon atoms due to the calculation capacity of the computer used and rigidness of porous graphitic carbon.

The molecular interactions between tetrabutyl or methylphosphate ions and three model layers of graphitic carbon were calculated using the CAChe program. The size of model layers was decided by the calculation capacity of the computer. Polycyclic aromatic hydrocarbon layers were constructed with 31 (PAH31) and 83 (PAH83) aromatic rings. A double layer of polycyclic aromatic hydrocarbon layer was constructed with two layers of 22 aromatic rings (PAH22×2). The localized electron of the PAH22×2 layer did not affect the adsorption of a cation as observed on tetrabutyl-methylphosphate ion-pair formation and had little effect on methylphosphate ion. Van der Waals energy showed the greatest contribution to their adsorption as summarized in Table 2.

Table 1
Change of molecular properties due to PAH7 pair formation

Properties	Single PAH7	Pair of PAH7s	Balance
Final heat of formation/kcal	808.939	1618.322	0.444
Total energy/eV	−3042.349	−6084.679	−0.019
Electronic energy/eV	−20786.277	−61868.544	−20295.990
Core–core repulsion/eV	17743.928	55783.865	20296.009
Ionization potential/eV	9.420	9.491	−9.349

Balance = pair of PAH7s-2(single PAH7)

Table 2
Adsorption of $(CH_3)_4N^+$ and $CH_3HPO_4^-$ on PAH22×2 layer

	PAH22×2	$(CH_3)_4N^+$/PAH22×2	$CH_3HPO_4^-$/PAH22×2
Stretch	187.711	188.150	188.710
Stretch bend	0	0.146	−2.051
Improper torsion	13.258	13.258	13.258
Electrostatic	0	0	2.244
Angle	0	0.636	6.598
Dihedral angle	0	0.001	0.078
Van der Waals	104.622	102.340	102.065
Hydrogen-bond	0	0	−1.365
Final	305.5911	304.5301	309.5363

unit: kcal/mol

Furthermore, the adsorption site effect of ion–dipole type interaction was studied by adsorption of methylphosphate ion on the larger polycyclic aromatic hydrocarbons PAH31 and PAH83. The electron density of PAH83 could not be calculated because of the calculation capacity of the computer used. As demonstrated before using the smaller polycyclic aromatic hydrocarbons PAH14 and PAH22, the electronic charge of the center carbons of PAH83 is less than that of PAH31. The contribution of electrostatic energy must be eliminated for adsorption of such small ions at the center of PAH83, but electrostatic energy may contribute a little at the edge of PAH. The changes in energy values by adsorption at both the center and edge of PAH31 and PAH83 are summarized in Table 3.

The above results indicated that the adsorption site did not affect the electrostatic energy or Van der Waals energy even in cases in which the molecular sizes of adsorbents (PAH) are quite different. Ion–dipole interaction was negligible unlike ion-pair formation of tetrabutyl and methylphosphate ions, with van der Waals energy showing the main contribution for adsorption [36].

Guanidino compounds are polar and retained on a graphitic carbon layer without ion-pair reagent, and several guanidino compounds were separated in potassium citrate buffer at pH 4.5. Arginine retained strongly on a graphic carbon has two ionic groups, anionic carboxyl groups and a cationic guanidyl group. The molecular interactions between arginine and three model layers of graphitic carbon were analyzed using the CAChe program. The size of model layers was determined by the calculation capacity of the computer. A polycyclic aromatic hydrocarbon layer was constructed with 31 aromatic rings (PAH31). A double polycyclic aromatic hydrocarbon layer was constructed with two layers of 22 aromatic rings (PAH22×2). A carbon layer was constructed by combining two layers of 32 cyclic

Table 3
Adsorption of methylphosphate ion on PAH31 and PAH83

	PAH31	C/PAH31	E/PAH31	PAH83	C/PAH83	E/PAH83
Stretch		123.067	123.080	316.552	317.233	317.136
Stretch bend	0.000	−2.041	−2.048	0.000	−2.046	−2.034
Improper torsion	0.000	0.023	0.025	0.000	0.027	0.020
Electrostatic	0.000	2.195	2.191	0.000	2.189	2.197
Angle	0.000	6.599	6.605	0.000	6.607	6.603
Dihedral angle	0.000	0.050	0.056	0.000	0.057	0.047
Van der Waals		948.223	948.052	2426.760	2420.650	2420.623
Hydrogen-bond	0.000	−0.849	−0.870	0.000	−0.870	−0.849
Final		1077.267	1077.091	2426.760	2743.847	2743.743

unit: kcal/mol, C/PAH31 and C/PAH83: adsorption of methylphosphate ion at center of PAH31 and PAH83, E/PAH31 and E/PAH83: adsorption of methylphosphate ion at edge of PAH31 and PAH83.

rings (C32×2). The maximum retention of arginine was observed on a graphitic carbon column with potassium citric buffer, pH 4.5, and sodium phosphate buffer, pH 12.90, as the eluent [37]. Therefore, the molecular interactions were calculated using both cationic and anionic forms of arginine. Fig. 5A shows that PAH22×2 interacts with the guanidyl group of arginine at the side, and Fig. 5B shows that PAH22×2 interacts with the carboxyl group of arginine at the side. Arginine adsorbs on C32×2 phase. Fig. 6A is the top view and Fig. 6B is the side view.

Molecular interaction analysis indicated no clear ion–dipole interaction for the retention of arginine on polycyclic aromatic hydrocarbons even if the localization of electrons occurs at the edge of the molecule. A cationic guanidino group seemed to be involved in adsorption. Very weak hydrogen bonding or ion–dipole interaction may contribute through affecting molecular structure or anionic carboxyl groups on a graphitic carbon, but the main forces were hydrophobic interactions (van der Waals interactions) as demonstrated by analysis of adsorption of tetrabutylammonium and methylphosphate on polycylic aromatic hydrocarbons.

In conclusion, saccharides and ions show similar retention on graphitic carbon as an ion-exchange liquid chromatography on a graphitic carbon. However, computational chemical analysis using model polycyclic aromatic hydrocarbons as models of graphitic carbons indicated that the contribution of electrostatic effect is negligible but that of hydrophobic effect related to van der Waals energy change is predominant for the retention. These observations indicated that an oxygenated surface group, –COOH [38], will contribute to the retention of cations by ion–ion interaction, but chromatographic behavior of guanidino compounds indicated that cation-exchange is negligible as demonstrated by computational chemical calculation. The retention of guanidino

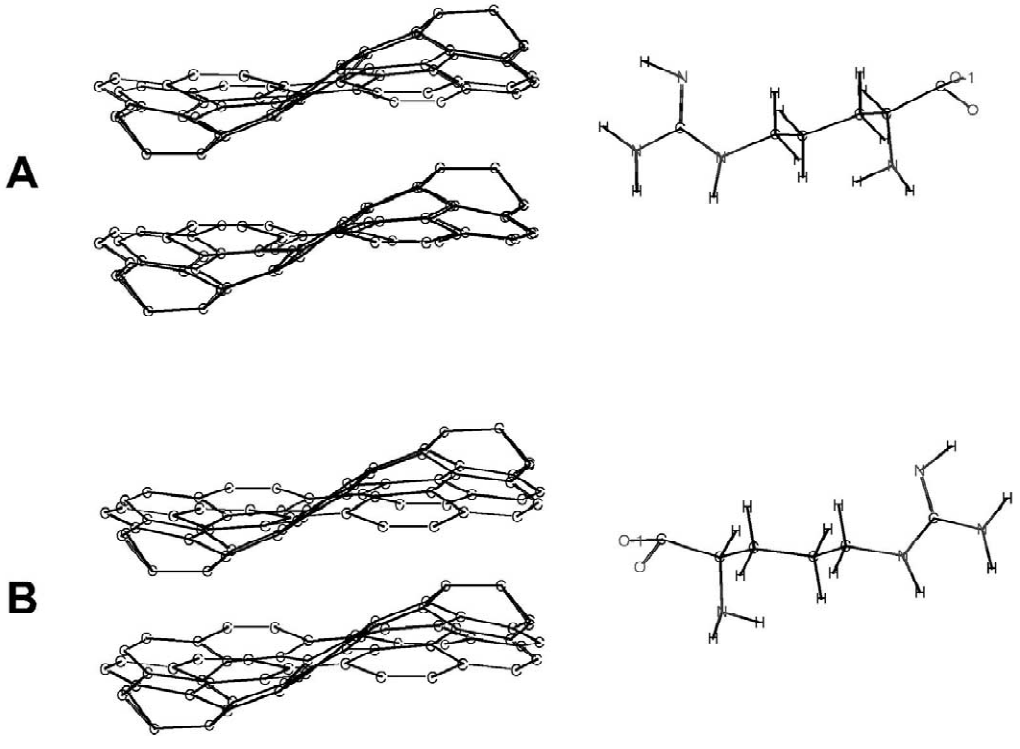

Fig. 5. Molecular interaction between arginine and PAH22×2 phase in high pH. A: complex between guanidyl group and PAH22×2 at side position, B: complex between ionized group and PAH22×2 at side position.

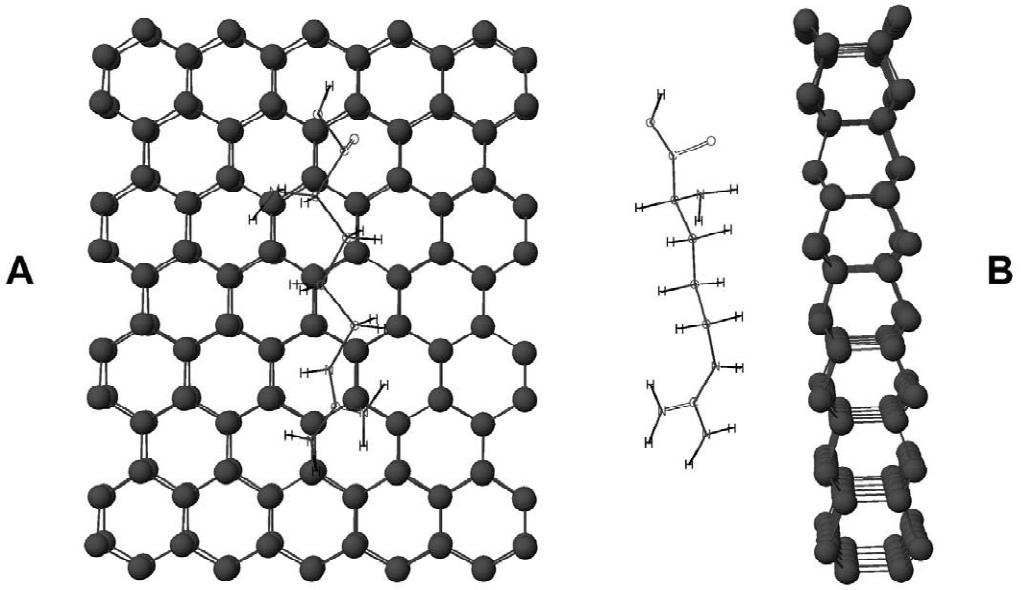

Fig. 6. Molecular interaction between arginine and C32×2 phase in low pH. A: top view, B: side view.

compounds in the molecular form was stronger than that of their ionic form. Therefore, hydrophobic interactions seemed to be predominant.

3. Synthesis of porous graphitic carbon

There are three types of porous graphitic carbons. Carbonization of the organic precursors below 1000 °C results in an amorphous pyrolytic carbon containing micropores and mesopores. It possesses an oxygenated surface bearing various functional groups such as –OH, –COOH, –C–O–C–, etc. When pyrolytic carbon is heated to about 1500 °C, amorphous glassy carbon is produced. Carbon heated above 2000 °C has the atomic structure of two-dimensional graphite. These materials show different adsorption properties [38].

In 1978, Knox and Gilbert patented a method of making porous carbon [9]. In this method, a high-porosity HPLC silica gel is impregnated with a phenol-formaldehyde resin. The resin is carbonized at 1000 °C in nitrogen or argon, and the silica particles dissolved out with alkali. They called this material ''porous glassy carbon'' (PGC). The material proved to be microporous, and its LC performance was poor. On heating to 2000~2800 °C, the micropores closed and the material became graphitized [11]. This material was called porous glassy carbon, and marketed under the trade name Hypercarb™. Obayashi, Ozawa and Kawase of Tonen Corporation [39] made porous graphites by an entirely different procedure. In their method, a roughly 50/50 mixture of low molecular mass pitch (MW~300) and a polymerizable monomer, such as styrene and/or divinylbenzene, along with a suitable initiator, is suspended in water and the monomer polymerized. The beads are then separated and heated in stages to 1100 °C, and finally to around 2800 °C. The typical material so produced has pores with diameters from 200 to 500 Å. The material is marketed under the trade name BTR Carbon column by BioTech Research Co. (Kawagoe, Japan). Another method was developed by Ichikawa, Yokoyama, Kawai, Moriya, Komiya and Kato of Nippon Carbon Company and Tosoh Corporation [40]. In this procedure, equal amounts of carbon black colloid particle with a diameter of about 300 Å and a phenolic resin are dissolved in methanol, made into a slurry, and then spray-dried to give roughly spherical particles in the range of 3–100 μm. The polymerization of the phenolic resin is completed at about 140 °C, and the

particles heated at a controlled rate in nitrogen to 1000 °C and then to 2800 °C. The final materials have specific surface areas ranging from 20 to 120 m^2/g, and specific pore volumes from 0.3 to 1.0 ml/g. The material is marketed under the trade name TSKgel Carbon-500 by Tosoh.

Rittenhouse and Olesik developed a low temperature graphitized porous carbon [34]. Poly-(phenylene diethyl) compounds are first synthesized at low temperature (200–800 °C), and then slowly heated to produce glassy carbon. If the LTGC is treated at low processing temperatures (i.e., 200 °C), then its surface specificity is similar to those of bonded phenyl polysiloxane stationary phases [41]. As the processing temperature is increased, conjugation within the LTGC increases and the dipolarity/polarizability of the solute becomes more important. At processing temperatures close to 550 °C, the LTGC shows chemical specificity and retention very similar to those of PGC.

Hirayama et al. prepared spherical carbon packing materials from spherical cellulose particles. They described the retention mechanism from the π-electron and the steric selectivity [42].

4. Applications

4.1. Use as matrix for dynamic coating on porous graphitic carbon

Since the surface of graphite consists essentially of gigantic aromatic molecules, it contains no surface functional groups. Porous graphites are therefore expected to be highly reproducible materials, the adsorptive properties of which should be independent of their mode of manufacture. In addition, graphite is extremely unreactive and unaffected by aggressive eluents such as strong acids and bases. Bare graphite behaves as a strong reversed-phase packing material [31]. Since the derivatization is very difficult, the only way to alter its chromatographic properties is by adsorption of suitable modifiers.

Porous graphitic carbon is very hydrophobic, and therefore it is suitable to prepare different phases after dynamic coating of the surface. Wan et al. used N-substituted L-phenylalanine chiral selectors for separation of amino acid enantiomers [43]. After coating the surface of porous graphitic carbon with a series of N-substituted L-phenylalanine chiral selectors, enantioselectivity of 36 amino acid enantiomers was studied. The coated PGC phases all showed appreciable enantioselectivity for both non-polar and acidic amino acids but basic amino acids were predominantly unretained. The order of retention of a pair of amino acid enantiomers was L>D on the alkyl-L-phenylalanine phases but a reversed retention order (D>L) was observed on the aryl-substituted L-phenylalanine phases. N-Blocked dipeptides [44] and losalorid [45] were also used as chiral selectors. The coated PGC demonstrated excellent chiral selectivity and column stability for chiral ligand-exchange liquid chromatography. The use of PGC as a support matrix for chiral ligand-exchange liquid chromatography has enabled more effective investigation of the retention mechanism of enantioselectivity without undesirable interference from secondary interactions observed with silica-based supports. Knox and Wan also used an enantiomeric modifier for separation of enantiomers of amino acids and hydroxy acids [46].

Okamoto, Isozaki and Nagashima studied elution conditions for the determination of anions by suppressed ion-interaction chromatography using a graphitized carbon column. A combination of tetrabutylammonium hydroxide and acetonitrile have been investigated to optimize the separation of common anions, F^-, Cl^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-}, HPO_4^{2-} and I^- [47]. Cetyltrimethyl ammonium ion [48] and alkylammonium-*o*-phosphate [49] were used for the separation of anions.

Knox and Wan [50] prepared three types of weak anion-exchangers by adsorption of polyethyleneimine (PEI) onto porous graphite (PGC): PGC coated dynamically with PEI, and PGC coated with a cross-linked polymeric PEI. Using the model solutes iodate, bromide, nitrate and nitrite, dissolved in aqueous buffers, the modified PGC showed typical ion-exchange behavior, and exhibited chromatographic performance similar to that of bonded ion-exchange silica gel. The properties of an anion-exchanger may be conferred on porous graphitic carbon by adsorption of polyethyleneimine from aqueous solution. This may be achieved dynamically using a solution of 0.1% PEI, by forming an insoluble monolayer (deposited dynamically but fixed by passage of phosphate buffer in which PEI is

insoluble). The insoluble monolayer coating gives the best performance and shows excellent stability with no change in retention after passage of 8000 column volumes of eluent [50]. Monser and Greenway used heptane sulfonic acid for separation of amines [51]. Nitrobenzoyl- and hexylbenzoyl-modified graphitic carbon were used for the separation of substituted phenols and pharmaceutical agents, hexobarbital, oxazepam, nitrazepam [52].

Direct liquid chromatographic analysis of drugs in serum was performed after filling pores of carbon with cross-linked dextran [38]. This method can be applied for analysis of relatively polar drugs, retention of which on ordinary inter-hydrophobic phases for quantitative analysis is usually poor.

4.2. Direct separation of polar compounds on porous graphitic carbon

The strong hydrophobicity of graphite permits reversed-phase type chromatography of polar compounds. Porous graphitic carbon is stable in strong acid and alkaline solutions and is extremely hydrophobic, and the retention time of ordinary compounds were quite long compared to those of commonly used octadecyl-bonded silica gels. Therefore, porous graphitic carbon is suitable for the chromatography of very polar compounds such as saccharides, ions and guanidino compounds.

Guanidino compounds are very polar and usually exist in ionic form due to the high pK_a value of the guanidino group. Separation is carried out by ion-exchange liquid chromatography [53–56] or reversed-phase ion-pair liquid chromatography [57,58], but to date the separation efficiency has not been satisfactory. Pre-derivatization was applied using benzoin [59,60] and ninhydrin [61]. However, their separation and reproducibility was not satisfactory due to the instability of packing materials and the complicated separation systems required.

The retention capability of a graphitic carbon column for guanidino compounds is shown in Fig. 7, where their retention times were measured in 50 m*M* sodium citrate buffer at ambient on an octadecyl-bonded silica gel column (Luna C_{18}, 150×4.6 mm I.D.) and a graphitic carbon column (TSKgel Carbon 500, 100×4.6 mm I.D.). Guanidino compounds were not retained on the octadecyl-bonded column even

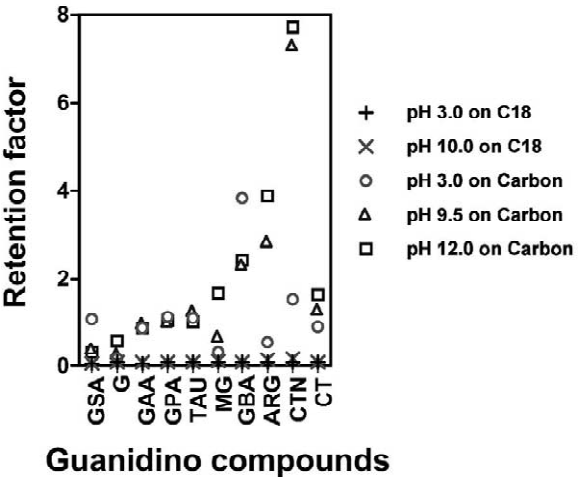

Fig. 7. Retention factors of guanidino compounds on an octadecyl-bonded silica gel column and a graphitic carbon column. Column C_{18}: Phenomenex Luna C_{18} (150×4.6 mm I.D.), Carbon: TSKgel Carbon 500 (100×4.6 mm I.D.), Eluent: 50 m*M* sodium citrate; GSA: guanidino succinate, G: guanidine, GAA: guanidino acetate, GPA: guanidino propionate, TAU: taurocyamine, MG: methylguanidine, GBA: guanidino butyrate, ARG: arginine, CTN: creatinine, CT: creatine.

under low and high pH solutions. Several guanidino compounds were separated on the graphitic carbon column. Total separation was performed by addition of ion-pair reagent in pH 4.5 sodium citrate buffer, and the chromatogram is shown in Fig. 8 [37]. This separation system was successfully applied to analyze sera from diabetic patients. The addition of ion-pair reagent in sodium citrate buffer increased retention on an octadecyl-bonded silica gel column. The difference in hydrophobic retention capacity of octadecyl-bonded silica gel and graphitic carbon columns was effectively applied to separate ten guanidino compounds using isocratic elution with a column switching system. The chromatogram is shown in Fig. 9 [62]. This parallel separation of guanidino compounds with different polarity using isocratic elution shortened the analysis time. The number of samples was doubled from the gradient elution. This type of column selection will be useful to shorten the time required for separation of complex mixtures.

Saccharides are separated by ion-exchange liquid chromatography and normal-phase liquid chromatography using very polar phases such as anion-ex-

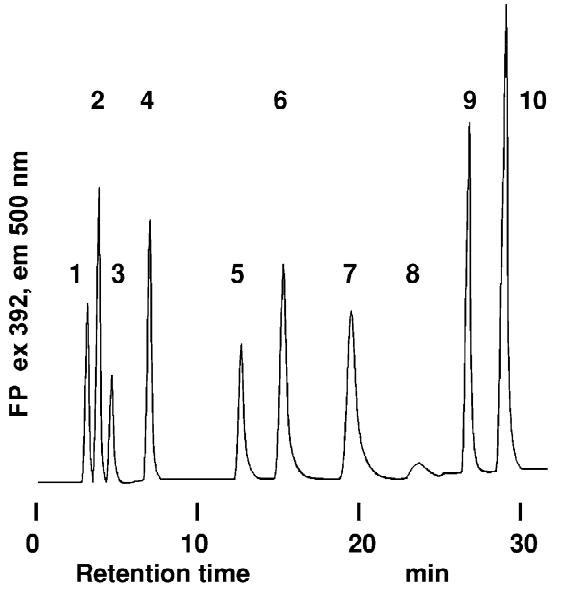

Fig. 8. The separation of ten guanidino compounds on a porous graphitic carbon column in ion-pair liquid chromatography. Column: BTR carbon, 50×4.6 mm I.D. packed 3.5 μm graphitic carbon, Eluent: four step-wise gradient from 10 m*M* sodium citrate buffer (pH 4.50) containing 20 v% acetonitrile, Flow-rate: 0.8 ml/min at 40 °C. Fluorescence detection: ex. 392 and em. 500 nm using 0.6% ninhydrin and 1 *M* NaOH solutions at flow rate of 0.2 ml/min. Peak 1: guanidino succinate, 2: taurocyamine, 3: creatine, 4; guanidino acetate, 5: guanidine, 6: guanidino propionate, 7: methylguanidine, 8: creatinine, 9: arginine, 10: guanidino butyrate.

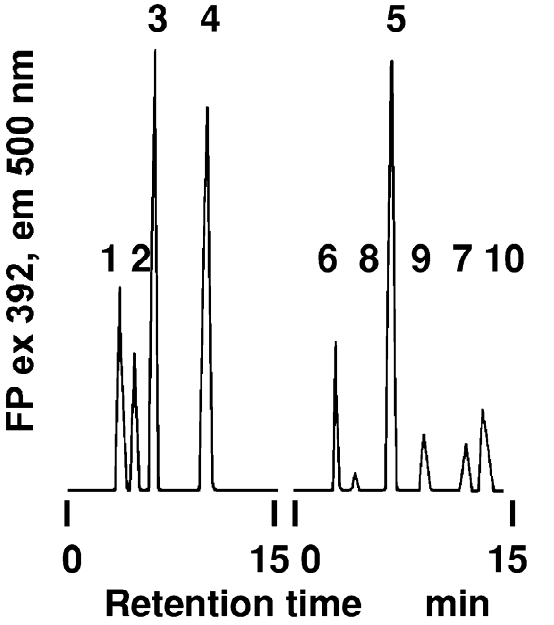

Fig. 9. Column-switching and isocratic separation of ten guanidino compounds in ion-pair liquid chromatography. Column A: TSK super ODS, 50×4.6 mm I.D. packed 2 μm ODS silica. Peaks 1–10 and detection conditions: see Fig. 8.

changers, amino and amido-phases and guanidino-phases in highly concentrated aqueous acetonitrile. Saccharides are also separated on graphitic carbon columns in basic solution in which saccharides are ionized as in ion-exchange liquid chromatography [63–74].

Gu and Lim separated [23] anionic and cationic compounds of biomedical interest, TcO4- and ReO4-, using trifluoroacetic acid as a component of eluent. Takeuchi et al. also used graphitic carbon column for the separation of anions [22,75]. Forgacs and Cserhati studied the chromatographic behavior of sixteen monoamine oxidase inhibitory drugs (proparlgylamine derivatives) using ethanol–water mixtures as eluents [26]. Forgacs isolated an anti-cancer drug, taxol, from *Taxus baccata* using a porous graphitized carbon column with aqueous dioxane as the eluent [76].

Nazir et al. used a porous graphitic carbon column for high temperature separation of cyclosporin A and U using *tert.*-butylmethylether and methanol mixture as the eluent [77]. Wilson used superheated water as the mobile phase for the separation of drugs [78]. Isosorbide-5-mononitrate in Imdur tablets was analyzed by super critical fluid chromatography using methanol–carbon dioxide [79]. Yamaki et al. also used PGC for high temperature separation of peptides [80]. Li et al. reported chromatography of diphosphine-bridged complexes with heteronuclear Au–M(M = Mn, Re) bonds, 1,1′-bis(diphenylphosphine)ruthenocene, bis(diphenylphosphine)methane and triphenylphosphine-substituted heterometallic Au–Mn or Au–Re carbonyl complexes. The eluents were aqueous acetonitrile, dichloromethane or tetrahydrofuran and hexane mixtures. Molecular size and electronegativity influenced the elution order [81]. Elfakir and Lafosse separated alkylglycosides which are biological non-ionic surfactants widely used in membrane biochemistry using aqueous acetonitrile or methanol as the eluent [82]. Chaimbault, Elfakir and Lafosse concluded that porous graphitic carbon was

better than octadecyl bonded phases for chromatography of polyethoxylated alcohols [83]. Petro et al. prepared graphitic carbon and separated *cis–trans* isomers of α-irone in aqueous acetonitrile after deactivation by hydrogenation [38]. Karlsson, Berglin and Charron obtained better separation of alprenolol and related substances on a porous graphitic carbon column than an octyl bonded silica gel column in alkaline methanol without ion-pair reagent. Metoprolol and related substances were also separated on a porous graphitic carbon column [84]. Yamaki et al. [85] studied the chromatographic behavior of 133 peptides, with from 1 to 148 amino acid residues on a carbon column eluted with a linear gradient of acetonitrile [10–70%(v/v), 30 min] in 0.1% TFA solution. Peptides with aromatic residues retained stronger adsorption on the carbon than on the ODS column by an interaction based on the aromatic or graphitic nature of the surface of the microspherical carbon. Perfluorocarboxylic acids were used for the separation of free amino acids and small peptides [86–90].

Ting and Porter [91] used a porous graphitic carbon column for separation of corticosteroids using electrochemically modulated liquid chromatography. Changes in the voltage applied to the column markedly affected the efficiency as well as the elution order of the separation, with the mixture fully resolved at large negative values of applied potential. Mechanistic aspects in terms of the influence of changes in the applied voltage on the extent of the interactions between these analytes and the stationary phase were dependent on the strength of the donor–acceptor interactions between the analytes and PGC.

In general, trifluoroacetic acid and acetonitrile mixtures are used as eluents for chromatography of a variety of compounds such as creatinine and creatin in urine [23], remoxipride [23], alditols and glycopeptides [92], glucosinolates and desulfoglucosinolates [93], peptides including angiotensin [85], *O*-tetraacetyl-β-D-glucopyranosyl isothiocyanate derivatized amino acids [94] and phosphonic acids in a spiked tap water [95]. Trifluoroacetic acid and methanol mixture was used for the analysis of degradation products of 5-fluorouracil [96]. Trifluoroacetic acid and tetrahydrofuran mixture was used for the separation of polar drug metabolites. The polar drug conjugates from their parent compounds and from endogenous material present in urine were sufficiently separated on a PGC column [97]. Aqueous acetonitrile containing ammonium hydroxide or sodium hydroxide has been used as the eluent for chromatography of oligosaccharides [98] and carbohydrates [63].

A variety of polar pharmaceutical compounds were analyzed by liquid chromatography using graphitic carbons. These are 5-alkyl-2′-deoxyuridine derivatives [99], barbituric acid derivatives [100], tetracycline antibiotics and 6-Epi-doxycycline [101], ceramide [102], paracetamol and related compounds [103], L-DOPA and the metabolites [104] and phosphopeptides [105].

Arsenite and arsenare were directly separated on a graphitic phase [106]. Selenium-containing glutathion S-conjugates in a yeast extract [107] and Se-methyl-selenocysteine, selenomethionine, selenocysteine, selenothione and selenocysteine were also separated on a graphitic phase [108]. Graphitic carbon phase was used to study the role of the Na^+ ion on phenol derivatives-hydroxypropyl-β-cyclodextrin complex formation in water and methanol mixtures [109].

The very hydrophobic phase, graphitic carbon, has been used for the separation of a variety of compounds such as polyglycerols, precursor of polyglyceryl fatty acids [110,111], fatty acid methylesters [112], PCB [113], terpene derivatives [114], polar phenolic compounds in olive oil mill waste water [115], ethoxylated laurylalcohols [116], cyanuric acid in swimming pool water [117a,117b], nonylphenol and selected nonylphenol polyethoxylates [118], 3,N4-etheno-2′-deoxycytidine in crude DNA hydrolyzates [119] and acrylamide in cooked foods [120].

Graphitized carbon black was used for extraction of polar pesticides in water [121]. A variety of graphitized carbon phases have been used for solid-phase extraction [122].

5. Conclusions

The physical and chemical stability of graphitic carbons performed a variety of applications in both chromatography and extraction, especially for polar compounds, those are difficult on both silica-based and organic polymer-based packing materials. The disadvantage is difficult desorption of non-polar

compounds adsorbed on the surface. The development of 3.5 μm particles improves the separation power of graphitic carbon columns with the high theoretical plate number.

References

[1] T. Hanai, R. Miyazaki, J. Suzuki, T. Kinoshita, J. Liq. Chromatogr. Rel. Technol. 20 (1997) 2941.
[2] R. Miyazaki, T. Hanai, J. Suzuki, T. Kinoshita, J. Liq. Chromatogr. Rel. Technol. 21 (1998) 2887.
[3] T. Hanai, R. Miyazaki, T. Kinoshita, Anal. Chim. Acta 378 (1999) 77.
[4] H. Hosoya, Kotaibuturi 33 (1998) 181.
[5] CAChe™ program from Fujitsu.
[6] C.-K. Lim, Adv. Chromatogr. 32 (1992) 1.
[7] J.H. Knox, P. Ross, Adv. Chromatogr. 37 (1997) 73.
[8] J.H. Knox, P. Ross, Adv. Chromatogr. 37 (1997) 121.
[9] J.H. Knox, M.T. Gilbert, UK Patent 2035282 (1978).
[10] M.T. Gilbert, J.H. Knox, B. Kaur, Chromatographia 16 (1982) 138.
[11] J.H. Knox, B. Kaur, J. Chromatogr. 352 (1986) 3.
[12] B.J. Bassler, R. Kaliszan, R.A. Hartwick, J. Chromatogr. 461 (1989) 139.
[13] J.P. Weber, P.W. Carr, Anal. Chem. 62 (1990) 2620.
[14] R. Kaliszan, K. Osmialowski, B.J. Bassler, R.A. Hartwick, J. Chromatogr. 499 (1990) 33.
[15] T.M. Engel, S.V. Olesik, Anal. Chem. 63 (1991) 1554.
[16] Y. Cui, S.V. Olesik, Anal. Chem. 63 (1991) 1812.
[17] T.M. Engel, S.V. Olesik, Anal. Chem. 63 (1991) 1830.
[18] T.M. Engel, S.V. Olesik, J. High Resolut. Chromatogr. 14 (1991) 99.
[19] W. Larkins Jr., S.V. Olesik, J. Microcol. Sep. 5 (1993) 543.
[20] J. Kriz, E. Adamcova, J.H. Knox, J. Hora, J. Chromatogr. A 663 (1994) 151.
[21] C. Lepont, A.D. Gunatillaka, C.F. Poole, Analyst 126 (2001) 1318.
[22] T. Takeuchi, T. Kojima, T. Miwa, Chromatography 21 (2000) 147.
[23] G. Gu, C.K. Lim, J. Chromatogr. 515 (1990) 183.
[24] M.-C. Hennion, V. Coquart, S. Guenu, C. Sella, J. Chromatogr. A 712 (1995) 287.
[25] Q.-H. Wan, P.N. Shaw, M.C. Davies, D.A. Barrett, J. Chromatogr. A 697 (1995) 219.
[26] E. Forgacs, T. Cserhati, J. Chromatogr. B 681 (1996) 197.
[27] D.A. Barrett, M. Pawula, R.D. Knaggs, P.N. Shaw, Chromatographia 47 (1998) 667.
[28] P. Chaimbault, C. Elfakir, M. Lafosse, J. Chromatogr. A 797 (1998) 83.
[29] Q.-H. Wan, M.C. Davies, P.N. Shaw, D.A. Barrett, Anal. Chem. 68 (1996) 437.
[30] E. Leira, A. Botana, R. Cela, J. Chromatogr. A 724 (1996) 67.
[31] J.H. Knox, B. Kaur, High performance liquid chromatography, in: R.A. Hartwick, P.J. Brown (Eds.), 1989, p. 189.
[32] J.H. Knox, B. Kaur, G.R. Millward, J. Chromatogr. 352 (1986) 3.
[33] K. Gaudin, P. Chaminade, A. Baillet, J. Chromatogr. A 973 (2002) 61.
[34] C.T. Rittenhouse, S.V. Olesik, J. Liq. Chromatogr. Relat. Technol. 19 (1996) 2997.
[35] P.T. Jackson, P.W. Carr, J. Chromatogr. A 958 (2002) 121.
[36] T. Hanai, Proceeding 21st Int. Sym. Cap. Chromatogr. Electropho., June 20–24, 1999, p. 83.
[37] Y. Inamoto, S. Inamoto, T. Hanai, M. Tokuda, O. Hatase, K. Yoshi, N. Sugiyama, T. Kinoshita, J. Chromatogr. B 707 (1998) 111.
[38] M. Petro, F. Belliardo, I. Novak, D. Berek, J. Chromatogr. B 718 (1998) 187.
[39] Obayashi, Ozawa, Kawase, Tonen Corporation, European Patent, 0458548A (1990).
[40] Ichikawa, Yokoyama, Kawai, Morita, Komiya, Kato, Nippon Carbon Co. Ltd., and Tosoh Corporation, European Patent, 0458176A (1990).
[41] T.M. Engel, S.V. Olesik, M.R. Callstrom, M. Diener, Anal. Chem. 65 (1993) 3691.
[42] C. Hirayama, S. Nagaoka, T. Matsumoto, H. Ihara, J. Nonbo, H. Kurisaki, S. Ikegami, J. Liq. Chromatogr. 18 (1995) 1509.
[43] Q.-H. Wan, P.N. Shaw, M.C. Davies, D.A. Barrett, J. Chromatogr. A 765 (1997) 187.
[44] A. Karlsson, O. Karlsson, J. Chromatogr. A 905 (2001) 329.
[45] A. Tornkvist, P.J.R. Sjoberg, K.E. Markides, E. Heldin, Chirality 14 (2002) 653.
[46] J.H. Knox, Q.-H. Wan, Chromatographia 40 (1995) 9.
[47] T. Okamoto, A. Isozaki, H. Nagashima, J. Chromatogr. A 800 (1998) 239.
[48] H. Nagashima, T. Okamoto, J. Chromatogr. A 855 (1999) 261.
[49] M.C. Gennaro, E. Marengo, V. Gianotti, J. Liq. Chromatogr. Relat. Technol. 23 (2000) 2599.
[50] J.H. Knox, Q.-H. Wan, Chromatographia 42 (1996) 83.
[51] L.I. Monser, G.M. Greenway, Anal. Chim. Acta 322 (1966) 63.
[52] J.A. Harnisch, D.B. Garzda, J.W. Anderegg, M.D. Porter, Anal. Chem. 73 (2001) 3954.
[53] Y. Hiraga, T. Kinoshita, J. Chromatogr. 342 (1985) 269.
[54] Y. Hung, M. Kai, H. Nohta, Y. Ohkura, J. Chromatogr. 305 (1984) 281.
[55] B. Marescau, L.A. Qureshi, P. De Deyn, J. Letarte, R. Ryba, A. Lowenthal, Clin. Chim. Acta 146 (1985) 21.
[56] B. Marescau, P. De Deyn, L. Van Gorp, A. Lowenthal, J. Chromatogr. 377 (1986) 334.
[57] Y. Kobayashi, H. Kubo, T. Kinoshita, Anal. Sci. 3 (1987) 363.
[58] L.E. Webb, J. Chromatogr. 381 (1986) 406.
[59] M. Kai, T. Miyazaki, Y. Ohkura, J. Chromatogr. 311 (1984) 257.
[60] M. Kai, T. Miyazaki, Y. Yamaguchi, Y. Ohkura, J. Chromatogr. 268 (1983) 417.
[61] V.K. Boppana, G.R. Rhodes, J. Chromatogr. 506 (1990) 279.
[62] Y. Inamoto, S. Inamoto, T. Hanai, M. Tokuda, O. Hatase, K. Yoshi, N. Sugiyama, T. Kinoshita, Biomed. Chromatogr. 12 (1998) 239.
[63] K. Koizumi, Y. Okada, M. Fukuda, Carbohydr. Res. 215 (1991) 67.

[64] K.A. Thomsson, N.G. Karlsson, G.C. Hansson, J. Chromatogr. A 854 (1999) 131.
[65] N. Kawasaki, M. Ohta, S. Hyuga, O. Hashimoto, T. Hayakawa, Anal. Biochem. 269 (1999) 297.
[66] N. Kawasaki, M. Ohta, S. Hyuga, M. Hyuga, T. Hayakawa, Anal. Biochem. 285 (2000) 82.
[67] N. Kawasaki, Y. Haishima, M. Ohta, S. Itoh, M. Hyuga, S. Hyuga, T. Hayakawa, Glycobiology 11 (2001) 1043.
[68] S. Itoh, N. Kawasaki, M. Ohta, M. Hyuga, S. Hyuga, T. Hayakawa, J. Chromatogr. A 968 (2002) 89.
[69] N.G. Karlson, N.H. Packer, Anal. Biochem. 305 (2002) 173.
[70] E.F. Hounsell, M.J. Davies, K.D. Smith, Protein Protocols Handbook, J.M. Walker (Ed.), 2nd ed., Humana Press, Totowa, N.J., USA, (2002) 819
[71] T. Tanimoto, A. Ikuta, M. Sugiyama, K. Koizumi, Chem. Pharm. Bull. 50 (2002) 280.
[72] N. Kawasaki, M. Ohta, S. Itoh, M. Jyuga, S. Hyuga, T. Hayakawa, Biologicals 30 (2002) 113.
[73] Y. Daali, S. Cherkaoui, X. Cahours, E. Varesio, J.-L. Veuthey, J. Sep. Sci. 25 (2002) 280.
[74] I. Clarot, D. Cledat, L. Boulkanz, E. Assidjo, T. Chianea, P.L.P. Cardot, J. Chromatogr. Sci. 38 (2000) 38.
[75] T. Takeuchi, T. Kojima, T. Niwa, J. High Res. Chromatogr. 23 (2000) 590.
[76] E. Forgacs, Chromatographia 39 (1994) 740.
[77] T. Nazir, L.A. Gould, C. Marriott, G.P. Martin, M.B. Brown, Chromatographia 46 (1997) 628.
[78] I.D. Wilson, Chromatographia 52 (2000) S28.
[79] O. Gyllenhaal, A. Karlsson, J. Biochem. Biophys. Methods 43 (2000) 135.
[80] S. Yamaki, T. Isobe, T. Okuyama, T. Shinoda, J. Chromatogr. A 728 (1996) 189.
[81] C.-H. Li, P.M.N. Low, S. Li, H.K. Lee, T.S.A. Hor, Chromatographia 44 (1997) 391.
[82] C. Elfakir, M. Lafosse, J. Chromatogr. A 782 (1997) 191.
[83] P. Chaimbault, C. Elfakir, M. Lafosse, J. Chromatogr. A 797 (1998) 83.
[84] A. Karlsson, M. Berglin, C. Charron, J. Chromatogr. A 797 (1998) 75.
[85] S. Yamaki, T. Isobe, T. Okuyama, T. Shinoda, J. Chromatogr. A 729 (1996) 143.
[86] K. Petritis, P. Chaimbault, C. Elfakir, M. Dreux, J. Chromatogr. A 896 (2000) 253.
[87] A.A. Adoubel, S. Guenu, C. Elfakir, M. Dreux, J. Liq. Chromatogr. Rel. Technol. 23 (2000) 2433.
[88] C. Desportes, M. Charpentier, B. Duteurtre, A. Maujean, F. Duchiron, J. Chromatogr. A 893 (2000) 281.
[89] P. Chaimbault, K. Petritis, C. Elfakir, M. Dreux, J. Chromatogr. A 870 (2000) 245.
[90] K. Petritis, P. Chaimbault, C. Elfakir, M. Dreux, J. Chromatogr. A 896 (2000) 253.
[91] E.-Y. Ting, M.C. Porter, Anal. Chem. 69 (1997) 675.
[92] M. Davis, K.D. Smith, A.-H. Harbin, E.F. Hounsell, J. Chromatogr. 609 (1992) 125.
[93] C. Elfakir, M. Dreux, J. Chromatogr. A 727 (1996) 71.
[94] W.C. Chan, R. Micklewright, D.A. Barrett, J. Chromatogr. A 697 (1995) 213.
[95] J.-P. Mercier, Ph. Morin, M. Dreux, A. Tambute, J. Chromatogr. A 849 (1999) 197.
[96] L.K. Liem, L.H.L. Choong, K.T. Woo, Clin. Biochem. 35 (2002) 181.
[97] J. Ayrton, M.B. Evans, A.J. Harris, R.S. Plumb, J. Chromatogr. B 667 (1995) 173.
[98] J.-Q. Fan, A. Kondo, I. Kato, Y.C. Lee, Anal. Biochem. 219 (1994) 224.
[99] G.A. Csiktusnadi-Kiss, G. Sagi, Z. Tegyei, Chemia Analityczna 45 (1999) 795.
[100] E. Forgacs, T. Cserhati, Anal. Lett. 32 (1999) 1867.
[101] L. Monser, F. Darghouth, J. Pharm. Biomed. Anal. 23 (2000) 353.
[102] K. Gaudin, P. Chaminade, D. Ferrier, A. Baillet, J. Liq. Chromatogr. Rel. Technol. 23 (2000) 387.
[103] L. Monser, F. Darghouth, J. Pharm. Biomed. Anal. 27 (2002) 851.
[104] P. Koivisto, A. Tornkvist, E. Heldin, K.E. Markides, Chromatographia 55 (2002) 39.
[105] P.O. Vacratsis, B.S. Phinney, D.A. Gage, K.A. Gallo, Biochemistry 41 (2002) 5613.
[106] S. Mazan, G. Cretier, N. Gilon, J.-M. Mermet, J.-L. Rocca, Anal. Chem. 74 (2002) 1281.
[107] T. Lindemann, H. Hintelmann, Anal. Chem. 74 (2002) 4602.
[108] T. Lindemann, H. Hintelmann, Anal. Bioanal. Chem. 372 (2002) 486.
[109] Y.C. Guillaume, Y.T. Truong, J. Millet, L. Nicod, J.C. Rouland, M. Thomassin, J. Chromatogr. A 955 (2002) 197.
[110] P. Chaimbault, S. Cassel, S. Claude, C. Debaig, T. Benvegnu, D. Plusquellec, P. Rollin, M. Lafosse, Chromatographia 50 (1999) 239.
[111] S. Cassel, P. Chaimbault, C. Debaig, T. Benvegnu, S. Claude, D. Plusquellec, P. Rollin, M. Lafosse, J. Chromatogr. A 918 (2001) 96.
[112] C. Viron, P. Andre, M. Dreux, M. Lafosse, Chromatographia 49 (1999) 137.
[113] M.C. Pietrogrande, A. Benvenuti, S. Previato, F. Dondi, Chromatographia 52 (2000) 425.
[114] I. Clarot, D. Cledat, S. Battu, P.J. Cardot, J. Chromatogr. A 903 (2000) 67.
[115] J. Vial, M.-C. Hennion, A. Fernandez-Alba, A. Aguera, J. Chromatogr. A 937 (2001) 21.
[116] V. Nemeth-Kiss, A. Jacob, E. Forgacs, Chemia Analityczna 46 (2001) 613.
[117] R. Cantu, O. Evans, M.L. Magnuson, Chromatographia 53 (2001) 454;
R. Cantu, O. Evans, F.K. Kawahara, L.J. Wymer, A.P. Dufour, Anal. Chem. 73 (2001) 3358.
[118] J.L. Gundersen, J. Chromatogr. A 914 (2001) 161.
[119] C.W. Roberts, M.I. Churchwell, F.A. Beland, J.-L. Fang, D.R. Doerge, Anal. Chem. 73 (2001) 303.
[120] J. Rosen, K.-E. Hellenas, Analyst 127 (2002) 880.
[121] A.D. Corcia, R. Samperi, A. Marcomini, S. Stelluto, Anal. Chem. 65 (1993) 907.
[122] M.-C. Hennion, J. Chromatogr. A 885 (2000) 73.