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### Prospects for Carbon as Packing Material in High-Performance Liquid Chromatography

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PROSPECTS FOR CARBON AS PACKING MATERIAL  
IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Early work on use of carbons for liquid chromatography shows that special carbons must be developed if carbon is to be useful in HPLC. The processes used to make carbons, the properties of such carbons and the requirements for HPLC carbons are discussed. The preparation of HPLC carbons is reviewed in detail and the results which have been obtained are assessed in terms of their kinetic and thermodynamic performance. Applications are reviewed.

It is concluded that none of the existing HPLC carbons combine all the desired features of particle rigidity, adequate surface area and uniformity of surface chemistry but that there is no reason to doubt that such a material can eventually be prepared. The kinetic performance of existing materials is probably adequate but peaks are often wide due to inadequate control of the surface chemistry. Carbons from different sources exhibit similar but not identical selectivity. Broadly

carbons exhibit different selectivity from reversed phase silica gels. In particular they show greater discrimination of isomers and members of homologous series but less discrimination of functional groups. Carbons show much stronger retention with any eluent than do reversed phase silica gels but different eluents (other than water) show rather a small range of eluotropic strength.

### 1. INTRODUCTION

Active carbon adsorbents have for many years played an important role in industrial purification and refinement processes (1). Carbon was therefore an obvious choice for the early fundamental studies in column liquid chromatography by Tiselius and co-workers (2,3). The carbons used at that time and indeed until quite recently, were made from industrial products and were not specifically designed for chromatography. Amongst the substances separated on carbon using polar eluents were oligosaccharides and their methylated derivatives (4-7), amino acids (8-10), fatty acids and fatty alcohols (11) and even 5'-nucleotides obtained from the enzymatic digestion of RNA and DNA respectively (12). Analysis of retention patterns revealed that aromatic derivatives were more strongly adsorbed than aliphatic derivatives: this was attributed to the graphite-like surface of active carbons. In order to prevent irreversible adsorption of the solutes, carbons were often pre-treated with modifiers, e.g. stearic acid, n-octadecane, n-octadecylamine etc. (10, 13). With stearic acid as deactivating reagent, for example, aromatic amines could be eluted from carbon columns with high recoveries (10). Detailed studies were carried out on the effect of molecular size and eluent composition on the adsorption of methyl derivatives of pyridine (14,15). During this period the main theoretical interest in liquid chromatography centred on the treatment of adsorption phenomena and the evaluation of separation techniques: the status of carbon in this context was reviewed by Snyder in 1968 (16).

During the years following the early chromatographic experiments on carbon, manufacturing processes for carbons were extended and improved; carbon blacks in various grades and with defined properties were produced in large quantities for the rubber industry; the high-temperature treatment of carbon materials, including graphitization, was thoroughly examined. Concurrently gas chromatography was developed as a potential separation technique (17, 18). In gas-solid chromatography active carbon was employed as adsorbent for hydrocarbon separations (19) and graphitized carbon blacks were chosen as model adsorbents for examining gas-solid interactions (20,21), due to their non-polar and homogeneous surfaces. Graphitized carbon blacks were also employed as supports in gas-liquid chromatography (22,23) and as stationary phases in capillary columns, (24,25). Research between 1960 and 1970 in gas chromatography resulted in excellent separations of volatile hydrocarbons and provided a much better understanding of the phenomena occurring at the gas-solid interface.

For completeness' sake it may also be noted that a few experiments have been performed on active carbon as a support in thin-layer chromatography (26,27). The black colour of the plates, however, made a simple visualization of the spots difficult, although the drawback could be avoided by using radio-labelled solutes or by subsequent elution from the carbon into a silica layer.

Early in the 1970's a new era in chromatography opened with the development of high performance column liquid chromatography for which microparticulate silicas and their surface modified derivatives rapidly became the preferred column packings. However, in the search for new supports showing different selectivities and greater resistance to hostile eluents, especially alkalis, attention has once again turned to carbon. In contrast to 30 years ago, highly developed technologies now exist for producing various kinds of carbon: active carbons,

pyrolytic carbons, glassy carbons, carbon blacks and graphitized carbons. Between them these materials possess a wide range of surface and pore structures. Nevertheless, such carbons still, in the main, have been developed for purposes other than chromatography, e.g. as adsorbents in water treatment, as coatings, as fibres, as fillers etc. It is not therefore surprising that the development of carbon packings for HPLC has had to be carried out independently. The present review describes the work of the last decade and concludes with a critical discussion of what has been achieved.

## 2. PREPARATION OF CARBON PACKINGS

### 2.1. Desirable Characteristics for a Column Packing Material

To be applicable as a packing in HPLC a particulate carbon should possess the following characteristics: (i) sufficient hardness to withstand high pressures: (ii) a well-defined, reproducible and stable surface which shows no change during chromatographic work or storage: (iii) a specific surface area in the range of 50 to 500m<sup>2</sup>/g to give adequate retention of solutes and to maintain a reasonable linear sample capacity: (iv) a mean pore size not less than 10nm and an absence of micropores in order to ensure rapid mass transfer of solutes into and out of the particles: (v) ease of preparation by a simply controlled, low-cost process.

The extent to which these often conflicting characteristics can be achieved will be dependent upon the type of starting material, upon the procedure chosen for preparation of the product and upon the conditions under which it is used. Evidently numerous routes can be taken in the synthesis of tailor-made porous carbon products and much work is still required to provide carbons with optimum properties for HPLC.

## 2.2. Processes involved in Carbon Formation

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.

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 700 to 1000 K 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, the two limiting cases.

Carbonization covers a number of processes including coking, charring and reaction with oxidising gases such as oxygen, carbon dioxide and water vapour. It is carried out between 1000 and 1500 K (28). Carbonization increases the percentage 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 pyrolysed between 1300 and 2000 K to yield dense non-porous layers of pyrolytic carbon (29).

Active carbons may still contain inorganic impurities such as sulphur and silica depending upon their origin. These can be removed by volatilization at 1500 to 2000 K. 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 2000 K to 3200 K. Such heat treatment brings about densification with concurrent removal of structural defects, and forms a three dimensionally ordered graphitic

structure (30). The degree of graphitization of any carbon brought about by high temperature treatment depends strongly on its initial source and structure. Thus treatment of some active carbons at temperatures as low as 1500 K 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 3200 K.

### 2.3. Consideration Relevant to the Synthesis of Carbon Packings for HPLC

#### Particle Size and Shape

With a granular starting material of sufficient hardness, angular microparticles can readily be obtained by milling and size-grading into narrow cuts (31). Alternatively soft carbons such as carbon black agglomerates can be reinforced by depositing pyrolytic carbon as binder (32,33). Spherically shaped carbon particles in the 5-200 $\mu$ m size range can be obtained by thermal treatment of polymer beads (34-36). With all pre-sized materials note must be taken of the extent to which consecutive treatments may change the shape, size and size distribution of the original particles.

An essential requirement of any final material for HPLC is that it must possess sufficient hardness to withstand high pressures and high eluent flow rates during column packing and operation. The hardness of carbon is directly related to its molecular and pore structures: thus layer-type materials such as graphite are soft whereas amorphous glassy carbons possess high mechanical strength due to their microcrystalline mosaic structure. Between these two limiting cases a large number of structural intermediates exists. One of them is the so-called turbostratic structure proposed by Biscoe and Warren (37). The layers in such carbon materials are 'arranged roughly parallel and equidistant but are not otherwise mutually oriented'. It may be concluded that because of their low

mechanical strength highly ordered graphite-like carbons are less likely to be suited as packings in HPLC than microcrystalline or amorphous carbons.

Particle porosity also has an important effect on mechanical strength. In general particles are too fragile for HPLC when the particle porosity exceeds about 70% (a typical silica gel, for example, has a particle porosity of about 55%). A complicated situation arises with carbons of composite structure, e.g. reinforced carbon blacks. These have an inhomogeneous molecular structure as well as being porous. No predictions can be made for such materials: they must be tested under experimental conditions.

#### Surface composition

Apart from its primarily aromatic character the carbon surface carries a variety of functional groups. The majority are acidic groups such as carbonyl, carboxyl and phenolic hydroxyl but some basic groups (38,39) may also be present. These functional groups are located at defect sites which occur randomly at the edges of crystallites. Thus the less crystalline the product the higher will be the concentration of polar surface functional groups, although even crystalline graphite rapidly chemisorbs oxygen when exposed to the atmosphere. The polar surface functional groups can be eliminated by annealing at about 750 K (40) but some will immediately reform on exposure to air. A more effective means of removal is hydrogenation at 1300 K. Alternatively, more specific surface reactions can be carried out to mask the polar functional groups, as is done in surface modification of silica (41). Graphitization followed by chemical reduction of the surface will apparently offer the best chances of success in preparing a good adsorbent surface.

However, given the considerations of the last two sections, it becomes apparent that there are serious difficulties in



making robust carbons with stable and well defined surfaces for, while graphitization may improve surface properties, it will reduce the particle strength. A compromise may have to be accepted, or alternatively a composite structure may be required.

#### Origin of Porosity, Pore Size and Specific Surface Area.

The origin and characterization of porosity in carbons including graphite has been the subject of several detailed studies (42-45). Evans and Marsh (42) claim that the "shape, size and distribution of porosity are intimately associated with the (molecular) structure of carbons and graphites". Isotropic and non-graphitizable carbons are microporous, whereas anisotropic graphitizable carbons generally exhibit little microporosity. Pore size and shape can be observed by optical microscopy, scanning electron microscopy and phase-contrast high resolution electron microscopy. Characteristic parameters describing the size and volume of micropores of below 3nm diameter are derived from sorption measurements (44). Apart from open and accessible pores carbon may also contain closed pores which are indirectly evidenced by comparing the apparent density measured by helium with that of pure graphite.

Each population of pores contributes to the total specific surface area. Micropores can potentially contribute surface areas exceeding  $1000\text{m}^2/\text{g}$ . So-called supermicropores will provide even higher areas although by this stage the concept of surface area loses its physical meaning. In general active carbons have high surface areas whereas graphitized materials have low surface areas. Carbons having high surface areas due to micropores are not suitable for use in column liquid chromatography on account of slow mass transfer, high activity in adsorption, heterogeneity of surface activity and too high retention. Undoubtedly one of the major problems in the manufacture of porous carbons suitable for HPLC arises from the extreme difficulty of eliminating micropores.

The above discussion indicates that it will be very difficult to obtain an ideal material having the correct combinations of particle rigidity, adequate surface area, uniformity of pore structure and uniformity of surface composition. Inevitable compromises will have to be accepted. The best solution may rest with a composite material having a hard microcrystalline framework with 10nm pores, the internal surface of which is coated with a thin dense layer of graphite.

#### 2.4. Preparation Procedures

The most extensive work on the preparation and characterisation of carbons for HPLC has been that of Guiochon and co-workers (32,33) who have devoted much effort to the modification of carbon black agglomerates. These agglomerates are made up from independent spherical particles of submicron size: they possess a specific surface area of between 10 and 200m<sup>2</sup>/g (46) and significant porosity. Owing to their origin, carbon blacks consist of small crystallites of graphitic structure, sometimes containing intrusions of amorphous carbon (47), and they possess a variety of functional groups chemically bound to the surface which give rise to their oxygen and hydrogen content. Heat treatment at about 3000 K in an inert atmosphere removes these surface groups. It also leads to an intense growth of crystals, and improves their mutual arrangement and orientation. The density of particles of graphitized thermal carbon blacks (GTCB) approaches that of graphite and the surface becomes non polar and homogeneous (47). However, the aggregates of these polyhedral microparticles are mechanically very fragile. Reinforcement of aggregated GTCB particles can be performed by depositing a thin film of pyrolytic carbon through pyrolysis of benzene at 1200 K (32,33,48). The hardness and specific surface area of pyrocarbon-modified carbon black (PMCB) are closely related to the amount of pyrocarbon deposited (32,33). While coating is traditionally performed in fluidized beds (49), Colin et al (32,33) used a quartz tube containing a crucible filled with carbon powder.

Inert gas containing a low partial pressure of benzene was passed while the tube was heated in a furnace at about 1200 K. The properties of pyrolytic carbon, namely its anisotropy, apparent density, crystallite size and microstructure, are strongly influenced by the temperature, the gas composition, the contact time and the bed surface area (49), but in general it possesses a turbostratic structure. For low surface area carbon blacks, e.g. Sterling FTFF, the amount of pyrocarbon deposited was proportional to the contact time and the concentration of benzene circulated through the furnace. The load could be increased to 50% w/w or more, but high loading resulted in a sharp drop from the initial surface area and in a heterogeneous surface (49). The optimum deposit was about 15-20% (w/w) which still provided sufficient mechanical strength. Surface purification was achieved by hydrogen treatment at 1300 K or by thermal treatment at 3000 - 3300 K. Surprisingly such treatments gave only slight improvement in chromatographic properties, such as peak profile and loadability (32,33,50). In producing materials for HPLC, problems arose in sizing batches of 8-10 $\mu$ m of carbon blacks, in coating these agglomerates and in sizing the resulting powder. Consequently, most studies were performed on particles with  $d_p > 15\mu$ m. Later Colin and Guiochon (51,52) adopted the benzene pyrolysis procedure to deposit pyrocarbon on the surface of silica as developed by Bebris et al (53, 54). Thermally pre-treated silica gels were employed and again the optimum carbon deposit for avoiding particle agglomeration and low chromatographic performance was about 15% (w/w). Unfortunately the coating of the silica surface was not continuous and free of defect sites, and the retention of solutes was not the same as with pure carbon.

The pyrolysis of organic compounds on porous silica was further examined by Leboda (55-58), using aliphatic and aromatic alcohols and dichloromethane. Two-stage pyrolysis was sometimes used. The carbon-silica adsorbents were characterized by

sorption-measurements, infrared spectroscopy and gas chromatography. However, no separation by liquid-solid chromatography was carried out.

A novel carbon material has recently been developed by Czechoslovakian researchers (59,60). It is unusual in being prepared not by high-temperature carbonization but by room temperature reduction of polytetrafluoroethylene (PTFE). Non-porous angular microparticles of PTFE were first degreased and dried under vacuum at 423 K. The resulting crumbs were then treated with lithium-amalgam at 293 K, forming lithium fluoride and a carbon residue. The lithium fluoride was extracted and the product, called JADO-carbon, dried under vacuum at 423 to 623 K. Samples were prepared partially reduced (59), completely reduced (60), loaded with lithium fluoride, and with LiF fully extracted. Elemental analysis (by weight) of the dry product gave C 86%; H 1%; oxygen (residue) 13% (59). The apparent (helium) density was  $2.17\text{g/cm}^3$ , the specific surface area according to the BET method employing argon  $S_{\text{BET}}$  was  $2500\text{m}^2/\text{g}$ . Uptake of benzene at 298 K amounted to  $1.25\text{ml/g}$ , indicating a high particle porosity of  $\epsilon = 0.73$ . Although the molecular structure of JADO-carbon is amorphous, the micro-structure comprises fibres joining nodules. The nodules are  $1.1 \pm 0.35\text{nm}$  in diameter and the distance between them is  $1.9 \pm 0.3\text{nm}$ . The resulting cavities in the carbon skeleton are  $2.3 \pm 0.3\text{nm}$  in diameter. The final structure is determined by the primary structure of PTFE.

Attempts were subsequently made to decrease the specific surface area and oxygen content by various treatments (61): (i) after leaching the lithium fluoride the microparticles were dried under vacuum at 573 K and annealed at 1223 K under helium giving:  $S_{\text{BET}} = 2000\text{m}^2/\text{g}$ ; (ii) the LiF loaded carbon was heated at 1223 K under helium. After cooling the material was immersed in a 0.1% w/w solution of aqueous detergent. Excess of solution was removed, the sample washed, dried and heated at 973 K in

hydrogen giving  $S_{\text{BET}} = 1300\text{m}^2/\text{g}$ ;  $\epsilon = 0.76$ ; oxygen 0.5%;  
(iii) the LiF loaded carbon was heated at 1223 K in helium.  
After washing out the LiF residue and drying under vacuum at  
573 K the product was annealed at 2673 K under argon giving:  
 $S_{\text{BET}} = 20\text{m}^2/\text{g}$ ;  $\epsilon \approx 0$ ; oxygen 0.

By a procedure analogous to that of Plzak et al (59), Zwier and Burke (62) prepared a high-strength carbon packing by reduction of the fluorocarbon Kel-F 300 LD with lithium amalgam. As the surface of this KFD carbon exhibited polar behaviour due to the presence of hydroxyl, carbonyl and carboxyl groups the material was treated (i) with trimethylchlorosilane to mask the hydroxyls and (ii) with thionylchloride followed by reacting the chlorinated carbon with the Grignard reagent  $\text{C}_8\text{H}_{17}\text{MgBr}$ , giving an octyl bonded carbon. The effect of silane and Grignard modification was monitored by means of ESCA measurements. The surface modified KFD carbon showed behaviour intermediate between that of the unmodified KFD carbon and typical silica based reverse phase materials.

Recognising the disadvantages of PMCB, Ciccioli et al (63) examined a commercial graphitized carbon black, Carbopack B marketed by Supelco and made from saran active carbon (64). The material was first ground to a particle size of around  $20\mu\text{m}$  and the fraction of  $d_p > 20\mu\text{m}$  used as packing. Although the material was extremely fragile and required very careful handling its LC properties were encouraging, and confirmed that the graphitic surface is likely to be the optimum for HPLC applications if it can be provided on a more robust structure.

Unger et al (31,65) employed cokes and active carbons as precursors. The starting materials had adequate hardness for HPLC and could be milled and fractionated to give narrow cuts of the desired particle size. Batches were then treated to remove the non-carbonaceous constituents:

- (i) by leaching of inorganic impurities with HCl/HF mixtures and subsequent annealing at 2073 to 3073 K,
- (ii) by annealing at 2073 to 2873 K without extraction,
- (iii) by annealing according to (ii) followed by treatment with iron salts, partial degassing at 1273 K, removal of added salts and final reduction by hydrogen at 673 K.

Procedures (i) and (ii) resulted in products having only meso- and macropores but with very low surface area. Procedure (iii) gave  $S_{\text{BET}}$  values in the range of 50 to  $200\text{m}^2/\text{g}$ , but the products still contained a proportion of micropores.

Another form of carbon has recently been described by Knox and Gilbert (66). The material is a porous glassy carbon (PGC) and is made by pyrolysing a phenol formaldehyde mixture within the pores of a template material (66) such as silica gel. The composite polymer/silica gel is gradually heated from 400 K to 1300 K to carbonize the polymer. The silica template is then dissolved out by alkali or by hydrofluoric acid and the resulting porous carbon heated to a temperature in excess of 2500 K. The area of the porous carbon when initially prepared at  $1300^\circ\text{C}$  is 50 to  $500\text{m}^2/\text{g}$  depending upon the surface area and pore dimensions of the silica gel initially used. After heating to  $> 2500\text{ K}$  the area is reduced to between a half and a quarter of the original value. Carbon produced in this way from a typical silica gel has a rather high porosity of around 80% and is structurally weak. However, by using a more porous gel, materials capable of withstanding high pressures can be produced. The chromatographic characteristics of the PGC's so far produced are somewhat similar to those of other high temperature carbons, especially the pyrolytic carbons of Guiochon (32,33,50). It is also applicable in gas chromatography where its chromatographic properties are similar to those of Carbo-pack B except that the material itself is very much more robust.

### 3. CHROMATOGRAPHIC STUDIES ON CARBON PACKINGS

#### 3.1. Kinetic Performance

The term "kinetic performance" implies a combination of speed of elution, plate efficiency, and economy of pressure drop. It is best measured by the separation impedance  $E$  (67) defined by equation (1):

$$E \equiv h^2 \phi' = \frac{N}{t_m} \times \frac{N}{\Delta p} \times \frac{1}{\eta} \quad (1)$$

where  $h$  = reduced plate height,  $\phi'$  = flow resistance factor,  $N$  = number of theoretical plates to which column is equivalent,  $t_m$  = elution time of unretained fully permeating solute,  $\Delta p$  = pressure and  $\eta$  = eluent viscosity. The flow resistance factor  $\phi'$  is dimensionless and given by equation (2)

$$\phi' = \Delta p d_p^2 / u \eta L \quad (2)$$

where  $d_p$  = mean particle diameter,  $u$  = linear velocity of eluent and  $L$  = column length. Where the mean particle size is well established,  $\phi'$  is found to be in the range 500-1000. For well behaved liquid chromatography the reduced plate height  $h$  is found to obey the approximate equation

$$h = B/v + A v^{1/3} + C v \quad (3)$$

The best columns show  $B \approx 2$ ,  $A \approx 1$ ,  $C \approx 0.1$  and have minimum  $h$  of about 2-3 at  $v$  in the range 2-5 (67,68).

The performance of any material packed into a column is summarized by quoting its  $\phi'$ -value and either showing the  $(h, v)$  plot, giving values of  $A$ ,  $B$  and  $C$ , or giving optimum values of  $h$ .

Implicit in the term "well behaved liquid chromatography", however, is the assumption that peak dispersion arises entirely from the kinetic effects covered in equation (3). For the carbon adsorbents so far tested this is rarely the case. Peaks are often asymmetric as a consequence of non-linear isotherms for the adsorption of solutes from eluent onto the surface. While it may be useful to treat the data according to equation (3) it is not necessarily legitimate to interpret A, B and C in terms of the kinetic processes with which they are usually associated. The goodness-of-packing of a column largely determines the parameter A. Microparticulate carbons have usually been packed by the well-known slurry technique (31,32,67, 69) using suspension media adapted to the wetting characteristics of carbon. Mixtures of dibromoethane and acetonitrile (32), and of dioxane and paraffin oil (31) have been used. Recently PGC's have been successfully packed using a 1% aqueous solution of the nonionic detergent Tween 20 as suspension medium (70). Ciccioli et al (63), however, dry packed their columns using Carbo-pack B.

Colin et al (33) determined column efficiencies of PMCB using particles of 15 $\mu$ m and above. Optimum performance was achieved with a 20% loading of pyrocarbon. Optimum h-values of 3-7 were found for solutes with k'-values up to 1.1. However, for k'-values above 2 column efficiency generally dropped rapidly, probably due to surface inhomogeneity and overloading (52). Graphitization of PMCB affected a very small improvement in lowering A, B and C for an unretained solute (50) but significant improvement for retained solutes (32). Other carbons (31,61) showed similar effects. One of the best chromatograms published using PMCB is shown in Figure 1. Figure 2 shows a separation on carbon produced by purification and high temperature treatment of active carbon (31).

By contrast to the hard carbons, Carbo-pack B studied by Ciccioli et al (62) with particle size of 20 $\mu$ m gave  $h = 4$  at  $k' = 7$  and excellent peak symmetry as shown by Figure 3.



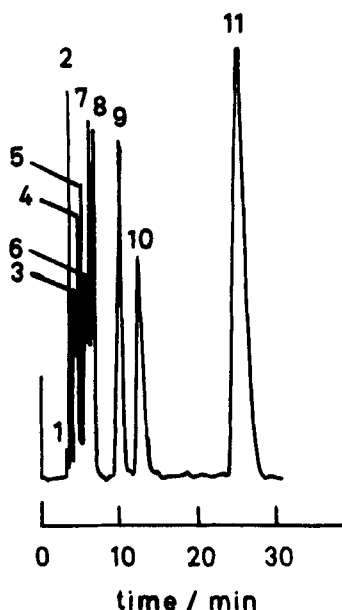


FIGURE 1. Separation of methyl benzenes. Column 550mm. Packings: 25-31 $\mu$ m, Black Pearls L + 44% pyrocarbon, graphitized,  $\sim 40\text{m}^2/\text{g}$ . Eluent: acetonitrile 0.5ml/min. Detector: UV.  $h$  for last peak = 15. Solutes: 1 = unretained; 2 = benzene; 3 = 1,3 dimethylbenzene (DMB); 4 = 1,2 DMB; 5 = 1,3,5 trimethylbenzene (TMB); 6 = impurity; 7 = 1,2,4 TMB; 8 = 1,2,3 TMB; 9 = 1,2,4,5 tetramethyl benzene (TeMB); 10 = 1,2,3,4 TeMB; 11 = pentamethylbenzene. (from ref. 33).

PGC falls between the PMCB and Carbopack and gives reasonably symmetrical peaks at  $k'$ -values up to 15, as shown in Figure 3, but  $h$  appears to be large at about 25 with the 5 $\mu$ m particles used.

The permeability of carbon columns is much as expected with  $300 < \phi' < 1000$  provided that well sized fractions free of fines are used and that the material is sufficiently robust to resist compression.

In some studies (31, 71) column loadability was studied using Snyder's parameter  $\theta_{0.1}$  (16), defined as the mass of solute injected per gram of packing material which produces a 10%

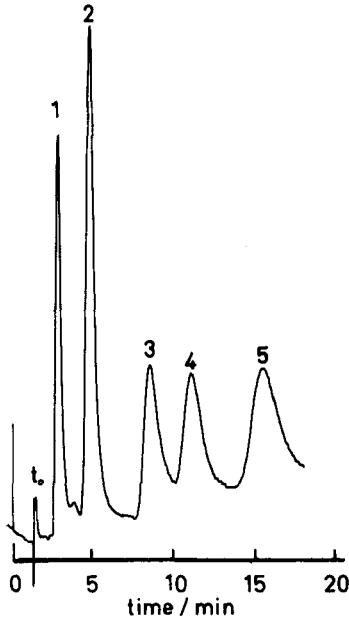


FIGURE 2. Separation of benzene carboxylic acids. Column: 100 x 10mm. Packings: 9 $\mu$ m active carbon calcined 2073 K,  $\sim$  5m<sup>2</sup>/g. Eluent: methanol/water (30/70 v/v), pH = 6.0 containing 5mM tetrabutylammonium, 2.7ml/min. Detector: UV. h for last peak = 45. Solutes: 1 = pentacarboxybenzene; 2 = 1,4 dicarboxybenzene-3 = 1 methyl 3 carboxy benzene; 4 = 1,3,5 tricarboxybenzene, 5 = 1,2 dihydroxy, 3 carboxybenzene (ref. 71).

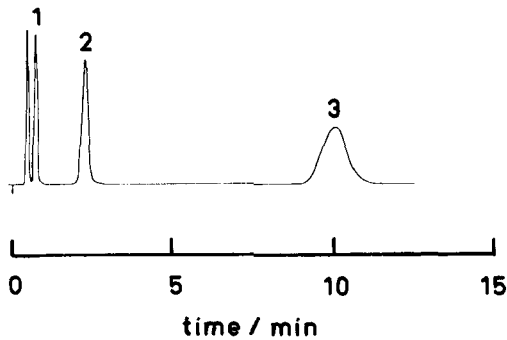


FIGURE 3. Separation of analgesics. Column: 250 x 2mm. Packing: 25-33 $\mu$ m Carbopack B,  $\sim$  80m<sup>2</sup>/g. Eluent: Methanol, 1ml/min. Detector: UV. h for last peak = 10. Solutes: 1 = phenylacetamide; 2 = phenacetin; 3 = caffeine (from ref. 63).

decrease in  $k'$ . For silicas and reversed-phase silicas  $\theta_{0.1}$  normally lies between 100 and 1000  $\mu\text{g}/\text{g}$ . An alternative measure  $\theta_{0.5}'$  is the mass of solute per gram of packing which produces a 50% reduction in the plate efficiency of the column. Both  $\theta_{0.1}$  and  $\theta_{0.5}'$  depend upon the type of adsorbent, the surface heterogeneity, the nature of the solute and of its interaction with the adsorbent surface, and the nature of the eluent.

Colin et al (71) reported  $\theta_{0.5}'$  values of 10-60  $\mu\text{g}$  for PMCB with acetonitrile as solvent. Unger et al (31) obtained values of around 10  $\mu\text{g}/\text{g}$  on carbons of very low surface areas ( $\sim 5\text{m}^2/\text{g}$ ). Higher values more in line with those for silica gels would be anticipated from carbons of higher specific surface areas. They also showed that  $\theta_{0.1}$  and  $\theta_{0.5}'$  values were different for different esters using methanol as eluent.

It may be concluded that carbons can be produced to give good chromatographic efficiencies which in some cases are comparable to those obtained with silica gels. However, the efficiency in general is significantly less as is the column loading. These two features are closely associated; both probably arise from the as-yet unsatisfactory surface chemistry of the present carbons. However, there is every reason to suppose that high efficiency carbons can be produced which will eventually compete on equal terms with existing silica gel based materials.

### 3.2. Retention and Selectivity

Solute retention on carbon arises from adsorption of solutes onto the carbon surface. Solvent molecules adsorbed at the surface are thought of as being displaced by solute molecules during elution, the balance between solvent and solute adsorption providing the observed retention. According to Snyder (16) the column capacity ratio for an adsorbent can be expressed by equation (4):

$$\log_{10} k' = \log_{10} \frac{V_s}{V_m} + \beta (S^{\circ} - A_s \epsilon^{\circ}) \quad (4)$$

where  $V_s$  = volume of a monolayer of eluent covering the active surface,  $V_m$  = volume of eluent,  $\beta$  = surface activity factor,  $S^{\circ}$  = dimensionless free energy of adsorption of solute ( $\Delta G/2.303RT$ ) from a standard eluent,  $A_s$  = molecular cross sectional area of that part of the solute in contact with the surface measured in units of  $8.5\text{\AA}^2$ ,  $\epsilon^{\circ}$  = dimensionless free energy of adsorption of a quantity of eluent sufficient to cover  $8.5\text{\AA}^2$ . Both  $S^{\circ}$  and  $\epsilon^{\circ}$  are measured relative to the value for a standard eluent since absolute values are not directly measurable. In the case of silica gel, pentane is taken as the standard having  $\epsilon^{\circ} = 0$ , giving  $\epsilon^{\circ}$  values in the range 0 to 1. The parameters  $V_s$  and  $\beta$  are affected by the water coverage of the surface. For carbons, methanol has been taken as the standard eluent with  $\epsilon^{\circ} = 0$  and in the light of the very limited data available on surface activity  $\beta$  may be taken as unity. With these conventions  $\epsilon^{\circ}$ -values for carbon are positive for solvents such as dichloromethane, alkanes, benzene, which are stronger than methanol, and are negative for eluents containing water. The extension of the series towards water and neat aqueous buffers is, however, limited by the fact some carbons are not wetted by water.

Eluotropic series have been reported on charcoal (16), PMCB (33) and carbons made from thermally treated cokes and active carbons (31) and are listed in Table 1. It is noted that there are significant differences in the sequence which can probably be attributed to the different surface characteristics of the materials and the different test solutes used.

Values for  $\epsilon^{\circ}$  have been calculated by Colin et al (71,72) and a selection of these values are shown in Figure 5. The range of eluotropic strength is substantially less on carbon than on silica gel especially if aqueous mixtures are discounted. For methanol/water mixtures,  $\log_{10} k'$  increases

TABLE 1  
Eluotropic Series for Carbons  
(in order of increasing solvent strength)

Charcoal (16)	PMCB  (for alkyl benzenes) (33)	Thermally treated coke and active carbon  (for naphthalene) (31)
water		
methanol ●	methanol ●	methanol ●
ethanol	acetonitrile ●	hexane ●
acetone	ethylacetate ●	tetrachloromethane ●
propanol	hexane ●	acetonitrile ●
diethylether		dimethylsulphoxide
butanol	dichloromethane ●	tetrahydrofuran ●
ethylacetate ●	butylchloride ●	trichloromethane ●
hexane ●	tetrahydrofuran ●	dichloromethane ●
	heptane	
	octane	
	nonane	
benzene ●	benzene ●	benzene ●

linearly with water content as in reversed-phase chromatography to a maximum water content of about 50% corresponding to the composition at which carbon ceases to be wetted by this mixture (31,71). As found in classical adsorption chromatography on carbons, modifiers such as aromatic hydrocarbons, bases and acids are often necessary to improve peakshape. With PGC for example (see Figure 4) the addition of terphenyl (70) was necessary

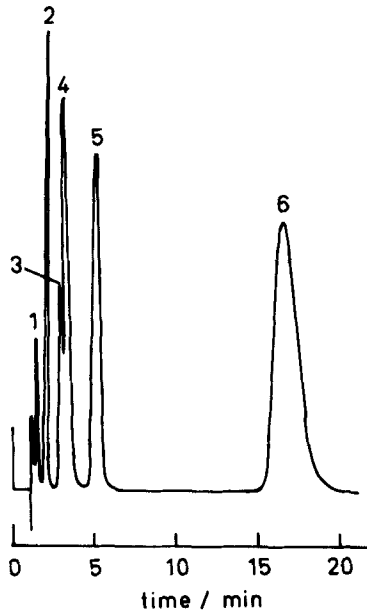


FIGURE 4. Separation of methylbenzenes. Column: 100 x 2mm. Packing: 5 $\mu$ m porous glassy carbon PGC26 heated to 2600 K. 380m<sup>2</sup>/g. Eluent: methanol. Detector: UV. h for last peak = 25. Solutes: 1 = benzene; 2 = toluene + 1,4 DMB; 3 = 1,3 DMB; 4 = 1,2 DMB; 5 = 1,2,4 TMB; 6 = 1,2,4,5 TeMB. (ref. 70).

to give symmetrical elution peaks for the more retained alkyl benzenes and polynuclear aromatics while the addition of acetic acid was necessary for successful elution of aromatic acids. Similar behaviour was observed with thermally treated active carbons (73). It seems likely that the modifier occupies specific active sites on the surface of the carbons, such sites being responsible for surface heterogeneity and the extreme curvature of the adsorption isotherm at low surface coverages. The situation is familiar to those involved in adsorption chromatography with silica gel (16). Intelligent control of eluent composition is evidently necessary with currently available carbons if good peak shape is to be obtained.

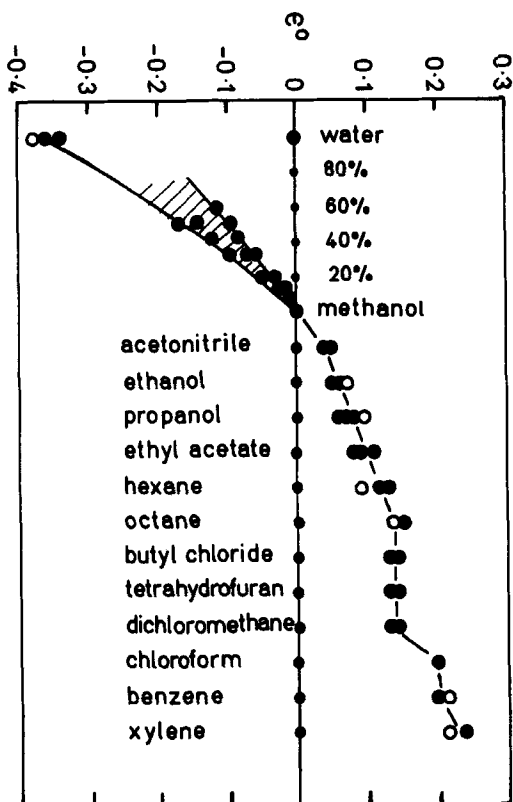


FIGURE 5. Mean eluotropic strength of organic eluents on PMCB using alkyl benzenes, methyl benzenes, methyl phenols and polynuclear aromatics: ● determined by chromatography, ○ calculated from interfacial surface tension data. (Data selected from Table 3 of ref. 72).

Carbons offer a special advantage over silica-based materials in that they can be used over a much wider range of pH. JADO carbon was used with neat buffers of pH from 1 to 8 (61,74) while Unger et al (31) separated alkaloids using methanol/water buffers with pH from 6-12. An example is shown in Figure 6. As with bonded silicas, separations based on ion-pair formation are also possible as seen from Figure 2.

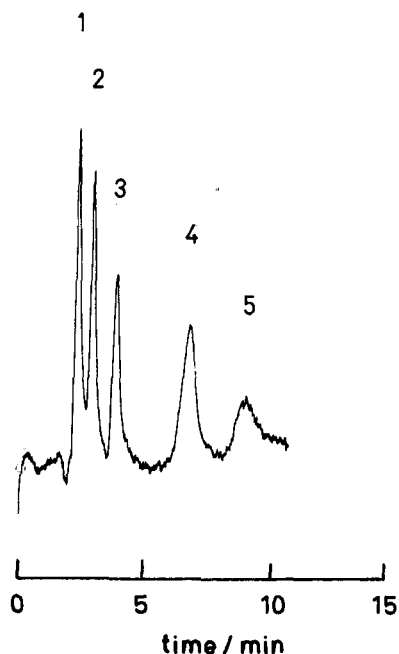


FIGURE 6. Separation of alkaloids. Column 100 X 10mm. Packing: 9 $\mu$ m active carbon calcined at 2073 K,  $\sim 5\text{m}^2/\text{g}$ . Eluent: methanol/water (80/20 v/v), pH = 11.2, 1.5ml/min. Detector: UV. h for last peak = 20. Solutes: 1 = ephedrine; 2 = codeine + anaestasin; 3 = theobromine; 4 = caffeine; 5 = strychnine. (from ref. 31).

A widely used method of quantitating the hydrophobic balance between a stationary and mobile phase is to plot  $\log_{10} k'$  for members of a homologous series against the number of carbon atoms,  $n$ , in the molecule or alkyl chain. A linear relationship is often found. Such measurements in reversed-phase systems give a scale of elutropic strength for the  $\text{CH}_2$  group, as can be seen by differencing equation (4) for two successive homologues.

$$\alpha' \equiv \log_{10}(k'_{n+1}/k'_n) = (S_{\text{CH}_2}^0 - A_{\text{CH}_2} \epsilon^0) \quad (5)$$



where  $S^{\circ}_{\text{CH}_2}$  is the dimensionless free energy of adsorption of a  $\text{CH}_2$  group from the standard eluent, and  $A_{\text{CH}_2}$  is the area occupied by a  $\text{CH}_2$  group on the surface. Several authors (32,33,61) have shown linear plots of  $\log_{10} k'_n$  against  $n$ , from which the values of  $\alpha'$  may be calculated. These values are collected in Table 2. They are substantially greater than those obtained in reversed phase silica packings with the same eluent composition (31, 65). For example, Colin et al quote  $\alpha' = 0.15$  for alkyl benzenes eluted by methanol from Partisil ODS-2 whereas the values for carbon are about 0.25.

The  $\alpha'$ -values for different carbons are self consistent and clearly show the increasing elutropic strength (decreasing  $\alpha'$  value) from methanol and acetonitrile to pentane and heptane. Typical  $\alpha'$ -values for the quasi-homologous series of methyl substituted benzenes are 0.4 to 0.45 (33, 70).

Increase in the number of conjugated rings in the solute molecule leads to a marked increase in retention as seen from the data of Table 3. It is noted that in spite of a very wide range of surface area within the carbons studied the elution order is always the same. Values of  $k'$  relative to fluorene are generally comparable and when plotted against the number of carbon atoms in the solute give a fair correlation as shown in Figure 7. The gradient gives an  $\alpha'$ -value of 0.32 for addition of each carbon atom. This falls closer to the value for addition of  $\text{CH}_2$  groups (0.25) than to the value for addition of  $\text{CH}_3$  groups (0.4 to 0.45) and indicates that contrary to general belief graphitized carbons do not show any special selectivity towards conjugated rings.

The behaviour of carbon is distinctly different from that of reversed phase silicas (76,77) in regard to retention of aromatic compounds carrying polar functional groups. As shown by Figure 8, polar derivatives of benzene, especially those having large dipoles, are much more strongly retained by carbons

TABLE 2

Values of  $\alpha' = \log_{10}(k'_{n+1}/k'_n)$  for Homologous Series eluted from Carbons

Form of Carbon	Homologous Series	Eluent	range of n (1)	$\alpha'$	Ref.
PMCB 15% Pyrocarbon on Sterling Carbon Black - 6m <sup>2</sup> /g	alkyl benzenes	acetonitrile/water 46:54 v/v	9 - 15	0.26	32
	n-alkanois	acetonitrile	5 - 11	0.25	32
PMCB 55% Pyrocarbon on Black Pearls - 40m <sup>2</sup> /g	alkyl benzenes	acetonitrile	7 - 15	0.24	33
	bromo-n-alkanes	"	3 - 9	0.24	"
	chloro-n-alkanes	"	4 - 7	0.24	"
	n-alkanois	"	5 - 15	0.24	"
Carbon II from PTFE (1300 m <sup>2</sup> /g)	n-alkanes	diethyl ether	12 - 14	0.13	61
	n-alkanes	pentane	8 - 16	0.14	
		methanol	8 - 16	0.32	
Carbon I from PTFE (2000 m <sup>2</sup> /g)	n-chloroalkanes	heptane	7 - 16	0.11	61
	n-alkenes	"	10 - 18	0.11	
	n-alkanes	"	8 - 19	0.12	
Carbon III from PTFE (20 m <sup>2</sup> /g)	n-alkanois	methanol		0.32	61
		acetonitrile		0.24	
		dichloromethane		0.20	
		diethyl ether		0.13	
		pentane		0.14	
	heptane		0.11		
Porous glassy carbon 16 380 m <sup>2</sup> /g	alkyl benzenes	methanol	4 - 9	0.23	70

Notes: (1). n is total number of carbon atoms in molecule.

TABLE 3  
Absolute values of  $k'$  and values relative to fluorene (bracketed) for elution of  
Polynuclear Aromatic Hydrocarbons from Carbons

Form of Carbon	Eluent	$k'$ or $k'/k'$ fluorene (bracketed)							Ref.
		benzene	naphthalene	acenaphthene	fluorene	phenanthrene	anthracene		
PMCB + 15% pyrocarbon on sterling CB. $\sim 6m^2/g$	acetonitrile		0.4 (0.08)			5.0 (1.0)			32
PMCB + 55% pyrocarbon on Black Pearls $\sim 40m^2/g$	benzene		0.06 (0.18)	0.25 (0.8)	0.33 (1.0)	0.8 (2.4)	1.0 (3.0)	33	
Purified coke heated 3073 K 3 hrs $\sim 5m^2/g$	methanol		0.07 (0.21)		0.35 (1.0)	0.52 (1.5)		31	
Porous Glassy Carbon 7 $350m^2/g$	methanol acetonitrile ethyl acetate hexane dichloromethane	1.3 (0.02)	4.9 (0.08)	24 (0.4)	61 (1.0)			70	
		0.7 (0.04)	1.5 (0.09)	6.3 (0.4)	17 (1.0)				
			0.6	2.1					
			0.6 (0.12)	1.7 (0.3)	5.5 (1.0)				
			0.6 (0.25)	1.0 (0.4)	2.4 (1.0)	8.5 (3.5)	16 (6.7)		
Porous Glassy Carbon 26 $380m^2/g$	dichloromethane	0.04 (0.003)	1.7 (0.13)	4.7 (0.4)	13 (1.0)			70	
PGC26 + adsorbed terphenyl	dichloromethane methanol	0.04 (0.005)	1.0 (0.13)	3.2 (0.4)	8 (1.0)			70	
		0.2	16						
Number of Carbon Atoms in Solute		6	10	12	13	14	14		

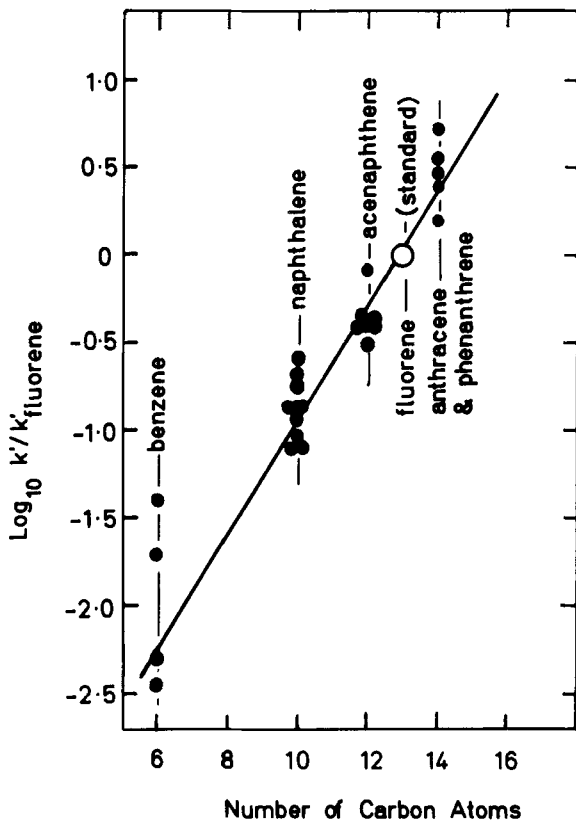


FIGURE 7. Dependence of  $\log_{10} k'/k'_{\text{fluorene}}$  upon carbon number,  $n$ , for elution of polynuclear aromatic hydrocarbons from carbons. Data from Table 3.

than benzene itself whereas the opposite is true with reversed-phase bonded silica gels. In general, however, the effect of added groups whether polar or not gives approximately the same increase in retention when considered on the basis of the number of heavy atoms (C,N,O) in the group, provided that the stereochemistry of the adsorbate does prevent adsorption of all the groups. This again emphasises the importance of dispersive rather than polar interactions at the carbon surface.

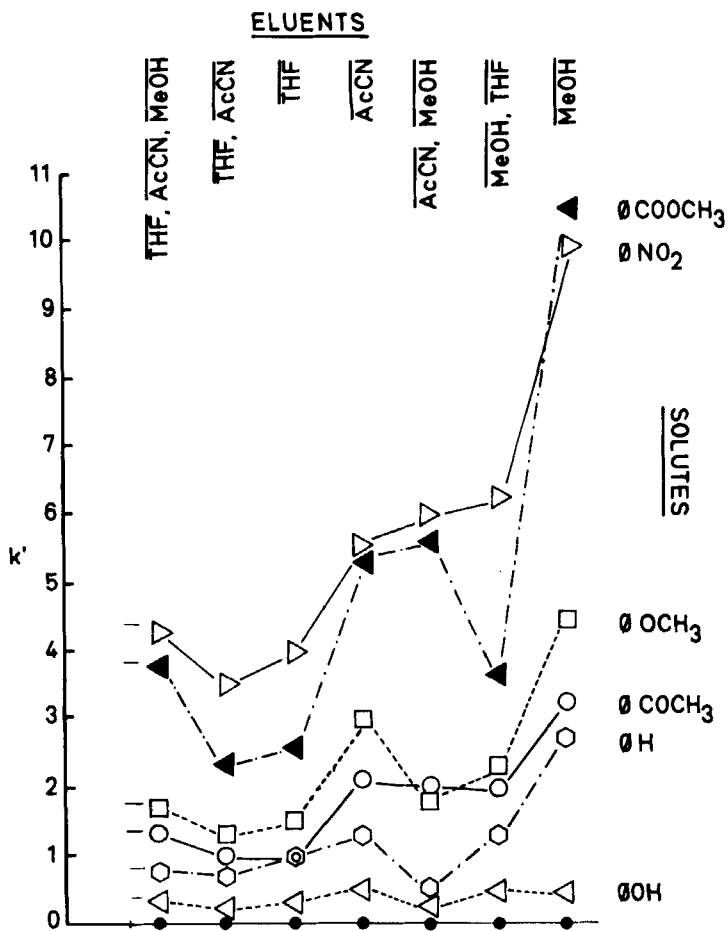


FIGURE 8. Solvent selectivity for different eluents on carbon. Column and packing: as for Figures 2 and 6. Eluents:  $\overline{\text{MeOH}}$  = methanol/water (70/30 v/v);  $\overline{\text{THF}}$  = tetrahydrofuran/water (40/60 v/v);  $\overline{\text{AcCN}}$  = acetonitrile/water (50/50 v/v). Eluents marked  $\overline{\text{THF}}$ ,  $\overline{\text{MeOH}}$  or  $\overline{\text{THF}}$ ,  $\overline{\text{AcCN}}$ ,  $\overline{\text{MeOH}}$  etc. are made by mixing equal volumes of the individual eluents. Solutes: monofunctional derivatives of benzenes as shown (ref. 73).

The discriminating behaviour of carbons towards solutes of different polarity is relatively poor. On the other hand discrimination on the basis of molecular size appears to be higher than that of reversed phase silicas. Carbon also offers superior selectivity with respect to positional isomers such as cis-trans, aromatics containing hetero atoms etc. Figure 4 illustrates this selectivity in the separation of xylenes using PGC. While the differences in eluotropic strength of the common eluents on carbon seems relatively small (Figure 5), the rather special selectivity of carbons requires detailed examination and may well show strong dependence on eluent composition. This area requires much more study. The results of Colin et al (79) indicate that such work is likely to be rewarding.

In summary, carbon packings offer selectivity characteristics which appear to be significantly different from those of alkyl-bonded silicas. In particular they show greater selectivity for members of homologous series and isomeric compounds and lower selectivity in respect of functional groups of similar molecular weight.

#### 4. APPLICATION OF CARBON PACKINGS

Although carbons for HPLC are not yet available as commercial products, a great diversity of solute types have been separated on carbons. A unique study was carried out by Telepchak (78) who employed a column packed with 10 $\mu$ m diamond dust by resolve aromatic hydrocarbons. Bebris et al (53) used methanol/water mixtures and pyrocarbons modified silica gel (PMS) to separate monoalkyl benzenes, polymethyl benzenes, alkylnaphthalenes, methyl phenols and benzoic acid esters.

The most comprehensive studies have been carried out by Guiochon and co-workers on PMCB and PMS. Aromatic hydrocarbons and methyl benzenes were well separated using acetonitrile as eluent, naphthalenes required a stronger solvent, dichloromethane,

and diphenols required ethylacetate. Other compounds subsequently separated were polychlorinated biphenyls (PCB's), steroids, sulphur and nitrogen-containing compounds (71, 79). The selectivity of PMCB towards isomers was examined with mixtures of undecen-1-ol isomers, isomeric aromatics, and isomeric compounds used in the synthesis of liquid crystals (52). Methanol/water and acetonitrile/water were used to separate adamantanes (80).

Hanai and Walton (81) applied PMCB's and PMS's to the separation of PCB's and other pesticides using methanol/water as eluent and also suggested their use for trace enrichment and preconcentration of pesticides in environmental samples. This suggestion was exploited by Frei et al (82) who compared the effectiveness of ODS-silica and carbons of different origins in the enrichment of samples containing traces of chlorinated phenols.

The Czechoslovakian group (61) found JADO carbon suitable for separation of amino acids in neat aqueous buffers. They carried out detailed studies on the effect of the pH and ionic strength of the eluent to predict optimum separation conditions (74). Ciccioli et al (62) obtained excellent separations of alkyl substituted benzenes and naphthalene derivatives, triazine isomers, phthalate esters, analgesics and amino acids on Carbopack B.

Unger et al (31) demonstrated the utility of carbon in highly alkaline solution by resolving alkaloids using a methanol/water buffer at pH 11-12. They also discriminated various carboxylic acids using reversed-phase ion-pair chromatography (75).

Carbon packings have thus been used for a wide range of chemically different solutes ranging from non-polar to highly polar and ionic. It appears to be possible to elute compounds

containing virtually all common functional groups if the eluent is appropriately chosen. However, a serious limitation of most of the carbons is that their peak capacity is limited by growing asymmetry as  $k'$  increases. However, certain carbons do show promise which suggests that this problem can be overcome.

### 5. CONCLUSIONS

Carbons have been produced having some but not all of the following desirable features:

- (a) adequate hardness
- (b) appropriate particle size
- (c) good surface characteristics

The hard carbons are superior in regard to (a) and (b) and less good in regard to (c) whereas Carbo-pack B, a soft carbon, is superior in regard to (c) but unsatisfactory in regard to (a). There is no reason to believe that a carbon cannot be developed which possesses all features to a sufficient degree to be useful in routine HPLC.

Carbon offers a unique selectivity which is different from that of reversed phase alkyl-bonded silica gels. Although it has been applied to a fair range of compounds its application to biochemistry has hardly begun, and an enormous field remains to be explored.

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