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GRAPHITIZED CARBON BLACK COLUMNS FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Commercially available graphitized carbon black, after a fractionation process to obtain particles of various sizes can be used for the preparation of columns for high-performance liquid chromatography where it behaves as a natural reversed phase. The chromatographic and packing performances of these columns have been determined. Reduced plate heights of less than four have been obtained for retained compounds ($k' = 7$), coupled with a satisfactory permeability. A critical evaluation of the advantages and limitations of these columns is made. Some practical applications are reported to allow a direct comparison between graphitized carbon black and other reversed-phase chromatographic supports.

INTRODUCTION

Since the pioneering work of Kiselev and co-workers[1,2], graphitized carbon black (GCB) has increasingly been applied in the gas chromatography of complex organic mixtures[3]. The versatility of this material is mainly due to the characteristics of its surface which is almost free of unsaturated bonds and polar active sites so that adsorption of various molecules occurs non-specifically. These features have been exploited[2–4] in gas–solid chromatography (GSC) as well as in gas–liquid–solid chromatography (GLSC).

Although GCB is particularly suitable as a reversed phase in liquid chromatography and in high-performance liquid chromatography (HPLC), no direct use of GCB has been reported mainly because, as pointed out by Colin *et al.*[5], practical application is prevented owing to its poor mechanical properties. Those authors tried to overcome this limitation by hardening the GCB surface by benzene pyrolysis in an inert gas stream. This treatment, which yields a deposit of pyrolytic carbon, renders the particles hard enough to stand the high pressures commonly required

when the so-called balanced-density slurry packing method[6] is employed. However, drastic changes are produced in the properties of the original particles: the surface area is decreased and the material is not as inert and homogeneous[5,7]. Tailing is frequently observed in peaks with a rather high capacity ratio, and a further graphitization process has been proposed[5].

Since both the hardening and the graphitization processes require careful control of some parameters (such as temperature, time and benzene flow-rate) in order to obtain a sufficiently reproducible support, an investigation has been carried out with commercially available GCB in an attempt to obtain HPLC columns. This paper describes the preparation procedure and an evaluation of the performance of the columns in terms of plate height as a function of mean particle diameter and capacity ratio, k'. Some practical applications are presented, together with a comparison between GCB and the most widely used materials in reversed-phase chromatography. The limitations and advantages of GCB in HPLC are also discussed.

EXPERIMENTAL

Column preparation

Carbopack B (80–100 mesh) supplied by Supelco (Supelco, Bellefonte, PA, U.S.A.) surface area *ca.* 80 m^2/g, was selected because of its mechanical stability compared to other GCB. The material was ground on a mechanical sieving machine with rubber balls. The resulting particles were then placed on the top of a set of metal screens (200, 150, 88, 75, 54, 45, 33, 25, 15 μm) having a diameter of 22 cm and acetone was continuously added to the top screen. This preliminary treatment breaks down those particles formed by simple agglomeration of microparticles present in the original material. The frictional forces due to the flow of acetone dislocate the particles as their cohesion is reduced by the decrease of the interparticular electrostatic interactions; consequently microcrystallites from the core of the particles are washed out. After this treatment the various GCB sizes were dried and the dried material was sieved again. Examination under the microscope showed that particles having mean diameters greater than 20 μm are well defined and almost spherical, whereas those having mean diameters less than 20 μm appear to be mainly comprised of irregularly shaped microparticles. Only particles in the former category were selected for our investigation. Because we wished to check the packing arrangement during the packing process as well as during the chromatographic analysis, glass columns, 25–35 cm in length, instead of stainless steel were employed. Glass tubes (6 mm O.D., 1.6 mm I.D.) can be used even above 200 kg/cm^2, although the column capacity is reduced.

Columns were prepared by a dry packing technique similar to that employed for packing GCB micropacked columns commonly used in gas chromatography[8]. GCB was continuously placed on the top of the column with constant vibration of the walls as well as of end of the column. As the use of fritted metal ends was found to damage the GCB particles, the column ends were separated from the packing by means of 180–200 μm metal particles. These were kept in place by a 10-μm metal screen supported on a PTFE O-ring placed in contact with the internal wall of the metal fitting. Polyamide ferrules were used to ensure a tight metal–glass connection and were found to be stable above 200 kg/cm^2.

Apparatus

To obtain a low dead volume, a home-made injector was employed. This device permits observation of the sample injection and prevents column plugging caused by the syringe needle; it is quite useful especially when soft particles are used.

The columns were set into a Varian Model 4100 liquid chromatograph (Varian Aerograph, Walnut Creek, CA, U.S.A.) equipped with a variable-wavelength Variscan UV detector and fed with a liquid of low viscosity (pentane) by slowly increasing (2 h) the flow-rate from 0.1 to 2 ml/min. As this procedure generally causes a slight rearrangement and a small decrease (*ca.* 2 cm) of the height of the packed material, additional particles are required to fill the extra volume. For column safety, it is suggested that GCB of larger particle size (120–180 μm) be added. A schematic diagram of the injection device and of the connections of the glass columns with the liquid chromatograph is shown in Fig. 1.
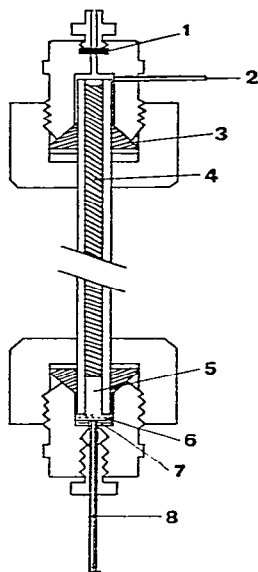

Fig. 1. Scheme of the injector and its connections to the column. 1 = Septum; 2 = liquid inlet; 3 = polyamide ferrules; 4 = glass column filled with Carbopack B particles; 5 = 180–200 μm metal particles; 6 = 10-μm metal screen; 7 = 1 mm thick PTFE O-ring; 8 = stainless-steel capillary tube (0.2 mm I.D.).

RESULTS AND DISCUSSION

GCB column performance

Three sets of columns filled with particles with mean diameters corresponding to 75–88, 33–45 and 25–33 μm respectively were prepared. Each column was then connected to the chromatographic apparatus and its efficiency was measured for compounds having a capacity ratio ranging from about 0.01 to 7. The test mixture injected contained benzene, 1,3,5-trimethylbenzene, naphthalene, methylnaphthalene and 3,6-dimethylnaphthalene and the elvent was pentane. Fig. 2 shows typical plots of HETP *vs.* linear velocity for two of the most retained compounds ($k' = 3.07$ and
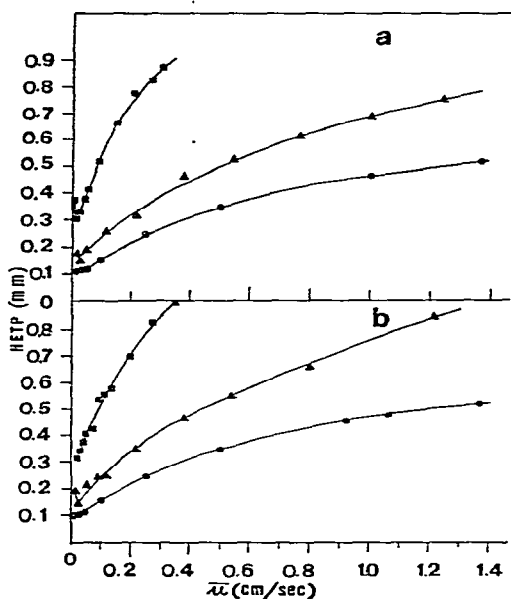
Fig. 2. HETP *vs.* linear velocity for glass columns (25 cm × 1.6 mm I.D.) packed with Carbopack B particles of different sizes. Eluent: pentane. Particle size: ■, 75–88 μm; ▲, 33–45 μm; ●, 25–33 μm. Compounds: a = methylnaphthalene ($k' = 3.07$); b = 3,6-dimethylnaphthalene ($k' = 7.01$).

7.01 respectively). The variations of HETP values *vs.* increasing values of the capacity ratio for various compounds are shown in Fig. 3a. All HETP values correspond to the of minimum values observed in the HETP *vs.* linear velocity curves. The dashed line refers to the HETP values measured at a linear velocity of 1 cm/sec with a column filled with 25–33 μm particles.
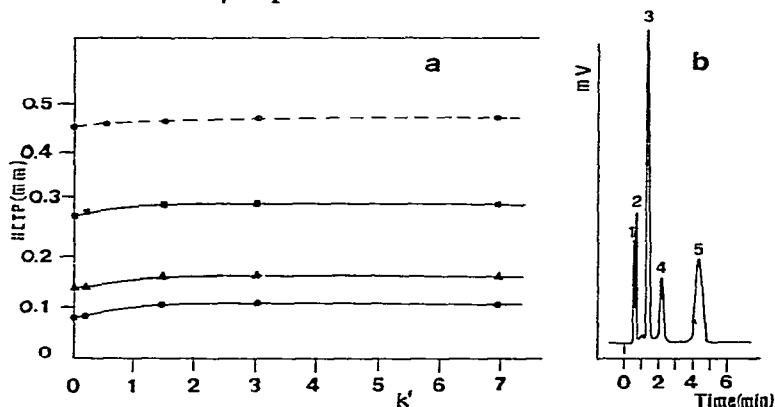

Fig. 3. a, HETP *vs.* capacity ratio calculated at a linear velocity corresponding to the minimum HETP value for Carbopack B particles of different sizes (see Fig. 2). The dashed line corresponds to the HETP value measured at a linear velocity of 1 cm/sec. b, Typical chromatogram obtained with a glass column (25 cm × 1.6 mm I.D.) packed with Carbopack B (particle size, 25–33 μm). Peaks: 1 = benzene; 2 = 1,3,5-trimethylbenzene; 3 = naphthalene; 4 = methylnaphthalene; 5 = 3,6-dimethylnaphthalene.

When the above fractionating procedure described was carefully followed, the differences in HETP between the various columns in each set never exceeded the values in Figs. 2 and 3 by more than 10%. Fig. 3b shows a typical chromatogram obtained with a 25-cm column filled with 25–33 μm particles and working at a linear velocity of 1 cm/sec.

From the results reported in Figs. 2 and 3 the following conclusions can be made:

(1) The average minimum HETP values obtained for retained compounds (k' from 3 to 7) are almost equal to four times the particle diameter (reduced plate height ranging from 3.7 to 4.0). This indicates that quite efficient columns can be obtained with GCB. If the results obtained at low k' with GCB do not differ appreciably from those obtained with hardened carbon black particles[5], the slope of the HETP vs. $\bar{u}$ plots obtained at high linear velocity appears to be less with GCB than with pyrolytic carbon.

(2) Plots of HETP vs. k' are almost flat for different values of the mean particle diameter and for the range of linear velocities employed. This means that efficiency losses arising from non-linearity of the adsorption isotherm are negligible and there is only slight tailing of the peaks due to the dead volume. This explains why the values obtained at $k' = 0$ are slightly higher than expected on the basis of plate height theory. It should be added, however, that whereas the value of the minimum HETP at $k' = 0$ is of theoretical interest, in practice of greater importance is the HETP value measured for a fairly strongly retained compound ($k' = 3$) when the linear velocity approaches the values commonly used during analytical separations (0.5–1 cm/sec). In this content, GCB columns show a comparable or better performance than those filled with the commonly used reversed-phase packings, having the same particle diameter.

The above considerations demonstrate the advantage in using Carbopack B columns in comparison with pyrolytically hardened carbon black particles. The high efficiency observed with the former at high values of the capacity ratio should undoubtedly be attributed to its greater homogeneity in comparison with the latter. The pyrolytic treatment produces an increase of the number of active sites on the carbon surface having an intermediate structure between amorphous and graphitized carbon, and therefore a lower degree of homogeneity[9].

The packing performance of GCB columns has been evaluated also by measuring the permeability exhibited by each set of columns characterized by different particle size. Column permeabilities, φ, were calculated from the equation[10] $\varphi = \Delta P d_p^2 / L u \eta$, where ΔP is the column pressure drop, L the column length, u the mobile phase velocity and η the eluent viscosity. Values ranging from 650, when $d_p > 33$ μm, to 870 when $d_p < 33$ μm, have been calculated. Although the packing permeability is not as good as that calculated for pyrolysed carbon black, the values obtained indicate that GCB may be suitable for HPLC.

It has to be stressed, however, that Carbopack B columns have a more limited field of application than those packed with modified carbon black. As they require solvents of viscosity less than 0.7 cP, any attempt to use water–methanol (30:70) or even pure ethanol as eluent has been unsuccessful. Although this limitation might in practice be overcome by operating with eluents of low viscosity, which in the case of carbon black cover almost the entire scale of eluotropic strength[7], care has to be taken to prevent columns plugging when viscous eluents are needed in connection with rapid gradients.

Moreover it has so far been impossible to prepare GCB particles smaller than 20 μm, a size available for other packing materials.

Practical application of GCB columns

Some analytical applications which can be performed rapidly without the use of gradients or viscous solvents have been examined to provide a direct comparison with the packings commonly used in reversed-phase liquid chromatography.

Fig. 4b shows the separation of a mixture of chlorinated pesticides and Fig 4a the fingerprint of a commercial PCB mixture (Fenclor 64). As the former sample was analyzed by using a more selective but also weaker eluent (methanol) than the latter, it is suggested that GCB columns might fruitfully be employed for sample fractionation.
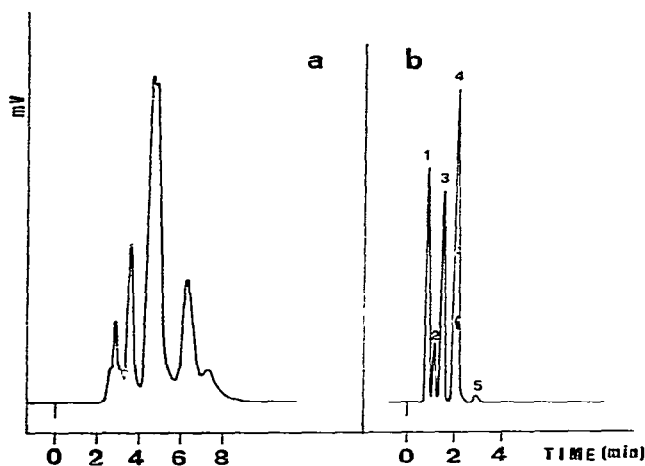

Fig. 4. a, Separation of a PCB mixture (Fenclor 64) on a glass column (25 cm × 1.6 mm I.D.) packed with Carbopack B (25–33 μm). Eluent: pentane. Flow-rate: 1 ml/min. b, Analysis of a mixture of chlorinated pesticides on the same column as in a. Eluent: methanol. Flow-rate: 0.5 ml/min. Peaks: 1 = chlordane; 2 = aldrin; 3 = pp'-DDT; 4 = Metoxichlor; 5 = unknown.

Fig. 5a shows the chromatogram of a mixture of alkyl phthalates commonly used as plastifiers, and Fig. 5b illustrates the analysis of an environmental sample consisting of a water condensate collected from the gaseous emission of an urban waste incinerator. Although these analyses can be performed with many other packings, it is worth noting the selectivity exhibited by GCB in the separation of diisobutyl and di-*n*-butyl phthalate isomers.

Fig. 6a shows the separation of atrazine, a known herbicide, from some of its metabolites produced by bacterial degradation[11].

Fig. 6b illustrates the direct analysis of the products formed during an "*in vitro*" bacterial degradation of an atrazine solution.

Fig. 7 shows a typical pharmaceutical analysis, which is generally carried out by reversed-phase chromatography: the separation of active components of an analgesic pharmaceutical product consisting of phenylacetamide, phenacetin and caffeine. Acetylsalicylic acid, if present in such drugs, is eluted after the phenylacetamide peak.
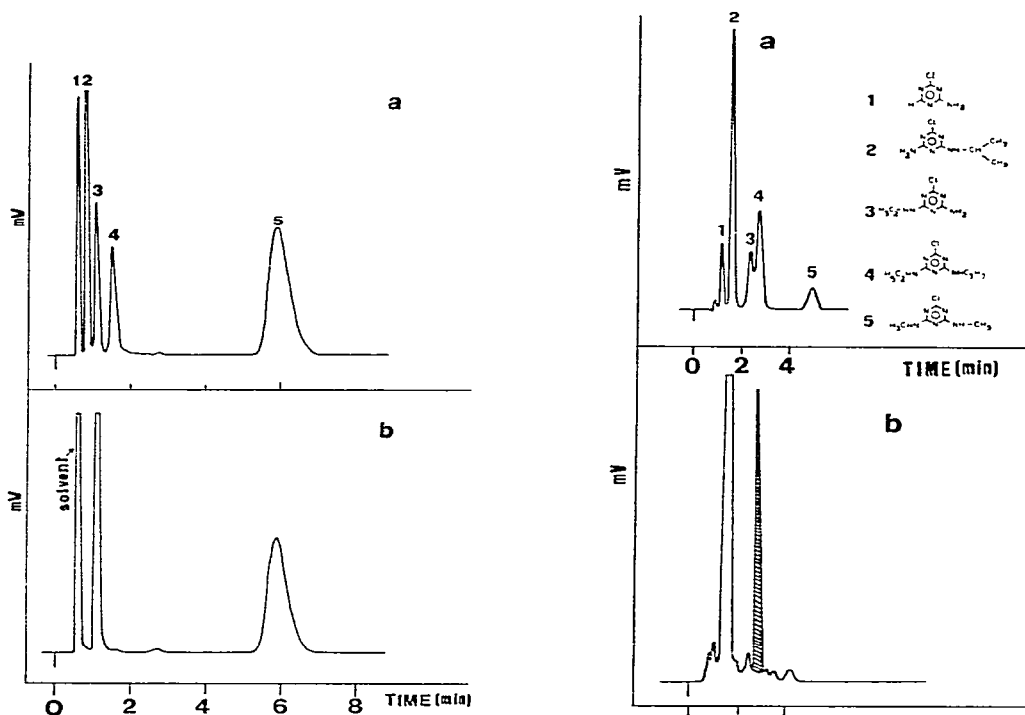
Fig. 5. Separation of a mixture of plastifiers on a glass column (25 cm × 1.6 mm I.D.) packed with Carbopack B (25–33 μm). Eluent: methanol. Flow-rate: 1 ml/min. a, Chromatogram of a standard mixture. Peaks: 1 = dimethyl phthalate; 2 = diethyl phthalate; 3 = Diisobutyl phthalate; 4 = di-*n*-butyl phthalate; 5 = di-*n*-octyl phthalate. b, Water condensate from urban waste incinerator.

Fig. 6. Analysis of some triazine isomers. Column and eluent: same as in Fig. 5. Flow-rate: 0.5 ml/min. a, Pure standards; b, sample collected from an "*in vitro*" bacterial degradation of atrazine.
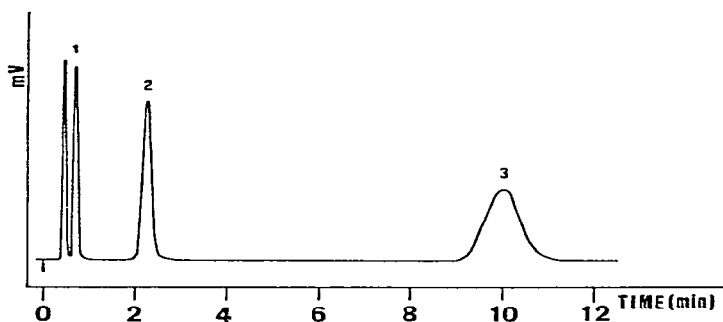

Fig. 7. Separation of some constituents of a pharmaceutical preparation (analgesic). Conditions as in Fig. 5. Peaks: 1 = phenylacetamide; 2 = phenacetin; 3 = caffeine.

Finally Fig. 8 shows the separation of two UV absorbing free amino acids in methanol solution: phenylalanine and tryptophan. This separation should be regarded only as an attempt to demonstrate the versatility of GCB for the analysis of such fractionations.
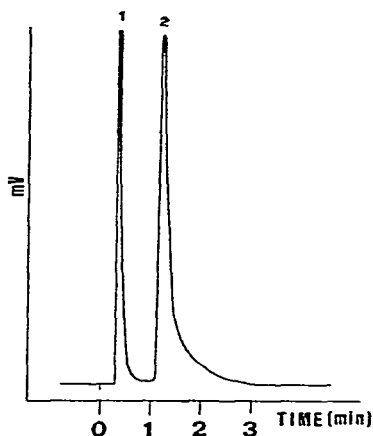
Fig. 8. Separation of two UV absorbing amino acids. Conditions as in Fig. 5. Peaks: 1 = phenylalanine; 2 = tryptophan.

CONCLUSIONS

Commercially available GCB can successfully be used for the preparation of HPLC columns providing the material is fractionated into various sizes. Although there are some limitations related to the viscosity of the eluents, GCB, which is a natural reversed phase, may be specific in the analysis of polar low boiling isomers as in the separation of complex mixtures, especially when particles having small diameters can be employed.

ACKNOWLEDGEMENTS

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