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Development and use of carbon adsorbents in the liquid chromatographic separation of isomers

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SUMMARY

Porous carbonaceous sorbents, prepared by a replicate method on a silica template, were examined under the liquid chromatographic conditions. The carbons were obtained by pyrolysis of two different organic precursors (a phenol-formaldehyde resin and saccharose) at 600°C. Columns packed with 15–20- μm particles were obtained by means of high-viscosity slurry techniques. The separation of some pairs of *E/Z* diastereomers is described and discussed.

INTRODUCTION

Significant progress in the preparation of carbonaceous adsorbents as stationary phases for high-performance liquid chromatography (HPLC) has taken place in the last decade^{1–10}. Many varieties of carbonaceous materials have been developed and applied to a fair range of chemically different solutes ranging from non-polar to highly polar and ionic. However, the chromatographic behaviours of such carbonaceous stationary phases were different, owing to a variety of factors; some of them have new properties and offer a unique hydrophobic and hydrophilic selectivity which is different from that of reversed-phase alkyl-bonded silica. Unfortunately, most of the carbons produced have some but not all of the desirable requirements for good chromatographic phases, such as the correct combination of particle rigidity, high surface area, uniformity of the pore structure and uniformity of the surface composition.

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An ideal carbonaceous column packing material, showing high chemical stability over a wide pH range, adequate particle hardness to withstand high pressures, high surface area, defined functional groups homogeneously distributed on the particle surface and a mean pore size larger than 10 nm to ensure a rapid mass transfer to solutes is very difficult to obtain, and inevitably compromises will have to be made. Despite this, some good-quality carbons have recently been developed and tested¹¹. Critical reviews of the progress and problems in the field of carbonaceous adsorbents for LC have been published^{12,13}. Columns with high-efficiency carbon packings have only just become commercially available (Hypercarb; Shandon, Runcom, U.K.). The superior selectivity of the surface of some carbons towards positional isomers such as *E/Z* is one of the most impressive properties compared with conventional HPLC phases.

This paper describes the results obtained with two different carbon adsorbents in the separation of *E/Z* isomers of α -chlorochoalcone (2-chloro-1,3-diphenyl-1-propen-1-one), safrole-(*E/Z*)-isosafole and -(*E/Z*)-stilbene.

EXPERIMENTAL

Materials

The LC separations were carried out with carbon CF-18 (derived from phenol-formaldehyde resin) and CF-19 (from saccharose), prepared at the Institute of Polymers of the Slovak Academy of Science (Bratislava, Czechoslovakia), using a replicate method involving the pyrolysis of precursors on a microparticulate silica matrix¹⁴⁻¹⁶.

The physical and chemical characterizations of the carbonaceous adsorbents, performed through particle-size analysis, shape determination via electron microscopy, surface area determination, thermal analysis and Fourier transform (FT) IR spectroscopy have been described previously¹⁷.

Both carbons used in this work have a sponge-like structure with good mechanical strength. Scanning electron micrographs of CF19 and CF18 are shown in Figs. 1 and 2. The determined physical characteristics of the two packing materials are summarized in Table I.

The rough materials with particle size ranging from 5 to 90 μm , were directly sieved into narrow-mesh size fractions using an ASTM-DIN 5- μm IG/1 microsieve shaker (Giuliani, Turin, Italy). The stainless-steel chromatographic columns (250 \times 4.6 mm I.D.) were packed using the slurry-packing technique. The slurry contained about 8% (w/w) of carbon adsorbent in a mixture of dibromomethane and acetonitrile. The density of the mixture was adjusted in order to avoid considerable floating and sedimentation of carbon particles. After treatment in an ultrasonic bath for 5 min, the slurry was pressed into the column with acetonitrile under a pressure of 20-30 MPa.

Equipment

The analyses were performed on a Perkin-Elmer Series 3B liquid chromatograph equipped with a Rheodyne 7125 injection valve and a Perkin-Elmer LC-100 column oven. Component elution was monitored with a Perkin-Elmer LC-75 variable-wavelength detector coupled with a Perkin-Elmer LC-75 Autocontrol.

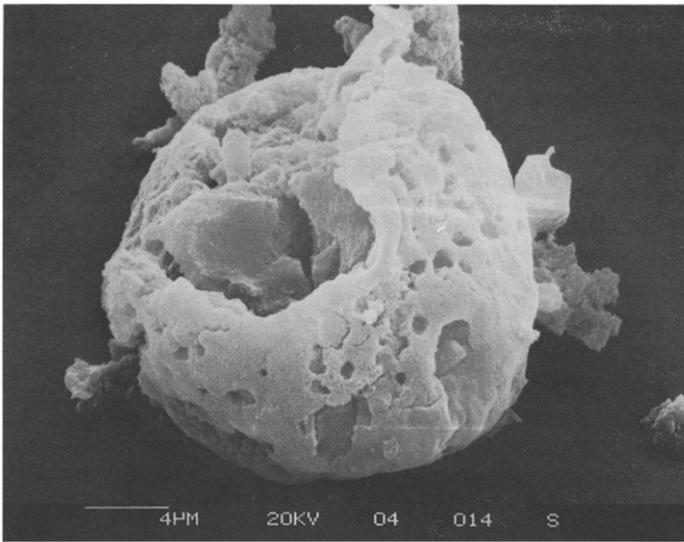


Fig. 1. Scanning electron micrograph of carbon CF18.

The separations were achieved under the following conditions. For (*E/Z*)- α -chlorochoalcone, the mobile phase was acetonitrile–water–chloroform (70:10:2), flow-rate 2 ml min^{-1} , temperature 35°C and wavelength 260 nm . For safrole–(*E/Z*)-isosafrole, the mobile phase was acetonitrile–water–chloroform (70:6:5), flow-rate 2 ml min^{-1} , temperature 35°C and wavelength 254 nm . For (*E/Z*)-stilbene, the mobile phase was acetonitrile–chloroform–2-propanol (50:40:10), flow-rate 2 ml min^{-1} , temperature 35°C and wavelength 290 nm . Peak areas were measured with a Perkin-Elmer LCI 00 laboratory computing integrator.



Fig. 2. Scanning electron micrograph of carbon CF19.

TABLE I

PHYSICAL CHARACTERISTICS OF THE PYROLYTIC CARBONS

 d_n = number-average particle diameter; d_w = weight-average particle diameter.

Sample	Surface area (m^2/g)	Bulk density (g/cm^3)	Residual silica (%)	d_n μm	d_w μm	d_w/d_n
CF18	770	0.26	10	26.7	32.9	1.23
CF19	735	0.45	2	22.4	32.7	1.46

For peak assignments, the UV spectra of the separated *E/Z* isomers were measured in the stopped-flow mode on the LC-75 Autocontrol and compared with reference spectra.

RESULTS AND DISCUSSION

The diffuse reflectance (DR)-FT-IR spectra of the two carbons used show a number of absorptions due to the presence of several functional groups; the charring process did not reach the stage of complete destruction of the starting structure. At the temperature used in the preparation of the two carbon adsorbent samples (600°C), a carbonaceous polymer network was formed which still maintained some of the chemical features of the initial material, together with new functional groups generated in the carbonization process. The carbon obtained by pyrolysis of saccharose is different to that from phenol-formaldehyde; both types of carbons, however, show a mixed behaviour owing to the presence of polar and non-polar groups. In CF19 carbon the charring of saccharose proceeded with extensive condensation of the starting molecules and aromatization of the resulting structure; oxygen-containing groups were still substantially present either as OH residues or as ether links or as newly formed C=O groups¹⁷.

As expected, the carbonaceous packing is suitable for the separation of homologous series of compounds, which depend on hydrophobic selectivity, and for the separation of isomers which depend on stereoselective surfaces.

In preliminary experiments, chromatographic separations were achieved using a binary solvent mixture of acetonitrile and water. Although mixtures of acetonitrile and water can generally be used to obtain mobile phases with a wide range of eluotropic strength, the results were not satisfactory. By increasing the strength parameter (ϵ) by adding chloroform to the mobile phases, the efficiency and the selectivity were considerably improved.

The preliminary studies performed on CF19 confirmed that this material was more suitable than CF18 for the resolution of positional isomers, and therefore we used columns packed with the former phase in subsequent work.

Fig. 3 shows the separation of *E/Z* isomers of α -chlorochoalcone on a CF19 column. Figs. 4 and 5 illustrate the separation of safrole - (*E/Z*)-isosafrole and (*E/Z*)-stilbene in the same column.

Although the particle batches of carbon CF19 used were relatively large ($d_p > 15 \mu m$) and irregularly shaped and the size distribution was not perfectly uniform, which is generally prejudicial to efficiency, the *E/Z* isomers tested were very easily

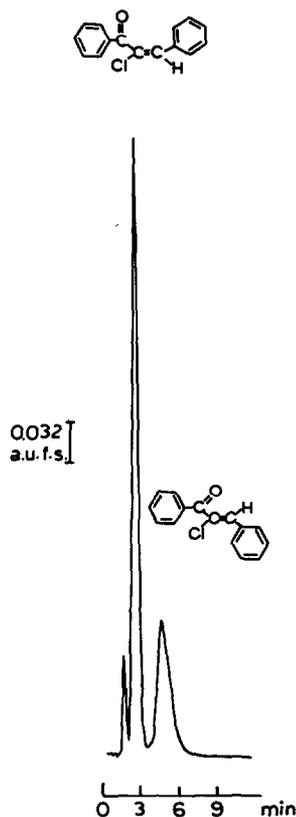


Fig. 3. Separation of (*E/Z*)- α -chlorochalcone. The peak at a retention time of 1.77 min is an impurity. Column, CF19, 15–20 μm (250 \times 4.6 mm I.D.); mobile phase, acetonitrile–water–chloroform (70:10:2); flow-rate, 2 ml min^{-1} ; temperature, 35°C; wavelength, 260 nm.

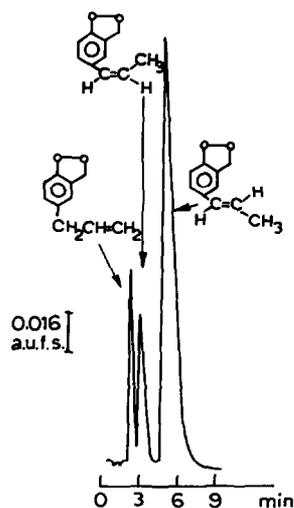


Fig. 4. Separation of safrole and (*E/Z*)-isosafrole. Column, CF19, 15–20 μm (250 \times 4.6 mm I.D.); mobile phase acetonitrile–water–chloroform (70:6:5); flow-rate, 2 ml min^{-1} ; temperature, 35°C; wavelength, 254 nm.

separated and the chromatographic behaviour of the carbon column was satisfactory. The peak broadening that can be observed on the chromatograms may be due to the presence of narrow pores on the carbon particle surface (very low mass transfer in such pores), to the irregular shape and size range of the particles or to incorrect packing.

Even though in solute–sorbent interactions charge transfer (π -complexes) and polarization phenomena play an important role, the greatest contribution to absorption on the carbonaceous surface seems to be associated with dispersion forces of the sorbate molecules¹⁸. The structural effect of the sorbate molecules is non-linear and depends on the difference in the geometric structure of the molecules.

For example, in the (*E/Z*)- α -chlorochalcon separation, the molecule of the *E*-isomer is less planar than the *Z*-isomer molecule, which has a higher contact surface area with the carbonaceous adsorbent. The *Z*-isomer, which is more planar, is therefore more strongly retained than the *E*-isomer, in which the smaller surface area of contact reduces the absorption contribution.

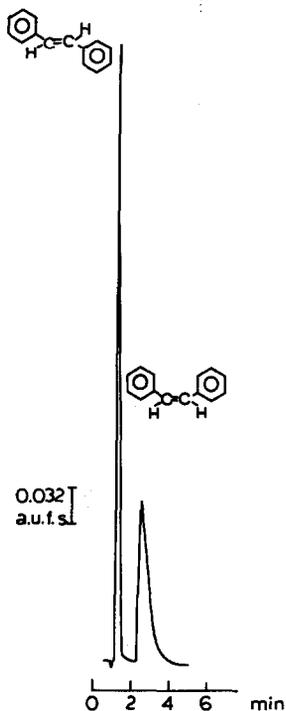


Fig. 5. Separation of (*E/Z*)-stilbene. Column, CF19, 15–20 μm (250 \times 4.6 mm I.D.); mobile phase, acetonitrile–chloroform–2-propanol (50:40:10); flow-rate, 2 ml min^{-1} ; temperature, 35°C; wavelength, 290 nm.

CONCLUSION

Despite the fact that the separation of *E/Z* isomers was performed with a medium-efficiency column, the initial results are very promising. Dispersion forces play the major role in the adsorption on the surface of the carbonaceous particles, and this is shown by stereoselectivity in the separation of positional isomers, particularly when changes in the substituent positions have a considerable effect on the space configuration of molecules having the same mass.

To conclude, the carbonaceous adsorbents tested revealed a very high resolving power for isomer separations compared with conventional packing materials such as silica and silica-bonded phases, on which the resolution of these molecules is very difficult to achieve.

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