REVERSED-PHASE LIQUID–SOLID CHROMATOGRAPHY ON MODIFIED CARBON BLACK

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SUMMARY

The preparation of carbon adsorbents suitable for reversed-phase high-performance liquid chromatography is described. The effect of the amount of carbon deposited by benzene pyrolysis on thermal carbon blacks on the hardness and specific surface area of the particles obtained is discussed. Optimum performances are obtained with 20% of pyrocarbon. The chromatographic performance of the columns is discussed in terms of coefficients of the Knox and Giddings equations and a scale of solvent eluotropic strengths is established. Efficiencies corresponding to HETP values of 3 and 7 times the particle diameters are obtained for unretained ($k' = 0$) and retained ($k' = 1.1$) compounds. The polarity of the solvent has little influence on the retention but affects the solubility in the mobile phase. The weakest solvents are low-molecular-weight, polar molecules such as water, methanol and acetonitrile, and the strongest solvents are heavy and/or aromatic molecules, such as chloroform, benzene and xylene. Homologous compounds are easy to separate, as well as geometrical isomers. Some examples of separations are described and discussed.

INTRODUCTION

The advantages of the reversed-phase technique in liquid chromatography and especially in high-performance liquid chromatography (HPLC) are well known and have recently been reviewed[1-4]. Up to now, only chemically bonded stationary phases have been available for carrying out reversed-phase HPLC. Excellent results are obtained when the phases are stable[5-7], and recently reactions have been found for synthesizing phases that resist solvolysis by most liquid phases, including water and methanol. They are still difficult to prepare and very expensive, however. The organic groups are bonded to a silica surface, which permits generally the use of the very fine particles available. These groups are mainly octyl, octadecyl and phenyl[4]. The exact retention mechanism is not yet known in detail, but it certainly involves non-polar dispersion forces between the solute molecules and the groups bonded to the silica

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surface. These groups exhibit complete disorder in their positions and in their orientation above the surface. Consequently, the selectivity that can be expected is the same as that of a non-polar liquid.

In true adsorption chromatography, selectivity to geometrical isomers is exhibited if adsorbents of regular structure are used, as is well known in gas–solid chromatography, using graphitized carbon black. The use of carbon adsorbents in HPLC could thus offer a non-polar, inexpensive stationary phase, with a large specificity for geometrical isomers and a high thermal stability, which makes it easy to cleaning from its surface heavy compounds trapped on it that are not soluble in the mobile phase. The direct use of thermal carbon blacks, either graphitized or not, is impossible in HPLC, however; their mechanical stability is much too poor as the particles are in fact aggregates of polycrystalline microparticles 100–5000 Å in size. These assemblages are destroyed by shear forces due to the liquid flow during column operation or which appear during packing procedure or even during sieving. Hardening of these particles to overcome a similar but much less serious problem, limiting their gas chromatographic (GC) performance, has been described[8]. An analogous procedure has been used by us. The conditions are different as a much greater particle hardness should be achieved, the shear forces in liquid chromatographic (LC) columns being much larger than in GC columns. Good results have been obtained in the preparation of the particles, as described in a previous paper[9]. The procedure has been slightly modified to increase the production capacity. Smoother, more homogeneous particles are obtained as a consequence of the changes and better chromatographic results are achieved.

This paper deals mainly, however, with chromatographic results, applications to various analyses and the derivation of the eluotropic strength of a number of solvents, allowing their classification in an eluotropic series. This series, analogous to that derived by Snyder for silica and alumina, is very useful in helping the analyst to choose the best solvent. Results on the permeability and efficiency of the columns are also given, but these are preliminary, as the parameters of the preparation procedure have not yet been optimized.

PREPARATION OF THE CARBON PHASE

Carbon blacks are commercially available in two forms, a very fine powder, mostly microparticles of average diameter between 100 and 5000 Å, and larger agglomerates (50–1000 μm), made by pelletization of these microparticles. The powder is too fine to be used in HPLC and the agglomerates are too coarse and unstable[9]. The packing of good, efficient HPLC columns requires hard spherical particles, with an average diameter between 5 and 30 μm and a very narrow size range[10–12]. Hence there are three steps in the preparation of the adsorbent: the preparation of small agglomerates, hardening of these particles and size sorting.

Sieving of carbon blacks

Direct sieving of the original material (Black Pearls L from Cabot, Boston, Mass., U.S.A.) is not the method of choice as the aggregates are too crumbly, assemble in larger agglomerates under the effect of mechanical shaking and contain too small a proportion of the desirable fine particles (5–7% below 25 μm). The rough material

is therefore crushed over a 50-μm mesh sieve using a thick sheet of paper and the particles are forced through the sieve. This gives a good yield of 15–30-μm particles, which are then sieved during a short time to prevent their assembly into larger aggregates.

Hardening of the particles

The particles are hardened by pyrocarbon resulting from the pyrolysis of benzene[8,9]. The experimental arrangement has been described previously[9]; some modifications were made in order to increase the production to more than a few grams a day.

Basically, the particles are heated at 900° in a stream of nitrogen saturated with benzene vapour at 30° by passing it through a temperature-controlled bubbler. The partial pressure of benzene is 0.21 atm. Previous study has shown that the amount of pyrocarbon made on the carbon black is proportional to the total amount of benzene circulated through it, independent of the flow-rate[9]. Typical experimental conditions and results are given in Table I. The carbon yield is almost constant.

Size sorting

The particles are now hard enough to withstand all methods of size sorting. Sieving into narrow-mesh size fractions and elutriation are possible. These procedures allow the elimination of carbon black resulting from benzene pyrolysis[13] or from the layer of pyrocarbon on the furnace wall that is peeled off during cooling. The procedure gives mainly particles of size between 20 and 40 μm, and work is in progress to prepare smaller particles.

Characteristics of the carbon and optimization of the procedure

The carbon obtained is metallic grey, whereas carbon black is deep black. The most important properties, however, are the hardness, the chemical nature of the surface and the specific surface area of the particles.

The hardness is measured using the method describing by Barmakova et al.[8]. After preliminary sieving, about 2 g of the 80–125-μm fraction are placed on an 80-

TABLE I

PREPARATION OF HARDENED CARBON BLACKS

Original carbon black	Flow-rate of nitrogen (ml/min)	Temperature of the C_6H_6 (°C)	Reaction time (min)	Pyrocarbon coated (%, w/w)	Pyrolysis yield (%)
Black Pearls 46 (654 m²/g)	65.2	33.5	10	44	64
	60	33.25	15	67	70
	64	33.25	20	94	69
Black Pearls 800 (250 m²/g)	64.1	28.5	5	16	72
	65.2	28.0	14	41	74
	68	28.0	25	75	71
Black Pearls L (110 m²/g)	65.2	27.75	5	9.5	70
	63.8	28.0	10	19	70
	63.8	28.0	20	37	70

μm sieve together with 12 steel balls, each 8 mm in diameter. The sieve is shaken for 10 min on a sieve shaking machine, and the amount of powder that is crushed and falls through the sieve is weighed. Fig. 1 shows the influence of the amount of pyrocarbon on the particle hardness. This result is in agreement with the fact that columns packed with carbon are stable at moderate flow-rates only when the amount of pyrocarbon exceeds 15–20%[9]. The determination of the shear force necessary to break a sizable fraction of the particles through the variation of permeability with pressure gradient would certainly be more significant[14]. This is more difficult and time consuming, and will be done later.
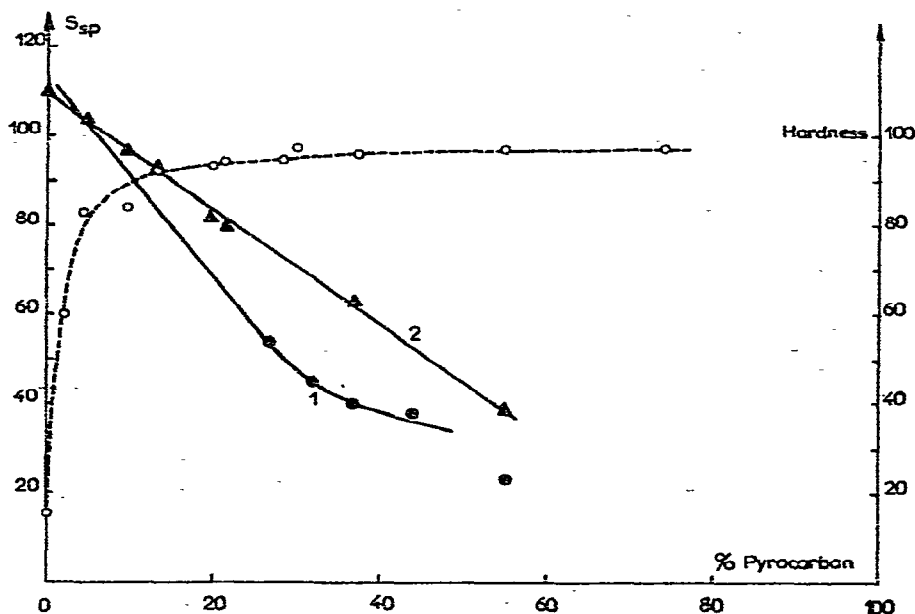
Fig. 1. Influence of the amount of pyrocarbon (gram per gram of carbon black treated) on the physical properties of carbon black (Black Pearls L). Solid lines and ▲: specific surface area (S_{Sp}, m²/g). 1 = Results obtained with the previous procedure[9]; 2 = results given by the new procedure. The point for untreated carbon black is identical with the origin of the solid lines and to that given by the manufacturer. Broken lines and ○: particle hardness (wt.% of uncrushed particles, see text).

Benzene pyrolysis occurs mainly on the carbon surface. The phenomenon is similar to the coating of a solid support by a liquid stationary phase in GC[15,16]. A large decrease in the specific surface area with an increase in the amount of pyrocarbon made can be expected and is indeed observed (cf., Fig. 1). We lack data on carbon blacks with larger specific surface areas. However, in most instances the adsorption on carbon is very strong, and so optimal retention is observed using carbon powder with a low or moderate specific surface area (see below).

Only some of the experimental parameters have been investigated, and are far from optimized. Most analyses were carried out with carbon blacks coated with about 40% of pyrocarbon.

PACKING PERFORMANCE

The packing performance can be measured by the column efficiency and to a lesser extent by the permeability[7,11,17]. The column efficiency is best shown as the variation of the reduced plate height ($h = H/d_p$) with the reduced velocity ($v = ud_p/D_m$) for non-retained and retained compounds. In the first instance, the quality of the packing itself is studied, while in the second the quality of the adsorbent becomes critical.

The column permeability is characterized by the value of

$$\varphi' = \frac{\Delta P \, d_p^2}{L \, u \, \eta} \tag{1}$$

where ΔP is the column pressure drop, L the column length, u the mobile phase velocity and η the viscosity. In practice, φ' is between 500 and 1000 (ref. 18). The smaller h and φ' are, the better is the column. Experimental results are given in Table II.

Usually, h and v are related by the Knox equation[5-7]:

$$h = \frac{B}{v} + A \, v^{0.33} + C \, v \tag{2}$$

when B accounts for the effect of axial diffusion and is typically between 1.5 and 2, while C accounts for the resistance to mass transfer in the stationary phase. For uniformly porous spheres[7]:

$$C = \frac{1 + k' - \alpha}{30 \, (1 + k')^2} \cdot \frac{D_m}{D_s} \tag{3}$$

where α is the fraction of the eluent in interparticle space, k' the column capacity ratio and D_s the average diffusion coefficient of the solute between particles. For good silica columns[4,7,11], C is small, typically $1-2 \cdot 10^{-2}$. The resistance to mass transfer in the mobile phase outside the particles is accounted for by A and is closely related to the quality of the packing.

Our experimental results have been fitted to eqn. 2, using a least-squares fit technique. The optimum values of the parameters are listed in Table II. As some coefficients are negative, we have used the original rigorous equation[17]:

$$h = \frac{B'}{v} + \frac{A'}{D' + \dfrac{1}{v}} + C' v \tag{4}$$

Furthermore, as it is difficult, imprecise and time consuming to measure column efficiency at the very low flow-rate corresponding to values of the reduced velocity below a few units when particle sizes of 20–40 μm are used, we have assumed $B = B' = 2$ in all instances in eqns. 2 and 4. The diffusion coefficients are derived from the classical Wilke and Chang equation[17], taking 1.5 as the association parameter of acetonitrile.

TABLE II

EFFICIENCY OF DIFFERENT COLUMNS PACKED WITH CARBON

Mobile phase: acetonitrile.

Characteristics	Column			
	A	B	C	D
Length (cm)	70	70	55	54
d_p (μm)	31.5–40	15–20	25–31.5	25–31.5
Permeability (φ')	1810	1610	740	650
Efficiency				
$k' = 0$*:				
A	23.79	16.24	1.82	1.34
C	−0.54	−0.16	0.023	0.027
A'	21.6	17.4	1.42	1.28
D'	0.38	0.39	0.243	0.20
C'	0.15	0.17	0.045	0.028
v_{opt}**	0.35	0.40	1.60	1.70
h_{min}**	12.40	11.05	2.93	2.85
$k' = 1.15$***:				
A	—	23.7	10.0	11.0
C	—	0.75	0.31	0.23
A'	—	57.3	8.4	7.7
D'	—	1.34	0.44	0.51
C'	—	1.91	0.48	0.35

* Benzene.
** Calculated for $k' = 0$ by eqn. 4.
*** 2,3,6-Trimethylphenol.

Columns A and B, packed with insufficiently hardened material, are very poor, as shown by the very large value of the coefficients A and A'. In fact, from the value of φ' it can be assumed that the particles have been broken during packing and the average particle size reduced from 35 μm to about 20 μm. In column C, the fine dust has not been removed from the carbon material. The efficiency is slightly improved when this is done, as shown by the data for column D.

The almost 3-fold reduction in efficiency when k' increases from 0 to 1.10 is unexpected, unexplained and somewhat disappointing. A similar effect has been observed on silica or alumina particles[19], when large particles were used in about the same size range as our carbon particles, but the silica or alumina columns were used at much larger reduced velocities. Part of the effect, at least, may be attributed to the formation of bottle-neck micropores during benzene pyrolysis. This explanation, however, is largely conjectural at present and has to be investigated in more detail. It does not seem, however, that this effect could result from column overloading and peak deformation due to a non-linear isotherm. Similar results have been observed on columns packed with treated carbon blacks of widely different specific surface area, the amounts injected being always the same.

RETENTION IN HOMOLOGOUS SERIES

It is well known that the size of alkyl groups, which usually is of little importance in conventional liquid–solid chromatography (LSC), greatly influences the re-
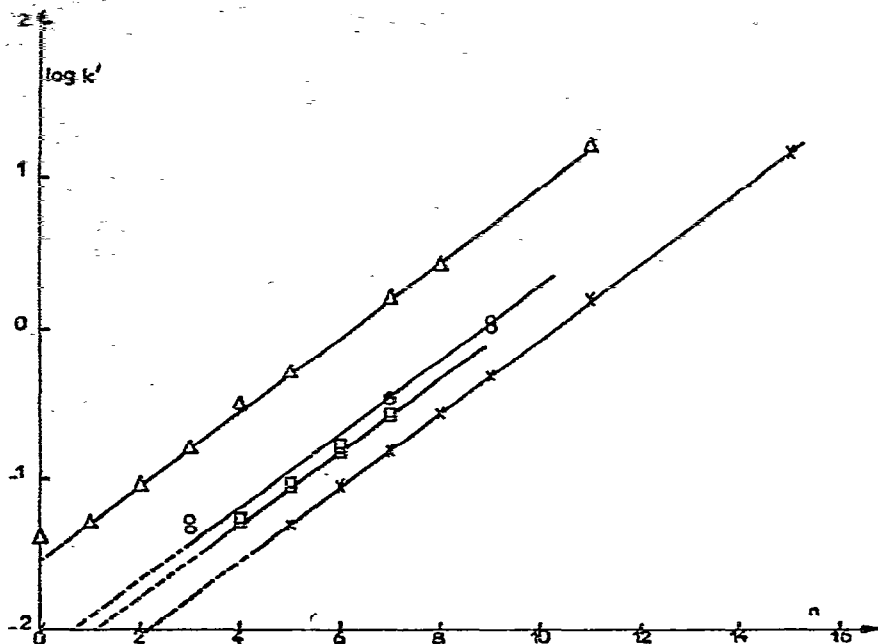
Fig. 2. Variation of log k' with the number of carbon atoms (n) in homologous series. $\triangle$ = C$_6$H$_5$–(CH$_2$)$_n$–CH$_3$; $\bigcirc$ = Br–(CH$_2$)$_n$–CH$_3$; $\square$ = Cl–(CH$_2$)$_n$–CH$_3$; $\times$ = HO–(CH$_2$)$_n$–CH$_3$. Column A (cf., Table II), Black Pearls + 55% of pyrocarbon. Solvent, acetonitrile; detector, UV and RI.

tention volume in reversed-phase chromatography. Fig. 2 shows the variation of the column capacity factor for homologous series of linear 1-alkanols and 1-chloro-, 1-bromo and 1-phenylalkanes as a function of the chain length. The plot obtained is similar to those derived from GC data. Similar results are obtained with other solvents and, as might be expected, the slope of the log k' versus number of CH$_2$ groups in the chain decreases with increasing solvent strength (see the next section).

The column capacity ratios, $k'_{i,j}$ and $k'_{i,k}$, for a given compound, i, in two different solvents, j and k, is given by[20]

$$\log k'_{i,j} = \log k'_{i,k} + \log \left(\frac{V_j}{A_j} \cdot \frac{A_k}{V_k} \right) + \frac{A_i}{2.3\,RT} (\sigma_j^0 - \sigma_k^0) + \log \left(\frac{\gamma_{i,j}^b \gamma_{i,k}^S}{\gamma_{i,j}^S \gamma_{i,k}^b} \right) \quad (5)$$

where A_j, V_j and σ_j^0 are the molar surface area, molar volume of species j, and the interfacial tension between the adsorbent and species j, respectively, while $\gamma_{i,j}^b$ and $\gamma_{i,j}^S$ are the activity coefficients at infinite dilution of solute i in solvent j in the bulk phase and on the surface, respectively. Furthermore, it has been shown[20] that $(\sigma_j^0 - \sigma_k^0)/2.3\,RT$ is proportional to the difference between the strengths of solvent j and solvent k, as defined by Snyder[21].

Assuming that the molar surface area, A_i, of the compounds i of a given homologous series is a linear function of the number, n, of CH$_2$ groups, and neglecting

the last term in eqn. 5, which accounts for the secondary effect due to activity coefficients, it can be derived that

$$\frac{\varepsilon_j^0}{\varepsilon_k^0} = \frac{l_j - l_R}{l_k - l_R} \tag{6}$$

where l_j is the slope of the plot of $\log k'_{i,j}$ versus n_i and R represents the reference solvent with $\varepsilon_R^0 = 0$.

Other interesting series of compounds are the polymethyl aromatic hydrocarbons. The variation of the column capacity factor with the number of hydrogen atoms replaced by a methyl group is shown on Fig. 3 for benzene, phenol and naphthalene derivatives. Although adsorption on a carbon surface is very selective for geometrical isomers, so that different points are obtained for all polysubstituted derivatives, there is a general trend showing again that, to a first (rough) approximation, $\log k'$ increases linearly with the number of methyl groups. It is also observed that the OH group behaves in a similar manner to a methyl group, although o-cresol and o-xylene can be easily resolved, demonstrating that dispersion forces play the major role in adsorption on carbon blacks. It is also worth noting that the substitution of four CH groups,
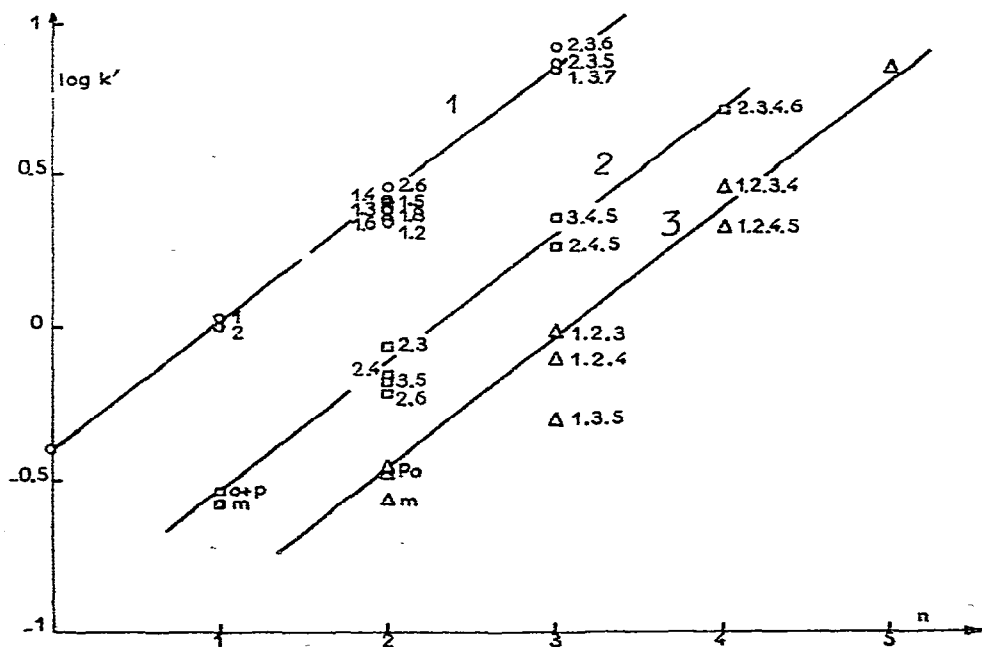
Fig. 3. Effect on the retention of the substitution of a methyl group on the aromatic ring. 1, ○ = Methylnaphthalene; 2, □ = methylphenol; 3, △ = methylbenzene. Solvent, acetonitrile; detector, UV. 1 = Column B, Sterling FTFF; 2 and 3 = column A (cf., Table II).

which transforms benzene into naphthalene, gives the same increase in retention as the substitution of 1.3 CH_2 groups, while the effect of a CH_3 substitution on an aromatic ring is 3.5 times greater than the increase of one CH_2 group in a linear alkyl chain. Very similar results are observed in GC.

Finally, it should be mentioned that isopropylbenzene is eluted before ethylbenzene (relative retention 0.6). This is in apparent contradiction with the experimental results discussed above. The molecular weight, molar volume and area of isopropylbenzene are, of course, larger than those of ethylbenzene, and so is the adsorption energy but not necessarily the adsorption free energy. This illustrates the fact that the adsorption entropy is also important although often overlooked. A similar situation is again observed in GC; the results of Kiselev and Yashin[22] indicate that ethylbenzene has a larger retention volume than isopropylbenzene below 95 °C, although its adsorption potential is smaller[23]. The order of elution is reversed above 95 °C as the adsorption entropy of isopropylbenzene is greater than that of ethylbenzene.

ELUOTROPIC STRENGTH

In conventional LSC, it is generally accepted that the more polar the solvent, the larger its eluotropic strength. As adsorption on carbon surfaces is essentially controlled by dispersion forces, polarity will be of secondary importance in reversed-phase chromatography on carbon. Although the weakest solvents are really the most polar, which is the opposite situation to that in classical LC, an investigation of the behaviour of 15 solvents and several mixtures showed that the phenomenon is more complex than it appeared at first[9], and that it would be misleading just to reverse the polarity scale obtained for polar adsorbents. The confusion is due to the fact that the most polar solvents are also those of smallest molecular weight, volume and area. These last properties determine the retention volumes much more than polarity. Thus, for instance, benzene is much less retained than phenol when using acetonitrile.

The polarity of the solvent, however, is not devoid of importance as it influences the solubility of the solute in the mobile phase, so that the last term in eqn. 5 can no longer be neglected. This is especially important for mixtures of solvents of different polarity when strong interactions can take place between the solute and the polar solvent. It has been shown, for example, that 1,3,4-trimethylbenzene, which is eluted before 3,4,5-trimethylphenol in acetonitrile–water mixtures of low water content, is eluted after the phenol in mixtures that contain more than 30% (v/v) of water[9]. Of course, the solubility of phenols in aqueous solutions increases rapidly with the water concentration owing to the formation of hydrogen bonds. If the water concentration increases, the solvent–adsorbent interactions decreases while the solvent–solute interactions are enhanced for polar solutes. The result is that the eluotropic strength of the solvent mixture seems to decrease less rapidly for polar than for non-polar solutes (Fig. 4). There is, of course, only one eluotropic strength for a given solvent, pure or in mixtures, but failure to take the last term in eqn. 5 into account results in scales of apparent eluotropic strengths that differ with the nature of the compounds used to measure it, thereby restricting their usefulness. If the predictive value of these scales is limited from the quantitative point of view, however, they can still be used qualitatively with advantage to select rapidly the best solvent to perform a particular analysis.
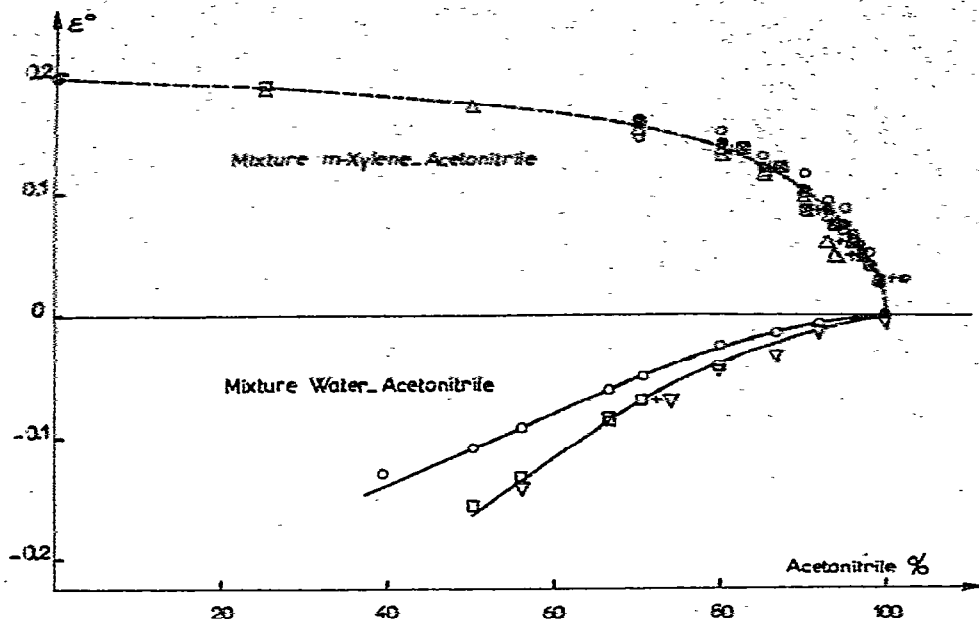
Fig. 4. Eluotropic strength of solvent mixtures as a function of acetonitrile concentration (volume-%). Broken line: mixture of *m*-xylene and acetonitrile: □ = pyrene; △ = phenanthrene; △ = 1,8-dimethylnaphthalene; ○ = acenaphtene; ⊠ = 2-methylnaphthalene, ◐ = naphthalene. Solid lines: water–acetonitrile mixtures (calculated from data in ref. 9): ▽ = 2,3,4,6-tetramethylbenzene; □ = 1,3,4-trimethylbenzene; ○ = 3,4,5-trimethylphenol. Column, C: Black Pearls, 55% pyrocarbon-graphitized. Detector, UV.

The eluotropic strength can be defined as

$$\log \left(\frac{k'_{i,j}}{k'_{i,k}} \right) = A_i \left(\varepsilon_k^0 - \varepsilon_j^0 \right) + B_{jk} \tag{7}$$

where the contributions of activity coefficient is neglected, and

$$B_{j,k} = \log \left(\frac{V_j}{A_j} \cdot \frac{A_k}{V_k} \right) \tag{8}$$

Eqn. 7 is analogous to eqn. 7a, given by Snyder[21], when taking into account the solvent effect $(B_{j,k})$:

$$\log \left(\frac{k'_{i,j}}{k'_{i,k}} \right) = A_i \left(\varepsilon_k^0 - \varepsilon_j^0 \right) \tag{7a}$$

The molar area is obtained by[24]

$$A_i = V_i^{\frac{2}{3}} N^{\frac{1}{3}} \tag{9}$$

where N is Avogadro's number.

Methanol was chosen as the reference solvent, R. As it is obvious that

$$\varepsilon_{J,R}^0 = \varepsilon_{J,S}^0 + \varepsilon_{S,R}^0 \tag{10}$$

it is possible to derive and use one scale of eluotropic strength while the retention of strongly adsorbed solutes cannot be measured using methanol.

Eluotropic strengths for a number of solvents have been measured using eqn. 7 applied to five different series of compounds: n-alkyl- and polymethylbenzenes, poly-methylphenols, polymethylnaphthalenes and fused-ring polynuclear aromatics (ace-naphtene, fluorene, phenanthrene and pyrene). The results are given in Table III. The differences in the calculated values have been previously explained. It has been mentioned[9] that for a series of homologous compounds

$$\log k_{i,J}' = \alpha_J + n \beta_J \tag{11}$$

and, on the other hand, we can assume that

$$A_i = a_i + b_i n \tag{12}$$

Hence eqn. 7 gives

$$\alpha_J - \alpha_k + (\beta_J - \beta_k) n = (a_i + b_i n)(\varepsilon_k^0 - \varepsilon_J^0) + B_J k \tag{13}$$

which should be valid for any value of n. This equation is equivalent to

$$\alpha_J - \alpha_k = a_i (\varepsilon_k^0 - \varepsilon_J^0) + B_{J,k} \tag{14}$$

and

$$\beta_J - \beta_k = b_i (\varepsilon_k^0 - \varepsilon_J^0) \tag{15}$$

For two solvents of the same eluotropic strength, eqns. 14 and 15 give

$$\beta_J = \beta_k \tag{16}$$

$$\alpha_J - \alpha_k = B_{J,k} \tag{17}$$

The slopes of the plots of $\log k_{i,J}'$ versus n are the same, but $k_{i,J}'$ and $k_{i,k}'$ are different, as $B_{J,k}$ is usually different from zero, unless the molar volumes are the same. Although the retention times of the compounds of a homologous series are different, the shift is proportional and the relative retention of two compounds remains the same.

As pointed out at the beginning of this section, eqn. 7, which neglects the bulk and surface activity coefficients, is only a rough approximation of eqn. 5. For ex-ample, the eluotropic strengths of methanol and acetonitrile are identical when eqns. 5 and 6 are used and different when derived from eqn. 7. Furthermore, eqn. 9 neglects interactions in the adsorbed layer and considers molecules as small cubes.

The data in Table III, however, are qualitatively very useful for solvent selec-

TABLE III

ELUOTROPIC STRENGTH OF VARIOUS SOLVENTS

Solvent*	Molecular weight	Log $\frac{V^0}{A^0}$	A^0 (m^2)	ε^0					Viscosity (cP)
				Alkyl-benzene	Methyl-benzene	Methyl-phenol	Methyl-naphthalene	Fused aromatic	
Methanol	32.04	−7.312	9.957·10⁴	0	0	0	0	0	0.597
Acetonitrile	41.05	−7.275	10.180·10⁴	0.038	0.048	0.025	0.039	0.039	0.364
Ethanol	46.07	−7.259	12.707·10⁴	0.049	0.051	0.058	0.051	0.051	1.200
n-Hexane	86.18	−7.142	21.749·10⁴	0.123	0.128	—	0.091	0.086	0.326
Ethyl acetate	88.11	−7.184	17.932·10⁴	0.095	0.105	0.080	0.091	0.091	0.455
n-Heptane	100.21	−7.125	23.474·10⁴	0.137	0.142	—	0.110	0.119	0.409
Butyl chloride	92.57	−7.174	18.727·10⁴	0.132	0.141	—	0.115	0.112	0.469**
n-Octane	114.23	−7.077	25.159·10⁴	0.151	0.149	—	0.138	0.139	0.542
Tetrahydrofuran	72.10	−7.211	15.835·10⁴	0.133	0.141	—	0.139	0.139	—
Methylene dichloride	84.83	−7.240	13.458·10⁴	0.127	0.137	—	0.132	0.133	0.449**
n-Nonane	128.26	−7.097	26.796·10⁴	0.157	0.181	—	0.161	0.161	0.711
Chloroform	119.38	—	—	—	—	—	—	—	0.580
Benzene	78.11	−7.198	16.821·10⁴	—	—	—	0.200	0.204	0.652
m-Xylene	106.16	−7.231	20.802·10⁴	—	—	—	—	0.240	0.520

* Classification by order of decreasing K'.
** At 15°.

tion. These data show, for example, that the range of solvent strengths is not very large. The strongest solvents seem to be aromatic compounds with large molecules. Owing to the necessity of working with solvents of small viscosity, the choice is limited. The use of the UV absorption detector is ruled out, which does not make these solvents very attractive. The consequence is that the analysis of strongly adsorbed compounds such as fused-ring polynuclear aromatics is possible only on carbon blacks of low specific surface area such as Sterling FTFF.

Using eqn. 7, and taking no account of B_{jk}, we have calculated the eluotropic strength of mixtures of water and acetonitrile (cf., Fig. 4). As this equation is an approximation, different values are obtained from the data pertaining to different compounds, as explained above. Much more consistant results are obtained for mixtures of the less polar m-xylene and acetonitrile (Fig. 4). The variation in eluotropic strength is very rapid at low concentrations of xylene, because xylene is much more strongly adsorbed than acetonitrile on carbon black. This phenomenon is similar to the variation in the eluotropic strength of mixtures of polar and non-polar compounds in conventional LC[20]. This effect can be utilized to adjust the polarity of the solvent mixture to optimize the sample solubility. For example, Fig. 4 shows that the eluotropic strength of a 1:1 mixture m-xylene and acetonitrile differs by less than 15% from that of pure m-xylene. The polarity of these mixtures varies widely and can be adjusted for large solubility of either polar or non-polar compounds.
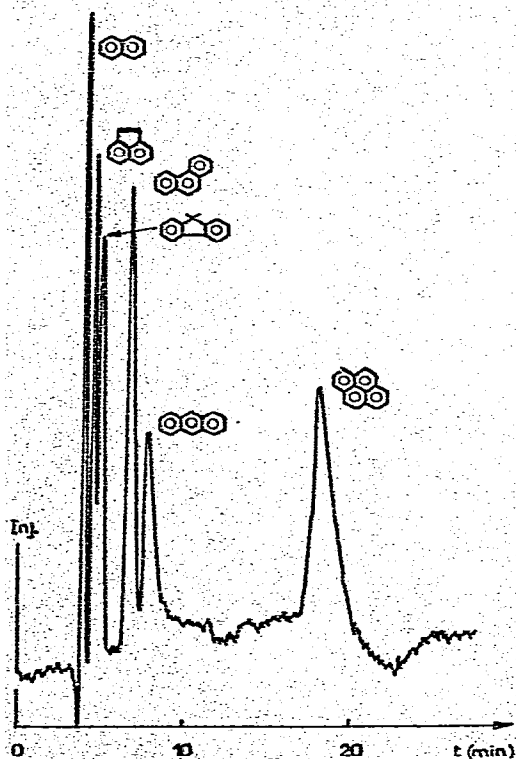
Fig. 5. Separation of polynuclear aromatic hydrocarbons. Column, C; solvent, benzene; detector, R.I.; flow-rate, 0.15 ml/min.

ANALYSIS ON CARBON COLUMNS

Reversed-phase chromatography must not be considered as a specific tool for non-polar compounds. It is true that many non-polar solutes that are eluted together in classical HPLSC can be separated by the reversed-phase technique. The separation of polar solutes, however, can also be performed using reversed-phase HPLSC because, as previously discussed, polarity does not have a major influence on retention. Thus, we have performed separations of linear aliphatic alcohols (C_5–C_{12}) and of linear alkylbenzenes (methyl to nonyl) using the same column, with the same eluent (acetonitrile). For a given number of carbon atoms (10), the retention of an alcohol ($C_{10}H_{21}OH$, $k' = 0.53$) is greater than that of an alkylbenzene [C_6H_5–$(CH_2)_3$–CH_3, $k' = 0.05$].

We think that pure reversed-phase HPLSC must be considered as a powerful technique for the separation of solutes that have only small differences in their molecular weights and have the same active groups, such as homologous series. Obviously, selectivity for positional isomers can be less than in classical HPLSC, as small changes in substituent positions can give rise to important variations in polarity and polarizability, at constant molecular weight. As we show later, however, such separations are often possible. On the other hand, when changes in physical properties are not sufficiently important to provide a successful separation by classical HPLSC, the reversed-phase technique can give good results.
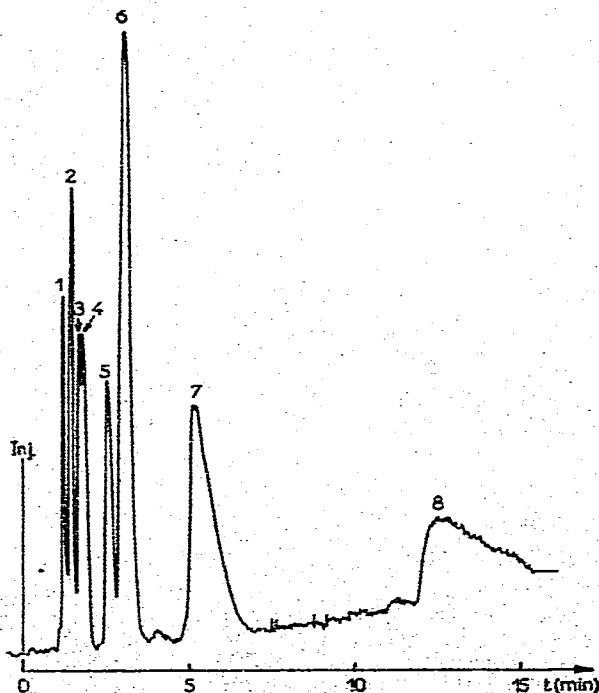
Fig. 6. Separation of aromatic hydrocarbons. Column, B; solvent, acetonitrile; detector, UV; flow-rate, 1.15 ml/min. Peaks: 1 = benzene; 2 = styrene; 3 = indene; 4 = tetralin; 5 = naphthalene; 6 = diphenyl; 7 = 2-methylnaphthalene; 8 = 1,8-dimethylnaphthalene.

Figs. 5 and 6 show the separation of mixtures of polyaromatic hydrocarbons. The first chromatogram was obtained using benzene as eluent, which is not really practical because of its toxicity and the impossibility of using the classical UV detector.

Furthermore, the elution of compounds with more than five or six aromatic rings seems to be impossible. The use of m-xylene, the strongest solvent found so far, would reduce the retention of pyrene only four-fold. The elution of these compounds was also achieved on a carbon with a smaller specific surface area, Sterling FTFF modified with 14% of pyrocarbon (9.5 m²/g), using n-nonane as eluent. In any event, carbon should not be chosen for the separation of large polyaromatic compounds, as silica gives excellent results. On the other hand, the separation of alkyl-substituted aromatic hydrocarbons is easier, as shown in Fig. 6. A similar chromatogram obtained using silica, with an excellent separation, has been published[24], but it seems that with this last adsorbent naphthalene and 2-methylnaphthalene will be eluted together. Similarly, the separation of positional isomers is illustrated by Figs. 7 and 8.

Fig. 7 shows the elution of almost all of the methylbenzenes. Several interesting observations can be made. Firstly, the order of elution follows the order of increasing molecular weight, as in GC. Secondly, all groups of isomers except o- and p-xylene are almost completely resolved. p-Xylene is less retained than o-xylene but, as in GC[22], their separation is difficult. It has been partially achieved using a weaker solvent ($k_0' = 1.65$, $k_p' = 1.55$, $\alpha = 1.065$, $N = 11,200$ for $R = 1$). Thirdly, the elution order
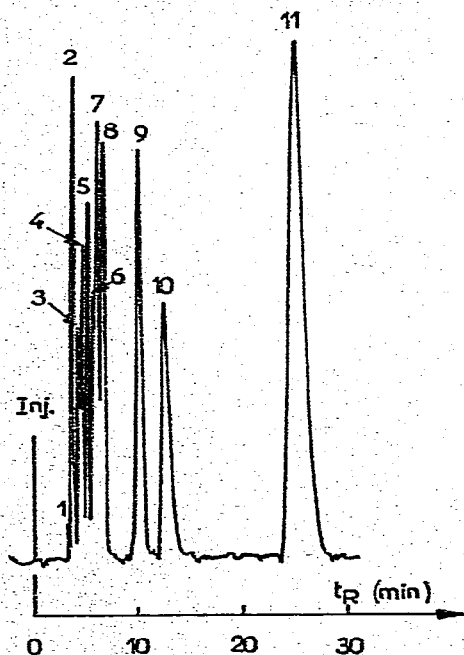
Fig. 7. Separation of methyl- and polymethylbenzenes. Column, D: Black Pearls L, 44% pyrocarbon-graphitized. Solvent, acetonitrile; detector, UV; flow-rate, 0.5 ml/min. Peaks: 1 = unretained; 2 = benzene; 3 = m-xylene; 4 = o-xylene; 5 = 1,3,5-trimethylbenzene (TMB); 6 = impurity in pentamethylbenzene; 7 = 1,2,4-TMB; 8 = 1,2,3-TMB; 9 = 1,2,4,5-tetramethylbenzene (TeMB); 10 = 1,2,3,4-TeMB; 11 = pentamethylbenzene.

TABLE IV

RETENTION OF AROMATIC HYDROCARBONS IN GC AND LC ON CARBON BLACKS

Compound	Gas chromatography[*]		Liquid chromatography		Specific gravity	Refractive index
	$Log_{10} U_A$	t (°C)	k'	Solvent[**]		
m-Xylene	0.384	190	0.27	Acetonitrile	0.8684	1.4973
o-Xylene	0.437	190	0.33	(column I)	—	—
p-Xylene	0.450	190	0.35		0.8968	1.5058
1,3,5-TMB	0.251	240	0.50		0.8642	1.4998
1,2,4-TMB	0.343	240	0.78		0.889	1.5044
1,2,3-TMB	0.381	240	0.96		0.944	1.5139
1,2,4,5-TeMB	0.366	280	2.10		0.8380	1.4790
1,2,3,4-TeMB	0.410	280	2.83		0.9010	1.5187
m-Cresol			0.26	Acetonitrile	1.0336	1.5398
p-Cresol			0.29	(column I)	1.0347	1.5395
o-Cresol			0.29		1.0465	1.4453
2,6-DMP			0.60		—	—
3,5-DMP			0.65		—	—
2,4-DMP			0.69		1.0276	1.5420
2,3-DMP			0.86		—	—
2 4,5-TMP			1.81		—	—
3,4,5-TMP			2.27		—	—
1-MMN	0.735	280	1.06	Acetonitrile	1.0287	—
2-MMN	0.737	280	1.00	(column II)	1.0290	—
1,2-DMN	1.344	280	2.19		1.018	—
1,6-DMN	1.327	280	2.23		—	—
1,8-DMN	1.263	280	2.44		—	—
1,3-DMN	1.298	280	2.48		1.006	1.609
1,5-DMN	1.271	280	2.54		—	—
1,4-DMN	1.281	280	2.60		1.019	1.6158
2,6-DMN	1.367	280	2.85		—	—
1,3,7-TMN	1.780	280	7.01		—	—
2,3,5-TMN	1.815	280	7.39		—	1.608
2,3,6-TMN	1.848	280	8.33		—	—

[*] After refs. 25 and 26.

[**] Column I: $L = 20$ cm, $d_c = 3.5$ mm, $d_p = 30$ μm; BPL, 33% pyrocarbon. Column II: $L = 70$ cm, $d_c = 2$ mm, $d_p = 40$ μm; Sterling FTFF, 14% pyrocarbon.
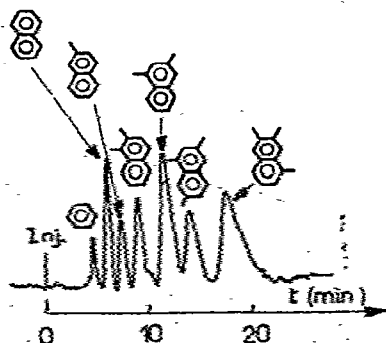
Fig. 8. Separation of naphthalene and polymethylnaphthalenes. Column, A; solvent, methylene dichloride; detector, UV; flow-rate, 0.32 ml/min.
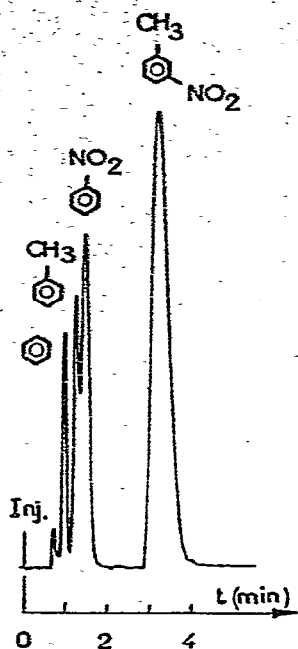
Fig. 9. Separation of a mixture of compounds with very different polarities. Column, B; solvent, 41 % acetonitrile–water; detector, UV; flow-rate, 1.0 ml/min.

follows the order of increasing density and, generally, refractive index. The same effect occurs in the elution of phenols (cf., Table IV). The order of elution is the same as in GC using GTCB[26,27], with some notable exceptions for the methylnaph-thalenes (see Table IV). Fig. 8 shows the separation of some naphthalene derivatives. Of course, it is obvious that for such separations HPLSC using carbon black is not competitive with GC.
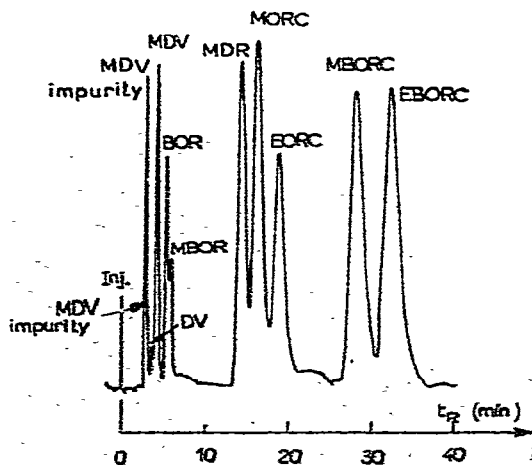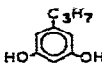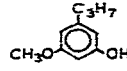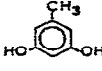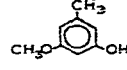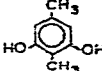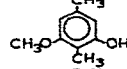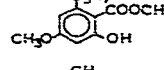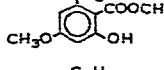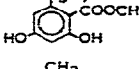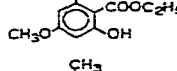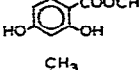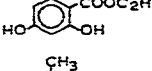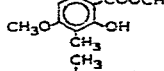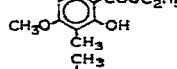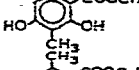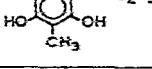

Fig. 10. Separation of a mixture of substituted diphenol derivatives (cf., Table V). Column, D; solvent, ethyl acetate; detector, UV; flow-rate 0.6 ml/min.

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TABLE V

RETENTION CHARACTERISTICS OF SOME DIPHENOL DERIVATIVES

Compound	Formula	Symbol	k'	HETP (mm)
Divarinol	C_3H_7 ring, $HO-$, $-OH$	DV	0.51	0.37
Monomethoxydivarinol	C_3H_7 ring, CH_3O-, $-OH$	MDV	0.56	1.08
Orcinol	CH_3 ring, $HO-$, $-OH$	OR	0.59	0.41
Monomethoxyorcinol	CH_3 ring, CH_3O-, $-OH$	MOR	0.65	0.41
β-Orcinol	CH_3 ring, $HO-$, $-OH$	BOR	1.14	0.46
Monomethoxy-β-orcinol	CH_3, CH_3 ring, CH_3O-, $-OH$	MBOR	1.29	0.50
Methyl divaricatinate	C_3H_7, $COOCH_3$ ring, CH_3O-, $-OH$	MDC	3.44	0.36
Methyl everninate	CH_3, $COOCH_3$ ring, CH_3O-, $-OH$	ME	3.95	0.54
Methyl divarate	C_3H_7, $COOCH_3$ ring, $HO-$, $-OH$	MDR	4.33	0.68
Ethyl everninate	CH_3, $COOC_2H_5$ ring, CH_3O-, $-OH$	EE	4.63	0.72
Orcinol methyl carboxylate	CH_3, $COOCH_3$ ring, $HO-$, $-OH$	MORC	5.15	0.68
Orcinol ethyl carboxylate	CH_3, $COOC_2H_5$ ring, $HO-$, $-OH$	EORC	6.10	0.63
Methyl rhizonate	CH_3, $COOCH_3$ ring, CH_3O-, $-OH$, CH_3	MR	8.19	0.58
Ethyl rhizonate	CH_3, $COOC_2H_5$ ring, CH_3O-, $-OH$, CH_3	ER	9.63	0.41
β-Orcinol methyl carboxylate	CH_3, $COOCH_3$ ring, $HO-$, $-OH$, CH_3	MBORC	9.75	0.45
β-Orcinol ethyl carboxylate	CH_3, $COOC_2H_5$ ring, $HO-$, $-OH$, CH_3	EBORC	11.25	0.48

This example has been chosen only as a test of the applicability of carbon liquid chromatography.

The chromatogram in Fig. 9 shows that it is possible to separate simultaneously compounds with very different polarities (benzene and nitrobenzene) and of identical polarity (benzene and toluene) in a short time. The resolution of toluene and nitrobenzene is not very good, but can easily be improved with a more efficient column; that used in this work has less than 600 theoretical plates.

The chromatogram in Fig. 10 illustrates the separation of dihydrobenzene derivatives. These compounds have at least two polar functional groups (see Table V for abbreviations used and formulae). The separation of these compounds on silica is very difficult if not impossible. The range of polarity is very large and it is not possible to elute all of these solutes with the same solvent. On the other hand, the gradient technique is of limited help as under isocratic conditions several compounds are hardly separated. GC is difficult to apply because of the low volatility and high polarity of the samples.

A complete separation has not yet been achieved on carbon black, but it could easily be improved by using a more efficient column than that used in this work, which exhibits less than 1800 theoretical plates for each compound. Decreasing the flow-rate will make the time of analysis too long. The positions of some of the peaks in the chromatograms are surprising. If it is normal that MR and MBORC are eluted before ER and EBORC, respectively, it is abnormal that MR (and ER) are eluted before MBORC (and EBORC). Comparison between the couples MDC and MDR, ME and MORC, EE and EORC, MR and MBORC, and ER and EBORC suggests that the retention is decreased when an OH group is replaced with a CH_3O group, whereas the reverse is true for the couples BOR and MBOR and DV and MDV. Moreover, it is extremely surprising that DV is eluted before OR.

Some characteristic features of adsorption on graphite can be noticed. Firstly, replacing a methyl with an ethyl group in the ester compounds (ME, EE, etc.) increases the capacity ratio in the same proportion for the different couples (1.17, 1.18, 1.18, 1.15). Secondly, replacing a hydrogen atom with a methyl group on the aromatic ring increases the retention in a larger proportion, 1.95 and 1.98, a value that is smaller than that for the non-hindered groups shown in Fig. 5[2–7]. It is also encouraging to observe that the decrease in efficiency with retention is not very large. It seems that the HETP is maximal at about $k' = 4$–5.

CONCLUSION

It is now possible to use with advantage modified carbon blacks to pack efficient columns and obtain good analyses. Several problems have yet to be solved. The first is the preparation of finer particles, to obtain better efficiency and hopefully a smaller decrease in efficiency with increasing retention. Attempts to coat fine silica particles by pyrocarbon have been very successful (Results will be reported later). Optimization of the preparation is not yet complete, and it is not known whether a different pyrolysis temperature could give better results, or to what extent. Graphitization improves the column loadability. Also, the study of the eluotropic strength of solvents has to be refined, and the extent of the specificity for geometrical isomers needs to be known with better precision. Work is in progress in these fields.

ACKNOWLEDGEMENTS

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