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COMPARISON OF SOME PACKINGS FOR REVERSED-PHASE HIGH-PERFORMANCE LIQUID–SOLID CHROMATOGRAPHY

I. SOME ANALYTICAL CONSIDERATIONS

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SUMMARY

A comparison is made between non-polar chemically bonded phases (CBP), pyrocarbon-modified silica gel (PMS) and pyrocarbon-modified carbon black (PMCB). The retention mechanism is probably an adsorption process in all instances but the contribution of the solute–solvent interactions to the retention is larger on CBP than on PMS or PMCB, resulting in smaller retentions and a greater selectivity for polar compounds. Retentions in homologous series show that the group selectivity is better for PMS (or PMCB) than for CBP. The selectivity for geometrical isomers is also better for PMS. However, if important differences in the polarities of isomers appear, CBP can also be very selective phases.

Changing the water content of the solvent mixture generally does not provide important variations in selectivity. Such variations are better achieved by changing the modifier and are more important for CBP than for PMS.

A comparison of the loadability of columns is very difficult when single compounds are injected. If separation problems are to be dealt with, the most important parameter is the selectivity of the chromatographic system. The choice of the best packing therefore depends to a great extent on the separations in question.

INTRODUCTION

Reversed-phase high-performance liquid–solid chromatography (RPHPLC) is a powerful analytical technique and, as recently noted by Molnar and Horváth[1], its popularity is due to its relative simplicity and its high degree of reproducibility. The advantages and disadvantages of RPHPLC have been discussed earlier[2]. The method permits the separation of non-polar or polar solutes with very good efficiency and selectivity. The most popular of the various reversed-phase (RP) packings is probably ODS-silica, in which the silanol groups of the silica gel have been modified to non-polar moieties by the addition of chemically bonded $-(CH_2)_{17}CH_3$ groups. Other types of packings are also available, such as silica gel modified by a chemically bonded short *n*-alkyl chain or ethylphenyl groups, silica and carbon black modified by pyrocarbon, and styrene–divinylbenzene copolymers[2].

This paper describes a comparison of the analytical properties of different

types of ODS-silica, phenylethyl-silica and pyrocarbon-modified silica (PMS). The work of other groups, *e.g.*, Hemetsberger *et al.*[3], has shown that there are no important differences in behaviour between the bonded short-chain *n*-alkane packings and the ODS types, and for this reason the former packings were not included in this study. In the second part of this work[4], we deal with some theoretical aspects of comparisons between packings and considered retention mechanisms, HETP curves, effects of temperature on retention and solvent strength. The results of this work indicates that the mechanism of retention on RP packings can probably best be explained in terms of an adsorption process. This conclusion is not completely certain and this is likely a most unusual type of adsorption. However, for the purposes of this paper, we shall speak of a solute being adsorbed by the packing rather than being partitioned between the solvent and the stationary phase. In addition, the packing will often be referred to as the adsorbent.

This part of our study is concerned with a comparison of the retentions of polar and non-polar solutes on the various packings, a comparison of the selectivities of these packings for compounds in homologous and pseudo-homologous series, geometrical isomers and compounds with similar structures but with different polar groups. The change in selectivity exhibited when the aqueous organic modifier and the water content of the solvent are varied is studied, and the loadabilities of different columns are compared. Finally, some examples of separation problems are given.

EXPERIMENTAL

The columns used and their characteristics are given in Table I and they will subsequently be referred to by the numbers given there. Various combinations of liquid chromatographic pumps and detectors were used. The pumping systems included a Waters Assoc. (Milford, Mass., U.S.A.) Model M 6000 pump, an Orlita (Giessen, G.F.R.) Model DMP-1515 pump, a Tracor Model 995 constant-flow isochromatographic pump and a Micromeritics (Atlanta, Ga., U.S.A.) Model 7000 liquid chromatograph. UV-adsorbing solutes were detected with either a Micromeritics Chromonitor 785 variable-wavelength detector or a Micrimeritics UV monitor, both operating at 254 nm. Non-adsorbing solutes were detected with an experimental refractive index detector made by the Institut d'Optique (Paris, France). Injections were made with 5-μl Hamilton Model HP 305 high-pressure syringes into low-volume, home-made, septum-type injector ports. Servotrace recorders (Sefram, Paris, France) were used to record the chromatograms.

Solvent mixtures were made with double-distilled water. The methanol (MOH), acetonitrile (ACN), dioxane (DX) and tetrahydrofuran (THF) organic modifiers were of pro analisi grade from Merck (Darmstadt, G.F.R.) and were used without further distillation.

Pesticide solutions were made with analytical standards from Polyscience Corp. (Evanston, Ill., U.S.A.). The polychlorinated biphenyl (PCB) samples were a gift from the Food and Drug Administration (Washington, D.C., U.S.A.). The other solutes were of analytical-reagent grade.

TABLE I

CHARACTERISTICS OF THE COLUMNS USED

Column No.	*Packing*	*Manufacturer*	*Column length (cm)*	*Particle size (μm)*
1	Partisil ODS	Whatman	25	10
2	Partisil ODS 2	Whatman	25	10
3	μBondapak C_{18}	Waters Assoc.	30	10
4	μBondapak fatty acids	Waters Assoc.	30	10
5	Pyrocarbon on silica	Home-made with Spherosil XOB 75 (Rhône-Poulenc)	50	15–20
6	Pyrocarbon on carbon black	Home-made with Black Pearls L	65	15–20
7	Pyrocarbon on silica	Home-made with Spherosil XOB 75	50	15–20
8	Pyrocarbon on silica	Home-made with Spherosil XOB 030	140	20–30
9	Pyrocarbon on carbon black	Home-made with Sterling FT FF	75	30–40
10	Pyrocarbon on silica	Home-made with Spherosil XOB 75	115	20–30

RESULTS AND DISCUSSION

The selectivity of the packing and of the mobile phases and the column loadibility are discussed in the following sections. Finally, the findings are illustrated by some analytical results.

Selectivity of the packing

The retention of solutes on RP columns is mainly due to non-specific interactions between the solute molecules and the stationary phase. These interactions probably have different origins when the packing material is made of pyrocarbon (PMS and PMCB) and when it is a non-polar chemically bonded silica[4]. The retention of a solute is defined in terms of its capacity factor (k'), which is related to the thermodynamic adsorption constant (κ) by the equation

$$k' = \kappa \cdot \frac{A_s}{V_0} = \kappa \cdot \frac{d_R}{\varepsilon_T} \cdot S_{sp} \tag{1}$$

where A_S is the total surface area of the adsorbent in a column of dead volume $V_0 \cdot d_R$ is the packing density of the stationary phase which has a specific surface area of S_{sp}. The total porosity of the column is ε_T. Most often, for a given solute and solvent, κ is larger for the pyrocarbon packings. However, eqn. 1 also shows that k' is proportional to the specific surface area of the packing and is independent of the column geometry assuming that d_R and ε_T are only related to the packing procedure. The PMS that we used in this work has a low specific surface area (less than 60 m^2/g) and the retentions in a given solvent are similar to or greater than those obtained with

the n-C_{18} silica giving the greatest capacity factors (in this work it is often the μBondapak from Waters Assoc.). Working with n-nonylbenzene and methanol as solvent for instance, the capacity factors for columns 2, 3 and 5 (see Table I) are 0.13 1.30 and 4.86, respectively. Moreover, it should be noted that, when using PMCB with larger specific surface areas (Black Pearls L, 150 m^2/g; Black Pearls 800, 250 m^2/g), it is possible to obtain much larger k' values if necessary. If similar retentions are expected with a PMS column and a C_{18} one, the solvent associated with the bonded silica column will be generally most polar (larger water content). This increase in the eluent polarity is very often associated with a larger selectivity of the C_{18} packing for the separation of polar and non-polar solutes.

This does not mean, however, that the carbon adsorbents cannot be used for the separation of such compounds, as will be demonstrated later.

Calculations of k' values are very sensitive to the value determined for the retention time of an unretained solute (t_0). Horváth and Lin[5] noted that the mobile phase hold-up volume (or column dead-volume), V_0, may vary with the choice of unsorbed tracer solutes, *i.e.*, the measured hold-up volume will depend on a particular "unretained solute's" ability to penetrate the intraparticular porosity of the packing material, V_i. Their expression:

$$V_0 = V_e + \varphi V_i \tag{2}$$

where V_e is the interparticular fluid volume of the column, introduces the coefficient φ ($0 < \varphi < 1$), which accounts for the extent of intraparticular pore penetration by a particular solute. Thus, for valid comparisons to be made between different columns and packings, a constant definition of which solute will be considered to be "unretained" must be decided upon. RP solvents are usually mixtures of water and an organic modifier. For the purposes of this study, we assumed that the peak produced from the injection of a sample of pure water defined the time t_0 (it is not obvious that the injection of a sample of pure organic modifier into a column being operated with an aqueous solution of that modifier will result in a peak of $k' = 0$ if water is chosen as the unretained solute). The small water molecules can be considered to exhibit the same degree of intraparticular pore penetration (constant φ) no matter what the size of the molecules of the organic modifier may be. However, problems were encountered in identifying the chromatographic signal due to water, especially in the solvent systems acetonitrile–water (ACN–W), dioxane–water (DX–W), and tetrahydrofuran–water (THF–W), because of the oscillating positive and negative absorbance signals produced upon the injection of pure water. The interference of the negative signal due to water (essentially momentarily diluted solvent) and the positive signal due to a temporary higher concentration of organic modifier (or some other reflex signal) prevents one from fixing accurately the point of time t_0. The smallest detectable amount of water should be injected in order to minimize this problem. Ideally, a dead volume marker should be determinable for each injection, so as to minimize errors in calculated k' values due to pressure and flow fluctuations. We feel that this problem deserves further attention. Future work could deal with the injection of isotope-labelled solutes or deuterated water to obtain a clearer picture of what is called the column "dead" volume.

Homologous and pseudo-homologous series. A characteristic feature of RPC is

the linear dependence of log k' on the number of carbon atoms in a homologous series[2-4]. A true homologous series is obtained by adding the same $-CH_2-$ increment in a linear chain, *e.g.*, *n*-alkylbenzenes. In this work we have also considered as pseudo-homologous series the polymethylbenzenes and the polymethylphenols.

Results obtained for alkyl- and methylbenzenes and methylphenols are reported in Figs. 1 and 2. The expected quasi-linear plots are obtained. It is noted that for a given number of carbon atoms it seems difficult to separate an alkyl- and a polymethylbenzene with CBP. Such separations are easily achieved with either the PMS or PMCB carbon packings. Columns 1, 3 and 4 were operated with the solvent mixture methanol–water (50:50), and columns 2 and 5 with methanol–water (85:15) and pure methanol, respectively, owing to excessive retentions with the previous mixture. The slopes of the plots corresponding to *n*-alkyl- and polymethylbenzenes are similar for columns 1, 3 and 4. The slope decreases for column 2 because of the decrease in selectivity resulting from the larger concentration of organic modifier in the solvent.

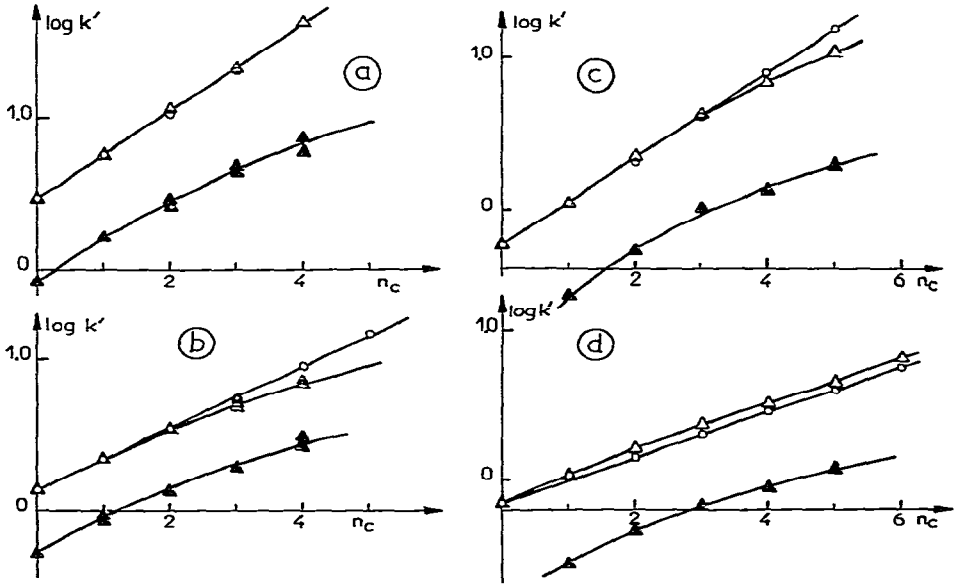


Fig. 1. Variation of the logarithm of the capacity factor in homologous series for bonded-phase packings. (a) Column 3, solvent MOH–W (1:1); (b) column 4, solvent MOH–W (1:1); (c) column 1, solvent MOH–W (1:1); (d) column 2, solvent MOH–W (85:15). △, Methylbenzenes; ○, alkylbenzenes; ▲, methylphenols.

In Fig. 2, the slopes for *n*-alkyl- and methylbenzenes are very different and a similar effect is also observed for PMCB (Fig. 2a and ref. 7) and in gas–solid chromatography on graphitized carbon black. This result demonstrates that adsorption on carbon packings is dependent on the presence of aromatic rings and their degree of substitution, probably due to a kind of specific interaction[8]. Such behaviour leads to both advantages and disadvantages in separation studies, *i.e.*, good selectivity for geometrical isomers and the near impossibility of eluting polyaromatic conjugated

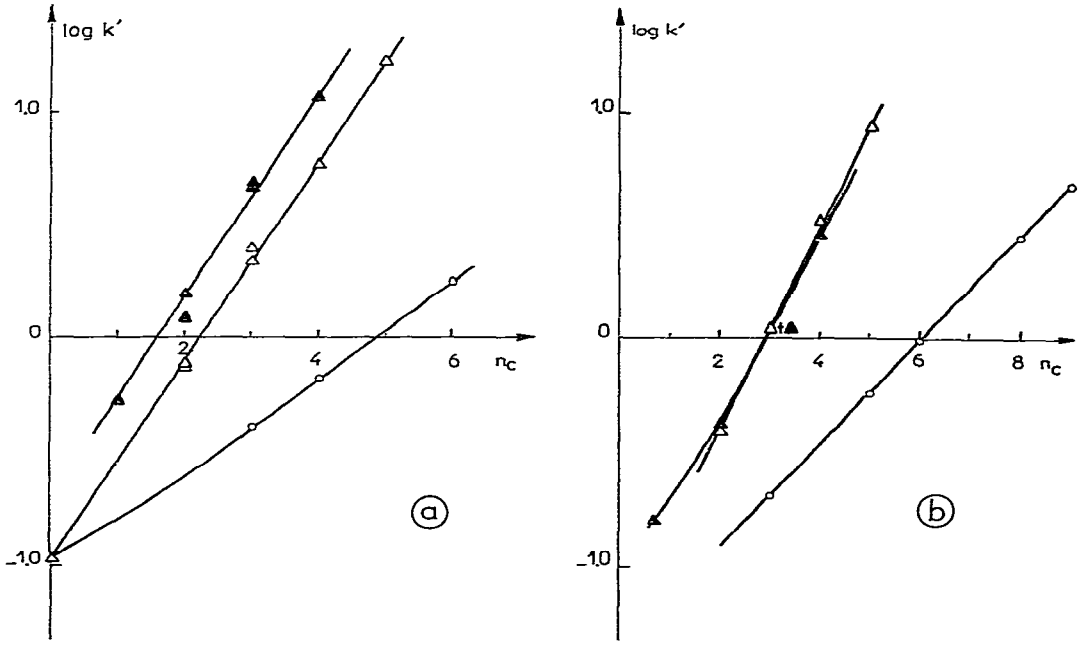


Fig. 2. Variation of the logarithm of the capacity factor in homologous series for carbon packings. (a) Column 6, solvent MOH; (b) column 5, solvent MOH. Symbols as in Fig. 1.

solutes larger than pyrene or benzopyrene because of their excessive adsorptions. Moreover, the selectivity for homologues, given as

$$\alpha = \frac{k'_{n+1}}{k'_n}$$

where n is the number of carbon atoms in the molecule, is better for PMS than for the ODS packing when using the same solvent. For example, when eluting methylbenzene, we obtained values of 2.71 and 1.41 for columns 5 and 2, respectively, with methanol as solvent.

For the polymethylphenols chromatographed on CBP, the linearity of the plots is poorer than that observed for the polymethylbenzene series. The plots have a slightly convex curvature but the average slopes are nevertheless similar to those obtained with the other series. Whereas the separations between methylbenzenes and methylphenols are good when using CBP (Fig. 1), they seem to be difficult to obtain with PMS (Fig. 2b), which again is similar to the results in gas chromatography. However, the use of a carbon black modified packing with a larger specific surface area allows a sufficient increase in the retention of the polar compounds relative to the non-polar ones and thus permits the separation. This is illustrated in Fig. 2a, and suggests that the selectivity of PMS and PMCB can be different, probably because of the slihtly polar character of the PMS packings (the surface can be incompletely covered by the film of pyrocarbon).

We have also investigated the effect of increasing the number of different polar

substituents (Cl-, OH-, NO_2-) on a phenyl ring. Each series of compounds (chlorobenzenes etc.) cannot be considered as an homologous series as it is likely that the effect of the second and further substituents on the first one is too great and modifies the character of the compound.

The plots of log k' *versus* the number n of substituents are linear for the chlorobenzene derivatives, at least for $n \leqslant 3$, as shown in Fig. 3a. The most selective system seems to be PMS with acetonitrile as solvent. It should be noted that with a solvent that gives retentions similar to those observed with columns 2 and 4 the slope of the line corresponding to column 5 would have been even larger. However, the separation of these three chlorobenzenes is possible with all of the columns tested. Although the k' values are similar for columns 2 and 4, the selectivity is slightly better with the former.

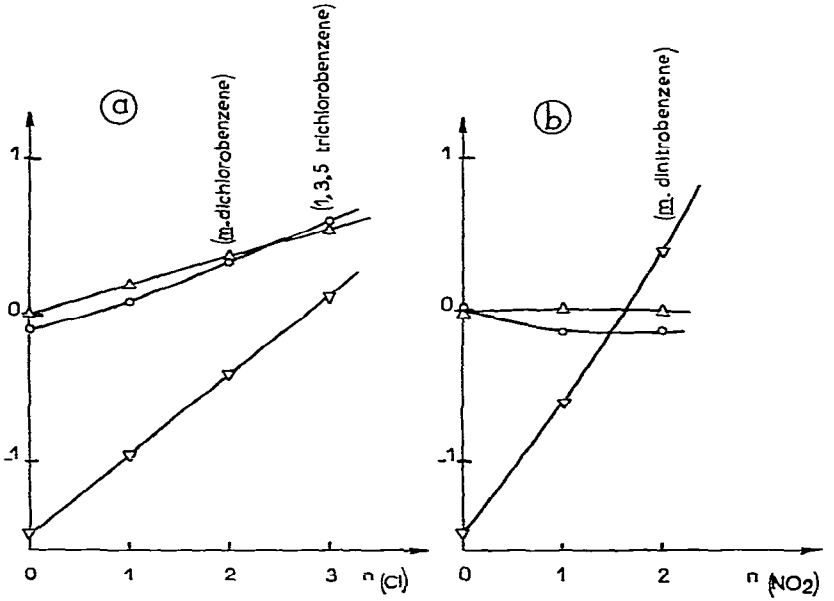


Fig. 3. Variation of the logarithm of the capacity factor in different series. (a) Chlorobenzenes; ○, column 2, solvent MOH–W (58:42); △, column 4, solvent MOH–W (85:15); ▽, column 5, solvent ACN; (b) nitrobenzenes; ○, column 2, solvent MOH–W (80:20); △, column 4, solvent MOH–W (58:42); ▽, column 5, solvent ACN.

The situation is very different when aromatic hydrogen atoms are replaced with nitro or hydroxy groups. Fig. 3b suggests that the plot for mono- and dinitrobenzene chromatographed on column 5 is linear (however, with only three experimental values, (n = 0,1,2) an absolute conclusion on this point cannot be drawn). For the two other columns, the capacity factors for benzene, nitro- and *m*-dinitrobenzene are very similar and their separation is impossible, at least when using methanol as the organic modifier in the solvent mixture. In fact, it is possible that changing the modifier would result in a change in selectivity. This question will be considered in a subsequent section. However, as the most commonly used organic solvent for RPC with CBP

is methanol, the major part of this work was carried out with water–methanol mixtures.

The results observed for hydroxybenzenes (phenol, resorcinol and pyrogallol) with the three types of packings are also very different. On PMS, the capacity factors increase in the order given but the plots are not linear (the group selectivity decreases with increasing numbers of hydroxyl groups). For the ODS and ethylphenyl packings, the k' values decrease from benzene to pyrogallol and negative k' values have been obtained for trihydroxybenzene[4]. A possible explanation for such a phenomenon is the formation of large associations of molecules by hydrogen bonding, which would not be able to penetrate completely the intraparticular porous structure of the packing ($\varphi < 1$). It would be similar to exclusion chromatography.

The comparisons between the retentions of methyl-, chloro-, nitro- and hydroxybenzenes confirm that the interactions between solute and stationary phase are less important for bonded phases than for carbon packings. When working with CBP, if the solute–solvent interactions become particularly strong (hydroxy derivatives), the effect of the increasing solute solubility in the mobile phase is more important than the increase in solute–adsorbent interactions due to larger molecular areas, the net result being a smaller k' value. To date, we have never observed such a phenomenon with PMS or PMCB when chromatographing simple molecules. Hence it seems that CBP will give better packings for the separation of very large and polar molecules than carbon packings, on which retentions will be too large. This is a serious problem with carbon adsorbents as the adjustment of k' values for very large molecules by varying the specific surface area of the packing can result in poor peak shape and efficiency (S_{sp} smaller than 10 m^2/g). In addition, the elution of very polar solutes on PMS often gives unsymmetrical peaks with poor efficiency. A possible explanation of this phenomena is the presence of the unshielded polar groups at the surface of the PMS. These polar groups can act as active sites for very polar compounds, resulting in poor peak shape.

Effect of unsaturation. The introduction of double-bond unsaturation in a molecule generally decreases its retention. This effect was observed for all of the RP packings studied in this work. The plots of log k' *versus* carbon number for *n*-alkanes and *n*-alkenes are straight lines with identical slopes, the line for *n*-alkenes being located below that for *n*-alkanes. The separation of two compounds with the same number of carbon atoms in these series is easy with columns 2 and 6 ($\alpha = 1.78$ and 1.56, respectively, in methanol), more difficult with column 4 ($\alpha = 1.2$ in methanol–water (65:35)). In the following, for all the examples of separation, chromatograms were obtained for one column of C_{18} silica (No. 2), the column of ethylphenyl-silica (No. 4) and a pyrocarbon column. We should also point out that the analysis times will often be larger for PMS or PMCB columns than for the CBP columns because of the use of large particles. Indeed, in order to obtain good efficiencies with such columns, it is necessary to work at low flow-rates.

We used the series ethylbenzene, styrene and phenylacetylene in comparing the retentions of compounds that differ only by a single, a double or a triple bond at one point in the molecule. The separations we obtained with columns 2, 4 and 6 are shown in Fig. 4. In all instances the most retained compound is phenylacetylene but the order of elution of ethylbenzene and styrene is different on the bonded phases and on the pyrocarbon packing. Such results would at first seem to contradict the results

obtained for *n*-alkanes and *n*-alkenes. However, in this case it should be pointed out that the unsaturated bond is conjugated with an aromatic ring, which results in the unsaturated solutes being planar and having a polarizability different to that of ethylbenzene.

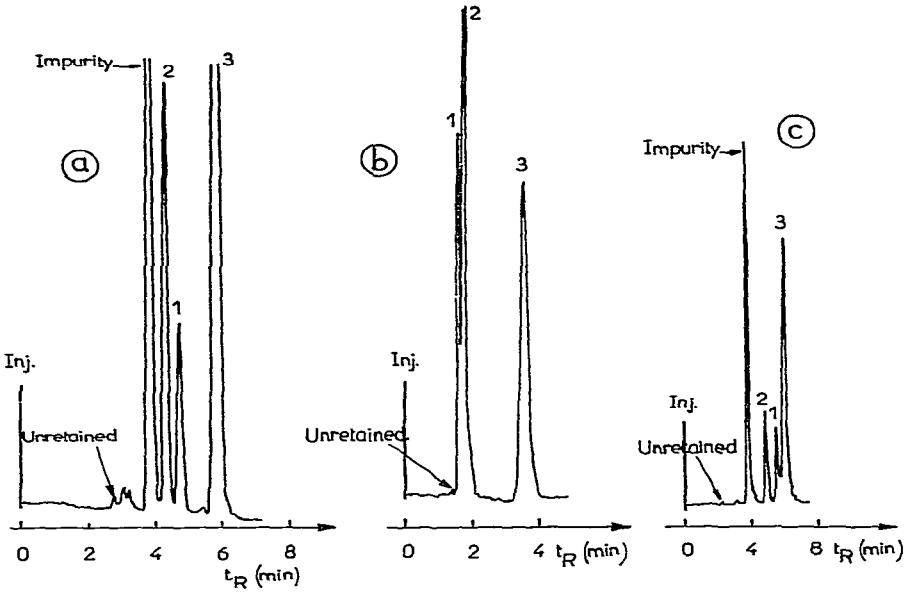


Fig. 4. Separation of ethylbenzene (1), styrene (2) and phenylacetylene (3). (a) Column 4, solvent MOH–W (65:35), flow-rate 1 ml/min; (b) column 6, solvent ACN, flow-rate 1.1 ml/min; (c) column 2, solvent MOH–W (85:15), flow-rate 1 ml/min. UV detection at 254 nm.

The best separation is obtained with the phenyl packing (column 4). Column 6 exhibits a poor resolution of ethylbenzene and styrene, and column 2 gives a poor resolution of styrene and phenylacetylene. It is likely that the resolution could be improved by increasing the capacity factors, especially for column 6. However, owing to time considerations, we feel that it is better to maintain relatively low capacity factors ($k' < 5$) when working with mixtures that contain only a few solutes. This is especially true when a given analysis can be performed more easily by adjusting the parameters of the chromatographic system, phase ratio or solvent composition. This philosophy was applied to all of the separations presented in the last section of this paper.

Geometrical isomers. The selectivity of the various packings towards the separation of geometrical isomers has been studied. Carbon adsorbents (PMS or PMCB) generally give good results because of the close dependence between the adsorption energy and the structure of the adsorbed molecule. As for CBP, the separation of geometrical isomers will be generally easy if these solutes exhibit significant differences in solubility in the mobile phase.

Fig. 5 shows that positional isomers on an aromatic ring such as the three trimethylbenzenes are very difficult to separate on bonded phases, whereas on PMS the separation is accomplished very easily. The complete resolution of the isomers is

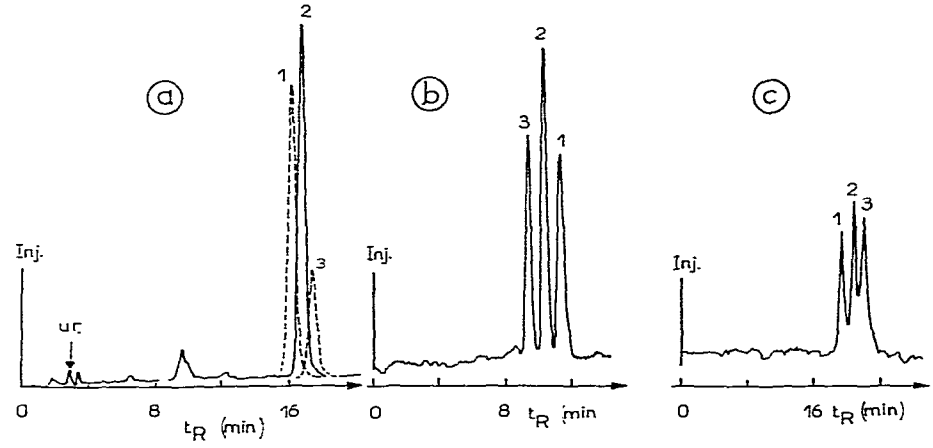


Fig. 5. Separation of 1,2,3- (1), 1,2,4- (2) and 1,3,5- (3) trimethylbenzenes. (a) Column 4, solvent MOH–W (50:50), flow-rate 1 ml/min; (b) column 7, solvent ACN, flow-rate 1 ml/min; (c) column 2, solvent MOH–W (72:28), flow-rate 1 ml/min. UV detection at 254 nm.

probably possible with Partisil ODS 2 but the k' values would have to be increased, thereby significantly lengthening the time of analysis (the capacity factors for Fig. 5c are 7.3 ,7.9 and 8.4). If should also be noted that the order of elution on PMS is the inverse of that on CBP.

When dealing with polar positional isomers it is probable that the polar solvent–solute interactions of *o*-, *m*- and *p*-bromonitrobenzene, for example, are different. The separations of these isomers are shown in Fig. 6. Complete resolution is

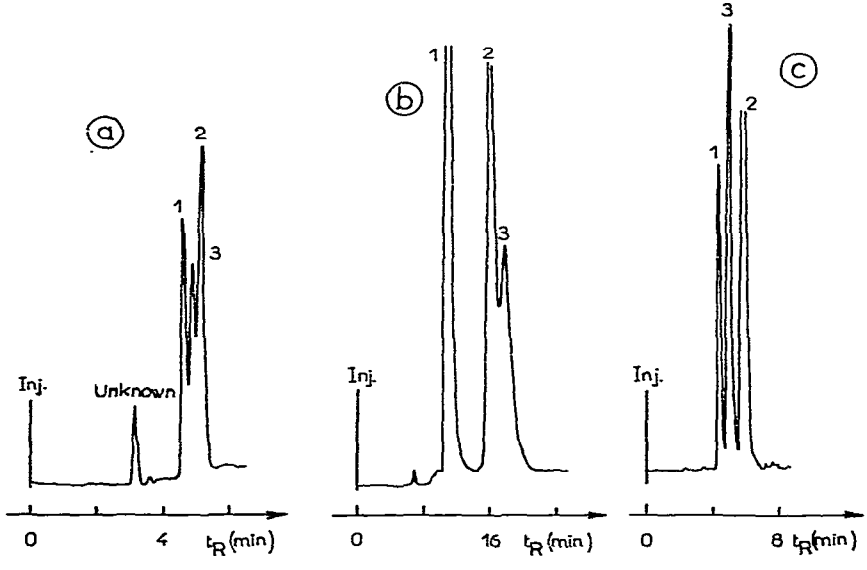


Fig. 6. Separation of *o* (1), *m*- (2) and *p*- (3) bromonitrobenzenes. (a) Column 4, solvent MOH–W (60:40), flow-rate 1.48 ml/min; (b) column 5, solvent ACN, flow-rate 0.74 ml/min; (c) column 2, solvent MOH–W (80:20), flow-rate 1 ml/min.

obtained only with column 2. Column 4 would require 14,500 theoretical plates for a complete separation of the *p*- and *m*-isomers with the solvent system that we used. The PMS column efficiency (Fig. 6b) is much lower for these polar compounds than was previously noted with the non-polar trimethylbenzenes. The separation between the

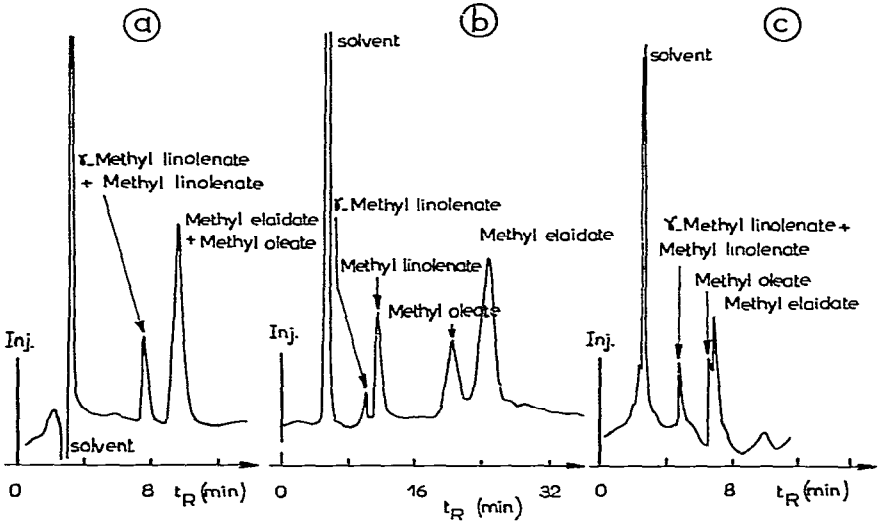


Fig. 7. Separation of γ-methyl linolenate (1), methyl linolenate (2), methyl oleate (3) and methyl elaidate (4). (a) Column 4, MOH–W (82:18), flow-rate 0.95 ml/min; (b) column 8, solvent chloroform, flow-rate 1 ml/min; (c) column 2, solvent MOH, flow-rate 1 ml/min. Refractive index detection.

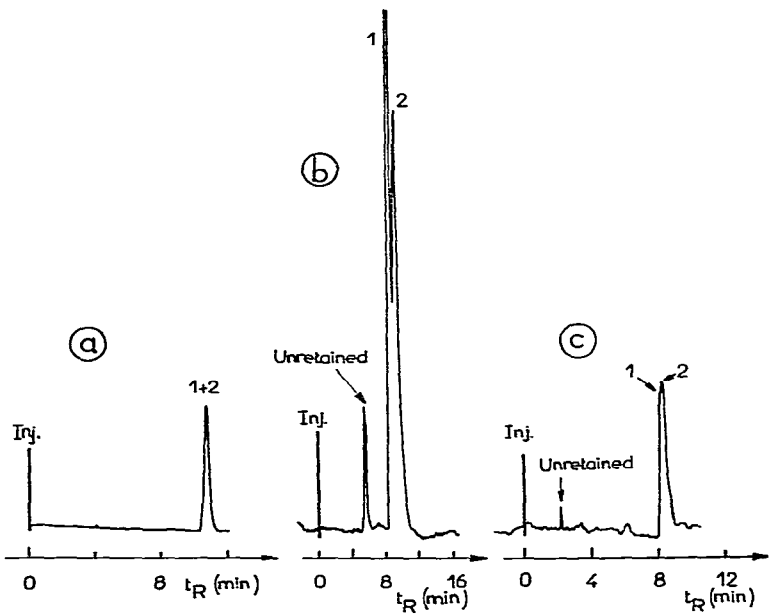
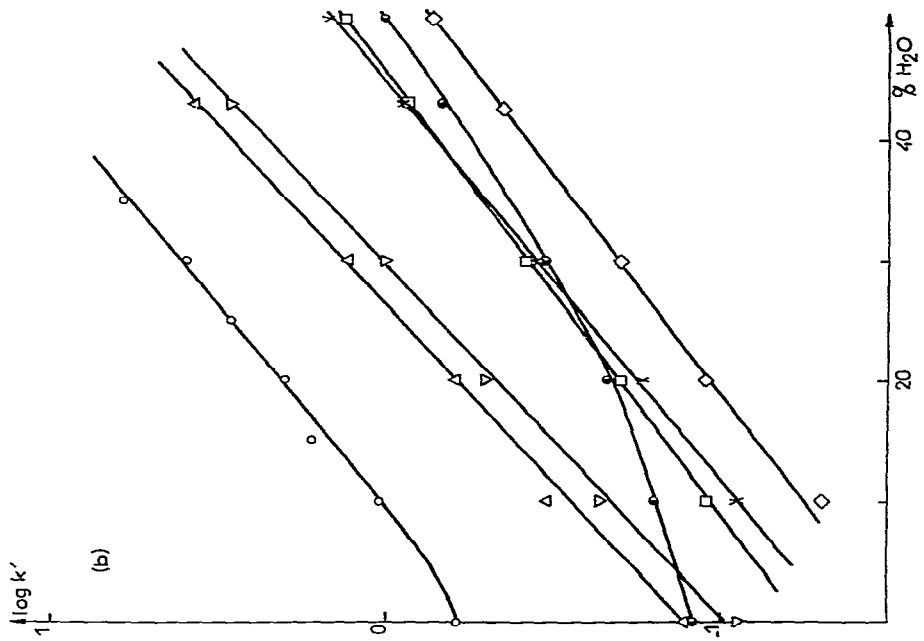
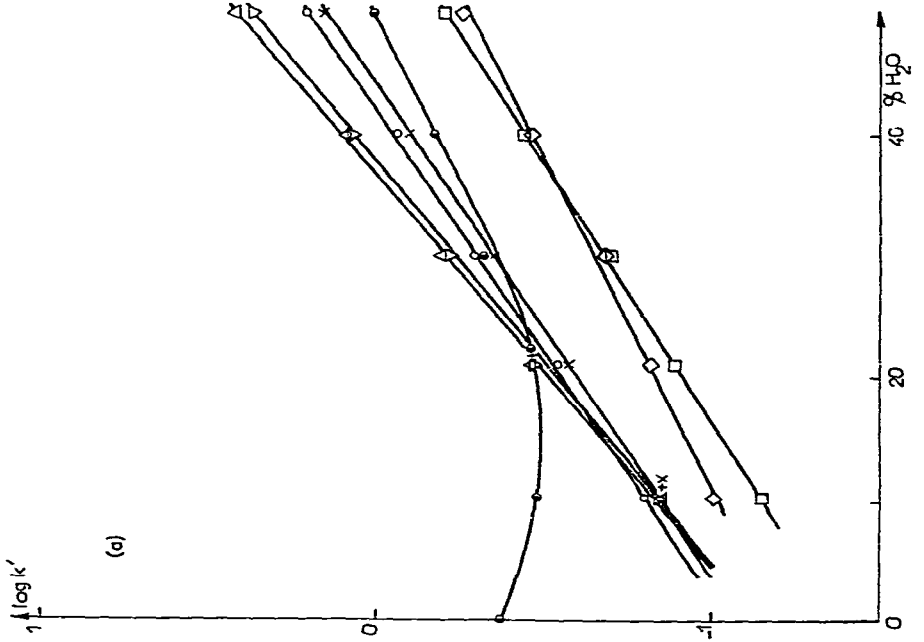


Fig. 8. Separation of α- (1) and β-methylstyrene (2). (a) Column 4, solvent MOH–W (63:35), flow-rate 1 ml/min; (b) column 6, solvent ACN, flow-rate 0.3 ml/min; (c) column 2, solvent MOH–W (80:20), flow-rate 1 ml/min. UV detection at 254 nm.


log k'
1
0
-1
(b)
20
40
% H2O


log k'
1
0
-1
(a)
0
20
40
% H2O

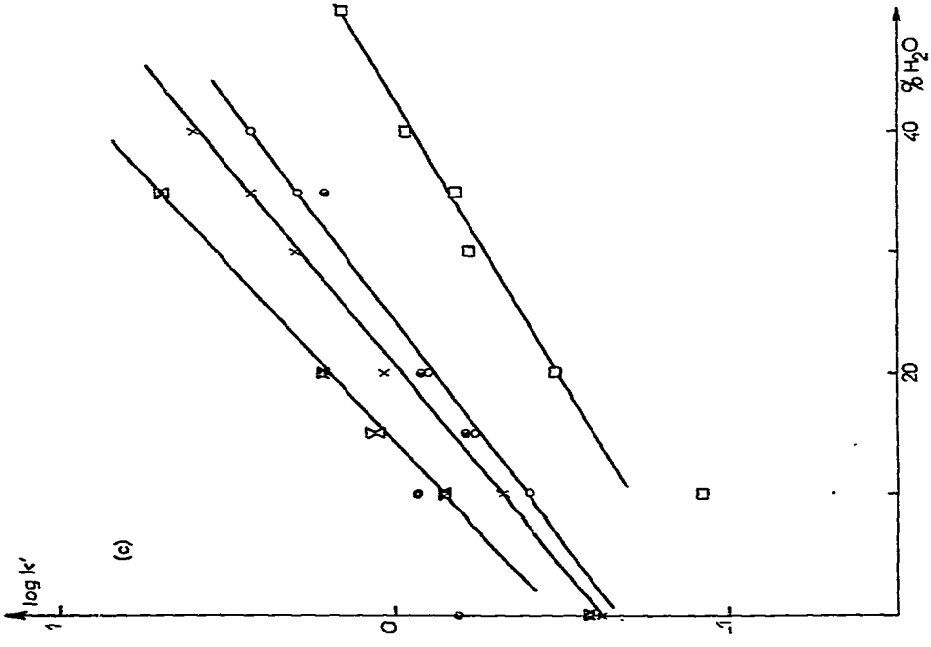


Fig. 9. Variation of the logarithm of capacity factors with the composition of the methanol–water mixture. (a) Column 4; (b) column 5; (c) column 2. ○, Nitrobenzene; △, chlorobenzene; ▽, toluene; □, phenol; ×, benzene; ◑, pyridine; ◇, aniline.

m- and *p*-isomers is again particularly difficult. Note the very important difference between the retention times of *o*- and *m*-bromonitrobenzene ($\alpha = 2.38$) on PMS.

Another interesting problem is the separation of *cis*- and *trans*-isomers. The selectivity of columns 2, 4 and 8 towards such isomers was tested with the separation of methyloleate (*cis*) and methylelaidate (*trans*). The chromatograms shown in Fig. 7 represent the elution of a mixture of methyl esters of different unsaturated fatty acids, each with 18 carbon atoms. It appears that the best system for this separation is the carbon packing, which provides a resolution of the *cis*- and *trans*-isomeric forms of greater than 1.4. A comparable resolution could perhaps be obtained on column 2 in the same time. It is also possible to separate methyl linolenate and γ-methyl linolenate with the PMS column. We feel that the carbon packings are particularly suited to these types of separations.

The last example of isomer separation is that of α- and β-methylstyrene. One can predict that this separation will be difficult with CBP because *a priori* the positioning of the methyl group at the α- or β-position will not greatly affect the solubility of the solute in the mobile phase. As can be seen in Fig. 8, the separation is not possible with column 4. Resolution of the two peaks is only just beginning on the Partisil ODS 2 column. A complete separation under the same conditions would require the use of a column about four times as long. The separation seems less difficult with PMCB (a resolution of 1.2 requires about 4000 theoretical plates).

Selectivity of the mobile phase

We have considered in this work only binary eluents composed of water and an organic modifier.

Effect of the water concentration. Retention and selectivity depend on both the water content and the nature of the organic modifier used in the solvent mixture. The solvent strength (ε°) of the solvent system methanol–water was studied in part II[4]. We used alkyl- and methylbenzenes and methylphenols as solutes. It is found that there is good agreement between the ε° values calculated from the retentions of different compounds in a homologous series. However, the results are slightly different for the benzene and phenol derivatives. In order to see if important changes in selectivity can result from variations in the water content of the eluent, the retentions of seven compounds with various polarities (nitrobenzene, chlorobenzene, toluene, benzene, phenol, pyridine and aniline) were measured in different methanol–water mixtures. The results are reported in Fig. 9. Because of excessively large retentions it was not possible to use mixtures containing more than 50% (v/v) of water.

The results indicate that the solute for which interactions with the solvent are the most important is pyridine. Indeed in this case the k' values first decrease and then increase for columns 2 and 4; no initial decrease in k' is observed for column 5. Working with a PMCB column in the solvent system acetonile–water, such a phenomenon has been observed for both pyridine and quinoline[9]. This particular behaviour of pyridine is probably due to its reaction with water, pyridine being a relatively strong base. It is noticeable that the elution profiles of pyridine are very unsymmetrical on the three packings studied (we have observed the same phenomenon on silica gel columns). No attempt has been made to change the pH of the solvent.

On increasing the water content of the eluent, the eluotropic strength of the solvent decreases (either because of a smaller solvent adsorption or a larger solvo-

phobic effect). On the other hand, the solubility of polar solutes in the mobile phase can increase. The balance between these two opposite trends determines the retention. As a result, the capacity factors can sometimes decrease with decreasing ε°. This point is illustrated in Fig. 9, where the k' values of very polar solutes (phenol and aniline) increase more slowly with decreasing solvent strength than those of non-polar or weakly polar compounds (toluene, benzene and chlorobenzene). It should be noted that this can explain the minimum observed in the curve of pyridine.

It is also interesting to compare the relative positions of the nitrobenzene curve. This solute has the largest molecular area because of the bulky nitro group. On the carbon packings, the adsorption of this molecule increases considerably with decreasing ε°, although at the same time the solubility in the eluent of this polar compound also increases. Nitrobenzene has the largest retention of the seven test solutes on column 5, but the solvent strength that we can calculate from its retention volume is smaller than that derived from the retention of toluene, for which mobile phase effects are almost non-existent.

The retention of nitrobenzene on CBP is always less than those of chlorobenzene and toluene because of the greater solute–solvent interactions experienced by nitrobenzene. On all columns, the behaviours of chlorobenzene and toluene are very similar; the separation of these two compounds would be difficult with the bonded phase packings, whereas it is easy on PMS.

Changing the water content of the eluent does not have a strong effect on the selectivity. For some pairs of solutes with similar retentions (phenol and aniline on column 4, phenol and benzene on column 5), inversions in the elution order can occur but they do not lead to very important changes in selectivity. The separation of all seven solutes seems possible only with column 5, although the resolution of benzene, pyridine and phenol is difficult at all concentrations, partly as a result of the large capacity factor that must be attained for the elution of nitrobenzene. In practice, such

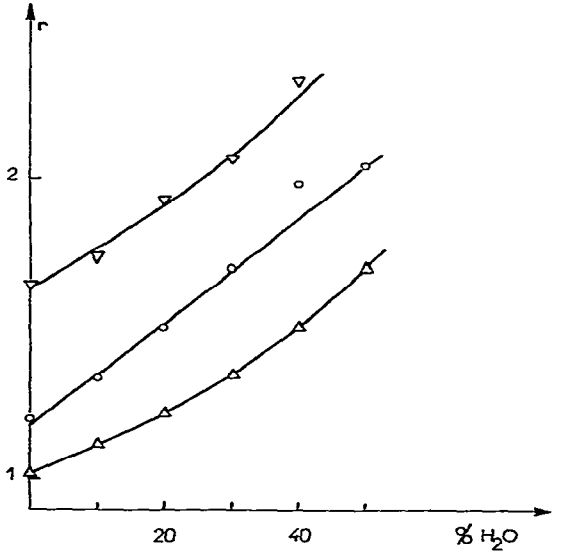


Fig. 10. Variation of group selectivity (α) for *n*-alkylbenzenes with the composition (%, v/v) of the methanol–water mixture. ▽, Column 5; ○, column 2; △, column 4.

a separation predicted from k' values may prove difficult to achieve, owing to the poor peak shape and low efficiency for polar compounds, particularly for pyridine.

The selectivity (α) in homologous series is obviously dependent on the water content of the solvent[10]. Results are reported in Fig. 10. The best packing for the resolution of such homologous compounds is PMS. Equivalent group selectivities are obtained for the PMS column using pure methanol, the Partisil ODS 2 column with methanol–water (75:25) and the ethylphenyl-silica column operated with methanol–water (50:50). However, the group selectivity of the PMS packing in any given solvent could be considerably increased (by a factor of as much as 3) by using modified silica with a larger specific surface area. This option makes possible an increase in the peak capacity of a column and could prove helpful to the chromatographer interested in separation problems such as those concerning linear and branched chains with the same carbon number[11].

Effect of the organic modifier. The effect of the modifier in the solvent upon selectivity has been studied with methanol, acetonitrile, tetrahydrofuran and dioxane as the organic component of the solvent system. Experiments were performed under normalized conditions for the capacity factor of nitrobenzene. This means that, when

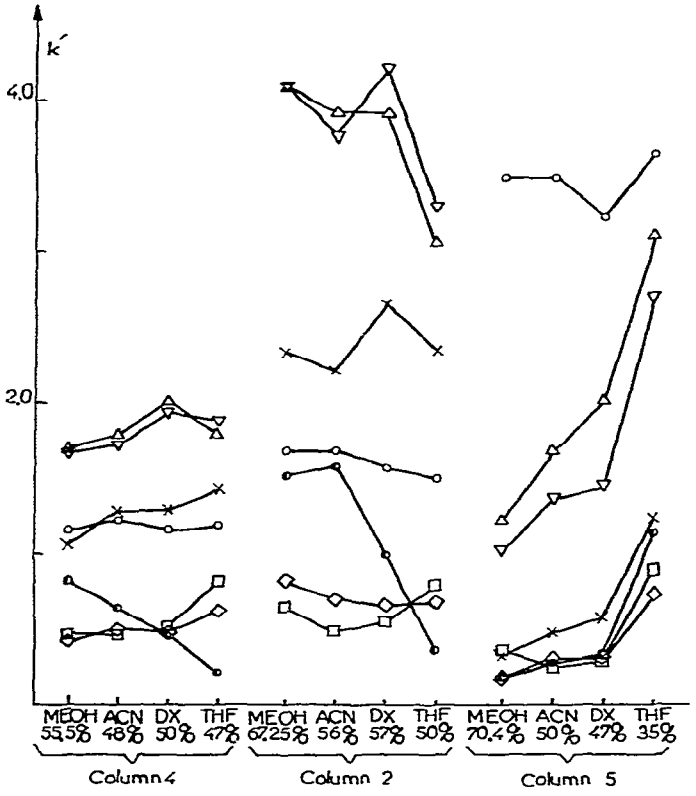


Fig. 11. Variation of the capacity factors under conditions of k' normalization for nitrobenzene. ○, Nitrobenzene; △, chlorobenzene, ▽, toluene, □, phenol, ×, benzene, ◑, pyridine, ◇, aniline.

the modifier was changed from methanol to acetonitrile and in turn tetrahydrofuran and dioxane, the water concentration was varied in order to obtain a constant k' value for nitrobenzene. Results are reported in Fig. 11 for the seven compounds in question. The normalization of the k' values of nitrobenzene on each column was successful to within a relative standard deviation of less than 5%.

Changing the modifier has more important consequences for the selectivity than changing the water content of the eluent. The importance of changing the modifier has also been reported by Karger *et al.*[10].

The separation of the mixture could be performed with column 2 if it were able to produce 8000 theoretical plates. Such a plate number would just be attainable when working at the minimum of the h–curve, where the efficiency is maximized. This relatively high efficiency is required for the separation of toluene and chlorobenzene.

It is much more difficult to achieve the overall separation with the PMS column, because even under the best conditions, tetrahydrofuran–water (35:75), it would require 11,500 plates owing to the very similar retentions of benzene and pyridine. The separation of the components benzene, pyridine, aniline and phenol on this column would have to be carefully controlled, especially as the k' values are all smaller than 2. Of these solutes the most retained is benzene, and inversions in the order of elution of the others occur when changing the modifier, possibly because of fluctuation in the k' value of nitrobenzene. It should also be noted that for all of the compounds except phenol, the capacity factors increase when the modifier is changed in the arbitrary order chosen from methanol to tetrahydrofuran, holding the k' value of nitrobenzene constant. The separation of the seven test compounds would be easier with methanol–tetrahydrofuran (65:35), corresponding to a larger k' value for nitrobenzene (*cf.*, Fig. 9b).

With column 4 the separation appears to be impossible, requiring more than 30,000 plates for the separation of toluene and chlorobenzene. Again, however, the organic modifier which results in the best resolution of the solute mixture is tetrahydrofuran. For this column, despite the changes in selectivity observed on changing the modifier, the major problems in the separation are consistently those of resolving the three solute pairs toluene–chlorobenzene, benzene-nitrobenzene and phenol–aniline (note again the odd behaviour of pyridine for both columns 4 and 2).

From this experiment, it can be seen that at present it is difficult to make predictions of the retention behaviour of a solute on changing the modifier. As each modifier has a characteristic adsorption on the packing material, when the modifier is changed the water concentration of the solvent must also be adjusted so as to maintain the same range of k' values. It is probable that the overall effects of this procedure will have different consequences for different solutes. The order of increasing eluotropic strength is probably methanol $<$ acetonitrile $<$ dioxane $<$ tetrahydrofuran. The results shows that the CBP packings perform better than the pyrocarbon-modified types for the separation of polar compounds, in the sense that larger variations in selectivity on changing the modifier are observed with the former. Indeed, one can take better advantage of the specific solute–solvent interactions with CBP packings. Other measurements of retention times have been made with the solvent system methanol–water for solutes of high and medium polarity (benzaldehyde, benzoquinone, bromobenzene, anisole, etc.), yielding the same conclusions. As previously stated, poor chromatograms are obtained for very polar solutes on PMS. Work is in progress to

improve our understanding of this phenomenon and to develop a means of improving the peak profiles, such as by using a further treatment of the PMS packing with trimethylchlorosilane to neutralize most active sites left exposed following pyrocarbon deposition, or working at higher temperatures.

Loadability

The property that poses the most difficult problem for making comparisons between packings and chromatographic systems is the column loadability. What is the best measurement of the linear capacity (θ) of a chromatographic system? For Snyder[12] it is the amount of solute injected per gram of packing that produces a 10% decrease in the k' value; for others[13] it is the amount injected that results in an increase of 0.1 mm in the HETP (it should also be noted that increasing the amount of solute injected can increase the k' value if the adsorption isotherm is concave). The use of small particle packings has shown that even "small" injections, which do not affect the capacity factor, may cause important changes in the efficiency of the column[13].

We believe that the measurement of linear capacity by the injection of increasing amounts of a single compound does not really provide the information that the chromatographer requires. Experiments with single injections of a solute yield a very interesting picture of the thermodynamic properties of column loading and provide the chromatographer with useful information. However, the main problem for the analyst is to establish the maximum amount of a solute mixture that can be injected in order to keep the resolution of two peaks of interest larger than, *e.g.*, 1.2. The answer to this question depends on three parameters; the selectivity and the efficiency of the system and the isotherm of adsorption (or partition) of the solutes. The linear capacity of a column for one compound cannot be considered to be a characteristic of the packing. If we want simply to establish the role of the packings, experiments must be performed with the same solute for which the capacity factors obtained with each column using the same solvent are equal (due to the dependence of efficiency on retention). If the packings are different, it is unlikely that it will be possible to attain criteria for valid comparison. Therefore, θ must be regarded as a characteristic of the paired parameters packing–solvent and, of course, of the solute. In addition, increasing the specific surface area of the packing (or the amount of the stationary phase) by a given factor will affect θ in a complex manner, as the k' value of the solute will also change if the solvent is kept unchanged and θ is a function of k'.

Two series of experiments were performed. Firstly, θ was determined for "single-solute" injections of 1,2,4-trimethylbenzene (TMB) and 2,3,5-trimethylphenol (TMP) on different columns. Secondly, increasing amounts of different mixtures were injected on the same columns.

Column capacity for single solutes. Figs. 12 and 13 summarize the results obtained for the variation of the reduced HETP (h) with increasing amounts of 1,2,4-TMB and 2,3,5-TMP for columns 1, 2, 3 and 4 (Fig. 12) and the three TMB isomers for the PMS (column 7, Fig. 13). In his thorough study of the linear capacity of columns, Done[13] chose to define the quantity θ as being that weight of solute which results in an increase of 0.1 mm in the HETP from the extrapolated "zero-weight" injection intercept of an HETP *versus* normalized injection weight plot. Such a definition has the drawback of involving a much too close relationship between θ and the particle size. In the light of the facts mentioned earlier about the difficulty in establishing the

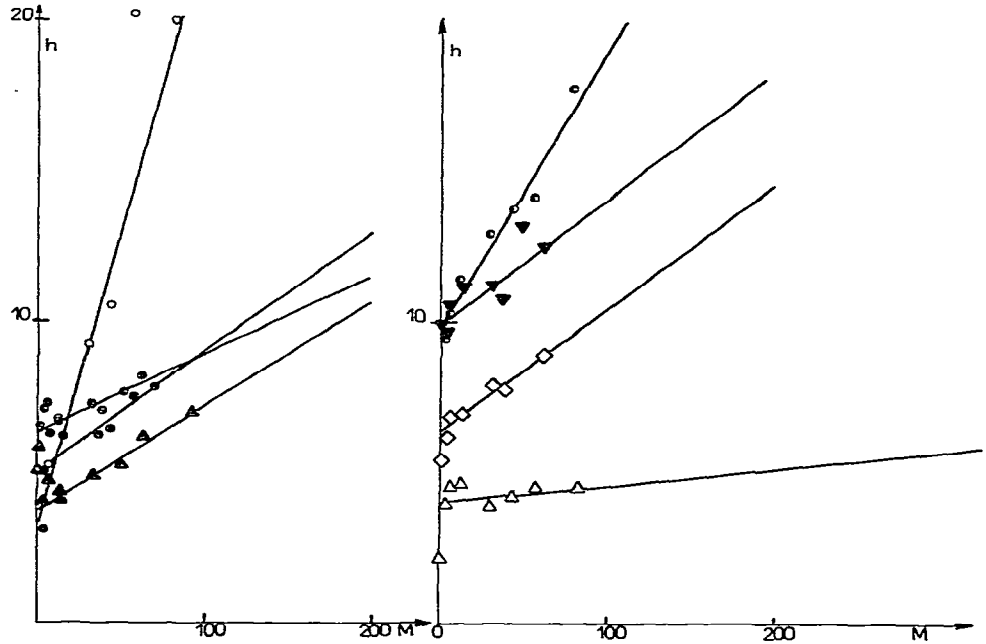


Fig. 12. Change in reduced HETP (h) with the amount of solute injected (M in μg of solute per g of packing) for bonded packings. ◇, ▲, ◑ and △, 1,2,4-trimethylbenzene on columns 4, 3, 2 and 1, respectively; ○, ●, ▼ and ⊗, 2,3,5-trimethylphenol on columns 4, 3, 2 and 1, respectively.

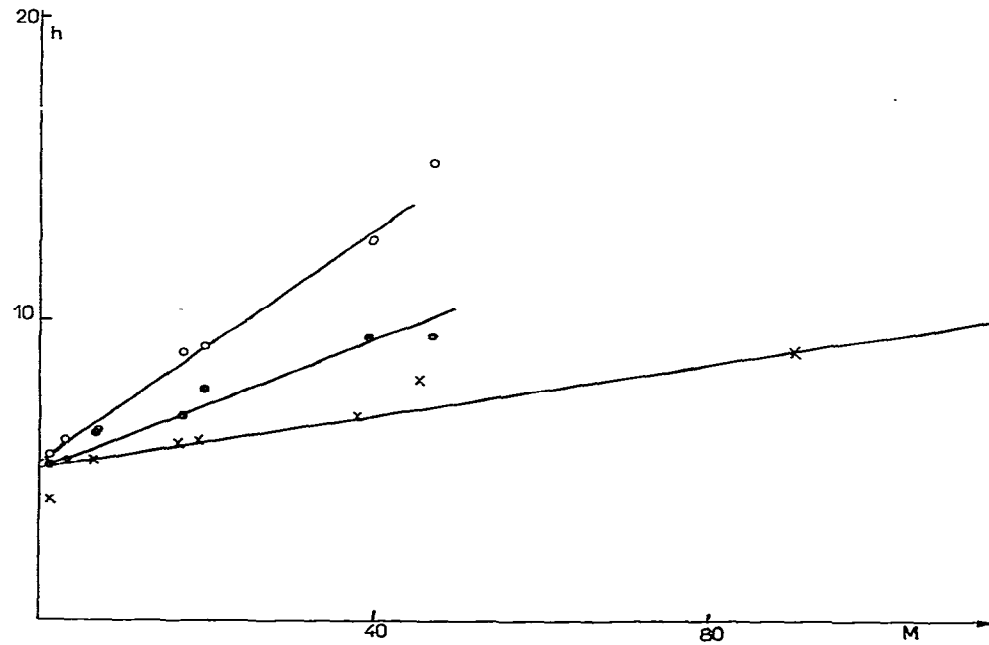


Fig. 13. Change in reduced HETP (h) with the amount of solute injected for pyrocarbon-modified silica gel (column 7) See Fig. 12 and Table II. ○, ● and × represent 1,2,3-, 1,2,4- and 1,3,5-trimethylbenzenes, respectively.

TABLE II
LINEAR CAPACITIES OF COLUMNS (θ)

Injection	*Solute*	*Column*	*Solvent*	k'	θ (μg/g)*
Single-solute injection	2,3,5-TMP	4	MOH–W (50:50)	3.20	119
		3	MOH–W (60:40)	3.22	62
		2	MOH–W (85:15)	0.68	117
		1	MOH–W (50:50)	0.90	120
	1,2,4-TMB	4	MOH–W (50:50)	8.65	9.0
	—	3	MOH–W (60:40)	13.75	56
		2	MOH–W (85:15)	2.33	356
		1	MOH–W (50:50)	3.97	46
		7	ACN	0.34	24
	1,2,3-TMB	7	ACN	0.48	15
	1,3,5-TMB	7	ACN	0.22	59
Mixture injection	1,3,5- + 1,2,3- + 1,2,4-TMB	7	ACN	—	50
	Parathion + methylparathion	7	ACN	—	10.5
		2	MOH–W (80:20)	—	> 600
	Testosterone + progesterone	2	MOH	—	60

* For single solute injection, injected amount providing a 50% increase in h_0 (reduced HETP extrapolated at zero sample amount); for mixture injection, injected amount providing a resolution of 1.3.

proper experimental conditions for valid comparisons of linear capacities between columns, we chose an arbitrary measure of θ as being that weight of solute per gram of packing which results in a 50% increase in h from the extrapolated "zero-weight" injection. Linear capacities calculated using this definition are given in Table II.

The results for the PMS column illustrate clearly that even though three isomers are eluted within a k' range of 0.25 capacity factor units, the column capacities can be very different. All columns except Partisil ODS 2 exhibit a larger linear capacity for the earlier eluted 235-TMP peak, supporting the notion that preparative work should be performed at small k'-values where adequate resolution of the peaks of interest is possible. The very high capacity of the Partisil ODS 2 column for 1,2,4-TMB at $k' = 2.33$ is not readily explained. Improvement of the experimental design for these determinations would consist in injecting much larger amounts of solute at a wavelength that would avoid detector saturation. In this way, the validity of linear extrapolations to very high concentrations of solutes (as with the column 2 plot) could be verified. Another determination of θ for 1,2,4-TMB on the phenyl column was made using methanol–water (60:40) instead of a 50:50 mixture. The k' value was reduced by a factor of 2 while a 20-fold increase in θ was observed with the stronger solvent mixture. This result supports the idea that the column capacity is so closely related to other separation parameters that it can hardly be considered as an inherent characteristic of a particular packing or column. Note that peak shape distortion, with a steep peak tail, due to a concave adsorption isotherm for 1,2,4-TMB on the phenyl column, was observed at high concentrations of solute (above 43 μg/g). Most solutes have a

convex adsorption isotherm. In this particular case, the peak distortion resulted in increasing k' values with increasing amounts of injected solute.

Column capacity for mixtures. This series of experiments deals with the true chromatographic problem: for a given separation, what packing allows the maximum injection of solute? In this instance, the selectivity of the system is the most important parameter. If the resolution of the two peaks in a simple two-component mixture is greater than, *e.g.*, 3 under analytical conditions, it is possible that even with an increase of 50% in HETP and a 30% change in capacity factors the separation will still be accomplished with a resolution better than 1.3 (which corresponds to a baseline separation). Thus, a column which has a small capacity determined by the injection of single compounds may indeed be more useful if its selectivity is larger.

Let us consider, for example, the separation of parathion and methylparathion. Results obtained with columns 7 and 2 are given in Figs. 14 and 15, respectively, and in Table II. The resolution is better than 1.3 even when more then 0.6 mg per gram of packing is injected with column 2. Under analytical conditions, the k' values of methylparathion and parathion are 1.04 and 1.76, respectively ($\alpha = 1.7$). While the same separation is easy with the PMS column when small amounts are injected ($k' = 5.76$ and 6.57, $\alpha = 1.14$), the resolution decreases much faster and it is not possible to inject more than 10 μg of the mixture per gram of packing if a resolution better than

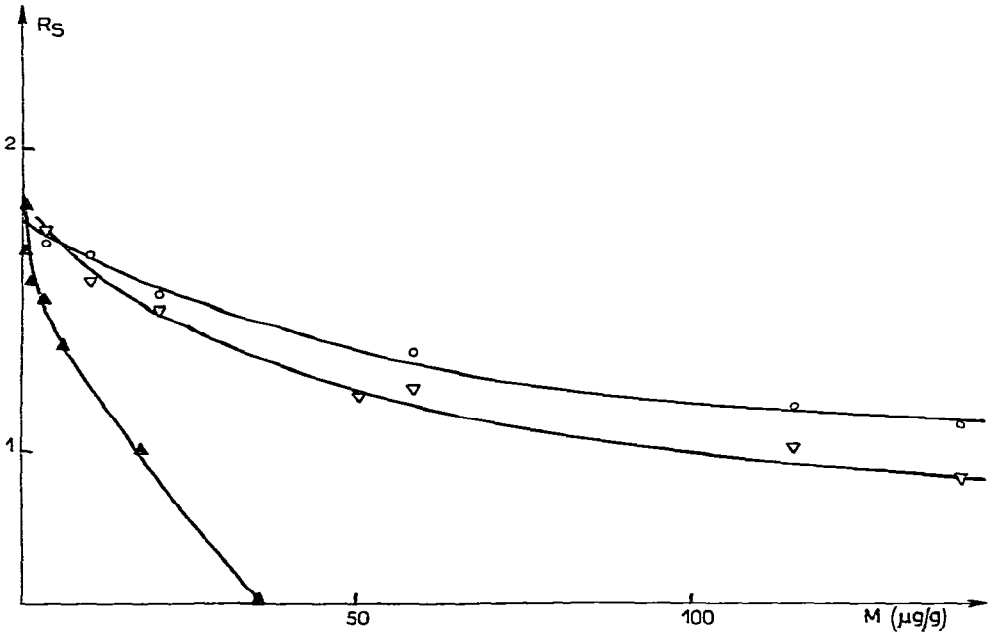


Fig. 14. Change in resolution with increasing amounts of solute mixture injected on column 7. Solvent, acetonitrile. ○, 1,3,5- and 1,2,4-trimethylbenzenes; △, 1,2,4- and 1,2,3-trimethylbenzenes. Capacity factors: 0.22, 0.34 and 0.48 ($\alpha = 1.55$ and 1.41). For a resolution of 1.3, $N = 3300$ and 3000, respectively. ▲, Methylparathion and parathion. Capacity facotrs: 5.76 and 6.57. $\alpha = 1.14$, $N = 2400$. See Table II. M is the amount of mixture (1,2,4- + 1,2,3- + 1,3,5- +TMB or parathion + methylparathion) injected (μg/g). In each mixture the weight fraction is the same for the different components.

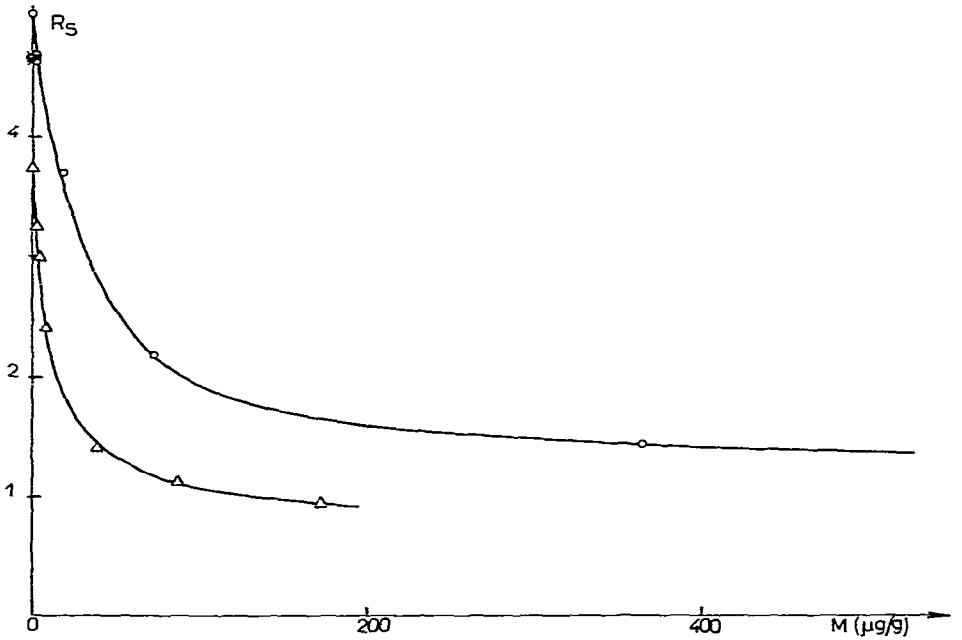


Fig. 15. Change in resolution with increasing amount of solute mixture injected on column 2. ○, Methylparathion and parathion. Capacity factors: 1.04 and 1.76. $\alpha = 1.7$, $N = 400$. Solvent, MOH–W (80:20). △, Testosterone and progesterone. Capacity factors: 0.87 and 1.42. $\alpha = 1.63$, $N = 520$. Solvent, MOH. See Fig. 14 and Table II.

1.3 is expected. Note that with a different carbon column (No. 8) the separation conditions were better ($k' = 0.80$ and 0.94, $\alpha = 1.18$). With the phenyl packing, the capacity factors are 2.19 and 3.86 ($\alpha = 1.76$) with the mixture methanol–water (65:35); the capacity of this column is probably even better than that of column 2.

If the separation problem is the resolution of the three trimethylbenzene isomers, we have seen that only a carbon packing can be used. The variation of the resolution of these compounds with the amount injected is illustrated in Fig. 14. It appears that if a resolution better than 1.3 is expected, a maximal amount of 40 μg of each solute per gram of packing can be injected. The linear capacity determined by the injection of 1,2,3-TMB as a single solute was 15 μg/g. The k' values for 1,3,5-, 1,2,4- and 1,2,3-TMB with acetonitrile as solvent were 0.22, 0.34 and 0.48, respectively.

Experiments were also performed with the mixture progesterone–testosterone. Their capacity factors obtained by using column 9 with chloroform as solvent were 0.68 and 1.49, respectively. The selectivity of this system was so important that even very large injections (> 200 μg/g) of the mixture did not inhibit the separation. On the other hand, with column 2 (Fig. 15) it was found that the maximal amount that could be injected while maintaining adequate resolution was 40 μg/g even though the resolution under analytical conditions was very good (resolution better than 3.5).

In conclusion, it seems that the best strategy when planning a preparative separation is to choose the chromatographic system that provides the best resolution for the solutes of interest, while maintaining their capacity factors at low values (smaller

than 3). This is probably the most desirable procedure even if, for the packing in question, the linear capacity as determined from injections of single compounds is smaller than that for another adsorbent with poorer resolution. We are conscious of the fact that this scheme results in larger capacities for the more efficient columns and for the longer columns. Retention time is a parameter that has been neglected in this discussion.

Some examples of separation problems

Some simple separation problems were investigated in order to compare the performances of the different columns, using similar sulphur and nitrogen-containing compounds, various pesticides (including PCBs) and steroids. It should be pointed out that no literature search was carried out on the separation problems investigated. The purpose of this work is not to solve important separation problems, but to make a broad comparison between the different materials. It is obvious from the following that no optimization has been tried on the different examples we have worked with. It is likely that a careful study of the solvent composition (pH, ion pairing, etc.) could have improved some results for both CBP and PMS. The packings have just been operated with the simplest solvent possible, that is mixtures of water and methanol or acetonitrile. It is also possible that RP systems may not even be the most suitable for some of these examples. The problems are investigated here strictly for the purpose of comparison.

Similar nitrogen- and sulphur-containing compounds. This separation problem demonstrates the selectivity of each system for cyclic compounds in which an aromatic

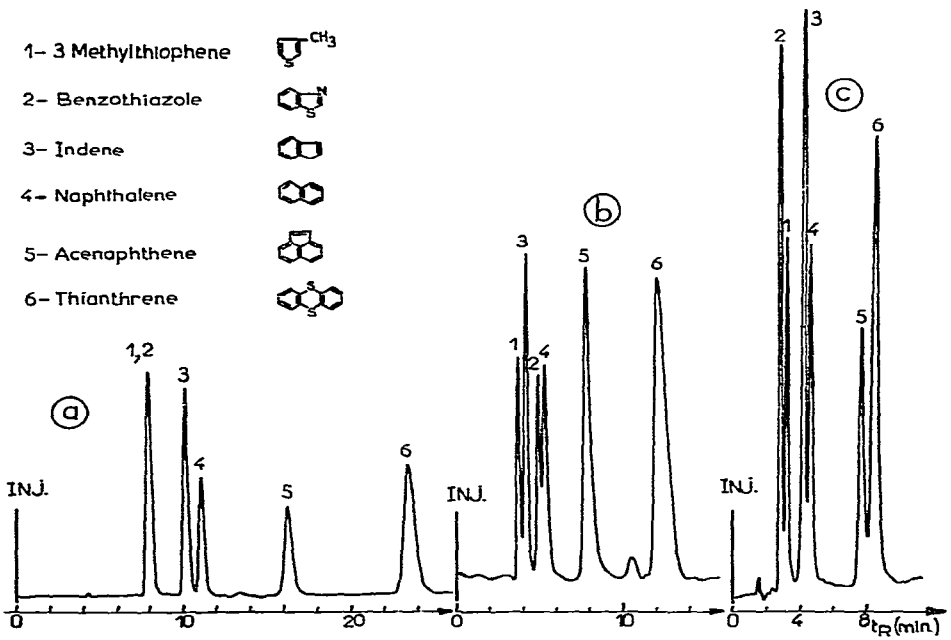


Fig. 16. Separation of sulphur- and nitrogen-containing compounds. (a) Column 4, solvent MOH–W (65:35), flow-rate 0.67 ml/min; (b) column 9, solvent ACN, flow-rate 0.5 ml/min; (c) column 2, solvent MOH–W (83:17), flow-rate 1.35 ml/min.

carbon atom has been replaced with a nitrogen or sulphur atom. Good selectivity would prove very useful in the separation of petroleum fractions by RPHPLC. Examples of chromatograms are given in Fig. 16. Good results are obtained with columns 2 and 9. The resolution exhibited by column 9 is slightly better (two partially unresolved pairs instead of three). Note that the separations on columns 2 and 9 are made within k' ranges of 0.8–4 and 0–2.3, respectively. Such ranges of retention correspond to excellent working conditions. The elution order on the PMCB column parallels the order of increasing solute molecular weight and area. This fact is not surprising as the mobile phase (acetonitrile) is not very polar and the solute–solvent interactions would be much smaller than with the methanol–water mixtures used with the other columns. On column 4, 3-methylthiophene and benzothiazole are eluted together. This is unfortunate because the selectivity of the packing is very good for the other solutes (the range of k' values is 1–4.45). As far as peak symmetry is concerned, the results are good for all the packings. A small tailing is observed for peaks 5 and 6 on column 9. We have previously reported[8] that the elution of conjugated aromatic rings can give peaks with poor symmetry, but they are acceptable in this instance.

Pesticides and chlorinated hydrocarbons. Two groups of compounds were analysed: pesticides containing sulphur, phosphorus and chlorine and polychlorinated biphenyls (PCBs).

TABLE III

CAPACITY FACTORS OF SOME PESTICIDES

Type	*Compound*	*Column 2 MOH–W (83:17)*	*Column 4 MOH–W (65:35)*	*Column 5 (ACN)*	*Column 5 (MOH)*
Containing phosphorus and sulphur	Ronnel (1)*	3.94	5.78	3.38	> 15
	Ethion (2)	3.00	2.08	0.39	1.44
	Coral	1.76	7.15	0.33	1.18
	Guthion (3)	1.82–2.97	0.13–1.68–2.34	0.00	0.00
	Phosdrin	< 0**	< 0**	0.00	0.00
	Naled	0.28–0.56	0.38–1.21	0.00	0.00
	Methyltrithion (4)	2.57	6.70	1.39	—
	Cygon	0.22–0.75–133	(10 peaks)	0.15***	0.01
	Malathion (5)	0.72	2.64	0.16	0.32
	Parathion (6)	1.10	3.86	0.94	2.15
	Methylparathion (7)	0.72	2.19	0.80	1.85
	Phorate	1.94	4.14	0.02	0.30
Containing chlorine	DDT	6.66	—	0.17	0.26
	BHC	2.11	2.81	0.10	0.15
	TDE	3.54–4.06	8.84–9.67	0.17	0.28
	Perthane	2.54	4.16	0.52	0.87
	Endrin	2.50–4.44	3.16–7.21	0.13–0.21	0.24
	Lindane	2.06	2.49	0.36	0.92
	Methoxychlor	3.37	8.43	0.32	0.63
	Heptachlor	5.74	10.71	0.11	0.09
	Dieldrin	4.42	6.71	0.14	0.14
	Endosulfan	1.43	2.23	0.20	0.00

* Figures in parentheses refer to peak numbers in Fig. 17.
** Solute peak was eluted before solvent.
*** Very large peak. k' at the maximum of the peak.

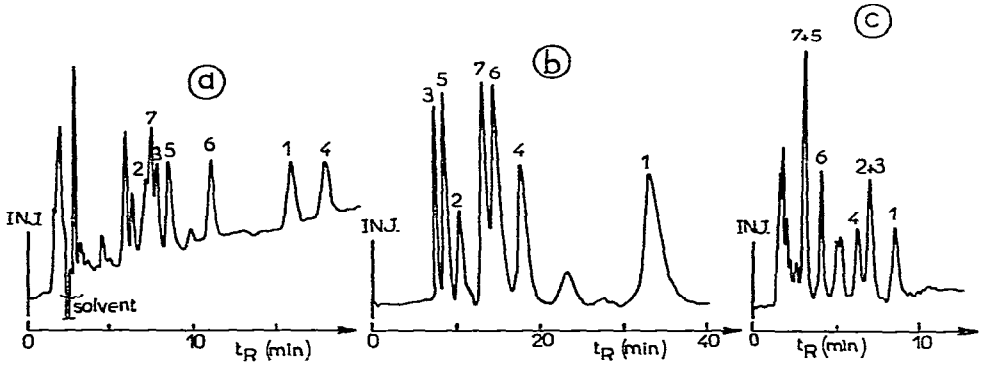


Fig. 17. Separation of sulphur- and phosphorus-containing pesticides. (a) Column 4, solvent MOH–W (65:35), flow-rate 1.05 ml/min; (b) column 8, solvent ACN, flow-rate 0.7 ml/min; (c) column 2, solvent MOH–W (83:17), flow-rate 1.35 ml/min. Refractive index detector. For identification of compounds, see Table III.

Capacity factors for the first group are reported in Table III. Some injections produced several peaks, especially Cygon, which generates ten peaks on column 4. Because of the lower efficiency of the PMS column, the elution of such standards generally produces a broad unresolved band.

None of the chromatographic systems is able to resolve the 22 compounds listed in Table III. The separations that pose particular problems for each column are generally different. It was interesting to attempt the separation of some pesticides containing sulphur or phosphorus in the same mixture (guthin, malathion, ethion, methylparathion, parathion, methyltrithion, and ronnel). The chromatograms in Fig. 17 show that good results were obtained with PMS. This is not to say that PMS or PMCB is the most suitable packing for all pesticides separations. For example, CBP packings seem to be more useful when dealing with chlorinated pesticides.

Each PCB sample (Aroclor 1248, 1221, etc.) is a mixture of numerous compounds, including isomers and separations of such mixtures are difficult. It is probable that carbon packings will prove very useful for these separations. Two examples are considered here: the relatively simple Aroclor 1221 and the more complex Aroclor 1248. Chromatograms are presented in Figs. 18 and 19, respectively. The best results are obtained with PMS, although Partisil ODS 2 and μBondapak-phenyl packings also give good separations. The elution of Aroclor 1248 under the most suitable gradient conditions derived from the isomatic chromatogram in Fig. 19c would considerably increase the resolution of the early peaks. For columns 2 and 4, as incompletely resolved peaks are present all over the chromatograms, the use of gradient elution for the resolution of these peaks would be more difficult.

However, no attempt was made to optimize these isocratic separations. The use of a solvent modifier other than methanol with the CBP packings could give better results than with PMS. The long analysis time on column 10 is a result of the length of the column (115 cm) and the large particle size (20–30 μm). With particles of this size, good efficiency is attainable only at very low velocities. For example, if one were to work at the minimum of the *h versus v* curve ($v = 2.5$), the

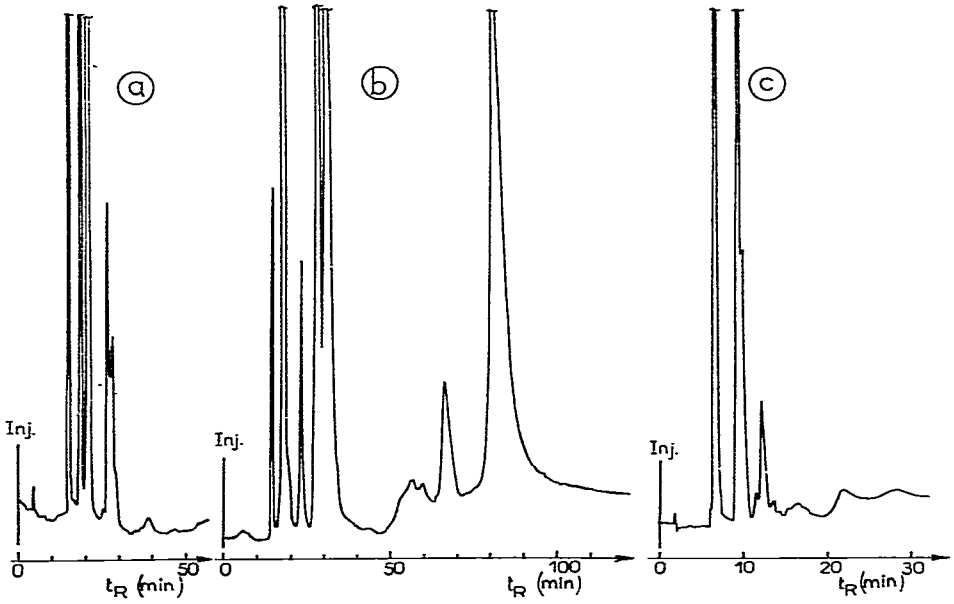


Fig. 18. Separation of Aroclor 1221. (a) Column 4, solvent MOH–W (65:35), flow-rate 0.67 ml/min; (b) column 10, solvent MOH, flow-rate 0.3 ml/min; (c) column 2, solvent MOH–W (83:17), flow-rate 1.65 ml/min. UV detection at 254 nm.

retention time of an unsorbed solute would be 75 min. Under our experimental conditions, $\nu \approx 19$ and more than 7000 theoretical plates are produced ($h \approx 6.5$).

Steroids. Separations of steroids pose rigorous chromatographic tests as they often have very similar structures. The resolution of α- and β-type geometrical isomers is particularly difficult. The compounds studied are listed in Table IV with their corresponding capacity factors. The separation of the two pairs of geometrical isomers 5β- and 5α-androstane-3-17-dione and 5β- and 5α-androstan-3α-ol-17-one is good with PMS, difficult with μBondapak C_{18} and nearly impossible with the phenylethyl packing. These results are in agreement with the general tendency already noted, of the superiority of carbon packings for the separation of geometrical isomers, at least when the compounds do not exhibit major differences in polarity. The separation of all four solutes in the same mixture would require 8300 plates for column 5, 190,000 plates for column 2 and 76,000 plates for column 4. In practice, 8000 plates would be difficult to attain with the PMS column for such solutes as their peak shapes are sometimes poor. Such efficiency could perhaps be achieved with a long column (50 cm) packed with 10-μm particles.

The increasing order of retention for the two solutes pregnanetriol and pregnanediol is the same on all three columns. Prediction of this elution order can be rationalized for the CBP packings (greater solvent–solute interactions for the more polar pregnanetriol), but the reverse would be expected for the PMS packing as pregnanetriol has a higher molecular weight and the specific interactions with the low-polarity solvent used (chloroform) are necessarily weak. This indicates that even if relatively confident predictions of elution order can be made when chromato-

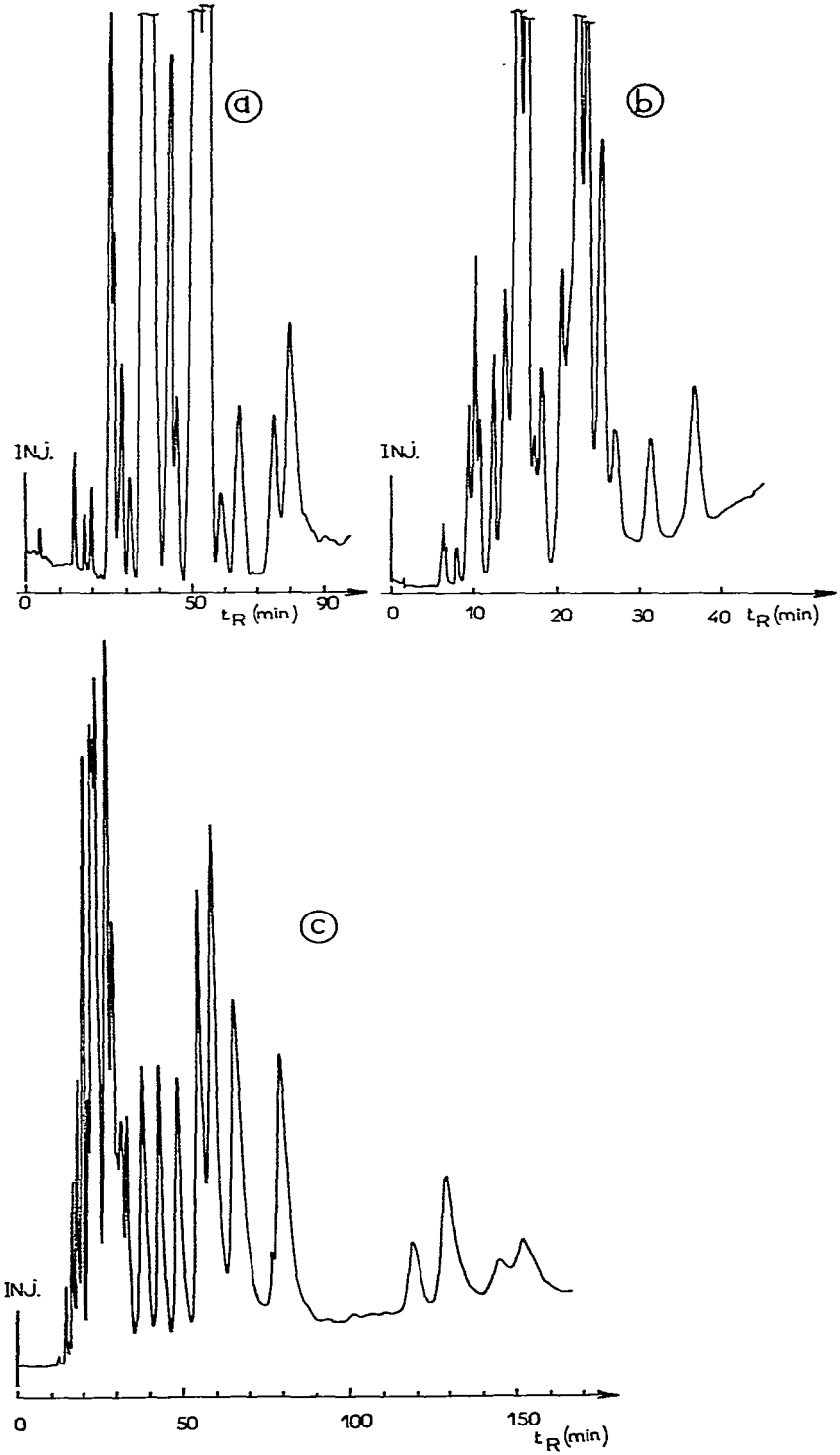


Fig. 19. Separation of Aroclor 1248. (a) Column 4, solvent MOH–W (65:35), flow-rate 0.67 ml/min (b) column 2, solvent MOH–W (83:17), flow-rate 1.65 ml/min; (c) column 11, solvent MOH, flow-rate 0.3 ml/min. UV detection at 254 nm.

TABLE IV

CAPACITY FACTORS OF SOME STEROIDS

Compound	*Column 2 (MOH)*	*Column 4 MOH–W (65:35)*	*Column 5 (Chloroform)*
5β-Androstane-3,17-dione	1.51	3.59	0.11
5α-Androstane-3,17-dione	1.71	3.50	0.26
5β-Androstan-3α-ol-17-one	1.74	3.78	0.41
5α-Androstan-3α-ol-17-one	2.03	3.96	0.33
Etiocholan-3α-ol-17-one	1.69	3.71	0.38
Estrone	0.91	2.44	0.64
Androsterone	2.00	3.88	0.32
Testosterone	1.40	2.61	1.49
Progesterone	2.51	5.33	0.68
Pregnanediol	3.63	6.08	0.98
Pregnanetriol	2.77	4.76	0.33

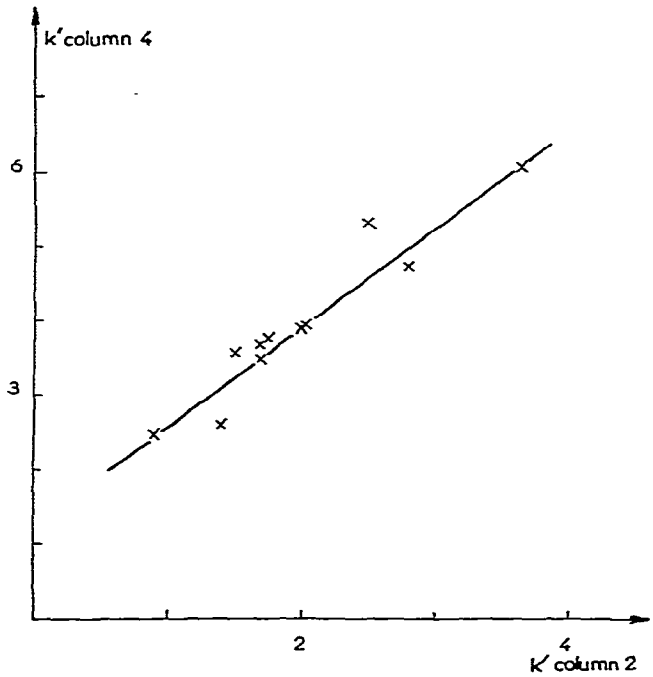


Fig. 20. Relationship between the capacity factors of steroids (listed in Table III) eluted on columns 2 and 4.

graphing simple molecules (methylbenzenes or phenols), the situation becomes much more complicated when the geometry of the solute is complex and it is difficult to visualize the overall interactions. With pregnanetriol, the presence of the third hydroxy group is likely to affect in an important manner the orientation of the molecules adsorbed on the pyrocarbon surface, compared with that of pregnanediol, and hence the adsorption energy. It is noted that the selectivities of the two CBPs are very similar

for all of the steroids studied, as shown by Fig. 20. No valid explanation has been found. No similar correlation exists with the retention data on column 5.

CONCLUSION

Although the retention on non-polar bonded phases and pyrocarbon-modified packings are only due to non-specific forces, the contribution of specific solvent–solute interactions to the overall retention is greater for the former materials. This statement is supported by the greater selectivity observed for similar compounds that have different polarities on CBP. As carbon packings are generally used with less polar solvents than CBP, the selectivity in this instance results essentially from the differences in the adsorption of the solutes. When using CBP with polar solvent mixtures, the selectivity is largely due to the solubility of compounds in the mobile phase[14]. On the other hand, the selectivity for solutes in a homologous series and also for geometrical isomers is generally better for PMS or PMCB. The two types of packings thus seem to be complementary.

More important changes in selectivity are observed on changing the modifier of the water–organic solvent system than on changing the water content. In most instances, very symmetrical elution peaks are obtained with the CBP, which is not always true for PMS.

As far as the loadability of a column is concerned, it is very difficult to make meaningful comparisons between different packings. However, when dealing with the separation of mixtures, the selectivity of the chromatographic system is of prime importance.

REFERENCES

1 I. Molnar and C. Horváth, *Clin. Chem.*, 22 (1976) 1497.
2 H. Colin and G. Guiochon, *J. Chromatogr.*, 141 (1977) 289.
3 H. Hemetsberger, W. Maasfeld and H. Ricken, *Chromatographia*, 7 (1976) 303.
4 H. Colin and G. Guiochon, *J. Chromatogr.*, in press.
5 C. Horváth and H.-J. Lin, *J. Chromatogr.*, 126 (1976) 401.
6 K. Karch, I. Sebestian and I. Halász, *J. Chromatogr.*, 122 (1976) 3.
7 H. Colin, C. Eon and G. Guiochon, *J. Chromatogr.*, 122 (1976) 223.
8 H. Colin and G. Guiochon, *J. Chromatogr.*, 137 (1977) 19.
9 H. Colin and G. Guiochon, unpublished results.
10 B. L. Karger, J. R. Grant, A. Hartkoff and P. H. Weiner, *J. Chromatogr.*, 128 (1976) 65.
11 H. Colin and G. Guiochon, in preparation.
12 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968.
13 J. N. Done, *J. Chromatogr.*, 125 (1976) 43.
14 D. C. Locke, *J. Chromatogr. Sci.*, 12 (1974) 433.