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Comparison of stationary phases for packed-column supercritical fluid chromatography

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SUMMARY

A study of the applicability of different stationary phases for packed-column supercritical fluid chromatography is described. The compatibility of these phases with carbon dioxide and with a number of test solutes was established qualitatively from the observed peak shapes. Retention and selectivity differences between the different columns were studied quantitatively. The stationary phases studied include chemically modified silicas, polysiloxane-coated silicas, modified and unmodified porous graphitic carbon and modified and unmodified poly(styrene–divinylbenzene) copolymers. It is concluded that differences in selectivity between different stationary phases are often due to interactions between the solute molecules and active sites on the surface. Such interactions lead to poor peak shapes and are therefore undesirable. For most of the solutes studied, with the notable exception of polyaromatics, a poly(ethylene glycol)-coated carbon phase provided the best results. Owing to the very high retentivity of unmodified carbon, this phase turned out to be very stable. The coating of different types of polymers on solid surfaces may be used to create stationary phases without residual active adsorption sites, but with considerable differences in selectivity.

INTRODUCTION

Carbon dioxide is the preferred solvent for supercritical-fluid chromatography (SFC)¹. It has a number of great advantages, including its favourable critical properties ($T_c \approx 31^\circ\text{C}$; $P_c \approx 73$ bar), favourable safety and toxicity characteristics, availability in sufficient purity at low cost and compatibility with a variety of chromatographic detectors. Unfortunately, carbon dioxide is essentially a non-polar solvent². Non-polar solutes, such as hydrocarbons, can be eluted as sharp, symmetrical peaks from many different stationary phases. Polar solutes, however, are often eluted as broad, asymmetric peaks, or are not eluted at all^{3–5}. To improve this situation, more polar mobile phases can be used. Among the possible pure solvents, ammonia is the only realistic choice². Although ammonia has been used occasionally in SFC⁶, its routine application is likely to involve major problems. Therefore, it is

almost inevitable to use mixtures when polar mobile phases are required. The common practice is to add a small amount ($\leq 20\%$) of a polar organic solvent (a "modifier") to a non-polar mobile phase such as carbon dioxide. Although the critical temperatures of such mixtures will be higher than that of pure carbon dioxide, the values will be well within practical reach and far below the values for the pure polar solvents. Therefore, these mixtures can be used at supercritical (or near-critical) conditions, resulting in lower viscosities and higher diffusion coefficients as compared with liquids.

The addition of organic modifiers to the mobile phase will affect retention in SFC in three different ways^{7,8}: by increasing the mobile phase polarity, by increasing the mobile phase density (at constant temperature and pressure) and by deactivation of active sites on the surface. The last factor is thought to be the most important in packed-column SFC. In contrast to the first two factors, it may cause the peak shape to improve dramatically on addition of very small amounts ($< 1\%$) of modifiers, because active sites on the surface have been held responsible for causing a detrimental mixed retention mechanism in packed-column SFC⁹.

In open-tubular (capillary) SFC, the third effect of adding modifiers is much less significant than it is in packed-column SFC^{10,11}. As a consequence, a larger number of (classes of) solute molecules can be eluted from wall-coated open-tubular columns than from packed columns using pure carbon dioxide as the mobile phase. This indicates that an alternative way of dealing with polar solutes in SFC is to prepare homogeneous stationary phases without active adsorption sites.

There are some good reasons to try and avoid the use of modifiers. These include technical problems in accurately adding small amounts of modifier, column stability, instrument reliability and, especially, detection compatibility. Therefore, stationary phases for SFC are desirable which allow the elution of the largest possible variety of solutes as sharp, symmetrical peaks, using pure carbon dioxide as the mobile phase, and provide sufficient selectivity for a variety of separations. This second aspect of SFC stationary phases implies that a choice is needed between different phases that show substantial differences in selectivity. This situation is similar to that in gas chromatography (GC), but contrary to that in liquid chromatography (LC). In the latter technique, selectivity is usually varied by varying the nature and composition of the mobile phase, while keeping the stationary phase the same. If we try to use unmodified carbon dioxide as the mobile phase for SFC, we will need to achieve all variations in the selectivity by varying the stationary phase.

In this paper, we describe a study of a number of stationary phases, which were first evaluated qualitatively. Thereafter, five were selected for a quantitative evaluation. We selected a group of 25 test solutes, all of fairly low molecular weight, but with a range of different functional groups, to judge the applicability of the different phases to different classes of compounds.

EXPERIMENTAL

Columns

An octadecylsilane (ODS)-modified silica (Rosil C₁₈) was obtained from Alltech (Eke, Belgium). A poly(styrene-divinylbenzene) (PS-DVB) column (Rogel) was also obtained from Alltech. Two alkyl-modified PS-DVB (ACT-1) columns were obtained from Bètron (Rotterdam, The Netherlands). Polysiloxane-coated silica columns were

obtained from Keystone Scientific (Bellefonte, PA, U.S.A.). A γ -aminopropyl-modified silica column was obtained from Chrompack (Middelburg, The Netherlands).

Totally porous graphitic carbon was provided by Professor J. H. Knox (University of Edinburgh, U.K.) and was packed into columns at the Technical University of Delft (The Netherlands). Columns packed with this material are commercially available from Shandon (Runcorn, U.K.). A column packed with porous carbon was coated *in situ* with poly(ethylene glycol) with an average molecular weight of 14 000 (Aldrich, Milwaukee, WI, U.S.A.), resulting in what we describe as a "carbonwax" column. Coating was performed by deposition from a concentrated solution in chloroform, followed by slow evaporation of the solvent. After coating, a very high pressure drop over the column was observed. On opening the column, a considerable amount of coated stationary phase (about 1 cm in length, *i.e.*, about 10% of the content of the column) was pressed out of the column ("toothpaste effect"). Remarkably, after reclosure an efficient and, under SFC conditions, very stable column remained. According to the supplier of commercial carbon materials (Shandon), the toothpaste problem could occur with "older" batches of carbon. In recent carbon packings the porosity has been reduced, so that the toothpaste problem will no longer occur.

Several additional chemically modified silicas⁹ have been evaluated previously, but none of these was thought to be sufficiently good (homogeneous) to be included in this study. All columns were 150 mm \times 4.6 mm I.D., except the carbon columns, which had a length of 100 mm. The instrumentation used for packed-column SFC has been described in detail elsewhere^{12,13}.

Quantitative study

The quantitative study was performed on ODS, Deltabond-C₁ and -C₈, Carbowax-modified carbon ("carbonwax") and γ -aminopropyl-modified silica columns. At a constant temperature of 50°C the pressure was adapted so that the capacity factor of *sec.*-butylbenzene relative to dichloromethane was approximately 0.5. In each instance, the pressure drop over the column was adjusted to about 10% of the inlet pressure. The resulting pressures are listed in Table I.

TABLE I
SUMMARY OF THE DIFFERENT COLUMNS USED IN THE QUANTITATIVE STUDY

All columns were used at 50°C. The inlet pressures listed are such that k (eqn. 1) for *sec.*-butylbenzene was *ca.* 0.5. $\bar{\rho}$ is the average density of carbon dioxide in the column, V_c is the volume of the empty column and V_{DM} is the retention volume of dichloromethane; ϵ_{DM} is the ratio of the two volumes.

Stationary phase	P_{in} (bar)	P_{out} (bar)	$\bar{\rho}$ (g/ml)	V_c (ml)	V_{DM} (ml)	ϵ_{DM}
ODS	142	128	0.654	2.49	1.76	0.71
Deltabond-C ₈	123	111	0.555	2.49	2.75	1.08
Deltabond-C ₁	117	106.5	0.515	2.49	3.07	1.22
Carbonwax	145	131.5	0.666	1.66	1.93	1.16
Amino	117	106.8	0.517	2.49	3.51	1.41

The test solutes used are listed in Table II. The first compound listed is dichloromethane (No. 1), which was used both as a solute and as the solvent for the other 25 test solutes (Nos. 2–26). Solute were obtained from various sources. All solutes were of such purity as to show only a single major peak in the chromatogram. Only solutes that could be observed with a UV detector were included.

Capacity factors were calculated assuming dichloromethane to be unretained, *i.e.*,

$$k_{i, \text{DM}} = \frac{t_i - t_{\text{DM}}}{t_{\text{DM}}} \quad (1)$$

where $k_{i, \text{DM}}$ is the capacity factor of solute i with respect to dichloromethane (DM), t_i is the retention time of the solute and t_{DM} that of dichloromethane. The consequences of using eqn. 1 will be considered below.

Retention of dichloromethane

The retention volume can be calculated by measuring the flow-rate leaving the system (*i.e.*, at a pressure of 1 bar) and by calculating the average density of the mobile phase in the column. The retention volumes of dichloromethane on the five different columns are given in Table I where they are compared with the volume of the empty column. The column porosity, ε , can be defined as

$$\varepsilon = V_0/V_c \quad (2)$$

where V_0 is the elution volume of an unretained component and V_c is the volume of the empty column. Reasonable values for ε for columns packed with porous particles are in the range 0.6–0.8. Assuming that dichloromethane is an unretained component, we can calculate the porosity from

$$\varepsilon_{\text{DM}} = V_{\text{DM}}/V_c \quad (3)$$

The values obtained for ε_{DM} are also listed in Table I. It appears from these values that dichloromethane does not behave as an unretained solute on all columns. It appears to be approximately unretained on the ODS column, but on both polysiloxane (Deltabond) columns, on the amino column and on the carbonwax column it is significantly retained. Moreover, the retention of a component such as dichloromethane is a strong function of the operating conditions. This is illustrated in Fig. 1, which shows the variation of the "true" capacity factor of dichloromethane *vs.* the column inlet pressure on an ODS column at 45°C. These "true" capacity factors were calculated using a column porosity of 0.578, which was obtained by the gravimetric method¹⁴. It is seen that a typical sigmoidal curve is obtained for retention *vs.* pressure in SFC, similar to what is observed for other solutes¹⁵. Clearly, even on the ODS column dichloromethane is *not* an unretained solute. At inlet pressures of *ca.* 150 bar or higher it is approximately unretained (at 45°C; see Table I), but at lower pressures its retention increases. The other columns all show much higher ε values (*i.e.*, relative retention volumes) than the ODS column, indicating that dichloromethane is more retained.

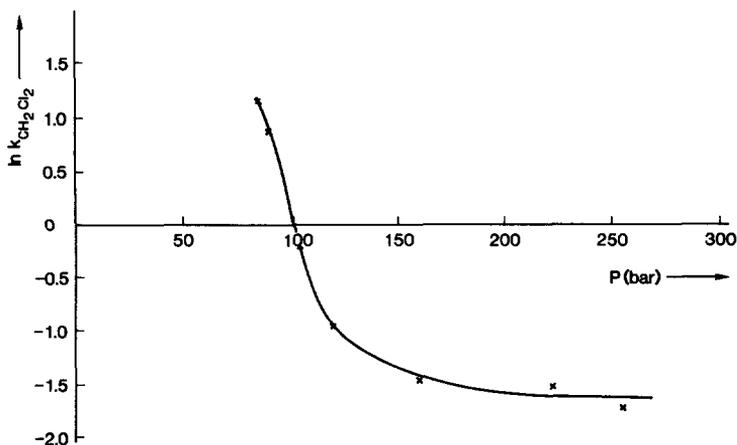


Fig. 1. Variation of the capacity factor of dichloromethane with pressure on the ODS column. Conditions: temperature, 45°C; $P_{out} = 0.9 P_{in}$.

With the unmodified carbon column, the retention is probably due to adsorption at the surface of the stationary phase. With the polysiloxane-type and poly(ethylene glycol) phases, retention is more likely to be the result of penetration of dichloromethane into the polymeric layer, where it may be retained. Both effects may play a role on PS-DVB-type columns, on which dichloromethane was also found to be significantly retained.

Despite the observation that dichloromethane is not an unretained solute, it was used as such for the present study for several reasons: it is difficult to use the gravimetric method, and for some columns, specifically the carbonwax column, it is almost impossible, because the column will change on flushing with a liquid solvent; the use of a supposedly unretained solute such as dichloromethane is a practical method for establishing a reasonable value; and provided that data on the actual retention volume of dichloromethane are available (as they are in Table I), the capacity factors presented in this paper can easily be recalculated using a different value for V_0 or ϵ by using the following equations:

$$k_i = (k_{i, DM} + 1)(V_{DM}/V_0) - 1 \quad (4)$$

or

$$k_i = (k_{i, DM} + 1)(\epsilon_{DM}/\epsilon) - 1 \quad (5)$$

The main problem is that the capacity factors reported here and in many other papers are not the real thermodynamic values, defined by

$$k = q_s/q_m \quad (6)$$

where q_s and q_m are the total amounts of solutes present in the stationary and mobile phase, respectively. In obtaining thermodynamic data from SFC experiments and, indeed, from LC experiments, considerable care must be taken to obtain correct values for V_0 .

RESULTS AND DISCUSSION

Qualitative evaluation of columns

Several of the columns were discarded from the quantitative study after an initial qualitative evaluation, in which the approximate efficiency and the peak shapes for some simple test solutes (alkylbenzenes, naphthalene and biphenyl) were considered, in addition to the column retentivity and its stability during several days or weeks of operation.

Unmodified graphitic carbon column. This column generally yielded peaks with better symmetry than, for example, the ODS column. However, this was accompanied by excessively long retention times for many solutes. For example, even at 35°C, where the densities are higher than at the temperature of 50°C used in this study, and even when the pressure was increased to 250 bar the capacity factor of *n*-butylbenzene was still above 3. Under these conditions, naphthalene could not be eluted. Because it appeared to us that the use of the unmodified carbon column in SFC would be restricted to solutes readily amenable to GC, we excluded this column from the quantitative study.

Alkyl-modified PS-DVB column. After carefully conditioning an ACT-1 column in the SFC instrument, the results were disappointing. Broad, asymmetric and even split peaks were observed. On opening the column, we found that the packed bed had collapsed and signs of flow channeling along the sides of the column were apparent even without magnification. Re-equilibration of the column with an organic solvent to allow its use in LC was not successful. Hence, the column degradation process appeared to be irreversible. A second column was purchased, installed and conditioned even more carefully, but the results were similar. Therefore, we concluded that this column packing material is not stable towards supercritical carbon dioxide, probably owing to considerable differences in the degree of swelling of the polymeric material in liquid solvents such as methanol and supercritical fluids such as carbon dioxide.

Unmodified PS-DVB column. The Rogel column showed a very high retentivity, similar to that observed with the unmodified carbon column. In both instances, the interaction with the surface appears to be stronger for aromatic solutes. After the problems we experienced with the ACT-1 columns, we also re-inspected the peak shapes obtained with the Rogel column. In the latter instance, the peaks were reasonably symmetrical, but some fronting could often be observed. Initially, we ascribed this to possible overloading of the column. Now, however, we are no longer convinced of the stability of this type of column under SFC conditions. Even though the problem is much less than it was for the ACT-1 columns, we decided not to include a PS-DVB-type column in the quantitative study.

Other columns. The remaining five columns could be used under reasonable conditions (see Table I) for a substantial portion of the test solutes and yielded reproducible retention data over longer periods of time. Therefore, these columns were included in the quantitative study.

Table II provides a summary of the peak shapes obtained for the 26 test solutes (including the solvent dichloromethane) on these five columns. In each instance pure carbon dioxide was used as the eluent. A distinction is made between sharp, symmetrical peaks (symbol +), significantly broadened and possibly asymmetrical

TABLE II

SUMMARY OF THE ELUTION CHARACTERISTICS OF VARIOUS TEST SOLUTES FROM DIFFERENT STATIONARY PHASES USING PURE CARBON DIOXIDE AS THE ELUENT

Temperature, 50°C. Pressure adapted to yield $k = 0.5$ for *sec.*-butylbenzene relative to dichloromethane. Sample concentrations, *ca.* 1 mg/ml for aromatic solutes, *ca.* 10 mg/ml for aliphatic solutes. Sample volume, 1 μ l. For identification of stationary phases see Experimental. + = Eluted as a sharp peak; ● = eluted as a broadened peak; - = eluted as a very broad, non-symmetrical peak; x = not eluted.

No.	Solute	ODS	Deltabond-C ₈	Deltabond-C ₁	Carbonwax	Amino
1	Dichloromethane	+	+	+	+	+
2	Benzene	+	+	+	+	+
3	Toluene	+	+	+	+	+
4	Ethylbenzene	+	+	+	+	+
5	<i>n</i> -Propylbenzene	+	+	+	+	+
6	<i>sec.</i> -Butylbenzene	+	+	+	+	+
7	Phenol	-	●	●	+	x
8	<i>p</i> -Nitrophenol	x	x	x	x	x
9	<i>o</i> -Nitrophenol	-	●	-	+	-
10	2,4-Dinitrophenol	x	-	x	x	x
11	Benzoic acid	x	x	x	x	x
12	Bromobenzene	+	+	+	+	+
13	Chlorobenzene	+	+	+	+	+
14	<i>N,N</i> -Dimethylaniline	x	x	x	+	●
15	<i>o</i> -Nitrotoluene	+	+	+	+	+
16	Benzaldehyde	-	●	●	+	x
17	Benzyl alcohol	x	x	x	+	x
18	Methyl benzoate	-	●	●	+	+
19	Nitrobenzene	+	+	+	+	+
20	Dimethyl phthalate	x	●	●	+	x
21	Naphthalene	+	+	+	●	+
22	Biphenyl	+	+	-	-	+
23	2-HEMA ^a	x	-	-	+	x
24	Diheptyl ether	+	+	+	+	+
25	2-Tridecanone	-	●	●	●	x
26	Cyclohexanone	-	-	-	● ^b	x
<i>Total score</i>						
	+	13	13	13	19	14
	●	0	6	5	3	1
	-	6	3	3	1	1
	x	7	4	5	3	10

^a 2-Hydroxyethyl methacrylate.

^b Co-elutes with solvent (dichloromethane).

peaks (●) and very broad, non-symmetrical peaks (-). Components that are not eluted with a capacity factor of 30 or less are indicated by x. The boundaries between "good", "reasonable" and "bad" peak shapes are necessarily vague. When the peak shapes are "good", the combination of the stationary phase and carbon dioxide as the mobile phase can be applied successfully for the solute concerned. This may still be the case in the "reasonable" situations, although in this instance the retentions (capacity factors) are likely to be affected by the amount of solute injected⁹. With really "bad" peak shapes or non-eluted solutes, we consider that the stationary phase cannot be

applied for these compounds with pure carbon dioxide as the mobile phase. Of course, the results in Table II represent only one set of pressure-temperature conditions for each column. However, it is our experience that the quality of the observed peaks is not a strong function of pressure and temperature. Also, because all the test solutes are of low molecular weight, very long retention times are probably the result of a particularly strong interaction.

The ODS column yields good results for 13 out of the 26 solutes, including hydrocarbons, halogenated hydrocarbons, nitro-substituted hydrocarbons and di-*n*-heptyl ether. Based on previous work³, we can add cyano-substituted hydrocarbons to this short list. The good peak shape observed for di-*n*-heptyl ether does not necessarily imply good results for other ethers. For example, in methoxy or ethoxy groups the oxygen atom may be much more accessible for interactions³. The ODS column cannot be applied to any of the other test solutes without modifying the mobile phase.

The Deltabond-C₈ and -C₁ columns yield essentially identical results in terms of peak shapes for 24 out of 26 solutes. Only for *p*-nitrophenol and 2,4-dinitrophenol does the Deltabond-C₈ column perform better. This is in contrast to the substantial improvement achieved when a silica phase is chemically reacted with long-chain silanes (such as octyl or octadecyl) compared with the use of trimethylsilyl-modified silicas⁹. Apparently, long alkyl chains connected to a polysiloxane backbone, which may shield the Si-O-Si bonds, are less beneficial than long alkyl chains connected to the silica, which may shield surface silanols.

The Deltabond columns may be used to elute some, but not all, of the phenols, esters, aldehydes and ketones. However, in all instances the observed peak shape and efficiency are substantially worse than what is achieved with, for example, hydrocarbons. In other words, there seems to be some degree of improvement relative to the ODS column, but the nature of the problem seems to remain the same. According to the supplier of the columns, the amount of stationary phase per square metre of silica surface is about three times higher than that on a typical ODS column. This would imply that only a very thin layer of polysiloxane is coated on the surface. This may cause surface silanols to play a smaller, but still significant, part in the retention mechanism. A thicker layer may result in excessively long retention times, but this problem can be avoided by starting with a parent silica with a lower specific surface area.

That a much thicker layer of a polymeric coating on a solid adsorbent is not necessarily detrimental to the column performance is demonstrated by the results obtained with the carbonwax column. The exact amount of polymer deposited on the particles is unknown, but is believed to be considerable. This column yielded very good results for 19 out of 26 solutes. Only *p*-nitrophenol, 2,4-dinitrophenol and benzoic acid could not be eluted. The polar (Carbowax) character of the stationary phase makes it useful for the elution of many polar solutes, including solutes such as *o*-nitrophenol, *N,N*-dimethylaniline, benzyl alcohol, dimethyl phthalate and 2-hydroxyethyl methacrylate, none of which could be handled successfully with any of the other columns. However, there is still evidence of the carbon character, because solutes with two aromatic rings, such as naphthalene and biphenyl, show very long retention times and asymmetric peaks, which may be indicative of a mixed retention mechanism, involving both the polymeric coating and the underlying surface. This may also be the reason for the broad peaks observed for ketones. Because the coating process has not been

optimized, the coating may not be uniform throughout the column. Therefore, it may be possible to improve the performance of this type of column.

Carbowax has been used extensively as a stationary phase in SFC, its use dating back to the early work of Sie and Rijnders¹⁶ and Rijnders¹⁷. However, the stationary phase may dissolve in the supercritical mobile phase¹⁸, leading to poor reproducibility and ageing of columns. Because of the very high retentivity of unmodified porous graphitic carbon, it may be a much better surface to retain a deposited layer of a polymeric material, without any cross-linking or chemical bonding to the surface. At least in this study, excellent stability of the carbonwax column was observed during several weeks of operation.

In our study, the γ -aminopropyl-modified silica did not perform well. Only for methyl benzoate and N,N-dimethylaniline did it perform better than the ODS and Deltabond columns. For all other solutes it appeared to behave similarly or worse. At first sight this seems to be in contrast with the results of Ashraf-Khorassani and Taylor⁵, who studied the applicability of a number of stationary phases for the elution of basic components and found amino-modified silicas to perform better than octadecyl-modified silicas. However, basic components were not strongly represented in our set of test solutes, so that our results do not contradict those of ref. 5. It is interesting that for the most basic solute in our test set, dimethylaniline, the carbonwax rather than the amino column yielded the best results.

Quantitative study

Table III summarizes the results of the quantitative study of the retention of our set of test compounds on the five selected columns. Values obtained from poorly shaped peaks are given in parentheses. In these cases, the capacity factor is expected to decrease with increasing amount of sample⁹ and hence the value found with one particular injection is not really meaningful. In some instances, a peak could only be observed if the sample size was increased. This was not done to enhance the detectability, but rather to reduce the retention and to sharpen the peak. When larger amounts of solutes were injected, this is indicated in the table.

There appears to be little difference in the selectivities of the stationary phases towards alkylbenzenes (methylene-group selectivity). Only for benzene do the values differ considerably, but the capacity factors are very small for this solute. As all values for benzene are larger than zero, it is (even) less suitable than dichloromethane as an "unretained" solute. The strangely high capacity factor for propylbenzene was reproducible, but could not be explained.

For biphenyl and naphthalene the carbonwax column showed large capacity factors (and poor peak shapes). The amino column also showed a relatively large retention for these solutes. For most of the other solutes, the ODS and Deltabond columns showed few differences, while the carbonwax and amino columns yielded much higher capacity factors for a number of polar solutes. These selectivity differences will be investigated in more detail in the next section.

Stationary phase selectivity

Fig. 2 shows a comparison of the capacity factors obtained on the ODS and Deltabond-C₈ columns. The logarithms of the capacity factors obtained on the different columns are plotted on the horizontal and vertical axes. If all points were to

TABLE III

CAPACITY FACTORS OF THE TEST SOLUTES ON THE FIVE DIFFERENT STATIONARY PHASES

Values obtained from distorted peaks are given in parentheses. Conditions as in Table II.

No.	Solute	ODS	Deltabond-C ₈	Deltabond-C ₁	Carbonwax	Amino
1	Dichloromethane	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
2	Benzene	0.16	0.14	0.12	0.06	0.08
3	Toluene	0.26	0.23	0.20	0.24	0.20
4	Ethylbenzene	0.32	0.32	0.30	0.34	0.30
5	<i>n</i> -Propylbenzene	0.44	0.42	0.40	0.52	0.41
6	<i>sec.</i> -Butylbenzene	0.46 ^b	0.49 ^b	0.48 ^b	0.49 ^b	0.46 ^b
7	Phenol	(3.88) ^c	(1.02)	(1.43)	6.84	—
8	<i>p</i> -Nitrophenol	—	—	—	—	—
9	<i>o</i> -Nitrophenol	(3.15) ^c	(0.70)	(0.92)	4.50	—
10	2,4-Dinitrophenol	—	(1.34)	—	—	—
11	Benzoic acid	—	—	—	—	—
12	Bromobenzene	0.56	0.52	0.49	0.94	0.57
13	Chlorobenzene	0.38	0.36	0.34	0.52	0.38
14	<i>N,N</i> -Dimethylaniline	(2.55) ^c	—	—	2.63	9.41
15	<i>o</i> -Nitrotoluene	0.72	0.68	0.81	3.06	4.13
16	Benzaldehyde	(2.53)	(0.74)	(0.78)	1.24	—
17	Benzyl alcohol	(19.9) ^c	—	—	2.68	—
18	Methyl benzoate	(1.85)	(0.82)	(0.83)	2.45	6.26
19	Nitrobenzene	0.71	0.62	0.78	2.46	4.46
20	Dimethyl phthalate	(7.85) ^c	(3.28)	(2.79)	6.82	—
21	Naphthalene	1.24	1.16	0.70	(9.90)	2.06
22	Biphenyl	1.59	1.58	1.40	(15.29)	3.18
23	2-HEMA ^d	—	(5.06)	(3.85) ^c	0.99	—
24	Diheptyl ether	0.82	0.78	0.71	4.33	0.58
25	2-Tridecanone	(3.17) ^c	(1.81) ^c	(1.68) ^c	(4.48) ^c	—
26	Cyclohexanone	(3.69) ^c	(1.28) ^c	(1.00)	0.08	—

^a By definition.^b Normalized value achieved by varying the pressure.^c Obtained with a 10-fold increased sample concentration.^d 2-Hydroxyethyl methacrylate.

fall on a straight line with unit slope, such as that drawn, this would indicate that there are no differences in selectivity. A straight line with a different slope indicates that the selectivity is systematically higher for all solutes on one of the phases, but that the order of elution will never change. Deviations from the straight line illustrate differences in selectivity for certain compounds and possible variations in elution order.

In Fig. 2 only four solutes, indicated by the half-black squares, deviate significantly from the straight line. For all four compounds, benzaldehyde, methyl benzoate, tridecanone and cyclohexanone, the peak shape is very bad on the ODS column. For these solutes, retention will decrease with increasing amount of sample injected, so that the points will approach the straight line. This is illustrated for two solutes in Fig. 3. The type of curves obtained in these figures has been interpreted in terms of a mixed retention mechanism⁹. In this model, the capacity factor obtained for large amounts of sample injected is thought to be due to interactions between the solute molecules and the C₁₈ chains on the surface. The rise in capacity factors observed with

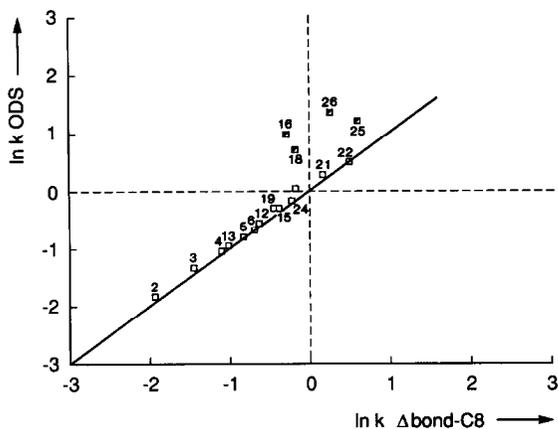


Fig. 2. Comparison of the retention of the test solutes on the ODS and Deltabond-C₈ phases (Δ bond-C8). \square = (Reasonably) good peak shapes on both columns; \blacksquare = bad peak shape on the column indicated on the vertical axis. For conditions see Tables I and II.

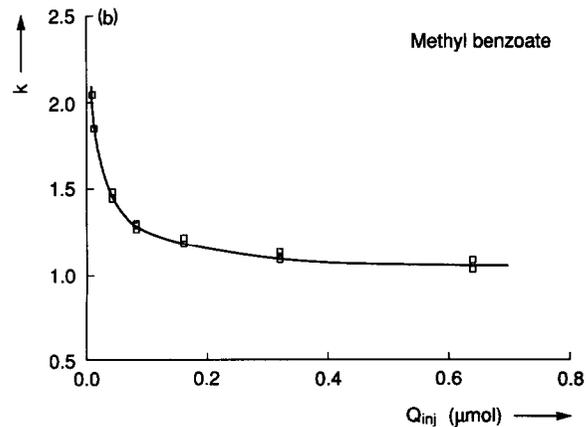
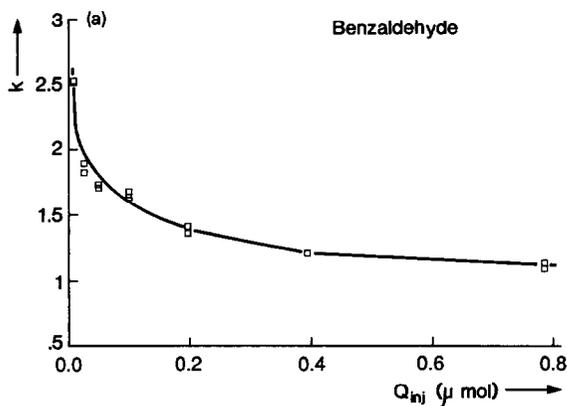


Fig. 3. Variation of the capacity factor for (a) benzaldehyde and (b) methyl benzoate on the ODS column as a function of the injected amount of solute. For conditions see Tables I and II.

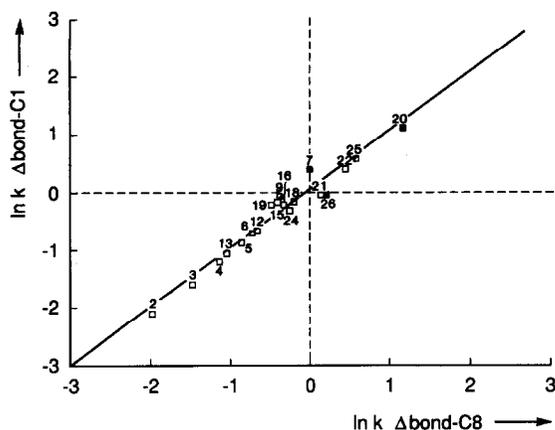


Fig. 4. Comparison of the retention of the test solutes on the Deltabond-C₁ and -C₈ phases. ◻ = Bad peak shape on the column indicated on the horizontal axis; ◼ = bad peak shapes on both columns. Other symbols and conditions as in Fig. 2.

decreasing sample size is thought to be caused by interactions between the solute molecules and residual silanols at the surface. The capacity factors obtained for large injected amounts can be seen to be approximately 1.1 for benzaldehyde, and 1.0 for methyl benzoate. These values fall very close to the straight line around which all sharp peaks are located. This suggests that any difference in selectivity observed between the ODS and Deltabond-C₈ columns are due to silanol group effects, which are undesirable, because of their detrimental influence on the peak shapes.

In Fig. 4 the retentions on the two Deltabond columns are compared. All the points are approximately situated on a straight line, so that there are hardly any selectivity differences between the two "different" phases. Considering the similar behaviour of the two columns in both qualitative (Table II) and quantitative senses (Fig. 4), one of these columns is likely to be sufficient. Also, the selectivity is identical with that of ODS columns, unless surface silanols play a role. This would cause

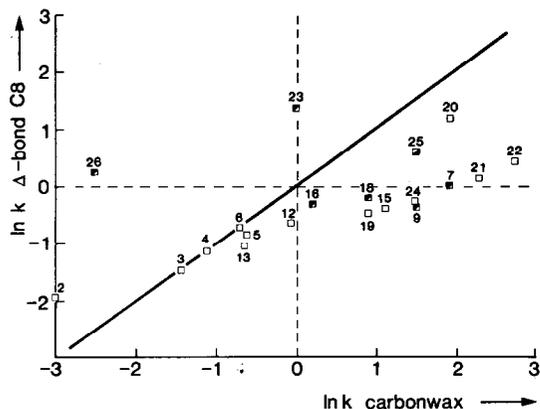


Fig. 5. Comparison of the retention of the test solutes on the carbonwax and Deltabond-C₈ phases. Symbols and conditions as in Figs. 2 and 4.

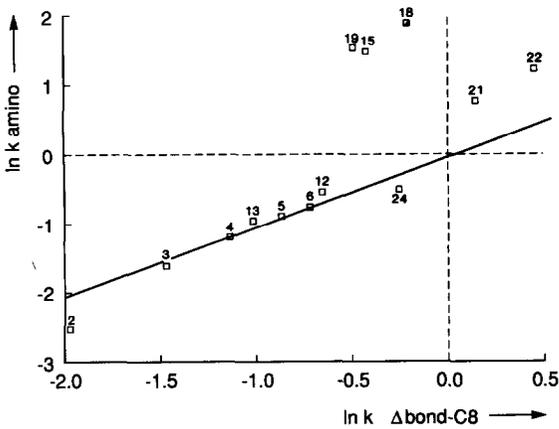


Fig. 6. Comparison of the retention of the test solutes on the amino and Deltabond-C₈ phases. Symbols and conditions as in Figs. 2 and 4.

increased retention on ODS columns (and hence selectivity differences), but also poor peak shapes and efficiencies. This will usually be too high a price to pay for an increase in selectivity.

Figs. 5 and 6 illustrate that the carbonwax and amino columns do show significant differences in selectivity for certain solutes in comparison with the non-polar phase. All solutes that can be connected by a line with a negative slope show a change in the elution order on the two different phases. It is seen that some dramatic changes are possible for these phases. In Fig. 7 the two polar phases are mutually compared.

Fig. 8 illustrates the differences in selectivity that may result from the application of different stationary phases in packed-column SFC. In this example, the selectivity for the three compounds [nitrobenzene (19), naphthalene (21) and di-*n*-heptyl ether (24)] was different on the Deltabond-C₈ (Fig. 8a) and carbonwax columns (Fig. 8b),

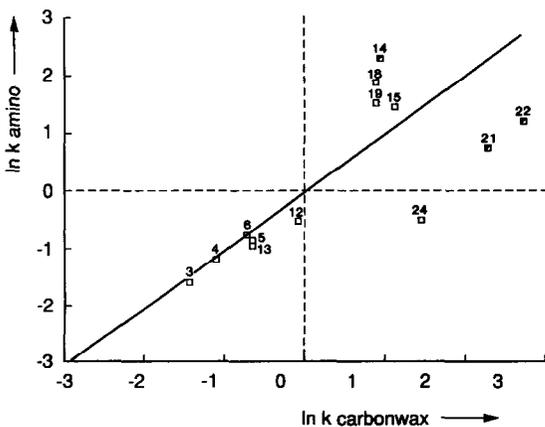


Fig. 7. Comparison of the retention of the test solutes on the carbonwax and amino phases. Symbols and conditions as in Figs. 2 and 4.

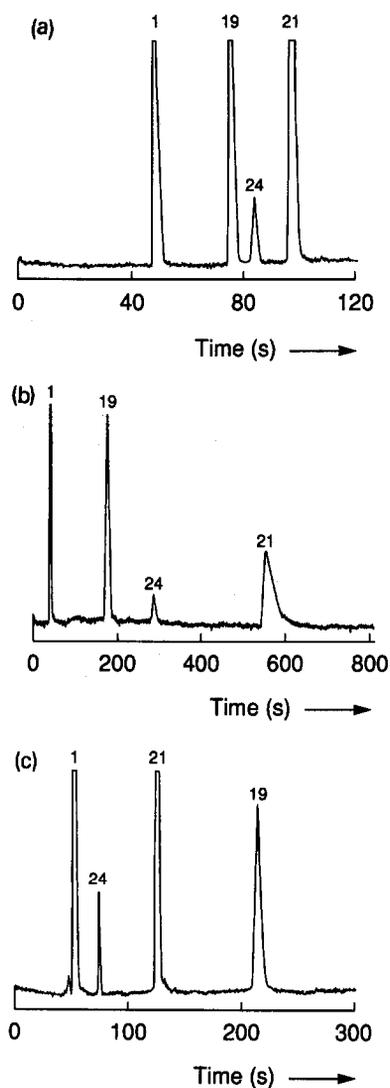


Fig. 8. Illustration of the possible differences in stationary phase selectivity in packed-column SFC. Solutes, dichloromethane (solvent, 1), nitrobenzene (19), naphthalene (21) and di-*n*-heptyl ether (24). Stationary phases: (a) Deltabond-C₈; (b) carbonwax; (c) amino. Conditions as in Fig. 2.

but the elution order was identical. On the amino column, even the elution order was different (Fig. 8c). It should be noted that only solutes that show reasonable or good peak shapes are included in this example. Fig. 8 serves to illustrate the differences in selectivity between the different columns, rather than their applicability. For the latter, Table II provides a better indication.

CONCLUSIONS

There is a requirement in SFC for stationary phases from which as many different (classes of) solutes as possible can be eluted as sharp, symmetrical peaks, using unmodified carbon dioxide as the mobile phase. Moreover, several such phases with different selectivities will be required. Octadecyl-modified silica (ODS) columns are applicable to non-polar solutes, such as hydrocarbons, but only to a very small number of polar solutes. Polysiloxane-coated silica (Deltabond) columns perform better than ODS for certain classes of solutes. However, for many polar solutes peak shapes and efficiencies still leave to be desired.

A very stable column was prepared by coating poly(ethylene glycol) on porous graphitic carbon. The resulting "carbonwax" column showed the best results for polar solutes of all columns tested. Improvements may be possible by optimizing the coating process and probably by packing the column after depositing the stationary phase on the particles.

A γ -aminopropyl-modified silica phase did not perform much better than an ODS column for our set of test solutes, in which few basic solutes were present. ODS, Deltabond-C₁ and -C₈ columns showed virtually no differences in selectivity. Significant selectivity differences were observed between these three non-polar columns and the "carbonwax" and amino columns, and between the two polar columns mutually.

The results indicate that by preparing homogeneous stationary phases, the number of solutes that can be eluted with SFC using pure carbon dioxide as the mobile phase can be greatly increased. A promising method for preparing phases is to coat relatively thick layers of a polymeric material on solid particles. To avoid excessive retention times, the specific surface area of the particles may be reduced. When coating poly(ethylene glycol) with an average molecular weight of 14 000 on porous graphitic carbon, no cross-linking or chemical bonding turned out to be necessary.

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REFERENCES

- 1 P. J. Schoenmakers and L. G. M. Uunk, *Adv. Chromatogr.*, 30 (1989) 1-80.
- 2 P. J. Schoenmakers and L. G. M. Uunk, *Eur. Chromatogr. News*, 1 (1987) 14.
- 3 P. J. Schoenmakers, F. C. C. J. G. Verhoeven and H. M. van den Bogaert, *J. Chromatogr.*, 371 (1986) 121.
- 4 T. Greibrokk, B. E. Berg, A. L. Blilie, J. Doehl, A. Farbrot and E. Lundanes, *J. Chromatogr.*, 394 (1987) 429.
- 5 M. Ashraf-Khorassani and L. T. Taylor, in C. M. White (Editor), *Modern Supercritical-Fluid Chromatography*, Hüthig, Heidelberg, 1988, pp. 115-134.
- 6 J. C. Keui, K. E. Markides and M. L. Lee, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 257.
- 7 J. G. M. Janssen, P. J. Schoenmakers and C. A. Cramers, in P. Sandra (Editor), *Proceedings of the 10th International Symposium on Capillary Chromatography (Riva del Garda)*, Hüthig, Heidelberg, 1989, p. 1383.

- 8 J. G. M. Janssen, P. J. Schoenmakers and C. A. Cramers, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 12 (1989) 645.
- 9 P. J. Schoenmakers, L. G. M. Uunk and P. K. de Bokx, *J. Chromatogr.*, 459 (1988) 201.
- 10 C. R. Yonker and R. D. Smith, *J. Chromatogr.*, 361 (1986) 246.
- 11 S. M. Fields, K. E. Markides and M. L. Lee, *J. Chromatogr.*, 406 (1987) 223.
- 12 P. J. Schoenmakers and F. C. C. J. G. Verhoeven, *Trends Anal. Chem.*, 6 (1987) 10.
- 13 P. J. Schoenmakers and F. C. C. J. G. Verhoeven, *J. Chromatogr.*, 352 (1986) 315.
- 14 G. E. Berendsen, P. J. Schoenmakers, L. de Galan, G. Vigh, Z. Varga-Puchony and J. Inczedy, *J. Liq. Chromatogr.*, 3 (1981) 13.
- 15 P. J. Schoenmakers, *J. Chromatogr.*, 315 (1984) 1.
- 16 S. T. Sie and G. W. A. Rijnders, *Anal. Chim. Acta*, 38 (1967) 31.
- 17 G. W. A. Rijnders, *Chem.-Ing.-Tech.*, 49 (1970) 890.
- 18 J. J. Czubryt, M. N. Myers and J. C. Giddings, *J. Phys. Chem.*, 74 (1970) 4260.