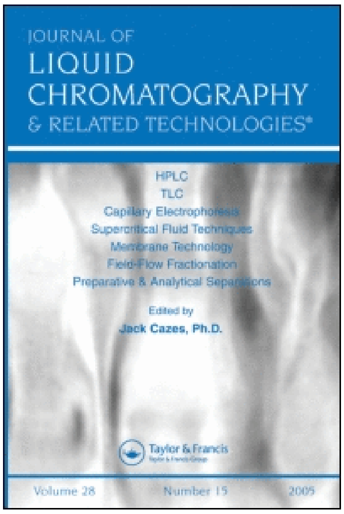

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A COMPARISON OF THE RETENTION OF HOMOLOGOUS SERIES AND OTHER TEST SOLUTES ON AN ODS COLUMN AND A HYPERCARB CARBON COLUMN

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ABSTRACT

When compared with an ODS column having good surface coverage and operated with pure methanole eluent, the Hypercarb column exhibits similarities in some respects and dissimilarities in others. Methylene homologues separations look similar on ODS and on Hypercarb. Their ln k' increase linearly with carbon number, although much steeper on Hypercarb. The free sorption enthalpy of methylene groups is about three times higher on the carbon column. The apparent phase ratio is lower with Hypercarb by a factor of about 100. Consequently, the retention of smaller size solutes is lower than on ODS, and the sample load capacity is smaller, too. n-Alkanes and other long chain alkyl compounds exhibit an unusual behavior on Hypercarb. With increasing sample size, their peaks show a strongly fronting profile, possibly indicating a change in retention mechanism, and the peak maximum shifts to longer times. Steric exclusion effects could not be detected with Hypercarb. Selectivity with respect to various substituents is entirely different from that on ODS, most probably due to some adsorption type retention mechanism on Hypercarb. With diluted samples, Hypercarb can yield separations with very high selectivities and excellent peak shape.

INTRODUCTION

Many samples must be separated with water- free eluents. If homologues have to be analyzed, a RP system is almost unavoidable, but, among the more readily available eluents, methanole sets the limit for polarity and, in most cases, selectivity. Since Hypercarb is a special variety of graphitized pure carbon, it was assumed to be comparable to ODS as far as its response to solute solvophobicity is concerned but, possibly, might have better separation power. The objective of this work was to compare the separation of various homologous series and other test solutes having characteristic properties on a densely grafted ODS phase with that obtained on Hypercarb using purely methanolic eluent. Furthermore, it shall be tried to describe the separation characteristics of this relatively new phase in somewhat more general terms.

EXPERIMENTAL

The apparatus for one set of data was a modified Varian 5000 pump with RI and UV detectors from Knauer (Berlin). The data system was Trio. The injector was a forced air driven Rheodyne valve with a 7.8 μl loop. Eluent flow was 1ml / min 100% MeOH.

Columns were : 1. Inertsil ODS II, 5 μm, 125x4.6 mm, #901.026 from VDS optilab.

2. Hypercarb , 7 μm, 100x4.7 mm, # B 0176 from Shandon

Samples contained 20 μl of each solute in 5 ml MeOH, unless otherwise indicated. The extra column dead volume V_{extra}= 0.051 ml was accounted for. The column dead volume V_m was calculated for both columns according to the (n+1)/n - method from n-alkane rention data [1-5]. For Inertsil, V_m = 1.100 ml and for Hypercarb, V_m = 1.321 ml was found. This data was used for all other solutes. Between 5 and 7 runs were performed with each sample, the average was used for calculations. This refers to experiments performed at room temperature (24±1°C) using the Varian 5000 pump.

In a second set of experiments, the somewhat more precise Varian 8500 pump was used, the column was water-thermostated at 35° ± 0.1°C unless otherwise stated, the injector was replaced by a compressed helium driven Valco C6W valve (20 msec switchung time), and the data system was a CR3A (Shimadzu). In this set, preferably the Hypercarb column was investigated. With these presumably more precise data, a column dead volume of Vm = 1.264 ml at 35°C was found as the average over the whole range from C_6 to C_{15}.

Eluent methanole (MeOH) was HPLC grade from Riedel -deHaen, water was taken from a Millipore-Waters purification set. Solute chemicals were obtained from Aldrich.

RESULTS AND DISCUSSION

Experiments at 24°C

Homologous Series

Retention times were converted into log capacity factors ln k′ using the above dead volumes. On both columns, a plot of ln k' over carbon number yielded reasonably good straight lines, as shown for n-alkanes in figure 1 and for the 1-chloro-alkanes in figure 2.

For the determination of parameters a and b of the ln k' equation,

$$(1)\quad \ln k' = \underline{a} + \underline{b} \cdot n_C$$

data from low series members having extremely small retention was omitted. The parameters are summarized in table 1. Current experiments using a refined apparatus suggest that deviations from linearity of small solutes result from insufficient data precision and the use of one single V_m for all solutes.

Slope b on Inertsil shows that this phase is densely grafted and very solvophobic. Many of the more common ODS columns have logarithmic methylene selectivities b on the order of 0.16 to 0.17 for n-alkanes in the same eluent/solutes system, indicating a somewhat lower solvophobicity. The apparent free sorption enthalpy was calculated from the b value to be -0.5 kJ/mole per CH_2-group (24°C). This figure comprises both the real free enthalpy change and a change of effective phase ratio caused by a decrease of the actually available pore volume [6-8]. The phase ratio of the Inertsil column was calculated as

$$(2)\quad \ln \Phi = \underline{a} - 1.12 \cdot \underline{b} = -1.61$$

or $\Phi = 0.2$. Similar values are quite common for ODS/-MeOH - systems [8]. From $\Phi = 0.2$ and the column dead volume, the volume of the stationary phase is calculated to be $V_{stat} = 220\ \mu l$.

The b value for Hypercarb is unusually high. The free sorption enthalpy of -1.5 kJ/mole per CH_2 group indicates a different sorption mechanism. A tentative

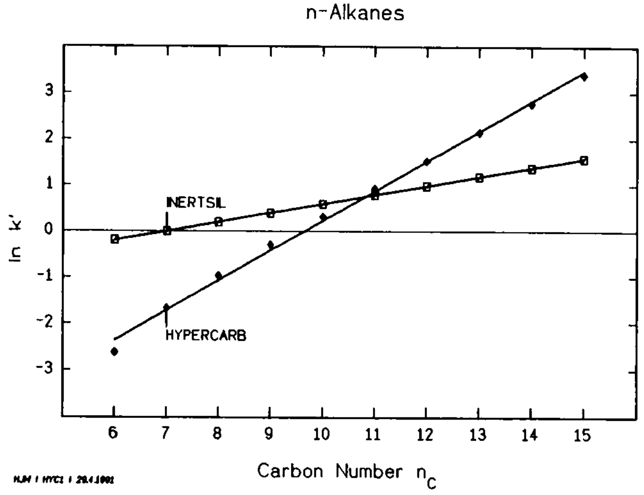

FIGURE 1
Ln k' of n-alkanes on Hypercarb and on Inertsil at 24°C with pure MeOH eluen

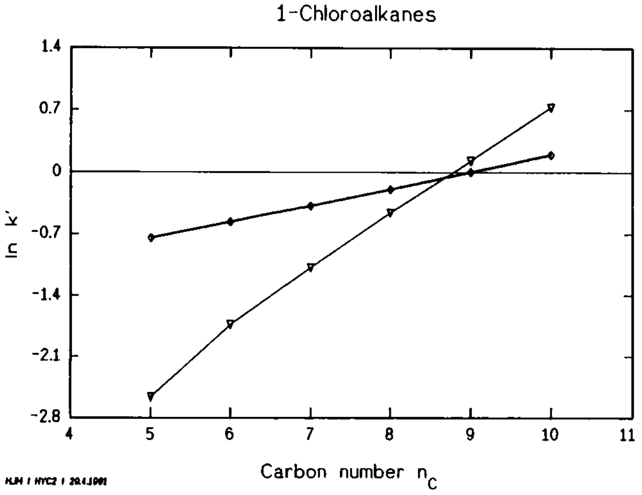

FIGURE 2
Ln k' of 1-chloro-alkanes on Hypercarb and on Inertsil at 24°C with pure MeOH eluent

TABLE 1A
INERTSIL
Retention of Homologous Series on Inertsil at 24°C. Solute class; parameters a and b of ln k′ equation; r= correlation coefficient; n = number of solutes in the series. Eluent 100% MeOH.

Solutes	a	b	r	n
n-Alkanes	-1.3883	0.1982	0.99999	10
Phenylalk.	-1.0594	0.1876	0.99922	9
Chloroalk.	-1.6915	0.1881	0.99987	6
Bromoalk.	-1.5897	0.1891	0.99994	6
Iodoalk.	-1.3541	0.1842	0.99987	6
Dibromoalk	-1.8493	0.1874	0.99988	7
n-Alcohols	-2.6274	0.1916	0.98750	3
Me-ketones	-2.6665	0.1893	0.99987	3
Nitriles	-2.8694	0.2120	0.99671	13

TABLE 1B
HYPERCARB
Retention of Homologous Series on Hypercarb Same conditions as table 1A.

Solutes	a	b	r	n
n-Alkanes	-5.8867	0.6186	0.99994	9
Phenylalk.	-2.8081	0.5726	0.99982	9
Chloroalk.	-5.3006	0.6122	0.99982	7
Iodoalk,	-4.3874	0.5944	0.99996	5
Dibromoalk	-4.0635	0.5810	0.99996	5
n-Alcohols	-5.9637	0.5961	0.99850	7
Me-ketones	-6.0216	0.5860	0.99999	5
Nitriles	-5.7732	0.5959	0.99968	5

phase ratio calculation similar to eq. (2) yields ln Φ = -6.58 or Φ = 0.0014. The Hypercarb phase ratio is roughly 100 times smaller than the ODS phase ratio. Using the column dead volume, the "volume of the stationary phase" is calculated to be $V_{stat} \sim 2$ µl. Obviously, this cannot be a volume in the usual sense. The attachment of solutes at the stationary phase is probably adsorptive in character. Then, V_{stat} in Φ has to be replaced by a sorption surface area, and C_s in the distribution coefficient K by a surface concentration [9].

The steeper rise of ln k′ with increasing solute size on Hypercarb as compared to Inertsil is caused not only by a more negative sorption free enthalpy. All silica based RP materials containing meso- and micropores exhibit partial exclusion effects which depend on pore and solute size [6,8]. The larger solutes are, the more is retention reduced by these effects. In order to get an estimate of the magnitude of this effect, both columns were subject to the procedure for pore size distribution determination introduced by Halász [10].

For Inertsil, with THF as eluent and polystyrene standards (molecular weight ranging from 580 to $2.95 \cdot 10^6$) plus benzene as solutes, a lognormal pore size distribution with a most abundant pore diameter of 99 Å and a standard deviation $\sigma_{pore} = 0.65$ (^{e}log) was found. Elution data ranged from 0.60 ml for the totally excluded polymer to 1.182ml for benzene. This latter figure is a little higher than V_m.

On Hypercarb, elution volumes of benzene (1.290 ± 0.015 ml) and PS $2.95 \cdot 10^6$ (1.295 ± 0.01 ml) were identical and very close to V_m. Consequently, exclusion effects cannot be shown to be important in Hypercarb, and a ln k′ decrease due to a change of the effective phase ratio as found in ODS materials does not occur. This finding does not agree with manufacturers data claiming a mean pore diameter of 250 Å as determined from BET data [11]. We tentatively suggest that exclusion effects can occur only with materials the main surface of which is located in tube-shaped pores. The main surface of Hypercarb seems to be on the outside of the carbon whiskers of the Hypercarb "porcupine" structure, which may well give rise to nitrogen condensation or mercury penetration, but still lets large molecules use the full eluent volume.

The investigated homologous series were chosen to cover a polarity range as wide as possible. Substituents were phenyl, chlorine, bromine, iodine, hydroxyl and acetyl, all in 1 position, and bromine in 1,ω positions. On Inertsil, only the three largest available members of the strongly polar series could by used since smaller ones did not have enough retention. ln k' equations based on such data are expected to be affected by larger errors.

The logarithmic methylene selectivities $\underline{b} = \ln \alpha$ are all close to 0.19 on Inertsil and to 0.59 on Hypercarb. Within a particular series, they are constant in the limits of data precision. This seems to indicate that both Inertsil/MeOH and Hypercarb/MeOH behave like typical RP-systems as far as retention of paraffinic chains is concerned.

The influence on retention of the substituent which appears in intercept $\underline{a}$, is of particular interest. In order to isolate the substituent contribution, ln k' was incremented as shown below :

(3) $\ln k' = \underline{a} + \underline{b} \cdot n_C = 2 \cdot \lambda_{exc} + n_C \cdot \lambda_2 + \ln \Phi$

{ n-alkanes }

(4) $\ln k' = \lambda_{exc} + n_C \cdot \lambda_2 + \lambda_R + \ln \Phi$

{ $CH_3-(CH_2)_{n-1} - R$ }

(5) $\ln k' = 2 \cdot \lambda_R + n_C \cdot \lambda_2 + \ln \Phi$

{ $R - (CH_2)_n - R$ }

(6) $\ln k' = \lambda_{exc} + (n_C - 1) \cdot \lambda_2 + \lambda_{CN} + \ln \Phi$

{ $CH_3-(CH_2)_{n-1}-CN$ }

These equations are only first approximations since they do not account for partial exclusion, mutual influence of molecular constituents on each other, and the generation of new dipole moments. λ_R is the ln k'-increment of the substituent, λ_{exc} accounts for the

TABLE 2
Approximate Substituent Contributions λ_R to ln k'

Group	Inertsil	Hypercarb	Difference
Phenyl	0.45	3.45	3.00
Cl-	-0.19	0.96	1.15
Br-	-0.09	1.34	1.43
J-	0.15	1.87	1.72
$(Br-)_2$	2·(-0.17)	2·1.26	2·1.43
CN-	-1.17	1.08	2.25
OH-	-1.12	0.30	1.42

excess of the methyl increment over the methylene increment λ_2, Φ is the phase ratio. For λ_R, data in table 2 has been calculated. The substituent increments in the Inertsil/MeOH system are very similar to values observed in other ODS systems. Phenyl and iodine produce retention, the other groups decrease retention, CN- and OH- being particularly effective. This can be explained by a distinct prevalence of partition effects in non-aqueous ODS systems which, in turn, are governed by solubility effects in first place.

In the Hypercarb/MeOH system, all substituents produce retention. This again suggests that it is mainly adsorption which is responsible for retention on Hypercarb. Obviously, the interaction of highly polar groups with the basically aromatic structure of Hypercarb is so strong that it overcompensates the increase of solubility in the mobile phase. If λ_R(Inertsil) is taken as a rough measure for interaction with the eluent, the difference {λ_R(Hy)- λ_R(In)} may be used to describe the adsorptive force on Hypercarb in a first approximation. It seems that polarizability plays an important rôle in the interaction with the graphitic adsorbent, because the phenyl group with its large π

TABLE 3A

Retention Volumes and Retention Indices of Linear and Branched Alkanes on Inertsil and on Hypercarb

V_{ms} = gross retention volume, I_K = retention index, abbreviations e.g. 2233tetMeButane = 2,2,3,3-Tetramethyl-Butane. Column at 24°C. Eluent 100% MeOH.

INERTSIL

Solute	n_C	V_{ms}	I_K	ΔI
23diMeButane	6	2.06	554	-46
22diMeButane	6	2.01	525	-75
223triMeButane	7	2.16	614	-86
2233tetMeButane	7	2.28	674	-26
2MePentane	6	2.08	566	-34
22diMePentane	7	2.16	614	-86
23diMePentane	7	2.23	649	-51
24diMePentane	7	2.17	618	-82
223triMePentane	8	2.35	709	-91
234triMePentane	8	2.38	720	-80
3MeHexane	7	2.27	670	-30
25diMeHexane	8	2.34	702	-98
2255tetMeHexane	10	2.48	764	-236
22diMeHexane	8	2.32	695	-105
2MeHeptane	8	2.47	757	-43
3MeHeptane	8	2.47	759	-41
4MeHeptane	8	2.46	755	-45
3MeOctane	9	2.73	855	-54
4MeOctane	9	2.70	846	-54
26diMeOctane	10	2.87	899	-101
27diMeOctane	10	2.88	903	-97
2MeNonane	10	3.04	951	-49

system and the cyano group with its triple bond produce much retention.

Branched Alkanes

Retention data for the separation of branched alkanes is given in tables 3A and 3B. The retention index is based on the n-alkane series [8, 12] as $I_K = \frac{100}{\underline{b}_{alk}} \cdot (\ln k' - \underline{a}_{alk})$. The retention index changes observed when going from the n-alkane to the branched

TABLE 3B
HYPERCARB

Solute	n_C	V_{ms}	I_K	ΔI
22diMeButane	6	1.58	568	-32
223triMeButane	7	1.52	463	-237
2233tetMeButane	7	1.51	447	-253
2MePentane	6	1.50	427	-173
22diMePentane	7	1.53	478	-122
23diMePentane	7	1.57	559	-141
24diMePentane	7	1.53	495	-205
223triMePentane	8	1.58	563	-237
234triMePentane	8	1.62	613	-187
3MeHexane	7	1.61	602	-98
25diMeHexane	8	1.61	605	-195
2255tetMeHexane	10	1.94	790	-210
22diMeHexane	8	1.60	594	-206
2MeHeptane	8	1.75	707	-93
3MeHeptane	8	1.74	702	-98
4MeHeptane	8	1.72	690	-110
3MeOctane	9	1.95	794	-106
4MeOctane	9	1.96	795	-105
26diMeOctane	10	1.98	802	-198
27diMeOctane	10	2.02	814	-185
2MeNonane	10	2.50	913	-87

alkanes having the same number of carbon atoms is shown in tables 3A,B as ΔI :

$$(7) \quad \Delta I = I_K - 100 \cdot n_C$$

On both columns, retention of branched isomers is lower than that of the n-alkanes. However, the index decrease on Hypercarb does not parallel that on the ODS column, again indicating a different mechanism. In most cases, the selectivity of Hypercarb for isomer separation is clearly superior despite the fact that retention volumes are small. Most of the ΔI on Inertsil range between -30 and -100, while on Hypercarb, all ΔI except two are between -100 and -250. There was only one solute pair hard to separate on Hypercarb with purely methanolic eluent (4-methyl and 3-methyl octane).

TABLE 4
Retention of Homologous Series on Hypercarb at 35°C

Solute class; n_{sol}, number of solutes in the series; s%, average percent standard deviation of retention times for 6 runs; parameters a and b of ln k′ equation; r, correlation coefficient.

Solutes	n_{sol}	s%	a	b	r
R-H	9	0.25	-5.5387	0.5652	0.99984
R-Cl	9	0.15	-4.8469	0.5466	0.99998
R-Br	7	0.25	-4.5430	0.5464	0.99998
R-J	7	0.10	-4.0452	0.5363	0.99994
Br-R-Br	8	0.30	-3.7183	0.5351	0.99998
R-OH	7	0.30	-5.4296	0.5467	0.99999
HO-R-OH	7	0.30	-5.4844	0.5432	0.99995
R-CN	7	0.08	-5.7900	0.5444	0.99997
R-Ph	8	0.13	-2.4969	0.5107	0.99990
R-S-R	7	0.70	-4.7673	0.5068	0.99995
R-O-R	6	0.30	-5.6736	0.5258	0.99995
Me-CO-R	8	0.25	-5.2436	0.5548	0.99996
Ph-CO-R	6	0.20	-0.7587	0.5113	0.99750
nPr-S_x-nPr	7	0.50	-2.4306	0.7002	0.99978

Experiments at 35°C

Homologous Series

This set of data is assumed to be more precise because the pump delivers a more constant flow, the injector operates faster (about 20 msec), and the column was more carefully thermostated. Data on some series is summarized in table 4.

As expected from theory, retention is lower at 35°C. Retention of 1,ω-diols which is almost negligible in the ODS/MeOH system, does not differ much from that of mono-alcohols on Hypercarb. Sulfur atoms in dipropyl polysulfide chains produce about 1.3 times as much ret-

TABLE 5
Retention of Non-Linear Solutes on Hypercarb

Solute	t_{msr}	ln k'	I_K
Cyclo-octasulfur	16.978	2.5175	1425
Thianthrene	18.830	2.6292	1445
Dibenzothiophene	8.852	1.7860	1296
Fluorene	9.631	1.8844	1313
1,4Dithiane	1.531	-2.0822	612
Benzene	1.442	-2.2920	574
Diphenyl	7.627	1.5992	1263
Naphtalene	6.252	1.3510	1219
Diphenyl methane	2.324	-0.2844	930
1,2Diphenyl ethane	6.760	1.4501	1237
2,2Diphenyl propane	2.014	-0.6790	860
1,4Diphenyl butane	11.652	2.0962	1351
Adamantane	1.545	-2.0000	626
Cyclohexane	1.426	-3.1804	417
Methyl cylohexane	1.495	-2.3417	566
1,2Dimethyl cyclohexane	1.598	-1.7278	674
1,3Dimethyl cyclohexane	1.616	-1.6506	688
1,4Dimethyl cyclohexane	1.603	-1.7057	678
1,3,5Trimethyl cyclohexane	1.821	-1.0380	796
Ethyl cyclohexane	1.577	-1.8260	657
Butyl cylohexane	2.588	-0.0396	973
Dicyclohexyl ethane	3.747	0.6305	1092
Cycloctane	1.56	-1.9132	641
Cyclodecane	2.442	-0.1675	950
Cyclododecane	12.100	2.1389	1358

ention as methylene groups, which coincides with their behavior in ODS/MeOH systems [13].

Exept for some scatter at very low values, ln k' intercepts a at 35°C and a at 24°C are linearly related. There is also a decrease of slope b of eq. 1 with rising temperature, but the correlation is not nearly as good.

Non-Linear Solutes

Table 5 shows retention data of compounds strongly deviating from a chain shape. The retention index of S_8

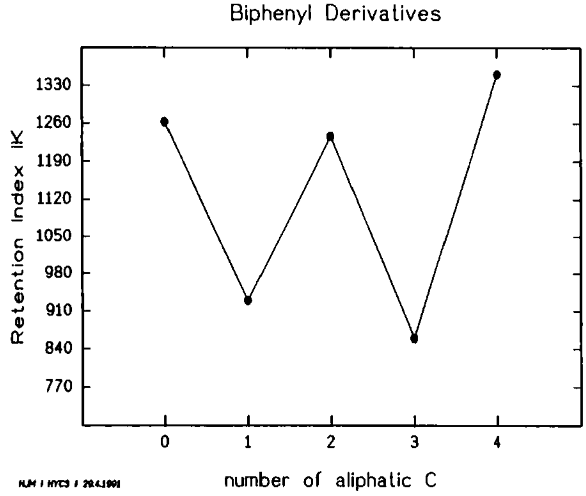

FIGURE 3
Retention index of biphenyl and diphenylalkanes on Hypercarb

(cyclo-octasulfur) is high due to the extreme solvophobicity of this dipole-free homonuclear compound. Basically the same effect is observed in ODS systems [14].

The methylene index increment in cyclo-alkanes increases non-linearly with increasing ring size to an unusually high value. It might be that the larger rings can attach more closely to the stationary phase surface.

Benzene has higher retention than cyclohexane on Hypercarb which is the opposite of what is found on ODS. The reason is almost certainly an increased interaction with the graphitic surface of the carbon phase, overriding the increased solubility in the eluent.

The effect of inserting alkyl groups between the aromatic rings of biphenyl is demonstrated in fig.3. From biphenyl to diphenylmethane there is a retention loss of over 300 index units. In an ODS system, the CH_2 group under consideration produces distinctly less retention than a CH_2 in an n-alkane, due to its fairly high acidity, but its ln k′ contribution is still positive [12]. We assume that, on Hypercarb, the shape of the diphenylmethane molecule is the deciding factor. Because of the approximately tetrahedral arrangement around the methylene carbon, only one ring can accommodate close enough to the stationary phase.

The next step to diphenylethane increases the index by ~300 units, far too much as to be attributed to a CH_2 group proper. The step to diphenylbutane finally arrives at a retention which is ~90 units above that of biphenyl itself. This seems to indicate that, with increasing alkyl chain length, the benzene rings can get closer to the stationary surface, and that the inner CH_2 groups, being less activated by the rings, can produce some retention. In the propyl compound, both rings are bonded in 2 position of the C_3 chain. Since retention is even lower than with diphenylmethane although there are two additional methyl groups, one may conclude that sterical hindrance in attaching to the stationary surface has increased.

Chlorobenzenes

Data from a comparison of retention of chloro benzenes on Hypercarb and on Inertsil is given in table 6. Data for hexachlorobenzene on Hypercarb is not available due to high retention of about 4 hours (extrapolated from ln k′).

It is seen that, in this case, selectivity for isomer separation is relatively poor on both columns. Figure 4

TABLE 6
Retention of Chlorobenzenes on Inertsil and Hypercarb

	HYPERCARB			INERTSIL		
Solute	t_{msr}	ln k'	I_K	t_{msr}	ln k'	I_K
none	1.391	-2.2920	574	1.523	-0.7769	204
1	1.732	-1.1069	784	1.590	-0.6607	272
1,3	2.626	0.0336	986	1.757	-0.3837	434
1,4	2.772	0.1430	1005	1.681	-0.5001	366
1,2	2.652	0.0568	990	1.684	-0.4957	368
1,3,5	5.481	1.1931	1191	2.113	0.0318	677
1,2,3	6.143	1.3407	1217	1.878	-0.2217	528
1,2,4,5	18.930	2.6349	1446	2.261	0.1640	754
1,2,3,4	20.512	2.7209	1461	2.180	0.0936	713
1-5	72.818	4.0359	1694	2.789	0.5301	968
1-6	-	-	-	3.606	0.9179	1195

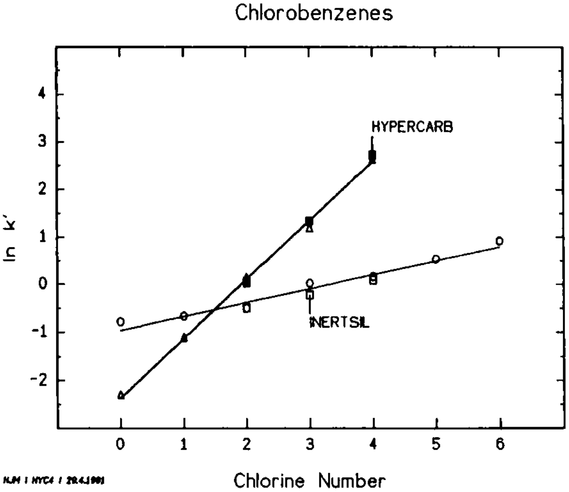

FIGURE 4
Chlorobenzenes : ln k' as function of chlorine atom number. Chlorine positions :triangles, circels: 0; 1; 1.4; 1.3.5; 1,2,4,5; 1-5; 1-6. full, open squares: 1.2; 1.2.3; 1.2.3.4. Not resolved: 1.3.

shows that ln k′ over chlorine number is roughly linear. On the average, substitution of one hydrogen by a chlorine atom increases the sorption free energy by -0.74 kJ/mole on Inertsil and -3.24 kJ/mole on Hypercarb. While the former figure mainly reflects an increase in cavity energy, the latter suggests an unusually strong interaction with the carbon phase.

It should be noted that, even on the ODS phase, aromatic chloro substitution increases retention, while substitution of aliphatic hydrogen lowers retention, as shown in table 2. This latter retention decrease has previously been observed with other aliphatic hetero substitutions and was attributed to the formation of local polar centers in the vicinity of the bond between CH_2 or CH_3 and the hetero atom [15-17].

Sample Amount and Peak Shape

Overloading a column with sample generally causes peak tailing because the sorption isotherm tends to change to a Langmuir shape. Under these circumstances, the more loosely sorbed majority of solute molecules moves to the peak front, and the peak maximum appears at retention times shorter than those observed with linear behavior at infinite dilution.

Fig. 5 shows a chromatogram of 7.8 μl of a concentrated n-alkanes solution on Inertsil. The peaks are slightly tailing which is entirely regular behaviour. The same injection on Hypercarb produces very strange peak profiles, as can be seen from fig.6. If a diluted sample is run on Hypercarb, an almost normal chromatogram with more or less tailing peaks is obtained (fig.7).

The higher concentration peak profile is characterized by strong fronting which becomes more pronounced the

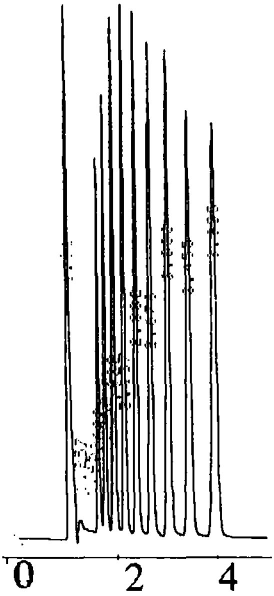

FIGURE 5
Chromatogram of n-alkanes on Inertsil
7.8 µl injected. Sample C_6 - C_{14} , 50µl each in 5 ml MeOH.

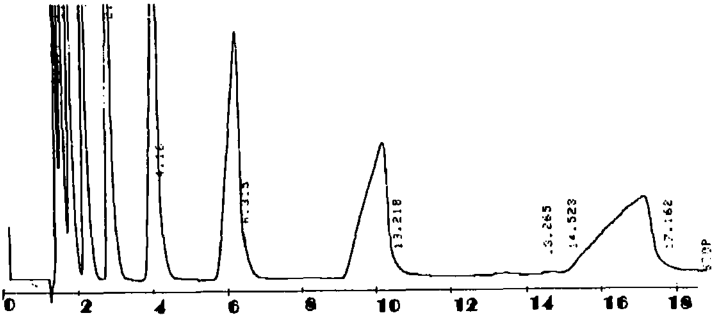

FIGURE 6
Chromatogram of n-alkanes on Hypercarb
7.8 µl injected. Sample C_6 - C_{14} , 50µl each in 5 ml MeOH.

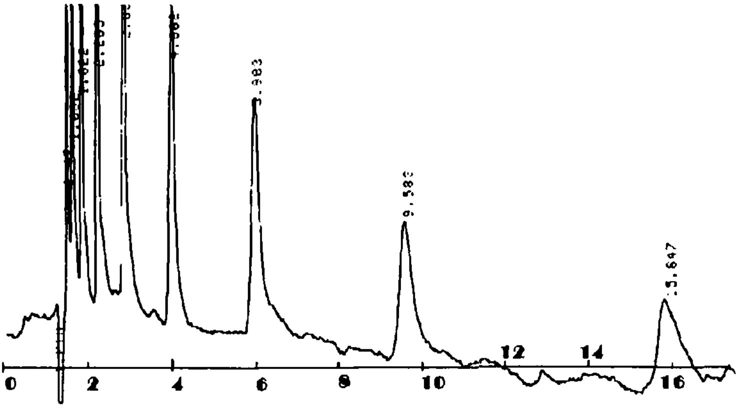

FIGURE 7
Chromatogram of n-alkanes on Hypercarb
7.8 µl injected. Sample C_6 - C_{14} , 3µl each in 5 ml MeOH.

longer the alkyl chains are. At intermediate concentrations, peaks of shorter chain solutes exhibit regular profiles with a slight tailing while peaks above C_{14} already show fronting.

It should be noted that the time scale is 2 min per tick in figs. 5 - 7. In each chromatogram, the last peak is C_{14}, which appears after 3.95 min on Inertsil, after 15.85 min on Hypercarb at low concentration, and after 17.16 min at high concentration. These times refer to the peak maximum. Basically identical results were found if smaller volumes of the more concentrated solution were injected. Retention times found with a larger or more concentrated sample are longer than with a smaller or less concentrated one. From fig.8 it is seen that the retention difference increases with increasing carbon number in the alkane from practically zero at C_6 to as much as 1 min at C_{14}. This effect indicates that the methylene selectivity slowly increases with increasing sample amount. Indeed, slope $\underline{b}$ of

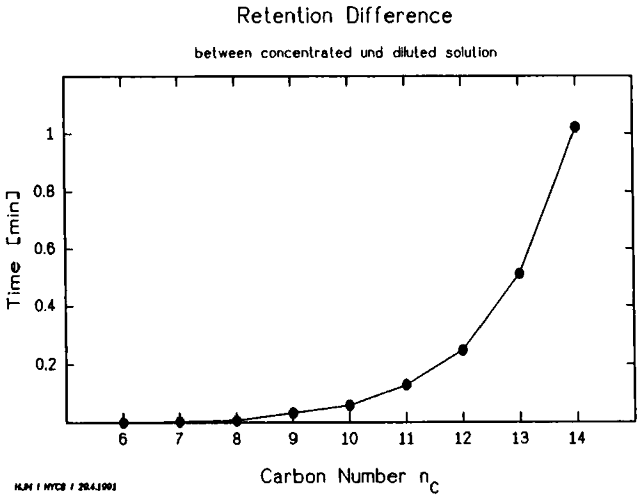

FIGURE 8
n-Alkanes on Hypercarb. Retention time observed with concentrated sample minus time with diluted sample.

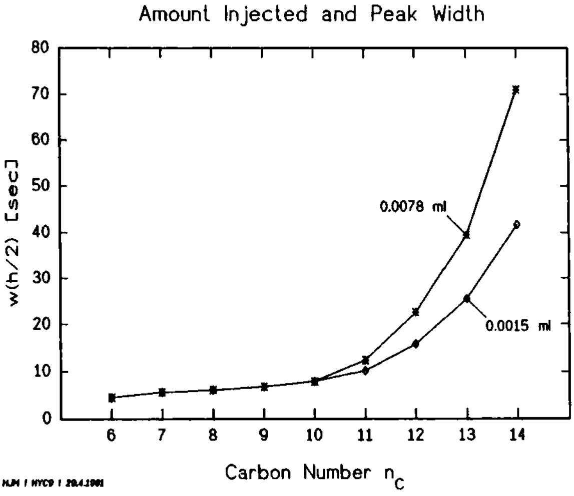

FIGURE 9
Peakwidth at half height of n-alkanes on Hypercarb for 1.5μl and 7.8μl injection.

the ln k' equation is 0.5608 with a 1.5 μl sample and 0.5779 with a 7.8 μl sample.

The peak width at half hight (read from the CR3A integrator) increases more strongly with the larger sample as seen from fig.9. This cannot be due to the increased injection volume, since the peak broadening contribution of a 7.8 μl injection is about 0.5 sec in this system.

The tailing factor TF was determined manually at half hight and at 20% height using a calibrated magnifying glass. TF at ($h_{max}/2$) is shown in fig.9 as a function of sample size. It drops from TF~1.2 (normal tailing) for small injections to TF~0.3 (strong fronting) for larger injections. Practically the same result was obtained at $h_{max}/5$. Similar results were found with chloroalkane solutes (C_6-C_{10},C_{12},C_{14}). A 100% solute concentration increase from 25 to 50 μl/4 ml MeOH worsened the tailing factor from 0.43 to 0.28 at half height of the tetradecyl chloride peak. Simultaneously, the peaks get both higher and wider, but the strange shape remains the same. With n-alcohols, it takes very high sample loads to produce fronting. At 150 mg/4 ml MeOH, pentadecanole produces a distinctly broadened, tailing peak, while the hexadecanole peak is broad and severely distorted with excessive fronting.

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