

CHROM. 12,219

COMPARISON OF LINEAR AND BRANCHED NON-POLAR CHEMICALLY BONDED STATIONARY PHASES IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

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SUMMARY

Non-polar chemically bonded stationary phases having branched or unbranched structure were synthesized, and their specific surface areas, pore size distributions and carbon contents were estimated. Based on these data the number of silane molecules bonded to unit surface area of the original silica was calculated for each phase.

Retentions of selected solute groups were measured in four mobile phases of various elution strength, prepared from methanol-water mixtures at various ratios. Dependences of k' on the length of the "bristle", on the size and structure of solute molecule and the elution strength of mobile phase were plotted.

Results are compared for pairs of phases having different structures but either the same total number of carbon atoms in the bonded molecule or the same length of the "bristle".

INTRODUCTION

The significance of bonded phases is demonstrated by the growing number of publications dealing with them. Different preparation procedures of bonded phases have been studied and developed in the past. These experiments suggest that the best results are achieved when reacting silica with a haloalkylsilane (or alkoxyalkylsilane)¹⁻⁴. In recent years this method of preparation of bonded phases has been used exclusively. Although these packings are widely used, their structure and separation mechanism are still not completely understood¹.

Kirkland¹ and Uihlein and Halász⁵ pointed out that the separation mechanism is very complicated and that many processes are involved. The majority of articles on separation mechanism on bonded phases deal with non-polar phases. In 1974, Locke⁶ expressed the selectivity of these packings by means of thermodynamic equations. The separation mechanism on such phases has been discussed in connection

* Part of the Ph.D. Thesis of Vladimír Řehák, Charles University, Prague, 1979.

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with their preparation and properties⁷⁻⁹, and in terms of structure¹⁰⁻¹³. The separation mechanism itself was dealt with in another series of papers¹⁴⁻¹⁷. These different approaches to the same problem indicate the complexity of the separation mechanism. Despite the fact that very useful and interesting results have been reported, definite conclusions still cannot be drawn.

Our attention has been focused mainly on one factor influencing the retention on chemically bonded phases, *i.e.*, the bonded phase structure. Two groups of packings were synthesized, one having branched structures, the other unbranched phases. All packings are listed in Table I.

TABLE I
LIST OF PREPARED PHASES

Packing (bonded alkyl)	Abbreviation	C atoms		Reference packings
		in chain	total	
2,4,4-Trimethylpentyl	2,4,4	5	8	<i>n</i> -pentyl; <i>n</i> -octyl
4-Butyloctyl	C ₈ —<	8	12	<i>n</i> -octyl; <i>n</i> -dodecyl
1-Ethyladamantyl	adam		12	<i>n</i> -dodecyl
<i>n</i> -Butyl	C ₄	4	4	—
<i>n</i> -Hexyl	C ₆	6	6	—
<i>n</i> -Octyl	C ₈	8	8	—
<i>n</i> -Dodecyl	C ₁₂	12	12	—
<i>n</i> -Octadecyl	C ₁₈	18	18	—
Methyl	HMDS	1		—

EXPERIMENTAL

Chemicals

Silica gel LiChrosorb SI 100, particle diameter 10 μm (irregularly shaped) was obtained from E. Merck (Darmstadt, G.F.R.), *n*-butyltrichlorosilane and trihexylchlorosilane from Alfa Products (Danvers, Mass., U.S.A.), *n*-dodecyltrichlorosilane from Peninsular Chemresearch, a subsidiary of Calgon Corp. (Gainesville, Fla., U.S.A.) and *n*-octadecyltrichlorosilane from E. Merck. 2,4,4-Trimethylpentylmethyl-dichlorosilane, *n*-octylmethyldichlorosilane and 4-butyloctyltrichlorosilane were synthesized by the authors. 1-Ethyladamantyltrichlorosilane was purchased from Petrarch Systems (Levittown, Pa., U.S.A.).

All other chemicals, *i.e.*, solvents, solutes, reagents, etc., were obtained from E. Merck, Merck-Schuchardt (Hohenbrunn bei München, G.F.R.), Riedel de Haën (Hannover, G.F.R.), Aldrich (Milwaukee, Wisc., U.S.A.), Fluka (Buchs, Switzerland) and Lachema (Brno, Czechoslovakia).

Apparatus

A home-made liquid chromatograph consisting of a M3S4 pump (Orlita, Giessen, G.F.R.), pulse damping device, septum injector, stainless-steel column (25 cm \times 4.6 mm I.D.) and UV detector at 254 nm (Universität des Saarlandes, Saarbrücken, G.F.R.), refractometric detectors R4 and R401 (Waters Assoc., Milford, Mass., U.S.A.) was used. An LC 8500 (Varian Aerograph, Walnut Creek, Calif., U.S.A.) was also employed.

Preparation of packings

A 10-g amount of silica (LiChrosorb SI 100 pretreated with HCl-HNO₃ (9:1) at 140° for 4 h, washed with distilled water to neutrality and dried in vacuum oven at 150° and 1 Torr for at least 8 h) was suspended in dry toluene (dried over molecular sieve) in a three-necked, round-bottomed flask equipped with a reflux condenser, mechanical stirrer and dropping funnel. After a short period of stirring, a haloalkylsilane (0.03 mol) was introduced into the flask and the equivalent amount of triethylamine (Schmidt¹⁸) was added dropwise. The reaction mixture was again refluxed for 3 h. The whole procedure was carried out with strict exclusion of moisture to prevent polymerization. The product was filtered off and washed with three 200-ml portions of benzene, methylene chloride, methanol, methanol-water (1:1), methanol and methylene chloride. The dry packing was then silanized with hexamethyldisilazane (HMDS) (10% solution in toluene) in the same apparatus as used in the preparation. The reaction between silica and haloalkylsilane was monitored by both the C,H analysis and methyl-red adsorption test for the presence of unreacted silanols¹⁹. Small samples of the reaction mixture were taken at intervals of 30 min. It was found that, under these conditions, the reaction is complete in 2 h as shown in Fig. 1.

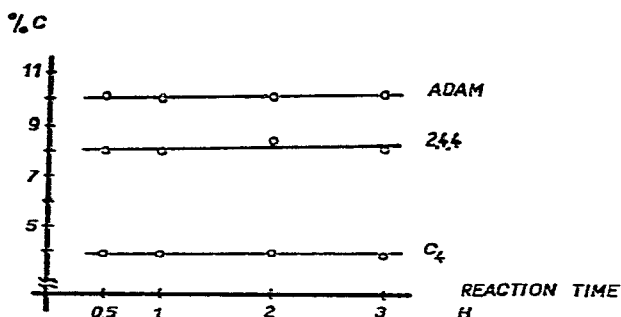


Fig. 1. Dependence of carbon content on the duration of reaction between silica and haloalkylsilane.

Synthesis of commercially unavailable haloalkylsilanes

The reaction of a silane with the double bond of an olefin²⁰, sometimes called hydrosilylation, was used for the synthesis of 2,4,4-trimethylpentylmethylchlorosilane, and 4-butyloctyltrichlorosilane. When catalyzed by ultraviolet light²¹, H₂PtCl₆ · H₂O²² and by Pt/C²³ the reaction proceeds contrary to the Markovnikov rule. This is very important for the further use of prepared compounds. In this way are prepared the haloalkylsilanes with the silyl group bonded to the last atom of the hydrocarbon chain of the original olefin. We used the catalysis by H₂PtCl₆ · H₂O and the course of the reaction was checked by infrared spectroscopy (band at 1660–1600 cm⁻¹ for C=C—disappears and in the case of methylchlorosilane a new band at 1245 cm⁻¹ for Si-CH₃ appears). The position of the silyl group on the hydrocarbon chain was determined by nuclear magnetic resonance spectroscopy. 2,4,4-trimethyl-1-pentene was obtained from Merck and 4-butyl-1-octene was synthesized in our laboratory. (Its synthesis will be described elsewhere.)

Characterization of packings

C,H analysis. The results show that branching of the longest alkyl does not

influence the reactivity of haloalkylsilane. All three packings having twelve C atoms in bonded alkyl (1-ethyladamantyl, *n*-dodecyl, 4-butyloctyl) had comparable carbon content between 12.5 and 13.3% (after silanization). In contrast, the packing with only 6% of carbon has been reproducibly prepared by bonding of trihexylchlorosilane (eighteen C atoms in the molecule), in accordance with Schmidt¹⁸. The packing with ca. 16% of carbon was obtained by bonding of octadecyltrichlorosilane (eighteen C atoms in the molecule). The shielding of the chlorine atom by the hexyl chains causes a decrease in reactivity of trihexylchlorosilane.

Specific surface area measurements. The method of thermal desorption, introduced by Nelsen and Eggertsen²⁴, was employed, using apparatus by Grubner²⁵. The results in Table II (together with results of C,H analysis) show that the specific surface area decreases by bonding of an alkyl. This decrease ranges between 35 and 52% (of the value for unmodified silica) for the packings described in this work. Values above 50% were obtained for bonded *n*-octadecyl and for silica modified directly with HMDS. For all other packings, values between 35 and 42% were found. The differences in decrease of specific surface area between branched and corresponding unbranched phases are small. In all cases the values are slightly lower for the branched packing; however, the difference is comparable with the accuracy of the method.

TABLE II

C,H ANALYSIS DATA AND SPECIFIC SURFACE AREAS OF PREPARED PHASES

The values of % of carbon before silanization with HMDS are given.

Packing (bonded alkyl)	% of C	Amount of silica in 1 g of packing	Corresponding surface area (m ² /g)	Area after bonding	% of decrease
2,4,4-Trimethylpentyl	9.29	0.8633	292.0	166.5	42.98
4-Butyloctyl	11.74	0.8391	281.3	180.3	35.93
1-Ethyladamantyl	12.49	0.8341	279.7	281.3*	
Methyl	3.74	0.9526	314.0	149.8	52.29
<i>n</i> -Octyl	10.88	0.8400	286.0	170.3	40.45
<i>n</i> -Dodecyl	12.43	0.8300	277.7	162.0	41.60
<i>n</i> -Octadecyl	16.32	0.7874	264.0	175.1	52.65

* For bonded 1-ethyladamantyl there is no decrease in specific surface area.

Pore size distribution. Our presumptions that bonding of an alkyl decreases the main pore diameter were confirmed by Martin's method²⁶. The decrease of the main pore diameter is in very good agreement with the value calculated for each packing using the simplest possible model—a cylinder, to whose inner walls maximum stretched alkyls are bonded. Table III lists the calculated and measured values. The results for 1-ethyladamantyl cannot be correlated with the theory apparently because its structure differs greatly from those of the other alkyls. It cannot be excluded that only this structure enables specific interactions leading to the deviation observed.

Surface concentration of bonded molecules. Based on data given in Table II, the values of surface concentrations of bonded molecules were calculated. The results presented in Table IV show that all packings described in the present work were prepared under the condition of maximum coverage²⁷. The site requirements of bonded

TABLE III
PORE SIZE DISTRIBUTION MEASUREMENTS: THE MEAN PORE DIAMETERS

Packing (bonded alkyl)	Approx. maximum length of the alkyl chain (Å)	Mean pore diameter (Å)	
		Calculated	Measured
2,4,4-Trimethylpentyl	3.6	90.0	91.2
4-Butyloctyl	8.4	83.0	84.1
1-Ethyladamantyl	8.0	84.0	120.0*
<i>n</i> -Butyl	3.6	92.6	93.3
<i>n</i> -Pentyl	4.8	90.0	—
<i>n</i> -Hexyl	6.0	88.0	89.7
<i>n</i> -Octyl	8.4	83.0	86.6
<i>n</i> -Dodecyl	13.2	74.0	78.0
<i>n</i> -Octadecyl	20.0	60.0	65.7
Trihexyl	6.0	88.0	93.3

* Measured value for bonded 1-ethyladamantyl is higher than for original silica (100 Å).

molecules were found to be between 40 and 57 Å², roughly corresponding to the data reported by Hemetsberger *et al.*⁸. The values are strongly dependent on the accuracy of both surface area measurement and C,H analysis, so that the agreement can be interpreted as good. Comparison of values for pairs of the corresponding branched and unbranched packings suggests that the coverage of branched phase is slightly lower.

TABLE IV
SURFACE CONCENTRATIONS OF BONDED MOLECULES AND SITE REQUIREMENTS FOR EACH PHASE

Packing (bonded alkyl)	μmol of silane bonded to 1 m ²	No. of silane molecules per 100 Å ²	Site requirement of 1 molecule
2,4,4-Trimethylpentyl	3.40	2.05	48.87
4-Butyloctyl	2.94	1.77	56.38
1-Ethyladamantyl	3.15	1.90	52.66
<i>n</i> -Butyl	3.21	1.93	51.70
<i>n</i> -Hexyl	3.30	1.99	50.25
<i>n</i> -Octyl	4.09	2.46	40.61
<i>n</i> -Dodecyl	3.15	1.90	52.66
<i>n</i> -Octadecyl	2.90	1.75	57.11
Methyl	3.31	1.99	50.25

Chromatography

Packing of columns. All the columns were packed by the same method—balanced density slurry packing using tetrabromoethane and carbon tetrachloride-dioxane (1:1). The packed column was connected to the slurry reservoir through a tube (5 cm, I.D. as the column to be packed). A 20-ml volume of pure solvent mixture (used for slurry preparation) was introduced into the apparatus, the slurry and the reservoir were filled with pressurising liquid (*n*-heptane) and then the column was packed continuously in the vertical position. The quality of the columns was checked using methylene chloride as mobile phase. The columns which gave satisfactory results (*i.e.*, symmetrical elution curve for benzene, at linear velocity of 5 mm/sec show-

ing relative band broadening of $50 \mu\text{m}$ or less and lastly, having permeability better than $1 \cdot 10^{-9} \text{ cm}^2$) were used for further experiments.

Loadability of columns estimated as described by Karch²⁸ was found to be comparable for both branched and unbranched packings. Values between $5 \cdot 10^{-4}$ and $1.5 \cdot 10^{-5} \text{ g}$ per gram of packing were found, depending on the carbon percentage and on the solute used for this estimation.

The rate of equilibration after a change in mobile phase composition is also comparable for both types of packings. If the methanol content in a new mobile phase is greater than 1%, constant capacity factors (k') are reached very quickly —after 15–20 ml have been pumped through the column. Using pure water as a new mobile phase, the equilibrium is not reached even after pumping of several litres of water through the column. This can be explained probably by non-wettability of apolar phases by water.

The excellent reproducibility of packing and the efficiency of the prepared columns were the same for both branched and unbranched phases. No differences were found in their loadability nor in the rate of equilibration after the mobile phase composition had been changed. These facts enable us to compare different bonded phases and to interpret the influence of bonded phase structure on retention.

Retention. The dependence of retention (described in terms of k') on several

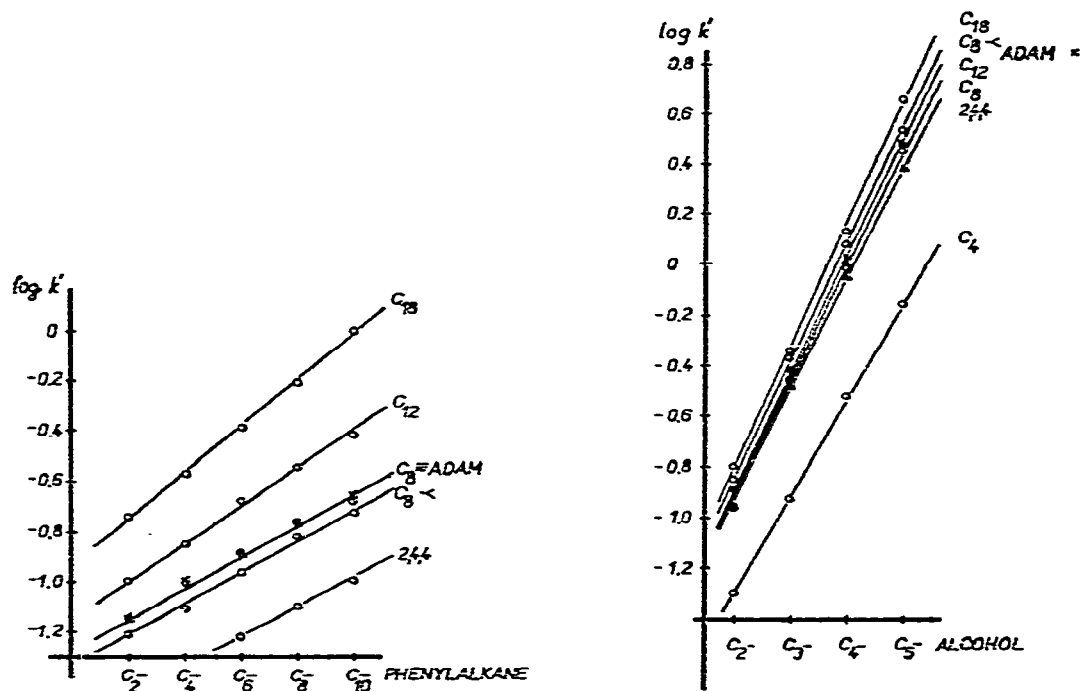


Fig. 2. Influence of the bristle length on the retentions of phenylalkanes. Eluent: methanol. Samples: ethylbenzene, butylbenzene, hexylbenzene, phenyloctane and phenyldecane. Stationary phases as in Table I.

Fig. 3. Influence of the bristle length on the retentions of n -alcohols. Eluent: methanol–water (30:70). Samples: ethanol, propanol, butanol and pentanol. Stationary phases as in Table I.

parameters (length and structure of bonded molecule, the size and structure of solute molecule and the mobile phase elution strength) were studied. The measured k' values were normalized to 1 μmol of a bonded molecule in order to enable the structure effect on the retention to be determined with a greater sensitivity. Figs. 2 and 3 show examples of dependences of $\log k'$ on the length of bonded molecule (on the bristle length). For branched packings, the length of the bonded molecule is represented by that of the longest chain. For both types of phases, the value of k' is proportional to the bristle length.

The following differences in retention were found. For a given solute, the k' value and especially the ratio of k' on branched to k' on corresponding unbranched packing are determined by mobile phase elution strength and the solute molecule structure. In mobile phases possessing high elution strengths the k' values for given solutes are always higher on unbranched packings. This difference decreases with decreasing elution strength and the ratio of k' values can be reversed for many solutes. The retention on branched phases is then greater than on the corresponding unbranched phases. The solute structure seems to be the principal factor determining the conditions under which this levelling or reversal of the retention occurs. Figs. 4-6 illustrate this. They show the retention of different solutes measured in the same mobile phase (influence of the mobile phase elution strength is excluded). While, in all cases, methyl derivatives of benzene have lower k' on branched than on corresponding unbranched phases, the retention of dialkyl phthalates is quite different from phase to phase. Their retention on bonded 2,4,4-trimethylpentyl and 4-butyloctyl is comparable with that on corresponding unbranched phases. The retention of these

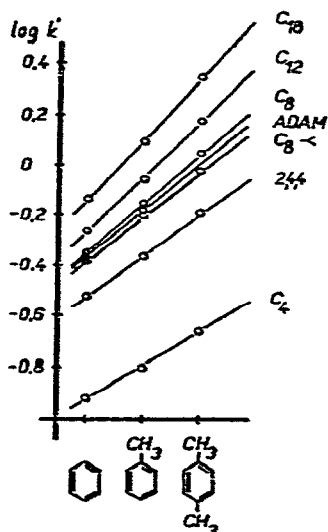


Fig. 4. Influence of solute molecule size and structure on the retentions of benzene methyl derivatives. Eluent: methanol-water (70:30). Samples: benzene, toluene and *p*-xylene. Stationary phases as in Table I.

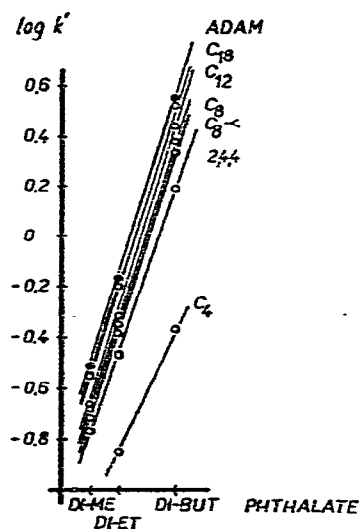


Fig. 5. Influence of solute molecule size and structure on the retentions of dialkyl phthalates. Eluent: methanol-water (70:30). Samples: dimethyl, diethyl and dibutyl phthalate. Stationary phases as in Table I.

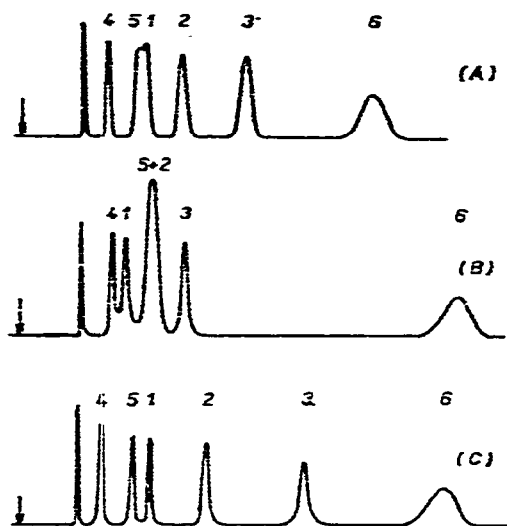


Fig. 6. Separation of a mixture of benzene methyl derivatives and dialkyl phthalates. Eluent: methanol-water (70:30). Samples: 1 = benzene, 2 = toluene, 3 = *p*-xylene, 4 = dimethyl, 5 = diethyl and 6 = dibutyl phthalate. Stationary phases: A = *n*-dodecyl, with 13.35% of carbon after the silanization; (B) = 1-ethyladamantyl, with 12.88% of carbon after silanization; C = *n*-octadecyl, with 16.79% of carbon after silanization.

solutes on bonded 1-ethyladamantyl is greater than on *n*-dodecyl (corresponding unbranched phase) or *n*-octadecyl (the largest unbranched packing).

The special selectivity of bonded phase for a solute which has the same structure was also studied. 1-Ethyladamantyl was chosen as a striking example. The relative retention of adamantane to *n*-decane in methanol was measured. This value was found to be lower than 1 on all packings except bonded 1-ethyladamantyl. On this phase the retention of both the C_{10} hydrocarbons is equal.

CONCLUSIONS

The differences observed in retention on branched and unbranched phases are caused exclusively by the structure of bonded molecules because other factors can be neglected. The basic features of packings of both types, *i.e.*, the carbon content, pore size distribution, coverage, specific surface area, the quality of columns packed with these phases, loadability and rate of equilibration are almost the same.

The differences in retention on phases of different structure are more pronounced in mobile phases of lower elution strength. The k' values are governed by the solute structure. All these results suggest that comparison of retention on branched and unbranched phases could be also used for solving more general problems of the separation mechanism, based on the study of intermolecular interactions. The phases with branched structure could also be useful in extending the applications of high-performance liquid chromatography.

ACKNOWLEDGEMENTS

The authors are grateful to S.E.A. for the support of this work, to all members of Institut für Angewandte Physikalische Chemie, Universität des Saarlandes, Saarbrücken (Professor I. Halász, Head), to all members of Laboratorium voor Instrumentele Analyse, Technische Hogeschool Eindhoven, Eindhoven (Dr. Ir. C. A. Cramers, Head) for the effective cooperation and many useful discussions. Many thanks also to Drs. B. Máca and V. Sváta from Department for Organic Chemistry, Faculty of Science, Charles University, Prague for their help in the synthesis of unavailable olefins.

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