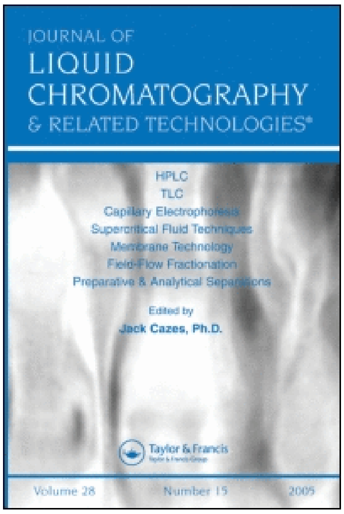

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SEPARATION OF FULLERENES WITH NOVEL STATIONARY PHASES IN MICROCOLUMN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Newly designed bonded phases have been synthesized and evaluated for the separation of fullerene molecules. Further investigations about the retention behaviour of fullerenes with these phases have been carried out systematically.

In this study we describe the separation of fullerenes with novel bonded stationary phases in microcolumn high performance liquid chromatography (micro-HPLC) and also propose the basic separation mechanism of those molecules obtained from the preliminary chromatographic observations. The results indicate that the octadecyldiphenyl bonded silica phases, which have been synthesized from octadecyldiphenylchlorosilane as the silanization reagent, possess a better retentivity for fullerenes than the octadecyldimethylsilica phase (i.e. monomeric ODS phase) having a similar surface coverage value.

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INTRODUCTION

Many reports about the chromatographic separation of C_{60} and C_{70} fullerenes have been published [1-3] because the isolation or collection of a specific fullerene is one of the first series in characterization. More recently, the separation of so-called higher-fullerenes, such as C_{76}, C_{78}, C_{82} and C_{84} etc., has become an important subject for chromatographers [4-5]. The molecular features of C_{60} and C_{70} are dissolved enough to be distinguished easily, however, there are only slight size and shape differences among higher-fullerene molecules having similar or identical carbon atom numbers in their chemical structures. Therefore, the liquid chromatographic separation of fullerene molecules with molecular size and shape recognition capabilities of bonded phases can be considered as the most powerful separation techniques for these purposes, and it is important to investigate the separation mechanism of fullerenes based on the analysis of their retention behaviors with various bonded phases in HPLC.

To investigate the retention behaviour of fullerenes with chemically bonded silica phases, we have introduced various stationary phases such as, commercially available ODS phases [5-8], multi-legged phases [9], multi-methoxy phases [6,10] and multi-phenyl phases [11], in the last few years. This is due to the fact that fullerene molecules can be seen as the very large polycyclic aromatic hydrocarbons (PAHs) and, therefore, each fullerene molecule is regarded as a good sample to study the retention mechanism in HPLC. On the other hand, if the chromatographic separation mechanism of fullerenes with the stationary phases is elucidated, a newly designed stationary phase which can effectively separate them will be developed by using the concept based on that mechanism.

In this study we introduced three alkyldiphenyl bonded silica phases having both alkyl-chains and two phenyl-rings in their chemical structures, and studied the

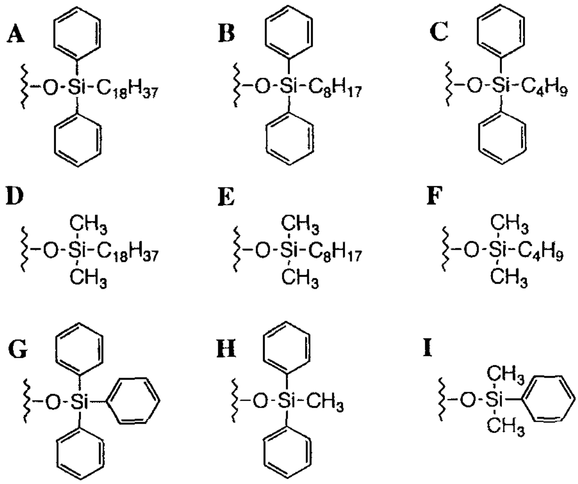

FIGURE 1 Structures of the chemically bonded phases used in this study. (A)C18Diph, (B)C8Diph, (C)C4Diph, (D)C18, (E)C8, (F)C4, (G)Triph, (H)Diph and (I)Monoph.

chromatographic retention behaviors of C_{60} and C_{70} fullerenes with these stationary phases in HPLC. Systematic analysis of the retention behaviors was also carried out by comparison of the results with alkyldiphenyl phases to those of corresponding alkyldimethyl phases and some phenyl-derivatized bonded phases described earlier [11].

EXPERIMENTAL

Bonded Phase Synthesis

Chemical structures of bonded phases investigated were shown in Figure 1. These bonded phases were synthesized in a similar manner as described previously [10,12], and the basic characteristics of these phases are summarized in Table 1.

Table 1 The characteristics of bonded phases.

	bonded phase	pore size (Å)	carbon content (%)	surface coverage (μmol/m^2)	ligand interval (Å)
A	C_{18}Diph	120	8.48	1.54	10.4
B	C_8Diph	120	5.89	1.56	10.3
C	C_4Diph	120	4.15	1.35	11.1
D	C_{18}	120	9.08	2.52	8.1
E	C_8	120	5.08	2.68	7.9
F	C_4	120	1.73	1.50	10.5
D	C_{18} (Develosil ODS-5)*	100	20	3.31	7.1
D	C_{18} (Develosil ODS-N-5)*	100	16	2.47	8.2
D	C_{18} (Develosil ODS-P-5)*	100	11	1.57	10.3
G	Triph 70 Å	70	11.2	1.14	12.1
H	Diph 70 Å	70	13.7	2.20	8.7
I	Monoph 70 Å	70	10.8	3.40	7.0
G	Triph 150 Å	150	4.79	1.19	11.8
H	Diph 150 Å	150	6.11	2.16	8.8
I	Monoph 150 Å	150	5.35	3.81	6.6

*commercially available ODS phases (Nomura Chemical, Seto, Japan).

Three commercially available ODS phases, Develosil ODS-5, Develosil ODS-N-5 and Develosil ODS-P-5, were also used for comparison. These phases were obtained from Nomura Chemicals (Seto, Japan) as gifts.

Chromatographic Measurements.

The bonded phases were packed using a slurry method into a fused-silica capillary (Tokyo Chemical Industries, Tokyo, Japan) of 0.53 mmi.d. x 200 mm length. The microcolumn HPLC system consisted of a microfeeder MF-2 pump (Azuma Electric, Tokyo, Japan), a Rheodyne 7520 injector (Cotati, CA, USA) with a 0.2 μL injection volume and a Uvidec 100-III UV detector (Jasco, Tokyo, Japan) set at 320 nm. The mobile phase was guaranteed reagent grade n-hexane (Kishida Chemical, Osaka, Japan). The typical flow-rate was 4 μL/min., and the mixture of toluene and cyclohexane was used as the sample solvent. The chromatographic measurements were done at least three times. The column temperature was controlled using a modified Hewlett-Packard Model 5820-II Gas Chromatographic oven (Yokogawa Analytical Systems, Musashino, Tokyo, Japan).

RESULTS and DISCUSSION

Figure 2 shows the chromatograms for the separation of C_{60} and C_{70} fullerenes with three alkyldiphenyl bonded silica phases under the same conditions. The retentivity of the C_{18}Diph bonded phase is larger than those of the C_8Diph and C_4Diph phases. This suggests the contribution of the long alkyl chains in the bonded phase to the fullerenes retention, because of almost comparable surface coverage values of these three phases shown in Table 1. In order to make this behaviour clear, the separation of C_{60} and C_{70} was carried out with various bonded phases shown in Figure 1. These retention data have also been summarized in Table 2. Develosil ODS-5 has the best separation performance

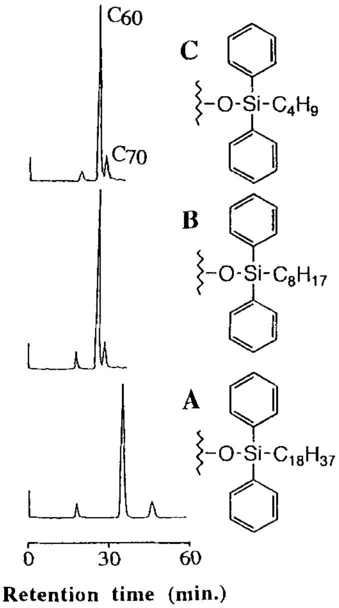

FIGURE 2 Chromatograms for the separation of C60 and C70 with three alkyldiphenyl bonded phases. (A)C18Diph, (B)C8Diph and (C)C4Diph; mobile phase, n-hexane; column temperature, 30 °C.

among various ODS phases investigated previously [7], similarly, the C_{18}Diph phase gives the best separation for fullerenes as can be seen from these data.

By comparison of the retention data with C_{18}Diph and those with Develosil ODS-P-5, it can be said that two phenyl-rings in the bonded phase structure contribute significantly to the retention value of fullerene molecules. Similar trends are also observed by comparing the data of C_4Diph phase with those of C_4 phase. In spite of the lower surface coverage value of C_{18}Diph phase to C_{18} phase, the C_{18}Diph phase possesses the stronger retentivity toward fullerene molecules than the C_{18} phase. The results also indicate the contribution of the phenyl-rings to the retention of fullerenes and these results have a good agreement with the data

Table 2 Retention data for the separation of C_{60} and C_{70} with various bonded phases. Mobile phase, n-hexane; column temperature, 30 ℃.

	bonded phase	capacity factor (k')		separation factor
		C_{60}	C_{70}	α(k' C_{70}/k' C_{60})
A	C_{18}Diph	1.02	1.67	1.64
B	C_8Diph	0.42	0.57	1.36
C	C_4Diph	0.41	0.56	1.37
D	C_{18}	0.52	0.84	1.63
E	C_8	0.086	0.124	1.44
F	C_4	0.061	0.087	1.43
D	C_{18} (Develosil ODS-5)*	0.87	1.60	1.84
D	C_{18} (Develosil ODS-N-5)*	0.81	1.32	1.64
D	C_{18} (Develosil ODS-P-5)*	0.44	0.64	1.45
G	Triph 70 Å	2.28	3.59	1.58
H	Diph 70 Å	1.85	2.89	1.56
I	Monoph 70 Å	0.86	1.24	1.44
G	Triph 150 Å	0.45	0.63	1.39
H	Diph 150 Å	0.45	0.64	1.41
I	Monoph 150 Å	0.27	0.37	1.36

*commercially available ODS phases (Nomura Chemical, Seto, Japan).

obtained with Triph, Diph and Monoph phases [11], where a smaller retentivity of Monoph phases than Triph and Diph phases having two phenyl-rings in their structures has been observed for the separation of fullerenes.

It can also be said from the comparison of the separation factors with C18 (120 Å pore size, 9.08 C%) and those with Develosil ODS-N-5 (100 Å pore size, 16 C%), where both phases have very similar surface coverage values, that the capacity factors for the fullerenes with these two bonded phases are different according to the difference of the silica gel properties.

On the other hand, the effect of alkyl chain length can be seen among C_{18}Diph, C_8Diph and C_4Diph, and corresponding alkyldimethyl phases. With the C_{18}Diph phase, larger capacity factors for fullerenes and better separation factors for fullerenes were found in comparison to those observed with C_8Diph and C_4Diph phases. This observation indicates that the length of alkyl chains in the bonded phases contribute to the retention of fullerenes, and that C_8 and C_4 chains are too short to interact effectively with fullerene molecules. These results have a good agreement with the trends seen in our previous investigation [7].

Table 3 shows the retention data for the separation of C_{60} and C_{70} with the C_{18}Diph phase at various column temperatures, and the corresponding van't Hoff plots are shown in Figure 3. For both C_{60} and C_{70}, linear van't Hoff plots are obtained over the temperature range investigated (from 0 °C to 80 °C), however, the separation factor between these fullerenes decreases more slowly than Develosil ODS-P-5 when the column temperatures are elevated. As the Develosil ODS-P-5 phase possesses an almost identical surface coverage value as the C_{18}Diph phase. This phenomenon can be explained by the uniformity of the ligand interval (about 10.4 Å). That is to say, C_{18}Diph phase can show better uniformity of the ligand interval than that of the ODS-P-5 in order to interact effectively with fullerenes.

Although further consideration about the retention mechanism of fullerenes with chemically bonded stationary phases is needed, it can be concluded from the above systematic evaluations that:

1) two phenyl-rings at the bottom part of the bonded phase contribute to effective interaction between the bonded phase ligands and fullerene solutes;

Table 3 Retention data for the separation of C_{60} and C_{70} with C_{18}Diph at various column temperatures. Mobile phase, n-hexane.

column tmperature (℃)	capacity factor (k')		separation factor α (k' C_{70}/k' C_{60})	
	C_{60}	C_{70}		
0	1.24	2.13	1.72	[1.54]*
20	1.09	1.81	1.66	[1.44]*
30	1.02	1.67	1.64	
40	0.971	1.58	1.63	[1.42]*
50	0.916	1.48	1.62	
60	0.852	1.38	1.62	[1.38]*
70	0.827	1.32	1.60	
80	0.767	1.23	1.60	

*the data with Develosil ODS-P-5 in the previous study [8].

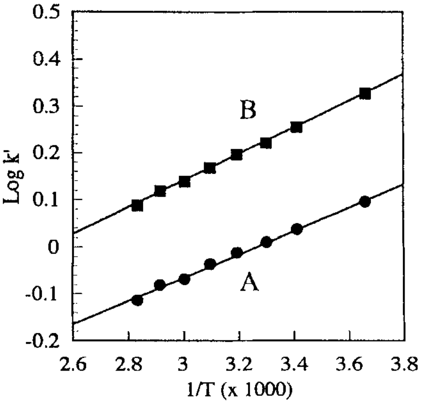

FIGURE 3 van't Hoff plots for C60 and C70 fullerenes with the C18Diph phase. (A)C60 and (B)C70; mobile phase, n-hexane.

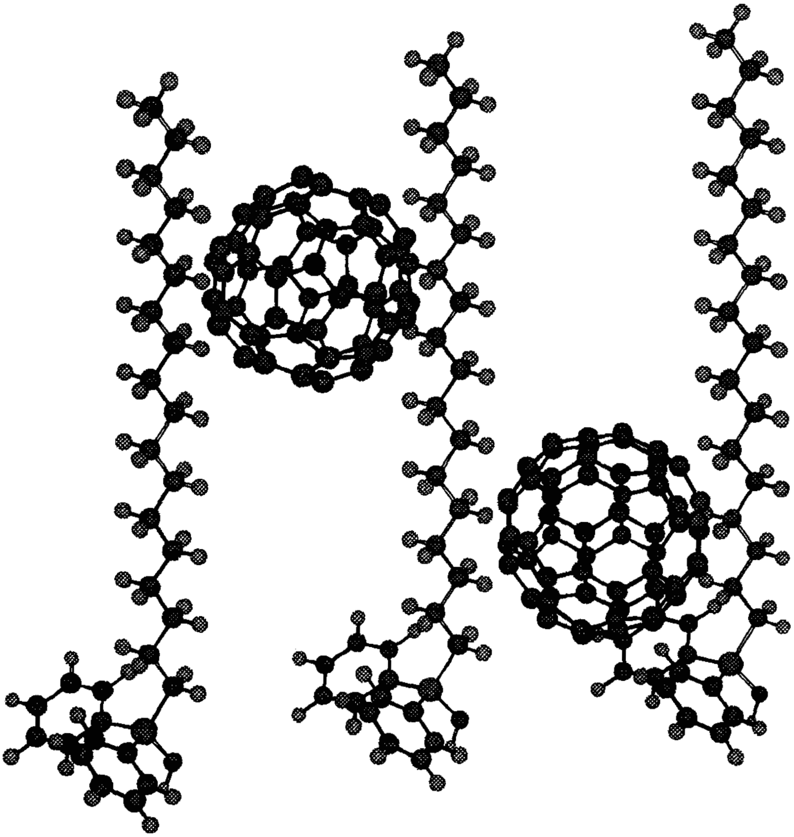

FIGURE 4 Schematic diagram of the interaction between C60 and the C18Diph bonded phase. The model was depicted by Chem3D Plus software (Cambridge Scientific Computing Inc., MA, USA). For convenience by the software, the bonded phase was drawn with hydroxyl group (-OH) as the residual functional substituent bonded to the silicon atom in the silanization reagent.

2) the phenyl rings bonded to the silicon atom induce the uniformity of the interval of alkyl chains, and therefore, the good retentivity can be obtained (the interaction model between C_{60} and C_{18}Diph phase based on this concept is shown in Figure 4), because the phenyl rings work as the spacer of bonded phase ligands on the silica support;

3) there is a possibility that a critical chain length exists to interact effectively with fullerenes, though it seems longer is better.

Some additional studies are currently undergoing in our laboratory in order to elucidate the separation mechanism of fullerenes using other novel stationary phases such as liquid-crystal bonded phases [13,14].

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