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Nano-scale design of novel stationary phases to enhance selectivity for molecular shape and size in liquid chromatography

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Abstract

The design of novel stationary phases in liquid chromatography (LC) which offer enhanced selectivity or better resolution of fullerenes is described. Fullerene molecules have been chosen due to their recent popularity and importance in both chemistry and the materials science fields. From the previous studies on octadecyl modified silica (ODS) stationary phases in LC an important structural requirement for stationary phases that can effectively separate fullerenes has been identified. The distance between each bonded functional group on the silica surface that is best-fit to the fullerene diameter (e.g. C₆₀ has ca. 7 Å diameter and inter-ligand distance 7.1 Å of ODS phase is the best-fit size) is the key to improve the separation performance. A phenyl ligand contribution to fullerenes retention has also been found to be important. The size and shape of the cavity-like multilegged phenyl bonded phases is the dominant parameter for the structural recognition of fullerenes. By utilizing these factors a novel stationary phase structure is proposed.

1. Introduction

The design of novel stationary phases which can offer enhanced selectivity for structural isomer separations in liquid chromatography (LC) is one of the most important tasks for separation chemists. The huge demands for such stationary phases to solve practical separation problems are needed in pharmaceutical, medicinal, biochemical and materials science fields. In order to establish such scheme and concept, we have to know the nano-scale molecular–molecular interactions between bonded phases and solute molecules. We have selected a very special class of

carbon compounds—fullerenes as our sample probe for this investigation [1–5].

In this study, three tactics are targeted to propose a novel stationary phase which can offer good resolution and selectivity for fullerene separations. First, we have evaluated commercially available octadecylsilica (ODS) stationary phases in fullerene separations; the results provide us the basic understanding and knowledge for designing novel stationary phases. Second is the design and synthesis of novel phases using the knowledge obtained by ODS evaluation. We can then confirm that these novel stationary phases are applicable to enhance fullerene selectivity or not. Finally, we compare the performance of these novel phases to several newly

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developed multilegged bonded phases in the same application.

2. Experimental

Two LC systems were used in this study. For the basic evaluation of bonded phases, a micro-LC system consisted of a Microfeeder MF-2 (Azuma Electric, Tokyo, Japan) and an Uvidec-100 UV detector (Jasco, Tokyo, Japan) with a Rheodyne 7520 injector (0.2 μ l, CA, USA). The stationary phases were packed into the fused-silica capillaries of 150 mm \times 0.53 mm I.D. length and the mobile phase flow-rate was 2 μ l/min. For the conventional column evaluation, a Jasco 880-PU pump coupled with a Jasco MD-915 photodiode array detector (200–900 nm detectable range) using a Rheodyne 7125 injector. The column size was 250 mm \times 4.6 mm I.D. length and the mobile phase flow-rate was 1 ml/min.

Most of the stationary phases were synthesized by the methods reported previously [6,7]. The diphenylalkyl bonded phases were monomerically synthesized by using alkyl-diphenylchlorosilanes such as butyl, octyl and octadecylsilanes as the starting materials in the usual way. The alternative alkyl bonded phases were also synthesized by using alkylmonochlorosilanes as the starting material. The synthesized materials had been evaluated in their quality by elemental analysis and solid-state NMR spectroscopy. Multilegged phases were also synthesized by a similar method reported in our previous publications [8–10]. The basic difference between these phases and other conventional phases is the structure on the silica surface. The multilegged phases have a unique structure which covers the silica surface horizontally, whereas typical phenyl and ODS phases are attached vertically to the silica surface by siloxane bonding. As the bidentate or tridentate reagents synthesized in the laboratory can form two or three bonds to the silica surface for each molecule of silane, these multi-bonds produce a multi-membered ring with the silica structure (i.e., they can produce cavity-like structure on the surface).

Develosil ODS phases were obtained from Nomura Chemicals (Seto, Japan) as gifts.

The mobile phase solvents and other chemicals were all commercially available except for the fullerenes samples. The mixture of fullerenes and pure C₆₀ were obtained by the carbon soot which was produced at Science Core in Toyohashi (Japan). Solvent extraction and column chromatography were used to pre-purify those samples.

3. Results and discussion

3.1. ODS study

Polymeric and monomeric ODS phases

Two different types of ODS phases were evaluated in this study. The first are the monomeric ODS phases which were synthesized using monochlorosilane as a starting material. The second are polymeric ODS phases which were produced from trichlorosilane. These two types of phases differ in their molecular shape and size recognition capability, especially for polycyclic aromatic hydrocarbons (PAHs) [11–15]. This difference can be found in fullerene separations as well [16–18]. In our previous work [16,18], the separation of fullerenes with monomeric and polymeric ODS phases using *n*-hexane or toluene–acetonitrile as the mobile phase has been discussed. Fullerene retention with the monomeric ODS is greater than that with the polymeric phase and the resolution for higher fullerenes with the monomeric is better than the polymeric phase. The elution order of higher fullerenes is also different between these two phases. For example, using a toluene–acetonitrile mobile phase with the monomeric ODS, the elution is C₇₆, C_{78-C2v}, C_{78-C2v} and D₃ together, and then C₈₂ and C₈₄. However, the elution order of the fullerenes with the polymeric ODS phase is C_{78-C2v}, C₇₆, C_{78-C2v}, D₃, C₈₄ and C₈₂. Since the polymeric ODS phase has better capabilities of recognizing molecular shape and size than the monomeric ODS phase, the fullerene elution order with the polymeric phase is strongly dependent on the shape difference of the

fullerene molecules. More experiments have been performed using the monomeric ODS phases in fullerene separations to elucidate the retention mechanism.

Influence of physical parameters

A number of physical parameters can change the retention characteristics of fullerenes within monomeric ODS phases, such as pore diameter, surface area, surface coverage and the distance between the adjacent C_{18} functional groups, etc. Using the same silica gel, these parameters can be minimized. In our recent work [18], three monomeric ODS phases were carefully studied. The results of this work are summarized in Table 1. Three ODS phases having varying surface coverages made from the same silica gel are compared in the separation performance of C_{60} and C_{70} . The retention data show that the higher surface coverage ODS phases provided longer retention and separation power for C_{60} and C_{70} . A temperature dependence study of C_{60} and C_{70} retention on these three different phases also showed that the higher coverage ODS is better than its low coverage counterparts at higher temperature. It has been clearly demonstrated that a maximum retention existed at a given temperature for each phase. The high coverage phase gave the longest retention around -20°C , medium coverage was about -30°C and the low coverage phase was about -50°C . The highest coverage ODS gave the highest maximum retention temperature for C_{60} and C_{70} which contradicts the general rule of retention of PAHs in reversed-phase LC where the lower the temperature is the longer the retention time will be.

A mixture of PAHs and C_{60} were chosen a test probes with these three monomeric ODS phases in order to determine the uniqueness of the separation mechanism needed to resolve C_{60} . For PAHs, decreasing temperature resulted in increasing their retentions. However, when the test temperature is below -20°C , the retention of the C_{60} would not increase even with decreasing temperature. It is apparent that the recognition mechanism of the monomeric ODS phase with the fullerene molecule is different from that applied to PAHs. It has also been found from a plot of the separation factors of C_{60} and C_{70} against temperature that the separation factors for C_{60} and C_{70} are monotonically increasing with decreasing temperature with a lower surface coverage ODS phases, when the temperature is lower than -20°C the separation factors are almost constant while the retentions of both C_{60} and C_{70} are decreasing with decreasing temperature. The separation factors with the highest carbon load Develosil ODS-5 at temperatures less than -20°C showed similar values to those of the polymeric ODS phase. This means the physical property of the high surface coverage monomeric ODS phase is very similar to the polymeric phase at very low temperatures.

The results can be easily explained by the drawings shown in Fig. 1, using the concept of different inter-ligand distance among the bonded C_{18} functional groups. At ambient and/or higher temperature, the C_{18} functional groups are easily relaxed and movable so they can interact with the solute C_{60} without any restriction. With a high surface coverage phase, the distance between each C_{18} group is about 7.1 \AA which is

Table 1
Retention data of C_{60} and C_{70}

Stationary phase	C (%)	Surface coverage ($\mu\text{mol}/\text{m}^2$)	Capacity factor (k')		Separation factor C_{70}/C_{60}
			C_{60}	C_{70}	
Develosil ODS-5	20	3.31	0.87	1.61	1.85
Develosil ODS N-5	16	2.47	0.81	1.32	1.63
Develosil ODS P-5	11	1.57	0.44	0.64	1.45

Mobile phase: *n*-hexane at room temperature.

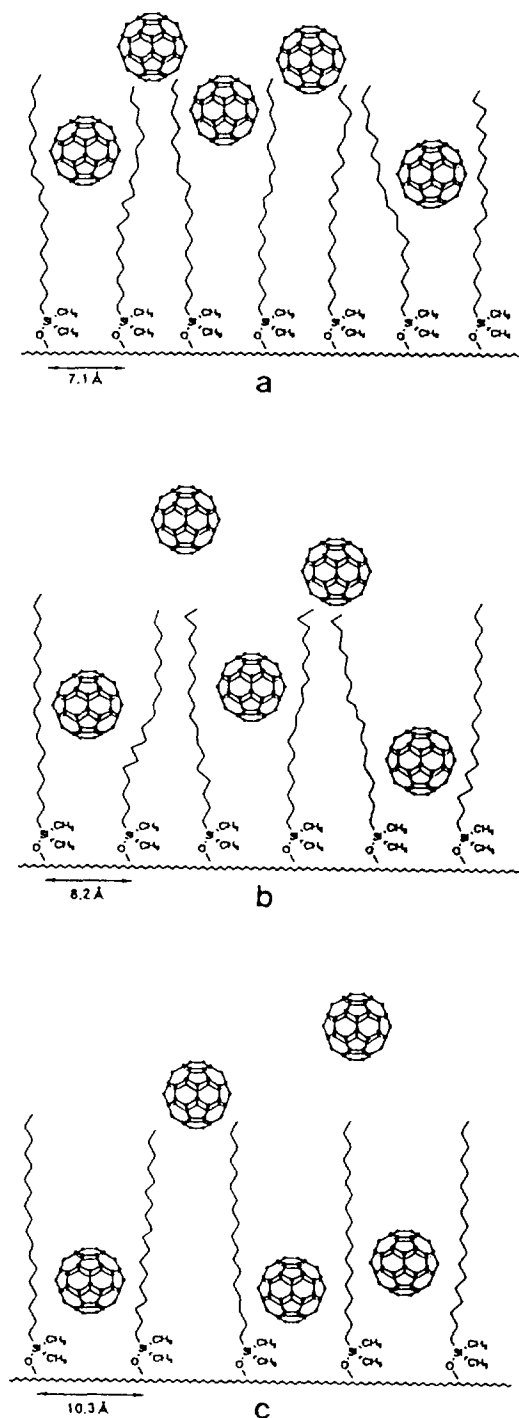


Fig. 1. Schematic diagram of the ODS bonded phase on a silica surface when interaction occurs between the C₁₈ phase and C₆₀. (a) Develosil ODS-5; (b) Develosil ODS N-5; (c) Develosil ODS P-5.

very similar to the diameter of the C₆₀ molecule which results in very efficient interactions. However, decreasing the surface coverage of ODS results in increasing the distance between C₁₈ functional groups which results in less interaction with the C₆₀ molecule. At high temperatures, all the C₁₈ ligands of the three bonded phases have freedom to move and there is not much difference in C₆₀ recognition among these phases caused by difference in distance, but the possibility that interaction occurs is dominant. The C₁₈ ligands become more rigid and ordered at lower temperatures and the ligand distance becomes very crucial enabling them to interact with the C₆₀ molecule. Any mismatch of ligand distance and diameter of the C₆₀ molecule results in a weak interaction and similar retentions. As a conclusion, the ODS ligand distance between each adjacent pair is the most important aspect in obtaining a better selectivity for fullerenes in reversed-phase LC.

3.2. Newly synthesized stationary phases study

There are other important factors at the surface of the bonded phases, such as the length of alkyl chain in conjunction with other functional groups. When phenyl groups were present, they expected to form strong π - π interactions with fullerenes. Therefore several novel phases have been synthesized, these are depicted in Fig. 2. The phases consist of a monoalkyl chain such as ODS, octyl (C₈) and butyl (C₄) phases with two phenyl or two methyl groups.

Retention data obtained from these phases are summarized in Table 2. The data for phases A, B, C, D, E and F reveal the contribution of the alkyl chain length and phenyl groups versus their dimethyl counterparts. Since A, B and C have the same surface coverage, the distance between the functional groups are almost identical. Any discrimination of fullerene selectivities results from the different alkyl chain lengths. When comparing phases A, B and C to their dimethyl counterparts, it is clearly demonstrated that the diphenyl phases exhibit much better separation than those of dimethyl phases. It also appeared that the phenyl group contribu-

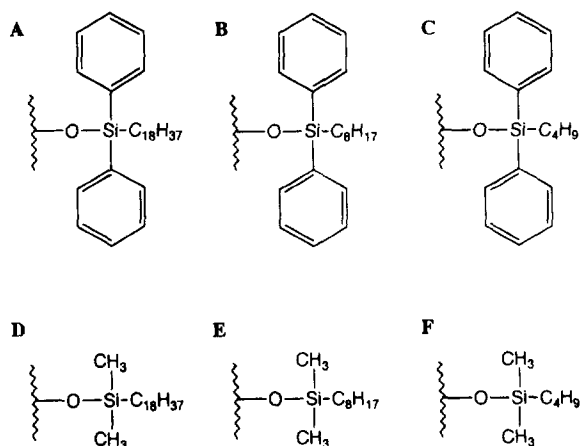


Fig. 2. Novel stationary phases investigated in this work. (A) C_{18} Diph; (B) C_8 Diph; (C) C_4 Diph; (D) C_{18} ; (E) C_8 ; (F) C_4 .

tion to the fullerene separations is larger than the ligand distance contribution if one compares the data for phases C and F which have a similar ligand distance.

3.3. Multilegged stationary phases study

Basic concept of multilegged phases

Another conclusion that can be drawn from the examinations described above is that the phenyl group is a key ingredient for fullerene separations in addition to shorter inter-ligand distance and longer alkyl chain length. All the stationary phases we evaluated so far are vertical types of stationary phases. The typical example of the vertical type of chemically bonded phase is shown in the upper part of Fig. 3. We can design

the phase from a different approach such that the bonded phase is situated in the horizontal position relative to the silica surface. This example is shown in the right part of Fig. 3. As described above the phenyl groups at the bottom part of the ODS bonded phase are contributing to the retention of fullerenes. It seems to be a requirement to insert phenyl groups in the bonded phase to enhance the retention and the selectivity for fullerene separations. To investigate this, we have designed new phases as shown in Fig. 4. These phases are called multilegged bonded phases. Each phenyl moiety is situated in a parallel way in the cavity-like structure on the silica surface. The walls of the cavity can be made by several methyl groups attached to two or three silicon atoms which are bonded to the silica surface and to phenyl groups such as phenyl, biphenyl, benzyl and anthracene groups. BP, BMB and BBB were made to investigate the contribution to the retention of the biphenyl group, methylene groups and the varying size of the cavity. An anthracene moiety was also made to investigate a different geometrical contribution to the retention.

For the separation of C_{60} and C_{70} TP has been tried first, because it has been shown in our work that TP was very effective for the separation of planar and non-planar small PAHs [8]. The size of its cavity is the most suitable for the retention of non-planar small PAHs. Compared to ODS phases, TP has been found to have a reversed-elution order in *o*-terphenyl and triphenylene. Such retention behaviour is reasonably explained by the molecular modelling interpretation.

Table 2

Basic characteristics of bonded phases and retention data for C_{60} and C_{70} with these phases. Mobile phase; n-hexane at 30°C

Bonded phase	Pore size (Å)	C (%)	Surface coverage ($\mu\text{mol}/\text{m}^2$)	Ligand interval (Å)	k'		Separation factor	
					C_{60}	C_{70}		
A	C_{18} Diph	120	8.48	1.54	10.4	1.02	1.67	1.64
B	C_8 Diph	120	5.89	1.56	10.3	0.42	0.57	1.36
C	C_4 Diph	120	4.15	1.35	11.1	0.41	0.56	1.37
D	C_{18}	120	9.08	2.52	8.1	0.52	0.84	1.63
E	C_8	120	5.08	2.68	7.9	0.086	0.124	1.44
F	C_4	120	1.73	1.50	10.5	0.061	0.087	1.43

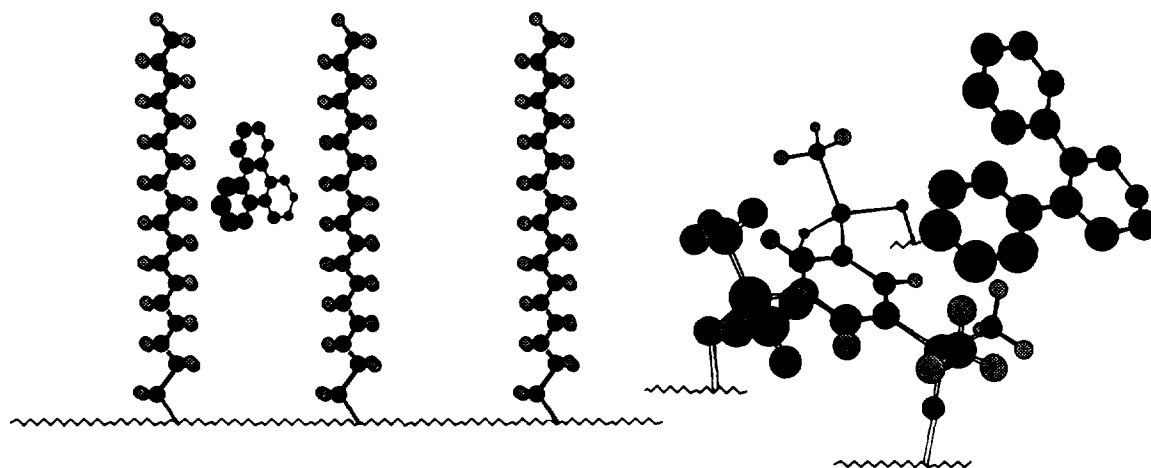


Fig. 3. Schematic diagram of two different dimensional interactions between bonded phase and solute. (Left) vertical situation, C₁₈; (right) horizontal situation, TP.

Planar triphenylene can interact with several methyl groups attached to the silicon atoms which have a bond to the silica gel surface, but the solute can not reach the phenyl group at the bottom part of the cavity in the case of TP phase. However, *o*-terphenyl which is a non-planar molecule, can interact with methyl groups and also the phenyl group at the bottom. The shape of *o*-terphenyl is very suitable allowing it

to be included into the cavity-like structure by a size-fit mechanism. By this concept one can expect the size-fit mechanism for a C₆₀ and C₇₀ separation with multilegged bonded phases. The cavity-size of the TP phase is relatively small and not large enough to include even the smaller C₆₀ molecule. Therefore both C₆₀ and C₇₀ are not included into the cavity and two solutes can not be retained and separated. However the BP

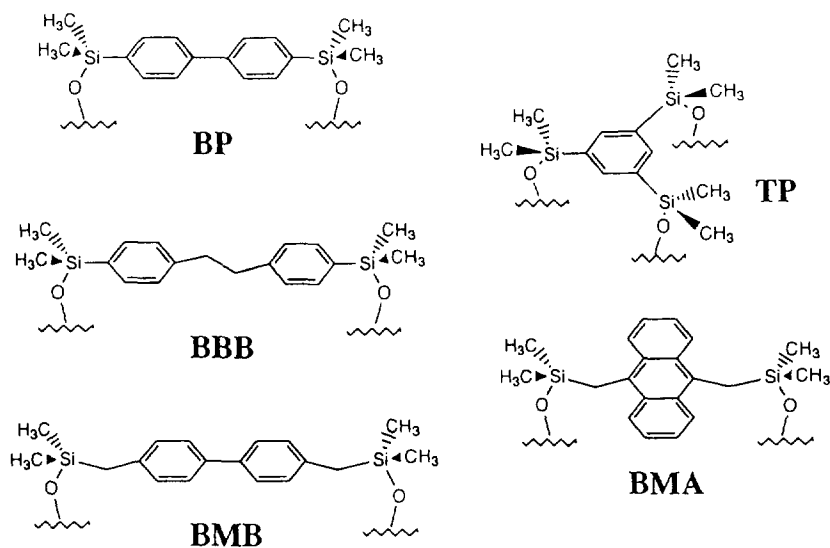


Fig. 4. Various multilegged bonded phases synthesized in this work.

Table 3
Surface coverages of multilegged bonded phases

Stationary phase	C (%)	Surface coverage ($\mu\text{mol}/\text{m}^2$)
BBB	14.2	3.11
BMB	13.4	2.88
BMA	11.6	2.16

phase produces a different situation. The cavity size of the BP phase is about 9–10 Å and the size of C_{60} is 7 Å, and C_{70} is 7×9 Å. Therefore, the size of C_{70} makes it a better fit to the cavity of the BP phase. The retention of C_{70} should be larger than C_{60} and the separation should be improved.

Retention behaviours of fullerenes with multilegged phases

Based on this concept other multilegged phases were also evaluated and the results have indicated that this mechanism works very well for fullerene separations. Table 3 shows the basic properties of several multilegged stationary phases such as BBB, BMB and BMA. The big difference of the basic properties of those phases are surface coverage. BMA has the smallest coverage. And this induces the lowest retention for C_{60} and C_{70} even though it has a large selectivity.

The retention data are summarized in Table 4. BBB and BMB phases retain C_{60} and C_{70} more than either BP or ODS phases. The BMB phase

Table 4
Retention data for C_{60} and C_{70} with various stationary phases

Stationary phase	Capacity factor, k'		Separation factor C_{70}/C_{60}
	C_{60}	C_{70}	
BP	0.75	1.22	1.63
BBB	2.36	4.26	1.80
BMB	5.36	12.0	2.23
BMA	0.24	0.96	2.21
Develosil ODS-5	0.59	0.96	1.65

Mobile phase: *n*-hexane.

shows the largest retention and good selectivity for C_{60} and C_{70} . The difference between BBB and BMB is the location of the methylene groups in their structures. BMB has a diphenyl group in its structure and this group can easily interact with fullerenes because the movement of the diphenyl group is allowed due to two methylene groups between the diphenyl and the silicon atom which can work as a buffer for such movement. Such movement can interact with fullerenes through π - π interaction. The benzyl group of the BBB phase does not have the ability to move freely because one edge is bonded to a silicon atom. The BP phase also does not have such freedom of movement found in the BMB phase. Therefore BMB is the most suitable stationary phase for fullerene separations. The BMA phase also has freedom of movement because the anthracene moiety is not connected to the silicon atom and the phase showed very high selectivity. However, its low surface coverage does not give a large retention because of difficulty of bonding chemistry.

Using BMB another practical possibility has been evaluated. As fullerenes have low solubilities in most organic solvents the mobile phase for preparative scale separations can be selected from only a very few solvents (e.g. toluene). However, pure toluene will not make a good separation, therefore we need to use the mobile phase with toluene used only as a modifier. In

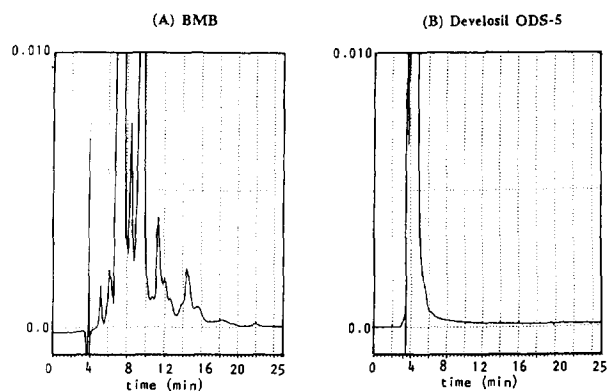


Fig. 5. Chromatograms for the separation of a fullerenes mixture with BMB (A) and Develosil ODS-5 (B). Mobile phase; *n*-hexane-toluene (70:30), flow-rate; 1 ml/min.

order to know the practicality of using BMB as the stationary phase we have tried to use a toluene–*n*-hexane mixture as the mobile phase. As a result two chromatograms were obtained as shown in Fig. 5. *n*-Hexane–toluene (70:30) BMB can give a good separation and selectivity for higher fullerenes but monomeric ODS can only give one peak for all of the fullerenes. So BMB can be useful for preparative scale separations.

3.4. Proposing novel stationary phase

As a conclusion, in order to design a novel stationary phase for enhancing selectivity for fullerenes the following items are required. (i) If the bonded phase is a non-aromatic moiety longer alkyl chains are preferable and the distance between the bonded moieties is crucial to enhancing selectivity (vertical situation). (ii) If the separation mechanism based on size-fit and

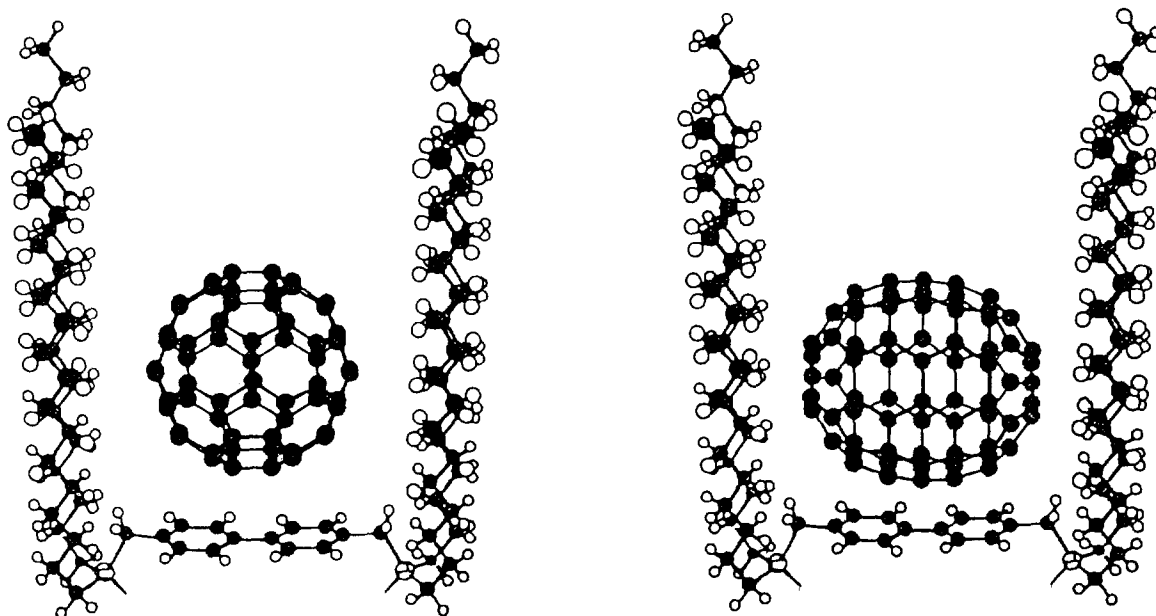
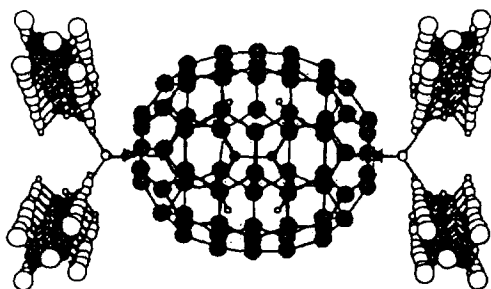


Fig. 6. A novel stationary phase proposed by this investigation. The phase should be made by a silane compound called 4,4'-bis(dioctadecylchlorosilylmethyl)biphenyl.

shape-fit is expected, multilegged phases are the most preferred phases because they can produce a cavity-like structure on the silica surface. The diphenyl group should be included in the structure (horizontal situation). (iii) Combining (i) and (ii) will produce the most desirable novel phase which has long alkyl chains and diphenyl groups at the bottom part of the cavity-like structure. Utilizing these assets a novel phase can be designed as in Fig. 6, where the schematic picture of the most desirable phase is shown. A long alkyl chain (C_{18}) can catch fullerenes and can also form the cavity walls. Biphenyl group at the bottom part can catch fullerenes by $\pi-\pi$ interaction when the solutes enter the cavity. The biphenyl group and methylene group also work to control the distance among each alkyl chain. This novel phase will be synthesized and evaluated in our laboratory in the near future. The discussion in this communication is only focusing on fullerene separations but this concept can be useful for designing new stationary phases to solve other separation problems which require enhanced selectivity.

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