Retention Mechanism of Fullerenes Separation in Liquid Chromatography with Alkyl Bonded Stationary Phases

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DOCTOR OF ENGINEERING

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アルキル鎖化学結合型固定相を用いた液体クロマトグラフィにおけるフラーレン分離の保持メカニズム

（和文要旨）

液体クロマトグラフィ（ＬＣ）は化学のみならず、医、薬、生化学など様々な分野において広く用いられている。ＬＣでは固定相による溶質保持の差により混合物から個々の成分を分離することができる。しかしながら、溶質の保持をコントロールする機構、特に溶質の保持に関する固定相の寄与については詳細な解明は未だ十分ではない。そこで一般的な固定相であるオクタデシルシリカ（ＯＤＳ）などのアルキル鎖化学結合型固定相を用いたＬＣでの保持メカニズムに対して、立体的な形状を有するフラーレン類と平面的な形状を有する多環芳香族炭化水素（ＰＡＨ）を溶質に用いて解析を試みた。

液体クロマトグラフィにおける温度効果については、多くの報告があり、一般的には、温度の低下とともに溶質の保持は、増加するとされている。ところが、我々はＯＤＳを使用したフラーレンの分離において通常とは異なる温度効果のあることを見い出した。ｎ－ヘキサンを移動相として使用し、カラム温度を変化させてフラーレンの保持を測定すると、ある温度で保持が最大となり、平面的な溶質の保持が、温度低下に対して単調に増加する傾向を有するのとは異なる傾向が存在することがわかった。この原因を解析するため、被覆率やアルキル鎖長の異なる固定相を用いフラーレンの保持の温度に対する変化を測定することとした。固体ＮＭＲスペクトル法を用いてＯＤＳとその他のアルキル鎖化学結合型固定相のスペクトルの温度変化も測定した。その結果を基に、溶質保持をコントロールする機構について考察した。固体ＮＭＲでの３０ｐｐｍ付近のオクタデシル鎖の中ほどの－ＣＨ$_2$（メチレン）による信号に着目すると、緩和時間の最小になる温度がフラーレンの保持が最大になる温度と非常によく一致し、固定相アルキル鎖のコンフォメーションの温度変化がフラーレン類の保持に大きく寄与していることがわかった。

本論文の構成は以下の通りである。

第１章では、本研究を行うに至った背景および本研究の目的を述べる。

第２章から４章では、種々のＯＤＳ固定相を用いてフラーレン類の分離を行った結果を述べる。第２章でモノメリックタイプとポリメリックタイプの２種類のＯＤＳ固定相を用いてフラーレンの分離を行った結果について述べる。ここでは、特にポリメリックタイプの固定相の有する分子形状認識能に着目してフラーレン異性体の分離を試みた。第３章では市販の種々のＯＤＳ固定相を用いてフラーレン分離を行った結果を述べ、第４章では特にＣ$_{84}$以降のハイヤーフラーレンの分離を行った結果を示す。

第５章ではメトキシフェニルプロピル固定相を用いたフラーレンの分離を行った結果を述べる。アルキル鎖からのみからなるポリメリックタイプＯＤＳ固定相とは異なった分子形状認識能について述べる。

第６章から８章ではアルキル鎖化学結合型固定相を用いたフラーレン類の分離における温度効果に着目し、その保持機構を考察した。第６章では異なる被覆率のＯＤＳ固定相を用い、温度を変化させてフラーレン類の分離を行い、低温下でのフラーレン類の特異な保持挙動の原因について考察した。第７章では固体ＮＭＲスペクトル法を用いて固定相アルキル鎖結合相の温度変化に対するスペクトル変化を調べた結果を述べる。そしてこの変化がＬＣでのフラーレン類の保持挙動とうまく対応すること示す。第８章では種々のアルキル鎖長の固定相を用い、フラーレン類のＬＣ分離における温度効果並びにアルキル鎖長の効果、アルキル鎖間距離の効果、フェニル基の効果について調べた結果を述べる。アルキル鎖長の違い、根元のフェニル基の有無によりフラーレンの保持に差を生ずることを示し、それをＮＭＲの結果と合わせて、アルキル鎖化学結合型固定相を用いたＬＣにおけるフラーレン類の保持をコントロールする機構についての考察を行った。

最後に９章では、本論文の結論を述べる。

Summary

In chemical, medical, pharmaceutical sciences, high performance liquid chromatography (LC) is one of the most widely used technique to separate various components from complex mixture. Nevertheless the retention mechanism in the chromatographic process has not been clear well. The aim of this study is to understand the retention mechanism of fullerenes separation with alkyl bonded stationary phases, especially octadecylsilica (ODS), at relatively low temperature region.

The temperature effect on the retention of fullerenes in LC was examined using ODS and other alkyl bonded stationary phases. In LC with ODS phases, in general, solute retention is inversely related to temperature, i.e., retention decreases with increasing temperature. However, It was found that the maximum retention temperature of bulky and spherical fullerenes lies around - 10 °C with the higher surface coverage ODS phase. In contrast, planar solutes such as polycyclic aromatic hydrocarbons (PAHs) maintained the expected behavior, i.e. reduction of the column temperature resulted in a monotonic increase in retention. Solid state CP-MAS NMR spectrometric measurements of the stationary phases have indicated that the relaxation time of the 30 ppm methylene signal changes with the temperature and it has a minimum relaxation time at the temperature which closely matches the maximum retention temperature observed by chromatographic measurements. This strong correlation between the changes in relaxation time and retention indicate that the retention of fullerenes with alkyl bonded silica stationary phases is dependent on the rigidity and orderliness of the ligand as well as the interval between adjacent moieties. The basic interpretation of retention mechanism with various alkyl bonded stationary phases, especially at low temperatures, was performed using both NMR spectra and LC chromatographic retention data.

In Chapter 1, general introduction and the aim of this dissertation are described.

In Chapter 2, the separation of fullerenes with monomeric and polymeric ODS bonded phases was studied. It was found that the functionality of ODS is a very important factor in controlling the elution of higher fullerenes; i.e., the polymeric ODS is more powerful in

separating higher fullerenes than the monomeric ODS by their shape difference, while the latter can separate them by the molecular weight or size.

In Chapter 3, various ODSs were evaluated for the separation of fullerenes with toluene-based mobile phase systems.

In Chapter 4, the existence of several higher fullerenes larger than C_{86} was confirmed and their UV/Vis spectra were obtained by the photodiode array detection coupled to the analytical LC. The identification of higher fullerenes larger than C_{86} was accomplished by Liquid Chromatography-Mass Spectrometry (LC-MS) measurements.

In Chapter 5, fullerenes were separated using dimethoxyphenylpropyl (DMP) bonded stationary phases with n-hexane as the mobile phase. It was found that the DMP exibit a relatively smaller dependence on temperature while maintaining the resolution. For the separation of higher fullerenes, DMP offers faster analysis at higher temperatures while maintaining its high resolution.

In Chapter 6-8, the temperature dependency of the separation of fullerenes in LC has been examined using various alkyl bonded stationary phases. The retention behavior of fullerenes in low temperature region on the alkyl bonded stationary phases is discussed using information obtained by CP-MAS solid-state NMR spectroscopy and LC.

In Chapter 6, the effect of column temperature, especially at low temperatures, on the separation of fullerenes with monomeric and polymeric ODS bonded phases was studied. Though, decreasing the ODS column temperature induces an increase in selectivity, the maximum retention temperature of fullerenes was found for both types of ODS phases with n-hexane eluent. It was found that the selectivity for higher fullerenes on monomeric phases becomes similar to that on polymeric phases at low temperature. It was also found that as the carbon content of monomeric phases is increased, the selectivity also becomes similar to that with the polymeric phases.

In Chapter 7, both CP-MAS NMR spectra and LC chromatographic data are interpreted. The temperature effect on the retention of fullerenes in the range 80 ˚C to -70 ˚C in LC has been examined using ODS. It was found that the maximum retention temperature lies around - 10 ˚C with the higher surface coverage ODS phase. CP-MAS NMR measurements on the stationary phases indicated that the relaxation time at the 30 ppm methylene signal changes with the temperature and has a minimum relaxation time at the temperature which closely matches the maximum retention temperature observed by LC.

In Chapter 8, several phases which have different ligand intervals and chain lengths was examined, and then the effect on the retention of fullerenes of column temperature, the alkyl chain length and the ligand interval between each alkyl chain was discussed. It was found that a maximum retention temperature exists with long alkyl bonded stationary phases, whereas there is no similar effect with the newly synthesized alkyl bonded phases which have two phenyl groups at the base of the bonded phase. The retention behavior of fullerenes in the low temperature region on alkyl bonded stationary phases is discussed using information obtained by CP-MAS NMR spectroscopy and LC.

In Chapter 9, the conclusion of this dissertation is described.

Contents

Chapter 1

Introduction

Alkyl bonded stationary phases, especially octadecylsilica (ODS), have been one of the most powerful and widely used stationary phases in high performance liquid chromatography (LC). To understand the retention mechanisms, many efforts have been expended. However, the contribution of the stationary phase is generally not known well yet. The purpose of this dissertation is to clarify the contribution of the stationary phase to the mechanism.

Since the existence of C_{60} was confirmed, the unique-shape carbon molecules, so-called "fullerenes", have been attracting attention in a wide variety materials science. In 1970, the possibility of the existence of a 60-carbon cluster molecule was predicted by Osawa [1], and then in 1985 a stable carbon molecular ion consisting of 60 atoms was observed in the time-of-flight mass spectrometric measurements of laser ablated graphite [2]. In 1990, Kratschmer et al. [3] found an excellent synthetic method of carbon clusters. In order to do scientific investigation on fullerenes, various chromatographic separation techniques are required to separate and purify these molecules. LC is probably the best method one can choose for this application. The number of LC stationary phases for this purpose has been increased tremendously over the past few years.

In LC separation of fullerenes, the popular stationary phase called "ODS" is generally used with n-hexane as the mobile phase[4-8], although other novel stationary phases have also been used for this purpose[9-11]. Two main reasons why ODS is popular as the primary stationary phase are: i) ODS is easily commercially available, and ii) optimization of the separation is easier than other novel stationary phases. Therefore, it is reasonable to consider that for higher fullerenes separations ODS should be first tested before the evaluation of other novel phases.

The ODS phases can be divided into two different types based on their bonding chemistry used for the preparation of the phases. One is monomeric type and another is polymeric type. The monomeric type is synthesized by monochlorosilane and the polymeric type is synthesized by trichlorosilane as the starting material. The difference of both ODS phases is defined by the C_{18} alkyl chains on the silica surface, the former has the isolated single C_{18} chains but the latter has the possibility to have the networked chains (this is the reason why this phase is so-called "polymeric type").

Wise and Sander have examined this topic intensively [12-15] and they have found that with polymeric ODS the tendency to recognize the molecular planarity of PAHs is stronger than with the monomeric ODS. They have concluded that the structure of the polymeric ODS is more rigid and slit-like (they called "slot-model") than that of the monomeric ODS. Thus the polymeric ODS cannot interact well with non-planar PAHs because of its slit-like structure, while planar solutes can be retained more strongly, because they more easily enter the slits.

Various ODS stationary phases were evaluated for the separation of fullerenes (Chapter 2-4). The fullerenes are nonplanar shape aromatic molecules, it is easily supposed that the functionality of ODS is a very important factor in controlling the elution of fullerenes. Polymeric ODS is more powerful in separating higher fullerenes than monomeric ODS by their shape difference, while the latter can separate them by the molecular weight or size. However, it is the big question arised what is the actual difference between the bonded phases of polymeric ODS and the ones of monomeric ODS. Therefore, this dissertation is focusing on the model of interaction between solutes and stationary phases structures, especially between fullerenes and alkyl bonded stationary phases.

In this study on fullerenes separation (Chapter 6), the maximum retention temperature of fullerenes was found for an ODS phase with an average ligand interval shorter than the approximate diameter of fullerenes. This result supports the idea that the configurational change of the alkyl chains with temperature is the most important factor in controlling the retention of solutes in LC. Large bulky solutes such as fullerenes can apparently magnify this effect. In order to give the most reasonable explanation, the following experiments and evaluation have been performed (Chapter 7-8).

In this study, it was also observed that NMR spectral changes as a function of temperature for the ODS phase (Chapter 7). The drastic changes of spectra , especially with the signals around 30 ppm, are induced by temperature variations. The signals are typically assigned to methylene(CH_2) groups in the middle part of the alkyl chains [15-19]. These results suggested that a more careful examination of the NMR spectra would yield insights into the configuration changes of the alkyl chains with temperature. Then it was supposed that these aspect should provide an explanation of the chromatographic behavior observed above.

In order to confirm the importance of average ligand interval and chain length, it is necessary to investigate new phases which have different ligand intervals and chain lengths. For this purpose, we have synthesized and investigated several phases which have different alkyl chain lengths and have two phenyl groups at the base of the bonded phases (Chapter 8). Then, the effect on the retention of fullerenes of column temperature (especially in the low temperature region), the alkyl chain length of the bonded stationary phases, the ligand interval between each alkyl chain and the contribution of the phenyl groups was discussed by using liquid chromatographic and NMR spectroscopic information.

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Chapter 2

Separation and Identification of Higher Molecular Weight Fullerenes by High-Performance Liquid Chromatography with Monomeric and Polymeric Octadecylsilica Bonded Phases*

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*Modified from: *Anal.Chem.*, **65**, 2650-2654 (1993).

2.1 Abstract

The separation of fullerenes with monomeric and polymeric ODS bonded phases has been studied and the existence of three C_{78} isomers confirmed. It has been found that the elution order of higher fullerenes is controlled by their molecular size and shape and that polymeric ODS resolves three C_{78} isomers, one being eluted faster than C_{76}. Monomeric ODS cannot resolve two of the C_{78} isomers, although a lower column temperature of 10 °C gives its partial separation. UV/Visible spectral assignments were confirmed by FAB-MS measurements of the fractionated samples.

2.2 Introduction

Separation of fullerenes in the soot extract is an important analytical problem, considering the increasing attention paid to these new compounds of potential importance in materials science, superconductivity, and other related technologies. LC approaches have been reported to separate typical fullerenes such as C_{60} and C_{70}, and other higher molecular weight fullerenes [1-5]. Stationary phases which have had some success in separating fullerenes, such as Pirkle type [6], multi-legged phenyl bonded phase [7], and multi-methoxyphenylpropyl phases [8] indicate the need to study in more detail the retention mechanism of fullerene LC separations as based on molecular recognition. However, those approaches still need development before they could be useful for industrial or large laboratory-scale separations. Therefore, the main approach at present for separation of various sizes of fullerenes is to use ODS bonded phases with toluene-based mobile-phase systems, which increase the solubility of fullerenes in the mobile phase and hence the amount injectable into the separation system. Although several investigators have reported the use of ODS stationary phases for this analysis [9-18], basic discussions on whether polymeric-type or monomeric-type phases are a better choice have not appeared in the literature. In this chapter, we investigate which is the better stationary phase for fullerene separation, monomeric or polymeric, focusing on isomer resolution. It has been found that monomeric ODS phase is the more powerful stationary phase for the isomer separations, based on the difference in their molecular weights, but polymeric ODS is better for the separation based on molecular shape difference.

2.3 Experimental

Instrumentation

The mass spectrometer (MS) used in this work was a JEOL JMS-SX102 A (Akishima, Japan). The ionization was performed by fast atom bombardment (FAB) using Xe (5 kV) as the primary beam and *m*-nitrobenzylalcohol (*m*-NBA) plus 1 % toluene as the matrix. The samples were introduced into the MS system using a flow injection method.

Basic studies on LC separations were performed using a 880 PU LC pump (Jasco, Hachioji, Japan) combined with a UV detector (Shodex M315, Showa Denko, Tokyo, Japan) set at 320 nm or a Jasco MD-920 UV/Vis photodiode array detector. Mobile phases were toluene-methanol and toluene-acetonitrile mixtures. A 4.6 mm i.d. x 250 mm Develosil ODS-5 column (monomeric ODS, 5 μm, Nomura Chemicals, Seto, Japan) and the same size Wakosil II 5C18AR column (polymeric ODS, 5 μm, Wako Chemicals, Tokyo, Japan) were used in the evaluation. The flow rate of the mobile phases was always 1 mL/min. The same Develosil ODS-5 stationary phase packed into a 20 mm i.d. x 250 mm column was used for the semipreparative-scale separations together with a Jasco 880 PU pump connected to a Jasco 880 UV detector. The mobile phase for the sample fraction collection process was a toluene-methanol mixture which was optimized using an analytical scale column with a flow rate of 10 mL/min. Column temperature was controlled by a LAB-Thermo Model LH-1000E(Toyo Seisakusho, Tokyo, Japan) and Tosoh RE-8000 oven (Tokyo, Japan).

Sample Preparation

Carbon soot was produced by an arc discharge in an inert gas environment. The soot was first extracted with toluene (fraction A in Figure 2-1, where the sample preparation process is schematically summarized), and then the residue was extracted with trichlorobenzene in order to obtain a high concentration of fullerenes larger than C_{70} (fraction B in Figure 2-1). The

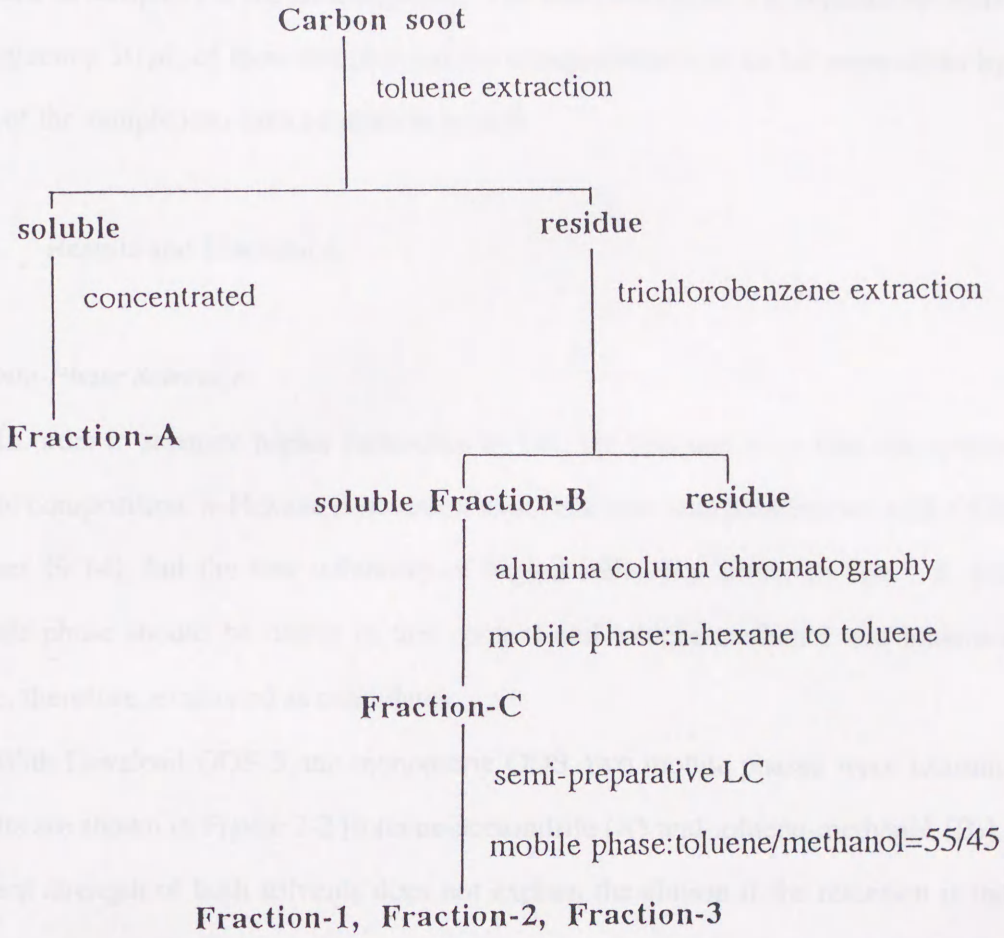

Figure 2-1. Schematic diagram of the sample preparation process.

remaining C_{60} and C_{70} in fraction B was eliminated by passing through an alumina column with an *n*-hexane and toluene gradient elution (fraction C in Figure 2-1). Fraction C was used as the sample for the MS measurements after preparative-scale LC separation and fractionation (Fraction 1-3). Those several fractions were evaporated to dryness and then dissolved again in toluene as samples for the investigation. The analytical-scale LC separations were performed by injecting 10 μL of these samples and the semipreparative scale LC separations by injecting 1 mL of the sample into each separation system.

2.4 Results and Discussion

Mobile-Phase Selection

In order to separate higher fullerenes by LC, the first task is to find the optimum mobile-phase composition. *n*-Hexane is common in several previous publications with ODS stationary phases [9-14], but the low solubility of higher fullerenes limits its use. A toluene-based mobile phase should be useful in this context and toluene-methanol and toluene-acetonitrile were, therefore, evaluated as candidates.

With Develosil ODS-5, the monomeric ODS, two mobile phases were examined and the results are shown in Figure 2-2 [toluene-acetonitrile (A) and toluene-methanol (B)]. Although solvent strength of both solvents does not explain the elution if the retention is induced by a typical reversed-phase mechanism, both give relatively good separations for fullerenes higher than C_{70}. One can choose the countersolvent in toluene on the following basis: (1) if a better separation for higher fullerenes is desired, acetonitrile is the choice; (2) if faster analysis with a high-resolution column is required, methanol should be used. As this investigation was using conventional-size columns for analytical separation, toluene-acetonitrile was preferred. Experiments to find the optimum composition of toluene and acetonitrile in mobile phase showed 40-60 % acetonitrile in toluene to give the best compromise in resolution, analysis time, and solubility of the samples.

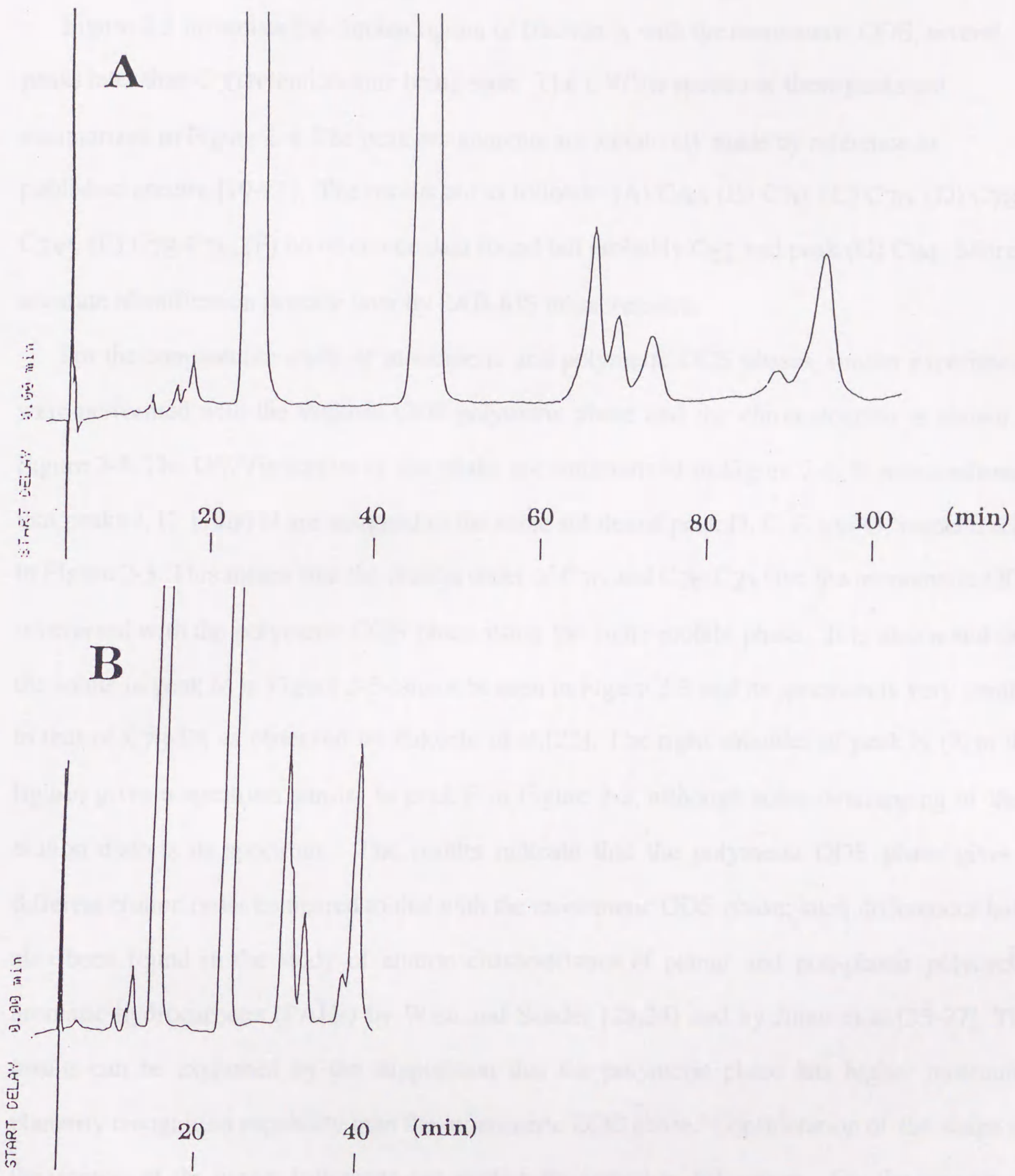

Figure 2-2. Chromatograms of fraction A with toluene-acetonitrile and toluene-methanol mobile phase systems: (A)toluene/acetonitrile=50/50, (B)toluene/methanol=50/50. column, Develosil ODS-5; temperature; room temperature (ca. 15-20 °C), mobile-phase flow rate, 1 mL/min; detector; UV at 325 nm.

Comparison of Monomeric and Polymeric ODS phases for Higher Fullerene Separation

Figure 2-3 illustrates the chromatogram of fraction A with the monomeric ODS, several peaks later than C_{70} retention time being seen. The UV/Vis spectra of these peaks are summarized in Figure 2-4. The peak assignments are tentatively made by reference to published spectra [19-22]. The results are as follows: (A) C_{60}, (B) C_{70}, (C) C_{76}, (D) C_{78}-C_{2v}', (E) C_{78}-C_{2v}, (F) no reference data found but probably C_{82} and peak (G) C_{84}. More accurate identification is made later by FAB-MS measurements.

For the comparative study of monomeric and polymeric ODS phases, similar experiments were performed with the Wakosil ODS polymeric phase and the chromatogram is shown in Figure 2-5. The UV/Vis spectra of the peaks are summarized in Figure 2-6. It was confirmed that peaks J, K, L and N are assigned to the same solutes of peak D, C, E and G, respectively, in Figure 2-3. This means that the elution order of C_{76} and C_{78}-C_{2v}' for the monomeric ODS is reversed with the polymeric ODS phase using the same mobile phase. It is also noted that the solute in peak M in Figure 2-5 cannot be seen in Figure 2-3 and its spectrum is very similar to that of C_{78}-D_3 as observed by Kikuchi et al.[22]. The right shoulder of peak N (Z in the figure) gives a spectrum similar to peak F in Figure 2-3, although some overlapping of their elution distorts its spectrum. The results indicate that the polymeric ODS phase gives a different elution order compared to that with the monomeric ODS phase; such differences have also been found in the study of elution characteristics of planar and non-planar polycyclic aromatic hydrocarbons (PAHs) by Wise and Sander [23,24] and by Jinno et al.[25-27]. The results can be explained by the supposition that the polymeric phase has higher molecular planarity recognition capability than the monomeric ODS phase. Consideration of the shape of the isomers of the higher fullerenes can explain the retention differences. For the isomers of C_{78}, C_{78}-C_{2v}' has the longest of the shorter diameter (most bulky), C_{78}-C_{2v} has a little different symmetry, and D_3 has a narrower shape than the other two isomers. The polymeric ODS elutes bulkier C_{2v}' first and the more cylindrical D_3 late by virtue of its planarity recognition capability. The reversed elution order of C_{84} and C_{82} with the polymeric ODS (as tentatively assigned, peak F in Figure 2-3 and the right shoulder of peak N (Z in Figure 2-5)) can be also explained the same way. The C_{82} shape is considered to be longer and narrower

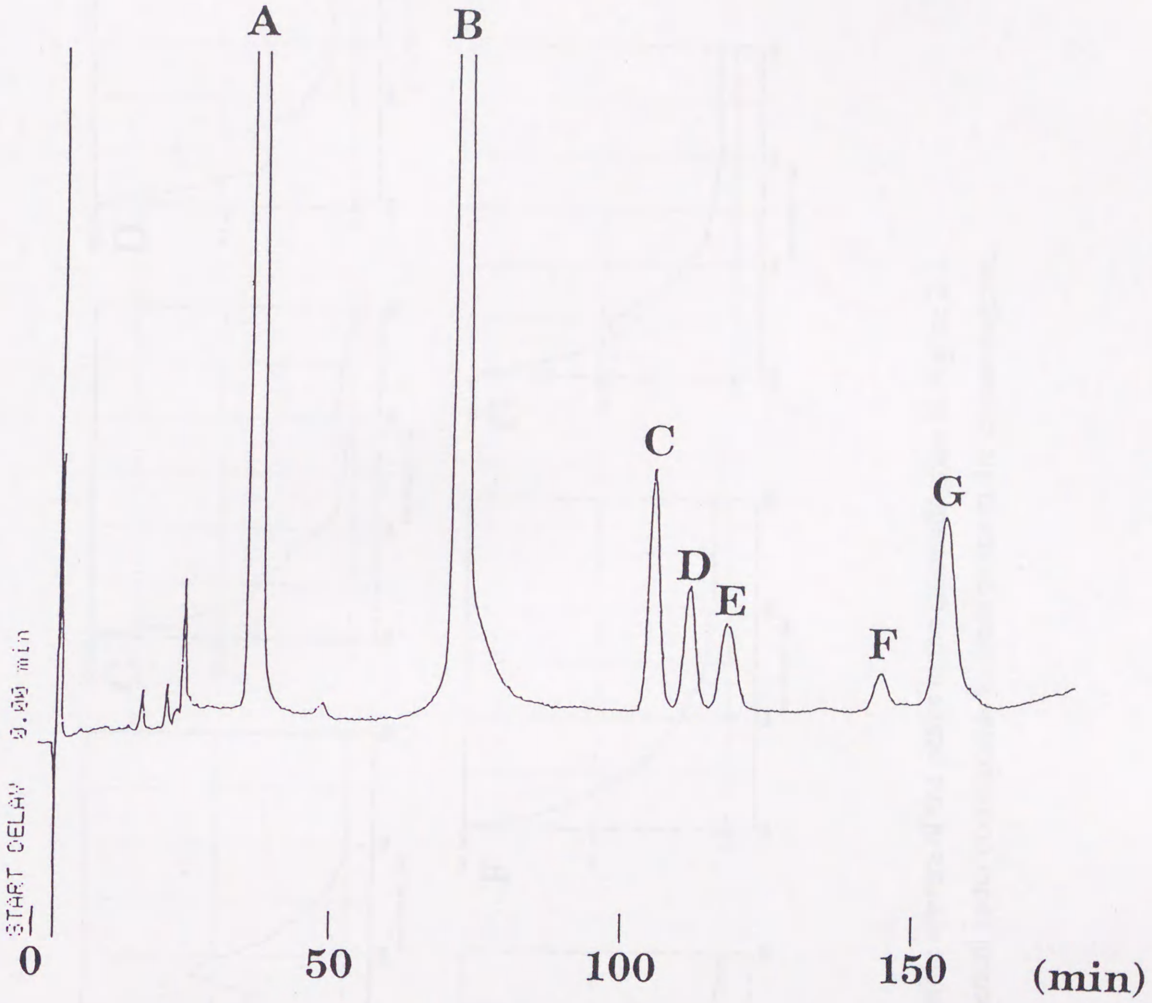

Figure 2-3. Chromatogram of fraction A with the monomeric ODS. mobile phase, toluene/acetonitrile=45/55; flow rate, 1 mL/min, detection, UV at 325 nm; temperature, room temperature.

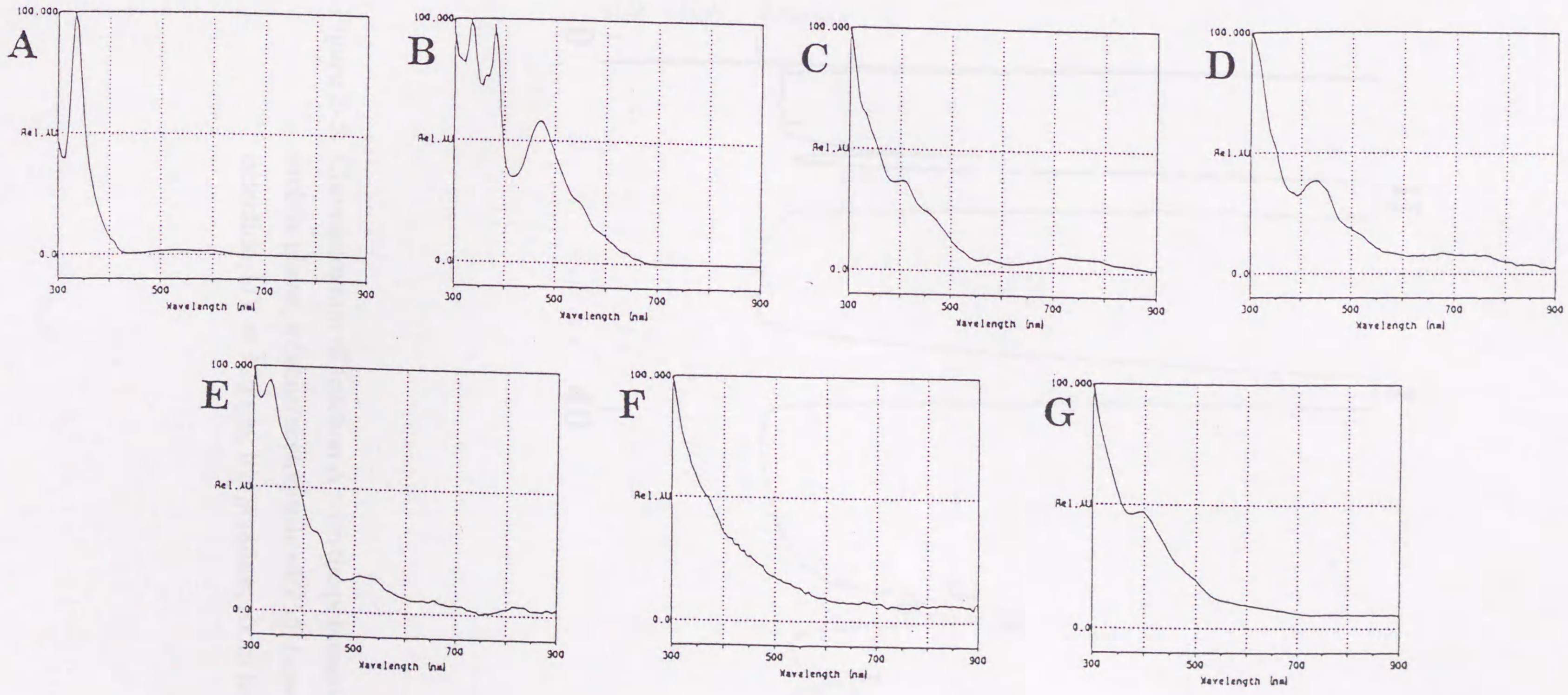

Figure 2-4. UV-Visible spectra of the peaks in the chromatogram in Figure 2-3.
Alphabetical order corresponds to these peaks in the chromatogram.

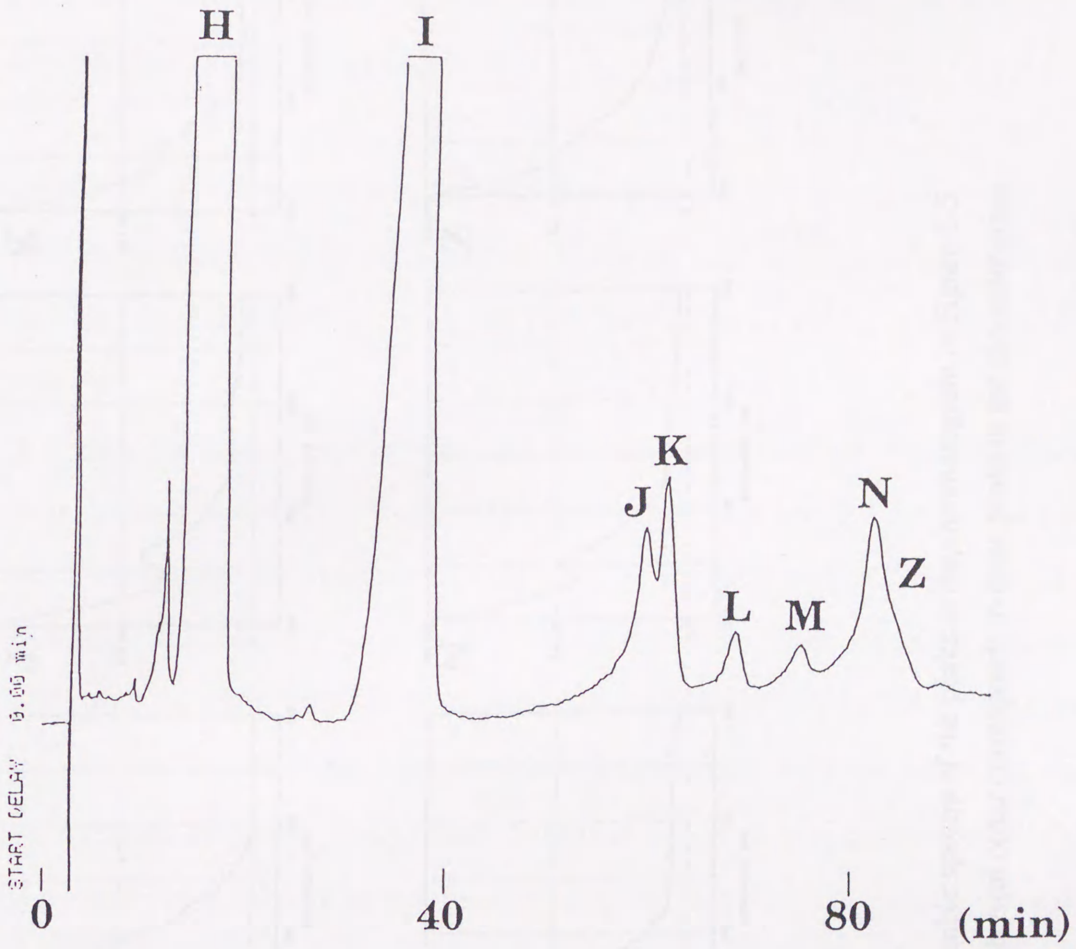

Figure 2-5. Chromatogram of fraction A with the polymeric ODS: mobile phase, toluene/acetonitrile=45/55; flow rate, 1 mL/min, detection, UV at 325 nm; temperature; room temperature.

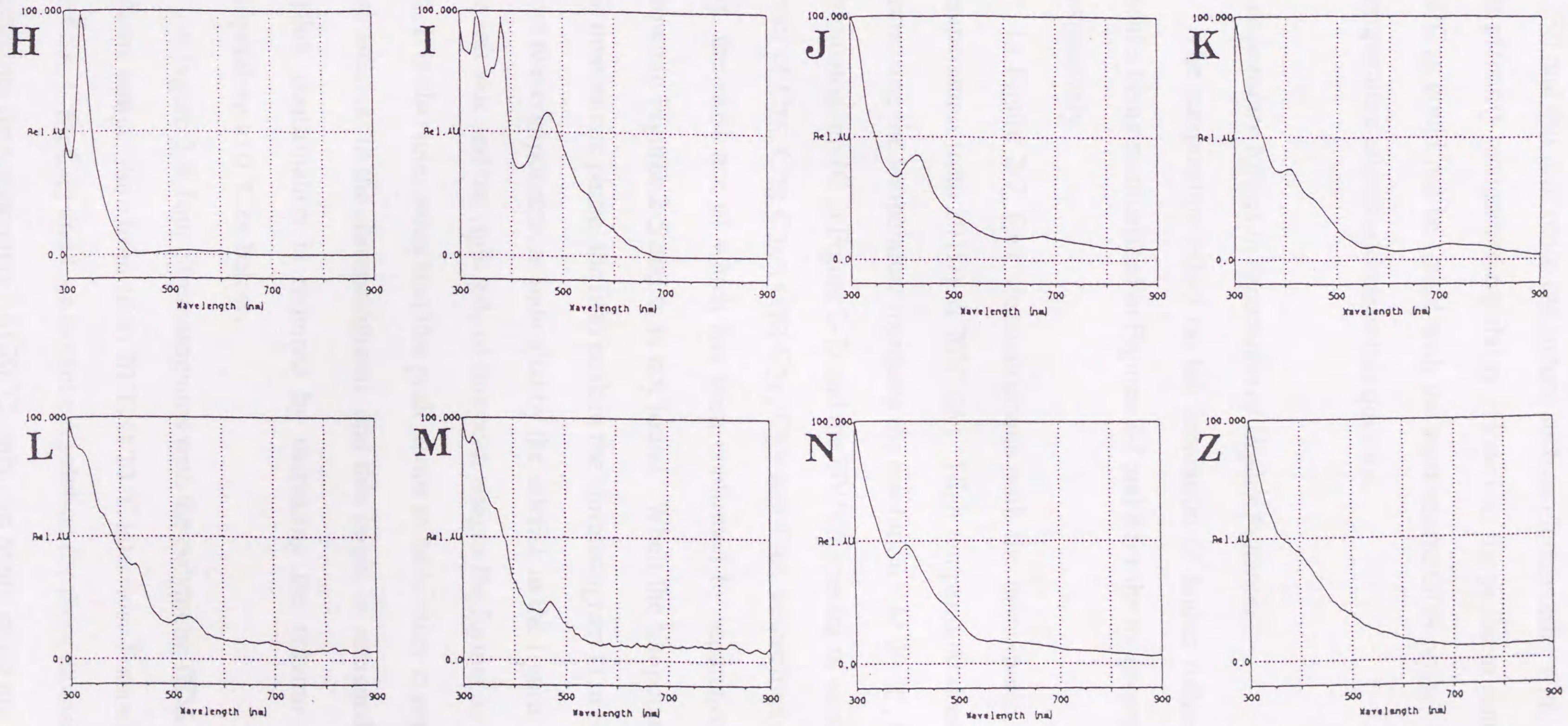

Figure 2-6. UV-visible spectra of the peaks in the chromatogram in Figure 2-5. Alphabetical order corresponds to these peaks in the chromatogram.

than C_{84} (although there are possibilities for at least three isomers of this fullerene to exist [28]) and this difference can induce such an elution order with the polymeric phase because of its planarity recognition capability. However, the problem remained as to why the D_3 isomer of C_{78} could not be found with the monomeric ODS phase. The following investigation of temperature effects addresses this question.

Temperature Effects in Separation of Higher Fullerenes

The temperature effect on the separation of higher fullerenes has been investigated, the results being summarized in Figures 2-7 and 8 for the monomeric and the polymeric phases, respectively.

In Figure 2-7, four chromatograms with the monomeric ODS are illustrated at column temperatures from 10 (D) to 70°C (A). High temperature does not give good resolution, and decreasing the temperature increases the resolution. At 30 °C, five clear peaks are seen in the chromatogram (C in Figure 2-7) and the UV/Vis spectra of each peak assign the solutes in the order of C_{76}, C_{78}-C_{2v}', C_{78}-C_{2v}, C_{82} and C_{84}, respectively. However, the peak for C_{78} D_3, the existence of which has been confirmed by separation with the polymeric phase as shown in Figures 2-5 and 6, is not found. When the temperature is decreased to 10 °C with the monomeric phase, the fifth peak in the chromatogram D in Figure 2-7 appears to include at least two components, as indicated by the asterisk in the figure. UV/Vis spectral matching for the left side and the right side of this peak assigns the former as C_{78}-C_{2v} and the latter as C_{78}-D_3. It is also interesting that this peak seems to have other components coeluted (as shown by two asterisks in the chromatogram), and this might be assigned as the second isomer of C_{84}; further confirmation is required by increasing the column efficiency or decreasing the temperature to 0 °C or below.

In Figure 2-8, four chromatograms with the polymeric ODS are shown with changing column temperature from 10 to 70 °C at 20 °C intervals. Focusing on the elution profiles for the C_{76}, C_{78}, C_{82} and C_{84} isomers, resolution for those isomers is also improved upon decreasing the temperature. At 70 °C, only two major peaks are found and the higher fullerenes are eluted at the shoulder of the second peak; however, at 50 °C, four major peaks

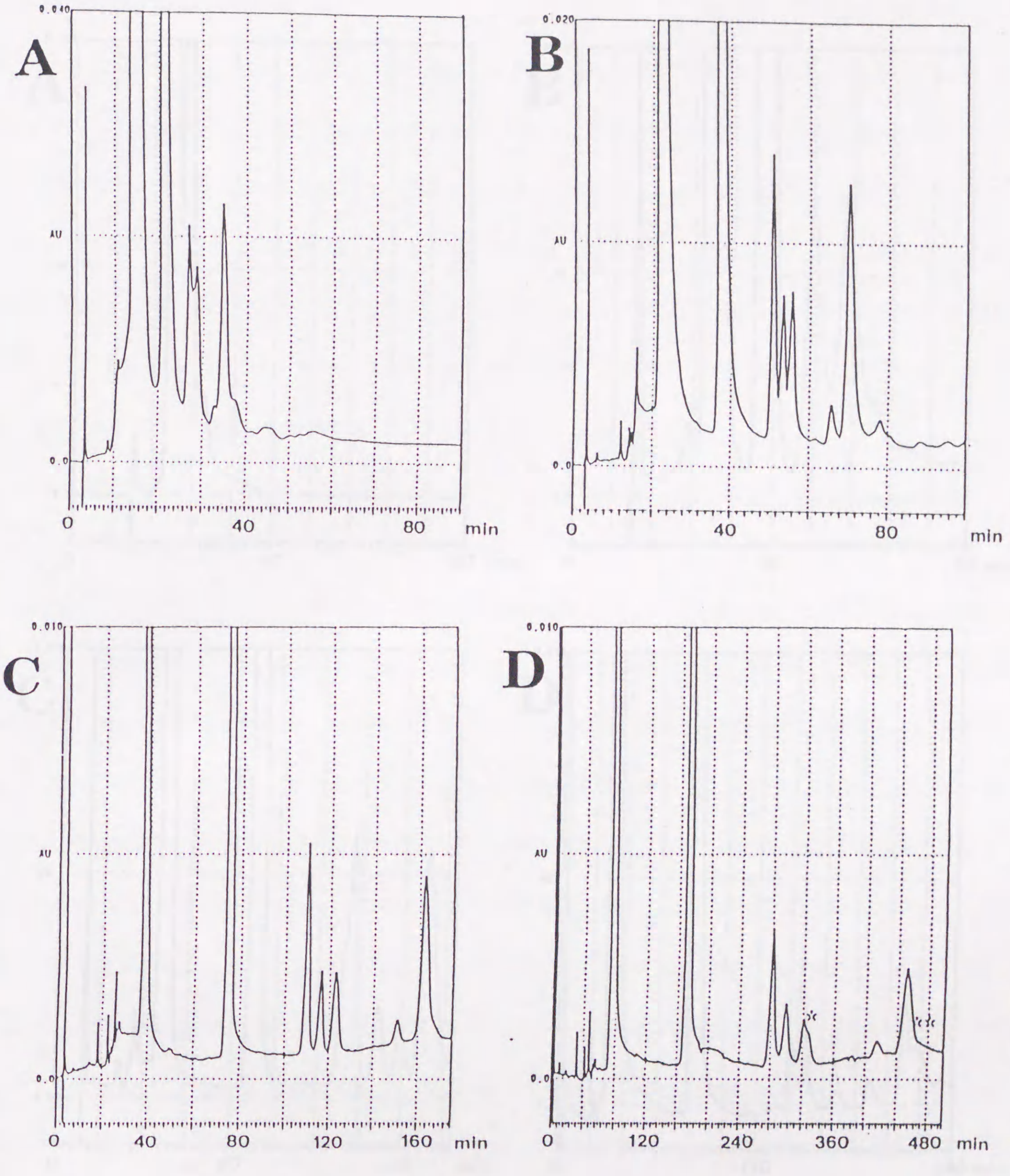

Figure 2-7. Chromatograms of fraction A with the monomeric ODS at different column temperatures: (A) 70, (B) 50, (C) 30 and (D) 10 °C. Mobile phase, toluene/acetonitrile=45/55; flow rate, 1 mL/min; detection; UV at 325 nm.

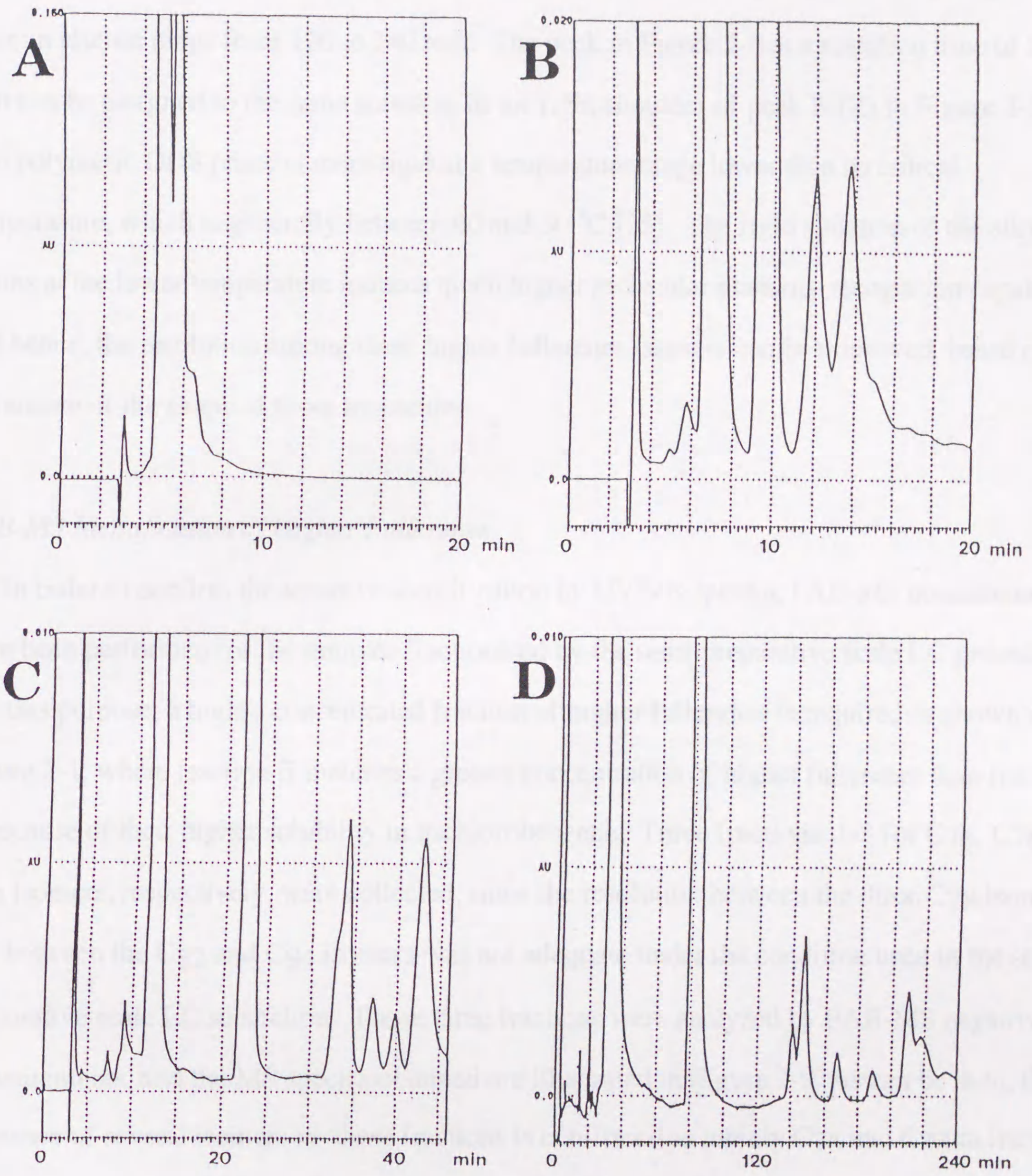

Figure 2-8. Chromatograms of fraction A with the polymeric ODS at different column temperatures: (A) 70, (B) 50, (C) 30 and (D) 10 ˚C. Mobile phase, toluene/acetonitrile=45/55; flow rate, 1 mL/min; detection; UV at 325 nm.

are seen. At 30 °C, clear peaks for the higher fullerenes isomers are found and eight peaks are resolved at 10 °C. The elution order at 10 °C can be defined by the UV/Vis spectral measurements and that is C_{78}-C_{2v}', first, C_{76}, C_{78}-C_{2v}, C_{78}-D_3, C_{84} and probably C_{82}, over an elution range from 120 to 240 min. The peak in Figure 2-8 at a retention time of 220 min can be assigned to the same solute as in the right shoulder of peak N (Z) in Figure 2-5. The polymeric ODS phase is more rigid at a temperature range lower than its critical temperature, which is generally between 40 and 50 °C [25]. The rigid situation of the alkyl chains at the lower temperature induces much higher molecular planarity recognition capability, and hence, the resolution among these higher fullerenes isomers can be improved, based on the difference of the shape of those molecules.

FAB-MS Identification of Higher Fullerenes

In order to confirm the tentative identification by UV/Vis spectra, FAB-MS measurements have been performed for the samples fractionated by the semi-preparative scale LC procedure. For this purpose, a highly concentrated fraction of higher fullerenes is required as shown in Figure 2-1, where fraction B includes a greater concentration of higher fullerenes than fraction A because of their higher solubility in trichlorobenzene. Three fractions, 1-3 for C_{76}, C_{78} and C_{84} isomers, respectively, were collected, since the resolution between the three C_{78} isomers and between the C_{82} and C_{84} isomers was not adequate under the condition used in the semi-preparative scale LC separation. Those three fractions were analyzed by FAB-MS negative ion measurements, and the MS spectra obtained are illustrated in Figure 2-9. As can be seen, the existence of several isomers in these fractions is confirmed as mainly C_{76} and C_{78} in fraction-1, C_{78} and small amounts of C_{76} in fraction 2, and C_{82} and C_{84} in fraction 3. There are some signals caused by isomers oxides whose existence can be explained by two major reasons: (1) they may be produced in the ionization process of the FAB-MS measurements and (2) it is well-known that if fullerenes are left in a solution which is exposed to air, oxidation of these materials occurs. The assignments by UV/Vis spectral measurements are now confirmed. The UV/Vis spectra of peak F in Figure 2-3 and the right shoulder of peak N (Z) were thus confirmed as C_{82}, because C_{82} signals are clearly seen in the mass spectrum of fraction 3.

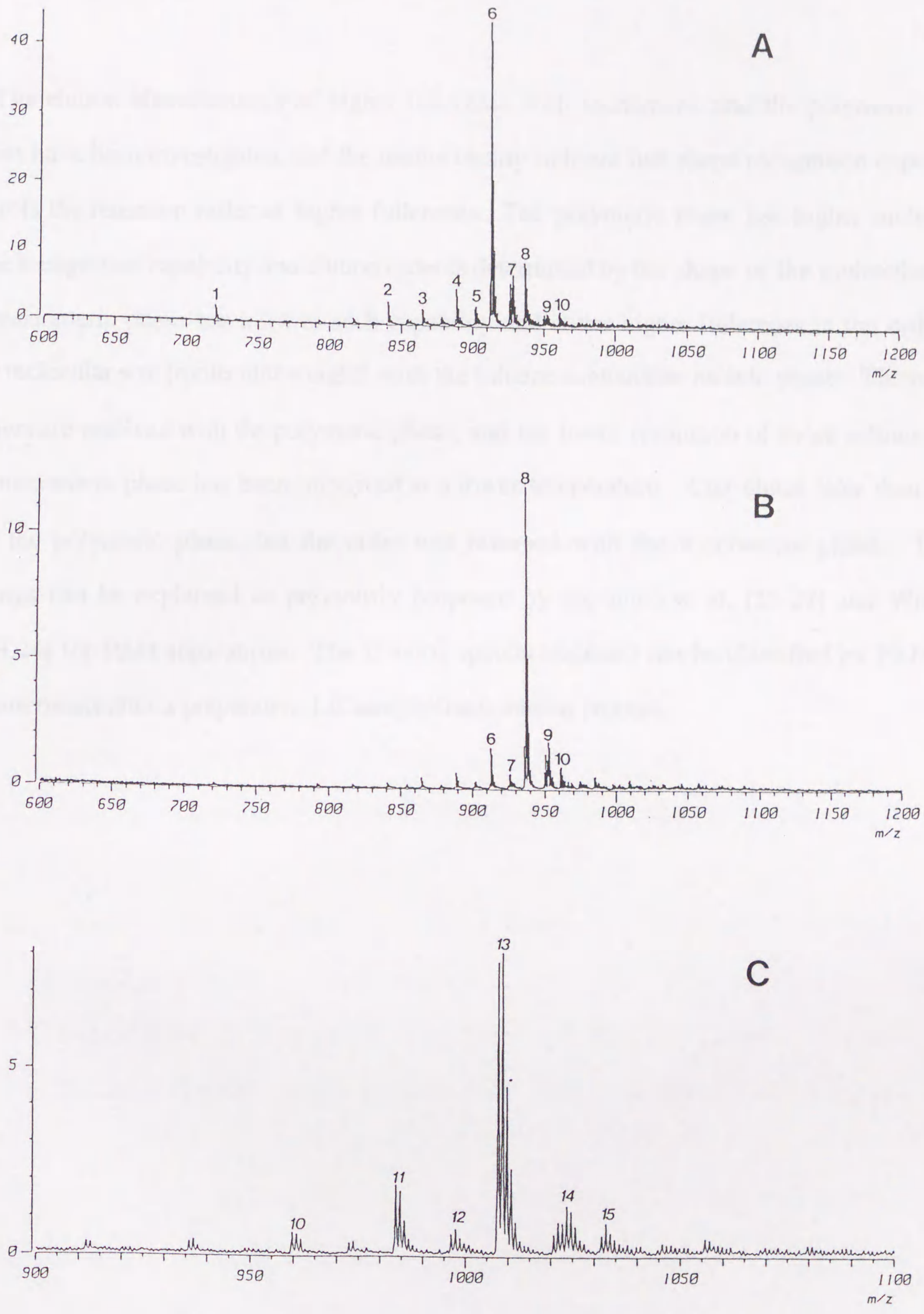

Figure 2-9. FAB-MS spectra for three fractions: (A) fraction 1, (B) fraction 2 and (C) fraction 3. Mass number tentative assignments: (1) C_{60}, (2) C_{70}, (3) C_{72}, (4) C_{74}, (5) $C_{74}O$, (6) C_{76}, (7) $C_{76}O$, (8) C_{78}, (9) $C_{78}O$, (10) C_{80}, (11) C_{82}, (12) $C_{82}O$, (13) C_{84}, (14) $C_{84}O$ and (15) C_{86}.

2.5 Conclusion

The elution characteristics of higher fullerenes with monomeric and the polymeric ODS phases have been investigated, and the results clearly indicate that shape recognition capability controls the retention order of higher fullerenes. The polymeric phase has higher molecular shape recognition capability and elution order is determined by the shape of the molecules, but the monomeric phase has a lower such capability and elutes higher fullerenes in the order of their molecular size (molecular weight) with the toluene-acetonitrile mobile phase. Three C_{78} isomers are resolved with the polymeric phase, and the lower resolution of those solutes with the monomeric phase has been improved at a lower temperature. C_{82} elutes later than C_{84} with the polymeric phase, but the order was reversed with the monomeric phase. These findings can be explained as previously proposed by the Jinno et al. [25-27] and Wise et al.[23,24] for PAH separations. The UV/Vis spectra obtained can be identified by FAB-MS measurements after a preparative-LC sample fractionation process.

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Chapter 3

Liquid Chromatographic Separation of Higher Fullerenes with Octadecylsilica Stationary Phase*

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*Modified from: *Chimica Oggi*, **12** (No. 9), 19-24 (1994).

3.1 Abstract

Various ODS stationary phases were evaluated for the separation of fullerenes with toluene-based mobile phase systems. As the results demonstrate, it has been found that the functionality of ODS is a very important factor in controlling the elution of higher fullerenes; i.e., polymeric ODS is more powerful in separating higher fullerenes than monomeric ODS by their shape difference, while the latter can separate them by the molecular weight or size. In this chapter, the effects of the surface coverage and pore size are also found in the separation of higher fullerenes.

3.2 Introduction

Closed-caged carbon molecules, so-called "fullerenes", have recently attracted attention in a wide variety materials science[1-5]. There have been many publications on the characteristics of fullerenes smaller than C_{70} which were investigated using instrumental analytical techniques such as mass spectrometry [6-8], nuclear magnetic resonance spectrometry [9-12], infrared spectrometry [13-15], ultraviolet spectrometry [16] and other spectrometric measurements [17,18]. In order to pursue such studies on characterization of higher fullerenes, isolation of the components from the soot is the most important and urgent task. It is the appropriate target for further studies on higher fullerenes to develop a separation technique such as LC which is generally used for the isolation and preparation of C_{60} and C_{70}.

In LC separation of C_{60} and C_{70}, the popular stationary phase called "ODS" is generally used with n-hexane as the mobile phase [19-23], although other novel stationary phases have also been used for this purpose [24-26]. Two main reasons why ODS is popular as the primary stationary phase are: i) ODS is easily commercially available, and ii) optimization of the separation is easier than other novel stationary phases. Therefore, it is reasonable to consider that for higher fullerene separations ODS should be first tested before evaluation of other novel phases. In this chapter, different types of ODS phases were evaluated for the separation of higher fullerenes by using toluene-based mobile phase systems in order to increase the solubility of those fullerenes in the mobile phase, and the experimental results are discussed.

3.3 Experimental

Carbon soot was produced by a system CC-A/1-1/3 Type-3 (Shinku Riko, Tokyo, Japan), set at Toyohashi Science Core, Toyohashi, Japan, with a usual arc discharge in an Ar inert gas environment. The soot was firstly extracted by toluene (fraction-A in Figure 3-1, where the sample preparation process is summarized), and then residue was extracted by 1,2,4-trichrolobenzene in order to obtain a high concentration of higher fullerenes than C_{70} (fraction-B in Figure 3-1). LC separation were performed using an 880 PU LC pump (Jasco, Hachioji, Japan) combined with a Jasco MD-920 UV/Vis photodiode array detector. Mobile phase were n-hexane, toluene/methanol and toluene/acetonitrile mixtures, and the flow rate was 1 mL/min. Conventional 4.6 mm i.d. commercially available columns were used for the evaluation and the phases used are listed in table I with their basic characteristics. The exception Nova-pak and Bondasphere have 8 and 3.9 mm i.d. respectively. Column temperature was typically controlled at 30 °C by a LAB-Thermo Model LH-1000 E (Tokyo Seisakusho, Tokyo, Japan).

3.4 Results and discussion

Separation of C_{60} and C_{70}

As the first step of this evaluation, separation of C_{60} and C_{70} with n-hexane mobile phase was carried out using various ODS stationary phases with different characteristics as summarized in Table 3-I. In this study, fraction-A was used as the sample injected into the LC system. The capacity factors of C_{60} and C_{70} and the separation factors were calculated and the obtained data are summarized in Table 3-II. From these results, it appears that the separation factors obtained with polymer-based octadecyl phase (Asahipak ODP) and polymeric ODS phases are very high values. However, in the chromatograms obtained with such phases the observed peaks had severe tailings and did not give a baseline separation (e.x. ODP) with even

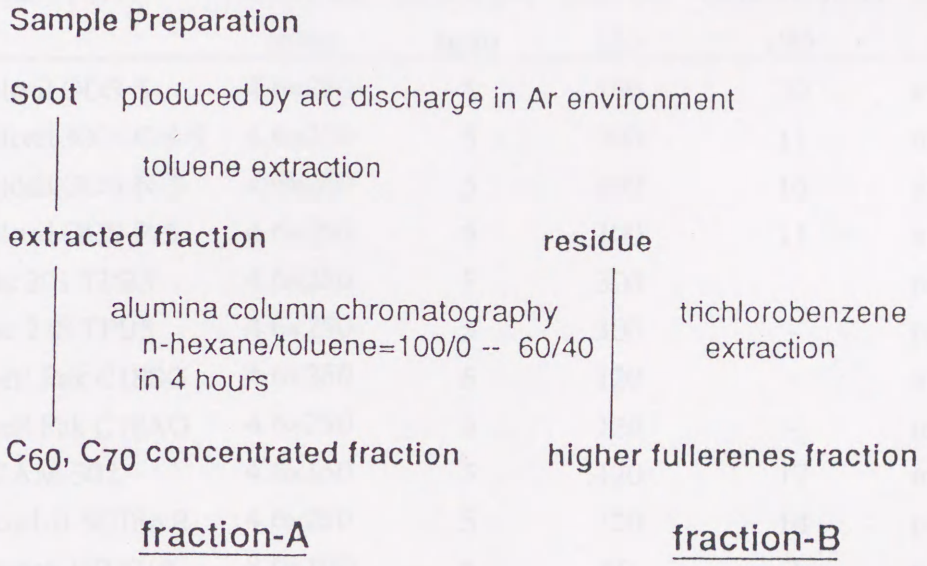


Figure 3-1. Sample preparation process.

Table 3-I. Stationary phases and their characteristics evaluated in this work.

No.	stationary phase	column size (mm)	particle size (μm)	pore size (Å)	carbon content (%)	functionality
1	Develosil ODS-5	4.6×250	5	100	20	monomeric
2	Develosil 300 ODS-5	4.6×250	5	300	11	monomeric
3	Develosil ODS-N-5	4.6×250	5	100	16	monomeric
4	Develosil ODS-P-5	4.6×250	5	100	11	monomeric
5	Vydac 201 TPB5	4.6×250	5	300	-	polymeric
6	Vydac 218 TPB5	4.6×250	5	300	-	polymeric
7	Capcell Pak C18SG	4.6×250	5	120	-	monomeric
8	Capcell Pak C18AG	4.6×250	5	120	-	monomeric
9	YMC AM-302	4.6×150	5	120	17	monomeric
10	Wakosil-II 5C18AR	4.6×250	5	120	18	polymeric
11	Nova-pak HR C18	8.0×100	5	60	7	monomeric
12	Asahipak ODS-50	4.6×150	5	-	-	polymer-based
13	Bondasphere 5μ C18	3.9×150	5	100	17	monomeric

Develosil: Nomura Chemicals, Capcell Pak: Shiseido, Polymer coated type,
Vydac: Separations Group, YMC: Yamamura Chemicals, Wakosil: Wako Chemicals,
Nova-pak and Bondasphere: Waters, Asahipak: Asahi Chemicals.

Table 3-II. Retention data of C_{60} and C_{70} separation with n-hexane.

No.	Stationary phase	Retention factor, k'		Separation factor of C_{70}/C_{60}
		C_{60}	C_{70}	
1	Develosil ODS-5	0.87	1.61	1.85
2	Develosil 300 ODS-5	0.48	0.89	1.85
3	Develosil ODS-N-5	0.81	1.32	1.63
4	Develosil ODS-P-5	0.44	0.64	1.45
5	Vydac 201 TPB5	0.25	0.39	1.56
6	Vydac 218 TPB5	0.22	0.45	2.05
7	Capcell Pak C18SG	0.34	0.53	1.56
8	Capcell Pak C18AG	0.41	0.62	1.51
9	YMC AM-302	0.66	1.12	1.72
10	Wakosil-II 5C18AR	0.25	0.53	2.11
11	Nova-pak HR C18	0.66	1.16	1.75
12	Asahipak ODS-50	5.10	12.3	2.41
13	Bondasphere 5μ C18	0.66	1.12	1.70

such high separation factors. It is apparent that monomeric ODS phases gave the best results for the separation. Especially, Develosil ODS-5 is the best of all phases evaluated. The carbon content of this phase is ca. 20%, and therefore it seems that the surface coverage is one of the important factors for getting high resolution of C_{60} and C_{70} with ODS phases, although other factors such as base-silica properties may have an influence on the separation power.

The effect of the surface coverage (carbon content) of the stationary phases on the C_{60}/C_{70} separation can be examined using the retention data with Develosil ODS-5, ODS-N-5, ODS-P-5 of which carbon contents are 20, 16 and 11 %, respectively. The obtained chromatograms shown in Figure 3-2 and the close examination of the data in Table 3-II suggest that the higher carbon content of the ODS phase, the better separation factors obtained and the decrease of the surface coverage induces smaller retention for both fullerenes. One can also compare the effect of the pore size of the base silica on the separation power using the data with Develosil ODS-5 and Develosil 300 ODS-5.

The separation factors with two columns are nearly the same values as shown in Table 3-II, but it has been found that the resolution and the retention with 300 ODS-5 were very poor as demonstrated in Figure 3-3, where two chromatograms obtained with both phases are compared. It is questionable whether the difference of pore size or surface coverage effects the different separation power. However, this fact indicates that the stationary phase with high surface coverage is more effective in giving better separation. It can be concluded that high loaded monomeric ODS phases with high specific surface area are the best choice for the separation of C_{60} and C_{70} with n-hexane mobile phase.

Separation of higher fullerenes

In order to separate higher fullerenes by LC (fraction-B is the sample in this case), the first task is to find the optimum mobile phase composition. As described above for the separation of C_{60} and C_{70}, n-hexane is common in several previous publications, but the low solubility of higher fullerenes limits its use. Higher solubility in toluene induces the idea to use toluene-based mobile phase [27-30]. By several trials with various ODS phases using

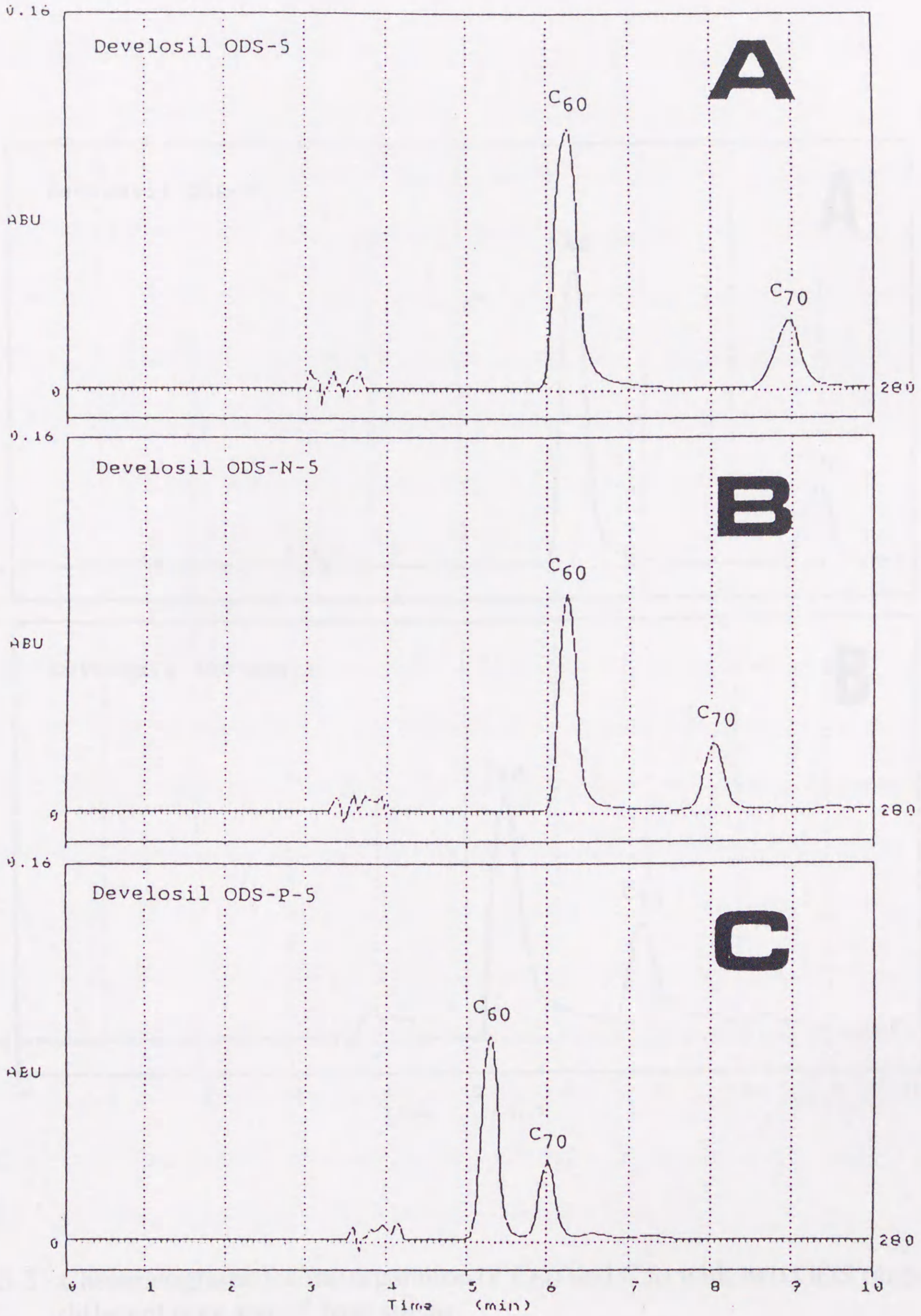


Figure 3-2. Chromatograms for the separation of C_{60} and C_{70} with three ODS phases with different surface coverage.
(A) Develosil ODS-5, (B) Develosil ODS-N-5, (C) Develosil ODS-P-5
Mobile phase: n-hexane, 1mL/min; detection: UV at 280 nm.

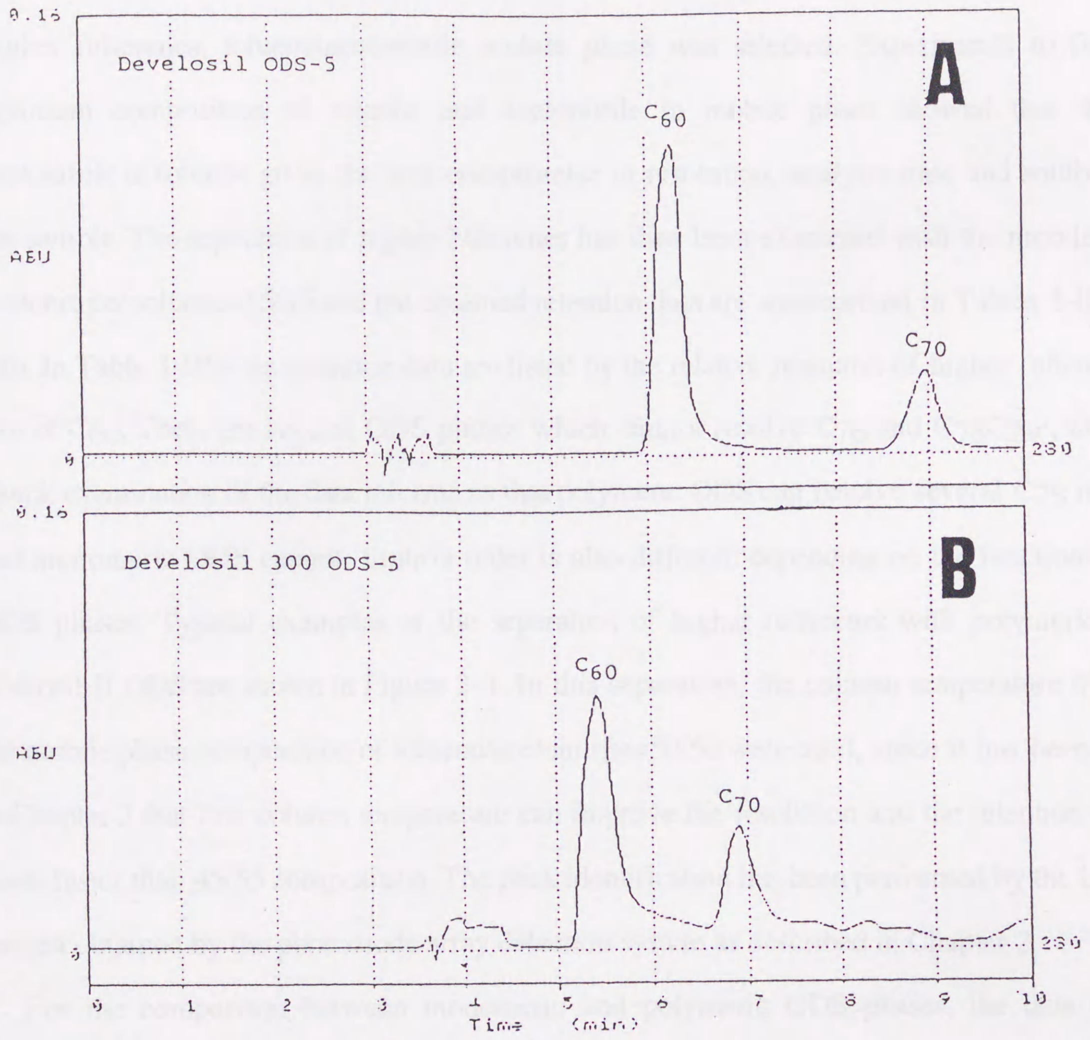


Figure 3-3. Chromatograms for the separation of C_{60} and C_{70} with two ODS phases with different pore size of base silicas.
(A) Develosil ODS-5, (B) Develosil 300 ODS-5
Mobile phase: n-hexane, 1mL/min; detection: UV at 280 nm.

toluene/acetonitrile and toluene/methanol mobile phase systems, the following conclusions are also obtained as described in Chapter 2: i) if a better separation for higher fullerenes is desired, acetonitrile is the choice; ii) if faster analysis with high resolution column is required, methanol should be used. Because this work is focusing on analytical purpose to get better separation of higher fullerenes, toluene/acetonitrile mobile phase was selected. Experiments to find the optimum composition of toluene and acetonitrile in mobile phase showed that 40-60% acetonitrile in toluene gives the best compromise in resolution, analysis time and solubility of the sample. The separation of higher fullerenes has then been examined with the mobile phase acetonitrile/ toluene=55/45 and the obtained retention data are summarized in Tables 3-IIIa and IIIb. In Table 3-IIIb the retention data are listed by the relative retention of higher fullerenes to that of C_{60}. There are several ODS phases which cannot resolve C_{76} and $C_{78}C_{2v}'$, and D_3. Quick examination of the data informs us that polymeric ODS can resolve several C_{78} isomers and monomeric ODS cannot. Elution order is also different depending on the functionality of ODS phases. Typical examples of the separation of higher fullerenes with polymeric ODS Wakosil-II ODS are shown in Figure 3-4. In this separation, the column temperature 0°C and the mobile phase composition of toluene/acetonitrile=50/50 were used, since it has been found in Chapter 2 that low column temperature can improve the resolution and the retention can be made faster than 45/55 composition. The peak identification has been performed by the UV/Vis spectra obtained by the photodiode array detection system as described in Chapter 2.

For the comparison between monomeric and polymeric ODS phases, the data clearly indicated the different elution order of higher fullerenes. In this chapter, though many ODS phases are evaluated, monomeric ODS phases generally elute C_{76}, C_{78} C_{2v}', C_{78} C_{2v}+D_3, C_{82} and C_{84}, while polymeric phases retain as C_{78} C_{2v}', C_{76}, C_{78} C_{2v}, C_{78} D_3, C_{84} and C_{82}. The elution order difference between monomeric and polymeric phases can be easily explained by their molecular planarity recognition capability. For PAHs polymeric ODS has higher retention for planer solutes than non-planar solutes and monomeric ODS does not notably have such behaviour.

Therefore, if one considers the shape and size of higher fullerene isomers it is easy to explain the elution order with the polymeric ODS. As shown in Figure 3-5, the isomer C_{78}

Table 3-III(a). Retention factors of higher fullerenes on various stationary phases.

	Stationary phase	k'C_{60}	k'C_{70}	k'C_{76}	K'C_{78} -$C_{2v'}$	k'C_{78} -C_{2v}	k'C_{78} -D_3	k'C_{82}	k'C_{84}
1	Develosil ODS-5	12.20	24.98	37.03	39.18	41.33	41.33	51.09	54.96
2	Develosil 300 ODS-5	5.38	11.13	16.57	17.57	18.57	18.57	23.20	24.89
3	Develosil ODS-N-5	12.78	24.40	34.60	37.17	38.46	38.46	47.22	51.67
4	Develosil ODS-P-5	5.42	9.13	12.02	12.90	13.14	13.14	15.62	16.85
5	Vydac 201 TPB 5	1.33	2.69	4.05	4.05	4.41	4.71	5.37	5.37
6	Vydac 218 TPB 5	1.69	3.34	4.89	4.89	5.38	5.58	6.40	6.40
7	Capcell Pak C18 SG120	4.57	8.05	10.96	10.96	10.96	10.96	15.09	15.09
8	Capcell Pak C18 AG120	5.60	9.96	13.56	14.21	14.85	14.85	17.62	18.75
9	YMC-PACK AM-302	9.27	18.00	25.56	27.24	28.47	28.47	34.83	37.73
10	Wakosil-II 5C18 AR	3.12	6.79	10.83	10.46	11.90	12.91	14.02	14.02
11	Nova-pak HR C18	19.38	37.97	55.16	61.37	61.37	61.37	74.96	80.96
12	Ashahipak ODS-50	9.35	18.45	26.93	30.04	30.04	30.04	37.40	40.78
13	BONDASHERE 5μ C18	9.43	18.35	26.17	28.07	29.18	29.18	36.10	39.00

Toluene/acetonitrile = 45/55; detection at UV 325 nm.
Stationary phase 5,7 and 8 did not give any separations of C_{60} and C_{70}.

Table 3-III(b). Relative retention of higher fullerenes to C_{60}.

	Stationary phase	C_{70}	C_{76}	C_{78}-$C_{2v'}$	C_{78}-C_{2V}	C_{78}-D_3	C_{82}	C_{84}
1	Develosil ODS-5	2.048	3.305	3.211	3.388	3.388	4.188	4.505
2	Develosil 300 ODS-5	2.069	3.080	3.266	3.452	3.452	4.312	4.626
3	Develosil ODS-N-5	1.909	2.707	2.908	3.009	3.009	3.695	4.043
4	Develosil ODS-P-5	1.685	2.218	2.380	2.424	2.424	2.882	3.109
5	Vydac 201 TPB 5	2.023	3.045	3.045	3.316	3.541	4.038	4.038
6	Vydac 218 TPB 5	1.976	2.893	2.893	3.183	3.302	3.787	3.787
7	Capcell Pak C18 SG120	1.176	2.398	2.398	2.398	2.398	3.302	3.302
8	Capcell Pak C18 AG120	1.779	2.421	2.538	2.652	2.652	3.146	3.348
9	YMC-PACK AM-302	1.942	2.757	2.939	3.071	3.071	3.757	4.070
10	Wakosil-II 5C18 AR	2.176	3.471	3.353	3.814	4.138	4.494	4.494
11	Nova-pak HR C18	1.959	2.846	3.167	3.167	3.167	3.868	4.178
12	Ashahipak ODS-50	1.973	2.880	3.213	3.213	3.213	4.000	4.361
13	BONDASHERE 5μ C18	1.946	2.775	2.977	3.094	3.094	3.828	4.136

Toluene/acetonitrile = 45/55; detection at UV 325 nm.

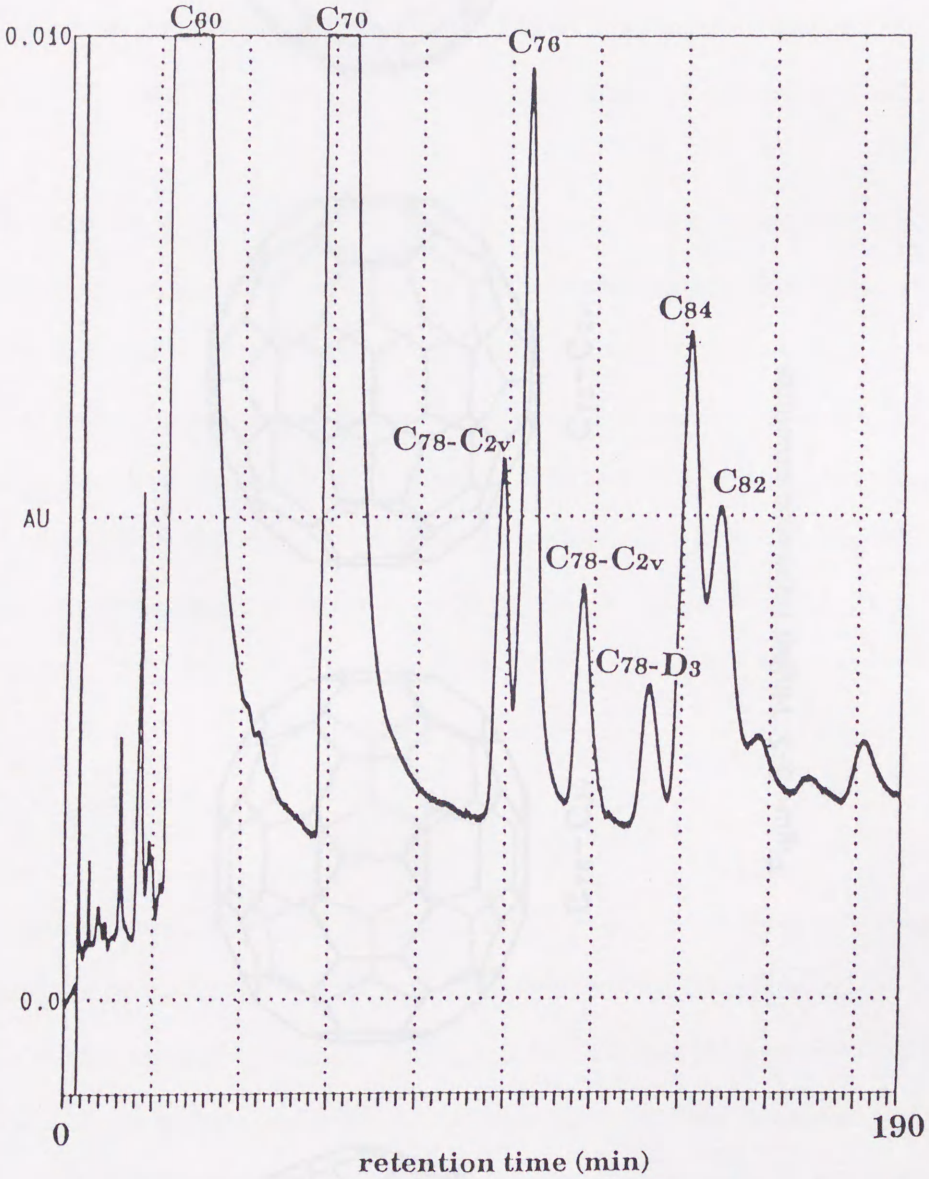
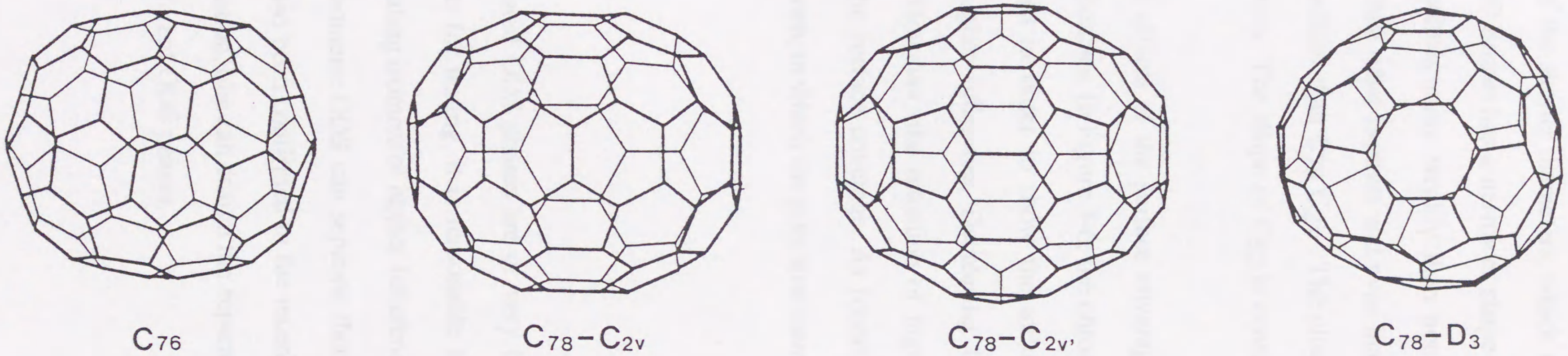


Figure 3-4. Chromatogram for the separation of higher fullerenes with polymeric ODS Wakosil-II.
Mobile phase: toluene/acetonitrile=50/50, 1mL/min; temperature: 0 °C; detection: UV at 280 nm.



C_{76} $C_{78}-C_{2v}$ $C_{78}-C_{2v'}$ $C_{78}-D_3$

Figure 3-5. Higher fullerenes structures.

C_{2v}' has the longest of the shorter diameters, which means it is the most bulky of the three C_{78} isomers, and the D3 isomer has a narrower shape and is the least bulky. Polymeric ODS phases retain bulkier solutes more weakly than non-bulky solutes and then C_{78} C_{2v}' is retained more weakly than other isomers and even than C_{76}, which is a smaller molecule in molecular weight but bulkier than C_{78} C_{2v}'. The elution order of C_{82} and C_{84} can also be explained in a similar way. The shape of C_{82} is considered to be longer and narrower than C_{84}.

In this chapter, the effects of the surface coverage and pore size are also found in the separation of higher fullerenes. In Figure 3-6, the chromatograms with three Develosil ODS-5 phases are demonstrated in order to show the influence of the surface coverage on the separation power of higher fullerenes. Decreasing the surface coverage, the retention is reasonably decreased. However, the retention of higher fullerenes separation is also worse with the decrease of the surface coverage. As found in the case of C_{60}/C_{70} separation, a similar trend was also seen, in which the pore size seems not to have so much influence on the resolution.

3.5 Conclusion

Although the evaluated ODS phases are of very limited availability because we are just using Japanese products for testing, it is reasonable to conclude that the polymeric ODS is more powerful in separating isomers of higher fullerenes than monomeric ODS by their shape difference, and the monomeric ODS can separate them by their molecular size or molecular weight. This fact has also been confirmed in the recent publication by Anacleto and Quilliam [31]. Using this information, one can design the separation system of higher fullerenes by the combination of two different ODS phases.

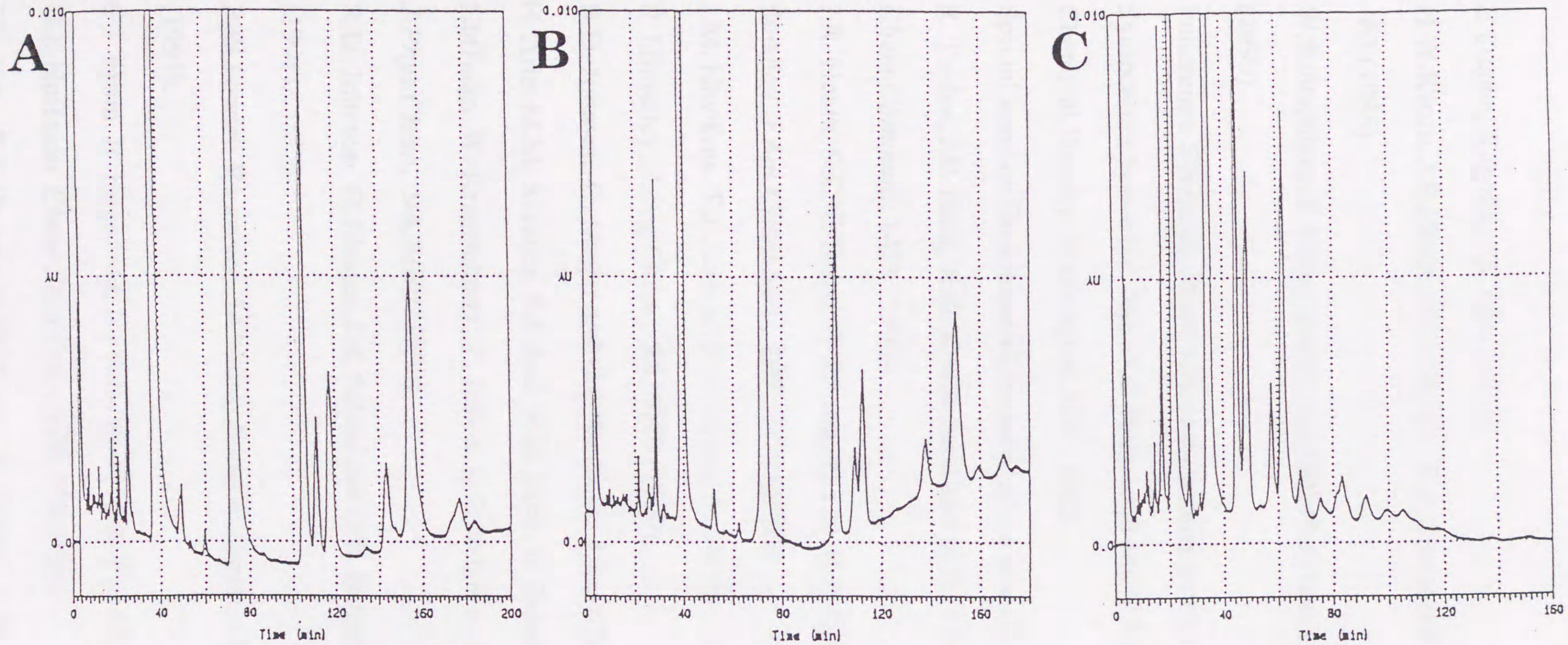


Figure 3-6. Chromatograms for the separation of higher fullerenes with three ODS phases with different surface coverage.
(A) Develosil ODS-5, (B) Develosil ODS-N-5, (C) Develosil ODS-P-5
Mobile phase: toluene/acetonitrile=45/55, 1mL/min; temperature: 30 °C; detection: UV at 325 nm.

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Chapter 4

Separation and Identification of Higher Fullerenes in Soot Extract by Liquid Chromatography-Mass Spectrometry *

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*Modified from: *Chromatographia,* **41**, 353-360 (1995).

4.1 Abstract

Two-step LC has been applied to the soot extract and the identification of higher fullerenes has been accomplished by LC-MS measurements using ESI interface. The first separation step is the preparative-scale LC using a 50 mm i.d. column packed with monomeric ODS because elution is mainly controlled by relative molecular mass. 39 batches of five fractions each were collected and then as the second separation step each fraction was analysed by analytical-scale LC using a conventional column of a polymeric ODS phase which can elute fullerenes according to shape and structure. This stationary phase can also separate many isomers of higher fullerenes, consequently the existence of several higher fullerenes larger than C_{86} has been confirmed and their UV/Vis spectra were obtained by the photodiode array detection system coupled to the analytical LC.

4.2 Introduction

The development of methods for the production of large quantities of the so-called fullerenes has led to increasing interests in the isolation, identification and characterization of these molecules. The major components present in soot produced by the vaporization of graphite, C_{60} and C_{70}, have been well characterized by various spectroscopic methods after LC separations [1-10]. However, the fullerene-rich soots also contain relatively smaller amounts of higher fullerenes than C_{70} which are much more difficult to isolate by conventional LC methods. There are a number of isomers of those fullerenes, although the isolation and identification of them is also difficult. Several papers focusing on the separation and identification of large fullerenes by LC methods have been published and the most recent results indicated that there should exist compounds at least to C_{90} in large fullerenes fractions extracted from the soot [11,12]. To confirm their existence we need to use an analytical approach which can give multi-dimensional information in one chromatographic run. Hyphenated techniques [13] are the most convenient and suitable methods for this purpose, LC-MS especially would be the most powerful way to accomplish it [11, 14]. LC-MS combines LC and MS via an interfacing devices which will not induce any disturbance in mass spectral measurements caused by the mobile phase solvents in LC. Typical interfacing devices are ESI (electro-spray ionization), API (atmospheric ionization) and FAB (fast atom bombardment ionization); ESI is the most suitable ionization method for fullerenes analysis using currently available LC-MS interfacing techniques[15]. Here we describe the LC-MS approach using the ESI interface for isolation and identification of higher fullerenes. The results have shown that this approach is sufficiently powerful to identify large fullerenes in soot extract and the largest fullerene found in this work is C_{98}. Interesting elution behaviour of C_{98} using polymeric ODS as the stationary phase is also found and the reason for this result can be interpreted by the molecular planarity recognition mechanism of the ODS phase.

4.3 Experimental

The soot was obtained by arc discharge in an inert gas environment using the system CC-A/1-1/3 Type-3 (Shinku Riko, Tokyo, Japan) set at Toyohashi Science Core, Toyohashi, Japan. The soot was first extracted by toluene and the residue was then extracted by 1,2,4-trichlorobenzene in order to obtain a high concentration of fullerenes larger than C_{70}. The trichlorobenzene solution was vaporized by nitrogen and the solvent was substituted to toluene-methanol=55:45 mixture used as the mobile phase for preparative-scale separations. The solution was then filtrated by an PTFE membrane filter (1 μm pore, Toyo Roshi, Tokyo, Japan) and the solution obtained was used as the sample for the preparative separations. Isolation and identification of the large fullerenes have been performed using two-step LC separations, first preparative-scale separation for large fullerenes fractions and the second, analytical-scale to identify large fullerenes in each fraction by LC-MS. For preparative separations we used a monomeric ODS stationary phase because it elutes fullerenes according to their molecular mass. As described previous chapter, for analytical separations we used a polymeric ODS as the separation medium because it elutes fullerenes according to their shape and structure [11, 12, 16-21]. Therefore using the monomeric ODS one can collect fractions containing fullerenes having different molecular masses. Each collected fraction can then be separated to identify isomers by recognizing their shape and structure with the polymeric ODS.

Preparative separations were by the LC system consisted of 887 PU pump (Jasco, Tokyo, Japan), Jasco UV-970 UV detector at 350 nm with a cell for preparative use (45 μL), and a Rheodyne 7125 injector with 40 mL sample loop (Cotati, CA, USA) coupled with Develosil Lop ODS column (500 × 50 mm i.d. packed with monomeric ODS, 30 μm, Nomura Chemicals, Seto, Japan). Separations were performed at ambient temperature at 40 mL min^{-1} of toluene-methanol=55:45. A typical chromatogram is shown in Figure 4-1. Fraction collection was done manually and five fractions as indicated in Figure 4-1 were accumulated in 39 times. Information about each fraction and the assumed components are summarized in Table 4-I. In this table the volumes collected are also listed. Solvent was evaporated to reduce the total sample and the reduced volumes of each fraction are also tabulated.

Analytical scale separations for three fractions such as Sample Nos.3,4 and 5 have been using the following instrumentation: two pumps Jasco PU-980, a high-pressure mixer PU-980-30, detector UV-970, degasser DG-980-50, Rheodyne 7725 injector and a 865-CO oven for temperature control at 30 °C. The LC separations were using Wakosil II5C18AR (250 × 4.6 mm i.d. column of ODS phase (5 μm), Wako Chemicals, Tokyo, Japan) with acetonitrile/toluene = 55:45 or 50:50 mobile phase. The flow rate was 1 mL min^{-1}. Samples for analysis were 10 μL each of the three fractions. LC-MS was by a VG Biotech PLATFORM instrument with ESI interface (Jasco, Tokyo, Japan). The MS conditions were: mass range measured; 600-1600, capillary voltage; 3.4 kV, ion-source temperature; 70 °C, split ratio; 1:20. To obtain the UV/Vis spectra of each higher fullerene ($>C_{86}$) identified by LC-MS, LC with photodiode array detection was performed for Sample No. 5. The system used comprised a Jasco PU-980 pump, Hewlett-Packard 1040A photodiode array detector (Yokogawa Analytical Systems, Tokyo, Japan) controlled by HP 9000 computer and a Rheodyne 7125 with 20 μL loop for injection. The column temperature was controlled by Tosoh RE-8000 oven at 30 °C. Other separation conditions were the same as those in LC-MS measurements.

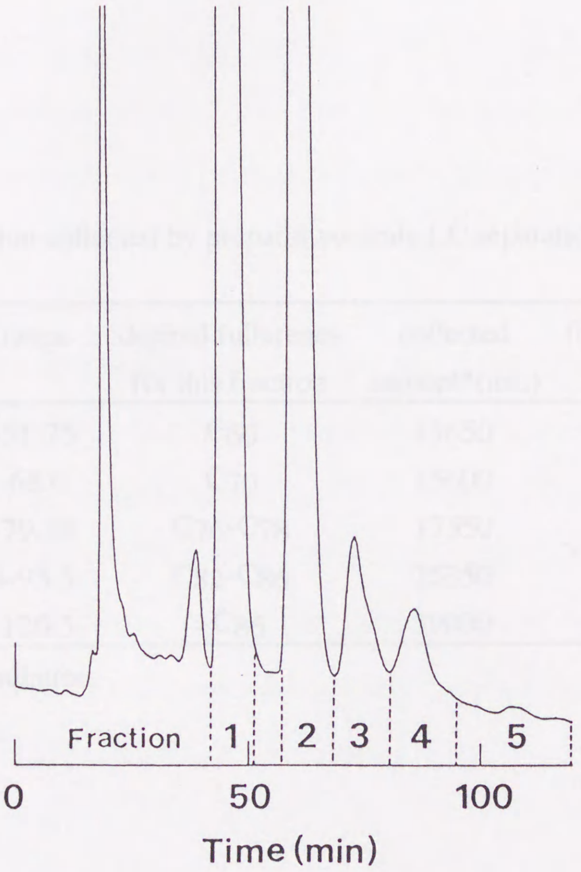

Figure 4-1. Chromatogram of the soot extract by preparative-scale separation. 39 batches of five fractions each were collected by 40 mL each injection. Other conditions described in the text.

Table 4-I. Fraction collected by preparative-scale LC separations.

fraction	time range	desired fullerenes for this fraction	collected amount*(mL)	final amount (mL)	Sample No. for analysis
1	43.0-51.75	C_{60}	13650	45	-
2	58.0-68.0	C_{70}	15600	50	-
3	68.0-79.25	C_{76}-C_{78}	17550	30	No.3
4	79.25-95.5	C_{82}-C_{86}	25350	35	No.4
5	95.5-120.5	>C_{86}	39000	7	No.5

*39 times accumulation.

4.4 Results and Discussion

Sample No. 3

This fraction should contain C_{76} and C_{78} fullerenes. It has been found according to recent publications [11, 12, 16, 17, 21-25] that C_{76} has a chiral isomer and C_{78} has at least three isomers. It is hard to separate each C_{76} chiral isomer but three C_{78} isomers have been well characterized: C_{78}-C_{2v}', C_{78}-C_{2v} and C_{78}-D_3. Using the polymeric ODS the elution order of those fullerenes is different from that with the monomeric ODS [11, 12, 16, 17, 22, 23], because the monomeric ODS essentially elutes fullerenes by relative molecular mass but the polymeric elutes them by molecular shape and structure. The chromatograms measured by UV at 325 nm and MS by total ion monitoring included four clear peaks and one of them has a small shoulder on the early eluting part. Therefore five positions in the retention time have been referred to their mass spectra and the results are shown in Figure 4-2. As a result the five peaks can be assigned as C_{60}, C_{78}, C_{76}, and two C_{78} isomers. The elution order found for this fraction with the polymeric ODS is determined as C_{78}-C_{2v}', C_{76}, C_{78}-C_{2v} and C_{78}-D_3 by using information from Chapter 2 where the isomers of C_{78} were identified by photodiode array UV/Vis detection. LC-MS information clearly indicated that our previous findings on the elution order with the polymeric ODS were correct.

Sample No. 4

This fraction should contain C_{82}, C_{84} and some larger fullerenes. The UV spectra and MS chromatograms contained three major peaks and one of them is broad with a shoulder. We have referred these peaks as peak Nos. 1, 2, 3 and 4 and peak No. 2 and No.3 create the same peak. The identification of each peak is easy by the mass spectra as shown in Figure 4-3. The elution order of C_{84} and C_{82} is interesting because larger molecular mass C_{84} is eluted faster than C_{82} on the polymeric ODS. Because the polymeric ODS recognizes the molecular planarity so well the non-planar generally elutes faster than the planar counterpart. The shape and structure of C_{82} is less bulky than C_{84} and the former elutes later than the latter. Any isomers of C_{84} are not found in this fraction. The important is that C_{86} is found in this

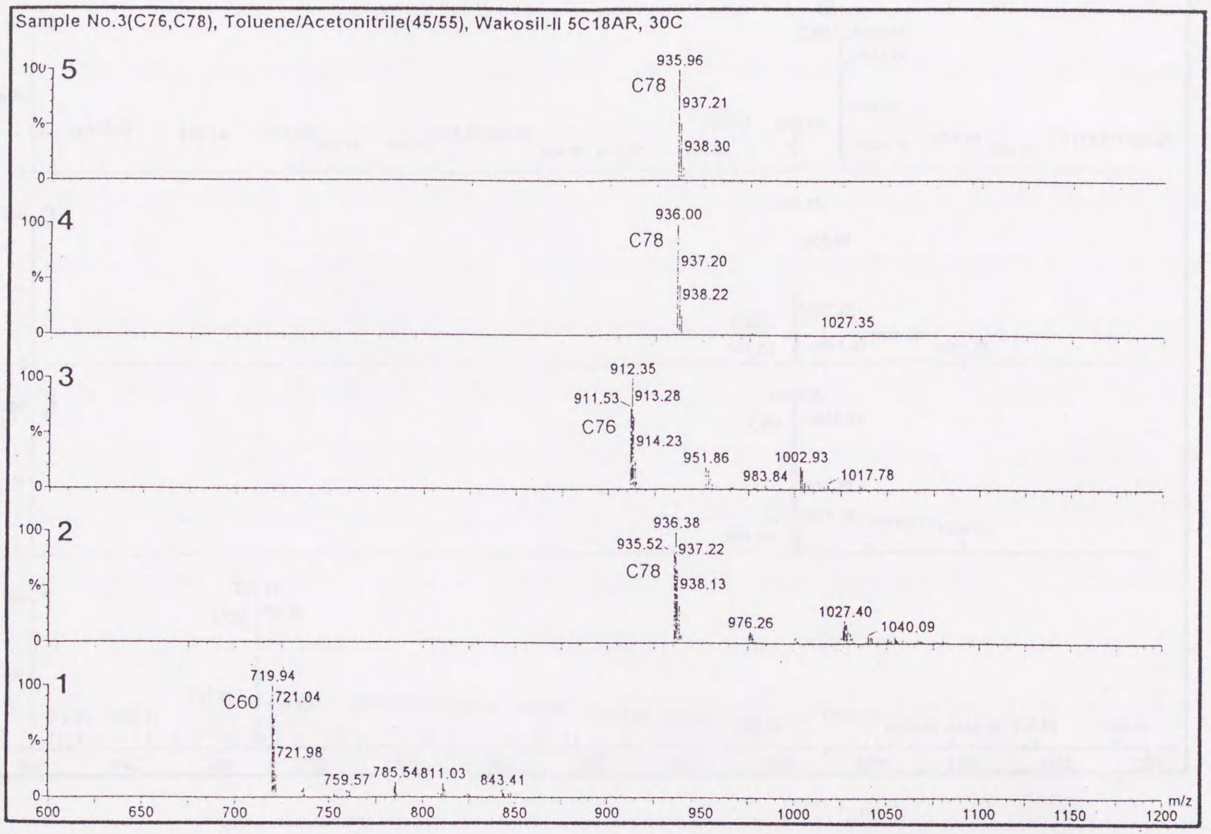

Figure 4-2. Mass spectra for each peak in chromatograms of sample No.3.
1: 10.4 min retention time, 2: 28.8 min, 3: 29.3 min, 4: 32.3 min, 5: 34.5 min.

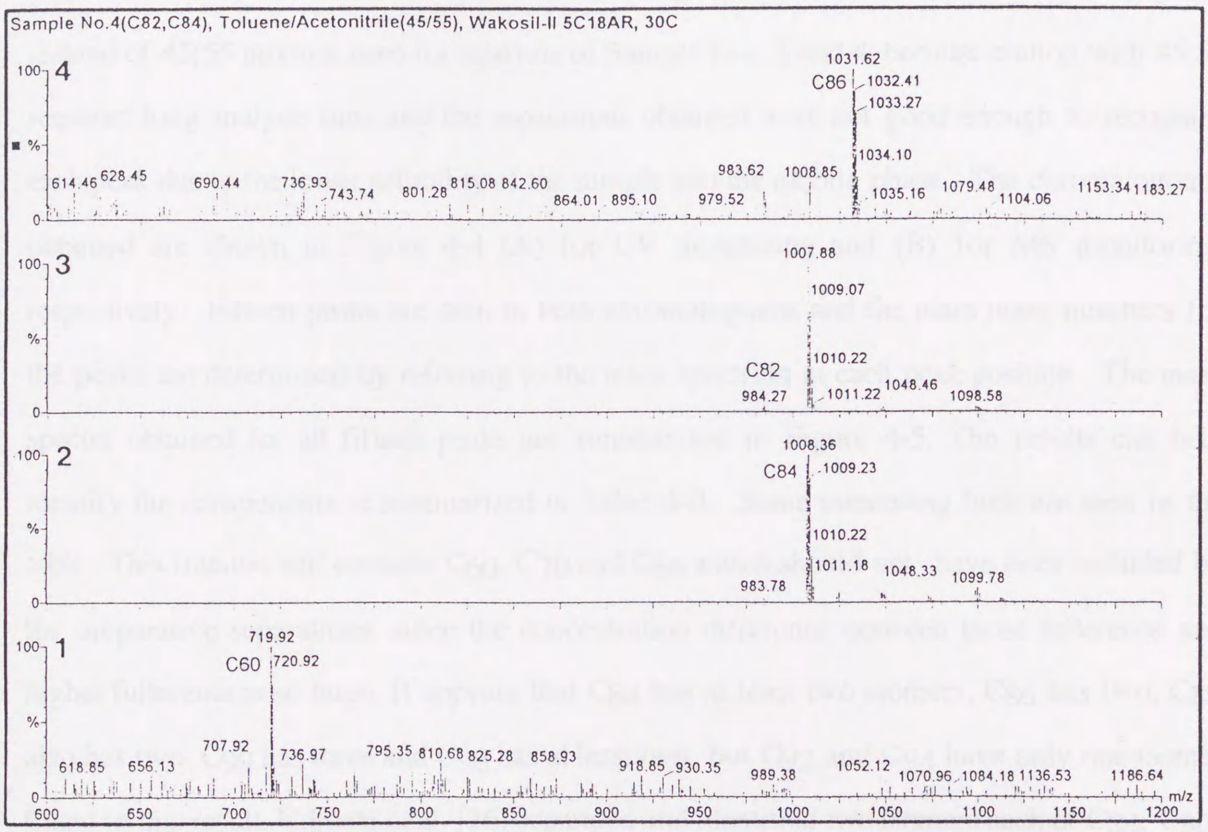

Figure 4-3. Mass spectra for each peak in chromatograms of sample No.4.
1: 10.2 min retention time, 2: 36.6 min, 3: 37.3 min, 4: 42.5 min.

fraction and elutes later than C_{84}. This means C_{86} structure is not unusual because if the structure were unusual it should elute faster or much later than C_{84}.

Sample No. 5

The most exciting results were obtained for this fraction which should contain larger than C_{86}. The analysis of this fraction was by using toluene-acetonitrile=50:50 mobile phase instead of 45:55 mixture used for analysis of Sample Nos. 3 and 4, because elution with 45:55 required long analysis time and the separations obtained were not good enough to recognize each peak due to the lower solubility of the sample into the mobile phase. The chromatograms obtained are shown in Figure 4-4 (A) for UV monitoring and (B) for MS monitoring, respectively. Fifteen peaks are seen in both chromatograms and the main mass numbers for the peaks are determined by referring to the mass spectrum at each peak position. The mass spectra obtained for all fifteen peaks are summarized in Figure 4-5. The results can help identify the components as summarized in Table 4-II. Some interesting facts are seen in the table. This fraction still contains C_{60}, C_{70} and C_{84} which should not have been included by the preparative separations, since the concentration difference between those fullerenes and higher fullerenes is so huge. It appears that C_{84} has at least two isomers, C_{86} has two, C_{88} also has two, C_{90} has three and C_{98} has at least two, but C_{92} and C_{94} have only one isomer found in this result. Kikuchi et al. [26] separated and identified two isomers each of C_{84}, C_{90}, C_{92}, C_{94} and C_{96} and one isomer for C_{88} using an expensive, commercially available preparative column (Buckyprep, Nacalai Tesque, Kyoto, Japan) by off-line MS measurements for the collected fractions. Our results are not totally consistent with Kikuchi's results but on-line LC-MS information should be more accurate than off-line measurements. Factors that might induce such differences include the different soot sources and the production methods. Further works is required to confirm the number of isomers for higher fullerenes with LC-MS on-line detection. Another important fact seen in the results is the elution time for two isomers of C_{98} that are unusually fast-faster than C_{84}. As the monomeric ODS discriminates fullerenes by their molecular weight and the polymeric discriminates by shape and structure, the shape and structure of two C_{98} isomers should be very bulky and unusual type. This sort

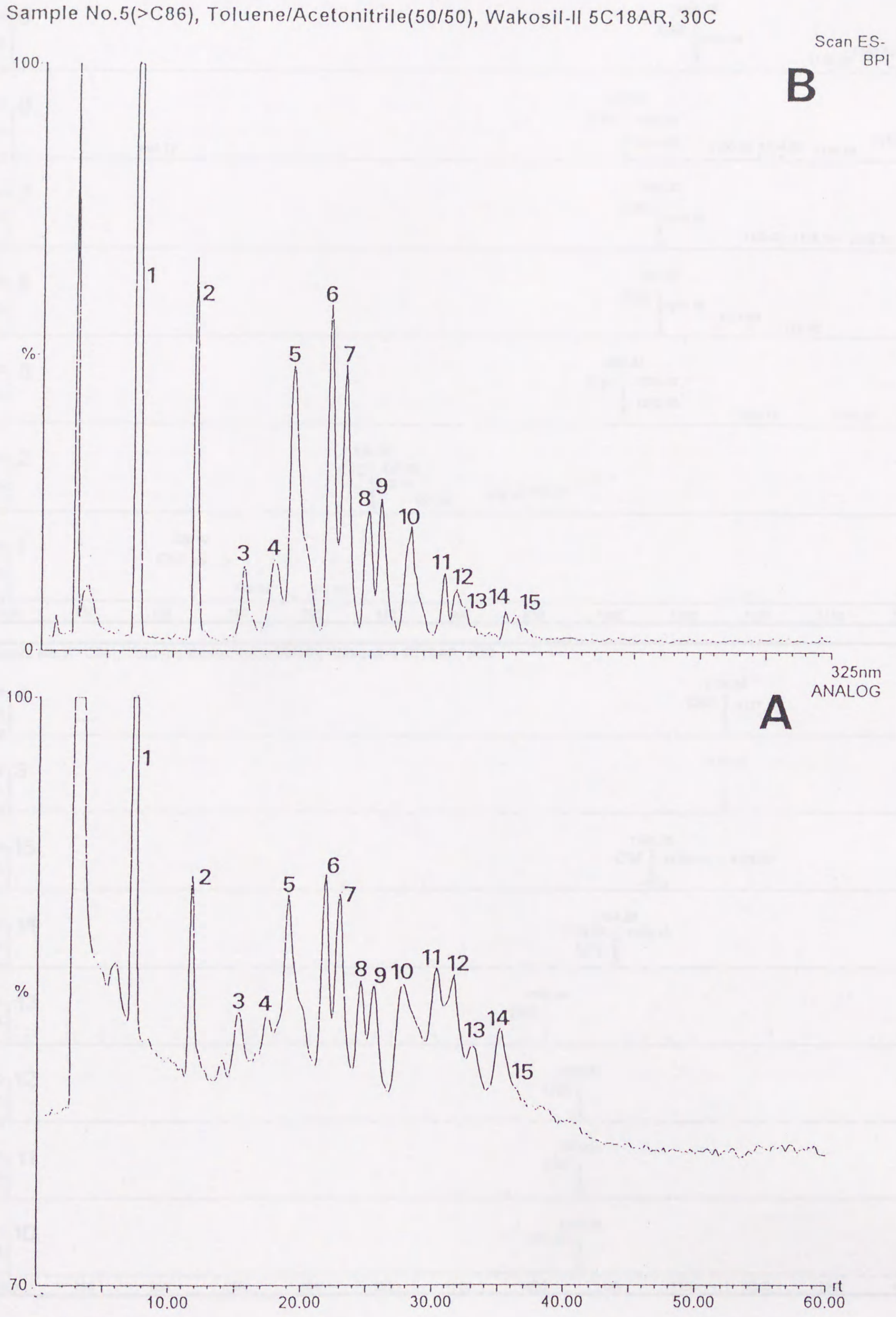

Figure 4-4. Chromatograms of Sample No. 5 by the LC-MS measurement.
(A) UV chromatogram at 325nm,
(B) MS chromatogram by total ion monitoring.

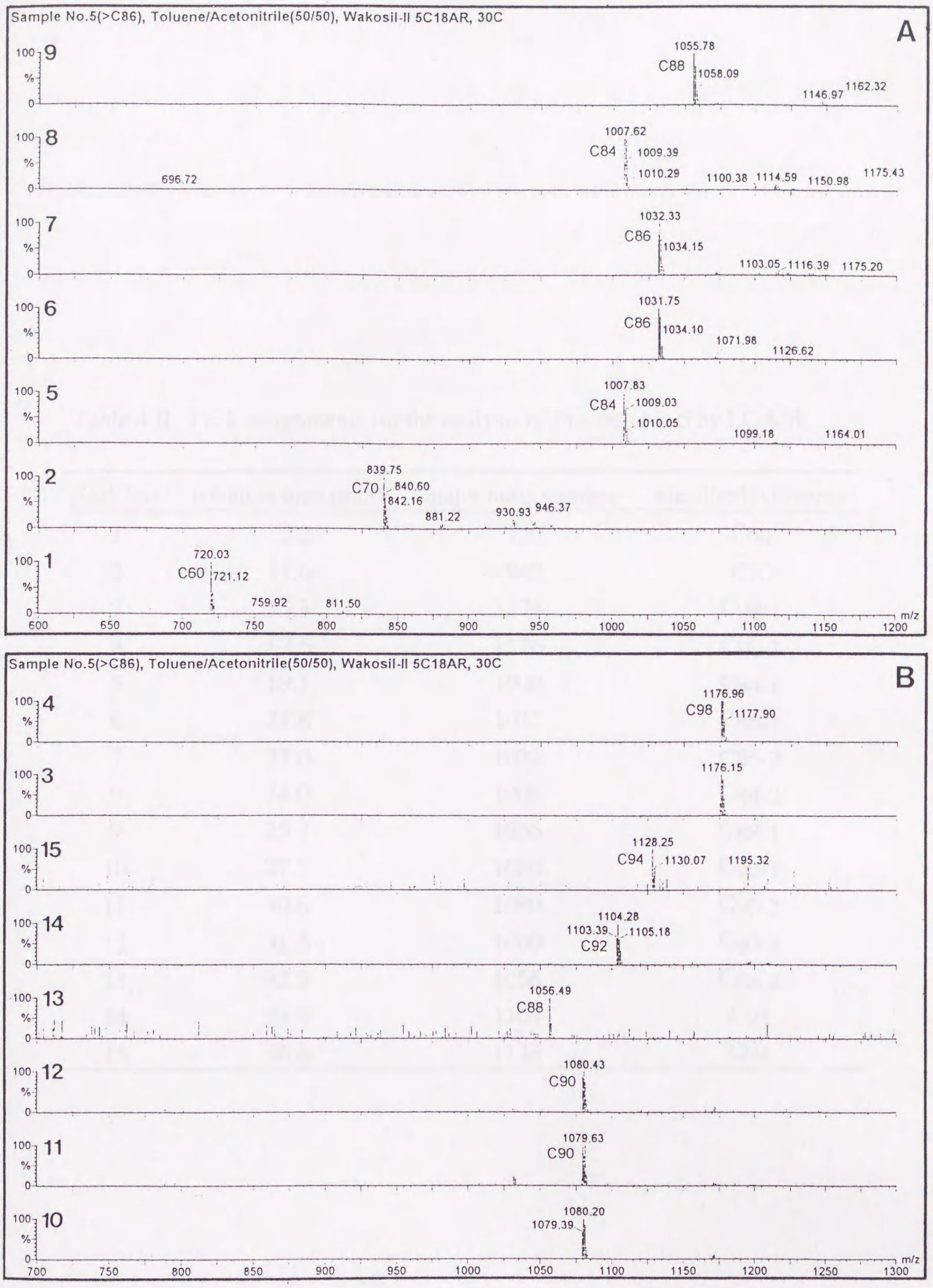

Figure 4-5. Mass spectra obtained for each peak in chromatograms in Figure 4-4.

(A) for peaks 1-9 (except 3 and 4)

1: 7.2 min, 2: 11.6 min, 5: 19.1 min, 6: 21.8 min, 7: 23.0 min, 8: 25.0 min, 9: 25.7 min.

(B) for peaks 10- 15, and 3 and 4

10: 27.7 min, 11: 30.6 min, 12: 31.5 min, 13: 32.5 min, 14: 35.9 min, 15: 36.6 min, 3: 15.3 min, 4: 17.5 min.

Table 4-II. Peak assignments for the analysis of Fraction No.5 by LC-MS.

peak No.	retention time (min)	major mass number	identified fullerenes
1	7.2	720	C_{60}
2	11.6	840	C_{70}
3	15.3	1176	C_{98}-1
4	17.5	1176	C_{98}-2
5	19.1	1008	C_{84}-1
6	21.8	1032	C_{86}-1
7	23.0	1032	C_{86}-2
8	24.0	1008	C_{84}-2
9	25.7	1056	C_{88}-1
10	27.7	1080	C_{90}-1
11	30.6	1080	C_{90}-2
12	31.5	1080	C_{90}-3
13	32.5	1056	C_{88}-2
14	35.9	1104	C_{92}
15	36.6	1128	C_{94}

of information will help to characterize higher fullerenes and further developments of new stationary phases in LC can give such information about shape and structure of higher fullerenes based on different molecular recognition capabilities.

In order to obtain the UV/Vis spectra of higher fullerenes identified by LC-MS LC separation using the photodiode array detection has again been performed. The UV/Vis spectra for each peak obtained are summarized in Figure 4-6. The spectra in Figure 4-6 will be very valuable for further investigations on isolation and identification of higher fullerenes, because LC-photodiode array detection is much easier to perform than LC-MS measurements in general laboratory.

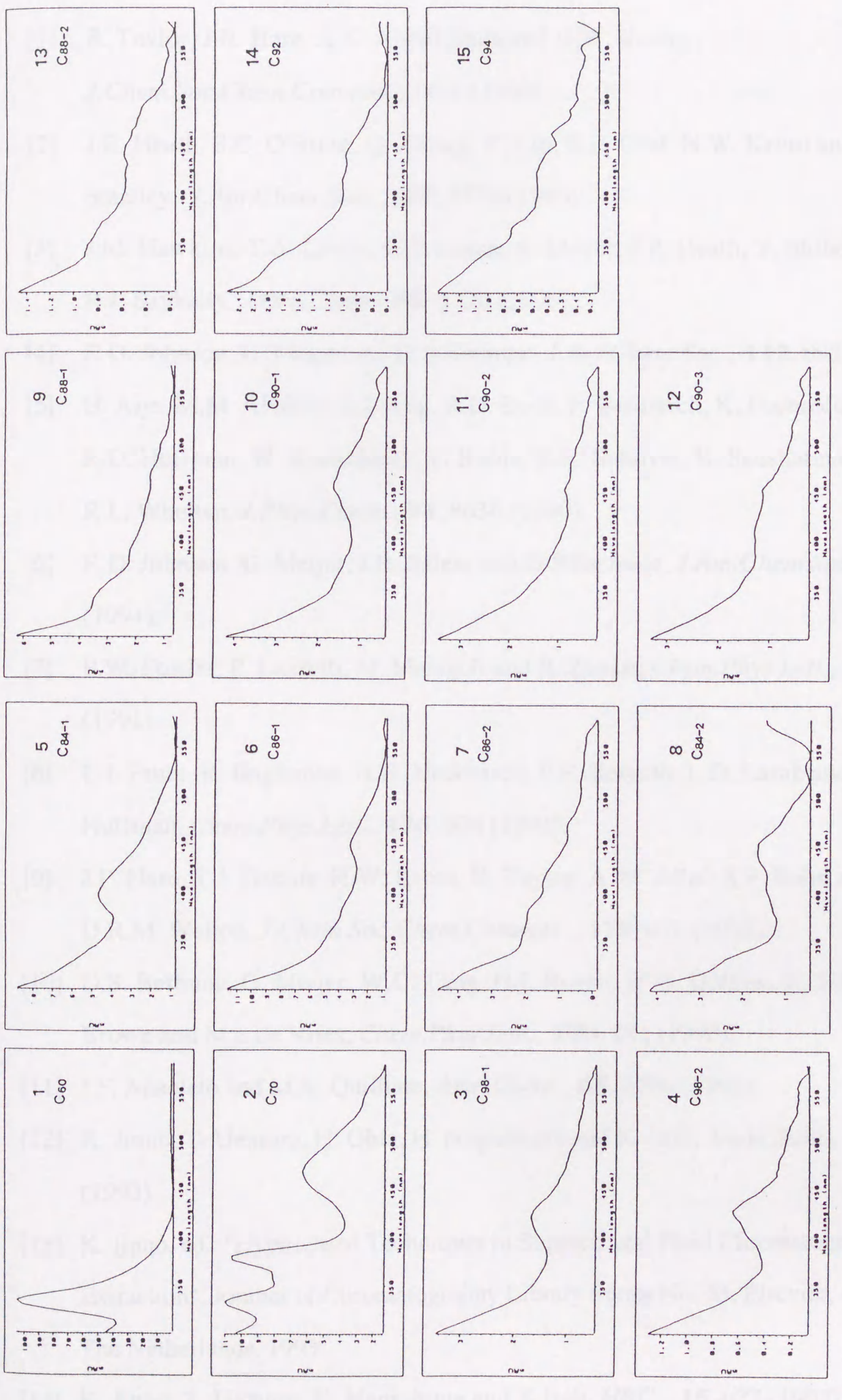

Figure 4-6. UV/Vis spectra for higher fullerenes by LC-photodiode array detection.

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Chapter 5

Dimethoxyphenylpropyl Bonded Silica Phase for Higher Fullerenes Separation by High-performance Liquid Chromatography*

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*Modified from: *J.Chromatogr.*, **648**, 71-77 (1993).

5.1 Abstract

Fullerenes were separated using three chemically bonded phases, dimethoxyphenylpropyl (DMP), monomeric and polymeric ODSs with n-hexane as the mobile phase. DMP and the monomeric ODS are the best choices for the separation of C_{60} and C_{70} compounds, while DMP is the only phase that has high temperature stability while maintaining the resolution. For the separation of higher fullerenes, DMP offers faster analysis at higher temperatures while maintaining its high resolution, whereas ODS phases can not provide similar run times while offering the same resolution. In conclusion, DMP is the most suitable and promising stationary phase for fullerenes analysis because of the short run time and its superior separation efficiency.

5.2 Introduction

Since the existence of all-carbon molecules such as C_{60} and C_{70} was confirmed in 1985, there have been many publications dealing with the separation and analysis of these compounds, called fullerenes, using various analytical techniques [1-18]. The unique properties and structures of these molecules have been widely studied, and now studies on their usefulness in materials science fields are the driving force that attract many scientists to this field of chemistry. In order to further scientific investigations of fullerenes, chromatographic separation techniques are required to separate and purify these molecules. LC is probably the best method one can choose for this application.

Although novel stationary phases based on nitrophenyl groups have proved to be useful for C_{60} and C_{70} separation and a few are now commercially available [19-21], a basic approach to optimize separation systems for higher fullerenes is still urgently required. Studies of the separation of C_{60} and C_{70} using multi-legged biphenyl bonded phase (BP)[22], commercially available ODS phases (polymeric and monomeric phases) [23-26] and a novel DMP bonded phase [27] have been previously conducted in our laboratory. The separation of higher fullerenes such as C_{76}, C_{78} and C_{84} with ODS phases was also reported in previous chapters [25,26]. These investigations indicated that there is still potential for further studies to obtain higher selectivity with the above-mentioned stationary phases using their characteristic chromatographic properties. In this chapter, we describe an approach to enhance the selectivity of separation with varying column temperature in order to get better resolution for C_{60} and C_{70} and other higher fullerenes using various stationary phases, especially DMP phase.

5.3 Experimental

The LC system used consisted of Tosoh CCPE pump(Tokyo, Japan), a Hewlett-Packard 1040 A photodiode array detector (Yokogawa Analytical Systems, Musashino, Japan) controlled by HP 9000 computer, and a Rheodyne 7520 injector (Cotati, CA, USA) with 20 μl loop volume for injection. The column temperature was controlled by a Tosoh RE-8000 oven at temperatures between 30 and 80 °C and LAB-Thermo Model LH-1000E (Toyo Seisakusho, Tokyo, Japan) at temperatures lower than 30 °C. Separation columns evaluated herein were as follows: Develosil ODS-5 (monomeric type, 5 μm, Nomura Chemicals, Seto, Japan) 250 mm x 4.6 mm i.d.; Wakosil II 5C18AR (polymeric type, 5 μm, Wako Chemicals, Tokyo, Japan) 250 mm x 4.6 mm i.d.; and DMP (monomeric type, 5 μm, laboratory made), 150 mm x 4.6 mm i.d. In this work, DMP was used as the primary stationary phase and others were used as references for the comparison of the chromatographic performance for fullerenes separations, since it has been found previously [27] that DMP is very promising stationary phase for fullerenes separation (C_{60} and C_{70}). The mobile phase was n-hexane at a flow-rate of 1 ml/min.

Carbon soot was produced by a common arc discharge in an inert gas environment. The soot was extracted with toluene in order to obtain the C_{60} and C_{70} fraction and the residue was extracted with 1,2,4-trichlorobenzene to get highly concentrated higher fullerenes [25]. The solution was then evaporated to dryness and then re-dissolved into toluene for use as the test sample for the injection.

5.4 Results and Discussion

Separation of C_{60} and C_{70}

In general, in LC, high temperature results in lower retention and high resolution. However, Pirkle and Welch [19] reported an unusual temperature dependence of their special column (the so-called Pirkle column) for C_{60} and C_{70} separation, *i.e.* higher temperature causes higher retention and high resolution. In this chapter, therefore, the first step was to separate C_{60} and C_{70} with various stationary phases and varying column temperature. In Figure 5-1, the separation factors for C_{60} and C_{70} are plotted against temperature. With the polymeric ODS phase, the separation factors changed drastically with the column temperature: decreasing the temperature increased selectivity. Low temperature makes it possible to separate both fullerenes with low retention and resolution. At temperatures greater than 40 °C, the same separation cannot be achieved because of very low retention and co-elution of C_{60} and C_{70}. This means that polymeric ODS is not suitable for separating C_{60} and C_{70} even at low column temperatures, because the retentions of two fullerenes are very short and the separation efficiency is very sensitive to small change in temperature. With the monomeric ODS phase, the change of the separation factors with temperature is not as drastic as found when using the polymeric ODS phase, but the tendency for the selectivity to increase at lower temperatures is clearly seen. From the practical point of view, the monomeric phase is preferable to the polymeric, although at low temperatures the polymeric ODS phase can offer much higher separation factors than the monomeric ODS. Actual retention values with the polymeric phase are always lower than those with the monomeric, and this fact demonstrate that the monomeric material is the better stationary phase for fullerenes separations. As previously reported by Pirkle and Welch [19] different retention mechanism are affected to varying degrees by temperature change. With the DMP phase, the temperature dependency is very minimal, not as low as the Pirkle column, but the lowest temperature dependency of the three phases studied.

Higher fullerenes separation

In Chapters 2-3, polymeric and monomeric ODS phases with toluene-acetonitrile as the

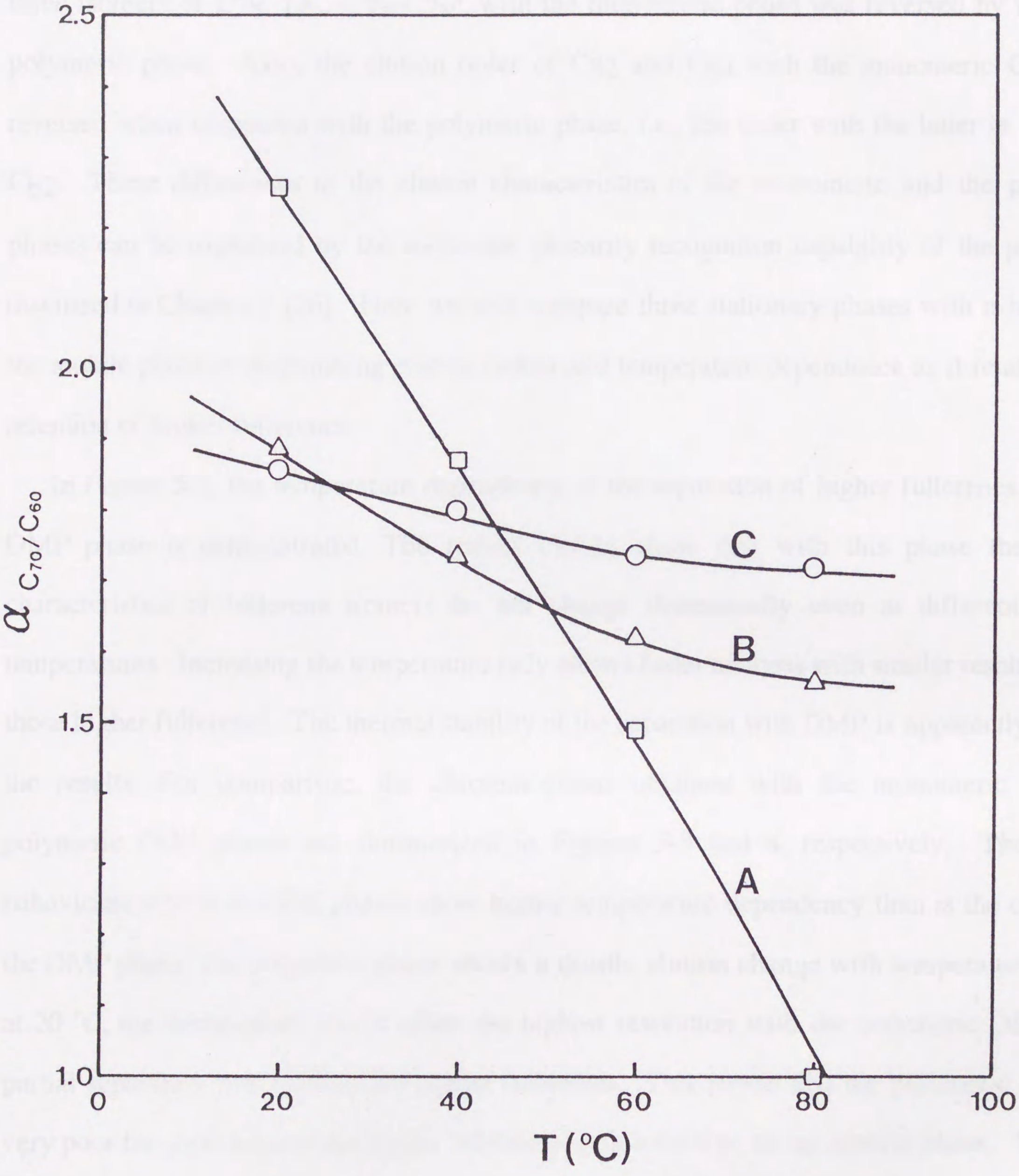

Figure 5-1. Relationships between separation factors (α) for C_{60} and C_{70} with four different stationary phases and column temperature. (A) polymeric ODS, (B) monomeric ODS, (C) DMP.

mobile phase were evaluated for the separation of higher fullerenes such as C_{76}, C_{78}, C_{82} and C_{84}. Interesting results were obtained in that work: the elution order of C_{76} and one of three isomers of C_{78}, *i.e.*, C_{78}-C_{2v}', with the monomeric phase was reversed by using the polymeric phase. Also, the elution order of C_{82} and C_{84} with the monomeric ODS was reversed when compared with the polymeric phase, *i.e.*, the order with the latter is C_{84} and C_{82}. These differences in the elution characteristics of the monomeric and the polymeric phases can be explained by the molecular planarity recognition capability of the phases, as discussed in Chapter 2 [26]. Here we will compare three stationary phases with n-hexane as the mobile phase in determining elution orders and temperature dependence as it relates to the retention of higher fullerenes.

In Figure 5-2, the temperature dependency of the separation of higher fullerenes with the DMP phase is demonstrated. The results clearly show that with this phase the elution characteristics of fullerene isomers do not change dramatically even at different column temperatures. Increasing the temperature only allows faster analysis with similar resolution for those higher fullerenes. The thermal stability of the separation with DMP is apparently seen in the results. For comparison, the chromatograms obtained with the monomeric and the polymeric ODS phases are summarized in Figures 5-3 and 4, respectively. The elution bahaviours with both ODS phases show higher temperature dependency than is the case with the DMP phase. The polymeric phase shows a drastic elution change with temperature. Even at 20 ˚C, the temperature which offers the highest resolution with the polymeric ODS, only partial separation was realized for higher fullerenes. This means that the polymeric phase is very poor for separation of the higher fullerenes with n-hexane as the mobile phase. With the monomeric phase the resolution is a little better. At 20 ˚C with the monomeric ODS, only three major peaks which range from C_{76} to C_{84} in the chromatogram appear, and these peaks merge into two major peaks at 80 ˚C. Therefore, the monomeric phase is better than the polymeric phase but neither is good for higher fullerenes separations.

Two important and conclusive facts are obtained from the experimental results: (1) the resolution of higher fullerenes with the DMP phase is better than with other phases using n-hexane as the mobile phase and (2) increasing the temperature with the DMP phase results in

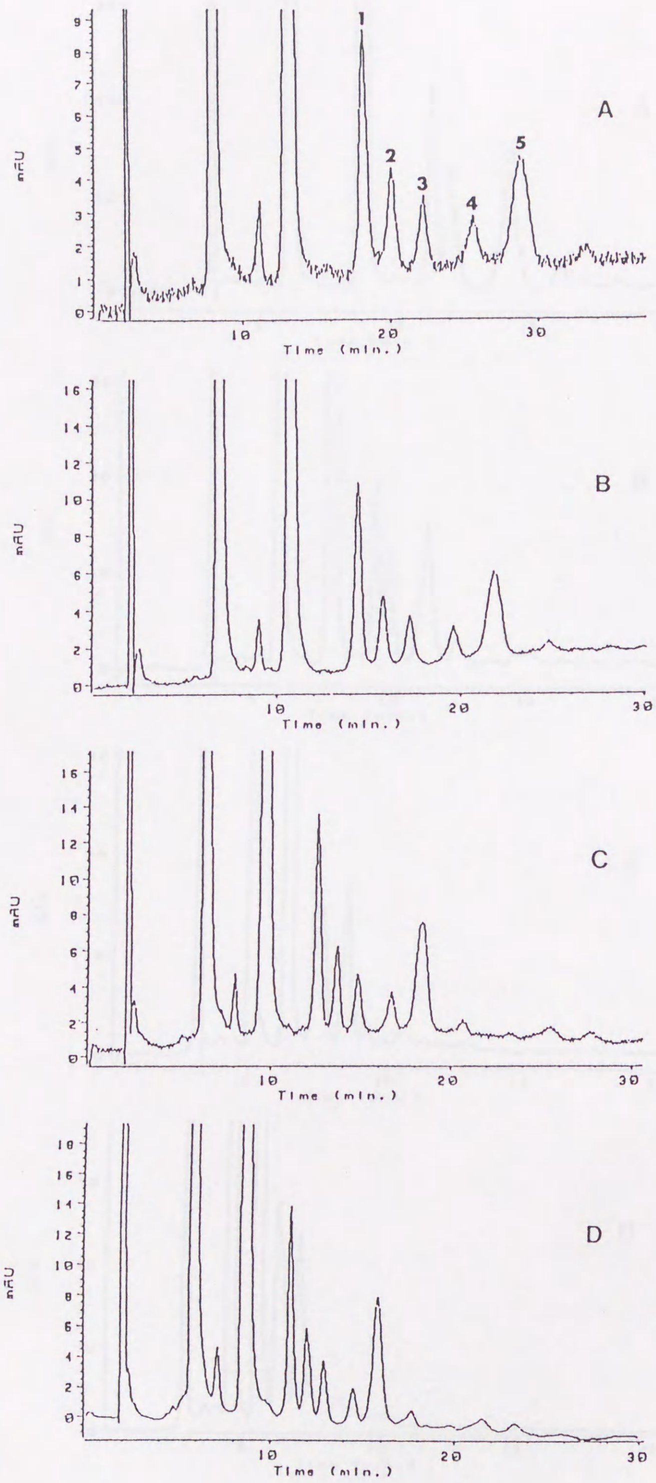

Figure 5-2. Separations of higher fullerenes with the DMP phase at different temperatures. (A) 20 °C, (B) 40 °C, (C) 60 °C and (D) 80 °C. Peak assignments are described in the caption of Figure 5-5.

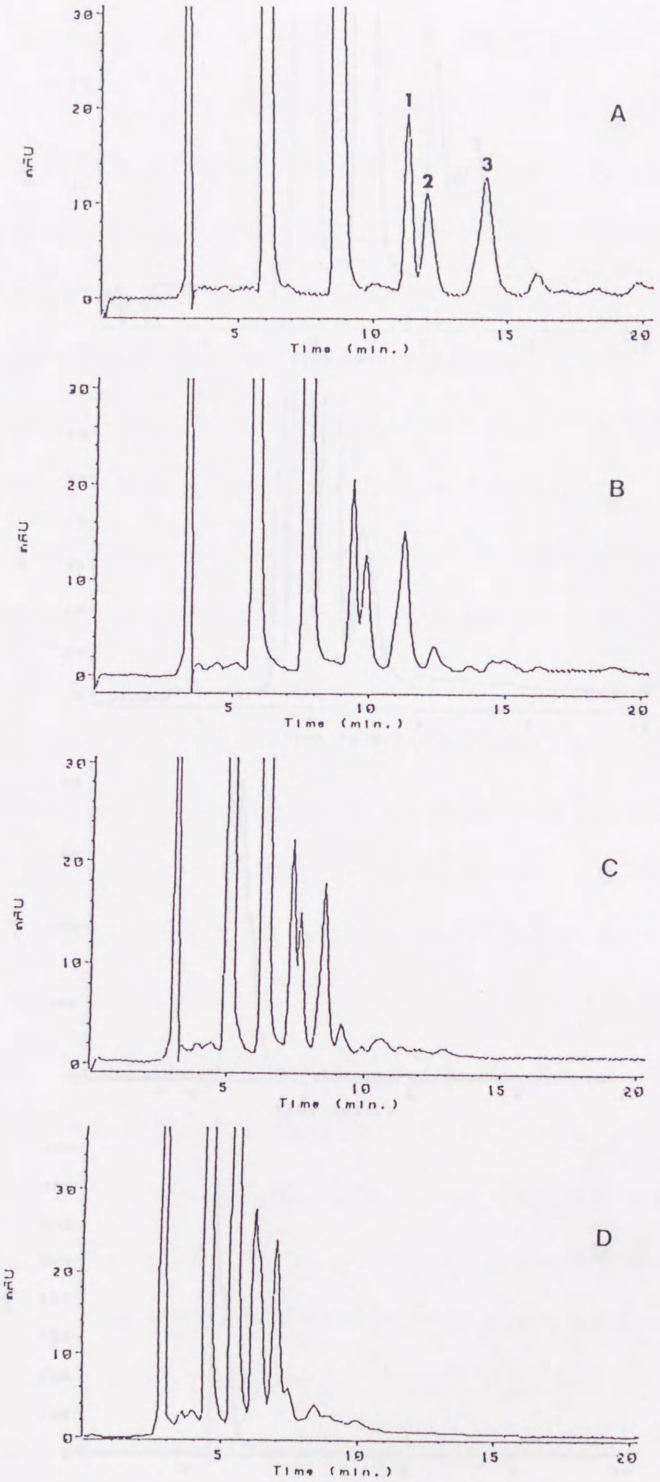

Figure 5-3. Separations of higher fullerenes with the monomeric ODS phase. (A) 20 °C, (B) 40 °C, (C) 60 °C and (D) 80 °C. Peak assignments are found in the text.

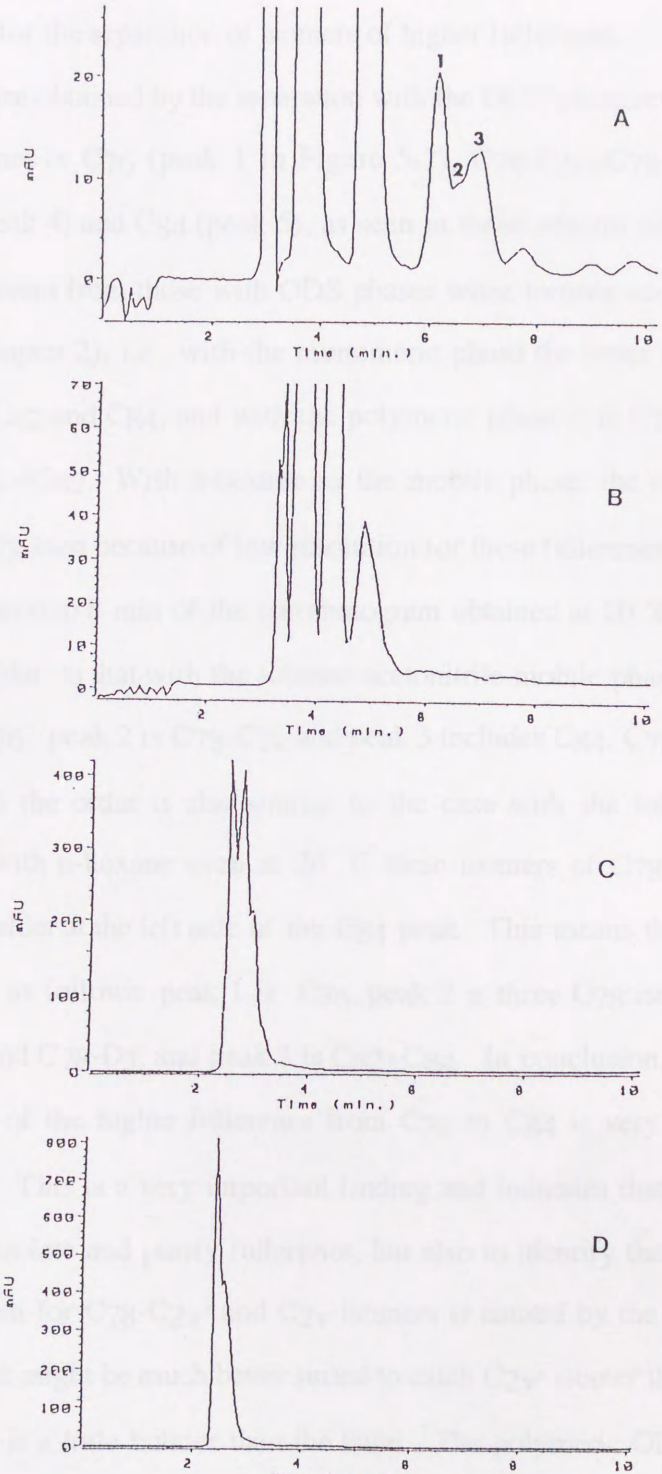

Figure 5-4. Separations of higher fullerenes with the polymeric ODS phase. (A) 20 °C, (B) 40 °C, (C) 60 °C and (D) 80 °C. Peak assignments are found in the text.

slightly faster analysis without loss of resolution. It is clear that the DMP phase is the best stationary phase for the separation of isomers of higher fullerenes.

UV-Vis spectra obtained by the separation with the DMP phase reveal that the elution order of higher fullerenes is C_{76} (peak 1 in Figure 5-2), C_{78}-C_{2v}+C_{78}-D_3 (peak 2), C_{78}-C_{2v}' (peak 3), C_{82} (peak 4) and C_{84} (peak 5), as seen in those spectra summarized in Figure 5-5. This order is different from those with ODS phases when toluene-acetonitrile was used as the mobile phase (Chapter 2), *i.e.*, with the monomeric phase the order is C_{76}, C_{78}-C_{2v}', C_{78}-C_{2v} + C_{78}-D_3, C_{82} and C_{84}, and with the polymeric phase it is C_{78}-C_{2v}', C_{76}, C_{78}-C_{2v}, C_{78}-D_3 and C_{84} +C_{82}. With n-hexane as the mobile phase, the order with the polymeric phase is not clearly seen because of low resolution for these fullerenes. By meaning spectra at several points from 6 to 8 min of the chromatogram obtained at 20 °C, it was determined that the order was similar to that with the toluene-acetonitrile mobile phase. Peak 1 in Figure 5-4 is C_{78}-C_{2v}' + C_{76}, peak 2 is C_{78}-C_{2v} and peak 3 includes C_{84}, C_{78}-D_3 and C_{82}. With the monomeric phase the order is also similar to the case with the toluene-acetonitrile mobile phase, although with n-hexane even at 20 °C three isomers of C_{78} elute together and C_{82} makes a little shoulder at the left side of the C_{84} peak. This means that the peak assignments in Figure 5-3 are as follows: peak 1 is C_{76}, peak 2 is three C_{78} isomers in the order C_{78}-C_{2v}', C_{78}-C_{2v} and C_{78}-D_3, and peak 3 is C_{82}+C_{84}. In conclusion, it has been found that the elution order of the higher fullerenes from C_{76} to C_{84} is very different with different stationary phases. This is a very important finding and indicates that LC separations can be used not only to isolate and purify fullerenes, but also to identify their size and shape. The different order seen for C_{78}-C_{2v}' and C_{2v} isomers is caused by the cavity-like shape of the DMP phase, which might be much better suited to catch C_{2v}' isomer than C_{2v} isomer because the former isomer is a little bulkier than the latter. The polymeric ODS phase, which has the largest molecular planarity recognition capability, retains C_{2v}' less than even C_{76}, and these small differences of shape and structure of the higher fullerenes isomers can be recognized by different types of stationary phases in LC. This indicates that LC separations are performed under molecular level interactions such as solute-stationary phase interaction. If one can choose a series of appropriate stationary phases and determine the elution order of unknown

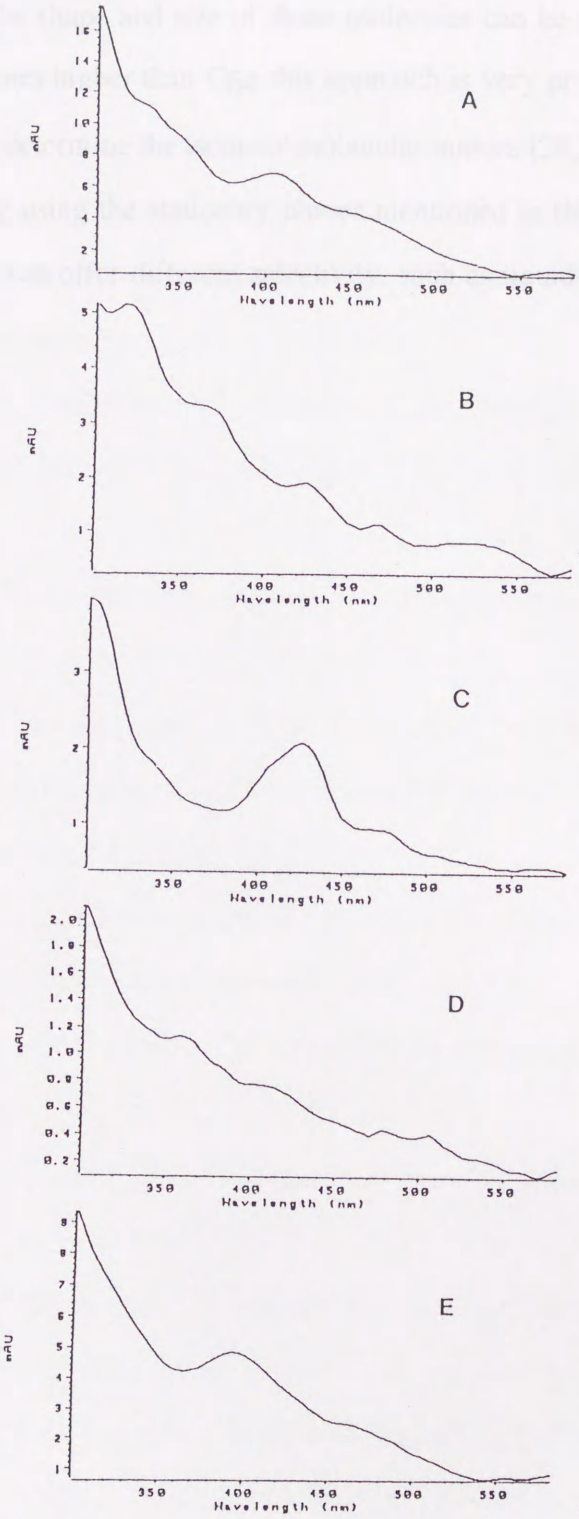

Figure 5-5. UV/Vis spectra of the peaks in the chromatograms shown in Fig. 5-2. (A) peak 1; (B) peak 2; (C) peak 3; (D) peak 4; and (E) peak 5. By careful examination the spectra can be assigned as follows: (A) C_{76}; (B) C_{78}-C_{2v}+C_{78}-D_3; (C) C_{78}-C_{2v}'; (D) C_{82}; (E) C_{84}.

fullerenes isomers, the shape and size of those molecules can be predicted by systematic LC analyses. For fullerenes higher than C_{84} this approach is very promising if one can combine LC-MS technique to determine the isomers' molecular masses [28,29]. This research is under way in our laboratory using the stationary phases mentioned in this work in addition to other different types which can offer different selectivity, such as liquid-crystal bonded phases [30-32].

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Chapter 6

Temperature Effect in Separation of Fullerene by High-performance Liquid Chromatography*

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*Modified from: *Chromatographia*, **39**, 453-459 (1994).

6.1 Abstract

The effect of column temperature, especially at low temperatures, on the separation of fullerenes on monomeric and polymeric ODS bonded phases has been studied. Decreasing the column temperature induces an increase in selectivity. The best temperature for the separation of fullerenes was determined for both types of ODS phase with n-hexane eluent. The selectivity for higher fullerenes on monomeric phases becomes similar to that on polymeric phases at low temperature. It has been found that as the carbon content of monomeric phases is increased, the selectivity also becomes similar to polymeric phases.

6.2 Introduction

Since the existence of fullerenes was confirmed, owing to their unique properties and structures, there have been many publications dealing with the preparation, separation and analysis of these compounds [1-20]. In Chapter 2~5 and the paper of Saito et al, the separations of fullerenes on monomeric and polymeric ODS phases [21,23], DMP bonded phase [22] and triphenyl bonded phase [24] were reported. These investigations have indicated that while triphenyl and DMP bonded phase have high temperature stability neither ODS bonded phases has such temperature stability. The temperature stability of triphenyl and DMP bonded phases arise from the different retention mechanism from that with ODS phases. In the former system, molecular recognition with π–π interaction is predominant, but distribution is dominant in the latter system. In LC with ODS phases, in general, solute retention is inversely related to temperature, i.e., retention decreases with increasing temperature. A linear relationship between the logarithm of retention factor and the reciprocal of absolute temperature (log k' vs 1/T, van't Hoff plot) is usually found. However the existence of phase transitions in the bonded phase was observed by Morel et al.[25]. Also IR spectral changes resulting from crystal lattice ordering were observed by Sander et al.[26]. Overall bonded phase ordering resulting from a reduction in gauche-gauche, gauche-trans-gauche, and end gauche conformation was observed at low temperatures by them. They also reported that column selectivity was found to vary continuously with temperature and the shape recognition ability of the ODS phase to separate closely related isomers was observed to be greatest at subambient temperatures [27,28]. Hence a better separation of fullerene isomers is also expected to be achieved at subambient temperature with ODS bonded phases.

In this chapter, the effect of column temperature, especially at low subambient temperature, on the selectivity of fullerene separation with various ODS stationary phases in LC will be discussed.

6.3 Experimental

The LC system consisted of Jasco PU-980 pump (Tokyo, Japan), Hewlett-Packard 1040 A photodiode array detector (Yokogawa Analytical Systems, Musashino, Japan) controlled by HP 9000 computer, and Rheodyne 7125 injector (Cotani, CA, USA) with 20 μL loop for injection. The column temperature was controlled by Tosoh RE-8000 oven (Tokyo, Japan) between 30 °C and 80 °C and LAB-Thermo Model LH-1000E (Toyo Seisakusho, Tokyo, Japan) between 0°C and 30°C. Lower column temperature below freezing point was controlled with methyl alcohol and dry ice. A 250 × 4.6mm i.d. Develosil ODS-5 (5 μm, 20 C%, Nomura Chemicals, Seto, Japan), Develosil ODS N-5 (16 C%) and Develosil ODS P-5 (11 C%) were used as the monomeric type ODS columns. A similar Wakosil II 5C18AR column (5 μm, 18 C%, Wako Chemicals, Tokyo, Japan) and Vydac 218TP54 (5 μm, 8-9 C%, Separations Group, Hesperia, CA, USA) were used as polymeric type columns. The mobile phase was n-hexane with a flow rate 1mL min^{-1}.

Carbon soot was produced by a common arc discharge in an inert gas environment. The soot was extracted with toluene in order to obtain the C_{60} and C_{70} fractions and the residue was extracted with 1,2,4-trichlorobenzene to obtain highly concentrated higher fullerenes. The solution was then evaporated to dryness and redissolved in toluene for use as the test sample for injection. The following hydrocarbons were used as PAH solutes: phenanthrene, anthracene, pyrene, triphenylene and chrysene; all are commercially available. Each peak identification was performed by UV-Vis spectral matching with published spectra.

6.4 Results and Discussion

In Figures 6-1, several chromatograms using three monomeric ODS phase with n-hexane as the mobile phase are illustrated, changing the column temperature between 20 °C and -70 °C. In addition to the fact that the higher surface coverage gives better separation, it appears that high temperatures can not give good resolution but decreasing the temperature induces increase in resolution. In Figure 6-1, the best separation of fullerene isomers is achieved at -20 °C with the monomeric ODS bonded phase, Develosil ODS-5 (20 C%). In isomer separation, the best resolution is obtained at -20 °C with n-hexane mobile phase. At temperatures lower than -20°C it is found that the peak of C_{78}-$C_{2v'}$ is almost merged with the peak of C_{76} (peak No. 1 in the figure), since C_{78}-$C_{2v'}$ elutes at almost the same retention time of C_{76}. This means the retention of C_{78}-$C_{2v'}$ tends to be shorter than that of C_{76} with decreasing temperature. A change in elution order of C_{82} (peak No. 5) and C_{84} (peak No. 6) is also observed with decreasing temperature. In Chapters 2~4, we have shown that the elution order of higher fullerenes is controlled by their molecular size and shape with monomeric and polymeric ODS bonded phases using toluene-acetonitrile eluent [21]. The polymeric ODS resolves three C_{78} isomers, one of them being eluted faster than C_{76}. The monomeric ODS can not resolve two of the three C_{78} isomers but a column temperature of 10 °C induces partial separation. This behavior is assumed to be the result of the better shape recognition ability of the polymeric ODS. It has also been reported in another previous paper of Jinno et al.[29] that the difference between the surface structural characteristics of both ODS bonded phases revealed by NMR and FT-IR can explain why the functionality of ODS phases can result in different molecular planarity recognition capabilities. Sander et al. reported that at low temperatures phase selectivity is more "polymeric-like" for both ODS phase types [28]. So it is considered that the best separation of three isomers of C_{78} at -20 °C is also due to the superior shape recognition ability of the monomeric ODS bonded phase. However below -20 °C, and contrary to expectation, decreasing temperature induces decreasing resolution and retention. It is thought that lower column temperature changes the structure of the stationary phase to rigid and ordered and, because of a more "slit-like" structure at the surface, better

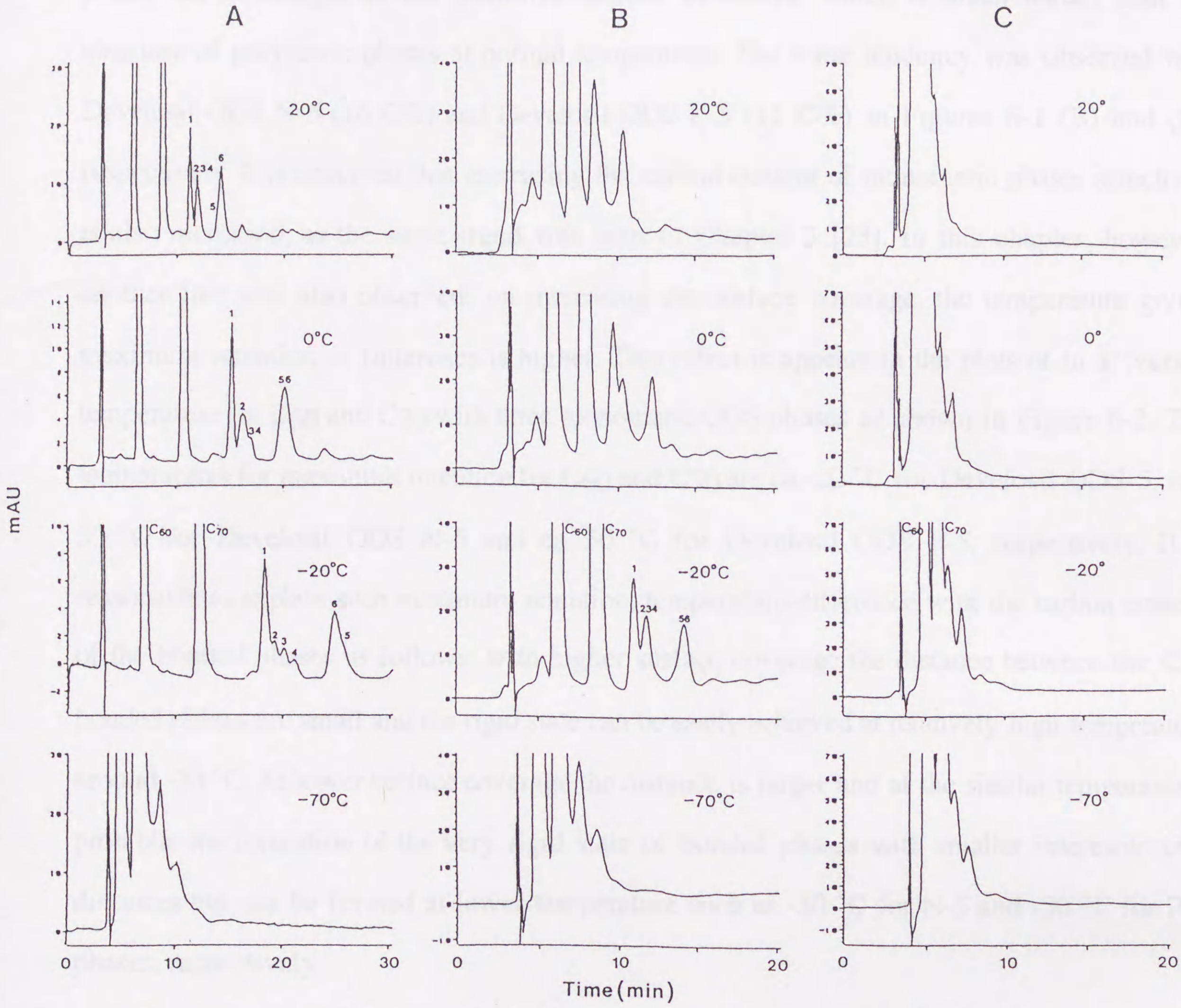

Figure 6-1. Separations of higher fullerenes with monomeric ODS phases at various temperatures. A:Develosil ODS-5, 20 C%; B:Develosil ODS N-5, 16 C%; C:Develosil ODS P-5, 11 C%; mobile phase, n-hexane; flow rate, 1 ml min^{-1}; detection, 312nm. peaks 1=C_{76}, 2=C_{78}-$C_{2v'}$, 3=C_{78}-C_{2v}, 4=C_{78}-D_3, 5=C_{82}, 6=C_{84}.

separation of higher fullerenes is achieved at -20 °C. At this stage, the monomeric phase behaves as like polymeric one. Then below that temperature, it is also supposed that the bonded phases become more rigid and ordered, finally the phases can scarcely retain the bulky and globular fullerenes, when retention of all of fullerenes decreased. This state of the bonded phase can be thought as the solid-like surface structures, which is much harder than the structure of polymeric phases at normal temperature. The same tendency was observed with Develosil ODS N-5 (16 C%) and Develosil ODS P-5 (11 C%) in Figures 6-1 (B) and (C), respectively. It is observed that increasing the carbon content of monomeric phases selectivity is also increased, as the same trend was seen in Chapter 3 [23]. In this chapter, however, another fact was also observed: on increasing the surface coverage, the temperature giving maximum retention of fullerenes is higher. This effect is appears in the plots of ln k' versus temperature for C_{60} and C_{70} with three monomeric ODS phases as shown in Figure 6-2. The temperatures for maximum retention for C_{60} and C_{70} are ca.-20 °C for Develosil ODS-5, ca.-30 °C for Develosil ODS N-5 and ca.-50 °C for Develosil ODS P-5, respectively. It is reasonable to explain such maximum retention, temperature-difference with the carbon content of the bonded phases as follows: with higher surface coverage the distance between the C_{18} bonded phases are small and the rigid state can be easily achieved at relatively high temperature around -20 °C. At lower surface coverage the distance is larger and at the similar temperatures prohibits the formation of the very rigid state of bonded phases with smaller intermolecular distances but can be formed at lower temperature such as -30 °C for N-5 and -50 °C for P-5 phases, respectively.

The chromatograms using polymeric ODS phases and the plots of ln k' vs temperature are in Figures 6-3 and 4. It appears that the retention with polymeric ODS is smaller than with the monomeric kind. With Wakosil ODS, the elution order can be determined at 0 °C, and the order is a quite different from the monomeric phases. That is C_{78}-C_{2v}', C_{76}, C_{78}-C_{2v}, C_{84}, C_{78}-D_3 and C_{82}, and this order is unusual compared to the monomeric phases at the same temperature around 0 °C. This fact can be explained by the good molecular-shape recognition of the polymeric phase. The maximum retention temperatures of fullerenes on polymeric ODS is higher than for the three monomeric ODS phases, and is because the intermolecular distance

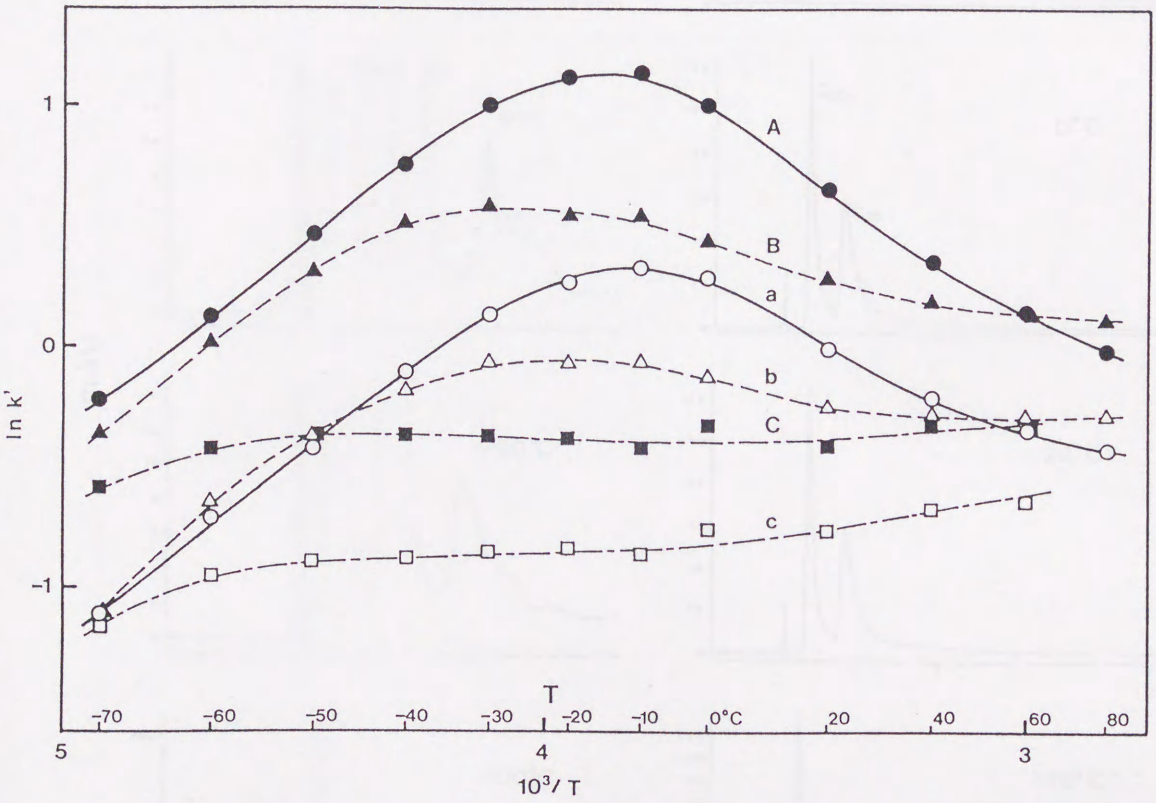

Figure 6-2. ln k' versus temperature for C_{60} and C_{70} for three different carbon-content, monomeric ODS phases. a-○: C_{60}, Develosil ODS-5 (20 C%); A-●: C_{70}, Develosil ODS-5. b-△: C_{60}, Develosil ODS N-5 (16 C%); B-▲: C_{70}, Develosil ODS N-5. c-□: C_{60}, Develosil ODS P-5 (11 C%); C-■: C_{70}, Develosil ODS P-5.

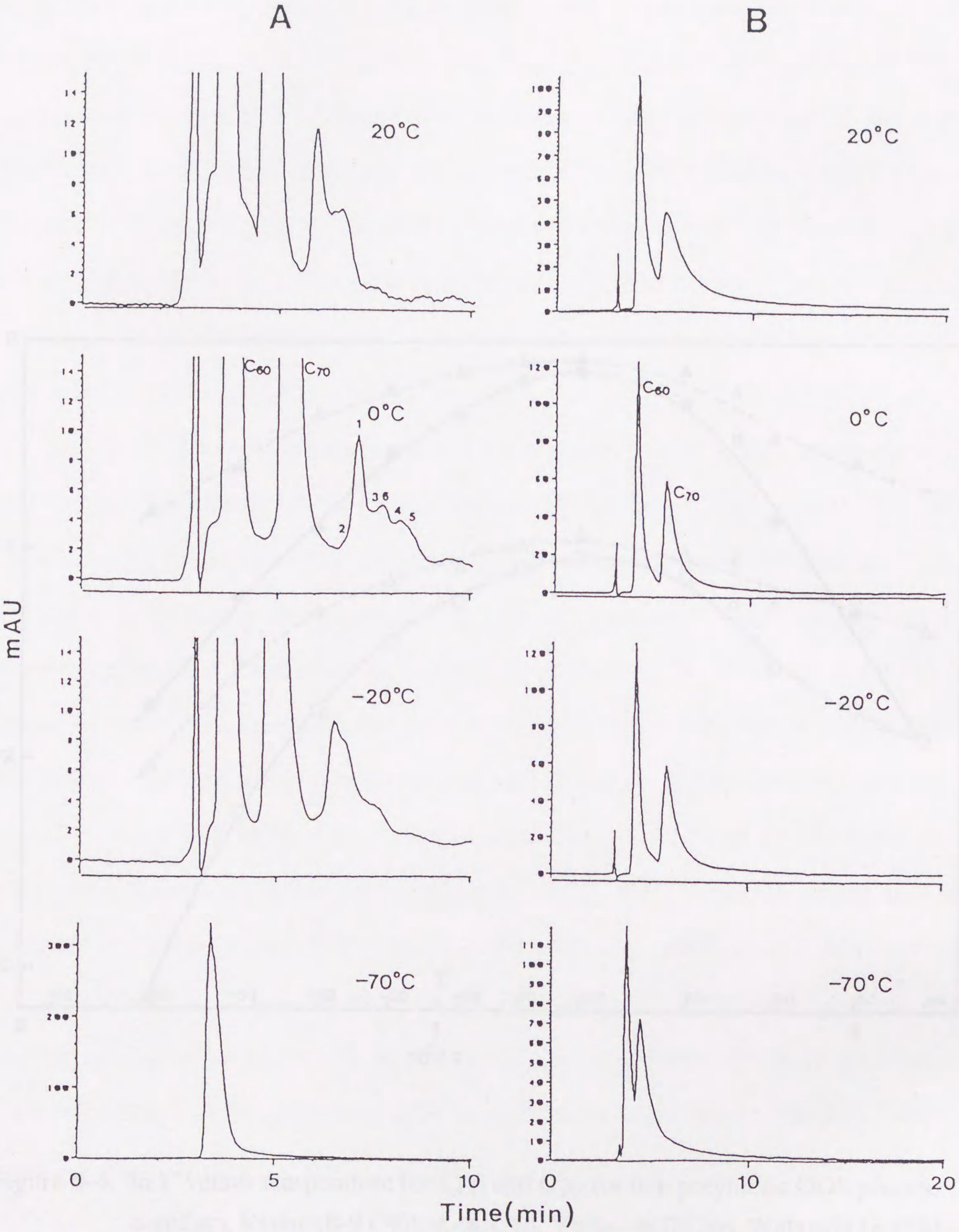

Figure 6-3. Separations of higher fullerenes on the polymeric ODS phase at various temperatures. A:Wakosil, B:Vydac; mobile phase, n-hexane; flow rate, 1 ml min^{-1}; detection, UV at 312nm.
peak 1:C_{76} 2:C_{78}-$C_{2v'}$ 3:C_{78}-C_{2v} 4:C_{78}-D_3 5:C_{82} 6:C_{84}.

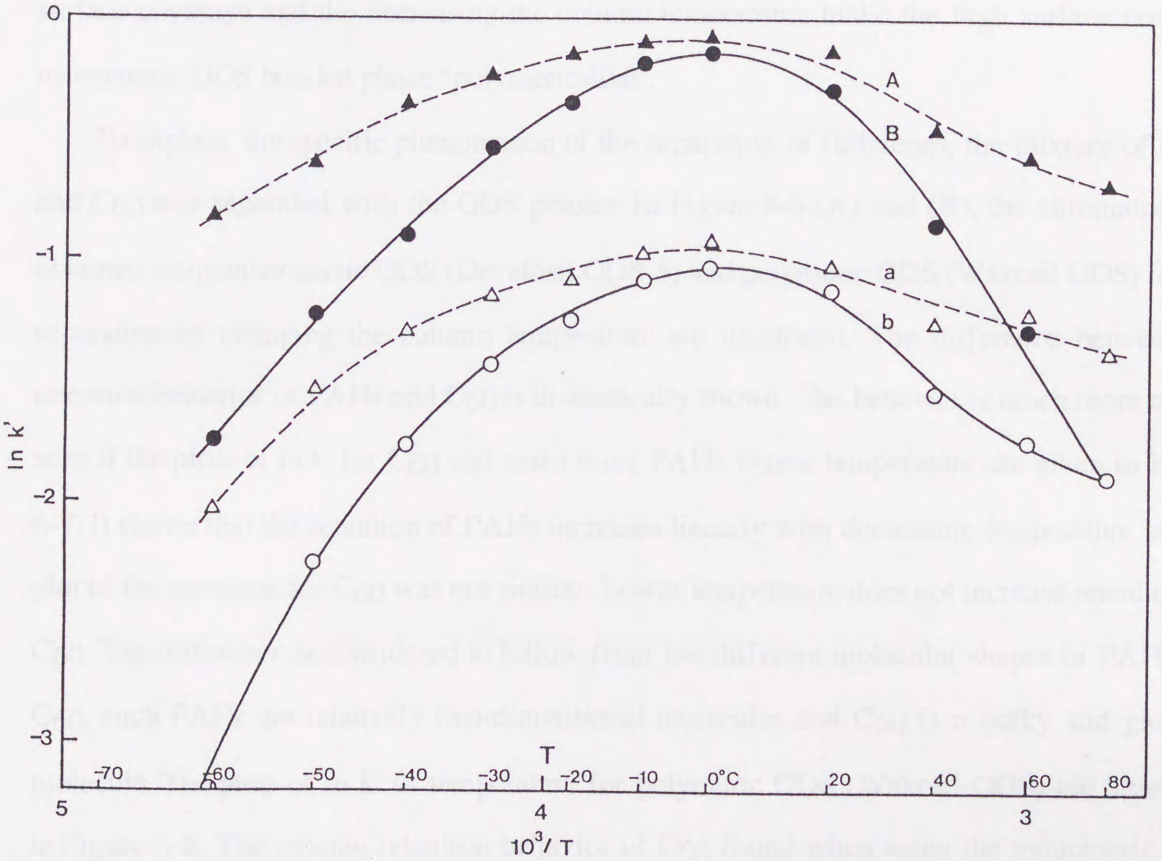

Figure 6-4. ln k' versus temperature for C_{60} and C_{70} for two polymeric ODS phases. a-△:C_{60}, Vydac (8-9 C%); A-▲:C_{70}, Vydac. b-○:C_{60}, Wakosil (18 C%); B-●:C_{70}, Wakosil.

of C_{18} bonded phases in associated states of the polymeric phase is smaller than that in monomeric ones. For comparison of monomeric and polymeric ODS phases, relationships between separation factors for C_{60} and C_{70} with four different stationary phases and column temperature are illustrated in Figure 6-5. Increasing the surface coverage of the monomeric phases improves the separation factor for C_{60} and C_{70}. With Develosil ODS-5, separation of C_{60} and C_{70} above 0 °C is similar to other monomeric ODS phases but below that temperature the separation factor becomes similar to polymeric ODS. The result suggests that increasing the surface coverage and the decreasing the column temperature make the high surface coverage monomeric ODS bonded phase "polymeric-like".

To explain the specific phenomenon of the separation of fullerenes, the mixture of PAHs and C_{60} was separated with the ODS phases. In Figure 6-6 (A) and (B), the chromatograms obtained using monomeric ODS (Develosil ODS-5) and polymeric ODS (Wakosil ODS) for the separation by changing the column temperature are illustrated. The difference between the retention behavior of PAHs and C_{60} is dramatically shown. The behavior is much more clearly seen if the plots of ln k' for C_{60} and main three PAHs versus temperature are given in Figure 6-7. It shows that the retention of PAHs increases linearly with decreasing temperature but the plot of the retention for C_{60} was not similar. Lower temperature does not increase retention for C_{60}. The difference is considered to follow from the different molecular shapes of PAHs and C_{60}, such PAHs are relatively two-dimensional molecules and C_{60} is a bulky and globular molecule. The plots of ln k' vs temperature for polymeric ODS (Wakosil ODS) are illustrated in Figure 6-8. The strange retention behavior of C_{60} found when using the monomeric ODS apparently could not be observed for the C_{60} retention on polymeric materials because of its small retention but the good shape recognition for PAHs is clearly seen because of the extreme difference in retention behavior of PAHs and C_{60}. Retention of the bulky, globular fullerene, C_{60}, at low temperature is difficult with the polymeric ODS phase since the rigid and ordered bonded phase works to exclude the molecule. The above investigation clearly indicates that decreasing temperature induces an increase in the rigid and ordered "slit-like" structure on the ODS surface.

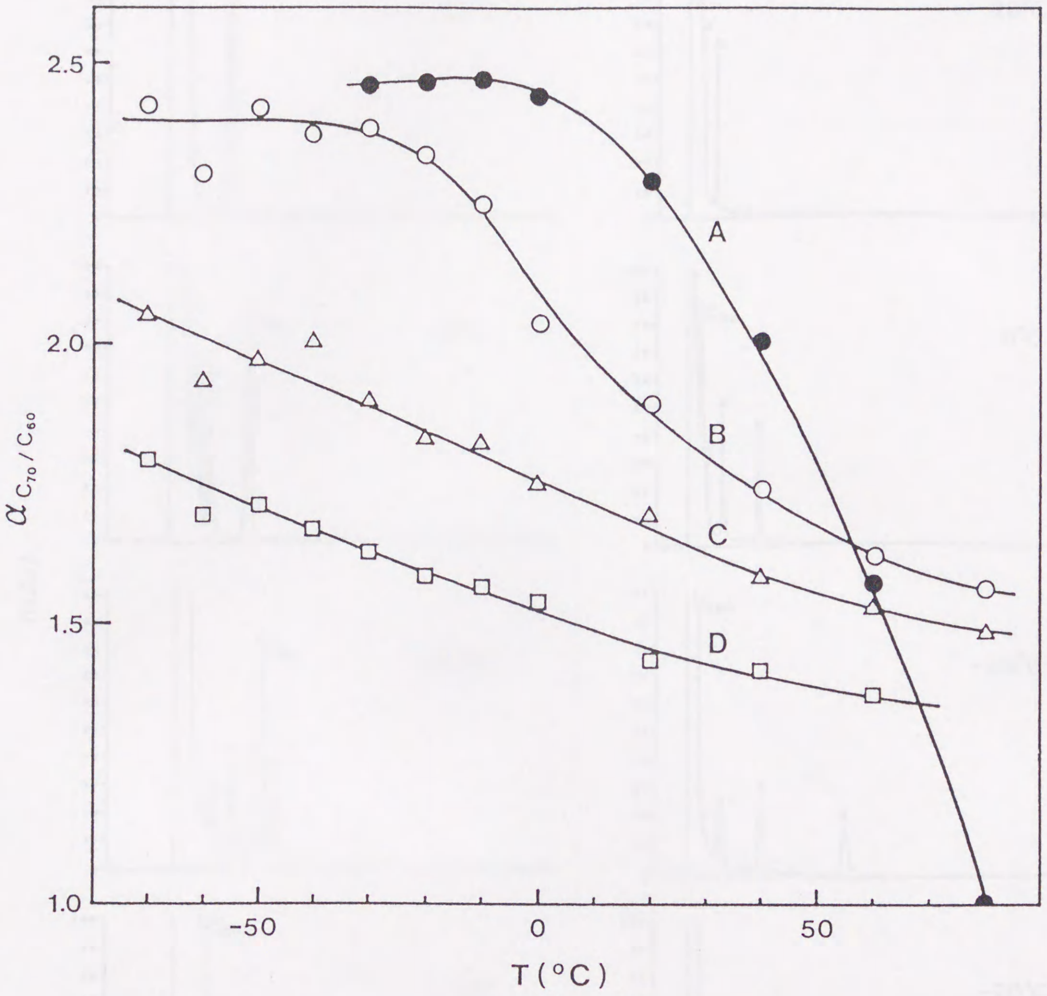

Figure 6-5. Separation factors versus column temperature for C_{60} and C_{70} with four stationary phases.
A-●: Wakosil (polymeric type), B-O:Develosil ODS-5 (monomeric type, 20 C%), C-△:Develosil ODS N-5 (monomeric type, 16 C%), D-□:Develosil ODS P-5 (monomeric type, 11 C%)

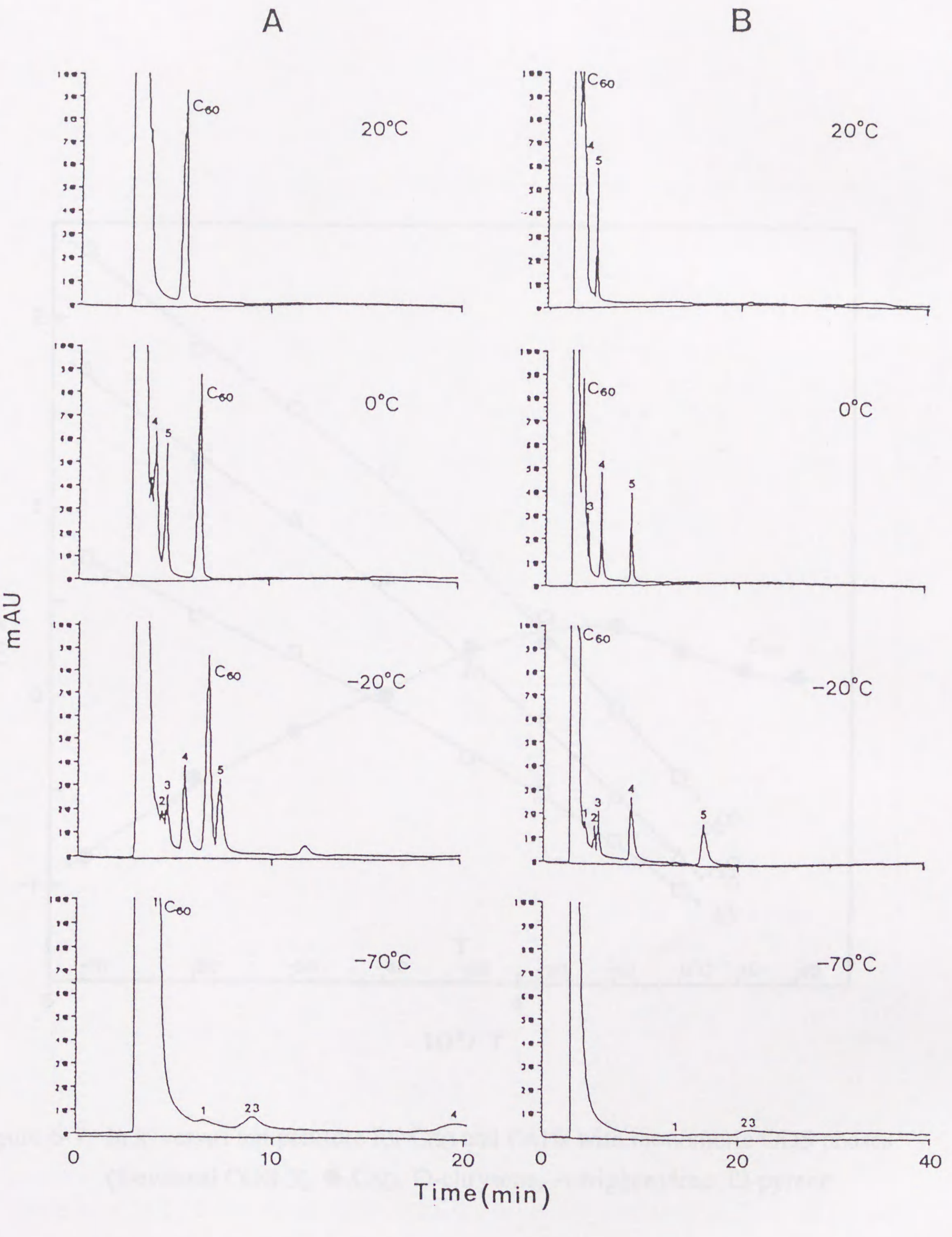

Figure 6-6. Separations of C_{60} and PAHs with monomeric ODS and polymeric ODS phases at various temperatures.

A:Develosil ODS-5, B:Wakosil; mobile phase, n-hexane; flow rate, 1 ml min $^{-1}$; detection, 254nm. Peaks 1=phenanthrene, 2=anthracene, 3=pyrene, 4=triphenylene, 5=chrysene.

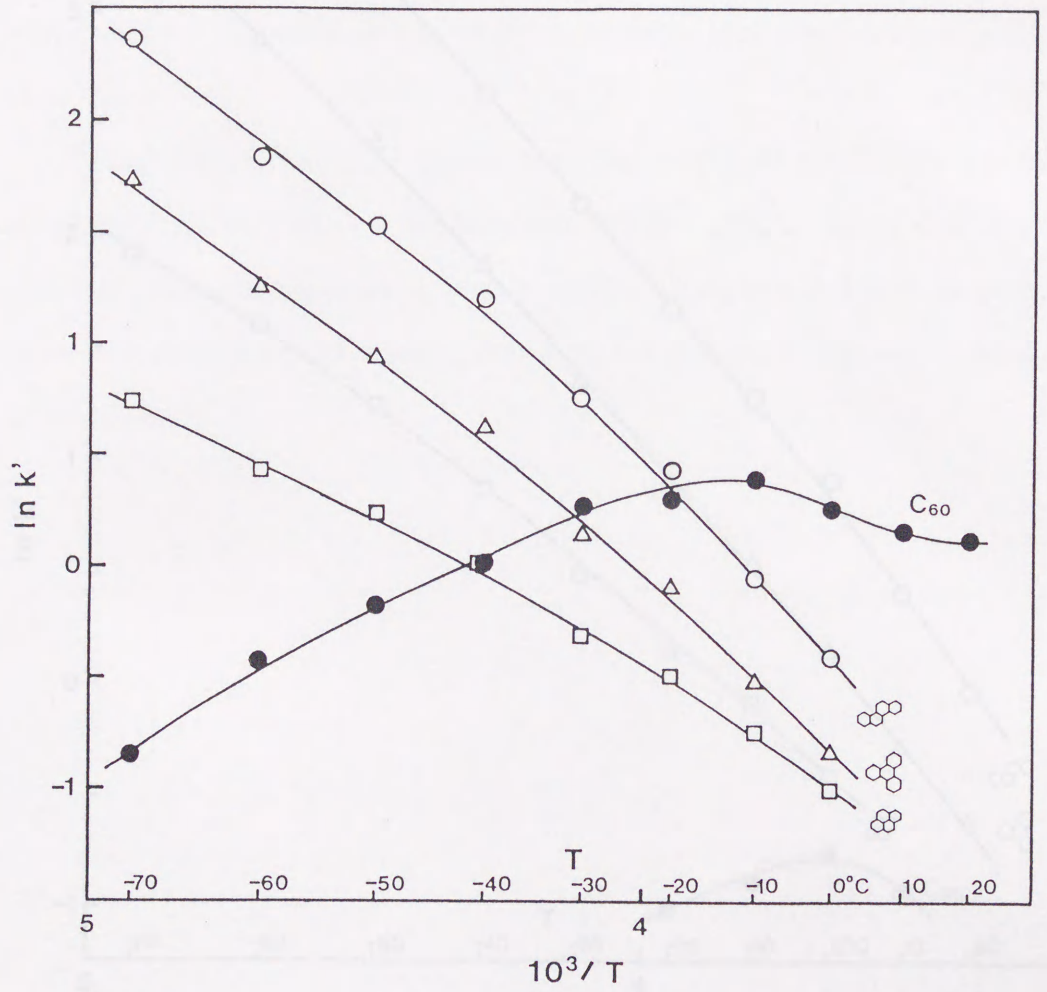

Figure 6-7. ln k' versus temperature for C_{60} and PAHs with monomeric ODS phases (Develosil ODS-5). ●-C_{60}, ○-chrysene, △-triphenylene, □-pyrene.

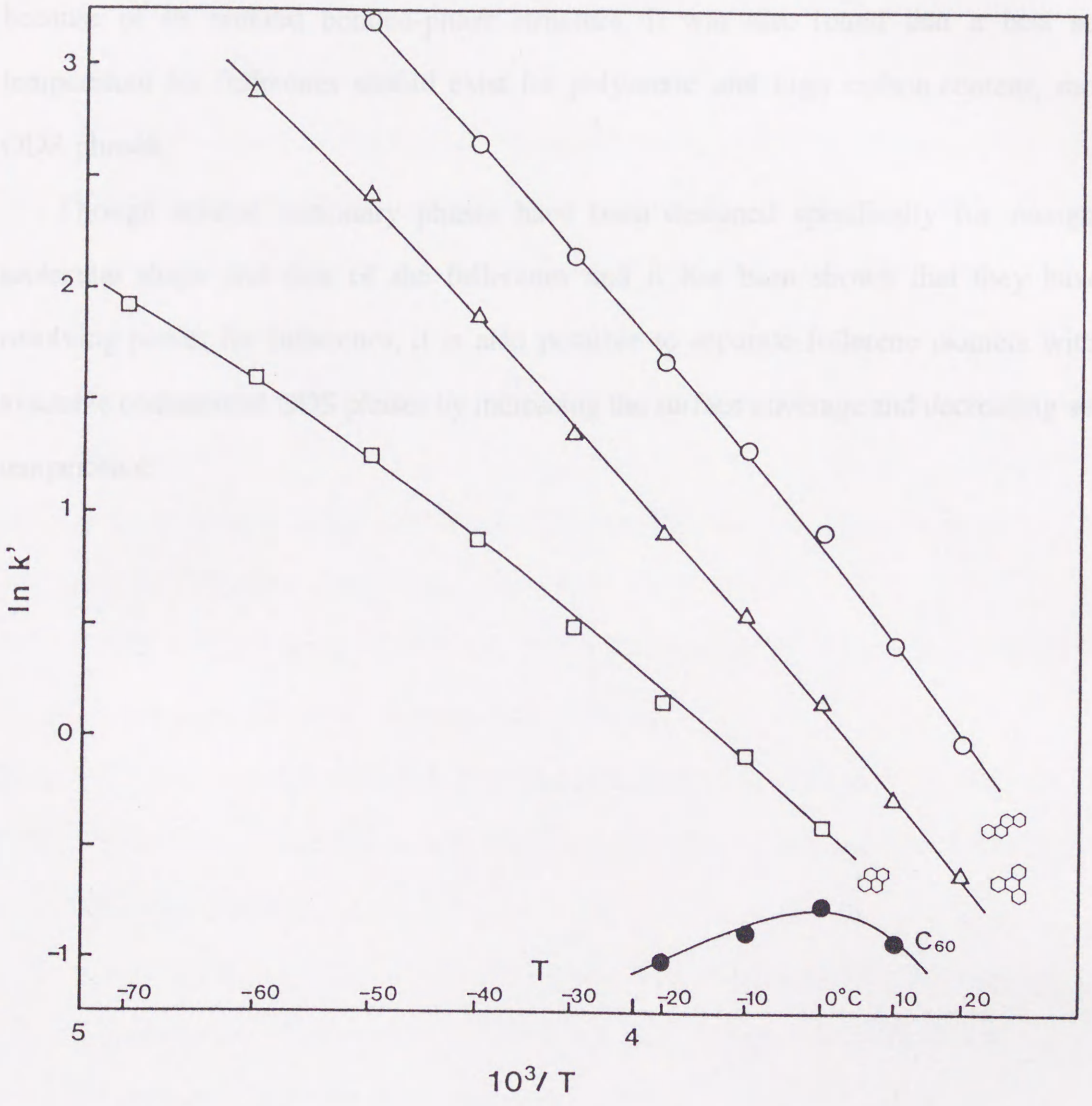

Figure 6-8. ln k' versus temperature for C_{60} and PAHs with polymeric ODS phases (Wakosil). ●-C_{60}, O-chrysene, △-triphenylene, □-pyrene.

6.5 Conclusion

Better separation of fullerene isomers was achieved at subambient temperatures with ODS bonded phases. Molecular planarity recognition was confirmed at low subambient temperature because of its ordered bonded-phase structure. It was also found that a best separation temperature for fullerenes should exist for polymeric and high carbon-content, monomeric ODS phases.

Though several stationary phases have been designed specifically for recognition of molecular shape and size of the fullerenes and it has been shown that they have strong resolving power for fullerenes, it is also possible to separate fullerene isomers with widely available commercial ODS phases by increasing the surface coverage and decreasing separation temperature.

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Chapter 7

Retention of Fullerenes by Octadecylsilica. Correlation with NMR Spectra at Low Temperatures*

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*Modified from: *Chromatographia,* **40**, 507-512 (1995).

7.1 Abstract

The temperature effect on the retention of fullerenes in the range 80 °C to -70 °C in LC has been examined using ODS stationary phases. It has been found that the maximum retention temperature lies around - 10 °C with a highly carbon loaded ODS phase. Solid state CP-MAS NMR measurements on the stationary phases indicated that the relaxation time at the 30 ppm methylene signal changes with the temperature and has a minimum relaxation time at the temperature which closely matches the maximum retention temperature observed in chromatography. The interpretation of both NMR spectroscopic and LC chromatographic data are discussed.

7.2 Introduction

The temperature dependency of LC retention has been studied by several authors [1-5] and the general trend observed is that retention increases with decreasing column temperature. If peak symmetry is retained at reduced temperatures, the resolution or separation factor also improves, primarily because of longer retention. However, some exceptions to these general observations are found. Pirkle et al. have found [6] both the retention of fullerene C_{60} and the separation factor between C_{60} and C_{70} increases with increasing column temperature when using their special stationary phase. Jinno et al. have reported little change in retention of C_{60} and C_{70} with column temperature when using either a multilegged biphenyl bonded phase (BP) [7] or a DMP phase (Chapter 5) [8]. These results might suggest that, in comparison with typical solute such as PAHs, somewhat different molecular interactions are induced between fullerenes and unique or unusual stationary phases. However, we have reported (Chapter 6) [9] an unusual temperature dependency while studying the separation of fullerenes with conventional, commercially available ODS. We observed that a highly loaded monomeric ODS increasingly retained C_{60} and C_{70} fullerenes with decreasing column temperature until a maximum retention temperature (-10 °C) was reached. Retention of these solutes then decreased with further lowering of the column temperature. In contrast, PAHs maintained the expected behaviour with decreasing column temperature (80 to -70 ˚C), i.e., a monotonic increase in retention. We also observed that the maximum retention temperature for a particular ODS phase depends on the loading density of that phase, i.e. -10 ˚C for a highly loaded, -30 ˚C for a moderately loaded and -50 ˚C for a lightly loaded ODS phases, respectively. These observations led us to probe for variations of the stationary phase structure with changing temperature, using both spectroscopic (NMR) and chromatographic techniques. The results are described in this communication.

7.3 Experimental Section

Apparatus

The LC system consisted of a Jasco PU-980 pump (Tokyo, Japan), a Hewlett-Packard 1040 A photodiode array detector (Yokogawa Analytical Systems, Musashino, Japan) controlled by a HP 9000 computer, a Rheodyne 7125 injector (Cotati, CA, USA) with a 20 μL loop for injection and a 250 x 4.6 mm i.d. column packed with the stationary phase under study. Column temperatures between 80 °C and 30 °C were controlled by a Tosoh RE-8000 oven (Tokyo, Japan) and a Lab-Thermo Model LH-1000E (Toyo Seisakusho, Tokyo, Japan) for temperatures between 30 and 0 °C. Column temperatures below 0 °C were controlled with a methanol-dry ice slush bath.

Solid state NMR spectra, cross-polarization (CP) with magic angle spinning (MAS) were obtained on a Bruker MSL 300 spectrometer (Billerica, MA, USA).

Reagents

Toluene and n-hexane were obtained from Tokyo Kasei, Tokyo, Japan. Develosil ODS-5 (5 μm, 100 Å pore size, 20 % C load), Develosil ODS-N-5 (16 % C load) and Develosil ODS-P-5 (11 % C load) were obtained from Nomura Chemicals, Seto, Japan. A C_8-phase (5 μm, 120 Å pore size, 5.1 % C load) used as a reference was synthesized by J&W Scientific (Folsom, CA, USA). The C_{60} and C_{70} fullerenes were isolated from carbon soot by a process described in our previously [7-9]. Chrysene, a four-ring PAH, was used as reference (from Wako Chemicals, Osaka, Japan).

Procedures

CP-MAS NMR Measurements.

Samples of 200-300 mg stationary phases were spun at 5 KHz in a double bearing ZrO_2 rotor. For ^{13}C spectra, the contact time was 5 ms with a repetition rate of 5 s. Chemical shifts were referred to tetramethylsilane by using the carbonyl signal of glycine as a standard. Simulated spectra were generated using the LINESIM software package provided with the spectrometer by Bruker Instruments. Line widths were determined by three methods: manually from the experimental spectrum, by a visual estimation using LINESIM and by a least squares fit using LINESIM.

Chromatographic Measurements.

The mobile phases were n-hexane and a toluene/n-hexane mixture with a typical flow rate of 1 mL min^{-1}. Peak identification was by UV-Vis spectral match to known spectra.

7.4 Results and Discussion

Chromatographic Studies.

Figure 7-1 summarizes typical changes seen for the retention of C_{60} and C_{70} as a function of temperature when using n-hexane mobile phase and any one of the ODS-5 stationary phases. From high to low column temperatures the separation performance alters substantially. The retention factors (ln k') of C_{60} and C_{70} are plotted versus the column temperature in Figure 7-2(A) for ODS-5, (B) for ODS-N-5 and (C) for ODS-P-5, respectively. In Figure 7-2(A) the retention data of chrysene are also plotted as a reference. Maximum retention temperatures can be seen at ca. -10 ˚C with ODS-5, ca. -20~-30 ˚C with N-5 and ca. -50 ˚C (not clearly seen) with P-5. In contrast, the retention of chrysene increases monotonically with decrease in temperature using ODS-5. Figure 7-2(D) shows that similar chromatographic behavior is observed when using a n-hexane/toluene mobile phase with ODS-5, demonstrating that such retention behavior is much less dependent on mobile phase composition than stationary phase properties.

NMR Studies. Figure 7-3 shows NMR spectral changes as a function of temperature for the ODS-5 phase. Drastic spectral changes are induced by temperature variations, especially the signals around 30 ppm, which are typically assigned to methylene(CH_2) groups in the middle part of the alkyl chain [10-13]. These initial results suggested that a more careful examination of the NMR spectra would yield insights into configuration as changes in the alkyl

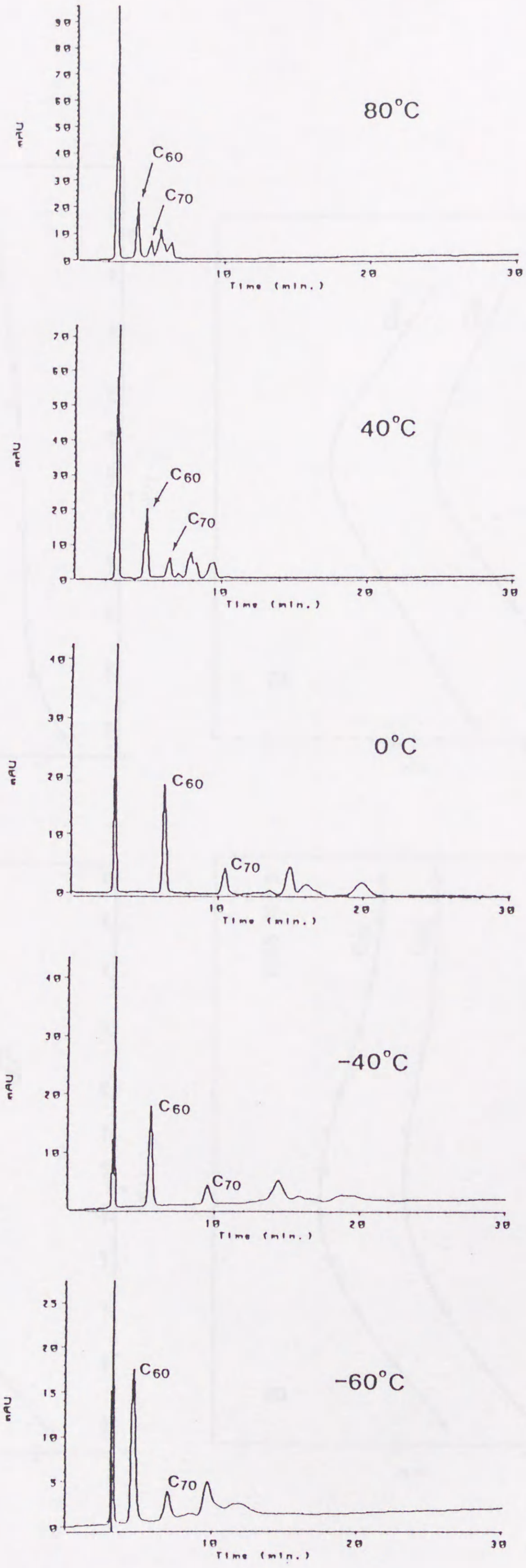

Figure 7-1. Separations of C_{60} and C_{70} with Develosil ODS-5.
mobile phase:n-hexane, 1 mL/min, detection: UV at 312 nm.

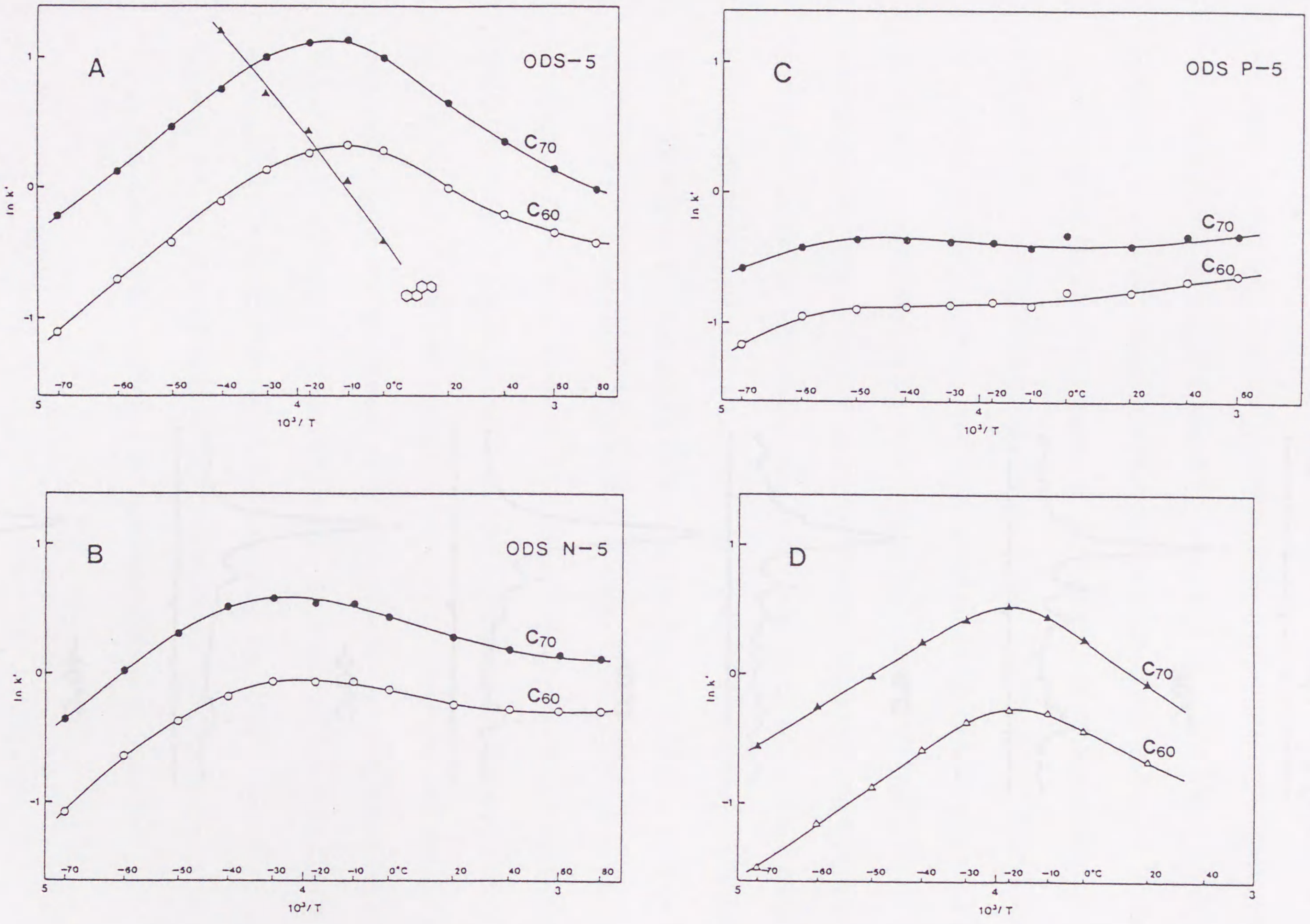

Figure 7-2. Plots of ln k' vs. temperature for C_{60}, C_{70} and chrysene (only in (A)) with (A) ODS-5, (B)ODS-N-5, (C)ODS-P-5 using n-hexane mobile phase, and (D) ODS-5 using n-hexane/toluene=85/15 mobile phase.

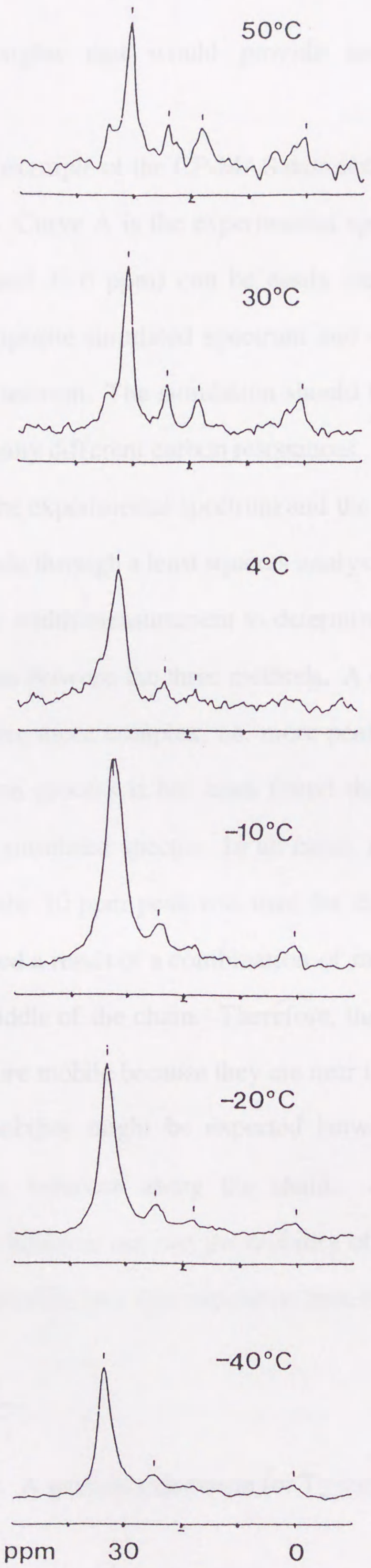

Figure 7-3. CP-MAS ^{13}C NMR spectra of Develosil ODS-5 at different temperatures.

chains with temperature, insights that would provide an explanation of the above chromatographic behavior.

Figure 7-4 shows a typical example of the CP-MAS data obtained for all three phases over the temperature range studied. Curve A is the experimental spectrum of the P-5 phase at 30 °C. Three peaks (30.0, 32.0 and 33.6 ppm) can be easily identified in this portion of the spectrum. Curve B is the composite simulated spectrum and curve C shows the individual components of the simulated spectrum. The simulation should be tested in this case since the actual peak is a composite of many different carbon resonances. Simulation is first performed by a visual matching between the experimental spectrum and the proposed computer-generated spectrum. A close match is made through a least squares analysis. These two approaches are chosen along with a direct line width measurement to determine how well they all agree. In our case there is close agreement between the three methods. A direct measurement would not be possible if the spectrum were more complex, i.e. more peaks with close chemical shifts. As the results of this simulation process it has been found that there is an excellent match between the experimental and simulated spectra. In all cases, i.e. at each temperature for all three phases, the line width of the 30 ppm peak was used for the calculation of the relaxation time. The 30 ppm peak is indeed a result of a combination of many carbon atoms. However, all of these are located in the middle of the chain. Therefore, they are not overly restricted by being close to the surface or more mobile because they are near the end of the alkyl chain.

While some differences in mobility might be expected between C_4 or C_5 and C_{15}, the measurement presents average behavior along the chain. Solute retention is probably controlled by the average chain behavior not just the mobility of a particular carbon site in the chain. The line width is related to the spin-spin relaxation time by

$$\text{line width} = \frac{1}{\pi T_2}$$

where T_2 is the relaxation time. A general expression for T_2 can be written as follows:

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_{2(\text{inhomo})}} + \frac{1}{T_{2(\text{diff})}}$$

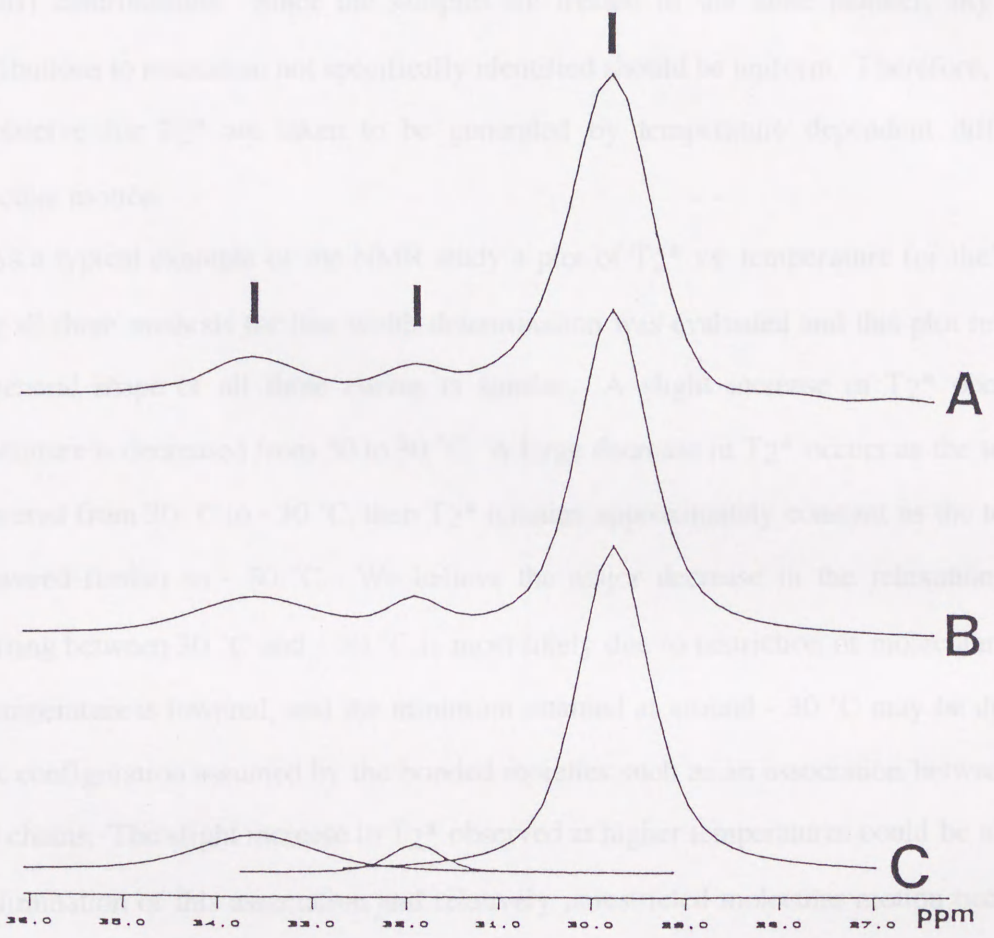

Figure 7-4. CP-MAS ^{13}C NMR spectrum of ODS-P-5 around 30 ppm region. (A) measured spectrum, (B) composited simulated spectrum, (C) individual components of the simulated spectrum.

where T_2(inhomo)=contributions to relaxation from an inhomogeneous magnetic field and T_2(diff)=contributions to relaxation from diffusion effects. The value obtained is an apparent spin-spin relaxation time (T_2*) which can include effects other than molecular motion such as field inhomogeneities. Since the lines are already quite broad, it is unlikely that inhomogeneity contributions will be significant. The molecules are not freely diffusion, which minimizes T_2(diff) contributions. Since the samples are treated in the same manner, any additional contributions to relaxation not specifically identified should be uniform. Therefore, the change we observe for T_2* are taken to be generated by temperature dependent differences in molecular motion.

As a typical example of the NMR study a plot of T_2* vs. temperature for the N-5 phase using all three methods for line width determination was evaluated and this plot revealed that the general shape of all three curves is similar. A slight increase in T_2* occurs as the temperature is decreased from 50 to 30 °C. A large decrease in T_2* occurs as the temperature is lowered from 30 °C to - 30 °C, then T_2* remains approximately constant as the temperature is lowered further to - 50 °C. We believe the major decrease in the relaxation time T_2* occurring between 30 °C and - 30 °C is most likely due to restriction of molecular motion as the temperature is lowered, and the minimum attained at around - 30 °C may be due to some stable configuration assumed by the bonded moieties such as an association between adjacent C-18 chains. The slight increase in T_2* observed at higher temperatures could be attributed to the elimination of this association and relatively unrestricted molecular motion occurs. In all cases the measurements are reversible; i.e., after raising or lowering the temperature, the same value for line width is obtained when we return to the original temperature.

Figure 7-5 is a plot of T_2* vs. temperature for the three methods of calculation applied to all three phases. Similar plots are obtained for each calculation method. The curves all show a decrease in the relaxation time from higher to lower temperature, with a minimum at some point of the curve. In the higher temperature region, the T_2* values obtained for the packings are ordered ODS-P-5>ODS-N-5>ODS-5. The temperature where a minimum in T_2* is reached are ordered ODS-5>ODS-N-5>ODS-P-5. Both of these observations parallel the loading density of C-18 bonded phase, which is ordered ODS-5>ODS-N-5>ODS-P-5. Since the

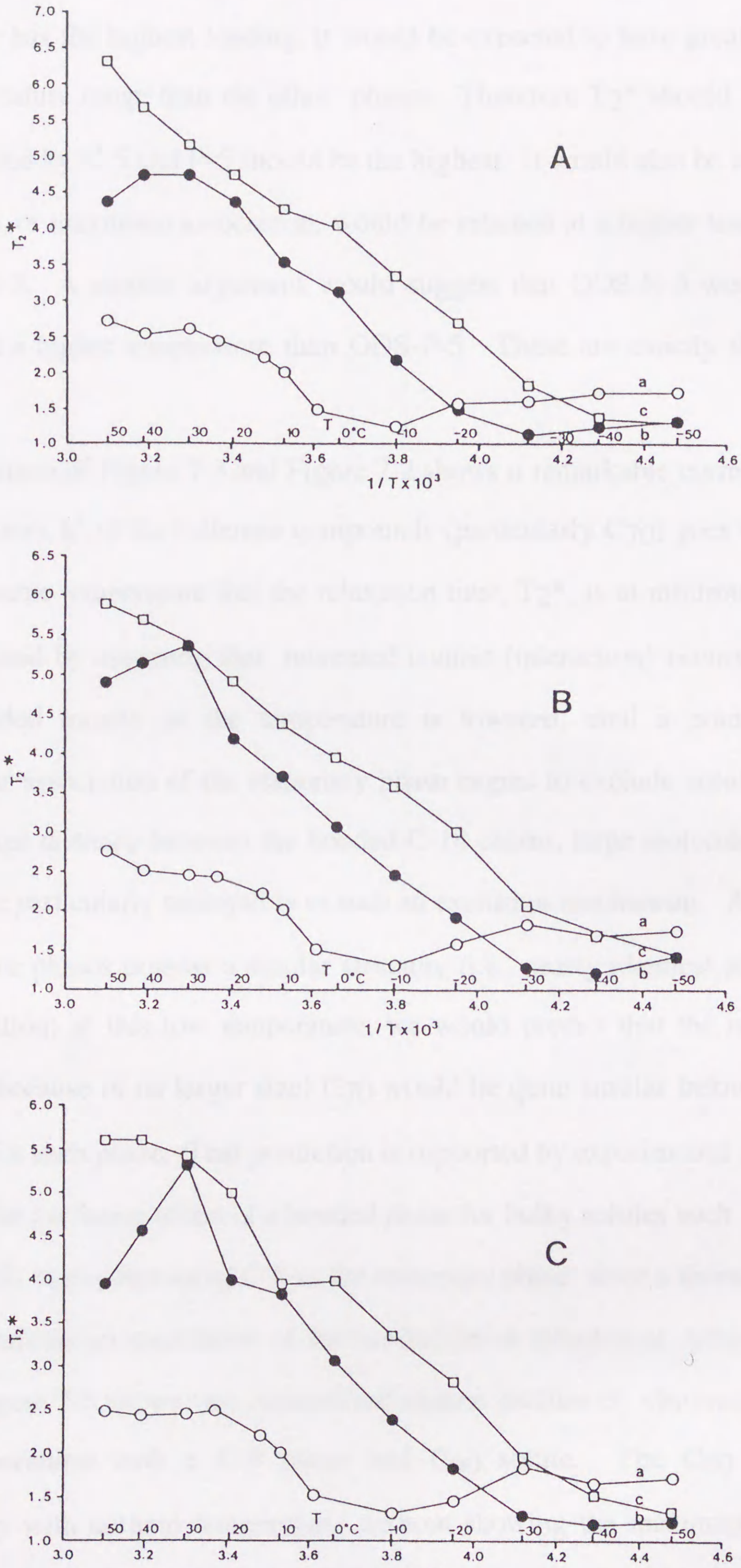

Figure 7-5. Plots of T_2* vs. temperature.
(A) manual calculation, (B) initial Linesim calculation,
(C) final Linesim calculation.
a: ODS-5, b: ODS-N-5, c: ODS-P-5.

ODS-5 phase has the highest loading, it would be expected to have greater association in the higher temperature range than the other phases. Therefore T_2* should be the least for this phase, followed by N-5 and P-5 should be the highest. It would also be expected that a stable configuration, or maximum association, would be retained at a higher temperature for ODS-5 relative to N-5. A similar argument would suggest that ODS-N-5 would retain maximum association at a higher temperature than ODS-P-5. These are exactly the results which are observed.

A comparison of Figure 7-5 and Figure 7-2 shows a remarkable correlation. The retention (retention factor), k', of the fullerene compounds (particularly C_{70}) goes through a maximum at about the same temperature that the relaxation time, T_2*, is at minimum. This correlation can be explained by assuming that increased contact (interaction) occurs between the solute and the bonded moiety as the temperature is lowered, until a point is reached where intramolecular association of the stationary phase begins to exclude solute. Considering the relative average distance between the bonded C-18 chains, large molecules such as C_{60} and C_{70} would be particularly susceptible to such an exclusion mechanism. A related observation is that all three phases possess a similar structure (i.e., nearly identical accessible surface for solute interaction) at this low temperature, we would predict that the retention of C_{60} and (particularly because of its larger size) C_{70} would be quite similar below the T_2* minimum temperature for each phase. That prediction is supported by experimental results.

Finally, the exclusion effect of a bonded phase for bulky solutes such as fullerenes should be more clearly seen when using C-8 as the stationary phase, since a shorter alkyl chain length more easily induces an association of the bonded phase moieties at temperatures higher than for C-18. Figure 7-6 shows two dimensional elution profiles of chromatograms generated at various temperatures with a C-8 phase and C_{60} solute. The C_{60} retention decreases monotonically with column temperature, without showing the maximum retention behavior observed with C-18 phases. Below - 20 °C the retention observed for C_{60} is shorter than the solvent peak (toluene), an observation most clearly seen in the profile obtained at - 70 °C. In this case, a total exclusion effect is dominating the retention of C_{60}. The results obtained for the C-8 phase clearly support the model described above for the ODS bonded phase, where

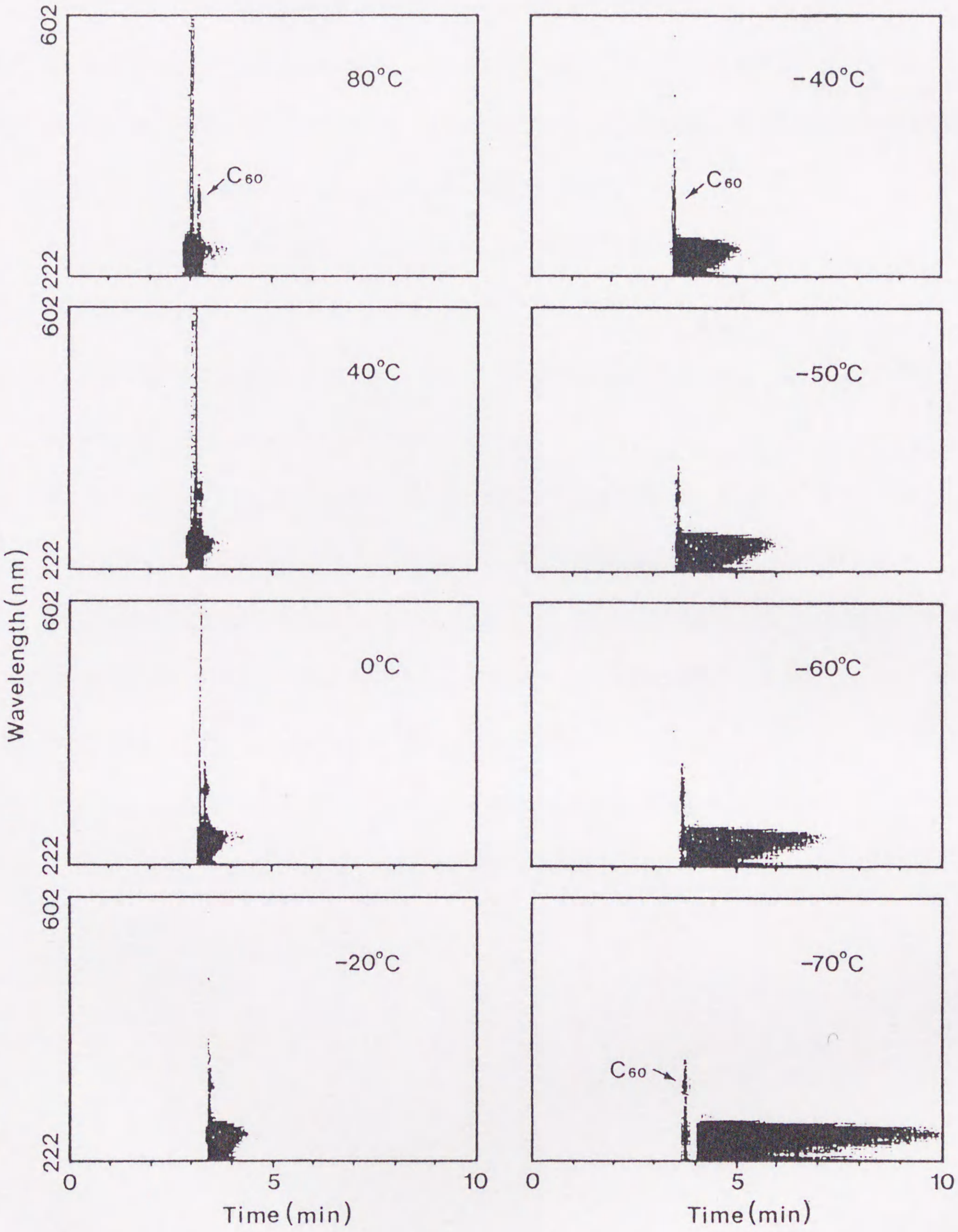

Figure 7-6. Two dimensional elution profiles of C_{60} and the solvent (toluene) at various temperatures with the C-8 stationary phase.
mobile phase: n-hexane, 1 mL/min.

bonded phase association and the configurational change with temperature are the most important factors controlling retention in LC. Large bulky solutes such as fullerenes can apparently magnify this effect.

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Chapter 8

Effect of Temperature on the Mechanism of Retention of Fullerenes in Liquid Chromatography using Various Alkyl Bonded Stationary Phases*

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*Modified from: *Chromatographia*, **42**, 56-63 (1996).

8.1 Abstract

The temperature-dependency of the separation of fullerenes in LC has been examined using various alkyl bonded stationary phases. It has been found that a maximum retention temperature exists with long alkyl bonded stationary phases, whereas there is no similar effect with the newly synthesized alkyl bonded phases which have two phenyl groups at the base of the bonded phase. The interpretation of the retention behavior of fullerenes in the low temperature region on alkyl bonded stationary phases is discussed using information obtained by CP-MAS solid-state NMR spectroscopy and LC.

8.2 Introduction

Alkyl bonded stationary phases, especially ODS materials have been mainly used for reversed-phase liquid chromatographic separations. The temperature-dependency of retention has been investigated by several authors [1-7]. The existence of a transition in the bonded phase has been observed by Morel *et al.* [1-3]. It was suggested that the nature of this phenomena is a function of the microstereochemistry. IR spectral changes resulting from crystal lattice ordering were also observed by Sander *et al.* [8-11]. They found that the selectivity of the column varied continuously with temperature. The ability of ODS phases to recognize shapes in the separation of closely related isomers was also observed to be highest at subambient temperatures. They suggested that the selectivity of a monomeric ODS phase is more polymeric-like at low temperatures. Cole and Dorsey suggested that a phase transition in reversed-phase materials was observed at a bonding density greater than 2.84 μmol m^{-2} [5].

In Chapter 6 and 7 [12,13], it was observed that a highly loaded monomeric ODS column, i.e. a phase with an average ligand interval shorter than the approximate diameter of fullerenes, results in the exclusion of those molecules when the ligand has a rigid and ordered structure at low temperature. In contrast, solutes such as PAHs maintained the expected behavior, i.e. reduction of the column temperature resulted in a monotonic increase in retention. We have, therefore, suggested that the configurational change of the alkyl chains with temperature is the most important factor in controlling the retention of solutes in LC. Large bulky solutes such as fullerenes can apparently magnify this effect. So, it is expected that the best separation of fullerenes should be achieved at low temperature with the ligand which has an appropriate space between each bonded moiety. To confirm the importance of ligand interval and chain length, it is necessary to investigate new phases which have ligand intervals and chain lengths to best retain and recognize the shape and size of fullerenes. For this purpose, we have synthesized several phases which have different alkyl chain lengths and which have two phenyl groups at the base of the bonded phases. In this chapter, the effect on the retention of fullerenes of column temperature, the alkyl chain length of the bonded stationary phases, and the ligand interval between each alkyl chain will be discussed by using liquid chromatographic and NMR spectroscopic information.

8.3 Experimental

The LC system consisted of a Jasco PU-980 pump (Tokyo, Japan), a Hewlett-Packard 1040 A photodiode array detector (Yokogawa Analytical Systems, Musashino, Japan) controlled by an HP 9000 computer. A Rheodyne 7125 injector (Cotati, CA, USA) with a 20 μL loop was used for injection. Column temperatures between 80 and 30 °C were controlled by means of a Tosoh RE-8000 oven (Tokyo, Japan) and between 30 and 0 °C by a Lab-Thermo Model LH-1000E system (Toyo Seisakusho, Tokyo, Japan); temperatures below 0 °C were maintained with a mixture of methanol and dry ice.

Solid state NMR spectra with cross-polarization (CP) and magic angle spinning (MAS) were obtained on a Bruker MSL 300 spectrometer (Billerca, MA, USA) with a variable temperature unit. Sample (200 - 300 mg) was placed in double bearing ZrO_2 rotor. The spinning rate was between 4700 and 5200 KHz depending on the temperature. A contact time of 5 ms and a repetition rate of 5 s were used for all spectra.

n-Hexane and toluene were obtained from Tokyo Kasei (Tokyo, Japan). The stationary phases used, C-18, C-8, C-4, C-18 diphenyl (C-18 Diph), C-8 diphenyl (C-8 Diph) and C-4 diphenyl (C-4 Diph), are shown in Figure 8-1 and Table 8-I and were synthesized in this

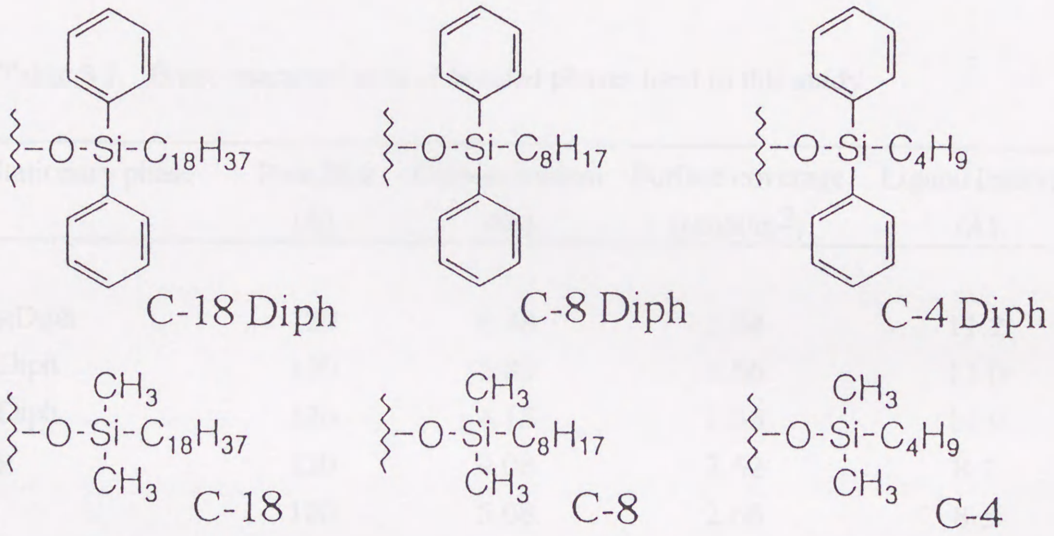

Figure 8-1. Chemical structures of bonded phases synthesized (A) C-18 Diph, (B) C-8 Diph, (C) C-4 Diph, (D) C-18, (E) C-8 and (F) C-4 bonded phases.

Table 8-I. Basic characteristics of bonded phases used in this study.

Stationary phase	Pore Size (Å)	Carbon content (%)	Surface coverage (μmol/m^2)	Ligand Interval (Å)
C_{18}Diph	120	8.48	1.54	11.2
C_8Diph	120	5.89	1.56	11.0
C_4Diph	120	4.15	1.35	11.9
C_{18}	120	9.08	2.52	8.7
C_8	120	5.08	2.68	8.5
C_4	120	1.73	1.50	11.2

laboratory. The C-18, C-8, C-4 stationary phases were packed into 25 cm x 4.6 mm i.d. columns, the others into 10 cm x 4.6 mm i.d. columns. A C-30 phase was used for the reference column. The material was Develosil 300 C-30 (Nomura Chemicals, Seto, Japan) packed in a 25 cm x 4.6 mm i.d. stainless-steel column. It was synthesized via a trichlorosilane; the carbon content was 11 % with 300 Å. C_{60} and C_{70} fullerenes were isolated from carbon soot produced by the process described in previous chapters [14-16]. The PAHs, chrysene, triphenylene and pyrene (obtained from Wako Chemicals, Osaka, Japan) were used as references. The mobile phase was n-hexane with a typical flow rate of 1 mL/min.

8.4 Results and Discussion

Figure 8-2 shows two-dimensional chromatograms for C_{60}, C_{70}, and the solvent (toluene) at various temperatures with C-18 and C-18 Diph stationary phases. It was observed that the C-18 phase gave better separation of C_{60} and C_{70} with decreasing column temperature until a maximum in retention was achieved at a temperature of about -30°C. The retention of these solutes then decreased with further reduction of the column temperature. In contrast, the retention of the solvent (toluene) increased monotonically with decreasing column temperature. It is clearly apparent that the order of elution of the three components changes from solvent, C_{60}, C_{70} to C_{60}, C_{70}, solvent at a temperature of about -50°C. This kind of elution order change was not, however, observed with the C-18 Diph phase. C_{60} and C_{70} gave the expected elution behavior with decreasing column temperature (80° to -70°C), i.e. a monotonic increase in retention for the two solutes. It has been postulated in Chapters 6 and 7 [12,13] that a configurational change of the ligand as a function of temperature is the most important factor influencing fullerene retention in LC and that this change is caused by an exclusion effect of the rigid alkyl chains which do not have enough space for fullerenes to fit in-between adjacent bonded moieties. Large bulky solutes such as fullerenes can apparently magnify this exclusion temperature effect. This means that bonded phases which have enough space and possess a shape to match that of the fullerenes should retain such molecules to a

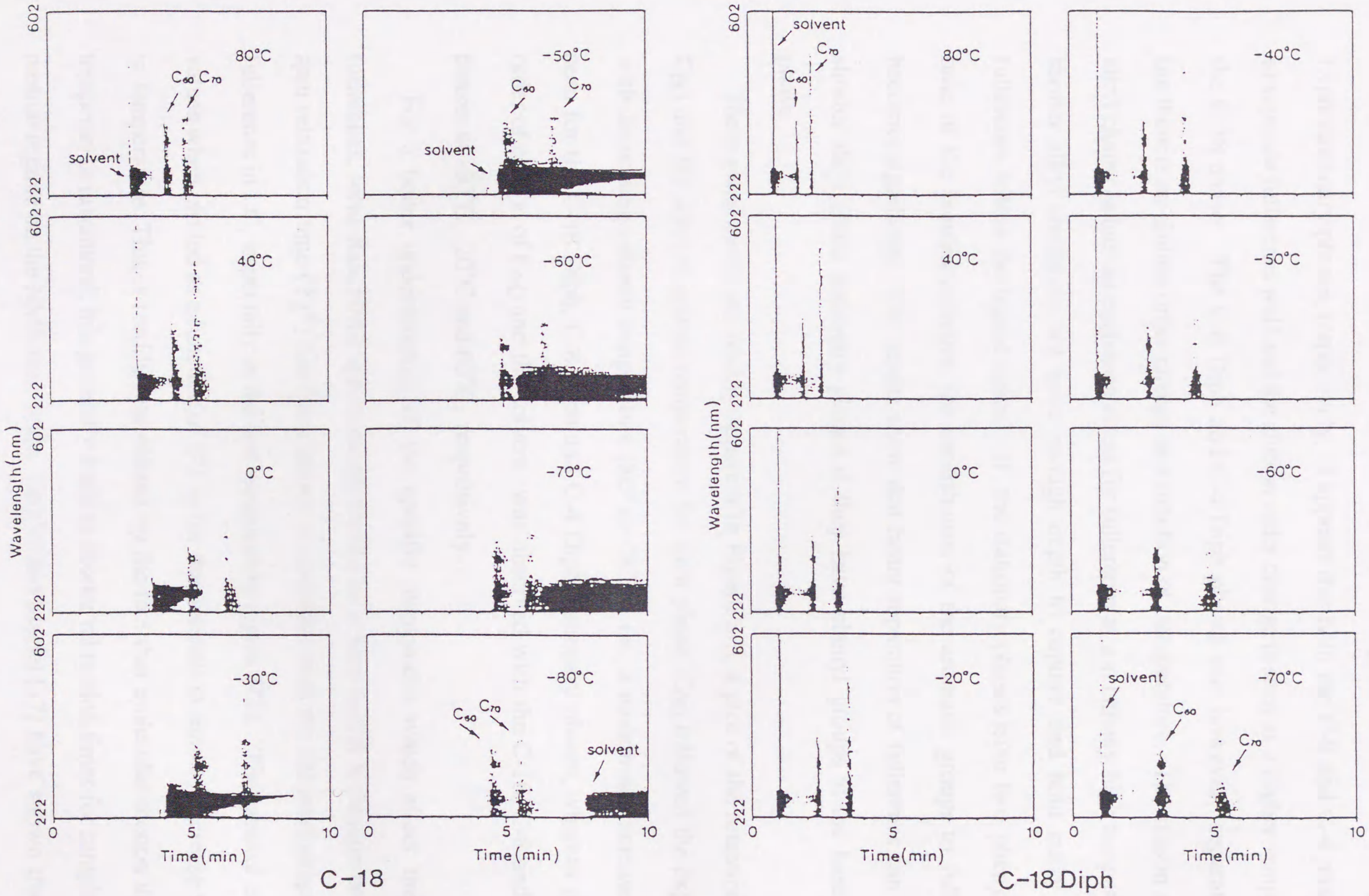

Figure 8-2. Two-dimensional chromatograms for C_{60} and C_{70} at various temperatures with C-18 bonded phases.
mobile phase: n-hexane, solvent: toluene, flow rate: 1mL/min.

greater extent even at lower temperatures.

Figures 8-3 and 4 show two-dimensional chromatograms for C-8/C-8 Diph and C-4/C-4 Diph stationary phases, respectively. It appears that both the C-8 and C-4 phases are not able to separate fullerenes well and the elution order change is seen at a higher temperature than for the C-18 phase. The C-8 Diph and C-4 Diph phases can, however, separate the fullerenes but there is no elution order change as a function of temperature. The reason why the shorter alkyl chains induce an exclusion effect for fullerenes at a relatively high temperature is that the shorter alkyl chains do not have enough depth to capture and hold bulky molecules like fullerenes within the ligand space. If the stationary phases have two phenyl groups at the base of the bonded moieties, the contribution of the aromatic groups to fullerene retention becomes significant. The results show that better separation of fullerenes can be achieved by shorter alkyl chain stationary phases if they have phenyl groups at the base of the bonded phase.

These phenomena are readily apparent in Figure 8-5, a plot of the retention ratio between C_{60} and the solvent against temperature for each phase. C_{60} followed the expected behavior with decreasing column temperature (80° to -70°C), i.e., a monotonic increase of retention is seen for the C-18 Diph, C-8 Diph and C-4 Diph stationary phases, whereas a change in the order of elution of C_{60} and the solvent was observed with the C-18, C-8 and C-4 stationary phases at -50°C, -20°C and 60°C, respectively.

For a better understanding of the specific phenomena which affect the separation of fullerenes, solid-state NMR spectroscopy should be a very useful technique because the spin-spin relaxation time (T_2*) has been shown to correlate with the chromatographic behavior of fullerenes in LC, especially in the low temperature region [13]. The general trend of the T_2* values when plotted as a function of 1/T is for this relaxation time to decrease with a decrease in temperature. This is readily rationalized by the fact that molecular motion decreases as the temperature is reduced; this generally leads to shorter relaxation times for samples in the limited motion regime on the NMR time scale. Zeigler and Maciel [17] have shown that the real spin-spin relaxation time differs from T_2* determined according to equation :

$$\Delta W = \frac{1}{T_2^*}$$

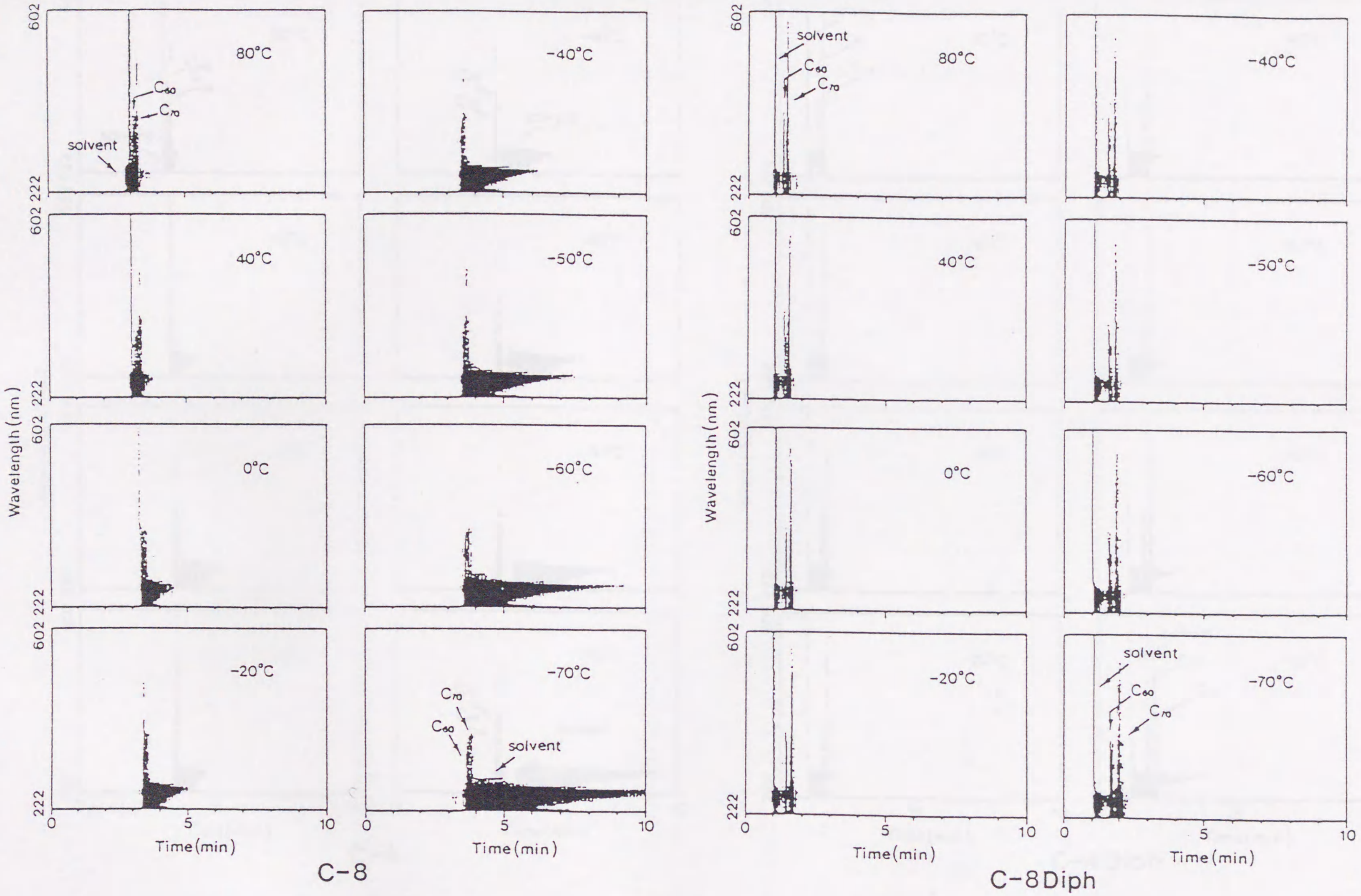

Figure 8-3. Two-dimensional chromatograms for C_{60} and C_{70} at various temperatures with C-8 phases.
mobile phase: n-hexane, solvent: toluene, flow rate: 1mL/min.

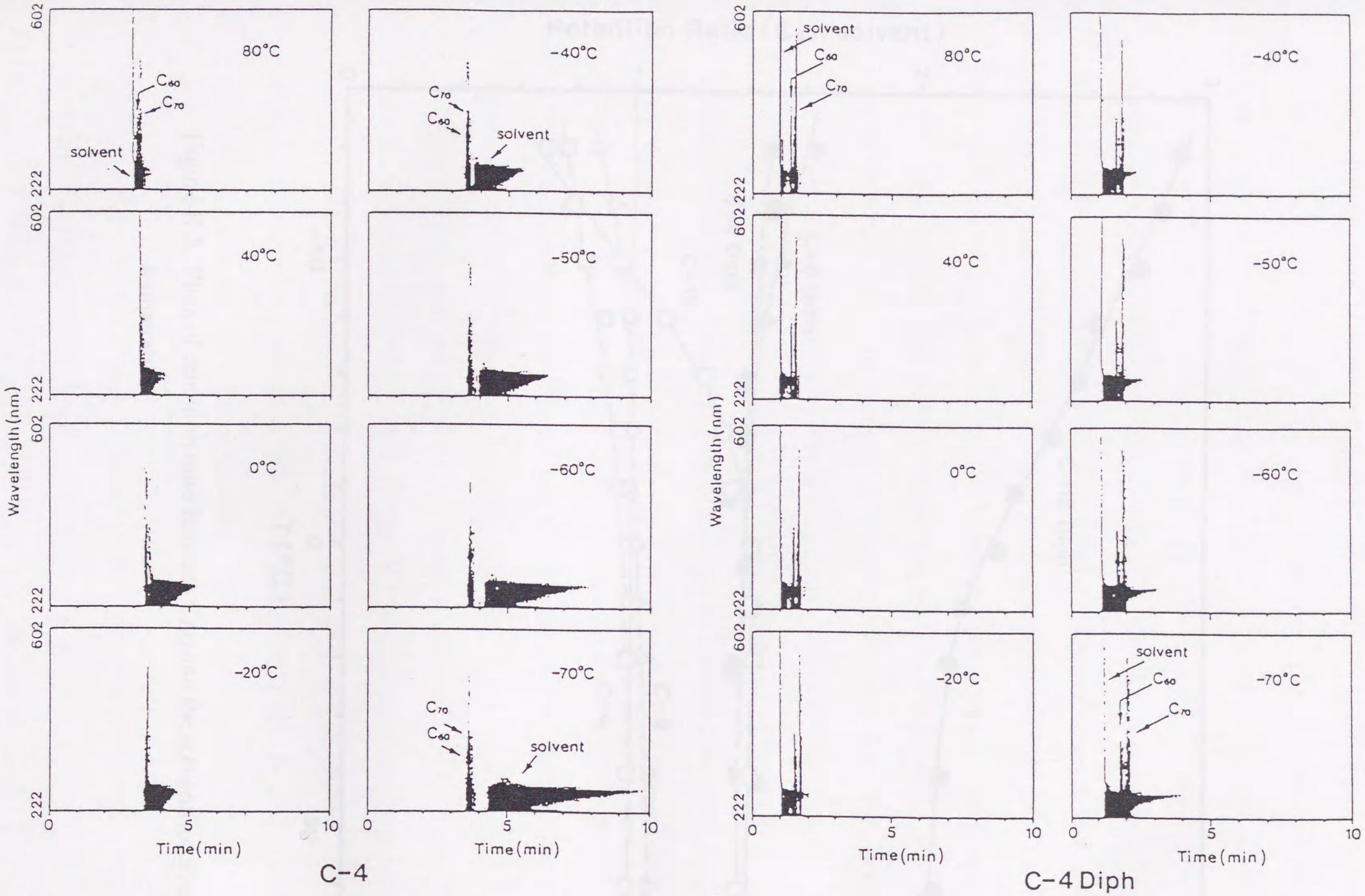

Figure 8-4. Two-dimensional chromatograms for C_{60} and C_{70} at various temperatures with C-4 bonded phases.
mobile phase: n-hexane, solvent: toluene, flow rate: 1mL/min.

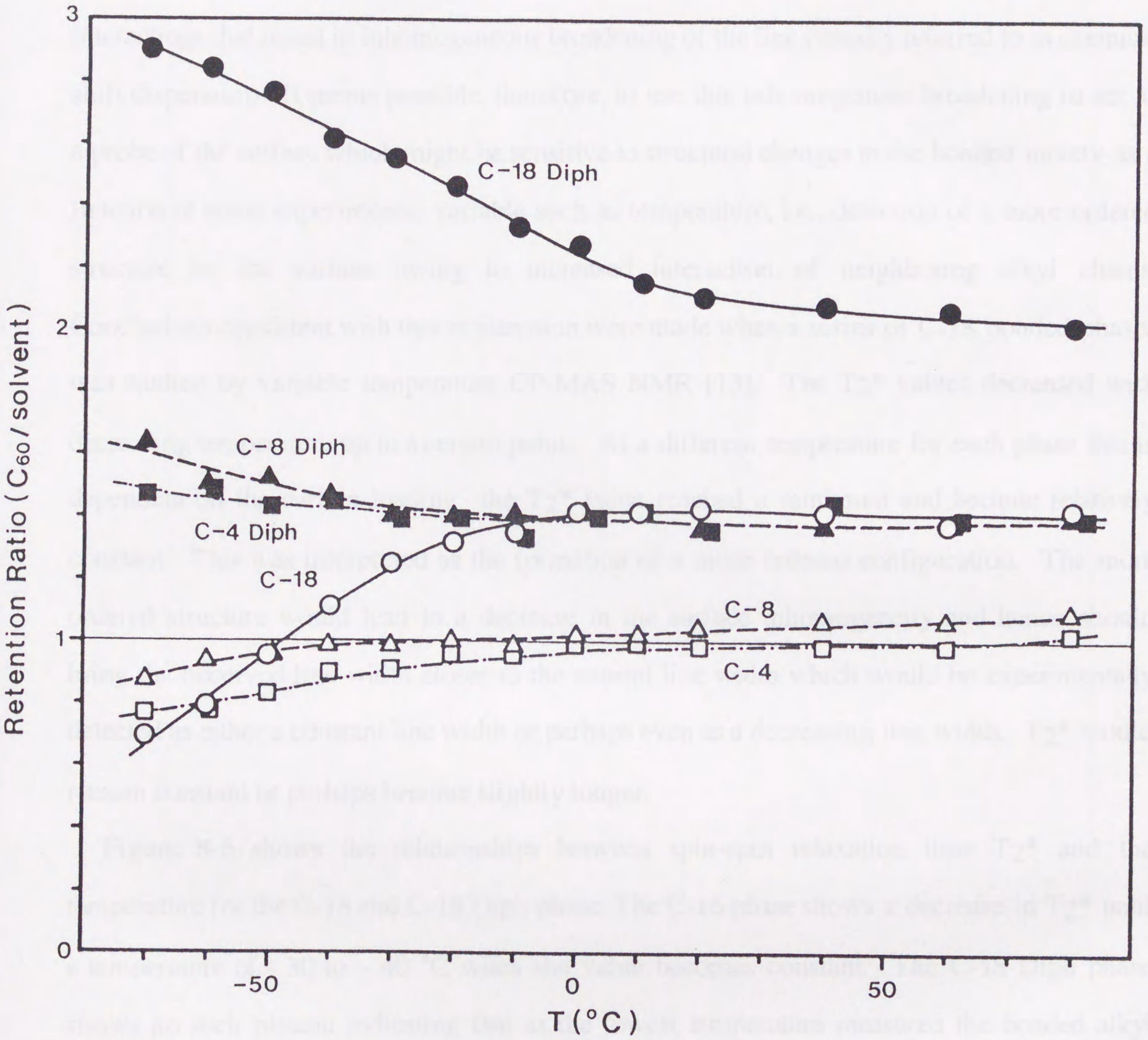

Figure 8-5. Plots of retention ratio between C_{60} and the solvent(toluene) versus temperature.

by a factor which can vary from 2.6 to 48 for C-18 silica depending on the loading of the phase and the position of the carbon in the alkyl chain. The difference between the natural T_2* and that calculated from the line width has been attributed to variations in the surface topography of the silica which lead the alkyl chains to assume a variety of conformations and interactions that result in inhomogeneous broadening of the line (usually referred to as chemical shift dispersion). It seems possible, therefore, to use this inhomogenous broadening to act as a probe of the surface which might be sensitive to structural changes in the bonded moiety as a function of some experimental variable such as temperature, i.e., detection of a more ordered structure on the surface owing to increased interaction of neighboring alkyl chains. Conclusions consistent with this explanation were made when a series of C-18 bonded phases was studied by variable temperature CP-MAS NMR [13]. The T_2* values decreased with decreasing temperature up to a certain point. At a different temperature for each phase that is dependent on the carbon loading the T_2* value reached a minimum and became relatively constant. This was interpreted as the formation of a more ordered configuration. The more ordered structure would lead to a decrease in the surface inhomogeneity and hence should bring the observed line width closer to the natural line width which would be experimentally detected as either a constant line width or perhaps even as a decreasing line width. T_2* would remain constant or perhaps become slightly longer.

Figure 8-6 shows the relationships between spin-spin relaxation time T_2* and the temperature for the C-18 and C-18 Diph phase. The C-18 phase shows a decrease in T_2* until a temperature of - 30 to - 40 °C when the value becomes constant. The C-18 Diph phase shows no such plateau indicating that at the lowest temperature measured the bonded alkyl chain had not achieved any ordered structure. It is very interesting to note that the relaxation time change for the C-18 phase correlates well with the plots of ln k' against temperature for C_{60} and the three PAHs; Figure 8-7 shows that there is a large difference between the retention behavior of PAHs and C_{60}. For the C-18 phase it is seen that the retention of PAHs increased linearly with decreasing temperature but there is a curvature in the plot at approximately 30 °C. It is also apparent that the retention of C_{60} decreases drastically below approximately -30°C. It was suggested in Chapter 7 [13] that the major decrease in the relaxation time T_2* occurring

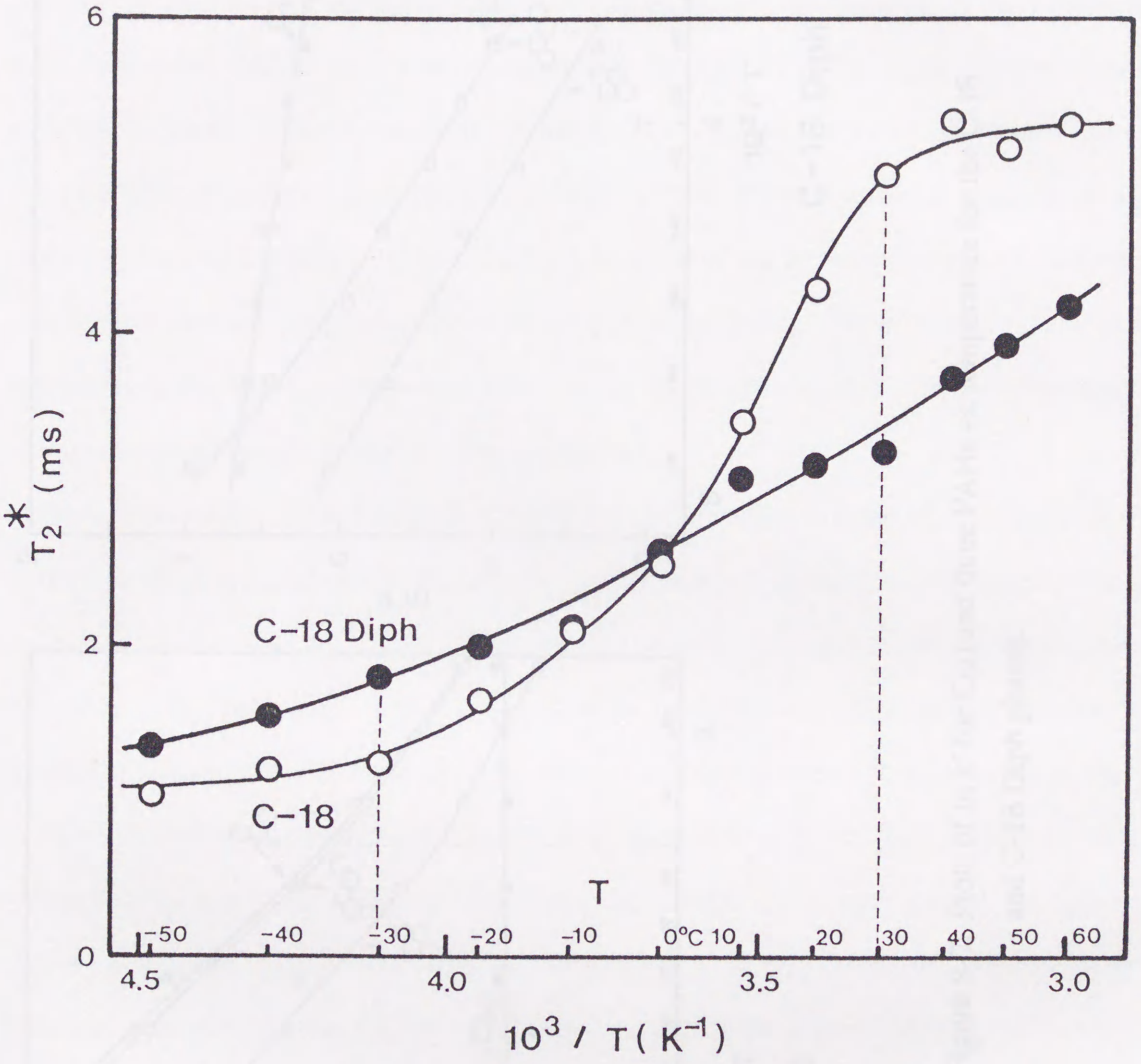

Figure 8-6. Plots of spin-spin relaxation time T_2* vs. temperature for the C-18 and C-18 Diph phases.

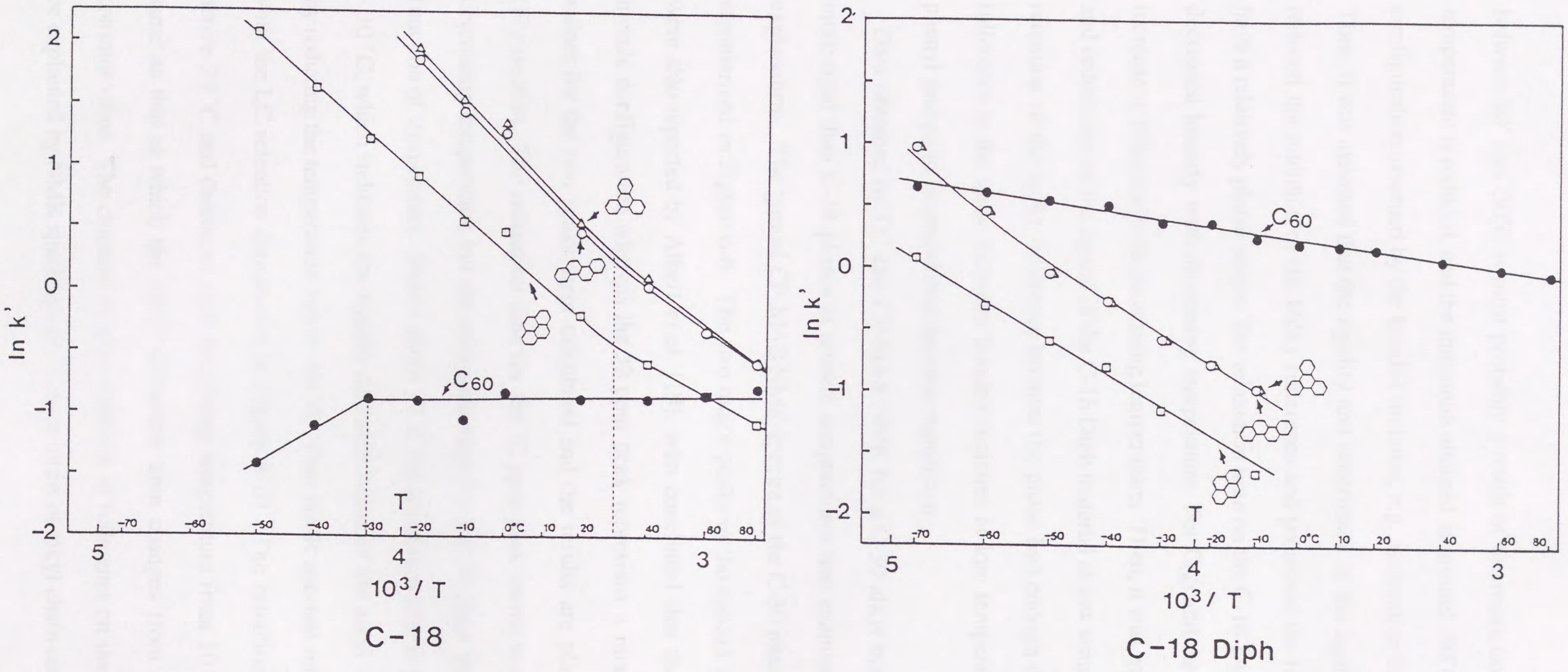

Figure 8-7. Plots of ln k' for C_{60} and three PAHs vs. temperature for the C-18 and C-18 Diph phases.

between 30° and -30°C is most probably a result of the restriction of molecular motion as the temperature is reduced, and the minimum attained at around -30°C may be because of a stable configuration assumed by the bonded moieties, e.g. association between adjacent C-18 chains. Then, it was assumed that the rigidity and orderliness of the ligand at low temperature greatly reduced the retention of the bulky fullerenes and promoted the retention of the PAHs, which have a relatively planar shape. The relaxation time on the C-18 Diph phase, on the other hand, decreased linearly with decreasing temperature. For C_{60} and the PAHs there was monotonic increase in retention with decreasing temperature. Then, it was assumed that the lower rigidity and orderliness of the ligand of the C-18 Diph material at low temperature greatly facilitated the retention of the bulky fullerenes because the phase had enough depth to trap and hold bulky fullerenes in the space between bonded moieties at low temperature; the contribution of the phenyl groups to retention then become significant.

Data obtained by LC and CP-MAS NMR for a C-30 alkyl bonded phase which should be more rigid than C-18 phases at normal temperatures was examined to substantiate the above explanation. The typical CP-MAS NMR spectra of the C-30 phase at various temperatures are summarized in Figure 8-8. The two major peaks in the spectra are at 30 and 32 ppm; these were also reported by Albert *et al.* [18]. who concluded that the 30 ppm peak is the more mobile configuration whereas the 32 ppm peak represents a more rigid structure. The T_2* values for the two peaks were calculated and the results are plotted against the temperature (Figure 8-9). The relaxation time for the 32 ppm peak seems to decrease monotonically with decreasing temperature but the relaxation time for the 30 ppm peak behaves differently as a function of temperature. Below about 10 °C the relaxation time is practically constant down to - 30 °C, which indicates the rigidity and orderliness of the alkyl chains is not much changed by reducing the temperature below 10 °C. This NMR spectral information is quite consistent with the LC retention data shown in Figure 8-10. The retention of C_{60} is almost constant above 20 °C and decreases with decreasing temperature from 10 °C. This temperature is the same as that at which the NMR relaxation time changes from a monotonic decrease to a constant value. The chromatographic behavior of fullerenes on the C-30 phase can, therefore, be explained by NMR spectroscopic data in terms of alkyl chain configurational changes.

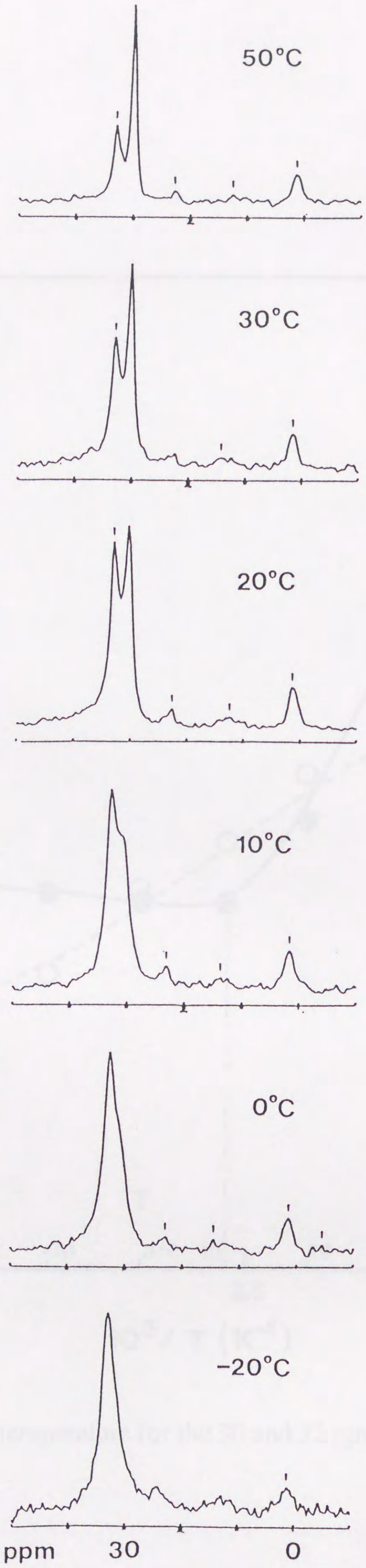

Figure 8-8. CP-MAS spectra of C-30 bonded phase at various temperatures.

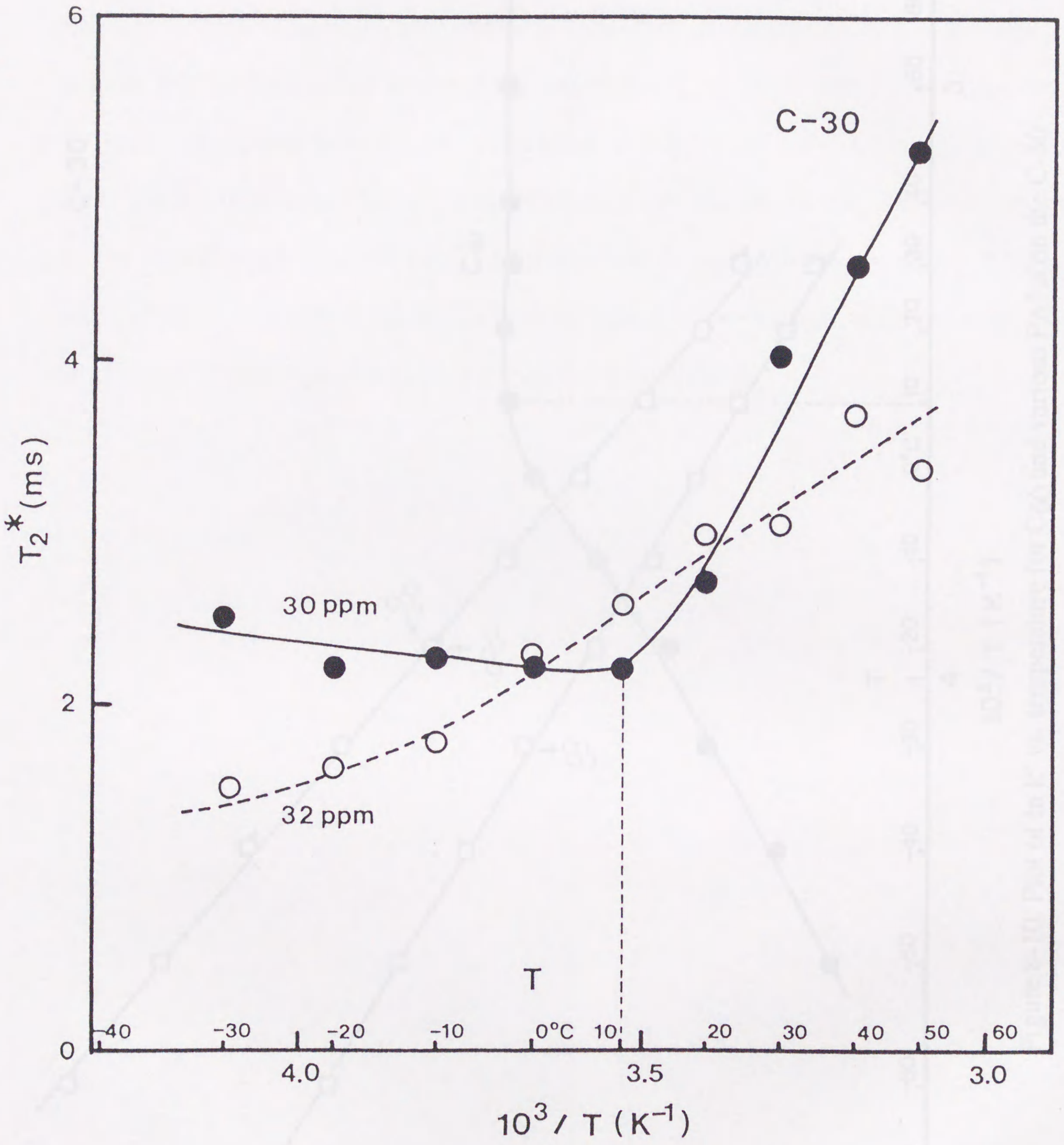

Figure 8-9. Plot of T_2* vs. temperature for the 30 and 32 ppm peaks of C-30 phase.

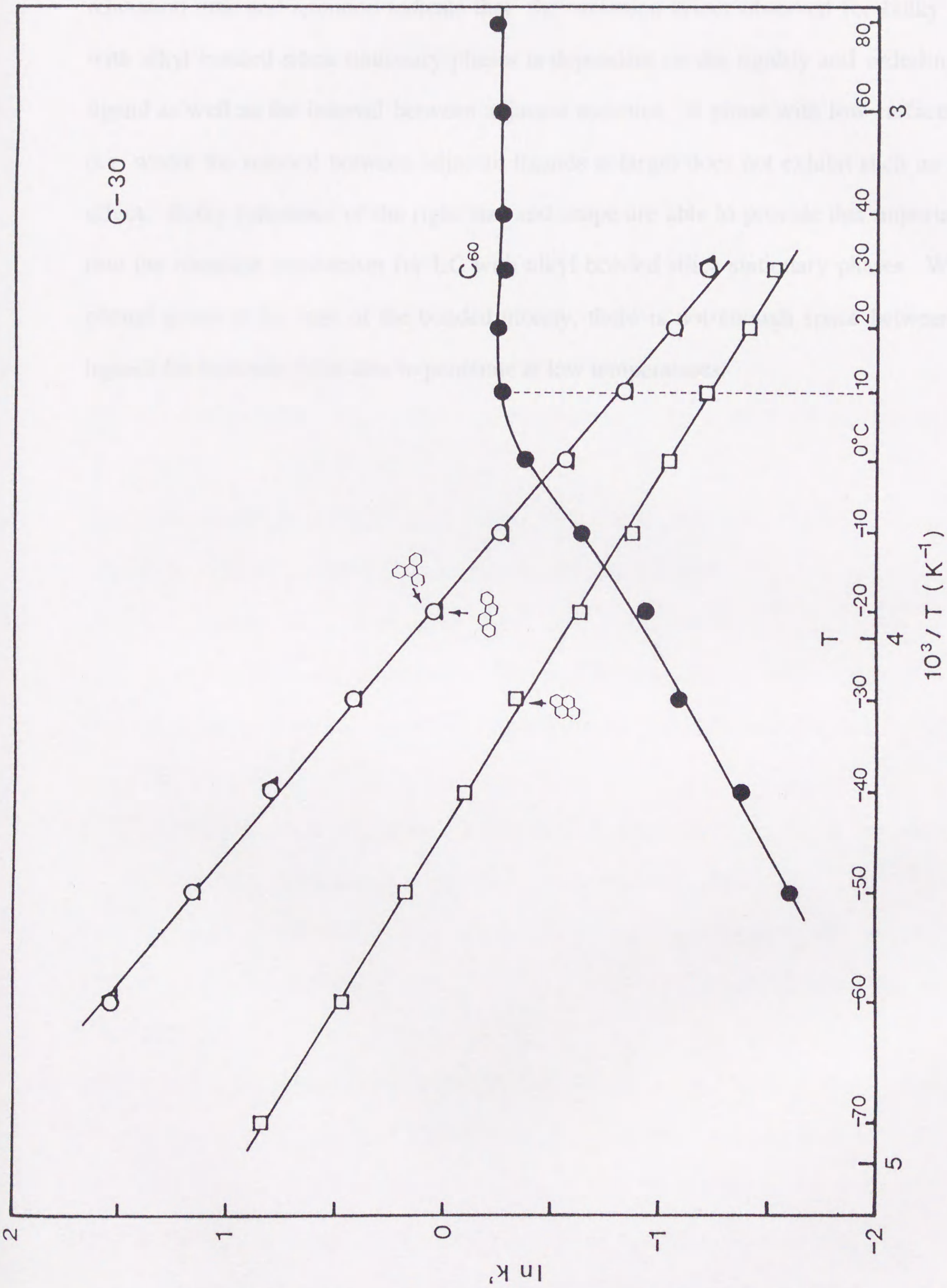

Figure 8-10. Plot of ln k' vs. temperature for C$_{60}$ and various PAHs on the C-30 bonded phase.

From these results, it appears that the spin-spin relaxation time is a good indicator of the LC retention behavior of fullerenes. The strong correlation between the changes in spin-spin relaxation time and retention indicate that the exclusion effect observed for bulky fullerenes with alkyl bonded silica stationary phases is dependent on the rigidity and orderliness of the ligand as well as the interval between adjacent moieties. A phase with low surface coverage (i.e. where the interval between adjacent ligands is large) does not exhibit such an exclusion effect. Bulky fullerenes of the right size and shape are able to provide this important insight into the retention mechanism for LC with alkyl bonded silica stationary phases. Without the phenyl group at the base of the bonded moiety, there is not enough space between adjacent ligands for fullerene to be able to penetrate at low temperatures.

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Chapter 9

Conclusion

In LC separation, the retention mechanisms are generally not known well yet. In order to design the best separation system for the particular problem, the mechanism should be known and the interactions in the molecular level also should be interpreted. Because the interactions are so complex and the part of the interactions is sometimes dominant and sometimes non-dominant to control the retention, to realize such conditions is hard task particularly in LC systems. In such case one can make the model interaction in chromatographic separation process by using information of solutes and stationary phases structures. Molecular recognition mechanism is such one in separation process. The molecule can be recognized by the stationary phase and the separation process can be induced by different molecular-level interactions between the stationary phase and the molecules which have a different shape and size each other. This dissertation is focusing on this view in chromatography.

In this dissertation, the elution characteristics of higher fullerenes with monomeric and the polymeric ODS phases were firstly investigated (Chapter 2,3), and the results clearly indicate that the shape recognition capability controls the retention order of higher fullerenes. The polymeric phase has higher molecular shape recognition capability and the elution order is determined by the shape of the molecules, but the monomeric phase has a lower such capability and elutes higher fullerenes in the order of their molecular size (molecular weight) with the toluene-acetonitrile mobile phase. Three C_{78} isomers are resolved with the polymeric phase, and the lower resolution of those solutes with the monomeric phase was improved at a lower temperature. C_{82} elutes later than C_{84} with the polymeric phase, but the order was reversed with the monomeric phase. It is also described the LC-MS approach using the ESI interface for isolation and identification of higher fullerenes. The results have shown that this approach is sufficiently powerful to identify large fullerenes in soot extract (Chapter 4) and the largest fullerene found in this work is C_{98}. Interesting elution behaviour of C_{98} using polymeric ODS is also found and the reason for this result can be interpreted by the molecular planarity recognition mechanism of the ODS phase. The UV/Vis spectra obtained can be identified by FAB-MS measurements after a preparative-LC sample fractionation process.

From the experimental results of separating isomers of higher fullerenes with DMP phase (Chapter 5), two important facts are obtained: (1) the resolution of higher fullerenes with the

DMP phase is better than with other phases using n-hexane as the mobile phase and (2) increasing the temperature with the DMP phase results in slightly faster analysis without loss of resolution. For the separation of higher fullerenes, DMP offers faster analysis at higher temperatures while maintaining its high resolution.

For understanding more details on the retention mechanism of these alkyl bonded stationary phases, the retention of fullerenes at subambient temperatures was investigated (Chapter 6). Better separation of fullerene isomers was achieved at subambient temperatures with ODS bonded phases. It was also found that the best separation temperature for fullerenes should be existed for polymeric and high carbon-content, monomeric ODS phases. It was observed that a highly loaded monomeric ODS column, i.e. a phase with an average ligand interval shorter than the approximate diameter of fullerenes interact efficiently with fullerenes, and finally results in the exclusion of fullerene molecules when the ligand has a rigid and ordered structure at low temperature (Figure 9-1). In contrast, solutes such as polycyclic aromatic hydrocarbons (PAHs) maintained the expected behavior, i.e. reduction of the column temperature resulted in a monotonic increase in retention. It was found that the selectivity for higher fullerenes on monomeric phases becomes similar to that on polymeric phases at low temperature. It was also found that as the carbon content of monomeric phases is increased, the selectivity for fullerenes also becomes similar to that with the polymeric phases.

The effect on the retention of fullerenes of column temperature and the ligand interval between each alkyl chain was discussed by using LC and NMR spectroscopic information (Chapter 7). From these results, it appears that the spin-spin relaxation time is a good indicator of the LC retention behavior of fullerenes. The strong correlation between the changes in spin-spin relaxation time and retention indicate that the exclusion effect observed for bulky fullerenes with alkyl bonded silica stationary phases is dependent on the rigidness and orderliness of the ligand as well as the interval between adjacent moieties (Figure 7-5 and 7-2). A low surface coverage phase (i.e. where the interval between adjacent ligands is large) does not exhibit such an exclusion effect. Bulky fullerenes which possess the right size and shape are able to provide this important insight into the retention mechanism in LC for alkyl bonded silica stationary phases.

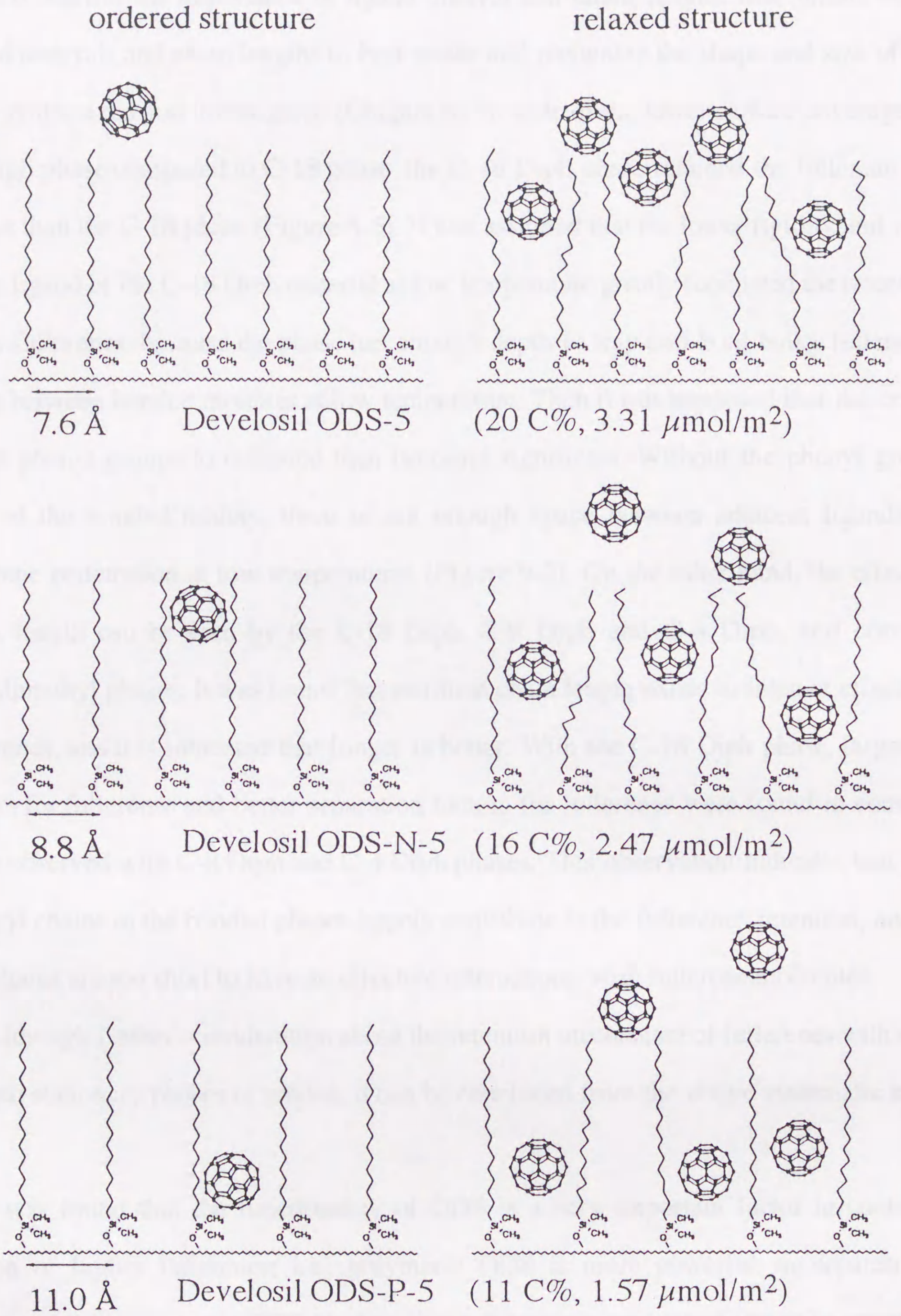

Figure 9-1. Schematic for the interaction between three different inter-ligand distance C-18 bonded phases and fullerenes.
ordered structure: at low temperature, relaxed structure: at high temperature

To confirm the importance of ligand interval and chain length, new phases which have ligand intervals and chain lengths to best retain and recognize the shape and size of fullerenes were synthesized and investigated (Chapter 8). In spite of the lower surface coverage of the C-18 Diph phase compared to C-18 phase, the C-18 Diph phase retained the fullerene molecules longer than the C-18 phase (Figure 8-5). It was assumed that the lower rigidity and orderliness of the ligand of the C-18 Diph material at low temperature greatly facilitated the retention of the bulky fullerenes, because the phase has enough depth to trap and hold bulky fullerenes in the space between bonded moieties at low temperature. Then it was supposed that the contribution of the phenyl groups to retention then becomes significant. Without the phenyl group at the base of the bonded moiety, there is not enough space between adjacent ligands to allow fullerene penetration at low temperatures (Figure 9-2). On the other hand, the effect of alkyl chain length can be seen by the C-18 Diph, C-8 Diph and C-4 Diph, and corresponding alkyldimethyl phases. It was found that a critical chain length exists to interact effectively with fullerenes, and it is indicated that longer is better. With the C-18 Diph phase, larger retention factors for fullerenes and better separation factors for fullerenes were found in comparison to those observed with C-8 Diph and C-4 Diph phases. This observation indicates that the length of alkyl chains in the bonded phases largely contribute to the fullerenes retention, and C-8 and C-4 chains are too short to have an effective interactions with fullerene molecules.

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) It was found that the functionality of ODS is a very important factor in controlling the elution of higher fullerenes; i.e., polymeric ODS is more powerful in separating higher fullerenes than monomeric ODS by their shape difference, while the latter can separate them by the molecular weight or size.

2) It was found that the surface coverage is also a very important factor in controlling the elution of higher fullerenes; i.e., as surface coverage of monomeric phases is increased, the selectivity becomes similar to that with the polymeric phase and then molecular shape

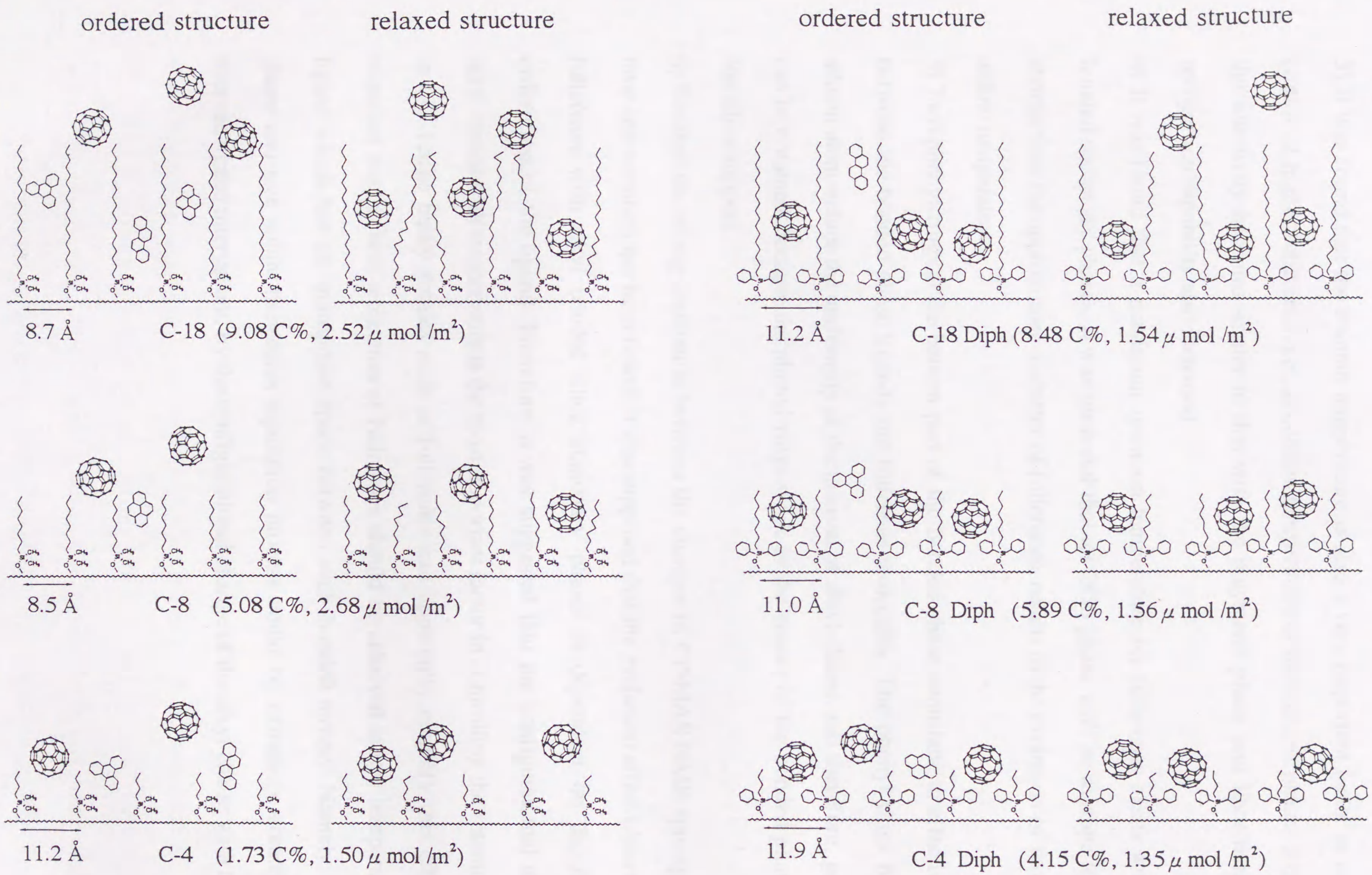

Figure 9-2. Schematic for the interaction between the alkyl bonded phases and fullerenes.
ordered structure: at low temperature, relaxed structure: at high temperature

recognition capability increased.

3) It was found that the column temperature is also a very important factor in controlling the elution of higher fullerenes; i.e., as column temperature of monomeric phases is decreased, the selectivity becomes similar to that with the polymeric phase and then molecular shape recognition capability also increased.

4) It was found that a maximum retention temperature for fullerenes exists with long alkyl bonded stationary phases. It was observed that an ODS phase with an average ligand interval shorter than the approximate diameter of fullerenes, results in the exclusion of those molecules at low temperature.

5) Two phenyl-rings at the bottom part of the bonded phase contribute to effective interaction between the bonded phase ligands and fullerene molecules. The phenyl rings bonded to the silicon atom induce the uniformity of the interval of alkyl chains, and therefore, good retention can be obtained, because the phenyl rings work as the spacer of the bonded phase ligands on the silica support.

6) Because the strong correlation between the changes in CP-MAS NMR spin-spin relaxation time and retention has been found, it was supposed that the exclusion effect observed for bulky fullerenes with alkyl bonded silica stationary phases is dependent on the rigidness and orderliness of the ligand. Therefore, it was suggested that the configurational change of the alkyl chains with temperature is the most important factor in controlling the retention of solutes in LC. Large bulky solutes such as fullerenes can apparently magnify this effect. So, it is expected that the best separation of fullerenes should be achieved at low temperature with the ligand which has an appropriate space between each bonded moiety. Namely, the different shape and size solute molecules separation process could be extremely controlled with an average ligand interval and by the configurational change of the alkyl chains with temperature.

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Publications

Papers Related to This Dissertation

Original Research Papers

1. "Dimethoxyphenylpropyl bonded silica phase for higher fullerenes separation by high-performance liquid chromatography"
   K.Jinno, H.Ohta, Y.Saito, T.Uemura, H.Nagashima, K.Itoh, Y.-L.Chen, G.Luehr, J.Archer, J.C.Fetzer and W.R.Biggs,
   J.Chromatogr., **648**, 71-77 (1993).

2. "Separation and identification of higher molecular weight fullerenes by high-performance liquid chromatography with monomeric and polymeric octadecylsilica bonded phases"
   K.Jinno, T.Uemura, H.Ohta, H.Nagashima and K.Itoh,
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3. "Liquid chromatographic separation of higher fullerenes with octadecylsilica stationary phase"
   K.Jinno, T.Uemura, H.Ohta, H.Nagashima and K.Itoh,
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4. "Temperature effect in fullerenes separation by high-performance liquid chromatography"
   H.Ohta, Y.Saito, K.Jinno, H.Nagashima and K.Itoh,
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5. "Retention of fullerenes by octadecyl silica. Correlation with NMR spectra at low temperatures"
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   Chromatographia, **40**, 507-512 (1995).

6. "Separation and identification of higher fullerenes in soot extract by liquid chromatography-mass spectrometry"
   K.Jinno, H.Matsui, H.Ohta, Y.Saito, K.Nakagawa, H.Nagashima and K.Itoh,
   Chromatographia, **41**, 353-360 (1995).

7. "Effect of temperature on the mechanism of retention of fullerenes in liquid chromatography using various alkyl bonded stationary phases"
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Other Contributions

Original Research Papers

1. "Microcolumn liquid chromatography combined with computer-assisted retention prediction system for polycyclic aromatic hydrocarbons in an extract from diesel particulate matter"
   K.Jinno, H.Ohta, Y.Hirata, S.Sasaki and H.Abe,
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2. "Separation of C_{60} and C_{70} fullerenes with a triphenyl bonded silica phase in microcolumn liquid chromatography"
   Y.Saito, H.Ohta, H.Nagashima, K.Itoh, K.Jinno, M.Okamoto, Y.-L.Chen, G.Luehr and J.Archer,
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   J.Liq.Chromatogr., **18(10)**, 1897-1908 (1995).

4. "Nano-scale design of novel stationary phases to enhance selectivity for molecular shape and size in liquid chromatography"
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5. "Separation of C_{60} and C_{70} fullerenes with liquid crystal bonded silica phases in microcolumn high performance liquid chromatography"
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6. "Separation of polycyclic aromatic hydrocarbons with a C_{60} bonded silica phase in microcolumn liquid chromatography"
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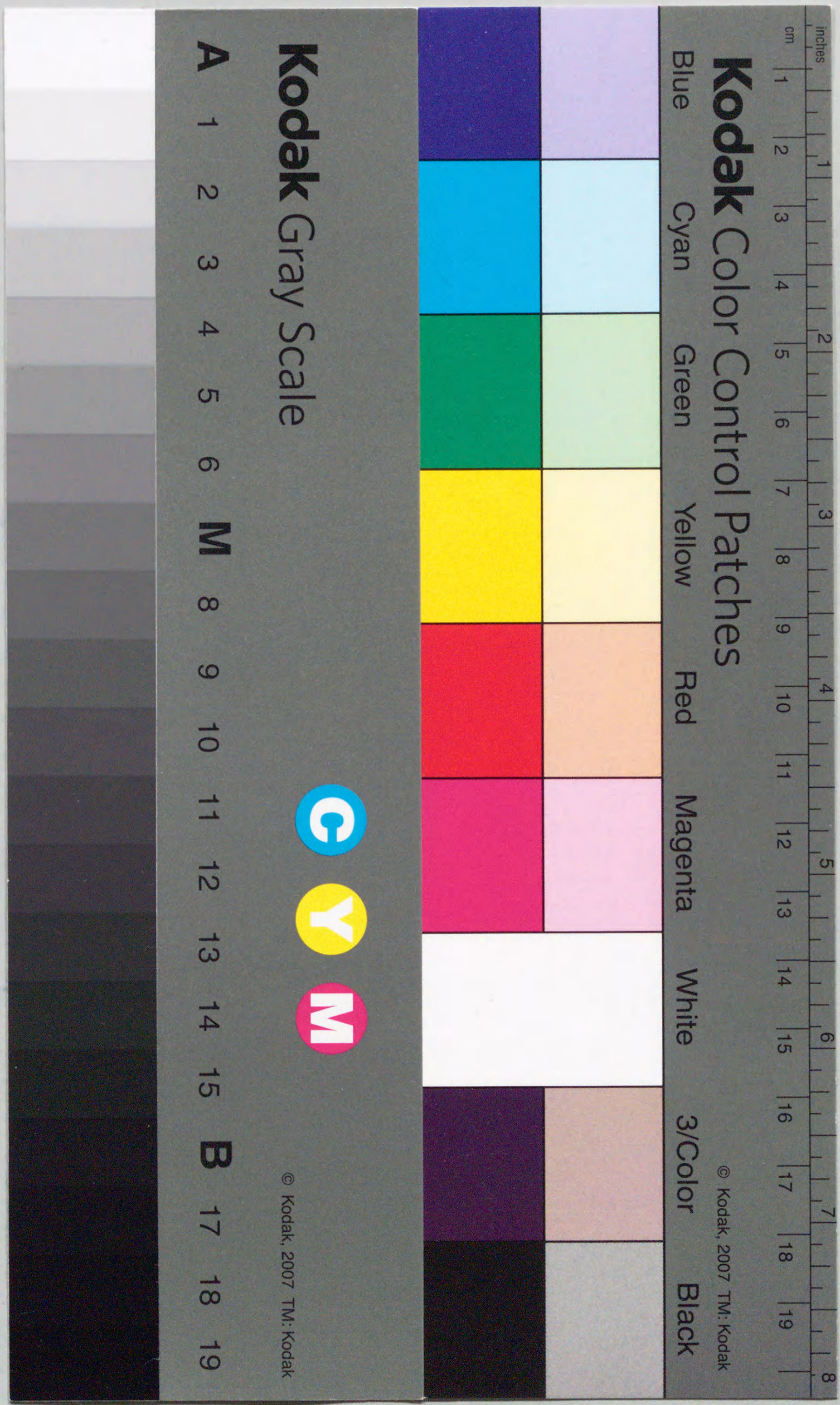
7. "Copper-phthalocyanine stationary phases (Cu-PCs) for fullerenes separation in microcolumn liquid chromatography"
K.Jinno, C.Kohrikawa, Y.Saito, H.Ohta, J.Haginaka and Y.Saito,
J.Microcol.Sep., **8**, 13-20 (1996).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