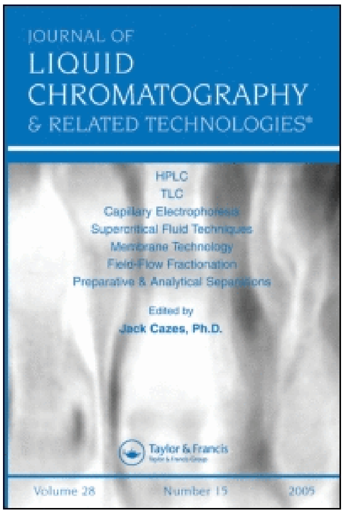

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INFLUENCE OF PORE SIZE AND PORE SIZE DISTRIBUTION OF POLYMER-BASED PACKING MATERIALS ON CHROMATOGRAPHIC SEPARATION OF CARBON CLUSTERS

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ABSTRACT

Size monodispersed poly(styrene-divinylbenzene) particles with a variety of pore size and pore size distribution were prepared by a multi-step swelling and polymerization method and utilized as packing materials in high performance liquid chromatography (HPLC). As an application of these packing materials, separation of carbon clusters C_{60} and C_{70} were examined in a mixture of hexane and benzene and an influence of the pore size and the pore size distribution of the polymer-based packing materials on the separation was investigated. Although carbon clusters are sterically bulky molecules, packing materials with smaller pores showed better separation than those with larger pores. Completely separated peaks and the good peak shape suggest possible isolation of carbon clusters on relatively cheap polymer-based packing materials.

INTRODUCTION

Analytical separation as well as preparative separation of carbon clusters such as C_{60} and C_{70} (Figure. 1) has been an important subject in both analytical and physical chemistries, because chemical and physical properties of the carbon clusters and their derivatives recently interest a lot of chemists and physicists. In fact, a lot of unique techniques have been examined to get better resolution [1]. In spite of unestablished separation mechanism, specially designed packing materials involving appropriate cavity for a carbon cluster [2] are utilized for the separations to afford nice results based on special sterical interactions, however, from the view point of actual isolation process and/or preparative separation, stable, cheap, and readily available packing materials are clearly more suitable.

Based on our recent work, the use of silica-based stationary phase including π-acidic ligand such as phenyl or pyrenyl group enhanced loading capacity of carbon clusters [3]. In that sense, poly(styrene-divinylbenzene) gel seems to be also an effective medium for preparative separation of carbon clusters. In practice, poly(styrene-divinylbenzene) gel was commercially introduced to a preparative separation of C_{60} and C_{70} in an appropriate mixture of hexane and benzene, however, the reported separation factor was not so satisfactory (1.42) and serious peak tailing was observed [4]. Since the polymer-based packing materials are easy to prepare and clearly cheaper and more stable than some of specially designed silica-based packing materials [2], optimizations for the separation conditions such as suitable pore structures or column packing stability have been still very important and urgent necessity. In addition, since a chemical stability of packing materials strictly affects the purity of the separated compounds especially in a preparative separation, chemically stable polymer-based packing materials should be greater in this point. A contamination of the separated compounds through a cleavage of stationary ligand or chemical decomposition of stationary phase is unavoidable problem of alkylsilylated silica-based packing materials and even small amount of impurity should

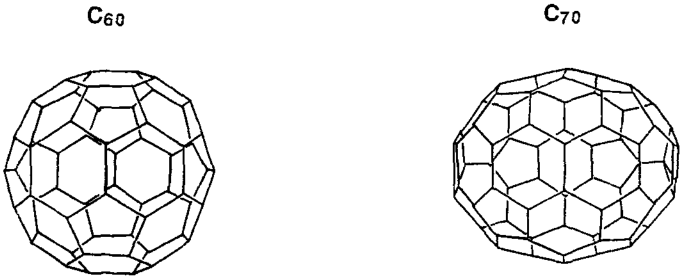

FIGURE 1. Carbon clusters of C_{60} and C_{70}.

affect exact physical properties of carbon clusters and be avoided. These must be a great responsibility of separation science.

Recently, size monodispersed polymer-based packing materials were prepared by a multi-step swelling and polymerization method (two step micro emulsion method) and utilized in a variety of separation mode [5,6]. Compared with size polydispersed packing materials prepared mainly by traditional suspension polymerization method, the monodispersed packing materials show excellent column efficiency, moreover, column pressure drop is much lower than that of corresponding polydispersed particles based on their size monodispersity without any fines. This is a very important advantage to mechanically brittle and unstable polymer-based packing materials in both analytical and preparative separations to obtain good reproducibility through the repeated examinations.

Here, we wish to report the separation of C_{60} and C_{70} using readily prepared size monodispersed poly(styrene-divinylbenzene) packing materials with a variety of pore size and pore size distribution, and compare stability of packed columns, separation factor (α), and peak shape with a commercial wide pore packing material. Although a separation of larger carbon cluster such as C_{84} and larger including their derivatives is now more important, an optimization of separation conditions for general carbon clusters using polymer-based packing materials is also important, because the mixture of a variety of carbon clusters including C_{60}, C_{70}, and larger should be produced through the preparation process of carbon clusters [7]. It is relatively easy to investigate a retention mechanism of carbon clusters using a mixture of only C_{60} and C_{70}.

EXPERIMENTAL

Materials; Carbon clusters were gifts from Kyoto University and pre-treatment took place to obtain the fractions including C_{60} and C_{70} by the use of silica gel open column chromatography in a mixture of hexane and benzene. The identifications of each products were confirmed by the reported physical data [7]. Size monodispersed poly(styrene-divinylbenzene) packing materials were prepared in our laboratory using a typical multi-step swelling and polymerization method reported elsewhere [8]. The compositions of monomers and porogenic solvents are as follows; the particle for column 1; commercial divinylbenzene = 50 vol% and toluene = 50 vol%, the particle for column 2; styrene = 20.6 vol%, commercial divinylbenzene = 29.6 vol%, and toluene = 50 vol%, and the particle for column 3; styrene = 16.3 vol%, commercial divinylbenzene = 23.7 vol%, and cyclohexanol = 60 vol%. The yields of all prepared particles were better than 90 % based on the amount of the used monomers. The particle size of the monodispersed particles was set at 5.5 μm and their size-monodispersity was quite good as depicted in Figure 2. The polymer-based packing materials were packed into stainless steel column (4.6 mm ID X 100 mm) under a wet condition. A commercial packing material PLRP S300 was purchased from Polymer Laboratory Inc., and the mean particle size was at 10 μm. All chromatographic grade solvents were purchased from Nacalai Tesque (Kyoto, Japan) and used without further purifications.

Equipments : Chromatography was carried out with a Jasco 880-PU intelligent HPLC pump equipped with a Rheodyne 7125 valve loop injector. Both UV detector set at 254 nm or 308 nm of a Jasco UVIDEC-100-III and RI detector of a Waters Differential Refractometer R401 were used for peak monitor. Separation of carbon clusters was carried out at 30 ± 0.1 °C in a normal phase mode, while examinations for

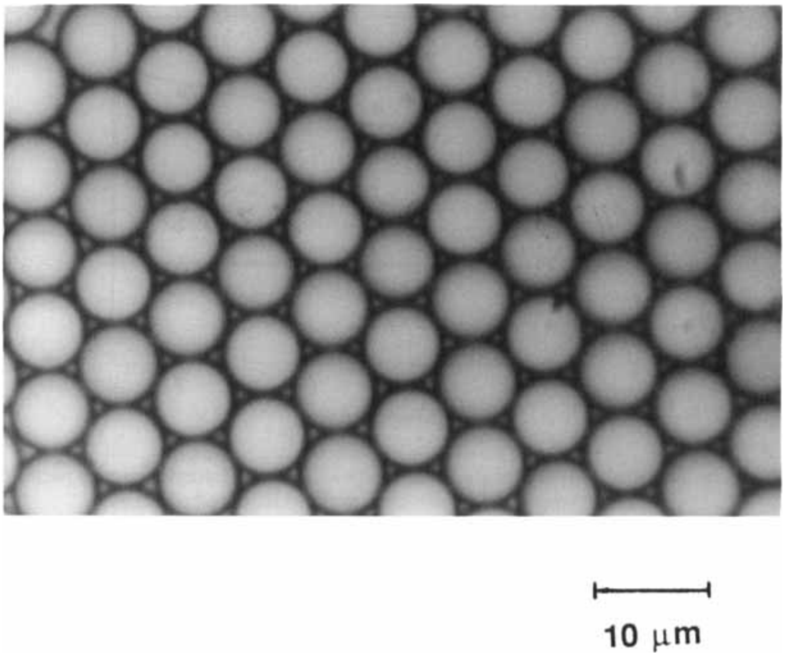

FIGURE 2. Optical micrograph of prepared size-monodispersed particles.

stability of packed columns took place in a reversed-phase gradient mode. Benzene employed as a solvent for carbon clusters was utilized for the t_0 measurement, while uracil was employed as a nonretained sample in the reversed-phase mode and capacity factor k' was calculated based on the actual retention time determined using a Shimadzu C-R4A Chromatopac. Reproducibility of data in duplicate was better than 2 %.

RESULTS AND DISCUSSION

Stability of Packed Columns

Figure 3 depicted a comparison of stability of columns packed with size monodispersed particles and polydispersed particles prepared by the multi-step swelling

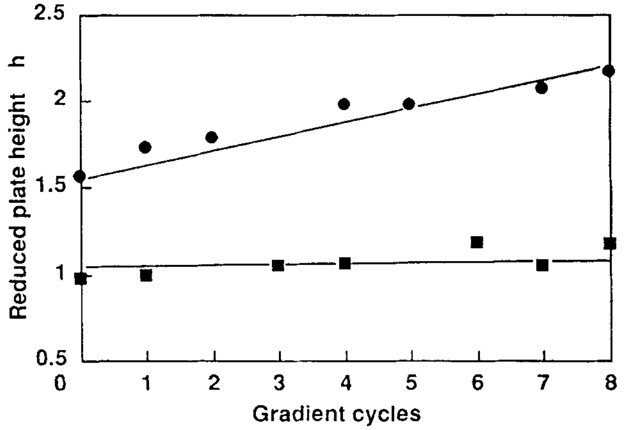

FIGURE 3. Stability of columns packed with poly(styrene-divinylbenzene) particles.
■; Monodispersed particles, ●; Polydispersed particles
Mobile phases: A: 20% aqueous acetonitrile
B: 60% aqueous acetonitrile
Gradient: from 100% A to 100% B in 10 min.
Flow rate: 2 ml/min.
Column check: sample: toluene in 60% aqueous acetonitrile

and polymerization method and the corresponding suspension polymerization method, respectively. This experiment was examined in a reversed-phase gradient mode because a column pressure drop was much higher in the employed mode than in a normal phase mode utilized for separation of carbon clusters as described later. After 8 cycles of the liner gradient, the column packed with size polydispersed packing materials loss column efficiency based on the relative reduced plate height (h) (eq.1) of toluene in 60% aqueous acetonitrile more rapidly (ca. 30%), while the column packed with size monodispersed packing materials was stable.

$$h = H / d_p \quad (1)$$

H = plate height, d_p = particle size

These findings mean that the column packed with the size monodispersed packing materials is much more stable than that with polydispersed packing materials even if another severe separation conditions have to be required. In addition, as described later, size monodispersed polymer-based packing materials show not only better column efficiency but also very low column pressure drop which leads longer column life-time. So that, these advantages of size monodispersed particles must realize actual repeated separation and isolation.

Pore Size Distribution

Usually polymer-based packing materials show bimodal pore size distribution [9] and a balance of the contribution of micropores (< 2 nm), mesopores (2 - 50 nm), and macropores (50 nm <) determines retention selectivity of the packing materials [10]. A greater contribution of micropores (< 2 nm) of polymer-based packing materials tends to show preferable retention toward rigid and compact molecules such as aromatic compounds even if the polymer-based packing materials do not include any aromatic functional groups [11].

We examined size exclusion chromatography (SEC) in tetrahydrofuran (THF) using polystyrene standard samples as well as alkylbenzenes. The calibration curves on the poly(styrene-divinylbenzene) packing materials are depicted in Figure 4. To clear the contribution of the each pores, volume % of pores was plotted against logarithm of molecular weight of the solutes (log MW) because pore volumes were different each other.

All the calibration curves reveal pore size distribution without clear exclusion limit on lower molecular weight side, but we can classify those into mainly two different types of pore size distributions. When a good solvent with the monomers, toluene was utilized as porogenic solvent (column 1 and column 2), relatively small pores were produced as reported [10] and the packing materials prepared from only commercial divinylbenzene (55 % of isomers of divinylbenzene and 45 % of ethylstyrene etc. were included) as the monomer involved slightly larger contribution of the smaller pores (column 1), than that including styrene as a co-monomer (column 2) which may be explained based on higher

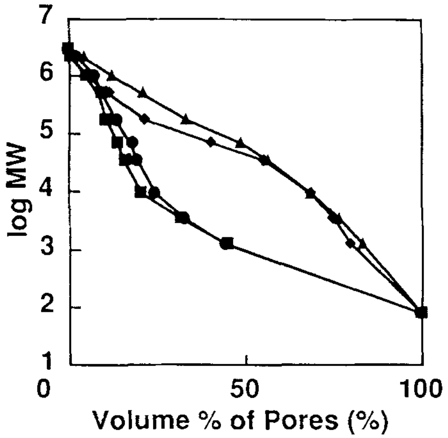

FIGURE 4. Calibration curves on poly(styrene-divinylbenzene) particles
Mobile phase; tetrahydrofuran (THF)
Flow rate; 0.5 ml/min.
Detection; UV 254 nm
Samples; Polystyrene standards
■; Column 1, ●; Column 2, ▲; Column 3, ◆; PLRP S300

TABLE 1. **Separation of C_{60} and C_{70} on Poly(styrene-divinylbenzene) Columns**[a)]

Column	$k'C_{60}$	$k'C_{70}$	$\alpha(C_{70}/C_{60})$	Pressure (MPa)
1	1.64	2.70	1.64	0.4
2	1.95	3.14	1.61	0.6
3	0.34	0.51	1.49	0.2
PLRP S300	0.41	0.64	1.56	1.0

a) Mobile phase; benzene:hexane=50:50, Flow rate; 0.5 ml/min, Detection; UV 308 nm.

crosslinking density on column 1 [10], while cyclohexanol gave relatively large pores (column 3) which were similar to that of commercial wide pore column PLRP S300 but slightly greater contribution of larger pores was observed on column 3.

Separation of Carbon Clusters

The results on separations of carbon clusters are summarized in Table I. A mixture of hexane and benzene was utilized as a mobile phase, because it is very easy to isolate the clusters from the solution by a simple evaporation. Column 1 and 2 must involve large surface area due to the large contribution of smaller pores which may reflect larger k' values, while column 3 and PLRP S300 are similar wide pore columns and k' values are smaller. Interestingly, column 1 with the largest contribution of smaller pores provided smaller k' value than column 2 in spite of lager pore volume (1.53 ml for column1 and 1.34 ml for column 2).

Usually, on the polymer-based packing materials, bulky molecules tend to be excluded from micropores to result in preferential retention for rigid and compact molecules [10,11]. However, in these cases, slightly bulkier molecules C_{70} was retained longer on all the columns than more compact C_{60} which was observed in most of another reported separations of carbon clusters [1,2]. If size exclusion effect works dominantly, Column 1 which mainly involves the small pores should afford worse separation, however, column 1 gave slightly better separation factor α than column 2 with slightly smaller k' value in practice. These findings strongly suggest possible dominant separation mechanisms of carbon clusters in normal phase mode are another interactions such as dispersion force and/or charge transfer interaction. Since a diameter of C_{60} is reported to be ca. 7 Å [7] which is much smaller than the mean pore diameter of the prepared particles (ca. 10^2 Å on column 1 measured by a BET method) and smaller pores have more rapid curvature of the internal surface of pores, slightly flat C_{70} molecules contact with the adsorbent better and are retained longer on packing materials with the smaller pores to result in better separation, while loose contact between solutes and flatter curvature of internal surface in wide pore packing materials affords worse separation factor. On the other hand, if ratios of k' values on column 1 and 2 are calculated, k'(C_{60} on column 1) / k'(C_{60} on column 2) is 0.84, while that for C_{70} is 0.86. These values mean on column 1, smaller carbon cluster C_{60} is also excluded from the pores to afford better separation factor α with smaller k' value. Therefore, this exclusion effect toward even small molecules from the pores is also important factor for the separation.

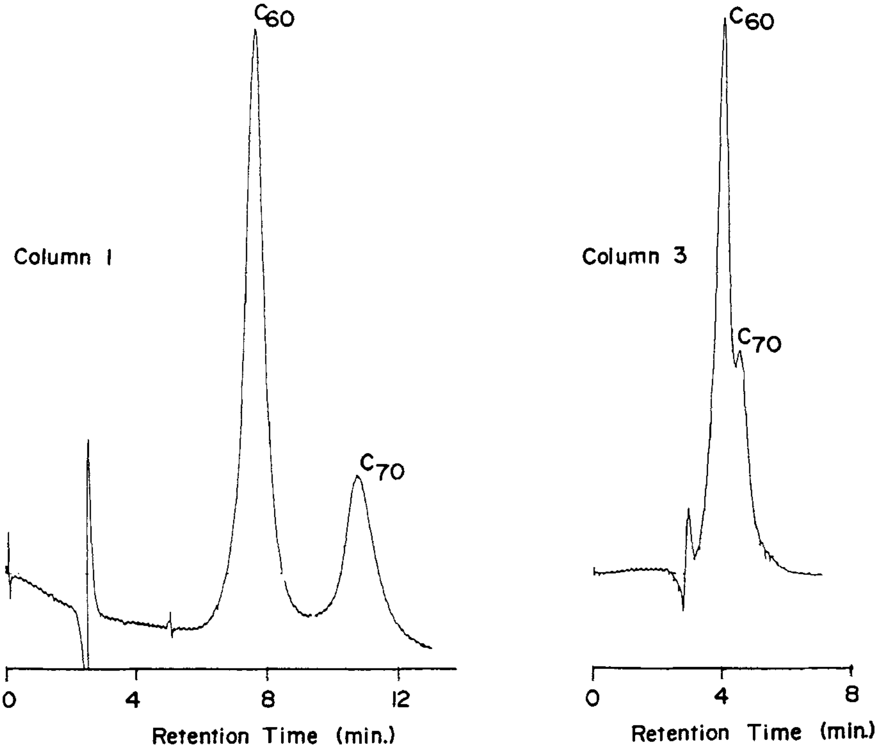

FIGURE 5. Separation of a mixture of C_{60} and C_{70}
Mobile phase; hexane:benzene=50:50 (volume)
Flow rate; 0.5 ml/min.
Detection; UV 308 nm

Usually, polymer-based packing materials with small pores tend to show a peak broadening or tailing due to slow mass transfer within the pores which provides poorer resolution, so that the wide pore columns are thought to be suitable especially for the separation of bulky solutes. However, column 1 and 2 unexpectedly provided better separations with good peak shape than the wide pore columns where both peaks were not separated completely each other as depicted in Figure 5. The addition of more hexane should make the retention time longer to result in complete separation finally, but

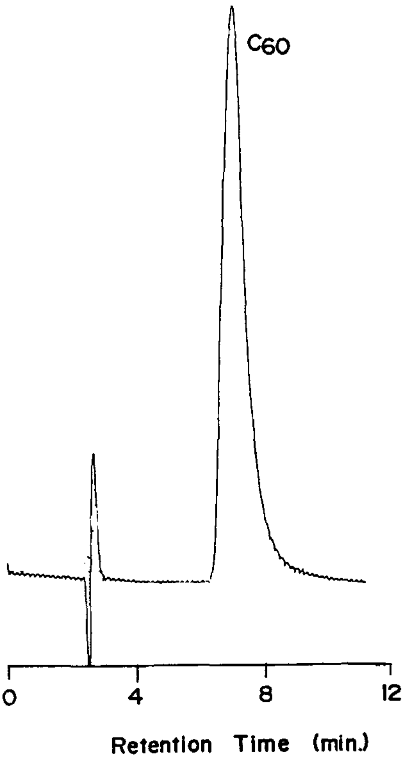

FIGURE 6. A chromatogram of the isolated C_{60} on column 1.
Mobile phase; hexane:benzene=50:50
Flow rate; 0.5 ml/min.
Detection; UV 308 nm

that is not so good idea because hexane is not a good solvent for carbon clusters which leads lower loading amount.

In addition, even under the normal phase condition, column 1, 2, and 3 showed much lower column pressure drop than 10 µm PLRP S300. The high column pressure drop should become a more serious problem in a larger and longer preparative scale column when large amount sample is injected at once. Actually, column 1 was utilized for separation of the mixture of C_{60} and C_{70} and an isolation was examined by a simple fractionation. A chromatogram of the isolated C_{60} indicated a complete isolation was achieved on column 1 (Figure 6).

From the view point of the separation of carbon clusters, some nice columns are now available (e.g. k' > 3), however, a rapid progress in the chemistry of carbon clusters should require more preparative separation of one carbon cluster from the soot extract to make the price down. If an eclectic idea between a good separation and cheap materials is required for the preparative separation of carbon clusters, the use of poly(styrene-divinlybenzene) packing materials is one of the most eclectic ideas because of the cheap materials and a medium separations. Of course, size polydispersed poly(styrene-divinylbenzene) packing materials prepared by a suspension polymerization method are also useful for this purpose, however, monodispersed packing materials may make a great contribution to the good reproducibility in the repeated separations based on good column mechanical stability derived from the size monodispersity and low column pressure drop to be applicable for the separations. We utilized three kinds of size monodispersed poly(styrene-divinylbenzene) packing materials and unexpectedly, microporous column showed better separations with low column pressure drop. In a near future, these findings will be very important for an application of the size monodispersed polymer-based packing materials for actual use. Further investigation of such kind of work is now under progress. Finally, partial funding by the National Institute of Health (GM44885-01) is gratefully acknowledged.

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