**Development of a C₆₀-fullerene bonded open-tubular
capillary using a photo/thermal active agent for liquid
chromatographic separations by π – π interactions**

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22 Abstract

23 This short communication describes a newly developed open-tubular capillary which was coated
24 with C₆₀-fullerene by a covalent bonding via a photo/thermal active agent. We utilized perfluorophenyl
25 azide (PFPA) as an active agent, which can be used for the “photo click” coupling of the carbon
26 materials. The inner wall of a fused silica capillary was treated with silane conjugated PFPA, and then
27 C₆₀-fullerene was chemically modified by a photoreaction or a thermal reaction. Through evaluations of
28 the capillaries by liquid chromatography, the separation characteristics of three polycyclic aromatic
29 hydrocarbons (PAHs) were confirmed in both capillaries. With comparison of the retention behavior to
30 a commonly used C₁₈ column, the prepared capillaries showed the specific separation ability based on
31 the π - π stacking by C₆₀-fullerene. The capillary prepared by the thermal reaction provided the base line
32 separation of phenanthrene, triphenylene, and benz[a]pyrene within 3 min at 18.8 cm capillary length.

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34 *Keywords:* C₆₀-fullerene, perfluorophenyl azide, liquid chromatography, π - π interaction, polycyclic
35 aromatic hydrocarbons

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47 1. Introduction

48 Carbon nano-materials such as a fullerene, graphene, and nanotube have been widely studied in the
49 fields of semiconductors, conductive transparent films, medicines, etc because of their excellent thermal
50 and/or electrical conductivity, physical strength, and specific surface area [1-4]. These carbon materials
51 consist of a π -conjugated system from sp^2 carbons and π electrons that are diffusely distributed within
52 the molecule. The π electrons provide the dipole interaction based on the London dispersion force and
53 allow the π - π interaction in regard to the aromatic compounds. According to the specific interaction, the
54 carbon nano-materials have been applied for the separation media in liquid chromatography (LC),
55 capillary electrochromatography (CEC), capillary electrophoresis, and solid phase extraction [5-11]. In
56 common cases of these applications, the introduction of the various functional groups to the surface of
57 the carbon materials were necessary for the immobilization onto the substrate or improvement of
58 solubility/dispersibility. However, the introduction of the functional groups provided opportunity for
59 other interactions, such as hydrogen bonding and ionic interaction. Therefore, in order to obtain the
60 separation medium having characteristic π - π interactions, another technique is required to immobilize
61 the carbon materials.

62 Perfluorophenylazide (PFPA) is a photo/thermal active molecule for the conjugation of carbon
63 materials, polymers, and carbonhydrates [12-16], PFPA is effective for reaction with the conjugated
64 double bond because of the occupied fluorine atoms, therefore the unexpected reactions such as a
65 dimerization and cyclization via the azide-based molecules occur less frequently [17,18]. We also
66 previously reported the effective immobilization of the graphene flakes, alkyl chain-modified
67 nanoparticles, polymer thin films, and small molecules by the PFPA derivatives [19,20]. The aim of this
68 study is to achieve simple yet effective preparation of the carbon-based separation medium via the
69 PFPA derivatives. A fused silica capillary modified with C_{60} -fullerene, which has higher
70 solubility/dispersibility compared to the other carbon nano-materials and suitable reactivity based on its
71 spherical morphology [21-23], was evaluated by capillary LC. We examined the effects against the

retention **behavior** by C₆₀-fullerene and achievement of selective separations of **several** polycyclic aromatic hydrocarbons (PAHs) by the π - π interaction. In this communication, we propose a simple and effective method for the preparation of the C₆₀-fullerene immobilized separation medium.

2. Experimental Section

2.1. Chemicals and instruments

Deionized water was obtained by Milli-Q (Direct-Q 3UV) and all solvents were purchased from Wako Chemicals (Osaka, Japan). C₆₀-fullerene was purchased from SES Research (Huston, TX, USA). The other reagents for the solutes in LC, the synthesis of PFPA derivatives, and the mobile phases were purchased from Nacalai Tesque (Kyoto, Japan). A fused-silica capillary (25 μ m i.d., 375 μ m o.d.) was purchased from Polymicro Technologies (Phoenix, AZ, USA). A transparent fused-silica capillary (25 μ m i.d.) was purchased from GL Science (Tokyo, Japan).

Capillary LC experiments were performed with a P/ACE MDQ system (Beckman Coulter, Fullerton, CA, USA) equipped with a UV absorbance detector. For Fourier transform infrared spectroscopy (FTIR), a Nicolet iS5 FT-IR (ThermoScientific, Waltham, MA, USA) was used for the definition of the synthesized compounds.

2.2. Preparation of a C₆₀-fullerene bonded capillary

N-(3-trimethoxysilylpropyl)-4-azido-2,3,5,6-tetrafluorobenzamide (PFPA-silane) was synthesized according to the previous studies [24,25]. The synthesis procedures including the scheme and FT-IR spectra of products are summarized in Supplementary data (Scheme S1, Fig. S1). All the capillaries were washed with 1.0 M HCl aq., 1.0 M NaOH aq., and water to activate silanol groups of capillary inner wall. After drying with N₂ purge, PFPA-silane solution in methanol (12.6 mM) was passed through the Capillary-A, -B, -D, and -E at 10 μ L /h for 24 h. Then, methanol, toluene, and C₆₀-fullerene in toluene (36 mM) was filled in Capillary-A, -B, and -D, respectively.

For Capillary-C, PFPA-silane in toluene (12.6 mM) and C₆₀-fullerene in toluene (36 mM) were mixed and treated by UV irradiation for 20 min in advance. The mixture was filled into the activated capillary. The capillary was folded for 24 h in ambient.

Additionally, in order to obtain a capillary which was modified with C₆₀-fullerene at higher density, the thermal reaction was employed for the immobilization of C₆₀-fullerene instead of UV irradiation. As shown in Table 1, Capillary-D' was prepared by same the method as Capillary-D except with a smaller diameter. For Capillary-E, C₆₀-fullerene saturated in toluene was filled into the activated capillary (25 μm i.d.) and dried under vacuum to evaporate the solvent (× 3). The capillary was treated at 170 °C for 5 h. Then, dichlorobenzene was filled into the capillary and treated by sonication for 5 min to remove the unreacted C₆₀-fullerene.

After each reaction, all the capillaries were washed with dichloromethane and methanol by pump to remove the unreacted C₆₀-fullerene. The reaction schematic for Capillary-D is shown in Fig. 1. Also, the preparation conditions of all the capillaries are summarized shown in Table 1.

The LC evaluations were carried out under the following conditions: capillary size, 50 μm i.d. or 25 μm i.d. mobile phase, water/acetonitrile or water/methanol/tetrahydrofran (THF); pressure, 50 mbar; injection, 50 mbar-3 s; detection, UV 200 or 254 nm; temperature, 25 °C. Other conditions are listed in each figure caption. The retention factor is defined as follows;

Retention factor, $k = (t_R - t_0) / t_0$, t_R : retention time of solute, t_0 : retention time of acetone

3. Results and discussion

3.1. Effect of the modification with C₆₀-fullerene

To examine the effect of the modification with C₆₀-fullerene, Capillaries-A to -D were evaluated by capillary LC using acetone and benzene as solutes. The chromatograms in each capillary are shown in Fig. 2. From these results, only Capillary-D allowed separation ability of the two solutes. Both of Capillary A and B were prepared without C₆₀-fullerene, so that we confirmed the solvents did not affect

the retention of benzene. Regarding Capillary-C, we tried to synthesize the C₆₀-fullerene attached PFPA-silane by direct silane coupling to capillary inner wall. However, the specific retention based on C₆₀-fullerene was not observed. We assumed that the reaction between C₆₀-fullerene and PFPA was not complete, so that the retention property of the capillary was similar to Capillary-B. On the other hand, the base-line separation of acetone and benzene was achieved in Capillary-D. The result suggests bonding of C₆₀-fullerene under these conditions.

3.2. Optimization of the preparation of the C₆₀-fullerene bonded capillary

As shown in above results, the C₆₀-fullerene bonded capillary showed the retention ability based on the interaction with immobilized C₆₀-fullerene. However, the absolute retentions were not enough to separate several solutes such as PAHs. We considered that the density of the bonded C₆₀-fullerene was too low. Therefore we carried out the other approaches to enhance the retention ability by C₆₀-fullerene. In the previous study, the solubility of C₆₀-fullerene was much higher in dichlorobenzene (24.6 mg/mL) than in toluene (2.9 mg/mL) [23]. Thereupon another capillary was prepared by the similar procedure using dichlorobenzene as a solvent of C₆₀-fullerene under the UV reaction (Capillary-F). The results are shown in Fig. S2 and S3. Although the suitable separations of PAHs were achieved in the Capillary-F, the similar separation was also observed in the Capillary-G, which was prepared without C₆₀-fullerene. In brief, dichlorobenzene itself reacted with PFPA the reactivity of dichlorobenzene was higher than that of toluene due to the effect of the electron-accepting group, chlorine. Consequently, in order to avoid the effects of the solvent, we prepared another capillary without the solvents by the thermal reaction using a narrower capillary (Capillary-E). As shown in the Experimental, the capillary was washed with dichlorobenzene by sonication so that any unreacted C₆₀-fullerene was removed completely. Additionally, to compare the separation ability, Capillary-D' was also prepared using a narrower capillary. Here, we employed THF as a part of mobile phase because THF can provide the sharper peak under the π - π interaction [26,27]. As a result, the Capillary-E provided the better

147 separation ability compared to the Capillary-D' (Fig. 3.), although the elution time was different in each
148 column because of the difference of the column length. Furthermore, the typical separation of PAHs by
149 LC using Capillary-E is shown in Fig. 4. As shown in this chromatogram, **Capillary-E** allowed the
150 effective base-line separation of **three PAHs** within 5 min, the plate number estimated by using
151 phenanthrene was at 3300. Additionally, the repeatability of the capillary preparation was examined
152 with another capillary prepared by same method as Capillary-E. As a result, the relative standard
153 deviation of the retention of phenanthrene was estimated at 0.342 ($n = 2$).
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155 3.3.Examination of the retention *behavior* in the C₆₀-fullerene bonded capillary

156 To clarify the retention **differences** of the bonded C₆₀-fullerene, the alkylbenzenes as the hydrophobic
157 solutes and PAHs acceptable by the π - π interaction were evaluated in both the C₆₀-fullerene bonded
158 capillary and C₁₈ packed column. Here, we considered the relationship of the octanol-water partition
159 coefficient $\log P_{o/w}$ of each solute against the logarithmic retention factor, k [28,29]. In typical analysis
160 on the reversed phase mode (C₁₈-based column), the separation ability depended only on the
161 hydrophobic interaction, especially for hydrocarbons. Therefore, **if the solute-column interaction of the**
162 **C₆₀-fullerene bonded capillary were similar to the C₁₈ column,** the retention tendency should be also
163 similar. However, as shown in Fig. 5, the slope of the plots based on alkylbenzene and PAHs are not
164 similar. In fact, the slope of alkylbenzenes in the C₁₈ column (0.50) was higher than that of the C₆₀-
165 fullerene bonded capillary (0.47) even though the absolute retentions were pretty low in the C₆₀-
166 fullerene bonded capillary, so that the retention of ethyl benzene could not be estimated. On the other
167 hand, the slope of PAHs in the C₆₀-fullerene bonded capillary (0.67) was much higher than that of the
168 C₁₈ column (0.46). Here, the plot of naphthalene in Capillary-E was removed because of low retention
169 as well as ethylbenzene. **Thus the hydrophobic interactions were the major cause of the retention in C₁₈**
170 **column, whereas the additional interaction, which was believed to be from the “ π - π interaction”, was**
171 **mainly worked for the retentions of PAHs in the C₆₀-fullerene bonded capillary.** Hence, we strongly

172 suppose that the effective separation based on the π - π interaction using the newly developed C₆₀-
173 fullerene bonded capillary prepared with PFPA derivatives was confirmed.

175 4. Conclusion

176 In this study, we developed a novel capillary which was modified with C₆₀-fullerene using a
177 photo/thermal reactive agent, PFPA. The prepared capillaries provided the retention ability based on the
178 interaction with the bonded C₆₀-fullerene. Also, we showed that the retention property of the C₆₀-
179 fullerene bonded capillary was affected by the specific interaction between the solutes and the bonded
180 C₆₀-fullerene. Finally, we successfully achieved the base-line separation of three PAHs using the C₆₀-
181 fullerene bonded capillary by LC. Although we used an open-tubular capillary to examine the
182 possibilities of PFPA/C₆₀-fullerene conjugation and the separation ability of the C₆₀-fullerene bonded
183 capillary in this study, we believe that similar concept is applicable for the several formats such as
184 monolithic columns. We expect that the additional improvement of the preparation of the column using
185 the carbon nano-materials will provide new separation media in LC and CEC.

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238 Table 1. Contents of the prepared capillaries.

	step 1	step 2
Capillary-A	PFPA-silane / methanol	methanol without C ₆₀ (UV)
Capillary-B	PFPA-silane / methanol	toluene without C ₆₀ (UV)
Capillary-C	PFPA-silane + C ₆₀ / toluene (UV)	C ₆₀ - PFPA-silane / toluene (silane coupling)
Capillary-D	PFPA-silane / methanol	C ₆₀ / toluene (UV)
Capillary-D'	PFPA-silane / methanol	C ₆₀ / toluene (UV)
Capillary-E	PFPA-silane / methanol → Filling C ₆₀ / toluene and dry (×3) → Thermal reaction (no UV)	

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240 Capillaries-A, -B, UV irradiation with only the solvent in capillary; Capillary-C, synthesis of PFPA
 241 conjugated C₆₀-fullerene and silane coupling in the capillary at 2nd step; Capillary-D, following as Fig.
 242 1.; Capillary-E, by thermal reaction without any UV reactions.

243 Column size, Capillary-A, B, C, D, 50 μm i.d.; Capillary-D', E, 25 μm i.d.

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260 Figure captions

261 Fig. 1. Schematic image of the preparation of the C₆₀-fullerene bonded capillary.

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263 Fig.2. Chromatograms in the prepared capillaries.

264 capillary size, 50 μm i.d. $\times$ total length 65 cm (effective length 56.5 cm); mobile phase,
265 acetonitrile/water = 1/9 (v/v); pressure, 50 mbar; injection: 50 mbar- 3 s; detection, UV 200 nm; solutes,
266 acetone, benzene (1.0 mg/mL).

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268 Fig. 3. Separation of PAHs using the prepared capillaries.

269 capillary, Capillary-D' (capillary size, 25 μm i.d. $\times$ effective length 30.0 cm), Capillary-E (capillary size,
270 capillary size, 25 μm i.d. $\times$ effective length 28.5 cm); mobile phase, water/methanol/THF =
271 5.00/4.75/0.25 (v/v/v); pressure, 1.5 psi; injection, 5.0 psi, 4.0 s; detection, UV 254 nm; temperature,
272 25 °C.

273 solutes, phenanthrene (25 mg/L), triphenylene (25 mg/L)

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275 Fig. 4. Separation of PAHs using the C₆₀-fullerene bonded capillary by LC

276 LC conditions

277 capillary, Capillary-E (capillary size, 25 μm i.d. $\times$ effective length 18.8 cm); mobile phase,
278 water/methanol/THF = 4.85/4.65/0.50 (v/v/v); pressure, 50 mbar; injection, 50 mbar-3 s; detection, UV
279 200 or 254 nm; temperature, 25 °C.

280 solutes, phenanthrene (25 mg/L), triphenylene (25 mg/L), benzo[a]pyrene (50 mg/L).

281

282 Fig. 5. Plots of the logarithmic k vs $\log P_{\text{o/w}}$ of alkylbenzenes and PAHs

283 LC conditions

284 capillary, Capillary-E (capillary size, 25 μm i.d. $\times$ effective length 30.0 cm); mobile phase,
285 water/methanol/THF = 5.00/4.75/0.25 (v/v/v); pressure, 50 mbar; injection, 50 mbar-3 s; detection, UV
286 200 or 254 nm; temperature, 25 $^{\circ}\text{C}$.

287 C_{18} column: LC system (Shimadzu, Kyoto, Japan); column, YMC-Pack ODS (4.6 mm i.d. $\times$ 75 mm);
288 mobile phase, water/methanol/THF = 5.00/4.75/0.25 (v/v/v); flow rate, 1.0 mL/min; injection, 5.0 μL ;
289 detection, UV 200 or 254 nm; temperature, 40 $^{\circ}\text{C}$.

290 Solutes, benzene, butylbenzene, hexylbenzene, octylbenzene, naphthalene, phenanthrene, triphenylene
291 (50 mg/L).

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Fused silica capillary

*PFPA-silane
/ methanol*

1st step

*fullerene
/ toluene*

hν

2nd step

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.