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High-Performance Liquid Chromatography Stationary Phases Based on π–π Electron Interaction. Aminopropyl Silica Gels Modified with Metal Phthalocyanines

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Aminopropyl silica gels were modified with two porphyrin derivatives and five phthalocyanine derivatives. The modified silica gels were evaluated for the usefulness as high-performance liquid chromatography stationary phases for the separation of π-electron-rich polyaromatic hydrocarbons (PAHs) such as naphthalene and phenanthrene. As a result of the modification, the aminopropyl silica gels acquired a function of π–π electron interaction with PAHs. In particular, the aminopropyl silica gel modified with copper phthalocyanine (Cu-PCS) showed the strongest π–π electron interaction with PAHs. It was the most suited as a stationary phase for the separations of PAHs among the modified silica gels tested. The Cu-PCS column could be utilized for the separation of π-electron-rich compounds including medicines, mutagens, and pollutants.

The separation of π-electron-rich medicines, mutagens, and pollutants are often conducted by high-performance liquid chromatography (HPLC). For this purpose, many investigators developed new stationary phases, of which the separation mode was mainly based on hydrophobic, π–π electron, dipole–dipole, and/or electrostatic interactions between a substance(s) and the stationary phase.[1—3] Among these interactions, π–π electron interaction (π–π interaction) seemed to be most effective for the separation of π-electron-rich compounds. However, only a few stationary phases based on π–π interaction has been reported. One of them is a stationary phase based on a pyrene derivative, which has wide π-electron clouds.[4—8] To develop new stationary phases based on π–π interaction, we were interested in porphyrin and phthalocyanine derivatives that have wider π-electron clouds than a pyrene derivative. If porphyrin or phthalocyanine can be fixed on silica gel, a new stationary phase capable of exhibiting much stronger π–π interaction than "the pyrene stationary phase" will become available for the separation of π-electron-rich compounds.

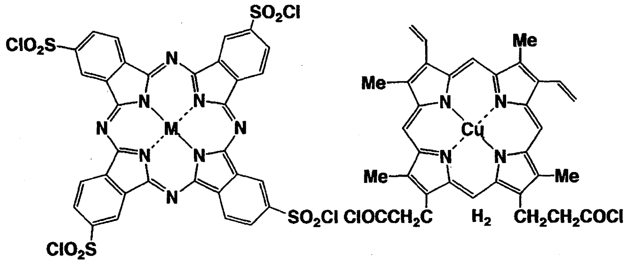
In a previous communication, we reported that a silica gel modified with copper phthalocyanine (Fig. 1) could be a good stationary phase based on π–π interaction for the separation of polyaromatic hydrocarbons (PAHs).[9] The present paper deals with full details of the study, including the examinations carried out for the establishment of a practically useful stationary phase for the separation of π-electron-rich compounds.

Experimental

Chemicals and Reagents. Metal phthalocyanines (M-PC, M = Cu^{2+}, Mn^{3+}, Co^{3+}, Fe^{3+}, and H_2) were obtained from commercial sources (Kanto Kagaku and Tokyo Kasei). Tetrakis (4-carboxyphenyl)porphyrin (H_2-TCPP) and protoporphyrin (H_2-PP) were purchased from Wako Pure Chemical Industries and Tokyo Kasei, respectively. Methyl alcohol (MeOH) used as a mobile phase was of special grade for HPLC. Dioxane of special grade was dried with molecular sieves for the preparations of modified silica gels. The π-electron-rich compounds such as benzene, naphthalene, anthracene, phenanthrene, *o*-terpheny and triphenylene (see Fig. 2), were of reagent grade (Tokyo Kasei and Wako Pure Chemical Industries). Other reagents were of analytical grade or reagent grade.

Silica Gels. As aminopropyl silica gels, Develosil NH_2-5 (Dev-5) and Develosil NH_2-10 (Dev-10) (particle size, 5 μm and 10 μm, respectively) and LiChrosorb-NH_2 (Lic-10) (particle size, 10 μm) were purchased from Nomura Chemicals Co. Ltd and Merck AG, respectively.

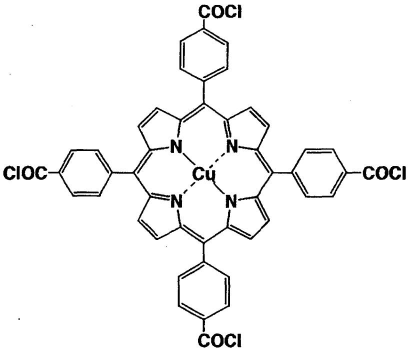
Preparation of Metal Phthalocyaninetetrasulfonyl Chloride (M-PCSCl). By following a method in the literature,[10] we prepared Cu-PCSCl (see Fig. 1). One gram of M-PC was dissolved in 12 g of chlorosulfuric acid, and the solution was heated at 135–150 °C for 2 h with stirring. The reaction mixture was cooled to room temperature, and was poured over crushed ice with stirring. The resulted precipitate of M-PCSCl was filtered off, washed several times with 1.0 $\mathrm{mol\,dm^{-3}}$ hydrochloric acid, and dried to remove hydrochloric acid under vacuum on sodium hydroxide. The for-



M = H_2, Cu^{2+}, Mn^{3+}, Co^{3+} and Fe^{3+}

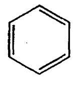
Acid chloride of M-phthalocyaninetetrasulfonic acid (M-PCSCl)

Acid chloride of Cu-protoporphyrin (Cu-PPCl)

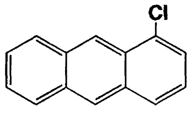


Acid chloride of Cu-tetrakis(4-carboxyphenyl)porphyrin (Cu-TCPPCl)

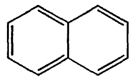
Fig. 1. Structures of acid chlorides of metal phthalocyanines and copper porphyrin.



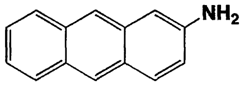
Benzene



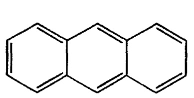
1-Chloroanthracene



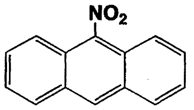
Naphthalene



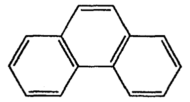
2-Aminoanthracene



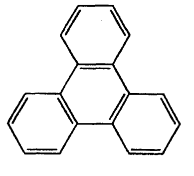
Anthracene



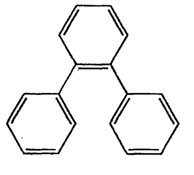
9-Nitroanthracene



Phenanthrene



Triphenylene



o-Terphenyl

Fig. 2. Structures of samples used in this study.

mation of Cu-PCSCl was confirmed by estrifying the resulting Cu-PCSCl with MeOH and measuring the FAB-mass spectrum of the obtained methyl ester (mother ion; 1116 amu).

Preparation of Acid Chlorides of Cu-PP and Cu-TCPP (Cu-PPCl and Cu-TCPPCl). H_2-TCPP and H_2-PP were metalized by the conventional method[11] with copper (II) acetate to give Cu-TCPP and Cu-PP, respectively. Cu-TCPPCl and Cu-PPCl (see Fig. 1) were prepared by chlorocarbonylation of Cu-PP and Cu-TCPP with thionyl chloride in dry dioxane at 70 °C. Excess thionyl chloride and dioxane were removed under vacuum, and solid Cu-PPCl and Cu-TCPPCl were washed with dry dioxane and allowed to stand under vacuum to remove the remaining thionyl chloride.

Preparation of Silica Gel Modified with M-PCS, Cu-TCPP, and Cu-PP. For binding the porphyrins and phthalocyanines to a support, a mixture of the acid chloride (39 μmol, M-PCSCl, Cu-PPCl, and Cu-TCPPCl) and dry silica gel (6 g) in dry dioxane were refluxed until the mixture became colorless. Thus, each metal phthalocyanines and copper porphyrins (6.5 μmol) bound 1 g of silica gel. Silica gels modified with metal phthalocyanines and copper porphyrins were filtered, washed with water and then acetone, and dried under vacuum.

Metal porphyrins and metal phthalocyanines bound to the silica gel were not eluted by water, MeOH and dioxane, since they were bound firmly to the silica gels through the covalent bond. $-CONH-$ or $-SO_2NH-$.

In the present paper, subscripts "D" and "L" denoted the silica gels Deverosil- and LiChrosorb-NH_2, modified with metal phthalocyanines, and subscripts "5" and "10" denoted particle sizes of silica gel. For an example, Cu-PCS_{D5} denoted that Develosil NH_2, whose particle size was 5 μm, was modified with Cu-PCS.

Columns Packed with the Modified Silica Gels. The modified silica gels were packed into stainless steel columns (4 mm×150 mm) by conventional slurry packing method (slurry solvent A conc, Chemco Co,). For comparison, Dev-5, Dev-10, and Lic-10 were packed into a column in the same manner as described above.

Sample Solutions. As sample solutions, 500 $\mu g\,cm^{-3}$ benzene, 10 $\mu g\,cm^{-3}$ naphthalene, 1 $\mu g\,cm^{-3}$ anthracene and 2.5 $\mu g\,cm^{-3}$ phenanthrene in MeOH–water (80 : 20, v/v) were used. o-Terphenyl and triphenylene (2.5 $\mu g\,cm^{-3}$) were used for evaluation of the planar recognition ability of a column.

Apparatus. The HPLC system was constructed with a Shimadzu LC-9A pump (Shimadzu Seisakusho), a Rheodyne model 7125 sample injector (USA) with a 20 mm^3 loop and a Shimadzu photodiode array detector SPD-M6A (Shimadzu Seisakusho) with a personal computer (Epson-286VS, Seiko-Epson) as a data station. The column was allowed to stand at room temperature, around 20 °C.

As a mobile phase, a mixture of MeOH and water (80:20, v/v) was used at a flow rate of 0.5 $cm^3\,min^{-1}$. The eluent was monitored between 230 and 270 nm.

Evaluation of Interaction of Modified Silica Gels and Samples. The difference of retention time of a sample ($\Delta RT = RT_{Samp} - RT_{Ref}$) between the modified silica gel (RT_{Samp}) and unmodified silica gel (RT_{Ref}) columns was used for evaluation of the interaction between stationary phases and samples. The value of ΔRT could be proportional to an increment of interaction between a sample and metal porphyrins or metal phthalocyanines by modification of silica gels. Dead volume (t_0) was decided by the injection of MeOH.

Results and Discussion

Comparison of Metal Phthalocyanines. The retention data obtained using Dev-5 and M-PCS$_{D5}$ columns are summarized in Table 1. The retention times for the tested PAHs with the unmodified silica gel (Dev-5) column showed almost the same value. This result suggested that PAHs usually did not interact with the Dev-5 column. However, the M-PCS$_{D5}$ columns exhibited a more or less positive ΔRT value, suggesting that PAHs interacted with the M-PCS on the silica gel. In Table 1, it should be noted that the ΔRT values depend upon the central metal ions of M-PCS$_D$ and the spread of π-electron clouds of the samples.

The ΔRT value decreases in the order of Cu-, H$_2$-, Co-, Mn-, and Fe-PCS$_{D5}$. In particular, the largest extension of the retention time among tested PAHs was obtained for phenanthrene. Further, Cu-PCS$_{D5}$ seemed to have the strongest interaction with π-electron-rich compounds. We take into consideration that many coordinated complexes including a copper(II) ion frequently take a square planar conformation. Thus Cu-PCS on the silica gel also takes a square planar conformation, and therefore π-electron-rich PAHs easily come close to the π-electron clouds of Cu-PCS. Since, in the case of Co-, Mn-, and Fe-PCS$_{D5}$, the metal ions take axial ligand(s) to neutralize charges, the PAHs is not able to approach closely the π-electron clouds of M-PCS due to the steric hindrance of the axial ligand(s). In the case of H$_2$-PCS$_{D5}$, ΔRT values are not comparable with those with Cu-PCS$_{D5}$, although H$_2$-PCS is also expected to take a planar conformation. The ΔRT difference between H$_2$- and Cu-PCS$_{D5}$ may be caused from differences in distribution of π-electron clouds on the phthalocyanine ring, that is, π-electrons on H$_2$-PCS are localized while keeping a two hold axis (C_2 axis) perpendicular to the ring plane, in contrast with macroaromatic π-electrons of Cu-PCS, as seen in Fig. 3.

Retention times and ΔRT were increased in the order of benzene, naphthalene, anthracene, and phenanthrene. A sample with a more aromatic ring, i.e., a larger spread of π-electrons, had a tendency to have the longer retention time for all the modified silica gels, as seen in Table 1. This is because the wider π-electron clouds interact more strongly with wide clouds of M-PCS on the silica gel.

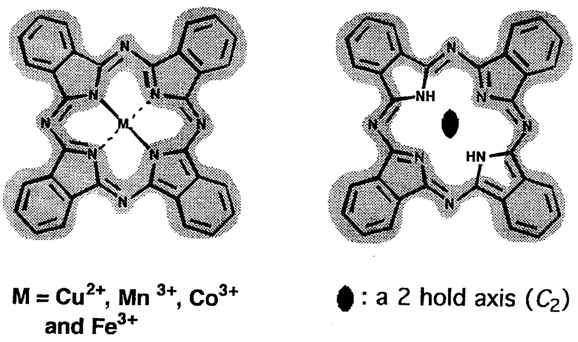


Fig. 3. Schematic feature of π-electron clouds of M-PCS and H$_2$-PCS.

To investigate the planar recognition ability of the M-PCS$_{D5}$, the separation for triphenylene and *o*-terphenyl was checked. As shown in Table 1, the order of the planar recognition ability of M-PCS$_{D5}$ coincides with the order of magnitude of ΔRT, since triphenylene has wide π-electron clouds, in contrast with *o*-terphenyl which has small clouds. The separation factor (α) (about 190) on Cu-PCS$_{D5}$ is much larger than the α values on the other M-PCS$_{D5}$. Thus, we concluded that Cu-PCS$_{D5}$ is a stationary phase exhibiting an excellent planar recognition ability for PAHs such as triphenylene.

As described above, Cu-PCS$_{D5}$ gave the highest ΔRT value for all PAHs among the M-PCS$_{D5}$ tested, as shown in Table 1, and copper(II) ion was the best central metal ion for the separation of the PAHs.

Comparison of Copper Porphyrins and Copper Phthalocyanine. As discussed above, copper(II) ion was superior to the other metal ions as the central metal ion of M-PCS. To check the effect of spread of π-electron clouds of the modified silica gel on the retention of PAHs, we investigated the retention of the PAHs on Cu-PP$_{D5}$ and Cu-TCPP$_{D5}$. These porphyrins include copper(II) ion as the central metal ion and have smaller π-electron clouds than Cu-PCS. The retention data obtained are summarized in Table 2. In the cases of both Cu-PP$_{D5}$ and Cu-TCPP$_{D5}$, the values of ΔRT for naphthalene, anthracene and phenanthrene were smaller than those with Cu-PCS$_{D5}$, suggesting that the π–π interaction between

Table 1. Retention Time (RT)$^{a)}$ and ΔRT of PAHs on M-PCS$_{D5}$ Columns$^{b)}$

Samples	Retention time (ΔRT)					
	Dev-5	Cu-PCS$_{D5}$$^{c)}$	Mn-PCS$_{D5}$$^{c)}$	Co-PCS$_{D5}$$^{c)}$	Fe-PCS$_{D5}$$^{c)}$	H$_2$-PCS$_{D5}$$^{c)}$
Benzene	3.2_0	3.3_4 (0.1_4)	3.2_6 (0.0_6)	3.2_0 (0.0_0)	3.2_3 (0.0_3)	3.2_2 (0.0_2)
Naphthalene	3.1_9	3.4_8 (0.2_9)	3.2_8 (0.0_8)	3.2_5 (0.0_5)	3.2_3 (0.0_4)	3.5_7 (0.1_8)
Anthracene	3.1_9	5.1_3 (1.9_4)	3.4_7 (0.5_6)	3.7_5 (0.5_6)	3.3_2 (0.1_3)	3.9_0 (0.7_1)
Phenanthrene	3.1_9	5.5_9 (2.4_0)	3.4_9 (0.3_0)	3.7_9 (0.6_0)	3.3_2 (0.1_3)	4.0_2 (0.8_3)
o-Terphenyl	3.1_5	3.1_8 (0.0_3)	3.1_5 (0.0_0)	3.2_3 (0.0_9)	3.1_5 (0.0_0)	3.2_5 (0.1_0)
Triphenylene	3.1_6	25.8_3 (22.6_9)	4.8_8 (1.7_2)	8.0_2 (4.8_6)	3.7_3 (0.5_7)	9.3_8 (6.2_3)
$\alpha^{d)}$	1	189	20	29	6	33

a) The Units of retention time and ΔRT are minutes. Parentheses indicate ΔRT. b) HPLC conditions: Mobile phase; MeOH–water (80 : 20, v/v), Flow rate; 0.5 cm^3 min^{-1}. c) M-PCS bound amount on silica gel: 6.5 μmol per 1 g silica gel. d) $\alpha = k'_{\text{Triphenylene}}/k'_{o-\text{Terphenyl}} = (RT_{\text{Triphenylene}} - t_0)/(RT_{o-\text{Terphenyl}} - t_0)$, where t_0 is 3.0_6 min by MeOH.

Table 2. ΔRT of PAHs on Cu-TCPP$_{D5}$ and Cu-PP$_{D5}$ Column[a)]

Stationary	ΔRT			
phases	Benzene	Naphthalene	Anthracene	Phenanthrene
Cu-TCPP$_{D5}$[b)]	-0.0_2	0.1_0	0.7_9	0.6_4
Cu-PP$_{D5}$[b)]	0.0_7	0.1_5	0.6_4	0.5_5

a) HPLC conditions: Mobile phase; MeOH–water (80 : 20, v/v) Flow rate; 0.5 cm^3 min^{-1}. b) Cu-porphyrins bound amount on silica gel: 6.5 μmol per 1 g silica gel.

PAHs and porphyrin ring was weaker than that between PAHs and Cu-PCS. Furthermore, it should be noted that the values of ΔRT for phenanthrene were smaller than those for anthracene, as seen in Table 2. The porphyrin ring has smaller π-electron clouds than those on the phthalocyanine ring, which results in sufficient π–π interaction with anthracene but not with phenanthrene, in contrast with the case of the phthalocyanine ring. On the other hand, as shown in Fig. 4, Cu-PP having only two caboxyl groups is perpendicularly bound to the surface of the silica gel, while Cu-TCPP clings in parallel with the surface through the four carboxyl groups. Thus, Cu-PP$_{D5}$ may exhibit different retention property with Cu-TCPP$_{D5}$ and Cu-PCS$_{D5}$.

In the present study, we selected a phthalocyanine ring as the macroaromatic ring having an appropriate spread of π-electron clouds for the separation of a wide variety of PAHs.

Effect of Type and Particle Size of Silica Gels on the Column Efficiency. To check the effect of the particle size of silica gel on the retention of PAHs, we tried to modify Dev-10, which differs in the particle size from Dev-5. As a result, the retention times and theoretical plate numbers (N) of all the PAHs were essentially the same between Cu-PCS$_{D5}$ and -PCS$_{D10}$ (see Table 3 and Fig. 5), and thus the particle size of silica gel may be concluded not to affect the retention times and N of PAHs.

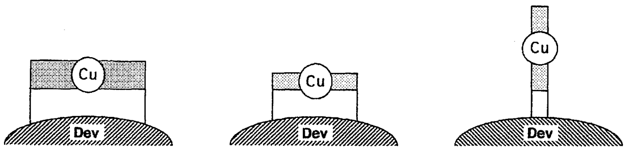


Fig. 4. Proposed scheme of Cu-PCS, Cu-TCPP, and Cu-PP bound to silica gels.

Table 3. ΔRT and Theoretical Plate Number (N) Values of PAHs on Cu-PCS$_{D5}$, Cu-PCS$_{D5}$, and Cu-PCS$_{L10}$ Column[a)]

Stationary	ΔRT (N)[b)]			
phases	Benzene	Naphthalene	Anthracene	Phenanthrene
Cu-PCS$_{D5}$[c)]	0.1_4 (3000)	0.2_9 (3400)	1.9_4 (3800)	2.4_0 (3600)
Cu-PCS$_{D10}$[c)]	0.1_4 (2900)	0.2_6 (3300)	1.9_4 (3600)	2.4_2 (3500)
Cu-PCS$_{L10}$[c)]	0.0_7 (2400)	0.1_6 (2900)	1.9_3 (2800)	2.4_7 (2700)

a) HPLC conditions: Mobile phase; MeOH–water (80 : 20, v/v), Flow rate; 0.5 cm^3 min^{-1}. b) Parentheses indicate theoretical plate number. Theoretical plate number $(N) = 5.45 \times (RT/W_{0.5})^2$, RT: Retention time of sample, $W_{0.5}$: Half height width. c) M-PCS bound amount on silica gel: 6.5 μmol per 1 g silica gel.

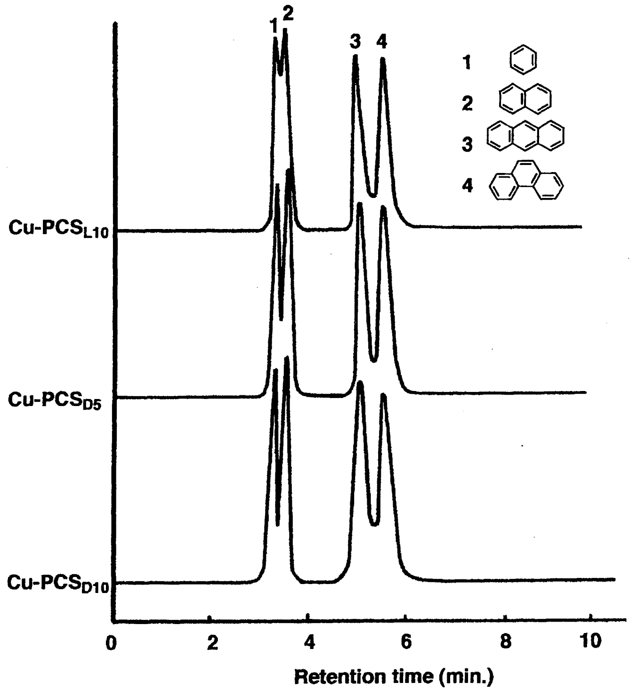


Fig. 5. Chromatograms of benzene, naphthalene, anthracene, and phenanthrene on Cu-PCS$_{D5}$, Cu-PCS$_{D10}$, and Cu-PCS$_{L10}$ columns. HPLC conditions: Mobile phase; MeOH–water (80:20, v/v), Flow rate; 0.5 cm^3 min^{-1}. M-PCS bound amount on silica gel: 6.5 μmol per 1 g silica gel.

Lic-10 is a irregular silica gel, while Dev-5 and Dev-10 are of a spherical type. Retention data obtained from Lic-10 modified with Cu-PCS (Cu-PCS$_{L10}$) and those from Cu-PCS$_{D10}$ were compared. The values of ΔRT with Cu-PCS$_{L10}$ for all the samples were not different from those with Cu-PCS$_{D10}$, as shown in Table 3. This result indicates that the shape and the particle size of silica gel do not seriously affect the π–π interaction as far as the retention data are concerned. However, Cu-PCS$_{D5}$ and Cu-PCS$_{D10}$ afford better separation for benzene and naphthalene than Cu-PCS$_{L10}$ as shown in Fig. 5, since N values with Cu-PCS$_{D10}$ and -PCS$_{D5}$ are slightly larger than those with Cu-PCS$_{L10}$ as shown in Table 3.

In the present paper, we decided to modify Dev-5 because of its large N values and its easy packing into a column.

Bound Amount of Cu-PCS on the Silica Gel. Figure 6 represents the relationship between ΔRT and the bound amount of Cu-PCS on a silica gel. For four PAHs, as the bound amount of Cu-PCS increased from 3.5 to 13.0 μmol g^{-1} (μmol of bound Cu-PCS per 1 g of Dev-5), the ΔRT increased linearly, although the slopes were different from one another with each sample, as seen in Fig. 6. This linearity to the bound amount suggested that only Cu-PCS on the silica gel interacts with the PAHs. These data indicated that the separation of PAHs could be improved by increasing the bound amount of Cu-PCS. However, the bound amount

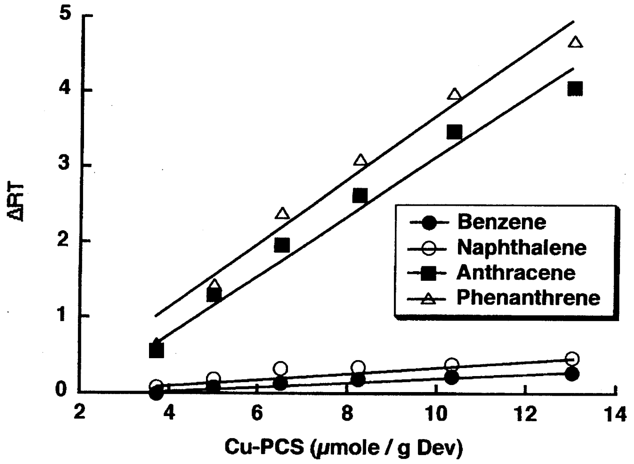


Fig. 6. Relationship between Δ*RT* and bound amount of Cu-PCS on silica gels. HPLC conditions: Mobile phase; MeOH–water (80:20, v/v), Flow rate; 0.5 cm^3 min^{-1}.

affected not only Δ*RT* but also *N*, and *N* took the maximum value at 6.5 of a silica gel for all the PAHs tested, as shown in Table 4. Thus, we decided to modify Dev-5 (1.0 g) with 6.5 μmol of Cu-PCS.

Effect of Eluent on the Retention of PAHs. MeOH–water (80 : 20, v/v) mobile phase offered the good separation for the PAHs as described above. Thus, we decided to use MeOH–water (80 : 20, v/v) as a mobile phase and did not extensively examine the type of organic modifier. With regard to the content of MeOH, MeOH–water (50 : 50, v/v) was not suitable for the PAHs because it took about 60 min for the elution of phenanthrene. In 100% MeOH, the π–π interaction was weakened and the separation for the PAHs was not enough in comparison with 80% MeOH.

Application. To examine the applicability of Cu-PCS$_{D5}$ to the other PAHs derivatives, we tried to separate 9-nitro-, 1-chloro-, and 2-amino-anthracenes from one another; the result is shown in Fig. 7. The π-electron density on the ring of each of these anthracenes is expected to be slightly different, since the substituent moieties of these anthracenes should exhibit different electron-attractive or -donating abilities. In fact, Cu-PCS$_{D5}$ recognized a slight difference of π-electron cloud among these anthracenes and gave a different retention time for each sample. The good separation of these anthracenes suggests that Cu-PCS$_{D5}$ is an excellent stationary phase, based on the π–π interaction with samples. Based on these results, Cu-PCS$_{D5}$ column may be applicable to separation of various PAHs, π-electron rich medicines and those derivatives.

Table 4. Relationship between Theoretical Plate Number (*N*) of PAHs and Bound Amount Cu-PCS on Dev-5[a)]

Cu-PCS	Theoretical plate number			
amount[b)]	Benzene	Naphthalene	Anthracene	Phenanthrene
3.5	2300	2300	2500	2600
5.2	3000	3000	2600	2700
6.5	3000	3400	3800	3600
8.3	2600	2400	2100	2100
10.3	2100	2000	1600	1700
13.0	1600	2500	2100	2300

a) HPLC conditions: Mobile phase; MeOH–water (80 : 20, v/v), Flow rate; 0.5 cm^3 min^{-1}. b) μmol of bound amount of Cu-PCS per 1.0 g of Dev-5 (μmol g^{-1}).

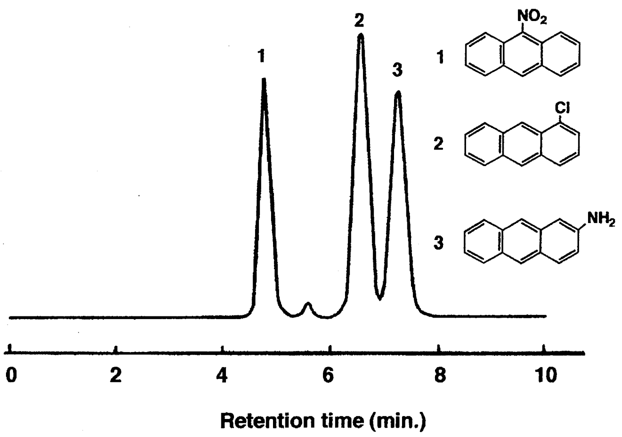


Fig. 7. Chromatogram of anthracene derivatives on Cu-PCS$_{D5}$ column. HPLC conditions: Mobile phase; MeOH–water (80:20, v/v), Flow rate; 0.5 cm^3 min^{-1}. M-PCS bound amount on silica gel: 6.5 μmol per 1 g silica gel.

A commercially available aminopropyl–silica gel can be converted by the modification with Cu-PCS into a functional silica gel exhibiting π–π interaction with PAHs. The silica gel modified with Cu-PCS is useful as an HPLC stationary phase for separation of π-electron rich compounds and is applicable not only to analytical purposes but also to a wide range of fields.

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