

HPLC Retention Behavior of Poly-Aromatic-Hydrocarbons on Aminopropyl Silica Gels Modified with Cu(II)- and Ni(II)-Phthalocyanine Derivatives in Non-polar Eluent

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A comparative study was conducted to elucidate the mechanism underlying the separation of poly-aromatic-hydrocarbons (PAHs) and related compounds thereof on a column packed with silica gels modified with Ni(II)- or Cu(II)-phthalocyanine derivatives (PCS) (Ni- or Cu-PCS_D column) and commercially available PYE[®] and NPE[®] columns with a non-polar eluent, such as *n*-hexane. It has been revealed that the dominant interaction responsible to the separation of PAHs on the Cu-PCS_D and the PYE[®] columns with *n*-hexane is the π - π interaction; however, in the separation of PAHs having 4 rings such as pyrene on the Ni-PCS_D column, participation of π - d interaction was indicated. The predominant role of π - π interaction in the separation of PAHs of less than three rings on the Ni-PCS_D column was demonstrated using anthracene. All the columns possessed planar recognition ability and were estimated to be potentially useful in the separation and the analysis of PAHs.

Key words phthalocyanine; Cu complex; Ni complex; HPLC; non-polar eluent; π - π interaction

High performance liquid chromatography (HPLC) has been widely used for separating and analyzing substances having π electron system such as environmental pollutants, mutagenic substances and pharmaceuticals.^{1–7} Researches have been focused on the development of stationary phases capable of making good use of the π - π interaction between π electrons of a sample and a ligand, immobilized on a support, having π electrons.^{8–12} There are commercially available columns of which separation mechanism is mainly based on the π - π interaction, such as PYE[®] column, wherein 2-(1-pyrenyl)ethyl group is immobilized on silica gels. It has been reported that such commercial columns are useful in the separation/analysis of poly-aromatic-hydrocarbons (PAHs) or the like.^{8,9} On the other hand, it has been reported that a silica-based M-PCS_D column, wherein metal phthalocyanine sulfonic acid derivative (M-PCS) is immobilized on silica gels, and of which separation mechanism is principally based on the π - π interaction in a polar solvent (e.g., methanol–water system), is useful for separations of PAHs.^{13–16} In the M-PCS_D, metal phthalocyanine is immobilized on silica gels in a horizontal manner, and the immobilization makes greater planar recognition ability than that of the PYE column.^{14,15} Generally, PAHs having many aromatic rings are slightly/poorly soluble in a polar solvent, and therefore, PAHs are usually extracted with a non-polar eluent before applying to HPLC. Thus, a comparative study has been carried out to elucidate the mechanism underlying the separation of PAHs with the M-PCS_D columns and other columns including the PYE[®] column in a non-polar eluent, such as *n*-hexane. It was revealed that the dominant interaction between the M-PCS_D column and PAHs is π - π interaction even in a non-polar solvent, as will be hereinafter described in detail.

Experimental

Reagents Ni-phthalocyanine (Ni-PC) and Cu-phthalocyanine (Cu-PC)

were purchased from Aldrich, U.S.A. Phthalocyanine (H₂-PC) was obtained from Tokyo Kasei, Japan. As a supports, Develosil NH₂-5[®] (hereinafter, referred to as “Dev”, Nomura Kagaku, Japan) which is a spherical aminopropyl silica gels (particle size, 5 μ m; specific surface area, 350 m²/g; average pore size, 120 Å) was used. As an eluent, *n*-hexane for HPLC (Nacalai Tesque, Japan) was mainly used, and as a solvent for packing M-PCS_D, slurry solvent A Conc. (Chemco Scientific, Japan) was used. The commercially available PYE[®] column and NPE[®] (nitro-phenylethyl silica gels) column (Nacalai Tesque, Japan) were used for comparison to elucidate the underlying separation mechanism of the M-PCS_D column.

M-PCS_D Columns Metal-phthalocyanine tetrasulfonyl chloride (M-PCSCl: M=H₂, Ni²⁺ and Cu²⁺, see Fig. 1) was synthesized from H₂-PC, Cu-PC and Ni-PC as described by Nadler *et al.*¹⁷ The resultant Ni-PCSCl (5.6 mg), Cu-PCSCl (5.6 mg) or H₂-PCSCl (5.3 mg) was each dissolved in dioxane (150 ml), following the addition of Dev (1.5 g), and was refluxed for 2 h. After standing to cool, M-PCS_D was separated by filtration, and washed with methanol (100 ml), dried thoroughly *in vacuo* over diphosphorous pentoxide. The M-PCS_D column was then prepared by packing the obtained M-PCS_D into a stainless-steel column (ϕ 4.6×150 mm) for HPLC by a conventional slurry packing method. Throughout the process, the filtrate and the like were clear and colorless, and the absorption spectra thereof were free from characteristic absorption band(s) of M-PCSCls or M-PCSs. From these evidences, it was concluded that M-PCS was completely immobilized on silica gels. The amount of M-PCS immobilized was also estimated from the absorption spectrum of the initial M-PCS solution in dioxane. M-PCS_D

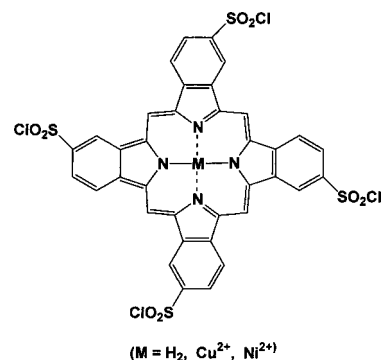


Fig. 1. Structure of M-PCSCl (M=H₂, Cu²⁺ and Ni²⁺)

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columns were remained unchanged during use for at least one year.

Apparatus The HPLC system was mainly constructed with a Shimadzu LC-6A pump, a Shimadzu SPD-6AV detector, a Shimadzu Chromatopac C-R6A recorder and a Rheodyne model 7125 sample injector (Rheodyne, U.S.A.). Typical HPLC conditions are as follows: column temperature; ambient, detection; 264 nm in principle, eluent; 100% *n*-hexane at a flow rate of 0.5 ml/min.

Samples Sample compounds include 1- to 4-membered polycyclic aromatic hydrocarbons (PAHs, Kanto Kagaku, Tokyo Kasei, Nacalai Tesque and Wako Pure Chemical Ind. Ltds., Japan), anthracene derivatives (Aldrich, Tokyo Kasei and Wako Pure Chemical Ind. Ltds.) and naphthalene derivatives (Tokyo Kasei). Heterocyclic PAHs, carbazole and norharman (Aldrich and Tokyo Kasei) were also used. Biphenyl, biphenylene, diphenylmethane, fluorene, *o*-terphenyl, triphenylene, 1,1'-binaphthyl and peryrene (Aldrich, Tokyo Kasei and Nacalai Tesque) were used for evaluating the planar recognition ability. Each sample was used after dissolving in *n*-hexane, which also served as an eluent. Sample solution(s) contained 25 or 250 $\mu\text{g/ml}$ benzene and/or 5–20 $\mu\text{g/ml}$ PAH(s) in *n*-hexane.

Results and Discussions

To obtain primitive information on the mechanism underlying the separation on M-PCS_D column in a non-polar eluent, HPLC was carried out using unsubstituted 1- to 4-membered aromatic compounds (benzene, naphthalene, anthracene, phenanthrene, pyrene and triphenylene) in *n*-hexane. Figure 2 shows the chromatograms obtained from the Cu- and the Ni-PCS_D columns; the chromatograms revealed that both columns are able to separate these PAHs in proportion to the number of aromatic rings, indicating that there is some interaction between M-PCS_D and PAHs even in the non-polar eluent, such as *n*-hexane. The interaction was then examined using Ni-PCS_D.

Effect of Amount of Immobilized M-PCS To elucidate whether immobilized M-PCS participates in the interaction between PAHs and M-PCS_D as described above, the effect of amount of M-PCS per Dev (1.0 g) on the retention factor (*k*) was examined. Specifically, the retention behaviors of Ni-PCS_D columns containing a different amount of immobilized Ni-PCS were compared using 1- to 4-membered PAHs. Figure 3 shows the relationships between the amounts of Ni-PCS immobilized and *k* value of each PAH. In all the PAHs, the *k* value increased as the amount immobilized increased, indicating that Ni-PCS plays a leading role in the interaction between Ni-PCS_D and PAHs in a non-polar eluent. Dev alone hardly retained PAHs and was unable to separate PAHs tested.

Effect of PAH in *n*-Hexane on *k* Values of Sample PAHs

It was estimated that π electrons of PAHs were mainly responsible for the interaction between simple PAHs, such as pyrene, and various stationary phases. To examine whether π electrons participate in the interaction between M-PCS_D and PAHs, HPLC was carried out using *n*-hexane containing benzene as an additive having π electrons and the *k* value of a sample PAH was obtained. If π electrons of the PAH added in the eluent is responsible for the interaction with M-PCS, the PAH should compete with a sample PAH(s), thereby affecting *k* value thereof. The examination was carried out using the Ni-PCS_D column, 9-nitroanthracene (9-NA) as a sample PAH, and *n*-hexane containing 1–70 w/v% of benzene (additional PAH) as an eluent. 9-NA, which is not "simple PAH", was used as the sample because an additional PAH (in this case, benzene) in the eluent may disturb the detection of sample PAH. Results are shown in Fig. 4. The *k* value of 9-NA decreased and the elution of 9-NA was accelerated as

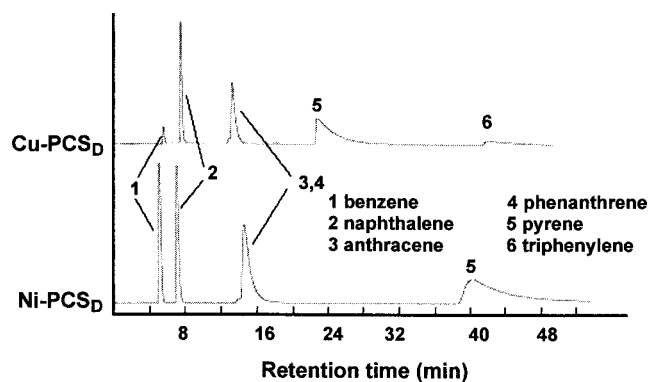


Fig. 2. Chromatograms of PAHs on the Cu- and Ni-PCS_D Columns⁽⁷⁾

1; benzene (25 $\mu\text{g/ml}$ in the above chart and 250 $\mu\text{g/ml}$ in the below chart), 2; naphthalene, 3; anthracene, 4; phenanthrene, 5; pyrene, 6; triphenylene. HPLC conditions: column temperature; ambient, detection; 264 nm in principle, eluent; 100% *n*-hexane at a flow rate of 0.5 ml/min. a) Anthracene and phenanthrene could be separated with each other in a polar eluent.⁽¹³⁾

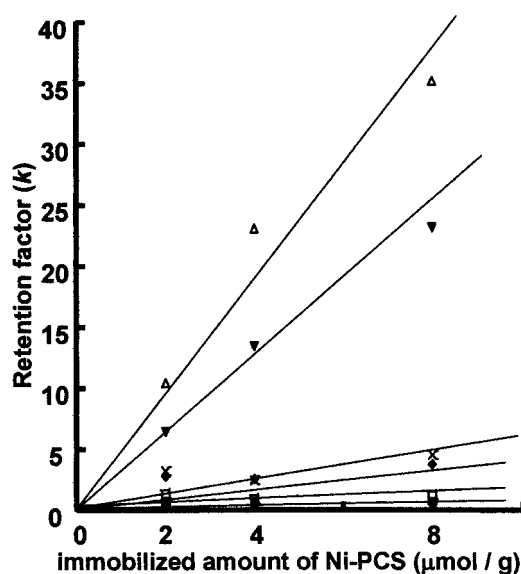


Fig. 3. Effect of the Amount of Ni-PCS Immobilized on Retention Factor (*k*)

●; benzene, □; naphthalene, ◆; anthracene, ×; phenanthrene, ▼; pyrene, Δ; triphenylene. HPLC conditions: column temperature; ambient, detection; 264 nm in principle, eluent; 100% *n*-hexane at a flow rate of 0.5 ml/min.

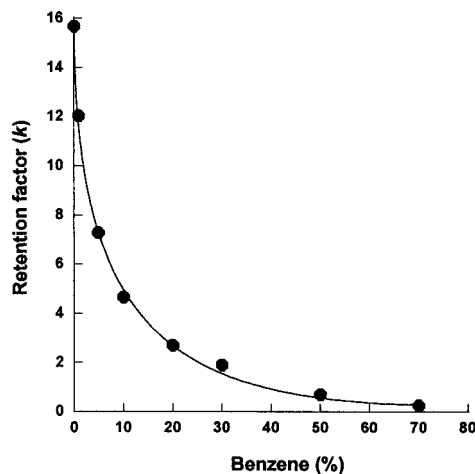


Fig. 4. *k* Values of 9-Nitroanthracene in *n*-Hexane Containing Benzene

HPLC conditions: column temperature; ambient, detection; 400 nm, eluent; *n*-hexane containing various v/v% benzene at a flow rate of 0.5 ml/min.

the concentration of benzene increased. The result suggested that by competitive interaction of benzene in the eluent and sample 9-NA, benzene disturbed the interaction between 9-NA and Ni-PCS_D. The competitive examination was further conducted in the same manner as described above using *n*-hexane containing naphthalene and anthracene which differ from each other in the ability to interact with Ni-PCS_D. The obtained *k* value of 9-NA dropped by about 25 and 30% in the presence of 0.03 mol/l of naphthalene and anthracene in *n*-hexane, respectively, compared to the presence of the same amount of benzene (data not shown). The indicated concentration (0.03 mol/l) corresponds to 2.4% in Fig. 4. It was revealed that the greater the interacting ability of additional PAH, the stronger the competitive interaction and the greater the drop of *k* value of 9-NA. Based on these results, we conceived that immobilized M-PCS and π electron of PAHs are both involved in the interaction between M-PCS_D and PAHs even in a non-polar eluent.

Retention Behavior of M-PCS_D Columns for PAHs in Non-polar Eluent (1) *Effect of Central Metal on M-PCS_D Column:* The interactions between PAHs and M-PCS_D in a non-polar solvent includes electrostatic interaction, π - π interaction, π -*d* interaction, and the like. Among them, the electrostatic interaction has been considered to be relatively dominant in a non-polar solvent. The metal positioned at the center of the metal porphyrin is generally expressed on paper as if it has a charge to facilitate understanding. This can be a trigger of the meaningless argument involving the cationic property of the central metal, as seen in the case of In-porphyrin derivative-immobilized column.¹⁰ Actually, in said compound, two lone electron pairs of N⁻ coordinate to the central metal; Cu-PCS or Ni-PCS, therefore, cannot have a bivalent cation. However, there may be some partial charge on the central metal. The retention behavior of the Cu- and the Ni-PCS_D columns for PAHs was compared with that of the H₂-PCS_D column which is thought to be almost free from partial charge. As shown in Fig. 5, there were no significant differences among the H₂-, the Cu- and the Ni-PCS_D columns when the ring number of PAHs is from 1 to 3, indicating that the central metal does not have a large partial charge, and that the electrostatic interaction involving π electrons is small.

In the cases of 4-membered PAHs such as pyrene and triphenylene, however, Cu-PCS_D gave slightly higher *k* value than H₂-PCS_D. It seemed to reflect the difference in the distribution of π electrons. One of reasons of this would be that H₂-PCS in a non-polar eluent takes the same form as that in an aqueous phase, *i.e.*, a tautomer having 2-fold-axis vertical to the ring, while Cu-PCS takes the resonance structure having 4-fold-axis, indicating that it is due to the difference in π electron distribution. On the other hand, the *k* value of pyrene or triphenylene on the Ni-PCS_D column was about 3.7-fold of that on the H₂-PCS_D column, suggesting the existence of another interaction, such as π -*d* interaction between Ni-PCS and pyrene, in addition to the π - π interaction. Further examination was conducted using columns which had been well investigated with regard to such an interaction.

(2) *Comparison of M-PCS_D Columns with PYE[®]- and NPE[®]-Columns:* The PYE[®] column is known to be a column of which main separation mechanism is based on the interaction involving π electrons, and the NPE[®] column is known to

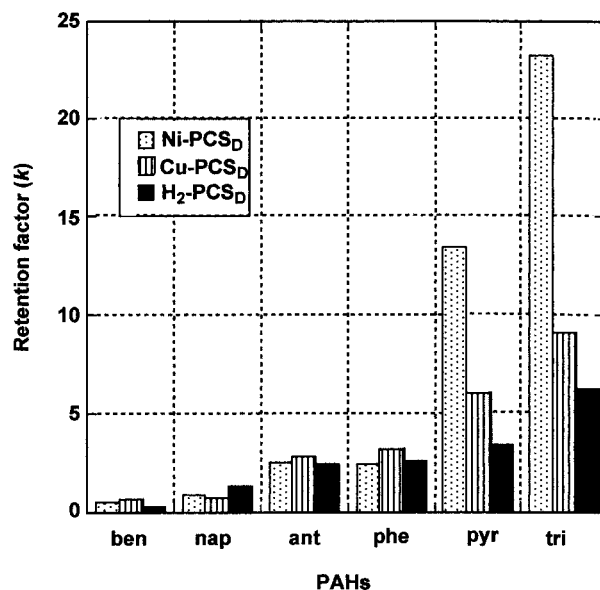


Fig. 5. *k* Values of PAHs on H₂-, Cu- and Ni-PCS Columns

ben; benzene, nap; naphthalene, ant; anthracene, phe; phenanthrene, pyr; pyrene, tri; triphenylene. HPLC conditions: column temperature; ambient, detection; 264 nm in principle, eluent; 100% *n*-hexane at a flow rate of 0.5 ml/min.

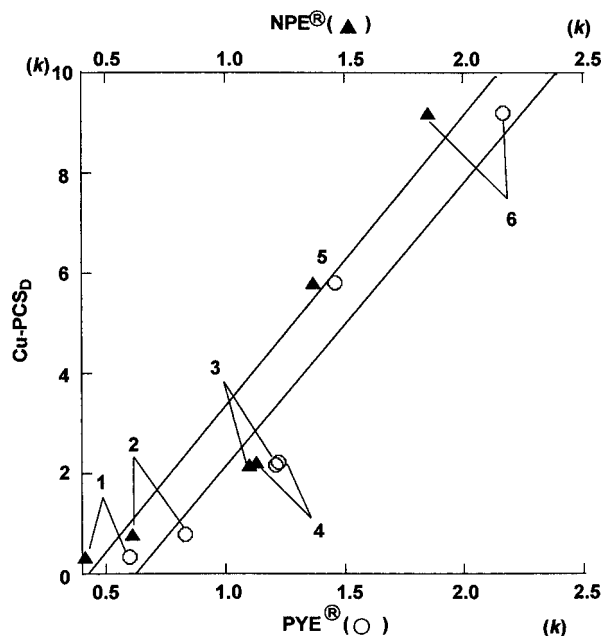


Fig. 6. Correlation of *k* Values on Cu-PCS_D, PYE[®] and NPE[®] Columns

1; benzene, 2; naphthalene, 3; anthracene, 4; phenanthrene, 5; pyrene, 6; triphenylene. HPLC conditions: column temperature; ambient, detection; 264 nm in principle, eluent; 100% *n*-hexane at a flow rate of 0.5 ml/min.

make use of interactions involving dipoles as well as π electrons. These columns were used for elucidation of separation mechanism of the Cu- and the Ni-PCS_D columns, and compared in regard to separation behavior for PAHs. First, the *k* values of PAHs on the Cu-PCS_D column were compared with those on the PYE[®] and NPE[®] columns. The correlation diagram regarding retention factor (*k*) for respective columns is given in Fig. 6. The PAHs having no dipole moment are separated only by virtue of π - π interaction involving π -electrons on both the PYE[®] and the NPE[®] columns. Accordingly, the correlation diagram is expected to show a linear correla-

tion, if underlying separation mechanism of the Cu-PCS_D column for PAHs is the π - π interaction, by analogy with the case of anion-exchange silica gels modified with Cu-phthalocyanine tetrasulfonate.¹⁸⁾ As shown in Fig. 6, the k values on the Cu-PCS_D column were almost in a linear correlation with those on both of the PYE[®] and the NPE[®] columns for respective PAHs. Accordingly, it was demonstrated that, in the separation on the Cu-PCS_D column, the electrostatic interaction is not involved and the π - π interaction plays a dominant role as long as simple PAHs are concerned.

In the case of the Ni-PCS_D column, as shown in the correlation diagram with the PYE[®] and the NPE[®] columns (Fig. 7), the k values of 1 to 3-membered PAHs were in a linear correlation with those obtained from the PYE[®] and the NPE[®] columns. The k values of pyrene and triphenylene, which have four rings, however, were particularly high in Ni-PCS_D, deviating greatly from the correlation. These results indicated the involvement of an interaction other than π - π interaction in the cases of pyrene and triphenylene. It is known that Cu²⁺ mainly takes the planar four-coordination geometry (a planar conformation), while Ni²⁺ also takes the six-coordination geometry (an octahedral conformation) as well as the planar four-coordination geometry. We, then, expected the contribution of a π - d interaction, which is the interaction between d orbital of Ni atom and π electrons of a sample. As shown in Fig. 8, which depicts the correlation diagram between the Ni- and the Cu-PCS_D columns, the k values for pyrene and triphenylene deviated from the correlation. The π - d interaction is not observed in the cases of small PAHs, such as anthracene. This would be because the π - π interaction generated between the pyrrole ring and the like hampers π electrons of such a small molecule from accessing the surface of Ni atom. In the case of PAHs, such as pyrene, which have broader π electron cloud than anthracene, when the molecules come close due to π - π interaction, however, the π electrons easily access to Ni atom and bring about π - d interaction. As the result, it was revealed that the interaction between M-PCS_D column and PAHs in a non-polar eluent is related to not only π electrons but also central metal and the like, and that M-PCS_D column may show different retention behavior for PAHs depending on the central metal.

(3) *Retention Behavior for Anthracene and Naphthalene Derivatives:* As mentioned above, the π electron interaction is the main interaction between M-PCS_D and naphthalene or anthracene even in a non-polar solvent. To elucidate the involvement of any interactions other than π electron interaction, anthracene and naphthalene derivatives having various substitutes were applied to four columns, and the k values were obtained. The correlation diagram of the k values is provided in Fig. 9. Contrary to the expectation, the k values of the derivatives having functional groups such as chloro-, methyl- or bromo- showed almost a linear correlation among all the columns. This indicated that, as far as the samples shown in Fig. 9 are concerned, the Ni- and the Cu-PCS_D columns, similarly to the PYE[®] and the NPE[®] columns, interact with a sample *via* π electron. Some derivatives having nitro- or amino-group or PAHs having heterocyclic ring, however, were more retained on the M-PCS_D columns and could not be eluted within 150 min (data not shown), indicating the possible existence of other interactions between M-PCS_D and nitro or amino group in a non-polar eluent.

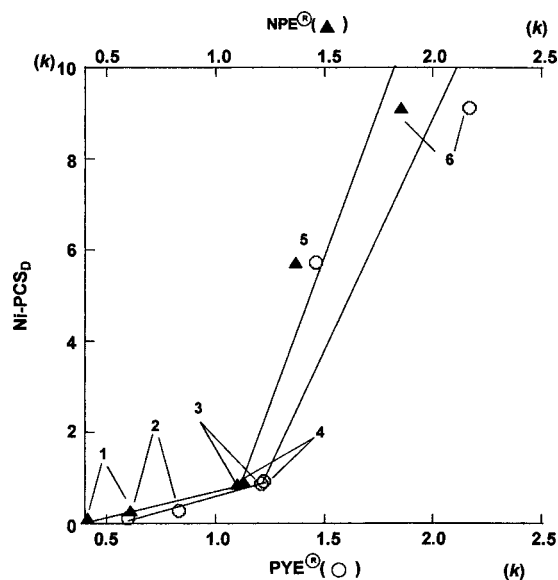


Fig. 7. Correlation of k Values on Ni-PCS_D, PYE[®] and NPE[®] Columns

1; benzene, 2; naphthalene, 3; anthracene, 4; phenanthrene, 5; pyrene, 6; triphenylene. HPLC conditions: column temperature; ambient, detection; 264 nm in principle, eluent; 100% *n*-hexane at a flow rate of 0.5 ml/min.

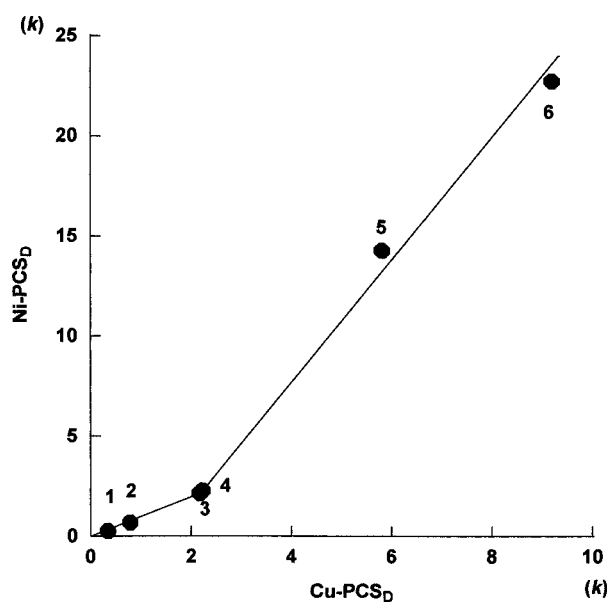


Fig. 8. Correlation of k Values on Cu- and Ni-PCS_D columns

1; benzene, 2; naphthalene, 3; anthracene, 4; phenanthrene, 5; pyrene, 6; triphenylene. HPLC conditions: column temperature; ambient, detection; 264 nm in principle, eluent; 100% *n*-hexane at a flow rate of 0.5 ml/min.

Planar Recognition Ability of M-PCS_D It has been reported that the M-PCS_D column exhibits an excellent plane recognition ability in a polar eluent.^{14,15)} It has also been known that many substances from environmental pollutants and mutagens are plane-type molecules having π electrons. The planar recognition ability of the M-PCS_D column was evaluated using, as a sample, pairs of substances having different planarity, A (biphenylene/biphenyl), B (fluorene/diphenylmethane) and C (triphenylene/*o*-terphenyl). Table 1 shows the structure of respective compounds, and the separation factors (α) of pairs of compounds on the Ni-PCS_D, the Cu-PCS_D, the PYE[®] and the NPE[®] columns, respectively.

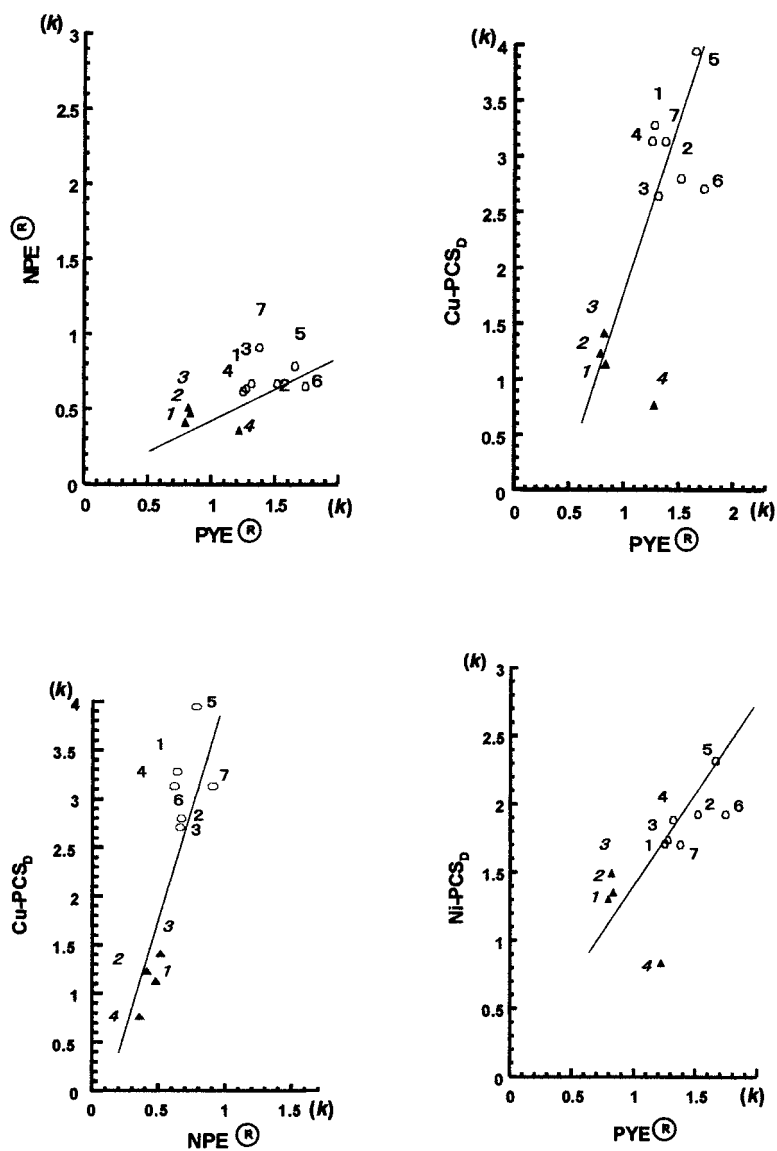
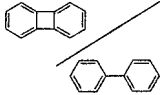
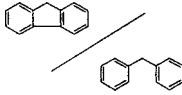
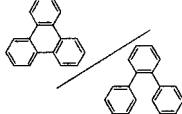


Fig. 9. Correlation of k Values of Naphthalene and Anthracene Derivatives

▲: naphthalene derivatives: 1; 1-CH₃, 2; 1-Cl, 3; 1-Br, 4; 1-COOH. ○: anthracene derivatives: 1; 1-CH₃, 2; 2-CH₂CH₂, 3; 9-C₆H₅, 4; 2-Cl, 5; 9-Br, 6; 9,10-Cl, 7; 1-COOH. HPLC conditions: column temperature; ambient, detection; 264 nm in principle, eluent; 100% *n*-hexane at a flow rate of 0.5 ml/min.

Table 1. Structures of Pairs and Separation Factor (α)

	Column	Ni-PCS _D	Cu-PCS _D	PYE [®]	NPE [®]
A		1.26	1.21	0.99	1.06
B		1.32	1.29	1.33	1.18
C		7.19	9.01	2.19	1.41

HPLC conditions: column temperature; ambient, detection; 264 nm, eluent; 100% *n*-hexane at a flow rate of 0.5 ml/min.

Each sample consisted of two compounds which are almost the same in regard to the molecular size but quite different in regard to planarity, as will be understood from the structure. As shown in Table 1, the separation factor (α) in the PYE[®] and the NPE[®] columns for the pair A consisting of compounds of small molecular size is too small for the practical application. On the contrary, the Cu- and the Ni-PCS_D columns have separation ability for all the samples tested as indicated by α values. That is, as for the pair A, α is about 1.2, which is larger than those obtained by the PYE[®] and the NPE[®] columns; as for the pair B, α is equivalent to that obtained with the PYE[®] column that is known to have an outstanding planar recognition ability among commercially available columns; and as for the pair C, α is as large as 3-fold of that obtained using the PYE[®] column. These facts show that the Cu- and the Ni-PCS_D columns have excellent ability to recognize the magnitude and stretch of π electron cloud of large PAHs even in a non-polar eluent with a remarkable planar recognition ability, although the ability is rather smaller than that in a polar solvent. Accordingly, these columns are potentially useful in the separation of environmental pollutants, mutagens, and the like, which comprises many planer molecules.

Conclusion

The retention behaviors of the Cu- and the Ni-PCS_D columns for PAHs were compared with those of PYE[®] and NPE[®] columns. The result revealed that the Cu- and Ni-PCS_D columns have an excellent ability to recognize the number of aromatic ring of PAHs even in a non-polar solvent such as *n*-hexane through π - π interaction. Furthermore, Ni-PCS_D column can separate PAHs by virtue of the π -*d* interaction in addition to the π - π interaction.

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