# Aminopropyl-Silica Gel Modified with Nickel(II)-Phthalocyanine for Separation of π-Electron Rich Compounds by High Performance Liquid Chromatography

Masaki Mifune,[a] Midori Onoda,[b] Tsutomu Takatsuki,[a] Takemine Kanai,[b] Akimasa Iwado,[b] Noriko Motohashi,[c] Jun Haginaka,[d] and Yutaka Saito*,[a]

*Faculty of Pharmaceutical Sciences, Okayama University,[a] Tsushima-Naka, Okayama 700–8530, Japan, The Graduate School of Natural Science and Technology, Okayama University,[b] Tsushima-Naka, Okayama 700–8530, Japan, and Kobe Pharmaceutical University,[c] Motoyamakita-machi, Higashinada-ku, Kobe 658–0003, Japan, and Faculty of Pharmaceutical Sciences, Mukogawa Women's University,[d] Koshien, Nishinomiya 663–8179, Japan.*
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Aminopropyl-silica gels modified with a nickel(II)–phthalocyanine derivative (Ni-PCS$_D$) were evaluated as HPLC-stationary phases for the separation of π-electron rich compounds such as polyaromatic hydrocarbons (PAHs) and mutagens. A column packed with Ni-PCS$_D$ retains some π-electron rich compounds longer than a column packed with copper(II)–phthalocyanine derivatives (Cu-PCS$_D$), suggesting that a π–π electron interaction and an additional interaction between Ni-PCS and the compound. On the basis of the retention data of four-ring PAHs, anthracene derivatives and heterocyclic aromatic hydrocarbons, we propose that the additional interaction would be due to an interference of the π- or n-electrons would interact and with the d-orbital of the Ni atom in Ni-PCS. Further, the Ni-PCS$_D$ column is expected to be superior to the Cu-PCS$_D$ column for the separations of drugs, mutagens and pollutants which have both π-electrons and ligand atom(s).

**Key words** Nickel(II)–phthalocyanine; π–π electron interaction; π–d electron interaction; HPLC

Previously, we demonstrated that commercially available aminopropyl-silica gel can be converted by means of a copper(II)-phthalocyanine derivative (Cu-PCS) into functional silica gel (Cu-PCS$_D$) capable of exhibiting π–π interactions with polyaromatic hydrocarbons (PAHs) and that Cu-PCS$_D$ would be useful as a HPLC stationary phase for the separation of π-electron rich compounds.[1—3] In addition, we show that, in comparison with pyrenylethyl-silica gel (PYE),[2] the characteristics of Cu-PCS$_D$ as a stationary phase is attributable only to the π–π interaction between samples and Cu-PCS$_D$.[3] In contrast to copper(II) ion in the planar Cu-PCS molecule, the nickel(II) ion has eight electrons in the 3d orbital and frequently undergoes coordination with ligands. Thus, a nickel complex takes a square-planar ($dsp^2$, low spin) and/or an octahedral ($sp^3d^2$, high spin) configuration. If a $dsp^2$ type complex of Ni-PCS can be fixed on silica gel, we should be able to develop with a new stationary phase, which is characterized by the interaction between π- or n-electrons and the d-orbital ($π(n)$–d interaction), in addition to the strong π–π interaction needed for the separation of π-electron rich compounds.

In the present paper, we report that silica gel modified with Ni-PCS derivative (Ni-PCS$_D$, Fig. 1) is a good stationary phase, based on the π–π and the $π(n)$–d interactions, for the separation of PAHs. We also report the practical usefulness of Ni-PCS$_D$ as the stationary phase for the separation of π-electron rich compounds containing nitrogen atom(s), which are of pharmaceutical interest.

### Experimental

**Reagents** Aminopropyl-silica gel, Develosil NH$_2$-5 (Dev, particle size: 5 μm) was purchased from Nomura Chemicals Co., Ltd. Nickel(II)–phthalocyanine (Ni-PC) was obtained from a commercial source (Kanto Kagaku Co., Ltd.). Methanol, used as mobile phase, was of special grade for HPLC. Dioxane of special grade was dried with molecular sieves for the preparation of the modified silica gel. π-Rich compounds that serve as samples (see Fig.

2), *i.e.*, benzene, naphthalene, anthracene, phenanthrene, o-terphenyl and triphenylene were of reagent grade (Tokyo Kasei or Wako Junyaku Co., Ltd.). Heterocyclic aromatic hydrocarbons, fluorene, carbazole and norharman, were purchased from Aldrich or from Tokyo Kasei Co., Ltd. Mutagens, Trp-P-1, Trp-P-2, Glu-P-1 and Glu-P-2, were purchased from Wako Junyaku Co., Ltd. Other reagents were of analytical grade or of reagent grade.

**Preparation of Nickel-Phthalocyanine Tetrasulfonylchloride (Ni-PCSCl)** Ni-PCSCl (see Fig. 1) was prepared in accordance with a literature[4] method. Ni-PC (0.5 g) was dissolved in 6 ml chlorosulfonic acid, and the solution was refluxed at 150 °C for 1.5 h with stirring. The reaction mixture was cooled to room temperature, and poured slowly over crushed ice with stirring. The resulted precipitation was filtered off, washed several times with 1.0 mol/l hydrochloric acid, and dried under vacuum to remove hydrochloric acid and obtain Ni-PCSCl. The formation of Ni-PCSCl was confirmed by treating the product obtained with methanol and measuring the FAB-mass spectrum of the resulting methyl-ester.

**Preparation of Ni-PCS$_D$ and Cu-PCS$_D$** For linking Ni-PCS to silica gel, a mixture of Ni-PCSCl (39 μmol) and dry aminopropyl-silica gel (6 g) in dry dioxane were refluxed until the mixture became clear. Thus, 6.5 μmol/g Ni-PCS bound to 1 g of silica gel. The resultant product was filtered, washed with water and then acetone, and dried under vacuum to obtain Ni-PCS$_D$.

Cu-PCS$_D$ (6.5 μmol Cu-PCS/g) as prepared in the same manner as described above and reported in our previous papers[1—3].

**Column Packed with the Modified Silica Gel** Ni-PCS$_D$ (1.5 g) was packed into a stainless steel column (4 mm × 150 mm) by a conventional slurry method (slurry solvent A conc, Chemco Co., Ltd.). As controls, 1.5 g Cu-PCS$_D$ was packed into stainless steel column (4 mm × 150 mm) in the
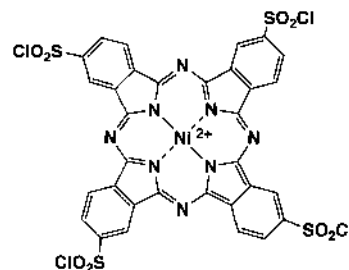


Fig. 1. Structures of Acid-Chlorides of Nickel(II)–Phthalocyanines

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## 1) Simple PAHs

**1** benzene **2** naphthalene **3** anthracene **4** phenanthrene

## 2) Four-ring PAHs

**1** benz[a]anthracene **2** chrysene **3** pyrene **4** triphenylene

## 3) Anthracene derivatives

8 9 1
7        2
6        3
5 10 4

group A    9-methyl-anthracene,
            2-ethyl-anthracene,
            9,10-dimethyl-anthracene

groupB     9-nitro-anthracene,
            1-amino-anthracene,
            2-amino-anthracene,
            1-chloro-anthracene,
            9,10-dichloro-anthracene,
            9,10-dibromo-anthracene

## 4) Heterocyclic aromatic hydrocarbons

fluorene    carbazole    norharman

Fig. 2.    Structures of Samples



Fig. 3.    Resonance Raman Spectra of an Aqueous Solution of Ni-TMPyP (A) and a Suspension of a Cation-Exchange Resin Modified with Ni-TMPyP in 50% Methanol (B)

④, Characteristic bands due to square-planer Ni-TMPyP; ⑥, characteristic bands due to octahedral Ni-TMPyP.
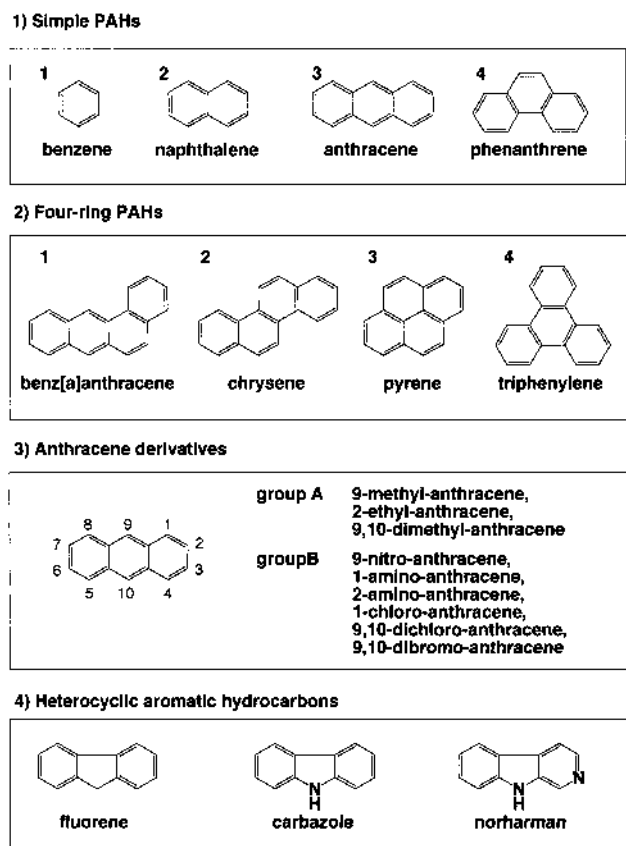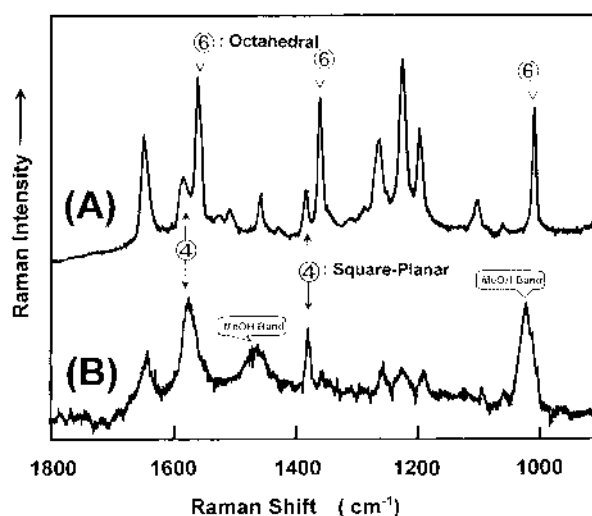
same manner. Thus, both the Ni- and Cu-PCS$_D$ columns were essentially similar except for the central metal ion.

**Sample Solution**   As sample solutions, $500\,\mu g/ml$ benzene, $10\,\mu g/ml$ naphthalene, $1\,\mu g/ml$ anthracene and $2.5\,\mu g/ml$ phenanthrene in 80% methanol were used because of the low sensitivity of the photodiode array detector. $o$-Terpheny and triphenylene ($2.5\,\mu g/ml$) were selected as a suitable pair for evaluating of the planarity recognition ability of the column. Other sample solutions were 1—3 $\mu g/ml$ in methanol.

**Apparatus**   The HPLC system was consisted of a Shimadzu LC-9A pump, a Rheodyne sample injector with a $20\,\mu l$ fix loop (model 7125) and a Shimadzu photodiode array detector SPD-M6A with a personal computer (Epson-286VS, data station). The column was allowed to stand at room temperature around 20 °C. The mobile phase, a mixture of methanol and water (9 : 1), was mainly used at a flow rate 0.5 ml/min for the Ni-PCS$_D$ column. The eluent was monitored between 230 and 270 nm.

Resonance Raman spectra (RRS) were recorded using a rotational cell on a JASCO NR-1000 laser Raman spectrophotometer equipped with NEC Ar$^+$ ion laser (4W).

## Results and Discussion

**Estimation of Ni-PCS Configuration**   To obtain information about the configuration of Ni-PCS on the silica gel, we investigated the RRS of nickel-porphyrin, nickel-tetrakis(methylpyridinium-4-yl)porphine (Ni-TMPyP), since square-planar and octahedral configuration of Ni-TMPyP were expected to be easily distinguishable by resonance Raman spectroscopy.[5] Ni-TMPyP in water is in equilibrium between a square-planar and a octahedral configurations, where no water and two water molecules, respectively, are coordinated to a nickel atom. However, when a water-miscible organic solvent such as acetone is added to the aqueous solution, the equilibrium is disrupted and only the square-planar Ni-TMPyP can exit in the mixture.[6] We then com-

pared with the RRS of aqueous solution of Ni-TMPyP and 30% acetone solution of Ni-TMPyP with a suspension of a solid cation-exchange resin modified with Ni-TMPyP in 50% methanol to obtain fundamental information about the nickel(II) ion on Ni–porphyrin fixed to supports. This showed that the square-planar Ni-TMPyP is much common than the octahedral Ni-TMPyP when fixed to the cation-exchange resin (Fig. 3). This result suggested that the coordination of the water molecule to the nickel ion in the porphyrin ring is disturbed by the support, since Ni-TMPyP clings parallel to the surface of the support through the four ionic bonds between the $N$-methylpyridyl and cation-exchange groups. With regard to the Ni-PCS$_D$, it was difficult to obtain detailed RRS measurements in our laboratory, since Ni-PCS lacks enough electron transitions to resonance around the emission lines of the Ar$^+$ ion-laser. Thus, by analogy with Ni-TMPyP, we assumed that Ni-PCS on silica gel adapts a square-planar configuration around the nickel(II) ion, since Ni-PCS clings parallel to the surface of the silica gel through four sulfonamide-bonds, similar to the above-mentioned case of Ni-TMPyP. This assumption was also supported by the strong $\pi-\pi$ interaction of Ni-PCS$_D$ with the $\pi$-rich PAHs as described below.

**Separation Characteristics of the Ni-PCS$_D$ Column**   In this study, the retention characteritics of the Ni-PCS$_D$ column were mainly compared with those of the Cu-PCS$_D$ column which had reported previously to exhibit the strongest $\pi-\pi$ interactions with PAHs among modified silica gels examined.[2] Also, silica gels were developed[7,8] by Kibbey and Meyerhoff after the publication of our previous communication.[1] These were modified In- and Sn-[5-($p$-carboxyphenyl)-10,15,20-triphenylporphine] (In- and Sn-CPTPPs), where In- and Sn-CPTPP are bound perpendicularly to the surface of silica gels as in the case of pyrenylethyl-silica gel (PYE)[9] and silica gel modified with Cu-protoporphyrin (Cu-PP$_D$)[2] columns. The authors reported that the immobilized In- and Sn-CPTPP interact with aromatic-sulfonates and carboxylates mainly through the anion-exchange properties of

Table 1. Retention Times (Rt, min), Retention Factor ($k'$) and Theoretical Plate Numbers ($N$) of Simple PAHs

| | Cu-PCS$_D$ | | | Ni-PCS$_D$ | | |
|---|---|---|---|---|---|---|
| | Rt | $k'$ | $N$ | Rt | $k'$ | $N$ |
| Benzene | 3.28 | 0.072 | 2285 | 3.18 | 0.000 | 2359 |
| Naphthalene | 3.45 | 0.127 | 1383 | 3.42 | 0.074 | 1904 |
| Anthracene | 5.40 | 0.765 | 1643 | 6.33 | 0.991 | 2266 |
| Phenanthrene | 5.97 | 0.951 | 1485 | 6.97 | 1.192 | 1839 |



Fig. 5. Chromatograms of Benz[a]anthracene (1), Chrysene (2), Pyrene (3) and Triphenylene (4) Using the Cu- and Ni-PCS$_D$ Columns

HPLC conditions: Mobile phase, 80% and 90% methanol; flow rate, 0.5 ml/min.

In addition, we should note that the theoretical plate numbers ($N$) for these PAHs increase by about 30% when the central metal ion of Cu-PCS was changed to Ni$^{2+}$. This improvement indicated that the Ni-PCS$_D$ column is superior to the Cu-PCS$_D$ column for the separation of simple PAHs.

**Separation of Four-Ring PAHs** Figure 5 shows the chromatograms for four-ring PAHs. Unexpectedly, these four PAHs are more strongly retained on the Ni-PCS$_D$ column under the same HPLC conditions than on the Cu-PCS$_D$ column as seen in Fig. 5. This strong retention of the PAHs on Ni-PCS$_D$ cannot be attributed solely to the $\pi$–$\pi$ interaction between the PAHs and Ni-PCS on silica gel, since the $\pi$-electron clouds of phthalocyanine ring are unlikely to increase very much simply by changing the central metal ion of Cu-PCS from copper(II) to nickel(II). Rather, the strong interaction indicated the existence of a $\pi$–$d$ interaction between the $d$ orbital of the nickel ion and the $\pi$-electron of the aromatic ring (so-called $\pi$-complexes) are well-known in coordination chemistry.[10]

When the polarity of the mobile phase for the Ni-PCS$_D$ was reduced from 80% methanol to 90% methanol, the retention times of the PAHs on Ni-PCS$_D$ approached those on Cu-PCS$_D$ as shown in Fig. 5. It should be noted that the peaks due to chrysene and pyrene could be separated from each other, when eluted with 90% methanol, although this was not completely satisfactory. Further, the retention behavior of pyrene on the Ni-PCS$_D$ column was very different from that on the Cu-PCS$_D$ column. Thus, the pyrene peak was close to the triphenylene peak on the Ni-PCS$_D$ column while it overlapped the chrysene peak on the Cu-PCS$_D$ column. If the retention of PAHs on Ni-PCS$_D$ is due merely to the $\pi$–$\pi$ interaction, the retention data should be similar to those on Cu-PCS$_D$, and the chrysene peak should also be close to the pyrene peak. The result shown in Fig. 5 suggested an additional interaction, such as the $\pi$–$d$ interaction, plays a part in the retention of PAHs on Ni-PCS$_D$. In addition, the $\pi$-electrons interacting with the $d$-orbitals of Ni-atom are restricted in their movement by $\pi$–$d$ interaction. Thus, the molecular shape recognition ability of the Ni-PCS$_D$ column may be different from that of the Cu-PCS$_D$ column, since the $\pi$–$d$ interaction may affect the recognition ability arising from the $\pi$–$\pi$ interaction.

**Separation of Anthracene and Its Substituted Derivatives** To investigate the interaction between the $n$ electron-
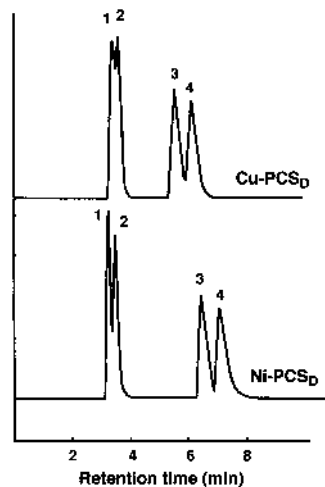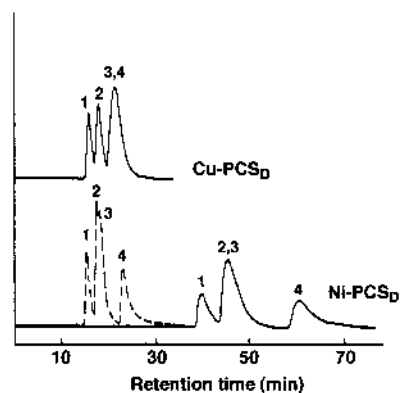


Fig. 4. Chromatograms of Benzene (1), Naphthalene (2), Anthracene (3) and Phenanthrene (4) Using the Cu- and Ni-PCS$_D$ Columns

HPLC conditions: Mobile phase, 80%methanol; flow rate, 0.5 ml/min.

the central metals in the porphyrin-core.[7] Since no anion-exchange poperties are expected for Ni-PCS having no extra positive-charge, we did not compare the retention characteristics of the Ni-PCS$_D$ column with those of In- and Sn-CPTPP columns in this study.

**Separation of Benzene and Simple PAHs** The retention data obtained using the Cu- and Ni-PCS$_D$ columns for benzene and three PAHs are summarized in Table 1 and Fig. 4. The retention times for the PAHs on the unmodified aminopropyl-silica gel column were essentially similar, showing that there is little interaction between the PAHs tested and the unmodified silica gel.[2] The Cu- and Ni-PCS$_D$ columns exhibit a positive value of retention factor ($k'$), indicating that the $\pi$–$\pi$ interaction favors the separation of PAHs. The increase in the retention times with increasing ring number suggested that the larger PAHs interacted more strongly with the Cu- and Ni-PCS on the silica gel. These retention data also indicated that anthracene and phenanthrene interact more strongly with Ni-PCS than with Cu-PCS. We previously reported that silica gel modified with metal-porphyrins, which have axial ligands and are in an octahedral configuration, interacted much more weakly with the PAHs than the Cu-PCS$_D$ did. This is because that the PAHs are not able to gain closely access to the $\pi$-electron clouds of octahedral Co$^{3+}$- and Fe$^{3+}$-PCS due to the steric hindrance of the axial ligands which coordinate with the metal ion to neutralize charges. The results shown in Table 1 suggested that the Ni-PCS on silica gel adopted in a square-planar configuration because no steric hindrance of the axial ligands was observed as in the case of the Cu-PCS$_D$ column.

Table 2.　Retention Times (Rt, min) and Separation Factors ($\alpha$) of Anthracene Derivatives

| | Cu-PCS$_D$ (80% Methanol)[a] | | Ni-PCS$_D$ (90% Methnol)[a] | |
|---|---|---|---|---|
| | Rt | $\alpha_{ant}$ | Rt | $\alpha_{ant}$ |
| Anthracene | 5.00 | 1.00 | 5.25 | 1.00 |
| Group A | | | | |
| 9-Methyl-anthracene | 7.59 | 2.35 | 7.72 | 2.18 |
| 2-Ethyl-anthracene | 6.17 | 1.62 | 6.53 | 1.62 |
| 9,10-Dimethyl-anthracene | 12.36 | 4.80 | 12.35 | 4.43 |
| Group B | | | | |
| 9-Nitro-anthracene | 4.88 | 0.96 | 5.43 | 1.17 |
| 1-Amino-anthracene | 5.91 | 1.47 | 6.95 | 1.82 |
| 2-Amino-anthracene | 6.96 | 2.01 | 7.47 | 2.07 |
| 1-Chloro-anthracene | 6.96 | 2.01 | 7.63 | 2.15 |
| 9,10-Dichloro-anthracene | 14.90 | 6.10 | 17.45 | 6.89 |
| 9,10-Dibromo-anthracene | 13.05 | 5.15 | 14.72 | 5.57 |

a) Parentheses indicate mobile phase (elutant).

Table 3.　Retention Times (Rt, min) and Separation Factors ($\alpha$) of Hetrocyclic Aromatic Hydrocarbons

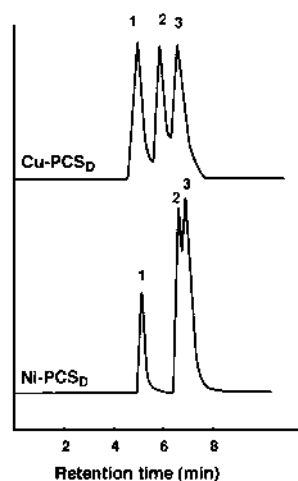| | Cu-PCS$_D$ (50% Methanol)[a] | | Ni-PCS$_D$ (57% Methnol)[a] | |
|---|---|---|---|---|
| | Rt | $\alpha_{het}$ | Rt | $\alpha_{het}$ |
| Fluorene | 8.52 | 1.00 | 8.40 | 1.00 |
| Carbazole | 9.48 | 1.17 | 9.99 | 1.30 |
| Norharman | 8.11 | 0.92 | 9.85 | 1.28 |

a) Parentheses indicate mobile phase (elutant).



Fig. 6.　Chromatograms of Anthracene (1), 1-Amino-anthracene (2) and 1-Chloro-anthracene (3) using the Cu-PCS$_D$ and Ni-PCS$_D$ Columns

HPLC conditions: Mobile phase, 80% and 90% methanol; flow rate, 0.5 ml/min.

pair(s) of a sample and the $d$ orbital of the nickel(II) ion, we evaluated the retention data of anthracene derivatives having the substitutent(s) lacking a ligand atom, such as a methyl group (group A), or containing ligand atom(s) such as amino, nitro or halogen groups (group B). Under the same HPLC conditions, the retention times of all the samples on the Ni-PCS$_D$ column are much longer than that on the Cu-PCS$_D$ column, similar to the aforementioned case of the four-ring PAHs. We, therefore, reduced the polarity of the mobile phase for the Ni-PCS$_D$ column so as to obtain similar retention time for anthracene on the Cu- and Ni-PCS$_D$ columns. The results are summarized in Table 2 and parts of chromatograms are shown in Fig. 6, where the peaks on the Ni-PCS$_D$ column are narrower than on the Cu-PCS$_D$ column.

The separation factors of group A, $\alpha_{ant}$, remain constant or decreased when the central metal ion is changed from copper(II) to nickel(II), suggesting that alkyl groups, such as methyl, confers no additional interaction but possibly changes only slightly the $\pi$-electron clouds of the anthracene ring. In contrast to the group A derivatives, $\alpha_{ant}$ values of the

group B derivatives, including one or more ligand atoms, ($n$-electron pair donor), are about 1.1 and 1.3-fold greater on the Ni-PCS$_D$ column than those on the Cu-PCS$_D$ column. Further, when the retention time of 9-nitroanthracene is compared with that of anthracene, it is shorter on the Cu-PCS$_D$ column ($\alpha_{ant} < 1$) but longer on the Ni-PCS$_D$ column ($\alpha_{ant} < 1$). These results indicated that an additional interaction involving the $n$-electron-pair occurs on changing the central metal ion from copper(II) to nickel(II).

**Separation of Heterocyclic Aromatic Hydrocarbons** To clarify the effect of the $n$-electron-pair-donor in heterocyclic ring(s) on the retention of heterocyclic, three molecules having a similar structure, fluorene, carbazole and northarman (see Fig. 2), were examined using both the Cu- and Ni-PCS$_D$ columns. As shown in Table 3, the retention times of norharman and carbazole swing back and forth, respectively, to that of fluorene in the case of the Cu-PCS$_D$ column, indicating that the the $\pi$-electron density of the whole molecule is under the influence of the nitrogen atom(s) in the ring(s). However, in the case of the Ni-PCS$_D$ column, the retention times of norharman and carbazole are longer than that of fluorene, despite the presence of nitrogen atom. This difference can be explained by the nitrogen atom of the pyrrole ring being able to coordinate with the nickel(II) ion in Ni-PCS$_D$. We should note that the $\alpha_{het}$ values of norharman and carbazole on the Ni-PCS$_D$ column are also greater than those in the Cu-PCS$_D$ column, as shown in Table 3.

**Planarity Recognition Ability** As discussed previously,[3] one of the beneficial features of Cu-PCS$_D$ is the good planarity recognition ability for PAHs. We, therefore, investigated the planarity recognition ability of the Ni-PCS$_D$ column using $o$-terphenyl and triphenylene pairs and comparing the result with that of the Cu-PCS$_D$ column. The Ni-PCS$_D$ column gave a much greater $\alpha_{plane}$, *i.e.*, $k'_{triphenylene}/k'_{o-terphenyl}$, value than the Cu-PCS$_D$ column does (see Fig. 7). This is because the $\pi$-electrons of the planar molecule could easily interact as a whole with the $\pi$-electron clouds and the $d$ orbital of Ni-PCS. However, Ni-PCS$_D$ retains the non-planar molecule as an extent similar to the Cu-PCS$_D$ column, on which the non-planar molecule hardly interacts with the $\pi$-electron clouds of Ni- or Cu-PCS, because only part of the molecule can participate in such interactions.

Retention on the Cu-PCS$_D$ column is governed only by the $\pi$–$\pi$ interaction as mentioned previously.[3] On Ni-PCS$_D$, the $\pi$–$d$ interaction can occur in addition to the $\pi$–$\pi$ interaction. Thus, the better planarity recognition ability of the Ni-PCS$_D$ column is due to effective $\pi$–$\pi$ and $\pi$–$d$ interactions.

**Applications** To evaluate the practical applications of the Ni-PCS$_D$ column, we studied the separation of mutagens,
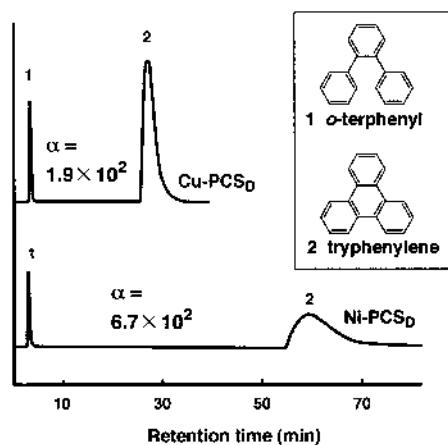
Fig. 7. Chromatograms of *o*-Terphenyl (1) and Triphenylene (2) Using the Cu-PCS$_D$ and Ni-PCS$_D$ Columns

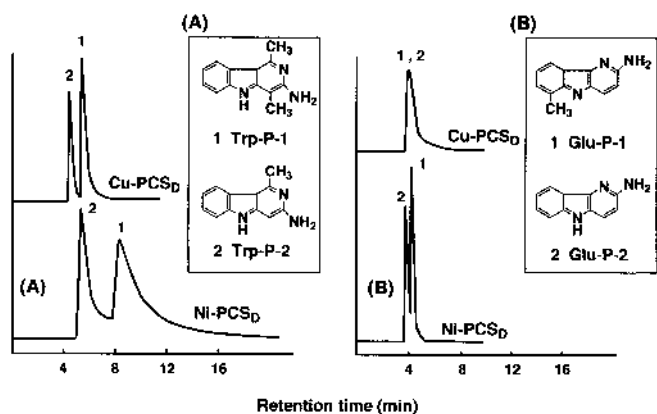HPLC conditions: Mobile phase, 80% methanol; flow rate, 0.5 ml/min.



Fig. 8. Chromatograms of Mutagens Using the Cu-PCS$_D$ and Ni-PCS$_D$ Columns

HPLC conditions: Mobile phase, 100% methanol; flow rate, 0.5 ml/min.

models of pharmaceutically interesting compounds, on Cu- and Ni-PCS$_D$ columns. The mutagens shown in Fig. 8 possess not only $\pi$-electrons but also *n*-electron-pair-donors (nitrogen atoms) in both the ring and substituents. As seen in Fig. 8A, Trp-P-1 and Trp-P-2 can be separated from each other by both the Cu- and Ni-PCS$_D$ columns. Although strong tailing of the peaks was observed on the Ni-PCS$_D$ column, the retention times are longer than those on the Cu-PCS$_D$ column, suggesting that the *n–d* interaction may be involved in retention on the Ni-PCS$_D$ column. As shown in Fig. 8B, Glu-P-1 and Glu-P-2, whose peaks overlap on the Cu-PCS$_D$ column, give two sharp peaks because of the *n–d* interaction, indicating that the interactions between the test compounds and Ni-PCS$_D$ play a significant role in the separation of these two mutagens. In addition, we should note that one of the advantages of these columns is that both Cu- and Ni-PCS$_D$ columns require only a mixture of water and methanol as the mobile phase.

In conclusion, nickel ion is superior to copper ion as the central metal ion of metal-phthalocyanine derivatives for the separation of some PAHs and mutagens. We expect that the Ni-PCS$_D$ column will prove useful for the separation of compounds such as drugs, mutagens and pollutants which have both $\pi$-electrons and ligand atom(s).

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