Separation Characteristics of Aminopropyl Silica Gels Modified with Copper-Phthalocyanine as High Performance Liquid Chromatography Stationary Phase

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Separation characteristics of aminopropyl silica gels modified with copper-phthalocyanine (Cu-PCSD) were investigated in comparison with those of a pyrene stationary phase (PYE). By the use of alkylbenzenes as samples, we have found that the π - π electron interaction is dominant in the separation mode of a column packed with Cu-PCSD, while the hydrophobic interaction with alkyl groups participates in the separation mode of a PYE column. The separation factor ($\alpha_{n'+1}$) of a polyaromatic hydrocarbon (PAH) having $n'+1$ aromatic rings by a PAH having n' aromatic rings is larger than that obtained from the PYE column. In particular, when n' is 3, $\alpha_{n'+1}$ is more than 6 times that of the PYE column in the maximum value. In addition, the planar recognition ability of the Cu-PCSD column is much better than that of the PYE column. The above results indicate that the separation characteristics of Cu-PCSD are mainly due to the π - π electron interaction based on Cu-PCS bound in parallel to the surface of the aminopropyl silica gel.

Keywords Separation characteristics, copper-phthalocyanine-based phase, modification, aminopropyl silica gel, π - π electron interaction, polyaromatic hydrocarbon

Previously, we have reported that aminopropyl silica gels modified with copper-phthalocyanine (Cu-PCSD) is effective as an HPLC-stationary phase for separation of π -electron rich polyaromatic hydrocarbons (PAHs).^{1,2} In the previous reports, we proposed that the separation is caused from the π - π electron interaction (π - π interaction) between PAHs and Cu-PCS on the silica gels. For wide applications of Cu-PCSD column, it is indispensable to elucidate the separation characteristics of Cu-PCSD. In the present paper, we tried to reveal the separation mode of Cu-PCSD in comparison with that of a column packed with a pyrene stationary phase (PYE column), which was reported to separate various PAHs based on the strong π - π interaction.^{3,4} Consequently, the π - π interaction is revealed to be more essential for the separation mode of the Cu-PCSD than that for the PYE column.

Experimental

Reagents

An aminopropyl-silica gel, Develosil NH₂-5 (Dev-5, particle size; 5 μ m), was purchased from Nomura Chemicals Co. Ltd. Copper-phthalocyanine (Cu-PC)

was obtained from Kanto Kagaku Co. Ltd. Methanol as mobile phase of special grade for HPLC was purchased from Ishizu Seiyaku Co. Ltd. Dioxane of special grade for HPLC was dried with molecular sieves and used for the preparations. Other reagents were of analytical grade or reagent grade.

Packed columns

The preparation of silica gels modified with a copper-phthalocyanine derivative (Cu-PCSD) was carried out by the method reported previously.¹ Briefly, in dry dioxane, Dev-5 (6 g) and copper(II)-phthalocyanine tetrasulfonyl chloride (Cu-PCSCl, 39 μ mol, see Fig. 1), which were prepared from Cu-PC and chlorosulfonic acid, were refluxed until the mixture become clear. Cu-PCSD (6.5 μ mol Cu-PCS/g Dev-5) was filtered off, washed with water and dry acetone in sequence, and dried under vacuum.

The modified silica gels (Cu-PCSD) and Dev-5 were packed into stainless-steel columns (4 mm $\times$ 150 mm) by the slurry packing method (slurry solvent A conc, Chemco Scientific Co. Ltd.). As a PYE column, COSMOSIL 5PYE column (particle size; 5 μ m, 4 mm $\times$ 150 mm), purchased from Nacalai Tesque Co. Ltd., was used.

Fig. 1 Structure of copper(II)-phthalocyanine tetrasulfonyl chloride (Cu-PCSCl).

Apparatus

The HPLC system was constructed with a Shimadzu LC-9A pump, a Model 7125 Rheodyne sample injector with 20 μ l fixed sample loop and a Shimadzu photodiode-array detector SPD-M6A with an Epson-286VS personal computer (Seiko-Epson) as a data station. The columns were allowed to stand at room temperature around 20°C.

As a mobile phase, a mixture of methanol and water (4:1 by volume) was mainly used at a flow rate of 0.5 ml/min, since this component was suitable for estimation of the π - π interaction. The eluent was monitored between 230 and 270 nm.

Samples

Samples used in this study are classified into the three groups (groups A, B and C) shown in Fig. 2. They were all of reagent grade and were purchased from Tokyo Kasei or from Aldrich Co. Ltd. As sample solutions, their methanol/water (80:20 v/v%) solutions (5 – 500 μ g/ml) were used.

Results and Discussion

In the case of the Cu-PCS_D column, Cu-PCS would be expected to be bound in parallel with the surface of the silica gel through the four sulfonyl-groups, as shown in Fig. 3. On the other hand, in the case of the PYE column, a pyrenylethyl group is bound perpendicularly to the surface of silica gel in contrast with the Cu-PCS_D column. The difference in the surface structure of π -rich aromatic rings, in particular the orienta-

Fig. 3 Scheme of surface structures of Cu-PCS_D and PYE.

Fig. 2 Structures of samples.

tion of π -electron clouds, would result in different separation modes of the Cu-PCS_D and the PYE columns. To investigate the difference, our interest was focused on three sample groups shown in Fig. 2 A - C. Alkylbenzenes with different carbon numbers (group A) gives information about the hydrophobic interaction.^{5,6} Furthermore, pairs of PAHs each having n' and $n'+1$ aromatic rings (group B) are expected to indicate the strength of the π - π interaction through separation factors ($\alpha_{n'+1}$), and pairs of planar and non-planar PAHs each having the same number of aromatic rings (group C) are expected to provide detailed knowledge about the planar recognition ability of the columns.^{7,8}

On the other hand, silica gels modified with In- and Sn-porphyrins (In- and Sn-CPTPPs), where In- and Sn-porphyrins are bound perpendicularly to the surface of silica gels similarly to the cases of PYE^{3,4} and Cu-PP_D columns², were reported^{9,10} after our previous communication.¹ The immobilized In- and Sn-porphyrins were reported to interact with aromatic-sulfonates and -carboxylates through anion-exchange properties of the central metals in the porphyrin-core.⁹ Since Cu-PCS on the silica gels has no extra charge, the anion-exchange property is not expected. Furthermore, aromatic sulfonates are retained strongly with aminopropyl silica gels under our HPLC conditions. Thus, no Coulomb interaction between Cu-PCS_D and aromatic sulfonates was examined in this paper.

Comparison of PYE and Cu-PCS_D columns

Figure 4 represents the chromatograms of PAHs on the PYE and the Cu-PCS_D columns. Six PAHs having one to four aromatic rings seem to be separated from one another on both columns. However, the retention characteristics, *i.e.*, the order of elution of PAHs, is different between the two columns, suggesting that they do not share the same separation mode. In addition, the longer retention of PAHs on the PYE column indicates that not only the π - π interaction but also the hydrophobic interaction may play an important role in the separation of PAHs on the PYE column. Then, we tried to clarify the separation characteristics of the Cu-PCS_D

Fig. 4 Chromatograms of some PAHs on Cu-PCS_D and PYE columns: 1, benzene; 2, naphthalene; 3, anthracene; 4, phenanthrene; 5, chrysene; 6, pyrene. HPLC conditions: mobile phase, methanol/water (80/20, v/v); flow rate, 0.5 ml/min.

column in more detail.

Separation characteristics of Cu-PCS_D column

Alkylbenzenes in Fig. 2-A are expected to exhibit the interactions due to both aromatic and alkyl groups under suitable conditions. In the case of the interaction due to an alkyl group(s), it is well known that the longer alkyl chain gives the larger retentivity.^{5,6} If Cu-PCS_D has an interaction concerned with alkyl group in addition to the π - π interaction, the capacity factors of alkylbenzenes become longer as the number of alkyl chains increases. Thus, we examined the relationship between the capacity factor and the number of alkyl chains (n). Consequently, as shown in Fig. 5, the logarithms of the retention volumes ($\log k'$) tend to decrease linearly with an increase of n from 0 to 9. This result indicates that Cu-PCS_D does not interact with an alkyl group through a hydrophobic interaction such as the alkyl- π interaction. The slight decrease of $\log k'$ can be attributed to the weak interference of alkyl chain to the π - π interaction. Contrary to the case of Cu-PCS_D, $\log k'$ values obtained from the PYE column increase linearly with an increase of n , suggesting that the hydrophobic interaction contributes to the retention of alkylbenzenes. The hydrophobic interaction could be caused from the interaction with pyrenylethyl groups that are perpendicularly bound in a tangle on the surface of the silica gel.

As discussed above, in the case of Cu-PCS_D, only the π - π interaction is dominant for the retention of alkylbenzenes and no interaction concerned with alkyl groups was observed; that is, only the aromatic ring of alkylbenzene interacts with the π -electron clouds of Cu-PCS on Cu-PCS_D.

π - π interaction and number of ring of PAHs

The number of rings of PAHs, that is, the size of the

Fig. 5 Relationships between $\log k'$ and carbon number (n) of alkylbenzene on Cu-PCS_D and PYE columns. HPLC conditions: mobile phase, methanol/water (80/20, v/v); flow rate, 0.5 ml/min.

Fig. 6 Separation factors ($\alpha_{n'+1}$) on Cu-PCSD and PYE columns. HPLC conditions: mobile phase, methanol/water (80/20, v/v); flow rate, 0.5 ml/min.

π -clouds, could affect their retention data. We studied separation factors (k' of $n'+1$ ring compound/ k' of n' ring compound : $\alpha_{n'+1}$) using PAHs pairs each having n' and $n'+1$ rings for the Cu-PCSD and the PYE columns. All $\alpha_{n'+1}$ values obtained using the Cu-PCSD column are larger than those obtained using the PYE column, as seen in Fig. 6. In particular, the $\alpha_{n'+1}$ values, anthracene/naphthalene and pyrene/anthracene, are much larger than those of the PYE column. These results indicate that the Cu-PCSD column is more effective for the separation of three and four ring-PAH derivatives than the PYE column.

The difference between Cu-PCSD and PYE columns could be explained by the difference in the size of π -electron clouds. The π -electron clouds of three and four ring PAHs are of more suitable sizes to interact strongly with those of Cu-PCS than those of pyrene, which has smaller π -electron clouds than Cu-PCS does.

Planar recognition ability

As suggested previously¹, one of the advantageous features of Cu-PCSD is the good planar recognition ability for PAHs. Thus, the planar recognition ability of the Cu-PCSD column was investigated in detail by using the four pairs of planar and non-planar compounds shown in Fig. 2-C. The separation factor (k' of planar compound/ k' of non-planar compound: α_{plane}) of these pairs are summarized in Table 1. In the case of the Cu-PCSD column, the capacity factors of non-planar molecules increase a little with an increase in the number of aromatic rings, but those of planar molecules become much larger. Consequently, the Cu-PCSD column gives larger α_{plane} values than the PYE column in all cases (see Table 1). Particularly, we should note the case of biphenylene and diphenyl, in which the difference in the characteristics of Cu-PCSD and the PYE columns is typically observed. As shown in Fig. 7,

Table 1 The separation factors (α_{plane}) of planar PAH by non-planar PAHs

Column	Biphenylene/ diphenyl ^a	Fluorene/ diphenyl- methane ^b	Triphenylene/ o-terphenyl ^b	Peryrene/ binaphthyl ^c
PYE	0.8	1.4	2.7	3.6
Cu-PCSD	2.3	3.8	189	191

HPLC conditions: mobile phase, (a) 70% methanol, (b) 80% methanol, (c) 100% methanol; flow rate, 0.5 ml/min.

Fig. 7 Chromatograms of diphenyl (1) and biphenylene (2) on Cu-PCSD and PYE columns. HPLC conditions: mobile phase, methanol/water (70/30, v/v); flow rate, 0.5 ml/min.

more planar biphenylene is retained longer than non-planar diphenyl on the Cu-PCSD column, contrary to the case of the PYE column. Since the surface of Cu-PCSD could be covered flatly with π -electron clouds of Cu-PCS, a π -electron-rich planar molecule could easily interact as a whole therewith. However, since the pyrene ring on the PYE column is not parallel to but perpendicular to the surface of silica gel, as shown in Fig. 3, a planar molecule could interact incompletely with the π -clouds. Further, Cu-PCSD retains the non-planar molecule less compared to a planar molecule, since the non-planar molecule can interact partially with π -electron clouds of Cu-PCS through only one of plane rings of the molecule. Furthermore, in the case of PYE column, non-planar biphenylene interacts with pyrenylethyl group not only through the π - π interaction but also through hydrophobic interactions, and is more retained with the PYE column than the Cu-PCSD column. As mentioned above, the π - π interaction is dominant for the retention of Cu-PCSD, and only a little hydrophobic interaction on its surface covered with π -electron clouds is expected. Thus, the better planar recognition ability of the Cu-PCSD column compared to the PYE column is caused from the separation characteristics of Cu-PCSD, where the π - π interaction is dominant and the hydrophobic interaction makes almost no contribution.

In conclusion, the characteristic of Cu-PCSD as a stationary phase is based on the π - π interaction between

samples and π -electron clouds of Cu-PCS. This characteristic is thought to render all the advantageous features to the Cu-PCS_D column.

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