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Effect of octabromination of a tetrakis(4-carboxyphenyl)porphine derivative bound to silica gels on HPLC retention behaviors of polyaromatic hydrocarbons

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Abstract

The effect of bromination of Cu-porphyrin-derivative-immobilized silica gels (Cu-$TCPP_D$) was examined by comparing the retention behaviors of polyaromatic hydrocarbons (PAHs) on Cu-$TCPP_D$ and Cu-octabromotetrakis(4-carboxyphenyl)porphine-derivative-immobilized silica gels (Cu-$OBTCPP_D$) columns. It was revealed that bromination affects strongly the π–π electron interactions caused from hydration energy in a polar eluent (80% methanol) possibly as a result of a destruction of planar structure of porphine-ring by bromination. It was also revealed that bromination enhances π–d interactions as well as the π–π electron interactions in a broad sense (e.g., dispersion forces) in a non-polar eluent (*n*-hexane). However, the bromination did not exert much influence on electrostatic interactions caused from polarization of mono-halogenated benzenes.

Keywords: HPLC stationary phase; π–d Interaction; π–π Interaction; Bromination of porphyrin; Dispersion force; Polyaromatic hydrocarbons

1. Introduction

In the separation and/or analysis of mutagens and environmental pollutants, such as polyaromatic hydrocarbons (PAHs) characterized by the presence of π electrons, HPLC stationary phases which achieve separation through π electron interactions have proved to be useful [1–5]. Typical columns of this type are a PYE column containing a stationary phase having 2-(1-pyrenyl)-ethyldimethylsilyl group and analogues thereof [6]. Besides, columns on which macrocyclic compounds with a wide spread of π electron-cloud such as Cu-phthalocyanine derivatives [7,8] or In-protoporphyrin [9,10] have been developed and reported to be useful for the separation of PAHs and the like. The Cu-phthalocyanine-derivative-immobilized silica gels columns are known to exert different interactions depending on an eluent due to the presence of macrocyclic aromatic ring. That is, in a polar eluent, it exerts strong π–π electron interactions, and, in a non-polar eluent, interactions involving π electron wherein π electrons of PAHs coordinate to Cu atom, or the like [8,11]. These stationary phases are characterized by a large planar π electron-cloud structure. On the other hand, it has been known that octabromination of porphine-rings destroys the planar structure [12,13]. In the present research, we attempted to evaluate the effect of bromination of porphine-ring on the retention behavior of PAHs, etc., in comparative experiments using Cu-tetrakis(4-carboxyphenyl)porphine- and octabromo-analogue-immobilized silica gels ("Cu-$TCPP_D$" and "Cu-$OBTCPP_D$") columns.

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2. Experimental

2.1. Chemicals and reagents

Tetrakis(4-carboxyphenyl)porphine (H_2-TCPP) was obtained from Tokyo Kasei Co. Ltd., Japan. As a support, Develosil NH_2-5, which is spherical aminopropyl-silica gel (particle size, 5 μm; specific surface area, 250 m^2/g; average pore size, 12 nm) was purchased from Nomura Kagaku, Japan. As eluents, purified water by Labo IonPure-12 (Millipore, USA), methanol and *n*-hexane of an HPLC-grade (Nacalai Tesque, Kyoto, Japan) were mainly used. As a solvent for packing Cu-$TCPP_D$ and Cu-$OBTCPP_D$, slurry solvent A Conc. (Chemco Scientific, Japan) was used. The commercially available 2-(1-pyrenyl)ethyldimetylsilyl silica gels (PYE) column was purchased from Nacalai Tesque. Other reagents were of analytical or reagent grade.

2.2. Preparation of Cu-$TCPP_D$ and -$OBTCPP_D$ columns

Cu-TCPP and Cu-OBTCPP (see Fig. 1) was synthesized according to the method described in literature [14–16]. Acid chlorides of Cu-TCPP and Cu-OBTCPP (Cu-TCPPCl and Cu-OBTCPPCl, see Fig. 1) was synthesized from dry Cu-TCPP and Cu-OBTCPP as described by Iwado et al. [17]. To prepare the Cu-porphyrin-immobilized silica gels, Cu-TCPPCl or Cu-OBTCPPCl (ca. 4 mg) was dissolved in dry dioxane (10 ml). After adding Develosil NH_2-5 (ca. 1.5 g), the mixture was refluxed for 2 h and allowed to cool. Cu-$OBTCPP_D$ or Cu-$OBTCPP_D$ (15 μmol Cu-porphyrin/g Dev) was filtered off and washed with methanol (100 ml), dried thoroughly in vacuo over P_4O_{10}. The Cu-$TCPP_D$ and Cu-$OBTCPP_D$ columns were prepared by packing the resulting silica gels into a stainless steel column (4.6 mm i.d. × 150 mm) by a conventional slurry packing method. The amount (15 μmol/g) of Cu-TCPPCl and Cu-OBTCPPCl immobilized was estimated from the absorption spectrum of the initial Cu-TCPPCl and Cu-OBTCPPCl solution in dry dioxane.

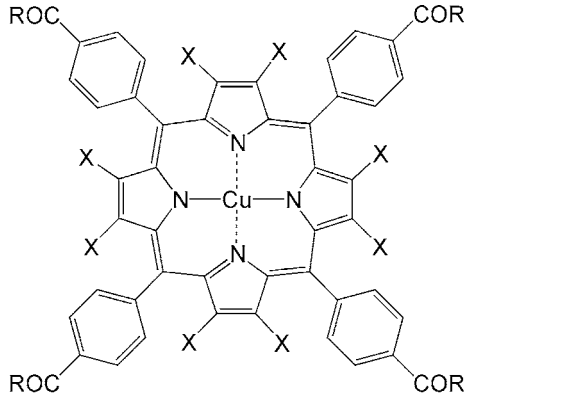

Fig. 1. Structures of Cu-porphyrins.

2.3. Apparatus

The HPLC system consisted of a Shimadzu LC-10AT pump, a Shimadzu SPD-10A detector, a Shimadzu Chromatopac C-R6A recorder (Shimadzu Co., Japan) and a Rheodyne model 7161 sample injector (Rheodyne, USA). Typical HPLC conditions are as follows: column temperature, ambient; detection, 264 nm in principle; an eluent, 50–90% methanol or 100% *n*-hexane at a flow rate of 0.5 ml/min.

2.4. Samples

Sample compounds include one to four-membered PAHs shown in Fig. 2 (Kanto Kagaku, Tokyo Kasei, Nacalai Tesque and Wako Junyaku, Japan), fluorobenzene, chlorobenzene, iodobenzene (Nacalai Tesque), bromobenzene, benzonitrile (Ishidzu Pharm. Co. Ltd., Japan) and nitrobenzene (Katayama Chem. Ind., Japan). Sample solutions were 250 μg/ml benzene and its derivatives, and 5–20 μg/ml PAHs solutions in 80% methanol or 100% *n*-hexane.

3. Results and discussion

In both of the Cu-$TCPP_D$ and Cu-$OBTCPP_D$, Cu-porphyrins are supposed to be bound in parallel with the surface of the silica gel through the four amide bonds. In such a situation, a sample having π electrons is expected to show interactions involving π electrons with Cu-porphyrins having a wide spread of π electron. Therefore, bromination that destroys the planarity of Cu-TCPP may affect the interactions with a sample involving π electrons. From this viewpoint, comparative experiments were conducted to elucidate the effect of bromination on the retention behaviors of PAHs. Specifically, the retention behaviors of PAHs, etc., on the Cu-$OBTCPP_D$ and Cu-$TCPP_D$ columns were compared and the change of interactions was elucidated.

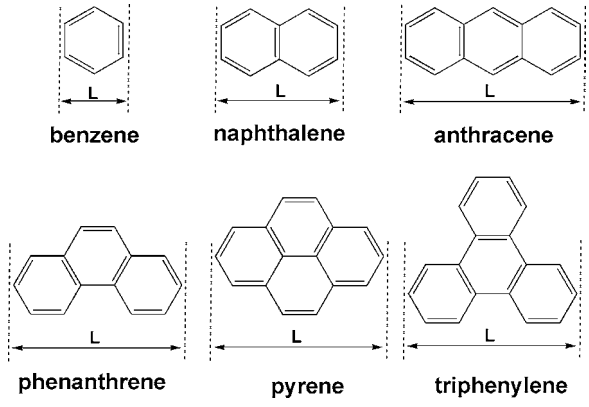

Fig. 2. Structures of polyaromatic hydrocarbons (PAHs). L: the longest molecular length, see Section 3.1.

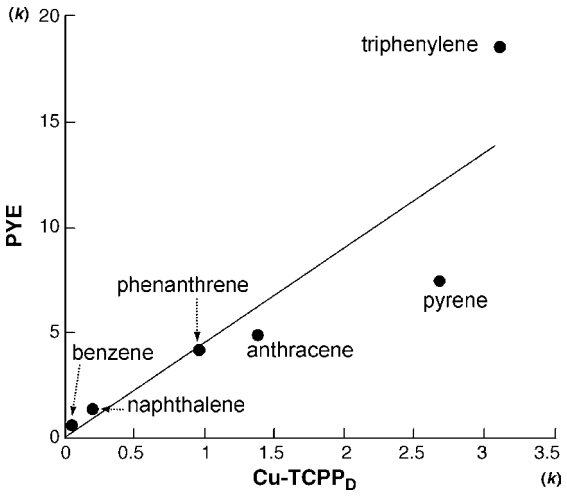

Fig. 3. Correlation of retention factor (k) of PAHs on PYE and Cu-TCPP$_D$ columns in 80%MeOH.

3.1. π–π Electron interaction in polar eluent

Firstly, the interaction(s) through the Cu-TCPP$_D$ column functions was evaluated by comparing the retention factors of PAHs with those on the PYE column in 80%methanol as a polar eluent. The Cu-TCPP$_D$ and PYE columns both exerted π–π electron interactions as shown in Fig. 3. As can be seen from Fig. 3, the both columns were almost in linear correlation regarding k values. This means that the Cu-TCPP$_D$ column, like the PYE column, retains PAHs through the π–π electron interaction based on hydration (solvation) energy in a polar eluent. The difference in k values between the Cu-TCPP$_D$ and PYE columns is attributable to the fact that the former contains less amount of immobilized Cu-TCPP.

We then obtained the mutual relation of k values of PAHs on the Cu-TCPP$_D$ and Cu-OBTCPP$_D$ columns. The results are provided in Fig. 4. Fig. 4 shows that the both columns are in linear correlation regarding benzene, naphthalene, phenanthrene and anthracene, and not triphenylene and pyrene. Fig. 4 also shows that the Cu-OBTCPP$_D$ column could not distinguish phenanthrene, triphenylene and pyrene from each other as indicated by dashed line parallel to the abscissa axis. As can be seen from Fig. 4, the k values of the three compounds are almost in parallel with the dashed line. These facts mean that, as we previously suggested [18], porphyrin ring of the Cu-OBTCPP$_D$ column, of which ring has been deprived of planarity by bromination, can recognize the PAHs through the interactions with a limited part of π electron spread as illustrated in Fig. 2. In other words, the Cu-OBTCPP$_D$ column is able to recognize the longest length of PAHs ("L", see Fig. 2), and not the broadening of PAHs. It was concluded that the destruction of planarity of porphine ring by bromination affects strongly the π–π electron interactions in a polar eluent.

3.2. Interactions involving π electron in non-polar eluent

To evaluate the retention behaviors of PAHs on a Cu-TCPP$_D$ column in a non-polar eluent, the mutual relation with a PYE column was obtained as described in Section 3.1. The results are provided in Fig. 5. As can be seen from Fig. 5, a good linear correlation was observed for k values of PAHs in n-hexane as a non-polar eluent between the Cu-TCPP$_D$ and PYE columns. This means that the Cu-TCPP$_D$ column, like PYE column, exerts the π–π electron interactions in a broad sense (including dispersion forces of π electrons). What is of interest is that, different from 80% methanol, the Cu-TCPP$_D$ column retains PAHs in n-hexane with k values almost equivalent to the PYE column, despite the less immobilized amounts.

We then elucidated the effect of bromination by comparing the retention properties of Cu-TCPP$_D$ and Cu-OBTCPP$_D$ columns on the basis of k values of simple PAHs. The results are provided in Fig. 6. Fig. 6 shows that the Cu-TCPP$_D$ and Cu-OBTCPP$_D$ columns are in good linear correlation regarding k values, which means that Cu-OBTCPP$_D$ column, like the Cu-TCPP$_D$ column, also exerts interactions involving π electrons. However, detailed comparison revealed that the Cu-OBTCPP$_D$ column gives much bigger k values than the Cu-TCPP$_D$ column, indicating that, the Cu-OBTCPP$_D$ column can

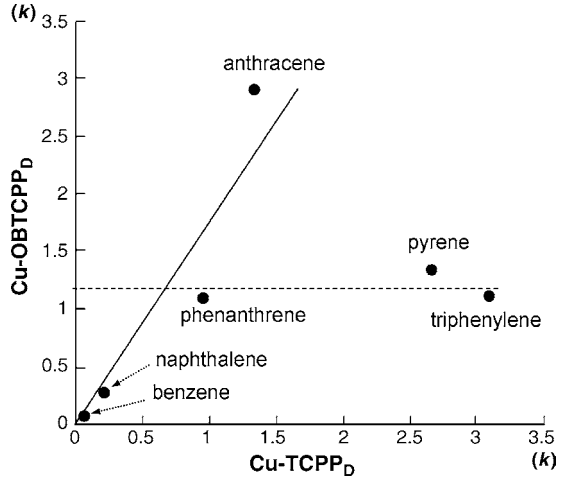

Fig. 4. Correlation of retention factor (k) of PAHs on Cu-OBTCPP$_D$ and Cu-TCPP$_D$ columns in 80%MeOH.

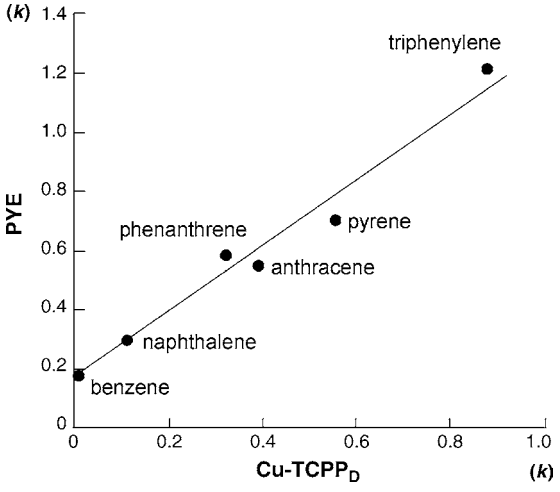

Fig. 5. Correlation of retention factor (k) of PAHs on PYE and Cu-TCPP$_D$ columns in n-hexane.

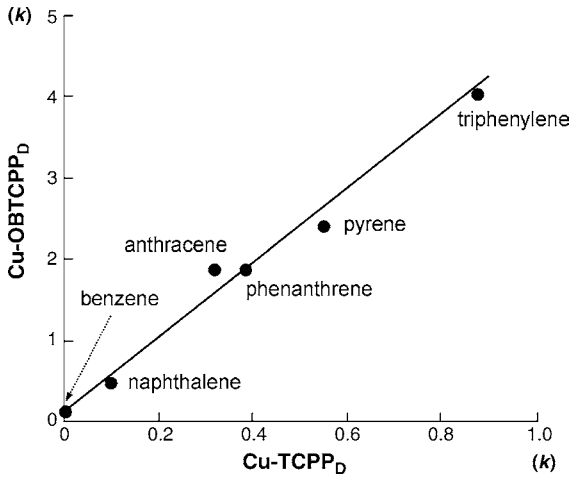

Fig. 6. Correlation of retention factor (k) of PAHs on Cu-OBTCPP$_D$ and Cu-TCPP$_D$ columns in n-hexane.

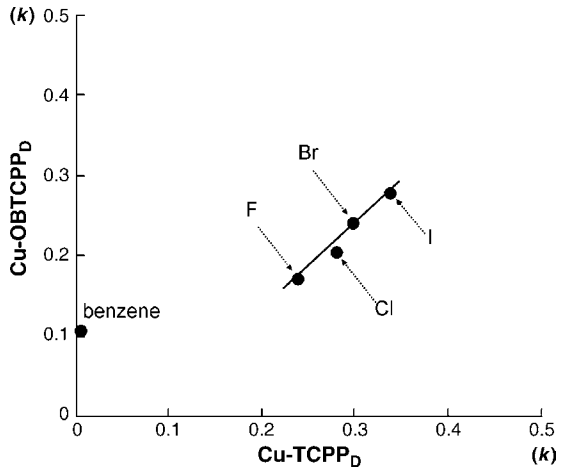

Fig. 7. Correlation of retention factor (k) of mono-halogenated benzenes on Cu-OBTCPP$_D$ and Cu-TCPP$_D$ columns in n-hexane.

exert stronger interactions involving π electrons than the Cu-TCPP$_D$ column that maintains a planar macrocyclic aromatic ring structure. Since Cu-TCPP has a broad planar structure while Cu-OBTCPP lacks planar structure of porphyrin ring as a result of bromination, at least the dispersion forces due to a sample and Cu-porphine of stationary phase must be reduced in the latter. Considering the above, it would be reasonable to attribute the increase of k value after bromination to the enhancement of the interaction between a copper atom and π electrons of a sample, specifically, the interaction based on π electron coordinates to Cu (π–d interaction). Such enhancement can be explained by that the destruction of planarity of porphine-ring facilitated π electrons of a sample to coordinate to copper. It was concluded that bromination of Cu-TCPP enhances the interactions involving π electrons in a non-polar eluent.

3.3. Electrostatic interactions

As previously reported in the literature [19], a pentabromobenzyloxy- propylsilyl silica gels (PBB) column having a brominated benzene ring recognizes fluoro-, chloro-, bromo- and iodo-benzenes in polar and non-polar eluents [19]. We examined k values of these samples on the Cu-TCPP$_D$ and Cu-OBTCPP$_D$ columns in 80% methanol. As shown in Table 1, the Cu-TCPP$_D$ column hardly recognizes fluoro-, chloro-, bromo- and iodo-benzenes in a polar eluent. Unexpectedly, the recognition ability was not improved even by bromination.

We then prepared a correlation chart from k values of benzene, fluoro-,chloro-, bromo- and iodo-benzenes on Cu-TCPP$_D$ and Cu-OBTCPP$_D$ columns in a non-polar eluent (Fig. 7). As can be seen from Fig. 7, the Cu-OBTCPP$_D$ and Cu-TCPP$_D$ columns showed linear correlation regarding halogenated benzenes. However, the k values are too small to assume that the π–π electron and electrostatic interactions are dominant in these cases. The results demonstrate that, as far as halogenated benzenes are concerned, the electrostatic interactions are almost unchanged by bromination. The Cu-TCPP$_D$ column seems to retain the samples slightly stronger than the Cu-OBTCPP$_D$ column in n-hexane, which may suggest the possibility of generation of repulsive force between a bromine atom of Cu-OBTCPP and a halogen atom of a sample.

The retention behaviors of nitrobenzene and benzonitrile having a dipole on the Cu-OBTCPP$_D$ and Cu-TCPP$_D$ columns were then compared. Table 2 shows k values of nitrobenzene and benzonitrile on Cu-OBTCPP$_D$ and Cu-TCPP$_D$ columns in respective eluents. As can be seen from Table 2, the Cu-OBTCPP$_D$ and Cu-TCPP$_D$ columns hardly retained these samples in a polar eluent. However, when a non-polar eluent was employed, retention for the two compounds on both stationary phase-types was rather strong. In addition, the k values of nitrobenzene and benzonitrile on the Cu-OBTCPP$_D$ column were rather bigger

Table 2
Correlation of retention factor (k) of nitrobenzene and benzonitrile on the Cu-TCPP$_D$ and Cu-OBTCPP$_D$ columns

	80% Methanol		n-Hexane	
	Nitrobenzene	Benzonitrile	Nitrobenzene	Benzonitrile
Cu-TCPP$_D$	0.03	0.11	2.99	6.16
Cu-OBTCPP$_D$	0.11	0.11	3.16	8.25

Table 1
Correlation of retention factor (k) of mono-halogenated benzenes on the Cu-TCPP$_D$ and Cu-OBTCPP$_D$ columns in 80% methanol

Column	Fluorobenzene	Chlorobenzene	Bromobenzene	Iodobenzene
Cu-TCPP$_D$	0.05	0.08	0.09	0.11
Cu-OBTCPP$_D$	0.08	0.10	0.11	0.15

than the Cu-$TCPP_D$ column regarding. These results indicate that bromination slightly enhanced the dipole–dipole interaction [20]. It is supposed that a pyrrole ring commonly present in both Cu-$TCPP_D$ and Cu-$OBTCPP_D$ columns participates in the dipole–dipole interaction.

4. Conclusion

It was revealed that octabromination of Cu-$TCPP_D$ leads to enhancement of interactions involving π electrons especially in a non-polar eluent. In particular, the π–d interaction was found to be dominant in non-polar eluent between Cu-OBTCPP and PAHs. Thus, the resultant Cu-$OBTCPP_D$ column would be a superior column capable of recognizing the spreading π electron-cloud of a sample in a non-polar eluent. The Cu-$OBTCPP_D$ column can be a promising column for separation and/or analysis of mutagens and medicines characterized by the presence of π electrons.

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