HPLC Retention Behaviors of π -Electron Rich Compounds on Ni^{2+} - and Cu^{2+} -Phthalocyanine Derivatives Bound to Silica Gels in Polar Eluents

Ikuko TSUKAMOTO,^{*1} Madoka SAITO,^{*1,*2} Minako YAMANE,^{*2} Kenji KAWATA,^{*2} Yuji KITAMURA,^{*2}
Youji KITAMURA,^{*3} Masaki MIFUNE,^{*3†} Yutaka SAITO,^{*3} and Jun HAGINAKA^{*4}

^{*1} Faculty of Medicine, Kagawa University, Ikenobe, Miki-cho, Kagawa 761-0793, Japan

^{*2} Department of Pharmaceutical Chemistry, Graduate School of Natural Science and Technology, Okayama University, Tsushima-Naka, Okayama 700-8530, Japan

^{*3} Department of Pharmaceutical Sciences, Graduate School of Medicine and Dentistry and Pharmaceutical Sciences, Okayama University, Tsushima-Naka, Okayama 700-8530, Japan

^{*4} Faculty of Pharmaceutical Sciences, Mukogawa Women's University, Koshien Kubancho, Nishinomiya 663-8179, Japan

The effect of the central metal of columns packed with silica gels binding Ni^{2+} - and Cu^{2+} -phthalocyanine derivatives (Ni- and Cu-PCS_Ds) on the retention behavior of poly-aromatic-hydrocarbons (PAHs) thereof in a polar eluent was examined. The retention factors of PAHs on the Ni- and Cu-PCS_Ds in 80% methanol showed a good linear correlation. The Cu-PCS_D column exhibited the π - π interactions for PAHs, while the Ni-PCS_D column exhibited the π - d interactions for PAHs in addition to the π - π interaction for PAHs. Further, an investigation of the retention behavior of anthracene derivatives having different substituents revealed that the Ni- and Cu-PCS_D columns could recognize the differences of substituents only in a polar eluent.

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Introduction

HPLC has been widely used for separations and analyses of substances having a π -electron system, such as environmental pollutants, mutagenic substances and pharmaceuticals.¹⁻⁶ Research has 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.⁷⁻¹¹ We have developed a silica-based Cu-PCS_D column wherein a copper phthalocyanine sulfonic acid derivative (Cu-PCS, see Fig. 1) is bound on silica gels and evaluated to be useful for separations of PAHs.¹²⁻¹⁴ A further investigation was conducted to elucidate the HPLC retention behavior of poly-aromatic-hydrocarbons (PAHs) on Cu- and Ni-PCS_D columns in a non-polar eluent, which revealed that the separation of PAHs is dominated by interactions involving a π electron.¹² In the present study, in order to elucidate the separation mechanism of PAHs on the Cu- and Ni-PCS_D columns, we examined the effects of the central-metal of Cu- and Ni-PCS_D on the retention behavior of certain compounds having π electrons in a polar eluent.

Experimental

Reagents

Ni^{2+} -phthalocyanine and Cu^{2+} -phthalocyanine were purchased

from Sigma-Aldrich Inc. (St. Louis, USA). As a support, Develosil NH₂-5[®] (hereinafter, referred to as "Dev", Nomura Kagaku, Japan), which is a spherical aminopropyl silica gels (particle size, 5 μm ; specific surface area, 350 m^2/g ; average pore size, 12 nm), was used. As eluents, methanol, *n*-hexane and chloroform of HPLC grade (Nacalai Tesque, Japan) were mainly used, and a slurry solvent for packing M-PCS_D, slurry solvent A Conc. (Chemco Scientific, Japan) was employed.

M-PCS_D columns

Ni- and Cu-PCS_Ds were prepared from M-PC (M = Ni^{2+} and Cu^{2+}) via M-PCSCl (see Fig. 1), as described in a previous paper.¹² The M-PCS_D column was then prepared by packing the obtained M-PCS_D into a stainless-steel column (4.6 mm i.d. $\times$ 150 mm) for HPLC by a conventional slurry packing method. Throughout the process, the filtrate and the like were clear and

Fig. 1 Structure of M-PCSCl (M = Ni^{2+} and Cu^{2+}).

[†] To whom correspondence should be addressed.
E-mail: mifune@pharm.okayama-u.ac.jp

Fig. 2 Correlation of k values of PAHs on Cu-PCSD and Ni-PCSD columns in 80% methanol. HPLC conditions: column temperature, ambient; detection, 264 nm in principle; eluent, 80% methanol at a flow rate of 0.5 ml/min.

Fig. 4 Correlation of k values of PAHs on the Cu-PCSD columns in 80% methanol and n -hexane. HPLC conditions: column temperature, ambient; detection, 264 nm in principle; a flow rate of 0.5 ml/min.

Fig. 3 Correlation of k values of PAHs on the Ni-PCSD columns in 80% methanol and n -hexane. HPLC conditions: column temperature, ambient; detection, 264 nm in principle; a flow rate of 0.5 ml/min.

colorless, and the absorption spectra thereof were free from the characteristic absorption band(s) of M-PCSCls or M-PCSSs. From this evidence, it was concluded that M-PCS was completely immobilized on silica gels. The amount of M-PCS immobilized was also estimated to be 4 $\mu\text{mol/g}$ from the absorption spectrum of the initial M-PCSCl solution in dioxane. The M-PCSD columns 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, USA). Typical HPLC conditions were as follows: column temperature, ambient; detection, 264 nm, in principle; eluent, 80% methanol at a flow rate of 0.5 ml/min.

Samples

Sample compounds include 1- to 4-membered PAHs (Kanto Kagaku, Tokyo Kasei, Nacalai Tesque and Wako Junyaku, Japan), and anthracene derivatives (Aldrich, Tokyo Kasei and Wako Junyaku, Japan).

Results and Discussion

Metal porphyrin analogs are members of macrocyclic compounds and have a wide-spread π electron cloud. As we previously reported, Ni- and Cu-PCSD columns show different HPLC retention properties in a non-polar eluent, such as n -hexane.¹² However, in a polar eluent, a π - π electron interaction related to the hydration energy (solvation energy) possibly occurs between the column and a simple PAH, which unlikely occurs in a non-polar eluent. To investigate the effect of the central-metal on the possible π - π electron interaction, we compared the retention factors (k) of simple PAHs on the Cu- and Ni-PCSD columns in 80% methanol. The results are shown in Fig. 2. As can be seen, the k values of PAHs on these columns become larger as the ring number increases. In contrast to the results in a non-polar eluent, the k values on these columns have a linear correlation. However, the k value on the Ni-PCSD column is about 2-fold larger than that on the Cu-PCSD column. Because Cu- and Ni-PCSDs have almost the same scale of electron clouds, the above difference indicates the presence of an additional interaction other than the π - π electron interaction between Ni-PCS and PAHs. In view of coordination chemistry, Cu^{2+} and Ni^{2+} differ in certain aspects. Although both metals can form six-coordinate complexes, they both formed four-coordinate bonds in the present Cu- and Ni-PCSDs. It is well known that Cu^{2+} generally forms four-coordinate bonds in a majority of complexes, and hardly forms six-coordinate bonds due to "Jahn-Teller effect".¹⁵ On the other hand, Ni^{2+} is known to readily form four or six-coordinate bonds.¹⁶ Accordingly, in the case of Ni-PCS, which contains Ni^{2+} and is readily coordinated, π electrons can easily coordinate to Ni^{2+} , which brings about a strong π - d interaction. Since the π - d interaction is of the electrostatic type, the said interaction is unlikely occur in a polar eluent in a direct manner. However, once the Ni-PCS and PAHs bind through the π - π interaction, a hydrophobic environment would appear between them and allow the π - d interaction to occur. If this hypothesis is correct, there must be a linear correlation between the k values in 80% methanol and those in n -hexane. We obtained the k values in these eluents and carefully examined them. As can be seen from Fig. 3, the k values of PAHs in 80% methanol (a polar eluent) and those in n -hexane (a non-polar eluent) had a linear correlation. This result supported the above hypothesis. Considering the fact that no linear correlation exists between Ni-PCSD and 2-(1-pyrenyl)ethyl-dimethylsilyl-silica gel (PYE)

Fig. 5 Correlation of k values of anthracene derivatives on the Cu-PCS_D columns in 80% methanol and *n*-hexane. 2-Cl, 2-chloro-anthracene; 2-Et, 2-ethylanthracene; 1-Me, 1-methylanthracene; 9-Br, 9-bromo-anthracene; 9,10-Cl, 9,10-dichloroanthracene. HPLC conditions: column temperature, ambient; detection, 264 nm in principle; a flow rate of 0.5 ml/min.

Fig. 6 Correlation of k values of anthracene derivatives on the Ni-PCS_D columns in 90% methanol and *n*-hexane. 2-Cl, 2-chloro-anthracene; 2-Et, 2-ethylanthracene; 9-Me, 9-methylanthracene; 9,10-Me, 9,10-dimethylanthracene; 9,10-Cl, 9,10-dichloroanthracene; 9,10-Br, 9,10-dibromoanthracene. HPLC conditions: column temperature, ambient; detection, 264 nm in principle; a flow rate of 0.5 ml/min.

columns in *n*-hexane,¹² Ni-PCS exerts a π - d interaction in 80% methanol as well as in *n*-hexane. The k values in a polar eluent are smaller than those in a non-polar eluent, demonstrating that the π - d interaction is weaker in a polar eluent. This means that the interaction between a ligand and a molecule having a π electron and taking a planar structure is unexpectedly stronger in *n*-hexane compared to 80% methanol. These results are of great interest and highly suggestive when we deal with the interactions of biological molecules, for example, "stacking and/or intercalation" in a hydrophobic environment.

On the other hand, the k values in 80% methanol and those in *n*-hexane were almost in linear correlation in the case of the Cu-PCS_D column, mainly based on the π - π electron interaction, as shown in Fig. 4. Considering that the k values on the Cu-PCS_D column showed a linear correlation with those on the PYE column lacking a central metal in *n*-hexane,¹² the above results indicated that the π - π electron interaction is dominant in 80% methanol.

As we previously reported,¹² anthracene derivatives having a

Table 1 k values of anthracene, 1-amino- and 2-nitro-anthracenes on Cu-, Ni-PCS_D and Develosil columns

Column	Eluate	Anthracene	2-Nitro-anthracene	1-Amino-anthracene
Cu-PCS _D	<i>n</i> -Hexane	2.8	14.3	> 50
	80% methanol	0.7	0.7	1.4
Ni-PCS _D	<i>n</i> -Hexane	2.6	13.7	> 50
	90% methanol	1.3	1.3	1.5
Dev	<i>n</i> -Hexane	~0	1.3	0.1

substituent(s) show similar k values, and cannot be separated in a non-polar eluent. We therefore investigated the retention behavior of anthracene derivatives on Cu-PCS_D and Ni-PCS_D in a polar eluent, *i.e.*, 80% or 90% methanol. The results are shown in Figs. 5 and 6. It is clear that the Cu-PCS_D retained 9,10-dichloro-anthracene, and the Ni-PCS_D retained three two-substituted anthracene derivatives in a polar eluent more strongly than in *n*-hexane. The k values of the retained anthracene derivatives are 3- to 5-fold larger than that of anthracene. These results can be explained as follows: (1) The introduction of a simple substituent(s) into anthracene does not greatly affect the π -electron system, and therefore an interaction involving π electrons is hardly changed in a non-polar eluent (*n*-hexane). (2) In a polar eluent, when the molecular size is enlarged by introducing the introduction of a substituent(s), more polar molecules are required for hydration or the like, which eventually increases the energy. (3) M-PCS_D has a π electron system of sufficient size. It is of great interest that the tested columns can separate anthracene derivatives in a polar eluent (an aqueous methanol) depending on the molecular size, which are inseparable in a non-polar eluent (*n*-hexane).

In a previous study, where a non-polar eluent was used,¹² samples having a substituent(s) capable of providing a dipole moment were not examined. In the present study, we conducted an examination using samples having a substituent(s) capable of providing a dipole moment in both polar and non-polar eluents. Table 1 gives the results. In general, a dipole-dipole interaction, one involving electrostatic interactions, is considered to be ineffective in a polar eluent. As can be seen from Table 1, both the Cu- and Ni-PCS_D columns give k values of 2-nitro- and 1-amino-anthracenes not greatly different from that of anthracene in 80% or 90% methanol. However, the k values of 2-nitro- and 1-amino-anthracenes in *n*-hexane are 10-fold larger than that of anthracene. Table 1 also shows that a column packed with only the support, Dev, fails to strongly retain these compounds. The results indicate that the strong retention by the Cu- and Ni-PCS_D columns is attributable to the interaction with Cu- and Ni-PCS, and not with silica gels. These facts also indicate that the dipole-dipole interaction occurs between 2-nitro-anthracene and M-PCS only in a non-polar eluent. In this situation, the dipole of M-PCS is probably derived from a bias of charge between the metal and ligand, *i.e.*, $M^{\delta+}$ - $N^{\delta-}$ (pyrrole ring).

Conclusion

The present study revealed that, in a polar eluent, the Cu-PCS_D column exerts a π - π electron interaction, and that the Ni-PCS_D column exerts both the π - π electron interaction and the π - d interaction. It also revealed that both columns have a molecular size-recognition ability in a polar eluent.

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