

## Bidentate and Tridentate Heterocyclic Azo Compounds Having Long Alkyl Chains as Coating Reagents for Ion Chromatography of Manganese, Zinc, and Cadmium Ions

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Bidentate and tridentate heterocyclic azo compounds with and without a long alkyl chain were prepared and examined for cation exchange chromatography of manganese, zinc, and cadmium; these ions could not be separated by reversed phase HPLC following precolumn derivatization with heterocyclic azo compounds owing to the dissociation of the complexes. The newly prepared azo compounds having a long alkyl chain favorably orientate in the reversed-phase stationary phase such that the coordinating parts of the ligand may make contact with metal ions in the mobile phase. Bidentate ligands showed sharp peaks but almost no resolution of manganese and cadmium. A tridentate ligand strongly retained all the three metal ions, which could be separated within 10 min by a competing ligand and by optimizing the pH.

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### Introduction

One of the effective methods for simultaneous determination of metal ions is reversed-phase HPLC separation coupled with spectrophotometry. The intrinsically high performance of reversed-phase stationary phases developed for the separation of organic compounds is applicable to inorganic cations after precolumn derivatization. Various derivatizing reagents, such as dithiocarbamates,<sup>1-3</sup> 8-quinolinol,<sup>4,5</sup> porphyrins,<sup>6-8</sup> and heterocyclic azo dyes,<sup>9-11</sup> have been developed and examined considering the sample compositions. We have also synthesized a series of heterocyclic azo compounds and extensively studied the retention behavior of the reagent and their metal complexes.<sup>12-18</sup> With this type of reagent, the complexes of iron, cobalt, nickel, and copper are sufficiently stable to enable separation in the column. In contrast, the complexes of manganese, zinc, and cadmium are dissociated in the column after separation from excess reagent and usually can not be detected.<sup>19</sup> This lability, however, suggests the possibility of cation exchange chromatography of these weakly interacting metal ions.

Chromatographic separation of metal ions using complexation has been developed in order to achieve different selectivity patterns from that of conventional ion chromatography. Stationary phases obtained by physical adsorption of chelating reagents on the surface of high-performance reversed-phase substrates are especially versatile.<sup>20-25</sup> Continuous supply from the mobile phase is necessary for reagents of less lipophilicity (dynamic coating),<sup>22-25</sup> while durable coating is possible for highly lipophilic reagents (precoating).<sup>20-23</sup> In this study, we have synthesized a series of azo reagents with and without a long alkyl chain which enhanced the distribution of the reagent into the stationary phase and have evaluated their performances as a precoating reagent on an octadecylsilyl column for the separation of manganese, zinc, and cadmium ions.

### Experimental

#### Apparatus

The HPLC system consisted of two Model PU-980 intelligent pumps, a Model 9725i sample injector with a 10- $\mu$ l sample loop, a Model 965-CO column oven, and a Model MD-915 multiwavelength UV-Vis detector (wavelength range 200–800 nm) equipped with a Model DP-L915 data processing system (Jasco, Tokyo, Japan). A Model CD-5 conductometric detector (Showa Denko, Tokyo, Japan) was also used to monitor the elution of metal ions. Two C8 bonded silica gel columns (Model Unisil Q C8 and Model Inertsil C8, GL Science, Tokyo, Japan) and one C18 bonded silica gel column (Model L-column ODS, Chemical Evaluation Research Institute, Tokyo, Japan) of the same dimension (4.6 mm i.d.  $\times$  250 mm, particle diameter 5  $\mu$ m) were employed as a stationary phase. The absorption spectra were recorded with a Model UV-2200A spectrophotometer (Shimadzu, Tokyo, Japan). <sup>1</sup>H-NMR spectra were measured with a Model Gemini 300 spectrometer (300 MHz, Varian, CA, USA).

#### Reagents

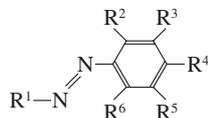
The structures of compounds used in the present study are shown in Table 1. Among the parent compounds, 2-(2-pyridylazo)-1-naphthol ( $\alpha$ -PAN)<sup>26</sup> and 4-(2-pyridylazo)-1-naphthol ( $p$ -PAN)<sup>27</sup> were prepared by condensation of 2-hydrazinopyridine with 1,2-naphthoquinone and 1,4-naphthoquinone, respectively. 1-(2-Pyridylazo)-2-naphthol ( $\beta$ -PAN)<sup>28</sup> was synthesized by coupling a 2-pyridinediazonium salt with 2-naphthol, while 4-(2-thiazolylazo)-1-naphthol ( $p$ -TAN)<sup>29</sup> was synthesized by coupling a 2-thiazolediazonium salt with 1-naphthol. Each crude compound was purified by extraction of their chloroform solutions with acid and alkali and by recrystallization from ethanol-water. 2-(2-Pyridylazo)-4-methylphenol (PAC)<sup>30</sup> was used as obtained.

One of the long alkyl derivatives, 2-(2-pyridylazo)-4-(octyloxy)-phenol (PAOOP), was synthesized by coupling 2-pyridinediazonium salt with 4-(octyloxy)phenol. The isomers were separated by extraction of the hexane solution with 0.01 mol dm<sup>-3</sup> sodium hydroxide in acetone-water (50:50, v/v). Other

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Table 1 Dissociation constants of heterocyclic azo compounds (ethanol-water, 50:50 (v/v);  $I = 0.1 \text{ mol dm}^{-3} \text{ KCl}$ )

| Abbreviated name      | Substituent              |   |   | $\text{p}K_{\text{a(NH)}}$ | $\text{p}K_{\text{a(OH)}}$ |
|-----------------------|--------------------------|---|---|----------------------------|----------------------------|
| Long alkyl derivative |                          |   |   |                            |                            |
| PAOOP                 | $\text{R}^1$ : pyridyl   | $\text{R}^3$ : $\text{OC}_8\text{H}_{17}$ | $\text{R}^6$ : OH                         | 1.9                        | 10.3                       |
| $\alpha$ -PAOON       | $\text{R}^1$ : pyridyl   | $\text{R}^4, \text{R}^5$ : 1,3-butadienyl | $\text{R}^6$ : $\text{OC}_8\text{H}_{17}$ | 3.9                        | —                          |
| $\beta$ -PAOON        | $\text{R}^1$ : pyridyl   | $\text{R}^2, \text{R}^3$ : 1,3-butadienyl | $\text{R}^6$ : $\text{OC}_8\text{H}_{17}$ | 5.4                        | —                          |
| $p$ -PAOON            | $\text{R}^1$ : pyridyl   | $\text{R}^2, \text{R}^3$ : 1,3-butadienyl | $\text{R}^4$ : $\text{OC}_8\text{H}_{17}$ | 2.8                        | —                          |
| $p$ -TAOON            | $\text{R}^1$ : thiazolyl | $\text{R}^2, \text{R}^3$ : 1,3-butadienyl | $\text{R}^4$ : $\text{OC}_8\text{H}_{17}$ | <1                         | —                          |
| Parent compound       |                          |   |   |                            |                            |
| PAC                   | $\text{R}^1$ : pyridyl   | $\text{R}^3$ : $\text{CH}_3$              | $\text{R}^6$ : OH                         | 1.8                        | 10.5                       |
| $\alpha$ -PAN         | $\text{R}^1$ : pyridyl   | $\text{R}^4, \text{R}^5$ : 1,3-butadienyl | $\text{R}^6$ : OH                         | 2.4                        | 10.7                       |
| $\beta$ -PAN          | $\text{R}^1$ : pyridyl   | $\text{R}^2, \text{R}^3$ : 1,3-butadienyl | $\text{R}^6$ : OH                         | 2.0                        | 12.3                       |
| $p$ -PAN              | $\text{R}^1$ : pyridyl   | $\text{R}^2, \text{R}^3$ : 1,3-butadienyl | $\text{R}^4$ : OH                         | 3.6                        | 9.6                        |
| $p$ -TAN              | $\text{R}^1$ : thiazolyl | $\text{R}^2, \text{R}^3$ : 1,3-butadienyl | $\text{R}^4$ : OH                         | <1                         | 7.4                        |



long alkyl derivatives were prepared by Williamson ether synthesis with 1-bromooctane and the parent pyridylazo- and thiazolylazonaphthols.<sup>31,32</sup> A 30% sodium carbonate solution containing cetyltrimethylammonium bromide was used for 2-(2-pyridylazo)-1-(octyloxy)naphthalene ( $\alpha$ -PAOON) and 1-(2-pyridylazo)-2-(octyloxy)naphthalene ( $\beta$ -PAOON), while sodium hydride in *N,N*-dimethylformamide was used for 4-(2-pyridylazo)-1-(octyloxy)naphthalene ( $p$ -PAOON) and 4-(2-thiazolylazo)-1-(octyloxy)naphthalene ( $p$ -TAOON). The crude materials were purified by chromatography on silica-gel column, by extraction with acid and alkali, or by precipitation from ethyl acetate solution by the addition of *n*-hexane. The purities of the compounds were confirmed by  $^1\text{H}$  NMR and reversed-phase HPLC.

Each compound was dissolved in ethanol or ethanol-water to give a  $1 \times 10^{-3} \text{ mol dm}^{-3}$  solution for column-coating and  $2.5 \times 10^{-5} \text{ mol dm}^{-3}$  solution for the batch experiments. Standard stock solutions ( $1.0 \times 10^{-2} \text{ mol dm}^{-3}$ , pH 2) of zinc, manganese, and cadmium were prepared by dissolving the analytical-reagent grade nitrates.

#### Chromatographic procedure

The reversed-phase columns were coated with each compound by passing the  $1 \times 10^{-3} \text{ mol dm}^{-3}$  ethanol-water solutions for 2 h, followed by washing with water for 1 h at a flow rate of  $0.3 \text{ cm}^3 \text{ min}^{-1}$  and at a column temperature of  $40^\circ\text{C}$ . A  $10\text{-}\mu\text{l}$  aliquot of  $1 \times 10^{-3} \text{ mol dm}^{-3}$  metal ion solution was placed on the column via an injection valve and eluted with a mobile phase at a flow rate of  $0.7 \text{ cm}^3 \text{ min}^{-1}$  and at a column temperature of  $40^\circ\text{C}$ . An aqueous solution ( $1 \times 10^{-4} \text{ mol dm}^{-3}$ , approximately pH 10) of 2-(2-thiazolylazo)resorcinol (TAR) or 2-(2-pyridylazo)resorcinol (PAR) as a postcolumn derivatizing reagent was merged with the eluent, and the absorbance was monitored at 200–800 nm. The void volumes used to calculate  $k'$  values were determined by injecting pure water into each mobile phase.

## Results and Discussion

#### Reactions with metal ions in solution and spectral changes

The acid dissociation constants of five newly synthesized compounds as well as their parent compounds were spectrophotometrically determined at an ionic strength of  $0.1 \text{ mol dm}^{-3} \text{ KCl}$  in ethanol-water (50:50, v/v), which allowed the solubilization of these reagents in the presence of metal ions over a wide pH range of 3–11 (Table 1). The introduction of an octyl group enhanced the basicities of nitrogen atoms of  $\alpha$ -PAN and  $\beta$ -PAN but not for PAC,  $p$ -PAN, and  $p$ -TAN. The basicity decreased in the order of:  $\beta$ -PAOON >  $\alpha$ -PAOON >

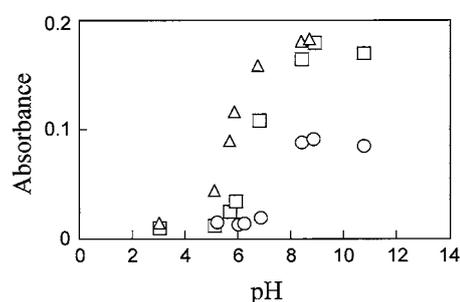


Fig. 1 Effect of pH on absorbance of binary systems consisting of PAOOP and metal ions. Metal,  $1 \times 10^{-5}$ ; PAOOP,  $1 \times 10^{-5}$ ; buffer,  $1 \times 10^{-2} \text{ mol dm}^{-3}$ . Ethanol-water (50:50, v/v);  $25^\circ\text{C}$ . Metal, wavelength:  $\Delta$ , Zn, 586;  $\square$ , Cd, 581;  $\circ$ , Mn, 575 nm.

$p$ -PAOON > PAOOP >  $p$ -TAOON. The resulting compounds showed the absorption maximum at a longer wavelength than the parent compounds; e.g., 545 nm for  $\alpha$ -PAOON compared with 485 nm for  $\alpha$ -PAN and 538 nm for  $\beta$ -PAOON compared with 470 nm for  $\beta$ -PAN.

The reactivities of these compounds to three metal ions ( $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Cd}^{2+}$ ) were estimated from the change in absorption spectrum. Practically no changes were obtained for bidentate ligands of  $\alpha$ -PAOON,  $\beta$ -PAOON,  $p$ -PAOON, and  $p$ -TAOON, while appreciable changes were observed for tridentate PAOOP. The absorption spectrum of binary systems consisting of PAOOP and  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Cd}^{2+}$  was measured at varying pH values. The absorbances at wavelengths characteristic of each metal ion are shown as a function of pH in Fig. 1. The changes in absorbance caused by complexation were found at pH > 5 for  $\text{Zn}^{2+}$ , at pH > 6 for  $\text{Cd}^{2+}$ , and at pH > 7 for  $\text{Mn}^{2+}$ . The pH profiles indicated that the reactivity of PAOOP to metal ions was in the order of:  $\text{Zn}^{2+} > \text{Cd}^{2+} > \text{Mn}^{2+}$ .

The reactivity of  $p$ -PAOON, as a representative of bidentate ligands, to metal ions was examined by  $^1\text{H}$  NMR in acetone- $d_6$ - $\text{D}_2\text{O}$  (70:30, v/v). All the aromatic protons were unambiguously assigned as shown in Fig. 2(A). Although the addition of  $\text{Zn}^{2+}$  caused no chemical shift of these protons due to the lability of the complex, a pronounced broadening of protons adjacent to the azo group was observed (Fig. 2(B)). Cadmium ion also caused the broadening to a lesser extent, but manganese did not. The broadening indicated that the reactivities of bidentate ligands were also in the order of:  $\text{Zn}^{2+} > \text{Cd}^{2+} > \text{Mn}^{2+}$ .

#### Preliminary studies on reagents and columns

The L-column ODS was coated with six reagents by adjusting



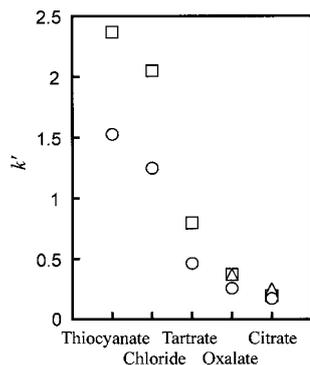


Fig. 5 Effect of additives on retention of metal ions. Column, Unisil Q C8 coated with PAOOP; eluent,  $6 \times 10^{-3}$  mol dm $^{-3}$  additive solution, pH 6.5; flow-rate,  $0.7$  cm $^3$  min $^{-1}$ ; column temperature,  $40^\circ$ C. Metal:  $\Delta$ , Zn;  $\square$ , Cd;  $\circ$ , Mn.

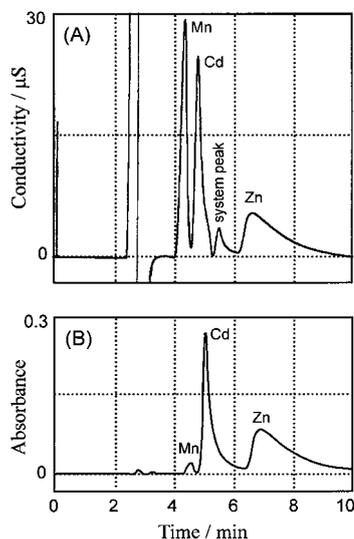


Fig. 6 Typical chromatograms of metal ions with coating of PAOOP. (A) Conductometric detection; (B) spectrophotometric detection. Column, Unisil Q C8; eluent,  $1 \times 10^{-3}$  mol dm $^{-3}$  oxalate containing  $1 \times 10^{-3}$  mol dm $^{-3}$  ethylenediammonium ion, pH 6.5; flow-rate,  $0.7$  cm $^3$  min $^{-1}$ ; column temperature,  $40^\circ$ C.

Heterocyclic azo compounds were coated on the reversed-phase HPLC columns, which were examined for ion-exchange chromatographic separation of metal ions. During the procedure, none the compounds with or without a long alkyl chain leaked from the column with aqueous eluent and exhibited retention behavior characteristic of metal ions. A long alkyl chain not only enhanced the distribution of the azo compounds but oriented the donor atoms suitably for the interaction with metal ions in the mobile phase.

In this study, manganese with small affinities to the heterocyclic azo compounds was retained by the interaction of the residual silanol group. Another possibility for the retention of manganese is the introduction of anion-exchange sites such as sulfonates on the present heterocyclic azo compounds.

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