Application of Adduct Formation and Masking Effect to Separation of Some Metal Chelates of 3-Mercapt-1,5-diphenylformazane in Reversed-Phase High-Performance Liquid Chromatography

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The adduct formation and the masking effect due to the addition of neutral ligands to mobile phase were applied to improve the mutual separation of some metal chelates of 3-mercapt-1,5-diphenylformazane (dithizone) in reversed-phase high-performance liquid chromatography. The addition of trioctylphosphine oxide and triethanolamine (TEA) was effective for controlling the retention time of some metal dithizonates. The addition of TEA made it possible to separate the overlapped peaks of nickel(II) and zinc(II) dithizonates from each other. On the other hand, 1,10-phenanthroline (phen) masks Ni^{II} and Zn^{II} selectively depending upon stabilities of their charged phen complexes. The degree of the masking effect of phen was predictable to some extent from the stability constants of the metal dithizonates and the metal–phen complexes.

Recently, we have proposed the utilization of adduct formation and masking effect for improving the separation of metal chelates in reversed-phase high-performance liquid chromatography (RP-HPLC).[1,2] The addition of neutral ligands such as 4-methylpyridine (4-MP), trioctylphosphine oxide (TOPO), 1,10-phenanthroline (phen), and 2,2′-bipyridyl (bpy) to the mobile phase caused the change of retention time of metal chelates (adduct formation) or depression of their peaks (masking effect) in RP-HPLC of metal 4-(2-thienyl)-4-thioxo-1,1,1-trifluoro-2-butanonates(STTA) and 4,4,4-trifluoro-1-(2-thienyl)-1,3-butanedionates(TTA). Such effects can be explained by their synergistic extraction constants in the chelate extraction.[2]

In this study, the adduct formation and the masking effect due to the addition of neutral ligands were applied to the mutual separation of metal chelates of 3-mercapt-1,5-diphenylformazane (dithizone, H_2dz) on an octadecyl-bonded silica gel column. In particular, phen showed the strong masking effect, and the peaks of $Ni(Hdz)_2$ and $Zn(Hdz)_2$ could be selectively suppressed by changing phen concentration.

Experimental

Reagents. Individual standard stock solutions of metal ions (8.0×10^{-3} M, M=mol dm^{-3}) were prepared by dissolving acetates of cobalt(II), nickel(II), copper(II), zinc(II), and cadmium(II) (Wako Pure Chemicals) in water. These individual standard solutions were mixed together, and diluted to 4.0×10^{-4} M (each metal ion concentration) with water. Dithizone (Wako Pure Chemicals) was used without further purification. Additives of 4-MP, phen, bpy, triethanolamine (TEA), and TOPO (Wako Pure Chemicals and Dojin Labs.) were also used without further purification. These additives were dissolved in methanol to give required concentration before use. Chloroform and methanol described above were of HPLC grade (Wako Pure Chemicals) and all other chemicals used were of guaranteed grade. Deionized water was used throughout.

Apparatus. A liquid chromatograph consisted of a Shimadzu Model LC-9A pump, a Hitachi Model L-4200 UV-vis detector, and a Shimadzu Model CTO-2A column oven. A sample injector was a Rheodyne Model 7010 sample injection valve with a Rheodyne Model 7012 loop filler port and a Rheodyne Model 7020 5-μl sample loop. A data processor was a Shimadzu Model Chromatopac C-R1B. Shakes on extraction were made with a Iwaki KM type shaker. Residual metal ions in the aqueous phase after extraction were determined with a Shimadzu Model AA-670A flame atomic absorption spectrometer.

Procedure. Preparation of Metal Dithizonates Sample: Twenty cm^3 of an aqueous solution containing 1.0×10^{-4} M individual metal ions, whose pH was adjusted to 6.7 with aqueous ammonia and 0.1 M KH_2PO_4–0.01 M $Na_2B_4O_7$ buffer, was placed in a separatory funnel. An equal volume of chloroform solution containing 5.0×10^{-3} M H_2dz was added; the mixture was then shaken vigorously for 30 min. After the phases were allowed to separate, aliquots (15 μl) of the organic phase were injected into the HPLC. All the HPLC measurements were repeated at least three times.

HPLC Conditions: An analytical column for all experiments was a 5 μm particle diameter Wako Pure Chemicals Wakopak Wakosil-II 5C18-HG column (150 mm×4.6 mm i.d.). The temperature of the column was maintained at 35 °C with the column oven throughout the measurements. The mobile phase was an aqueous methanol [methanol–water (82 : 18, v/v)] which was saturated with a small excess of H_2dz at room temperature. When necessary, various concentrations of an additive were added to the solution. This was filtrated to remove precipitate, and de-gassed under reduced pressure in an ultrasonic bath prior to pump. It was prepared every time before use. The flow rate of the mobile phase was set at 0.8 cm^3 min^{-1} throughout the measurements. The absorption spectrophotometric detection was carried out at 520 nm.

Results and Discussion

Separation of Some Metal Dithizonates on Octadecyl-Bonded Silica Gel Column. Ohashi et al. tried to separate five metal dithizonates from each other on an octadecyl-bonded silica gel column. Although $Co(Hdz)_3$ gave an intensive peak, mutual separation of

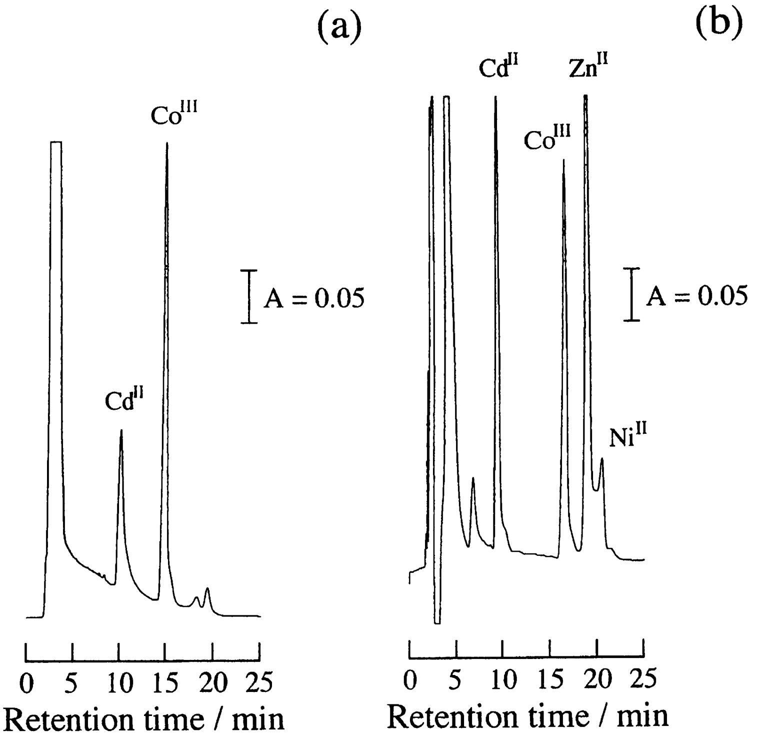

Fig. 1. Chromatogram of metal dithizonates. Column: Wakopak Wakosil-II 5C18-HG (5 μm; 150 mm×4.6 mm i.d.). Mobile phase: (a) methanol–water (82 : 18, v/v), (b) Dithizone (saturated concentration) was added to the mobile phase of (a). See text for further chromatographic conditions.

three metal dithizonates, Ni^{II}, Zn^{II}, and Pb^{II}, could not be achieved. Moreover, the peak of Cd(Hdz)$_2$ was not observed.[3] We also tried to separate some metal dithizonates with similar HPLC conditions. As shown in Fig. 1(a), our results were basically the same as those of Ohashi et al. except that Cd(Hdz)$_2$ gave a sharp peak. The results of Fig. 1(a) could be explained to some extent by stability constants ($\log \beta_2$) of metal dithizonates (Table 1). Since Ni(Hdz)$_2$ and Zn(Hdz)$_2$ have smaller stability constants than that of Cd(Hdz)$_2$, those complexes may easily be dissociated in the column. Although Cu(Hdz)$_2$ has the largest stability constant, its peak was unidentified under these conditions. Ohashi et al. has been reported that the chloroform solution of primary copper dithizonate [Cu(Hdz)$_2$] in the presence of methanol showed the absorption spectral change due to the formation of secondary copper dithizonate [Cu(dz)] and caused the decrease in the absorbance along with subsequent decomposition.[5] Thus, methanol and water in the mobile phase may be responsible for the disappearance of the Cu(Hdz)$_2$ peak in RP-HPLC. As for cobalt, initially prepared Co^{II} was oxidized during extraction[6] and the inert Co(Hdz)$_3$ was separated.[3] For improving the degree of separation, we have tried to add H$_2$dz to the mobile phase in order to prevent those complexes from dissociation in the column. Since H$_2$dz is slightly soluble in a methanol–water mixed solution, the mobile phase was saturated with H$_2$dz at room temperature. With the addition of H$_2$dz to the mobile phase, the peaks of Ni(Hdz)$_2$ and Zn(Hdz)$_2$ appeared as shown in Fig. 1(b). In particular, Zn(Hdz)$_2$ and Cd(Hdz)$_2$ gave sharp and intensive peaks. On the other hand, the peak intensity of Co(Hdz)$_3$ decreased slightly and the Cu(Hdz)$_2$ peak was not identified. The detection limits of the present method for Co^{II} and Cd^{II} were 1.2 ng and 2.0 ng (S/N=3), respectively. The HPLC conditions of Fig. 1(b) were applied throughout the following experiments.

Table 1. Stability Constants of Metal Dithizonates and Metal–phen Complexes

Metal ion	Dithizone[a)] $\log \beta_2$	phen[b)] $\log \beta_3$
Ni^{II}	14.17±0.05	24.8
Cu^{II}	19.18±0.07	21.35
Zn^{II}	13.96±0.08	17.55
Cd^{II}	15.10±0.15	14.92

a) From Ref. 4: Spectrophotometry, Ionic strength 0.1 M, Temperature 25±1°C. b) From Ref. 10: Potentiometry, Ionic strength 0.1 M, Temperature 20 °C.

Application of Adduct Formation to Separation of Metal Dithizonates. In the separation of metal–STTA and TTA chelates, the addition of 4-MP and TOPO to the mobile phase caused the adduct formation, that resulting in the change of the retention time and/or the enhancement of the peaks, while phen and bpy showed the masking effect.[2] Thus, 4-MP and TOPO were also tested as a synergist in this study. The effect of 4-MP was not remarkable. On the other hand, the addition of TOPO to the mobile phase caused the change of the retention time of metal dithizonates. Figure 2 shows the dependence of the retention time of metal dithizonates on TOPO concentration in the mobile phase, and Fig. 3 shows the chromatogram of metal dithizonates with TOPO in the mobile phase. The change of the retention time was the largest for Cd(Hdz)$_2$, and Zn(Hdz)$_2$ was in the next place. The results were consistent with data of chelate extraction; the degree of adduct formation of Cd(Hdz)$_2$ with TOPO has been reported to be larger than that for Zn(Hdz)$_2$.[7,8] As for Co(Hdz)$_3$ and Ni(Hdz)$_2$, the adduct formation with TOPO has not clearly been observed in chelate extraction. However, the addition of TOPO increased the retention time of Co(Hdz)$_3$, while it has no effect on that of Ni(Hdz)$_2$.

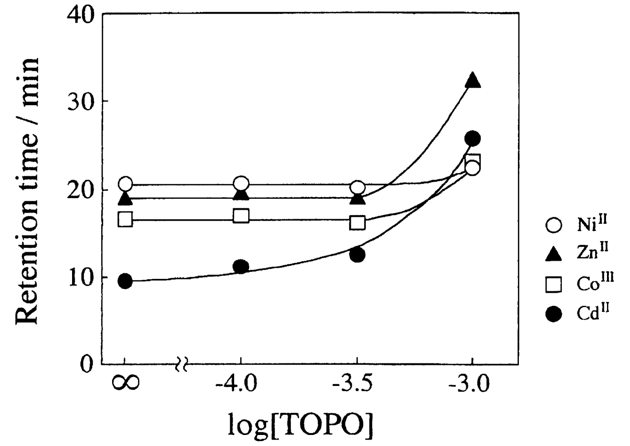

Fig. 2. Effect of TOPO on the retention time of metal dithizonates. The various concentrations of TOPO were added to the mobile phase shown in Fig. 1(b).

Moreover, the effect of TEA as a hydrophilic additive on the separation was also tested. Shibata et al. reported that the addition of TEA to the mobile phase reduced the retention of rare earth octaethylporphyrins in RP-HPLC because of its hydrophilic nature.[9] As shown in Fig. 4, the retention time of the adducts was also shorter than that of host chelates. Although the peaks of Ni(Hdz)$_2$ and Zn(Hdz)$_2$ were overlapped without TEA as shown in Fig. 1(b), the addition of TEA made it possible to separate from each other, in which the retention time of Ni(Hdz)$_2$ decreased more steeply. The detection limit for Zn^{II} was 1.2 ng (S/N=3) under these conditions.

Application of Masking Effect to Separation of Metal Dithizonates. When phen or bpy was added to the mobile phase shown in Fig. 1(b), masking of metal ions was observed. The effects of phen and bpy were essentially the same. Since the effect of phen was more remarkable than that of bpy, only the results on phen will be discussed. With the mobile phase containing 1.0×10^{-4} M phen [Fig. 5(a)], the peak of Ni(Hdz)$_2$ disappeared. Furthermore, with the mobile phase containing 1.0×10^{-3} M phen [Fig. 5(b)], the peak of Zn(Hdz)$_2$ disappeared. That is, Ni^{II} and Zn^{II} were selectively masked with the addition of phen. Dithizone in the host chelate may be displaced with phen to form charged metal–phen complex in the column. Such charged complex may hardly be retained on octadecyl-bonded phase. The masking effect of phen on

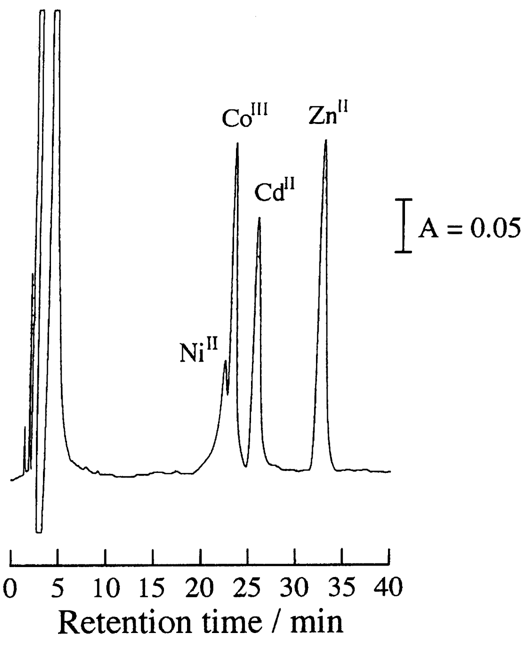

Fig. 3. Chromatogram of metal dithizonates with the addition of TOPO. TOPO (1.0×10^{-3} M) was added to the mobile phase shown in Fig. 1(b).

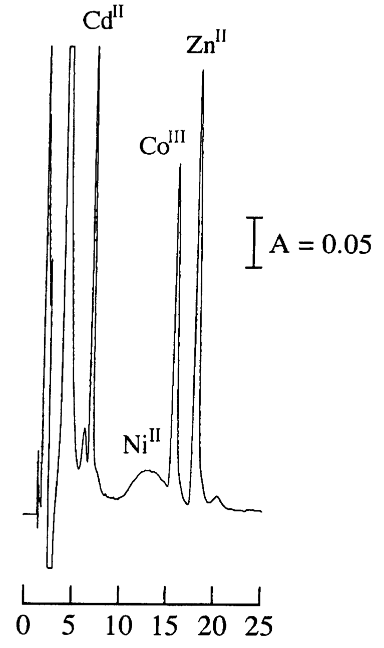

Fig. 4. Chromatogram of metal dithizonates with the addition of TEA. TEA (1.0×10^{-4} M) was added to the mobile phase shown in Fig. 1(b).

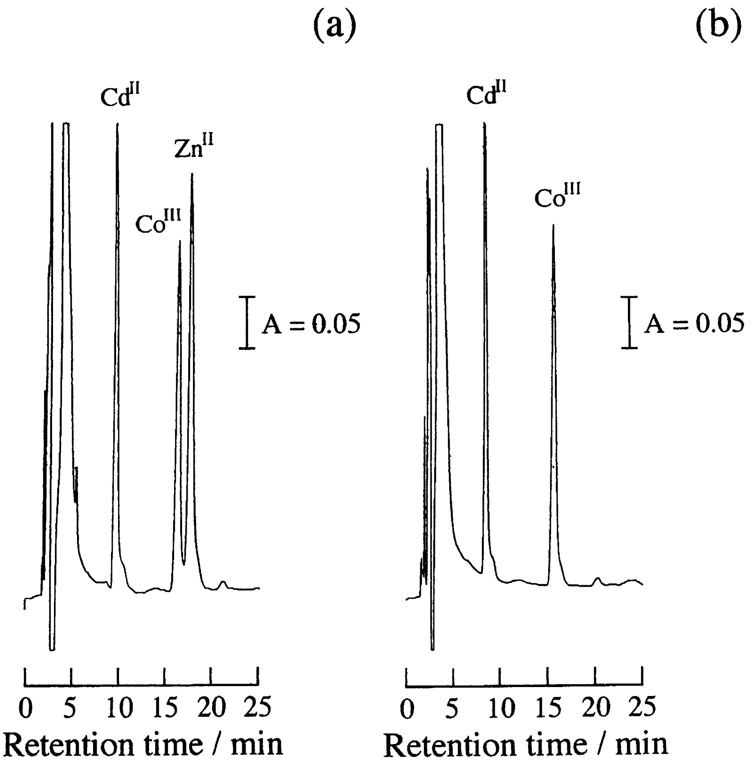

Fig. 5. Chromatogram of metal dithizonates with the addition of phen. Phen was added to the mobile phase shown in Fig. 1(b). Phen concentration: (a) 1.0×10^{-4} M, (b) 1.0×10^{-3} M.

metal dithizonates could be explained by stability constants of metal dithizonates and metal–phen complexes (Table 1). The stability constants of metal–phen complexes are $Ni^{II} > Zn^{II} > Cd^{II}$, and those of metal dithizonates are $Cd^{II} > Ni^{II} \approx Zn^{II}$. Thus, the order in the ease of replacement of dithizone in the metal dithizonates with phen should be $Ni^{II} > Zn^{II} > Cd^{II}$. This order corresponds to the experimental results on the vanishing order of the metal dithizonate peaks ($Ni^{II} > Zn^{II} > Cd^{II}$).

In chelate extraction of metal–STTA, TTA, and dithizone systems, phen and bpy act not only as masking agents but also as synergists.[11—14] However, we found that phen and bpy mostly behave as masking agents in RP-HPLC of metal–STTA, TTA,[2] and dithizone systems. The degree of the distribution of phen and bpy may be greater in the mobile phase (methanol–water mixture) of RP-HPLC than in an aqueous phase of chelate extraction because phen and bpy are much more soluble in alcohol than in water.[15] Therefore, the formation of a charged complex (i.e. metal–phen complex) may be more favorable in RP-HPLC than in chelate extraction.

Conclusion Additives such as TOPO and TEA were effective for improving the mutual separation of metal dithizonates. Moreover, phen could mask Ni^{II} and Zn^{II} selectively. The degree of the masking effect was predictable to some extent from the stability constants of the metal-chelating agent and the metal-additive. Thus, the masking effect of phen would be useful to depress interfering peaks in RP-HPLC of metal chelates.

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