Utilization of Adduct Formation and Masking Effect for Separation of Some Metal 4-(2-Thienyl)-4-thioxo-1,1,1-trifluoro-2-butanonates and 4,4,4-Trifluoro-1-(2-thienyl)-1,3-butanedionates by Reversed Phase HPLC

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(Received September 1, 1992)

The separation behavior of Co^{III}-, Ni^{II}-, Cu^{II}-, and Zn^{II}-STTAs [4-(2-thienyl)-4-thioxo-1,1,1-trifluoro-2-butanone] and Co^{II}-, Ni^{II}-, and Cu^{II}-TTAs [4,4,4-trifluoro-1-(2-thienyl)-1,3-butanedione] in reversed phase high performance liquid chromatography was investigated when various additives were added to the mobile phase. The adduct formation or the masking effect was observed upon addition of additives. When trioctylphosphine oxide was added to the mobile phase, mutual separation of those metal chelates was especially remarkable due to the adduct formation. Moreover, the decomposition of some metal chelates in the HPLC column was also prevented by the adduct formation. On the other hand, 2,2′-bipyridyl and 1,10-phenanthroline showed the masking effect. Such effects of additives were consistent with their synergistic extraction constants in chelate extraction.

High performance liquid chromatography (HPLC) of metal chelates has widely been studied, and recognized as one of the promising techniques for trace metal determination.[1—7] In particular, reversed phase HPLC (RP-HPLC) has often been applied to the separation of metal chelates because of its versatility.[2,5,7] However, we sometimes encounter various problems such as difficulty in mutual separation of metal chelates and their decomposition in separation column in RP-HPLC of metal chelates.

In solvent extraction of metal chelates, synergistic effect, that is, the adduct formation, has been used for the improvement of the extractability of an analyte, increase of extraction rate, stabilization of metal chelates, and so on. In RP-HPLC, the separation of analytes is supposed to be based on their partition between mobile phase (polar solvent) and stationary phase (nonpolar solvent).[8] Such mechanism is analogous to that of solvent extraction. Thus, we have been interested in the use of adduct formation for the HPLC separation of metal chelates which are commonly encountered in solvent extraction. In RP-HPLC of metal chelates, the adduct formation may provide a new technique to control the separation, i.e., to change the retention time of metal chelates, and to prevent them from decomposition in separation column. Although Igarashi et al. and Saitoh et al. have reported adduct formation in the separation of some metal-porphine derivatives by RP-HPLC,[9,10] no systematic study has been done on this subject so far.

We primarily reported the separation of Co^{III}, Ni^{II}, Cu^{II}, Zn^{II}, and Cd^{II} 4-(2-thienyl)-4-thioxo-1,1,1-trifluoro-2-butanonates [STTA] and their pyridine base adducts by RP-HPLC, in which the adduct formation should be mainly responsible for the change in the retention of metal-STTA chelates.[11] In the present paper, we summarized the effect of the addition of various additives to the mobile phase on the mutual separation of metal-STTA chelates in RP-HPLC. Here, additives include bidentate bases, 2,2′-bipyridyl (bpy) and 1,10-phenanthroline (phen), oxygen-containing monodentate bases, tributylphosphate (TBP) and trioctylphosphine oxide (TOPO) and a pyridine base, 4-methylpyridine (4-MP). Moreover, this approach was extended to the separation of some metal 4,4,4-trifluoro-1-(2-thienyl)-1,3-butanedionates [TTA, (β-diketone analog of STTA)].

Experimental

Reagents. Individual standard stock solutions (8.0×10^{-3} M, M=mol dm^{-3}) were prepared by dissolving acetate salts of cobalt(II), nickel(II), copper(II), and zinc(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. STTA and TTA (both from Dojin Labs.) were used without further purification. For extraction, STTA and TTA were dissolved in chloroform to give 5.0×10^{-3} M. STTA and TTA stock solutions were freshly prepared every two days and a week respectively. For the mobile phase of HPLC, the STTA stock solution was made by dissolving it in methanol and acetonitrile to give 5.0×10^{-3} M, and the TTA stock solution was made by dissolving it in methanol and acetonitrile to give 5.0×10^{-2} M. Those STTA and TTA stock solutions were diluted to required concentration with suitable solvent before use. Additives of 4-methylpyridine (4-MP), 2,2′-bipyridyl (bpy), 1,10-phenanthroline (phen), tributylphosphate (TBP), and trioctylphosphine oxide (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, methanol, and acetonitrile described above were of HPLC grade (Wako Pure Chemicals) and all other chemicals used were of guaranteed grade. Deionized water was used throughout.

Apparatus. An HPLC system consisted of a Shimadzu Model LC-9A pump, 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 Shimadzu Model CTO-2A column oven, a Shimadzu Model SPD-1 UV-visible spectrophotometric detector, and a Shimadzu Model Chro-

matopak C-R6A chromatographic data processor. Measurements of pH were made with a Denki Kagaku Keiki Model HGC-10 pH meter and a glass electrode. 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 Chelate Samples: Twenty cm^3 of an aqueous solution containing 1.0×10^{-4} M of those individual metal ions, whose pH was adjusted to 6.5 for the STTA system and 7.2 for the TTA system by ammonium hydroxide and 0.1 M KH_2PO_4–0.01 M $Na_2B_4O_7$ buffer respectively, was placed in a separatory funnel. An equal volume of chloroform solution containing 5.0×10^{-3} M STTA or 5.0×10^{-3} M TTA was added; the mixture was then shaken vigorously for 30 min. After the phases were allowed to separate, an aliquot of the organic phase (15 μl) was injected into RP-HPLC. All the HPLC measurements were repeated at least three times.

HPLC Condition: An analytical column for all experiments was a 5-μm particle diameter Shimadzu Shim-pack CLC-ODS(M) 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 for the STTA system was methanol–water–acetonitrile (72:18:10, v/v) containing 8.2×10^{-5} M STTA and various concentrations of an additive. That for the TTA system was methanol–water–acetonitrile (68:22:10, v/v) containing 1.2×10^{-3} M TTA and various concentrations of an additive. When necessary, pH of the mobile phase was measured. The effect of organic solvent on the pH measurement was not corrected in this study. The mobile phase was de-gassed under reduced pressure in an ultrasonic bath before use. The flow rate of the mobile phase was set at 0.8 $cm^3\ min^{-1}$ throughout the measurements. The spectrophotometric detection was carried out at 370 nm for metal-STTAs and 340 nm for metal-TTAs respectively.

Results and Discussion

Separation of Some Metal-STTA and TTA Chelates on ODS Column. For the separation of Co^{III}-, Ni^{II}-, Cu^{II}-, and Zn^{II}-STTA chelates on the ODS column, the chromatographic conditions in the previous paper[11] were used in the present study with minor modification as shown in Fig. 1, in which 8.2×10^{-5} M STTA was added to the mobile phase. In the absence of STTA in the mobile phase, on the other hand, only the peak of Co^{III}-STTA chelate appeared at the same retention time as that in Fig. 1, while the peaks of all the other chelates disappeared completely. In spite of using bivalent Co^{II}, Co^{II} was oxidized during extraction[12,13] and the inert trivalent Co^{III}-STTA was separated. When 1.5×10^{-5} M STTA was added to the mobile phase, the peaks of Cu^{II}-, Ni^{II}-, and Co^{III}-STTAs appeared, although the peak of Ni^{II}-STTA was small and diffused. With the addition of 8.2×10^{-5} M STTA as shown in this figure, Ni^{II}-STTA gave the sharp and intensive peak and the peak of Zn^{II}-STTA was also obtained. The retention time of Ni^{II}-STTA decreased with the increase in the STTA concentration (1.5×10^{-5}—8.2×10^{-5} M) in the mobile phase.

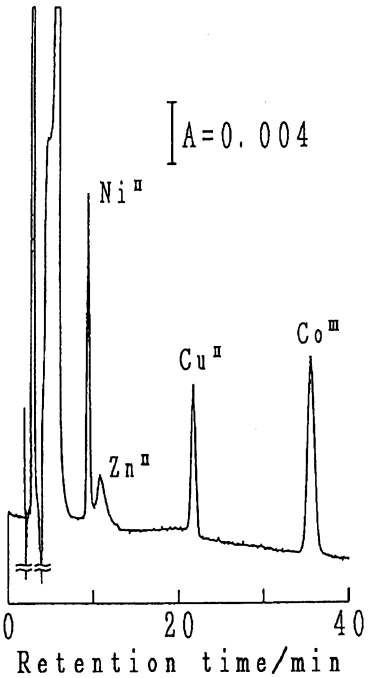

Fig. 1. Chromatogram of metal-STTA chelates. Column: Shim-pack CLC-ODS(M) (5 μm; 150 mm×4.6 mm i.d.). Mobile phase: methanol–water–acetonitrile (72:18:10 v/v) containing 8.2×10^{-5} M STTA. See text for further chromatographic conditions.

As for metal-TTAs, Saitoh and Suzuki performed the separation of some metal-TTAs with poly(vinyl acetate) gel.[14] However, since the ODS column has not been applied so far to the separation of these chelates, chromatographic conditions were investigated for their separation on the ODS column. Figure 2 shows the chromatogram of metal-TTAs where 1.2×10^{-3} M TTA was added to the mobile phase. Ni^{II}-, Cu^{II}-, and Co^{II}-TTAs were separated on the chromatogram. In the TTA system, cobalt remained bivalent according to the data of solvent extraction.[15] When 8.2×10^{-5} M TTA was added to the mobile phase, on the other hand, only small unresolved peak was obtained for the three TTA chelates. With the increase in the TTA concentration, the peaks of the TTA chelates became sharper and more intensive. Moreover, the retention times of those TTA chelates decreased with the increase in the TTA concentration (8.2×10^{-4}—1.6×10^{-3} M). In spite of the addition of excess TTA (1.6×10^{-3} M) to the mobile phase, the peak of Zn^{II}-TTA was hardly recognized on the chromatogram. The absence of Zn^{II}-TTA on the chromatogram was probably due to dissociation of the chelate in the column. The addition of further TTA to the mobile phase caused severe interference with spectrophotometric detection due to the increase of background absorption.

Effects of Various Additives on Separation of Metal-STTA Chelates. 4-MP, bpy, phen, TBP, and

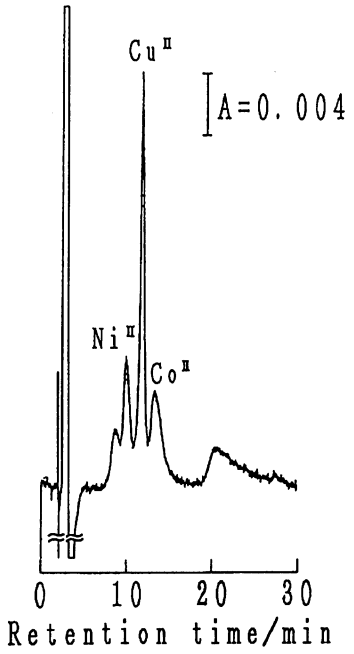

Fig. 2. Chromatogram of metal-TTA chelates. Mobile phase: methanol–water–acetonitrile (68:22:10 v/v) containing 1.2×10^{-3} M TTA. Other chromatographic conditions were the same as those of Fig. 1.

TOPO at various concentrations were added to the mobile phase of Fig. 1, respectively, and their effects on the separation of metal-STTA chelates were investigated as follows:

4-MP. Figure 3 shows the dependence of the retention time of metal-STTA chelates on 4-MP concentration. Although the effects of 4-MP on the STTA system have already been reported in the previous paper,[11] this compound was examined again as a typical pyridine base to make it possible to compare between the previous and the present works. The retention time of Zn^{II}- and Ni^{II}-STTA chelates increased with the increase in the 4-MP concentration, which may be due to the adduct formation as shown in the previous paper.[11] Although pH change in the mobile phase might cause the increase in the retention time, it may not be a prominent factor in this study according to the following results: The retention time of Zn^{II}-TTA was prolonged ca. 5 min by the addition of 4-MP at the same pH (pH=6.0). Moreover, the addition of 4-MP was much more effective on the change of the retention time than that of 2-MP, which has about the same pK_a value as 4-MP but weaker coordination ability because of the steric hindrance caused by methyl group of 2-position.[11] On the other hand, the retention time of Cu^{II}- and Co^{III}-STTA chelates was not changed with the addition of 4-MP. Such results agreed with the previous results.[11] In addition, Fig. 4 shows the dependence of the peak intensity (peak area) of each metal-STTA chelate upon 4-MP concentration in the mobile phase. The peak intensity of Zn^{II}-STTA increased along

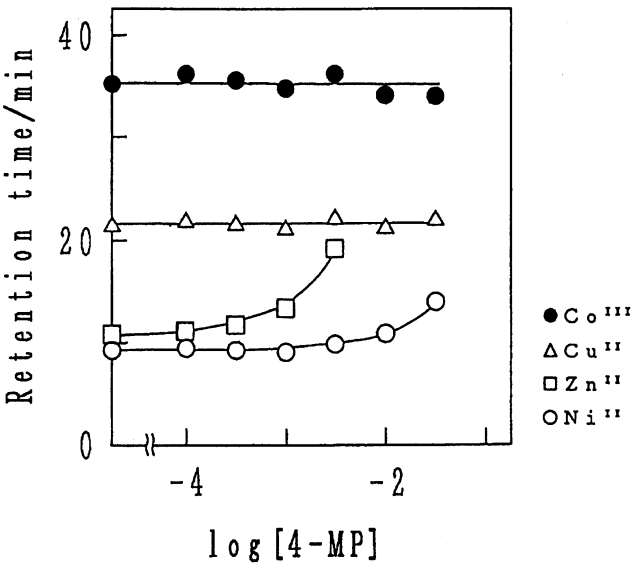

Fig. 3. Effect of 4-MP on the retention time of metal-STTA chelates. The various concentrations of 4-MP were added to the mobile phase of Fig. 1.

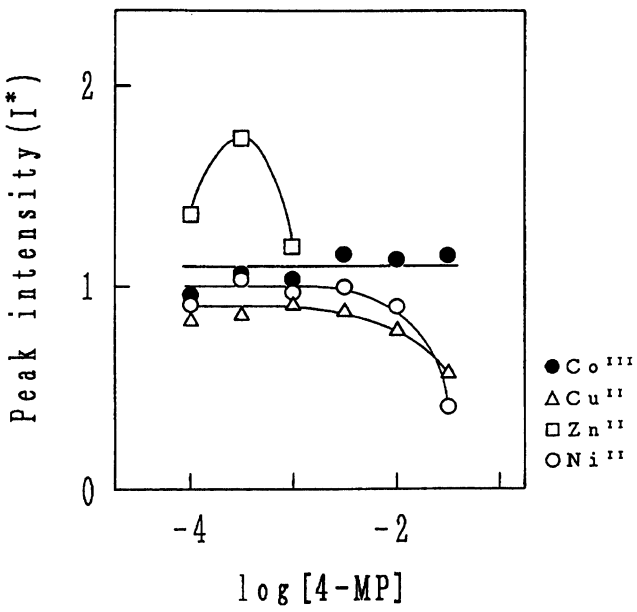

Fig. 4. Effect of 4-MP on the peak intensity of metal-STTA chelates.

$$I^* = \frac{\text{Peak area with 4-MP}}{\text{Peak area without 4-MP}}$$

with the addition of 4-MP, which may be due to the prevention of the chelate against decomposition in the separation column.

Bpy and Phen. Figure 5 shows the results on phen. The retention time of Ni^{II}- and Zn^{II}-STTA chelates did not change remarkably with the increase in phen concentration. The peak of those chelates gradually declined and faded out. That is, phen might act as masking agent (see below).[16] As is seen from the figure, on the other hand, the retention time of Cu^{II}-

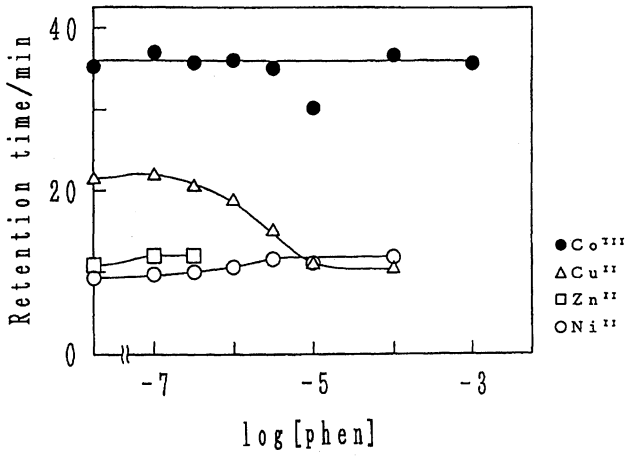

Fig. 5. Effect of phen on the retention time of metal-STTA chelates. The various concentrations of phen were added to the mobile phase of Fig. 1.

STTA chelate decreased gradually with the increase in the phen concentration. Moreover, the peak intensity of the Cu^{II} chelate also decreased. As to Co^{III}-STTA chelate, its retention time also remained constant as well as in the case of 4-MP. Similar results were obtained on bpy addition.

TBP and TOPO. In solvent extraction, TBP and TOPO are the most popular monodentate neutral ligands containing oxygen as a donor atom. When TBP was added to the mobile phase, no remarkable effect was observed on the retention time of STTA chelates. As TBP is a weaker base, the adduct formation might not occur remarkably. Figure 6 shows the change of retention time of metal-STTA chelates with the addition of TOPO which has much higher hydrophobicity and basicity than TBP. The retention time of all the STTA chelates increased with the increase in TOPO concentration. In particular, the separation between Zn^{II}- and Ni^{II}-STTAs were improved with the addition of TOPO as shown in the figure. This situation was also shown in the chromatogram of metal-STTAs with and without TOPO in Figs. 7 and 1, respectively. Moreover, Fig. 8 summarizes the change of peak intensity of STTA chelates with the addition of TOPO to the mobile phase. The peak area of Zn^{II}-STTA increased with the addition of TOPO. The degree of the increase in the peak area of Zn^{II}-STTA with the addition of TOPO was rather greater than that with 4-MP. The enhancement of the Zn^{II}-STTA peak may be due to stabilization of the metal chelate by the adduct formation. On the other hand, higher concentration of TOPO depressed the peak intensities of all the STTA chelates tested. In particular, the peak intensity of Cu^{II}-STTA decreased drastically. The retention time of Co^{III}-STTA chelate, which was not changed with the addition of the other bases, increased with the addition of TOPO as shown in Fig. 6. This may be due to the adduct formation of CoR_3S_2 (R=STTA, S=TOPO), as suggested in the solvent extraction.[17]

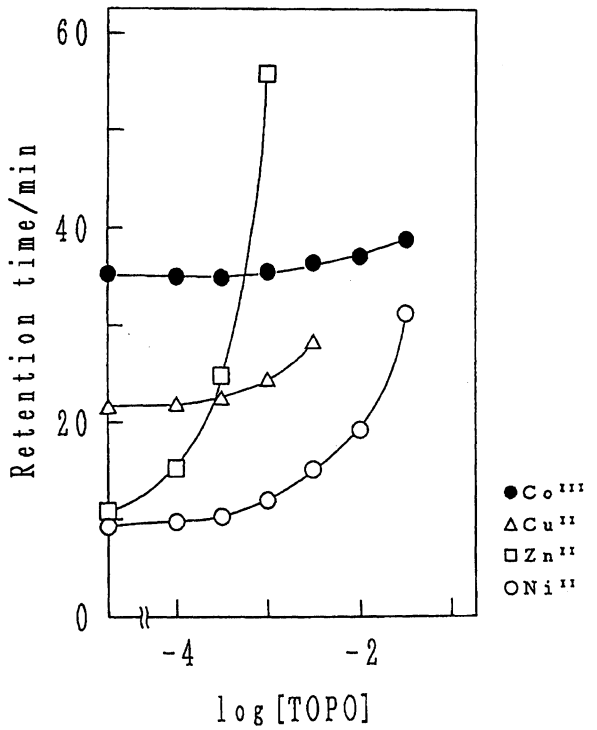

Fig. 6. Effect of TOPO on the retention time of metal-STTA chelates. The various concentrations of TOPO were added to the mobile phase of Fig. 1.

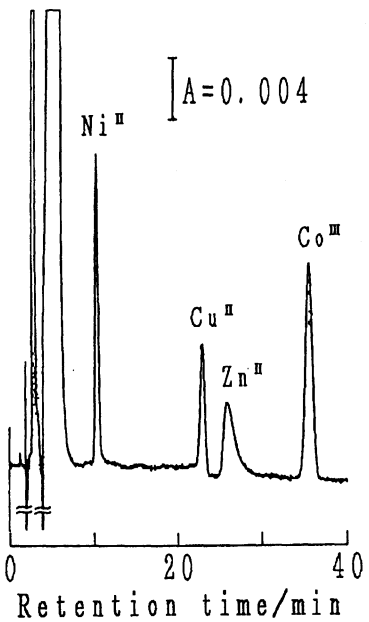

Fig. 7. Chromatogram of metal-STTA chelates with the addition of TOPO to the mobile phase. TOPO (3.0×10^{-4} M) was added to the mobile phase of Fig. 1.

Effects of Various Additives on Separation of Metal-TTA Chelates. Effects of the addition of various additives on the TTA system were also investigated as follows:

4-MP. Figure 9 shows the change of the retention time of metal-TTAs with the addition of 4-MP. The decrease in the retention time of Co^{II}-TTA chelate with the addition of 4-MP was observed, while that of Cu^{II}-TTA remained constant. Although the retention time of Ni^{II}-TTA decreased in lower concentration range of 4-MP, it changed to increase in higher concentration. These retention behavior differed from those of the STTA system in Fig. 3. The peak intensities (peak area) of those three chelates remained constant. The decrease in the retention time of the TTA chelates was also observed with the mobile phase of higher pH (pH=5.7), which was adjusted with ammonia instead of 4-MP. That is, the decrease of the retention time should be mainly due to the change in pH of the mobile phase rather than the adduct formation with 4-MP. On the contrary, the adduct formation for Ni^{II}-TTA might occur to some extent in higher concentration range of 4-MP.

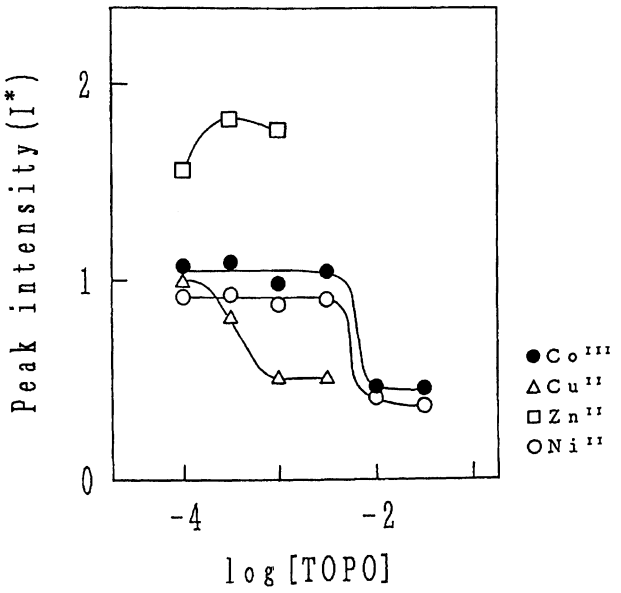

Fig. 8. Effect of TOPO on the peak intensity of metal-STTA chelates.

$$I^* = \frac{\text{Peak area with TOPO}}{\text{Peak area without TOPO}}$$

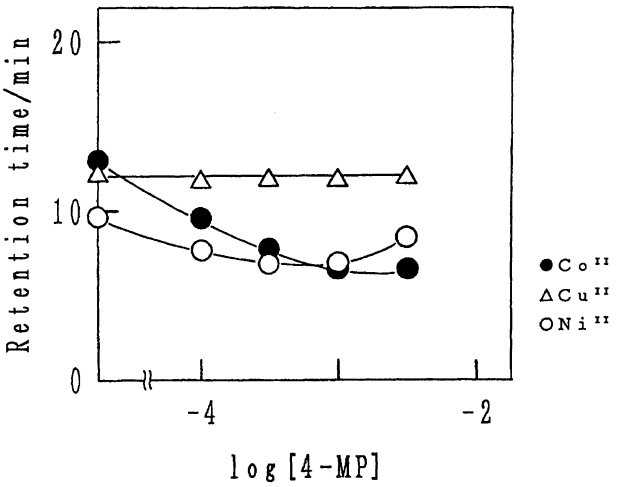

Fig. 9. Effect of 4-MP on the retention time of metal-TTA chelates. The various concentrations of 4-MP were added to the mobile phase of Fig. 2.

Bpy and Phen. Figure 10 shows the change in the retention time of metal-TTAs with the addition of phen. The peak intensity of Ni^{II}- and Cu^{II}-TTA chelates gradually decreased with the increase in phen concentration and the peaks finally faded out. The peak of Co^{II}-TTA vanished even with the addition of 1.0×10^{-7} M phen. Thus, phen may act as a masking agent for those TTA chelates. On the other hand, the retention time of Co^{II}-TTA chelate increased and its peak intensity remained constant with the addition of bpy. The difference between bpy and phen on the retention behavior of Co^{II}-TTA chelate may be ascribed to the difference in stability constants of bpy and phen toward Co^{II} (see below). Since phen is a stronger ligand than bpy,[18] phen might displace the TTA in Co^{II}-TTA chelate, and formed charged complex, $[Co(TTA)(phen)_n]^+$ or $[Co(phen)_n]^{2+}$. The retention behavior of Cu^{II}-TTA chelate with the addition of phen and bpy differed very much from that of Cu^{II}-STTA chelate (see Fig. 5). Since the Cu^{II}-TTA chelate is much less stable than that of STTA, Cu^{II}-TTA chelate is likely to be masked more strongly by phen or bpy.

TOPO. The retention time of Co^{II}-, Ni^{II}-, and Cu^{II}-TTA chelates increased with an increase in TOPO concentration as is seen in Fig. 11. These results were similar to those on the STTA system with TOPO. The peak of Zn^{II}-TTA chelate was not detected even with the addition of TOPO. Figure 12 shows the chromatogram of metal-TTA chelates with TOPO in the mobile phase. The degree of mutual separation of the metal-TTA chelates could be improved in comparison with that without TOPO (Fig. 2). Although the peak

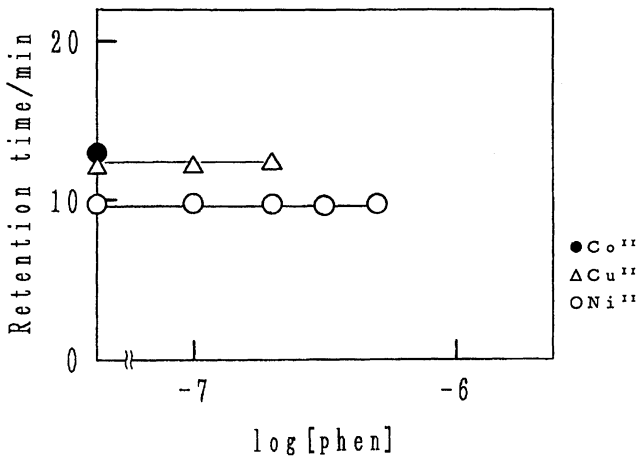

Fig. 10. Effect of phen on the retention time of metal-TTA chelates. The various concentrations of phen were added to the mobile phase of Fig. 2.

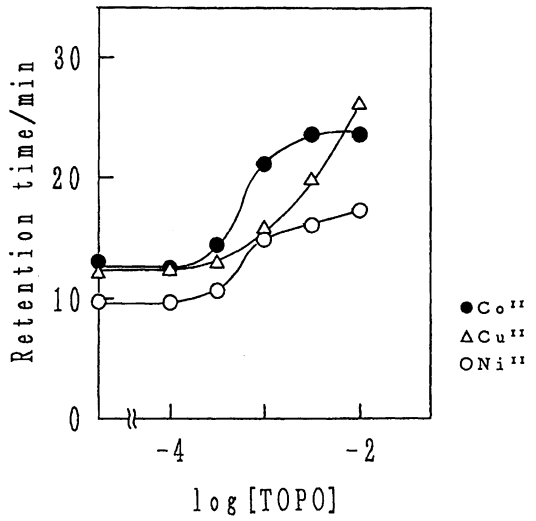

Fig. 11. Effect of TOPO on the retention time of metal-TTA chelates. The various concentrations of TOPO were added to the mobile phase of Fig. 2.

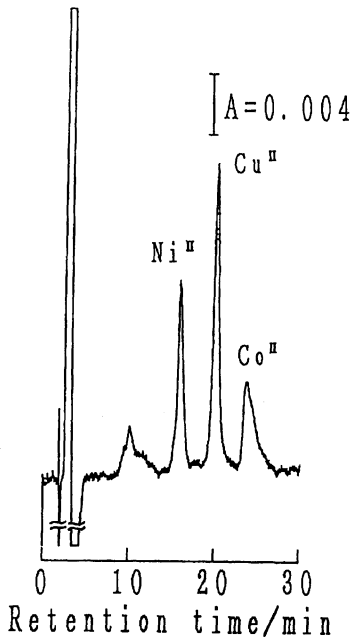

Fig. 12. Chromatogram of metal-TTA chelates with the addition of TOPO to the mobile phase. TOPO (3.0×10^{-3} M) was added to the mobile phase of Fig. 2.

intensity of Cu^{II}-TTA decreased with the increase in TOPO concentration, those of Ni^{II}- and Co^{II}-TTAs increased.

Separation of Metal Chelates in RP-HPLC and Their Extraction Constants in Solvent Extraction. Table 1 summarizes the synergistic extraction constants on various metal chelate systems studied in this paper. Although all the constants in the table were for water–chloroform system, they may still be useful to estimate the stabilities of the metal chelates in HPLC column. The stability of the host

Table 1. Synergistic Extraction Constants on Various Additives[a)]

Aditive	Host chelate	log $K_{ex,0}$	log $K_{ex,1}$	log $\beta_{,1}$
	Ni^{II}-STTA	−2.69	0.41	3.10
	Cu^{II}-STTA	4.10	—[b)]	—[b)]
	Zn^{II}-STTA	−4.48	−0.81	3.67
4−MP				
	Co^{II}-TTA	−7.97	−5.14	2.83
	Ni^{II}-TTA	−8.37	−3.54	4.83
	Cu^{II}-TTA	−2.30	0.42	2.72
	Zn^{II}-TTA	−8.11	−3.59	4.52
	Ni^{II}-STTA	−2.92	—[b)]	—[b)]
	Cu^{II}-STTA	4.60	—[b)]	—[b)]
	Zn^{II}-STTA	−4.71	−1.66	3.05
TOPO				
	Co^{II}-TTA	−8.11	−4.86	3.25
	Ni^{II}-TTA	−8.52	−5.62	2.90
	Cu^{II}-TTA	−1.95	—[b)]	—[b)]
	Zn^{II}-TTA	−8.52	−3.98	4.54
	Ni^{II}-STTA	−2.85	9.00	11.85
	Cu^{II}-STTA	4.10	8.16	4.06
	Zn^{II}-STTA	−4.90	1.70	6.60
phen				
	Co^{II}-TTA	−8.15	2.12	10.27
	Ni^{II}-TTA	−8.52	—[b)]	—[b)]
	Cu^{II}-TTA	−2.25	2.32	4.57
	Zn^{II}-TTA	−8.58	0.05	8.68

a) Unpublished data by Kawamoto and Akaiwa.
b) Ambiguous for adduct formation.

chelates in the mobile phase should be estimated by log $([MR_2]_{aq}/[M^{2+}]_{aq})$, in which,

$$\log([MR_2]_{aq}/[M^{2+}]_{aq}) = \log K_{ex,0} + 2\log[HR] + 2pH - 2\log K_{DR}$$

(see Appendix for derivation of the equation). Since the pH of the mobile phase was ca. 4.3 in both STTA and TTA systems, [HR] was 8.2×10^{-5} M for STTA and 1.2×10^{-3} M for TTA, and log K_{DR} is 0.63 for STTA and 1.73[19)] for TTA respectively, we can get the log $([MR_2]_{aq}/[M^{2+}]_{aq})$ values for our HPLC experiments as summarized in Table 2. As shown in Figs. 1 and 2, Ni^{II}-, Cu^{II}-STTAs, and Cu^{II}-TTA, whose log $([MR_2]_{aq}/[M^{2+}]_{aq})$ values exceed ca. −4.0, gave sharp and intensive peaks. Although no data are available for Co^{III}-STTA, Co^{III} compounds are well known as very inert against the ligand exchange reaction. Thus Co^{III}-STTA also gave very intensive peak. On the other hand, Zn^{II}-STTA and Co^{II}- and Ni^{II}-TTAs gave rather small peaks. Their log $([MR_2]_{aq}/[M^{2+}]_{aq})$ values fall in the range of −9.2 to ca. −5.0. Although Zn^{II}-TTA has almost the same value as those of Co^{II}- and Ni^{II}-TTAs, its chromatogram peak could not be found. These phenomena might be attributable to the difference in the ligand exchange rate in those chelates. For example, the dissociation rate of metal-*N*,*N*,*N*′,*N*′-tetrakis(2-aminoethyl)ethylenediamine chelates is reported to be

Table 2. Values of log $([MR_2]_{aq}/[M^{2+}]_{aq})$[a)]

Metal ion	STTA	TTA
Co^{2+}	—	−8.78
Ni^{2+}	−3.65	−9.17
Cu^{2+}	3.44	−2.87
Zn^{2+}	−5.53	−9.10

a) The following conditions were used for the calculation; STTA system: pH=4.3, $[HR]=8.2\times10^{-5}$ M, log $K_{DR}=0.63$. TTA system: pH=4.3, $[HR]=1.2\times10^{-3}$ M, log $K_{DR}=1.73$.

$Zn^{II}\approx Cu^{II}>Co^{II}\gg Ni^{II}$.[20] This order in the dissociation rate of metal chelates might explain the absence of Zn^{II}-TTA peak in our study. That is, Zn^{II}-TTA may easily be decomposed in the column, while Ni^{II}-TTA may be preserved because of its inertness against ligand exchange. The importance of the kinetic characteristics of metal chelates in RP-HPLC separation was also pointed out by Yotsuyanagi and Hoshino in their kinetic differentiation mode-HPLC.[21]

Effects of Additives on the HPLC Separation and Their Synergistic Extraction Constants in Solvent Extraction. As mentioned above, the adducts formation was observed with the addition of 4-MP and TOPO. On the contrary, when phen or bpy was added, the masking effect, i.e., the decrease of the retention time or the fading-out of the chromatographic peaks, was observed. In the case of 4-MP, the adduct formation on the Zn^{II}-STTA was the most prominent. As is shown in Table 1, the log $\beta_{,1}$ for Zn^{II}-STTA is considerably large. We could also observe the adduct formation on Ni^{II}-STTA, whose log $\beta_{,1}$ is 3.10, although the degree of the effect of adduct formation was much less in Ni^{II}-STTA than in Zn^{II}-STTA. This may be due to the fact that Ni^{II} is inert against ligand exchange. As for the TTA system, only Ni^{II}-TTA, whose log $\beta_{,1}$ value is the largest in Table 1, may form the adduct with 4-MP, although the other metal-TTAs tested are also known to form the adducts in solvent extraction (see Table 1).

The peak intensities as well as the retention times of Zn^{II}-STTA, Co^{II}-TTA, and Ni^{II}-TTA increased with the addition of TOPO, these facts indicating the prominent adduct formation. All of those chelates are known to form the adducts with TOPO in solvent extraction (see Table 1). On the other hand, the peak intensities of Ni^{II}-STTA, Cu^{II}-STTA, and Cu^{II}-TTA decreased with the addition of TOPO, although their retention times were prolonged with the increase in TOPO concentration. These facts might suggest that both the adduct formation and the masking effect with TOPO occurred to some extent at the same time. The synergistic extraction constants with TOPO for Ni^{II}-STTA and Cu^{II}-STTA have not been reported, while that for Cu^{II}-TTA (log $\beta_{,1}=3.98$) has been reported on cyclohexane as solvent.[22] However, the adduct formation with TOPO for these three chelates was ambiguous in our study (see Table 1). It is widely acknowledged in solvent extraction that the extraction constant (log $K_{ex,1}$) of a chelate increases, while the stability constant (log $\beta_{,1}$) of the corresponding adduct decreases.[23] This general rule may explain both the results of RP-HPLC and the data on TOPO in Table 1. Since TOPO is highly hydrophobic and bulky, the TOPO adducts may increase their affinity to the stationary phase remarkably compared with their host chelates. Thus, the effect of the addition of TOPO on the retention time may be much greater than that of 4-MP.

The synergistic extraction constants of phen as an additive are also shown in Table 1. Very large log $\beta_{,1}$ value for phen has been reported in solvent extraction as is shown in this table. Since phen has strong coordination ability to tested metal ions,[18] STTA or TTA in the host chelates may be displaced with phen to form $[MRS_n]^+$ or $[MS_n]^{2+}$ (R=STTA or TTA, S=phen) in the HPLC column. This phenomenon is also known in solvent extraction.[16] Such charged complexes may be stuck to the active site of the stationary phase, causing the fading-out of peaks of those metal-STTA or TTA chelates. The degree of the effect of phen may be partly explained on the basis of ligand exchange rate in the metal chelates. As shown in Figs. 5 and 10, the peaks of Zn^{II}-STTA, Co^{II}-TTA, and Cu^{II}-TTA were faded out in lower concentration range of phen. The ligand exchange rate of Zn^{II}, Co^{II}, and Cu^{II} chelates is known to be relatively fast as mentioned earlier. On the other hand, Ni^{II}-STTA, in which Ni^{II} is inert against ligand exchange, is somewhat resistant with the addition of phen. The peak of Ni^{II}-TTA faded out at ca. 1.0×10^{-6} M phen, which may be due to lower stability of the host chelate (Ni^{II}-TTA) compared with Ni^{II}-STTA. Although Cu^{II} is labile against ligand exchange, Cu^{II}-STTA host chelate is very stable as is shown in Table 2. The coordination of STTA to Cu^{II} is much stronger than that to Zn^{II} and Ni^{II}. Thus, the STTA molecule might not be lost from the Cu^{II} chelate even with the addition of phen. However, due to the Jahn–Teller effect, phen may occupy the equatorial sites and STTA might act as unidentate at the axial position.[24—26] In other words, with the addition of phen the adduct might become distorted and unstable, causing the decrease in its affinity to the stationary phase in RP-HPLC. Similar discussion can be applied to the addition of bpy.

Conclusion

The effects of the addition of various additives to the mobile phase on the separation of metal chelates by RP-HPLC should be understood in terms of the adduct formation and the masking effect. It is possible to change the retention time of the metal chelates by the adduct formation. Moreover, the peak intensities of some metal chelates were enhanced by the adduct formation, which prevented these chelates from decompo-

sition in the column. In particular, the effect of TOPO, which is highly hydrophobic and bulky, was remarkable in this study. On the other hand, phen and bpy showed masking effects. These effects were consistent with their synergistic extraction constants obtained in the chelate extraction systems, although the kinetic aspect in ligand exchange should also be taken into account for the prediction of the effects of the additives in RP-HPLC. Conclusively, the addition of various additive will provide a new technique for controlling the separation of metal chelates and/or enhancing their sensitivities in RP-HPLC.

Appendix

Synergistic Extraction Constant. By assuming that main species extracted with chelating reagent (HR) and additives (S) are MR_2S_n (M=Co, Ni, Cu, Zn, and n=0, 1), the extraction reaction can be expressed by

$$M^{2+} + 2HR_{org} + nS_{org} \rightleftharpoons MR_2S_{n,org} + 2H^+, \quad (1)$$

where the subscript org denotes the organic phase. The extraction constant is defined by Eq. 2.

$$K_{ex,n} = \frac{[MR_2S_n]_{org}[H^+]^2}{[M^{2+}][HR]^2_{org}[S]^n_{org}}. \quad (2)$$

Moreover, the value of log $\beta_{,1}$ is defined by Eq. 3.

$$\log \beta_{,1} = \log K_{ex,1} - \log K_{ex,0}. \quad (3)$$

log ($[MR_2]_{aq}/[M^{2+}]_{aq}$). When n is zero in Eq. 2, $K_{ex,0}$ can be expressed by

$$K_{ex,0} = \frac{[MR_2]_{org}[H^+]^2}{[M^{2+}][HR]^2_{org}}. \quad (4)$$

Then, the distribution coefficient of MR_2 is defined by Eq. 5.

$$K_{DM} = \frac{[MR_2]_{org}}{[MR_2]_{aq}}. \quad (5)$$

Substituting $K_{DM}[MR_2]_{aq}$ in Eq. 5 for $[MR_2]_{org}$ in Eq. 4 and rearranging Eq. 4 gives

$$\frac{[MR_2]_{aq}}{[M^{2+}]_{aq}} = \frac{K_{ex,0}[HR]^2_{org}}{[H^+]^2 K_{DM}}. \quad (6)$$

Then, the K_{DM} value can be assumed to be almost equal to ${K_{DR}}^2$,[27] which is

$${K_{DR}}^2 = \frac{[HR]^2_{org}}{[HR]^2_{aq}}. \quad (7)$$

Here, Eq. 6 can be rewritten as

$$\log ([MR_2]_{aq}/[M^{2+}]_{aq}) = \log K_{ex,0} + 2\log [HR]_{org} + 2pH - 2\log K_{DR}. \quad (8)$$

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