Determination of Co(II) Ion as a 4-(2-Thiazolylazo)resorcinol or 5-Methyl-4-(2-thiazolylazo)resorcinol Chelate by Reversed-Phase Capillary High-Performance Liquid Chromatography

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Received August 20, 2003

Determination of Co(II) ion as a 4-(2-thiazolylazo)resorcinol(TAR) or 5-methyl-4-(2-thiazolylazo)resorcinol (5MTAR) chelate was accomplished by reversed-phase capillary high-performance liquid chromatography (RP-Capillary-HPLC) using a Vydac C_4 column and MeCN-water mixture as mobile phase. The effect of change in pH and MeCN percentage of the mobile phase on the retention factor. k and peak intensity were evaluated. It was found that 30% MeCN (v/v) of pH 5.60 or 7.20 was adequate as mobile phase when TAR or 5MTAR is used. Detection limit (D.L., S/N=3) in each case was 2.0×10^{-7} M (11.8 ppb) and 3.0×10^{-7} M (17.7 ppb). The Co(II) ion in mineral and waste water was determined with the optimum column and mobile phase.

Key Words : Capillary-HPLC. Co(II) ion. 4-(2-Thiazolylazo)resorcinol(TAR). 5-Methyl-4-(2-thiazolylazo)resorcinol(5MTAR)

Introduction

Capillary high-performance liquid chromatography (Capillary-HPLC) has been developed from 1990. and its utility was reported in many analytical journals.[1-3] Although k and sensitivity of a compound in Capillary-HPLC were similar with universal HPLC. a small injection volume (100 nL) and very small quantity of waste production by its lowest flow rate (flow rate: 4.0 ± 0.1 μL/min.) during the experiment were its great advantages.

Metal ions were generally determined with atomic absorption spectrophotometry (AAS) or inductively coupled plasma-atomic emission spectrophotometry (ICP-AES). But. separation and simultaneous determination of mixtures of metal ions as their metal-chelates with an organic chelating reagent by reversed-phase high-performance liquid chromatography (RP-HPLC) had been accepted in inorganic analysis in recent years.[4-8] The organic chelating reagents. were 4-(2-pyridylazo)-resorcinol(PAR).[8,9] 4-(2-thiazolylazo)-resorcinol (TAR).[4,7] 2-(2-thiazolylazo)-p-cresol(TAC),[10] 1-oxa-4,7,10,13-tetraaza-cyclopentadecane (OTAP),[11] and 2-(5-bromo-2-pyridylazo)-5-[N-propyl-N-(3-sulfopropyl)amino]phenol (5BrPSAA).[5] etc. TAR is the one of the popular chelating reagents. It forms either neutral or ionic chelates with many metal ions according to the pH.[13,14,20] Metal-TAR chelates have high molar absorptivity (about 10^5 order ε) and is water soluble. But. determination of metals as metal-TAR by RP-Capillary-HPLC has not been investigated. Even. determination of metals as metal-5MTAR by universal HPLC has not yet been studied. Although metal ion determination with preconcentration processes were reported.[8,9,11,16,17] it was not applied in this study. The objective of this study was selective separation and determination of Co(II) ion as Co(II)-4-(2-thiazolylazo)-resorcinol chelate(Co(II)-TAR) or Co(II)-5-methyl-4-(2-

thiazolylazo)resorcinol chelate(Co(II)-5MTAR) by RP-Capillary-HPLC. To accomplish the purpose. the effects of change in pH and MeCN percentage of the mobile phase on the k and peak intensity of Co(II)-TAR or Co(II)-5MTAR were evaluated. and the optimum mobile phase condition and column were found. Also. the detection limit (D.L.) of Co(II) ion was measured with the optimum mobile phase and column. and Co(II) ion in mineral and waste water were determined.

Experimental Section

Capillary High-Performance Liquid Chromatography (Capillary-HPLC) System and Other Instruments. Capillary-HPLC system was introduced in our previous study.[11] Column and detector which were different from the former apparatus[11] were a Vydac C_4 capillary column (300 μm(i.d.) $\times$ 15 cm(L). 5 μm particle. LC Packings, Netherlands) (denoted as 'C$_4$' hereafter) and a Wellchrom K-2501 UV-visible spectrophotometric detector made by Knauer Co. (Germany. cell volume: 70 nL).

As shown in Figure 1, most of mobile phase was recycled (flow rate: 4.0 mL/min. by M510 Waters HPLC pump) and a small fraction of it flows (flow rate: 4.0 ± 0.1 μL/min.) through the injector, capillary column and detector. and it becomes waste.
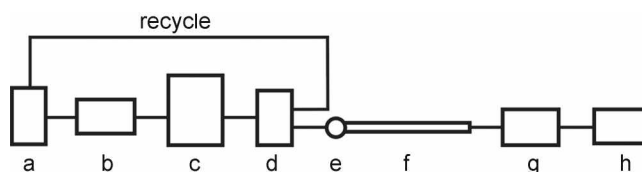

Figure 1. Schematic diagram of Capillary-HPLC system. a: Reservoir, b: Degasser, c: Waters HPLC pump, d: Splitter, e: Injector (6 way valve), f: Capillary column, g: UV-Visible detector, h: X-Y recorder.

The analytical wavelengths (values of λ_{max}) and absorptivities of the compounds were determined by TU-1800 UV-Visible spectrophotometer (P. Genral Instrument Co.). The pH of the mobile phase was measured by Orion 420A pH meter.

Chemicals. The MeCN used as a component of mobile phase was HPLC grade from Tedia Chemical Co. and all other chemicals were obtained from Aldrich and Tokyo Kasei Chemical Co. and used as supplied throughout the experiments. Doubly distilled water was used as a solvent for the preparation of all solutions.

Methods. The concentration of stocked Co(II) ion solution was 1.00 mM and other metal ion solutions were 5.00 mM in 1.00 mM hydrochloric acid solution. The above metal solutions and 2.00 mM TAR or 5MTAR solution dissolved in 50%(v/v) MeCN-water mixtures were used to prepare 2.0×10^{-7} M~5.0×10^{-5} M Co(II)-TAR, Co(II)-5MTAR, other metal(II)-TAR and metal(II)-5MTAR chelate solutions with diluting by the doubly distilled water. These sample solutions were sonicated with USS-1 sonicator (Nihonseki, Japan) and filtered by passing through a Whatman PVDF syringe filter membrane (pore diameter 0.45 μm, 13 mm).

The mobile phases were MeCN-water mixtures (MeCN : H_2O 20 : 80-30 : 70) containing 0.05 M sodium acetate (pH 4.00-5.00), or malonic acid, disodium salt (pH 3.00-3.90 or 5.10-6.50), or tris(hydroxymethyl)aminomethane, hydrochloride (pH 7.00-8.30); to 10.0 mL of 0.5 M the salt solution, 70.0-60.0 mL of the doubly distilled water added, and mixed with 20.0-30.0 mL of MeCN (total volume was 100 mL). The solution was made to the desired pH again by adding a few drops of concentrated hydrochloric acid or 10 M sodium hydroxide solution, sonicated, and filtered by Milli-Pore filtering apparatus containing Milli-pore FH organic filter (pore size: 0.45 μm).

Each components dissolved in an injected sample solution was eluted through the C_4 with the above mobile phase. The k values of the compounds were calculated by the following equation[4,11]

$$k = (t_R - t_0) / t_0$$

where t_R is a retention time(min.) of a compound and t_0 is the retention time of mobile phase (2.20 min.).

The optimum mobile phase condition and column for Co(II) ion determination could be found from the observation of the effects of change in pH and MeCN percentage of mobile phases for the k and peak intensity of the Co(II)-TAR or Co(II)-5MTAR. With the mobile phase of optimum condition, calibration curve was prepared and D.L. (concentration at S/N=3) of Co(II) ion was measured from the peak intensities on the resulting chromatograms of the successively diluted sample solutions.

Co(II) ion-spiked mineral water from the Chojong area and waste water from Cheongju Industrial Environmental Facilities Management Corporation (CIEFMC) located in Cheongju-city, Korea were used as the sample solutions for an application. The sample solutions were pretreated as follows: 20 μL of 1.00 mM Co(II) ion solution and 100 μL of 2.00 mM TAR or 5MTAR in 50%(v/v) MeCN-water were added to 8.0 mL of the mineral or waste water filtered by a Whatman PVDF syringe filter, and it was diluted to 10 mL by the filtered mineral or waste water.

Results and Discussion

Capillary-HPLC System and Detection Wavelengths. A specific characteristics of this system is the role of mobile phase splitter. The splitter plays a role of the best damper[3] and splits the mobile phase by two. It was illustrated in experimental section.

As can be seen in Table 1, λ_{max} of Co(II)-TAR was 483 nm and that of Co(II)-5MTAR was 450 nm. Therefore, 483 nm or 450 nm was used when Co(II) ion determined as Co(II)-TAR or Co(II)-5MTAR, individually. The molar absorptivities of TAR and 5MTAR were 10^4 order, but those of the Co(II)-chelates were 10^5 order.

The Effect of pH on the k and Peak Intensity of the Chelates. Figure 2 shows the effect of pH of the mobile phase on the k values and peak intensities of Co(II)-TAR and Co(II)-5MTAR, TAR, and 5MTAR when the compounds eluted through C_4. The elution order of the compounds from pH 3.5 to pH 7.0 was Co(II)-TAR < TAR < Co(II)-5MTAR < 5MTAR.

It could be observed in Figure 2 from pH 6.0 to 7.2 that k values of TAR, 5MTAR, and Co(II)-5MTAR were abruptly decreased, but the k of Co(II)-TAR was appeared as constant. And, their peak intensities including Co(II)-TAR were abruptly increased in the pH range. This phenomena was explained with the pK values and chelate stability. The pK_1, pK_2, and pK_3 of TAR were reported as 0.59, 6.54, and 11.8, and those of 5MTAR were 0.93, 6.12, and 12.8.[18] Therefore, it could be expected that the abrupt decrease in k values and abrupt increase peak intensities of the compounds at the pH range is due to the ionization of the compounds around the pH that is close to pK_2. And, the considerable increasing of peak intensities of Co(II)-TAR and Co(II)-5MTAR at the pH range is due to the increased quantities of chelates by their increased stability by the ionization of the ligands.

It was thought that most metal-TAR or metal-5MTAR would be dissociated on the stationary phase[8] and didn't appear on the chromatograms at the acidic and neutral solution (pH 2.0-8.0). For example, the following metals did not give any peak in the pH range: Na(I), Mg(II), Ca(II), Al(II), Cr(III), Mn(II), Cu(II), Zn(II), Pb(II), and Cd(II). They

Table 1. Analytical wavelenths (λ_{max}) and molar absorptivities (ε mol $L^{-1}cm^{-1}$) of TAR, 5MTAR, and their metal-chelates at pH 8.5 and 25 °C

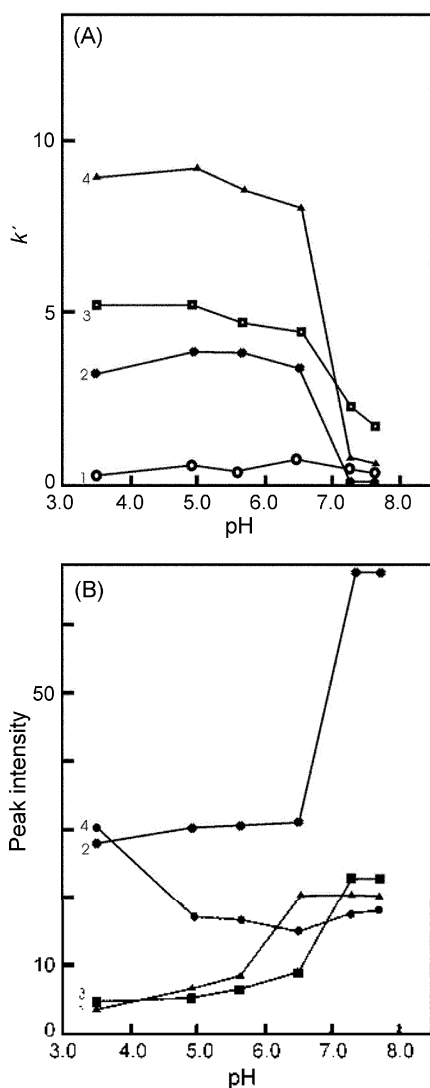| Compound	λ_{max}	ε/mol $L^{-1}cm^{-1}$
TAR	437	20,000
Co(II)-TAR	483	262,000
Ni(II)-TAR	492	110,000
5MTAR	420	17,000
Co(II)-5MTAR	450	199,000
Ni(II)-5MTAR	456	104,400

Figure 2. The effect of pH for the *k* (A) and peak intensity (B) of the compounds on C_4 at 15 °C." "Concentration of the compounds: [TAR] & [5MTAR]: 1.4×10^{-4} M, [Co(II)-TAR]: 1.0×10^{-5} M, [Co(II)-5MTAR]: 2.0×10^{-5}. Identification: 1: Co(II)-TAR. 2: TAR. 3: Co(II)-5MTAR. 4: 5MTAR. Mobile phase: 22.5% MeCN.

Figure 3. Separation chromatograms of Co(II)-TAR and TAR. and Co(II)-5MTAR and 5MTAR by using 22.5%(v/v) MeCN as mobile phase at various pH I." "Column. MeCN percentage in mobile phase. concentration of each compounds in sample solutions. and temperature are same as in Figure 2. Identification: A: Co(II)-TAR. B: TAR. C: Co(II)-5MTAR. D: 5MTAR.

gave no detectable peaks or co-eluted with TAR or 5MTAR.

The effect of pH was also described with Figure 3. The sensitivity of the chelates increased with increasing pH of the mobile phase, and when the pH was increased from pH 6.5 to pH 7.2 on C_4, the elution order between Co(II)-TAR and TAR, or Co(II)-5MTAR and 5MTAR were inversed.

The Effect of MeCN Concentration in Mobile Phase on *k*, Peak Intensity, and D. L. The effect of changing MeCN concentration in mobile phase from 20 to 30% was observed. As can be seen in Figure 4, the *k* values of the compounds were decreased with increasing the MeCN percentage in mobile phase, but peak intensities were increased. Therefore, the lowest D.L. could be obtained with increased MeCN percentage in mobile phase. In this case, the maximum sensitivity of Co(II) ion as Co(II)-TAR or as Co(II)-5MTAR could be obtained at 30% MeCN in mobile phase. This pheomenon was explained in earlier studies.[11,19]
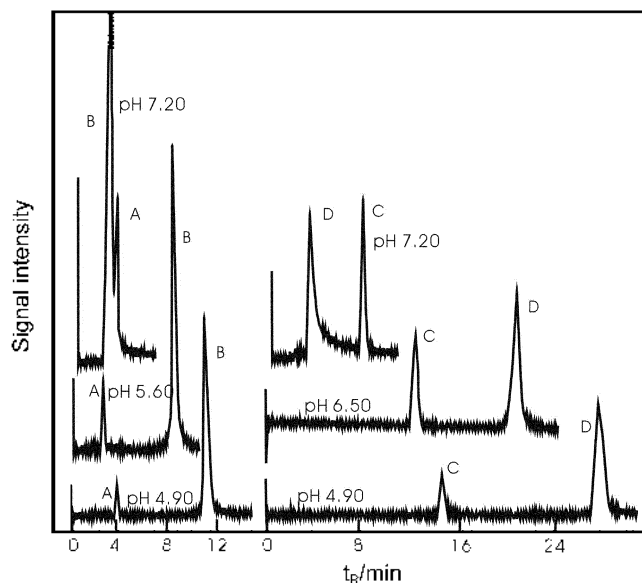
The MeCN percentage in mobile phase was limited from 20 to 30%, because the *k* values of the TAR and 5MTAR were so large when MeCN percentage in mobile phase was less than 20%, and the peak by ligand (TAR or 5MTAR) overlapped with that of metal-chelate(Co(II)-TAR or Co(II)-5MTAR) when MeCN in mobile phase was more than 30%.

It was found that maximum sensitivity of Co(II) ion as Co(II)-TAR could be obtained at pH 5.6 and that as Co(II)-5MTAR could be obtained at pH 7.2. Figure 4 shows the chromatograms of Co(II)-TAR or Co(II)-5MTAR separation from the added excess TAR or 5MTAR. Although pH 6.50 looks like optimum in Figure 3, the peak of TAR interfered that of Co(II)-TAR with the increased MeCN percentage (30%).

The peak intensity by Co(II)-5MTAR with 22.5% MeCN (pH 7.2) as mobile phase was 22.0 for the concentration of 2.0×10^{-5} M (1.18 ppm) in Figure 4. but the value was increased to 48.0 by 30% MeCN at same pH and same concentration. The peak intensity of 1.0×10^{-5} M (0.59 ppm) Co(II)-TAR by 22.5% MeCN at pH 5.60 as mobile phase was 11.0 and 31.5 by 30.0% MeCN at the same pH. Therefore. it was expected that sensitivity by Co(II)-TAR is better than that of Co(II)-5MTAR. It was found that 30%(v/v) MeCN solution at pH 5.60 or 7.20 were the optimum mobile phase when it was measured as Co(II)-TAR or Co(II)-5MTAR, individually.

D.L. was determined with the chromatograms obtained by successive dilution of the standard stock solution of Co(II) ion. With the method, D.L. values of 2.0×10^{-7} M (11.8 ppb) or 3.0×10^{-7} M (17.7 ppb) were obtained when Co(II) ion was measured as Co(II)-TAR or Co(II)-5MTAR by elution with the optimum mobile phase.
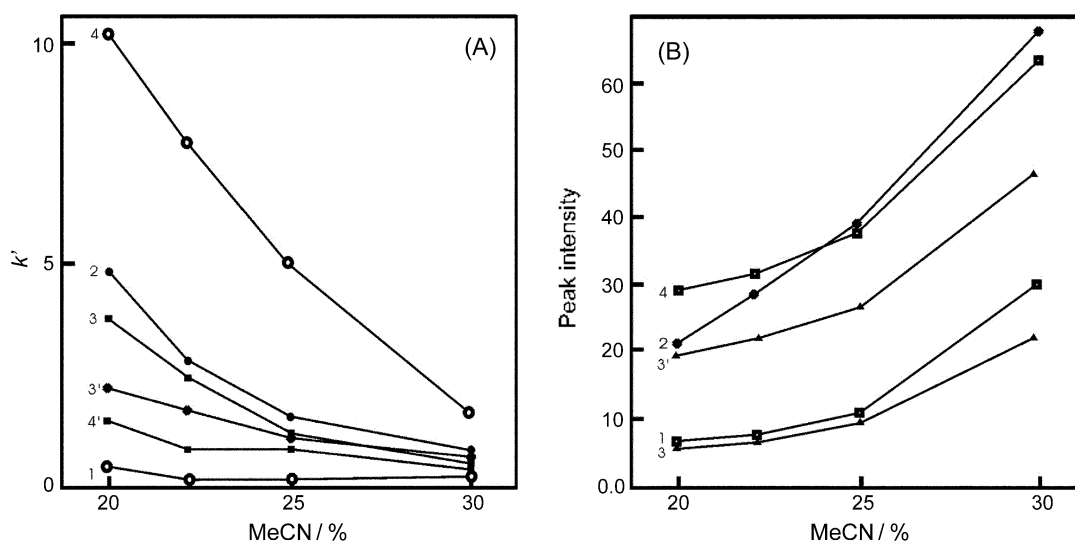
Figure 4. The effect of MeCN percentage in mobile phase for the *k* and peak intensity of the compounds on C₄ at pH 5.60 and 7.20.[a] [a]Concentration of each compounds and temperature are same as in Figure 2. Identification: 1: Co(II)-TAR at pH 5.60. 2: TAR at pH 5.60. 3: Co(II)-5MTAR at pH 5.60. 3': Co(II)-5MTAR at pH 7.20. 4: 5MTAR at pH 5.60. 4': 5MTAR at pH 7.20.

Table 2. Determination of Co(II) ion spiked in mineral and waste water as Co(II)-TAR(A) or Co(II)-5MTAR(B)[a]

Sample solution	Co(II) ion spiked/ppb	Measured compound	Found ppb[b]	Found Recovery %
Mineral water	117.2 (2.0 × 10⁻⁶ M)	A	119.0 ± 5.5 (123.6 ± 8.5)[c]	101.5 (105.5)[c]
		B	122.5 ± 5.5	104.5
Waste water	117.2 (2.0 × 10⁻⁶ M)	A	121.5 ± 5.5 (110.5 ± 8.5)[c]	103.7 (94.3)[c]
		B	118.0 ± 5.5	100.7

[a]Column: C₄. Mobile phase: 30% MeCN at pH 5.6 for A and at pH 7.2 for B. Temperature: 15 °C. [b]Averagerelative ± standard deviation. [c]GF-AAS method.

Co(II) ion Determination in Mineral and Waste water.

Co(II) ion spiked in mineral and waste water were prepared as described in the experimental section. Table 2 shows the recovery percentage by the procedure. The recovery percentage was 100 ± 5.0% (R.S.D. < 5.0%). Therefore, it was thought that Co(II) ion existing as ppb level in mineral and waste water could be determinated as Co(II)-TAR or Co(II)-5MTAR with UV-Visible spectrophotometric detection by RP-Capillary-HPLC.

Conclusions

Determination of Co(II) ion as Co(II)-TAR or Co(II)-5MTAR by RP-Capillary-HPLC was possible without any interferences by universal metal ions, such as Na(I), Ca(II), Mn(II), Fe(II), Fe(III), Ni(II), Cu(II), Zn(II), Cd(II), and Pb(II) ion. The analytical wavelengths were 483 or 450 nm, and the optimum mobile phases were 30% MeCN at pH 5.6 or 7.2 when the Co(II) ion determined as Co(II)-TAR or Co(II)-5MTAR, respectively. In each case, the D.L. values were 11.8 or 17.7 ppb. The Co(II) ion existing as ppb level in mineral and industrial waste water were favorably determined as Co(II)-TAR or Co(II)-5MTAR with C₄ and the mobile phase.

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