Ion-Pair Liquid Chromatographic Elution Behavior of Mercury(II)-4,7,13,18-Tetraoxa-1,10-diazabicyclo[8,5,5]-eicosane Complex Using Sodium 1-Octanesulfonate and Benzyltrimethylammonium Chloride in a MeOH-Water Solution as the Mobile Phase

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Because of its toxic effect, separation and determination of mercury in environmental samples has been an important research area.[1,3] There have been some reports related to separation and determination of mercury after pretreatment and preconcentration of the samples; Garcia *et al.* extracted mercuric ions in the sea water by diethyldithiocarbamate sodium salt, pyrrolidin-1-yldithioformate, or dithizone in solid sorbent and determined its amount with cold vapour atomic absorption spectrometry.[2] Determination of mercury in environmental substances using on-line microwave digestion and atomic flourescence spectrometry was studied by Morales-Rubio *et al.*,[3] and use of arylidenerhodamines for determination of mercury was tried by Tarek *et al.*[4]

On the other hand, metal ions form stable complexes with macrocyclic polyethers. The stability of these complexes depends upon the relative size of the ionic radius of the cation to the cavity in the macrocyclic polyether. As an example, the mercuric ion could be selectively extracted with ionizable dibenzobistriazolo-crown ether in supercritical carbon dioxide: Wang *et al.*[5] used the crown ether as an extractant in methanol(5%) modified CO_2 to extract the ion quantitatively from sand and cellulose-based filter paper at 60 ℃ under 200 atm.

Separation of mercuric ion illustrated above was performed by extracting mercuric ions with a chelating agent dissolved in an organic solvent or supercritical CO_2, and determination, by AAS[2], AFS[3], or NAA[6]. There has been no study on ion-pair liquid chromatographic elution behavior of Hg(II)-cage-type bicyclodiazacrown ether complex. Compared to monocyclic diazacrown ether, cage-type bicyclic diazacrown ether, whose two bridgeheads consist of two nitrogen connected groups, bind metal ions tightly into the space of the lattice with higher ion selectivity and greater stability.[7] For this reason, considerable attention has been paid to these compounds, as well as monocyclic crown and azacrown ethers.

Studying the ion-pair liquid chromatography of metal ion-diazacrown ether complexes, we found that Hg(II)-4,7,13,18-tetraoxa-1,10-diaza[8,5,5]-eicosane(TODABE) complex has a specific elution behavior.[10] TODABE is a cage-type bicyclodiazacrown ether. This paper reports the results.

Experimental

Apparatus. Ion-pair liquid chromatographic elution behavior of Hg(II)-TODABE complex was observed with a Waters Associates high performance liquid chromatograph (HPLC) composed of a M510 dual pump, an U6K injector, a M441 discrete wavelength UV-VIS spectrometric detector, a M740 data recorder, and a temperature control system (TCS). A Supelco LC-18 separation column (4.6 mm×15 cm, 5 μm particle) was used to elute metal ion-TODABE complexes. Void volume of the column is 1.02 mL. Temperature of the column was controlled by the TCS. Dissolved gases in mobile phases were eliminated by sonicating with a Branson M2200 sonicator. Eluents were filtered through a Millipore 0.5 μm organic filter before use. The flow rate of the mobile phase was controlled to 1.0 mL/min throughout. For pH measurements, a Chemcadet M5894 pH meter was used.

Reagents. All of the solutions were prepared with ultrapure water which was obtained by a Milli-Q Water purification System (Millipore Co.). All reagents, such as TODABE, sodium 1-octanesulfonate (1-OSANa), benzyltrimethylammonium chloride (BTMACl), and metal salts, were of analytical reagent grade. They were obtained from Aldrich Chemical Co., and used as supplied. Standard solutions of Cd(II), Cu (II), Hg(II), Ni(II), Fe(III) and Zn(II) were prepared from their chloride salts and dilute hydrochloric acid (pH 2.0). Accurate concentrations of these solutions were determined by complexometric titration with EDTA.

Standard procedure. In a 10 mL volumetric flask, an aliquot of the mercuric ion solution (0.2-1.0 mL of 5.0×10^{-3} M) and 1.0 mL of 5.0×10^{-3} M TODABE solution were pipetted. The flask was filled to the mark with water, tightly stoppered, and allowed to stand at ambient temperature for 24 hrs to ensure complete Hg(II)-TODABE complex forming.

The mobile phases were pH controlled methanol-water (30 : 70, 40 : 60, or 50 : 50) solutions containing 1-OSANa (2.0×10^{-4}-2.0×10^{-2} M) and BTMACl (2.0×10^{-4}-4.0×10^{-3} M). The mobile phase was eluted through a Supelco LC-18 column with a flow rate of 1.0 mL/min untill the absorbance appeared to be constant at a wavelength of 254 nm. A frontal chromatogram was recorded while the mobile phase eluted. From the frontal chromatogram, adsorption equilibrium time of the ion-pairing reagents on the stationary phase was determined. The effective time to obtain constant absorbance was found 1 hr. When the column equilibrium was completed, 10-20 μL of the sample solution was injected. The capacity factor (k') of Hg(II)-TODABE complex was calculated by the following equation:

$$k' = (t_r - t_o)/t_o$$

where t_r is the retention time of the complex and t_o is that of the mobile phase. The concentration of the Hg(II) ion in the sample solution was determined by measuring the peak area. System peaks were confirmed by injecting 20 μL of 40% MeOH solution including 0.003 M BTMACl and 40% MeOH one after another. After k' values were measured or separation experiments performed in a given condition, the column was washed with a methanol-water (80 : 20) solution.

Determination of spiked mercury in artificial sea water and industrial waste water. The mercuric ion spiked artificial sea water sample was prepared as follows; 3.0 g of sodium chloride, 0.45 g of magnesium chloride, 0.30 g of calcium chloride, and 0.54 g of mercuric chloride were dissolved in 50 mL of distilled water, put into a 100 mL

volumetric flask, and water was filled to the mark. On the other hand, heavy metal ions were also spiked in the industrial waste water because it includes them under the detection limits. The solution was prepared as follows; 0.54 g of mercuric chloride, 0.10 g of lead nitrate, 0.10 g of ferric chloride, and 0.10 g of cadmium nitrate were dissolved in 100 mL of industrial waste water from the Cheongju industrial waste water treatment area.

Results and Discussion

Effect of methanol percentage in the mobile phase on the k'. The k' value of Hg(II)-TODABE complex was measured over the methanol composition range in the mobile phase from 30 to 50%(v/v). The k' value decreased with increase of the methanol percentage due to decreased interaction between the complex and the stationary phase (Table 1). The Hg(II)-TODABE complex has smaller k' values than those of Cu(II) and Pb(II)-TODABE complex.

Effect of temperature on the k'. The k' value of the Hg(II)-TODABE complex was measured at various column temperatures from 25 ℃ to 50 ℃. The plot of 1/T *vs.* k' is given in Figure 1. The calculated value of adsorption enthalpy (ΔH) based on the slope of the line in the figure is −2.57 kJ/mol.

Effect of the concentration of 1-OSANa and BTMACl in mobile phase on the k'. In this study, combined effects of varying concentrations of anionic and cationic ion-pairing reagents on the elution behavior of Hg(II)-TODABE complex were examined. Selected anionic and cationic ion-pairing reagents are 1-OSANa and BTMACl. We can assume that BTMACl acts as an indirect detection reagent[8,9,10] as well as a competitive ion-pairing reagent for the Hg(II)-TODABE complex with the 1-OSANa. Figure 2 shows the effect of the concentration of 1-OSANa and BTMACl on the k' of Hg(II)-TODABE complex. The k' value decreases as the concentration of BTMACl increases while the concentration of 1-OSANa is kept 0.010 M, and increases as the concentration of 1-OSANa increases while the concentration of BTMACl is kept 0.003 M. Also, the retention time and peak band width decrease upon increasing the concentration of BTMACl from 2×10^{-4} M to 3×10^{-3} M while the concentration of 1-OSANa is kept 0.010 M (Figure 3 (A, B, C, D)). S_1 and S_2 in Figure 3 are system peaks caused by water in the sample solution and detection reagent (in this case BTMACl).[10] These phenomena were simply explained by the fact that the adsorbed ion-paired complex with the 1-OSANa on the stationary phase can be exchanged with the higher concentration of BTMACl more effectively than with the lower one. When the concentration of 1-OSANa was increased in the eluent, the adsorbed quantity on the stationary phase would be increased and the complex can be adsorbed more efficiently to give a larger k' value, because it is cationic.

Although k' value decreased upon decrease of 1-OSANa concentration, it took more time to obtain stable base line (more than 2 hrs). Hence, we chose an eluent containing 0.010 M 1-OSANa and 0.003 M BTMACl for separation and determination of Hg(II)-TODABE complex.

Metal ions, such as Cd(II), Cu(II), Fe(III), Ni(II), Pb(II), and Zn(II), did not interfere. Their retention times were different from that of Hg(II) (Table 1); The metal ions-TODABE complexes were eluted near the first system peak (S_1 in Figure 3) and Hg(II)-TODABE complex was eluted near the

Table 1. Effect of MeOH percentage in mobile phase containing 0.010 M 1-OSANa and 0.003 M BTMACl (pH 5.5) on the k' of Hg(II)-TODABE

Metal ion-TODABE complex	MeOH, %		
	30	40	50
Hg(II)	10.2(36.5)[a]	5.6(12.0)	4.4(5.5)
Cu(II)	12.0(30.0)	7.0(7.50)	4.5
Pb(II), Cd(II) Fe(III), Ni(II), Zn(II)	12.0	7.0	4.5

[a] 0.0004 M BTMACl in mobile phase.

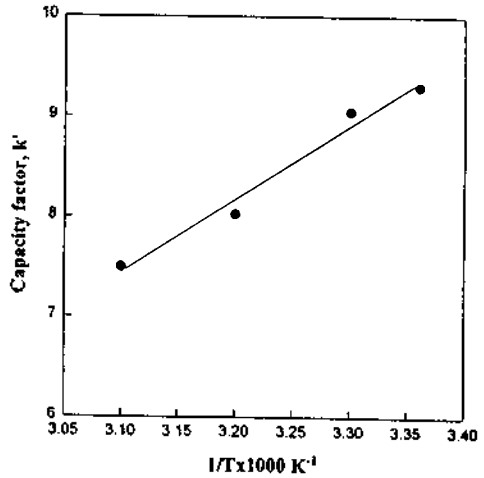

Figure 1. Plot of 1/T *vs.* k'.

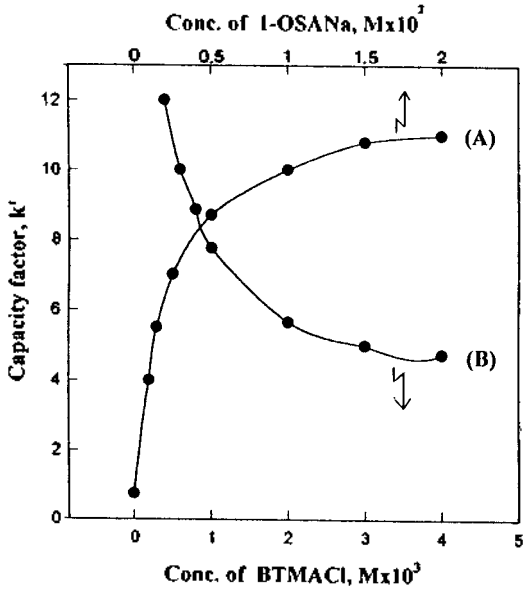

Figure 2. Effects of the concentration of 1-OSANa(A) and BTMACl(B) on the k' of the Hg(II)-TODABE complex. Mobile phase; 0.003 M BTMACl in 30%(v/v) MeOH, pH 5.5(A), 0.010 M 1-OSANa in 40%(v/v) MeOH, pH 5.5(B).

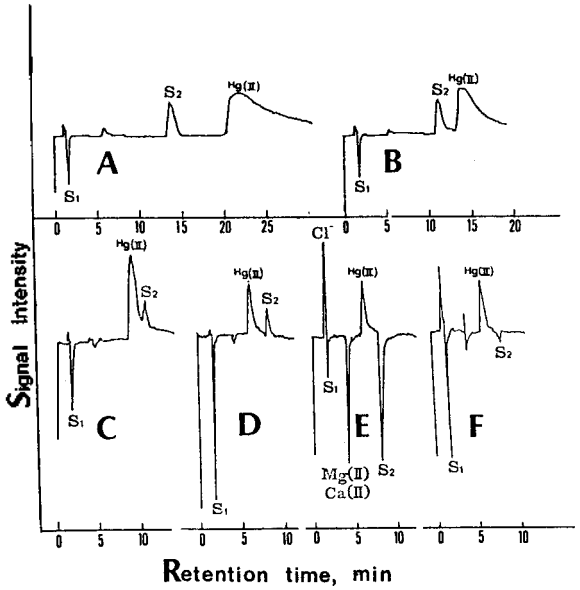

Figure 3. Chromatograms of Hg(II)-TODABE complex. Mobile phase; 0.010 M 1-OSANa and 0.0002 M(A), 0.0003 M(B), 0.0010 M(C), 0.0030 M(D, E, F) BTMACl in 40% MeOH(v/v), pH 5.5. Sample; 20 μg of Hg(II)(A), 10 μg of Hg(II)(B, C), 5 μg of Hg(II) (D) in 10 μL of aqueous solution, 5 μg of Hg(II) in 10 μL of artificial sea water (E) and industrial waste water (F). S_1 and S_2: system peaks.

Table 2. Recovery of Hg(II) ion spiked in artificial sea water and industrial waste water

Sample	Number	Hg(II) injected, μg	Hg(II) determined, μg[a]	Recovery, %
Hg(II) spiked	1	4.00	3.88	97.0± 3.5
artificial sea	2	7.00	7.05	100.7± 2.3
water	3	10.00	10.30	103.0± 4.2
Cd(II), Cu(II),	1	4.00	3.80	97.5± 4.5
Pb(II), Zn(II)	2	7.00	7.20	103.0± 2.8
spiked industrial	3	10.00	9.60	96.0± 4.5
waste water				

[a] Average of triplicate determinations.

second system peak (S_2 in Figure 3).[10]

Determination of spiked mercury(II) ion in artificial sea water and industrial waste water. The artificial sea water spiked with the mercuric ion and industrial waste water spiked with Cd(II), Cu(II), Pb(II), Zn(II), Fe(III), and Hg(II) ions were treated with standard procedures and injected in the HPLC system. One of the results is shown in Figure 3(E, F) and analyses of spiked Hg(II) ion in artificial sea water and industrial waste water are shown in Table 2 for 3 different concentrations of spiked Hg(II). The recovery percentages of Hg(II) in each sample ranged from 96% to 103% and R.S.D. values were 2.8-4.5%.

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Structure-Specific Cleavage of Yeast $tRNA^{phe}$ by (η^6-mesitylene) manganese(I) tricarbonyl hexafluorophosphate

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Determination of the tertiary structure of RNA molecule is essential in understanding its biological function. Recently, several synthetic redox-active coordination complexes have been used as chemical nucleases in probing the secondary and tertiary structures of nucleic acids.[1] Some of these complexes exhibit structure-specific cleavage patterns. 1,10-phenanthroline copper(I) [$(OP)_2$-Cu(I)], for example, exhibits a strong scission preference for the single-stranded loops of stem-loop structure in tRNA[2]; methidium propyl-EDTA iron (II) [MPE-Fe(II)] has a reactivity pattern with tRNA that is complementary to $(OP)_2$-Cu(I), preferring double-stranded regions.[3] Recently, we have shown that MPE-Fe(II) exhibits a scission preference for terminal regions of helices and/or for unstable helical regions of 5S rRNA.[4]

Here we report that an octahedral coordination complex (η^6-mesitylene) manganese(I) tricarbonyl hexafluorophosphate [MTH-Mn(I)][5] exhibits a structure-specific cleavage

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