

Ion-Pair High Performance Liquid Chromatographic Retention Behavior of Mercury(II)-4,7,13,18-tetraoxa-1,10-diazabicyclo[8,5,5]icosane Complex and Selective Determination of the Mercuric Ion in Waste Water by Forming of the Complex

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Because of its toxic effect on human health, various methods of determination of Hg(II) ion in aqueous solutions have been developed.¹⁻⁶ The studies are mostly related with atomic absorption spectrophotometry (AAS) after preconcentration by a chelating resin; Wang *et al.* found the method of preconcentration of mercuric ion in aqueous solution by using two sep pak C₁₈ cartridge columns and sodium dimercaptopropane-1-sulfonate as chelating agent and determination of the adsorbed mercuric ion by graphite furnace AAS after the elution with small volume of MeOH.¹ A study on the sample flow injection in-line sorbent extraction coupled with continuous cold vapour AAS for mercuric ion detection was performed by Garcia *et al.*² Das *et al.* developed the preconcentration method of mercuric ion in industrial and laboratory waste water by a column packed with styrene-divinylbenzene copolymer derivatized by quinaldinic acid amide group as stationary phase and determined mercuric ion in MeOH eluate by AAS.⁴ But, these works were sometimes interfered by heavy metal ions.

In the previous studies, we found the combination effect of sodium 1-octanesulfonate (SOS) and benzyltrimethyl ammonium chloride (BTMACl) in the mobile phase on the retention of the Hg(II)-4,7,13,18-tetraoxa-1,10-diazabicyclo[8,5,5]icosane (Hg(II)-TODABE) complex in ion-pair high performance liquid chromatography (IPHPLC) with indirect spectrometric determination.⁷ The unsymmetrical cage-type bicyclic diazacrown ether, TODABE, has specific selectivity on mercuric ion and the complex shows special retention for the μ -Bondapak CN stationary phase compared to monocyclic diazacrown ethers.⁸ But, the peak by the complex was a little interfered by the system peak.

The aim of this work was selective determination of mercuric ion in waste water by IPHPLC. The mercuric ion in the sample solution was changed to Hg(II)-TODABE complex by adding TODABE and the mobile phase was MeCN solution containing sodium dodecyl sulfate (SDS) and sodium 1-naphthalenesulfonate (SNS) as an ion-pairing and a detection reagent at the indirect spectrometric detection, respectively. Hence, the combination effect of SDS and SNS instead of SOS and BTMACl in the former work was observed. A best peak of the complex could be obtained.

Experimental Section

Apparatus. The observation of the retention behavior of

Hg(II)-TODABE complex was accomplished with a high performance liquid chromatograph made by Waters Associate Co. equipped with a M510 dual piston pump, an U6K universal injector, a M441 UV/Visible spectrophotometric detector, and a M740 recorder. A μ -Bondapak CN column (3.9 mm \times 15 cm, 5 μ m particle) was used to separate Hg(II)-TODABE complex from co-existing chemical species. The column was temperature controlled with Waters Temperature Control System consisting of a temperature control module and a column heater.

A DP-215M pH meter made by Dongwoo Medical System Co. (Korea) was used for the pH measurement of the mobile phase during the study. Dissolved gases in the mobile phase were expelled by sonicating with a M220 sonicator made by Nihonseiki Kaisha Co. and filtering through a 0.45 μ m Nylon-66 membrane filter in a Waters filtering set.

Reagents. All reagents, such as TODABE, SDS, SNS, etc., were obtained from Aldrich or Lancaster Chemical Co. and used as supplied without further purification. MeOH and MeCN used as a component in the mobile phase were purchased from Tedia Chemical Co. Inc. and used also as supplied. Distilled water prepared by a Büchi Fontavapor M201 distilling apparatus was deionized by passing through a Waters Milli-Q water purification system and used throughout the experiment. Standard solution of Hg(II) ion was prepared from their chloride salt and dilute nitric acid (pH 2.0).

Standard Procedure. The standard Hg(II)-TODABE complex solutions were prepared as follows; 54.30 mg of mercuric chloride (F.W.: 271.5) was weighed, put into 100 mL volumetric flask, dissolved, and diluted to the volume with water (2.0 mM). 0.5-2.0 mL of the 2.0 mM Hg(II) ion solution was pipetted into 10 mL volumetric flasks, 6 milliliters of 2.0 mM TODABE solution were added into each mercuric ion solutions, diluted to the volume with water, and let stand for 1 h.

The mobile phases were pH controlled MeCN solution (MeCN : H₂O 20 : 80) containing SDS (5.0-20 mM), SNS (0.2 mM), and CH₂ClCOOH (pH 2.0-3.5, 25 mM) or ammonium acetate (pH 4.0-8.0, 25 mM). The mobile phase was passed through the μ -Bondapak CN column at a flow rate of 1.0 mL/min until SDS is equilibrated on the stationary phase (1-1.5 h)⁷. The complex was detected by the M441 UV/Visible spectrophotometric detector at the wavelength of 254 nm.

The k' values were calculated by the following equation. $k' = (t_R - t_M) / t_M$, where t_R is the retention time of the complex

and t_M is that of solvent in the mobile phase.

Waste water from Environmental Facilities Management Corporation (EFMC) in Cheongju city was used as a sample solution for application. For the examination of the possibility of the mercuric ion determination in the waste water by calibration method with accuracy test, it was pretreated as follows; 27.15 mg of $HgCl_2$ and 57.68 mg of TODABE (F.W.: 288.84) were weighed, put into 100 mL volumetric flask, and diluted to the volume with the waste water (200.6 ppm). The solution was successively diluted with waste water by 20, 4, and 2 times (10.03, 50.15, and 100.3 ppm, respectively), and filtered by a 0.45 μm Nylon-66 membrane filter in a Waters sample filtering set.

Results and Discussion

The Effect of pH. The k' of the complex was measured with changing the pH of the mobile phase from pH 2.0 to 8.0. The peak by the Hg(II)-TODABE complex obtained on the chromatogram was single and sharp (Figure 1). The effect of pH on the k' is shown in Figure 2. The k' decreases as the pH of the mobile phase increases from pH 2.0 to 4.5, whereas increases as the pH increases from pH 4.5 to 8.0. This phenomena were explained as follows; The strength of positive charge of the nitrogen atoms in the ligand ring of the complex increases as the pH of the mobile phase decreases from pH 4.5 to 2.0. Therefore, the ion-pairing of the complex with the SDS on the stationary phase is increased at the lower pH and it makes the k' large. On the other hand, the increase of pH from 4.5 to 8.0 makes the ring neutral and the attraction of the ligand by the neutral stationary phase increases while the attraction by ion-pairing of the metal ion in the complex with SDS on the stationary phase is similar.

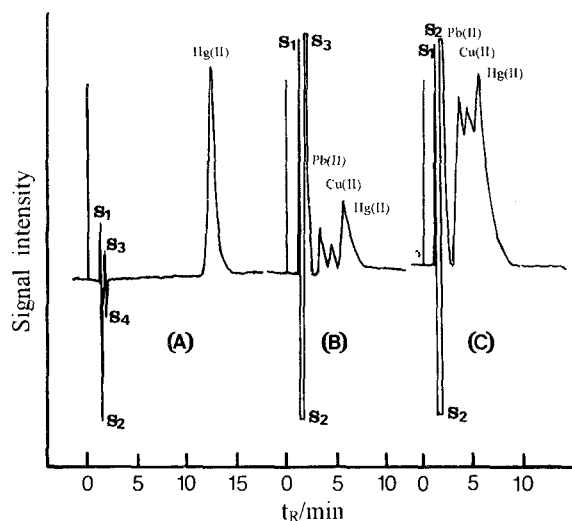


Figure 1. Chromatograms of Cu(II), Hg(II), and Pb(II)-TODABE complexes at pH 2.8 (A) and pH 6.5 (B and C). Mobile phase; MeCN solution (MeCN : H₂O 20 : 80) containing SDS (10 mM) and SNS (0.2 mM). Peak identification; S₁-S₄: System, Metals injected; (A): 4.00 μg respectively (Cu(II), Pb(II), and Hg(II)), (B): 20.7 μg of Pb(II), 6.35 μg of Cu(II), and 1.3 μg of Hg(II), (C): 62.1 μg of Pb(II), 19.1 μg of Cu(II), and 4.00 μg of Hg(II).

In Figure 2, the effect of pH on the k' of Hg(II)-1-oxa-4,7,10,13-tetraazacyclopentadecane (Hg(II)-OTACP) complex is compared with that on Hg(II)-TODABE. The OTACP is another azacrown ether which is synthesized by Kim *et al.* in one of our previous studies.^{9,12} It was found that the peak by the Hg(II)-OTACP complex didn't appear at the lower pH (pH < 4.5), the k' of the complex increases as the pH of the mobile phase increases from 4.5 to 6.0, and appears similar value at pH 6.5 and 8.0. This means the complex is dissociated at the lower pH.

The Effect of SDS Concentration on k' . As can be seen in Figure 3, k' of the Hg(II)-TODABE complex was measured with increasing the SDS concentration from 5.0 mM to 20 mM at pH 2.8 and 6.5. The k' of the complex increases as the concentration increases from 5.0 to 7.5 mM, whereas decreases as the concentration increases from 7.5 to 20 mM. Kim *et al.* illustrated the phenomena as an ion-pairing on the stationary phase for the increasing region of the k' and an interaction of the SDS micelle in the mobile phase with sample molecule for the decreasing region.¹⁰ For the increasing region of the k' , the positively charged complex attracted strongly by the negatively charged SDS on the stationary phase by the electrostatic force as the adsorbed quantity of SDS monomers on it increases by the increased concentration of the SDS in the mobile phase.

On the contrary, for the decreasing region (SDS concentration > 7.5 mM), the number of the SDS micelles are increasing as the concentration of SDS is increasing. Therefore, the electrostatic attraction of the positively charged complex with the increased number of SDS micelles in the mobile phase is increasing. Because the SDS micelles are more negatively charged than the SDS monomers, the positively charged complex is more attracted to SDS micelles in the mobile phase than to the SDS monomers adsorbed on the CN stationary phase. Hence, the k' is decreased.

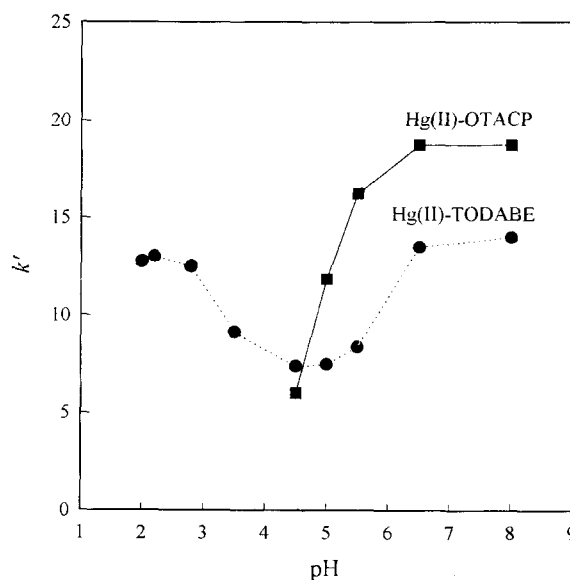


Figure 2. Effect of pH on the k' of mercury(II)-azacrown ether complexes. Mobile phase; MeCN solution (MeCN : H₂O 20 : 80) containing SDS (10 mM) and SNS (0.2 mM) at 25 °C.

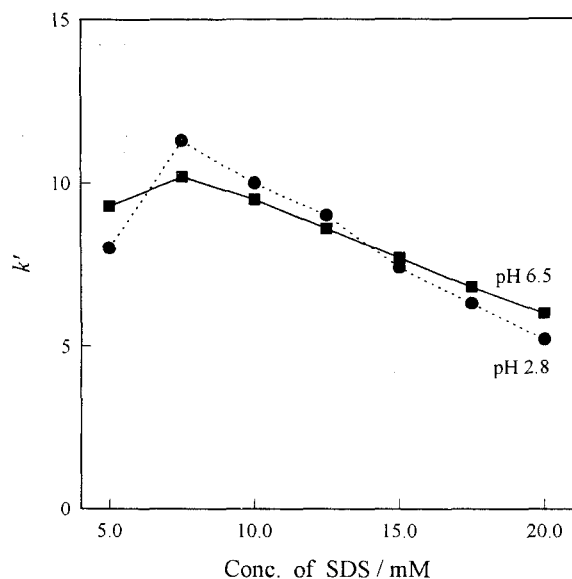


Figure 3. Effect of the SDS concentration on the k' of Hg(II)-TODABE complex.

The maximum value of k' of the complex is appeared at the boundary point concentration of SDS illustrated as the ion-pair forming on the stationary phase and the attraction of the complex by the micelles formed in the mobile phase. The boundary point concentration of SDS is specific to each compound; 5.5 mM for nucleosides,¹⁰ 7.5 mM for the Hg(II)-TODABE complex (this study), and 10 mM for the noble metal-thiacrown ether complexes.¹¹

Separation of the Metal Ions-TODABE Complexes and Indirect Spectrophotometric Detection. The system peaks (peaks of MeCN, water, and SNS) in this work appeared within 3 min after injection of the sample solution, as can be seen in Figure 1. It was great advantage of this work, because system peaks in indirect spectrophotometric detection method sometimes interfere the sample peak.^{7,12} The negatively charged SNS cannot be attracted by the neutral stationary phase strongly or same negatively charged SDS on it.

The heavy metal ion-TODABE complexes peaks on the chromatogram were not found at the lower pH (pH < 4.5) (Figure 1(A)). It was expected that Cu(II)- and Pb(II) ions couldn't form complexes with TODABE at pH 2.8 and was eluted with system peaks. Although the pH of the mobile phase is 6.5, for example, Pb(II)- and Cu(II)-TODABE complex elute before the Hg(II)-TODABE complex and interfere the sample peak at high concentration (Figure 1(B) and (C)). The resolution value of Cu(II) and Hg(II)-TODABE complex peak was calculated as 0.9.

In the previous study, we found that the MeCN solution (MeCN : water 20 : 80) was proper mobile phase to elute Hg(II)-TODABE complex.⁷ The proper k' value could be obtained with the SDS concentration of 12.5 mM (Figure 3). The complex was base line separated with system peaks and the retention time was not so large at the concentration. On the other hand, the SNS concentration didn't influence the k' value and detection limit of the Hg(II)-TODABE complex.

Table 1. Accuracy tests for the proposed method

| Hg(II) ion concentration/ppm | | Accuracy ^b /% |
|------------------------------|----------------------------|--------------------------|
| Prepared ^a | Found (Means) ^b | |
| 10.03 | 9.910±0.19 | 98.80±1.9 |
| 50.15 | 50.75±1.95 | 101.2±3.9 |
| 100.3 | 98.49±2.80 | 98.20±2.8 |

^aSpiked concentration into the waste water after adding excess of 2.00 mM TODABE, Injected: 30 μ L, ^bMean of three determinations with relative standard deviation (s).

The proper absorbance value by SNS in mobile phase for indirect spectrophotometric detection could be obtained with 0.2 mM. Therefore, MeCN solution (MeCN : H₂O 20 : 80) containing SDS (12.5 mM) and SNS (0.2 mM) at pH 2.8 was selected as optimum mobile phase.

Determination of Trace Mercuric Ion in a Waste Water.

The calibration curve was obtained in the concentration range from 0.050 mM (10.03 ppm) to 0.833 mM (167 ppm) in case of 30 μ L of sample solution was injected. The calibration graph was fitted to $y=0.31x-5.05$. The correlation coefficient (r) was 0.9990. The relative standard deviation for five replicate injections of 50.15 ppm Hg(II) solution was calculated as 1.30%. The detection limit (signal to noise ratio of 3) was determined with successive dilution of the 1.00 mM Hg(II)-TODABE complex solution. The detection limit was 0.025 mM (5.015 ppm).

In Table 1, the Hg(II) ion spiked in the waste water was determined for the accuracy test. We obtained the 98-101% of accuracy.

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