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Speciation of Trace Metals in Pond Water as Studied by Liquid Chromatography/Inductively Coupled Plasma Mass Spectorometry

Akihide Itoh, Chisen Kimata, Hajime Miwa, Hideyuki Sawatari, and Hiroki Haraguchi*

Department of Applied Chemistry, School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-01

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An on-line separation-detection system, liquid chromatography coupled with inductively coupled plasma mass spectrometry (ICP-MS), has been developed and applied to the study on speciation of trace metals in pond water. First, the total concentrations of dissolved metal ions in pond water were determined by ICP-MS after chelating resin preconcentration. Then, pond water was preconcentrated by ultrafiltration using a filter with a molecular weight permeation limit of 10000, providing size exclusion chromatography. Large organic molecules which had combined with trace metals in the preconcentrated samples were separated with a gel filtration column and detected by UV absorption and ICP-MS, sequentially. In consequence, large organic molecules with the molecular weight of $\geqq$300000 and 10000—50000 could be observed. A large number of trace metals (41 elements) were found in those organic molecules. Furthermore, the percentages of metal ions in the forms of large organic molecule–metal complexes were estimated from their concentrations as determined by the flow injection method.

In recent years, chemical speciation of various trace metals have received attention,¹⁾ because metallic elements even at the trace or ultratrace level have different physicochemical or biological properties and functions, depending on their chemical forms. This means that only the determination of the total content of each element in the sample is not sufficient to elucidate chemical or biological functions, for example, in environmental chemistry and toxicology. In the previous paper,(2) we reported the study on speciation of large organic molecule–metal complexes in pond water, using ICP-AES (inductively coupled plasma atomic emission spectrometry) as a simultaneous multielement detector for size exclusion chromatography together with UV absorption detection. In the experiment, 12 elements were detected in the chromatograms for the preconcentrated pond water samples. The detection sensitivity of ICP-AES, however, was not sufficient to figure out the behaviors of trace metals in natural water. ICP-MS (inductively coupled plasma mass spectrometry) can provide the detection limits lower by 2—3 orders of magnitude than those available with ICP-AES.(3) As discussed elsewhere,(1,4—7) the LC/ICP-MS system may be one of the most useful techniques for identification of different chemical forms of the elements including non-metallic ones such as phosphorus and sulfur. Similar systems were applied to biological samples(8,9) and toxic compounds.(10) However, the studies on speciation of trace elements in natural water has been performed only by HPLC/ICP-AES.(2,10) In the present research, we have thus developed a real time data acquisition system for liquid chromatography with multielement detection by ICP-MS and applied this system to the study of speciation of trace metals in pond water.

Experimental

Instrumentation. In the size exclusion chromatography (SEC) system, HPLC (Model LC-9A, Shimadzu, Kyoto) was used together with a gel filtration column (Superose 12, Pharmacia LKB, Uppsala, Sweden) and an UV absorption detector (Model 870-UV, JASCO, Tokyo), which was similar to one used previously.(2) The filtration range of the gel filtration column used was 1000—300000 in molecular weight (MW). In the present experiment, 50 mM (M = mol dm^{-3}) tris-HNO$_3$ buffer solution (pH 7.3) was used as the mobile phase at the flow rate of 0.5 ml min^{-1}. Tris-buffer solution was used because the continuous nebulization of high concentrated inorganic salt buffer solution might cause clogging of the torch and the sampling cone in the ICP-MS instrument. The molecular weight calibration of the gel filtration column was performed by using standard proteins with different molecular weights.(2) The sample injection volume for the SEC measurement was 200 μl. For the detection of trace metals by ICP-MS after UV absorption detection, a capillary tubing of the ICP nebulizer was connected with a piece of Teflon® tubing to the outlet of the UV absorption detector.

The ICP-MS instrument (Model SPQ 8000A, Seiko Instrument Co., Tokyo) was operated under the following conditions. RF output power 1.0 kW, carrier argon flow rate 0.5 dm^3 min^{-1}, auxiliary argon flow rate 1.0 dm^3 min^{-1}, coolant argon flow rate 15 dm^3 min^{-1} and sampling depth 12 mm above load coil. In the measurement of the chromatograms with ICP-MS detection, data were acquired in multielement mode by peak hopping over 15 *m*/*z* positions for 5 s measurement time. In the present computer program, the ion counts at each *m*/*z* over 15 *m*/*z* positions were measured two times for 5 s, where the dwell time at each *m*/*z* was 100 ms in every measurement. The averaged ion counts of the pair of measurements at each *m*/*z* was taken as one point in the chromatogram for each element examined. Therefore, the chromatogram of each *m*/*z* was drawn by plotting the data for one point per 5 s measurement. Total acquisition time was 2700 s, which resulted in 540 points measurement for each *m*/*z*. In the present real-time data acquisition system, 15 elements within the 120 *m*/*z* mass-range were detected simultaneously. These experimental conditions of dwell time and mass-range scanning were the critical limits in the present chromatogram measurement because the shorter dwell time for each *m*/*z* (element) deteriorated the sensitivity in the ICP-MS measurement and the wider mass-range scanning resulted in incorrect peak profile measurements in chromatography. The chromatograms observed were smoothed by taking an average of 10 points.

Sample Pretreatment. The pond water sample collected from the Kagami-ga-ike pond in the campus of Nagoya University was first filtered with a glass filter, and then with a membrane filter (pore size: 0.45 μm). The filtered sample was preconcentrated by 500-fold in volume by ultrafiltration using a filter of the molecular weight permeation limit of more than 10000. The ultrafiltration system (MILLIPORE, Pericon Cassette system) used here was of a tangential flow type. In the present ultrafiltration procedure, one liter of the pond water sample was finally reduced to 2 ml, and this 500-fold preconcentrated solution was provided to the chromatographic measurement.

Determination of Metal Ion Concentration in Pond Water. Before analyzing organic molecule-metal complexes by SEC/ICP-MS, we determined the total concentrations of dissolved metal ions in pond water by ICP-MS. In this experiment, the water sample was filtered with a membrane filter (pore size 0.45 μm) and then acidified to pH ca. 1 by adding HNO_3. Since the concentrations of trace metals were extremely low, trace metals were preconcentrated by a chelating resin method, where Chelex 100 from Bio Rad Laboratory (USA) was used. The chelating resin preconcentration method similar to those reported in the previous work[11] was employed with some modification. In the present preconcentration procedure, 0.3 g of Chelex 100 was added into a 500 ml water sample, and the pH of the solution was adjusted to 5, using 25% ammonia solution and acetic acid solution. After stirring for 2 h, the solution was filtered with a glass filter to collect the resin. Then, the resin on the glass filter was carefully rinsed with 15 ml of 1 M ammonium acetate. This procedure was necessary to reduce Mg and Ca adsorbed on the resin. Finally, metal ions adsorbed on the chelating resin were dissolved with 10 ml of 2 M HNO_3. Thus, 50-fold preconcentration in volume was achieved.

Results and Discussion

Total Concentrations of Metal Ions Dissolved in Pond Water. The total concentrations of dissolved metal ions in pond water obtained are summarized in Table 1. As is seen in Table 1, 17 elements were determined in the present experiment. The concentrations of Fe, Al, and Mn were significantly high, while other elements were at the level of a few ng ml^{-1} (ppb) or lower than ng ml^{-1}. In Table 1, the recovery values for each metal ion was also shown. These were obtained by the chelating resin preconcentration method with addition of 5 or 50 ppb spiked element, followed by the ICP-MS measurement. The recovery values for Fe, Mo, V, Ti, and U were lower than 50%, and their analytical values are shown in parentheses. In the present experiment, the simultaneous preconcentration was carried out at pH 5 for the simultaneous multielement determination by ICP-MS.

Table 1. Concentrations of and Recovery Values of Dissolved Metal Ions in Kagami-ga-ike Pond Determined by ICP-MS after Preconcentration Using Chelating Resin

Element	Concn[a]/ng ml^{-1}	Recovery[b]/%
Fe	930±110	45.3
Al	14±0.9	77.8
Mn	14±0.9	78.4
Zn	5.2±1.4	106
Ni	4.2±0.2	112
Cu	1.6±0.1	94.0
Sn	0.77±0.09	116
Pb	0.52±0.11	82.9
Mo	0.47±0.03	19.8
V	0.33±0.03	39.6
Ti	0.15±0.03	2.4
Ce	0.10±0.01	80.9
Co	0.056±0.005	114
Cd	0.036±0.003	111
Y	0.032±0.002	77.7
La	0.032±0.013	74.7
U	0.024±0.002	16.3

a) The standard deviation was estimated from 4-times measurements (repeated preconcentration procedures). b) In the recovery test, 5 or 50 ppb (ng ml^{-1}) metal ions were spiked in the pond water sample, and the analytical procedure similar to that described in the section of Experimental was carried out.

Concentration Factor in Ultrafiltration Preconcentration. The pond water sample was preconcentrated by 500-fold in volume in ultrafiltration preconcentration using a filter of molecular weight permeation limit over 10000, as mentioned earlier. It is noted here that the ultrafiltration filter used can retain about 90% of the molecules with molecular weight more than 10000. Furthermore, significant loss of large organic molecules may occur due to their adsorption on the tubing wall of the ultrafiltration system and on the ultrafiltration filter. Thus, the actual concentration factor in ultrafiltration procedure was estimated by measuring the carbon concentrations in the untreated, preconcentrated and filtered pond waters by ICP-AES. The concentration of carbon were 17.6 ppm, 502 and 14.1 ppm in the untreated, preconcentrated and filtered samples, respectively. From these results, the actual concentration factor in ultrafiltration was estimated to be 143.

Speciation of Trace Metals Combined with Large Organic Molecules in Pond Water. In Figs. 1 and 2, as some examples, the chromatograms of 12 kinds of trace metals detected by ICP-MS were shown, along with the chromatograms of large organic molecules detected in UV absorption at 254 nm. In addition to the chromatograms shown in Figs. 1 and 2, those for other 29 trace metals were observed in the present experiments. All trace metals detected are summarized in Table 2.

In general, the chromatograms in Figs. 1 and 2 are very similar to those observed by ICP-AES in the previous work,[2] although the retention times for organic molecules were somewhat different from each other. In particular, the retention times of both peaks were longer, compared to those in the previous experiment. This may be ascribed to the use of a different buffer solution. In the previous work by LC/ICP-AES, the phosphate buffer solution was used as the

Table 2. Distributions of Trace Elements in Large Organic Molecules with Different Molecular Weights

Retention time	750	1500—1900			≧ 1950
s		1600	1750	1800	
Molecular weight	≧ 300000	50000—10000			≦ 10000
Elements detected by ICP-MS	P Al Ti V Fe Ni Cu Zn Ga Sr Y Zr Ag Sn I Ba REEs[a] W Pb Hg U As Cd	Zr REEs[a] Cd	Co Ni Cu I Pb S Ti Zn Sr Y W Mo	Sb As	I Br Ag As
Elements detected by ICP-AES[b]	Al Si Ca Mn Fe Zn Sr Ba Cu		Mn Zn Cu Mo		Na Mg Ca Sr Ba Si

a) REEs indicate the following rare earth elements; La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu. b) Cited from Ref. 2. As mentioned in the text, the retention times of the elements detected by ICP-MS and ICP-AES were slightly different, maybe, because the different buffer solutions were used as the mobile phase.

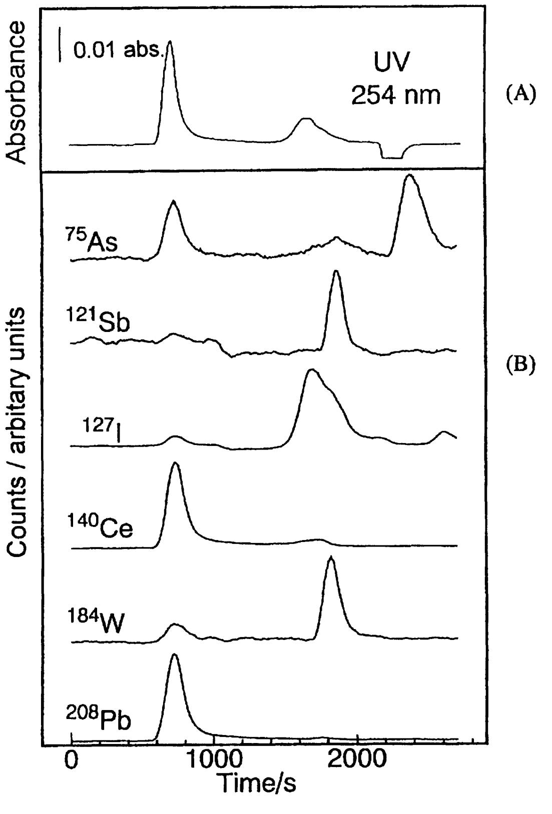

Fig. 1. Chromatograms of organic molecules (A) and trace metals (B) in concentrated Kagami-ga-ike pond water (500-fold) with UV absorption and ICP-MS detection, respectively.

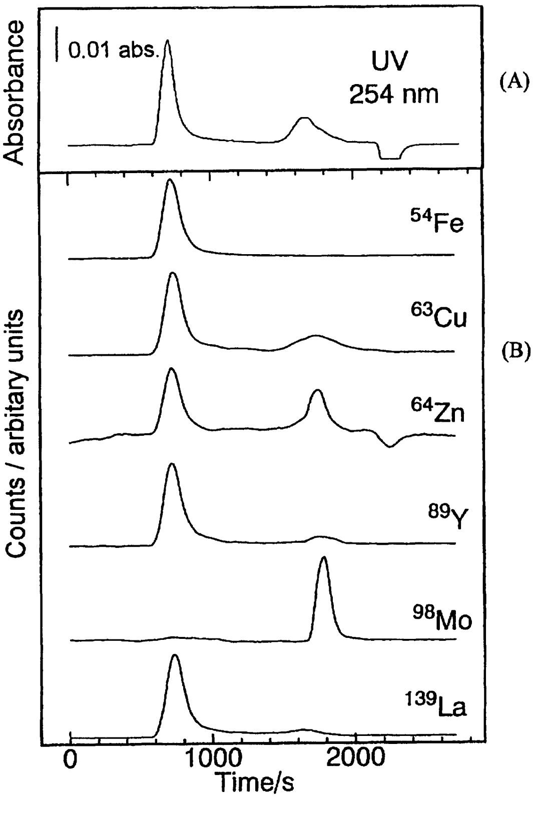

Fig. 2. Chromatograms of organic molecules (A) and trace metals (B) in concentrated Kagami-ga-ike pond water (500-fold) with UV absorption and ICP-MS detection, respectively.

mobile phase.[2] In the UV absorption chromatograms shown in Figs. 1 and 2, two peaks are observed, at the retention times of ca. 750 s and 1500—1900 s. According to the molecular weight calibration, these peaks correspond to the molecular weights of ≧300000 and 10000—50000, respectively. In Figs. 1 and 2, the peak intensities of the chromatograms detected by ICP-MS are shown in arbitrary units by normalizing the larger peak for each element to 1.0 to show the relative peak intensities for each element, because the elements examined were in a wide concentration range.

In Fig. 1, the chromatograms for Fe, Cu, Zn, Y, Mo, and La show the different behaviors of each element. As is seen, only one peak was observed for Fe and Mo at the shorter and longer retention times, respectively. On the contrary, other elements provided two peaks, although the two peak intensities were different from each other. In Fig. 2, the chromatograms for As, Sb, I, Ce, W, and Pb are illustrated. It is noted here that all these elements provided the peaks at both shorter and longer retention times and their peak positions in the retention time range of 1500—1900 s were slightly different. In general, all elements in the chromatograms obtained by ICP-MS detection (Figs. 1 and 2) were observed at the retention times in coincidence with the UV absorption peaks observed in the UV-detected chromatograms shown in the upper parts in Figs. 1 and 2. These facts indicate that the chemical forms of these elements were in some large organic molecule–metal complexes.

The UV-absorption peaks for large organic molecules detected at the retention time corresponding to MW 10000—50000 are quite broad, with several shoulder peaks. On the other hand, as is clearly seen in Fig. 2, the peak positions of trace elements in this retention time range provide the different retention times in the chromatograms measured by ICP-MS. These facts suggest that the UV-absorption peak for MW 10000—50000 consists of three or four different organic molecules, but they are not well separated in the chromatograms detected with UV absorption. This indicates that ICP-MS may work as a better high-resolution detector of HPLC than the UV absorption detector.

In the present experiment, 41 elements in total were detected in the chromatograms for the preconcentrated pond water samples. A large number of metal ions were contained in the organic molecules with MW ≧300000, which were observed near the retention time of ca. 750 s, and some of them were also observed in the fraction of organic molecules with MW 10000—50000. The trace metals detected by the present system are summarized in Table 2. As is seen in Table 2, they are classified into five groups, depending on the different retention times of trace metals, which correspond to different molecular weights of large organic molecules in pond water. These results indicate that several different organic molecules may exist in pond water, which have characteristic binding sites for metal ions.[13]

According to our previous work,[14] organic molecules observed in Figs. 1 and 2 are likely humic acids or their derivatives. Since humic acid substances have complicated chemical structures, it is difficult to elucidate the chemical forms of the organic molecule–metal complexes in pond water at present. Even so, the findings in the present experiment are important to discuss the chemical forms for fate analysis of trace metals in natural water.

Alkali and alkaline earth elements except for strontium and barium were not examined in the present experiment, because the concentrations of these elements were too high for the ICP-MS measurement. Furthermore, for example, as is seen in Fig. 2, As and I provided the LC peaks at the retention time larger than 1950 s, which corresponded to a molecular weight smaller than 10000. In ultrafiltration, only the molecules with molecular weight larger than 10000 are preconcentrated, while the molecules and ions whose molecular weights are smaller than 10000 remain in the preconcentrated solutions at almost the original concentration level. The chromatogram peaks for As and I may be ascried to rather smaller molecules or ions which exist in pond water. As is seen in Table 2, Br and Ag were also detected at the retention time larger than 1950 s.

Table 3. Total Concentrations of Trace Metals in Preconcentrated Pond Water and the Percentages of Trace Metals in the Large Organic Molecule–metal Complexes

Element	Concentration ng ml^{-1}	Percentage of complex forms/%
Fe	4600	18
Ni	16	16
Cu	47	45
Zn	81	33
Cd	0.41	28
La	1.0	47
Ce	2.9	45
Pr	0.2	—[b]
Al	275	37
Ti	96	—[b]
V	1.5	18[a]
Pb	48	80
Mn	11	7.4
Mo	3.3	22
Sn	1.7	12
Y	0.72	40
U	0.016	6.8[a]

a) Recovery of the ion in the chelating resin preconcentrated procedure was under 50%. b) Not estimated because of too poor recovery value.

Concentrations of Trace Metals in Preconcentrated Solution. At present, it is difficult to determine the concentration of trace metals observed in the chromatograms detected by ICP-MS, because there are no appropriate standard chemicals for calibration. Thus, the concentrations of trace metals in the pond water preconcentrated by ultrafiltration were determined by the flow injection method using ICP-MS as a detector. In this case, the concentrations of trace metals in the preconcentrated pond water were obtained as the total values. The results for some trace metals are summarized in Table 3. The percentages of trace metals in large organic molecules–metal complexes are also shown in Table 3. These were estimated from the concentrations of dissolved metal ions, shown in Table 1, and those of metal ions in the sample preconcentrated by ultrafiltration, where the concentration factor of 143 was described earlier, were used for calculation.

References

1) N. P. Vela, L. K. Olson, and J. A. Caruso, *Anal. Chem.*, **65**, 585A (1993).

2) A. Itoh, M. Aikawa, H. Sawatari, A. Hirose, and H. Haraguchi, *Chem. Lett.*, **1993**, 1017.

3) A. Montaser and D. W. Golightly, "Inductively Coupled Plasmas in Analytical Spectrometry," VCH Publisher, New York

(1992).

4) S.-J. Jiang and R. S. Houk, *Spectrochim. Acta, Part B*, **43B**, 405 (1988).

5) I. S. Krull, "Trace Metal Analysis and Speciation," Elsevier Science Publishers B. V., Amsterdam (1991).

6) H. Hamanaka, A. Itoh, S. Itoh, H. Sawatari, and H. Haraguchi, *Chem. Lett.*, **1995**, 363.

7) Z. Mason, S. d. Storms, and K. D. Jenkins, *Anal. Biochem.*, **186**, 187 (1990).

8) K. Takatera and T. Watanabe, *Anal. Sci.*, **1992**, 469.

9) A. Al-Rashdan, D. Heitkemper, and J. A. Caruso, *J. Chromatogr. Sci.*, **29**, 98 (1991).

10) W. S. Gardner, P. F. Landrum, and D. A. Yates, *Anal. Chem.*, **54**, 1198 (1982).

11) H. Sawatari, T. Toda, T. Saizuka, C. Kimata, A. Itoh, and H. Haraguchi, *Bull. Chem. Soc. Jpn.*, **68**, 3065 (1995).

12) A. Itoh, C. Kimata, H. Miwa, Shan Ji, and H. Haraguchi, unpublished data.

13) E. Tipping and M. A. Hurley, *Geochim. Cosmochim. Acta*, **56**, 3627 (1992).

14) A. Itoh and H. Haraguchi, *Chem. Lett.*, **1994**, 1627.