Chemical Speciation of Trace Metals in Natural Water by Ultrafiltration/Size Exclusion Chromatography/ UV Absorption/ ICP-MS

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Abstracts: A study on elemental speciation of trace metals in lake water (Lake Biwa in Japan) has been carried out by a size exclusion chromatography (SEC) / inductively coupled plasma mass spectrometry (ICP-MS) system. Before analysis, the water sample was preconcentrated with a ultrafiltration technique, where the large molecules with molecular weight larger than 10,000 were concentrated. Then the preconcentrated water samples (500-1000 fold) were analyzed by a SEC/ICP-MS system. Most trace metals were found at the UV absorption peaks corresponding to the molecular weights of ca. 300,000 and 10,000-50,000, where trace metals were on-line detected by ICP-MS. The results suggest that many of trace metals exist as the large organic molecules-metal complexes in natural water.

Keywords: Chemical speciation, trace metals, natural water, size exclusion chromatography, inductively coupled plasma mass spectrometry

1. Introduction

In last 3 decades, various methods of analytical atomic spectrometry such as atomic absorption spectrometry, atomic fluorescence spectrometry, inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) have been explored as the sensitive and precise techniques for trace and ultratrace analysis, and extensively used in many scientific fields [1,2]. As the result of such development, the following

analytical features are now obtainable especially with use of ICP-MS [3];

   a) Ultratrace analysis with the detection limit at the ppt (10^{-12} g/ml) or sub-ppt level,

   b) simultaneous multielement detection,

   c) accurate and precise determination by isotope ratio measurement using stable isotopes,

In consequence, some new analytical research fields or concepts have been created in relation with trace elements. "Chemical or elemental speciation" is one of such fields [4].

   In general, total concentrations of trace metals as the bulk concentration have been determined in water analysis. However, as in the cases of mercury, arsenic, tin etc., it has been found that individual or definite chemical compounds have different physicochemical or biochemical properties, which result in biological defects to human beings and other living matters like microorganisms, animals and plants [5]. Therefore, more extensive studies on speciation of trace metals are now required in many fields [6], including development of some new analytical techniques [4].

   In the present study, elemental speciation of trace metals in lake water has been carried out by using a combined or hyphenated system of liquid chromatography and ICP-MS. Here, a real time data acquisition system for liquid chromatography with ICP-MS detection was developed and applied to the simultaneous multielement detection of trace metals in the eluent of size exclusion chromatography by ICP-MS. In addition, the total concentrations of dissolved trace elements in lake water were also determined by ICP-MS after chelating resin preconcentration by 50-fold.

2. Experimental

Sample collection and pretreatment: The surface water samples were collected in Lake Biwa (biggest lake in Japan located near Kyoto). The water samples were pretreated as follows, in similar manner to the previous

work [7]. The samples were first filtered with a glass filter and then with a membrane filter (pore size 0.45 μm) immediately after sampling. The filtered samples were further applied to ultrafiltration in order to preconcentrate large molecules (molecular weight (MW) larger than 10,000). In ultrafiltration, a filter of the molecular weight permeation limit more than 10,000 was used. Finally the preconcentrated water samples were provided to liquid chromatographic analysis.

   Instruments: An HPLC (model LC-9A, Shimadzu, Kyoto, Japan) equipped with an UV absorption detector (model 870-UV, JASCO, Tokyo, Japan) was used with a gel filtration column (Superose 12, Pharmacia LKB, Uppsala, Sweden), and thus the present HPLC system worked as size exclusion chromatography. The gel filtration column used had a size exclusion limit of 10,000 (in MW). Tris-HNO$_3$ buffer solution (pH 7.3) was used as a mobile phase instead of inorganic salt buffer in order to avoid clogging of the nebulizer and torch in the ICP-MS system. The sample volume injected into the LC column was 200 μl. Other experimental conditions for HPLC are summarized in *Table 1*.

Table 1. Instrumental components and experimental conditions

HPLC	LC-9A	Shimadzu
column		Superose-12(Pharmacia LKB)
mobile phase		50 mM Tris-HNO$_3$ (pH 7.3)
flow rate		0.5 ml/min
sample volume		200 μl
UV absorption		254 nm
ICP-MS	SPQ 8000A	Seiko
RF power		1.0 kW
carrier gas		0.5 l/min Ar
auxiliary gas		1.0 l/min Ar
coolant gas		1.5 l/min Ar
sampling depth		12 mm above load coil
date acquisition		peak hopping mode
measurement points		15 m/z per 1 mass-range scanning
dwell time		100 ms at each m/z
data plot		1 point per 5 s
measurement time		1700 s (540 points)

An ICP-MS instrument was of model SPQ 8000A from Seiko Instrument Co. (Tokyo, Japan). The operational conditions for the ICP-MS measurements are also shown in *Table 1*. When the present ICP-MS instrument was used for LC detection, data acquisition was performed in multielement mode by peak hopping over 15 m/z positions for 5 s measurement time. Using this real-time data acquisition system, thus, 15 elements were sequentially detected in one mass-range scanning for the chromatogram measurement. The chromatogram measured by ICP-MS detection was smoothed by taking an average of 10 points.

3. Results and Discussion

Concentrations of dissolved metal ions in Lake Biwa

Before analyzing large molecular species combined with metal ions, the concentrations of dissolved metal ions in lake water collected in Lake Biwa were determined by ICP-MS. In this experiment, the water sample filtered with a membrane filter (pore size 0.45 μm) was used for analysis, where the water sample was acidified to pH ca. 1 with adding HNO_3. Since the concentrations of most metal ions were extremely low, the 50-fold preconcentration using chelating resin (Chelex-100 purchased from Bio-Rado Laboratories, USA) was performed. The preconcentration procedure with Chelex-100 was almost similar to those described earlier [8-10]. Actually, 500 ml of lake water was preconcentrated at pH 6 by using 0.5 g of chelating resin, and finally the metal ions adsorbed on the resin were dissolved with 10 ml of 1 M HNO_3. Then the preconcentrated solution was provided to the analysis by ICP-MS. In the ICP-MS measurement, the concentrations of metal ions were determined by a pneumatic nebulization technique (direct nebulization), where the experimental conditions for ICP-MS operation were the same as those shown in *Table 1*.

The analytical results are summarized in *Table 2*.

As is seen in *Table 2*, 19 elements in total were determined in the concentration range from 3.0 ppb (ppb = 10^{-9} g/ml) for Fe down to 0.0021 ppb for La.

In *Table 2*, the literature values for 11 elements are also listed, which were cited from the literatures [11,12]. No concentrations for Sn, Zr, V, Y, Co, Ce and La have been reported for Lake Biwa water. Several characteristic features are seen in the results shown in *Table 2*. The concentrations of Fe, Al and Ti obtained in the present experiment are rather lower than the literature values. For Zn, Mn, Cu, Mo and Ni, the present concentrations are in the ranges of the concentrations reported for these elements in the literatures. In addition, the concentrations of V, Pb, and Cd are almost close to or at the same concentration levels as those of the literature values. In general, it is stated here that the present results are quite reasonable as the dissolved concentrations of trace metal ions in less

Table 2. Concentrations of dissolved metal ions in lake water of Lake Biwa determined by chelating resin preconcentration and ICP-MS

element	concentration found ng/ml	literature value[a] ng/ml
Fe	3.0	17-20
Al	1.4	21
Zn	2.2	0.20-32
Mn	0.75	0.6-11
Cu	0.66	0.18-7.1
Mo	0.36	0.42-5.3
Ni	0.28	0.027-13
V	0.20	0.17
Sn	0.10	—
Pb	0.046	0.069
Ti	0.044	0.740
W	0.036	—
Zr	0.021	—
U	0.020	—
Y	0.012	—
Co	0.0051	—
Cd	0.0041	0.0017
Ce	0.0031	—
La	0.0021	—

a) Cited from Refs. (11) and (12). The sign — indicates no literature value.

polluted lake water. The different concentrations for Fe, Al and Ti may be caused by the different sample pretreatment in each experiment. The higher concentrations of Fe, Al, Ti and Mn are often obtained, when some acid (HCl or HNO_3) is added just after sampling and then filtered with a membrane filter before the determination [8]. Maybe, this is explained by the fact that most of metallic ions are adsorbed on or combined with the suspended particulates in natural waters and they are partly dissolved in the solution phase when acid is added in the water samples. Since no acid was added before filtration with a membrane filter in the present experiment, rather lower values might be obtained for most of metallic ions observed here.

Elemental speciation of trace metals in lake water

The lake water sample filtered with a membrane filter was further concentrated by 500-fold with an ultrafiltration filter with the MW permeation limit larger than 10,000. In this procedure, only molecules with MW larger than 10,000 were preconcentrated for analysis by SEC. The sample volume injected into the SEC column was 200 μl. As described earlier, the eluent from SEC was detected first by an UV absorption detector at 250 nm and then by ICP-MS.

The SEC chromatograms are shown in *Figs. 1* and *2*. In each figure, the chromatogram detected by UV absorption at 254 nm is shown on the upper part, and the chromatograms detected by ICP-MS on the lower part. As is seen in *Figs. 1* and *2*, mainly two peaks are observed at the retention times of ca. 750 s and 1500-1900 s in the UV absorption-detected chromatograms. According to the molecular weight calibration using standard proteins [7], the retention times of ca. 750 s and
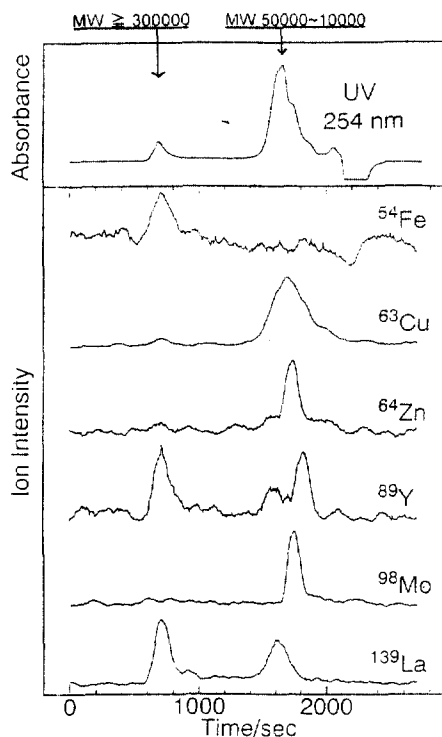
Fig.1. SEC chromatogram of organic molecules (MW ≧ 10,000) and trace metals in concentrated lake water (Lake Biwa) with ICP-MS detection. Preconcentration factor by ultrafiltration; 500-fold
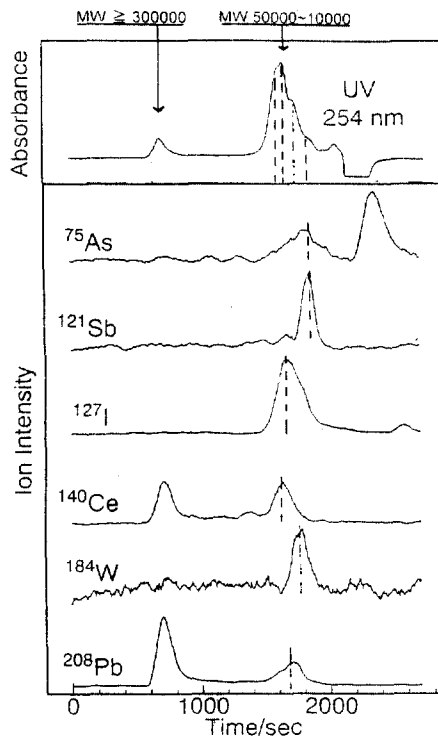
Fig.2. SEC chromatogram of organic molecules (MW ≧ 10,000) and trace metals in concentrated lake water (Lake Biwa) with ICP-MS detection. Preconcentration factor by ultrafiltration; 500-fold

Table 3. Elements found in the SEC chromatograms detected by ICP-MS.
    Sample: Lake water from Lake Biwa

| retention time/s | 750 | 1500-1900 | | | >1950 |
		1600	1750	1800	
MW[a]	300,000	50,000-10,000			$\leqq 10,000$
	Al, Mn, Fe, Y, La, Ce , Pr, Pb, Ba	Mn, Cu, I, Pb			Cl, Br, As
		Y, La, Ce,	S, Zn, Mo, W	Y, Sb, As	

a) MW = molecular weight

1500-1900 s are corresponding to MW >300,000 and MW 50,000-10,000, respectively, in the gel filtration column used.

It is interesting, as is seen in *Figs. 1* and *2*, that metallic and iodine elements were observed only at the retention times corresponding to the peaks detected by UV absorption. Iron was detected only at the position of MW $\geqq$ 300,000. On the other hand, Cu, Zn, As, Sb, I and W were detected only at the position with the smaller molecular weight, although the peak positions for these elements are slightly different from each other. Furthermore, Y, La, Ce and Pb are clearly detected at both of the peak positions. In addition, As provided another peak at the position with MW < 10,000. This result suggests that some arsenic species with MW < 10,000 exists besides the species combined with large molecule whose molecular weight is larger than 10,000.

In *Table 3*, all the elements detected by the present SEC/ICP-MS system are summarized in the groups with the different retention times or molecular weights. According to our previous work[13], the UV absorption chromatogram was very similar to that for humic acid substances. Thus it is considered that the large molecules detected in the UV absorption chromatogram shown in *Figs 1* and *2* are originated mainly from humic acids (HA). Thus these results indicate that most metallic elements as well as non-metallic elements such as S, Cl, Br and I are dissolved in the chemical forms combined with humic acid substances in lake water. Unfortunately, it is difficult to estimate exactly the amounts of metallic ions combined with humic acid

substances quantitatively because of some loss of metallic ions during ultrafiltration due to adsorption. However, it can be approximately said that more than 50% of Fe, Cu, Zn and rare earth elements (La, Ce) exist in the HA-metal complex forms.

It is further noted here that, among dissolved metal ions determined in *Table 2*, Ni, V, Sn, Ti, Zr, U, Co and Cd were not found in the large molecular complexes shown in *Table 3*. Instead, La, Pr, Ba, S, Sb, As, Cl, Br and I were newly found in the large molecular complexes with humic acids. These results suggest that Ni, V, Sn, Ti, Zr, U, Co and Cd may exist as the small molecular species in lake water. On the other hand, significantly large amounts of La, Pr, Ba, Sb, As, S, Cl, Br and I in lake water are dissolved in the forms combined with humic acids.

It is also pointed out that the elements detected in the range of retention time between 1500-1900 s are classified into 4 groups, as is seen in *Table 3*. The first group is for Mn, Cu, I and Pb, which provided the rather broad peaks in the chromatograms detected by ICP-MS. Other three groups are distinguished by the different retention times, as is seen in *Table 3*. Indeed, the elements in these three different groups were found at the different peak positions in the chromatograms shown in *Figs. 1* and *2*. These facts indicate the possibility that the organic molecules detected in the retention time range of 1500-1900 s consist of at least 3 different components or species.

Although some direct evidences can not be given from the present investigation, it is suggested that the large

molecules with MW larger than 300,000 may be deeply related to some particle formation by coaggulation of humic acid substances, in which metallic ions may play important roles [13]. As for particle formation, further investigation should be required in relation with scavenging effects often observed in natural waters.

Conclusion

In the present study, the hyphenated LC/ICP-MS system for on-line chromatographic measurement with capability of simultaneous multielement detection has been developed and applied to the elemental speciation of trace metals in lake water. With use of the LC (SEC)/ICP-MS system, it has been elucidated that metallic ions in lake water of Lake Biwa exist as the dissolved forms of humic acid-metal complexes with several different molecular weights. Although it has been well known that many kinds of metallic elements are contained in humic acid substances in solid phase extracted from soils or sediments, the humic acid-metal complexes in the dissolved state are directly observed for the first time in the present study by using the on-line LC/ICP-MS system. These findings may be useful for further study on solution chemistry of trace elements and their geochemical cycles in aquatic environment.

Acknowledgment

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