CHEMISTRY LETTERS, pp. 1017-1020, 1993.

Chemical Speciation of Large Molecular Metal Complexes in Pond Water

Akihide ITOH, Masahide AIKAWA, Hideyuki SAWATARI,
Akio HIROSE, and Hiroki HARAGUCHI*
Department of Applied Chemistry, School of Engineering,
Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464

The concentrated large molecules in pond water were separated by size exclusion chromatography, where organic molecules and trace metals were on-line detected by UV absorption and ICP-AES, respectively. It was found that some metal ions combined with large molecular substances of molecular weight larger than 10000.

Chemical speciation of trace metals in natural waters such as seawater, river water and pond/lake water is the subject of interest because the chemical species in natural waters have not generally been elucidated well. In the previous papers,[1,2] it has been reported that some metalloenzymes such as alkaline phosphatase, carboxypeptidase, nitrogenase and so forth exist in seawater and lake water, and they often play important roles, for example, for phosphorus cycles in lake water.[3] It was also suggested that other large molecular complexes with trace metals may exist in natural water,[4] although it is difficult to elucidate their chemical forms. Hence in the present study a new approach has been tried for chemical speciation of trace metals in natural water.

Size exclusion chromatography (SEC) was employed for the separation of macromolecules. A UV absorption detector (Model UVIDEC-100II, Jasco, Tokyo) and an inductively coupled plasma atomic emission spectrometer (ICP-AES; Model Plasma Atomcomp MkII, Jarrell-Ash, Franklin, MA, USA) were used for the detections of organic molecules and trace metals, respectively. For SEC, a high performance liquid chromatograph (Model LC-9A, Shimadzu, Kyoto) was used with a gel filtration column (Superose 12, Pharmacia, Uppsala, Sweden) that had filtration range of molecular weight 1000-300000. Generally 16 mM (M = mol/dm^3) dipotassium hydrogenphosphate-nitric acid buffer solution (pH 7.3) as the mobile phase was eluted at the flow rate of 0.5 ml/min. The sample injection volume was 400 µl. The UV detector and ICP-AES were connected to the elution tubing from the separation column in series. The UV absorption was detected at 280 nm. The ICP-AES instrument was operated under the following conditions; RF output power 1.0 kW, carrier argon gas flow rate 0.48 l/min, auxiliary gas flow rate 20 l/min, outer gas flow rate 1.0 l/min, and observation height 18 mm above load coil. The present ICP-AES measurement system had a computer software for time-sequence measurement, which allowed to determine up to 39 elements simultaneously. In the time-sequence measurement program, at each time-interval in the range of 0.1-10 s the emission signals for the elements examined were acquired and the 1600 data points as the total could be measured for each chromatogram (retention time range; 2.66-266 min).

In order to evaluate the present measurement system, the chromatograms were obtained by the UV and ICP-AES detections in terms of alkaline phosphatase (MW 140000), which was obtained from calf intestine (Sigma Chemical Co., St. Louis, MO, USA). The results are shown in Fig. 1. The calibration curve for molecular weight is also given in Fig. 1, which was measured by using Blue Dextran (MW 2000000), alcohol dehydrogenase (150000), alubumin (66000), carbonic anhydrase (29000), and cytochrome-C (12400). As is seen in Fig. 1, alkaline phosphatase was detected at the retention time of ca. 20 min as the UV absorption and zinc emission peaks. The retention time of ca. 20 min corresponded to about MW 140000 from comparison with the MW calibration curve, shown in the upper part of Fig. 1.

The pond water was collected from the Kagami-ga-ike pond in the campus of the Nagoya University and Aichi-Yosui pond in Togo-cho, Aichi Prefecture. The pond water was filtered first with a glass fiber filter and then with a membrane filter (pore size: 0.45 μm) after sampling. The filtered samples were further concentrated by 1500-2500 fold with ultrafiltration using a filter with the molecular weight permeation limit more than MW 10000. The chromatograms obtained by the UV detector and ICP-AES for the pond water from the Kagami-ga-ike pond are shown in Fig. 2. The concentrations of dissolved metal ions in the pond water determined before preconcentration by ICP-AES were as follows [concentration is shown in the parenthesis in the unit of ppb (10^{-9} g/ml)]; Na(13000), K(2500), Mg(3000), Ca(11000), Sr(59), Ba(52), Si(10000), Mn(81), Fe(79), Cu(5.0), Zn(17).

In the chromatogram (Fig. 2(a)) obtained by the UV detection, three main peaks were observed together with the broad absorption in the whole. As can be seen in the chromatogram for trace metals (Fig. 2(b)), several peaks were observed at the retention times corresponding to Peaks 1 and 3. Some other metal ions were also detected after the separation limit of the

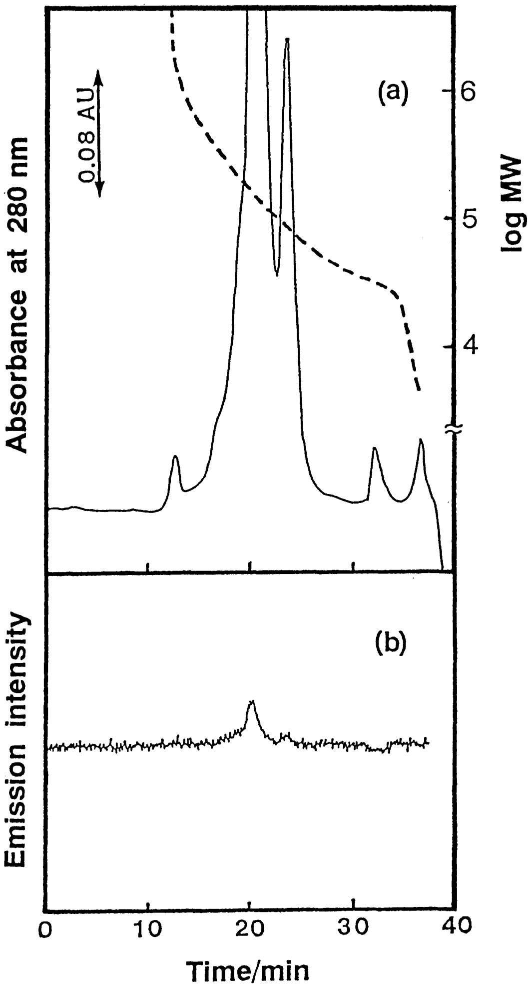

Fig. 1. Chromatograms for alkaline phosphatase detected by UV absorption (a) and ICP-AES(b), and calibration curve for molecular weight (----).

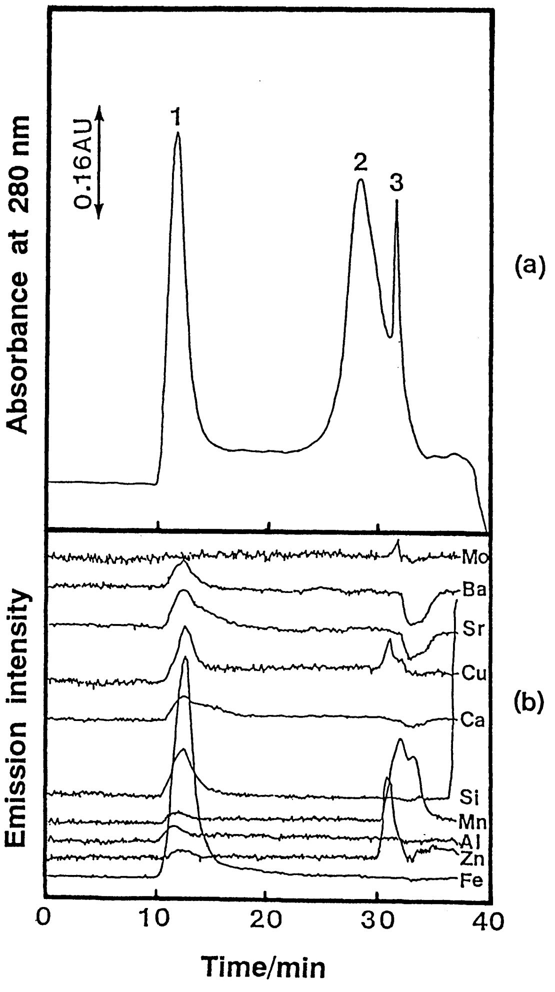

Fig. 2. Chromatograms for the concentrated pond water from Kagami-ga-ike pond, detected by UV absorption(a) and ICP-AES(b).

present separation column used, although their peaks are not shown in the chromatogram in Fig. 2. The metal ions detected by the present method are summarized in Table 1, where they are classified into four groups depending on the molecular weight ranges. As is seen in Table 1, no metal ions were detected in the molecular weight range corresponding to Peak 2.

In the chromatogram shown in Fig. 2, some small absorptions are observed in the molecular range between Peaks 1 and 2, where no metal ion is apparently observed. According to the previous paper,[2)] alkaline phosphatase was detected at the retention time of ca. 20 min, when the

Table 1. Metal ions detected in the chromatograms for pond waters

Molecular weight fraction	Pond water	
	Kagami-ga-ike	Aichi yosui
Peak 1 (MW > 300000)	Al, Si, Ca Mn, Fe, Zn Sr, Ba	Al, Si, Fe Sr, Ba
Peak 2 (MW ca. 45000)	ND[a]	ND[a]
Peak 3 (MW 1000-30000)	Mn, Zn, Cu Mo	Mn, Zn
After separation limit (MW < 1000)	Na, Mg, Ca, Sr, Ba, Si	Na, Mg, Ca, Sr, Ba, Si

a) ND: Not detected.

enzymatic activity detection was utilized for the Kagami-ga-ike pond water. These facts suggest that various large molecular compounds are possibly contained in the pond water, although they can not be identified because of poor sensitivities and chromatographic resolution of the analytical methods employed in the present experiment. There is some possibility that such large molecules may be humic acids or their derivatives. Further experiment is now in progress to elucidate the large molecules and their metal complexes.

The present research was supported by the Grant-in-Aid for the Scientific Research (No. 02453060 and No. 04202123) from the Ministry of Education, Science and Culture.

References

1) K. Kobayashi and H. Haraguchi, Nippon Kaisui Gakkaishi, 42, 155 (1988); other references are therein.

2) A. Hirose, Y. Esaka, M. Ohta, and H. Haraguchi, Chem. Lett., 1993, 307.

3) K. Kobayashi, K. Iwase, S. Hashimoto, H. Ueda, K. Fujiwara, A. Otsuki, K. Fuwa, and H Haraguchi, Bull. Chem. Soc. Jpn., 60, 925 (1987).

4) K. Kobayashi, T. Akagi, and H. Haraguchi, Bull. Chem. Soc. Jpn., 63, 554 (1990).

(Received March 29, 1993)