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Formation of Large Molecular Humic Acid with Addition of Zinc Ion as Elucidated by Liquid Chromatography/ICP-AES

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A size exclusion chromatography system with UV absorption detection and simultaneous multielement detection by ICP-AES has been used to elucidate that humic acid forms some large molecules with molecular weight larger than 300000 in aqueous solution, when zinc is added in humic acid solution as the ionic form.

In general, humic substances are classified into three groups, i. e., humic acid, fulvic acid and humin, which are known to be undecompositional organic compounds originated from biomass. It is also known that humic substances are widely distributed in soil, sediment, and natural water.1) However, physicochemical properties and chemical characteristics of humic substances have not been well understood because of their complicated structures. Many approaches from electrochemical method,2) complexing capacity,3) estuarine chemistry4) and chemical modeling5,6) have been made in the research on humic acid and trace metals existing in it. But little information is available for detailed interaction between trace metal ions and humic acid molecules. Even so, it is generally considered that various chemical species produced by the interaction between trace metal ions and large organic molecules, which may be mainly humic acid, play an important role in the cycle of dissolved trace metals in aquatic environment.7) Recently chemical speciation for trace metals existing in natural water has been received great interest.8) In previous paper,9) we developed an on-line size exclusion chromatography system with simultaneous multielement detection by ICP-AES (inductively coupled plasma atomic emission spectrometry) for chemical speciation. In the experiment, it was found that most of large organic molecules including metal ions in natural water might be humic acid or its derivatives. Therefore, in the present study, we have applied the same measurement system to elucidation of the interaction between some metal ions and humic acid, and it has been found that some formation of larger molecular humic acid occurs, when zinc ion is added in humic acid solution.

The size exclusion chromatography (SEC) system for molecular separation was almost similar to one used previously.9) High performance liquid chromatography (HPLC; Model LC-9A, Shimadzu, Kyoto) was used with a gel filtration column (Superose 12, Pharmacia LKB, Uppsala, Sweden) and an UV absorption detector (UVIDEC II, JASCO, Tokyo). The filtration range of the present gel column was 1000-300000 as molecular weight (MW). An inductively coupled plasma atomic emission spectrometer (ICP-AES; Model Plasma Atomcomp Mk II, Jarrell-Ash, Franklin, MA, USA) was used as the element-selective multielement detector for SEC, and trace elements involved in large organic molecules were detected simultaneously in a time-sequence mode. Generally, 16 mM (M= mol/dm^3) dipotassium hydrogenphosphate-nitric acid buffer solution (pH 7.3) was used as the mobile phase and eluted at the flow rate of 0.5 ml/min. The sample injection volume was 200 μl. The UV detector and ICP-AES were connected to the elution tubing from the separation column in series. In this experiment, organic molecules were detected by the UV absorption 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 1.0 l/min, outer gas flow rate 20 l/min, and observation height 18 mm above load coil. The present ICP-AES system had a computer program of time-sequence data acquisition for chromatogram measurement, which allowed to detect 39 elements simultaneously. In the time-sequence measurement program, the emission signals of analyte elements examined were acquired at each time-interval of 2 s and the 1600 data points as the total were measured for each chromatogram, which resulted in the whole retention time range of 53.3 min.

A commercially-available humic acid reagent purchased from Tokyo Kasei Co. (Tokyo) was used without further purification. The humic acid solution was prepared by dissolving 2 g of the reagent in 1 l of pure water. After taking water-soluble component by filtration with a membrane filter (pore size: 0.45 μm), the sample solution of humic acid obtained was injected into the SEC/UV/ICP-AES system. The chromatograms of humic acid solution measured by this system are shown in Fig. 1. In the chromatogram obtained by the UV detection in Fig. 1 (A), three main peaks were observed as a broad one (Peak 1) and two relatively narrow ones (Peaks 2 and 3). As can be seen in the chromatograms for trace metals in Fig 1.(B), Mn was observed only at the retention time corresponding to Peak 1, and Fe and Al at the retention times corresponding to Peak 1 and Peak 2. It is noted here that these trace metals of Mn, Fe and Al were originally contained in the humic acid reagent. The retention times and shapes of Peak 2 and 3 observed in Fig. 1 (A) were almost similar to those of the peaks in the chromatogram of pond water sample,[9] although the retention time of Peak 1 was different from that of pond water sample. This result indicates that humic acid examined here contains some compounds similar to large organic molecules in natural water. Thus the effect of metal ions on dissolved humic acid in natural water was further investigated.

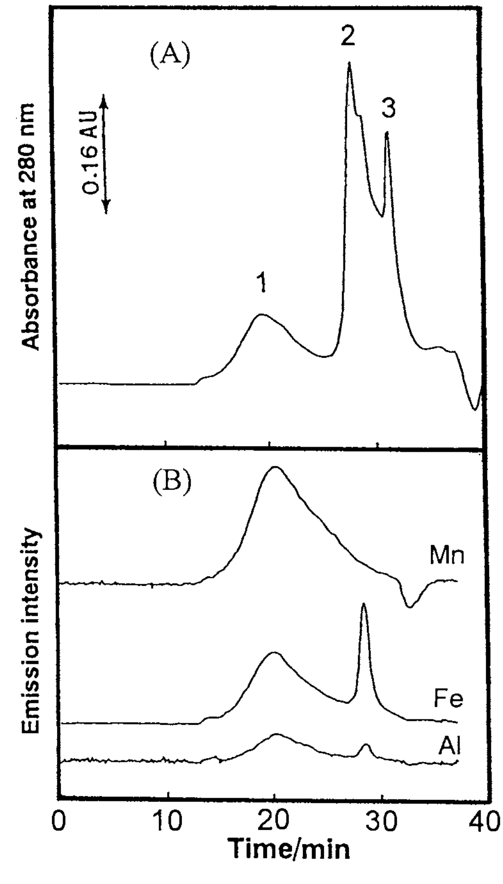

Fig. 1. Chromatograms for humic acid solution detected by (A) UV absorption [280 nm] and (B) ICP-AES [emission wavelength; Mn 257.6 nm, Fe 259.9 nm, Al 308.2 nm].

The interaction between humic acid and metal ion was investigated by the SEC/UV/ICP-AES system with addition of zinc ion at several concentration levels. In previous paper,[9] it was elucidated that zinc ion was one of the elements included in large organic molecules in natural water, and so zinc ion was chosen as a metal ion added to humic acid solution. In this experiment, the zinc standard solution for atomic emission spectrometry (Wako Pure Chemical Industries, Osaka) was used for the added solution, in which the zinc concentration was adjusted so as to be 0, 10, 100 and 1000 ppm (ppm = 10^{-6} g/ml) in 0.1 M nitric acid, respectively. Then the added solution was mixed with the humic acid solution in the ratio of 1 : 9, and these mixed solutions were measured by the SEC/UV/ICP-AES system. The chromatograms observed are shown in Fig. 2. Comparing the UV-detected chromatograms in Fig. 2 (A), it is seen that the peak near the retention time of ca. 20 min (Peak 1) was shifted to one near the retention time of 12 min with increase of zinc concentration, while the retention times of Peak 2 and Peak 3 were not changed. According to the molecular weight calibration for the present size exclusion column,9) the retention times of 12 and 20 min corresponded to MW $\geqq$ 300000 and 150000, respectively. Thus it should be noted that the addition of zinc ion in the humic acid solution promoted the formation of larger molecules for humic acids.

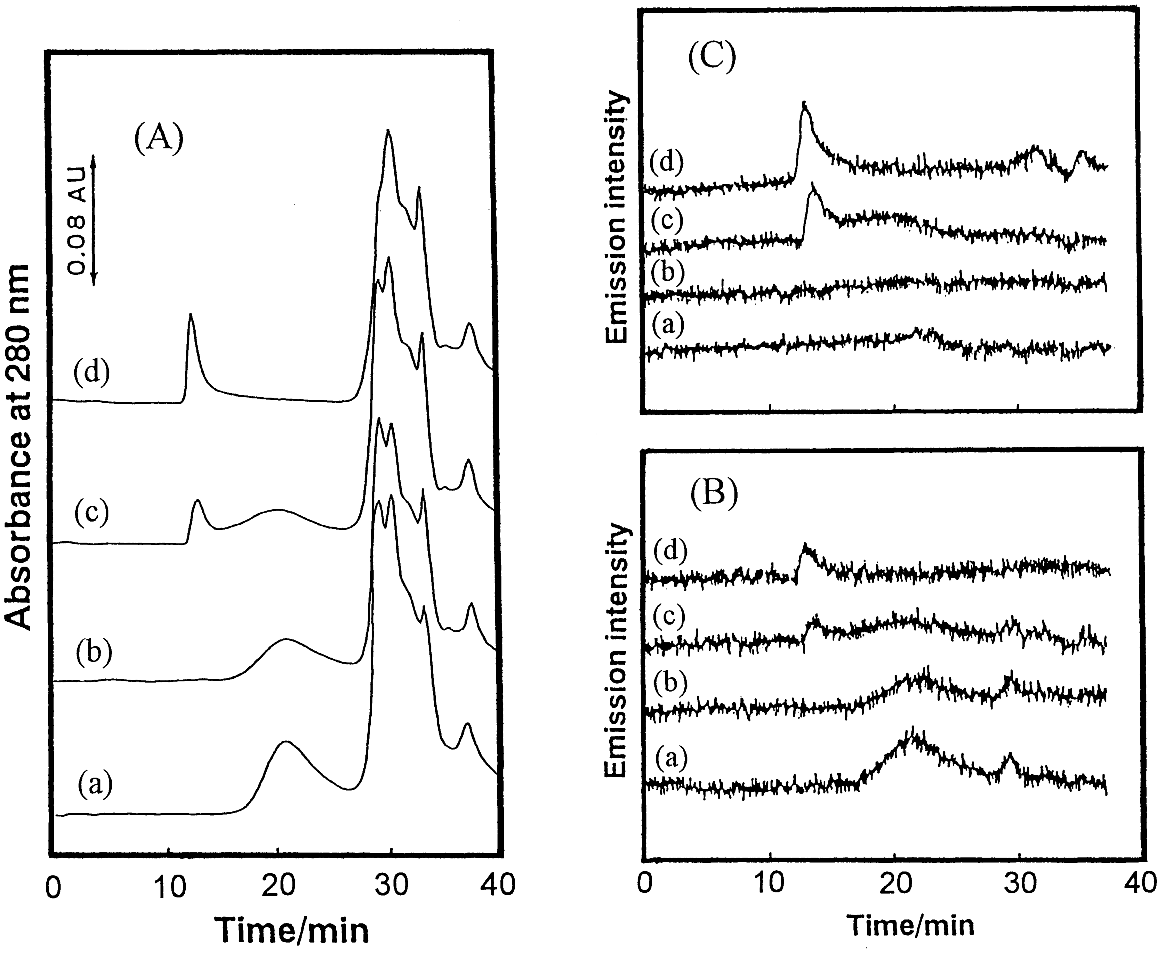

Fig. 2. Chromatograms for humic acid solution with addition of zinc ion detected by UV absorption (A) and ICP-AES for Al (B) and for Zn (C). In all the chromatograms, (a) Zn not added, (b) Zn 1 ppm added, (c) Zn 10 ppm added, (d) Zn 100 ppm added. The wavelengths for UV absorption and ICP-AES detections are the same as in Fig. 1, except for emission wavelength of Zn 213.8 nm.

Aluminum existing in humic acid and zinc added in humic acid solution were also detected by ICP-AES, and the chromatograms are shown in Fig. 2 (B and C), respectively. As for aluminum, which was originally contained in humic acid with iron and manganese, its retention time for Peak 1 in Fig. 2 (B) was also shifted to the shorter retention time with the formation of large molecular humic acid by addition of zinc ions. The chromatograms for iron and manganese showed almost similar changes to those for aluminum. It is also noted that zinc ion, which was not originally contained in humic acid examined, was incorporated into the fractions for Peak 1 and Peak 2. Since Peak 1 at the retention time of 12 min corresponds to the molecule with MW $\geqq$ 300000, these results may indicate that some part of humic acids combined with zinc ions and formed larger molecules in aqueous solution. Furthermore, it should be noticed that the chromatogram for humic acid solution with addition of 100 ppm zinc ion was almost similar to that for preconcentrated pond water,9) in both cases of the UV absorption-detected and ICP-AES detected chromatograms.

Similar experiment was performed by the SEC/UV/ICP-AES measurement with addition of alkali and alkaline earth ions, which were major elements dissolved in natural water. Such large molecular formation could not be observed in the case of alkali and alkaline earth ions. The present experimental results suggest that large organic molecules in natural water, which may be humic acid or its derivatives, are major compounds to form some complexes with heavy metal ions and play significantly important roles in the presence of dissolved trace metals in acquatic envirnment. Further experiment using natural water samples is now in progress to prove the determining factors for dissolved trace metals in natural waters.

References

1) "Humic substances in soil, sediment, and water," ed by G. R. Aiken, D. M. Mcknight, R. L. Wershaw, and P. Maccarthy, John Wiley & Sons (1985).
2) T. M. Florence, *Talanta*, **29**, 345 (1982).
3) T. Midorikawa, E. Tanoue, and Y. Sugiura, *Anal. Chem.*, **62**, 1737 (1990).
4) E. R. Sholkovitz and D. Copland, *Geochim. Cosmochim. Acta*, **45**, 181 (1981).
5) J. Vuseta and J. J. Morgen, *Environ. Sci. Technol.*, **12**, 1302 (1978).
6) W. Fish, D. A. Dzombak, and F. M. Morel, *Environ. Sci. Technol.*, **20**, 676 (1986).
7) M. Hiraide, *Anal. Sci.*, **8**, 453 (1992).
8) "Metal Speciation," *Anal. Chim. Acta*, **284**, No.3 (Special issue), (1994).
9) A. Itoh, M. Aikawa, H. Sawatari, A. Hirose, and H. Haraguchi, *Chem. Lett.* **1993**, 1017.

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