Chromatography with pH-Gradient Elution of Dissolved Humic Substances in River Water

Kazuya OHGA, Yuji ARITOMI and Hiroshi OHTSU

Department of Environmental Chemistry and Engineering, Faculty of Engineering, Oita University, Dannoharu, Oita 870-11, Japan

Keywords Dissolved humic substances, pH-gradient elution, river water, iron(III) ion, solid adsorbent

Many researchers have obtained general knowledge of the chemical properties of aquatic humic substances.¹ But the analytical methods commonly used involve skillful but time-consuming techniques for isolating the quite dilute humic substances.² On the other hand, a wide-ranging search for changes in the concentration and properties of the humic substances often requires analysis of a great number of samples. In such cases, an uncomplicated analytical method having no isolation step is needed. We wish to report herein that a chromatographic method with pH-gradient desorption effectively serves as a means of quickly knowing the composition and relative concentration of dissolved humic substances in river water.

Hydrophobic adsorption onto a nonionic macroporous resin, Amberlite XAD-8, at pH 2 has been generally considered the best method for isolation of humic substances from natural waters, since the resin has the great adsorption capacity and the humic substances adsorbed are mostly recovered by eluting with a basic solution, without the risk of chemical alteration.²⁻⁴ Soil humic acids have also been successfully fractionated by pH-gradient elution. This is based on the mechanism that as the pH of an eluting solution increases, components having larger pK_a values are progressively ionized and thus desorbed.^{5,6} The chromatographic method employed here is a simple combination of the adsorption onto the resin and the pH-gradient desorption and gave information about differences hitherto unknown in the composition and concentration of dissolved humic substances between upper and lower reaches of a river. In addition, the combination showed not only the well-known decrease in the concentration induced by coprecipitation with a Fe^{3+} ion⁷ but also a change in the composition.

Experimental

Water was purified with a Millipore Milli-RO15

water purification system and an activated charcoal column. XAD-8 resin was purified according to the procedure reported by Thurman and Malcolm.³ Glass columns packed with the resin (Fig. 1) were washed alternately several times with 0.1 M (1 M=1 mol dm⁻³) HCl and 0.1 M NaOH prior to use. River water samples were taken from the Oita river (Oita, Japan) about 7 km upstream from the river mouth and from the Shirataki river, a small river in which the Oita river originates. The water samples were filtered through a 0.5 μ m glass-fiber filter (Toyo Roshi GC50) and were then adjusted to pH 2.0 with phosphoric acid. The acidified samples (500 cm³) were pumped onto the columns at a flow rate of 1.3 cm³ min⁻¹. Before chromatographic runs, an acidic mixture of phosphoric, acetic, and boric acids (0.01 M, respectively) was passed through the columns for 1.5–2 h until a flat base-line had been obtained. A pH-gradient solution was prepared by titrating the acidic solution with a basic

Fig 1 Block diagram for fractionation of humic substances by pH-gradient elution. 1, acidic solvent; 2, basic solvent; 3, solvent gradient system (linear mode); 4, peristaltic pump; 5, XAD-8 precolumn (8 mm i.d.×20 mm); 6, XAD-8 column (10 mm i.d.×300 mm); 7, sample water; 8, UV detector; 9, pH electrode with a flow cell.

mixture of 0.01 M trisodium phosphate, 0.01 M sodium acetate, 0.005 M sodium borate, and 0.005 M NaOH using a JASCO GP-A30 solvent gradient system (linear mode). The solution was passed through at a flow rate of $0.9 \text{ cm}^3 \text{ min}^{-1}$ with the help of a peristaltic pump (Tokyo Rikakikai MP-3). The column effluent was monitored with absorbance at 400 nm using a Shimadzu UV-140 spectrophotometer equipped with a flow cell. The pH was also monitored using a flow-through pH electrode (TOA Electronics GS-80). A water sample in which Fe^{3+} was intentionally dissolved was stirred at room temperature for 1 h, filtered, and then subjected to the same chromatography. The small precolumn was renewed after each run.

Results and Discussion

Curves a and b in Fig. 2 show a typical elution pattern of a water sample (dissolved organic carbon (DOC), 2.4 mg dm^{-3}) taken from the Oita river on July 18, 1988 and the pH profile of the eluate, respectively. The elution pattern was reproducible to $\pm 3.5\%$ in the total band area on repeated runs. The purified-water blank (curve c) had no peak, though it gave slightly positive absorbances at basic pHs.

The DOC in natural waters which is adsorbed onto XAD-8 resin at pH 2 and desorbed with 0.1 M NaOH has been designated the hydrophobic acid fraction.^{3,4,7} For river waters, this fraction is commonly composed of humic substances and trace amounts of hydrophobic low-molecular-weight acids.³ The above elution pattern was obtained at 400 nm, a relatively long wavelength where almost all of the low-molecular-weight acids have little absorption, and was thus regarded primarily as the elution pattern of the dissolved humic substances. Also, it can be pointed out that components eluting in the low pH region have higher carboxyl group contents, while components above pH 10 have higher phenolic-hydroxyl group contents.^{5,6,8}

The total elution-band area of a water sample (DOC, 1.7 mg dm^{-3}) taken from the Shirataki river on the same day (curve d in Fig. 2) was smaller than that of the Oita river (curve a), indicating that the Shirataki river sample contained smaller amounts of dissolved humic substances. In addition, the band area in the pH 3–5 region (elution volume, 65–120 cm^3) was quite small and a large band appeared above pH 11.5 (elution volume > 225 cm^3); that is, dissolved humic substances in the Shirataki river were richer in phenolic groups than those in the Oita river.

It is well known that Fe^{3+} efficiently induces aggregation of humic substances and hence is effective in precipitating them from dilute solutions.^{7,9} In fact, the addition of $1.0 \times 10^{-5} \text{ M Fe}^{3+}$ to 500 cm^3 of the Oita river water sample induced a decrease of 35% in the total band area. Interestingly, the area ratio of the bands below pH 8 to those above pH 8 also increased from 0.93 to 1.38.

Fig 2 Fractionations of water samples by pH-gradient elution. Samples from the Oita river (a), the Shirataki river (b) and the purified-water blank (c). Curve b is the pH profile of the eluate.

Because of their lower acidities and relatively high molecular weights, humic acids are generally adsorbed onto XAD-8 resin at pH 7, whereas fulvic acids are not adsorbed.⁸ Components eluting above pH 8 are therefore abundant in humic acids and components below pH 8 abundant in fulvic acids. Thus, the more efficient decrement of components above pH 8 may be explained at least in part on the basis of the fact that metal complexes of humic acids precipitate more readily than those of fulvic acids.⁹

This work was supported by the Nippon Life Insurance Foundation.

References

1. R. F. Christman and E. T. Gjessing, "Aquatic and Terrestrial Humic Materials", Ann Arbor Science Publishers, Ann Arbor, 1983.
2. G. R. Aiken, in "Humic Substances in Soil, Sediment, and Water", pp. 363–385, ed. G. R. Aiken, D. M. McKnight, R. L. Wershaw and P. MacCarthy, John Wiley & Sons, New York, 1985.
3. E. M. Thurman and R. L. Malcolm, *Environ. Sci. Technol.*, **15**, 463 (1981).
4. J. A. Leenheer, *Environ. Sci. Technol.*, **15**, 578 (1981).
5. M. A. Curtis, A. F. Witt, S. B. Schram and L. B. Rogers, *Anal. Chem.*, **53**, 1195 (1981).
6. P. MacCarthy, M. J. Peterson, R. L. Malcolm and E. M. Thurman, *Anal. Chem.*, **51**, 2041 (1979).
7. J. A. Leenheer, in Ref. 2, pp. 409–429.
8. E. M. Thurman and R. L. Malcolm, in Ref. 1, pp. 1–23.
9. F. J. Stevenson, "Humus Chemistry", pp. 309–354, John Wiley & Sons, New York, 1982.

(Received November 29, 1988)

(Accepted February 8, 1989)