Letters to the Editor

Determination of Humic and Fulvic Acids in River Waters by Concentration with Anion Exchanger Followed by Centrifugation

Tsutomu NOMIZU, Masaki SANJI, Masataka HIRAIDE and Atsushi MIZUIKE

Faculty of Engineering, Nagoya University, Nagoya 464, Japan

Keywords Humic acid, fulvic acid, river water, anion exchange, centrifugation

Humic substances are the major organic constituents of fresh waters and play many important roles. For example, they interact strongly with toxic heavy metals¹ and may be precursors of trihalomethanes formed during water treatment with chlorine.² Humic substances in water are usually divided into two fractions: humic acid, which is soluble in alkaline solutions but precipitates in acid solution, and fulvic acid, which is soluble in both alkaline and acid solutions.

A rapid method based on coprecipitation and flotation was proposed by the present authors³ for the separation and determination of humic acid in fresh waters, but it is not applicable to fulvic acid. Humic and fulvic acids can be simultaneously concentrated by low-energy physical sorption on macroreticular non-ionic polymers or by anion exchange⁴, and then humic acid alone is separated at pH 1 by centrifugation⁵, although the operational details have not been reported.

In the present work, the optimum conditions are established for the separation of humic and fulvic acids in river water by centrifugation after preconcentration by anion exchange.

was prepared by dissolving the purified humic acid with 0.01 M sodium hydroxide, neutralizing and diluting; its absorption coefficient was 0.096 cm^{-1} at 350 nm at pH 7. A standard fulvic acid solution ($100 \mu\text{g ml}^{-1}$) was prepared from fulvic acid extracted from soil and purified;⁶ its absorption coefficient was 0.170 cm^{-1} .

Recommended procedure

A 500-ml water sample is filtered through a $0.45 \mu\text{m}$ -pore Millipore filter (47-mm diameter) and passed through a DEAE-cellulose column, 1-cm diam. $\times$ 1 cm, at a flow rate of 5 ml min^{-1} to sorb humic substances. They are then desorbed by backward-flow elution with 10 ml of 0.1 M sodium hydroxide at a flow rate of 1 ml min^{-1} . The collected effluent is acidified to pH 1 with 11 M hydrochloric acid to precipitate humic acid and divided into two centrifuge tubes. Then centrifugation is performed at 14000 min^{-1} for 72 min. The supernatant solution, 4.5 ml, in each tube is carefully removed with a pipet; then the two solutions are combined and neutralized with 10 M sodium hydroxide, and the absorbance(*A*) of the solution is measured at 350 nm at pH 7 against distilled water as reference.⁷ To the remaining 0.5 ml solution in each tube, about

Experimental

Apparatus and reagents

Figure 1 shows the concentration column, which consists of a sorbent bed and two Pyrex glass parts. A Shimadzu UV-180 spectrophotometer was used with 1- and 5-cm cells. A Kubota KH-180 centrifuge was employed with a swing-bucket rotor to carry four 7-ml centrifuge tubes, except for 50000 min^{-1} with a Hitachi 65P-7 ultracentrifuge with three 5-ml centrifuge tubes.

Diethylaminoethyl(DEAE)-cellulose(60–100 mesh, Eastman Kodak) was sequentially washed in 0.5 M hydrochloric acid (1 h), water, 0.5 M sodium hydroxide (1 h) and water, and stored in water at pH 7.

Humic acid (Aldrich Chemical, extracted from peat soil) was purified by repeated dissolution in 0.1 M sodium hydroxide and precipitation in 0.1 M hydrochloric acid. A standard humic acid solution ($5 \mu\text{g ml}^{-1}$)

Fig. 1 Concentration column.

0.1 ml of 1 M sodium hydroxide is added to redissolve humic acid, and the solutions in both tubes are combined, neutralized with 1 M hydrochloric acid and diluted to 10 ml, and the absorbance (B) of the solution is measured at pH 7. The absorption coefficients due to fulvic and humic acids in the original river water are obtained by $A/50l$ and $(B - 0.1A)/50l$, respectively, where l is the optical path length of a cell. The concentrations are calculated by reference to the standard solutions described previously.

Results and Discussion

Concentration of humic substances by sorption

The XAD-7 resin (macroreticular nonionic polymer) was investigated first.⁸ In this case, 500 ml of water samples have to be acidified to pH 2 and passed through a column longer than 10 cm at a flow rate of 2 ml min⁻¹ for satisfactory sorption of humic substances. Desorption recoveries of humic acid were only 70–80%, though those of fulvic acid were reported to be more than 90%.⁸

Dextran gel and cellulose anion exchangers were investigated next; here humic substances in 500 ml of water were quantitatively sorbed on a 1-cm column at a flow rate of 5 ml min⁻¹ at neutral pH. Their desorption depended on the functional groups and matrix compositions of anion exchangers and on the eluent composition. DEAE-Sephadex A-25 is a weak-base anion exchanger previously used by the present authors for the separation of heavy metals associated with humic substances in fresh waters;⁹ it gave a desorption recovery of *ca.* 100% for humic substances with 10 ml of 0.1 M sodium hydroxide–0.5 M sodium iodide. But this eluent was not suitable for the present work, because iodine produced near pH 1 interferes with the spectrophotometric determination of humic substances. Desorption recoveries with 10 ml of 0.1 M and 0.5 M sodium hydroxide were only 13 and 21% for humic acid, respectively. DEAE-cellulose, a weak-base anion exchanger recommended by Miles *et al.*¹⁰, was finally selected, because desorption recoveries of humic and fulvic acids sorbed on the column were 91 and 95%, respectively, with 10 ml of 0.1 M sodium hydroxide.

Backward-flow elution improved the recoveries of humic substances.

Iron in the sample, which interferes with the spectrophotometric determination of humic substances⁷, is satisfactorily removed during this step.

Separation of humic and fulvic acids by centrifugation

Figure 2 shows that humic acid remaining in the supernatant solution (pH 1) decreases with decreasing sedimentation coefficient, which is $\ln(x_2/x_1)/\omega^2t$, where x_1 and x_2 are the initial and final distances of the particle from the axis of rotation, ω is angular rotation velocity, and t is the centrifugation time. Humic acid was quantitatively separated by centrifugation at 14000 min⁻¹ for 72 min, while fulvic acid remained in the solution. Better results may be obtained by centrifugation at 50000 min⁻¹, but an ultracentrifuge has to be used.

Application to river water

The proposed method was applied to the determination of humic substances in river water, with the results shown in Table 1. The blank value through the whole procedure was negligible. Fulvic acid is predominant in this river water. The analytical results for humic acid

Fig. 2 Sedimentation of humic and fulvic acids at pH 1 by centrifugation: (●) humic acid, 5 µg ml⁻¹; (○) fulvic acid, 100 µg ml⁻¹.

Table 1 Separation and determination of humic and fulvic acids in river water (Shōnai River, Nagoya)

Technique used	Absorption coefficient of river water/ 10 ⁻⁴ cm ⁻¹	Concentration in river water/ µg ml ⁻¹
Anion exchange and centrifugation		
Fulvic acid	39	2.3
	38	2.2
Humic acid	5.1	0.027
	6.5	0.034
Coprecipitation and flotation		
Humic acid	7.1	0.037
	8.6	0.045

obtained by the proposed method are in fairly good agreement with those obtained by coprecipitation with hydrated iron(III) oxide followed by flotation, considering that there are differences in separation mechanism between the two.

References

1. T. M. Florence and G. E. Batley, *CRC Crit. Rev. Anal. Chem.*, **9**, 219 (1980).
2. K. Urano, H. Wada and T. Takemasa, *Water Res.*, **17**, 1797 (1983).
3. M. Hiraide, F. Ren, R. Tamura and A. Mizuike, *Mikrochim. Acta* [Wien], **1987 II**, 137 (1988).
4. S. Boggs, Jr., D. G. Livermore and M. G. Seitz, *Rev. Macromol. Chem. Phys.*, **C25**, 599 (1985).
5. E. M. Thurman and R. L. Malcolm, *Environ. Sci. Technol.*, **15**, 463 (1981).
6. S. Arai and K. Kumada, *Soil Sci. Plant Nutr.*, **29**, 543 (1983).
7. T. Nomizu, M. Sanji and A. Mizuike, *Anal. Chim. Acta*, **211**, 293 (1988).
8. G. R. Aiken, E. M. Thurman, R. L. Malcolm and H. F. Walton, *Anal. Chem.*, **51**, 1799 (1979).
9. M. Hiraide, S. P. Tillekeratne, K. Otsuka and A. Mizuike, *Anal. Chim. Acta*, **172**, 215 (1985).
10. C. J. Miles, J. R. Tuschall, Jr., and P. L. Brezonik, *Anal. Chem.*, **55**, 410 (1983).

(Received December 28, 1988)

(Accepted April 1, 1989)