

CHROM. 16,817

Note

Reversed-phase liquid chromatography of molecular weight-fractionated sedimentary fulvic acid

KOHI HAYASE* and HIROYUKI TSUBOTA

Faculty of Integrated Arts and Sciences, Hiroshima University, Higashisenda-machi, Naka-ku, Hiroshima 730 (Japan)

(First received March 8th, 1984; revised manuscript received March 26th, 1984)

Reversed-phase liquid chromatography (RPLC), where retention is governed by solvophobic interactions between the solute and the stationary phase^{1,2}, has been demonstrated to be effective in the separation and isolation of many compounds of geochemical interest³⁻⁷. By the use of RPLC, Templeton and Chasteen⁸ investigated organic matter extracted from anoxic sediment, and discussed the polarity of the samples. Therefore, the application of RPLC to fulvic acid (FA), which is one of the humic substances and has a large molecular weight range, is useful and meaningful. In our previous study of humic substances⁹, the relationship between surface activity, which is correlated to solvophobic interactions, and molecular weight was discussed. For the characterization of FA, it is significant to study the effect of molecular weight on the hydrophilicity of FA, by use of RPLC.

For the first time, in this work, RPLC was applied to the characterization of FA, which was fractionated into five molecular weight ranges by ultrafiltration procedures. The effect of molecular weight on the hydrophilicity of FA is reported.

EXPERIMENTAL

Preparation of FA

The sediment sample was collected from Tokyo Bay in 1974 at 35°15'N and 139°48'E during the cruise of R. V. Tansei Maru (KT-74-18) of the Ocean Research Institute, University of Tokyo. The FA was extracted from the sediments with aqueous alkaline solutions as described previously⁹.

Molecular weight fractionation of FA

The molecular weight fractionation of FA was carried out by using a series of Amicon Diaflo Ultrafilters of various molecular weight retentions, and Amicon filtration procedures. The resulting FA fractions were classified as: FA(K), MW < 10,000; FA(10K), 10,000-50,000; FA(50K), 50,000-100,000; FA(100K), 100,000-300,000; FA(300K), > 300,000.

RPLC measurements

RPLC measurements were carried out at room temperature on a 6000A pump

and 440 Type UV detector (Waters Assoc., Milford, MA, U.S.A.) with a Radial Pak μ Bondapak C_{18} column (100×8.0 mm I.D.). Water and water-2-propanol were used as the eluents, the flow-rate being 1.0–1.5 ml/min. The injection volume was 2–10 μ l. The pressure was varied between 400 and 1300 p.s.i., and the wavelength of the detector was 254 nm. Chromatograms were displayed on a 730 data module (Waters Assoc.) and elution time was printed out digitally.

RPLC of benzenecarboxylic acids (benzoic acid, phthalic acid, trimellitic acid and pyromellitic acid) was also performed for comparison with FA. The aqueous solution of each FA and benzenecarboxylic acid was adjusted to pH 8 with 0.1 M sodium hydroxide and the concentration of injected solutions was 80 mg/l.

RESULTS AND DISCUSSION

Chromatograms of various molecular weight fractions of FA, eluted with water and water-2-propanol, exhibited sharp peaks, the shape of which was independent of the flow-rate. Representative chromatograms of FA(10K) are shown in Fig. 1. Fig. 2 displays a plot of the elution volumes of various molecular weight fractions of FA against the 2-propanol content in the mobile phase. The elution volumes increase with increasing 2-propanol content. FA is generally hydrophilic,

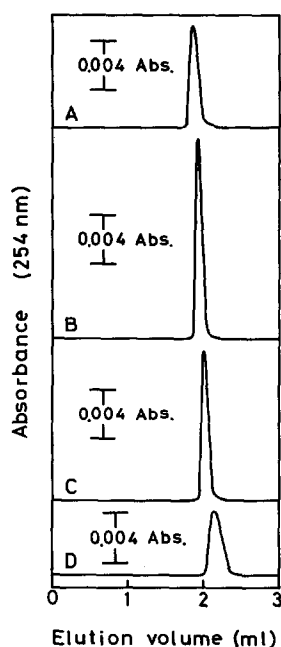


Fig. 1. Chromatograms of FA(10K), molecular weight range 10,000–50,000. Eluents: A, water; B, water-2-propanol (75:25); C, water-2-propanol (50:50); D, water-2-propanol (25:75). Flow-rate: 1.0 ml/min. Injection volume: 2 μ l. Concentration: 80 mg/l.

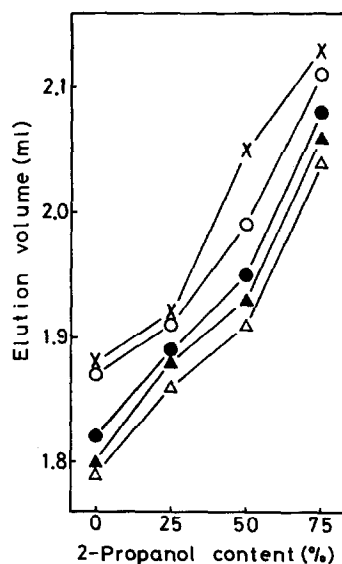


Fig. 2. Plots of the elution volumes of various molecular weight fractions of FA against the 2-propanol content of the mobile phase. Column: Radial Pak μ BondaPak C_{18} . Flow-rate: 1.0 ml/min. Fractions: \times , Fa(K); \circ , FA(10K); \bullet , FA(50K); \triangle , FA(100K); \blacktriangle , FA(300K).

since the elution is retarded by increasing the hydrophobicity of the mobile phase.

From Fig. 2 it is seen that the elution volume increases with decreasing molecular weight of FA, and that the effect of molecular weight on the elution volume is independent of the 2-propanol content. FA(300K) is an exception to this, for reasons which are not known at present. In general, lower-molecular-weight FA exhibits a larger elution volume.

In a study of the RPLC of some amino acids on LiChrosorb RP-8 (octyl silica), Molnar and Horvath¹⁰ reported that the elution volume increased with increasing partition coefficient in octanol-water, corresponding to the increasing hydrophobicity. That is, a lower elution volume represents a greater hydrophilicity. For the benzenecarboxylic acids in Table I, similar decreases in elution volume with increasing hydrophilicity (from benzoic acid to pyromellitic acid) are observed.

TABLE I
ELUTION VOLUMES OF BENZENECARBOXYLIC ACIDS

Compound	Eluent (ml)		
	Water	Water-2-propanol (75:25)	Water-2-propanol (50:50)
Benzoic acid	3.95	2.45	2.24
Phthalic acid	2.11	2.06	2.09
Trimellitic acid	1.95	1.98	2.01
Pyromellitic acid	1.91	1.95	1.99

From the above results, it is suggested that sedimentary FA exhibits increasing hydrophilic character with increasing molecular weight. This is in agreement with the results obtained by Visser¹¹ that higher-molecular-weight fractions of aquatic FA are richer in oxygen than materials of lower molecular weight, although the origins of the FA differ. RPLC is effective for the hydrophobic-hydrophilic characterization of humic substances.

REFERENCES

- 1 Cs. Horvath and W. Melander, *J. Chromatogr. Sci.*, 15 (1977) 393.
- 2 H. Colin and G. Guiochon, *J. Chromatogr.*, 141 (1977) 289.
- 3 J. B. Derenbach, M. Ehrhardt, C. Osterroht and G. Petrick, *Mar. Chem.*, 6 (1978) 351.
- 4 G. L. Mills and J. G. Quinn, *Mar. Chem.*, 10 (1981) 93.
- 5 J. Lee, *Water Res.*, 15 (1981) 507.
- 6 G. L. Mills, A. K. Hanson, J. G. Quinn, W. R. Lammela and N. D. Chasteen, *Mar. Chem.*, 11 (1982) 355.
- 7 D. J. Mackey, *Mar. Chem.*, 13 (1983) 169.
- 8 G. D. Templeton, III and N. D. Chasteen, *Mar. Chem.*, 10 (1980) 31.
- 9 K. Hayase and H. Tsubota, *Geochim. Cosmochim. Acta*, 47 (1983) 947.
- 10 I. Molnar and Cs. Horvath, *J. Chromatogr.*, 142 (1977) 623.
- 11 S. A. Visser, *Water Res.*, 17 (1983) 1393.