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Note

Application of reversed-phase liquid chromatography to dissolved organic matter in estuarine seawater

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The chemical characterization of dissolved organic matter (DOM) in seawater is important from environmental, biochemical and geochemical viewpoints. Up to the present time, much work on gel chromatography has been carried out on the characterization of DOM in natural waters[1–4]. Recently, reversed-phase liquid chromatography (RPLC) has been utilized in the isolation and separation of many organic compounds from seawater[5–9]. Since RPLC is based on solvophobic interactions between the solute and the stationary phase[10,11], information on the hydrophilicity of organic materials can be obtained by this technique.

The effectiveness of RPLC in the study of sedimentary fulvic acid has already been reported[12]. In that study, we showed that the sedimentary fulvic acid exhibited increasing hydrophilic character with increasing molecular weight. In research on fresh-water organic matter by RPLC, Lee[7] found three kinds of fractions, the hydrophobicities of which differed from each other. Brown *et al.*[13] reported a RPLC study on low-molecular-weight, polar organic compounds dissolved in sewage.

Although no method can be used to separate quantitatively all the DOM in seawater, various methods, such as solvent extraction, and adsorption on columns of Sep-Pak, XAD resin, anion-exchange resin or activated charcoal, have been applied at different stages in the separation of DOM from seawater. Since the hydrophobicity is correlated with the adsorptivity on adsorbents and with the efficiency of extraction by organic solvent, investigation of DOM with RPLC should prove useful and significant for the isolation and concentration of DOM. In the present study, DOM is extracted into chloroform from estuarine seawater for the hydrophilic–hydrophobic characterization. Slowey *et al.*[14] reported that copper–organic complexes in seawater were extracted into chloroform without any chelating agent.

Since fluorescence reflects the aromatic character of DOM, more detailed information on the qualitative nature of DOM may be obtained by the use of a fluorescence detector in addition to an absorption detector. Preliminary results on the application of RPLC with double detectors (fluorescence and absorption) to the analysis of DOM in estuarine seawater are reported in this paper.

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EXPERIMENTAL

Sample

A surface seawater sample was collected on June 11th, 1983, from Hiroshima Bay (34°21.1'N, 132°24.2'E) in a 5-l nitric acid-cleaned polyethylene sampling bottle and was filtered through a nitric acid-cleaned 0.4-μm Nuclepore filter in a Class 100 clean room, with use of a nitrogen-pressurized PTFE in-line filtration apparatus. The salinity and temperature of the sample seawater were 29.31‰ and 18.8°C, respectively.

Extraction of DOM

Ultra fine grade chloroform (Nakarai) was used after distillation. DOM was extracted from a 1-l seawater sample, at pH 8 or 3, into 20 ml of chloroform in a 1-l PTFE separatory funnel. The sample at pH 3 was prepared by addition of distilled nitric acid. This extraction process was carried out on four aliquots. All of the above treatments were carried out in the clean room.

RPLC measurements

The chloroform extracts containing DOM were combined and evaporated to nearly 1 ml at *ca.* 40°C. After addition of 20 ml of propan-2-ol to the residue, the solvent was evaporated again to *ca.* 1 ml. The resulting solution was passed through a 0.5-μm PTFE filter, and the filtrate was used for RPLC measurements.

The RPLC measurements were carried out at room temperature (23–25°C), with a 6000A pump and 440 Type UV detector (Waters Assoc., Milford, MA, U.S.A.) and a Hitachi 650-10 LC fluorescence detector, on a Radial Pak μBondapak C_{18} column (100 × 8.0 mm I.D.). Mixtures of propan-2-ol and water in different ratios were used as eluents. The injection volume was 2–10 μl. The flow-rate was 1.5–2.0 ml/min, at a pressure of 600–1600 p.s.i. (40–110 kg/cm^2). The wavelength of the UV detector was 254 nm, and the excitation and emission wavelengths of the fluorescence detector were 320 and 420 nm, respectively. The UV and fluorescence detectors were serially connected, and the delay of elution volume between the two detectors was *ca.* 0.2 ml. Chromatograms were displayed, and elution times were digitally printed out, on a 730 data module (Waters Assoc.). The reproducibility of elution volume was within 0.02 ml, and elution was continued until 60 ml of eluent had been passed.

RESULTS AND DISCUSSION

Chromatograms obtained for DOM extracted into chloroform at pH 8 are shown in Fig. 1. The eluents used were water and water–propan-2-ol (70:30 and 50:50). Each chromatogram in Fig. 1 has only one peak with the UV detector. Although the same amounts of sample were injected into the column, the peak height of the fraction 1-X in Fig. 1a is much lower than those in Fig. 1b and c. It is thought that the major components of fraction 1-X are not eluted from the column with water as eluent. The elution volume of the fraction 1-X decreased with increasing propan-2-ol content. We have already reported that the elution volume of benzoic acid, which is a relatively hydrophobic substance, decreased and that of pyromellitic acid, which is relatively hydrophilic, increased with increasing propan-2-ol content[12].

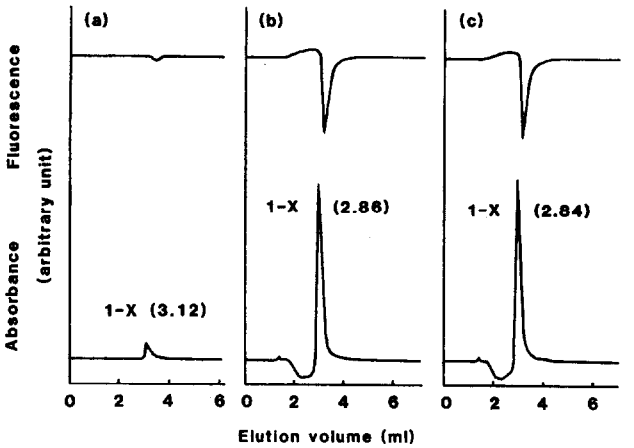

Fig. 1. Chromatograms of DOM extracted with chloroform from seawater at pH 8. Numbers in parentheses are elution volumes (ml). Detectors: absorption (254 nm) and fluorescence (excitation and emission wavelengths at 320 and 420 nm, respectively). Eluent (flow-rate in parentheses): (a) water (2.0 ml/min); (b) water–propan-2-ol (70:30) (2.0 ml/min); (c) water–propan-2-ol (50:50) (1.5 ml/min).

Thus, it can be said that the fraction 1-X consists of relatively hydrophobic organic matter. Each chromatogram detected by fluorescence (excitation and emission wavelengths at 320 and 480 nm, respectively) has also one peak in Fig. 1. From the delay of elution volume, each peak detected by fluorescence corresponds to that by UV detection. Since fluorescence can result from aromatic groups of organic molecules, it is suggested that fraction 1-X has some aromatic characteristics.

Fig. 2 shows chromatograms of DOM, which was extracted into chloroform from seawater at pH 3. Although there are three peaks obtained by UV detection in Fig. 2b and c, only one small peak is observed in Fig. 2a. A larger amount of the fraction 2-X and all of the fractions 2-Y and 2-Z are shown to be adsorbed on the column, when the eluent is water. The elution volume of the fraction 2-X decreased with increase in propan-2-ol content just as with fraction 1-X, and the fraction 2-X also consists of relatively hydrophobic organic matter. The chromatogram shown in Fig. 2b shows finer resolution than that in Fig. 2c. Three kinds of organic substances exhibiting different degrees of hydrophilicity were observed in Fig. 2b and c. Lee[7] also employed RPLC for the separation of fresh-water organic materials into fractions of different polarity, and found three fractions of organic compounds. However, in these studies, the hydrophilicity of the DOM cannot be compared with that of Lee, because the eluents are different. In our previous study[12], it was reported that a lower elution volume represents a greater hydrophilicity on this column. It is suggested then, that the degree of hydrophilicity of the three fractions decreases from 2-X to 2-Y to 2-Z.

In Fig. 2b and c, the chromatogram obtained by fluorescence detection has only one peak. From the elution volume, each peak corresponds to the peak 2-X, and the fractions 2-Y and 2-Z did not exhibit fluorescence. Because fluorescence is attributed to aromatic groups of molecules, it is suggested that the fraction 2-X is more aromatic character than the fractions 2-Y and 2-Z. Fluorescence/absorbance peak ratios of the fractions 1-X and 2-X have the same values (0.43) in Fig. 1 and

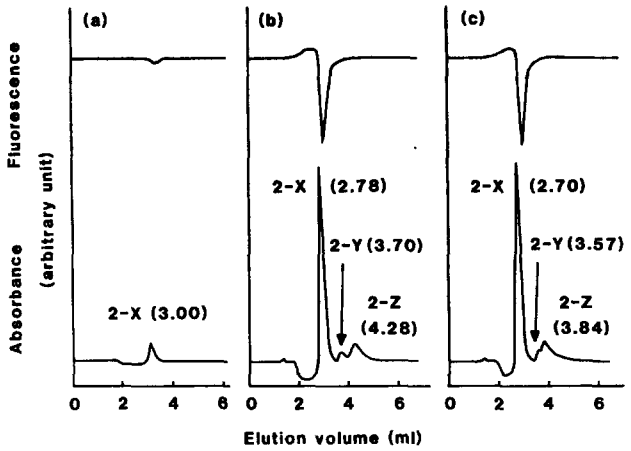

Fig. 2. Chromatograms of DOM extracted chloroform from seawater at pH 3. Numbers in parentheses are elution volumes (ml). Conditions as in Fig. 1.

2, and thus, these fractions are considered to be identical (hence forward, designated fraction X). This fraction X is observed to be slightly more hydropohobic at pH 8 than at pH 3, because, the elution volume of fractions 1-X and 2-X are 2.86 and 2.78 ml in Figs. 1b and 2b, respectively. The above fact can be also recognized for Figs. 1c and 2c. It can be said that fraction X became slightly hydrophilic by protonation at lower pH. Thus, the fraction X is considered to be a neutral or a weakly basic DOM.

Although three peaks were obtained by UV detection in Fig. 2b, only one peak was observed in Fig. 1. This fact means that the fractions 2-Y and 2-Z were not extracted into chloroform from seawater at pH 8. It is suggested that fractions 2-Y and 2-Z are more hydrophilic than fraction X at pH 8, since chloroform is a hydrophobic solvent. On the other hand, the fractions 2-Y and 2-Z are less hydrophilic than the fraction X at pH 3 in Fig. 2b. This increase in hydrophobicity of fractions 2-Y and 2-Z from pH 8 to 3 may be attributed to deionization of their functional groups by decrease in pH. It is reasonable to think that fractions 2-Y and 2-Z contain more functional groups (such as –COOH and phenolic –OH), the ionization of which is easily neutralized by decrease in pH, than fraction X. The fractions 2-Y and 2-Z are thought to be acidic DOM as shown by actual RPLC chromatograms.

Though the peaks detected by absorbance and fluorescence are thought to be due to organic substances, the amount of sample eluate is too small to analyse for organic matter directly. The following experiment was therefore performed. The sample extract was evaporated and kept at 250°C for 10 h to decompose organic matter, and the residue was dissolved in propan-2-ol. This solution was analysed by RPLC and no peak was observed. This result implies, although indirectly, that the peaks in Fig. 1 and 2 are due to organic matter, which was decomposed at temperatures lower than 250°C.

From seawater, the DOM with elution volumes in the range between 2.70 and 4.28 could be isolated. We have already studied the RPLC of fulvic acid extracted with aqueous alkaline solutions from the sediment of Tokyo Bay, and their elution

volumes were *ca.* 1.9 ml on this column[12]. The three fractions of the DOM extracted from the seawater sample were less hydrophilic than the sedimentary fulvic acid. Fractions with elution volumes larger than the fraction 2-Z and smaller than the fraction X were not observed in the chromatograms. It is not known whether such fractions do not exist in seawater or that they cannot be extracted into chloroform. Further investigations should be concerned with developing methods for concentrating DOM to a maximum degree.

Here, the hydrophilic–hydrophobic balance and aromatic character of the DOM in seawater were actually represented on the chromatograms. The application of RPLC with double detectors (absorption and fluorescence) appeared to be effective in the characterization of DOM in seawater.

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