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Note

Application of high-performance aqueous gel permeation chromatography to humic substances from marine sediment

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High-performance gel permeation chromatography (GPC) is a type of liquid chromatography in which the separation is based on the molecular size of the solute, usually in organic solution. On the other hand, conventional gel chromatography used in aqueous systems has been often utilized in the measurements of molecular distribution and weight, the isolation, purification, and fractionation of bio-macromolecular compounds over an extremely wide range of molecular weights. Therefore, the application of gel chromatography to humic substances (humic and fulvic acids) with polydispersity is useful and meaningful. In the past, the gels used in the gel chromatography of humic substances[1–4] were sagarose, agarose, Bio-Gel and particularly Sephadex gel. However, since these soft gels are operated by low pressure drops (low flow-rates), the separation is often time-consuming and the resolution is not very sharp.

For the first time, in our laboratory, a recently developed high-performance aqueous GPC column (TSK-GEL, Type-SW; Toyo Soda, Tokyo, Japan) was applied to the separation and characterization of humic substances. The GPC column packed with microspheres of hydrophilic gels (grain size, 10 $\pm$ 2 μm) was operated by high pressure in aqueous systems (as in gel chromatography), and had more than 5000 theoretical plates/ft. According to the TSK-GEL catalogue (Toyo Soda), the fractionation performance of the molecular weight by the column is 1000–300,000 on proteins and 1000–100,000 on dextrans.

EXPERIMENTAL

Preparations of samples

The samples of humic and fulvic acids were extracted with 0.1 *M* $Na_4P_2O_7$ + 0.1 *N* NaOH solution from the marine sediment of the Sagami Bay. The sediment was collected at 34°59′5″N, 139°07′3″E at a depth of 88 meters and at a distance of 11 km from the shore. The humic acid was purified by centrifugation, redissolution, reprecipitation, Millipore filtration (0.22 μm) and cation exchange. The fulvic acid was purified by activated carbon, Millipore filtration and cation exchange. The acidic solutions of the purified humic and fulvic acids were neutralized to pH 7 with 0.1 *N* NaOH, were Millipore filtered and the samples were injected into the GPC column.

The concentrations of the humic and fulvic acids samples were 532 and 307 ppm as total organic carbon, respectively.

Apparatus and conditions

GPC measurements were carried out at room temperature on a Tri-Rotor pump and Uvidec-100 Type UV detector (Japan Spectroscopic) with TSK-GEL G 3000 SW column (60 cm × 0.75 cm I.D.). Pure water and 0.1 *M* NaCl aqueous solution were used as the eluent, the flow-rates were 0.5 and 1.0 ml/min, and the injection volumes were varied between 0.025 and 0.1 ml. The pressure operated was approximately 30 atm for 1.0 ml/min of flow-rate. The wavelength of detector was 254 nm. The void volume (V_0) and total effective column volume ($V_0 + V_i$) of the column were determined using Blue dextran 2000 and acetone, respectively.

RESULTS AND DISCUSSION

Fig. 1 shows chromatograms of the humic and fulvic acids eluted with 0.1 *M* NaCl. The chromatograms were nearly independent of flow-rates and the amounts injected. However, using pure water as an eluent, the large part of the humic and fulvic acids eluted at V_0, namely the higher-molecular-weight range. Orange G (mol.wt. 452) also eluted at V_0 in the water system. In these cases, the exclusive effect would be caused by the coulombic repulsion between the gel and the solute. As shown in Fig. 1, with 0.1 *M* NaCl as an eluent, all the peaks of the humic and fulvic acids appeared between V_0 and $V_0 + V_i$. Unlike Sephadex gel[5,6], the retardation based on adsorption forces was not observed. To separate the higher-molecular-weight part of the humic acid, the experiment with G 4000 SW column which fractionates higher molecular weights than G 3000 SW is now under investigation.

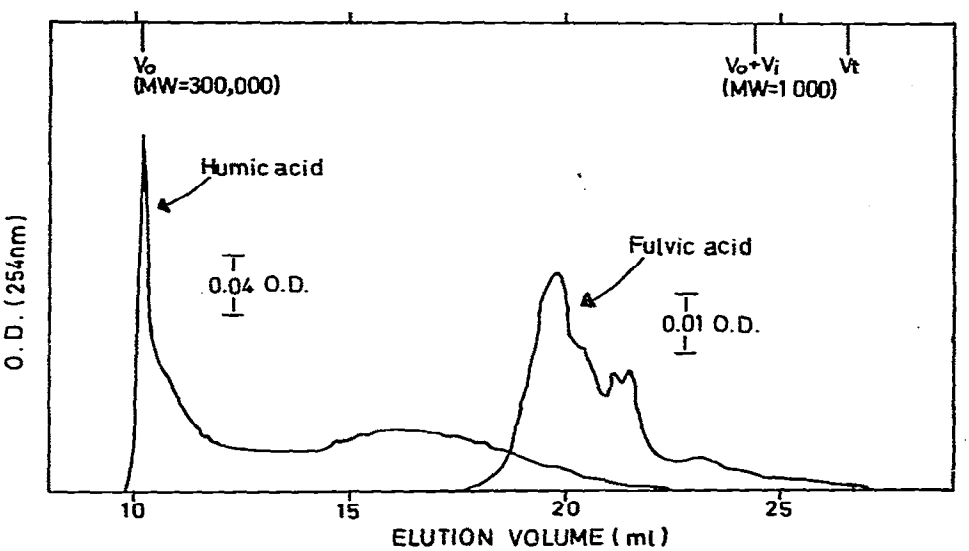

Fig. 1. Chromatograms of the humic and fulvic acids extracted from marine sediment. Eluent, 0.1 *M* NaCl; flow-rate, 1.0 ml/min; amount injected, 0.05 ml. V_t is the total column volume of the column.

From these results, TSK-GEL Type SW series for high-performance aqueous GPC are suggested to be extremely effective for the fractionation based on molecular size and estimation of molecular weight of humic substances.

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