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Superheated Water Chromatography of Phenols Using Poly(styrene-divinylbenzene) Packings as a Stationary Phase

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A method for the superheated water chromatography of phenols was developed using a poly(styrene-divinylbenzene) (PSDVB) stationary phase. The stationary phase of superheated water chromatography must be stable against the attack of water. A durability test for PSDVB packings and octadecylsilyl (ODS)-silica gels indicated that PSDVB packings were stable in superheated water in the temperature range of 100 – 150˚C, whereas octadecylsilyl groups of ODS-silica gels cleaved even at 100˚C. The retention of phenols on the PSDVB stationary phase decreased with an elevation of the column temperature. The retention mechanism was characterized using a thermodynamic theory that has been used for describing retention in conventional RP-HPLC. The application of the present method to an environmental analysis was also demonstrated, in which a suitable separation with good peak shape was obtained for *p*-chlorophenol in river-water samples.

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Reversed-phase high-performance liquid chromatography (RP-HPLC) is a very popular technique for the analysis of nonvolatile or semivolatile organic compounds, and is employed in a wide variety of chemical laboratories. In RP-HPLC, mixtures of relatively polar organic solvents (*i.e.*, methanol or acetonitrile) and water are generally used as a mobile phase; the retention time of analytes can be easily controlled by changing the composition of the mobile phase. However, most organic solvents are not environmentally friendly and are potentially toxic to the operator. In addition, the use of an organic solvent involves a higher running cost. As a consequence, minimizing the amount of the required organic solvents or the development of an organic solvent-free analytical technique in RP-HPLC is of great interest.

Water is a nonhazardous, inexpensive, and environmentally friendly solvent. The solubility of organic compounds to ambient water is so poor that the use of water alone as the mobile phase in RP-HPLC is unsuitable. However, the solubility of organic compounds increases with an elevation in the temperature under modulated pressure to maintain the liquid state of water. This solubility change can be interpreted in terms of the change in the polarity of water. For example, elevating the temperature and the pressure of water from 25˚C and atmospheric pressure to 200˚C and 5 MPa decreases the dielectric constant from 78 to 35.[1,2] The latter value is nearly equal to that of ambient methanol.[1] In fact, an elevation of the temperature and pressure remarkably enhances the solubility of hydrophobic compounds in water.[3]

HPLC with water at elevated temperatures and pressures (so-called “superheated water” chromatography) is considered to be capable of replacing conventional RP-HPLC with organic solvents. Some researchers have already proven the usefulness of this technique.[4–16] In most cases, poly(styrene-divinylbenzene) (PSDVB) packings were used as the stationary phase,[4–15] because of their thermal-stability in size-exclusion chromatography and conventional RP-HPLC. Octadecylsilyl (ODS)-silica gels have also been employed at column temperatures above 100˚C.[4–6] For example, the separations of phenols, anilines and alkylbenzenes were performed on an ODS-silica gel stationary phase, and their retention factors were 2 – 3 times smaller than those on a PSDVB stationary phase.[6] On the other hand, Wilson pointed out the lesser thermal stability of ODS-silica gels.[7] No published studies to date have evaluated the durability of these stationary phases.

In this study, we developed superheated water chromatography of phenols using a PSDVB stationary phase. The stability of PSDVB packings was evaluated by means of a durability test, in which superheated water was passed through the separation column for 144 h. Having investigated the retention behavior of phenols in this system, we succeeded to apply superheated water chromatography to an environmental sample analysis.

Experimental

Reagents

The deionized water which we used was prepared in our laboratory using a Millipore (San Jose, CA, USA) Milli-Q Gradient system at an output of 18.2 MΩ cm.

All of the test compounds were purchased from Wako Pure Chemical (Osaka, Japan). Sample solutions of these compounds were prepared by dissolving them into HPLC-grade methanol obtained from Kanto Chemical (Tokyo, Japan).

Two kinds of river-water samples were collected in Ibaraki prefecture. Each sample was filtered with a 0.45 μm membrane filter and then *p*-chlorophenol (12.5 μg l^{-1}) was added into it.

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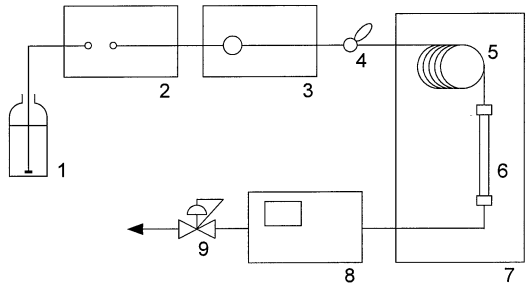

Fig. 1 Schematic diagram of the superheated water chromatographic system. 1, deionized water; 2, degasser; 3, pump; 4, injector; 5, preheat coil; 6, separation column; 7, oven; 8, UV-VIS detector; 9, back-pressure regulator.

Superheated water chromatography

A schematic diagram of a superheated water chromatographic system is shown in Fig. 1. The apparatus used for the durability test consisted of a Shodex (Tokyo, Japan) Degas KT25 degasser, a Shimadzu (Kyoto, Japan) LC-6A pump, a Rheodyne (Cotati, CA, USA) Model 7725i sample injector with a 10-μl sample loop, a preheat coil (stainless-steel, 5 m × 0.5 mm i.d.), a separation column, an oven from a Shimadzu GC-7A gas chromatograph, a Shimadzu SPD-6A UV-VIS detector, and a JASCO (Tokyo, Japan) 880-81 back-pressure regulator. The separation columns were made in our laboratory by packing either Hamilton (Reno, NV, USA) PRP-1 (particle size, 10 μm) or Fuji Silysia (Kasugai, Japan) Chromatorex DU0010TT (particle size, 10 μm) into an empty stainless-steel column (50 mm × 3.0 mm i.d.). All other experiments were performed using another apparatus consisting of an Agilent Technologies (Wilmington, DE, USA) 1100 HPLC system (a degasser, a quaternary pump and a UV-VIS detector), a Rheodyne Model 7125 sample injector with a 10-μl sample loop, a preheat coil (stainless-steel, 10 m × 0.25 mm i.d.), a Hamilton PRP-1 separation column (150 mm × 2.1 mm i.d., particle size, 5 μm), an oven from a Shimadzu GC-7A gas chromatograph, and a JASCO 880-81 back-pressure regulator.

The chromatographic conditions were as follows: column temperature, 100 – 150˚C; pressure of mobile phase, 10 MPa; flow rate, 0.2 ml min^{-1} for the column with inner diameters of 2.1 mm and 0.4 ml min^{-1} for the columns with inner diameters of 3.0 mm; detection wavelength, 220 nm; injection volume, 0.4 – 2.0 μl.

Determination of carbon contents of ODS-silica gels

A CE instruments (Milan, Italy) EA 1110 elemental analyzer was used to obtain the carbon content of the ODS-silica gels. Two determinations were made and the average values were taken.

Preparation of river-water samples

The river-water samples were prepared by solid-phase extraction. An outline of the procedure is as follows. A Waters (Milford, MA, USA) PS-2 cartridge packed with PSDVB was conditioned with 10 ml of acetone and 10 ml of deionized water. After a portion of river-water samples was acidified to pH 2 with 1 M hydrochloric acid, 500 ml of them was loaded onto the SPE cartridge at a flow rate of 10 ml min^{-1}. Then, the SPE cartridge was washed with 50 ml of deionized water and dehydrated for 10 min. The adsorbed compounds were eluted with 10 ml of acetone. Finally, the eluate was reduced to 1.0 ml under a gentle stream of nitrogen.

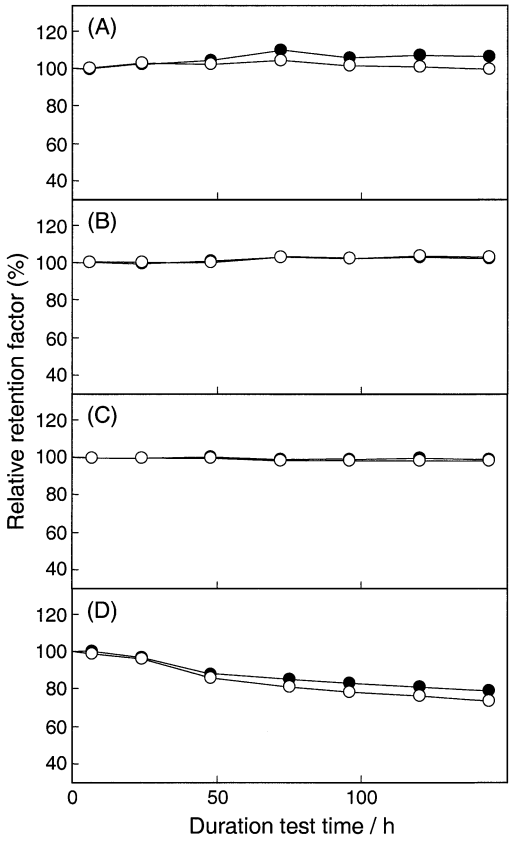

Fig. 2 Duration test of packing materials. Test conditions: (A) PSDVB at 150˚C, (B) PSDVB at 125˚C, (C) PSDVB at 100˚C, (D) ODS-silica gels at 100˚C. Symbols: (●) phenol, (○) *p*-cresol.

Results and Discussion

The stationary phase of superheated water chromatography must be stable against an attack of water, especially since the reactivity of water increases with an elevation in the temperature. The stability of the PSDVB packings was evaluated by means of a following duration test: superheated water was passed through the separation column under constant conditions for 144 h, and the retention factors of phenol and *p*-cresol were measured. Uracil was used to measure t_0. The stability of the PSDVB packings was evaluated in the temperature ranges of 100 – 150˚C, as shown in Fig. 2(A) – (C). The retention factors of phenol and *p*-cresol were almost constant at each temperature. The peak shapes of phenol and *p*-cresol were also unchanged during the durability test. Therefore, PSDVB packings were considered to be stable against superheated water, and were suitable as the stationary phase for superheated water chromatography. The stability of ODS-silica gels was also evaluated. The retention factors of phenol and *p*-cresol on ODS-silica gels decreased with time even at 100˚C, as shown in Fig. 2(D). During the duration test, the carbon content of ODS-silica gels decreased from 21.7% to 18.7%. The change in the carbon contents was probably due to the cleavage of ODS groups from the silica-gel surface. These results suggested that ODS-silica gels could not withstand prolonged use in superheated water chromatography. Therefore, the following investigations were carried out with PSDVB packings as the stationary phase.

Typical chromatograms of phenol, *p*-cresol and *p*-

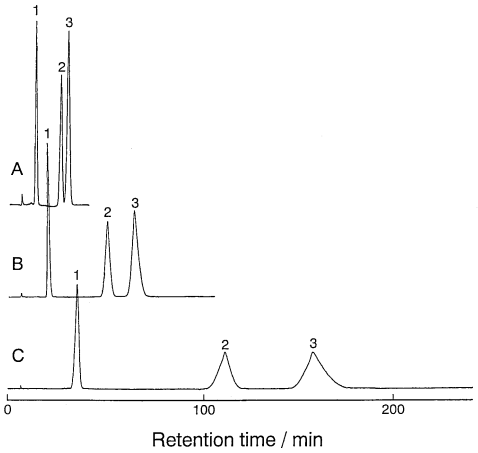

Fig. 3 Superheated water chromatograms of phenols. Column temperature: (A) 150˚C, (B) 125˚C, and (C) 100˚C. Other chromatographic conditions: pressure, 10 MPa; flow rate, 0.2 ml min^{-1}; separation column, PRP-1; detection wavelength, 220 nm. Peaks: 1, phenol; 2, *p*-cresol; 3, *p*-chlorophenol.

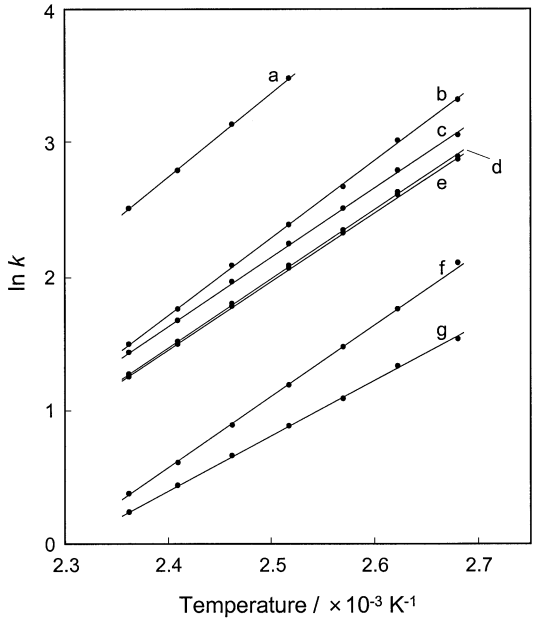

Fig. 4 Effect of the temperature on the retention factor of phenols. Symbols: (a) 2,4-xylenol, (b) *p*-chlorophenol, (c) *o*-cresol, (d) *p*-cresol, (e) *m*-cresol, (f) *p*-nitrophenol, (g) phenol. Pressure: 10 MPa.

chlorophenol are shown in Fig. 3. The elution time of all phenols decreased with an elevation in the column temperature: reasonable elution was obtained when the column temperature was set near to 150˚C. The repeatabilities (n = 8) obtained with respect to the retention time and peak area of phenol at 150˚C were 0.21% and 1.9%, respectively. Although the apparatus used was a laboratory-made system, these results were fairly good for the accurate analyses of phenols.

RP-HPLC separations of phenols were performed in order to compare the retention of phenols in superheated water chromatography with that in conventional RP-HPLC. The same apparatus as superheated water chromatograph, except for removing the column oven and the back-pressure regulator, was employed with the acetonitrile/water mobile phase. When the concentration of acetonitrile was set to 26% (v/v), the elution time of *p*-chlorophenol was 29.3 min, which was almost equal to that in superheated water chromatography at 150˚C. Under these conditions, the detection limits (S/N = 3) of *p*-chlorophenol at the detection wavelength of 220 nm were 0.39 ng in superheated water chromatography and 0.45 ng in conventional RP-HPLC: no remarkable difference was observed between these techniques.

The retention mechanism in superheated water chromatography was investigated using a thermodynamic theory used for describing retention in conventional RP-HPLC. The temperature dependence for the retention of solutes can be given by

$$\ln k = -\Delta H^\circ/RT + \Delta S^\circ/R + \ln \phi \quad (1)$$

where k is the retention factor of the solute, ΔH° is the enthalpy of transfer of the solute from the mobile phase to the stationary phase, ΔS° is the entropy of the transfer of the solute from the mobile phase to the stationary phase, R is the gas constant, T is the absolute temperature, and ϕ is the phase ratio of the separation column. The effect of the column temperature on the retention factor of seven phenols in superheated water chromatography was investigated at 100 – 150˚C under a mobile-phase pressure of 10 MPa. A van't Hoff plot was obtained by plotting ln k *vs*. 1/T, as shown in Fig. 4. As generally observed in conventional RP-HPLC,[17] linear relationships were observed with correlation coefficients greater than 0.999. These linear relationships suggested that ΔH° and ΔS° were independent of the temperature. That is, the retention mechanism of phenols in superheated water chromatography was unchangeable under these experimental conditions.

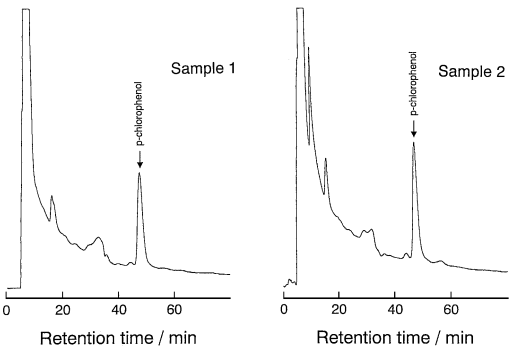

Fig. 5 Superheated water chromatograms of river-water samples. Chromatographic conditions: flow rate, 0.2 ml min^{-1}; separation column, PRP-1; column temperature, 140˚C; pressure, 10 MPa; detection wavelength, 220 nm.

The applicability of the present method to river-water sample analyses was evaluated with *p*-chlorophenol as a target analyte. SPE was used for extracting *p*-chlorophenol from the samples. A recovery test was performed for evaluating the recovery rate in the SPE procedure, as follows: *p*-chlorophenol (12.5 μg l^{-1}) was added into 500 ml of deionized water, and was then extracted by SPE under the same conditions as described in the experimental section. As the recovery rate approached 90%, this procedure was considered to be sufficient for the extraction of *p*-chlorophenol in aqueous samples.

Superheated water chromatograms of the SPE extracts of two river-water samples are shown in Fig. 5. A suitable separation concerning *p*-chlorophenol was observed for each sample.

Moreover, the peak shapes of *p*-chlorophenol were quite good. However, the resolution of the peaks was not adequate; a selective detection, such as mass spectrometric detection, was needed for a simultaneous determination of phenols.

In this study, superheated water chromatography of phenols was investigated. Phenols could be separated reproductively for a long time when PSDVB packings were used as a stationary phase; their retention could be controlled by changing the column temperature. In addition, the present method was shown to be applicable to environmental analysis. These results lead to the conclusion that not only is superheated water chromatography environmentally friendly, it is also possible to substitute for conventional RP-HPLC of phenols.

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