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Notes

Superheated Water Chromatography of Low Molecular Weight Polyethylene Glycols with Ultraviolet Detection

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Superheated water chromatography (SWC) with ultraviolet detection was applied to the separation of low molecular weight polyethylene glycols (PEGs). PEG oligomers could be detected sensitively when the detection wavelength was set at 190 nm. The effect of column temperature on the separation of PEG oligomers was investigated. The elution time of all PEG oligomers decreased with increase in the column temperature; linear relationships were obtained between ln k and $1/T$. A temperature-programmed SWC separation enabled the baseline separation of a PEG 200 sample within 50 min.

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Reversed-phase high-performance liquid chromatography (RP-HPLC) is a very popular analytical technique for semivolatile and nonvolatile compounds, and is employed in a wide variety of chemical laboratories. In RP-HPLC, organic solvents are generally used as components of the mobile phase. Since most organic solvents are not environmentally friendly and are potentially toxic to the operators, minimizing the amount of organic solvents or the development of organic solvent-free techniques in RP-HPLC is of great interest.

Superheated water, water at elevated temperatures under modulated pressure to maintain its liquid state, has less polarity in comparison with ambient water.[1] The use of superheated water as the mobile phase in HPLC (the so-called "superheated water chromatography (SWC)" technique) has recently been demonstrated to be a viable substitute for conventional RP-HPLC with organic solvents.[2,3] SWC can potentially eliminate the consumption of organic solvents and decrease the hazards to the operators. In addition, it has the advantage of permitting sensitive detection. For example, flame ionization detection can be performed without large modification.[4-8] Moreover, Smith and Burgess pointed out that the lower self-absorption of water might produce successful ultraviolet (UV) detection at shorter wavelengths.[9]

Polyethylene glycols (PEGs), which consist of repeating ethoxy units between two terminal hydroxyl groups, are important classes of synthetic oligomers. The applicability of RP-HPLC to the characterization of PEGs has been evaluated, and it was found that RP-HPLC could achieve high resolutions in the separation.[10] On the other hand, the detection of PEGs in RP-HPLC is difficult because of a lack of chromophores in PEG molecules.[10] The UV detection of these compounds could be performed with some success by setting the detection wavelength to 190 nm.[11,12] However, few kinds of organic solvents can be used as the components of the mobile phase due to their UV cutoff.

In this study, we applied SWC to the characterization of low molecular weight PEGs. PEG oligomers could be detected successfully by setting the detection wavelength at 190 nm. Having investigated the effect of the column temperature on the retention of PEG oligomers, we succeeded in separating a PEG 200 sample by programming the column temperature.

Experimental

Reagents

The deionized water was prepared in our laboratory using a Millipore (San Jose, CA, USA) Milli-Q gradient system at an output of 18.2 MΩ cm^{-1}. Triethylene glycol (TriEG), tetraethylene glycol (TeEG), pentaethylene glycol (PeEG) and hexaethylene glycol (HexEG) were obtained from Aldrich (Milwaukee, WI, USA). PEG 200 was purchased from Tokyo Kasei (Tokyo, Japan). The other reagents were of reagent or HPLC grade and were used as received.

Superheated water chromatography

The SWC system employed in this study consisted of a Shimadzu (Kyoto, Japan) DGU-12AM degasser, a Shimadzu LC-10ADvp pump, a Rheodyne (Cotati, CA, USA) Model 7725i sample injector with a 5-μL sample loop, a preheat coil (stainless-steel, 5 m × 0.25 mm i.d.), an oven from a Hitachi (Tokyo, Japan) G-3000 gas chromatograph, a Shimadzu SPD-10 Avp UV-VIS detector, and a Jasco (Tokyo, Japan) 880-81 back-pressure regulator. A separation column (100 mm × 3 mm i.d.) was prepared in our laboratory by packing Hamilton (Reno, NV, USA) PRP-1 packings (particle size, 10 μm).

All sample solutions were prepared by dissolving PEGs into deionized water. The SWC separations of PEGs were carried out under the following conditions: back-pressure of mobile

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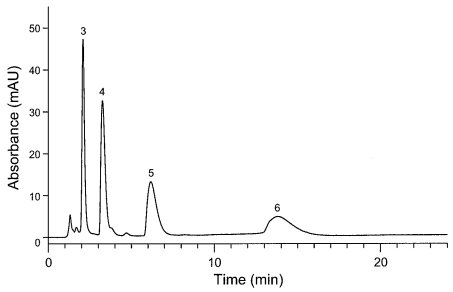

Fig. 1 Superheated water chromatogram of standard solution. Column temperature, 125˚C. Other chromatographic conditions as in Experimental. The numbers of repeating units of oligomer are on the tops of the peaks.

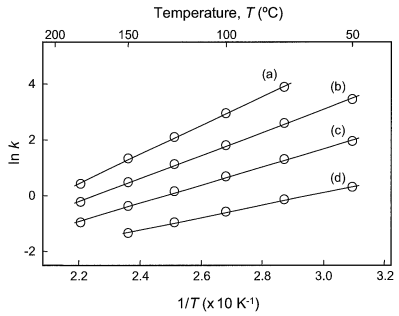

Fig. 2 Effect of the column temperature on the retention factor of PEGs. Symbols: (a) hexaethylene glycol, (b) pentaethylene glycol, (c) tetraethylene glycol, (d) triethylene glycol. Other chromatographic conditions as in Experimental.

phase, 3 MPa; flow rate, 0.6 mL min^{-1}. The UV detection wavelength was set to 190 nm.

Results and Discussion

Because the reactivity of water increases with an elevation in the temperature, the chromatographic system including the stationary phase must be stable against an attack of superheated water. Some research groups including ours already reported that poly(styrene-divinylbenzene) (PSDVB) packings were highly stable as the stationary phase in SWC.[13,14] In this study, therefore, the separation column packed with PRP-1 was used. In the range of column temperature up to 180˚C, no variation in the retention of PEGs under the same chromatographic conditions was observed during the experiment.

UV detection of PEGs

A standard solution containing TriEG, TeEG, PeEG and HexEG was subjected to SWC. Figure 1 shows an SWC chromatogram obtained at 125˚C under the UV detection at 190 nm. A stable baseline with low fluctuations was provided. Four target PEG oligomers could be detected and separated from each other. PEGs having more repeating units retained strongly on the stationary phase; the elution order was based on the degree of the polymerization, as is generally observed in conventional RP-HPLC.[10]

The effect of the detection wavelength on the UV detection of PEGs was investigated in terms of the detection limit (S/N = 3) for TeEG. The detection limits obtained at 190 nm, 195 nm and 200 nm were 0.16 μg, 0.33 μg and 1.2 μg, respectively; the detection at 190 nm provided the lowest detection limit. Since this wavelength is shorter than the UV cutoff of most of the organic solvents used as the mobile phase component in RP-HPLC,[15] SWC with UV detection at 190 nm is advantageous for the sensitive detection of PEGs.

Effect of column temperature on retention of PEGs

The effect of the column temperature on the retention of TriEG, TeEG, PeEG and HexEG was investigated. The column temperature was varied from the vicinity of that conventional RP-HPLC (50˚C) to superheated conditions (180˚C). The elution time of all oligomers decreased with an elevation in the column temperature. On the other hand, the elution order of PEG oligomers was constant. A van't Hoff plot is presented by plotting ln k *vs.* 1/T, as shown in Fig. 2. Linear relationships with correlation coefficients greater than 0.998 were obtained for all oligomers; it seems that the enthalpy and entropy of the transfer of the solute from the mobile phase to the stationary phase were independent in this temperature range.

To compare with the temperature effect in SWC, conventional RP-HPLC separations of these oligomers were performed at 30 – 75˚C using acetonitrile/water mixtures (acetonitrile concentration, 5% and 10%, v/v) as the mobile phase. The apparatus used was the same as that in the SWC system except that the back-pressure regulator was removed; it was employed with UV detection at 195 nm. An increase in the column temperature produced less retention of the oligomers. When 10% acetonitrile was used in the mobile phase, for example, the retention time of HexEG decreased from 5.4 min to 3.4 min with the increase in the column temperature from 30˚C to 75˚C. A van't Hoff plot showed linear relationships under both mobile phase conditions; the value of the enthalpy for individual oligomer decreased as the concentration of acetonitrile was decreased.

Separation of PEG 200

As an application of SWC to the characterization of commercially available PEGs, isothermal separations of PEG 200 were demonstrated in the column temperature of 100 – 180˚C. The typical chromatograms are shown in Fig. 3. The peaks were identified by comparison with those of TriEG, TeEG, PeEG and HexEG. The elution times of all oligomers, including those other than these four oligomers, decreased with increase in the column temperature. All of the oligomers detected could be eluted within 20 min when the column temperature was set to 180˚C. However, most of them had less retention on the stationary phase and a baseline separation of PEG oligomers having five or fewer repeating units could not be achieved. At the column temperature of 100˚C, on the other hand, the peak resolutions of the oligomers was improved. However, those having more than eight repeating units could not be eluted within 120 min. That is, the separations at lower temperatures were advantageous to produce a higher resolution of PEGs, especially for those having lower molecular weights. However, higher temperatures were needed to elute PEGs having higher molecular weights.

Then, SWC separations were demonstrated under temperature-programmed conditions, in which the column temperature was varied during a chromatographic run. Figure 4 shows a chromatogram obtained for the separation of PEG 200. The column temperature was programmed as follows: the column oven was initially maintained at 100˚C for 2 min, then the temperature was raised to 180˚C at 2.5˚C min^{-1}, and it was then maintained for 25 min. The baseline rose with the

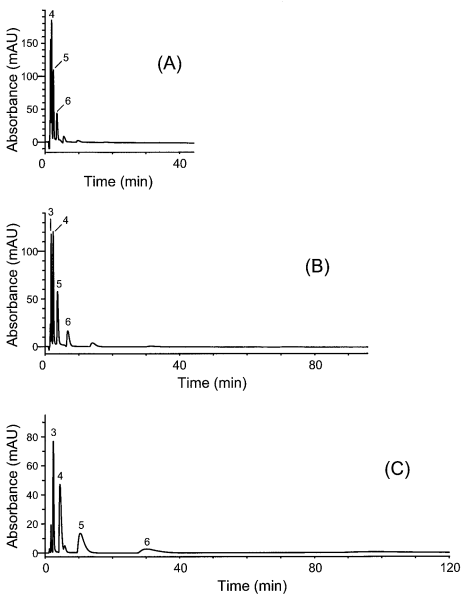

Fig. 3 Superheated water chromatograms of PEG 200 obtained at the column temperature of (A) 180˚C, (B) 150˚C and (C) 100˚C. Other chromatographic conditions as in Experimental. The numbers of repeating units of identified oligomers are on the tops of the peaks.

elevation in the column temperature, probably due to the variation of refractive index of the mobile phase.[16] It could be adjusted based on the chromatogram obtained without the sample injection. The baseline separation could be achieved for all PEG oligomers including those having five or fewer repeating units. On the other hand, the elution times of PEG oligomers having more repeating units became shorter than those at 100˚C as a result of the rise in the column temperature to 180˚C. All of the oligomers contained in PEG 200 could be eluted within 50 min. The repeatabilities (RSD, $n = 4$) obtained with respect to the retention time of these compounds were 0.08 – 0.37%. Although the apparatus used was a laboratory-made system, these results were fairly good for the peak identification. The relative peak area of TriEG, TeEG, PeEG and HexEG to all oligomers contained were 17.71 ± 0.14%, 26.61 ± 0.15%, 23.54 ± 0.12% and 14.74 ± 0.10% (mean ± SD, $n = 4$), respectively. The peak sensitivities of PEG oligomers were not equal. But the results in the relative peak are suitable for the characterization of PEGs. Further accurate quantification of the individual oligomers will be achieved if one uses a PEGs reference material, such as NMIJ CRM 5005-a.

The results we obtained demonstrate that SWC with UV detection is suitable for the characterization of low molecular weight PEGs. The present method is expected to be useful for the analyses of low molecular weight oligomers other than PEGs. The results also suggest that SWC separations under

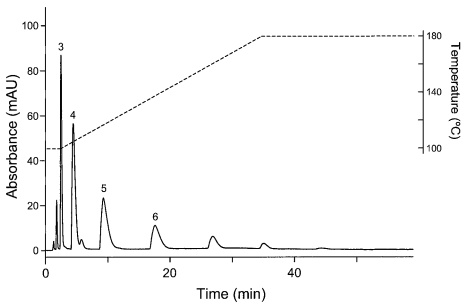

Fig. 4 Superheated water chromatogram of PEG 200 under the temperature-programmed conditions. Other chromatographic conditions as in Experimental. The numbers of repeating units of identified oligomer are on the tops of the peaks.

higher column temperature than we operated are required for the characterization of high molecular weight PEGs. Further investigations leading to the development of more thermally stable equipment will be of great interest.

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