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Polymer molecular weight characterization by temperature gradient high performance liquid chromatography

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We report a novel method of temperature gradient interaction h.p.l.c. for the characterization of molecular weight distribution of macromolecules. A very fine and reproducible control of interaction between polymer chains and the packing material can be achieved by altering the column temperature. A mixture of 10 polystyrene standard samples (molecular weight range: 1700–2 890 000) of narrow molecular weight distribution are analysed by this method. Near complete separation down to the baseline is achieved with the use of a single C18 bonded silica column. This method is thus proven to provide a much superior resolution to the conventional size exclusion chromatography. Copyright © 1996 Elsevier Science Ltd.

(Keywords: polymer characterization; temperature gradient; high performance liquid chromatography)

Introduction

Analysis of macromolecules, both synthetic and biological, by high performance liquid chromatography (h.p.l.c.) has been an area of intense research activity. Two of the most popular methods are size exclusion chromatography (s.e.c.) and interaction chromatography (i.c.). S.e.c. is a simple but universal method that provides a separation based on the partition equilibrium of solute molecules between the same solvent located in the mobile phase and in the pores of the packing material (the stationary phase). Therefore it utilizes the entropic effect in the partition equilibrium so as to separate the macromolecules in terms of their molecular size. Due to the separation mechanism of s.e.c., it is necessary that a series of columns with various pore size (or mixed bed column) needs to be used to separate the solutes by the hydrodynamic volume in the solvent.

On the other hand, i.c. has been widely used to analyse copolymers in terms of their chemical composition[1–3]. But i.c. can be used to separate homopolymers in terms of the molecular weight as well[4–9]. The separation mechanism of i.c. relies on the enthalpic interaction between solute molecules and the stationary phase, which depends on the molecular weight of the solute molecules. Since the magnitude of such interaction is a very strong function of the molecular weight, the solvent gradient elution technique needs to be adopted to optimize the chromatographic separation[5–9]. According to the basic thermodynamics, however, the partition coefficient can also be altered by varying the temperature[10]. In this communication, we would like to report that a new method, temperature gradient h.p.l.c., can be a very efficient tool for the characterization of molecular weight distribution of macromolecules.

Experimental

A typical isocratic h.p.l.c. apparatus was used for both s.e.c. and i.c. except for the columns. The s.e.c. system included four divinylbenzene gel columns, 10^5A, 10^3A, 10^2A and mixed bed (Alltech, Jordi DVB, 250 × 10 mm). For the i.c. system a single C18 bonded silica column (Alltech, Nucleosil, 100 Å pore, 250 × 4.5 mm) was used. Particle size of both column packing materials was 5 μm. Ten standard polystyrenes (PS) of the following average molecular weight and polydispersity index were obtained from Polymer Labs (UK), Daelim Industrial Co. (Korea) and Tosoh Co. (Japan): 1700 (M_w/M_n = 1.06), 11 600 (1.03), 22 000 (1.03), 37 300 (1.04), 68 000 (1.03), 114 000 (1.05), 208 000 (1.05), 502 000 (1.04), 1 090 000 (1.08), 2 890 000 (1.09). In i.c. experiments, the column was put in a jacket connected with a bath/circulator so that the column temperature was controlled in a pre-programmed manner. The mobile phase was a mixture of tetrahydrofuran/acetonitrile (THF/ACN) at a composition of 49/51 (v/v). In s.e.c. measurements, the column temperature was kept at 40°C and the mobile phase was THF. The flow rate for both experiments was 0.5 ml min^{-1}. A mixture of 10 PS standards was made at the concentration of 1 mg ml^{-1} for each polymer and the injection volume was 50 μl. The wavelength of the u.v./vis. detector was set at 260 nm.

Results and discussion

In *Figure 1* the isothermal i.c. chromatograms of the PS mixtures at different temperatures are displayed. Contrary to s.e.c., lower molecular weight polymer is eluted at lower retention volume (V_R) in i.c., which is confirmed by separate injection of each PS standard. At 5°C only four low mol. wt. polymers are eluted in the V_R range shown. The small shoulder at V_R near 3 ml is the solvent peak. As the temperature is raised, eluted peaks are shifted to lower V_R revealing that the partition of PS at stationary phase is getting smaller. At 37°C all the PS peaks merged into a single peak near the solvent peak position. This is the point where the entropic (size exclusion) and enthalpic (interaction) contribution for the separation mechanism are cancelled out[10–13]. Above this temperature, size exclusion effect starts to dominate

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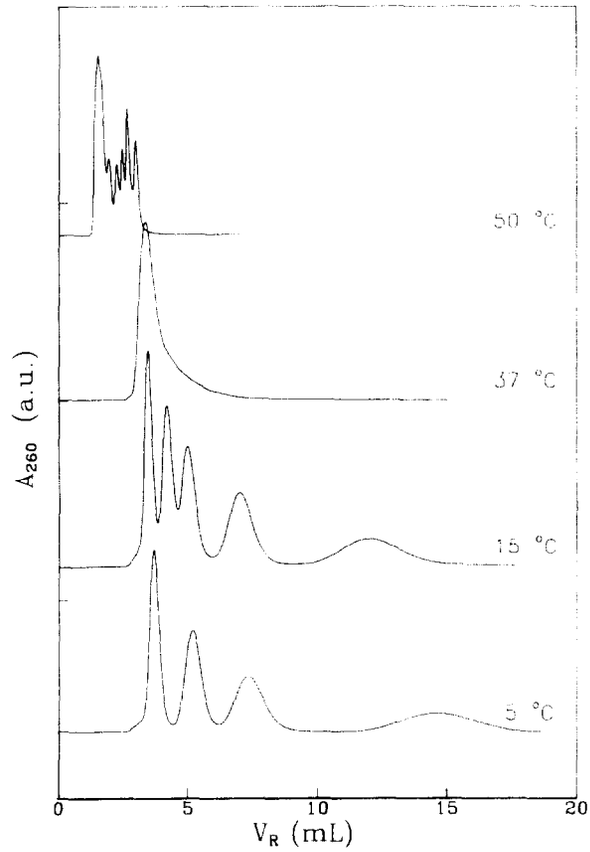

Figure 1 Isothermal i.c. chromatograms of 10 PS standards (mol. wt. range 1700–2 890 000) at four different temperatures. Contrary to s.e.c., lower molecular weight polymer is eluted at lower V_R. At 5°C only four low mol. wt. polymers are eluted in the V_R range shown. The small shoulder at V_R near 3 ml is the solvent peak. As the temperature is raised, eluted peaks are shifted to lower V_R. At 37°C, all the PS peaks merged into a single peak near the solvent peak position. Above this temperature, size exclusion effect starts to dominate so that polymers are eluted earlier than the solvent and the elution sequence in mol. wt. is reversed at 50°C

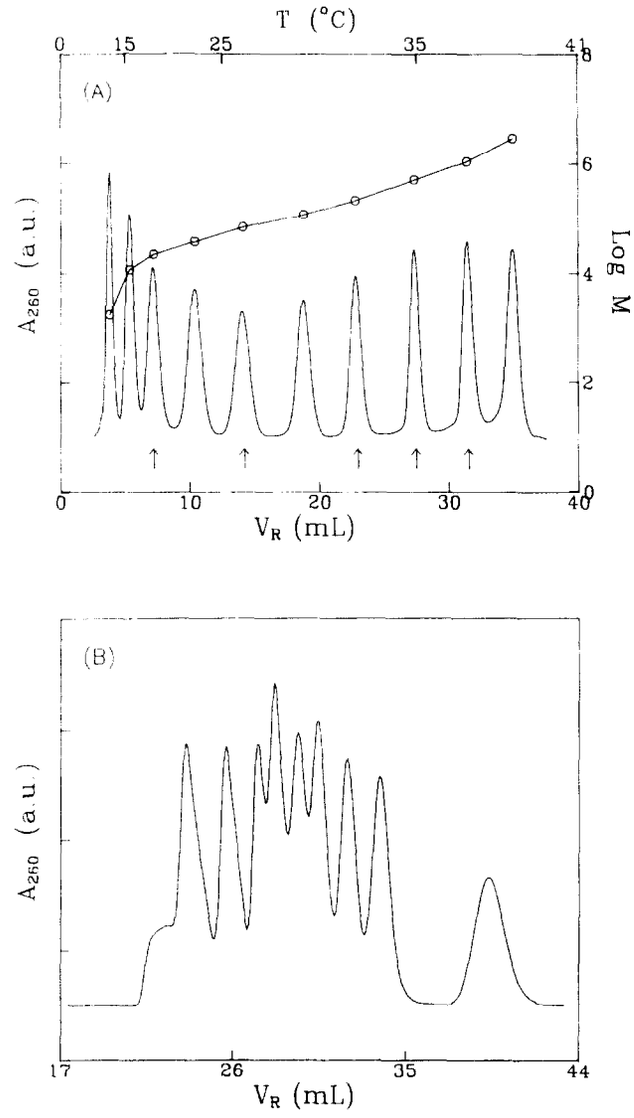

Figure 2 Comparison of temperature gradient i.c. (A) and s.e.c. (B) chromatograms for 10 PS standards. In (A), the upper abscissa represents the temperature of circulating fluid in the column jacket and the calibration curve of log M vs. V_R is also shown. In comparison with the s.e.c. chromatogram in (B), it is clear that the resolution of i.c. is far superior to s.e.c. Arrows in (A) indicate the polymers used for further analysis as summarized in *Table 1*

so that polymers are eluted earlier than the solvent and the elution sequence in mol. wt. is reversed at 50°C.

Taking advantage of this temperature effect in i.c., temperature gradient i.c. analysis of the same set of PS standards is carried out and the chromatogram is shown in *Figure 2A*. In this figure, the upper abscissa represents the temperature of circulating fluid in the column jacket. There must be some temperature difference between the mobile phase and the circulating fluid. Measurement of the real column temperature was not attempted. Soon after the sample is injected at 0°C the heating of circulating water is started. The heating rate is 1.5°C min^{-1} from 0 to 15°C, 1°C min^{-1} from 15 to 25°C, 0.333°C min^{-1} from 25 to 35°C, and 0.25°C min^{-1} from 35 to 44°C. The resolution is so remarkable that a near complete separation of 10 PS standards down to the baseline is achieved. The detailed separation mechanism remains to be verified, but it is certainly not a fractional dissolution type since all the PS samples are completely soluble at this solvent composition except for the highest molecular weight (M_w : 2 890 000) at low temperature. The cloud point of the high molecular weight polymer is determined as 7 ± 1°C. Therefore the solubility may briefly play a role at the beginning of elution for the high molecular weight case, but the major separation mechanism has to be the interaction with the stationary phase. As a matter of fact, we lower the temperature in order to enhance the resolution of the low molecular weight samples. For the polymers of molecular weight higher than *ca.* 10 000, a comparable resolution can be obtained by programming the temperature gradient above 10°C. In addition, the chromatogram changes dramatically when a column with different packing materials is used.

The calibration curve of log M vs. V_R, similar to that commonly used in s.e.c. is also shown in *Figure 2A*. For the sake of comparison, an s.e.c. chromatogram of the same set of PS standards using four s.e.c. columns is displayed in *Figure 2B*. It is clear that the resolution of i.c. is far superior to s.e.c. For a more critical comparison, molecular weight distribution of five PS standards (indicated by arrows in *Figure 2A*) are measured independently by use of the calibration curve and the results are summarized in *Table 1*. While the M_w obtained by both methods are nearly the same, M_w/M_n by i.c. is much smaller than that by s.e.c. For an anionically polymerized polymer it is

Table 1 Characterization of standard PS by s.e.c. and i.c.

$M_w(\times 10^3)/M_w/M_n$	
Manufacturer's[a]	I.c.
22.0/1.03	20.8/1.01
68.0/1.03	62.6/1.008
208/1.05	192/1.005
502/1.04	478/1.004
1090/1.08	1083/1.007

[a] Provided by Polymer Lab. Inc., and Tosoh Co.

expected theoretically that the polydispersity index gets smaller as the degree of polymerization increases[14]. Usually this prediction has not been experimentally supported for high polymers by s.e.c. analysis, which is also apparent in *Table 1*. From the i.c. analysis in this study, however, the M_w/M_n value of high polymers is at least comparable to the smaller molecular weight values. In this regard we believe that s.e.c. has, so far, overestimated the M_w/M_n value of high polymers. This was previously pointed out from the result of a field flow fractionation experiment[15].

We close our report by pointing out the merits and shortcomings of the new method. In comparison with s.e.c., i.c. provides a much better resolution at a low column cost. Considering the inevitable temperature gradient across the column cross section in this experimental geometry, which should aggravate the resolution, we may expect an even better resolution. We are currently working with microbore columns to see if we can improve the resolution further. Also, the sample capacity of i.c. appears higher than s.e.c. considering the size of the columns used in this study. On the other hand, i.c. is not so universal as s.e.c. that an optimized condition has to be found for the specific polymer to be characterized. It includes the choice of packing material, eluent, temperature gradient and so on. If one needs a critical evaluation of the molecular weight distribution or repeatedly deals with one kind of polymer, however, this new method is able to provide a strong edge over conventional s.e.c. Also, it seems quite promising for a preparative scale application. We are currently working on more details of this method, namely the nature of the interaction, pore size effect, loading capacity, and so on.

As mentioned earlier, the molecular weight analysis of polymers can be done by the solvent gradient i.c.[4]. We found that the fine tuning of the interaction strength between the polymer chains and the packing materials is easier and more reproducible in the temperature gradient method than in the solvent gradient method. In addition, the isocratic elution in the temperature gradient method allows us high versatility in choosing detectors such as the refractive index detector or the light scattering detector which is not useful for the solvent gradient h.p.l.c. method.

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