The Measurement of Molecular Weight Distribution of some Polydisperse Polystyrenes by Reversed Phase and Size Exclusion Chromatography

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Key Words
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Summary
The methods of size exclusion chromatography and reversed phase gradient elution high performance liquid chromatography were compared for the analysis of the molecular weight distributions of six general purpose polydisperse polystyrenes. The results indicated that for samples within the exclusion limit of the size exclusion columns the agreement between the number, weight and z-average molecular weights for the size exclusion and reversed phase methods was good. The reversed phase method appears less sensitive to concentration effects than the size exclusion method and offers possibilities of better resolution.

Introduction
Polymer molecular weight characterisation by size exclusion chromatography is useful because it yields information on the number, weight and z-average molecular weights simultaneously. Other methods such as ebulliometry, vapour pressure lowering, osmotic pressure, end group assay, solution viscosity, and ultracentrifugation do not give distribution information [1] unless additional fractionation steps are undertaken. As such, the size exclusion method has become the method of choice for routine molecular weight studies.

Armstrong and Bui [2, 3] developed molecular weight separations of polystyrenes in a dichloromethane-acetonitrile solvent system that also compared favourably to size exclusion separations [5-9]. However, these comparisons have only been qualitative and no distribution parameters using reversed phase methods have been published. To compare the methods of size exclusion and reversed phase chromatography we have undertaken a preliminary investigation into the molecular weight characterisation of a series of general purpose polystyrene samples. The number, weight and z-average molecular weights are calculated, and the advantages and disadvantages of each technique are summarized.

Experimental
Acetonitrile and dichloromethane (HPLC grade) were obtained from Mallinkrodt Australia Pty Ltd. The monodisperse polystyrene standards used were molecular weights 110k and 200k (Waters Associates, Milford, MA, USA), and 17.5k, 50k, 410k, and 929k (Polysciences Inc., PA., USA).

The manufacturer's descriptions of the general purpose polystyrene samples that were analysed were: Sample 1, 1683 standard polymer Mw 250 000, Mn 100 000, Mw/Mn = 2.5, (Dow chemical (Australia) Limited, Altona, Victoria); Sample 2, STYRON 685M, melt flow rate 1.6 g/10 min, ultimate tensile strength 44.1 MPa, Vicat Softening point 108 °C, (Dow Chemical (Australia) Limited); Sample 3, STYRON 678A, melt flow rate 12 g/10 min, ultimate tensile strength 32.4 MPa, Vicat Softening point 90 °C, (Dow Chemical (Australia) Limited); and Sample 4, molecular weight approximately 100 000, (BDH Chemicals Limited, Poole, England). Samples 1809 and 1417 were prepared by radical initiated solution polymerization techniques (C.S.I.R.O., Division of Chemicals and Polymers, Clayton, Victoria, Australia). Sample 1417 was prepared using a mercaptan chain transfer agent. The molecular weight distributions of the last three samples were unknown.
Reversed phase chromatographic experiments were performed using two M6000A pumps, a 660 solvent programmer, a U6K injector and a 740 data module, (Waters Associates, Milford, MA, USA). The detector used was a variable wavelength UVIS 200 set at 262 nm, (Linear Instruments Corp. Nevada, USA). The reversed phase column used was a Hibar C18 cartridge column, 12.5 cm length, 4.0 mm I.D., 5 µm particle size, nominal pore size 60 Å, (E. A. Merck, Darmstadt, Germany). Column temperature was maintained at 25.0 °C in a thermostated waterjacket. Calibration of the reversed phase system was achieved by injecting a standard polymer solution (5 µg in a 5 µl injection volume) containing a mixture of monodisperse polystyrene standards of molecular weights 17.5k, 50k, 110k, 200k, 410k and 929k. An initial mobile phase of φl = (46 : 54) dichloromethane-acetonitrile followed by gradient elution using a convex gradient profile previously described [4], φf = (66 : 34) dichloromethane-acetonitrile was used. A five hour runtime was selected on the gradient programmer but the analysis was stopped after the elution of the last peak. The flow rate was 1.0 ml min⁻¹. To avoid sample migration of the lower molecular weight polystyrenes prior to the influence of the gradient [10], all samples and standards were injected 4 minutes after gradient initiation. This standard injection was repeated 3 times during the analysis of the polydisperse samples. The general purpose polydisperse samples were analysed as for the monodisperse standards except that the sample load was 100 µg per 5 µl injection volume.

The size exclusion experiments used SE 100, SE 500 and SE 1000 columns connected in series (Dupont Co., Wilmington, DE, USA) were carried out using the same instrumentation as for the reversed phase method. Each of the SE columns were 25 cm in length, 6.2 mm I.D. and 8 µm particle size. Calibration of the size exclusion system was carried out in two ways. Initially, the various molecular weight monodisperse polystyrenes were injected at several different sample loads in the range of 0.5 µg-25 µg (using a 5 µl injection volume) and peak positions were extrapolated to zero sample load. A second calibration was based on triplicate injection of the monodisperse polystyrenes at a sample load of 1 µg and then at a sample load of 5 µg. The general purpose polystyrene samples were analysed by injecting a 5 µg sample in 5 µl. The mobile phase was 100 % dichloromethane at a flow rate of 1.0 ml min⁻¹. All polymer samples analysed by the reversed phase method and the size exclusion method using the SE columns were prepared in 100 % dichloromethane.

Size exclusion experiments were also performed using a set of six Ultrastyragel size exclusion columns of pore sizes 100 Å, 500 Å, 1 · 10⁵ Å, 1 · 10⁴ Å, 1 · 10³ Å and 1 · 10⁲ Å (Waters Associates, Milford, MA, USA). The instrumentation used for these analyses consisted of a Waters 150-C GPC system with a Waters 840 software package. The mobile phase was tetrahydrofuran at a flow rate of 1.0 ml min⁻¹ at a temperature of 30 °C. The sample load used for the calibration and the analysis of polydisperse samples was 100 µg and the injection volume was 50 µl as recommended by Waters Associates.

The number, weight and z-average molecular weight distributions were calculated by measuring the area under the peak profile at 0.2 ml intervals for the reversed phase method and 0.1 ml intervals for the size exclusion method for which the three columns were used. The Waters 840 software package was used to estimate these averages using the six column Ultrastyragel size exclusion set and the 150-C GPC system.

**Results and Discussion**

Calibration curves for both the size exclusion and reversed phase methods were prepared using a series of monodisperse polystyrene standards. For the reversed phase system a linear relationship between log (molecular weight) and the elution volume was used over the entire molecular weight range and is shown in Figure 1a. The relationship between log (molecular weight) and the elution volume for the three column size exclusion system followed a second order polynomial relationship, as shown in Figure 1b (note the size exclusion calibration curve illustrated was obtained from the elution volumes extrapolated to zero sample load). The calibration curve...