A New Chip-Based Viscometer for Size Exclusion Chromatography

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Introduction

Size exclusion chromatography (SEC), is a well-established technique for determining the molecular weight distribution of polymers on the basis of size in solution. Typically, during the analysis, molecular sizes are converted into molecular weights by use of a calibration curve, established for a series of well defined polymers of known molecular weight. However, in many situations, this method is unsatisfactory as the results obtained are very dependent on the chemistry of the samples under investigation, and the number and type of polymer standards is quite limited. To counter this problem, new methodologies have been developed utilizing detectors that respond directly to molecular properties and remove the dependence on sample chemistry. One such detector is the flow viscometer, which responds to the intrinsic viscosity of polymers and gives molecular weight via the so-called universal calibration technique.

The drawback of traditional designs of viscometers is the large effective cell volume and resulting excessive system dispersion. This high dead volume leads to peak broadening that can strongly influence the accuracy of calculated results. Also, most commercial viscometers are not suited to low flow rates and applications where sample size is limited. To counter these issues, a new, miniaturized, chip-based viscometer has been designed for polymer analysis. The chip-based system has a much-reduced, effective cell volume compared to traditional detectors and is capable of operating at extremely low flow rates. Use of the new detector leads to a significant reduction in system dispersion, giving more accurate peak shapes and a significant improvement in the accuracy of results. This poster introduces the chip-based viscometer and illustrates the application of the device in polymer analysis.

Size Exclusion Chromatography (SEC)

In SEC, a column packed with porous beads of a pre-defined pore size is used to separate polymer molecules on the basis of their molecular size in solution. The sample under investigation is dissolved and introduced into the column in a flowing stream of solvent. Smaller molecules (relative to the packing material pore size) are able to penetrate a large number of pores while larger molecules are unable to access as many. As a result, the elution of small molecules is retarded compared to the larger molecules and a separation based on size is generated.

In conventional SEC employing a single concentration detector, the elution profile of a sample is converted into a molecular weight distribution by comparison to a calibration of molecular weight as a function of elution time. This calibration is generated from measuring the elution behaviour of a series of narrow standards of known molecular weight. Assuming that the standards used for calibration and the unknown sample are of the same chemistry, accurate molecular weights are obtained. However, if the standards and sample are not of the sample chemistry, discrepancies in the calculated molecular weights result due to differences in the molecular size of the two polymers. In this case, accurate molecular weights cannot be generated from this approach and relative values are obtained. Figure 1 shows a schematic of the SEC technique.

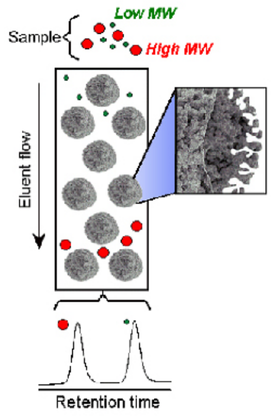

Figure 1: Schematic Representation of the SEC Technique

SEC with Viscometry and the Universal Calibration

Employing a viscometer in the system allows accurate molecular weights to be calculated regardless of the chemistry of the sample and the standards. This is through use of the following relationship:

Molecular Size (hydrodynamic volume) = Intrinsic viscosity [η] x Molecular weight M

During the SEC experiment, sample concentrations are determined through the use of a concentration detector (typically differential refractive index) and specific viscosities (ηsp) are measure by the viscometer. At high dilution, intrinsic viscosities can be calculated using the following approximation:

Intrinsic viscosity [η] = Specific viscosity ηsp / Concentration C

A series of narrow standards are run and a calibration is defined as a relationship between elution volume and the product of intrinsic viscosity [η] and molecular weight M, the equivalent of molecular size. As the column is separating on the basis of molecular size, a single calibration results regardless of the chemistry of the standards employed, a 'universal calibration'. Using this calibration then generates accurate molecular weights for unknown samples regardless of chemistry, as long as the polymer elutes under pure SEC conditions. Figure 2 shows an example of the universal calibration generated for a wide range of polymer chemistries in tetrahydrofuran.

Figure 2: The Universal Calibration, Reference: Grubisic, Rempp, Benoit, J. Polym. Sci., Part B, Polym. Lett., 5:753 (1967)

Viscometer Design

The most popular design of viscometer is the four-capillary bridge system. In this design, four balanced capillaries are set on two separate flow paths or 'arms' such that flow from the columns is split in a one to one ratio down each arm. Flow of large polymer molecules down the restricted capillary channels generates a pressure that can be measured across the system. After the first capillary on the first arm, the polymer molecules flow through a second capillary and then out to waste. However, on the second arm, a small column with a large internal volume is placed before the second capillary. On entering this column the polymer molecules are delayed, resulting in pure solvent flowing though the capillary on the second arm while polymer molecules elute through the capillary in the first arm. This resulting pressure imbalance is measured and easily converted into a viscosity measurement after suitable calibration. Figure 3 shows a schematic representation of the four-capillary bridge viscometer.

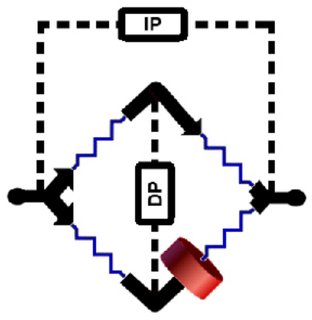

Figure 3: Schematic Representation of the Four-Capillary Bridge Viscometer

A major drawback of this design of viscometer is that the system is large with an appreciable effective cell volume. Combined with the cell volume of the differential refractive index detector and any light scattering detector used in the system, this can result in excess dispersion giving significant broadening of sample peaks. This effect has made viscometers unsuitable for the analysis of very narrow polydispersity samples and limits utility of the technique. Also, considerable sample volume is required with conventional viscometers due to the large effective cell volume and the high flow rates required. This limits the use of viscometry in applications where sample size is limited, such as is found with biological samples.

A Miniaturised Viscometer

The major drawbacks of viscometry in SEC are related to the large size of the device and the dispersion caused from the large effective cell volume. Therefore, to increase the utility of viscometry, a miniaturised version of the detector has been developed. The device is based on the established four-capillary bridge design, replacing the tubing and capillaries with channels etched into the surface of a silicon chip. Figure 4 shows the chip and a schematic of the chip's flow path.

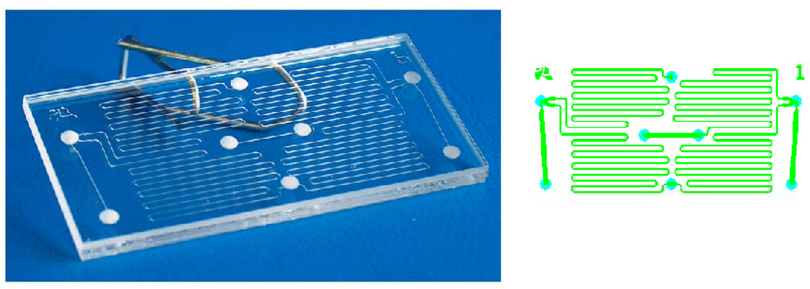

Figure 4: Image and Flow Path Schematic of the Chip-based Viscometer

The chip was housed and the pressure transducers and hold-up column attached as shown in Figure 5.

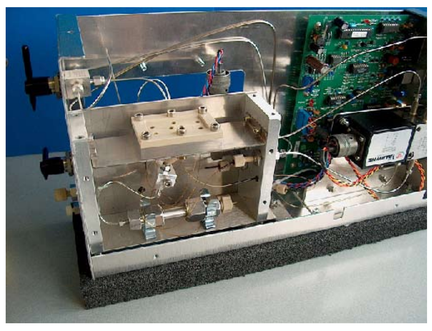

Figure 5: Housing of the Chip-based Viscometer and Hold-up Volume

A comparison of the analytical viscometer design (employing the tubular capillaries) and the chip-based design is shown in Table 1. The chip viscometer operates at significantly reduced solvent flow than the analytical system, and clearly, the effective capillary volume of the chip system is significantly reduced, minimising dispersion in the system. Other operational parameters such as the shear rate and noise are similar.

	Analytical	Chip-based
Configuration	4-capillary bridge	4-capillary bridge
Capillary / channel	250µmx600mm	260µmx60µmx140mm
Effective cell volume	35µl	2µl
Operation flow rate	1.0ml/min	100µl/min
Shear rate	3000s-1	3000s-1
Typical noise	<0.5mV	<0.5mV

Table 1. Comparison of the Analytical and Chip-Based Viscometer Systems

The chip-based viscometer system was evaluated by the analysis of a series of polymers in tetrahydrofuran and the results were compared to a typical analytical system.

Performance in Tetrahydrofuran (THF)

The performance of the chip-based viscometer in tetrahydrofuran was assessed in comparison to an analytical viscometer system. The systems were set up as follows:

System: Analytical
Solvent: Tetrahydrofuran
Flow Rate: 1.0ml/min
Detectors: Conventional DRI + analytical viscometer
Alignment: Detectors plumbed in parallel, 0.5ml/min through each
Inj Vol: 100µl
Column: PLgel 5µm MIXED-C, 300x7.5mm

System: Chip-based
Solvent: Tetrahydrofuran
Flow Rate: 1.0ml/min
Detectors: Conventional DRI + chip-based viscometer
Alignment: Flow split, 0.9ml/min through DRI, 0.1ml/min through viscometer
Inj Vol: 100µl
Column: PLgel 5µm MIXED-C, 300x7.5mm

Polystyrene

A series of polystyrene standards were analysed using both systems. Figure 6 shows the Mark-Houwink plot obtained for the standards using the chip-based system.

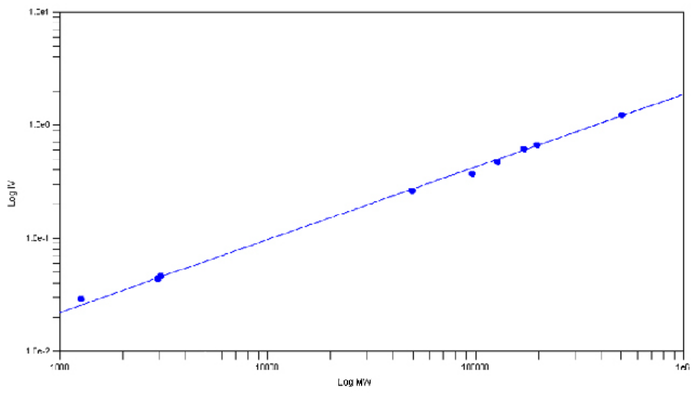

Figure 6: Mark-Houwink Plot for Polystyrene Standards in THF

A straight line plot was obtained as expected and gave Mark-Houwink constants of K = 16.1 x 10-5 and alpha = 0.717, very close to the literature values.

The two systems were then used to analyse a sample of broad polystyrene. Figure 7 shows an overlay of the chromatograms obtained. The chip-based viscometer in combination with the DRI detector gave a similar peak width to the analytical viscometer alone, and was much improved over the peak obtained on the analytical viscometer with DRI, which showed some band broadening effects.

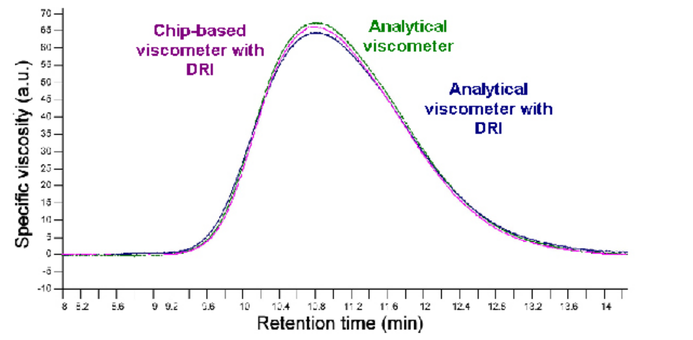

Figure 7: Overlaid Chromatograms for the Broad Polystyrene Sample in THF Obtained on an Analytical Viscometer with and without a DRI Detector, and on the Chip-Based Viscometer with DRI Detector

Polymethylmethacrylate

The systems were then used to analyse a series of polymethylmethacrylate standards in tetrahydrofuran. Figure 8 shows an overlay of the chromatograms obtained for one of the narrow standards on the two systems.

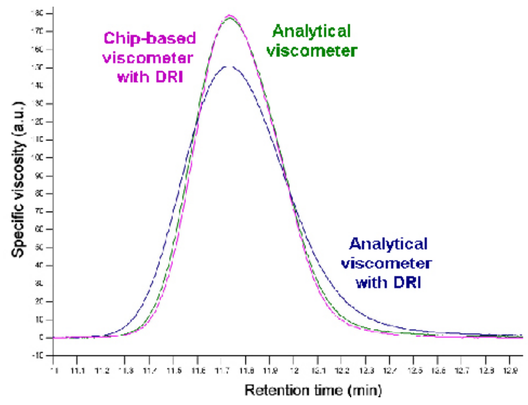

Figure 8: Overlaid Chromatograms for a Polymethylmethacrylate Narrow Standard in THF

The chip-based viscometer in combination with the DRI detector gave a very narrow peak width, comparable to the analytical viscometer alone, and was far superior to the peak obtained on the analytical viscometer with DRI. The extra dispersion of the analytical system resulted in increased band broadening and an artificially broad peak, whereas the chip-based viscometer gave a far better reflection of the true peak width. Figure 9 shows an overlay of the Universal Calibration obtained with the polymethyl methacrylate and the polystyrene narrow standards, illustrating the quality of the data obtained with the chip-based viscometer.

Summary

Size exclusion chromatography employing viscometers is fast becoming a common tool for the analysis of polymers. Using the viscometer and a concentration detector such as a refractive index detector, accurate molecular weights can be calculated via the universal calibration approach that are not reliant on using standards and samples of the same chemistry. The drawback of analytical viscometers is that they have large, effective cell volumes and operate at high flow rates, making them less suitable for narrow polydispersity polymers or systems where only small sample volumes are available.

To remedy this drawback a new chip-based viscometer has been developed. Based on an etched silicon chip, this device has greatly reduced effective cell volumes and can operate at far lower flow rates than an analytical viscometer. As a result far lower band broadening is observed and the chip-based systems gives a far truer reflection of the molecular weight distribution of samples than an analytical system.

Presented at SCM-3 2007
CS1009/C/1.07

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