

Evaluation of a Single-Capillary Viscometer Detector On Line to a SEC System Used with a New Pulse-Free Pump

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ABSTRACT: A single-capillary viscometer (SCV) used as an on-line viscosity-sensitive detector for size exclusion chromatography (SEC) presents considerable interest. Unfortunately, the commercially available version, which uses a traditional HPLC pump and a pulse dampener, presents serious problems and can give poor performance. Flow rate fluctuations are the main problems in the use of the on-line SCV detector. The pulse dampener resolves the flow fluctuations problems that are intrinsic to a reciprocating pump. Unfortunately, the pulse dampener introduces secondary, yet variable, flow fluctuations that make the SCV unreliable. In the past, several solutions to the problem have been described. Our proposal is to use the SCV on-line detector with a new commercial pulse-free pump without a pulse dampener. A detailed evaluation of this new SEC–SCV system has been performed using various polymers. Molar mass distribution, intrinsic viscosity distribution, and constants of the Mark–Houwink–Sakurada relationship have been determined. The used polymers were soluble both in organic solvent (PS, PMMA, PVAc, PVC, polyalkylthiophene) and in aqueous solvent (PEO, PEG, pullulan, and hyaluronan). The results obtained are encouraging. © 1998 John Wiley & Sons, Inc. *J Appl Polym Sci* 68: 1651–1659, 1998

Key words: single-capillary viscometer; size exclusion chromatography; flow fluctuations; intrinsic viscosity; Mark–Houwink–Sakurada constants

INTRODUCTION

Since its introduction in 1972,¹ the on-line viscosity-sensitive detector for size exclusion chromatography (SEC) has become an interesting technique for the determination of molar mass distribution (MMD), intrinsic viscosity distribution (IVD), and constants of the Mark–Houwink–Sakurada relationship. In the 1980s, two commercial on-line SEC viscometers were introduced. The first one, in 1984,² was the differential viscometer (DV) from Viscotek. The second one, in 1989,³ was the single-capillary viscometer (SCV) from Waters. The Viscotek DV detector uses four capillary tubes in a hydraulic Wheastone bridge con-

figuration and two differential pressure transducers. In the Waters SCV detector a single differential transducer measures the pressure drop across a stainless steel capillary tube. For its relative simplicity SCV has been stressed immediately as an interesting viscometer. After some positive SCV evaluations,^{3–5} some “problematic” evaluations⁶ and lately also a negative evaluation have been reported.⁷ To overcome the flow fluctuations problem Lesec has initially proposed to decrease the flow resistance of the polymer solution in the concentration detector cell⁸ using semicapillary tubes. Recently,⁹ Lesec has proposed to use of a second pressure transducer, located before the injector, for the correction of the flow fluctuations.

Our idea is to use the SCV on-line detector with a new commercially available pulse-free HPLC pump, Alliance 2690 separations module from Waters, without pulse dampener. For 6 years in

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our laboratory a Waters SCV detector integrated in a GPC 150CV system has been in operation. In this study we have replaced the pump, dampener, and autoinjector of the 150CV system with a pump and autoinjector of the pulse-free 2690 pump. A detailed evaluation of this new SEC–SCV system has been performed using various polymers. Molar mass distribution (MMD), intrinsic viscosity distribution (IVD), and, in particular, the constants of the Mark–Houwink–Sakurada relationship have been determined. In the evaluation we have used polymers soluble both in organic solvent, THF and chloroform, and in aqueous solvent. Polymers soluble in organic solvent were polystyrene (PS), poly(methyl methacrylate) (PMMA), poly(vinyl acetate) (PVAc), poly(vinyl chloride) (PVC), and polyalchylthiofene (PAT). Polymers soluble in aqueous solvent were poly(ethylene oxide) (PEO), poly(ethylene glycol) (PEG), and linear polysaccharides as pullulan and hyaluronan (HA).

EXPERIMENTAL

Materials

Several narrow MMD standards and several broad MMD samples from different sources were used. Narrow MMD standards were purchased, respectively: PS by Polymer Standards Service (Germany); PEO and pullulan by Showa Denko (Japan); PEG by Polymer Laboratories (UK). Broad MMD samples were purchased respectively: PS NBS 706 by National Bureau Service (USA), PS Edistir 1380 by Montedison (Italy); PMMA and PVC by BDH (USA); PEO and PAT by Aldrich (USA). The PVAc sample was obtained from Mapei (Italy). HA samples were generously obtained from Dr. Soltes (S.A.S. Slovak Republic) and from Pharmacia and Upjohn (Italy). Tetrahydrofuran (THF) from Labskan and chloroform from Aldrich solvents were of HPLC grade. Water solvent was of MilliQ (Millipore) grade. BHT from Aldrich and NaCl from Sigma were of analytical grade.

Chromatographic System

An original multidetector SEC system (Fig. 1) was used. The system was composed of an Alliance 2690 separations module, pump, and autoinjector, from Waters (USA); and the SCV and the refractive index (DRI) detectors integrated in the SEC 150CV chromatographic system. In the aque-

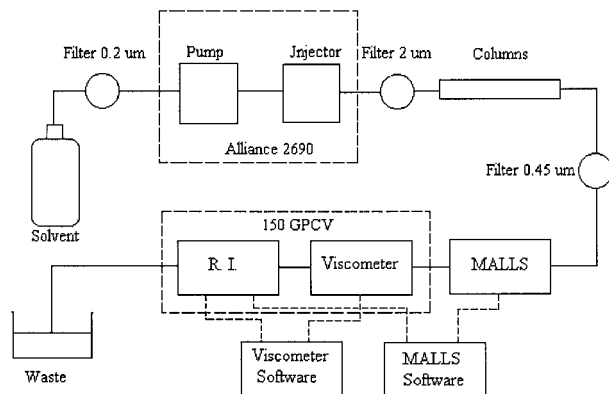


Figure 1 Scheme of the new SEC–MALLS–SCV multidetector system.

ous solvent an additional multiangle laser light scattering (MALLS) detector from Wyatt (USA) was used. The description of the SEC–MALLS–SCV multidetector system and related problems have been reported previously.^{10,11} In THF the experimental conditions were 0.8 mL/min of flow rate, 35°C of temperature, and four Ultrastaygel Waters columns (10^6 – 10^5 – 10^4 – 10^3). In chloroform the experimental conditions were 0.6 mL/min, 35°C, and two PLGel Mixed C columns from Polymer Laboratories. In the aqueous mobile phase the experimental conditions were 0.15M NaCl as the mobile phase, 0.8 mL/min, 35°C, a precolumn, and two columns (KB806–KB805) from Shodex (Japan).

Viscometry

SCV data acquisition and analysis software was Millennium 2.15 from Waters. Details of the SCV analysis software have been described elsewhere.^{3–5} The signal of the viscometer detector depends on the specific viscosity of the solution. Hence, to obtain a constant signal-to-noise ratio the concentration of the samples has been calculated so that $[\eta] \times c = 0.1$. On-line SCV detection is based on the concept of the universal calibration.¹² The universal calibration curve, polynomial third-order fit, in the organic mobile phase was generated by 16 narrow PS standards with the molar mass ranging from 162 to 5.48×10^6 g/mol. On-line SCV detection in the aqueous mobile phase presents two additional serious problems. First, the validity of the universal calibration in the aqueous mobile phase is quite questionable. In the aqueous mobile phase a fractionation of the macromolecules not exclu-

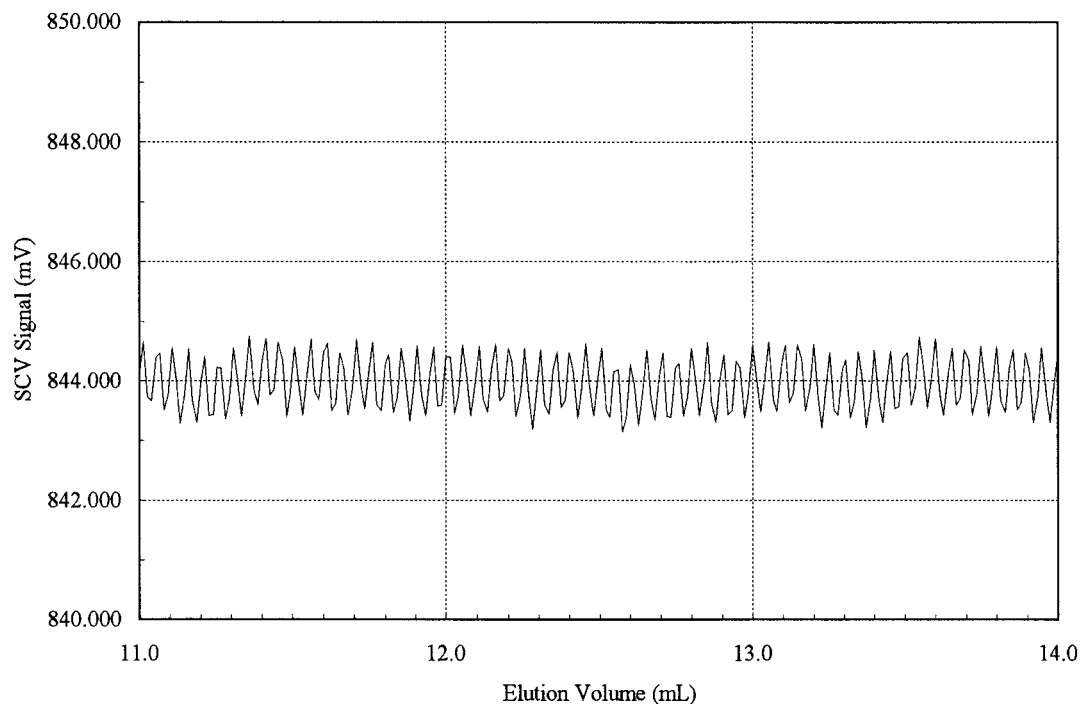


Figure 2 Signal (baseline) of the new SCV detector. Experimental conditions: THF as mobile phase, 0.8 mL/min of flow rate, 35°C.

sively steric is fairly usual. The second problem concerns the insufficient molar mass range, approximately up to 1×10^6 g/mol, of the narrow standards Pullulan or PEO/PEG. Therefore, in the aqueous mobile phase we have followed a different strategy. We have used a three-detector system, SEC–MALLS–SCV. In this system the molar mass, by MALLS, and the intrinsic viscosity, by SCV, has been measured directly. Subsequent to the acquisition by means of the Millennium and MALLS software the raw data have been exported and elaborated with personal software.

The intrinsic viscosity $[\eta]$ of some samples was measured also in the static off-line mode by an Ubbelohde viscometer. Off-line $[\eta]$ values have been used as reference for SCV on-line values. Off-line viscosity data analysis has been performed by the usual Huggins relationship.

Light Scattering

MALLS on-line detector Dawn DSP-F from Wyatt, He-Ne laser 632.8 nm of wavelength, measures the intensity of the scattered light at 18 fixed-angular locations ranging, in THF, from 12.3 to 159.7°. Data acquisition and analysis software was Astra 4.2 from Wyatt. MALLS hard-

ware and analysis software have been described in detail elsewhere.¹³ The calibration constant was calculated using toluene as the standard, assuming a Rayleigh factor $R_\theta = 1.406 \times 10^{-5} \text{ cm}^{-1}$. The photodiodes angular normalization was performed measuring the scattering intensity of a high concentrated solution of an isotropic scatterer. The characteristics of the scatterer used in the normalization were (1) soluble in the mobile phase; (2) low value of the gyration radius ($\langle s^2 \rangle^{1/2} \leq 3 - 4 \text{ nm}$); (3) uniform or almost very narrow MMD. Selected molecules that match most of the characteristics above listed were a PS narrow standard, 10 K of molar mass, dispersity $D = 1.03$, in organic solvents, and a BSA globular protein in the aqueous solvent. The experimental methodology to get reliable results from an on-line MALLS detector have been described previously.¹¹

RESULTS AND DISCUSSION

Theory, experimental variables, and data analysis algorithms of the SCV detector have been described in detail in the past^{4-6,14} and will not be reported herein. On-line viscometers are highly flow-rate sensitive. For this reason the key element of the SCV detector, integrated in the Wa-

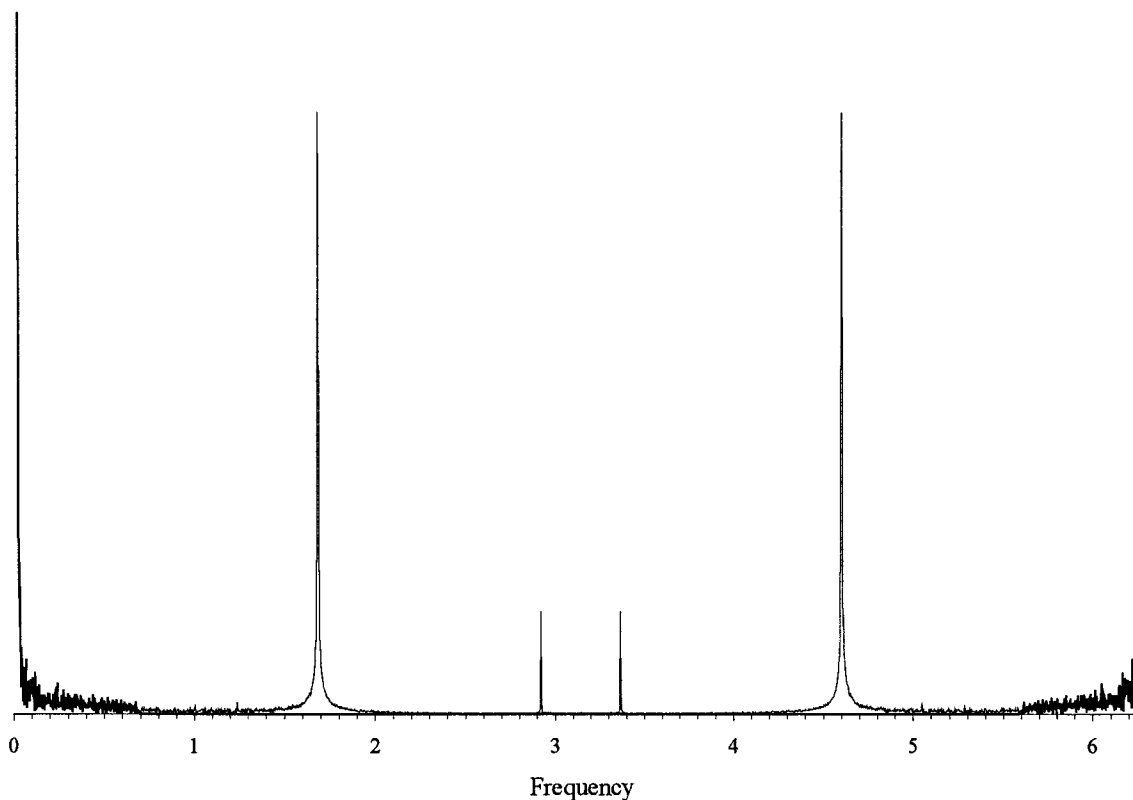


Figure 3 Frequency signal spectrum of the new SCV detector for a broad Edistir 1380 PS sample.

ters GPC 150CV system, is the so-called baseline optimization box (BOB). The BOB consists of a series of eight dampeners and restrictors in alternate order that reduce the flow fluctuations, intrinsic to a reciprocating dual piston pump, by a factor of more than 100.³ In reality, the baseline of the on-line SCV detector with the BOB dampener is very stable. Problems arise during the elution of the polymers. BOB behaves as a hydraulic "capacitance"⁶ and when the solution meets resistance to the flow introduces secondary, yet variable, flow fluctuations. Every time the polymeric solution meets an obstacle (frits of the columns, detector cell, etc.) flow rate goes to a down and up cycle.⁶ As a consequence, the slope of the Mark-Houwink-Sakurada equation estimated by the "conventional" SCV detector using a broad MMD sample is underestimated. The phenomenon, "Lesec effect," is well known and has been extensively studied in the past.⁴⁻⁶ To overcome of the flow fluctuations problem we suggest use of the SCV on-line detector with the new pulse-free pump without a dampener.

Flow Rate Fluctuations of a 2690 Pump

At constant mobile phase viscosity the SCV detector is a very sensitive flowmeter. Figure 2 shows a portion of the baseline of the new SCV detector, without dampener: the experimental conditions were THF as the mobile phase, 0.8 mL/min of flow rate, 35°C of temperature. We can see a residual oscillation, noise, with a period of about 4 s. The amplitudes of these oscillations depend on the flow rate and a little on the solvent: approximately 1.2 mV in THF, 1.1 mV in chloroform, 1.6 mV in DMF, and 1.8 mV in water.

It is worth noting that the oscillations, period, and amplitude are very constant. For this reason it is relatively simple to eliminate it. For example, we can use a fast Fourier transform (FFT) filter as proposed from Provder et al.¹⁴ The frequency signal spectrum, for a broad PS sample NBS706, is shown in Figure 3. Instead, Figure 4 shows the signals before and after the FFT filter. For convenience, the signal before the FFT filter has stayed shifted of +3 mV. In the FFT filtra-

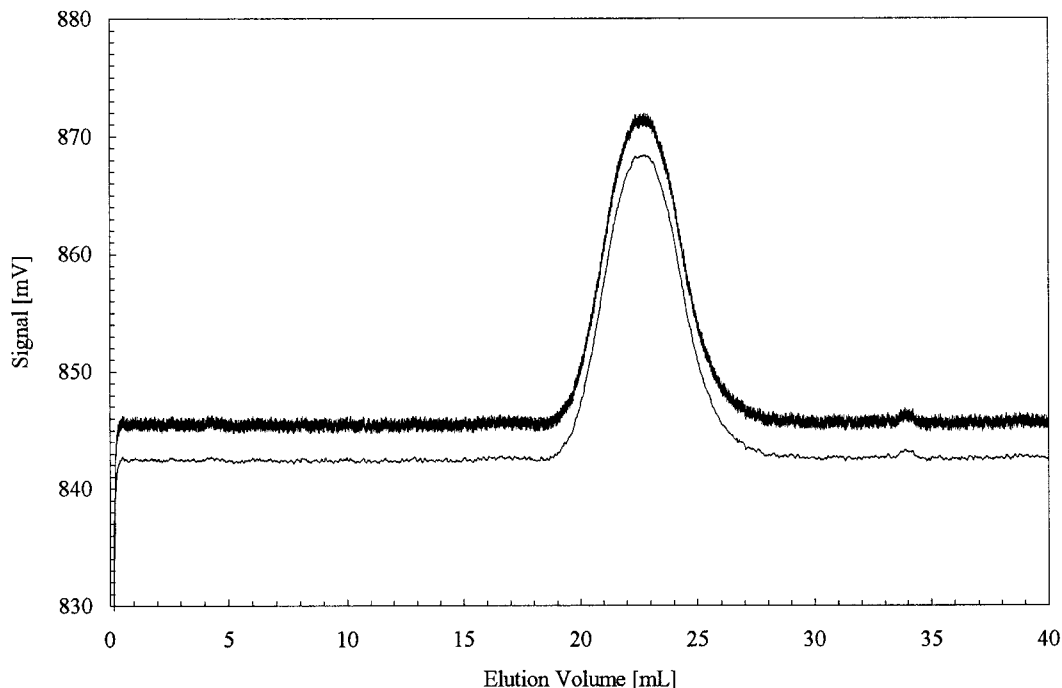


Figure 4 SCV detector signals before and after the FFT filtration for a broad PMMA sample. The signal before the FFT filtration has shifted to +3 mV.

tion¹⁴ we have set to zero the amplitude of the frequency between 0.7 to $2\pi \cdot 0.7$.

An alternative to the FFT filter consists in the use of the Savitsky–Golay smoothing algorithm¹⁵ implemented in the Millennium 2.15 software. In the following elaboration we have used the Savitsky–Golay algorithm, 21 points, and the final smoothed chromatograms were very similar to that obtained by the FFT digital filter. After filtration the noise decreases to approximately 0.1 – 0.2 mV (Fig. 4). The Savitsky–Golay

smoothing algorithm is simple, reliable, and does not distort the chromatographic peak. The areas of the chromatographic peak of the sample, before and after the digital filter, are identical.

Interdetector Delay Volume

A correct value of the interdetector SCV–DRI delay volume, for the alignment of the signals, must be accounted for. It is well known that the values of the Mark–Houwink–Sakurada constants re-

Table I $[\eta]$ Data Obtained from the New On-Line SCV Detector for PEO, PEG, Pullulan Narrow Standards in $0.15M$ NaCl at $35^\circ C$

	PEO		PEG		Pullulan	
	M_p (g/mol)	$[\eta]$ (dL/g)	M_p (g/mol)	$[\eta]$ (dL/g)	M_p (g/mol)	$[\eta]$ (dL/g)
1	860,000	4.1988	23,000	0.3326	853,000	1.6487
2	570,000	3.0268	12,600	0.2524	380,000	0.9829
3	270,000	2.0231	7100	0.1776	186,000	0.6095
4	160,000	1.2872	4100	0.1221	100,000	0.4041
5	85,000	0.8352	1470	0.0689	48,000	0.2557
6	45,000	0.5384	960	0.0563	23,700	0.1564
7	21,000	0.3188	600	0.0446	12,200	0.1035
8					5800	0.0655

Table II $[\eta]$ Data Obtained from the New On-Line SCV Detector for Some PS Narrow Standards in THF and Chloroform Solvents

	M_p (g/mol)	$[\eta]$ THF (dL/g)	$[\eta]$ CHCl ₃ (dL/g)
1	5,480,000	8.1597	—
2	900,000	2.2975	2.1495
3	105,560	0.5245	0.5196
4	10,900	0.0987	0.0979
5	1060	0.0328	0.0317

covered from a single broad MMD sample have influenced notably from the value of the interdetector delay volume.^{4,5,8,14,19} The delay volume value used in our data reduction software, 80 μ L, was approximately equal to the calculated physical delay volume, connecting tubes plus the detectors cell of the system. We retain that this datum is very important. In fact, in the “conventional” 150 GPCV system to obtain reliable Mark–Houwink–Sakurada constants from a single broad MMD sample it is necessary to use a lower value of the delay volume, and in some cases also a negative value, to compensate for the flow fluctuations problem.⁸ The value of the interdetector delay volume used in the data reduction software has been determined by a numerical optimization of the best superimposition between the experimental $[\eta] = f(V)$ SCV calibration, from a broad MMD linear sample, and the classical $[\eta] = f(V)$ calibration from some narrow standards. Our method is very similar to the method reported by Balke et al.¹⁹

Narrow MMD Standards Characterization

Aqueous Solvent

For a first evaluation of the new SCV system in the aqueous mobile phase, we have used some

PEO, PEG, and pullulan narrow standards. Table I reports $[\eta]$ data obtained by the new SCV system for the PEO, PEG, and pullulan narrow standards in the 0.15 M NaCl mobile phase at 35°C of temperature. $[\eta]$ results are in a good agreement with the data reported in literature.^{16,17}

Using $[\eta]$ data reported in Table I and the molar mass of the standards declared from the vendor, we have obtained the following Mark–Houwink–Sakurada constants: $k = 3.32 \times 10^{-4}$, $a = 0.694$ for PEO; $k = 1.13 \times 10^{-3}$, $a = 0.568$ for PEG; and $k = 2.31 \times 10^{-4}$, $a = 0.65$ for pullulan. Here and in the following, $[\eta]$ values are expressed in dL/g. The constants “ k ” and “ a ” of the Mark–Houwink–Sakurada equation in the aqueous solvent are very close to the values reported in the literature.¹⁶ Data were in good agreement despite our mobile phase, 0.15 M NaCl, being a little different from that used, 0.1 M NaNO₃, in the cited article.¹⁶

Organic Solvent

Table II reports some results obtained by means of the new SCV detector for PS narrow standards both in THF and in the chloroform mobile phase. Again, $[\eta]$ results are congruent with the data reported in the literature.^{14,18} Using the data reported in Table II for the PS polymer in the THF solvent we obtain the following Mark–Houwink–Sakurada constants: $k = 1.4 \times 10^{-4}$ dL/g, $a = 708$. The regression has stayed calculated using the linear portion of the log-log plot, disregarding the lower molar mass standard. The Mark–Houwink–Sakurada constants for the PS polymer in the THF solvent were very close to the values reported previously.^{14,18}

Broad MMD Samples Characterization

Table III reports a comparison between $[\eta]$ data obtained by the new on-line SCV detector and by

Table III Comparison Between $[\eta]$ Results Obtained by the New SCV Detector and by an Off-Line Ubbelohde Viscometer for Some PS and PEO Broad Samples

Sample	Solvent	M_w (g/mol)	D	$[\eta]^a$ (dL/g)	$[\eta]^b$ (dL/g)
PS NBS 706	THF	267,600	2.45	0.9406	0.9358
PS Edistir 1380	THF	230,700	2.38	0.9025	0.8922
PEO Sample A	0.15 M NaCl	114,600	3.66	1.0054	1.0119
PEO Sample B	0.15 M NaCl	207,400	3.42	1.6413	1.6486
PEO Sample C	0.15 M NaCl	434,700	3.84	3.0403	3.0575

^a SEC–SCV system.

^b Off-line Ubbelohde viscometer.

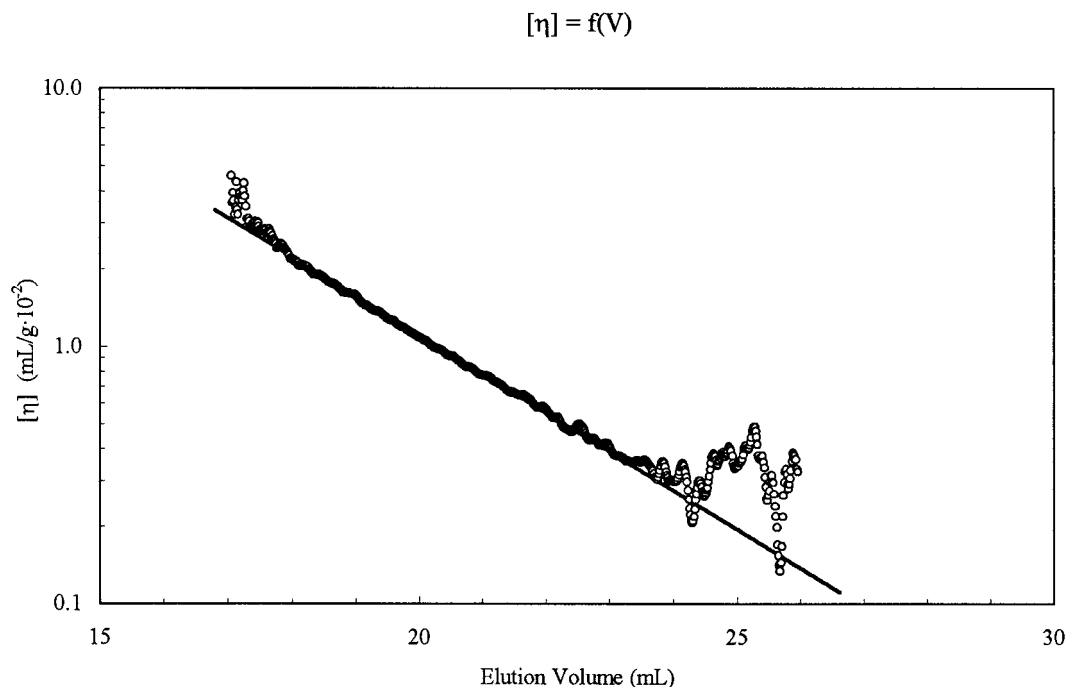


Figure 5 Experimental $[\eta] = f(V)$ calibration of the new SEC-SCV system obtained by a broad PS sample.

the off-line Ubbelohde viscometer. The agreement between on-line and off-line $[\eta]$ data was very good. In fact, the difference between on-line and off-line $[\eta]$ values was lower than 1%. Besides, the agreement between our M_w and dispersity (D) values and the nominal values reported by the vendors was very good.

Figure 5 shows the experimental $\text{Log}([\eta]) = f(V)$ calibration of the SEC-SCV system, where V is the elution volume, obtained by the broad MMD PS sample NBS 706. The signal-to-noise

ratio was very good, and with the exception of the extremities of the chromatogram, the local $[\eta]_i$ values were very accurate.

Table IV reports some constants “ k ” and “ a ” of the Mark-Houwink-Sakurada equation obtained by the new on-line SCV detector with some broad MMD samples both in organic and in aqueous solvents. As we can see, these results were in a good agreement with the results obtained using narrow standards and with the values reported in the literature.^{14,16-21} PVAc Mark-Houwink-

Table IV Mark-Houwink-Sakurada Constants, Obtained From the New On-Line SEC-SCV System, Using Broad MMD Samples Both in Organic and Aqueous Solvent at 35°C

Polymer	Sample	Solvent	k dL/g	a
PS	Edistir1380	CHCl_3	0.92×10^{-4}	0.752
PS	NBS706	THF	1.40×10^{-4}	0.708
PS	Edistir1380	THF	1.58×10^{-4}	0.703
PMMA	BDH	THF	1.14×10^{-4}	0.699
PVAc	Mapei	THF	1.01×10^{-4}	0.760
PVC	BDH	THF	3.96×10^{-4}	0.701
PAT	Aldrich	THF	1.20×10^{-4}	0.727
PEO	Aldrich	0.15M NaCl	3.94×10^{-4}	0.699
HA ($M_w \approx 6.5 \times 10^5$)	Soltes	0.15M NaCl	3.67×10^{-4}	0.785
HA ($M_w \approx 2.0 \times 10^6$)	Pharmacia and Upjohn	0.15M NaCl	4.13×10^{-3}	0.612

$$[\eta] = f(M)$$

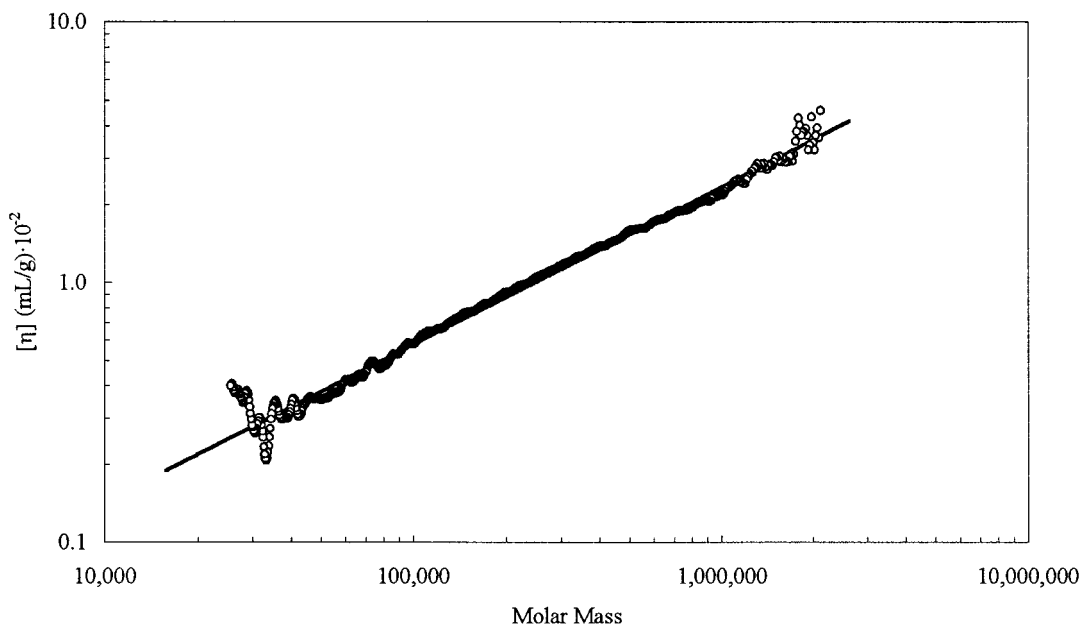


Figure 6 Experimental Mark-Houwink-Sakurada plot, $[\eta] = f(M)$, obtained by the new SCV detector for a broad PS sample in THF solvent.

Sakurada constants in THF solvent have been calculated from the linear portion of the $[\eta] = f(M)$ plot. The PAT sample was a commercial polyoctylthiophene polymer from Aldrich and the obtained Mark-Houwink-Sakurada constants were close to that obtained on our previously study using different PAT samples.²⁰ The difference between the two HA samples of different molar mass is noteworthy. The M_w average of the first HA sample, from Soltes, was approximately 6.5×10^5 g/mol, while the M_w average of the second HA sample, from Pharmacia and Upjohn, was approximately 2.0×10^6 g/mol. The obtained constants were in good agreement with the values reported from Bothner et al.²¹ for HA polymers in 0.2M NaCl with molar mass respectively lower or higher than 1×10^6 g/mol.

Finally, Figure 6 shows the experimental Mark-Houwink-Sakurada plot for the Edistir 1380 PS broad MMD sample in the THF solvent at 35°C obtained by the new SEC-SCV on-line system. Disregarding the extremities of the plot where the signal-to-noise ratio was low, the obtained Mark-Houwink-Sakurada constants for this PS broad MMD sample were $k = 1.58 \times 10^{-4}$ and $a = 703$. Again, these values were in a good agreement with the reported values for the PS polymer in the THF solvent.

CONCLUSIONS

An original multidetector SEC system composed of an Alliance 2690 pulse-free pump, without a dampener, SCV, and DRI detectors, has been tested. A number of narrow MMD standards and broad MMD samples both in organic and in aqueous solvent have been used for a general evaluation of the new SEC-SCV system. The new on-line viscometer shows a residual noise that could be easily eliminated by means of a digital filter.

The new system provides accurate intrinsic viscosity and MMD results. Besides, the Mark-Houwink-Sakurada constants obtained using both narrow standards and broad MMD samples are very close to the reported values in the literature. The interdetector delay volume, between the SCV and the concentration detector, obtained by an optimization method, was approximately the physical interdetector delay volume of the system. This fact confirms that the flow rate upset problem, typical of a "conventional" SEC-SCV system, has been resolved or at least minimized.

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