International Journal of Polymer Anal. Charact., 17: 381–393, 2012

ISSN: 1023-666X print/1563-5341 online
DOI: 10.1080/1023666X.2012.669529

CAVEATS REGARDING THE "SOLVENT/NON-SOLVENT" AND SELS APPROACHES IN SIZE-EXCLUSION CHROMATOGRAPHY AND RELATED METHODS

Kelsey E. McNeel,[1] Dustin J. Richard,[1] and André M. Striegel[2]

[1]*Department of Chemistry and Biochemistry, Florida State University, Tallahassee, Florida, USA*

[2]*Analytical Chemistry Division, National Institute of Standards and Technology, Gaithersburg, Maryland, USA*

A rarely documented, yet industrially popular, approach to the size-exclusion chromatography (SEC) and related analysis of polymers that do not dissolve in common solvents (i.e., those solvents that are employed as mobile phases in high-throughput SEC systems) is to dissolve these analytes in a different solvent and to then inject this solution onto an SEC system that employs a non-solvent as mobile phase. A variant of this approach, known as solvent enhanced light scattering (SELS), is employed to compensate for low optical contrast between analyte and mobile phase. Both the "solvent/non-solvent" and SELS approaches can provide results for a given sample in the form of chromatograms from which, using an in-place calibration curve, molar mass averages and distributions may be calculated. There appear to be no reports, however, on the accuracy of the solvent/non-solvent and SELS approaches. To this effect, the former were evaluated here (with an eye on implications for the latter), using well-characterized narrow dispersity polystyrene (PS) and poly(methyl methacrylate) standards and broad dispersity PS samples. A variety of approaches to the solvent/non-solvent method were employed, most with disastrous results, except for the trivial case when both solvent and mobile phase are good solvents for the polymer, are miscible with each other, and there is little difference in the specific refractive index increment of the analyte in each. This last case notwithstanding, based on the results shown here it is recommended that the solvent/non-solvent and SELS approaches be abandoned immediately, as they are likely to provide a false sense of confidence in inaccurate results.

***Keywords*:** Industrial approach; Size-exclusion chromatography; Solvent enhanced light scattering; Solvent/non-solvent

Submitted 1 February 2012; accepted 17 February 2012.

The authors would like to thank Drs. Imad Haidar Ahmad, Amandaa Brewer, and Catherine Rimmer, Ms. Mallory Morris, and Mr. Steven Rowland for their assistance and for helpful discussions. Commercial products are identified to specify adequately the experimental procedure. Such identification does not imply endorsement or recommendation by the National Institute of Standards and Technology, nor does it imply that the materials identified are necessarily the best available for the purpose.

Correspondence: André M. Striegel, National Institute of Standards and Technology, 100 Bureau Dr., Mail Stop 8392, Gaithersburg, MD 20898, USA. E-mail: andre.striegel@nist.gov

INTRODUCTION

During the past half century, size-exclusion chromatography (SEC) has become the premier method through which to obtain, in a single analysis, the molar mass (M) averages and distributions of both natural and synthetic macromolecules.[1–4] While it has long been recognized that SEC with on-line static light scattering detection (when employed in conjunction with concentration-sensitive detection) is generally the most accurate way to determine these averages and distributions, it is quite common for industrial laboratories to possess one or several high-throughput SEC (and/or field-flow fractionation) systems, each employing a single, concentration-sensitive detector (most commonly a differential refractometer or DRI). Using these systems, several thousand or tens of thousands of chromatographic runs may be performed each year. Molar mass averages and distributions are obtained with these systems through the application of peak-position or calibrant-relative calibration curves (where oftentimes, it should be noted, the calibrants bear little if any chemical and/or architectural resemblance to the analytes themselves). Currently, if a particular analyte does not dissolve in the solvent/mobile phase in a high-throughput system (most commonly tetrahydrofuran for the analysis of synthetic polymers or water for the analysis of biopolymers), the possibility of rinsing and purging the front end of the system, the columns, and the detector, and of establishing a new calibration curve usually constitutes unacceptable downtime due to the length (on the order of days) needed to complete these procedures and to the sample backlog consequently generated. An oft-employed solution to this predicament is the ''solvent/non-solvent'' approach, explained below.

In the solvent/non-solvent approach, a sample that does not dissolve in the mobile phase in the high-throughput SEC (i.e., the SEC mobile phase is a non-solvent for this particular analyte) is dissolved in a solvent that does dissolve the sample. A fraction of this polymer solution is then injected into the SEC system, which still operates using its traditional mobile phase. The resultant chromatogram is analyzed using the calibration curve already in place to obtain calibrant-relative M averages and distributions, as would be done for any of the other thousands of samples analyzed on this system each year. In the solvent/non-solvent approach, it is usually recommended that the solvent and the non-solvent be miscible with each other. Sometimes, this approach is also used for samples with low optical contrast with the mobile phase, i.e., when the specific refractive index increment $\partial n/\partial c$ of the sample solutions is close to zero. In said cases, the sample is dissolved in a solvent where the $\partial n/\partial c$ will be higher and this solution is injected into the SEC system operating with its usual mobile phase. The latter approach has been given the term solvent enhanced light scattering or SELS.[5–7]

While very little has been published on the solvent/non-solvent or SELS approaches (nothing, it seems, in the peer-reviewed literature), ample anecdotal evidence exists as to the widespread application of these approaches in industrial settings. As best as we can ascertain, however, no concerted effort has been made to determine the accuracy of the solvent/non-solvent or SELS approaches. These methods will yield results, but what is the accuracy of the latter, even if only as compared to traditional calibrant-relative results from the same system (i.e., even if the M data are not absolute, as would be if determined using SEC/light scattering)?

The SELS approach and, by implication, the solvent/non-solvent approach, was questioned recently by Held and Kilz.[8]

To attempt to answer the above question, we present here the results of a series of studies in which the solvent/non-solvent approach was applied to a series of well-characterized narrow and broad dispersity standards. Several variants of the solvent/non-solvent approach were employed and, in all cases, results were compared to those obtained when the samples were analyzed using an SEC system in which the mobile phase was also a solvent for the polymers. As will be seen, the utmost caution should be employed when using the solvent/non-solvent and SELS approaches, to the point where abandoning these questionable approaches is recommended.

EXPERIMENTAL SECTION

Materials

Polystyrene (PS) and poly(methyl methacrylate) were obtained from Agilent/Polymer Laboratories and from PSS Polymer Standards Service. Solvents were obtained from Fisher Scientific. All materials were used as received, without further purification.

SEC/DRI

For all samples, $1\,\mathrm{mg\,mL^{-1}}$ solutions were prepared, shaken gently by hand, and allowed to solvate at least overnight. After this, $100\,\mu\mathrm{L}$ of unfiltered solution were injected onto a Waters 2695 Separations Module with on-line degasser and in-line pre-injector filter, connected to a $300\,\mathrm{mm} \times 7.5\,\mathrm{mm}$ PLgel column packed with $5\,\mu\mathrm{m}$ particles of 10^4 Å nominal pore size from Agilent. Detection was performed using an Optilab rEX differential refractometer from Wyatt Technology Corp. System temperature was $30° \pm 1°\mathrm{C}$. Toluene was added as a flow rate marker to all samples, to help correct for minor pump pulsations. All experiments were conducted at least in triplicate. Data acquisition and analysis were performed using Clarity software (version 2.4.1.91) from DataApex.

The calibration curve in Figure 1 was constructed by analyzing narrow dispersity ($M_w/M_n \leq 1.06$) linear PS standards using dimethyl formamide (DMF) as both solvent and mobile phase. As reported by the manufacturers, the peak-average molar masses M_p of the standards (determined in tetrahydrofuran at room temperature), in g $\mathrm{mol^{-1}}$, were: 8450, 19760, 70950, 102000, 355000, 538000, and 775000. To construct the calibration curve, each standard was analyzed in triplicate and the retention volume of each run of each standard was adjusted, using the toluene flow rate marker peak relative to the retention volume of this marker peak as averaged over all injections of all calibration standards. The curve is the result of a non-weighted, third-order least-squares fit, with $r^2 = 0.997$.

RESULTS AND DISCUSSION

The solvent/non-solvent approach was evaluated using polystyrene (PS) standards as analytes and the solvents and mobile phases detailed in Table I.

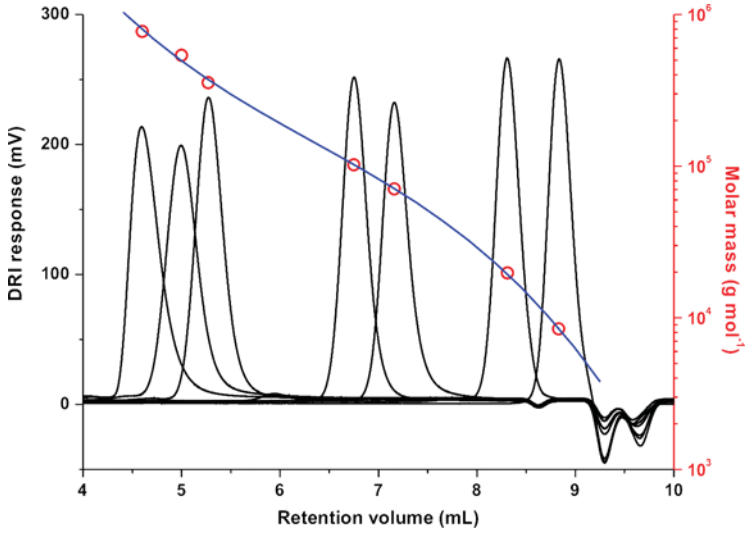

Figure 1. SEC chromatograms of narrow dispersity PS standards, as monitored with the DRI detector (signal in millivolts, mV). Circles denote the intersection of the retention volume of the peak apex and the peak-average molar mass M_p of each standard; each point represents the average of triplicate injections, with standard deviations much smaller than data markers and, therefore, not shown. Solid line constitutes a third-order least-squares non-weighted fit to the data, with $r^2 = 0.997$. Solvent: DMF; mobile phase: DMF; temperature: 30°C. See Experimental section and discussion of experiment set #1 for details (color figure available online).

An additional set of experiments (experiment set #6 in Table I) using poly(methyl methacrylate) (PMMA) standards is described at the end of this section.

Experiment Set #1

Solvent: DMF (S)
Mobile phase: DMF (S)
Mutual miscibility: Miscible

Table I. Solvent and mobile phase combinations used when evaluating the solvent/non-solvent approach for analyzing PS standards

Experiment set #	Solvent	Mobile phase	Mutual miscibility of solvent and mobile phase
1	DMF (S)	DMF (S)	M
2	THF (S)	DMF (S)	M
3	DMF (S)	DMSO (NS)	M
4	THF (S)	DMSO (NS)	M
5	DMF (S)	Hexane (NS)	I
6[a]	Acetone (S)	Acetonitrile (NS)	M

DMF: *N,N*-dimethyl formamide; THF: tetrahydrofuran; DMSO: dimethylsulfoxide; S: solvent for PS; NS: non-solvent for PS; M: miscible; I: immiscible.

[a]Used for analyzing PMMA standards. For this experiment set, S and NS refer to solubility of PMMA.

Table II. Molar mass averages and dispersity of PS calibration standards

M_p (g mol^{-1})	M_n (g mol^{-1})	M_w (g mol^{-1})	M_w/M_n	Retention volume (mL)
8500 ± 0^a	8200 ± 0	8500 ± 0	1.04 ± 0.01	8.83^b
19400 ± 0	18800 ± 0	19400 ± 0	1.03 ± 0.01	8.31
71200 ± 0	68100 ± 400	69700 ± 100	1.02 ± 0.01	7.16
102000 ± 0	99100 ± 100	101000 ± 100	1.02 ± 0.01	6.75
370000 ± 0	355000 ± 0	363000 ± 200	1.02 ± 0.01	5.27
495000 ± 900	463000 ± 7200	483000 ± 3000	1.04 ± 0.02	5.00
795000 ± 2000	686000 ± 1800	728000 ± 1000	1.06 ± 0.01	4.60

Obtained using DMF as both solvent and mobile phase and calibration curve shown in Figure 1. Uncertainties represent 1 standard deviation based on at least triplicate determinations (see Experimental section for details).

[a]Where standard deviation is given as 0, corresponds to less than ± 60 g mol^{-1}.

[b]In all cases, standard deviation less than ± 0.01 mL.

In this set of experiments, a series of narrow dispersity PS standards were dissolved in DMF and injected into an SEC system that also employed DMF as mobile phase. The calibration curve generated using these standards is shown in Figure 1, as are the chromatograms of the standards themselves. This curve was used to calculate the M averages in II–V and the molar mass distributions (MMDs) in Figures 2 and 4.

The various M averages of the PS standards in Figure 1 are given in Table II. Because DMF is a thermodynamically good solvent for PS at the experimental temperature[9,10] and is also compatible with the styrene/divinylbenze PLgel column packing material, and because both analytes and calibrants possess the same repeat unit chemistry and polymeric architecture, the data in Table II can be regarded as benchmarks to which other experimental results may be compared.

Given in Table III are the results, using DMF as both solvent and mobile phase and applying the calibration curve in Figure 1, for two broad PS samples, denoted here Broad PS1 and Broad PS2. These results will be compared to those using tetrahydrofuran (THF) as solvent next.

Experiment Set #2

Solvent: THF (S)
Mobile phase: DMF (S)
Mutual miscibility: Miscible

Table III. Molar mass averages and dispersity of broad PS samples (experiment sets #1 and #2)

Sample	Solvent	M_n (g mol^{-1})	M_w (g mol^{-1})	M_z (g mol^{-1})	M_w/M_n
Broad PS1	DMF	102000 ± 8000	183000 ± 5000	428000 ± 77000	1.81 ± 0.18
	THF	107000 ± 4000	178000 ± 1000	344000 ± 12000	1.66 ± 0.06
Broad PS2	DMF	344000 ± 21000	565000 ± 5000	773000 ± 1000	1.65 ± 0.09
	THF	365000 ± 22000	573000 ± 5000	748000 ± 1000	1.57 ± 0.08

In all cases, mobile phase was DMF. Results calculated by applying calibration curve in Figure 1. Uncertainties represent 1 standard deviation based on at least triplicate determinations (see Experimental section for details).

Table IV. Molar mass averages and dispersity of narrow PS standards (experiment sets #1 and #2)

Sample	Solvent	M_n (g mol^{-1})	M_w (g mol^{-1})	M_z (g mol^{-1})	M_w/M_n
Narrow PS1	DMF	50200 ± 200	51300 ± 300	52300 ± 500	1.02 ± 0.01
	THF	49400 ± 200	51300 ± 200	53300 ± 500	1.04 ± 0.01
Narrow PS2	DMF	194800 ± 700	200500 ± 1100	205600 ± 1300	1.03 ± 0.01
	THF	200200 ± 1000	205600 ± 200	210700 ± 100	1.03 ± 0^a
Narrow PS3	DMF	617000 ± 10000	645000 ± 2300	677000 ± 5700	1.04 ± 0.02
	THF	598000 ± 12000	642000 ± 5900	686000 ± 2700	1.07 ± 0.02

In all cases, mobile phase was DMF. Results calculated by applying calibration curve in Figure 1. Uncertainties represent 1 standard deviation based on at least triplicate determinations (see Experimental section for details).

[a]Standard deviation of zero corresponds to less than ± 0.01.

Table V. Molar mass averages and dispersity of two narrow PS standards (experiment set #3)

Sample[a]	M_n (g mol^{-1})	M_w (g mol^{-1})	M_z (g mol^{-1})	M_w/M_n
PS 8500	13000 ± 1000	21800 ± 5000	45200 ± 17000	1.67 ± 0.40
PS 19400	13600 ± 3600	17600 ± 1200	24200 ± 500	1.30 ± 0.36

Results calculated by applying calibration curve in Figure 1. Uncertainties represent 1 standard deviation based on at least triplicate determinations (see Experimental section for details).

[a]Samples are denoted as per M_p given in Table II.

The first comparison involved dissolving the samples in THF and analyzing the solutions using the DMF mobile phase. THF and DMF are mutually miscible. They are also both good solvents for PS and, as such, from a ''real world'' perspective this type of scenario would be employed only for a SELS-type approach, i.e., when the optical contrast between analyte and mobile phase are relatively low, as

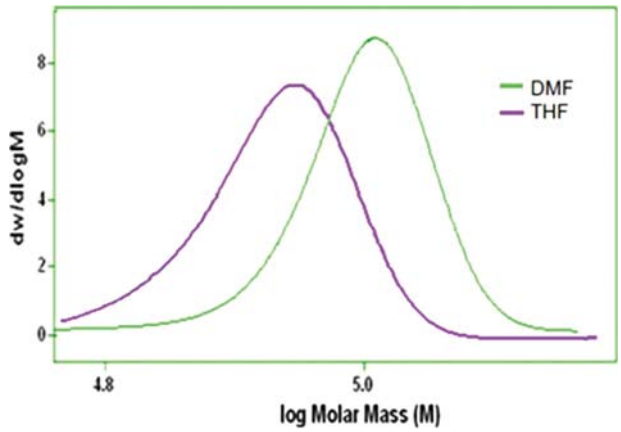

Figure 2. Differential molar mass distribution (MMD) of PS 102000 narrow dispersity standard. Green curve: Both solvent and mobile phase are DMF. Purple curve: Solvent is THF, mobile phase is DMF. See discussion of experiment set #2 for details (color figure available online).

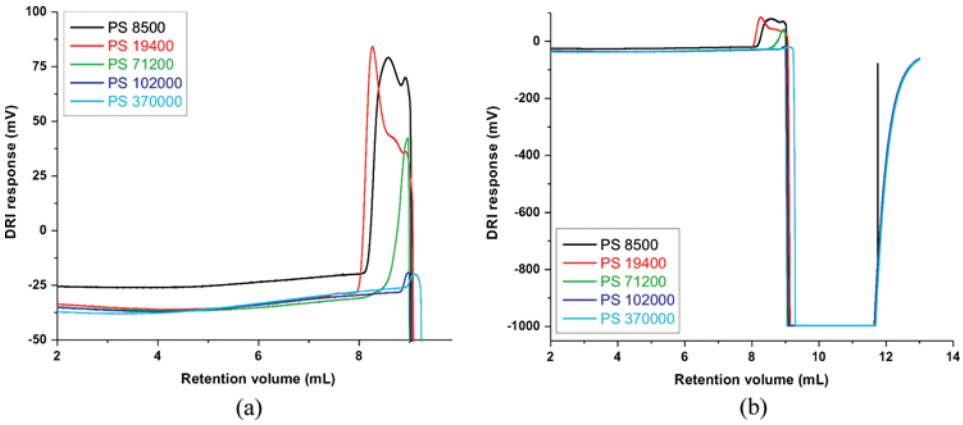

Figure 3. (a) SEC chromatograms of narrow dispersity PS standards, using DMF as solvent and DMSO as mobile phase. (b) Expanded view, showing the DMF solvent peak (which goes off-scale at −1000 mV). See discussion of experiment set #3 for details (color figure available online).

compared to the optical contrast between analyte and solvent (not the case here, as the $\partial n/\partial c$ of PS in either DMF or THF is $>0.150\,\mathrm{mL\,g^{-1}}$ at the experimental conditions).[11] Here, we have studied this particular solvent/mobile phase combination for comparison to other two-component scenarios in Table I in which the mobile phase may be a non-solvent for the analyte or in which the solvent and mobile phase are both solvents for the analyte but are immiscible with each other.

Results from this set of experiments are given in Figure 2 and Table IV for the narrow standards studied, and in Table III for the broad samples. As expected, given that both THF and DMF are good solvents for PS and miscible with each other, for all cases results are fairly similar when using DMF as both solvent and mobile phase as compared to when using THF as solvent and DMF as mobile phase. In most cases, the largest differences are observed when comparing the M_z obtained by both approaches, followed in magnitude and frequency by differences in M_n. No pattern could be found in the differences among the two data sets.

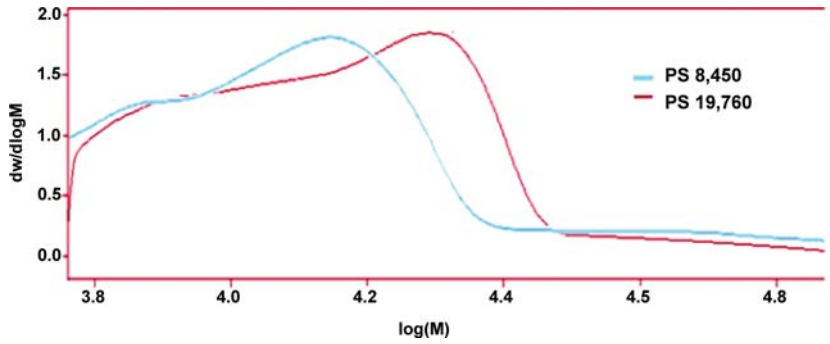

Figure 4. Differential MMD of PS 8500 and PS 19400 narrow dispersity standards, using DMF as solvent and DMSO as mobile phase. See discussion of experiment set #3 for details (color figure available online).

The above lends some confidence to the SELS approach *when used for determining the M range of the sample,* and as long as it involves the use of a solvent/mobile phase combination where both of these are solvents for the analyte and miscible with each other. As explained in the Introduction, when the $\partial n/\partial c$ of a solution of analyte in the mobile phase is low, SELS employs a solvent (different from the mobile phase) in which the $\partial n/\partial c$ of the analyte will be large, thereby yielding a high signal-to-noise ratio and increased precision in the determination of the M averages and MMD of the polymer. As shown by our comparison, the M range of the sample does not appear to be affected by this approach. Because the specific refractive index increments of solutions of PS in THF and of PS in DMF are similar (~15% difference) to each other, and because the response of the DRI is proportional to the product of concentration and $\partial n/\partial c$ (i.e., DRI $\propto c \times \partial n/\partial c$), little difference is expected between the M averages and MMDs determined by both methods using these two solvents. However, because M averages are determined via:[1]

$$M_\beta = \frac{\sum_i c_i M_i^x}{\sum_i c_i M_i^{x-1}} \text{ when } x = 0,\ \beta = n;\ \text{when } x = 1,\ \beta = w;\ \text{when } x = 2,\ \beta = z \quad (1)$$

where c_i and M_i are, respectively, the concentration and molar mass at each SEC elution slice i, and because the individual c_i are determined from the DRI response, larger differences in M averages and MMD might be expected when the $\partial n/\partial c$ of the analyte in the solvent is extremely different from the $\partial n/\partial c$ of the analyte in the mobile phase. In other words, even when using a solvent/mobile phase combination in which both of these are solvents for the analyte and miscible with each other, the SELS approach might increase the precision of the analysis at the expense of diminished accuracy. Further experiments in this regard certainly appear warranted.

Experiment Set #3

Solvent: DMF (S)
Mobile phase: DMSO (NS)
Mutual miscibility: Miscible

Our next comparison involved using a solvent/mobile phase combination where both of these were mutually miscible, but in which the mobile phase was a non-solvent for the polymer. To this effect, DMF was used as the solvent and dimethyl sulfoxide (DMSO) as the mobile phase. This general scenario resembles that in which the solvent/non-solvent approach is used in industrial high-throughput labs. Results from these experiments are shown in Figures 3 and 4 and in Table V (compare M averages and dispersity to results in Table II).

As can be seen in the figures and table, this did not prove to be a particularly successful approach to determining the M averages and distributions of polymers. Of the five polymers analyzed, only the three lowest-M ones appeared to elute from the column, as observed in Figure 3(a). (The elution of DMF is seen in the large negative peak in Figure 3(b). The peak is negative because the refractive index of DMF,

1.4305, is lower than that of DMSO, 1.4783. All refractive index data given in the article are from Higgins and Klinger[12].) The two largest-M polystyrenes either precipitated onto the column packing material and did not desorb over a noticeable time frame, or they co-eluted with the solvent peak. In either case, no useable data were obtained for the two largest-M polystyrenes. However, even the chromatograms of the three polystyrenes that did elute are not promising (see Figure 3(a)). That of PS 71200 yielded no useful information. The M averages and MMDs of PS 8500 and PS 19400 are given in Table V and Figure 4 (note that these results were determined using the calibration curve in Figure 1, as PS is insoluble in DMSO). These clearly show the danger of the solvent/non-solvent approach, which in many cases will yield results, whose accuracy is highly questionable. While the M_n and M_w of PS 19400 show some resemblance to the results for this same polymer as given in Table II, the chromatogram in Figure 3(a) and the MMD in Figure 4 show this resemblance to likely be coincidental, as there is little similarity between this chromatogram and MMD and those of a narrow dispersity PS standard (compare to the shape of chromatogram of the same sample in Figure 1). It should be remembered that even vastly different MMDs can have similar, or even identical, M averages.[1] At any rate, if one were to look at the information in Table V and in Figure 4, a highly distorted picture of the analytes would emerge, with most likely unfortunate consequences for any decisions made based upon this information (e.g., decisions regarding sample batch quality or process conditions).

The possibility of viscosity differences between solvent and mobile phase being responsible for the observed behavior is discussed below for experiment set #6. Next we examine the same type of scenario, but employing a thermodynamically better solvent for PS.

Experiment Set #4

Solvent: THF (S)
Mobile phase: DMSO (NS)
Mutual miscibility: Miscible

This is the same scenario as in experiment set #3. A different solvent (THF) was employed to see if better results could be obtained than when using DMF as solvent (as THF is, thermodynamically, a somewhat better solvent for PS than is DMF at the experimental temperature). The results were worse, however, as no analytes eluted from the column (or they co-eluted with the solvent) in experiment set #4.

The possibility of viscosity differences between solvent and mobile phase being responsible for the observed behavior is discussed in experiment set #6. Otherwise, no further discussion of this solvent/non-solvent scenario appears warranted at this point.

Experiment Set #5

Solvent: DMF (S)
Mobile phase: Hexane (NS)
Mutual miscibility: Immiscible

While we have received no anecdotal evidence as to the use of this scenario (and, as with most of the above, there appear to be no reports in the peer-reviewed literature), it seemed intriguing to employ a solvent/mobile phase combination where both these components were immiscible with each other to examine the possibility of delivering the analyte to the detector by "encapsulating" it in a microdroplet of solvent within the mobile phase medium. To this end, DMF was employed as solvent and hexane as mobile phase.

Again, results were not encouraging. The same samples as in experiment set #3 were studied. Archetypal behavior was shown by PS 19400, as seen in Figure 5(a) in which nothing is observed to elute within the size-exclusion separation volume of the column. (For reference, the negative peak at ≈9.8 mL is a system peak, and the somewhat larger positive peak at ≈11.3 mL is from the toluene added as a flow rate marker to all samples. See Experimental section for details.) Unlike experiment set #3, however, in which the solvent eluted within the exclusion volume of the column (Figure 3(b)), in the present case the solvent is seen to elute well after the total permeation volume (Figure 5(b)). This result appears to be a consequence of the large difference in polarities between DMF and hexane (6.4 and 0.1, respectively, according to the P′ polarity index of Snyder),[12] resulting in elution of DMF via a normal phase separation mechanism. (This peak is positive, as compared to the negative solvent peak in Figure 3(b), due to the refractive index of DMF, 1.4305, being higher than that of hexane, 1.3749.) Whether or not the PS analytes are co-eluting with the DMF is not known, as their presence is expected to imperceptibly change the size of the solvent peak. Because hexane is a non-solvent for PS, it is also possible that the analytes precipitated onto the column.

We attempted a variant on this experiment set, employing DMF as solvent and cyclohexane as mobile phase, i.e., using a solvent/mobile phase combination in which both components are known solvents for PS and immiscible with each other. Unfortunately, dissolving the PS standards in cyclohexane and maintaining them in solution throughout the experiment proved to be an insurmountable challenge.

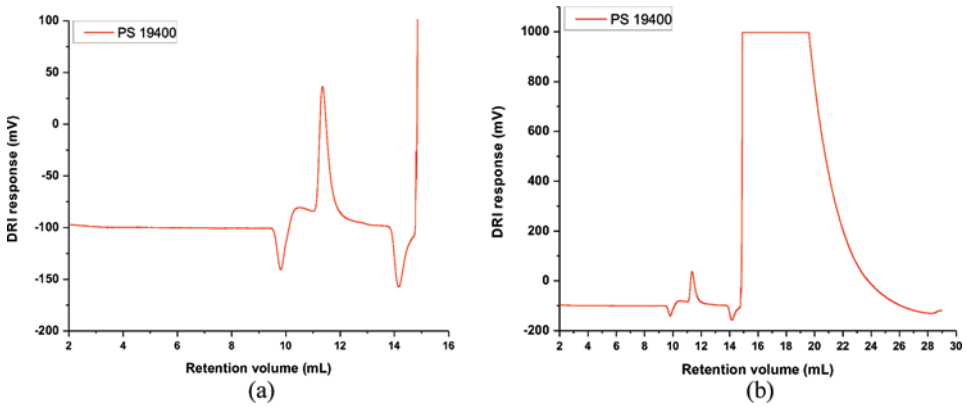

Figure 5. (a) SEC chromatograms of narrow dispersity PS 19400 standard, using DMF as solvent and hexane as mobile phase. (b) Expanded view, showing the DMF solvent peak (which goes off-scale at 1000 mV). See discussion of experiment set #5 for details (color figure available online).

The theta (θ) temperature for PS in cyclohexane is reportedly between 34° and 35°C.[13,14] Even setting the temperature controllers in our system to 40°–50°C, no peaks were observed for most cases and, when peaks were observed, they were irreproducible. These results are likely due to "cold spots" in the equipment (e.g., interconnecting tubing, valves, etc.), where the polymer solution is exposed to temperatures below the theta temperature, leading to precipitation of the analyte.

Experiment Set #6

Solvent: Acetone (S)
Mobile phase: Acetonitrile (NS)
Mutual miscibility: Miscible
Analyte: PMMA

Both experiment sets #3 and #4 involved a solvent/mobile phase combination in which both components were miscible with each other, but in which the mobile phase was a non-solvent for the PS analytes. When discussing the results from these experiments, it was postulated that large viscosity differences between solvent and mobile phase may have been responsible for the negative results. We wished to test this proposition using a solvent/mobile phase combination in which both components were miscible with each other, in which the mobile phase was a non-solvent for the analyte, but in which the viscosity difference among solvent and mobile phase was small. While we were unable to find this type of solvent/mobile phase combination for PS, using acetone as solvent and acetonitrile as mobile phase satisfies all the requirements when PMMA is the analyte. As such, we proceeded with our studies of PMMA.

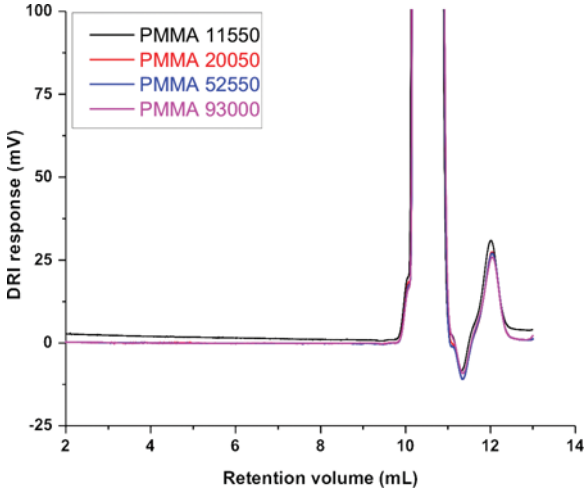

Figure 6. SEC chromatograms of narrow dispersity PMMA standards, using acetone as solvent and acetonitrile as mobile phase. Numbers denote the M_p of the standards. See discussion of experiment set #6 for details (color figure available online).

The room temperature viscosity of DMSO is 2.24 cP, while that of DMF is 0.92 cP and that of THF is 0.55 cP.[12] Relatively large differences in viscosity existed in experiment set #3 between the solvent (DMF) and the mobile phase (DMSO), and also in experiment set #4, where THF was the solvent and DMSO was the mobile phase. In our PMMA experiments, however, the viscosity difference between solvent and mobile phase was less than 5%, as the viscosity of acetone is 0.36 cP and that of acetonitrile is 0.375 cP.[12] Regardless of this small difference, no elution was observed in this solvent/non-solvent experiment for narrow dispersity PMMA standards over nearly an order of magnitude in M, as observed in the chromatograms in Figure 6 (where the names of the standards denote their respective M_p). As evidenced by the location of the solvent peak, which elutes between approximately 10 and 11 mL (the smaller peak at ≈12.0 mL is a system peak), the acetone solvent appears to elute via a predominantly size-exclusion mechanism, with minimized enthalpic contributions to the separation. As hypothesized earlier, either the PMMAs are all co-eluting with the solvent, regardless of M, or they have precipitated onto the column. Regardless, viscosity differences among solvent and mobile phase do not appear responsible for the behavior observed for the PMMAs, nor for the PSs in experiment sets #3 and #4.

CONCLUSIONS

We have attempted here a systematic evaluation of the solvent/non-solvent approach popular in industrial high-throughput SEC and field-flow fractionation (FFF) laboratories and also employed in SELS. Our experiments employed well-characterized narrow and broad PS standards and narrow PMMA standards, covered a broad molar mass range, and employed a variety of solvent/mobile phase combinations. Except when the solvent and mobile phase were both solvents for the polymer and miscible with each other, no other approach yielded accurate results (this accuracy was also contingent upon the $\partial n/\partial c$ of polymer solutions in the solvent being similar to the $\partial n/\partial c$ of polymer solutions in the mobile phase). While disappointing from an experimental point of view, those cases in which no evidence of the analytes could be seen in the chromatograms are less troubling in that an analyst would be limited with respect to the incorrect conclusions that could be drawn about the sample. More troubling are those cases such as seen in Figures 3 and 4 and Table V, in which results are obtained, but the results are highly inaccurate. For the case of well-characterized standards, such as those studied here, the inaccuracy is obvious. This is not necessarily so in the case of samples for which the M averages and distribution are not known a priori, i.e., exactly the type of cases where the solvent/non-solvent and SELS approaches is normally employed. The consequences of incorrect decisions based on erroneous data derived from these types of solvent/non-solvent or SELS experiments can be far-reaching, with deep legal and financial repercussions.

It should be noted that the most common variant of the SELS approach is one in which both solvent and mobile phase are solvents for the sample and miscible with each other, but where the optical contrast of the sample with the mobile phase is small, while the optical contrast of the sample with the solvent is much larger. This approach does appear to be able to characterize the molar mass *range* of the sample

with reasonable accuracy, though the M averages and MMD obtained may be suspect due to the large differences in $\partial n/\partial c$ between solutions of the analyte in the solvent vis-à-vis in the mobile phase.

In conclusion, use of the solvent/non-solvent and SELS approaches to SEC, FFF, hydrodynamic chromatography, and related methods should be done with the utmost caution, if at all. In general, the authors believe these approaches should be abandoned (with the exception of characterization of the M range via SELS, as explained in the previous paragraph), as they are likely to do more harm than good by providing inaccurate data while simultaneously obscuring this inaccuracy.

REFERENCES

1. Striegel, A. M., W. W. Yau, J. J. Kirkland, and D. D. Bly. 2009. *Modern Size-Exclusion Liquid Chromatography*, 2nd ed. New York: Wiley.
2. Striegel, A. M., ed. 2005. *Multiple Detection in Size-Exclusion Chromatography*, ACS Symp. Ser. 893. Washington, D.C.: American Chemical Society.
3. Striegel, A. M. 2008. Size-exclusion chromatography: Smaller, faster, multi-detection, and multi-dimensions. *Anal. Bioanal. Chem.* 390: 303–305.
4. Striegel, A. M. 2005. Multiple detection in size-exclusion chromatography of macromolecules. *Anal. Chem.* 77: 104A–113A.
5. Wong, W. S., C. Mirley, and S. Daughtry. 2008. SELS – Solvent enhanced light scattering. *LC GC Application Notebook* September: 45.
6. Sanchez, J. C., W. S. Wong, C. Mirle, and S. Daughtry. 2009. Solvent enhanced light scattering (SELS) for GPC. *Polym. Prepr.* 50(1): 91.
7. Brousseau, J. L., and W. S. Wong. 2009. Analysing synthetic polymers with solvent enhanced light scattering. *Sep. Sci.* 1: 24–28.
8. Held, D., and P. Kilz. 2009. How to choose a static light-scattering technique for molar mass determination. *The Column* 5: 28–32.
9. Kurata, M., and Y. Tsunashima. 1999. Viscosity-molecular weight relationships and unperturbed dimensions of linear chain molecules. In *Polymer Handbook*, 4th ed., eds. J. Brandup, E. H. Immergut, and E. A. Grulke, pp. VII/1–VII/83. New York: Wiley-Interscience.
10. Bloch, D. R. 1999. Solvents and nonsolvents for polymers. In *Polymer Handbook*, 4th ed., eds. J. Brandup, E. H. Immergut, and E. A. Grulke, pp. VII/497–VII/545. New York: Wiley-Interscience.
11. Michielsen, S. 1999. Specific refractive index increments of polymers in dilute solution. In *Polymer Handbook*, 4th ed., eds. J. Brandup, E. H. Immergut, and E. A. Grulke, pp. VII/547–VII/627. New York: Wiley-Interscience.
12. Higgins, R. S., and S. A. Klinger, eds. 1990. *Solvent Guide*, 3rd ed. Muskegon, Mich.: Burdick & Jackson.
13. Elias, H.-G. 1999. Theta solvents. In *Polymer Handbook*, 4th ed., eds. J. Brandup, E. H. Immergut, and E. A. Grulke, pp. VII/291–VII/326. New York: Wiley-Interscience.
14. Smith, M. J., I. A. Haidar, and A. M. Striegel. 2007. Measuring the size of polymers with negative radii using MALS/QELS: An exploration of the thermodynamic radius. *Analyst* 132: 455–460.