



## Absolute molar mass determination in mixed solvents. 2. SEC/MALS/DRI in a mix of two “nearly-isovirial” solvents

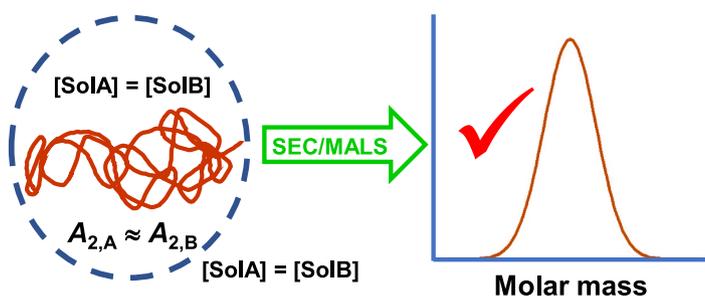
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### HIGHLIGHTS

- Absolute molar mass determination in mixed solvents.
- Accuracy of determination compromised by preferential solvation.
- Use of a nearly isovirial solvent pair can solve this problem.
- Successfully tested employing size-exclusion chromatography.
- On- and off-line static light scattering and refractometry are also needed.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

#### Keywords:

Size-exclusion chromatography  
Mixed solvents  
Preferential solvation  
Light scattering  
Absolute molar mass  
Second virial coefficient

### ABSTRACT

The accurate determination of polymer molar mass ( $M$ ) averages and distributions via size-based separation methods employing mixed solvents remains one of the great macromolecular characterization challenges. This is due to the possibility for preferential solvation, whereby the region in the immediate vicinity of the polymer in solution becomes enriched in one of the solvents as compared to this solvent's fractional amount in the mix. In such cases, the chromatographic baselines of differential detectors such as light scattering photometers and differential refractometers no longer provide a quantitative reflection of the solvents' contribution to the heights of individual peak slices, thereby introducing error into molar mass calculations. The problem is addressed here through the use of a “nearly-isovirial” solvent pair. The second virial coefficient ( $A_2$ ) of both solvents being nearly equal for the polymers analyzed means that preferential solvation is obviated. The accuracy of this approach is shown via analysis by size-exclusion chromatography with on-line multi-angle static light scattering and differential refractometry detection (SEC/MALS/DRI), for a trio of narrow-dispersity polystyrene (PS) standards covering a nearly 40-fold range in  $M$ . In a mix of two nearly isovirial solvents, namely tetrahydrofuran (THF) and toluene, calculated molar mass averages and distributions are shown to be essentially identical across the range of solvent ratios. Polymer size is likewise shown to be constant with solvent ratio in this scenario. This is contrasted with the same polymers dissolved in a mix of the non-isovirial, non-isorefractive solvents THF and dimethylformamide (DMF). Results from this latter set of experiments show the large error in calculated  $M$  that results from preferential solvation, which can be as high as  $\approx 1 \times 10^5 \text{ g mol}^{-1}$  for an  $8 \times 10^5 \text{ g mol}^{-1}$  narrow-dispersity polystyrene. It is determined that, for a 25:75 THF:DMF mix, the solvent ratio in the immediate vicinity of the polymers examined “flips” to  $\approx 75:25$  THF:DMF due to preferential solvation.

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<https://doi.org/10.1016/j.aca.2022.340369>

Received 13 June 2022; Received in revised form 1 September 2022; Accepted 6 September 2022

Available online 25 September 2022

0003-2670/Published by Elsevier B.V.

## 1. Introduction

For macromolecules, molar mass ( $M$ ) is known to influence a variety of important processing and end-use properties, such as adhesion, elongation, tensile strength, etc. As such, accurate determination of the various  $M$  averages and the associated molar mass distribution (MMD) is essential in product and process development, in product failure assessment, and for ascertaining the accuracy and completeness of new synthetic routes. The preeminent technique by which to obtain this information is size-exclusion chromatography (SEC [1–3]), though other size-based methods have also been employed and are especially useful for polymers not amenable to analysis by SEC (these methods, which include hydrodynamic chromatography and flow field-flow fractionation [4–6], are henceforth implied when discussing SEC). A variety of approaches, involving various detector combinations, has been employed for molar mass determination by SEC. The approach generally considered most accurate is a combination of on-line static light scattering (SLS), in particular multi-angle static light scattering (MALS), with a concentration-sensitive detector, most commonly a differential refractometer (DRI) [7]. With SEC/MALS/DRI, one can determine “absolute,” calibrant-independent, molar masses. Polymer size can also be determined with this approach, provided the analytes display sufficient angular dissymmetry at the experimental conditions, *i.e.*, that the polymers scatter light in a sufficiently different way at one angle versus another [8].

Size-exclusion chromatography experiments are performed in neat solvents, or in solvents containing only a modest amount of added salt or some other modifier [9,10]; experiments are generally not conducted in mixed solvents. The reason for the latter is that, in mixed solvents, macromolecules can undergo preferential solvation. In preferential solvation, the solvent region in the immediate vicinity of the polymer is enriched in one solvent versus the other as compared to the solvent ratio outside this region [11,12]. By way of generic example, if a macromolecule is dissolved in a 50:50 mix of solvents A and B, in the region of the hydrodynamic volume in the vicinity of the polymer the solvent ratio may be 50:50, but it also could possibly be 80:20, 25:75, or any other non-50:50 ratio. SLS and DRI are both differential detectors (as are, it should be noted, on-line viscometers and ultraviolet/visible spectrophotometers, among others), where the solvent baseline is subtracted from each peak slice signal so that the contribution to scattering, refraction, etc. of the dissolved analyte may be determined after subtraction of the contribution from the solvent itself. Preferential solvation means that the solvent baseline no longer accurately represents the solvent contribution to each slice of a chromatographic peak. Classic experiments by Kratochvíl and colleagues demonstrated how preferential solvation led to the weight-average molar mass ( $M_w$ ) determined by off-line, batch-mode SLS being underestimated by as much as 44% in some cases and overestimated by as much as 23% in others [13,14].

In other, non-size-based macromolecular methods, such as many of those classified as interaction polymer chromatography [15], where enthalpic contributions generally dominate the separation, the use of mixed solvents is common. In these techniques, an evaporative mass detector is usually used. In the case of so-called evaporative light scattering detectors, solvents evaporate within the heated drift tube (evaporator) portion of the detector. Not only are these destructive detectors, but they also suffer from non-linearity of response, from allowing loss of oligomers along with solvent evaporation, and from analyst having an at-best empirical understanding of their operation and response. When detectors such as UV/visible or fluorescence spectrophotometers are used, being differential detectors their response in mixed solvents suffers from the same preferential solvation issue at play with MALS and DRI detection. Absolute molar masses are thus rarely reported in IPC, with calibrant-relative  $M$  usually being given, instead.

We recently introduced a method through which to obtain the accurate molar mass of macromolecules employing SEC/MALS/DRI in select mixed-solvent cases [16]. The particular cases involve the use of

an isorefractive solvent pair as the solvent mix, *i.e.*, the mix is composed of a pair of solvents with equal refractive indices as each other at the experimental conditions; equality was defined as a refractive index difference of no more than 0.001. Other requirements for this isorefractive approach to succeed include the solvents being miscible with one another in all proportions, the mix being a solvent for the analytes examined, the solutions possessing sufficient optical contrast with the solvent mix to generate chromatographic peaks with high and reproducible signal-to-noise ratios, and the solvent mix being a good chromatographic mobile phase that does not induce noticeable non-size-exclusion interactions between analytes and column packing material. This method works because, even if preferential solvation is present, its effects are nullified by the two solvents having the same refractive index: The specific refractive index increment ( $\partial n/\partial c$ ), a parameter vital to both the DRI and SLS responses and one which depends on solvent refractive index (among other experimental variables; see Ref. [17] for a more thorough discussion of this parameter), is unaffected by preferential solvation in the isorefractive case.

The isorefractive approach, while successful, is somewhat restrictive; it is difficult to find isorefractive solvent pairs which also meet the additional requirements described above. Another way to obviate preferential solvation in mixed solvents is to avoid it. This can be done by choosing solvent pairs wherein both solvents are equally “liked” by the macromolecule at the experimental conditions. If a macromolecule does not have a preference for one solvent over the other in this type of pair, then the solvent ratio in the immediate vicinity of the polymer should be the same as the ratio far away from the macromolecule. In such mixed solvent cases, the solvent baselines in SEC/MALS/DRI chromatograms will accurately represent the solvent contribution to each chromatographic peak slice.

How, then, to determine how much a macromolecule “likes” a particular solvent? One indicator is the thermodynamic quality (“goodness”) of a solvent, which is given quantitative meaning through the second virial coefficient  $A_2$  of a solution [18]. This coefficient measures the excess chemical potential, or excess Gibbs free energy of dilution, between macromolecule and solvent molecules in solution. In general, a value of  $A_2 > 0$  denotes a good solvent, a value close to zero denotes a poor solvent,  $A_2 < 0$  corresponds to a non-solvent, and  $A_2 = 0$  denotes the theta state of a solution (a thermodynamically pseudo-ideal state equivalent to the Boyle temperature for gases). At a given temperature, a macromolecule having very similar values of  $A_2$  in two different solvents should correspond to the polymer liking each of the two solvents almost identically. Work, especially chromatography, at poor and theta conditions is quite challenging, as one risks macromolecular precipitation from solution or unwanted enthalpic, non-size-exclusion interactions between analyte and column packing material. Consequently, if one seeks a pair of so-called “isovirial” solvents for the type of SEC/MALS/DRI experiment delineated here, it is best these be good solvents at the experimental temperature.

This paper details studies into performing SEC/MALS/DRI in a mix of two non-isorefractive, but “nearly isovirial” solvents. It investigates PS standards covering a 40-fold range in molar mass and augments the chromatography experiments with off-line MALS and DRI measurements. It also examines the consequences of performing SEC/MALS/DRI analysis in a mix of two non-isorefractive, non-isovirial solvents. We hope the work presented helps broaden the list of tools available for macromolecular separations, not only size- but also interaction-based, by providing an additional route to the determination of accurate molar masses in mixed solvents, and that the results help inform those investigating the thermodynamics of dilute polymer solutions [19].

## 2. Experimental

### 2.1. Materials

Narrow-dispersity linear polystyrene (PS) standards were purchased

from Agilent/Polymer Laboratories (Amherst, USA) and PSS Polymer Standards Service (Mainz, Germany). HPLC-grade tetrahydrofuran (THF), toluene, and dimethylformamide (DMF) were from Fisher Chemical (Fair Lawn, USA). As reported by the manufacturers, PS 18 K had a nominal  $M$  of  $1.8 \times 10^4 \text{ g mol}^{-1}$ , PS, PS 420 K a nominal  $M$  of  $4.20 \times 10^5 \text{ g mol}^{-1}$ , and PS 800 K a nominal  $M$  of  $8.00 \times 10^5 \text{ g mol}^{-1}$ . In all cases, the reported molar mass dispersity  $D \leq 1.02$ .

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.

## 2.2. SEC/MALS/DRI

Size-exclusion chromatography experiments were performed using an Agilent 1260 isocratic HPLC pump and autosampler (Agilent Technologies, Santa Clara, USA), the column oven of a Waters 2795 separations module (Waters Corp., Milford, USA), an on-line degasser connected to a PLgel Mixed-A SEC column packed with  $20 \mu\text{m}$ -diameter porous styrene/divinylbenzene (S/DVB) particles, followed by a DAWN HELEOS-II MALS detector (Wyatt Technology Corp., Santa Barbara, USA) and an Optilab T-rEX DRI (Wyatt). A  $0.2 \mu\text{m}$  Teflon filter (Whatman) was placed after the pump and before the injector. Flow rate was  $1 \text{ mL min}^{-1}$ ; injection volume was  $100 \mu\text{L}$ ; autosampler, column, and detector temperatures were  $25 \text{ }^\circ\text{C}$ . Experiments in 25:75 THF:DMF were also conducted at  $0.5 \text{ mL min}^{-1}$  and  $0.25 \text{ mL min}^{-1}$ . According to manufacturer specifications, the SEC column separates linear PS in THF at room temperature over the range of  $2 \times 10^3 \text{ g mol}^{-1}$  to  $4 \times 10^7 \text{ g mol}^{-1}$ . The MALS unit measured scattered light simultaneously at 15 different angles, ranging from  $28^\circ$  to  $147^\circ$ . The vacuum wavelength of operation ( $\lambda_0$ ) of the MALS detector is  $658 \text{ nm} \pm 4 \text{ nm}$ ; that of the DRI detector matches this value to within  $7 \text{ nm}$ . In all solvent ratios, nominal concentrations were  $10 \text{ mg mL}^{-1}$  for PS 18 K,  $2 \text{ mg mL}^{-1}$  for PS 420 K, and  $1 \text{ mg mL}^{-1}$  for PS 800 K; a higher concentration (but still well below the critical overlap concentration  $c^*$ ) was used for PS 18 K because this polystyrene was also used for normalization of MALS photodiodes as well as for determination of interdetector delay and interdetector band broadening. In each solvent, each sample was analyzed at least in triplicate. Dissolution was achieved through mixing by gentle inversion, allowing overnight for full dissolution and solvation. Column recoveries were greater than 93% in all cases. Data acquisition and processing was performed using Astra software (Wyatt, version 7.3.2.21). In all solvent ratios PS 18 K data were processed using the Debye formalism in Astra while PS 420 K and PS 800 K data were processed using a Berry formalism.

When switching from one solvent or solvent ratio to another, after finishing experiments in one solvent (solvent ratio is hereby implied; all solvent ratios are volume-to-volume or  $v/v$ ), system flow was slowly reduced to  $0 \text{ mL min}^{-1}$ , then the inlet reservoir was switched to the new solvent. The pump was purged of the old solvent according to manufacturer instructions then, with the DRI set on Purge, system flow was slowly increased to  $1 \text{ mL min}^{-1}$  in  $0.1 \text{ mL min}^{-1}$  increments. The entire system was purged at  $1 \text{ mL min}^{-1}$  overnight, usually for at least 18 h, *i.e.*, the system was purged in each case with at least 1 L of the new solvent (far more than necessary, but overnight purging was convenient; in most cases, purging with ten column volumes should suffice). The next day, first the absolute refractive index (aRI) was measured (see Section 2.3 below) to determine if it had achieved a steady value and to note this value, then the DRI was taken off Purge and its baseline was allowed to stabilize (because now flow was through both the sample and reference sides of the DRI cell) before injecting several solvent blanks followed by sample injections. Solvent blanks were also run at the end of each sample queue, to verify system performance and to note if any appreciable baseline drift had occurred during a sample queue; the latter was shown to not be the case.

## 2.3. Determination of absolute refractive index (aRI)

To measure the absolute refractive index (aRI) of a liquid using the same Optilab T-rEX refractometer employed in the SEC experiments described above, the liquid must be present in both the sample and reference chambers of the detector. The purge valve should be ON and both chambers should be carefully flushed with the liquid to be measured. The detector was maintained at a temperature of  $25.0 \text{ }^\circ\text{C} \pm 0.1 \text{ }^\circ\text{C}$ . With the purge valve ON, the aRI of the liquid in the flow cell is displayed in the “aRI” tab on the Optilab T-rEX front panel. After purging the refractometer with the solvent for at least 10 min at  $1 \text{ mL min}^{-1}$ , the absolute refractive index was measured. This was done for each solvent composition, about 30 min–60 min before starting the SEC experiments to allow for sufficient time for the DRI baseline to stabilize after turning off the detector flow cell Purge once each aRI measurement was completed. The detailed procedure for performing this measurement is described in Ref. [20]. The obtained aRI values are given in Table 1.

## 2.4. Off-line, batch-mode DRI and MALS: determination of $\partial n/\partial c$ and $A_2$ , respectively

The specific refractive index increment  $\partial n/\partial c$  and second virial coefficient  $A_2$  were determined for PS 420 K and PS 800 K, in each solvent or solvent mix, using in each case at least five dissolutions of each polymer, ranging from  $0.5 \text{ mg mL}^{-1}$  to  $5 \text{ mg mL}^{-1}$ . The off-line, batch-mode DRI and MALS experiments were performed in conjunction with each other, with both detectors coupled to each other off-line from the SEC system; the coupled-detector method is described in detail in Ref. [21]. Detector temperatures were maintained at  $25.0 \text{ }^\circ\text{C}$  with a precision of  $\pm 0.1 \text{ }^\circ\text{C}$  or better. Each sample dissolution was injected into the detectors using a Pump 11 Pico Plus Elite syringe pump (Harvard Apparatus, Holliston, USA) at a flow rate of  $0.1 \text{ mL min}^{-1}$ . Data acquisition and processing were performed using Astra software (Wyatt). For  $A_2$  calculations, the same light scattering formalisms as employed in on-line MALS were used respectively for each polymer. Off-line analysis of PS 420 K in 25:75 THF:DMF was not performed due to insufficient sample.  $\partial n/\partial c$  values in the various solvent ratios are given in Table 1.

## 3. Results and discussion

### 3.1. Choosing a nearly isovirial solvent pair

The first step in the search for near-isovirial solvents for PS at or near room temperature entailed sifting through the list of  $A_2$  values given in Ref. [22]. Once an appropriate solvent pair was found, it was important to ensure that: (1) Both individually and in all proportions these solvents dissolved PS; (2) both solvents possessed sufficient optical contrast with the polymer to generate SEC peaks with high signal-to-noise ratios (S/N); if the individual solvents met this criterion, then so also would the mixes; (3) the neat solvents or mixes did not induce noticeable

**Table 1**

Absolute refractive index (aRI) of various solvent compositions and specific refractive index increment ( $\partial n/\partial c$ ) of polystyrene at these compositions.

Solvent	aRI	$\partial n/\partial c$
100% THF	1.4028	$0.194 \pm 0.002$
50:50 THF:Toluene	1.4495	$0.149 \pm 0.003$
25:75 THF:Toluene	1.4701	$0.134 \pm 0.002$
100% Toluene	1.4873	$0.116 \pm 0.006$
25:75 THF:DMF	1.4224	$0.168 \pm 0.001$

Uncertainties in aRI are  $\leq 0.0001$  in all cases.  $\partial n/\partial c$  values are in  $\text{mg mL}^{-1}$  and associated uncertainties correspond to instrumental standard deviations. Temperature:  $25.0 \text{ }^\circ\text{C} \pm 0.1 \text{ }^\circ\text{C}$ ;  $\lambda_0 = 658 \text{ nm}$ . For  $\partial n/\partial c$ , at least five different sample dissolutions were measured, across a concentration range of approximately  $0.5 \text{ mg mL}^{-1}$  to  $5 \text{ mg mL}^{-1}$ ; see Sections 2.3 and 2.4 for details.

**Table 2**

Molar mass averages of PS 18 K in various solvent ratios, as determined by SEC/MALS/DRI.

	$M_n$	$M_w$	$M_z$
100% THF	18000	18000	18000
50:50 THF:Toluene	17900	17900	17900
25:75 THF: Toluene	17600	17600	17600
100% Toluene	17600	17600	17600
25:75 THF:DMF	19800 ± 600	19900 ± 700	20500 ± 600

Molar mass averages in  $\text{g mol}^{-1}$ . Averages and standard deviations based on at least triplicate analyses; where no standard deviation is given, this corresponds to a standard uncertainty of less than  $\pm 100 \text{ g mol}^{-1}$ . See Section 2 for details.

non-size-exclusion interactions between PS and the column packing material; and (4) the solvents were not isorefractive with each other at the experimental conditions. The last point ensured that we were studying a different case than that described in Ref. [16]. It should be noted that the  $A_2$  values obtained from the literature were merely used as an initial guide. Actual  $A_2$  values at our experimental conditions were measured in our laboratory by off-line, batch-mode MALS, as described in Section 2.4.

Based on the above considerations, THF and toluene were chosen as solvents. For linear PS, their literature values for  $A_2$  at 25 °C are quite similar; they are both well-known to be good solvents for PS, as denoted by their  $A_2$  and Mark-Houwink exponent values, and good chromatographic mobile phases when employing SEC columns packed with S/DVB particles [1]; and their refractive indices, while quite different from each other (see Table 1), can still provide for high detector S/N due to their large difference with respect to the refractive index of PS [23]. Quantitative “fine-tuning” of  $A_2$  by changing the experimental temperature, while possible, is highly impractical given the large amount of sample needed for any single  $A_2$  determination at any one particular temperature via off-line, batch-mode MALS. Solubility tests employing a PS with nominal  $M$  of  $2 \times 10^7 \text{ g mol}^{-1}$  showed that the solvents readily dissolved PS in the proportions shown in Table 1. For analysis, we chose three narrow-dispersity linear polystyrenes covering a 40-fold range in molar mass, from  $1.8 \times 10^4 \text{ g mol}^{-1}$  to  $8 \times 10^5 \text{ g mol}^{-1}$ .

### 3.2. Absolute molar mass averages and distributions, and radii of gyration

As can be seen from Table 3 through 4, the  $A_2$  values of PS 420 K and PS 800 K in neat THF and toluene, while not identical, are quite similar to each other, confirming that these solvents are near-isovirial for PS at the experimental temperature. In both cases, the  $A_2$  in toluene is slightly lower than that in THF; this reflects, for both polymers, in a slight

**Table 3**

Molar mass averages, radii of gyration, and second virial coefficients of PS 420 K in various solvent ratios, as determined by SEC/MALS/DRI.

	$M_n$	$M_w$	$M_z$	$R_{G,z}$	$A_2 (\times 10^{-4})$
100% THF	414000	416000	419000	28	4.11 ± 0.25
50:50 THF: Toluene	417000 ± 1000	418000 ± 1000	420000 ± 1000	27	3.86 ± 0.62
25:75 THF: Toluene	413000 ± 1000	414000 ± 1000	415000 ± 1000	26	3.55 ± 0.56
100% Toluene	412000 ± 1000	413000 ± 1000	414000	27	3.17 ± 0.08
25:75 THF: DMF	461000 ± 4000	462000 ± 3000	464000 ± 3000	24	nd

Molar mass averages in  $\text{g mol}^{-1}$ ,  $R_{G,z}$  in nm,  $A_2$  in  $\text{cm}^3 \text{ mol g}^{-2}$ . Averages and standard deviations based on at least triplicate analyses; where no standard deviation is given, this corresponds to a standard uncertainty of less than  $\pm 1000 \text{ g mol}^{-1}$  in molar mass and less than  $\pm 1 \text{ nm}$  in radius. For  $A_2$ , precision represents instrumental standard deviation. nd: Not determined. See Section 2 for details.

**Table 4**

Molar mass averages, radii of gyration, and second virial coefficients of PS 800 K in various solvent ratios, as determined by SEC/MALS/DRI.

	$M_n$	$M_w$	$M_z$	$R_{G,z}$	$A_2 (\times 10^{-4})$
100% THF	791000 ± 6000	800000 ± 4000	805000 ± 3000	40	3.19 ± 0.14
50:50 THF: Toluene	802000 ± 2000	805000 ± 2000	807000 ± 2000	40	2.96 ± 0.03
25:75 THF: Toluene	795000 ± 2000	796000 ± 2000	798000 ± 2000	39	2.77 ± 0.01
100% Toluene	800000 ± 4000	802000 ± 3000	803000 ± 3000	39	2.51 ± 0.06
25:75 THF: DMF	878000 ± 4000	890000 ± 5000	902000 ± 9000	36	1.87 ± 0.01

Molar mass averages in  $\text{g mol}^{-1}$ ,  $R_{G,z}$  in nm,  $A_2$  in  $\text{cm}^3 \text{ mol g}^{-2}$ . Averages and standard deviations based on at least triplicate analyses; where no standard deviation is given, this corresponds to a standard uncertainty of better than  $\pm 1 \text{ nm}$  in  $R_{G,z}$ . For  $A_2$ , precision represents instrumental standard deviation. See Section 2 for details.

diminution of  $A_2$  of as a function of increasing toluene content in the mixed solvent cases [24].

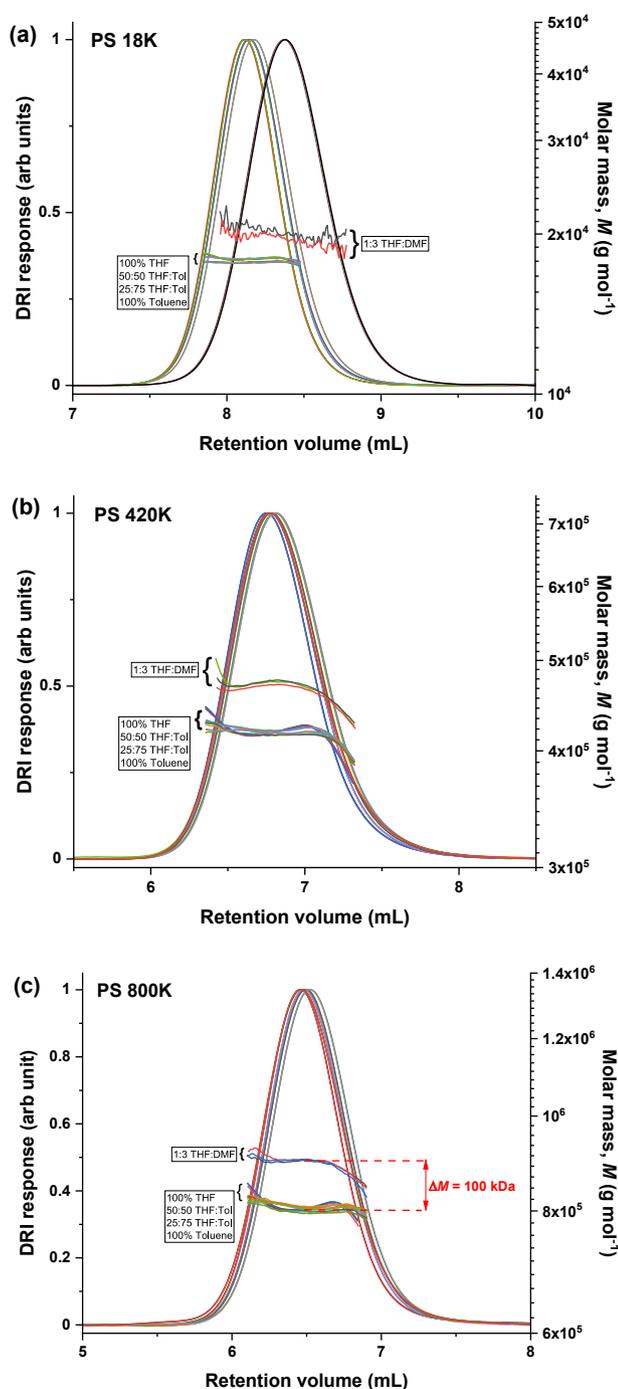
Results for the three PS samples examined are given in Table 2 through 4, which list the number-, weight, and z-average molar masses ( $M_n$ ,  $M_w$ , and  $M_z$ , respectively) of the polymers. For PS 420 K and PS 800 K, the z-average radius of gyration ( $R_{G,z}$ ) and second virial coefficient  $A_2$  are also given. For PS 18 K, no  $R_G$  results are reported due to a lack of sufficient angular dissymmetry by this sample at the experimental conditions (which is why this sample was employed for normalization of the MALS photodiodes; see Section 2.2). No  $A_2$  values are given for this sample because it was not subjected to off-line, batch-mode analyses (these analyses are quite sample-intensive and most of the PS 18 K had already been used to prepare the relatively high-concentration SEC samples of this polymer which, as explained earlier, was used for MALS normalization purposes as well as for determination of interdetector delay and band broadening parameters).

As can be seen in Table 2 through 4, the  $M_n$ ,  $M_w$ , and  $M_z$  of each of the PS standards examined are essentially invariant to the ratio of THF to toluene. This is also seen in Fig. 1a–c, which overlay the molar mass across the chromatograms of each sample. Note the chromatographic data and molar mass results in these figures do not constitute averages or “best picks” among the experiments; rather, every single run of each sample has been plotted in the overlays, along with every single calculated  $M$ . For each of the three samples, the minuscule spread among the  $M$  versus retention time relations as a function of THF:toluene ratio is remarkable. That the calculated  $M$  is invariant to solvent ratio for these two solvents can also be seen in Fig. 2, which overlays the differential MMDs of each sample in each solvent or solvent mix.

That THF and toluene have nearly identical solvating power for PS is also seen, in Tables 3 and 4 for PS 420 K and PS 800 K, respectively, in the relatively constant size of each polymer, as given by  $R_{G,z}$ , as a function of toluene content in THF. This constancy in  $R_G$  is shown graphically in Fig. 3a and b, where it is observed that the  $R_G$  of PS 420 K all fall within  $\approx 1 \text{ nm}$  of each other, and those of PS 800 K within  $\approx 1.5 \text{ nm}$  of each other, across all THF:toluene ratios.

### 3.3. SEC/MALS/DRI in a mix of non-isovirial, non-isorefractive solvents

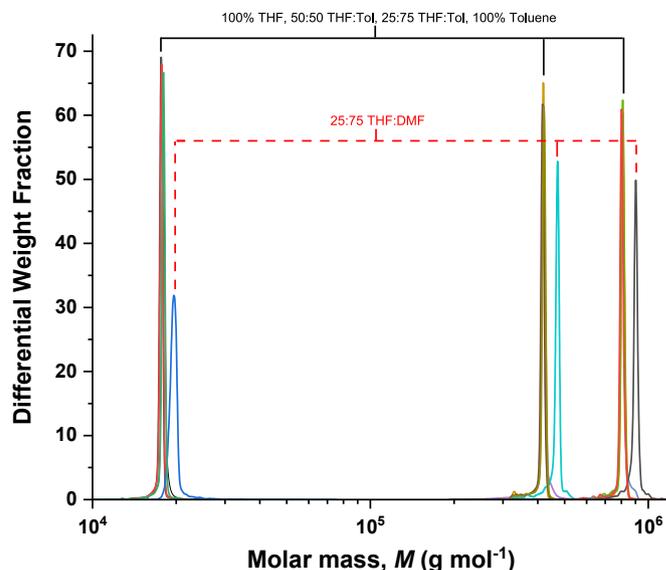
We have shown here that accurate molar mass averages and distributions can be obtained by SEC/MALS/DRI in a mix of non-isorefractive but near-isovirial solvents, regardless of solvent proportions. We have shown previously [16] that these quantities can also be accurately derived using the same technique employing a mix of isorefractive, non-isovirial solvents. The question naturally arises as to what happens if one uses a mix of non-isovirial, non-isorefractive solvents in SEC/MALS/DRI. To address this uncertainty, we examined the same three PS



**Fig. 1.** Molar mass, as determined by SEC/MALS/DRI, across DRI chromatograms, for all solvent compositions examined. (a) PS 18 K, (b) PS 420 K, (c) PS 800 K. Plots include every run of every sample in each solvent or solvent mix. See Section 2.2 for experimental details.

standards as above using a 25:75 ratio of THF:DMF at the same experimental conditions as the THF:toluene experiments. These solvents were chosen because they both dissolve PS [25], but their refractive indices are quite different from each other (an  $\approx 20$  times greater difference than in the isorefractive cases previously examined [26]), and the difference in their  $A_2$  values is substantially greater ( $\approx 40\text{--}50\%$  difference) than that between THF and toluene [22,27]. At the experimental temperature, DMF is a poorer solvent for PS than is THF (or toluene) [22].

The  $\partial n/\partial c$  and  $A_2$  of PS 800 K in 25:75 THF:DMF were determined by



**Fig. 2.** Differential molar mass distributions, determined by SEC/MALS/DRI, of PS 18 K, PS 420 K, and PS 800 K, for all solvent compositions examined. See Section 2.2 for experimental details.

off-line, batch-mode analysis as described in Section 2.4 (no off-line measurements were performed on the other polystyrenes due to insufficient sample); the absolute refractive index of the solvent mix was measured as per Section 2.3 and is given in Table 1, along with the  $\partial n/\partial c$ .

As can be seen in Table 2 through 4, the molar mass averages determined in THF:DMF are higher than those in neat THF, neat toluene, or the THF:toluene mixes. For PS 18 K, the difference is only a couple thousand  $\text{g mol}^{-1}$ . This difference increases to  $\approx 5 \times 10^4 \text{ g mol}^{-1}$  for PS 420 K and to nearly  $1 \times 10^5 \text{ g mol}^{-1}$  for PS 800 K, not an insignificant amount, especially in the case of narrow dispersity standards. As observed in Fig. 1a–c, for all three polymers there is a large difference between the molar masses across the chromatograms in 25:75 THF:DMF as compared to the THF:toluene cases. This difference is also observed in the respective MMDs, as seen in Fig. 2.

As noted in Table 1, we have determined the  $\partial n/\partial c$  of PS in neat THF and in the 25:75 THF:DMF mix. The  $\partial n/\partial c$  of PS in neat DMF, at the same temperature and similar wavelength (633 nm), is given in several different sources as  $0.16 \text{ mL g}^{-1}$  [28,29]. If, for each PS standard, we incrementally change its  $\partial n/\partial c$  in the Astra software, we find that a value of  $(0.185 \pm 0.001) \text{ mL g}^{-1}$  yields molar mass averages in agreement with those obtained in either neat THF or toluene. With this and the other  $\partial n/\partial c$  values, one can calculate an approximate solvent ratio within the hydrodynamic volume in the vicinity of the polymers, via:

$$0.185 \text{ mL g}^{-1} = (1-x)(0.194 \text{ mL g}^{-1}) + x(0.16 \text{ mL g}^{-1})$$

For this relation we find that  $x = 0.26$ . This means that, while in the solvent region away from the various PS in solution the solvent ratio is 25:75 THF:DMF, in the region in the immediate vicinity of the polymers the ratio is  $\approx 75:25$  THF:DMF! (It merits repeating that this is an approximate relationship, given the potential for non-linearity in  $\partial n/\partial c$  as a function of solvent ratio for solvents which present non-ideal mixing, etc.). This essential reversal of solvent ratios near versus away from the polymers in solution shows the preferential solvation that can occur in mixed solvents. The result was independent of flow rate, proving identical within experimental error at  $0.5 \text{ mL min}^{-1}$  and  $0.25 \text{ mL min}^{-1}$  as compared to  $1 \text{ mL min}^{-1}$ . The consequences of this phenomenon, as noted earlier, are seen in the last row of Table 2 through 4 and in Fig. 1a–c and Fig. 2.

The presence of DMF, even upon solvent ratio reversal, results in the mix being a poorer solvent at the experimental temperature than is neat

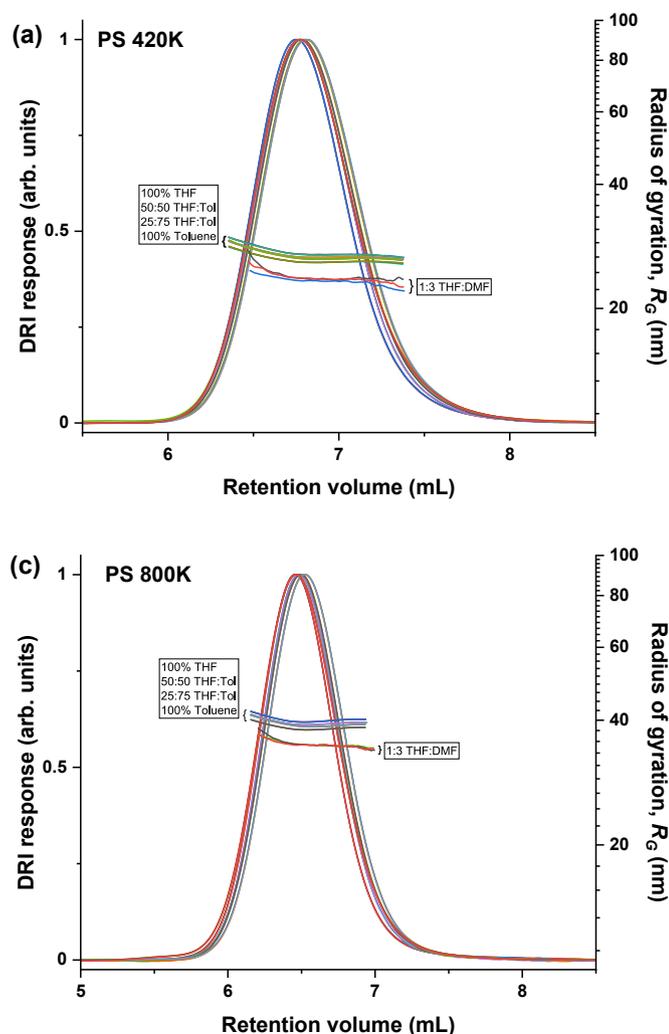


Fig. 3. Radius of gyration, as determined by SEC/MALS/DRI, across DRI chromatograms, for all solvent compositions examined. (a) PS 420 K, (b) PS 800 K. Plots include every run of every sample in each solvent or solvent mix. See Section 2.2 for experimental details.

THF, as given by the  $A_2$  value of PS 800 K in the mix (Table 4). This means that the polymers adopt a more compact conformation in THF:DMF than in THF alone, a fact reflected in the smaller  $R_G$  of PS 420 K and PS 800 K in the former versus the latter solvents (see Tables 3 and 4 and Fig. 3a and b).

#### 4. Conclusions

Demonstrated here is a novel approach by which to obtain accurate molar mass averages and distributions for macromolecules using SEC/MALS/DRI in mixed solvents. The method relies on a mix of near-isovirial solvents. This obviates the problem of preferential solvation by ensuring equality in the solvent ratios in the immediate vicinity and away from a polymer in dilute solution. These experiments complement our previous, but more restrictive, approach to the problem of obtaining accurate  $M$  information in mixed solvents, which employed a mix of isorefractive solvents [16].

THF and toluene solvent compositions were investigated because, at the experimental temperature, the  $A_2$  values of PS dissolved in the neat forms of these solvents are very similar.

Molar mass averages and distributions for several narrow-dispersity PS standards covering a nearly 40-fold range in  $M$  were shown to be statistically identical across a range of THF:toluene ratios and in

agreement with values determined in the neat solvents themselves. The virtually equal solvating power of both solvents was reflected in the invariance in polymer size, as given by the radius of gyration, as a function of solvent ratio.

Results for all three PS standards dissolved in a mix of non-isorefractive, non-isovirial solvents are likewise striking, for the opposite reason as above: Preferential solvation led to  $M$  values being off by anywhere from several thousand  $\text{g mol}^{-1}$  for the lowest- $M$  standard to nearly  $1 \times 10^5 \text{ g mol}^{-1}$  for PS 800 K. Calculations showed that, for a 25:75 mix of THF:DMF, preferential solvation led to the solvent mix in the immediate vicinity of the polymers being  $\approx 75:25$  THF:DMF, an essential reversal of the solvent ratio away from the macromolecules in solution.

The results presented here offer a new way of obtaining accurate molar masses in mixed solvents by SEC/MALS/DRI. The method is also applicable to other size-based techniques such as hydrodynamic chromatography and flow field-flow fractionation and should be of great interest to practitioners of the various interaction polymer chromatography methods, wherein mixed solvents and solvent gradients are regularly employed. The results should also serve to inform ongoing discussions regarding the thermodynamics of dilute polymer solutions.

#### CRediT authorship contribution statement

**André M. Striegel:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

All relevant data are included in the manuscript.

#### Acknowledgements

The author is most grateful to Prof. Paul S. Russo (Georgia Institute of Technology) for reviewing a draft of this manuscript and for his helpful comments.

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