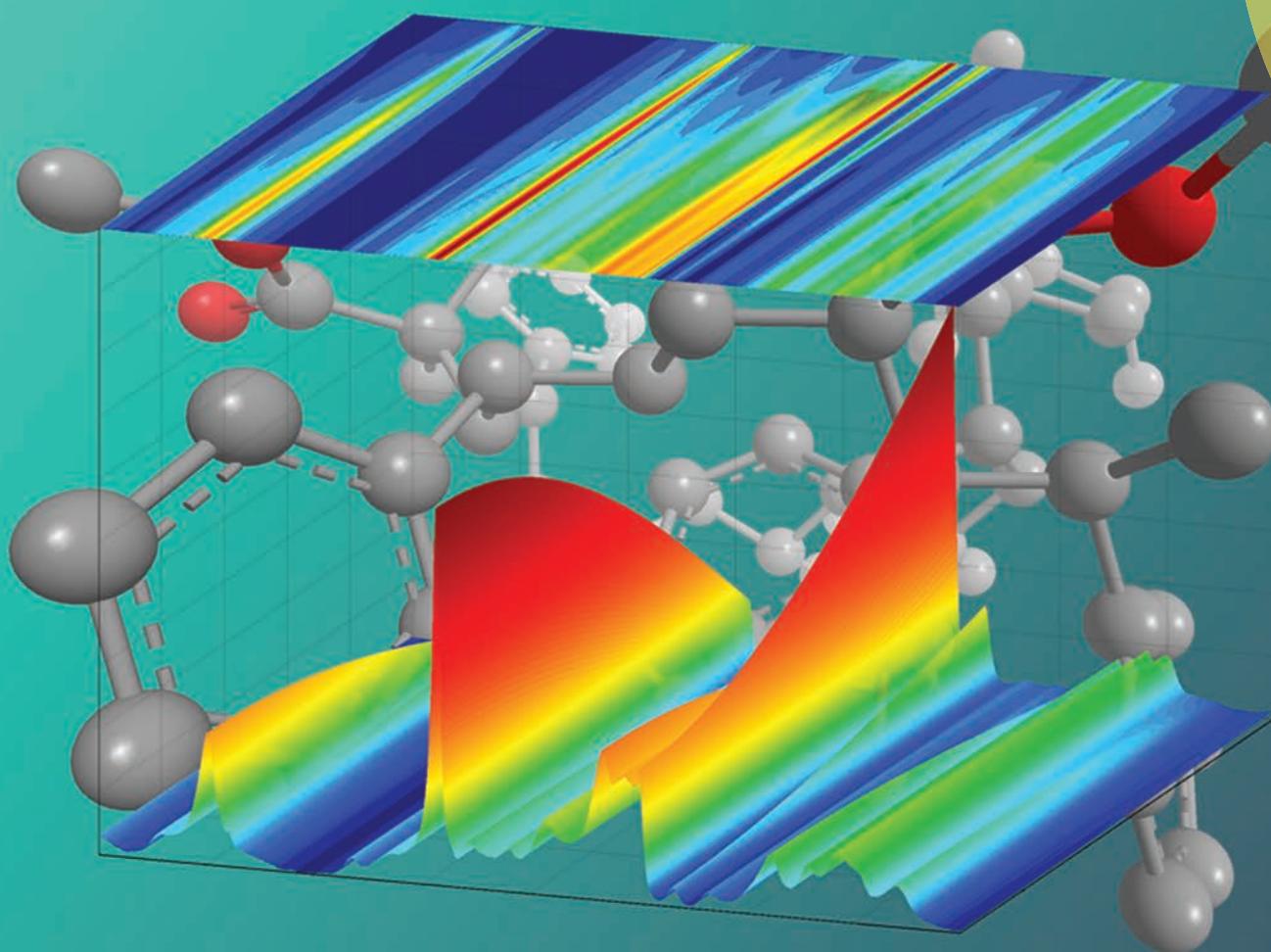


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On the feasibility of determining polymer chemical heterogeneity by SEC with continuous off-line Raman detection

Leena Pitkänen,^a Aaron A. Urbas^{*b} and André M. Striegel^{*a}

Examined here is the feasibility of employing Raman spectroscopy as a detection method in size-exclusion chromatography (SEC) and related macromolecular separations, for the purposes of determining the chemical heterogeneity of copolymers. To this effect, heart-cutting fractions from the SEC peak of a gradient random copolymer of styrene (S) and methyl methacrylate (MMA) were collected, at concentrations resembling those of eluting slices in an analytical SEC experiment, and subsequently analyzed by off-line Raman. The amount (weight or mole percent) of styrene in each fraction was quantitated by Raman by applying a calibration curve constructed through the analysis of well-characterized block, alternating, and random S-MMA copolymers. The weight percentages of S (w% S) obtained from SEC with off-line Raman analysis of the gradient copolymer were compared to results previously obtained using SEC with on-line multi-angle static light scattering, differential refractometry, and ultraviolet absorption detection. Not only did Raman rank the w% S of the fractions in the correct order but, on average, Raman results differed by 3% or less as compared to the values obtained *via* multi-detector SEC. The results from this study provide proof of principle of both the feasibility of continuous off-line Raman detection for macromolecular separations and of the possibility of employing for this purpose the same or similar hardware to that currently used for continuous off-line Fourier transform infrared detection.

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1. Introduction

Polymers are known to possess various macromolecular distributions and heterogeneities, the best known and most commonly determined of which is the molar mass distribution (MMD), as its breadth or narrowness is known to affect properties such as elongation, tensile strength, and adhesion.^{1–3} In the case of copolymers, processing and end-use properties such as toughness, brittleness, and biodegradability are also affected by chemical heterogeneity, defined as the average chemical composition of the copolymer as a function of the molar mass (*M*) of the latter.³ Chemical heterogeneity is usually measured through separation science, capitalizing upon the advantages of employing multiple detectors with different sensitivities in size-based one-dimensional (1D) sep-

arations, or upon separation orthogonality in two-dimensional liquid chromatography (2D-LC).^{4–15}

The power of macromolecular 2D-LC, as regards the present discussion, lies in its ability to determine the combined chemical composition and molar mass distributions of a copolymer, and a number of examples of the applicability of these methods can be found in the literature.^{4–6} For polyolefin characterization, in particular, 2D-LC with both composition-sensitive and quantitative detection has proved to be a powerful approach.¹⁶ Difficulties in implementing these 2D techniques stem from complications inherent to method development, especially in the enthalpically-controlled or thermodynamically pseudo-ideal dimension, from low and/or biased analyte recovery, and from the need for the comprehensive transfer of all fractions of oftentimes broadly distributed analytes from the first to the second dimension (in contrast to small-molecule 2D-LC, where interdimensional transfer is usually of a multiplicity of narrow peaks).¹⁴ For logistical reasons, the development of 2D techniques is usually preceded by that of the individual 1D methods.^{7,8}

In the case of size-based 1D separation methods such as size-exclusion chromatography (SEC),³ hydrodynamic chromatography (HDC),^{17,18} or flow field-flow fractionation,^{19–22} deter-

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mination of the chemical heterogeneity of copolymers has followed two approaches (neither of which, it should be noted, accounts for local polydispersity effects):^{23,24} One relies upon a multidetector approach that combines *M*-sensitive detection with dual concentration-sensitive detectors where, for the latter, the chemical selectivity and/or specificity of each detector differs with respect to each component of interest in the copolymer.^{25,26} In addition to the chemical heterogeneity of the copolymer, this approach provides the chemical-heterogeneity-corrected *M* averages, distribution, and solution conformation. The second approach relies on the combination of separation methods such as SEC, HDC, or field-flow fractionation, and detection methods such as nuclear magnetic resonance (NMR) spectroscopy, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, or Fourier-transform infrared spectroscopy (FT-IR) to determine the chemical heterogeneity.^{6,9,27} Molar mass averages and distributions obtained in the literature *via* the latter approach are generally both calibrant-relative and uncorrected for chemical heterogeneity.

Of the methods mentioned in the previous paragraph, the coupling of size-exclusion chromatography to FT-IR is probably the most common, with the detector being used in either online (*i.e.*, employing a flow-through cell) or continuous off-line mode.^{28,29} One condition of the online approach is that the eluent should be transparent in the spectral range of interest. The potential for spectral interference from the solvent is eliminated in continuous off-line mode where, after the SEC separation, the eluate is directed through a heated nozzle for solvent evaporation and the subsequent deposition of sample onto a rotating germanium or zinc selenide disk. Sample spots on the disk are then analyzed by FT-IR off-line to obtain spectra for each eluted and deposited fraction. Because the rate of rotation of the disk is known, as is the chromatographic flow rate, each spot on the disk can be correlated to a particular peak, or to a particular position on a peak, in a given chromatogram. The continuous off-line approach has thus found broader applicability, and has been found to have greater sensitivity, than the online approach.^{4,10}

While SEC/FT-IR has been used for determination of chemical heterogeneity of copolymers for nearly 20 years,^{3,6,27,30} little interest has been paid to Raman spectroscopy as a macromolecular chromatography detection method. The exception for polymers is a single attempt, over two decades ago, by Edwards and coworkers to couple both conventional and FT-Raman online and off-line with SEC to determine the microstructure of polybutadienes dissolved in tetrahydrofuran.³¹ Even in this case, quantitative information could not be obtained because the instrumentation available at the time suffered from insufficient sensitivity and from a slow response time. To our knowledge, no further attempts to couple Raman spectroscopy with SEC or related size-based separation techniques have been reported in the macromolecular literature. (It should be noted that SEC has been successfully coupled to Raman, in both on- and off-line mode, for the

characterization of carbon nanotube and graphene flake suspensions, capitalizing upon the very large Raman scattering cross-section of these colloidal analytes).^{32–35} This appears to be a missed opportunity, given the complementarity of Raman and IR. Here, we attempt to address this oversight. In general, Raman is more sensitive to unsaturation and vibrations associated with the molecular backbone. Raman spectra tend to be less crowded than IR spectra, which can facilitate interpretation in some applications. In addition, Raman is readily applicable at high temperatures and also has distinct advantages over IR when operating in water.

The purpose of the present proof-of-principle study is to evaluate the feasibility of coupling SEC (or related size-based separation methods) to Raman spectroscopy for the purposes of determining the chemical heterogeneity of copolymers, and to also evaluate the accuracy and precision of the determinations. As mentioned above, one of the main advantages of Raman over IR detection lies in the area of water-soluble polymers due to the almost complete transparency of water in the Raman, but not IR, range of the electromagnetic spectrum. However, to assess the accuracy of the method it is advantageous to initially study a copolymer with well-characterized chemical heterogeneity, and most of the latter are soluble only in organic solvents. Also, the type of study performed here is aimed at demonstrating the feasibility of SEC with continuous off-line Raman detection where, akin to SEC with continuous off-line FT-IR detection, the solvent in the eluate would be evaporated through a heated nozzle prior to analyte deposition onto an appropriate sample holder. If successful, not only would the suitability of Raman detection be demonstrated for characterizing chemical heterogeneity of copolymers, but also the potential dual-purpose suitability of continuous off-line FT-IR detection hardware for Raman detection purposes, *i.e.*, collecting a continuous chromatographic trace on Ge or ZnSe or related plates, for subsequent Raman analysis. (It should be noted that, contrary to the case of FT-IR detection, most Raman experiments of this sort would probably be performed in a 180° backscattering geometry, so that the chemical identity of the plate material with respect to transparency in the spectral region where the measurements are being performed is not a primary concern).

To address the above objectives, the present study employs a well-characterized styrene-methyl methacrylate gradient random copolymer, which allows us to compare its chemical heterogeneity as previously determined by SEC with on-line multi-angle static light scattering (MALS), differential refractometry (DRI), and ultraviolet spectroscopy (UV) detection to a heart-cutting approach to off-line Raman detection (*i.e.*, this comparison to results from a well-established technique allows us to establish the accuracy of the SEC-Raman method). In the latter, the fractional concentrations employed were chosen so as to resemble those normally found in a typical chromatographic slice. Both methods of determining chemical heterogeneity are contrasted to each other qualitatively and quantitatively. As shall be seen, the agreement between the two sets of results provides confidence in the ability to

perform continuous off-line Raman detection in size-based macromolecular separations, and also to employ commercial FT-IR eluate transfer hardware for this purpose.

2. Experimental

2.1 Materials and reagents

A gradient random copolymer of styrene (S) and methyl methacrylate (MMA), (poly(styrene-*co*-methyl methacrylate) or more briefly P(S-*co*-MMA)) was obtained from Polymer Source Inc. (Montreal, Canada). Polystyrene (PS) homopolymer (nominal M of 200 000 g mol⁻¹, $D = 1.06$) was from Pressure Chemical (Pittsburg, PA) and poly(methyl methacrylate) (PMMA) homopolymer (M_p 138 500 g mol⁻¹, $D = 1.06$) from Agilent/Polymer Laboratories (Amherst, MA). Two block copolymers, poly(styrene-*b*-methyl methacrylate)s, one having 25% (mol%) of styrene (S) and the other 75% S, and an alternating copolymer, poly(styrene-*alt*-methyl methacrylate), were also purchased from Polymer Source Inc. The chemical composition (*i.e.*, the amount of styrene and methyl methacrylate) of the block and alternating copolymers was verified by ¹H NMR spectroscopy. Unstabilized tetrahydrofuran (THF) was purchased from Burdick & Jackson (Muskegon, MI).

2.2 SEC with off-line Raman analysis

Size-exclusion chromatography instrumentation included an Agilent 1260 isocratic HPLC pump (Agilent Technologies, Santa Clara, CA), an autosampler (Agilent 1260), a DAWN-HELEOS-II MALS detector (Wyatt Technology Corp., Santa Barbara, CA), and a T-rEX DRI (Wyatt Technology Corp.). Three 7.5 mm × 300 mm Agilent/Polymer Laboratories PLgel 10 μm particle size Mixed-B columns connected in series were employed for separation of the gradient random copolymer. The MALS detector was normalized with a narrow M dispersity ($D \leq 1.06$) PS with nominal M of 30 000 g mol⁻¹. Interdetector delays and interdetector band broadening were determined with that same narrow dispersity sample. A sample concentration of 5 g L⁻¹ in unstabilized tetrahydrofuran was used, to ensure sufficient polymer amount in each collected fraction. Although 5 g L⁻¹ is somewhat higher than commonly used in SEC, the previously published intrinsic viscosity data for this same polymer indicate that it is at least three times below the critical overlap concentration of the most abundant species of the copolymer,²⁵ a precaution which is further augmented by the dilutory effect of the SEC separation itself. Additionally, molar mass averages, distributions, and chromatograms obtained at 5 g L⁻¹ in the present study were well within experimental error of the same data previously obtained at 1 g L⁻¹ (ref. 25). The injection volume was 100 μL. Copolymer fractions were collected manually from the outlet tubing of the last detector (DRI). The delay time from the DRI detector cell to the end of the tubing was calculated from the flowrate (1 mL min⁻¹), inner diameter of the tubing, and length of the tubing. The peak was divided in five fractions with approximately equal mass of copolymer in each. Based on division

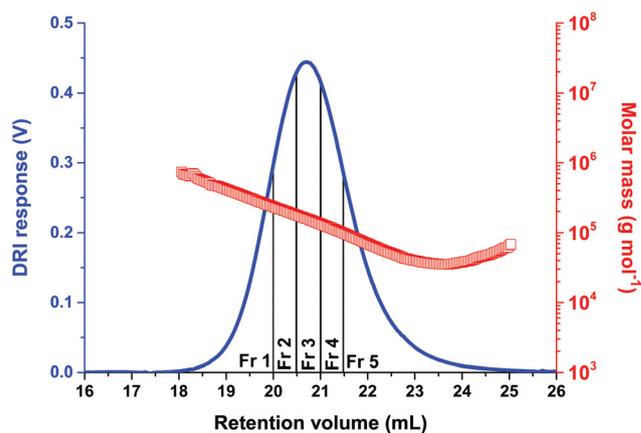


Fig. 1 Chromatogram (DRI response, in volts V, blue curve) and molar mass (open red squares) for styrene-methyl methacrylate gradient random copolymer. The sections of peak separated by black vertical lines represent the collected fractions Fr 1 to Fr 5 (see section 2.2 for detailed information about fraction collection).

into equal mass fractions, the collected fractions in terms of elution volume range were as follows: Fraction (Fr) 1, 17.5 mL to 20.0 mL; Fr 2, 20.0 mL to 20.5 mL; Fr 3, 20.5 mL to 21.0 mL; Fr 4, 21.0 mL to 21.5 mL; Fr 5, 21.5 mL to 25.0 mL (Fig. 1). Each fraction contained ≈ 100 μg of copolymer, though it is likely that as little as 20 μg would have sufficed, and probably even less than this had an efficient solvent-elimination interface been employed which could deposit analyte into smaller areas.

For Raman analysis, the solvent in each SEC fraction was allowed to evaporate completely and the copolymer residue was re-dissolved in 50 μL of fresh unstabilized tetrahydrofuran. Twenty μL aliquots of these solutions were deposited on cleaned aluminum foil substrates fixed on glass microscope slides. The copolymer was distributed throughout the dried deposits, which were 5 to 10 mm in diameter, but the sample was more concentrated in the periphery. Spectra were acquired from various locations within each deposit. It should be noted that, given the size of the sample deposits and the laser focal volume, a very small fraction of the deposited copolymer was actually measured in the Raman sampling. For the styrene and methyl methacrylate copolymer and homopolymer reference samples, solutions of approximately 1 g L⁻¹ were prepared and deposited in the same manner as were the SEC fractions. Raman spectra were acquired from the dried residues using a Renishaw S1000 micro-Raman spectrometer (Renishaw, Gloucestershire, UK) consisting of a Leica DMLM microscope coupled to a 250 mm focal length imaging spectrograph with a proprietary deep depletion, thermoelectrically cooled (-70 °C) charge-coupled device. For this work, a 532.2 nm continuous wave laser (Model 142, Lightwave Electronics, Mt. View, CA), holographically ruled 1800 grooves mm⁻¹ grating, and 50× objective were used. The excitation laser was focused to a line approximately 50 μm long at the sample position and aligned to the spectrograph entrance slit to maximize throughput. The

line focus was utilized to reduce laser power density and increase the amount of sample illuminated in a single acquisition. Laser power at the sample was typically 1 mW to 10 mW. Spectra were acquired using a continuous grating scan mode in the region 200 cm^{-1} to 3200 cm^{-1} with a 10 s per pixel integration time and consisted of 2332 data points. The spectral resolution was approximately 3 cm^{-1} ; this is a typical operating resolution on this instrument and was not specifically optimized for the present application. For each sample, spectra were acquired from 24 to 30 distinct locations on the dried deposits. Raman imaging was not performed; however, the motorized translation stage and Raman mapping capabilities in the spectrometer control software (WiRE 3.1, Renishaw, Gloucestershire, UK) were utilized to automate spectral acquisition within a sample. Varying degrees of background luminescence were observed among samples and pre-acquisition "photobleaching" exposures up to 30 s were used to mitigate the impact. All Raman spectra were corrected for relative intensity using SRM 2242.^{36,37}

2.3 Determination of specific refractive index increment ($\partial n/\partial c$)

The $\partial n/\partial c$ values were experimentally determined for linear homopolymer samples of PS (M_w of $200\,000\text{ g mol}^{-1}$) and PMMA (M_w of $138\,500\text{ g mol}^{-1}$). The $\partial n/\partial c$ of the bulk gradient copolymer was calculated based on the $\partial n/\partial c$ values of these homopolymers and the weight fractions (w) of the respective monomers in the copolymer as reported by the manufacturer, using (eqn (1)):^{25,38,39}

$$\left(\frac{\partial n}{\partial c}\right)_{\text{P(S-co-MMA)}} = \left(\frac{\partial n}{\partial c}\right)_{\text{PS}} w_{\text{S}} + \left(\frac{\partial n}{\partial c}\right)_{\text{PMMA}} w_{\text{MMA}} \quad (1)$$

The samples were dissolved in THF at concentrations varying from 1 g L^{-1} to 6 g L^{-1} and each dilution was injected directly into Optilab T-rEX DRI cell (vacuum wavelength of light $\lambda_0 = 658\text{ nm}$) using a Razel model A-99EJ syringe pump at a flow rate of 0.1 mL min^{-1} and a temperature of $25\text{ }^\circ\text{C}$. The samples and neat THF were filtered before measurement through $0.22\text{ }\mu\text{m}$ Teflon syringe filters (VWR). ASTRA software (Wyatt, version 6.1.1.17) was employed for data collection and processing. The differential refractive index data of PS and PMMA were plotted against concentration and the $\partial n/\partial c$ values were obtained from the slope of these plots. The $\partial n/\partial c$ of PS was $0.196 \pm 0.002\text{ mL g}^{-1}$ and that of PMMA $0.090 \pm 0.006\text{ mL g}^{-1}$. The $\partial n/\partial c$ of the gradient random copolymer containing 24.6 weight % of styrene was $0.116 \pm 0.006\text{ mL g}^{-1}$, as calculated with eqn (1).

3. Results and discussion

In this study, we collected SEC-separated fractions of a styrene-methyl methacrylate gradient random copolymer and determined the relative weight percentage of both monomers quantitatively for each collected fraction using Raman spectroscopy. Our results aim to demonstrate how the coupling of Raman

detection to an SEC (or related size-based) separation can be employed for quantitative determination of the chemical heterogeneity of copolymers. This study also intends to show the potential of Raman as a continuous off-line detection method for SEC and other macromolecular separation techniques, similarly to the way FT-IR is used. To demonstrate the accuracy of the present approach, we employed a copolymer the chemical heterogeneity of which had been previously determined employing SEC with MALS and two concentration-sensitive detectors, differential refractive index (DRI) and ultraviolet absorption spectroscopy (UV) at $\lambda_0 = 262\text{ nm}$.²⁵ Because styrene absorbs at this wavelength but methyl methacrylate does not, the ratio of the UV and DRI responses yields information on the chemical composition for each eluted slice, given that the DRI response is due to the concentration of both MMA and S while the UV response at the given wavelength is due only to the concentration of S.

It should be mentioned that it has not been unambiguously demonstrated that the particular multi-detector SEC method described above provides the most accurate determination of chemical heterogeneity in all cases of copolymers with one or more chromophoric groups. As a matter of fact, as shall be seen later the method's inability to determine the existence of tacticity heterogeneity in a copolymer may be partially responsible for the small determinate error observed when comparing multi-detector SEC to SEC-Raman in the present case. We also note here that initial attempts to analyze by SEC-Raman one of the very few well-characterized water-soluble gradient random copolymers, poly(acrylamide-co-N,N-dimethylacrylamide), met with failure due to the large salt concentration ($\approx 0.5\text{ mol L}^{-1}$) needed to maintain the copolymer in solution without aggregation or sorption onto the SEC columns.²⁶ When attempting to analyze the dried heart-cut SEC fractions by Raman, the copolymer was found to be too strongly diluted within the salt matrix for accurate characterization.

The SEC peak of the S-MMA gradient random copolymer was divided in five equal mass fractions, as shown in Fig. 1; each fraction contained $\approx 100\text{ }\mu\text{g}$ of polymer. From these SEC fractions, $40\text{ }\mu\text{g}$ were deposited on microscope slides for Raman analysis. The mass of copolymer in each fraction was sufficient for Raman detection, giving reason to believe that continuous off-line coupling should be possible when using concentrations typical in SEC analyses ($\leq 1\%$). In the upcoming sections, the quantitative determination of the average chemical composition of copolymer fractions with Raman is discussed and results are compared to ones obtained previously using SEC coupled online with MALS, DRI, and UV detection. Different types of styrene and methyl methacrylate copolymers (block, random, alternating) were used to construct the calibration model that was employed for quantitative determination of styrene content in the gradient random copolymer fractions separated by SEC. The bulk chemical compositions of these different types of copolymers were determined using ^1H NMR spectroscopy and the determined styrene content for each copolymer is collected in Table 1.⁴⁰

Table 1 Styrene content (as both mole and weight percentages), weight-average molar mass (M_w), and molar mass dispersity ($D \equiv M_w/M_n$) of copolymers used in this study

| Polymer samples | Styrene content (mol%) from manufacturer | Styrene content (wt%) from $^1\text{H NMR}$ | M_w^a (g mol^{-1}) | D^a |
|-------------------------------------|--|---|---------------------------------|-------|
| PS | 100 | 100 | 200 000 | 1.06 |
| PMMA | 0 | 0 | 265 000 ^b | <1.12 |
| P(S- <i>b</i> -MMA) | 25 | 25.2 | 45 900, 130 000 ^c | 1.16 |
| P(S- <i>b</i> -MMA) | 75 | 74.9 | 130 700, 46 000 ^c | 1.14 |
| P(S- <i>ran</i> -MMA) | 20 | 19.1 | 205 000 | 1.62 |
| P(S- <i>ran</i> -MMA) | 25 | 21.5 | 292 300 | 1.60 |
| P(S- <i>alt</i> -MMA) | 50 | 50.6 | 436 000 | 1.86 |
| P(S- <i>co</i> -MMA) ^{d,e} | 24.4 ^e | 24.8 | 200 000 | 1.43 |

^a From manufacturer. ^b Peak apex molar mass M_p . ^c Number-average molar mass of styrene block (first number), and methyl methacrylate block (second number). ^d Gradient random copolymer. ^e Average value. wt% S in this copolymer extends from $\approx 30\%$ at the low- M end of the MMD to $\approx 20\%$ at the high- M end (see Fig. 5 and also ref. 25).

3.1 Qualitative comparison of Raman spectra and peak assignments

Presented in Fig. 2 are the Raman spectra of the bulk gradient random copolymer and of PS and PMMA homopolymers, with band positions identified. Table 2 lists the indicated Raman band positions of the gradient random copolymer, with band assignments based on the respective homopolymer spectra when the origin was clearly apparent. Tables 3 and 4 list the indicated Raman band positions of the PS and PMMA homopolymers, respectively. In all cases, Raman bands are assigned according to ref. 41–43. For qualitative comparisons of the SEC fractions, the collection of Raman spectra from each fraction was averaged, baseline-corrected using a cubic spline fit to user defined baseline regions, and normalized by the carbonyl stretch of PMMA at 1730 cm^{-1} . The reference sample set of PS and PMMA homopolymers, as well as styrene/methyl methacrylate copolymers, comprised a range of monomer ratios and monomeric arrangements (block, random, and alternating) and the associated Raman spectra were assessed to identify the most useful spectral bands for quantifying the relative quantities of styrene and methyl methacrylate. A number of potentially suitable bands were either poorly resolved or did not correlate well with composition. This latter trend may be attributable to differences in monomeric arrangement.⁴⁴ The ring stretching modes of PS at 1583 cm^{-1} and 1604 cm^{-1} and the carbonyl stretch of PMMA at 1730 cm^{-1} were found to correlate strongly with copolymer composition and were well resolved from other bands (for the gradient random copolymer, the signal-to-noise ratio of the 1604 cm^{-1} band was 244 ± 94 and that of the 1730 cm^{-1} band was 97 ± 33 , as calculated from the individual spectra from all five fraction, *i.e.*, from a total of 144 spectra). A similar finding was reported when correlating styrene and methyl methacrylate copolymer composition to the relative absorbance of the phenyl and carbonyl

bands at the corresponding frequencies in infrared spectra.⁴⁵ That work also revealed that a number of other bands in the infrared were dependent to some extent on the neighboring monomer units in the sequence.

Shown in Fig. 3 are several spectral regions from the SEC fractions of the gradient copolymer where differences in contributions from styrene and methyl methacrylate are most apparent. In this figure, all spectra have been normalized by the 1730 cm^{-1} band area; however, each panel was scaled independently to facilitate comparison. The spectra of PS and PMMA homopolymers have been included to identify associated bands. The trend in all regions indicates that the styrene composition increases with decreasing fraction number. The first fraction exhibits some spectral features that are not observed in other fractions, but no impurities were identified in the solvent that could give rise to these bands. For the first fraction, the CH region (panel E) shows marked differences compared to the remaining fractions and several additional, but less significant, unique bands were observed in other regions of the spectrum including a small peak near 1670 cm^{-1} that is evident in panel D of Fig. 3. These distinct spectral features were evident in all spectra collected from this fraction.

3.2 Raman quantitation of chemical composition of gradient random copolymer fractions

To develop a calibration model for the weight percent of styrene (wt% S) in the copolymers (a model which, it should be noted, could as easily be developed for the mole percent of styrene), the areas of Raman bands associated with the phenyl ring stretching modes of styrene (integrated from 1560 cm^{-1} to 1640 cm^{-1}) and carbonyl stretch of methyl methacrylate (integrated from 1690 cm^{-1} to 1780 cm^{-1}) were determined after applying local linear baseline corrections. The two styrene bands were integrated together due to overlap. Band area ratios from the reference sample set, calculated from the mean spectra, were used to develop a calibration model according to equation (eqn (2)):

$$\%S = 100\% \times \frac{r}{c + r} \quad (2)$$

where r is the measured S/MMA band area ratio, and c is a coefficient representing the relative band intensity difference between the two monomeric species. This choice of model is inherent to the use of a band area ratio and the assumption that the band areas are directly proportional to the relative amounts of each monomer in a copolymer. The one-parameter model was fit to the band area ratio of the reference samples ($n = 6$) using nonlinear least-squares in Matlab and yielded a coefficient of $c = 4.64$ ($r^2 = 0.994$, RMSE = 1.66%). The reference percent composition values for this model were determined by $^1\text{H NMR}$ spectroscopy for all samples (Table 1).

The reference sample data, fitted model, and associated 95% confidence intervals are shown in Fig. 4. The largest absolute residual, 2.5%, corresponds to the 74.9% styrene block copolymer, while the remaining samples were all within

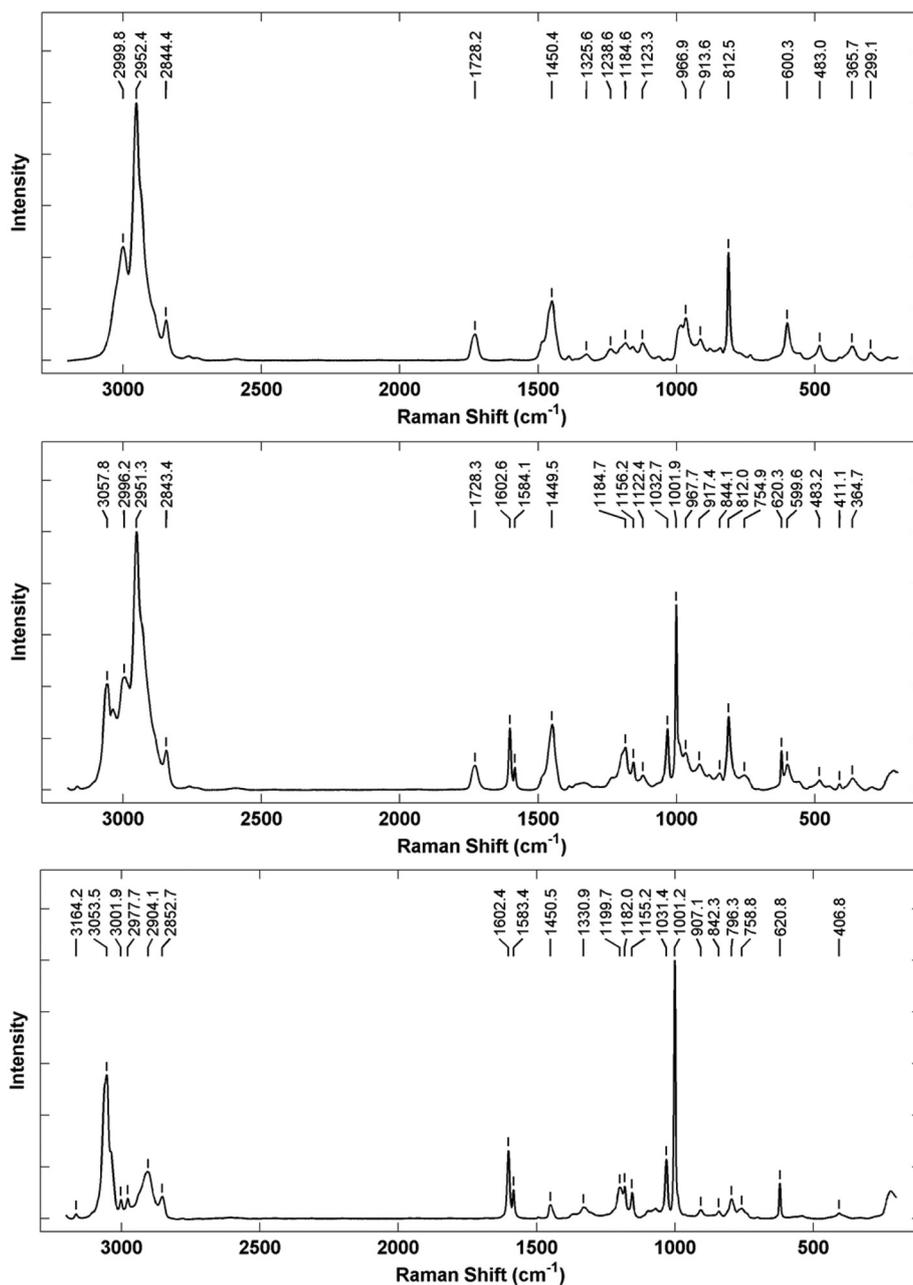


Fig. 2 Raman spectra (532 nm excitation) of PMMA (top), S-MMA gradient random copolymer (middle), and PS (bottom).

1.6%. The notable increase in scatter of the band area ratios for the 74.9% styrene sample is likely attributable to the impact of noise on the integration, rather than on sample heterogeneity (*i.e.*, the increased scatter is not due to compositional differences between locations in the dried deposit where Raman spectra were obtained), as the methyl methacrylate carbonyl band intensity decreases. Both the precision and the accuracy of the predictions based on this calibration model will be better for low styrene *versus* low methyl methacrylate content samples, owing to the significantly greater relative intensity ($c = 4.64$ from the fit to eqn (2)) of the styrene

ring stretching modes to the methyl methacrylate carbonyl band. This contrasts, as expected, with composition determination owing to the strong carbonyl and weak phenyl bands in the IR.⁴⁵ For the present investigation the resulting model was deemed suitable.

It should be noted that the use of blended PS and PMMA homopolymers, at various ratios, to generate reference data for composition prediction was investigated. While this approach was not thoroughly explored, it was observed that the dispersion in the Raman band area ratios from location to location in the dried deposits of the homopolymer blends was

Table 2 Raman band assignments for styrene-methyl methacrylate gradient random copolymer^{41,42}

| Raman shift (cm ⁻¹) | Origin | Band assignments ^a |
|---------------------------------|--------|---|
| 365 | PMMA | C–C skeletal def. of CC ₄ |
| 411 | | |
| 483 | PMMA | C–C skeletal def. of CC ₄ |
| 600 | PMMA | O–C=O def. |
| 620 | PS | In-plane ring def. |
| 755 | PS | Ring str. |
| 812 | PMMA | Symmetric CC ₄ str. |
| 844 | PMMA | C=O def. coupled to methyl rocking |
| 917 | | |
| 968 | PMMA | Main chain C–C str. |
| 1002 | PS | In-plane ring def. + out-of-plane CH def. |
| 1033 | PS | In-plane CH def. |
| 1122 | PMMA | C–O str. coupled to methyl rocking |
| 1156 | PS | In-plane CH def. |
| 1185 | | |
| 1450 | PMMA | CH ₂ def. |
| 1584 | PS | Ring str. |
| 1603 | PS | Ring str. |
| 1728 | PMMA | C=O str. |
| 2843 | PMMA | CH ₂ symmetric str. |
| 2951 | PMMA | CH ₃ symmetric str. |
| 2996 | PMMA | CH ₃ symmetric str. of O–CH ₃ |
| 3058 | PS | Aromatic CH str. |

^a def.: deformation; str.: stretching.

Table 3 Raman band assignments for polystyrene^{41–43}

| Raman shift (cm ⁻¹) | Band assignments ^a |
|---------------------------------|---|
| 406.8 | |
| 620.8 | In-plane ring def. |
| 758.8 | Ring str. |
| 796.3 | Out-of-plane CH bending |
| 842.3 | |
| 907.1 | Out-of-plane CH def. |
| 1001.2 | In-plane ring def. + out-of-plane CH def. |
| 1031.4 | In-plane CH def. |
| 1155.2 | In-plane CH def. |
| 1182.0 | In-plane CH def. |
| 1199.7 | In-plane CH def. |
| 1330.9 | CH ₂ wag |
| 1450.5 | CH ₂ bending |
| 1583.4 | Ring str. |
| 1602.4 | ring str. |
| 2852.7 | CH ₂ symmetric str. |
| 2904.1 | Tertiary CH str. |
| 2977.7 | |
| 3001.9 | Aromatic CH str. |
| 3053.5 | Aromatic CH str. |
| 3164.2 | |

^a def.: deformation; str.: stretching.

considerably greater than it was in the copolymer reference samples described above. This dispersion may be attributable to heterogeneous distribution on drying, especially given the immiscibility of PS and PMMA, but was not explored further. In addition, a regression model was investigated after exclud-

Table 4 Raman band assignments for poly(methyl methacrylate)^{41,42}

| Raman shift (cm ⁻¹) | Band assignments ^a |
|---------------------------------|---|
| 299.1 | C–C skeletal def. of CC ₄ |
| 365.7 | C–C skeletal def. of CC ₄ |
| 483.0 | C–C skeletal def. of CC ₄ |
| 600.3 | O–C=O def. |
| 812.5 | Symmetric CC ₄ str. |
| 913.6 | |
| 966.9 | Main chain C–C str. |
| 1123.3 | C–O str. coupled to methyl rocking |
| 1184.6 | C–C degenerate str. of CC ₄ |
| 1238.6 | C–C degenerate str. of CC ₄ |
| 1325.6 | |
| 1450.4 | CH ₂ def. |
| 1728.2 | C=O str. |
| 2844.4 | CH ₂ symmetric str. |
| 2952.4 | CH ₃ symmetric str. |
| 2999.8 | CH ₃ symmetric str. of O–CH ₃ |

^a def.: deformation; str.: stretching.

ing the 74.9% styrene sample and the resulting fit gave styrene content predictions that differed by $\approx 0.1\%$ compared to those presented below.

The spectra comprising the Raman data set for each SEC fraction were integrated individually, instead of taking the average, and the model described above was used to provide quantitative estimates of styrene content. Table 5 shows the number of spectra and mean and standard deviation (S.D.) of the predicted styrene content from the model for each fraction. Examination of the spectra suggests that the result for fraction 1 could be suspect, because some spectral contributions arising from the unknown impurity are partially interfering with both the S and MMA integration regions (see Fig. 3, panels C & D). The impact is difficult to quantify, but qualitative comparisons of the spectra in Fig. 3 suggest that the amount of S for fraction 1 could be lower than the predicted value reported in Table 5. These caveats are counterweighed, however, by the very reasonable agreement between the Raman- and multi-detector SEC-determined values of w% S for this fraction. As seen in Table 5, the Raman results for fraction 1 show only a 2.8% difference as compared to the SEC results averaged over the entirety of this fraction, a difference comparable to that found for the other four fractions examined.

3.3. Comparison of methods for determination of chemical heterogeneity in copolymers: SEC with off-line Raman versus SEC/MALS/UV/DRI

As already mentioned in the Introduction, the chemical composition of the same gradient random copolymer as used in this study was determined previously using a method in which SEC was coupled on-line to MALS, UV, and DRI detection (on-line viscometry detection augmented the experimental set-up of the previous study, to help shed light on how chemical heterogeneity can influence determination of the dilute solution conformation of copolymers).²⁵ Fig. 5 shows the styrene content as a function of molar mass determined both with

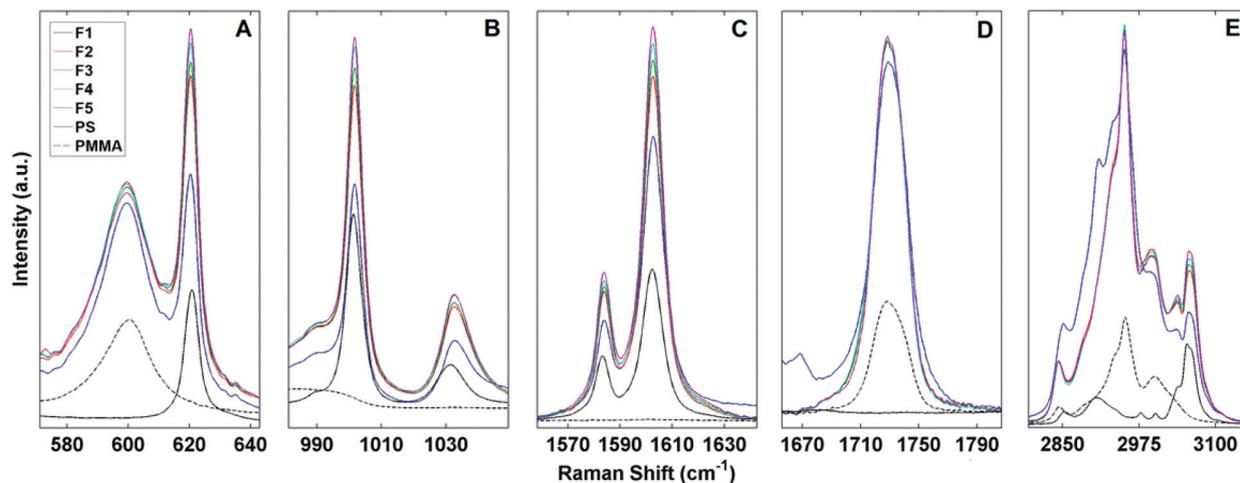


Fig. 3 Regions of the Raman spectra of the gradient copolymer SEC fractions where differences in copolymer composition are expected, all of which exhibit a consistent trend of increasing styrene content with decreasing fraction number (*i.e.*, styrene content decreases as molar mass increases). The SEC fraction spectra have been normalized by the area of carbonyl band of MMA at 1730 cm^{-1} (panel D). The spectra of PS and PMMA homopolymers have been overlaid for comparison purposes, to show the origin of the Raman bands in the copolymer. (See Tables 2–4 for individual band assignments and Fig. 2 for full spectra of the gradient random copolymer and of PS and PMMA homopolymers).

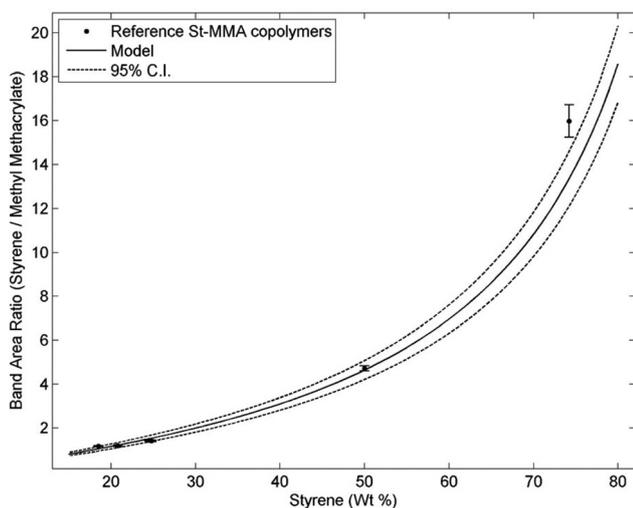


Fig. 4 Band area ratio (styrene ring stretching modes at 1583 cm^{-1} and 1604 cm^{-1} to the MMA carbonyl stretch at 1730 cm^{-1}) of the reference sample Raman data (filled circles) and the fitted model (solid line) of eqn (2) with associated 95% confidence intervals (95% C.I., dotted lines). Error bars for the reference sample data are drawn at ± 1 S.D.

SEC/MALS/UV/DRI and by off-line Raman for the collected SEC fractions discussed above. In the SEC/MALS/UV/DRI method, the styrene weight percentage was determined for every SEC elution slice i using following equation (eqn (3)):

$$(w\% S)_i = \frac{Z_i \left(\frac{\partial n}{\partial c} \right)_{\text{PMMA}}}{F \left(\frac{\partial n}{\partial c} \right)_{\text{PS}} - Z_i \left[\left(\frac{\partial n}{\partial c} \right)_{\text{PS}} - \left(\frac{\partial n}{\partial c} \right)_{\text{PMMA}} \right]} \times 100\% \quad (3)$$

where F is the ratio of the UV detector signal to that of the DRI detector for a PS homopolymer (assumed to be constant), and Z_i is the ratio of these signals (UV/DRI) for the gradient copolymer at each elution slice i . The derivation of the equation is given elsewhere.^{25,26,46} The placement of the Raman data on the molar mass axis in Fig. 5 was chosen to coincide with the weight-average molar mass of each of the five collected fractions. As can be seen from the figure and from Table 5, where SEC results are also provided as average w% S for each collected fraction and as the range in w% S for each fraction, the off-line Raman and multi-detector SEC results agree closely with each other, differing by at most 4% and, on average, by 3% or less. It should be noted that the bias in the results is always toward slightly lower %S values by Raman than by multi-detector SEC. Possible causes for this determinate error could be band broadening of the heart-cutting fractions occurring in the post-column tubing; uncertainties in the determination of interdetector delays and interdetector band broadening parameters in the multi-detector SEC experiments; uncertainties in the integration of NMR peaks (interpretation of the NMR spectra of the block copolymers is simpler than for the alternating copolymer which, in turn, is simpler than for the random copolymers; the same can also be said of the Raman spectra of the various types of copolymers); and, as recently demonstrated by Hiller and Hehn, potential tacticity heterogeneity within a given copolymer sample, not observable with the detectors employed in the present comparison.⁴⁷

The fact that the %S determined by off-line Raman analysis of the SEC-separated fractions closely agrees with the same datum as determined by an independent method, namely the online, continuous MALS/DRI/UV detection of SEC slices, provides strong support for, and confidence in, the use of Raman as a detection method in SEC and related techniques.

Table 5 Styrene content, as w% S, of gradient random copolymer as determined from Raman spectra of SEC dried deposits, and comparison to w% S by SEC/MALS/DRI/UV^a

| Fraction # | # Spectra | w% S (Mean \pm S.D.) | | | |
|------------|-----------|------------------------|------------------------------------|-------------------------------------|------------------------------------|
| | | Off-line Raman | SEC (<i>M</i> slice) ^b | SEC (fraction average) ^c | SEC (<i>M</i> range) ^d |
| 1 | 24 | 19.1 \pm 0.9 | 19.7 \pm 1.0 | 21.9 \pm 1.5 | 19.0 to 23.2 |
| 2 | 30 | 22.1 \pm 0.7 | 23.6 \pm 1.2 | 23.9 \pm 0.5 | 23.2 to 24.8 |
| 3 | 30 | 23.4 \pm 0.8 | 25.2 \pm 1.3 | 25.4 \pm 0.4 | 24.8 to 25.9 |
| 4 | 30 | 23.8 \pm 0.7 | 26.5 \pm 1.3 | 26.9 \pm 0.5 | 25.9 to 27.7 |
| 5 | 30 | 25.6 \pm 1.6 | 27.8 \pm 1.4 | 27.1 \pm 1.0 | 25.6 to 28.9 |

^a All SEC results are from SEC/MALS/DRI/UV analysis, as described in ref. 25. All SEC means and S.D.s are based on duplicate injections from two separate sample dissolutions. All Raman means and S.D.s are based on the number of spectra given in the “# Spectra” column, individually integrated as detailed in the text. ^b w% S, by SEC, for same *M* slice as Raman results in Fig. 4. ^c Average (with accompanying S.D.) w% S in each fraction, as determined by SEC. ^d Range of average (mean) w% S, by SEC, for each fraction analyzed by Raman.

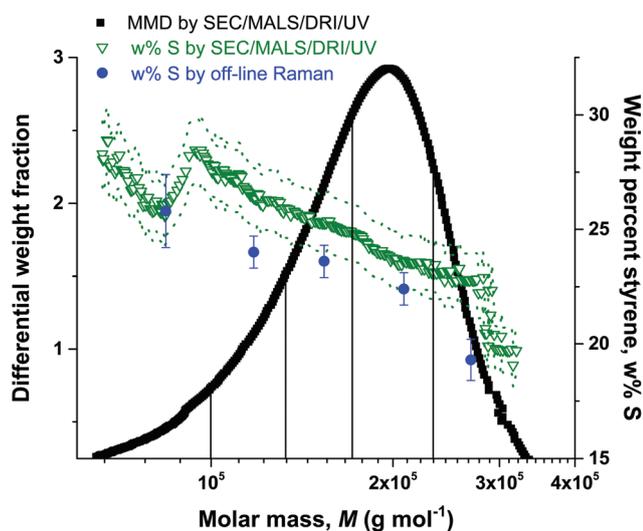


Fig. 5 Comparison of styrene content (as weight percent styrene, w% S) in gradient random copolymer P(S-co-MMA), as determined by Raman spectroscopy (filled blue circles) for each of five individual fractions (denoted by the fractions of the MMD separated by black vertical lines) and, continuously, by SEC/MALS/UV/DRI (open inverted green triangles) (SEC data adapted from ref. 25). For SEC data, green dotted lines correspond to 1 S.D. based on quadruplicate analyses, two injections each from two separate dissolutions (see ref. 25 for details). For Raman data, error bars correspond to 1 S.D.. Location of Raman data on the abscissa corresponds to weight-average *M* of each fraction. MMD determined by SEC/MALS/UV/DRI and corrected for chemical heterogeneity, as described in ref. 25.

Additional support is provided by the fact that the copolymer concentrations in the Raman-analyzed fractions were similar to the concentrations of an eluting species in an SEC experiment. This means that the type of equipment (or the same equipment!) employed for continuous off-line FT-IR detection in SEC and other macromolecular LC methods, wherein the solvent in the eluate is removed by evaporation and nebulization leaving a spot of analyte deposited on a Ge or ZnSe plate, should also be suitable for performing continuous off-line

Raman analysis. Raman spectroscopy enables the study of complementary vibrational information to IR. In addition, it is anticipated to have an advantage in the study of water-soluble polymers, where residual solvent can be problematic for IR detection due to spectral window masking by water solvent bands. As mentioned earlier, however, accurate evaluation of the potential of Raman detection when using aqueous solvents will necessitate well-characterized water-soluble gradient copolymers that do not require a high salt content to remain in solution and to prevent sorption onto chromatographic columns or field-flow fractionation membranes, or where efficient post-separation desalination can be effected.

4. Conclusions

The present study successfully demonstrates the feasibility of continuous off-line Raman spectroscopy as a detection method in SEC and related macromolecular separation techniques, in particular for determining the chemical heterogeneity of copolymers. This conclusion is based on our comparison of the chemical heterogeneity of a gradient random copolymer of styrene and methyl methacrylate, as previously determined continuously across the MMD of the copolymer utilizing SEC with on-line MALS, DRI, and UV detection, to the chemical heterogeneity of heart-cutting fractions of the SEC peak of this copolymer, determined by off-line Raman. We describe here the qualitative and quantitative interpretation of the Raman data. We also show that not only do the Raman results rank the weight (or mole) percent styrene of the fractions in the same order as does the SEC/MALS/DRI/UV method, but that both sets of results agree closely with each other, on average differing by less than 3%. Because the concentrations of the SEC peak fractions collected for Raman analysis in this study closely resemble the concentrations of continuously eluting SEC fractions, the present provides proof-of-principle that continuous off-line Raman detection can be performed similarly to continuous off-line FT-IR detection, the latter having been in use for several decades now, and using similar, and perhaps even the same, hardware for the continu-

ous collection of fractions onto a sample deposition plate for subsequent off-line spectroscopic analysis. This type of automated analysis should also provide an improvement over the present approach, not only in terms of the amount of data generated (continuous characterization of chemical heterogeneity across the chromatographic peak, as contrasted to the present heart-cut approach), but also *via* improvements in sample deposition focusing. Advantages of Raman over IR detection lie in the ability to access different absorption bands in the former as compared to the latter, and in the insensitivity of Raman to residual solvent in the analysis of water-soluble copolymers.

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