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Determining the Chemical-Heterogeneity-Corrected Molar Mass Averages and Distribution of Poly(styrene-*co*-*t*-butyl methacrylate) Using SEC/MALS/UV/DRI

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Abstract

Chemical heterogeneity, defined as the change (or lack thereof) across the molar mass distribution (MMD) in the monomeric ratio of a copolymer, can influence processing and end-use properties such as solubility, gas permeation, conductivity, and the energy of interfacial fracture. Given that each parent homopolymer of the copolymer monomeric components has a different specific refractive index increment (n/c) from the other component, chemical heterogeneity translates into n/c heterogeneity. The latter, in turn, affects the accuracy of the molar mass (M) averages and distributions of the copolymers in question. Here, employing size-exclusion chromatography coupled on-line to multi-angle static light scattering, ultraviolet absorption spectroscopy, and differential refractometry detection, the chemical heterogeneity (given as mass percent styrene) was determined for a poly(styrene-*co*-*t*-butyl methacrylate) copolymer. Also determined were the chemical-heterogeneity-corrected M averages and MMD of the copolymer. In the present case, the error in molar mass incurred by ignoring the effects of chemical heterogeneity in the M calculations is seen to reach as high as 53,000 g mol⁻¹ at the high end of the MMD. This error could be much higher, however, in copolymers with higher M or with larger difference among component n/c values, as compared to the current analyte.

Keywords

Chemical heterogeneity; Copolymer; Multi-detector size-exclusion chromatography; Specific refractive index increment; Molar mass; Poly(styrene-*co*-*t*-butyl methacrylate)

Introduction

tert-Butyl methacrylate (*t*-BMA) is copolymerized with styrene (St) to, among other things, impart functionality into styrenic blocks of the resultant copolymer [1]; to produce

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Compliance with Ethical Standards

Conflict of interest The authors have no potential conflicts of interest to declare. The research presented here did not involve human participants and/or animals.

copolymers that can act as macroinitiator precursors in controlled radical polymerizations [2]; and to produce polymers to be employed when studying the effect of copolymer sequence length heterogeneity on dilute solution conformation [3]. As is generally true with macromolecules, accurate determination of the molar mass of poly(St-*co*-*t*-BMA), including both the molar mass (M) averages and the molar mass distribution (MMD), is paramount for optimizing the processing and end use of these materials. These M parameters are most commonly obtained using size-exclusion chromatography (SEC [4–7] or, less commonly, other size-based separation methods such as hydrodynamic chromatography [7, 8] or flow field-flow fractionation [9–11]) coupled to a single, concentration-sensitive detector (most commonly a differential refractometer or DRI) and applying a calibration curve constructed from the chromatograms of commercially available narrow M dispersity standards. The M results obtained using this type of calibration curve approach will not be “absolute” but, rather, relative to the standards used, standards which themselves may have little or no chemical and/or architectural resemblance to the copolymer being analyzed.

In general, absolute results are obtained with SEC by employing, in conjunction with the concentration-sensitive detector, an on-line static light scattering (SLS) photometer. In lieu of the latter, an on-line viscometer can also be used, applying Benoit et al.’s concept of universal calibration [12]. The described methods of determining M , including the absolute ones, will, however, provide inaccurate results if the ratio of comonomers in the copolymer is non-constant across the MMD, such as in the case of gradient random copolymers [13] (thus, the term “chemical heterogeneity” refers to the relative amount of a particular monomer in a copolymer as a function of copolymer molar mass). The reason for this inaccuracy stems from the non-constancy in specific refractive index increment (n/c) as a function of M . Because details about the n/c and its determination have been elaborated upon recently in this journal, the reader is referred to Ref. [14] for a more thorough discussion thereof. For present purposes, it shall suffice to say that the response of the DRI (S_{DRI}) depends on n/c as per:

$$S_{\text{DRI}} = k_{\text{DRI}} \times c \times \frac{\partial n}{\partial c}, \quad (1)$$

where k_{DRI} is the instrument constant for the particular piece of hardware being employed and c is the concentration of analyte in solution.

If an on-line SLS photometer is being employed to determine M (and even if an off-line SLS is used to determine only the weight-average molar mass M_w), the n/c enters, additionally, via the K^* parameter of the Rayleigh–Gans–Debye equation [4, 5, 15]:

$$\frac{\Delta R(\theta)}{K^* c} = M_w P(\theta) [1 - 2A_2 c M_w P(\theta)], \quad (2)$$

where $R(\theta)$ is the excess Rayleigh scattering ratio (the amount of light scattered at a given angle θ by an analyte, polymeric or otherwise, solution in excess of that scattered by the

solvent), A_2 is the second virial coefficient of the solution, $P(\theta)$ is the particle scattering factor (otherwise known as the intramolecular interference function and related to the root-mean-square radius, or radius of gyration, of the analyte at the experimental conditions), and

$$K^* = \frac{4\pi^2 n_0^2 (\partial n / \partial c)^2}{\lambda_0^4 N_A}, \quad (3)$$

where, in turn, n_0 is the refractive index of the solvent at the experimental temperature and wavelength, λ_0 is the vacuum wavelength of the incident radiation, and N_A is Avogadro's number.

In a universal calibration experiment [4, 6], where the molar mass is obtained from a calibration curve of $\log \{M \times [\eta]\}$ versus retention volume, $[\eta]$ is the intrinsic viscosity and provides the gateway for n/c to affect the results via [4, 5, 16]:

$$[\eta] \equiv \lim_{c \rightarrow 0} \frac{\eta_{sp}}{c}, \quad (4)$$

where η_{sp} , the specific viscosity of the solution, is determined by the on-line viscometer and c is determined by the concentration-sensitive detector (again, most commonly a DRI).

Given the above discussion, it becomes clear how accurate determination of the n/c of each slice eluting from an SEC column (or from other size-based separation devices, which will henceforth remain implied when SEC is being discussed) is needed to ensure accurate determination of M averages and of the MMD, within the limits established by local polydispersity considerations during elution. Over the years, the corresponding author's group has devoted extensive resources to this problem, employing either quadruple- or quintuple- detector SEC with two concentration-sensitive detectors, DRI and UV/Vis, in the study of gradient copolymers in which either one or both component monomers absorb in the UV region of the electromagnetic spectrum [3, 17, 18]. Additional work in this regard has been elegantly performed by Hiller's group, also employing multi-detector SEC in the study of random copolymers [19] (it should be noted that Ref. [17] also discusses, more extensively than is done here, the effects of n/c heterogeneity upon the calculated M averages, MMD, and dilute solution conformation of copolymers).

In this Short Communication, we explore the application of the on-line coupling of multi-angle static light scattering (MALS), DRI, and UV detection to size-exclusion chromatography for determining the molar mass averages and distribution of a poly(styrene-*co*-*t*-butyl methacrylate) copolymer. Employing SEC/MALS/UV/DRI, we are able to determine absolute, chemical-heterogeneity-corrected values for the number-, weight-, and z -average molar masses (M_n , M_w , and M_z , respectively) and for the MMD of this copolymer, as well as determining the chemical heterogeneity itself.

Experimental

Materials

Narrow dispersity ($\mathcal{D} \leq 1.02$) linear polystyrene (PS) standard was obtained from Agilent/Polymer Laboratories (Amherst, MA, USA); poly(styrene-*co-t*-butyl methacrylate) copolymer, or P(St-*co-t*-BMA), was from Polymer Source (Dorval, Canada); unstabilized tetrahydrofuran (THF) was from EMD (Gibbstown, NJ, USA).

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.

SEC/MALS/UV/DRI Analysis

For the SEC/MALS/UV/DRI experiments, a 1 mg mL⁻¹ solution of copolymer in THF was prepared and left on a laboratory wrist-action shaker overnight to ensure dissolution. For increased precision, two different solutions, each of approximately the same concentration, were prepared and, from each dissolution, two injections were performed for a total of four injections. The SEC system consisted of a Waters 2695 Separations Module (Waters, Milford, MA, USA), three PLgel 10 μ m particle size Mixed-B SEC columns (Agilent/Polymer Laboratories), and three detectors connected in series, a DAWN EOS MALS photometer (Wyatt Technology Corp., Santa Barbara, CA, USA), followed by a Model 166 UV detector (Beckman-Coulter, Fullerton, CA, USA), followed by an Optilab rEX differential refractometer (Wyatt). The wavelength of the lamp of the UV detector was set to 260 nm, a wavelength at which styrene absorbs preferentially over *t*-butyl methacrylate (the latter has virtually negligible absorption at this wavelength). Flow rate was 1 mL min⁻¹; column, sample compartment, injector compartment, and detector temperatures were maintained at 25 °C.

A 31,400 g mol⁻¹ narrow dispersity ($\mathcal{D} \leq 1.02$) linear PS was employed for normalization of the MALS photodiodes, as well as for calculating interdetector delays and for interdetector band broadening correction [20]. Data acquisition was performed using Wyatt's ASTRA V software (V.5.3.2.13), plotting and calculations were performed with OriginPro (V.7.5885, OriginLab Corp., Northampton, MA, USA).

Specific Refractive Index Increment $\partial n / \partial c$ Determination

The specific refractive index increment, $\partial n / \partial c$, of the bulk copolymer (understanding that this value, as we shall soon see, changes as a function of M , due to chemical heterogeneity) and of two parent PS homopolymers (M_w of 49,000 and 133,000 g mol⁻¹ [14]) in THF at 25 °C, were (0.128 ± 0.002) mL g⁻¹ for poly(St-*co-t*-BMA) and (0.191 ± 0.002) mL g⁻¹ for PS (average value for the two PS homopolymers). The samples were dissolved in THF and left overnight on a wrist-action shaker to ensure full dissolution and solvation. For off-line $\partial n / \partial c$ determination, six dilutions of each sample, ranging from 0.5 to 5.0 mg mL⁻¹ for the copolymer, and from 1.0 to 6.0 mg mL⁻¹ for PS, were injected directly into the Optilab rEX differential refractometer (Wyatt) using a Razel model A-99EJ syringe pump at a flow rate

of 0.08 mL min⁻¹. Sample solutions were filtered gently through 0.45 µm Teflon syringe filters, neat THF for baseline determination through a 0.02 µm Teflon syringe filter. The wavelength of the refractometer is filtered to match, within ≈ 5 nm, the vacuum wavelength of the laser in the MALS photometer ($\lambda_0 = 685$ nm). Data acquisition and processing were done with Wyatt's ASTRA V software (V.5.3.2.13). Because of our inability to acquire poly(*t*-BMA), the $\partial n / \partial c$ of this homopolymer was calculated, using Eq. (6), from those of the bulk copolymer and PS homopolymer and using the *t*-BMA and styrene mass percentage compositions of the copolymer as determined by the manufacturer employing nuclear magnetic resonance spectroscopy. The $\partial n / \partial c$ value for poly(*t*-BMA) thus obtained was 0.0564 mL g⁻¹.

Results and Discussion

Combining the information obtained from, individually, the DRI and UV detectors for each SEC separation slice i , the mass percentage of styrene at each slice, (%St) _{i} , was calculated employing Eq. (5):

$$(\%St)_i = \frac{Z_i \left(\frac{\partial n}{\partial c} \right)_{P(t-BMA)}}{F \left(\frac{\partial n}{\partial c} \right)_{PS} - Z_i \left[\left(\frac{\partial n}{\partial c} \right)_{PS} - \left(\frac{\partial n}{\partial c} \right)_{P(t-BMA)} \right]} \times 100\%, \quad (5)$$

where $F = \frac{S_{UV,PS}}{S_{DRI,PS}} = \text{constant}$ and $Z_i = \frac{S_{UV,i}}{S_{DRI,i}}$. As such, F is the ratio of the signal from the UV detector to that of the DRI detector for the PS homopolymer ($S_{UV,PS}$ and $S_{DRI,PS}$, respectively); Z_i is the ratio of the UV and DRI signals for the copolymer at each elution slice i ($S_{UV,i}$ and $S_{DRI,i}$, respectively); and $(\partial n / \partial c)_{PS}$ and $(\partial n / \partial c)_{P(t-BMA)}$ are the specific refractive index increments of polystyrene and poly(*t*-butyl methacrylate), respectively. It should be noted that this equation, which is for a copolymer in which only one constituent monomer (namely, in the present case, styrene) absorbs in the UV, constitutes a special case of equation (2) in Ref. [18] (the derivation of which is given in the Supporting Information of Ref. [18]) for a copolymer in which both constituent monomers absorb in the UV. Also note that the chemical heterogeneity determined using Eq. (5) is uncorrected for local polydispersity effects in SEC, i.e., for the coelution of copolymer molecules which may differ from each other in %St, but which may, because of conformational effects resultant from sequence length heterogeneity [3], occupy identical hydrodynamic volumes in solution. This is not a great concern in the present case, however, as chemical local polydispersity effects are generally (though not always) considered to be substantially less than the architectural local polydispersity effects encountered when analyzing branched polymers.

The mass percentage of styrene across the MMD (i.e., the chemical heterogeneity of the copolymer), calculated as per the above, is shown as the inverted green triangles in Fig. 1 a, b. As seen in the figures, the %St originally drops rather quickly, from 63 to 50%, as M increases from 33,000 to 61,000 g mol⁻¹. The %St then continues to increase relatively slowly as a function of increasing M , reaching a value as high as 68% when $M = 610,000$ g

mol^{-1} . This behavior, the origin of which remains unexplained for this particular copolymer, is in contradistinction to that of, e.g., alternating copolymers, in which the monomeric ratio is constant across the MMD; or gradient random copolymers, in which the monomeric ratio changes gradually and unidirectionally across the MMD.

Once the relative mass percentages of each monomer have been calculated for each SEC elution slice [note that the mass percent of poly(*t*-BMA) at each slice, $\%P(t\text{-BMA})_i$ is simply $100\% - (\%St)_i$ with $(\%St)_i$ determined via Eq. (5)], the $\partial n / \partial c$ of each copolymer elution slice, $(\partial n / \partial c)_{P(\text{St-co-t-BMA}), i}$, is calculated using Eq. (6):

$$\left(\frac{\partial n}{\partial c}\right)_{P(\text{St-co-t-BMA}), i} = \left(\frac{\partial n}{\partial c}\right)_{PS} (\%St)_i + \left(\frac{\partial n}{\partial c}\right)_{P(t\text{-BMA})} (\%P(t\text{-BMA}))_i. \quad (6)$$

With this value, one can now calculate the n/c -corrected molar mass at each elution slice, $M_{\text{corrected}, i}$ from:

$$M_{\text{corrected}, i} = M_{\text{uncorrected}, i} \times \frac{\left(\frac{\partial n}{\partial c}\right)_{P(\text{St-co-t-BMA}), \text{bulk}}}{\left(\frac{\partial n}{\partial c}\right)_{P(\text{St-co-t-BMA}), i}}. \quad (7)$$

In Eq. (7), $M_{\text{uncorrected}, i}$ corresponds to the molar mass of each slice as determined by SEC employing the information from the MALS and DRI detectors and without correcting for $\partial n / \partial c$ differences among slices (i.e., it employs the $\partial n / \partial c$ value of the bulk copolymer, determined in “Specific Refractive Index Increment (n/c) Determination”); and $(\partial n / \partial c)_{P(\text{St-co-t-BMA}), \text{bulk}}$ corresponds to the $\partial n / \partial c$ of the bulk copolymer (a negligible contribution from the second virial coefficient A_2 to the calculation of M is assumed. This assumption appears quite reasonable given the low analyte concentrations employed, the additional dilutory effects of the chromatographic process itself, and the fact that, in Eq. (2), the term $2A_2cM_w \ll 1$).

Finally, to obtain the n/c -corrected M averages and MMD of the copolymer, corrected concentration values ($c_{\text{corrected}}$) need to be calculated for each slice. This is done by incorporating into Eq. (1), in slice-wise fashion, the n/c values calculated using Eq. (6). From here, the corrected molar mass averages are calculated as per:

$$M_{\beta, \text{corrected}} = \frac{\sum_i c_{\text{corrected}, i} M_{\text{corrected}, i}^x}{\sum_i c_{\text{corrected}, i} M_{\text{corrected}, i}^{x-1}}, \quad (8)$$

noting that when $x = 0$, $\beta = n$; when $x = 1$, $\beta = w$; and when $x = 2$, $\beta = z$.

Corrected and uncorrected values of the number-, weight-, and *z*-average molar masses (M_n , M_w , and M_z , respectively), and of the molar mass dispersity ($\mathcal{D} \equiv M_w / M_n$), are given in Table

1. As can be seen, there is substantial difference between the corrected and uncorrected values, from a difference of 10,000 g mol⁻¹ for M_n to 19,000 g mol⁻¹ for M_w , to 27,000 g mol⁻¹ for M_z . That the difference increases with increasing statistical moment is not a coincidence, because, as seen from Eq. (9), the magnitude of the error in molar mass, $M_{\text{error},i}$ increases as a function of molar mass (see last column of Table 1):

$$|M_{\text{error},i}| = |M_{\text{corrected},i} - M_{\text{uncorrected},i}| \quad (9)$$

$$= M_{\text{uncorrected},i} \times \left| \frac{\left(\frac{\partial n}{\partial c}\right)_{\text{PS(St-co-t-BMA), bulk}}}{\left(\frac{\partial n}{\partial c}\right)_{\text{PS(St-co-t-BMA), i}}} - 1 \right|.$$

Figure 1a, b contrasts the chemical-heterogeneity-corrected and uncorrected MMDs of the copolymer. In accordance with Eq. (9), the difference between these MMDs increases with increasing M . For ease of visualization of this effect, Fig. 1 a plots the MMD with the abscissa on a linear scale, while Fig. 1b shows the abscissa in the more traditional logarithmic scale. It should be noted that the molar mass at the highest end of the corrected and uncorrected MMDs differs from each other by 53,000 g mol⁻¹.

Conclusions

A method has been presented by which to experimentally determine the chemical heterogeneity in a styrene-*co*-*t*-butyl-methacrylate copolymer. The amount (mass percent) of styrene in the copolymer was initially observed to decrease by 13% over a relatively modest 30,000 g mol⁻¹ range, rising again by 18% over the remainder of the MMD (a broader range of $\approx$ 550,000 g mol⁻¹). Via SEC/MALS/UV/DRI, not only is it possible to perform these measurements, but also to determine the chemical-heterogeneity-corrected molar mass averages and distribution of this copolymer. How to obtain such information has been detailed herein, as well. As seen, the error in molar mass stemming from ignoring the effects of chemical heterogeneity in the M calculations can reach over 50,000 g mol⁻¹ at the highest end of the MMD of this copolymer. The magnitude of this error will increase for copolymers other than that studied here with either higher M , with larger difference among the n/c of the component monomers, or when both these factors combine. We hope that the method presented can be of assistance not only to those studying and working with P(St-*co*-*t*-BMA), but also to anyone interested in accurate molar mass data for copolymers in which one monomeric component absorbs in the UV, while the other does not. We also hope that the information presented provides a caveat to those working with, and analyzing, copolymers as regards the need to pay particular attention to the effects of chemical heterogeneity when determining copolymer molar mass and to the processing and end-use properties this affects, the latter of which include melting point, gas permeation, conductivity, interfacial fracture energy, and solubility.

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Fig. 1. Differential molar mass distribution of P(St-co-tBMA), corrected and uncorrected with respect to chemical heterogeneity. **a** With abscissa on a linear scale. **b** With abscissa on a logarithmic scale. In both cases, black filled squares correspond to uncorrected distribution, red open squares to corrected distribution, and green open inverted triangles to the mass percent of styrene

Table 1

Molar mass averages and dispersity of P(St-*co*-*t*-BuMA) copolymer, uncorrected and corrected for chemical heterogeneity

	Uncorrected	Corrected	% Change in M
M_n	$161,500 \pm 700$	$151,500 \pm 700$	6.60 ± 0.04
M_w	$224,500 \pm 1200$	$205,500 \pm 1100$	9.24 ± 0.07
M_z	$281,100 \pm 1800$	$254,000 \pm 1600$	10.7 ± 0.10
$\mathcal{D}$	1.39 ± 0.01	1.36 ± 0.01	–

All molar mass data in g mol^{-1} . Uncertainties based on quadruplicate injections, two each from two separate copolymer dissolutions, represent 1 standard deviation about the mean. See “Experimental” for details