Chromatographia (2017) 80:989–996
DOI 10.1007/s10337-017-3294-2

SHORT COMMUNICATION

Specific Refractive Index Increment ($\partial n/\partial c$) of Polymers at 660 nm and 690 nm

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Received: 13 January 2017 / Revised: 6 March 2017 / Accepted: 14 March 2017 / Published online: 23 March 2017

Abstract The specific refractive index increment ($\partial n/\partial c$) is an essential datum for the accurate quantitation of molar mass averages and distributions (inter alia) of macromolecules when refractometry, static light scattering, and/or viscometry detection are coupled on-line to size-based separation techniques. The latter include methods such as size-exclusion and hydrodynamic chromatography, and asymmetric and hollow-fiber flow field-flow fractionation. The $\partial n/\partial c$ is also needed for accurate determination of the weight-average molar mass of polymers by off-line, batch-mode multi-angle static light scattering. However, not only does $\partial n/\partial c$ differ among chemical species, it also depends on experimental conditions such as solvent, temperature, and wavelength. For the last 17 years, the author's laboratories have measured the $\partial n/\partial c$ of a variety of natural and synthetic polymers, at both 690 nm and, more recently, 660 nm, under a variety of solvent and temperature conditions. In all cases, this has been done by off-line, batch-mode differential refractometry, not by assuming 100% analyte column recovery and 100% accurate peak integration. Results of these determinations are presented here, along with the relevant experimental data.

Keywords Specific refractive index increment · $\partial n/\partial c$ · Polymers · Refractometry · Light scattering · Size-exclusion chromatography · Hydrodynamic chromatography · Field-flow fractionation

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Introduction

The most common, though not generally the most accurate, method for determining the molar mass (M) averages and distributions of macromolecules is size-exclusion chromatography (SEC) with a single, on-line concentration-sensitive detector, where the latter is usually a differential refractometer (DRI) [1–3]. More accurate determination of the M averages and of the molar mass distribution (MMD) is achieved by addition of an on-line viscometer (VISC) to this set-up, in the form of SEC/VISC/DRI and applying Benoit's classic concept of universal calibration [1–6] or by addition of an on-line static light scattering (SLS) detector, in the form of SEC/SLS/DRI, where the SLS detector may be of either the low-angle (LALS) or multi-angle (MALS) variety (see Sect. 9.3 of Ref. [3]). Yet another approach involves the use of on-line DRI, VISC, and right-angle (90°) static light scattering (RALS), where the combination SEC/RALS/VISC/DRI has traditionally been denoted as SEC^3 (see Sect. 9.6 of Ref. [3], and also [7]).

To ensure accuracy in the determination of M averages and of the MMD, each of the above experiments requires careful attention to a number of factors, most beyond the topic of this publication (detailed discussion of these factors can be found in chapters 8 and 9 of Ref. [3]). One requirement for accuracy in all the abovementioned SEC scenarios, however, is knowledge of the specific refractive index increment, or $\partial n/\partial c$, of the particular polymer being examined at the particular experimental conditions, the latter being solvent, temperature, and wavelength of the determination. The reason for this requirement is that the $\partial n/\partial c$, which can be thought of as the refractometry analog of the absorptivity in absorption spectroscopy, makes its presence felt in both the single- and multi-detector SEC experiments

described. In DRI, the relation between the refractive index of the solution, n, and the concentration of analyte in the solution, c, is given by (see Sect. 9.2 of Ref. [3]):

$$n \propto n_0 + (n_p - n_0)c, \tag{1}$$

where n_0 and n_p are the refractive indices of the solvent and the polymer, respectively. The signal from the DRI concentration-sensitive detector, S_{DRI}, is thus:

$$S_{DRI} = k_{DRI} \times c \times \frac{\partial n}{\partial c}, \tag{2}$$

where k_{DRI} is the instrument constant for the particular piece of hardware employed. Accurate integration of S_{DRI} thus requires knowledge of the instrument constant, which is independent of the analyte and the experimental conditions, and of $\partial n/\partial c$, which depends on both the latter factors. Given that differential refractometry is, as the name implies, a differential measurement, it measures the change in refractive index of an analyte solution, as compared to the refractive index of the solvent used to make the solution, in the limit of infinite dilution. This provides the definition of $\partial n/\partial c$ as:

$$\frac{\partial n}{\partial c} \equiv \lim_{c \to 0} \frac{n - n_0}{c} \tag{3}$$

Factors which affect refractive index will therefore also affect $\partial n/\partial c$. These include the chemical identity of the solvent employed to prepare a solution, the temperature at which the experiment is conducted, and the vacuum wavelength of the radiation source in the refractometer. As mentioned at the end of this section, it also depends on molar mass or, more accurately, on the degree of polymerization, in the oligomeric region of polymers. (While a universal definition of this region does not yet exist, for the purposes of the present discussion it may be regarded as the molar mass region in which $\partial n/\partial c$ changes, either increasing or decreasing, from one degree of polymerization to the next. See Sect. 13.2 of Ref. [3] for a more involved discussion of this topic.) It is because of this dependence of $\partial n/\partial c$ on a variety of experimental conditions, as well as because of differences in the accuracy of the various methods employed to determine $\partial n/\partial c$ (as detailed in the last paragraph of this section), and chiefly because all the necessary (for scientific reproducibility) experimental conditions are rarely reported along with the determination method, that few reliable literature sources exist for $\partial n/\partial c$ values and why values reported in the literature (see e.g., Ref. [8]) are often difficult, if not impossible, to reproduce.

In a universal calibration experiment, a calibration curve is constructed by plotting the logarithm of the product of the intrinsic viscosity, $[\eta]$, and molar mass of a set of standards versus the retention volume of these [1–4]. This calibration curve is then employed to determine "absolute", i.e., calibrant-independent M averages and MMDs of analytes. In this approach, which involves an SEC/VISC/DRI set-up, the $\partial n/\partial c$ now enters through the definition of the intrinsic viscosity:

$$[\eta] \equiv \lim_{c \to 0} \frac{\eta_{sp}}{c}, \tag{4}$$

where the specific viscosity, η_{sp}, is determined by the on-line viscometer and the concentration c by the on-line refractometer. As shown above, accurate determination of c by DRI requires accurate knowledge of $\partial n/\partial c$.

When on-line SLS detection is employed in the form of SEC/SLS/DRI, this detector measures the excess Rayleigh scattering ratio, $\Delta R(\theta)$, defined as the amount of light scattered by the analyte solution in excess of that scattered by the solvent at a given angle θ (see Sect. 9.3 of Ref. [3], and also [9]). In the limit of zero angle and near-infinite dilution, i.e., when $\theta = 0°$ and contributions from the second virial coefficient A_2 can be neglected (noting that the latter is a consequence of, but not the definition of, near-infinite dilution), the relation between $\Delta R(\theta)$ and the molar mass of the analyte, specifically the weight-average molar mass, M_w, is given by:

$$\frac{\Delta R(\theta)}{K^* c} = M_w \tag{5}$$

Here again, $\partial n/\partial c$ enters the picture, this time through both the determination of c using DRI detection and through the optical constant K^*, defined as:

$$K^* = \frac{4\pi^2 n_0^2 (\partial n / \partial c)^2}{\lambda_0^4 N_A}, \tag{6}$$

where λ_0 is the vacuum wavelength of the incident radiation and N_A is Avogadro's number (readers should note the importance of the SLS and DRI portions of the experiment being conducted at the same, or very similar, wavelengths as each other. Failure to do so will result in reduced accuracy in the determination of M_w by this dual-detector on-line method).

Lastly, in an SEC^3 experiment, $\partial n/\partial c$ enters through the response of both the DRI and the RALS detector, the latter being a type of SLS detector (see Sect. 9.6 of Ref. [3]).

It should be noted that all the above considerations for an SEC experiment apply equally to other types of size-based separations using the detector set-ups described. These separations include hydrodynamic chromatography (HDC [10, 11]) and flow field-flow fractionation, the latter as both asymmetric flow field-flow fractionation (AF4 [12, 13]) and hollow-fiber flow field-flow fractionation (HF5 [14]). Given the importance and ubiquity of $\partial n/\partial c$ in the determination of molar mass (and related parameters, such

as [η]) by SEC and related size-based techniques, its accurate determination is paramount.

In general, the determination of $\partial n/\partial c$ has proceeded amongst two different routes: the first, generally less accurate approach assumes that 100% of the injected analyte elutes from the column and also assumes that integration of the chromatogram (or fractogram, in the case of field-flow fractionation; henceforth, the inclusion of this technique will be assumed in the discussions) encompasses all, without exceeding, the contribution from the analyte. A second, more laborious, albeit generally more accurate route involves what is commonly referred to as an off-line, batch-mode DRI experiment (the on-line approach may offer certain advantages over its off-line counterpart when degassing of the solvent is needed, and/or when highly hydroscopic solvents are employed and exposure to ambient moisture causes random excursions in the DRI signal). Here, a series of solutions of different (carefully measured) concentrations of a given analyte in a given solvent are injected, usually in order of either increasing or decreasing concentration, directly into the DRI detector when the latter has been de-coupled from the separation system (i.e., the detector is now off-line). Solvent without any dissolved analyte is injected, as well, to obtain a baseline solvent value. This experiment yields a plot such as shown in Fig. 1a, where each "step" corresponds to the differential refractive index of a dilution of a different concentration of analyte. These measured differential refractive indices are then plotted (subsequent to solvent baseline subtraction) versus the concentration of each dilution, as shown in Fig. 1b and the points are fitted to a straight line (a non-weighted first-order fit, without forcing the fit line through the origin). The slope of this fitted line is the specific refractive index increment $\partial n/\partial c$ of the particular analyte at the solvent, temperature, and wavelength conditions of the experiment [3]. While $\partial n/\partial c$ has been found to change with molar mass in the oligomeric region, due to end group effects (see Sects. 9.2.1.3 and 13.3.2 of Ref. [3], and Ref. [15] for more detailed discussions of these effects), outside this region only minor variations are observed as a function of molar mass for any given polymer at a given set of experimental conditions.

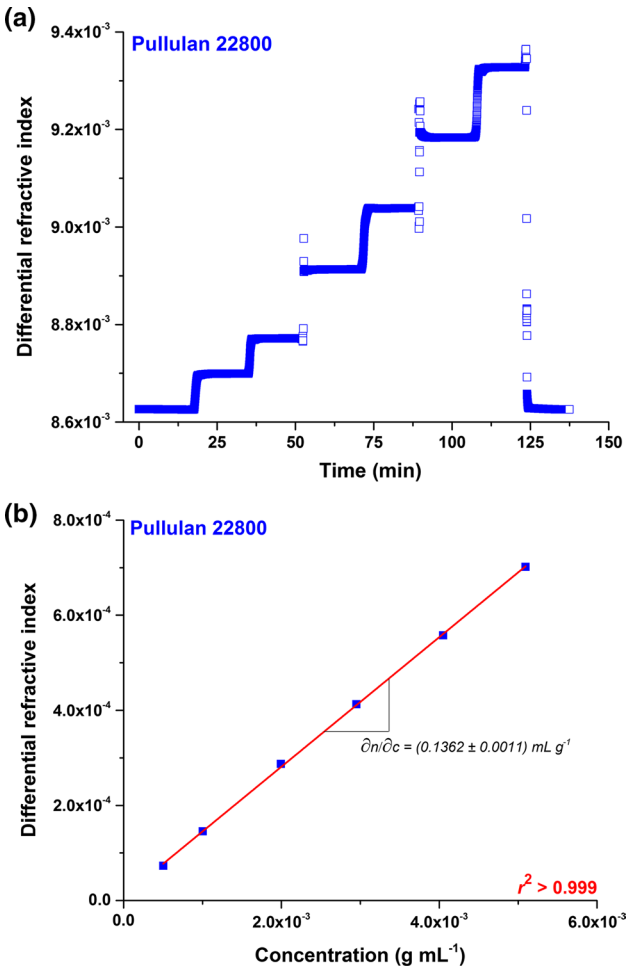

Fig. 1 Measuring the specific refractive index increment ($\partial n/\partial c$) of a polymer. Sample: pullulan with M_w = 22,800 g mol^{-1}; solvent: H_2O + 0.02% NaN_3; temperature: 25 °C; λ_0: 660 nm; flow rate: 0.1 mL min^{-1}; concentration range: 0.5–5 mg mL^{-1}. **a** Differential refractive index of solutions, before solvent baseline subtraction, of six dissolutions of increasing concentration, as a function of time. First and last plateaus correspond to solvent without added analyte. **b** Differential refractive index, after solvent baseline subtraction, of pullulan dissolutions as a function of analyte concentration in solution. The slope of the plot (*solid red line*), determined by a non-weighted first-order fit of the data without forcing through the origin, corresponds to the $\partial n/\partial c$ of pullulan at the solvent, temperature, and wavelength conditions of the experiment. The instrumental standard deviation of each data point is much smaller than the size of the data markers and is, therefore, not shown

Copolymers

The $\partial n/\partial c$ of copolymers is, in general, best determined as for homopolymers, using the off-line, batch-mode DRI method described above. However, the $\partial n/\partial c$ of copolymers (random, alternating, and block) can usually be calculated from the $\partial n/\partial c$ values of the component homopolymers along with the mass fraction of each homopolymer in the copolymer. For a generic AB copolymer, its $(\partial n/\partial c)_{AB}$ is calculated via the equation [8]:

$$\left(\frac{\partial n}{\partial c}\right)_{AB} = w_A\left(\frac{\partial n}{\partial c}\right)_A + w_B\left(\frac{\partial n}{\partial c}\right)_B, \quad (7)$$

where w_A and w_B are the mass fractions of the individual homopolymeric components A and B in the AB copolymer, and $(\partial n/\partial c)_A$ and $(\partial n/\partial c)_B$ are the specific refractive index increments of these same components at the same

experimental conditions at which the copolymer is being analyzed. The equation naturally extends to terpolymers, etc. It has been found to be particularly useful when attempting to accurately calculate, employing multi-detector SEC or a related size-based separation method, the M averages, MMD, and dilute solution conformation properties of copolymers where the monomeric ratio is non-constant across the MMD (e.g., as is the case for gradient random copolymers) [16, 17]. Additionally, the analysis of copolymers in which one of the monomeric components has $\partial n/\partial c = 0$ at the experimental conditions presents special advantages with respect to characterization of the remaining component, e.g., allowing measurement of the tracer diffusion coefficient of the "visible" component of the copolymer [18, 19].

Polyelectrolytes

While somewhat beyond the scope of this paper, a brief discussion regarding the determination of the $\partial n/\partial c$ of polyelectrolytes is included here for the sake of completeness. The most accurate way to measure the specific refractive index increment of this type of polymer is, again, through the off-line, batch-mode approach previously described. However, in the case of polyelectrolytes this procedure is complicated by the fact that the polymer solution needs to be dialysed against the solvent employed to make the solution, until a constant chemical potential (osmotic equilibrium) is obtained. After such potential has been reached, the liquid on the non-polymer side of the dialysis membrane is used as the solvent for baseline subtraction in the off-line, batch-mode experiment [20].

An alternative, oftentimes equally accurate approach employs the SEC column as a type of "dialysis chamber." Given the large number of theoretical plates over which a polyelectrolyte partitions during an SEC experiment, the $\partial n/\partial c$ values determined from the area of the DRI detector peak have often been found equivalent to those obtained by the more involved off-line, batch-mode dialysis approach [21–23]. It is very important to note, however, that for this equivalency to hold, the instrument constant for the differential refractometer [k_{DRI} in Eq. (2)] needs to be accurately known, 100% of the analyte must have eluted from the SEC column, and the DRI peak must be defined accurately with respect to the placement of both baseline and integration limits. If it is suspected that complete dialysis has not occurred during the timeframe of the SEC experiment, then the experiment should be repeated at increasingly lower flow rates, until the value obtained is flow-rate independent.

For the results presented here, the above considerations apply in cases such as polyacrylamide and poly(*N*,*N*-dimethyl acrylamide) dissolved in H_2O + NaCl + acetic acid (both in Table 1). For these, it was found that $\partial n/\partial c$ values determined by off-line, batch-mode DRI agreed closely with the same values as determined assuming 100% analyte recovery from the columns and 100% accurate peak integration. This indicates that, at the given experimental conditions, the polyelectrolytic behavior of these polymers was, at best, minimal [17].

Determining M_w by Off-line, Batch-Mode MALS

Off-line, batch-mode MALS experiments are performed in a manner virtually identical to their off-line, batch-mode DRI counterpart, save for employing a MALS detector instead of a DRI (see Sect. 9.3.3 of Ref. [3], and also Ref. [9]). In this type of MALS experiment, the scattering from several different polymer dissolutions, each of a different concentration and each injected individually into the light scattering photometer, is measured at a series of scattering angles (again, as in off-line, batch-mode DRI, in the case of each dissolution the concentration of analyte in solution must be measured carefully). The most common way of plotting the resulting data is in what is known as a "Zimm plot", where the ordinate is $K^*c/\Delta R(\theta)$ and the abscissa is $\sin^2(\theta/2) + kc$, with k being a number, with dimensions of reciprocal concentration (i.e., volume mass^{-1}), chosen to give a good visual distribution of data points on the plot, while the other symbols retain their same meaning as above (resultant data may also be plotted in formats other than a Zimm plot, e.g., a Berry plot or a Debye plot, the choice of plot depending on certain sample and solution characteristics such a long-chain branching, solution thermodynamics, etc.). In a Zimm plot, the slope of the line constructed by extrapolating the angular data to zero concentration is proportional to the z-average radius of gyration of the analyte in solution, $R_{G,z}$, while the slope of the line constructed by extrapolating the concentration data to zero angle is proportional to the second virial coefficient of the polymer solution, A_2. The common y-intercept of the two extrapolated lines equals the reciprocal of the weight-average molar mass, M_w^{-1}.

We now have two methods of determining the M_w of a polymer using static light scattering, SEC/SLS/DRI and off-line, batch-mode MALS. In each case, the specific refractive index increment $\partial n/\partial c$ enters into the calculations. In SEC/SLS/DRI, M_w is obtained by ratioing the signals from the SLS detector, S_{SLS}, and the DRI detector, S_{DRI}, at each chromatographic elution slice i after correction for interdetector delay (if the detectors are connected in series) or for split ratio (if connected in parallel), as per:

$$M_{w,i} \propto \frac{S_{SLS,i}}{S_{DRI,i}} \propto \frac{\Delta R(\theta)_i}{c_i} \tag{8}$$

Table 1 Specific refractive index increment ($\partial n/\partial c$) values of select polymers at 690 nm

Polymer	$\partial n/\partial c$ (mL g^{-1})[a]	N[b]	Concentration range (mg mL^{-1})	Solvent	Temperature (°C)[c]	DRI type[d]	References[e]
Alternan	0.066 ± 0.002[f] 0.072 ± 0.003[g]	7	0.4–3	DMSO	50	D	[26, 27]
Arabinogalactan[h]	0.096 ± 0.001	6	0.1–1	DMAc/LiCl	35	I	
Brominated polystyrene (PSBr)	0.110 ± 0.001	5	0.3–3	DMAc/LiCl	35	I	[28]
	0.137 ± 0.002	6	0.3–3	THF	25	I	
Polyacrylamide	0.162 ± 0.009	7	0.2–2	H_2O + NaCl + acetic acid[i]	30	D	[17]
Poly(acrylic acid)	0.0838 ± 0.0025	6	0.3–3	DMAc/LiCl	35	I	
Polyacrylonitrile	0.0757 ± 0.0011	6	0.2–2	DMAc/LiCl	35	I	
Poly(γ-benzyl-L-glutamate)	0.104 ± 0.001	5	0.3–3	DMAc/LiCl	35	I	[28]
Polycarbonate	0.1577 ± 0.0057	6	0.1–1	DMAc/LiCl	35	I	
	0.182 ± 0.001	5	0.5–5	THF	25	D	
Poly(*N,N*-dimethyl acrylamide)	0.150 ± 0.003	7	0.2–2	H_2O + NaCl + acetic acid[i]	30	D	[17]
Poly(ethylene glycol)	0.0617 ± 0.0015	7	0.3–3	DMAc/LiCl	35	I	
Poly(methyl methacrylate) (atactic)	0.052 ± 0.001	5	0.3–3	DMAc/LiCl	35	I	[28]
	0.087 ± 0.001 (M_w 3.50 × 10^5)[j]	6	1–6	THF	25	D	[29]
	0.086 ± 0.002 (M_w 4.95 × 10^5)	6	1–6	THF	25	D	[29]
	0.095 ± 0.002	7	0.5–5	*n*-Butyl chloride	32.6 and 35	D	[30]
	0.130 ± 0.001	7	0.5–5	Acetonitrile	29, 29.2, and 30	D	[30]
Poly(methyl methacrylate) (isotactic)	0.056 ± 0.001	5	0.3–3	DMAc/LiCl	35	I	[28]
Poly(methyl methacrylate) (solid sphere latex)	0.141 ± 0.003	7	0.2–2	HDC eluent[k]	25	D	[31]
Polystyrene (atactic)	0.146 ± 0.001 (M_w 3.05 × 10^5)	6	0.3–3	DMAc/LiCl	35	I	[28]
	0.190 ± 0.002 (M_w 4.9 × 10^4) 0.192 ± 0.002 (M_w 1.33 × 10^5) 0.195 ± 0.002 (M_w 1.99 × 10^5 and 1.51 × 10^5) 0.194 ± 0.002 (M_w 5.60 × 10^5) 0.198 ± 0.004 (M_w 7.59 × 10^5)	6	1–6	THF	25	D	[29]
	0.131 ± 0.004	7	0.5–5	Cyclohexane	24, 28, 32, 34 (θ),[l] 35	D	[30]
Polystyrene (solid and hollow sphere latexes)	0.237 ± 0.004	7	0.2–2	HDC eluent[k]	25	D	[32]
Poly(styrene-*co*-acrylonitrile)	0.132 ± 0.001 (23.6% acrylonitrile)	6	0.1–1	DMAc/LiCl	35	I	
Poly(styrene-*alt*-methyl methacrylate)	0.136 ± 0.002 (50.6% styrene)	6	1–6	THF	25	D	[29]
Poly(styrene-*b*-methyl methacrylate)	0.109 ± 0.001 (25.2% styrene) 0.168 ± 0.001 (74.9% styrene)	6	1–6	THF	25	D	[29]
Poly(styrene-*ran*-methyl methacrylate)	0.108 ± 0.004 (19.1% styrene) 0.111 ± 0.002 (21.5% styrene)	6	1–6	THF	25	D	[29]
Poly(vinyl acetate)	0.0401 ± 0.0011	6	0.3–3	DMAc/LiCl	35	I	
Poly(vinyl alcohol)	0.1033 ± 0.0008	6	0.3–2	DMAc/LiCl	35	I	
Poly(vinyl butyral)	0.0570 ± 0.0010[m] 0.0642 ± 0.0014[n]	6	0.3–3	DMAc/LiCl	35	I	[33][m]
Poly(*p*-vinylbenzyl chloride)	0.1818 ± 0.0009	7	1–6	THF	25	D	[34]
Poly(vinyl chloride)	0.112 ± 0.002	6	1–6	THF	25	D	[34]

Table 1 continued

Polymer	$\partial n/\partial c$ (mL g^{-1})[a]	N[b]	Concentration range (mg mL^{-1})	Solvent	Temperature (°C)[c]	DRI type[d]	References[e]
Silica (colloidal)	0.083 ± 0.002	6	0.2–2	H_2O/NaN_3	25	D	[35]
Silica (string-of-pearls)	0.079 ± 0.007	6	0.5–2	H_2O/NaN_3	25	D	[36]

Solvent abbreviations: DMSO: dimethylsulfoxide; DMAc/LiCl: *N,N*-dimethyl acetamide + 0.5% LiCl; THF: tetrahydrofuran; H_2O/NaN_3: H_2O + 0.02% NaN_3

[a] All uncertainties represent instrumental standard deviations

[b] Number of dissolutions, each of a different concentration, used for obtaining $\partial n/\partial c$

[c] In all cases, temperature was maintained to within ±0.01 °C

[d] I: Interferometric DRI (Optilab DSP, Wyatt Technology Corp., Santa Barbara, CA), D: Deflection-type DRI (Optilab rEX, Wyatt Technology Corp.)

[e] Where no reference given, refers to unpublished work

[f] Produced by *Leuconostoc mesenteroides* NRRL B-21297 alternansucrase

[g] Produced by *Leuconostoc mesenteroides* NRRL B-1355 mixed enzymes, twice-precipitated

[h] Type II, from wood larch

[i] 0.5 mol L^{-1} NaCl and 0.5 mol L^{-1} acetic acid

[j] All molar mass data in g mol^{-1}

[k] Aqueous solution of 0.002 mol L^{-1} HNa_2PO_4, 0.2% Brij-35, 0.2% formaldehyde, and 0.05% sodium dodecyl sulfate, at pH 7.5

[l] Denotes theta solvent/temperature conditions for 1.89 × 10^5 g mol^{-1} PS

[m] Mass percentages: vinyl butyral 79.3, vinyl alcohol 18.7, vinyl acetate 2.0

[n] Mass percentages: vinyl butyral 70, vinyl alcohol 30

Because the signal from the SLS detector is proportional to $\partial n/\partial c$ squared ($S_{SLS} \propto (\partial n/\partial c)^2$), whereas the signal from the refractometer is proportional to the first power of $\partial n/\partial c$ ($S_{DRI} \propto \partial n/\partial c$), the M_w obtained by SEC/SLS/DRI is also proportional to the first power of $\partial n/\partial c$. This means that an error of E % in $\partial n/\partial c$ will translate into an error of E % in M_w, when the latter is determined by SEC/SLS/DRI.

When M_w is determined by off-line, batch-mode MALS, however, the concentrations of the individual dissolutions have been determined "manually" (e.g., gravimetrically) by the analyst and are not measured by on-line DRI. Because M_w is now based only on the signals from the MALS photodiodes (or charge-coupled devices, in some instruments), it is proportional to $(\partial n/\partial c)^2$. Any error in $\partial n/\partial c$ propagates in off-line, batch-mode MALS as $[1 - (1 - E)^2] \times 100\%$ in molar mass error so that a, e.g., 10% error in $\partial n/\partial c$ corresponds to a $[1 - (1 - 0.1)^2] \times 100\% = 19\%$ error in M_w when the latter is determined by off-line, batch-mode MALS (as an aside, we note here that coupling the two detectors, DRI and MALS, to perform both off-line, batch-mode experiments with one set of sample dilutions, reduces sample consumption, disposables, time, and the generation of waste by a factor of two, while producing results—$\partial n/\partial c$, M_w, A_2, $R_{G,z}$—of comparable precision and accuracy to those obtained when each detector is decoupled from the other. See Ref. [24] for a more detailed discussion).

As can be seen from all the above discussions, accurate knowledge of $\partial n/\partial c$ is critical for obtaining accurate molar mass and related data for macromolecules natural and synthetic. Because of this, our group has generally tended to measure $\partial n/\partial c$ rather than relying on literature data, especially because in the latter all relevant experimental conditions are rarely stated. Additionally, our measurement technique of choice has been off-line, batch-mode DRI, rather than assuming 100% column recovery and 100% accurate peak integration. We have performed these types of measurements, originally at 690 nm and, more recently, at 660 nm, for the last 17 years (in both cases, values denote vacuum wavelengths of the incident radiation, with a standard deviation of ±10 nm). In this short communication, the author has attempted to compile all those results, along with any information relevant to use and/or reproduction. These $\partial n/\partial c$ values follow in Table 1 (for 690 nm) and Table 2 (for 660 nm). For informational purposes, it has been noted whether the 690 nm experiments involved an interferometric differential refractometer (denoted "I") or a deflection-type instrument (denoted "D"), though this datum should not have an effect on the calculated $\partial n/\partial c$ values (the reader is referred to Sect. 9.2.1 of Ref. [3] for a detailed discussion of the differences among these two types of differential refractometer). All 660 nm experiments were performed with a

Table 2 Specific refractive index increment (∂n/∂c) values for select polymers at 660 nm

Polymer	$\partial n/\partial c$ (mL g^{-1})[a]	N[b]	Concentration range (mg mL^{-1})	Solvent	Temperature (°C)[c]	References[d]
Bis(2-ethylhexyl adipate)	0.044 ± 0.002	6	0.2–3	THF	25	
Dextran	0.1350 ± 0.0004 (M_w 1.7 × 10^4)[e]	6	1–6	H_2O/NaN_3	25	[14]
	0.1288 ± 0.0016 (M_w 1.7 × 10^4)	6	1–6	H_2O + NaCl + acetic acid[f]	30	
Polyacrylamide	0.167 ± 0.006 (M_w 1.14 × 10^6)	6	0.5–5	$H_2O/NaNO_3$	25	
Polydimethylsiloxane	0.0016 ± 0.0006	6	1–7	THF	20	[25]
Poly(methyl methacrylate)	0.0853 ± 0.0015 (M_p 9.0 × 10^4)	5	0.5–5	THF	25	[37]
	0.0852 ± 0.0003 (M_w 8.4 × 10^5)	5	0.5–5	MIAK	25	
Polystyrene	0.1964 ± 0.0019 (M_w 2 × 10^5)	5	1–5	THF	25	[37, 38]
	0.194 ± 0.004 (M_w 4.3 × 10^5)	5	0.5–5	THF	25	
	0.194 ± 0.004 (M_w 4.3 × 10^5)	5	0.5–5	MIAK	25	
Poly(vinyl chloride)	0.0961 ± 0.0012 (M_w 1.2 × 10^4)	7	0.5–6	THF	25	
Pullulan	0.1362 ± 0.0011 (M_w 2.3 × 10^4)	6	0.5–5	H_2O/NaN_3	25	[14]

All measurements performed with deflection-type DRI (Optilab T-rEX, Wyatt Technology Corp.). Solvent abbreviations: H_2O/NaN_3: H_2O + 0.02% NaN_3; $H_2O/NaNO_3$: H_2O + 0.05 mol L^{-1} $NaNO_3$. MIAK: methyl isoamyl ketone; THF: tetrahydrofuran

[a] All uncertainties represent instrumental standard deviations

[b] Number of dissolutions, each of a different concentration, used for obtaining $\partial n/\partial c$

[c] In all cases, temperature was maintained to within ±0.01 °C

[d] Where no reference given, refers to unpublished work

[e] All molar mass data in g mol^{-1}

[f] 0.5 mol L^{-1} NaCl and 0.5 mol L^{-1} acetic acid

deflection-type instrument. All values were obtained by first-order fits of the data, without forcing through the origin. All Pearson's r values were 0.997 or greater, except for polydimethylsiloxane (PDMS), for which the value was 0.798 due to the vanishingly low $\partial n/\partial c$ of PDMS at the given experimental conditions (so low, in fact, that PDMS can be considered to be "spectroscopically invisible" at these conditions [25]).

The author hopes that the information presented here will be useful to the macromolecular characterization community, at large and, in particular, to macromolecular separation scientists. He also recommends that, if readers cannot find a literature value for the $\partial n/\partial c$ of their analyte at the exact conditions (solvent, temperature, wavelength) of their experiment, they should measure it themselves, most preferably by off-line, batch-mode DRI.

Acknowledgements While most of the measurements were performed by the author not all were and, as such, he benefitted from the work of many members of his laboratories over the years. These people include: Harriet Totoe, Michelle J. Smith, Imad A. Haidar Ahmad, Amandaa K. Brewer, Samantha L. Isenberg, Steven M. Rowland, Mallory J. Morris, Leena Pitkänen, and Pritish Sinha. 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.

Compliance with Ethical Standards

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

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